

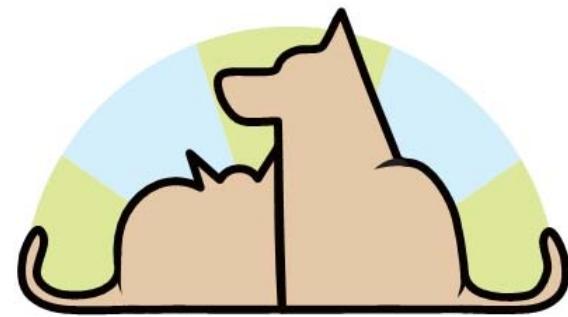
BAUER RESEARCH FOUNDATION

## Nutritional Based Anti-tumor therapy:

### Designing a “Trojan Horse” for the targeted destruction of tumor cells

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# Background



# Vitamin B12 concentrates in tumors

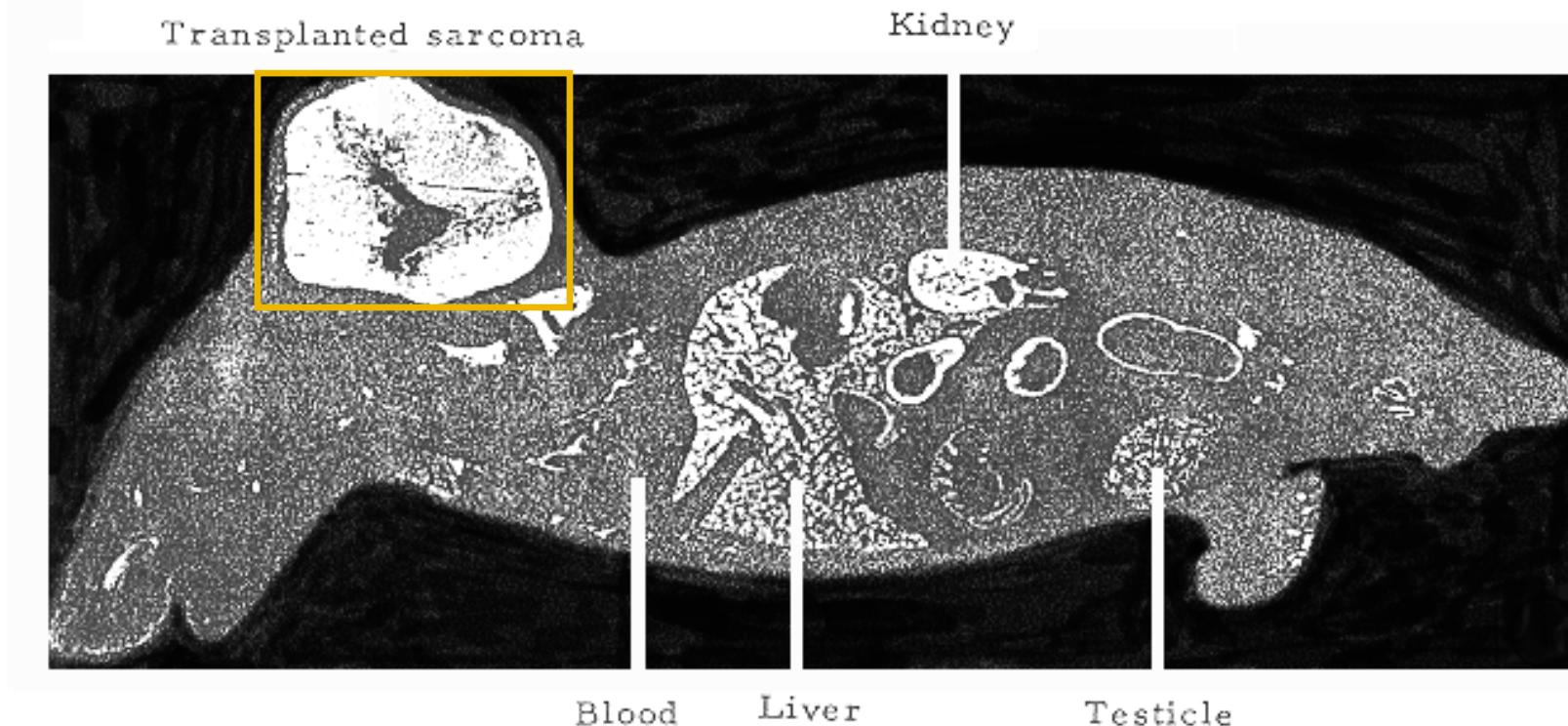


FIGURE 1

Autoradiogram of a mouse carrying a large transplanted  $^{90}\text{Sr}$ -induced soft fibroblastic osteosarcoma in the neck region. The animal was killed 4 days after an intravenous injection of vitamin B<sub>12</sub>-- $^{60}\text{Co}$ . Note high accumulation in the actively growing peripheral parts of the sarcoma. Accumulation is also seen in the gastric and intestinal mucosa, renal cortex and testicle.

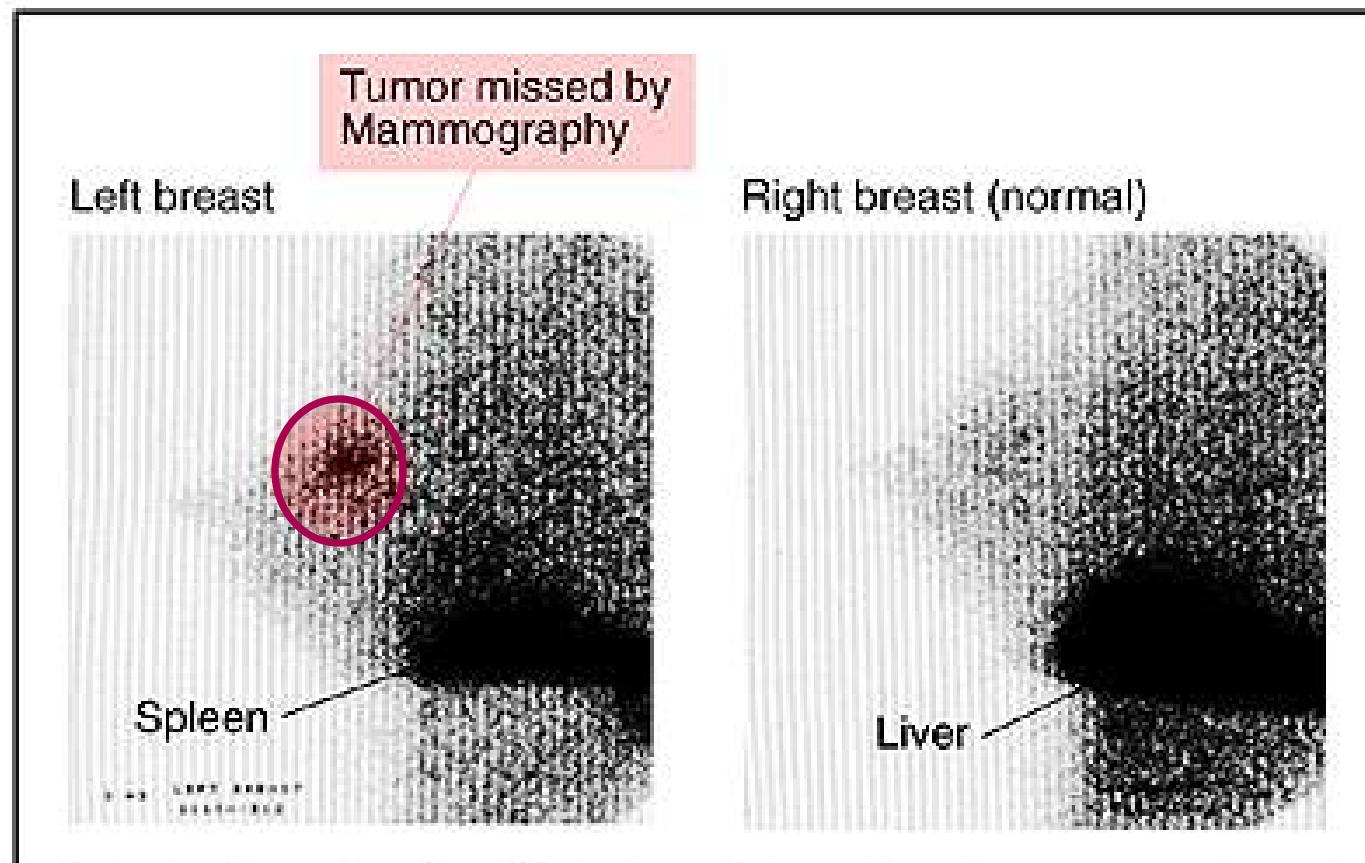
**Flodh and Ullberg Int J Cancer 3:694, 1968**



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Radiolabeled vitamin B12 as a tumor imaging agent



Smaller dark area shown in left B12 enhanced image shows breast tumor undetected by mammogram. Right image shows normal breast. Both images are side views.

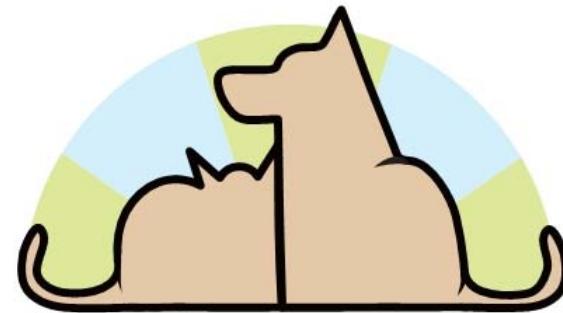
Collins DA et al. Mayo Clin Proc 2000; 75: 568-80



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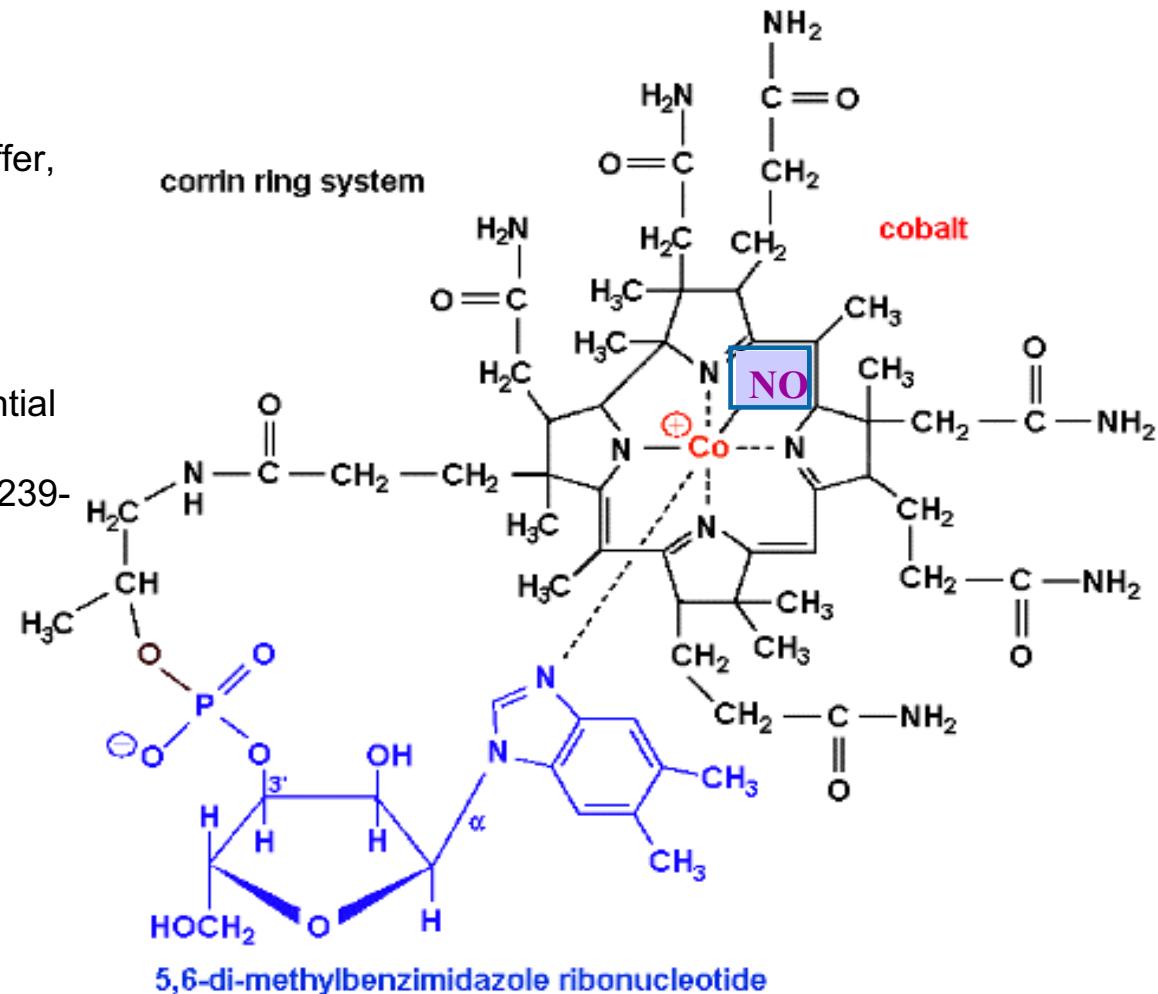
Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

