**RET Fusion Factsheet**

Accelerate RET inhibitor research with novel RET fusion PDX models and derived early drug development resources

Constitutively active RET fusion oncoproteins are important targets for oncology research. RET is a proto-oncogene that encodes a receptor tyrosine-protein kinase involved in numerous cellular mechanisms including cell proliferation, migration, and differentiation. Fusions of RET with different nearby genes on chromosome 10 (at 10q11.2) result in a constitutively active gene – permanently switched on to drive cell growth and tumor development. RET fusion oncoproteins are therefore highly ‘druggable’ and important targets for cancer research.

Historically, RET fusions have been found in 10% to 20% of sporadic papillary thyroid cancers(5); however, more recently a range of RET fusions were found in approximately 1% of lung adenocarcinomas(6,7). This incidence can increase to as much as 15% in never-smokers with lung adenocarcinomas that lack other known driver oncogenes(8). RET fusions (specifically CCDC6-RET and NCOA4-RET) have also been identified in patients with advanced colorectal cancer (CRC). Within CRC, RET fusions represent a novel class of oncogenic drivers, which occur at 0.2% frequency and without other known driver mutations e.g. KRAS, NRAS, BRAF, PIK3CA, or other fusion tyrosine kinases(9).

Currently, a specific RET-only inhibitor is not available. Multi-targeted tyrosine kinase inhibitors which are known to have anti RET activity are already approved for use in medullary thyroid cancer (e.g. cabozantinib and vandetanib), certain leukemias (e.g. ponatinib), and renal cell carcinoma (e.g. sunitinib and sorafenib). Many of these agents are now in clinical trials for cancer types including RET-rearranged NSCLC. Targeted therapies against RET fusions may provide novel treatments for a patient subset lacking other treatment options, particularly in advanced CRC where there is an unmet need for molecularly directed therapies(10).

**CrownBio RET Fusion Resources**

CrownBio has a range of xenograft and cell line RET fusion platforms, to provide a critical link between *in vitro* testing and clinical efficacy in drug development.

- **CrownBio RET Fusion PDX Models:** CR1520 and CR2518
- **HuPrime RET Fusion PDX Models:** CR1520 and CR2518
- **Model Background:**
  - The available patient background information, tumor pathology diagnosis, and completed sequencing data for CR1520 and CR2518 are shown in **Table 1**. The mutation status of 10 common oncogenes and tumor suppressor genes has also been verified, and the genes that have been evaluated for each specific model are included in **Table 1**. Both PDX are negative for hotspot mutations in KRAS, BRAF, and PI3K (CR2518 has a mutation in CTNNB1 identified by RNAseq), suggesting that RET fusion may be the primary oncogenic driver in these tumors. The pathology diagnosis for each tumor was QCD by CrownBio, and also established for the PDX model at a range of low passage numbers. QC data are included in **Table 1** and representative H&E stain images (400x) are included within **Table 2**; further images from other PDX passage numbers are available within HuBase.

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**Table 1:**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>Negative</td>
</tr>
<tr>
<td>BRAF</td>
<td>Negative</td>
</tr>
<tr>
<td>PI3K</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Table 2:**

Representative H&E stain images (400x) are included within HuBase.
# RET Fusion Factsheet

