A Trial of Intrapleural Adenoviral-mediated Interferon-α2b Gene Transfer for Malignant Pleural Mesothelioma

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ONLINE DATE SUPPLEMENT

Methods:

Anti-adenoviral Nab assessment. Nabs were assessed as previously described (10) and expressed as 1/serum dilution that inhibited 50% of the gene transfer induced by an adenovirus expressing the luciferase gene used to infect A549 lung cancer cells.

Immunoblots. To detect Ad.IFN-α2b-induced humoral responses against tumor antigens, immunoblotting against purified proteins and extracts from mesothelioma cell lines was performed using pre- and post-gene transfer sera. Purified SV40 large T-antigen protein was purchased from Chimerx (Milwaukee, WI). Purified mesothelin was provided by Drs. M. Ho and I. Pastin (National Cancer Institute). In some cases, cell lines were derived from patient pleural fluid samples and were grown in culture as previously described (9). Extracts from cells or purified proteins were prepared and immunoblotted with patient serum (diluted at 1:1500) from time points before treatment, and 6 weeks to 6 months after treatment as previously described (9).

Phenotypic characterization of innate immune cells by flow cytometry

Cryopreserved peripheral blood mononuclear cells (PBMC) collected prior to treatment and two daysafter gene transfer, were thawed and natural killer cell (NK) subsets and their activation status, were assessed with mAbs against CD3, CD14, CD19, CD20, CD56, CD16, CD69 and IFNaR. All mAbs were from BD Biosciences (San Diego, CA).

Briefly, PBMC samples were thawed, adjusted to $3x10^6$/ml in culture media and 100□l cells ($3x10^5$ cells) were placed into sterile FACS tubes (one for each stain/condition) and incubated overnight at 37°C. The next day, cells were incubated for 10 min at RT with 10% human serum and 10% serum corresponding to each of the Abs used, and then stained with CD3 PECy7, CD14 APC-H7, CD19 APC-H7, CD20 APC-H7, CD56 V450, CD16 PerCPCy5.5, CD69 FITC, IFN□R PE, or corresponding surface isotype mAb (IgG1k PECy7, IgG1k APC-H7, IgG1k V450, IgG1k PerCPCy5.5, IgG1k FITC, IgG1k PE) for 30 min on ice. Cells were then washed with FACS washing buffer (1xPBS supplemented with 0.1% BSA and 0.02% NaN_3, supplemented with 10% human serum and 10% serum corresponding to each of the Abs used), incubated with 1mL BD FACS Lysing solution (BD Biosciences) for 10 min at 37°C, washed with 2ml FACS washing buffer, re-suspended in 100μl FACS washing buffer and analyzed using LSRII. Analysis was done by collecting 100000 live lymphocytes (defined by size and granularity in FSC and SSC). Dead cells were excluded by manual gating in FSC/SSC. Detection thresholds were set according to isotype-matched negative controls. Results were expressed as Mean Fluorescent Intensity (MFI) and percent (%) of lymphocytes. Data analysis was performed using FloJo software (Tree Star, San Carlos, CA).

Supplemental Table 1. Adverse Events

The table below summarizes all adverse events as graded using the National Cancer Institute Common Terminology Criteria for Adverse Events v 3.0

Protocol: IRB 808806 UPCC 18508 IBC 08-292 CTRC 1176 IDS P-390					
	GRADE (Number of Events)				
Adverse Event	**1**	**2**	**3***	**4***	**Total**
Immunology/Allergy					
Recurrent ascites secondary to inflammation from vector	1		1		**2**
BLOOD					
Anemia	3	10	1		**14**
Leukopenia	7	6	1		**14**
Lymphopenia	4	3	7	3	**17**
Neutropenia		1	2		**3**
Thrombocytopenia	2	3			**5**
CARDIAC					
Hypotension-transient		2			**2**
COAGULATION					
PT	2				**2**
PTT-elevated	2	1			**3**
PTT-low	3				**3**
CONSTITUTIONAL					**0**
Fatigue (* note: all patients with cytokine release syndrome had fatique as part of that syndrome)			1		**1**
Insomnia	1	1			**2**
DERMATOLOGY					**0**
Erythema at pleurx site	1	1			**2**
Erythema - dressing margin	3	1			**4**
Rash-pleural catheter Site	1				**1**
Bruising-pleural catheter site	2				**2**
GI					
Nausea		2			**2**
Vomiting	3				**3**
Constipation	2	1			**3**
Diarrhea	1				**1**
Ulceration-stress induced	1	1			**2**
INFECTION					
Skin-pleural catheter site		1			**1**
Pleural Catheter Site	1				**1**
Staph Aureus-Pleural Cath Site			1		**1**
HEMORRHAGIC					
Melena	1				**1**
Epistaxis	1				**1**
LYMPHADEMA					
Bipedal	1				**1**
METABOLIC					
Hypoalbumenia	5	11	1		**17**
Alkaline phosphatase-elevated	1				**1**

