RET-related *in vivo* models

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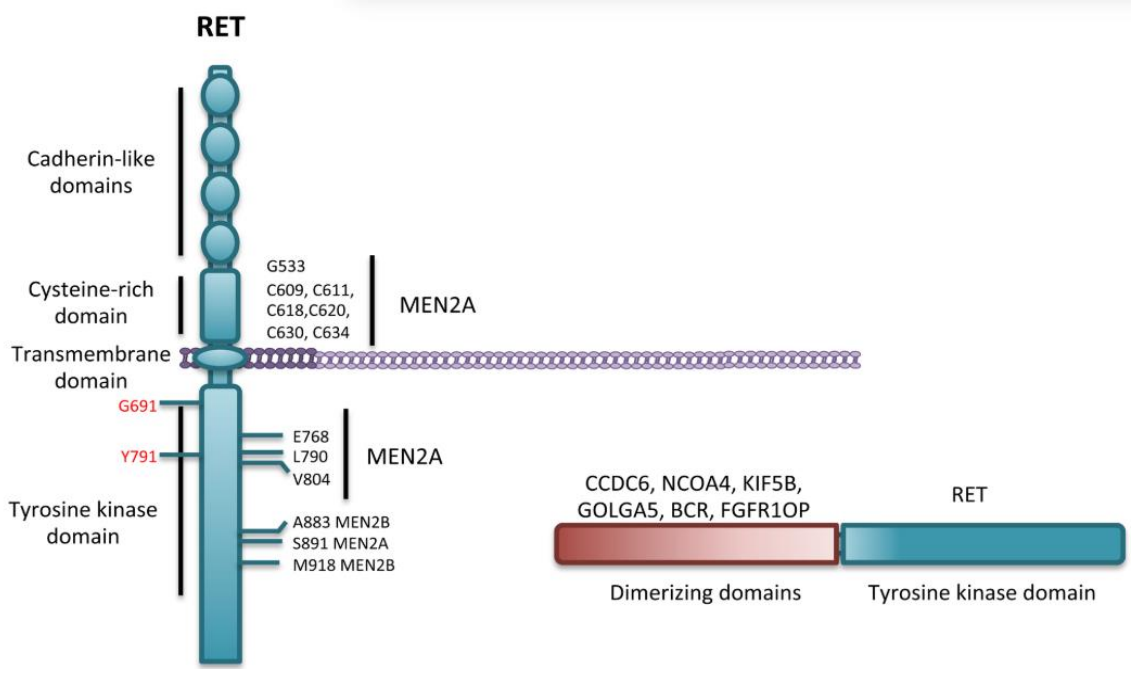
Outline

- **RET background as a drug target**
  - Structure and function
  - Signaling pathway
  - RET in cancer
  - RET-targeted inhibitors
- **RET-related CDX models**
- **Ba/F3 engineered cell lines for RET fusions**
  - CCDC6-RET engineered Ba/F3 cell lines
  - KIF5B-RET engineered Ba/F3 cell lines

