(Revised 07/09)

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(please insert ref. above)

NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Novel strategies to overcome ATP-binding cassette drug efflux transporters-mediated multidrug resistance in cancer cells
克服ABC藥物轉運泵介導腫瘤細胞多藥抗藥性的新策略

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. To Kin Wah	Prof. Fu Liwu
Post	Assistant Professor	Professor & Director
Unit / Department / Institution	School of Pharmacy, The Chinese University of HK	Cancer Institute, Cancer Center, Sun Yat-Sen University, Guangzhou, China
Co-investigator(s) *(with title)*	Prof. Lin Ge School of Biomedical Sciences, The Chinese University of HK	Nil

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	Jan 1, 2011	N/A	
Project Completion date	Dec 31, 2013	N/A	
Duration *(in month)*	36	N/A	

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To systematically investigate selected tyrosine kinase inhibitors (TKIs) for their inhibitory effects on ABCG2 or P-gp and thus the reversal of transporter-mediated multidrug resistance (MDR)

2. To study the MDR reversal effect of TKIs in vivo and in putative cancer stem-like cells (CSCs)

3. To investigate whether the TKIs interact with CYP isozymes, thereby causing pharmacokinetic interference with anticancer drugs

4. To study the possible cause of resistance to TKIs by overexpression of ABCG2 or P-gp

5.2 Revised Objectives

Date of approval from the RGC: N/A

Reasons for the change: _____

_____

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

Objective 1 (Systematic evaluation of potential reversal of MDR by TKIs):

A few promising TKIs were selected for evaluation (including afatinib, apatinib, axitinib, CUDC-101, crizotinib, pelitinib, vatalanib and volasertib). In cancer cell line models with defined overexpression of the major MDR transporters (P-gp, ABCG2 and MRP1), all of these TKIs were found to reverse MDR to different extent. CUDC-101 was also found to circumvent resistance mediated by MRP2 (a less commonly studied MDR efflux transporter and is conferring specifically platinum drug resistance). The detailed mechanism for MDR reversal was elucidated as direct transporter inhibition, inhibition of ATPase activity, or gene regulation. The findings have been or are to be reported in all 8 manuscripts listed in Part C.

Objective 2 (Study of MDR reversal by TKIs *in vivo* and in cancer stem-like cells):

Four TKIs demonstrating MDR reversal *in vitro* (afatinib, apatinib, axitinib and crizotinib) from Objective 1 were also found to exhibit potent MDR reversing effect in resistant cancer cells-bearing mice. Another three TKIs (apatinib, axitinib and vatalanib) were also investigated for MDR reversal in CSCs. Our data revealed that these TKIs have more specific transporter-inhibiting and MDR-reversing effect on the CSCs than the remaining cell population. Therefore, these TKIs may have the potential to eradicate the entire tumor and prevent recurrence. The findings have been or are to be reported in the 1st, 3rd and 5th manuscripts listed in Part C.

Objective 3 (Interaction of TKIs with metabolic enzymes):

Data from the *in vitro* screening assay indicated that CUDC-101 is a moderate inhibitor for both CYP3A4 and CYP2D6 whereas vatalanib is a weak inhibitor for CYP3A4 and a moderate inhibitor for CYP2D6. In a follow-up single-dose pharmacokinetic assay evaluating the combination of CUDC-101 and irinotecan

(CYP3A4 substrate), the result revealed that CUDC-101 leads to about 1.5-fold increase in the AUC of irinotecan. The information will be useful for clinical study as to whether dose adjustment is needed for the combination therapy. The results are included in two prepared manuscripts listed as the 6th and 8th items in Part C, which will be submitted for publication very soon.

Objective 4 (Possible MDR transporters-mediated resistance to TKIs):

Data from the specific transporter-mediated efflux assay were analyzed by the Dixon plot to categorize the tested TKIs as competitive, non-competitive or uncompetitive inhibitor of the individual transporter. We found that CUDC-101 is an uncompetitive inhibitor for both MRP1 and MRP2 whereas vatalanib is a competitive inhibitor for ABCG2. Monolayer drug transporter assay revealed that CUDC-101 is not transported by MRP1 or MRP2 whereas vatalanib is a substrate transported by ABCG2. Importantly, the overexpression of either MRP1 or MRP2 did not mediate resistance to CUDC-101 but overexpression of ABCG2 may confer a low level of resistance to valatanib. The results are included in two manuscripts listed as the 6th (CUDC-101) and 8th (vatalanib) items in Part C, which will be submitted for publication very soon.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

