LAPORAN AKHIR GERAN USM JANGKA PENDEK

DEVELOPMENT OF COMPARATIVE GENOMIC HYBRIDIZATION (CGH) TECHNIQUE FOR THE STUDY OF NASOPHARYNGEAL CARCINOMA (NPC)

Nama Penyelidik Utama :
PROF MADYA DR ZILFALIL BIN ALWI

Nama Co-Researcher:
DR SHAMIM AHMED KHAN
PROF MADYA DR HASNAN JAAFAR
DR NARAZAH MOHD YUSOFF
DR JAMES ASHMAN
DR HJH FAUZIAH MOHD IDRIS

No Geran :
304/PPSP/6131449

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

~~The specific biomarkers identified in this study can be used for earlier diagnosis and~~ prognosis of NPC

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana: NATASYA NAILI BT MUHAMAD NOR
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

IJAZAH SARJANA (GENETIK MANUSIA)-ONGOING

* Natasha B currently doing the remaining part of this project as her ongoing MSc and will complete the objective 3.

ii) Lain-lain:
Others

9. **Peralatan yang Telah Dibeli:**
Equipment that has been purchased

TIADA

Tandatangan Penyelidik
Signature of Researcher

17/5/09

Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

This project report has been assessed by an independent assessor and being reviewed by the research committee. The report is acceptable despite some limitation which has been highlighted to the researchers.

The output is also reasonable with one (1) publication in Jurnal of Science and Technology beside three oral presentations at national and international conference.

PROFESSOR AHMAD SHARI HALIM
Chairman of Research Commitee
School of Medical Sciences
Health Campus
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16150 Kubang Kerian, Kelantan.

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN/PUSAT
Signature of Chairman
[Research Committee of School/Centre]

9/9/09
Tarikh
Date

ABSTRACT

NPC is a disease in which malignant cells are formed in the tissue of nasopharynx. It is a highly prevalent disease in Southern China and Southeast Asia including Malaysia. CGH is a molecular cytogenetic technique which is used to identify imbalanced genetic alterations in this malignancy. Twenty eight samples were obtained. Out of this; twelve tumors were extracted from twelve NPC biopsies while twelve references DNA was extracted from twelve normal controls peripheral blood. Tumor DNA and normal DNA was labeled by nick translation method with green and red fluorescent dyes. These were hybridized at metaphase chromosomes DNA and counterstained with DAPI. Finally, the image was captured and analyzed. Chromosomal gains that were found in this study were 4q26, 11q13-q14, 9p13, 8q13-q22 and 10q22-q26 while chromosomal losses were found at region 20p12 and 13q21-q31. We believe this study has provided the platform for further investigations to locate possible tumor-suppressor genes and oncogenes in our NPC patients.

BORANG LAPORAN HASIL PENYELIDIKAN
PPSP

Tajuk geran: Development of Comparative Genomic Hybridization (CGH) Technique for the Study of Nasopharyngeal Carcinoma (NPC)
Penyelidik: Dr Shamim Ahmed Khan
Prof Madya Dr Hasnan Jaafar
Dr Narazah Mohd Yusoff
Dr James Ashman, Dr Hjh Fauziah Mohd Idris
Jenis geran: Geran Jangka Pendek
Tempoh geran: 2 tahun

Jenis laporan: Laporan Kemajuan ☐ Alatan di beli ☐ Ya:nyatakan.....
Laporan Akhir*: ☑ ☑ Tidak

OBJEKTIF SPESIFIK KAJIAN (sama spt dalam proposal asal)	**SECARA RINGKAS TERANGKAN PENCAPAIAN/HASIL**	**OBJEKTIF TERCAPAI ATAU TIDAK**
1. Untuk menggunakan teknik CGH pada pesakit NPC	semua sample NPC telah didiagnosakan melalui teknik CGH	Objektif tercapai
2. untuk mengetahui paten perubahan genetic pada pesakit NPC menggunakan teknik CGH	Didapati terdapat 4 pesakit daripada 12 pesakit mempunyai perubahan genetik	Objektif tercapai

- *Laporan Akhir perlu disertakan salinan manuskrip dan surat yang dihantar kepada mana-mana jurnal untuk penerbitan.*

Nama Penyelidik Utama (PI): Prof Madya Dr Zilfalil Alwi t.t.: Zilfalil
Tarikh: 17 Mei 2009