RET: structure and function

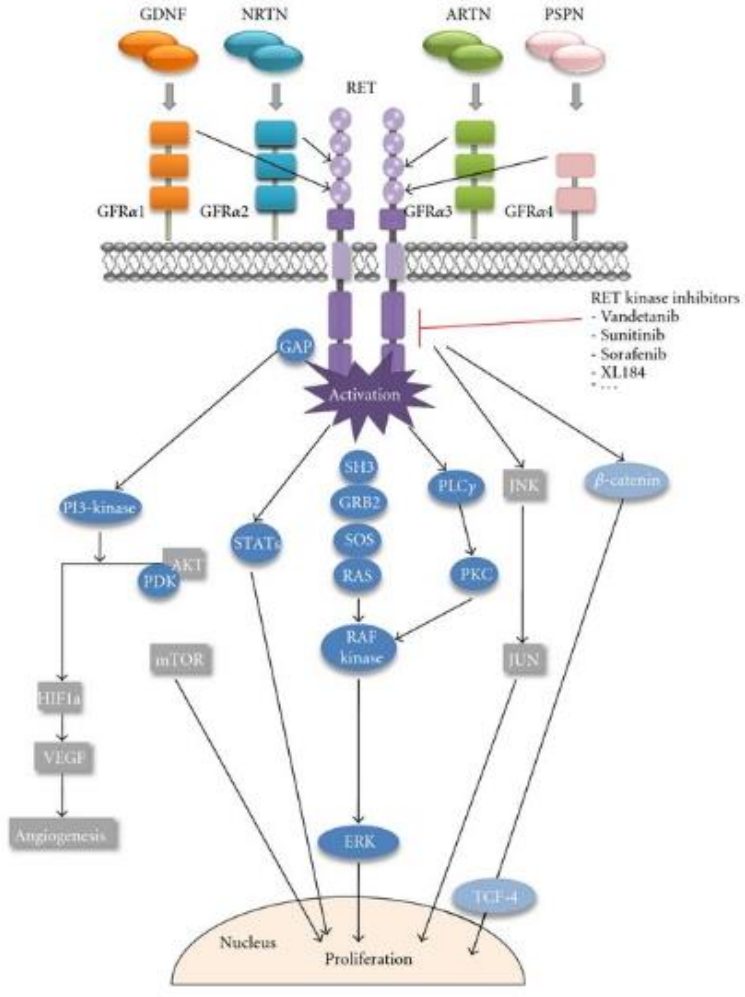
- RET (REarranged during Transfection) is a receptor tyrosine kinase which spans the plasma membrane, with a large extracellular domain containing cadherin-like repeats and a cysteine-rich region important for protein structure and ligand interactions. The RET intracellular tyrosine kinase domain is required for autophosphorylation and phosphorylation of substrates that promote RET downstream signals through multiple pathways.
- RET is required for the development of the kidney and for proliferation, differentiation and survival of central and peripheral nerve lineages, particularly those of the enteric nerve plexus, and other neuroendocrine cells, notably of the thyroid, adrenal and pituitary glands.
- Aberrant activation of the RET receptor (RET-mutation or RET-fusion) has been associated with multiple tumorigenesis.



The RET receptor tyrosine kinase and its oncogenic mutations. Schematic diagram showing locations of the common RET mutations identified in MEN2 patients (left) and RET rearrangements found in multiple cancers (right). The locations of RET mutations that give rise to MEN2 and the associated disease phenotypes are indicated.

RET signaling pathway

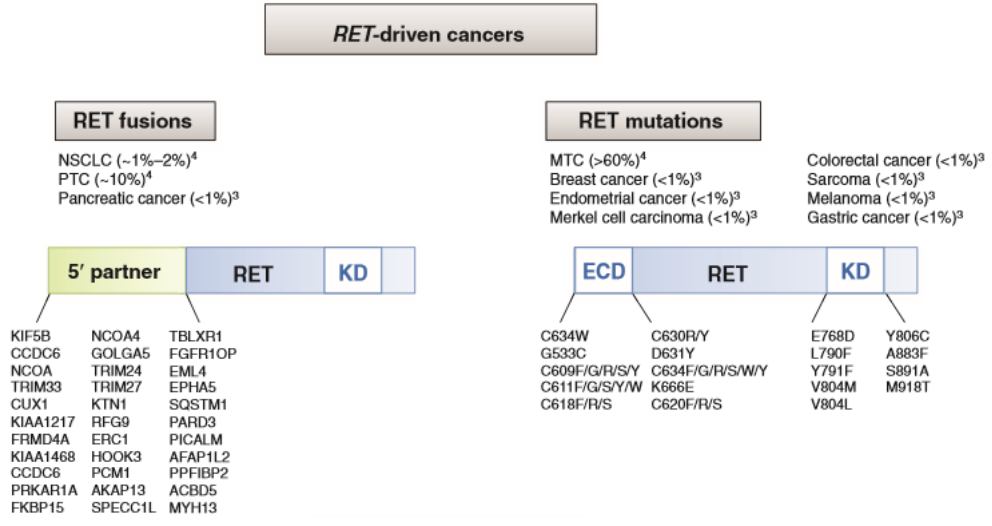
- RET forms a heterodimeric complex with GDNF (glial cell line-derived neurotrophic factor) family ligands GDNF, NRTN (Neurturin), ARTN (Artemin), PSPN (Persephin) and GDNF family co-receptors GFRα1-4. This leads to autophosphorylation of the intracellular tyrosine kinase domain, leading to activation of signaling pathways such as RAS/mitogen activated protein kinase (MAPK), RAS/extracellular signal-regulated kinase(ERK), phosphatidylinositol 3-kinase (PI3K)/AKT, and c-Jun N-terminal kinase (JNK).

