

**Protocol No: BC-08-01**

**Phase 1/2a, Dose-Escalation, Safety, Pharmacokinetic, and  
Preliminary Efficacy Study of Intraperitoneal Administration of  
DTA-H19 in Subjects with Advanced Stage Ovarian Cancer**

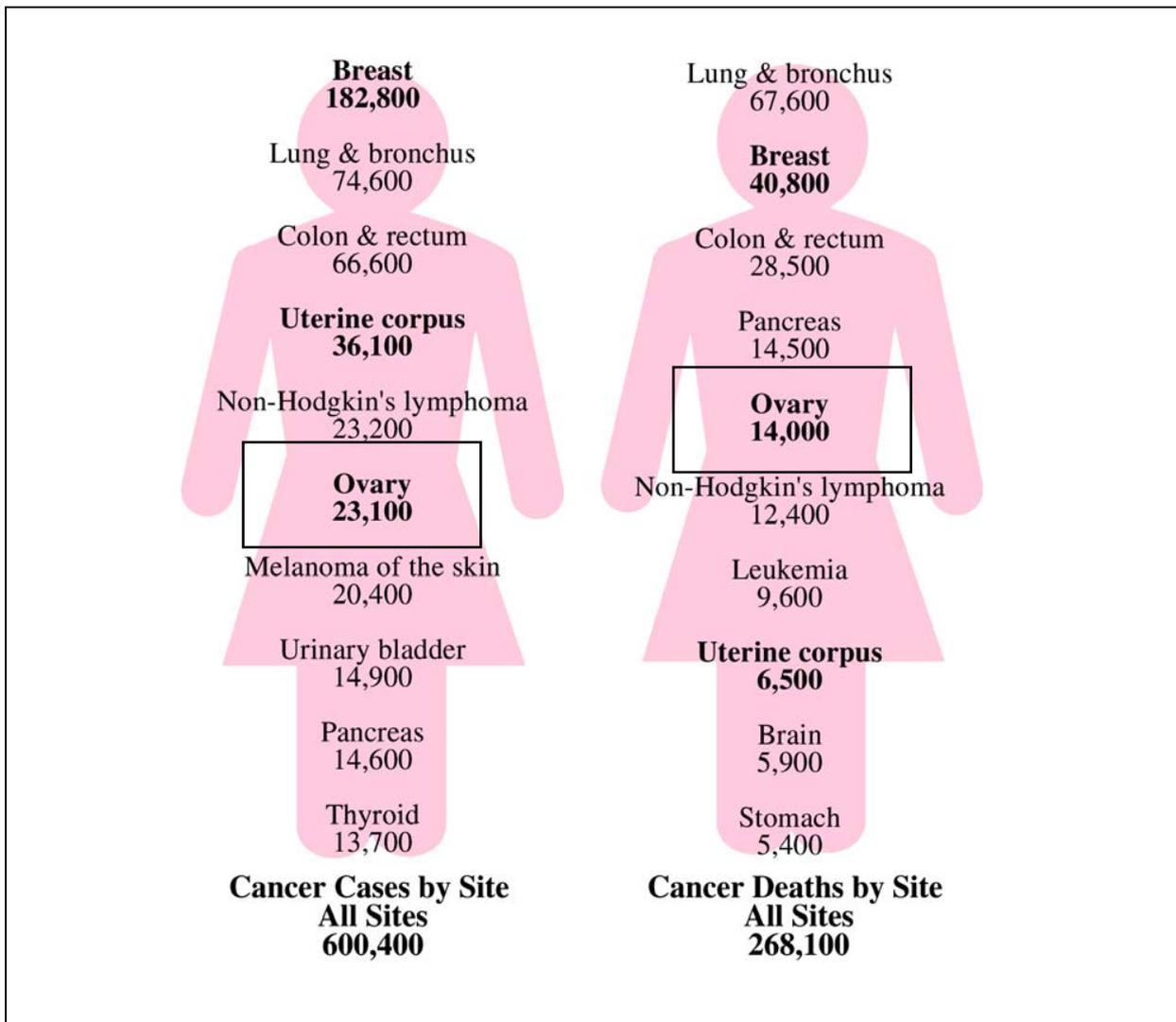
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**Presentation to the Recombinant DNA Advisory Committee  
March 4, 2009**

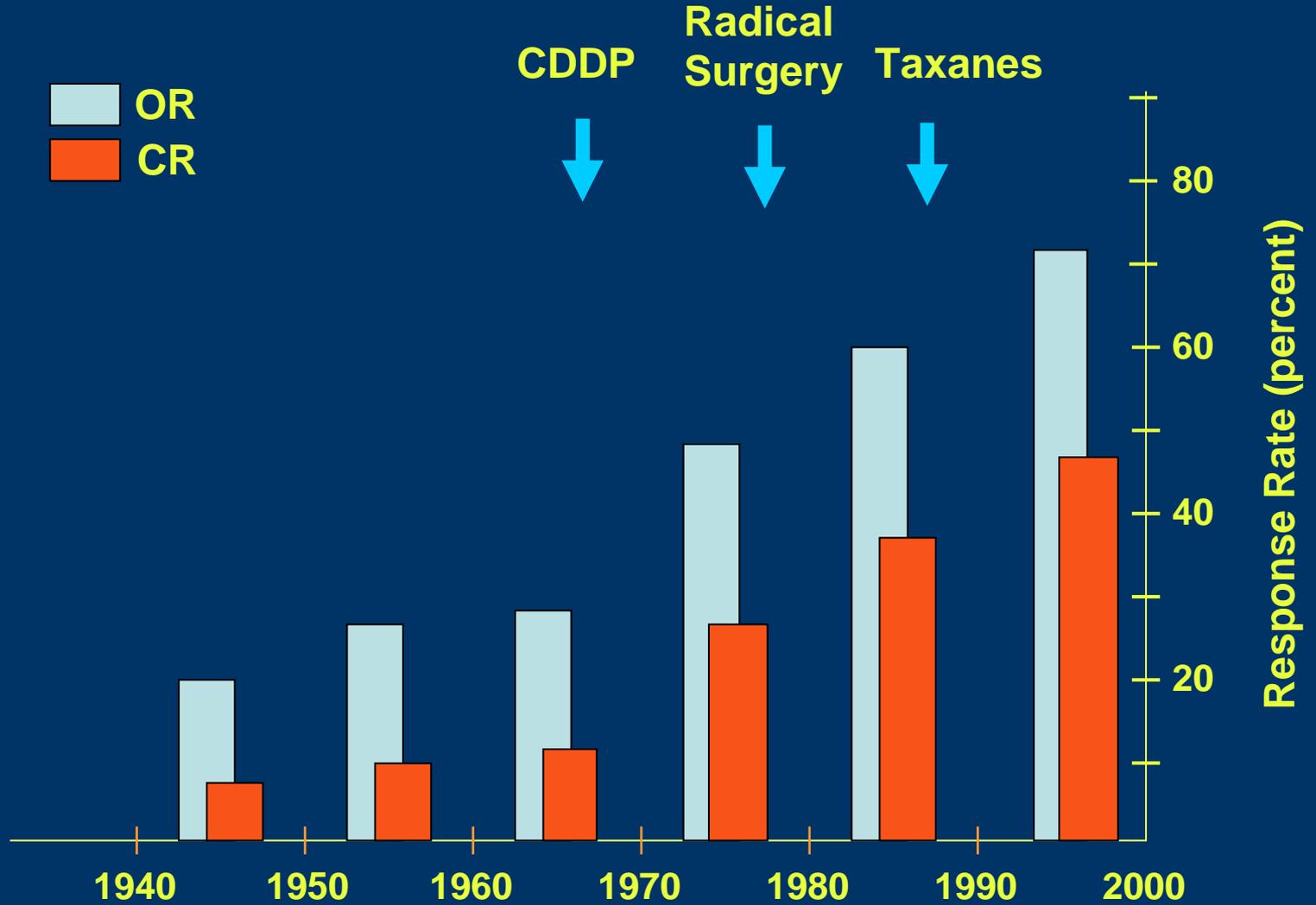
**By**

**George Coukos, M.D., Ph.D.  
Associate Professor  
Department of Obstetrics and Gynecology  
University of Pennsylvania Medical Center**

