Ng-3 (K103), Ng-4 (B119), Ng-5 (B116), Ng-6 (B117), Ng-7 (K202), Ng-10 (K105), Ng-22 (B105), OF-2 (B118), OF-3 (B112), OF-7(B101)

THE EFFECT OF 10 COMPOUNDS ON THE VIABILITY AND PROLIFERATION OF HT-29, COLO 205, C2BBe1 (CaCo2 CLONE), MPanc-96 & HFF CELL LINES - SCREENING ASSAY

Study No: IDL/006/CTX

Technical Report

Submitted to:
Idealiza Ltd.

May 26, 2009

DATA PAGE

Protocol Signed by the Study Director:	May 7, 2009
Initiation of Study:	May 10, 2009
Completion of Study:	May 14, 2009
HBI Study No.:	IDL/006/CTX
Archive Location:	Harlan Biotech Israel Kiryat Weizmann, Bldg. 13B Rehovot 76326, Israel
Sponsor:	Idealiza Ltd. Israel
Sponsor Monitor:	Dr. Michael Kutushov
Testing Facility:	Harlan Biotech Israel Kiryat Weizmann, Bldg. 13B Rehovot 76326, Israel

Study Director: _____ _____
R. Uhlmann, B. Sc Date

Total No. of Pages: 10

1. OBJECTIVE

The objective of this study is to assess the effect of 10 Test Items on the viability and proliferation of 5 different cell lines using the Alamar Blue detector.

2. TEST MATERIALS

2.1 Test Items:

Test Item No.	Test Item	Physical State	Batch Number	Molecular Weigh/ Concentration	Supplied By	Storage Conditions	Expiry Date
1	Ng-3	Powder	K103	612.84	Idealiza	RT	NA
2	Ng-4	Powder	B119	290.32	Idealiza	RT	NA
3	Ng-5	Powder	B116	318.87	Idealiza	RT	NA
4	Ng-6	Powder	B117	305.83	Idealiza	RT	NA
5	Ng-7	Powder	K202	319.86	Idealiza	RT	NA
6	Ng-10	Powder	K105	578.37	Idealiza	RT	NA
7	Ng-22	Powder	B105	369.94	Idealiza	RT	NA
8	OF-2	Powder	B118	407.99	Idealiza	RT	NA
9	OF-3	Powder	B112	927.0	Idealiza	RT	NA
10	OF-7	Powder	B101	479.02	Idealiza	RT	NA

2.2 Reference Item:

Name: **Doxorubicin**
Catalog No.: LO1DB
Lot. No.: 713474
Physical state: Liquid
Supplied by: EBEWE Pharm
Concentration: 2 mg/ml
Storage conditions: 2-8^{o}C
Sterility: Non-Sterile
Expiry Date: November 2011

2.3 Adjunct Materials:

2.3.1 Name: **AlamarBlueT^{M}**
Catalog no. BUF012B
Batch No.: 030908B
Physical state: Liquid
Supplied by: Serotec
Storage conditions: 5±3^{o}C
Expiry Date: September 2009

2.3.2	Name:	**DMSO**
	Batch No.:	078K0692
	Catalog No.:	D-5879
	Supplied by:	Sigma
	Physical state:	Liquid
	Storage conditions:	Room temperature
	Expiry Date:	August 2010

2.4 Culture Growth Medium:

2.4.1	Name:	**Growth Medium**[(a)]
	Batch:	300409
	Content:	DMEM supplemented with 10% FBS (Fetal Bovine Serum), 2mM glutamine, 1mM Na pyruvat, 1mM non-essential amino acid, 1mM Penicillin/Streptomycin
	Components supplied by:	Biological Industries, Israel
	Storage conditions:	5±3°C
	Expiry Date:	300509

2.5 Samples Preparation:

Test Items were dissolved in 100% DMSO to obtain stock solutions of 10 mM. Since Test Item #4 – Ng 6 did not dissolved properly, full dissolving was achieved using water bath sonicator for about 5 minutes. Stock solutions were diluted ×500 with Growth Medium to achieve working solutions of 20µM. The Vehicle Control solution contains 0.2% DMSO in Growth Medium.

