Standard Operating Procedure

Cancer Collaborative Biobank (CCB)

AZA-MDS-003 Processing Method

SOP 2017/##
Version No. 1.0

1 PURPOSE

The purpose of this method is to describe the AZA-MDS-003 PK processing methodology for the four (4) required collection time points and ten (10) possible optional time points (per patient) for the Celgene CC-AZA-MDS-003 Trial. There will be four (4) required time points for each patient (screening, C1D1, C3D1 and C6D1) with up to two (2) collections for the following time points: C1D1, C3D1 and C6D1. Each of these 3 time points requires sample processing for final off site analysis.

2 SCOPE

AZA-MDS-003 sample storage and processing will be performed by CCB staff for all Princess Alexandra patients enrolled in the Phase 3, Multicenter, Randomized, Double-blind Study to Compare the Efficacy and Safety of Oral Azacitidine Plus Best Supportive Care versus Placebo Plus Best Supportive Care in Subjects with Red Blood Cell Transfusion-dependent Anemia and Thrombocytopenia due to IPSS Lower-risk Myelodysplastic Syndromes.

The CCB is involved in processing and storage of the SM (Specimen)1/ PK plasma samples as well as storage only of the SM2/ PAXgene and SM3/Buccal swab samples collected for the **AZA**-MDS-003 trial.

- PAH Trial Coordinator: Louise Knopp Ph: Ext 2091
- PAH Trial Investigators Dr Tony Mills

It is expected that 3 patients will be accrued to this trial at Princess Alexandra Hospital over a 5 year period.

3 PRINCIPLE

Primary Objective:

To evaluate RBC transfusion independence in the 2 treatment arms (oral azacitidine plus best supportive care versus placebo plus best supportive care) in subjects with RBC transfusion-dependent anaemia and thrombocytopenia (platelet count ≤ 75 x 109/L) due to IPSS lower-risk MDS.

Secondary Objectives:

To evaluate in both treatment arms

- overall survival (OS);
- haematologic improvement-platelet response (HI-P);
- duration of RBC transfusion independence and time to RBC transfusion independence;
- progression to acute myeloid leukaemia (AML), and time to AM progression;

- haematologic improvement-erythroid response (HI-E);
- platelet-transfusion independence, duration of platelet transfusion independence, and time to platelet transfusion independence;
- hematologic response;
- clinically significant bleeding events;
- safety;
- health-related quality-of-life (HRQoL); and
- health care resource utilization.

Exploratory Objectives:

- To determine plasma concentration of azacitidine and explore exposure-response relationships of efficacy and safety endpoints;

  CC-486 (Oral Azacitidine)

- To evaluate molecular and cellular markers in the bone marrow at baseline that may provide further prognostic classification of MDS subtypes and potentially impact azacitidine efficacy;

To evaluate molecular and cellular markers in the bone marrow and/or peripheral blood at baseline and during therapy that may provide information on azacitidine mechanism of action and on-therapy markers predictive of response or relapse.

4 DEFINITIONS

PAH	Princess Alexandra Hospital
AML	Acute Myeloid Leukaemia
EDTA	Ethylenediaminetetraacetic acid
THU	Tetrahydrouridine
AZA	Azacitidine

5 REQUIREMENTS

5.1 Reagents and Consumables

- Lyophilized THU (10mg) (provided by Trial Coordinator)
- Methanol (e.g 2.5L Methanol by Analar Normapur Product#:20847-320)
- Tuberculin syringe (provided by Trial Coordinator)
- Distilled Water (e.g. UltraPure Distilled water by GIBCO Invitrogen, Reference #10977-015 500ml, DNA, RNAse free)
- Serological Pipettes (2ml and 5ml)
- 1000ul pipette
- 1000ul pipette tips
- 10mL polypropylene capped vial (provided by Trial Coordinator)

5.2 Equipment

- IKA Vortex Mixer
- Indelible pen Lumocolour Permanent Black Fine
  SDS Cat: 318-9
  Product code: 267790

6 SAFETY

Standard Personal protective equipment (PPE) should be worn at all times when handling biological specimens including, gloves, goggles and a laboratory gown.

7 TRIAL DESIGN (optional heading as needed)

Each time a new PAH patient is registered to the trial you will be notified by the PAH trials nurse.

The PAH trials nurse will also alert you of the incoming samples and will personally walk the samples to the CCB Laboratory as the samples must be processed and frozen within 30minutes of collection.

At the end of this trial an invoice for the appropriate number of patients will be sent to PAH Oncology.

