

Clinical/Translational Research

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Types of Clinical Trials

- **Treatment trials**
 - Test new treatments
- **Prevention trials**
 - Test new approaches
- **Screening trials**
 - Test the best way to find cancer, especially in its early stages.
- **Quality of Life trials**
 - Explore ways to improve comfort and quality of life for cancer patients.

PROTOCOL NB1011-1001

PHASE I/II STUDY OF NB1011
ADMINISTERED IV DAILY FOR 5 DAYS
EVERY 4 WEEKS IN FLUOROPYRIMIDINE-
RESISTANT METASTATIC OR RELAPSED
CANCERS

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Los Angeles*

Before getting into the Clinic...

Preclinical Studies:

Drug metabolism

- hepatic microsomes

- metabolism by cytochrome system

Cell based assays

- erythrocyte hemolysis

Human tissue analysis

Non-clinical toxicology

- dose escalation

- two mammalian species

- analysis of toxicology

- including histopathologic analysis of all tissues

Clinical Trials: Phase I

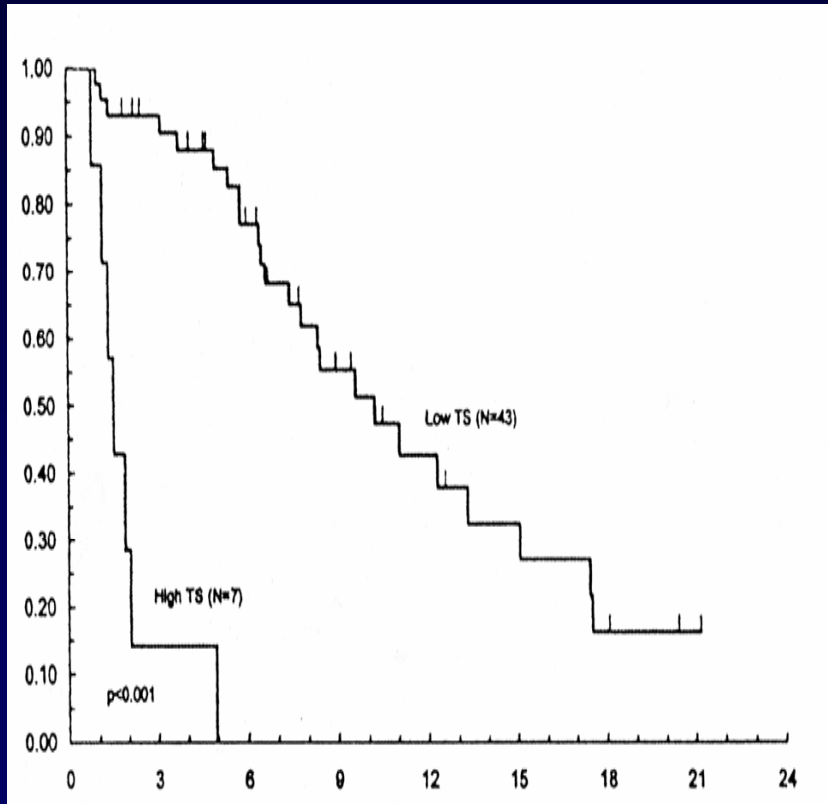
- Phase I clinical trials
 - “First in man”
 - Goals
 - to determine the maximum tolerated dose (MTD)
 - to determine the recommended phase II dose
 - may be minimum effective dose
 - to study pharmacokinetics/pharmacodynamics
 - Patient selection
 - normal volunteers
 - end stage disease
 - Study design
 - empiric dose escalation
 - beginning at 1/10th of the MTD in most sensitive species
 - 3 patients per cohort, unless DLT – then expand to six patients
 - dose escalation
 - » modified Fibonacci sequence (0,1,1,2,3,5,8,13,21,34,...)

Phase I Endpoints, Defined

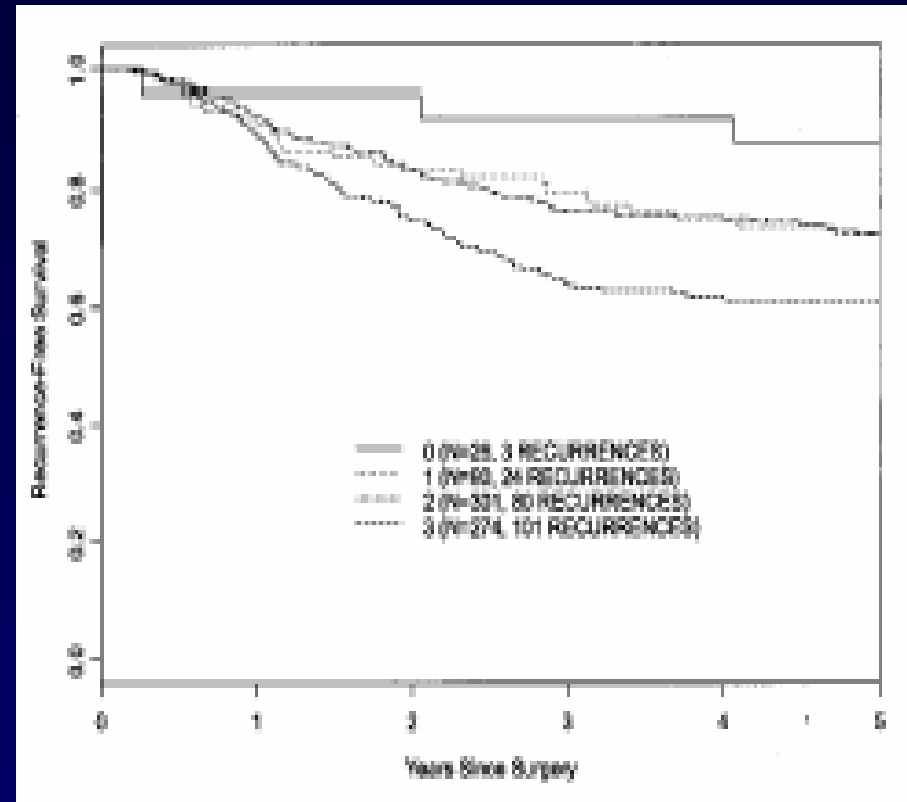
- Dose limiting toxicity
 - drug-related adverse events which are severe or life threatening
 - DLT in 2 of 6 subjects – MTD exceeded
- Maximum tolerated dose
 - often one dose level below the DLT
 - may not be theoretical ideal dose
 - more is not necessarily better

Higher TS Associated with Adverse Prognosis

Estimated Probability of Survival



Months After Treatment Start

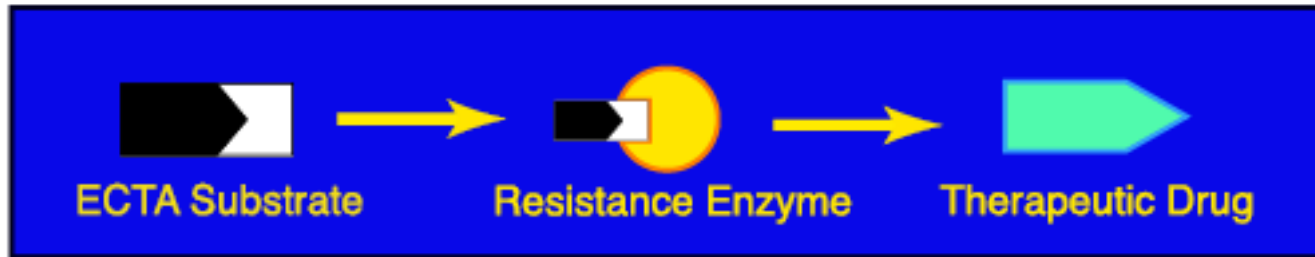


Source: *J. Clin. Oncol.* 19(23):4298-4304, 2001

Carmen J. Allegra, et al., *J Clin Oncol* 21:241-250. © 2003

Enzyme Catalyzed Therapeutic Agents

ECTA: The Basic Principle



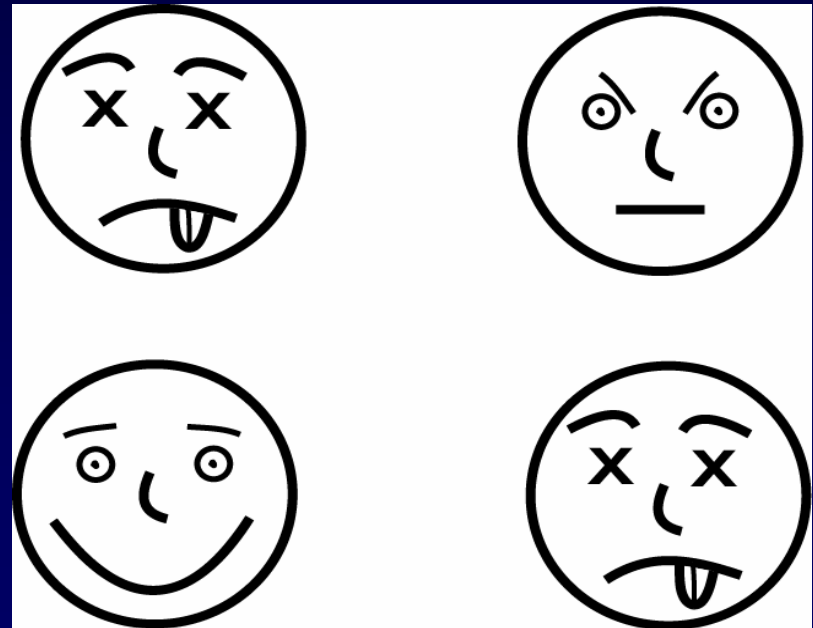
What is the purpose of the prodrug approach?

Traditional ChemoRx
("Inverted" Therapeutic Index)

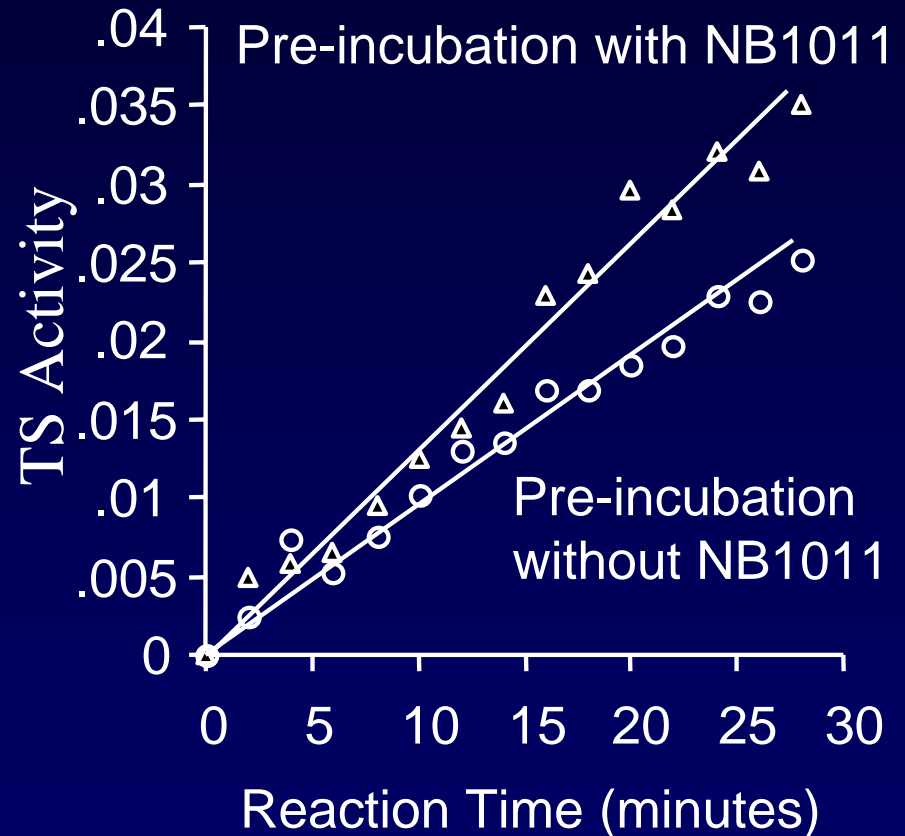
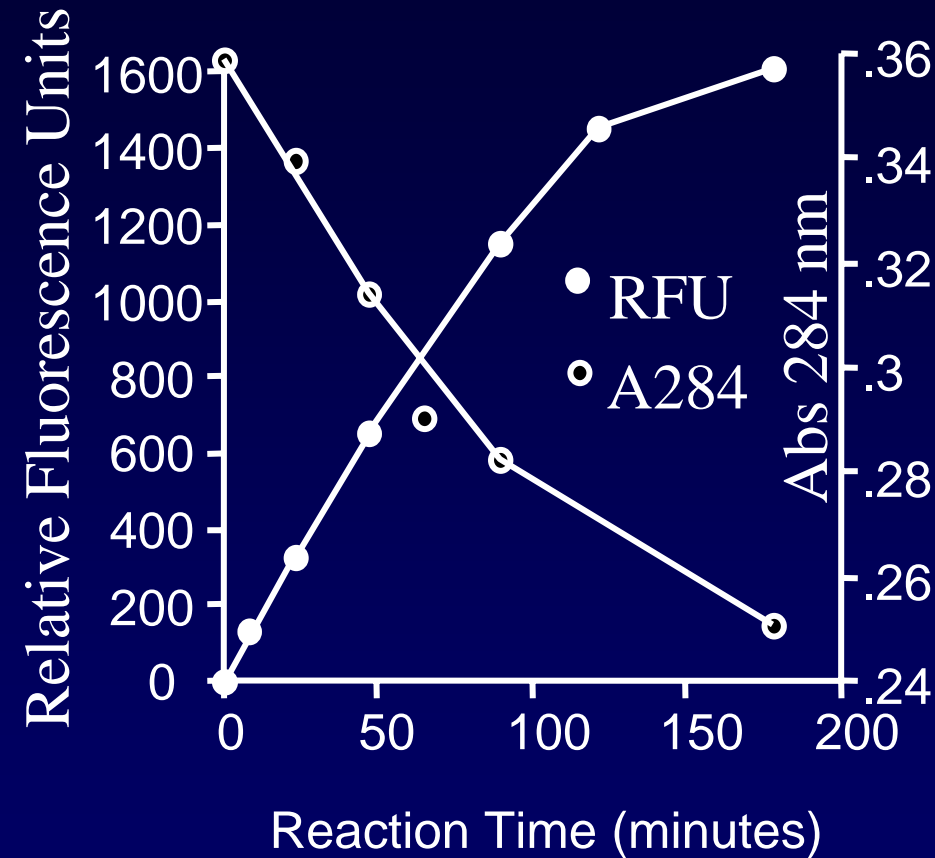
Successful Prodrug
("Positive" Therapeutic Index)