## Synthesis and Characterization



# Nitrosylcobalamin

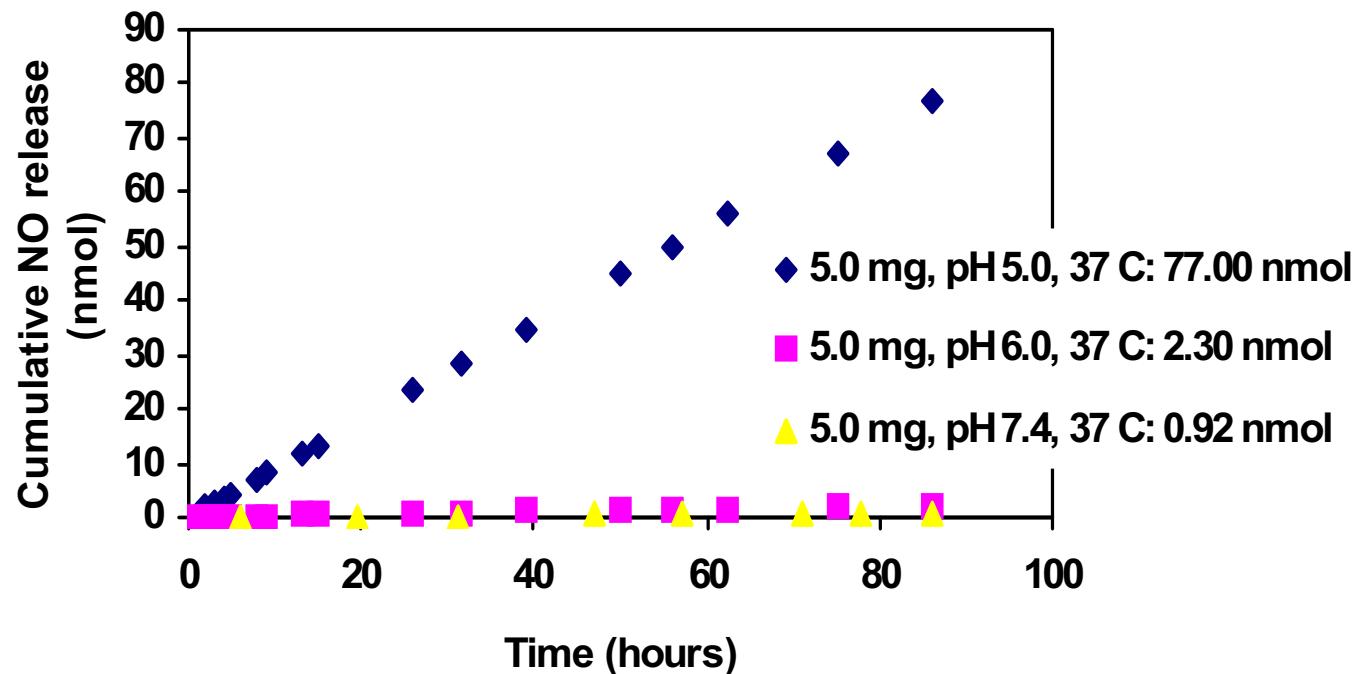
- Half-life ( $t_{1/2}$ ) in acetate buffer, pH 4.9 is 210 h at 37°C.
- Bauer JA. Synthesis, characterization and nitric oxide release profile of nitrosylcobalamin: a potential chemotherapeutic agent. *Anticancer Drugs* 1998;9:239-44.



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# pH- dependent NO release profile of nitrosylcobalamin (chemiluminescence headspace-gas analysis)



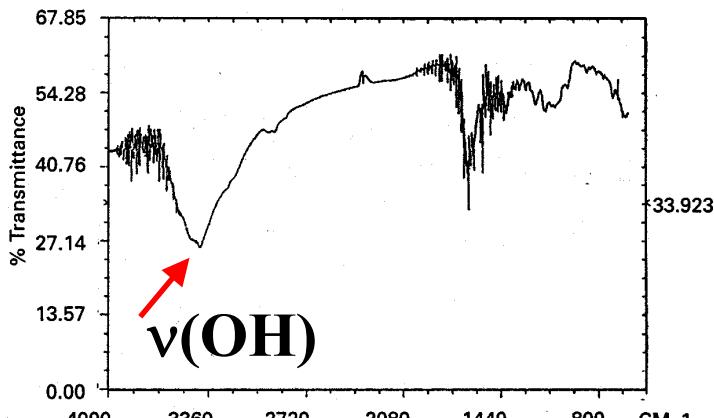
Braman RS, Hendrix SA. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium (III) reduction with chemiluminescence detection. Anal Chem 1989;61:2715-8.



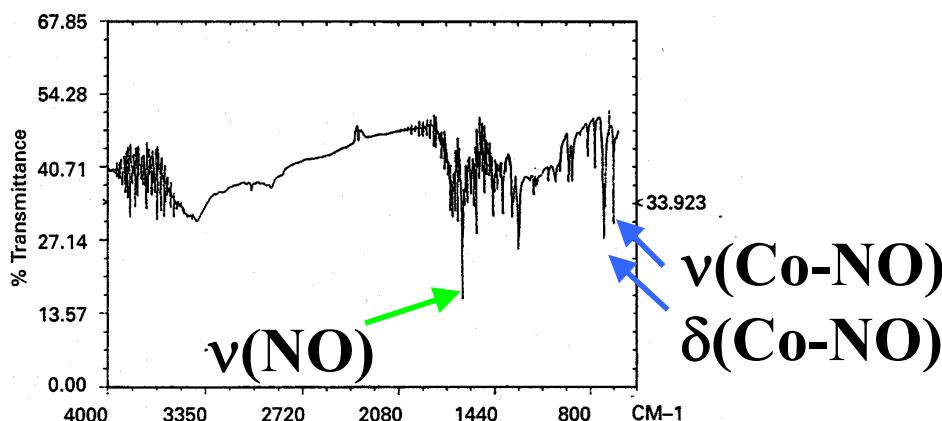
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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# IR spectra



The IR spectra of hydroxocobalamin (KBr pellet).



The IR spectra of nitrosylcobalamin (KBr pellet).

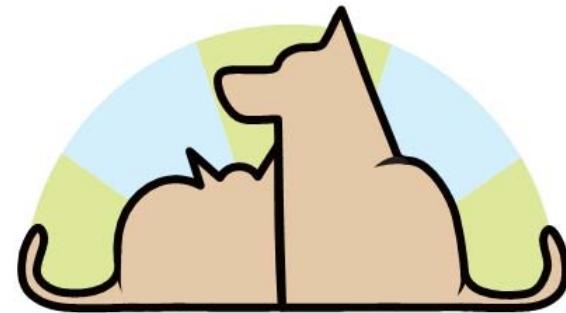
Assignments based on: Nakamoto K. Infrared and Raman Spectra of Inorganic and Coordination Compounds. New York: John Wiley and Sons; 1997.



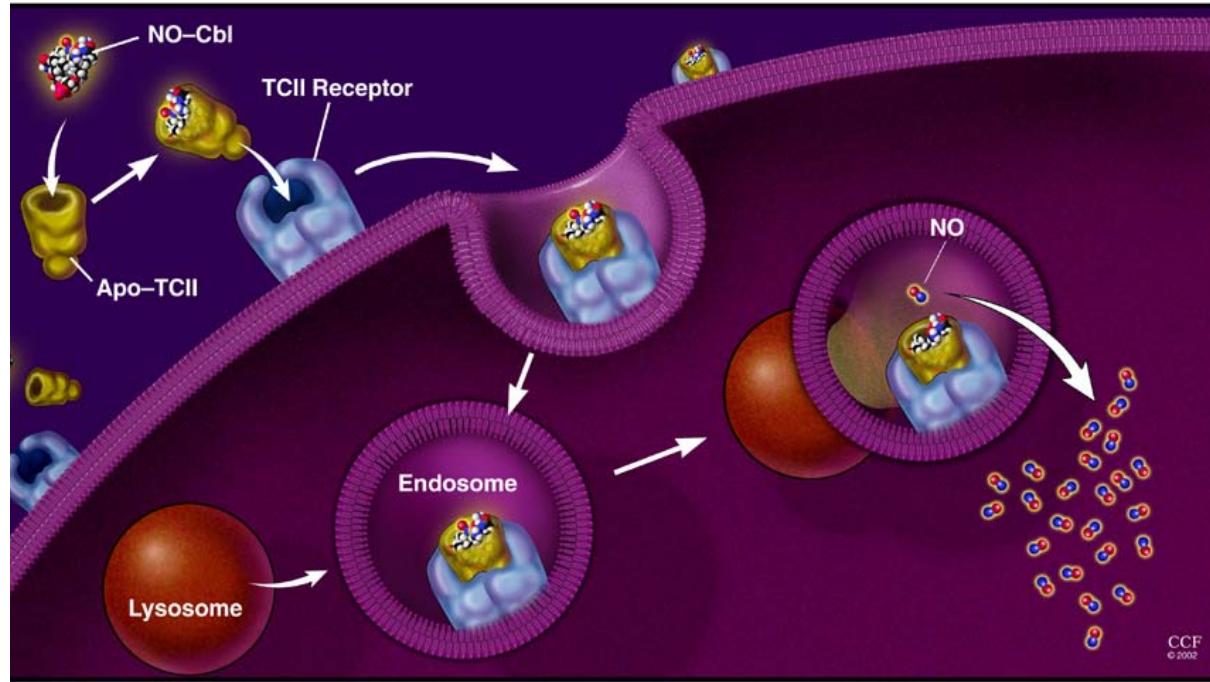
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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