LIST OF PUBLICATION AND PRESENTATIONS

Publication

[1]HASNITA BT CHE HARUN, [2]SHAMIM AHMED KHAN, [3]HASNAN JAAFAR, [4]FAUZIAH MOHD IDRIS, [5]NARAZAH MOHD YUSSOF, [6]JAMES ASHMAN, [7]ZULKIFLEE SALEHUDDIN, [8]MOHD NIDZAM B MD TAHIR AND [1]ZILFALIL ALWI.
Title: Identification of Genetic Imbalances of Nasopharyngeal Carcinoma (NPC) by Comparative Genomic Hybridization (CGH)
Tentatively scheduled for publication in Pertanika Journal
Volume 17, Number 1 (Jan 2009) issue
(please see attachment)

Oral Presentations

[1]HASNITA BT CHE HARUN, [2]SHAMIM AHMED KHAN, [3]HASNAN JAAFAR, [4]FAUZIAH MOHD IDRIS, [5]NARAZAH MOHD YUSSOF, [6]JAMES ASHMAN, [7]ZULKIFLEE SALEHUDDIN AND [1]ZILFALIL ALWI.
Title: Identification of Genetic Imbalances of Nasopharyngeal Carcinoma (NPC) by Comparative Genomic Hybridization (CGH): A Preliminary Report.
Presented at 7th National Congress on Genetics
5-7 May 2007 at Kota Bharu Kelantan.
(please see attachment)

NATASYA NAILI M N[1] , SHAMIM A K[2] , HASNAN J[3], NIDZAM M M T[4], ZILFALIL B A[1]
Title: Characteristic and Trends of Nasopharyngeal Carcinoma (NPC) in Hospital Universiti Sains Malaysia (HUSM).
Presented at !3th National Conference On Medical Sciences 2008
22-23 May, 2008 at Health Campus, USM, Kelantan
(please see attachment)

International

[1]NATASYA NAILI MN, [2]SHAMIM AHMED KHAN, [3]HASNAN JAAFAR, [4]FAUZIAH MOHD IDRIS, [5]NARAZAH MOHD YUSSOF, [6]JAMES ASHMAN, [7]ZULKIFLEE SALEHUDDIN AND [1]ZILFALIL ALWI.
Title: screening of Malay Patients with Nasopharyngeal Carcinoma (NPC) Using Comparative Genomic Hybridization (CGH) Technique for Identification of Specific Genetic Alterations
Presented at International Joint Symposium
24-25 November 2008 at Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia.
(please see attachment)

UPM
UNIVERSITI PUTRA MALAYSIA
RESEARCH MANAGEMENT CENTRE (RMC)
Pusat Pengurusan Penyelidikan

PERTANIKA *UPM's Refereed Journal*

Our Ref.: UPM/PERJ/AUTHR-REVISED/ACK/JST/17/2007 **Priority Mail**
2 NOVEMBER 2007

Mrs. Hasnita bt Che Harun
Human Genome Centre
School of Medical Sciences
USM Health Campus
16150 Kubang Kerian
KELANTAN

Dear Mrs. Hasnita,

Pertanika JST/17/2007 — Receipt of Amended Manuscript

I thank you and really appreciate your submission of the amended manuscript entitled, **"Identification of Genetic Imbalances in Nasopharyngeal Carcinoma (NPC) By Comparative Genomic Hybridization (CGH)"** to my office on 22 October 2007 for publication in Pertanika.

I must emphasize that you have done a very good job responding to the three reviewers' concerns for the manuscript in the revision as suggested by them. I congratulate you for a job well done.

Your amended manuscripts will now be sent to the JST Editorial Board for their endorsement and will then be forwarded to the publisher. It is anticipated that your articles will be tentatively scheduled for publication in Volume 17, Number 1 (Jan) 2009) issue.

Thank you once again for considering Pertanika as your preferred journal.

Sincerely,

DR. NAYAN DEEP S. KANWAL
Executive Editor *(Pertanika)*

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2. Prof. Dr. Zulkifli Idrus, Director,
   Reseach Management Centre (RMC), UPM, 43400 UPM, Serdang, Selangor

Identification of Genetic Imbalances of Nasopharyngeal Carcinoma (NPC) By Comparative Genomic Hybridization (CGH)

Hasnita Bt Che Harun[1], Shamim Ahmed Khan[2], Hasnan Jaafar[3], Fauziah Mohd Idris[4], Narazah Mohd Yussof[5], James Ashman[5], Zulkiflee Salehuddin[6], Mohd Nidzam B Md Tahir[7], Zilfalil Alwi[1].