Normal Cells

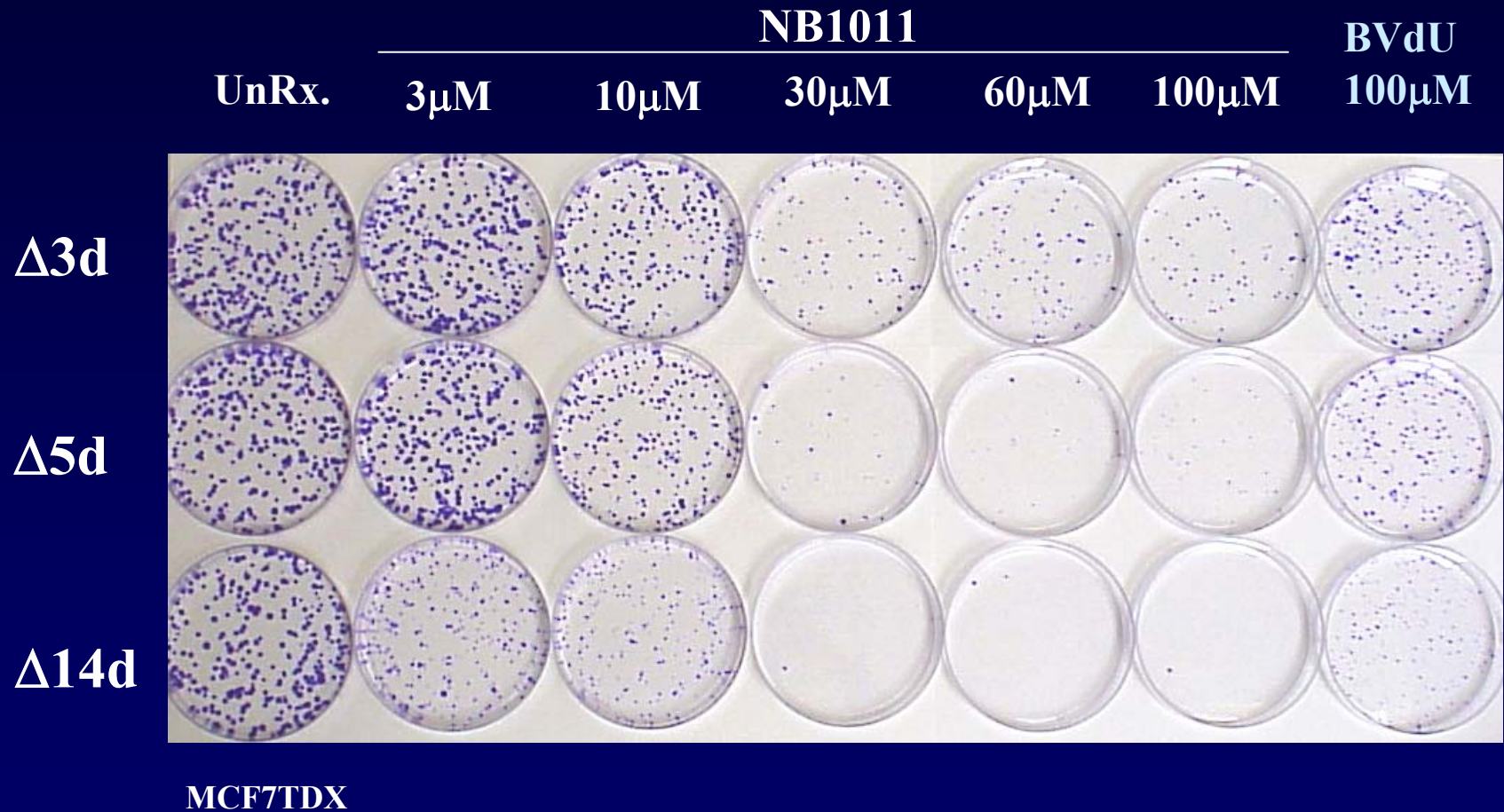
Tumor Cells



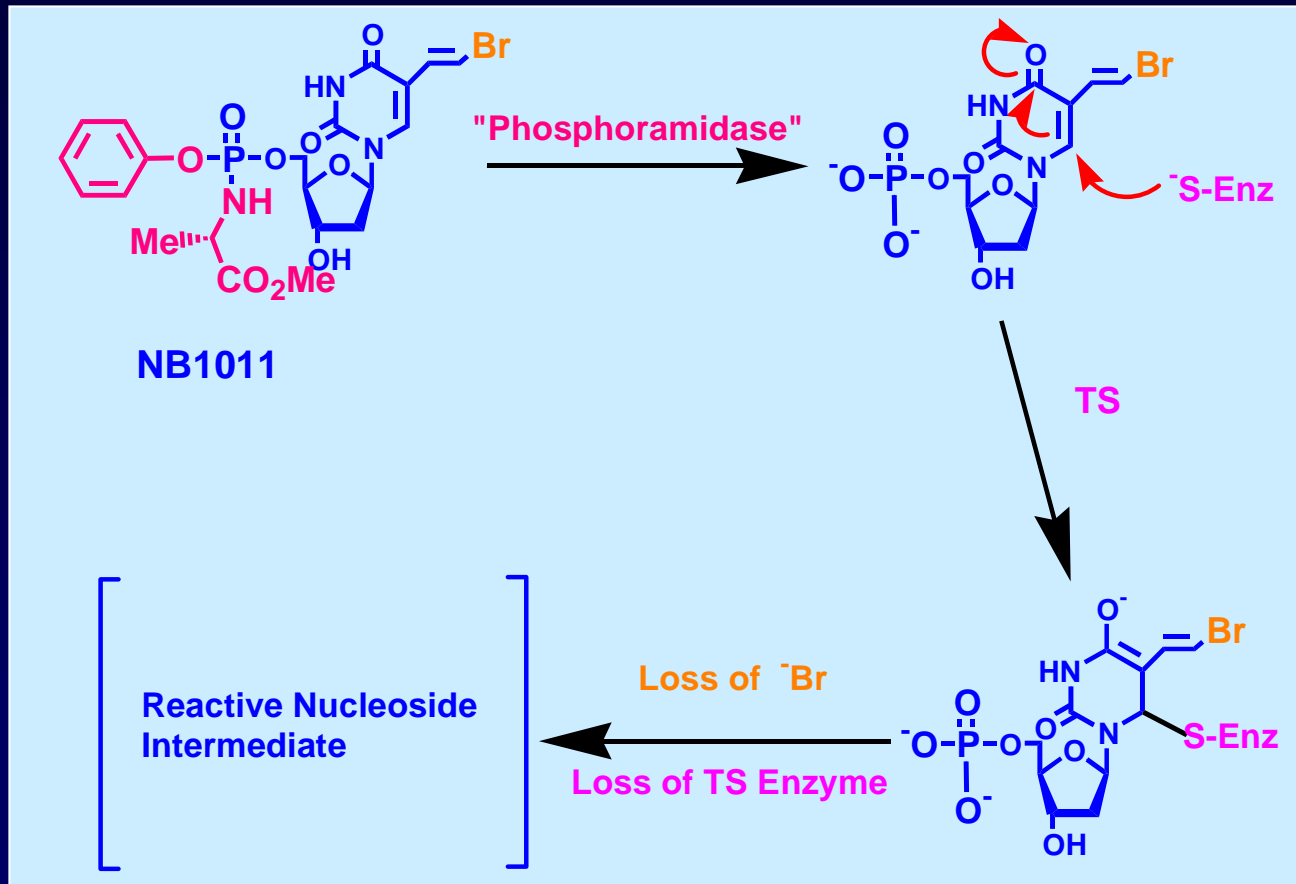
Enzymatic Activation of NB1011 by rhuTS does not inhibit TS



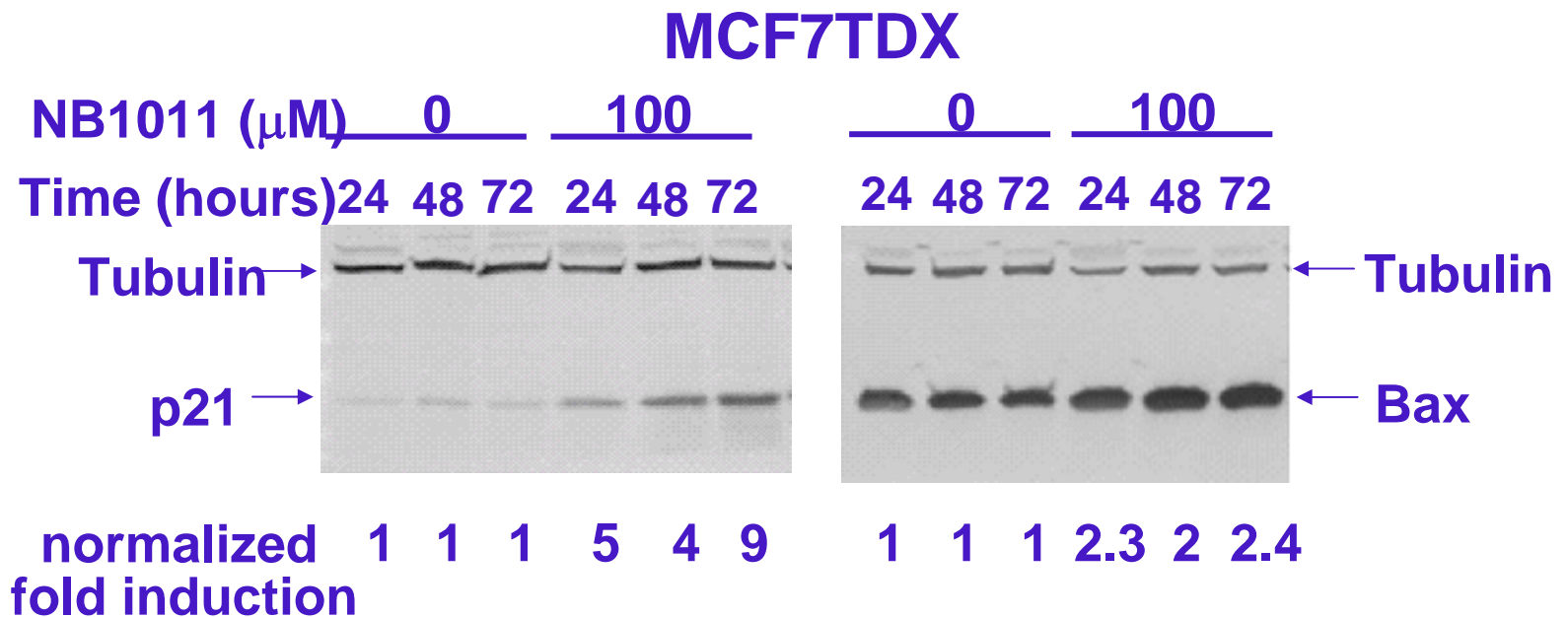
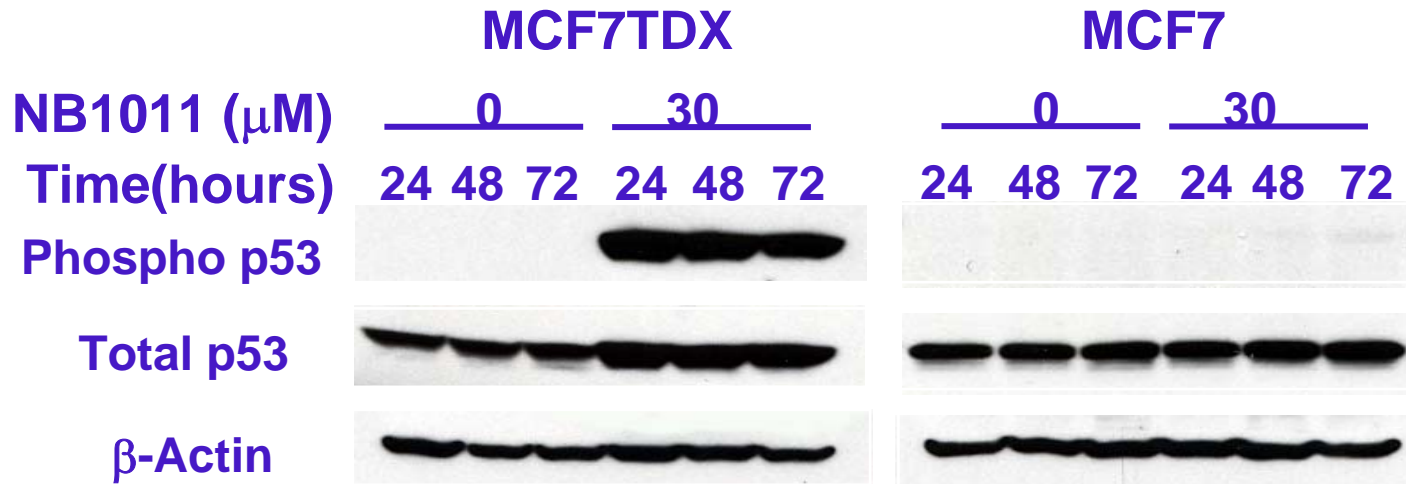
Clonogenic Assays



