Abstract #7266: VGLL4 is the target of the 3p25 homozygous deletion and presents a novel therapeutic vulnerability for TEAD1/4 but not TEAD1 inhibitors

Florian Muller*, Eliot Behr#, Selvi Kunnimalaiyaan*, Parth Mangrolia*, Jill Olson* and Stephen Rubino*
*Sporos BioDiscoery, @JLABS Suite 201, 2450 Holcombe Blvd, Houston, TX 77021, USA; #Department of Cancer Biology; MD Anderson Cancer Center; Houston; TX

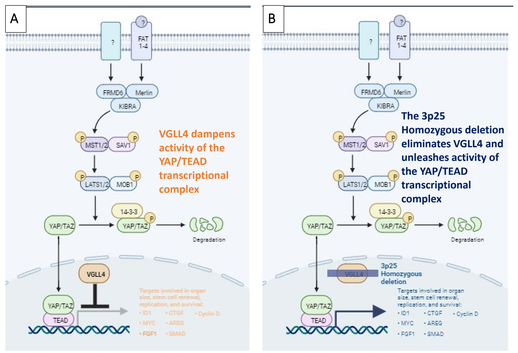
Poster #27

SPOROS BIODISCOVERY

Abstract

- The family of four TEAD transcription factors drive execution of the Hippo pathway and TEAD inhibitors have been developed which show strong anti-proliferative activity in vitro and in vivo but the genetic alterations that best identify responder populations have yet to be fully clarified.
- Sporos undertook a broad bioinformatic analysis of Hippo pathway components and regulatory genes to identify genetic alterations that drive TEAD activity and potentially act as targetable vulnerabilities.
- A key finding from this analysis is that the negative regulator of the YAP/TEAD complex; VGLL4; is the tumor suppressor target of the 3p25 locus homozygous deletion, which was previously thought to be VHL. VGLL4 is in the only gene in the peak of GISTIC significance in lung squamous cell carcinoma and VGLL4 and ATG7 are both in the peak in Renal Clear Cell Carcinoma (RCC) supporting VGLL4 deletion as a major oncogenic driver.
- Because VGLL4 is the major negative regulator of the YAP/TEAD transcriptional complex – we hypothesize that cancers with VGLL4-homozygous deletions would be YAP/TEAD hyperactive and sensitive to TEAD inhibitors.
- To test this hypothesis, we evaluated anti-tumor efficacy of TEAD inhibitors in a *VGLL4* homozygous deleted PDX (CrownBio KI2552; VHL-null; no other established driver mutations)
  - No anti-tumor activity observed with the TEAD1-isoform specific inhibitor VT103
  - *Strong anti-tumor activity including a >75% prolongation of survival with the TEAD1/4 inhibitor SPR1*
- Conclusion: *VGLL4*-homozygous deletions present a previously unrecognized driver alteration that can expose sensitivity to TEAD1/4 but not TEAD1-specific inhibitors

VGLL4 is a major negative regulator of the oncogenic YAP/TAZ-TEAD transcriptional complex

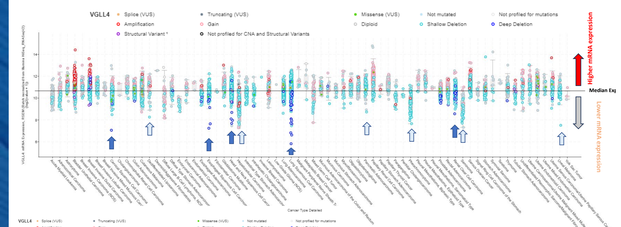


- The co-activators YAP/TAZ associate with the TEAD transcription factors to drive expression of genes that promote cancer through stimulation of cell proliferation; de-differentiation and cell survival
- The wingless co-activators (VGLL1-4) also associate with TEADs but instead drive expression of a differentiation transcriptional program and directly compete with YAP/TAZ for binding to TEAD - antagonizing Hippo pathway oncogenic activity
- GEMM studies show that *VGLL4* knockout directly antagonizes YAP1-driven proliferation

