A Randomized Stratified Multicentre Phase II Clinical Trial of Single Agent ADI-PEG 20™ (Pegylated Arginine Deiminase) in Patients with Malignant Pleural Mesothelioma

ADAM (Arginine Deiminase And Mesothelioma)

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DATE: 12/01/2013
PRINCIPAL INVESTIGATOR DECLARATION:

I am in receipt of this protocol/amended page[s]/ instructions to amend existing copies of the above named study protocol. I have read and understood this trial protocol and will adhere fully to ICH GCP Guidelines in accordance with The Declaration of Helsinki.

In addition I agree to:

- alert the Chief Investigator to any Serious Adverse Events within 24hrs of knowledge of the event,
- make no additions or changes to any aspect of the protocol without consent of the Chief Investigator or written consent from the Sponsor except when necessary to protect the safety of subjects,
- I agree to maintain accurate records according ICH GCP and make those records available for inspection as requested by the study,
- I agree to inform any trial subjects that the drugs are being used for investigational purposes and will fully explain the requirements of the study and obtain full written informed consent before enrolling subjects onto the study.

PRINCIPAL INVESTIGATOR: …………………………...…………………………..     TEL: …………..…………..…

SIGNATURE: ………………………………………………….…………………… DATE: …....……..………..…

ADDRESS:

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SITE NAME: …………………………………………………………………….… SITE NUMBER: ………………..

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- Lung Cancer and Mesothelioma Fund, Barts and the London Charity

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</thead>
<tbody>
<tr>
<td><strong>Short Title</strong></td>
<td>ADAM (Arginine deiminase &amp; Mesothelioma)</td>
</tr>
<tr>
<td><strong>Protocol Version Number and Date</strong></td>
<td>Version 9.0; 14&lt;sup&gt;th&lt;/sup&gt; September 2012</td>
</tr>
<tr>
<td><strong>Methodology</strong></td>
<td>Phase II randomized controlled clinical trial: Best-Supportive Care (Arm A) versus ADI-PEG 20™ and Best Supportive Care (Arm B)</td>
</tr>
<tr>
<td><strong>Study Duration</strong></td>
<td>The total duration of the study will be up to 36 months (18 months for recruitment, 6 months for treatment and at least 6 months follow up for each patient and 6 months for study closure and reporting). The ‘end of trial’ is defined as 6 months after the end of treatment for the last patient recruited. Long-term follow up for overall survival will continue.</td>
</tr>
<tr>
<td><strong>Study Centres</strong></td>
<td>Multi-centre</td>
</tr>
</tbody>
</table>
| **Objectives** | **Primary Objective:** To examine progression free survival in Arms A and B  
**Secondary Objectives:** To examine response rate, overall survival, time to progression and toxicity |
| **Number of Subjects/Patients** | 66 patients: 22 in Arm A and 44 in Arm B |
| **Main Inclusion Criteria** | Patients with advanced (non-resectable) malignant pleural mesothelioma:-  
- ASS-negative MPM by immunohistochemistry  
- CT-assessable disease by modified RECIST criteria  
- Chemo-naïve (i.e. first-line) or prior platinum-based combination chemotherapy.  
- Performance status 0-1  
- Adequate haematological status and hepatic function  
- Willing to give consent |
| **Sample size** | 22 patients recruited into arm A and 44 patients recruited into Arm B. ADI-PEG 20™ would be worth considering in a phase III trial if it were associated with a progression-free survival hazard ratio of 0.60 or smaller. |
# Glossary of Terms and Abbreviations

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>ADI-PEG 20™</td>
<td>Pegylated arginine deiminase (ADI)</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse Event</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Transaminase</td>
</tr>
<tr>
<td>ANC</td>
<td>Absolute Neutrophil Count</td>
</tr>
<tr>
<td>AR</td>
<td>Adverse Reaction</td>
</tr>
<tr>
<td>ASS</td>
<td>Argininosuccinatesynthetase (also, ASS1 and AS)</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Transaminase</td>
</tr>
<tr>
<td>ASR</td>
<td>Annual Safety Report</td>
</tr>
<tr>
<td>BSA</td>
<td>Body Surface Area</td>
</tr>
<tr>
<td>BSC</td>
<td>Best supportive care</td>
</tr>
<tr>
<td>CA</td>
<td>Competent Authority</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete Blood Count</td>
</tr>
<tr>
<td>CECM</td>
<td>Centre for Experimental Cancer Medicine</td>
</tr>
<tr>
<td>CI</td>
<td>Chief Investigator</td>
</tr>
<tr>
<td>CR</td>
<td>Complete Response</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CRO</td>
<td>Contract Research Organisation</td>
</tr>
<tr>
<td>CR-UK</td>
<td>Cancer Research UK</td>
</tr>
<tr>
<td>CTA</td>
<td>Clinical Trial Authorisation</td>
</tr>
<tr>
<td>CT</td>
<td>Computerized tomography</td>
</tr>
<tr>
<td>CTC</td>
<td>Common Toxicity Criteria (Adverse Events)</td>
</tr>
<tr>
<td>CTAAC</td>
<td>Clinical Trials Advisory &amp; Awards Committee</td>
</tr>
<tr>
<td>CTIMP</td>
<td>Clinical Trial of Investigational Medicinal Product</td>
</tr>
<tr>
<td>IDMC</td>
<td>Independent Data Monitoring Committee</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EMEA</td>
<td>European Medicines Agency</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>EUCTD</td>
<td>European Clinical Trials Directive</td>
</tr>
<tr>
<td>EudraCT</td>
<td>European Clinical Trials Database</td>
</tr>
<tr>
<td>EudraVIGILANCE</td>
<td>European database for Pharmacovigilance</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GAFREC</td>
<td>Governance Arrangements for NHS Research Ethics</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GMP</td>
<td>Good Manufacturing Practice</td>
</tr>
<tr>
<td>IB</td>
<td>Investigator Brochure</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median 50% Inhibitory Concentration</td>
</tr>
<tr>
<td>ICF</td>
<td>Informed Consent Form</td>
</tr>
<tr>
<td>IDMC</td>
<td>Independent Data Monitoring Committee</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>i.m.</td>
<td>Intramuscular (injection)</td>
</tr>
<tr>
<td>IMP</td>
<td>Investigational Medicinal Product</td>
</tr>
<tr>
<td>IMPD</td>
<td>Investigational Medicinal Product Dossier</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>ISRCTN</td>
<td>International Standard Randomized</td>
</tr>
<tr>
<td>JRO</td>
<td>Joint R&amp;D Office</td>
</tr>
<tr>
<td>MA</td>
<td>Marketing Authorisation</td>
</tr>
<tr>
<td>MHRA</td>
<td>Medicines and Healthcare products Regulatory Agency</td>
</tr>
<tr>
<td>MPM</td>
<td>Malignant Pleural Mesothelioma</td>
</tr>
<tr>
<td>MR</td>
<td>Minor Response</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Council</td>
</tr>
<tr>
<td>MS</td>
<td>Member State</td>
</tr>
<tr>
<td>Main REC/IRB</td>
<td>Main Research Ethics Committee</td>
</tr>
<tr>
<td>NCRN</td>
<td>National Cancer Research Network</td>
</tr>
<tr>
<td>NHS R&amp;D</td>
<td>National Health Service Research &amp; Development</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Health and Clinical Excellence</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamic(s)</td>
</tr>
<tr>
<td>PD rate</td>
<td>Progressive Disease Rate</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PH Eur</td>
<td>European Pharmacopoeia</td>
</tr>
<tr>
<td>PI</td>
<td>Principle Investigator</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetic(s)</td>
</tr>
<tr>
<td>PR</td>
<td>Partial Response</td>
</tr>
<tr>
<td>PFS</td>
<td>Progression Free Survival</td>
</tr>
<tr>
<td>QA</td>
<td>Quality Assurance</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>QP</td>
<td>Qualified Person for release of trial drug</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>QMUL</td>
<td>Queen Mary University of London</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Control Trial</td>
</tr>
<tr>
<td>REC/IRB</td>
<td>Research Ethics Committee</td>
</tr>
<tr>
<td>SAR</td>
<td>Serious Adverse Reaction</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SD</td>
<td>Stable Disease</td>
</tr>
<tr>
<td>SDV</td>
<td>Source Document Verification</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedures</td>
</tr>
<tr>
<td>SmPC</td>
<td>Summary of Product Characteristics</td>
</tr>
<tr>
<td>SMRP</td>
<td>Mesothelin-related peptides</td>
</tr>
<tr>
<td>SSA</td>
<td>Site Specific Assessment</td>
</tr>
<tr>
<td>SSAR</td>
<td>Suspected Serious Adverse Reaction</td>
</tr>
<tr>
<td>Subject</td>
<td>An individual who takes part in a clinical trial</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected Unexpected Serious Adverse Reaction</td>
</tr>
<tr>
<td>TMG</td>
<td>Trial Management Group</td>
</tr>
<tr>
<td>TSC</td>
<td>Trial Steering Committee</td>
</tr>
<tr>
<td>TTP</td>
<td>Time to Disease Progression</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopoeia</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cells</td>
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</tbody>
</table>
1. INTRODUCTION

1.1 Background - Mesothelioma

Malignant pleural mesothelioma (MPM) is a rapidly lethal cancer arising from the parietal pleural mesothelium, and is associated with exposure to asbestos. MPM is characterized by local invasion of adjacent structures including the chest wall, mediastinum, diaphragm and pericardium resulting in progressive shortness of breath. Median survival with best supportive care alone is approximately 9-12 months. The incidence of mesothelioma is rising in the United Kingdom and Europe. A projected doubling of cases has been predicted within the next two decades [1].

There is no standard of chemotherapy for mesothelioma [2]. Systemic therapy with cytotoxic drugs is the mainstay of treatment. Despite the existence of a large number of phase II clinical studies previously conducted, there exists no robust randomized data confirming a survival advantage over best supportive care. For example, the recently completed MSO-1 study conducted by the MRC Clinical Trials Unit, randomizing patients to vinorelbine versus mitomycin/vindesine/cisplatin versus active symptom control (ASC) failed to show a benefit for chemotherapy when added to ASC in terms of overall survival or improvement in quality of life [3]. Objective response rates (PR and CR) for single cytotoxic agents range from 10 to 20%. For combination therapy, objective response rates typically range between 20-40%, as documented with the NICE-approved systemic chemotherapy for MPM, pemetrexed and cisplatin, with response rates double that of single-agent cisplatin (40% versus 20%) [4].

Cytotoxic therapy relies in part on the induction of programmed cell death or apoptosis for efficacy. Anti-cancer activity is typically limited by intrinsic apoptosis resistance in incurable, metastatic solid tumours [5]. MPM typically exhibits significant insensitivity to induction of apoptosis, however the molecular basis of this phenotype has not been fully elucidated. There is an urgent need for novel, active treatments for MPM. It is likely that conventional cytotoxic therapy alone has reached a therapeutic plateau [6, 7]. Better understanding of MPM biology, coupled to targeted therapies with rationally designed mechanism of action has the potential to improve the outlook of patients with this malignancy. However, the rapid evaluation of such promising compounds is essential.

1.2 Investigational Medicinal Product (IMP) – ADI-PEG 20™

ADI-PEG 20™ (or ADI-SS PEG 20,000 mw) is a pegylated form of the enzyme arginine deiminase derived from the bacterium, Mycoplasma hominis. ADI-PEG 20™ has obtained orphan drug approval in the US the treatment of patients with hepatocellular carcinoma and
malignant melanoma and in Europe for the treatment of hepatocellular carcinoma (HCC) [8].

