

## LETTER TO THE EDITOR

Dear Sir,

### A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice

Models based on athymic nude mice have been used for human cancer research. However, *s.c.* or *i.m.* xenografts have failed to metastasize, or have only metastasized at low rates, even when they were derived from tumors that were highly metastatic in the patient (Fidler, 1990).

Recent reports from a number of laboratories have indicated that implanting human tumor cells orthotopically in the corresponding organs of nude mice results in much higher metastatic rates. Metastases have developed after orthotopic implantation of cell lines, including colon cancer, bladder cancer, melanoma, breast cancer, head-and-neck cancer, and pancreatic cancer [see Fidler (1990) for review].

Our approach is to avoid disruption of tumor integrity and to orthotopically implant histologically-intact patient tumor tissue directly after surgery or biopsy. Such a model should better reflect the original properties of human cancer and could be of great value in the development of new drugs and treatment strategies. We have therefore constructed metastatic models of human colon cancer in nude mice, making direct use of surgical specimens which can exhibit the varied clinical behavior that occurs in human subjects (Fu et al., 1991a). We have also constructed metastatic models of human bladder cancer utilizing orthotopic transplantation of intact tissue (Fu et al., 1991b).

A number of xenograft models have been developed for human lung cancer. These include *s.c.*-implant models and implantation under the renal capsule, but these models have not been sufficiently representative of the clinical situation (McLemore et al., 1988). The studies of McLemore et al. (1987, 1988) have utilized the orthotopic concept to develop more relevant lung-tumor models in nude mice. The first model developed by McLemore et al. (1987) utilized the growth of human lung cancer cell lines in the bronchioloalveolar region of the right lung of nude mice implanted via an intrabronchial injection. Suspensions of disaggregated fresh tumor specimens were also implanted intrabronchially (*i.b.*) by this group. These tumors grew intrabronchially much more extensively than the same tumors inoculated *s.c.* However, most of the tumors propagated *i.b.* were localized to the right lung, with only 1% metastasizing to the left lung, 2% to the trachea, 6% to the peritracheal area and only 3% spreading distantly to lymph nodes, liver or spleen. McLemore et al. (1988) subsequently developed a second model by injecting lung tumor cells via an intrathoracic route into the pleural space. This model seems similar to the intrabronchial model in that extensive local growth occurs with little metastatic spread.

We now describe a method that utilizes histologically intact tumor tissue implanted into the left lung by a thoracotomy procedure. Our observations thus far indicate that this method results not only in extensive local growth in nude and SCID mice, but also in development of regional and distant metastases. This corroborates the findings from other tumor

models developed by our group for bladder (Fu et al., 1991b) and colon (Fu et al., 1991a) cancer, in which the orthotopic transplantation of intact tissue leads to extensive local growth and metastasis, as in the clinical situation.