2.6 Test System

Five different cell lines were used in this test:
H-29 - Human colorectal adenocarcinoma, ATCC # HTB-38
COLO 205 - Human colorectal adenocarcinoma, ATCC # CCL-222
C2BBe1 (clone of CaCo2) – Human colorectal adenocarcinoma, ATCC # CRL-2102
MPank-96 – Human pancreatic adenocarcinoma, ATCC # CRL -2380
HFF - Human foreskin fibroblast (BJ), ATCC # CRL -2522

2.7 Test procedure:

Cells were thawed and passed at least once. Exponentially growing cultures of each cell line were harvested, centrifuged, counted and seeded each at the density of 5000 cells/100µl/ well in pre-warmed Growth Medium in 4 rows of 96-well tissue culture plates. For the 5 cell lines a total of 3 plates were used (plates 1-3). An additional plate (plate 4) with one row of each cell line was seeded to serve as Time 0 (T_0). The Plates were incubated until the next day at 37±1°C, 5±0.5% CO_2 and 95±5% humidity, to enable cells adherence to the well.

At the following day the following procedures were performed:

Plate 4- T_0 plate: 100µl of fresh Growth Medium were added to each well followed by the addition of 22µl AlamarBlue™. The fluorescent signal (Excitation

(a) The same Growth Medium was used for the five Cell Lines

544nm/Emission 590nm) was measured following an appropriate incubation time (4 hours and 24 hours).

Plates 1-3 – treatment plates: 100µl of the various treatment solutions were added to the cells according to the following plate plan. During treatments addition, on plate 3 seeded with BJ (HFF) cells, TI-2 was added by mistake on TI-1. The treatments were immediately removed, the cells were washed twice with Growth Medium and TI-1 treatment was added properly.

Plate 1-3 – Test Items:

	1	2	3	4	5	6	7	8	9	10	11	12	
A	TI-1	TI-2	TI-3	TI-4	TI-5	TI-6	TI-7	TI-8	TI-9	TI-10	RI	VC	1st Cell Line
B													
C													
D													
E	TI-1	TI-2	TI-3	TI-4	TI-5	TI-6	TI-7	TI-8	TI-9	TI-10	RI	VC	2nd Cell Line
F													
G													
H													

Plate 5 – Blank: In an additional plate, individual wells were filled with 100µL/well Growth Medium and 100µL/well of the different treatments (8 wells each). This plate served as fluorescent signal blank.

Following 48 hours of incubation, 22µL/well of AlamarBlue™ were added to plates 1-3 and plate 5 and the fluorescent signal (Excitation 544nm/Emission 590nm) was measured following an appropriate incubation time (4 hours and 24 hours).

3. DATA EVALUATION

3.1 Signals from blank wells (plate 5) were averaged for each treatment and subtracted from the treatments optical density (O.D.) values.

3.2 Average optical density (O.D.) was calculated for each set of replicate wells.

3.3 Cell growth was expressed as (percentage):

a) Percent of t_0:

$$\frac{\text{Averaged O.D} \times 100}{\text{Averaged O.D at } t_0}$$

b) Percent of Control:

$$\frac{(\text{Averaged O.D} - \text{to}) \times 100}{(\text{Averaged O.D Vehicle} - \text{to})}$$

Negative value (<0%) indicates cytotoxic effect

Positive value (0-99%) indicates cytostatic effect.

3.4 Viability (%) was expressed as:

$$\frac{\text{Averaged O.D (treatment)} \times 100}{\text{Averaged O.D (Vehicle)}}$$

4. RESULTS:

The effect of 10 Test Items on the viability and proliferation of 5 different cell lines was evaluated using the AlamarBlue Assay.

