UK-Malaysia Bilateral Medical & Health Research Collaboration

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Hotel Istana
NIH is the research arm of the Ministry of Health (MOH) Malaysia (MOH: 141 hospitals, 1039 health clinics, 1821 community clinics, 2013)
Role of NIH: Carry out and translate research into clinical and public health practice
Institute for Medical Research

Founded as the Kuala Lumpur Pathological Institute in 1900

VISION

To be a world renowned institution for biomedical research

MISSION

Our Mission is to improve health by:

1. Carrying out quality biomedical research to address national health priorities
2. Providing specialized diagnostic services
3. Building national capacity through technology transfer and consultative services

Functions

- Research (biomedical)
- Specialised Diagnostic
- Training & Consultancies
NASOPHARYNGEAL CARCINOMA (NPC) Dr Alan Khoo

a major cancer in Malaysia but is less studied in the world

Ten most frequent cancers, all residence, Malaysia 2007

Breast
Colorectal
Trachea, Bronchus, Lung
Nasopharynx
Cervix, Uteri
Lymphoma
Leukaemia
Ovary
Stomach
Liver

Percentage of cancers (%)

Source: National Cancer Registry (2007)

National Cancer Institute (United States) Research Funding for Major Cancer Types (2012)

Breast Ca
Colorectal
Lung
NPC
Uterus/Cervix
Leukemia
Ovary
Liver

Funding (USD)

NB. Major cancer types – most frequent cancers in Malaysia
Source: FY 2012 Research Funding by Cancer Type, NCI

Total Clinical Trials by Major Cancer Types Registered at clinicaltrials.gov website

Breast Ca
Colorectal
Lung
NPC
Uterus/Cervix
Leukemia
Ovary
Liver

number of clinical trials

NB. Major cancer types – most frequent cancers in Malaysia
Source: www.clinicaltrials.gov (June 2013)

Total World Publications in PubMed on Major Cancers of Malaysia

Breast Ca
Colorectal
Lung
NPC
Uterus/Cervix
Leukemia
Ovary
Liver

Number of publications

NB. Total number of publications as determined by PubMed search for selected cancers as of November 2006. Results subject to limitations of search methodology
Nasopharyngeal carcinoma affects vulnerable groups

Odds ratio of risk of NPC (Lowest social class vs all others) = 4.1 (95% CI: 2.2-7.1)
Highly pronounced and significant even after adjustment for ethnic subgroup and diet. (NPC study in Malaysia - Armstrong et al., 1998)

Extremely high incidence rate in Malaysia

National Cancer Registry 2006, Ministry of Health Malaysia

High incidence in natives

Isolated Communities

Bidayuh in Sarawak, Malaysia

Zhuang, China

Naga, India

Paawen, Taiwan

All these communities have the highest NPC incidence in the world

NPC top cancer among 14-49 year age males

Figure 14: Ten most frequent cancers in males by age groups. Peninsular Malaysia 2006: Age 14-49 years old

Affects working men

Wee et al 2010

NPC

Working age group
# Nasopharyngeal carcinoma: needs for collaboration

<table>
<thead>
<tr>
<th>Established platforms / Preliminary findings / Available resources / known</th>
<th>Research questions</th>
<th>Needs for collaboration</th>
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<tbody>
<tr>
<td>• GAP: Extremely high incidence rate in natives of Borneo (e.g. Bidayuh) – cause unknown</td>
<td>• Risk and protective HLA types in local non-Chinese NPC?</td>
<td>Need experts in the HLA and cancer field to identify the risk and protective HLA types for NPC</td>
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<tr>
<td>• &gt;90% NPC cases are EBV+</td>
<td>• In order to develop cost effective screening programmes, the high risk groups should be identified. Is it possible to identify high risk groups using HLA typing?</td>
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<tr>
<td>• Established non-invasive screening tools for NPC utilizing EBV related markers</td>
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<td>• Association with specific HLA types are reported in Chinese NPC and EBV+ Hodgkin lymphoma.</td>
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<tr>
<th>GAP: EBV sequence variation in Malaysia not known</th>
<th>What are the EBV genome sequences associated with NPC?</th>
<th>Need experts in EBV and next-generation sequencing</th>
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<tbody>
<tr>
<td>• Recent literature suggests EBV in NPC may be distinct</td>
<td>• Are NPC associated EBV genome sequences different from those in other EBV associated malignancies like EBV+ Hodgkin lymphoma, EBV+ gastric cancer etc?</td>
<td>EBV pulldown assays which require low quantity DNA</td>
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<td>• EBV positive NPC tissue samples collected from a network of hospitals in Malaysia</td>
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Network of specimen and data collection sites for NPC studies

