Cancer Biology 2

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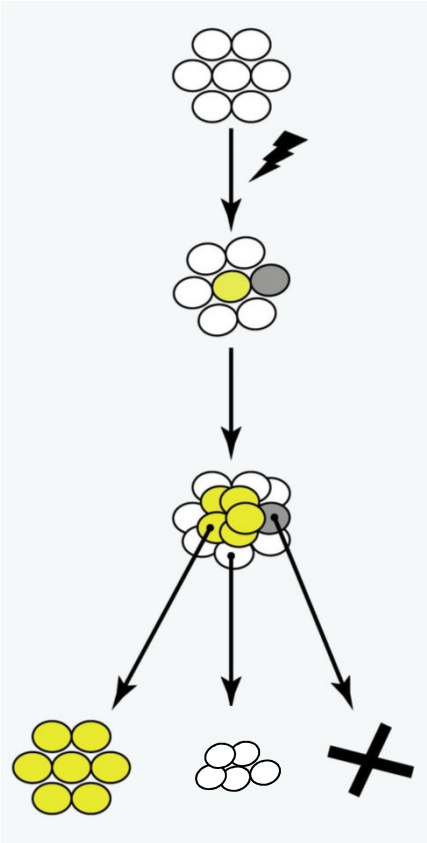
week	date	teacher	topic
1	2025-02-17	Joerg Huelsken	Cancer Stem Cells
2	2025-02-24	Joerg Huelsken	Metastasis
3	2025-03-03	Joerg Huelsken	Cell death
4	2025-03-10	Joerg Huelsken	Cancer Signalling Pathways
5	2025-03-17	Joerg Huelsken	Tumor Histology
6	2025-03-24	Joerg Huelsken	Tumor Histology
7	2025-03-31	Joerg Huelsken	exam
8	2025-04-07	Joerg Huelsken	Adaptive Immunity (T cells)
9	2025-04-14	Miki de Palma	Tumor Angiogenesis
10	2025-04-28	Miki de Palma	Tumor Angiogenesis
11	2025-05-05	Joerg Huelsken	Innate Immunity (Myeloid)
12	2025-05-12	Joerg Huelsken	Innate Immunity (NK cells)
13	2025-05-19	Joerg Huelsken	Cancer Metabolism
14	2025-05-26	Joerg Huelsken	exam

Learning objectives

- Hierarchical organization in normal tissues and cancer
- Definition and functional properties of Cancer Stem Cells (CSCs)
- Assays and methods to detect CSCs
- In vivo evidence for CSCs
- Prospects of Targeting CSCs

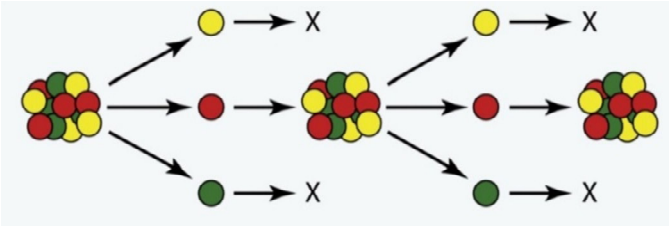
Intra-tumoral Heterogeneity

Genetic Evolution

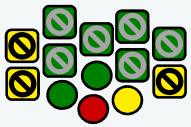


Epigenetic and Environmental Adaptations

Cancer Stem Cells

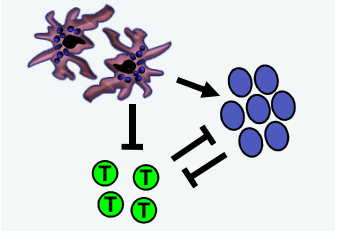


Senescence

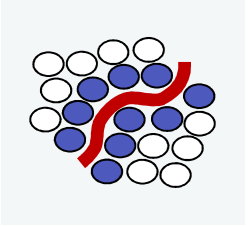


Dormancy

Immune Evasion/Suppression

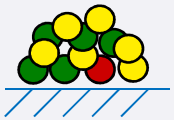


Metabolic Adaptation



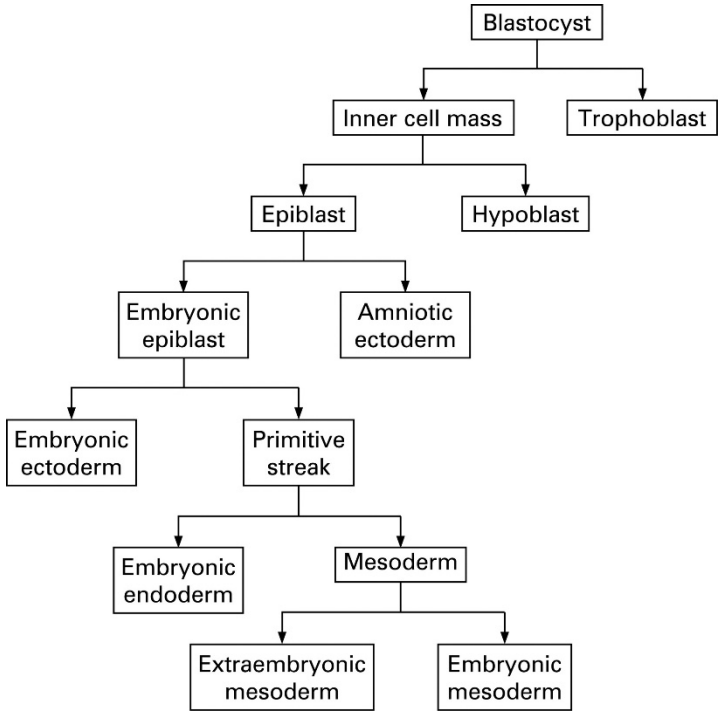
Stroma Interactions

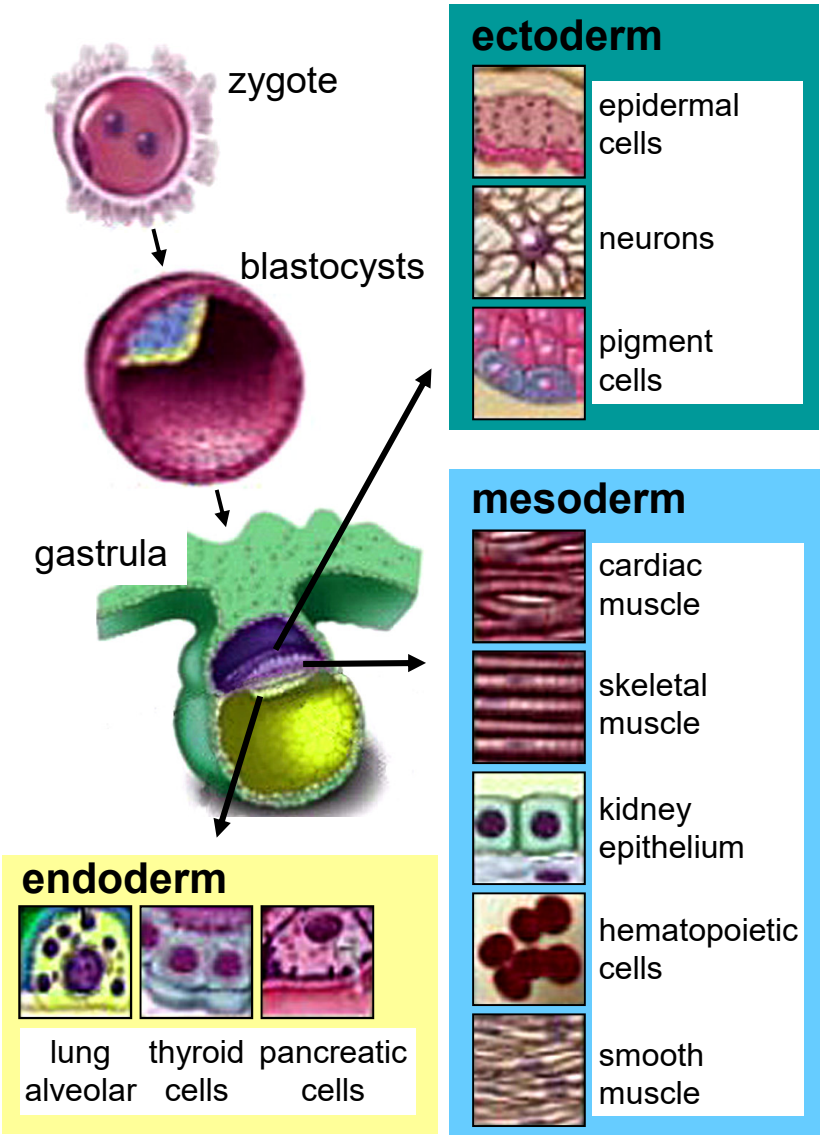
ECM codes



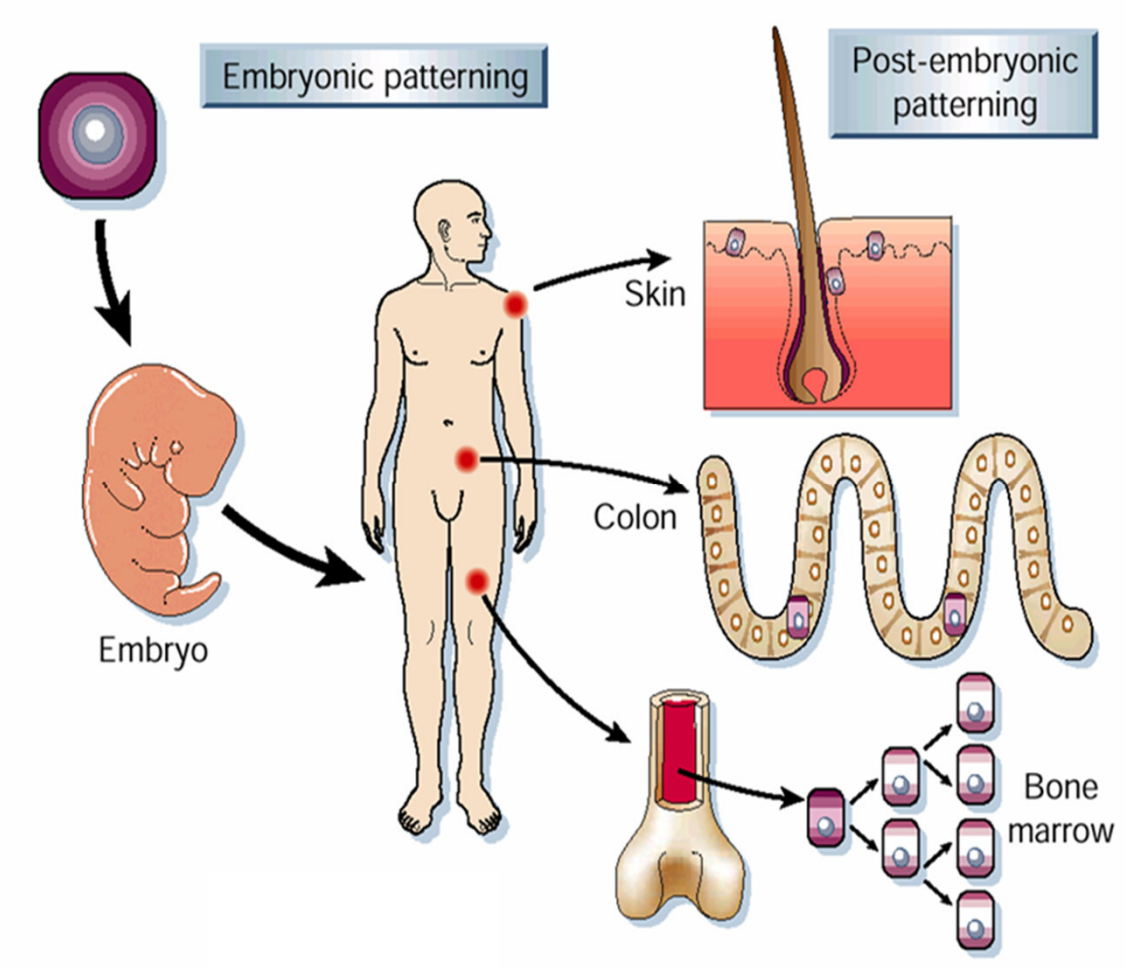
Hierarchical organization principles in development

