

# Safety and Toxicity of Catheter Gene Delivery to the Pulmonary Vasculature in a Patient with Metastatic Melanoma

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## ABSTRACT

**One approach to gene therapy for human cancer is transcatheter injection of DNA liposomes into tumor masses. To determine the feasibility of selective delivery of recombinant genes by a catheter to the pulmonary vasculature in humans, a patient with melanoma received two treatments of HLA-B7 plasmid DNA complexed to cationic liposomes into a right posterior basal pulmonary artery associated with a mass lesion. The treatments were well tolerated. No adverse respiratory, cardiac, immunologic, or other organ toxicities were detected. The delivery of recombinant genes by catheter may be a useful modality to treat human malignancy and other diseases.**

## OVERVIEW SUMMARY

**Transcatheter delivery of HLA-B7 DNA and cationic liposomes into a segment of a pulmonary artery was safely performed in 1 patient with tumor nodules in the lung. No immunologic or organ toxicities were observed. Percutaneous catheter gene delivery has been performed in humans. Further refinements of this approach may lead to useful treatments for a variety of human diseases.**

## INTRODUCTION

**O**NE APPROACH TO CANCER IMMUNOTHERAPY is the direct transmission of recombinant genes into established tumors to modulate the immune response directed against tumor growth (Plautz *et al.*, 1993). Recombinant genes can be directly introduced into tumors by injection of subcutaneous tumor nodules or by catheter delivery into the vasculature of parenchymal masses (Nabel *et al.*, 1993a). Therapeutic drugs are routinely delivered to selective sites in the circulation by catheters to treat vascular diseases (Kennedy *et al.*, 1985; Ambrose *et al.*, 1992) and parenchymal tumors (Ensminger, 1993). Transcatheter introduction of recombinant genes and vectors to selective re-

gions of the vasculature has been established in preclinical animal models (Nabel *et al.*, 1990; LeClerc *et al.*, 1992; Barr *et al.*, 1994), but the toxicities and safety of catheter-based gene delivery in humans has not been explored. We report here the delivery of a class I major histocompatibility complex (MHC) gene in cationic liposomes by transcatheter injection into the right posterior basal pulmonary artery and surrounding right lower lobe tumor mass in a patient enrolled in a human gene therapy protocol for immunotherapy of melanoma. The findings suggest that transcatheter delivery of genetic material in this patient appears safe and well tolerated. This approach may be applicable to the genetic treatment of cancer and vascular diseases.

## MATERIALS AND METHODS

Informed consent was obtained according to the Committee to Review Grants for Clinical Research and Investigations Involving Human Beings of the University of Michigan Medical School, the Recombinant DNA Advisory Committee of the National Institutes of Health, and the Food and Drug Administration.

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### Vector production

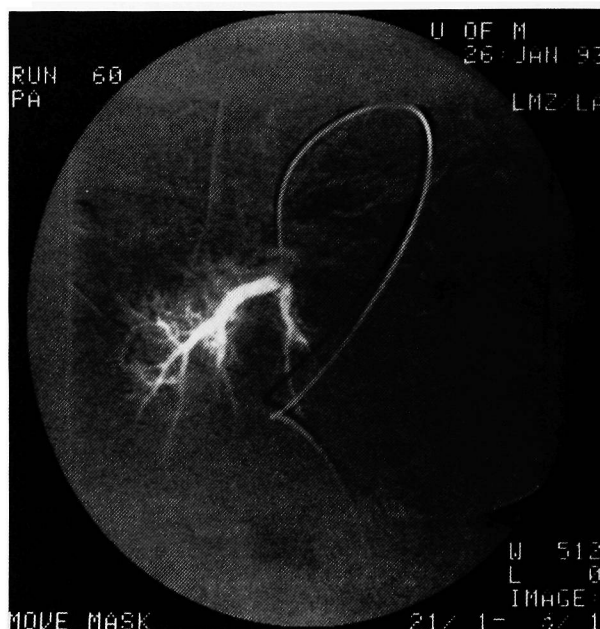
A eukaryotic expression vector plasmid, pHLA-B7, was prepared by insertion of an HLA-B7 cDNA into the Rous sarcoma virus  $\beta$ -globin plasmid as previously described (Nabel *et al.*, 1993a). HLA-B7 DNA-liposome complexes were prepared just prior to catheterization by adding 0.1 ml of lactated Ringer solution into a sterile vial of HLA-B7 plasmid DNA (20  $\mu$ g/ml; 0.1 ml). An aliquot of this solution (0.1 ml) was added to 0.1 ml of 150  $\mu$ M dioleoyl phosphatidylethanolamine/3b-[N-N',N'-dimethylaminoethane] carbamoyl] cholesterol liposome (Gao and Huang, 1991) in lactated Ringer solution in a separate sterile vial. The solution was allowed to incubate for 15 min at room temperature, and 0.5 ml of sterile lactated Ringers was added to the vial and mixed. The DNA, liposomes, and sterile vials were prepared in accordance with Food and Drug Administration guidelines and quality control procedures, as has been previously described (Nabel *et al.*, 1993a).

