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Preclinical characterization of THE-630, a next-generation inhibitor for KIT-mutant gastrointestinal stromal tumors (GIST)

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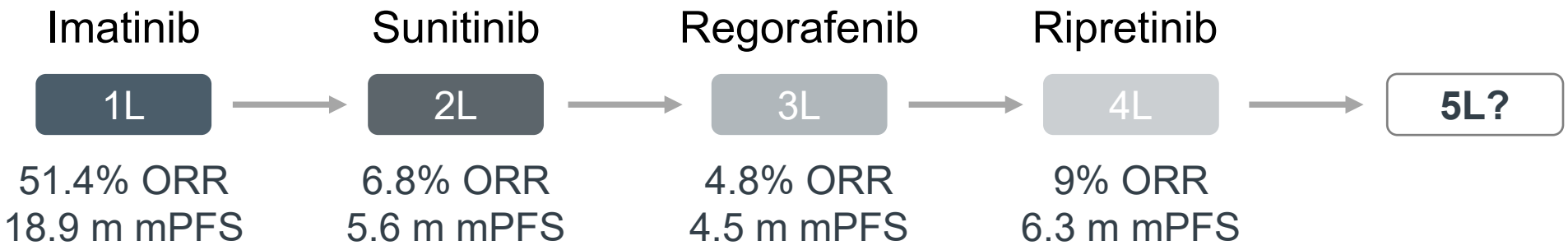
Gastrointestinal stromal tumors (GIST): Treatment landscape and unmet needs



- Most common sarcoma of the GI tract
  - ~4,000-6,000 new GIST cases in US each year
- ~80% of cases driven by activating mutations in KIT
- Outcomes are poor in 2L and beyond
- Unmet needs: 5L – and superior option in earlier lines

*Avapritinib is approved in the US for GIST patients with PDGFRA exon18 mutations only

Abbreviations: ORR (objective response rates); mPFS (median progression free survival); m (months); L (line)

Sources: GLEEVEC [package insert]; SUTENT [package insert]; STIVARGA [package insert]; QINLOCK [package insert]

There remains a need for a pan-KIT inhibitor for advanced GIST patients

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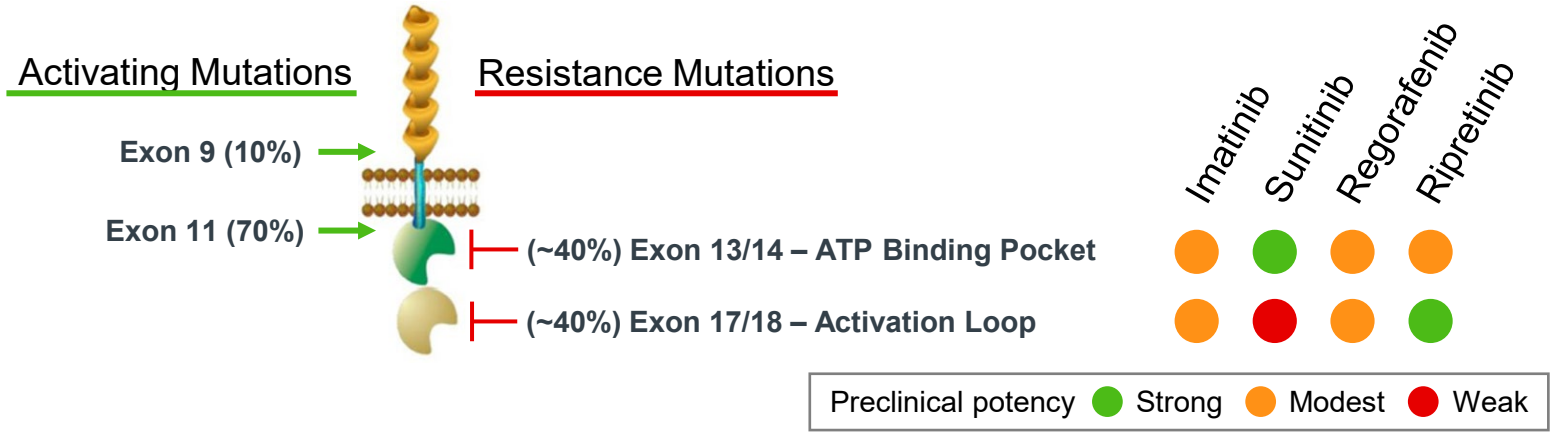
Genetics of drug-sensitivity are well understood

