Project number: T12-401/16-W

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *1 December 2016*
Project completion date: *30 November 2021*

1. Project Title: Plasma DNA as a platform technology for cancer detection

2. Names and Academic Affiliations of Project Team Members[#]

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the whole project period
Project Coordinator (PC)	LO Yuk-ming Dennis / Li Ka Shing Professor of Medicine	Department of Chemical Pathology, CUHK	10
Deputy Project Coordinator and Co-PI	CHIU Wai-kwun Rossa / Choh-Ming Li Professor of Chemical Pathology	Department of Chemical Pathology, CUHK	10
Co-Principal Investigator(s)	MOK Shu-kam Tony / Li Shu Fan Medical Foundation Professor of Clinical Oncology	Department of Clinical Oncology, CUHK	3
	CHAN Tak-cheung Anthony / Li Shu Fan Medical Foundation	Department of Clinical Oncology, CUHK	3

	Professor of Clinical Oncology		
	KWONG Yok-lam / Chui Fook-Chuen Professorship in Molecular Medicine	Chair of Haematology and Haematological Oncology, University of Hong Kong	3
	CHAN Kwan Chee / Professor	Department of Chemical Pathology, CUHK	10
	VAN HASSELT Charles Andrew / Professor	Department of Otorhinolaryngology, Head and Neck Surgery, CUHK	3
	SUN Hao / Professor	Department of Chemical Pathology, CUHK	3
	NG Siu-man Simon / Professor	Department of Surgery, CUHK	3
	CHAN Lik-yuen Henry / Professor#	Department of Medicine and Therapeutics, CUHK	3
	WONG Wai Sun Vincent / Mok Hing Yiu Professor of Medicine	Department of Medicine and Therapeutics, CUHK	3
	MA Buig-Yue Brigette / Professor	Department of Clinical Oncology, CUHK	3
	CHAN Lam Stephen / Professor	Department of Clinical Oncology, CUHK	3
	JIANG Peiyong / Associate Professor	Department of Chemical Pathology, CUHK	8

Please highlight the approved changes in the project team composition and quote the date when the RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

Prof. Henry Chan had resigned effective on 31 October 2020. Prof. Vincent Wong, also a world-renowned hepatologist, has replaced Prof. Chan to continue the collaborative role in this project. The RGC approval date was 2 November, 2020

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. To derive the maximum amount of diagnostic information from cell-free nucleic acids in plasma, urine and other body fluids by studying the genomic, methylomic and transcriptomic aberrations associated with malignancies	1. 100%	1. Completed
1. Using the molecular profiles of cell-free nucleic acids to detect and determine the tissue of	2. 100%	2. Completed

Objectives*	Percentage achieved	Remarks**
origin of malignancy		
3. To generate new insights into the biology of plasma nucleic acids	3. 100%	3. Completed

* *Please highlight the approved changes in objectives and quote the date when the RGC granted approval of such changes.*

****** *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

6.1 What are the most exciting research accomplishments of the project?
(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.))

1. Pioneering the fragmentomics analysis of cell-free DNA
Our discovery of differential size profiles between tumour-derived and non-tumour-derived DNA and between maternal and fetal DNA in the circulation, as reported in our earlier TRS and AoE reports (T12-404/11 and AoE/M-04/06), has suggested nonrandom fragmentation of cell-free DNA. This has prompted us to further study the biology of fragmentation of cell-free DNA, which we describe as 'fragmentomics' analysis. In the current project, our work has led to the development of a number of fragmentomics markers with diagnostic implications for cancer detection and informing tissue of origin. These fragmentomics markers include preferred end, end motif and jagged end. Plasma DNA preferred end refers to the genomic locations genomic locations at which the plasma DNA were preferentially cleaved (Jiang et al. *Proc Natl Acad Sci U S A* 2018;115(46),E10925–E10933). End motif refers to the few nucleotides at plasma DNA ends regardless of the site of origin within the genome (in contrast to the plasma DNA preferred ends). Using the hepatocellular carcinoma (HCC) model, we have demonstrated a distinct plasma DNA end motif profile of HCC patients from healthy control subjects and patients with chronic hepatitis B infection. Specifically, the 4-mer end motif CCCA was reduced in patients with HCC compared to healthy controls. We have also validated the change in the plasma DNA end motif profile in other types of cancer including lung and colorectal cancer (Jiang et al. *Cancer Discov* 2020:10(5),664–673). Jagged end of plasma DNA refers to the presence of single-stranded protruding ends in double-stranded DNA molecules. We have developed a protocol to analyse the jaggedness of plasma DNA (or jagged index) through bisulfite sequencing and again demonstrated its diagnostic potential in the HCC model (Jiang et al. *Genome Res* 2020:30(8),1144–1153). Taken together, we have pioneered a new dimension of analysis of cell-free DNA with its research and clinical values being increasingly recognised. Recently, we have been invited by the journal Science for submission of a Review article to discuss the fragmentomics analysis, together with the epigenetic and topologic analyses, of cell-free DNA (Lo et al. *Science* 2021;372(6538):eaaw3616)

2. Development of a second-generation nasopharyngeal carcinoma (NPC) screening test that has already been launched for clinical service
Our previous large-scale study, supported by the earlier TRS funding (T12-404/11), has validated the utility of plasma Epstein-Barr virus (EBV) DNA for screening of NPC (Chan et al. New Engl J Med 2017; 377:513-522). The benefits of screening including early cancer detection and survival improvement were illustrated. This is the first demonstration in a prospective study that a liquid biopsy-based test could effectively achieve early detection of cancer with survival benefit. Our study has also served as a model study for evaluation of the clinical utility of circulating tumour DNA (ctDNA)-based screening test. With the funding support, we have carried out re-screening of the whole 20,000-subject cohort, which would address the important issue of the optimal screening interval using plasma EBV DNA. A manuscript on the findings of the re-screening study has been submitted and is now under review.

