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海外特別研究員最終報告書

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（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地： New York （国名： USA ）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
アポトーシスに着眼したEGFR阻害薬の耐性を克服する新たな肺癌治療の開発

3. 派遣期間： 2018年 4月 1日 ～ 2020年 3月 31日

4. 受入機関名及び部局名
Memorial Sloan Kettering Cancer Center, Human Oncology and Pathogenesis Program

5. 所期の目的の遂行状況及び成果…書式任意 **書式任意（A4判相当3ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式10－別紙1～4に記入の上、併せて提出すること。

[Background]

The discovery of targetable molecular alterations in genes, such as epidermal growth factor receptor (EGFR), has driven the evolution of targeted therapies for non-small cell lung cancer (NSCLC) and shifted treatment paradigms for the disease. As a result, the treatment of lung cancer represents the vanguard of personalized cancer medicine. However, the clinical success of EGFR tyrosine kinase inhibitors (TKI) in *EGFR*-mutant NSCLC is limited by the eventual development of acquired resistance. Among the resistance mechanisms, a "second-site mutation" in EGFR, T790M, accounts for ~50-60% of acquired resistance after the first- and second-generation EGFR-TKI treatment. To overcome this major resistance mechanism, the third-generation EGFR inhibitors, including CO-1686 (rociletinib) and AZD9291 (osimertinib) have been developed that inhibit both the activating and T790M mutations of EGFR while sparing wild-type EGFR. Osimertinib is not only effective in T790M-aquired cases, but also superior to earlier generation EGFR-TKIs as first-line therapy, resulting in its approval by the FDA as first-line treatment for patients with advanced *EGFR*-mutant NSCLC. Unfortunately, acquired resistance to osimertinib inevitably occur and the resistance mechanisms are heterogeneous, among which the most frequent C797S secondary mutation being identified only in a subset of patients (~26%). Overall, it has become evident that targeting EGFR mutations alone is unlikely to cure *EGFR*-mutant lung cancer patients.

Acquired resistance to EGFR-TKIs occurs through the selection of pre-existing clones as well as the evolution of drug-tolerant persister cells that survive treatment through adaptive mechanisms, allowing cells to evade apoptosis and survive. Over time, the drug-tolerant cells can acquire resistance through mutational or non-mutational mechanisms. We hypothesize that enhancing apoptosis through combination therapies will eradicate cancer cells including the preexisting resistant clones and thereby reduce the emergence of drug-tolerant and resistant clones during treatment. In this study, we sought to develop cell death mechanism-based combination therapies that enhance the proapoptotic effect of osimertinib to

eradicate cancer cells using an integrated approach combining high-throughput drug screening and mechanistic elucidation.

[Results]

To identify the best combination to enhance the apoptotic activity of the third-generation EGFR inhibitor osimertinib in *EGFR*-mutant lung cancer, we have performed HTS of a custom chemical library that is composed of inhibitors of 200 targets involved in more than 20 signaling pathways, and covers most of the FDA-approved anti-cancer agents (Fig. 1a,b). Among the promising candidate agents, Aurora kinase and IGF1R inhibitors synergized with osimertinib to enhance apoptosis in *EGFR*-mutant H1975 cells (Fig. 1c). Four out of eight Aurora kinase inhibitors (the enhancer group) screened in HTS induced BIM- and PUMA-mediated apoptosis and completely killed the 'osimertinib-persister' clones, which required p-AURKB inhibition in H1975 cells (Fig. 2a-d). *AURKB*-knockdown with siRNA also enhanced BIM- and PUMA-mediated apoptosis in combination with osimertinib treatment (Fig 2e,f). Mechanistically, AURKB inhibition stabilized BIM protein through Ser87 de-phosphorylation (Fig. 3a-f), whereas enhanced *PUMA* transcription via FOXO1/3 (Fig. 3g,h). Importantly, osimertinib-resistant *EGFR*-mutant lung cancer cells due to epithelial mesenchymal transition were highly sensitive to Aurora kinase inhibitors, and exhibited efficacy *in vivo* (Fig. 2h,i). Although combined EGFR and AURKB inhibition cooperatively suppress tumor growth of H1975 xenograft bearing mice, the tumors exhibited re-growth three weeks after treatment discontinuation (Fig. 2g).

