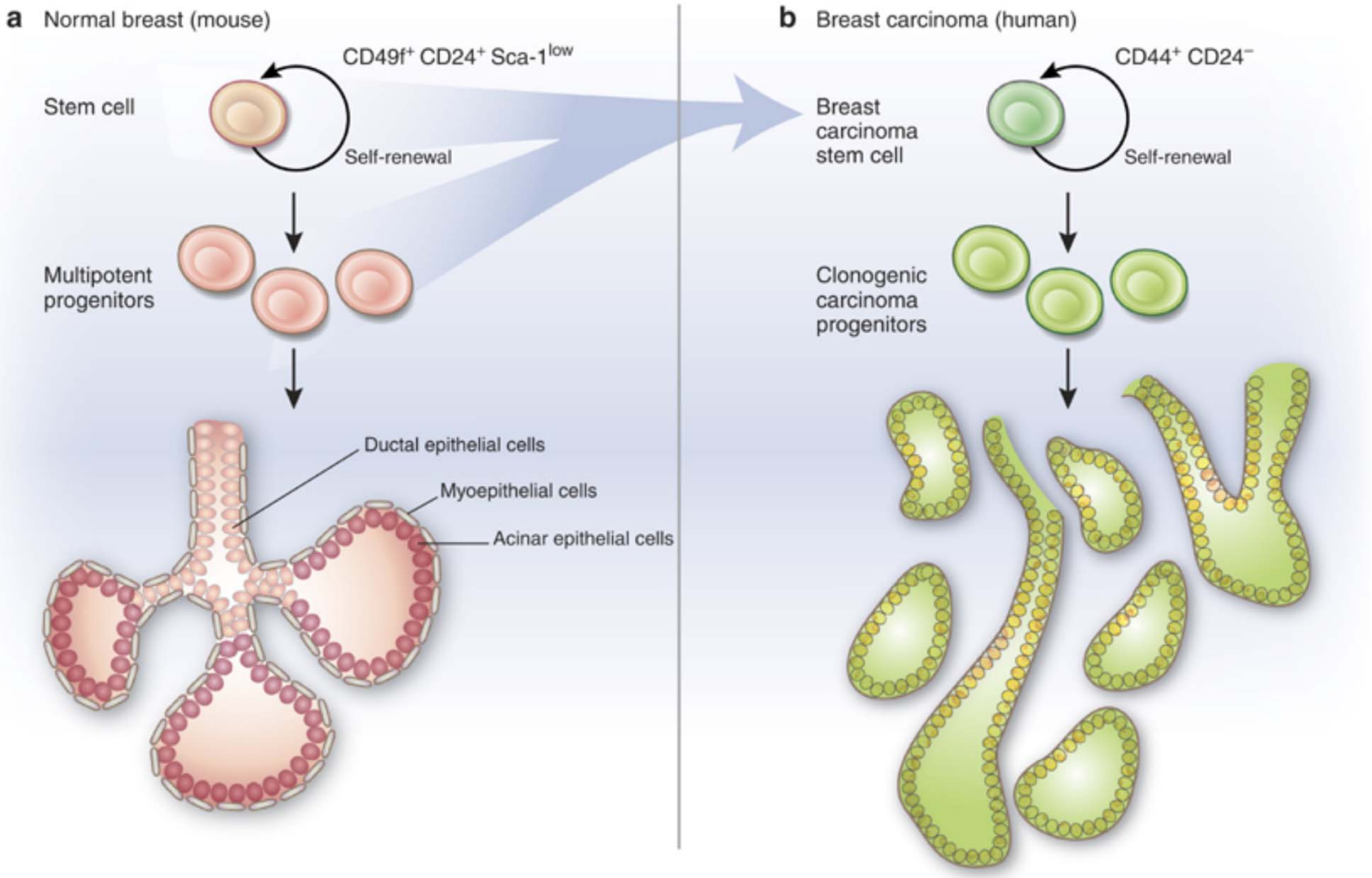
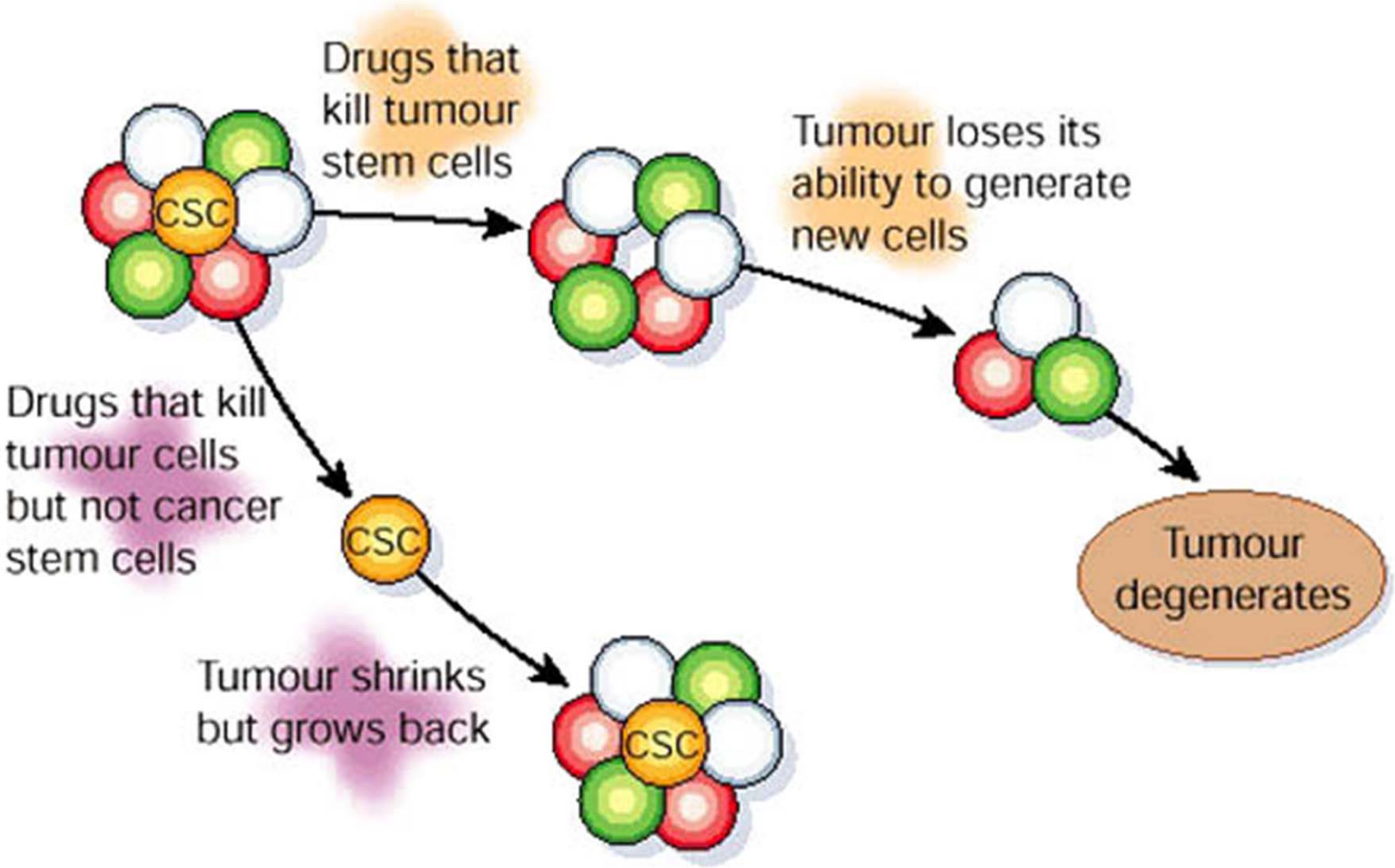
CCR Breast Cancer Stamp Fund Program

Barbara K. Vonderhaar, PhD

Co-Chair Breast & Gynecologic Malignancies Faculty

Program Coordinating PI


a
Normal breast (mouse)
CD49f+ CD24+ Sca-1low
Stem cell
Self-renewal
Multipotent progenitors
Ductal epithelial cells
Myoepithelial cells
Acinar epithelial cells
b
Breast carcinoma (human)
CD44+ CD24−
Breast carcinoma stem cell
Self-renewal
Clonogenic carcinoma progenitors


Drugs that kill tumour stem cells
CSC
Tumour loses its ability to generate new cells
Drugs that kill tumour cells but not cancer stem cells
CSC
Tumour degenerates
Tumour shrinks but grows back
CSC

- **Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer**

- **Project 2: Development and Characterization of Affibody®-Based Bioconjugates for Molecular Imaging and Targeted Therapy of HER2-Positive Breast Cancers**

- **Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer**

  - **Hypothesis:** Breast cancer stem cells can be characterized by unique cell surface markers that can be used for targeting molecular imaging probes and directing molecular therapy.

