Experimental ‘loss-of function’ annotation of STK11 mutations with prognostic and therapeutic implications

Abstract #5584

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Introduction

Loss of function (LOF) mutations in Serine Threonine Kinase 11 (STK11) occur in 15% of lung adenocarcinoma and have been shown to drive resistance to immune checkpoint blockade clinically, as well as in preclinical models. Though STK11 is commonly inactivated in human cancer with strong implications for treatment outcomes, few STK11 mutations identified from tumor samples have been functionally characterized.

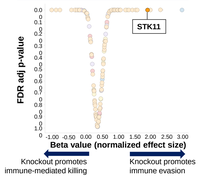
TNG260 is an inhibitor of CoREST that is currently being investigated in combination with pembrolizumab for the treatment of STK11-mutant cancer (NCT05887492). Patients are eligible for enrollment in the TNG260 phase 1/2 trial if their tumor contains a deleterious STK11 mutation. To begin classifying non-annotated variants, over 2,000 distinct mutations in the STK11 gene were identified from STK11 literature or public repositories of tumor sequencing data such as AACR Project GENIE and ClinVar. Where possible, loss-of-function annotations were captured from literature or predictive tools such as PolyPhen-2. However, many STK11 variants, particularly missense mutations, have never been functionally characterized.

We developed a functional screening approach to characterize STK11 alterations using the lung adenocarcinoma cell line A549. A549 cells contain homozygous loss of STK11 via a truncation mutation at Q37, and re-expression of wild-type STK11 in these cells strongly impairs their growth in vitro and in vivo. We created a library of STK11 variant cDNAs, each containing a unique barcode. This library was expressed in A549, and cells were maintained in vitro or in vivo to allow for positive selection of STK11 loss-of-function variants and depletion of variants that behave like wild-type STK11. At the end of the screen, variants were quantified by NGS using each mutant cDNA’s unique barcode and compared to well-annotated controls.

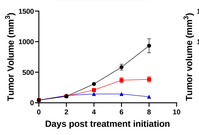
These data were assembled to generate TNG260mutationfinder.com -- the first website to curate STK11 variants with functional annotations.

STK11 deficiency causes resistance to immune checkpoint blockade

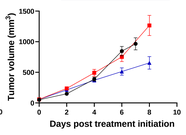
In vivo CRISPR screen identifies STK11 as driver of α-PD1 resistance



MC38 - Control



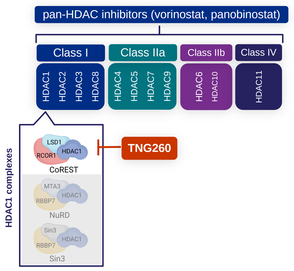
MC38 - STK11 Deficient



Immune deficient
Immune competent
Immune competent + α-PD1

STK11-mutated lung adenocarcinoma patients do not respond as well to immune checkpoint blockade as STK11 wild-type

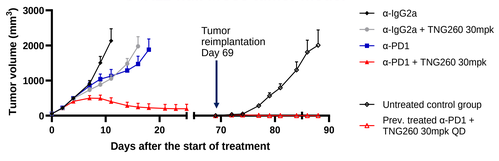
TNG260 is a CoREST complex inhibitor that reverses α-PD1 resistance caused by loss of STK11



TNG260 is CoREST-selective

		CoREST	NCoR	NuRD	Sin3
Pan	Vorinostat	0.06	0.53	0.13	0.16
Class I	Tucidinostat	0.12	0.20	1.40	>100
	Domatinostat	0.09	0.19	30.0	>100
CoREST	TNG260	0.17	>100	>100	>100

STK11-null MC38 tumor model

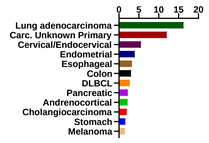


5/8 mice had complete tumor regression by day 34

5/5 mice with complete regression rejected tumor reimplantation

Verifying STK11 loss-of-function status is critical for determining the preferred approach for patient treatment

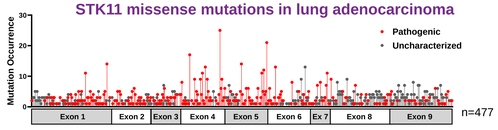
% Frequency of STK11 mutations



STK11 variants in lung adenocarcinoma

Variant Type	Frequency
Missense	22%
Nonsense	20.8%
Frameshift (indel)	31.1%
Splicing	7.7%
Deletion	17%
Rearrangement	1.4%

STK11 missense mutations in lung adenocarcinoma



Existing computational algorithms do not agree on pathogenicity of STK11 missense mutations identified from the literature

Alpha Missense Pathogenicity score v. Ensemble score

Design of a functional enrichment screen to annotate patient-derived STK11 mutations for TNG260 clinical trial

Category	# of cDNAs
EGFP	3
STK11 wild-type	3
Benign mutants	3
Annotated pathogenic mutants	20
Mutants with conflicting data	15
Truncations near end of sequence	3
Pathogenic computational score, but no supporting data	252

A549 3D Culture

Cells expressing STK11 LOF mutations will enrich

A549 Xenografts

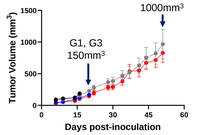
Cells expressing STK11 LOF mutations will enrich