Putative Mechanism of Action of NB1011



NB1011 Treatment Activates p53 Selectively in MCF7TDX Cells

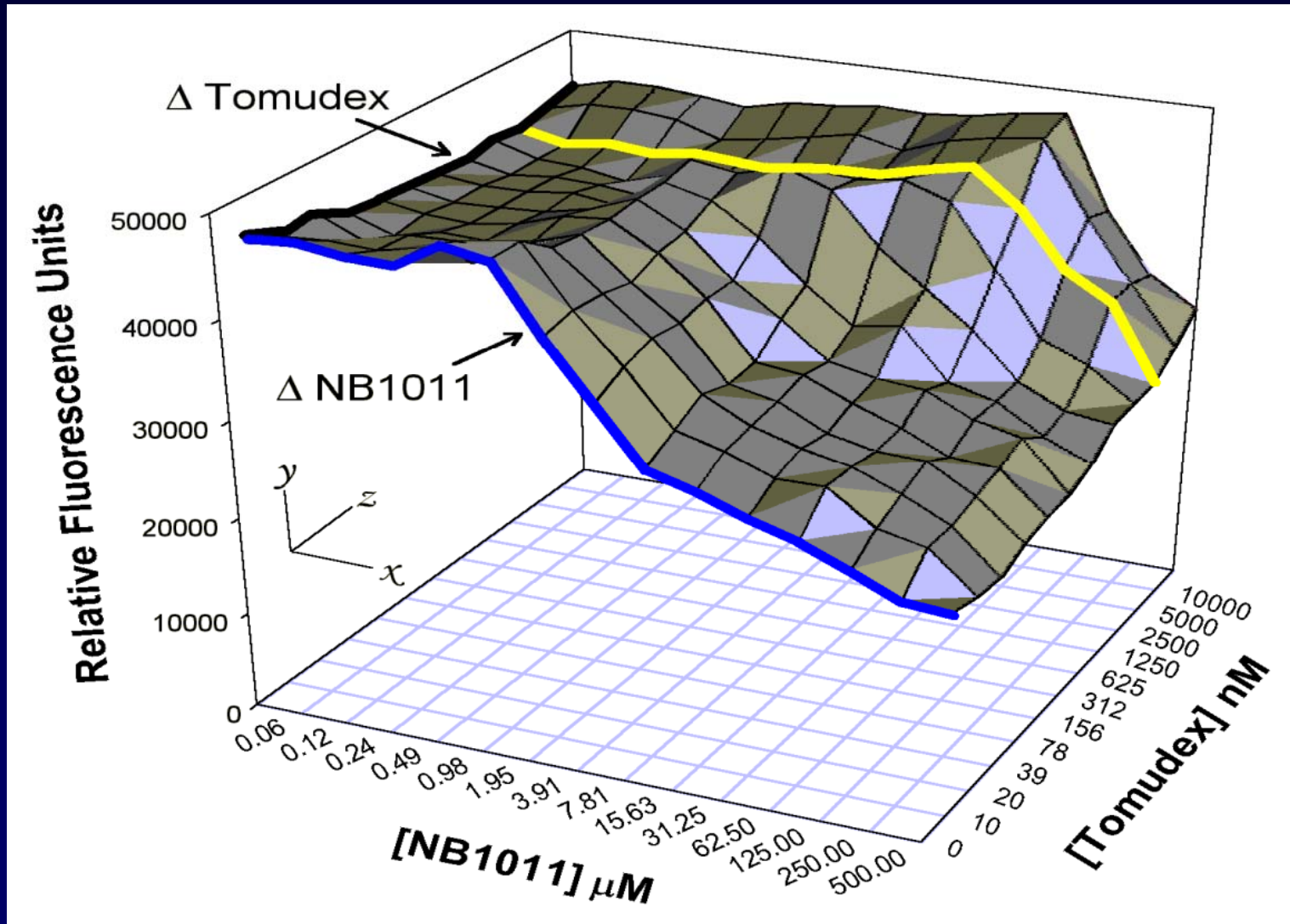


NB1011 Cytotoxicity Correlates with TS

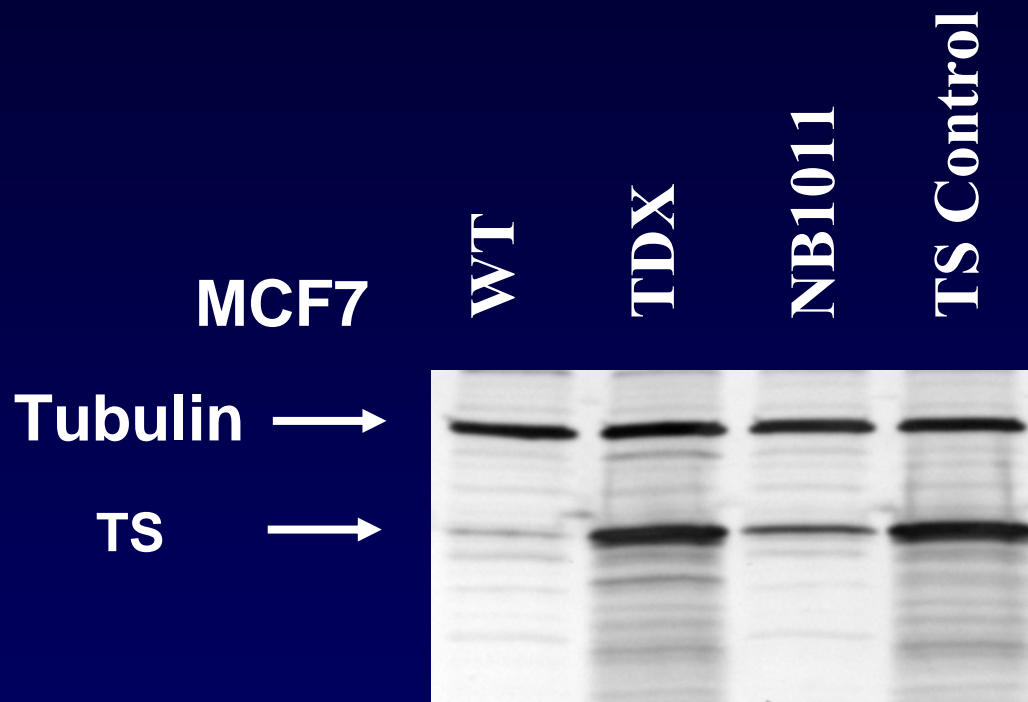
<u>Cell Line</u>	<u>TS Protein Level</u>	<u>IC-50 μM</u>	<u>Rs*</u>
HT1080 V	173	393 +/- 62	- 0.997
HT1080 TS1	411	258 +/- 12	
HT1080 TS2	468	199 +/- 73	
HT1080 TS3	667	62 +/- 41	
HT1080 TS4	678	65 +/- 16	
SKBR3 V	64	412 +/- 23	-1
SKBR3 TS1	170	252 +/- 65	
SKBR3 TS2	340	67 +/- 16	
SKBR3 TS3	590	13 +/- 4	
SW527 P	50	332 +/- 28	-1
SW527TDX1	800	55 +/- 16	
SW527TDX2	1000	13 +/- 3	

* Rs = Spearman Rank Order Correlation Coefficient

Enzymatic Activation of NB1011 is Inhibited by TS Inhibitor Tomudex

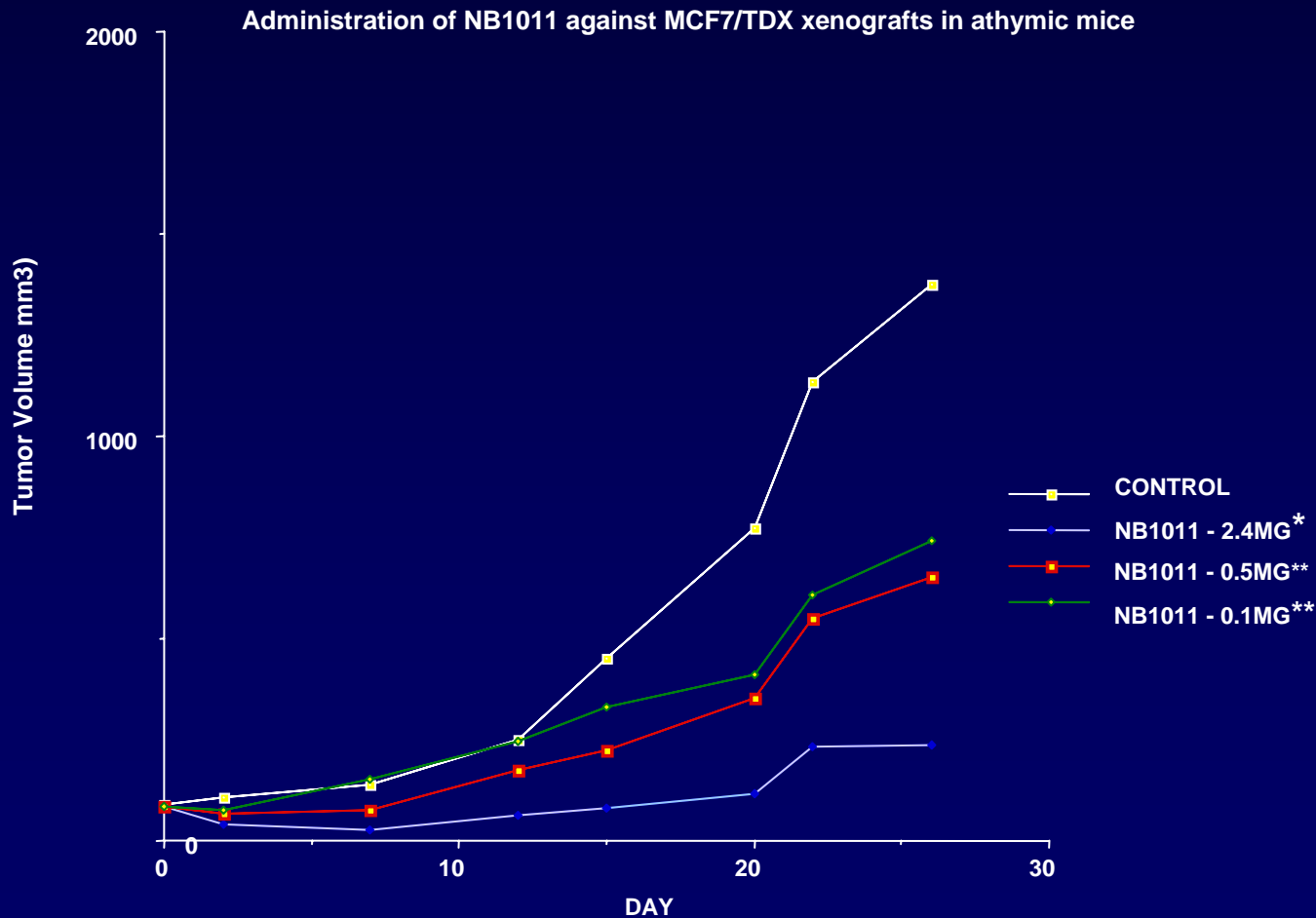


NB1011 selects for cells with decreased levels of Thymidylate Synthase



IC₅₀(uM)	Tomudex	.026	>10	.041
	NB1011	291	2	240

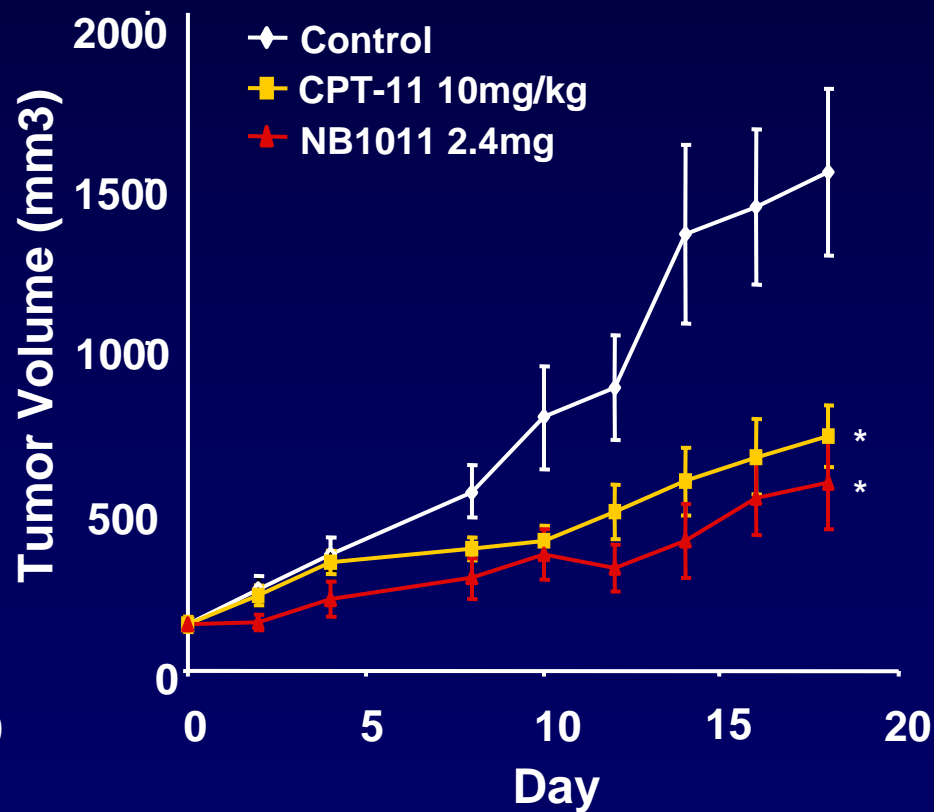
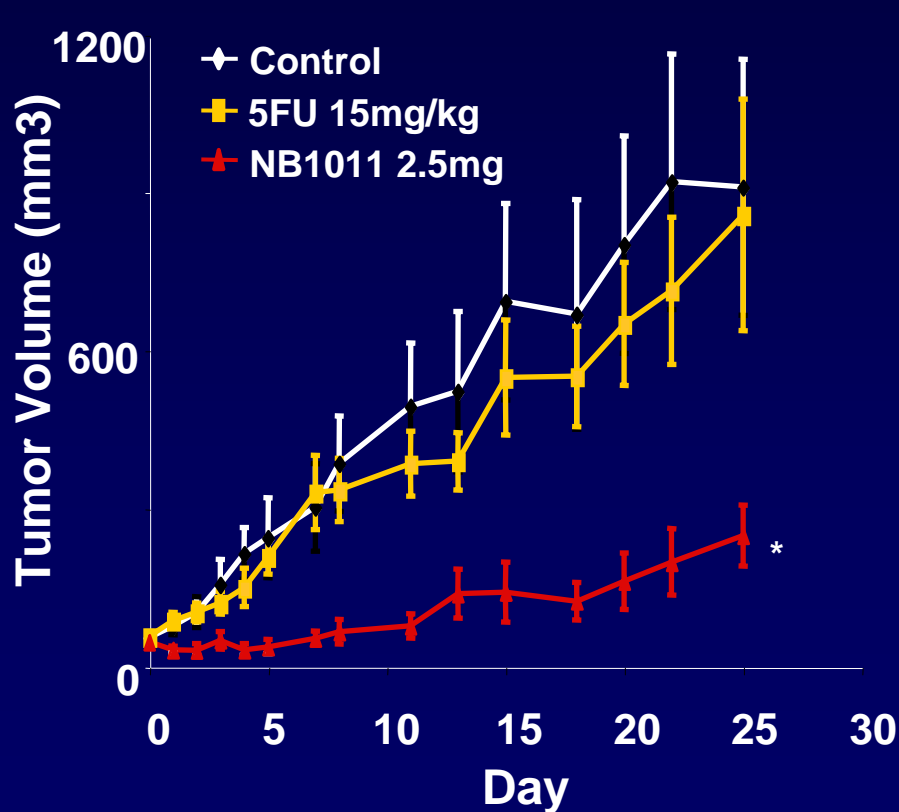
Preclinical Efficacy of NB1011 Against Tomudex-resistant MCF7 Breast Cancer Xenografts in Athymic Mice



