Assessing combinations of chemotherapy agents in Alveolar Soft Part Sarcoma cell lines

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THE SUNSHINE PROJECT
FUNDED BY THE PEDIATRIC CANCER FOUNDATION
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Introduction

Alveolar Soft Part Sarcoma (ASPS) is a slow growing, cheomotherapy refractory, frequently metastasizing and rare soft tissue sarcoma subtype occurring mainly in children and young adults.
ASPS is characterized by an unbalanced translocation between the ASPSCR1 gene (involved in glucose transport) and the TFE3 gene (a transcription factor).

Methods

1. **Selected FDA approved or clinically evaluated agents incorporating human pharmacokinetic parameters**
2. **Test agents at clinically achievable concentrations and durations across two cell lines (ASPS-1 and ASPS-KY) harboring ASPSCR1:TFE3.**
3. **CellTiter-Glo at 72 hrs post treatment.**
4. **Select most active agents**
5. **Test 2 drug combinations**
6. **Additional testing of combinations using 3D spheroid models of ASPS.**

Single Agent Activity

Epigenetic		TKI		Conventional	
5-Azacitadine	Methylation	Dasatinib	SRC	Pemetrexed	Anti-folate
Belinostat	HDAC	Axitinib	VEGF1/2, PDGFRB, kit	Temozolomide	Alkylator
(+)-JQ1	BRD(BET family) inhibitor	Sorafenib	VEGF2/3. PDGFRB, FLT3, BRAF, R	GSK923295A	CENP-E inhibitor
Romidepsin	HDACi (class I, II)	Pazopanib	VEGF1/2/3, PDGFRAB, FGF1/3, L	Gemcitabine	Nucleoside analogue
Entinostat	HDAC	Crizotinib	ALK/met	SN-38	Topo I
EPZ5676	DOT1L inhibitor	Cabozantinib	AXL, FLT3, KIT, MET, RET, TIE-2,	Ixabepilone	Microtubule
EPZ6438	EZH2 inhibitor	Vandetanib	VEGF2, EGF, RET	Doxorubicin	DNA, topo II
HCI2509	LSD1 inhibitor	Regorafenib	VEGF2, Tie-2	Etoposide	Topo II
Panobinostat	HDACi	**Tivantinib (ARQ197)**	c-Met	Palifosfamide	Alkylator
		Cediranib (AZD2171)	TKI	Docetaxel	Microtubule
Metabolic		**Other**		**RAS**	
Pioglitazone	Insulin sensitizer	Triapine	RnR inhibitor; Fe chelator	Everolimus	mTOR
Na Dichloroacetate	Activates oxidative metab	17-AAG (Tanespimyci	HSP90	Temsirolimus	mTOR (binds FKBP-12)
Metformin	AMPK?, Glucose	Plerixafor 8HCl	CXCR4	BKM120	PI3K inhibitor
Perhexiline	inhibits carnitine channel	Carfilzomib	Proteosome	Trametanib	MEK
Simvastatin	Statin	Birinapant	SMAC mimetic/IAP	Perifosine	AKT inhibitor
		Selinexor (KPT-330)	CRM1 inhibitor	SAR245408 (XL147	PI3K (&MEK/ERK?)
Cell Cycle		Omacetaxine	Protein Synthesis	BEZ235	PI3K and mTOR inhibitor
AZD1775 (MK1775)	Wee1	PF-03084014	γ-secretase (Notch)		
Dinaciclib (SCH727965	CDK1,2,5,9	TRC102	Base-Excision-Repair Inhibitor		
		Vismodegib	Hedgehog pathway, PgP		
		Alisertib (MLN8237)	Aurora kinase inhibitor		

Table 1. Agents Tested. All agents were tested at Cmax, 20% Cmax, and 4% Cmax in DMEM with 15% FBS.