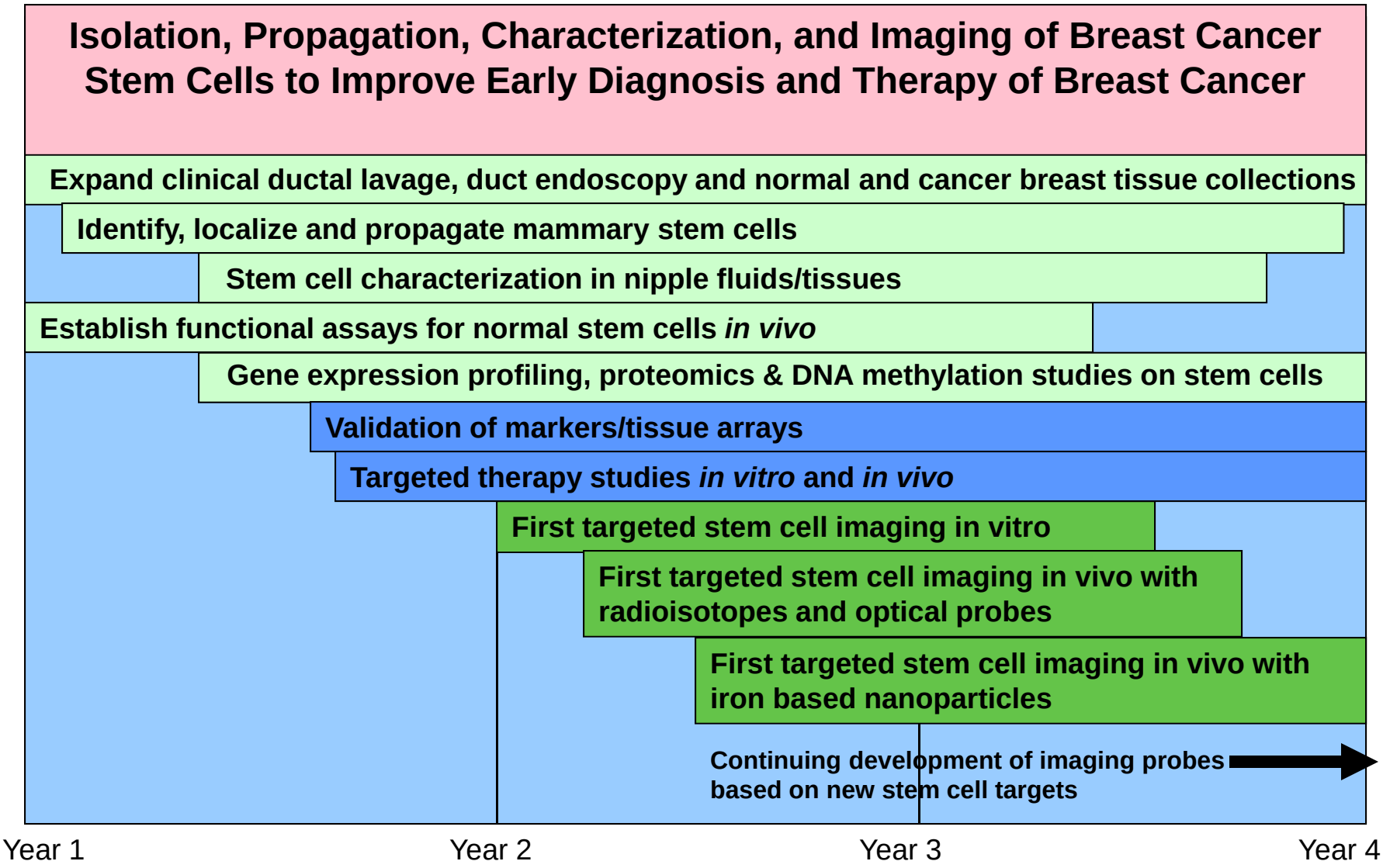
- **Project 2: Development and Characterization of Affibody®-Based Bioconjugates for Molecular Imaging and Targeted Therapy of HER2-Positive Breast Cancers**

  - **Hypothesis:** Delivery of therapeutic substances to HER2-positive breast cancers can be optimized using conjugates of HER2-specific Affibody® molecules with multifunctional thermosensitive liposomes.

Jacek Capala, PhD
Robert Blumenthal, PhD
Peter Choyke, MD

- **Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer**

- **Specific Aims**
  - Identify, localize and characterize stem/progenitor cells in human breasts from normal and high-risk women, as well as those from malignant neoplasms,
  - Define a rigorous functional assay for the normal stem cell and its niche in humanized mouse mammary fat pads,
  - Develop targeted imaging methods that will allow the detection of breast cancer stem cells within tumors at high resolution, as a prelude to developing targeted treatments, and
  - Develop improved chemotherapy of breast cancer by targeting the breast cancer stem cell niche.


Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer
Expand clinical ductal lavage, duct endoscopy and normal and cancer breast tissue collections
Identify, localize and propagate mammary stem cells
Stem cell characterization in nipple fluids/tissues
Establish functional assays for normal stem cells in vivo
Gene expression profiling, proteomics & DNA methylation studies on stem cells
Validation of markers/tissue arrays
Targeted therapy studies in vitro and in vivo
First targeted stem cell imaging in vitro
First targeted stem cell imaging in vivo with radioisotopes and optical probes
First targeted stem cell imaging in vivo with iron based nanoparticles
Continuing development of imaging probes based on new stem cell targets
Year 1
Year 2
Year 3
Year 4

- **Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer**
- **PIs**
  - **Breast Stem Cells:** Barbara K. Vonderhaar Ph.D., NCI (Co-Chair BGMF), Gilbert Smith, Ph.D., NCI, John Ortaldo, Ph.D., consultant, NCI retired, David Salomon, Ph.D., NCI, Robert Callahan, Ph.D., NCI, Ron McKay, Ph.D., NINDS; Michael Dean, Ph.D., NCI, Joshua Zimmerberg, M.D., Ph.D., NICHD, Leonid Margolis, Ph.D., NICHD, Michael Gottesman, M.D., NCI, John Niederhuber, M.D., NCI;
  - **Clinical/Translational/Therapy:** Sheila Prindiville, M.D., M.P.H., NCI, Sandra Swain, M.D., NCI, David Danforth, M.D., NCI, Jennifer Eng-Wong, M.D.,NCI,Stan Lipkowitz, M.D., NCI, Elise Kohn, M.D., NCI (Co-Chair BGMF), Susan Bates, M.D., NCI, Tito Fojo, M.D., NCI, Larry Maxwell, M.D., Walter Reed;
  - **Imaging:** Peter L. Choyke, M.D., NCI, Martin Brechbiel, Ph.D., NCI, Hisataka Kobayashi, M.D., Ph.D., NCI, Catherine Chow, M.D., CC, David Thomasson, Ph.D., CC, Brad Wood, M.D., CC, Eva Baker, M.D. Ph.D., CC.

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- **Our immediate goals for the first year are to:**
  - **Catalog existing samples from various protocols, amend existing protocols and write new protocols to expand the tissue collection to include core biopsies, ductal lavage and duct endoscopy samples from normal and high risk patients. This includes establishing a database to collect clinical and biological data from the patient samples;**
  - **Standardize processing of samples and preparation of dispersed cells from tissues, tumors and pleural effusions;**

- **Our immediate goals for the first year are to:**
  - **Optimize growth conditions in vitro for human breast stem cell (all sources) as mammospheres, as monolayers at clonal density and in the rotating bioreactor;**
  - **Optimize the in vivo growth conditions for normal breast epithelial cells in humanized NOD/SCID mouse mammary fat pads and for cells from pleural effusions and primary breast tumors in NOD/SCID mice; and**
  - **Validate the practicality of amplification of cDNA from 100, 1000 and 10,000 cells for expression array analysis and establish a plan to analyze protein expression analysis of total and cell surface proteins.**

Sources of Tissue

- Suburban Hospital (SH)
- NIH Clinical Center (CC)
- Bethesda Naval Hospital
- (Walter Reed Hospital)

