Project number: _T12-401/13R___

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *1st January 2014*
Project completion date: *31st December, 2018*

---

1. Project Title: Systematic Development of Molecular Targets for Nasopharyngeal Carcinoma

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)	Kwok-Wai Lo	Dept. of Anatomical & Cellular Pathology, CUHK	15 hours
Co-Principal Investigator(s)	Ka-Fai To	Dept. of Anatomical & Cellular Pathology, CUHK	4.5 hours
	Anthony Tak-Cheung Chan	Dept. of Clinical Oncology, CUHK	4 hours
	Qian Tao	Dept. of Clinical Oncology, CUHK	6 hours
	Charles Andrew van Hasselt	Dept. of Otorhinolaryngology, Head and Neck Surgery, CUHK	2 hours
	Alice Sze-Tsai Wong	School of Life Sciences, HKU	6 hours
	Ting-Fung Chan	School of Life Sciences, CUHK	2 hours
	Kevin Yuk-Lap Yip	Dept. of Computer Science and Engineering, CUHK	6 hours
	Ying-Rui Li	Beijing Genomics Institute and iCarbonX	0.5 hours

Co-Investigator(s)	Sai-Wah Tsao	School of Biomedical Sciences, HKU	6 hours
	Nathalie Wong	Dept. of Anatomical & Cellular Pathology, CUHK	2 hours
	John Kong-Sang Woo	Dept. of Otorhinolaryngology, Head and Neck Surgery, CUHK	4 hours
	Brigette Buig-Yue Ma	Dept. of Clinical Oncology, CUHK	5 hours
	Siu-Tim Cheung	Dept. of Surgery, CUHK	5 hours
	Vivian Wai-Yan Lui (Approved on 24 April, 2015)	School of Biomedical Science, CUHK	8 hours
	Edwin Pun Hiu (Approved on 24 April, 2015)	Dept. of Clinical Oncology, CUHK	4 hours
	Jason Ying Kuen Chan (Approved on 24 April, 2015)	Dept. of Otorhinolaryngology, Head and Neck Surgery, CUHK	4 hours
Collaborators	Pierre Busson	Gustave Roussy Institute, CNRS (National Center for Scientific Research), France	N.A.
	Peter S. Hammerman (Approved on 15 August, 2015)	Department of Medicine, Dana Farber Cancer Institute, Harvard Medical School, USA	N.A.
	Jeongsun Seo (Approved on 15 August, 2015)	Dept. of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Korea	N.A
	Angela Kwok-Fung Lo (Approved on 15 August, 2015)	Dept. of Anatomical & Cellular Pathology, CUHK	N.A
	Richard Kwong-Wai Choy (Approved on 15 August, 2015)	Dept. of Obstetrics & Gynaecology, CUHK	N.A
	Nai-Ki Mak (Approved on 11 July, 2017)	Dept. of Biology, HKBU	N.A
	Ka-Leung Wong (Approved on 11 July, 2017)	Dept. of Chemistry, HKBU	N.A
	Guang Zhu (Approved on 11 July, 2017)	Division of Life Science, HKUST	N.A
	Angela Ruchao Wu (Approved on 11 July, 2017)	Division of Life Science & Division of Biomedical Engineering, HKUST	N.A.

 # 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.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. To establish the mutational landscape and transcription signature of NPC through large-scale massive parallel sequencing of clinical specimens	1. 100%	Completed
2. To define driver mutations, molecular and druggable targets and recurrent somatic alterations of NPC through integrative informatic analysis.	2. 100%	Completed
3. To elucidate the functional roles of potential somatic variants and the antitumor efficiency of potential targeting therapeutic approaches in NPC models	3. 100%	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.))

Nasopharyngeal carcinoma (NPC) poses serious health problem in South China including Hong Kong despite its rare occurrence in other parts of the world. The aim of this TRS project is to prolong survival and reduce mortality of NPC patients through the systematic discovery of tumor biomarkers and novel molecular targets and thereof the development of innovative therapeutic strategies. Our TRS research team has successfully achieved the project objectives and made important accomplishments in molecular basis and translational research of NPC, impacting the clinical management of this deadly cancer.

(1) Establishment of comprehensive genomic landscape of nasopharyngeal carcinoma (NPC)

With collaborative effort among our TRS team members, the genomic profiles of 175 NPC including cell lines, PDXs and microdissected tumor samples have been comprehensively investigated by either whole-exome and/or whole-genome sequencing. Firstly, we have performed whole-exome sequencing (WES) of 111 microdissected tumor specimens, with 15 cases subjected to whole-genome sequencing (WGS) to determine its mutational landscape. A median mutational rate of 1.9/Mb was found in the cohort of 78 primary and 33 recurrent/metastatic tumors. The key findings reported includes: **(1)** Increasing mutational burden in primary tumors was negatively correlative with patients' overall and disease-free survival. In addition, a small subgroup of tumors (~3%) with hypermutator phenotypes and inactivating mutations of mismatch repair (MMR) gene were detected. **(2)** The COSMIC mutational signatures for deamination of 5-methyl-cytosine process and defective DNA mismatch repair signature are predominant in NPC. A potential role of impaired DNA mismatch repair activity in NPC tumorigenesis was unvieled. **(3)** We discovered high frequencies of somatic genomic aberrations (41%) of multiple negative regulators (*CYLD, TRAF3, NFKBIA* and *NLRC5*) involved in NF-κB signalling pathways. The driver roles of multiple *CYLD* and *TRAF3* mutations were confirmed by various functional studies in EBV-positive NPC cells. Notably, as a potent activator of NF-κB signalling pathways, EBV-encoded LMP1 was overexpressed in a total of 25.7% of the cases. We revealed mutual exclusivity among tumours with somatic NF-κB pathway aberrations and LMP1-overexpression, suggesting that activation of inflammatory NF-κB pathway by both somatic and viral events is a key oncogenic driver for NPC pathogenesis. **(4)** Here we are the first one to report the discovery of high rate of MHC class I gene aberrations (*NLRC5, HLA-A, HLA-B, HLA-C*) in NPC. Inactivating alterations of these genes were identified in 30% of NPC patients who are with poor outcome. These somatic changes may lead to impaired antigen presentation mechanisms, subsequently interfering the clinical efficacy of immune checkpoint inhibitor or other immunotherapies in NPC patients. **(5)** We have also confirmed other reported somatic changes including recurrent mutations of *TP53*, *NRAS* and various cancer genes in chromatin modifying and PI3K/MAPK pathways.

Recently, we have built up a comprehensive catalogue of genomic alterations of 79 NPC samples including 17 cell lines/xenografts and 62 microdissected tumor specimens by WGS. Through integrative informatics analysis with transcriptome and ATAC-sequencing datasets, we have identified somatic alterations in both coding and noncoding regions. In this WGS study, we have further revealed a new double-strand break repair (DSB) mutational signature and the critical roles of structural variants (SVs) in targeting driver events and promoting tumorigenesis. We have defined genetic and epigenetic inactivation of *TGFBR2* at chromosome 3p22 as a driver event for tumor initiation. Functional study has demonstrated a key role of attenuating *TGFBR2/SMAD3* signaling in the persistent EBV latent infection and clonal expansion of NPC cells. The genomic study also allows us to identify novel mechanisms for activation NF-κB (e.g. *BIRC2/BIRC3* deletions) and disruption of antigen presentation pathway (e.g inactivation MHC-class II genes and aberrant EBV gene expression), implying a new NPC tumorigenesis model in which a co-evolution

of EBV latency and acquired genomic changes during tumor progression. This new co-evolution model for NPC tumorigenesis have been presented in our recent reviews in *Lancet* and *Seminar in Cancer Biology*. The WGS dataset has also contributed to construct a new whole-genome reference panel for Asians, NARD for facilitating precision medicine in Asian population.

- Li YY, Chung GT, Lui VW, To KF, Ma BB, Chow C, Woo JK, Yip KY, Seo J, Hui EP, Mak MK, Rusan M, Chau NG, Or YY, Law MH, Law PP, Liu ZW, Ngan HL, Hau PM, Verhoeft KR, Poon PH, Yoo SK, Shin JY, Lee SD, Lun SW, Jia L, Chan AW, Chan JY, Lai PB, Fung CY, Hung ST, Wang L, Chang AM, Chiosea SI, Hedberg ML, Tsao SW, van Hasselt AC, Chan AT, Grandis JR, Hammerman PS, Lo KW. Exome and genome sequencing of nasopharynx cancer identifies NF-κB pathway activating mutations. ***Nat Commun***. 2017, 8:14121.
- Tsang CM, Lui VWY, Bruce JP, Pugh TJ, Lo KW. Translational genomics of nasopharyngeal cancer. ***Semin Cancer Biol***. 2019 Sep 12. pii: S1044-579X (19)30284-6.
- Yoo SK, Kim CU, Kim HL, Kim S, Shin JY, Kim N, Yang JSW, Lo KW, Cho B, Matsuda F, Schuster SC, Kim C, Kim JI, Seo JS. NARD: whole-genome reference panel of 1779 Northeast Asians improves imputation accuracy of rare and low-frequency variants. ***Genome Med***. 2019 Oct 22;11(1):64.