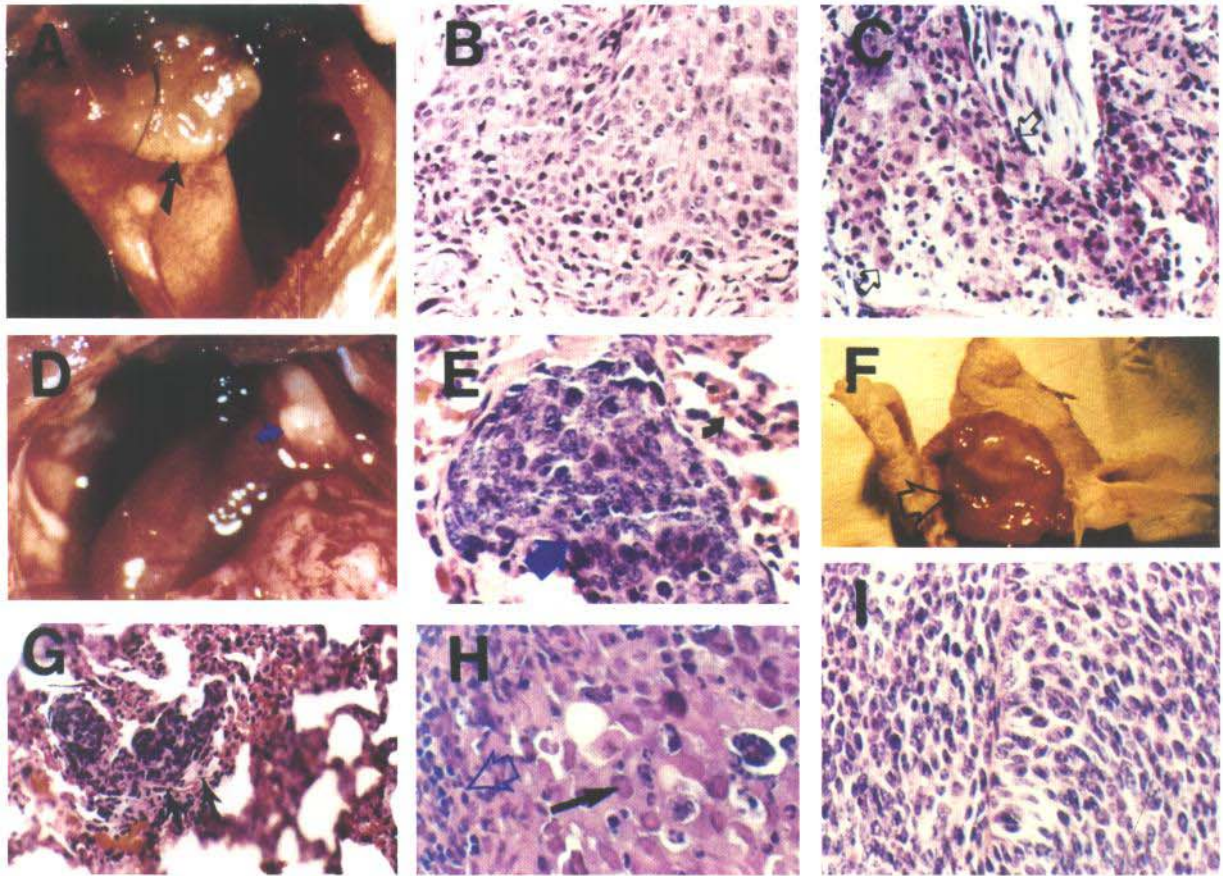
Male and female athymic *nu/nu* and SCID mice, approximately 4–6 weeks of age, were put in a glass chamber and anesthetized with an inhalation anesthetic, isoflurane. When fully anesthetized, they were removed from the chamber and continued on inhalation anesthesia via a glass tube containing the anesthetic. Tumors were transplanted into the left lung in all these experimental animals. The left lung was used for 2 reasons: (1) the loss of lung function is relatively smaller than in right-lung operations, and the left-lung-operated animals survive better; and (2) the left lung in mice has only one lobe, and tumors can develop easily after implantation.

Before starting the operation, 1–6 tumor pieces (1–1.5 mm<sup>3</sup> per piece) were sewn together with a 7-0 nylon surgical suture and fixed by making one knot. Putting all pieces of the tumor on one suture in advance can ensure a quick operation, which is necessary for the survival of the animals. Experimental animals were put in a position of right lateral decubitus, with the 4 limbs properly fixed. A 0.8-cm transverse incision of skin was made in the left chest wall. Chest muscles were separated by a sharp dissection, and costal and intercostal muscles were exposed. An intercostal incision of 0.4–0.5 cm on the third or fourth costa on the chest wall was made and the chest wall was opened. The left lung was lifted with a forceps and the tumor was sewn into the left upper lung with one suture. Two knots were made and the rest of the thread was cut off. All the lung tissue was returned into the chest cavity and the chest-wall incision was closed with a 6-0 surgical suture. The closed condition of the chest wall was then examined immediately. (If a leak exists, it must be closed by adding sutures until the incision is closed completely.) After this, an intrathoracic puncture was made with a 3-ml syringe and 25 G11/2 needle to withdraw the remaining air from the chest cavity. After the air had been withdrawn, a completely inflated lung could be seen through the thin chest wall. The skin and chest muscles were finally closed with 6-0 surgical suture in one layer. All the procedures described above were performed with a 7× magnifying microscope.

Table I shows that when poorly-differentiated large-cell-squamous-cell tumor 2268 was transplanted orthotopically to the left lung as histologically-intact tissue directly from surgery, 5 out of 5 mice produced locally-grown tumors averaging 8.2 mm in diameter, in an average time of 61 days. Opposite-lung metastases occurred, as well as lymph-node metastases (Table I). Figure 1 shows both the gross nature and histopathology of the primary tumor and metastases, with the tumor faithfully maintaining its large-cell-squamous-cell morphology. When grown *s.c.*, this tumor grew only locally in 2 of 4 animals and no metastases were observed.

