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Revised Clinical Study Protocol	
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A Phase II, Double-Blind, Randomised Study to Assess the Efficacy of AZD6244 (Hyd-Sulfate) in Combination with Dacarbazine Compared with Dacarbazine Alone in First Line Patients with *BRAF* Mutation Positive Advanced Cutaneous or Unknown Primary Melanoma

Sponsor:

AstraZeneca AB, 151 85 Södertälje, Sweden

AstraZeneca Research and Development site representative

The following Amendment(s) and Administrative Changes are included in this revised protocol:

Amendment No.	Date of Amendment	Local Amendment No.	Date of local Amendment
1			
2			

Administrative change No.	Date of Administrative Change	Local Administrative change No.	Date of local Administrative Change
1			

AstraZeneca

PROTOCOL SYNOPSIS

A Phase II, Double-Blind, Randomised Study to Assess the Efficacy of AZD6244 (Hyd-Sulfate) in Combination with Dacarbazine Compared with Dacarbazine Alone in First Line Patients with *BRAF* Mutation Positive Advanced Cutaneous or Unknown Primary Melanoma

International Co-ordinating Investigators

Study centre(s) and number of patients planned

Approximately 80 first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma will be recruited from approximately 50 centres from countries including France, Germany, Netherlands, Norway, Spain, Sweden, Switzerland, UK, Czech Republic, Hungary, Brazil, USA and Australia. Approximately 60% of all patients will be recruited from European Union countries.

Study period		**Phase of development**
Estimated date of first patient enrolled	Q2 2009	II
Estimated date of last patient completed	Q3 2011	

The end of this study is defined as the date when all patients receiving AZD6244 have been followed for a minimum period of 12 months since start of treatment, or the date of the final analysis of the data, whichever is the later. At this time point, the clinical study database will close to new data. Patients are, however, permitted to continue to receive any study treatment beyond the closure of the database if, in the opinion of the investigator, they are continuing to derive clinical benefit from study treatment, in the absence of significant toxicity.

Objectives

Primary objective	Outcome variables
To assess the efficacy in terms of Overall Survival (OS) of AZD6244 in combination with dacarbazine, compared with dacarbazine alone, in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma	Overall Survival

Secondary objectives	Outcome variables
To further assess the efficacy of AZD6244 in combination with dacarbazine, compared with dacarbazine alone, in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma	- Alive and Progression Free at 6 months (APF6) - Progression Free Survival (PFS) - Objective Response Rate (ORR) - Duration of Response (DoR) - Change in Tumour Size at 12 weeks
To assess the safety and tolerability profile of AZD6244 in combination with dacarbazine	- Adverse Events - Clinical chemistry, haematology and urinalysis - Vital signs (including weight) - Physical examination - Echocardiogram - Electrocardiogram (ECG) - Ophthalmologic examination
To investigate the use of plasma and serum as a potential source of circulating free tumour DNA (CFDNA) for the analysis of *BRAF* mutation status	Correlation of *BRAF* mutation status derived from plasma, serum and tumour material.
To investigate the pharmacokinetics of AZD6244 and N-desmethyl AZD6244 when AZD6244 is administered in combination with dacarbazine	Where the data allow, derived PK parameters for AZD6244, N-desmethyl AZD6244 and any other known metabolites will be produced which may include, but not be restricted to, C_{max}, AUC and $t_{1/2.}$

Exploratory objectives	Outcome variables
To assess the prevalence, severity and change over time of advanced cutaneous or unknown primary melanoma specific symptoms in patients receiving AZD6244 in combination with dacarbazine and dacarbazine alone	Total Melanoma Specific Symptom questionnaire (MSSQ) score
To explore potential biomarkers in residual tumour, plasma and/or serum, taken for *BRAF* mutational analysis, which may influence development of advanced cutaneous or unknown primary melanoma (and associated clinical characteristics) and/or response (optional)	Correlation of biomarkers to response and/or development of advanced cutaneous or unknown primary melanoma
To investigate the relationship between AZD6244 and/or N-desmethyl AZD6244 and any other known metabolite plasma concentrations/exposure and clinical outcomes, efficacy, AEs and/or safety parameters if deemed appropriate	Output from both graphical and/or appropriate PK/PD modelling techniques.
To collect and store DNA, derived from a blood sample, for future exploratory research into genes that may influence response eg, distribution, safety, tolerability and efficacy of AZD6244 and/or agents used in combination and/or as comparators (optional)	Correlation of host polymorphisms with variation in PK, PD, safety or response parameters observed in patients treated with AZD6244 (and/or agents used in combination or as comparators)

Study design

This is a Phase II, double-blind, randomised, placebo-controlled study comparing the efficacy of AZD6244 (75 mg, orally uninterrupted twice daily) in combination with dacarbazine (1000 mg/m^2 iv infusion over at least 60 minutes on day 1 of each 21 day cycle) against dacarbazine alone, in first line patients with advanced cutaneous or unknown primary melanoma.

Patients will be selected on the basis of *BRAF* mutation positive status of their tumour sample and will be randomised in a ratio of 1:1 to receive AZD6244 or placebo in combination with dacarbazine. Following randomisation on Day 1, patients will attend for visits on Day 8, 15, 22, 29, 36, 43 and every 3 weeks thereafter for as long as they are receiving study treatment. Tumour evaluation according to RECIST guidelines will be performed at screening, Week 12 and every 12 weeks thereafter, relative to the date of randomisation. Up until the time of data cut off (DCO) for the analysis of PFS, patients must be followed until evidence of RECIST-defined progression (regardless of reason for treatment discontinuation). It is important that

patients are assessed according to the intended scanning schedule to prevent the bias in analysis that can occur if one treatment group is assessed more often or sooner than the other.

If a patient discontinues study treatment (AZD6244/placebo to AZD6244 [referred to as placebo hereafter] and dacarbazine) for reasons other than objective disease progression, RECIST assessments will continue according to the original schedule until objective disease progression. RECIST measurements will be used to derive the secondary variables APF6, PFS, ORR, DoR and change in tumour size at 12 weeks.

Patients will be permitted to continue to receive any study treatment after objective disease progression if, in the opinion of the investigator, they are continuing to derive clinical benefit from study treatment, in the absence of significant toxicity. Such patients will attend scheduled 3-weekly visits until discontinuation of the last study treatment, or until the end of the study, whichever occurs first.

Once a patient has had objective disease progression recorded, and discontinued all study treatment, they are to be followed up for survival status every 8 weeks until death, withdrawal of consent or the end of the study, whichever occurs first.

Approximately 80 patients (40 per treatment arm) will be randomised into this study. In order to recruit 80 evaluable first line *BRAF* mutation positive advanced cutaneous or unknown primary melanoma patients, it is expected that approximately 310 patients will be screened.

Target patient population

Male and female first line patients aged 18 and over with *BRAF* mutation positive advanced (inoperable stage III and stage IV) cutaneous or unknown primary melanoma. Eligible patients are those who would be candidates to receive dacarbazine as a standard of care for advanced cutaneous or unknown primary melanoma. Patients must have measurable disease (using CT/MRI) as defined by RECIST guidelines (Appendix D) and WHO performance status of 0-1.

Investigational product, dosage and mode of administration

AZD6244 Hyd-Sulfate (75 mg) will be administered orally as capsules. The Hyd-Sulfate formulation will be used in this study and unless otherwise specified is the formulation referenced throughout this document. AZD6244 will be administered orally uninterrupted twice daily, in combination with dacarbazine 1000 mg/m^2 iv, administered on day 1 of each 21 day cycle.

Comparator, dosage and mode of administration

Placebo will be administered orally uninterrupted twice daily, in combination with dacarbazine 1000 mg/m^2 iv, administered on day 1 of each 21 day cycle.

Duration of treatment

Patients are expected to receive AZD6244/placebo orally uninterrupted twice daily until RECIST-defined disease progression, in the absence of significant toxicity.

Patients are expected to receive up to 8 cycles of dacarbazine in the absence of significant toxicity. Investigators may decide to reduce the number of cycles of dacarbazine if significant toxicity develops. Further cycles of dacarbazine may be administered at the investigator's discretion if they feel it to be beneficial and it does not contravene local practice.

Patients who continue to derive clinical benefit from study treatment, in the opinion of the investigator, may continue to receive any study treatment after RECIST-defined progression, until the end of the study.

At the defined end of study (see Section 10.5), patients will be permitted to continue to receive AZD6244 if, in the opinion of the investigator, they are continuing to derive clinical benefit in the absence of significant toxicity. At the end of the study, any such patients will be unblinded and those receiving AZD6244 may begin open-label AZD6244 treatment. Any patients ongoing with study treatment beyond the end of study will be followed up according to the investigational site standard of care and investigator judgement. Investigators must continue to report all SAEs to AstraZeneca Patient Safety Department until 30 days after the last study treatment is discontinued and in accordance with Sections 7.3.3 and 7.3.4.

Statistical methods

The primary objective of this study will be to compare the efficacy of AZD6244 in combination with dacarbazine, versus dacarbazine alone, in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma, by assessing OS.

The secondary objective of this study will be to further assess the efficacy of AZD6244 in combination with dacarbazine, versus dacarbazine alone, in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma, by assessing the secondary variables of APF6, PFS, ORR, DoR and change in tumour size at 12 weeks.

The OS analysis will be performed when approximately 58 death events have occurred. If the true Hazard Ratio (HR) is 0.57 (likely to correspond to a 75% prolongation of OS), this analysis will have approximately 80% power to demonstrate a statistically significant difference for OS, assuming a 1-sided 10% significance level. This trial has been sized using a 1-sided significance level of 10% as it is a Phase II study looking for a signal of improved efficacy. If a 1-sided $p<0.1$ is observed for the comparison of OS between AZD6244 in combination with dacarbazine, versus dacarbazine alone, the results will be regarded as promising (but not definitive) as there is a less than 1 in 10 probability that such a result could have been detected if there was truly no treatment effect. Assuming 58 events occur, an observed HR of <0.71 will achieve a 1-sided p-value <0.1 within the trial.

Assuming non-uniform recruitment and a median OS of 9 months (Patel 2008) for dacarbazine, if approximately 80 patients (40 per arm) are recruited over 12 months it is

predicted that 58 events will occur approximately 16 months following recruitment of the last patient.

An analysis of PFS, APF6, ORR, DoR and change in tumour size at 12 weeks will be performed 6 months after the last patient has been enrolled in the study when it is considered the PFS data is sufficiently mature, APF6 will be available and the vast majority of patients will have discontinued randomised therapy. OS data may also be assessed at this time.

Efficacy data will be analysed on an intention-to-treat basis using randomised treatment.

PFS, ORR, DoR, change in tumour size at 12 weeks and APF6 will be assessed using RECIST measurements (RECIST assessments to be carried out at baseline, Week 12 and every 12 weeks thereafter relative to randomisation).

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study protocol.

Abbreviation or special term	Explanation
AE	Adverse event (see definition in Section 7.3.1)
AJCC	American Joint Committee on Cancer
ALP	Alkaline phosphatase
ALT/SGPT	Alanine aminotransferase
ANC	Absolute Neutrophil Count
ANCOVA	Analysis of covariance
APF6	Alive & Progression Free at 6 months
ARMS™	Amplification Refractory Mutation System
AST/SGOT	Aspartate aminotransferase
AZD6244 Hyd-Sulfate	AZD6244 hydrogen sulphate salt
AUC	Area under plasma concentration time curve
BD	Twice Daily
BP	Blood pressure
bpm	Beats per minute
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CFDNA	Circulating free tumour deoxyribonucleic acid
C_{max}	Maximum plasma concentration
CNS	Central Nervous System
CR	Complete Response
CRF	Case Report Form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA4	Cytotoxic T-Lymphocyte Antigen 4
DAEs	Discontinuation due to Adverse Events
DCO	Data Cut Off
DLT	Dose-limiting toxicity
DMPK	Drug metabolism and pharmacokinetics
DNA	Deoxyribonucleic acid

Abbreviation or special term	Explanation
DoR	Duration of Response
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
ERK	Extracellular signal-regulated kinase
Ethics Committee	Synonymous to Institutional Review Board and Independent Ethics Committee
EU	European Union
FACT-G	Functional Assessment of Cancer Therapy - General
FACT-MEL	Functional Assessment of Cancer Therapy - Melanoma
FFPE	Formalin-fixed paraffin-embedded
FSH	Follicle Stimulating Hormone
γGT	Gamma glutamyl transferase
GCP	Good Clinical Practice
GM-CSF	Granulocyte macrophage colony-stimulating factor
GMP	Good Manufacturing Practice
HDPE	High Density Polyethylene
HIV	Human immunodeficiency virus
HR	Hazard Ratio
IATA	International Air Transport Association
ICH	International Conference on Harmonisation
INR	International Normalised Ratio
International Co-ordinating Investigator	If a study is conducted in several countries the International Co-ordinating Investigator is the Investigator co-ordinating the investigators and/or activities internationally.
IPS	Investigational Products, AstraZeneca
ITT	Intention To Treat
iv	Intravenous
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
LD	Longest Diameter
LDH	Lactate Dehydrogenase
LH	Luteinising Hormone

Abbreviation or special term	Explanation
LPI	Last Patient In (enrolled into the study)
LVEF	Left Ventricular Ejection Fraction
MedDRA	Medical Dictionary for Regulatory Activities
MEK	Mitogen-Activated Protein Kinase Kinase
MRI	Magnetic resonance imaging
MSSQ	Melanoma Specific Symptom Questionnaire
MTIC	5-(3-dimethyl-1-triazenyl) imidazole-4-carboxamide
NSCLC	Non-small cell lung cancer
NTL	Non-target lesion
NYHA	New York Heart Association
OAE	Other Significant Adverse Event (ie, adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the patient from study treatment; see definition in Section 12.1.2.
OD	Once Daily
ORR	Objective Response Rate
OS	Overall Survival
p	Probability
PCR	Polymerase chain reaction
PD	Progression of Disease
pERK	Level of ERK phosphorylation
PFS	Progression Free Survival
PK	Pharmacokinetics
PPV	Positive predictive value
PR	Partial Response
PRO	Patient Reported Outcomes
QoL	Quality of Life
QTcF	QT corrected using Fridericia's formula
RECIST	Response evaluation criteria in solid tumours
RPLS	Reversible Posterior Leucoencephalopathy Syndrome
SAE	Serious adverse event (see definition in Section 7.3.2)
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software

Abbreviation or special term	Explanation
SD	Stable Disease
SDV	Source Data Verification
SPC	Summary of Product Characteristics
Study treatment	AZD6244/placebo or dacarbazine
$t_{1/2}$	Terminal elimination half life
t_{max}	Time to reach the maximum plasma concentrations
UACR	Urinary albumin:creatinine ratio
ULN	Upper Limit of Normal
WBDC	Web Based Data Capture
WHO	World Health Organisation

1. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

1.1 Medical emergencies and AstraZeneca contacts

The Principal Investigator(s) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such, see Section 7.3.4**

In the case of a medical emergency the investigator may contact the Study Delivery Team Leader. If the Study Delivery Team Leader is not available, contact the Study Delivery Team Physician at the AstraZeneca Research and Development site shown below.

Name	**Role in the study**	**Address & telephone number**
contact persons below		

Name	Role in the study	Address & telephone number
Local contact persons can be added in wet-ink.		

1.2 Overdose

- An overdose with associated adverse events (AEs) is recorded as the AE diagnosis/symptoms on the relevant AE modules in the CRF and on the Overdose CRF module
- An overdose without associated symptoms is only reported on the Overdose CRF module.

1.3 Pregnancy

All outcomes of pregnancy must be reported to AstraZeneca.

1.3.1 Maternal exposure

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the patient was discontinued from the study.

If any pregnancy occurs in the course of the study, the investigator or other site personnel must inform appropriate AstraZeneca representatives immediately, but no later than the end of the next business day, of when he or she becomes aware of it. The designated AstraZeneca representative works with the investigator to ensure that all relevant information is provided to the appropriate AstraZeneca patient safety data entry site within 30 calendar days.

The PREGREP module in the CRF is used to report the pregnancy and the PREGOUT is used to report the outcome of the pregnancy

1.3.2 Paternal exposure

The outcomes of any conception occurring from the date of the first dose, until 30 days after last dose, must be followed up and documented in the "Pregnancy Outcome Report" form.

Male patients must refrain from fathering a child during the study and **16 weeks** following the last dose of AZD6244/placebo, since the potential for chromosomal aberrations in male gametes, and possible teratogenic effects thereof, has not yet been thoroughly investigated. Male patients should be advised to take contraceptive measures for **6 months** after cessation of dacarbazine treatment.

Pregnancy of the patients' partner is not considered to be an AE. However, the outcome of all pregnancies (including spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented.

2. INTRODUCTION

2.1 Background

2.1.1 Melanoma

Melanoma is the most clinically significant, potentially life-threatening form of skin cancer. The incidence rate of melanoma continues to increase by approximately 3% per year (American Cancer Society 2006). Despite the advances in prevention, early detection and treatment of the patients with melanoma, it still accounts for approximately 8000 deaths annually in the US (American Cancer Society 2006), more than 1600 in the UK (UK Skin Cancer Mortality Statistics 2006) and more than 800 in Australia (National Cancer Statistics Clearing House and National Mortality Database, AIHW 2008).

Melanoma disease staging is based upon TNM criteria, with T representing tumour thickness and ulceration, N representing regional lymph node involvement or intra-lymphatic regional metastases, and M representing distant metastatic spread; and is split into four stages by the American Joint Commission for Cancer (AJCC; Balch et al 2001).

If detected and treated at early stage, when melanoma is amenable to surgical excision, the disease has overall favourable outcome with the cure rate of approximately 90% (Kim et al 2002). In contrast, patients with metastatic (Stage IV) melanoma have poor a prognosis with an average 5-year survival rate of <5%, with a median survival period of 6-9 months (Eggermont and Kirkwood 2004).

The standard treatment for patients with metastatic melanoma has not been defined and the available treatments are limited. Current therapeutic approaches in use and under investigation include chemotherapy (mono and poly-chemotherapy), immunotherapy, biochemotherapy, vaccines and gene therapy. However, none of the existing treatments has so far yielded significant improvement in response rates or has been shown to improve overall survival (Danson and Middleton 2001), which in part reflects low activity and efficacy of available agents.

2.1.2 Mitogen activated protein kinase kinase (MEK)

The intracellular Ras regulated Raf/MEK/ERK protein kinase signal cascade is a key pathway involved in cellular proliferation, and there is a strong link between deregulation of this pathway and uncontrolled cell proliferation and survival (Chow et al 2005).

It is anticipated that inhibition of MEK activity should inhibit transduction of the mitogenic signals from multiple pathways, resulting in an effect on tumour proliferation, differentiation

and survival. Activation of the Ras/Raf/MEK/ERK cascade in melanoma has been shown in the number of studies (Fecher et al 2007).

2.1.3 AZD6244

AZD6244 is a potent, selective, uncompetitive inhibitor of MEK, licensed for development by AstraZeneca Pharmaceuticals from Array BioPharma. Array BioPharma was responsible for the first administration into man study, performed at three centres in the United States. The remainder of the clinical development programme for oncology indications is the responsibility of AstraZeneca. Phase II monotherapy studies commenced in 2006.

2.1.3.1 Pre-clinical experience with AZD6244

The pharmacological effects of AZD6244 have been studied extensively by examining the anti-tumour activity of AZD6244 in different in vivo and in vitro tumour models. Overall, AZD6244 had strong anti-tumour activity in multiple non-clinical models, including human melanomas (LOX and A375v), human breast carcinomas (Zr-75-1 and MDA-MB-231), human pancreatic tumours (BxPC3, AsPC1, HPAC, MIA PaCa-2 and PANC 1), human lung cancer tumours (A549) and human colon carcinomas (HT-29, Colon 26 tumours, Colo205, SW620, Lovo and HCT116).

AZD6244 has demonstrated potent inhibition of *BRAF* or *KRAS*-positive cell line viability, and inhibition of xenograft growth both as monotherapy and in combination with a number of cytotoxic and targeted agents, including temozolomide. Temozolomide has the same active metabolite (MTIC) as dacarbazine, but is metabolised via an alternative route that is more reproducible in pre-clinical models than dacarbazine. The detailed information on pre-clinical studies is presented in the Investigator Brochure.

2.1.3.2 Clinical experience with AZD6244

The formulation taken into the Phase I clinical study by Array BioPharma (Study # ARRY-0401) was an extemporaneous preparation of an oral suspension of AZD6244 as the free-base in an aqueous solution of sulphobutylether β-cyclodextrin (SBE-CD, Captisol®), referred to as the free-base suspension formulation. The monotherapy Phase II clinical programme also utilised this formulation. Subsequent formulation development resulted in a capsule formulation of AZD6244 as the hydrogen sulphate salt (hereafter referred to as AZD6244 Hyd-Sulfate).

Phase II monotherapy programme with AZD6244 free-base suspension

The programme of AstraZeneca-sponsored Phase II AZD6244 free-base monotherapy studies is complete.

In study D1532C00003 (melanoma) there was no difference in efficacy between AZD6244 and temozolomide for the primary endpoint of progression free survival, however anti-tumour activity was detected (partial responses, especially in *BRAF* mutation positive melanomas, and prolonged stable disease; Dummer et al 2008).

Phase I monotherapy study with AZD6244 Hyd-Sulfate (D1532C00005)

Study D1532C00005 is a Phase I, open-label study to assess the safety, tolerability and pharmacokinetics of AZD6244 Hyd-Sulfate in patients with advanced solid tumours. According to the criteria predefined in the protocol, the 75 mg twice daily dose has been designated as the maximum tolerated monotherapy dose for the AZD6244 Hyd-Sulfate formulation. The emerging safety profile of AZD6244 Hyd-Sulfate is broadly consistent with observations for the AZD6244 free-base suspension formulation.

The information about the most frequently reported all-causality AEs reported at 75 mg twice daily in study D1532C00005 is outlined in Table 1, and further described in the Investigator Brochure.

Table 1 Most frequently reported all-causality AEs in study D1532C00005

AE preferred term	Number (%) of patients at AZD6244 75 mg twice daily N=35	
Fatigue	23	(65.7)
Dermatitis acneiform	21	(60.0)
Nausea	17	(48.6)
Diarrhoea	19	(54.3)
Oedema peripheral	17	(48.6)
Cough	15	(42.9)
Dry skin	15	(42.9)
Dyspnoea exertional	11	(31.4)
Anorexia	10	(28.6)
Constipation	9	(25.7)
Vomiting	8	(22.9)
Abdominal pain	8	(22.9)
Pruritus	7	(20.0)

Phase I combination study (D1532C00004)

Study D1532C00004 is a Phase I, open label study to assess the safety, tolerability and pharmacokinetics of AZD6244 Hyd-Sulfate in patients with advanced solid tumours when administered in combination with other anti-cancer agents. As of 25 March 2009, 13 patients have been dosed with AZD6244 (7 patients received 50 mg twice daily and 6 patients received 75 mg twice daily) in combination with 1000 mg/m^2 dacarbazine (iv infusion over 60 minutes on day 1 of each 21 day cycle) in the dose-escalation phase of this study. There were 6 evaluable patients in each cohort. One patient in the AZD6244 50 mg twice daily plus dacarbazine cohort experienced a dose-limiting toxicity (DLT) of CTCAE grade 4

thrombocytopenia. None of the patients receiving AZD6244 75 mg twice daily plus dacarbazine experienced an AE which would be classed as a DLT according to the protocol criteria. Therefore, the recommended Phase II dose for the combination of these agents is 75 mg twice daily AZD6244 Hyd-Sulfate and 1000 mg/m^2 dacarbazine (iv infusion over 60 minutes on day 1 of each 21 day cycle). The safety, tolerability and anti-tumour activity of the determined combination dose of AZD6244 will be further explored in the expansion phase of study D1532C00004.

The preliminary PK data suggest there are no measurable interactions that significantly affect the plasma exposure of either AZD6244 or dacarbazine.

The study is currently ongoing and the safety data presented below are preliminary and unvalidated. All 13 patients dosed with AZD6244 in combination with dacarbazine developed at least one AE. There were no AEs resulting in discontinuation of the study treatment. The most frequently reported AEs, regardless of dose cohort, severity, causality and seriousness, were: nausea (7/13 patients, 53.8%), diarrhoea (5/13 patients, 38.5%), dysgeusia, constipation, neutropenia, rash (each reported by 4/13 patients, 30.8%), anaemia, thrombocytopenia, oedema peripheral, chills, pyrexia and asthenia/fatigue (each reported by 3/13 patients, 23.1%). In addition to rash, an AE of dermatitis acneiform was reported by one patient. The majority of AEs corresponded to CTCAE grade 1 to 2. CTCAE grade 3 diarrhoea, grade 4 dyspnoea (associated with malignant pleural effusion), grade 4 neutropenia and grade 4 thrombocytopenia were experienced by a single patient each, while 2 patients experienced CTCAE grade 3 anaemia.

The number and types of AEs reported in patients receiving the combination of AZD6244 with dacarbazine were consistent with the nature of study treatments and the disease under investigation. It was concluded that AZD6244 did not add significant toxicity to the dacarbazine side effect profile. Further information on the most common causally-related AEs reported in this study and discussion of the AEs of this combination can be found in the Investigator Brochure.

2.1.4 Dacarbazine

Dacarbazine is an imidazole dimethyltriazene alkylating agent that has been approved for use in the treatment of metastatic malignant melanoma and Hodgkin's disease since the 1970s. In the treatment of melanoma it has been used as both a monotherapy and as a combination therapy with other cytotoxic agents, tamoxifen and/or immunomodulatory agents.

Dacarbazine still remains a routine treatment for melanoma outside of clinical trials. Overall response rates to dacarbazine in large Phase III trials have been reported at approximately 10%, with a further 20% of patients having stable disease. Complete responses are rare with the duration of 3 to 6 months (Bedikian et al 2006, Middleton et al 2000, Patel 2008).

Dacarbazine is generally well tolerated, with the most common side effects being nausea and vomiting, both of which are managed effectively by the use of anti-emetic agents such as steroids and serotonin antagonists (Lens and Eisen 2003). Other adverse effects reported

include haematopoetic depression (predominantly neutropenia and thrombocytopenia), diarrhoea, flu-like symptoms, alopecia, impairment of renal function and photosensitivity. Potentially fatal hepatic vein thrombosis with hepatocellular necrosis has been reported in 0.01% of patients, but usually when administered concomitantly with other cytotoxic agents. Dacarbazine does not cross the blood-brain barrier and so is ineffective in the subpopulation of patients with CNS metastases.

2.2 Research hypothesis

AZD6244 (150 mg/day) in combination with dacarbazine (iv 1000 mg/m^2 per cycle) shows improved efficacy compared to dacarbazine alone in a placebo-controlled study for first line patient with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma.

2.3 Rationale for conducting this study

Extensive research in melanoma biology and its molecular pathways has unfortunately not translated into novel effective treatments. The survival of patients with metastatic melanoma has not improved in the last 30 years, indicating significant unmet clinical need (Serrone et al 2000).

Recent studies have resulted in a clearer picture of molecular events in melanoma development, with MAPK signal transduction pathway raising particular interest due to high frequency of gain-of-function *BRAF* mutations in cutaneous melanoma (Fecher et al 2007). *BRAF* mutations have been identified in more than 60% of malignant melanomas (Davies et al 2002) and their oncogenic role suggests that inhibition of activated MAPK signal pathway could be of potential therapeutic benefit.

Studies have demonstrated the evidence of exquisite MEK sensitivity in cell lines harbouring *BRAF* mutations (Solit et al 2006). In line with this, AZD6244, the selective uncompetitive inhibitor of MEK, has also been shown to produce more profound responses in *BRAF* mutation positive tumours. Although there was no statistically significant difference between AZD6244 and temozolomide for the primary endpoint of PFS (as described in Section 2.1.3.2), anti-tumour activity was detected with AZD6244 (partial responses, particularly in the *BRAF* mutation positive sub-group, and prolonged stable diseases). Five out of six partial responses achieved with AZD6244 in study D1532C00003, and one complete response confirmed in study D1532C00005, were in patients with cutaneous melanoma harbouring *BRAF* mutation.