## *In vitro* anti-tumor activity of NO-Cbl



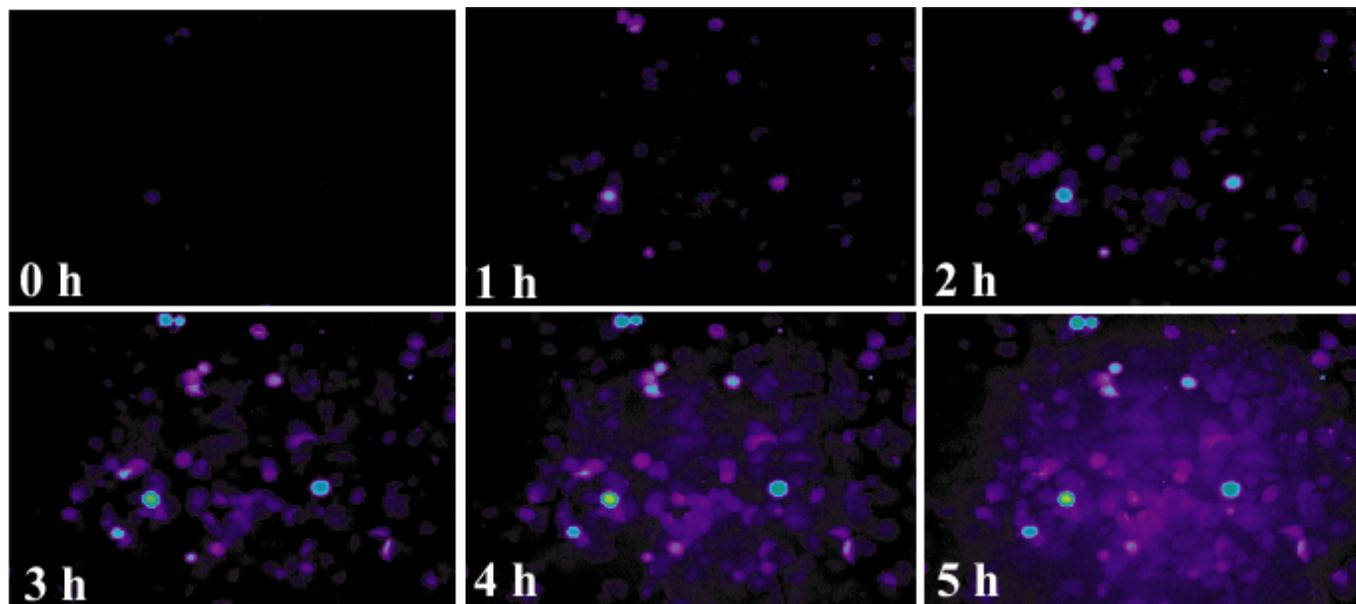
# Receptor mediated uptake of NO-Cbl



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Nitric oxide released in NIH-OVCAR-3 cells from NO-Cbl: 5 h time lapse



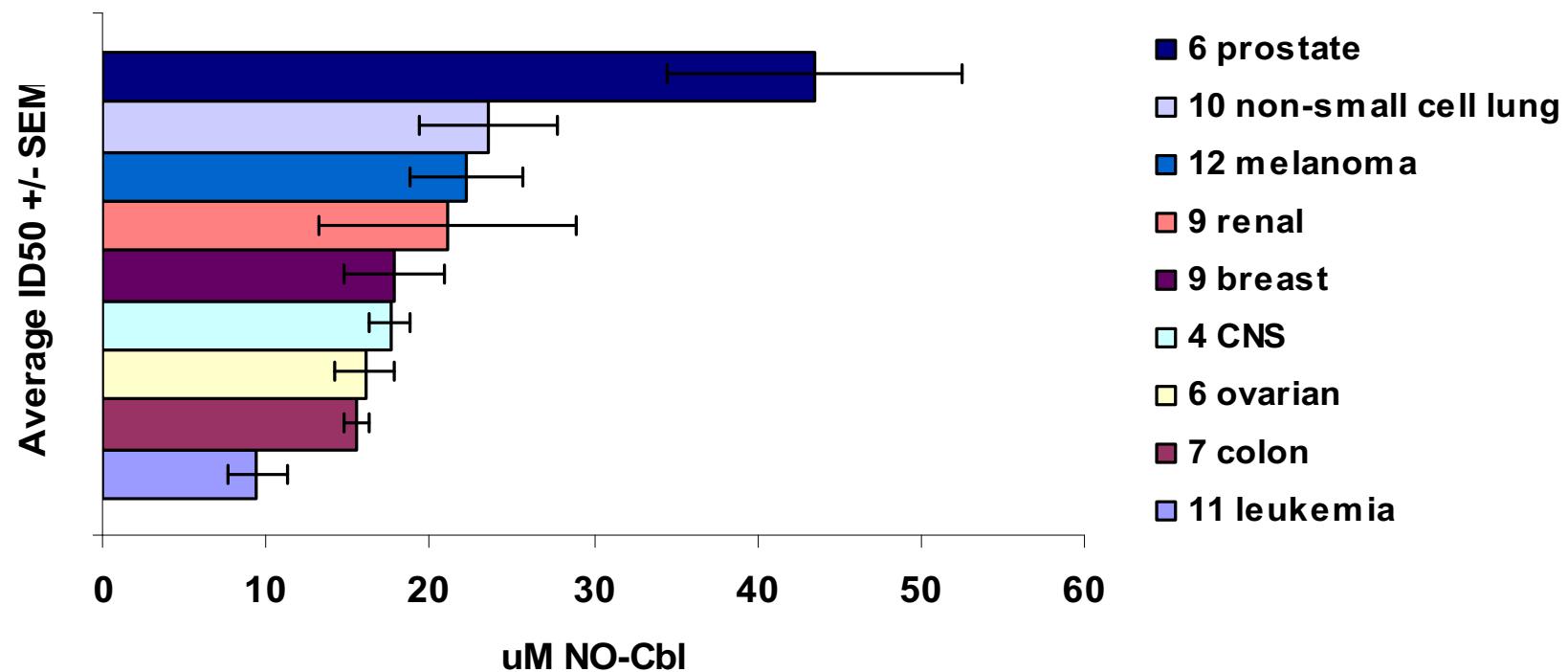
Method: Confocal Microscopy with fluorescence detection  
DAF-FM indicator dye (Molecular Probes)



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Nitrosylcobalamin (NO-Cbl): a vitamin B12  
based anti-tumor agent

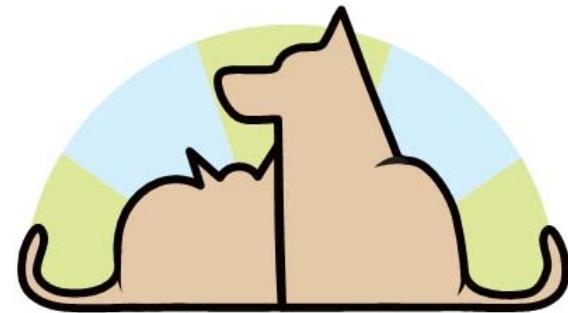
# NCI/DTP program *in vitro* testing results



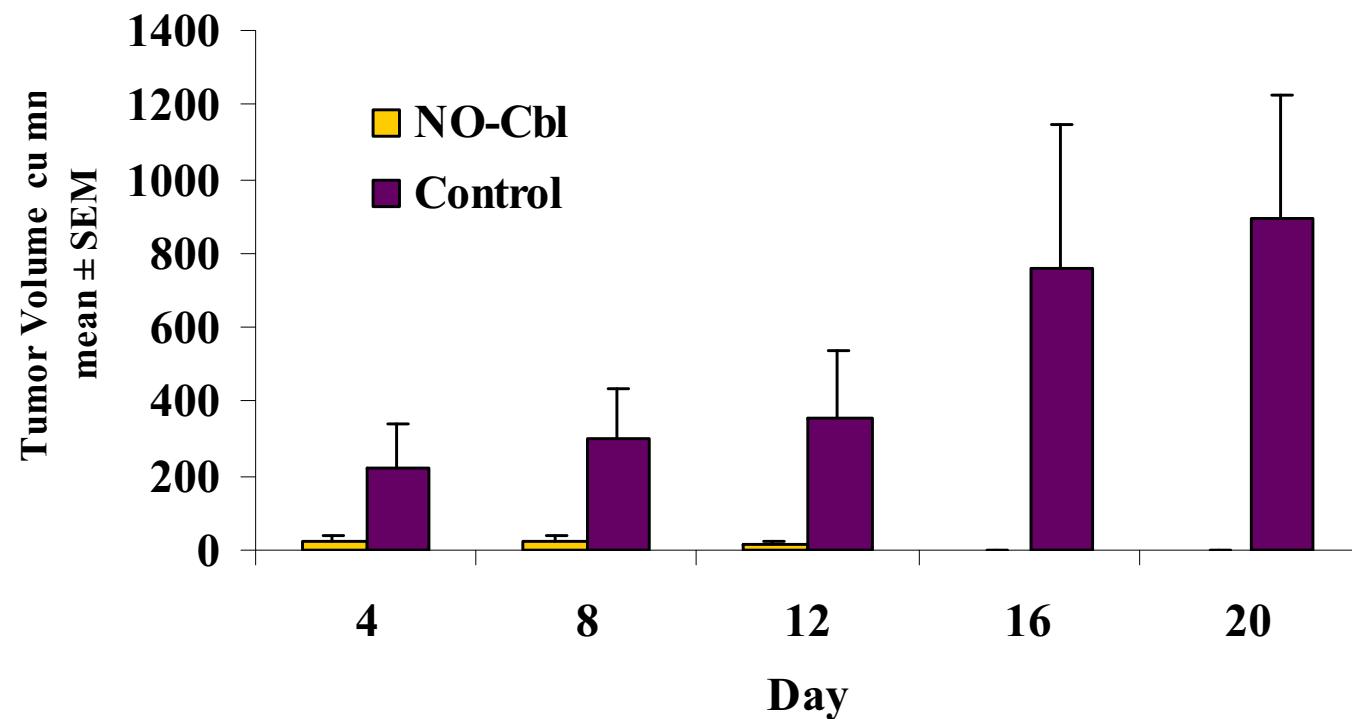
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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

