

# 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**<sup>\*</sup>

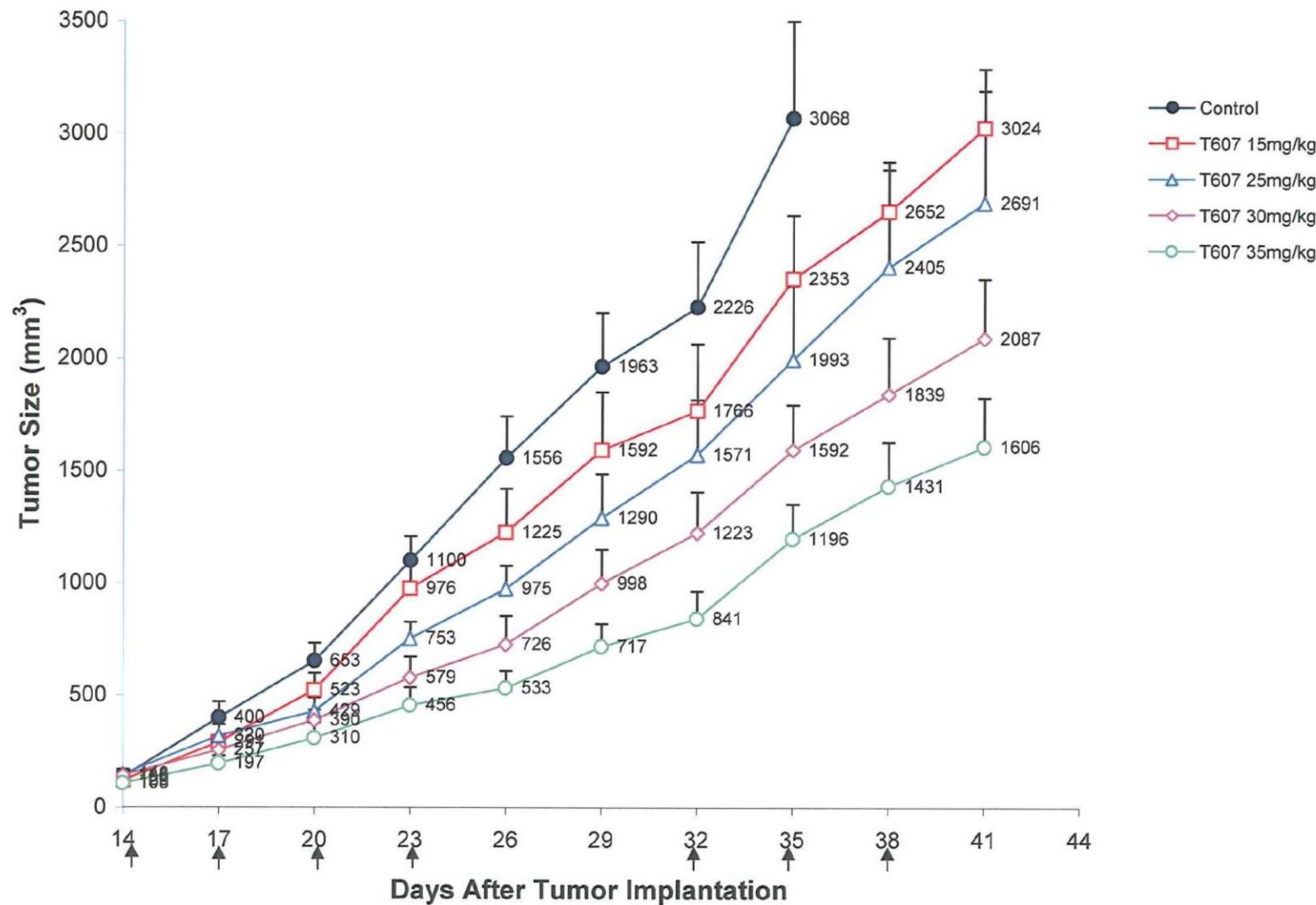
Therapy	T-706	Taxotere	Combination	Control
Dose-range	15, 25, 30, 35 mg/kg	3, 5, 6, 7 mg/kg	(15+3), (25+5), (30+6), (35+7) mg/kg	Vehicle (DMSO)
Route	i.v. injection	i.v. injection	i.v. injection	i.v. injection
Schedule	Q3Dx4	Q3Dx4	Q3Dx4	Q3Dx4
Mice (x doses)	5(x4) = <b>20</b>	5(x4) = <b>20</b>	5(x4) =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