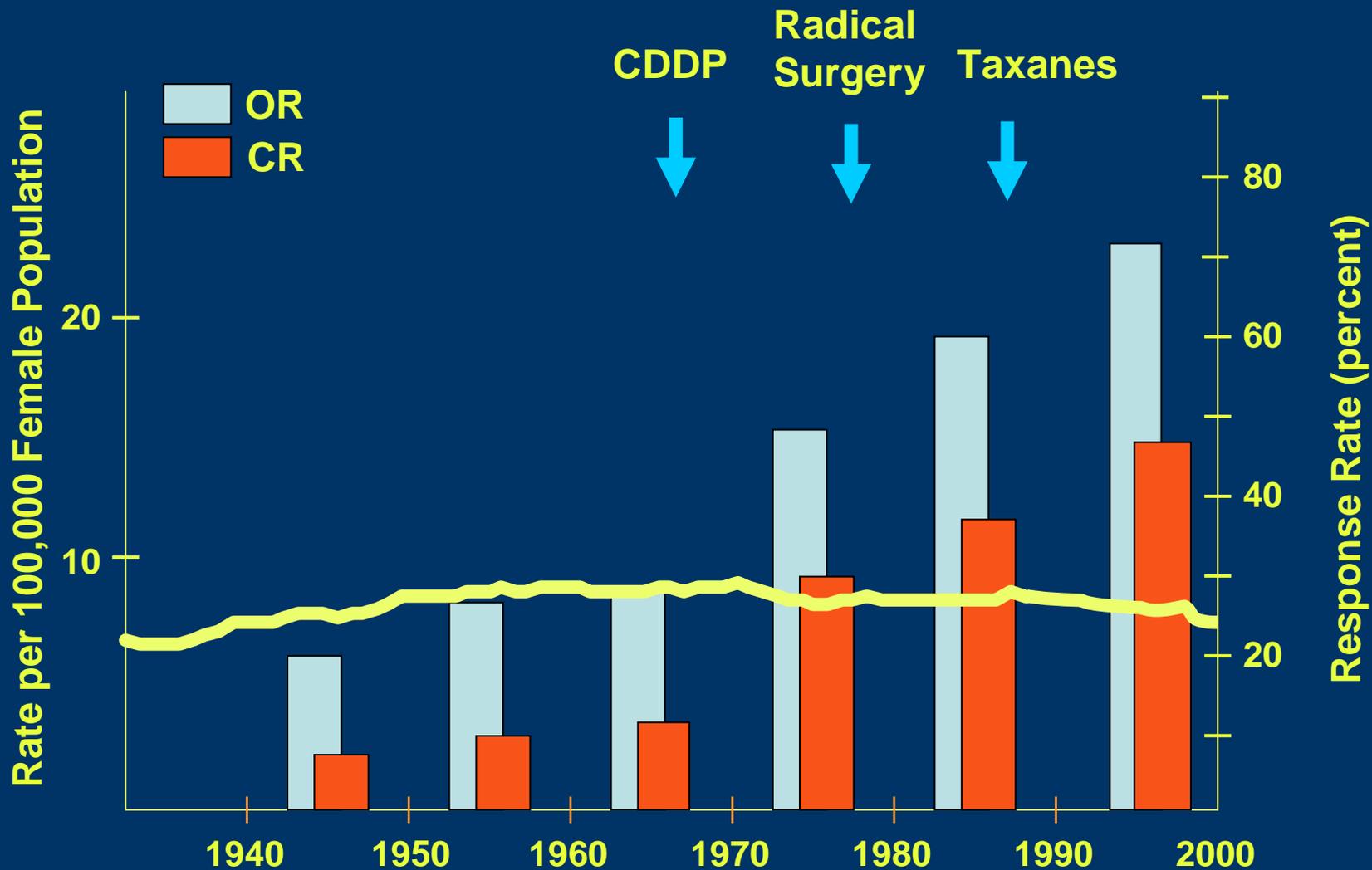
# American Cancer Society Female Cancers 2000 Statistics



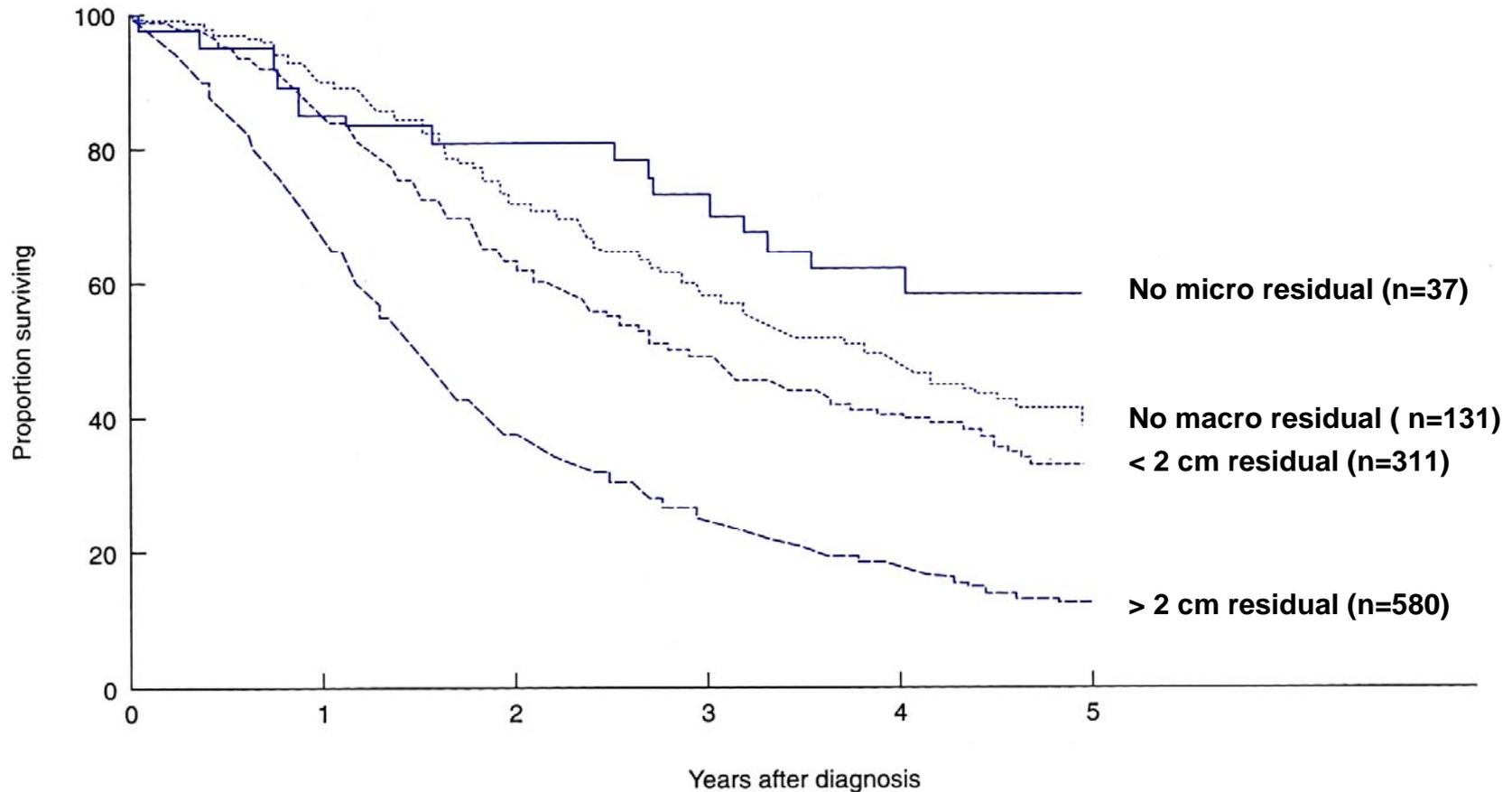
# Response Rates in U.S. Women with Ovarian Cancer

