London Health Sciences Centre
London Regional Cancer Program

Schulich
MEDICINE & DENTISTRY

Imaging cells as they metastasize

Ann F. Chambers, PhD

Canada Research Chair in Oncology
Distinguished Oncology Scientist, London Regional Cancer Program
Director, Translational Breast Cancer Research Unit
London Ontario Canada






Ann Chambers **Ian MacDonald** **Alan Tuck** **Alison Allan** **John Lewis** **David Rodenhiser**

Current lab members:

Technicians: David Dales, Carl Postenka, Nicole Hague, Joseph Andrews, Wendy Kennette, Carmen Simedrea

Graduate students: Jason Townson, Jenn Kirstein, Michael Lizardo, Lesley Souter, Laura Caria

Postdoctoral Fellows/Research Associates: Waleed Al-Katib, Pieter Anborgh, Terlika Sharma, Brigitte Goulet, Patricia McGowan

Key Collaborators: Alan Groom *(IVVM)*

Paula Foster, Brian Rutt, Chris Heyn, Patricia Steeg *(brain mets, MRI)*

Jim Lacefield, Aaron Fenster, Lauren Wirtzfeld *(3D Ultrasound)*

Dalit Barkan, Jeff Green *(in vitro models of dormancy)*

Past lab members …… including: G. Naumov, S. Vantyghem, B. Hedley

Funding sources include: ***Canadian Institutes for Health Research***
Ontario Cancer Research Network
Canadian Breast Cancer Research Alliance
US Department of Defense Breast Cancer Program






Ann Chambers **Ian MacDonald** **Alan Tuck** **Alison Allan** **John Lewis** **David Rodenhiser**

Current lab members:

Technicians: David Dales, Carl Postenka, Nicole Hague, Joseph Andrews, Wendy Kennette, Carmen Simedrea

Graduate students: Jason Townson, Jenn Kirstein, Michael Lizardo, Lesley Souter, Laura Caria

Postdoctoral Fellows/Research Associates: Waleed Al-Katib, Pieter Anborgh, Terlika Sharma, Brigitte Goulet, Patricia McGowan

Key Collaborators: Alan Groom *(IVVM)*

Paula Foster, Brian Rutt, Chris Heyn, Patricia Steeg *(brain mets, MRI)*

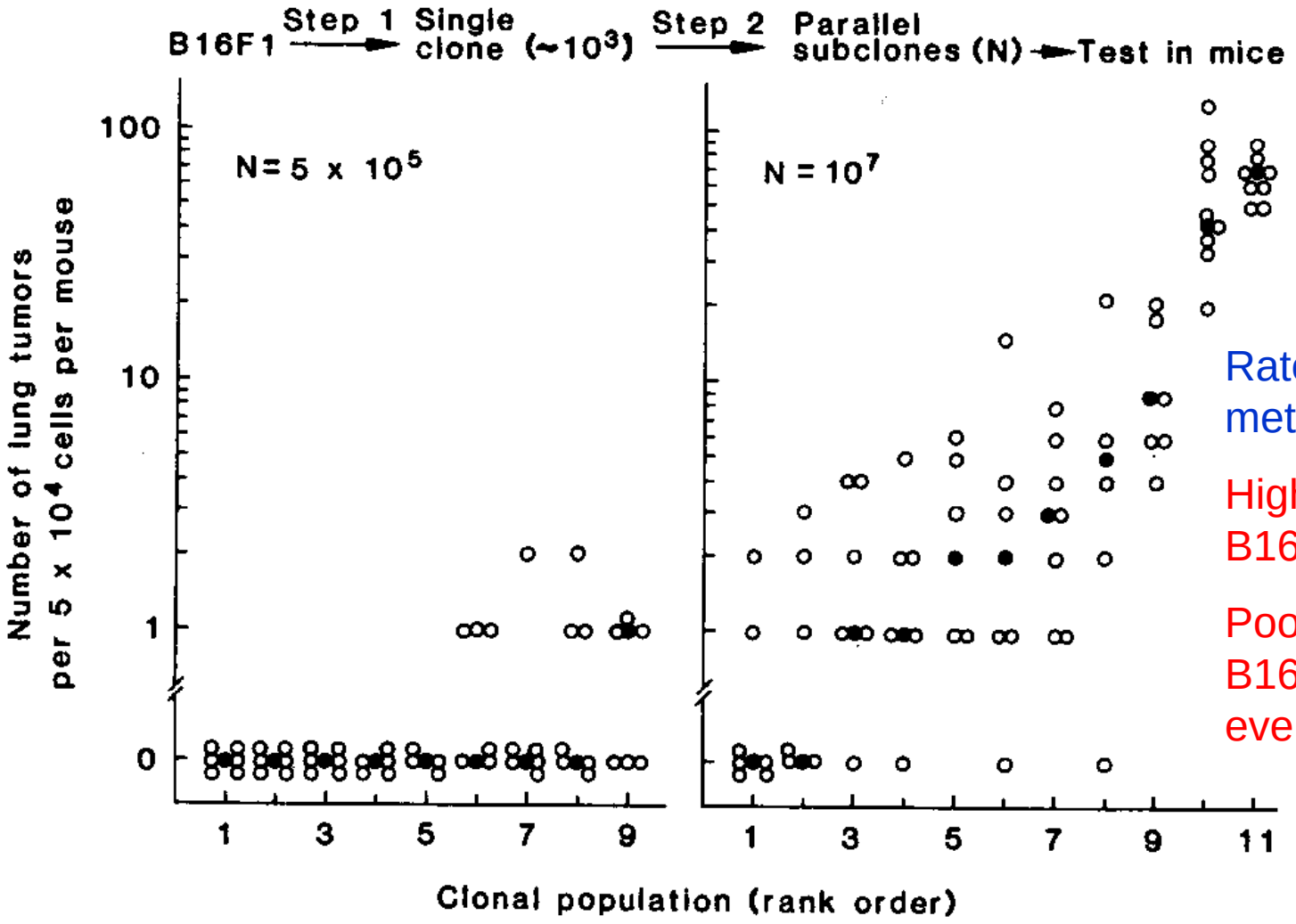
Jim Lacefield, Aaron Fenster, Lauren Wirtzfeld *(3D Ultrasound)*

Dalit Barkan, Jeff Green *(in vitro models of dormancy)*

Past lab members …… including: G. Naumov, S. Vantyghem, B. Hedley

Funding sources include: ***Canadian Institutes for Health Research***
Ontario Cancer Research Network
Canadian Breast Cancer Research Alliance
US Department of Defense Breast Cancer Program

B16F1 clones - grown to small population size have few metastatic clones - grown to large population size have many metastatic clones



Rates of generation of metastatic variants

Highly metastatic B16F10: $\mu = 5 \times 10^{-5}$

Poorly metastatic B16F1: $\mu = 1 \times 10^{-5}$ events/cell/generation

Hill, Chambers, Ling, Harris. Dynamic heterogeneity: Rapid generation of metastatic variants in mouse B16 melanoma cells. Science 224: 998-1001, 1984

The Problems

- Most cancer deaths are due to **METASTASIS**
- Most **DRUGS** ultimately fail in the metastatic setting
- Metastases can occur years after apparently successful primary treatment – **TUMOR DORMANCY**

How does metastasis occur – biologically, molecularly, physically?

Can metastasis be prevented, or treated more effectively?

What is responsible for tumor dormancy (and re-awakening)?

Can release from dormancy be prevented?

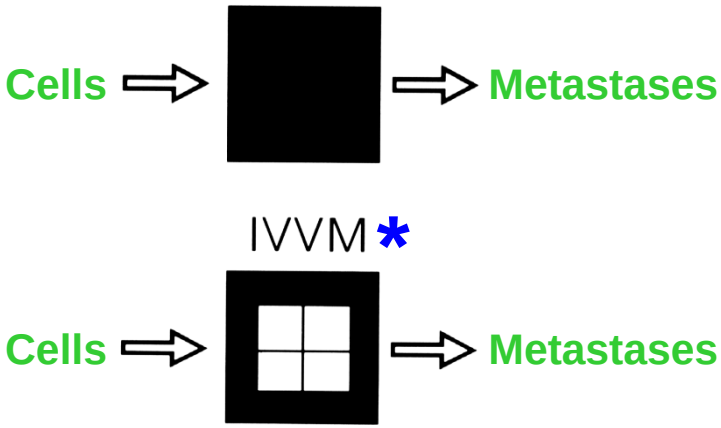
… What is the difference between "cured" and "tumor dormancy"??