ADI-PEG 20\textsuperscript{TM} degrades arginine, an essential amino acid for the growth of tumour cells that lack expression of the rate-limiting enzyme for arginine production, argininosuccinate synthetase (ASS). Although arginine deprivation therapy as an anticancer strategy has been investigated for several decades [9-13], it is only recently that arginine depletion has shown encouraging activity in patients with specific tumour types [14]. The arginine degrading drug, ADI-PEG 20\textsuperscript{TM}, with its prolonged half-life and ability to safely and specifically suppress detectable levels of circulating arginine, has been assessed in both preclinical models and in patients with malignant melanoma and hepatocellular carcinoma. Since cells in our body express ASS ubiquitously, ADI-PEG 20\textsuperscript{TM} selectively targets ASS negative tumour cells (i.e. arginine auxotrophs), such as melanoma and HCC, for destruction [15]. We have recently extended these observations to malignant mesothelioma showing that arginine depletion triggers tumour cell apoptosis or programmed cell death [16a,16b]. Although the mechanism remains unclear, ADI-PEG 20\textsuperscript{TM} may affect several arginine-dependent processes within ASS-negative tumours, including the production of polyamines and nitric oxide, generation of tumour stroma and angiogenesis [17, 18]. Thus, ASS loss may represent a form of metabolic reprogramming favouring tumorigenesis and a worse phenotype, whilst at the same time revealing an Achilles' heel susceptible to arginine deprivation with ADI-PEG 20\textsuperscript{TM} [19].

1.3 Preclinical Data

Pharmacokinetic/PharmacodynamicProfile

Initial \textit{in vitro} studies of arginine deiminase (ADI) revealed that this agent was effective in killing HCC and melanoma cells by depleting arginine found in the culture medium. ADI degrades arginine into citrulline and ammonia [20, 21]. Although the pharmacodynamic (PD) endpoint of arginine depletion could be readily measured \textit{in vivo} (using high performance liquid chromatography), ADI had poor availability in mammals due to a short circulating half-life (~5hr) and high antigenicity, being derived from mycoplasma [22]. The pharmacokinetic (PK) profile of ADI was substantially improved upon pegylation [23]. Thus, 5U of i.m. ADI-PEG 20\textsuperscript{TM} lowered plasma arginine to undetectable levels for ~7 days in the mouse, whereas 5U i.m. of the native (non-pegylated) compound lowered plasma arginine by 40-50% at 24hrs, returning to baseline by 48hrs [15]. Superiority of ADI-PEG 20\textsuperscript{TM} compared to the native (non-pegylated) drug in suppressing tumour growth and extending survival was confirmed in xenograft murine models of both human HCC and melanoma cell lines [15]. Furthermore, the latter studies, and more recent work with MPM cell lines and
human pancreatic and prostate cancer xenograft models, confirmed that lack of ASS expression equated with the therapeutic effect of ADI-PEG 20™ [16, 24, 25].

**Toxicology**

ADI-PEG 20™ selectively depletes arginine, a non-essential amino acid for many mammalian organisms, including humans, due to the ubiquitous expression of the biosynthetic enzyme ASS. However, for some carnivores such as the cat and ferret, arginine is an essential dietary component, and depletion of this amino acid leads to encephalopathy and death [18, 26]. No adverse effects of arginine depletion using ADI-PEG 20™ were noticed in the xenograft tumour studies discussed above [15]. The only toxic byproduct of ADI activity is ammonia and is readily deactivated by the liver via the urea cycle.

**1.4 Clinical Data to Date**

**Human Pharmacokinetics/Pharmacodynamics**

The clinical pharmacology characterization of ADI-PEG 20™ has been determined from two published Phase I/II studies in subjects with hepatocellular cancer and melanoma.

**Efficacy**

Izzo et al reported a 47% (complete and partial) response rate in a phase I/II study of patients with advanced hepatocellular carcinoma [27], and Ascierto et al documented an overall 25% (complete and partial) response rate in patients with advanced metastatic melanoma treated with the same drug [28]. Several patients with prolonged response were observed in both studies, with more recent phase II data replicating the efficacy seen in patients with advanced melanoma (Dr Lynn Feun, Miami, US, personal communication). The results to date emphasize the need for further randomized studies in patients with ASS-negative tumours. Of note, many epithelial tumours are not amenable to this approach due to an abundance of ASS expression [30-32].

Recent preclinical and clinical studies indicate that higher doses of ADI-PEG 20™ may be more effective without compromising safety. A recent animal xenograft model of human small cell lung cancer (SCLC) presented by Kelly et al revealed that there is better disease control with higher doses of ADI-PEG 20™. Also, 320 IU/m² (and higher i.e. 640 IU/m²) has already been used safely in trials of patients with hepatocellular cancer (HCC) and melanoma. The most recent ADI-PEG 20™ trial in patients with melanoma presented by Feun et al at ASCO 2010 recommended that further investigation of ADI-PEG 20™ is warranted at the higher dose (ie 320 IU/m² or higher). All the objective responses in this trial were observed in patients who received the 320 IU/m² dose compared with none in the
160IU/m² dose. Similarly, in view of these updated preclinical and clinical results, the 320 IU/m² (=36.8 mg/m²) dose will be applied in the present mesothelioma study [38-41].

Finally, recent data has emerged from preclinical and clinical studies indicating that ASS1 negativity may be a marker of platinum resistance in malignancy [42, 43]. Overexpression of ASS1 conferred up to a 10-fold increase in ovarian cancer cell line sensitivity to platinum drugs whilst conferring resistance to arginine deprivation. In contrast, ASS1 suppression using RNA interference increased tumour cell sensitivity to ADI-PEG 20™ but led to a decrease in platinum sensitivity. Two independent clinical datasets of patients with ovarian cancer treated with platinum-containing regimens support these preclinical observations, with de novo platinum chemoresistance and a decreased relapse-free interval linked to downregulation of ASS1 [42, 43]. Thus, we will also evaluate the role of ASS1 as a biomarker of sensitivity to ADI-PEG 20™ in patients previously exposed to platinum containing regimens; in practice this will include prior pemetrexed/platinum and/or gemcitabine/platinum exposure.

1.5 Rationale and Risk/Benefits

Rationale

MPM is a profoundly apoptosis resistant malignancy with the benefit of palliative chemotherapy confined to a subgroup of patients (<40%). The rationale underlying arginine depletion with ADI-PEG 20™ is that arginine auxotrophic tumours undergo programmed cell death or apoptosis, due to an absence of the rate-limiting enzyme for arginine biosynthesis, ASS. We have documented that ASS is commonly absent in patients with MPM and that ADI-PEG 20™ induces death of ASS negative MPM tumours. This study seeks to define the role of ADI-PEG 20™ in patients with confirmed ASS-negative MPM, whilst avoiding the known difficulties inherent in MPM response assessments, with a best supportive care control arm. Patients who are either chemonaive (i.e. ‘window of opportunity’ stratification) or who have been exposed to platinum-based combination chemotherapy (i.e ‘prior treatment’ stratification) will be randomized into this study.

Risks

With regard to safety no significant toxicities were observed in the published studies of ADI-PEG 20™ in patients with hepatocellular and melanoma, with local i.m. injection site reactions (swelling and tenderness) subsiding within 24-48 hrs of administration of the drug [27, 28]. Several instances of grade 1 and II elevated biochemical abnormalities were noted in these phase I/II studies with no clear correlation between severity and dose of ADI-PEG
Hyperuricaemia, which occurred only in ADI-PEG 20™ responding patients with hepatocellular carcinoma, was considered to be secondary to tumour lysis and was promptly treated with allopurinol or uricase.

Following on from the responses noted in the two published studies above, over 300 patients have now been treated with ADI-PEG 20™ in the US, Europe and Taiwan with advanced hepatocellular cancer and melanoma. The most recent experience is with an ongoing phase I/II study in patients in New York (US) with advanced metastatic melanoma [29], following on from a previously published study performed jointly in Europe (Naples, Italy) and the US (Miami, Fl) [28]. Both studies have confirmed previous findings that ADI-PEG 20™ is largely well tolerated, with few side effects compared to conventional cytotoxic chemotherapy. The Naples/Miami trial has been without significant adverse events noting local i.m. injection site reactions only, whereas two patients on the Memorial Sloan Kettering Cancer Centre and New York University study developed grade III seizures that resolved without sequelae, and which were considered possibly and/or likely ADI-PEG 20™ related adverse events.

**Benefits**

ADI-PEG 20™ is a novel investigational agent in cancer and may be effective in certain patients with arginine auxotrophic tumours. Phase I/II clinical studies performed in patients with liver cancer and melanoma have shown signs of encouraging clinical activity in a proportion of patients. Patients with MPM currently have very few options and testing ADI-PEG 20™ in ASS-negative disease may provide efficacy data (with a safer toxicity profile than traditional cytotoxic therapy) permitting further exploration in a randomized phase III study.

## 2 STUDY AIMS AND OBJECTIVES

### 2.1 AIMS OF THE TRIAL

To examine whether the arginine depleting drug, ADI-PEG 20™, might be effective as a targeted therapy in patients with ASS-negative malignant pleural mesothelioma.

### 2.2 Primary objectives

To examine the progression free survival in those receiving ADI-PEG 20™, and in the control group. The primary endpoint is progression-free survival.

### 2.3 Secondary objectives

The following will be assessed in control and ADI-PEG-20™ groups:

1. To measure the response rate (stable disease or SD, partial response or PR and complete response or CR).
ii) To measure overall survival  
iii) To measure time to progression  
iv) To assess safety (adverse events).

2.4 Translational and Exploratory Objectives

Translational Objectives:

i) To measure peripheral blood pharmacodynamics (arginine and citrulline levels) and immunogenicity (antibodies to ADI-PEG 20\textsuperscript{TM}), using a vial of blood obtained each week in clinic. This testing will be performed by Polaris Group as previously published [27, 28].

ii) To measure serum mesothelin as a tumour marker for the management of patients with MPM.

Exploratory Objectives:

The analyses specified below are the current plans for these samples. It is recognised that during the course of this study new scientific data and techniques may become available which may result in adding to or changing the specified analyses. Any changes in these analyses will not require a protocol amendment in their own right, but will be included in the next substantial amendment.

i) Pharmacogenomics

Patients who progress, having initially responded to ADI-PEG 20\textsuperscript{TM}, will be approached to obtain a tumour biopsy using US or CT-guided sampling (Informed Consent Form C). Samples will be stored in paraffin and a cDNA library established to enable microarray analysis. Supervised and unsupervised classification of genes that predict resistance, progression free survival, and overall survival to ADI-PEG 20\textsuperscript{TM} will be conducted to establish clinically relevant biomarkers.

