IK-930, a TEAD Paralog Selective Inhibitor for Treating YAP/TAZ-TEAD Dependent Cancers

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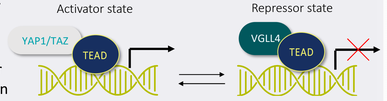
INTRODUCTION

- The Hippo pathway is frequently mutated in human cancers and mediates resistance to therapies targeting key oncogenic pathways
- The ultimate step of the Hippo pathway is mediated by the TEAD transcription factors which consist of four related paralogs TEAD1-4
- TEADs bound to YAP1 or TAZ *induce* expression of pro-growth and anti-apoptotic genes, whereas TEADs bound to VGLL4 suppress gene expression (Figure 1)
- Given the essential roles of the Hippo pathway in renal physiology, it may be beneficial to selectively target a TEAD paralog to minimize on-target toxicity, while maintaining anti-tumor efficacy
- Although highly homologous, the lipid-binding pocket of TEADs harbor some sequence divergence, highlighting an opportunity to design paralog-specific compounds
- IK-930 is a TEAD1 selective inhibitor that has demonstrated anti-tumor activity as a monotherapy in Hippo pathway mutated cancers and when combined with targeted therapies in multiple experimental models (see poster #3852)
- IK-930 is currently in Phase 1 development (NCT05228015)

Figure 1: TEADs function in two opposing states

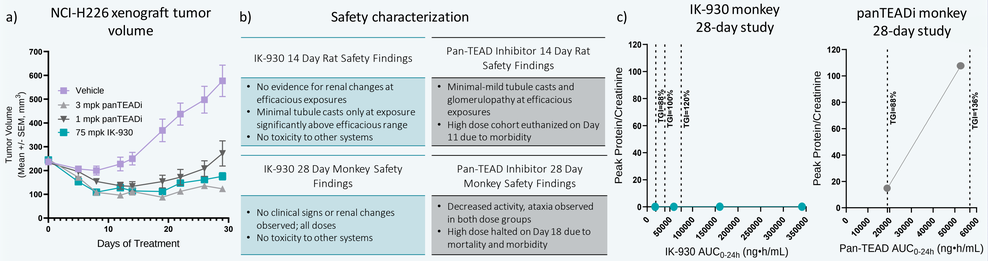
- TEAD binding partners determine its function:
  - Activator with YAP1 or TAZ (TEAD palmitoylation dependent)
  - Repressor with VGLL4 (palmitoylation independent)
    - VGLL4 repression is TEAD dependent (TEAD-binding deficient mutants do not repress transcription)
    - VGLL4 competes with YAP1/TAZ to inhibit transcription



(Chan P et al. Nat. Chem. Bio. 2016; Li Q et. Cell Stem Cell. 2020; Zhang W et al. Cell Res. 2014)

IK-930 has Superior Tolerability over panTEADi and Greater Therapeutic Window

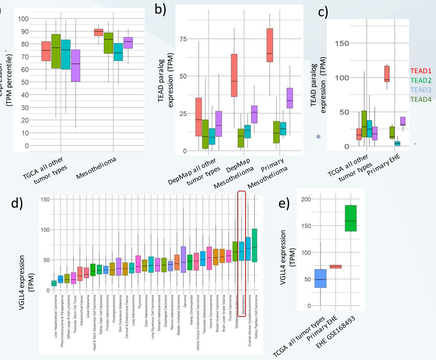
Figure 3: Renal safety/therapeutic window comparison of IK-930 versus panTEAD inhibitor



Anti-tumor activity in the NF2 deficient mesothelioma tumor NCI-H226 grown as a xenograft in mice (a). Morbidity and histopathology in rats and monkeys for IK-930 and panTEAD inhibitor (b). Average urinary protein-to-creatinine ratios relative to exposures in monkeys treated with IK-930 or a panTEAD inhibitor. Average IK-930 or panTEAD inhibitor exposures associated with different percent tumor growth inhibition (TGI) of NCI-H226 xenografts in mice (vertical line) (c).