>>New ways to study the metastatic process and tumor dormancy are needed

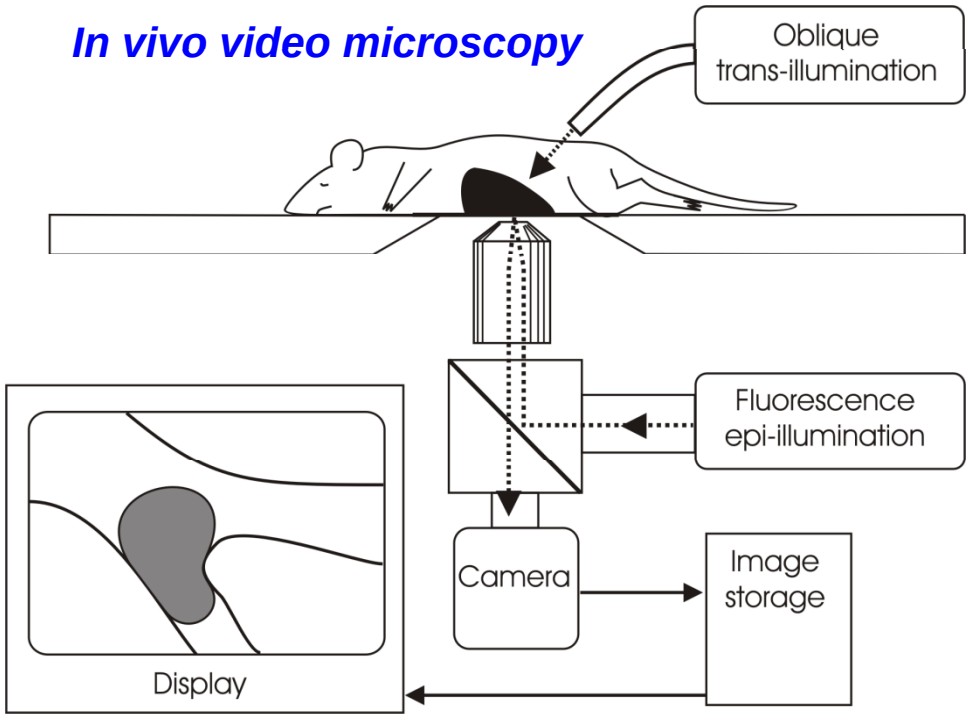
ULTIMATELY NEED TO TRANSLATE THIS INFORMATION TO BENEFIT PATIENTS

Imaging the Metastatic Process:

IVVM puts a “window” in metastasis assays



In vivo video microscopy



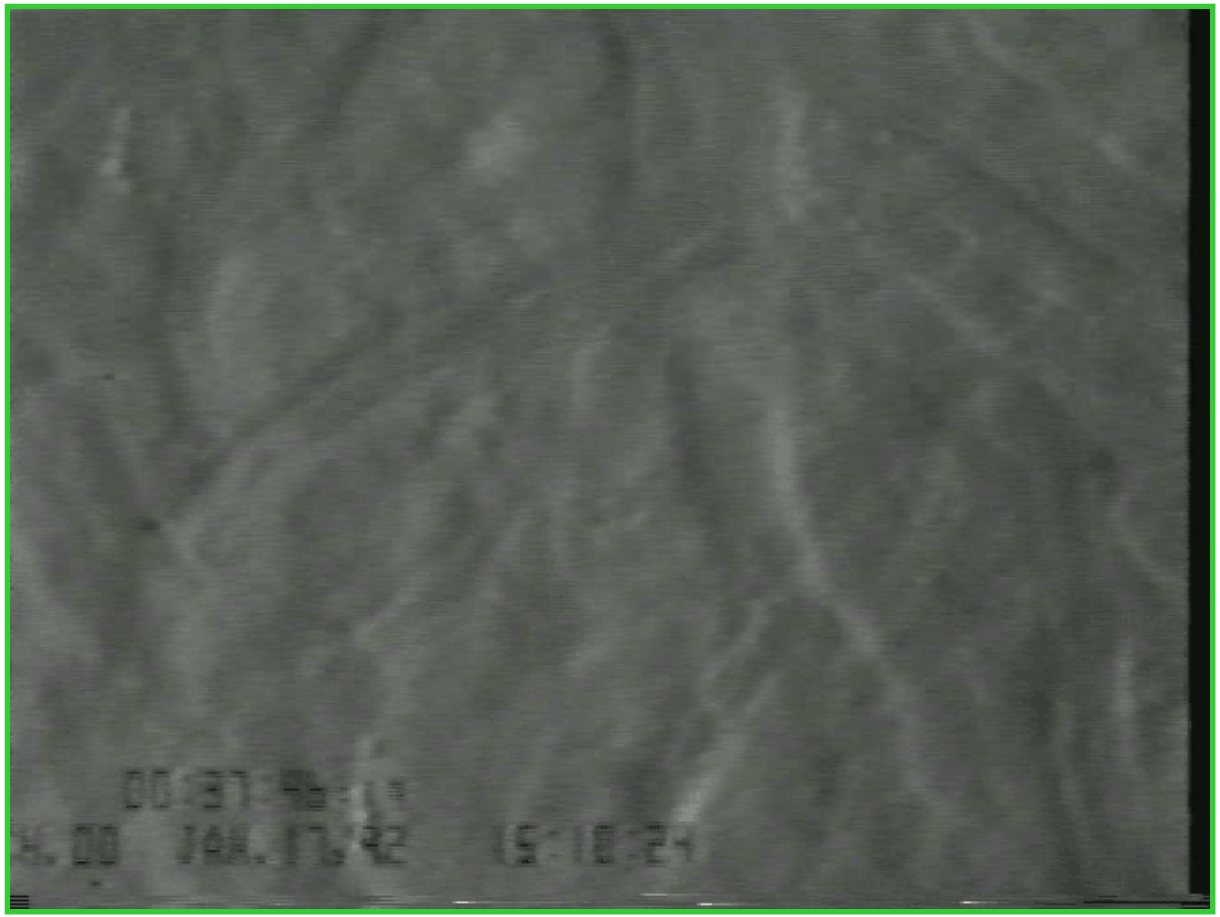
**** In vivo video microscopy***

…or other non-invasive imaging modalities – High Frequency Ultrasound, microCT, Magnetic Resonance Imaging

MacDonald et al., BioEssays 24: 885-893, 2002

Breast cancer cell arrested in liver sinusoid immediately after mesenteric vein i.v. injection

Most circulating cancer cells arrested in 1st capillary bed encountered – most do not circulate freely



Fluorescently labeled mammary carcinoma cell

Implications for the biology of organ-specific metastasis?

IVVM: high-resolution & kinetics

Extravasated melanoma cell wrapping pseudopodial projections around arteriole in chick CAM

Calcein-AM fluorescent labeling: added to cells before injection ('exogenous')

20μm

Video by Sahadia Koop

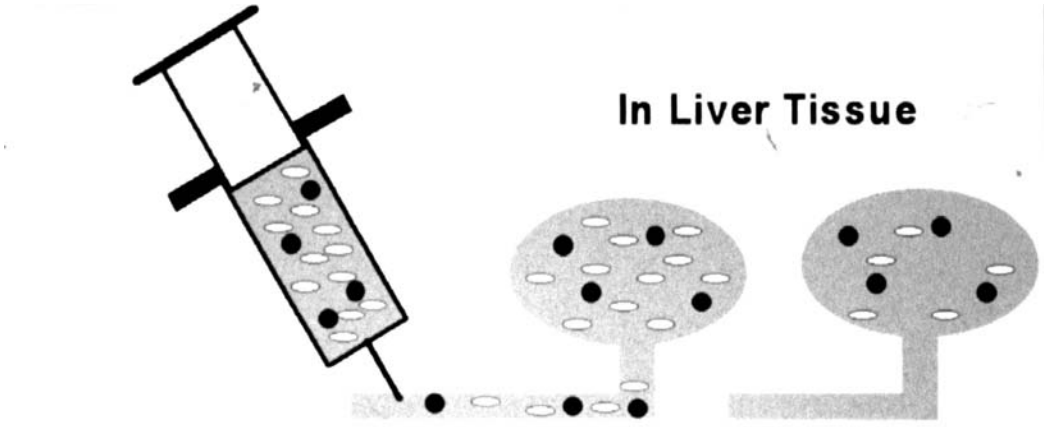
IVVM: 3D structural information

Melanoma micrometastasis growing as perivascular collar around pre-existing vessel

