

# Complete Remissions in Chemotherapy of Lung Cancer Models Induced in Mice by Asbestos

WILLIAM E. SMITH, M.D.<sup>1†</sup>, DORAS D. HUBERT, B.A.<sup>1</sup>, EBRAHIM YAZDI, B. PHARM.<sup>1</sup>, STEPHEN M. HOLIAT, D.V.M.<sup>1</sup>, ELIZABETH CUTLER BISSELL, B.A.<sup>1</sup>, LAUREL MELCHER WATTS, B.A.<sup>1</sup>, RAYMOND A. BAYLOUNY, PH.D.<sup>2</sup>, AND HAROLD J. SOBEL, M.D.<sup>3</sup>

## Abstract

Chemotherapy trials were run with mice bearing transplants of carcinomas originally induced from fetal mouse lung cells by asbestos. After treatments with a nitrosourea (PCNU)\*, mice bearing transplants of a large-cell carcinoma (ASB XIV) had complete remissions (CR) in 13 of 20 animals on a 15 mg/kg regimen, in 6 of 20 on an 8 mg/kg regimen, and in none of 10 on a 4 mg/kg regimen. With comparable total doses, treatment was most effective when PCNU was given in a few large doses. In groups where CRs occurred, continued PCNU treatment of animals without CRs prolonged survival but yielded no additional CRs. No CRs of ASB XIV occurred in 80 mice treated with eight other anticancer agents or in 50 controls injected with 0.9% NaCl solution.

In mice bearing transplants of a squamous cell carcinoma (ASB XIII), treatments with PCNU were followed by CRs in 3 of 38 animals on 15 mg/kg regimens and in 3 of 28 animals on 8 mg/kg regimens. In groups of 6 mice fed a retinoid (Ro 10-9359) and treated with PCNU, CRs of ASB XIII occurred in 3 animals in each of two trials and in none in a third trial. Ro 10-9359 inhibited growth of transplants of squamous cell carcinoma LC 12 that had been induced from fetal mouse lung cells by a polycyclic hydrocarbon.

In trials of four other anticancer agents vs. ASB XIII, CRs occurred only with cyclophosphamide (CPA). There were 7 CRs among 8 mice treated with CPA 100 mg/kg × 3, no CRs in 10 after 100 mg/kg × 2, one CR in 8 after 50 mg/kg × 3, and no CRs in 6 after 50 mg/kg × 4. With the 50 mg/kg × 4 regimen of CPA and 7.5 mg/kg PCNU on the same days, there were 5 CRs in 8 mice. As a single agent, aziridinybenzoquinone (AZQ) increased life span but gave no CRs. There were CRs of ASB XIII in all of 8 mice after toxic combined therapy with PCNU and AZQ. There were no CRs in 66 control mice bearing ASB XIII.

**Key Words:** Lung cancer, nitrosourea, retinoids, asbestos cancer.

---

\*1-(2-chloroethyl)-3-(2,6-dioxo-piperidyl)-1-nitrosourea

## Background

IN THE 1960s, DR. IRVING J. SELIKOFF saw patients with lung cancers or pleural mesotheliomas who had worked in a New Jersey factory handling as-

bestos. At that time, one of us (Dr. William Smith) had facilities for experimenting with animals at Fairleigh Dickinson University's Health Research Institute in Madison, New Jersey. The owner of the factory introduced Dr. Selikoff and Dr. Smith, gave them a sample of asbestos (amosite) used in his factory, and suggested they test whether it would induce tumors in animals. It did. Their report (1) provided some of the early evidence for carcinogenicity of asbestos.

Epidemiological studies by Dr. Selikoff and his group at Mount Sinai established carcinogenicity of asbestos for humans (2–3). Dr. Smith became an assistant clinical professor in Dr. Selikoff's group at Mount Sinai, and animal experiments with asbestos were continued by Dr. Smith and his colleagues at the Health Research Institute.

---

<sup>1</sup>Retired, Health Research Institute and <sup>2</sup>Department of Chemistry, Fairleigh Dickinson University, Madison, NJ; and <sup>3</sup>University of Medicine and Dentistry of New Jersey, Newark, NJ.

<sup>†</sup>Deceased.

Address all correspondence to Mrs. Doras Hubert, 2 North Road, Glens Falls, NY 12801.

Support for this work was provided by U.S. Public Health Service Grant UI 00454 and grants from the Johns Manville Fund, the Institute of Occupational and Environmental Health (Montreal), Hoffmann-LaRoche, Inc., the Fannie E. Rippel Foundation, the George A. Ohl, Jr. Cancer Fund, and the Ann Earle Talcott Fund.

Accepted for publication July 2004.

## Introduction

Tumors resembling human mesotheliomas were found in hamsters after a single intrapleural injection of the amosite or chrysotile varieties of asbestos (1). Incidence of such tumors was found to be related to size of fibers and dose (4–7). Chemotherapy trials were run with hamsters bearing transplants of an epithelial mesothelioma induced by asbestiform tremolite, a sarcomatous mesothelioma induced by chrysotile, and a mixed epithelial-sarcomatous mesothelioma induced by crocidolite. Partial responses (increased lifespan) but no complete remissions (CR) were achieved (8–10). Cryopreserved cells from these tumors, identified respectively as mesotheliomas 10–24, H 12 and H 75, are available from Tumor Bank, National Cancer Institute, Bethesda, MD.

Since inhalation exposures of experimental animals require elaborate equipment and intratracheal injections are difficult to perform, we used the “tissue transplant technique” for experiments with mice (11). By this technique minced lungs from near-term BALB/c mouse fetuses are mixed with a material to be tested for carcinogenicity and implanted by trocar through skin slits into posterior thigh muscles of young adult mice of the same strain. Implants of minced fetal mouse lungs produce growths of alveolar lung tissue and bronchiolar-like structures. When these implants are made with polycyclic hydrocarbon carcinogens there is adenomatoid change, adenocarcinomas, squamous metaplasia and squamous cell carcinomas (12). The “tissue transplant technique” was used for tests for inhibition of carcinogenesis by vitamin A. A diet high in vitamin A increased, rather than decreased, induction of cancers from fetal mouse lung tissue by methylcholanthrene, a polycyclic hydrocarbon carcinogen (13).