**TABLE I** – GROWTH AND METASTASIS OF THE HUMAN PATIENT SQUAMOUS-CELL TUMOR 2268 TRANSPLANTED ORTHOTOPICALLY AS INTACT TISSUE VIA THORACOTOMY VS. SUBCUTANEOUS TRANSPLANTATION IN NUDE MICE

Transplantation route	Number of mice	Number of mice with local growth	Number of mice with metastases	Size of implanted tumor	Average diameter of primary tumor	Average growth time	Regional metastasis	Distant metastatic sites
Orthotopic	5	5	4	1–1.5 mm <sup>3</sup> , 6 pieces	8.2 mm	61 days	Mediastinum, chest wall pericardium.	Metastases were found throughout left lung, right lung, cervical lymph node, and left and right mediastinal lymph nodes
Subcutaneous	4	2	0	1–1.5 mm <sup>3</sup> , 10–30 pieces	15.5 mm	85 days	None	None



**FIGURE 1** – Growth and metastasis of human lung tumor in nude mouse after orthotopic transplantation via thoracotomy. (a) Implanted human lung tumor, indicated by black arrow, grown in left lung of nude mouse. (b) Histopathology of the tumor shown in (a). (c) Histopathology of the human lung tumor after removal from patient and before implantation. Arrows indicate that the original tumor is similar to the tumor growing in the mice. (d) Left mediastinal lymph node of the nude mouse involved with human lung tumor (blue arrow). (e) Histopathology of metastatic human lung tumor in contralateral lung of the mouse. Blue arrow indicates tumor, black arrow indicates normal lung. (f) Metastasis of human lung tumor in left cervical lymph node of mouse (arrow). (g) Histopathology of metastatic human lung tumor (arrows) in operated lung of nude mouse. (h) Histopathology of metastasis of human lung tumor in left mediastinal lymph node of nude mouse. Black arrow indicates tumor, blue arrow indicates lymphocytes. (i) Histopathology of metastasis of human lung tumor in left cervical lymph node of nude mouse.

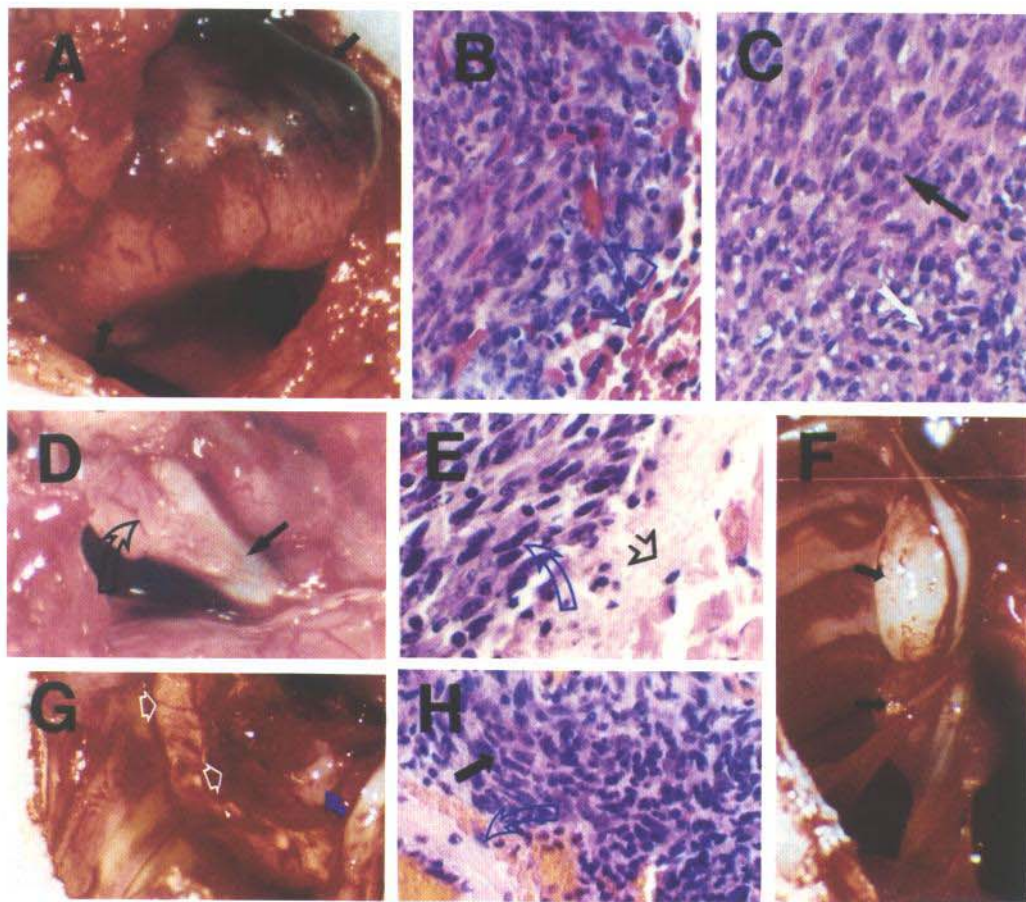
When the histologically intact human small-cell lung-carcinoma cell line Lu-24 was transplanted into the left lung of nude mice via thoracotomy after harvesting of s.c.-growing tissue from nude mice, 5 out of 5 mice produced locally-growing tumors averaging 10 mm in diameter within 24 days (Table II). All 5 mice produced regional metastases, including tumor invasion of the mediastinum, chest wall and pericardium, and distant metastases involving the right lung, esophagus, diaphragm, parietal pleura and lymph nodes. These 5

