UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

# GENOME-WIDE ANALYSIS FOR GENOMIC ALTERATION IN ACUTE PROMYELOCYTIC LEUKAEMIA


PENYELIDIK

PROFESOR DR. ROSLINE HASSAN

PENYELIDIK BERSAMA

PROF. DR. ABDUL AZIZ BABA
PROF. DR. WAN ZAIDAH ABDULLAH
PROF. DR. HASNAN JAAFAR
PROF. MADYA DR. MUHAMMAD FARID JOHAN
PROF. MADYA DR. ADNAN MANSOR
DR. SARINA SULONG
DR. ZUBAIDAH ZAKARIA
DR. AZLAN HUSIN


2017

# RU GRANT
# FINAL REPORT FORM

Please email a softcopy of this report to rcmo@usm.my

| | |
|---|---|
| **A** | **PROJECT DETAILS** |
| i | **Title of Research:**<br><br>**Genome-wide Analysis for Genomic Alteration in Acute Promyelocytic Leukaemia** |
| ii | **Account Number:**<br>**1001/PPSP/812105** |
| iii | **Name of Research Leader:**<br><br>**PROF. DR. ROSLINE HASSAN** |
| iv | **Name of Co-Researcher:**<br><br>1. Prof Dr Abdul Aziz Baba<br>2. Prof Dr Wan Zaidah Abdullah<br>3. Prof Dr Hasnan Jaafar<br>4. Assoc. Prof. Dr Muhammad Farid Johan<br>5. Associate Professor Dr Adnan Mansoor<br>6. Dr Sarina Sulong<br>7. Dr Zubaidah Zakaria<br>8. Dr Azlan Husin |
| v | **Duration of this research:**<br><br>a) Start Date : 01 March 2012<br>b) Completion Date : 28 February 2015<br>c) Duration : 36 Months<br>d) Revised Date (*if any*) : 1. 01 March 2015 – 28 February 2016<br>2. 01 March 2016- 31 August 2016 |
| **B** | **ABSTRACT OF RESEARCH** |
| | *(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English. This abstract will be included in the Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)*<br><br>**Please refer to the Technical Report** |

## C BUDGET & EXPENDITURE

i

**Total Approved Budget :**

**Total Expenditure :**

**Balance :**

**Percentage of Amount Spent (%) :**

***# Please attach final account statement (eStatement) to indicate the project expenditure***

ii **Equipment Purchased Under Vot 35000**

| No. | Name of Equipment | Amount (RM) | Location | Status |
|---|---|---|---|---|
| | -NA- | | | |
| | | | | |
| | | | | |

***# Please attach the Asset/Inventory Return Form (Borang Penyerahan Aset/Inventori) – Appendix 1***

## D RESEARCH ACHIEVEMENTS

i **Project Objectives** *(as stated/approved in the project proposal)*

| No. | Project Objectives | Achievement |
|---|---|---|
| 1 | To characterize the loss of heterozygosity (LOH) and chromosome copy number (CN) in APML patients using SNP-array | Completed |
| 2 | To determine the FLT3 gene mutations in these patients | Completed |
| 3 | To investigate the role of miRNA expression in these subgroups of APML patients | Completed |
| 4 | To determine the underlying genomic signature of poor risk APML patients | Completed |
| 5 | To determine the association between the laboratory and genetic features giving rise to the outcome difference in these known good prognosis patients. | Completed |

ii

**Research Output**

**a) Publications in ISI Web of Science/Scopus**

| No. | Publication (authors,title,journal,year,volume,pages,etc.) | Status of Publication (published/accepted/ under review) |
|---|---|---|
| 1. | Characterizing *PML-RARα* isoforms of acute promyelocytic leukaemia (APL) in Malay patients. Rosline H, Abu Dzarr A, Azlan H, Rapiaah M, WZaidah A, Selamah G, Ang CY, Baba AA. Bangladesh Journal of Medical Science Vol. 13 No. 03 July 14. Page: 311-315 DOI: http://dx.doi.org/10.3329/bjms.v13i3. | Published |
| 2. | MiR-100 as potential biomarker in leukemias: a scoping review. **(Will be published in Leukemia Research).** | In progress |
| 3. | Characterization of microRNAs expression profiles and *in silico* identification of targeted genes and pathway involves in acute promyelocytic leukemia patients. **(Will be published in Experimental Haematology Journal).** | In progress |