(2) <u>Development of innovative bioinformatics tools for genomic studies.</u>

In this TRS project, our bioinformatics team has developed multiple innovative tools for analysing complex genomic features, aberrant enhancer methylation, enhancer-target networks and unique viral and cellular transcripts in NPC and other human cancers. We have characterized the complex genome structure of NPC cells by genome sequencing and a new nanochannel-based optical maps. Through developing a comprehensive SV-calling pipeline and corresponding open-source software, OMSV, precise structural breakpoints and uncovering novels sequences in the structural variants (SVs) in NPC cells were successfully identified. OMSV was demonstrated as a powerful tool for accurately calling of large and complex SVs in cellular and EBV genomes. Using the OMSV pipeline developed by this TRS project, the bioinformatics team and collaborators have revealed population-specific patterns of SV across 26 human population (Levy-Sakin, *et al*. Nature Commun, 2019). KY Yip has also developed a new method for inferring enhancer-target interactions by integrating epigenomic and transcriptomic data from various tumor samples. We have applied this method to reconstruct the enhancer-target networks and identify differentially methylated enhancers in liver cancer and NPC (Xiong, *et al.* Nat Commun 2019). The new approach allows us to identify novel epigenetic regulated driver genes in human cancers. Recently, we have established PSIRC (pseudo-alignment identification of circular RNAs), the first method that can detect and quantify circular RNA transcript isoforms of all lengths from RNA sequencing. PSIRC can accurately quantify circRNA full-length transcripts with high sensitivity and specificity. Applying PSIRC on RNA-seq data of NPC samples allows us to discover many differentially expressed circRNA isoforms with potential oncogenic roles in cancer development.

- Li L, Leung AK, Kwok TP, Lai YYY, Pang IK, Chung GT, Mak ACY, Poon A, Chu C, Li M, Wu JJK, Lam ET, Cao H, Lin C, Sibert J, Yiu SM, Xiao M, Lo KW, Kwok PY, Chan TF, Yip KY. OMSV enables accurate and comprehensive identification of large structural variations from nanochannel-based single-molecule optical maps. ***Genome Biol***. 2017 Dec 1;18(1):230.
- Cao Q, Anyansi C, Hu X, Xu L, Xiong L, Tang W, Mok MTS, Cheng C, Fan X, Gerstein M, Cheng ASL, Yip KY. Reconstruction of enhancer-target networks in 935 samples of human primary cells, tissues and cell lines. ***Nat Genet***. 2017 Oct;49(10):1428-1436.

(3) <u>Establishment and characterization of native EBV-positive NPC cell lines, patient-derived xenografts and tumor organoid models for basic and translational studies.</u>

By transcriptome sequencing and short tandem repeat (STR) profiling, we and other have previously shown that a number of cell lines commonly used in NPC research are EBV-negative and cross-contamination of HPV-18 positive HeLa cells, casting doubt on the relevance of these cell lines for basic and preclinical studies, particularly as preclinical models for evaluation of therapeutic agents. For the past three decades, only limited authenticated EBV-positive cell line (C666-1) and patient-derived xenografts (PDXs; xeno-2117, xeno-666, C15, C17, C18) established

by team members and collaborators are available for NPC studies. Lack of well-characterized patient-derived EBV-positive tumor models is a major obstacle hampering research progress in NPC. For the past few years, our team member SW Tsao have successfully established a large panel of new NPC cell lines (NPC43, C17C, NPC53) and PDXs (xeno-23, -32, -38, -47, and -72). Based on these new resources, we then established a repository containing a full spectrum of cell lines and PDXs from different clinical stages of NPC progression, including primary (C666-1, xeno-666, xeno-2117, xeno-32, C15, C18), recurrent (NPC43, xeno-23, xeno-47, xeno-76) and distant metastatic tumors (C17C, C17). All these NPC models have been authenticated and well characterized. Importantly, majority of these NPC models contain EBV episomes and express type II latent genes. Whole genome landscape, cellular and viral transcriptome, methylome and histone modification profile of these NPC models have been constructed. Representative tumor models for various molecular subclasses, such as MMR-mutated (C666-1, xeno-666, NPC38), NF-κB-mutated (NPC43, C17, xeno-76, xeno-32) and LMP1-driven (C15) were defined. Importantly, these EBV+ve cell lines were shown to be capable of undergoing lytic EBV reactivation, providing representative NPC models for unveiling the latent-to-lytic switch mechanisms and related cellular factors. These invaluable resources allow us to develop efficient EBV-targeting and innovative oncolytic therapies. Furthermore, these well-characterised patient-derived tumor models will serve as preclinical models in high-throughput drug screening and evaluating the efficacy of novel therapeutic agents. Recently, we have developed new protocols for the establishment of tumor organiods from the PDXs and patient's tumor biopsies. The tumor organoids will further empower our capability for drug screening and predict treatment responses of cancer drugs of NPC patients.

- Lin W, Yip YL, Jia L, Deng W, Zheng H, Dai W, Ko JMY, Lo KW, Chung GTY, Yip KY, Lee SD, Kwan JS, Zhang J, Liu T, Chan JY, Kwong DL, Lee VH, Nicholls JM, Busson P, Liu X, Chiang AK, Hui KF, Kwok H, Cheung ST, Cheung YC, Chan CK, Li B, Cheung AL, Hau PM, Zhou Y, Tsang CM, Middeldorp J, Chen H, Lung ML, Tsao SW. Establishment and characterization of new tumor xenografts and cancer cell lines from EBV-positive nasopharyngeal carcinoma. ***Nat Commun***. 2018 Nov 7;9(1):4663.
- Yip YL, Lin W, Deng W, Jia L, Lo KW, Busson P, Vérillaud B, Liu X, Tsang CM, Lung ML, Tsao SW. Establishment of a nasopharyngeal carcinoma cell line capable of undergoing lytic Epstein-Barr virus reactivation. ***Lab Invest***. 2018 Aug;98(8):1093-1104.

(4) Unveiling the unique roles of EBV in NPC pathogenesis

Persistent EBV infection drives neoplastic transformation and plays key role in the progression of NPC. For the past three decades, our team members and others have unveiled the unique properties of EBV in nasopharyngeal epithelial cells. In this TRS project, we have systematically characterized the EBV transcriptomes and elucidated the functional roles of EBV-encoded proteins and non-coding RNAs in NPC. Through transcriptome sequencing, we have uncovered the expression of unique latent genes, isoforms and various lytic transcripts in the tumor cells. The discovery of novel latent genes implies new research directions for EBV carcinogenesis, especially their potential roles in immune evasion and viral latency. Extensive studies of the new EBV latent genes have been initiated through the support of two recently funded CRF projects (C4001-18G, C7027-16G). In addition, through constructing EBV transcriptome maps, we have defined a group of EBV-encoded *miR-BARTs* predominantly expressed in NPC. Strikingly, these *miR-BARTs* negatively regulate the expression of a key DNA double-strand breaks (DSBs) repair gene, *ATM*, thereby controlling DNA damage repairs and inhibiting lytic reactivation. The finding provided new evidences for EBV-induced genomic instability, supporting the occurrence of a new mutational signature associated with failure of DNA double-strand break-repair by homologous recombination. As the first research team to report the EBV-mediated metabolism reprogramming in NPC, we have defined the driver roles of EBV-encoded LMP1 and its downstream oncogenic pathways including NF-κB and mTORC/AKT signalling cascades in inducing this important cancer hallmark. Our results also indicate the potential of repurposing lipogenesis inhibitors in clinical treatment of locally advanced or metastatic NPC. Through characterization of the EBV-positive NPC cell lines, we further confirmed the continued presence of episomal EBV genomes and expression of multiple

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 funding amount and duration of 5 years of TRS allowed us to conduct a large scale prospective genomic study on NPC and to validate the potential biomarkers and therapeutic targets identified. With the support of this TRS funding, our project team have established a number of invaluable resources including the largest WES (111 tumors) and WGS (79 tumors) datasets. In addition to promote genomic studies of NPC, the dataset has also contributed to construct a new whole-genome reference panel for Asians, NARD for facilitating precision medicine in Asian population. The TRS project has established the genomic landscape, transcriptome profile and epigenetic features of a large panel of authenticated EBV-positive NPC cell lines and PDXs by multiple genomic sequencing studies. This invaluable resource will greatly enhance the progress of basic and translational research of NPC. Importantly, through TRS funding, we have initiated long-term collaborative relationship with a number of international leading scientists and research groups on genomics, viral carcinogenesis, preclinical and clinical studies of NPC.