Moving forward, the clinical development in melanoma will investigate the efficacy of AZD6244 in combination with other anti-cancer agents. The personalised medicine approach of pre-selecting only patients with *BRAF* mutation offers an opportunity to target treatment to the most appropriate patients, therefore maximising the chance of identifying therapeutic benefit (Royal Society 2005).

Dacarbazine is the routine therapy for metastatic melanoma outside of clinical trials and has been used as a standard of care in clinical trials. Cell lines resistant to dacarbazine have been shown to express significantly higher levels of pERK and to slow down their proliferation

rates in response to treatment with combination of MEK inhibitor and dacarbazine, suggesting that ERK inhibition makes cells more responsive to dacarbazine (Lev et al 2004). Pre-clinical studies with combination of AZD6244 and temozolomide (which shares the same metabolite with dacarbazine, namely MTIC) have also indicated additive effect of therapies and resulted in more dramatic responses as compared to either of the agents alone (Wilkinson et al 2008).

The objective of this clinical study is to look for a signal of improved efficacy by combining AZD6244 with dacarbazine, compared to dacarbazine alone, in *BRAF* mutation positive first line advanced melanoma patients. As this study will involve the first exposure of a significant number of first line advanced melanoma patients to the combination of AZD6244 and dacarbazine, this study will assess the pharmacokinetic, safety and tolerability profile of the combination, compared to dacarbazine alone, by regular monitoring of adverse events, symptom and safety assessments, and blood sampling for pharmacokinetic analysis. As part of this study, tumour mutation status will be determined as a key entry criterion. However, the acquisition of tumour samples from this patient population can be difficult and therefore an investigation into the concordance between the mutation status derived from a tumour biopsy and that from a blood sample (circulating free tumour DNA; CFDNA) will be performed. Data from blood samples collected for CFDNA analysis will be generated at a later date and will not be used as an entry criterion for the study.

2.4 Benefit/risk and ethical assessment

Evidence of AZD6244 anti-tumour activity in advanced melanoma has been demonstrated in a Phase II monotherapy study and Phase I study with AZD6244 Hyd-Sulfate. It is therefore clear that some patients may derive benefit from therapy with AZD6244. Further Phase II investigations, utilising AZD6244 both as monotherapy and in combination with other anti-cancer agents, for which there is a strong preclinical rationale, are required to understand the activity profile of AZD6244.

Based on a signal coming from the pre-clinical and clinical data, and to maximize the potential effect of treatment, this will be the first study directed specifically at investigating the combination of AZD6244 and dacarbazine in the first line *BRAF* mutation positive advanced melanoma patient population.

Dacarbazine has a well documented safety profile in melanoma patients. The 1000 mg/m^2 dose of dacarbazine has been used in clinical practice over recent years and tested in several clinical studies without raising additional safety concerns (McDermott et al 2008, Patel 2008).

The potential safety issues observed to date in the AZD6244 monotherapy programme are outlined in Section 2.1.3.2 and are clarified further in the Investigator Brochure. Guidance documents (algorithms) were developed to facilitate investigators with management and/or investigation of dermatological events, dyspnoea, and decrease in left ventricular ejection fraction (LVEF) and are included in the protocol for reference (see Section 4.1.10.3, Appendix H and Appendix J respectively).

The safety and tolerability profile of AZD6244 in combination with dacarbazine has been studied in 13 patients with solid tumours and was shown to be consistent with the two individual monotherapy profiles (see Section 2.1.3.2). Evaluation of the combination safety and efficacy is ongoing, as patients are being enrolled into the expansion phase of study D1532C00004.

In case of any intolerable and severe AEs, the investigator should follow the instructions provided in Section 4.1.10, and mitigate the risk to a patient by applying the suggested dose reduction/adjustment algorithm for AZD6244 and/or adjusting dacarbazine dosing accordingly.

AstraZeneca believes that the investigation of AZD6244 in combination with dacarbazine in the first line *BRAF* mutation positive advanced melanoma setting is justified, based on the emerging safety profile of AZD6244 Hyd-Sulfate alone, and in combination with dacarbazine, the lack of highly effective alternative treatments available to this subset of patients, their limited life expectancy, and the strength of the scientific hypothesis under evaluation.

As a part of this study the patients will undergo standard clinical evaluations, including physical examinations, registration of vital signs and basic biometric parameters, safety laboratory evaluations (haematology, chemistry, urinalysis), regular pregnancy tests (where applicable), ECGs, computed tomography (CT)/magnetic resonance imaging (MRI) scans and echocardiogram scans. Diagnostic procedures and assessments mandated by the study protocol have been designed with the consideration of safety profiles of both treatments. The types and frequency of assessments are aligned to the current healthcare standards with additional precaution and increase in examinations during the first two 3-weekly cycles of treatment to ensure timely detection of any early treatment emergent safety signals.

Adverse events related to visual function have been reported at a low frequency in most monotherapy studies with AZD6244. There were no specific clinical findings reported from patients that underwent ophthalmological evaluation after reporting the adverse event of visual disturbance. To further assess and document any clinical effects that may be linked to development of visual function adverse events in patients receiving AZD6244, a full ophthalmological examination should be conducted at baseline, at Week 6 and on the occurrence of any visual disturbance adverse event.

One patient receiving monotherapy with AZD6244 at 75 mg twice daily experienced grade 2 blurred vision, followed by a serious adverse event of convulsion, which occurred two days after insertion of a biliary stent. CT and MRI scans showed no intracerebral metastases, but gave a differential diagnosis of Reversible Posterior Leucoencephalopathy Syndrome (RPLS) in combination with pre-existing vascular abnormalities. As a result of this finding, patients experiencing blurred vision (CTCAE ≥grade 2) concurrent with neurological symptoms suggestive of possible RPLS (eg, seizure, altered mental status, headaches) will be additionally required to undergo an MRI scan (alternatively, if MRI scan is medically contraindicated or not available at site, a CT scan) of the brain.

Some patients receiving AZD6244 have been observed to develop asymptomatic decreases in LVEF in the absence of confounding comorbidities. AstraZeneca continues evaluation of LVEF changes in patients receiving AZD6244 and collects baseline and sequential measurements of LVEF and end systolic and diastolic ventricular diameters on echocardiogram.

3. STUDY OBJECTIVES

3.1 Primary objective

Primary objective	Outcome variables
To assess the efficacy in terms of Overall Survival (OS) of AZD6244 in combination with dacarbazine, compared with dacarbazine alone, in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma	Overall Survival

3.2 Secondary objectives

Secondary objectives	Outcome variables
To further assess the efficacy of AZD6244 in combination with dacarbazine, compared with dacarbazine alone, in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma	- Alive and Progression Free at 6 months (APF6) - Progression Free Survival (PFS) - Objective Response Rate (ORR) - Duration of Response (DoR) - Change in Tumour Size at 12 weeks
To assess the safety and tolerability profile of AZD6244 in combination with dacarbazine	- Adverse Events - Clinical chemistry, haematology and urinalysis - Vital signs (including weight) - Physical examination - Echocardiogram - Electrocardiogram (ECG) - Ophthalmologic examination
To investigate the use of plasma and serum as a potential source of circulating free tumour DNA (CFDNA) for the analysis of *BRAF* mutation status	Correlation of *BRAF* mutation status derived from plasma, serum and tumour material.

Secondary objectives	Outcome variables
To investigate the pharmacokinetics of AZD6244 and N-desmethyl AZD6244 when AZD6244 is administered in combination with dacarbazine	Where the data allow, derived PK parameters for AZD6244, N-desmethyl AZD6244 and any other known metabolites will be produced which may include, but not be restricted to, C_{max}, AUC and $t_{1/2.}$

3.3 Exploratory objectives

Exploratory objectives	Outcome variables
To assess the prevalence, severity and change over time of advanced cutaneous or unknown primary melanoma specific symptoms in patients receiving AZD6244 in combination with dacarbazine and dacarbazine alone	Total Melanoma Specific Symptom questionnaire (MSSQ) score
To explore potential biomarkers in residual tumour, plasma and/or serum, taken for *BRAF* mutational analysis, which may influence development of advanced cutaneous or unknown primary melanoma (and associated clinical characteristics) and/or response (optional)	Correlation of biomarkers to response and/or development of advanced cutaneous or unknown primary melanoma
To investigate the relationship between AZD6244 and/or N-desmethyl AZD6244 and any other known metabolite plasma concentrations/exposure and clinical outcomes, efficacy, AEs and/or safety parameters if deemed appropriate	Output from both graphical and/or appropriate PK/PD modelling techniques.
To collect and store DNA, derived from a blood sample, for future exploratory research into genes that may influence response eg, distribution, safety, tolerability and efficacy of AZD6244 and/or agents used in combination and/or as comparators (optional)	Correlation of host polymorphisms with variation in PK, PD, safety or response parameters observed in patients treated with AZD6244 (and/or agents used in combination or as comparators)

4. STUDY PLAN AND PROCEDURES

This Clinical Study Protocol has been subject to a peer review according to AstraZeneca standard procedures.

4.1 Overall study design and flow chart

This is a Phase II, double-blind, randomised, placebo-controlled study comparing the efficacy of AZD6244 (75 mg, orally uninterrupted twice daily) in combination with dacarbazine (1000 mg/m^2 iv infusion over at least 60 minutes on day 1, of each 21 day cycle) against dacarbazine alone, in first line patients with *BRAF* mutation positive advanced (inoperable stage III and stage IV) cutaneous or unknown primary melanoma.

Patients will be selected on the basis of the *BRAF* mutation positive status of their tumour sample and will be randomised in a ratio of 1:1 to receive AZD6244 or placebo in combination with dacarbazine (see Figure 1). Following randomisation on Day 1, patients will attend for visits on Day 8, 15, 22, 29, 36, 43 and every 3 weeks thereafter for as long as they are receiving study treatment. Tumour evaluation, according to RECIST guidelines, will be performed at screening, Week 12 and every 12 weeks thereafter, relative to the date of randomisation. Up until the time of DCO for the analysis of PFS, patients must be followed until evidence of RECIST-defined progression (regardless of reason for treatment discontinuation). It is important that patients are assessed according to the intended scanning schedule to prevent the bias in analysis that can occur if one treatment group is assessed more often or sooner than the other.

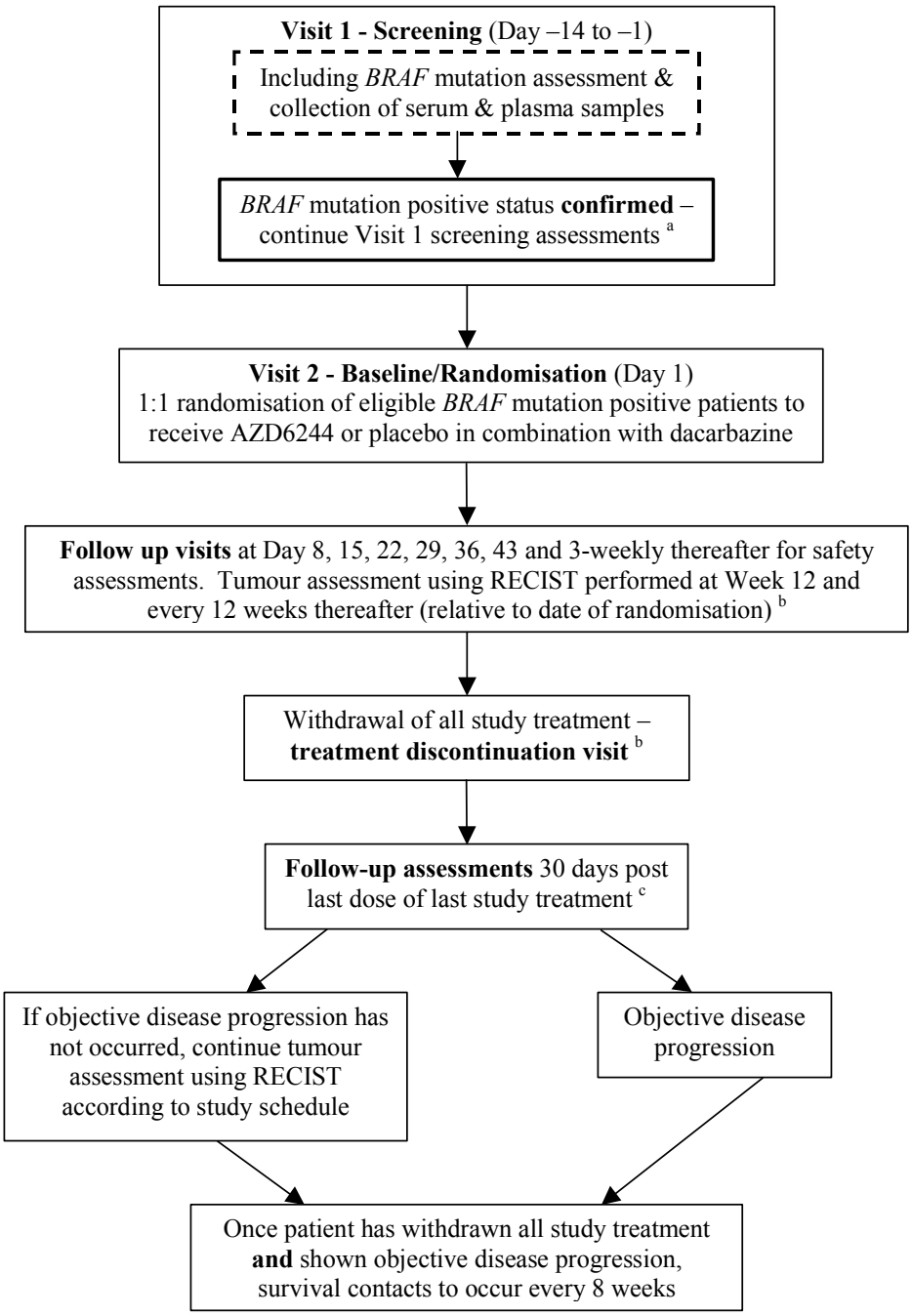
If a patient discontinues study treatment (AZD6244/placebo and dacarbazine) for reasons other than objective disease progression, RECIST assessments will continue according to the original schedule until objective disease progression. RECIST measurements will be used to derive the secondary variables of APF6, PFS, ORR, DoR and change in tumour size at 12 weeks.

Patients will be permitted to continue to receive any study treatment after objective disease progression if, in the opinion of the investigator, they are continuing to derive clinical benefit from study treatment, in the absence of significant toxicity. Such patients will attend scheduled 3-weekly visits until discontinuation of the last study treatment, or until the defined end of the study (see Section 10.5), whichever occurs first. Following the end of the study, patients receiving active AZD6244 will be permitted to continue treatment, if in the opinion of the investigator, they are deriving clinical benefit. Any such patients will be followed up according to the investigational site standard of care and investigator judgement.

Once a patient has had objective disease progression recorded and discontinued all study treatment, they are to be followed up for survival status every 8 weeks until death, withdrawal of consent or the end of the study, whichever occurs first.

Approximately 80 patients (40 per treatment arm) will be randomised into this study. In order to recruit 80 evaluable first line *BRAF* mutation positive advanced cutaneous or unknown primary melanoma patients, it is expected that approximately 310 patients will be screened from countries including France, Germany, Netherlands, Norway, Spain, Sweden, Switzerland, UK, Czech Republic, Hungary, Brazil, USA and Australia. Assessments planned at each visit are detailed in Table 2 and Sections 4.1.1, 4.1.2, 4.1.3, 4.1.4, 4.1.5, 4.1.7 and 4.1.8.

Figure 1 Study flow chart



a Screening assessments can be performed in a stepwise process, or in parallel with the *BRAF* mutation assessment
b RECIST assessments to be continued until objective disease progression (regardless of reason for treatment discontinuation)
c Last dose of study treatment = last dose of AZD6244/placebo or dacarbazine

Table 2 Study plan

Visit	**1**	**2**	**3**	**4**	**5**	**5.1**	**5.2**	**6**	**7**	**8+** [a]	**Discontinuation from treatment**		
Visit Description	Screening	Baseline/ Randomisation	Treatment Period								Treatment Discontinuation Visit	30 days post last dose of last study treatment	Progression & Survival Follow-up [b]
Day	-14 to –1	1	8	15	22	29	36	43	64	85	N/A	N/A	N/A
Week	-2 to 0	0	1	2	3	4	5	6	9	12	N/A	N/A	N/A
Visit Window (compared to Day 1)	N/A	N/A	±2 days	±2 days	±2 days	±2 days	±2 days	±1 wk	±1 wk	±1 wk	N/A	±7 days (compared to trt disc visit)	N/A
Mutation status informed consent	X												
Main study informed consent	X												
Enrol partient via IVRS/IWRS	X												
Additional screening procedures [c]	X												
Optional host genetic consent & sample [d]		X											
MSSQ		X			X			X	X	X	X		
Adverse events [e]	X	X	X	X	X	X	X	X	X	X	X	X [f]	X [f]
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X [g]
Vital signs including weight	X	X	X	X	X			X [h]	X	X [h]	X	(X) [i]	
Clinical chemistry/haematology	X	X	X	X	X	X [j]	X [j]	X	X	X	X	(X) [k]	
Urinalysis [l]	X	X	X	X	X			X	X	X	X		
Pregnancy test (pre-menopausal females only)	X	X							X [a]				
Plasma sample collection for PK [m]		X			X								

Table 2 Study plan

Visit	**1**	**2**	**3**	**4**	**5**	**5.1**	**5.2**	**6**	**7**	**8+** [a]	**Discontinuation from treatment**		
Visit Description	Screening	Baseline/ Randomisation	Treatment Period								Treatment Discontinuation Visit	30 days post last dose of last study treatment	Progression & Survival Follow-up [b]
Day	-14 to –1	1	8	15	22	29	36	43	64	85	N/A	N/A	N/A
Week	-2 to 0	0	1	2	3	4	5	6	9	12	N/A	N/A	N/A
Visit Window (compared to Day 1)	N/A	N/A	±2 days	±2 days	±2 days	±2 days	±2 days	±1 wk	±1 wk	±1 wk	N/A	±7 days (compared to trt disc visit)	N/A
ECG [n]	X	X			X			X [h]		X [h]	X	(X) [i]	
WHO performance status	X				X			X	X	X	X		
Echocardiogram [h]	X							X		X		(X) [i]	
Tumour evaluation by RECIST [o]	X									X			X
Physical examination	X								X [a]		X		
Ophthalmologic examination [p]	X							X					
IVRS/IWRS: dispense AZD6244/placebo		X			X			X	X	X			
AZD6244/placebo dosing [q]		twice-daily dosing →											
Dacarbazine infusion [r]		X			X			X	X	X			
Check returned study medication					X			X	X	X	X		
Survival status													X

a From Visit 8 (Week 12) onwards patients to attend clinic visits every 3 weeks until discontinuation of treatment with assessments matching those at Week 12, with the exception of RECIST assessments (every 12 weeks until objective disease progression), echocardiogram (every 12 weeks), pregnancy test, and physical exam (every 9 weeks from Week 9)

b If a patient discontinues study treatment for reasons other than objective disease progression RECIST assessments will continue according to the original schedule until objective disease progression or until the time of DCO for the analysis of PFS. Following objective disease progression or discontinuation of all study treatment (whichever is the later date) patients must be contacted every 8 weeks to establish survival status

c Additional screening procedures will include: confirmation of *BRAF* mutation status using patient's tumour, collection of serum and plasma sample for CFDNA, collection of demography data, medical and surgical history, previous anti-cancer treatment, disease staging, smoking status, height, eligibility check. Baseline CT scan can be performed up to 14 days prior to randomisation. Refer to Section 4.1.1 for the detailed list of screening assessments and procedures

d Optional pre-dose blood sample from consented patients only (for details see Appendix E). If a sample is not taken pre-dose the sample can be taken at any time during the study

e All cardiorespiratory AEs with no obvious diagnosis should be assessed with an echocardiogram, single ECG, vital signs and weight. Asymptomatic decreases in LVEF should be investigated according to the algorithm provided in Appendix J. All new dyspnoea AEs or worsening of pre-existing dyspnoea AEs should be followed up according to the dyspnoea algorithm provided in Appendix H. If a patient experiences an AE of visual disturbance (including blurring of vision) a complete full ophthalmological examination, including a slit-lamp examination, must be performed. In case of blurred vision (grade ≥2) concurrent with neurological symptoms suggestive of RPLS, patients are required to undergo MRI (or CT) scan of the brain. Scans should be repeated upon resolution of neurological symptoms or upon clinically significant neurological deterioration.

f Collection of AEs/SAEs will continue until 30 days after the last dose of the last study treatment. Patients who discontinue study treatment for reasons other than disease progression, and are continuing to have RECIST assessments, following the 30 days after the last dose of the last study treatment they will only have study procedure-related SAEs captured until the patient is considered to have progressive disease, and therefore will have no further RECIST assessments. For patients who continue to receive treatment beyond the defined end of study, investigators will continue to report all SAEs until 30 days after treatment is discontinued

g Following discontinuation from all study treatment, only details of any treatment for advanced-melanoma (including surgery) will be collected until end of study

h Vital signs (including weight) and single ECG must be repeated each time an echocardiogram is performed and as described in footnote e. These assessments should not be repeated during the relevant visit if already performed as part of the echocardiogram procedure

i Patients who have a drop in LVEF >10% from baseline (measured at screening assessment) at time of discontinuation of AZD6244/placebo should have a follow up echocardiogram, single ECG and vital signs (including weight) performed 30 days after discontinuation of AZD6244/placebo in order to document reversibility

j Only haematology samples to be collected 7 days and 14 days after the second infusion of dacarbazine

k All patients with an AST (SGOT), ALT (SGPT) or bilirubin value >ULN at time of the last dose of AZD6244/placebo should have a further liver chemistry profile (AST, ALT, bilirubin and ALP) performed 30 days (±7days) after permanent discontinuation of AZD6244/placebo

l For urinalysis, a single-spot early morning urine specimen will be collected on the day of the scheduled visit, where the local laboratory is able to determine urine albumin and urine creatinine concentrations from a single-spot urine specimen.

m On PK sampling days dosing should not occur until pre-dose PK samples have been taken. Sparse PK sampling for all patients: 4 blood samples collected on Days 1 and 22 at each of the following intervals: pre-dose, 15 minutes-1 hour, 1.5-2.5 hrs, and 3-8 hr post-dose; Optional comprehensive PK sampling: 8 blood samples collected on Days 1 and 22 at each of the following intervals: pre-dose, 30 minutes, 1, 1.5, 2, 4, 6 and 8 hrs post-dose

n Single ECG at Visit 1, treatment discontinuation visit and each time an echocardiogram is performed; triplicate ECGs pre-dose, 2 hours and 4 hours post-dose of AZD6244/placebo at Visit 2, and 2 hours post-dose of AZD6244/placebo at Visit 5

o RECIST assessment will be performed using CT or MRI scans of chest, abdomen and pelvis. All baseline tumour assessments must be able to adequately assess tumour burden and should be performed no more than 14 days before the start of study treatment Follow-up assessments will be

performed at Week 12 and every 12 weeks relative to date of randomisation. Any other sites at which new disease is suspected should also be appropriately imaged. Up until the time of DCO for the analysis of PFS, patients must be followed until evidence of RECIST-defined progression (regardless of reason for treatment discontinuation). If an unscheduled scan was performed and the patient has not progressed, every attempt should be made to perform the subsequent scans at their scheduled time points

p Full ophthalmological examination should include a slit-lamp examination. Also see footnote e

q Patients will receive AZD6244/placebo for as long as, in the opinion of the investigator, they are receiving clinical benefit in the absence of significant toxicity. AZD6244/placebo treatment should continue after dacarbazine treatment has finished. Patients should continue to receive AZD6244/placebo until at least RECIST-defined progression and may continue to receive treatment after progression at the investigator's discretion

r Patients are expected to receive up to 8 cycles of dacarbazine in the absence of significant toxicity. Further cycles of dacarbazine may be administered at the Investigator's discretion if they feel it to be beneficial and it does not contravene local practice.

4.1.1 Screening

The consent process can either be conducted in a step-wise manner (mutation status screening informed consent first, main study informed consent given once *BRAF* mutation status has been confirmed), or both consents can be performed at the same time, depending on investigator preference and local practice. The following procedures will be conducted for each patient:

- Contact interactive voice response system (IVRS)/interactive web response system (IWRS) to enrol the patient
- Demography (date of birth, sex, race) will be collected
- Collection of AEs will start after signing the first consent form.

4.1.1.1 Confirmation of *BRAF* mutation status

A mutation status screening informed consent will be obtained. This will cover consent for confirmation of the *BRAF* mutation status of the patient's tumour and collection of a plasma and serum sample for analysis of *BRAF* mutations status in CFDNA. A biological samples research addendum to the mutation status screening informed consent will be used to collect consent for the optional use of residual samples for other biomarker research.

A tumour sample for determination of *BRAF* mutation status will be collected. This tumour sample may be an archival sample, or a fresh biopsy may be taken if the investigator deems this to be appropriate. Samples collected from primary or metastatic tumour deposits will be accepted. Blood samples for serum and plasma CFDNA assessment will be collected for all patients, regardless of mutation status.

In order to participate in this study, each patient must have the *BRAF* mutation status of a tumour sample confirmed in one of the following ways:

- By an AstraZeneca appointed central laboratory (see Figure 2 and Section 7.8.1)

or

- By an AstraZeneca approved local laboratory using agreed methodology.

Note: AstraZeneca must agree the use of any local laboratory to determine *BRAF* mutation status in this study prior to the use of such data for entry of patients into the study.

The *BRAF* mutation status of the patient's tumour may have been confirmed at any time prior to randomisation.

For central laboratory analysis of *BRAF* mutation status, if the *BRAF* mutation is not detected from the initial tumour biopsy sample, and histopathological review shows it to be of a poor quality, a second tumour biopsy sample can be submitted for re-testing. In order to optimise the chance of obtaining sufficient good quality DNA to identify the *BRAF* mutation status, it

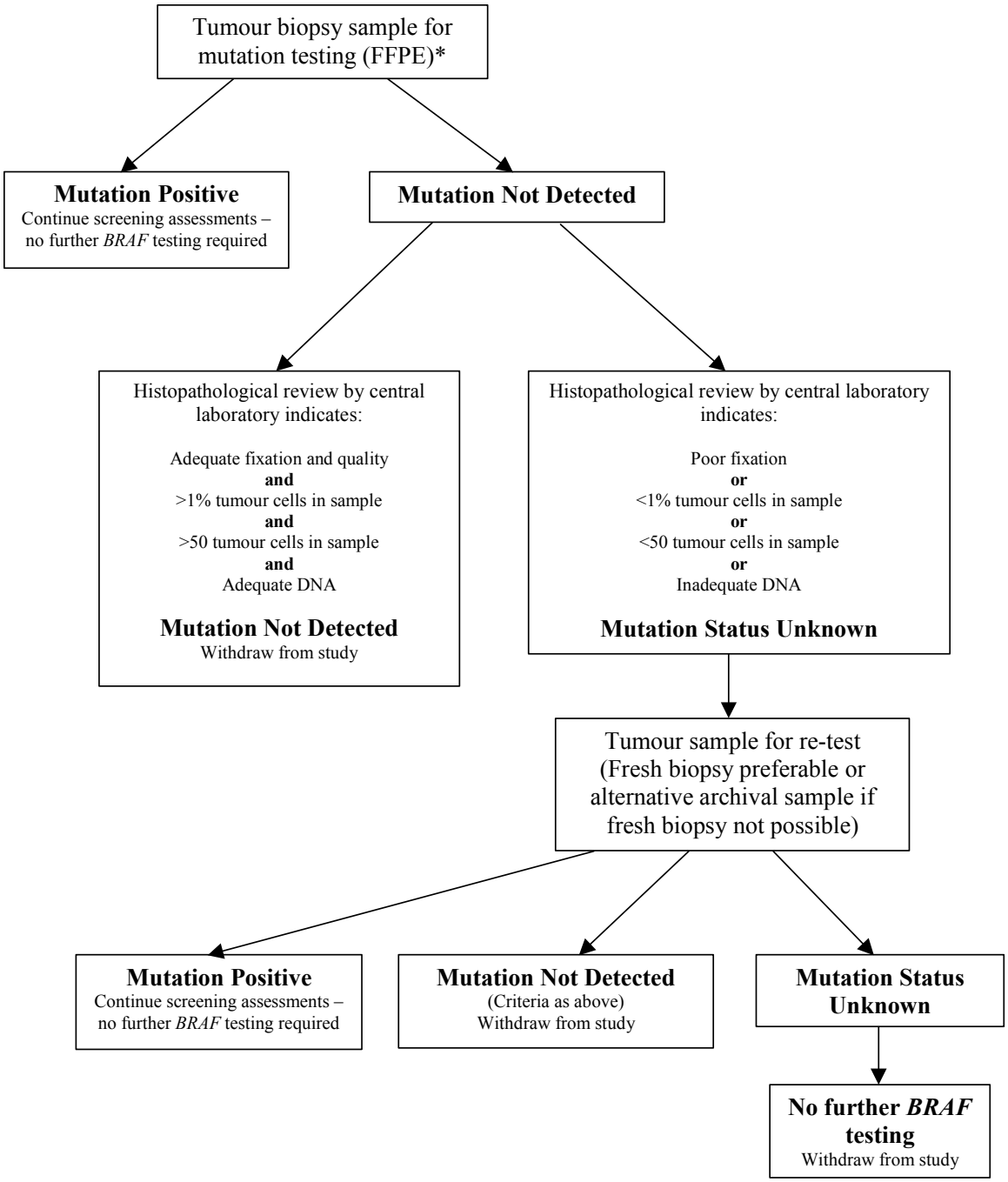
is recommended that the investigator submits a fresh biopsy sample, where possible. In case of a failure to demonstrate *BRAF* mutation positive status following one re-test at the central laboratory (ie, 'mutation is not detected' or 'mutation status unknown'), no further testing will be permitted and such patients will not be eligible. See Figure 2 for further details.