Fueled by our encouraging data about the reversal of MDR by TKIs *in vitro* and *in vivo*, the Mainland team will look into the possibility of initiating clinical trials to evaluate the possible MDR reversal in cancer patients. Our current data will provide useful information to facilitate these clinical investigations, such as starting dose and whether dose adjustment will be needed for the drug combination. Meanwhile, the Hong Kong team, through another collaboration with Department of Chemistry, has attempted to incorporate the chemical structure of selected TKIs into the structure of conventional anticancer drugs. The idea is to make use of the TKI moiety of the new compounds to help circumvent or prevent drug resistance. Some preliminary data has been generated to support a RGC General Research Fund application (2014/15) submitted by the Hong Kong PI.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Multidrug resistance (MDR) is a major obstacle limiting the efficacy of cancer chemotherapy. It is cause by the overexpression of ABC drug efflux transporters on cancer cell surface. We found that molecular targeted tyrosine kinase inhibitors (TKIs) are able to restore sensitivity of MDR cells to conventional anticancer drugs. By using cell models with defined overexpression of three major MDR transporters (P-gp, ABCG2 and MRP1), we have studied the mechanisms for MDR reversal by a few promising TKIs. Importantly, our data indicate that some TKIs can specifically target the cancer stem-like cells (CSCs) and enhance their sensitivity to conventional anticancer drugs, thereby potentially eliminating cancer recurrence. We have also investigated the potential

interaction of the TKIs with metabolic enzymes and interference with pharmacokinetics of other concurrently administered anticancer drugs. Our data revealed mild inhibition of two major metabolic enzymes by selected TKIs, which could provide guidance for clinical use of the novel TKI-anticancer drug combinations. Moreover, we found that those TKIs inhibiting MDR transporters through a competitive manner may be more prove to cause their own resistance. Collectively, our results form the basis for the rational use of TKIs in combination with conventional anticancer drugs for MDR reversal.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project
(Please attach a copy of each 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.))

The Latest Status of Publications				Author(s) *(bold the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*					
2012				**Tong XZ, Wang F, Liang S, Zhang X, He JH, Chen XG, Liang YJ, Mi YJ, To KK, Fu LW***	Apatinib (YN968D1) enhances the efficacy of conventional chemotherapeutical drugs in side population cells and ABCB1-overexpressing leukemia cells. Biochem Pharmacol 2012, vol 83: 586-597	2012	Yes	Yes
2012				**Zhou WJ, Zhang X, Cheng C, Wang F, Wang XK, Liang YJ, To KK, Zhou W, Huang HB, Fu LW***	Crizotinib (PF-02341066) reverses multidrug resistance in cancer cells by inhibiting the function of P-glycoprotein	2012	Yes	Yes
2012				**Wang F, Mi YJ, Chen XG, Wu XP, Liu Z, Chen SP, Liang YJ, Cheng C, To KK, Fu LW***	Axitinib targeted cancer stemlike cells to enhance efficacy of chemotherapeutic drugs via inhibiting the drug transport function of ABCG2	2012	Yes	Yes

2013				**To KK*, Poon DC, Chen XG, Fu LW**	Volasertib (BI6727), a novel polo-like kinase inhibitor, reverses ABCB1 and ABCG2-media ted mulitdrug resistance in cancer cells	No	Yes	Yes
		✓		**Wang XK, He JH, Xu JH, Ye S, Wang F, Zhang H, Huang ZC, To KK, Fu LW**	Effect of afatinib on enhancing the efficacy of conventional chemotherapeu tical agent via eradicating cancer stem-like cells	No	Yes -abstract attached	Yes
		✓		**To KK*, Poon DC, Wei YM, Chen XG, Lin G, Fu LW**	CUDC-101, a hybrid molecular targeted agent, reverses resistance to Pt anticancer drugs via multiple mechanisms	No	Yes -abstract attached	Yes
			✓	**To KK*, Poon DC, Wang F, Lin G, Fu LW**	Pelitinib (EKB-569) targets the upregulation of ABCG2 and potentiates cytotoxic effect of ABCG2 substrate anticancer drugs in hyperthermia	No	Yes -abstract attached	Yes
			✓	**To KK*, Poon DC, Wang F, Lin G, Fu LW**	Vatalanib circumvents ABCG2-media ted multidrug resistance in hypoxia	No	Yes -abstract attached	Yes

9. Recognized International conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*
Apr/2012/ Chicago, USA	Crizotinib (PF-02341066) reverses multidrug resistance in cancer cells by inhibiting the function of P-glycoprotein	American Association of Cancer Research 2012 Annual Meeting	2012	Yes	Yes
Apr/2013/ Washington DC, USA	CUDC-101, a hybrid molecular targeted agent, reverses multiple mechanisms of drug resistance	American Association of Cancer Research 2013 Annual Meeting	No	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Daniel Poon (Prof. Kenneth To, PI of this grant, is his direct supervisor)	Ph.D.	Initially registered for M.Phil on Aug 1, 2011; subsequently transferred to Ph.D. in Sept 2012	Expected year of graduation: 2015
Yuming Wei (Prof. Kenneth To, PI of this grant, is her co-supervisor)	Ph.D. (Department of Chemistry)	Aug 1, 2011	Expected year of graduation: 2015

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

Not applicable