## *In vivo* anti-tumor activity of NO-Cbl



# MCF-7 human breast carcinoma grown in athymic nude mice (n=4)



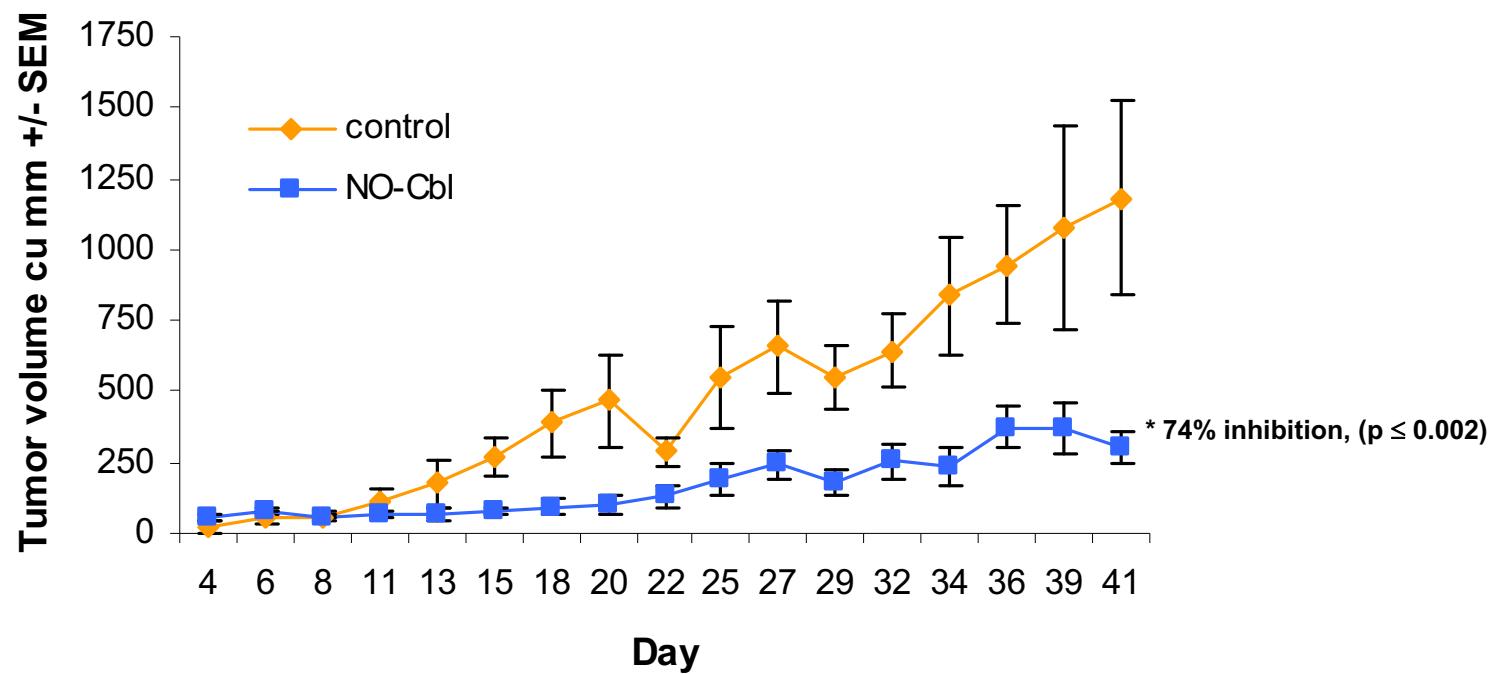
dose = 3 mg NO-Cbl (100 mg/kg) or vehicle i.p. q.d. starting day 2



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# DU145 human prostate carcinoma grown in athymic nude mice (n=4)



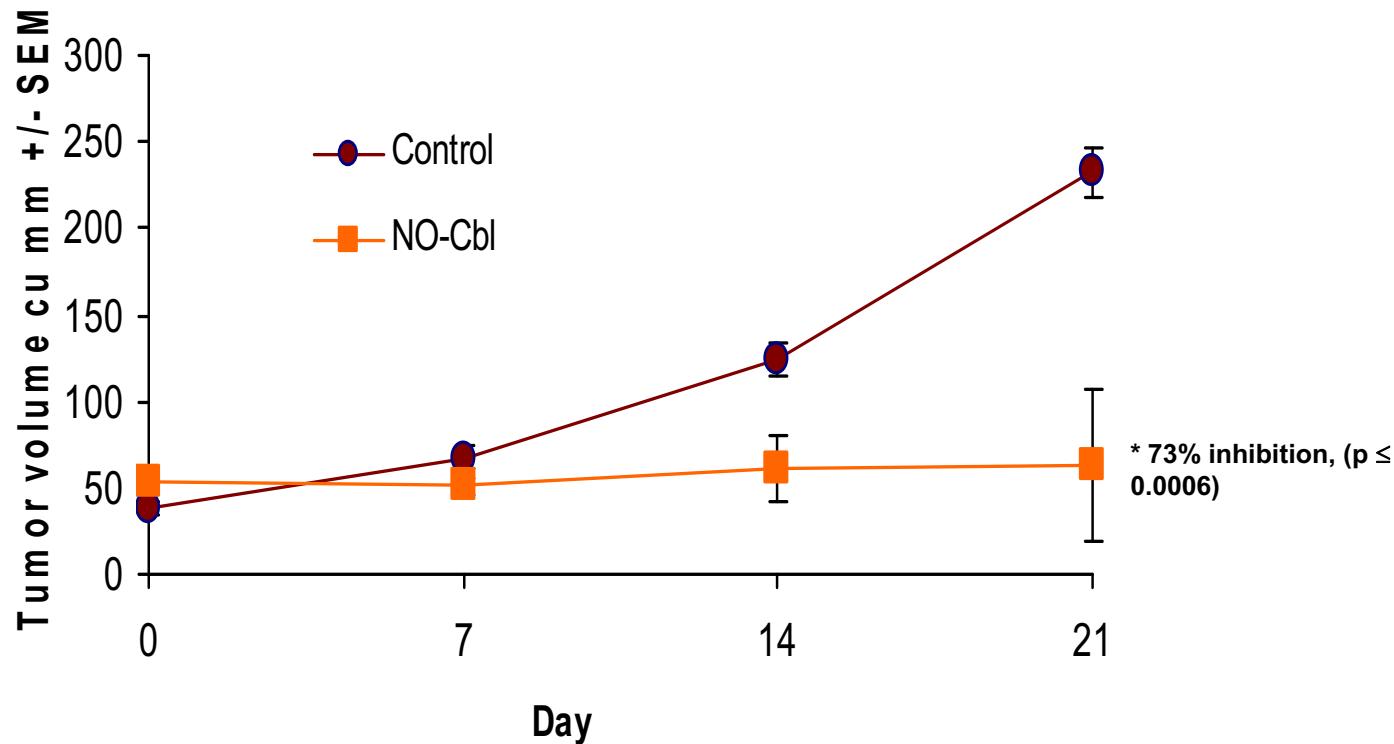
5 mg NO-Cbl, (165 mg/kg) i.p. q.d., starting day 2



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# C4-2 prostate carcinoma grown in athymic nude mice (n=20)



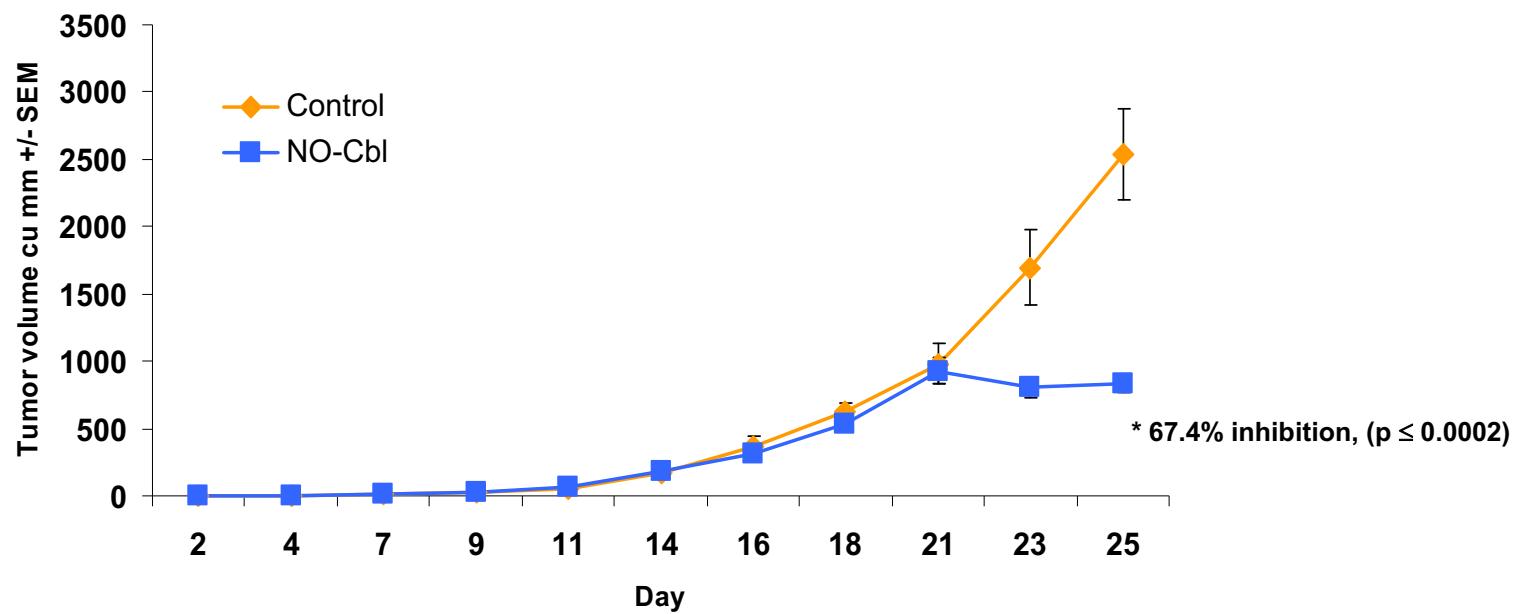
5 mg NO-Cbl, (165 mg/kg) i.p. q.d., starting day 2



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Effect of NO-Cbl on A375 human melanoma xenografts (n=8)



5 mg NO-Cbl, (165 mg/kg) i.p. b.i.d, starting day 2



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Bichon Frise Case Study – Tufts CVM & Angell Animal Med Center

Oscar

Male, age 10, weight 8 kg

Bichon frise

Diagnosis: anal sac adenocarcinoma

Treatment with NO-Cbl resulted in 43% reduction of the primary tumor and 90% reduction of the iliac lymph node.



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Bichon Frise Case Study – Tufts CVM & Angell Animal Med Center

Treatment began on July 26, 2004  
initial dosage was 28 mg/kg, or 255 mg/day

## Bichon Frise

	Week	3 8/13/2004	5 8/31/2004	10 9/30/2004	12 10/14/2004	15 11/5/2004	21 12/17/2004	24 1/5/2005	26 1/22/2005	29 2/14/2005	33 3/16/2005	35 3/30/2005	37 4/13/2005	39 4/28/2005
ALKP(5-131 U/L)		148	121	83	na	70	88	83	85	93	69	166	n/a	114
ALT (12-118 U/L)		246	263	130	na	103	121	137	104	115	122	89	n/a	122
AST (15-66 U/L)		52	43	30	na	29	42	49	43	40	39	54	n/a	41
BUN (6-25 mg/dL)		49	74	92	74	80	86	77	70	81	81	55	82	51
Creatinine (0.5-1.6)		0.8	1	1.1	1	1	1.1	1	1	1.2	1.3	1.1	1.2	1.5
Calcium (8.9-11.4 mg/dL)		10.5	10.5	11	10.6	10.6	10.9	10.3	10.2	10.6	10.3	9.9	10.4	10.1
Glucose (71-120 mg/dL)		97	83	96	na	103	92	85	92	94	83	87	n/a	105
Sodium (146-155 mEq/L)		152	151	150	151	154	153	149	149	150	151	150	151	152
Potassium (3.7-5.1 mEq/L)		4.3	4.1	3.9	3.9	4.4	4.6	4.1	4.7	4.5	3.9	4.4	4.3	3.9
Chloride (106-118 mEq/L)		116	117	114	116	116	112	113	113	111	115	115	113	116
Co2 (16.1-28.2 meq/L)		17.1	17	18.7	16.9	23	23	18.9	19.4	22	19.8	16.3	17.1	n/a
Cholesterol (119-341 mg/dL)		238	203	212	na	216	254	206	210	236	191	235	n/a	230
Magnesium 1.6-2.5 mEq/L		2.2	2.3	2.7	na	2.6	2.8	2.7	2.9	3	3	3	n/a	2.8
WBC (6.0-14.3 x10^3/ul)		10.4	7.9	6.1	na	8.6	7.4	na	9.9	7.8	6.7	9.2	8.5	8.8
RBC (5.8-8.9x10^6/ul)		6.27	6.54	6.51	na	6.03	6.29	na	6.26	5.76	6.12	5.73	5.9	5.72
lymphocytes (1.0-3.9x10^3/ul)		1	0.9	0.8	na	0.681	0.666	na	1	0.227	0.7	0.9	1	1.232
monocytes (0.1-0.9 x 10^3/ul)		0.6	0.4	0.5	na	0.511	0.222	na	0.6	0.303	0.5	0.8	0.6	0.616
eosinophils (0.0-1.2 x 10^3/ul)		0.2	0.4	0.3	na	0.596	0.148	na	0.5	0.227	0.3	0.2	0.4	0.176
basophils (0.0-0.1 x 10^3/ul)		0	0	0	na	0.085	na	na	0	na	0	0	0	0
platelet count (161-513 x 10^3/ul)		469	523	466	na	537	478	na	550	509	502	494	549	487