mice were implanted with only one 1.5-mm<sup>3</sup> piece of tissue. Three other mice were implanted with much more tissue, with subsequent similar tumor growth and metastases (data not shown).

Three severe-combined-immunodeficient (SCID) mice were also implanted orthotopically with histologically-intact Lu-24 tissue via thoracotomy. All 3 animals produced locally-growing tumors averaging 7.5 mm<sup>2</sup> in diameter within 17 days (Table II). All 3 SCID mice also developed regional me-

**TABLE II** – GROWTH AND METASTASIS OF THE HUMAN SMALL-CELL LUNG CARCINOMA CELL LINE LU-24 TRANSPLANTED ORTHOTOPICALLY AS INTACT TISSUE VIA THORACOTOMY IN NUDE AND SCID MICE

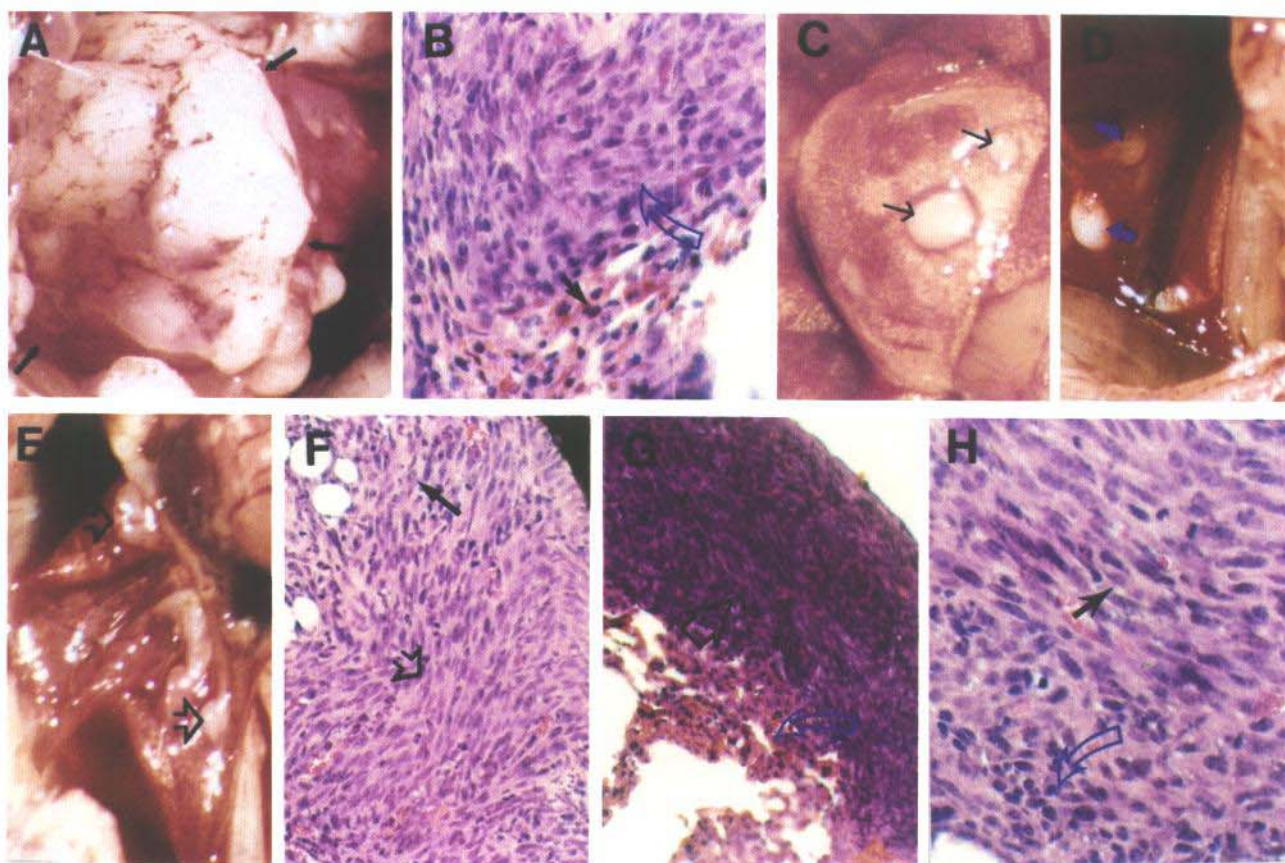
Type of mouse	Number of mice	Number of mice with local growth	Number of mice with metastases	Size of implanted tumor	Average diameter of primary tumor	Average growth time	Regional metastasis	Distant metastatic sites
Nude	5	5	5	1.5 mm <sup>3</sup>	10 mm	24 days	Mediastinum, left chest wall pericardium.	Metastases in right lung, parietal pleura, esophagus, diaphragm, left paraesophageal lymph node
SCID	3	3	3	1.5 mm <sup>3</sup>	8.7 mm	17 days	Mediastinum, left chest wall, pericardium.	Metastases in right lung, right chest wall, diaphragm, parietal pleura, lower mediastinal lymph node, paraesophageal lymph node, and hilar lymph node