Tumour and peripheral blood leukocyte genomic DNA will be obtained to enable prospective pharmacogenomic studies. These may include analysis of gene copy number by microarray-based comparative genomic hybridization (CGH) analysis and single nucleotide polymorphism (SNP) microarray analyses. Methylation-specific (MS)-PCR of the ASS promoter, and/or pyrosequencing, and methylation analysis using the Illuminar Methylation 500K assay.

ii) Metabolomics

Serum will be sampled pre-treatment and monthly during the first 6 months of the study. Plasma and serum will be banked and utilized for the identification of predictive and
prognostic serum markers using 1H-NMR, Mass Spectrometry and HPLC with appropriate supervised and unsupervised classification algorithms.

iii) **Inflammatory pathway analysis**
The effect of ADI-PEG 20™ on cytokine levels will be measured using plasma and serum samples collected during the first 6 months of the treatment phase of the study.

iv) **Molecular Pathology**
Formalin-fixed, paraffin embedded diagnostic tissue blocks will be centralized for 0.6mm tissue core sampling to enable manufacture of a tissue microarray. This will be performed using tumour biopsy material obtained in addition to that tested for ASS expression and will enable rapid testing of pathways relevant to mesothelioma tumorigenesis and arginine metabolism to facilitate further developments in this novel area. For patients who have had multiple consecutive biopsies e.g. repeat resections, samples of these tissue blocks may also be requested for analysis.

v) **Nitrate and dimethylarginine levels**
We will measure nitrate, nitrite and dimethylarginine levels using patient blood samples (as per (ii) above).

vi) **Radiology**
CT image files will be banked digitally and archived.

vii) **Pleural Fluid**
Patients with accessible pleural fluid prior to the ‘Treatment Phase’ of the study may have this drained following informed consent. Tumour cells will be extracted, propagated ex vivo and studied to improve our understanding of the role of arginine depletion in mesothelioma tumorigenesis. (For indicated sites only)

viii) **Clinical Dataset**
The clinical database will be anonymised for use in correlative analyses in conjunction with pharmacogenomic, proteomic, molecular pathological and cell biological studies.
3 INVESTIGATIONAL PLAN

3.1 Overall Design

3.1.1 Study Groups

This is a randomised, multicentre phase II study in subjects with malignant pleural mesothelioma. Patients will be treated in the first line setting or following platinum-based combination chemotherapy.

3.1.2 Treatment Duration

There is a pre-screening period during which consent for the study is taken (Informed Consent Form A) and a biopsy sent for ASS status confirmation in order to confirm eligibility. Subsequently, there is a 28 day screening period during which patient eligibility is confirmed further and randomisation is completed (Informed Consent Form B). The duration in both arms of the study will be for a period of 24 weeks (i.e. 6 months). All patients will then be followed up from the end of study treatment, in order to evaluate overall survival. All fit patients will be offered systemic chemotherapy upon progression (if chemo-naïve), an alternative trial, or standard management according to local policy. Continuation of treatment for patients on Arm B (BSC + ADI-PEG 20™) who have stable disease or better after 6 months of treatment will be considered on an individual case basis and is at the discretion of the Chief Investigator, Polaris and the patient’s local clinical team.

3.1.3 Study Duration

The total duration of the study will be 36 months (18 months for recruitment, 6 months for treatment and at least 6 months follow up for each patient; and 6 months for study closure and reporting). Long-term follow up for overall survival will continue.

The end of the trial is defined as 6 months after the end of treatment for the last patient recruited or, if he/she has died before then, for the last patient alive. After this, all patients will continue to be followed up for the safety and efficacy endpoints (as an observational study).

3.2 Clinical Trial Schema

- Study Initiation (Pre-Treatment) Phase

Patient Consent, Registration and Screening (see Section 4) will be performed prior to enrolment onto the study treatment phase of the trial outlined below:
Table 1: Study Assessment Schedule

<table>
<thead>
<tr>
<th>Study Phase</th>
<th>Screening Phase</th>
<th>Treatment Phase</th>
<th>Follow-up Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-screening</td>
<td>Screening</td>
<td>Month 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Months 1, 2</td>
<td>Months 1, 2</td>
</tr>
<tr>
<td>Day 0</td>
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<td>Week 4</td>
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<tr>
<td>Week 12</td>
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<tr>
<td>Week 20</td>
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<tr>
<td>Week 28</td>
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<td>Week 36</td>
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<td>Week 44</td>
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<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Week 52</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

1. If patient has received chemotherapy following the initial diagnostic biopsy, the biopsy sent for assessment should be from post-chemotherapy (see Section 5.2 for further details).
2. Haemoglobin, WBC, Platelets, ANC, ALT, AST, Alkaline Phosphatase, Serum albumin, Urea, Uric Acid, Calcium (corrected), Creatinine, Phosphate, Potassium, Sodium.
3. Only for patients at indicated sites.
4. Serum Uric Acid result must be checked prior to each dose of ADI-PEG 20 (Arm B only) on Day 1 of each cycle.
5. Ideally within 2 weeks prior to randomisation (up to 4 weeks will not be considered a deviation) – results must be obtained and reviewed prior to randomisation.
6. To be performed between Day 22 of cycle and Day 1 of subsequent cycle.
7. To be performed between 2nd and 4th week after commencing treatment.
8. To be performed if a scan has not been taken within 4 weeks of completion of treatment.
9. To be performed 6 months after completion of treatment or sooner if ‘clinically appropriate’ i.e. at signs of disease progression.
10. For females of child bearing potential only.
11. To be recorded at baseline, +20, +40 and +60 minutes post dosing for patients on Arm B.

See Attachment F for a study assessment schedule for patients who continue treatment beyond 6 months (See section 3.1.2)
3.3 Overview of Study population

This study will enrol patients with a confirmed diagnosis of malignant pleural mesothelioma on histology in either the first-line setting (chemo-naive) or following exposure to platinum-based combination chemotherapy, with at least a 4 week interval from the last treatment episode. Only patients with a good performance status (PS 0 or 1) will be considered. Those patients whose biopsy is confirmed as ASS negative will be eligible to proceed through to the screening phase of the study.

3.4 Target Accrual

Sixty-six subjects will participate in this study (see section 10, Statistical methods). The study will enroll patients into two groups: Arm A, a control group consisting of best supportive care (BSC) with 22 patients; and Arm B, consisting of ADI-PEG-20™ and BSC with 44 patients.

4. SUBJECT SELECTION

4.1 Inclusion Criteria

1. Males and Females aged 18 years and older. (There is no upper age limit)
2. Histopathological evidence of ASS-negative MPM. All biopsies will be reviewed for ASS expression using immunohistochemistry. Central lab confirmation is required before randomization.
3. Performance status ECOG ≤1. Life expectancy should be greater than 3 months (see Appendix D).
4. Chemo-naive patients

   OR,

   Patients who have been previously treated with platinum-based combination chemotherapy with progressive disease at entry. In the event of a baseline diagnostic ASS-positive test, a repeat biopsy confirming loss of ASS expression will be required post platinum-based combination chemotherapy, with at least a 4 week interval from the last treatment episode.
5. CT measurable disease by modified RECIST criteria (see Appendix B)
6. Adequate bone marrow function, or supported through treatment:
1. Haemoglobin 10g/dl or greater.
2. White cell count 2 x 10^9/L or greater, neutrophil count 1.5 x 10^9/L or greater
3. Platelets 75 x 10^9 /L or greater.
7. Adequate hepatic function (AST and ALT < 3 x upper limit of normal; bilirubin<1.5 x upper limit of normal)
8. Creatinine clearance > 30ml/min
9. Able to give written informed consent to participate.

4.2 Exclusion Criteria

1. Participation in another clinical trial using an investigational agent.
2. Patients with surgically resectable disease
3. Recurrent pleural effusion (not pleurodesed)
4. Receipt of extensive radiation (hemi-thorax) therapy within 6 weeks before enrollment. Radiation to chest port sites following thoracotomy is permitted.
5. A history of prior malignant tumour, unless the patient has been without evidence of disease for at least three years, or the tumour was a non-melanoma skin tumour or in-situ cervix carcinoma.
6. Symptomatic or known brain or leptomeningeal metastases
7. Uncontrolled or severe cardiovascular disease including myocardial infarction within 6 months of enrollment.
8. New York Heart Association (NYHA) Class III or IV heart failure (Attachment 10, NYHA Classification of Cardiac Disease), uncontrolled angina, clinically significant pericardial disease, or cardiac amyloidosis.
9. Serious medical (e.g. uncontrolled diabetes, hepatic disease, infection, uncontrolled gout) or psychiatric illness likely to interfere with participation in this clinical study.
11. Patients of child-bearing age must not become pregnant. Females of childbearing potential must have a negative pregnancy test within 7 days prior to being registered for protocol therapy. All patients enrolled on the study must agree to use acceptable birth control measures whilst on the study; using both barrier and hormonal methods. Patients that are surgically sterile are also eligible to participate in this study.
12. Females must not be breastfeeding.

14. Preplanned surgery or procedures that would interfere with the study protocol.

15. Allergy to pegylated products

16. Exposure to another investigational drug within 4 weeks prior to start of study treatment

17. Patients currently receiving Low Molecular Weight Heparin or Warfarin

### 4.3 Subject Recruitment and Screening

Potential patients will be identified at local multidisciplinary team thoracic oncology meetings. Eligible patients will be approached in the outpatient clinic by a member of the research team. To be considered for this trial a patient must have an ECOG performance status of 0-1 (see Attachment D) with an advanced CT-measurable malignant pleural mesothelioma that on subsequent testing is ASS negative. Patients that are chemo-naive or patients who have been previously treated with platinum-based combination chemotherapy may be considered for enrollment.

### 4.4 Withdrawal of Subjects

#### 4.4.1 When and How to Withdraw Subjects

The duration of the study treatment period for a patient is 24 weeks. Further treatment is at the discretion of the treating physician in consultation with the Chief Investigator and Polaris Pharmaceuticals Inc.

Subjects who do not receive any treatment for any reason will not be considered to be evaluable. Any patient who has entered the treatment phase of the study will be considered evaluable.

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care.

A subject will be withdrawn from the study for any of the following reasons:

- Withdrawal of consent.
- Lost to follow-up.
- The investigator believes that for safety reasons (e.g. adverse event) it is in the best interest of the subject to stop treatment.
- The subject becomes pregnant.
- The subject has disease progression (on CT or FDG-PET/CT).
- Non–compliance.
• Protocol violations.
• Intercurrent illness.
• Patient with CR who is treated for at least 2 cycles post CR.
• Missed or delayed doses of ADI-PEG 20™ (see section 6.7).

When a subject withdraws before completing the study, the reason for withdrawal is to be documented on the CRF and in the source documents. The patient does not need to give a reason for withdrawal, but the Principal Investigator should make a reasonable effort to document a reason.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

All patients who withdraw from the trial protocol for any reason above will be followed up for survival data only. Data will be collected regarding any subsequent treatments or procedures.

5. STUDY PROCEDURES AND SCHEDULE OF ASSESSMENTS

5.1 Informed consent procedures

It is the responsibility of the Investigator, or a person delegated by the Investigator to obtain written informed consent (Informed Consent Form A) from each subject prior to participation in this study, following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. Ample time (at least 24 hours) must be given for consideration by the patient before taking part. The PI must record when the patient information leaflet (PIL) has been given to the patient.

The Investigator or designee must explain that subjects are completely free to refuse to enter the study or to withdraw at any time during the study, for any reason.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects, including those already being treated, should be informed of the new information, giving a copy of the revised form and give their consent to continue in the study.

5.2 Study Procedures: Registration and Screening Phase

To enrol a patient onto the ADAM study the following procedures must take place:

a) Patient provides written informed consent (Informed Consent Form A)
b) Patient is registered with the ADAM Trial Co-ordinator
c) Central lab confirmation of ASS negative tumour
d) Patient undergoes all screening procedures as per assessment schedule (Table 1)
e) Patient provides written informed consent (Informed Consent Form B) to be
randomised, if applicable, (see Randomisation Procedure below)

Refer to the ADAM Patient Registration and Randomisation Manual for further details
on the patient registration and randomisation procedures.

