A Novel RET TKI Inhibits Growth of Patient-Derived Xenografts with RET Fusion and Overcomes Ponatinib-Induced Resistance

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Abstract

We established two CRC PDX models carrying two different types of RET fusions, i.e. CCDC6-RET (CR2518) and NCOA4-RET (CR1520), as revealed by RNAseq/Sanger sequencing. These two PDXs were found to be sensitive to ponatinib (AP24534), a TKI with high RET inhibitory activity1-3. Most importantly, ponatinib treatment caused rapid induction of drug resistance in the CR2545 PDX, hallmarking by introduction of a gate keeper mutation, V804M4-5. We set out to test a novel RET-TKI, R101 (it inhibits the wild-type enzyme at IC50 of 1.6 x 10^-7M) for overcoming the ponatinib induced resistance. R101 demonstrated similar potency as ponatinib (EC50 0.56 vs. 4.7 uM) in inhibiting in vitro 2D proliferation of CR1520 PrimePanel cell line, while it resulted significantly less pot in HUVEC normal endothelial cells (EC50 5.0 vs. 0.25uM). In vivo efficacy study showed strong antitumor activity of R101 in both CR2518 and CR1520. Interestingly, the ponatinib resistant CR2545 also demonstrated robust response to R101, with greatly extended overall survival of tumor bearing mice following treatment. Our data suggest that R101 could potentially be a good candidate for the treatment of tumors with RET gene fusion, and ponatinib/vandetanib resistant cases.

Conclusions

1. CRC-PDXs carry RET oncogene fusions: CR1520 (NCOA4-RET) and CR2518 (CCDC6-RET) and overexpress RET.
2. Both models are initially sensitive to the RET inhibitor ponatinib.
3. A Novel RET-TKI, R101, showed strong antitumor activity in both models.
4. R101 overcame ponatinib (AP24534) induced resistance in model CR2545.
5. R101 inhibits the wild-type RET enzyme with IC50 of 6.7e-8M. R101 inhibits RET at IC50 of 5.6e-7M (similar to ponatinib) in 2D assay on the PrimePanel CR1520. R101 inhibits HUVEC at IC50 of 5e-6M (20x less toxic than ponatinib).
6. RNAseq reveals that a classic gate keeper mutation is introduced into the ponatinib resistant CR2545 model after ponatinib exposure.

References

2. Mengmeng Yang JC, Sheng Guo, Jean Pierre Wery, and Henry Q.X. Li. Rapid conversion to resistance, of a colon PDX with RET-fusion, by ponatinib treatment could potentially be attributed to the introduction of the gate keeper mutation, V804M. AACR-Annual Conference 2015

Figure 3. CR1520 is sensitive to ponatinib treatment

Figure 4. CR2518 is sensitive to ponatinib treatment

Figure 5. CR2545 is resistant to ponatinib treatment

Figure 6. R101 showed strong antitumor activity in CR1520

Figure 7. R101 showed strong antitumor activity in CR2518

Figure 8. R101 overcame ponatinib induced resistance (CR2545)

Figure 9. CR1520 survival curve (cutoff: 500mm³)

Figure 10. CR2518 survival curve (cutoff: 500mm³)

Figure 11. CR2545 survival curve (cutoff: 500mm³)

Table 1. Patients information

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Age</th>
<th>Pathology diagnosis</th>
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<tbody>
<tr>
<td>CR1520</td>
<td>M</td>
<td>82</td>
<td>Moderately poorly differentiated adenocarcinoma (Pa, F2)</td>
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<tr>
<td>CR2518</td>
<td>F</td>
<td>82</td>
<td>Poorly differentiated adenocarcinoma (Pa, P3)</td>
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</tbody>
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Figure 12. RET kinase gate keeper V804M mutation is introduced in the ponatinib resistant CR2545