3-day melanoma micrometastasis in chick CAM

Endogenous label: melanin

Cell accounting: 10 μm microspheres to quantify cell survival and metastatic inefficiency



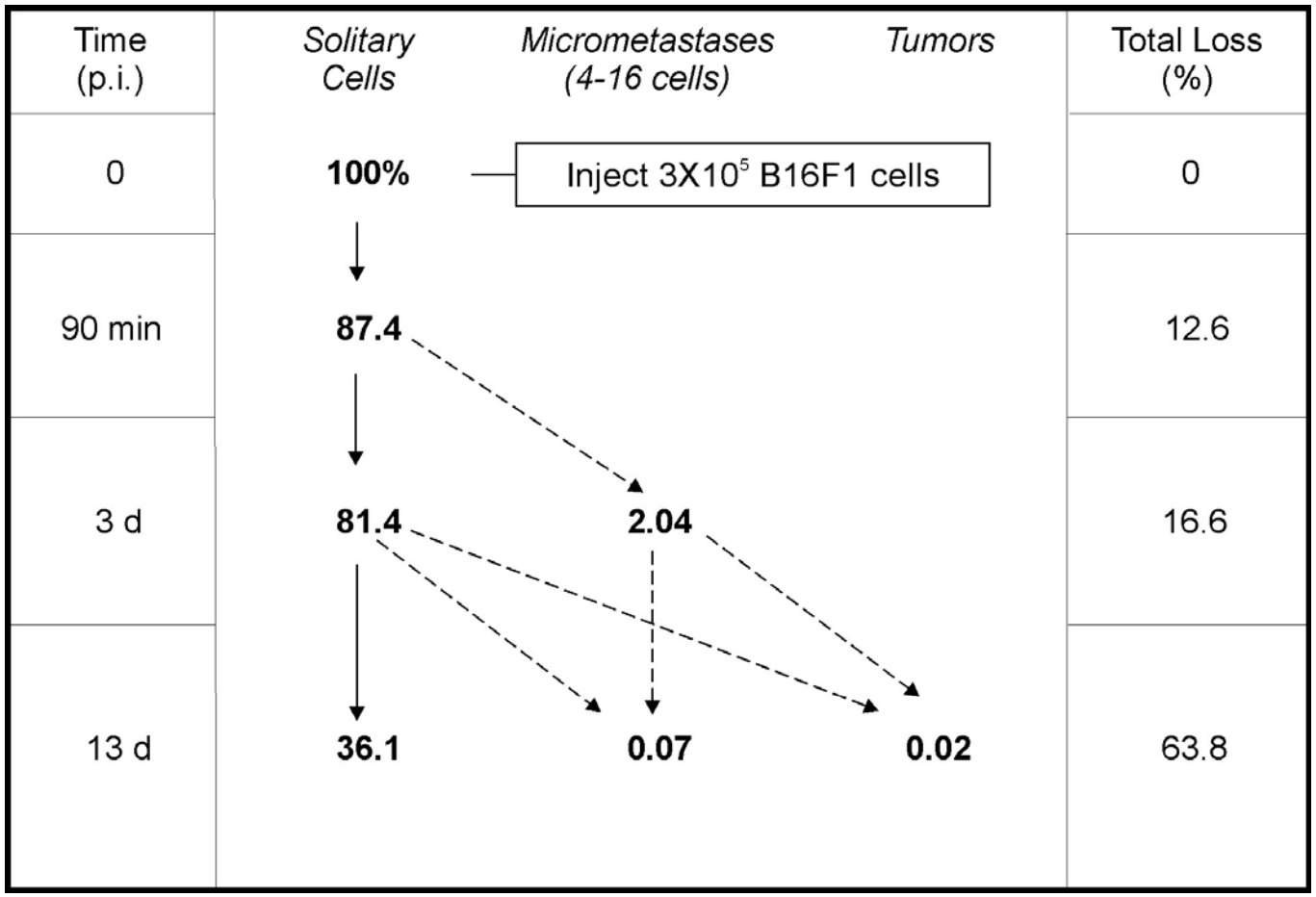
	Before Injection	1 Hour	14 Days
# cells	10	8	5
# microspheres	1	1	1
Cell survival	**100%**	**80%**	**50%**

Need to know both numerator *AND* denominator:

Cells still present / Cells that originally arrived in the organ

Chambers et al., Breast Cancer Res 2: 400-407, 2000

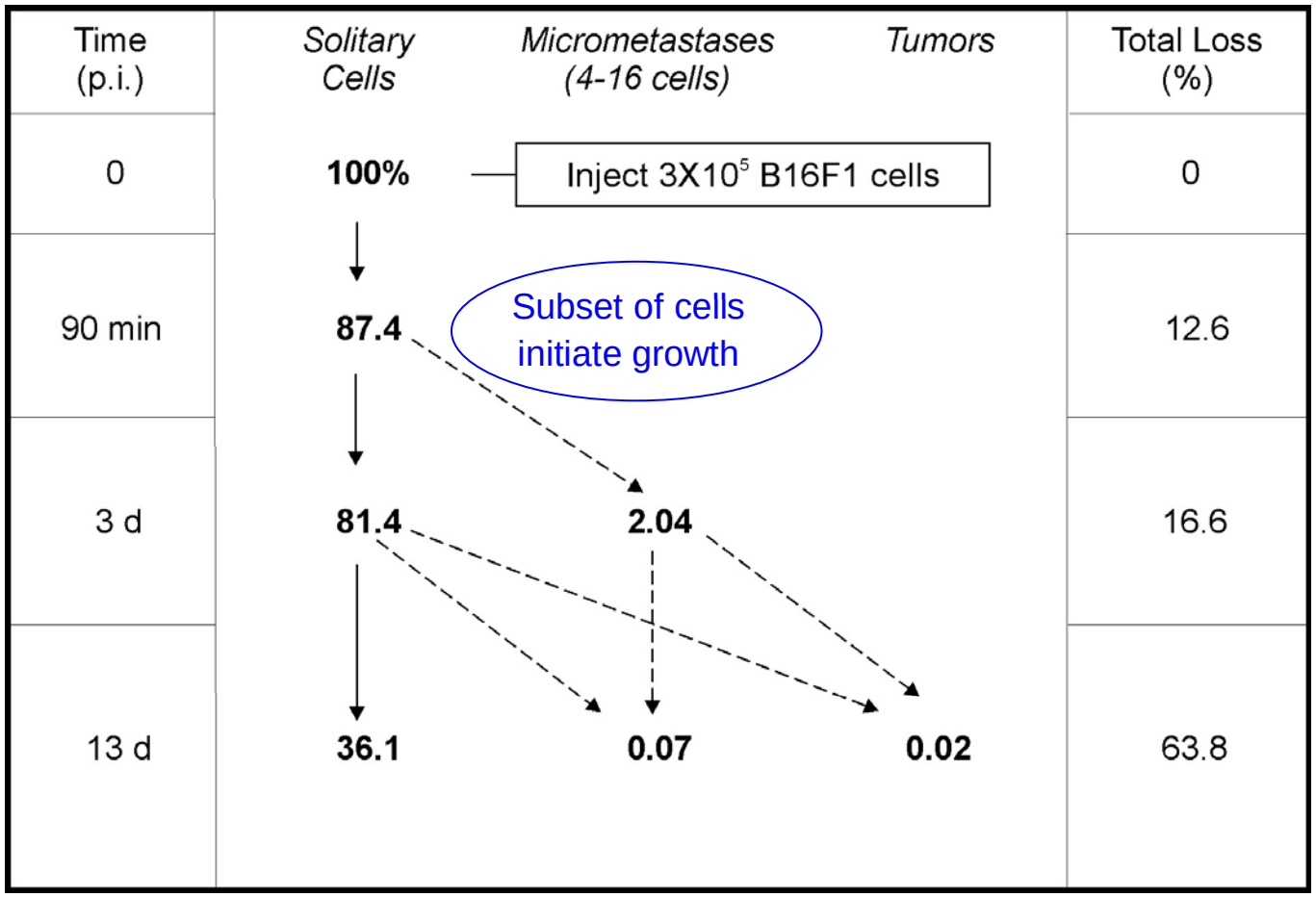
Metastases form from small subset of cells delivered to secondary site