[1]Human Genome Centre, [2]Department Of Otorinolaringology, [3]Department Of Pathology, [4]Department Of Microbiology, School Of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan.

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ABSTRACT

Nasopharyngeal Carcinoma (NPC) is highly prevalent in Southern China and Southeast Asia. To unveil the molecular basis of this endemic disease, Comparative Genomic Hybridization (CGH) technique was used to identify imbalanced genetic alterations in this malignancy. Chromosomal gains that were found in this study were 4q26 (20%) and 11q13- q14 (20%), while chromosomal losses were 20p12 (40%) and 13q21-q31 (20%).

These preliminary results suggest that there may be activation of oncogene in the gain regions and suppression of tumor suppressor gene in the loss regions.

Introduction

Nasopharyngeal Carcinoma (NPC) is a common cancer in Southeast Asia and its prevalence is clearly affected by genetic background. More than 80% of patients achieve long term survival, if they are treated in the early stages. However, majority are diagnosed at late stage which makes treatment less effective and difficult. Unveiling the molecular basis of this cancer is important, in order to derive targets for novel therapeutic strategies and control of this disease. Investigations on susceptibility genes would be of interest. Some genetic changes such as allelic loss, chromosomal gains or losses and specific gene alterations were reported to occur frequently in NPC. However, little is known about specific gene(s) related to the genesis or progression of NPC. Chromosomal imbalances identifiable through Comparative Genomic Hybridization (CGH) may shed some light on common genetic alterations that may be of relevance to the onset and progression of NPC. So, a study was designed to identify the genetic imbalances in NPC employing CGH technique. This technique permits a rapid screening of genetic imbalances throughout the entire tumor genome without the need of prior knowledge of the genetic alteration to be investigated. Since its development, CGH has been applied to analyze genomic abnormalities in many types of solid tumours. Many previously unrecognized sites of recurrent genomic alterations which may harbor oncogenes or tumour suppressor genes have been detected by this approach, providing useful information on candidate chromosomal regions for further study.

Materials and methods

Patients with Nasopharyngeal Carcinoma (NPC), diagnosed clinically and histopathologically were enrolled into this study. Tumor DNA was extracted from NPC biopsies while reference DNA was extracted from normal controls peripheral blood. Then, tumor DNA and normal reference DNA were labeled by nick translation method with green and red fluorescent, respectively. Hybridization of red and green labeled DNA to metaphase spread was performed. DNA was counterstained with 4',6-diamidino-2-phenylindole (DAPI). Finally, the image was captured and then analyzed by Cytovision software (Applied Imaging).

Results and Discussion

Among our study subjects, we observed that Malays were higher than Chinese and there were no Indian patients. All the samples were Type I NPC which was Poorly Differentiated Carcinoma. The cases were all males with age between 55 and 65 years old. We also found that pattern of NPC in Kelantan population is in contrast with study that have been done by Nicholls et al in North American and Southern Chinese population. In North American population, 25% were type I, 12% were type II and 63% were type III. In Southern Chinese population, only 2% were type I, 3% were type II and 95% were type III. These finding may be due to the different population distribution in Kelantan. But larger sample size is needed to confirm this result.

