Abstract #1254

Predictive Models for Tumor Cell Targeting with Plinabulin, Derived from *In Vitro* Screening and Affymetrix mRNA Expression Data

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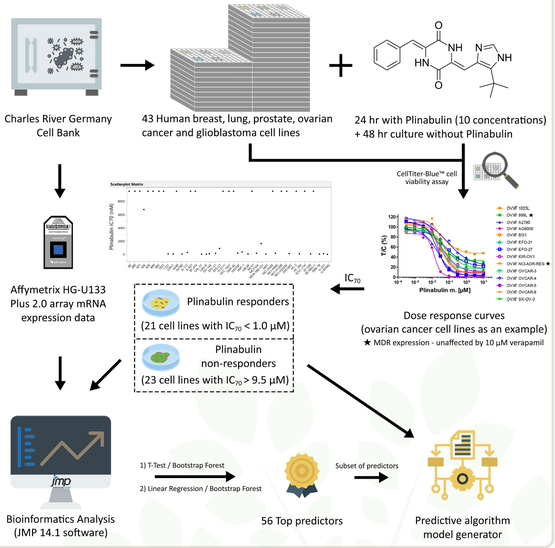
Experimental flow chart



Aim and methods

Aim

Tubulin binding drugs are approved for the treatment of many cancer types, without the use of molecular markers to select patients likely to respond. Plinabulin binds β-tubulin at a differentiated site and is being tested in a Phase 3 clinical study for the treatment of NSCLC. Additional cancer indications are being considered for plinabulin and an algorithm for selecting especially sensitive cancers and patient subgroups would be of significant value.

Summary of Methods

Affymetrix HG-U133 Plus 2.0 array mRNA expression data for 43 human breast, lung, prostate, ovarian or CNS cancer cell lines was utilized to develop mathematical models to predict *in vitro* plinabulin potency against the same cell lines. To characterize potency, cells were treated for only 24 hours with plinabulin and then cultured for another 48 hours without plinabulin. Viable cell number was then measured with a Cell Titer-Blue Assay, and the plinabulin concentration causing a 70% reduction in signal (IC_{70}) versus vehicle treated controls was derived. Cell lines were clearly separable into plinabulin Active (21 cell lines with IC_{70} < 1.0 μM) and Inactive (IC_{70} > 9.5 μM) groups. Log2 transformed Affymetrix gene probeset signal values, preprocessed with the GeneChip robust multi-array average analysis algorithm, were then used to select the top predictor probesets, utilizing JMP 14.1 statistical software.

TTest-Predictor Method: The probesets ranked among the top 200 predictors with a bootstrap forest partitioning technique were compared by t-test in Active versus Inactive tumor cell lines. For those reaching $p < 0.05$, the annotated genes for these probesets, if available, were noted. Next, all of the probesets in the array that are mapped to the same noted genes were identified. Jetset scoring methods to assess each probeset for specificity, splice isoform coverage, and robustness against transcript degradation have been shown to be valuable tools in assessing the value of each probeset, in particular correlating with protein expression (Li et al., 2011). At this point therefore, the probeset with the highest Jetset score that mapped to each noted gene, with a p value < 0.01 for Active versus Inactive values, was selected. In addition, probesets without a mapped gene, with a p value < 0.01 for plinabulin Active versus Inactive values, were also selected. 40 top probesets (HITs) were selected in this manner.

Correlation-Predictor Method: Probesets with correlation $p < 0.01$ versus plinabulin IC_{70} were ranked for their ability to predict plinabulin Active versus Inactive 3 times. For those with an average rank < 50 (low score = high rank) and not already picked up with the TTest-Predictor method above, the gene annotation was evaluated. 16 "HIT" probesets that were non-annotated or had the highest Jetset score for each identified gene, and had differential expression between plinabulin Active versus Inactive cell lines ($p < 0.01$), were selected.

The 56 HITs were ranked 4 times as predictors. Models (mathematical algorithms) were constructed from the 56 HIT gene probesets in JMP to identify plinabulin responding cell lines, utilizing either one-layer TanH multimode fit neural networks or binary logistic regression.

