Comparing TEAD Palmitoylation Inhibitors with Differential TEAD Selectivity in Combination Efficacy with Targeted Therapies and in Renal Safety

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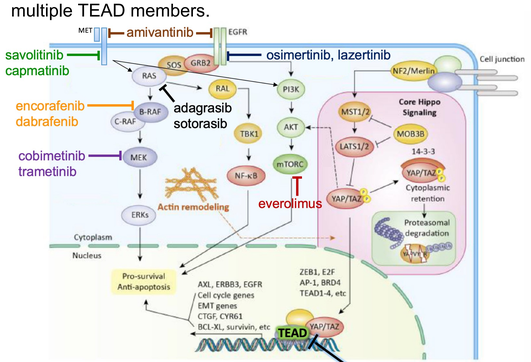
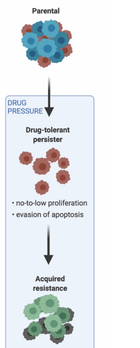
Background

- The Hippo-YAP/TAZ pathway is involved in the regulation of cell proliferation, survival, and cell migration. Genetic alterations of the Hippo signaling pathway components resulting in YAP/TAZ activation have been reported in a variety of human malignancies. YAP/TAZ activation and functional requirement have also been linked to resistance to targeted therapies by providing the essential survival signal in drug-tolerant persister/dormant cells.
- TEAD transcription factors are the major effectors of the Hippo-YAP/TAZ pathway. There are four members in the TEAD family: TEAD1, TEAD2, TEAD3, and TEAD4. All four members have a conserved cysteine residue that gets auto-palmitoylated and a highly conserved central pocket in which the palmitate is buried. TEAD auto-palmitoylation is required for TEAD interaction with coactivator YAP/TAZ and transcriptional activity.
- We have discovered and developed highly potent and selective TEAD auto-palmitoylation inhibitors that interact directly with TEAD by occupying the central palmitate pocket, disrupt YAP/TAZ-TEAD protein interaction, suppress TEAD transcriptional activity, and selectively block *NF2*-deficient mesothelioma proliferation *in vitro* and inhibit *NF2* mutant tumor growth *in vivo* (Tang *et al. Mol Cancer Ther*, 2021).
- One of these compounds, VT3989, is being evaluated in an ongoing phase 1 clinical trial, where partial responses in mesothelioma patients have been demonstrated, showing for the first time that the Hippo pathway is druggable and that the Hippo pathway is now a validated target for cancer therapy (Yap *et al.* AACR Annual Meeting 2023).
- It remained a question, however, which TEAD members are more important and whether it would be safer and as efficacious to inhibit one TEAD member than multiple TEAD members.



Adapted from Nguyen and Yi, Trends Cancer. 5(5): 283–296 (2019)



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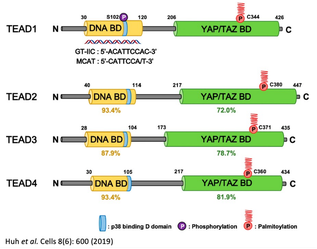
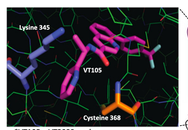
VT3989
Vivace TEAD Inhibitors

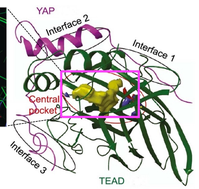


Huh *et al.* Cells 8(6): 600 (2019)



*VT105 = VT3989 analog

Tang et al., *Mol Cancer Ther*, 2021
Chan et al., *Nat Chem Biol*, 2016
Noland et al., *Structure*, 2016
Pobatti et al., *Structure*, 2015