\* Treatment with i.v. injection started on Day 14 after tumor implantation.

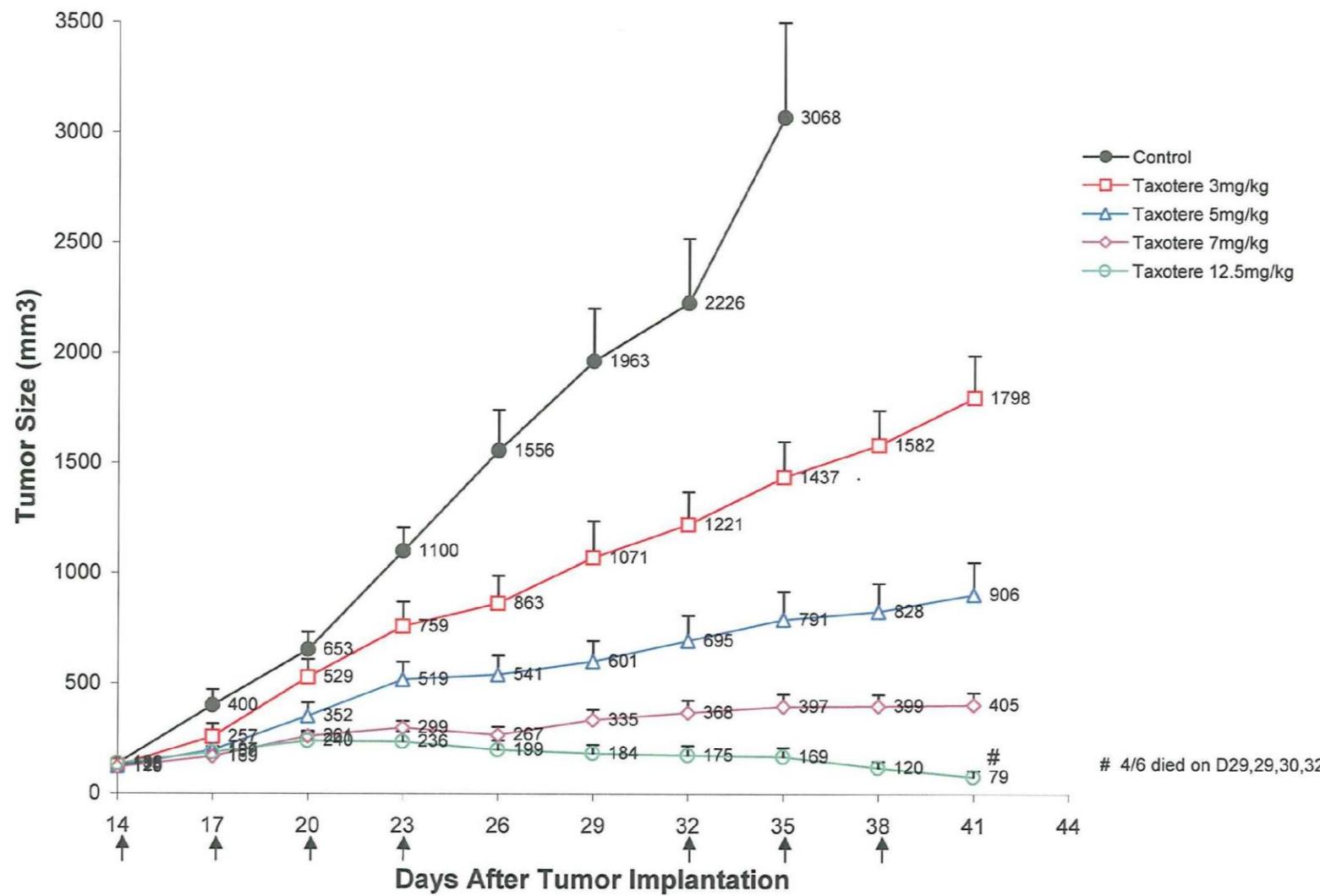
Measurement of **tumor size (mm<sup>3</sup>)** and **body-weight (gm)** every other day.

