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ANALYSIS OF RESISTANCE MECHANISMS TO PRALSETINIB (BLU-667) IN PATIENTS WITH *RET* FUSION–POSITIVE NON-SMALL CELL LUNG CANCER (NSCLC) FROM THE ARROW STUDY

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DISCLOSURES

Commercial interest	Relationship(s)
Agios, Amgen, Ariad/Takeda, Array Biopharma, AstraZeneca, Blueprint Medicines Corporation, Bristol-Myers Squibb, Genentech, Gilead, Incyte, Loxo/Lilly, Merck, Novartis, Oncorus, Pfizer, and Regeneron Pharmaceuticals	Consultant or honoraria
Ariad/Takeda, Genentech/Roche, and Novartis	Research support
Adaptimmune, Alexo, Array Biopharma, Blueprint Medicines Corporation, Bristol-Myers Squibb, Jounce, Merck, Moderna, Novartis, and Tesaro	Institutional research support
Ironwood Pharmaceuticals	Immediate family member who is an employee

Pralsetinib is approved by the U.S. Food and Drug Administration (FDA) for the treatment of adults with metastatic *RET* fusion–positive NSCLC. Pralsetinib is not approved for the treatment of any other indication in the USA by the FDA or for any indication in any other jurisdiction by any other health authority.

ACQUIRED RESISTANCE TO TKIs IN NSCLC

- Targeted therapies against oncogenic drivers (e.g., EGFR, ALK) have demonstrated high response rates in NSCLC;[1,2] however, treatment resistance is common[3]
- Potential mechanisms of acquired resistance across oncogenic drivers include on-target secondary mutations and off-target bypass signaling pathways[4,5]
- The *EGFR* T790M gatekeeper mutation accounts for approximately 60% of cases of acquired resistance to first- and second-generation EGFR TKIs in patients with *EGFR*-mutant NSCLC[6]
  - On-target resistance mutations have also been observed for other oncogenic drivers (e.g., ALK and ROS1)[5,7]
- Gaining insight into mechanisms of resistance will help inform treatment strategies in NSCLC (e.g., targeting gatekeeper mutations, combination therapy)

1. Maemondo M et al. *N Engl J Med*. 2010;362:2380–2388; 2. Peters S et al. *N Engl J Med*. 2017;377:829–838; 3. Lin JS, Shaw AT. *Trends Cancer*. 2016;2:350–364; 4. Toyokawa G, Seto T. *Oncol Res Treat*. 2015;38:291–298; 5. Le X et al. *Clin Cancer Res*. 2018;24:6195–6203; 6. Klempner SJ et al. *Lung Cancer*. 2015;89:357–359; 7. Michels S et al. *J Thorac Oncol*. 2019;14:1266–1276.
ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; ROS1, c-ros oncogene 1; TKI, tyrosine kinase inhibitor.

