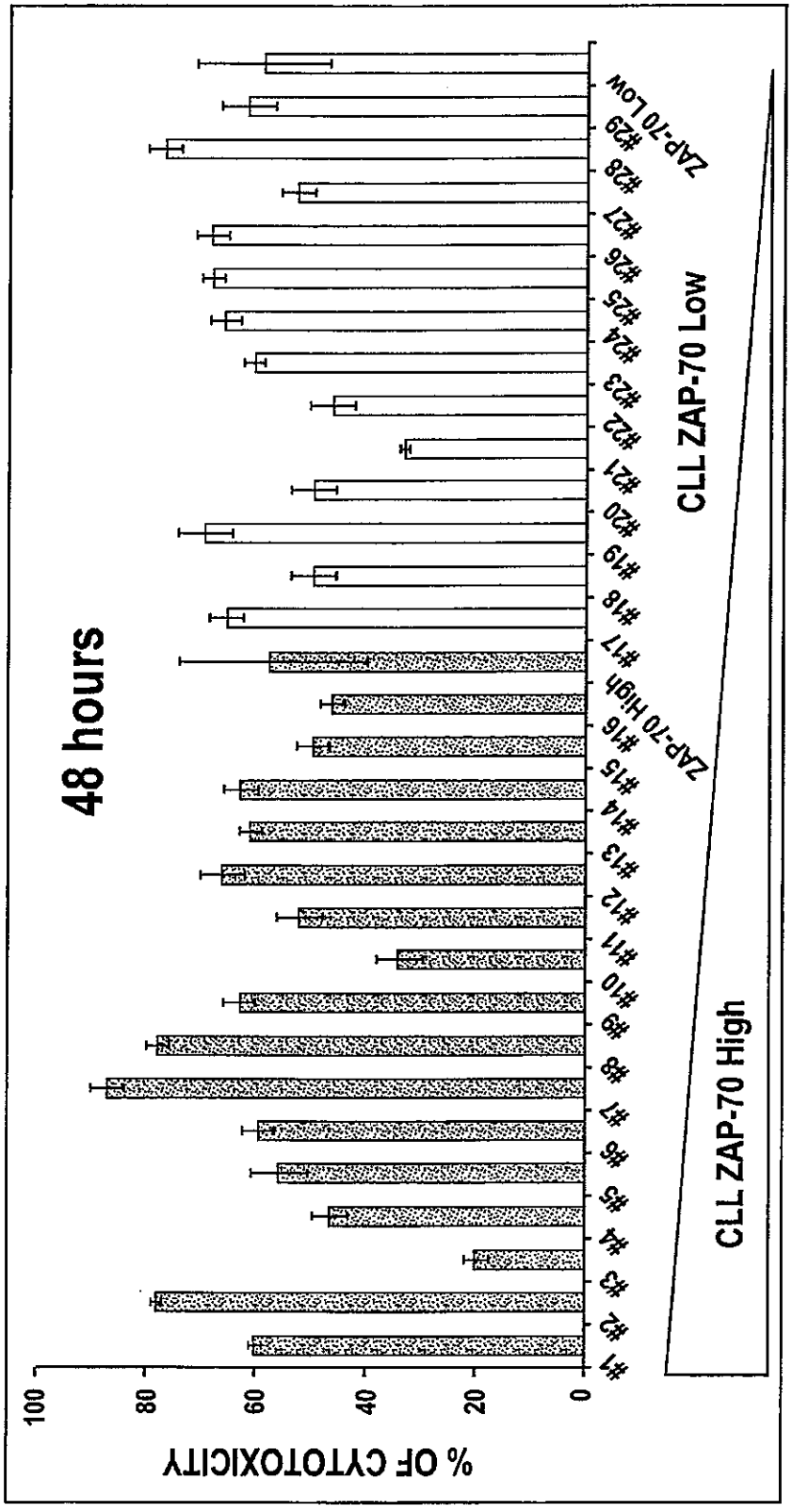
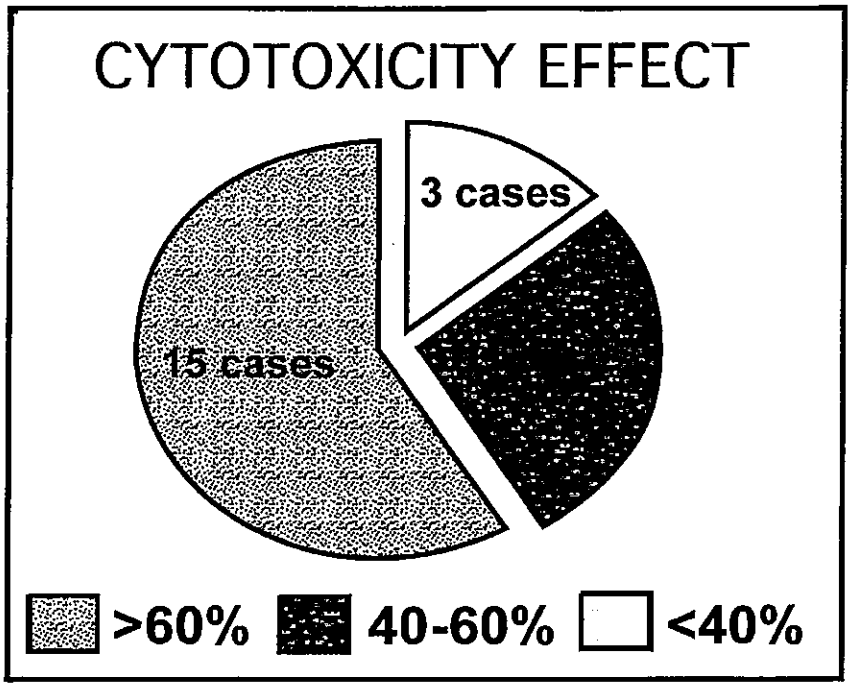