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

6.4 (a) Peer-reviewed journal publication(s) arising <u>directly</u> from this project:
(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference. Please mark the symbol "#" next to the publications involving inter-institutional collaborations)

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
			2019	Bruce JP, To, KF, Chung GT, Lui VW, Yip KY, Woo JK, Ma BB, Hui EP, et al. Tsao SW, Chan AT, Pugh TJ, Lo KW.	Whole-genome landscape of EBV-associated nasopharyngeal carcinoma. (will submit to Nature Commun) #	2019	No	Yes	No
			2019	Lechner M, Schartinger VH,*, Steele CD, Nei W, Ooft ML,Schreiber LM, Pipinikas CP, Chung GT, Chan YY, Wu F, To KF, *et al*., Busson P, Lo KW, Wollmann G, Pillay N, Vanhaesebroeck B*, Lund VJ*	Key role of Somatostatin receptor 2 in nasopharyngeal cancer and its association with EBV: impact on prognosis, imaging and therapy. (will submit to Nature Medicine) #	2019	No	Yes	No
2019				Tsang CM, Lui VW, Bruce JP, Pugh TJ, Lo KW*	Translational Genomics of Nasopharyngeal Cancer. Semin Cancer Biol. pii: S1044-579X(19)30284-6, 2019. #	2019	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2019				Yoo SK, Kim CU, Kim HL, Kim S, Shin JY, Kim J, Yang JS, Lo KW, Cho B, Matsuda F, Schuster SC, Kim C, Kim J, Seo JS*.	NARD: whole-genome reference panel of 1,779 Northeast Asians improves imputation accuracy of rare and low-frequency variants. Genome Med.; 11:64, 2019. #	2019	Yes	Yes	Yes
2019				Rickinson AB*, Lo KW	Nasopharyngeal Carcinoma: A History. In: Nasopharyngeal Carcinoma From Etiology to Clinical Practice. Eds. Lee AWM, Lung ML, Ng WT. Pg. Academic Press (2019), pp1-16.#	2019	Yes	No	Yes
2019				Yip YL, Lin WT, Deng W, Tsang CM, Tsao SW	Establishment of Nasopharyngeal Carcinoma Cell Lines, Patient-Derived Xenografts, and Immortalized Nasopharyngeal Epithelial Cell Lines for Nasopharyngeal Carcinoma and Epstein–Barr Virus Infection Studies. In: Nasopharyngeal Carcinoma From Etiology to Clinical Practice. Eds. Lee AWM, Lung ML, Ng WT. Academic Press (2019), pp85-107.	2019	Yes	Yes	Yes
2019				Pang PS, Liu T, Lin W, Tsang CM, Yip YL, Zhou Y, Guan XY, Chan RC, Tsao SW*, Deng W*	Defining early events of Epstein-Barr virus (EBV) infection in immortalized nasopharyngeal epithelial cells using cell-free EBV infection. J Gen Virol. 100: 999-1012, 2019.	2019	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2019				Tsang CM, Lo KW, Nicholls JM, Huang SCM, Tsao SW.	Pathogenesis of Nasopharyngeal Carcinoma: Histogenesis, Epstein–Barr Virus Infection, and Tumor Microenvironment. In: Nasopharyngeal Carcinoma From Etiology to Clinical Practice. Eds. Lee AWM, Lung ML, Ng WT. Academic Press (2019), pp45-64.	2019	Yes	Yes	Yes
2019				Ho EY, Cao Q, Gu M, Chan RW, Wu Q, Gerstein M, Yip KY*	Shaping the nebulous enhancer in the era of high-throughput assays and genome editing. Brief Bioinform. pii: bbz030, 2019	2019	Yes	Yes	Yes
2019				Zhang J, Jia L, Liu T, Yip YL, Tang WC, Lin W, Deng W, Lo KW, You C, Lung ML, Lung HL, Cheung AL, Tsao SW*, Tsang CM*	mTORC2-mediated PDHE1α nuclear translocation links EBV-LMP1 reprogrammed glucose metabolism to cancer metastasis in nasopharyngeal carcinoma. Oncogene. 38: 4669-4684, 2019.	2019	Yes	Yes	Yes
2018				Ma BBY, Lim WT, Goh BC, Hui EP, Lo KW, Pettinger A, Foster NR, Riess JW, Agulnik M, Chang AYC, Chopra A, Kish JA, Chung CH, Adkins DR, Cullen KJ, Gitlitz BJ, Lim DW, To KF, Chan KCA, Lo YMD, King AD, Erlichman C, Yin J, Costello BA, Chan ATC*.	Antitumor Activity of Nivolumab in Recurrent and Metastatic Nasopharyngeal Carcinoma: An International, Multicenter Study of the Mayo Clinic Phase 2 Consortium (NCI-9742). J Clin Oncol. 36:1412-1418, 2018. #	2019	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2018				Lo AK, Lung RW, Dawson CW, Young LS, Ko CW, Yeung WW, Kang W, To KF, Lo KW*.	Activation of sterol regulatory element-binding protein 1 (SREBP1)-mediated lipogenesis by the Epstein-Barr virus-encoded latent membrane protein 1 (LMP1) promotes cell proliferation and progression of nasopharyngeal carcinoma. J Pathol. 246: 180-190, 2018. #	2019	Yes	Yes	Yes
2018				Ma J, Kala S, Yung S, Chan TM, Cao Y, Jiang Y, Liu X, Giorgio S, Peng L, Wong AST*.	Blocking Stemness and Metastatic Properties of Ovarian Cancer Cells by Targeting p70S6K with Dendrimer Nanovector-Based siRNA Delivery. Mol Ther. 26:70-83, 2018. #	2019	Yes	Yes	Yes
2018				Lung RW, Hau PM, Yu KH, Yip KY, Tong JH, Chak WP, Chan AW, Lam KH, Lo AK, Tin EK, Chau SL, Pang JC, Kwan JS, Busson P, Young LS, Yap LF, Tsao SW, To KF, Lo KW*	EBV-encoded miRNAs target ATM-mediated response in nasopharyngeal carcinoma. J Pathol. 244: 394-407, 2018. #	2019	Yes	Yes	Yes
2018				Yip YL, Lin WT, Deng W, Jia L, Lo KW, Busson P, Liu XF, Tsang CM, Lung ML, Tsao SW*	Establishment of a nasopharyngeal carcinoma cell line capable of undergoing lytic Epstein-Barr virus reactivation Lab Invest. 98: 1093-1104, 2019. #	2017	Yes	Yes	Yes
2018				Wong CH, Ma BBY, Hui CWC, Lo KW, Hui EP, Chan ATC.	Preclinical evaluation of ribociclib and its synergistic effect in combination with alpelisib in non-keratinizing nasopharyngeal carcinoma. Sci Rep. 8:8010, 2018.	2019	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2018				Lin W, Yip YL, Jia L, Deng W, Zheng H, Dai W, Ko JMY, Lo KW, Chung GTY, Yip KY, et al, Chan CK, Li B, Cheung AL, Hau PM, Zhou Y, Tsang CM, Middeldorp J, Chen H, Lung ML, Tsao SW*	Establishment and characterization of new tumor xenografts and cancer cell lines from EBV-positive nasopharyngeal carcinoma. Nat Commun. 9:4663, 2018. #	2019	Yes	Yes	Yes
2018				Yap LF*, Lo KW	Epstein-Barr virus and epithelial carcinogenesis In DNA Tumour Viruses:Virology, Pathogenesis and Vaccines. Caister Academic Press (2018) pp.139- 162. #	2019	Yes	Yes	No
2018				Li L, Xu J, Qiu G, Ying J, Du Z, Xiang T, Wong KY, Srivastava G, Zhu XF, Mok TS, Chan AT, Chan FK, Ambinder RF, Tao Q.	Epigenomic characterization of a p53-regulated 3p22.2 tumor suppressor that inhibits STAT3 phosphorylation via protein docking and is frequently methylated in esophageal and other carcinomas. Theranostics. 8:61-77, 2018. #	2019	Yes	Yes	Yes
2018				Li L, Ma BBY, Chan ATC, Chan FKL, Murray P, Tao Q*.	Epstein-Barr Virus-Induced Epigenetic Pathogenesis of Viral-Associated Lymphoepithelioma-Like Carcinomas and Natural Killer/ T-Cell Lymphomas. Pathogens.7. pii: E63, 2018. #	2019	Yes	Yes	Yes
2018				Lui VWY, To KF, Lo KW*.	Genomic profiles of nasopharyngeal carcinoma: The importance of histological subtyping and Epstein-Barr virus in situ assays. Cancer. 124:434-435, 2018.	2019	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2018				Huang SCM, Tsao SW, Tsang CM*	Interplay of Viral Infection, Host Cell Factors and Tumor Microen-vironment in the Pathogenesis of Nasopharyngeal Carcinoma. Cancers.10. pii: E106, 2018.	2019	Yes	Yes	Yes
2018				Ngan HL, Wang L, Lo KW, Lui VWY*	Genomic Landscapes of EBV-Associated Nasopharyngeal Carcinoma vs. HPV-Associated Head and Neck Cancer. Cancers. 10. pii: E210, 2018.	2019	Yes	Yes	Yes
2017				Cao Q, Anyansi C, Hu X, Xu L, Xiong L, Tang W, Mok MTS, Cheng C, Fan X, Gerstein M, Cheng ASL, Yip KY*.	Reconstruction of enhancer-target networks in 935 samples of human primary cells, tissues and cell lines. Nat Genet. 49: 1428-1436, 2017. #	2019	Yes	Yes	Yes
2017				Tsao SW*, Tsang CM, Lo KW*.	Epstein-Barr virus infection and nasopharyngeal carcinoma. Philos Trans R Soc Lond B Biol Sci. 372. pii: 20160270, 2017.	2019	Yes	Yes	Yes
2017				Zhu X, Zhang Q, Ho ED, Yu KH, Liu C, Huang TH, Cheng AS, Kao B, Lo E, Yip KY*.	START: a system for flexible analysis of hundreds of genomic signal tracks in few lines of SQL-like queries. BMC Genomics. 18:749, 2017.	2019	Yes	Yes	Yes
2017				Zhang J, Jia L, Tsang CM, Tsao SW*	EBV Infection and Glucose Metabolism in Nasopharyngeal Carcinoma. Adv Exp Med Biol. 1018:75-90,2017	2019	Yes	Yes	Yes
2017				Hau PM*, Tsao SW.	Epstein-Barr Virus Hijacks DNA Damage Response Transducers to Orchestrate Its Life Cycle. Viruses. 9. pii: E341, 2017	2019	Yes	Yes	Yes