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)**

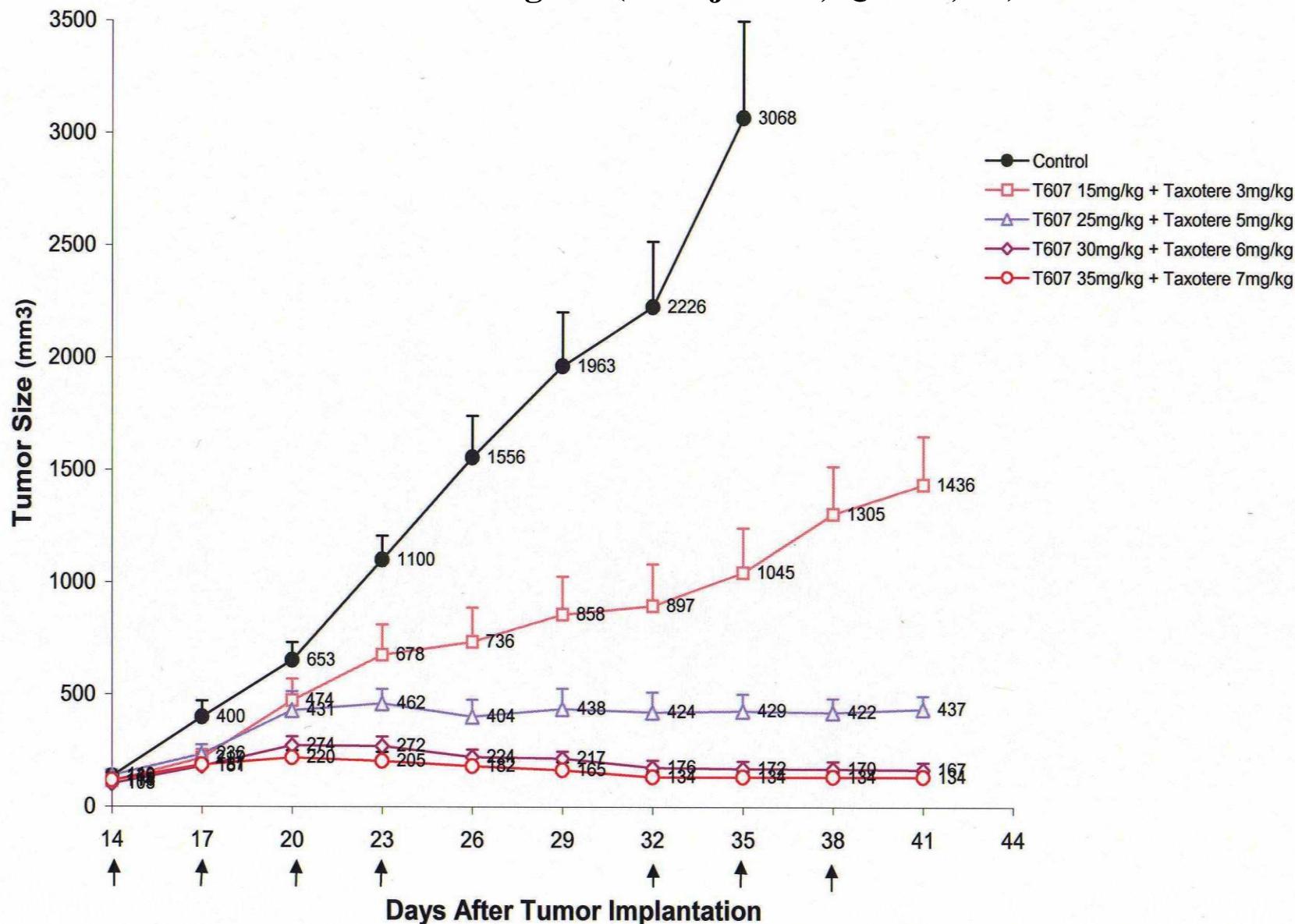


**II-B-3 Therapeutic effect of Taxotere in nude mice bearing human colon carcinoma HCT-116 xenograft**  
 (i.v. injection, Q3Dx4, x3)



## 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])

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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

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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