NOD/SCID mice

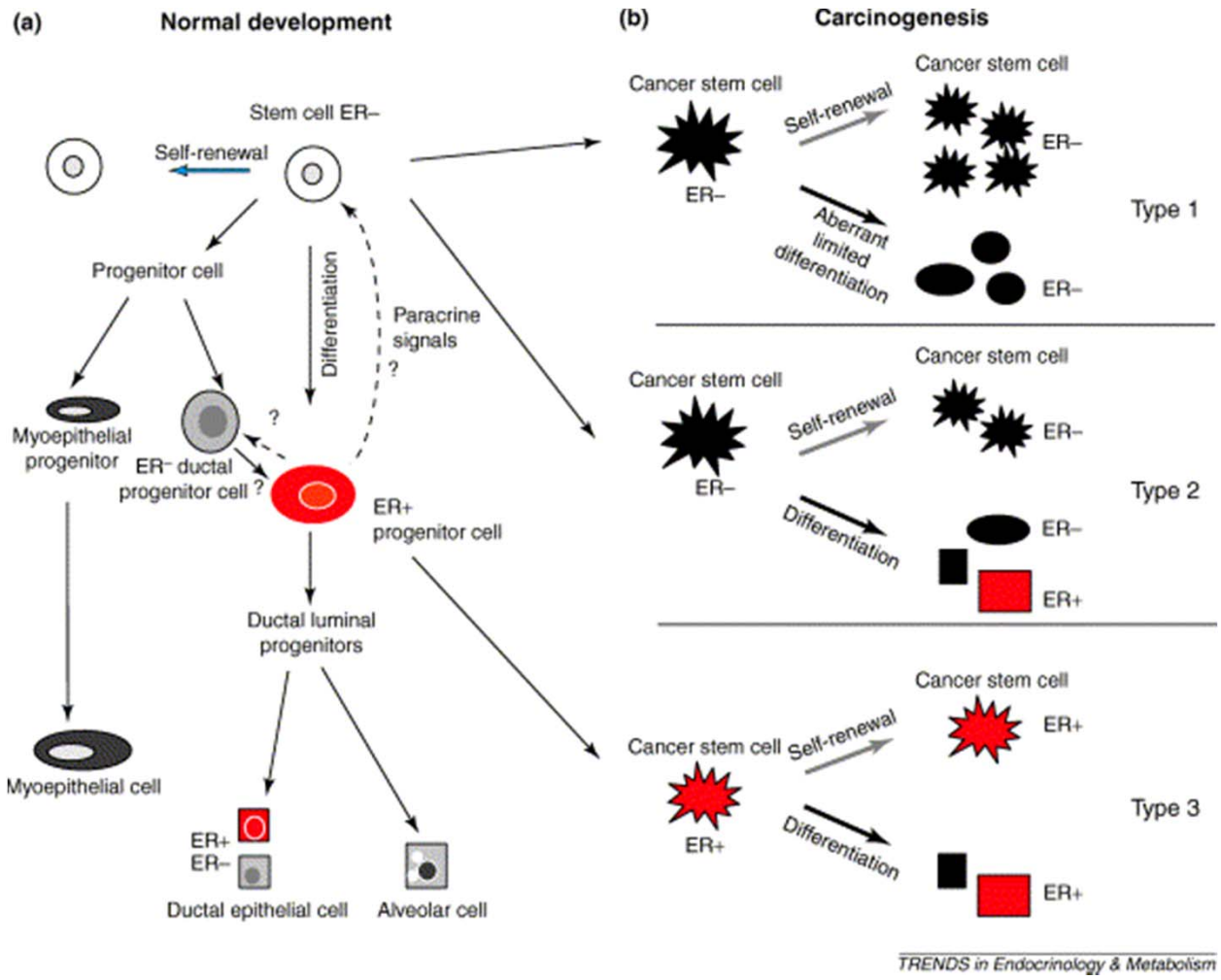
- NIH colony

Suburban Samples- Updated 5/22/06 ** from biopsy

Case	SB #	Age	Race	Age @ Menarche	Age @ 1st Pregnancy	Age @ 1st Child Birth	Age @ Menopause	Taken Birth Control or HRT	# of Prior Biopsies	# of Prior Biopsies w/ Atypical Displasia	# of 1st Degree Relatives w/ B.C.	# of other family members w/ B.C.
UPN-2		58	B	11	21	21	48	YES	0	N/A	0	0
UPN-4		64	C	10	20	20	50	NO	0	N/A	0	0
UPN-8		36	B	11	23	23	N/A	YES	0	N/A	0	0
UPN-30		18	C	12	N/A	N/A	N/A	NO	0	N/A	0	0
UPN-32		24	B	12	N/A	N/A	N/A	YES	0	N/A	0	0
UPN-33		32	B	18	25	25	N/A	YES	0	N/A	0	0
UPN-35	10	62	C	13	26	26	50	YES	0	N/A	0	0
UPN-38	11	57	C	12	N/A	N/A	55	YES	0	N/A	0	0
UPN-48	12	50	B	16	20	20	N/A	NO	1	N/A	0	?
UPN-49	13	37	H	13	26	27	N/A	YES	0	N/A	0	?
UPN-65	14	34	B	11	21	21	N/A	NO	0	N/A	0	0
UPN-66	15	40	C	12	22	22	N/A	NO	0	N/A	0	0
UPN-67	16	66	O	11	23	23	50	DonÕt know	0	N/A	0	0
UPN-70	17	25	B	13	N/A	N/A	N/A	YES	0	N/A	0	0
UPN-74	18	32	C	11	N/A	N/A	N/A	NO	0	N/A	0	0
UPN-77	19	30	C	13	25	25	N/A	NO	0	N/A	0	0
UPN-79 *from biopsy*	20	60	C	12	20	21	47	NO	2	Unknown	0	0
UPN-80	21	53	C	10	26	26	N/A	NO	1	Unknown	0	0
UPN-81	22	19	C	12	N/A	N/A	N/A	NO	0	N/A	0	0
UPN-85	23	18	C	10	N/A	N/A	N/A	NO	0	N/A	0	0
UPN-93 **	24	86	C	10	22	23	51	NO	2	Unknown	1	1
UPN-102	25	25	C	12	N/A	N/A	N/A	YES	0	N/A	0	0
UPN-107 **	26	45	???	12	38	38	N/A	YES	1	Unknown	0	0
UPN-110	27	40	C	11	23	23	N/A	NO	0	N/A	0	0
UPN-113 **	28	49	B	11	23	23	N/A	NO	1	Unknown	1	0
UPN-116	29	32	B	12	28	28	N/A	NO	0	N/A	0	0
UPN-90	30	56	B	14	N/A	N/A	49	YES	0	N/A	0	0
UPN-115 **	31	53	B	14	21	21	50	NO	1	Unknown	0	0

STEM CELLS

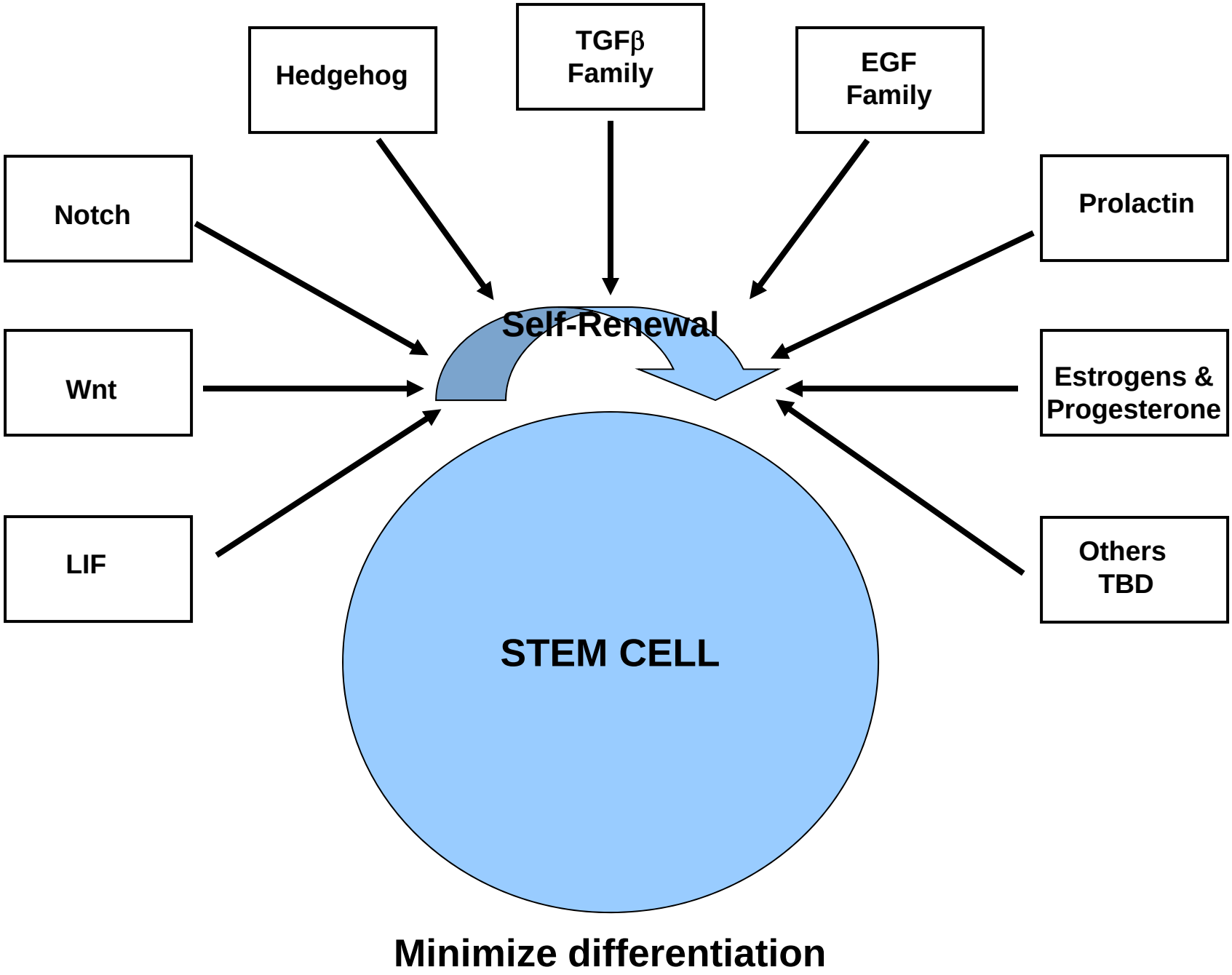
- Currently defined by function
- Plan to define at molecular level
  - Cancer
  - Normal



TRENDS in Endocrinology & Metabolism

Big Challenge

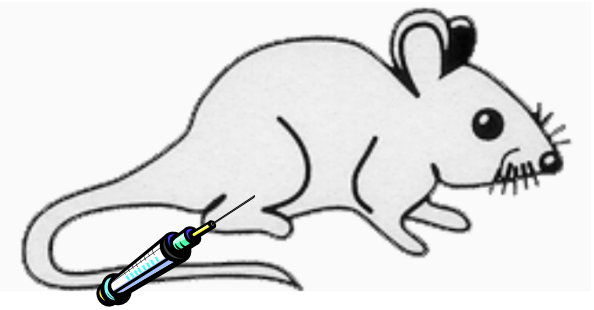
How to maintain and/or expand the stem cell population


Hedgehog
TGFβ Family
EGF Family
Notch
Prolactin
Self-Renewal
Wnt
Estrogens & Progesterone
LIF
Others TBD
STEM CELL
Minimize differentiation

