Weill Cornell Medical College

Ex Vivo Model for Lung Cancer Metastasis

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Weill Cornell Medical College
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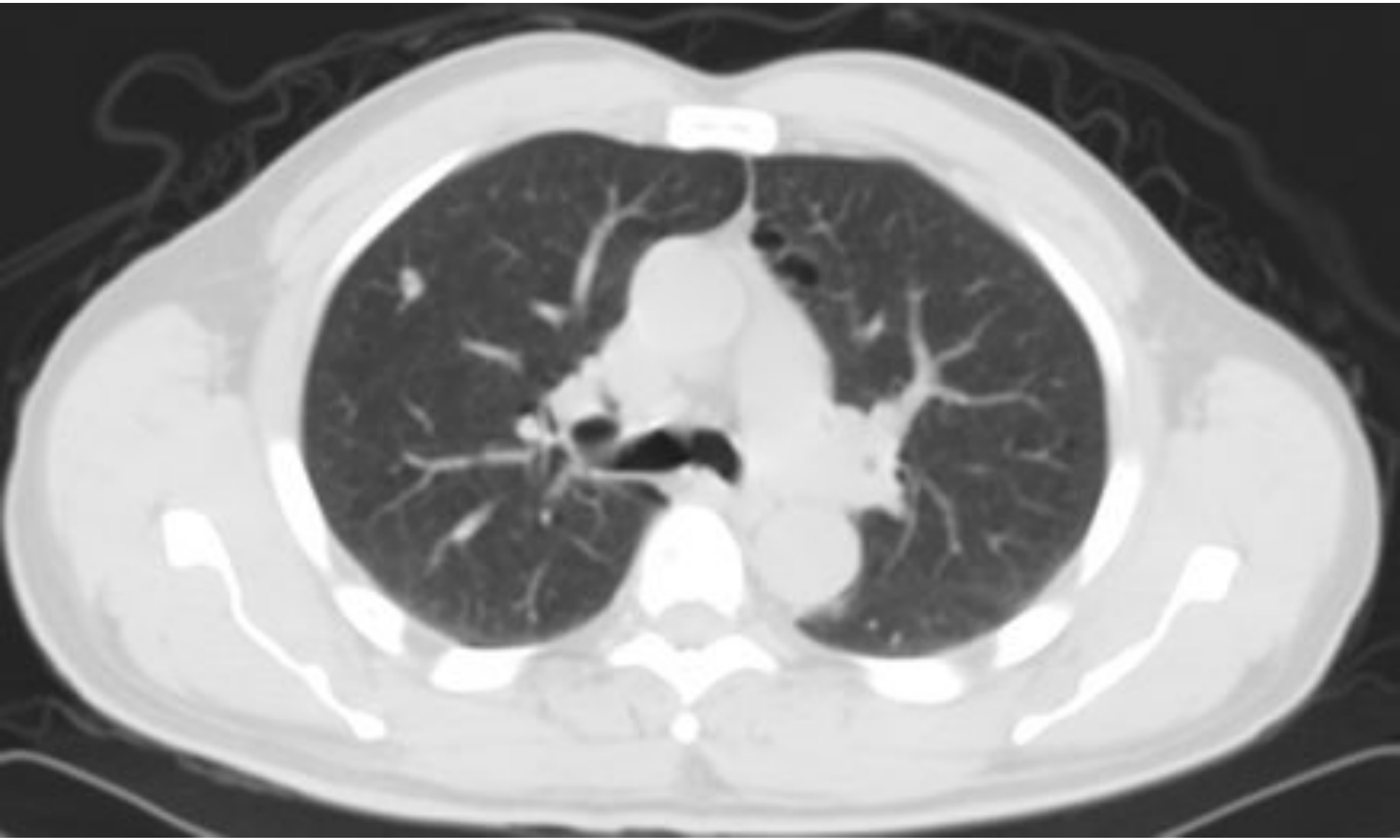
May 3, 2013

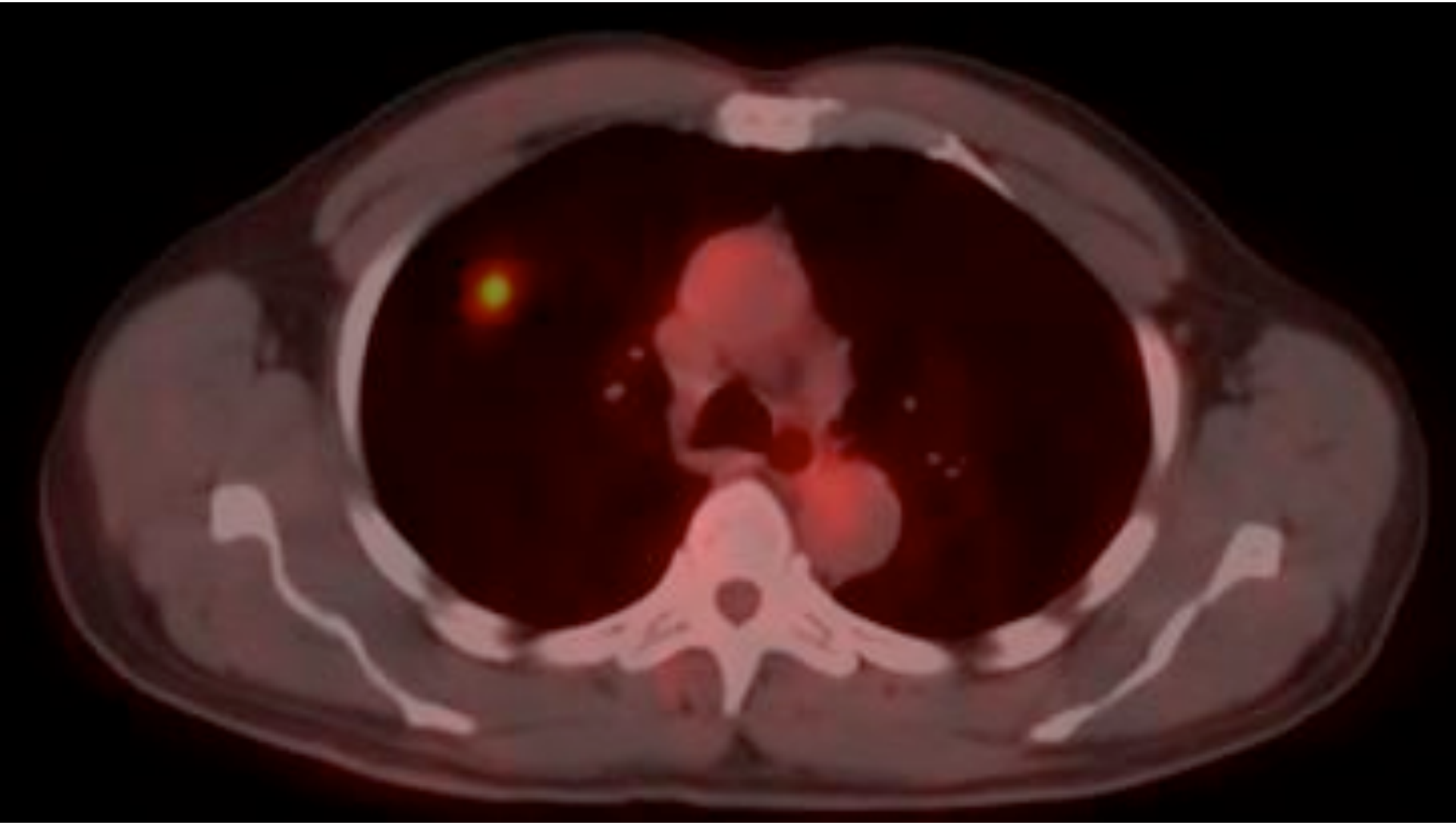
Objective

- Lung Cancer in Patients
- Mechanism behind metastasis
- *Ex Vivo* Lung Cancer Model

Lung Cancer Patient

72 year old gentleman with
60 pack year smoking history
presents with a cough.