PRALSETINIB RETAINS POTENCY AGAINST *RET* GATEKEEPER MUTATIONS

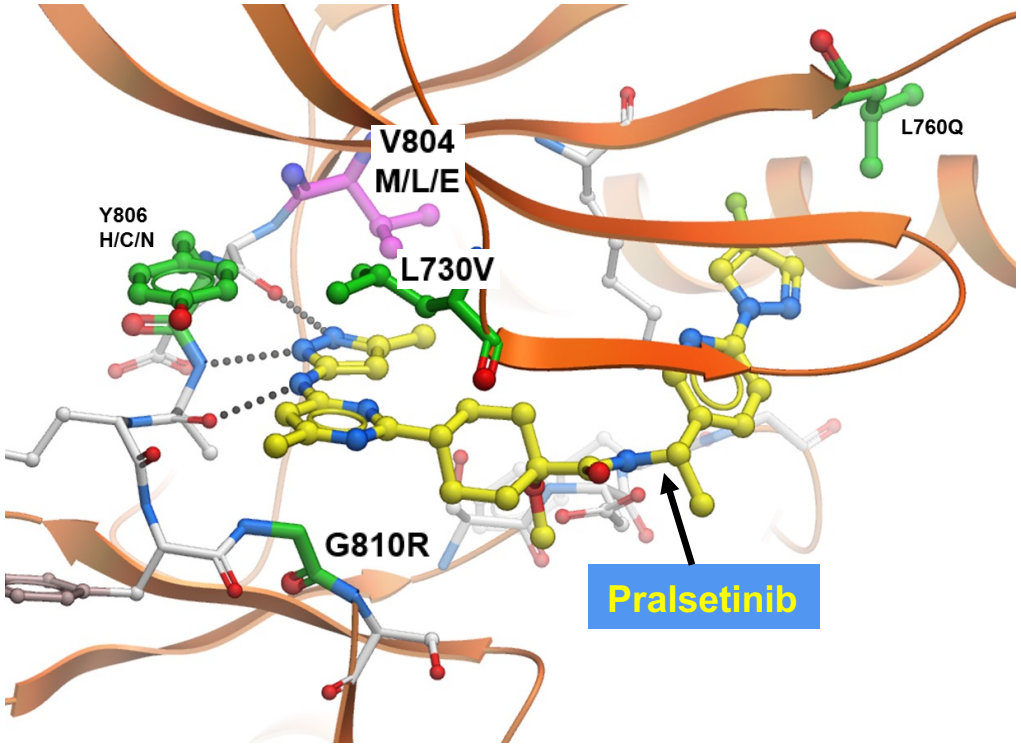
- Pralsetinib is an investigational agent being developed for the treatment of patients with solid tumors harboring *RET* alterations including *RET* fusions in metastatic NSCLC and other solid tumors, and *RET* point mutations and short insertions/deletions in MTC
- Pralsetinib was designed as a potent and selective RET inhibitor with limited off-target kinase activity and potency against *RET* V804 gatekeeper mutations

	Biochemical IC_{50} (nM)	
	Pralsetinib	**Vandetinib**
CCDC6-RET	0.4	21
RET V804L	0.4	4014
RET V804E	0.7	>10,000
RET V804M	0.4	726
VEGFR2	35	4.8

	Cell proliferation IC_{50} (nM)[a]		
	Pralsetinib	**Vandetinib**	**Selpercatinib**
KIF5B-RET	12	544	11
KIF5B-RET V804L	11	8800	34
KIF5B-RET V804M	10	7862	88
KIF5B-RET V804E	15	8340	114
VEGFR2	80	62	87

[a]IC_{50} proliferation assays were conducted in BaF3 cells with a KIF5B-RET background for all RET variants; a VEGFR2 phosphorylation assay was conducted in HUVEC cells.
IC_{50}, half maximal inhibitory concentration; MTC, medullary thyroid cancer; RET, rearranged during transfection; VEGFR, vascular endothelial growth factor receptor 2.

