

Appendix II, Example II-B

Two-Drug Combination Against Human Xenograft Tumor In Nude Mice

Slides from Chou TC, et al. Proc AACR 46: 1167, 2005 (Mini-symposium)

see also: Chou TC, Pharmacol. Rev. 58: 621-681, 2006 (P.669)

Chou TC, Cancer Res. 70: 440-446, 2010 (P.441)

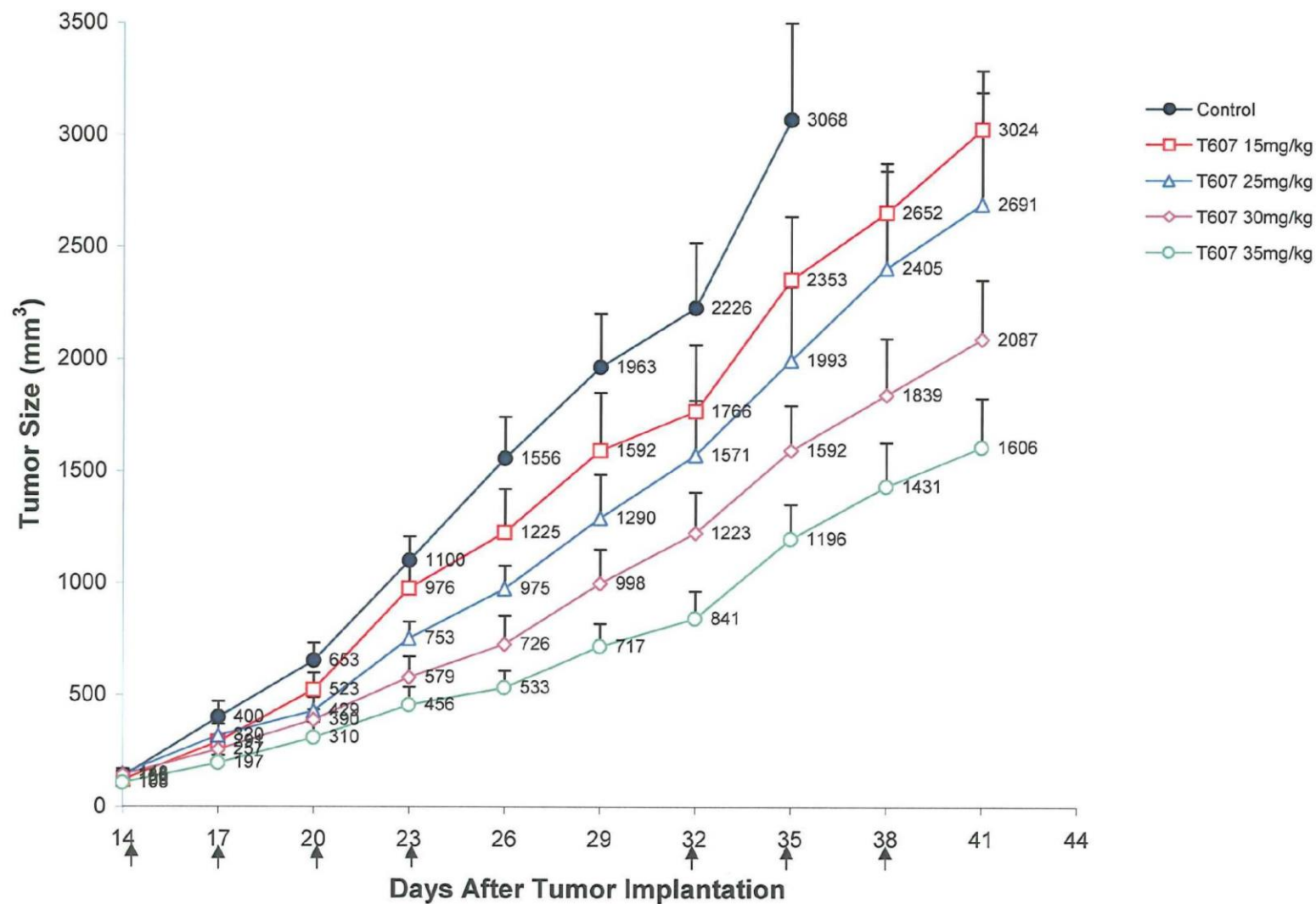
Chou TC, Am J Cancer Res. 1: 925-954, 2011 (P. 944)