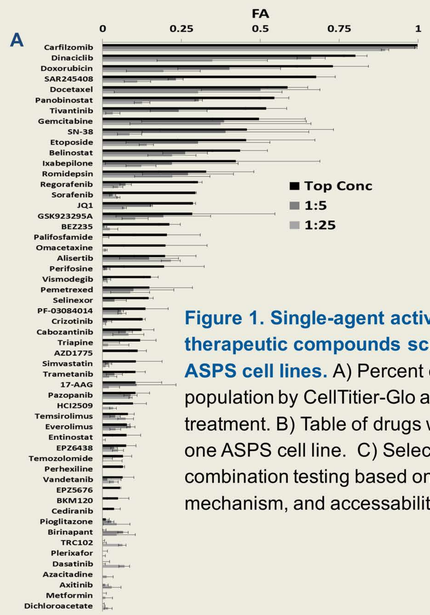

Figure 1. Single-agent activities of 54 therapeutic compounds screened against 2 ASPS cell lines. A) Percent of affected cell population by CellTitier-Glo at 72 hrs post drug treatment. B) Table of drugs with FA>30% in at least one ASPS cell line. C) Selected 8 agents for combination testing based on activity, diversity of mechanism, and accessability

B

		CellTiter-Glo Assay - Affected Population (%)								
		Average			ASPS-1			ASPS-ky		
Top (ng/ml)	Drug Name	Top Conc	1:5	1:25	Top Conc	1:5	1:25	Top Conc	1:5	1:25
4232	Carfilzomib	99.7	99.3	89.5	99.8	99.7	90.7	99.6	98.9	88.3
525	Dinaciclib	80.2	66.1	34.75	76.6	61.6	17.3	83.8	70.6	52.2
3880	Doxorubicin	73	40.15	19.3	61.7	23.9	7.6	84.3	56.4	31
13900	SAR245408	67.7	23.2	11.05	61.7	25.6	15.2	73.7	20.8	6.9
2000	Docetaxel	58.6	50.1	30.3	52.2	31.3	3.7	65	68.9	56.9
21	Panobinostat	54.5	30.5	12.5	50	29.3	10	59	31.7	15
1459	Tivantinib	51.85	24.1	3.25	45.3	15.1	1	58.4	33.1	5.5
41000	Gemcitabine	49.55	38.5	37.4	34.8	12.4	8.7	64.3	64.6	66.1
30	SN-38	45.7	38.95	8.55	18.2	12.5	4.7	73.2	65.4	12.4
20000	Etoposide	45.55	30.25	13.9	23.8	7.4	11.6	67.3	53.1	16.2
32124	Belinostat	43.6	26.15	21.95	34.9	19.1	14.3	52.3	33.2	29.6
100	Ixabepilone	42.2	21.95	12.25	15.5	0.9	7.5	68.9	43	17
377	Romidepsin	32.8	27.05	22.1	17.7	12.6	10.2	47.9	41.5	34
7120	GSK923295A	28.45	19.3	10.35	2.3	4.3	6.2	54.6	34.3	14.5
10000	Palifosfamide	20.45	0	0	9.9	0	0	31	2	0
96	Omacetaxine	19.9	0	1.1	6.7	0	1.4	33.1	0	0.8
6000	Perifosine	19.5	1.4	0	32.2	2.3	0	6.8	0.5	0

C

Agent	Mechanism/Target	cMax (ng/ml)	IC50 (ng/ml) Single Agent Study ASPS-KY	Single Agent Study ASPS-1	5x5 Studies ASPS-KY	5x5 Studies ASPS-1	*ASPS-KY* Average	*ASPS-KY* StdErr	*ASPS-1* Average	*ASPS-1* StdErr	% of cMax ASPS-KY	% of cMax ASPS-1	Agent
Belinostat	*HDACi*	**32124**	452	433	600	306	526	*74*	370	*63*	**1.6**	**1.2**	**Belinostat**
Dinaciclib	*CDK1,2,5,9*	**525**	6.8	35.5	14.8	49.3	10.8	*4.0*	42.4	*6.9*	**2.1**	**8.1**	**Dinaciclib**
Doxorubicin	*DNA, topo II*	**3880**	372	3700	396	5610	384	*12*	4660	*955*	**9.9**	**120**	**Doxorubicin**
Gemcitabine	*Nucleoside analogue*	**41000**	22	977000	37	1380000	29.2	*7.5*	1180000	*202000*	**0.071**	**2878**	**Gemcitabine**
SAR245408	*PI3K/MEK/ERK*	**13900**	163000	229000	94000	166000	129000	*34500*	198000	*31500*	**928**	**1424**	**SAR245408**
Selinexor	*CRM1 inhibitor*	**621**	4540	4600	3740	2590	4140	*400*	3600	*1010*	**667**	**580**	**Selinexor**
SN38	*Topo I*	**30**	2.39	845.5	1.00	3800	1.70	*0.70*	2330	*1480*	**5.7**	**7767**	**SN38**
Tivantinib	*c-Met*	**1459**	317	2120	822	4570	569	*253*	3350	*1230*	**39**	**230**	**Tivantinib**