Luzzi et al, Am J Pathol 1998, 153:865-873

also ***Cameron et al, Cancer Res 2000, 60:2541-2546***

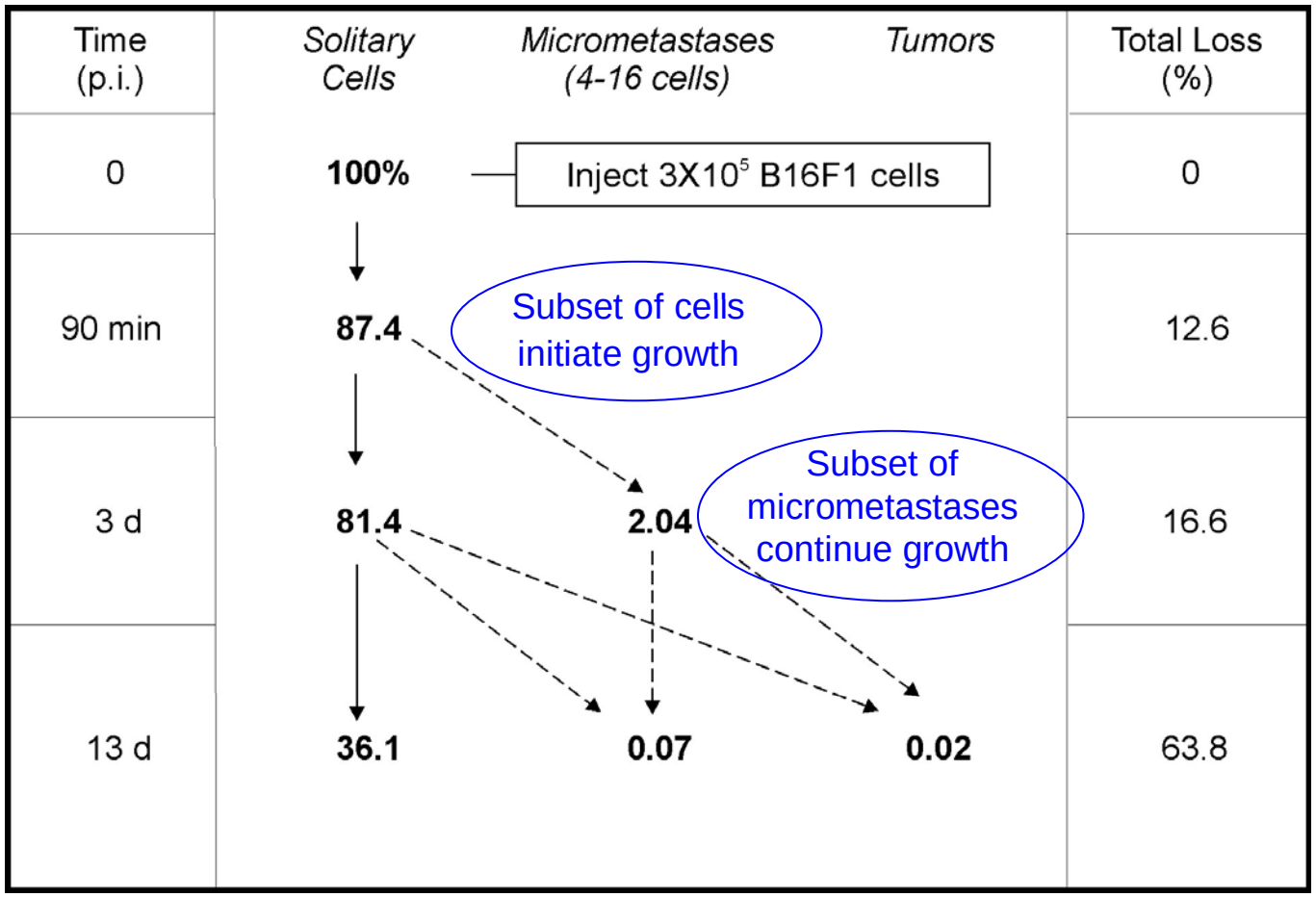
Metastases form from small subset of cells delivered to secondary site



Luzzi et al, Am J Pathol 1998, 153:865-873

also ***Cameron et al, Cancer Res 2000, 60:2541-2546***

Metastases form from small subset of cells delivered to secondary site

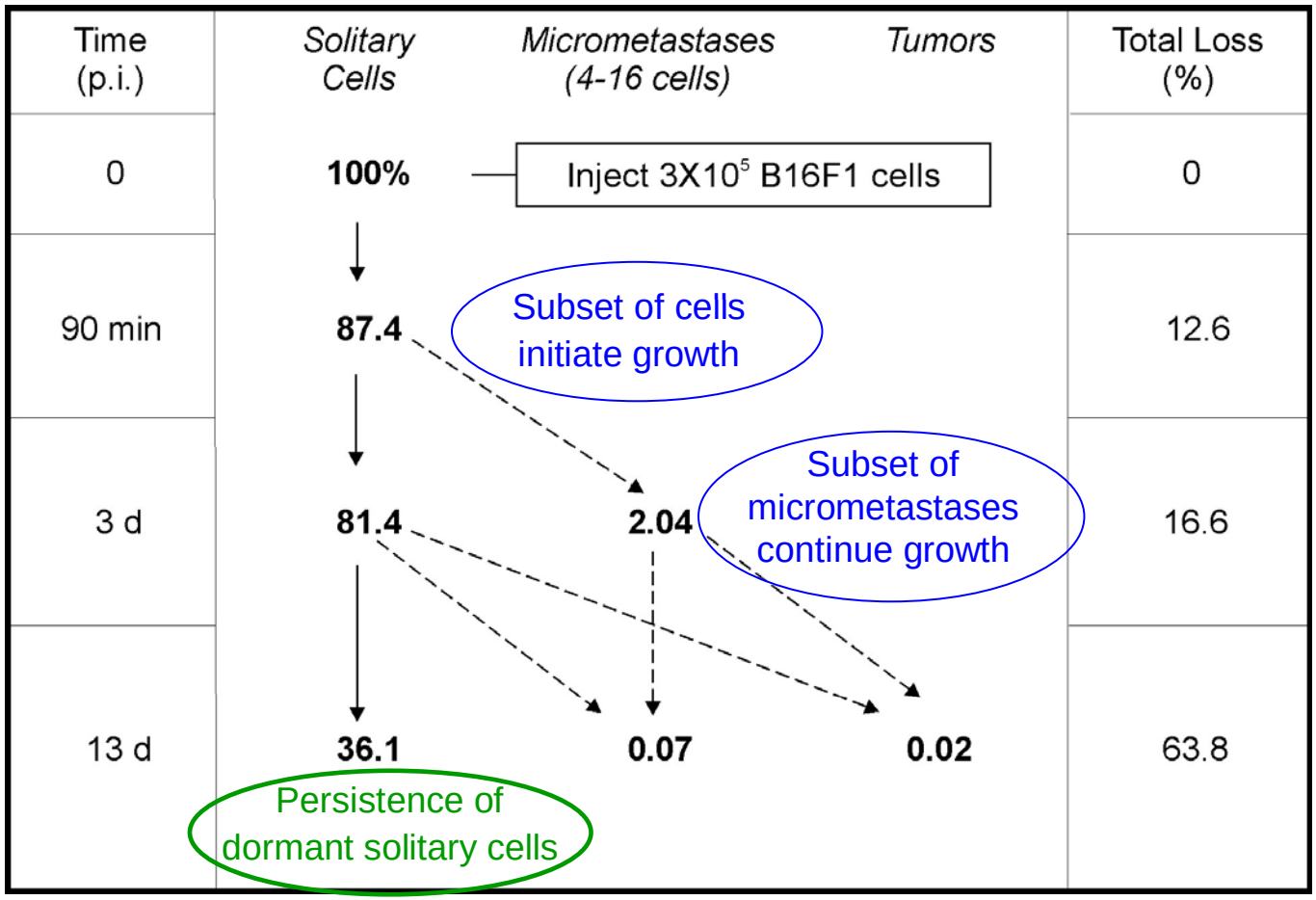


Luzzi et al, Am J Pathol 1998, 153:865-873

also ***Cameron et al, Cancer Res 2000, 60:2541-2546***

Metastases form from small subset of cells delivered to secondary site

Large population of potentially dormant cells identified



Luzzi et al, Am J Pathol 1998, 153:865-873

also ***Cameron et al, Cancer Res 2000, 60:2541-2546***

How do highly and poorly metastatic populations differ?