This technique was also used to test for the carcinogenicity of various preparations of asbestos. At 27 sites where implants of fetal mouse lung tissue were made together with a preparation of harsh chrysotile asbestos, there was fibrosis, adenomatoid change and squamous metaplasia. At 9 of these sites, there were lesions with gross and histologic characteristics of neoplasia: 4 epidermoid carcinomas, 2 adenocarcinomas and 3 sarcomas (14). The sarcomas may have arisen from tissues of the new hosts rather than from cells in the implants. Two of the carcinomas were maintained in serial generations by transplants into thigh muscles of new hosts for chemotherapy trials.

The preparation of asbestos used with these implants was a harsh chrysotile with a broad range of fiber sizes. Average fiber length was 36 microns, as measured at 100 (by optical microscopy (15)). When this preparation was milled to reduce the length of

most fibers to below 5 microns, it did not induce carcinomas from the implants of fetal mouse lung cells (16). And no carcinomas arose from such implants made with long-fiber preparations of other varieties of asbestos: soft chrysotile, International Union Against Cancer (UICC) Standard Reference Samples of crocidolite, amosite and anthophyllite (16). Histologic sections show that the samples that did not induce carcinomas in these experiments caused little fibrosis at the intramuscular sites in mice, in contrast to the extensive fibrosis that surrounded implants made with the long-fiber preparation of harsh chrysotile. This is evidence that carcinogenicity of asbestos is related to its fibrogenicity.

The UICC Standard Reference Samples and samples of soft chrysotile were found by others to induce carcinomas of the lungs and pleural mesotheliomas after inhalation exposures of rats (17). Other inhalation experiments with chrysotile, amosite and crocidolite found that these induced lung tumors in rats but not in rabbits, guinea pigs or gerbils (18). Papillary bronchial carcinomas developed in two mice in those experiments, but a similar tumor was found in a control mouse that had not been exposed to asbestos. That humans are a susceptible species is shown by epidemiological studies associating exposure to asbestos with carcinomas of lungs and mesotheliomas in humans (3).

Except for the work reported in references 11 and 12, all of the above experiments by our group were carried out at Fairleigh Dickinson University's Health Research Institute in Madison, New Jersey, which closed in 1983. Some results of our chemotherapy trials with mice were briefly reported (19–21). As treatment of lung cancers remains a challenging subject, the above trials and previously unreported trials are detailed in the present paper.

## Materials and Methods

Animals from a breeding colony of BALB/c mice, maintained in our laboratory, were fed Purina Mouse Chow #5015 and water ad libitum. For serial passage for chemotherapy trials, we used two lines of carcinomas that had been induced from fetal mouse lung cells by the above-described preparation of long-fiber, harsh chrysotile (14). Tumors were removed aseptically. Living tissue was selected and minced with scalpels. Approximately 2 mm<sup>3</sup> of the mince was transplanted by trocar through skin slits wiped with an antiseptic (Betadine) into right posterior thigh muscles of young adult BALB/c males.

Body weights of animals were recorded at the time of transplant and at weekly intervals thereafter. Treatments were started on Day 7 after transplant. Control animals received intraperitoneal (ip) injec-

tions of sterile 0.9% NaCl solution on the same schedule as that of the most extensively treated group in each experiment. Dosage of drugs included LD<sub>10</sub> values for mice (22). Drugs were supplied by the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD, and were:

- NSC-95466 — PCNU: [1-(2-chloroethyl)-3-(2,6-dioxo-piperidyl)-1-nitrosourea]
- NSC-301739 — dianhydroanthracenedione dihydrochloride
- NCS-119875 — cis-DDPt [cis-diamine-dichloroplatinum]
- NSC-139105 — triazinate
- NSC-132313 — dianhydrogalactitol
- NSC-182986 — AZQ: [aziridinylbenzoquinone]
- NSC-259272 — ara-A-5'-phosphate
- For use with ara-A-5'-phosphate, an adenosine deaminase inhibitor (pentostatin) was provided by Parke, Davis and Company, Detroit, MI.
- Other drugs utilized were: cyclophosphamide (Cytoxan [CPA]), Mead Johnson Laboratories, Evansville, IN.; doxorubicin HCl (Adriamycin), Adria Laboratories, Inc., Wilmington, DE; and 5-fluorouracil and retinoids, Hoffman-La Roche, Inc., Nutley, NJ.

Sizes of tumors at implant sites in test and control animals were measured with calipers and compared at Day 28 after transplant. Percent inhibition of tumor size (ITS%) was calculated for a treated group and its control group by the following formula:

$$\frac{\text{Average controls} - \text{Average treated}}{\text{Average Controls}} \times 100$$

The number of days that each animal lived was recorded. Average survival of mice in a treated group was compared with average survival of its control group, and any increased lifespan (ILS%) was calculated. In a group where complete remissions occurred, usually by 28 days, ITS% was calculated for all animals in that group but ILS% was calculated only for those without remissions. Except where stated, each experiment consisted of a control group and 2–6 treatment groups, with 10 or 6 male mice per group. All animals were examined by necropsy with special attention to presence or absence of metastases. Tissues from representative lesions were fixed in 10% neutral buffered formalin. Sections were stained with hematoxylin and eosin.

## Results

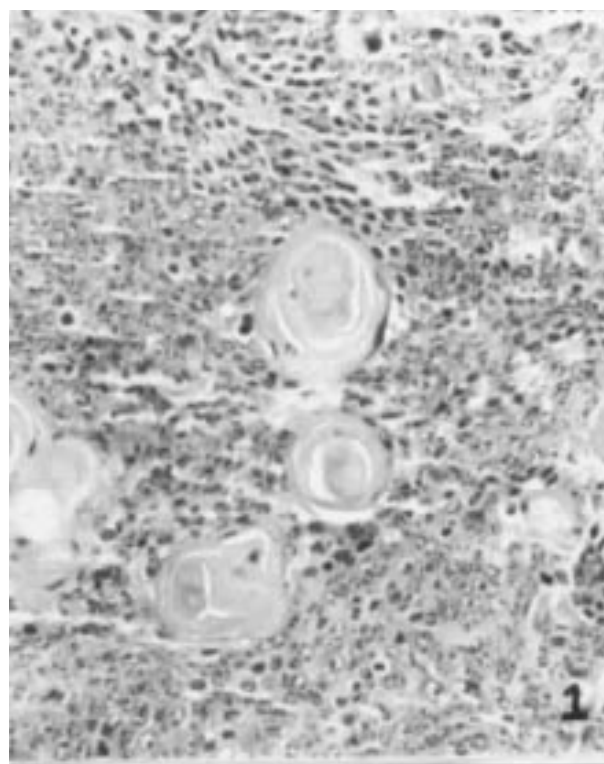
Transplants of each of the 2 tumor lines, identified as ASB XIII and ASB XIV, resulted in progres-

sive growths in essentially all new hosts, leading to death of controls within about 2 or 3 months, with large tumors at the sites of transplantation. The line designated as ASB XIII was a squamous cell carcinoma (Fig. 1). It was locally invasive, but no metastases were found. The line designated ASB XIV was a large cell carcinoma (Fig. 2). It metastasized widely, notably to the lungs (Fig. 3).

