Pre-clinical characterization of potent and selective next-generation RET inhibitors

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Pre-clinical characterization of potent and selective next-generation RET inhibitors

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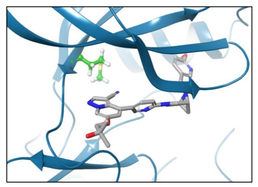
Background

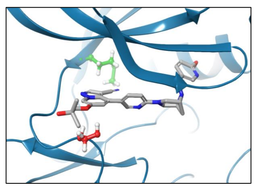
- In May 2020, selpercatinib became the first FDA-approved selective RET inhibitor, indicated for patients (pts) with RET fusion-positive NSCLC and thyroid cancer as well as RET-mutant medullary thyroid cancer.[1,2]
- Mutations at the solvent front of the ATP pocket have been identified as a mechanism of acquired resistance to RET inhibitors selpercatinib and pralsetinib, due to a steric clash created at the RET G810 position and result in a loss of binding potency.[3,4]
- Maintaining potency against RET V804 mutations is important to prevent emergence of resistance and treat patients with concurrent RET V804 gatekeeper and G810 solvent front mutations.
- Therefore, a next-generation RET inhibitor should maintain potency against both solvent front and gatekeeper resistance mutations individually and when co-expressed.

Table 1. RET G810 solvent front mutations confer resistance to approved RET inhibitors

	Selpercatinib IC_{50} (nM)	Pralsetinib IC_{50} (nM)
Founder alterations		
CCDC6-RET	10	5
KIF5B-RET	3	3
RET M918T	9	1
Acquired resistance mutations		
KIF5B-RET G810S	53	81
KIF5B-RET V804L	5	2
KIF5B-RET V804M	7	10
KIF5B-RET G810S+V804M	434	370
RET M918T+G810S	39	141



Crystal structure of selpercatinib (gray) bound to RET V804M (V804M gatekeeper residue shown in green). Selpercatinib is a potent inhibitor of RET V804M.



Model of G810S solvent front mutation (in red) shows Ser810 close to the space where selpercatinib binds, consistent with a potency loss in KIF5B-RET G810S+V804M.

Table 2. Next-generation RET inhibitors are very potent against RET alterations in cell-based assays

	LOX-18228 IC_{50} (nM)	LOX-19260 IC_{50} (nM)
Founder alterations		
CCDC6-RET	4	4
KIF5B-RET	1	1
RET M918T	4	6
Acquired resistance mutations		
KIF5B-RET G810S	8	11
KIF5B-RET V804L	56	21
KIF5B-RET V804M	71	34
KIF5B-RET G810S+V804M	35	36
RET M918T+G810S	6	8

RET-mutant inducible HEK-293 cell lines were generated and treated with LOX-18228 or LOX-19260 for 1 hr. Phospho-RET levels were measured using an In Cell Western protocol, and IC_{50} values were calculated using a 4-parameter fit.

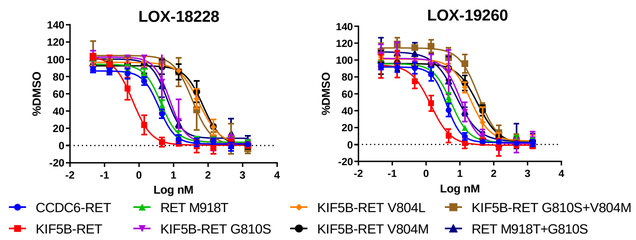
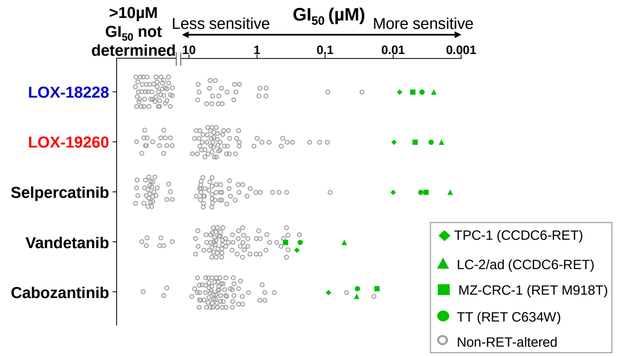


