A phase 2 study of the monocyte-targeted histone deacetylase inhibitor Tefinostat (CHR-2845) in chronic myelomonocytic leukaemia (CMML)

ISRCTN: ISRCTN17394489

EudraCT: 2015-002281-23

Approved by: Dr Steven Knapper

Signature:

Date: 28th March 2017

Version: 3.0

PROTOCOL

Study Title:	A PHASE TWO STUDY OF THE MONOCYTE-TARGETED HISTONE DEACETYLASE INHIBITOR TEFINOSTAT (CHR-2845) IN CHRONIC MYELOMONOCYTIC LEUKAEMIA (CMML)
Protocol Number:	SPON 1345-14
Indication:	Chronic Myelomonocytic Leukaemia (CMML)
Development Phase:	Phase II
Sponsor:	Cardiff University Research & Commercial Division 30-36 Newport Road Cardiff CF24 0DE UK
Sponsor's Responsible:	Dr Steven Knapper
Date:	28th March 2017
Version:	3.0
EUDRACT Number:	2015-002281-23
REC number (UK):	15/WA/0391

The protocol describes a trial which is being conducted by the Cardiff University Centre for Trials Research (CTR) under the sponsorship of Cardiff University. It provides information about procedures for the entry, treatment and follow-up of patients. It is not intended that this protocol should be used as an *aide-memoire* or guide for the treatment of other patients. Every care has been taken in its drafting, but corrections or amendments may be necessary. Centres are required to complete a registration process with CTR and to confirm acceptance of the terms of sponsorship required by Cardiff University. Before entering patients into the trial, centres must ensure that the trial protocol has received approval from the host institution's Research Governance procedures.

<u>Governance Statement:</u>

This study will be conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996) and the principles of Good Clinical Practice (GCP) as described in International Conference on Harmonisation guidelines.

The protocol has been approved in the UK by Wales REC 3 (REC) and the Medicines and Healthcare Products Regulatory Agency (MHRA).

Clinicians are required to read the whole protocol before commencing treatment

TRIAL SYNOPSIS

Title

A phase 2 study of the monocyte-targeted histone deacetylase inhibitor tefinostat (CHR-2845) in chronic myelomonocytic leukaemia (CMML)

Trial Design and Objectives

Single-arm, multi-centre, two-stage phase 2 clinical trial with the dual primary objectives of evaluating the safety and clinical efficacy of tefinostat monotherapy in CMML.

Outcome Measures

Primary Outcome Measures

- Safety and tolerability of tefinostat defined as the proportion of patients experiencing CTC grade 3-4 non-haematological toxicity or death thought to be at least possibly related to tefinostat during the first two cycles of treatment.
- Overall clinical response rate (according to International Consortium MDS/MPN Response Criteria)

Secondary Outcome Measures

- Incidence and duration of complete remission / partial remission / clinical benefit (according to International Consortium MDS/MPN Response Criteria)
- Overall clinical response rate (according to Wattel and modified IWG criteria)
- Achievement of red blood cell and platelet transfusion independence
- Overall survival
- Progression-free survival
- Incidence of transformation of CMML to AML (and time to AML transformation)
- Duration of tefinostat therapy
- Quality of life
- Biological correlates including hCE-1 expression and changes in protein acetylation

Patient population

The trial is open to patients with CMML-2 and those CMML-1 patients with symptomatic bone marrow failure or myeloproliferation who require treatment based on blood cytopenias / leucocytosis, disease symptoms or higher-risk prognostic score (CPSS).

Sample Size

Approximately 40 patients will be recruited over 12-18 months at 10-20 centres in the United Kingdom.

Main Eligibility Criteria

Inclusion Criteria

- All CMML-2 patients are eligible. For those patients classified as CMML-1, the following must be present:
  - Symptomatic bone marrow failure / myeloproliferation defined as *one or more of*: red cell transfusion dependence with pre-transfusion Hb<90g/l, symptomatic anaemia (and Hb<115g/l), thrombocytopenia (platelets<$50x10^9$/l), symptomatic bleeding due to platelet function defect or disseminated intravascular coagulation, white blood cell count>$50x10^9$/l

and/or

- CMML-specific Prognostic Score (CPSS) of intermediate-2 or high risk

and/or

- Systemic symptoms including weight loss with no alternative explanation (10% of baseline weight within previous 6 months)
- Symptomatic splenomegaly
- Symptomatic extramedullary involvement, e.g. skin infiltration, serous effusions

- Age ≥18 years
- ECOG performance status of 0-2 at study entry
- Women of childbearing potential must have a negative urine pregnancy test within 7 days prior to starting study drug and must use at least two effective methods of contraception throughout the study and for three months following the last dose of the study drug
- Men whose partner is a woman of childbearing potential must use at least two effective contraceptive methods throughout the study and for three months following the last dose of study drug

Exclusion Criteria

- CMML with eosinophilia and 5q33 abnormality
- CMML patients who are considered suitable for allogenic stem cell or bone marrow transplantation
- Previous chemotherapy for CMML except hydroxycarbamide and 5-azacitidine
- Creatinine concentration >2x the institutional upper limit of normal range
- Liver transaminases (AST / ALT) >3x the institutional upper limit of normal range or serum bilirubin >4x the institutional upper limit of normal range
- Pregnant or lactating females
- Use of experimental drug or therapy within 28 days of registration
- Other malignancy within 3 years other than curatively-treated basal cell or squamous cell skin cancer, carcinoma in situ of the cervix, organ-confined or treated non-metastatic prostate cancer with negative prostate specific antigen, in situ breast carcinoma after complete surgical resection or superficial transitional cell bladder carcinoma. Cases of CMML seen in association with systemic mastocytosis (systemic mastocytosis with associated clonal haematological non-mast cell lineage disease) *may* be included if the CMML is considered to be the clinically predominant disease.
- Known seropositivity for HIV infection, hepatitis B or C
- Uncontrolled inter-current illness including, but not limited to, ongoing infection, psychiatric illness or social situation that the treating physician judges would limit compliance with study requirements

Study Treatment

Tefinostat will be administered orally on a continuous basis, starting at a once daily dose of 360mg. The dose of tefinostat may be escalated or de-escalated for reasons of tolerability and clinical efficacy. Cycles of tefinostat therapy will be repeated every 28 days for at least 6 cycles unless the patient withdraws their consent for the study, disease progression or loss of response occurs or therapy is stopped on grounds of tolerability.

Following completion of the sixth cycle of treatment patients will undergo a formal response assessment. Responding patients, defined as those achieving: clinical benefit (or better) by International Consortium MDS/MPN Response Criteria may continue tefinostat until loss of response, disease progression or the development of unacceptable toxicity.

CONTENTS

List of Abbreviations

Abbreviation	Definition
AE	Adverse Event
ALT	Alanine Aminotransferase
AML	Acute Myeloid Leukaemia
ANC	Absolute Neutrophil Count
API	Active Pharmaceutical Ingredient
APTT	Activated Partial Thromboplastin Time
AR	Adverse Reaction
AST	Aspartate transaminase
CHO	Chinese hamster ovary cells
CMML	Chronic myelomonocytic leukaemia
CPSS	CMML-specific Prognostic Scoring System
CR	Complete response
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTR	Centre for Trials Research
CV	Curriculum Vitae
DIC	Disseminated Intravascular Coagulation
DLT	Dose-limiting toxicity
DLTs	Dose related toxicities
DSUR	Development safety update report
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
ESM	‘Esterase sensitive motif’
FAB	French American British classification
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
Hb	Haemoglobin
HCC	Hepatocellular carcinoma
hCE-1	Human carboxylesterase

HDACi	Histone deacetylase inhibitor
hERG	Human Ether-à-go-go-Related Gene
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IDMC	Independent Data Monitoring Committee
IMP	Investigative Medicinal Product
ISF	Investigator Site File
IWG	International Working Group
LDH	Lactate dehydrogenase
MAD	Maximum administered dose
MCV	Mean corpuscular volume
MDS	Myelodysplastic syndrome
MHRA	Medicines and Healthcare Products Regulatory Agency
MPN-SAF	Myeloproliferative Neoplasms Symptom Assessment Form
MTD	Maximum tolerable dosage
NICE	National Institute for Health and Clinical Excellence
NOAEL	No observable adverse effect level
PR	Partial Remission
PT	Prothrombin time
QP	Qualified Person
QT_c	Corrected QT interval
R&D	Research and Design
REC	Research Ethics Committee
rEPO	Recombinant Erythropoietin
SAE	Serious Adverse Event
SOP	Standard operating procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAM	Tumour associated macrophages
TK	Toxicokinetics
WBC	White blood cell
WHO	World Health Organisation

TRIAL MANAGEMENT GROUP

Chief Investigator **Dr Steven Knapper**
Clinical Reader & Consultant Haematologist
Division of Cancer and Genetics
Cardiff University
Heath Park
Cardiff CF14 4XN
Tel: 02920 745379
Fax: 02920 744655
E-mail: knappers@cardiff.ac.uk

TRIAL GOVERNANCE GROUP:		
Professor David Bowen **(Co-Investigator)** Haematology Department Leeds Teaching Hospitals NHS Trust St James University Hospital Level 03 Bexley Wing Beckett Street Leeds LS9 7TF Tel: 0113 206 8465 Fax: 0113 206 8177 Email: d.bowen@nhs.net	**Dr Mark Drummond** **(Co-Investigator)** Haematology Department Beatson West of Scotland Cancer Centre 1053 Great Western Road Glasgow, G12 0YN Tel: 0141 301 7712 Fax: 0141 301 7718 Email: mark.drummond@ggc.scot.nhs.uk	**Dr Mike Dennis** **(Co-Investigator)** Haematology and Transplant Unit Christie Hospital NHS Trust Wilmslow Road Manchester M20 4BX Tel: 0161 446 8430 Fax: 0161 446 3941 E-mail: mike.dennis@christie.nhs.uk
TRIAL STATISTICIAN	**TRIAL MANAGEMENT AND DATA MANAGEMENT**	**CTR LEAD**
Melissa Wright Trial Statistician Centre for Trials Research College of Biomedical & Life Sciences 4th Floor, Neuadd Meirionnydd Cardiff University Heath Park Cardiff, CF14 4YS Tel: 029 2068 7231 Email: Wright10@cardiff.ac.uk	**Trials Team** Centre for Trials Research Cardiff University Heath Park Cardiff, CF14 4XN Tel: 02921 847909 Fax: 02920 742289 Email: monocle@cardiff.ac.uk	Prof Robert Hills Professor of Translational Statistics Centre for Trials Research College of Biomedical & Life Sciences Cardiff University Heath Park Cardiff CF14 4XN Tel: 02920 744647 Fax: 02920 744655 Email: hillsrk@cardiff.ac.uk

LABORATORY STUDIES AND SAMPLE BANKING	MOLECULAR GENETICS	ACETYLOMIC STUDIES
Dr Marie Gilmour Department of Haematology School of Medicine Cardiff University Heath Park Cardiff CF14 4XN Tel: 02920 743482 Fax: 02920 744655 Email: gilmourm@cardiff.ac.uk	**Dr Catherine Cargo** Haematological Malignancies Diagnostic Service (HMDS) Leeds Cancer Centre Bexley Wing Beckett Street Leeds LS9 7TF Tel: 0113 20 67979 Fax: 0113 2067883 Email:catherine.cargo@leedsth.nhs.uk	**Professor Anthony Whetton** The University of Manchester Wolfson Molecular Imaging Centre, Floor 1 27 Palatine Road Withington Manchester M20 3LJ Tel: 0151 275 0038 Email: tony.whetton@manchester.ac.uk

ADMINISTRATION AND FOLLOW-UP

CENTRE FOR TRIALS RESEARCH (CTR)

DEPARTMENT OF HAEMATOLOGY,

SCHOOL OF MEDICINE

CARDIFF UNIVERSITY

HEATH PARK

CARDIFF, CF14 4XN

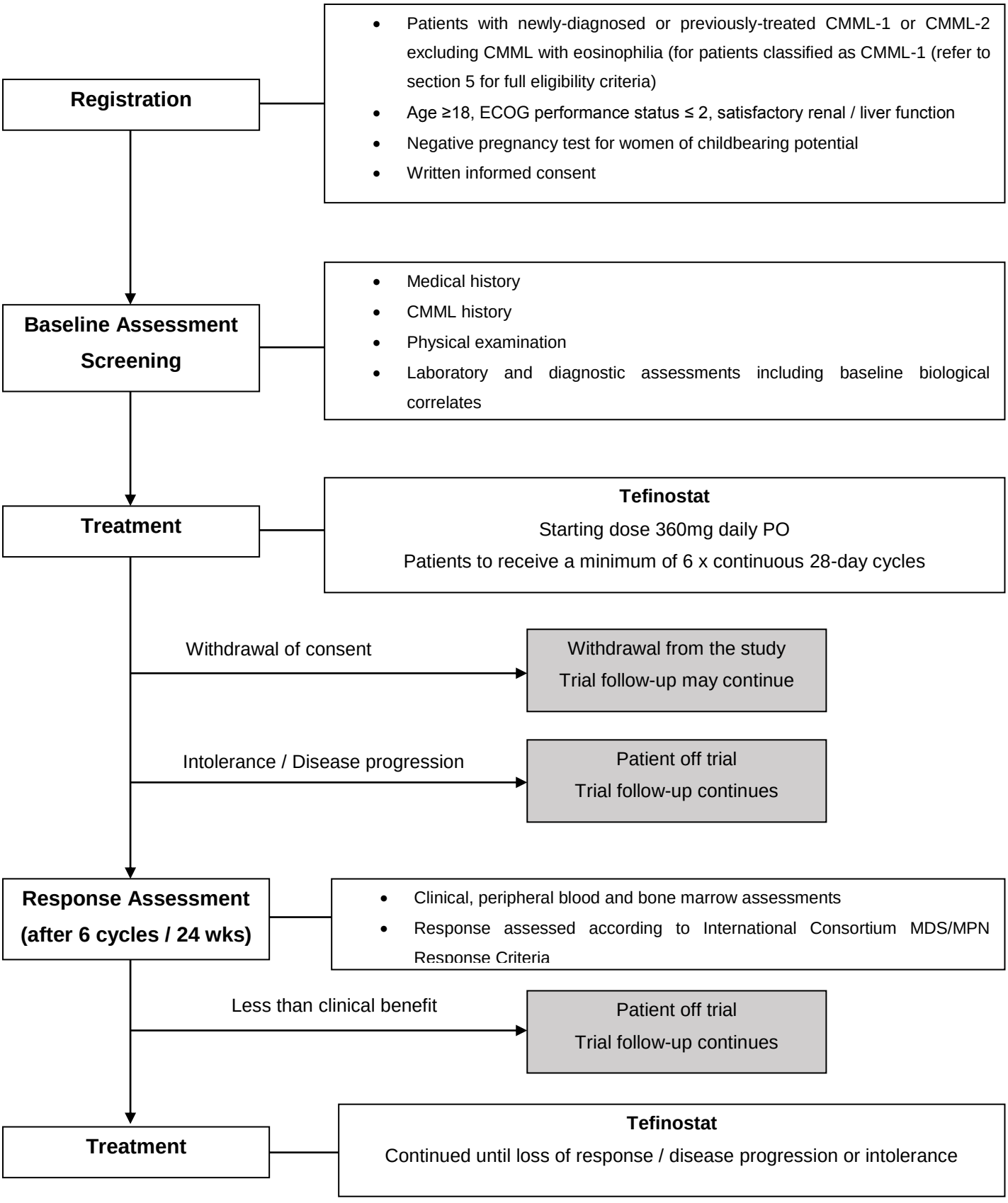
TEL: 029 2074 5397

FAX: 029 2074 2289

Website for Trial Information/ Data recording:

https://medic.cardiff.ac.uk/monocle

1 TRIAL FLOW DIAGRAM



2 BACKGROUND

2.1 Chronic Myelomonocytic Leukaemia (CMML)

Chronic myelomonocytic leukaemia (CMML) is a myelodysplastic / myeloproliferative neoplasm with a high median age of presentation (75 years) and a poor prognosis; the median survival from diagnosis remains only 11-17 months.[1-3] The incidence of CMML is poorly defined, although large population-based studies estimate that CMML constitutes approximately 10% of cases of myelodysplastic syndrome (MDS) where the overall incidence of MDS is approximately 4 cases per 100,000 per year and rises to a level of >30 / 100,000 / year beyond the age of 70.[2,3] Due to its relative rarity, its clinical and morphological heterogeneity and its overlap with other myeloid neoplasms, CMML has been a historically under-researched disease entity, with few clinical studies addressing it in isolation.

CMML has a highly heterogeneous disease phenotype with individual patients showing varying degrees of 'dysplastic' and 'proliferative' disease features. It is characterised by peripheral blood monocytosis (>1x10^9/l), bone marrow failure and accompanying blood cytopenias, sometimes in association with leucocytosis and other myeloproliferative features such as hepatosplenomegaly, constitutional symptoms and infiltration of the skin and other organs. In 1982, CMML was included as one of five MDS categories within the French-American-British (FAB) classification on the basis of marrow dysplasia, clonal cytogenetic abnormalities and a propensity for progression to acute myeloid leukaemia (AML).[4] Since 2001, the WHO classification has moved CMML into a separate category of 'mixed myelodysplastic / myeloproliferative neoplasms' where the disease has been additionally sub-divided into CMML-1 (with <10% bone marrow blasts) and CMML-2 (with 10-19% blasts) based on the powerful prognostic weighting of the bone marrow blast count.[5] A rare separate entity, the category of 'CMML with eosinophilia' has also been created; this is associated with translocations involving 5q33 (platelet-derived growth factor receptor) and a high degree of therapeutic sensitivity to imatinib mesylate therapy; CMML with eosinophilia is not representative of the bulk of CMML cases and falls outside the scope of the Monocle study.