II-B-1 Combination Therapy of T-706 and Taxotere against HCT-116 Xenograft Tumors in Nude Mice*

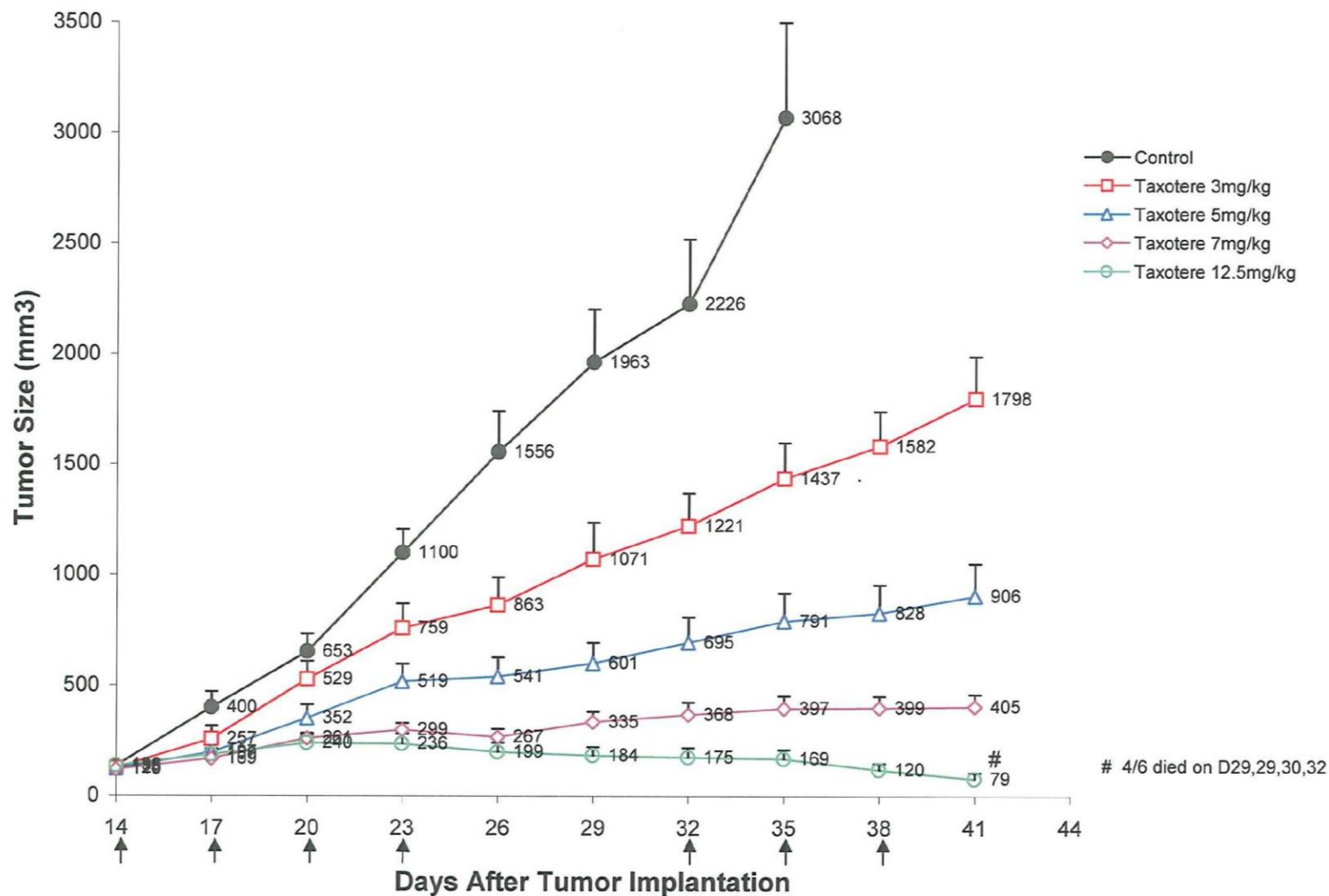
Therapy	T-706	Taxotere	Combination	Control
Dose-range	15, 25, 30, 35 mg/kg	3, 5, 6, 7 mg/kg	(15+3), (25+5), (30+6), (35+7) mg/kg	Vehicle (DMSO)
Route	i.v. injection	i.v. injection	i.v. injection	i.v. injection
Schedule	Q3Dx4	Q3Dx4	Q3Dx4	Q3Dx4
Mice (x doses)	5(x4) = 20	5(x4) = 20	5(x4) = 20	5(x1) = 5

