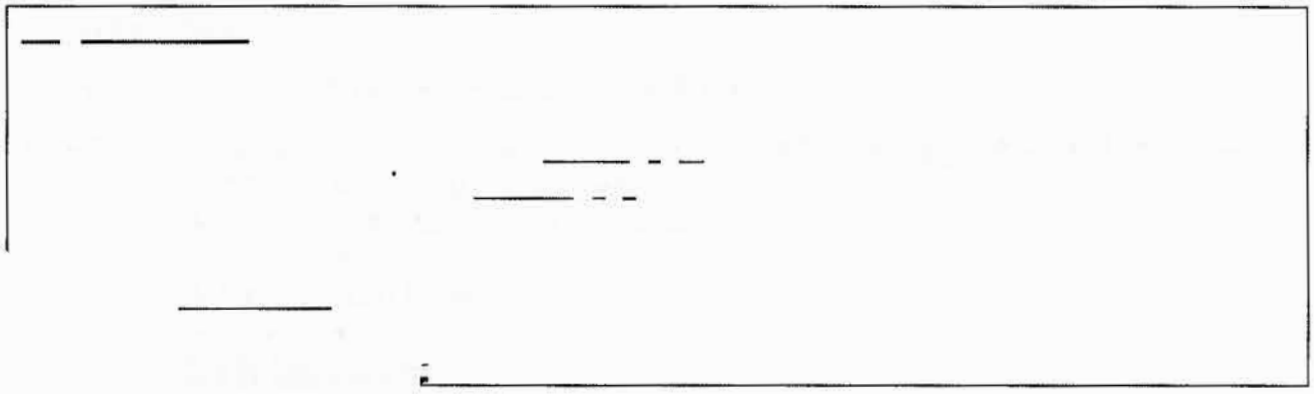
Project number: T12-701/17-R

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *Nov 1, 2017*
Project completion date: *Oct 31, 2022*



1. Project Title: Translational studies for elucidating the tumor heterogeneity and molecular evolution in metastatic gastrointestinal tract cancers for personalized medicine

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)	Maria Li LUNG/ Chair Prof	Clinical Oncology/HKU	8
	#Simon LAW/Chair Prof	Surgery/HKU	3
Co-Principal Investigator(s)	Dora KWONG/Clin Prof	Clinical Oncology/HKU	3
	Alfred LAM/Prof/Head	Pathology/Griffith University	3
	Kaon LAM/Clin Asst Prof	Clinical Oncology/HKU	3
	Chwee Teck LIM/Chair Prof	Biomed Engineering/NUS	3
	Pak SHAM/Chair Prof	Psychiatry/HKU	3
Co-Investigator(s)	Anthony LO/ Clin Assoc Consultant	Anatomical Pathology/QMH	3
Collaborator(s)	N.A.	N.A.	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.

 # Prof M Lung retired from HKU June 30, 2022. Prof S Law became PC July-Oct 2022.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. Decipher the molecular basis of ESCC metastasis and tumor heterogeneity	1. 100%	1.
2. Elucidation of predictive biomarkers for chemoresistance	2. 100%	2. *Oct 2017 approval to cut discovery set from 100 to 50 patients for each group
3. Improve treatment outcomes for GIT cancer patients by non-invasive real-time monitoring for early relapse	3. 100%	3. *Oct 2017 approval to reduce CRC patients from100 to 50 patients and not include gastric cancers
4. Establish new patient-derived metastatic CTC models for drug testing and functional studies	4. 100%	4. *Oct 2017 approval to confine to test studies in ESCC only, establish short term cultures, perform pilot studies for long-term cell cultures and PDXs, select a few candidate genes of interest in previous projects for functional ESCC analysis and not include TMA construction in project

* *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.))

The team's best research accomplishments include the following research outputs.