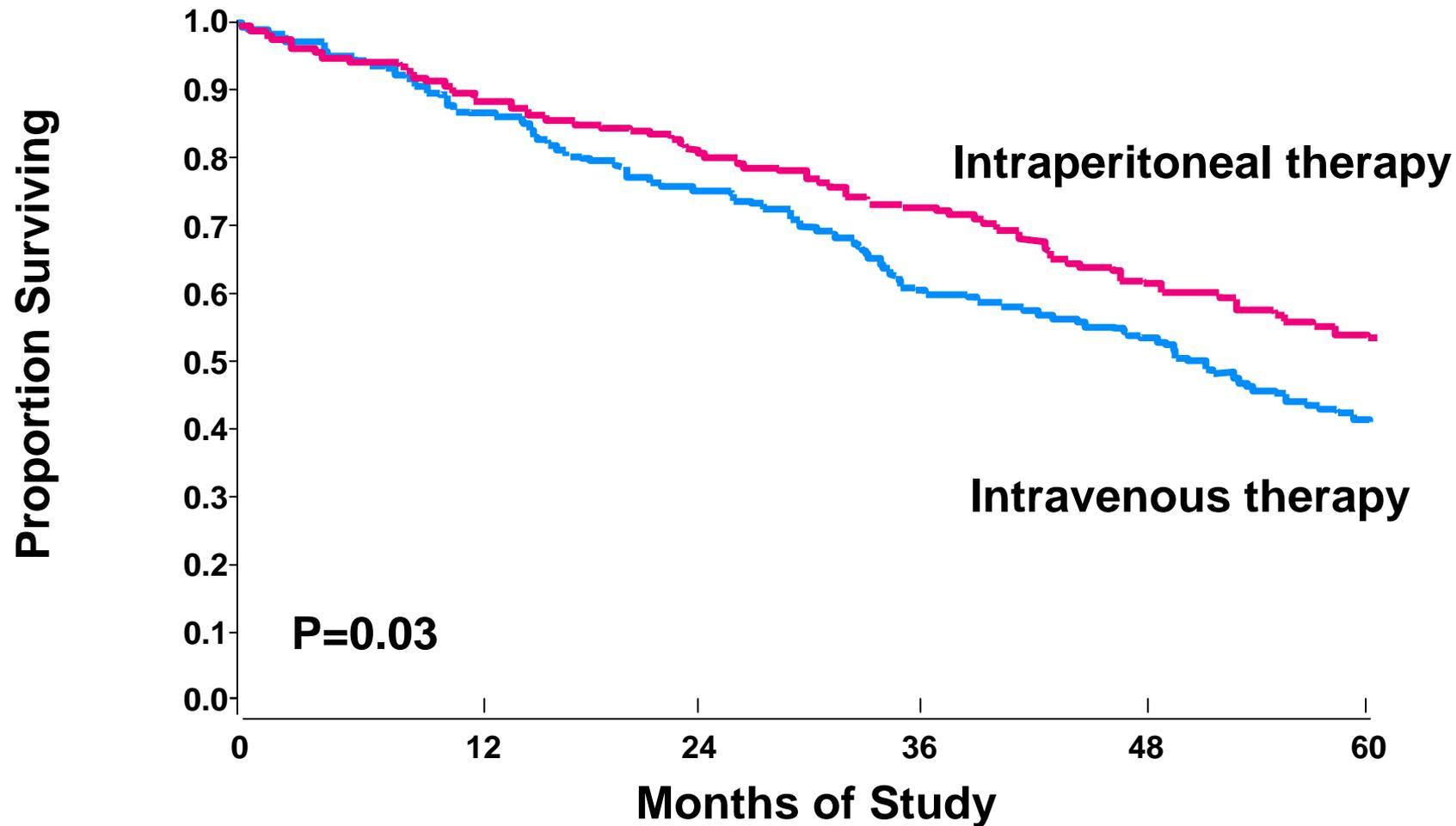


# Age-Adjusted Cancer Death Rates vs. Response Rates in U.S. Women with Ovarian Cancer



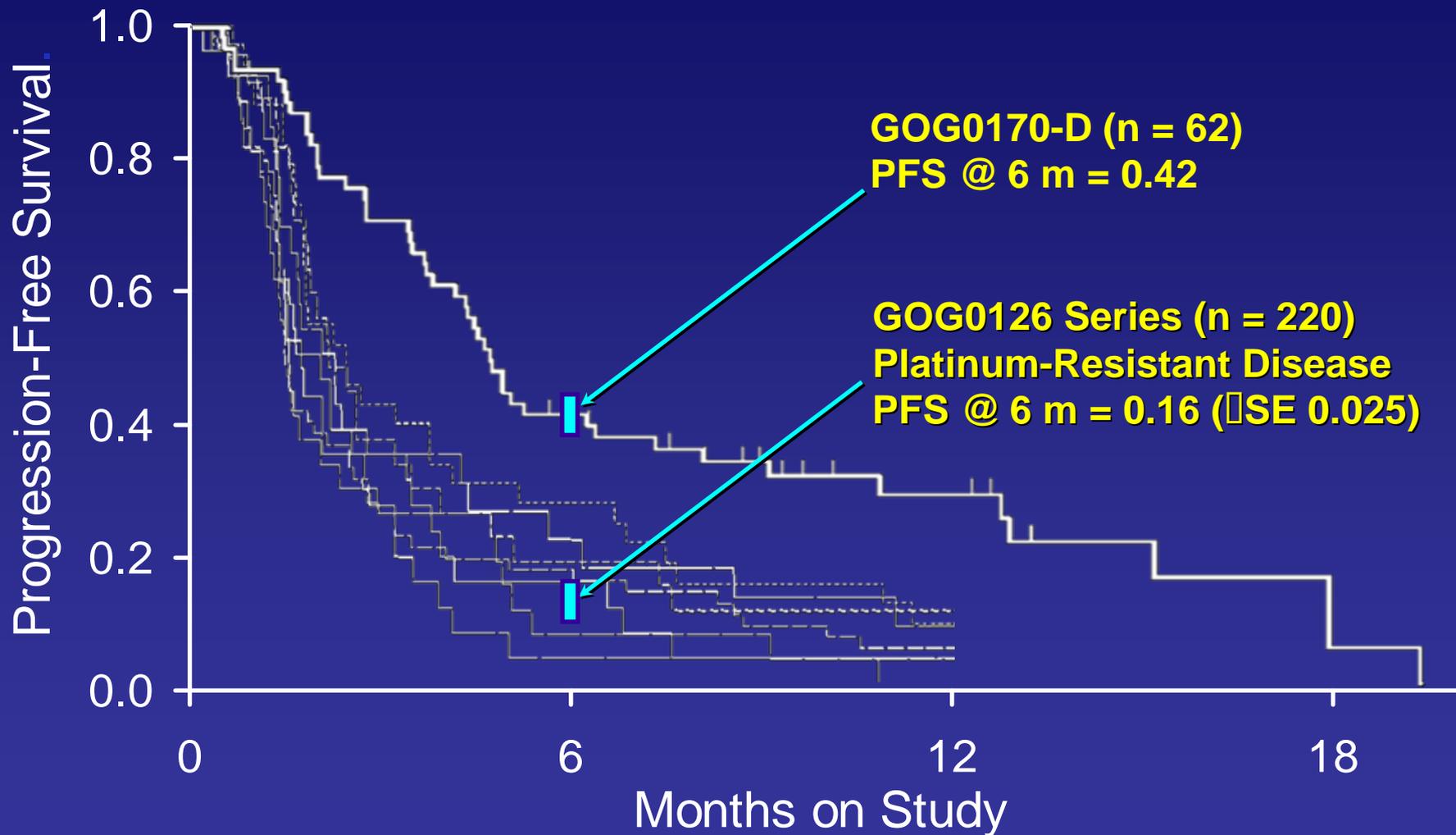
# 5-yr Survival in Stage IIIc Ovarian Ca (1059 Patients Treated in 1992-1994)



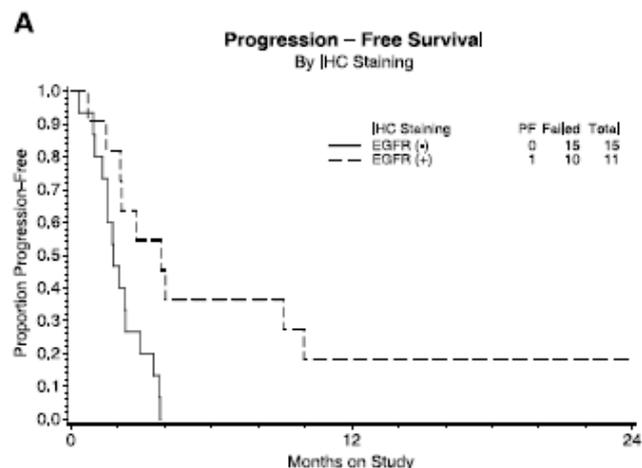
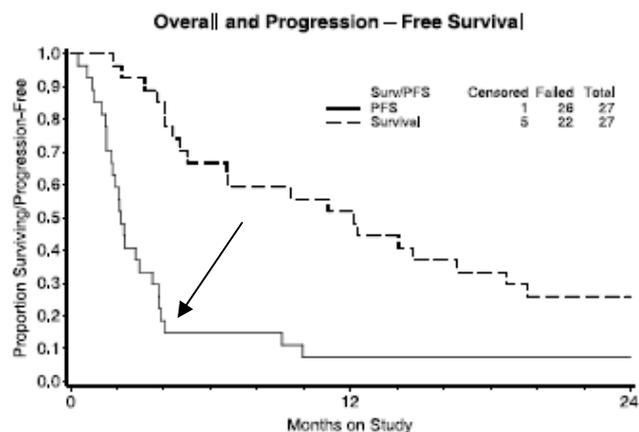


<u>No. at Risk</u>						
Intravenous therapy	210	183	157	123	106	63
Intraperitoneal therapy	205	183	165	142	114	77

# GOG0170D: Bevacizumab Phase II



# Phase II Study of Gefitinib in Patients with Relapsed or Persistent Ovarian or Primary Peritoneal Carcinoma and Evaluation of Epidermal Growth Factor Receptor Mutations and Immunohistochemical Expression: A Gynecologic Oncology Group Study



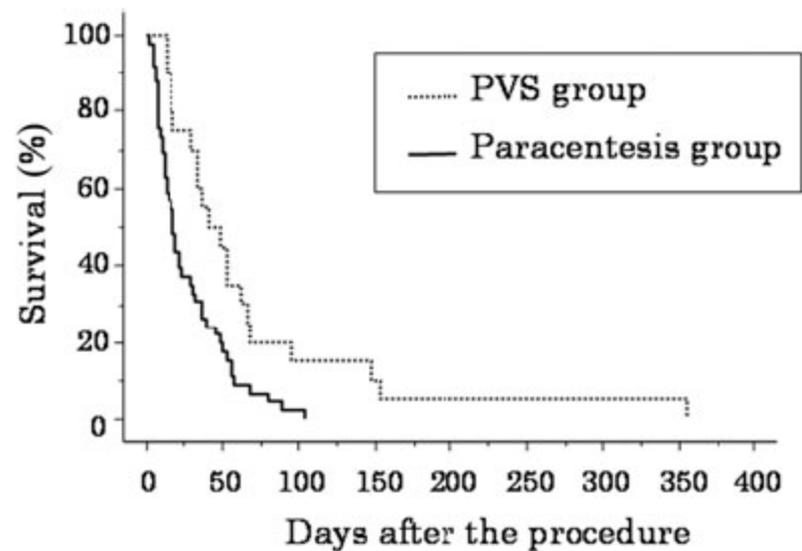
**Table 4.** Relationship between toxicities and progression-free survival