(b) Recognised international conference(s) in which paper(s) related to this project was/were delivered:

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)*
March/2019/ Atlanta, USA	Establishment and characterization of new tumor xenografts and cancer cell lines from EBV positive nasopharyngeal carcinoma	AACR Annual Meeting 2019	2019	Yes	Yes	No
July-August/ 2018/ Madison, USA	Activation of SREBP1-Mediated Lipogenesis by the Epstein-Barr Virus-Encoded LMP1 Promotes Cell Proliferation and Progression of Nasopharyngeal Carcinoma	International Conference for EBV and KSHV 2018	2019	Yes	Yes	No
April/2018/ Chicago, USA	M2-polarized macrophages increase invasiveness of EBV-associated nasopharyngeal carcinoma by inducing invadopodia formation	AACR Annual Meeting 2018	2019	Yes	Yes	No
April/2018/ Chicago, USA	Epstein-Barr virus-encoded microRNAs regulate the expression of BRCA1 in nasopharyngeal carcinoma	AACR Annual Meeting 2018	2019	Yes	Yes	No
April/2018/ Chicago, USA	Epstein Barr virus-encoded LMP1 activates the mTORC2 signaling pathway to reprogram glucose metabolism in nasopharyngeal epithelial cell	AACR Annual Meeting 2018	2019	Yes	Yes	No
April/2018/ Chicago, USA	Epstein Barr virus-encoded LMP1 reprograms glucose metabolism to enhance cell motility in nasopharyngeal epithelial cell	AACR Annual Meeting 2018	2019	Yes	Yes	No
April/2018/ Chicago, USA	Epstein-Barr virus-encoded miRNAs target ATM-mediated response in nasopharyngeal carcinoma	AACR Annual Meeting 2018	2019	Yes	Yes	No
April/2018/ Chicago, USA	Promotion of in vivo growth of Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma by miR-BARTs	AACR Annual Meeting 2018	2019	Yes	Yes	No
April/2018/ Chicago, USA	PD-L1 expression associated with treatment responses in colorectal cancer patients with XELOX/ FOLFOX chemotherapy: potential of checkpoint blockage and natural killer cell-based immunotherapy.	AACR Annual Meeting 2018	2019	Yes	Yes	No
June/2018/ Hong Kong	Acquired Genomic Changes in Nasopharyngeal Carcinoma (by Kwok Wai Lo)	Gordon Research Conference: Nasopharyngeal Carcinoma	2019	No abstract. Oral presentation only	Yes	No
June/2018/ Hong Kong	Immune Checkpoint Inhibitor in the Treatment of Nasopharyngeal Carcinoma: A Clinical Overview (by Brigette Buig-Yue Ma)	Gordon Research Conference: Nasopharyngeal Carcinoma	2019	No abstract. Oral presentation only	Yes	No
September/2017/ Mainz, German	Natural killer cell anergy in liver cancer patients and potential of adoptive cell transfer.	Third CRI-CIMT-EATI- AACR International Cancer Immunotherapy Conference	2019	Yes	Yes	No

(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.

The PC, KW Lo and an external advisor broad member, Prof Alan B Rickinson have recently

published a book chapter on the history of NPC research: “Nasopharyngeal Carcinoma: A History” in which the key findings of this TRS project were included as milestones in the field. Though the book chapter, the impacts of our TRS project were highlighted and disseminated to the scientific community and general public although no special acknowledgement of funding supports to this “history” chapter have been made.

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

Under the coordination of PC (KW Lo) and Executive Management committee (EMC), the team members from the CUHK and HKU have closely collaborated to achieve the three objectives of this TRS project. The members of basic research, bioinformatics and clinical sciences teams have had close communications and collaborations through the EAB, EMS and general meetings, as well as additional research group meetings among investigators. The collaborative efforts have already been demonstrated in joint publications, patents and grant applications described in sections 6.4, 6.7 and 7.1 respectively. The TRS project team members are also closely collaborated with other EBV/NPC researchers in Hong Kong, especially the investigators from Centre for Nasopharyngeal Carcinoma Research (CNPC), an Area of Excellence (AoE) project led by Prof Maria Lung. The team members of the TRS (KW Lo, SW Tsao) and AoE projects have worked closely on unveiling the pathological role of EBV and have successfully awarded a RGC-CRF grant entitled: “Regulation and pathogenic role of latent infection of Epstein-Barr virus in nasopharyngeal carcinoma” (C7027-16G) on 2016. During the late stage of TRS project, we have recruited a multi-disciplinary team of experts from HKUST (G Zhu, AR WU) and HKBU (KL Wong, NK Mak) as our local collaborators to develop the EBV targeting therapeutic strategies on 2017. Our joint efforts resulted in initiating a new CRF project “Targeting EBV in nasopharyngeal carcinoma: from mechanistic study to novel therapeutic development” (C4001-18G).