- 2 major classes of activating mutations
  - Imatinib, but not sunitinib, has reduced activity against Ex9 class
- ~80% of relapse cases driven by secondary resistance mutations in KIT
  - Most occur in ATP binding pocket (Exon 13/14) or Activation loop (Exon 17/18)
  - Most patients have more than 1 resistance mutation
- No drug has potent activity against all classes of activating and resistance mutations



Sources: Corless, C. et al. (2014) Mod. Pathol 27, S1-S16; Preclinical potency assessment also based on studies reported in this poster

Methods - 1

Predictive preclinical modeling of clinical drug sensitivity using engineered BaF3 cells

- TKI potency was evaluated using a panel of BaF3 cell lines engineered to express KIT variants shown in the Table (next slide)
- Viability assays (72 h) were performed using culture medium supplemented with physiological concentrations of human serum albumin (341 µM) and α1-acid glycoprotein (1 mg/mL) – to incorporate functional effects that binding to human serum proteins has on target inhibition
- Ripretinib and its active metabolite DP-5439 were tested separately and results averaged
- IC_{50}s were related to average TKI concentration (C_{av}; AUC/24 h) in patients at the approved dose
  - Imatinib[1] C_{av} 3377 nM
  - Sunitinib[2] C_{av} 136 nM
  - Ripretinib[3] C_{av} 2126 nM (sum of ripretinib and DP-5439 levels)
- Cellular IC_{50} in presence of human serum proteins related to patient C_{av} to predict clinical efficacy
  - KIT variants inhibited with IC_{50}s substantially below the patient C_{av} are predicted to be highly sensitive
  - KIT variants inhibited with IC_{50}s substantially above the C_{av} are predicted to be highly resistant
  - KIT variants inhibited with IC_{50}s close to the C_{av} (± ~2-fold) are predicted to be associated with stable disease, but ultimate tumor progression

Sources: [1]Peng et al., (2005) Clin. Pharmacokinet. 44:879-894;; [2]SUTENT [package insert]; [3]QINLOCK [package insert]

Methods - 2

Genotype of KIT variants in BaF3 cells

KIT Variant Abbreviation	Primary Mutation		Secondary Mutation	
	Location	Genotype	Location	Genotype
Ex11Del	Exon 11 (JM)	Del 557_558	-	-
Ex11Del + V654A			Exon 13 (ATP pocket)	V654A
Ex11Del + T670I			Exon 14 (ATP pocket)	T670I
Ex11Del + D816G			Exon 17 (A-loop)	D816G
Ex11Del + D816H				D816H
Ex11Del + D820A				D820A
Ex11Del + D820G				D820G
Ex11Del + N822K				N822K
Ex11Del + Y823D				Y823D
Ex11Del + A829P			Exon 18 (A-loop)	A829P
Ex9Ins	Exon 9 (ECD)	Ins (502AY)	-	-
Ex9Ins + V654A			Exon 13 (ATP pocket)	V654A
Ex9Ins + D816H			Exon 17 (A-loop)	D816H

JM (justamembrane domain); ECD (extracellular domain); ATP pocket (ATP binding pocket); A-loop (activation loop)

Kinase assays (Nanosyn)

- Single point (0.1 μM) screen was performed on 300 kinases (Km of ATP)
- IC_{50}s were determined for kinases inhibited by >50% (1 mM ATP)