* significantly different from day 26 control

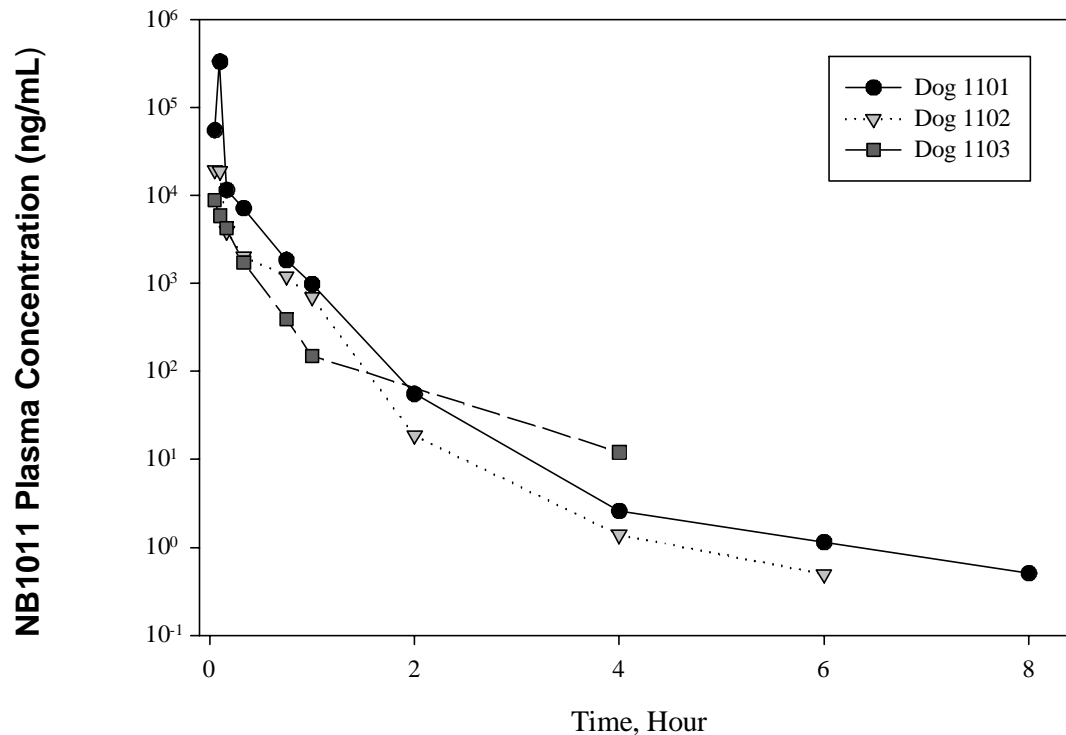
** significantly different from day 26 NB1011 2.4mg/day

Comparison of NB1011 vs. CPT-11 Against H630R-10 Colon Cancer Xenografts



*P<0.05 compared to control

Plasma concentrations of NB1011 in 3 dogs dosed at 25mg/kg



- Dogs -- Dose of 200 mg/kg (4000 mg/m²) well tolerated (1-hour infusion)
- Rats -- No dose limiting toxicity at 400 mg/kg (2400 mg/m²)

NB1011-1001 *Clinical Protocol*

Phase I Study Design

- First in Human Study
- Non-randomized, open-label, dose-escalation study, 5-FU-resistant CRC
- Investigative sites: UCLA and USC
- Phase I: 11 cohorts with doses ranging from 200-3000 mg/m²/day (completed)
 - cohort expanded at 2500 mg/m²/d for efficacy assessment based on PK data

Phase I: Key Eligibility/Exclusion Criteria:

Eligibility

- **Advanced recurrent or metastatic colorectal cancer, histologically confirmed.**
- **Progression within 6 months of fluoropyrimidine-based chemotherapy.**
- **Archival tumor tissue available.**
- **Evaluable/measurable disease.**
- **KPS \geq 70, life expectancy $>$ 3 months.**
- **Normal laboratory parameters.**

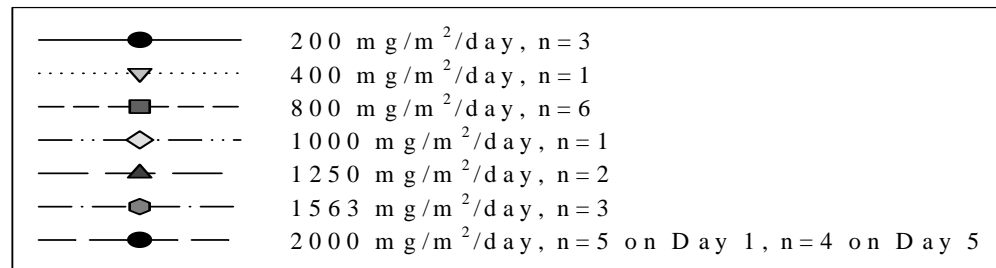
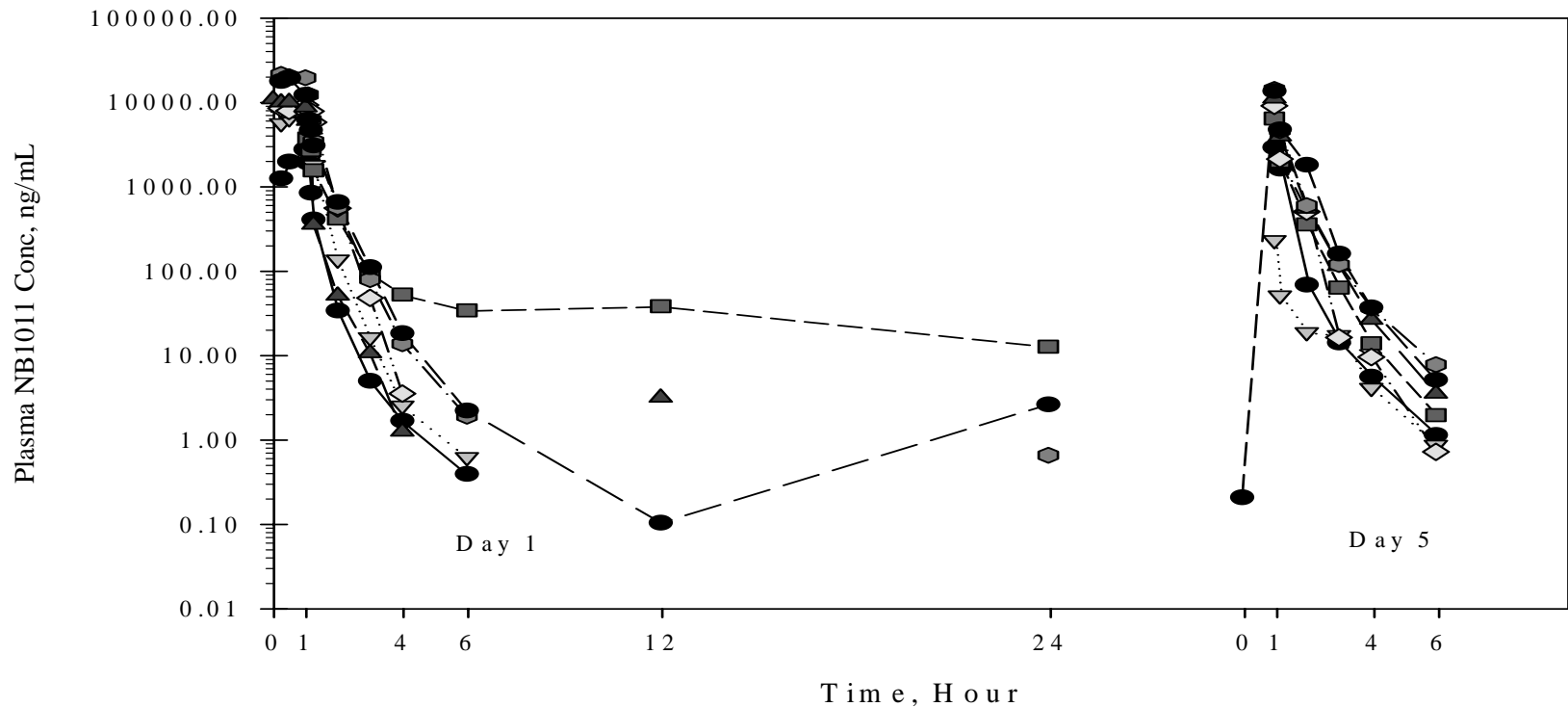
Exclusions

- **Concomitant fluoropyrimidines or TS inhibitors**
- **Allergy to cremaphor**
- **Uncontrolled infection**
- **Pregnancy/lactation**
- **CNS metastasis**
- **Use of Antabuse or alcohol dependency**

Patient Characteristics

- Heavily pre-treated group (N = 45)
- Average # of prior therapies = 4.3
- Approximately 60% of patients had previously received oxaliplatin
- Approximately 20% of patients had previously received an investigational agent

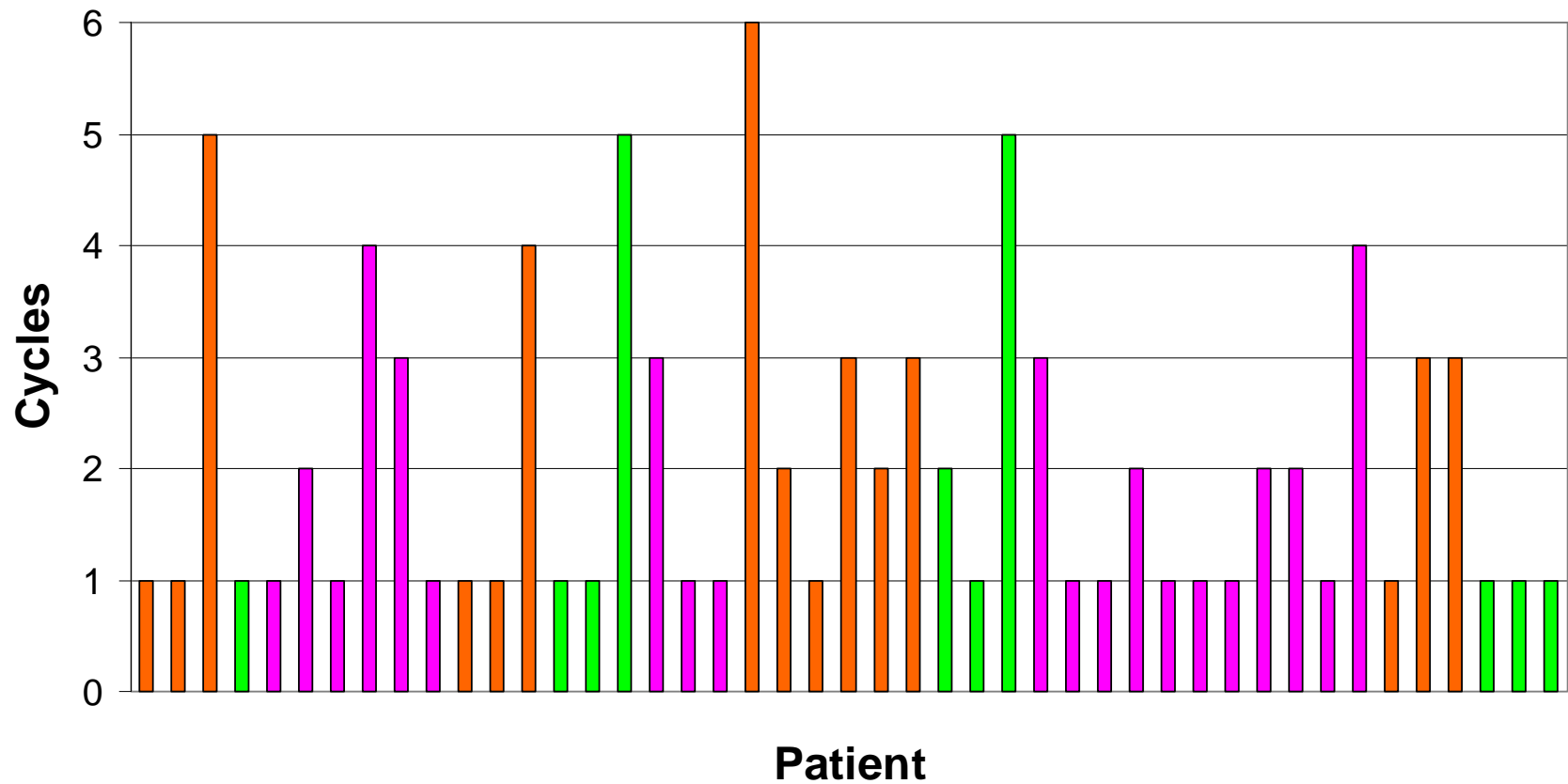
Mean Plasma NB1011 Concentration Profiles (Log Scale)



