1) **R’s Bleomycin induced lung fibrosis protocol** (For I.T. Bleomycin.)

Prepare Bleomycin stock at 10U/ml.

(10 U/ 1000 μL, 1 U/100 μL, 0.25 U/25 μL, 0.0178-0.0156 U/g, 17.8-15.6 U/kg) mice 6-8 weeks, female, 14-16 g/mouse (male 1 time 20 g/mouse)

Use 25 microliter of Bleomycin stock + 25 microliter PBS (so 0.2 Follow weight every other day 8 days). Sacrifice mice at day 8, and harvest Lung, skin for histology (masson trichrome stain and H&E) or qPCR analysis.

For OP (osmotic pump), fill 200 microliter of Bleomycin stock solution for each pump (so 2U/MOUSE).

Implement the pump on the back of mice and follow fibrosis for 15 days.

2) **UUH protocol**

Bleomycin (1.5U/kg body weight) in 60ul volume or saline intratracheally injection

1.5U/ 1000g, 0.0015 U/g, 0.03/ 20g (1 mouse, 8week)

(Technician)’s Method

- 1.5mg/kg = 1.5 U/kg, 0.0015 U/g
- 20g/mouse, 0.03/ 20g
- 30 μg/mouse

BLM preparing

- 0.5 mg/ml → 1 ependorf tube stock in -20 °C
- 60 μl from stocked BLM = 30 μg/60 μL

3) **AJRCMB :** Wild type male and female C57BL/6 mice (10-20 weeks old)

Doses of bleomycin were body weight adjusted to either 1 mg/kg or 2 mg/kg. These doses correspond to approximately 1.5 Units/kg and 3 Units/kg, meaning that a 30 g mouse received approximately 0.045 Units or 0.090 Units of bleomycin, respectively. The 1 mg/kg dose
was used throughout the study unless explicitly stated otherwise. Following bleomycin administration, mice were observed daily for signs of morbidity and weighed on days 3, 7, 10, and 14. Mice were fed mashed food to help prevent excess weight loss, and all lung function assessments and tissue collections were performed 21 days post-dosing unless explicitly stated otherwise. (Male Sex Hormones Exacerbate Lung Function Impairment After Bleomycin-Induced Pulmonary Fibrosis, AJRCMB Articles in Press. Published on February 14, 2008 as doi:10.1165/rcmb.2007-0340OC)

4) **Bleomycin Treatment.** Age- and sex-matched, 8- to 16-week-old 129_Sv MMP-7___ (The Jackson Laboratory) and MMP-7___ mice (a gift from L. M. Matrisian, Vanderbilt University School of Medicine, Nashville), and 14- to 23-week-old C57BL_6 MMP-7___ (Charles River Breeding Laboratories) and MMP- 7___ (L. M. Matrisian) mice were maintained in a specific pathogen-free environment. Mice were anesthetized by methoxyflurane, and a 24-gauge needle was inserted into the trachea by means of the oral cavity. Fifty microliters of bleomycin (0.05–0.08 units in 0.9% saline, Sigma) or saline was slowly injected. Mice were killed 14 or 21 days after bleomycin or saline injection, and lungs were collected for either hydroxyproline determination or histology. (PNAS 2002, www.pnas.org/cgi_doi_10.1073_pnas.092134099)

5) **Bleomycin (3 mg/kg;** Nippon Kayaku Co., Tokyo, Japan) was intratracheally administered in 60 μl saline to the male C57BL/6 mice (8–10 wk old; Japan Clea, Tokyo, Japan). On Days 3, 7, and 14 after bleomycin treatment, the animals were killed and the lungs were removed en bloc.

Animals were allocated to seven groups, as follows: (1) intratracheal saline + vehicle givenally, (2) intratracheal saline + 200 mg/kg of oral gefitinib, (3) intratracheal bleomycin + oral vehicle, (4) intratracheal bleomycin + 20 mg/kg of oral gefitinib, (5) intratracheal bleomycin + 90 mg/kg of oral gefitinib, (6) intratracheal bleomycin + 200 mg/kg of oral gefitinib, (7) intratracheal bleomycin + 12 mg/kg of intraperitoneal AG1478. Gefitinib suspension in 1% Tween 80 (0.2 ml) was given daily by gavage from Day 1 to Day 13; AG1478 was given intraperitoneally at a daily dose of 12 mg/kg in dimethyl sulfoxide solution from Day 1 to Day 13. For the saline and the bleomycin control groups (groups 1 and 3), a daily dose of vehicle (1% Tween 80 solution) was given orally.


1.5 U/kg = 1mg/kg, [4.5 U/kg = 3mg/kg, 0.0045 U/g, 0.1124 U/25g]