Summary and Conclusions

- **Utilizing our TEAD inhibitors with differential TEAD selectivity, we found that TEAD1-selective TEAD palmitoylation inhibitors are less efficacious than pan-TEAD/multiple-TEAD inhibitors in combination studies with targeted therapies.**
- **Multi-TEAD inhibitors have wider spectrum of efficacy than TEAD1-selective inhibitors in mesothelioma (in vitro cell proliferation assays).**
- **In 14-day/28-day rat studies, TEAD1-selective TEAD inhibitors also exhibited proteinuric nephropathy with evidence of podocyte injury by electron microscopy similar to that observed with pan-TEAD/multiple-TEAD inhibitors.**
- **Based on our findings, we can conclude that TEAD1-selective TEAD palmitoylation inhibitors can have similar on-target effect on kidneys as TEAD inhibitors with broader TEAD selectivity while having reduced anti-tumor efficacy and durability of response in combination with targeted therapies.**

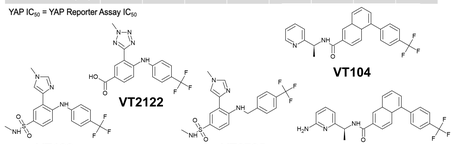
Comparing TEAD inhibitors with differential TEAD selectivity in *in vitro* cell proliferation

A.

TEAD Selectivity	TEAD1			Multi-TEAD / Pan-TEAD				
Compound ID	VT103	VT2122	VT3642	VT104	VT107	VT3989	VT3994	Inactive Analog
YAP IC_{50} (nM)	1.0	5.0	14.5	10.4	4.9	4.9	8.8	>3uM
TEAD1 ΔTm (ºC)	8.3	7.6	9.6	8.6	12.1	11.1	9.6	0.2
TEAD2 ΔTm (ºC)	4.1	1.8	1.3	5.4	10.1	8.8	11.0	0.5
TEAD3 ΔTm (ºC)	1.0	1.5	2.8	8.2	10.4	8.6	9.2	0.2
TEAD4 ΔTm (ºC)	1.9	0.7	2.7	4.3	8.9	5.1	6.7	0.2

YAP IC_{50} = YAP Reporter Assay IC_{50}

B.



C.

Compound	TEAD Selectivity	YAP Reporter IC_{50} (nM)	NCI-H226 Meso NF2 deficient 5-day	NCI-H226 Meso NF2 deficient 7-day	NCI-H2052 Meso NF2 mutant	NCI-H2452 Meso NF2 wildtype	Mero-14 Meso Merlin null	ZL55 Meso Merlin null	NCI-H1975 NSCLC EGFR mutant	Detroit 562 H&N YAP CNV=9
VT3989	TEAD1 (TEAD2) TEAD3	5	28 (80%)	5 (104%)	44 (78%)	>3000 (14%)	60 (94%)	48 (87%)	>3000 (33%)	>3000 (28%)
VT103	TEAD1	1	15 (76%)	1 (102%)	41 (62%)	>3000 (19%)	>10000	>10000	>3000 (24%)	>3000 (28%)
VT2735	TEAD1	9	596 (56%)	6 (94%)	>3000 (31%)	>3000 (8%)	Not tested	Not tested	>3000 (11%)	>3000 (22%)
IAG933	Pan-TEAD	Not tested	32 (100%)	10 (109%)	37 (99%)	435 (81%)	Not tested	Not tested	233 (100%)	443 (91%)

IC_{50} values in nM
(Max % Inhibition)

D.