Top predictors for Plinabulin response

Avg. Rank	Gene Symbol	△Active	Biology/Function
1.00	CALD1	↑	Caldesmon; calmodulin/actin binding; Regulate cell morphology and motility
2.50	UBXN8 (REP8/UBXD6)	↑	p97 ATPase complex cofactor; Lipid droplet biogenesis; Misfolded proteins repair
2.50	CDCA5	↓	Sororin; Sister chromatid cohesion, separation
5.75	Unannotated	↑	
6.50	ERI1	↑	RNA exonuclease; Histone mRNA degradation; rRNA processing
7.25	SEC14L1P1	↑	SEC14 like 1 pseudogene 1
7.25	SECISBP2L (SLAN)	↑	SECIS Binding Protein 2; Centrosome maturation; Retard proliferation, inhibit Aurora A
7.50	WDR20	↓	USP12-UAF1 deubiquitinating enzyme complex; Tumor suppressor inhibiting ERK & AKT
8.50	LGR5	↑	GPCR with ligand R-spondin; Intestinal stem cell activation; Wnt signaling
10.75	ADIPOR2	↓	Adiponectin receptor protein 2; Insulin resistance or hepatic fat accumulation
11.00	RUFY2	↑	RUN and FYVE domain containing 2; Detected in brain, lung, testis
11.25	COL5A2	↑	Fibrillar forming collagen; High expression in bladder cancer, breast cancer, CRC
12.25	YTHDC2	↑	Oncogene induced by c-Jun, ATF2; Regulate mRNA transcripts
14.75	RPL12	↓	Bind to 26S ribosomal RNA; Regulate translation interacting with eEF1A and eEF2
18.50	MTMR9	↑	Myotubularin-related protein; Obesity, hypertension; PtdIns(3)P regulation
18.50	Unannotated	↑	
18.50	Unannotated	↑	
18.75	TM9SF3	↑	Bind to β-adrenergic ligands; Tumor invasion; Transporter in spermatogenesis
21.00	CALB2	↓	Calretinin; calcium-binding; Ca^{2+} homeostasis; Apoptosis; Cell adhesion, cell growth
21.25	Unannotated	↑	
21.25	WDR92	↓	Protein with 2 WD40 repeat domains; Modulator of apoptosis and dynein assembly
22.75	DGUOK	↓	Deoxyguanosine Kinase; Hypercerebral mitochondrial DNA depletion syndrome
23.00	CTNNB1	↑	β-catenin; Wnt signaling; Cell adhesion and gene transcription; Oncogene
23.25	FKBP4	↓	Immunophilin protein family; Enhance progestin receptor-mediated transcription
23.25	BRPF3	↓	Histone H3 acetyltransferase activity; DNA replication and H3K14 acetylation (S phase)
23.50	DENND2D	↓	GEF; Regulate Rab9a/b GTPases and lysosome distribution
26.00	TMEM47	↑	Regulate epithelial cell junction; Associate actomyosin structures, cell morphology
27.25	RPS19	↓	Pre-rRNA processing (40S subunits)

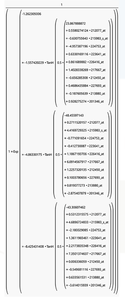
Avg. Rank	Gene Symbol	△Active	Biology/Function
28.00	Unannotated	↑	
29.75	AUP1	↓	Lipid droplet clustering → VLDL↑; Degradation of misfold ER proteins
30.50	ZFX	↑	Transcriptional coactivator; Cell proliferation; Apoptosis
34.75	MRPL30	↑	Mitochondrial ribosomal protein (39S subunit); Mitochondrial translation
35.25	TRAK1	↑	Mitochondrial motility, trafficking
35.50	RCCD1	↓	Histone demethylase activity; Regulate microtubule stability; Cell cycle repression
36.50	Unannotated	↑	
37.25	ZMAT3 (WIG1)	↑	TP53-dependent pathway; Cell cycle arrest; MYC-N activation; Tumor suppressor
37.75	GEMIN7	↓	Part of pre-mRNA splicing complex (survival of motor neuron complex)
38.00	ZNF106 (ZFP106)	↑	Sensory and motor neuron maintenance; mitochondrial electron transport chain
39.00	Unannotated	↑	
39.25	GLT8D1	↑	Glycosyltransferase family; Schizophrenia risk gene
42.25	CASC4	↑	Splicing; Upregulated in HER-2/Neu overexpressed cell line; Breast & Ovarian Cancer
42.50	FAM98B	↑	tRNA-splicing ligase complex; Correlated with PRMT1 (arginine methyltransferase)
42.75	NME1-NME2	↓	Nucleoside diphosphate kinase; DNA cleavage; DNA-binding transcription factors
44.00	HOOK3	↑	Microtubule-dependent intracellular vesicle & protein trafficking; Centrosome assembly
44.50	CSTF3 (CSTF77)	↑	Polyadenylation, pre-mRNA cleavage
44.50	ACTR3	↑	Component of ARP2/3 complex; Formation of the mitotic actin cluster
45.50	Unannotated	↑	
46.00	RPL38	↓	60S ribosomal protein; Regulate axial skeletal patterning (Hox protein) in mice
46.75	Unannotated	↑	
46.75	PLOD1	↑	Collagen fibrils cross-linking; Specify ECM facilitating organs & tissues morphogenesis
48.25	MRAS	↑	GTPase in TNF-α, MAPK signaling; Regulate RAF kinase activation
49.25	ZNF441	↑	Transcriptional regulation; Downregulated in ERG high tumors
51.00	RELB	↓	NF-κB subunit; Classic DC development and myelopoiesis
54.75	NLE1	↓	Notch activity; Axial skeletal formation in mice model
54.75	Unannotated	↓	
55.50	MRPS23	↓	Mitochondrial ribosomal protein (28S subunit); Cancer cell proliferation and metastasis