### Gene transfer

Percutaneous right heart catheterization was performed from the right femoral vein using sterile techniques. An 8 French sheath was inserted into the right femoral vein, and a 7 French Van Aman pigtail catheter was advanced into the right pulmonary artery under fluoroscopic guidance. After pressure measurement, selective catheterization of the right posterior basal segment artery was performed, and the Van Amen catheter was exchanged for a 5 French occlusion balloon catheter (Medi-Tech, Watertown, MA) over a 0.025-inch exchange guidewire. After balloon inflation, digital subtraction angiography was performed to confirm catheter position and arrest of blood flow (Fig. 1). Confirmation that blood flow was arrested was determined by inflation of the balloon and injection of intravenous contrast dye into the vascular space. No diffusion of the contrast material was noted over a 5-min period, thus ensuring that delivery of DNA and liposomes would proceed antegrade in the vasculature and not immediately admix with blood to inactivate the DNA-liposome complex. The posterior basal segment artery was rinsed with 30 ml of sterile saline, and the HLA-B7 DNA-liposome solution, 1.43  $\mu$ g of DNA in a volume of 0.6 ml, was instilled followed by an additional 1.0 ml of sterile saline. This solution was injected through the end hole of the catheter into the posterior basal segment artery. An additional 3.0 ml of sterile saline was instilled through the catheter, and the HLA-B7 DNA-liposome solution incubated for 20 min to achieve transduction of the local microcirculation. The balloon was deflated, and the catheter and femoral sheath were removed.

### Hemodynamic and biochemical monitoring

To determine the effects of DNA-liposome delivery to the pulmonary vasculature, hemodynamic and biochemical monitoring was performed. The patient's electrocardiogram (ECG) (six-lead) was continuously recorded prior to, during, and after injection of the DNA-liposome solution. Pulmonary artery, pulmonary capillary wedge pressure, and systemic blood pressure were measured 5 min prior to gene transfer and 20 min after delivery of DNA and liposomes. Biochemical para-



**FIG. 1.** Digital subtraction angiography of right posterior basal segment artery. After selective catheterization of the right posterior basal artery, a digital subtraction angiogram was performed. The intravenous contrast agent demonstrates displacement of the posterior basal segmental arteries by the tumor. The contrast agent was removed by instillation of 30 ml of sterile saline solution followed by the introduction of 1.43  $\mu$ g of pHLA-B7 plasmid DNA complexed to DC-cholesterol liposome.

meters were also measured 1 day before and 1 day after each treatment.

## RESULTS

A 68-year-old male with stage IV metastatic melanoma was enrolled in a human gene therapy clinical protocol. "Immunotherapy of malignancy by *in vivo* gene transfer into tumors" (Nabel *et al.*, 1992), based upon clinical guidelines. This patient had undergone previous treatment for his melanoma, including a wide local excision, lymph node dissection, chemotherapy with DTIC, *cis*-platinum, and tamoxifen, BCG and IL-2 immunotherapy, interferon- $\gamma$  (IFN- $\gamma$ ) immunotherapy, and radiation. The patient was refractory to these treatments, and had recurrent melanoma on the chest wall and back, axillary lymph nodes, and lungs, confirmed by biopsy, thoracentesis, and post mortem examination. This patient was HLA-B7<sup>-</sup>, HLA-1<sup>+</sup>, and haplotype B5, 57, and W4<sup>+</sup>.