The TRS project team has established extensive collaborations with the world-renowned genomics, NPC and EBV research groups. For example, the landmark NPC genomic study published in Nature Communication (Li YY *et al*. Nat Commun. 2017) was the result of a collaborative effort among our TRS project team, Prof Hammerman PS (from Danna-Farber Cancer Institute, Harvard Medical School), Prof Grandis JR (from University of California San Francisco) and Prof Seo JS (from Genomic Medicine Institute, Seoul National University). The TRS team worked closely with Prof Pugh TJ (Princess Margaret Cancer Centre, University Health Network, University of Toronto) and Prof Seo JS (Seoul National University) on the whole-genome sequencing of NPC tumors. Additionally, the PC was also joined an international genome project entitled “Discovering rare variants and deciphering population structure of 386 Mongolian individuals by whole-genome sequencing” led by Prof Jeong-Sun Seo, Seoul National University, Korea. A whole-genome reference panel NARD was recently established and published in Genome Medicine (Yoo SK et al, Genome Med. 2019). Notably, the PC and ATK Chan (co-PI, leader of Clinical Sciences team) have joined a project “Ending EBV Cancer” for the application of a £20M Grand Challenge funding from Cancer Research UK on 2016. The project was led by Prof Rickinson AB (University of Birmingham, UK) and included an international expert team (Prof Lieberman P from the Wister Institute, Philadelphia; Cohen J from National Institutes of Health, Rooney C from Baylor College of Medicine; Clevers H from Hubrecht Institute, Netherlands; Munz C from University of Zurich; Lehner P from Cambridge Institute for Medical Research, Cambridge Biomedical Campus; Strasser A from The University of Melbourne; Kaneda A from Chiba University, Japan). Furthermore, our members have also extended our partnerships with the international EBV research groups led by Prof Lawrence S Young (Warwick Medical School, UK), Prof Henri-Jacques Delecluse (DKFZ, Germany), Pierre Busson (Institut de Cancérologie Gustave Roussy, University Paris-Sud 11, France) and Lee-Fah Yap (University of Malaya, Malaysia).

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Wu, Man	PhD (KW Lo)	March, 2017	February, 2020 (expected)
Yu, Hung On Ken	PhD (KW Lo)	August, 2014	September, 2019
Siu, Pui Kei Sharie	PhD (KW Lo)	August, 2013	August, 2018
Huang, Tingting	PhD (KW Lo)	August, 2010	December, 2014
Lam, Ka Hei	MPhil (KW Lo)	August, 2017	August, 2019
Law, Hung Nam	MPhil (KW Lo)	August, 2014	August, 2016
Liu, Ming Ting Alyssa	MPhil (KW Lo)	August, 2018	July, 2020 (expected)
Chan, Sin Man	MPhil (KW Lo)	April, 2012	July, 2014
Mak, Ka Yan	MPhil (KW Lo)	August, 2012	October, 2014
Chau, Shuk Ling	PhD (KF To)	August, 2015	February, 2020 (expected)
Zhang, Yuen	PhD (Q Tao)	January, 2014	January, 2017
Li, Le	PhD (KYL Yip)	August 2014	October, 2018
Lau, Ka Ho	PhD (KYL Yip)	August, 2015	July, 2019
Lin, Weitao	PhD (SW Tsao)	November, 2012	November, 2016
Zhang, Jun	PhD (SW Tsao)	September, 2014	August, 2018
Ma, Jing	PhD (AST Wong)	July, 2014	June, 2018
Kala, Shashwati	MPhil (AST Wong)	July, 2012	August, 2014
Fung, Sze Wai Katie	PhD (ST Cheung)	November, 2013	October, 2017
Siu, Hon Lam Elaine	PhD (ST Cheung)	August, 2016	July, 2019
Leung, Chim Yan	PhD (ST Cheung)	August, 2012	July, 2015
Hui, Shinyee	MPhil (ST Cheung)	August, 2017	July, 2019
Ngan, Hoi-Lam Jason	MPhil (VWY Lui)	July, 2015	June, 2017

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

The PC (KW Lo) and KF To have identified a novel fusion gene as new molecular target of NPC and received an US Patent no. 9,464,327 B2 - "Recurrent transforming UBR5-ZNF423 fusion gene in EBV-associated nasopharyngeal carcinoma" on October 2016. Alice CT Wong and KW Lo have recently received an US Patent application on "BCL3 siRNA amphiphilic dendriplexes for effective and potent nasopharyngeal carcinoma treatment" (US Patent No. 10,421,968 B2). The whole-genome sequencing dataset developed by our TRS team was included in the Northeast Asian Reference Database (NSRD), a high-quality population-specific reference panel for the genetic studies and precision medicine in Northeast Asia (https://nard.macrogen.com/). The bioinformatics team led by TF Chan and KY Yip have established an alignment tool (OMBlast) and a software package (OMTools) for mapping and processing the data generated by optical mapping technology. Optical mapping has been widely applied in assisted-scaffolding in sequence assemblies and detection of structural variations. This powerful technology was used to accurately delineate the long distance structure variants in NPC genome. The team also has developed a Signal Track Query Language (STQL) for analysing a large number of genomic signal tracks in a generic way. Importantly, SW Tsao has established two new EBV-positive NPC cell lines (C17 and NPC43) and more than four EBV-positive NPC patient-derived xenografts (xeno-23, xeno-32, xeno-47, xeno-76) that will greatly enhance the studies on the role of EBV in NPC tumorigenesis and the development novel EBV-targeting therapeutic strategies.

6.8 Other education activities and/or training programmes developed:

In this TRS project, the team members provided a comprehensive training grounds for postgraduate students, junior research staffs and post-doctoral fellows. The basic science and bioinformatics teams have supervised more than 12 postdoctoral fellows in the areas of cancer genomics, bioinformatics, virology and cellular biology. KW Lo (PC) is a director of the MSc Programme in Medical Laboratory Science, CUHK and organized the courses for modern molecular diagnostics and genome technologies. As the chief of Diagnostic Molecular Pathology Laboratory, he has arranged the training programme of the molecular diagnostic tests to the medical

staffs and technologists from Hospital Authority. KF To and KW Lo have established a "Core Utilities of Cancer Genomics and Pathobiology" and provided training for next generation sequencing, bioinformatic analysis, imaging and various molecular and pathological technologies to young researchers in CUHK. As the Director of Organizing Committee, George SW Tsao is organizing an Croucher Summer Course 2017 – Advanced Imaging-Single Molecule & Super resolution Microscopy in Biomedical Research in Hong Kong on 20-26 August, 2017. The members of Clinical Science group, ATC Chan and Hui EP, have prepared a chapter in the educational book and an online video on "Epstein-Barr virus as a paradigm in nasopharyngeal cancer: from lab to clinic" in 2014 ASCO Annual Meeting, May 30 – June 3, 2014, Chicago, USA.

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.

The TRS project is meticulously managed and all deliverables were achieved as indicated in the endorsed project implementation timetable.

Project Management

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

The PC (KW Lo) and other members of Executive Management committee (EMC) had shared the responsibility in overseeing the project progression, budget allocations and management. The Executive Management Committee (EMC) includes the PC (KW Lo), leaders of Basic Research (AST Wong), Clinical Science (ATC Chan, KF To) and Bioinformatics (KYL Yip) TRS project team, the PC has played a leadership role in directing the 3 teams to focus on their respective projects and facilitating the collaborative studies. Through the 20 EMC and general meetings, the PC and EMC members coordinate communications, allocate resource and share project information and among team members. The PC also organized multiple joint research meetings among team members for exchange updated findings and idea. Through holding these meetings, the PC and investigators could ensure the progression, identify potential problems and explore new directions of the TRS projects. To strengthen the project management, the PC also organized 5 annual International Advisor Broad Meetings and invited experts in the field of EBV carcinogenesis (Prof. Paul Farrell), viral immunology (Prof Alan B Rickinson) and clinical therapies and translational studies of NPC (Prof Fei-Fei Lui) to access the project progress and provide critical advice to the TRS team members. To enhance the collaboration among NPC researchers, the PC and Prof Maria Lung (PC of AoE-CNPC project) have held a Joint NPC AoE and TRS Thematic Meeting for sharing information and initiating new collaborative projects on September, 2015. Through the NPC AoE-TRS joint meeting and various informal meetings (e.g. EBV workgroup meetings), he introduced new directions and initiated collaborative studies with the EBV and NPC research communities in Hong Kong and worldwide. At 2016, the PC and ATC Chan have joined as collaborator and co-PI of an international teams leading by Prof Alan B. Rickinson (University of Birmingham, UK) to submit an application entitled "Ending EBV Cancer" for £20M Grand Challenge funding from Cancer Research UK. In addition to establish international collaboration with experts in their fields, the PC and team leaders are also responsible to disseminate the important findings of TRS project to the public and scientific communities.