Combination Synergy Analysis

Table 2. Top combination results for ASPS and their FA and CI values by cell line.

Chou and Talalay Synergy Calculations:

CI is calculated using the isobologram equation for mutually nonexclusive drugs with different modes of action:
$CI = (D)_1/(Dx)_1 + (D)_2/(Dx)_2 + (D)_1(D)_2/(Dx)_1(Dx)_2$,

$(Dx)_1$ and $(Dx)_2$ are concentrations for drug 1 and drug 2 alone that provide x% inhibition; $(D)_1$ and $(D)_2$ are concentrations of drug 1 and drug 2 in combination with x% inhibition

CI	Description
<0.1	Very strong synergism
0.1-0.3	Strong synergism
0.3-0.7	Synergism
0.7-0.85	Moderate synergism
0.85-0.90	Slight synergism
0.90-1.10	Nearly additive
1.10-1.20	Slight antagonism
1.20-1.45	Moderate antagonism
1.45-3.3	Antagonism

Fraction Affected (FA) > 0.75 in either ASPS-KY or ASPS-1									
Agent		Target/Mechanism		ASPS-KY ng/ml		ASPS-1 ng/ml		ASPS-KY		ASPS-1	
Tx1	Tx2	Tx1	Tx2	Tx1	Tx2	Tx1	Tx2	FA	CI	FA	CI
Belinostat	**Dinaciclib**	HDACi	CDK1,2,5,9	900	60	1200	100	**0.707**	*1.84*	**0.880**	*0.85*
Belinostat	**Dinaciclib**	HDACi	CDK1,2,5,9	900	30	1200	50	**0.711**	*1.19*	**0.822**	*1.23*
Belinostat	**Dinaciclib**	HDACi	CDK1,2,5,9	900	3.75	1200	6.25	**0.399**	*3.91*	**0.770**	*1.51*
Belinostat	**Dinaciclib**	HDACi	CDK1,2,5,9	450	60	600	100	**0.736**	*1.36*	**0.863**	*0.61*
Belinostat	**Dinaciclib**	HDACi	CDK1,2,5,9	450	30	600	50	**0.710**	*0.91*	**0.762**	*1.03*
Belinostat	**Dinaciclib**	HDACi	CDK1,2,5,9	225	60	300	100	**0.689**	*1.51*	**0.836**	*0.53*
Belinostat	**Dinaciclib**	HDACi	CDK1,2,5,9	113	60	150	100	**0.693**	*1.41*	**0.827**	*0.44*
Belinostat	**Dinaciclib**	HDACi	CDK1,2,5,9	56.5	60	75	100	**0.678**	*1.45*	**0.794**	*0.48*
Belinostat	**Doxorubicin**	HDACi	DNA, topo II	900	150	1200	238	**0.679**	*0.74*	**0.792**	*1.33*
Belinostat	**Doxorubicin**	HDACi	DNA, topo II	113	2400	150	3800	**0.780**	*0.13*	**0.717**	*0.93*
Belinostat	**Gemcitabine**	HDACi	Nucleoside analogue	900	75	1200	41000	**0.633**	*1.07*	**0.911**	*0.48*
Belinostat	**Gemcitabine**	HDACi	Nucleoside analogue	900	37.5	1200	20500	**0.625**	*1.06*	**0.864**	*0.78*
Belinostat	**Gemcitabine**	HDACi	Nucleoside analogue	900	18.8	1200	10300	**0.620**	*1.06*	**0.844**	*0.91*
Belinostat	**Gemcitabine**	HDACi	Nucleoside analogue	900	9.4	1200	5150	**0.601**	*1.16*	**0.824**	*1.06*
Belinostat	**Gemcitabine**	HDACi	Nucleoside analogue	900	4.7	1200	2580	**0.597**	*1.18*	**0.812**	*1.14*
Belinostat	**Gemcitabine**	HDACi	Nucleoside analogue	450	75	600	41000	**0.435**	*2.44*	**0.757**	*0.80*
Belinostat	**SAR245408**	HDACi	PI3K/MEK/ERK	900	13000	1200	13000	**0.597**	*1.24*	**0.881**	*0.68*
Belinostat	**SAR245408**	HDACi	PI3K/MEK/ERK	900	6500	1200	6500	**0.553**	*1.56*	**0.880**	*0.68*
Belinostat	**SAR245408**	HDACi	PI3K/MEK/ERK	900	815	1200	815	**0.570**	*1.38*	**0.872**	*0.72*
Belinostat	**Selinexor**	HDACi	CRM1 inhibitor	900	620	1200	620	**0.616**	*1.40*	**0.812**	*1.39*