Management

Needle biopsy: adenocarcinoma of the lung

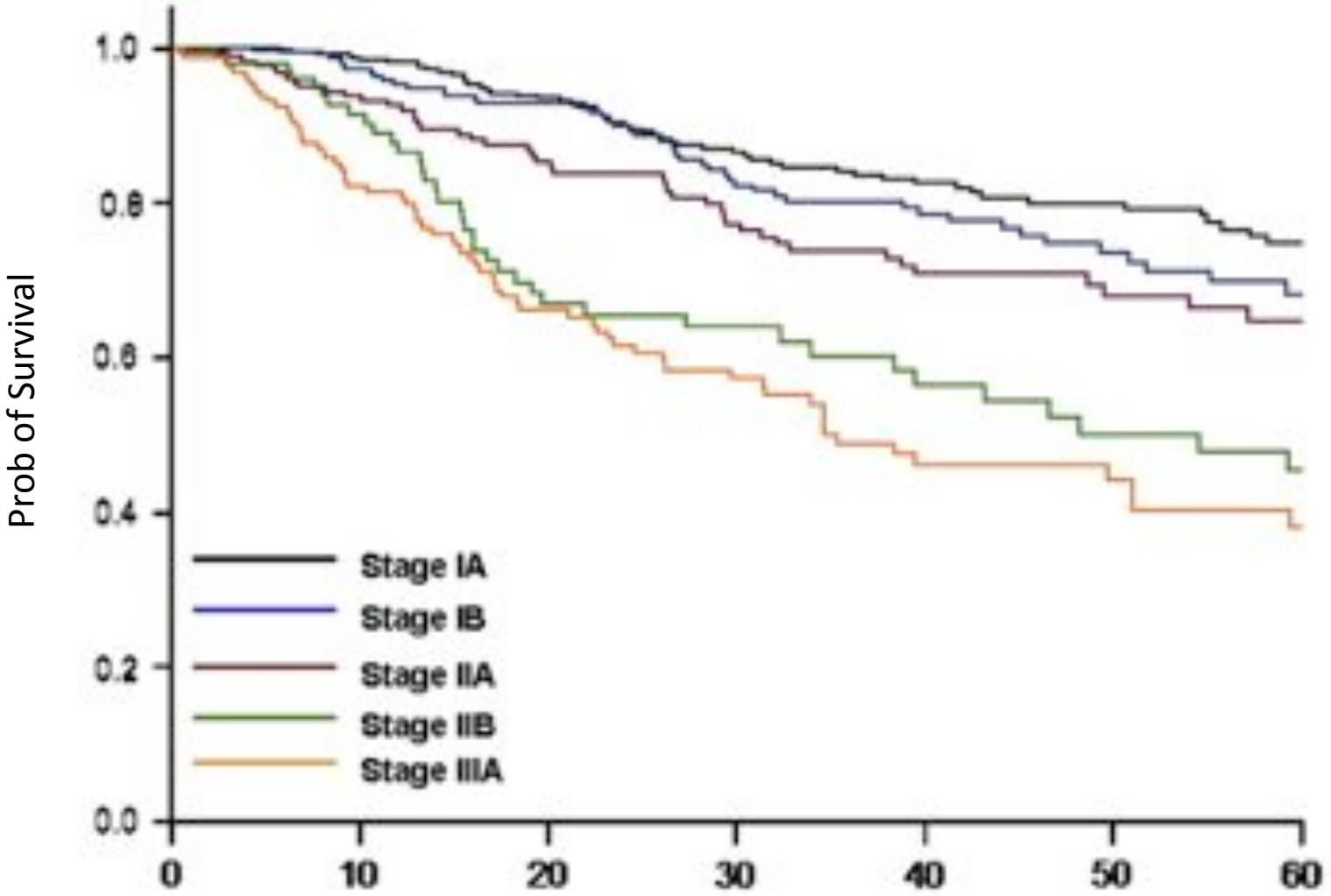
Clinical: Stage IA

Treatment:

- Minimally Invasive Right upper lobectomy
- Mediastinal lymph node dissection

Pathology: Stage IA adenocarcinoma of the lung

Survival by Stage



Kassis 2009

6 month later

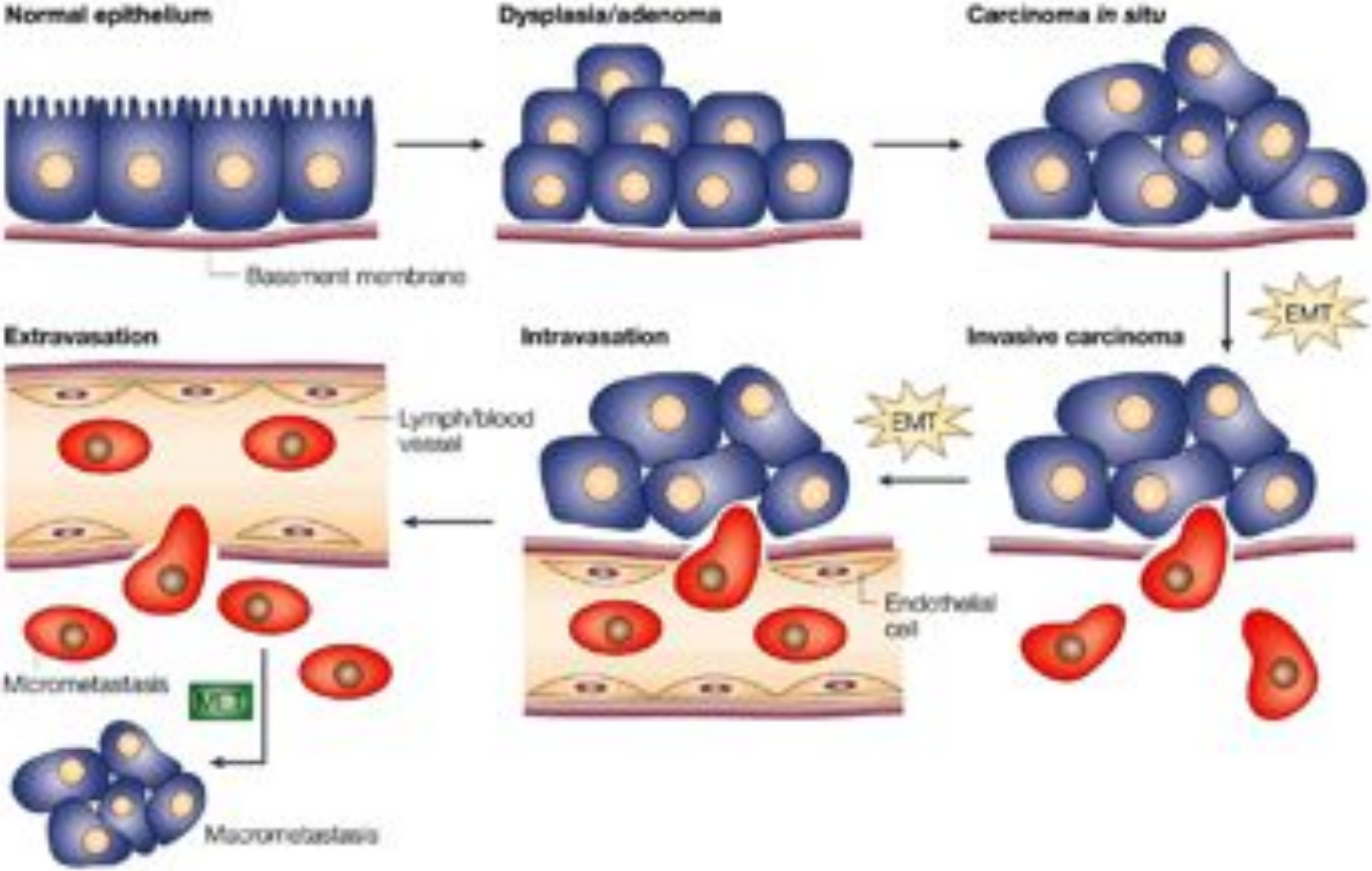
Image size: 512 x 512
View size: 879 x 879
WL: -500 WW: 1452
35404152 (72 y , 71 y)
5.3 Chest without contrast — Lung
3917
3
A
R
L
P
226
-500
-1226
Zoom: 172% Angle: 0
Im: 1/62 S (S → I)
Uncompressed
Thickness: 5.00 mm Location: 25.50 mm
5/14/12 9:17:40 AM
Made in OsiriX

Lung Cancer Patients

- Lung cancer cells form a nodule
- Blood vessels bring nutrients to the lung cancer nodule
- The nodule grows over time at the primary site
- Lung cancer spread to distant organs
- Metastatic disease lead to organ dysfunction and death

Hypothesis

- Epithelial to Mesenchymal Transition (EMT)
  - Tumor cells in a nodule break cell-cell and cell-matrix interaction and enter the circulation
- Mesenchymal to Epithelial Transition (MET)
  - Tumor cell in circulation enter the distal organ and form tumor



Nature Reviews | Cancer

Currently there is no *in vitro* or *ex vivo* model that can isolate the tumor cells at different phases of growth and metastasis

Modeling Human Lung Cancer

1. Lung cancer cells form a nodule
2. Lung cancer nodule grow in size over time
3. Lung cancer nodule can be perfused with nutrients and oxygen
4. Lung cancer cells escape the nodule through lymphatics or vascular circulation
5. Lung cancer cells in circulation grow in distant site

Cell Culture (2D)

Tumor Growth (4D)

Circulating Tumor Cells (CTC)

Metastatic Lesion (Met)

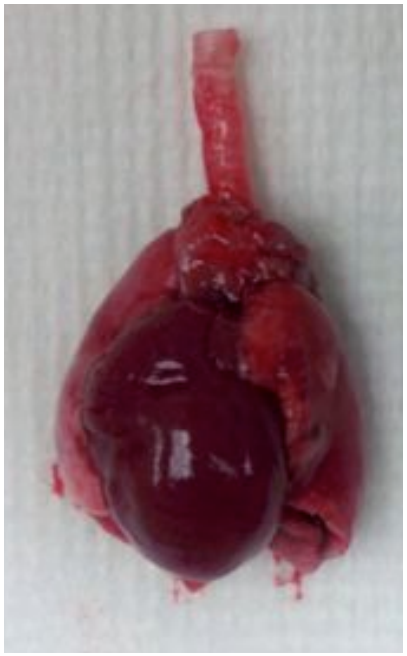
Cell Culture (2D)