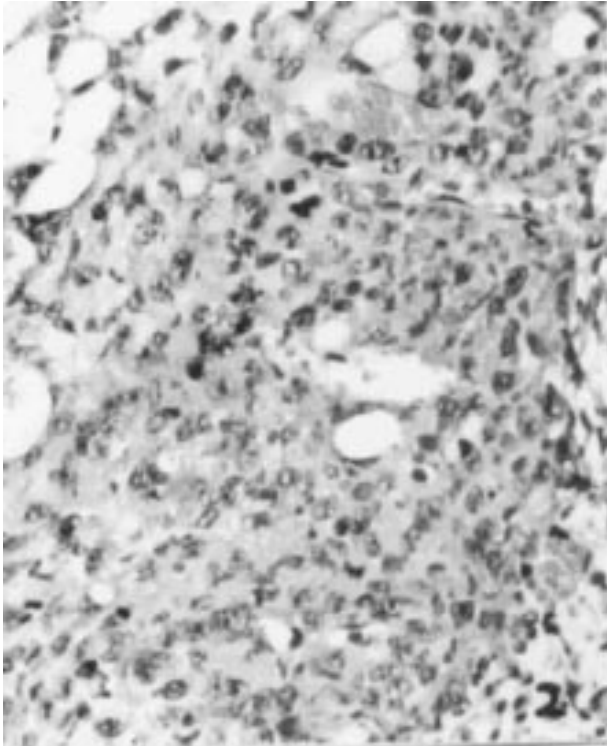
Doses and regimens of tested drugs are detailed in tables, which show inhibition of tumor size (ITS) at Day 28 after transplant, any increase in lifespan (ILS) of treated animals, and any complete remissions (CRs).

Data from trials with ASB XIV (10 animals in each group) are presented in Table 1, from which it can be seen that in 5 control groups all hosts died between 34 and 59 days (average: 43 days). All of these 50 control animals had large tumors at their transplant sites. Numerous metastases were found in lungs of the control hosts that lived more than 6 weeks after transplant.

As shown in groups of 10 animals (Table 1) and briefly reported (19), best results with ASB XIV followed treatments with PCNU. CRs occurred in 2 of 10 animals after 8 mg/kg 1 × q week × 3, in 4 of 10 animals after 8 mg/kg 1 × q week × 5. The other 14



**Fig. 1.** Section of ASB XIII tumor growing in thigh muscles of a mouse of the 128th transplant generation. This tumor was removed 50 days after transplant. It was a 30 mm spherical mass with moist keratin in center and rugose firm tissue around periphery. It is a squamous cell carcinoma. Note prominent "pearl" formation. (Hematoxylin and eosin × 288.)



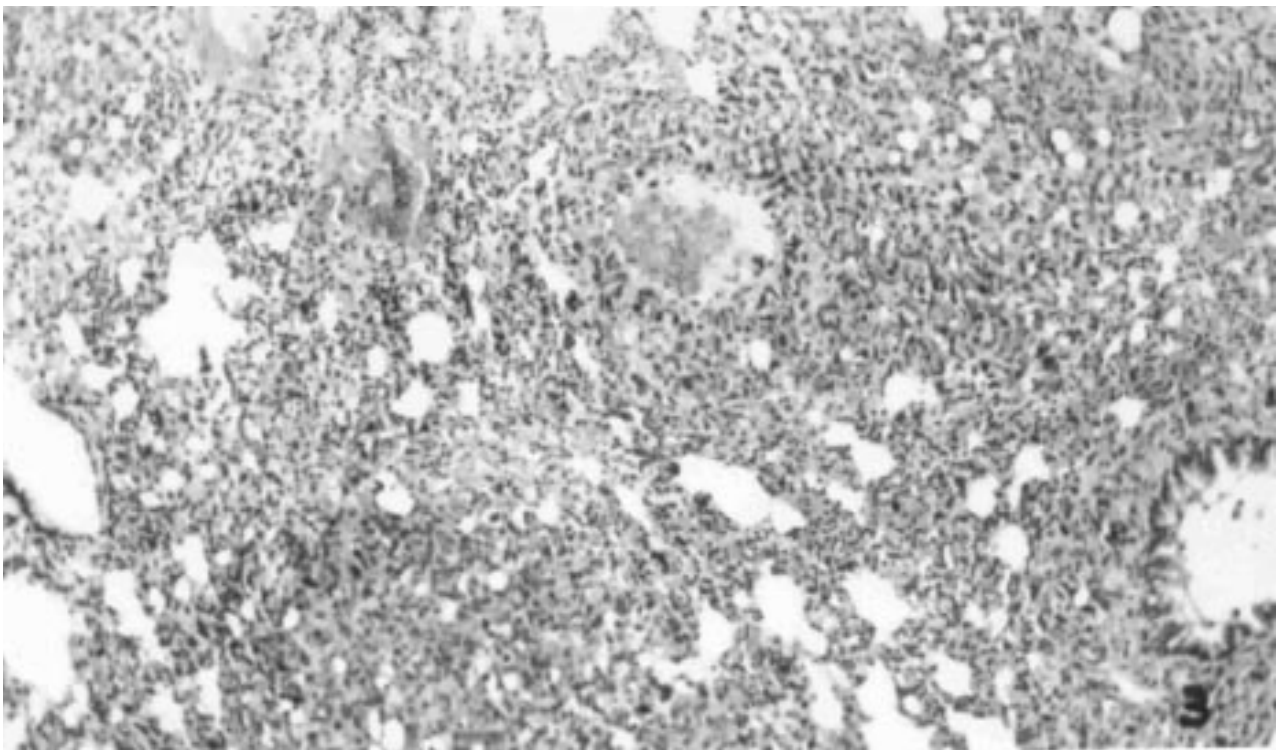
**Fig. 2.** Section of ASB XIV tumor growing in thigh muscles of a mouse of the 170th transplant generation. This tumor was removed 43 days after transplant. It was a 20 mm spherical mass with necrotic center and soft living tissue around periphery. It is a large cell carcinoma with prominent mitotic activity. (H & E  $\times$  384.)

animals in these two groups continued on treatments with PCNU 8 mg/kg 1  $\times$  q week up to a total of 112 mg/kg. Their survival was prolonged, but all of these 14 mice developed progressively growing cancers that proved fatal.