8 SAMPLING and SAMPLE PREPARATION

Patient sample collections (SM1/AZA PK, SM2/PAXgene and SM3/Buccal swab) need to be organised to coincide with CCB opening hours. For SM1/AZA PK sample, though the patient's 1st and 2nd blood collections must be taken 2 hours apart, it is critical that any 1st post dose' blood collections are only scheduled to be taken after 9.00 AM and only up to 2.30 PM. This collection period allows for CCB to be on site, allows sufficient time for processing to occur and ensures a patients 2nd post dose' collection can still be taken and processed within CCB business hours, (Monday to Friday only not including public holidays)

8.1 Sample Collection Requirements

Sample to be collected	Time points[a]	Processing requirements
SM1/AZA PK Peripheral Blood in 1 x 3mL EDTA tube (with THU-Solution to be added by Trial	1. Cycle 1, Day 1 (required) a. 1st Post Dose Collection b. 2nd Post Dose Collection 2. Cycle 3, Day 1 (required) a. 1st Post Dose Collection b. 2nd Post Dose Collection 3. Cycle 6, Day 1 (required) a. 1st Post Dose Collection	Each time point will require: 1 x Primary PK plasma sample and; 1 x Backup PK plasma sample

Coordinator immediately after collection)	b. 2nd Post Dose Collection 4. Unscheduled (optional)[a] a. 1st Post Dose Collection b. 2nd Post Dose Collection	
SM2/PAXgene Peripheral Blood in 1 x 8.5mL PAXgene tube	Screening and 10 other optional[a] time points	Storage of collection tube
SM3/Buccal Swab DNA Genotek Oragene Saliva Collection Tube	Screening and 1 other optional[a] time point	Storage only

Note: 1st and 2nd Post Dose Collections should be collected at least 2 hours apart between 0.5 hours and 6 hours post dose.

Peripheral blood should be collected in EDTA tubes and must be processed and frozen as soon as possible- **within 30 minutes of collection**.

[a]See time point table (Section 8.2) for the required and optional time points for each sample

8.2 Time point Table

Possible Sample Time Points Arriving for the **AZA-**MDS-003 Trial

Time Point	Required Time Points			Optional Time Points		
	SM1 AZA PK Plasma	SM2 DNA PAXgene (Whole Blood)	SM3 Buccal Swab	Optional SM1 AZA PK Plasma	Optional SM2 DNA PAXgene (Whole Blood)	Optional SM3 Buccal Swab
Screening		√	√			
C1D1 -1st Post Dose	√					
C1D1 -2nd Post Dose	√					
C3D1 -1st Post Dose	√					
C3D1 -2nd Post Dose	√					
C6D1 -1st Post Dose	√					
C6D1 -2nd Post Dose	√					
C3 D1					√	
C3 D15					√	
C6 D1					√	
C6 D15					√	
C12 D1					√	
C12 D15					√	

C18 D1					√	
C24 D1					√	
C30 D1					√	
Unscheduled 1st post dose				√		
Unscheduled 2nd post dose				√		
Unscheduled No time given						√

9 PROCEDURE HAZARDS (optional heading as needed)

Nil

10 CALCULATIONS AND RESULTS (optional heading as needed)

Nil

11 PROCEDURE

NOTE: Timing is critical in this process.

11.1 Preparation of THU-Solutions

1 THU-Solution A

THU-Solution A is stored at -30℃ and expires two months from date of preparation and thus can be made up in advance.

a. Add 2mL methanol to the lyophilized THU using a tuberculin syringe. (The final concentration of THU-Solution A is 5mg/mL)

NOTE: Methanol is very hydroscopic (attracts water very readily) which reduces its organic solvent properties. Use fresh methanol to ensure THU is completely dissolved.

b. Mix vigorously and thoroughly using a vortex mixer for a few minutes until THU is completely dissolved.

c. Label the tube as below:
   - THU-Solution A
   - Date of Preparation:
   - Date of Expiry: (two months after preparation date)
   - Initials:

d. Store at -30˚C.

e. Fill out **AZA**-MDS-003 Reagent Log (Appendix 1)

THU-Solution A is then used to make THU-Solution B

2 THU-Solution B

THU-Solution B is stored at 4℃ and expires one day from date of preparation and thus must only be made up once the collection time and date is confirmed by the PAH trial coordinator.

a. Transfer 5mL of distilled water to the 10mL polypropylene capped vial.
b. Using a pipette add 300ul (0.3mL) of THU-Solution A to the 5mL of distilled water (The final concentration of THU-Solution B is 0.3mg/mL)
c. Label the tube as below:
    - THU-Solution B
    - Date of Preparation:
    - Date of Expiry: (i.e , date of expiration = date preparation +one day)
    - Initials:
f. Store at 4˚C.
g. Fill out **AZA**-MDS-003 Reagent Log (Appendix 1)

11.2 SM1/PK Plasma Samples

NOTE: Timing is critical in this process.