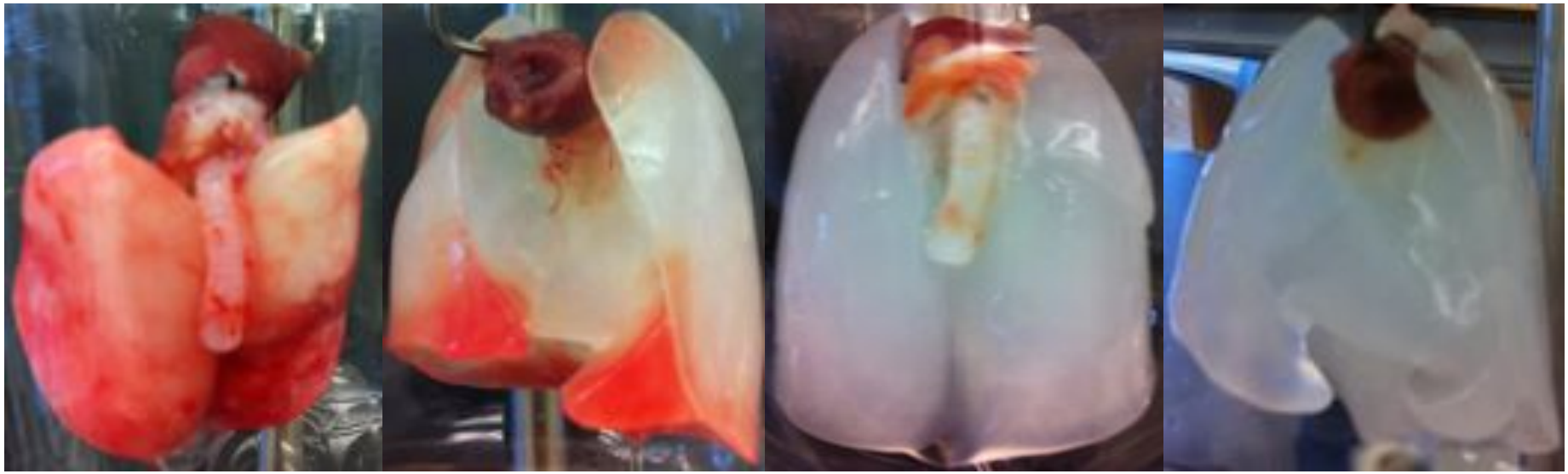
Tumor Growth (4D)

Circulating Tumor Cells (CTC)

Metastatic Lesion (Met)