Belinostat	**Selinexor**	HDACi	CRM1 inhibitor	900	310	1200	310	**0.613**	*1.24*	**0.798**	*1.38*
Belinostat	**Selinexor**	HDACi	CRM1 inhibitor	900	155	1200	155	**0.570**	*1.47*	**0.786**	*1.41*
Belinostat	**Selinexor**	HDACi	CRM1 inhibitor	900	77.5	1200	77.5	**0.539**	*1.69*	**0.780**	*1.43*
Belinostat	**SN38**	HDACi	Topo I	900	15	1200	30	**0.640**	*2.07*	**0.857**	*0.82*
Belinostat	**SN38**	HDACi	Topo I	900	7.5	1200	15	**0.596**	*2.34*	**0.826**	*1.04*
Belinostat	**SN38**	HDACi	Topo I	900	3.75	1200	7.5	**0.564**	*2.37*	**0.805**	*1.20*
Belinostat	**SN38**	HDACi	Topo I	900	1.88	1200	3.75	**0.576**	*1.71*	**0.777**	*1.42*
Belinostat	**SN38**	HDACi	Topo I	900	0.94	1200	1.88	**0.526**	*2.19*	**0.774**	*1.45*
Belinostat	**Tivantinib**	HDACi	c-Met	900	363	1200	363	**0.716**	*0.82*	**0.766**	*1.53*
Belinostat	**Tivantinib**	HDACi	c-Met	900	182	1200	182	**0.669**	*0.91*	**0.752**	*1.64*
Belinostat	**Tivantinib**	HDACi	c-Met	900	91	1200	91	**0.660**	*0.88*	**0.777**	*1.42*
Dinaciclib	**Doxorubicin**	CDK1,2,5,9	DNA, topo II	30	2400	50	3800	**0.844**	*0.36*	**0.738**	*0.94*
Dinaciclib	**Doxorubicin**	CDK1,2,5,9	DNA, topo II	30	1200	50	1900	**0.822**	*0.40*	**0.662**	*0.89*
Dinaciclib	**Doxorubicin**	CDK1,2,5,9	DNA, topo II	30	600	50	950	**0.809**	*0.42*	**0.614**	*0.87*
Dinaciclib	**Doxorubicin**	CDK1,2,5,9	DNA, topo II	30	300	50	475	**0.779**	*0.48*	**0.569**	*0.93*
Dinaciclib	**Doxorubicin**	CDK1,2,5,9	DNA, topo II	15	2400	25	3800	**0.809**	*0.25*	**0.550**	*1.63*
Dinaciclib	**SAR245408**	CDK1,2,5,9	PI3K/MEK/ERK	60	3250	100	3250	**0.736**	*1.13*	**0.757**	*0.54*
Dinaciclib	**Selinexor**	CDK1,2,5,9	CRM1 inhibitor	60	310	100	310	**0.744**	*1.23*	**0.813**	*0.47*
Dinaciclib	**Selinexor**	CDK1,2,5,9	CRM1 inhibitor	60	155	100	155	**0.749**	*1.13*	**0.816**	*0.40*
Dinaciclib	**Selinexor**	CDK1,2,5,9	CRM1 inhibitor	60	77.5	100	77.5	**0.739**	*1.15*	**0.812**	*0.38*
Dinaciclib	**Selinexor**	CDK1,2,5,9	CRM1 inhibitor	30	620	50	620	**0.752**	*0.79*	**0.651**	*0.84*
Gemcitabine	**Doxorubicin**	Nucleoside analogue	DNA, topo II	75	2400	41000	3800	**0.757**	*0.17*	**0.599**	*1.03*
Gemcitabine	**Doxorubicin**	Nucleoside analogue	DNA, topo II	37.5	2400	20500	3800	**0.771**	*0.12*	**0.550**	*1.19*
SN38	**Dinaciclib**	Topo I	CDK1,2,5,9	7.5	30	15	50	**0.766**	*0.56*	**0.527**	*0.98*
SN38	**Dinaciclib**	Topo I	CDK1,2,5,9	3.75	30	7.5	50	**0.754**	*0.56*	**0.569**	*0.79*
Tivantinib	**Dinaciclib**	c-Met	CDK1,2,5,9	1450	30	1450	50	**0.784**	*1.30*	**0.488**	*1.51*
Tivantinib	**Dinaciclib**	c-Met	CDK1,2,5,9	725	30	725	50	**0.752**	*1.00*	**0.507**	*1.23*
Tivantinib	**Dinaciclib**	c-Met	CDK1,2,5,9	363	30	363	100	**0.727**	*1.43*	**0.797**	*0.40*
Tivantinib	**Dinaciclib**	c-Met	CDK1,2,5,9	182	60	182	100	**0.721**	*1.33*	**0.795**	*0.40*
Tivantinib	**Dinaciclib**	c-Met	CDK1,2,5,9	91	60	91	100	**0.714**	*1.29*	**0.791**	*0.42*