TEAD1 and VGLL4 Highly Expressed in EHE & Mesothelioma

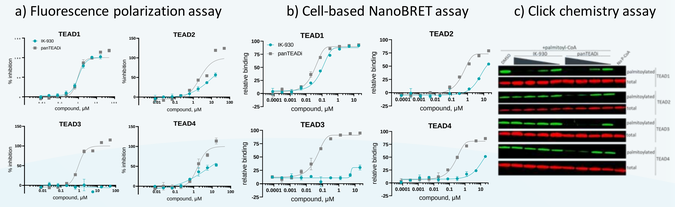
Figure 8: TEAD1 and VGLL4 are highly expressed in mesothelioma and EHE tumors



TEAD paralog expression in mesothelioma versus all other tumor types from TCGA (a). TEAD paralog expression in primary mesothelioma cell lines (n=19), mesothelioma cell lines in DepMap and all other cell lines in DepMap (b). TEAD paralog expression in EHE tumor samples (n=6) and all tumors in TCGA (c). VGLL4 expression by tumor type in TCGA (d). VGLL4 expression in EHE tumors samples (n=6), in EHE samples from GSE168493 (Seavey et al., Genes Development 2021) or in all tumors in TCGA (e).

IK-930 is a TEAD1 Selective Palmitoylation Inhibitor

Figure 2: IK-930 is a TEAD1 selective palmitoylation inhibitor

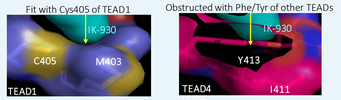


d) Summary of biochemical and cellular data

IK-930	TEAD1	TEAD2	TEAD3	TEAD4
FP (IC_{50} µM)	0.88 ± 0.22	9.23 ± 1.80	> 50	6.58 ± 0.93
Click/Chem(IC_{50} µM)	0.2-0.5	>20	>20	>20
Nanobret (IC_{50} µM)	0.091 ± .002	15.53 ± 1.32	> 20	> 20

panTEADi	TEAD1	TEAD2	TEAD3	TEAD4
FP (IC_{50} µM)	0.92 ± 0.25	2.29 ± 0.51	1.18 ± 0.52	1.38 ± 0.58
Click/Chem(IC_{50} µM)	0.2-0.5	2	0.5	2
Nanobret (IC_{50} µM)	0.030 ± .004	0.51 ± .022	0.041 ± .001	0.32 ± .081

e)

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TEAD1 C405
TEAD1 VVTNRDTQETLLCMACVFEVSNSEHGA 416
TEAD2 VVTNRDTQELLLCTAYVFEVSTSERGA 437
TEAD3 VVTSRDSQETLLVIAFVFEVSTSEHGA 425
TEAD4 VVTNRDTQETLLCIAYVFEVSASEHGA 424
                    TEAD4 Y413
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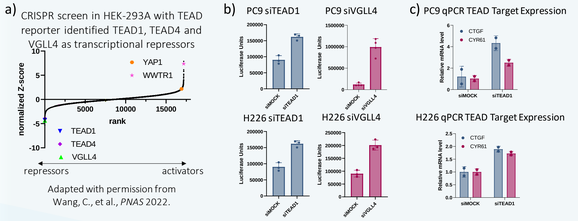
Binding of IK-930 or a panTEAD inhibitor to TEAD1-4 using a BODIPY-palmitate Fluorescence Polarization (FP) competition assay (a) or a NanoBRET assay (b). (c) Blocking of alkyl-palmitoyl-CoA binding by IK-930 or a panTEAD inhibitor to recombinant TEAD1-4 YAP1-binding domain using a click chemistry assay. (d) Table summarizing the relative potency of IK-930 or a panTEAD inhibitor in biochemical and cellular assays. (e) Structural representation of TEAD1 and TEAD4 palmitoylation pockets highlighting rationale for TEAD1 selective binding of IK-930.

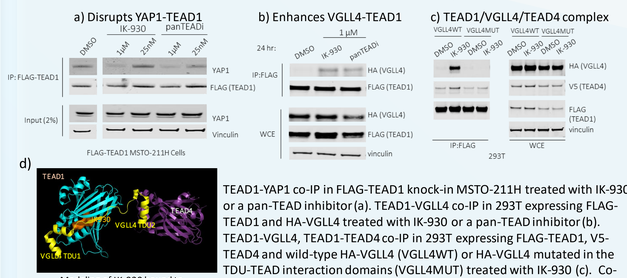
TEAD-VGLL4 Binding Functions as Transcriptional Repressor; IK-930 Promotes VGLL4 Binding to TEAD1