1) Poster at Health Research Symposium 2021 (Hong Kong)
Lung M.L., Ko J.M.Y., Ng B.H.Y., Lam K.O., Chiu K.W.H., Kwong D.L.W., Fong H.C.H., Wong I.Y.H., Chan F.S.Y., Wong C.L.Y., Chan K.K., Law T.T., Lam C.C.S., Dai W., Law S.Y.K. Clinical Application of Enumeration and Genomic Characterization for Non-invasive Detection and Real-time Monitoring of Circulating Tumor Cells for Esophageal Carcinoma
Best Poster Award, Nov 23, 2021

2) Invited oral presentation at the Journal Club of the Health Bureau, 2023 February 10, Video conference (By Zoom). Clinical application of enumeration and genomic characterization for non-invasive detection and real-time monitoring of circulating tumor cells for esophageal carcinoma.

3) Invited oral presentation at 18th ISDE World Congress for Esophageal Diseases, 2022 September 26-28, Virtual.
Ko JMY, Guo C, Liu C Ning L, Dai W, Tao L, LO AWI, Wong CWY, Wong IYH, Chan FSY, Wong CLY, Chan KK, Law TT, Lee NPY, Liu Z, Jiang H, Li Z, Law S, Lung ML. Clonal relationship and alcohol consumption-associated mutational signature in synchronous hypopharyngeal tumors and esophageal squamous cell carcinoma.

4) Invited oral presentation at 18th ISDE World Congress for Esophageal Diseases, 2022 September 26-28, Virtual.
Ng H.Y., Ko J.M.Y., Wong I.Y.H., Wong C.L.Y., Chan S.Y., Chan K.K., Law T.T., Lam K.O., Kwong D.L.W., Law S., Lung M.L. Clinical utility of ctDNA in longitudinal monitoring of treatment responses and relapse in metastatic esophageal squamous cell carcinoma

5) Publication in top surgery journal shows the importance of our longitudinal real-time monitoring of ctDNAs as prognostic markers for ESCC.

Ng, HY, Ko, JMY, Lam, KO, Kwong, DLW, Lo, AWI, Wong IYH, Wong, CLY, Chan, SY, Chan, KK, Law, TT, Dai, W, Fong, HCH, Choy, FSF, Lo, CK, Chen, C, Law, S, and Lung, ML, *Circulating tumor DNA dynamics as prognostic markers in locally advanced and metastatic esophageal squamous cell carcinoma.* JAMA Surgery, 2023. **Sep 20. doi: 10.1001/jamasurg.2023.4395. Online ahead of print. (Impact factor† = 16.685, JIF Rank: 1/282 (Surgery), JIF Quartile/Percentile: Q1/99.82)**

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 added value of TRS funding basically allowed larger-scale projects in terms of budget and people involved and supported, as well as extending the timeline for the project. Our aims and deliverables could not be delivered in a 3-year period, as is the case for both GRF and CRF support.
The TBRS allowed our team to be inclusive of clinicians in Hong Kong, as well as external collaborators, pathologists, bioinformaticists, bioengineers, and molecular/cellular biologists. This large team was able to provide the needed clinical specimens and patient information and to alert the team of important needs of the patients that might be addressed. The scientists had a varied background making it possible to conduct CTC and ctDNA liquid biopsy studies, as well as NGS expertise for single cell RNA analysis, whole exome sequencing, and whole genome sequencing. We had the bioinformatics expertise to analyze the large datasets and the pathologists to find patient FFPE tumors for molecular confirmation of mutations observed in liquid biopsies.
In addition, with TRS funding for a 4-5 year period, this allowed the accruement of the needed patient cohorts for the studies. Having an international advisory group to comment on our approaches and findings was also value-added.

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

N/A

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).*

SEE APPENDIX 1.

The Latest Status of Publications	Author(s) *(denote*	Title and	Submitted	Attached	Acknow-	Accessibl

Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*	*the corresponding author with an asterisk*)*	journal/book *(with the volume, pages and other necessary publishing details specified)*	to the RGC *(indicate the year ending of the relevant progress report)*	to this report *(Yes or No)*	ledged the support of RGC *(Yes or No)*	e from the institutional repository *(Yes or No)*
2020				Ko JMY, Ng HY, Lam KO, Chiu KWH, Kwong DLW, Lo AWP, Wong JC, Lin RCW, Fong HCH, Li JYK, Dai W, Law S, Lung ML*	Liquid biopsy serial monitoring of treatment responses and relapses in advanced esophageal squamous cell carcinoma. *Cancers* 2020, 12, 1352, doi:10.3390/cancers12061352.	2020	Yes	Yes	Yes
2021				Kam NW, Wu KC, Dai W, Wang Y, Yan LYC, Shakya R, Khanna R, Qin Y, Law S, Lo AWI, Lee VHF, Guan XY*, Kwong DLW*	Peritumoral B cells drive proangiogenic responses in HMGB1-enriched esophageal squamous cell carcinoma. *Angiogenesis* 2021 Oct 6;1-23. doi: 10.1007/s10456-021-09819-0		Yes	Yes	Yes
2021				Choi S, Ko JMY, Yu VZY, Lvwen N, Lung M*	Differentiation-related *ZNF750* suppresses cell growth in esophageal squamous cell carcinoma. *Oncology Letters.* 2021, 22: 513. PMID: 33986873		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)*						
2022				Ko JMY, Guo C, Liu CH, Ning L, Dai W, Tao L, Lo AWI, Wong CWY, Wong IYH, Chan FSY, Wong CLY, Chan KK, Law TT, Lee NYP, Liu ZC, Jiang HY, Li ZG*, Law S*, Lung ML*	Clonal relationship and alcohol consumption-associated mutational signature in synchronous hypopharyngeal tumours and oesophageal squamous cell carcinoma. *Br J Cancer*. 2022 Dec;127(12):2166-2174. doi: 10.1038/s41416-022-01995-0. Epub 2022 Oct 19.		Yes	Yes	Yes
2023				Ko JMY*, Lam, KO, Kwong DLW, Wong IYH, Chan FSY, Wong CLY, Chan KK, Law TT, Chiu KWH, Lam CCS, Wong JC, Fong HCH, Choy, FSF, Lo A, Law S*, Lung ML*	Circulating Tumor Cell Enumeration for Serial Monitoring of Treatment Outcomes for Locally Advanced Esophageal Squamous Cell Carcinoma. *Cancers* (Basel). 2023 Jan 29;15(3):832. doi: 10.3390/cancers15030832.		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)*						
2023				Ng HY, Ko JMY, Lam KO, Kwong DLW, Lo AWI, Wong IYH, Wong CLY, Chan SY, Chan KK, Law TT, Dai W, Fong HCH, Choy FSF, Lo CK, Chen CC, Law S, Lung ML*	Circulating Tumor DNA Dynamics as Prognostic Markers in Locally Advanced and Metastatic Esophageal Squamous Cell Carcinoma. *JAMA Surg*. 2023 Sep 20. doi: 10.1001/jamasurg.2023.4395. Online ahead of print.		Yes	Yes	Yes
		2023		Dai W*, Ko JMY, H ZZ, Chow LKY, Chung MKY, Yu VZY, Ng BHY, Wong CWY, Leung KK, Chen CC, Wong IYH, Law S, Lo AWI, Lam AKY, Lung ML*	Characterizing Chromosome Instability Reveals Its Association with Lipid-Associated Macrophages and Clonal Evolution of Lymph Node Metastasis in Esophageal Squamous Cell Carcinoma. Submitted.		Yes	Yes	No
		2023		Yu VZY, So SS, Lung BCC, Hou GZZ, Wong CWY, Chung MKY, Wong IYH, Wong CLY, Chan KK, Chan FSY, Law TT, Xu KY, Tan ZT, Chow LKY, Lam KO, Lo AWI, Lam A, Kwong DLW, Ko JMY, Law S, Lung ML*	*ΔNp63*-restricted viral mimicry response impedes cancer cell viability and promotes antitumor immunity in esophageal squamous cell carcinoma. , Submitted.		Yes	Yes	No