* Treatment with i.v. injection started on Day 14 after tumor implantation.
 Measurement of **tumor size (mm³)** and **body-weight (gm)** every other day.
 Observation ended on Day 41

II-B-2 Therapeutic effect of **T607** in nude mice bearing human colon carcinoma **HCT-116** xenograft (i.v. injection, Q3Dx4, x3)

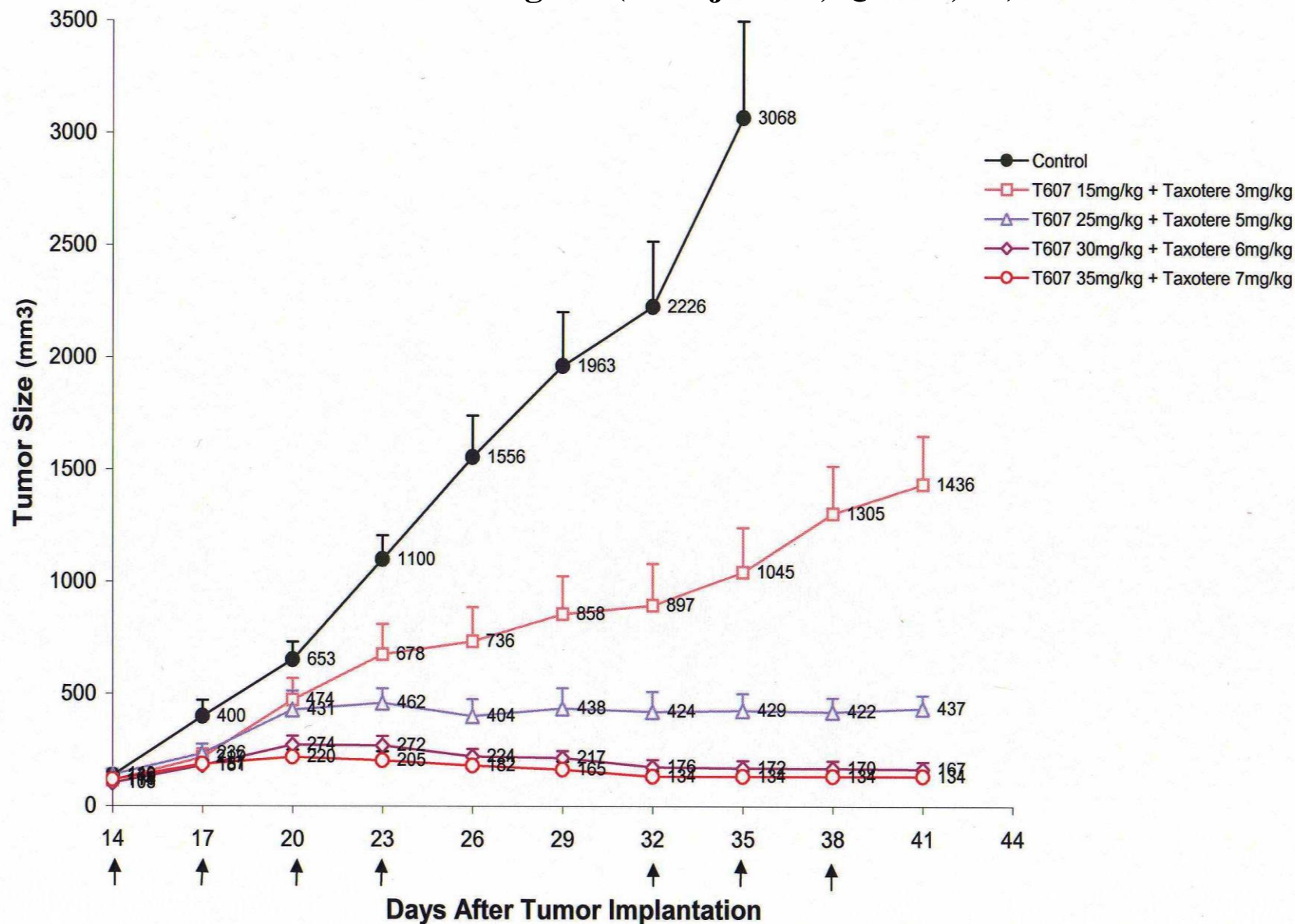


II-B-3 Therapeutic effect of **Taxotere** in nude mice bearing human **colon carcinoma HCT-116** xenograft (i.v. injection, Q3Dx4, x3)



II-B-4 Therapeutic effect of **T607** & **Taxotere** in nude mice bearing human colon carcinoma

HCT-116 xenograft (i.v. injection, Q3Dx4, x3)



II-B-5**CompuSyn Report**

Experiment Name: T-607 + Taxotere In Vivo
Date: 3/28/2008
File Name: A:\82902D41.cse