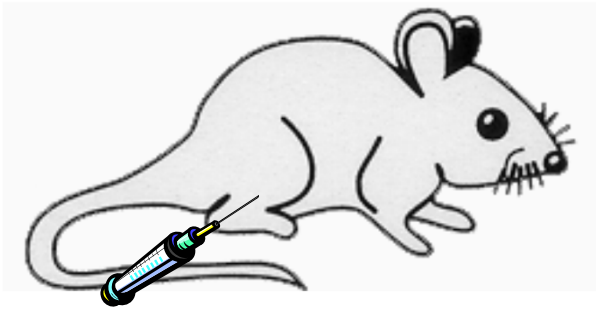
Normal Tissue

Functional assay

Humanize the mouse mammary fat pad



4 weeks

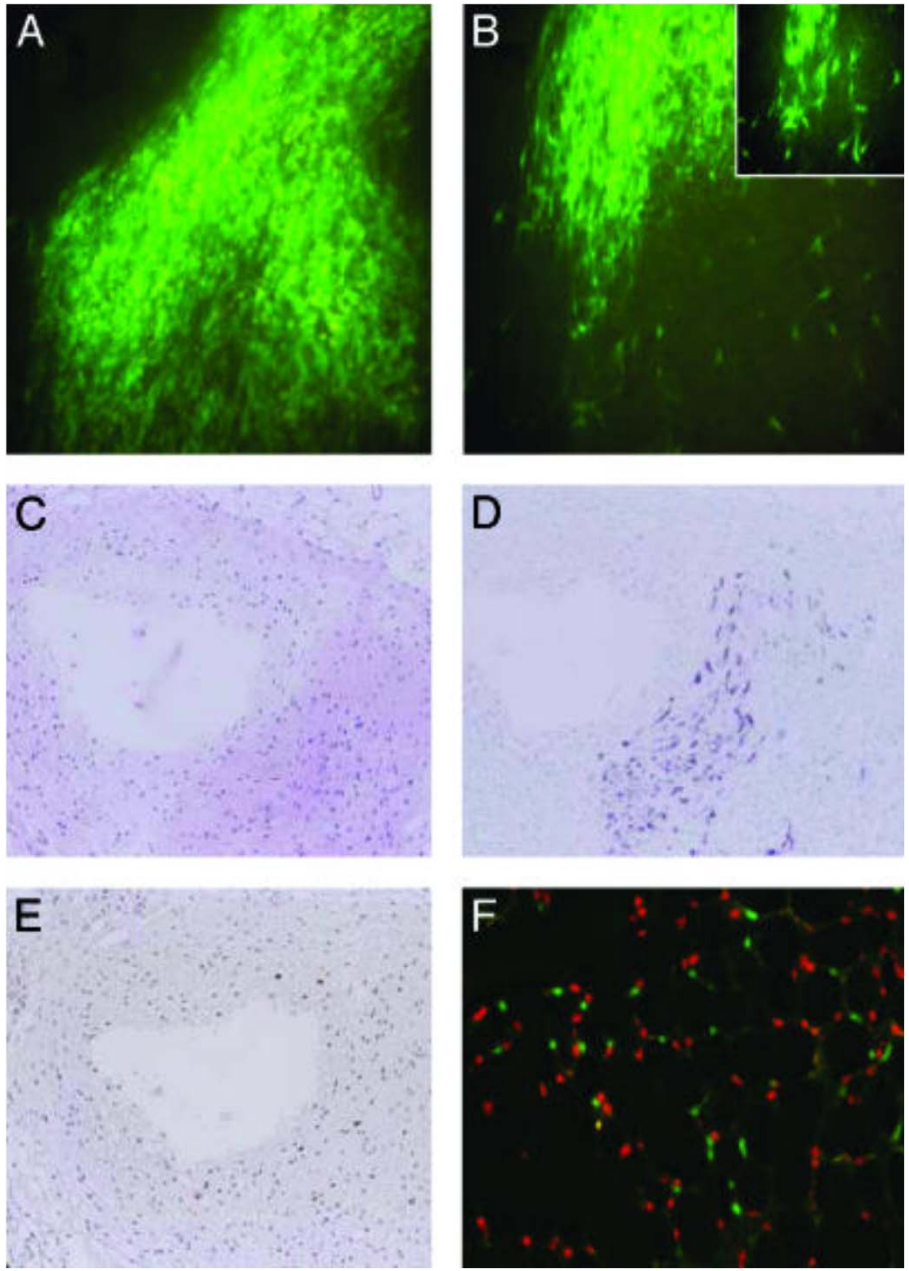


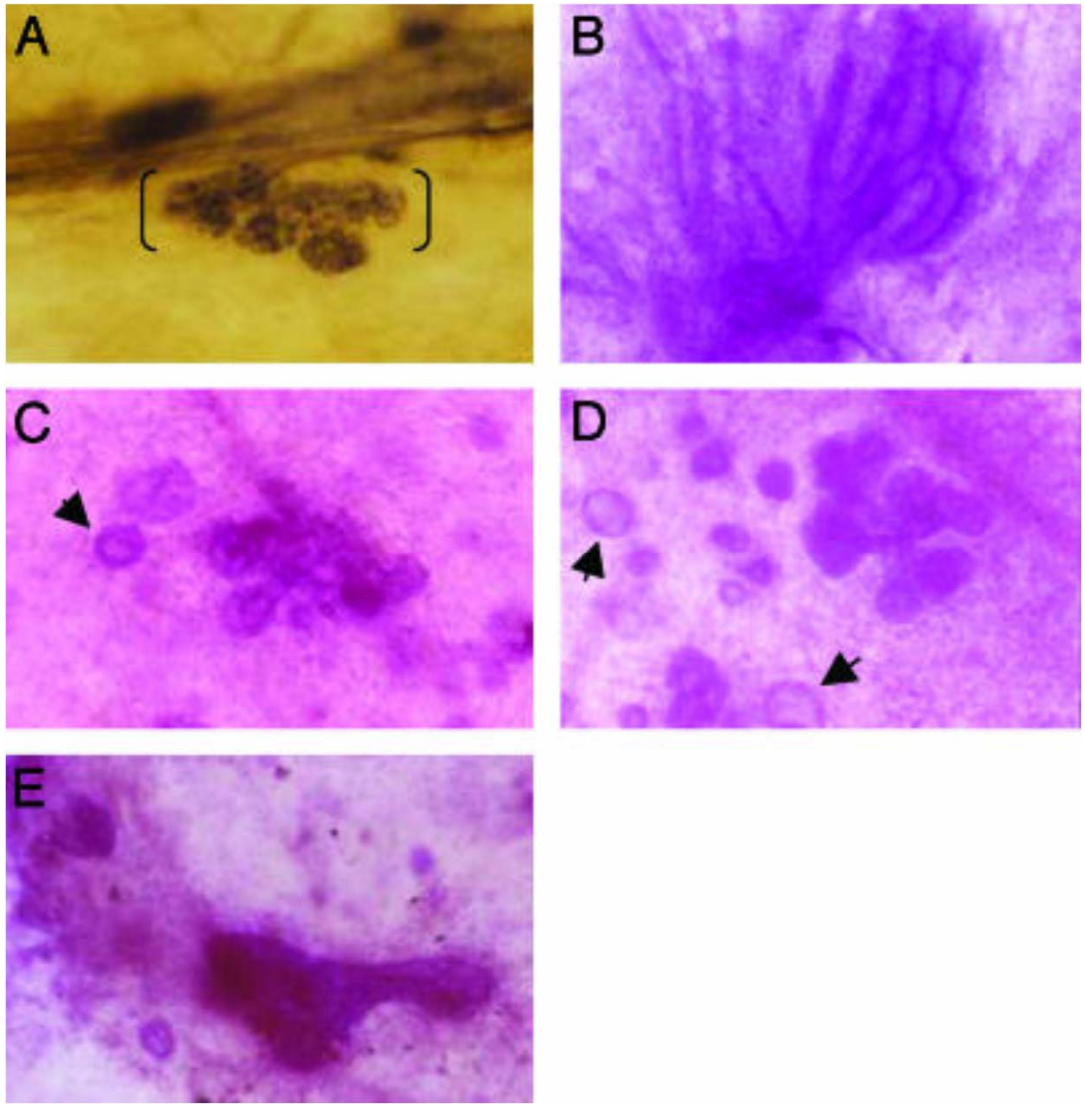
Fibroblasts
(into cleared fat pad)
Immortalized-GFP

Epithelial cells & Fibroblasts

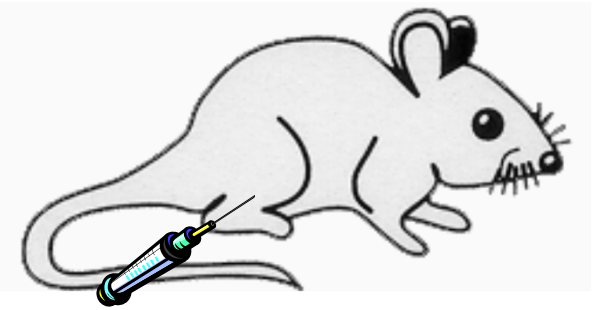
8-12 weeks

Outgrowth/isolation molecular analysis

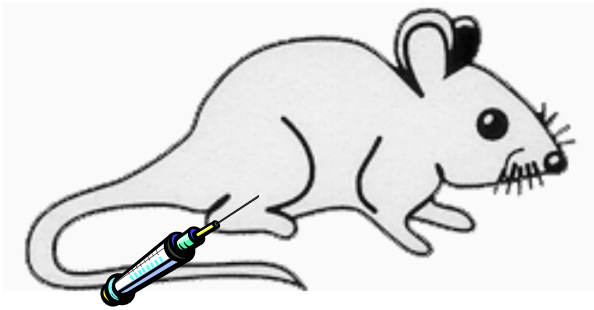

A
B
C
D
E
F


A
B
C
D
E

Humanize the mouse mammary fat pad



4 weeks



Fibroblasts
(into cleared fat pad)

Immortalized-GFP

Normal

- **Intralobular**
- **Interlobular**

Tumor

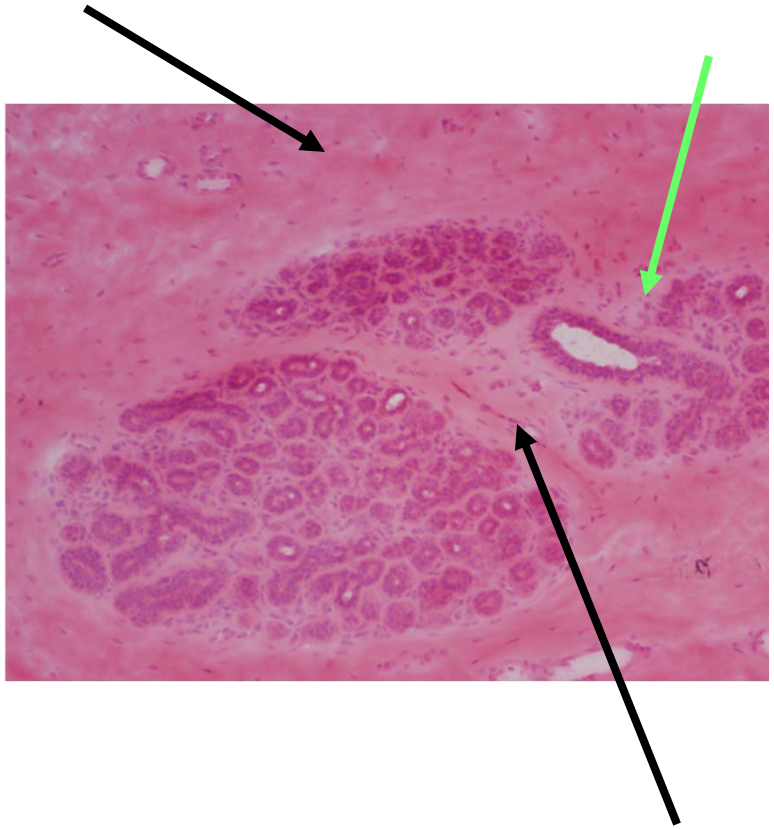
High risk tissue?