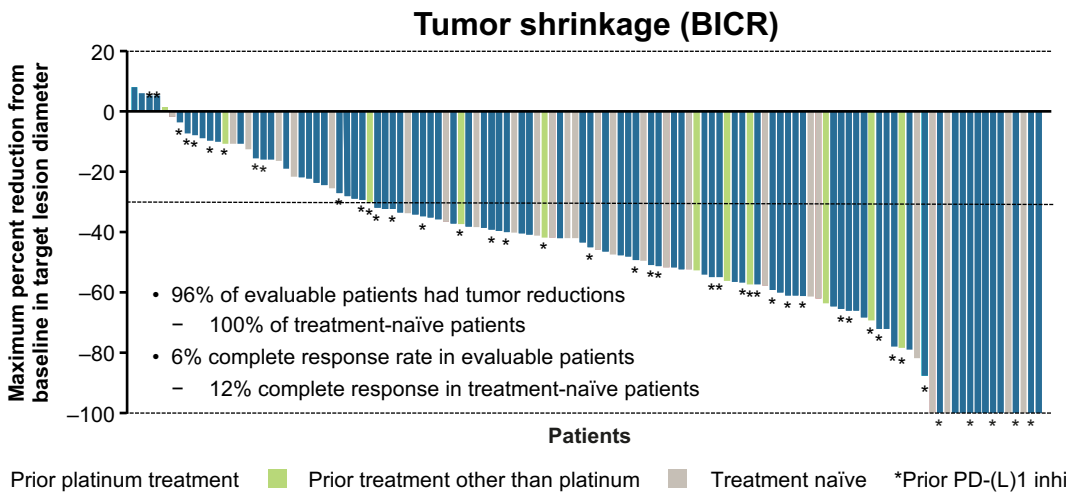
PREDICTED MECHANISMS OF PRALSETINIB RESISTANCE

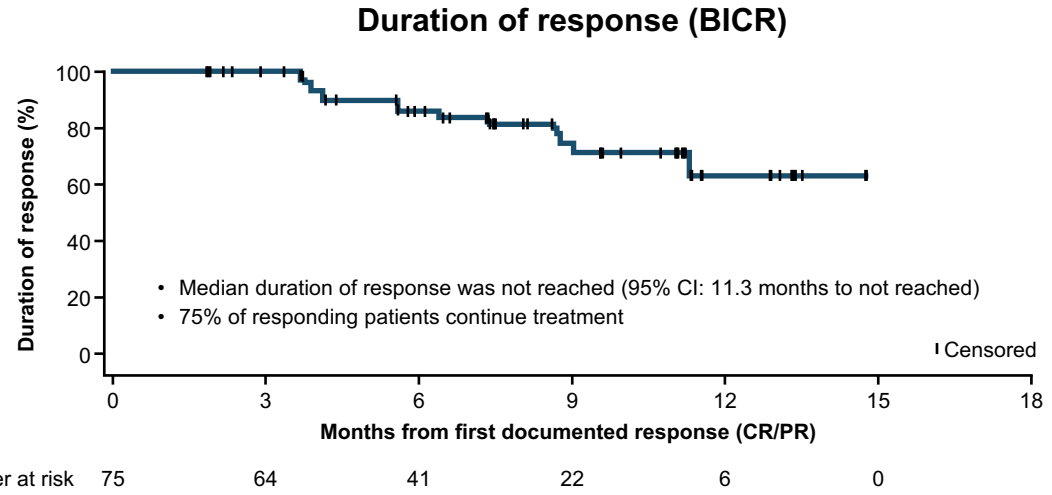


- Based on *in vitro* resistance screens, mutations were identified at the *RET* G810 and L730 positions with reduced pralsetinib potency
  - *RET* G810 is at the “solvent front”
  - *RET* L730 is in the “roof” region of the ATP binding site
- Mutations at the *RET* V804 “gatekeeper” position were not seen using *in vitro* resistance screens

ATP, adenosine triphosphate.

PHASE 1/2 ARROW TRIAL (NCT03037385) OF PRALSETINIB IN PATIENTS WITH ADVANCED *RET* FUSION–POSITIVE NSCLC[a]





- 65% overall response rate,[b] including 6% complete responses, in all response evaluable patients with *RET* fusion–positive NSCLC
- Well-tolerated across tumor types, with predominantly Grade 1–2 treatment-related adverse events

[a]Gainor JF et al. IASLC NACLC 2020 [Poster 37]. [b]Data cut-off: November 18, 2019; includes two patients still on treatment with partial responses pending confirmation.
BICR, blinded independent centralized review; CI, confidence interval; CR, complete response; PD-(L)1, programmed cell death/programmed cell death ligand-1; PR, partial response.

METHODS: FULL CODING AND SPECIFIC EXON ANALYSIS OF *RET* AND OTHER GENES IMPLICATED IN TKI RESISTANCE

- Plasma sampling for ctDNA analysis
  - Dose escalation: C1D1, C1D15, C2D1, C3D1, each restaging visit and EOT
  - Expansion: C1D1, each restaging visit and EOT
- ctDNA analysis performed using Personal Genome Diagnostics PlasmaSELECT™ 64 RUO
  - Next-generation sequencing panel
  - SNVs and indels in 58 genes
  - Full coding region analysis of *RET*
  - Amplification analysis for 18 genes
  - Rearrangements for 17 genes
  - Microsatellite analysis