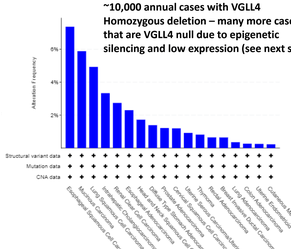
=> So far no studies have reported *VGLL4* homozygous deletion in human tumors

Recurrent homozygous deletions and low expression of VGLL4 in TCGA solid tumors

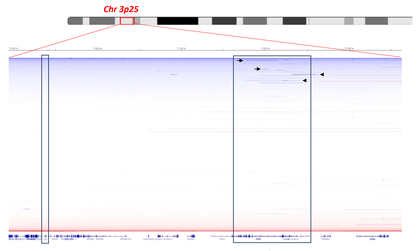
Recurrent homozygous deletions and low expression of *VGLL4* in squamous cell carcinomas

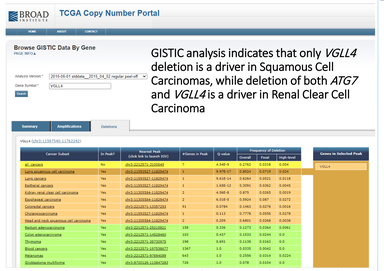


~10,000 annual cases with VGLL4 Homozygous deletion – many more cases that are VGLL4 null due to epigenetic silencing and low expression (see next slide)



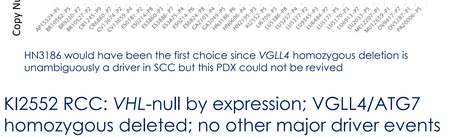
Homozygous deletions at the 3p25 locus center on *VGLL4* and *ATG7* - not *VHL*



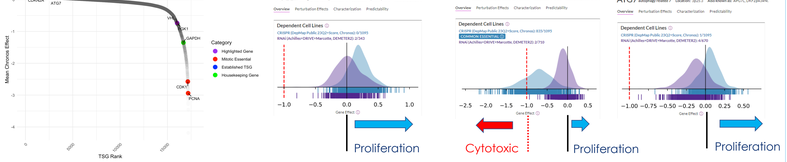


GISTIC analysis indicates that only *VGLL4* deletion is a driver in Squamous Cell Carcinomas, while deletion of both *ATG7* and *VGLL4* is a driver in Renal Clear Cell Carcinoma

***VGLL4* is among the most frequently deleted genes in Lung Squamous Cell Carcinoma**



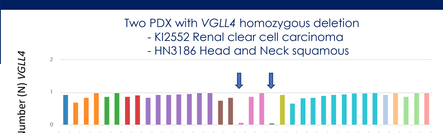
VGLL4 ranks amongst the strongest tumor suppressor genes in DepMap



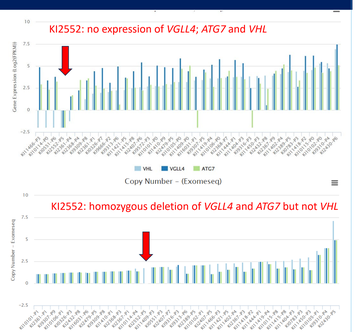
Gene	TCGA Primary Tumors: Mutations (Hot-spot)	TCGA Primary Tumors: OncoPrint Deep Deletions	TCGA Primary Tumors: ASCAT Homozygous Deletions (Focal)	CrownBio PDX: Homozygous Deletions (of 2548)	DepMap Cell Lines (23Q2): Median CRISPR Score Chronos	DepMap Cell Lines (23Q2): Dependent cell lines (out of 1095)
VHL	207 (58)	28	6 (1)	0	-0.75	835
ATG7	120 (0)	74	35 (30)	13	0.08	1
VGLL4	66 (0)	80	37 (32)	2	0.24	0

- *VHL* CRISPR deletion is cytotoxic in most cancer cell lines
- *ATG7* CRISPR deletion is fitness neutral
- *VGLL4* CRISPR deletion is fitness enhancing in most cancer cell line