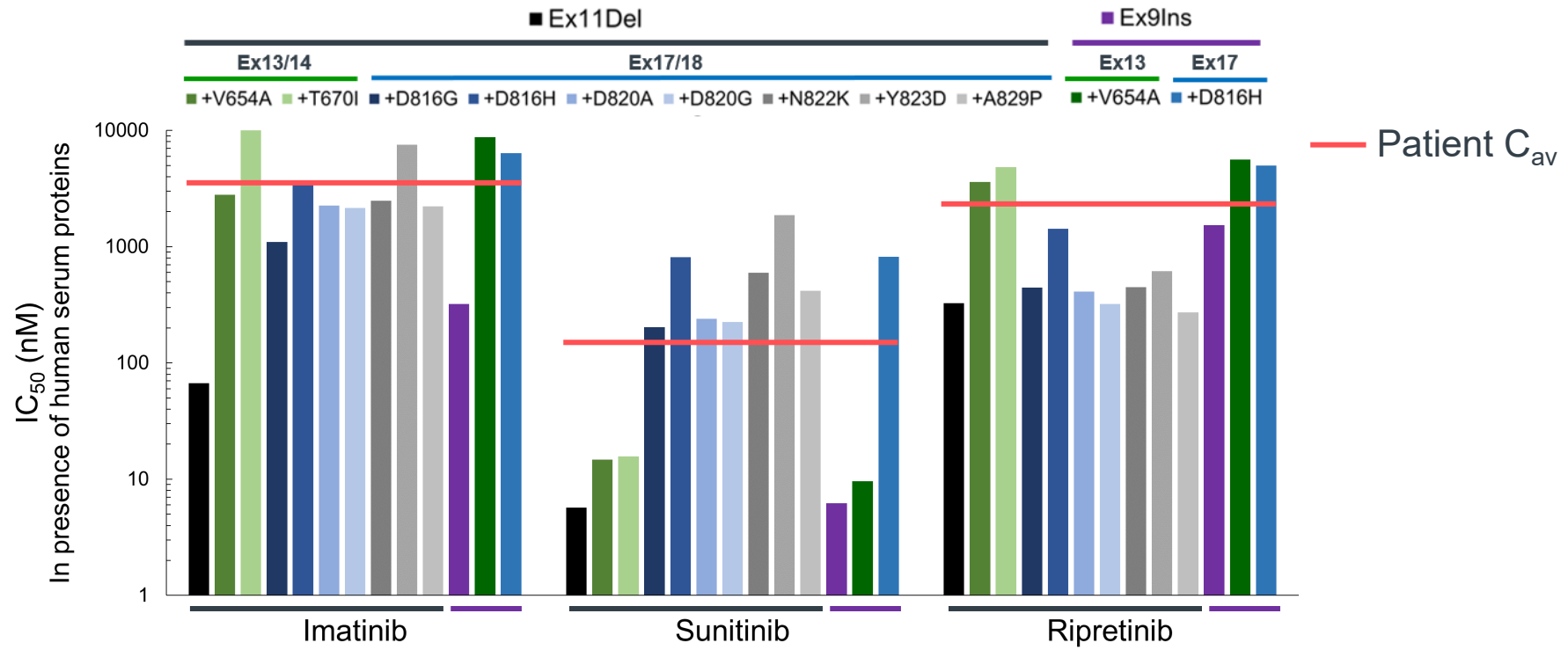
Viability/signaling assays in cancer cell lines

- TKI potency in cancer cell lines and parental BaF3 cells was evaluated using standard cell culture medium
- Immunoblot analyses were performed on GIST-T1 cells treated for 2 hr

Efficacy studies

- GIST-T1 or engineered BaF3 cells were implanted subcutaneously into the right flank of female CB-17 SCID mice and TKIs administered orally when tumors reached ~140 mm^3
  - Sunitinib 20 mg/kg QD
  - Ripretinib 50 mg/kg BID
  - THE-630 10-25 mg/kg QD
- All dose levels were well-tolerated with no clinical signs observed

Predictive preclinical modeling of clinical drug sensitivity using engineered BaF3 cells