## Table 1: Summary of HuPrime RET Fusion PDX Models Background

<table>
<thead>
<tr>
<th>HuPrime Identifier</th>
<th>Patient Background</th>
<th>Tumor Pathology Diagnosis</th>
<th>PDX Tumor Pathology QC</th>
<th>Genomic Profiling</th>
<th>IHC</th>
<th>Treatment History</th>
<th>Examples of Oncogene Mutation Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1520 (NCOA4-RET)</td>
<td>Asian male, aged 82 years</td>
<td>Moderately-poorly differentiated adenocarcinoma</td>
<td>Pa, P2: Moderately-poorly differentiated adenocarcinoma</td>
<td>Affy U219 and SNP 6.0 data (P2) RNAseq (P3)</td>
<td>NA</td>
<td>Naive</td>
<td>WT: AKT, BRAF, c-MET, CTNNB1, EGFR, ERK, KRAS, PIK3CA, PTEN, TP53</td>
</tr>
<tr>
<td>CR2518 (CCDC6-RET)</td>
<td>Asian female, aged 82 years</td>
<td>Adenocarcinoma of ascending colon, ulcerative type, poorly differentiated, invaded through intestinal wall to peripheric adipose tissue. No malignant cells adjacent to superior and inferior stump. Regional lymph nodes (LN): paraintestinal LN (0/17)</td>
<td>Pa, P3: Poorly differentiated adenocarcinoma</td>
<td>Affy U219 and SNP 6.0 data (P3) RNAseq (P2)</td>
<td>CK7(+/-) CK20(++) Syn(+++) CD56(-) Ki-67(60%+) CerbB-2(+) VEGF(-) D2-40 (lymphatic vessel +)</td>
<td>Naive</td>
<td>WT: AKT, BRAF, EGFR, ERK, KRAS, PIK3CA, PTEN, TP53</td>
</tr>
</tbody>
</table>

## Table 2: HuPrime RET Fusion PDX Models Pathology

H&E stained pathology images (400x) from the original patient tumor, and from a low passage number PDX model.

<table>
<thead>
<tr>
<th>HuPrime Identifier</th>
<th>CR1520</th>
<th>CR2518</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Tumor</td>
<td><img src="CR1520_Patient_Tumor" alt="Image" /></td>
<td><img src="CR2518_Patient_Tumor" alt="Image" /></td>
</tr>
<tr>
<td>PDX</td>
<td><img src="CR1520_PDX_P10" alt="Image" /></td>
<td><img src="CR2518_PDX_P3" alt="Image" /></td>
</tr>
</tbody>
</table>

## HuPrime RET Fusion PDX Model Expression Levels

The expression levels of RET in PDX models CR1520 and CR2518 are shown in **Figure 1**, in comparison with all HuPrime CRC models, and just showing CRC models with expression levels >3. **Figure 1** shows Affymetrix Human Genome U219 Array Plate expression data, RNAseq expression data is also available in HuBase. Both models show high expression of RET, as does model CR2545 which is derived from model CR2518.

## HuPrime RET Fusion PDX Model Genomic Profiling

RET fusions within the PDX models were identified by RNA sequencing (full genomic and sequencing data for the models and the fusions are available within HuBase). The CR1520 model has an in-frame NCOA4-RET fusion at exon 9 of the NCOA4 gene and exon 12 of the RET gene, detailed in **Figure 2**. RT-PCR analysis of the patient tumor and passage 3 of CR1520, and direct sequencing of the model confirmed the fusion protein was present (**Figure 3**). The CR2518 model has an in-frame CCDC6-RET fusion at exon 8 of the CCDC6 gene and exon 12 of the RET gene, detailed in **Figure 4**. RT-PCR analysis on passage 2 and 5 of CR2518, and direct sequencing of the model confirmed the fusion protein was present (**Figure 5**).
Figure 1: HuPrime RET Fusion PDX Model Expression Data
Gene expression levels of RET (using Affymetrix Human Genome U219 Array Plate, probeset 5979_at) in all HuPrime CRC PDX models, and CRC models with expression levels >3 (overlaid panel).

Figure 2: HuPrime RET Fusion PDX Model Genomic Profiling: CR1520 Gene Fusion
The chromosome 10 position of the NCOA4-RET fusion and associated fusion protein sequence.

Figure 3: HuPrime RET Fusion PDX Model Genomic Profiling: CR1520 RT-PCR Analysis and Sequencing
RT-PCR and direct sequencing of CR1520 confirms the presence of the NCOA4-RET fusion protein.

Figure 4: HuPrime RET Fusion PDX Model Genomic Profiling: CR2518 Gene Fusion
The chromosome 10 position of the CCDC6-RET fusion and associated fusion protein sequence.
Figure 5: **HuPrime RET Fusion PDX Model Genomic Profiling: CR2518 RT-PCR Analysis and Sequencing**

RT-PCR and direct sequencing of CR2518 confirms the presence of the CCDC6-RET fusion protein.