7. Awards and Recognition

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

RGC Collaborative Research Fund (x 4, total amount: HK$22,757,693)

KW Lo (PC), KF To and ***AST Wong:*** Targeting Epstein-Barr virus in nasopharyngeal carcinoma: from mechanistic study to novel therapeutic development. Project no.: C4001-18G. (1/2/2019 – 28/2/2022; HK$6,269,185);

TF Chan (PC), KW Lo and ***KYL Yip:*** A nanochannel-based next-generation mapping system for

the study of complex genomic feature and variation for biotechnological and biomedical applications. Project no.: C4057-18EF. (2019-2022; HK$2,173,431);
SW Tsao (PC) and ***KW Lo:*** Regulation and pathogenic role of latent infection of Epstein-Barr virus in nasopharyngeal carcinoma. Project no. - C7027-16G. (1/1/2017-31/12/2010; HK$ 6,315,125);
SW Tsao (co-PI) and ***AST Wong (co-PI):*** Biomimetic 3D microsystem to study tumor survival and drug responses. Project no. - C1013-15G. (1/1/2016-31/12/2019; HK$7,999,952);

RGC Research Impact Fund (x 1, total amount: HK$9,800,000)

N Wong (PC) and ***KL Wong:*** Patient-Derived Preclinical Models for Translational Cancer Research: a Hong Kong-based Biotechnology Centre for Genomic Medicine. Project no.: R4022-18. (2019-2022; HK$9,800,000).

RGC General Research Fund (x 16, total amount: HK$14,478,622)

KW Lo (PI): Studies on the role of SREBP1 in EBV-induced metabolic reprogramming in NPC. Project no.: 14117316. (1/1/2016-31/12/2019; HK$1,088,950);
SW Tsao (PI): Pathogenicity of EBV isolated from nasopharyngeal carcinoma. Project no.: 17114818. (2018/2019; HK$ 972,000); Deciphering multiple mechanisms underlying the retention of Epstein-Barr viruses in nasopharyngeal carcinoma cells. Project no.: 17104617. (2017 / 18; HK$ 1,350,602); Immortalization of nasopharyngeal epithelial cells by the EBV-encoded LMP1. Project no.: 17161116. (1/7/2016-30/6/2019; HK$ 1,256,240);
KF To (PI): Target inhibition of ATM-mediated homologous recombination repair by Epstein-Barr virus miR-*BARTs* in nasopharyngeal carcinoma: molecular mechanisms and clinical implications. Project no.: 14138016. (1/9/2016-31/8/2018; HK$ 811,383); Study on the role of Esptein-Barr Virus (EBV)-encoded microRNAs in controlling DNA damage repairs in EBV-associated nasopharyngeal carcinoma. Project No.: 14104415 (1/1/2016-31/12/2018; HK$1,187,649);
KYL Yip (PI): Towards complete modeling of gene regulation in a cell type. Project no.: 14203119. (1/1/2019-31/12/2022; HK$518,999); Large-scale inference of DNA contact maps for studying genome structures and their functional significance. Project no.: 14170217. (2017/18; HK$ 600,000); Reconstruction of enhancer-target networks and simultaneous modelling of the quantitative effects of multiple targeting enhancers on gene expression across hundreds of human cell and tissue types. Project no.: 14145916. (1/11/2016-31/10/2018; HK$ 685,930);
VWY Lui (PI): MAPK1 Genomic Alterations as Predictor of Treatment Response and Driver for Tumor Growth in Head and Neck Cancer. Project no: 17114814. (2014/15; HK$ 661,203); RAC1 Genomic Alterations Drive Head and Neck Cancer Progression. Project no: 14168517. (2017/18; HK$ 1,229,089); ALK/PIK3CA Genetic Coupling: Implications for Progression and Personalized Therapy in Head and Neck Cancer. Project no.: 17121616. (1/1/2016-31/12/2019; HK$840,664);
AST Wong (PI): Metadherin: a novel target for b-catenin and a critical mediator of the positive feedback loop between highly metastatic tumor cells and macrophages? Project no.: 17141216. (2016/17; HK$1,092,092); Nuclear p70 S6 kinase: a molecular determinant of early tumor metastasis? Project no.: 17103417. (2017/18; HK$ 905,046);
JYK Chan (PI): A multicenter evaluation of a combination of nasopharyngeal brush biopsy and plasma for EBV DNA in detecting local failures in nasopharyngeal carcinoma. Project no.: 14108818. (2018/19; HK$678,775);
BBY Ma (PI): Prospective evaluation of predictive biomarkers of response to the immune-checkpoint inhibitor nivolumab in patients with recurrent or metastatic nasopharyngeal carcinoma. Project no.: 14161317. (2017/18; HK$ 600,000).

RGC NSFC/RGC Joint Research Scheme (x 1, total amount: HK$1,164,858)

SW Tsao (PI): A mechanistic and clinicopathological study on the impact of invadosomes in promoting nasopharyngeal carcinoma (NPC) metastasis under the interplay of stromal macrophages and EBV infection. Project No.: N_HKU735/18. (2018/19; HK$1,164,858).

Health and Medical Research Fund (x 1, total amount: HK$1,199,944)

SW Tsao (PI): Preclinical evaluation of therapeutic use of a selective cdk4/6 inhibitor (PD-332991) in nasopharyngeal carcinoma. Project no.: 04151726. (2017-2019; HK$1,199,944)

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

The NPC TRS team members have been invited to present the findings of the project in multiple international conferences. They have also played key roles in organizing the Gordon Research Conferences of NPC in Hong Kong at 2016, 2018 and 2020, and various cancer meetings.

Invited speakers or organisers:

KW Lo: **(1)** "NPC Genetics: Progressive Genomic Changes". Gordon Research Conference: Nasopharyngeal Carcinoma. June 26-July 1, 2016, Hong Kong. **(2)** "Genomic landscape of EBV-associated nasopharyngeal carcinoma". Cancer 2016: Frontiers in Cancer Research. November 1, 2016, Hong Kong**. (3)** "Whole exome and genome sequencing identifies frequent NF-κB pathways activating mutations in EBV-associated nasopharyngeal carcinoma" 17th International symposium on Epstein-Barr virus and associated disease. August 8-12, 2016, Zurich. **(4)** "Genomic Landscape of EBV-associated nasopharyngeal carcinoma". The 76th Annual Meeting of the Japanese Cancer Association, September 28-30, 2017. Yokohama Kanagawa, Japan. **(5)** "Genomics and Novel Targeted Pathways" in a NCI Naso-Pharyngeal Cancer Clinical Trials Planning Meeting organized by National Cancer Institute on January 27-28, 2018 in Phoenix, USA. **(6)** "The genome landscape of EBV-associated nasopharyngeal carcinoma". International Head and Neck Cancer Symposium. 20-21 January, 2018. **(7)** "Acquired Genomic Changes in Nasopharyngeal Carcinoma". Gordon Research Conference: Nasopharyngeal Carcinoma. June 24-29, 2018, Hong Kong. **(8)** "Translational genomics of nasopharyngeal carcinoma". 2019·Nasopharyngeal Carcinoma Guangzhou Summit and Founding Meeting of Professional Committee of NPC and NPC Minimally Invasive Surgery Course, 8-10 November, 2019, Guangzhou. **(9)** Organiser of the meeting – Cancer 2016: Frontiers in Cancer Research. November, 2016 and Cancer 2017: Translating Cancer "Omics' to Precision Medicine, June 22, 3027, Hong Kong. **(10)** Chair of "Molecular basis of virus-associated tumors and their therapeutic targets" section, The 76th Annual Meeting of the Japanese Cancer Association, September 28-30, 2017. Yokohama Kanagawa, Japan. **(11)** "Exome and Genome Alterations in Nasopharynx Cancer". Gordon Research Conference: Translational Cancer Genomics. June 30 – July 5, 2019, Hong Kong. **(12)** Chair of organizing committee of Cancer 2019, November 21, 2019, Hong Kong. Vice Chair and Chair of organizing committee of Gordon Research Conferences: Nasopharyngeal Carcinoma 2018 and 2020 respectively, Hong Kong.

SW Tsao: **(1)** "Establishment of new NPC xenografts and cell lines for EBV studies". Gordon Research Conference: Nasopharyngeal Carcinoma. June 26-July 1, 2016, Hong Kong. **(2)** "Establishment and characterization of newly established nasopharyngeal carcinoma xenografts and in vitro NPC cell lines for EBV studies". 17th International symposium on Epstein-Barr virus and associated disease. August 8-12, 2016, Zurich. **(3)** Vice Chair of Gordon Research Conferences: Nasopharyngeal Carcinoma, June 26- July 1, 2016, Hong Kong. **(4)** Chair of organizing committee of Gordon Research Conferences: Nasopharyngeal Carcinoma, June 24-29, 2018, Hong Kong.