Registration Procedures

Once a patient has consented to participate in this trial by signing Informed Consent Form
A, the Trial Co-ordinator at the Centre for Experimental Cancer Medicine will be notified
using the Registration form.

Registration Telephone: +44 (0) 20 7882 8764
Registration Fax: +44 (0) 20 7882 8409
Monday – Friday, 9am – 5pm
(excluding Bank Holidays)

Each patient will be allocated a unique patient screening number and the site personnel
informed of this by fax.

Refer to the ADAM Patient Registration and Randomisation Manual for further details.

Pre - Screening

Pre-screening can only occur after Informed Consent Form A has been signed and the
patient has been registered with the Centre for Experimental Medicine. During the pre-
screening period tumoral ASS status will be assessed by Central Lab:

A paraffin tissue block (which will be returned once cut), or if this is not readily available, at
least 8 unstained paraffin sections, of the patient’s mesothelioma tumour must be provided
for this assessment. Please see ADAM Site Lab Manual for further details.

Confirmation of ASS MPM tumoral negativity by Central Lab will be forwarded to the Trial
coordinator, who will communicate the result to the participating site. Patients will then be
informed of the result and undergo subsequent screening procedures if the biopsy is
negative for ASS and they consent to do so.
In addition, all patients in the pre-screening phase of the trial will have research blood and urine samples collected for pharmacodynamic and metabolomic analysis.

Should a patient ‘fail’ pre-screening as their tumour is ASS positive, they may be reconsented at a later date and have a new biopsy retested. In these instances patients should be registered in full and a new screening number will be assigned.

**Screening:**

Upon confirmation that a patient has an ASS negative tumour they should be asked to sign Informed Consent Form B. Then, the following procedures will need to be completed prior to randomisation:

1. Complete medical history recorded

2. Serum β-hCG (screening) and urine pregnancy (Day 1 before dosing) tests will be performed for all women of childbearing potential; if results are positive the subject will not be enrolled on the study.

3. A baseline CT and FDG-PET/CT scan within 2 weeks prior to randomisation. *NOTE: Only NCRI accredited PET centres may perform FDG-PET/CT scans in this trial.*

4. Physical examination, vital signs, ECG and ECOG performance status assessment.

5. Standard Biochemistry (including uric acid tests) and Haematology

6. LCSS Assessment

Please refer to the Assessment Schedule (Table 1) for a complete list of screening procedures.

### 5.3 Method for assigning subjects to treatment group

After eligibility has been confirmed, patients will be randomised to the trial. Randomisation will be performed centrally by the Centre for Experimental Cancer Medicine.
Each patient will be allocated a unique patient trial number and the site personnel informed of the patient’s treatment allocation by fax.

Minimisation (2:1) will be used with gender, histological subtype of mesothelioma (non-sarcomatoid and sarcomatoid), prior treatment (chemo-naive and prior treatment with platinum-based combination chemotherapy) and recruitment site as stratification factors.

Refer to the ADAM Patient Registration and Randomisation Manual for further details on the patient registration and randomisation procedures.
5.4 Treatment procedures – Schedule and Drug Administration

For details of all assessments to be completed during the study please refer to the Assessment Schedule (Table 1).

For patients on **Arm A** (receiving best supportive care only) the following assessments will be completed (patients are required to attend study visits once a month):

- **Monthly:**
  - Routine blood test (haematology and biochemistry, 5ml each)
- **Screening phase, D1 in Cycle 1, Cycle 3, Cycle 5 and first follow-up visit:**
  - Research blood sample (1x10ml) for SMRP, cytokine and metabolomic analysis
- **Screening phase, D1 in Cycle 1 and first follow-up visit:**
  - Urine sample (for metabolomic analysis using Mass Spectrometry, HPLC and 1H-NMR)

For patients on **Arm B** (receiving best supportive care and ADI-PEG 20™) the following assessments will be completed:

- **Weekly:**
  - Routine blood test (haematology and biochemistry, 5ml each) to be available prior to administration. (Plasma/serum urate level on each day of dosing of ADI-PEG 20™ prior to administration.)
- **Screening phase, Day 1, 8, 15 and 22 in Cycles 1 and 2 ; Day 1 in Cycle 3 to Cycle 6 and first follow-up visit:**
  - Research blood sample (1x10ml) for SMRP, cytokine and metabolomic analysis
- **Day 1 in cycles 1, 2, 3, and 5 and first follow-up visit:**
  - Urine sample (for metabolomic analysis using Mass Spectrometry, HPLC and 1H-NMR)

Additional tests from the research blood/urine sample mentioned above:

- Research blood tests (1x10ml) to assess the levels of arginine and citrulline, levels of ADI-PEG 20 and the levels of antibodies to ADI-PEG 20.
As part of the translational component of this study, patients receiving ADI-PEG 20 will undergo a FDG-PET/CT scan (at available sites) during the second - fourth week of the study.

Should progression occur following a period of response to ADI-PEG 20 (SD, PR or CR), patients will be approached for an US or CT guided biopsy to determine the level of ASS expression (Informed Consent C).

For details of all assessments to be completed during the study please refer to the Assessment Schedule (Table 1). For details of assessments to be completed during the extension phase please refer to Attachment F.

**Sample Shipment**

All research blood and urine samples that are being collected will be shipped to the Centre for Experimental Cancer Medicine, Barts Cancer Institute, Queen Mary University of London during the course of this study. All samples will be stored at Queen Mary University of London until analysis. The appropriate signed informed consent form, approved by the ethics committee, will be obtained from patients for research sample testing.

Refer to the ADAM Site Lab Manual for further details on sample collection, handling and shipment.

**Drug Administration**

The amount in mg of ADI-PEG 20™ to be administered will be determined based on body surface area (BSA) using the standard BSA calculation provided in section 6.3 (although sites are permitted to use the BSA calculation in accordance with local practice).

Instructions including calculation of the subject’s dose, preparation and handling of the ADI-PEG 20™ injection, discarding of used study drug supplies, and precautions and care if contact with skin are provided in section 6.4, Instructions for the Preparation and Handling of ADI-PEG 20™ Injections.
Patients’ vital signs (Pulse, BP, RR, O2 Sats and Temp) will be monitored at baseline and at 20, 40 and 60 minutes post-ADI-PEG 20™. Thus, patients will remain in the clinic for 1 hr following administration of the ADI-PEG 20™ to exclude any anaphylactic or adverse cardiovascular effects [26, 27].

Local site pain may be experienced with i.m. injections with the recommended dose of ADI-PEG 20™, which may reach volumes of 6.4ml (for patients with a BSA of 2.0 m²).

To minimize pain at the injection site, it is recommended that all i.m. injections are split across (at least) two sites.

Dose reductions should not be made on the basis of pain. Instead the number of injection sites should be increased in an attempt to maintain the dose.

In the event of Grade 3 or 4 toxicity, please contact the Chief Investigator to discuss if a dose reduction can be considered.

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5.5 Flow diagram:

*For patients who complete 6 months of treatment and do not progress (or who are withdrawn for any reason other than disease progression), there should be eight weekly review for up to 12 months in out-patient clinic, then review of notes up to 18 months for subsequent treatment and survival data collection. For those who progress, their notes should be reviewed for subsequent treatment and survival data collection only.
5.6 Follow up procedures

For ASS negative patients who receive trial treatment:
All patients who receive any treatment on the trial will have a follow-up visit 7 days after their last day of treatment.

If patients have been withdrawn for disease progression, then following this visit, their notes should be reviewed for subsequent treatment and survival data collection only.

If patients have been withdrawn for a reason other than disease progression, they should be reviewed on an 8-weekly basis in the out-patient clinic for a period of six months or until progression. Notes should then be reviewed for subsequent treatment and survival data collection.

Patients who complete 6 months of trial treatment without progression of their disease will be reviewed on an 8-weekly basis in the out-patient clinic for a period of six months after completion of their treatment. Notes should then be reviewed for subsequent treatment and survival data collection.

For ASS positive patients:
Notes should be reviewed for subsequent treatment and survival data collection only and a record made in the CRF.

5.7 Laboratory Assessments

- Haematology and routine biochemistry (U&Es, LFTs, Bone profile) results obtained no more than 48 hours prior to therapy must be available and reviewed by the investigator before subsequent ADI-PEG 20\textsuperscript{TM} dosing to evaluate for possible toxicity. Any abnormal blood results (e.g. high urate) will require normalisation prior to redosing with ADI-PEG 20\textsuperscript{TM}. Platelets and neutrophil count should be above 75×10\textsuperscript{9} /L and 1.0×10\textsuperscript{9} /L respectively prior to each IMP re-administration.

- Serum ß-hCG (screening) and urine pregnancy (Day 1 before dosing) tests will be performed for all women of childbearing potential; if results are positive the subject will not be enrolled or allowed to continue in the study.

- The maximum volume of blood drawn at each visit for laboratory evaluations throughout this study is approximately 30 mL for each subject (approximately 10 mL for
research, 10 mL for special studies {pharmacodynamics and immunogenicity} and 10 mL for safety laboratory evaluations). See Laboratory manual.

- Pharmacodynamics and immunogenicity testing will be performed to assess if ADI-PEG 20™ treatment is depleting peripheral blood arginine, and if antibodies are being generated to the drug.

### 5.8 Radiology Procedures

See Appendices A and B for full details of radiology procedures.

- **Objective Response**

Assessment of CT radiology will employ the response evaluation criteria in solid tumours (modified RECIST) guidelines using spiral CT [33, 34]. Measurable lesions up to a maximum of five will be recorded and measured, with the thickness of the circumferential pleural disease measured at three separate levels on transverse sections.

FDG-PET/CT for functional response according to EORTC criteria will also be used [35-37].

The RECIST Quick Reference, as posted on the NIH website, and the EORTC criteria reference are provided in Appendix B.

- **Durability of Response and Survival**

Progression free survival (PFS, or the length of time during and after treatment in which a patient is living with a disease that does not get worse) and overall survival (time from first treatment to death) will be used as standard measures of treatment efficacy. Patients will be followed up in clinic for 12 months from treatment completion undergoing regular 8 weekly clinical review and a further CT scan 6 months after completion of the treatment period, in responding patients (SD/PR/CR) to confirm whether a response is maintained.

**NOTE:** PET/CT will only be performed at Centres with facility available, whose PET Centres are part of an accreditation scheme.
5.9 Pharmacodynamics and Immunogenicity

Pharmacodynamics and immunogenicity testing will be performed to assess if ADI-PEG 20™ treatment is depleting peripheral blood arginine, and if antibodies are being generated to the drug.

6. INVESTIGATIONAL MEDICINAL PRODUCT(S)

Refer to the ADAM IMP Handling Manual for further information.

6.1 Definition of IMP

ADI-PEG 20™ (Pegylated Arginine deiminase for injection) is an antineoplastic agent available for i.m. use only that induces apoptosis of arginine auxotrophic tumour cells. Each single dose vial contains 3.5 ml of ADI-PEG 20™ as a sterile solution at a concentration of ~100IU/ml (11.5 mg/ml). The ADI-PEG 20™ is provided as a sterile solution consisting of: arginine deiminase, polyethylene glycol, monobasic sodium phosphate, USP, dibasic sodium phosphate, USP, sodium chloride, USP, pH 6.8-7.0, water for injection QS to 3.5 ml.