4.1.1.2 Other screening procedures

All the additional screening assessments detailed below must be conducted within the 14 days prior to first study treatment, unless specified otherwise:

- Main study informed consent will be obtained prior to all other study procedures
- The following information will be collected for each patient:
  - Disease staging
  - Medical and surgical history
  - Concomitant medications and previous anti-cancer therapy
  - Smoking status.
- Tumour evaluation according to RECIST guidelines. RECIST assessment will be performed using CT or MRI scans of chest, abdomen and pelvis. Any other sites where disease is suspected or known at baseline must be also imaged. All baseline tumour assessments must be able to adequately assess tumour burden and should be performed no more than 14 days before the start of study treatment
- Assessment of WHO performance status
- Physical examination
- Vital signs (resting blood pressure [BP], pulse rate), weight and height
- Blood samples for haematology and clinical chemistry, including lactate dehydrogenase (LDH)
- Urinalysis (at sites where the local laboratory is able to determine the required parameters, see Section 7.3.5)
- Pregnancy test for female pre-menopausal patients
- Single ECG
- Full ophthalmologic examination, including slit-lamp examination

Figure 2 Algorithm for *BRAF* mutation testing (central laboratory analysis only)



* Formalin-fixed paraffin-embedded

- Echocardiogram (can have been performed up to 28 days prior to first study treatment)
- Collection of AEs will start after signing the first consent form (the mutation status screening informed consent and/or the main study informed consent)
- Overall assessment of patient eligibility for the study, including *BRAF* mutation status.

Upon confirmation of eligibility, patients will be invited to attend the randomisation visit (Visit 2, Day 1).

4.1.2 Randomisation visit (Visit 2, Day 1)

During this visit eligible patients will be randomised into two treatment groups in a ratio of 1:1 to commence treatment with either AZD6244 or placebo in combination with dacarbazine.

Patients must not be randomised unless all eligibility criteria have been met. Prior to dosing with AZD6244/placebo, patients will undergo the following assessments and procedures (with the exception of the collection of post-dose PK samples and post-dose ECGs):

- Complete Melanoma Specific Symptom questionnaire (MSSQ)
- Collection of AEs
- Changes to concomitant medications
- Vital signs (resting BP, pulse rate) and weight
- Triplicate ECGs (5 minutes apart) pre-dose, 2 hours and 4 hours post-dose of AZD6244/placebo (see Section 7.3.7)
- PK sampling (see Table 2 and Section 7.5):
  - Sparse sampling schedule: pre-dose, 15 minutes-1 hour, 1.5-2.5 hours, 3-8 hours post dose
  - Comprehensive sampling schedule (optional): pre-dose, 30 minutes, 1, 1.5, 2, 4, 6 and 8 hours post-dose
- Blood samples for clinical chemistry (including LDH) and haematology
- Urinalysis (at sites where the local laboratory is able to determine the required parameters, see Section 7.3.5)
- Pregnancy test for female pre-menopausal patients

- For those patients who agree to have a blood sample stored for future host genetic analysis, host genetics research informed consent will be obtained and a pre-dose blood sample collected (for details see Appendix E)

- Contact IVRS/IWRS to randomise the patient and dispense double-blind medication. The patient will commence dosing with AZD6244/placebo on Visit 2, Day 1

- Dacarbazine will be administered intravenously to all patients on Visit 2, Day 1.

4.1.3 Follow-up visits (Visit 3, Day 8 onwards)

Patients will attend follow-up visits on Day 8, 15, 22, 29, 36, 43 and every 21 days thereafter. At each of these visits patients will undergo the following assessments:

- Collection of AEs. Please note:

  – All cardiorespiratory AEs with no obvious diagnosis should be assessed with an echocardiogram, single ECG, vital signs (resting BP, pulse rate) and weight.

  – Asymptomatic decreases in LVEF should be investigated according to the algorithm provided in Appendix J

  – All new dyspnoea AEs, or worsening of pre-existing dyspnoea AEs, should be followed up according to the dyspnoea algorithm provided in Appendix H

  – If a patient experiences an AE of any visual disturbance, a complete ophthalmologic examination, including slit-lamp examination, must be performed. Any AE of blurred vision of CTCAE grade ≥2 concurrent with neurological symptoms suggestive of possible RPLS (seizure, altered mental status, headache) must be followed up with MRI scan (or if MRI scan is medically contraindicated or not available at site, CT scan) of the brain. Scans should be repeated upon resolution of neurological symptoms or upon clinically significant neurological deterioration.

- Changes to concomitant medications

- Blood samples for clinical chemistry (including LDH) and haematology. Note: only collect haematology samples 7 days and 14 days after the second infusion of dacarbazine.

Additional assessments will be performed as follows:

- RECIST evaluations using CT (or MRI) of chest, abdomen and pelvis to be performed at Week 12 and every 12 weeks thereafter relative to date of randomisation. Up until the time of DCO for the analysis of PFS, patients must be

followed until evidence of RECIST-defined progression (regardless of reason for treatment discontinuation)

- Vitals signs (resting BP, pulse rate) and weight on Days 8, 15, 22 and every 3 weeks thereafter (please note that vital signs should not be repeated if they have already been taken at the time of the echocardiogram at the same visit)
- Urinalysis on Days 8, 15, 22 and every 3 weeks thereafter (at sites where the local laboratory is able to determine the required parameters, see Section 7.3.5)
- Triplicate ECGs (5 minutes apart) 2 hours post-dose of AZD6244/placebo on Day 22 (see Section 7.3.7)
- Day 22 PK sampling - patients must withhold taking the morning dose of AZD6244/placebo until pre-dose PK sample is collected (see Table 2 and Section 7.5):
  - Sparse sampling schedule: pre-dose, 15 minutes-1 hour, 1.5-2.5 hours, 3-8 hours post-dose
  - Comprehensive sampling schedule (optional): pre-dose, 30 minutes, 1, 1.5, 2, 4, 6 and 8 hours post-dose
- Complete MSSQ at Week 3, Week 6 and at every scheduled visit thereafter
- Assessment of WHO performance status at Week 3, Week 6 and at every scheduled visit thereafter
- Patients are expected to receive up to 8 cycles of dacarbazine administered intravenously on day 1 of every 21 day cycle in the absence of significant toxicity. Investigators may decide to reduce the number of cycles of dacarbazine if significant toxicity develops. Further cycles of dacarbazine may also be administered at the investigator's discretion, if they feel it to be beneficial and it does not contravene local practice. If dacarbazine dosing is withheld for any reason, subsequent cycles will be deferred to maintain the interval, and the 3-weekly cycle will resume from the date that the dacarbazine is administered
- Contact IVRS/IWRS to dispense AZD6244/placebo medication at Week 3 and at every scheduled visit thereafter
- Check AZD6244/placebo returned medication at Week 3 and at every scheduled visit thereafter
- Echocardiogram at Week 6, Week 12 and every 12 weeks thereafter. Vital signs (resting BP, pulse rate), weight and a single ECG must also be recorded at the time of every echocardiogram assessment

- A full ophthalmologic examination will be performed at Week 6 and on occurrence of any AE of any visual disturbance
- Physical examination at Week 9 and every 9 weeks thereafter
- Pregnancy test for female pre-menopausal patients performed at Week 9 and every 9 weeks thereafter.

Patients will be permitted to continue to receive any study treatment after objective disease progression if, in the opinion of the investigator, they are continuing to derive clinical benefit from study treatment, in the absence of significant toxicity.

4.1.4 Treatment discontinuation visit

The treatment discontinuation visit will be conducted as soon as possible after the patient has received the last dose of the last study drug. This will either be after the last dose of AZD6244/placebo or dacarbazine, depending on which treatment is discontinued last.

During this visit patients will undergo the following assessments:

- Complete MSSQ
- Assessment of WHO performance status
- Vital signs (resting BP, pulse rate) and weight
- Physical examination
- Blood samples for clinical chemistry (including LDH) and haematology
- Urinalysis (at sites where the local laboratory is able to determine the required parameters, see Section 7.3.5)
- Single ECG
- Collection of AEs
- Changes to concomitant medications
- Check AZD6244/placebo returned medication
- Contact IVRS/IWRS to register patient's discontinuation of AZD6244/ placebo.

Following discontinuation of all study treatment for any reason, patients may receive any subsequent therapy for advanced melanoma at the discretion of the investigator. Details of such treatment (including surgery) are to be recorded in the electronic case report form (eCRF).

Collection of AEs/SAEs will continue until 30 days after the last dose of the last study treatment. Any AEs that are unresolved at the patient's last AE assessment in the study will be followed up by the investigator for as long as medically indicated, but without further recording in the eCRF.

4.1.5 Thirty days after last dose of last study treatment

Thirty days (±7 days) after permanent discontinuation of the last study treatment (AZD6244/placebo or dacarbazine) a treatment discontinuation follow-up contact should be performed to collect the following for all patients:

- AEs
- Changes to concomitant medications (following this visit, only anti-cancer treatment [including surgery] will be collected).

4.1.6 Additional assessments thirty days after last dose of AZD6244/placebo

Thirty days (±7 days) after the last dose of AZD6244/placebo has been taken, the following assessments should be performed, where necessary:

- All patients with an aspartame aminotransferase (AST), alanine aminotransferase (ALT) or bilirubin value above ULN at time of the last dose of AZD6244/placebo should have a further liver chemistry profile (AST, ALT, bilirubin and alkaline phosphatase [ALP]) performed
- Patients who have an LVEF decrease >10% from baseline (measured at the screening vist) at time of discontinuation of AZD6244/placebo should have a follow up echocardiogram performed in order to document reversibility, single ECG and vital signs (including weight).

When AZD6244/placebo is the last study drug to be discontinued, these assessments should be performed in addition to the assessments described in Section 4.1.5.

If AZD6244/placebo is discontinued before dacarbazine, these assessments should be performed 30 days (±7 days) after the last dose of AZD6244/placebo. The assessments described in Sections 4.1.4 and 4.1.5 should then be performed when dacarbazine is permanently discontinued.

4.1.7 Progression follow-up

If a patient discontinues study treatment (AZD6244/placebo and dacarbazine) for reasons other than objective disease progression, RECIST assessments will continue according to the original schedule until objective disease progression, up until the time of DCO for the analysis of PFS. For such patients, following the 30 days after the last dose of the last study treatment, only study procedure-related SAEs will be captured until the patient is considered to have progressive disease, and therefore will have no further RECIST assessments.

4.1.8 Survival follow-up

Patients will attend a visit, or will be contacted, every 8 weeks from the date of objective disease progression, or discontinuation of the last dose of the last study treatment (whichever is the later date), to assess survival status. Details of any treatment for advanced melanoma (including surgery) post the last dose of study treatment must be recorded in the eCRF. In addition, all patients will be contacted in the week following data cut-offs to confirm survival status.

All patients will be followed until death, withdrawal of consent, or the end of the study.

4.1.9 Open-label AZD6244 treatment following the end of the study

The end of this study is defined as the date when all patients receiving AZD6244 have been followed for a minimum period of 12 months since start of treatment, or the date of the final analysis of the data, whichever is later. Patients are, however, permitted to continue to receive any study treatment beyond this point if, in the opinion of the investigator, they are continuing to derive clinical benefit from study treatment, in the absence of significant toxicity.

Following the end of the study, any such patients will be unblinded and if they have been receiving AZD6244 they can begin open-label AZD6244 75 mg twice daily (or permanently reduced dose if applicable). Patients who do continue to receive study treatment beyond the defined end of study will be followed up according to the investigational site standard of care and investigator judgement. Investigators must continue to report all SAEs to the AstraZeneca Patient Safety department until 30 days after study treatment is discontinued, in accordance with Section 7.3.4.

4.1.10 Management of study treatment-related toxicity

The immediate management of any adverse event should be according to standard clinical practice for that event; for example anaemia should be managed by blood transfusion, and hypertension should be treated with appropriate anti-hypertensive medication. Subsequent management of treatment-related adverse events should be guided by the investigator's assessment of causality.

4.1.10.1 Dacarbazine-related toxicity

Adverse events considered related to administration of dacarbazine are listed in the dacarbazine product information. One of the common toxicities with dacarbazine is haemopoietic depression which primarily involves leukocytes and platelets, although, anaemia may sometimes occur. Treatment with dacarbazine should be withheld on occurrence of the following toxicities, if considered due to dacarbazine:

- Haematological toxicity:
  - Neutropenia CTCAE grade ≥3 for more than 7 days

  - Febrile neutropenia (eg, Absolute Neutrophil Count (ANC) <0.5 x 10^9/L associated with temperature ≥38.5°C)

  - Thrombocytopenia CTCAE grade ≥3

- Severe (CTCAE grade 3 or 4) non-haematological toxicities.

Therapy with dacarbazine can be re-started upon the resolution of the toxicity to CTCAE grade 1 or baseline, and may continue at a permanently reduced dose 750 mg/m^2. Dose reductions should be considered in case of more than one delay due to myelosuppression.

Patients developing severe toxicities (CTCAE grade 3 and 4) related to dacarbazine (as described above), despite dose reduction to 750 mg/m^2 and adequate supportive care, must resume treatment with dacarbazine upon resolution of the adverse events to grade 1 or baseline. A final dose reduction to 500 mg/m^2 is permitted. Patients requiring dose reduction below 500 mg/m^2 should be discontinued from dacarbazine therapy.

Patients experiencing toxicity attributable to dacarbazine will be allowed to continue AZD6244 therapy if chemotherapy is interrupted or terminated.

4.1.10.2 AZD6244-related toxicity

Adverse events considered related to administration of AZD6244 are listed in Section 2.1.3.2 and in greater detail in the Investigator Brochure. If any adverse events of dyspnoea or asymptomatic decreases in LVEF occur, that are considered at least partly due to administration of AZD6244, algorithms for the investigation and management of these events are provided in Appendices H and J respectively. For all adverse events reported in this study that are considered at least partly due to administration of AZD6244, the following dose reduction/adjustment guidance should be applied.

Treatment with AZD6244/placebo should be withheld if one of the following toxicities considered related are observed, despite optimal supportive care:

- Any intolerable adverse event regardless of grade

- Any adverse events CTCAE grade ≥3 (except for neutropenia CTCAE grade 3 continuing for ≤7 days).

AZD6244/placebo treatment may not be restarted until the toxicity improves to CTCAE grade 1 or baseline, except for rash where patients with CTCAE grade 2 rash may restart treatment. Additional information on the management of skin toxicity is provided in Section 4.1.10.3.

Treatment may be resumed at the original dose or at a permanently reduced dose (75 mg once daily) at the discretion of the investigator. See Figure 3.

If a patient experiences occurrence of a new toxicity requiring treatment interruption once having restarted on treatment, study medication should again be withheld until the toxicity improves to CTCAE grade 1 or baseline, except for rash where CTCAE grade 2 rash is acceptable. Upon recovery, treatment may resume at the previous dose level or the dose can be reduced either to 75 mg once daily (if no reduction has yet occurred) or further adjusted to 50 mg twice daily (if dose reduction to 75 mg once daily has already taken place).

However, if a patient experiences recurrence of the same toxicity as that causing a previous dose interruption and/or dose reduction, study medication should be withheld until the toxicity improves to CTCAE grade 1 or baseline, except for rash where CTCAE grade 2 rash is acceptable. Upon recovery, treatment should resume at a permanently reduced or adjusted dose (see Figure 3):

- 75 mg once daily: if no dose reduction has yet occurred
- 50 mg twice daily: dose adjustment if dose reduction to 75 mg once daily has already occurred
- 50 mg once daily: dose reduction if dose reduction to 75 mg once daily followed by dose adjustment to 50 mg twice daily has already occurred.

Therefore, the dose reduction/adjustment algorithm in the study allows for 3 steps: 75 mg twice daily (initial dose) → 75 mg once daily (the first reduction) → 50 mg twice daily (the dose adjustment) → 50 mg once daily (the final dose reduction). Once reduced/adjusted, the dose cannot be returned to the previous level.

If a patient experiences recurrence of any toxicity that has already contributed to dose reductions down to the lowest dosing schedule for this study (50 mg once daily), the patient must discontinue AZD6244/placebo treatment.

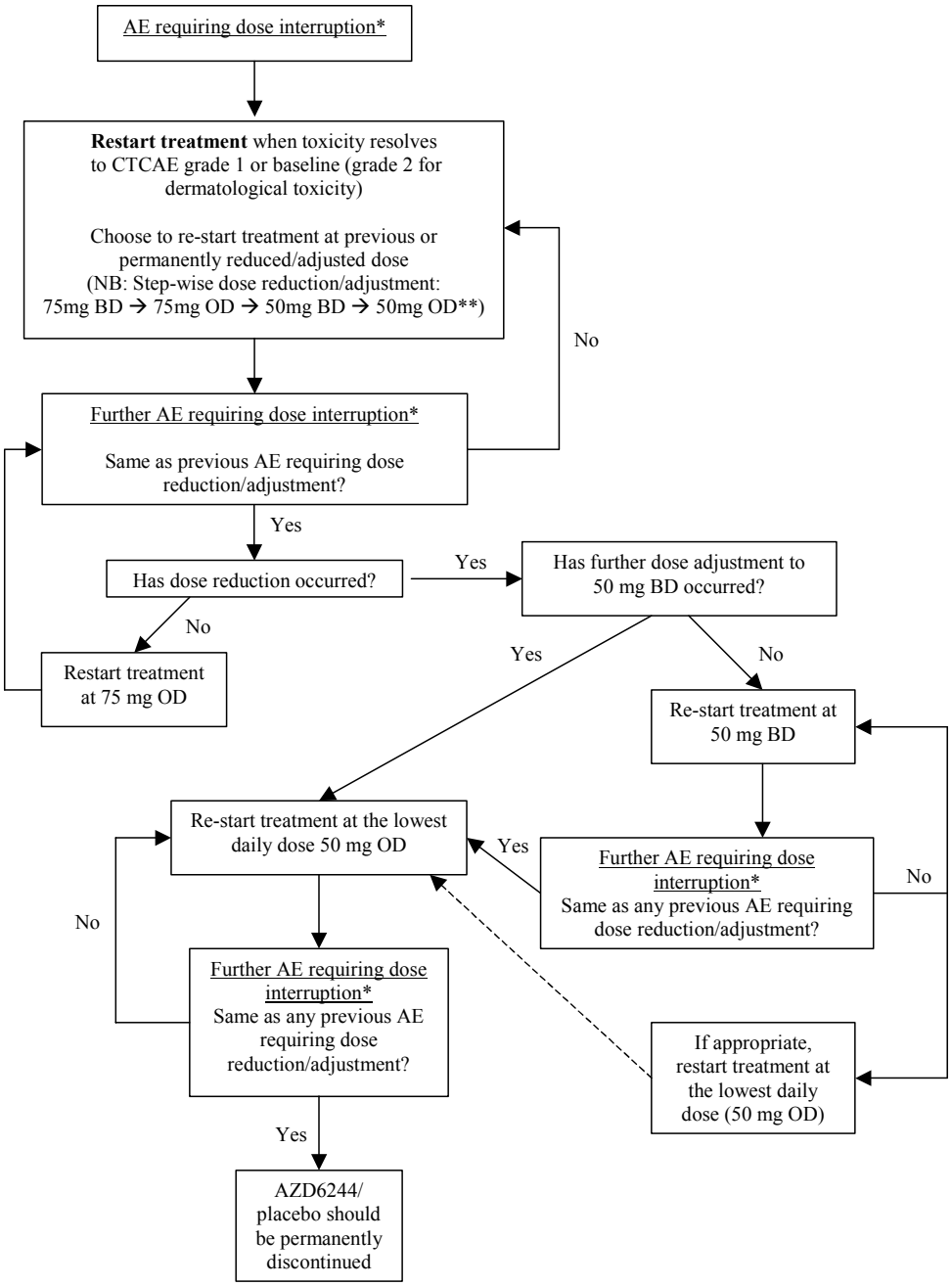
If a patient receiving the lowest dosing schedule (50 mg once daily) experiences a novel toxicity that cannot be adequately managed by dose interruption and medical interventions then the patient must discontinue AZD6244/placebo treatment, as no further dose reductions/adjustments are permitted.

All dose delays, reductions and adjustments will be recorded in the eCRF.

Dose re-escalation of AZD6244/placebo is not permitted in this study.

In the event of any dose delay/reduction/adjustment, patients should continue to follow the assessments schedule as described in Table 2 relative to baseline.

Figure 3 Algorithm for AZD6244/placebo dose reduction/adjustment



* All AEs should be managed according to standard clinical practice
** Once reduced/adjusted, dose cannot be returned to the previous level

4.1.10.3 Management of skin toxicity

The aetiology of skin toxicities associated with the use of AZD6244 is uncertain and there are no established algorithms for rash management. An algorithm based on dermatology best practices for other contemporary targeted agents that cause skin toxicity is offered as guidance to managing skin toxicities seen in patients being treated on this protocol (Pérez-Soler et al 2005).

The algorithm suggests a step-wise approach to rash management. If the rash is CTCAE grade 1, consider starting with topical steroids (eg, bethamethasone), topical antibiotics such as clindamycin gel, or no treatment if the patient is asymptomatic. Use of topical steroid cream with higher potency may be considered early in patients with moderate rash on the face.

If the rash is CTCAE grade 2, continue topical steroid or pimecrolimus cream and consider adding an oral tetracycline or a similar agent.

If the rash reaches CTCAE grade 3 or above, dose interruption and/or dose reduction (for details see Figure 3), coupled with the addition of topical steroids is recommended.

Pruritus of any grade may be treated with an antihistamine, such as diphenhydramine or hydroxyzine hydrochloride.

Xerosis can be treated with classical emollients.

Secondary infection may complicate or worsen skin toxicity. To reduce the likelihood of nasal infection, intranasal mupirocin may be considered. Infected rash may be treated with a short course of an oral tetracycline, such as doxycycline. Sun exposure should be avoided in patients receiving doxycycline or other tetracycline antibiotics.

If there is a clinical diagnosis of impetigo, or an infection with *Staphylococcus aureus* is confirmed, topical mupirocin might be used. Infected lesions suspected to be treatment-resistant should be cultured. If there is no improvement after two weeks of treatment, therapy for the rash should be considered ineffective and discontinued.

All required treatment information and adverse event information for rash should be recorded in the eCRF.

4.2 Rationale for study design, doses and control groups

This Phase II study will be randomized, double blind and placebo-controlled in order to minimise bias when assessing whether AZD6244 plus dacarbazine shows better efficacy (OS, PFS, ORR, DoR, change in tumour size at 12 weeks and APF6) when compared to placebo plus dacarbazine in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma. The primary endpoint of OS is chosen as it is recognised as a universally accepted direct measure of benefit. The secondary endpoints of PFS, ORR, DoR, change in tumour size at 12 weeks and APF6 will be assessed using RECIST criteria which are recognised measures of anti-tumour activity (Therasse et al 2000).

An analysis of PFS and APF6 will occur 6 months after the last patient is recruited and the primary analysis of OS will be performed when approximately 58 death events have occurred. If the true HR is 0.57, this analysis will have approximately 80% power to demonstrate a statistically significant difference for OS, assuming a 1-sided 10% significance level. This trial is designed as a randomised screening trial to quantify the level of risk entailed for further development. Thus the Type I and Type II errors have been adjusted to be less constrained, so that the targeted treatment benefit may be appropriate while the sample size remains reasonable (as discussed by Rubinstein et al 2005). If a 1-sided $p<0.1$ is observed for the comparison of OS between AZD6244 in combination with dacarbazine, versus placebo in combination with dacarbazine, the results will be regarded as promising (but not definitive) as there is a less than 1 in 10 probability that such a result could have been detected if there was truly no treatment effect.

Based on pre-clinical data (Lev et al 2004, Wilkinson et al 2008), it is hypothesised that patients who are most likely to receive the greatest benefit from the combination of AZD6244 and dacarbazine would be *BRAF* mutation positive melanoma patients as combination activity was dramatically increased in cell lines with activated MEK pathway. In addition, of the seven confirmed AZD6244 responses reported in both the Phase II advanced melanoma study (Dummer et al 2008) and the Phase I relative bioavailability study, six have been in patients with *BRAF* mutation positive advanced melanoma.

Assessment of *BRAF* mutation positive patients in Phase II provides the optimum way of determining activity of the combination whilst exposing as few patients as possible to the study drug ie, if the combination does not have sufficient activity in *BRAF* mutation positive patients then it is unlikely to be more efficacious in *BRAF* mutation negative patients, thus upfront selection in Phase II provides the optimum decision-maker for Phase III.

The population for this study will consist of first line cutaneous and unknown primary melanoma patients for whom dacarbazine is an appropriate therapeutic option, as dacarbazine is the only treatment licensed for advanced melanoma in most participating territories. The optimal treatment and dose schedule for dacarbazine has never been fully established, with there being no evidence that response rates or duration of response are affected by either. Since the introduction of more effective anti-emetic agents, the use of single-dose regimens administered once per cycle have improved both patient comfort and clinic utilization time (Eggermont and Kirkwood 2004).

The 1000 mg/m^2 dose of dacarbazine has been used in clinical practice over recent years and tested in several clinical studies (McDermott et al 2008, Patel 2008). This dose has also been co-administered with AZD6244 in the ongoing Phase I study (D1532C00004) and the preliminary safety results suggest that AZD6244 does not add significant toxicity to the profile seen with dacarbazine alone (Section 2.1.3.2). Investigators will be made aware of emerging safety information arising from this study and advised on management approaches for adverse events.

Patients may continue to receive AZD6244/placebo whilst, in the opinion of the investigator, they are continuing to receive clinical benefit in the absence of unacceptable toxicity. It is

expected that AZD6244 will be more effective when given in combination with chemotherapy. Therefore, patients who continue to tolerate combination therapy following completion of 8 chemotherapy cycles will be allowed to continue dacarbazine infusions until disease progression as long as this does not contravene local medical practice. There will be no crossover of treatment arms permitted, as this would necessitate unblinding of the randomisation code.