Adverse Events

- MTD not yet defined in phase I
- NB-1011-related AEs include the following:
 - Diarrhea (grade I)
 - Nausea and vomiting (grade I/II)
 - Rash (grade III)
 - Transaminitis (grade I)
- No alopecia or significant hematologic toxicity

Treatment Summary

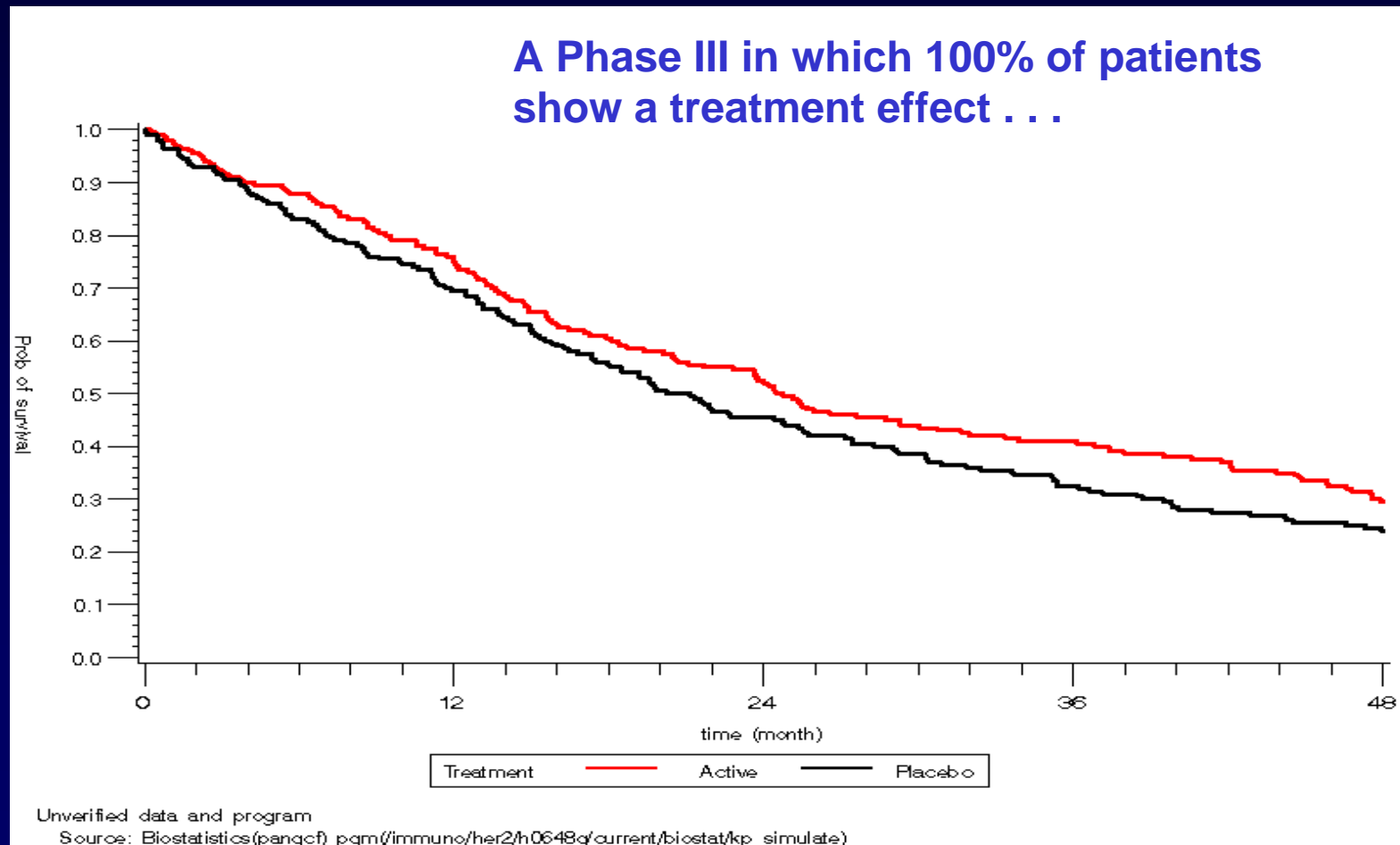


NB1011 Clinical Development

Progress to Date (Summary)

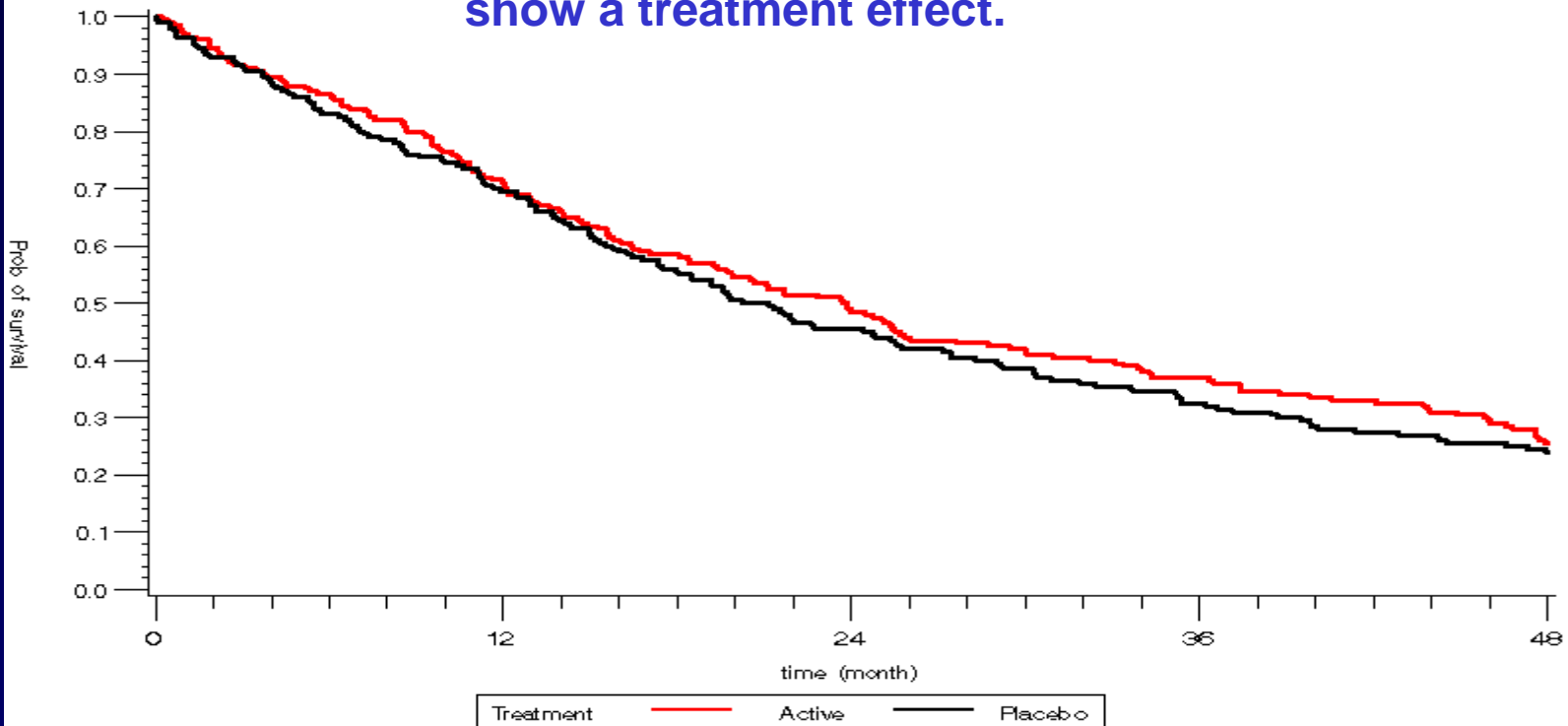
- NB1011-1001
 - Half-life of NB1011 just under one hr, BVDU approx. 22 hrs
 - Majority of patients in this trial, in retrospect, had low TS in archival samples
 - Stable disease in 7/45 patients (15.6%)
 - 1 patient completed 6 cycles – still alive at 1 year
 - 1 patient completed 5 cycles - high TS (7 fold overexpression by RT-PCR in Fresh biopsy)

Patient Selection – Without selection, a potentially active new therapy could be missed.



Patient Selection – Without selection, a potentially active new therapy could be missed.

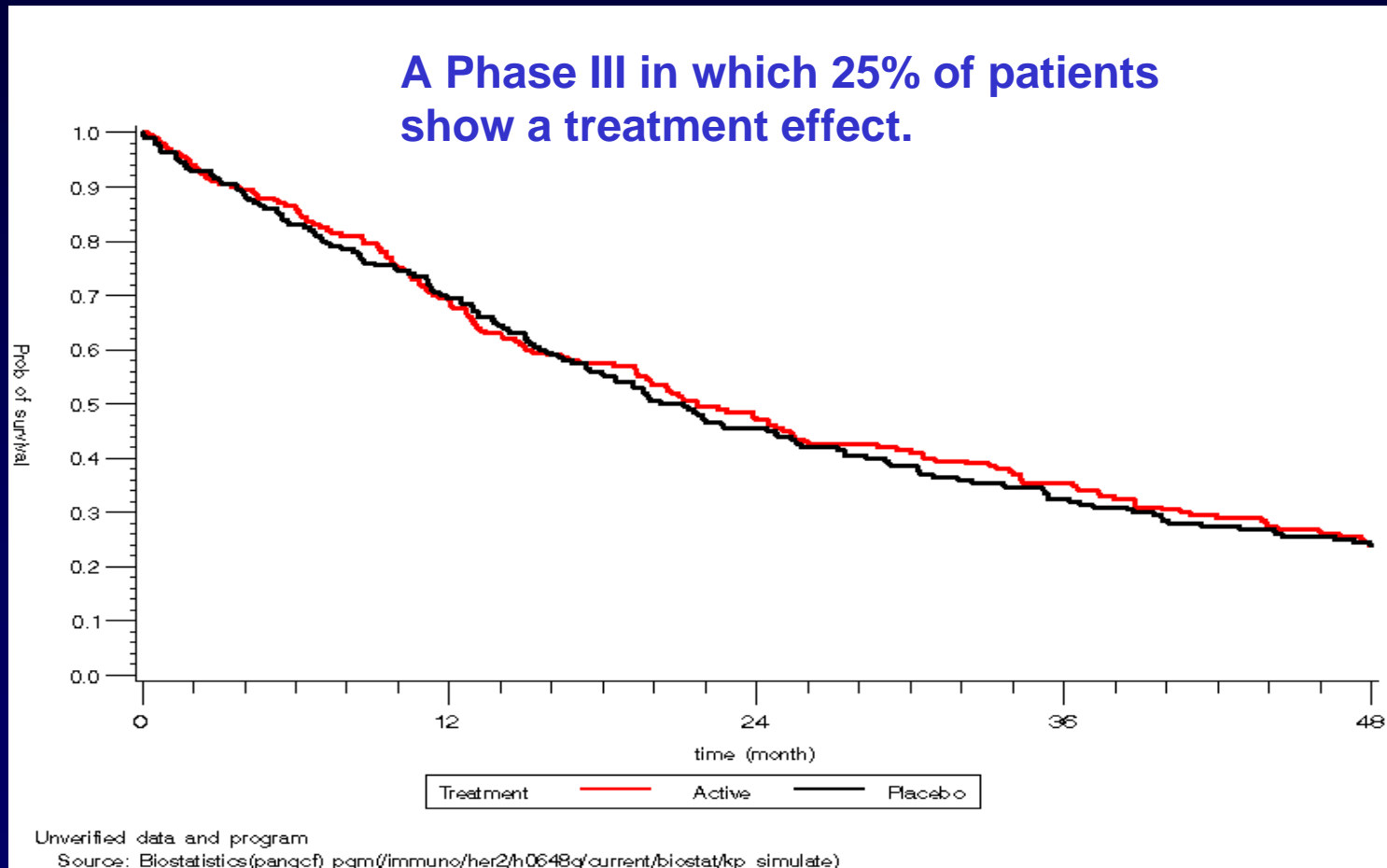
1 A Phase III in which 50% of patients
show a treatment effect.



Unverified data and program

Source: Biostatistics(pangcf) pgm/immuno/her2/h0648g/current/biostat/kp_simulate)

Patient Selection – Without selection, a potentially active new therapy could be missed.



Target certain patients

Creates a barrier to enrollment

Example: First Line MBC (median survival ~ 22 months)

Expected Benefit	Required Sample Size And Study Duration	Target Prevalence	Required "Screened" Population
↑ 5 months (22.7%)	1250 → 52 mos	100%	1250
		50%	2500
		25%	5000

* *Need a specimen, wait for test results.*

* *Need to screen many patients.*

Next Steps for NB1011 Clinical Evaluation

- Enroll High TS expressers
 - Sensitivity of NB1011 increases with high TS
 - Screen patients for TS prospectively
- Investigate alternative regimen
 - Prolonged infusion in colorectal cancer
 - Relatively short half-life of NB1011
 - Preclinical research clearly shows that longer exposure times increase potency and cytotoxicity
- Broaden entrance criteria to all tumor types that express high TS and easily biopsied

Ideal Therapeutic Drug

- Patient's perspective
 - Cures everyone
 - No side effects
 - Taken orally (preferably just once)
 - Free

Ideal Therapeutic Drug

- Industry perspective
 - Prolongs survival indefinitely, but...
 - Cures no one
 - Requires daily life-long therapy
 - No side effects
 - Expensive
 - Too complicated to make generic

Ideal Therapeutic Drug

- Medical Doctor's Perspective
 - Prolongs survival indefinitely
 - Cures no one
 - Administered intravenously (preferably daily)
 - Except on weekends or holidays
 - Can be given by nurses
 - Not so toxic that patients don't want it, but...
 - Just toxic enough to stop surgeons from giving it

Acknowledgements



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- Ginny Paton
- Gloria Meng
- Mark Sliwkowski

U.S. Dept of Defense Breast Cancer Research Program

Thanks!



