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Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Montefiore-Einstein
Center for
Cancer Care

Nanoparticles as Therapeutic Platforms

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Clinical Research Perspective

- What do clinical researchers need to know about nanotechnology?
- What tests must nano-materials themselves undergo?
- What are the potential and known risks with the use of nanotechnology and how are unknowns being addressed?
- Are there real advantages to nanotechnology?

Proc. Nat. Acad. Sci. USA
Vol. 72, No. 9, pp. 3666–3670, September 1975
Immunology

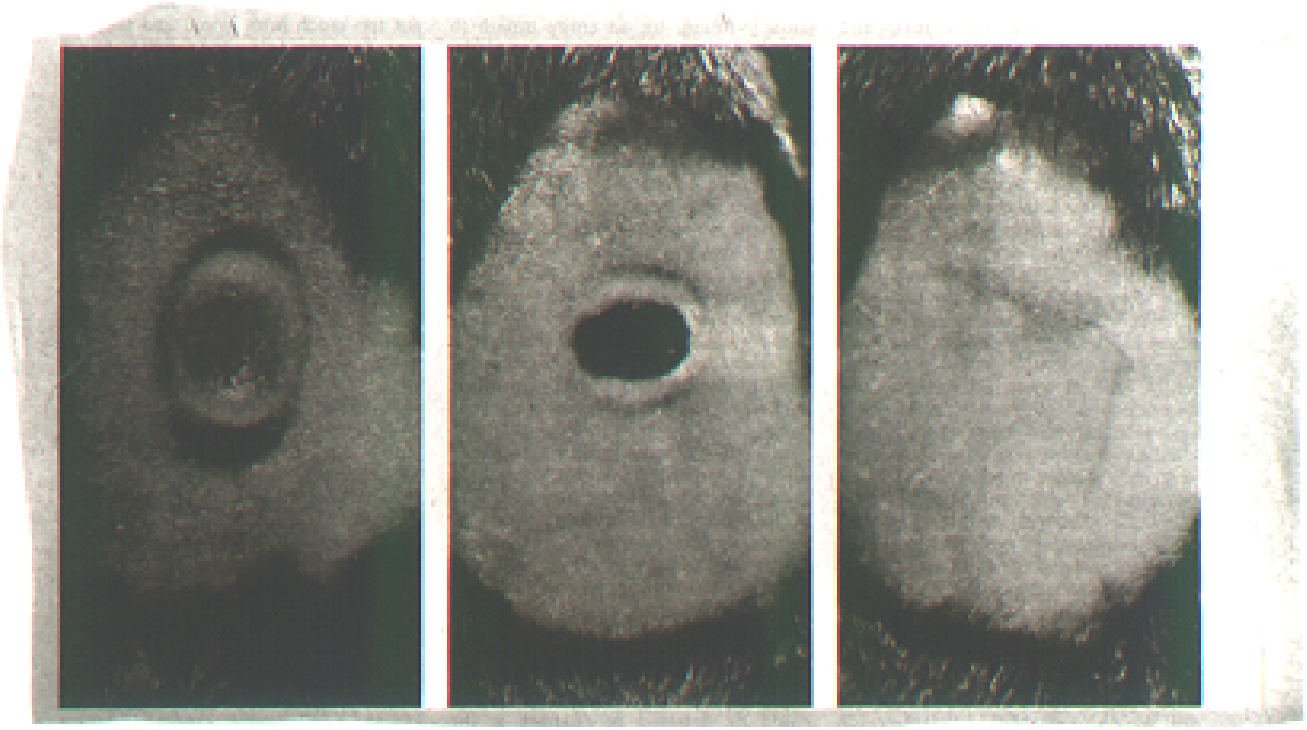
An endotoxin-induced serum factor that causes necrosis of tumors

(activated macrophage)

E. A. CARSWELL, L. J. OLD, R. L. KASSEL, S. GREEN, N. FIORE, AND B. WILLIAMSON

Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

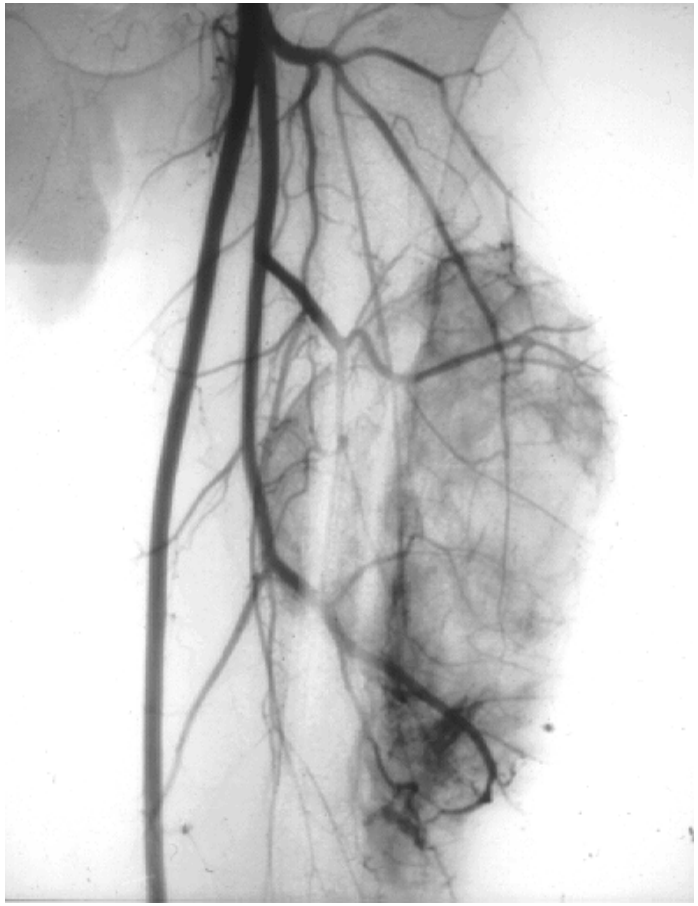
Communicated by Lewis Thomas, June 23, 1975



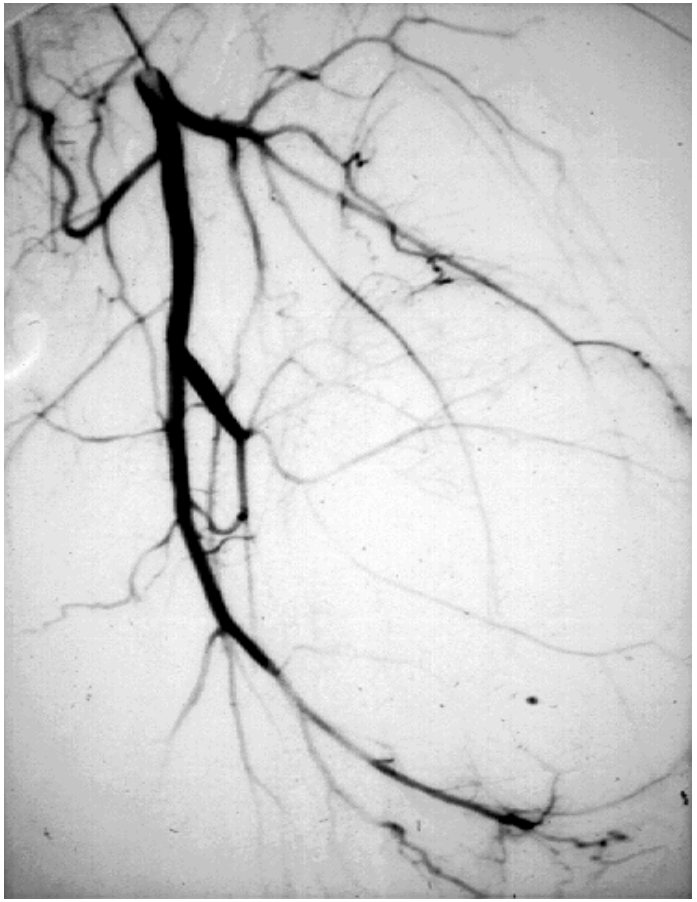
Tumor Necrosis Factor (TNF)

- Cytokine with potent anti-tumor effect
- Utility in humans currently limited by side effects and cannot be administered systemically
- Used clinically in regional perfusion setting
- Good candidate for targeted therapy as high doses may be delivered to the tumor while limiting systemic toxicity
- Anti-tumor effect mediated by effects on vascular endothelium
  - TNF induces vascular permeability & hypercoagulability