Decellularized Matrix





15 min

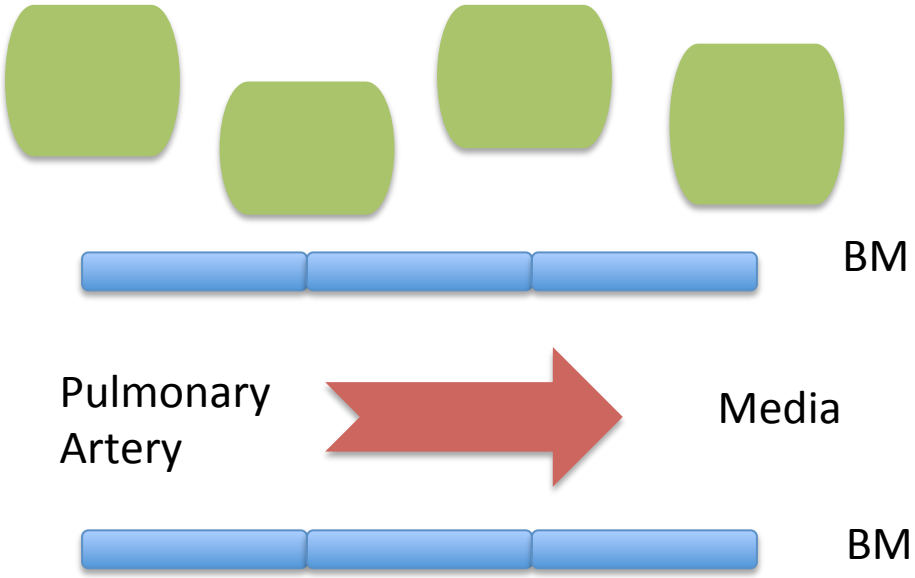
30 min

60 min

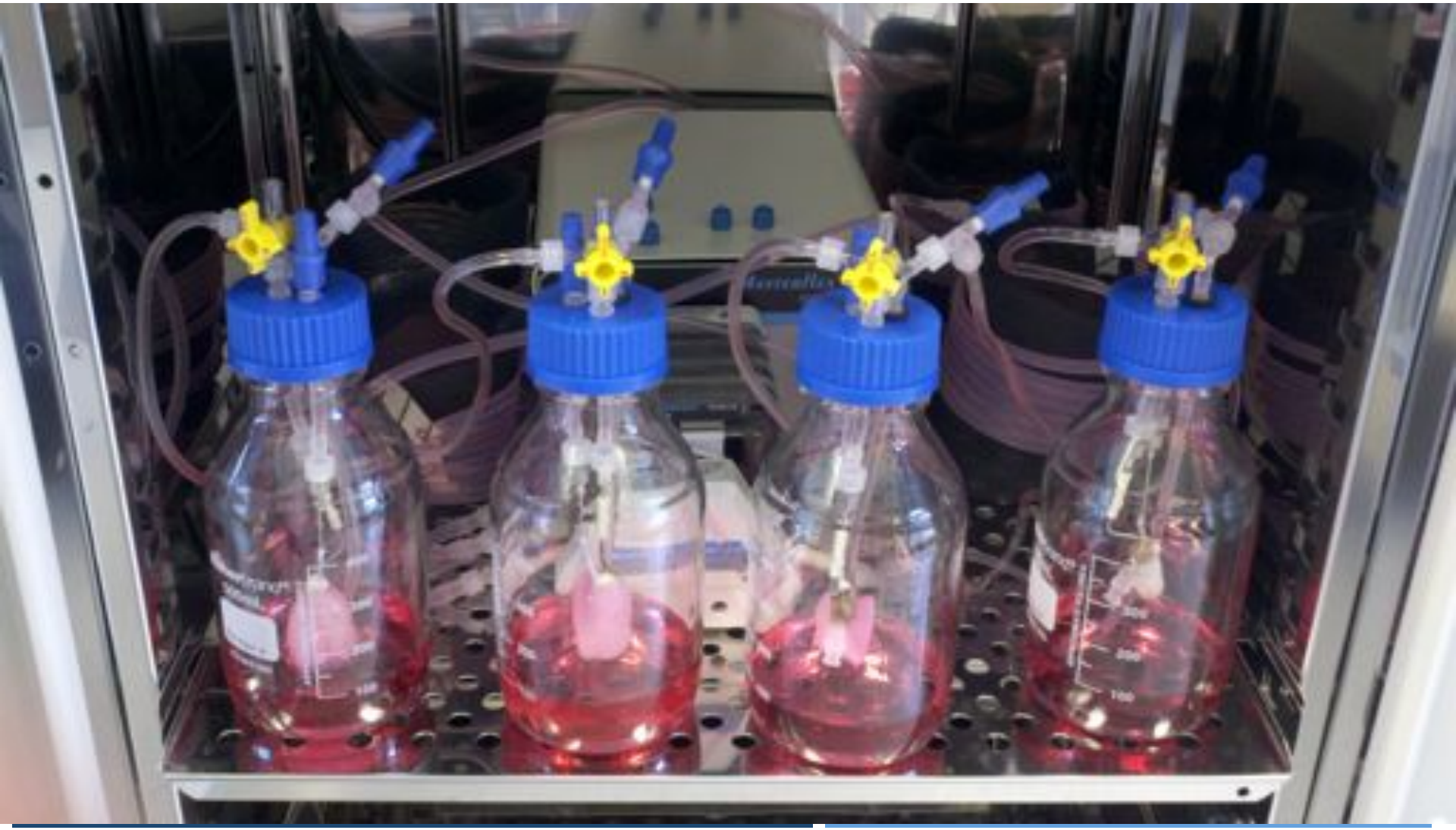
120 min

Recellularization with Tumor Cells

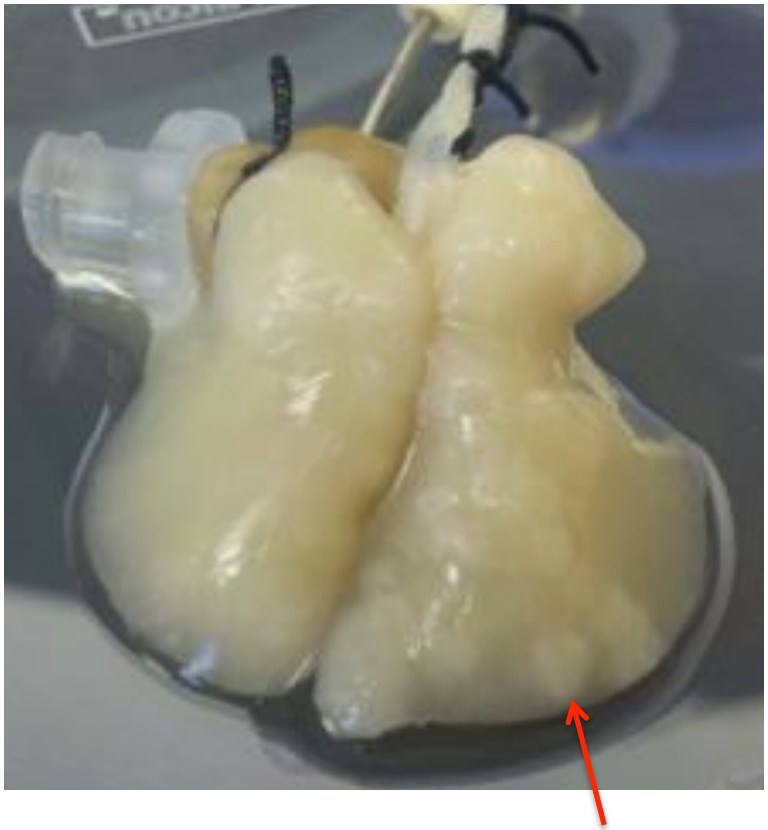
Trachea:
A549
Human lung cancer cells



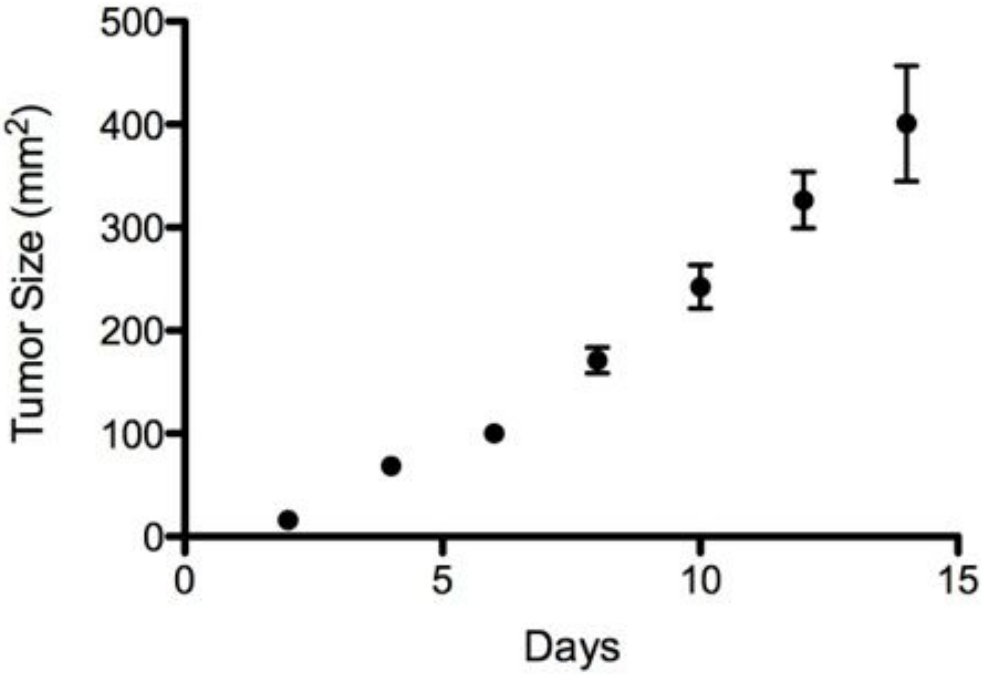
Bioreactor

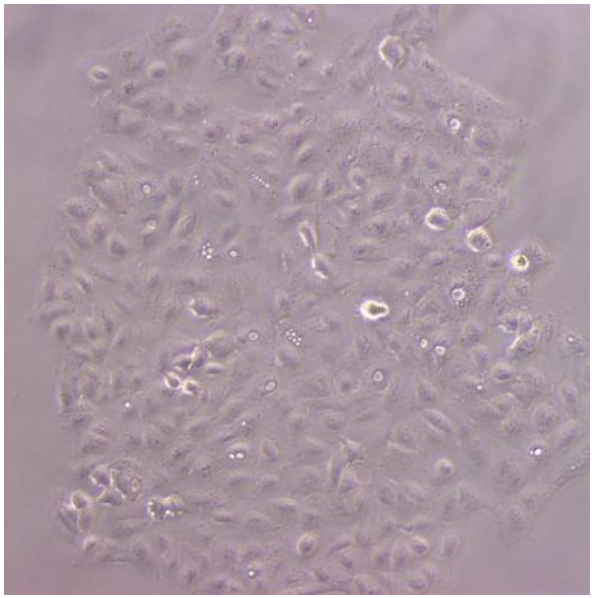


Tumor Nodule Formation and Growth

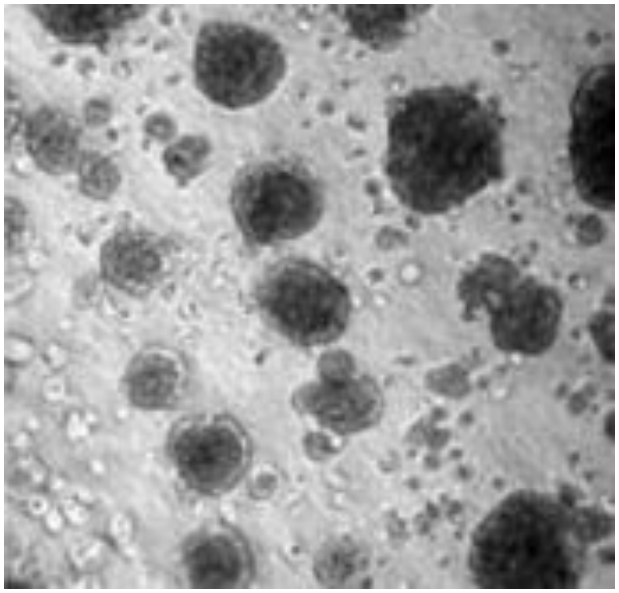


Nodule

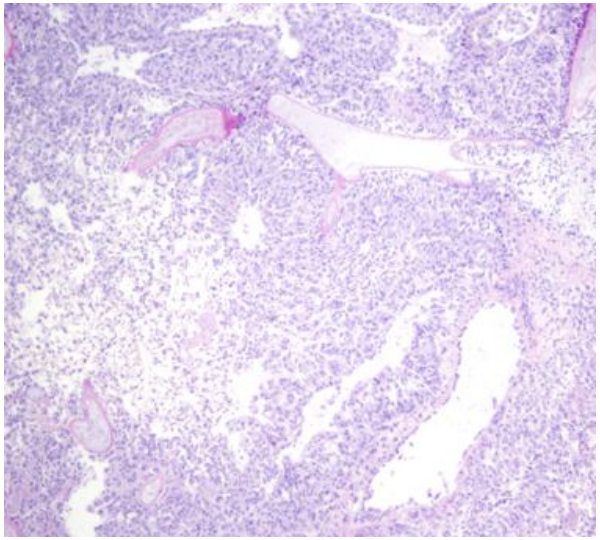


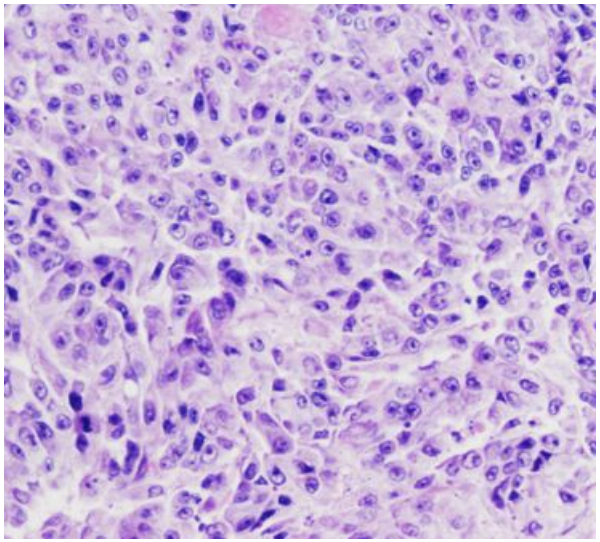


2D



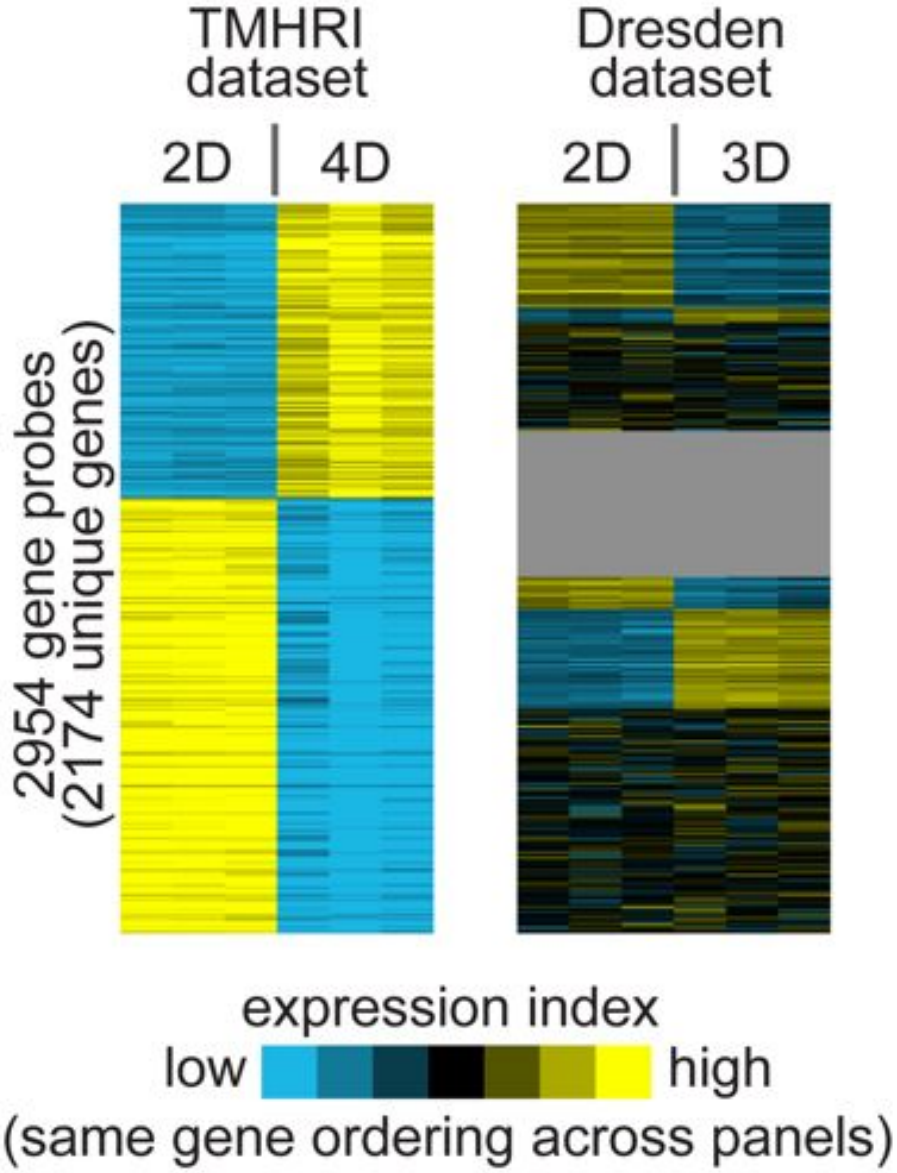
3D*



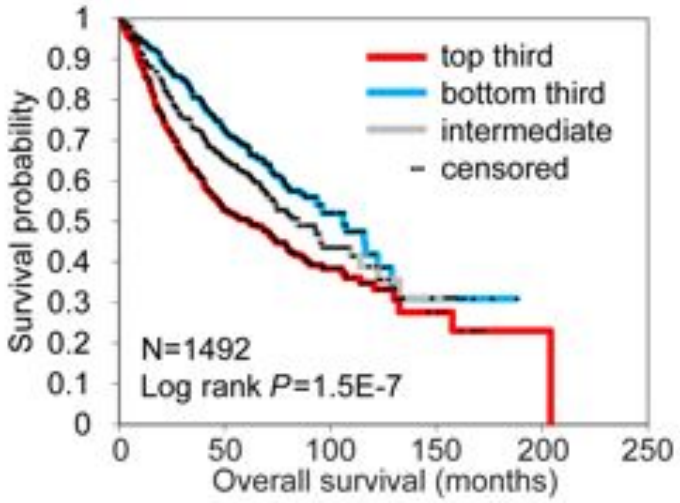


4D

*Zschenker *PLOS ONE* 2012


TMHRI dataset
Dresden dataset
2D
4D
2D
3D
2954 gene probes (2174 unique genes)
expression index
low
high
(same gene ordering across panels)

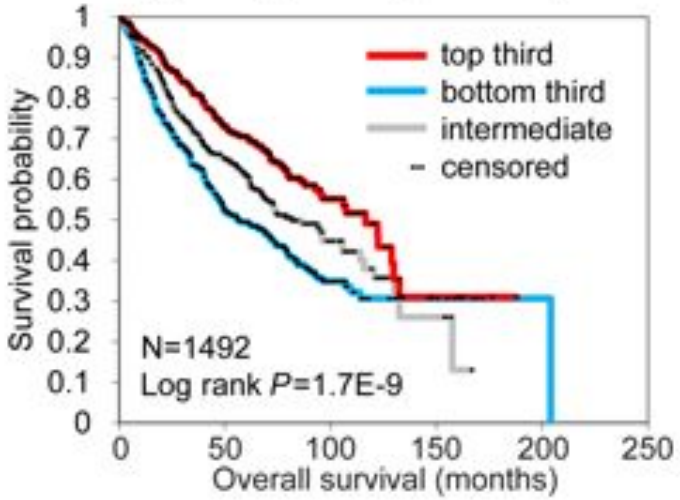