BBY Ma: **(1)** "Novel targets for NPC treatment". Gordon Research Conference: Nasopharyngeal Carcinoma. June 26-July 1, 2016, Hong Kong. **(2)** "Checking in on checkpoint inhibitors – impact on management of head and neck cancer". Immuno-Oncology Hong Kong 2016. November 19-20, 2016, Hong Kong. **(3)** "Multicenter phase II study of nivolumab in previously treated patients with recurrent and metastatic non-keratinizing nasopharyngeal carcinoma - Mayo clinic Phase 2 Consortium P2C-MN026, NCI9742, NCT02339558". AACR annual meeting, April 1-5, 2017, Washington. **(4)** "Immune Checkpoint Inhibitor in the Treatment of Nasopharyngeal Carcinoma: A Clinical Overview". Gordon Research Conference: Nasopharyngeal Carcinoma. June 24-29, 2016, Hong Kong.

ATC Chan: **(1)**"New drugs for metastatic disease". 2016 ASCO Annual meeting. June 3-7, 2016, Chicago. **(2)** Chairman of the meeting "Immuno-Oncology Hong Kong 2016". November 19-20, 2016, Hong Kong. **(3**)"Immuno-oncology in nasopharynx cancer". ESMO Immuno-Oncology Congress 2017, 7-10 December, 2017, Geneva Switzerland. **(4)** "The role of immunotherapy in NPC". ESMO Immuno-Oncology Congress 2017, 7-10 December, 2017, Geneva Switzerland. **(5)**

"Overview of recent developments in immunotherapy for H&N squamous cell cancer (HNSCC) and nasopharyngeal cancer (NPC). ESMO Immuno-Oncology Congress 2018, 13-16 December, 2018, Geneva Switzerland. **(6)** "How to address the research questions on nasopharyngeal carcinoma: The case for an Asian/European partnership". ESMO Immuno-Oncology Congress 2018, 13-16 December, 2018, Geneva Switzerland.

EP Hui: "Axitinib in recurrent or metastatic nasopharyngeal carcinoma (NPC): final result of a phase 2 clinical trial with pharmacokinetic (PK) correlation". ESMO 2016 Congress. October 7-11, Copenhagen, Denmark.

Q Tao: "Epigenetic targets" European Society for Medical Oncology (ESMO)-Asia 2016, 18-21 Dec 2015, Singapore.

ST Cheung: **(1)** "Rational Design to Combine with Checkpoint Immunotherapy". Immuno-Oncology Summit Europe 2019. March 18-22, 2019. London, UK; **(2)** Rational Combination of PD-L1/PD1 Targeting and Natural Killer Cells: Hepatocellular Carcinoma Preclinical Study. Immuno-Oncology Summit. August 27-31, 2018. Boston, USA;

VWY Lui: **(1)** "Clinically relevant omics in head and neck cancer: are we digging deep enough". Cancer 2017–Translating Cancer "Omics" to Precision Medicine, 22 June 2017, Hong Kong; **(2)** "Developing precision medicine and targeted therapies for Head and Neck Cancer". ENT Conference, December 2018, Hong Kong. **(3)** "Research Advances in Translational Oncology". Cancer 2018, November, 2018; **(4)** "Translational Genomics Landscape in NPC". Nasopharyngeal Cancer Workshop, January 2019, Singapore.

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

KW Lo is a member of International Expert Panel of National Medical Research Council (NMRC), Singapore, a translational Co-Chair of a new phase III trial (LOI # HN1854, NRG Oncology HN007) 'An Open-label, Phase III Study of Platinum- Gemcitabine with or without Nivolumab in the First-line Treatment of Recurrent or Metastatic Nasopharyngeal Carcinoma', and an academic editor of PLOS ONE,

ATC Chan is Vice-President of the Hong Kong College of Physicians. He is an associate editor of Annals of Oncology and Chinese Journal of Cancer and editorial board member of International Journal of Cancer.

BBY Ma is president of the Hong Kong Head and Neck Society (2016-17), a council member (2016-2019) of WICR (Women in Cancer Research) in AACR, member of NCI Head and Neck Cancer Steering Committee (HNSC) (2017), Scientific Committee of American Association for Cancer Research (2016) and ESMO GI 2017 at Barcelona. She serves in the International Advisory Board of the Lancet Oncology and editorial board of the *Asian-Pacific Journal of Clinical Oncology* and *South Asian Journal of Oncology*. She is a member of the Joint Scientific Committee for Phase 1 Clinical Trials, Centre of Health Protection, Hong Kong Hospital Authority and the vice chair of the Hong Kong Nasopharyngeal Cancer Study Group. Recently, she serves as the Study Chair/PI of the new phase III trial (LOI # HN1854, NRG Oncology HN007).

KF To is member of Advisory Boards of Novartis APECHO2015, MSD Asia Pacific NSCLC & Biomarker and MSD Asia Pacific Oncology Biomarker.

KYL Yip is a member of the editorial board of Journal of Biomedical Informatics. He is a member of organizing committees of Croucher Foundation Advanced Study Institute on Genetic Variation and Genome Architecture in Development, Heath and Disease and the 21st Annual International Conference on Research in Computational Molecular Biology (2017).

TF Chan is Associate Editor of the journals Frontiers in Genetics and Frontiers in Plant Science.

Q Tao is Vice-President of the international Epigenetics Society and panel member for National Natural Science Foundation of China (NSFC). He is an academic Editor of PLOS ONE and editorial board member of Chinese J Cancer, Journal of Clinical Epigenetics and Epigenetic Diagnosis & Therapy.

AST Wong is an editorial Board Member of the journal Current Medicinal Chemistry and panel

member of State Key Laboratory for Oncogenes and Related Genes grant, Shanghai Cancer Institute, China.
SW Tsao is an academic editor of PLOS ONE and editorial board member of the journal Current Cancer Drug Target.
N Wong is an associate Editor of Journal of Pathology and panel member of RGC GRF and ECS - Biology and Medicine Panel (2013-2018).

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

The NPC genome datasets established in this TRS project were delivered in public databases, dbGAP (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001244.v1.p1) and ENA (https://www.ebi.ac.uk/ena/browser/view/PRJEB12830). The WGS dataset from our TRS project has also contributed to the development of a whole-genome reference panel of Northeast Asians (NARD), which yields the greatest imputation accuracy of rare and low-frequency variants compared with the existing panels (e.g. 1000 Genomes Project Phase 3) (Yoo SK *et al.* Genome Med. 2019 Oct 22;11(1):64.) The multiple alignment algorithm for optical mapping including OMSV and OMMA pipelines developed by our TRS Bioinformatics team was applied for establishment of population-specific patterns of structural variation across 26 human population (Levy-Sakin M, *et al.* Nat Commun. 2019 Mar 4;10(1):1025.) and accurately reconstructs the phylogenomic relationships and identifies functional elements among different populations (Leung AK, *et al.* Gigascience. 2019 Jul 1;8(7).pii: giz079). The biomarkers and immunotherapy strategies for NPC developed by our TRS team members were presented and discussed in a National Cancer Institute (NCI) sponsored clinical trial planning meeting (CTPM) focusing on immunotherapy in NPC on February 27-28, 2018, in Phoenix, AZ, USA. The details of this meeting and application of our findings in planning of new clinical trial for NPC were documented in a recent article published in J Natl Cancer Inst. (Le QT, *et al*. J Natl Cancer Inst. 2019 Mar 26.).

7.5 Other awards and recognitions as a result of this project (please specify):
KW Lo: Outstanding fellow of the Faculty of Medicine 2013-2018, 2018-2023, CUHK; ***KF To***: Outstanding fellow of the Faculty of Medicine 2014-2019, CUHK; ***N Wong:*** Outstanding fellow of Faculty of Medicine 2017-2022, CUHK; ***KYL Ip***: Outstanding fellow of Faculty of Engineering 2019-2024, CUHK; ***KYL Ip***: Young Researcher Award, Faculty of Engineering 2019, CUHK.

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 this TRS project will significantly advance the current clinical management of NPC patients to achieve excellent control of this Asian cancer by prolonging disease-free survival, minimising treatment toxicities and improving quality of life of patients. To improve the patient's outcome, the newly identified biomarkers (e.g. loss of MHC class I) have been applied in recent clinical trials of immune checkpoint inhibitors. For developing efficient treatment, we have already received patents for targeting novel oncogenic fusion genes and innovative nanovector-siRNA against unique NF-κB signal in NPC while a patent application for novel EBV-targeting oncolytic treatment is in preparation. These innovative preclinical studies provide opportunities for effective control of this common cancer in our community. Our established comprehensive genome datasets and representative EBV-positive NPC models have major impact in maintaining the leading position of Hong Kong in genomic and translational research of NPC. The genomic and functional findings contribute to the development of new tumorigenesis models and unveil the crucial role of EBV in initiation and progression of NPC. Through this TRS project, our term members have established new collaborations with the world-renowned EBV and NPC research groups. We were invited to join international expert teams of various projects for targeting EBV cancers, defining reference genome of Asian population and conducting NIH funded clinical trials.