Predictive model development

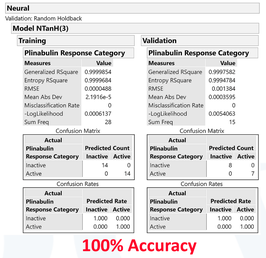
JMP 14.1 software

Model Validation

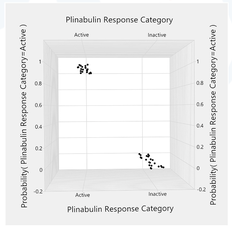
Model Generation



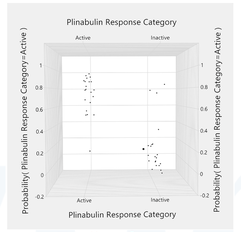
Model 1



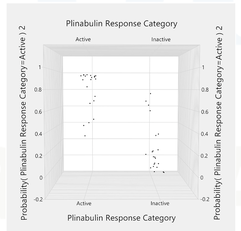
100% Accuracy



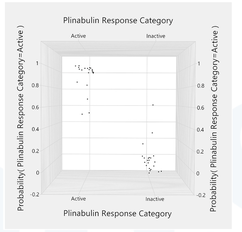
Model 1: Top 10 probesets



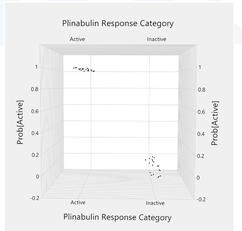
Top 2 probesets



Bottom 10 probesets



Top 5 probesets



Logistic regression
(using CALD1, SLAN, UBXN8, CDCA5, AUP1)

Plinabulin activity for different cancer cell lines

Cancer Type	Plinabulin Activity	43 Cell Lines Tested for Predictive Model Development	Additional Cell Lines Tested
CNS	Active	A-172, SF-539, SNB-78, U-251, U-87MG	LN-229, SF-268
CNS	Inactive		M059K (IC_{70} > 10 μM, IC_{50} = 87 nM)
Lung	Active	Calu-6, LXFL1121, NCI-H460	
Lung	Inactive	LXFA289, LXFA526, LXFA629, LXFA983, A549, HOP-62, NCI-H322M, NCI-H226, SK-MES-1, A427, NCI-H1299, H2171, SCLC-21H, LXFE66NL	
Breast	Active	CAL-51, HS578T, JIMT-1, MCF10A, MX1	
Breast	Inactive	MAXFTN401, BT-474, HCC-1937, MCF7, MDA-MB-231	
Ovarian	Active	A2780, EFO-21, EFO-27, OVCAR3	
Ovarian	Inactive	OVXF899, OVXF1023	
Prostate	Active	22Rv1, DU-145, LNCaP, PC-3M	
Prostate	Inactive	PC-3	

Model 1 predicted all 3 to be active

Predictors of Plinabulin activity grouped by biological/cellular function

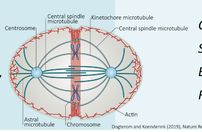
Mitosis

Actin / Myosin

CALD1
CALB2
CTNNB1
TMEM47
ACTR3

Mitotic spindle

CDCA5
SECISBP2L/SLAN
BRPF3
RCCD1



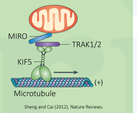
mRNA regulation

ERI1	*YTHDC2*	*FKBP4*
ZFX	*ZMAT3 (WIG1)*	*CASC4*
FAM98B	*NME1-NME2*	*CSTF3 (CSTF77)*
ZNF441	*RELB*	

Organelle transport

TM9SF3
WDR92
TRAK1

DGUOK
DENND2D
HOOK3



Ribosomal function

40S subunit	60S subunit	Mitochondrial
RPS10	*RPL12* #	MRPL30 #
RPS19 #	*RPL38* #	MRPS23 #
RACK1	*RPL13P5*	
	RPL18	
	RPL8	

★ All 40S/60S subunits are lower in Plinabulin responding cell lines

Among 56 top predictors

Conclusion

Predictive models:

- Models have been developed that predict the ability of plinabulin to target cancer cells *in vitro* with high confidence utilizing mRNA expression data

Future steps:

- Test algorithms for the ability to predict plinabulin activity *in vitro* with additional cancer cell lines, as well as plinabulin *in vivo* activity
- Utilize top predictors to better understand plinabulin's MOA

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