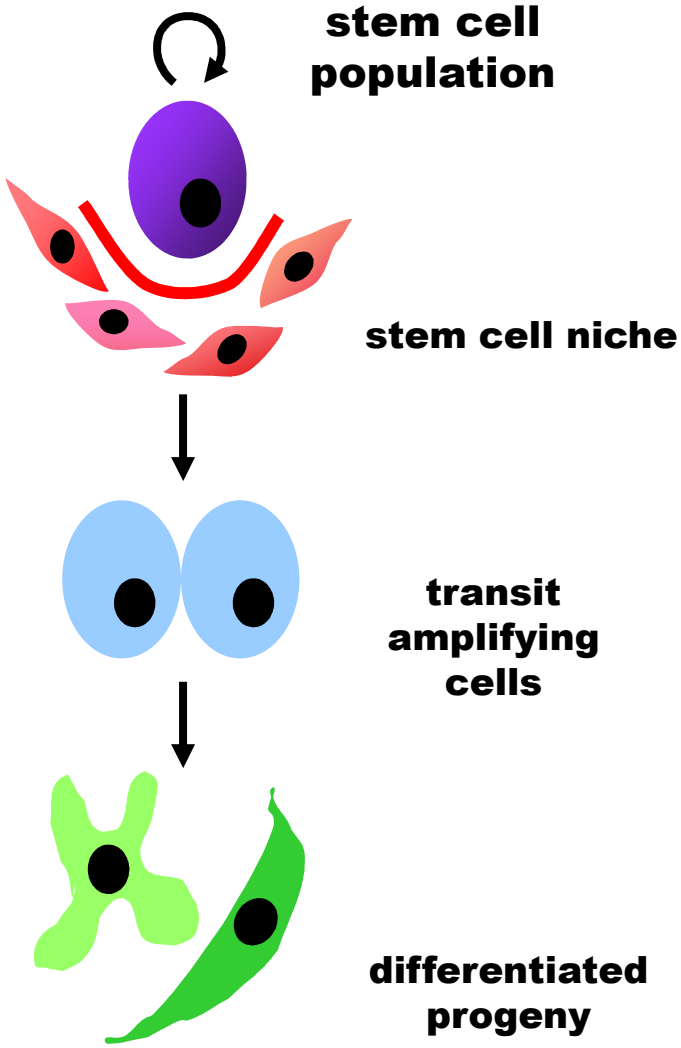
- hierarchical organization
- transient progenitor populations with increasingly narrowed potential
- successive patterning and specification events
- repeated use of few, conserved signaling pathways (Wnt, BMP, FGF, HH, Notch, ...)





Hierarchical organization in tissue homeostasis





CSC in AML: the first study

ARTICLES

Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell

DOMINIQUE BONNET & JOHN E. DICK

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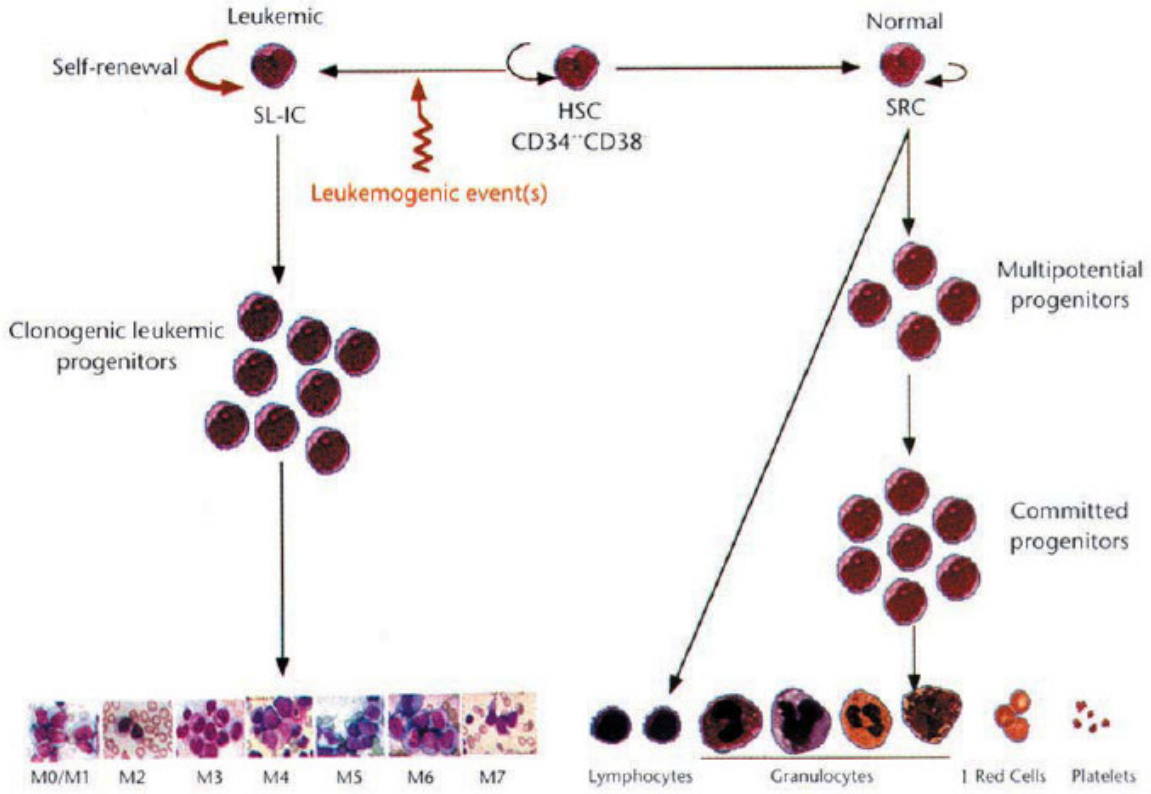
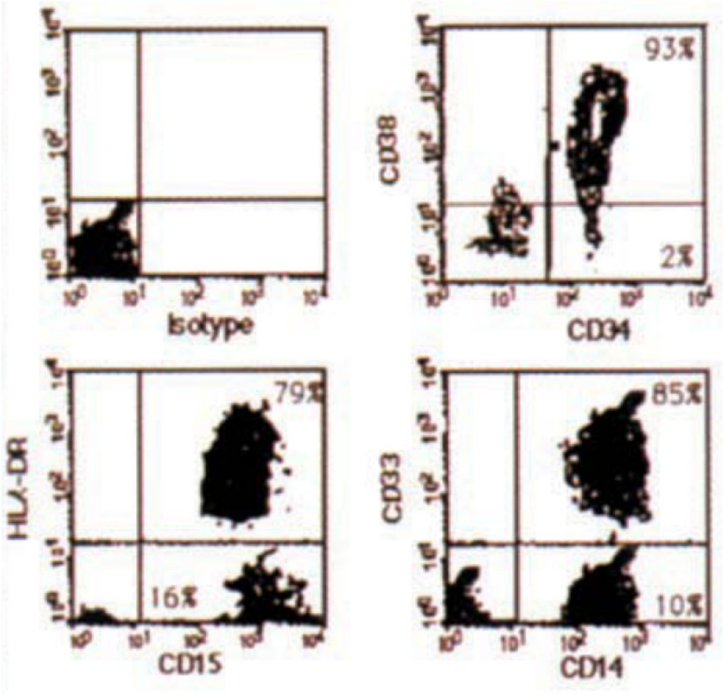


CSC in AML: the first study frequency of CSCs and their multipotency

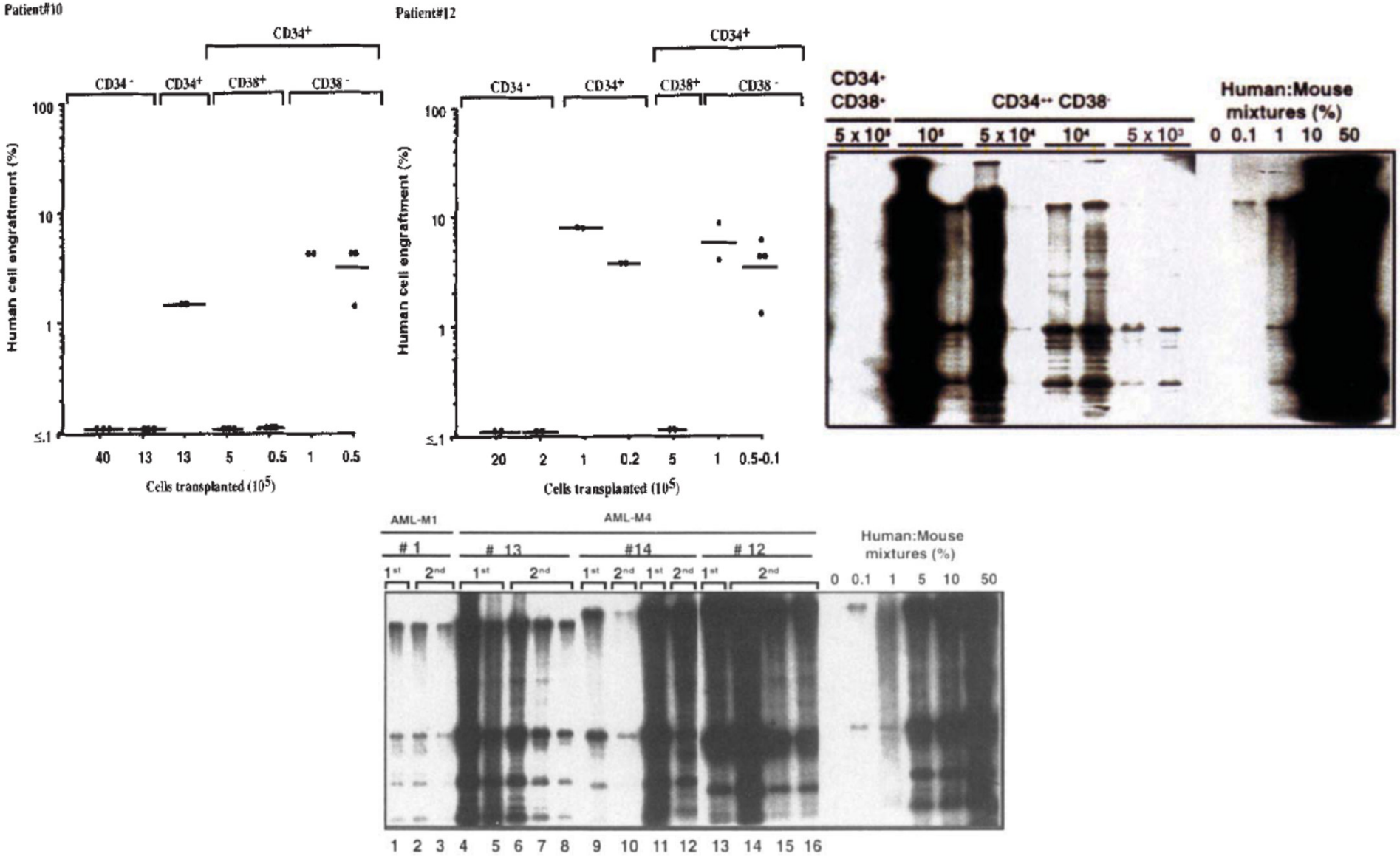
Table 1 Patient-to-patient heterogeneity in expression of CD34 and CD38 antigens