Figure 4: TEAD transcription factors can function as transcriptional repressors



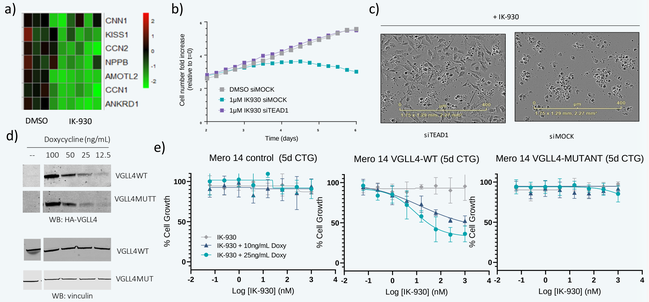
CRISPR screen in HEK-293A TEAD luciferase reporter (a). TEAD luciferase reporter in cells treated with TEAD1 and VGLL4 siRNAs (b). *CTGF* and *CYR61* expression in cells treated with TEAD1 siRNAs (c).

Figure 5: IK-930 disrupts YAP1/TEAD1 binding, stabilizes VGLL4/TEAD1 binding, and can form a TEAD1/VGLL4/TEAD4 heterotrimeric complex



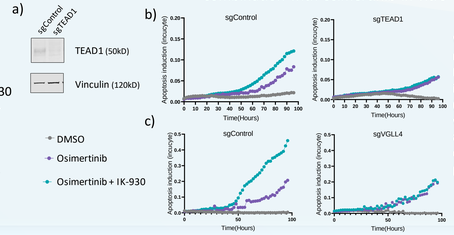
TEAD1-YAP1 co-IP in FLAG-TEAD1 knock-in MSTO-211H treated with IK-930 or a pan-TEAD inhibitor (a). TEAD1-VGLL4 co-IP in 293T expressing FLAG-TEAD1 and HA-VGLL4 treated with IK-930 or a pan-TEAD inhibitor (b). TEAD1-VGLL4, TEAD1-TEAD4 co-IP in 293T expressing FLAG-TEAD1, V5-TEAD4 and wild-type HA-VGLL4 (VGLL4WT) or HA-VGLL4 mutated in the TDU-TEAD interaction domains (VGLL4MUT) treated with IK-930 (c). Co-crystal structure of IK-930/TEAD1 modeled with VGLL4 and TEAD4 (d).

Figure 6: TEAD1 and VGLL4 drive anti-tumor activity of IK-930 in vitro in mesothelioma cell lines



TEAD target gene down regulation in primary mesothelioma cells treated with 2 IK-930 concentrations (3x) (a). Primary mesothelioma cell number change with TEAD1 siRNAs and/or IK-930 (b). Images showing differential effect of IK-930 +/- siTEAD1 (c). Western measuring doxycycline-induced expression of VGLL4WT or VGLL4MUT in Mero14 (d). IK-930 effect on growth of Mero14 expressing doxycycline induced VGLL4WT or VGLL4MUT (e).

Figure 7: TEAD1 and VGLL4 mediate anti-tumor activity of IK-930 in combination with osimertinib in vitro

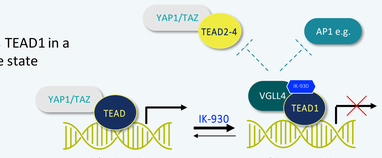


Western blot measuring CRISPR-mediated TEAD1 knockout in EGFR mutant PC9 (a). Apoptosis induction in PC9 sgTEAD1 cells (b) or sgVGLL4 cells (c) treated with osimertinib or osimertinib and IK-930 measured using a caspase-activated fluorescent probe.

IK-930 Rebalances TEAD1 Transcriptional Activity Towards a Repressive State

Figure 9: Schema representing TEAD activator and transcriptional repressive states in presence or absence of IK-930

- IK-930 preferentially keeps TEAD1 in a transcriptionally repressive state
- IK-930 / TEAD1 / VGLL4 complex potentially blocks chromatin access of other transcription factors



CONCLUSIONS

- IK-930 selectively inhibits TEAD1
- IK-930 achieved equivalent efficacy to panTEAD inhibitors with improved therapeutic index in preclinical species
- Preclinical data support that IK930 can be clinically tested to be dosed daily at efficacious exposures without significant toxicities
- IK-930 shifts the equilibrium of TEAD1 towards a repressive transcriptional state, by disrupting its interaction with YAP1 and enhancing its interaction with VGLL4
- TEAD1 is the most highly expressed paralog in mesothelioma and EHE tumors, VGLL4 is highly expressed in these tumors as well
- Collectively, these findings suggest that IK-930 drives TEAD1 into a repressive complex that functionally antagonizes pro-tumorigenic transcriptional machinery (Figure 9)