Reproductive toxicology data indicate that AZD6244 can have adverse effects on embryofoetal development and survival, at dose levels that do not induce maternal toxicity in mice. Dacarbazine has been shown to be mutagenic, teratogenic and carcinogenic in animals. Consequently restrictions requiring the use of adequate contraceptive measures in patients of child-bearing potential and their partners, and the exclusion of patients who are pregnant or actively breast feeding, have been incorporated into the design of the study.

5. PATIENT SELECTION CRITERIA

Patient population should be selected without bias.

Investigator(s) must keep a record of patients who entered pre-trial screening but were never enrolled eg, subject screening log. Each patient must meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be exceptions to this rule.

5.1 Inclusion criteria

For inclusion in the study patients must fulfil the following criteria:

1. Provision of signed, written and dated informed consent prior to any study specific procedures

2. Male or female, aged 18 years or older

3. Histological or cytological confirmation of advanced (inoperable stage III and stage IV) cutaneous or unknown primary melanoma

4. WHO performance status 0-1

5. At least one lesion, not previously irradiated, that can be accurately measured at baseline as ≥10 mm in the longest diameter (LD; except lymph nodes which must have a short axis ≥15 mm) with CT or MRI, and which is suitable for accurate repeated measurements

6. Tumour sample confirmed as *BRAF* mutation positive (Note: sample must be available upon enrolment to ship to the AstraZeneca appointed central laboratory, or mutation status confirmed locally at an AstraZeneca approved local laboratory using agreed methodology

7. Evidence of post-menopausal status, or negative urinary or serum pregnancy test for female pre-menopausal patients. Post-menopausal status is defined as:

   – natural menopause with menses >1 year ago

   – radiation-induced oophorectomy with last menses >1 year ago

   – chemotherapy-induced menopause with 1 year interval since last menses

   – serum FSH, LH and plasma oestradiol levels in the postmenopausal range for the institution

   – bilateral oophorectomy or hysterectomy

8. Serum creatinine clearance >50 ml/min, by either Cockcroft-Gault formula or 24-hour urine collection analysis

9. Patients should be able to swallow AZD6244/placebo capsules.

5.1.1 Host genetics research study (optional)

For inclusion in the optional host genetics research study patients must fulfil the following criteria:

1. Provision of optional host genetics research informed consent.

If a patient declines to participate in the host genetics research, there will be no penalty or loss of benefit to the patient. A patient who declines host genetics research participation will not be excluded from any other aspect of the main study.

5.1.2 Biomarker research study (optional)

For inclusion in the optional biomarker research study patients must fulfil the following criteria:

1. Provision of optional consent for use of residual tumour and CFDNA serum and plasma samples, following *BRAF* mutation testing, for additional biomarker research.

If a patient declines to participate in the optional biomarker research, there will be no penalty or loss of benefit to the patient. Any patient who declines to participate in the optional biomarker research will not be excluded from any other aspect of the main study.

5.2 Exclusion criteria

Patients must not enter the study if any of the following exclusion criteria are fulfilled:

1. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study site)

2. Previous randomization of treatment in the present study

3. Having received an investigational drug within 30 days of starting treatment, or have not recovered from side effects of an investigational drug

4. Diagnosis of uveal or mucosal melanoma

5. Any prior Investigational therapy comprising inhibitors of Ras, Raf or MEK

6. Any prior cytotoxic chemotherapy or biochemotherapy for advanced melanoma

   - patients who previously received adjuvant biochemotherapy will be eligible, unless their treatment included temozolomide or dacarbazine

   - patients with a treatment history including isolated limb perfusion or isolated limb infusion will be eligible, unless the perfusion or infusion was performed with dacarbazine

7. Any other investigational non-chemotherapeutic therapy for advanced melanoma, with the exception of prior monotherapy with interleukin-2, cytokines (eg, α-interferon or GM-CSF) or vaccine, which are permitted

   - use of anti-CTLA4 monoclonal antibodies will be allowed in adjuvant settings

8. Any non-systemic therapy (except focal palliative radiotherapy) for advanced melanoma within 30 days of starting study treatment

9. Any unresolved toxicity above CTCAE grade 2 from previous anti-cancer therapy, apart from alopecia

10. Brain metastases or spinal cord compression unless asymptomatic, treated and stable off treatment (eg, steroids) for at least 3 months

11. Laboratory values as listed below (from laboratory results at Visit 1):

    - ANC $<1.5 \times 10^9$/L (1,500 per mm^3)

    - Platelets $<100 \times 10^9$/L (100,000 per mm^3)

    - Haemoglobin ≤9.0 g/dL

    - Serum bilirubin >1.5xULN

    - AST or ALT >2.5xULN

12. LDH ≥2xULN (from the Visit 1 laboratory result)

13. Cardiac conditions as follows:

    - Uncontrolled hypertension (BP≥150/95 despite optimal therapy)
    - Heart failure NYHA Class II or above
    - Prior or current cardiomyopathy
    - Baseline LVEF ≤50%
    - Atrial fibrillation with heart rate >100 bpm
    - Unstable ischaemic heart disease (myocardial infarction within 6 months prior to starting treatment, or angina requiring use of nitrates more than once weekly)

14. Major surgery within 4 weeks prior to starting study treatment

15. Hypersensitivity to AZD6244, or dacarbazine or any excipient of these agents

16. Patients with a history of another primary malignancy within 5 years prior to starting study treatment, except adequately treated basal or squamous cell carcinoma of the skin, or carcinoma of the cervix in situ and the disease under study

17. Any evidence of severe or uncontrolled systemic disease, active infection, active bleeding diathesis or renal transplant, including any patient known to have hepatitis B, hepatitis C or human immunodeficiency virus (HIV)

18. Refractory nausea and vomiting, chronic gastrointestinal diseases (eg, inflammatory bowel disease), or significant bowel resection that would preclude adequate absorption

19. Female patients who are breast-feeding or patients of reproductive potential who are not employing an effective method of birth control

20. Clinical judgement by the investigator that the patient should not participate in the study.

5.2.1 Host genetics research study (optional)

Exclusion criteria for participation in the optional host genetics research component of the study:

1. Previous allogeneic bone marrow transplant

2. Whole blood transfusion within 120 days of the date of host genetic sample collection (except for leukocyte-depleted blood transfusion, which is allowed).

5.3 Procedures for handling incorrectly included patients

Patients that do not meet the inclusion/exclusion criteria for a study should not, under any circumstances, be enrolled into the study– there can be no exceptions to this rule.

Where patients that do not meet the study criteria are enrolled in error, incorrectly randomised, or where patients subsequently fail to meet the criteria for the study post enrolment, a discussion must occur between the AstraZeneca Study Team Physician and the investigator regarding whether to continue or discontinue the patient from the study treatment. The AstraZeneca Study Team Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the patient should have their study therapy stopped. Those patients randomised in error should remain in the study and be followed for progression free survival and overall survival where possible.

5.4 Withdrawal of patients

5.4.1 Criteria for discontinuation of study treatment

Patients may be discontinued from study treatment at any time. Specific reasons for discontinuing study treatment are:

- Adverse events
- Voluntary discontinuation by the patient who is at any time free to discontinue his/her study treatment, without prejudice to further treatment
- Risk to patients as judged by the investigator and/or AstraZeneca
- Severe non-compliance to protocol as judged by the investigator and/or AstraZeneca
- Condition under investigation worsened
- A female patient becoming pregnant.

5.4.2 Procedures for discontinuation of a patient from study treatment

A treatment discontinuation visit will be conducted as soon as possible after the patient received the last dose of the last study drug (AZD6244/placebo or dacarbazine). See Section 4.1.4 for details of the assessments to be performed at this visit.

Collection of AEs/SAEs will continue until 30 days after the last dose of study treatment.

Thirty days after permanent discontinuation of the last study treatment (AZD6244/placebo or dacarbazine), a treatment discontinuation follow-up contact should be performed. See Section 4.1.5 for details of the assessments to be performed at this contact.

All patients with an AST, ALT or bilirubin value above ULN at the time of the last dose of AZD6244/placebo should have a further liver chemistry profile (AST, ALT, bilirubin and ALP) performed 30 days (±7days) after permanent discontinuation of AZD6244/placebo.

If a patient discontinues study treatment for reasons other than disease progression, and therefore continues to have tumour assessments using RECIST, following the 30 days after the last dose of the last study treatment only study procedure-related SAEs will be captured until the patient is considered to have progressive disease, and therefore will have no further RECIST assessments.

Patients will attend for a visit, or will be contacted, every 8 weeks from the date of objective disease progression or discontinuation of the last dose of the last study treatment (whichever is the later date) to assess survival status. See Section 4.1.8 for details of the assessments to be performed at this contact. In addition, all patients will be contacted in the week following data cut-offs to confirm survival status.

Patients who have a drop in LVEF >10% from baseline at time of discontinuation of AZD6244/placebo should have a follow-up echocardiogram performed 30 days after permanent discontinuation of AZD6244/placebo in order to document reversibility.

5.4.3 Criteria for withdrawal from the study

Patients may be withdrawn from study assessments at any time. Specific reasons for discontinuing a patient are:

- Voluntary withdrawal by the patient who is at any time free to withdraw his/her participation in the study, without prejudice to further treatment
- Incorrectly enrolled patients (ie, the patient does not meet the required inclusion/exclusion criteria and is therefore not randomised into the study)
- Patient lost to follow-up.

5.4.4 Procedures for withdrawal of a patient from the study

A patient that withdraws from the study will always be asked about the reason(s) for withdrawal and the presence of any adverse events. If possible, they will be seen and assessed by an investigator(s). Adverse events will be followed up (see Sections 7.3.3 and 7.3.4), symptom questionnaires and study drug should be returned by the patient.

Collection of AEs/SAEs will continue until 30 days after the last dose of study treatment. Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the

eCRF. AstraZeneca reserves the right to ask for further information/clarification on any AE that may be considered of interest.

See Appendix E for details of withdrawal from optional host genetic research. See Appendix F for details of withdrawal from optional biomarker research.

6. STUDY CONDUCT

6.1 Restrictions during the study

- Female patients of child-bearing potential will be required to use reliable methods of contraception for the duration of the study and until 4 weeks after the last dose of AZD6244/placebo and 3 months after the last dose of dacarbazine. Male patients will be required to use reliable methods of contraception for the duration of the study and until 16 weeks after the last dose of AZD6244 /placebo. Male patients should also be advised to take contraceptive measures for 6 months after cessation of dacarbazine treatment. Reliable methods of contraception should be used consistently and correctly. Acceptable methods include implants, injectables, combined oral contraceptives (which must all be combined with barrier methods of contraception), some IUDs and vasectomised partner. Sexual abstinence is also an acceptable method of contraception according to ICH Guideline M3

- AZD6244/placebo should be taken on an empty stomach (no food or drink other than water for 2 hours prior to dosing and 1 hour after dosing), as described in Section 6.4.2. On clinic days on which PK samples are scheduled (Visits 2 and 5), dosing should be delayed until arrival at the clinic and until the pre-dose PK sample has been taken and until the pre-dose ECG has been performed (Visit 2 only)

- Patients should avoid excessive sun exposure and use adequate sunscreen protection if sun exposure is anticipated

- Please refer to Section 6.5 for all restrictions relating to concomitant medications.

- During treatment in the study, patients may receive palliative radiotherapy at the site of bone metastases that were present at baseline providing the investigator does not consider that the bone pain is indicative of clinical disease progression. If a patient has further bone pain for which a second course of palliative radiotherapy is considered, the patient should be discussed with the AstraZeneca Study Team Physician to decide if it is necessary for the patient to be discontinued from study therapy. The need for radiotherapy to any other site should be discussed with the AstraZeneca Study Team Physician and any decisions will be made on a case-by-case basis.

6.2 Patient enrolment and randomisation

The Principal Investigator will:

1. Obtain signed, written and dated informed consent from the potential patient before any study specific procedures are performed

2. Contact IVRS/IWRS to assign potential subject a unique enrolment number E00NNXXX, with NN being the 2-digit centre number, and XXX being the patient enrolment code at the centre. Enrolment codes will start at 001 in each centre and will be assigned sequentially (eg, at Centre 01, patients will be assigned E-codes E0001001, E0001002, etc)

3. Determine patient eligibility. See Sections 5.1 and 5.2

4. Contact IVRS/IWRS to assign eligible subject a unique randomisation code (subject number), starting at "001".

Randomisation codes will be assigned strictly sequentially by IVRS/IWRS as patients are eligible for randomisation.

If a patient withdraws from the study, then his/her enrolment and randomisation codes cannot be reused. If a patient withdraws from the study then they cannot re-enter into the study.

6.2.1 Procedures for randomisation

At Visit 2, patients who satisfy all the entry criteria will be centrally assigned by the IVRS/IWRS to receive AZD6244 or placebo in a ratio of 1:1, according to the randomisation scheme generated by the Biostatistics Group, AstraZeneca.

Every effort should be made to minimise the time between randomisation and starting treatment. Patients must not be randomised unless all eligibility criteria have been met.

6.2.2 IVRS/IWRS

IVRS/IWRS will be used for allocation of enrolment number, allocation of randomisation number, study medication assignment, discontinuation from study treatment, emergency code breaks and study drug shipment confirmation. The IVRS/IWRS technology will be managed and maintained by Clinphone. The system is accessible by telephone (IVRS) or via the web (IWRS) 24 hours a day, 7 days a week. The IVRS/IWRS user manual, which has full details of the operation and use of the IVRS/IWRS, will be provided to each centre.

6.3 Blinding and procedures for unblinding the study

6.3.1 Methods for ensuring blinding

The active and placebo capsules will appear identical and be presented in the same packaging to ensure blinding of the medication. Medication will be labelled using a unique material pack code which is linked to the randomisation scheme. IVRS/IWRS will allocate randomisation numbers sequentially when sites call IVRS/IWRS to randomise an eligible patient. IVRS/IWRS will allocate the medication pack code to be dispensed to the patient.

6.3.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomisation for each randomised patient, will be available to the investigator(s) or pharmacists from the IVRS/IWRS. Routines for this will be described in the IVRS/IWRS user manual that will be provided to each centre.

The treatment code must not be broken except in medical emergencies when the appropriate management of the patient necessitates knowledge of the treatment randomisation. If the treatment code is broken then the investigator(s) must document and report to AstraZeneca.

AstraZeneca retains the right to break the code for SAEs that are unexpected and are suspected to be causally related to an investigational product and that potentially require expedited reporting to regulatory authorities. Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual patient have been made and documented.

6.4 Treatments

6.4.1 Identity of investigational product(s)

- AZD6244 and matching placebo Hyd-Sulfate formulation capsules will be supplied in high-density polyethylene (HDPE) bottles. At each dispensing visit each patient will receive sufficient bottles for 21 days treatment coverage, plus overage.

- Dacarbazine will be sourced as marketed commercially available material/locally sourced or prescribed according to local regulations.

Table 3 Investigational product dosage form, strength and manufacturer

Investigational product	Dosage form and strength	Manufacturer
AZD6244	25 mg Hyd-Sulfate capsule	AstraZeneca
Placebo to match AZD6244	Capsule	AstraZeneca
Dacarbazine	100 mg, 200 mg, 600 mg powder for infusion	Sourced locally

6.4.2 Doses and treatment regimens

Patients will be randomised on a 1:1 basis, via IVRS/IWRS, to receive either AZD6244 75 mg twice daily, or matching placebo, in combination with dacarbazine.

(i) AZD6244/Placebo

AZD6244/ placebo will be supplied in bottles of 56 capsules of 25 mg strength. At each dispensing visit, sufficient AZD6244/placebo for 21 days treatment coverage, plus overage, will be dispensed. Individual bottles will be dispensed in accordance with the medication

identification numbers provided via the IVRS/IWRS. Patients will be provided with dosing instructions for the study.

Patients will receive three 25 mg AZD6244/placebo capsules twice daily, commencing on Day 1. Capsules should be taken whole and with approximately 240 mL water.

The initial dose of AZD6244/placebo can be reduced/adjusted under the circumstances described in Section 4.1.10.2.

All doses of AZD6244/placebo should be taken on an empty stomach (no food or drink other than water for 2 hours prior to dosing and 1 hour after dosing). On clinic days on which PK samples are scheduled to be taken (Visits 2 and 5), the dosing should be delayed until arrival at the clinic and until the pre-dose PK sample has been taken and until the pre-dose ECG has been performed (Visit 2 only).

The doses should be taken approximately 12 hours apart for example at 0800 and 2000, or at 0900 and 2100 (once daily doses should be taken approximately 24 hours apart). Wherever possible, doses should not be missed. If a patient misses taking a scheduled dose, they should take the next scheduled dose and the missed dose will not be made up.

Any deviations from dosing schedule, dose interruptions, dose reductions and dose adjustments should be recorded in the eCRF.

Patients are permitted to continue to receive AZD6244, following the end of the study (as defined in Section 10.5) if, in the opinion of the investigator, they are continuing to derive clinical benefit, in the absence of significant toxicity. At the end of the study any such patients will be unblinded and if found to be receiving active drug they can begin open-label AZD6244. Patients will be dispensed bottles of open-label AZD6244 25 mg capsules and will be instructed to take three capsules twice daily (or permanently reduced dose if applicable). Subjects will continue to receive open-label AZD6244 as long as they wish to remain on treatment, and they are benefiting from treatment in the opinion of the investigator, and they do not meet the criteria for discontinuation of study treatment (see Section 5.4.1).

(ii) Dacarbazine

On day 1 of each 21 day cycle, a 1000 mg/m^2 iv infusion of dacarbazine will be administered over at least 60 minutes. Patients are expected to receive up to 8 cycles of dacarbazine in the absence of significant toxicity. Administration of dacarbazine may continue beyond 8 cycles if deemed beneficial for a patient by the Investigator, and if it does not contravene local medical practice.

Dacarbazine dose reductions are to be performed as described in Section 4.1.10.1. Any deviations from dosing schedule, dose interruptions, or dose reductions should be recorded in the eCRF.

6.4.3 Additional study drug (not applicable)

6.4.4 Labelling

Each bottle of AZD6244 and matching placebo capsules will be labelled by Investigational Products (IPS), AstraZeneca or its designee.

All labels will comply with good manufacturing practice (GMP) regulations, and will state that the drug is for clinical use only or that it is the investigational drug and is to be used by qualified investigators only and should be kept out of reach of children. The following information will be pre-printed on the bottle label: study code, unique medication ID number, expiry date, contents of the bottle, dosing instructions and storage conditions. The labels will have blank spaces for the site personnel to complete the following at the time of drug dispensing: enrolment code, visit number and dispensing date.

Each bottle of AZD6244/placebo capsules will have a tear-off portion that will be removed at the time of dispensing and attached to the paper Drug Accountability Log.

Information on the bottle labels for open-label AZD6244 (only supplied following the end of study as defined in Section 10.5) will include the study code, expiry date, contents of the bottle, dosing instructions and storage conditions. The labels will have blank spaces for the site personnel to complete the following at the time of drug dispensing: enrolment code and dispensing date. Open-label bottles of AZD6244 will also have a tear-off portion that will be removed at the time of dispensing and attached to the Drug Accountability Log.

Dacarbazine will be supplied locally and will be dispensed in accordance with the local dacarbazine product information.

6.4.5 Storage

All study drugs must be kept in a secure place under appropriate storage conditions. The investigational product label on the bottle and the Investigator Brochure specifies the appropriate storage and shipment conditions.

6.5 Concomitant and post-study treatment(s)

The following treatment/drugs are restricted in this study:

- No other anti-cancer agents, or investigational drugs should be administered whilst patients are receiving study medication. The investigator may initiate any subsequent anti-cancer therapy only after the patient has discontinued all study treatment (AZD6244/placebo and dacarbazine)

- Caution must be taken with concomitant administration of dacarbazine in patients receiving phenytoin. Administration of benzodiazepine may be required to reduce risk of seizures, see dacarbazine product information for further details

- Concomitant administration of yellow fever vaccine, live attenuated vaccines, cyclosporin and tacrolimus should be avoided while the patient is receiving dacarbazine

- Patients who are taking coumarin anticoagulants should increase the frequency of assessment of anticoagulation, such as INR measurements, upon initiation of dosing with AZD6244/placebo

- Patients should not take vitamin E supplements or multivitamin supplements which provide a total daily dose in excess of 100% of the recommended daily dose of vitamin E. The maximum dose of vitamin E patients may receive from AZD6244 or placebo is approximately 210 mg/day

- Throughout the study, patients should avoid changes to, or the addition of all concomitant medications, in particular any that may affect the metabolism of AZD6244 (eg, CYP1A2 or 3A4 inhibitors/inducers), unless considered clinically indicated.

Other medication, which is considered necessary for the patient's safety and well-being, may be given at the discretion of the investigator.

Following discontinuation from study treatment for any reason, patients may receive any subsequent therapy for their disease at the discretion of the investigator. Details of such treatment are to be recorded in the eCRF.

6.6 Treatment compliance

The administration of all medication (including investigational products) must be recorded in the appropriate sections of the eCRF.

6.6.1 Accountability

The study drug provided for this study will be used only as directed in the study protocol. Patients must return all used and unused medication and packaging at Visit 5 and at every scheduled clinic visit thereafter. Drug accountability will be performed at Visit 5 and at every scheduled clinic visit thereafter. The study personnel will account for all drugs dispensed and returned.

Where appropriate facilities and procedures for drug destruction exist and prior approval from the site monitor has been received, site personnel will account for all unused drugs and for appropriate destruction. Certificates of delivery, destruction and return must be complete and signed.

Where such facilities do not exist, study site personnel/study monitor will return all unused drugs to AstraZeneca. Certificates of delivery and return must be completed and signed.

7. COLLECTION OF STUDY VARIABLES

7.1 Recording of data

The Principal Investigator will provide AstraZeneca with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timeliness of the data reported to AstraZeneca in the eCRF and in all required reports according to any instructions provided.

Data will be entered in the Web Based Data Capture (WBDC) system at the study site. Trained study personnel will be responsible for entering data specified in the protocol into the WBDC system and according to the eCRF instructions. When data have been entered reviewed, edited and Source Data Verification (SDV) performed by an AstraZeneca representative, the data will be frozen to prevent further editing. The Principal Investigator will be notified to sign the eCRF electronically as per the eCRF instructions. A copy of the eCRF data will be archived at the study site.

7.1.1 Electronic data capture at AstraZeneca clinical pharmacology units (not applicable)

7.2 Screening and demography procedures

Refer to Section 4.1.1.

7.2.1 Follow-up procedures

Refer to Sections 4.1.2, 4.1.3, 4.1.4, 4.1.5 and 4.1.8.

7.3 Safety

It is of the utmost importance that all staff involved in the study is familiar with the content of this section. The Principal Investigator is responsible for ensuring this.

7.3.1 Definition of adverse events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

7.3.2 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the patient or may require medical intervention to prevent one of the outcomes listed above.

For reporting purposes, any suspected transmission via a medicinal product of an infectious agent is also considered an SAE and is reported in an expedited manner. Any organism, virus or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent.

For further guidance on the definition of a SAE, see Appendix B to the Clinical Study Protocol.

7.3.3 Recording of adverse events

AEs/SAEs will be collected throughout the study from informed consent until 30 days after study treatment is discontinued.

If a patient discontinues study treatment for reasons other than disease progression, and therefore continues to have tumour assessments using RECIST, study procedure-related SAEs must be captured until the patient is considered to have progressive disease, and therefore will have no further RECIST assessments.

At the end of the study, following unblinding, patients who have been randomised to AZD6244 are permitted to continue to receive any study treatment if, in the opinion of the investigator, they are continuing to derive clinical benefit from study treatment, in the absence of significant toxicity. Patients who do continue to receive study treatment beyond the defined end of study will be followed up according to the investigational site standard of care and investigator judgement. Investigators must continue to report all SAEs to the AstraZeneca Patient Safety department until 30 days after AZD6244 is discontinued, in accordance with Section 7.3.4.

AEs will be graded according to the National Cancer Institute Common Terminology Criteria for AEs (CTCAE Version 3, June 2003).

Variables

The following variables will be recorded in the CRF for each AE:

- AE description

- Onset date
- Resolution date
- Maximum CTCAE grade
- Final action taken (no action, dose interruption, dose reduction, dose discontinuation)
- Treatments patient received for AE
- Outcome
- Causality due to AZD6244/placebo ("yes" or "no")
- Causality due to dacarbazine ("yes" or "no")
- Causality due to study procedure ("yes" or "no")
- Whether event constitutes an SAE.

AEs will be coded using MedDRA (Medical Dictionary for Regulatory Activities).

After study completion (ie, after any scheduled follow-up period has ended) there is no obligation to actively report information on new AEs or SAEs occurring in former study patients. However, if an investigator learns of any SAEs, including death, at any time after a patient has completed the study and he/she considers there is a reasonable possibility that the event is related to AZD6244, the investigator should notify AstraZeneca, Patient Safety department.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 7.3.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE.

The investigator will assess causal relationship between the Investigational Product or a study procedure and Adverse Events, and answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by AZD6244 or dacarbazine or a study procedure".

Causal relationship in cases where the disease under study has deteriorated due to lack of effect will be classified as no reasonable possibility.

A guide to the interpretation of the causality question is found in Appendix B to the Clinical Study Protocol.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the patient or reported in response to the open question from the study personnel: "Have you had any health problems since the previous visit?", or revealed by observation will be collected and recorded in the eCRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The reporting of protocol-mandated laboratory/vital sign abnormalities as both laboratory findings and AEs should be avoided. They should not be reported as AEs unless any criterion for an SAE is fulfilled, the laboratory/vital sign abnormality causes the patient to discontinue treatment with the investigational product, or if the investigator has a strong belief that it should be reported as an AE. All abnormalities from protocol-mandated laboratory/vital measurements will be summarised in the Clinical Study Report in the "Laboratory measurements and variables" section.

If an abnormal laboratory value/vital sign is associated with a diagnosis or clinical signs or symptoms, then the diagnosis, sign or symptom should be reported as an AE and the associated laboratory result /vital sign should be considered additional information to support the diagnosis. This applies to both protocol-mandated measurements and those that are measured outside of protocol requirements.

Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anaemia rather than low haemoglobin value).

Follow-up of unresolved adverse events

Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the eCRF. AstraZeneca retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Disease progression

Disease progression can be considered as a worsening of a patient's condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the disease and/or increases in the symptoms of the disease. Expected progression of the disease under study and/or expected progression of signs and symptoms of the disease under study, unless more severe in intensity or more frequent than expected for the patient's condition, should not be reported as an AE. The development of new, or progression of existing metastasis to the primary cancer under study should be considered as disease progression and not an AE. An event that is unequivocally due to disease progression must not be reported as an AE/SAE.

Lack of efficacy

Where there is deterioration in the condition for which the study treatment is being used, there may be uncertainty as to whether this is lack of efficacy or constitutes an AE. In such cases, unless AstraZeneca or the reporting physician considers that the study treatment contributed to the deterioration, or local regulations state to the contrary, the deterioration should be considered to be lack of efficacy and not an AE.

Handling of deaths

All deaths that occur during the study, or within the 30-day follow-up period after the administration of the last dose of study treatment, must be reported as follows:

- Death, which is clearly as a result of disease progression, should be communicated to the study monitor at the next monitoring visit and should be documented in the eCRF module, but should not be reported as a SAE

- Where death is not due (or not clearly due) to progression of disease under study the AE causing the death must be reported to the study monitor as an SAE within 24 hours. The report should contain a comment regarding the co-involvement of progression of disease, if appropriate, and should assign a single primary cause of death together with any contributory causes

- Deaths with an unknown cause should always be reported as a SAE but every effort should be made to establish a cause of death. A post-mortem may be helpful in the assessment of the cause of death, and if performed a copy of the post-mortem results (with translation of important parts into English) should be forwarded to AstraZeneca, Patient Safety within the usual timeframes.

New cancers

The development of a new cancer should be regarded as an AE and will generally meet at least one of the serious criteria. New cancers are those that are not the primary reason for the administration of the study treatment and have been identified after the patient's inclusion in this study. They do not include metastases of the original cancer. Symptoms of metastasis or the metastasis itself should not be reported as an AE/SAE as they are considered to be disease progression.