A group of 10 animals was treated with 15 mg/kg of PCNU on Days 7 and 14. All of these animals had palpable tumors 4–9 mm in diameter when examined at Day 14. Seven of these animals had no palpable tumors when examined at Day 28 or subsequently. These 7 survived until Day 160, when they were sacrificed and examined by necropsy. No living tumors were found at their transplant sites; no metastases were found. These 7 are regarded as cures. They and similar animals are listed in the tables as complete remissions.

The other 3 animals in this group had palpable tumors on Day 28 and were continued on weekly doses of 15 mg/kg PCNU until they died. Two of these animals developed progressively growing tumors and died on Day 89, one with multiple metastases in the lungs, the other with no metastases. The tumor in the third animal regressed and was no longer palpable at Day 92, but recurred thereafter despite continued treatment to a total of 275 mg/kg. This animal died on Day 139 with a large tumor at the transplant site but no metastases.

In another trial, 10 animals were treated with 15 mg/kg of PCNU on Days 7, 14, and 21. All had palpable tumors 4–9 mm in diameter on Day 14. No tumors were palpable in any of these animals on Day



**Fig. 3.** Section of lung from mouse bearing tumor shown in Fig. 2. All lobes of the lungs were largely replaced by metastases. (H & E  $\times$  144.)

**TABLE 1**  
*Trials with Large Cell Carcinoma ASB XIV*  
*10 Male Mice per Group*

Dose mg/kg	Total dose mg/kg	Treatment (days)	Average tumor size at day 28		Survival after transplants <sup>a</sup>			Complete remissions
			mm	ITS <sup>c</sup> (%)	Average (days)	Range (days)	ILS <sup>b</sup> (%)	
<b>PCNU [1-(2-chloroethyl)-3-(2,6-dioxo-piperidyl)-1-nitrosourea]</b>								
4	36	7–15	11	47	47	36–56	18	
8 <sup>d</sup>	40	7,14,21,28,35	3	82	93	86–98	116	4
8 <sup>d</sup>	24	7,14,21	7	58	70	58–101	63	2
15 <sup>d</sup>	45	7,14,21	0	100	60	38–84	40	6
15 <sup>d</sup>	30	7,14	1	94	89	89	106	7
<b>dianhydroanthracenedione dihydrochloride</b>								
1.5	9	7,11,14,18,21,25	16	–14	39	35–50	–10	
<b>cis-diaminedichloroplatinum (cis-DDPt)</b>								
6	18	7,11,14			20	9–44	–54	
3.33	16.65	7–11	15	–4	46	38–51	2	
<b>triazinate</b>								
51.3	461.7	7–11 + 14–17	13	11	44	15–66	–1	
<b>dianhydrogalactitol</b>								
4.75	23.75	7–11	15	–4	43	35–49	–5	
<b>ara-A-5'-phosphate</b>								
200	1800	7–11 + 14–17 together with pentostatin						
0.25	2.25	7–11 + 14–17	15	–9	41	21–50	–8	
<b>pentostatin</b>								
0.25	2.25	7–11 + 14–17	13	8	48	43–55	7	
<b>doxorubicin HCl</b>								
2	14	8,15,22,24, 30,36,43	15	9	52	43–60	10	
3	33	2 × q week	8	38	67	58–75	43	
<b>5-fluorouracil</b>								
50	550	2 × q week	13	19	50	37–64	16	
<b>cyclophosphamide</b>								
200	200	7						
25	225	2 × q week	16	1	45	35–53	6	
100	200	7,11						
25	250	2 × q week	16	1	44	42–46	5	
50	200	7,8,10,11						
25	200	2 × q week	17	–6	42	36–53	1	
<b>saline controls</b>								
		(5 groups)	16		43	34–59		

<sup>a</sup> Where complete remissions occurred, range, average survival and ILS refer to other animals in group.

<sup>b</sup> ILS = increase in lifespan.

<sup>c</sup> ITS = inhibition of tumor size.

<sup>d</sup> Animals without remissions at day 28 were continued on stated dosage. They had increased lifespan but no remissions.

28. There was one non-cancer-related death on Day 38. Another died of diarrhea on Day 43. Two developed progressively growing tumors and died with pulmonary metastases, on Days 75 and 84, despite continued weekly doses of 15 mg/kg of PCNU. The

other 6 animals received no further treatments after Day 21. Those 6 animals remained free of palpable tumors and survived until Day 160, when they were sacrificed for examination. Necropsies revealed no living tumors at transplant sites. There was no evidence of metastases.

As shown in Table 1, there were no complete remissions of ASB XIV after a total dose of 36 mg/kg PCNU delivered in doses of 4 mg/kg, whereas CRs occurred after a total dose of 24 or 30 mg/kg delivered in doses of 8 or 15 mg/kg.

In all above-cited trials with PCNU, necropsies of animals revealed no gross lesions attributable to toxicity of PCNU. Histologic or biochemical studies for evidence of toxic effects were not made. Changes in average body weights of animals in trials with PCNU were not greater than in controls.

Trials of other drugs versus ASB XIV, also detailed in Table 1, achieved no CRs. Treatments with doxorubicin at 2 mg/kg to a total of 14 mg/kg gave 10% ILS. At 3 mg/kg to a total of 33 mg/kg, there was 43% ILS ( $p < 0.001$ ). In a trial with 5-fluorouracil at 50 mg/kg to a total of 550 mg/kg, there was 42% ITS but only 16% ILS. Three regimens of cyclophosphamide had no notable effect on tumor sizes at Day 28 or on survival times. Nor were there notable effects of tested regimens of dianhydroanthracenedione dihydrochloride, triazinate, dianhydrogalactitol, or ara-A-5'-phosphate with pentostatin or pentostatin alone.