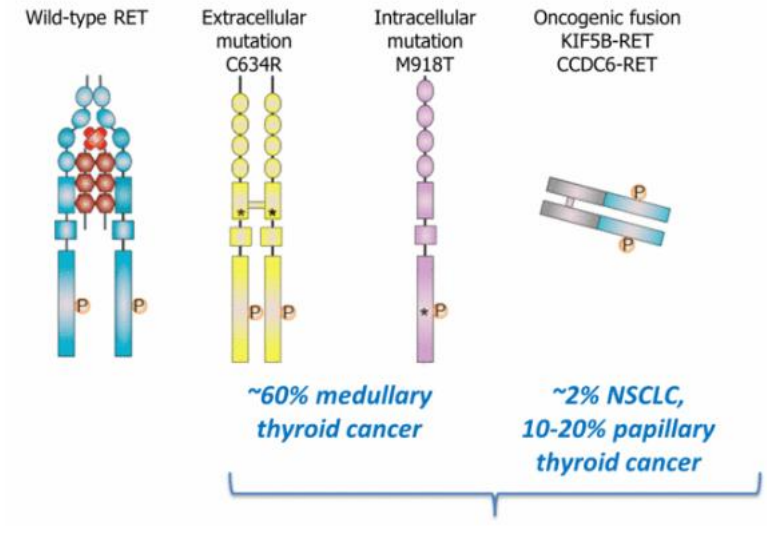


Outline of RET signaling pathways. The RET receptor tyrosine kinase is activated through indirect binding of GDNF family ligands ARTN, PSPN, or NRTN to GFR-α co-receptor, launching a cascade of cell signaling in several key pathways.

RET aberrations in cancer

- RET aberrations fall into two types: Point mutations and chromosomal rearrangements (fusions). RET mutations have been associated with tumor proliferation, invasion, and migration. RET fusions or rearrangements are somatic juxtapositions of 5' sequences from other genes with 3' RET sequences encoding tyrosine kinase that lead to constitutively active cytosolic chimeric proteins.
- RET mutations occur somatically in 40–65% sporadic medullary thyroid cancers (MTC). RET fusions have been found in 10–20% of papillary thyroid carcinoma (PTC), ~1–2% of non-small-cell lung carcinoma (NSCLC), and more recently, in 3% of Spitzoid tumors.
- The most common RET fusions are CDCC6-RET and NCOA4-RET in PTC and KIF5B-RET in NSCLC.





RET targeted inhibitors

- Tyrosine kinase inhibitors are drugs that target kinases such as RET in RET-driven (RET-mutation or RET-fusion-positive) disease. Several multikinase inhibitors (MKI) are FDA-approved for cancer therapy (sunitinib, sorafenib, vandetanib, cabozantinib, regorafenib, ponatinib, lenvatinib, alectinib) and non-oncologic diseases (nintedanib), and are undergoing clinical trials in RET-fusion-positive malignancies.

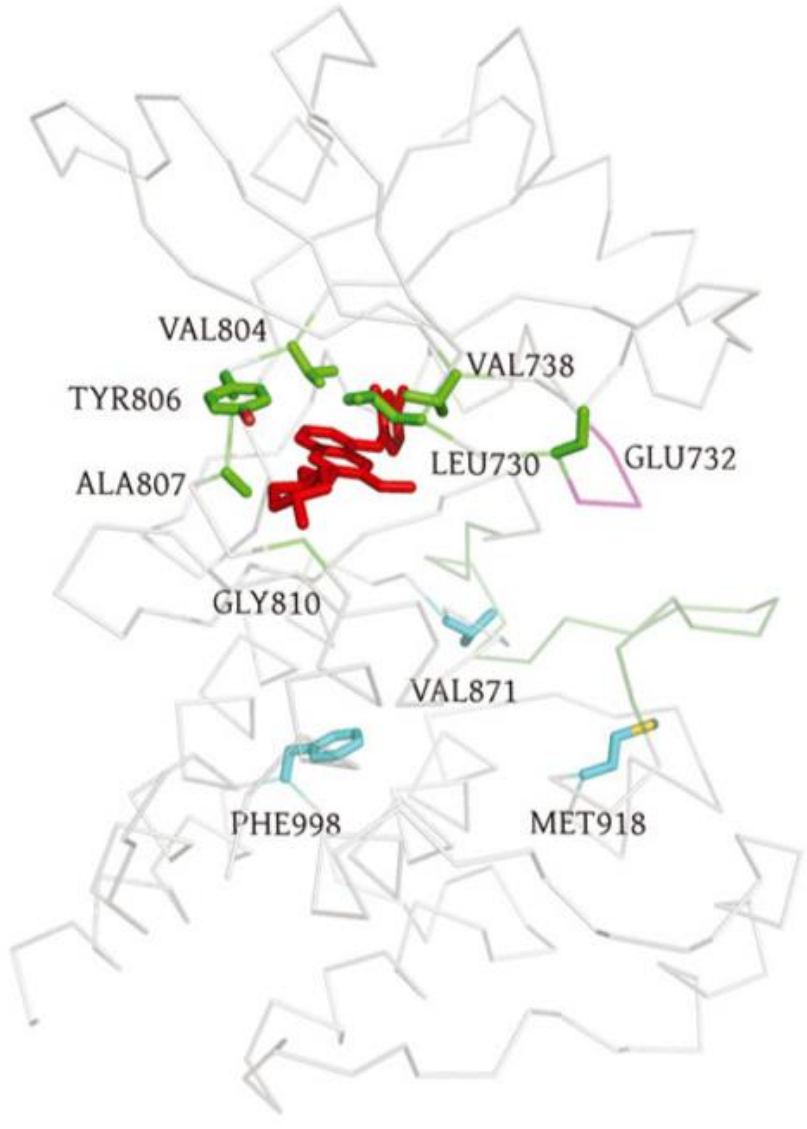
Studies on Therapies directed towards *RET*-rearranged Cancers.