STK11 Enrichment Screen

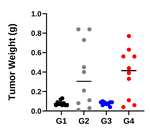
Cells with LOF variants will outcompete and enrich compared to benign variants

STK11 functional screen performed in 3D culture and xenografts

Xenograft Tumor Volume



Xenograft Tumor Weight

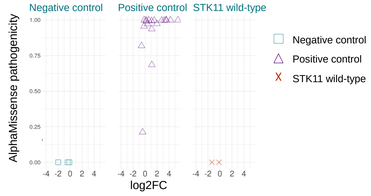


G1 - 150mm³ (10mil cells/mouse)
G2 - 1000mm³ (10mil cells/mouse)
G3 - 150mm³ (1mil cells/mouse)
G4 - 1000mm³ (1mil cells/mouse)

3D in vitro samples were collected at day 0, day 7 and day 14 of growth in 3D culture. In vivo xenograft samples were collected at 150mm³ and 1000mm³, representing “early” and “late” timepoints, respectively. Samples were prepared for NGS and sequenced for a unique barcode representing each STK11 variant.

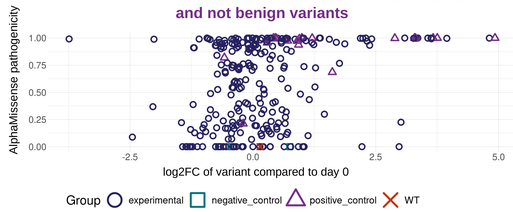
Functional screen enriches true loss-of-function STK11 mutations over benign variants

Functional screen enriches positive control loss of function variants compared to benign variants or wild-type STK11



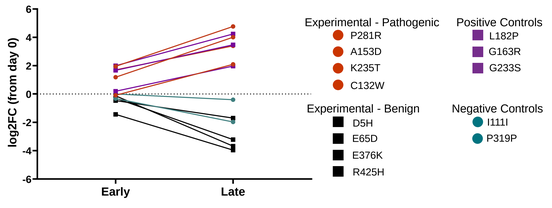
Each STK11 variant was compared to day 0 to determine the log2 fold change (log2FC) of each variant. Log2FC values that exceed the WT and benign controls indicate pathogenic variants. Pathogenicity scores from each missense mutation were determined by AlphaMissense and plotted against log2FC.

Screen enriches for true loss of function STK11 variants and not benign variants



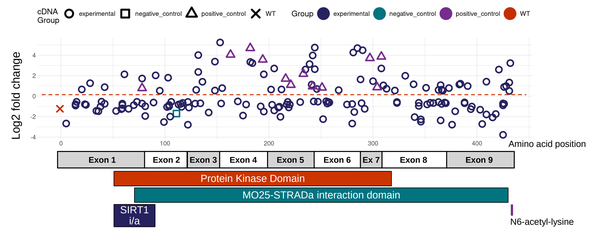
Each STK11 variant was compared to day 0 to determine the log2 fold change (log2FC) of each variant. Pathogenic variants are those with log2FC values that exceed wild-type STK11 and negative controls. Pathogenicity scores from each missense mutation were determined by AlphaMissense and plotted against log2FC.

Change in STK11 mutation frequency over time



Log2 fold change in representative STK11 variants between “early” and “late” timepoints. Early tumors were collected at 150mm³, and late timepoint tumors were collected at 1000mm³. Positive controls enriched during the screen, while negative controls were static or depleted. Experimental mutations were pathogenic if log2FC values increased over time, while experimental mutations were benign if they stayed static or depleted over time.

Annotation of novel pathogenic missense mutations in STK11



Each STK11 variant was compared to day 0 to determine its log2 fold change (log2FC). Each variant’s log2FC was plotted by amino acid sequence of STK11, shown with corresponding domains. Variants that fall above the red dotted line are pathogenic.

Benign Mutations		Pathogenic Mutations	
R405W	E376K	L290P	C132R
K81N	R409P	T244P	I300N
K122R	K175R	L285P	F148S
R415L	V34F	C132W	K235T
I99T	A205P	A153D	L182R
L252M	L105S	G242E	G61S
Y340H	G346S	P281R	
D5H	E65D	L184P	
P280L	C418Y	T244N	
D53N	G346V	W308R	
R425H		G288V	

TNG260mutationfinder.com, a searchable database of 2600 STK11 loss-of-function variants identified from patient tumors

TNG260 Mutation Finder

Summary

- **Tumors with STK11 loss of function are resistant to immune checkpoint blockade**
- **STK11 is altered in 15% of NSCLC, but STK11 missense mutations are poorly annotated**
- **The functional screen identified LOF mutations that had low pathogenicity scores, and vice versa – indicating that functional validation of in silico analyses are necessary for STK11 missense variants**
- **Data was assembled to generate TNG260mutationfinder.com, a repository of patient-derived STK11 alterations with pathogenic or benign calls**
- **Annotation of STK11 is critical for identifying patients with true STK11 LOF mutations which can help inform treatment options**
- **STK11 mutation is one criterion used to inform patient eligibility for NCT05887492, a clinical trial studying TNG260 + pembrolizumab in patients with STK11-mutated solid tumors**

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TCGA Research Network: www.cancer.gov/tcga