No therapeutic responses were seen in animals treated with a tested dosage of cis-diaminedichloroplatinum (cis-DDPt) (3.33 mg/kg daily to a total of 16.65 mg/kg). That group showed 15% loss in average body weight on Day 28. Administration of cis-DDPt at doses of 6 mg/kg on Days 7, 11 and 14 proved toxic: 26% weight loss by Day 14 and death before controls.

Trials with single agents for treatment of hosts bearing squamous cell carcinoma ASB XIII and treated with various drugs in groups of 10 male mice each are detailed in Table 2. Treatments were started Day 7 after transplant. PCNU 8 mg/kg  $1 \times q \text{ wk} \times 7$  gave 25% ITS and 19% ILS. With 15 mg/kg PCNU  $1 \times q \text{ wk} \times 7$  ITS was 49% and there was one CR. ILS was 33% for the other animals in this group.

After treatment with CPA 100 mg/kg on Days 7 and 11, there was 85% ITS but only 17% ILS and no CRs.

In dosage used for ASB XIV, there was no response of ASB XIII to doxorubicin or ara-A-5'-phosphate. There were no CRs in 20 controls.

Trials of combined therapies for squamous cell carcinoma ASB XIII are shown in Table 3, in which

**TABLE 2**  
*Single Agent Trials with Squamous Cell Carcinoma ASB XIII*  
*10 Male Mice per Group*

Dose mg/kg	Total dose mg/kg	Treatment <sup>a</sup> (days)	Average tumor size at day 28		Survival after transplants <sup>b</sup>			Complete remissions
			mm	ITS <sup>d</sup> (%)	Average (days)	Range (days)	ILS <sup>c</sup> (%)	
<b>PCNU [1-(2-chloroethyl)-3-(2,6-dioxo-piperidyl)-1-nitrosourea]</b>								
15	105	$1 \times q \text{ wk} - 7$	9	49	76	56-90	33	1
8	56	$1 \times q \text{ wk} - 7$	13	25	68	52-82	19	
<b>cyclophosphamide (im)</b>								
100	200	7,11 followed by:						
25	150	$2 \times q \text{ wk}$	2	85	75	60-89	17	
<b>doxorubicin HCl (im)</b>								
3	21	$2 \times q \text{ wk}$	14	9	53.1	37-62	-17	
<b>ara-A-5'-phosphate</b>								
200	2000	together with pentostatin						
0.25	2.5	QID $\times 7-11$ + 14-18	15	3	69	57-83	8	
<b>pentostatin</b>								
0.25	2.5	QID $\times 7-11$ + 14-18	17	-6	56	39-92	-13	
<b>saline controls</b>								
		(2 groups)	17	0	60	48-85		

<sup>a</sup> Treatments were started day 7 after transplant.

<sup>b</sup> Where complete remissions occurred, range, average survival and ILS refer to other animals in group.

<sup>c</sup> ILS = increase in lifespan.

<sup>d</sup> ITS = inhibition of tumor size.

**TABLE 3**  
*Combined Therapies with Squamous Cell Carcinoma ASB XIII*  
*Six Male Mice per Group*

Trial	Treatment <sup>a</sup>	Average tumor size at day 28		Survival after transplants <sup>b</sup>			Complete remissions
		mm	ITS <sup>d</sup> (%)	Average (days)	Range (days)	ILS <sup>c</sup> (%)	
<b>CM 31</b>							
#1	PCNU 15 mg/kg	2	86	43	41–44	–37	
#2	PCNU 8 mg/kg	8	43	74	58–85	25	
#3	PCNU 15 mg/kg Ro 10-9359	3	79	83	70–103	41	3
#4	PCNU 8 mg/kg Ro 10-9359	3	79	75	62–82	27	3
#5	Ro 10-9359	17	–21	47	40–68	–20	
#6	PCNU 15 mg/kg Ro 1-5488	3	79	83	70–97	41	1
#7	Ro 1-5488	14	0	51	37–71	–14	
#8	saline placebo	14		59	35–89		
<b>CM 37</b>							
#1	PCNU 8 mg/kg	10	41	83	64–100	50	1
#2	PCNU 8 mg/kg Ro 1-5488	11	35	70	36–79	21	
#3	Ro 1-5488	11	35	65	56–82	18	
#4	PCNU 8 mg/kg Ro 10-9359	8	53	47	18–77	–15	
#5	Ro 10-9359	15	12	50	39–56	–9	
#6	CPA 50 mg/kg	12	29	55	47–75	0	
#7	CPA 50 mg/kg Ro 10-9359	6	65	44	27–84	–20	1
#8	CPA 50 mg/kg Ro 1-5488	12	29	63	51–75	15	
#9	saline control	17		44	37–51		
#10	saline/placebo control	17		55 <sup>e</sup>	40–70		
<b>CM 33</b>							
<b>Eight Female Mice per Group</b>							
#1	PCNU 15 mg/kg	6	43	78	59–99	63	1
#2	PCNU 15 mg/kg CPA 100 mg/kg	0	100	76	66–84	58	4
#3	CPA 100 mg/kg	1	100	97	97	100	7
#4	AZQ 9 mg/kg	12	25	64	52–82	33	
#5	PCNU 15 mg/kg AZQ 9 mg/kg	0	100	33	13–104	–31	8
#6	saline control	17		4	35–76		
<b>CM 36</b>							
<b>Eight Female Mice per Group</b>							
#1	PCNU 15 mg/kg	2	88	58	24–11	4	
#2	PCNU 15 mg/kg CPA 50 mg/kg	2	88	69	26–111	25	
#3	CPA 50 mg/kg	10	3	59	49–76	5	1
#4	PCNU 7.5 mg/kg CPA 50 mg/kg	2	88	87	79–97	55	5
#5	saline control	16		56	41–86		

<sup>a</sup> PCNU was injected ip on Days 7, 14, 21, 28 in CM 31 and CM 37, and on Days 7, 14, 21 in CM 33 and CM 36. Other drugs were injected subcutaneously or im on same days: Ro 10-9359 or Ro 1-5488 were added to food to provide 10 mg/kg daily on Days 2 to 28.