6.2 Product sourcing manufacture and supply

ADI-PEG 20™ is manufactured in the US by DESIGNERX PHARMACEUTICALS INC., a subsidiary of Polaris Group, regulated by the State of California, US. ADI-PEG 20™ is an orphan medicinal product but does not currently have a marketing authorization from the MHRA. The IMP will be imported by Fisher Bioservices, Bishop’s Stortford, Herts, UK, employing a registered QP to undertake IMP batch release and importation.

ADI-PEG 20™ will be imported to the UK by Fisher Bioservices, and distributed directly to the pharmacy departments (or to a suitable location as agreed on a per site basis) at each of the centres by Fisher Bioservices.

6.3 Prescription of IMP

ADI-PEG 20™ will be prescribed by the Principal Investigator or designated nominee according to the body surface area (BSA) of the patient at a dose of 36.8 mg/m² (equivalent to 320IU/m²). BSA will be calculated based on body weight and height using the following standard calculation, or as per institutional policy:
BSA = $\sqrt{\frac{Ht(cm) \times Wt(kg)}{3600}}$

The ADI-PEG 20™ dose will not be corrected for obese subjects. The dose should be calculated on Day 1 of each cycle and remain consistent during an individual cycle. The dose administered should remain consistent across cycles unless a notable change in weight (i.e. loss or gain of ~10%) is documented during the Day 1 weight assessment of the cycle, in which case the subject’s dose should be recalculated at that time.

6.4 Pre-medication

No specific pre-medication is required prior to ADI-PEG 20™.

6.5 Preparation and Administration of IMP

Preparation and Handling of Solution
ADI-PEG 20™ is not a conventional cytotoxic. However, caution should be exercised when handling and preparing the ADI-PEG 20™ solution. Caution should be taken to avoid accidental ingestion, inhalation, and dermal or ocular contact when handling the test article. The pharmacist or designee will prepare the ADI-PEG 20 dose.

Study drug will be supplied in sterile, single-use vials containing 3.5 ml of ADI-PEG 20™ at a concentration of 11.5 mg/ml and on receipt will be stored in a designated freezer at -70°C or below. On the day of patient administration, ADI-PEG 20™ for i.m. injection should be thawed at room temperature for 30 minutes, and then drawn into the syringe. Alternatively, each vial can be taken out of the -70°C or below freezer and thawed overnight (14-20 hours) at 2-8°C prior to the day of administration. The study medication vial must be used within 24 hours after removal from the -70°C or below freezer. If the drug is not used immediately after thawing it is preferable to store the drug refrigerated until ready for use. The drug is more stable when stored refrigerated (4°C) as compared with stored at room temperature (25°C), and the temperature of a refrigerator is typically better regulated than the room temperature.

ADI-PEG 20™ is then ready for injection without reconstitution. The drug may be drawn up into a syringe by the pharmacy or the vials may be dispensed to a nurse (local policy allowing) who can draw up the required amount ‘at the bedside’.
**IMP administration**

Designated study site personnel, trained in the administration of i.m. injections, will administer ADI-PEG 20™ into the superolateral quadrant of the buttock (gluteus muscle). The administered sites and time of administration will need to be recorded in the case report form. ADI-PEG 20™ doses will be administered on a weekly basis (i.e. every 7 days, see schema on page 20). Local site pain may be experienced with I.M. injections with the recommended dose of ADI-PEG 20™, which may reach volumes of 6.4ml (for patients with a BSA of 2.0 m²). To minimize pain at the injection site, the injection may be split and administered into more than one site according to the volume of injection (with a recommended maximum volume of 5 ml).

Dose reductions should not be made on the basis of pain. Instead the number of injection sites should be increased in an attempt to maintain the dose.

In the event of Grade 3 or 4 toxicity, please contact the Chief Investigator to discuss if a dose reduction can be considered.

**Discarding of Vial and Syringe**

After administration, all materials that have been used for administration should be disposed of according to standard practices. Any unused or expired vials must be retained for reconciliation by the trial coordinator. For detailed instructions, please see the ADAM IMP Handling Manual. Once authorisation for destruction from the Sponsor has been received, a log must be kept of all disposed materials (drug only).

**Procedures to follow in case of skin contact**

Wash the affected area immediately and thoroughly with soap and water. In case of contact with mucous membranes, flush thoroughly with water. Always contact the investigator after any form of body contact.

**6.6 Prior and Concomitant Therapies**

If the administration of any concomitant therapy becomes necessary, it must be reported in the appropriate section of the CRF.
Permitted Therapy

The following medications and support therapies are examples of supportive care that may be used if needed during the study.

- Allopurinol
- Analgesics as required for the treatment of the mesothelioma.

Investigators must contact the CI before concomitant administration of any medications with potential anti-neoplastic activity.

Prohibited Therapy

The following medications are prohibited at all times:

- Any anti-neoplastic agent other than ADI-PEG 20™ with the exception of medications that may have anti-neoplastic activity but are taken for other supportive reasons e.g. cyclo-oxygenase-2 [COX-2] inhibitors, low dose steroid (eg. Prednisolone) and bisphosphonates.
- Any experimental agent other than ADI-PEG 20™.

If in doubt please contact the ADAM Clinical Trial Coordinator or the Chief Investigator. All concomitant medications must be recorded in the CRF.

6.7 Dose modification/reduction/delay

Dose modifications or significant delays in dosage administration are not expected. However, in the event of grade III or IV toxicity occurring, a dose reduction to 18.4mg (equivalent to 160IU/m²), may be considered after discussion and authorisation by the Chief Investigator.

Doses should be delivered at 7 day intervals (+24hrs only in the event of public holidays or emergencies). Dosing delays are not permitted unless first authorised by the Chief Investigator.

No missed doses are permitted. Any subject who misses a dose or experiences more than a 24hr delay (unless authorised by the Chief Investigator) will be withdrawn from the study.
6.8 Toxicity profiles

Before each dose of study drug, the subject will be evaluated for possible toxicities that may have occurred after the previous dose(s). Toxicities are to be assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE), Version 3.0 (see Appendix C). Previously observed toxicities related to ADI-PEG 20™ are to be managed as follows:

- Local injection site reactions to i.m. ADI-PEG 20™ are self-limiting and resolve within 48hrs. Higher doses of i.m. ADI-PEG 20™ (36.8mg/m²) used in the earlier phase I/II studies were painful due to the volumes administered.

- The development of hyperuricaemia should be monitored using a pre (on day 0) and post (on day 8) ADI-PEG 20™ blood test. Any clinically relevant elevation should be treated with allopurinol or urate oxidase, according to local policy. Treatment with ADI-PEG 20™ should be restarted with evidence that the urate blood level is normalising.

- Anaphylactoid reactions are considered very rare and if these occur may present with hypotension and should be managed as described in Appendix C.2

- Epilepsy: Grade 3 seizure with temporary loss of consciousness has been observed in <1% of patients treated with ADI-PEG 20™ and should be managed with i.m. benzodiazepines; seizures have resolved without sequelae in affected patients. See Appendix C.3 for further management details.

6.9 Labeling and Packaging

Study drug labels will contain information to meet the applicable regulatory requirements. ADI-PEG 20™ will be supplied in boxes of bulk vials (24 vials per box).

6.10 Receipt of IMP and Supplies/Storage

The IMP will be imported into the UK from the US by Fisher Bioservices and stored at its facility in Bishop’s Stortford, Herts, UK. The Fisher designated Qualified Person (QP) will release the IMP on an as required basis by each clinical facility. Each participating hospital pharmacy will arrange for storage of ADI-PEG 20™ in a -70°C or below freezer until required by a patient randomised to receive ADI-PEG 20™ on the protocol (Arm B). Individual institutions will be required to provide the sponsor with details of where the IMP will be stored at site, and provide details of who will be responsible for IMP accountability, monitoring of freezers, receipt of supplies etc, if this is not the pharmacist.
Each site will receive IMP according to the number of patients recruited at any one time. ADI-PEG 20™ will be received by the pharmacy department (or alternative as discussed above) at each of the host institutions. Departments should acknowledge receipt of the IMP as per paperwork received with the drug. Please ensure that ordering and delivery records for any ADI-PEG 20™ in this trial are retained in the Pharmacy Site File.

Drug accountability will be recorded on a study-specific IMP accountability log. Shipments of ADI-PEG 20™ received at the site will be logged onto the accountability log. The accountability log will be kept in the study-specific Pharmacy File.

6.11 Dispensing of the IMP

ADI-PEG 20™ will be dispensed by the pharmacy at each of the host institutions in accordance with a trial-specific prescription. Due to variations between sites and the use of electronic or paper prescribing systems, a sample prescription form will not be provided by the co-ordinating centre. However, all prescriptions must be approved by the Sponsor prior to site opening to recruitment.

Unopened vials of ADI-PEG 20™ will be stored at -70°C or below temperature. There is no reconstitution, as the vials contain ADI-PEG 20™ that is ready for injection after thawing.

ADI-PEG 20™ injections should be prepared and handled according to the instructions provided in Instructions for the Preparation and Handling of ADI-PEG 20™ Injections (see Section 6.5).

6.12 Return or destruction of IMP

Accountability for the study drug at the study site is the responsibility of the investigator. This responsibility may be delegated to the pharmacist, or another appropriate person. The investigator will ensure that the study drug is used only in accordance with this protocol. Unused ADI-PEG 20™ **MAY NOT** be used for any purpose other than that outlined in the protocol.

Drug accountability records indicating the drug’s delivery date to the site, inventory at the site, use by each patient, and disposal of the drug will be maintained by the clinical site. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable) and patient numbers.
All used, unused or expired study drug will be disposed of at the study site and documented following authorisation by the Sponsor. All material containing ADI-PEG 20™ will be treated and disposed of as hazardous waste in accordance with governing regulations.

7. **PHARMACOVIGILANCE**

7.1 **General definitions**

7.1.1 **Adverse Events (AE)**

An AE is any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of an Investigational Medicinal Product (IMP), **whether or not considered related to the IMP**.

7.1.2 **Adverse Reaction (AR)**

An AR is any untoward and unintended response in a subject to an Investigational Medicinal Product (IMP), which is related to any dose administered to that subject. All adverse events judged by either the reporting investigator or the CI as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

7.1.3 **Serious Adverse event (SAE) or Serious Adverse Reaction (SAR)**

**Serious Adverse Event (SAE)**

An SAE fulfils at least one of the following criteria:

- Is fatal – results in death (NOTE: death is an outcome, not an event)
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
Suspected Serious Adverse Reaction (SSAR)

An SSAR is an adverse reaction that is classed as serious and which is consistent with the information about the medicinal product as set out in the Summary of Product Characteristics (SmPC) or Investigator’s Brochure (IB) for that product.

7.1.4 Suspected unexpected Serious Adverse Reaction (SUSAR)

The definition of a SUSAR is any suspected unexpected adverse reaction related to an IMP that is both unexpected and serious. In this case the event is not outlined in the Summary of Product Characteristics (SmPC) or Investigator’s Brochure (IB) for that product.

7.2 Operational Definition – Serious Adverse Events (SAEs)

7.2.1 Events classed as non reportable SAEs

The following events will be recorded in the medical notes and the CRFs but a sponsor SAE form will not be completed or submitted to the coordinating centre.

Prolongation of hospitalisation for:

- Routine treatment or monitoring of the studied indications not associated with any deterioration in condition.

- Treatment which was elective or pre-planned, for a pre-existing condition not associated with any deterioration in condition.

- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions for serious as given above and not resulting in hospital admission.