Clonal cytogenetic abnormalities are seen in approximately 20-40% of CMML patients with most frequent alterations including trisomy 8, monosomy 7, del(7q) and abnormalities of chromosome 12p.[1] In recent years the advent of next generation sequencing has enabled the identification of molecular aberrations in approximately 90% of CMML patients including mutations of epigenetic regulator genes (EZH2, ASXL1, TET2, DNMT3A, IDH1/2), spliceosome pathway components (SF3B1, SRSF2, U2AF1), DNA damage response genes (TP53) and receptor tyrosine kinases and transcription factors (JAK2, RAS, CBL).[6] The frequency and sequence

of acquisition of somatic mutations in CMML appears distinct from that seen in MDS and the prognostic relevance of these lesions continues to be explored.[7,8]

Although the overall prognosis for CMML patients is poor, with median survival comparing unfavourably to that seen in MDS, there is considerable inter-patient variation. In MDS there has been consensus acceptance of the 1997 IPSS and 2012 IPSS-R risk stratification models which have allowed more consistent prognostication as well as greater uniformity of patient selection for clinical trials.[9,10] These models included relatively few CMML patients in their design cohorts, however, with neither allowing patients with 'proliferative CMML' (peripheral white blood cell count >12 $x10^9$/l), thus limiting their applicability in CMML. In recent years, several new CMML-specific risk stratification models have been created to include both the myelodysplastic and myeloproliferative CMML variants. The most well-established of these is the CMML-specific Prognostic Scoring System (CPSS), derived from a training cohort of 558 Spanish CMML patients and validated in an independent series of 274 German patients.[11] The CPSS separates CMML patients into 4 distinct risk groups, predicting both overall survival and risk of AML transformation based on blast percentage (WHO subtype), peripheral WBC count (FAB subtype), cytogenetic risk group and red cell transfusion dependency, and has been incorporated into the eligibility criteria of the Monocle study.

2.2 Current therapeutic options in CMML

Although allogeneic stem cell transplantation represents a potentially-curative treatment approach to CMML, it is associated with considerable treatment-related morbidity and mortality and is thus applicable to only a small minority of younger, fitter patients.[12,13] For the vast majority of CMML patients, treatment is given with predominantly palliative intent, comprising standard supportive care measures for cytopenias along with mild chemotherapy (usually hydroxycarbamide) to control the myeloproliferative component.[14] Recent years have seen increasing use of hypomethylating agents such as 5-azacitidine in cases where dysplastic features predominate. Azacitidine is currently approved for CMML-2 patients (blasts >10% in bone marrow or >5% in peripheral blood) who 'lack proliferative features' (WBC<13x10^9/l) based on registration studies in wider MDS that contained relatively few CMML patients.[15,16] A recent UK-based study of azacitidine in a wider CMML population (the CMML-201 study) has, however, demonstrated relatively modest response rates to the agent.[17] There remains a pressing need for therapeutic strategies in CMML that are capable of altering the natural history of the disease.

2.3 Tefinostat (CHR-2845)

Tefinostat (CHR-2845, Sixth Element Capital) is a novel type of histone deacetylase inhibitor (HDACi) which utilises 'esterase sensitive motif' (ESM) technology to target HDAC inhibition specifically within cells of the monocyte-macrophage lineage. Despite a significant pre-clinical rationale for HDAC inhibition in haematological

malignancies including myelodysplastic syndromes, the clinical uptake of HDAC inhibitors has so far been hindered by systemic toxicities including gastrointestinal disturbances, fatigue and insomnia.[18,19] Tefinostat is a cell-permeant ester that is cleaved to an active acid (CHR-2847) by an intracellular esterase (human carboxylesterase 1, hCE-1) that is found only in cells of monocyte lineage. CHR-2847, being a charged molecule, cannot readily leave cells and selectively accumulates, and is active within, hCE-1-expressing cells resulting in a 20- to 100-fold increase in anti-proliferative potency for monocytic over non-monocytic tumour cells. This selectivity creates a potentially-increased therapeutic window in haematological malignancies involving cells of monocyte lineage (AML-M4, AML-M5 and CMML) by targeting the HDACi effect within tumour cells and potentially sparing the systemic toxicological effects associated with non-selective HDAC inhibitors.[20] There is, in addition, increasing evidence that macrophages associated with progressive solid tumours (tumour-associated macrophages – TAMs) and some haematological tumours are reprogrammed by neighbouring tumour cells to induce immune suppression of host defences, facilitating progressive tumour growth and dissemination. Targeting TAMs with tefinostat could thus also potentially be of future clinical benefit in non-monocyte derived tumours.

2.3.1 Tefinostat: in vitro pre-clinical data

Tefinostat has an IC_{50} of 113nM when tested against HeLa cell nuclear extracts containing a number of HDAC isoforms while its corresponding acid, CHR-2847, has an IC_{50} of 160nM. Tefinostat has anti-proliferative effects against a wide range of cell lines but is extremely potent against human monocyte-derived cell lines: THP1 (monocytic leukaemia) IC_{50} 22nM, NOMO-1 (AML M5) 26nM and FUJ/P31 (AML M5) 64nM. CHR-2845 was approximately 10- to 20-fold more potent than the non-selective HDACi vorinostat in monocytoid cell lines. It should be noted that the active acid CHR-2847 (produced within myelomonocytic cells following cleavage of tefinostat by the esterase hCE-1) is inactive when added extracellularly to proliferating cells because the charged nature of this molecule prevents access into the cell.

In vitro studies of tefinostat in primary AML and CMML cells showed significant growth inhibitory/pro-apoptotic effects in monocytoid AML (M4/M5) and CMML in comparison to non-monocytoid FAB type AML. Tefinostat efficacy was demonstrated to correlate both with levels of cellular hCE-1 expression and with the induction of high levels of intracellular acetylation **(see Fig 1)**.[21]

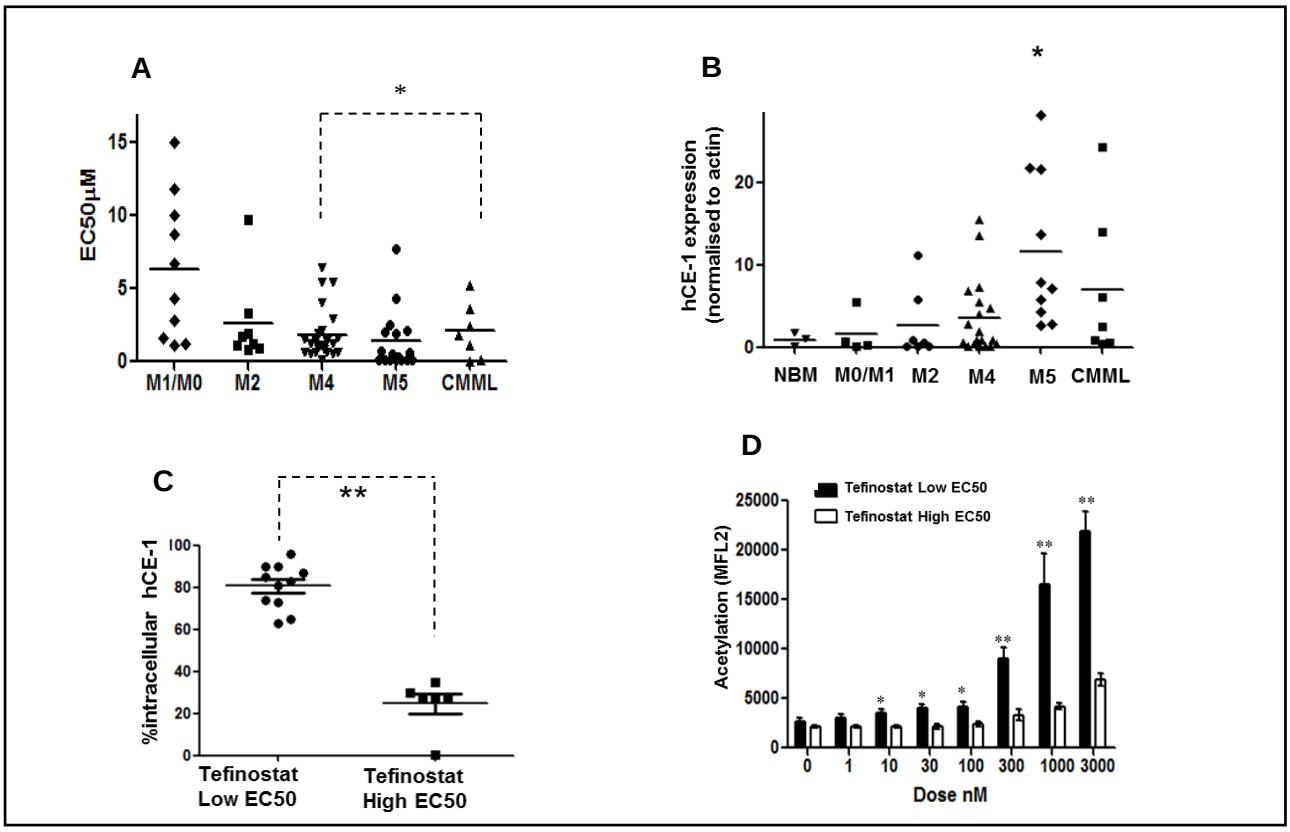


Fig 1: Selective *in vitro* efficacy of Tefinostat against primary leukaemia samples of monocytoid lineage. **(A)** MTS derived spread of EC50 values in a cohort of 66 primary AML and 7 primary CMML samples. (M4/M5/CMML vs M0/M1 *p=0.009 Spearman's correlation). **(B)** Comparison of levels of hCE-1 protein expression levels across FAB types. **(C)** Intracellular hCE-1 levels in EC50 high and low patients **P<0.001 Mann Whitney U. **(D)** Acetylation induction in Tefinostat sensitive (Black bars, n=8) compared to insensitive (high EC50) AMLs (White bars, n=5), *p<0.05, **p<0.005.

2.3.2 Tefinostat: Pre-clinical pharmacokinetic and toxicology data

Tefinostat is a poor inhibitor of the metabolism of cytochrome P450 model substrates in isolated human liver microsomes (IC_{50} >100µM), apart from the metabolism of nifedipine by CYP3A4 for which the IC_{50} is 2.67µM. The IC_{50} for inhibition of CYP3A4 using midazolam and testosterone as substrates is much weaker, with IC_{50}s of 75.3 and 41.1µM, respectively. CHR-2847, the major active metabolite of tefinostat, shows no inhibition (IC_{50} > 100µM) of any P450 isoforms tested, other than CYP3A4 metabolism of nifedipine (IC_{50} 55.6µM). Studies in human liver microsomes suggest that the major route of metabolism of tefinostat is hydrolysis to the acid CHR-2847 via a non-cytochrome-mediated pathway. There was no induction of cytochrome P450 enzymes following 28 days of dosing in the rat. Comparative metabolism in dog, rat, human and monkey hepatocytes showed a similar pattern of metabolites in all four species. The degree of human plasma protein binding of Tefinostat is approximately 99%.

The acute median lethal oral dose for male and female rats was estimated to be >2000mg/kg. During a 28-day oral toxicity study in the rat, animals treated at 300mg/kg/day were noted to have salivation and fur/coat staining in the peri-oral area. Reduced body weight gain associated with reduced food intake was noted for males treated at 100 and 300mg/kg/day. An increase in spleen weight at the end of the 28-day treatment period for animals dosed at 300mg/kg/day. The study identified a potential to induce a reversible mild regenerative anaemia at 300mg/kg/day. The no observable adverse effect level (NOAEL) for this study was considered to be 100mg/kg/day.

During a 28-day oral toxicity study in monkeys, males treated at 450mg/kg/day showed dehydration, whilst both males and females at this dose level showed a slight reduction in body weight, with recovery evident during the 28-day recovery period. As in the rat, there was evidence of a reversible, regenerative anaemia in all treated groups. Platelet count was increased for males and females treated at 150 and 450mg/kg/day and activated partial thromboplastin times were longer for animals treated at 450mg/kg/day. There was recovery from these changes following cessation of dosing. A decrease in lymphocytes in animals dosed at 450mg/kg/day was noted at the end of the treatment period. Although this was not statistically significant, it was still evident at the end of the 28-day recovery period. Clinical chemistry changes noted included increased total bilirubin (all dose levels), increased urea (females, all dose levels) increased aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase (450mg/kg/day), decreased cholesterol (150 and 450mg/kg/day) and increased creatinine (150 and 450mg/kg/day). Decreased thymus weight was apparent at the end of the treatment period for all dose levels and was still evident at the end of the 28-day recovery period for females. Moderate or marked thymic atrophy was noted at 450mg/kg/day with mild atrophy evident for individual animals dosed at 50 and 150mg/kg/day. This finding was still evident in females at a reduced severity on completion of the 28-day recovery period. Accumulation of haemosiderin (mild or moderate) in Kupffer cells was present in the liver of animals treated at 450mg/kg/day and females at 150mg/kg/day with minimal accumulation observed sporadically in the remaining treatment groups. The finding was still evident in females at a reduced severity on completion of the 28-day recovery period. Minimal or mild centrilobular hepatocyte hypertrophy or minimal or mild hepatocyte degeneration was evident for individual animals at 150 and 450mg/kg/day. These findings were not evident at the end of the 28-day recovery period. Mild periportal fatty vacuolation was observed at the end of the 28-day recovery period in one animal of each sex previously dosed at 450mg/kg/day. A NOAEL could not be defined for this study.

Tefinostat appears to be devoid of mutagenic potential in bacteria and whilst it has been shown to induce a higher than control incidence of chromosomal aberrations in cultured CHO cells this is only apparent at concentrations of 200 and 210µg/mL (in the presence of S9 mix), which are toxic to the test cells.

2.3.3 Tefinostat: Clinical experience to date

CHR-2845-001 study

In this first-in-man, '3+3' dose escalation study of once daily oral tefinostat (CHR-2845-001, EudraCT No. 2008-005241-27), 18 patients with refractory haematological malignancies were treated at continuous doses ranging from 20 to 640mg.[22] Tefinostat was well-tolerated and no dose-related toxicities (DLTs) occurred during the trial; dose escalation was halted at 640mg without identification of a maximum tolerated dose (MTD). Long term treatment was well-tolerated: 76 28-day treatment cycles were delivered in total; an average of 4.2 cycles per patient (range 1-15). The adverse event (AE) profile of tefinostat, where there was felt to be at least a *possible* relationship to the study drug, included nausea (7 patients), anorexia (5 patients), rash (4 patients), fatigue (4 patients), constipation (3 patients) and increased serum creatinine (2 patients). Nausea was seen in patients at all doses. Anorexia was seen in one patient at each of the 160mg and 640mg dose levels and in three patients at the 320mg dose level. Constipation (3 out of 5 patients) and increased serum creatinine (2 out of 5 patients) were only seen in patients treated at the 640mg dose level. Although no DLTs occurred during the trial, the tefinostat-related toxicities noted at 640mg (creatinine increase, constipation) indicated that plasma levels of tefinostat at that dose were high enough to begin to override monocyte-targeted activity leading to early signs of systemic toxicity.

Pharmacokinetic analyses in the CHR-2845-001 study confirmed that oral absorption of tefinostat was adequate and that metabolism to the active metabolite CHR-2847 was rapid. Mean plasma levels (Cmax and AUC0-4) of tefinostat and CHR-2847 were dose-proportional over the dose range studied although relatively high inter-individual variability was observed.

Pharmacodynamic analysis indicated that increases in protein acetylation (up to 8-fold) were restricted to monocytoid cells at doses between 40mg and 320mg, with non-specific acetylation effects in other cell lineages correlating with the non-specific toxicities seen at 640mg. Of 2 CMML patients treated in the study, one achieved a bone marrow complete response (CR) at relatively small doses of 20-80mg, with the other remaining stable for 1 cycle before withdrawing consent during cycle 2. A treatment-related decrease in peripheral and bone marrow blasts was noted in a further AML-M2 patient. The early signs of clinical efficacy in the absence of significant toxicity, with evidence of effective targeting of HDAC inhibitory activity to monocytes in the CHR-2845-001 trial indicated that effective HDACi activity may be achieved in the absence of the commonly observed systemic toxicities seen in clinical trials with other non-targeted HDACi and this provides a compelling rationale for the further evaluation of tefinostat in larger clinical studies in populations of patients with monocytoid malignancies.

In view of the inter-patient variability in pharmacokinetic data seen in the CHR-2845-001 trial which utilised the freebase formulation of tefinostat, a decision was taken to switch to the tefinostat tartrate salt formulation for future clinical studies. Tefinostat tartrate has improved dissolution and disintegration properties in comparison to the tefinostat freebase. A comparative monkey PK study showed equivalent tefinostat and CHR-2847 AUC with reduced variability between animals dosed with tefinostat tartrate capsules.