<sup>b</sup> Where complete remissions occurred, range, average survival and ILS refer to other animals in group.

<sup>c</sup> ILS = increased life span.

<sup>d</sup> ITS = inhibition of tumor size.

<sup>e</sup> This group (placebo control) was used to calculate ITS and ILS for treated groups.

AZO = aziridinybenzoquinone; CPA = cyclophosphamide

each group consisted of 6 male mice, except for experiments CM 33 and CM 36, in which there were 8 females per group. In experiments on combined therapies, all of 46 control mice given only ip injections of NaCl solution or fed placebo beadlets died of progressively growing ASB XIII cancers.

Trials were made to test whether efficacy of PCNU versus ASB XIII might be enhanced by addition of a retinoid to food. As described elsewhere (21), mice in our laboratory were found to eat an average of 4 grams of unpelleted food (Purina Mouse Chow) per day. For experiments described in the present paper, a retinoid was mixed into this food to provide 10 mg/kg for a 20-gram mouse per day from Day 2 through Day 28 after transplant. Retinoids tested were Ro 1-5488 (all-trans retinoic acid) and its ester Ro 10-9359 (trimethylmethoxyphenyl analog ethyl ester). Ro 10-9359 was received as a powder, Ro 1-5488 in gelatin beadlets. Placebo beadlets contained corn oil instead of a retinoid. Retinoids and placebo beadlets were supplied by Hoffmann-LaRoche, Inc., Nutley, NJ.

Findings in experiment CM 31 (6 male mice per group) are shown in Table 3. PCNU was given Days 7, 14, 21 and 28. There was no ITS, no increase in lifespan and no CRs after either retinoid alone; there were no CRs in controls. There was ITS but no CRs after PCNU without retinoids. There were 3 CRs after treatment with the 15 mg/kg regimen of PCNU plus Ro 10-9359 and 3 CRs after the 8 mg/kg regimen of PCNU plus Ro 10-9359, and a single CR after the 15 mg/kg regimen plus Ro 1-5488. This experiment was briefly reported (20). As reported, ILS for mice without CRs on 15 mg/kg regimens were 56% for PCNU plus Ro 1-5488 and 33% for PCNU plus Ro 10-9359. Those calculations are based on median survival. Based on average survival, ILS is 41% in both these groups.

Animals in the control group died between 35 and 89 days after transplant. The 7 CRs in this experiment lived until they were sacrificed at 127 days. In the group on 15 mg/kg PCNU without retinoids, all 6 mice died between 41 and 44 days after transplant. They were in one cage and their deaths were attributed to contemporaneous disease. In an experiment with 10 mice bearing ASB XIII, treatment with 15 mg/kg PCNU  $1 \times q$  week  $\times 7$  gave one CR; the 9 others survived 56–90 days (Table 2). Table 2 also shows that no CRs of ASB XIII occurred in 10 mice that survived 52–82 days after PCNU 8 mg/kg  $1 \times q$  week  $\times 7$ .

In experiment CM 37 (Table 3) groups of 6 mice were treated with 8 mg/kg PCNU on the same regimen as in CM 31 ( $1 \times q$  week  $\times 4$ ), with and without retinoids. In the group treated with PCNU without retinoids, there was 41% ITS, 50% ILS and one CR. In the group on PCNU plus Ro 1-5488, there was 35% ITS and 21% ILS. In the group on PCNU plus Ro 10-

9359, there was 53% ITS but lifespan was about the same as that of controls. In CM 37, there were no CRs in groups treated with retinoids, with or without PCNU.

As also shown in Table 3, experiment CM 37 included trials in groups of 6 mice treated with CPA 50 mg/kg im on Days 7, 14, 21 and 28, with and without retinoids (10 mg/kg) in food. In the group treated only with this regimen of CPA, ITS was 29% but average survival was the same as in saline/placebo controls. The group on CPA plus Ro 1-5488 had 29% ITS and 15% ILS. In the group on CPA plus Ro 10-9359, there was 65% ITS, but 3 animals in that group died before controls. A CR in that group cannot be attributed to Ro 10-9359, since a single CR occurred in experiment CM 36 after treatment with CPA 50 mg/kg  $\times 3$ .

In trials by others, mice bearing transplants of colon adenocarcinoma no. 51 responded to PCNU or to 6-thioguanine (6-TG) as single agents and cures were obtained by combined therapy with these drugs (23). Using groups of 6 male mice with ASB XIII, we ran trials of 6-TG subcutaneously on days 7, 14 and 21, with and without PCNU ip 4, 8 or 15 mg/kg on the same days. CRs in the groups on those regimens of PCNU without 6-TG were 0, 2 and 1, respectively. In groups on those regimens of PCNU plus 4 mg/kg 6-TG, CRs were 1, 0, 2, respectively. With 6-TG alone, there was 44% ILS. Trials of 6-TG at 8 or 12 mg/kg were toxic.

In CM 33, groups of 8 female mice were treated on Days 7, 14 and 21 with CPA 100 mg/kg or aziridinybenzoquinone (AZQ) 9 mg/kg, with and without PCNU 15 mg/kg, on the same days. With PCNU as a single agent there was one CR. With CPA as a single agent there were 7 CRs that lived until they were sacrificed, at 120 days. In the group treated with CPA and PCNU there were 4 CRs, but all mice in this group died between 66 and 84 days after transplant. This combined therapy cured the tumors, but toxicity may have killed their hosts. In the group treated with AZQ as a single agent there was 25% ITS at Day 28 and 33% ILS, but no CRs. In the group treated with PCNU plus AZQ, 7 animals died without tumors between 13 and 27 days after transplant. The one survivor in that group died without tumors at 104 days. Presumably, combined therapy at these doses cured the tumors but killed their hosts.

In CM 36, there were 8 female mice in each group. There was one CR after CPA as a single agent of 50 mg/kg im on Days 7, 14 and 21. There were 5 CRs after that regimen of CPA plus PCNU 7.5 mg/kg ip on these days. In another group in the same experiment, after treatment with the same regimen of CPA but with the addition of PCNU 15 mg/kg ip Days 7, 14 and 21, ITS was 80%, but ILS only 25% and there were no CRs.

