Supplementary Table 2. Test for the presence of synergistic interactions.

Survival fractions of single and combination treatments, fold increase of cytotoxicity, and fractional product of cells at each condition, were analyzed as in Linda Duska et al, J Natl Cancer Instit, 91 (18): 157-63, 1999.

Raw data is expressed as mean survival fractions (s.f.) from PDT alone, MTX alone, or the combination.
Cytototoxic fractions (c.f.) were calculated as 1 - s.f.
To test Bliss synergism, calculate the fractional product value as: c.f. [comb] / {c.f. [PDT] + c.f. [MTX] - (c.f. [PDT] x c.f.[MTX])}

If this product > 1, then Bliss synergism
If this product = 1, then Bliss additivity
If this product < 1, then Bliss antagonism

			SURVIVAL FRACTIONS (s.f.)						CYTOTOXIC FRACTIONS (c.f.)			
CELL TYPE	METHOTREX CONCENTR	n	**MTX alone** (at conc shown)		**PDT alone**		**PDT + MTX**		**MTX alone**	**PDT alone**	**PDT + MTX**	FRACTIONAL PRODUCT
	mg / liter		**MEAN**	SD	**MEAN**	SD	**MEAN**	SD				
NHEK	0	3	**0.936**	0.027	**0.815**		**n/a**					
NHEK	0.001	3	**0.852**	0.012	**0.815**	0.063	**0.786**	0.028	0.148	0.185	0.214	**0.700**
NHEK	0.01	3	**0.825**	0.021	**0.815**	0.063	**0.782**	0.026	0.175	0.185	0.218	**0.664**
NHEK	0.1	3	**0.736**	0.003	**0.815**	0.063	**0.752**	0.027	0.264	0.185	0.248	**0.620**
NHEK	1.0	3	**0.635**	0.013	**0.815**	0.063	**0.682**	0.021	0.365	0.185	0.318	**0.659**
SCC13	0	3	**0.800**	0.038	**0.837**	0.033	**n/a**					
SCC13	0.001	3	**0.852**	0.015	**0.837**	0.033	**0.629**	0.109	0.148	0.163	0.371	**1.293**
SCC13	0.01	3	**0.830**	0.042	**0.837**	0.033	**0.471**	0.017	0.170	0.163	0.529	**1.733**
SCC13	0.1	3	**0.763**	0.065	**0.837**	0.033	**0.496**	0.078	0.237	0.163	0.504	**1.395**
SCC13	1.0	3	**0.757**	0.015	**0.837**	0.033	**0.408**	0.080	0.243	0.163	0.592	**1.616**
HEK1	0	3	**0.958**	0.019	**0.897**	0.0	**n/a**					
HEK1	0.001	3	**0.877**	0.032	**0.897**	0.0	**0.500**	0.117	0.123	0.103	0.500	**2.344**
HEK1	0.01	3	**0.915**	0.015	**0.897**	0.0	**0.417**	0.059	0.085	0.103	0.583	**3.253**
HEK1	0.1	3	**0.810**	0.098	**0.897**	0.0	**0.298**	0.038	0.190	0.103	0.702	**2.567**
HEK1	1.0	3	**0.687**	0.072	**0.897**	0.0	**0.148**	0.030	0.313	0.103	0.852	**2.220**

Supplementary Table 2 (continued)

Bliss synergy using fractional products Method of Duska et al, JNCI, 91 (18): 157-63, 1999. (Paul C. Elson, Ph.D. 04/16/2008)

		Using Formulas from Duska et al (Synergy Defined as a Ratio)			Synergy in a Linear Form[6]		
Cell Line	**MTX Dose**	**Synergy ± s.e.[1,2]**	**p[3]**	**Interpretation**	**Synergy ± s.e.[7]**	**p[8]**	**Interpretation**
NHEK	0.001 mg/L	**0.70** ± 0.09	.08	No synergy/antagonism	-0.09 ± 0.04	.12	No synergy/antagonism
	0.01	**0.66** ± 0.08	.05	Antagonism	-0.11 ± 0.03	.09	No synergy/antagonism
	0.1	**0.62** ± 0.06	.03	Antagonism	-0.15 ± 0.04	.05	Antagonism
	1.0	**0.66** ± 0.04	.01	Antagonism	-0.16 ± 0.03	.03	Antagonism
	All doses combined[4]	**0.66** ± 0.02	<.001	Antagonism	-0.13 ± 0.02	.005	Antagonism
SCC13	0.001 mg/L	**1.30** ± 0.23	.33	No synergy/antagonism	0.08 ± 0.07	.32	No synergy/antagonism
	0.01	**1.73** ± 0.16	.05	Synergy	0.22 ± 0.03	.02	Synergy
	0.1	**1.40** ± 0.18	.16	No synergy/antagonism	0.14 ± 0.06	.13	No synergy/antagonism
	1.0	**1.62** ± 0.14	.05	Synergy	0.23 ± 0.05	.04	Synergy
	All doses combined[4]	**1.51** ± 0.10	.02	Synergy	0.17 ± 0.03	.02	Synergy
HEK1	0.001 mg/L	**2.34** ± 0.37	.07	No synergy/antagonism	0.29 ± 0.07	.05	Synergy
	0.01	**3.24** ± 0.25	.01	Synergy	0.40 ± 0.04	.008	Synergy
	0.1	**2.56** ± 0.48	.08	No synergy/antagonism	0.43 ± 0.06	.02	Synergy
	1.0	**2.22** ± 0.22	.03	Synergy	0.47 ± 0.04	.008	Synergy
	All doses combined[4]	**2.59** ± 0.23	.006	Synergy	0.40 ± 0.04	.002	Synergy

[1] Let a = mean cytotoxic fraction from PDT alone; b= mean cytotoxic fraction from MTX alone; c = mean cytotoxic fraction from the combination The calculation for Bliss synergy (Duska et al) is: c/[(a+b) – (ab)]. Values >1 indicate synergy; <1 implies antagonism; equality with 1 indicates independence

[2] Standard errors were estimated using a first order Taylor series expansion about the variance of c/[(a+b) – (ab)]

[3] p-values are from 2-sided, one-sample t-tests of H_0: Bliss synergy=1

[4] Counts synergy of each dose as one "observation"

[6] c-[(a+b) – (ab)]; values>0 indicate synergy; <0 implies antagonism; equality with 0 indicates independence

[7] Estimated variance using first order Taylor series

[8] p-values are from 2-sided, one-sample t-tests of H_0: Bliss synergy=0