- B16F1 / liver
  Luzzi, Am J Path, 1998
- B16F10 / lung
  Cameron, Cancer Res, 2000
- NIH3T3 +/- ras / liver
  Varghese, Cancer Res,2002
- D2A1, D2.OR / liver
  Naumov, Cancer Res, 2002
- MDA-MB-231 vs. 231BR / brain
  Heyn, Mag Res Med, 2006 & unpublished

<u>Constant</u>

High initial arrest & survival in 1st capillary bed: >85%

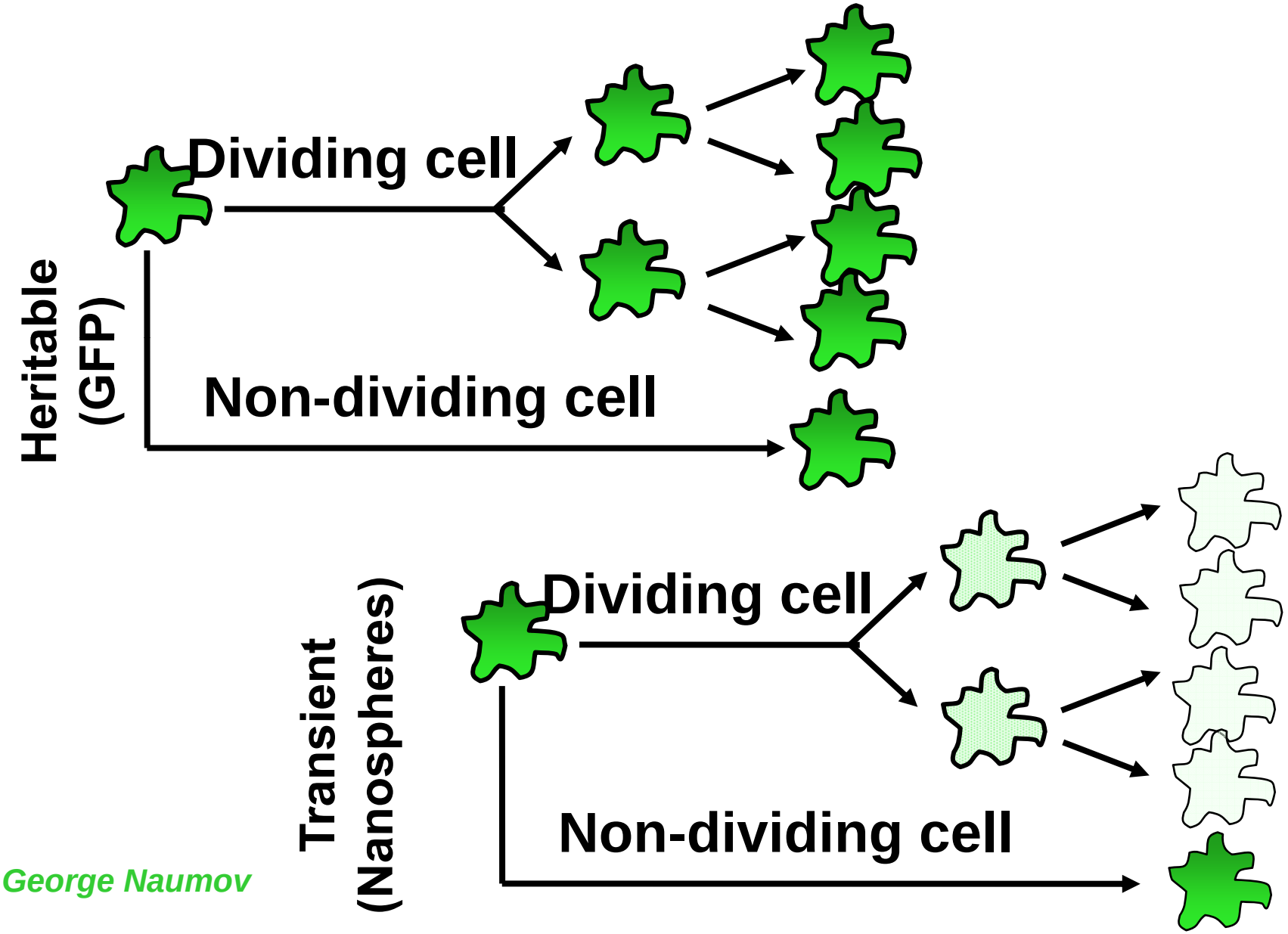
<u>Variable</u>

% of cells that:

- initiate growth
- persist in growth
- remain dormant
- *cancer stem cell %?*
  (Alison Allan – J Cell Mol Med, 2008)

Subset of cells responsible for metastasis

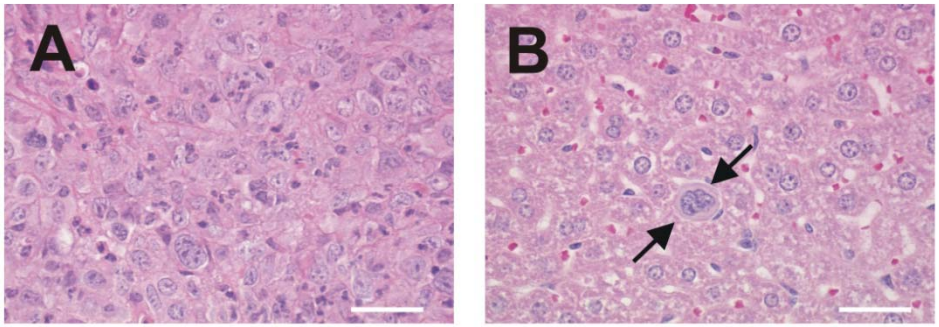
Heritable vs. transient cell labeling



George Naumov

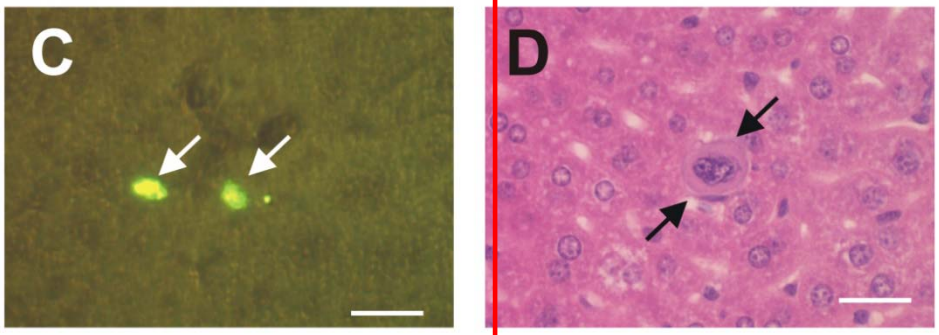
Large numbers of dormant mammary carcinoma cells persist in secondary sites

D2.OR mfp tumor, H&E



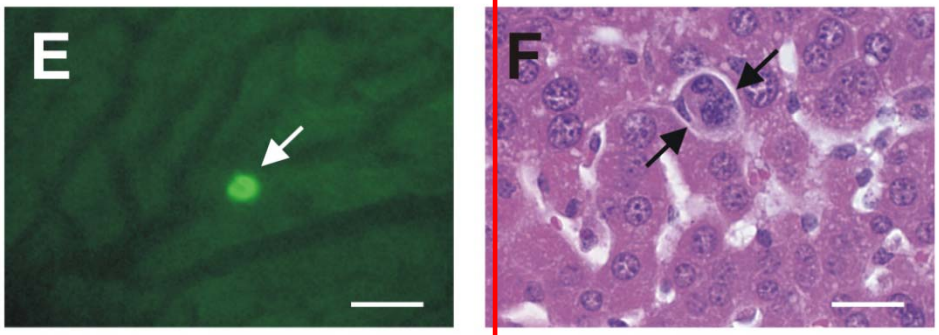
Liver, 25 d, solitary D2.OR cell, from mfp, H&E