Another squamous cell carcinoma (LC 12) was available in our laboratory for serial transplant to thigh muscles of BALB/c mice from our breeding colony. It was induced from fetal mouse lung cells by a polycyclic hydrocarbon (methylcholanthrene) and has been described and illustrated (13). Chemotherapy trials were run with groups of 6 male mice bearing transplants of LC 12. A first experiment with PCNU at 15 or 8 mg/kg  $1 \times q \text{ wk} \times 7$  showed no CRs and little effect on tumor size or survival. In a second experiment, there were no CRs in mice treated only with PCNU or in controls, but there was one CR in mice on a diet to which Ro 10-9359 had been added and one CR in mice on this diet plus ip injections of 8 mg/kg PCNU  $1 \times q \text{ wk} \times 4$ . Tumor size at Day 28 in the 6 mice on the Ro 10-9359 diet without PCNU was 0, 0, 8, 8, 9, 11 mm. In a saline/placebo control group at Day 28, it was 12, 13, 13, 15, 15, 18 mm. In a saline control group, it was 12, 13, 13, 14, 15, 15 mm. The single CR in the group on the Ro 10-9359 diet without PCNU lived until Day 120, when he was sacrificed. The other mice in his group died with tumors at 73, 84, 94, 97 and 111 days. Survival in the two control groups ranged from 65–90 days. The diet containing Ro 10-9359 for this experiment was prepared to provide 10 mg/kg, as in the experiments with ASB XIII, and was given through Day 28.

Cryopreserved cells, identified as pulmonary carcinoma LC 12 and pulmonary carcinoma ASB XIV, are available from the Tumor Bank, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. ASB XIII is not available, as it did not "take" regularly in BALB/c mice from sources other than our breeding colony.

### Discussion

In trials of nitrosoureas vs. transplants of a spontaneous anaplastic epidermoid carcinoma of the lung in mice (Lewis lung carcinoma), cures were achieved by treatment with PCNU (24). In clinical trials, however, PCNU was not effective for treatment of lung cancer (25). There were partial responses in only 2 of 22 adenocarcinomas, 2 of 11 large cell carcinomas, 2 of 15 small cell carcinomas and none of 13 squamous cell carcinomas (25). In these trials, PCNU was used as a single agent.

In experiments with mice detailed in the present article, PCNU as a single agent was effective for treatment of a large cell carcinoma (ASB XIV) but was seldom effective for treatment of a squamous cell carcinoma (ASB XIII). Response of ASB XIII was enhanced in some trials by combined therapies with PCNU and either Ro 10-9359, CPA or AZQ. PCNU produced no CRs for another squamous cell carcinoma (LC 12) but Ro 10-9359 caused marked growth

inhibition. There was one CR of LC 12 after PCNU plus Ro 10-9359 and one CR after Ro 10-9359 alone.

Ro 10-9359 was found to inhibit induction of papillomas and carcinomas by a polycyclic hydrocarbon carcinogen on skins of mice (26). Clinical trials were made with advanced squamous cell carcinomas of the lungs treated by combined therapy with bleomycin and a nitrosourea (CCNU) with and without Ro 10-9359 in a preparation marketed as etretinate (27). Partial remissions (25–50% reduction in tumor size) were found in 3 of 25 patients treated with these drugs without Ro 10-9359 and in 5 of 21 treated with these drugs plus Ro 10-9359. This difference was not statistically significant, nor was survivorship in either of these groups. In these trials Ro 10-9359 was given orally 1 mg/kg daily for 6 weeks. In experiments showing inhibition of chemically induced papillomas and carcinomas of the skins of mice, Ro 10-9359 was given by stomach tube 30 mg/kg five days a week for 36 weeks (28).

In experiments reported elsewhere (29), responses of ASB XIII and ASB XIV were not notably different in males and females, and there was no notable difference in survivorship between the treated or control groups.

Relationships between vitamin A and retinoids have been summarized (29). In 2,592 patients, of whom 60% had cancers of the oral cavity or larynx and 40% had non-small-cell cancers of the lungs, treatments were found not to gain effectiveness from daily intake of high doses of vitamin A (retinyl palmitate) over a two-year period (30). Large-scale epidemiologic studies have found that long-term continued intake of high doses of vitamin A increases, rather than decreases the incidence of lung cancer (31). Biologic effects of retinoids has been reviewed (32, 33), and toxicity, including teratogenicity, has been summarized (33). Findings of abnormalities in fetuses preclude use of isotretinoin (13-cis retinoic acid) and etretinate (Ro 10-9359) during pregnancy.

Combined therapy with 13-cis retinoic acid and interferon-alpha has been found to be effective in clinical trials for treatment of squamous cell carcinomas of the skin (34) and cervix (35). For treatment of lung cancers with this combination, clinical trials arranged by the National Cancer Institute are in progress.

### Conclusion

Chemotherapy trials were run with mice bearing transplants of carcinomas originally induced from fetal mouse lung cells by asbestos. In trials of nine drugs as single agents, complete remissions of a large cell carcinoma (ASB XIV) were obtained only with a nitrosourea (PCNU): 19 CRs in 40 mice. Treatment of a squamous cell carcinoma (ASB XIII) with PCNU was less effective: 6 CRs in 66 mice.

Response of ASB XIII was enhanced by combined therapy with PCNU and a retinoid (Ro 10-9359) in two of three trials. High doses of cyclophosphamide gave 7 CRs of ASB XIII in 8 mice. Combined therapy with low doses of cyclophosphamide and PCNU gave 5 CRs of ASB XIII in 8 mice. AZQ increased lifespan but gave no CRs of ASB XIII as a single agent. After toxic combined therapy with AZQ and PCNU, there were CRs in all of 8 mice. PCNU as a single agent gave no CRs of a squamous cell carcinoma (LC 12) originally induced from fetal mouse lung cells by a polycyclic hydrocarbon. Ro 10-9359 inhibited growth of LC 12.

### Acknowledgments

For advice on drugs and dosage we are indebted to the late Stephen Davis, M.D., formerly Chief, Oncology/Hematology, Veterans Administration Medical Center, East Orange, New Jersey. We thank Jane Boschert for preparing this manuscript.