High  
Low  
Carboplatin 150 mg

	Carboplatin			Carboplatin			Carboplatin			Carboplatin		
Week	42 5/19/2005	43 5/26/2005	44 6/2/2005	45 6/9/2005	48 6/29/2005	49 7/6/2005	50 7/11/2005	51 7/19/2005	52 7/28/2005	54 8/05/2005	55 8/10/2005	56 8/17/2005
ALKP(5-131 U/L)	n/a	148	167	n/a	n/a	107	106	n/a	n/a	n/a	n/a	n/a
ALT (12-118 U/L)	n/a	181	260	n/a	n/a	138	179	n/a	n/a	n/a	n/a	n/a
AST (15-66 U/L)	n/a	72	64	n/a	n/a	51	49	n/a	n/a	n/a	n/a	n/a
BUN (6-25 mg/dL)	n/a	129	98	n/a	n/a	80	82	n/a	n/a	n/a	n/a	n/a
Creatinine (0.5-1.6)	n/a	1.6	1.2	n/a	n/a	1.1	1.2	n/a	n/a	n/a	n/a	n/a
Calcium (8.9-11.4 mg/dL)	n/a	10.6	9.9	n/a	n/a	10	10.9	n/a	n/a	n/a	n/a	n/a
Glucose (71-120 mg/dL)	n/a	103	90	n/a	n/a	94	94	n/a	n/a	n/a	n/a	n/a
Sodium (146-155 mEq/L)	n/a	153	155	n/a	n/a	153	151	n/a	n/a	n/a	n/a	n/a
Potassium (3.7-5.1 mEq/L)	n/a	3.7	3.7	n/a	n/a	4.2	3.6	n/a	n/a	n/a	n/a	n/a
Chloride (106-118 mEq/L)	n/a	116	118	n/a	n/a	117	116	n/a	n/a	n/a	n/a	n/a
Co2 (16.1-28.2 meq/L)	n/a	18.3	18.1	n/a	n/a	20.1	20.6	n/a	n/a	n/a	n/a	n/a
Cholesterol (119-341 mg/dL)	n/a	246	223	n/a	n/a	207	202	n/a	n/a	n/a	n/a	n/a
Magnesium 1.6-2.5 mEq/L	n/a	3.5	2.9	n/a	n/a	3.2	2.8	n/a	n/a	n/a	n/a	n/a
WBC (6.0-14.3 x10^3/ul)	10.8	5.2	0.9	10.2	n/a	19.5	8.7	16.5	8.8	3	11.8	14.2
RBC (5.8-8.9x10^6/ul)	5.51	5.05	5.11	5.06	n/a	4.81	4.79	4.78	4.37	4.65	4.85	5.04
lymphocytes (1.0-3.9x10^3/ul)	1.1	0.5	0.5	0.8	n/a	1.2	0.8	2.1	0.4	1.08	0.4	1.6
monocytes (0.1-0.9 x 10^3/ul)	0.6	0.1	0.2	1	n/a	0	0.3	1.9	0.1	0.33	1.2	1.3
eosinophils (0.0-1.2 x 10^3/ul)	0.3	0.1	0	0.1	n/a	0.2	0.7	0.1	0.2	0.15	0.1	0.1
basophils (0.0-0.1 x 10^3/ul)	0	0	0	0	n/a	0	0	0.1	0	0	0	0
platelet count (161-513 x 10^3/ul)	411	392	240	706	n/a	884	150	720	434	462	677	682



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Bichon Frise Case Study – Tufts CVM & Angell Animal Med Center

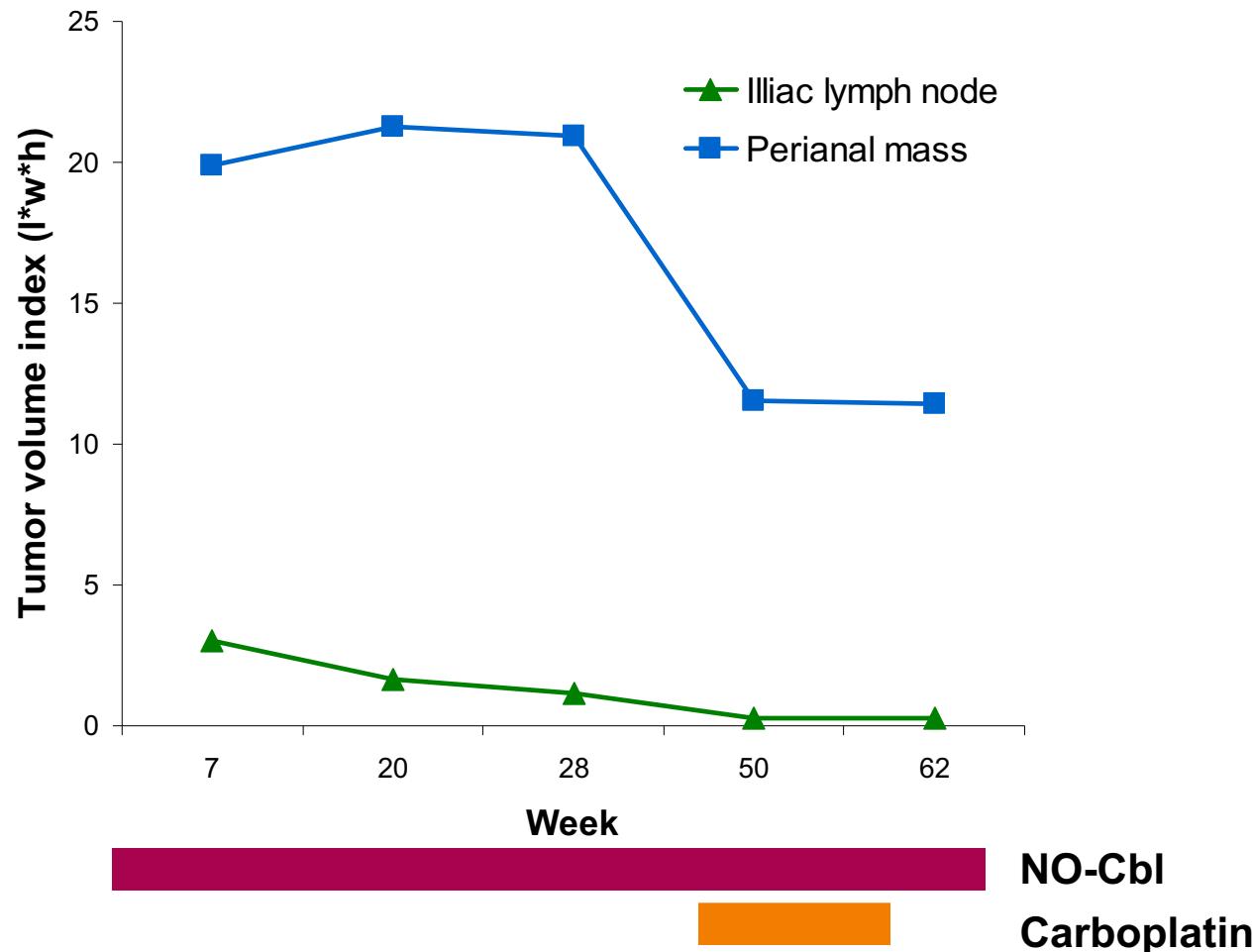
Week	57	58	60	62	63	69	77
	8/24/2005	9/1/2005	9/15/2005	9/28/2005	10/5/2005	11/16/2005	1/11/2006
ALKP(5-131 U/L)	124	n/a	133	101	n/a	100	78
ALT (12-118 U/L)	141	n/a	176	117	n/a	94	109
AST (15-66 U/L)	42	n/a	33	50	n/a	16	22
BUN (6-25 mg/dL)	61	n/a	86	74	n/a	79	102
Creatinine (0.5-1.6)	1.2	n/a	1.3	1.2	n/a	1.1	1.3
Calcium (8.9-11.4 mg/dL)	10	n/a	9.7	9.9	n/a	10.9	10.6
Glucose (71-120 mg/dL)	87	n/a	97	85	n/a	98	83
Sodium (146-155 mEq/L)	153	n/a	153	154	n/a	149	153
Potassium (3.7-5.1 mEq/l)	4.4	n/a	4.3	3.8	n/a	4	4
Chloride (106-118 mEq/L)	117	n/a	116	117	n/a	112	114
Co2 (16.1-28.2 meq/L)	17.4	n/a	21.4	19.5	n/a	22.6	18.9
Cholsterol (119-341 mg/dL)	181	n/a	199	183	n/a	231	226
Magnesium 1.6-2.5 mEq/L)	2.8	n/a	3.1	3	n/a	3.2	3.3
WBC (6.0-14.3 x10^3/ul)	6.8	2.2	11.7	5.9	4.3	8	8.1
RBC (5.8-8.9x10^6/ul)	4.96	4.93	5.08	5	5.12	5.05	5.79
lymphocytes (1.0-3.9x10^3/ul)	0.3	0.3	0.6	0.6	0.9	1.4	1.1
monocytes (0.1-0.9 x 10^3/ul)	0	0	1.5	0.1	0.8	0.6	0.7
eosinophils (0.0-1.2 x 10^3/ul)	1	1	0.4	0.3	0	0.3	0.7
basophils (0.0-0.1 x 10^3/ul)	0	0	0	0	0	0.1	0
platelet count (161-513 x 10^3/ul)	529	529	695	210	351	574	420



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

## Bichon Frise Case Study – Tufts CVM & Angell Animal Med Center

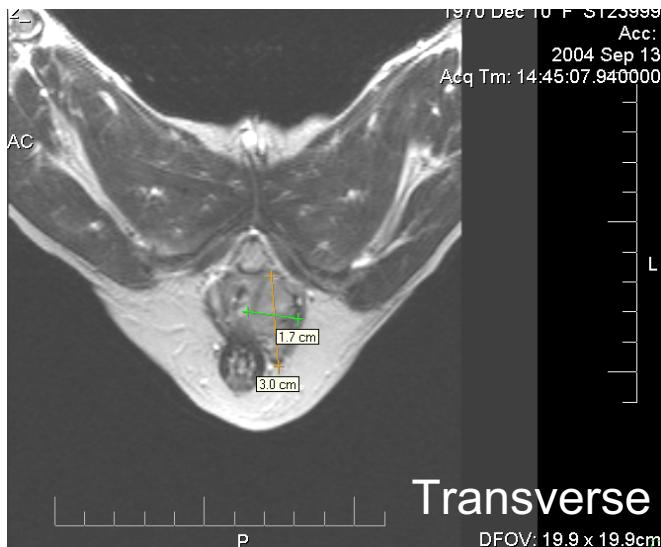


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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Bichon Frise Case Study – Tufts CVM & Angell Animal Med Center

A) Baseline (week 7)



B) Week 62



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Giant Schnauzer Case Study – Ohio State University CVM

Gertie

Female, Age 13, weight 38.5 kg

Giant Schnauzer

Diagnosis: thyroid adenocarcinoma

Ten months of treatment with NO-Cbl  
resulted in a 77% reduction in the primary  
tumor.