   In the previous prospective study, plasma EBV DNA testing was done via the conventional real-time polymerase chain reaction (PCR) and we have adopted a sequential testing

arrangement (4 weeks apart) to define screen-positivity in order to reduce the false positive rate. However, such testing arrangement would pose a logistic limitation. In the current project, we have explored the differentiating molecular characteristics of plasma EBV DNA between NPC and non-NPC subjects. We have identified distinct NPC-associated molecular profiles of plasma EBV DNA through targeted next-generation sequencing (NGS), including quantitation, size and methylation. Based on the discovery, we have developed novel diagnostic approaches that allow better differentiation of NPC and non-NPC samples. Through the quantitative and size analysis, we could reduce the false positive rate of NPC detection from 5.4% to 0.7% with a modelled positive predictive value (PPV) of 19.6% in the screening cohort, that is, almost double of that two time-point PCR-based testing reported before (11.0%) (Lam et al. *Proc Natl Acad Sci U S A* 2018;115(22):E5115-E5124). Importantly, this NGS-based test obviates the need for obtaining serial samples as adopted in the prospective screening study and facilitate population-wide testing. In addition, methylation analysis could further improve the false positive rate with a resultant modelled PPV of 35.1%, that is, triple of that two time-point PCR-based testing (Lam et al. *Nat Commun* 2019;10(1):3256). The novel technologies have been licensed to Take2 Health and the sequencing-based NPC screening test has now become clinically available and is branded as Prophecy™ (https://take2health.net/products-and-services/take2-prophecy/).

3. <u>Elucidated the model of nucleases in the fragmentation of circulating DNA</u>
With the realisation of non-random fragmentation of circulating DNA from the size analysis, we further studied the fragmentation biology of circulating DNA. We hypothesise that different types of nucleases may play a role in the generation and fragmentation of circulating DNA. To validate our hypothesis, we systematically evaluated the respective roles of deoxyribonuclease1-like 3 (DNASE1L3), deoxyribonuclease1 (DNASE1) and DNA fragmentation factor subunit beta (DFFB, also known as caspase-activated DNase) in circulating DNA fragmentation. We analysed the circulating DNA profiles between knockout mice deficient in each type of nucleases and their wildtype counterparts and proposed a stepwise model of plasma DNA fragmentation. We have demonstrated that the typical circulating DNA might be generated in two major steps: (1) intracellular DNA fragmentation by DFFB, intracellular DNASE1L3, and other nucleases, and (2) extracellular DNA fragmentation by serum DNASE1L3 and further degradation by DNASE1. Our work has established the link between nuclease biology and circulating DNA physiology with important implications to the field of liquid biopsy. This has demonstrated the potential of our proposed end motif analysis for investigating plasma DNA biology with diagnostic implications. Our work is published in the *American Journal of Human Genetics* (Han et al. *Am J Hum Genet* 2020;106(2):202-214) and it was selected as one of the 'Best of AJHG 2019 and 2020'

4. <u>Development of a novel methodology for direct detection of DNA methylation</u>
DNA methylation is a foundational biological process where methyl groups are covalently added to DNA molecules. DNA methylation is essential for normal development in mammals and is involved in disease pathogenesis including cancer development. The most common form of this process involves the generation of 5-methylcytosine (5mC). The conventional method of studying 5mC involves bisulfite treatment, followed by PCR or massively parallel sequencing (i.e. bisulfite sequencing (BS-seq)). Such method would, however, lead to severe DNA degradation. The project team developed a novel methodology to directly examine 5mC through single molecule real-time sequencing (SMRT). The so-developed holistic kinetic (HK) model holistically analysed kinetic signals of a DNA polymerase and sequence context for every base within a measurement window. A convolutional neural network was employed to train a methylation classification model for genome-wide 5mC detection. The sensitivity and specificity reached 90% and 94%, with a 99% correlation of overall methylation level with the conventional bisulfite sequencing. Importantly, this methodology has provided a robust system

for simultaneous genome-wide genetic and epigenetic analyses and could be applied on different biological samples including tumoural tissues. This study was published in the Proceedings of the National Academy of the United States of America (Tse et al. *Proc Natl Acad Sci U S A* 2021;118(5),e2019768118) and highlighted on the online news organisation GenomeWeb for its translational value (https://www.genomeweb.com/sequencing/hong-kong-team-develops-method-improve-pacbio-direct-cytosine-methylation-detection#.Y2tpPy8Rr9A).

5. Development of multiple novel technologies to inform the tissue of origin of plasma DNA
In the previous TRS project, we have developed the plasma DNA tissue mapping technology for deconvoluting the proportional contribution of different tissues or organs to the pool of plasma DNA (Sun et al. *Proc Natl Acad Sci U S A* 2015;112:E5503-12). In the current TRS project, we have further developed multiple technologies to infer the tissue of origin of plasma DNA. The above-mentioned fragmentomics marker, plasma DNA end motif, was shown to bear the tissue of origin information in the pregnancy and liver transplantation models. We have also studied the relationship between plasma DNA fragmentation and chromatin structure because of the tissue specificity of chromatin profiles of the tissues of origin. We have developed a novel approach termed orientation-aware fragmentation analysis of plasma DNA which includes the 5' and 3' end-orientation information of plasma DNA fragments for analysis. This was used to deduce the chromatin structure of the origin tissue (Sun et al. *Genome Res* 2019;29(3):418-427). Moreover, a novel method called Genetic-Epigenetic Tissue Mapping (GET-Map) analysis was developed (Gai et al. eLife 2021;10:e64356). In this GET-Map analysis, we incorporated the genotypic analysis into the methylation deconvolution to allow tissue deconvolution for each genetic component. This method might find its potential applications in cancer diagnostics and also transplantation medicine. In this collaborative work with Hannah Valentine of the National Heart, Lung and Blood Institute of USA, we evaluated such technology for detection of graft rejection in lung transplant recipients. In summary, we have developed multiple novel technologies for informing tissue of origin that is important for cancer diagnostics purpose.

6.2 What was the added value of the TRS funding, rather than standard project grant funding?
(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

The TRS funding support is crucial to maintaining the competitiveness of the project team in the hot field of circulating tumour DNA research, or liquid biopsy. Our previous work, also supported by the previous round of TRS funding, has demonstrated promising clinical values in cancer diagnostics and therefore attracted international commercial interests. As an example, our invention of genomewide cfDNA methylation as a generic method for cancer detection has been licensed to GRAIL Inc. Prof. Dennis Lo is a scientific co-founder of GRAIL. Using our core technology, GRAIL has launched the Galleri test, which is the first commercially launched multi-cancer early detection (MCED) test. The value of this technology can be seen by the acquisition of GRAIL by Illumina at the end of 2021 at a price of USD 8 billion.