Phase I/II study in Hepatocellular carcinoma

A phase I/II study of tefinostat is currently underway for the treatment of cancer-related inflammation in patients with advanced hepatocellular carcinoma (HCC) (EudraCT No. 2012-000326-22, sponsor Queen Mary University of London). HCC patients currently have very limited treatment options: sorafenib, the only registered treatment, provides a limited survival benefit of 2-3 months. Activated liver macrophages (Kupffer cells) are intrinsically linked to the pathogenesis of HCC; macrophage infiltration is associated with HCC progression, disease recurrence and poor survival after hepatectomy, providing a rationale for monocyte/macrophage-directed HDAC inhibitory therapy in that setting. The HCC study is investigating both once and twice-daily dosing of the tefinostat tartrate formulation, delivered via 120mg capsules. In a phase I dose escalation, up to 5 cohorts of 3-6 patients will be treated (360mg, 480mg once daily, then 240, 360 and 480mg twice daily) to further determine safety and tolerability, prior to treatment of up 39 further patients in a phase II dose expansion. Emerging safety and clinical information, pharmacokinetic and pharmacodynamic data from the HCC study will be made available to the Monocle study investigators (and Data Monitoring Committee) and will potentially inform amendments to the Monocle study tefinostat dosing schedule, particularly with regard to the future application of twice daily dosing.

2.4 Rationale for phase 2 clinical study of Tefinostat in CMML

CMML remains an area of significant unmet therapeutic need; very few clinical trials have specifically addressed this relatively rare and highly heterogeneous disease entity. For the majority of CMML patients in whom allogeneic stem cell transplantation is not a viable strategy, no currently-available treatments are capable of altering the natural history of the disease. Tefinostat, by delivering monocyte-targeted effects, carries the potential to exploit the significant pre-clinical rationale for HDAC inhibition in myelodysplastic syndromes, while avoiding the systemic toxicities associated with this class of agents and, as such, could represent a first disease-specific treatment entity in CMML. Pre-clinical data emphasise the selective HDACi-mediated anti-proliferative and pro-apoptotic effects of tefinostat in monocytoid leukaemias, while existing phase I clinical data suggest good tolerability along with pharmacodynamic evidence of monocyte-restricted protein acetylation effects at achievable doses with encouraging early signs of clinical efficacy, including one bone marrow complete response from two CMML patients treated to date.

A single-arm phase 2 study of tefinostat, to evaluate clinical efficacy in CMML while providing additional safety and tolerability data, is a logical next step in the development of this agent. Forty patients will be treated utilising a two-stage design including a midway stopping rule. Based on recruitment experiences in the recent UK-based CMML-201 trial [17] which used broadly similar eligibility criteria to those used here, it is anticipated that a majority of non-transplant-eligible, symptomatic CMML patients living within the catchment areas of 10-20 UK study centres will enter the trial. Correlative biological studies exploring biomarkers of clinical efficacy in individual patients including baseline hCE-1 expression levels and molecular profiling via targeted gene sequencing will potentially allow stratification of sub-groups of CMML patients most likely to derive future clinical benefit from this approach. If clinical efficacy, along with acceptable safety, of tefinostat is demonstrated by the Monocle study, it will be desirable for tefinostat to be taken forward into conventionally-powered randomised controlled trial assessment to further evaluate clinical activity. Given the relative rarity of CMML, such a larger study would likely require larger numbers of UK centres and/or international collaboration.

3 OBJECTIVES

The MONOCLE study is available to patients with newly-diagnosed or previously-treated CMML as defined by the WHO Classification 2008 (Appendix 1). The trial is open to patients with CMML-2 and to sub-groups of CMML-1 patients with symptomatic bone marrow failure or myeloproliferation who require treatment based on blood cytopenias / leucocytosis, disease symptoms or higher-risk prognostic score (CPSS) (refer to section 5 for full eligibility criteria).

3.1 Therapeutic questions to be addressed

The main research questions to be addressed are:

- Is tefinostat (CHR-2845) safe and tolerable for patients with CMML?
- Is tefinostat clinically effective in CMML?
- Do clinical responses to tefinostat correlate with baseline biological measures of disease including hCE-1 expression level and molecular mutational profile, and with differential effects of tefinostat on protein acetylation between monocytes and other haematological cell lineages?

3.2 Endpoints

3.2.1 Primary Endpoints

- Safety and tolerability of tefinostat
- Overall clinical response rate (according to International Consortium MDS/MPN Response Criteria)

3.2.2 Secondary Endpoints

- Incidence and duration of CR / PR / clinical benefit (according to International Consortium MDS/MPN Response Criteria)
- Overall clinical response rate (according to Wattel and modified IWG Criteria)
- Achievement of red blood cell and platelet transfusion independence
- Overall survival
- Progression-free survival
- Incidence of transformation of CMML to AML (and time to AML transformation)
- Duration of tefinostat therapy
- Quality of life
- Biological correlates including hCE-1 expression, changes in protein acetylation

For formal definitions of study endpoints refer to section 10.4.

3.3 Biological Correlates

Included within the MONOCLE study is a programme of translational laboratory work / correlative science that seeks to explore potential biomarkers of clinical efficacy in individual patients and to better-define the biology of clinical responses to tefinostat therapy.

Samples of blood and bone marrow for central analysis and biological studies will be obtained at trial registration, and upon completion of treatment cycles 3 and 6 (see section 8). hCE-1 expression will be quantified in baseline blood and bone marrow samples via flow cytometric analysis (Marie Gilmour, Cardiff University). To determine the genetic landscape of the study population, targeted gene sequencing of 26 commonly-mutated genes in myeloid malignancies will be performed on DNA extracted at baseline (Catherine Cargo, HMDS, Leeds).The sequencing panel will include *TET2, DNMT3A, IDH1/2, ASXL1, EXH2, SRSF2, SF3B21, U2AF1, ZRSR2, CSF3R, SETBP1, CBL, NRAS, KRAS, FLT3, KIT, JAK2, CALR, MPL, NPM1, BCOR, RUNX1, TP53, WT1 and STAG2.* Acetylomic analysis using histone peptide array mass spectrometry will be used to determine changes at specific histone peptide sites in response to tefinostat therapy (Anthony Whetton, Stem Cell and Leukaemia Proteomics Laboratory, University of Manchester). To further dissect the non-genomic molecular heterogeneity underlying patient responses, serial plasma samples will be profiled using the Luminex multiplex platform; inflammatory cytokine levels will be quantified before and after treatment to explore whether clinical efficacy is associated with changes in cytokine profile (Joanna Zabkiewicz, Cardiff University).

Additionally, serial analysis of the differential effects on protein acetylation between peripheral blood monocytes, lymphocytes and granulocytes will be performed at six timepoints during cycle 1 of tefinostat therapy (day 1 pre, 1hr, 2hr and 4hr-post dose, day 15 pre-dose and day 29 pre-dose – see Section 8) and correlated both with pharmacokinetic measurements and clinical responses (Marie Gilmour, Cardiff University).

4 TRIAL DESIGN

4.1 Type / design of trial

MONOCLE is a single-arm, non-randomised, open-label phase 2 study to establish the safety and efficacy of oral tefinostat therapy in CMML. Given that prior clinical experience of tefinostat in CMML is limited to only 2 patients who received the drug within a previous first in man phase I study of 18 patients with advanced haematological neoplasms [22] a randomised study is not yet felt to be justified. The MONOCLE trial follows a simple Bryant and Day two-stage design [26] which is based on Simon's two-stage design but incorporates toxicity as well as clinical efficacy (futility) considerations into sample size and early stopping rules in order to avoid unnecessary exposure of patients in the event of excessive toxicity or in the absence of evidence of clinical efficacy.

4.2 Study population and recruitment

Up to 40 patients will be recruited at 10-20 study centres within the United Kingdom. The recent CMML-201 trial, with a similar single-arm phase 2 design and very similar eligibility criteria to the MONOCLE study, recruited 32 CMML patients at 13 UK centres over a period of just over 6 months.[17] A similar number of UK sites, including sites that previously collaborated in the CMML-201 trial and several additional Leukaemia Lymphoma Research Trials Acceleration Programme centres, will participate in the MONOCLE study. Recruitment will be competitive with no fixed allocation of patient places per study site. Accrual of the planned 40 patients is predicted to take 12 months. The patient population to be studied requires continuous clinical care; consequently a low rate of non-compliance and loss to follow-up is anticipated.

4.3 Study treatment and follow-up

All MONOCLE patients will receive oral tefinostat on a continuous basis, treatment cycles being arbitrarily defined as 28 days (4 weeks) in length for the purposes of scheduling study procedures and response evaluations. Treatment will continue for 6 cycles (24 weeks) unless there is evidence of disease progression, the patient experiences unacceptable toxicity or the patient asks to be withdrawn. If, following 6 cycles, the patient is considered to be benefitting from tefinostat therapy (for definition see section 8.3) and the benefit/risk balance is considered acceptable, they may continue to receive tefinostat until the occurrence of disease progression or unacceptable toxicity.

Clinical response to tefinostat will be assessed primarily according to International Consortium MDS/MPN Response Criteria [28] based on peripheral blood assessment (full blood count and blood film / differential performed fortnightly during the first 6 cycles of therapy and every 4 weeks if therapy continues beyond 6 cycles) and bone marrow assessments performed following completion of 3 and 6 cycles of therapy. Bone marrow

assessment may also be repeated at the investigator's discretion in the event of suspected disease progression or premature cessation of trial therapy. Red cell and platelet transfusion requirements will be recorded at follow-up visits.

Patients will be followed up for a minimum of 18 months (to assess overall survival and progression free survival) following registration, or for 30 days after the last trial treatment if treatment exceeds 18 months. After the initial 24-week treatment period, clinicians will be asked to complete 6-monthly follow-up forms to give patient status in line with proposed DMEC meeting frequency.

4.4 Centre for Trials Research Monitoring

It is expected that site initiation training will be conducted via WebEx – copies of trial training material will also be provided to sites, to ensure ongoing training needs are met. Staff at sites will be required to sign off that they attended and completed the site initiation training.

Monitoring of sites will be conducted as per the trial monitoring plan and as defined by the Sponsor. Central monitoring processes will be in place, and participating sites will be informed of the requirements for any site monitoring during trial training and throughout the trial, as necessary. If significant problems are identified then a monitoring visit to site will be triggered. Further monitoring visits may be undertaken if requested by Sponsor, Chief Investigator, etc.

At the point of trial closure, participating sites will be provided with details of documentation held by the Sponsor including list of patients registered, SAEs, drug shipment details, etc. Any outstanding queries should be resolved prior to local trial closure. CTR will require confirmation that an investigator's site file is up to date, prior to authorising the archiving of trial documentation and data.

5 STUDY ELIGIBILITY

Patients ≥18 years of age with CMML will be screened for trial suitability and must meet the eligibility criteria below. No deviations from these criteria will be permitted.

5.1 Inclusion Criteria

Patients will be eligible for the MONOCLE study if **all** of the following apply:

1. Newly-diagnosed or previously-treated CMML meeting WHO criteria (Appendix 1) with the following characteristics:

   All CMML-2 patients are eligible.

   For patients classified as CMML-1, the following must be present:

   · Symptomatic bone marrow failure or myeloproliferation defined as ***one or more of***:

   red cell transfusion dependence with pre-transfusion Hb <90g/l

   symptomatic anaemia (Hb <115g/l)

   thrombocytopenia (platelets <50 x 10^9/l)

   symptomatic bleeding due to platelet function defect or DIC/fibrinolysis

   white blood cell count >50 x 10^9/l

   and/or

   · CMML-specific Prognostic Score (CPSS) of intermediate-2 or high risk [(11)] (see Appendix 2 for derivation of CPSS score)

   and/or

   · Systemic symptoms including weight loss with no alternative explanation (10% of baseline weight within previous 6 months)

   · Symptomatic splenomegaly

   · Symptomatic extramedullary involvement, e.g. skin infiltration, serous effusions

2. Subject is able and willing to sign the Informed Consent Form
3. Age greater than or equal to 18 years at the time of signing the Informed Consent Form
4. Willingness to undergo scheduled assessments as per the study protocol including stipulated bone marrow assessments
5. ECOG performance status of 0-2 at study entry (see Appendix 6)
6. Women of childbearing potential must have a negative urine pregnancy test within 7 days prior to starting study drug

7. Women of childbearing potential must use at least two effective contraceptive methods throughout the study and for three months following the date of the last dose of study drug (see Appendix 7)
8. Men whose partner is a woman of childbearing potential must use at least two effective contraceptive methods (see Appendix 7)

5.2 Exclusion Criteria

Patients will not be eligible for the MONOCLE study if ***any*** of the following apply:

1. CMML with eosinophilia and 5q33 abnormality
2. CMML patients who are considered suitable for allogenic stem cell or bone marrow transplantation
3. Previous chemotherapy for CMML except Hydroxycarbamide and 5-azacitidine
4. Creatinine concentration > 2x the upper limit of institutional normal range
5. Liver transaminases (AST / ALT) > 3x the upper limit of institutional normal range or serum bilirubin > 4x the upper limit of institutional normal range
6. Pregnant or lactating females
7. Use of experimental drug or therapy within 28 days of registration
8. Other malignancy within the last 3 years other than: curatively-treated basal cell or squamous cell skin cancer, carcinoma in situ of the cervix, organ-confined or treated non-metastatic prostate cancer with negative prostate-specific antigen, in situ breast carcinoma after complete surgical resection, or superficial transitional cell bladder carcinoma. *Cases of CMML seen in association with systemic mastocytosis (systemic mastocytosis with associated clonal haematological non-mast cell lineage disease) should be discussed with the Chief Investigator and **may** be included if the CMML is considered to be the clinically predominant disease.*
9. Known seropositivity for HIV infection or infectious hepatitis (type B or C)
10. Uncontrolled inter-current illness including, but not limited to, ongoing infection, psychiatric illness or social situation that the treating physician judges would limit compliance with study requirements

All queries relating to eligibility criteria should be directed to the Chief Investigator via Centre for Trials Research , Cardiff University (see contact details at front of protocol)

6 RECRUITMENT AND REGISTRATION

6.1 Centre Registration

Patients will be recruited from multiple research institutions around the United Kingdom. In order to be registered as a trial centre, an individual at each participating institution is required to act as the local Principal Investigator at that site. The following must be completed and returned to the Sponsor before the centre opens to recruitment:

- R&D approval
- Site added to Clinical Trials Authorisation
- Signed Site Agreement (including signed PI statement)
- Delegation log

For administrative purposes, investigators will also be asked to supply:

- Details of location and point of contact of their cytogenetic, immunophenotyping and pharmacy services
- E-mail addresses, CVs and evidence of GCP training for all personnel at site that will conduct trial procedures
- Relevant institutional normal laboratory normal ranges (including bilirubin, ALT, AST, creatinine)

6.2 Patient recruitment and informed consent process

Patients may only be recruited once a trial centre is fully registered. The assessment of eligibility and the informed consent process will be undertaken by authorised members of staff at research centres who are qualified by training and/or experience in taking informed consent to GCP standards. Informed, written consent for entry into the trial must be obtained prior to registration.

Patients will be approached during standard clinic visits for management of their disease and will be provided with verbal and written details about the trial (**Patient Information Sheet and Consent Form 1**). This document includes detailed information about the rationale, design and personal implications of the trial. Provision of information regarding the trial is permitted by any member of the patient's care team approved to do so by the Principal Investigator, although the Principal Investigator should be informed of any patients approached to participate by any other member of the patient's care team. Following information provision, patients will have as long as necessary to consider participation (normally a minimum of 24 hours) and will be given the opportunity to discuss the study with family and other healthcare professionals before confirming whether they are willing to take part in the study.

Assenting patients will then be formally assessed for eligibility and invited to provide informed, written consent. The Principal Investigator or other clinically qualified member of the trial team who has received GCP training and has been approved by the Principal Investigator as detailed on the delegation log is permitted to take informed consent. The right of the patient to refuse consent without giving reasons will be respected. Furthermore, the patient will be free to withdraw from the study at any time without giving reasons and without prejudicing further treatment.

A record of the consent process detailing the date of consent and all those present will be kept in the patient's notes. The original consent form will be filed in the Investigator Site File, a copy will be filed in the patient's notes and a copy of the form will be given to the patient.

6.3 Registration process

Following confirmation of eligibility and written, informed consent patients will be registered. Registration will be performed by the trials team. The research site should notify the trials team of any prospective patients, to ensure that an authorised member of staff will be in the office to register the patient. The research site should scan and email, or fax through the **Patient Eligibility Checklist / Registration Form (Form A)**. The trials team will then register the patient onto the database and send a Registration Email to all the relevant personnel at site. Once the database becomes outward facing, patient registration will still be performed by the trials team, but staff at the research site will be able to enter all of the other data on the database. :

After trial registration the research site will:

- provide each patient with a trial ID card and advise them to carry it at all times and present it to medical staff should they be admitted to hospital during their time on trial
- notify the patient's GP of their participation in the trial

6.4 Non-registration

Each research centre will complete a non-registration/screening log of all patients who are approached about the study but are not registered either because they fail to meet trial eligibility criteria or decline participation in the study. Anonymised information will be collected including:

- Age
- Gender

- Reason for ineligibility or declining participation

Non-registration logs will be requested by CTR on a quarterly basis.