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Nitrosylcobalamin (NO-Cbl): a vitamin B12  
based anti-tumor agent

# Giant Schnauzer Case Study – Ohio State University CVM

Giant Schnauzer Week	Treatment began on August 30, 2004				
	0 4/13/04	1 8/26/2004	3 9/7/2004	10 9/20/04	11/8/2004
ALKP(5-131 U/L)	135	163	154	149	175
ALT (12-118 U/L)	45	39	33	20	40
AST (15-66 U/L)	27	22	18	27	66
BUN (6-25 mg/dL)	18	17	14	16	19
Creatinine (0.5-1.6)	1.3	1.3	1.5	1.1	1.2
Calcium (8.9-11.4 mg/dL)	10.6	12.5	11.4	10.9	12.1
Glucose (71-120 mg/dL)	101	106	123	122	137
Sodium (146-155 mEq/L)	151	152	142	150	147
Potassium (3.7-5.1 mEq/l)	4.4	5	5.4	5.1	4.4
Chloride (106-118 mEq/L)	111	112	106	110	108
Co2 (16.1-28.2 meq/L)	na	na	na	na	na
Cholesterol (119-341 mg/dL)	273	325	283	250	313
Magnesium 1.6-2.5 mEq/L)	1.6	1.8	1.2	1.4	na
WBC (6.0-14.3 x10^3/ul)	na	7.9	10.1	10.7	na
RBC (5.8-8.9x10^6/ul)	na	6.8	6.4	6.8	7.2
lymphocytes (1.0-3.9x10^3/ul)	na	1.58	1.616	1.605	0.9
monocytes (0.1-0.9 x 10^3/ul)	na	0.632	0.606	0.856	0.4
eosinophils (0.0-1.2 x 10^3/ul)	na	0.158	0	0	1
basophils (0.0-0.1 x 10^3/ul)	na	0	0	0	0
platelet count (161-513 x 10^3/ul)	na	456	506	504	na
<b>High</b>					
<b>Low</b>					



**BAUER RESEARCH FOUNDATION**

Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Giant Schnauzer Case Study – Ohio State University CVM

A: Baseline



B: Post-therapy



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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

## Golden Retriever, Buddy – PetsDx, Pittsburgh PA

- 6 year old, neutered male
- Diagnosis: malignant peripheral nerve sheath tumor (MPNST)
- Received NO-Cbl 20 mg/kg for 15 months
- Resulted in 54% reduction in tumor volume



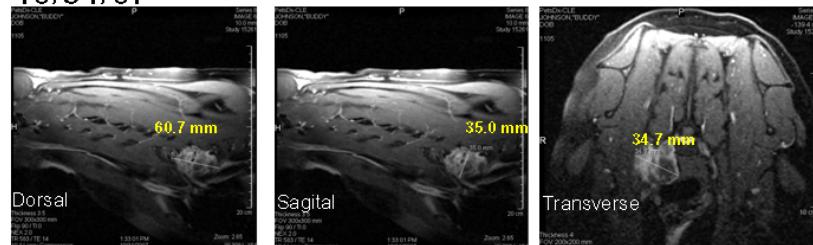
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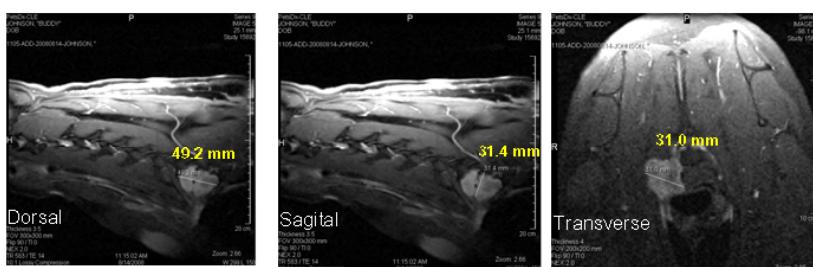
Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

# Golden Retriever, Buddy – PetsDx, Pittsburgh PA

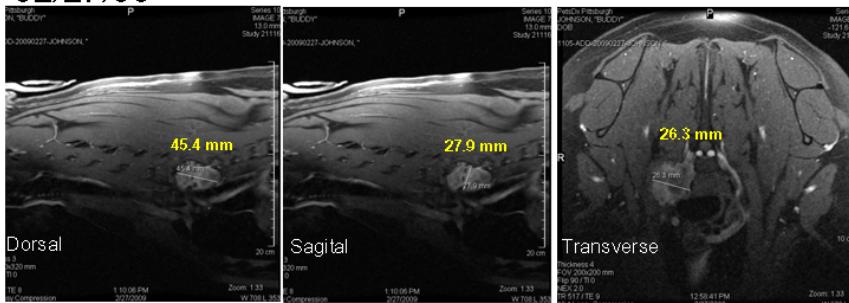
10/31/07



8/14/08



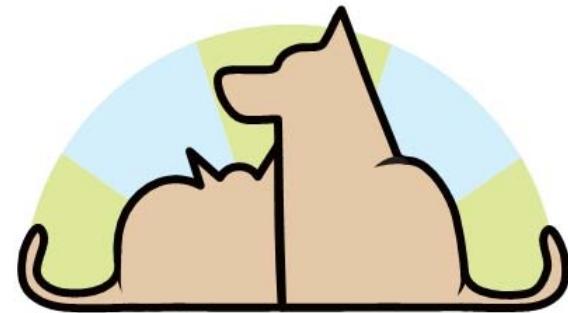
02/27/09

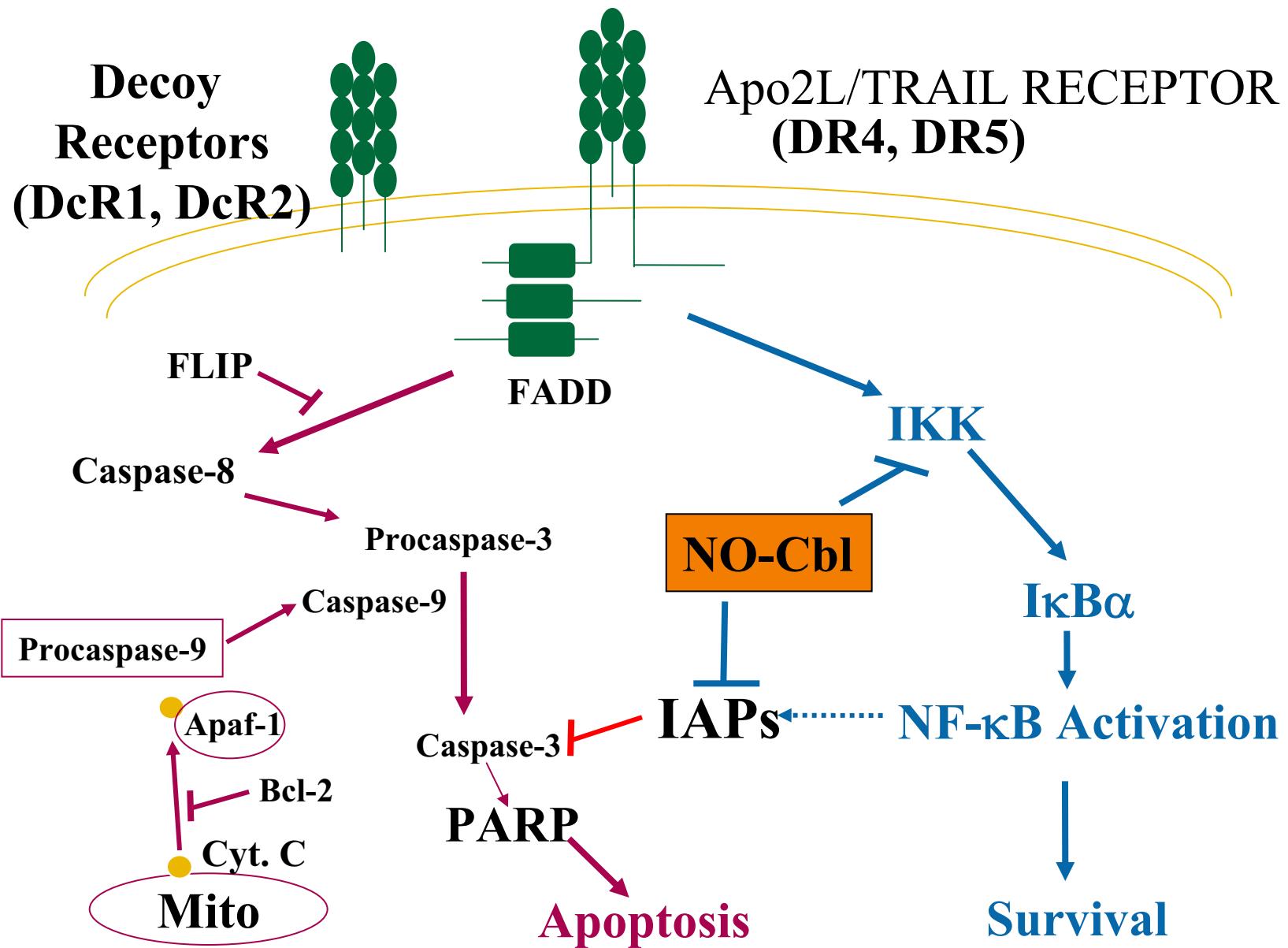


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Nitrosylcobalamin (NO-Cbl): a vitamin B12 based anti-tumor agent