Description: Combination of T-607 + Taxotere against HCT-116 Xenograft Tumors in Nude Mice. D41

Drug: T-607 (T-607) [mg/Kg]
Drug: Taxotere (TXT) [mg/Kg]
Drug Combo: T-607 + Taxotere (T-6+TX) (T-607+TXT [5:1])

Data for Drug: T-607 [mg/Kg]

Dose Effect

15.0	0.4212
25.0	0.4849
30.0	0.6005
35.0	0.6926

4 data points entered.

X-int: 1.32557

Y-int: -1.6540 +/- 0.53787

m: 1.24775 +/- 0.38265

Dm: 21.1629

r: 0.91743

Data for Drug: TXT [mg/Kg]

Dose Effect

3.0	0.6558
5.0	0.8266
7.0	0.9225

3 data points entered.

X-int: 0.35580

Y-int: -0.7587 +/- 0.17400

m: 2.13244 +/- 0.25198

Dm: 2.26884

r: 0.99309

Data for Drug Combo: T-6+TX (T-607+TXT [5:1])

Dose A Effect

15.0+	0.7252
25.0+	0.9163
30.0+	0.9681

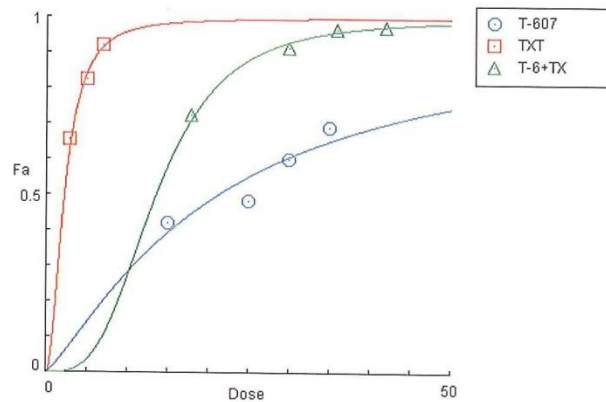
II-B-6'

35.0+ 0.9744

4 data points entered.