Full coding [a] and specific exon analysis for the regions of 58 well-characterized cancer genes Amplification analyses performed for 18 genes [b]				
AKT1	*CDK6*[a,b]	*GNAS*	*NPM1*	*PTEN*
ALK[a,b]	*CDKN2A*	*HRAS*	*NRAS*	*RB1*
AR[a,b]	*CTNNB1*	*IDH1*	*NTRK1*	*RET*[a]
ATM	*DNMT3A*	*IDH2*	*NTRK2*	*RNF43*
BRAF[a]	*EGFR*[a,b]	*JAK2*	*NTRK3*	*ROS1*[b]
BRCA1	*ERBB2*[a,b]	*KIT*[a,b]	*PALB2*	*TERT*
BRCA2	*ESR1*	*KRAS*[a]	*PIK3CA*	*TP53*[a]
CCND1[b]	*EZH2*	*MAP2K1*	*PIK3CB*	*TSC1*
CCND2[b]	*FGFR1*[b]	*MET*[b]	*PIK3R1*	*TSC2*
CCND3[b]	*FGFR2*[b]	*MTOR*	*POLD1*	*VHL*
CD274[a,b]	*FGFR3*[b]	*MYC*[b]	*POLE*	
CDK4[a,b]	*FLT3*	*MYCN*[b]	*PTCH1*	

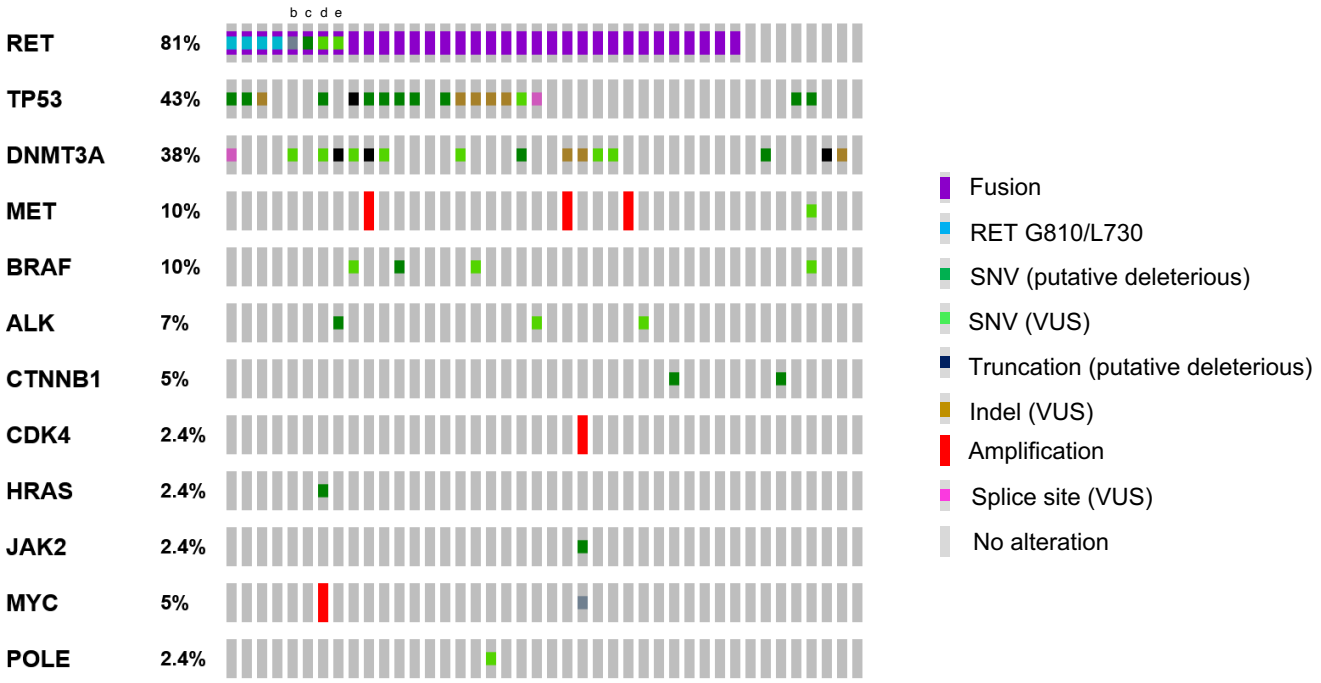
Rearrangement analyses for selected regions of 17 well-characterized cancer genes				
ALK	*ETV6*	*MYC*	*PDGFRA*	*RARA*
BCR	*FGFR1*	*NTRK1*	*PDGFRB*	*RET*
BRAF	*FGFR2*	*NTRK2*	*RAF1*	*ROS1*
EGFR	*FGFR3*			