![RT-PCR Image](image)

**HuPrime RET Fusion PDX Model Treatment Data**

Treatment data with 5-FU for CR1520, and 5-FU and irinotecan for CR2518 is stored within HuBase. The CR1520 model is sensitive to 5-FU, whereas CR2518 is only partially responsive to 5-FU and sensitive to irinotecan. The PDX models have also been treated with a RET inhibitor, with both CR1520 and CR2518 shown to be sensitive to the agent (Figure 6).

**Figure 6: HuPrime RET Fusion PDX Models CR1520 and CR2518 are Sensitive to RET Inhibitor Treatment**

A.

![Tumor Growth Curves](image)

Development of a RET Fusion PDX Model Resistant to RET Inhibition

As shown above, model CR2518 is responsive to RET inhibition. However, following consecutive cycles of treatment with the RET inhibitor AP24534, resistance to the agent is developed and the RET inhibitor resistant PDX variant CR2545 is produced (Figure 7). We have validated the model to confirm that the CCDC6-RET fusion is still present. RNAseq data revealed that the acquired resistance is due to a new mutation (V804M) at the RET kinase domain, that is known to cause significant changes in the binding domain for the inhibitor.

**Figure 7: HuPrime RET Fusion PDX Model CR2545 is Resistant to RET Inhibitor Treatment**

![Tumor Growth Curves](image)

PrimePanel RET Fusion Cell Line: CR1520

The PrimePanel CR1520 primary cancer cell line was generated from the CR1520 PDX model, and validated to confirm that the NCOA4-RET fusion is still present (Figure 8). The CR1520 cell line has been shown to be sensitive to a range of RET inhibitors (Figure 9).
Figure 8: **PrimePanel** RET Fusion Cell Line Genomic Profiling: CR1520 RT-PCR Analysis
NCOA4-RET fusion product (271bp) confirmed by RT-PCR.

![RT-PCR Image](image_url)

Figure 9: **PrimePanel** RET Fusion Cell Line CR1520 is Sensitive to RET Inhibitor Treatment

![Graphs showing sensitivity to inhibitors](graph_url)

<table>
<thead>
<tr>
<th>RET Inhibitor</th>
<th>Sorafenib</th>
<th>Sunitinib</th>
<th>XL-184</th>
<th>ZD6474</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</td>
<td>3.87</td>
<td>3.45</td>
<td>4.58</td>
<td>1.46</td>
</tr>
</tbody>
</table>

**PrimeXeno RET Fusion Xenograft: CR1520**

A **PrimeXeno** in vivo model has been developed from the CR1520 **PrimePanel** cell line. The subcutaneous xenograft model shows robust growth following inoculation (Figure 10).

![Growth Curve](growth_curve)

**Figure 10: PrimeXeno** RET Fusion Xenograft Model CR1520 Growth Curve

### Conclusions

The recent identification of RET fusions in CRC and lung cancer has led to a need for RET fusion models across all stages of drug discovery. CrownBio provides a range of xenograft and cell line RET fusion platforms, to provide a critical link between in vitro testing and clinical efficacy in drug development.

Our **HuPrime** PDX models provided the first description of oncogetic RET fusions in CRC patient samples, NCOA4-RET fusion and CCDC6-RET fusion, which have been further observed in patients with advanced CRC. Our models provide clinically relevant xenografts for evaluation of therapeutics targeting RET fusions, with models responsive and resistant to RET inhibitors available.

Our PDX models have also been used to derive a **PrimePanel** cell line to provide a corresponding in vitro cell culture system for cost-effective and high-throughput drug screening and model selection. The related **PrimeXeno** CDX model completes our full resource platform of cell line, CDX, and PDX for all research requirements around RET fusions.

CrownBio can be contacted at busdev@crownbio.com for any further questions or information required on our RET fusion resources, or for information on other CrownBio products and services.

### References

1. My Cancer Genome® RET Fusions in Thyroid Cancer [http://www.mycancergenome.org/content/disease/thyroid-cancer/ret/127 Accessed 08 April 2016].
7. Geogt JM, Chen T-H, Clackson T et al. RET fusions identified in colorectal cancer PDX models are sensitive to the potent RET inhibitor ponatinib [abstract]. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr S-9; San Diego, CA; Philadelphia (PA); AACR; Cancer Research 2014; 74(19 Suppl):Abstract nr 2726.