Drug	Cancer	Study Type
Alectinib	NSCLC, Thyroid Cancer	Phase I/II (NCT03131206)
Alectinib	NSCLC	Phase II (NCT03445000)
Alectinib	NSCLC	Phase II (NCT02314481)
Apatinib	NSCLC	Phase II (NCT02540824)
AUY922	NSCLC	Phase II (NCT01922583)
BLU-667	NSCLC, MTC, PTC, Colon Cancer, and other Solid Tumors	Phase I (NCT03037385)
BOS172738	NSCLC	Phase I (NCT03780517)
Cabozantinib	NSCLC	Phase II (NCT01639508)
Cabozantinib	NSCLC	Phase II (NCT03468985)
Dasatinib	Salivary Gland Cancer	Phase II (NCT00859937)
Dovitinib	Solid and Hematological Malignancies	Phase II (NCT01831726)
Lenvatinib	LADC	Phase II (NCT01877083)
LOXO-292	NSCLC, MTC, PTC, Colon Cancer, other Solid Tumors	Phase I/II (NCT03157128)
Ponatinib	NSCLC	Phase II (NCT01813734)
Regorafenib	Melanoma	Phase II (NCT02587650)
RXDX-105	NSCLC	Expanded Access (NCT03784378)
Sunitinib	Solid Tumors	Pilot Study (NCT02450123)
Sunitinib	Solid Tumors	Pilot Study (NCT02691793)
Sunitinib	MTC, PTC, FTC, Hurthle Cell Carcinoma	Phase II (NCT00381641)
Vandetanib	NSCLC	Phase II (NCT01823068)

J Clin Oncol. 2019,37:9008. Barcelona, Spain. Abstract. 2019,PL02.08. Cancer Treatment Reviews. 2019,81:101911

Emergence of acquired resistance to RET inhibitors

- A panel of TKI-resistant RET mutations were identified during the study of using multikinase inhibitors to treat thyroid cancer and RET fusion-positive non-small cell lung cancer.
- These TKI-resistant mutations are located in the Gly-rich loop (L730, E732 and V738), the gate-keeper residue (V804) or the hinge strand (Y806, A807 and G810) that comprise approximately two-thirds of the drug binding pocket in the co-crystal structure of RET.



OncoTargets and Therapy 2017:10 607–615. British Journal of Pharmacology. 2018,175:3504–3515

RET kinase domain mutations confer resistance to TKIs

IC50 (μM) of RET TKI against RET mutants.