7 TREATMENT DETAILS

7.1 Definition of the Investigational Medical Product

Within the Monocle trial, tefinostat is classified as the Investigational Medicinal Product (IMP). Tefinostat will be provided as gelatine capsules containing tefinostat.tartrate equivalent to 120mg tefinostat. Investigators should refer to the Investigator's Brochure (IB) for information regarding the physical and chemical properties of tefinostat and of tefinostat.tartrate capsules.

7.2 Tefinostat manufacture and supply

Tefinostat will be supplied free of charge by CRT Pioneer Fund LP acting through its General Partner CRT Pioneer GP Limited (CPF) and distributed directly to participating sites' NHS pharmacies. All supplies will be provided with a Monocle trial-specific label by Catalent Pharma Solutions. Manufacture of the tefinostat drug substance (API) will be performed by Aptuit (Oxford) Ltd. and the subsequent drug product manufacture, including encapsulation, will be performed by Patheon UK Ltd. Drug product assembly, labelling and Qualified Person (QP) Certification will be performed by Catalent Pharma Solutions.

A 'seeder' supply of tefinostat capsules sufficient to treat a single patient for 4 weeks (one course) will be supplied to each participating site after CTR have confirmed that all necessary approvals are in place. Subsequent supplies must be ordered by sites via CTR using the drug order form. CTR will then order further supply direct from Catalent Pharma Solutions to send to sites. Within the study, tefinostat will be dispensed to individual patients at 4-weekly intervals.

7.3 Drug storage and accountability

Tefinostat should be stored at between 2-8^{o}C in a secure area to which access is limited to authorised site staff. The principal investigator (or delegated individual in pharmacy) must ensure that the study drug is stored and dispensed in accordance with local standard operating procedures and applicable regulatory requirements and that, for each patient, accurate records are maintained of the quantities of tefinostat dispensed and used. All unfinished bottles of tefinostat will be returned to the trial pharmacist who will count and document any unused medication which may then be destroyed in accordance with local pharmacy practice. At the conclusion of the trial, comparison will be made between figures for the overall amount of tefinostat shipped to each site's pharmacy and local records of the amounts of tefinostat dispensed and destroyed with account being given of any discrepancy.

At home patients should store tefinostat in the refrigerator (between 2-8°C).

7.4 Tefinostat dosing and treatment schedule

Tefinostat will be administered orally once daily on a continuous basis. **Patients will commence tefinostat at a once daily dose of 360mg (i.e. 3 x 120mg tefinostat capsules)**. Treatment cycles will be defined as 28 days (4 weeks) in length for the purposes of scheduling study procedures and response evaluations. The dose of tefinostat may be escalated or de-escalated at the investigator's discretion for reasons of safety and clinical efficacy (see section 7.6).

Tefinostat capsules should be ingested with a sufficient quantity of water, preferably following a meal. In the event of a delayed dose patients may take the dose up to 6 hours after the scheduled dose time. If a dose is more than 6 hours late, the dose should be missed and the patient should continue with the next dose at the scheduled time. If a patient vomits following a dose, the dose should not be replaced.

Cycles of tefinostat therapy will be repeated every 28 days for at least 6 cycles unless the patient withdraws their consent for the study, disease progression or loss of response occurs (see Appendix 3) or therapy is stopped on grounds of tolerability.

Following completion of the sixth cycle of treatment patients will undergo a formal response assessment (see section 8.3). Responding patients, defined as: clinical benefit or better by International Consortium MDS/MPN Response Criteria (Appendix 3) may continue taking tefinostat until loss of response, disease progression or the development of unacceptable toxicity. Immediately upon completion of the sixth cycle a short extension of tefinostat therapy (maximum 14 days) will be permitted in order to allow adequate time for sites to fully assess week 24 response assessment investigations.

7.5 Patients with uncontrolled myeloproliferation

For patients who have uncontrolled myeloproliferation, the concomitant use of hydroxycarbamide is permitted. Patients who are already receiving hydroxycarbamide therapy at the time of trial entry will be allowed to continue hydroxycarbamide at the time of initiation of tefinostat treatment. The introduction of hydroxycarbamide is also permitted, at the discretion of local investigators, for WBC persistently ≥30 $x10^9$/l. Investigators should make every effort, according to individual patient circumstances, to wean and stop hydroxycarbamide as patients continue on tefinostat therapy. The concomitant use of hydroxycarbamide will not be permitted following completion of the third cycle of tefinostat (week 12) and patients with uncontrolled myeloproliferation beyond this time point will be withdrawn from the study.

7.6 Tefinostat dose modifications

Tefinostat dose adjustments (both escalations and reductions) will be made for purposes of safety and clinical efficacy. All dose changes should be recorded in the CRF. Investigators should maintain a low threshold for discussing planned dose changes with the chief investigator or trial management team.

7.6.1 Dose reductions for toxicities related to tefinostat

Toxicities will be graded using the NCI CTCAE version 4 (Appendix 8).

No modification in tefinostat dose is required for grade 1-2 toxicities.

In the event of grade 3 or 4 toxicity considered possibly, probably or definitely related to tefinostat, the study drug should be temporarily discontinued and the next treatment cycle delayed until the toxicity has resolved to grade 1 or less. Thereafter, the drug should be recommenced at a dose reduced by one dose level (120mg/day); patients previously taking 360mg should be reduced to 240mg, patients taking 240mg should be reduced to 120mg. The lowest permitted tefinostat dose is 120mg daily. Attempts to re-escalate the tefinostat dose following resolution of toxicity will be at the discretion of the investigator, but should not be made until the patient has completed a full 28-day cycle at the reduced dose without additional grade 3-4 toxicity; if the same AE occurs a second time further dose escalations will not be permitted.

If a grade 3 or 4 toxicity considered related to tefinostat does not resolve to grade 1 or less within 28 days, the patient will be removed from the study but should continue to undergo follow-up evaluations (please refer to section 8.5 for patient evaluations required in these circumstances).

7.6.2 Dose escalations for clinical efficacy

The dose of tefinostat may be increased by one dose level (120mg/day) up to a maximum daily permitted dose of 480mg for patients who have not achieved a maximal clinical response (see Appendix 3). Dose increases may be made at the start of a new 4-week cycle of tefinostat therapy in the absence of significant ongoing toxicity. The earliest permitted time for dose increase will be the beginning of treatment cycle 2 (week 5). The dose of tefinostat should not be increased to a higher level if the patient previously required a safety-related dose reduction.

7.7 Concomitant medication and therapy

7.7.1 Supportive care

Concomitant medications and therapies deemed necessary for the supportive care and safety of the patient should be given at the clinician's discretion as per local practice. Local supportive care includes transfusions of blood products and administration of antibiotics, analgesics, anti-pyretics and anti-emetics as necessary. G-CSF is not routinely recommended but may be administered at the investigator's discretion during the treatment of neutropenic sepsis. The use of rEPO is not recommended.

7.7.2 Other investigational agents or anti-cancer treatment

The use of other anti-cancer or investigational agents is not permitted within the trial. The concomitant use of hydroxycarbamide is permitted in patients with 'uncontrolled myeloproliferation' during the first three cycles of tefinostat therapy (see section 7.5).

7.7.3 Hepatotoxic and nephrotoxic medications

The metabolism of tefinostat is not known to be affected by CYP3A4 inducers and inhibitors. The principal route of elimination of tefinostat is hepatic metabolism. Increased creatinine has been observed at higher tefinostat doses (640mg daily). Caution is advised with potentially hepatotoxic or nephrotoxic medications; an increased frequency of monitoring is initially recommended in these patients.

8 SCHEDULE OF STUDY ASSESSMENTS

	Screening	Cycle 1			Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6			Treatment can be continued as long as response is maintained - maximum follow-up interval 4 weeks	Loss of response / Disease progression / Intolerability
Week		**1**	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**	**21**	**23**	**25**		
Day	**Day -28 to 0**	**1**	**2**	**15**	**29**	**43**	**57**	**71**	**85**	**99**	**113**	**127**	**141**	**155**	**169**		
Informed consent	✓																
Screening - eligibility criteria	✓																
Medical and CMML history	✓																
Demographic data	✓																
Concomitant medications	✓	✓			✓		✓		✓		✓		✓		✓	✓	✓
Transfusion history	✓	✓			✓		✓		✓		✓		✓		✓	✓	✓
Physical Examination	✓	✓			✓		✓		✓		✓		✓		✓	✓	✓
Vital Signs, Weight	✓	✓			✓		✓		✓		✓		✓		✓	✓	✓
Performance score (ECOG)	✓														✓		✓
Haematology	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Biochemistry	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Coagulation screen	✓																
Urinalysis	✓	✓		✓	✓		✓		✓		✓		✓		✓		✓
Blood and bone marrow for central correlative studies	✓								✓						✓		
Pregnancy test (1)	✓	✓			✓		✓		✓		✓		✓		✓	✓	
ECG	✓	✓			✓		✓		✓		✓		✓			✓	
Bone marrow assessment (2)	✓								✓						✓		✓
QoL questionnaires	✓								✓						✓		
Dispensation of study medication		✓			✓		✓		✓		✓		✓		✓	✓	
Blood samples for acetylation studies (3)		✓	✓	✓	✓												
Blood samples for pharmacokinetic studies (4)		✓	✓	✓	✓												
Adverse event assessment		Monitor during treatment and for 30 days after last dose of tefinostat															
Progression / AML transformation	Monitor from day of registration to end of trial																

(1) Pregnancy test (urine) should be performed within 1 week of each study cycle in women of childbearing potential
(2) Bone marrow examination should be performed at baseline, after 3 and 6 cycles of treatment and, for patients continuing beyond 6 cycles of tefinostat, at the time of stopping drug due to loss of response / disease progression or toxicity. At each of these time-points blood and bone marrow samples will be shipped for central biological correlative studies
(3) Blood for acetylation studies will be taken pre-dose on day 1, 1hrs, 2hrs and 4hrs post dose on day 1, pre-dose on day 2, pre-dose on day 15 and pre-dose on day 29 (day 1 of cycle 2)
(4) Blood for pharmacokinetic studies will be taken pre-dose on day 1, 1 hrs, 2hrs and 4hrs post dose on day 1 and pre-dose on day 2 , pre-dose on day 15 and pre-dose on day 29 (day 1 of cycle 2)

The procedures to be conducted at trial visits for each subject enrolled in the study are presented in tabular form in Section 8 above and described in the text that follows.

8.1 Baseline assessments (day -28 to 0)

The following screening and baseline assessments should be performed within the period of 28 days prior to commencing the first cycle of tefinostat therapy (days -28 to 0). Some of these assessments may have been conducted as routine clinical practice prior to the patient's entry into the trial.

The following will be recorded / obtained for all patients:

8.1.1 Medical history and demographics

- Demographic details - age, sex
- Past medical history to include history and treatment of past and concomitant diseases
- CMML history to include date of diagnosis, CMML subtype (at diagnosis and current), cytogenetic status at diagnosis, prior treatments received
- Transfusion history: red cell and platelet transfusions in the 8 weeks prior to study entry
- Concomitant medications
- ECOG performance status (Appendix 6)

8.1.2 Physical examination

- Full physical examination with particular attention to: liver and spleen size by palpation, lymph node enlargement, skin infiltration, gingival hypertrophy. (Spleen and liver measurement should be recorded in centimetres palpable below the costal margin in the mid-clavicular line, or assessed by ultrasound).
- Vital signs: pulse, blood pressure, temperature, respiratory rate, pulse oximetry
- Height and weight

8.1.3 ECG

- 12-lead electrocardiogram including assessment of QT_c interval

8.1.4 Pregnancy test and contraception

- A urine pregnancy test should be performed for all women of childbearing potential **within 7 days prior to starting tefinostat therapy**
- Female trial subjects of childbearing potential must have a negative pregnancy test and must agree to use two reliable forms of contraception during the trial and for at least 3 months after treatment has finished (Appendix 7)
- If female trial subjects are found to be pregnant this must be reported to CTR. Further information on this is in section 11.7.

- Male trial subjects must agree to use a reliable form of contraception during the trial and for at least 3 months after treatment has finished (Appendix 7)
- If a partner of male trial subject is found to be pregnant during their treatment, or within 3 months of the end of their treatment, this must be reported to CTR. Further information on this is in section 11.7

8.1.5 Haematology assessments

- Full blood count to include haemoglobin, differential white cell count (including monocyte count and blast %), platelet count, MCV
- Reticulocyte count
- Coagulation screen to include APTT, PT, fibrinogen
- Bone marrow aspiration and trephine biopsy. Analysis of bone marrow aspirate to include morphological assessment (with differential count), local immunophenotypic (to include blast and typical / atypical monocytoid populations) and cytogenetic assessments. Local assessment of trephine histology.

8.1.6 Biochemistry assessment

- Sodium, potassium, urea, creatinine (and eGFR), magnesium, calcium, phosphate, glucose, albumin, total protein, bilirubin, ALT/AST, alkaline phosphatase, LDH, uric acid

8.1.7 Urinalysis

- Using standard dipstick assessment (pH, protein, glucose, blood, ketones and leucocytes). This should be supplemented by formal quantification of any potentially-relevant abnormalities (eg. 24hr collection to quantify proteinuria).

8.1.8 Samples for central assessment and biological studies

- Separate samples of blood and bone marrow should be obtained at the time of the baseline assessment for correlative biological and biomarker studies including determination of molecular genetic profile, protein acetylation profile and hCE-1 expression level
- **4ml bone marrow and 20ml peripheral blood** should be placed in EDTA tubes clearly labelled with trial number, patient initials and date of birth
- Investigators will be supplied with a collection kit which should be sent to the reference laboratory at the address below using the enhanced Royal Mail delivery service and the account reference number provide with the collection kit:

Dr Marie Gilmour
Department of Haematology
School of Medicine
Cardiff University
Heath Park
Cardiff CF14 4XN
Tel: 02920 744524
Email: GilmourM@cardiff.ac.uk

- It is also intended to store excess patient material for future research. It is mandatory to obtain the patient's specific consent to do so. **For this purpose Patient Information Sheet 2 and Consent Form 2 should be used.** Copies of Consent Form 2 should be stored in the study file on site, in the patient's notes and a copy given to the patient. If a patient has ***not*** consented to storage of excess material, the local site team should inform the CTR staff who will arrange to have any remaining material destroyed.

8.1.9 Quality of life assessments

- Patients should complete the EuroQol EQ5D-5L and MPN-SAF questionnaires (see Appendix 9 and 10). Completed questionnaires should be returned to CTR using the pre-paid envelopes provided, or by scan and email.

8.2 Treatment assessments during tefinostat cycles 1-6

Each patient will receive a minimum of six 28-day cycles of tefinostat treatment, subject to tolerability. Cycles will run continuously. During cycles 1-6 (weeks 1-24), patients will be assessed fortnightly - on day 1 and on day 15 (+/- 2 days) of each cycle. The following will be assessed:

8.2.1 Clinical assessment (each cycle)

- Adverse events (day 1 and 15)
- Concomitant medications (day 1 only)
- Transfusion history – at day 1 of cycle 1 this should include any transfusions administered between screening and the start of cycle 1, for other cycles it should include any transfusions over the preceding 28 days.
- Physical examination including liver and spleen size by palpation, lymph nodes, skin infiltration, gingival hypertrophy(day 1 only)
- Vital signs and weight (day 1 only)

8.2.2 Laboratory assessments (each cycle)

- Full blood count to include haemoglobin, differential white cell count (including monocyte count and blast %), platelet count, MCV (days 1 and 15)Biochemistry assessment: sodium, potassium, urea, creatinine (and eGFR), magnesium, calcium, phosphate, glucose, albumin, total protein, bilirubin, ALT/AST, alkaline phosphatase, LDH, uric acid (days 1 and 15)

8.2.3 Pregnancy test (each cycle)

- For all women of childbearing potential a negative urine pregnancy test result should be confirmed within 7 days prior to the start each cycle of tefinostat therapy

8.2.4 Urinalysis

- Performed on days 1 and 15 (cycle 1 only), and day 1 (cycles 2-6). Using standard dipstick assessment (pH, protein, glucose, blood, ketones and leucocytes). This should be supplemented by formal quantification of any potentially-relevant abnormalities (eg. 24hr collection to quantify proteinuria).

8.2.5 Pharmacokinetic studies (cycles 1 and 2 only)

- Pharmacokinetic (PK) sampling will be performed over the initial period of tefinostat therapy.
- **2ml blood** samples should be placed in Na_2EDTA/NaF vacutainers (grey top) at the following time points:

  Cycle 1 Day 1 – pre-dose

  Cycle 1 Day 1 – 1 hour post-dose

  Cycle 1 Day 1 – 2 hours post dose

  Cycle 1 Day 1 – 4 hours post dose

  Cycle 1 Day 2 – pre-dose (24 hours post first dose)

  Cycle 1 Day 15 – pre-dose

  Cycle 2 Day 1 – pre-dose

- At each time point, the sample will be centrifuged at 1500g for 10 minutes at 4°C in a refrigerated centrifuge. Following centrifugation, the plasma should be divided into 2 approximately equal amounts, placed into 2ml cryovials and frozen as quickly as possible to below -65°C for storage until shipment. Please refer to the Laboratory Manual for precise details of processing and shipment of PK samples.