Translational Clinical Research

- **Traditional funding mechanisms for academic researchers**
- **NIH – RO1 grant**
 - very focused on a specific research question
 - cannot be too innovative or far reaching or else study sections will not fund the proposal
 - modest budgets
 - slow, incremental progress over many years
 - loved by institutions for high overhead rates
- **Foundation grants (Susan Komen, Milken, etc.)**
- **Philanthropic grants**
 - more potential for testing new ideas
 - minimal paperwork
 - less taken by the University as overhead

Translational Research

- Industry-sponsored research
- Less important to the institution (less overhead)
- Potential for investigator bias?
- Less paperwork than an NIH grant, but more legal hassles
- Often more immediate translational potential than typical RO1

**Combined Biologic Therapy of Breast
Cancer Using 2 Humanized Monoclonal
Antibodies Directed Against HER2 and
VEGF**

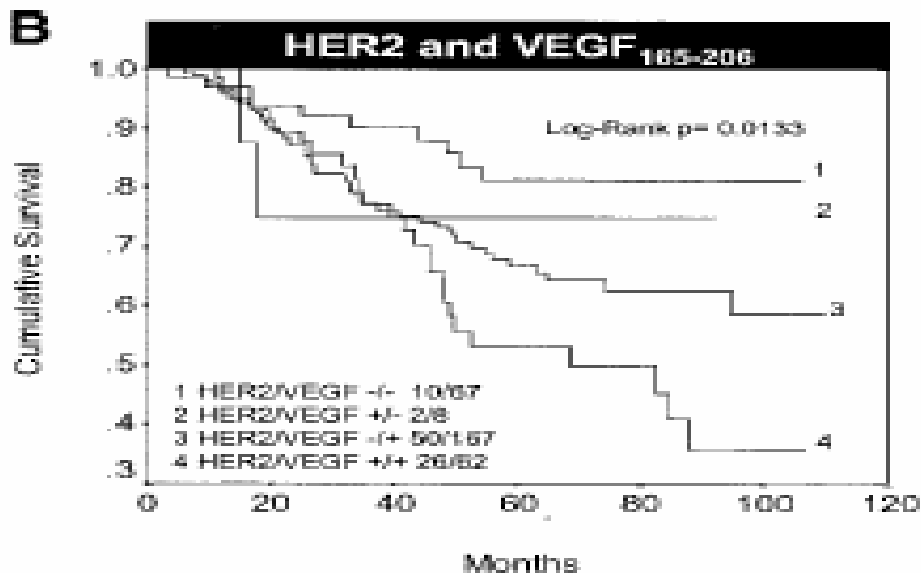
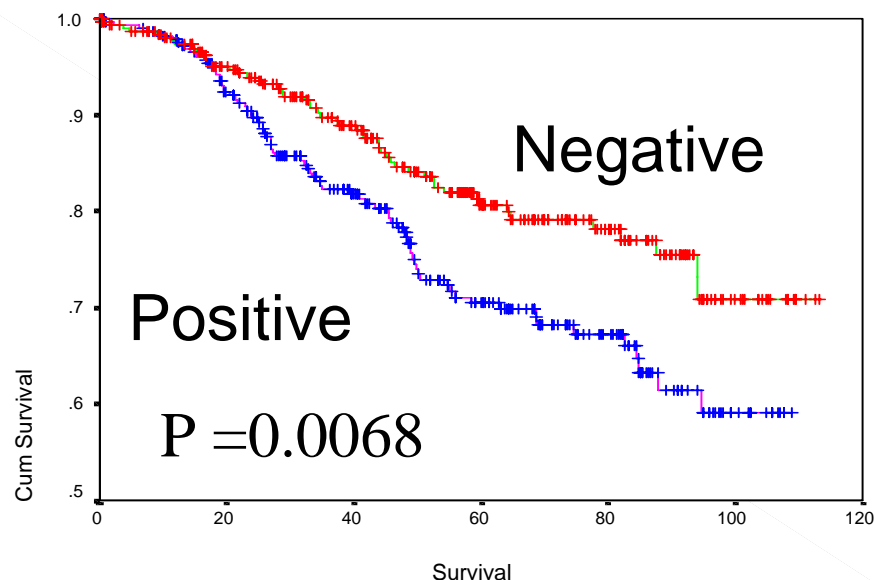
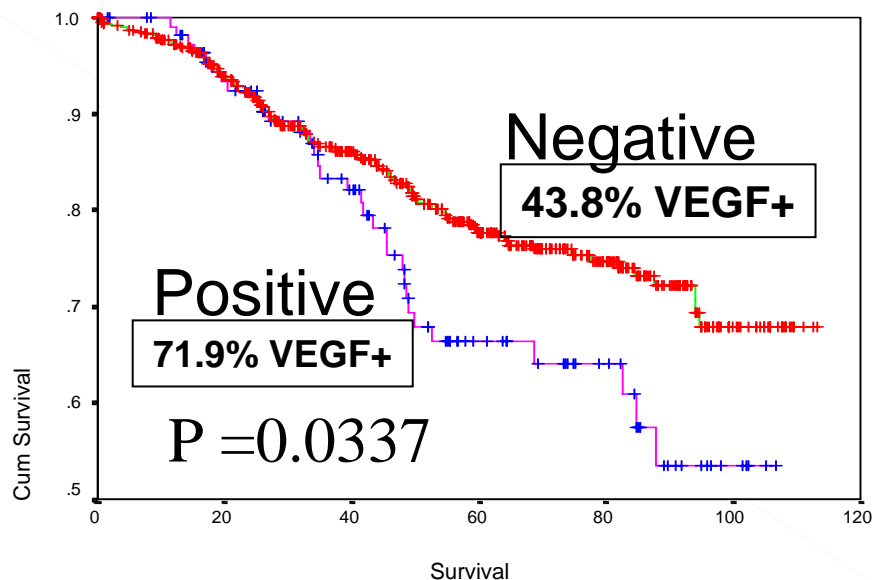
Mark Pegram

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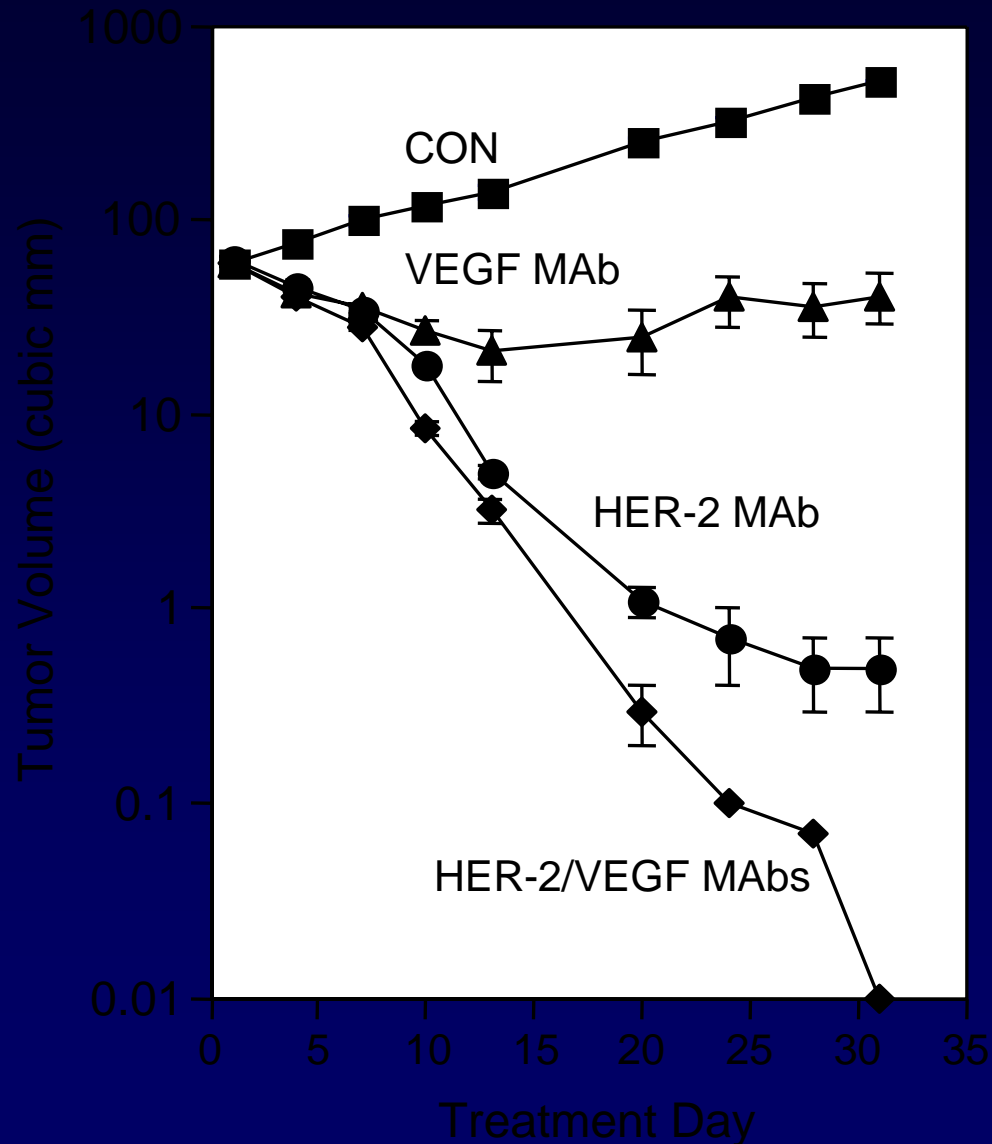
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Overall Survival in Primary Breast Cancers