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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

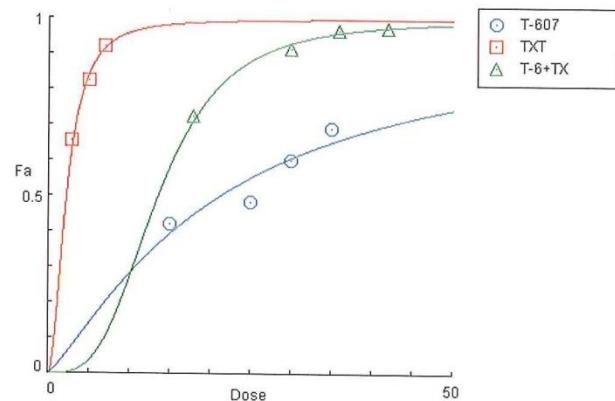
Y-int: -3.6954 +/- 0.50702

m: 3.26539 +/- 0.34155

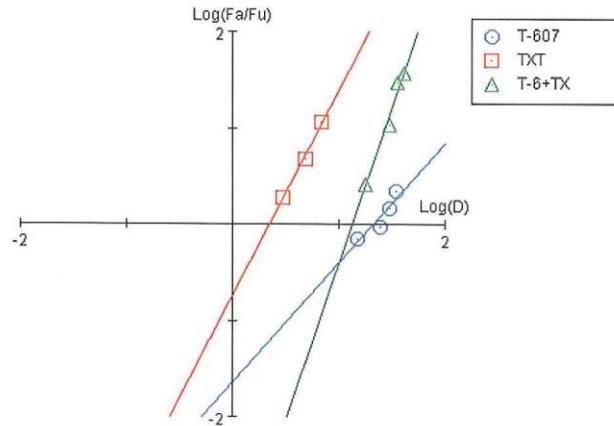
Dm: 13.5417

r: 0.98924

Dose-Effect Curve



Median-Effect Plot

**II-B-7**

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

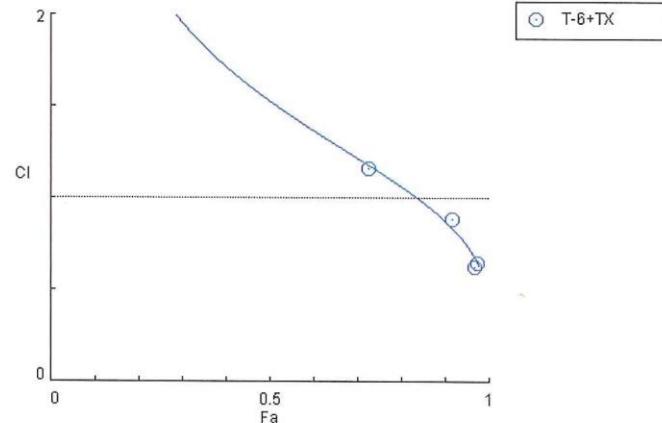
CI values for actual experimental points:

Total Dose    Fa    CI Value

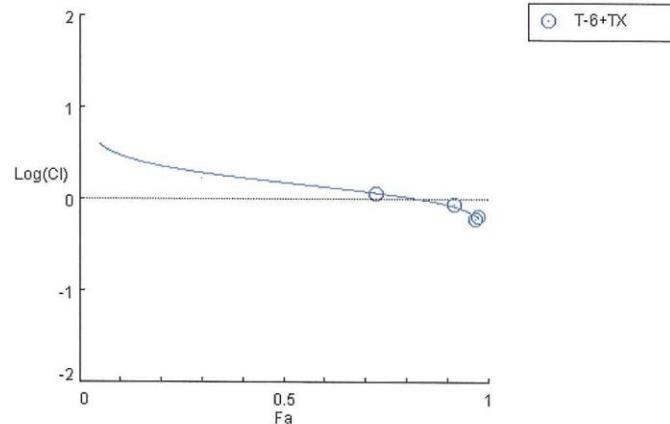
**II-B-8**

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



## II-B-9

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

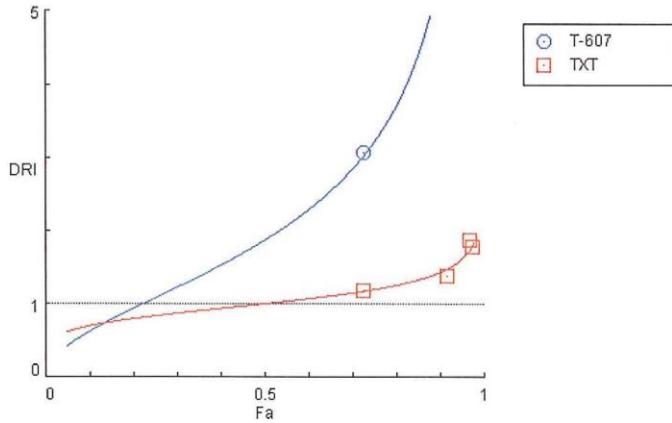
<u>Fa</u>	<u>Dose T-607</u>	<u>Dose TXT</u>	<u>DRI T-607</u>	<u>DRI TXT</u>
0.05	1.99861	0.57034	0.43636	0.62262
0.1	3.63752	0.80968	0.63175	0.70311
0.15	5.27019	1.00585	0.79439	0.75808
0.2	6.96721	1.18432	0.94394	0.80228
0.25	8.77384	1.35538	1.08846	0.84072
0.3	10.7316	1.52490	1.23271	0.87581
0.35	12.8858	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

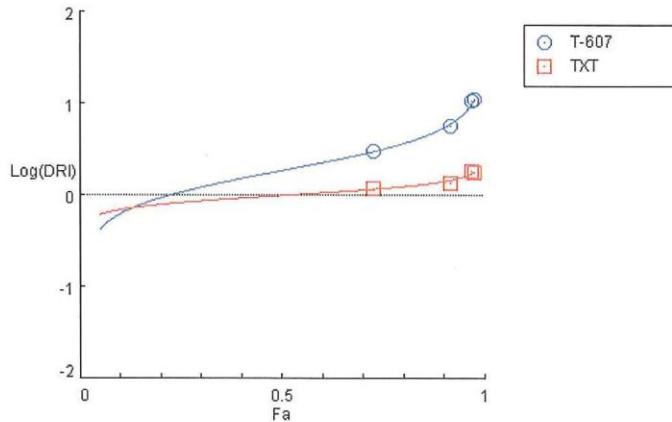
<u>Fa</u>	<u>Dose T-607</u>	<u>Dose TXT</u>	<u>DRI T-607</u>	<u>DRI TXT</u>
0.7252	46.0611	3.57633	3.07074	1.19211
0.9163	144.053	6.96925	5.76212	1.39385
0.9681	326.148	11.2420	10.8716	1.87367
0.9744	391.066	12.5018	11.1733	1.78597