% OF CYTOTOXICITY
100
80
60
40
20
0
48 hours
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
#18
#19
#20
#21
#22
#23
#24
#25
#26
#27
#28
#29
ZAP-70 Low
ZAP-70 High
CLL ZAP-70 Low
CLL ZAP-70 High

Fig. 1A



Mean Cytotoxicity:

CLL ZAP-70 High= 57.59%

CLL ZAP-70 Low= 59.27%

Fig. 1B

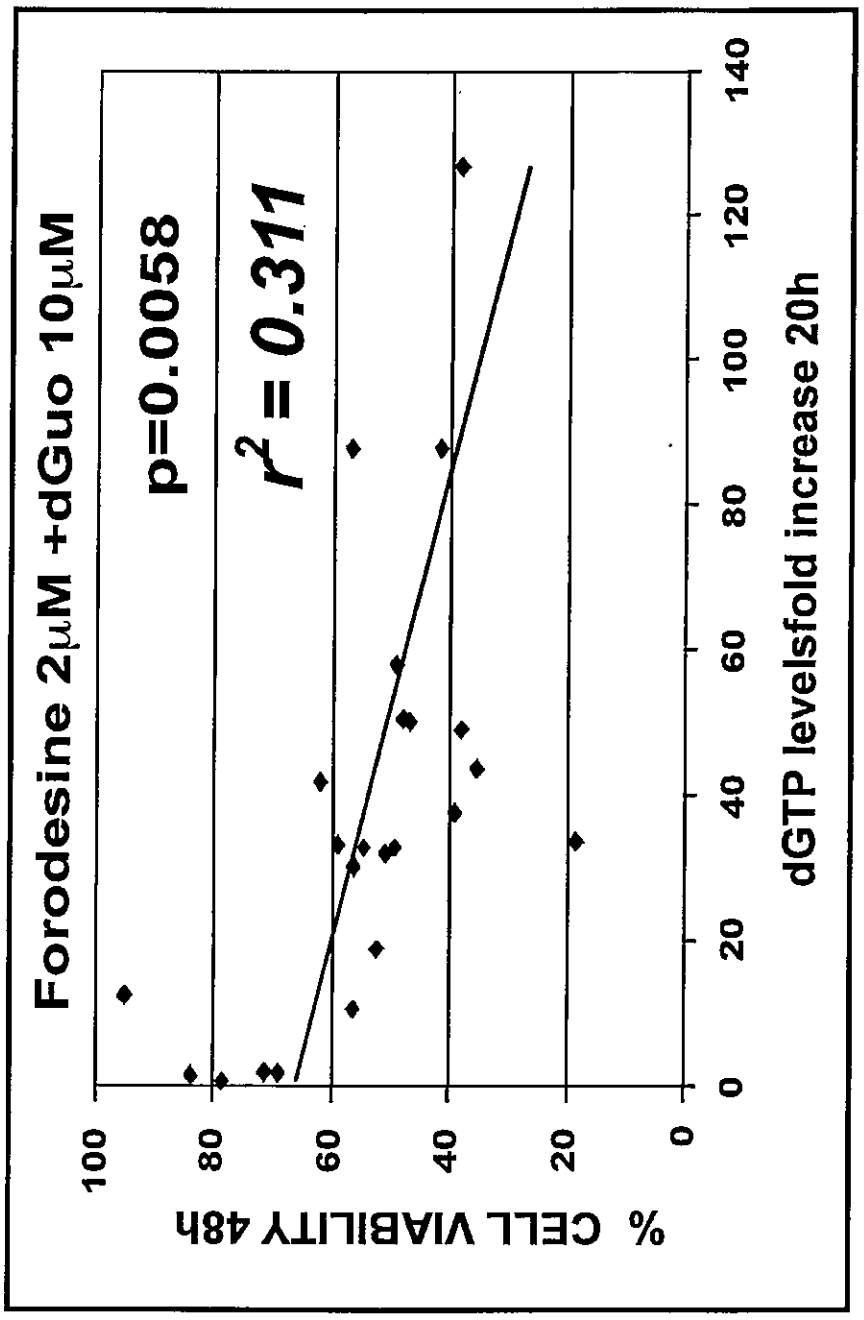

Forodesine 2μM +dGuo 10μM
p=0.0058
$r^2 = 0.311$
% CELL VIABILITY 48h
dGTP levelsfold increase 20h
0
20
40
60
80
100
120
140

Fig. 2

CLL #	% ZAP -70	ATM STATUS	12 trisomy (% of cells)	13q deletion (% of cells)	PREVIOUS TREATMENT	RESPONSE TO PREVIOUS TREATMENT	FORODESINE CYTOTOXICITY (2 μM+dGuo 20 μM)	BENDAMUSTINE CYTOTOXICITY (25 μM)	FLUDARABINE CYTOTOXICITY 1 μg/ml (7.5 μM)