1 <u>Immediately before each Collection:</u>

a. The Trial Coordinator will call 30 minutes before collection to inform CCB staff that collection is going ahead as planned.
b. After hearing from the Trial Coordinator, load tuberculin syringes (one for each PK blood collection tube - one per patient per collection) with 0.1mL of THU-Solution B.
c. Store on wet ice until picked up by the Trial Coordinator.
d. Instruct the Trial Coordinator that they should also keep the PK collection EDTA tubes on wet ice for at least 15 minutes prior to blood draw.
e. Instruct the PAH Trial Coordinator that they must walk the samples down to CCB immediately (within 10 minutes of the blood collection taking place) after collection, to allow sufficient time for processing.

Note: If multiple tubes for other tests are also to be taken during this collection, it is suggested that a second person is used to administer the THU solution B to the 3ml EDTA blood sample, mix by inversion and directed to hand deliver the sample to the CCB on wet ice, so that the PK sample is received within the nominated time frame. Additional time needed by the Trial Coordinator to finish taking all the other tubes, plus label each, clean up and then deliver the sample will not allow sufficient time for the laboratory processing component to be performed. If that is not possible, the CCB cannot meet the laboratory sample storage requirements indicated in the trial protocol.

2 After Collection:

Time period from draw to freezing of plasma must be less than 30 minutes.

a. Prior to centrifugation, press “FT” (fast temp) on the centrifuge to allow the centrifuge to drop quickly to the required temperature.

b. Separate plasma from red blood cells by centrifugation at 4˚C for 10 minutes at 2000xg (Program G, Eppendorf 5810R centrifuge).
c. Transfer approximately equivalent volume of plasma into the provided cryovials, labelled Primary (Prim) and Back-up (B-up). Check the other details on the cryovials correspond with the patient and time point being processed.
d. Freeze immediately at -80˚C.

11.3 SM2/PAXgene samples

NOTE: Draw the DNA PAXgene samples last during the phlebotomy session.

1 Collect blood in one 8.5mL DNA PAXgene tube until vacuum is exhausted and blood ceases to flow
2 Gently invert 8-10X.
3 Place tube in a vertical rack at RT for 2-3 hrs.
4 Invert 8-10 times.
5 Immediately freeze at -80ºC freezer in a rack.
6 Do not place in Styrofoam as tube may crack.
7 PAXgene DNA tube must be frozen for at least 24 hrs before shipping.
8 Ship on dry ice to Covance CLS upon sponsor's request.

11.4 SM3/Buccal swab samples

1 Collect saliva into the provided Genotek Oragene Saliva Collection tube
2 Store the DNA Genotek Oragene Saliva Collection tube at room temperature (15-30°C) until ambient shipment to Covance upon Sponsor's request.

11.5 Sample Processing details, registration and storage

1 Complete the CCB **AZA**-MDS-003 Sample Processing Form (Appendix 2)
2 Enter the patient and sample details in the following logs:
    i. 'Master Daybook'
    ii. 'TEMP BOX LOG'
3 Return completed CCB **AZA**-MDS-003 Processing Form (Appendix 2), along with any other documentation which came with the samples, to the CCB office.
4 Once paper work has been received in the office the CCB sample coordinator will enter the patient and sample details in the following spreadsheet and database:
    i. '**AZA**-MDS-003 Registered Patients' spreadsheet
    ii. '**AZA**-MDS-003 Trial Samples' spreadsheet
5 Sign and date on the CCB **AZA**-MDS-003 Processing Form.
6 The patient's paperwork will then be filed in the **AZA**-MDS-003 Trial folder.
7 Sign and date on the CCB **AZA**-MDS-003 Processing Form.

Once dispatch has occurred update the **AZA**-MDS-003 Trial samples spreadsheet with date of dispatch.