Pre-ILP



Post-ILP



Regression of Massive Melanoma After ILP with Melphalan and TNF

Phase II Trials of Intravenous TNF

Authors	Dose Schedule	Histology	Evaluable Patients (n)	Responses* CR	PR	MR/SD
Lenk et al.(120)	683-956 $\mu g/m^2$ q 8-12d	Mixed	18	0	0	4
Aboulafia et al.(121)	100 $\mu g/m^2$ q wk	Kaposi's sarc.	5	0	0	0
Kemeny et al.(122)	100-150 $\mu g/m^2$ qd x 5d	Colorectal	14	0	0	3
Whitehead et al.(123)	150 $\mu g/m^2$ qd x 5d	Colorectal	20	0	0	2
Schaadt et al.(124)	217-652 $\mu g/m^2$ 3x/wk	Colorectal	15	0	0	1
Budd et al.(125)	150 $\mu g/m^2$ qd x 5d	Breast	19	0	0	0
Brown et al.(119)	150 $\mu g/m^2$ qd x 5d	Pancreas	22	0	0	0
Skillings et al.(126)	150 $\mu g/m^2$ qd x 5d	Renal cell	22	1	1	0
Feldman et al.(127)	150 $\mu g/m^2$ qd x 5d	Melanoma	21	0	0	1
Total:			156	1	1	11

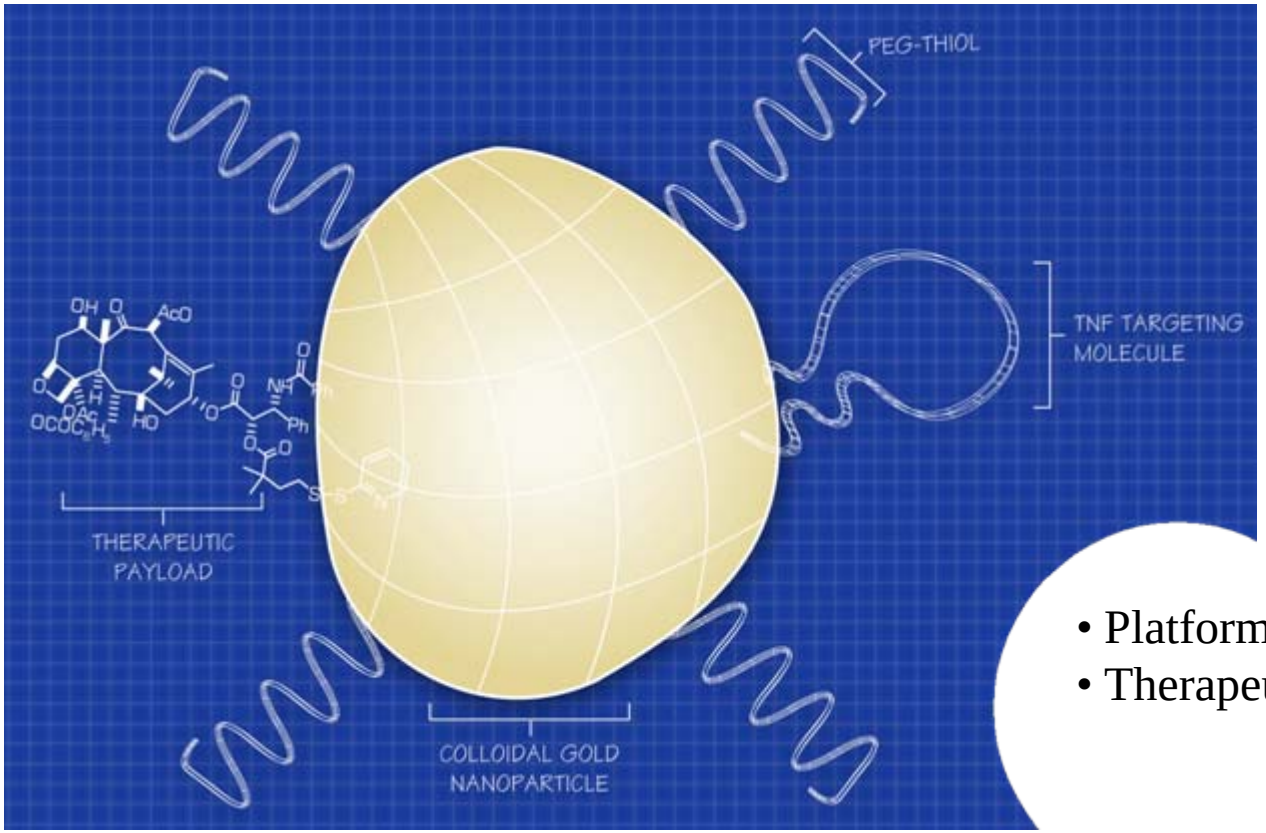
Dose-Limiting Toxicities in Phase I Trials of Intravenous TNF

	Number of Trials (%)		
	Bolus Intravenous	Cont. Intravenous	Total
Dose-Limiting Toxicity	(n=12)	(n=6)	(n=18)
Hypotension	11 (92)	2 (33)	13 (72)
Hepatotoxicity	5 (42)	1 (17)	6 (33)
Malaise, myalgia, fatigue	2 (17)	2 (33)	4 (22)
Thrombocytopenia	1 (8)	3 (50)	4 (22)
CNS toxicity, confusion	1 (8)	2 (33)	3 (17)
Leukopenia	1 (8)	1 (17)	2 (11)
Headache	0 (0)	2 (33)	2 (11)
Nausea	1 (8)	0 (0)	1 (6)
Fever, chills	0 (0)	1 (17)	1 (6)

Colloidal Gold As Nanomedicine:

- History of safety (colloidal gold used for 70+ years)
- Tumor targeted
- Improved biodelivery
- Increased efficacy with lower toxicities
- Highly versatile
- Ease of manufacturing

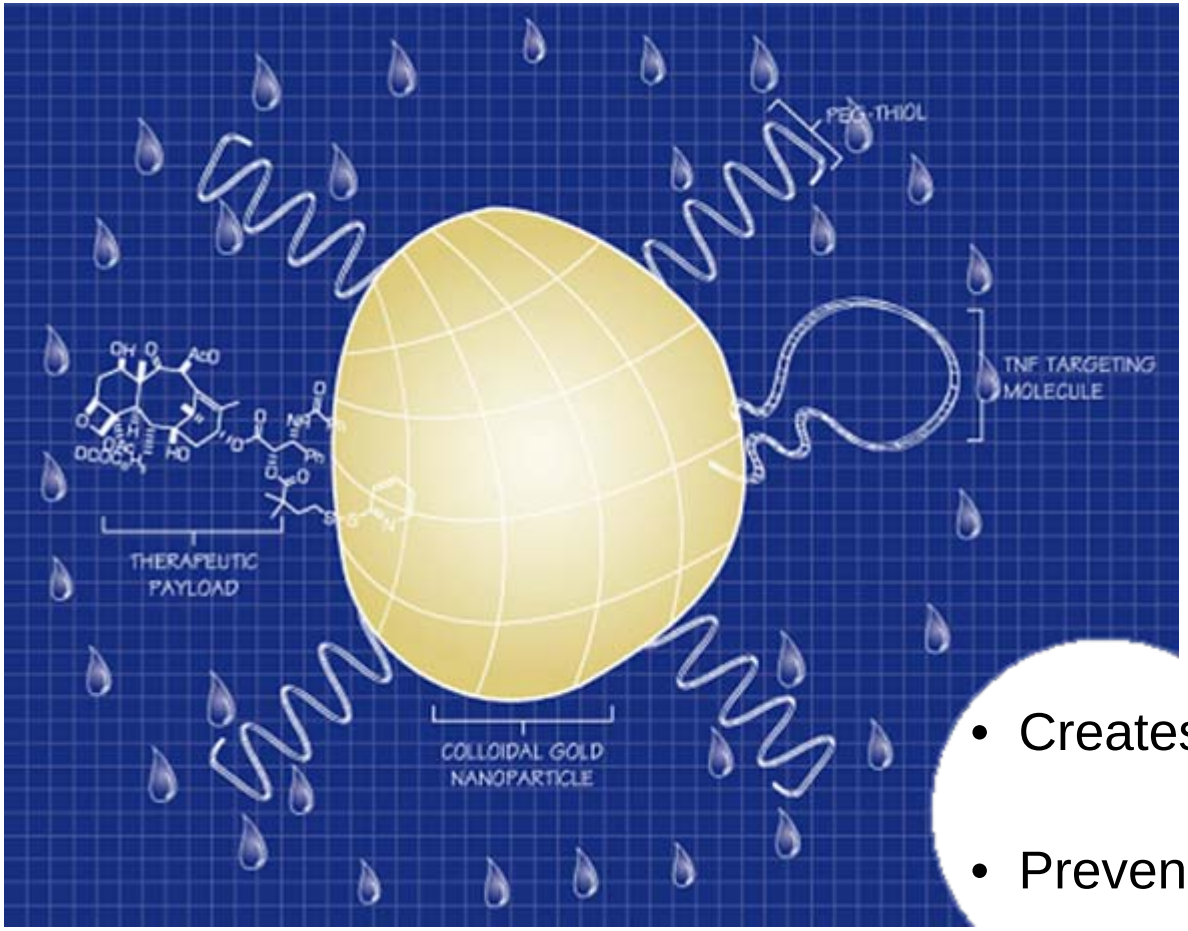
Cytimmune Core Technology:



- Platform for Targeting Ligand
- Therapeutic Payload

Cytimmune Core Technology:

PEG-THIOL Particle Hydration after Intravenous Injection



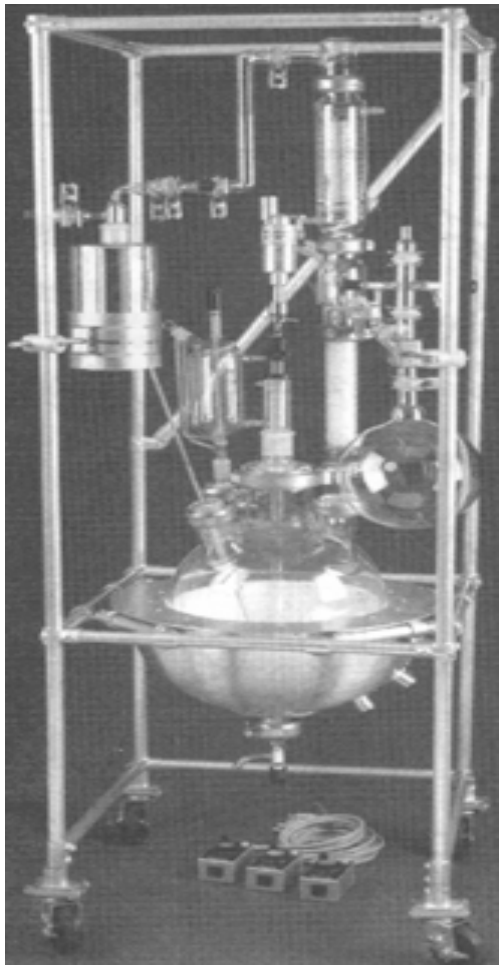
- Creates a Water Shield
- Prevents Immune Detection

Colloidal Gold Nanoparticle Synthesis

Gold Chloride

+

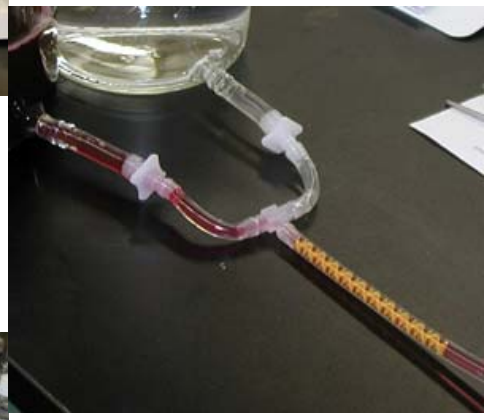
Sodium Citrate



Au^{3+} → Au^{0}

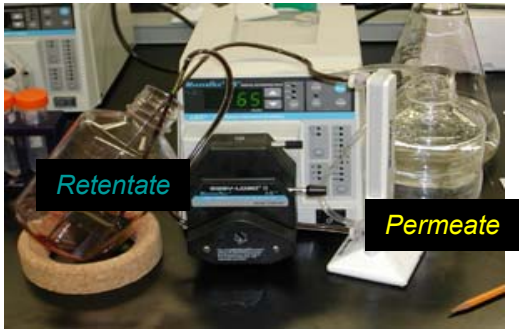
Manufacturing at a Glance

Current Manufacturing Process utilizes 1 pump to draw reagents into the in-line mixer. 1 Pump and or the Volume of mixer (<100 □l) may be limiting for large scale process