Patient	FAB subtype	Age/Sex	Level of engraftment of NOD/SCID mice with $10–20 \times 10^6$ MNCs	Percent of $CD34^+$ in MNCs	Percent of $CD34^{++}CD38^-$ in MNCs	Estimated frequency of SL-IC per 10^6 MNCs
1	M1	64/F	74 ± 10	43	0.8	100–200
8	M4	62/F	45 ± 8	80	1.0	1
10	M4	58/M	62 ± 5	11	0.75	0.2
12	M4	65/M	76 ± 6	2.0	0.2	49
13	M4	69/M	37 ± 7	95	2.0	0.2
14	M4	59/F	28 ± 9	1.1	0.2	2
18	M5	71/F	18 ± 6	0.3	0.02	0.2

FAB, French-American-British criteria[6] for subtypes; NOD/SCID mice, non-obese diabetic mice with severe combined immunodeficiency disease; MNCs, mononuclear cells; SL-IC, SCID leukemia-initiating cell.



CSC in AML: the first study
serial transplantation assays

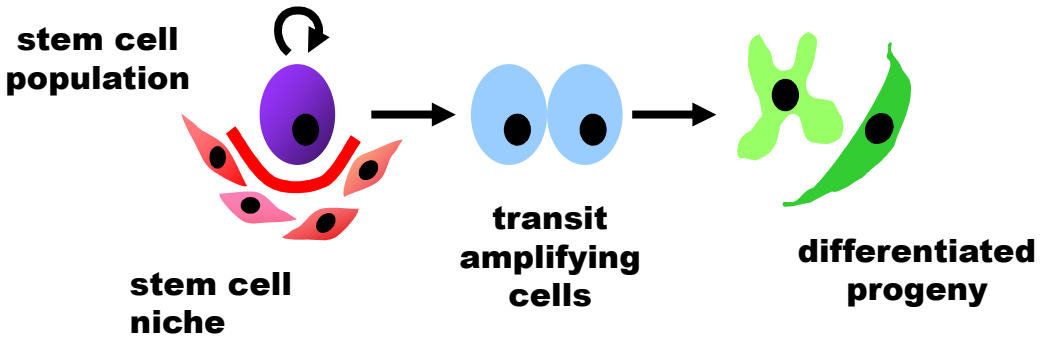


Definition of Tissue-specific vs. Cancer Stem Cells

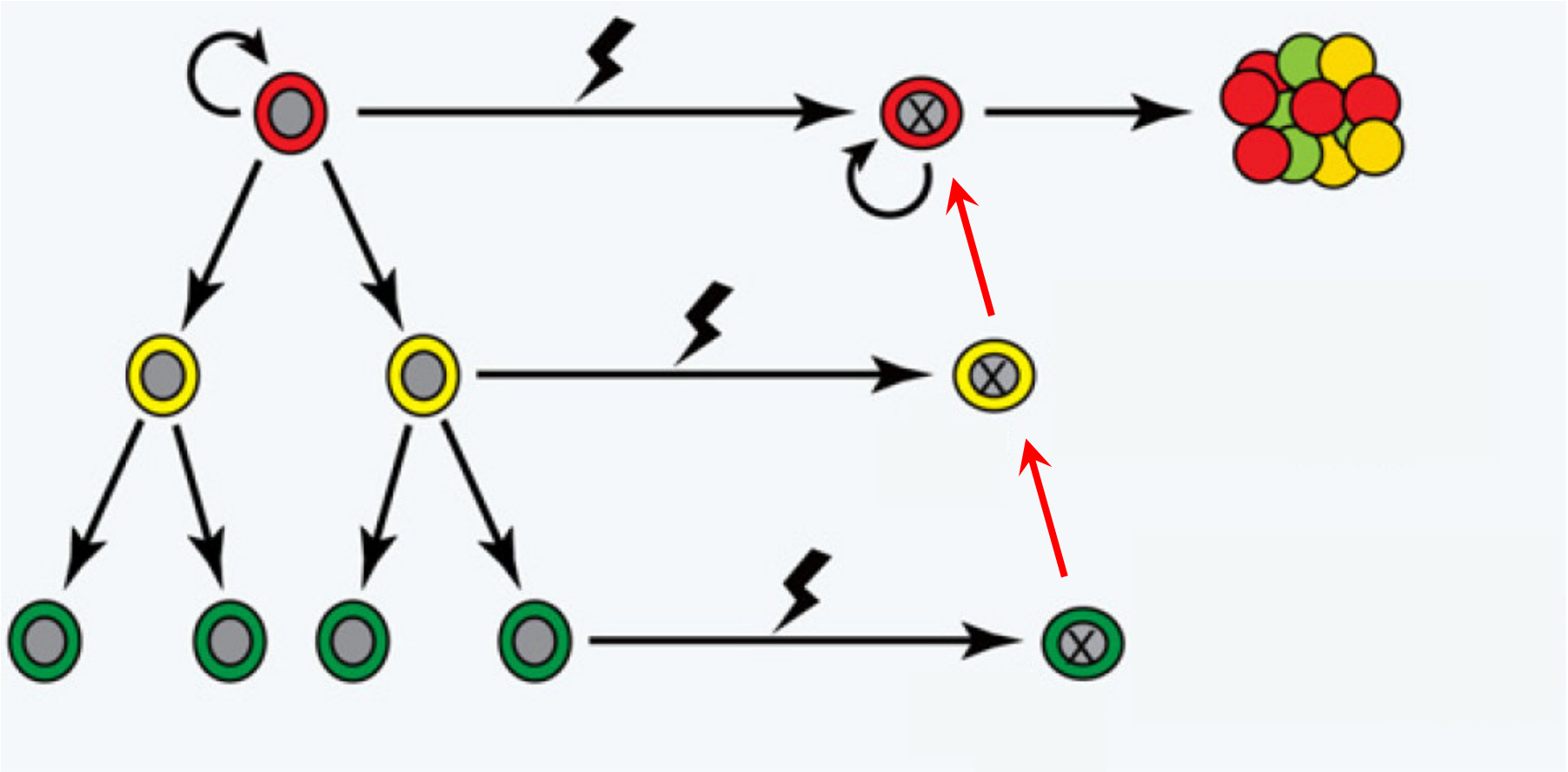
heterogeneous population of undifferentiated cells,

<u>defined by functional assays</u> and capable of:

1) **self-renewing/self-maintaining their population**
   self-renewing/self-maintaining their population
2) **production of differentiated, functional progeny (multipotency)**
   production of heterogeneic, (abberantly) differentiated progeny
3) **regenerating a functional tissue after injury or upon transplantation**
   regenerating an exact copy of the primary tumor upon transplantation
4) **interaction with an appropriate environment (niche), homing to the niche**
   self-sufficiency in growth stimulatory signals, evasion of growth-restrictive signal