8.2 Others (please specify):
NA

9. Sustainability of the Project

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

Yes. The findings of this TRS project greatly enhance our understanding of the molecular base and viral-host interplay in NPC tumorigenesis. The TRS project identified new genetic lesions in TGF-β signalling contribute to EBV persistent infection in epithelial cells and its critical role in tumor initiation. The co-evolution model of somatic alterations and EBV latent gene expression during tumor progression was proposed and evidenced by activating altered NF-κB signalling pathways by disrupting antigen-presentation mechanisms by somatic alterations and expression of various EBV latent genes in NPC. The findings also contribute to the development of novel biomarkers and new strategies of immunotherapy for NPC. We have reported the first oncogenic fusion gene *UBR5-ZNF423* and multiple key somatic changes (NF-κB related gene mutations, MTAP deletion) and their potential as molecular targets for NPC treatment. Furthermore, the TRS project has defined a number of actionable targets such as *MTAP* deletion and *BCL3* activation in this unique EBV-associated cancer. The nanosystem to deliver siRNA against key oncogenic transcription factors in *in vivo* NPC models was demonstrated as effective therapeutic strategies for this deadly epithelial malignancy.

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

Yes. As listed in section 7.1, three CRF projects evolved directly from this project were recently funded to investigate the pathogenesis role of EBV in epithelial cells (C7027-16G), to develop EBV-targeting therapies in NPC (C4001-18G) and to study of complex genomic feature and structural variations in human cancer genomes (C4057-18EF). This TRS project also allows our team members to establish new patient-derived preclinical models (e.g. PDXs, tumor organoids) for translational cancer research. A RGC funded Research Impact Fund project (R4022-18) led by N Wong has been initiated on 2019. Based on the results of this TRS project, BBY Ma and KW Lo are collaborating with a panel of international research teams to propose a new phase III trial (LOI # HN1854, NRG Oncology HN007) of immunotherapy for recurrent/metastatic NPC respectively. This new clinical trial was successfully approved and will start on 2020. The members of Clinical Science team are initiating multiple clinical trials of new therapies against EBV and molecular targets identified in this TRS project. In addition, as shown in section 7.1, our team members have also initiated several RGC funded GRF projects to follow up the new findings of this project.

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

Yes. A number of interesting studies have been established among the TRS team members and domestic/international collaborators as directly results from this project. For example, ***BBY MA*** and ***KW Lo*** currently serve as the Study Chair/PI and co-Chair of Translational Science of a new phase III trial (LOI # HN1854, NRG Oncology HN007) of immunotherapy for recurrent/metastatic NPC respectively. They are collaborating with a group of international experts including A Dimitriors Colevas (Stanford University, USA), Sara M Calkins (UCSF, USA), Lillian L. Siu (Princess Margaret Hospital, Canada), Boon Cher Goh and Wan TD Lim (National University Cancer Institute, Singapore), Danny Rischin (Peter MacCallum Cancer Centre, Australia), Bhumsuk Keam (Seoul National University Hospital, South Korea), and Chaosu Hu (Fudan University Shanghai Cancer Center). ***KW Lo*** and ***SW Tsao*** are collaborating with Prof LF Yap from University of Malaya and Prof LS Young from Warwick Medical School, UK to study the roles of TGF-β signal pathways on NPC tumorigenesis. They are also working with an international research group led by Prof Matt Lechner in UCL Cancer Institute, University of College London, UK on the development of imaging and novel therapy targeting somatostain receptor 2 in NPC patients. They are collaborating with Prof J Middldorp from VU University Medical Center, Netherlands and

Henri-Jacques Delecluse from German Cancer Research Centre (DKFZ), German to study the EBV persistent latent infection and lytic reactivation. ***Q Tao, SW Tsao*** and ***KW Lo*** are working with Profs Dong-Yan Jin and Honglin Chen from University of Hong Kong to identify the host restriction and dependency factors for EBV infection. ***KW Lo*** and ***AST Wong*** are collaborating with Dr Ling Peng at the French National Scientific Research Center (CNRS) to develop new nanosystems against unique molecular targets in NPC. ***KW Lo*** and ***KY Yip*** are working with Prof Alan Khoo Soo-Beng from Institute for Medical Research Malaysia to characterize a panel of NPC PDXs. ***TF Chan*** and ***KY Yip*** is collaborating with Prof Pui-Yan Kwok from University of California-San Francisco, USA to reveal population-specific patterns of structural variation across 26 human population by optical genomic mapping and their OMTools package. ***KW Lo*** has initiated a collaboration with Dr AW Cheng from the Jackson Laboratory for Genomic Medicine, USA on modulating EBV latent and lytic genes in NPC cells by CRISPR-based technology. He is also collaborating with Prof Ben Ko from Hong Kong Polytechnic University and Prof Haim Barr, Director of Chemical Genomics Laboratory of Weizmann Institute of Science, Israel, for high-throughput screening on EBV-positive NPC cell models.

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.

Based on the findings from this TRS project, we have successfully obtained funding to support three RGF CRF projects for defining the critical role of EBV and somatic gene alterations in NPC tumorigenesis and developing novel EBV-targeted therapies for this viral-associated cancer. A total of **HK$14,757,741** were received from Hong Kong Research Grant Council. These CRF projects include: (1) C4001-18GF – "Targeting Epstein-Barr virus in nasopharyngeal carcinoma: from mechanistic study to novel therapeutic development" led by KW Lo (PC); (2) C4057-18EF – "A nanochannel-based next-generation mapping system for the study of complex genomic feature and variation for biotechnological and biomedical applications" led by KF Chan (co-PI); and (3) C7027-16G-"Regulation and pathogenic role of latent infection of Epstein-Barr virus in nasopharyngeal carcinoma" led by SW Tsao (co-I) and KW Lo (PC). KW Lo and SW Tsao have also joined as co-PI of a research team led by Prof XY Guan (HKU) to request a total of HK$24,000,000 from RGC TBRS 2019/2020 to support a project entitled 'Precision Oncology for Nasopharyngeal Carcinoma-From Clinic to Bench and Back to the Clinic".

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	57	68	5	2	Patents	2

12. The Layman's Summary

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

As the most prevalent cancer in our middle-aged workforce population, NPC is a major health-care problem in Hong Kong. More than 60% of newly diagnosed NPC patients are presented with advanced stage disease and show poor clinical outcome. The key problems of these patients are distant failure and lack of efficient treatment for recurrent diseases. New clinical interventions to treat the disease, prolong disease-free survival and improve quality of life of patients are therefore of strategic importance. A comprehensive understanding of genetic changes involved in NPC tumorigenesis is expected to offer the basis for research to develop promising disease control strategies for this cancer. In this project, we have decoded the DNA sequence of entire NPC genome and thereby catalogue the whole spectrum of genomic changes involved in NPC tumorigenesis by massive parallel genome sequencing and newly developed bioinformatics tools. Our team has systematically defined the driver mutations and identified key "molecular targets" through large-scale genome and transcriptome sequencing, bioinformatic analysis and extensive validation in primary NPC samples. Functional studies have confirmed the oncogenic activities and biological significances of candidate driver mutations in NF-κB and TGF-β signalling pathways and various EBV-encoded oncogenic proteins and noncoding RNAs. Our newly announced NPC tumorigenesis model in which a co-evolution of EBV latency and acquired genomic changes during tumor progression has provided new insights for the molecular basis of this viral-associated cancer. To translate the genomic findings to clinical management, statistical analysis has been conducted to identify a number of new biomarkers such as tumor burden, LMP1 expression, TP53 mutation, 12p13 amplification and somatic alteration of MHC molecules for predicting patient's outcomes and response to immunotherapy. Importantly, a number of candidate molecular targets (e.g BCL3, MTAP) and their relevant tumour dependency were uncovered and elucidated for their therapeutic potentials in our unique panel of *in vitro* and *in vivo* NPC models. Our findings will provide important novel biomarkers and therapeutic targets for developing personalized cancer treatment strategies. We expect this project would make strategic breakthrough in molecular genetics of NPC and contribute significant impact to the control of this common cancer.