The patient received two courses of HLA-B7 gene treatment by direct intratumor injection into cutaneous melanoma nodules on the left lower chest (treatment #1) and left upper chest (treatment #2). He responded to these treatments, showing complete regression of both treated nodules, as well as a 3-cm pulmonary nodule (Nabel *et al.*, 1993a). No evidence of toxicity was observed in this patient or any others studied in this trial (Table 1; see also Nabel *et al.*, 1993a). However, the patient

TABLE 1. BIOCHEMICAL PARAMETERS IN TREATED PATIENTS

	HEMATOLOGY						CHEMISTRY												IMMUNOLOGY						
	Day	HCT	WBC	PLT	PT	PTT	Na	K	CL	CO2	BUN	CR	AST	ALT	LDH	CPK	AMY	LIP	α-B7	μ-DNA	CRP	ANA	ESR	CH50	
Pt. 1	0	44.2	6.9	225.0	11.7	21.6	134.0	3.5	96.0	26.0	11.0	0.6	35.0	56.0	197.0	47.0	67.0		Neg	<2.5	1.5	Neg	38.0	>200.0	
	2	40.4	6.0	205.0			135.0	3.5	96.0	33.0	12.0	0.7	30.0	45.0	214.0										
	14	39.5	6.6	241.0	11.5	21.8	139.0	3.5	98.0	32.0	20.0	0.7	44.0	59.0	205.0	47.0	71.0	44.0							
	28	41.7	6.3	171.0			135.0	3.7	97.0	27.0	14.0	0.6	44.0	57.0	210.0						<2.5				
	56	43.3	6.2	246.0	11.7	24.2	136.0	3.5	93.0	30.0	17.0	0.6	57.0	74.0	215.0				96.0	136.0	Neg	* <2.5	1.0	Neg	58.0
Pt. 2	0	46.1	5.5	224.0	11.1	22.4	138.0	4.0	94.0	34.0	3.0	0.6	54.0						Neg						
	2	46.8	6.9	210.0			139.0	4.6	98.0	25.0	8.0	0.5	25.0	21.0	232.0	<20.0	54.0	20.0							
	14	45.8	6.2	334.0	10.9	22.8	138.0	3.8	91.0	36.0	8.0	0.6	23.0	22.0	147.0			46.0	16.0						
	28	43.4	5.6	232.0	11.3	22.2	140.0	4.2	97.0	32.0	6.0	0.6	10.0								2.9			162.0	
	56	45.0	6.9	206.0	11.3	22.6	134.0	3.9	92.0	33.0	4.0	0.5	10.0	17.0	158.0			33.0	4.0	Neg	<2.5				>200.0
Pt. 3A	0	30.9	4.5	215.0	11.4	21.8	140.0	4.3	100.0	31.0	25.0	1.2	21.0	25.0	254.0	<20.0	78.0	26.0	Neg	3.8		Neg			>200.0
	2	32.3	5.2	217.0			140.0	4.2	103.0	31.0	26.0	1.0	18.0	22.0	265.0	<20.0	63.0	12.0							
	14	29.8	6.5		11.6	21.8	140.0	4.6	104.0	32.0	30.0	1.4	13.0	20.0	213.0			95.0	16.0						
	28	34.9	7.8	200.0	11.4	22.1	141.0	4.6	103.0	30.0	30.0	1.1	20.0	20.0	258.0			123.0	18.0		<2.5	<.60		59.0	>200.0
	56	33.8	5.6	264.0	11.5	22.6	140.0	4.3	100.0	29.0	31.0	1.1	19.0	21.0	278.0			73.0	11.0	Neg	<2.5	1.0	Neg		>200.0
Pt. 3B	0	32.8	6.4	240.0	11.6	22.2	141.0	4.5	104.0	31.0	32.0	1.3	24.0	23.0	239.0			93.0	16.0	Neg	5.1	<.60	Neg		179.0
	2	30.5	6.3	195.0			145.0	4.5	109.0	28.0	23.0	1.0	15.0	14.0	278.0	<20.0	69.0	12.0							
	14	31.3	6.3	227.0	11.9	21.5	139.0	4.2	102.0	28.0	31.0	1.1	8.0	15.0	241.0			81.0	14.0						
	28	32.6	5.4	221.0	11.5	21.8	142.0	4.0	106.0	28.0	29.0	1.3	14.0	21.0	257.0			84.0	30.0		<2.5	<.60	Neg		>200.0
	56	32.3	5.0	202.0	11.7	21.3	143.0	4.1	107.0	24.0	24.0	1.1	28.0	28.0	251.0			104.0	52.0	Neg	<2.5	<.60		65.0	182.0
Pt. 4	0	36.5	6.3	207.0	11.0	21.9	139.0	3.9	104.0	27.0	10.0	1.0	19.0	12.0	215.0			<30.0	40.0	Neg	<2.5	<.60	Neg	14.0	132.0
	2	39.0	7.3	181.0			139.0	3.9	99.0	26.0	13.0	0.9	33.0	20.0	544.0			<30.0	5.0						
	14	37.2	7.2	242.0	11.5	22.3	142.0	4.2	102.0	28.0	17.0	1.1	13.0	25.0	357.0										
	28	35.9	7.4	183.0	12.1	21.9	139.0	4.0	102.0	30.0	12.0	1.0	30.0	27.0	520.0			<30.0	26.0		<2.5	1.6	Neg	38.0	169.0
	56	37.6	7.7	205.0	12.2	21.6	138.0	4.1	96.0	30.0	7.0	1.0	28.0	24.0	666.0			<30.0	18.0	Neg	<2.5	2.2	Neg	29.0	163.0
Pt. 5	0	34.7	7.4	290.0	12.4	24.5	139.0	4.3	98.0	28.0	14.0	0.9	41.0				<20.0	47.0	18.0	Neg	<2.5	<.60	Neg		>200.0
	2	34.6	5.1	268.0			141.0	4.3	101.0	28.0	14.0	0.8	33.0	27.0	205.0	<20.0	43.0	18.0							
	14	32.3	4.2	319.0	11.9	25.8	141.0	4.2	102.0	26.0	13.0	0.9	37.0	24.0	262.0			36.0	24.0						
	28	28.5	4.4	274.0	11.9	24.5	142.0	4.7	104.0	29.0	15.0	0.9	39.0	26.0	284.0			30.0	16.0		<2.5	0.8	1:80	29.0	187.0
	56	33.1	7.4	403.0	12.3	27.1	139.0	3.9	97.0	26.0	14.0	0.8	48.0	22.0	473.0			34.0	18.0	Neg	4.7	4.7	Neg	38.0	>200.0