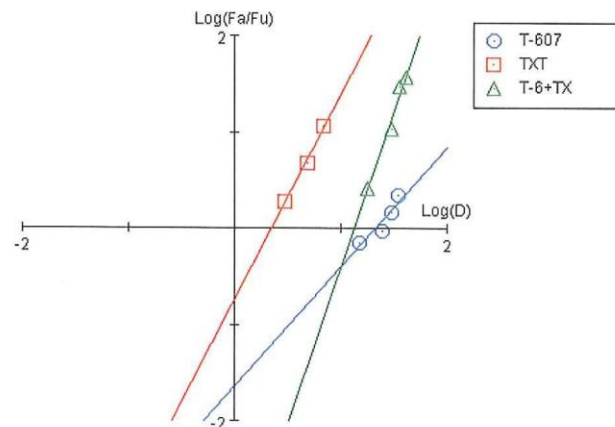
X-int: 1.13167**Y-int:** -3.6954 +/- 0.50702**m:** 3.26539 +/- 0.34155**Dm:** 13.5417**r:** 0.98924

Dose-Effect Curve



Median-Effect Plot

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CI Data for Drug Combo: T-6+TX (T-607 +TXT [5:1])

Fa	CI Value	Total Dose
0.05	3.89780	5.49623
0.1	3.00517	6.90946
0.15	2.57795	7.96109
0.2	2.30585	8.85722
0.25	2.10818	9.67295
0.3	1.95302	10.4468
0.35	1.82469	11.2032
0.4	1.71440	11.9604
0.45	1.61672	12.7346
0.5	1.52799	13.5417
0.55	1.44560	14.4000
0.6	1.36748	15.3321
0.65	1.29190	16.3684
0.7	1.21716	17.5535
0.75	1.14142	18.9578
0.8	1.06229	20.7038
0.85	0.97603	23.0343
0.9	0.87539	26.5401
0.95	0.74019	33.3643
0.97	0.66041	39.2638

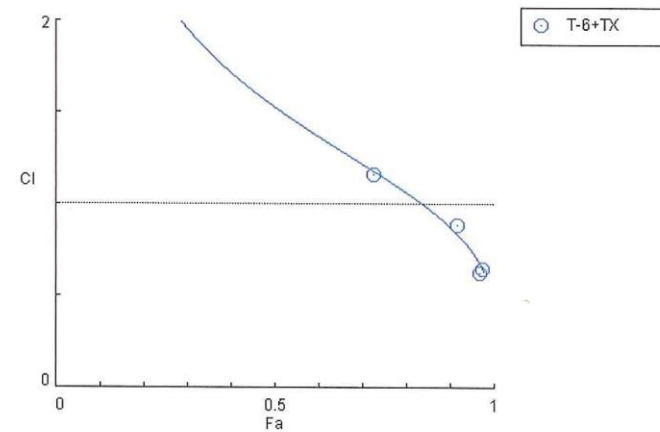
CI values for actual experimental points:

Total Dose	Fa	CI Value
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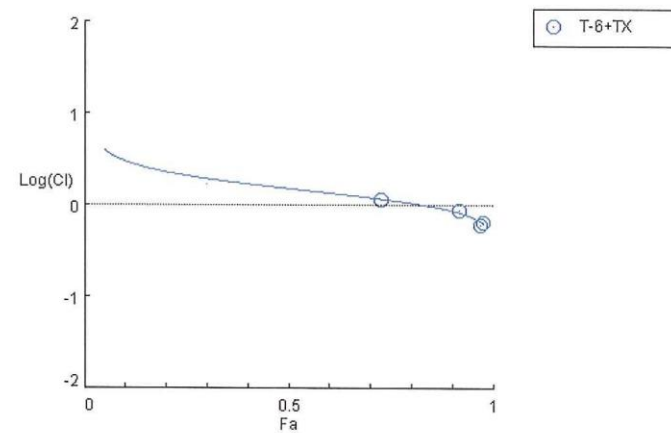
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18.0	0.7252	1.16450
30.0	0.9163	0.89098
36.0	0.9681	0.62570
42.0	0.9744	0.64942

Combination Index Plot



Logarithmic Combination Index Plot



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DRI Data for Drug Combo: T-6+TX (T-607 +TXT [5:1])