#7	60%	Normal	Normal	11.4 %	CdA / FCM	YES	87.2 ±3%	12.5 ±4%	2.1±2%
#28	1%	Normal	Normal	Normal	FC / R-F / CHOP	NO	77.1 ±5%	41.8 ±2%	43.3 ±3%
#8	53%	Normal	Normal	90%	-	-	77.9 ±2%	40.6 ±2%	31.1 ±2%
#2	91%	Normal	45 %	Normal	FCM	YES	78. 2±2%	65.2 ±1%	59.2 ±4%
#25	3%	Normal	Normal	5%	CLB	NO	68.3 ±4%	25.9 ±3%	25.9 ±3%
#9	50%	Normal	Normal	Normal	R-CHOP	NO	63.1 ±6%	n.d.	n.d.
#12	36%	Normal	Normal	29%	-	-	66.1 ±3%	26.47 ±5%	42.65 ±2%
#4	73%	n.d.	n.d.	n.d.	FCM / CHOP	NO	46.7 ±4%	52.7 ±4%	50.1 ±4%
#22	n.d.	Normal	Normal	Normal	-	-	46.5 ±2%	5.8 ±3%	1.1±2%
#21	7%	Normal	30.5 %	Normal	CHOP / R-CHOP	NO	33.4 ±3% * *	38.3 ±3%	40.1± 2%
#3	87%	Normal	Normal	90%	FCM/Camp/PDN	NO	20.1 ±5% **	19.2 ±2%	27.3 ±5%
						MEAN ±SD	60.4 ±21%	32.8 ±34%	33.3 ±36%