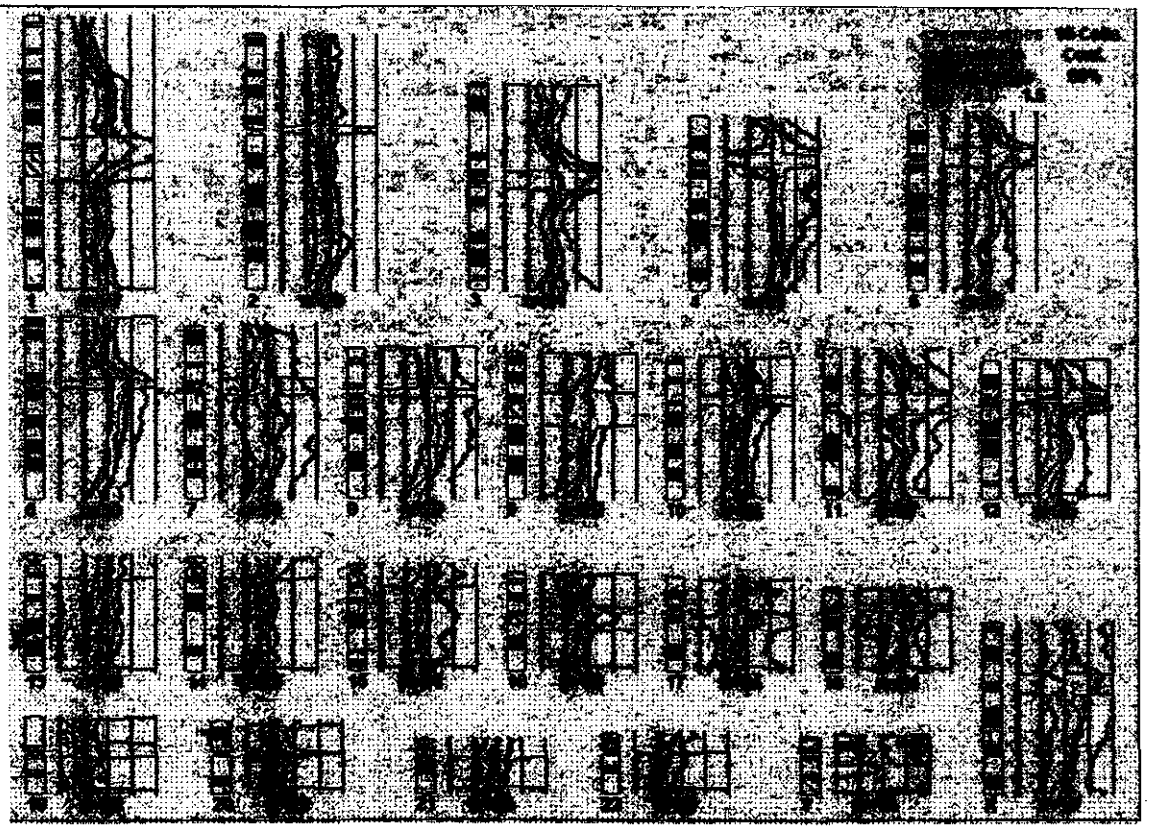

Figure 1: CGH profile of case 2. Fluorescent ratio profiles calculated are plotted alongside the chromosome ideogram. Red and green lines represent thresholds for chromosomal losses (0.75) and gains (1.25), respectively.

In this preliminary analysis, we identified chromosomal gains at 4q26 (20%) and 11q13 (20%) while chromosomal losses were identified at 20p12 (40%) and 13q22-q31 (20%) (Refer figure 1). It is presumed that there may be activation of oncogene in the gain regions and suppression of tumor suppressor gene in the loss regions. All chromosomal imbalances that were detected in this study were consistent with other study except for 20p12. Areas of gain such as 3p, 8q 12p, 12q or loss such as 14p and 14q were reported to represent earlier and more fundamental chromosomal aberrations that occur early during NPC oncogenesis. In Chen et al's (1999) study, on 51 NPC tumours, (25 primary

and 26 recurrent tumours) the most common copy number increases occurred on chromosome arms 12p (59%), 1q (47%), 17q (47%), 11q (41%) and 12q (35%). Chromosomal gain at 11q13 was the most frequent gain found in the study done by Hui et al (2005). Six oncogenes located at this region were MEN 1, CCND 1, FGF 3, EMS 1, GARP, and PAK 1 genes. In their study, CCND1 gene found to be the most frequent oncogene that was over express. In contrast, we found only 20% of the samples to have 11q13 gain. In our study, loss at 20p12 was the most frequent alteration. This finding maybe due to the different histological pattern found in our patient population. All the patients were keratinizing squamous cell carcinoma (Type I) NPC which is poorly differentiated subtype. In Hui et al study, most of the samples were Type III.

Conclusions

This preliminary analysis has demonstrated that CGH is an effective method to survey the NPC genome for chromosomal regions of variant DNA sequence copy numbers. This study has provided evidence for sites of several recurrent chromosomal abnormalilties including 4q26, 11q13, 20p12 and 13p22-q31. Further studies are warranted to identify regions most likely to contain genes of biological significance to NPC development and progression.