Alkaline phosphatase-low		1			**1**
ALT-low	7				**7**
AST-low	1	1			**2**
AST-elevated	1				**1**
Bilirubin, total-low	3				**3**
Hypocaclemia	8	3			**11**
Creatinine-low	2				**2**
Creatinine-elevated	1	2			**3**
Hyperglycemia	1	2	3		**6**
Hypoglycemia		1			**1**
Protein, Total, low	12				**12**
Hyperammonemia	1				**1**
BUN	3				**3**
Chloride	6				**6**
CO2	1				**1**
Hypomagnesemia		1			**1**
Potassium - low	2				**2**
Sodium - low	8				**8**
TSH, high	1				**1**
NEUROLOGY					
Mood Alteration-anxiety	2				**2**
PAIN					
Headache	1				**1**
Pleural catheter site	3	3			**6**
Infusion site	1	1			**2**
Tumor		3			**3**
Penile on urination (no infection)	1				**1**
PAC site-no infection		1			**1**
Back-worsening pre-existent		1			**1**
Episodic under Right breast		1			**1**
PULMONARY					
Chest congestion		1			**1**
Cough	1	2			**3**
Hypoxia		2	1		**3**
SYNDROME					
Cytokine Release	9	12			**21**
TOTAL	**124**	**83**	**18**	**3**	**228**

*Do not meet definition of DLT

Supplemental Table 2. Interferon-α concentrations were measured using an ELISA kit at the designated time points.

Supplemental Table 2A: Pleural Fluid IFN-α Levels **(ng/ml**)
* received only one dose, NA = not available

Patient	**Dose**	**Day 1 Pre 1st dose**	**Day 2**	**Day 3**	**Day 4 (pre 2nd dose)**	**Day 5**	**Day 6**	**Day 14**
	Viral Part.							
301	10^{12}	0.03	1906	NA	925	715	NA	NA
302*	10^{12}	0.02	203	116	44	5	2	NA
303	10^{12}	0.66	75	144	150	72	147	43.7
304	3×10^{11}	0.01	11	5	0.98	3	0.7	
307	3×10^{11}	1.07	11	4	2	2	1.6	0.7
308	3×10^{11}	0.07	11	2	2	11	NA	NA
309	3×10^{11}	0.02	127	54	37	21	NA	NA
312	3×10^{11}	0	0	2.2	0.9	1.2	NA	NA
313*	3×10^{11}	0	0.9	2.1	1.1	0.6	NA	NA

Supplemental Table 2B. **Serum** IFNα Levels (**ng/ml**)
* received only one dose, NA = not available