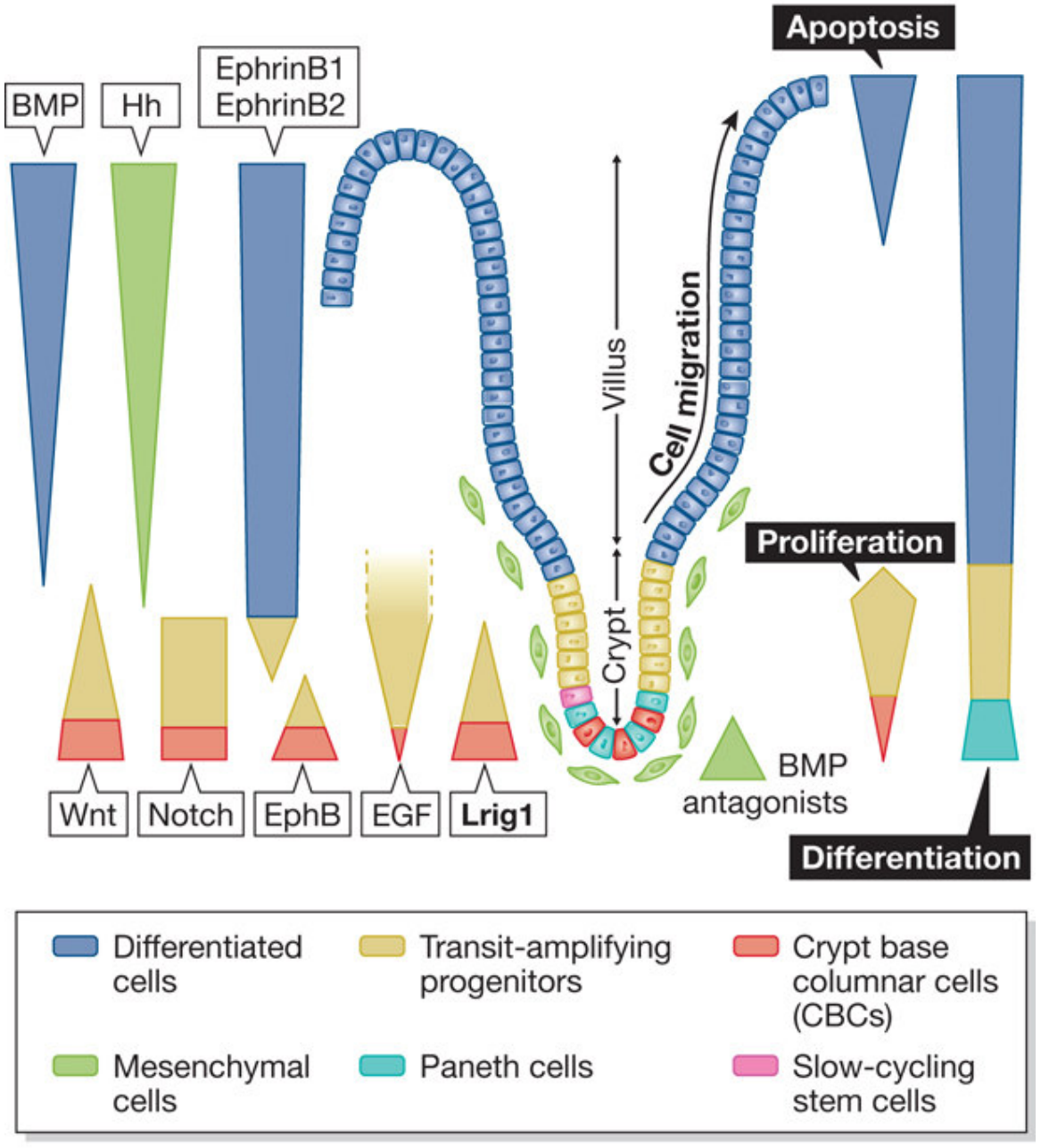


Cell of origin

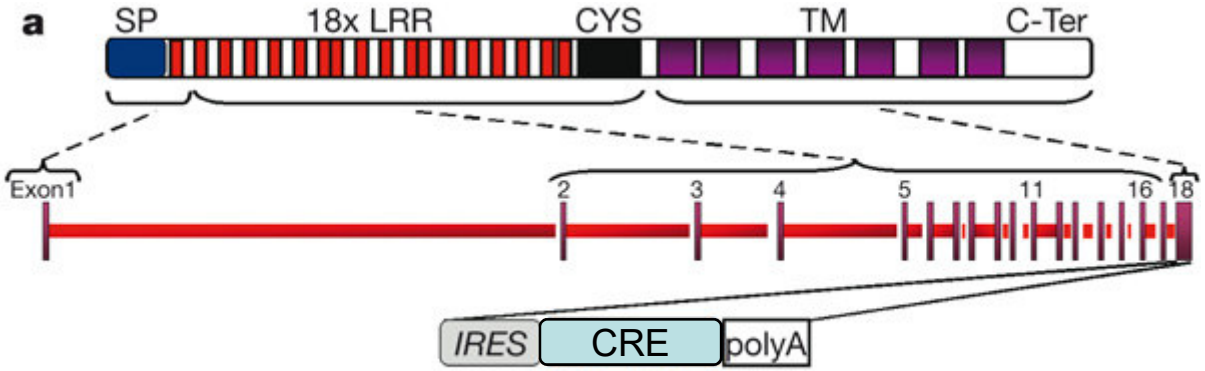


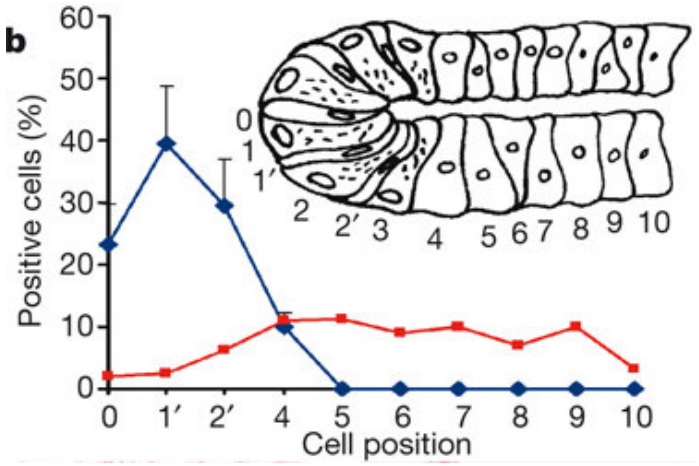
modified from Magee et al. ***Cancer Cell*** *2012*

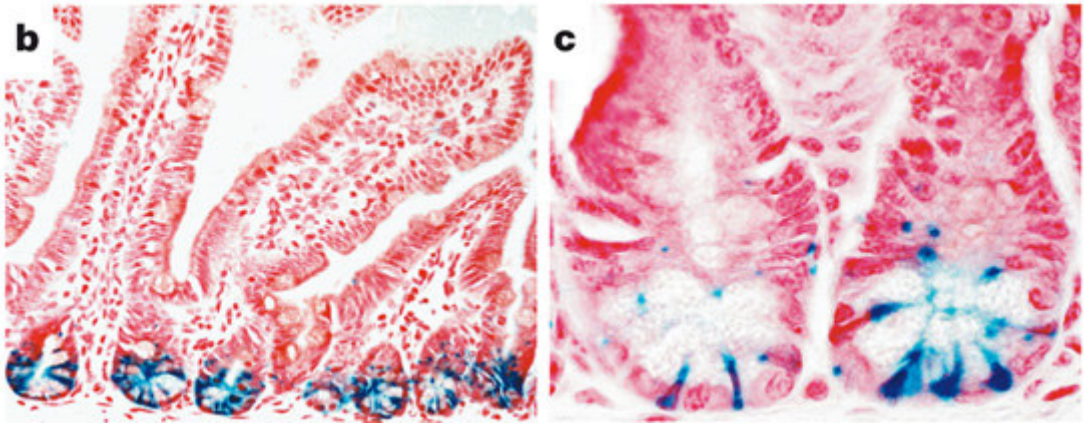
Cues controlling intestinal homeostasis

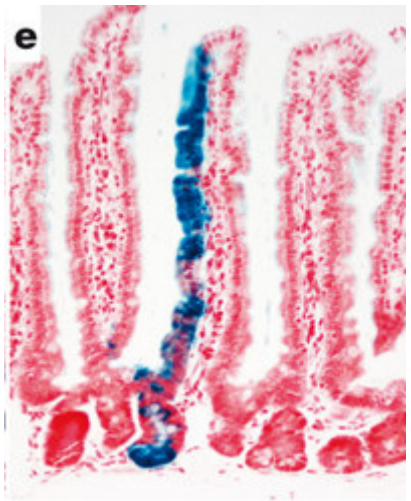


Intestinal stem cell specific gene modification (Lgr5-Cre)