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)*						
	2023			Tan Z, Ko JMY*, Yu VZY, Lam KO, Kwong DLW, Wong IYH, Chan FSY, Wong CLY, Chan KK, Law TT, Choy FSF, Ng HY, Law SYK, Lung ML*	Multigene Profiling of Circulating Tumor Cells in Esophageal Squamous Cell Carcinoma Identifies Prognostic Cancer Driver Genes Associated with Epithelial-Mesenchymal-Transition Progression and Chemoresistance. *Cancers*, accepted.		Yes	Yes	No
		2023		Ko JMY*, Guo C, Lo AWI, Tao L, Leung AKK, Chan SCY, Wong CWY, Law S, Wong IYH, Wong CLY, Chan FSY, Chan KK, Law TT, Lam KO, Kwong DLW, Dai W, Lam AKY, Lung ML	Oncogenic *NFE2L2* mutations in both plasma ctDNA and formalin-fixed paraffin-embedded specimens are prognostic of treatment outcome and survival in resectable esophageal squamous cell carcinoma patients. *Annals of Surgery*, submitted.		Yes	Yes	No
			2023	Wong CWY, Ko JMY, Ng HY, Yu VZ, Law S, Lung ML*	Characterisation of Heterogeneity of Oesophageal Squamous Cell Carcinoma Ecosystem by Single-Cell Transcriptomic Analysis. Manuscript in preparation.		Yes	Yes	No

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)*						
			2023	Ko JMY, CCS Lam, Xu KY, Lam KO, Kwong DLW, Lo AWI, Chiu KWH, Wong IYH, Chan FSY, Wong CLY, Chan KK, Law TT, Wong JC, Fong HCH , Wong CWY,Leung AKK, Ng HY, Dai W, Lee NP, Law S, Lung ML*	CTC genomic profiling for serial monitoring of treatment responses and relapse in advanced esophageal squamous cell carcinoma. Manuscript in preparation		Yes	Yes	No
			2023	Lung BC, Yu VZ*, Hou GZ, Dai W, Law S, Lung ML*	*Prolyl 3-hydroxylase 1* maintains selective polyunsaturated fatty acid metabolism and suppresses programmed cell death to sustain esophageal cancer development. Manuscript in preparation.		Yes	Yes	No
			2023	Lung BC, Yu VZ*, Wong IY, Wong CL, Chan DK, Chan FS, Law BT, Lam K, Kwong DL, Lo AW, Law S, Lung ML*	Pharmaceutical Csf1r inhibition reprograms the tumor microenvironment and suppresses esophageal squamous cell carcinoma. Manuscript in preparation.		Yes	Yes	No

(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)

SEE APPENDIX 2.

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)*

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)*
05/2019/Bergamo, Italy	Genomic Characterization of Tumor Heterogeneity of Esophageal Squamous cell Carcinoma (ESCC)-related Dual Cancers	EACR-ESMO Joint Conference on Liquid Biopsies	2020	Yes	Yes	No
06/2019, HK	Genomic Profiles to Identify the Key Events in Metastasis for Esophageal Squamous Cell Carcinoma	Gordon Research Conference, Multiomics-based cancer molecular typing, prognostication and treatment	2020	Yes	Yes	No
10/2019/Corfu, Greece	Comparative analysis of numbers of circulating tumor cells in nine tumor types	4th ACTC meeting on "Liquid biopsy: latest advances and future challenges	2020	Yes	Yes	No
10/2019/Corfu, Greece	Liquid biopsy serial monitoring of treatment response and relapse of metastatic esophageal squamous cell carcinoma	4th ACTC meeting on "Liquid biopsy: latest advances and future challenges	2020	Yes	Yes	No
4/2020/ San Diego, CA, USA	Establishment of patient-derived organoid culture and organoid-derived xenograft as models for esophageal squamous cell carcinoma study	111th American Association Cancer Research Annual Meeting, April 24-29 2020. San Diego, CA, USA. CANCELLED due to COVID	2020	Yes	Yes	No
6/2020/virtual USA	Real-time serial monitoring of liquid biopsies allows earlier detection of treatment responses and relapse for metastatic esophageal squamous cell carcinoma.	111th American Association Cancer Research Annual Meeting, April 24-29 2020. San Diego, CA, USA. CANCELLED Presented in virtual meeting June 2020	2020	Yes	Yes	No
4/2022/ New Orleans, LA, USA	Patient-derived tumor organoid cultures identify a chemo-resistant sub-population in esophageal squamous cell carcinoma.	American Association Cancer Research Annual Meeting, April 8 - 13, 2022 New Orleans, Louisiana, USA.		Yes	Yes	No