A 4D gene signature (TMHRI)



dataset	N	beta	p-value
Beer	86	pos	0.36
Bhattacharjee	84	pos	0.004
Bild	59	pos	0.13
Botling	106	pos	0.33
Chitale	193	pos	0.0009
Hou	40	pos	0.60
Okayama	204	pos	0.008
Shedden	442	pos	0.05
Tang	133	pos	0.004
Tomida	117	pos	0.001
Zhu	28	neg	0.38
compendium	1492	pos	<0.0001

(Univariate Cox beta "pos" denotes correlation with worse outcome.)

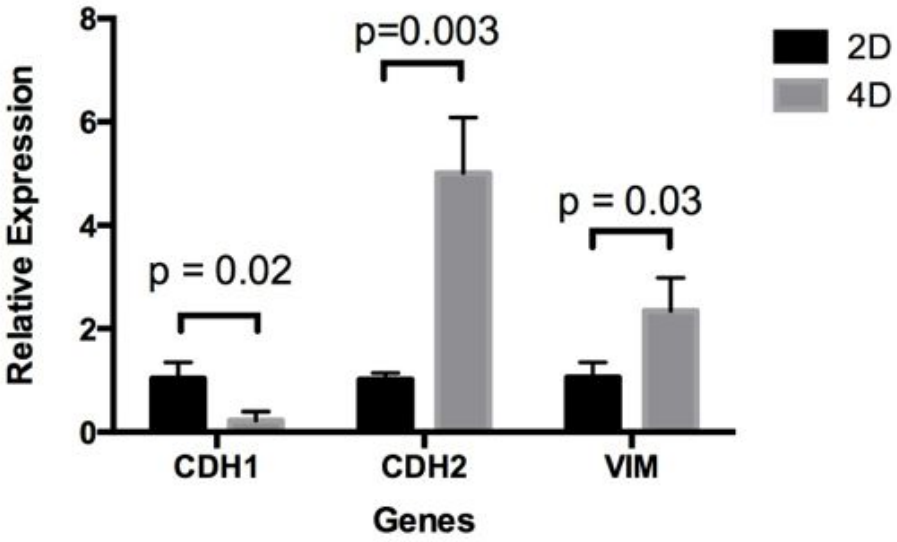
B 3D gene signature (Dresden)



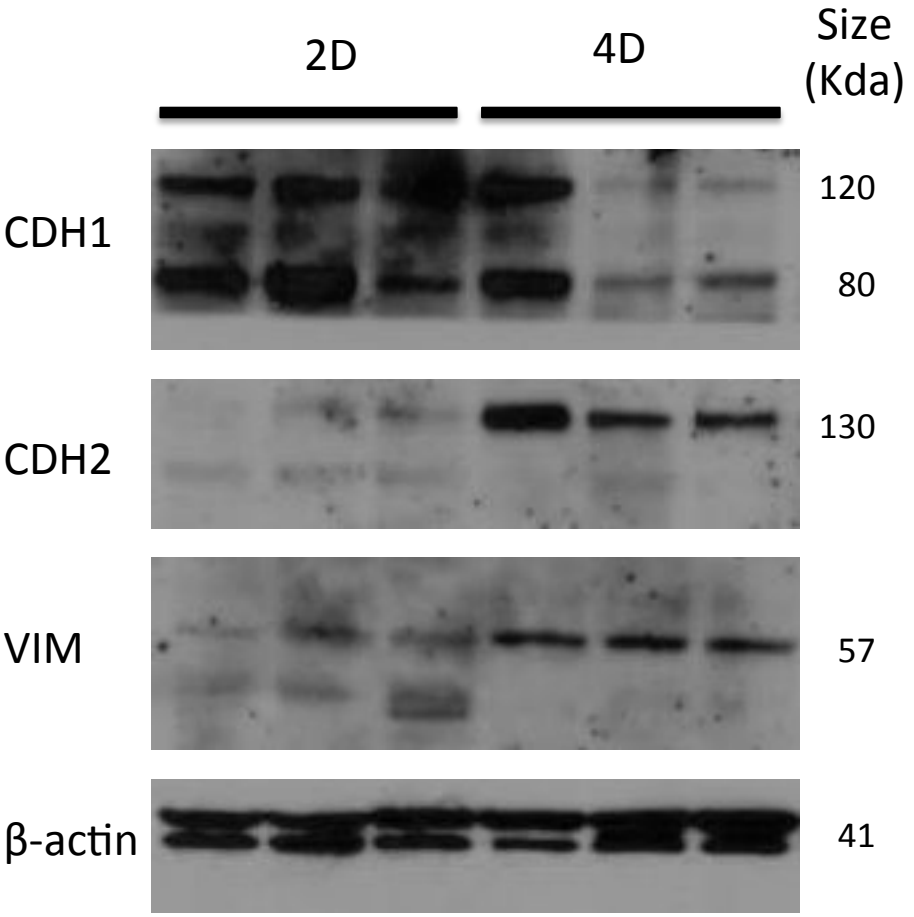
dataset	N	beta	p-value
Beer	86	neg	0.80
Bhattacharjee	84	neg	0.004
Bild	59	neg	0.01
Botling	106	neg	0.96
Chitale	193	neg	0.001
Hou	40	neg	0.19
Okayama	204	neg	0.0005
Shedden	442	neg	0.003
Tang	133	neg	0.07
Tomida	117	neg	0.01
Zhu	28	neg	0.59
compendium	1492	neg	<0.0001

(Univariate Cox beta "neg" denotes correlation with better outcome.)