Hematology, chemistry, and immunology toxicity profiles of patients treated by gene transfer of HLA-B7. Pt. 3 refers to the subject described in the present report. Five patients with stage IV melanoma refractory to all available therapies were enrolled based on guidelines of the clinical protocol (Nabel *et al.*, 1992a, 1993a). These patients were HLA-B7 negative, greater than 18 years of age, HIV negative, and infertile. Blank spaces indicate that values were not determined.

had at least two unresponsive pulmonary lesions, including a large right lower lobe mass identified by analysis of anterior-posterior and lateral views on chest film and computerized tomography of the chest. This mass was associated with the right posterior basal artery. Based on his previous response to this treatment, we considered the possibility of delivering the recombinant gene to the pulmonary nodule by catheterization. Permission to perform this procedure was sought and obtained from the Recombinant DNA Advisory Committee and the Food and Drug Administration.

Informed consent was obtained from the patient, and the patient underwent two percutaneous transcatheter treatments to the right posterior basal artery, 2 weeks apart (Fig. 1). During each treatment, 0.6 ml of DNA-liposome solution was administered followed by an additional total of 4 ml of sterile saline to distribute it to the local microcirculation. Both treatments were well tolerated, without acute complications. There were no ECG abnormalities or arrhythmias during both treatments. Analysis of hemodynamic parameters revealed no

TABLE 2. SYSTEMIC AND PULMONARY HEMODYNAMIC MEASUREMENTS DURING PULMONARY ARTERY GENE TRANSFER

	Treatment 1		Treatment 2	
	Pre	Post	Pre	Post
Systemic blood pressure (mm Hg)	121/71	128/76	111/74	116/78
Pulmonary artery pressure (mmHg)	28/8	27/8	22/4	23/5
Pulmonary capillary wedge pressure (mean) (mmHg)	15	16	10	11

Hemodynamic measurements of systemic and pulmonary artery pressures 5 min prior to and 20 min after catheter delivery of DNA-liposomes into the right posterior basal segment artery.

changes in pulmonary artery, pulmonary capillary wedge, or systemic arterial pressures measured 5 min before and 20 min after transcatheter injection (Table 2). The pulmonary artery pressure remained at 28/8 mmHg (treatment #1), and 22/4 mmHg (treatment #2), and the pulmonary capillary wedge pressure was 15 and 10 mmHg during treatments 1 and 2, respectively.