Fig. 3

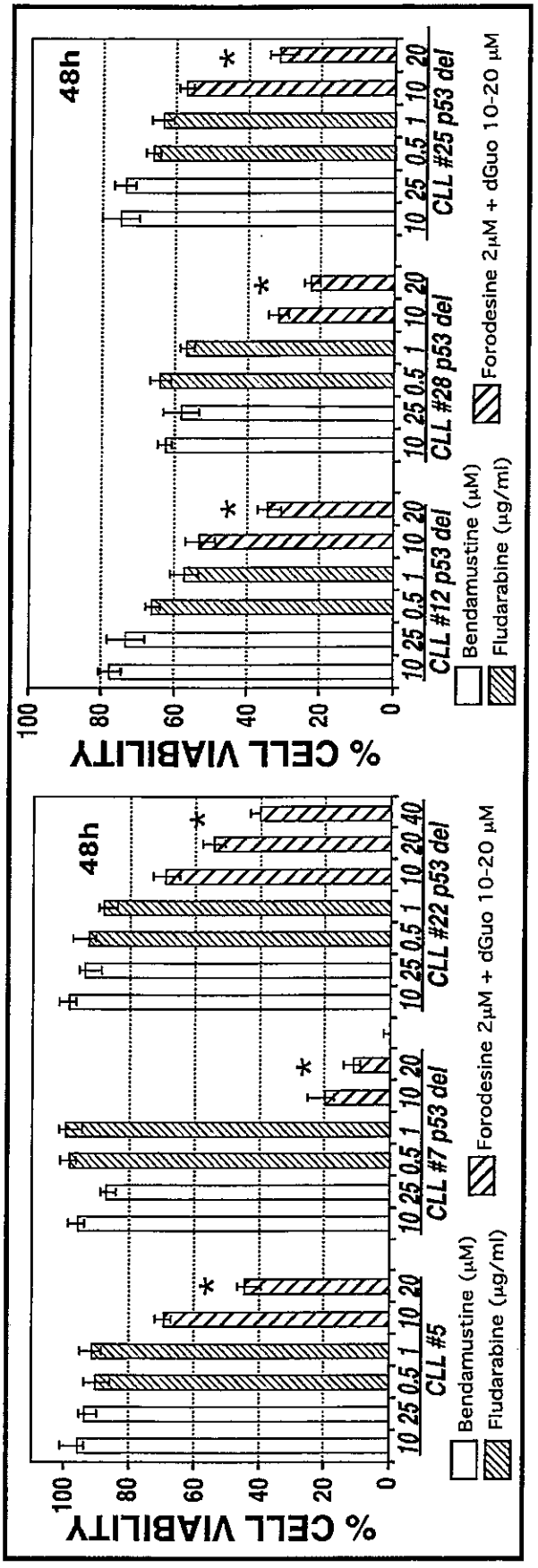
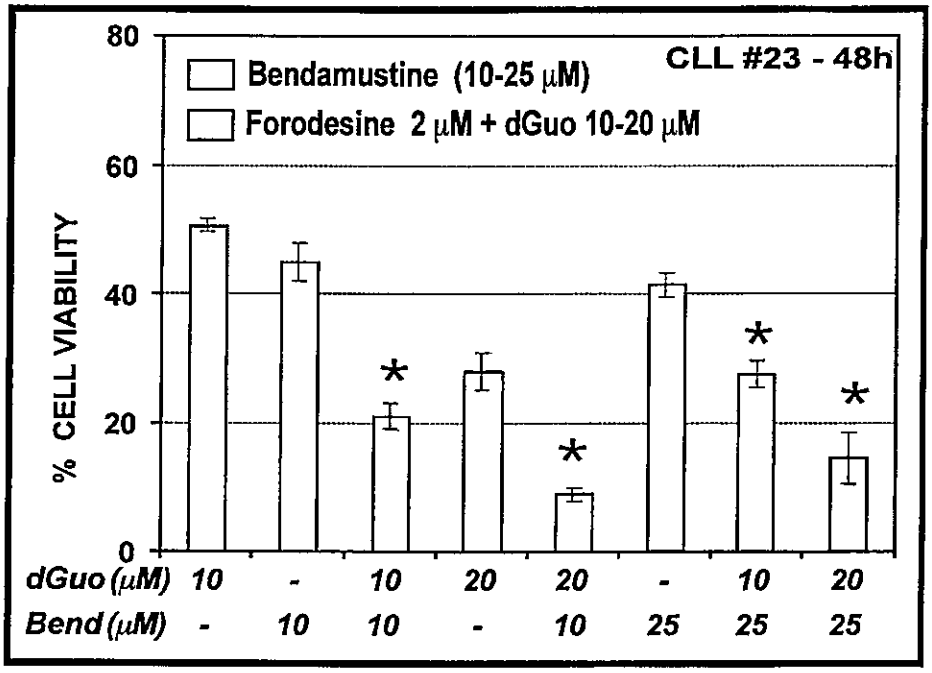