With the intense competition from newcomers and existing research groups from all over the world, our team has remained competitive and continue to develop novel tests with intellectual property (IP) protection through patent filing. With the TRS funding, we are able to build a strong team that is composed of clinician-scientists, molecular biologists and bioinformaticians that contribute to the development of novel technologies. The funding duration of 5 years also allowed the team to focus on the research without the need for repeated applications for grants of smaller funding amount. The substantial funding amount enhanced our efficiency in addressing multiple interrelated research goals in parallel, so that we were able to file many

patent applications in a timely manner. Our strong IP position has given us a competitive edge among the different research groups. The funding amount also allowed prompt validation of a biological discovery in large sample cohorts that was resource-demanding. As illustrated in the example of our second-generation NPC screening test, the process from test development to launching of clinical service occurred within the project period. In summary, the importance of TRS funding support could not be overstated.

6.3 If the project has not met its original objectives, why?

All of the objectives have been met.

6.4 (a) Please provide details of each peer-reviewed journal publication arising directly from this project by completing the table at **Annex** *(one for each publication)*.

(b) Please provide details of recognised international conference(s) in which paper(s) related to this project was/were delivered by completing the following table:
(Please attach a copy of each conference abstract)

Month/Year/ Place	Title	Conference name	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of the RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
(Please refer to Section 7.2)						

(c) RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/ papers, please indicate and provide explanations.

UGC funding has been acknowledged in all the publications.

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

This TRS project allowed the team to forge strong collaborative relationships with biomedical experts across multiple fields. All the studies require the contributions from chemical pathologists, oncologists, molecular biologists, genomic scientists and bioinformaticians. Our studies usually provide the proof-of-concept for cancer diagnostics which could subsequently be validated in various types of cancer. As illustrated in our work published in *Cancer Discovery* (Jiang et al. *Cancer Discov* 2020:10(5),664–673) as one example, we have our validated our novel fragmentomics marker in different types of cancer, including liver, lung, colorectal and nasopharyngeal cancer. This has involved the collaboration with oncologists, hepatobiliary, colorectal and head and neck surgeons and hepatologists. Our work on urinary cell-free DNA involved urologists and renal physicians. For our population screening projects on NPC, we have collaborated with otorhinolaryngologists, biostatisticians and epidemiologists.

Our proposed methodologies for tissue of origin analysis of plasma DNA has also found its utility in transplantation medicine. We have collaborated with Hannah Valentine of the National Heart, Lung and Blood Institute of USA on applying plasma DNA tissue mapping analysis to detect early graft rejection in lung transplant recipients.

In cell-free DNA fragmentation, we have demonstrated a few key nucleases including DNASE1L3 that contribute to the fragmentation process in murine models. We have subsequently collaborated with researchers from New York University (Boris Reizis), the Istituto Giannina Gaslini in Italy (Stefano Volpi) and the Hospital for Sick Children in Canada (Linda Hiraki) to analyse the fragmentation profiles of plasma DNA from human subjects disease-associated variations of *DNASE1L3*, who have developed familial systemic lupus erythematosus.

In addition, because of the translational value of our work, this project brought opportunities to the project team for working more closely with the commercial sector, the biotechnology industry and the Hong Kong Science and Technology Parks.

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Weng In Sam CHU	PhD	1/8/2015	17/9/2019
Wai Kei Jacky LAM	PhD	1/2/2016	30/9/2020
Haiqiang ZHANG	PhD	1/8/2016	30/9/2020
Ze ZHOU	PhD	1/8/2017	31/7/2021
Chiu Tung Don CHAN	PhD	1/8/2018	31/7/2022
Lizhen Mary Jane MA	PhD	1/8/2018	31/7/2022
Chen Spencer DING	PhD	1/8/2018	31/7/2022
On Yee Olivia TSE	PhD	1/8/2018	31/7/2022
Diana Siao Cheng HAN	PhD	1/10/2018	30/9/2022
Meihui Emma CHEN	PhD	1/11/2018	31/1/2022
Wai Fung Jack CHEUNG	MPhil	1/8/2019	31/8/2021
Lilian Pok Wa ZHONG	MPhil	1/8/2019	31/12/2021
Yuwei Daisy GAO	PhD	1/8/2019	Pending
Guangya WANG	PhD	1/12/2019	30/9/2022

The team has developed talents in biomedical sciences for both academia and industry. After graduation, Sam Chu became the chief information officer of the Sanomics Limited, which is a local biotechnology start-up and more recently, he takes up the post of general manager in Aurora Tele-Oncology. Jacky Lam has become an assistant professor at CUHK and continues research on molecular diagnostics as a clinician-scientist in the team. Haiqiang Zhang now serves as an associate research fellow of biotechnology and a cancer multi-omics clinical research scientist at the Beijing Genomics Institute (BGI), one of the world's largest genome research organisations. Ze Zhou, Mary Jane Ma and Spencer Ding are now postdoctoral fellows in the team. Don Chan is now a research associate in the CUHK/Prince of Wales Hospital Partnering Centre under the Hong Kong Genome Project, which is a large-scale, government-led genomic medicine project. Olivia Tse is now a scientific officer in the Queen

Mary Hospital. Diana Han is a clinical lecturer in the team. Emma Chen takes up the post of Scientist II in a biotechnology company called Crown Bioscience in China. Both Jack Cheung and Lilian Zhong are now research assistants in the team.