- Death due to disease progression (but not drug related)

- Hospitalisation or prolongation due to pleural effusions
Hospitalisation or prolongation due to chest infections

Hospitalisation or prolongation due to chest pain control

7.3 Investigators Assessment

7.3.1 Seriousness

Intensity for each adverse event will be determined by using the National Cancer Institute Common Toxicity Criteria (NCI CTC, version 3.0), wherever possible (see Appendix C.1). In those cases where the NCI CTC criteria do not apply, intensity should be defined according to the following criteria:

Mild: Awareness of sign or symptom, but easily tolerated.

Moderate: Discomfort enough to cause interference with normal daily activities.

Severe: Inability to perform normal daily activities.

Life Threatening: Immediate risk of death from the reaction as it occurred.

7.3.2 Causality

Relationship to study drug administration will be determined as follows:

None: No relationship between the experience and the administration of study drug; related to other aetiologies such as concomitant medications or patient’s clinical state.

Unlikely: The current state of knowledge indicates that a relationship is unlikely.

Possible: A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the patient’s clinical state or other modes of therapy administered to the patient.

Probable: A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the patient’s clinical state or other modes of therapy administered to the patient.
7.3.3 Expectedness

Investigators must assess the expectedness of each AE or AR, regardless of the causal relationship to trial treatment. The following are expected AEs (AR’s can be found in the IB):- These expected AE will be recorded in the CRF and medical records.

- Local tenderness at the site of the i.m. injection of ADI-PEG 20™
- Hyperuricaemia may occur in patients with ASS negative mesothelioma treated with ADI-PEG 20™ secondary to tumour lysis. However, this is considered a low risk event in view of the tumour volume in MPM.
- Grade 3 or 4 leucopoenia or thrombocytopenia has occurred (<1% of people overall exposed to this drug). This was also noted in pre-clinical toxicity testing.
- Epilepsy has been observed in 2 patients with metastatic melanoma treated with ADI-PEG 20™ (<1% of people overall exposed to this drug), and is at present subject to ongoing analysis.

7.4 Notification and reporting Adverse Events or Reactions

Details of all AE’s and ARs, whether observed by the investigator or reported by the patient, should be recorded on the toxicity section of relevant CRFs and returned to the Chief Investigator/Study Coordinator.

The severity of all non-serious ARs should be graded using the Common Terminology Criteria for Adverse Events (CTCAE) v3.0. These criteria can be accessed at [http://ctep.cancer.gov/forms/CTCAEv3.pdf](http://ctep.cancer.gov/forms/CTCAEv3.pdf)

All subjects will be reviewed for adverse events throughout the treatment period and for 30 days after administration of the last dose of ADI-PEG 20™.

7.5 Notification and Reporting of Serious Adverse Events/SUSAR

7.5.1 Serious Adverse Events (SAEs)
Serious adverse events need to be recorded in the medical record, CRF and sponsor SAE form (except for these outlined in 7.2.1). The SAE forms must to be sent to the Coordinating centre within one working day of local Investigator becoming aware. The Coordinating centre will only send completed and signed SAE forms to the sponsor. This will occur as soon as possible and within 1 week of the Coordinating centre having received the completed signed forms.

7.5.2 Suspected Unexpected Serious Events (SUSARs)

All SAEs assigned by the local investigator as both related to IMP and unexpected, will be recorded in the medical records, the CRF and the sponsor SAE form. These must be reported to the Coordinating centre immediately and within one working day of the local Investigator becoming aware of the event.

The co-investigators listed on the delegation log can be authorised to sign the sponsor SAE forms in the absence of the PI. The CI should not downgrade SAEs or SUSARs from the local Investigator, however the CI can upgrade an AE to a SAE or a SAE to a SUSAR.

The Coordinating centre should report SUSARs to the sponsor, immediately and within one working day of becoming aware of the event.

The Coordinating centre will request the sponsor to acknowledge receipt of the SUSAR. The Coordinating centre will report the SUSARs to the main REC and the other PIs as soon as possible. The sponsor will report the SUSARs to the MHRA as per regulation 33 of SI 2004/1031.

7.5.3 The Annual Safety Report (ASR)

Development Safety Update Report will be sent by the CI to the Sponsor, the MREC and the MHRA on the anniversary date, or within 1 month of, of the “notice of acceptance letter” from the MHRA, using the Sponsor’s DSUR form. The CI will carry out a risk benefit analysis of the IMPs encompassing all events having arisen on the trial.

7.5.4 Pregnancy

Due to the possible risks associated with the treatment, all women of childbearing potential (defined as women who have had any menstrual bleeding in the last 24 months and who
have not had a hysterectomy) should be informed of the potential risks to their unborn child should they fall pregnant whilst receiving treatment. Any woman who is pregnant at the time of eligibility assessment or is unwilling to use medically approved contraception whilst receiving treatment will be ineligible for entry to the study. All women of childbearing potential must use appropriate medically approved contraception before, during and for 3 months after the end of study treatment. Men whose partners are females capable of having children must use appropriate medically approved contraception during the study and for 3 months after the end of the study treatment, unless they are surgically sterile.

Pregnancies occurring in participants of the study, or participants' partners during the study may represent a safety issue and the Chief Investigator must be notified immediately. Site staff should notify the Study coordinator of a pregnancy in a trial subject or the partner of a trial subject and the estimated due date. Where a pregnancy is known, this will be followed for outcome and any adverse outcome of pregnancy assessed for causality to the treatment received. Pregnant partners of participants will be asked to give consent for the release of this information to the study team.

7.6 Overview of the Safety Reporting Process

The safety reporting process should be followed as described in the Sponsor’s SAE flow diagram. This can be found at:–

http://www.bartsandthelondon.org.uk/docs/saereportingform

The CI will notify POLARIS PHARMACEUTICALS INC. of all adverse events, in report format, at the end of the study.

7.7 Pharmacovigilance responsibilities

7.7.1 Local investigator:

1. Medical judgement in assigning:
   i. Seriousness
   ii. Causality
   iii. Expectedness

2. To ensure the SAE/SUSAR form is faxed to the Study Coordinator within one working day of becoming aware and to provide further follow-up information as soon as available.
3. The Investigator does not need to inform the MHRA of SAEs related to the study drugs as this will be done annually by the co-ordinating centre.

7.7.2 Chief Investigator (or nominated individual in CIs absence):

1. Chief Investigator to assign causality and expected nature of SAEs where it has not been possible to obtain local assessment.
2. Chief Investigator to undertake review of all SAE/SUSARs for seriousness/expectedness and causality.
3. In the event of disagreement between local assessment and Chief Investigator review with regards to SUSAR status, local assessment will not be overruled, but Chief Investigator may add comments prior to reporting to the MHRA.
4. Preparing safety and progress reports at least annually for the oversight bodies including the MHRA, main REC, and Sponsor, as required. Safety may be reported more frequently if appropriate.
5. Act upon information from the Independent Data Monitoring Committee (IDMC) and Trial Management Group (TMG).

7.7.3 Co-ordinating centre:

1. Expedited reporting of SUSARs to main REC and Sponsor within required timelines.
2. Notifying investigators of SUSARs or other safety issues which may compromise patient safety.
3. Expedited reporting of safety issues, including an increase in the rate of occurrence or change in severity of SARs to, main REC PIs and sponsor within required timelines.

Preparing reports including safety information at least annually for Polaris, the Trial Safety Committee, and Trial Management Group. Safety information may be reported more frequently if appropriate.

7.7.4 Sponsor:

Expedited reporting of SUSARs to MHRA within required timelines.
Expedited reporting of safety issues specifically reported by the CI to the sponsor, including an increase in the rate of occurrence or change in severity of SARs), to MHRA within required timelines.
7.8 **Trial Management Group:**

The TMG is made up of an independent chairperson, the Chief Investigator, trial coordination team, collaborators, and the trial statistician and will provide independent review of cumulative reports of all SAEs on a minimum 6 monthly basis to identify patterns or trends of events or identify safety issues, which would not be apparent on an individual basis.

8. **DATA HANDLING AND RECORD KEEPING**

8.1 **Confidentiality**

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational product(s) used in this study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

The investigator ensures that the personal data will be:

- processed fairly and lawfully
- collected for specified, explicit, and legitimate purposes and not further processed in a way incompatible with these purposes
- adequate, relevant, and not excessive in relation to said purposes
- accurate and, where necessary, kept current

Explicit consent for the processing of personal data will be obtained from the participating subject (or his/her legally acceptable representative) before collection of data. Such consent should also address the transfer of the data to other entities and to other countries.

The subject has the right to request through the investigator access to his/her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps should be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

In order to maintain patient privacy, all case report forms, study drug accountability records, study reports and communications will identify the patient by initials and the assigned subject number. The investigator will grant auditor(s) from regulatory authority(ies) access...
to the patient’s original medical records for verification of data gathered on the case report forms and to audit the data collection process. The patient’s confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Polaris Pharmaceuticals Inc., (including monitors on its behalf) will have access to pseudoanonymised data obtained from patients enrolled.

8.2 Study Documents

Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

Protocol Modifications

The investigator will conduct the study in accordance with the protocol, and will seek approval/favourable opinion by the CA and REC and the appropriate regulatory authority(ies). Changes to the protocol will require written CA and REC approval/favourable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The CA and REC may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favourable opinion for minor change(s) in ongoing studies that have the approval/ favourable opinion of the CA and REC. The investigator will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

Protocol amendments cannot be implemented without prior CA and REC approval. Any departures from the protocol must be reported as a protocol violation and fully documented in the case report forms and source documentation.

8.3 Case Report Forms

A Case Report Form (CRF) is required for each included subject. CRFs must be completed legibly in black ink. Subjects should be identified by initials and subject number only. All requested information must be entered on the CRF in the space provided. If an item is not applicable, it should be documented as such. **No blank spaces should be left on this form.**
Any change or correction to a CRF should be made by striking through the incorrect entry with a single line (not obscuring the original entry) and then entering the correct information adjacent to the incorrect entry. The correction must be initialed and dated by the person making the correction. The CRF must be promptly reviewed, signed and dated by the investigator or a delegated sub-investigator.

The investigator has ultimate responsibility for the accuracy, authenticity and timely collection and reporting of all clinical, safety and laboratory data entered on the CRF and any other data collection forms.

8.4 Record Retention and Archiving

At the end of the trial, all essential documents as defined by GCP will be securely archived by the coordinating and participating centres for a minimum of 20 years. Participating centres should retain the documents for a longer period, where so required by local policy. During this time, documents must remain available for inspection by the Sponsor or auditors and the Co-ordinating centre must be informed of the location of the archived material and the retrieval process. Following written authorisation from the Sponsor, arrangements for confidential destruction will then be made. If the investigator withdraws from the study, all responsibilities should be transferred to a designee acceptable to the Sponsor.

If the investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

The results of the study will be reported in a Clinical Study Report generated by the Chief Investigator and will contain all data from all investigational sites.

8.5 Compliance

Each investigator/participating centre research staff must adhere to the protocol as detailed in this document and will be responsible for enrolling only those patients who have met the eligibility criteria.

8.6 End of study definitions
The study will conclude once all the patients have been followed up for a period of 18 months (or until death), from end of study treatment.

9 GOVERNANCE ISSUES

To ensure responsibility and accountability for the overall quality of care received by patients during the study period, clinical governance issues pertaining to all aspects of routine management will be brought to the attention of the CI, Sponsor and, where applicable, to individual NHS Trusts.

9.1 Risk assessment:

This trial is a phase II trial. The Sponsor, together with the PI, has assessed this trial to be a Very High Risk (band D) according to their Risk Assessment SOP 28 (www.bartsandthelondon.nhs.uk/research/joint_rd_office_sops.asp).