**b) Publications in Other Journals**

| No. | Publication (authors,title,journal,year,volume,pages,etc.) | Status of Publication (published/accepted/ under review) |
|---|---|---|
| 1. | MicroRNAs As Potential Biomarkers in Acute Promyelocytic Leukaemia. Imilia Ismail, Sarina Sulong, and Rosline Hassan. New Journal of Science vol. 2014, Article ID 932342, 6 pages, 2014. doi:10.1155/2014/932342. | Published |
| 2. | Establishment of total RNA extraction method from formaline-fixed paraffin-embedded (FFPE) bone marrow sample for miRNA expression profiles. **(Will be published in Malaysia Journal of Applied Science).** | In progress |

**c) Other Publications**
*(book,chapters in book,monograph,magazine,etc.)*

| No. | Publication (authors,title,journal,year,volume,pages,etc.) | Status of Publication (published/accepted/ under review) |
|---|---|---|
| | | |

**d) Conference Proceeding**

| No. | Conference (conference name,date,place) | Title of Abstract/Article | Level (International/National) |
|---|---|---|---|
| 1. | The X$^{th}$ Malaysian National Haematology Scientific Meeting | Whole Genome Array-CGH Analysis in Acute Promyelocytic Leukaemia Patients: A Preliminary Study. | National |
| 2. | 6$^{th}$ International Symposium on Acute Promyelocytic Leukaemia | Acute Promyelocytic Leukaemia (APL) in Malays; A Single Centre Experience. | International |
| 3. | Human Genome Meeting (HGM) 2015 | MicroRNA Expression Profiles And Copy Number Alterations In Acute | International |

| | | | |
|---|---|---|---|
| | | Promyelocytic Leukaemia Patients: A Preliminary Study. | |
| 4. | 44th Annual Scientific Meeting International Society for Experimental Hematology (ISEH) | Differential Expression Profiles of MicroRNAs in Three Different Subtypes of Acute Promyelocytic Leukaemia Patients. | International |
| 5. | 13th Malaysian Society Of Haematology Annual Scientific Meeting | Identification Of Gene Expression Networks Associated With Acute Promyelocytic Leukaemia Patients By Pathway-Based Analysis. | National |
| 6. | 15th Annual Scientific Meeting, College of Pathologists, Academy of Medicine Malaysia | Over expression miR-100 and miR-125 and their role in the pathogenesis of Acute Promyelocytic Leukemia. | National |

***# Please attach a full copy of the publication/proceeding listed above***

**iii** **Other Research Ouput/Impact From This Project**
*(patent, products, awards, copyright, external grant, networking, etc.)*

| No. | Research Output |
|---|---|
| 1. | Cap dagangan (*trademark*) – Hemcopat Hematology Oncology Patient. |
| 2. | Provide a local database in the genetic of APML which can be used as the basis for future research and management of patients. |
| 3. | Jaringan penyelidikan dengan penyelidik luar, Prof. Olaf Heidenreich dari Newcastle University, United Kingdom. |
| 4. | External Grant - Geran Dana Penyelidikan Universiti (dari Universiti Sultan Zainal Abidin) **Title: Comparison of Gene Expression Profiles in acute and Chronic Myeloid Leukaemia Cell Lines by Using The Nanostring nCounter Analysis System** |
| 5. | Invited speaker at Nanostring Seminar & Workshop. Title of presentation: MicroRNA profiling in acute promyelocytic leukaemia using Nanostring nCounter system. |