Acknowlegement

This study was supported by short term grant (304/PPSP/6131449) and Fundamental Research Grant Scheme (FRGS) (203/PPSP/6171001). We would like to thank ENT Department of Hospital Universiti Sains Malaysia (HUSM) and ENT Department of

Hospital Raja Perempuan Zainab II (HRPZII). We also would like to thank Advanced Dental And Medical Institute.

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th National Congress on Genetics

5-7 MAY 2007

IDENTIFICATION OF GENETIC IMBALANCES OF NASOPHARYNGEAL CARCINOMA (NPC) BY COMPARATIVE GENOMIC HYBRIDIZATION (CGH): A PRELIMINARY REPORT

alita bt Che Harun, [2]Shamim Ahmed Khan, [3]Hasnan Jaafar, [4]Fauziah Mohd Idris, Narazah Mohd Yussof, [5]James Ashman, [6]Zulkiflee Salehuddin and [1]Zilfalil Alwi

[1]man Genome Centre, [2]Department Of Otorinolaringology, [3]Department Of Pathology, partment Of Microbiology, School Of Medical Sciences, Health Campus, Universiti Sains laysia, Kubang Kerian, Kelantan, [5]Advanced Dental & Medical Institute (Clinical Centre), versiti Sains Malaysia, Pulau Pinang, [6]Department Of Otorinolaringology , Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan.

parative Genomic Hybridization (CGH) is a molecular cytogenetic technique that was fied from *in situ* hybridization technique. This technique was designed to compensate the ulties present in conventional cytogenetic and Fluorescent *In Situ* Hybridization (FISH) s not dependent on cell culture. CGH can also be performed on archival material and it es no prior knowledge of the genetic aberrations. This technique can be used to identify anced genetic alterations such as deletions, gains and amplifications. Patients with opharyngeal Carcinoma (NPC), diagnosed clinically and histopathologically were enrolled his study. This study was conducted to identify the pattern of genetic imbalances in NPC alaysia. Tumor DNA was extracted from NPC biopsies while reference DNA was cted from normal controls peripheral blood. Then, tumor DNA and normal reference A were labeled by nick translation method with green and red fluorescent dyes, ctively. Hybridization of red and green fluorescent labeled DNA to metaphase spread was rmed. DNA was counterstained with 4',6-diamidino-2-phenylindole (DAPI). Finally, the ge was captured and then analyzed. Chromosomal gains that were found in this study were 6 (20%) and 11q13- q14 (20%). Chromosomal losses that were observed in this study were 12 (40%) and 13q21-q31 (20%). This preliminary study postulates that there may be ivation of oncogene in the gain regions and suppression of tumor suppressor gene in the loss gions. Our findings may also, in future, provide a comprehensive profile of chromosomal gions showing losses and gain in NPC within the Malaysian population.

UNIVERSITI SAINS MALAYSIA

13th NATIONAL CONFERENCE ON MEDICAL SCIENCES 2008

"HEALTHIER COMMUNITY THROUGH CONTINUOUS INNOVATION AND EDUCATION"

HEALTH CAMPUS, USM, KELANTAN 22-23 MAY, 2008

OS-16

CHARACTERISTIC AND TRENDS OF NASOPHARYNGEAL CARCINOMA (NPC) IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

Natasya Naili MN, [2]Shamim A K, [3]Hasnan J, [4]Azriani A R, [1]Zilfalil B A

[1]Human Genom Center, [2]Department of Otolaryngology, [3]Department of Pathology, [4]Department of Community Medicine, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objective: To evaluate the characteristic and trend of NPC in patients registered for treatment at Hospital Universiti Sains Malaysia (HUSM) from January 1999 to December 2007.

Patients and Method: 106 patients with confirmed NPC were reviewed at HUSM, Kelantan over the time period from January 1999 to December 2007. These patients were from Kelantan, Terengganu, Pahang, Perak, Johor, Kedah and Sabah. The patients included in this study had histologically proven NPC according to the World Health Organisation (WHO) classification and the Tumor, Node, Metastasis (TNM) staging. We observed great difference in time in trend and characteristic of NPC in the populations. Their clinical records were reviewed and clinical data collected.