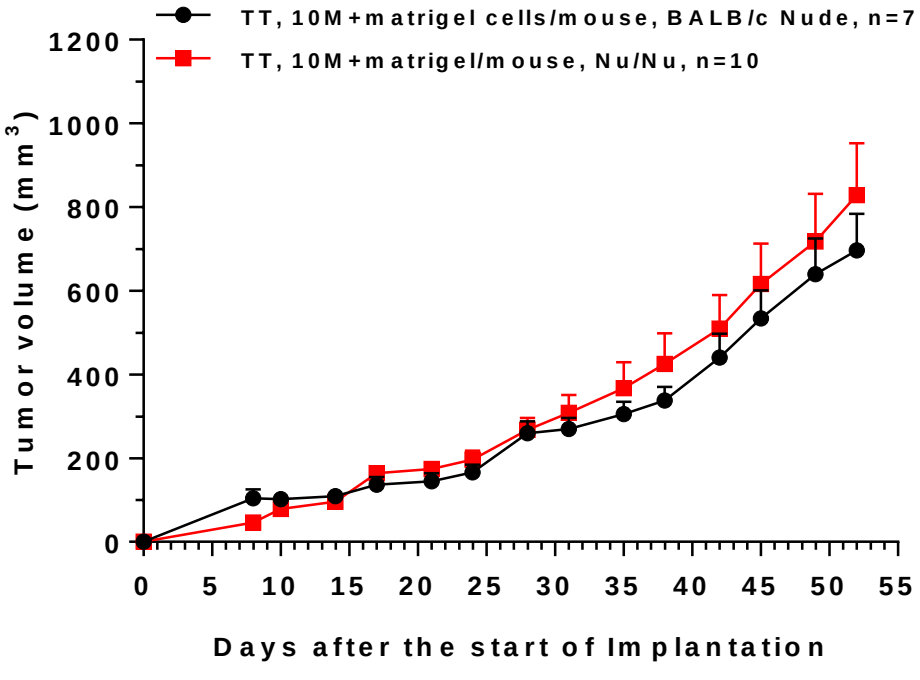
Mutation	IC_{50} (μM)[a]			
	Cabozantinib	Lenvatinib	Vandetanib	Nintedanib
WT	0.24 (0.21 to 0.27)	0.19 (0.18 to 0.21)	0.42 (0.35 to 0.48)	0.14 (0.13 to 0.15)
L730I	2.96 (2.80 to 3.13)	1.07 (1.03 to 1.12)	1.92 (1.79 to 2.06)	0.84 (0.81 to 0.89)
L730V	2.37 (2.28 to 2.47)	0.23 (0.21 to 0.25)	1.09 (1.04 to 1.14)	0.80 (0.77 to 0.84)
L730V/V804M	7.37 (7.21 to 7.53)	9.41 (7.93 to 10.89)	5.13 (4.89 to 5.16)	1.58 (1.48 to 1.68)
E732K	1.15 (1.08 to 1.21)	0.16 (0.15 to 0.17)	0.49 (0.44 to 0.55)	0.17 (0.16 to 0.18)
V738A	1.20 (1.16 to 1.25)	2.35 (2.26 to 2.45)	1.05 (0.95 to 1.14)	0.92 (0.86 to 0.98)
V804L	3.22 (3.00 to 3.45)	10.60 (9.19 to 12.01)	6.10 (5.82 to 6.34)	0.58 (0.51 to 0.64)
V804M	4.26 (4.01 to 4.50)	5.42 (4.92 to 5.93)	5.83 (5.57 to 6.09)	0.86 (0.80 to 0.92)
Y806N	4.76 (4.54 to 4.94)	1.93 (1.84 to 2.02)	5.86 (5.60 to 6.11)	0.91 (0.85 to 0.97)
A807V	0.57 (0.52 to 0.62)	0.54 (0.50 to 0.58)	1.05 (0.92 to 1.18)	0.12 (0.11 to 0.13)
G810A	0.22 (0.21 to 0.24)	0.11 (0.10 to 0.12)	2.76 (2.53 to 3.00)	0.13 (0.11 to 0.14)
G810S	1.05 (0.93 to 1.17)	0.67 (0.64 to 0.70)	5.47 (5.23 to 5.68)	0.56 (0.51 to 0.61)
V871I	1.07 (0.98 to 1.17)	0.52 (0.51 to 0.54)	1.00 (0.95 to 1.05)	0.18 (0.17 to 0.19)
F998V	0.74 (0.68 to 0.80)	0.87 (0.80 to 0.94)	0.77 (0.73 to 0.81)	0.14 (0.13 to 0.15)
M918T	1.57 (1.41 to 1.74)	1.42 (1.27 to 1.59)	1.83 (1.57 to 2.08)	0.24 (0.22 to 0.26)