Mesenchymal Characteristics



RT-PCR



Western

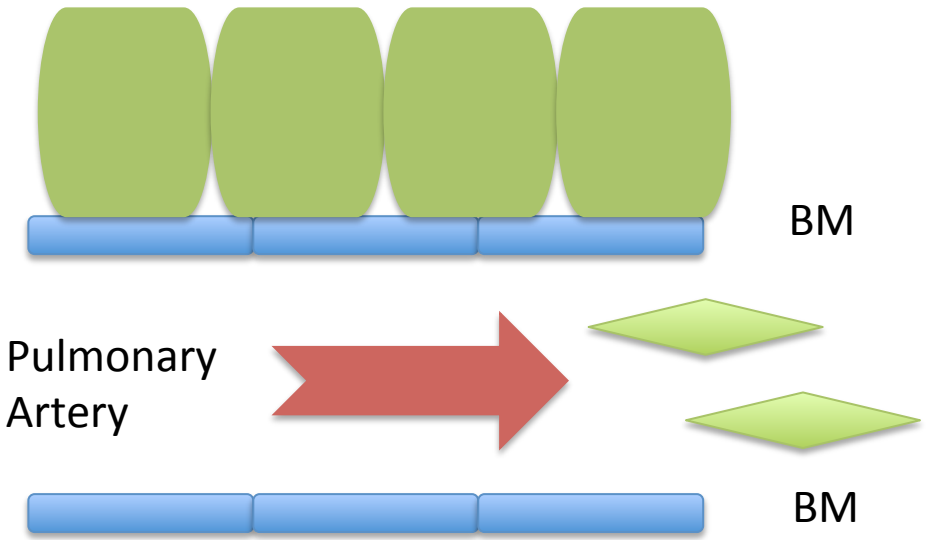
Cell Culture (2D)

Tumor Growth (4D)

Circulating Tumor Cells (CTC)

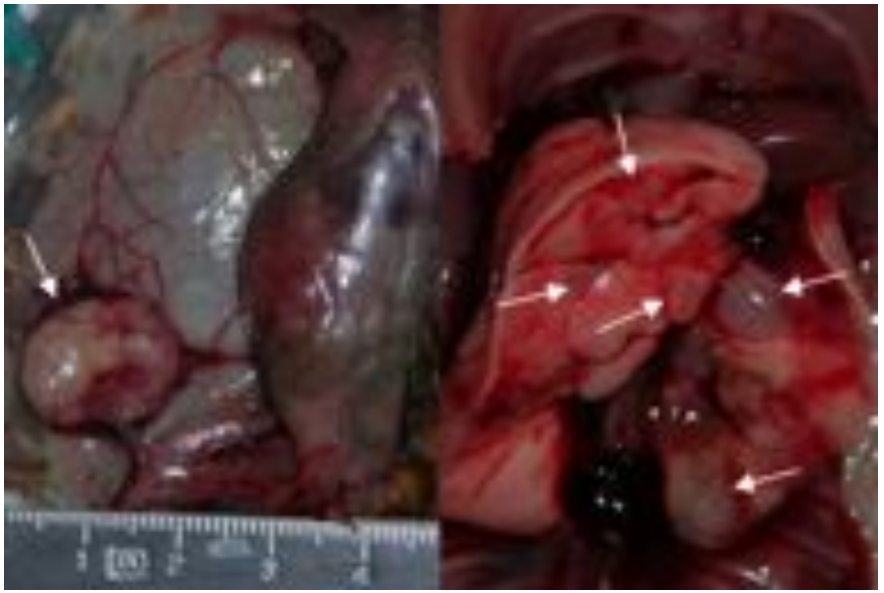
Metastatic Lesion (Met)

Trachea:
Lung Cancer Cells



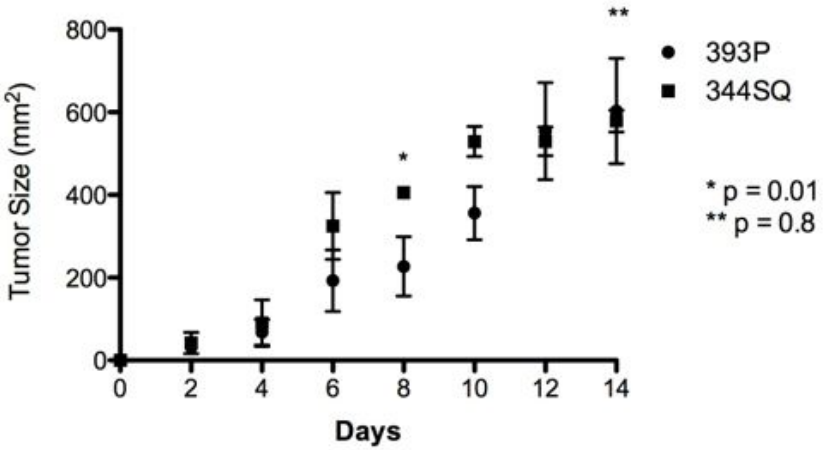
Mouse Lung Cancer Cell Lines

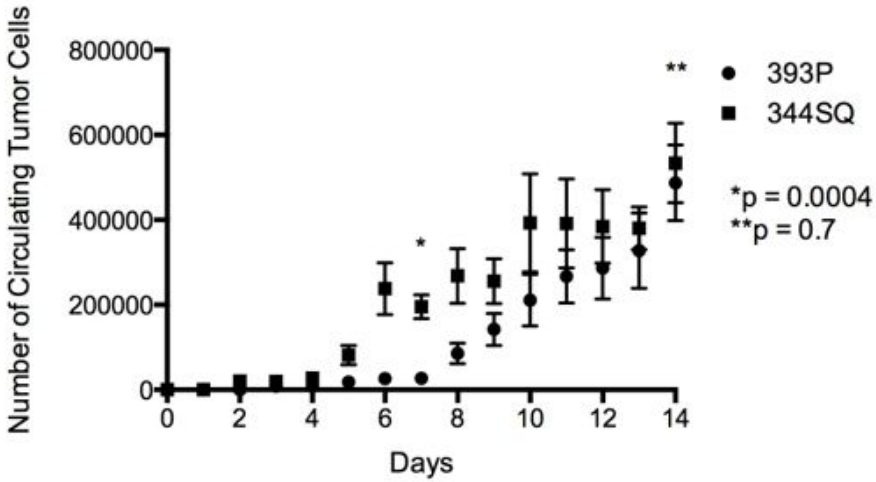
- Lung adenocarcinoma cell lines from $Kras^{LA1/+}$, $p53^{R172H\Delta G}$ Mice
- Inject 1 million cells SQ in mouse and euthanize at 6 weeks
  - 393P
    - Injection site – 7/10
    - Metastasis – 0/10
  - 344SQ
    - Injection site – 13/13
    - Metastasis – 13/13