	Progression-free survival $\geq 6$ mo ( $n = 4$ )	Progression-free survival $< 6$ mo ( $n = 23$ )	Log odds ratio 95% confidence interval
Skin toxicity, grade 3			
Yes	3 (75%)	1 (4%)	[0.689, 8.190]
No	1 (25%)	22 (96%)	
Skin toxicity, any grade			
Yes	4 (100%)	14 (61%)	[-0.759, $\infty$ ]
No	0 (0%)	9 (39%)	

## Treatment of malignant ascites in patients with advanced cancer: Peritoneovenous shunt versus paracentesis

Masahiro Seike, Iruru Maetani and Yoshihiro Sakai

Division of Gastroenterology, Department of Internal Medicine, Toho University Ohashi Medical Center, Meguro-ku, Tokyo, Japan

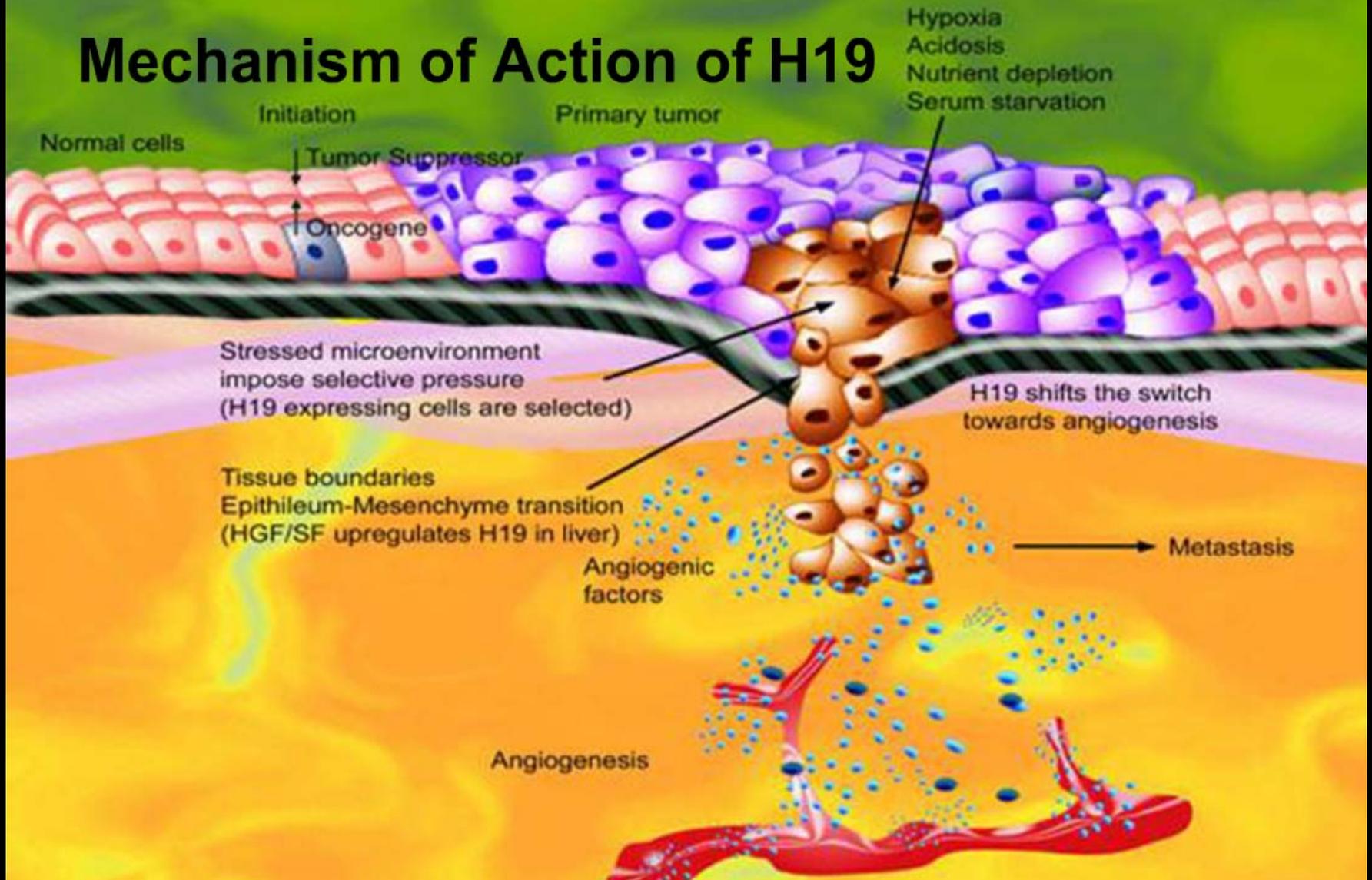


**Figure 6** Survival curves from the time of peritoneovenous shunt (PVS) placement or the first paracentesis until death.

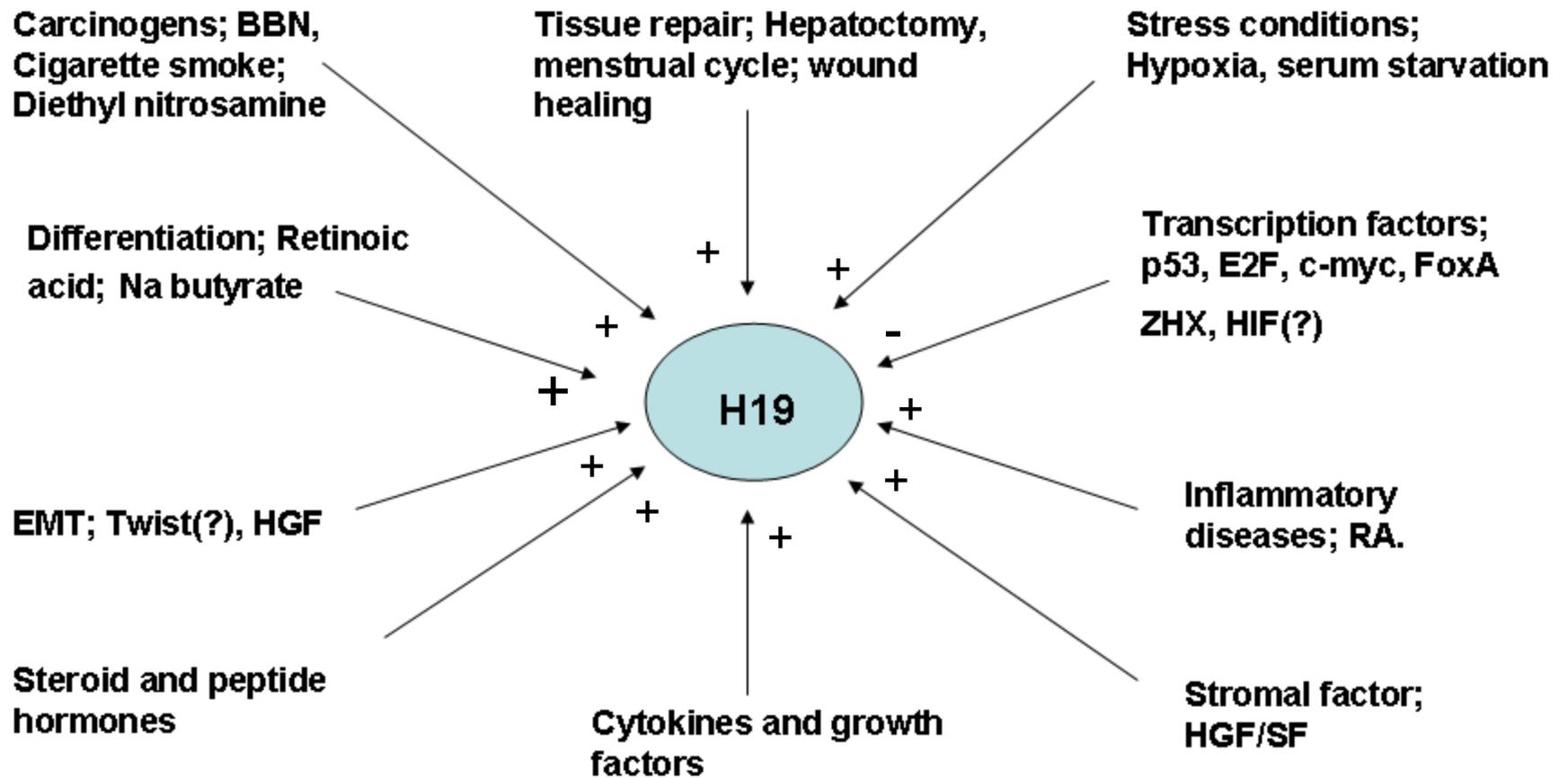
# Gene therapy approaches tested in ovarian cancer