KI2552: A PDX model with *VGLL4*-homozygous deletion in the CrownBio collection



HN3186 would have been the first choice since *VGLL4* homozygous deletion is unambiguously a driver in SCC but this PDX could not be revived



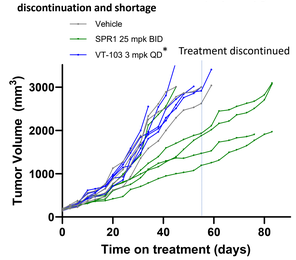
KI2552 RCC: *VHL*-null by expression; VGLL4/ATG7 homozygous deleted; no other major driver events

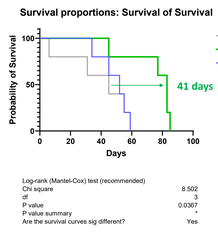
Model	Mut	CN
KI2552	EPHA2:p.G391R; MET:p.T1010I; ARFGEF1:p.G1137R; CNOT1:p.F1575L; TSC1:c.1029+2T>A; MGA:p.L2823V; BCR:p.D1106N; FRG1:p.S154G; FRG1B:p.A32T	DDX5:Amp; AFF4:Amp; CLTC:Amp

A PDX with a *VGLL4*-homozygous deletion responds to a TEAD1/4 but not a TEAD1 inhibitor

Experiment #1: Treatment with the TEAD1/4 inhibitor SPR1 delays tumor growth and extends survival of the KI2552 PDX but treatment with the TEAD1 inhibitor VT103 does not

Encouraging result (doubling of survival) especially given less than optimal experiment – due to drug discontinuation and shortage



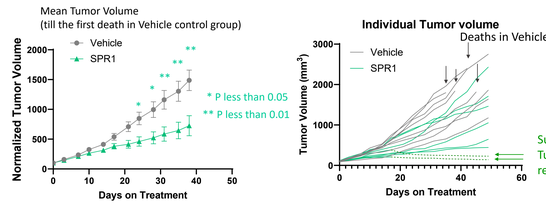


Log-rank (Mantel-Cox) test (recommended)

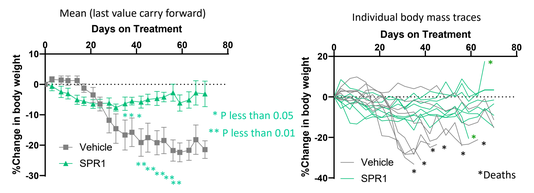
Chi square	8.502
df	3
P value	0.0367
P value summary	*
Are the survival curves sig different?	Yes

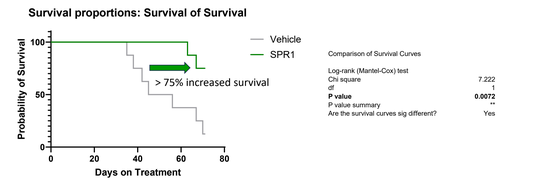
* VT103 and SPR1 were dosed to provide equal exposure (AUC) which yields the same anti-tumor efficacy in the TEAD1-dependent NCI-H226 xenograft model

Experiment #2: The TEAD1/4 inhibitor SPR1 slows tumor growth, attenuates cachexia, and dramatically extends survival



N = 8 +/- SEM SPR1 25 mg/kg PO BID





Comparison of Survival Curves	
Log-rank (Mantel-Cox) test	
Chi square	7.222
df	1
P value	**0.0072**
P value summary	**
Are the survival curves sig different?	Yes

Conclusion: The homozygous deletion of *VGLL4* is a previously unrecognized driver event in [squamous] carcinomas that may serve as a patient selection marker for TEAD1/4 but not TEAD1 inhibitors.

References: The puzzling case of the 3p25 homozygous deletion: the target is not *VHL* but *VGLL4* and *ATG7* doi: https://doi.org/10.1101/2023.11.06.565014

Acknowledgements
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