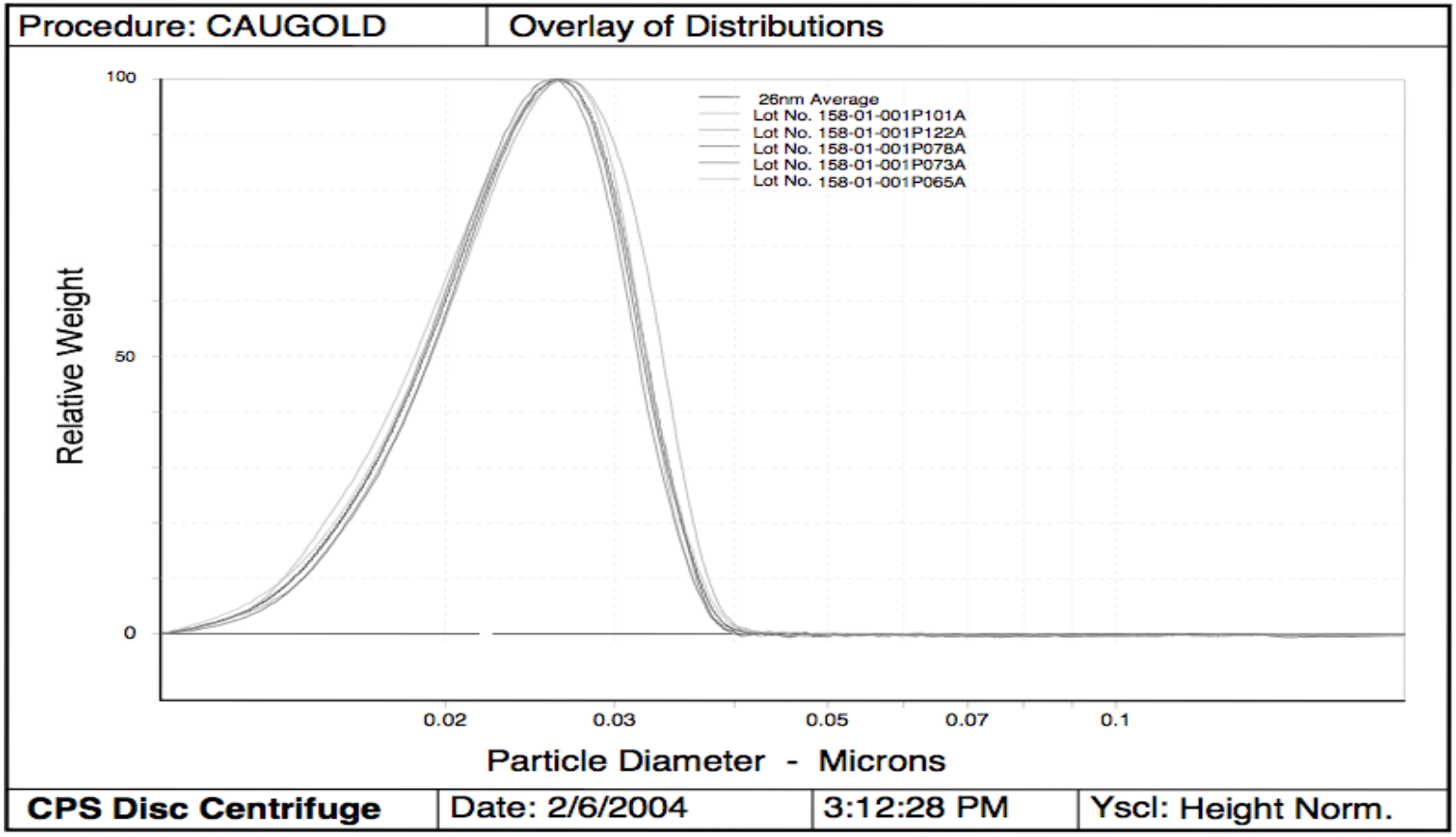


Addition of HSA follows complete mixing of regents

Concentration

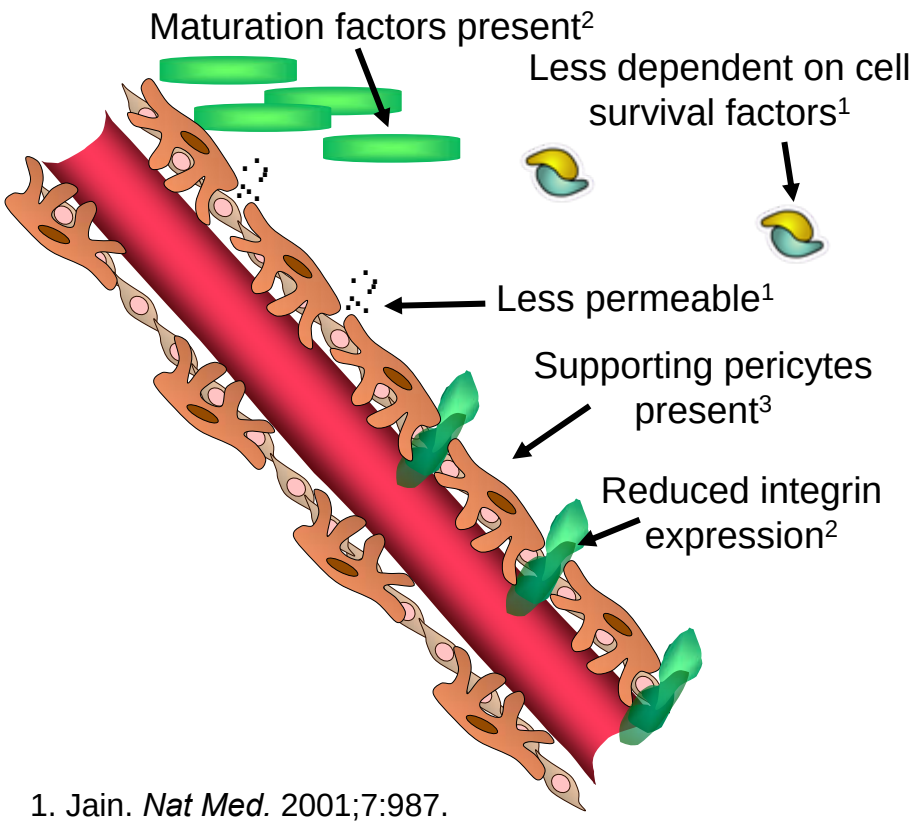


Reproducibility of Particle Synthesis

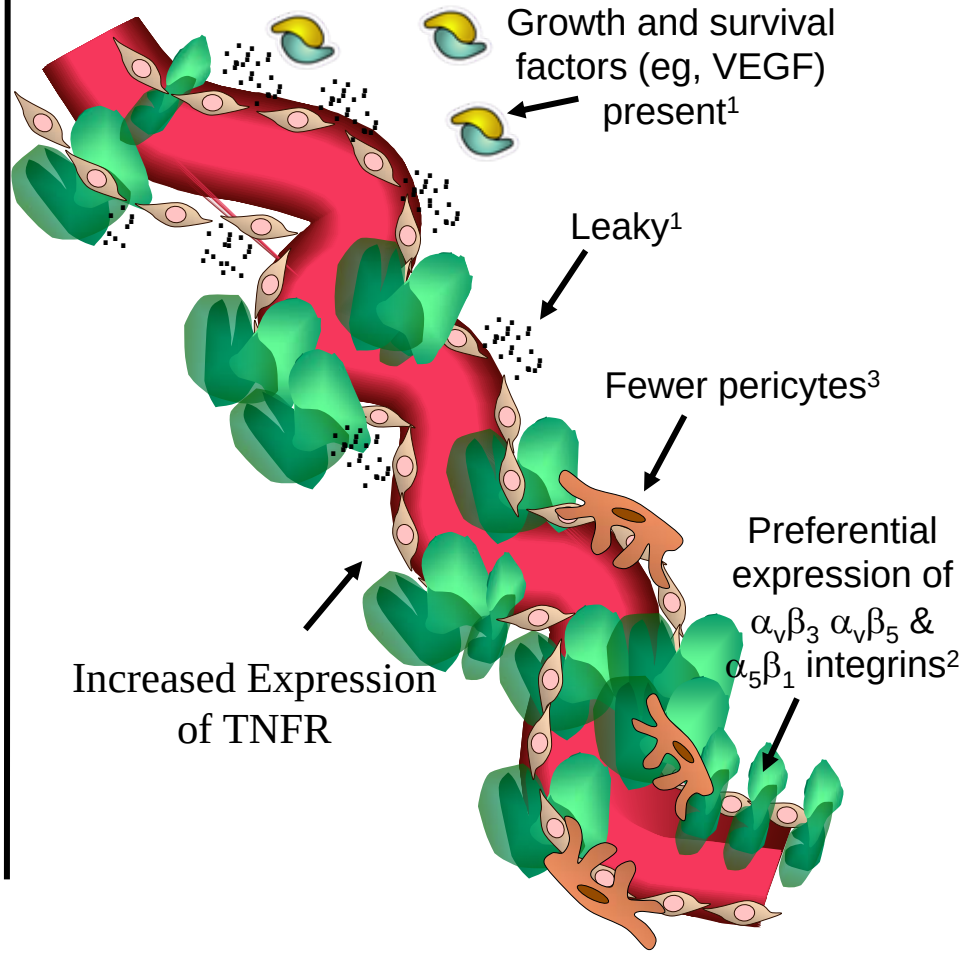


Normal and Tumor Vasculature

Normal Blood Vessels

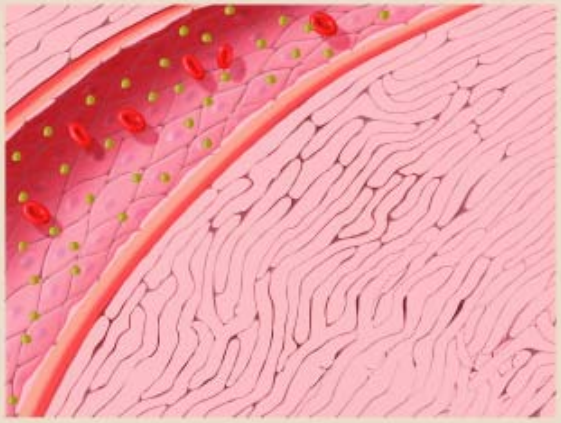


Tumor Blood Vessels

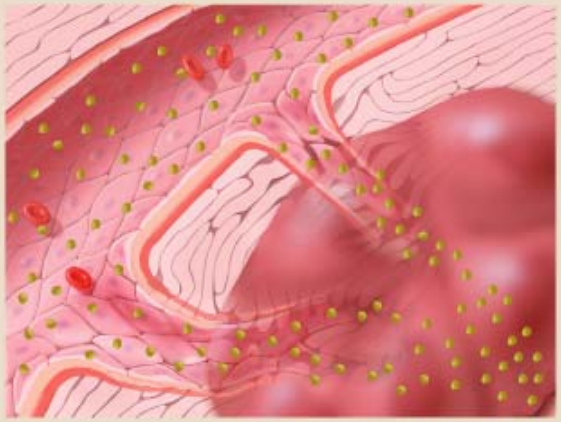


1. Jain. *Nat Med.* 2001;7:987.
2. Griffioen and Molema. *Pharmacol Rev*. 2000;52:237.
3. Blau and Banfi. *Nat Med*. 2001;7:532.

Nanoparticles Flowing Through:

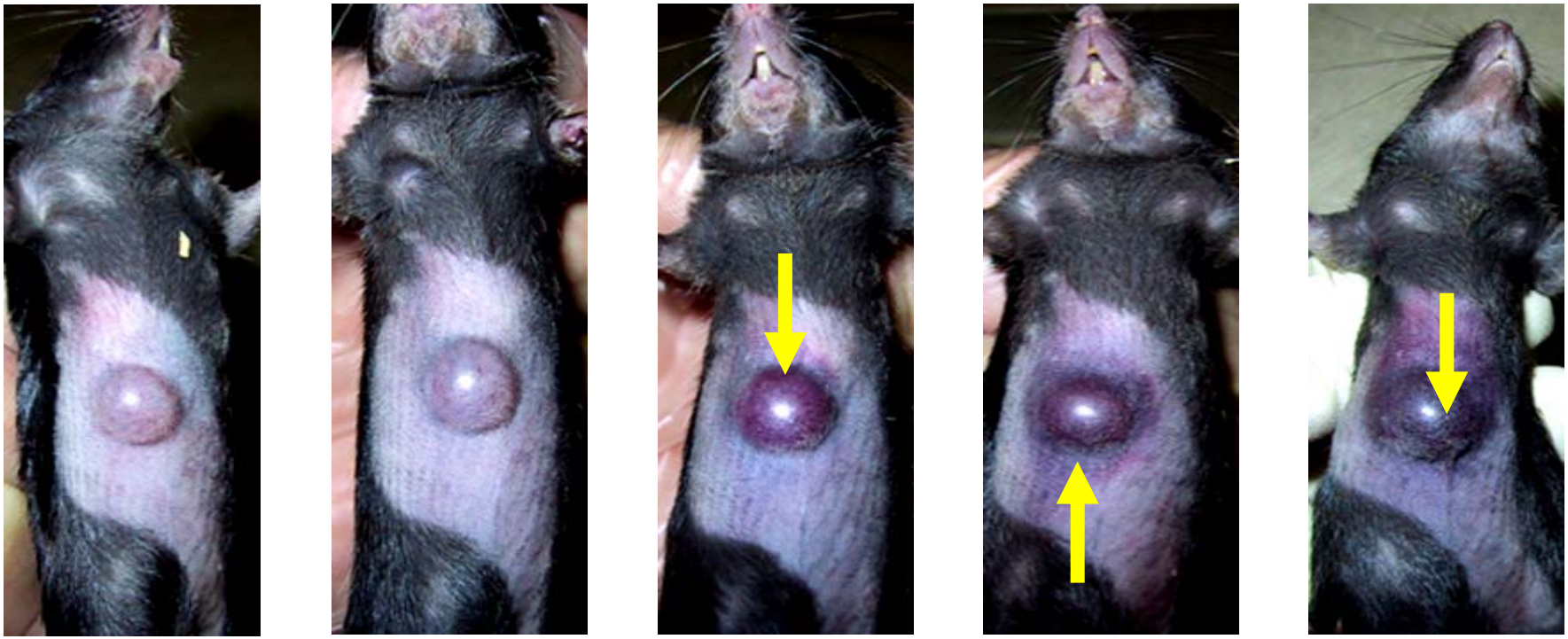


- Healthy blood vessels



- Leaky blood vessels **in** and **around** tumor

Trafficking of IV Injected CYT-6091 to a Colon Carcinoma Tumor Implanted in C57BL/6 Mice