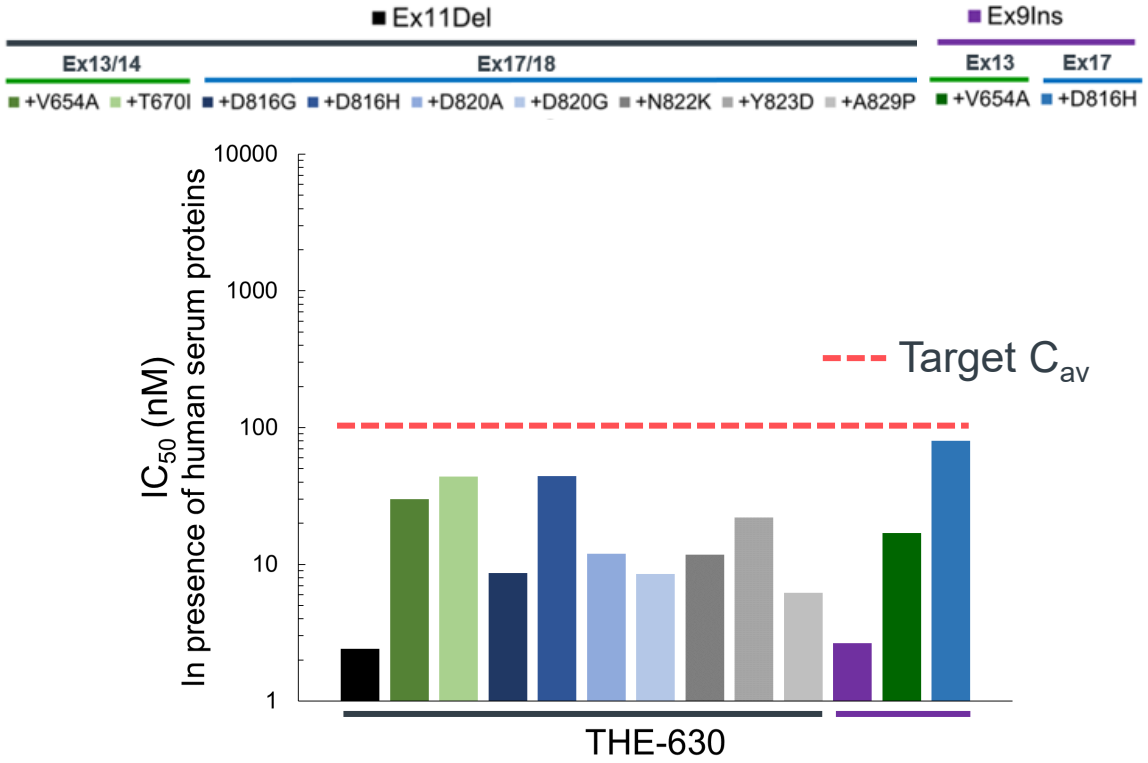


- Mutant classes predicted to be associated with resistance or minimal efficacy – ie, since exposure in patients (represented by C_{av}) does not substantially exceed the mutant IC_{50}
  - Imatinib: Ex13/14 and Ex17/18
  - Sunitinib: Ex17/18
  - Ripretinib: Ex13/14 and Ex9Ins primary ± secondary

THE-630 has the profile of a pan-KIT inhibitor for GIST in vitro

Pan-KIT inhibitory profile

- Potent cellular activity against
  - Ex11Del ± 9 resistance mutations (Ex13/14/17/18)
  - Ex9Ins ± 2 resistance mutations (Ex13/17)
  - PDGFRA V561D (IC_{50} <5 nM)
- 100 nM target C_{av} in patients for pan-KIT mutant activity



Selectivity profile

- 46/300 kinases inhibited by >50% at 0.1 µM
  - Ripretinib: 44/300
- 28 kinases inhibited with IC_{50} within 100-fold of KIT IC_{50} (0.5 nM)
  - Ripretinib: 27 (KIT IC_{50} 1.5 nM)
- THE-630 not designed to potently inhibit PDGFR D842V (GIST) and KIT D816V (mastocytosis) activating mutations
  - IC_{50} >100 nM in BaF3 cellular assays

THE-630 is highly active in cancer cell lines with activating mutations in KIT

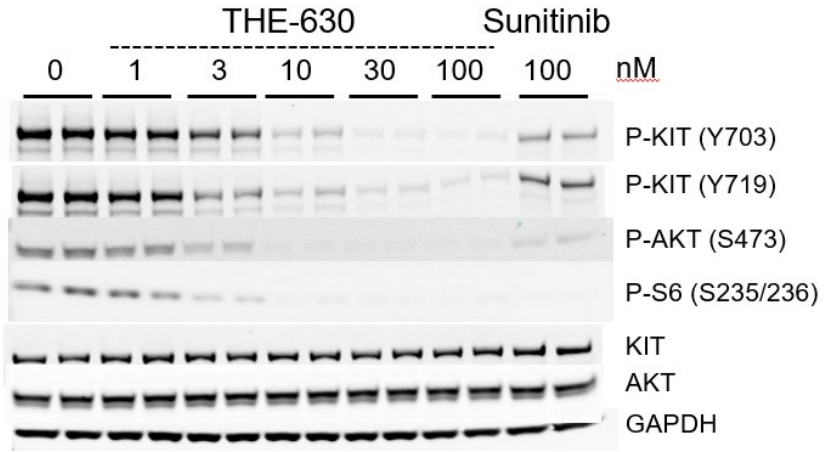
Potent activity against KIT Ex11Del, N822K (Ex17) and D816Y (Ex17) activating mutations