[Conclusions]

Our HTS and cell death mechanism-based studies revealed that AURKB is a crucial target to prevent initial adaptation upon osimertinib treatment by maximizing BIM- and PUMA-initiated apoptosis. Our data suggest that combined inhibition of EGFR and AURKB, two distinct pathways, is more effective in eliminating tumor cells than improving the inhibition of the same signaling axis, such as combined inhibition of EGFR and MEK or combined inhibition of EGFR and PI3K/AKT/mTOR. We identified PF as one of the best AURKB inhibitors in combination with osimertinib. A phase I trial of PF reported 19 solid tumor cases achieving stable disease with a clinically manageable adverse events profile, non-overlapping with those of EGFR-TKIs. Clinical evaluation will be needed to determine the efficacy and toxicity of this cell death mechanism-based therapeutic strategy for *EGFR*-mutant lung cancer.

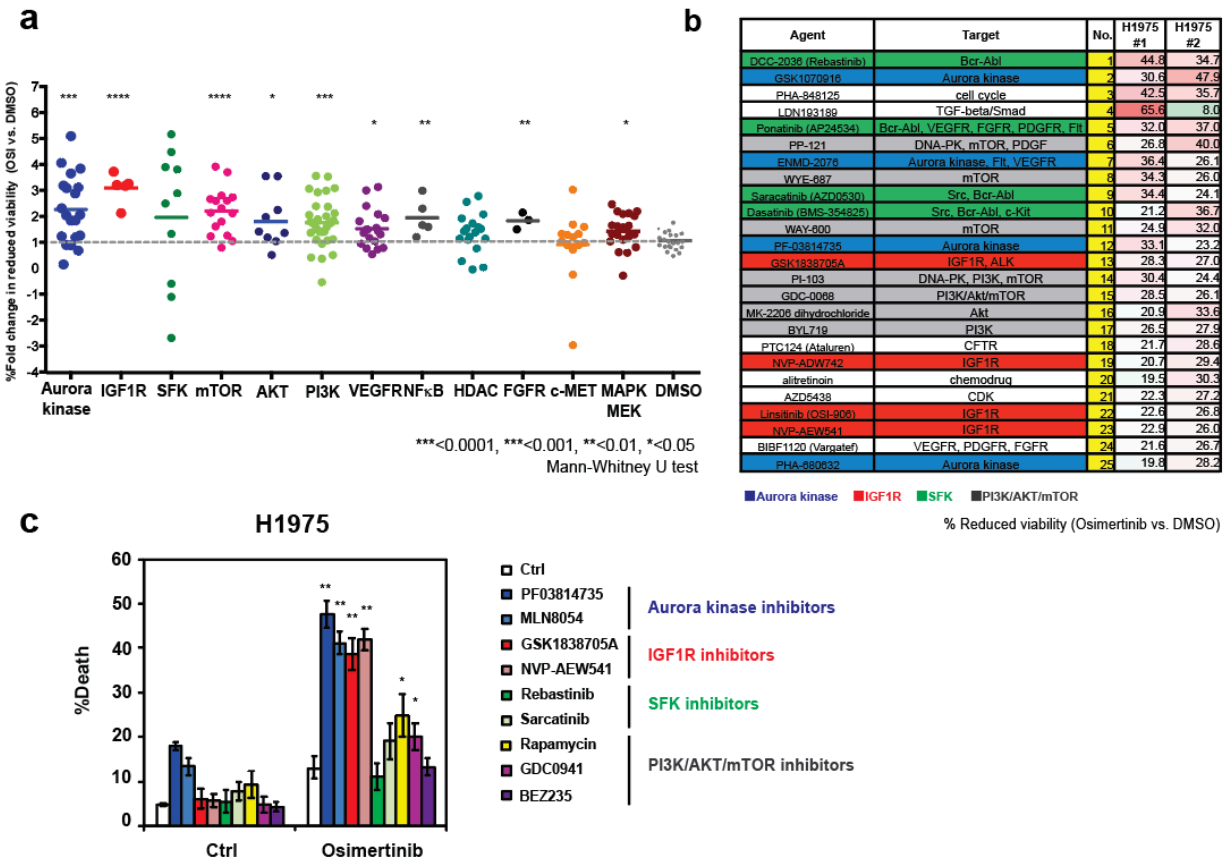
-This study is currently under revision

K Tanaka, S Han, S Yang, SD Selcuklu, YT Ganesan, A Moyer, et al. "Targeting Aurora B kinase prevents and overcomes resistance to EGFR inhibitors in lung cancer by enhancing BIM- and PUMA-mediated apoptosis" under revision in *Cancer Cell*

-This study was presented in the following meetings

K Tanaka, S Han, YT Ganesan, H Yu, MG Kris, JJ Hsieh, EH Cheng. "Enhancing apoptosis to overcome resistance to EGFR inhibitors in *EGFR*-mutant lung cancer" American Association of Cancer Research Annual Meeting, Poster, 3/29/2019