0 hour | 1 hour | 2 hours | 3 hours | 5 hours

Comparison of Colloidal Gold-TNF Formulations

Untreated

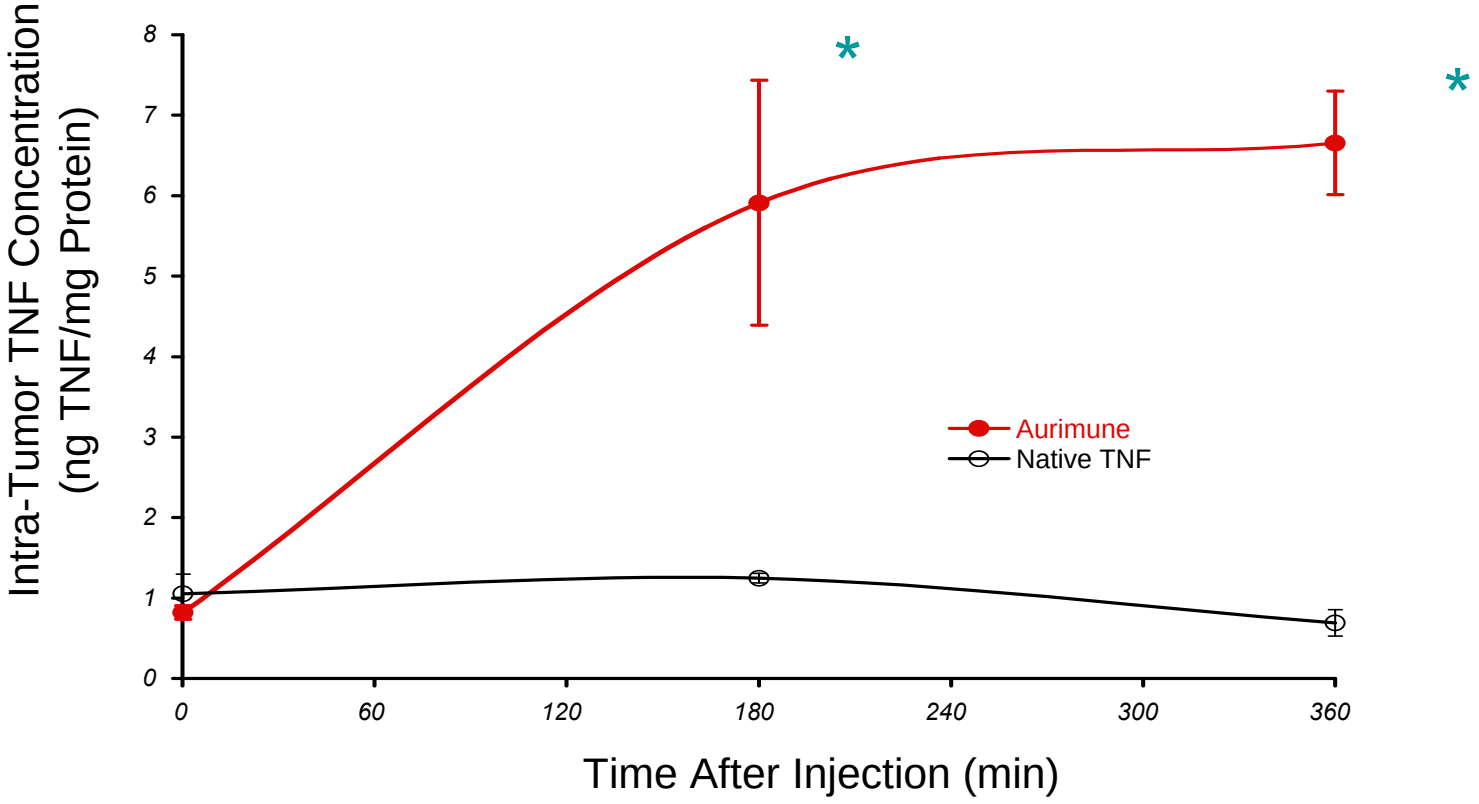
cAu-TNF

CYT-6091

cAu-TNF

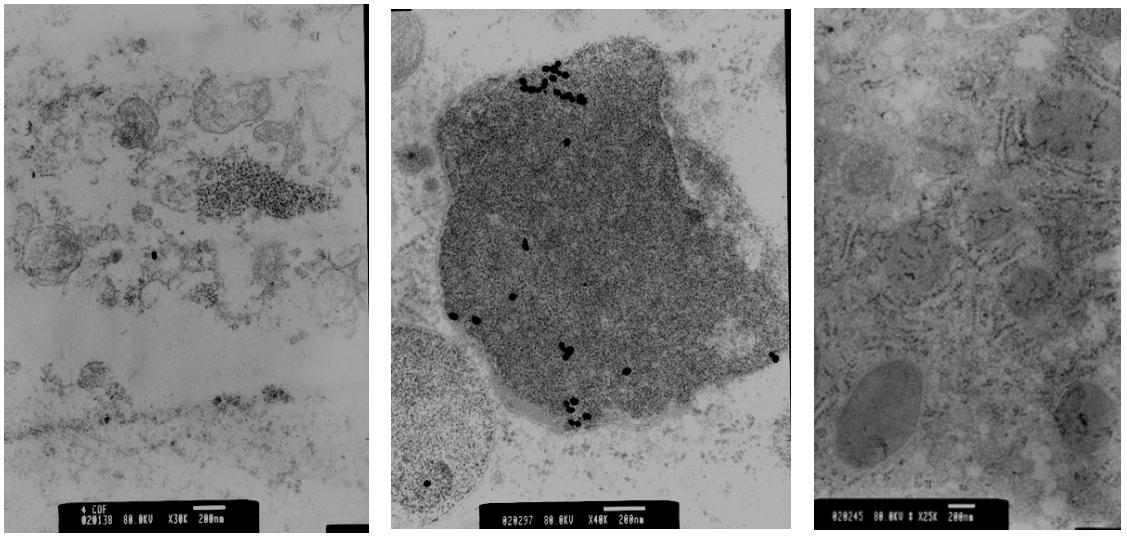
PEG-THIOL Bound
to cAu-TNF

Biodistribution: Tumor concentrations of TNF following IV Injection of Native TNF or CYT-6091 (Aurimune)