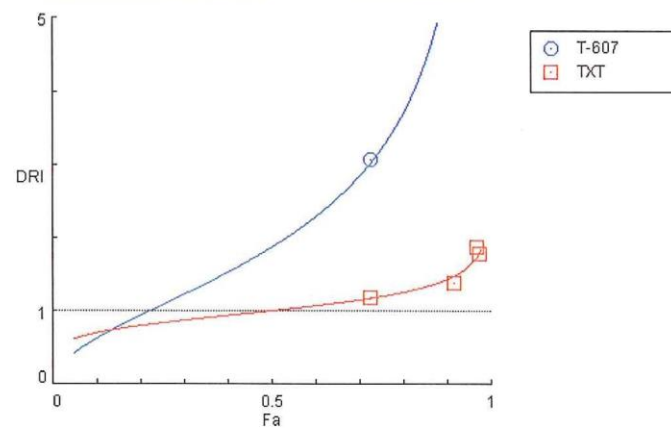
<u>Fa</u>	<u>Dose T-607</u>	<u>Dose TXT</u>	<u>DRI T-607</u>	<u>DRI TXT</u>
0.05	1.99861	0.57034	0.43636	0.62262
0.1	3.63752	0.80968	0.63175	0.70311
0.15	5.27019	1.00585	0.79439	0.75808
0.2	6.96721	1.18432	0.94394	0.80228
0.25	8.77384	1.35538	1.08846	0.84072
0.3	10.7316	1.52490	1.23271	0.87581
0.35	12.8858	1.69719	1.38023	0.90895
0.4	15.2914	1.87597	1.53420	0.94109
0.45	18.0189	2.06507	1.69795	0.97298
0.5	21.1629	2.26884	1.87535	1.00527
0.55	24.8554	2.49272	2.07128	1.03863
0.6	29.2888	2.74398	2.29236	1.07382
0.65	34.7567	3.03304	2.54808	1.11179
0.7	41.7336	3.37571	2.85301	1.15386
0.75	51.0457	3.79794	3.23111	1.20202
0.8	64.2821	4.34648	3.72582	1.25962
0.85	84.9811	5.11770	4.42720	1.33306
0.9	123.124	6.35759	5.56701	1.43728
0.95	224.089	9.02550	8.05971	1.62308
0.97	343.139	11.5811	10.4872	1.76973

DRI values calculated at experimental points

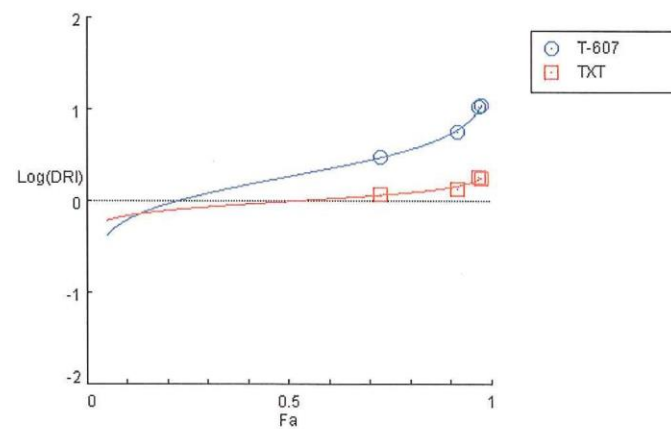
<u>Fa</u>	<u>Dose T-607</u>	<u>Dose TXT</u>	<u>DRI T-607</u>	<u>DRI TXT</u>
0.7252	46.0611	3.57633	3.07074	1.19211
0.9163	144.053	6.96925	5.76212	1.39385
0.9681	326.148	11.2420	10.8716	1.87367
0.9744	391.066	12.5018	11.1733	1.78597

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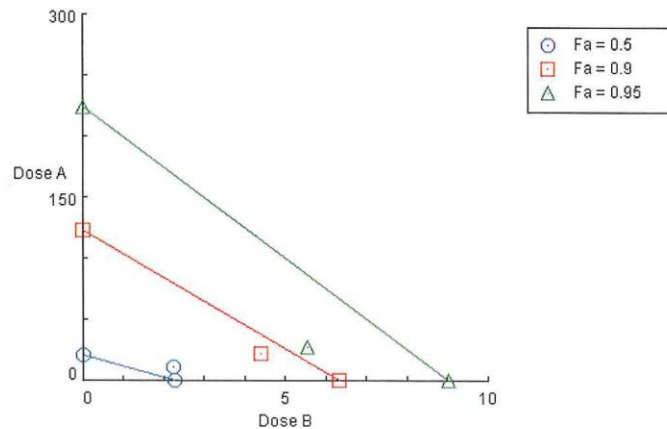
DRI Plot for Combo: T-6+TX (T-607 +TXT [5:1])