3D Spheroid Synergy Analysis

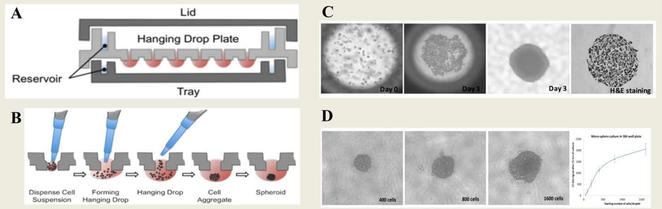

Figure 2. 3D Spheroid culture used for rapid combinatorial screening. Biomatrix Perfecta3D hanging drop plates were combined with Precision XS automated pipeting stations to generate spheroids and for drug application during combination screening. A) Hanging drop plate schematic. B) Spheriod formation schematic. C) ASPS cell line spheroid formation. H&E staining of 4μM cross-section slice at culture day 3. D) Spheroids generated from different starting cell density, CT-Glo assay can be used to detect differences in the cell number of spheroids.

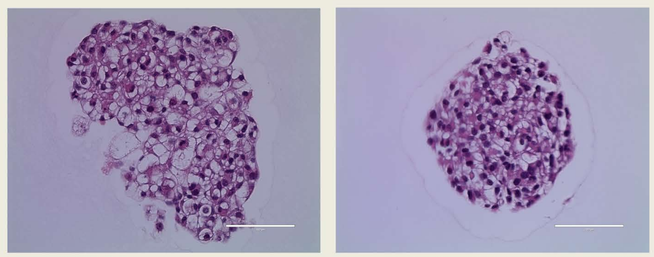

Figure 3. Different morphology of ASPS cell lines display in the 3D spheroid culture. H&E staining of 4 μM slices for A) ASPS-1 and B) ASPS-KY shows different cellular density within the spheroids.