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

- Novel bioinformatics algorithms to enable plasma DNA preferred end, end motif and jagged ends analyses
- Novel bioinformatics algorithms to enable plasma EBV DNA quantitative, size, methylation and genotypic analyses for nasopharyngeal cancer or other EBV-associated diseases diagnostics
  The sequencing-based NPC screening test described above has been licensed to Grail, Inc. and Take2 Health. Take2 Health is a new startup biotechnology company co-founded by Prof. Dennis Lo, Prof. Rossa Chiu and Prof. Allen Chan. Take2 Health has started to provide the NPC screening service in Hong Kong since May 2020.
- Novel methodology to enable direct detection of DNA methylation, called holistic kinetic (HK) model
- Novel bioinformatics algorithm to enable plasma DNA tissue of origin analysis using the orientation-aware nucleosome footprint approach
- Novel digital PCR assay for analysis of liver- and colon-derived plasma DNA
- Novel methodology termed as Genetic-Epigenetic Tissue Mapping (GET-Map) analysis to enable tissue of origin analysis of different genetic components of plasma DNA
- Novel methodology to enable topological analysis of plasma DNA

6.8 Other education activities and/or training programmes developed:

A symposium session each within the 22nd to the 24th Annual Scientific Symposia of the Hong Kong Cancer Institute and Immuno-Oncology Conference 2021 were organised and sponsored by this Theme-based Research Grant. The posters of these activities are included in the Appendix for reference.

In 2017, the 22nd Annual Scientific Symposium cum Breast Cancer Conference 2017 was on 11-12 November 2017 and the title of the sponsored session was “Translational Research in Breast Cancer”. The invited talks included “Preclinical data on a CDK inhibitor for treatment of triple negative breast cancer” by Jacqueline Jeruss, University of Michigan, USA and “Telomere length and breast cancer risk and prognosis” by Xiao-ou Shu, Vanderbilt University, USA.

In 2018, the 23rd Annual Scientific Symposium cum Immuno-Oncology Conference 2018 was on 10-11 November 2018 and the title of the sponsored session was “Basic Biology”. The invited talks included “Mechanisms of acquired resistance to immune checkpoint inhibitors” by Katerina Politi, Yale School of Medicine, USA, “Understanding the allele specific HLA loss and its implication on immune checkpoint inhibitor and cancer vaccine” by Charles Swanton, Cancer Research UK, UK and “Beyond immune checkpoint blockade: Emerging strategies” by Tak Mak, University of Toronto, Canada.

In 2019, the 24th Annual Scientific Symposium cum Breast Cancer Conference 2019 was on 19-20 October 2019 and the title of the sponsored session was “Translational Research in Breast Cancer”. The invited talks included “Gene expression signatures in ER+ node negative breast cancer” by Paul Pharoah, University of Cambridge, UK, “Immunotherapy in the neoadjuvant setting” by Hope Rugo, University of California, San Francisco, USA and “Risk

factors for breast cancer in Hong Kong women: a case control study" by Dr. Polly Cheung, Hong Kong Breast Cancer Foundation, HKSAR, China.

In 2020, the Immuno-Oncology Conference 2021 was on 17-18 April 2021. In the sponsored keynote session, the invited talks included "The birth of immunotherapy" by Gordon J Freeman, Dana-Farber Cancer Institute, USA and "Biological rationale personalized cancer vaccines in solid tumours" by Naiyer A Rizvi, Columbia University Irving Medical Center, USA.

In addition, departmental seminars have been organised and were well attended by academic staff and research postgraduate students at the Department of Chemical Pathology of CUHK. The details are as follows:

Date and Title of talk	**Speaker**
10 May 2017 "Treasure Hunt for the next big winner: Proteomics biomarkers for clinical unmet needs"	Professor Daniel W. Chan Professor of Pathology, Oncology, Radiology & Urology Director, Clinical Chemistry Division & Center for Biomarker Discovery and Translation Co-Director, Pathology Core Laboratories, Department of Pathology Johns Hopkins Medical Institutions, USA
21 July 2017 "There will be blood – a journey through genetics and epigenetics"	Professor Alexander Dobrovic Head, Translational Genomics and Epigenomics Laboratory, Olivia Newton-John Cancer Research Centre, Australia
14 December 2017 "Life at the single molecule level"	Professor Sunney Xie Mallinckrodt Professor of Chemistry and Chemical Biology, Department of Chemistry and Chemical Biology, Harvard University, USA
13 March 2018 "Human gene regulation: A journey from basic research to biomedical applications"	Professor Richard M Myers President, Science Director and Faculty Investigator, HudsonAlpha Institute for Biotechnology, USA
8 May 2018 "Ras GTPase-activating-like protein (IQGAP1) scaffolds intracellular signaling pathways – science and clinical implications	Professor David Barry Sacks Senior investigator, National Institutes of Health Clinical Professor of Pathology, George Washington University, USA
9 November 2018 "PREDICT: a Breast Cancer Prognostication and Treatment Benefit Model"	Professor Paul Pharoah Professor of Cancer Epidemiology, Strangeways Research Laboratory, University of Cambridge, UK
11 March 2019 "The surgical techniques and clinical application of nasal endoscopic nasopharyngectomy in nasopharyngeal carcinoma"	Professor Ming Yuan Chen Professor, Department of Nasopharyngeal Carcinoma Sun Yat-sen University Cancer Center, China
8 May 2019	Professor Allan Hildesheim

"Highlights of Findings from Nasopharyngeal Carcinoma (NPC) Studies in Taiwan and China"	Senior Investigator Division of Cancer Epidemiology & Genetics National Cancer Institute, USA
29 July 2019 "(Epi)Genomic predictors of disease biology in gastrointestinal cancer"	Professor Boon Ooi Patrick Tan Executive Director, Genome Institute of Singapore Director, SingHealth/ Duke-National University of Singapore, Precision Medicine Institute, Singapore

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

None

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

As mentioned in the previous review reports, the project team adopted the same management structure and scheme as the previous governance structure has been proven to be effective and efficient. The PC, Prof. Dennis Lo, led the project team, determined the project directions and set the priorities throughout the grant duration. Especially during the COVID outbreak period with social distancing requirements, the PC prioritised the different studies and adopted measures to minimise disruptions to laboratory work. Therefore, the team has remained productive despite the COVID outbreak. The PC held regular data review meetings on a frequent basis no less than once per week to monitor the research progress. While face-to-face meeting was used to be the main mode of communication between the PC and team members, it has been switched to electronic meeting after the COVID outbreak to ensure adequate supervision and guidance. Frequent communications with collaborators are conducted by the same means. Annually, all research team members and scientific advisory board (SAB) members are invited to attend a meeting (physical or video conference) where the project highlights of the year are presented. The SAB members for this project are:

* Richard M. Myers, PhD, President and Science Director, The HudsonAlpha Institute for Biotechnology, U.S.A.
* Patrick Tan, MD, PhD, Executive Director, Genome Institute of Singapore and Director, SingHealth/Duke-National University of Singapore Precision Medicine Institute, Singapore
* Toshikazu Ushijima, MD, PhD, Chief of the Division of Epigenomics, Senior Deputy Director, National Cancer Center Research Institute, Japan

Regarding knowledge transfer, the PC was active in disseminating the research findings by participating in many conferences and symposia around the world (see section 7.2). In addition, the PC was actively involved in the conceptualisation of new methodologies, patent filing and patent prosecution that has contributed to the team's strong IP position.