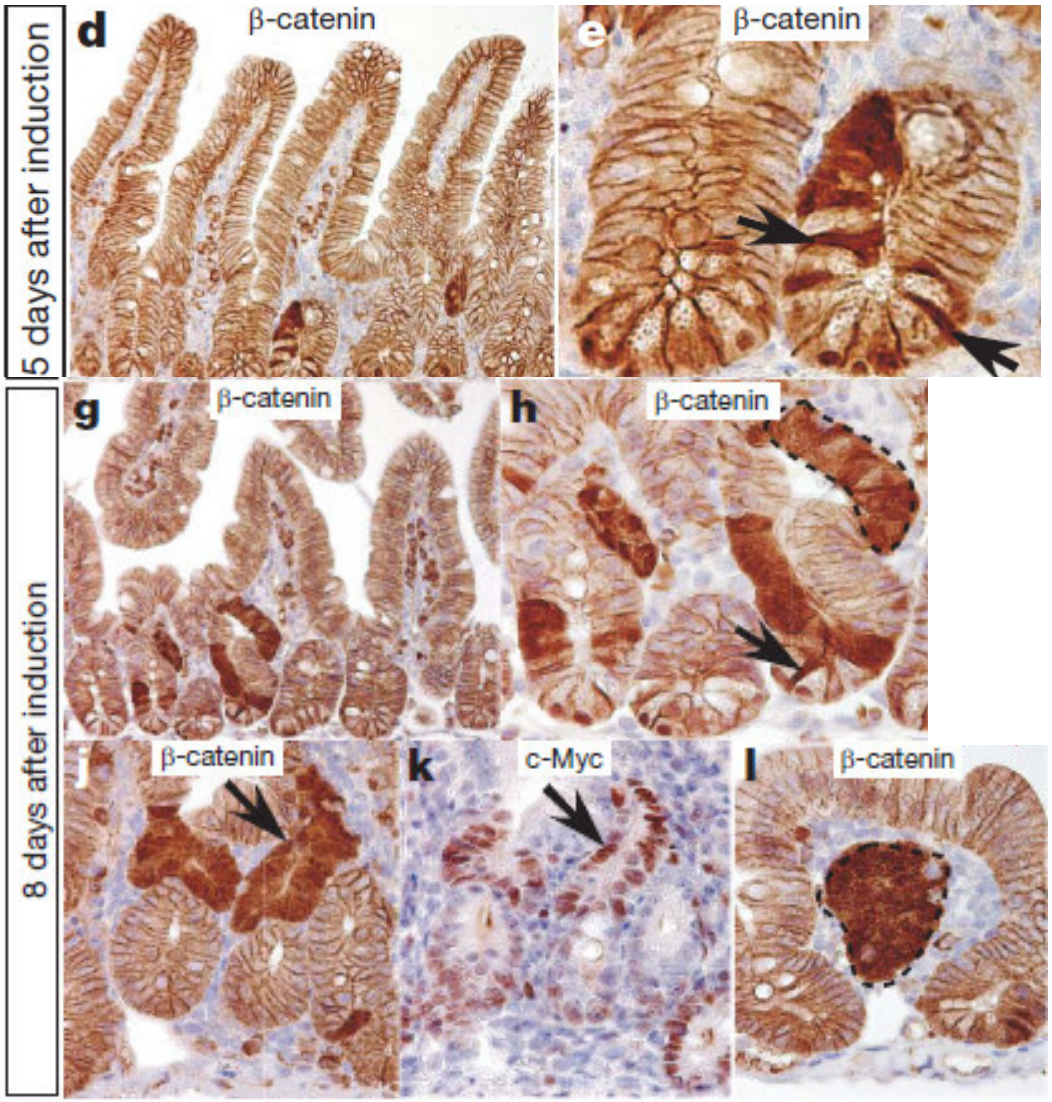


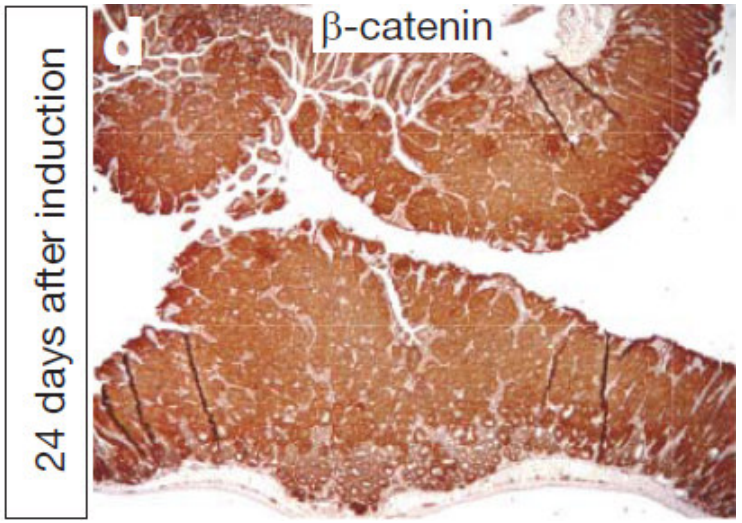




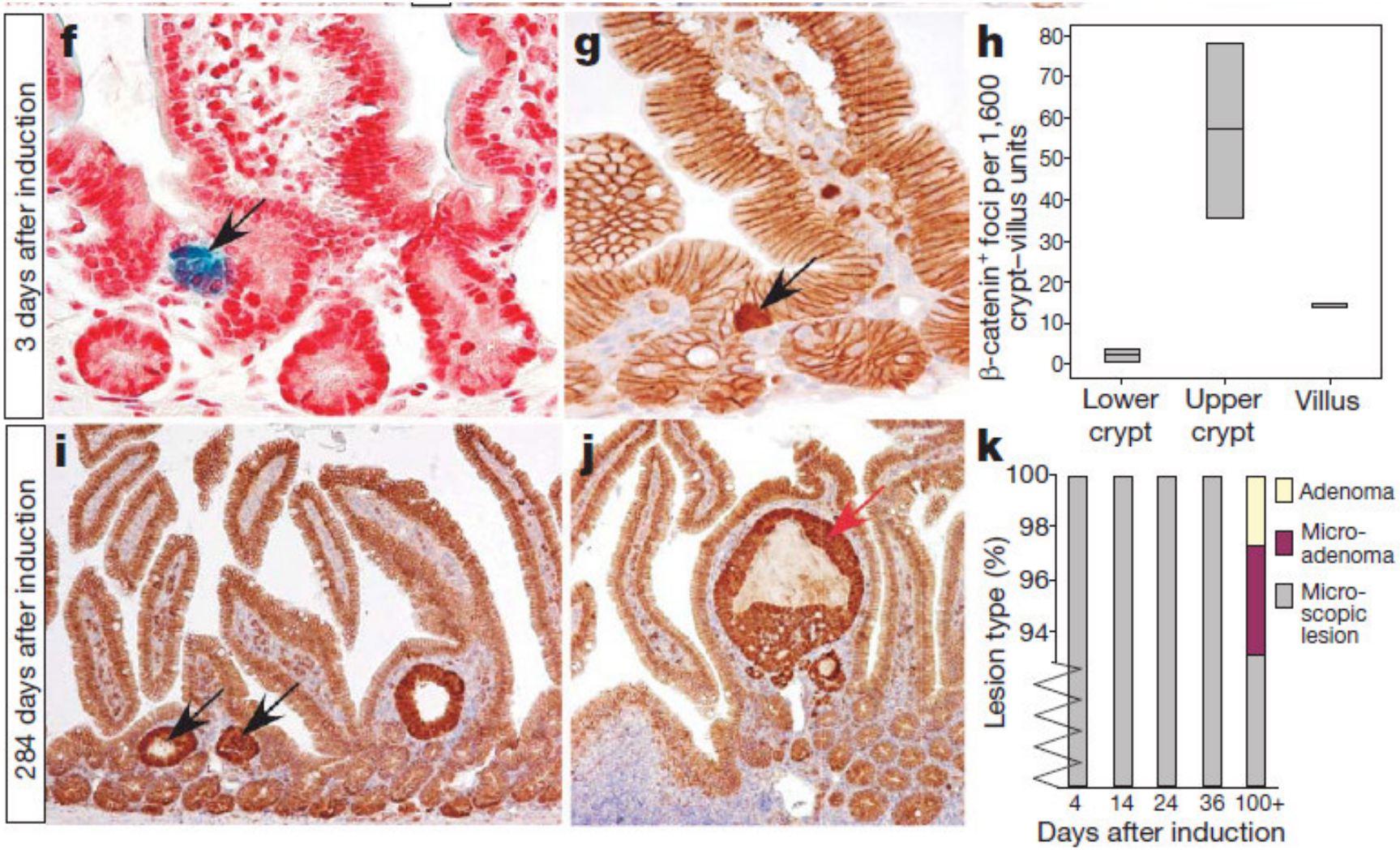
2 months

Stem cell specific induction of APC loss induces cancer (Lgr5-Cre x APC$^{lox/lox}$)

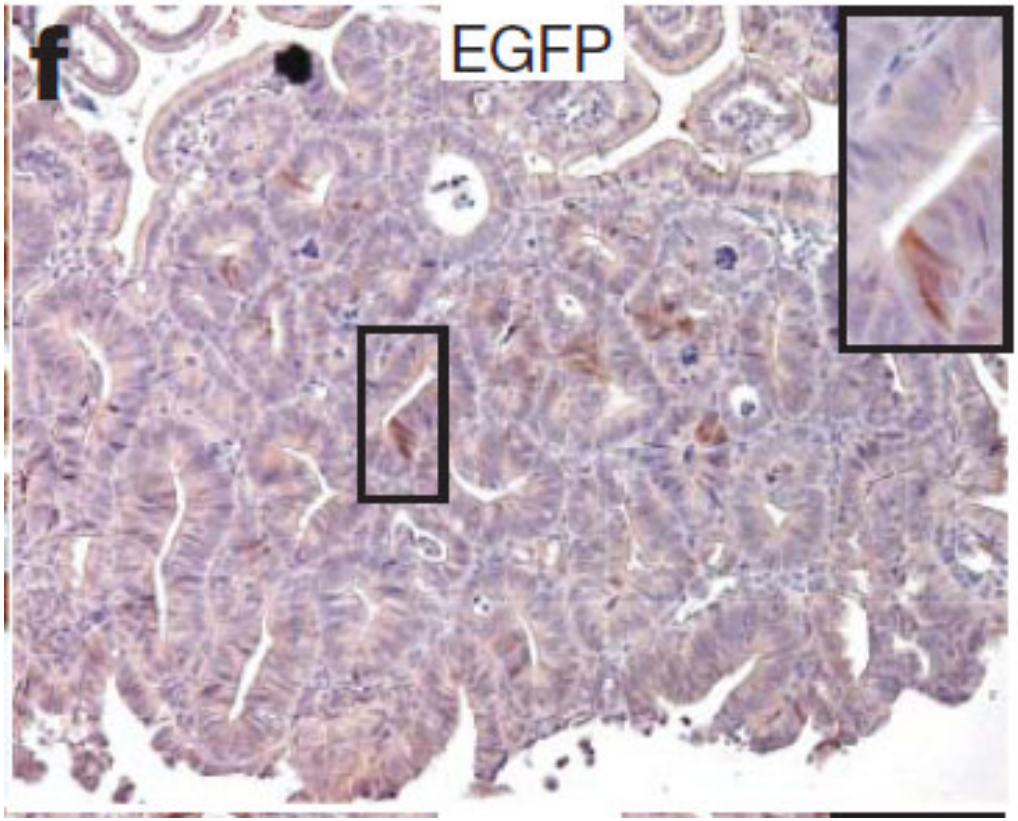


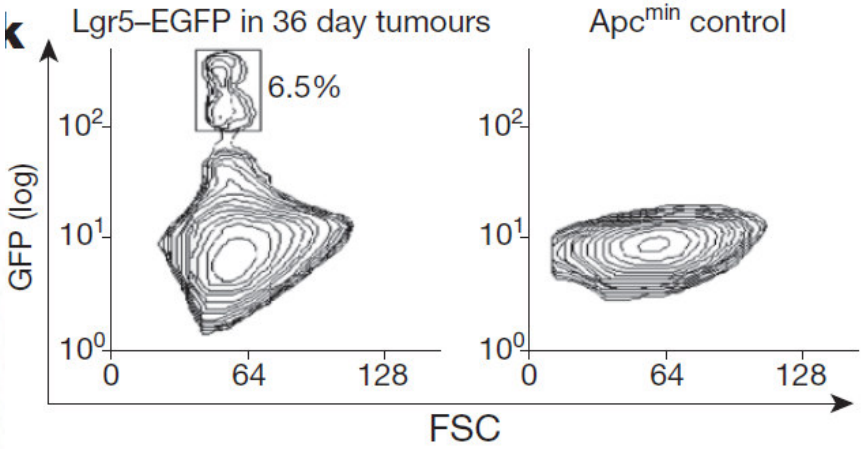


APC loss in any epithelial cell fails to induce cancer (Ah-Cre x APC $^{lox/lox}$)