9.2 Summary monitoring Plan

Refer to ADAM Trial Monitoring Plan for further information.

All sites will undergo an on-site initiation visit by the co-ordinating centre.

During the study all sites will undergo on-site monitoring visits by the coordinating centre and/or Sponsor within three months of enrolling their first patient and a minimum of every 6 months thereafter. In the event that the coordinating centre cannot perform an on-site monitoring visit, sites may be requested to complete a self-monitoring form at these time-points.

All sites will undergo an on-site close out visit by the coordinating centre.

The Sponsor will retain ultimate oversight of the monitoring process.

9.3 Audit and inspection

This study may be audited by representatives from the coordinating centre and the Sponsor. In addition, inspections may also be carried out by the Competent Authority at any time and the investigator should notify the Sponsor immediately if there are any such plans for an inspection.
Investigators are obliged to cooperate in any audit or inspection allowing the auditor/inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings or any issues. Audit or inspection may occur at any time during or after completion of the study.

9.4 Serious breaches in GCP or trial protocol

All investigators on the study will promptly notify the Chief Investigator or Sponsor of any potential serious breaches (as defined in Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 [Statutory Instrument 2004/1031], as amended by Statutory Instrument 2006/1928) that they become aware of.

A “serious breach” is a breach which is likely to effect to a significant degree:
(a) The safety or physical or mental integrity of the subjects of the trial; or
(b) The scientific value of the trial.

The CI should notify the sponsor (JRO) within a day of becoming aware of a potential serious breach.

Participating centres should contact the coordinating centre or CI for further information and guidance.

9.5 Quality Assurance

The trial will be conducted in accordance with the principles of Good Clinical Practice in clinical trials, the NHS Research Governance Framework and The Medicines for Human Use (Clinical Trials) Regulations (SI 2004/1031) and subsequent amendments through adherence to the sponsor standard operating procedures (SOPs).

9.6 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. An Independent Ethics Committee (IEC) will review all appropriate study documentation in order to safeguard the rights, safety and well being of patients. The study will only be conducted at sites where appropriate approval has been obtained.
The study co-ordinating team will inform the IEC of any changes to the conduct of the trial and seek approval for these changes and any amended patient materials. The Chief Investigator will maintain an accurate and complete record of all written correspondence to and from the IEC and will agree to share all such documents and reports with the sponsor.

The informed consent and any other documentation provided to subjects will be revised if important new information becomes available that is relevant to the subject’s consent. Amended documents will be approved by the IEC before distribution to patients.

**9.7 Trial Management Group**

The members of the **Trial Management Group (TMG)** are as follows:-

- Dr Peter Szlosarek (CI)
- Dr Jeremy Steele (Collaborator and Co-I)
- Dr Dean Fennell (Collaborator and PI)
- Dr Allan Hackshaw (Statistician)
- Dr Robin Rudd (Collaborator)
- Prof. Michael Sheaff (Collaborator)
- Trial coordinator and Senior Clinical Trial Coordinator

The TMG provides overall supervision for the execution of the trial and is advised by its independent chairman. The ultimate decision regarding continuation of the trial rests with the TMG. The TMG will report to regular meetings of the NCRI Lung Clinical Studies Group and the IDMC (see below).

The members of the **Independent Data Monitoring Committee (IDMC)** are as follows:-

- Dr Sanjay Popat (Consultant Medical Oncologist, Royal Marsden Hospital)
- Dr Rachel Benamore (Consultant Radiologist, Oxford Radcliffe NHS Trust)
- Caroline Bray (Senior Statistician, Cancer Research UK Clinical Trials Unit, Glasgow)

*NOTE: If there are changes regarding the Individual committee members during the course of the clinical study, written notification will be provided to the sponsor and will not require a protocol amendment(s)*
10 STATISTICS

10.1 Endpoints

10.1.1 Primary endpoint

Progression free survival, defined according to the modified RECIST criteria and measured from date of randomisation to the date when the disease starts to worsen or the patient has died (whichever is sooner).

10.1.2 Secondary Endpoints

The secondary efficacy parameters include the objective response rate, overall survival, time to progression and safety/toxicity.

10.2 Study definitions

Progression-free survival is measured from the date of randomisation until the date of progression or death, whichever occurs first.

The objective response rate, will be a proportion of evaluable subjects who achieve a confirmed SD, CR or PR per modified RECIST guidelines for plain CT and by EORTC guidelines for PET-CT. The number and percentage of subjects falling into each response category will be descriptively tabulated.

Safety/Toxicity will be assessed from adverse event and SUSAR reporting.

To assess pharmacodynamics and immunogenicity peripheral blood plasma will be tested for levels of arginine, citrulline, ADI-PEG 20 levels and antibodies to ADI-PEG 20.

10.3 Statistical considerations

10.3.1 Sample Size

66 subjects are anticipated, with a 2:1 randomization: Arm A (BSC only) (n=22) and Arm B (ADI-PEG 20™ and BSC) (n=44)

The prognosis of patients with malignant mesothelioma is very poor. About 40% of patients are expected to be alive and progression-free by 6 months (median PFS 4.5 months). ADI-PEG 20 would be worth considering in a phase III trial if it were associated with a hazard ratio of 0.60 (ie median PFS 7.5 months). To detect this with 80% power and one-sided
15% test of statistical significance, the sample size required is 66 patients (44 given ADI-PEG 20 and 22 in the control group); assuming a 2:1 allocation, 18 months recruitment and 12 months follow up after the end of recruitment (using Dupont WD and Plummer WD: PS power and sample size program available for free on the Internet. Controlled Clin Trials,1997;18:274).

10.3.2 Planned recruitment rate

A recruitment rate of 4 patients per month for randomisation is envisaged across the collaborating clinical centres (66 patients over 18 months). Based on the known expression of ASS in ~50% of mesothelioma tumours, a total of ~130 patients (or 7-8 patients per month) will need to be screened in total to identify 66 ASS negative patients.

10.4 Statistical Analysis

Progression-free survival with ADI-PEG 20™ (Arm B) will be compared with that in the control group (Arm A). We will obtain the PFS curves and the hazard ratio, 95% confidence interval and p-value. Toxicity would be presented as proportions at each grade (1 to 4) in each group, and compared using exact tests.

10.5 General considerations

Computer data processing, statistical analysis of data and the production of the final study report will be performed under the direction of the Cancer Research UK and UCL Cancer Trials Centre.

10.6 Frequency of Analysis

Safety will be monitored during the course of the trial (see Section 7). The primary endpoint, progression free survival, will be assessed at the end of the trial, though the Data Monitoring Committee may request this data earlier.

10.7 Interim Analysis

An interim analysis will be performed by the Independent Data Monitoring Committee (IDMC) to assess for safety and toxicity, after the first 22 patients (approximately a third) have been recruited onto the study and followed for a month; and again after 44 patients (approximately two-thirds) have been recruited. The IDMC will focus on the toxicity profile, including CTCAE grades, in each arm and compare between the treatment groups.
11 STUDY FINANCES

11.1 Funding Sources

This trial is investigator designed and led. It is funded by a Clinical Trials Advisory and Awards Committee (CTAAC) grant from Cancer Research UK. This study is a part of the National Institute for Health Research (NIHR) portfolio, as such it is envisaged that individual sites will apply to their Regional Comprehensive Local Research Networks (CLRN) for NHS service support costs.

The translational component of the study will be supported by a separate application to the Biomarker and Imaging Drug Development (BIDD) Committee at Cancer Research UK once samples have been collected for analysis. Polaris Group is providing ADI-PEG 20™ free of charge and will be performing the PD and PK analyses.

The Barts Lung Cancer and Mesothelioma Research Charity (Barts and The London Charity) is paying for IMP distribution within the UK.

Any additional sources of income will be highlighted to the REC without the need for a protocol amendment.

11.2 Subject expenses +/- payments

Payments are not available to cover patient expenses on this trial. Patients will be advised to speak to their doctor regarding any possibilities of local funding being available.

12 SPONSORSHIP AND INDEMNITY

This trial is an investigator-led trial. Dr Peter Szlosarek of Barts Cancer Institute, Queen Mary, University of London, is the Chief Investigator.

Barts Health NHS Trust is sponsoring the study and therefore providing indemnity.

13 PUBLICATION POLICY

This is an investigator-led study sponsored by the CI’s substantive employer, Barts Health NHS Trust. The data collected will not be used to licence/register any pharmaceuticals.
Authorship of the final manuscript(s), interim publications, or abstracts will be decided according to active participation in the statistical design, Trial Steering Committee, accrual of eligible patients and statistical analysis. Contributing centres (and participating investigators) will be acknowledged in the final manuscript, and the correct designation for this site is ‘Centre for Experimental Cancer Medicine, Barts Cancer Institute, Queen Mary University of London’. Representative for the sponsor will be added, as appropriate, as co-authors. No participant may present data from his/her centre separately from the rest of the study results unless approved by the Trial Steering Committee and the sponsor.
14 REFERENCES


15 APPENDICES

ATTACHMENT A: File Transfer

- All patients will have a copy of their CT and FDG-PET/CT scans burned to compact disc (CD).

- Average file size will be accommodated on a single CD.

- Files should be obtained for each patient at presentation, response assessments after two, four and six cycles, and confirmatory assessment at 6 months in responding patients at follow-up.

- The CDs should be clearly labeled with subject number, initials, date of birth and date of scan, and then shipped to the coordinating centre at the end of each complete cycle (2, 4, and 6 cycles).

- Files must be sent to the following address:

  ADAM Trial Coordinator
  Centre for Experimental Cancer Medicine
  Old Anatomy Building
  BARTS AND THE LONDON School of Medicine and Dentistry
  Charterhouse Square
  London EC1M 6BQ
  Phone: +44 (0) 207 882 8764
  Fax: +44 (0) 207 882 8409
ATTACHMENT B: CT and PET assessment

CT TECHNIQUE

CT technique is optimised with a dedicated pleural scan. The entire pleura needs to be imaged including the diaphragm. Pleural enhancement is best appreciated after the arterial phase, and a scan delay of 60 seconds with coverage from the lung apices to the inferior aspect of the liver should be used. Collimation will depend on scanner specification but ideally should be performed at 2 mm or less to allow the potential for subsequent 3 dimensional volume measurements. If this is not possible use minimum available collimation.

<table>
<thead>
<tr>
<th>Position</th>
<th>Arms abducted above head</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contrast</td>
<td>100-150 mls IV contrast if no allergy</td>
</tr>
<tr>
<td></td>
<td>3 mls per second</td>
</tr>
<tr>
<td>Delay</td>
<td>60 seconds</td>
</tr>
<tr>
<td>Direction</td>
<td>Cranio caudal</td>
</tr>
<tr>
<td>Coverage</td>
<td>From lung apices to inferior aspect of liver</td>
</tr>
<tr>
<td>Collimation</td>
<td>1.5 mm</td>
</tr>
<tr>
<td>Image reconstruction</td>
<td>5mm on lung windows</td>
</tr>
<tr>
<td></td>
<td>5 mm on mediastinal windows</td>
</tr>
<tr>
<td></td>
<td>2 mm mediastinal windows</td>
</tr>
<tr>
<td>Filming</td>
<td>Not required centrally</td>
</tr>
<tr>
<td>Archive</td>
<td>1. All images to be archived at centre</td>
</tr>
<tr>
<td></td>
<td>2. Two sets of all images should be copied to separate CD’s in a DICOM compatible format (coded)</td>
</tr>
</tbody>
</table>

Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference

Eligibility

- Only patients with measurable disease at baseline should be included in protocols where objective tumour response is the primary endpoint.