Table 1

The effect of Test Items on the viability and proliferation of 5 different cell lines

Test Item No.	Test Item Name	Cell Line	O.D (544/590nm) Av ±Std'	Percent of t_0 (%)	Percent of Control (%)	Viability (%)	Effect
1	Ng-3	**HT-29**	**198.43** *± 13.04*	119.82	177.70	107.80	proliferative
		COLO205	**204.83** *± 8.15*	108.21	63.76	95.87	cytostatic
		C2BBe1	**284.66** *± 9.62*	161.40	77.67	90.14	cytostatic
		MPanc	**213.29** *± 4.18*	148.52	98.16	99.39	cytostatic
		BJ (HFF)	**451.92** *± 9.60*	141.16	93.43	97.99	cytostatic
2	Ng-4	**HT-29**	**123.04** *± 2.12*	74.30	-230.38	66.84	cytotoxic
		COLO205	**210.95** *± 3.16*	111.44	88.84	98.73	cytostatic
		C2BBe1	**235.65** *± 3.26*	133.61	42.52	74.62	cytostatic
		MPanc	**201.24** *± 8.81*	140.14	81.19	93.78	cytostatic
		BJ (HFF)	**438.14** *± 12.96*	136.85	83.66	95.00	cytostatic
3	Ng-5	**HT-29**	**189.02** *± 37.26*	114.14	126.77	102.69	proliferative
		COLO205	**233.68** *± 9.42*	123.45	182.12	109.37	proliferative
		C2BBe1	**141.85** *± 11.55*	80.43	-24.76	44.92	cytotoxic
		MPanc	**184.11** *± 11.57*	128.20	57.05	85.79	cytostatic
		BJ (HFF)	**423.11** *± 3.99*	132.16	73.00	91.74	cytostatic
4	Ng-6	**HT-29**	**75.05** *± 3.47*	45.32	-490.21	40.77	cytotoxic
		COLO205	**7.92** *± 1.54*	4.18	-744.16	3.71	cytotoxic
		C2BBe1	**16.36** *± 1.41*	9.27	-114.77	5.18	cytotoxic
		MPanc	**109.42** *± 5.38*	76.20	-48.15	50.99	cytotoxic
		BJ (HFF)	**5.52** *± 2.70*	1.73	-223.10	1.20	cytotoxic

Test Item No.	Test Item Name	Cell Line	O.D (544/590nm) Av ±Std'	Percent of t_0 (%)	Percent of Control (%)	Viability (%)	Effect
5	Ng-7	**HT-29**	**137.18** *± 8.19*	82.84	-153.83	74.53	cytotoxic
		COLO205	**87.00** *± 1.91*	45.96	-419.69	40.72	cytotoxic
		C2BBe1	**94.34** *± 4.99*	53.49	-58.84	29.87	cytotoxic
		MPanc	**139.80** *± 5.34*	97.35	-5.36	65.15	cytotoxic
		BJ (HFF)	**211.06** *± 5.96*	65.92	-77.36	45.76	cytotoxic
6	Ng-10	**HT-29**	**86.53** *± 3.31*	52.25	-428.04	47.01	cytotoxic
		COLO205	**69.82** *± 3.06*	36.88	-490.20	32.68	cytotoxic
		C2BBe1	**89.07** *± 2.86*	50.50	-62.62	28.20	cytotoxic
		MPanc	**142.73** *± 6.92*	99.39	-1.23	66.51	cytotoxic
		BJ (HFF)	**79.03** *± 3.36*	24.68	-170.98	17.14	cytotoxic
7	Ng-22	**HT-29**	**94.95** *± 6.07*	57.34	-382.46	51.58	cytotoxic
		COLO205	**45.38** *± 2.51*	23.97	-590.45	21.24	cytotoxic
		C2BBe1	**66.55** *± 4.45*	37.73	-78.77	21.07	cytotoxic
		MPanc	**144.05** *± 10.91*	100.31	0.62	67.12	cytostatic
		BJ (HFF)	**0.70** *± 0.21*	0.22	-226.52	0.15	cytotoxic
8	OF-2	**HT-29**	**40.43** *± 3.17*	24.42	-677.58	21.97	cytotoxic
		COLO205	**7.40** *± 0.68*	3.91	-746.27	3.46	cytotoxic
		C2BBe1	**28.97** *± 3.15*	16.43	-105.72	9.17	cytotoxic
		MPanc	**105.85** *± 4.67*	73.71	-53.18	49.33	cytotoxic
		BJ (HFF)	**2.81** *± 0.24*	0.88	-225.02	0.61	cytotoxic