**FIGURE 2** – Growth and metastasis of human LU-24 small-cell lung tumor line in nude mouse after orthotopic transplantation via thoracotomy. (a) Implanted human tumor (arrows) grown in left lung of nude mouse. (b) Histopathology of the implanted human lung tumor in left lung of nude mouse. Hollow arrow indicates tumor and solid arrow indicates lung tissue. (c) Histopathology of metastasis of human lung tumor in paraesophageal lymph node of nude mouse. Black arrow indicates tumor, white arrow indicates lymphocyte. (d) Metastasis of human lung tumor to esophagus of nude mouse. Hollow arrow indicates tumor, solid arrow shows esophagus. (e) Histopathology of human lung tumor metastatic to the esophagus. Blue arrow indicates tumor, black arrow indicates muscle of esophagus. (f) Metastases of human lung tumor in paraesophageal lymph node of nude mouse. Each arrow indicates a lymph node. (g) Metastasis, in contralateral lung, of human lung tumor in nude mouse. Blue arrow indicates tumor, white arrows indicate tissue of right lung. (h) Histopathology of contralateral lung metastasis of human lung tumor in nude mouse. Black arrow indicates tumor, blue arrow indicates lung tissue.

tastases involving the mediastinum, left chest wall and pericardium and distant metastases involving the opposite lung, lymph nodes, parietal pleura and diaphragm (Table II). The time to morbidity in the nude mice after transplantation of Lu-24 via thoracotomy was 24 days, as mentioned above, but in the SCIDs it was only 17 days, with the tumor apparently

growing and metastasizing more rapidly in the SCIDs. Figure 2 demonstrates the gross and microscopic histopathology of Lu-24 in the nude and SCID mice, respectively. As can be seen, the tumor faithfully maintains its oat-cell morphology while growing locally, as well as metastasizing, in immunodeficient mice of both types. Thus, our results indicate a great



**FIGURE 3** – Growth and metastasis of human LU-24 small-cell lung tumor line in SCID mice. (a) Implanted human lung tumor grown in left lung of SCID mouse (arrows). (b) Histopathology of implanted human lung tumor in left lung of SCID mouse. Blue arrow indicates tumor, black arrow indicates lung tissue. (c) Metastases of human lung tumor in contralateral lung of SCID mouse. Each arrow indicates a separate metastasis. (d) Metastases of right lower mediastinal lymph nodes. Each arrow indicates a lymph node. (e) Metastases of human lung tumor to right upper mediastinal lymph nodes of SCID mouse. Each arrow indicates a lymph node. (f) Histopathology of metastases of human lung tumor to right upper mediastinal lymph nodes of SCID mouse. Hollow arrow indicates tumor, solid arrow indicates lymphocyte. (g) Histopathology of contralateral lung metastasis of human lung tumor of SCID mouse. Black arrow indicates metastatic tumor, blue arrow indicates lung tissue. (h) Histopathology of human lung tumor in right lower mediastinal lymph nodes of SCID mouse. Black arrow indicates tumor, blue arrow indicates lymphocyte.

potential for producing patient-like metastatic models of human lung cancer in immunodeficient mice. Such models should prove useful for drug evaluation and treatment of individual patients with lung cancer.

Yours sincerely,

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