K Tanaka, S Han, YT Ganesan, H Yu, MG Kris, JJ Hsieh, EH Cheng. "Enhancing apoptosis to overcome resistance to EGFR inhibitors in *EGFR*-mutant lung cancer" JMSA NY Life Science Forum, Poster, 4/20/2019



Agent	Target	No.	H1975 #1	H1975 #2
DCC-2036 (Rebastinib)	Bcr-Abl	1	44.8	34.7
GSK1070916	Aurora kinase	2	30.6	47.9
PHA-848125	cell cycle	3	42.5	35.7
LDN193189	TGF-beta/Smad	4	65.6	8.0
Ponatinib (AP24534)	Bcr-Abl, VEGFR, FGFR, PDGFR, Flt	5	32.0	37.0
PP-121	DNA-PK, mTOR, PDGF	6	26.8	40.0
ENMD-2076	Aurora kinase, Flt, VEGFR	7	36.4	26.1
WYE-687	mTOR	8	34.3	26.0
Saracatinib (AZD0530)	Src, Bcr-Abl	9	34.4	24.1
Dasatinib (BMS-354825)	Src, Bcr-Abl, c-Kit	10	21.2	36.7
WAY-600	mTOR	11	24.9	32.0
PF-03814735	Aurora kinase	12	33.1	23.2
GSK1838705A	IGF1R, ALK	13	28.3	27.0
PI-103	DNA-PK, PI3K, mTOR	14	30.4	24.4
GDC-0068	PI3K/Akt/mTOR	15	28.5	26.1
MK-2206 dihydrochloride	Akt	16	20.9	33.6
BYL719	PI3K	17	26.5	27.9
PTC124 (Ataluren)	CFTR	18	21.7	28.6
NVP-ADW742	IGF1R	19	20.7	29.4
alitretinoin	chemodrug	20	19.5	30.3
AZD5438	CDK	21	22.3	27.2
Linsitinib (OSI-906)	IGF1R	22	22.6	26.8
NVP-AEW541	IGF1R	23	22.9	26.0
BIBF1120 (Vargatef)	VEGFR, PDGFR, FGFR	24	21.6	26.7
PHA-680632	Aurora kinase	25	19.8	28.2

Fig 1. HTS identifies Aurora kinase and IGF1R inhibitor as the best apoptosis-inducing agents with osimertinib. (a) An overview of various categories of inhibitors identified from HTS that enhance osimertinib-induced growth inhibition of H1975 cells. Each dot represents a fold change of reduced viability (+osimertinib vs. +DMSO) for individual compound. **(b)** Top 25 agents that cooperate with osimertinib to reduce viability. **(c)** Cell death was quantified by annexin-V staining for H1975 cells treated with the four categories of promising candidate agents with or without osimertinib treatment (mean ± s.d., n=3). *, P<0.05; **, P<0.01 (Student's *t*-test).