HER2 N = 612 Total VEGF

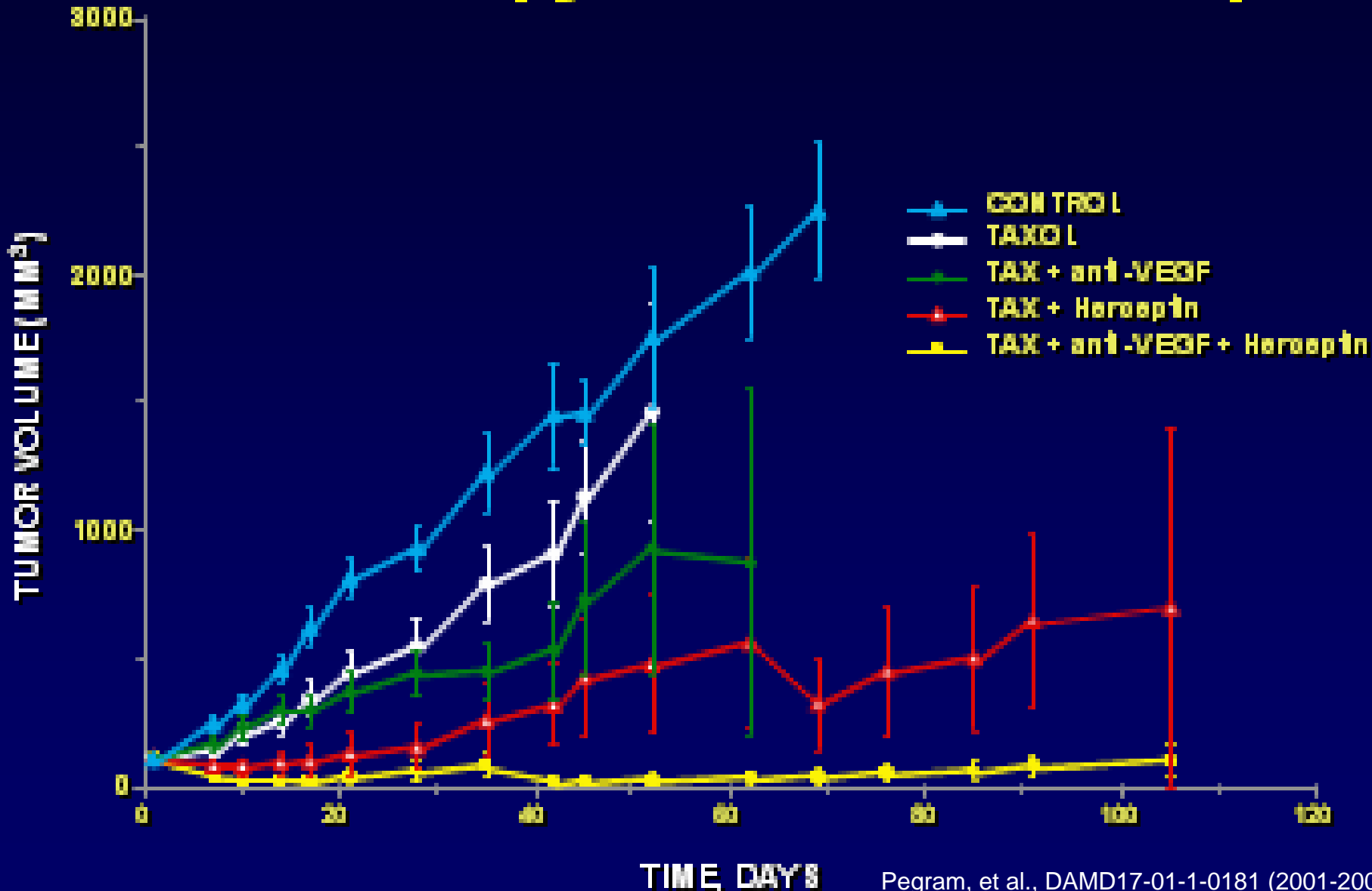


Trastuzumab and VEGF Antibody Inhibit Growth of MCF-7/HER-2 Xenografts In Vivo



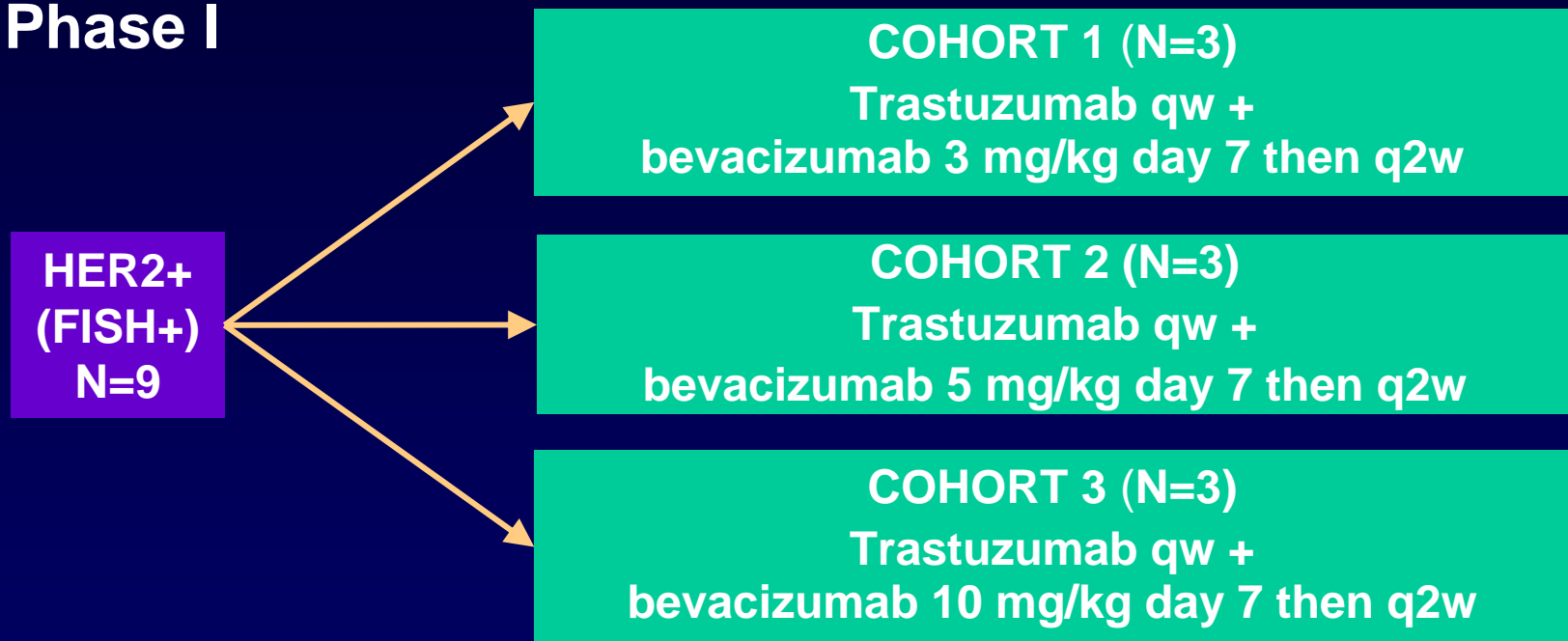
MCF7/HER2 XENOGRAFTS

Chemotherapy + Avastin + Herceptin



Phase I/II Trial of Trastuzumab + Bevacizumab in relapsed/MBC

Phase I



- Investigator-initiated, investigator held IND
- First report of 2 humanized MAbs in human subjects
- Primary endpoints: safety, PK

Key Eligibility Criteria

Inclusion Criteria

- Age 18-75
- ECOG 0,1,2
- Normal LVEF by ECHO or MUGA
- HER2 positive by FISH (any laboratory)
- O₂ saturation ≥ 90% on RA
- Bidimensionally measurable disease

Exclusion Criteria

- Four or more different organ sites of metastasis
- More than 50% parenchymal liver metastasis
- Symptomatic pulmonary metastasis
- CNS metastasis
- Therapeutic anticoagulation or ASA > 325 mg/day
- Uncontrolled HTN (SBP >160, DBP > 90)
- Clinically significant cardiovascular disease
- Proteinuria > 500 mg/24 hr

Phase I Dose Escalation FISH+ MBC: Bevacizumab + Trastuzumab

<u>Subject #</u>	<u>Sites of Metastasis</u>	<u>Prior Rx</u>
101	Bevacizumab: Liver	ACX4 → Tax X 4 Tamoxifen
102	3mg/kg Inflammatory	Surgery
103	Lung Bone	hormonal rx
104	Inflammatory Bone	ACX4
105	5mg/kg Pleura	TAX
106	Liver Lung Lymph Node	FAC ACX4
107	Mediastinum Bone	ACX4 → Tax X 4 radiation
108	10mg/kg Mediastinum	ACX4 → Taxotere X 4 XRT, tamox, navelbine Herceptin, faslodex
109	Breast/chest wall	FEC, hyperthermia Herceptin

Mean (SD) Pharmacokinetic Parameters for Bevacizumab and Trastuzumab

Molecule (N)	PK Parameters				MRT (days)
	Vc (L)	CL (L/day)	t _{1/2, α} (days)	t _{1/2, β} (days)	
Bevacizumab (N=7)	2.49 (0.829)	0.229 (0.0836)	1.39 (0.124)	19.3 (8.17)	25.0 (11.9)
Trastuzumab (N=6)	2.65 (0.412)	0.206 (0.0338)	2.08 (0.135)	22.2 (5.66)	27.7 (7.47)

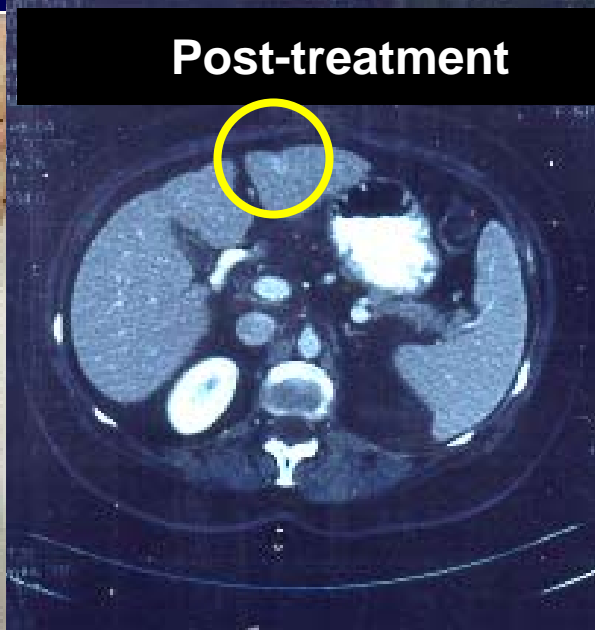
No evidence for a PK interaction between these 2 humanized MAbs

**Preliminary Data:
Adverse Events
Possibly Related
To Bevacizumab +
Trastuzumab**

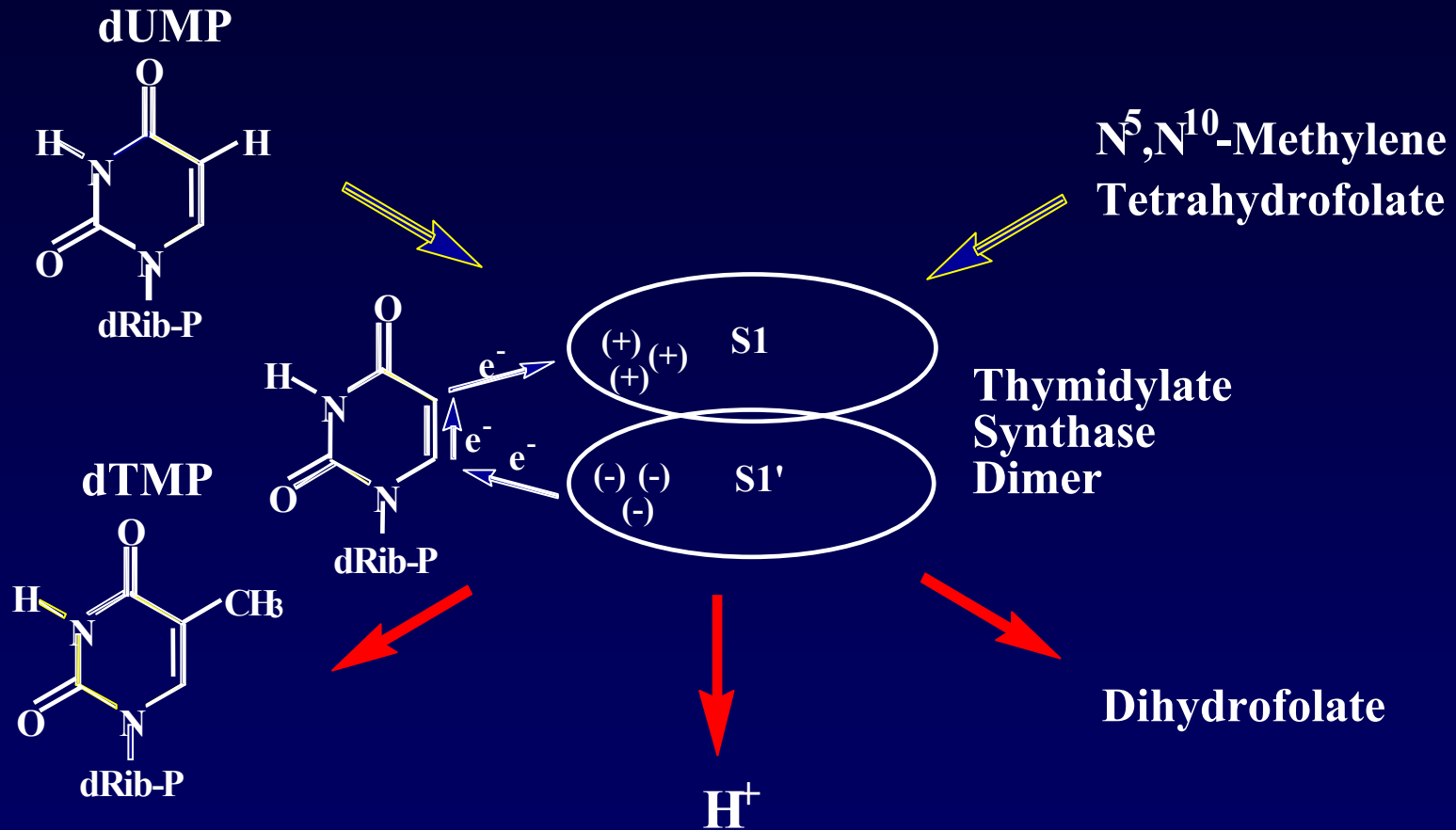
Adverse Event	Grade 1	Grade 2	Grade 3/4	Total
Alopecia	2	0	0	2
Anemia	2	0	0	2
Bleeding, esophageal varicies	0	0	1	1
Cardiac dysfunction	0	0	1	1
Chills	1	0	0	1
Cold sores	0	1	0	1
Diarrhea	1	1	0	2
Discoloration, Teeth	1	1	0	2
Ecchymosis (forearms)	1	0	0	1
Edema	0	2	0	2
Epistaxis	1	0	0	1
Fatigue	3	1	0	4
Gum bleeding, sensitivity, gingivitis	4	1	0	5
Hypertension	1	0	0	1
Mylagia	1	1	0	2
Nail changes	4	0	0	4
Nausea	1	1	0	2
Paresthesia, dysesthesia	1	1	0	2
Skin dryness, pigment, rash, psoriasis	3	2	0	5
Shortness of breath	0	1	0	1
Stomach Aches	1	0	0	1
Tachycardia	1	0	0	1
Thrombocytopenia	2	0	0	2

Phase I trastuzumab + bevacizumab clinical efficacy: 2CR, 3PR, 2SD (>6months)

Pegram, et al., SABCS (2004) #3039

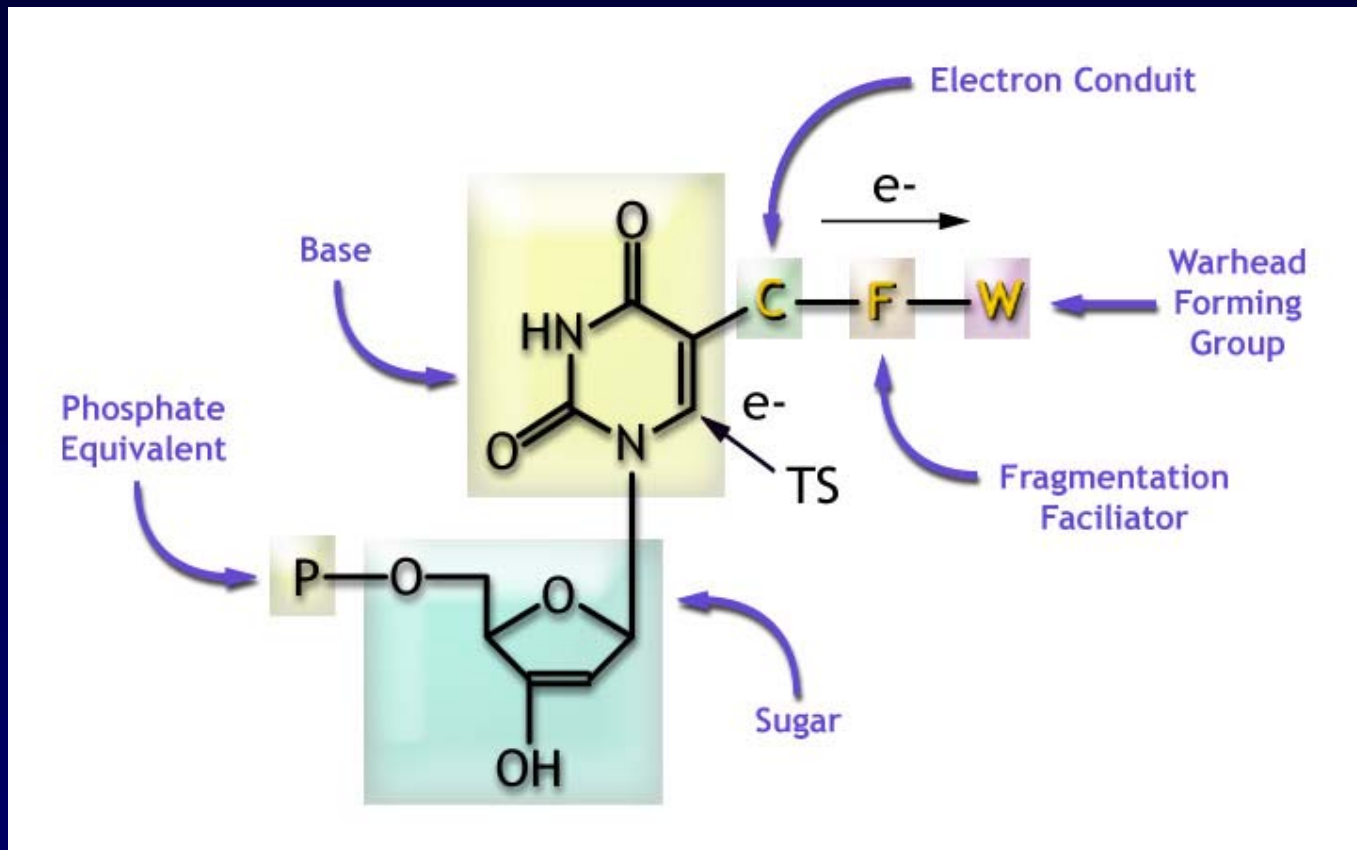


The Thymidylate Synthase Reaction



The key to TS activity is electron transfer from 6-to 5-position of uracil.