Results: The trends of NPC patients in HUSM are not constant. The number of patients shows a continuous rise and sudden drop. The Malay ethnic group showed highest number that attended HUSM. There were twice as many males as females. The highest mean age was in year 2000 which is 54.5 years. Majority of patients (46.2%) were from WHO type III classification which is different from previous study done in HUSM. Based on the TNM staging, 63.2% patients had reached stage IV. Most of the Kelantan patients (63.2%) were from Kota Bharu district which is the main district in Kelantan.

Discussion and Conclusion: Our result indicates that majority of the NPC patients attending HUSM were Malays. Over all number of new cases of NPC reporting to HUSM have significantly dropped from 2005-2007. The mean age for every year is between 40-55 years which is similar to many previous studies.

International Joint Symposium

Frontier In Biomedical Sciences: From Genes to Applications

Faculty of Medicine
Gadjah Mada University
Yogyakarta, Indonesia
24th and 25th November, 2008

PS – 02

Screening of Malay Patients With Nasopharyngeal Carcinoma (NPC) Using Comparative Genomic Hybridization (CGH) Technique for Identification of Specific Genetic Alterations

Natasya Naili M N[1] , Shamim A K[2] , Hasnan J[3], Fauziah M I[4], Narazah M Y[5], James A[5], Zulkiflee S[6], Zubaidah Z[7], Chin L P[7], Nidzam M M T[8], Zilfalil B A[1]
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Nasopharyngeal Carcinoma (NPC) is one of the most common cancers in Malaysia. Various genetic alterations including gain and losses in the chromosome of patients with NPC were reported in other population. However the patterns of chromosome aberration in Malaysian NPC patients have not been reported before. Comparative Genomic Hybridization (CGH) is a molecular cytogenetic technique that allows a rapid survey for regions of DNA sequences gain and loses. Using this technique we examined 8 samples with primary NPC tumors. The 8 samples were received from the hospitals at the north eastern region of Peninsular Malaysia. All patients were male and belong to Malay ethnic group. Based on WHO classification, 3 cases were classified type I NPC, 2 cases type II while 3 cases were type III. CGH was performed to screen the genetic alterations in NPC patients. We found chromosomal gains at region 4q26 (25%) and 11q13-q14 (25%) while chromosomal losses at region 20p12 (25%) and 13q21-q31 (25%) on patients 2. No chromosomes aberrations were found in other 7 samples. Contamination of tumor material with normal cells may be the cause of the failure to detect any abnormalities in other 7 samples. We believe this study has provided the platform for further investigations to locate any tumor-suppressor genes and oncogenes in our NPC patients.

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 31 JULAI 2008

Jumlah Geran:	RM	19,997.00	Ketua Projek:	DR. ZILFALIL ALWI
Peruntukan 2006 (Tahun 1)	RM	9,998.00	Tajuk Projek:	Development of Comparative Genomic Hybridazation(CGH) Technique for the study of Nasopharyngeal Carcinome (NPC)
		8,000.00		
Peruntukan 2007 (Tahun 2)	RM	1,999.00		
Peruntukan 2008 (Tahun 3)	RM	0.00	Tempoh:	15 Julai 06- 14 Julai 08
			No.Akaun:	304/PPSP/6131449

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSP	6131449		-	-	-	-	-	-	-
304	14000	PPSP	6131449		-	-	-	-	-	-	-
304	15000	PPSP	6131449		-	-	-	-	-	-	-
304	21000	PPSP	6131449		1,575.00	3,215.20	(1,640.20)	-	540.00	540.00	(2,180.20)
304	22000	PPSP	6131449		-	-	-	-	-	-	-
304	23000	PPSP	6131449		-	94.64	(94.64)	-	-	-	(94.64)
304	24000	PPSP	6131449		-	-	-	-	-	-	-
304	25000	PPSP	6131449		-	-	-	-	-	-	-
304	26000	PPSP	6131449		-	-	-	-	-	-	-
304	27000	PPSP	6131449		18,422.00	5,000.16	13,421.84	-	9,618.81	9,618.81	3,803.03
304	28000	PPSP	6131449		-	-	-	-	-	-	-
304	29000	PPSP	6131449		-	806.50	(806.50)	-	-	-	(806.50)
304	32000	PPSP	6131449		-	-	-	-	-	-	-
304	35000	PPSP	6131449		-	-	-	-	-	-	-
304	A11102	PPSP	6131449		-	-	-	-	-	-	-
					19,997.00	9,116.50	10,880.50	-	10,158.81	10,158.81	721.69