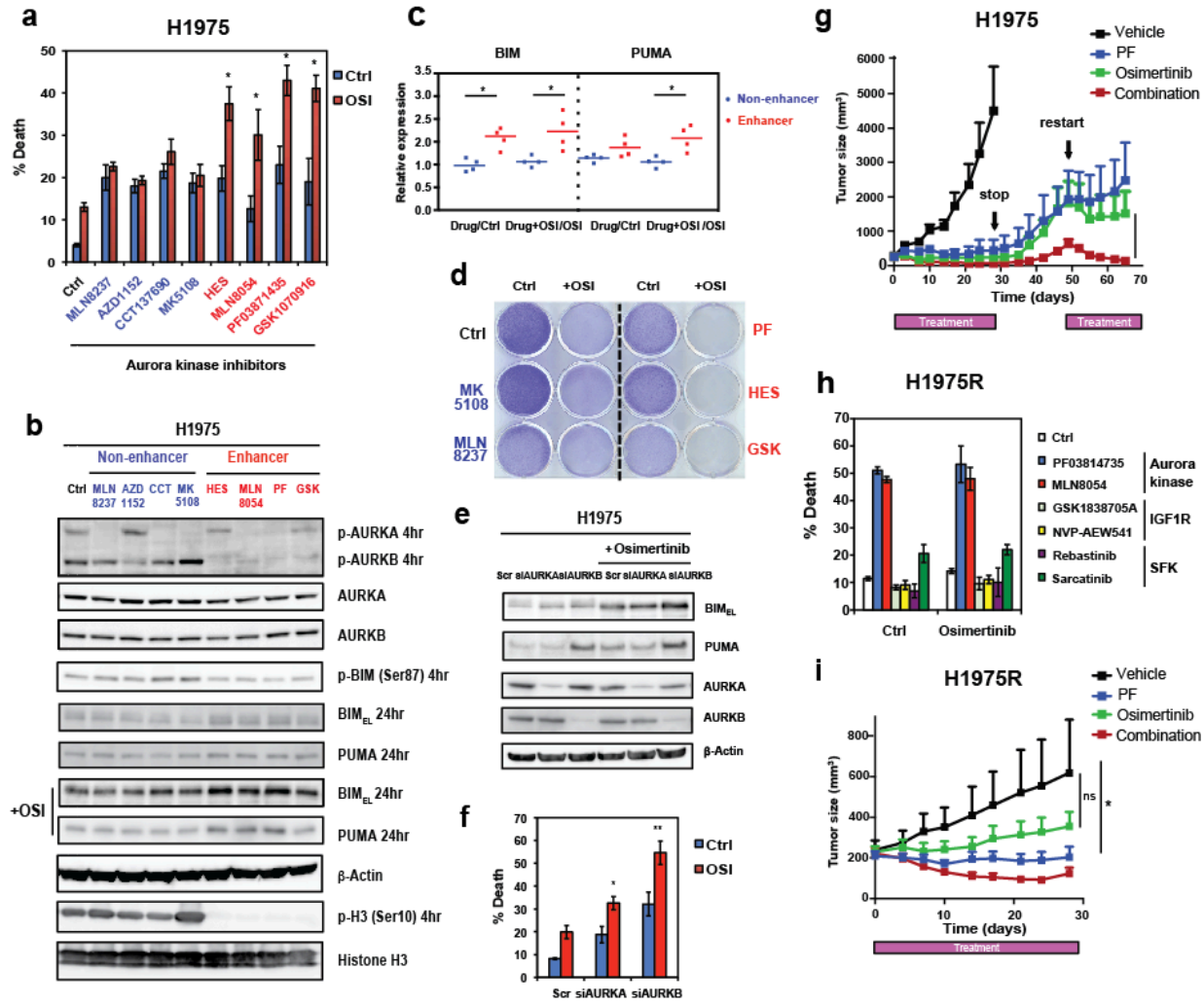


Fig 2. AURKB inhibition specifically induces BIM- and PUMA-mediated apoptosis in combination with osimertinib. (a) Aurora kinase inhibitors screened in HTS were divided into enhancer (red) and non-enhancer groups (blue) based on the combinational effect on apoptosis with osimertinib treatment (mean ± s.d., n=3). *, $P<0.05$ (Student's *t*-test). **(b,c)** H1975 cells treated with Aurora kinase inhibitors were assessed by immunoblot analysis. Enhancer group of Aurora kinase inhibitors blocked AURKB phosphorylation and Histone H3 (a substitute of AURKB), and enhanced BIM and PUMA induction with or without osimertinib treatment. **(d)** H1975 cells treated with the indicated agents for 14 days were assessed by clonogenic assays. Enhancer group of Aurora kinase inhibitors completely killed the residual colonies in combination with osimertinib. **(e, f)** H1975 cells, transfected with scrambled siRNA (*siSCR*), or siRNA against *AURKA* or *AURKB*, were treated with the indicated agents for 48h and subjected to cell death assays (mean ± s.d., n=3). *, $P<0.05$; **, $P<0.01$ (Student's *t*-test). **(g)** Nude mice bearing H1975 xenografts were treated with indicated agents for a total of 66 days including three weeks of treatment discontinuation. *, $P<0.05$ (two-way ANOVA). **(h)** Osimertinib-resistant H1975R cells due to EMT were treated with the indicated agents with or without osimertinib and cell death was quantified by annexin-V staining in (mean ± s.d., n=3). **(i)** Nude mice bearing H1975R xenografts were treated with indicated agents for 28 days. *, $P<0.05$ (two-way ANOVA).