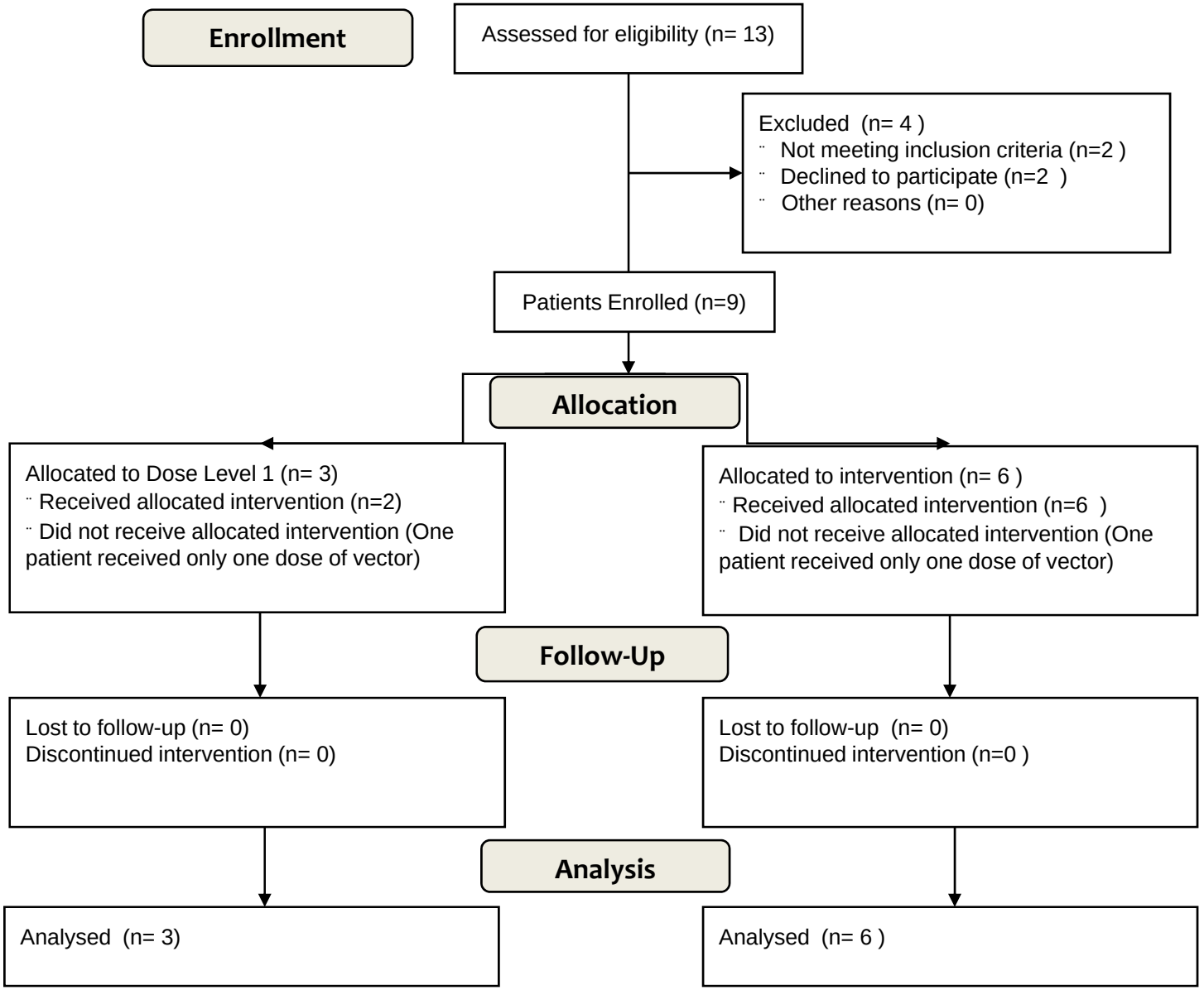
Patient	**Dose**	**Day 1 Pre 1st dose**	**Day 2**	**Day 3**	**Day 4 (pre 2nd dose)**	**Day 5**	**Day 8**	**Day 14**
	Viral Part.							
301	10^{12}	0	4.7	NA	0.5	0.4	NA	NA
302*	10^{12}	0.04	7.7	NA	1.9	NA	0.5	NA
303	10^{12}	3.3	3.7	NA	3.7	3.9	NA	NA
304	3×10^{11}	0	2.5	0.9	0.08	0.05	0	0
307	3×10^{11}	6.3	3.7	3.7	3.2	2.7	3.4	3.4
308	3×10^{11}	0.07	0.07	0.07	0.07	0.07	0.07	NA
309	3×10^{11}	0	0.5	0.16	0	0	0	0
312	3×10^{11}	0	0	0	0	0	0	0
313*	3×10^{11}	0	1.3	0.2	0.05	0	0	0