A

ASPS-1		Belinostat: (1:2)				
FA		31.3	62.5	125	250	500
Doxorubicin (1:2)	313	0.35	0.36	0.56	0.97	0.90
	625	0.44	0.40	0.26	0.88	0.88
	1250	0.22	0.29	0.21	0.79	0.80
	2500	0.42	0.28	0.23	0.33	0.80
	5000	0.51	0.41	0.38	0.48	0.82

ASPS-KY		Belinostat: (1:2)				
FA		31.3	62.5	125	250	500
Doxorubicin (1:2)	313	0.04	0.27	0.70	0.21	0.80
	625	0.48	0.84	0.51	0.49	0.63
	1250	0.67	0.69	0.67	0.57	0.69
	2500	0.46	0.83	0.67	0.66	0.75
	5000	0.45	0.81	0.90	0.70	0.75

ASPS-1		Belinostat: (1:2)				
CI		31.3	62.5	125	250	500
Doxorubicin (1:2)	313	0.624	1.1	0.81	0.038	0.327
	625	0.416	0.915	3.96	0.2	0.396
	1250	1.89	1.9	5.87	0.445	0.845
	2500	0.631	2.37	5.69	5.55	0.827
	5000	0.487	1.3	2.6	2.71	0.71

ASPS-KY		Belinostat: (1:2)				
CI		31.3	62.5	125	250	500
Doxorubicin (1:2)	313	7040	92.5	4.9	638	6.65
	625	9.79	0.552	24.6	52.6	34
	1250	2.24	3.15	6.48	30.3	20.6
	2500	21.4	0.733	7.15	14.9	11.8
	5000	39.3	1.07	0.447	11.2	12.4

B

ASPS-1		Belinostat: (1:2)				
FA		31.3	62.5	125	250	500
Tivantinib	125	0.97	0.54	0.86	0.54	0.59
	250	0.51	0.69	0.71	0.66	0.51
	500	0.34	0.52	0.75	0.60	0.57
	1000	0.35	0.29	0.40	0.60	0.55
	2000	0.54	0.48	0.57	0.42	0.58

ASPS-1		Belinostat: (1:2)				
CI		31.3	62.5	125	250	500
Tivantinib	125	0.0048	0.446	0.121	1.76	2.81
	250	0.289	0.206	0.38	0.969	4.13
	500	3.07	0.558	0.293	1.35	3.03
	1000	4.31	18.1	3	1.35	2.97
	2000	0.365	1.08	0.858	5.05	3.59

ASPS-KY		Belinostat: (1:2)				
FA		31.3	62.5	125	250	500
Tivantinib	125	0.78	0.49	0.52	0.81	0.63
	250	0.71	0.70	0.32	0.61	0.61
	500	0.83	0.64	0.66	0.66	0.58
	1000	0.61	0.70	0.59	0.45	0.53
	2000	0.61	0.74	0.65	0.52	0.52

ASPS-KY		Belinostat: (1:2)				
CI		31.3	62.5	125	250	500
Tivantinib	125	0.524	12.6	20.7	2.88	35.2
	250	1.14	2.34	101	20.4	38.8
	500	0.344	0.502	7.13	13.7	52.5
	1000	3.48	2.78	12.6	73.6	78.9
	2000	4.55	2.23	8.63	44	84.3

Figure 4. 3D spheroid synergy analysis of promising 2-drug combinations. A) Belinostat + Doxorubicin; B) Belinostat + Tivatinib

Conclusions/Future work

- **Our combination screening identified several promising drug combinations for alveolar soft-part sarcoma.**
- **3D spheroid cultures in 384-well format can be combined with automated pipetting station to perform combination drug screens in 5x5 matrix format.**
- **Additional testing of combinations *in vitro* are ongoing and we would propose confirmatory *ex vivo* and *in vivo* experiments towards a clinical trial.**

Acknowledgement

- **Funding provided by the Pediatric Cancer Foundation and the V Foundation.**