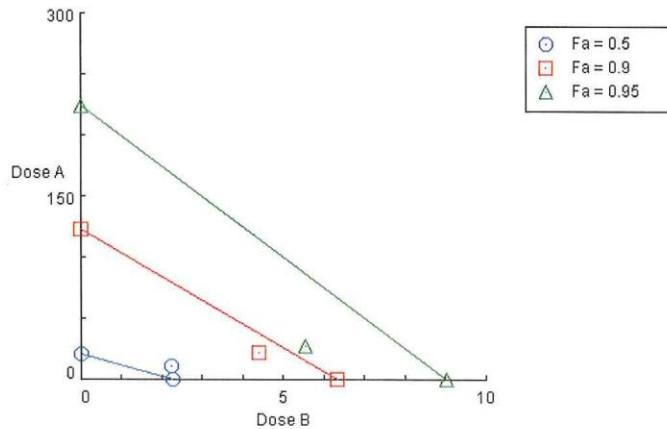
## II-B-10

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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**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])

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

## II-B-12

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

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

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Data for  $F_a = 0.9$

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

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Data for  $F_a = 0.95$

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

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Data for  $F_a = 0.97$

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

**II-B-13 Computerized quantitation of synergism for T-607 and Taxotere against HCT-116 xenografts in nude mice, Q3Dx4, x3, i.v. on Day 14, 17, 20, 23 and then 32, 35, 38.<sup>a</sup>**

Day of Measurement	ED <sub>50</sub> Value (mg/kg)	CI value <sup>b</sup> at				DRI value <sup>c</sup> at			
		ED <sub>50</sub>	ED <sub>75</sub>	ED <sub>90</sub>	ED <sub>95</sub>	ED <sub>50</sub>	ED <sub>75</sub>	ED <sub>90</sub>	ED <sub>95</sub>
<b>Day 29</b>									
T-607	29.8					2.1	2.3	2.4	2.5
TXT	3.30					1.2	1.3	1.4	1.6
T-607 + TXT	14.0 + 2.80	1.31	1.21	1.11	1.05				
<b>Day 32</b>									
T-607	32.4					2.4	2.9	3.3	3.7
TXT	3.2					1.2	1.4	1.6	1.8
T-607 + TXT	13.2 + 2.65	1.23	1.06	0.92	0.84				
<b>Day 35</b>									
T-607	30.5					2.5	3.1	3.9	4.5
TXT	2.87					1.2	1.4	1.6	1.8
T-607 + TXT	12.3 + 2.46	1.26	1.05	0.88	0.78				
<b>Day 38</b>									
T-607	25.2	1.46	1.14	0.90	0.77	2.1	3.0	4.3	5.5
TXT	2.47					1.0	1.2	1.5	1.7
T-607 + TXT	12.0 + 2.41								

<sup>a</sup> HCT-116 tumor tissue (50mg/mouse) was implanted s.c. into nude mice on Day 0. Treatment started on Day 14, Q3Dx4 and then started on Day 32, Q2Dx3 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 5:1.

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

<sup>c</sup> 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).

**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.<sup>a</sup>**

Day of Measurement	ED <sub>50</sub> Value (mg/kg)	CI value <sup>b</sup> at				DRI value <sup>c</sup> at			
		ED <sub>50</sub>	ED <sub>75</sub>	ED <sub>90</sub>	ED <sub>95</sub>	ED <sub>50</sub>	ED <sub>75</sub>	ED <sub>90</sub>	ED <sub>95</sub>
<b>Day 16</b>									
T-607	31.0					5.2	4.2	3.9	3.1
TXT	3.38					1.1	1.3	1.5	1.6
T-607 + TXT	6.0 + 3.0	1.08	1.01	0.97	0.95				
<b>Day 19</b>									
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				
<b>Day 22</b>									
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				

<sup>a</sup> 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.

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

<sup>c</sup> 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).