Cell line	KIT mutation	Exon	Tissue	Viability IC_{50} (nM)*			
				Imatinib	Sunitinib	Ripretinib	THE-630
GIST-T1	Ex11Del	11	Human GIST	30	13	12	4
Kasumi	N822K	17	Human AML	832	51	7	2
P815	D816Y	17	Murine mastocytoma	2599	195	40	30
BaF3**	N/A		Murine pro B cells	8434	2311	9545	2379

*Viability assays performed in the absence of human serum proteins

**Untransformed parental BaF3 cells grown in the presence of IL3

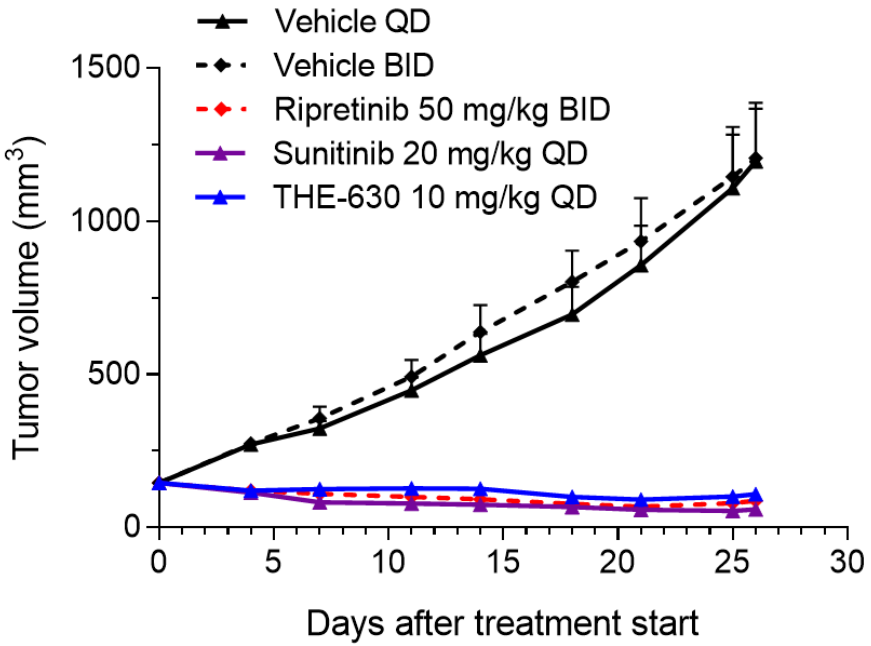
THE-630 cell killing associated with inhibition of KIT and downstream signaling



GIST-T1 cells treated for 2 hr

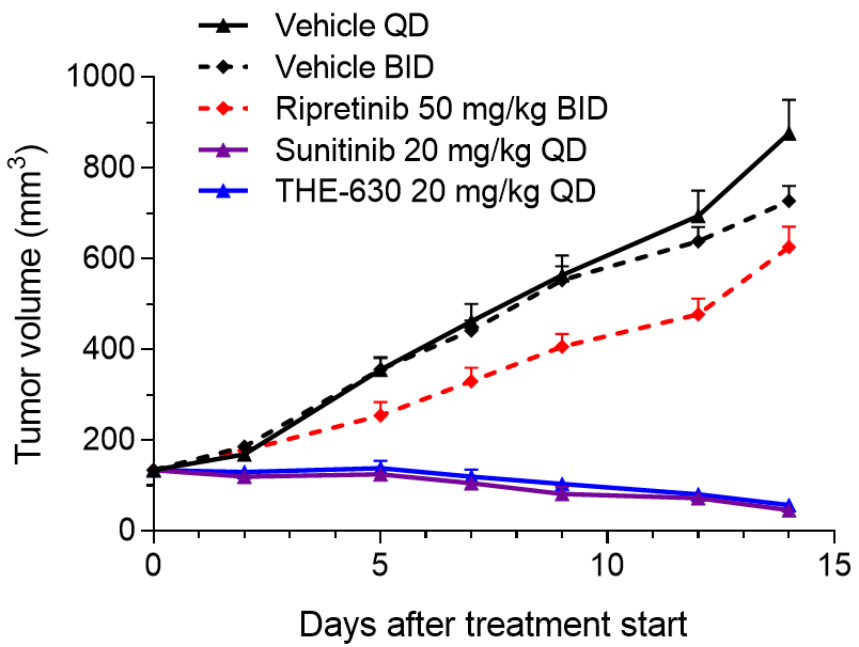
THE-630 is highly efficacious in tumor models containing KIT Ex11 and Ex9 activating mutations