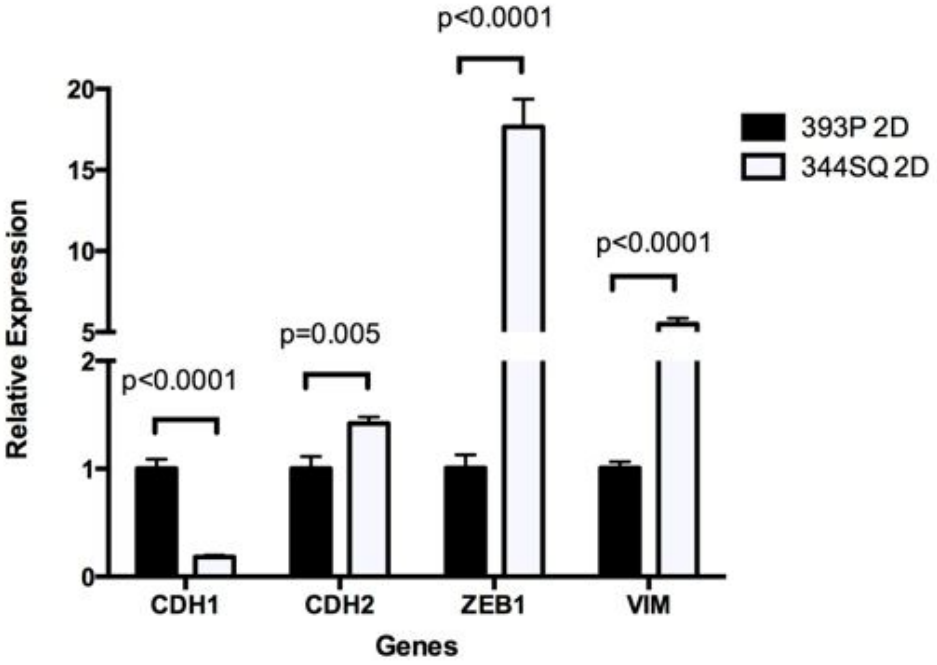
Gibbons *Genes Dev* 2009

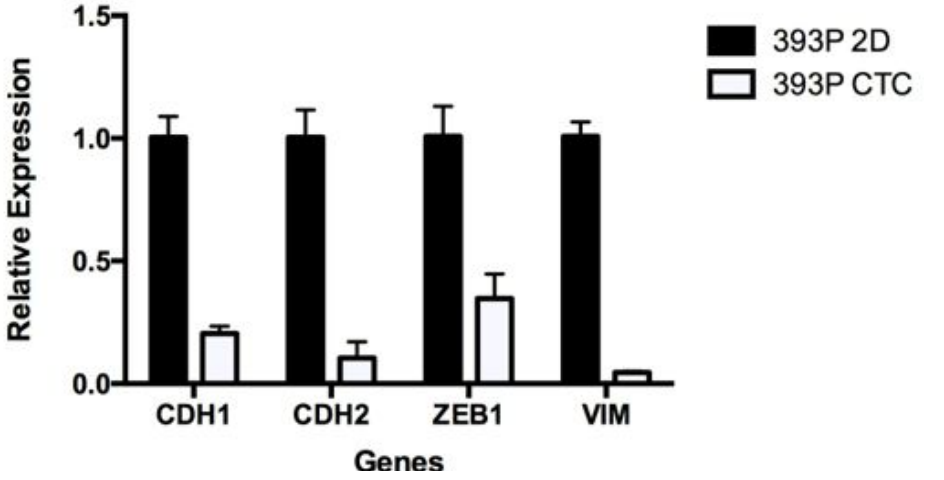
Tumor Growth and CTC in 4D model

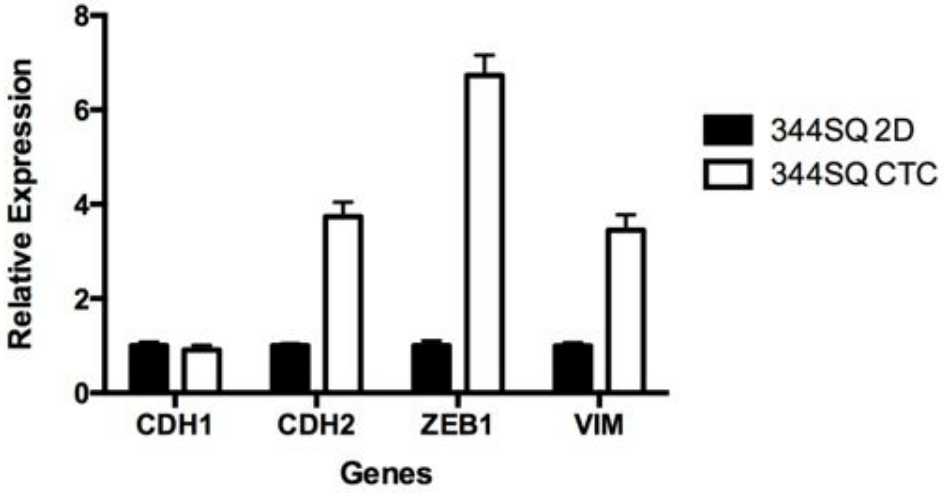




Mesenchymal Characteristics







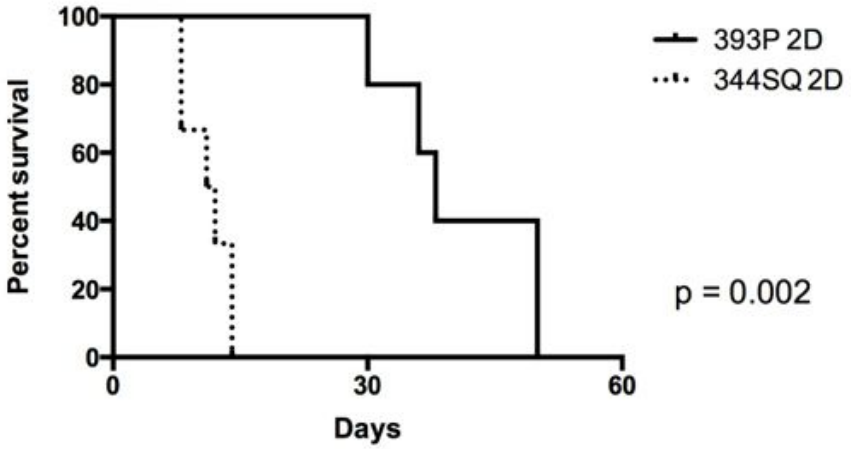
Tail vein injection

*

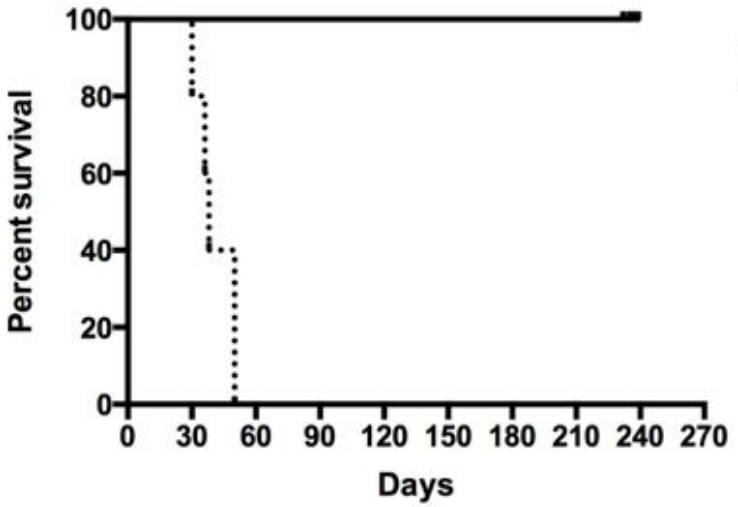
- Isolate 100,000 live cells in 2D vs CTC
- Inject cells
- Measure survival

*Benchmarks.cancer.gov

Mouse Survival



p = 0.002



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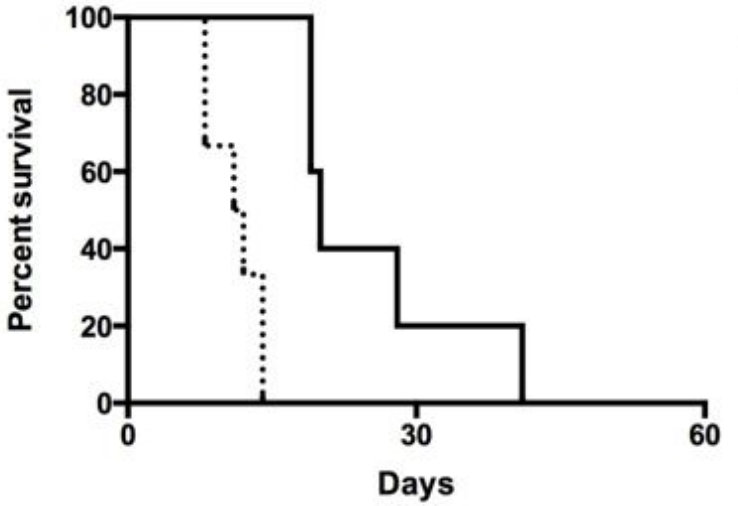


p = 0.002

Cell Culture (2D)

Tumor Growth (4D)

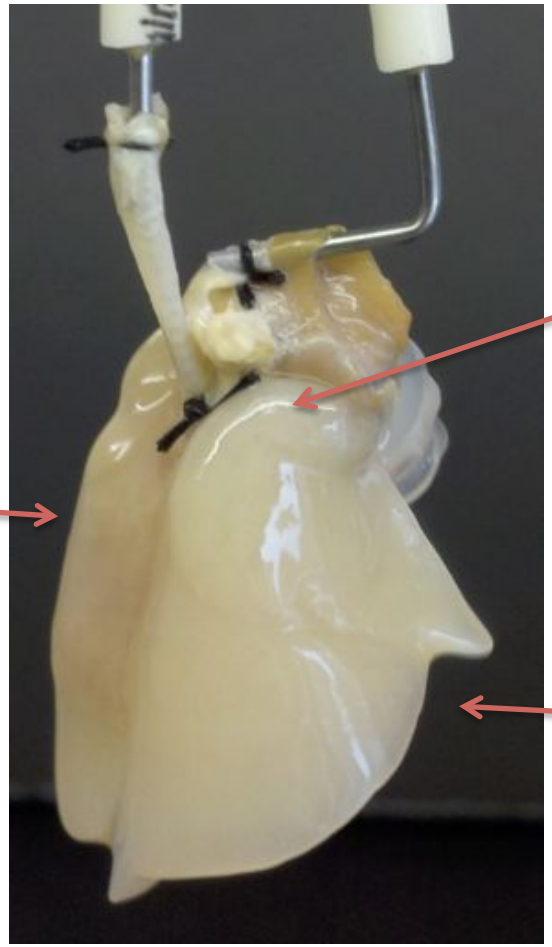
Circulating Tumor Cells (CTC)

Metastatic Lesion (Met)