- Suicide gene therapy or corrective gene therapy with adenovirus
  - thymidine kinase suicide gene
  - p53 gene
  - anti-erbB-2 single-chain antibody
  - interferon-beta
- Oncolytic viral therapy
  - oncolytic adenovirus ONYX
  - measles virus
- Liposomal DNA gene therapy
  - E1A gene
  - BRCA1 gene
- Gene-immune therapy
  - engineered T cells with chimeric immunoreceptor

# Mechanism of Action of H19

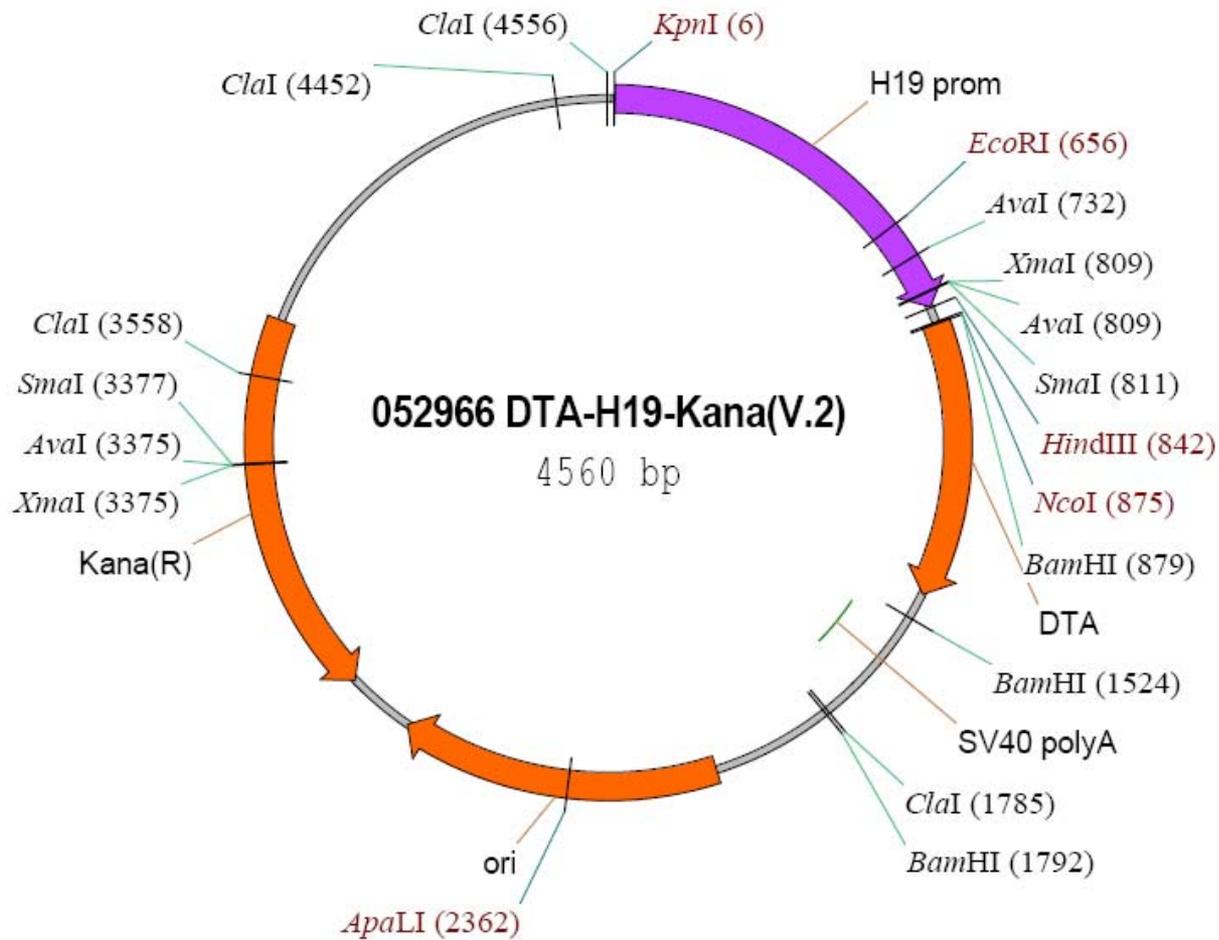


# Modulators of H19 Gene



# Expression of H19 in adult

- Normal tissues
  - Low levels in bone marrow
  - Corpus luteum
- Abnormal
  - Wound healing
  - Liver regeneration
  - Inflammatory sites
    - Atherosclerotic plaque
    - Rheumatoid arthritis synovial tissue
    - Airway of smokers