Cell Line	VT103 (TEAD1) GI50 (nM)	VT103 (TEAD1) Max inh %	VT104 (Multi-TEAD) GI50 (nM)	VT104 (Multi-TEAD) Max inh %	VT3989 (Multi-TEAD) GI50 (nM)	VT3989 (Multi-TEAD) Max inh %	VT107 (Pan-TEAD) GI50 (nM)	VT107 (Pan-TEAD) Max inh %	NF2 Mutation	LATS2 Mutation	Merlin Level
NCI-H226	1	99	16	99	9	101	9	101	very low NF2 transcript		Low/Undetectable
NCI-H2373	2	99	26	88	8	92	9	90	Homozygous deletion		Low/Undetectable
Mero-48a	5	78	88	99	38	109	34	108			Low/Undetectable
SDM103T2	2	68	60	85	26	82	22	86	Homozygous truncation		Low/Undetectable
NCI-H2052	10	94	33	91	<12	93	<12	90	Homozygous truncation		Low/Undetectable
ACC-MESO-1	5	76	20	81	15	84	26	83	Homozygous truncation		Low/Undetectable
ZL34	21	76	46	92	20	95	30	94			Low/Undetectable
JU77	232	62	70	76	74	82	41	84	Homozyous deleterious mut		Low/Undetectable
ZL55	>10000		101	78	48	87	46	85			Low/Undetectable
SPC212	>10000		>3000	56	60	72	>3000	64	Homozygous truncation		Low/Undetectable
Mero-14	>10000		124	82	60	94	47	88		Homozyous deleterious mut	Low/Undetectable
ZL5	>10000		236	78	178	80	107	79	Heterozygous truncation	Heterozygous truncation	Low/Undetectable
Mero-82	>10000		243	73	113	75	77	78		Heterozygous truncation	Low/Undetectable
ONE58	>2000		135	60	186	67	38	62			Low/Undetectable
Mero-83	>10000		214	68	118	75	79	75			Low/Undetectable
Mero-95	>10000		303	68	197	76	107	76			Low/Undetectable
Mero-41	>10000		984	56	260	71	135	57			Low/Undetectable
SPC111	>10000		1945	63	1511	64	206	80	Heterozygous truncation		Low/Undetectable
NO36	>10000		>3000	41	>3000	71	>3000	46			Low/Undetectable
Mero-84	>10000		>3000	45	>3000	33	>3000	48			Low/Undetectable
ACC-MESO-4	>10000		1098	62	879	67	312	73			Detected
Mero-25	>10000		>3000	52	2494	54	459	72			Detected
NCI-H28	>3000		>3000		>3000		>3000			Heterozygous tolerated mut	Detected
NCI-H2452	>3000		>3000		>3000		>3000				Detected
MSTO-211H	>3000		>3000							Heterozygous tolerated mut	Detected
HMMME			>3000		>3000		484	81			Detected

TEAD inhibitors with different TEAD selectivity
(A) TEAD selectivity was determined by thermal shift assays using purified recombinant TEAD proteins. The YAP reporter assay IC_{50} showed the potency of these TEAD inhibitors at blocking YAP/TAZ-TEAD transcriptional activity. (B) Chemical structures of selected TEAD inhibitors. TEAD1-selective and multi-/pan-TEAD inhibitors were tested against various cell lines (C) and a large panel of mesothelioma cell lines (D) in *in vitro* cell proliferation assays.

Efficacy and Pharmacokinetics in Mice

Compound ID	TEAD Selectivity	Minimal Efficacy Dose (NCI-H226 CDX)	Mouse PK (IV) Half-Life (hours)	Mouse PK (IV) Oral Availability (F)	Mouse 14-day PK (PO) Strain (Sex)	Dose (mg/kg)	Study Day	AUC_{0-24} (h*ng/mL)	Extrapolation
VT103	TEAD1	1mg/kg QD	13.2	75%	Swiss CD1 (M)	10	14	47900	1mg/kg ~ 4790
VT3989	TEAD1 (TEAD2) TEAD3	3mg/kg QD	15.7	75%	Balb/c nude (F)	10	14	18900	3mg/kg ~ 5670

➢ The exposure of VT103 and VT3989 at the minimal efficacy dose in mice is not very different (caveat: mice of different background strain and sex were used in the 14-day studies).