Microsatellite analyses				
BAT-25	*BAT-26*	*NR-21*	*NR-24*	*MONO-27*

C, cycle; ctDNA, circulating tumor deoxyribonucleic acid; D, day; EOT, end of treatment; SNV, single nucleotide variant.

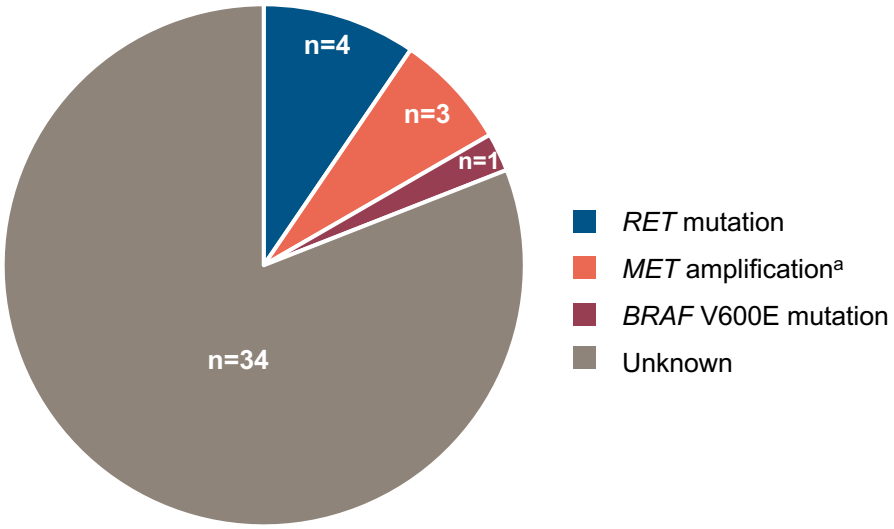
RET-MEDIATED RESISTANCE WAS UNCOMMON (1)

- Preliminary analysis from the ongoing ARROW study is based on an evaluation of paired baseline/on-treatment samples[a]
- Paired baseline/progression sample results available from 42 enrolled patients with a detectable *RET* fusion at baseline
- At progression
  - *RET* fusions were detectable in 34/42 cases
  - On-target mutations in *RET* were observed in 4/42 cases
  - Off-target alterations were observed in 4/42 cases
- *RET* V804 gatekeeper mutations were not seen as a potential mechanism of resistance
- No mechanism of acquired resistance was clearly defined in the remaining 34 cases



[a]Data cut-off: November 18, 2019. [b]*RET* W334* at progression but not at baseline. [c]*RET* Y791F at baseline (26.6%) and progression (33.1%). [d]*RET* S649_V650insLFF (1.4%) at progression but not baseline. [e]*RET* E459K at baseline (46.3%) and progression (46%).
VUS, variant of uncertain significance.