Liver, 11 wk iv, solitary D2.OR cells, thick tissue section



Liver, 11 wk iv solitary D2.OR cell, H&E

Liver, 21 d iv, solitary D2A1 cell, IVVM



Liver, 21 d iv, solitary D2A1 cell, H&E

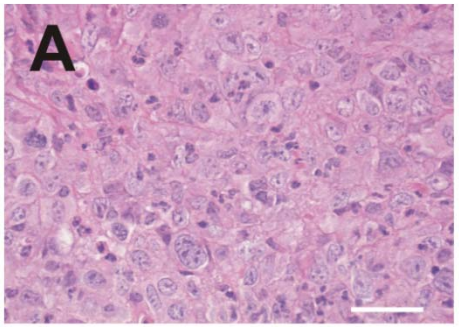
Dilutable label: fluorescent nanospheres (dilute with division)

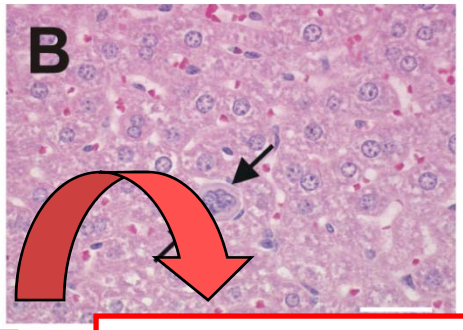
Naumov et al., Cancer Res. 62: 2162-2168, 2002

C:/george/manuscripts/d2paper/newfigures/fig1.cdr

Large numbers of dormant mammary carcinoma cells persist in secondary sites

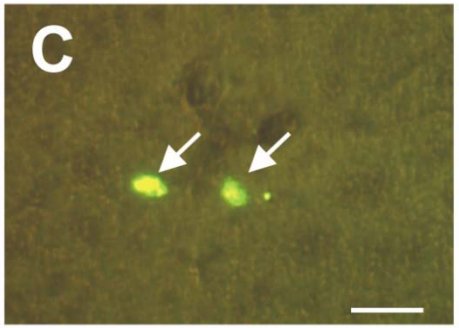
D2.OR mfp tumor, H&E





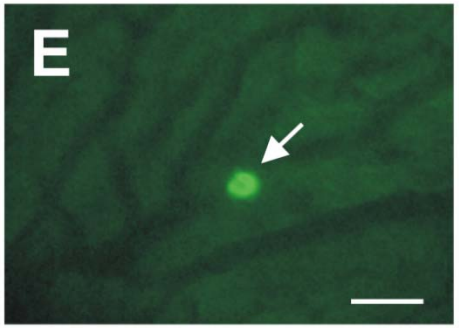
Liver, 25 d, solitary D2.OR cell, from mfp, H&E

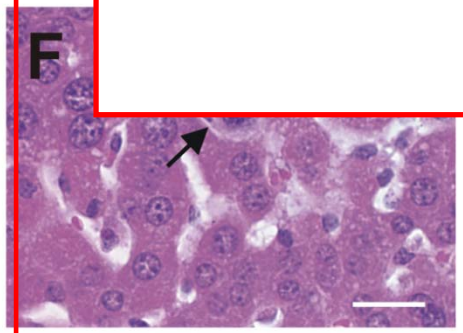
Liver, 11 wk iv, solitary D2.OR cells, thick tissue section



Viable cancer cells can be recovered from these livers

Liver, 21 d iv, solitary D2A1 cell, IVVM





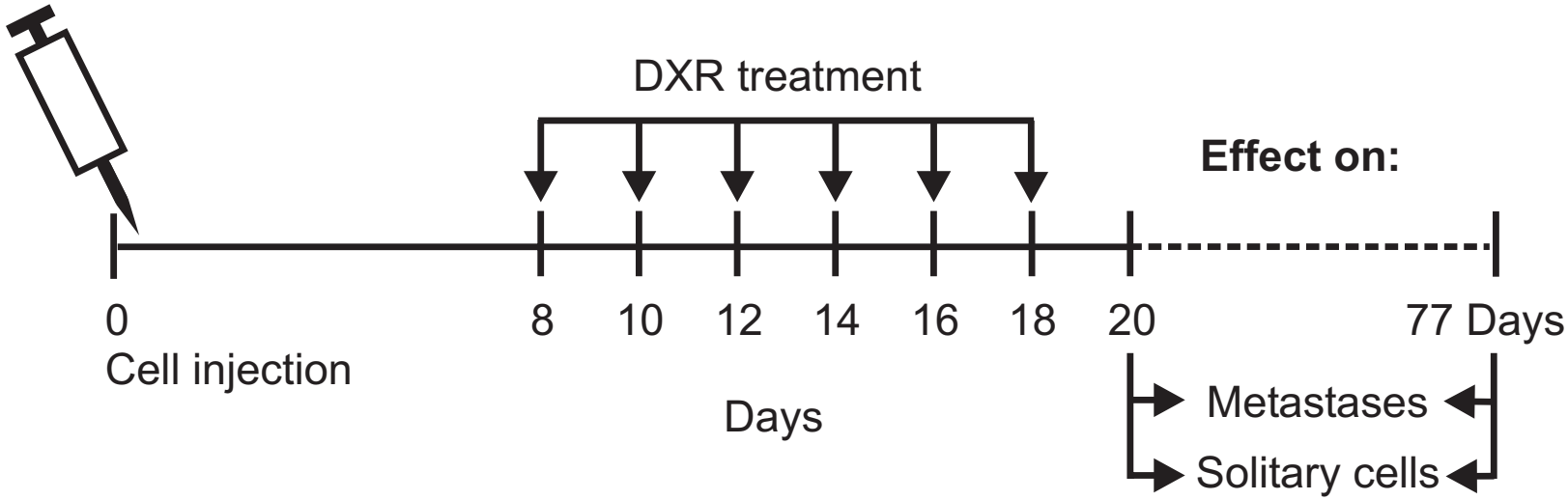
solitary D2A1 cell, H&E

Dilutable label: fluorescent nanospheres (dilute with division)

Naumov et al., Cancer Res. 62: 2162-2168, 2002

C:/george/manuscripts/d2paper/newfigures/fig1.cdr

Does cytotoxic chemotherapy affect numbers of dormant solitary cells?



Mammary carcinoma cells:

D2A1 metastatic

D2.0R poorly metastatic

DXR (doxorubicin) treatment (1 mg/kg), I.p.

PBS control treatment

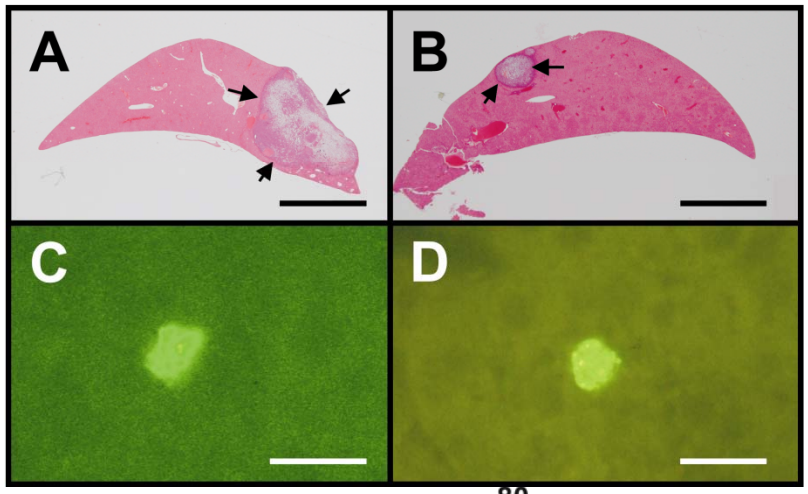
Injected iv (mesenteric vein) to target liver

Naumov et al. Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late-developing metastases. Breast Cancer Res Treat 82: 199-206, 2003