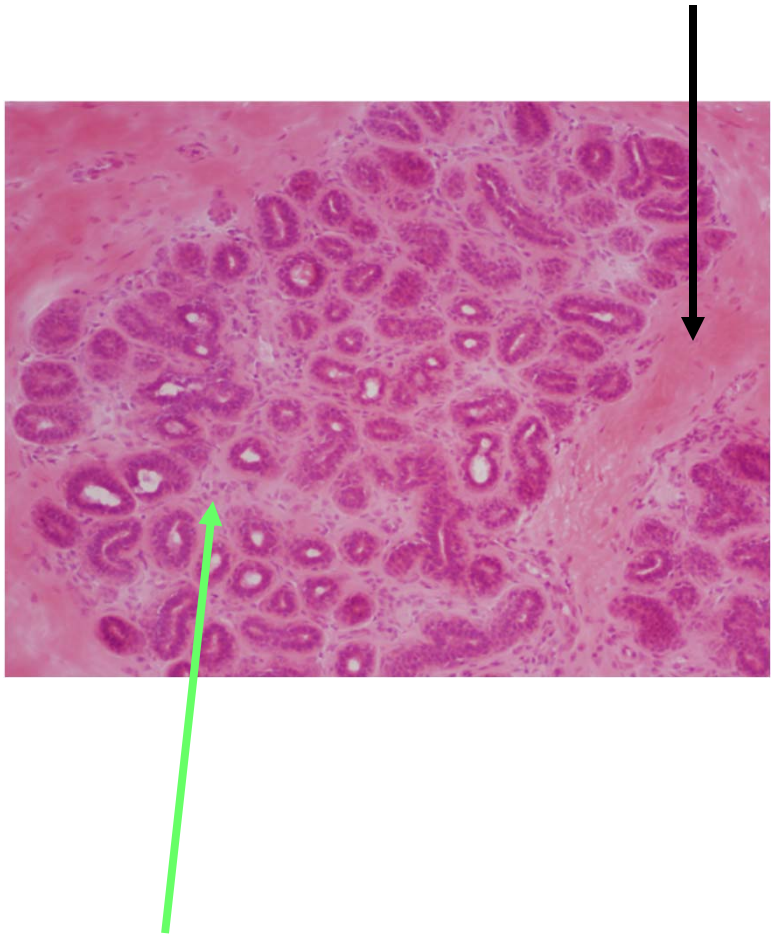
Epithelial cells & Fibroblasts

8-12 weeks

Outgrowth/outgrowth molecular analysis

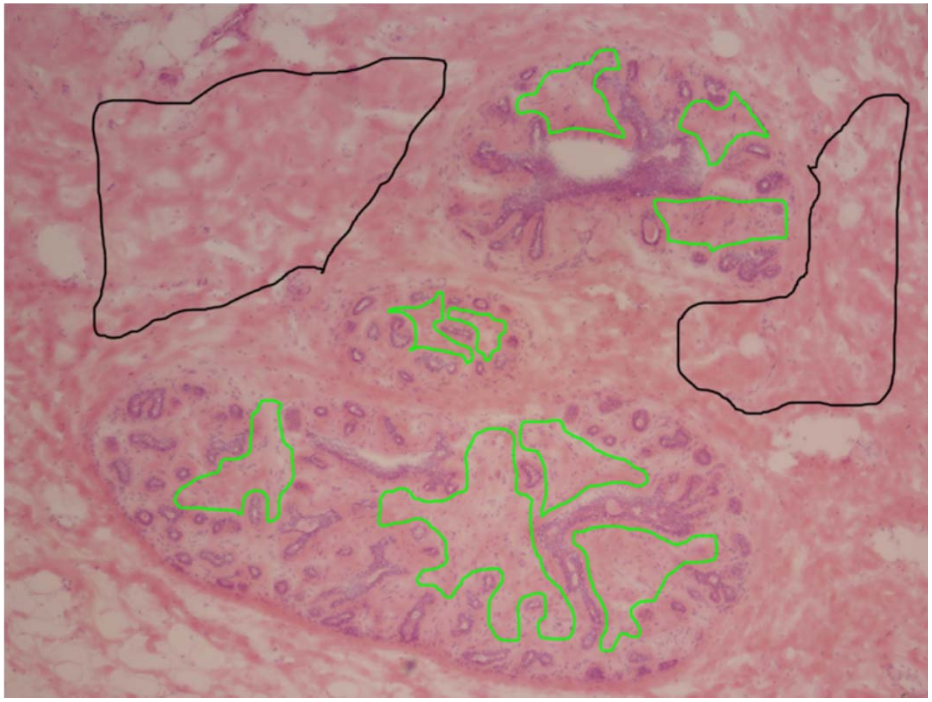
Normal Human Breast

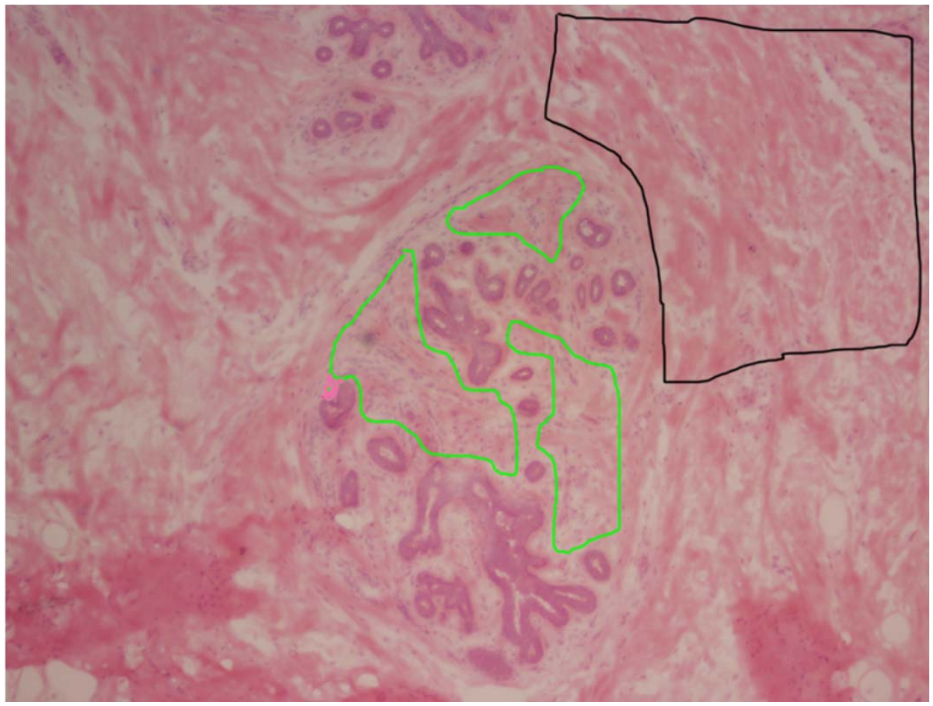


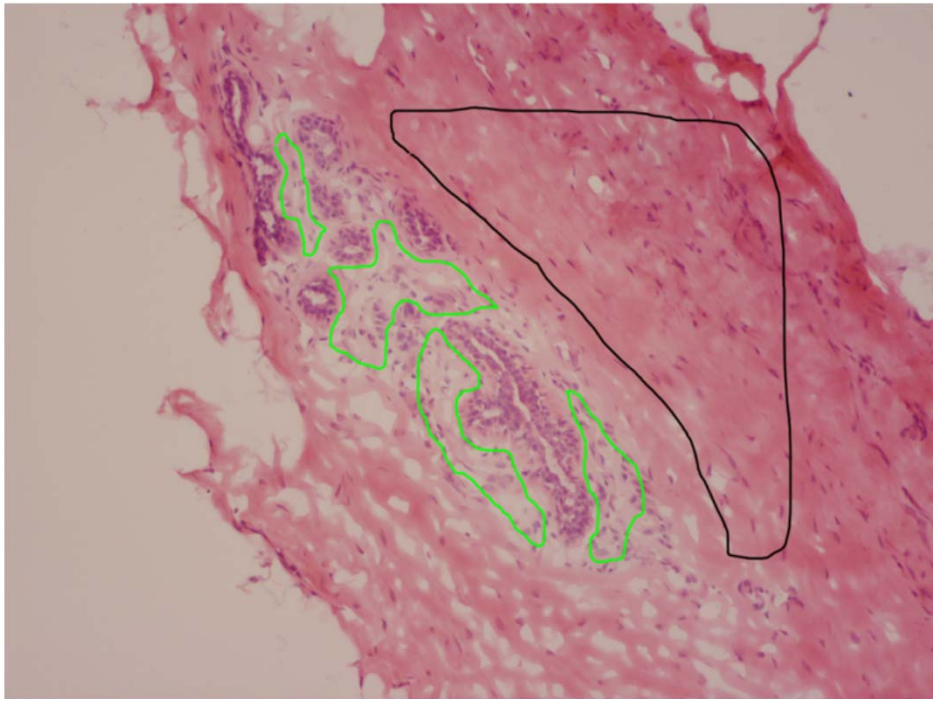


Intralobular stroma

Interlobular stroma







LCM

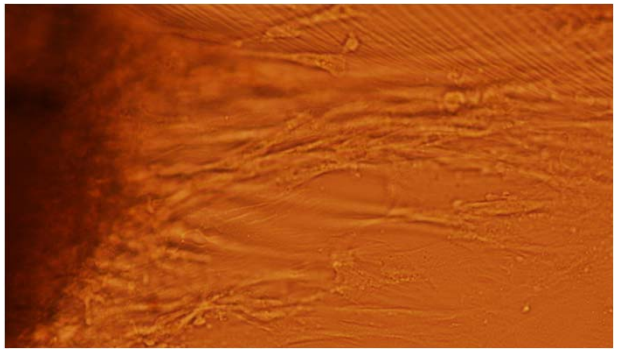
Microarrays

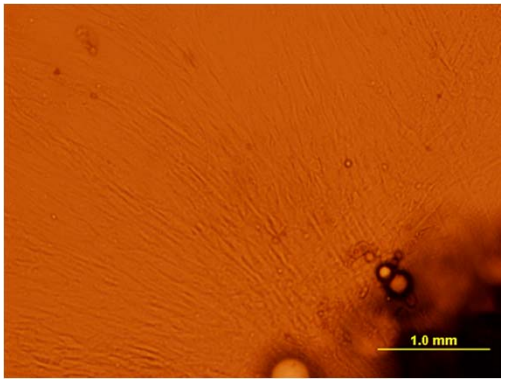
Surface markers
to distinguish fibroblast types

