Rapid conversion to resistance, of a colon PDX with ret-fusion, by Ponatibib treatment could potentially be attributed to the introduction of the gate keeper mutation, V804M

Mengmeng Yang[1], Jie Cai[1], Sheng Guo[1], Jean Pierre Wery[1], and Henry Q.X. Li[1,2*] , [1]Crown Bioscience, Inc., 3375 Scott Blvd, suite 108, Santa Clara, CA 95054 [2]State Key Laboratory of Natural and Biomimetic Drugs, Peking University. *: presenter

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Introduction

RET encodes a receptor tyrosine kinase (RTK) involved in cellular mechanisms of proliferation, migration and differentiation. Fusions of RET gene with different genes at 10q11.2 results in a constitutively activation and drive tumor development in various malignancies: 10–20% of sporadic papillary thyroid cancer (1), spitzoid neoplasms, CMML and 1% of lung adenocarcinomas (2, 3). Therefore, RET fusion proteins have been suggested to be important therapeutic targets. Ponatinib (AP24534), a multi-kinase inhibitor, has been approved for the treatment of Ph^+ chronic myeloid leukemia (CML) and acute lymphoblast leukemia (ALL), including CML with T315I/imatinib-resistance. Ponatinib has recently been found to potently inhibit the common NSCLC fusion variant, KIF5B-RET, at clinically-achievable concentrations.

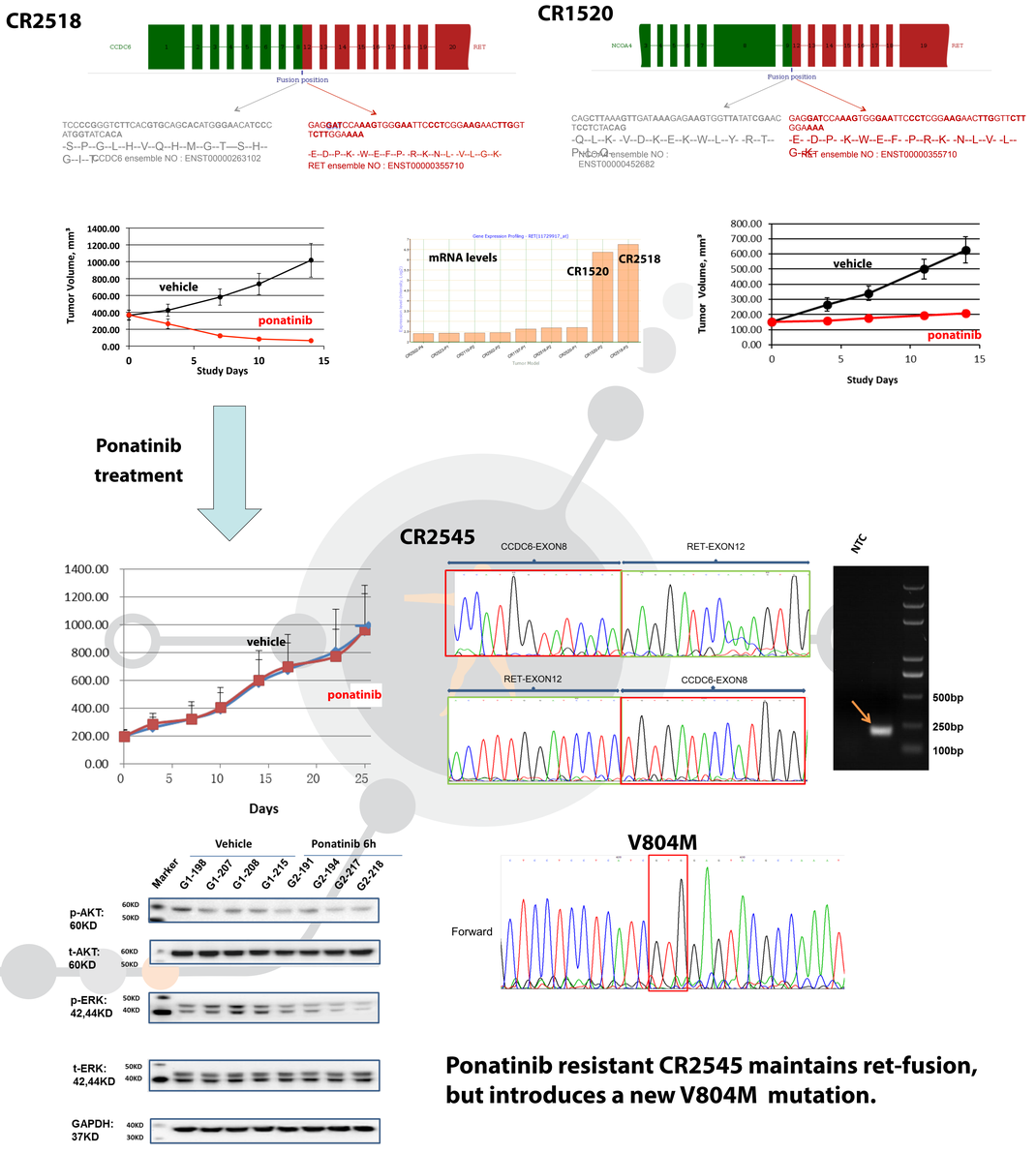
We have established large collection of colorectal patient derived xenografts (PDXs)(4, 5). 2 of the poorly differentiated adenocarcinoma, CR1520 and CR2518, contain two different chromosome 10 in-frame RET-fusions, CCDC6-RET (CR2518) and NCOA4-RET (CR1520), as revealed by RNAseq and Sanger Sequencing (6). Both models have also demonstrated over-expressing ret gene at mRNA levels. More importantly, they both responded Ponatinib significantly along with dephosphorylation of RET and downstream AKT, confirming these two types of Ret fusions as oncogenic drivers in these two models (6). Ponatinib could become an effective treatment of RET fusion driven diseases