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)*
9/2022/virtual Japan	Clonal relationship and alcohol consumption-associated mutational signature in synchronous hypopharyngeal tumors and esophageal squamous cell carcinoma	18th ISDE 2022 World Congress for Esophageal Diseases		Yes	Yes	No
9/2022/virtual Japan	Clinical utility of ctDNA in longitudinal monitoring of treatment responses and relapse in metastatic esophageal squamous cell carcinoma	18th ISDE 2022 World Congress for Esophageal Diseases		Yes	Yes	No
9/2022/virtual Japan	Patient-derived tumor organoids and xenografts as basic and preclinical translational esophageal squamous cell carcinoma models	18th ISDE 2022 World Congress for Esophageal Diseases		Yes	Yes	No
4/2023 Orlando, Florida, USA	Molecular detection of circulating cancer cells with cancer stem cell or mesenchymal characteristics in esophageal squamous cell carcinoma	AACR Annual Meeting 2023 in Orlando, Florida		Yes	Yes	No
4/2023 Orlando, Florida, USA	Modelling cancer cachexia using a comprehensive panel of heterogeneous esophageal squamous cell carcinoma xenografts for therapeutic application	AACR Annual Meeting 2023 in Orlando, Florida		Yes	Yes	No
4/2023 Orlando, Florida, USA	Characterisation of heterogeneity of esophageal squamous cell carcinoma ecosystem by single-cell transcriptomic analysis	AACR Annual Meeting 2023 in Orlando, Florida		Yes	Yes	No
4/2023 Orlando, Florida, USA	*p63* constrains cancer cell transposable element expressions and viral mimicry response to sustain esophageal cancer development and indicates therapeutic vulnerability.	AACR Annual Meeting 2023 in Orlando, Florida		Yes	Yes	No
9/2023 Toronto, Canada	*NFE2L2* mutations are predictive and prognostic of treatment outcome in resectable esophageal squamous cell carcinoma patients	19th ISDE 2023 World Congress for Esophageal Diseases		Yes	Yes	No
9/2023 Toronto, Canada	Modeling cancer cachexia using patient tumor organoid-derived xenografts in esophageal squamous cell carcinoma.	19th ISDE 2023 World Congress for Esophageal Diseases		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.

We have acknowledged the grant in all publications (as attached with report).

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

Some of the Co-PIs of this TBRS team were from Australia and Singapore. We have worked with them on the collaborative TBRS projects. A new collaboration with the Shanghai Hospital group was also established to extend our collection of synchronous ESCC and hypopharyngeal cancers for our tumor heterogeneity studies. Unfortunately, due to the COVID outbreak our later years of international meetings was suspended in 2020-2.

6.6 Research students trained (registration/awards):
SEE APPENDIX 3.

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Chen GUO	PhD	Sept 1 2016	Aug 2020
Shan Shan SO	PhD	Jan 1, 2019	Dec 2022
Zhen TAN	PhD	Sept 1, 2019	Aug 2023
Kaiyan XU	PhD	Nov 1, 2020	Expected Sept 2024
Bryan LUNG	PhD	Sept 1, 2021	Expected Aug 2025
Carissa WONG	PhD	Sept 1, 2021	Expected Dec 2025

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

N/A

6.8 Other education activities and/or training programmes developed:

N/A

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.

N/A

Project Management

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

The PC organized and coordinated the group meetings with different team members. She met regularly with TBRS team members to prepare for the RGC 2019 site visit and the visit/zoom meetings with our international advisory board (IAB). Due to the 2020 COVID pandemic, the planned 2020 IAB visit and research symposium activities were cancelled. In 2021 with the ongoing international travel restrictions and quarantines in Hong Kong still in place, the October 2021 IAB meeting was held virtually by Zoom. The TBRS project duration was extended by one year due to slowdown of clinical specimen collections for the TBRS projects

due to COVID pandemic disruption. The PC and team members continued to oversee project progress together with other team members to coordinate project activities.