Human Lung Cancer Cell Line

- NIH-H1299
  - Non small cell lung cancer derived from metastatic lymph node

Metastatic Model

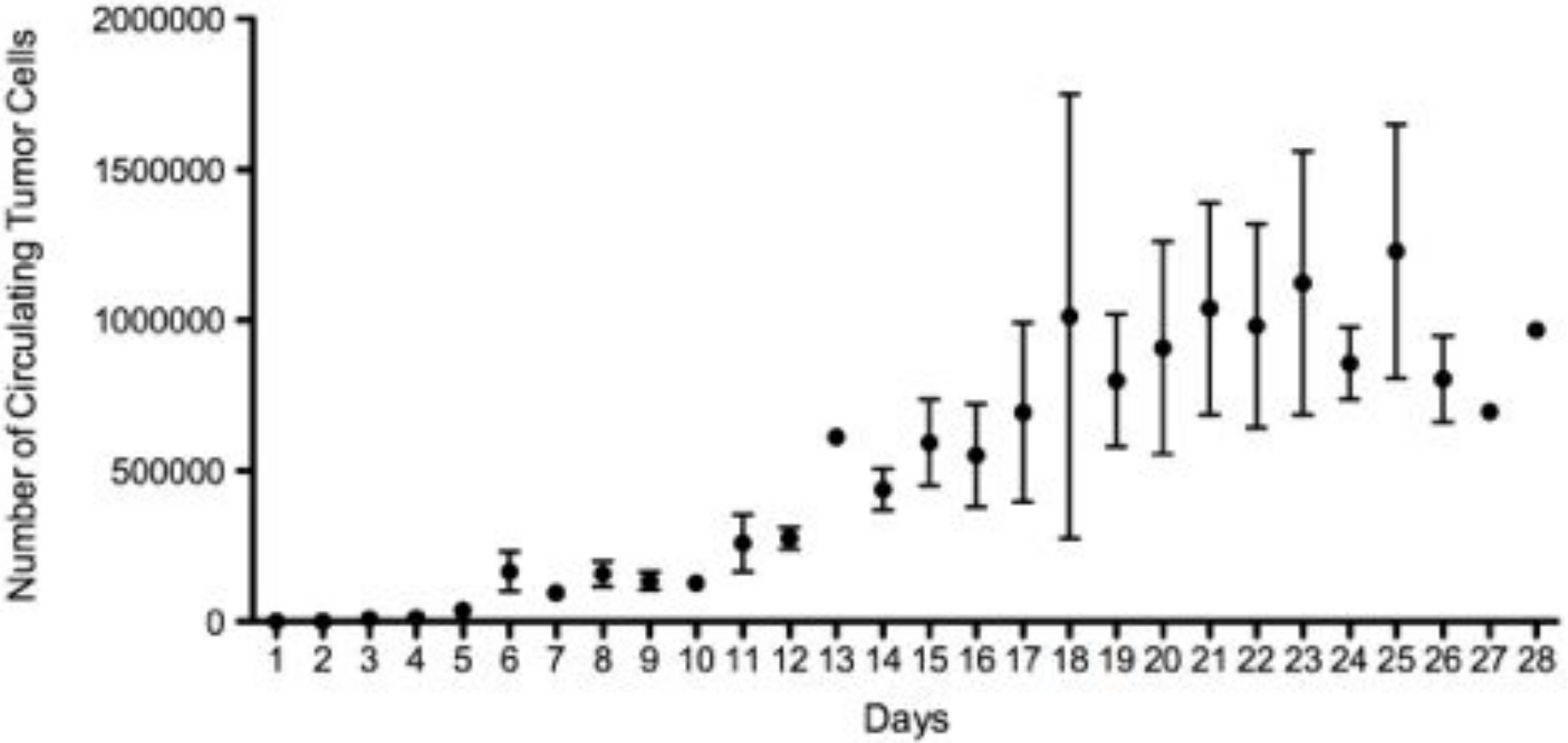


1. Tie off the right main bronchus

2. Seeding of primary tumor Into left lung via trachea

3. Tumor cells in right lung from CTC

Circulating Tumor Cells (CTC)

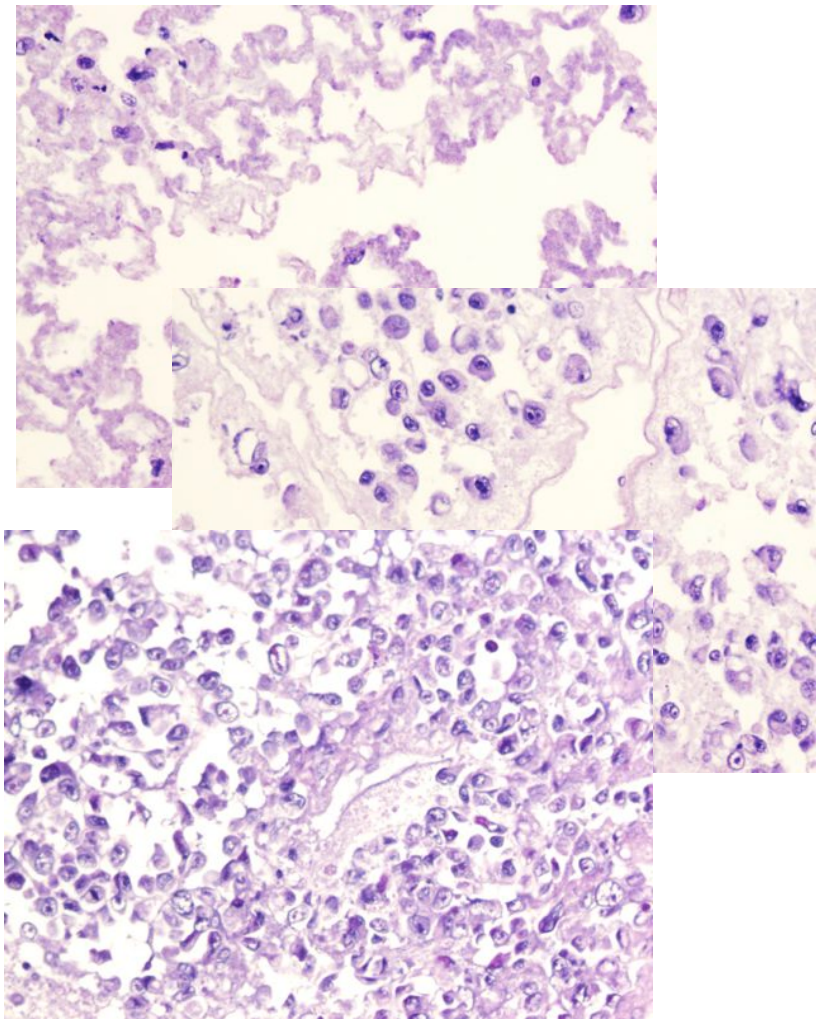


Metastatic Lesion (Met)

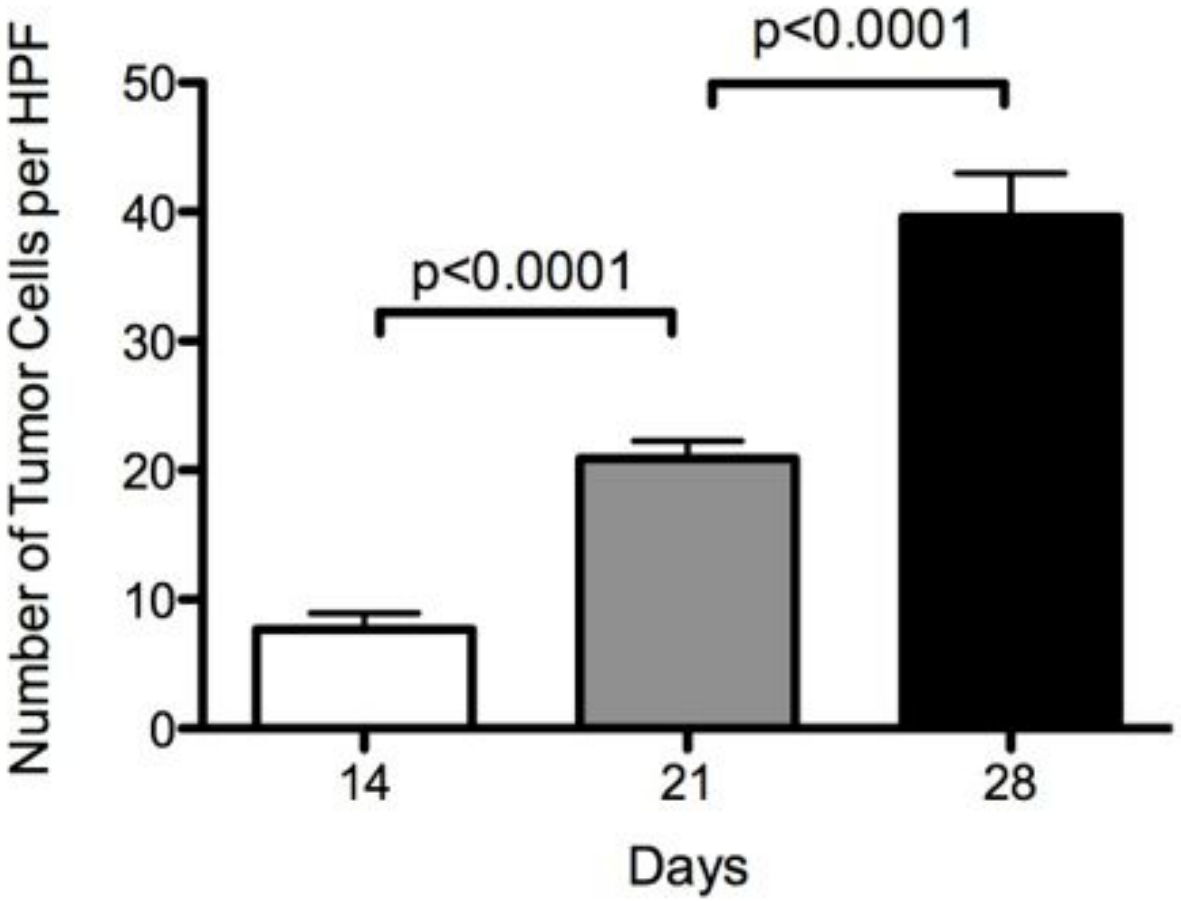
Day 14

Day 21

Day 28



Metastatic Lesion (Met)



Conclusion

- Ex Vivo 4D model may mimic the biology of lung cancer growth and metastasis occurring in patients with lung cancer.
- These models may provide better understanding of biology of tumor spread and potentially provide targets for novel treatment of lung cancer metastasis.