  - **Measurable disease** - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

  - **Measurable lesions** - lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques or ≥10 mm with spiral CT scan.

  - **Non-measurable lesions** - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan),
i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques; and.

- All measurements should be taken and recorded in metric notation, using a ruler, callipers or electronically. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.
- The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.
- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by colour photography, including a ruler to estimate the size of the lesion, is recommended.

Methods of Measurement

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumours of the chest, abdomen and pelvis. Head and neck tumours and those of extremities usually require specific protocols.
- When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumour lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.
- The utilisation of endoscopy and laparoscopy for objective tumour evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centres. Therefore, the utilization of such techniques for objective tumour response should be restricted to validation purposes in specialized centres. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.
• Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumour types such as germ cell tumours).

Baseline documentation of “Target” and “Non-Target” lesions

• All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs should be identified as target lesions and recorded and measured at baseline.

• Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

• A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumour.

• All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria

Evaluation of target lesions:

- **Complete Response (CR):** Disappearance of all target lesions
- **Partial Response (PR):** At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
- **Progressive Disease (PD):** At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
- **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of non-target lesions:

- **Complete Response (CR):** Disappearance of all non-target lesions and normalisation of tumor marker level
- **Incomplete Response / Stable Disease (SD):** Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
- **Progressive Disease (PD):** Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions
Although a clear progression of “non-target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

Evaluation of best overall response

- The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient’s best response assignment will depend on the achievement of both measurement and confirmation criteria.

<table>
<thead>
<tr>
<th>Target lesions</th>
<th>Non-Target lesions</th>
<th>New Lesions</th>
<th>Overall response</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>CR</td>
<td>No</td>
<td>CR</td>
</tr>
<tr>
<td>CR</td>
<td>Incomplete response/SD</td>
<td>No</td>
<td>PR</td>
</tr>
<tr>
<td>PR</td>
<td>Non-PD</td>
<td>No</td>
<td>PR</td>
</tr>
<tr>
<td>SD</td>
<td>Non-PD</td>
<td>No</td>
<td>SD</td>
</tr>
<tr>
<td>PD</td>
<td>Any</td>
<td>Yes or No</td>
<td>PD</td>
</tr>
<tr>
<td>Any</td>
<td>PD</td>
<td>Yes or No</td>
<td>PD</td>
</tr>
<tr>
<td>Any</td>
<td>Any</td>
<td>Yes</td>
<td>PD</td>
</tr>
</tbody>
</table>

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.

- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.
Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

- To be assigned a status of PR or CR, changes in tumour measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol.

Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of stable disease

- SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

- The clinical relevance of the duration of SD varies for different tumour types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Response review

For trials where the response rate is the primary endpoint it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study’s completion. Simultaneous review of the patients’ files and radiological images is the best approach.
Reporting of results

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients.

Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

The 95% confidence intervals should be provided.

In relation to the ADAM study the following should be observed:-

Modified RECIST criteria should be used where possible to measure response [28]. The thickness of circumferential pleural tumour is measured at three or more separate levels on transverse sections and the sum of the measurements is taken as the baseline sum longest diameter. A maximum of 6 measurements should be taken. Repeat measurements are taken at the same level and position on subsequent scans. Assessment may be made difficult following pleurodesis as this causes an inflammatory response causing pleural thickening, this may mimic progressive disease. Mesothelioma often affects previous surgical, biopsy and drain sites. There is a poor correlation between size of node and pathological involvement and measurement of nodes should not be used.

In addition to the modified RECIST criteria, a single measurement of maximal mesothelioma thickness should also be assessed and recorded for patients on the ADAM trial. Lastly, the DICOM data will also be processed using CT volumetric techniques being developed by Dr Sam Armato (University of Chicago, USA).
Evaluation of Response by SUV Change -EORTC Recommendations

Evaluation of response by SUV change-EORTC recommendations define the response categories as:

- **Progressive metabolic disease (PMD)** refers to an increase in $[^{18}\text{F}]-\text{FDG}$ tumour SUV of greater than 25% within the tumour region defined on the baseline scan, visible increase in the extent of $[^{18}\text{F}]-\text{FDG}$ tumour uptake (>20% in the longest dimension) or the appearance of new $[^{18}\text{F}]-\text{FDG}$ uptake in metastatic lesions.

- **Partial metabolic response (PMR)** classified as a reduction of a minimum of 15-25% in tumour $[^{18}\text{F}]-\text{FDG}$ SUV after one cycle of chemotherapy, and greater than 25% after more than one treatment cycle.

- **Complete metabolic response (CMR)** would be complete resolution of $[^{18}\text{F}]-\text{FDG}$ uptake within the tumour volume so that it was indistinguishable from surrounding normal tissue.

- **Stable metabolic disease (SMD)** is classified as an increase in tumour $[^{18}\text{F}]-\text{FDG}$ SUV of less than 25% or a decrease of less than 15% and no visible increase in extent of $[^{18}\text{F}]-\text{FDG}$ tumour uptake (>20% in the longest dimension).

These parameters are generalized guides for predicting response. The EORTC criteria are proposed criteria based upon retrospective literature review and are not specific to mesothelioma. See also Ceresoli et al [29].

All CT and PET scans in addition to local review at participating centres will also be subjected to a central review process.

Where possible, please ensure that the same radiographer conducts the local review of each patient’s scans throughout the duration of their treatment to minimise any variation due to interpretation.
ATTACHMENT C: CTC AND MANAGEMENT OF ADI-PEG 20™ TOXICITIES

(1) Common Terminology Criteria for Adverse Events

A copy of the NCI Common Terminology Criteria for Adverse Events v 3.0 (CTCAE) will be provided to all sites and may be obtained at:
http://ctep.cancer.gov
Published Date: 9th August 2006

(2) Potential and Management of Anaphylaxis

Previous clinical studies of ADI-PEG 20™ have been conspicuous for their lack of anaphylactic/anaphylactoid events (Izzo et al, Ascierto et al). However, since ADI-PEG 20™ is made using a foreign protein, the risk of anaphlaxis remains present and will be managed promptly using Resuscitation (UK) guidelines:
http://www.resus.org.uk/pages/reaction.htm

- Adrenaline 0.5 ml of 1:1000 i.m.; repeated after 5 minutes if poor response
- Chlorpheniramine (Piriton): 10-20 mg i.m.
- Hydrocortisone: 100-500mg i.m.
- Intravenous fluids (1-2L) of crystalloid if hypotension persists
- Oxygen
- Admission for observation

N.B Full cardio-pulmonary resuscitation procedures must be available on site in the event of further clinical deterioration.

(3) Potential and Management of Seizure

A recent study of ADI-PEG 20™ in patients with advanced malignant melanoma included two patients that developed grade 3 seizure possibly and probably related to ADI-PEG 20™. The overall risk of epilepsy with ADI-PEG 20™ administration is low (<1%) and will be monitored in this study. In the event of seizure/epilepsy occurring this will be treated promptly with local protocols using benzodiazepines. See also the NICE guidelines on http://www.nice.org.uk/Guidance/CG20 (October 2004).
## ATTACHMENT D: ECOG Performance Scale

<table>
<thead>
<tr>
<th>Grade</th>
<th>ECOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fully active, able to carry on all pre-disease performance without restriction</td>
</tr>
<tr>
<td>1</td>
<td>Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work.</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours</td>
</tr>
<tr>
<td>3</td>
<td>Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.</td>
</tr>
<tr>
<td>4</td>
<td>Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.</td>
</tr>
<tr>
<td>5</td>
<td>Dead.</td>
</tr>
</tbody>
</table>

ATTACHMENT E: ASS Histochemistry

(BD antibody 1:500 dilution)

ASS immunohistochemical (IHC) staining will be assessed by two investigators using a super sensitive IHC detection (biotin-free) system (BioGenex), replacing the original ‘ABC (‘avidin-biotin complex’) method. Biopsies are scored ASS negative (or ‘low expressors’) if there is either an absence and/or weak staining of ASS; in contrast biopsies are scored ASS positive (‘high expressors’) if there is moderate or strong ASS expression. In cases of heterogenous staining, the biopsy is scored negative if >50% cells are ASS deficient, whilst the biopsy is scored positive if >50% cells are moderate and/or strong ASS expressors. In the event of a repeat tumour biopsy, an increase in ASS deficiency compared to the baseline biopsy is required for a patient’s tumour to be deemed ASS negative / a low expressor.
ATTACHMENT F: Extension of ADI-PEG 20 Treatment beyond 6 months

Criteria for continuation of treatment:

Patients who have completed 6 months of treatment on Arm B (ADI-PEG 20 + Best Supportive Care) of the study and have stable disease or better, are entitled to continue treatment with ADI-PEG 20\textsuperscript{TM}. The decision to continue is at the discretion of both the patient’s clinician, the Chief Investigator and Polaris. Authorisation must be received from the Chief Investigator prior to continuing treatment.

Schedule:

<table>
<thead>
<tr>
<th>STUDY PROCEDURE</th>
<th>Extension Phase</th>
<th>End of treatment +7days</th>
<th>Follow-up After progression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Month X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Vital Signs (1)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Physical Exam</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ECG status</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematology</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Biochemistry (2)</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Blood sample for pharmacodynamic analysis</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood sample for metabolomic analysis</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine sample for metabolomic analysis</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeat tumour biopsy (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADI-PEG 20 i.m injection</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Concomitant Medications</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>AE assessment</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Survival data</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) To be collected at baseline, +26, +40 and +60 minutes
(2) Serum uric acid level to be checked prior to dosing on Day 1 of each cycle
(3) Optional (ICCF) - to be performed in event of patient progressing having previously shown response.

Patients receiving treatment on the extension phase may receive weekly treatment with ADI-PEG 20\textsuperscript{TM} until disease progression, unacceptable toxicity or withdrawal for up to two years from the last patient randomisation. Patients who are still on treatment at this time will be moved onto a compassionate care programme.

During the extension phase patients are required to continue to attend clinic weekly for the duration of their treatment. Within seven days of completing treatment patients will be required to have an end of treatment visit, thereafter they will be followed up for survival.

Dose modifications or significant delays in dosage administration are not expected. However, in the event of grade III or IV toxicity occurring, a dose reduction to 18.4mg (equivalent to 160IU/m\textsuperscript{2}), may be considered after discussion and authorisation by the Chief Investigator.
Doses should be delivered at 7 day intervals (±24hrs only in the event of public holidays or emergencies). Dosing delays are not permitted unless first authorised by the Chief Investigator.

No missed doses are permitted. Any subject who misses a dose or experiences more than a 24hr delay (unless authorised by the Chief Investigator) will be withdrawn from the study. Imaging should be performed as per Study Assessment Schedule on Page 19. (CT scan and PET-CT for patients on Arm B only performed at 6 months following completion of 6 months of treatment (i.e 12 months since randomisation) or before if felt to be ‘clinically appropriate’ i.e. at signs of disease progression.

**Data collection:**

Data for patients who enter the extension phase should be collected using the Extension Phase CRF Page. This form should be repeated for each month of treatment received as part of the extension phase.

**IMP requirements:**

Patients should be treated with ADI-PEG 20™ from the ADAM Trial supply.

Full accountability records and patient dispensing records should be maintained for the duration of their treatment on the extension phase.