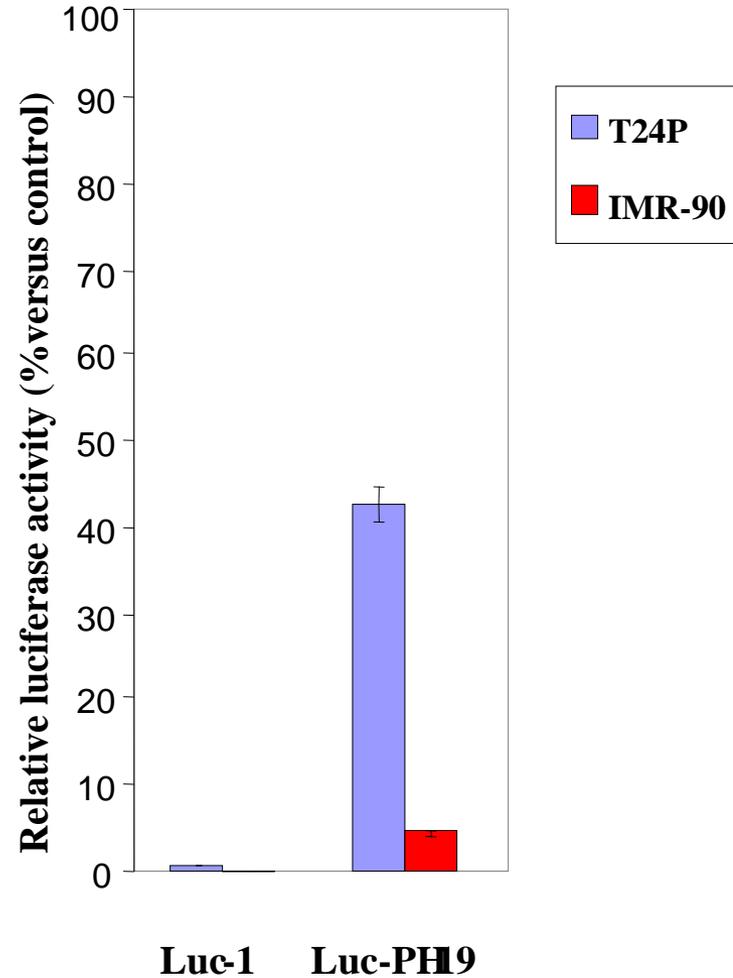


## **DTA-H19 DNA PLASMID**

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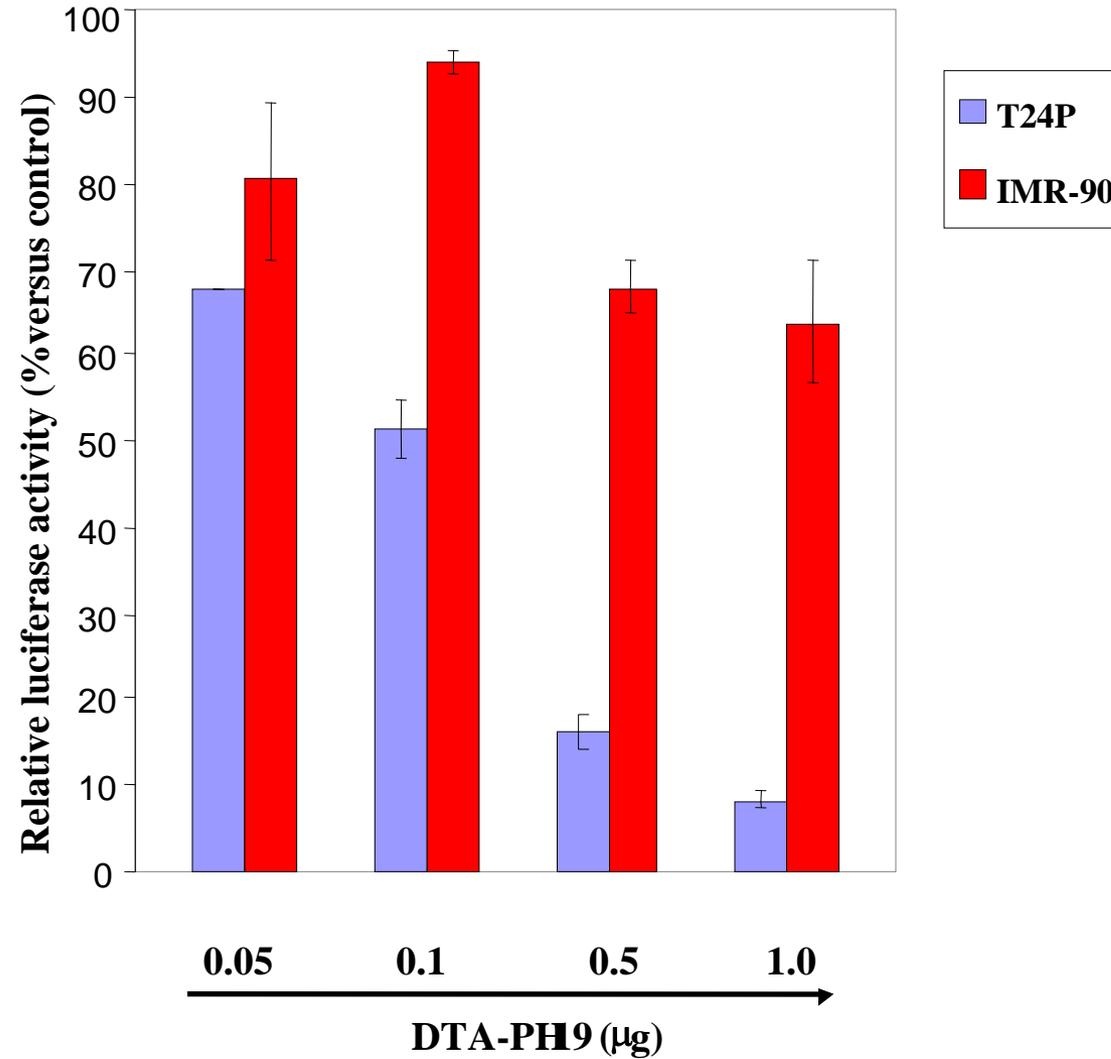
- **DTA-H19 is a double-stranded DNA plasmid that contains the gene for the diphtheria toxin A (DT-A) chain under the regulation of the H19 promoter**
- **The H19 gene is an oncogene that is overexpressed in malignant cells that is not seen in adult tissues under normal conditions**
- **The transcription factors acting on H19 promoter and upregulating H19 gene are activated in tumor cells**
- **DT-A chain encoded by DTA-H19 is expressed in tumor cells and inhibits protein synthesis and induces apoptosis**
- **DT-A protein cannot bind to cell surface receptors, thus DTA-H19 does not cause “bystander” effects**

## Specificity of H19 expression



Luciferase indicates expression of H19  
Regulatory sequences

## Specificity of DTA-H19 expression



## **SAFETY AND EFFICACY WERE SHOWN IN ANIMALS**

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**Animal efficacy studies included mouse, rat, and hamster syngeneic tumors and xenogeneic human tumors models:**

- **Intravesical administration of DTA-H19 caused regression of orthotopic tumor implanted in rat bladders (with no evidence of adverse pathology to normal bladder).**
- **Intraperitoneal administration of DTA-H19 in mice with human ascites tumor cells prolonged survival.**
- **Intratumoral administration and hepatic artery infusion of DTA-H19 in rats with liver metastases slowed tumor growth.**
- **Intratumoral administration of DTA-H19 in hamsters with pancreatic tumors implanted in the pancreas slowed tumor growth.**

**There were no adverse effects observed macroscopically or microscopically in the animal efficacy studies.**

# **GLP TOXICOKINETIC STUDIES IN MICE AND RATS**

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## **Repeat Dose Tox Study in Rats**

**6 intraperitoneal administrations of DTA-H19 up to 10 mg/kg 3x weekly for 2 weeks caused no adverse effects (clinical observations, weights, food consumption, histopathology, clinical labs)**

## **Repeat Dose Tox Study in Mice**

**6 intravenous administrations of DTA-H19 up to 10 mg/kg 3x weekly for 2 weeks caused no adverse effects (clinical observations, weights, food consumption, histopathology, clinical labs)**

## **PREVIOUS HUMAN EXPERIENCE WITH DTA-H19**

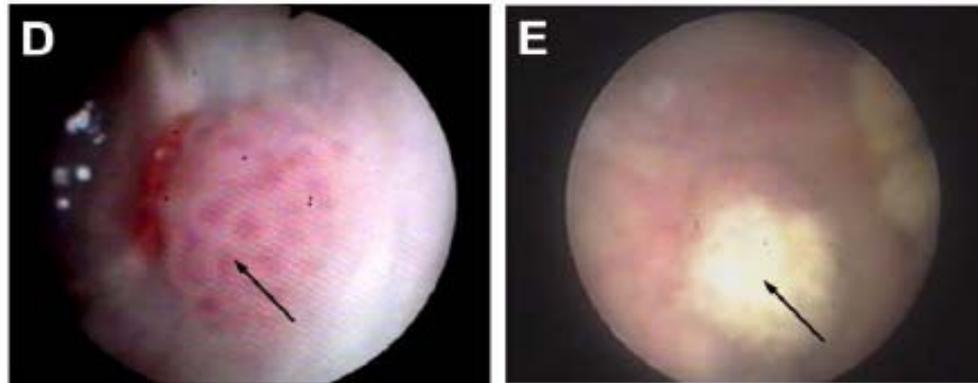
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- **Phase 1/2a Study of Superficial Bladder Cancer N=18 – completed**
- **Phase 2b Study of Superficial Bladder Cancer N=33 – ongoing**
- **Compassionate Treatment Patients**
  - **Two patients with hepatic metastases**
  - **Two patients with bladder cancer**
  - **One patient with ovarian cancer**

# TUMOR RESPONSE TO DTA-H19 IN BLADDER CANCER

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Videocystoscopy showing tumor before  
Intravesical administration



Videocystoscopy showing focal necrosis  
In area of tumor after 6  
intravesical administrations (2 mg DTA-H19)

2→20 mg DTA-H19/PEI  
Had failed bCG q wk X 3  
No DLT in 18 pts

## Possibly related AEs

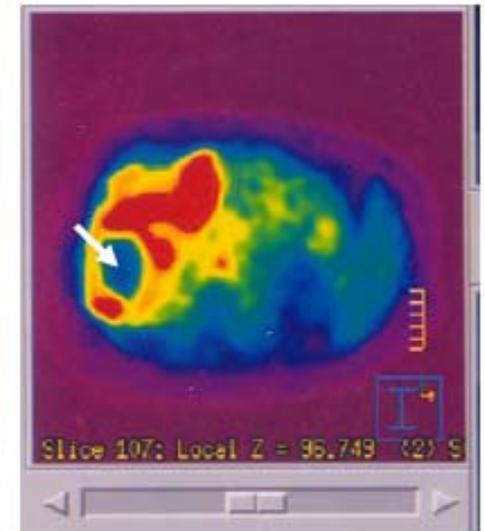
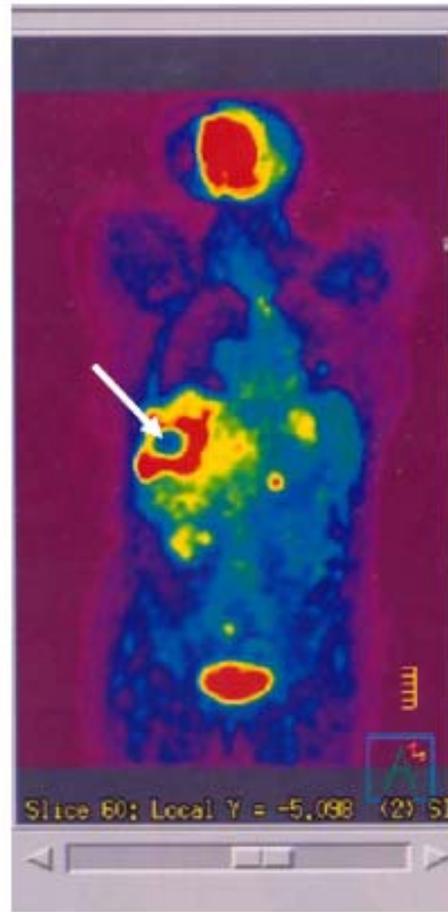
Grade 1 diarrhea (11%), HTN (16%), asthenia (11%), increased creatinine (22%), leukopenia (11%), increased LFTs (4%)  
1 case (6mg) grade 3 cystitis  
CR: 4/18, PR: 3/18, SD: 5/18

# COMPASSIONATE HEPATIC METASTASIS PATIENT TREATED WITH INTRATUMORAL DTA-H19



(A) CT scan of liver showing multiple metastatic lesions and rib involvement (arrow).