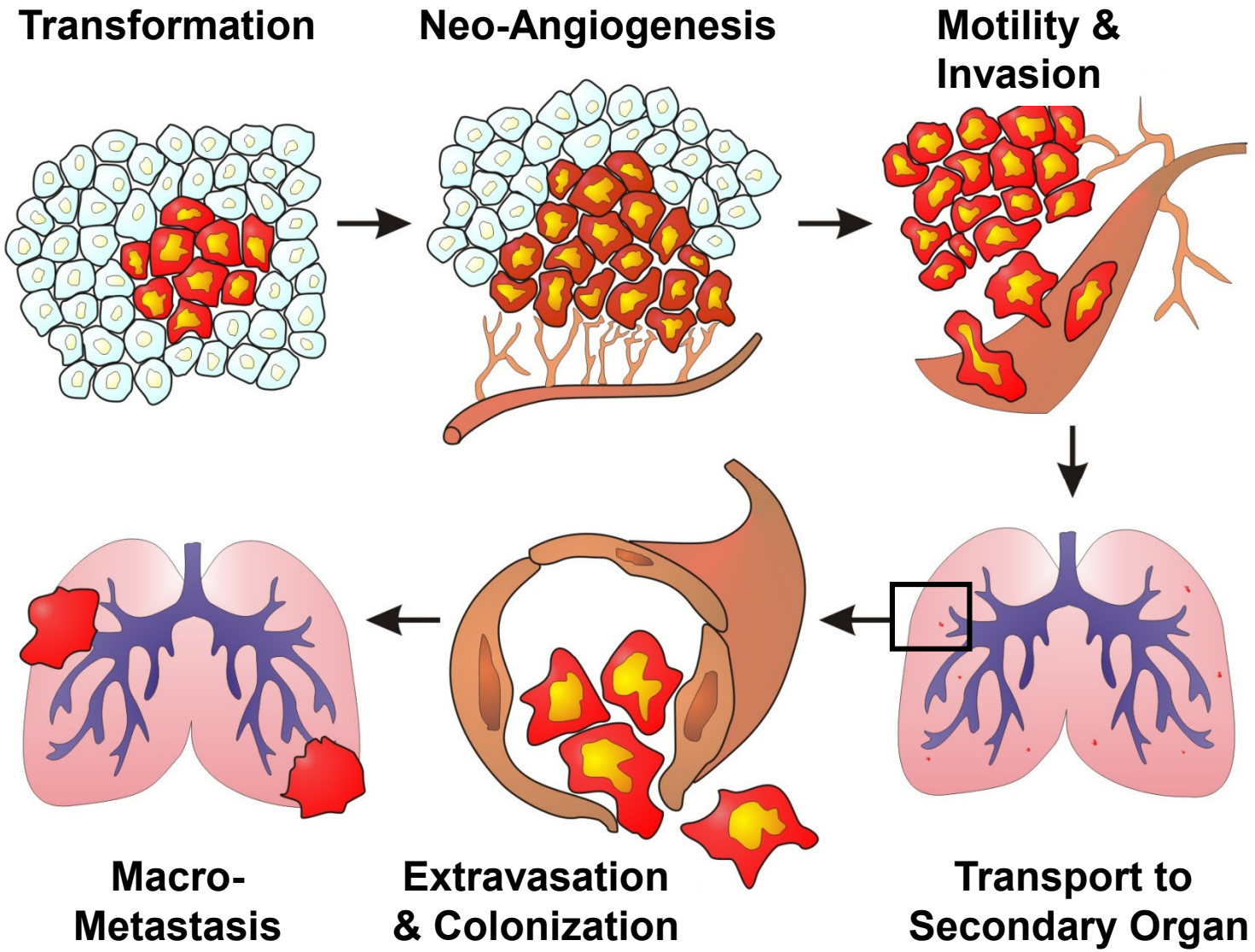
Rare Lgr5+ population in adenomas





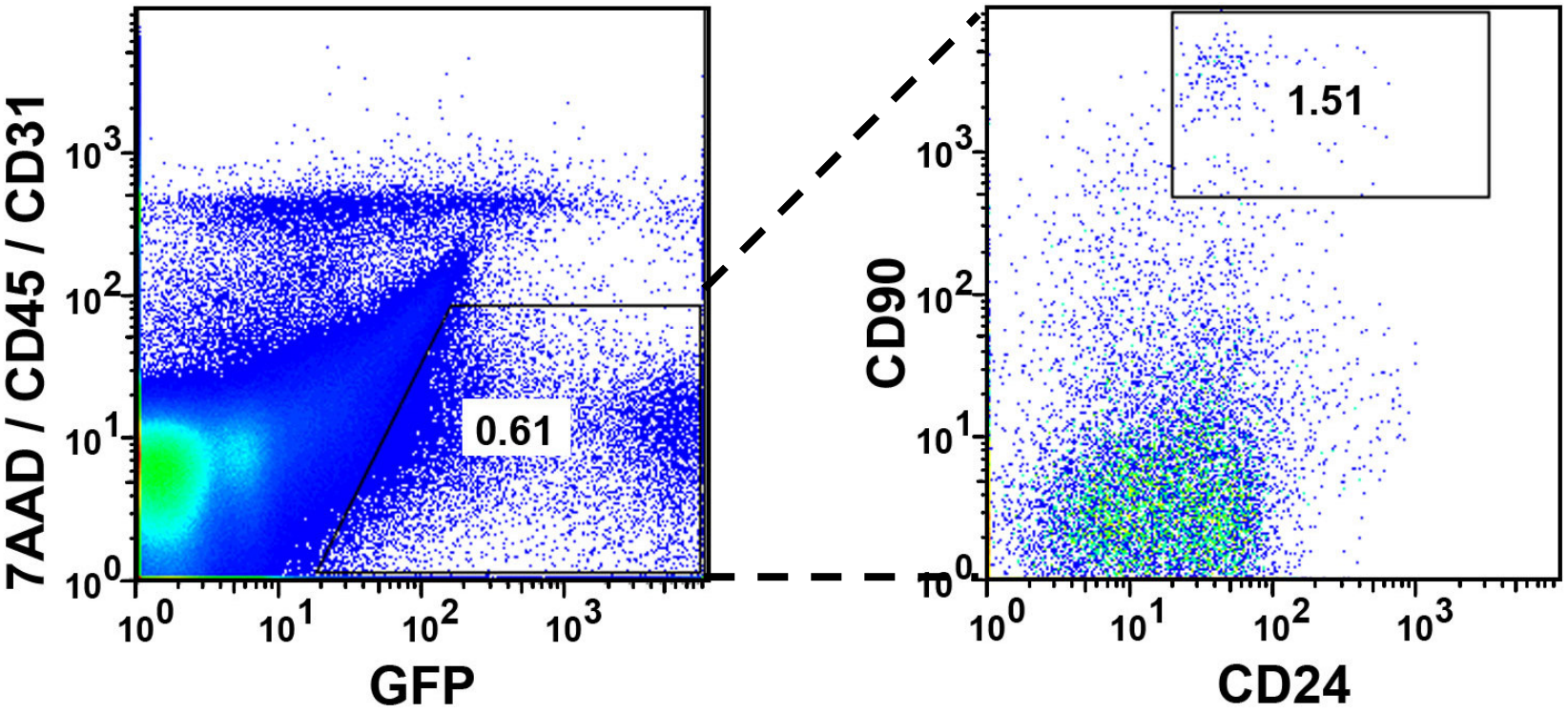
CSCs in cancer progression

Linear Cancer Progression Model



Murine breast cancer model: MMTV-PyMT

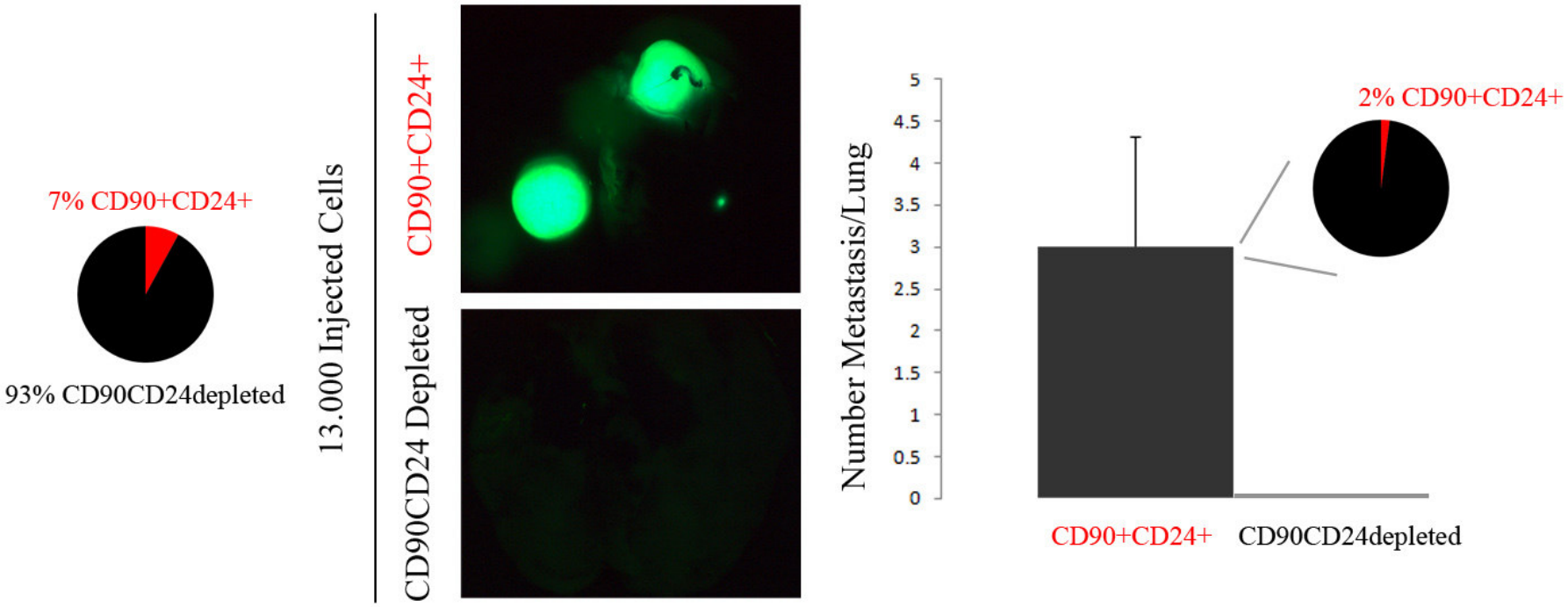
Isolation of $CD24^{+}CD90^{+}$ cancer stem cells



Malanchi et al., ***Nature 2012***

The CD24^{+}CD90^{+} population is responsible for metastasis initiation

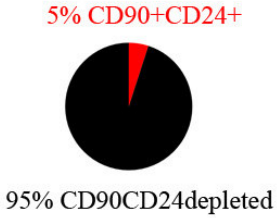
(cells isolated from primary tumors)

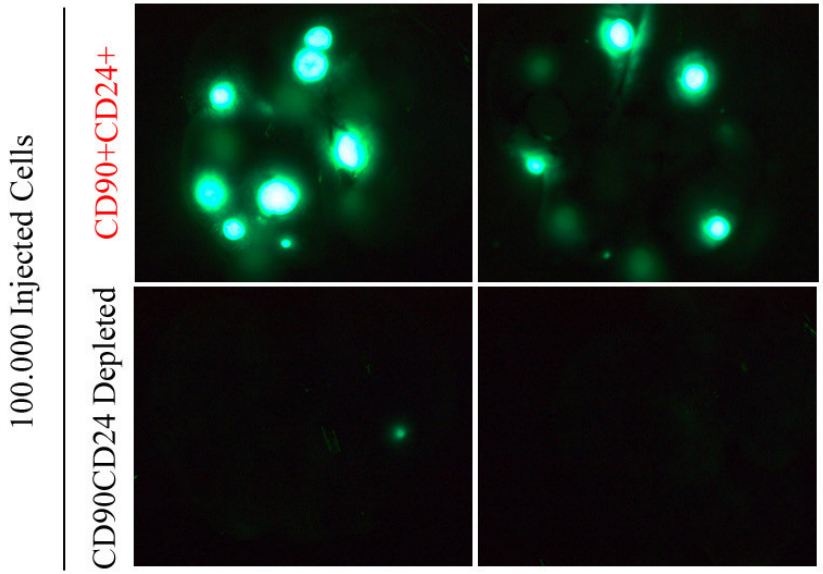


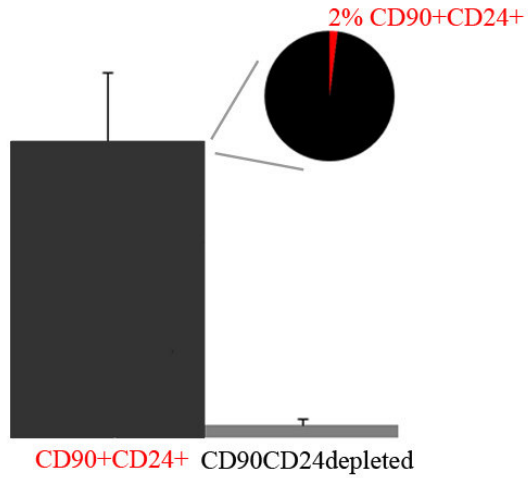
Malanchi et al., ***Nature 2012***

The CD24^{+}CD90^{+} population is responsible for metastasis re-initiation

(cells isolated from metastasis)