8.2.6 Acetylation studies (cycles 1 and 2 only)

- Studies of levels of histone acetylation in different haematological cell sub-populations will be performed over the initial period of tefinostat therapy
- **8ml blood** samples should be placed in EDTA at the following time points:

  Cycle 1 Day 1 – pre-dose

  Cycle 1 Day 1 – 1 hour post-dose

  Cycle 1 Day 1 – 2 hours post-dose

  Cycle 1 Day 1 – 4 hours post-dose

  Cycle 1 Day 2 – pre-dose

  Cycle 1 Day 15 – pre-dose

  Cycle 2 Day 1 – pre-dose

- Please refer to the Laboratory Manual for details of processing and shipment of histone acetylation samples

8.2.7 ECG (each cycle)

- 12-lead electrocardiogram including assessment of QT_c interval (day 1 only, pre-dose)

8.2.8 Bone marrow assessment (cycle 4 only)

- Bone marrow aspirate to be repeated on completion of 3 cycles of study treatment. This sample should be taken on (or within 7 days prior to) day 1 of cycle 4. Analysis to include morphological assessment (with differential count) and local immunophenotypic assessment (including blast and typical / atypical monocytoid populations).
- A bone marrow trephine biopsy is only necessary at this time point in patients for whom the baseline bone marrow aspirate sample was non-diagnostic, or in the event of an aparticulate aspirate at this time point.

8.2.9 Samples for central assessment and biological studies (cycle 4 only)

- Separate samples of blood and bone marrow should be obtained at the time of the pre-cycle 4 response assessment for central correlative biological and biomarker studies
- 4ml bone marrow and 20ml peripheral blood should be placed in EDTA tubes clearly labelled with trial number, patient initials and date of birth
- Investigators will be supplied with a collection kit which should be sent to the reference laboratory at the address listed in section 8.1.7 using the enhanced Royal Mail delivery service and the account reference number provide with the collection kit.

8.2.10 Quality of life assessment (cycle 4 only)

- Quality of life assessment will be repeated on day 1 of tefinostat cycle 4 (+/- 7 days)
- Patients should complete the EuroQol EQ5D-5L and MPN-SAF questionnaires (see Appendix 9 and 10). Completed questionnaires should be returned to CTR using the pre-paid envelopes provided.

8.3 Treatment efficacy assessment (end of cycle 6)

Patients will undergo a formal efficacy response assessment on day 29 of tefinostat cycle 6 (+/- 2 days)

8.3.1 Clinical assessment

- Adverse events
- Concomitant medications
- Transfusion history
- Physical examination including liver and spleen size by palpation, lymph nodes, skin infiltration, gingival hypertrophy
- Vital signs and weight
- ECOG performance status (Appendix 6)

8.3.2 Laboratory assessments

- Full blood count to include haemoglobin, differential white cell count (including monocyte count and blast %), platelet count, MCVBiochemistry assessment: sodium, potassium, urea, creatinine (and eGFR), magnesium, calcium, phosphate, glucose, albumin, total protein, bilirubin, ALT/AST, alkaline phosphatase, LDH, uric acid

8.3.3 Urinalysis

- Using standard dipstick assessment (pH, protein, glucose, blood, ketones and leucocytes). This should be supplemented by formal quantification of any potentially-relevant abnormalities (eg. 24hr collection to quantify proteinuria).

8.3.4 Bone marrow assessment

- Bone marrow aspiration and trephine biopsy. Analysis of bone marrow aspirate to include morphological assessment (with differential count), local immunophenotypic (to include blast and typical / atypical monocytoid populations) and cytogenetic assessments. Local assessment of trephine histology.

8.3.5 Samples for central assessment and biological studies

- Separate samples of blood and bone marrow should be obtained at the time of the end of cycle 6 response assessment for central correlative biological and biomarker studies
- 4ml bone marrow and 20ml peripheral blood should be placed in EDTA tubes clearly labelled with trial number, patient initials and date of birth
- Investigators will be supplied with a collection kit which should be sent to the reference laboratory at the address listed in section 8.1.7 using the enhanced Royal Mail delivery service and the account reference number provide with the collection kit.

8.3.6 Quality of life questionnaire

- Quality of life assessment will be repeated on day 29 of tefinostat cycle 6 (+/- 7 days)
- Patients should complete the EuroQol EQ5D-5L and MPN-SAF questionnaires (see Appendix 9 and 10).
- Completed questionnaires should be returned promptly to CTR using the pre-paid envelopes provided, or by scan and email (the MPN-SAF Total Symptom Score forms part of the International Consortium MDS/MPN Response Criteria – see appendix 3).

Following this efficacy assessment, a short extension of tefinostat therapy (maximum 14 days) will be permitted in order to allow adequate time for sites to fully assess week 24 response assessment investigations and discuss the results with trial subjects.

‘Clinical benefit’ from tefinostat will be defined as:

Clinical Benefit (or better) according to International Consortium MDS/MPN Response Criteria [28] in the absence of features of disease progression

(See Appendix 3).

This definition includes 'complete remission', 'complete cytogenetic remission',' partial remission', 'bone marrow responses' or 'clinical benefit' as defined within these criteria.)

Patients who are both benefitting clinically from tefinostat and continuing to tolerate tefinostat therapy will be permitted to continue treatment, pending the availability of study drug, until there is a loss of clinical response, disease progression or the development of unacceptable toxicity. Patients who are not deemed to be obtaining clinical benefit at the time of the end of cycle 6 efficacy assessment will be taken off tefinostat therapy.

8.4 Treatment assessments for patients continuing tefinostat therapy (cycle 7 and beyond)

For patients who have a satisfactory clinical response after completion of 6 cycles and continue on trial treatment, study visits are reduced in frequency to **4-weekly** until the point of loss of response / disease progression or the occurrence of unacceptable toxicity. At each visit the following will be assessed:

8.4.1 Clinical assessment

- Adverse events
- Concomitant medications
- Transfusion history
- Physical examination including liver and spleen size by palpation, lymph nodes, skin infiltration, gingival hypertrophy, cutaneous haemorrhages
- Vital signs and weight

8.4.2 Laboratory assessments

- Full blood count to include haemoglobin, differential white cell count (including monocyte count and blast %), platelet count, MCV
- Biochemistry assessment: sodium, potassium, urea, creatinine (and eGFR), magnesium, calcium, phosphate, glucose, albumin, total protein, bilirubin, ALT/AST, alkaline phosphatase, LDH, uric acid

8.4.3 ECG

- 12-lead electrocardiogram including assessment of QT_c interval (day 1 only of each subsequent cycle, pre-dose)

8.5 Assessment at disease progression or cessation of therapy due to unacceptable toxicity

For patients who stop tefinostat treatment at ***time points other than immediately following the post cycle 6 efficacy response assessment*** the following additional assessments should be performed. This will include patients in whom tefinostat is

withdrawn early (during cycles 1-5) due to unacceptable toxicity or clear disease progression (including those patients with uncontrolled myelosuppression who require concomitant hydroxycarbamide therapy beyond completion of tefinostat cycle 3 – see section 7.5) and also patients who stop tefinostat treatment due to disease progression or toxicity following a prolonged period of therapy extending beyond cycle 6.

8.5.1 Clinical assessment

- Adverse events
- Concomitant medications
- Transfusion history
- Physical examination including liver and spleen size by palpation, lymph nodes, skin infiltration, gingival hypertrophy, cutaneous haemorrhages
- Vital signs and weight
- ECOG performance status (Appendix 6)

8.5.2 Laboratory assessments

- Full blood count to include haemoglobin, differential white cell count (including monocyte count and blast %), platelet count, MCV
- Biochemistry assessment: sodium, potassium, urea, creatinine (and eGFR), magnesium, calcium, phosphate, glucose, albumin, total protein, bilirubin, ALT/AST, alkaline phosphatase, LDH, uric acid

8.5.3 Urinalysis

- Using standard dipstick assessment (pH, protein, glucose, blood, ketones and leucocytes). This should be supplemented by formal quantification of any potentially-relevant abnormalities (eg. 24hr collection to quantify proteinuria).

8.5.4 Bone marrow assessment

- Bone marrow aspirate and trephine biopsy. Analysis of bone marrow aspirate to include morphological assessment (with differential count), local immunophenotypic (blast and monocytoid populations) and cytogenetic assessments. Local assessment of trephine histology.

8.6 Early withdrawal from the trial

Participants have the right to withdraw their consent from the study at any time for any reason. Should a patient decide to withdraw from the study, all efforts should be made to report the reason for withdrawal as thoroughly as possible. The investigator should ascertain from which aspects of the trial the patient wishes to withdraw and record the details in the appropriate part of the CRF (Form F). If a patient chooses to withdraw from treatment only the patient should continue to be assessed in accordance with the protocol. If a patient wishes to withdraw from the trial (i.e. including trial-specific assessments)

but is willing for further data to be supplied to CTR, then further routine follow-up data such as disease response status, survival and further treatment, will continue to be supplied by the investigator to CTR until 18 months after registration.

The local investigator also has the right to withdraw patients from the study treatment for a number of justifiable reasons including unacceptable toxicity (an adverse event or events that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of the study drug), unforeseen events which in the opinion of the investigator make further treatment inadvisable, serious violation of the study protocol (including persistent patient non-attendance and non-compliance) or for clinical reasons not related to the study treatment. Patients withdrawn by the investigator should continue to undergo safety assessments as per the trial schedule and to receive supportive care as per local policy.

9 DATA RECORDING

Data recording for this trial will be via a web-based system. This will be a secure encrypted system accessed via a user-specific password which will comply with Data Protection Act standards. The system will be accessed on:

https://medic.cardiff.ac.uk/monocle

A user password will be supplied to investigators on completion of the procedures described in section 6.1.

For centres that are unable to use the internet system, a patient record book will be made available to download from the trial website: http://medic.cardiff.ac.uk/monocle. This can also be sent to the consultant in charge of the patient's management upon request to CTR following trial entry.

The Case Report Form (CRF) will comprise a set of forms capturing details of eligibility, baseline characteristics, treatment and outcome details. Forms should be completed via either the web-based system or patient record book. The CRF must be completed, signed/dated and returned to the Trials Office by the investigator or by an authorised member of the patient's care team (as delegated on the Site Signature and Delegation Log) within specific time frames (see 9.1). For sites using the paper patient record book system, CRF entries should be made in ballpoint pen, in blue or black ink, and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated.

Reported data should be consistent with the source data with any discrepancies being clearly explained. If information is not known, this should be clearly indicated on the form. All missing or ambiguous data will be queried. It is the responsibility of the investigator to ensure that the CRF has been completed correctly and that the data are accurate.

Over the course of the study, trial forms may be amended by CTR as appropriate. Whilst this will not constitute a protocol amendment, new versions of the form must be implemented by participating sites immediately upon receipt.

9.1 Data submission

Patient Eligibility Checklist / Registration (Form A) – should be completed at the time of patient registration and faxed to CTR (02920 742289)

Baseline Assessment (Form B) – to be completed and submitted when all diagnostic data requested are available (but no later than 1 month after trial entry)

Course 1-6 Reports (Form C) – comprise data collected at day 1 and 15 visits during each cycle of tefinostat therapy. To be completed and submitted following each patient review.

End of Course 6 Efficacy Assessment (Form D) – to be completed and submitted when all data (including bone marrow reports) from the post cycle 6 efficacy assessment are available (within 14 days of the course 6 day 29 visit)

Continuing Therapy Reports (Form E) – for patients continuing beyond 6 cycles of tefinostat therapy. Comprise data collected at day 1 of each subsequent course of tefinostat. To be completed and submitted following patient review.

Withdrawal from Therapy (Form F) – to be completed following cessation of tefinostat therapy at time points other than immediately following the results of the post-cycle 6 efficacy assessment. This will include patients who stop therapy early due to toxicity or clear disease progression and also patients who lose their clinical response after continuing therapy beyond cycle 6. To be completed and submitted when all data from the end of study assessment (including bone marrow reports) are available (within 14 days of cessation of therapy).

12-month Follow-up (Form G) – to be completed and submitted 12 months after trial entry

18-month Follow-up (Form H) – to be completed and submitted 18 months after trial entry

9.2 Quality of Life data collection

CMML has a heterogeneous clinical phenotype with a variable spectrum of disease-related constitutional symptoms. Previous studies of non-selective HDAC inhibitors have been associated with generalised systemic toxicities including gastrointestinal effects, fatigue and insomnia with resulting impacts on quality of life (QoL). QoL is thus an additional important outcome measure in assessing impact of tefinostat treatment in this patient population. QoL will be measured at baseline (prior to the start of treatment) and following 3 and 6 four-week cycles of tefinostat therapy. The instruments used will be the EuroQoL EQ-5D-5L (Appendix 9) and the Myeloproliferative Neoplasm (MPN) Symptom Assessment Form (MPN-SAF) (Appendix 10). The EQ-5D is a standardised instrument for use as a measure of health outcome. The EQ-5D-5L descriptive system comprises five dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety /depression). The

MPN-SAF is a 27-item validated tool which aims to assess the prevalence and severity of symptoms in patients with myeloproliferative neoplasms; its use in patients with myelodysplastic/myeloproliferative neoplasms including CMML is currently being validated. The MPN-SAF Total Symptom Score is currently included within the International Consortium MDS/MPN Response Criteria on an exploratory basis. [28, 29]

Patients should be able to undertake the assessment themselves, and the local trial team are asked to facilitate this and to return the responses promptly to CTR in the pre-paid envelopes.

9.3 Archiving

It is the responsibility of the Principal Investigator to ensure that all essential trial documentation and source records (e.g. signed Informed Consent Forms, ISFs, pharmacy files, patients' hospital notes) at their site are securely retained for at least 10 years after the end of the trial. Data held by CTR will be stored in a secure archive facility. Following authorisation from the sponsor, arrangements for confidential destruction will then be made.

9.4 Confidentiality and data protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act (1998). With the patient's consent, their full name, date of birth, National Health Service (NHS) number (or in Scotland the Community Health Index [CHI]), hospital number and general practitioner details will be collected at trial entry to allow tracing through the NHS Information Centre for Health and Social Care (service formerly provided by the Office for National Statistics) to assist with follow-up. Patients will be identified using only their unique trial number, initials, hospital number and date of birth on the CRF and in any correspondence between CTR and the participating site. Patients are asked, however, to give permission for CTR to be sent a copy of their signed Informed Consent Form which will not be anonymised. This will be used to perform in-house monitoring of the consent process. Where copies of source documents are required by CTR for central monitoring purposes, the patient's name must be obliterated by the site before sending.

The Investigator must maintain documents not for submission to CTR in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that patient confidentiality is protected.

CTR will maintain the confidentiality of all patient data and will not disclose information by which patients may be identified to any third party other than those directly involved in the treatment of the patient and

organisation for which the patient has given explicit consent for data transfer (e.g. Cancer Registries, laboratory staff). Representatives of the Monocle trial team may be required to have access to patients' notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times. If a patient withdraws consent from further trial treatment and/or further collection of data their information will remain on file. All collected data will be included in the final study analysis.

10 STATISTICAL CONSIDERATIONS

10.1 Sample size and early stopping rules

Up to 40 patients will be recruited to the study, all of whom will receive tefinostat therapy. The proposed trial design (Bryant and Day)[16] is based on Simon's two-stage design, but incorporates toxicity as well as clinical efficacy (futility) considerations into sample size and early stopping rules.

The primary clinical efficacy objective of the study is to determine overall response rate after 6 cycles of tefinostat. It is felt that if the overall response rate is below 10% the treatment should be rejected, and if above 30% the treatment is sufficiently promising to warrant further evaluation in a phase III trial. These figures reflect the minimum clinically relevant response rate seen in the CMML-201 study of azacitidine therapy in a similar CMML patient population. [17]

The primary safety and tolerability objective is to determine the proportion of patients who suffer an unacceptable level of toxicity as assessed during the first 2 cycles of tefinostat treatment. It is felt that if more than 40% of patients suffer unacceptable toxicity then the treatment should be rejected, and if fewer than 20% suffer some toxicity then the toxicity profile is considered acceptable. A maximum tolerated dose is often defined as the dose at or below the level at which one-third of patients experience toxicity and these figures correspond to that definition.

The significance level for both the efficacy and tolerability endpoints was chosen to be 10%. This is the bound of probability of recommending a treatment with either inadequate efficacy or unacceptable safety. The two-stage design was chosen to allow for early termination in the event of unacceptable toxicity or lack of efficacy. Under these conditions, and setting power at 85% for both the efficacy and safety endpoints, a maximum of 40 evaluable patients need to be recruited into the study. The first stage will recruit up to 19 patients and will require 6 or fewer to have unacceptable toxicity, with at least 3 patients showing clinical benefit (as defined in section 8.3) in order to proceed to the second stage.

Tefinostat will be considered for further randomised clinical evaluation if at least 7 of 40 patients respond according to the specified clinical response criteria, and fewer than 13 patients exhibit unacceptable toxicity (ie. at least 28patients are able to tolerate the drug. Those patients who are not known to have responded will be deemed non-responders. All patients who receive at least one dose of study drug will be considered evaluable, and as such non-compliance is expected to be minimal. There are no plans to replace patients that

withdraw from the study. Experience from the CMML201 study shows that compliance with follow-up is likely to be very good in this disease population. [17]

10.2 Data analysis

Statistical analysis will be overseen by Robert Hills, Professor of Translational Statistics at Cardiff University and CTR Lead. Analysis will be descriptive for the defined primary and secondary endpoints of the trial (proportions of patients with response/toxicity). The analysis population (patients evaluable for the primary endpoints) will include any patient starting one cycle of tefinostat therapy.