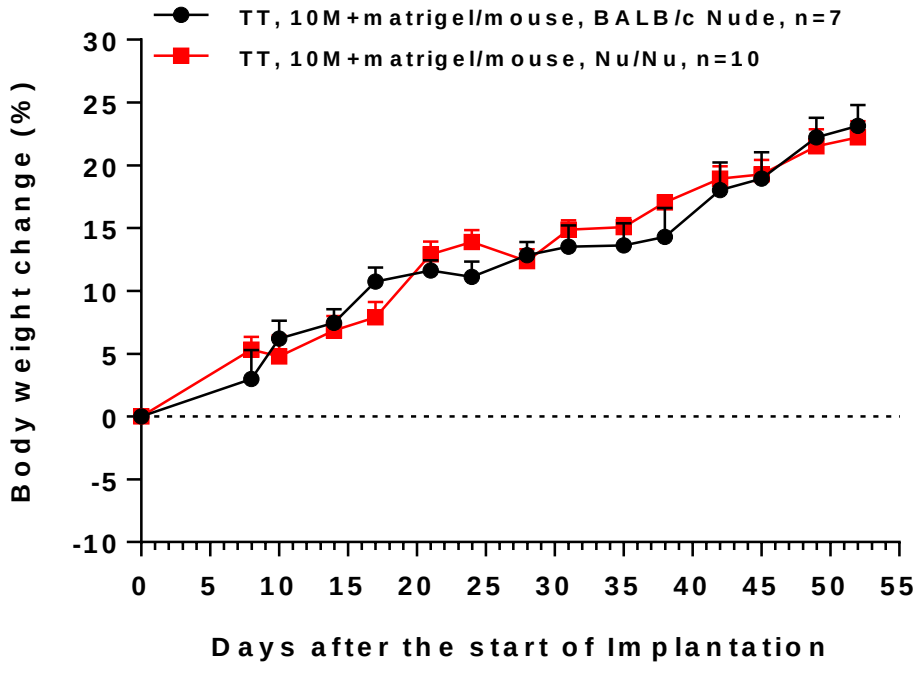
British Journal of Pharmacology. 2018,175:3504–3515

RET-related CDX models

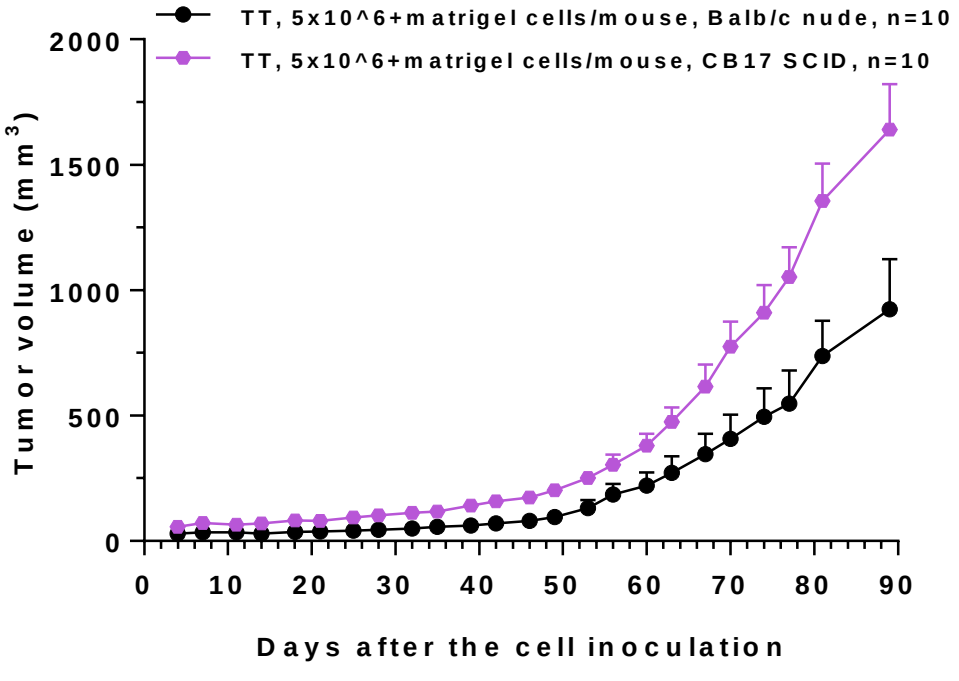
Cancer type	Cell line	Model genetics	Model growth curve
Thyroid	TT	RET C634W	Yes
Lung	LC-2/ad	CCDC6-RET fusion	Ongoing