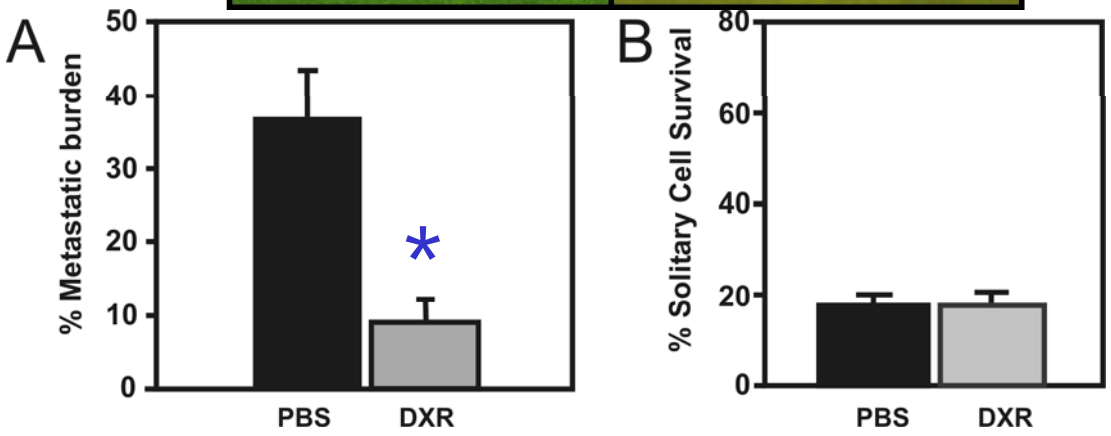
Cytotoxic chemotherapy inhibited metastatic growth but did *not* reduce numbers of solitary dormant cells

No treatment

Doxorubicin treatment



D2A1 mammary cancer cells in mouse liver

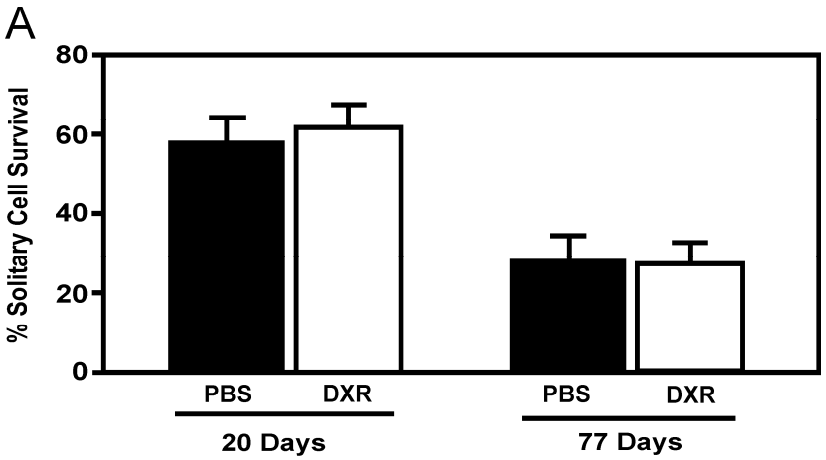


DXR ***inhibited*** D2A1 liver metastatic burden at 20 days

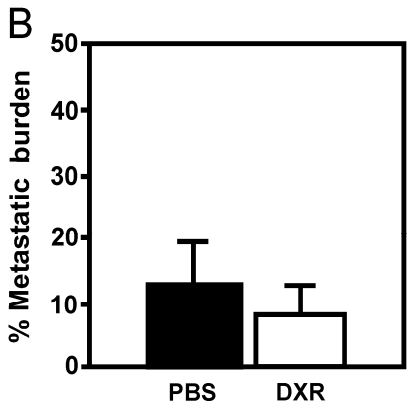
DXR had ***no effect*** on numbers of dormant solitary cells in liver

Naumov et al., Breast Cancer Res Treat 82:199-206, 2003

Cytotoxic chemotherapy did *not* affect numbers of dormant solitary cells or their late-developing metastases



DXR had *no effect* on numbers of dormant solitary D2.0R cells in liver at 20 or 77 days



DXR had *no effect* on D2.0R metastatic burden at 77 days

Naumov et al. Breast Cancer Res Treat 82:199-206,2003

Use of Magnetic Resonance Imaging to follow fate of breast cancer cells metastasizing to brain

- **MDA-MB-231BR cells form brain metastases after intracardiac injection in mice**

  Yoneda et al., J Bone Miner Res 16; 1486-1495, 2001

- **MDA-MB-231BR/EGFP cells – green fluorescent protein**

  Pat Steeg, Diane Palmieri, Julie Bronder

- **MDA-MB-231BR/EGFP cells labeled in vitro with MPIO**

  Paula Foster, Brian Rutt, Chris Heyn, Ann Chambers

  Micron-sized Iron Oxide Particles – taken up by cells in culture – detectable as signal voids in MRI

  Retained by cells until diluted by cell division

Funded by DoD - Patricia Steeg's Center of Excellence on Brain Metastasis of Breast Cancer

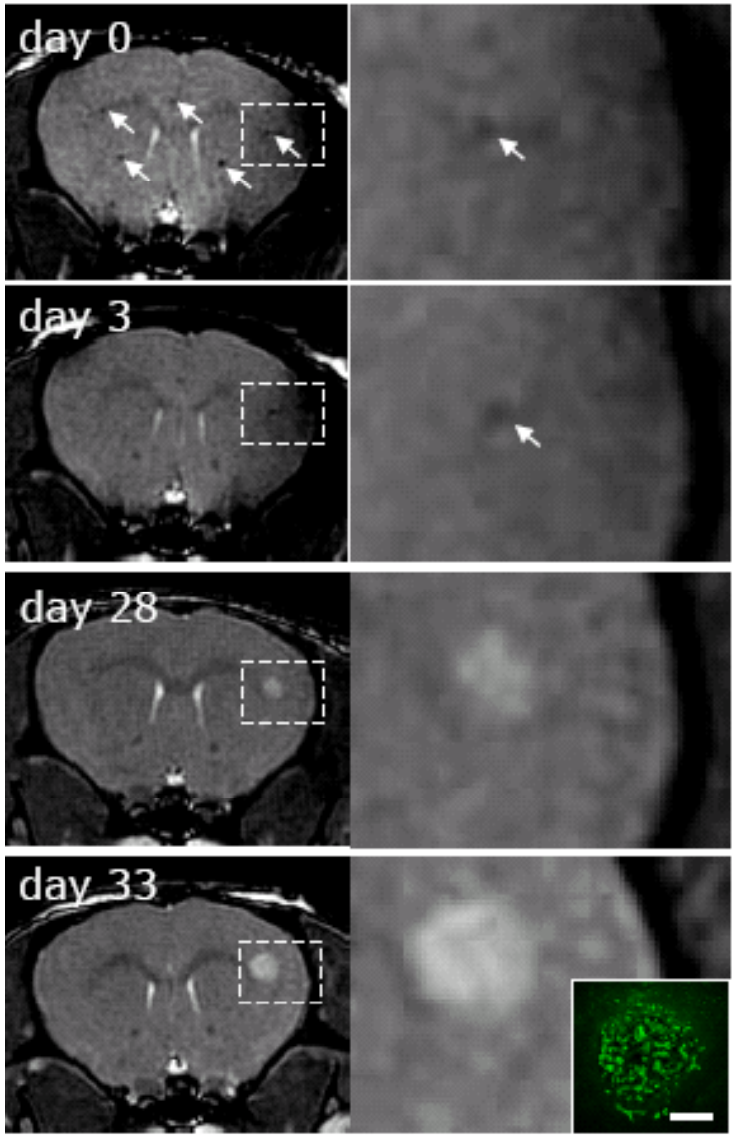
Single Cell MRI

- Clinical Field strength (1.5 - 3T)
- High strength gradient insert
- Custom-made RF coils
- FIESTA pulse sequence

Paula Foster and Brian Rutt

Heyn C et al., Magnetic Resonance in Medicine 55:23-29, and 56: 1001-1010, 2006

In vivo MRI to Monitor Fate of Breast Cancer Metastasis in Mouse Brain – 4D metastasis assay



MDA-MB-231BR human breast cancer cells (GFP)

Iron-loaded before injection (MPIO)