### References

1. Smith WE, Miller L, Churg J, Selikoff IJ. Mesotheliomas in hamsters following intrapleural injection of asbestos. *J Mt Sinai Hosp NY* 1965; 32:1–8.
2. Selikoff IJ, Churg J, Hammond EC. Asbestos exposure and neoplasia. *JAMA* 1964; 188:22–26.
3. Selikoff IJ, Lee DHK. Asbestos and disease. New York: Academic Press; 1978.
4. Smith WE, Hubert DD, Badollet MS, Churg J. Tests for threshold levels of carcinogenicity of asbestos. In: Internationale Konferenz über die biologischen Wirkungen des Asbestos. Deutsches Zentralinstitut für Arbeitsmedizin. Dresden: 1968; pp 240–242.
5. Smith WE. Experimental studies on biological effects of tremolite talc on hamsters. In: Symposium on Talc. Goodwin A, Comp. Information Circular 8639, U.S. Bureau of Mines. Washington, DC. 1974; pp. 43–48.
6. Smith WE, Hubert DD. The intrapleural route as a means for estimating carcinogenicity. In: Karbe E, Park JF, editors. Experimental lung cancer. Carcinogenesis and bioassays. Berlin: Springer Verlag; 1974; pp. 93–101.
7. Smith WE, Hubert DD, Sobel HJ. Dimensions of fibers in relation to biologic activity. WHO-IARC Symposium on Biological Effects of Mineral Fibers. Lyon, France. In: Wagner JC, editor. Biological effects of mineral fibres. IARC Sci Publ No. 30. 1980; pp. 357–360.
8. Smith WE, Hubert DD, Holiat SM, et al.. An experimental model for treatment of mesothelioma. *Cancer* 1981; 47:658–663.
9. Holiat SM, Smith WE, Hubert DD, Davis S. Chemotherapeutic trials with hamster mesothelioma 10-24: responses to azacitidine, aziridinylbenzoquinone, cisplatin, and PCNU. *Cancer Treat Rep* 1981; 65(11–12):1113–1115.
10. Holiat SM, Smith WE. Experimental models for treatment of mesotheliomas. Conf on Asbestosis. Inst. Immunology and Experimental Therapy. Polish Academy of Sciences. Wroclaw. Program Abstract. 1981.
11. Smith WE. The tissue transplant technic as a means of testing materials for carcinogenic action. *Cancer Res* 1949; 9:712–723.
12. Smith WE. The neoplastic potentialities of mouse embryo tissues. V. The tumors elicited with methylcholanthrene from pulmonary epithelium. *J Exp Med* 1950; 91:87–104.
13. Smith WE, Yazdi E, Miller L. Carcinogenesis in pulmonary epithelia in mice on different levels of vitamin A. *Environ Res* 1972; 5(2):152–163.
14. Smith WE, Yazdi E. Induction of carcinomas from mouse lung transplanted with asbestos [abstract]. *Proc Am Assn Cancer Res* 1969; 10:A 331.
15. Badollet MS, Gantt WA. Preparation of asbestos fibers for experimental use. *Ann N Y Acad Sci* 1965; 132 (1):451–455.
16. Smith WE, Cutler EL, Melcher L. Growth of pulmonary tissue exposed to different preparations of asbestos [abstract]. *Fed Proc* 1982; 41(4):A3833.
17. Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of the inhalation of asbestos in rats. *Brit J Cancer* 1974; 29:252–269.
18. Reeves AL, Puro HE, Smith RG. Inhalation carcinogenesis from various forms of asbestos. *Environ Res* 1974; 8:178–202.
19. Smith WE, Holiat SM, Cutler EL, et al. Response of asbestos-induced carcinomas to PCNU in BALB/c mice [abstract]. *Proc Am Assn Cancer Res* 1981; 22:A878.
20. Smith WE, Melcher L, Cutler EL, Baylouny RA. Response of a squamous cell carcinoma to combined treatment with PCNU and retinoids [abstract]. *Fed Proc* 1983; 42 (No. 7):A2063.
21. Hubert DD, Holiat SM, Smith WE, Baylouny RA. Inhibition of transplanted carcinomas in mice by retinoids but not by vitamin C. *Cancer Treat Rep* 1983; 67(12):1061–1065.
22. LD 10 Summary. Toxicity testing of anticancer drugs in small experimental animals. Southern Research Institute 1979; pp. 257.
23. Schabel FM Jr, Laster WR Jr, Trader MW, et al. Combination chemotherapy with nitrosoureas plus other anticancer drugs against animal tumors. In: Prestayko AW, Crooke S, Baker SK, et al. Nitrosoureas: current status and new developments. New York: Academic Press; 1981. pp. 9–26.
24. Montgomery JA, McCaleb GS, Johnston TP, et al. Inhibition of solid tumors by nitrosoureas. I. Lewis lung carcinoma. *J Med Chem* 1977; 20(2):291–295.
25. Ratanatharathorn V, Samson MK, Haas CH, et al. Phase II evaluation of 1-(2-chloroethyl)-3-(2,6-dioxo-(piperidyl)-1-nitrosourea (PCNU) (NSC-95466) in patients with advanced carcinoma of the lung. *Am J Clin Oncol (CCT)* 1983; 6(1):99–102.
26. Bollag W. Retinoids and cancer. *Cancer Chemother Pharmacol* 1979; 3:207–215.
27. Weber W, Arnold H, Drings P, et al. Etretnate (Ro 10-9359, Tigason) und CCNU (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) und Bleomycin versus CCNU und Bleomycin in der Behandlung des fortgeschrittenen Plattenepithel-Karzinoms des Bronchus (AIO\*-Studie BS 1/78) [German]. *Onkologie* 1983; 6(2):62–65.
28. Bollag W. Prophylaxis of chemically induced epithelial tumors with an aromatic retinoic acid analog (Ro 10-9359). *Europ J Cancer* 1975; 11:721–724.
29. Weber F, Cornish-Bowden A. Vitamin A and retinoids. *Brit J Nutr* 1995; 74: 869–870.
30. van Zandwijk N, Dalesio O, Pastorino U, et al. EUROSCAN, a randomized trial of vitamin A and N-acetylcysteine in patients with head and neck cancer or lung cancer. For the European Organization for Research and Treatment of Head and Neck and Lung Cancer Cooperative Groups. *J Natl Cancer Inst* 2000; 92(12):977–986.