Table 3. LOX-18228 and LOX-19260 are highly selective for RET

	LOX-18228		LOX-19260	
	Cell IC_{50} (nM)	Fold selectivity to WT KIF5B-RET	Cell IC_{50} (nM)	Fold selectivity to WT KIF5B-RET
FGFR1	1608	1780 x	1032	940 x
Flt3	>10000	>11000 x	280	250 x
KDR	684	760 x	>10000	>9000 x
KIT	376	410 x	772	700 x
PDGFRb	468	520 x	1467	1300 x
TrkC	384	420 x	43	40 x

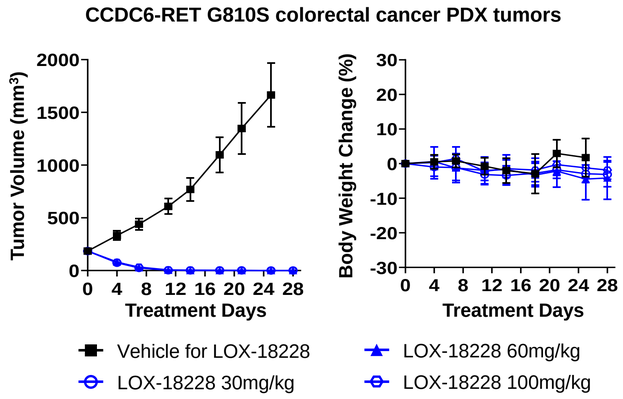
- LOX-18228 and LOX-19260 were highly selective against a panel of 374 WT kinases (Reaction Biology Corp) and cell-based kinase assays (performed at Eurofins or ProQinase).
- No concerning findings in Cerep Safety Screen 87 panel at 10 µM compound concentration.
- hERG IC_{50} >10 µM.

Fig 1. A cancer cell line screen demonstrates potency and selectivity of next-generation RET inhibitors



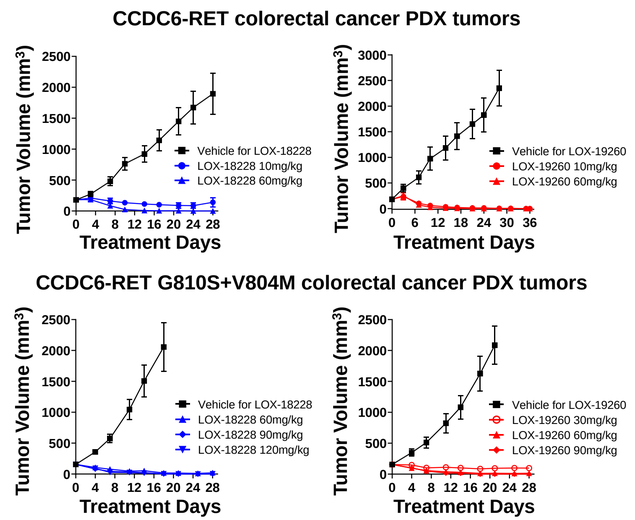
- The potency and selectivity of LOX-18228 and LOX-19260 are comparable to the selective RET inhibitor, selpercatinib.
- Multi-kinase inhibitors, vandetanib and cabozantinib, are examples of nonselective RET inhibitors.
- Top dose tested in this assay was 10 µM and all cell lines without GI_{50} values are assigned to the ">10 µM GI_{50} not determined" portion of the graph.

Fig 2. LOX-18228 is well-tolerated and causes complete tumor regression in a CCDC6-RET G810S PDX model



- LOX-18228 was dosed twice daily. Tumor volume data are mean ± SEM. Body weight data are mean % body weight change ± SD (n=8/group).
- The efficacy study to test LOX-19260 in the CCDC6-RET G810S PDX model is pending.
- All PDX studies were performed at Crown Biosciences.

Fig 3. LOX-18228 and LOX-19260 cause complete tumor regression in RET-altered PDX models



- LOX-18228 and LOX-19260 were dosed twice daily. Tumor volume data are mean ± SEM (n ≥ 6/group).
- LOX-18228 and LOX-19260 were well-tolerated (not shown).

Conclusions

- We have identified potent and selective next-generation RET inhibitors to address the emerging unmet need of patients who relapse on selective RET inhibitors.
- LOX-18228 and LOX-19260 represent promising next-generation RET inhibitor candidates that could be used to further extend durable disease control for patients with RET-altered cancers following the development of acquired resistance to current agents.
- An IND is planned for 2021.

References

1. Drilon A, et al. *N Engl J Med*. 2020;383:813-24.
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4. Lin JJ, et al. *Ann Oncol*. 2020;31:1725-33.