Ex11Del (primary mutation)



GIST-T1 model

Ex9Ins (primary mutation)



BaF3 model

THE-630 is highly efficacious in tumor models containing KIT Ex13, 17, and 18 resistance mutations

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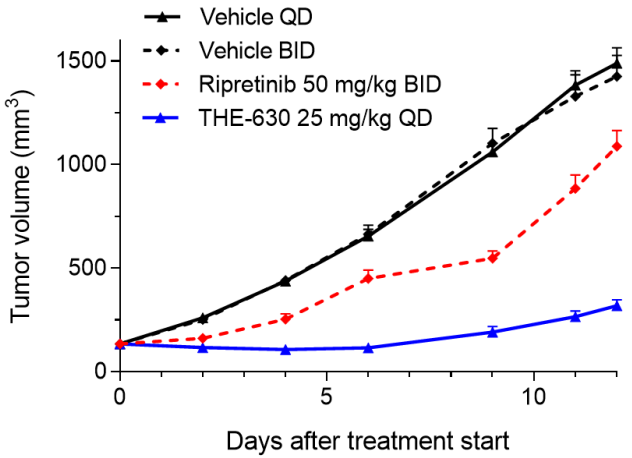
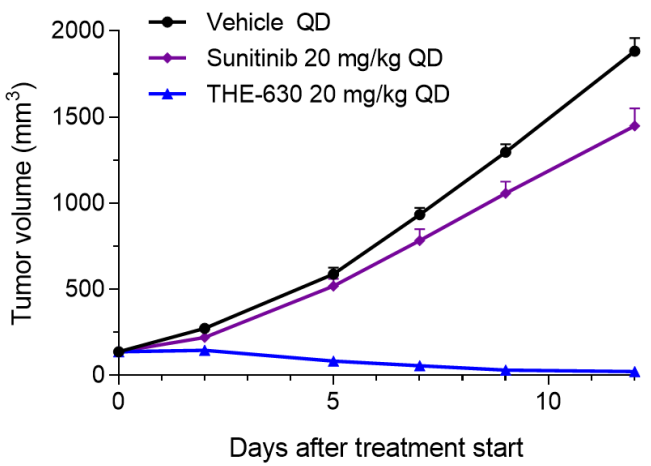
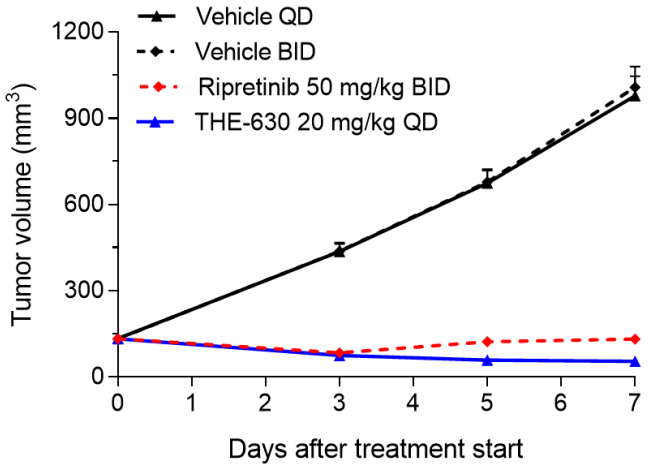
Ex11Del + V654A (Ex13)