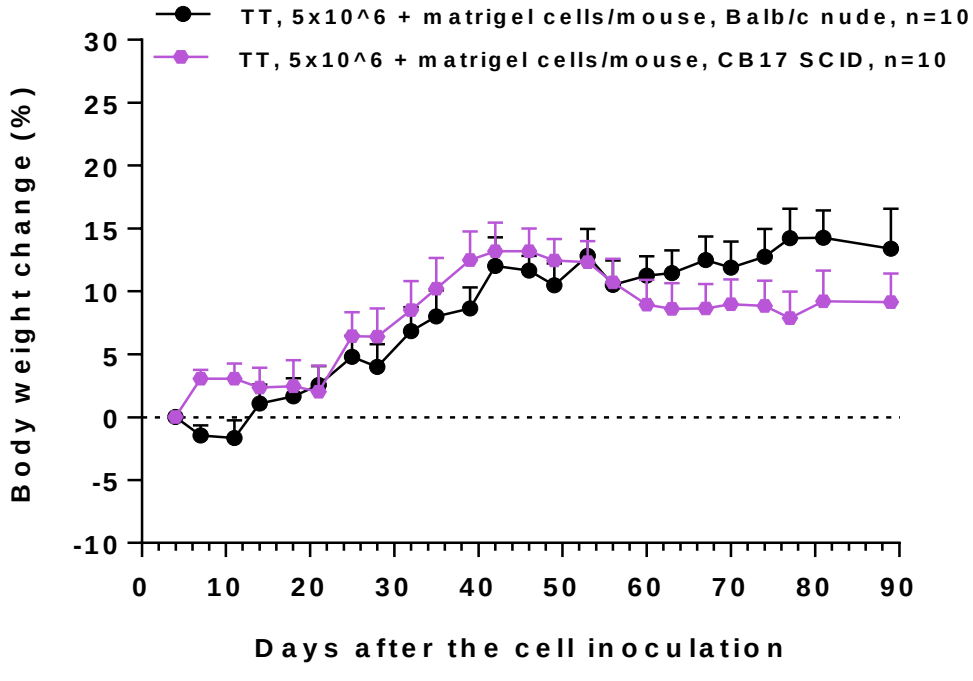
TT Thyroid Cancer CDX model





TT Thyroid Cancer CDX model

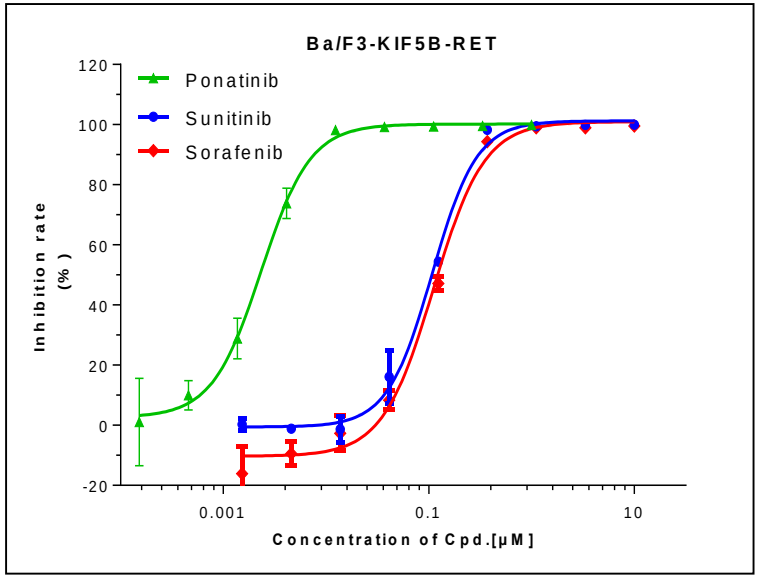


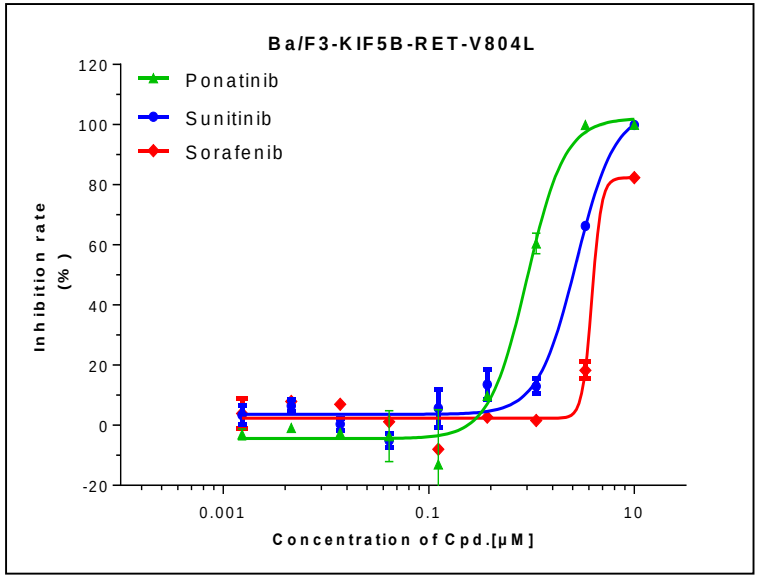


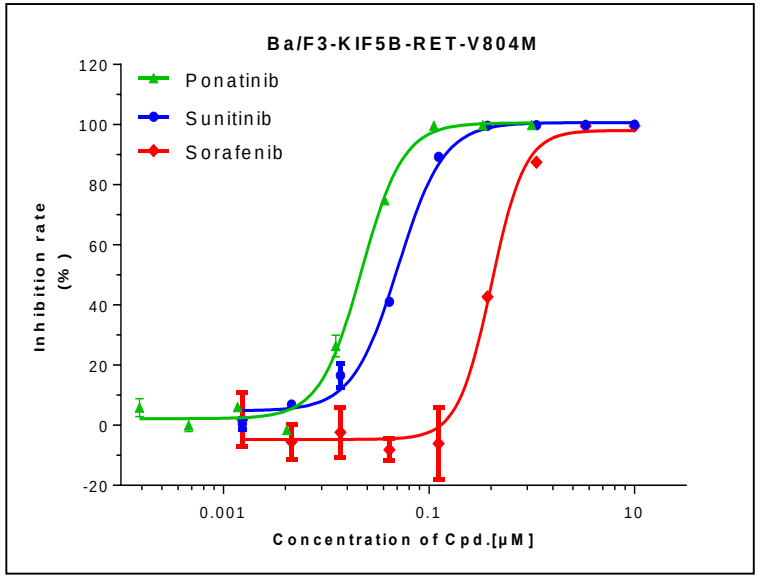
RET engineered Ba/F3 cell lines

Cancer type	Cell line	*In vitro*	*In vivo*
Pro-B cells	Ba/F3-KIF5B-RET-WT	Ready	Ongoing
	Ba/F3-KIF5B-RET-V804L		
	Ba/F3-KIF5B-RET-V804M		
	Ba/F3-CCDC6-RET-WT		
	Ba/F3-CCDC6-RET-V804M		
	Ba/F3-CCDC6-RET-V804L		
	Ba/F3-KIF5B-RET-G810R	Ongoing	
	Ba/F3-KIF5B-RET-G810S		

Relative IC50 of RET inhibitors in Ba/F3 KIF5B-RET cell lines







Cell Name	Compound	ReIC50 (μM)
Ba/F3-KIF5B-RET-WT	Ponatinib	0.0023
	Sunitinib	0.1075
	Sorafenib	0.1107
Ba/F3-KIF5B-RET-V804L	Ponatinib	0.9040
	Sunitinib	2.722
	Sorafenib	~3.864
Ba/F3-KIF5B-RET-V804M	Ponatinib	0.0222
	Sunitinib	0.0499
	Sorafenib	0.4032

Relative IC50 of RET inhibitors in Ba/F3 CCDC6-RET cell lines

Cell Name	Compound	Re IC50 (μM) Ave±SD