7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

As a result of the team's track record and scientific achievements, the team has received a contract research grant from GRAIL Inc. to enhance research support from September 2017 to August 2021. The grant covers the support for space, personnel of all levels, equipment, reagents and bioinformatics development.

In addition, the team has been awarded the InnoHK Research Grant of HK$483 million under the InnoHK Initiative of the Innovation and Technology Commission. The InnoHK is a major initiative by the HKSAR government to develop Hong Kong as the global innovation hub for research collaboration. With the funding support, we have set up the Centre for Novostics (novostics = novel diagnostics) that focuses on development of cutting-edge molecular diagnostics based on cell-free nucleic acids in blood and other bodily fluids. The novel diagnostic applications developed from the Centre will impact a wide range of fields, including oncology, prenatal medicine, transplantation and other diseases.

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Yes, the project team has participated in a total of 36 international meetings as invited speakers.

1. Lo YMD, "Breakthrough technology in cancer screening" in 2017 Oncology Research Symposium organized by Association of Clinical Research Professionals (ACRP), Hong Kong, 1 April 2017
2. Lo YMD, "Chromosomes to circulating DNA" in The 11th European Cytogenetics Conference, Florence, Italy, 1-4 July 2017.
3. Chiu RWK, Plenary: "Blood-based early detection of cancer – is it achievable?", Human Genome Society of Australasia 41st Annual Scientific Meeting, Australia, 5-8 August 2017.
4. Chiu RWK, "How to make a blood-based test for cancer screening", Australasian Society of Diagnostic Genomics Special Interest Group meeting, Brisbane, Australia, 5 August 2017.
5. Chiu RWK, "Teasing out the diagnostic information among circulating nucleic acids for cancer, prenatal and post-transplantation assessment", organized by the Australian Institute of Bioengineering and Nanotechnology, University of Queensland, Australia, 8 August 2017.
6. Lo YMD, "Cell-free DNA: from prenatal screening to molecular diagnosis of cancer", the 27th World Congress on Ultrasound in Obstetrics & Gynecology, Vienna, Austria, 16-19 September 2017.
7. Lo YMD, "Pushing forward the limit of plasma DNA-based molecular diagnostics" organized by Centre for Personalised Medicine, The Wellcome Trust Centre for Human Genetics, University of Oxford, UK, 25 September 2017.
8. Lo YMD, "Unlocking the power of circulating DNA for cancer detection", the 76th Annual Meeting of the Japanese Cancer Association organized by The Japanese Cancer Association, Yokohama, Japan, 28-30 September 2017.
9. Lo YMD, "Towards the use of plasma DNA for cancer screening", the 3rd Advances in Circulating Tumor Cells (ACTC): Liquid Biopsy in Clinical Practice, organized by University of Athens, Rhodes, Greece, 4-7 October, 2017.
10. Lo YMD, "Understanding circulating DNA through epigenomics", the International Human Epigenome Consortium (IHEC) Annual Meeting & Science Days 2017, Berlin, Germany, 12-14 October 2017.
11. Lo YMD, "Breaking the wall of early disease detection – How liquid biopsy can revolutionize medical diagnostics", Falling Walls Conference, Berlin, Germany, 8-9 November 2017.