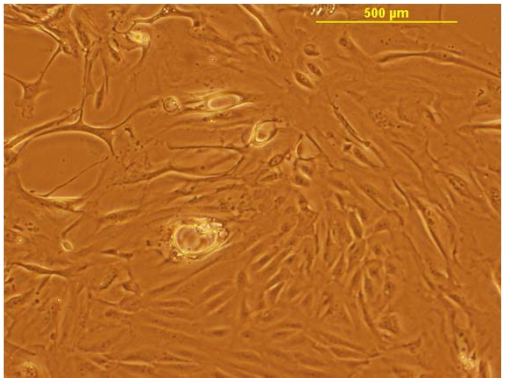
Fibroblasts

Normal Fibroblasts

Growing from chunk on plastic



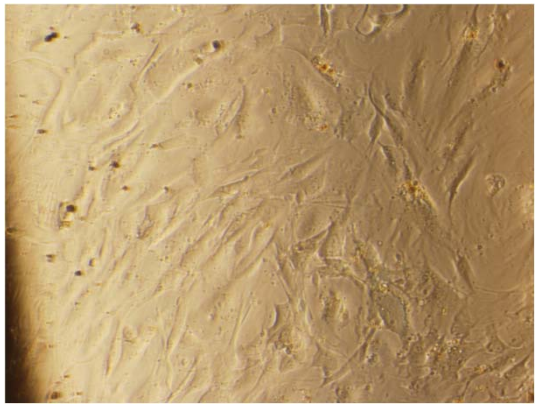




Passage 2 on plastic

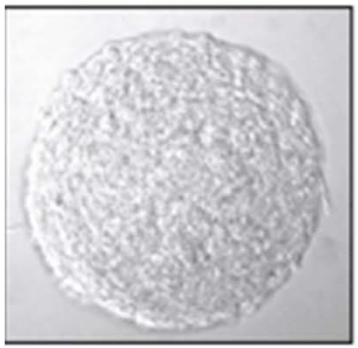
Tumor Fibroblasts

Growing from chunk on collagen



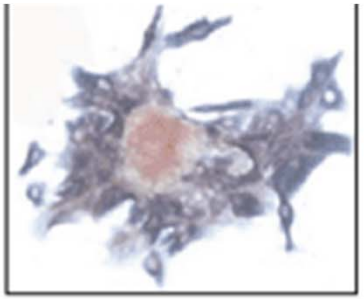
Big Question

- **Will fibroblasts growing on plastic express the surface markers that distinguish the two normal populations?**



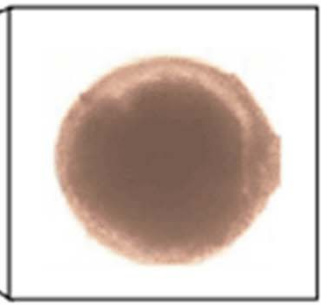
a. Mammosphere

Single cells

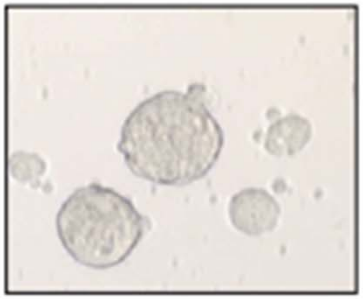


b. Differentiation along the three adult mammary cell lineages

c. Differentiation in 3D culture



d. Differentiation into functional structures



e. Self-renewal

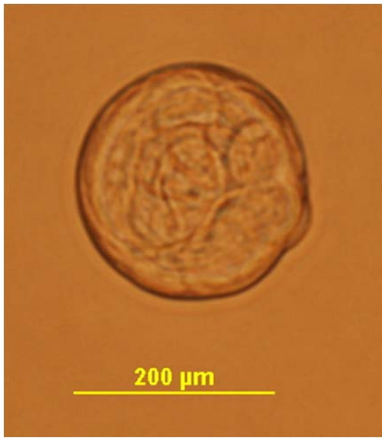
Experimental System:
Staged series of human breast-derived cell lines representing different steps in cancer progression

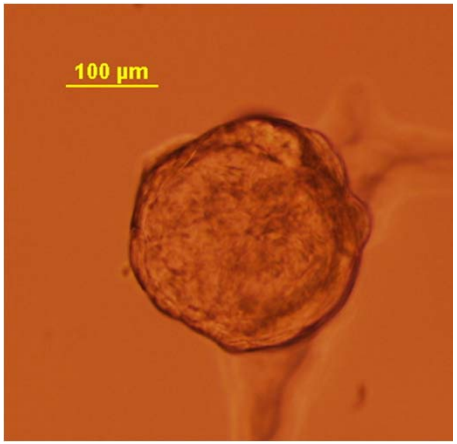
Dr. Fred Miller, Barbara Ann Karmanos Cancer Institute, Detroit

M-I	M-II	M-III	M-IV
"MCF10A" Spontaneously immortalized line from non-malignant breast epithelium	"MCF10AT1k.cl2 "Ha-ras transfected MCF10A	"MCF10Ca1h" Line derived from xenografts of MCF10AT	"MCF10Ca1a.cl1" Line derived from xenografts of MCF10AT
Normal	"Premalignant"	Well-differentiated tumor	Poorly diff. metastatic tumor