Malaysia — Highest in the world

Devi et al. 2004
### Nasopharyngeal carcinoma: needs for collaboration

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<td><strong>• GAP: Lack of new treatment for NPC</strong>&lt;br&gt;<strong>• GAP: High lymphocytic infiltrates in NPC suggests importance of microenvironment but not enough is known for development of therapy</strong>&lt;br&gt;<strong>• GAP: Lack of cell lines/xenographs for NPC research</strong>&lt;br&gt;  - No cell lines available commercially&lt;br&gt;  - most lines shared between researchers are suspected to be a fusion with Hela cells (ie not pure NPC cells)&lt;br&gt;  - the sole EBV positive cell line and xenograft line currently available internationally to researchers found to be possibly contaminated with exogenous virus&lt;br&gt;  - many attempts to culture cell lines/xenografts by many others failed&lt;br&gt;  - Unique new validated xenograft lines established from Malaysian NPC cases (not available elsewhere)&lt;br&gt;  - New <em>In vitro</em> and <em>in vivo</em> assays - including 3D and <em>in vivo</em> metastatic models adapted for xenograft cells&lt;br&gt;  - Laser capture microdissected samples of NPC microenvironment&lt;br&gt;  - NGS data of NPC</td>
<td><strong>• Role of tumour microenvironment in the pathogenesis of NPC</strong>&lt;br&gt;  - To understanding the biology (including viral, host and tumor interaction)&lt;br&gt;  - Identification of potential targets for therapy</td>
<td>Scientific collaboration with EBV/NPC experts</td>
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Nasopharyngeal carcinoma: needs for collaboration

Unique patient-derived xenograft (PDX) pair – NPC xenograft + EBV positive DLBCL (diffuse large B-cell lymphoma)-like xenograft from a single NPC specimen (HLA compatible pair) and new assays adapted for xenograft cells – preliminary work at IMR

Xenograft cells adapted for *in vitro* co-culture

NPC-GFP-luc2

3D spheroids

NPC 3D culture in 384 well-plate format

Orthotopic xenograft

Metastatic model

Subrenal capsule xenograft models for tissue interaction studies *in vivo*

and panel of NPC-derived EBV+ DLBCL-like xenografts
### Established platforms / Preliminary findings / Available resources / known

<table>
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<th>Chromosomal translocations in adult and childhood leukaemia</th>
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<td>- We received leukaemia diagnostic samples from all over the country (2008 – present)</td>
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<td>- Cases: Mainly AML, ALL and CML</td>
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<td>- Total number of samples: 2126</td>
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**Established Platform:**

1) **HemaVision kit HV01-28N**
   - Detects 28 chromosomal translocations and more than 145 breakpoints associated leukaemia

2) **AML mutation study**
   - Common AML mutation: FLT 3 ITD/D835 Mutation, NPM 1, c-KIT, CEBPA mutation
   - Received AML samples from all over the country (2014 – present)
   - Total number of samples: 

**Preliminary findings:**

1) Leukaemia translocations in Malaysia
3) Common genetic mutation in AML patients
4) High resolution array CGH/SNP/ exome sequencing

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- **Genomic characterization in adult and childhood leukaemia**
  - Whole exome sequencing: Implications for clinical assessment and prognostication to predict clinical outcome
  - Clonal heterogeneity and clonal evolution in Relapsed adult and childhood leukaemia

**Potential biomarkers**

1) Circulating MiRNAs and protein profiling as potential biomarkers

**Drug discovery and gene therapy**

1) Discovery of targeted novel drugs
2) Targeted oncogenes silencing in both adult and childhood leukaemia
3) Phenotypic screening and molecular signature of leukaemic stem cells for future targeted therapy