12 QUALITY CONTROLS AND ACCEPTANCE CRITERIA

Nil

13 REPORTING

Nil

14 RECORDS

Nil

15 REFERENCES

Covance Central Laboratory Services Manual**: AZA-**MDS-003 – dated 05-Dec-12, provided by the PAH trials nurse

16 ASSOCIATED DOCUMENTS

Nil

17 AMENDMENT HISTORY

QIS2 Edition			
Version	**Date**	**Updated by**	**Amendments**
1	Feb 2016	Jana Dracopoulos & Josefina Thomas	First Issue
2	Sep 2016	Josefina Thomas	1. Changed Trial Study name from MDS-AZA-003 to AZA-MDS-003 in the whole document. 2. Deleted all reference to ALLG Tissue Bank, replaced with PAH Tissue Bank where appropriate 3. Updated the Purpose Section – changed the number of required time points and added optional time points 4. Changed the number of patients to be accrued and the time period of accrual. 5. Updated 5.1 Reagents and consumables. 6. Added the following specimen reference: SM 1 for PK plasma, SM2 for PAXgene and SM3 for Buccal Swab 7. Updated section 8 and Table in Section 8.1. 8. Added section 8.2 – Time point table 9. Updated 9.2.1- stressed importance of submitting samples to the laboratory immediately after collection 10. Updated Section 18 and Appendices 1 – 2 11. Change to Metro South template and delete all reference to PAH Tissue Bank, replace with CCB where appropriate.

18 APPENDICIES

18.1 Reagent Log for **AZA**-MDS-003 THU solutions

18.2 CCB **AZA**-MDS-003 Processing Form

18.3 AZA-MDS-003 Covance Central Laboratory Manual (removed from this template example)

18.4 Quote for Trial (removed from this template example)

18.5 E-mail correspondence regarding storage (removed from this template example)

18.1 Appendix 1 – Reagent Log for AZA-MDS-003 THU Solutions

Expiry of Lyophilized THU	Batch Number of Lyophilized THU	Date THU Solution A Made:	Expiry of THU Solution A^:	Initials:

THU Solution A

^THU Solution A Date of Expiry: two months after preparation date

THU Solution B

Expiry of THU Solution A:	Date THU Solution B Made:	Expiry of THU Solution B*:	Initials:

*THU Solution B Date of Expiry: date preparation +one day

18.2 Appendix 2 – CCB AZA-MDS-003 Processing Form

CCB AZA-MDS-003 PROCESSING FORM

□ **New Trial Patient**
□ **Existing Trial**

Patient Name/Initials: _____

Trial ID Number: _____

Date of Birth: _____

UR number: _____

Details on tubes checked by: _____

No. of PB EDTA tubes received: _____~_____**(mls)**

SM1/AZA PK

Processed by: _____

Date of collection: _____

Time of collection: _____

Time of receipt: _____

Time of processing: _____

Time plasma taken off red cells: _____

Time plasma frozen: _____

Number of plasma vials made: _____□ Haemolysed

Required Collection Time Points

Tick appropriate time point

□ Baseline/Screening
□C1D1 – 1st Post Dose Collection
□ C1D1 – 2nd Post Dose Collection
□ C3D1 – 1st Post Dose Collection
□ C3D1 – 2nd Post Dose Collection
□C6D1 – 1st Post Dose Collection
□C6D1 – 2nd Post Dose Collection
□**Optional Collection Time Points**:
indicate the time point below:

_____

SM3/Buccal Swab

Date of collection: _____

Time of collection: _____

Time of receipt: _____

SM2/PAXgene

Date of collection: _____

Time of collection: _____

Time of receipt: _____

Has collection tube been left to stand at room temperature for 2-3 hrs? _____

Time tube frozen: _____

TEMP BOX LOCATION

M80-___ Temp Box: _____ (plasma)
M80-___ Temp Box: _____PAXgene
RT- ___ Temp Box: _____Buccal Swab

Reagents

Lyophilized THU: Batch: _____
THU solution A: Expiry: _____
THU solution B: Expiry: _____

SAMPLES HAVE BEEN ALLOCATED TO:

TRIAL: AZA-MDS-003 Trial

SM1/AZA PK PLASMA: _____ Anticoagulant: _____ ☐ Haemolysed

SM2/PAXgene tube: _____

SM3/Buccal Swab:

Sample Receipt & Registration Stamp

Patients TB consent checked by:	All sample details registered in TB database by:		All sample details checked on tube by:		All samples stored, temp box log updated & paperwork signed & filed by:	
	Trial Bank	Tissue Bank	Trial Bank	Tissue Bank	Trial Bank	Tissue Bank
n/a		n/a		n/a		n/a

STANDARD OPERATING PROCEDURE DETAILS

Standard Operating Procedure Number
SP 2015/## (assigned by Policy Officer)

Standard Operating Procedure Name
Standard Operating Procedure Name

Policy Reference
PL 2015/##
Policy Name

Procedure Reference
PR 2015/##
Procedure Name

Supersedes
Nil

Standard Operating Procedure Author
Name, Title

Portfolio Executive Director
Name, Title

Approving Officer
Name, Title

Approving Date
Date

Effective From
Date

Date of Last Review
Date

Date of Next Review
Date (within next 3 years)