Ba/F3-CCDC6-RET-WT	Cabozatinib (XL 184)	0.454±0.078
	Vandetanib (ZD6474)	0.470±0.045
	Lenvatinib (E7080)	0.190±0.021
	Sunitinib	0.394±0.049
	Regorafenib (BAY 73-45)	0.210±0.026
	Pralsetinib (BLU-667)	0.016±0.003
Ba/F3-CCDC6-RET-V804M	Cabozatinib (XL 184)	3.028±0.535
	Vandetanib (ZD6474)	4.752±0.212
	Lenvatinib (E7080)	2.005±0.774
	Sunitinib	0.263±0.040
	Regorafenib (BAY 73-45)	1.577±0.264
	Pralsetinib (BLU-667)	0.016±0.009
Ba/F3-CCDC6-RET-V804L	Cabozatinib (XL 184)	2.825±0.268
	Vandetanib (ZD6474)	4.285±0.178
	Lenvatinib (E7080)	3.542±0.717
	Sunitinib	0.494±0.052
	Regorafenib (BAY 73-45)	2.124±0.368
	Pralsetinib (BLU-667)	0.01±0.005

RET inhibitors	Note
Cabozatinib (XL 184)	1st generation
Vandetanib (ZD6474)	1st generation
Lenvatinib (E7080)	1st generation
Sunitinib	1st generation
Regorafenib (BAY 73-45)	1st generation
Pralsetinib (BLU-667)	2nd generation

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