Overdose

To date, no subject has experienced an overdose with AZD6244. There is currently no known antidote to AZD6244. The treatment of AEs associated with overdose should be supportive for the underlying symptoms. Doses of study treatment in excess of that specified in the clinical study protocol are considered to be an overdose.

In case of overdose with dacarbazine the site should follow standard local practice.

Should an overdose (accidental or deliberate) occur, it must be reported in accordance with the procedures described in Section 1.2 regardless of whether or not the overdose was associated with any symptom. All symptoms associated with the overdose should be reported as AEs.

Pregnancy

Should a pregnancy occur, it must be reported in accordance with the procedures described in Section 1.3. Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication.

7.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the eCRF. SAEs will be recorded from the time of informed consent.

The investigator and/or Sponsor are responsible for informing the Ethics Committee and/or the Regulatory Authority of the SAE, as per local requirements.

For studies in countries implementing the EU Clinical Trials Directive, Ethics Committees and Regulatory Authorities will be informed by AstraZeneca. If any SAE occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives immediately and no later than the end of the next **business day** of when he or she becomes aware of the SAE.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform appropriate AstraZeneca representatives of any follow-up information on a previously reported SAE immediately but no later than the end of the next **business day** of when he or she becomes aware of the SAE.

Investigators or other site personnel send automated email alert to the designated AstraZeneca representative.

If the WBDC system is not available, then the investigator or other study site personnel reports by telephone an SAE to the appropriate AstraZeneca representative.

The AstraZeneca representative will advise the investigator/study site personnel how to proceed.

The designated AstraZeneca representative works with the investigator to ensure that all the necessary information is provided to the appropriate AstraZeneca clinical drug safety data entry site within **one business day** for fatal and life-threatening events and within **five calendar** days for other SAEs. If the report arrives late in the day, it can be sent the following morning. If the report arrives during a weekend or public holiday, the information is

forwarded as early as possible on the first business day following the weekend or holiday. The clock start date is then the next business day.

The reference document for definition of expectedness/listedness is the Investigator Brochure for ADZ6244 and the EU Summary of Product Characteristics (SPC) for dacarbazine.

7.3.5 Laboratory safety assessment

The following clinical chemistry, haematology and urinalysis tests will be performed:

Table 4 Laboratory safety assessments

Clinical chemistry	Haematology	Urinalysis [a]
s-Albumin	Erythrocyte count	u-Albumin
s-ALT	Haemoglobin	u-Creatinine
s-AST	Platelet count	
s-ALP	Leukocyte cell count	
s-Total Calcium	Leukocyte differential count (absolute count):	
s-Creatinine	Neutrophils	
s-Gamma glutamyl transerase (γGT)	Eosinophils	
s-Glucose	Basophils	
s-Lactate dehydrogenase	Lymphocytes	
s-Magnesium	Monocytes	
s-Phosphate		
s-Potassium		
s-Sodium		
s-Total protein		
s-Total bilirubin		
s-Urea nitrogen		

a To be performed at sites where the local laboratory is able to determine urine albumin and urine creatinine concentrations from a single-spot specimen
s serum
u urine

All scheduled and unscheduled laboratory safety assessments will be analysed by the local laboratory.

Clinical chemistry, haematology and urinalysis testing will be repeated as clinically indicated as part of the routine management of the patient on the occurrence of AEs.

All patients with an AST, ALT or bilirubin value above ULN at time of the last dose of AZD6244/placebo should have a further liver chemistry profile (AST, ALT, bilirubin and ALP) performed 30 days (±7 days) after permanent discontinuation of AZD6244/placebo.

For blood volume see Section 8.1.

A single-spot early morning urine specimen will be collected on the day of the scheduled visit, at sites where the local laboratory is able to determine the concentration of urine albumin and urine creatinine from a single-spot urine specimen. Investigational sites unable to report these parameters will perform routine urinalysis according to the local standard of care.

7.3.6 Physical examination

A physical examination will be performed at screening, Week 9 and every 9 weeks thereafter. The last physical examination in the study will be performed at treatment discontinuation visit.

7.3.7 ECG

ECGs will be reviewed locally. Patients should be supine and at rest 10 minutes prior to recording the ECG.

Parameters including heart rate, duration of QRS complex, PR and QT intervals will be collected. RR interval and QTcF will be calculated by AstraZeneca from the data provided.

The investigator should review the paper copy of the ECGs on each study day and may refer to a local cardiologist if appropriate.

Any symptoms from the patient should be registered as a comment and if AE criteria are met, recorded as an AE.

7.3.7.1 Screening ECG

At screening all patients will have a single 12-lead ECG performed. The screening ECG can be conducted up to 14 days prior to Visit 2.

7.3.7.2 Treatment phase ECGs

Patients will have 12-lead ECGs captured in triplicate (5 minutes apart) pre-dose, 2 hours and 4 hours post-dose of AZD6244/placebo on Day 1, and 2 hours post-dose of AZD6244/placebo on Day 22. Single ECGs must also be performed at the time of every echocardiogram assessment and on occurrence of any cardiorespiratory adverse event. A single 12-lead ECG is also required at discontinuation of treatment.

7.3.8 Vital signs

Resting blood pressure and pulse rate will be measured using a semi-automatic blood pressure recording device with an appropriate cuff size. Vital sign assessments, including weight, will be performed at Visits 1, 2, 3, 4 and 5 and then 3-weekly thereafter, at discontinuation of the

last study treatment and at the time of any echocardiogram assessment. Height will be assessed at Visit 1 only.

Any changes in vital signs should be recorded as an AE if applicable.

7.3.9 Other safety assessments

7.3.9.1 Pregnancy test

A pregnancy test will be performed at screening, prior to starting treatment at Visit 2, and 9-weekly thereafter for female pre-menopausal patients.

7.3.9.2 Echocardiogram

An echocardiogram will be conducted at screening and at Week 6, Week 12 and at 12-weekly intervals on treatment. The screening echocardiogram can be conducted up to 28 days prior to Visit 2. A further echocardiogram should be performed as part of the assessment package for any cardiorespiratory adverse event with no obvious diagnosis (eg, not mandated in case of confirmed pulmonary embolus or myocardial infarction).

LVEF, end diastolic and end systolic right and left ventricular diameters should be recorded at each echocardiogram assessment. Patients experiencing an asymptomatic but clinically significant drop in LVEF should be managed according to the algorithm provided in Appendix J. Patients who have a drop in LVEF >10% from baseline (measured at Visit 1) at time of discontinuation of AZD6244/placebo should have a follow-up echocardiogram performed 30 days after permanent discontinuation of AZD6244/placebo in order to document reversibility.

7.3.9.3 Ophthalmologic examination

A complete ophthalmologic examination, including a slit-lamp examination, must be performed prior to first dose and at Week 6, and also when a patient experiences a visual disturbance AE.

7.3.9.4 MRI (or CT) scan of the brain

An MRI scan (or if MRI scan is medically contraindicated or not available at site, CT scan) of the brain must be performed when a patient experiences any AE of blurred vision of CTCAE grade ≥2 concurrent with neurological symptoms suggestive of possible RPLS (seizure, altered mental status, headache). Scans should be repeated upon resolution of neurological symptoms or upon clinically significant neurological deterioration.

7.3.9.5 WHO Performance Status

WHO performance status will be assessed at screening, Week 3, Week 6 and at every scheduled visit thereafter.

7.4 Efficacy

7.4.1 Tumour assessment by imaging techniques using RECIST

RECIST criteria will be used to assess patient response to treatment by determining PFS times. The modified RECIST guidelines for measurable, non measurable, target and non-target lesions and the objective tumour response criteria (CR [complete response], PR [partial response], SD [stable disease] or progression of disease) are presented in Appendix D.

The methods of assessment of tumour burden used at baseline (CT or MRI scans of chest, abdomen and pelvis) must be used at each subsequent follow-up assessment.

Following the baseline assessment, efficacy for all patients will be assessed by objective tumour assessments at Week 12 and every 12 weeks (±1 week) relative to date of randomisation. Up until the time of DCO for the analysis of PFS, patients must be followed until evidence of RECIST-defined progression, regardless of reason for discontinuation of treatment. If a patient dies prior to objective disease progression, then death will be regarded as progression for the analysis of PFS.

Following date of the DCO for the analysis of PFS, no further RECIST assessments will be required for this study. If RECIST assessments are performed after this DCO, the data will not be collected in the eCRF.

Progression due to "new lesions" in this study will be declared if one of the following occur:

- Appearance of new soft tissue lesions that are ≥10 mm in the longest diameter
- Appearance of new lymph nodes which must be ≥15 mm in the short axis
- Appearance of new bone lesion not documented at baseline
- Appearance of clinically significant effusion (that requires a change in drug therapy).

If the investigator is in doubt as to whether progression has occurred, particularly with response to non-target lesions (NTLs) or the appearance of a new lesion (see criteria above), it is advisable to continue treatment until the next scheduled assessment, or sooner if clinically indicated, and reassess the patient's status.

It is important to follow the assessment schedule as closely as possible. If an unscheduled scan was performed and the patient has not progressed, every attempt should be made to perform the subsequent scans at their scheduled time points. Please refer to the study plan (Table 2), Section 4.1.1 and Appendix D.

If a patient discontinues treatment (and/or receives a subsequent therapy) prior to progression, then the patient should still continue to be followed until either objective evidence of RECIST-defined progression, until the time of DCO for the analysis of PFS.

Categorisation of objective tumour response assessment will be based on the RECIST criteria of response: CR, PR, SD and progression of disease. Response will be calculated in comparison to the baseline tumour measurements obtained before starting treatment. Progression will be calculated in comparison to when the tumour burden was at a minimum (ie, smallest sum of diameters previously recorded since starting treatment).

The sponsor will determine the patient's overall visit response using the lesion assessments recorded on the eCRF pages.

RECIST assessments (including Target Lesion measurements) will be used to determine APF6, PFS, ORR, DoR and change in tumour size at 12 weeks.

7.4.2 Patient reported outcomes (PRO)

The methods for collecting PRO data are presented below. Symptoms will be assessed in both treatment groups using a Melanoma Specific Symptom questionnaire (MSSQ, see Appendix I) generated from specific questions from the Functional Assessment of Cancer Therapy–MEL (FACT-MEL) questionnaire.

The patient will complete all questionnaires at the scheduled clinic visit on Day 1 (pre-first dose) and every 3 weeks thereafter until discontinuation from study treatment. Each centre should allocate responsibility for the symptom questionnaires to a specific individual (eg, a research nurse). AstraZeneca will provide training for the relevant personnel in the administration of symptom questionnaires to help avoid the key problem of missing data. Before patients are randomised, they must be informed of the rationale for the study and the study details, including the symptom questionnaire. The patients should be instructed on how to complete the questionnaire and, if necessary, be assisted with completion of a training questionnaire that must be destroyed after completion.

7.4.2.1 Melanoma Specific Symptom Questionnaire (MSSQ)

The MSSQ consists of the Additional Concerns section, GP2 GP4 and GF1 from the FACT-MEL. The FACT-MEL consists of the FACT–G and an Additional Concerns domain. FACT-G was developed by Cella using a standardized approach to derivation and reduction (Cella et al 1993). It is a self-report instrument that measures multi-dimensional quality of life (QoL). The Additional Concerns section of the FACT-MEL was developed specifically for patients with melanoma and is used in addition to FACT-G to measure overall QoL in these patients. FACT-MEL was chosen as the basis for the MSSQ because it has good psychometric properties, has been shown to be responsive to change and known group differences (Cormier et al 2008), and is relatively simple and quick (up to 10 minutes) for patients to complete.

There will be no symptom questionnaire assessments in countries where an appropriate translation is not available.

7.4.2.2 Administration of PRO questionnaires

It is important that the value and relevance of QoL and symptom data is explained carefully to participating patients so that they are motivated to comply with data collection. There is research evidence that patients with breast cancer value the opportunity to provide information on their QoL (Fallowfield 1996). The research nurse, or appointed individual, should also stress that the information is confidential. Therefore, if the patient has any medical problems they should discuss them with the doctor or research nurse.

The instructions for completion of questionnaires are:

- The patient themselves must complete it
- It must be completed before any investigations or discussions about the status of the patient's disease with the clinic staff
- Help should not be given from relatives or clinic staff unless the patient is blind or illiterate
- Only one answer to every question should be checked
- Following completion, the nurse or appointed individual must confirm verbally with the patient that the questionnaires have been completed fully.

7.5 Pharmacokinetics

7.5.1 Collection of biological samples

Blood samples (2 mL) for determination of plasma concentrations of AZD6244 and N-desmethyl AZD6244 and any known metabolites will be collected from patients in either a sparse PK sampling schedule, or an optional comprehensive PK sampling schedule. Depending on emerging data/information, the timings and number of the PK samples may be altered, but the maximum total blood volumes given in Table 6 will not be exceeded. The actual sample date and time of all PK samples must be recorded in the eCRF.

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For blood volume see Section 8.1.

7.5.1.1 Sparse PK sampling

All patients will be asked to contribute at least 8 blood samples, one from each of the pre-defined time windows on Day 1 and Day 22:

- Pre-dose
- Between 15 minutes and 1 hour post-dose
- Between 1.5 and 2.5 hours post-dose

- Between 3 and 8 hours post-dose.

The actual sample date and time of all PK samples must be recorded in the eCRF.

7.5.1.2 Comprehensive PK sampling (optional)

Patients who choose to take part in the comprehensive PK sampling schedule will have samples taken:

- Pre-dose
- 30 minutes, 1, 1.5, 2, 4, 6 and 8 hours post-dose on Day 1 and Day 22.

The actual sample date and time of all PK samples must be recorded in the eCRF.

It is intended to conduct comprehensive PK sampling in approximately 20 patients.

7.5.2 Determination of drug concentrations in biological samples

Analysis of plasma samples for the determination of AZD6244 and N-desmethyl AZD6244 and any known metabolite concentrations will be the responsibility of Clinical Pharmacology & DMPK, Alderley Park, AstraZeneca, UK.

If warranted, the blood samples may also be used for analysis of additional metabolites, which may be reported separately from the Clinical Study Report.

7.6 Pharmacodynamics (not applicable)

7.7 Pharmacogenetics

See Appendix E for details of optional host genetic blood sample collection.

7.8 Biomarker Analysis

See Table 5 below, summarising all the biomarker samples to be collected for this study.

Table 5 Summary of biomarker analyses

Biomarker sample	Optional or mandatory	Screening or randomised population	Details in main protocol or appendix
Tumour biopsy for *BRAF* analysis	Mandatory	Screening	Main protocol
Residual tumour biopsy material (including extracted DNA)	Optional	Screening	Main protocol
Plasma sample for analysis of *BRAF* mutation in CFDNA	Mandatory	Screening	Main protocol
Serum sample for analysis of *BRAF* mutation in CFDNA	Mandatory	Screening	Main protocol

Table 5 Summary of biomarker analyses

Biomarker sample	Optional or mandatory	Screening or randomised population	Details in main protocol or appendix
Residual plasma sample	Optional	Screening	Appendix F
Residual serum sample	Optional	Screening	Appendix F
Blood sample for host genetic analysis	Optional	Randomised	Appendix E

7.8.1 Mandatory *BRAF* mutation assessment of tumour biopsy

As a requirement of the study, patients will provide consent for AstraZeneca to collect and analyse samples of their tumour material for analysis of *BRAF* mutation status. See Section 4.1.1.1 for further details. Samples collected from primary or metastatic tumour deposits will be accepted.

For sites using the central laboratory to analyse *BRAF* mutation status, the Principal Investigator will be asked to provide one of the following for each consenting patient, depending on which format is more convenient:

- Formalin-fixed, paraffin-embedded (FFPE) tumour tissue blocks
- 10 to 20 re-cut sections from FFPE tumour tissue block, presented on slides, including one stained with haematoxylin and eosin. Each section is to be 5 µm thick.

Sites should ship the tumour sample to the central laboratory as soon as it is available. If the central laboratory do not detect the *BRAF* mutation status from the initial tumour biopsy sample, and histopathological review shows it to be a poor quality sample, a second tumour biopsy sample should be submitted for re-testing. See Figure 2 for further details.

Samples will be collected, labelled, stored and shipped as detailed in the laboratory Manual.

Residual material may be used for optional exploratory biomarker research if consent has been obtained for this research.

If requested, unused tumour samples will be repatriated. For further details see the Laboratory Manual.

7.8.2 Mandatory collection of plasma and serum for exploratory analysis of CFDNA

All enrolled patients will be asked to provide a plasma and serum sample. These samples will be used for the extraction and analysis of CFDNA and subsequent analysis of *BRAF* mutation status. This area of mutation analysis is exploratory and it is hoped that such analyses will aid

the development of methodologies for analysis of tumour mutation status. It is hoped that this will lessen the burden for providing tumour biopsy samples for analysis in the future. Data generated from this analysis will not be used to aid patient recruitment in this study.

All patients who provide a tumour sample for analysis of *BRAF* mutation status will be required to provide the following:

- 4 mL blood sample for preparation of plasma
- 4 mL blood sample for preparation of serum.

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

Residual material may be used for optional exploratory biomarker research (eg, phosphatase and tensin homolog [PTEN] mutation status) if consent has been obtained for this research.

7.9 Health economics (not applicable)

Not applicable.

8. BIOLOGICAL SAMPLING PROCEDURES

8.1 Volume of blood

The total volume of blood that will be drawn from each patient in this study is as follows:

Table 6 Volume of blood to be drawn from each patient

Assessment		Sample volume (mL)	Number of samples	Total volume (mL)
Safety	Clinical Chemistry	5	13	65
	Haematology	2.6	15	39
Pharmacokinetic[a]		2	8	16
Plasma sample for CFDNA		4	1	4
Serum sample for CFDNA		4	1	4
Host genetics (optional)		10	1	10
Total				138

a For those patients participating in optional comprehensive PK sampling, number of PK samples=16, total volume of PK samples=32 mL, total number of all samples=55 and total volume of all samples=170 mL

The total volumes of blood given in Table 6 are based upon a patient remaining in the study for 6 months. At each 3-weekly visit after this time, a further total of 7.6 mL of blood will be

taken for clinical chemistry and haematology. Clinical chemistry and haematology samples will be analysed locally, therefore volumes may vary according to local practice.

8.2 Handling, storage and destruction of biological samples

The samples will be used up or disposed after analyses or retained for further use as described below.

Biological samples for future research will be retained at AstraZeneca, or its designee, for a maximum of 15 years following the finalisation of the Clinical Study Report. The results from future analysis will not be reported in the Clinical Study Report but will be reported separately in the bioanalytical method validation report

8.2.1 Clinical chemistry, haematology and urinalysis samples

All clinical chemistry, haematology and urinalysis samples will be analysed by the local laboratory and will be handled according to local laboratory practice.

The analyte stability limits defined by the local laboratory will be applied to all analyses performed for this study. Samples that fall outside these stability limits should not be analysed. Analytical data will not be reported if found to have been derived from a sample that fell outside these stability limits.

8.2.2 PK and/or PD samples

The long-term stability of the analyte(s) should be documented in method validation produced by AstraZeneca or its designee. Results from analyses of samples stored longer than the time period for which stability has been demonstrated should not be reported unless complementary analyte(s) stability data is acquired and amended to the relevant method validation report.

Samples will be disposed of after the Clinical Study Report has been finalised, unless retained for future analyses.

8.2.3 Mandatory tumour sample for *BRAF* mutation assessment

Residual material may be retained for the optional analysis of other biomarkers if consent has been obtained for this research. See Appendix F for further details.

8.2.4 Mandatory plasma and serum samples for exploratory analysis of CFDNA

Residual material may be retained for the optional analysis of other biomarkers if consent has been obtained for this research. See Appendix F for further details.

8.2.5 Optional host genetics research

Please see Appendix E for details of the optional host genetic research.

8.3 Labelling and shipment of biohazard samples

The Principal Investigator ensures that samples are labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B (materials containing or suspected to contain infectious substances that do not meet Category A criteria [see IATA 6.2 Regulations Guidance in Appendix C]).

Any samples identified as Infectious Category A materials are not shipped and further samples taken from the patient unless agreed with AstraZeneca and appropriate labelling, shipment and containment provisions are approved.

8.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

The Principal Investigator at each centre keeps full traceability of collected biological samples from the patients while in storage at the centre until shipment and keeps documentation of receipt of arrival.

The sample receiver keeps full traceability of the samples while in storage and during use until used, disposed or repatriated.

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

Samples retained for further use within in AstraZeneca will be registered in the AstraZeneca biobank system for the entire life cycle of the sample(s).

8.5 Withdrawal of informed consent for donated biological samples

If a patient withdraws consent to the use of biological samples donated, the samples will be disposed/destroyed, if not already analysed and documented.

The Principal Investigator:

- Ensures patients withdrawal of informed consent is notified immediately to AstraZeneca
- Ensures that biological samples from that patient, if stored at the study site, are immediately identified, disposed/destroyed and the action documented.
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed/destroyed and the action documented returned to the study site.

AstraZeneca ensures the central laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed/destroyed and the action documented is returned to the study site.

In the event that analysis/research has already been performed, AstraZeneca will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

If requested, unused tumour samples will be repatriated. For further details see the Laboratory Manual.

9. ETHICAL AND REGULATORY REQUIREMENTS

9.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP), applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

For studies including host genetic analysis special precautions are taken as described in Appendix E.

9.2 Patient data protection

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

9.3 Ethics and regulatory review

An Ethics Committee must approve the final study protocol, including the final version of the Informed Consent Form and any other written information to be provided to the patients. The investigator will ensure the distribution of these documents to the applicable Ethics Committee, and to the study site staff.

The opinion of the Ethics Committee must be given in writing. The investigator must submit the written approval to AstraZeneca before enrolment of any patient into the study.

The Ethics Committee must approve all advertising used to recruit patients for the study.

AstraZeneca must approve any modifications to the Informed Consent Form that are needed to meet local requirements.

If required by local regulations, the protocol must be re-approved by the Ethics Committee annually.

Before enrolment of any patient into the study, the final study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

The distribution of any of these documents to the national regulatory authorities will be handled by AstraZeneca.

AstraZeneca will provide Ethics Committees and Principal Investigators with safety updates/reports according to local requirements.

In the US the Principal Investigator is also responsible for providing the Ethics Committee with reports of any serious and unexpected adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the Principal Investigator.

Progress reports and notifications of serious and unexpected adverse drug reactions will be provided to the Ethics Committee according to local regulations and guidelines.

9.4 Informed consent

The Principal Investigator(s) at each centre will:

- Ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure that the patients are notified that they are free to withdraw from the study at any time
- Ensure that the patients are given the opportunity to ask questions and allowed time to consider the information provided
- Obtain and document the patient's signed and dated informed consent before conducting any procedure specifically for the study, including the following:
  - Collection of study blood samples and biopsies
  - Study ECGs
  - Study echocardiograms
- Ensure the original, signed Informed Consent Form is stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the patient.

9.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Co-ordinating Investigator and AstraZeneca.

If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol (Amended Protocol).

The amendment must be approved by each Ethics Committee and if applicable, also the national regulatory authority, before implementation. Local requirements must be followed for amended protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the protocol to each Principal Investigator(s). For distribution to Ethics Committee see Section 9.3.

If a protocol amendment requires a change to a centre's Informed Consent Form, AstraZeneca and the centre's Ethics Committee must approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by each Ethics Committee.

9.6 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, GCP, ICH guidelines and any applicable regulatory requirements. The investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at his or her centre.

10. STUDY MANAGEMENT BY ASTRAZENECA

10.1 Pre-study activities

Before the first patient is entered into the study, it is necessary for a representative of AstraZeneca to visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) (and other personnel involved with the study) their responsibilities with regard to protocol adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a Clinical Study Agreement between AstraZeneca and the investigator
- Discuss the specific requirements of the host genetic research with the investigator(s) (and other personnel involved with the study).

10.2 Training of study site personnel

Before the first patient is entered into the study, an AstraZeneca representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and also train them in any study specific procedures and the WBDC and IVRS/IWRS system(s) utilised and administration of the QoL questionnaires.

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

Before the first patient is entered into the study the investigational staff will have an opportunity to discuss the procedures associated with the collection of blood samples, extraction of DNA and host genetic research with AstraZeneca personnel or delegate. The ethical considerations specific to genotyping and the importance of the informed consent process will be made clear. The requirements for the collections of the patients' samples will also be made clear.

10.3 Monitoring of the study

During the study, an AstraZeneca representative will have regular contacts with the study site, including visits to:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the CRFs, and that investigational product accountability checks are being performed
- Perform source data verification (a comparison of the data in the CRFs with the patient's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating patients. This will require direct access to all original records for each patient (eg, clinic charts)
- Ensure withdrawal of informed consent to the use of the patient's biological samples is reported and biological samples are identified and disposed/destroyed accordingly, and the action is documented, and reported to the patient.

The AstraZeneca representative will be available between visits if the investigator(s) or other staff at the centre needs information and advice about the study conduct.

10.3.1 Source data

Refer to study specific Clinical Study Agreement for location of source data.

10.4 Study agreements

The Principal Investigator at each/the centre must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this study. In the event of any inconsistency between this Clinical Study Protocol and the Clinical Study Agreement, the Clinical Study Protocol shall prevail.

Agreements between AstraZeneca and the Principal Investigator must be in place before any study-related procedures can take place, or patients be enrolled.

10.5 Study timetable and end of study

The end of this study is defined as the date when all patients receiving AZD6244 have been followed for a minimum period of 12 months since start of treatment, or the date of the final analysis of the data, whichever is the later. At this time point, the clinical study database will close to new data. Patients are however, permitted to continue to receive any study treatment beyond the closure of the database if, in the opinion of the investigator, they are continuing to derive clinical benefit from study treatment, in the absence of significant toxicity. Following the defined end of the study, any such patients will be unblinded and if found to be receiving active drug they can begin open-label AZD6244 treatment.

Patients who do continue to receive study treatment beyond the defined end of study will be followed up according to the investigational site standard of care and investigator judgement. Investigators must continue to report all SAEs to the AstraZeneca Patient Safety department until 30 days after study treatment is discontinued, in accordance with Section 7.3.4. Additionally, as stated in Section 7.3.3, any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the eCRF. AstraZeneca reserves the right to ask for further information /clarification on any AE that may be considered of interest.

The study is expected to start in Q2 2009 and to be completed by Q3 2011.

The study may be terminated at individual centres if the study procedures are not being performed according to GCP, or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with AZD6244.

11. DATA MANAGEMENT BY ASTRAZENECA OR DELEGATE

Data entered in the WBDC system will be immediately saved to a central database and changes tracked to provide an audit trail. When the Principal Investigator has signed the eCRF electronically as per eCRF instructions, the subject's data will be locked.

All data management activities will be performed by the Cognizant Data Management Centre.

Medical coding will be performed using the AstraZeneca Autocoder application. The Data Management Centre Coding Team will perform coding using agreed coding conventions. AEs and medical and surgical history will be coded using the standard dictionary – MedDRA, and concomitant medication will be coded using the AstraZeneca Drug Dictionary.

SAEs will be entered into a global patient safety database for regulatory reporting purposes and be reconciled with the AEs in the clinical database.

Data associated with biological samples and PK assessments will be transferred to Cognizant as an electronic file and merged with study data as appropriate.

Data from external providers (eg, central laboratory) will be validated as appropriate to ensure that it is consistent with the clinical data and included in the final database.

Clean file will be declared for the database once all data have been received, entered, validated and all queries resolved. The database will be locked after clean file has been declared. Treatment codes will not be broken until after clean file. Following database lock, all data will be extracted as SAS (Statistical Analysis Software) data sets for the statistical analysis to be performed by AstraZeneca.

Some or all of the clinical datasets from the main study may be merged with the host genetic data in a suitable secure environment separate from the clinical database. The results from this host genetic research will be reported separately from the Clinical Study Report for the main study.

If assessed locally, the *BRAF* mutation status of the patient's tumour sample will be recorded in the eCRF. If assessed at the central laboratory, the *BRAF* mutation status of the patient's tumour sample will not be recorded in the eCRF, but will be transferred to Cognizant as an electronic file. The *BRAF* mutation status will be provided to investigators as part of the screening process, prior to a patient being randomised.