## Anti-tumor pathways and mechanisms

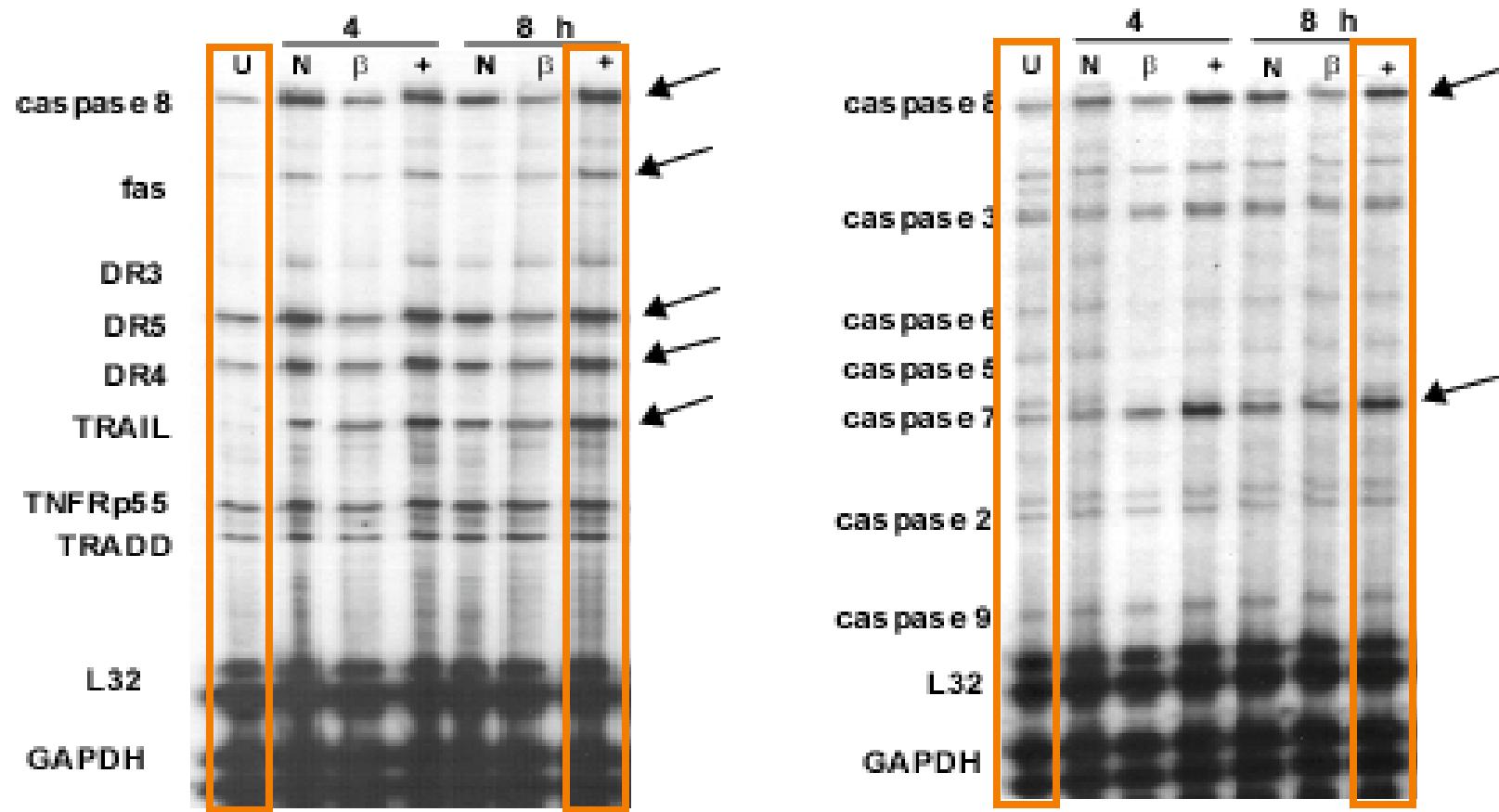




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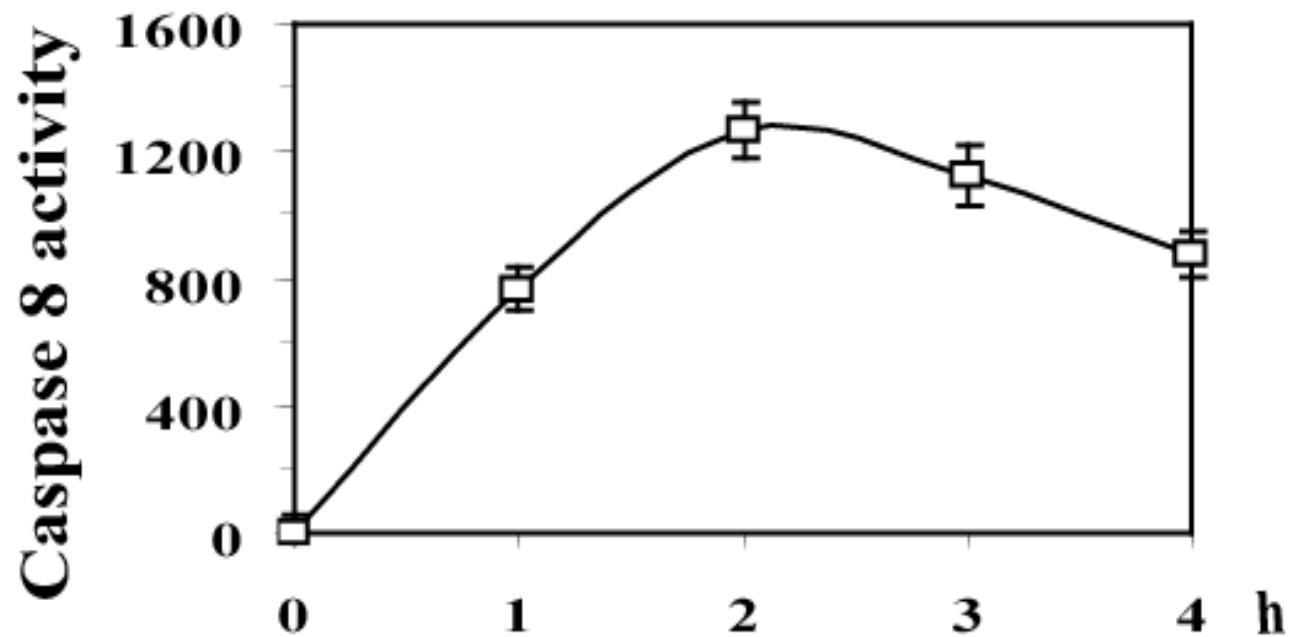
# Expression of death receptor/caspase mRNAs in NIH-OVCAR-3 cells



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## NO-Cbl 10 $\mu$ m activates Caspase-8



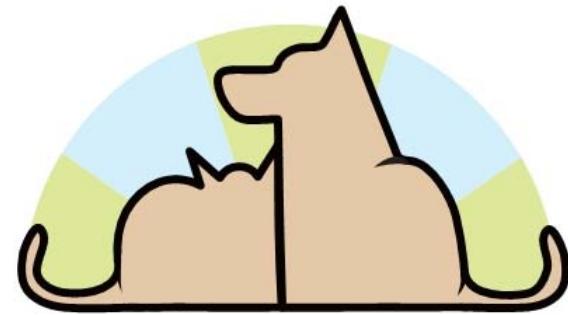
Method: Fluorometric analysis



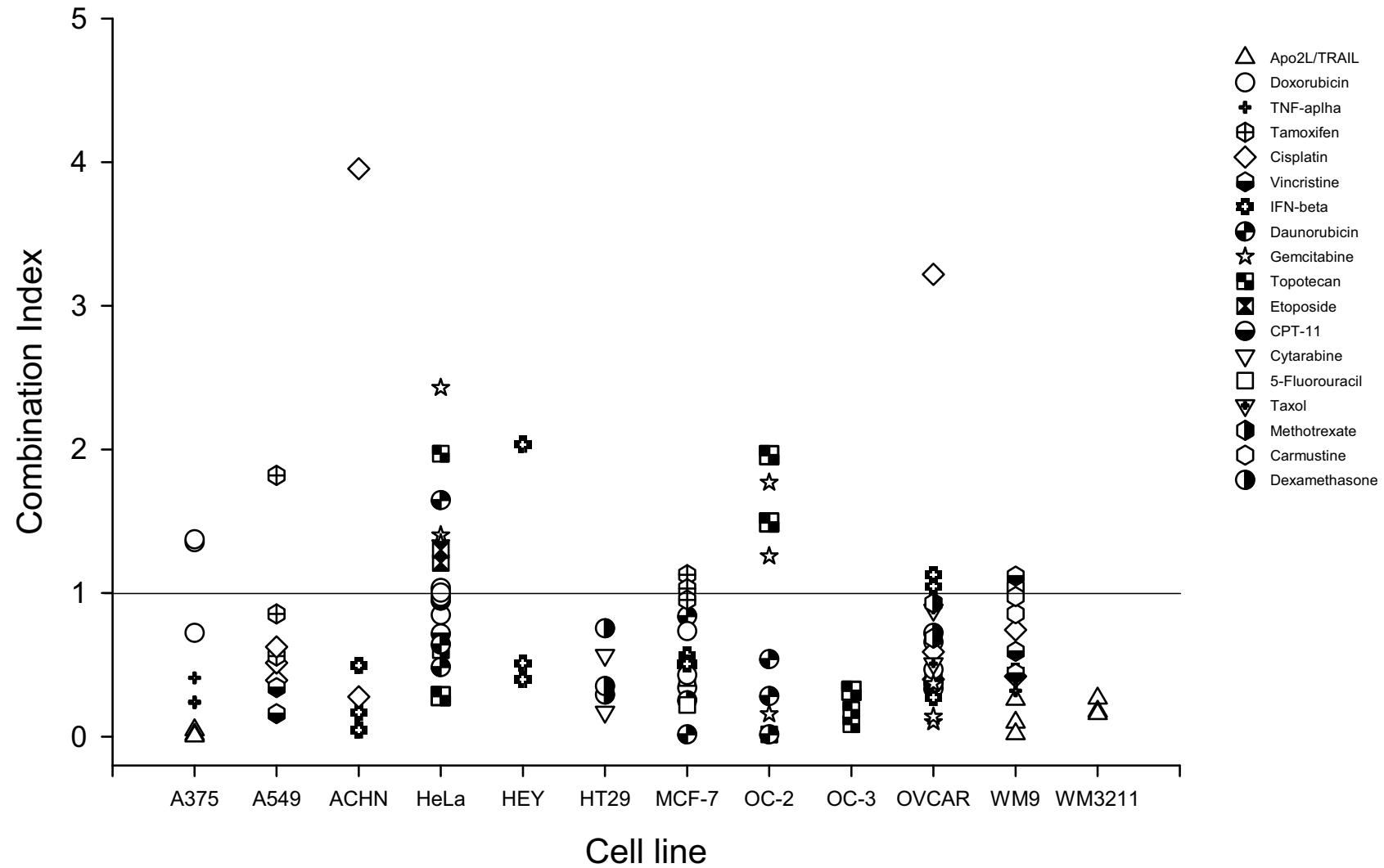
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## Combination therapy with other anti-tumor agents



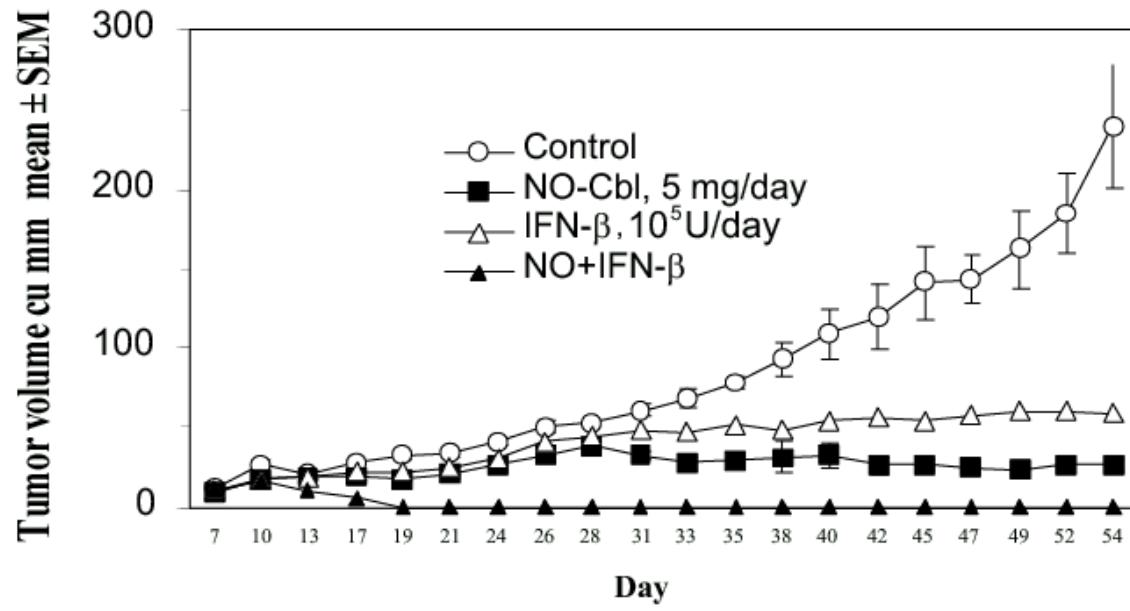
## Synergy---Chemopotentiating effects of NO-Cbl



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# Treatment of established NIH-OVCAR-3 xenografts



Day 0: 2 x 10<sup>6</sup> NIH-OVCAR-3 cells s.c (one tumor per mouse , n=8)