Differential Uptake of CYT-6091 by Tumor and Normal Tissue

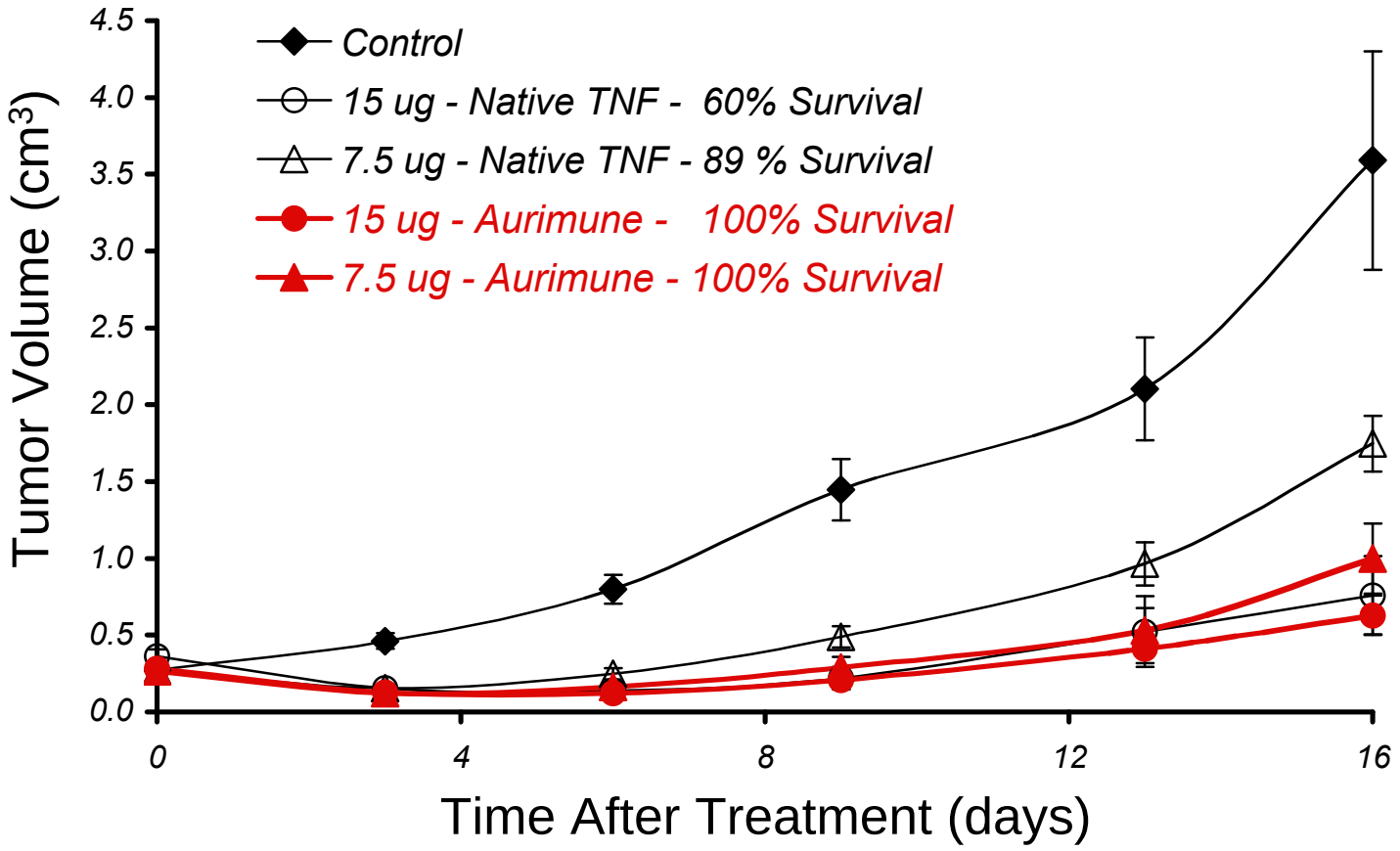
Spleen-9 Tumor-18 Liver-21



30,000X 40,000X 25,000X

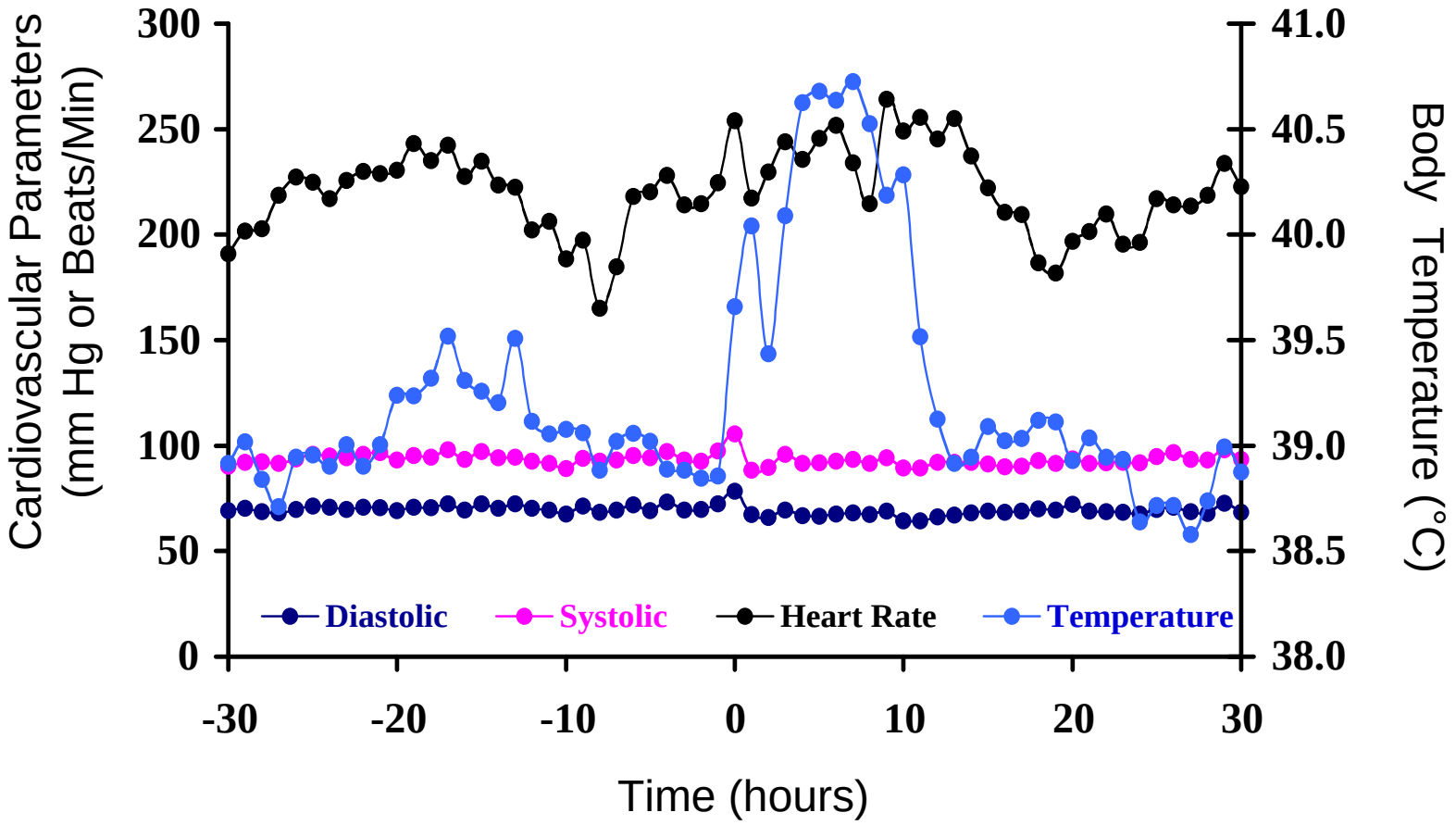
Bar = 200 nm

Comparison of Aurimune and Native TNF on Tumor Growth of MC-38 Tumors in C57BL/6 Mice



GLP Manufactured Lyophilized Aurimune for GLP Toxicology Study

Cardiovascular Observation in a single rabbit dosed with 25 µg/kg CYT-6091:
No hypotension observed



Phase I Clinical Protocol Design: Single Agent Trial

I. Dose Escalation:
   50 μg/m^2, 100 μg/m^2, 150 μg/m^2, 200 μg/m^2, 250 μg/m^2, 300 μg/m^2, 400 μg/m^2, 500 μg/m^2, 600 μg/m^2

II. Each Patient Receives Only One Dose Level

III. One Treatment Course = Treat on Day 0 and Day 15

IV. Three (3) Patients per Dose w/o DLT; Six (6) w/ one DLT in 1st cohort;
   Maximum Number of Patients in Dose Escalation = 36

V. Evaluation to Progress to Next Dose = Day 20

VI. Additional Patients (up to 12) may be recruited at MTD to increase N to a Maximum of 42 Patients

Demographics

Patients: 30

Gender: 12 M, 18 Female

Mean Age: 53.5 y (26.5-70.2)