Increasing malignancy

Mammospheres from MCF10A M-I cells



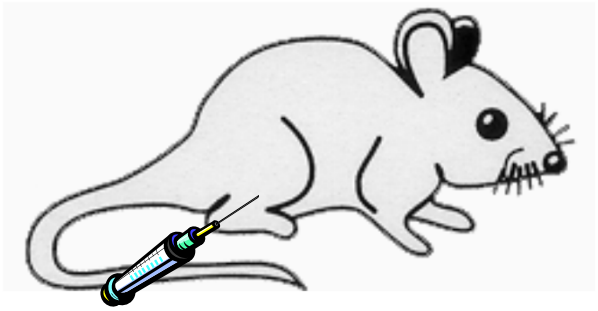


7-10 days in culture

Tumors & Pleural Effusions

Functional assay

-5 days: etoposide
& E2 pellet

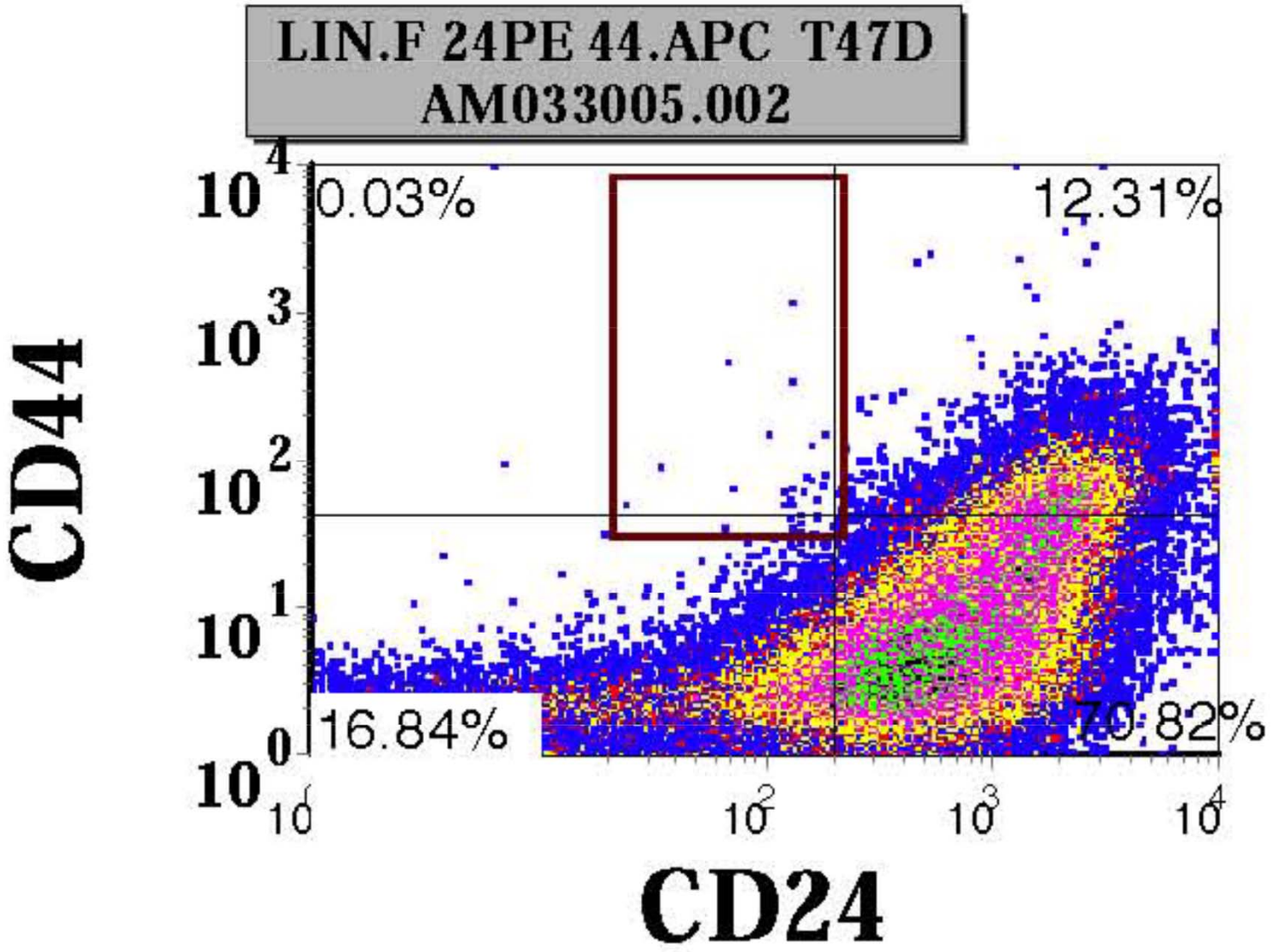


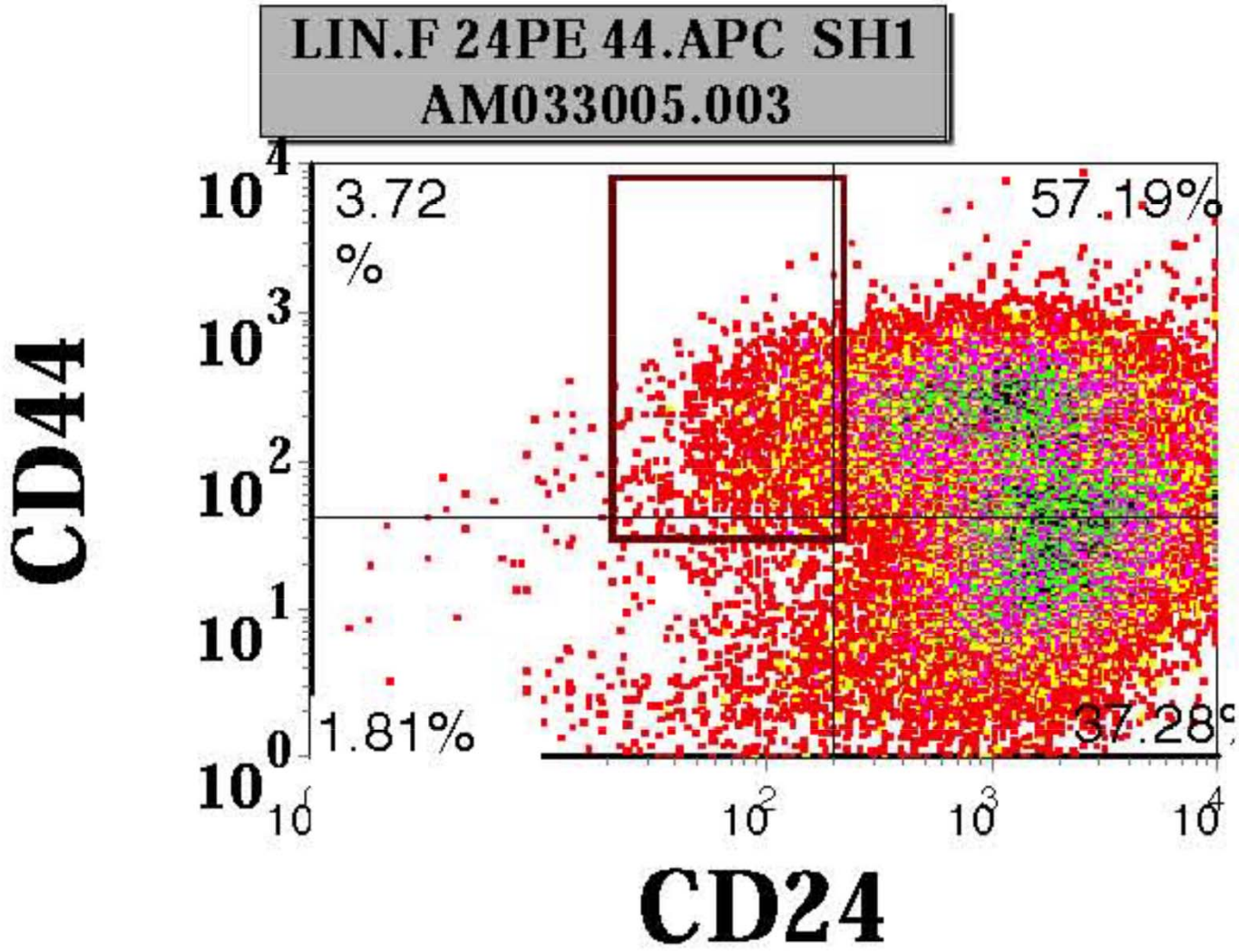
up to 8 months

Tumors/isolation
molecular analysis

3-5 x 10^6 cancer cells or PE

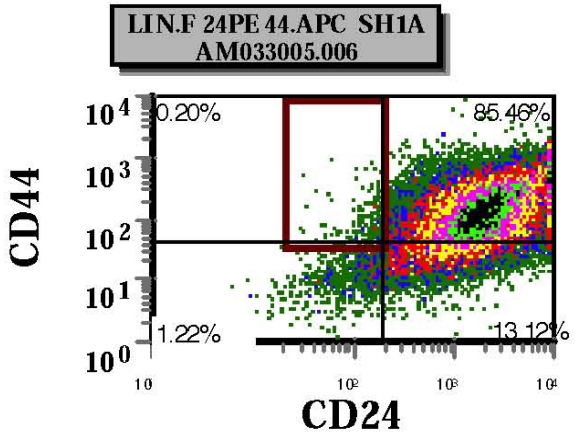
	Tumors
SH-1	3/5
CC-1	0/5
T47D	5/5


LIN.F 24PE 44.APC T47D
AM033005.002
CD44
CD24
0.03%
12.31%
16.84%
70.82%
10^4
10^3
10^2
10^1
10^0
10^1
10^2
10^3
10^4

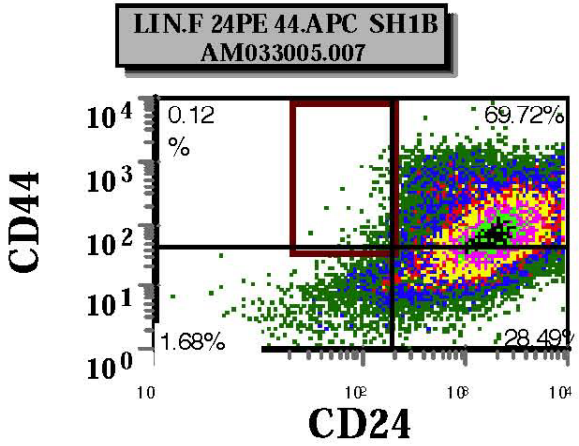

LIN.F 24PE 44.APC SH1
AM033005.003
CD44
CD24
3.72
%
57.19%
1.81%
37.28%
10^4
10^3
10^2
10^1
10^0
10^1
10^2
10^3
10^4

Cells isolated from SH1 tumors recapitulate the full spectrum of cell types

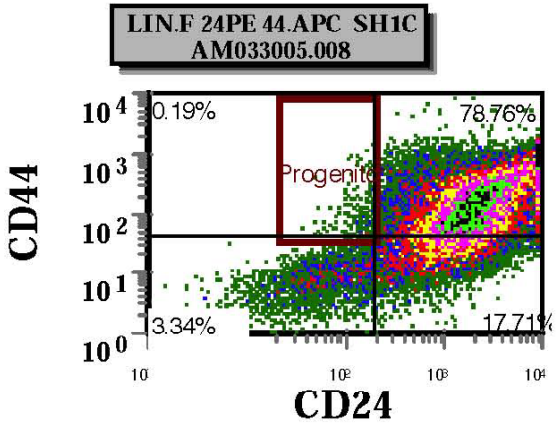
c.

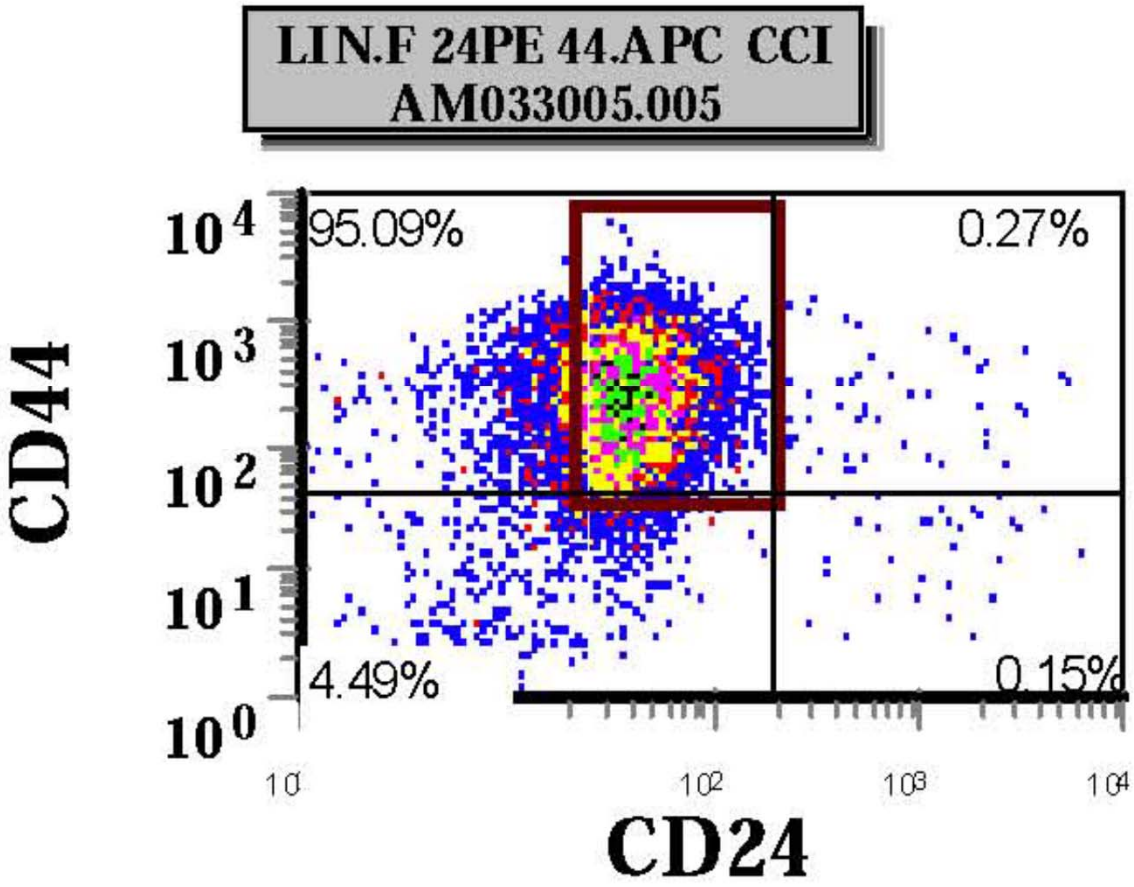


d.

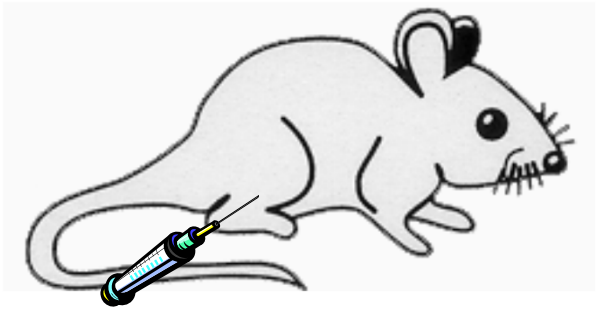


e.