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- Whole genome/exome sequencing and bioinformatics expertise
- Drug discovery
- Gene silencing
- Development of in vivo and in vitro leukemic stem cell model
Types of translocation in IMR: 2008 – 2014 (ALL, AML, CML)

- t(9;22) (q34;q11): 42%
- t(1;19) (q23;p13): 3%
- inv(16) (p13;q22): 4%
- t(6;9) (p23;q34): 1%
- t(6;11) (q27;q23): 3%
- t(8;21) (q22;q22): 12%
- t(15;17) (q24;q21): 12%
- t(11;19) (q23;p13.1): 1%
- t(11;19) (q23;p13.3): 1%
- t(10;11) (p12;q23): 2%
- t(4;11) (q21;q23): 2%
- del1 (p32): 2%
- t(6;11) (p22;q23): 2%
- t(9;9) (q34;q34): 1%
- t(9;11) (p22;q23): 2%
- t(9;12) (q34;p13): 1%
- t(11;19) (q23;p13.3): 1%

n = 526
Types of childhood leukaemia

- ALL: 40%
- AML: 18%
- CML: 7%
- OTHERS: 35%

Chromosomal translocation in childhood ALL: 2008 - 2011

- BCR-ABL1: 8.3%
- ETV6-RUNX1: 7.0%
- TCF3-PBX1: 1.7%
- STIL-TAL1: 2.6%
- MLL-MLLT10: 1.7%
- PML-RARA: 1.3%
- CBFB-MYH11: 1.3%
- RUNX1-RUNX1T1: 0.9%
- MLL-AFF1: 0.9%
- TCF3-HLF: 0.4%
- Negative: 73.8%

n=1141

n=229
Leukaemia translocation study: AML cases

AML cases: 2008-2015

AML Translocations: 2008-2015

- t (6;9) 2%
- t (8;21) 37%
- t (15;17) 48%
- inv (16) 13%

n= 847

AML mutations: 2014

- FLT3 ITD: 9.4%
- FLT D835: 3.3%
- NPM1: 15.6%
- C-KIT: 9%
- CEBPA: 34%

n=380
Leukaemia / lymphoma research in IMR

• **ALL**
  – Comprehensive Analysis for Cancer Predisposition Genes Involved in Adult ALL Using Multiple Ligation-Dependent Probe Amplification (MLPA)
  – High Resolution Genome-Wide Array Analysis of ALL and AML Based on Malaysian Genetic Profiles
  – Gene Expression Profiling in childhood ALL
  – Identification of Genomic Aberrations in Adult ALL using high Density SNP Arrays
  – Effect of TERT and TERC Gene Expressions in Regulating the Development and Progression of ALL Cells
  – Genomic Characterization and Whole Exome Sequencing of Philadelphia Positive in Adult ALL

• **AML**
  – Mutation detection in AML
  – High Resolution Genome-Wide Array Analysis of ALL and AML Based on Malaysian Genetic Profiles
  – Genome-Wide DNA Methylation Profiling on AML Patients in Malaysia
  – Identification of Clonal Evolution in Relapsed AML Using High Throughput Exome Sequencing

• **CML**
  – Gene Silencing of Multidrug Resistant Genes in Leukemia Cells
  – MicroRNAs in Adult Patients with CML in Response to Imatinib Treatment for Quantitative Expression Profiling

• **Lymphoma**
  – Gene Rearrangement using Fluorescence in situ Hybridization (FISH) in the diagnosis of Burkitt's Lymphoma.
  – Identification of Novel Biomarkers in B-Cell Lymphoma
• *E. denticulatum* species of red seaweed commonly known as *E. spinosum* and one of the primary sources of iota carrageenan that has commercial value and usually exploited for food products and cosmetic products
• It appears in a variety of shades from brown to green to red
• Exists naturally in the Philippines, tropical Asia, and the western Pacific
• Seaweed for this study was collected from East Coast of Malaysia (Semporna, Sabah)
Results