Demographics

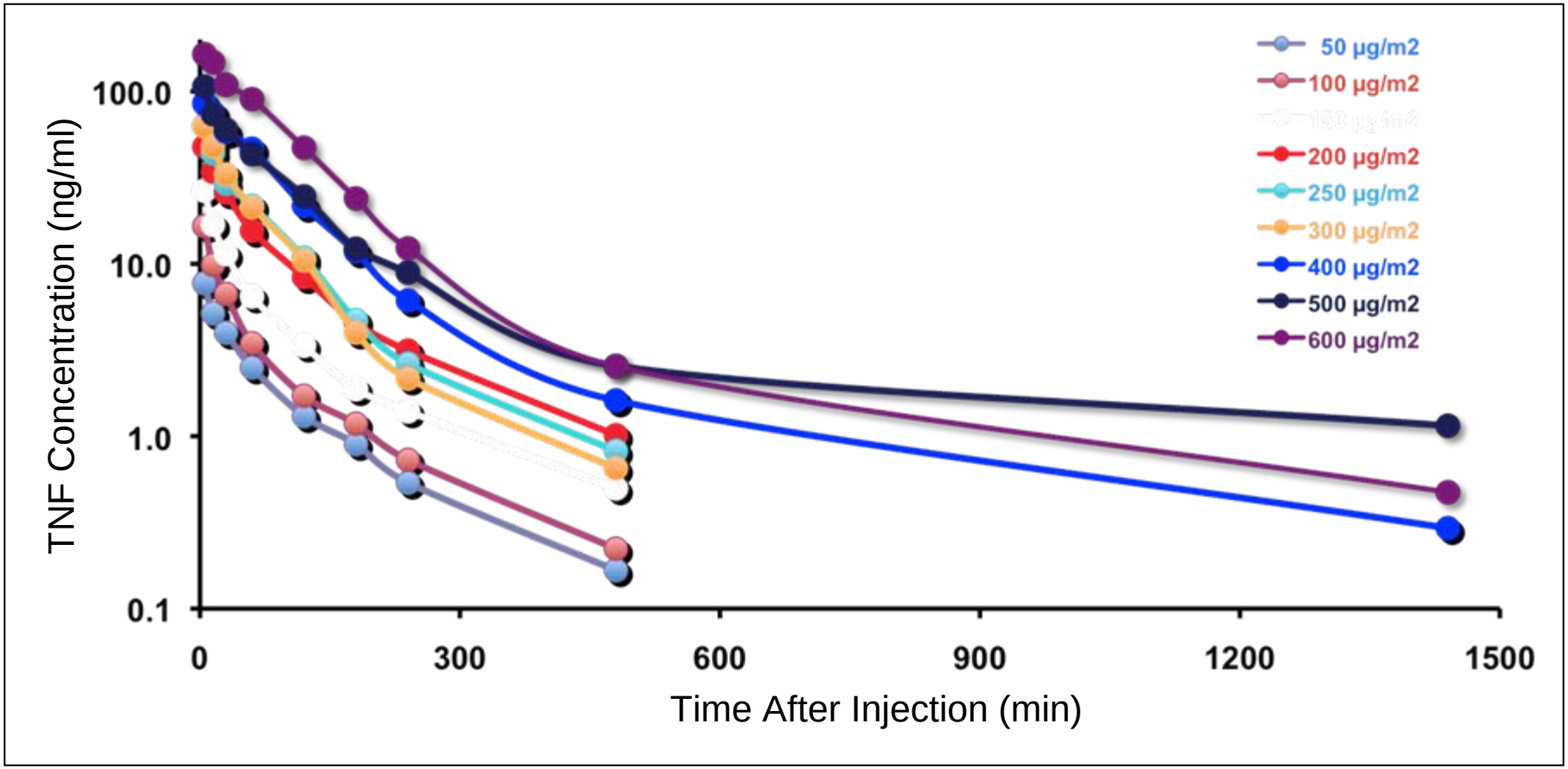
Study ID	Histology	Status
0601-01	Cutaneous melanoma	PD
0601-02	Colon Cancer (Adeno)	PD
0601-03	Ocular Melanoma	PD
0601-04	Colon Cancer (Adeno)	PD
0601-05	Colon Cancer (Adeno)	SD (2)
0601-06	Ocular Melanoma	PR (7)
0601-07	Adenocarcinoma of the Lung	PD
0601-08	Pancreatic Adenocarcinoma	PD
0601-09	Pancreatic Adenocarcinoma	PD
0601-10	Invasive Ductal Carcinoma	PD
0601-11	Leiomyosarcoma	PD
0601-12	Ocular Melanoma	PD
0601-13	Ocular Melanoma	SD (3)
0601-14	Pancreatic Adeno	no treatment given
0601-15	Pancreatic Adeno	PD
0601-16	Ocular Melanoma	PD

Demographics (cont.)

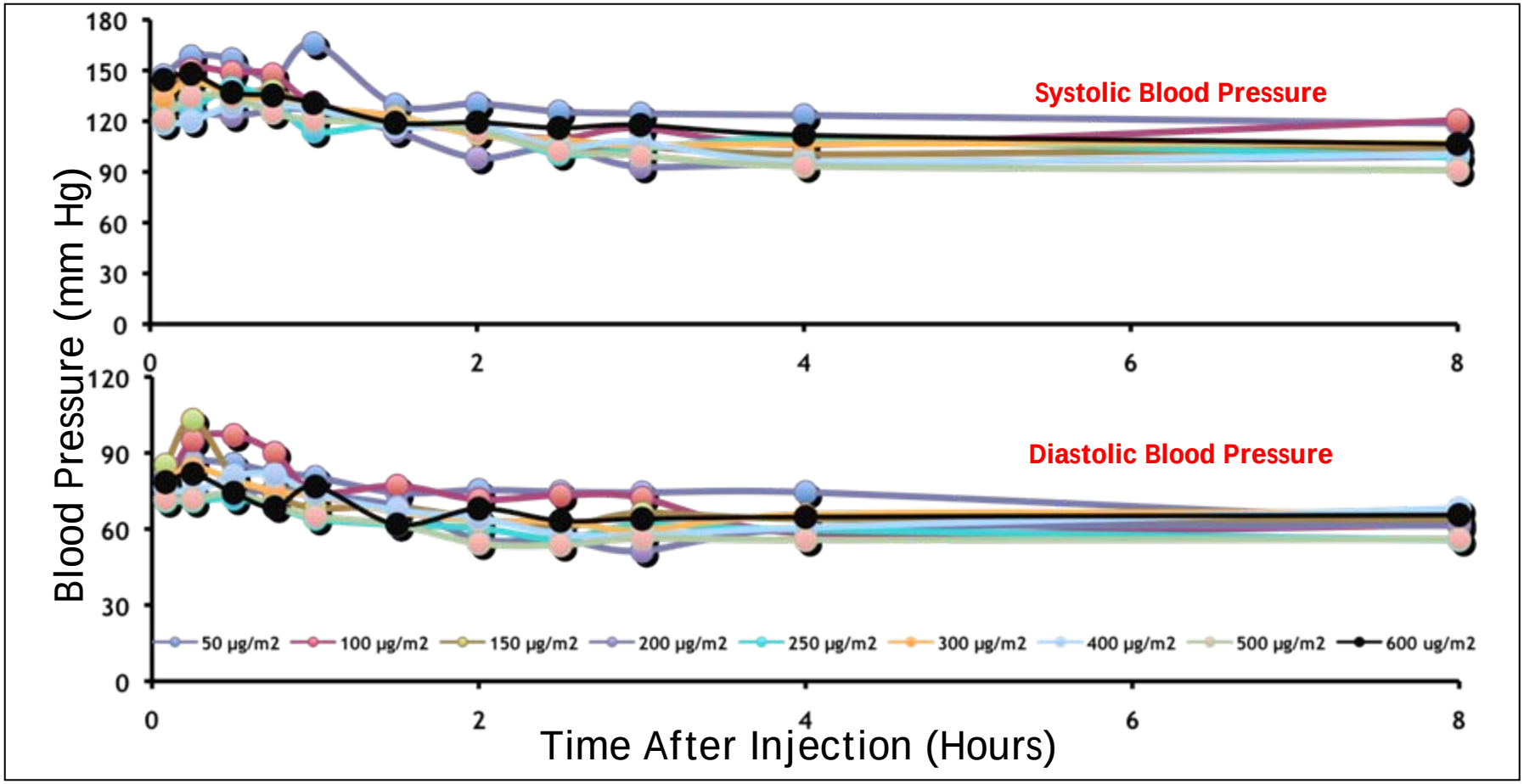
Study ID	Histology	Status
0601-17	Colon Cancer (Adeno)	PD
0601-18	Ocular Melanoma	PD
0601-19	Ocular Melanoma	PD
0601-20	Ocular Melanoma	PD
0601-21	Desmoplastic small round cell	PD*
0601-22	Rectal Adenocarcinoma	PD
0601-23	Colorectal Adenocarcinoma	PD
0601-24	Ocular Melanoma	PD
0601-25	Invasive Ductal Carcinoma	PD
0601-26	Colorectal Adenocarcinoma	PD*
0601-27	Desmoplastic small round cell	PD
0601-28	Colorectal Adenocarcinoma	PD
0601-29	Colorectal Adenocarcinoma	SD (2)
0601-30	Adrenocortical Carcinoma	PD