Intracardiac injection in mice

MRI every 1-4 days – whole brain

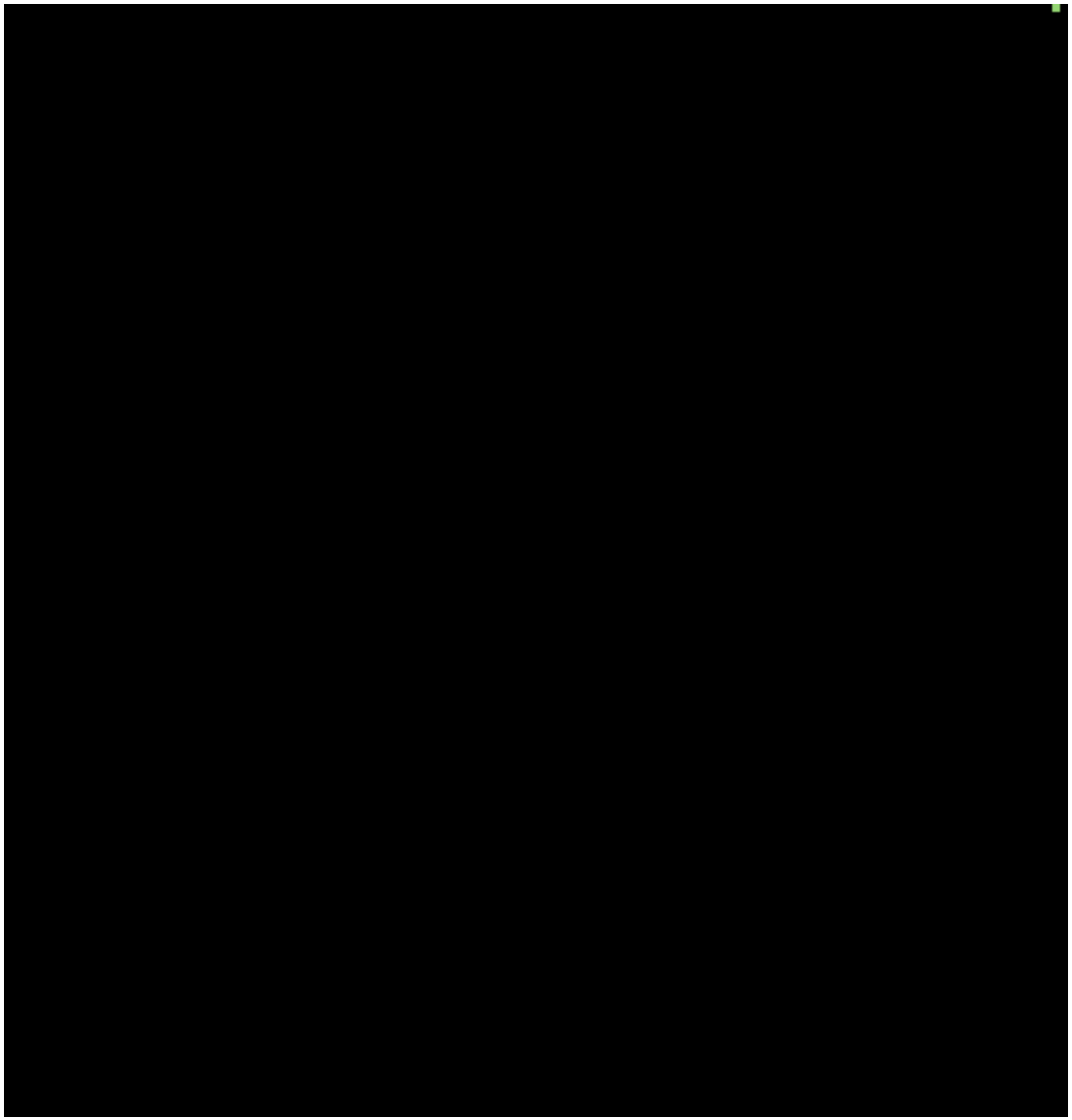
Clinical 1.5 T MR scanner with custom mouse coil – FIESTA pulse sequence

Single cells = MR signal voids (dark)

Metastases = MR hyperintensity (light) and GFP on histology

Heyn C et al., Magnetic Resonance in Medicine 56: 1001-1010, 2006

Fate of 231BR cells in mouse brain over 28 days after intracardiac injection - MRI



Red: Signal voids – iron-retaining cells – non-proliferating

Green: Signal hyperintensities – growing metastasis

Heyn C, et al., Magnetic Resonance in Medicine 56: 1001-1010, 2006

Fate of 231BR cells in mouse brain 28 days after intracardiac injection

	Day 28 MRI Void volume (mm3)	% of day 0 MRI Void volume (mm3)
'Transient' cells	33.5 ± 3.3	93.9 ± 0.7
Non-proliferating cells	1.6 ± 0.13	4.5 ± 0.8
Proliferating cells	0.56 ± 0.07	1.6 ± 0.06

Heyn C et al., Magnetic Resonance in Medicine 56: 1001-1010, 2006

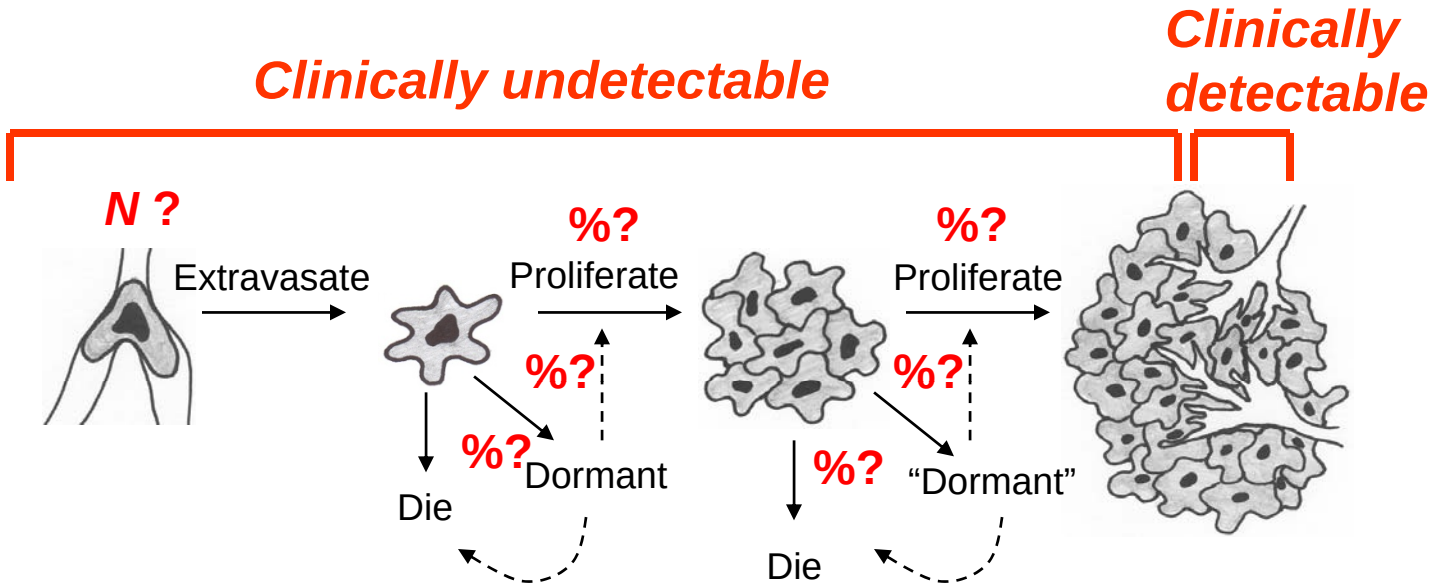
Fate of 231BR cells in mouse brain 28 days after intracardiac injection

	Day 28 MRI Void volume (mm3)	% of day 0 MRI Void volume (mm3)
'Transient' cells	33.5 ± 3.3	93.9 ± 0.7
Non-proliferating cells *	1.6 ± 0.13	4.5 ± 0.8
Proliferating cells	0.56 ± 0.07	1.6 ± 0.06

* ***Are (all? some of?) these dormant cells that can re-awaken? How kill them?***

Heyn C et al., Magnetic Resonance in Medicine 56: 1001-1010, 2006

Cancer cells in secondary organ may co-exist in functionally distinct states



- What affects *N* number of cells delivered to an organ?
- What affects % of dormant cells in different models?
- What affects the decision-point %'s?
- What causes cells to become dormant?
  (… genetics, epigenetics, microenvironment, host, cancer stem cells?)
- What awakens dormant cells?
- How can dormant cells be killed? *(… would that matter clinically?)*