2 doses = 12mg  
Palliative relief

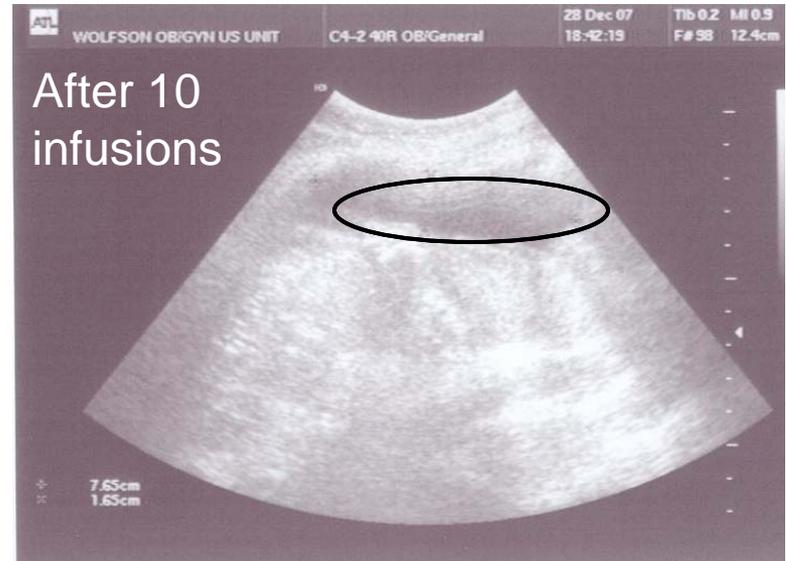
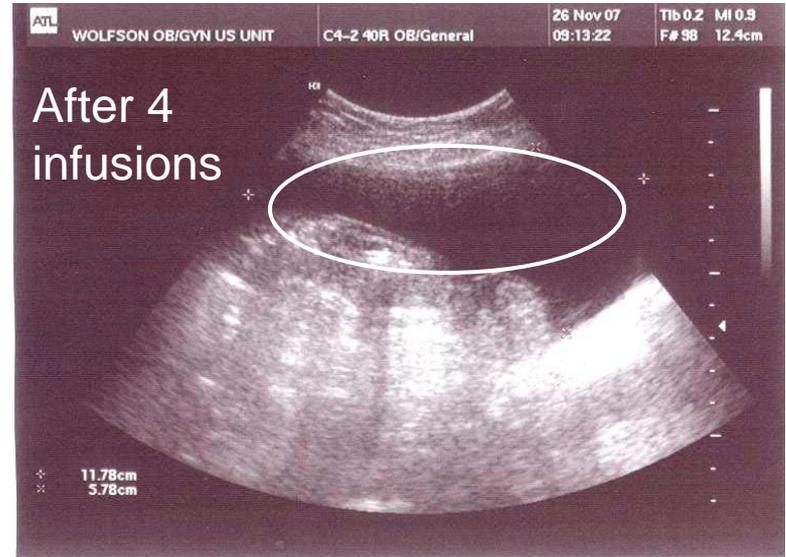
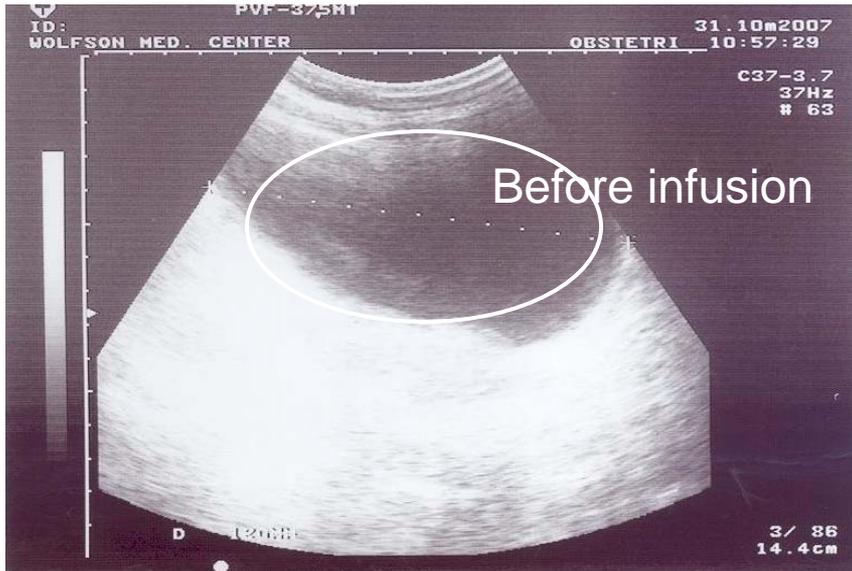


## **Second case: Intrahepatic intra-arterial DTA-H19 x 2 courses, 3 weekly 16→84 mg, stable dz x 2 months**

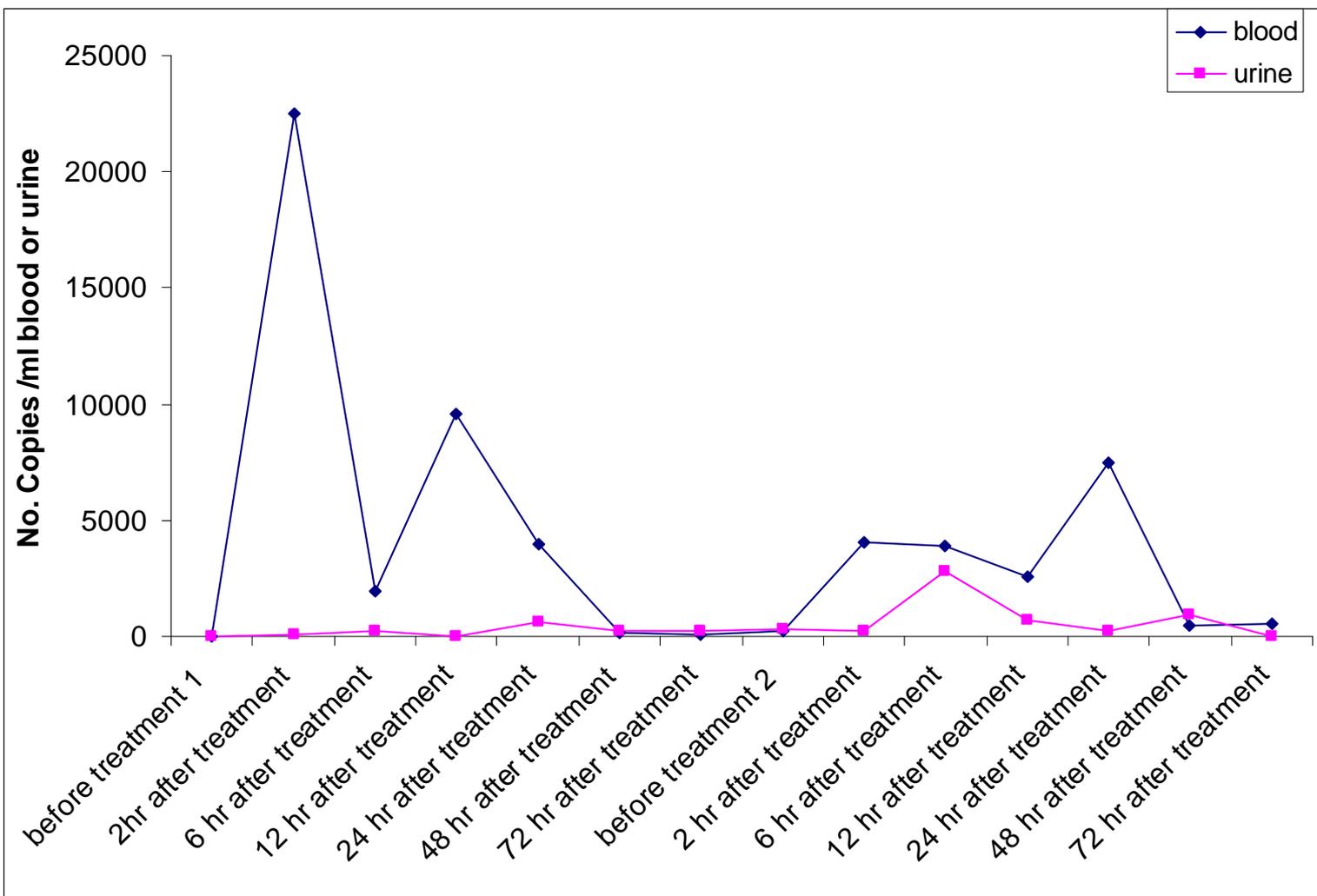
- During the 4th through the 6th rounds of infusions, elevations in ALT, AST, and GGT were observed, but lactate dehydrogenase and total bilirubin levels remained within normal limits throughout. These elevations were asymptomatic.
- Thrombocytopenia was reported once after the first round of HAI and was within normal limits for the duration of the rest of the 6 infusions. Hemoglobin fluctuated one time to 21% lower than the patient's baseline level.