Analysis of hematological and biochemical parameters revealed no systemic abnormalities in this patient, including measures of liver, renal, pancreatic, and cardiac function prior to or after the two transcatheter gene deliveries. Serum blood samples obtained during the pulmonary artery infusion were analyzed by the polymerase chain reaction (PCR) for the presence of plasmid DNA. In this patient, plasmid DNA was not detected in systemic blood during either treatment (sensitivity <2 pg/ml). Myocardial abnormalities were not detected by analysis of creatine kinase in the serum of this patient prior to, during, or after the treatments. Likewise, no anti-DNA antibodies were detected in the patient (Table 3). Two weeks following the second treatment, a lung biopsy was considered but was not performed due to poor respiratory status of the patient. A right pleural thoracentesis was performed to relieve a chronic pleural effusion from this lung. Cytologies revealed that the lesion represented a second independent malignancy, an adenocarcinoma, in the right lung distinct from the previously treated melanoma. The patient died 9 months later, and a post mortem examination confirmed the diagnosis of a second malignancy, an adenocarcinoma, in the right lung (Fig. 2).

## DISCUSSION

In this case report, a human HLA-B7 gene was delivered by catheter injection into the pulmonary artery associated with metastatic lung nodules of an HLA-B7-negative patient. The treatments were well tolerated. No apparent toxicities were observed. Pulmonary and systemic arterial pressures were normal, biochemical parameters were normal, and no anti-DNA antibodies were detected.

Local drug delivery, including site-specific gene transfer, offers several advantages compared with systemic drug therapy. Local administration of a drug or recombinant DNA can potentially result in high local and low systemic concentrations of an agent, which may minimize systemic toxicities. Several approaches to local delivery are feasible, including implantable subcutaneous pumps, encapsulation of drug within a polymer gel (Langer and Vacanti, 1993), and indwelling vascular catheters (Kennedy *et al.*, 1985; Ensminger, 1993). Prototype catheters have been developed and tested *in vivo* for local drug delivery into vascular thromboses and for vascular gene transfer, including double balloon catheters (Nabel *et al.*, 1990), porous balloons (Wolinsky and Thung, 1990), catheters coated with polymer gels (Riessen *et al.*, 1993), and autoperfusion catheters. These studies suggest that drugs, such as thrombolytic agents, can be delivered into focal vascular thromboses, achieving high local concentrations of drug and with less toxicity than might be incurred if equivalent doses were delivered systemically. These devices will require future modifications to optimize delivery, minimize local tissue damage, and permit vessel perfusion. Previous methods of local drug delivery are

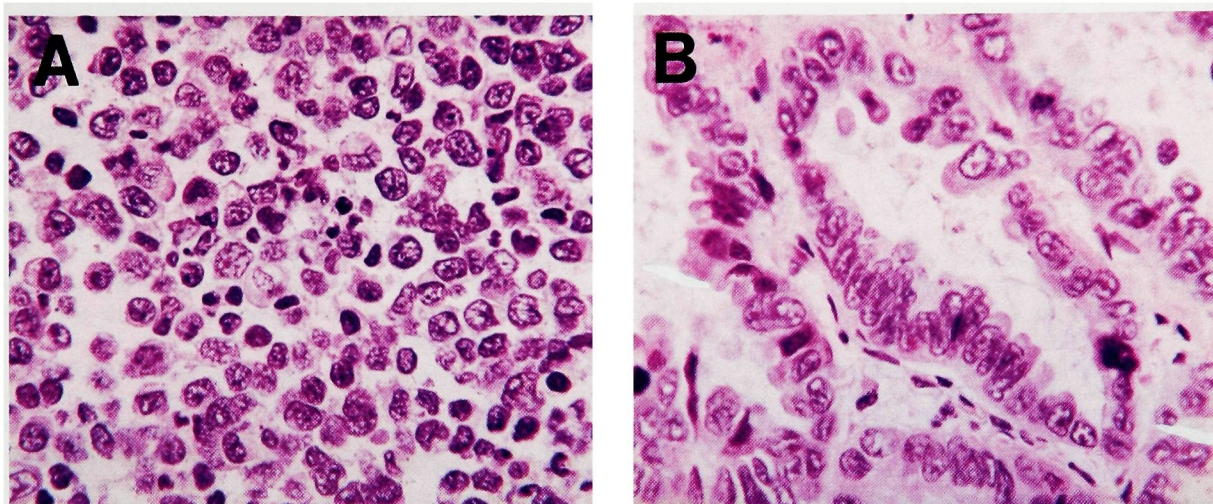
TABLE 3. HEMATOLOGY, CHEMISTRY, AND IMMUNOLOGY PARAMETERS BEFORE AND AFTER CATHETER GENE DELIVERY