General Structure of Thymectacin Compounds



* The 5' Extension Alters the Folate-binding Pocket of TS to Create Cofactor Independence

Various TS ECTA Compounds

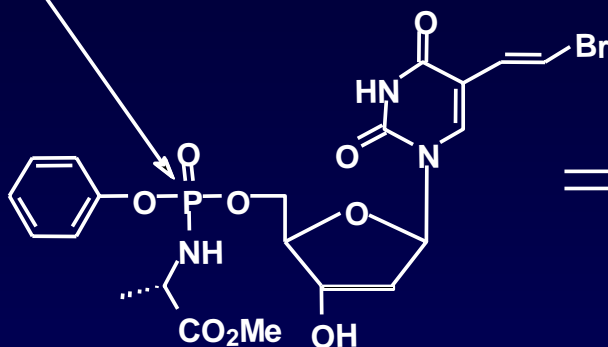


Compound	Y	R
NB1011	Phosphor- amidase	
NB1015	Nucleoside	
NB1017	Phosphor- amidase	
NB1024II	Nucleoside	
NB1018	Phosphor- amidase	
NB1022	Nucleoside	
NB1019	Phosphor- amidase	
NB1023	Nucleoside	

Conversion of NB1011 to Monophosphate Species

Cell membrane

phosphoramidate
prodrug required for
cell penetration

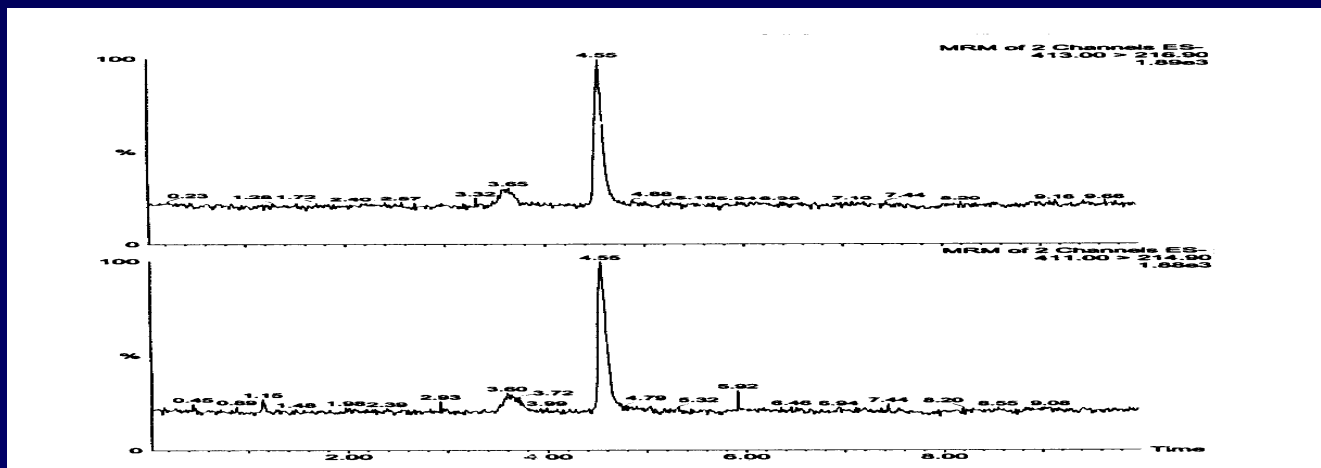


NB1011
(BVdUPA)

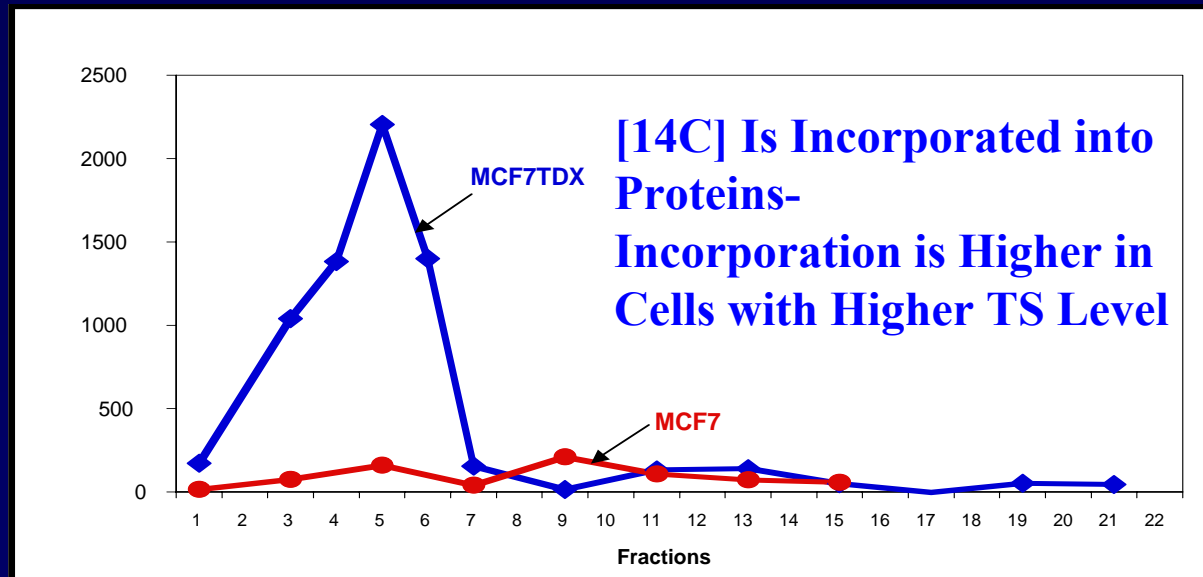
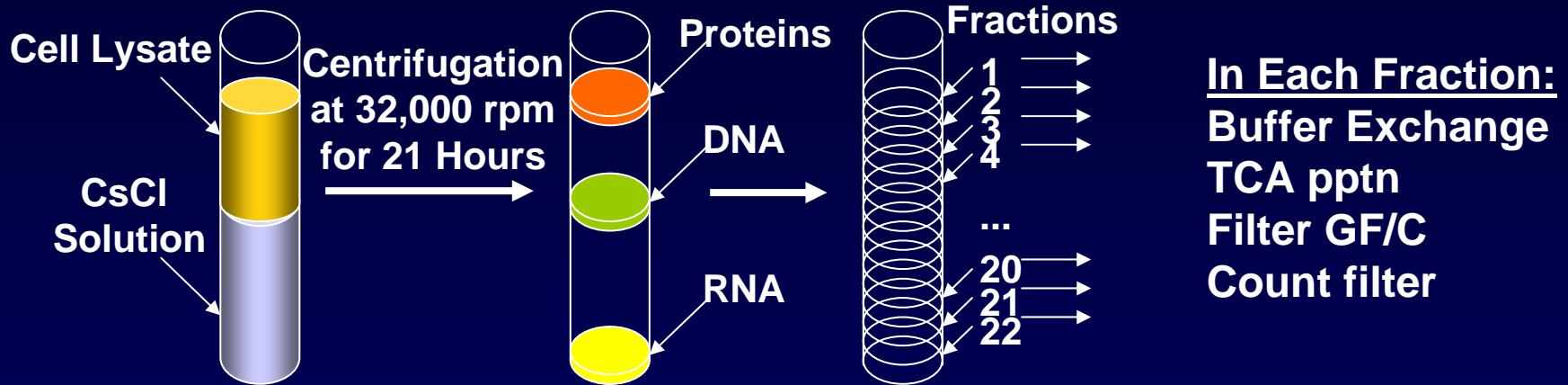
"Phosphor-
amidase"
(Carboxyl
Esterases)



BVdUMP
(TS-active form of NB 1011)



[¹⁴C]-NB1011 Treatment of MCF7 and MCF7TDX Cells

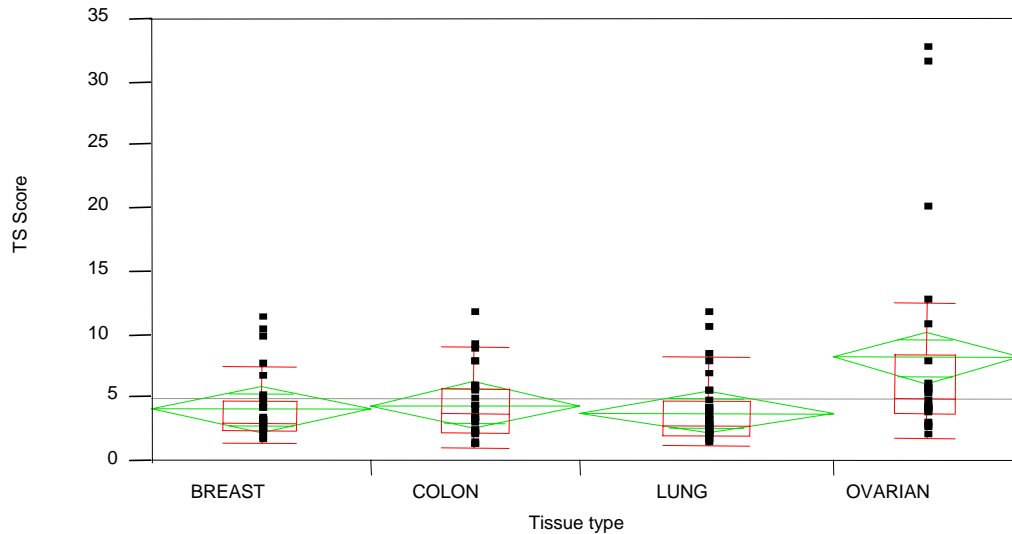


Study Treatment

- NB1011 given IV daily for 5 days q28d
- One cycle=28 days
- First cycle administered as inpatient
- Maximum of 6 cycles
- For doses ≤ 2000 mg/m²/d (cohorts 1-7), drug given over 1 hr
- For doses > 2000 mg/m²/d (cohorts 8-11), drug given over 2 hr

TS Levels by Cancer Tumor Type

Establishing Pre-screen TS “Score”



	Breast	Colon	Lung	Ovarian
Minimum	1.426419	1.143268	1.180992	1.831976
10%	1.883324	1.275744	1.491026	2.464566
25%	2.441145	2.154782	2.024752	3.728888
Median	3.023584	3.866089	2.900267	4.897696
75%	4.831233	5.804358	4.785620	8.404040
90%	9.733284	8.724613	8.228444	27.998810
Maximum	11.267740	11.566820	11.592280	32.525580

- Also, small intestine and esophageal cancer known to be very high in TS
- TS “Score”: is expressed as the mRNA ratio of TS to β -actin

Conclusions

- Novel use of well-established molecular target
- Lead compound (NB1011) has high therapeutic index in TS-overexpressing cells
- Demonstrated preclinical *in vivo* efficacy
- Toxicology and PK – preclinical and phase I completed
- Phase II trials – forthcoming '05

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Maria Sergeeva

Patricia Karjian

Qing Li

Jean Lee



Enroll all patients

Easy to miss an active treatment

Example: First Line MBC (median survival ~ 22 months)

Expected Benefit	Target Prevalence	Actual Benefit (All Patients)	Required Sample Size And Study Duration
↑ 5 months (22.7%)	100%	↑ 5 mos (22.7%)	1250 → 52 mos
	50%	↑ 2.5 mos (11.4%)	3500 → 108 mos
	25%	↑ 1.25 mos (5.7%)	11000 → 349 mos

* Easy to miss a potentially active new therapy as target prevalence decreases

Clinical Trials

- Components of a good clinical trial
 - Hypothesis
 - Protocol (and power calculation)
 - Ethical committee approval
 - Random allocation of patients
 - Double blind assessment of patients
 - Adequate resources
 - Data recording including adverse events
 - Ending trial and subsequent data analysis

Clinical Trials

- Ethics

- Is it an ethical study
- Is the protocol reasonable and feasible
- Informed consent and patient information
- Peer review
- Who is paying?

Clinical Hypothesis Testing

The patient

1. Start with the patient -- a clinical problem or question arises out of the care of the patient

The question

2. Construct a well built clinical research question derived from the case

The resources

3. Select the appropriate resource(s) and conduct a research study

The evaluation

4. Appraise that evidence for its validity (closeness to the truth) and applicability (usefulness in clinical practice)

The patient

5. Return to the patient -- integrate that evidence with clinical expertise, patient preferences and apply it to practice