Abstract

We are interested in exploring the potential resistance development of Ponatinib treatment. We prolong treated both models and also observed that the treatment rapidly drove CR2518 into resistance (The resistant derivative is called CR2545). To investigate the underlying mechanism of the resistance, we performed RNAseq analysis of CR2545, which revealed retaining of the ret-fusion, but also the introduction of a previously described gate-keeper mutation, V804M, at ret kinase domain. V804M, a mutation frequently found in familial medullary thyroid carcinoma (FMTC), introduces bulky amino acid at position 804 and found to resistant to vandetanib. This may contribute to the resistance. However, this notion apparently contradicts to the previous observation/claim that V804M is sensitive to Ponatinib (7), and Ponatinib should be used to treat RET-V804M/L cancers. It is possible that the described V804M containing vandtanib resistant cells are sensitive to Ponatinib, but via a non-RET mechanism for the multi-kinase inhibition nature of Ponatinib; or in our case, there are other non-RET Ponatinib resistant mechanisms been introduced in CR2545 where RET is no longer the key oncogenic driver. We also assessed pharmacodanamic (PD) parameters including pAKT and pERK, and the preliminary data seems still to show some drug effects on these biomarkers in the resistant model, but seemimgly to less degree as compared to parental sensitive model, which may explain the differences in efficacy.

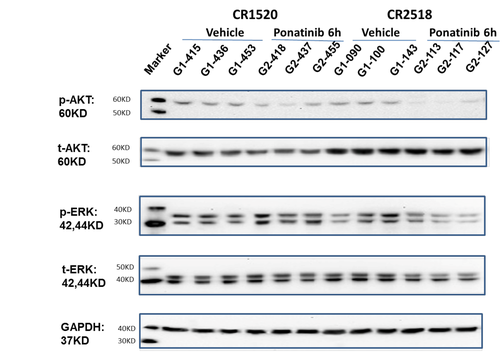
Contact: Henry Li, henryli@crownbio.com

Results



Ponatinib resistant CR2545 maintains ret-fusion, but introduces a new V804M mutation.

Results



Conclusions

1. **Treatment of a ret-fusion containing/ ponatinib sensitive a PDX-CRC model resulted in rapid development of resistance;**
2. **The resistant model still maintains the ret-fusion, but a new V804M gate-keeper mutation was found in the induced resistant model;**
3. **The resistant model seems to show less PD effects as compared to the sensitive parental model**

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