12. EVALUATION AND CALCULATION OF VARIABLES BY ASTRAZENECA

12.1 Calculation or derivation of safety variable(s)

12.1.1 Adverse events

Data from all cycles of randomised treatment will be combined in the presentation of safety data. AEs (both in terms of MedDRA preferred terms and CTCAE grade) will be listed individually by patient and treatment group. For patients who have a dose modification, all AEs (due to drug or otherwise) will be assigned to the initial treatment group.

Any AE occurring before treatment (ie, before study Day 1) will be included in the data listings but will not be included in the summary tables of AEs.

Any AE occurring within 30 days of study treatment discontinuation (ie, the last dose of AZD6244/placebo or dacarbazine) will be included in the AE summaries. Any events in this period that occur after a patient has received further therapy for cancer (following discontinuation of study medication) will be flagged in the data listings.

A separate data listing of AEs occurring more than 30 days after discontinuation of study medication will be produced. This will only include AEs 30 days after stopping all study treatments. These events will not be included in AE summaries.

A data listing of AEs that led to some kind of treatment interruption or dose reduction/adjustment (for AZD6224/placebo or dacarbazine) will also be presented.

A summary table of most common AEs will be produced. Most common will be defined as >10% of patients within any treatment group.

12.1.2 Other significant adverse events (OAE)

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs and Discontinuation due to Adverse Events (DAEs). Based on the expert's judgement, significant adverse events of particular clinical importance may, after consultation with the Patient Safety Physician, be considered OAEs and reported as such in the Clinical Study Report.

Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction/adjustment or significant additional treatment.

12.1.3 Vital signs, laboratory data, ECGs, echocardiogram, physical examination and ophthalmologic examination

For change from baseline summaries for vital signs, laboratory data, ECGs, echocardiogram and physical examination, the baseline value will be the latest result obtained prior to the start of study treatment. Change from baseline will be calculated programmatically by AstraZeneca using absolute change from baseline.

QTcF (Fredericia) will be calculated programmatically by AstraZeneca using the reported ECG values (heart rate).

R-R interval will be calculated by AstraZeneca using the heart rate values collected and the formula:

- R-R interval = 60/heart rate.

The urinary albumin/creatinine ratio (mg/mmol, UACR) will be calculated by AstraZeneca.

Corrected Calcium and Calcium Phosphate product will be calculated programmatically by AstraZeneca using the following formulas:

- Corrected Calcium (mmol/L) = Total Calcium (mmol/L) + ([40 – Albumin (G/L)] x 0.02)

- Calcium Phosphate (mmol/L) = Corrected Calcium (mmol/L) x Phosphate (mmol/L).

The denominator used in laboratory summaries will only include evaluable patients, in other words those who had sufficient data to have the possibility of an abnormality. For example:

- If a CTCAE criterion involves a change from baseline, evaluable patients would have both a pre-dose and at least one post-dose value recorded

- If a CTCAE criterion does not consider changes from baseline, to be evaluable the patient need only have one post dose-value recorded.

The denominator in vital signs data should include only those patients with recorded data.

Data from ophthalmologic examinations will be listed and not summarised.

12.2 Calculation or derivation of efficacy variable(s)

12.2.1 Overall survival (OS, time to death)

OS will be calculated as the interval from the date of randomisation to the date of patient death (any cause). Patients who have not died at the time of the final analysis, or who withdraw consent, will be censored at the last date the patient was known to be alive.

12.2.2 Progression free survival (PFS)

PFS is defined as the interval between the date of randomisation and the earlier date of objective disease progression per RECIST criteria or death due to any cause in the absence of progression. Patients who have not progressed or died at the time of analysis will be censored at the time of their latest objective tumour assessment. This includes patients who withdraw consent.

Up until the time of DCO for the analysis of PFS, patients are to have imaging assessments until objective progression, regardless of whether they discontinue their randomised therapy or take another anti-cancer therapy prior to progression. In light of the design, dates of progression and death will be defined as follows:

- The actual date of progression will be used in the analysis regardless of whether the patient had previously discontinued their randomised therapy, started another anti-cancer therapy, the event occurred between scheduled visits or previous visits were not evaluable due to partially, or had completely missing tumour assessments. This also applies to patients who die in the absence of progression

- If imaging assessments take place on multiple dates within a visit window then the date used for analysis will be defined as the earliest of:

  – Date of imaging assessment showing a new lesion (if progression is based on a new lesion)

  or

  – Date of last imaging assessment of measured lesions (if progression is based on increase in sum of measured lesions).

Criteria for calculating response where data for target lesions is missing will be pre-defined in the Statistical Analysis Plan (SAP).

12.2.3 Objective response rate (ORR)

Best overall response will be calculated as the best response recorded from date of randomisation (taking as reference for progressive disease the smallest measurements recorded since the treatment started) for each patient, and will be used for the summaries of objective response. Best overall response will be determined programmatically based on the RECIST criteria.

Objective response rate (ORR) is defined as the proportion of patients who have a best response of either CR or PR.

12.2.4 Duration of response (DoR)

DoR will be measured from the time measurement criteria for CR/PR (whichever is recorded first) are first met until the patient progresses or dies from any cause, regardless of whether the patient is still taking study medication. Patients that have not progressed at the time of the data cut-off for progression will be censored on the date of last evaluable disease assessment. Non-responding patients will be assigned a duration of zero.

12.2.5 Change in tumour size at 12 weeks

The tumour size is the sum of the longest diameters of the target lesions. The change in tumour size will be assessed using the ratio of the Week 12 tumour size over the baseline tumour size for each patient.

For those patients who progress at an unscheduled scan before Week 12, the tumour size from the progression assessment will be used instead of the Week 12 assessment.

For patients known to have progressed before Week 12, but for whom no progression RECIST target lesion assessment is available, change in tumour size will be imputed as a 20% increase.

For patients with missing baseline or missing Week 12 results but not known to have progressed, the data will be considered missing at random and the patients will be excluded from the analysis.

12.2.6 Calculation and derivation of patient reported outcome variables

The outcome variable to be assessed will be the total MSSQ score, which will consist of the total score of all individual questions within the MSSQ. This will be summarised by visit for both treatment arms.

12.3 Calculation or derivation of pharmacokinetic variables

The final PK analyses will be the responsibility of Clinical Pharmacology & DMPK, Alderley Park, AstraZeneca, UK. The actual PK sampling times will be used in the PK calculations.

Using appropriate PK software depending on whether comprehensive PK sampling or sparse PK sampling is obtained in practice, the PK data will be used to derive PK parameters such as, but not restricted to, C_{max}, AUC and $t_{1/2}$ for AZD6244, N-desmethyl AZD6244 and any known metabolites.

The AZD6244 and N-desmethyl AZD6244 and any known metabolite concentration-time profiles, along with the derived PK variables, will be listed for each patient per dose and dosing day and summarised appropriately, as described in the SAP.

Population PK models may be used to derive the PK parameters and will aim to characterise variability in the population by investigating the influence of covariates such as weight, age, smoking status and/or concomitant medications. In addition, if the data are suitable, potential relationships between plasma AZD6244 and N-desmethyl AZD6244 concentrations will be investigated using a graphical approach and/or appropriate PK/PD modelling techniques. A detailed PK analysis plan will be produced prior to any such investigations and will be reported separately.

12.4 Calculation or derivation of pharmacodynamic variables (not applicable)

12.5 Calculation or derivation of pharmacogenetic variables

See Appendix E for details of optional host genetic research variables.

12.6 Calculation or derivation of biomarker variables

12.6.1 *BRAF* mutation assessment of tumour biopsy

DNA extracted from the tumour samples will be analysed for mutations of *BRAF* using standard genetic analysis techniques. The central laboratory will assess *BRAF* mutation status using amplification refractory mutation system (ARMS™) analysis. Local laboratories may use agreed methodology for *BRAF* mutation testing as approved by AstraZeneca for the purposes of this study.

12.6.1.1 Optional analysis

If consent has been obtained, any residual tumour DNA or extracted DNA left over from the mutation analysis will be analysed for factors that may influence development of advanced

melanoma and/or response to AZD6244 (and/or agents used as comparators or as combinations). Methods of analysis may include, but are not limited to, investigation of genetic variability, gene expression profiling, protein expression profiling.

12.6.2 Analysis of CFDNA

CFDNA will be extracted from the plasma (and serum) samples for analysis of tumour specific mutations of *BRAF*. This will be undertaken using standard genetic analysis techniques. This be done will be using ARMS™ analysis initially, although other techniques may be used.

12.6.2.1 Optional analysis

If consent has been obtained, any residual plasma or serum or DNA extracted from plasma or serum left over from the mutation analysis will be analysed for factors that may influence development of advanced melanoma and/or response to AZD6244 (and/or agents used as comparators or as combinations). Methods of analysis may include investigation of genetic variability, gene expression profiling, protein expression profiling.

12.7 Calculation or derivation of health economics variables (not applicable)

13. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY ASTRAZENECA

13.1 Description of analysis sets

13.1.1 Analysis of safety population

Safety data will not be formally analysed. All patients who receive at least one dose of AZD6244/placebo or dacarbazine and for whom post-dose data are available will be included in the safety population. Throughout the safety results sections, erroneously treated patients (eg, those randomised to treatment A but actually given treatment B) will be accounted for in the actual treatment received group. Safety data will be summarised using treatment combination received.

All patients who receive at least one dose of AZD6244 or dacarbazine will be included in the assessment of AE profile (evaluable for safety population). Other safety data will be assessed in terms of physical examination, clinical chemistry, haematology, urinalysis, vital signs, echocardiogram, ophthalmological examinations and ECGs. At the end of the study, appropriate summaries of all safety data will be produced, as defined in the SAP.

Data from all cycles of initially randomised treatment will be combined in the presentation of safety data. AEs (both in terms of MedDRA preferred terms and CTCAE grade) will be listed individually by patient and treatment group. For patients who have a dose modification, all AEs (due to drug or otherwise) will be assigned to the initial treatment group. The number of patients experiencing each AE will be summarised by treatment group and severity.

13.1.2 Intention-To-Treat (ITT) population

Efficacy data will be analysed on an intention-to-treat basis using randomised treatment.

13.1.3 PK analysis set

PK data will be analysed according to treatment received. This population will comprise all patients who receive study treatment as per protocol and do not violate or deviate from the protocol in ways that would significantly affect the analyses. The population will be defined by the Study Team Physician, Pharmacokineticist and Statistician prior to any analyses being performed.

13.2 Methods of statistical analyses

A comprehensive SAP will be prepared before unblinding of the data.

RECIST will be used to assess tumours at screening, Week 12 and then every 12 weeks (relative to randomisation) until the earliest of disease progression, death, or withdrawal from consent. The outcome variable to be analysed will be the objective programmed assessment of response (based on the investigational site measurements and programmatically defined using the in-house algorithm) and not the investigators overall opinion, which will also be captured in the eCRF.

The primary objective of this study will be to compare the efficacy of AZD6244 in combination with dacarbazine, versus dacarbazine alone, in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma, by assessing OS.

The secondary objective of this study will be to compare the efficacy of AZD6244 in combination with dacarbazine, versus dacarbazine alone, in first line patients with *BRAF* mutation positive advanced cutaneous or unknown primary melanoma, by assessing the secondary variables of APF6, PFS, ORR, DoR, change in tumour size at 12 weeks.

The trigger for and approximate timings of the analyses are outlined in Table 7.

Table 7 Timing of planned efficacy analysis

Data Cut Off	Approximate time[a]	Trigger[b]	Outcome variables to be analysed
1	18 months	6 months post LPI	Secondary Variables: -PFS -APF6 -ORR -DoR -Change in tumour size at 12 weeks NB. Interim OS may also be performed

Table 7 Timing of planned efficacy analysis

Data Cut Off	Approximate time[a]	Trigger[b]	Outcome variables to be analysed
2	28 months	58 deaths	Primary Variable: -OS

a Times are from first patient entered. These times assume patients are recruited over 12 months according to a non-linear cumulative recruitment function and the median OS for dacarbazine is 9 months. If these assumptions do not hold, times will vary

b These criteria will determine the time analyses are performed

If a statistically significant difference (1-sided $p<0.1$) for the comparison of OS between AZD6244 in combination with dacarbazine, and dacarbazine alone is observed, the results will be regarded as promising (but not definitive) as there is a less than 1 in 10 probability that such a result could have been observed if there was truly no treatment effect. Confidence intervals for the treatment effects will be critical in interpretation of results from this trial. Results of secondary outcome variables need to be broadly consistent with the primary outcome of OS.

13.2.1 Overall survival (OS, time-to-death)

The analysis population for OS will be the intention-to-treat population. OS will be analysed using a Cox proportional hazards model. The model will allow for the effect of treatment and will include terms for WHO performance status (0 vs 1). The model will include these effects regardless of whether the inclusion of effects significantly improves the fit of the model.

The model will be fitted using PROC PHREG (in SAS Version 8.1) with the EXACT method to control for ties.

The hazard ratio (HR; AZD6244 in combination with dacarbazine: placebo in combination with dacarbazine) for treatment will be estimated together with its 80% confidence interval and 1 sided p-value (a HR less than 1 will favour AZD6244 in combination with dacarbazine). A Kaplan-Meier plot of OS and estimates of median OS will be presented by treatment group.

The assumption of proportionality will be assessed using plots of complementary log-log event times versus log time. If the assumptions of proportional hazards are shown not to hold for some of the baseline covariates then this will be explored but no action will be taken in the model fitting.

If there is a substantial departure in the proportional hazards assumption for treatment then the nature of non-proportionality will be explored and reported in the Clinical Study Report with the findings from the model. This information will be then used to aid the planning of the future direction of the AZD6244 program.

Whilst OS may initially be assessed at the time of the primary analysis of PFS, the final survival analysis will be performed when approximately 58 deaths have occurred. No

adjustment for multiplicity will be made in the OS analyses since any inference based on OS data will be made on the primary analysis occurring at 58 deaths.

13.2.2 Progression-free survival (PFS)

PFS will be analysed using a grouped Survival method for interval censored data (Whitehead 1989). The model will allow for the effect of treatment and will include terms for WHO performance status (0 vs 1). The model will include these effects regardless of whether the inclusion of effects significantly improves the fit of the model.

The model will be fitted using PROC GENMOD (in SAS), with a binomial distribution for the error term and complimentary log-log link function. The intervals (which will be derived according to the planned RECIST assessment schedule) and methodology for missing assessments will be detailed in the SAP.

The hazard ratio (HR; AZD6244 in combination with dacarbazine:placebo in combination with dacarbazine) for treatment will be estimated together with its 80% confidence interval and 1-sided p-value (a HR less than 1 will favour AZD6244 in combination with dacarbazine). A Kaplan-Meier plot of PFS, a Turnball Estimator plot and estimates of median PFS will be presented by treatment group.

An interval censored analysis will be performed as a sensitivity analysis to the grouped method to ensure robustness of results. Details of this analysis will be included in the SAP.

13.2.3 Objective response rate (ORR)

Summaries of best overall response and ORR by treatment arm will be produced. The ORR will be compared between AZD6244 in combination with dacarbazine vs. placebo in combination with dacarbazine using a multivariate logistic regression model and using the same covariates as used in the OS analyses, provided there are enough responses for a meaningful analysis. The results of the analysis will be presented in terms of an odds ratio together with its associated 80% confidence interval and 1-sided p-value. The ORR will be estimated for each treatment arm.

The variable used in the analysis will be the objective programmed assessment of response and not the investigator's overall opinion.

13.2.4 Alive and progression free at 6 months (APF6)

Kaplan-Meier estimates of APF6 will be calculated and compared between treatment groups. The analysis will adjust for the following factors: WHO performance status (0 vs 1). The log HR will be calculated using the difference in log (-log) of the Kaplan-Meier estimates of APF6. The variance of the log HR will be calculated using the corresponding logged version of Greenwood's formula for the standard error of a survival estimate (Hosmer & Lemeshow 1999). Results will be back transformed and presented as a HR together with its 80% confidence interval.

13.2.5 Duration of response (DoR)

Provided there are enough responses for a meaningful analysis, a Probability of Being in Response (PBR) function will be constructed. This function calculates the proportion of patients responding at each time point following randomisation. The PBR function will be used to display the data. Descriptive data will be provided for the DoR in responding patients, including the associated Kaplan–Meier curves (without any formal comparison or p-value attached). The expected DoR is derived for each treatment arm, along with the ratio of expected DoRs with 80% Confidence Interval, using the appropriate probability distribution for DoR in responding patients. The PBR function and the expected DoR will be calculated as described in the paper by Ellis et al 2008.

13.2.6 Change in tumour size at 12 weeks

Before any analysis is carried out, the distribution of the change in tumour size data will be looked at and if necessary, an appropriate transformation or non-parametric technique will be used.

Assuming no transformation, the effect of AZD6244 in combination with dacarbazine on changes in tumour size will be estimated from an analysis of covariance (ANCOVA) model including terms for treatment (AZD6244 in combination with dacarbazine or placebo in combination with dacarbazine) as well as a covariate for baseline tumour size. The results of the analysis will be presented in terms of adjusted means (least square means [lsmeans]) for each treatment together with their 2-sided 80% confidence intervals. An estimate of the treatment effect (difference* of the lsmeans; AZD6244 in combination with dacarbazine minus placebo in combination with dacarbazine) will be calculated together with its 2-sided 80% confidence interval.

*difference calculated in absolute terms e.g. 20% reduction vs 10% reduction from baseline gives a 10% reduction

Assumptions will be explored to validate the results of the main analysis.

Frequencies of non-target lesion progressions and new lesions at Week 12 will be presented together with the change in tumour size at 12 weeks results in order to put the change in tumour size results in perspective.

13.2.7 Patient reported outcomes (PRO)

Total MSSQ score will be summarised by visit for both treatment arms. Compliance rates will also be summarised.

13.2.8 PK data

The AZD6244 and N-desmethyl AZD6244 and any known metabolite concentration-time data, along with the derived PK parameters, will be listed and summarised appropriately.

13.2.9 PK/PD relationships

If the data are suitable, the relationship between the plasma AZD6244 and/or N-desmethyl AZD6244 concentrations/exposure and efficacy/safety parameters may be investigated graphically or using appropriate PK/PD software.

13.2.10 Biomarkers

To investigate agreement between serum/plasma *BRAF* mutation assessment and mandatory *BRAF* mutation assessment of tumour biopsy, a 2x2 table will be produced for those screened patients with matched samples (ie, samples for both tumour biopsy and serum/plasma samples) and the positive predictive value (PPV; the probability that the *BRAF* assessment of tumour biopsy is positive when the *BRAF* mutation assessment from the serum/plasma biopsy is positive) will be calculated.

13.3 Determination of sample size

The sample size for this study was selected to be consistent with the research hypothesis as described in Section 2.2.

This trial is designed as a randomised screening trial to quantify the level of risk entailed for further development. Thus the Type I and Type II errors have been adjusted to be less constrained, so that the targeted treatment benefit may be appropriate while the sample size remains reasonable (as discussed by Rubinstein et al 2005). The trial is of sufficient size to show that if AZD6244 in combination with dacarbazine is truly active, there is a high probability that it will demonstrate an effect sufficiently promising that it would warrant a follow up assessment in Phase III.

The OS analysis will be performed when approximately 58 death events have occurred. If the true HR is 0.57 (likely to correspond to a 75% prolongation of OS), this analysis will have approximately 80% power to demonstrate a statistically significant difference for OS, assuming a 1-sided 10% significance level. This trial has been sized using a 1-sided significance level of 10% as it is a Phase II study looking for a signal of improved efficacy. If a 1-sided $p<0.1$ is observed for the comparison of OS between AZD6244 in combination with dacarbazine, vs placebo in combination with dacarbazine, the results will be regarded as promising (but not definitive) as there is a less than 1 in 10 probability that such a result could have been detected if there was truly no treatment effect. Assuming 58 events occur, an observed HR of < 0.71 will achieve a 1-sided p-value < 0.1 within the trial.

Assuming non-uniform recruitment and a median OS of 9 months (Patel 2008) for the comparator, if approximately 80 patients (40 per arm) are recruited over 12 months it is predicted that 58 events will occur approximately 16 months following recruitment of the last patient. Overall recruitment and the overall number of progression events will be monitored on an ongoing basis, in order to predict time of analysis.

An analysis will be performed 6 months after LPI when it is considered the PFS data will be sufficiently mature, APF6 will be available and the vast majority of patients have discontinued randomised therapy. OS data may also be assessed at this time.

Screening rates

Assuming 50% of the total population have *BRAF* mutation positive advanced melanoma (seen in D1532C00003), and allowing for a 26% attrition rate (seen in study D1532C00003), it is expected that approximately 37% of patients screened will be determined to have *BRAF* mutation positive advanced melanoma.

13.4 Interim analyses

An analysis of the primary outcome variable OS may be performed at the time of the analysis of PFS. No adjustment for multiplicity will be made in the OS analyses since any inference based on OS data will be made on the primary analysis occurring at 58 deaths.

13.5 Data monitoring committee

No formal data monitoring committee will be set up during this Phase II study.

AstraZeneca will routinely monitor the safety data, and if any emerging clinically important events related to AZD6244 are identified then investigators will be informed in accordance with ICH guidelines.

14. LIST OF REFERENCES

American Cancer Society 2006
American Cancer Society. Cancer Facts and Figures 2006. Atlanta: American Cancer Society; 2006.

Balch et al 2001
Balch CM, Buzaid AC, Soong S-J, Atkins MB, Cascinelli N, Coit DG et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 2001;19(16):3635-3648.

Bedikian et al 2006
Bedikian AY, Millward M, Pehamberger H, Conry R, Gore M, Trefzer U et al. Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. J Clin Oncol 2006;24:4738-4745.

Cella et al 1993
Cella DF, Tulsky DS, Gray G, SaraBon B, Linn E, Bonomi A et al. The Functional Assessment of Cancer Therapy Scale: development and validation of the General Measure. J Clin Oncol 1993; 11(3): 570-9.

Chow et al 2005
Chow LQM, Eckhardt SG, Reid JM, Molina J, Hanson L, Piens J et al. A first in human dose-ranging study to assess the pharmacokinetics, pharmacodynamics, and toxicities of the MEK inhibitor ARRY-142886 (AZD6244) in patients with advanced solid malignancies. AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: Abstract C162.

Cormier et al 2008
Cormier JN, Ross MI, Gershenwald JE, Lee JE, Mansfield PF, Camacho LH et al. Prospective assessment of the reliability, validity, and sensitivity to change of the Functional Assessment of Cancer Therapy-Melanoma questionnaire. Cancer 2008;112(10):2249-57.

Danson and Middleton 2001
Danson S and Middleton M. Temozolomide: a novel oral alkylating agent. Expert Rev Anticancer Ther 2001;1(1):13-19.

Davies et al 2002
Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S et al. Mutations of the *BRAF* gene in human cancer. Nature 2002;417:949-954.

Dummer et al 2008
Dummer R, Robert C, Chapman P, Sosman J, Middleton M, Bastholt L et al. AZD6244 (ARRY-142886) vs temozolomide in patients with advanced melanoma: an open label, randomised, multi-centre phase II study. Presented at the 44th American Society of Clinical Oncology Annual Meeting, 30 May–3 June 2008. Abstract number 9033.

Eggermont and Kirkwood 2004
Eggermont AMM and Kirkwood JM. Re-evaluating the role of dacarbazine in metastatic melanoma: what have we learned in 30 years. Eur J Cancer 2004;40(12):1825-1836.

Ellis et al 2008
Ellis S, Carroll K, Pemberton K. Analysis of duration of response in oncology trials. Contemporary Clinical Trials 2008;29:456-465.

Fallowfield 1996
Fallowfield LJ. Quality of quality of life data. Lancet 1996;348:421.

Fecher et al 2007
Fecher LA, Cummings SD, Keefe MJ and Alani RM. Toward a molecular classification of melanoma. J Clin Oncol 2007;25:1606-1620.

Hosmer & Lemeshow 1999
Hosmer DW and Lemeshow S. Applied survival analysis: regression modeling of time to event date. John Wiley & Sons, New York; 1999. (Available upon request)

Kim et al 2002
Kim CJ, Reintgen DS and Balch CM. The new melanoma staging system. Cancer Control 2002;9(1):9-15.

Lens and Eisen 2003
Lens MB and Eisen TG. Systemic chemotherapy in the treatment of malignant melanoma. Expert Opin Pharmacother 2003;4(12):2205-2211.

Lev et al 2004
Lev DC, Onn A, Melinkova VO, Miller C, Stone V, Ruiz M et al. Exposure of melanoma cells to dacarbazine results in enhanced tumour growth and metastasis *in vivo*. J Clin Oncol 2004;22:2092-2100.

McDermott et al 2008
McDermott DF, Sosman JA, Gonzalez R, Hodi FS, Linette GP, Richards J et al. Double blind, randomised, Phase II study of the combination of sorafenib and dacarbazine in patients with advanced melanoma: a report from the 11715 study group. J Clin Oncol 2008;26:2178-2185.

Middleton et al 2000
Middleton MR, Grob JJ, Aaronson N, Fierlbeck G, Tilgen W, SeiterS et al. Randomized Phase III Study of Temozolomide Versus Dacarbazine in the Treatment of Patients With Advanced Metastatic Malignant Melanoma. J Clin Oncol 2000;18:158-166.

National Cancer Statistics Clearing House and National Mortality Database, AIHW 2008
National Cancer Statistics Clearing House and National Mortality Database, AIHW 2008. From website: http://www.aihw.gov.au/cancer/data/excel_tables/most_common_cancers.xls

Patel 2008
Patel PM. Extended schedule, escalated dose temozolomide versus dacarbazine in stage IV malignant melanoma: final results of the randomized Phase III study (EORTC 18032). Presented at EORTC's Perspectives of Melanoma XII, 2-4 October 2008.

Pérez-Soler et al 2005
Pérez-Soler R, Delord J, Halpern A, Kelly K, Krueger J, Sureda BM et al. HER1/EGFR inhibitor-associated rash: future directions for management and investigation outcomes from the HER1/EGFR inhibitor rash management forum. The Oncologist 2005;10:345-356.

Royal Society 2005
The Royal Society. Personalised medicines: hopes and realities. September 2005. ISBN 0 85403 620 2

Rubinstein et al 2005
Rubinstein LV, Korn EL, Freidlin B, Hunsberger S, Ivy SP, Smith MA. Design issues of randomized Phase II trials and a proposal for Phase II screening trials. J Clin Oncol 2005;23(28):7199-7206.

Serrone et al 2000
Serrone L, Zeuli M, Sega FM andCognetti F. Dacarbazine-based chemotherapy for metastatic melanoma: thirty-year experience overview. J Exp Clin Cancer Res. 2000;19(1):21-34.

Solit et al 2006
Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A et al. *BRAF* mutation predicts sensitivity to MEK inhibition. Nature 2006;439:358-362.

Therasse et al 2000
Therasse P, Arbuck SG, Eisenhauer, EA, Wanders J, et al. New guidelines to evaluate the response to treatment in solid tumors. J National Cancer Institute 2000;92:205-216.

UK Skin Cancer Mortality Statistics 2006
From Cancer Research UK website:
http://info.cancerresearchuk.org/cancerstats/types/skin/mortality/?a=5441

Wilkinson et al 2008
Wilkinson RW, Logié A, Holt SV, Haupt N, Curtis NJ, Hickinson DM et al. Activity of the MEK 1/2 inhibitor AZD6244 (ARRY-142886) in combination with standard and approved therapies: impact of *in vivo* sequencing of drug administration. Presented at the 99th American Association for Cancer Research Annual Meeting, San Diego; 12-16 April, 2008. Abstract number 4012.

Whitehead 1989
Whitehead J. The analysis of relapse clinical trials, with application to a comparison of two ulcer treatments. Stat Med 1989;8:1439-1454.

Clinical Study Protocol Appendix B

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Appendix B
Additional Safety Information

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Out-patient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse.

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a rechallenge.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?

- Is there a known mechanism?