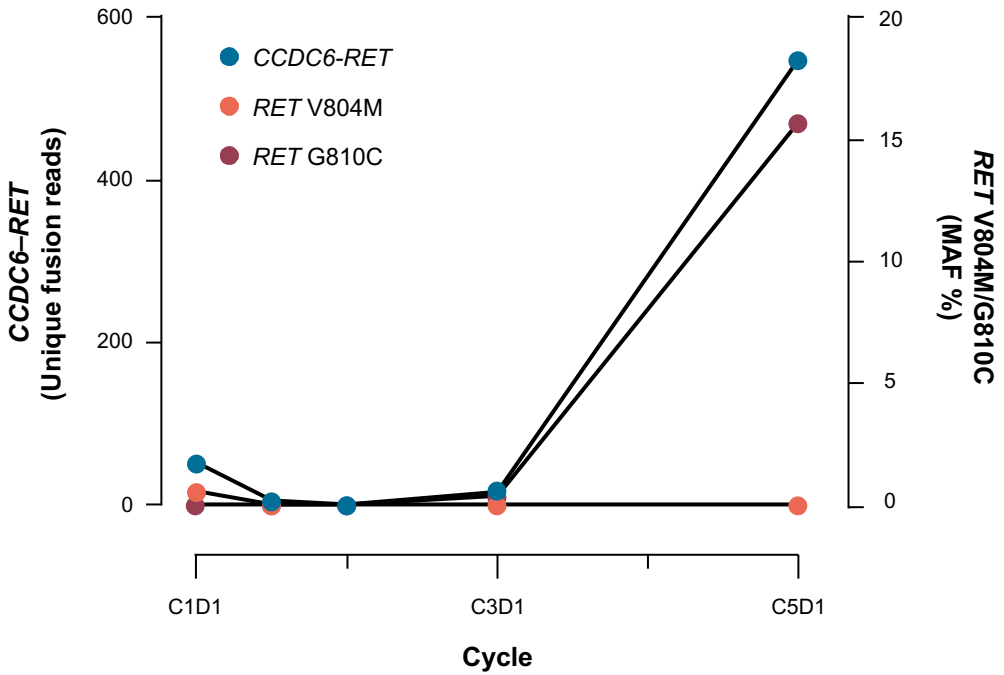
RET-MEDIATED RESISTANCE WAS UNCOMMON (2)



Patient	Prior MKI	Baseline	Progression	Best overall response	Duration of treatment (days)
1	Yes	*CCDC6-RET*	*CCDC6-RET* ***RET* G810S (0.36%)** ***RET* L730V (0.69%)**	PD	118
2	No	*KIAA1468-RET*	*KIAA1468-RET* ***MET* amp**	PR	195
3	No	*CCDC6-RET*	*CCDC6-RET* ***RET* G810C (3.76%)** ***RET* T729_L730insL (15.7%)**	PR	440
4	Yes	*CCDC6-RET* *RET* V804M	*CCDC6-RET* ***RET* G810C (15.8%)**	PD	161
5	No	*KIF5B-RET*	*KIF5B-RET* ***RET* L730V (0.81%)**	PR	171
6	No	*KIF5B-RET*	*KIF5B-RET* ***MET* amp**	PR	160
7	No	*KIF5B-RET* *RUNX1-RET*	*KIF5B-RET* *RUNX1-RET* ***MET* amp**	SD	160
8	No	*KIF5B-RET*	*KIF5B-RET* ***BRAF* V600E (13.3%)**	SD	181

[a]A gene is marked as amplified when coverage across the gene is observed at >1.25-fold relative to a background of normal controls. A significance test is applied to discard observations with *P*-values >0.01. Allelic imbalance at heterozygous sites are used to support the observation of a fold amplification when available.
BRAF, v-raf murine sarcoma viral oncogene homolog B1; MET, proto-oncogene tyrosine-protein kinase MET; MKI, multikinase inhibitor; PD, progressive disease; SD, stable disease.