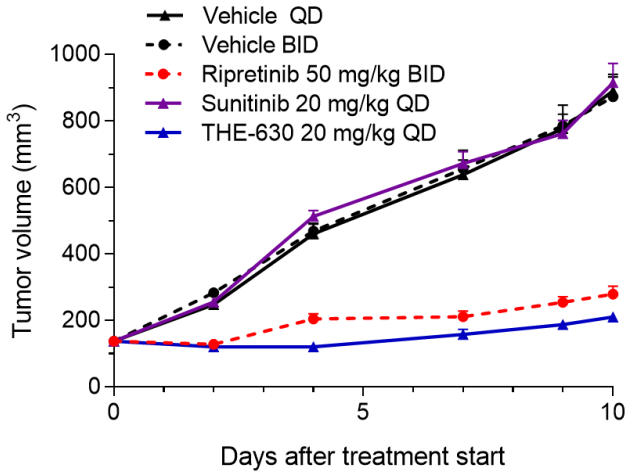
Ex11Del + N822K (Ex17)



Ex11Del + D820A (Ex17)



Ex11Del + A829P (Ex18)



All studies: BaF3 model

THE-630 is highly efficacious in tumor models containing all classes of KIT activating and resistance mutants

	Primary		Ex11Del + ATP pocket	Ex11Del + Activation loop		
	Ex9Ins	Ex11Del	V654A (Ex13)	N822K (Ex17)	D820A (Ex17)	A829P (Ex18)
Sunitinib	66% TR	59% TR	91% TR	25% TGI	ND	0% TGI
Ripretinib	17% TGI	41% TR	25% TGI	ND	1% TR	81% TGI
THE-630	58% TR	85% TR	86% TGI	83% TR	59% TR	90% TGI

TGI: tumor growth inhibition; TR: tumor regression; ND: Not determined

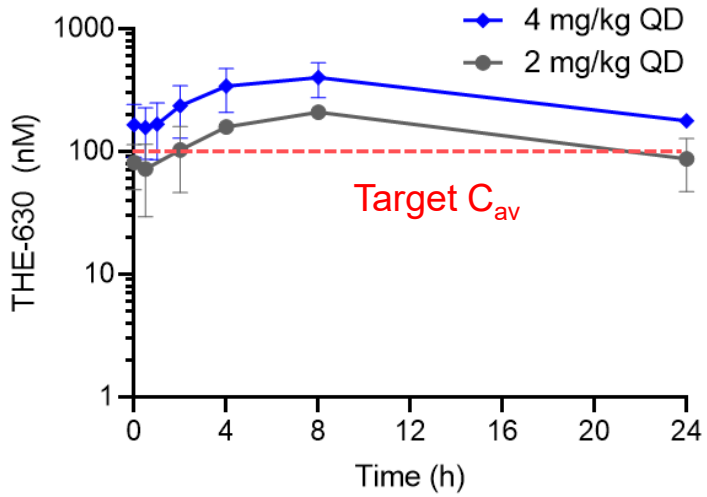
Preclinical Efficacy ● Stasis/regression ● No/minimal TGI

- THE-630 highly active against
  - Most common primary activating mutants (Ex11Del and Ex9Ins)
  - Most common ATP-binding pocket mutant (V654A)
  - All 3 activation loop mutants tested (N822K, D820A, A829P)
- THE-630 efficacy ≥ ripretinib against all mutants tested

THE-630 has favorable ADMET properties

ADME/Safety	THE-630
Plasma protein binding, 1 μM (Rat, Monkey, Human)	95, 90, 95%
Metabolic clearance (Rat, Monkey, Human LMs)	High, low/mod, low
CYP IC_{50}s (1A2, 2C9, 2C19, 2D6, 3A4)	>20 μM (no TDI)
hERG (IC_{50})	>10 μM

Toxicokinetics in NHPs after 14 days of oral daily dosing



- 14 daily doses were tolerated in NHPs at exposures that matched (2 mg/kg) or exceeded, by 2.5-fold (4 mg/kg), the target C_{av} for pan-KIT activity

Conclusions

- THE-630 has the profile of next-generation, pan-KIT inhibitor for GIST
  - Potent activity in cellular assays and strong efficacy in tumor models against both classes of activating mutations (Ex9 and Ex11) and both classes of resistance mutations (Ex13/14 and Ex17/18)
  - Good selectivity profile
- IND-enabling studies are underway based on promising nonclinical data
  - Favorable ADME profile
  - Target pan-KIT exposure levels achieved at tolerable doses in 14-day rat and NHP pilot studies
- A phase 1 clinical trial of THE-630 in patients with GIST is planned to begin in the second half of 2021

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