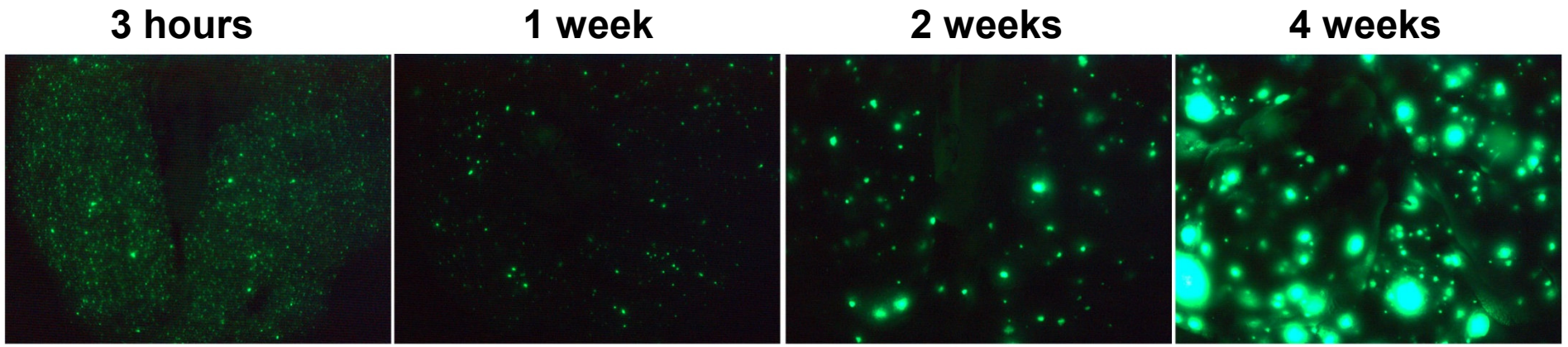


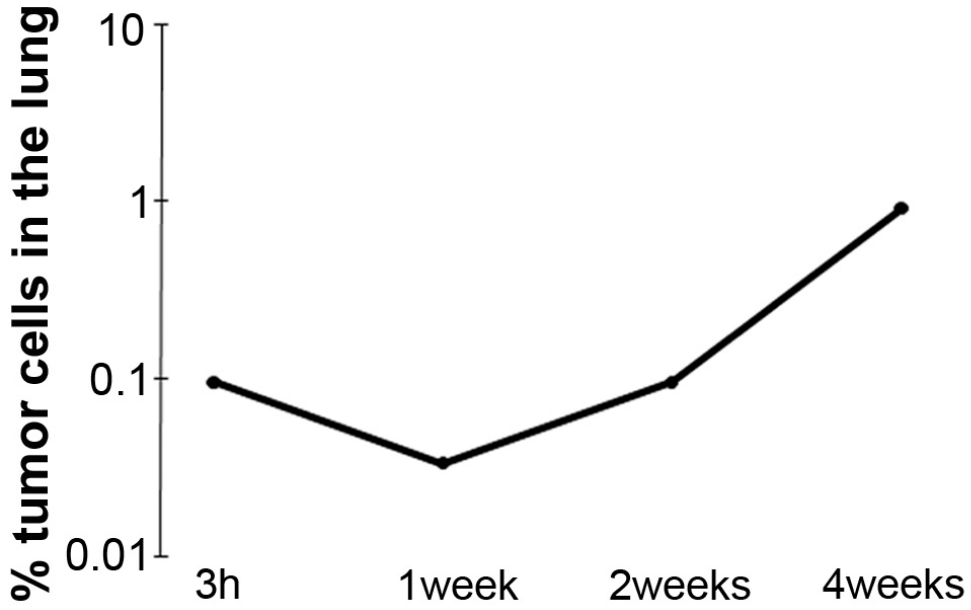




Malanchi et al., ***Nature 2012***

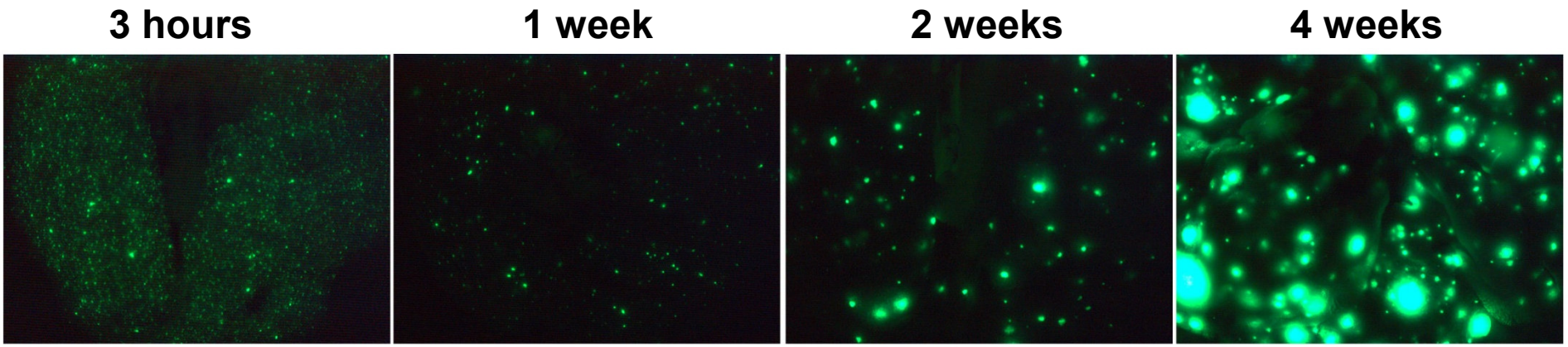
Early metastatic colonization relies on cancer stem cells

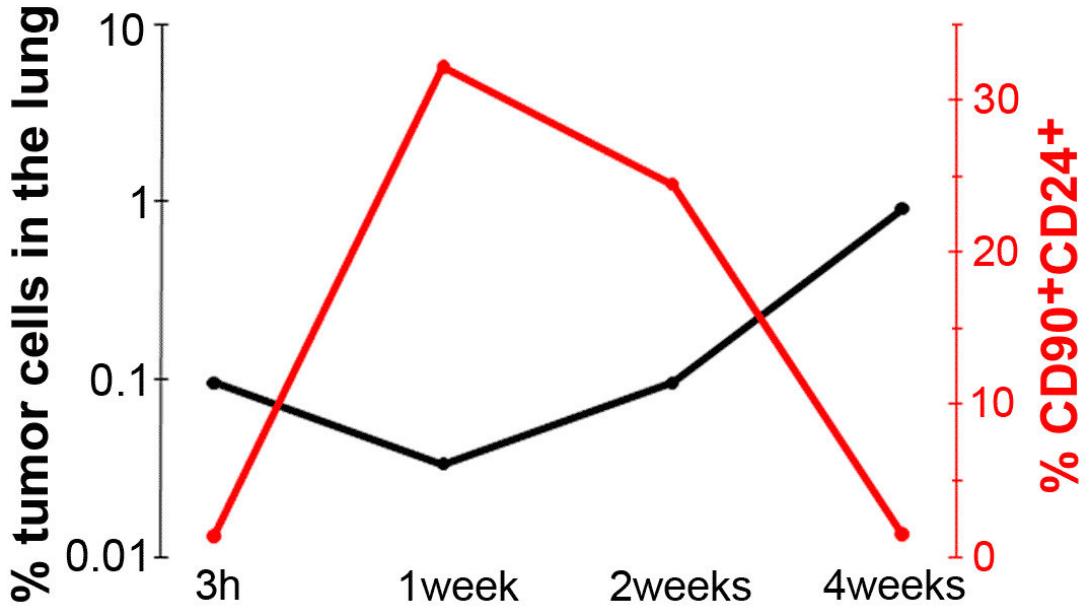




Malanchi et al., ***Nature 2012***

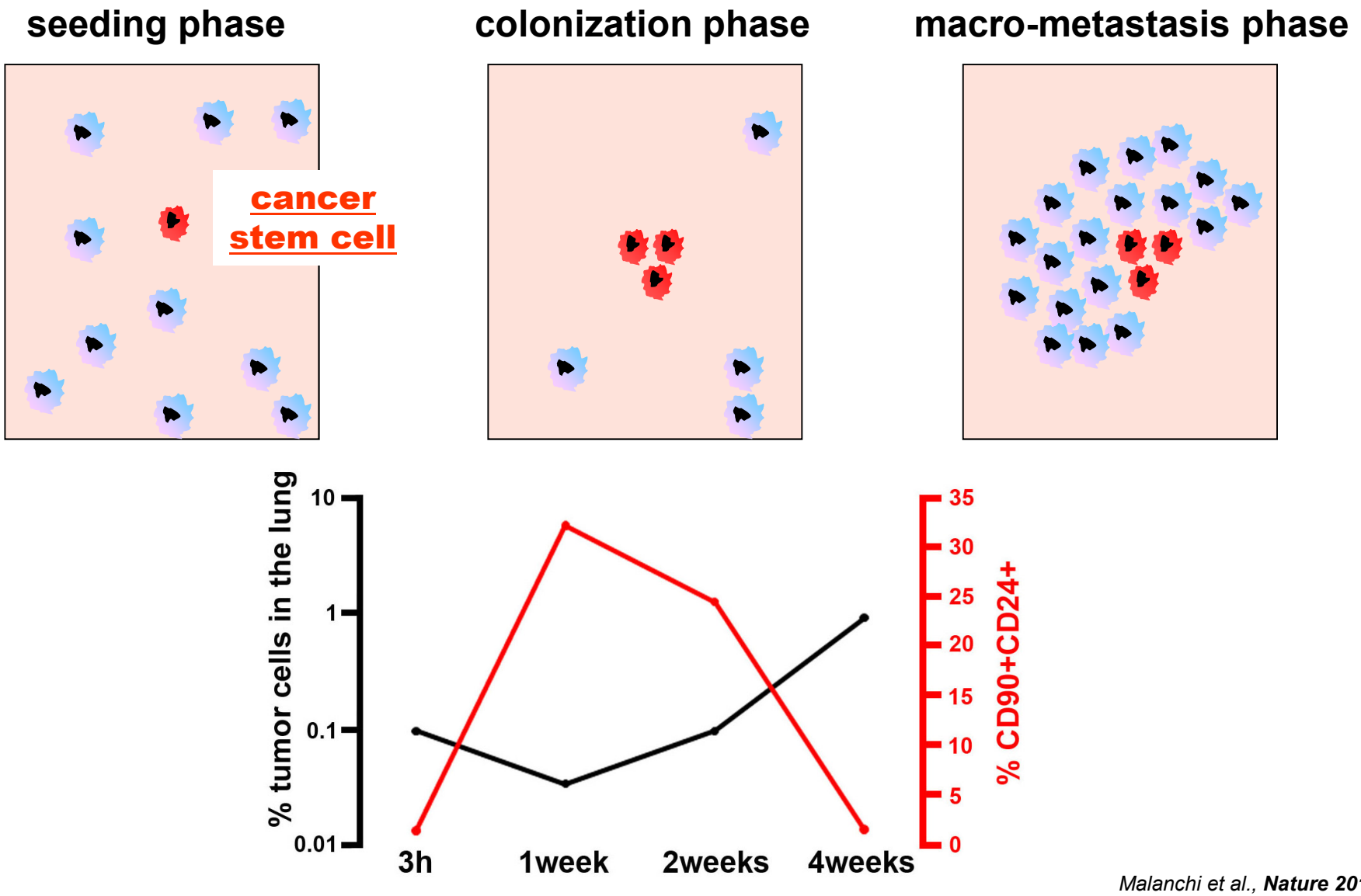
Early metastatic colonization relies on cancer stem cells





Malanchi et al., ***Nature 2012***

Changes in the Relative Amount of Cancer Stem Cells during Metastatic Progression



Malanchi et al., **Nature 2012**

Functional characterization of CSCs

Assaying CSCs: Transplantation

- Transplantation

  the assays to functionally identify a CSC population require transplantation into mice