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7. Awards and Recognition

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

HMRF Research grant 2018-2020 $1,199,732 PI: M Lung
CoAs: S Law, D Kwong, KO Lam
"Clinical application of enumeration and genomic characterization for non-invasive detection and real-time monitoring of circulating tumor cells for esophageal carcinoma"

HMRF Research grant 2019-2022 $1,484,832 PI: V Yu
CoAs: M Lung, S Law, D Kwong, A Lam
"Establishment of a patient-derived organoid in vivo drug testing model in ESCC and a pilot study for tumor-associated macrophage targeted therapy"

HMRF Research grant 2020-2023 $1,499,750 PI: J Ko
CoAs: M Lung, KO Lam, A Lo
"Molecular classification based on microsatellite instability and RAS pathway status as prognostic biomarkers for risk stratification of metastatic relapse in colon carcinoma patients"

HMRF Research grant 2022-2024 $1,500,000 PI: J Ko
CoAs: M Lung, KO Lam, A Lo, K Chiu
"Clinical significance of real-timing longitudinal monitoring of liquid biopsies for advanced colorectal cancer"

HMRF Research grant 2024-2026 $1,500,000 PI: J Ko
CoAs: M Lung, W Dai, S Law, D Kwong, I Wong, A Lo
"A clinical prognostic model incorporating longitudinal ctDNA genomic profiling for molecular residual disease and prediction of treatment response in esophageal squamous cell carcinoma"
Recommended for support

HMRF Research grant 2024-2026 $500,000 PI: V Yu
CoAs: M Lung, S Law, D Kwong, A Lam, I Wong, A Lo, W Dai, J Ko
"Feasibility assessment of an organoid culture-based ex vivo chemotherapy and immunotherapy sensitivity profiling platform to predict treatment response for esophageal squamous cell carcinoma patients"
Recommended for support

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

Due to COVID pandemic our team's international conference attendance and organization of meetings were greatly affected. Prof S Law organized the ISDE 2022 and 2023 World Congress for Esophageal Diseases and several of our TBRS members joined with oral or poster presentations.

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

Prof Law helped organize the ISDE World Congress for Esophageal Diseases. Prof Law was the president of ISDE 2023 World Congress for Esophageal Diseases.

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

No

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

HMRF Research Symposium Best Poster Award 2021 to M Lung

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.)?

The results of this project are expected to impact and inform clinicians of important timepoints and biomarkers that hallmark tumor treatment resistance and patient prognosis. Ongoing discussions and collaborations between clinicians and basic scientists in HKU continue.
This project on real-time monitoring of ESCC and CRC in Hong Kong provides an opportunity for biotechnology companies (like OncoSeek, which we had earlier set up in Hong Kong Science Park for developing biomarker and CTC tests for cancer), to capitalize upon.
We have established external collaborations on NGS analysis of ESCC and hypopharyngeal cancers in Shanghai, China that are ongoing.
The original findings and continued publications ensuing from the TBRS grant have led to new potential HMRF grants to continue the research collaborations established during the TBRS grant.

8.2 Others (please specify):

1. Identification of useful biomarkers for patient prognostication using serial blood testing at different timepoints of patient treatment by ctDNA and CTC analysis and from whole genome sequencing of primary and nodal metastatic tumors to identify potential biomarkers for high-risk ESCC patients with expected poor treatment outcomes.
2. Establishment of a bank of organoids and patient-derived xenografts for evaluation of useful drugs and development of animal models.

3. Assessment of dual cancers spatial inter- or intra-tumor heterogeneity. NGS data provided important implications for prognostication. Findings support multicentric independent clonal evolution and the theory of field cancerization, implicating an etiologic role of alcohol metabolism in dual ESC/HPC carcinogenesis.
4. scRNA Seq elucidated the complex immunosuppressive interactions of various stromal cell types and the epithelial tumors in the ESC tumor microenvironment and identified potential prognostic cancer cell transcriptomic signatures.
5. WGS data analysis allowed the discovery of the importance of genomic instability and nodal metastases in ESC, as potential biomarkers to identify the high-risk ESCC patients for early death post-treatment.
6. Analysis of the good and poor responders by comprehensive genomic profiling of FFPE tissues from patients receiving neoadjuvant CRT identified potential biomarkers associated with relapse and death.