Test Item No.	Test Item Name	Cell Line	O.D (544/590nm) Av ±Std'	Percent of t_0 (%)	Percent of Control (%)	Viability (%)	Effect
9	OF-3	**HT-29**	**49.89** *± 3.01*	30.13	-626.39	27.10	cytotoxic
		COLO205	**9.20** *± 0.88*	4.86	-738.91	4.30	cytotoxic
		C2BBe1	**10.27** *± 1.25*	5.82	-119.14	3.25	cytotoxic
		MPanc	**155.47** *± 9.78*	108.26	16.72	72.45	cytostatic
		BJ (HFF)	**74.36** *± 1.93*	23.23	-174.29	16.12	cytotoxic
10	OF-7	**HT-29**	**155.57** *± 16.69*	93.95	-54.28	84.52	cytotoxic
		COLO205	**20.48** *± 1.29*	10.82	-692.63	9.58	cytotoxic
		C2BBe1	**113.91** *± 6.04*	64.58	-44.80	36.07	cytotoxic
		MPanc	**239.21** *± 16.16*	166.57	134.67	111.47	proliferative
		BJ (HFF)	**306.14** *± 24.62*	95.62	-9.94	66.38	cytotoxic
RI	Doxorubicin	**HT-29**	**103.44** *± 5.58*	62.46	-336.52	56.19	cytotoxic
		COLO205	**149.62** *± 3.78*	79.04	-162.78	70.02	cytotoxic
		C2BBe1	**117.65** *± 3.65*	66.71	-42.12	37.26	cytotoxic
		MPanc	**199.63** *± 6.13*	139.01	78.92	93.03	cytostatic
		BJ (HFF)	**207.97** *± 10.79*	64.96	-79.55	45.09	cytotoxic

Figure 1

The effect of Test Items as percent of t_0

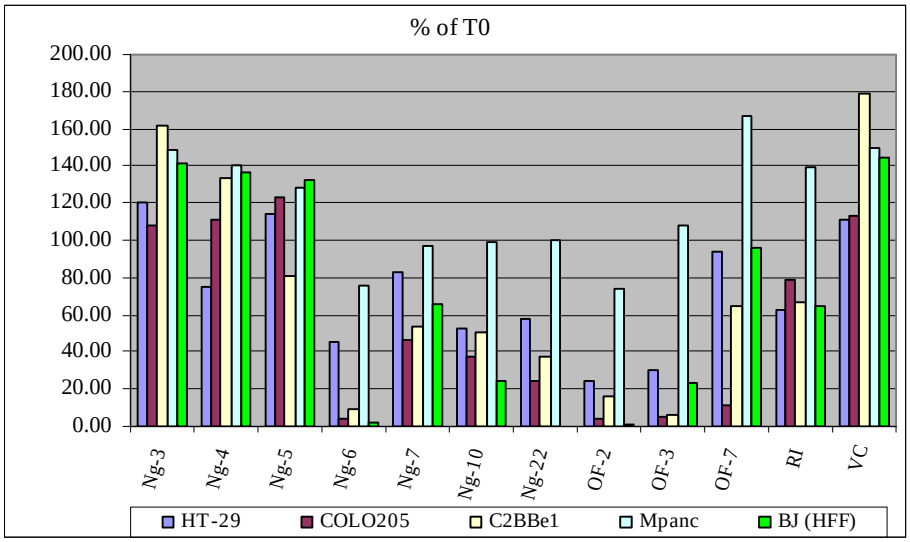


Figure 2

The effect of Test Items as percent of Vehicle Control (VC)