## E HUMAN CAPITAL DEVELOPMENT

**a) Graduated Human Capital**

| Student | Nationality (No.) | | Name |
|---|---|---|---|
| | National | International | |
| PhD | | | |
| MSc | | | |
| Undergraduate | | | |

b) **On-going Human Capital**

| Student | Nationality (No.) | | Name |
|---|---|---|---|
| | National | International | |
| PhD | 1 | | 1. Imilia Ismail |
| MSc | | | |
| Undergraduate | | | |

c) **Others Human Capital**

| Student | Nationality (No.) | | Name |
|---|---|---|---|
| | National | International | |
| Post Doctoral Fellow | | | 1.<br>2. |
| Research Officer | | | 1.<br>2. |
| Research Assistant | 1 | | 1. Nurul Ain Fathma Abdullah<br>2. |
| Others (.....) | | | 1.<br>2. |

## F COMPREHENSIVE TECHNICAL REPORT

Applicants are required to prepare a comprehensive technical report explaining the project. The following format should be used (this report must be attached separately):

- Introduction
- Objectives
- Methods
- Results
- Discussion
- Conclusion and Suggestion
- Acknowledgements
- References

## G PROBLEMS/CONSTRAINTS/CHALLENGES IF ANY

*(Please provide issues arising from the project and how they were resolved)*

| H | RECOMMENDATION |
|---|---|
| | *(Please provide recommendations that can be used to improve the delivery of information, grant management, guidelines and policy, etc.)* |

**Project Leader's Signature:**

| I | COMMENTS, IF ANY/ENDORSEMENT BY PTJ'S RESEARCH COMMITTEE |
|---|---|

Endorsed closure of grant.

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**Professor Aida Hanum Ghulam Rasool**
MBBS( Flinders), PhD (USM)
Head of Pharmacology Department
School of Medical Sciences
Health Campus, Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

.....

**Signature and Stamp of Chairperson of PTJ's Evaluation Committee**

18th April 2017

**Name :** PROFESOR (DR) ROSLINE HASSAN
Chairman Of Research commitee
School Of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

**Date :**

.....

**Signature and Stamp of Dean/ Director of PTJ**

**Name :** PROFESOR (DR) SHAIFUL BAHARI ISMAIL
Dekan
Pusat Pengajian Sains Perubatan
Kampus Kesihatan
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan

**Date :**

# RU GRANT
# FINAL REPORT CHECKLIST

Please use this checklist to self-assess your report before submitting to RCMO.
Checklist should accompany the report.

| NO. | ITEM | PLEASE CHECK (✓) | | |
|---|---|---|---|---|
| | | PI | JKPTJ | RCMO |
| 1 | Completed Final Report Form | ✓ | ✓ | ✓ |
| 2 | Project Financial Account Statement (e-Statement) | ✓ | ✓ | ✓ |
| 3 | Asset/Inventory Return Form<br>*(Borang Penyerahan Aset/Inventori)* | ✓ | ✓ | ✓ |
| 4 | A copy of the publications/proceedings listed in Section D(ii) (Research Output) | ✓ | ✓ | ✓ |
| 5 | Comprehensive Technical Report | ✓ | ✓ | ✓ |
| 6 | Other supporting documents, if any | ✓ | ✓ | ✓ |
| 7 | Project Leader's Signature | ✓ | ✓ | ✓ |
| 8 | Endorsement of PTJ's Evaluation Committee | | ✓ | ✓ |
| 9 | Endorsement of Dean/ Director of PTJ's | | ✓ | ✓ |

4/5/17

UNIVERSITI SAINS MALAYSIA

**BORANG PENYERAHAN ASET / INVENTORI**

==========

**A. BUTIR PENYELIDIK**

1. NAMA PENYELIDIK : PROF. DR. ROSLINE HASSAN
2. NO STAF : AM5042/4
3. PTJ : PUSAT PENGAJIAN SAINS PERUBATAN
4. KOD PROJEK : 1001/PPSP/812105
5. TARIKH TAMAT PENYELIDIKAN : 30/08/2016