9. Sustainability of the Project

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

With the established organoid and patient-derived xenograft cultures and animal models, new projects to use these important resources generated in the TBRS project to further study the development of chemoresistance during treatment will be ongoing. Since one of the major problems with treatment success is the development of drug tolerant persistence, these resources are expected to contribute to further research on the development of drug resistance that is common in ESCC chemotherapy treated patients. Our latest ESCC patient-derived organoid and xenograft biobank serves as authentic models for basic, translational, and preclinical studies.

ΔNp63 restricts retrotransposon expression and double-stranded RNA sensing, resulting in suppressed tumor-suppressive type I interferon (IFN-I) signaling in a STAT1/IRF1- and cGAS/STING-dependent manner; The ΔNp63/IFN-I signaling axis influences antitumor immunity, including cancer cell antigen presentation and tumor-infiltrating immune cell recruitment and reprogramming; ΔNp63 may serve as a predictive biomarker for cancer cell-targeted viral mimicry boosting treatment and immune checkpoint inhibitors.

Longitudinal monitoring of liquid biopsies including CTC enumeration and ctDNA mutational profiling are useful biomarkers for prognostication of relapse and survival, earlier detection of minimal residual diseases in advanced and metastatic ESCC patients. The current ctDNA NGS study identified *NFE2L2* from a panel of 77 oncology genes, future larger studies should expand to cover a custom ESCC-specific ctDNA panel for assessment of neoadjuvant and adjuvant therapy efficacy and clinical behaviour of recurrences for guiding clinicians on disease management. Our ctDNA analysis provides novel insights for future pharmacological and therapeutics option targeting *NFE2L2* signaling axis.

Our findings reveal a novel link between error-prone double-strand break (DSB) repairs and lipid-associated tumor-associated macrophages (LA-TAMs) through chromosome instability (CIN) in lymph node metastasis in esophageal squamous cell carcinoma (ESCC) and suggest targeting LA-TAMs may prevent metastasis and improve survival for ESCC. Further detailed investigation of functions of LA-TAMs in immunosuppression and cancer metastasis and evaluation of the feasibility of targeting these LA-TAMs are needed for prevention of cancer cell dissemination and improvement of patient survival in this deadly cancer.

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

Some of our HMRF NGS analysis of ESCC and drug testing using ESCC organoids developed protocols for CTC analysis evolved from our TBRS studies.

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

The Shanghai Hospital collaborations for the dual cancer project were developed and increased our sample size in our study, resulting in a joint publication and the plan to further the joint collaborations between Hong Kong and Shanghai.

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.

Two HMRF grant applications obtained in 2019 and 2020, involved our ESCC organoid work and CTC analysis, respectively, with close to $3M grant funding for these projects.

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	10, plus 4 others in preparation	16	0	0	NA	0

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

12. The Layman's Summary

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

Esophageal cancers are deadly cancers due to late diagnosis. We aimed to identify and characterize molecular changes associated with tumor metastasis to understand disease mechanisms and identify useful markers to improve diagnosis. Circulating tumor cells (CTCs)

from primary and metastatic tumors are shed into the blood stream and allow non-invasive real-time detection of cancers. We aimed to determine the usefulness of CTCs to understand tumor heterogeneity, identify useful markers hallmarking metastasis, patient stratification, and actionable targets for improved treatment, and to identify important immune and stromal contributors to cancer.

Retrospective and serial analysis of ESC patients undergoing treatment was performed to identify predictive markers associated with treatment outcomes. We aimed to identify genetic alterations in metastatic tumors of known good/poor chemoradiotherapy (CRT) responders from archived ESC patient tissues, as predictive biomarkers for patient stratification and improved guidance of treatment options. We determined the clinical utility of liquid biopsies for non-invasive real-time monitoring for metastasis and drug resistance for gastrointestinal tract cancers to expand treatment choices for precision medicine. We established a bank of organoid tissues directly from tumor tissues and established animal models to evaluate drug efficacy. These findings are expected to aid in identification of useful drugs for improved treatment of ESCC.