Log(DRI) Plot for Combo: T-6+TX (T-607 +TXT [5:1])



II-B-11 Isobologram for Combo: T-6+TX (T-607 +TXT [5:1])



Summary Table

Experiment Name: T-607 + Taxotere In Vivo
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Drug: Taxotere (TXT) [mg/Kg]
Drug Combo: T-607 + Taxotere (T-6+TX) (T-607 +TXT [5:1])

Drug/Combo	Dm	m	r
T-607	21.1629	1.24775	0.91743
TXT	2.26884	2.13244	0.99309
T-6+TX	13.5417	3.26539	0.98924

CI values at:

Combo	ED50	ED75	ED90	ED95
T-6+TX	1.52799	1.14142	0.87539	0.74019

Data for Fa = 0.5

Drug/Combo	CI value	Dose T-607	Dose TXT
T-607		21.1629	
TXT			2.26884

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T-6+TX	1.52799	11.2848	2.25695
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Data for Fa = 0.75

Drug/Combo	CI value	Dose T-607	Dose TXT
T-607		51.0457	
TXT			3.79794
T-6+TX	1.14142	15.7982	3.15964

Data for Fa = 0.9

Drug/Combo	CI value	Dose T-607	Dose TXT
T-607		123.124	
TXT			6.35759
T-6+TX	0.87539	22.1168	4.42336

Data for Fa = 0.95

Drug/Combo	CI value	Dose T-607	Dose TXT
T-607		224.089	
TXT			9.02550
T-6+TX	0.74019	27.8036	5.56072

Data for Fa = 0.97

Drug/Combo	CI value	Dose T-607	Dose TXT
T-607		343.139	
TXT			11.5811
T-6+TX	0.66041	32.7199	6.54397

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Additional Exp.

Computerized quantitation of synergism for T-607 and Taxotere against MX-1 xenografts in nude mice, Q3Dx4, i.v. on Day 7, 10, 13 and 16.^a

Day of Measurement	ED ₅₀ Value (mg/kg)	CI value ^b at				DRI value ^c at			
		ED ₅₀	ED ₇₅	ED ₉₀	ED ₉₅	ED ₅₀	ED ₇₅	ED ₉₀	ED ₉₅
Day 16									
T-607	31.0					5.2	4.2	3.9	3.1
TXT	3.38					1.1	1.3	1.5	1.6
T607 + TXT	6.0 + 3.0	1.08	1.01	0.97	0.95				
Day 19									
T-607	32.1					4.6	4.2	3.7	3.5
TXT	4.9					1.4	1.5	1.6	1.6
T-607 + TXT	7.0 + 3.5	0.93	0.92	0.91	0.90				
Day 22									
T-607	32.7					5.1	5.3	5.5	5.6
TXT	5.0					1.5	1.6	1.6	1.7
T-607 + TXT	6.4 + 3.2	0.84	0.82	0.80	0.79				

^a MX-1 tumor tissue (50mg/mouse) was implanted s.c. into nude mice on Day 0. Treatment started on Day 7, Q3Dx4 i.v. injection as indicated. Numerical values of tumor size were recorded prior to each treatment and were given in Table 1. The combination ratio for T-607: TXI was 2:1.

^b The combination index (CI) value of <, = and > 1 indicate synergism, additive effect and antagonism, respectively (2,3).

^c Dose reduction index (DRI) represent the order of magnitude (fold) of dose reduction that is allowed in combination for a given degree of effect as compared with the dose of each drug alone (2,3). All CI and DRI values are calculated on the basis of the classic isobologram equation using a computer software (4).