% CELL VIABILITY
48h
CLL #5
CLL #7 p53 del
CLL #22 p53 del
CLL #12 p53 del
CLL #28 p53 del
CLL #25 p53 del
Bendamustine (µM)
Fludarabine (µg/ml)
Forodesine 2µM + dGuo 10-20 µM

Fig. 4

Combination Index for Forodesine/Bendamustine (48h)

CLL	% ZAP-70	For 2+10 Bend 10	For 2+10 Bend 25	For 2+20 Bend 10	For 2+20 Bend 25	CLL	% ZAP-70	For 2+10 Bend 10	For 2+10 Bend 25	For 2+20 Bend 10	For 2+20 Bend 25
#24	2%	0.70	1.05	0.52	0.44	#6	30%	1.11	0.36	0.73	0.46
#19	12%	0.53	0.83	0.36	0.61	#100	100%	0.77	0.82	0.90	0.80
#23	0.4%	0.94	0.52	0.40	0.50	#74	64%	0.70	0.79	0.76	0.55
#20	19%	0.54	0.64	0.81	0.82	#42	72%	0.93	0.73	0.71	0.75
#27	3%	0.56	0.57	0.45	0.46	#18	29%	0.14	0.04	0.1	0.04
#21	4%	0.26	0.50	0.10	0.24	#17	41%	1.02	1.10	0.98	1.10
#12	10%	0.65	0.28	0.59	0.34	#10	30%	0.51	0.49	0.27	0.20
#15	25%	0.63	0.59	0.25	0.19	#36	42%	0.35	0.51	0.29	0.26
#50	1%	0.31	0.27	0.46	0.43	#16	32%	0.85	0.83	0.30	0.27
$ZAP\text{-}70^{Low}$ MEAN CI		**0.51**	**0.52**	**0.39**	**0.40**	$ZAP\text{-}70^{High}$ MEAN CI		**0.63**	**0.56**	**0.50**	**0.44**

Fig. 5A



Fludarabine/Forodesine combination (μM)

Mean CI = 1.96 (CI>1 is indicative of antogonic effect)

Combination Index of 6 representative CLLs (48h):

CLL	#5	#23	#13	#11	#12	#27
1. Forod 2 + dGuo10 + Fluda	3.75 → 1.62	3.09	1.56	1.31	2.31	1.78
2. Forod 2 + dGuo10 + Fluda	7.5 → 1.81	3.41	1.13	1.52	1.92	2.14
3. Forod 2 + dGuo20 + Fluda	3.75 → 1.29	1.37	1.31	1.45	1.96	1.51
4. Forod 2 + dGuo20 + Fluda	7.5 → 1.23	2.62	1.60	1.42	1.76	1.59

Fig. 5B

Combination index (CI) < 1 is indicative of synergism (Chou &Talalay's method).

Combinations analyzed:	CLL	#3	#74	#1	#87	#42	#6	#92
Rituximab 25 + Forod 2 + dGuo10:		0.64	0.73	0.59	0.13	0.88	0.46	0.72
Rituximab 50 + Forod 2 + dGuo10:		0.56	0.72	0.76	0.13	0.52	0.74	0.77
Rituximab 25 + Forod 2 + dGuo20:		0.51	0.55	0.49	0.12	0.63	0.35	0.60
Rituximab 50 + Forod 2 + dGuo20:								

Combination Index

CI mean = 0.53

Fig. 6