12. Lo YMD, “Biology and diagnostics applications of circulating nucleic acids in plasma”, the Sunney & Irene Chan Lecture In Chemical Biology 2017 organized by Department of Chemistry and School of Life Sciences of CUHK, Hong Kong, 13 November 2017
13. Lo YMD, “Circulating DNA for cancer screening”, the Human Genome in Healthcare Conference, London, UK, 23-24 November 2017.
14. Chiu RWK, TB Teoh 2017 Lecture: “From prenatal to cancer assessments: the power of Precision Diagnostics”, Hong Kong College of Pathologists, Hong Kong, 25 November 2017.
15. Chiu RWK, “Screening and identification of early cancers: Is it achievable?”, Global Congress on Biotechnology: Diagnostics and Therapeutics, Hong Kong Biotechnology Organization, Hong Kong, 23 November 2017.
16. Lo YMD, “Unlocking the hidden diagnostic potential of circulating DNA”, Institute of Biomedical Sciences Special Seminar, Academia Sinica, Taipei, Taiwan, 7 December 2017.
17. Lo YMD, “The epigenetics of plasma DNA and its applications” in The Joint Royal Society of Medicine (RSM) and Pathological Society 2018 Winter meeting: Epigenetics – understanding disease and guiding therapies, London, UK, 25-26 January 2018.
18. Lo YMD, “Unlocking the diagnostic potential of circulating DNA” in 2018 Lupus Research Alliance Human Immunology and Technology Meeting, Miami, USA, 28-30 January, 2018.
19. Lo YMD, “Early detection of cancer by circulating DNA” in Days of Molecular Medicine (DMM) 2018: “Emerging Asian Epidemic of Cancer and Heart Disease”, organized by the University of Hong Kong (Hong Kong), Days of Molecular Medicine Global Foundation (Cambridge, USA), Karolinska Institutet (Stockholm, Sweden), in collaboration with the Fondation IPSEN (Paris, France), Hong Kong, 1-2 March 2018.
20. Lo YMD, “Towards cancer screening using circulating DNA” in American Association for Cancer Research (AACR) Annual Meeting 2018, Chicago, USA, 17 April, 2018
21. Lo YMD, “Towards cancer screening using circulating DNA” in the 11th International Symposium on Minimal Residual Cancer (ISMRC 2018), Montpellier, France, 3-5 May 2018
22. Lo YMD, “Towards cancer screening using circulating DNA” in 2nd BCM-CUHK-Fudan Joint Symposium in Clinical Genetics and Birth Defects cum 14th Asia Pacific Congress in Maternal Fetal Medicine, Hong Kong, 19-20 May 2018.
23. Lo YMD, “Towards cancer screening using circulating DNA” in Future Forum Cities Summit – Shenzhen, Shenzhen, China, 26 May, 2018
24. Lo YMD, “Plasma EBV DNA for screening nasopharyngeal carcinoma” in The 2018 Gordon Research Conference on Nasopharyngeal Carcinoma, Hong Kong, 24-29 June 2018.
25. Lo YMD, “Pushing forward the frontiers of molecular diagnostics”, Keynote Lecture, The 2018 Global Innovation Summit and the 26th Annual Convention of Chinese Association for Science and Technology USA, New Haven, USA, 12-14 October 2018.
26. Lo YMD, “Pushing forward the frontiers of circulating DNA research and applications”, Keynote Lecture, Association of Chinese Geneticists in America (ACGA) 2018 Annual Meeting, San Diego, USA, 18 October 2018.
27. Lo YMD, “Unlocking the diagnostic potential for circulating DNA”, Keynote Lecture, 22nd Annual Meeting of the Sociedade Portuguesa de Genética Humana (Portuguese Society of Human Genetics)”, Porto, Portugal, 15-17 November 2018.
28. Lo YMD, “Screening for nasopharyngeal cancer using plasma Epstein-Barr virus DNA: Technology and clinical insights”, Plenary Lecture, American Association for Cancer Research (AACR) Annual Meeting, Atlanta, USA, 29 March – 3 April 2019.
29. Lo YMD, “Cell-free DNA in plasma: New knowledge in biology drives new applications”, Closing Plenary Lecture, Interdisciplinary Congress in Human Genetics, Madrid, Spain, 5 April 2019.
30. Lo YMD, “Pushing forward the diagnostic applications of cell-free DNA in plasma”, Plenary Lecture, 6th Macau Symposium on Biomedical Sciences 2019, Macau, 12-13 June 2019.

31. Lo YMD, “New developments in circulating DNA based molecular diagnostics”, Plenary Lecture, Advances in Genome Biology and Technology (AGBT) 2019 Precision Health Meeting, La Jolla, California, USA, 5-7 September 2019.
32. Lo YMD, “Plasma DNA fragmentomics and topologics: understanding of biology drives new applications”, Plenary Lecture, The 30th Annual Meeting of the Japanese Society for Gastroenterological Carcinogenesis, Yokohama, Japan, 7-8 November 2019.
33. Lo YMD, “Plasma DNA-based molecular diagnostics: fragments, circles and beyond”, Opening Keynote Lecture, AACR Special Conference on Advances in Liquid Biopsies, Miami, Florida, USA, 13-16 January 2020.
34. Chan KCA, “Screening for nasopharyngeal cancer in asymptomatic men by liquid biopsy”, 79th Annual Meeting of the Japanese Cancer Association, Hiroshima, Japan, 1-3 October 2020.
35. Lo YMD, "Recent developments in plasma DNA: fragments, circles and scissors" and "My journey from a medical student to a clinician-scientist", 創新系列雲上大講堂, Beijing Advanced Innovation Center for Structural Biology, Tsinghua University, 14 October 2020.
36. Lo YMD, "Screening for nasopharyngeal carcinoma using plasma Epstein-Barr virus DNA", The 4th International Head and Neck Cancer Symposium, 12 December 2020.

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?

Yes, Prof. Dennis Lo and Prof. Rossa Chiu are both Associate Editors of Clinical Chemistry, the No. 1 ranked journal with regard to impact factors by the Institute of Scientific Information in laboratory medicine. Prof. Lo is also a Senior Editor of *eLife* and an associate editor of a Nature partner journal, *Genomic Medicine*. Prof. Lo is an editorial board member of the *Journal of Pathology, Prenatal Diagnosis, Philosophical Transactions of the Royal Society B*, *Disease Markers, Journal of Genomes and Exomes*, and *Marrow*. Prof. Chiu is an editorial board member of *Clinical Chemistry, Critical Reviews in Clinical Laboratory Sciences, Prenatal Diagnosis, Clinical Biochemistry,* and *The Clinical Biochemist Reviews*. Prof. Allen Chan is an editorial board member of *Expert Review for Molecular Diagnostics*.

Prof. Dennis Lo is a Trustee of the Croucher Foundation and has been serving as a council member of the Research Council as well as the co-chairman of the Grant Review Board of the Food and Health Bureau of Hong Kong since 2012. Prof. Rossa Chiu was a member of the Research Grants Council biology and medicine panel (Joint Research Scheme) between 2012 to 2017. She is a member of the Innovation and Technology Fund research projects assessment panel (biotechnology), Innovation and Technology Commission, Government of HKSAR, since 2017. Prof. Allen Chan has been serving as a member of the Grant Review Board and the Expert Advisory Panel (Cancer) of the Food and Health Bureau of Hong Kong since 2015 and 2018, respectively

7.4 Any documentary proof of the application of technologies arising directly from this project?

The successful licensing of many of the team’s patents and patent applications on circulating tumour DNA analysis would be one important proof of the application of technologies developed. Examples include the above-mentioned invention of genomewide cfDNA methylation as a generic method for cancer detection which has been licensed to GRAIL Inc. Prof. Dennis Lo is a scientific co-founder of GRAIL. Using our core technology, GRAIL has launched the Galleri test, which is the first commercially launched multi-cancer early detection (MCED) test. Other examples include the second-generation NPC screening test and the novel methodology for direct detection of DNA methylation (Holistic Kinetic model) being licensed to Take2 Technologies, our commercial partner. The NPC screening service, branded as

Prophecy™, has been commercially launched in May 2020. (https://take2health.net/products-and-services/take2-prophecy/).