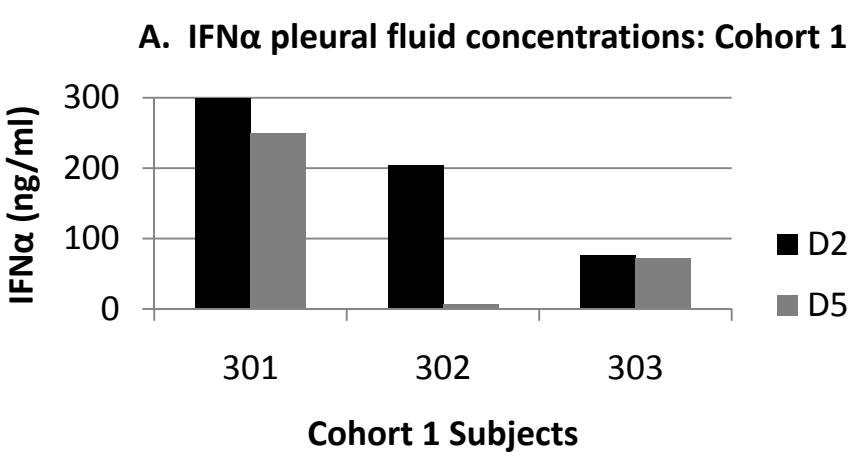
Supplemental Table 3: Anti-Ad Neutralizing Antibody Titers
Neutralizing antibody titers were determined as per Materials and Methods. The Nab titer was defined as the dilution of serum which inhibited gene transduction by 50% and is expressed as 1/titer.

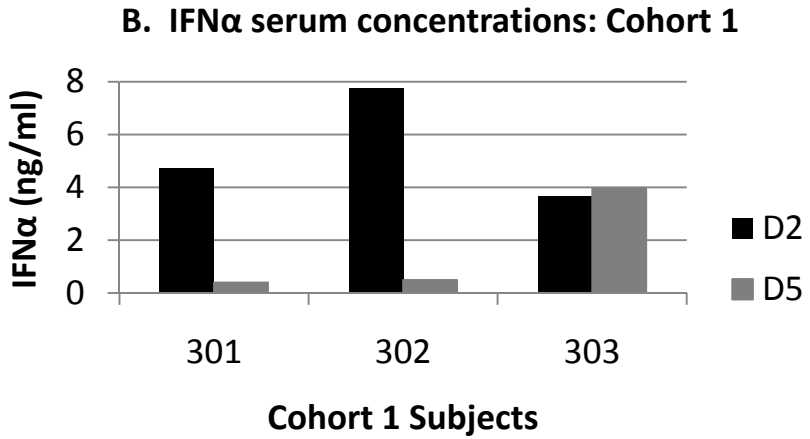
	Dose	**Day 1 (Pre 1st dose)**	**Day 2**	**Day 3**	**Day 4 (pre 2nd dose)**	**Day 5**	**Day 8**	**Day 14**	**Day 29**
	Viral Part.	1/titer							
301	10^{12}	<25	<50	NA	<50	100	NA	NA	NA
302*	10^{12}	<25	100	NA	300	400	19,200	25,600	>12,800
303	10^{12}	75	75	NA	100	100	NA	NA	2,400
304	3×10^{11}	<25	<25	<25	<25	200	>12,800	>12,800	>12,800
307	3×10^{11}	<50	<50	<50	<50	<50	3,200	12,800	>12,800
308	3×10^{11}	<25	300	150	25	50	12,800	NA	NA
309	3×10^{11}	<25	<25	<25	<25	800	>25,600	>25,600	NA
312	3×10^{11}	1:800	1:800	1:800	1:800	1:1000	>25,600	>25,600	>25,600
313*	3×10^{11}	1:400	1:400	1:400	1:400	1:400	>25,600	>25,600	>25,600

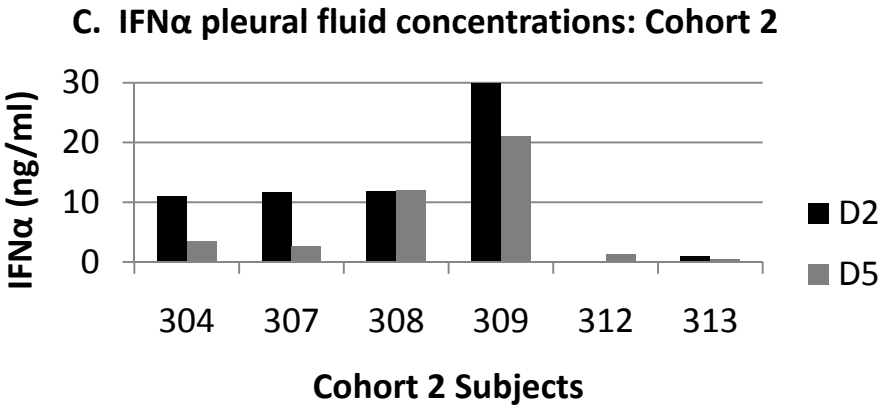
NA= not available
*Subject received only one dose