Day 7: NO-Cbl 100 mg/kg i.p., IFN- $\beta$  10<sup>5</sup> U s.c

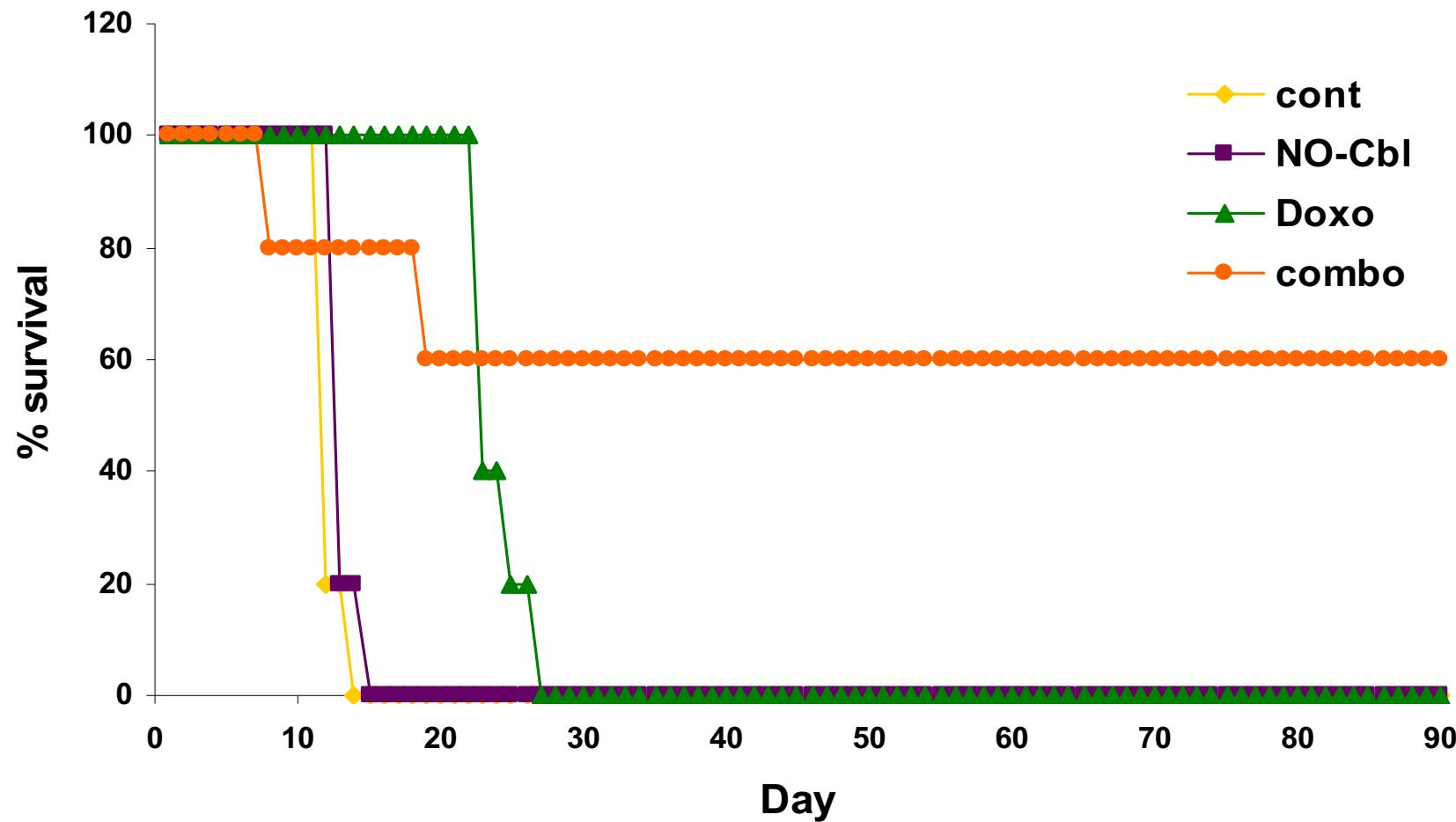
Day 25: combination treatment stopped, single agent treatment continued



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# Treatment of P388 murine leukemia in DBA/2 mice (n=5)



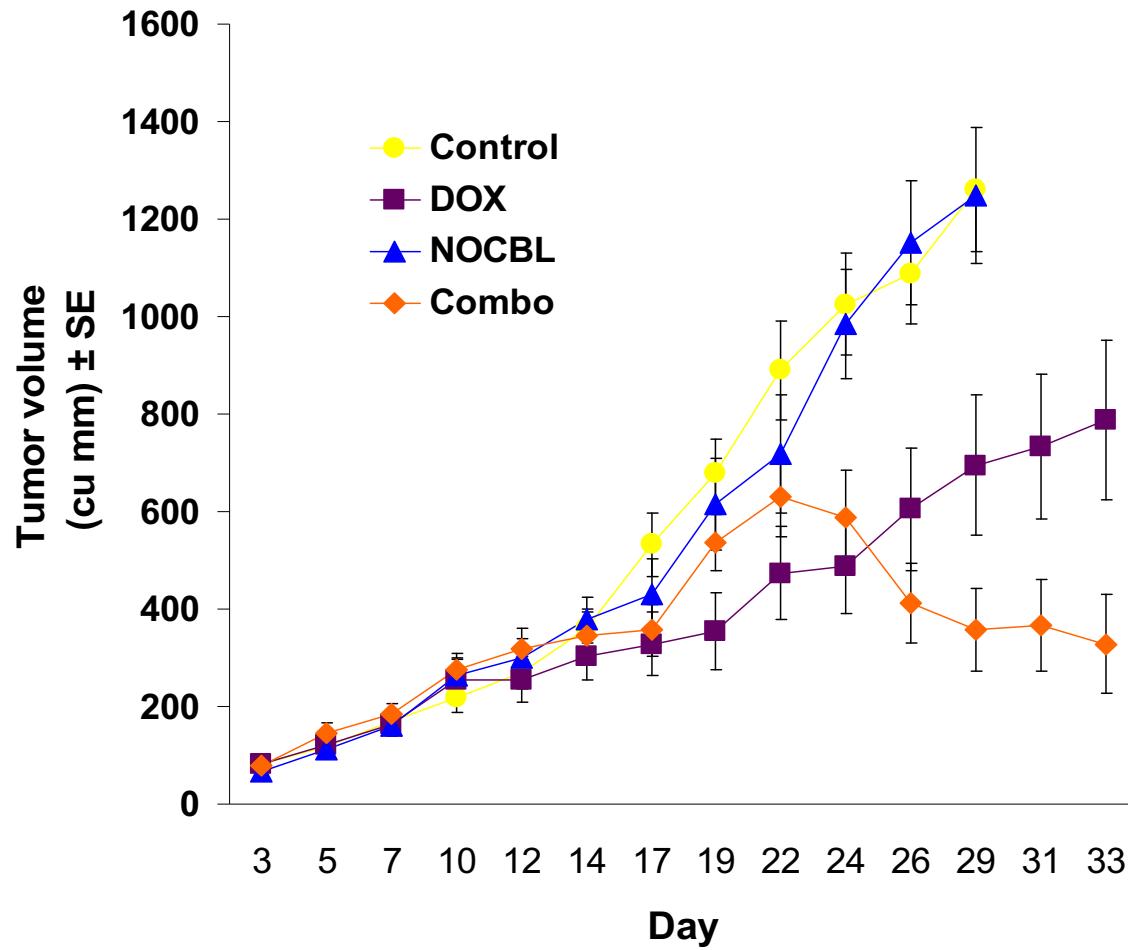
10 mg NO-Cbl, (330 mg/kg) i.p. b.i.d., doxorubicin 4 mg/kg i.p. once weekly



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# Treatment of HeLa cervical carcinoma xenografts (n=8)



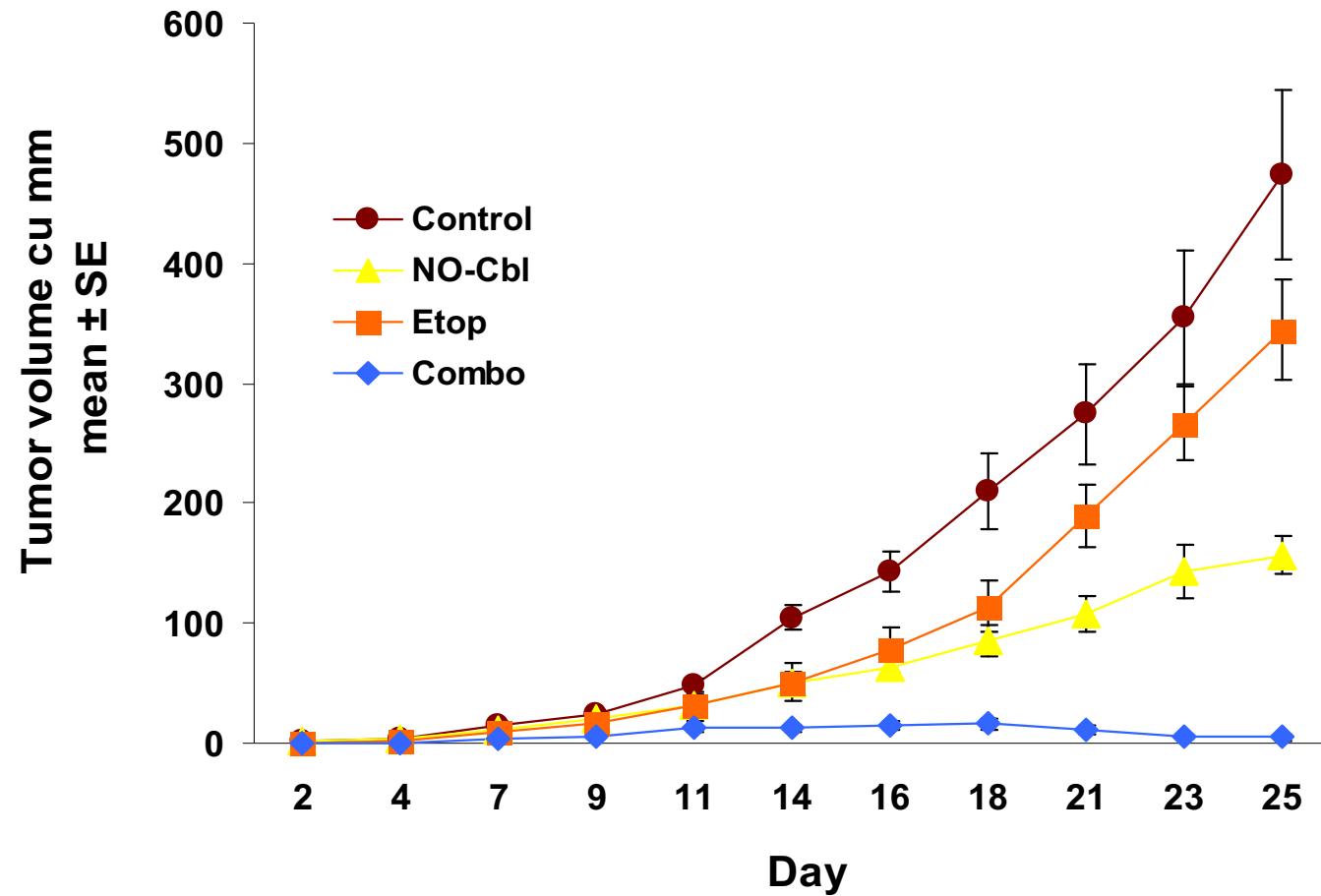
10 mg NO-Cbl, (330 mg/kg) s.c. q.d, doxorubicin 4 mg/kg i.p., once weekly



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# Effect of NO-Cbl and etoposide on NIH-OVCAR-3 ovarian carcinoma xenografts (n=8)



NO-Cbl 100 mg/kg i.p., q.d., etoposide 2 mg/kg i.p. b.i.w.



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