TEAD1-selective TEAD inhibitor shows renal toxicity

➢ Dose and exposure at which clinical signs of toxicity (decreased activity, reduced body weights and food consumption) were seen:

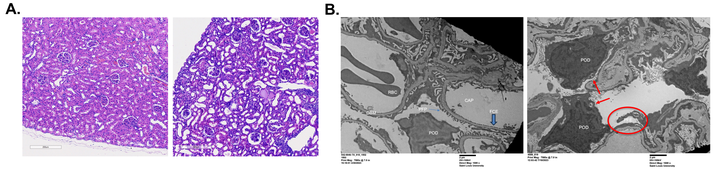
Compound ID	TEAD Selectivity	Dose (mg/kg/day)	C_{max} (ng/mL)	AUC_{0-24} (h*ng/mL)
VT103	TEAD1	≥ 0.6	382	6780
VT3989	TEAD1 (TEAD2) TEAD3	≥ 25	12,300	178,000
	Relative Ratio VT3989:VT103	42	32	26

➢ Similar magnitude of alterations in clinical pathology alterations reflective of renal injury only occurred at substantially higher doses and exposures of VT3989 than VT103.

➢ Increased kidney weights and proteinuric nephropathy of comparable severity occurred at substantially lower VT103 doses and systemic exposures than for VT3989.

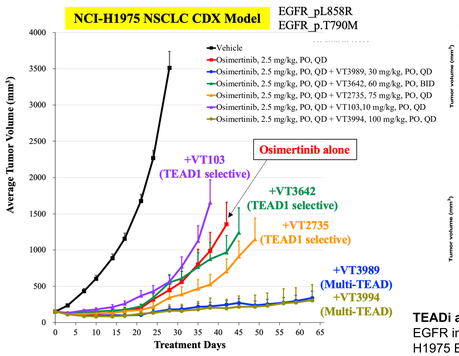
Parameter	Ratio of VT3989 to VT103 Values Dose	C_{max}	AUC_{0-24}
↓ Serum protein (>8%)	50	79	95
↓ Serum protein (>25%)	208	96	91
↓ Serum albumin (>50%)	125	125	125
↑ Urinary protein (>400X)	42	10	26
↑ Kidney weight	17	10	10
Proteinuric nephropathy – minimal to mild	50	79	95
Proteinuric nephropathy – minimal to marked	42	32	26

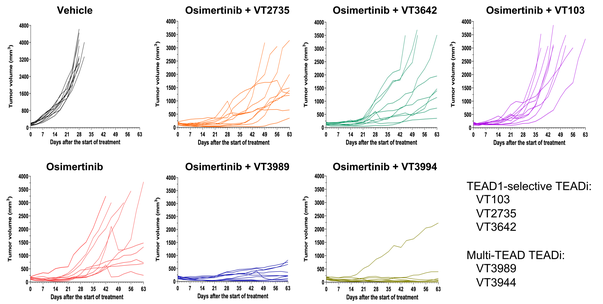
A. **B.**



Images of rat kidneys from 28-day female rat studies
(A) H&E images of control vehicle-treated rat kidney (left) and kidney from a female rat treated with VT103 at 0.6 mg/kg/day for 28 days (right). (B) EM images of control vehicle-treated rat kidney (left) and kidney from a female rat treated with VT103 at 0.4 mg/kg/day for 28 days (right). POD=podocyte cell body; PFE=podocyte foot process effacement (red oval); PFP=podocyte foot process (blue line arrow; GBM= glomerular basement membrane; FCE=fenestrated capillary endothelium; CAP=glomerular capillary; RBC=red blood cell.

TEAD1-selective TEAD inhibitors are less effective than TEAD inhibitors with broader TEAD selectivity in enhancing osimertinib efficacy and durability





TEAD1-selective TEADi:
VT103
VT2735
VT3642

Multi-TEAD TEADi:
VT3989
VT3944

TEADi and osimertinib combination study *in vivo*
EGFR inhibitor, osimertinib, was combined with TEAD1-selective or multi-/pan-TEAD TEAD inhibitors in NCI-H1975 EGFR mutant NSCLC CDX model. Treatments (>60 days) were continuous throughout the entire study.