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Clinical Study Protocol Appendix C

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Appendix C
IATA 6.2 Guidance Document

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm).** For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Cat A pathogens are eg. Ebola, Lassa fever virus

- are to be packed and shipped in accordance with IATA Instruction 602

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Cat B pathogens are eg. Hepatitis A, B, C, D, and E viruses, Human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Cat B or exempt under IATA regulations.
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm).**
- **Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content.**
- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable.

- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.

AstraZeneca

Clinical Study Protocol Appendix D	
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Appendix D
Guidelines for Evaluation of Objective Tumour Response Using RECIST Criteria (Response Evaluation Criteria in Solid Tumours)

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LIST OF TABLES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this appendix.

Abbreviation or special term	Explanation
CT	Computer tomography
CR	Complete Response
IR	Incomplete Response
iv	Intravenous
LD	Longest diameter
MRI	Magnetic Resonance Imaging
NA	Not Applicable
NE	Not Evaluable
NTL	New Target Lesions
PET	Positron emission tomography
PD	Progressive Disease
PR	Partial Response
RECIST	Response Evaluation Criteria in Solid Tumours
SD	Stable Disease
TL	Target Lesions

1. INTRODUCTION

This appendix details the implementation of RECIST Guidelines for the D1532C00006 study with regards to Investigator assessment of tumour burden, including protocol-specific requirements for this study.

2. DEFINITION OF MEASURABLE, NON-MEASURABLE, TARGET AND NON-TARGET LESIONS

Only patients with measurable disease at baseline should be included in this study. Measurable disease is defined by the presence of at least one measurable lesion which has not been previously irradiated. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable:

- At least one lesion, not previously irradiated, that can be accurately measured at baseline as ≥10 mm in the longest diameter (except lymph nodes which must have short axis ≥15 mm) with computed tomography (CT) or magnetic resonance imaging (MRI) and which is suitable for accurate repeated measurements.

Non-measurable:

- All other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan; longest diameter <10 mm or pathological lymph nodes with ≥10 mm to <15 mm short axis*).
- Soft tissue lesions assessed by clinical examination
- Truly non-measurable lesions include the following: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, cystic lesions.
- Previously irradiated lesions**
- Brain metastasis.

*Nodes with <10 mm short axis are considered non-pathological and should not be recorded or followed as NTLs

**Localised post-radiation changes which affect lesion sizes may occur. Therefore, lesions that have been previously irradiated will not be considered measurable and must be selected as Non-Target Lesions (NTL) at baseline and followed up as part of the NTL assessment.

Target lesions:

- A maximum of 10 measurable lesions (with a maximum of 5 lesions per organ), representative of all lesions involved suitable for accurate repeated measurement, should be identified as target lesions (TL) at baseline.

Non-Target lesions:

- All other lesions (or sites of disease) not recorded as TL should be identified as NTL at baseline.

3. METHODS OF MEASUREMENT

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.

A summary of the methods to be used for RECIST assessment is provided below and those excluded from tumour assessments for this study are highlighted, with the rationale provided.

Table 1: Summary of methods of assessment

Target Lesions	Non-Target lesions and New Lesions
CT (preferred)	CT (preferred)
MRI	MRI
	Clinical examination
	X-ray, Chest X-ray
	Ultrasound
	Endoscopy and laparoscopy

3.1 CT and MRI

CT and MRI are generally considered to be the best currently available and reproducible methods to measure TL selected for response assessment and to assess NTL and new lesions.

In the D1532C00006 study it is recommended that CT examinations of the chest, abdomen and pelvis will be used to assess tumour burden at baseline and follow-up time points. CT examination with intravenous (iv) contrast media administration is the preferred method. MRI should be used where CT is not feasible or it is medically contra-indicated. For brain lesion assessment, MRI is the preferred method.

3.2 Clinical examination

In the D1532C00006 study, clinical examination will not be used as part of RECIST assessment for TL. Clinically detected lesions can be selected as target lesions if they are

assessed by CT or MRI scans. Clinical examination can be used to assess NTL and to identify the presence of new lesions.

3.3 X-ray

3.3.1 Chest X-ray

In the D1532C00006 study, chest X-ray assessment will not be used as part of RECIST assessment for TL as they will be assessed by CT examination or MRI examination. Chest X-ray can, however, be used to assess NTL and to identify the presence of new lesions.

3.3.2 Plain X-ray

In the D1532C00006 study, plain X-ray may be used as a method of assessment for bone NTL and for confirmation of new bone lesions.

3.4 Ultrasound

In the D1532C00006 study, ultrasound examination will not be used as part of RECIST assessment for TL as it is not a reproducible method and does not provide an accurate assessment of tumour size. Ultrasound examination can, however, be used to assess NTL and to identify the presence of new lesions. If new or worsening clinical symptoms occur and an ultrasound is performed, then new lesions or progression of the existing lesions need to be confirmed by CT or MRI examination.

3.5 Endoscopy and laparoscopy

In the D1532C00006 study, endoscopy and laparoscopy will not be used as part of RECIST assessment for TL as they are not validated in the context of tumour measurements. However, endoscopy and laparoscopy can be used for assessment of NTL and to identify the presence of new lesions.

3.6 Tumour markers

In the D1532C00006 study, tumour markers will not be used as part of RECIST assessment.

3.7 Cytology and histology

In the D1532C00006 study, histology will not be used as part of the RECIST assessment.

Cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment is required when the measurable tumour has met criteria for response or stable disease. In such circumstances, the cytology is necessary to differentiate between response/stable disease (an effusion may be a side effect of the treatment) and progressive disease (if the neoplastic origin of the fluid is confirmed). Where cytology findings are not available, any effusion that significantly worsens (from trace to large) or appearance of clinically significant effusion (requiring change in drug therapy) during the study treatment, will be considered to be progression of NTL, or disease progression due to new lesions.

3.8 Imaging techniques not covered in the RECIST guidelines and not used in studies using RECIST tumour assessment

3.8.1 Isotopic bone scan

In the D1532C00006 study, isotopic bone scans will not be used to assess bone lesions as part of RECIST assessment due to insufficient specificity.

Bone lesions identified on an isotopic bone scan and confirmed by CT, MRI or X-ray at baseline should be recorded as NTL and followed by the same method as per baseline assessment. If new bone lesions or worsening bone symptoms occur and a bone scan is performed, then worsening of disease needs to be confirmed by CT, MRI or X-ray.

3.8.2 PET scan

In the D1532C00006 study PET scans will not be used for assessment of tumour response, as PET evaluations do not form part of the RECIST framework.

4. TUMOUR RESPONSE EVALUATION

4.1 Schedule of evaluation

All baseline tumour assessments must adequately assess tumour burden and should be performed no more than 14 days before randomisation and ideally should be performed as close as possible to the start of study of treatment. Any other sites at which new disease is suspected should also be adequately imaged at follow-up. Follow-up assessments will be performed at Week 12 and every 12 weeks (±1 week) after randomisation.

Bias in analysis can occur if one treatment group is examined more often or sooner than the other. If an unscheduled radiological and/or clinical tumour assessment is performed, and the patient has not progressed, the next scheduled tumour assessment should still be performed at the planned time (as detailed in the study plan). This schedule is to be followed in order to minimise any unintentional bias caused by some patients being assessed at a different frequency than other patients.

4.2 Target lesions (TL)

4.2.1 Documentation of target lesions

A maximum of 10 measurable lesions (with a maximum of 5 lesions per organ), representative of all lesions involved, should be identified as TL at baseline. Target lesions should be selected on the basis of their size (longest diameter for non-nodal lesions or short axis for lymph nodes) and their suitability for accurate repetitive measurements (by methods documented in Section 3 of this Appendix).

The site and location of each TL should be documented as well as the longest diameter (LD) of each TL (or short axis for lymph nodes). All measurements should be recorded in metric notation using a ruler, calipers, or electronic calipers etc. At baseline the sum of the diameters

for all TL will be calculated and reported as the baseline sum of diameters. At follow-up visits the sum of the diameters for all TL will be calculated and reported as the follow-up sum of diameters.

Special cases:

- If the slice thickness used for tumour assessment scans is >5 mm, the minimum size of measurable disease at baseline should be twice the slice thickness of the baseline scan
- If a TL splits into two or more parts, then record the sum of the diameters of those parts
- If two or more TL merge then the sum of diameters of the combined lesion should be recorded for one of the lesions and 0 mm recorded for the other lesion(s)
- If a TL becomes too small to measure accurately, then an estimate as close to the size as possible should be provided. The minimum size that can be recorded for a single lesion is 5 mm
- If a TL cannot be measured accurately due to it being too large, provide as close an estimate as possible of the size of the lesion
- For TL measurable in 2 or 3 dimensions, always report the longest diameter (short axis for lymph nodes)
- When a TL has had any intervention eg, radiotherapy, embolisation, surgery etc, during the study, the size of the TL should still be provided where possible.

4.2.2 Evaluation of target lesions

This section provides the definitions of the criteria used to determine objective tumour visit response for TL at the investigational site.

Table 2: Overall visit response for target lesions

Complete Response (CR)	Disappearance of all TL since baseline. Any pathological lymph nodes selected as target lesions must have a reduction in short axis to <10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of TL, taking as reference the baseline sum of diameters
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD
Progressive Disease (PD)	At least a 20% increase in the sum of diameters of TL, taking as reference the smallest sum of diameters recorded since the treatment started

Table 2: Overall visit response for target lesions

Not Evaluable (NE)	Only relevant if any of the TL were not assessed or not evaluable or had a lesion intervention

4.3 Non-target lesions (NTL)

4.3.1 Evaluation of non-target lesions

All other lesions (or sites of disease) not recorded as TL should be identified as NTL at baseline. Measurements are not required for these lesions, but their status should be followed at subsequent visits. At each visit an overall assessment of the NTL response should be recorded by the Investigator. This section provides the definitions of the criteria used to determine and record overall response for NTL at the investigational site at each visit.

If the Investigator is in doubt as to whether progression has occurred, with respect to NTL, it is advisable to continue treatment until the next scheduled assessment or sooner if clinically indicated and reassess the patient's status.

Table 3: Overall visit response for non-target lesions

Complete Response (CR)	Disappearance of all NTL since baseline All lymph nodes must be non-pathological in size (<10 mm short axis)
Incomplete Response (IR)/ Stable Disease (SD)	Persistence of one or more NTL
Progression (PD)	Unequivocal progression may be due to an important progression in one lesion only or in several lesions. In all cases the progression must be clinically significant for the physician to consider changing (or stopping) therapy
Not Evaluable (NE)	Only relevant when one or some of the NTL have not been assessed and in the Investigator's opinion they are not able to provide an evaluable overall NTL assessment
Not Applicable (NA)	Only relevant if there are no NTL at baseline

4.4 New Lesions

Details of any new lesions will also be recorded with the date of assessment.

New lesions in this study will be defined as:

- New lesions measuring ≥10 mm in longest diameter
- New lymph nodes lesions, ≥15 mm shorter axis.

4.5 Evaluation of Overall Visit Response and Best Overall Response

Table 4: Overall visit response

Target lesions	Non-Target lesions	New Lesions*	Overall response
CR	CR (or NA)	No	**CR**
CR	IR/SD	No	**PR**
PR	CR, IR/SD (or NA)	No	**PR**
SD	CR, IR/SD (or NA)	No	**SD**
PD	Any	Yes or No	**PD**
Any	PD	Yes or No	**PD**
Any	Any	Yes	**PD**
NE	Non-PD	No	**NE**
Non-PD	NE	No	**NE**

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease
IR = incomplete response, NE = not evaluable, NA = not applicable, * = see criteria above

The best overall response is the best response recorded from the start of treatment until disease progression.

Overall visit response and best overall response will be derived as part of the study analysis by the Sponsor from TL measurements, overall assessment of NTL and presence/absence of new lesions.

5. CONFIRMATION OF RESPONSE

According to RECIST Guidelines the main goal of confirmation of objective response is to minimize the risk of over-estimation of the response rate. This aspect of response evaluation is particularly important in non-randomized studies where response is the primary endpoint.

In the D1532C00006 study, confirmation of response (CR or PR) is determined by the study protocol to be performed at the next scheduled RECIST assessment (certainly no less than 4 weeks) following the date the criteria for response were first met. If the confirmation scan is performed earlier than the scheduled scan, every attempt should be made to perform the subsequent scans at their scheduled time points.

6. SPECIFICATIONS FOR RADIOLOGICAL IMAGING

These notes are recommendations for use in clinical studies. The use of standardized protocols for CT and MRI allows comparability both within and between different studies, irrespective of where the examination has been undertaken.

6.1 CT Scan

CT scans of the thorax, abdomen, and pelvis should be contiguous throughout the anatomical region of interest.

The type of CT scanner is important regarding the slice thickness and minimum sized lesions. 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 the thorax, abdomen, and pelvis.

CT examination with iv contrast media administration is the preferred method. This is to accentuate vascular structures from adjacent lymph node masses and to help enhance liver and other visceral metastases. The method of administration of iv contrast agents is variable. Contrast agent timing should be aimed at the portal-venous phase of the liver. In patients in whom the abdomen and pelvis have been imaged, oral contrast agents should be given to distinguish the bowel from other soft tissue masses. A consistent method should be used on subsequent examinations for any given patient.

If iodine contrast media is medically contraindicated at baseline, or at any time during the course of the study, then the recommended methods are CT thoracic examination without contrast and abdominal and pelvis MRI with contrast. If MRI cannot be performed then CT without iv contrast is an option for the thorax, abdomen and pelvis examination. For brain lesions assessment, MRI is the preferred method.

All window settings should be included in the assessment, particularly in the thorax where lung and soft tissue windows should be considered. When measuring lesions, the TL should be measured on the same window setting for repeated examinations throughout the study. All images from each examination should be included in the assessment and not “selected” images of the apparent lesion.

6.2 MRI Scan

MRI is entirely acceptable and capable of providing images in different anatomical planes. It is important therefore that when it is used, lesions must be measured in the same anatomical plane using the same imaging sequences on subsequent examinations. MRI scanners vary in the images produced. For a particular patient the same scanner should be used during the study assessment.

Moreover, many patients with advanced malignancy are in pain, so their ability to remain still for the duration of a scan sequence, in the order of 2-5 minutes is limited. Any movement during the scan time leads to motion artifacts, and degradation of image quality, such that the examination will probably be useless.

For these reasons, CT is the imaging modality of choice.

7. REFERENCES

Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumours. Journal of the National Cancer Institute. 2000 Feb 2;92(3):205-216

Clinical Study Protocol Appendix E	
Drug Substance	AZD6244
Study Code	D1532C00006
Appendix Edition Number	1
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Appendix E
Optional Host Genetic Research

HOST GENETICS RESEARCH SYNOPSIS

A Phase II, Double-Blind, Randomised Study to Assess the Efficacy of AZD6244 (Hyd-Sulfate) in Combination with Dacarbazine Compared with Dacarbazine Alone in First Line Patients with *BRAF* Mutation Positive Advanced Cutaneous or Unknown Primary Melanoma

The genetic research activities described in this appendix (including the collection and storage of genetic samples), are optional for study sites as well as for individual patients. These research activities will hereafter be referred to as "this genetic research." The clinical study protocol to which this document is appended will be referred to as "the main study". The term "genetic sample" means a blood sample collected for genetic research and/or DNA prepared from it.

This genetic research will be performed only after the appropriate Ethics Committee has approved it. Informed consent will be obtained using a form separate from that used for the main study. All sections of the protocol for the main study also apply to this genetic research.

Study centre(s) and number of patients who may be enrolled in this genetic research

As per main protocol.

Objectives

Objective	Outcome variables
To collect and store DNA, derived from a blood sample, for future exploratory research into genes that may influence response e.g. distribution, safety, tolerability and efficacy of AZD6244 and/or agents used in combination and/or as comparators	Correlation of host polymorphisms with variation in PK, PD, safety or response parameters observed in patients treated with AZD6244 (and or agents used in combination or as comparators).

Study design

It is proposed to collect an optional blood sample for genetic analysis. Provision of blood samples for genetic analysis will be optional for all patients entering the study and will involve a separate consent procedure. A patient's acceptance of this procedure will not be a requirement for his or her participation in the main study.

The blood sample and data for host pharmacogenetic analysis in this study will be coded. Each sample will be labelled with the study number and patient enrolment number (E-code). Only the investigator will be able to link the sample to the individual patient. The sample and data will not be labelled with a personal identifier.

Target population

All consenting patients in all centres participating in the main part of this study

Statistical methods

The number of patients who will agree to participate in this genetic research is unknown. It is therefore not possible to establish whether sufficient data will be generated. A statistical analysis plan will be prepared where appropriate.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
ADME	Absorption/Distribution/Metabolism and Excretion
AE	Adverse Event
CRF	Case report form
CSR	Clinical study report
DNA	Deoxyribonucleic acid
eCRF	Electronic case report form
EDTA	Ethylenediamine tetra-acetic acid
ICH	International Conference on Harmonisation
LIMS	Laboratory information management system
PD	Pharmacodynamic
PGx	Pharmacogenetics
PK	Pharmacokinetic
mL	Millilitre
UK	United Kingdom

1. BACKGROUND

AstraZeneca plan to include investigations into genetic variations and their effect on drug response as part of the drug development program for all projects where it is considered to be appropriate. By using this information, the aim is to better understand the impact of genetic variation and how it can be utilised to bring better drugs to the market.

To achieve this goal a systematic collection of deoxyribonucleic acid (DNA) for host genetic analysis (derived from blood samples taken from consenting study patients) will be implemented across a broad range of relevant clinical studies. The ability to acquire appropriate consent to collect blood samples to establish an archive and allow future meta-analysis of data derived from a number of studies for AZD6244 is of the utmost importance. This host genetic research forms part of this strategy.

1.1 Rationale for host genetic research

AstraZeneca intends to perform host genetic research in the AZD6244 clinical development programme to explore how genetic variations may affect the clinical parameters associated with AZD6244.

The benefits of being able to explore associations between genes and clinical outcomes within the AZD6244 programme are potentially many including the possibility to explain potential outliers, such as non-responders or to explain potential adverse reactions related to drug exposure.

2. HOST GENETIC RESEARCH OBJECTIVES

Genes that may be investigated include those which may be of relevance to the Absorption/Distribution/Metabolism/Excretion (ADME) of AZD6244 and/or agents used in combination or as comparators. Additional information on other genes important for this drug and for the response to AZD6244 (and or agents used in combination or as comparators) in cancers for which the drug is being developed will become available in the future and it is, therefore important to retain the possibility of investigating additional genes in the context of AZD6244 clinical program.

3. HOST GENETIC RESEARCH PLAN AND PROCEDURES

3.1 Host genetic research plan

This appendix to the Clinical Study Protocol has been subjected to peer review according to AstraZeneca standard procedures.

The patient will be asked to participate in this host genetic research during their enrolment or screening visit. If the patient agrees to participate a 10 mL blood sample will be collected into

a tube containing ethylenediamine tetra-acetic acid (EDTA). Blood samples will ideally be collected prior to first dosing of study treatment. Although genotype is a stable parameter, early sample collection is preferred to avoid introducing bias through excluding patients who may withdraw due to an adverse event (AE), such patients would be important to include in any host genetic analysis. If for any reason the sample is not drawn at Visit 2, it may be taken at any visit until the last study visit.

3.2 Selection of host genetic research population

3.2.1 Study selection record

All patients who take part in the study will be asked to participate in this host genetic research. Participation is voluntary and if a patient declines to participate there will be no penalty or loss of benefit. The patient will not be excluded from any aspect of the main study.

3.2.2 Inclusion criteria

For inclusion in this host genetic research, patients must fulfil all of the inclusion criteria described in the main body of the study protocol **and**:

1. Provide informed consent for the genetic sampling and analyses.

3.2.3 Exclusion criteria

Exclusion from this host genetic research may be for any of the exclusion criteria specified in the main study or any of the following:

1. Previous allogeneic bone marrow transplant

2. Whole blood transfusion within 120 days of the date of host genetic sample collection (except for leukocyte depleted blood transfusion, which is allowed).

3.2.4 Discontinuation of patients from this host genetic research

3.2.4.1 Criteria for discontinuation

Specific reasons for discontinuing a patient from this genetic are:

1. Withdrawal of consent for host genetic research. Patients may withdraw from this host genetic research at any time, independent of any decision concerning participation in other aspects of the main study. Voluntary discontinuation will not prejudice further treatment.

3.2.4.2 Procedures for discontinuation

Patients who discontinue from the main study should always be asked specifically whether they are withdrawing or continuing their consent for this host genetic research. It must be established whether the patient:

- Agrees to the host genetic samples and any DNA extracted from the sample being kept for host genetic research in the future

- Withdraws consent for the samples to be kept for host genetic research in the future and wishes the samples to be destroyed. Destruction of the samples (or the DNA extracted from the sample) will only be possible so long as the particular sample is traceable. In the event that host genetic research has already been performed, AstraZeneca will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

The principal investigator is responsible for providing written notification to AstraZeneca of any patient who has withdrawn consent for the use of the sample taken for host genetic research. AstraZeneca will provide written confirmation to the investigator of the actions taken with the sample, which must be filed in the investigator study file.

4. HOST GENETIC MEASUREMENTS AND CO-VARIABLES

4.1 Summary of host genetic objectives and analysis

The purpose of this host genetic research is to generate data for use in future retrospective or prospective analyses. Future analyses will explore host genetic factors, which may influence the disposition, efficacy, safety and tolerability to AZD6244 (and or agents used in combination or as comparators) and/or susceptibility to or prognosis of cancer. The results of this host genetic research will not form part of the clinical study report (CSR)for this study. The results may be pooled with host genetic data from other studies on AZD6244 to generate hypotheses to be tested in future studies.

4.2 Collection of samples for host genetic research

A 10 mL blood sample will be collected into a polypropylene tube containing EDTA and gently inverted a minimum of five times to mix thoroughly. The sample will then be stored at -20°C prior to shipment. Tubes will be identified with the protocol study number, centre number, enrolment code and/or randomisation number and date of sample collection. No personal identifiers (patient name, initials, or date of birth) will be placed on the tube or accompanying documentation. A record of the date of the patient consent to the host genetic research and the date of the blood sample collection will be recorded in the appropriate section of the electronic case report form (eCRF).

Genotype is a stable parameter, therefore if for any reason the blood sample is not drawn at their enrolment or screening visit, it may be taken at any visit until the last study visit. The host genetic blood sample should ideally be drawn through the same cannula used to draw blood samples required for the main study.

4.2.1 Sample processing and shipping

AstraZeneca, or its designee, will act as the central laboratory for sample logistics. This will include the supply of site material and all transport arrangements.

A single blood sample will be stored frozen (-20°C or below) at the site and sent to the central laboratory. The central laboratory will then send the samples to AstraZeneca, or its designated laboratory, for DNA extraction. Samples must remain frozen at all times. Further details on the processing of the samples are outlined in the Laboratory Manual.

Where possible samples should be shipped in batches and shipment will be co-ordinated with the receiving site to ensure samples arrive within working hours, on normal working days. Details of protocol study number, centre number, patient E-code, and/or randomisation number and date of sample collection should accompany the shipment.

4.2.2 Storage and coding of DNA samples

The following procedures refer to the processes adopted for the coding and storage of the samples for host genetic analysis. These processes are important to maintain patient confidentiality.

For all samples, irrespective of the type of coding used, the DNA will be extracted from the blood sample. The DNA sample will be assigned a unique number replacing the information on the sample tube. Thereafter, the DNA sample will be identifiable by the unique DNA number only. The DNA number will be used to identify the sample and corresponding data at the AstraZeneca genetics laboratories, or at the designated contract laboratory. No personal details identifying the individual will be available to any AstraZeneca employee working with the DNA.

The blood samples and data for host genetic analysis in this study will be coded. The link between the patient enrolment/randomisation code and the DNA number will be maintained and stored in a secure environment, with restricted access within the Clinical Genotyping Group Laboratory Information Management System (LIMS) at AstraZeneca. The link will be used to identify the relevant DNA samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit and to trace samples for destruction in the case of withdrawal of consent.

All DNA samples will be stored under secure conditions with restricted access at AstraZeneca or the contracted laboratory. The blood, DNA samples or data derived from the samples may be made available to groups or organisations working with AstraZeneca on this study or as part of the development drug project. However, the samples and any results will remain the property of AstraZeneca at all times. AstraZeneca will not give blood, DNA samples or data derived from the samples to any other parties, except as required by law.

5. MANAGEMENT OF HOST GENETIC RESEARCH DATA

Host genetic data, derived from blood DNA will not be reported in the CSR.

In the case of host genetic data, only the date the patient gave consent to participation in the host genetic research and the date the blood sample was taken from the patient will be recorded in the eCRF and database. The host genetic data will not be merged with the clinical dataset, residing within the clinical database, collected from the patient population for statistical analysis.

The host genetic data generated from the study will be stored in the AstraZeneca LIMS database or other appropriate system. This database is a secure database, which is separate from the database used for the main study. Some or all of the dataset from the main study may be duplicated within the AstraZeneca LIMS database for exploratory host genetic analysis.

5.1 Reporting of host genetic results

Results from this host genetic research will be reported separately from the CSR for the main study. AstraZeneca will not provide individual genotype results to patients, their family members, any insurance company, an employer, clinical study investigator, general physician or any other third party, unless required to do so by law. The patient's DNA will not be used for any purpose other than those described in the study protocol.

Individual patients will not be identified in any report or publication resulting from this work. The data and results of this study may be reviewed with collaborators and published, but neither the patient's name nor any other personal identifiers will appear in any publication or report.

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The number of patients who will agree to participate in the host genetic research is unknown. It is therefore not possible to establish whether a statistically relevant number of patients will consent to provide sufficient data to be collected to allow a formal statistical evaluation or whether only descriptive statistics will be generated. A statistical analysis plan will be prepared where appropriate.

7. STUDY MANAGEMENT

7.1 Monitoring

Before first patient entry into the study, a representative of AstraZeneca will visit the investigational study site. In addition to the requirements described in the main study, this host genetic research will be discussed.

During the study, a representative of AstraZeneca will have regular contacts with the investigational site. One of the purposes of these visits will be to perform source verification of the host genetic consent of participating patients and to ensure that the investigational team are adhering to the specific requirements of this host genetic research.

7.2 Training of staff

Before the first patient is entered into the study the investigational staff will have an opportunity to discuss the procedures associated with the collection of blood samples, extraction of DNA and host genetic research with a representative of AstraZeneca. The ethical considerations specific to host genetic analysis and the importance of the informed consent process will be made clear. The requirements for the collections of the patients' sample will also be made clear.

7.3 Changes to the protocol

Any changes to the host genetic research will comply with the principles described in Section 9.5 of the main body of the protocol.

7.4 Study agreements

The principal investigator at each centre must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this study. In the event of any inconsistency between this Clinical Study Protocol and the Clinical Study Agreement, the Clinical Study Protocol shall prevail. Specific reference to requirements relating to this host genetic research will be included in the study agreement(s).

8. ETHICS

8.1 Ethics review

In addition to documenting Ethics Committee approval of the main study, approval must be obtained for this host genetic research and the associated host genetic informed consent from the relevant Ethics Committee. It must be clearly stated in the approval that this host genetic research is approved. The investigator must submit written approval to AstraZeneca before any patient participates in this host genetic research.

8.2 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics.

For studies including genetic analysis special precautions are taken as described in Section 4.2.2 of this Appendix.

8.3 Informed consent

The host genetic component of this study is optional and the patient may participate in other components of the study without participating in the host genetic component. To participate in the host genetic component of the study which involves donation of blood samples, the patient must sign and date the consent forms for the main study and the consent form for the host genetic component of the study. Copies of all signed and dated consent forms must be given to the patient and the originals filed at the study centre. The principal investigator(s) is responsible for ensuring that consent is given freely and that the patient understands that they may freely discontinue from the genetic aspect of the study at any time.

8.4 Patient data protection

All data protection and confidentiality principles, described in the main study protocol, are applicable to this host genetic research.

Reference to participation in this host genetic research should not be recorded into the patients' general medical records. All notes should be kept within the clinical study records.