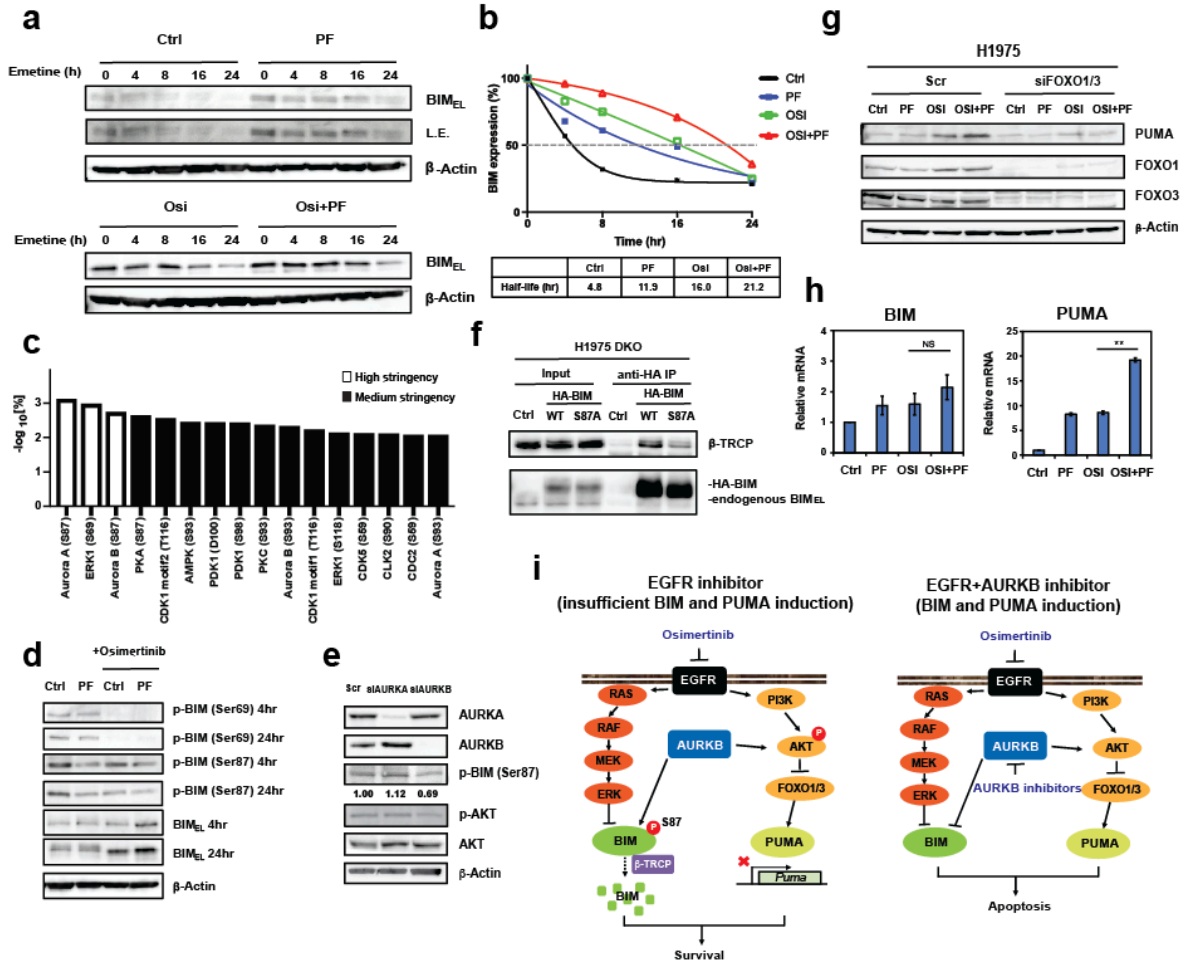


Fig 3. AURKB inhibition stabilizes BIM protein through de-phosphorylation at Ser87, and induces *PUMA* transcription via FOXO1/3. (a, b) In H1975 cells, BIM protein stability upon the indicated agents was assessed by immunoblots using the protein synthesis inhibitor emetine for the indicated time. **(c)** Computational prediction of BIM protein phosphorylation motif using the SCANSITE 4.0. **(d)** In H1975 cells, BIM was de-phosphorylated at Ser69 and Ser87 upon osimertinib and PF03814735, respectively. **(e)** H1975 cells, transfected with scrambled siRNA (*siSCR*) or siRNA against *AURKA* or *AURKB* were assessed by immunoblot analysis. **(f)** BAK- and BAX-knockout H1975 cells (H1975DKO) expressing HA-tagged wild-type or S87A mutant BIM were subjected to anti-HA immunoprecipitation. The input (5%) and immunoprecipitates were assessed by immunoblot analysis. **(g)** H1975 cells, transfected with scrambled siRNA (*siSCR*) or siRNA against *FOXO1*/3, were treated with the indicated agents for 48h. **(h)** Combined inhibition of EGFR and AURKB significantly enhanced mRNA expression of *PUMA*, whereas there was minimal change in *BIM* expression in H1975 cells assessed by qRT-PCR analysis (mean ± s.d., n=3). **, $P<0.01$ (Student's *t*-test). **(i)** A schematic model how AURKB inhibitors induce BIM- and PUMA-mediated apoptosis under EGFR inhibition.