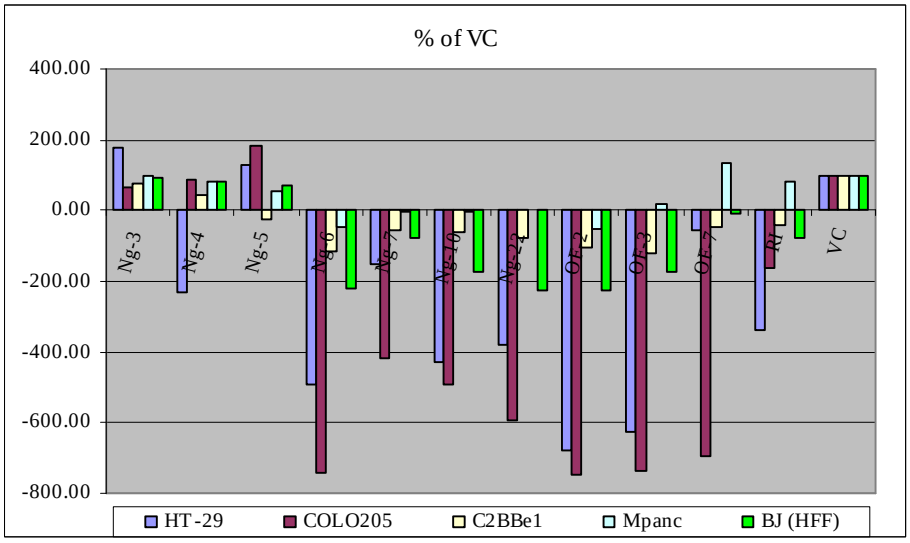
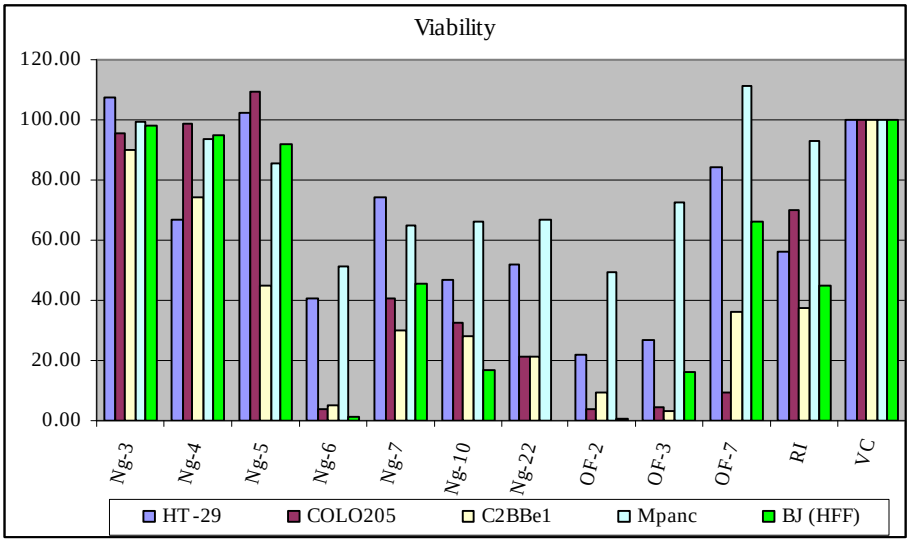


Figure 3

The effect of Test Items on the viability of cell lines



5. CONCLUSIONS:

The effect of the 10 Test Items on the viability and proliferation of the 5 different cell lines was assessed using the Alamar Blue detector.

The different Test Items showed various levels of cytotoxic, cytostatic and proliferative effects, on the different cancer cell lines (HT-29, COLO205, C2BBe1, MPanc) and on the normal cell line (BJ – HFF) relative to the Vehicle Control.

TI-1 – Ng3 did not show cytotoxic effect on any of the cell lines.