The team's strong IP position is reflected by the recognition of Prof. Dennis Lo as the "Top 20 Translational Researchers" in the world by the journal *Nature Biotechnology* for 5 consecutive years from 2016 to 2020.

7.5 Other awards and recognitions as a result of this project (please specify):

Prof. Dennis Lo, Prof. Rossa Chiu and Prof. Allen Chan have been named as one of the "Top 20 Translational Researchers" by the authoritative scientific journal *Nature Biotechnology* for 2020. It is the fifth consecutive year for Prof. Lo to receive this honour. These Top Translational Researchers were selected based on the number of granted patents awarded during the year. This honour reflects the importance of this TRS grant support in facilitating new innovation.

To highlight, Prof. Lo has won multiple prestigious international awards, including the 2022 Lasker~DeBakey Clinical Medical Research Award, the 2021 Breakthrough Prize in Life Sciences (also known as the 'Oscars of Science'), the 2021 Royal Medal in Biological Sciences by the Royal Society of London (Prof. Lo is the first Chinese scientist to receive the honour) and the inaugural Future Science Prize in Life Sciences in China in 2016.

Other awards won by the team members are listed below.

- Prof. Dennis Lo has been awarded the 2017 IFCC Distinguished Clinical Chemist Award, organized by International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), Durban, South Africa
- Prof. Dennis Lo has been nominated as the Sunney and Irene Chan Lecturer in Chemical Biology 2017, organized by Department of Chemistry and School of Life Sciences, The Chinese University of Hong Kong and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong
- Prof. Dennis Lo has been selected for the WuXi PharmaTech Life Science and Chemistry Awards – Outstanding Achievements Award, organized by WuXi AppTec, Beijing, China
- Prof. Rossa Chiu has been nominated as the 2017 TB Teoh Lecturer by the Hong Kong College of Pathologists
- Prof. Dennis Lo has been elected as Fellow ad eundem, organized by Royal College of Obstetricians and Gynaecologists, London, UK
- Prof. Dennis Lo and Prof. Rossa Chiu were named as Leaders of the Year 2017 - Commerce and Industry / Finance Category, organized by Sing Tao News Corporation Limited, Hong Kong
- Prof. Dennis Lo has been awarded the 2018 InnoStars Award, organized by Our Hong KonG Foundation, Hong Kong
- Prof. Dennis Lo and Prof. Allen Chan have been awarded The Chinese Society of Clinical
- Oncology (CSCO) Annual Achievement Award 2018
- Prof. Dennis Lo has been awarded the Extraordinary Scientific Innovation Award, The
- Chinese Association for Science and Technology, USA 2018
- Prof. Dennis Lo has been awarded the 2018 Excellence in Genetics Research Award,
- Association of Chinese Geneticists in America
- Prof. Dennis Lo has been awarded the 2018 Man of Hope (MOH) Award in the category of
- Health Advocate
- Prof. Dennis Lo has been elected as the Senior Fellow of the PHG Foundation, University of
- Cambridge.

- Prof. Dennis Lo and Prof. Allen Chan have been awarded the 2018 年度高等學校科學研究優秀成果獎（科學技術）發明獎二等獎
- Prof. Dennis Lo has been awarded The Fifth Biotechnology Get Together (2018) Award
- Prof. Dennis Lo has been awarded the Honorary Fellowship, the Hong Kong Academy of
- Medicine
- Prof. Dennis Lo has been nominated as the Doctor of Science, honoris causa, University of Macau
- Prof. Allen Chan has been awarded the Inaugural Ying Shek Chi Wai Foundation Meritorious Research Award and named as the Lo Ying Shek Chi Wai Foundation Scholar 2018
- Prof. Dennis Lo won the Biochemical Analysis Prize 2019, The German Society of Clinical
- Chemistry and Laboratory Medicine (DGKL) [DGKL - Deutsche Gesellschaft für Klinische
- Chemie und Laboratoriumsmedizin e. V.]
- Prof. Dennis Lo has been awarded the Fudan-Zhongzhi Science Award 2019
- Prof. Dennis Lo won 第二屆全國創新爭先獎 in 2020

8. Impacts

8.1 What are the current and expected impacts of the project on the long-term development of Hong Kong (social or economic development, e.g. patent, technology transfer, collaboration with external organisations, etc.)?

We believe that the achievements of this TRS project would contribute to the development of Hong Kong as a global innovation hub in biotechnology that is in line with the government policy. Through this project (and also the previous TRS project), we have established a strong intellectual property (IP) position. We have filed patent applications that cover a diverse array of methods for circulating tumour DNA analysis. Over the last two decades, we have over 1,500 patents filed in different jurisdictions and hold over 800 granted patents. These patents are divided in over 50 patent families. Prof. Dennis Lo, Prof. Rossa Chiu and Prof. Allen Chan were named as one of the Top 20 Translational Researchers by *Nature Biotechnology* for 2020 (announced in December 2021) based on the number of patents granted during the year. Our group has also been successful in exploring the commercial value of these intellectual properties, through licensing to industrial partners. One important patent portfolio related to cancer diagnostics has been licensed to GRAIL, the largest multi-cancer early detection (MCED) company by market value. Our partnership with GRAIL during the project period also indicates a vote of confidence on technologies developed in Hong Kong.

In addition, as mentioned above, the startup company Take2 cofounded by the team members, has started to provide the NPC screening service (Take2 Prophecy™) in Hong Kong in May 2020 through licensing the technology developed in this project. Take2 targets to extend the screening service to the Greater Bay Area and it is now setting up a state-of-the-art laboratory in the Guangdong Province. Currently Take2 has hired approximately 40 staff members from Hong Kong and Mainland China. Novel technologies developed from this project has served as a catalyst for building up talent pool in biotechnology.