*Patient received a single treatment before removal from study

Pharmacokinetics of CYT-6091 in Man



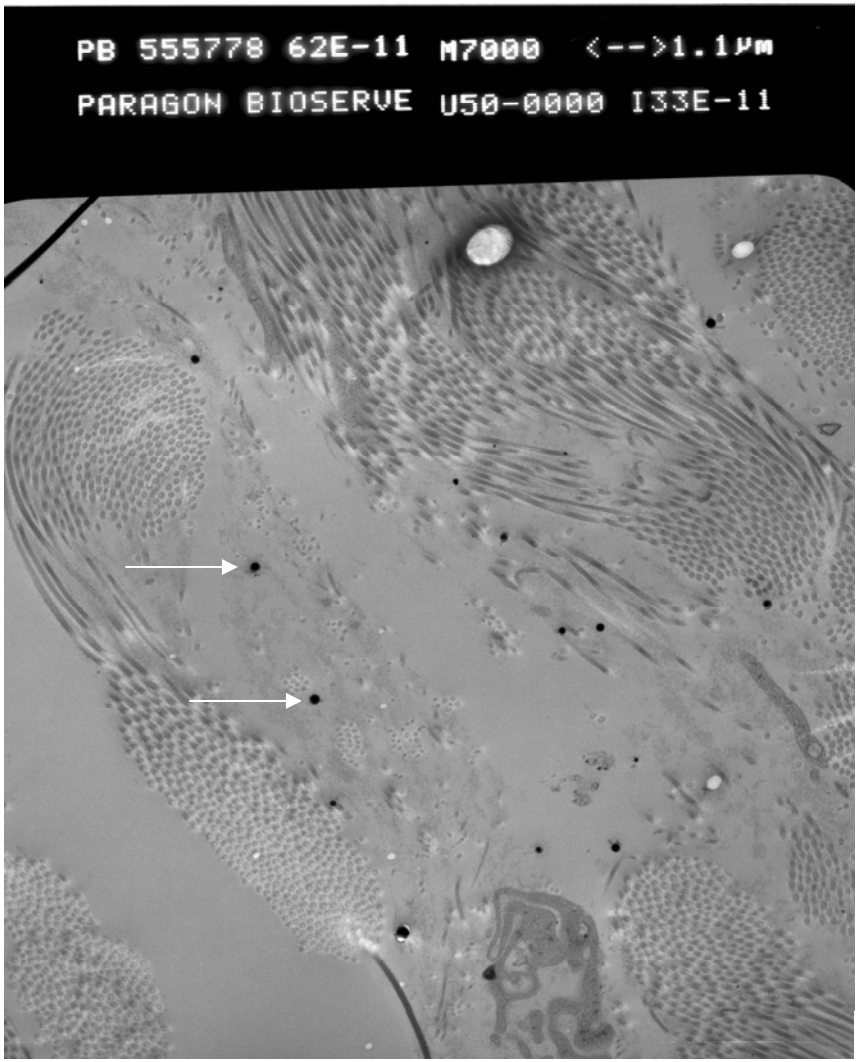
Effect of CYT-6091 on Blood Pressure



Adverse Event (CTCAE v3.0)	50-150 $\mu g/m^2$ (n=9)	200-300 $\mu g/m^2$ (n=10)	400-600 $\mu g/m^2$ (n=10)	Total (n=29)
Blood/Bone Marrow				
Lymphopenia	6	10	10	26
Leukocytes (total WBC)		1	1	2
Hemoglobin			2	2
Neutrophils/granulocytes			1	1
Metabolic/Laboratory				
Hypoalbuminemia	1	1	3	5
Hypokalemia	1	1	3	5
Hypercalcemia		1	1	2
AST/SGOT		3	2	5
ALT/ SGPT			1	1
Alkaline phosphatase		1		1
Hypophosphatemia		4	1	5
Hyperbilirubinemia			5	5
Adverse Events				
Fatigue	2	1		3
Pain, chest wall	1			1
Pain, liver	1			1
Pain, abdomen	2		2	4
Pain, bone		1		1
Pain, head			1	1
Pain, NOS			1	1
Nausea		1	1	2
Vomiting		1	1	2
Diarrhea			1	1
Infection, pneumonia		1		1
Infection, urinary		1	1	2
Anorexia		1		1
Thrombosis/embolism			1	1

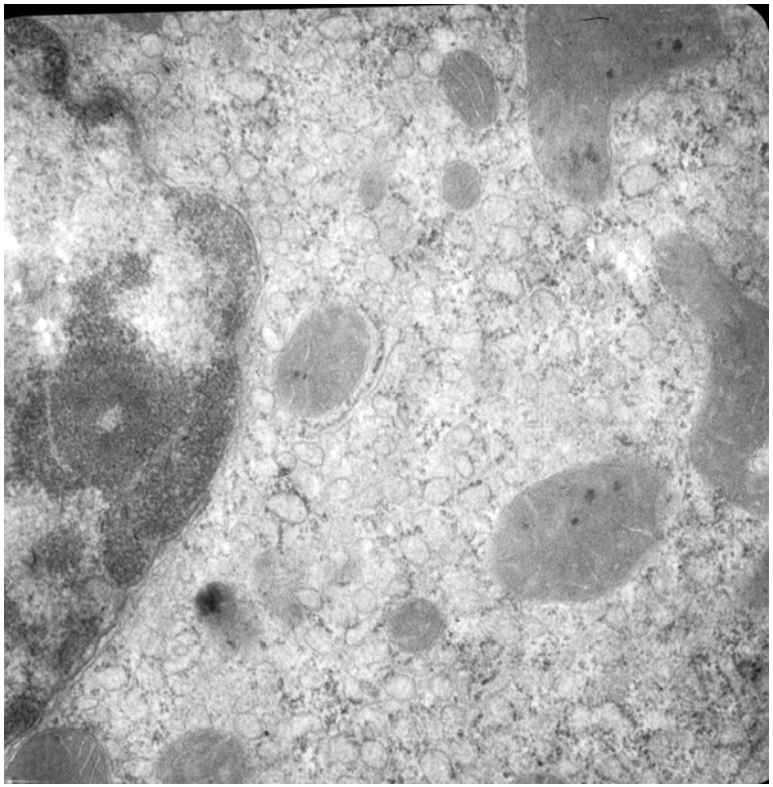
TEM on Tissue Biopsied 7 Hours Post Administration of CYT 6091 at Dose of 87 μg in Patient #1

Normal Tissue

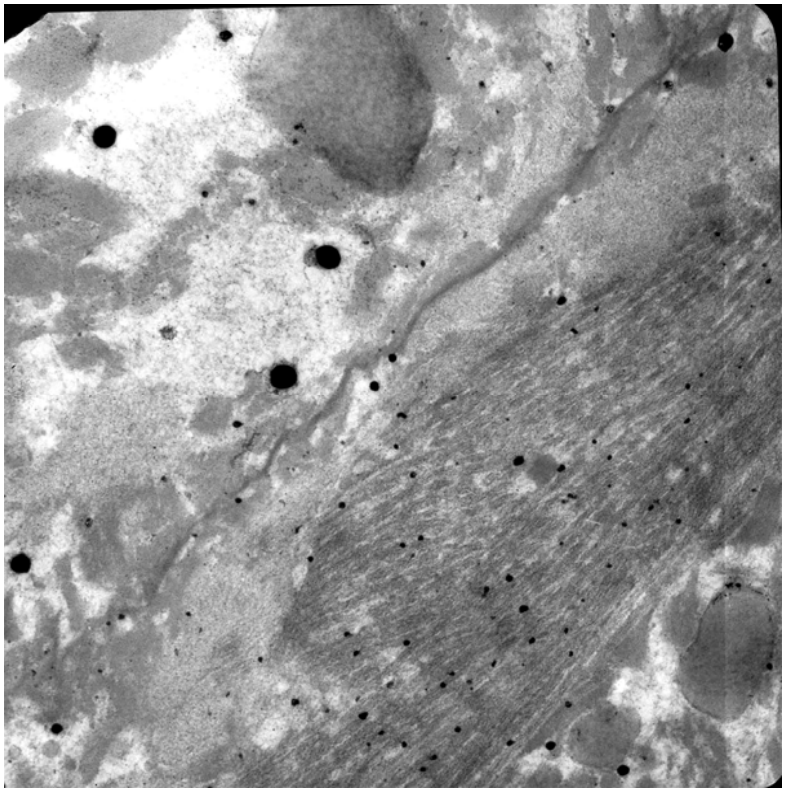


Tumor Tissue

TEM on Tissue Biopsied 24 Hours Post Administration of CYT 6091 at Dose of 225 mg in Patient #8



Normal Liver



Tumor

Summary

- CYT-6091 can be administered safely up to doses of 600 µg/m^2 by systemic injection.
- One partial response was seen in a patient with stage IV ocular melanoma.
- Particles appeared to traffic to tumors selectively as determined by EM.
- Phase II trials of CYT-6091 combined with chemotherapy are planned.

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