10.3 Independent Data Monitoring Committee (IDMC) and formal interim analysis

An Independent Data Monitoring Committee (IDMC) will review the accumulating safety and efficacy results of the trial on a continuous basis and a formal interim analysis will be performed after a sufficient number of evaluable patients have been treated to be able to determine whether the continuation criteria for progression to the second stage of the study have been met (see section 10.1). Recruitment to the study may thus need to be temporarily halted after the first 19 patients to allow sufficient safety and clinical efficacy data to accumulate. The IDMC will also have the power to request to suspend recruitment or stop the study at any time in the setting of emerging safety concerns. CTR will send safety and efficacy data, along with current recruitment figures to the IDMC whenever they are notified of a new treatment-related death or grade 3/4 non-haematological adverse reaction. To inform the decision of whether or not to proceed to the second stage of the study, overall response rates following completion of cycle 3 of tefinostat may be presented to the IDMC as an early indication of response. All analyses will be kept confidential until the end of the study.

10.4 Definition of outcome measures

10.4.1 Primary outcome measures

- Safety and tolerability of tefinostat will be defined as the proportion of patients experiencing a CTC grade 3-4 non-haematological toxicity[25] or death during the first two cycles of tefinostatthat is judged by either the principal investigator or the chief investigator (or his delegate) as having a reasonable suspected causal relationship to tefinostat.
- Overall clinical response rate will be defined as the cumulative proportion of patients achieving clinical benefit (or better) according to International Consortium MDS/MPN Response Criteria[28] at day 29 of the sixth or last cycle of tefinostat (whichever is the earliest).

10.4.2 Secondary outcome measures

- Incidence of CR / PR / clinical benefit will be defined according to the International Consortium MDS/MPN Response Criteria[28] (see Appendix 3) at day 29 of the sixth or last cycle of tefinostat (whichever is the earlier). Duration of response will be measured from the date of investigations confirming the achievement of response until the date on which it is confirmed that the patient is no longer sustaining response.
- Overall clinical response rate according to Wattel and Modified IWG criteria (see Appendix 4 and 5). Responses will be defined as the cumulative proportion of patients achieving minor response of better (according to Wattel criteria[14] and/or haematological improvement or better (according to the Modified IWG criteria)[23] at day 29 of the sixth or last cycle of tefinostat (whichever is the earliest).
- Red blood cell transfusion independence will be deemed to have been achieved if a patient who received red cell transfusion support in the eight weeks prior to baseline assessment remains free from red cell transfusion for a period of ≥ eight weeks
- Platelet transfusion independence will be deemed to have been achieved if a patient who received platelet transfusion support in the eight weeks prior to baseline assessment remains free from platelet transfusion for a period of ≥ eight weeks
- Overall survival is defined as the time from the date of trial registration to the date of death from any cause. Patients discontinuing the study, lost to follow-up or still alive at the end of study will be censored at the date of last follow-up.
- Progression-free survival is defined as the time from date of trial registration to the date of progression or death from CMML. Patients who die from a cause other than CMML prior to progression will be censored in the analysis. The date of progression is the date of the decision to investigate a clinical suspicion of progression which is subsequently confirmed.
- Time to AML transformation of CMML is defined as the time from trial registration to AML transformation of CMML or last follow-up. Patients in whom CMML does not transform to AML will be censored at the last date they were known to be alive and free from AML transformation of CMML. The date of AML transformation of CMML is the date of the decision to investigate clinical suspicion of AML transformation which is subsequently confirmed.
- Duration of tefinostat therapy is defined as the time elapsed between first and last doses of tefinostat therapy.
- Quality of life will be measured using the MPN-SAF and EuroQoL EQ-5D-5L questionnaires

10.5 End of trial definition

Patients will be followed up for a minimum of 18 months (to assess overall survival and progression free survival) following registration, or for 30 days after the last trial treatment if this exceeds 18 months. The end of trial will be 6 months after the last data capture. This will allow sufficient time for the completion of protocol procedures, data collection and data input. CTR will notify the MHRA and main REC that the trial has ended and will provide them with a summary of the clinical trial report within 12 months of the end of the trial.

11 PHARMACOVIGILANCE

The collection and reporting of Adverse Events (AEs) will be in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 and its subsequent amendments. Definitions of different types of AE are listed below. Investigators should assess the seriousness and causality (relatedness) of all SAEs experienced with reference to the Investigator's Brochure. Investigators at participating centres are obliged to report severe adverse events (SAEs) that occur in this trial to CTR in a timely manner.

11.1 Definitions of adverse events

11.1.1 Adverse event (AE)

An adverse event (AE) is any unintentional, unfavourable medical occurrence in a clinical trial subject which does not necessarily have a causal relationship with study treatment. This includes:

- new clinical signs or symptoms
- new illnesses or diseases (or the deterioration of an existing disease or illness)
- new clinically relevant deteriorations in any laboratory assessments or clinical tests

11.1.2 Adverse reaction (AR)

An adverse reaction (AR) is an untoward and unintended response to any dose of IMP administered, judged by either the reporting investigator or sponsor as having 'reasonable causal relationship' to the IMP

11.1.3 Serious adverse event (SAE)

A serious adverse event (SAE) is defined as an untoward (unfavourable) medical occurrence which is:

- Fatal or life-threatening*
- Requires or prolongs hospitalisation
- Is significantly or permanently disabling or incapacitating
- Constitutes a congenital anomaly or birth defect
- May jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above
- Development of a non-haematological toxicity of grade 3 (as defined by Common Terminology Criteria for Adverse Events (CTCAE) version 4 (see Appendix 8), which does not resolve to grade 2 or less within 7 days
- Development of any grade 4 non-haematological toxicity

*The term 'life-threatening' in the definition of a SAE refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.

The following events will ***not*** be recorded as SAEs within this trial:

- Hospitalisation for routine treatment or monitoring of CMML or other pre-existing condition that is not associated with any deterioration of the condition, for elective or pre-planned treatment including surgery or for social care
- Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions for serious as detailed above and not resulting in hospital admission
- Disease progression and/or death due to disease progression
- Deaths attributable to CMML beyond 30 days of the last administration of the study agent

11.1.4 Suspected unexpected serious adverse reaction (SUSAR)

A suspected unexpected serious adverse reaction (SUSAR) is an *adverse drug reaction* which both

- meets the definition of a serious adverse event
- is 'unexpected' in that the nature and severity of the event is not consistent with the applicable product information (i.e. Investigator's Brochure)
- Investigators will assess causality but do not assess expectedness, this is done by the nominated reviewer(s) on receipt of all SAEs. The reviewer will refer to the information supplied within the Investigator's Brochure and the CTR trial team will report events as SUSARs only if the nature, seriousness, severity and outcome is not consistent with the relevant product information.

The following events will be classified as **expected SAEs** related to CMML within this trial and therefore will not be reportable as SUSARs:

- Neutropenic sepsis
- Grade 4 haematological toxicity

11.2 Adverse event reporting requirements

11.2.1 Adverse events

Information about adverse events and reactions, whether volunteered by the patient, discovered by investigator questioning or detected through physical examination, laboratory test or other investigation will be collected and recorded for all patients from the time of start of protocol treatment until 30 days after the last dose of tefinostat therapy. All AEs should be captured on the Adverse Events Forms within the CRF. AEs will be reviewed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4 (see Appendix 8). For each adverse occurrence, the highest grade observed since the last visit should be recorded.

11.2.2 Serious adverse events

SAEs should be monitored from the time of patient registration until 30 days following the last administration of protocol treatment. On becoming aware that a patient has experienced an AE defined as serious (see 11.1.3), the event should be reported on a SAE form. The investigator (or delegate) must complete, date and sign the form which should be emailed (or faxed if email is not possible) to CTR using the contact details listed below **within 24 hours** of first becoming aware of the event. Please ensure that multiple events are not combined into one SAE form.

SAE Email: CTR-Safety@cardiff.ac.uk

SAE Fax Number: 0203 0432 376

When completing the form, the investigator will be asked to define the causality and severity of the SAE which should be documented using CTCAE version 4. They will not be asked to define expectedness, this will be carried out by the clinical reviewer.

It is accepted that a causality assessment is not always available at the time of the initial report – this opinion should be provided as soon as possible to ensure that regulatory reporting timelines are met.

Investigators should also report SAEs to their own Trust in accordance with local practice. All SAEs should be followed up until resolution or stabilisation of the event.

11.3 Trials office

CTR will assign each SAE a unique reference number which should be quoted on all correspondence and follow-up reports regarding the SAE. On receipt of an SAE form by CTR, seriousness and expectedness will be determined independently by a Clinical Coordinator (the Chief Investigator or his nominee). An SAE judged by the local investigator to have a reasonable causal relationship to the trial medication will be regarded as a Serious Adverse Reaction (SAR). The reviewer will assess all SARs and SAEs for expectedness. If the event meets the definition of a SAR that is unexpected it will be classified as a SUSAR.

11.4 Reference Safety Information

An overview of the current clinical experience and safety information pertaining to tefinostat is provided in section 2.3.3 of this protocol. More detailed summaries of pre-clinical and existing clinical information relating

to tefinostat can be found in appendices 11-12 and in the latest version of the tefinostat investigator's brochure.

11.5 Reporting to the regulatory authorities

CTR will report a minimal data set of all individual events categorised as fatal or life-threatening SUSARs to the MHRA, the main Research Ethics Committee (REC) and the sponsor within 7 days. Detailed follow-up information will be provided within an additional 8 days. All other events classified as SUSARs will be reported within 15 days.

CTR will report details of all SAEs and SARs (including SUSARs) to the MHRA and main REC annually from the date of Clinical Trial Authorisation, in the form of a Development Safety Update report. Details of all AEs can be reported to the MHRA on request.

The MHRA and main REC will be notified immediately if a significant safety issue is identified during the course of the trial.

Details of all SUSARs and any other safety issues which arise during the course of the trial will be reported to Principal Investigators. A copy of any such correspondence should be filed in the ISF.

The independent Data Monitoring Committee (DMEC) will assess toxicity (including all reported SAEs) as described in section 10.3

11.6 Deaths

Deaths must be reported as an SAE within 24 hours of becoming aware of the event if they meet the criteria outlined in 11.1.3 above. The date and cause of death will be collected. All deaths must also be recorded on the death report form, this information will also be collected in trial CRFs.

11.7 Pregnancies

Pregnancies occurring in study participants or participant's partners during tefinostat treatment or within 30 days of the last dose of study treatment may represent a safety issue. All pregnancies must be reported immediately to CTR using the pregnancy report form and, for pregnancies occurring in study participants, tefinostat treatment should discontinue.

Where a pregnancy is known, this should be followed for outcome and any adverse outcomes assessed for causality to the treatment received. There is a separate Patient Information Sheet and Informed Consent

Form that should be completed by the pregnant person to give CTR permission to follow their pregnancy to outcome.

12 TRIAL GOVERNANCE AND ETHICAL CONSIDERATIONS

12.1 Clinical governance and Quality Assurance

The Monocle trial will be conducted according to the protocol and in compliance with the principles of Good Clinical Practice in clinical trials as described in the International Conference on Harmonisation Guidelines, including the archiving of essential documents (CPMP/ICH/135/95). The trial must also conform to local Research Governance procedures; sites will not be permitted to enrol patients until written confirmation of local R&D approval is received by CTR. Centres are required to complete a registration process with CTR before recruitment is started and to confirm acceptance of the terms of sponsorship required by Cardiff University. It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary local approval.

CTR will manage the trial and will follow unit SOPs with regard to the handling of substantial amendments, archiving and all other generic clinical trial activities. CTR and the sponsor have systems in place to ensure that there is reporting and appropriate action taken in respect of:

- Serious breaches of GCP, the trial protocol and the Clinical Trial Authorisation
- Urgent safety measures
- Protocol violations

Investigators are required to promptly notify CTR if they become aware of a trial-related serious breach of GCP and/or the trial protocol. It is also the responsibility of individual clinicians to take immediate action if thought necessary to protect the health and interest of individual patients.

A ‘serious breach of GCP’ is defined as a breach which is likely to effect to a significant degree:

- The safety or physical or mental integrity of the subjects of the trial; or
- The scientific value of the trial

For further information the Investigator should contact the Trial-coordinator at CTR.

12.2 Ethical considerations

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, 1964 and amended at the 48th World Medical Association General Assembly, Somerset West, South Africa, 1996. Informed written consent must be obtained from patients prior to registration into the study. The right of a patient to refuse participation without giving reasons must be respected. The patient must remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment. The final study protocol, patient information sheets, consent forms and all other relevant study documentation will be submitted to and approved by a main Research Ethics Committee (Main REC) and the Medicines and Healthcare Products Regulatory Agency (MHRA) prior to circulation to sites and entering patients into the trial.

13 STUDY FINANCES

13.1 Funding sources

The Monocle trial is funded by Bloodwise.

Sixth Element Capital are supplying tefinostat free of charge for use in the study.

13.2 Patient expenses / payments

Participants will not receive payments for taking part in the Monocle study.

Patients will be able to claim reimbursement of any additional travel expenses and hospital parking costs that are incurred when attending Monocle study appointments that are considered over and above their routine care.

14 INSURANCE AND INDEMNITY

The Monocle trial is sponsored by Cardiff University. Cardiff University employees are indemnified by the University insurers for negligent harm caused by the design or co-ordination of the clinical trials they undertake whilst in the University's employment. Cardiff University cannot offer indemnity for non-negligent harm. Cardiff University is independent of any pharmaceutical company and, as such, is not covered by the Association of British Pharmaceutical Industry (ABPI) guidelines for patient compensation.

The NHS has a duty of care to patients treated at Trust and non-Trust hospitals, whether or not the patient is taking part in a clinical trial, and the NHS remains liable for proven clinical negligence and other negligent harm to patients under this duty of care.

15 PUBLICATION POLICY

Results of this trial will be submitted for peer reviewed journal publication. Manuscript(s) will be prepared by the Trial Management Group. Authorship of the final manuscript(s), interim publications or abstracts will be in accordance with ICMJE authorship policy.

To maintain the scientific integrity of the trial, data will not be released prior to the end of the trial, either for trial publication or presentation purposes, without the permission of the Chief Investigator. Any secondary publications and presentations prepared by investigators must be submitted to the TMG in a timely fashion and in advance of being submitted for publication. Individual collaborators must not publish data concerning their patients which is directly relevant to the questions posed in the trial until the main results of the trial have been published.

16 REFERENCES

1. Bacher U, Haferlach T, Schnittger S et al. Recent advances in diagnosis, molecular pathology and therapy of chronic myelomonocytic leukaemia. Br.J.Haematol. 2011;153:149-167.
2. Parikh SA, Tefferi A. Chronic myelomonocytic leukemia: 2013 update on diagnosis, risk stratification, and management. Am.J.Hematol. 2013;88:967-974.
3. Killick SB, Carter C, Culligan D et al. Guidelines for the diagnosis and management of adult myelodysplastic syndromes. Br.J.Haematol. 2014;164:503-525
4. Bennett JM, Catovsky D, Daniel MT et al. Proposal for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51(2):189-199
5. Swerdlow SH, Campo E, Lee Harris N. WHO classification of tumours of hematopoietic and lymphoid tissues. WHO Publications 2008.
6. Patnaik MM, Parikh SA, Hanson CA, Tefferi A. Chronic myelomonocytic leukaemia: a concise clinical and pathophysiological review. Br J Haematol 2014; 165: 273-286
7. Padron E, Steensma DP. Cutting the cord from myelodysplastic syndromes: chronic myelomonocytic leukemia-specific biology and management strategies. Curr Opin Hematol 2015; 22(2): 163-170
8. Itzykson R, Kosmider O, Renneville et al. Clonal arichitecture of chronic myelomonocytic leukemias. Blood 2013; 121(12): 2186-2198
9. Greenberg P, Cox C, Le Beau MM et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997; 89: 2079-2088
10. Greenberg PL, Tuechler H, Schanz J et al. Revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndromes. Blood 2012; 120: 2454-2465
11. Such E, Germing U, Malcovati L et al. Development and validation of a prognostic scoring system for patients with chronic myelomonocytic leukemia. Blood 2013; 121: 3005-3015.
12. Park S, Labopin M, Yakoub-Agha I et al. Allogeneic stem cell transplantation for chronic myelomonocytic leukemia: a report from the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. Eur J Haematol 2013; 90: 355-364
13. Eissa H, Gooley TA, Sorror ML et al. Allogeneic hematopoietic cell transplantation for chronic myelomonocytic leukemia: relapse-free survival is determined by karyotype and comorbidities. Biol Blood Marrow Transplant 2011; 17(6): 908-915
14. Wattel E, Guerci A, Hecquet B et al. A randomized trial of hydroxyurea versus VP16 in adult chronic myelomonocytic leukemia. Groupe Francais des Myelodysplasies and European CMML Group. Blood 1996;88:2480-2487.