No tumors after 8 months



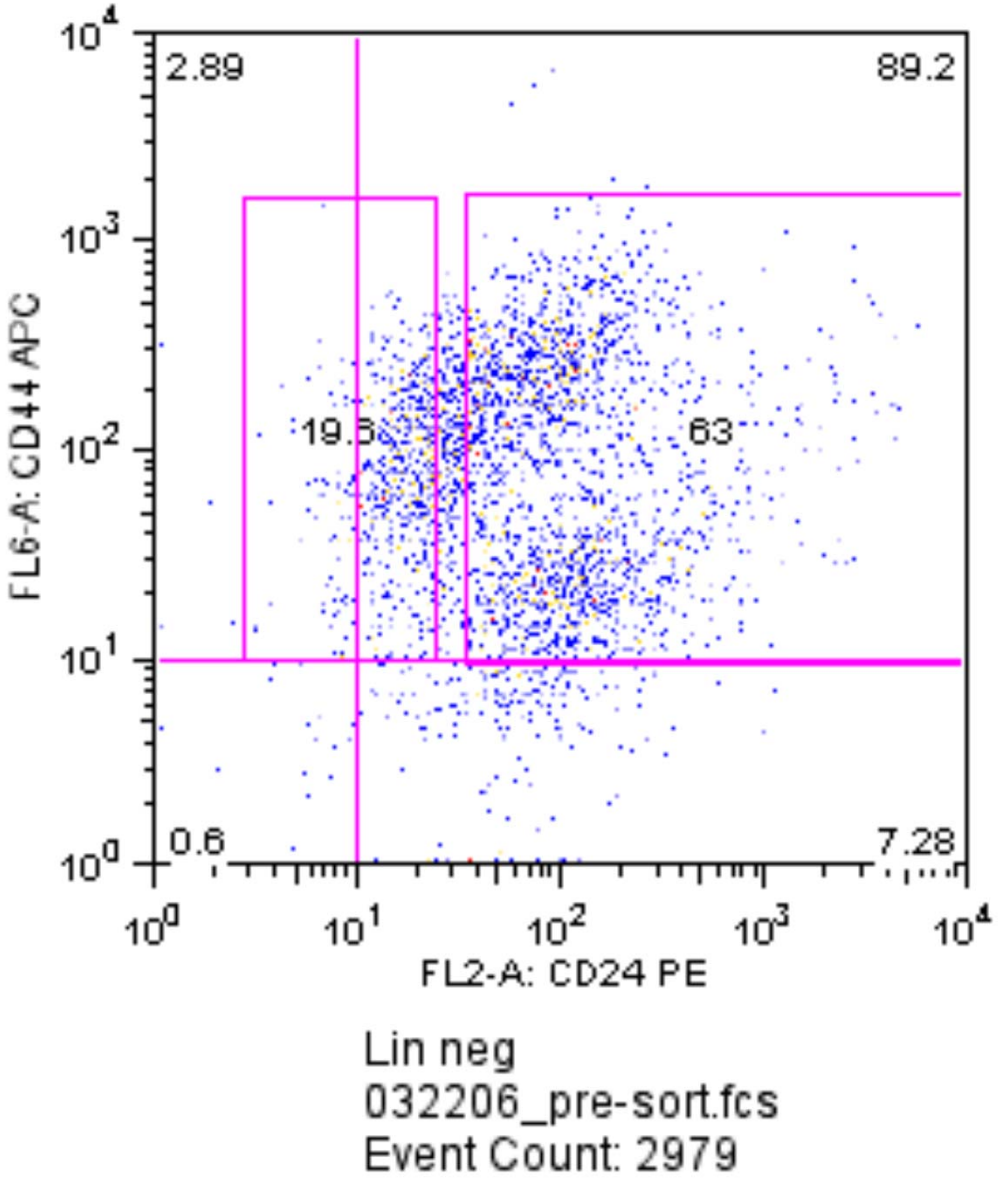
up to 6 months

Tumors/isolation
molecular analysis

Cancer cells or PE

Presort $CD44^{+}/CD24^{+}$ or $CD44^{+}/CD24^{-/low}$

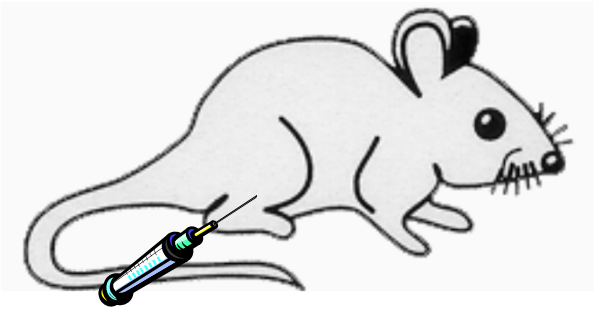
Cells
200
500
1K
10K



SH12

In vitro gives good tumorspheres

SH 12 after 5 months

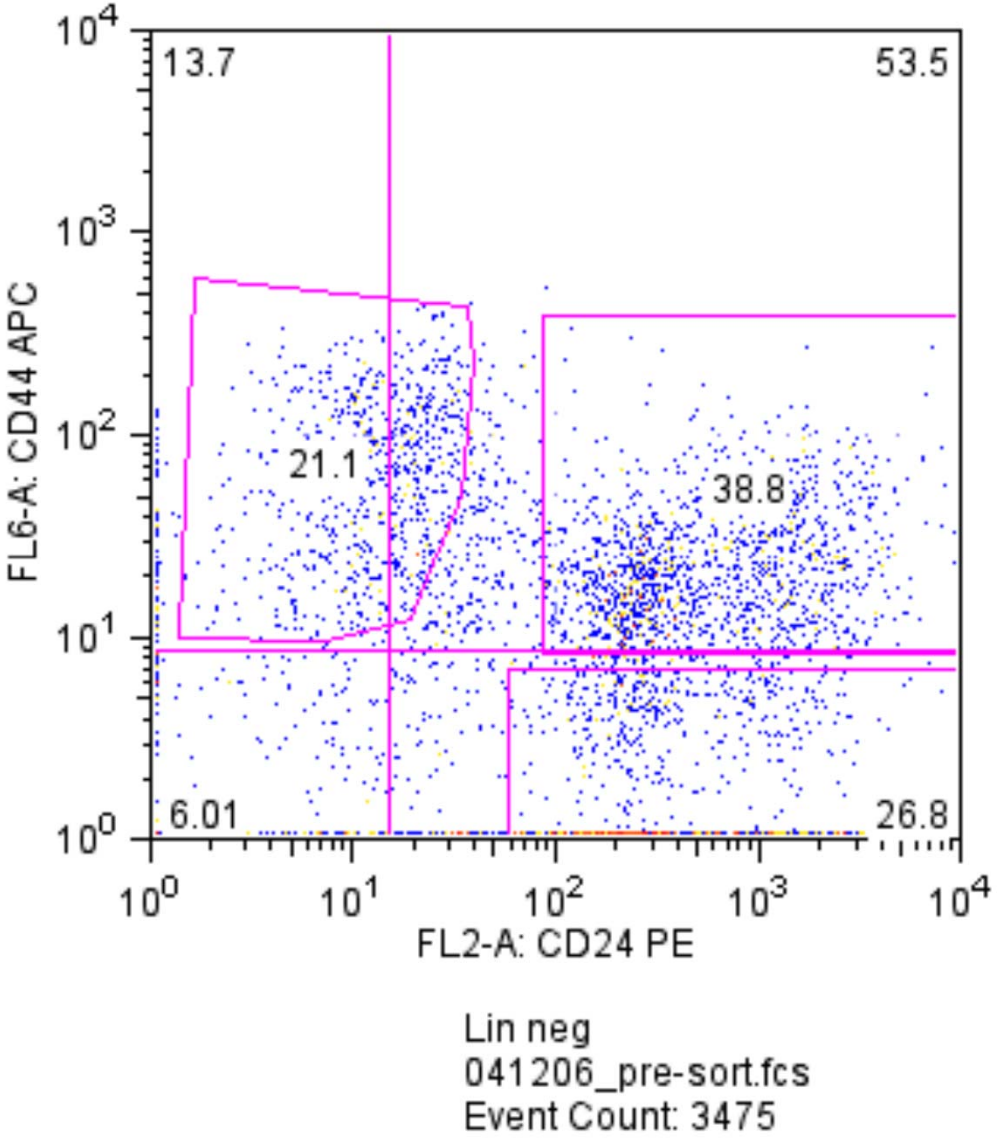


up to 6 months

Tumors/isolation
molecular analysis

Cancer cells or PE

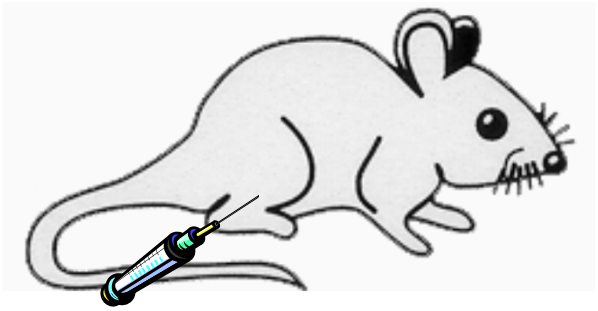
Presort	$CD44^+/CD24^+$	$CD44^+/CD24^{-/low}$
Cells		
200	0/6	2/6
500	0/6	2/6
1K	0/6	2/4
10K	0/6	3/4



SH8

In vitro gives good tumorspheres

SH 8 after 5 months



up to 6 months

Tumors/isolation
molecular analysis

Cancer cells or PE

Presort	$CD44^{+}/CD24^{+}$	$CD44^{+}/CD24^{-/low}$
Cells		
200	0/6	0/6
500	0/6	0/6
1K	0/6	0/6
10K	0/6	0/6

No tumors from $CD44^{-/low}/CD24^{+}$

MCF10A M-IV cells

- **Parental cells are a mix**
  - **$CD44^{+}/CD24^{+}$ and $CD44^{+}/CD24^{low/-}$**
- **In vitro gives good tumorspheres at 7-10days**
- **In tumorsphere selection media at 24hr cells are**
  - **$CD44^{+}/CD24^{+}$**
- **Into NOD/SCID mice**
  - **1K cells give 100% tumors in 3 weeks**
- **Grow out on plastic as colonies**
  - **$CD44^{+}/CD24^{+}$**
  - **$CD44^{+}/CD24^{low/-}$**

Conclusion

- **$CD44^{+}$ appears to be a marker for tumor formation in vivo**
- **$CD24^{low/-}$ does not appear to correlate with tumor formation in vivo**
- **New/additional markers are needed to better define the tumor stem cell on the molecular level**
  - **Tumorspheres from PE**
  - **Microarrays**
  - **MCF10A IV cells and known markers**