The success of plasma EBV DNA for screening of NPC has shed light on the use of liquid biopsy for screening of other types of cancer, which is a hot research topic from both the industry and the academia in recent years. There are a number of technical and clinical issues to be addressed with the use of ctDNA-based test for cancer screening. Our NPC screening study has also served as a model study for evaluation of the clinical utility of ctDNA-based screening tests. To illustrate, a prospective study conducted by the Johns Hopkins group (Lennon et al. *Science* (2020) 369;6499:eabb9601) on the use of ctDNA analysis for detection of multiple

cancer types has taken reference to our study and adopted a similar study design as our work published in the *New England Journal of Medicine* in 2017. This similarity has been discussed in our recent News and Views article in *Nature Review of Clinical Oncology* (Lo et al. *Nat Rev Clin Oncol* 2020;17(9):525-526).

8.2 Others (please specify):

Our work has established plasma EBV DNA as an effective screening tool of NPC which would lead to early cancer detection and survival benefit with a reduction of mortality by 90%. There is a strong need for early detection of NPC given the high disease burden in endemic regions including Hong Kong and southern parts of China. Screening with plasma EBV DNA could profoundly impact on the natural history of NPC. With the funding support, we have carried out re-screening of the whole 20,000-subject cohort, which would inform the optimal screening interval using plasma EBV DNA. Currently our team and international experts are drafting the first international recommendation guideline for NPC screening.

As mentioned, Take2 targets to offer the second-generation NPC screening test service in the Greater Bay Area. Compared to EBV antibody as another existing screening tool, this second-generation test reports a much lower false positive rate of less than 1%, which would imply a reduction in medical resources on confirmatory investigations. If a mass NPC screening program is launched for all men aged between 40 and 65 years old (~20 million men) in Guangdong as one of the provinces in China with the highest incidence rates of NPC, the second-generation NPC screening test would reduce the number of false positives by 10 fold, that is, 1.8 million subjects, compared to screening with EBV antibody. This is a huge impact from the public health point of view.

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

The work in the fragmentation pattern analysis pioneered by the project team has improved the understanding of the circulating DNA biology. In this regard, we have demonstrated that plasma DNA size, preferred ends, end motifs and jagged ends would be altered in different physiological and pathological conditions. We shall continue the investigation of these fundamental biological phenomena and aim to decipher the biological mechanisms underlying the production and degradation of circulating cell-free DNA. We would elucidate the interrelationships between the different aspects of plasma DNA fragmentation features and develop an integrated approach for the fragmentation analysis. At the same time, we shall study the perturbations of the plasma DNA fragmentation features among different diseases and aim to translate into clinical practice.

For the plasma EBV DNA analysis, we are planning to apply our developed technologies for molecular and genotypic analyses to other non-NPC EBV-related malignancies. Also, we may explore the genotypic analyses in NPC samples from other regions in order to study the prevalence of different virus strains in different endemic regions. Also, we shall explore similar concepts of molecular analyses in cancer types associated with other oncogenic viruses, e.g. hepatitis B virus.

9.2 Whether there are new projects evolved directly from this project?

There are new and ongoing projects on the analysis of the fragmentation profiles of circulating DNA as discussed above. We shall extend the concept to the analysis of cell-free DNA in other bodily fluids, e.g. urinary cell-free DNA. At the same time, we are exploring the relationship between the fragmentation profile of plasma DNA and epigenetic modifications.

For the plasma EBV DNA analysis, we are now evaluating our sequencing-based NPC screening tests in archived samples from a large Taiwan NPC cohort (the NPC Multicenter Case Control Study and the NPC Family Study). The data generated from this project would also allow genotypic comparison of EBV genotypes in NPC samples from different endemic regions.

9.3 Whether there are new collaborations developed directly from this project?

We have started collaboration with Hannah Valentine of the National Heart, Lung and Blood Institute of USA on applying our newly developed plasma DNA tissue mapping analysis to detect early graft rejection in lung transplant recipients.

After realisation of the nucleases that contribute to the fragmentation process in murine models, we have started collaboration with researchers from New York University (Boris Reizis), the Istituto Giannina Gaslini in Italy (Stefano Volpi) and the Hospital for Sick Children in Canada (Linda Hiraki) to analyse the fragmentation profiles of plasma DNA from human subjects disease-associated variations of *DNASE1L3*, who have developed familial systemic lupus erythematosus.

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

As mentioned, the team has been awarded the InnoHK Research Grant of HK$483 million under the InnoHK Initiative of the Innovation and Technology Commission. With the funding support, we have set up the Centre for Novostics (novostics = novel diagnostics) that focuses on development of cutting-edge molecular diagnostics based on cell-free nucleic acids and continue our work started under this TRS project. We shall continue to develop novel diagnostic applications that will impact a wide range of fields, including oncology, prenatal medicine, transplantation and other diseases.

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other sections of this report.)

	Peer-reviewed journal publications	Conference papers	Scholarly, books, monographs and chapters	Patents awarded	Other research outputs (please specify)	
					Type	No.
No. of outputs arising directly from this research project	26	36	1	220 filed		

11. Public Access of Completion Report

(PCs of projects funded in 2010/11 academic year or onwards are required to release the completion reports to the public through the RGC website. Completion reports containing

information such as abstracts in non-technical terms, objectives, research output including the list of conference papers / publications / journals and research findings and contact information of PCs should be open to public access.)

Please specify the information, if any, that cannot be provided for public access and give the reasons.

Information that cannot be provided for public access	Reasons
Nil	

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

Liquid biopsy, which is referred as the analysis of various cancer-derived biomarkers in blood or other body fluids, provides an avenue to delineate tumour characteristics non-invasively in contrast to the conventional tumour tissue biopsy. Using the nasopharyngeal cancer model, the research team established the clinical utility of plasma Epstein-Barr virus (EBV) DNA for screening of NPC through their prospective territory-wide study involving over 20,000 participants. The clinical benefits of screening including early cancer detection and survival improvement were demonstrated. The nasopharyngeal cancer model was also the first model to show that circulating DNA analysis could achieve early cancer detection. In addition, the team has developed different novel approaches of molecular analysis of circulating nucleic acids to enhance the applications in cancer diagnostics. Novel insights into the biology of circulating nucleic acids have been obtained and these have led to the discovery of new circulating tumour DNA biomarkers. Novel tests based on the developed technologies are now available for clinical use. Economically, the related technologies have been patented and licensed to biotechnology start-up companies and this has helped the build-up of talent pool.