- *E. denticulatum* inhibits Alpha-amylase (67-80%)
- Ethanolic, fiber and powder extracts showed potent lipase inhibitory activity
- Showed high antioxidant capacity by ORAC analysis
- *E. denticulatum* ethanolic extracts showed the presence of carotenoids, PUFAs and MUFAs
- Inhibited LPS/IFN induced IL-1beta, MCP-1, IL-6, TNF-alpha, nitric oxide on RAW 264.7 cell line
- Promotes glucose uptake in 3T3-L1 cell lines
- Enhanced adipogenesis activity in 3T3-L1
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| **1) E. denticulatum** ethanolic, powder, and soluble fibre extracts inhibited porcine pancreatic lipase enzyme (60-80%) | 1) Isolation of active compound  
2) The mechanism of pancreatic lipase and α-amylase inhibition by this seaweed extracts is unknown  
3) In-vivo studies would be required to evidence weight loss, glucose lowering effect, confirm acceptability, and record any side effects of seaweed-enriched foods | 1) To further on isolation of active compound in the red algae and followed by study on the mechanism of lipase and α-amylase inhibition  
2) To collaborate for in-vivo models |
| **2) E. denticulatum** ethanolic extract inhibited starch digestive enzyme, α-amylase (67-88%) | | |
| LC-MS/MS analysis of ethanolic extracts showed the presence of carotenoids, PUFAs and MUFAs | The are many unknown compounds present in the extract | Further study to characterize the unknown compounds |
Orthosiphon stamineus as a potential anti-diabetic drug in Gestational diabetes mellitus: Dr Husin and Dr Fara

- Orthosiphon stamineus (OS) is a popular folk medicines widely used to treat many diseases including diabetes in Malaysia.

- The anti-diabetic properties of OS noted in previous animal studies, however the mechanisms are not fully elucidated particularly in GDM.

- OS could indeed be a potential source of a specific oral hypoglycaemic agent to treat glucose intolerance in pregnancy and the findings may be important and useful for clinical trial and drug development with the aim to improve health outcomes for patients.
TOXICITY STUDIES ON OS CONDUCTED IN IMR

GENOTOXICITY

• OS is not mutagenic in the *in vitro* Salmonella/microsome assay and *in vivo* mouse bone marrow micronucleus assay.

PRENATAL DEVELOPMENTAL TOXICITY

• OS was found to be non-toxic to pregnant rats.
• OS did not cause embryofetal deaths, prenatal growth retardation.
• OS did produce some degree of structural ossification, however these variations do not affect the well being of the foetuses.
• NOAELs- Maternal toxicities: > 2000 mg/kg BW of OSAE
  Prenatal toxicities : > 1000 mg/kg BW of OSAE
# [DIABETES]: needs for collaboration

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<td>1. OS chloroform extract sub-fraction 2 on streptozotocin-induced diabetic rats significantly reduced the blood glucose level.</td>
<td>• To identify the anti-diabetic and toxicity effects of <em>Orthosiphon stamineus</em> extract in type 2 diabetic and Gestational diabetes mellitus streptozotocin-induced Sprague Dawley (SD) rat</td>
<td>• To learn and investigate the effect of OS on the levels of CD163 (macrophage-specific risk-predictor for developing Type 2 Diabetes mellitus) from placenta of Gestational Diabetic mellitus rats</td>
</tr>
<tr>
<td>2. OS ethanol extract and isolated sinensetin compound had inhibitory activities on α-glucosidase and α-amylase</td>
<td>• To understand the mechanisms of anti-diabetic properties associated with stimulation of insulin release, glucose utilization in target tissues and to identify the gene expression analysis.</td>
<td></td>
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</tbody>
</table>
Thank you
Biobank

Bar coding of specimens

Specimen storage systems

Database to track specimens
Molecular / Cell-based assay

InCell 2000 Analyzer (GE Healthcare)

Multimode microplate reader

Robotic liquid handling system (Hamilton)

Flow cytometry and sorter

Automated fluorescent microscopy, live cell imaging, spectral imaging, confocal microscopy, Metamorph software modules

Impedance based real time cell analyzer
Laser capture microdissection

Autostainer

Staining chamber

Upright microscope with Digital sight

Automated IHC scorer

Microtome, floating bath
Genomics Facility

Next Generation Sequencer - Miseq

Microarray scanner

PCR machines, Covaris, Qiacube automated DNA /RNA extraction machine
Animal Facility