TI-2 – Ng4 showed cytotoxic effect just on HT-29 cell line.

TI-3 – Ng5 showed cytotoxic effect just on C2BBe1 cell line.

TI-4 – Ng6 showed cytotoxic effect on all the cell lines including the normal cell line BJ(HFF).

TI-5 – Ng7 showed cytotoxic effect on all the cell lines including the normal cell line BJ(HFF).

TI-6 –Ng10 showed cytotoxic effect on all the cell lines including the normal cell line BJ(HFF).

TI-7 – Ng22 showed cytotoxic effect on all the cell lines except MPanc.

TI-8 – OF2 showed cytotoxic effect on all the cell lines including the normal cell line BJ(HFF).

TI-9 – OF3 showed cytotoxic effect on all the cell lines except MPanc.

TI-10 – OF7 showed cytotoxic effect on all the cell lines except MPanc.

RI – Doxorubicin showed cytotoxic effect on all the cell lines except MPanc.

RAW DATA

Plate 1 - Fluorescent signal (Excitation 544nm/Emission 590nm)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
HT-29	235.9	146.2	265.6	98.11	164.4	111.1	125.5	61.39	75.53	256.2	135.3	202.8
	203.9	146.6	182.7	92.72	159.9	105.8	114.0	55.46	70.64	232.6	133.5	225.2
	217.8	143.8	194.7	89.51	145.7	104.6	113.5	53.79	68.55	232.8	124.5	193.4
	220.1	142.2	199.5	93.97	157.3	110.9	113.3	57.16	70.33	233.7	125.3	201.5
COLO205	229.6	228.1	255.2	25.17	107.8	95.43	64.58	24.03	31.79	103.8	175.9	232.9
	215.6	234.4	249.5	28.33	108.0	88.93	65.80	23.29	29.69	105.3	177.6	249.6
	223.3	233.2	247.8	27.37	106.8	89.09	68.37	23.86	30.56	106.5	179.1	227.4
	235.2	234.7	269.0	25.32	104.1	92.33	70.15	24.94	31.20	106.9	170.6	231.5

Plate 2 - Fluorescent signal (Excitation 544nm/Emission 590nm)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
C2BBe1	314.6	258.5	176.5	33.40	110.7	113.3	82.23	50.12	31.80	197.8	148.8	347.1
	312.9	260.8	154.6	34.06	111.4	107.5	89.37	45.20	30.20	199.7	140.1	346.6
	300.3	256.8	153.0	36.33	121.7	112.6	88.04	43.02	31.46	198.5	144.0	335.4
	294.9	253.2	169.8	35.77	112.1	109.0	93.09	43.61	33.12	192.7	142.5	320.6
MPanc	239.3	216.4	213.5	124.8	156.4	170.9	164.0	121.0	181.2	319.7	225.0	244.1
	231.3	214.3	188.7	124.4	153.4	157.5	151.7	116.7	166.3	305.0	218.1	234.6
	236.1	230.7	212.5	127.1	164.0	159.2	170.3	127.6	188.4	328.9	227.1	229.2
	230.9	230.3	208.6	135.9	164.1	169.9	177.4	124.6	172.4	343.7	233.0	237.2

Plate 3 - Fluorescent signal (Excitation 544nm/Emission 590nm)

	Ng-3	Ng-4	Ng-5	Ng-6	Ng-7	Ng-10	Ng-22	OF-2	OF-3	OF-7	RI	VC
BJ (HFF)	485.6	471.1	447.5	26.62	237.3	105.1	22.43	19.50	94.08	418.2	245.8	478.9
	463.0	470.9	445.4	23.73	229.9	97.06	22.52	19.01	98.65	380.2	240.6	480.2
	474.8	447.3	447.4	20.26	232.6	100.3	22.23	19.18	95.17	374.8	227.1	475.3
	468.3	449.9	438.7	25.62	223.1	99.78	22.12	19.62	95.03	384.4	223.2	496.8