	Treatment 1		Treatment 2	
	Pre	Post	Pre	Post
<b>Hematology</b>				
Hemoglobin	10.5	10.6	10.4	11.5
Hematocrit (%)	31.8	31.3	29.4	33.7
White blood count	7.1	5.7	7.3	8.4
Platelet	258.0	232.0	276.0	317.0
Prothrombin time (sec)	13.3	12.2	12.8	12.9
Partial thromboplastin time (sec)	21.8	23.0	22.0	24.4
<b>Chemistry</b>				
Albumin (gm/dl)	3.1	3.3	3.1	3.3
Alkaline phosphatase (IU/l)	136.0	146.0	154.0	151.0
SGPT (IU/l)	21.0	24.0	22.0	23.0
Amylase (U/l)	52.0	57.0	64.0	49.0
SGOT (AST) (IU/l)	14.0	18.0	19.0	18.0
BUN (mg/dl)	23.0	18.0	27.0	24.0
Calcium (mg/dl)	9.2	9.0	8.9	9.4
Chloride (mEq/l)	100.0	101.0	98.0	98.0
Cholesterol (mg/dl)	176.0	200.0	193.0	205.0
CO <sub>2</sub> (mEq/l)	30.0	27.0	28.0	28.0
Creatine kinase (mg/dl)	<20	<20	<20	<20
Creatinine (mg/dl)	1.3	1.1	1.2	1.1
Glucose (mg/dl)	161.0	174.0	129.0	120.0
Lactate dehydrogenase (IU/l)	222.0	257.0	235.0	282.0
Phosphorous (mg/dl)	3.1	3.8	3.5	3.5
Potassium (mEq/l)	4.1	3.8	4.2	4.4
Total protein (gm/dl)	6.2	6.6	6.3	7.3
Sodium (mEq/l)	140.0	141.0	139.0	139.0
Total bilirubin (mg/dl)	0.2	0.4	0.3	0.4
Uric acid (mg/dl)	6.2	5.9	6.3	5.8
Angiotensin converting enzyme	22.6	27.5	26.5	24.2
<b>Immunology</b>				
αDNA	N/D	N/D	N/D	<2.5
ESR	N/D	N/D	N/D	121.0
CH50	N/D	N/D	N/D	>200
CRP	N/D	N/D	N/D	7.7

Hematology, chemistry, and immunology parameters in the patient 1 day before and 1 day after catheter delivery of DNA-liposomes into the right posterior basal segment artery. N.D. indicates values not determined. Although CH50 was elevated, this and other immunologic values were unchanged from baseline values (see Table 1).

limited, however, by the rapid clearance of drug following restoration of blood flow. Introduction of a recombinant gene into vascular tissue by catheter might provide for a sustained and localized effect of a therapeutic protein.

Transcatheter injection of recombinant genes into selective sites in the vasculature is one approach to the genetic treatment of cancer and/or vascular diseases that provides targeted expression *in vivo*. Endoluminal vascular gene transfer has been achieved in several animal models, including pigs (Nabel *et al.*, 1990, 1993b), dogs (Lim *et al.*, 1991; Chapman *et al.*, 1992), rabbits (LeClerc *et al.*, 1992; Barr *et al.*, 1994), and rats (Morishita *et al.*, 1993). Direct gene transfer to the vasculature has been well tolerated in animal models using cationic liposomes



**FIG. 2.** Histology of representative sections from melanoma lesions in the patient's left or right axilla (A) and adenocarcinoma from the right lower lung (B). Magnification, 400 $\times$ ; hematoxylin and eosin stain.

(Nabel *et al.*, 1992b; San *et al.*, 1993) with minimal toxicities. Recent studies from our laboratory suggest that catheter delivery of DNA to porcine pulmonary arteries using liposomes and adenoviral vectors results in gene expression in pulmonary arteries, capillaries and veins as well as distal alveoli (Muller *et al.*, 1994). While these studies have suggested that endovascular gene transfer is achievable by catheter delivery, its feasibility in humans was not previously tested. The findings in this case report suggest that transcatheter injection of DNA-liposomes into the pulmonary vasculature is a safe and nontoxic approach to the treatment of pulmonary malignancies and/or vascular diseases. These findings suggest that further development of catheters designed to treat focal diseases in humans is warranted and could be applied to the treatment of cancer and vascular diseases.

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