**B. MAKLUMAT ASET / INVENTORI**

| BIL | KETERANGAN ASET | NO HARTA | NO. SIRI | HARGA (RM) |
|---|---|---|---|---|
| | **-TIADA PEMBELIAN DIBUAT-** | | | |
| | | | | |
| | | | | |
| | | | | |

**C. PERAKUAN PENYERAHAN**

Saya dengan ini menyerahkan aset/ inventori seperti butiran B di atas kepada pihak Universiti:

PROFESSOR (DR.) ROSLINE HASSAN
Deputy Dean (Research)
School Of Medical Sciences
Health Campus
Universiti Sains Malaysia

( ) Tarikh: 30/3/2017

**D. PERAKUAN PENERIMAAN**

Saya telah memeriksa dan menyemak setiap alatan dan didapati :

☐ Lengkap
☐ Rosak
☐ Hilang : Nyatakan.....
☐ Lain-lain : Nyatakan .....

Diperakukan Oleh :

.....

Tandatangan Nama : .....
Pegawai Aset PTJ Tarikh : .....

***Nota** : Sesalinan borang yang telah lengkap perlulah dikemukakan kepada Unit Pengurusan Harta, Jabatan Bendahari dan Pejabat RCMO untuk tujuan rekod.

**UNIVERSITI SAINS MALAYSIA**
**JABATAN BENDAHARI**
**KUMPULAN WANG UNIVERSITI PENYELIDIKAN (RU)**
**PENYATA PERBELANJAAN SEHINGGA 28 MAC 2017**

RM

Jumlah Geran :

Peruntukan MAC 2012 :
(Tahun 1)

Peruntukan MAC 2013 :
(Tahun 2)

Peruntukan MAC 2014 :
(Tahun 3)

Ketua Projek : PROF. MADYA DR. ROSLINE HASSAN

Tajuk Projek: GENOME-WIDE ANALYSIS FOR GENOMIC ALTERATION IN ACUTE PROMYELOCYTIC LEUKAEMIA

Tempoh : 3 Tahun (01/03/2012-28/02/2015)
Lanjut Tempoh: 1 Tahun (01/03/2015-28/02/2016)

No. Akaun : 1001/PPSP/812105

| Kwgan | Akaun | PTJ | Projek | Peruntukan Projek | Perbelanjaan Terkumpul sehingga Tahun lalu | Peruntukan Semasa | Tanggungan Semasa | Bayaran Tahun Semasa | Belanja Tahun Semasa | Baki Projek |
|---|---|---|---|---|---|---|---|---|---|---|
| 1001 | 11000 | PPSP | 812105 | | | | | | | |
| 1001 | 14000 | PPSP | 812105 | | | | | | | |
| 1001 | 15000 | PPSP | 812105 | | | | | | | |
| 1001 | 21000 | PPSP | 812105 | | | | | | | |
| 1001 | 22000 | PPSP | 812105 | | | | | | | |
| 1001 | 23000 | PPSP | 812105 | | | | | | | |
| 1001 | 24000 | PPSP | 812105 | | | | | | | |
| 1001 | 25000 | PPSP | 812105 | | | | | | | |
| 1001 | 26000 | PPSP | 812105 | | | | | | | |
| 1001 | 27000 | PPSP | 812105 | | | | | | | |
| 1001 | 28000 | PPSP | 812105 | | | | | | | |
| 1001 | 29000 | PPSP | 812105 | | | | | | | |
| 1001 | 32000 | PPSP | 812105 | | | | | | | |
| 1001 | 35000 | PPSP | 812105 | | | | | | | |
| | 52000 | PPSP | 812105 | | | | | | | |