15. Fenaux P, Mufti GJ, Hellstrom-Lindberg E et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009; 10: 223-232.
16. Kaminskas E, Farrell A, Abraham S et al. Approval summary: azacitidine for treatment of myelodysplastic syndrome subtypes. Clin.Cancer Res. 2005;11:3604-3608.
17. Drummond, M. W, Pocock, C, Boissinot et al. A multi-centre phase 2 study of azacitidine in chronic myelomonocytic leukaemia. Leukemia. 2014. (EPub ahead of print)
18. Tan J, Cang S, Ma Y et al. Novel histone deacetylase inhibitors in clinical trials as anti-cancer agents. J.Hematol.Oncol. 2010; 3: 5.
19. Siitonen T, Timonen T, Juvonen E et al. Valproic acid combined with 13-cis retinoic acid and 1,25-dihydroxyvitamin D3 in the treatment of patients with myelodysplastic syndromes. Haematologica 2007; 92: 1119-1122.
20. Needham LA, Davidson AH, Bawden LJ et al. Drug targeting to monocytes and macrophages using esterase-sensitive chemical motifs. J.Pharmacol.Exp.Ther. 2011; 339: 132-142.
21. Zabkiewicz J, Gilmour M, Hills RK et al. The targeted histone deacetylase inhibitor tefinostat (CHR-2845) shows selective in vitro efficacy in monocytoid-lineage leukaemias. Oncotarget 2016; 7 (13): 16650-62
22. Ossenkoppele GJ, Lowenberg B, Zachee P et al. A phase I first-in-human study with tefinostat – a monocyte/macrophage targeted histone deacetylase inhibitor - in patients with advanced haematological malignancies. Br.J.Haematol. 2013; 162: 191-201.
23. Cheson BD, Greenberg PL, Bennett JM et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006; 108: 419-425.
24. A phase I/II dose escalation trial of HDAC inhibitor tefinostat (CHR-2845) for cancer associated inflammation in hepatocellular carcimoma. (Sponsor: Queen Mary Univesity of London, Chief investigator: Prof Thorsten Hagemann, EudraCT 2012-000326-22)
25. Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. National Institutes of Health, National Cancer Institute. 2009.
26. Bryant J, Day R. Incorporating toxicity considerations into the design of two-stage phase II clinical trials. Biometrics 1995; 51(4): 1372-83.
27. Oken MM, Creech RH, Tormey DC et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5(6): 649-655
28. Savona MR, Malcovatti L, Komrokji R et al. An international consortium proposal of uniform response criteria for myelodyplastic/myeloproliferative neoplasms (MDS/MPN) in adults. Blood 2015; 125(12): 1857-65

29. Scherber R, Dueck AC, Johansson P et al. The Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF): International Prospective Validation and Reliability Trial in 402 patients. Blood 2011; 118(2): 401-408

APPENDIX 1: WORLD HEALTH ORGANISATION CRITERIA FOR THE DIAGNOSIS OF CHRONIC MYELOMONOCYTIC LEUKAEMIA (2008) [5]

A diagnosis of CMML requires the following to be present:

1) Persistent peripheral blood monocytosis >1x10^9/l
2) No Philadelphia chromosome or *BCR-ABL1* fusion gene
3) No rearrangement of *PDGFRA* or *PDGFRB* (should be specifically excluded in cases with eosionophilia)
4) Fewer than 20% blasts* in the blood and in the bone marrow
5) Dysplasia in one or more myeloid lineages. If myelodysplasia or absent or minimal, the diagnosis of CMML may still be made if requirements 1-4 are met, ***and***:
   - an acquired, clonal cytogenetic or molecular gene abnormality is present in the haematopoietic cells, ***or***
   - the monocytosis has persisted at least 3 months ***and***
   - all other causes of monocytosis have been excluded.

(*Blasts include myeloblasts, monoblasts and promonocytes. Promonocytes are monocytic precursors with abundant light grey or slightly basophilic cytoplasm with few scattered, fine lilac-coloured granules, finely-distributed, stippled nuclear chromatin, variably prominent nucleoli, and delicate nuclear folding or creasing, and in this classification are equivalent to blasts. Abnormal monocytes which can be present both in the peripheral blood and bone marrow are excluded from the blast count.)

CMML is further subdivided into two sub-categories, depending on the number of blasts (plus promonocytes) found in the blood and bone marrow as follows:

CMML-1	Blasts (including promonocytes) <5% in the peripheral blood, <10% in the bone marrow
CMML-2	Blasts (including promonocytes) 5-19% in the peripheral blood or 10-19% in the bone marrow, or when Auer rods are present irrespective of the blast plus promonocyte count

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (2008)

APPENDIX 2: CMML-SPECIFIC PROGNOSTIC SCORING SYSTEM (CPSS) [11]

	Variable scores		
Variable	**0**	**1**	**2**
WHO subtype	CMML-1 Blasts (including promonocytes) <5% in peripheral bloods and <10% in bone marrow)	CMML-2 Blasts (including promonocytes) 5-19% in peripheral blood and 10-19% in bone marrow *or* presence of Auer rods	
FAB subtype	Myelodysplastic CMML (WBC <13 x10^9/l)	Myeloproliferative CMML (WBC ≥13 x10^9/l)	
CMML-specific cytogenetic risk classification	Low risk (Normal; Isolated –Y)	Intermediate risk (All other abnormalities)	High risk (Trisomy 8; Complex karyotype [≥3 abnormalities]; Abnormalities of Chromosome 7)
Red cell transfusion dependency	No	Yes	

Overall Score	**CPSS risk group**
0	Low
1	Intermediate-1
2-3	Intermediate-2
4-5	High

APPENDIX 3 : INTERNATIONAL CONSORTIUM MDS/MPN RESPONSE CRITERIA [28]

Complete Remission (*presence of ALL of the following improvements is required*)

- Bone marrow ≤5% myeloblasts (including monocytic blast equivalent in CMML) with normal maturation of all cell lines and return to normal cellularity *(persistent low-level dysplasia is permitted given the subjectivity of assignment of dysplasia)*
- Fibrosis ≤ grade 1 (mild reticulin fibrosis) – reassessment not required if fibrosis was not present on the baseline bone marrow biopsy
- Peripheral blood:
  - WBC ≤10 x 10^9/l
  - Platelets ≥100, ≤450 x 10^9/l
  - Blasts 0%
  - Monocytes ≤1 x 10^9/l
  - Hb ≥110 g/l
  - Neutrophils ≥1.0 x 10^9/l
  - Neutophil prescursors ≤2%
- Complete resolution of all extramedullary disease present before therapy (including cutaneous disease, disease-related serous effusions, palpable hepatosplenomegaly)

Complete Cytogenetic Remission

- Resolution of previously-present chromosomal abnormality known to be associated with MDS or MPN as seen on classic karyotyping (with minimum 20 metaphases) or by FISH

Partial Remission

- Normalisation of peripheral counts and hepatosplenomegaly with bone marrow blasts (and blast equivalents) reduced by 50%, but remaining >5% of cellularity (except in cases of MDS/MPN with ≤5% bone marrow blasts at baseline)

Marrow Reponses

- **Optimal Marrow Response**: Presence of all marrow criteria necessary for Complete Remission but without normalisation of peripheral blood indices (as above)
- **Partial Marrow Response**: Bone marrow blasts (and blast equivalents) reduced by 50%, but remaining >5% of cellularity (or reduction in grading of reticulin fibrosis from baseline on at least 2 bone marrow evaluations spaced at least 2 months apart)

Clinical Benefit (*requires 1 of the following in the absence of CR / PR / progression and independent of marrow response, resolution of abnormal peripheral blood counts must be based on at least 2 separate analyses over at least 8 weeks*)

- **Erythroid**
  - Hb increase by 20 g/dl
  - Transfusion independent for ≥8wks for patients requiring ≥4 units red cells in the previous 8wks
- **Platelets**
  - Transfusion independent for ≥8wks for patients requiring ≥4 platelet transfusions in the previous 8wks
  - *If pre-treatment platelets ≤20 x 10^9/l*: increase to >20 x10^9/l (and by at least 100%)
  - *If pre-treatment platelets 20-100 x10^9/l*: an absolute increase of ≥30 x 10^9/l
- **Neutrophils**
  - *If pre-treatment neutrophils ≤0.5 x 10^9/l*: increase to >0.5 x10^9/l (and by ≥ 100%)
  - *If pre-treatment neutrophils 0.5-1.0 x 10^9/l*: an absolute increase of ≥0.5 x10^9/l (and by at least 50%)
- **Spleen** - either a minimum 50% reduction in palpable splenomegaly of a spleen that is a least 10cm palpable at baseline or a spleen that is palpable at more than 5cm at baseline becoming not palpable
- **Symptoms** - improvement in symptoms as noted by a decrease of ≥50% in the MPN-SAF Total Symptom Score (only valid if baseline score>20)*

<u>Disease Progression</u> (*requires a combination of either 2 major, 1 major & 2 minor, or 3 minor criteria from the list below)*

Major Criteria of disease progression

- Increase in bone marrow blast count: - <5% blasts: ≥50% increase (and to >5% blasts)
  - 5-10% blasts: ≥50% increase (and to >10% blasts)
  - 10-20% blasts: ≥50% increase (and to >20% blasts)
- Evidence of cytogenetic evolution: - appearance of new cytogenetic abnormality
  - increase in cytogenetic burden of disease by ≥50%
- New extramedullary disease: - worsening splenomegaly (either from previously inpalpable to >5cm palpable, or >100% increase in previous 5-10cm splenomegaly, or ≥50% increase in previous >10cm splenomegaly)
  - extramedullary disease outside the spleen (including hepatomegaly, skin lesions, granulocytic sarcoma)

Minor Criteria of disease progression

- New transfusion dependence (at least 2u of red cells in the past month for Hb<85g/l that was not associated with clinically overt bleeding or felt to be associated with therapy
- Significant loss of maximal response of cytopenias: ≥50% decrement from maximum response in neutrophils or platelets (not felt to be associated with therapy)
- Reduction in Hb by 15g/l from best response or baseline (not felt to be associated with therapy)
- Increase in symptoms as noted by an increase of ≥50% in the MPN-SAF Total Symptom Score*
- Evidence of molecular clonal evolution

** The use of the MPN-SAF Total Symptom Score within the International Consortium MDS/MPN Response Criteria is currently considered exploratory. Validation among patients within MDS/MPN is currently under way.* [28]

APPENDIX 4: WATTEL ET AL RESPONSE CRITERIA IN CMML [14]

Clinical Remission

- Normal blood count: leukocytes 4 – 10 x10^9/l, monocytes <1 x10^9/l, neutrophils 1.5 – 7 x10^9/l, platelets >150 x10^9/l, haemoglobin >100 g/l (with no transfusion requirement)
- Normal bone marrow aspirate: blasts<5%, monocytes<5%, with absence of myelodysplastic features
- Normal bone marrow karyotype (if an abnormal clone was previously present)
- Absence of extramedullary leukaemia

Good Response

One of the following:

- Leukocytes <10 x10^9/l and platelets >100 x10^9/l and haemoglobin >100 g/l with no transfusion requirement and no extramedullary leukaemia
- Disappearance of prior visceral involvement (skin disease, pleural / pericardial / peritoneal effusion)
- In patients treated after progression to AML: reversion to chronic CMML phase

Minor Response

One of the following:

- >50% reduction of the leukocyte count and leukocytes <10 x10^9/l
- >50% reduction of the transfusion requirement or increase in haemoglobin >20 g/l
- Doubling of the platelet count (if initially >25 x10^9/l) or increase by > 50 x10^9/l (if initially <100 x10^9/l)
- Reduction in spleen size by more than 5cm
- Significant reduction (>50%) of cutaneous lesions or serous effusions

Progression

One of the following:

- Doubling of the leukocyte count (if initially >10 x10^9/l), or increase by more than 10 x10^9/l (if initially <10 x10^9/l)
- Drop in haemoglobin >20 g/l or >50% increase in transfusion requirement
- >50% reduction in platelet count, if initially between 50-200 x10^9/l
- Increase in spleen size by more than 5cm
- >10% increase in marrow blasts, or progression to AML
- Worsening (>50%) of cutaneous lesions or serous effusions

Stable Disease

- All other disease situations

APPENDIX 5: MODIFIED IWG RESPONSE CRITERIA IN MYELODYSPLASIA [23]

Complete Remission

- Bone marrow ≤5% myeloblasts with normal maturation of all cell lines
- Peripheral blood: Hb ≥110 g/l, Platelets ≥100 $x10^9$/l, Neutrophils ≥1.0 $x10^9$/l, No blasts

Partial Remission

- All CR criteria required except: bone marrow blasts decreased by ≥50% compared to pre-treatment, but still >5%. Bone marrow cellularity and morphology not relevant.

Haematologic Improvement†

Erythroid Response (if pre-treatment* Hb <110 g/l)

- Hb increase by 15 g/l
- Reduction in red cell transfusions by an absolute number of 4 units per 8 weeks compared with the pre-treatment transfusion number in the previous 8 weeks

Platelet Response (if pre-treatment* platelets <100 $x10^9$/l)

- If baseline count >20 $x10^9$/l: an absolute increase of ≥30 $x10^9$/l
- If baseline count <20 x 10^9/l: an increase to >20 $x10^9$/l, and by at least 100%

Neutrophil Response (if pre-treatment* neutrophils <1.0 $x10^9$/l)

- At least 100% increase and an absolute increase of >0.5 $x10^9$/l

Stable Disease

- Failure to achieve at least PR/HI but no evidence of progression

Progressive Disease

- For patients with: <5% blasts: ≥50% increase in blasts to >5% blasts
  5-10% blasts: ≥50% increase to >10% blasts
  10-20% blasts: ≥50% increase to >20% blasts
- At least 50% decrement from maximum response in neutrophils / platelets
- Reduction in Hb by ≥20 g/l
- New transfusion dependence

*Pre-treatment counts should be averages of at least 2 measurements (not influenced by transfusions) and ≥1 week apart

†Responses must last at least 8 weeks

APPENDIX 6: ECOG PERFORMANCE SCALE [27]

Grade	Activity Performance Description
0	Fully active, able to carry out all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg. light house work, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair.

APPENDIX 7: CONTRACEPTIVE METHODS

Female subjects

A 'woman of childbearing potential' (WCBP) is defined as:

- A sexually mature woman (ie. Any female who has ever experienced menstrual bleeding)

and

- Who has not undergone a hysterectomy or who has not been postmenopausal for at least 24 consecutive months (ie. Who has had menses at any time within the preceding 24 consecutive months)

WCBP must agree to use at least 2 effective contraceptive methods throughout the study and for 3 months following the date of the last dose of study drug.

The following are considered effective methods of contraception for WCBP:

- Contraceptive implant
- Levonorgestrel-releasing intrauterine system (IUS)
- Medroxyprogersterone acetate depot
- Tubal sterilisation
- Sexual intercourse with a vasectomised male partner only (vasectomy must be confirmed by 2 negative semen analyses)
- Ovulation inhibitory progesterone-only pills (ie. Desogestrel)
- Condoms and spermicidal jelly or Diaphragm and spermicidal jelly

Combined oral contraceptive pills are not recommended. Subjects using combined oral contraception should switch to one of the methods above. The increased risk of VTE continues for 4-6 weeks after stopping oral contraception

Male subjects

The following are considered effective methods of contraception for male subjects:

- Condoms and spermicidal jelly throughout study drug therapy, during any dose interruption and for to 3 months after cessation of study therapy if their partner is of childbearing potential
- Female partners must also use a method from the list above

APPENDIX 8: COMMON TERMINOLOGY CRITERIA FOR GRADING OF ADVERSE EVENTS [25]

Grading of toxicity and adverse events will be made according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4. The full CTCAE document is available in the Investigator Site File and on the National Cancer Institute (NCI) website. The following address was correct when this version of the protocol was approved:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

APPENDIX 9: EuroQoL EQ-5D-5L QUALITY OF LIFE QUESTIONNAIRE

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑

I have slight problems in walking about ❑

I have moderate problems in walking about ❑

I have severe problems in walking about ❑

I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑

I have slight problems washing or dressing myself ❑

I have moderate problems washing or dressing myself ❑

I have severe problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑

I have slight problems doing my usual activities ❑

I have moderate problems doing my usual activities ❑

I have severe problems doing my usual activities ❑

I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑

I have slight pain or discomfort ❑

I have moderate pain or discomfort ❑

I have severe pain or discomfort ❑

I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑

I am slightly anxious or depressed ❑

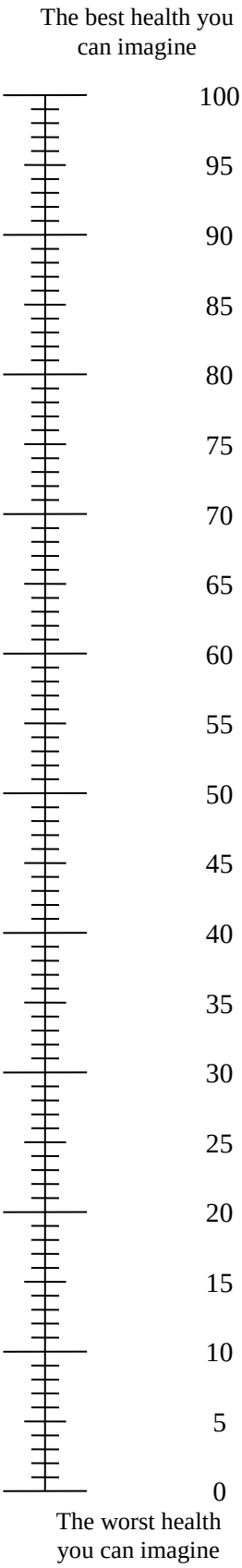
I am moderately anxious or depressed ❑

I am severely anxious or depressed ❑

I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the <u>best</u> health you can imagine.
  0 means the <u>worst</u> health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX 10: MPN-SAF QUALITY OF LIFE QUESTIONNAIRE

Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF)

Instructions: Please fill out all questions, as best able, reflecting how these symptoms affected you over the **LAST WEEK** unless directed otherwise. Complete forms until the STOP instruction toward the end of the packet.