Due to the exploratory nature of this host genetic research, there will be no routine communication of results to patients. AstraZeneca will not provide individual host genetic results to patients, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law.

Extra precautions are taken to preserve confidentiality and prevent host genetic data being linked to the identity of the patient. In exceptional circumstances, however, certain individuals might see both the host genetic data and the personal identifiers of a patient. For example, in the case of a medical emergency, an AstraZeneca Physician or an investigator might know a patient’s identity and also have access to his or her host genetic data. Also Regulatory authorities may require access to the relevant files, though the patient’s medical information and the host genetic files would remain physically separate.

9. REFERENCES

None.

AstraZeneca

Clinical Study Protocol Appendix F	
Drug Substance	AZD6244
Study Code	D1532C00006
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Appendix F
Optional Biomarker Research

OPTIONAL BIOMARKER RESEARCH SYNOPSIS

A Phase II, Double-Blind, Randomised Study to Assess the Efficacy of AZD6244 (Hyd-Sulfate) in Combination with Dacarbazine Compared with Dacarbazine Alone in First Line Patients with *BRAF* Mutation Positive Advanced Cutaneous or Unknown Primary Melanoma

The research activities described in this appendix (including the collection and storage of samples), are optional for study centres as well as for individual patients. These research activities will hereafter be referred to as "this research." The clinical study protocol to which this document is appended will be referred to as "the main study." The term "sample" means:

- Residual tumour biopsy material (including extracted DNA)
- Residual plasma samples
- Residual serum samples.

This research will be performed only after the appropriate Ethics Committee/IRB has approved it. Informed consent for this research will be obtained using a Biological Samples Research Addendum to the mutation status screening Informed Consent form, which is separate from the Informed Consent form used for the main study. All sections of the protocol for the main study also apply to this research.

Study centre(s) and number of patients who may be enrolled in this genetic research

As per main protocol

Objectives

Objective	Outcome variables
To explore potential biomarkers in residual tumour, plasma and/or serum, taken for *BRAF* mutational analysis, which may influence development of advanced cutaneous or unknown primary melanoma (and associated clinical characteristics) and/or response (optional)	Correlation of biomarkers to response and/or development of advanced cutaneous or unknown primary melanoma

Study design

It is proposed to collect a number of sample types (listed above). Provision of these samples will be mandatory in the main study, but the exploratory biomarker analysis will be optional for all patients entering the study, and acceptance of this procedure will not be a requirement for participation in the main study.

The samples and data for optional biomarker analysis in this research will be coded. Each sample will be labelled with the study number and patient enrolment number (E-code). Only the investigator will be able to link the sample to the individual patient. The samples and data will not be labelled with personal details.

Where genetic analysis will be undertaken the processes adopted for the coding and storage of samples will be more stringent in order to maintain patient confidentiality. As an added precaution for all samples the DNA sample will be assigned a unique number replacing the information on the sample tube. Thereafter, the DNA sample will be identifiable by the unique DNA number only. The DNA number will be used to identify the sample and corresponding data at the AstraZeneca genetics laboratories, or at the designated contract laboratory. No personal details identifying the individual will be available to any AstraZeneca employee working with the DNA.

Target population

All consenting patients in all centres participating in the main study.

Statistical methods

The number of patients who will agree to participate in this research is unknown. It is therefore not possible to establish whether sufficient data will be generated. A statistical analysis plan will be prepared where appropriate.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
eCRF	electronic Case Record Form
CSR	Clinical study report
DNA	Deoxyribonucleic acid
ICH	International Conference on Harmonisation
RNA	Ribonucleic acid

1. BACKGROUND

As part of the clinical drug development program for AZD6244, AstraZeneca plans to include investigations into variations in biomarker profiles and their relationship to drug effect. These biomarkers may be derived from deoxyribonucleic acid (DNA), ribonucleic acids (RNA), proteins and/or metabolites. By using this information, the aim is to better understand the impact of variation between individuals and how it can be utilised to bring better drugs to the market.

To achieve this goal a systematic collection of biological samples will be undertaken where appropriate. These are listed in the synopsis section above.

In addition to the above samples, a blood sample for host genetic analysis (optional) will also be taken. Details on the collection and use of this sample are given in Appendix E.

1.1 Rationale for research

AstraZeneca may perform optional biomarker research in the AZD6244 clinical development programme to explore how variations in biomarkers (eg, DNA, RNA and/or protein-based) may affect the clinical parameters associated with AZD6244 and/or agents used as comparators or as combinations.

Recent studies have demonstrated that it is possible to predict clinical outcomes by measuring gene expression in solid tumour samples and that complex gene expression measurements may be translated into simple gene or protein based tests (Paik et al 2004; Ring et al 2006). There are indications that these kinds of measurements may be used to select patients who will benefit from particular therapeutic approaches (Paik et al 2006). Similarly, profiling of proteins and small molecules enables the screening of a large number of molecules that may correlate with the activity/ benefit to patients of AZD6244 (Wulfkuhle et al 2003; Clayton et al 2006). Non-heritable, tumour specific alterations to DNA have also been linked to prognosis and to potential treatment benefit (Wu et al 2007, Yoshida et al 2007, Sunaga et al 2007, Massarelli et al 2007, Beauclair et al 2007).

The ability to acquire appropriate consent to collect biological samples to establish an archive and allow future meta-analysis of data derived from a number of studies for AZD6244 is of the utmost importance. This research forms part of this strategy.

The benefits of being able to explore associations between biomarker variations and clinical outcomes within the AZD6244 programme are potentially many including the possibility to identify patients most likely to benefit from treatment, explain potential outliers, such as non-responders or to explain potential adverse reactions related to drug exposure.

2. RESEARCH OBJECTIVES

Biomarker technologies enable the measurement of many different molecules, such as DNA, RNA, proteins and metabolites, within a sample. The objective of this research is to determine if associations exist between differences detected between individuals with the activity of AZD6244 (and/or agents used in combination and/or as comparators) and the benefit to the patient in receiving AZD6244.

3. RESEARCH PLAN AND PROCEDURES

3.1 Research plan

The patient will be asked to participate in this optional biomarker research during their enrolment or screening visit.

3.2 Selection of optional biomarker research population

3.2.1 Study selection record

All patients who take part in the study will be asked to participate in this optional biomarker research. Participation is voluntary and if a patient declines to participate in this optional biomarker research they will not be excluded from any aspect of the main study.

3.2.2 Inclusion criteria

For inclusion in this biomarker research, patients must fulfil all of the inclusion criteria described in the main body of the study protocol **and**:

1. Provide informed consent for the biomarker sampling and analyses.

3.2.3 Exclusion criteria

Exclusion from this biomarker research will be any of the exclusion criteria described in the main body of the study protocol.

3.2.4 Withdrawal of patients from this optional biomarker research

3.2.4.1 Criteria for withdrawal

Specific reasons for withdrawing a patient from this optional biomarker research are:

- Withdrawal of consent for optional biomarker research. Patients may withdraw from this optional biomarker research at any time, independent of any decision concerning participation in other aspects of the main study. Voluntary withdrawal will not prejudice further treatment.

3.2.4.2 Procedures for withdrawal

Patients who withdraw from the main study should always be asked specifically whether they are withdrawing or continuing their consent for this optional biomarker research. It must be established whether the patient:

- Agrees to the optional biomarker samples and any preparations derived from the sample being kept for research in the future

- Withdraws consent for the samples to be kept for optional biomarker research in the future and wishes the samples to be destroyed. Destruction of the samples (or the preparations derived from the samples) will only be possible so long as the particular samples are traceable. In the event that optional biomarker research has already been performed, AstraZeneca will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

The principal investigator is responsible for providing written notification to AstraZeneca of any patient who has withdrawn consent for the use of the sample taken for optional biomarker research. AstraZeneca will provide written confirmation to the investigator of the actions taken with the sample, which must be filed in the investigator study file.

4. MEASUREMENTS AND CO-VARIABLES

4.1 Summary of objectives and analysis

The purpose of this research is to generate data for use in future analyses. Future analyses will explore factors, which may influence the disposition, efficacy, safety and tolerability to AZD6244 (and/or agents used as comparators or as combinations) and/or susceptibility to or prognosis of cancer. The results of this research will not form part of the clinical study report (CSR) for the main study. The results may be pooled with data from other studies on AZD6244 to generate hypotheses to be tested in future studies.

4.2 Collection of samples for optional biomarker research

AstraZeneca or its designee will act as the central laboratory for sample logistics. Details of sample collection, processing, shipping and storage will be described in the Laboratory Manual.

The samples and data for analysis in this research will be coded and will not be labelled with any personal details. Each sample will be identified with the study number and patient enrolment number. In this way biomarker data may be correlated with clinical data, samples destroyed in the case of withdrawal of consent and regulatory audit enabled. However, only the investigator will be able to link the biomarker sample to the individual patient.

Where genetic analysis will be undertaken the processes adopted for the coding and storage of samples will be more stringent in order to maintain patient confidentiality. As an added

precaution for all samples the DNA sample will be assigned a unique number replacing the information on the sample tube. Thereafter, the DNA sample will be identifiable by the unique DNA number only. The DNA number will be used to identify the sample and corresponding data at the AstraZeneca genetics laboratories, or at the designated contract laboratory. No personal details identifying the individual will be available to any AstraZeneca employee (or employees of contract laboratories etc) working with the DNA.

The coded samples may be made available to groups or organisations working with AstraZeneca on this research or as part of the development drug project. However, the samples and any results will remain the property of AstraZeneca at all times. AstraZeneca will not give samples, sample derivatives or data derived from the samples to any other parties, except as required by law.

5. MANAGEMENT OF RESEARCH DATA

Some of the dataset from the main study may be duplicated within AstraZeneca for exploratory analyses in combination with the optional biomarker data. Neither the patient's name nor any other personal identifiers will be part of this dataset. Optional biomarker data will not be reported in the CSR. Only the date the patient gave consent to participation in the research and the date and time the biological sample(s) (if applicable) was taken from the patient will be recorded in the electronic CRF (eCRF) and database.

AstraZeneca will not provide optional biomarker research results to patients, their family members, any insurance company, an employer, clinical study investigator, general physician or any other third party, unless required to do so by law. The patient's samples will not be used for any purpose other than those described in the study protocol.

Individual patients will not be identified in any report or publication resulting from this work. The data and results of this research may be reviewed with collaborators and published, but neither the patient's name nor any other personal identifiers will appear in any publication or report.

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The number of patients who will agree to participate in the research and the number of whom will have any particular clinical outcome are unknown. It is therefore not possible to establish whether a statistically relevant number of patients will consent to provide sufficient data to be collected to allow a formal statistical evaluation or whether only descriptive statistics will be generated. A statistical analysis plan will be prepared where appropriate.

7. STUDY MANAGEMENT

7.1 Monitoring

Before first patient entry into the study, a representative of AstraZeneca will visit the investigational study centre. In addition to the requirements described in the main study, this biomarker research will be discussed.

During the study, a representative of AstraZeneca will have regular contacts with the investigational centres. One of the purposes of these visits will be to perform source verification of the informed consent of participating patients and to ensure that the investigational team are adhering to the specific requirements of this optional biomarker research.

7.2 Training of staff

Before the first patient is entered into the study the investigational staff will have an opportunity to discuss the procedures associated with the collection of samples and optional biomarker research with a representative of AstraZeneca. The requirements for the collections of the patients' sample will also be made clear.

7.3 Changes to the protocol

Any changes to the optional biomarker research will comply with the principles described in Section 9.5 of the main body of the protocol.

7.4 Study agreements

The principal investigator at each study centre must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this research. In the event of any inconsistency between this Clinical Study Protocol and the Clinical Study Agreement, the Clinical Study Protocol shall prevail. Specific reference to requirements relating to this optional biomarker research will be included in the study agreement(s).

8. ETHICS

8.1 Ethics review

In addition to documenting Ethics Committee/IRB approval of the main study, approval must be obtained for this optional biomarker research and the associated informed consent from the relevant Ethics Committee. It must be clearly stated in the approval that this optional biomarker research is approved. The investigator must submit written approval to AstraZeneca before any patient participates in this optional biomarker research.

8.2 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics.

8.3 Informed consent

The biomarker component of this research is optional and the patient may participate in other components of the study without participating in the optional biomarker component. To participate in the optional biomarker component of the study the patient must sign and date the consent forms for the main study and the consent form for the optional biomarker component of the study. Copies of all signed and dated consent forms must be given to the patient and the originals filed at the study centre in the investigator's study file. The principal investigator is responsible for ensuring that consent is given freely and that the patient understands that they may freely discontinue from the optional biomarker aspect of the study at any time

8.4 Patient data protection

All data protection and confidentiality principles, described in the main study protocol, are applicable to this optional biomarker research.

Due to the exploratory nature of this optional biomarker research, there will be no routine communication of results to patients. AstraZeneca will not provide individual results to patients, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law.

9. REFERENCES

Beauclair et al 2007
Beauclair S, Formento P, Fischel J, Lescaut W, Largillier R, Chamorey E, et al. Role of the HER2 [Ile655Val] genetic polymorphism in tumorogenesis and in the risk of trastuzumab-related cardiotoxicity. Ann Oncol 2007;18:1335-41.

Clayton et al 2006
Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, et al. Pharmaco-metabonomic phenotyping and personalized drug treatment. Nature 2006;440:1073-7.

Massarelli et al 2007
Massarelli E, Varella-Garcia M, Tang X, Xavier AC, Ozburn NC, Liu DD, et al. KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. Clin Cancer Res. 2007;13:2890-6.

Paik et al 2004
Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351:2817-26.

Paik et al 2006
Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol. 2006;24:3726-34.

Ring et al 2006
Ring BZ, Seitz RS, Beck R, Shasteen WJ, Tarr SM, Cheang MC, et al. Novel prognostic immunohistochemical biomarker panel for estrogen receptor-positive breast cancer. J Clin Oncol. 2006;24:3039-47.

Sunaga et al 2007
Sunaga N, Tomizawa Y, Yanagitani N, Iijima H, Kaira K, Shimizu K et al. Phase II prospective study of the efficacy of gefitinib for the treatment of stage III/IV non-small cell lung cancer with EGFR mutations, irrespective of previous chemotherapy. Lung Cancer. 2007;56:383-9.

Wu et al 2007
Wu YL, Zhong WZ, Li LY, Zhang XT, Zhang L, Zhou CC et al. Epidermal growth factor receptor mutations and their correlation with gefitinib therapy in patients with non-small cell lung cancer: a meta-analysis based on updated individual patient data from six medical centers in mainland China. J Thorac Oncol. 2007;2:430-9.

Wulfkuhle et al 2003
Wulfkuhle JD, Paweletz CP, Steeg PS, Petricoin EF 3rd, Liotta L. Proteomic approaches to the diagnosis, treatment and monitoring of cancer. Adv Exp Med Biol. 2003;532:59-68.

Yoshida et al 2007
Yoshida K, Yatabe Y, Park JY, Shimizu J, Horio Y, Matsuo K, et al. Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer. J Thorac Oncol. 2007;2:22-8.

Clinical Study Protocol Appendix G

Drug Substance	AZD6244
Study Code	D1532C00006
Edition Number	1
Date	

Appendix G
World Health Organisation Performance Status

Table 1 World Health Organisation Performance Status (Miller et al, 1981)

	Score
Fully active, able to carry out all usual activities without restrictions and without the aid of analgesia	0
Restricted in strenuous activity, but ambulatory and able to carry out light work or pursue a sedentary occupation. This group also contains subjects who are fully active, as in grade 0, but only with the aid of analgesics	1
Ambulatory and capable of all self-care, but unable to work. Up and about more than 50% of waking hours	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	3
Completely disabled, unable to carry out any self-care and confined totally to bed or chair	4

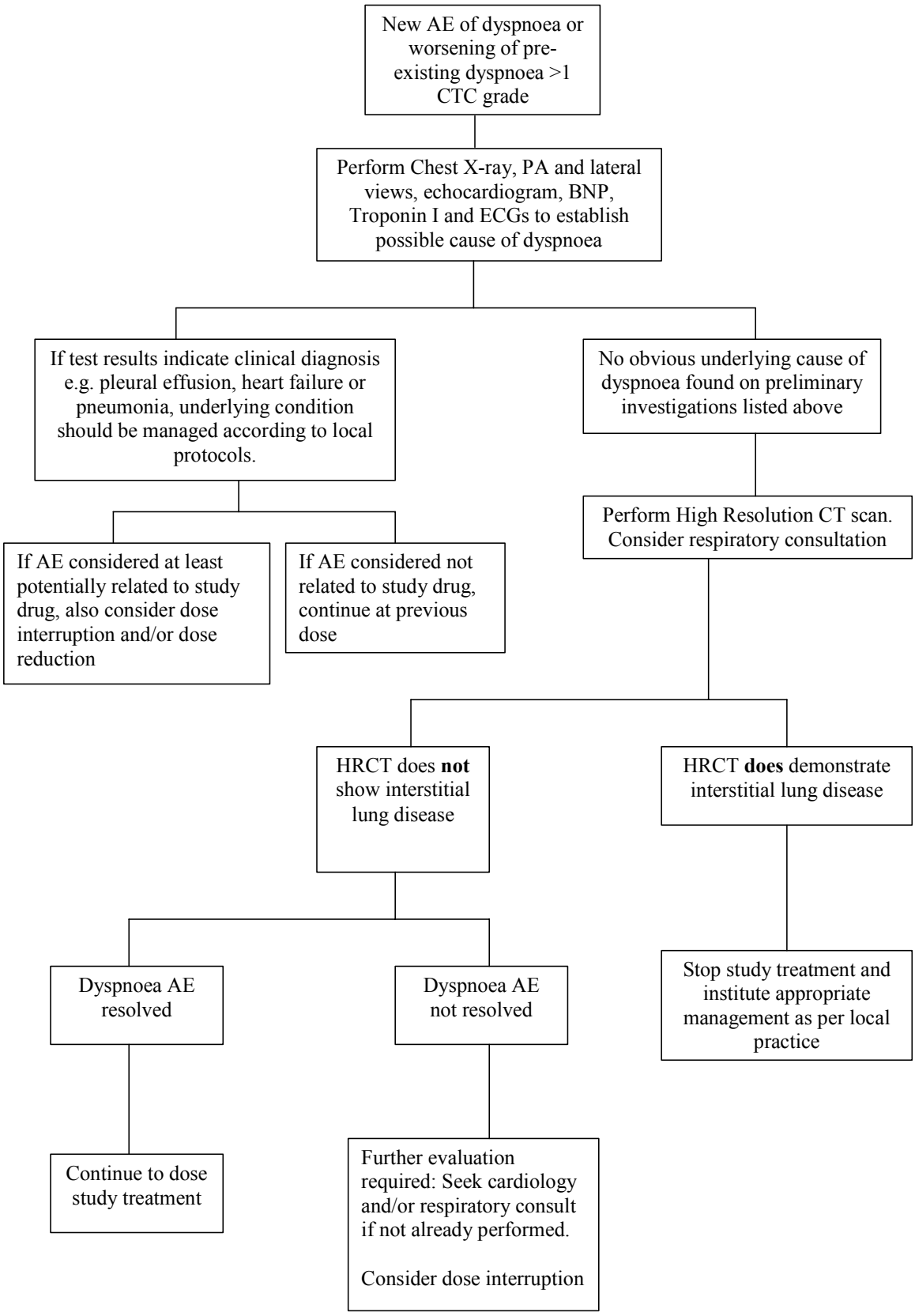
Reference

Miller et al, 1981
Miller AB, Hootstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47:207-214.

Clinical Study Protocol Appendix H

Drug Substance	AZD6244
Study Code	D1532C00006
Edition Number	1
Date	

Appendix H
Algorithm for Management of Dyspnoea


New AE of dyspnoea or worsening of pre-existing dyspnoea >1 CTC grade
Perform Chest X-ray, PA and lateral views, echocardiogram, BNP, Troponin I and ECGs to establish possible cause of dyspnoea
If test results indicate clinical diagnosis e.g. pleural effusion, heart failure or pneumonia, underlying condition should be managed according to local protocols.
No obvious underlying cause of dyspnoea found on preliminary investigations listed above
If AE considered at least potentially related to study drug, also consider dose interruption and/or dose reduction
If AE considered not related to study drug, continue at previous dose
Perform High Resolution CT scan. Consider respiratory consultation
HRCT does **not** show interstitial lung disease
HRCT **does** demonstrate interstitial lung disease
Dyspnoea AE resolved
Dyspnoea AE not resolved
Stop study treatment and institute appropriate management as per local practice
Continue to dose study treatment
Further evaluation required: Seek cardiology and/or respiratory consult if not already performed.
Consider dose interruption

Clinical Study Protocol Appendix I

Drug Substance	AZD6244
Study Code	D1532C00006
Edition Number	1
Date	

Appendix I
Melanoma Specific Symptom Questionnaire (MSSQ)

1. SUBJECT INFORMATION SHEET

1.1 About your questionnaire

We are interested in how patients feel whilst receiving treatment for your disease. Your doctor will ask you about your symptoms, but in addition, we would like you to fill in a brief questionnaire to give us your views on how you are feeling. The questionnaire has been designed specifically for people with your disease and asks you how you have been feeling during the past week.

All forms will be treated as confidential. Your doctor will not see this and your identity will not be revealed to anyone else involved in the study. If you have any worries about symptoms and side-effects of your disease or treatment, please discuss them with your doctor or nurse.

1.2 How to complete your questionnaire :

(a) Complete the questionnaire before you see your doctor

(b) Complete the questionnaire entirely on your own, rather than with a study nurse, relative, or friend

(c) It is important that you try and answer all the questions but do not spend too much time thinking about each one

(d) Indicate your answer to each question by selecting one answer that appears to be most appropriate for you

(e) There are no right or wrong answers

(f) If you have any questions, please ask the study nurse who will be happy to help

(g) The questionnaire should be handed over to the study nurse

THANK YOU FOR YOUR PARTICIPATION IN THIS STUDY

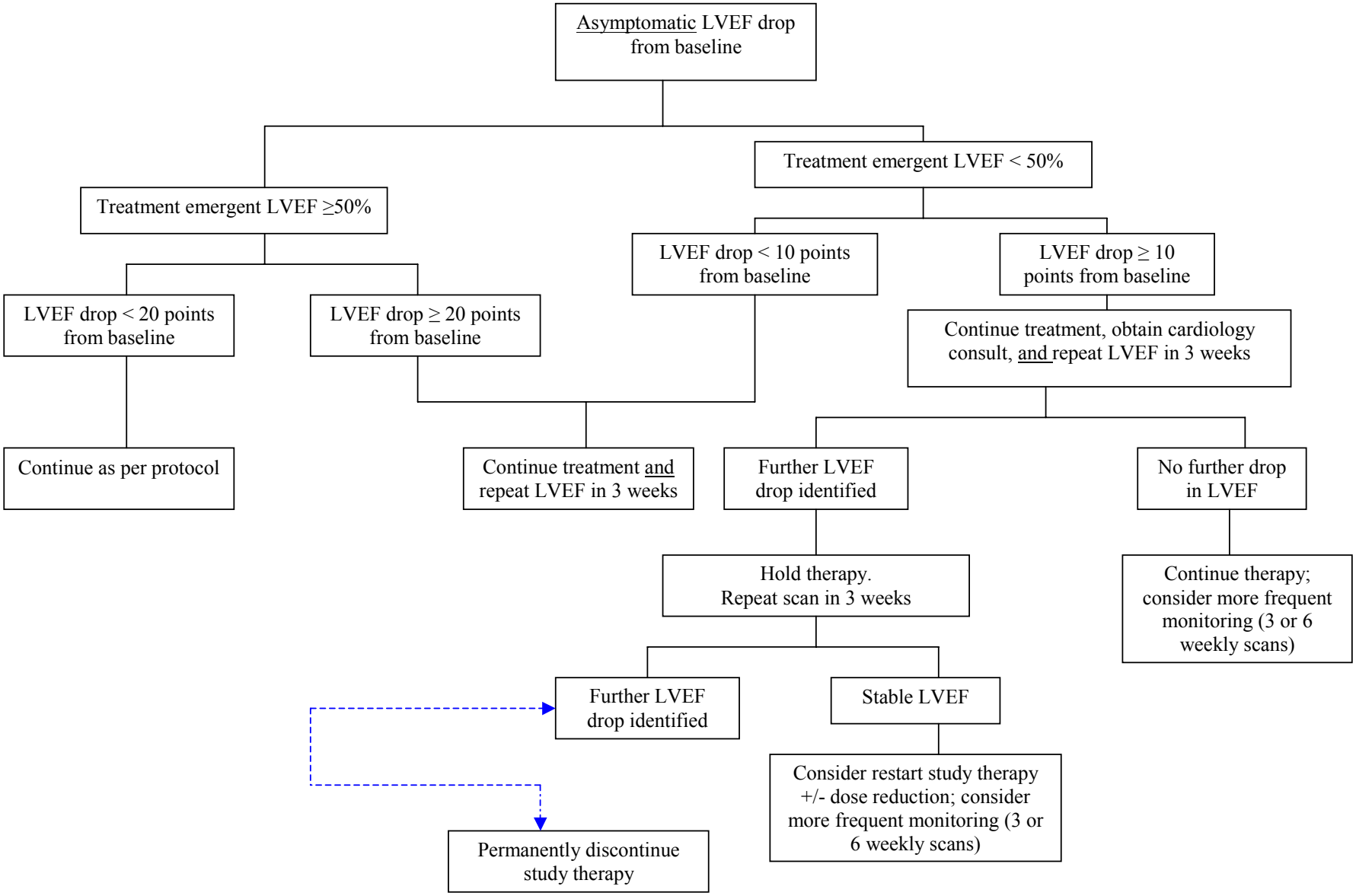
Melanoma Symptom Specific Questionnaire (MSSQ)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

	Not at all	A little bit	Somewhat	Quite a bit	Very Much
GP2 I have nausea…….……..	0	1	2	3	4
GP4 I have pain...……	0	1	2	3	4
GF1 I am able to work (include work at home).…..……...	0	1	2	3	4
M1 I have pain at my melanoma site or surgical site.……...	0	1	2	3	4
M2 I have noticed new changes in my skin (lumps, bumps, color)..	0	1	2	3	4
M3 I worry about the appearance of surgical scars..…….....	0	1	2	3	4
B1 I have been short of breath....…...	0	1	2	3	4
ITU4 I have to limit my physical activity because of my condition	0	1	2	3	4
An10 I get headaches…….	0	1	2	3	4
Hep3 I have had fevers….…….	0	1	2	3	4
C1 I have swelling or cramps in my stomach area.…….	0	1	2	3	4
C6 I have a good appetite……..	0	1	2	3	4
M5 I have aches and pains in my bones……...	0	1	2	3	4
M6 I have noticed blood in my stool...	0	1	2	3	4
ITU3 I have to limit my social activity because of my condition.……	0	1	2	3	4
MS8 I feel overwhelmed by my condition.……	0	1	2	3	4
M8 I isolate myself from others because of my condition.……	0	1	2	3	4
M9 I have difficulty thinking clearly (remembering, concentrating)…	0	1	2	3	4
HI7 I feel fatigued….…….….	0	1	2	3	4

Clinical Study Protocol Appendix J	
Drug Substance	AZD6244
Study Code	D1532C00006
Edition Number	1
Date	

Appendix J
Algorithm for Management of decrease in LVEF


Asymptomatic LVEF drop from baseline
Treatment emergent LVEF ≥50%
Treatment emergent LVEF < 50%
LVEF drop < 20 points from baseline
LVEF drop ≥ 20 points from baseline
LVEF drop < 10 points from baseline
LVEF drop ≥ 10 points from baseline
Continue treatment, obtain cardiology consult, and repeat LVEF in 3 weeks
Continue as per protocol
Continue treatment and repeat LVEF in 3 weeks
Further LVEF drop identified
No further drop in LVEF
Hold therapy. Repeat scan in 3 weeks
Continue therapy; consider more frequent monitoring (3 or 6 weekly scans)
Further LVEF drop identified
Stable LVEF
Consider restart study therapy +/- dose reduction; consider more frequent monitoring (3 or 6 weekly scans)
Permanently discontinue study therapy