# COMPASSIONATE OVARIAN PATIENT TREATED WITH INTRAPERITONEAL DTA-H19

Ultrasound showing area of ascites

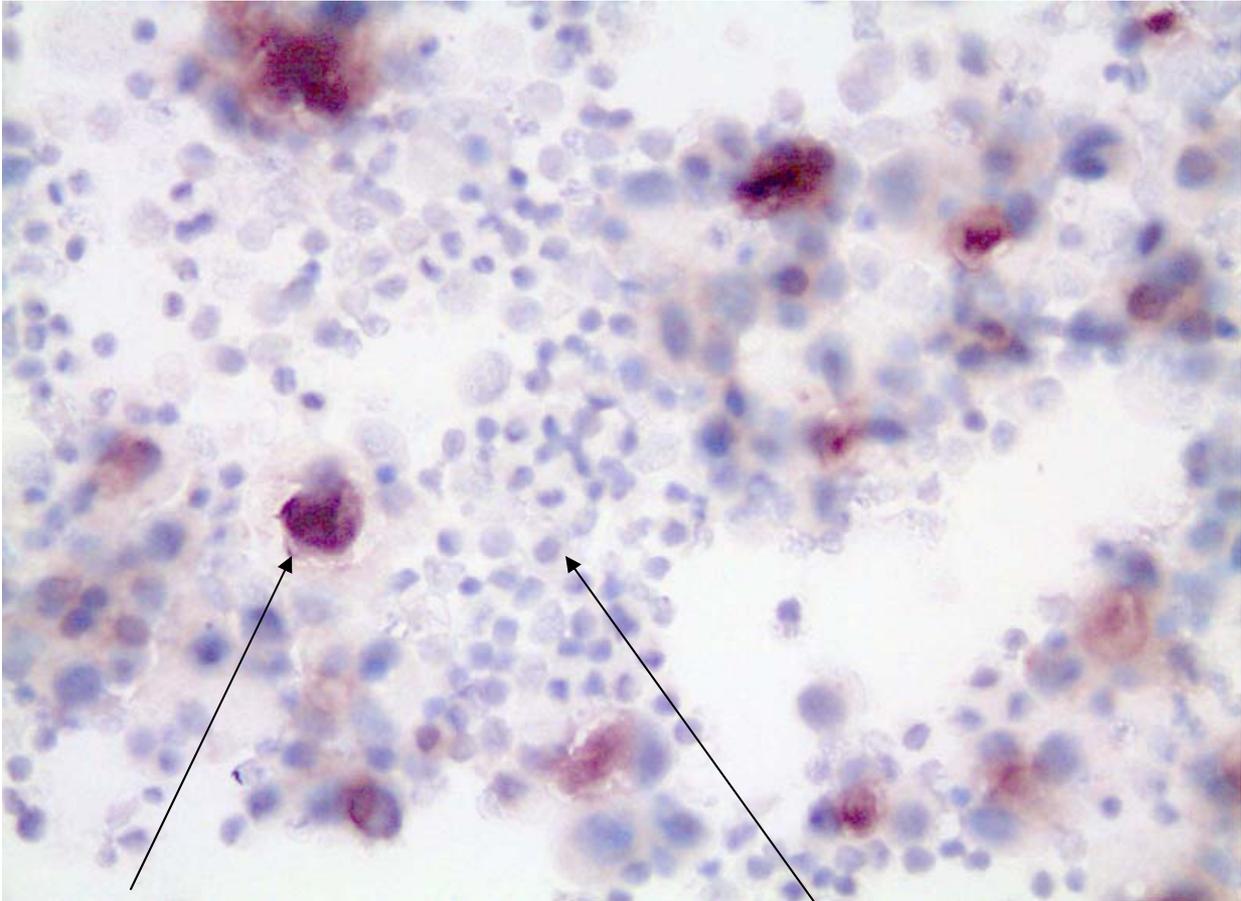


Treatment: 80 → 140 mg IP weekly x 10 then  
Combined with taxane weekly  
10/07 → 2/09, improved QOL, no AEs  
Nausea, fever, chills up to #7  
Total 1.7g in 17 IP injections as of Sept 08



# COMPASSIONATE OVARIAN PATIENT ASCITES TUMOR CELLS SHOWING H19 EXPRESSION

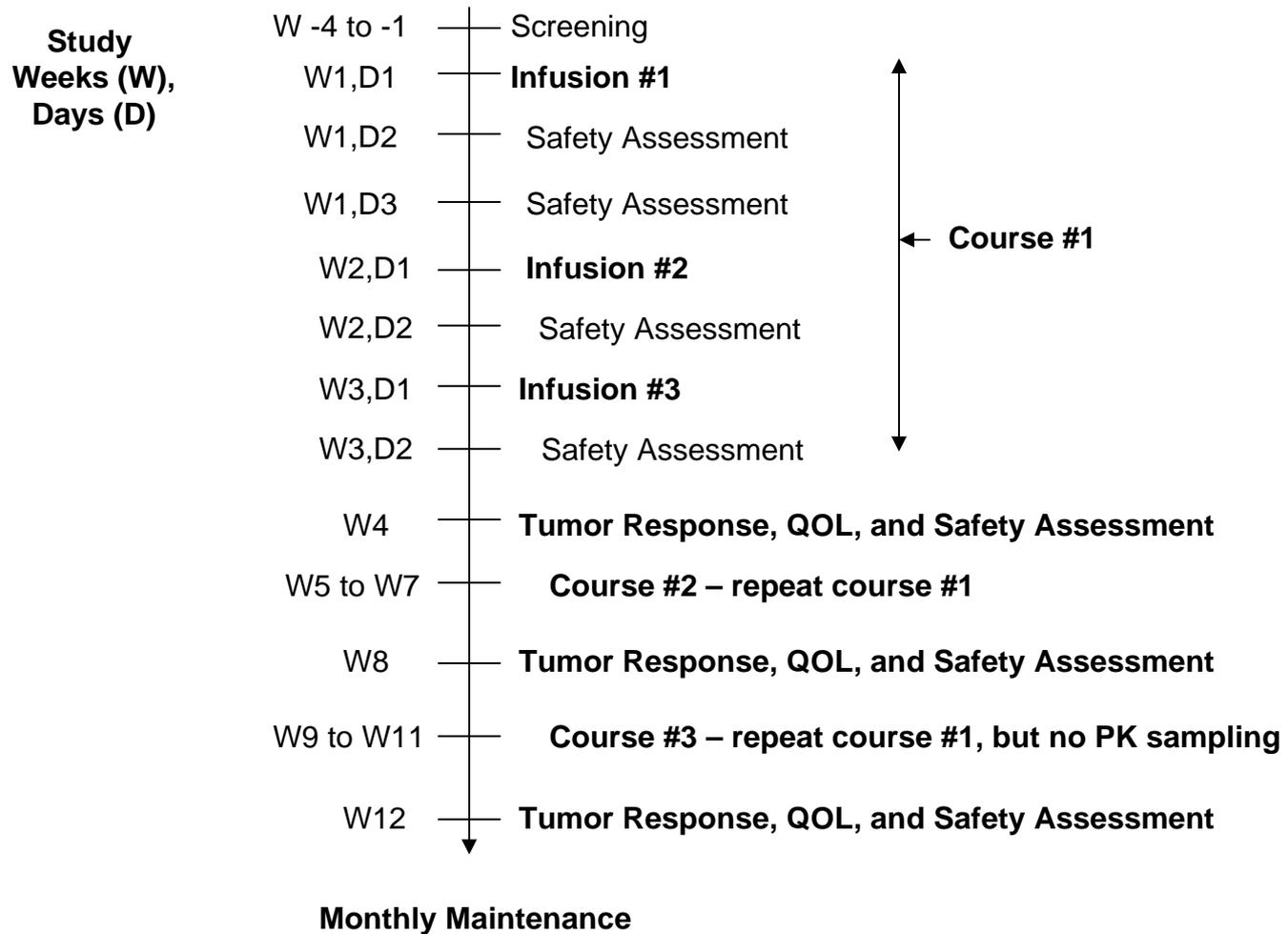
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Cytoplasmic staining of tumor cells

Absence of staining in leukocytic infiltrate

# Phase 1/2a Ovarian Cancer Study Schema



<b>Standard Dose Escalation Scheme</b>		
<b>Cohort #</b>	<b>Dose</b>	<b>Maintenance Dose</b>
1	60 mg IP weekly for 3 weeks, one week rest, then repeat for 2 more courses	60 mg IP once per month, for 6 months
2	120 mg IP weekly for 3 weeks, one week rest, then repeat for 2 more courses	120 mg IP once per month, for 6 months
3	240 mg IP weekly for 3 weeks, one week rest, then repeat for 2 more courses	120 mg IP once per month, for 6 months
4	480 mg IP weekly for 3 weeks, one week rest, then repeat for 2 more courses	120 mg IP once per month, for 6 months

<b>Example of the Modified Fibonacci Dose Escalation Scheme</b>		
<b>Cohort #</b>	<b>Dose</b>	<b>Maintenance Dose</b>
2	Increase by 60%: 100 mg IP weekly for 3 weeks, one week rest, then repeat for 2 more courses	100 mg IP once per month, for 6 months
3	Increase by approximately 40%: 140 mg IP weekly for 3 weeks, one week rest, then repeat for 2 more courses	100 mg IP once per month, for 6 months
4	Increase by approximately 25%: 175 mg IP weekly for 3 weeks, one week rest, then repeat for 2 more courses	100 mg IP once per month, for 6 months

# Study Design Features to Optimize Safety Monitoring

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- Staggered entry of patients into the study
- 2-fold dose escalation between cohorts
- Modified Fibonacci dose escalation (60%→40%→25%) if grade 2 AEs related to investigational product are observed
- 24-hour reporting of grade 2 or greater AEs to Medical Monitor + PI
- Continuous cardiac monitoring during infusions
- Clinical labs are assessed after every infusion
- AEs are assessed at each visit and before and after each infusion
- Pain scores are assessed before and after each infusion
- Physical exam is performed at each visit