Symptom	1 to 10 (0 if absent) ranking* 1 is most favorable and 10 least favorable
Please rate your fatigue (weariness, tiredness) by circling the one number that best describes your fatigue right NOW	(No Fatigue) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Please rate your fatigue (weariness, tiredness) by circling the one number that best describes your USUAL level of fatigue during past 24 hours	(No Fatigue) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Please rate your fatigue (weariness, tiredness) by circling the one number that best describes your WORST level of fatigue during past 24 hours	(No Fatigue) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Circle the one number that describes how, during the past 24 hours, fatigue has interfered with your	
• General activity	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
• Mood	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
• Walking ability	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
• Normal work (includes work both outside the home and daily chores)	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
• Relations with other people	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)
• Enjoyment of life	(Does not Interfere) 0 1 2 3 4 5 6 7 8 9 10 (Completely Interferes)

Circle the one number that describes how, during the past Week how much difficulty you have had with each of the following symptoms	
Filling up quickly when you eat (Early satiety)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Abdominal pain	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Abdominal discomfort	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Inactivity	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Problems with headaches	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Problems with concentration - Compared to prior to my MPD	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Dizziness/ Vertigo/ Lightheadedness	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Numbness/ Tingling (in my hands and feet)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Difficulty sleeping	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Depression or sad mood	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Problems with sexual desire or Function	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Cough	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Night sweats	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Itching (pruritus)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Bone pain (diffuse not joint pain or arthritis)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Fever (>100 F)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Daily)
Unintentional weight loss last 6 months	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
What is your overall quality of life?	(As good as it can be) 0 1 2 3 4 5 6 7 8 9 10 (As bad as it can be)

APPENDIX 11: SUMMARY OF NON-CLINICAL INFORMATION GUIDANCE FOR THE INVESTIGATOR

Tefinostat (CHR-2845) is an investigational compound in development for cancer.

Chemical and Physicochemical Description

CHR-2845 and CHR-2845.tartrate are white to pale yellow solids. CHR-2845 has low solubility in water (< 0.01 mg/mL), but is more soluble at pH 2.0 (10 mg/mL). Dry-fill, size 0, hard gelatin capsules containing 10, 40 and 80 mg CHR-2845 and 120 mg CHR-2845.tartrate are available.

Pharmacology

CHR-2845 is an HDACi with pleiotropic activity against a range of human cancer cells in vitro. It exerts profound anti-proliferative effects against monocytic tumour cell lines. As a consequence of the species specific cleavage of tefinostat by the monocyte-specific carboxylesterase and its instability in rodent plasma, evaluation of CHR-2845 in rodent models would not reflect its potential benefit in human disease.

Pharmacokinetics

The plasma concentration of CHR-2845 and its acid, CHR-2847, has been determined in PK studies. Bioavailability in the dog (47 %) is higher than in the monkey (6-13 %). Bioavailability in the monkey following administration of CHR-2845.tartrate is 9 % (equivalent to that with tefinostat freebase when administered as a suspension (10 %). CHR-2845 is rapidly converted to its acid metabolite, CHR-2847, in rodent plasma. The elimination half-life of CHR-2845 is 0.66 hours and 0.21 hours in the monkey and dog, respectively, and for CHR-2847 it is 1.23 and 1.1 hours for the monkey and dog, respectively. Following 28-day repeat dosing in the rat, CHR-2847 levels increased with dose (although not dose proportionally) and total exposure (AUC0-t) was approximately 50 % greater in females than males. Exposure to CHR-2847 doubled at the highest dose level (300 mg/kg/day) on Day 28 when compared to the Day 1 (lower doses were unaffected). Following 28-day repeat dosing in the monkey, CHR-2845 and CHR-2847 levels increased with dose (sub-dose proportionally in males and supra-dose proportionally in females), with total exposure (AUC0-t) 3-fold and 4-fold greater in the females for CHR-2845 and CHR-2847, respectively.

The data show that there is a sex difference in the TK parameters with greater exposure in females when dosed orally with CHR-2845 in monkeys. This pattern has not been observed in humans, although only a small number of patients have been evaluated to date.

Tefinostat is a moderate inhibitor (IC50 2.67 µM) of CYP3A4 with nifedipine as a substrate. Tefinostat inhibition of CYP3A4 was less potent when using testosterone and midazolam as substrates with IC50 values of 41.1 and 75.3 µM, respectively. Tefinostat did not inhibit the activity of any of the other CYP450 isoforms tested (IC50 > 100 µM). CHR-2847 is a weak inhibitor of CYP3A4 with nifedipine as a substrate, with an IC50 of 55.6 µM. CHR-2847 did not inhibit the activity of any of the other CYP450 isoforms tested (IC50 > 100 µM).

Based on these data, it is considered possible that tefinostat may participate in, or contribute to, drugdrug interactions which are mediated by CYP3A4.

Incubation of tefinostat with male CD-1 mouse, Sprague-Dawley rat, beagle dog, cynomolgus monkey and human hepatocytes resulted in measurable metabolism of tefinostat in all species. The rank order rate of metabolism of tefinostat was dog > rat > human > mouse > monkey. An examination of metabolic profiles by LC-MS/MS indicated that there is a similar pattern of metabolites in hepatocytes from all species investigated.

Toxicology

Toxicological data which are adequate to support early phase clinical studies have been generated. The available data comprise an evaluation of the potential to exert adverse pharmacological effects on the cardiovascular, respiratory and nervous systems, together with oral toxicity studies of 28 days duration in the rat and monkey, and evaluation of the potential to induce mutations in bacteria and chromosomal aberrations in cultured CHO cells.

There was no drug-related effect on hERG channel activity in transfected CHO cells. There were no adverse effects on a functional observational battery or motor activity in male albino rats given a single oral dose of up to 300 mg/kg. Single oral doses of up to 450 mg/kg were without effect on cardiovascular and respiratory parameters in conscious monkeys.

The acute median lethal oral dose for male and female rats is > 2000 mg/kg. During a 28-day oral toxicity study in the rat, animals treated at 300 mg/kg/day were noted to have salivation and fur/coat staining in the peri-oral area. Reduced body weight gain associated with reduced food intake was noted for males treated at 100 and 300 mg/kg/day. An increase in spleen weight at the end of the 28-day treatment period for animals dosed at 300 mg/kg/day. The study identified a potential to induce a reversible mild regenerative anaemia at 300 mg/kg/day. The no observable adverse effect level (NOAEL) for this study was considered to be 100 mg/kg/day.

During a 28-day oral toxicity study in monkeys, males treated at 450 mg/kg/day showed dehydration, whilst both males and females at this dose level showed a slight reduction in body weight, with recovery evident during the 28-day recovery period. As in the rat, there was evidence of a reversible, regenerative anaemia in all treated groups. Platelet count was increased for males at females treated at 150 and 450 mg/kg/day and activated partial thromboplastin times were longer for animals treated at 450 mg/kg/day. There was recovery from these changes following cessation of dosing. A decrease in lymphocytes in animals dosed at 450 mg/kg/day was noted at the end of the treatment period.

Although this was not statistically significant, it was still evident at the end of the 28-day recovery period. Clinical chemistry changes noted included increased total bilirubin (all dose levels), increased urea (females, all dose levels) increased asparatate aminotransferase, alanine aminotransferase and lactate dehydrogenase (450 mg/kg/day), decreased cholesterol (150 and 450 mg/kg/day) and increased creatinine (150 and 450 mg/kg/day). Decreased thymus weight was apparent at the end of the treatment period for all dose levels and was still evident at the end of the 28-day recovery period for females. Moderate or marked thymic atrophy was noted at 450 mg/kg/day with mild atrophy evident for individual animals dosed at 50 and 150 mg/kg/day. This finding was still evident in females at a reduced severity on completion of the 28-day recovery period. Accumulation of haemosiderin (mild or moderate) in Kupffer cells was present in the liver of animals treated at 450 mg/kg/day and females at 150 mg/kg/day with minimal accumulation observed sporadically in the remaining treatment groups. The finding was still evident in females at a reduced severity on completion of the 28-day recovery period. Minimal or mild centrilobular hepatocyte hypertrophy or minimal or mild hepatocyte degeneration was evident for individual animals at 150 and 450 mg/kg/day. These findings were not evident at the end of the 28-day recovery period. Mild periportal fatty vacuolation was observed at the end of the 28-day recovery period in one animal of each sex previously dosed at 450 mg/kg/day. A NOAEL could not be defined for this study.

Tefinostat appears to be devoid of mutagenic potential in bacteria and whilst it has been shown to induce a higher than control incidence of chromosomal aberrations in cultured CHO cells, this is only apparent at concentrations of 200 and 210 μg/mL (in the presence of S9 mix) which are toxic to the test cells.

The data suggest that subjects entered into clinical studies should be carefully monitored for evidence of haematological toxicity.

APPENDIX 12: SUMMARY OF CLINICAL INFORMATION GUIDANCE FOR THE INVESTIGATOR

Tefinostat is an orally-delivered histone deacetylase inhibitor targeted towards cells of the myelomonocytic lineage. Tefinostat was administered to humans for the first time in an open, uncontrolled, Phase I study. Cohorts of cancer patients with advanced or treatment refractory haematological diseases were administered increasing dose levels of tefinostat with the primary aim of assessing the safety and tolerability of the drug and finding a suitable dose and dosing regimen for future clinical studies.

The demographic characteristics of the patients suggest that they were broadly consistent with the patient group typically seen in clinical practice. The median age was 72 years (range 50 - 79). Eighteen patients entered the trial and all 18 patients completed at least the first 28-day cycle of treatment. The most common disease in this study was AML. There were four patients with diseases which might be expected to be driven by cells of the myelomonocytic lineage: two CMML patients and two AML-M5 patients.

Dose escalation followed steps of 100%, starting at 20 mg daily. There were no DLT during the study and dose escalation continued until the once-daily dose reached 640mg. This dose required patients to take 8 x 80 mg capsules and potential problems with patient compliance prevented any further dose escalations. In addition, there were early signs in the safety and pharmacodynamic data (increased plasma creatinine and decreased specificity of monocyte-targeting) that the dose of 640mg CHR-2845 resulted in exposure of levels of drug in tissues outside the hCE-1 expressing compartment that were capable of inhibiting HDAC. No MTD or MAD were identified in this study because dose escalation was halted prior to observation of DLT.

Dose-limiting toxicities that have been seen after exposure to non-targeted histone deacetylase inhibitors (e.g., fatigue, gastrointestinal toxicity, thrombocytopenia) were not seen after treatment with tefinostat, indicating that the targeted nature of the drug is able to provide maximal pharmacodynamic activity in the absence of significant toxicity.

Median duration of study treatment in this study was 58.5 days (mean 113) and ranged from 28 to 428 days. Ten patients were on study treatment for at least three cycles. As intra-patient dose escalation was allowed in this study, it is not possible to identify a single dose strength associated with extended treatment, however patients starting on 20, 40, 80, 160, 320 and 640mg all stayed on treatment for at least three cycles. Of the five patients who received 640mg (3 as an initial dose and 2 after dose escalation), 4 stayed on that dose for at least 3 cycles. The two patients that dose escalated to 640mg were treated for 5 and 6 cycles, respectively at 640mg.

In the haematology/biochemistry laboratory data, the only potentially dose-related signals observed were increases in plasma creatinine and magnesium at 640mg. The relatively small number of patients treated does not allow any firm conclusions to be drawn, except that tefinostat is well tolerated when taken once daily continuously for periods of up to 15 cycles, at doses of up to 320mg.

The adverse event (AE) profile of tefinostat during this trial, where there was at least a possible relationship to study drug included nausea (7 patients), anorexia (5 patients), rash (4 patients), fatigue (4 patients), constipation (3 patients) and increased blood creatinine (2 patients).

Nausea was seen in patients at all doses. Anorexia was seen in one patient at each of the 160 mg and 640 mg dose levels, and in three patients at the 320 mg dose level. Constipation (3 out of 5 patients) and increased blood creatinine (2 out of 5 patients) were only seen in patients treated with 640 mg.

Ten patients had a total of 22 SAEs during the study. Three of these patients had SAEs that were considered at least possibly related to study drug, 1 each in the 20mg (PR prolongation), 80mg (mucosal inflammation)

and 160mg (asthenia, anorexia, confusional state) cohorts. Given the small number of events observed, it was not possible to discern any pattern in the occurrence of the treatment-related SAEs.

Routine assessments of cardiac function were based on ECGs. Analysis of ECGs taken throughout the study did not identify any patterns of cardiac toxicity. PK analyses in this study confirmed that oral absorption of tefinostat was adequate and that metabolism to the active metabolite, CHR-2847, was rapid. The relatively short plasma half-life of the drug suggests that no accumulation should have been apparent with repeat dosing. The evidence of plasma accumulation following repeat daily doses of 160mg or more suggested that terminal elimination half-lives longer than those directly measured may have prevailed at higher doses. Mean plasma levels (Cmax and AUC0-4) of tefinostat and CHR-2847 were dose-proportional over the range studied, indicating the drug was well absorbed. Relatively high inter-individual variability was observed.

Monocyte-specific protein acetylation of up to 7-fold greater than baseline (median, 2.2; range 0.6-6.9-fold) was demonstrated over a range of doses, from 40mg to 320mg once daily. The cohort averages for protein acetylation was significantly increased at all doses greater than or equal to 40mg.

For all doses greater than or equal to 40mg, the cohort fold-increase was in the range of 2.8 to 4.5-fold greater than baseline, with no increase with dose being observed. At 640mg, while there was significant protein acetylation in monocytes, protein acetylation in lymphocytes and granulocytes was also increased, although to a lesser extent than observed in monocytes. This reduced selectivity for monocytes indicated that systemic distribution, driven by the high plasma levels of the parent drug achieved at 640mg, resulted in increased protein acetylation in non-hCE1-containing cells.

Eighteen patients were treated over a total of 76 treatment cycles at an average of 4.2 cycles per patient (SE = 0.9, range 1-15 cycles). Patients who had above normal numbers of monocytes at screening were treated for an average of 5.5 cycles, while patients with below normal monocytes at screening were treated for an average of 2.5 cycles, although this difference was not statistically significant.

Of the four patients with diseases characterised by monocytosis (two AML-M5 and two CMML), one CMML patient achieved a bone marrow CR (20-80mg) and the other CMML patient remained stable for one cycle before withdrawing consent. Neither of the two AML-M5 patients demonstrated any response to treatment.

One further patient, with AML-M2 (80mg), had a >50% reduction in bone marrow blasts and clearance of blasts from the periphery, but was not recorded as a formal PR due to the lack of recovery from CTCAE grade 3/4 neutropenia and thrombocytopenia.

A number of patients were treated for extended periods of up to 15 cycles. The apparent disease stability indicated by these extended treatment periods may represent either the natural course of the disease or a treatment effect of tefinostat.

Future clinical studies will employ tefinostat.tartrate capsules. Although no clinical studies have been undertaken in patients with tefinostat.tartate capsules to date, PK data show the bioavailability of tefinostat when delivered as tefinostat.tartrate in capsules to monkeys (9 %) is equivalent to that of tefinostat administration as a suspension (10 %). The CHR-2845.tartrate data in monkeys also show reduced variability.

A Phase I/II trial is currently ongoing in hepatocellular carcinoma (HCC) patients. These patients have only limited treatment options in the UK. Sorafenib is the only registered treatment which provides a limited survival benefit, but it is not recommended for use in HCC by the UK National Institute for Health and Clinical Excellence (NICE). Therefore, further therapy options are attractive for patients.

This trial has the normal limitations of a Phase I trial including potential toxicity and lack of proven efficacy, nevertheless, in view of the lack of treatment options and the fact that no patterns of toxicity were

identified in the first clinical trial that would particularly disadvantage HCC patients, the proposed trial will have an acceptable benefit:risk profile for the patients and can be carried out safely in institutions with experience in Phase I trials.

The initial dose for the hepatocellular carcinoma trial will be 360mg, 56% of the highest dose used previously (640mg; a dose which did not result in any DLT). Both once and twice daily dosing will be investigated, and as a result, the total daily dose may exceed the total daily dose reached in the haematological malignancy trial. However, it should be noted that a MTD was not defined in study CHR-2845-001 due to an absence of DLT, thus no dose ceiling has yet been identified. All dose escalation decisions will be taken with due consideration given to an assessment of benefit : risk, and all patients will have regular and detailed safety assessments throughout the trial period. The dose escalation/cohort expansion trial design proposed will allow the generation of further safety data in order to proceed in an appropriately cautious manner in this patient population.