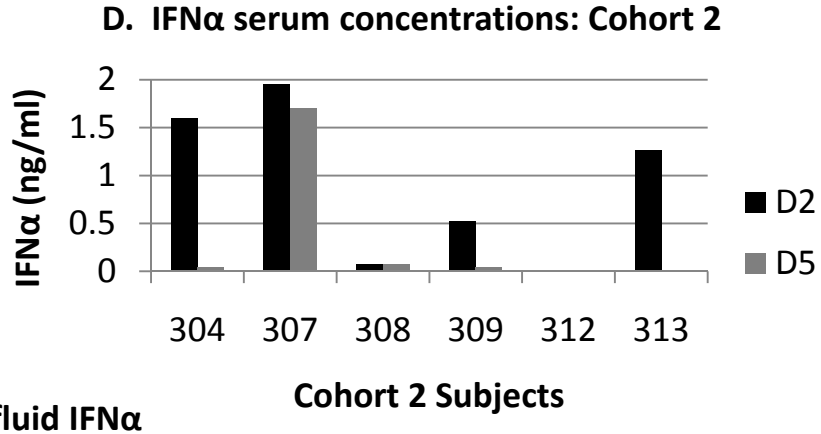
Supplemental Figure 1

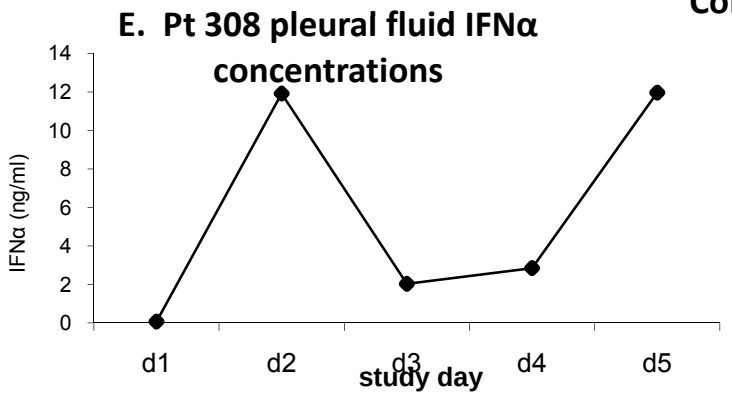



A. IFNα pleural fluid concentrations: Cohort 1
IFNα (ng/ml)
300
200
100
0
301
302
303
Cohort 1 Subjects
D2
D5


B. IFNα serum concentrations: Cohort 1
IFNα (ng/ml)
8
6
4
2
0
301
302
303
Cohort 1 Subjects
D2
D5


C. IFNα pleural fluid concentrations: Cohort 2
IFNα (ng/ml)
30
20
10
0
304
307
308
309
312
313
Cohort 2 Subjects
D2
D5


D. IFNα serum concentrations: Cohort 2
IFNα (ng/ml)
2
1.5
1
0.5
0
304
307
308
309
312
313
Cohort 2 Subjects
D2
D5


E. Pt 308 pleural fluid IFNα concentrations
IFNα (ng/ml)
14
12
10
8
6
4
2
0
d1
d2
d3
d4
d5
study day

Supplemental Figure 2. Pleural and Serum Interferon-α Concentrations

Panel A. Concentrations of IFN-α protein were measured (via ELISA) in pleural fluid from the three patients in Cohort 1 (1×10^{12} vps) 24 hours after the first Ad.IFN-α2b dose (Day 2) and 24 hours after the second Ad.IFN-α2b dose (Day 5). IFN-α concentrations on the Y-axis are expressed as ng/ml.

Panel B. Concentrations of IFN-α protein were measured in serum from the three patients in Cohort 1 (1×10^{12} vps) at the same time points as above. Subject 303 had higher than expected pre-therapy IFNα level in serum (refer to Supplemental Table 2)

Panel C Concentrations of IFN-α protein were measured in pleural fluid from the six patients in Cohort 2 (3×10^{11} vps) at the same time points as above.

Panel D. Concentrations of IFN-α protein were measured in serum from the six patients in Cohort 2 (3×10^{11} vps) at the same time points as above. Subject 307 had higher than expected pre-therapy IFNα level in serum (refer to Supplemental Table 2)

Panel E. Time course of pleural fluid IFN-a protein concentrations in the Subject 308. The vector was instilled on Day 1 and Day 4, after the pleural fluid samples had been taken. Note the increase on Day 5 after the second dose of vector on Day 4.