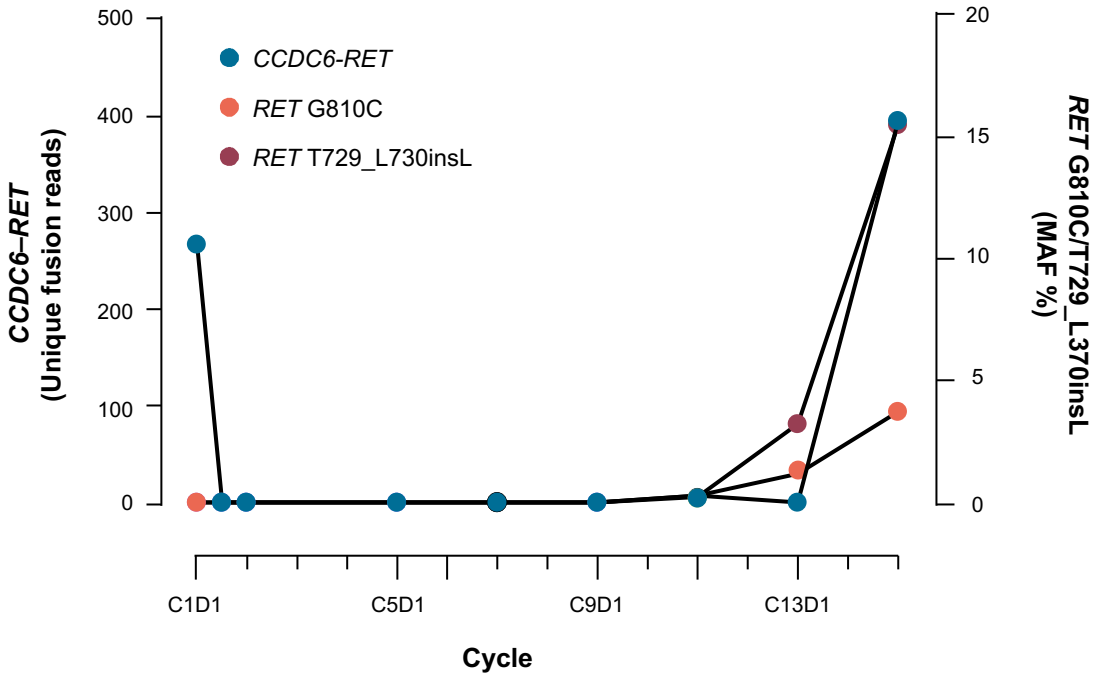
RAPID CLEARANCE OF *RET* V804M MUTATION



MAF, mutant allele fraction.

- Male in his late 50s with a *CCDC6-RET* fusion and metastatic disease
- Prior therapies included pemetrexed/carboplatin, vinorelbine, nivolumab (alone and with urelumab) and the MKIs sunitinib, ponatinib and vandetanib (with radiotherapy)
- *CCDC6-RET* and *RET* V804M detected at baseline, cleared from ctDNA by C1D15
- Progression at C3D1 with re-emergence of *CCDC6-RET* and detection of *RET* G810C, without re-emergence of *RET* V804M

DEVELOPMENT OF OLIGOCLONAL RESISTANCE



- Female patient in her early 40s with a *CCDC6-RET* fusion
- Prior treatment with a carboplatin/pemetrexed/bevacizumab followed by docetaxel
- Treatment with pralsetinib resulted in a partial response at first scan (~8 weeks) maintained through 6 months of treatment followed by radiographic progression at ~8 months
- *CCDC6-RET* observed in ctDNA at C1D1 with rapid clearance after 2 weeks of treatment
- *CCDC6-RET* re-emerged in ctDNA after progression as well as two independent *RET* mutations
  - G810C ‘solvent front’ mutation
  - T729_L730insL ‘roof’ mutation

CONCLUSIONS

- Pralsetinib is a selective RET TKI that has demonstrated clinical activity in patients with *RET*-altered NSCLC
- Preliminary results from plasma ctDNA analysis have identified potential on- and off-target mechanisms of resistance at progression in a subset of patients with NSCLC treated with pralsetinib
  - Potential on-target mutations have been observed at the “solvent front” (G810) and “roof” (L730)
  - Mutations at the *RET* V804 gatekeeper residue do not appear to mediate acquired resistance
  - Potential off-target mechanisms include amplification of *MET* and acquisition of *BRAF* V600E, consistent with observations with other TKIs in NSCLC
  - Oligoclonal resistance is observed
- In most cases, no putative mechanism of resistance was identified in ctDNA and additional analysis using tumor tissue obtained from progressing lesions are needed to fully define the landscape of acquired resistance to pralsetinib
- The full spectrum of resistance mutations may change with larger sample sizes, and additional insights from tissue biopsies will improve our understanding of resistance mechanisms and help inform potential next-generation RET inhibitor profiles and combination strategies