Technical Report

RU Grant

# Genome-wide Analysis for Genomic Alteration in Acute Promyelocytic Leukaemia


**Research Leader:**

**Prof. Dr. Rosline Hassan**

**Co Researcher(s):**

**Prof. Dr. Abdul Aziz Baba**

**Prof. Dr. Wan Zaidah Abdullah**

**Prof. Dr. Hasnan Jaafar**

**Assoc. Prof. Dr. Muhammad Farid Johan**

**Assoc. Prof. Dr. Adnan Mansoor**

**Dr. Azlan Husin**

**Dr. Sarina Sulong**

**Dr. Zubaidah Zakaria**

**SCHOOL OF MEDICAL SCIENCES**

**UNIVERSITI SAINS MALAYSIA**

## TABLE OF CONTENTS

## ABSTRAK

Leukemia promielositik akut merupakan sejenis leukemia mieloid akut dan dikaitkan dengan translokasi kromosom di mana ia menyebabkan terhasilnya protein PML-RARA. Walaupun translokasi kromosm ini telah disahkan sebagai penyebab kepada berlakunya leukemia, faktor-faktor lain seperti variasi dalam salinan nombor (CNV), ekspresi mikroRNA dan ekspresi gen mungkin memainkan peranan penting dalam patogenesis APL. Dalam kajian ini, kami menjalakan analisis CNV, analisis mutasi kepada gen FLT3, ekspresi profil miRNA dan ekspresi profil gen-berdasarkan analisis tapak jalan untuk memahami mekanisma disebalik perubahan genomik yang menyumbang kepada survival berbeza dalam sub kumpulan pesakit APL. Dalam analisis CNV, kehilangan sub band kromosom 5q13.2, 8p23.1 dan 16p12.3 didapati dalam enam daripada 8 kes (75%) dan pertambahan sub band kromosom 2p11.2 dan 14q32.33 didapati dalam semua kes (n=8). Analisis mutasi pada gen FLT3 tidak menunjukkan penemuan yang signifikan dalam semua sampel pesakit. Profil global miRNA dijalankan ke atas sampel APL menggunakan pendekatan mikroarray, diikuti oleh sistem Nanostring nCounter untuk mengesahkan keputusan yang diperolehi. Ekspresi miRNA daripada platform nCounter mendapati miR-100 adalah yang paling terekspresi dalam pesakit APL berbanding kontrol normal. Kami mengenalpasti kebanyakan gen yang diekspres adalah daripada tapak jalan RAS, MAPK, Apoptosis dan JAK STAT pada pesakit APL yang didiagnosa. Kesimpulannya, kajian ini menunjukkan mekanisma-mekanisma terlindung juga memainkan peranan penting dalam patogenesis APL. Dapatan ini memberi pandangan baru kepada peranan

miRNAs dan mRNA di dalam genetik APL dan mengetengahkan peranan mereka sebagai penanda aras kepada strata penyakit dan terapi target dadah.

## ABSTRACT

Acute promyelocytic leukemia (APL) is a subset of acute myeloid leukemias (AML), and is commonly associated with the presence of chromosomal translocations leading to the expression of the PML-RARA fusion protein. Although chromosomal translocation has been implicated in leukemogenesis, other underlying mechanisms such as copy number variation (CNV), microRNA (miRNA) expression and gene expression may also play important roles in the pathogenesis of APL. In this study, we performed CNV analysis, FLT3 mutation analysis, miRNA expression profiling and gene expression-pathway based analysis to understand the underlying mechanism of genomic alteration involve in contributing to the different survival in subgroups of acute promyelocytic leukaemia patients. In CNV analysis, chromosomal deletion on subband 5q13.2, 8p23.1 and 16p12.3 were commonly seen in six of eight cases (75%) and gain of 2p11.2 and 14q32.33 were found in all cases (n=8). Mutational analysis of FLT3 genes did not show any significant finding in all patients samples. Global miRNA profiling was performed on APL samples by using the microarray approach, followed by Nanostring nCounter system to validate the results. MiRNA expression profiles from nCounter platform revealed that miR-100 is most significantly upregulated in APL patients as compared to normal controls. We identified most differentially expressed genes in RAS signalling pathway, MAPK, Apoptosis and JAK STAT signalling pathway of APL at diagnosis patients. In conclusion, this study showed that other underlying mechanisms also play important roles in the pathogenesis of APL. These findings provide new insights into the role of miRNAs

and mRNA in the genetic origins of APL and highlight their potential as biomarkers for disease stratification and drug-targeted therapy.