- **which recipient strain?**

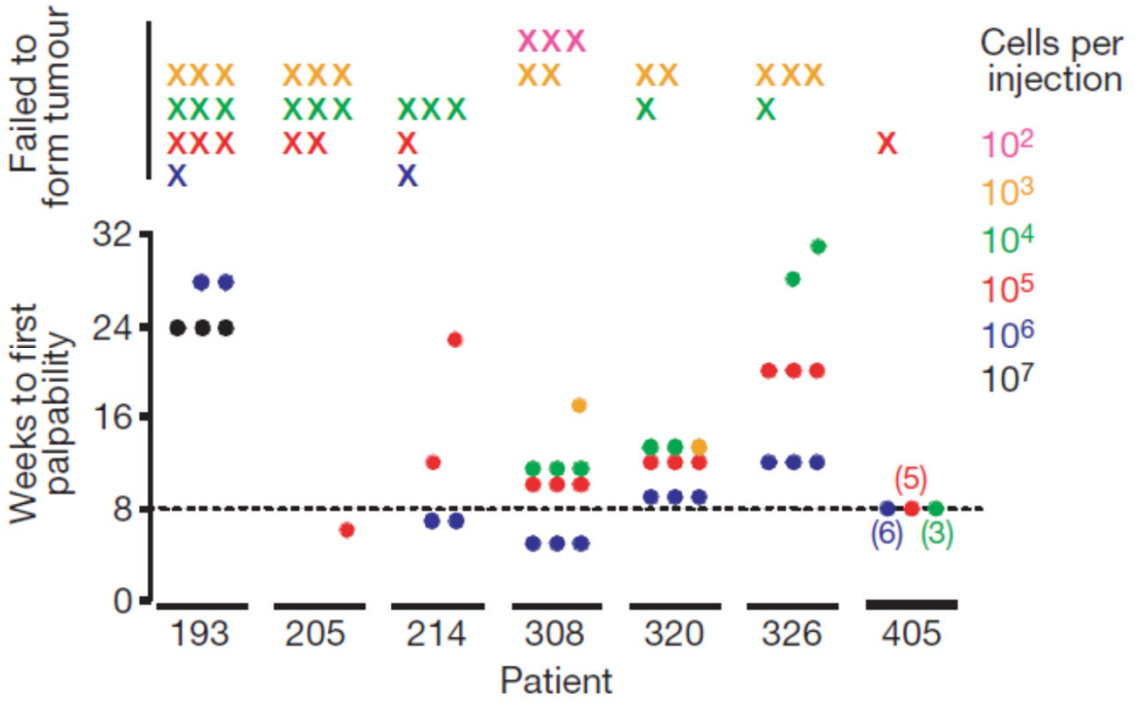
  immuno-deficient for xenotransplantation of human cells or mouse cells with foreign cDNAs
  - *Nude* (no thymus => hardly any $\alpha\beta$T cells, but B cells, $\gamma\delta$T cells and all other innate immune cells)
  - *RAG* or *NOD/SCID* (neither T nor B cells, but innate immune cells)
  - *RAG/cg* or *NOD/SCID/cg=NSG* (in addition lack of NK cells, less myeloid cells)
- **which route?**
  - subcutaneous (artificial morphology)
  - kidney capsule
  - orthotopic (good morphology and invasion possible, but often difficult to perform)
  - tail vein (metastasis-type of experiments, requires high cell numbers)
- **which other variables?**
  - cells embedded in ECM proteins

- **only selection for the most robust cells?**

- **only selection for cells which can cope with the species barrier (lack of factors)?**

Detection of CSCs can depend on the transplantation system

transplantation into *NOD/SCID*



Time after injection	Melanoma-initiating cell frequency (95% confidence interval)	
8 weeks	1/837,000	(1/512,000–1/1,370,000)
32 weeks	1/111,000 *	(1/67,000–1/185,000)

Detection of CSCs can depend on the transplantation system

transplantation into *NSG*

Patient	Mouse strain	Co-injection	Number of tumours / number of injections (cells per injection) 50,000	5,000	50	5	Melanoma-initiating cell frequency (95% confidence interval)	
481	NOD/SCID	Vehicle	0/3	0/6	0/3			(<1/60,000)
	NOD/SCID *Il2rg*$^{-/-}$	Matrigel			6/6	4/6	1/5*	(1/2–1/13)
491	NOD/SCID	Vehicle		0/3	0/6			(<1/5,100)
	NOD/SCID *Il2rg*$^{-/-}$	Matrigel			6/6	1/6	1/15*	(1/6–1/40)
492	NOD/SCID	Vehicle	3/3	3/6	0/6		1/7,300	(1/2,400–1/22,300)
	NOD/SCID *Il2rg*$^{-/-}$	Matrigel			6/6	2/6	1/11*	(1/4–1/31)
All	NOD/SCID	Vehicle	3/6	3/15	0/15		1/46,700	(1/19,600–1/110,900)
	NOD/SCID *Il2rg*$^{-/-}$	Matrigel			18/18	7/18	1/9*	(1/5–1/18)

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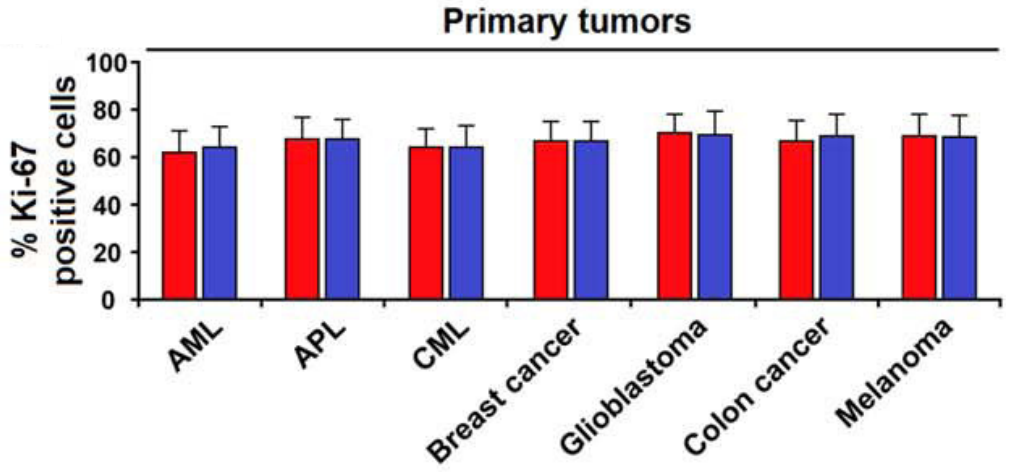
ARTICLES

Efficient tumour formation by single human melanoma cells

Elsa Quintana[1]*, Mark Shackleton[1]*, Michael S. Sabel[2], Douglas R. Fullen[3], Timothy M. Johnson[4] & Sean J. Morrison[1]

Proliferation is similar in CSCs and nonCSCs but tumor induction upon transplantation differs



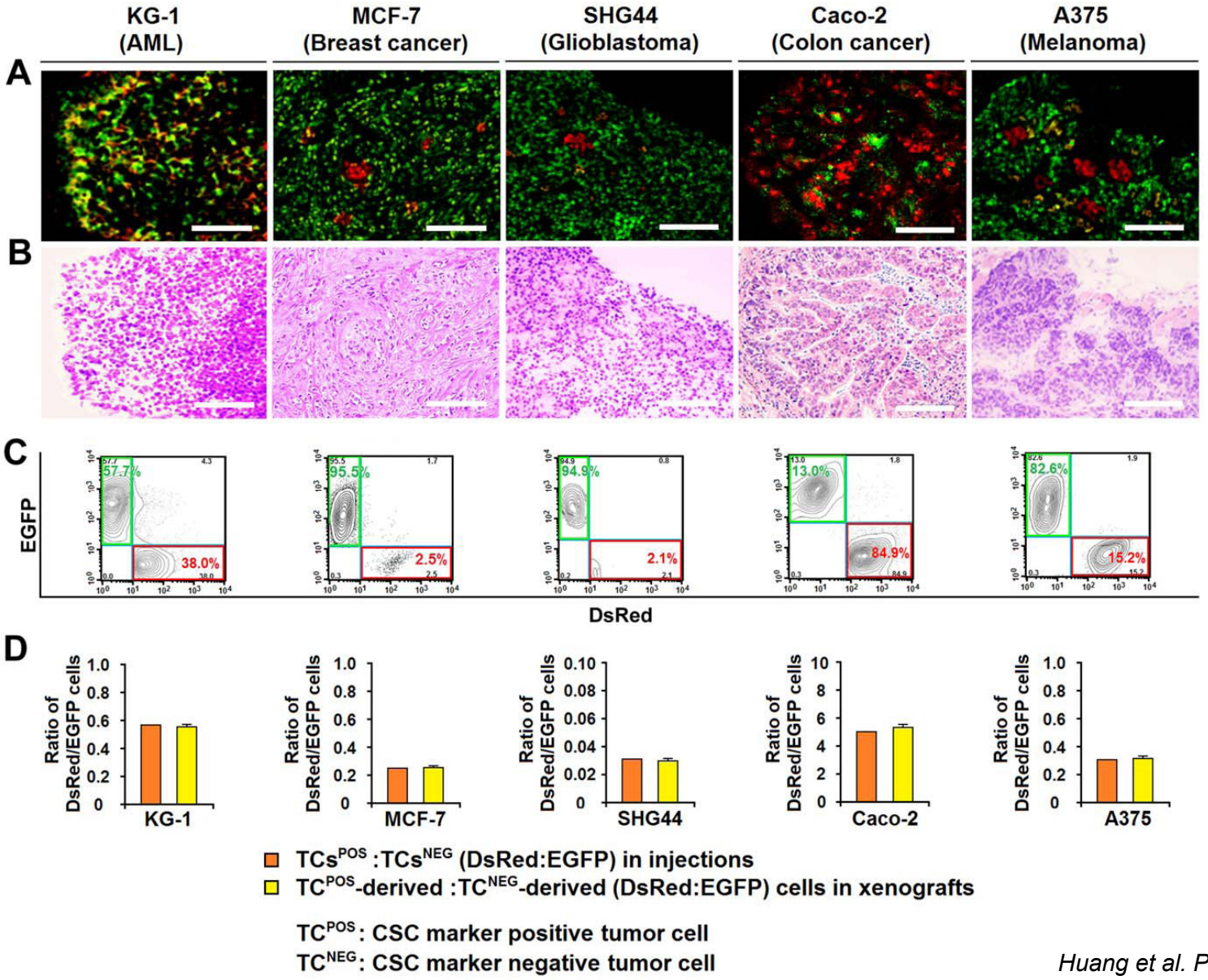
Cell line	KG-1		THP-1		HL60		K562	
Marker subset	CSC+	CSC-	CSC+	CSC-	CSC+	CSC-	CSC+	CSC-
Frequency of tumorigenic cells	1/2164	1/68078**	1/2164	1/280030**	1/4170	1/68078**	1/10720	1/135931**

Cell line	MCF-7		MDA-MB-231		SHG44		U251	
Marker subset	CSC+	CSC-	CSC+	CSC-	CSC+	CSC-	CSC+	CSC-
Frequency of tumorigenic cells	1/2164	1/280030**	1/4170	1/108957**	1/2164	1/135931**	1/2164	1/280030**

Cell line	HT-29		SW480		SW620		A375	
Marker subset	CSC+	CSC-	CSC+	CSC-	CSC+	CSC-	CSC+	CSC-
Frequency of tumorigenic cells	1/4170	1/280030**	1/2164	1/108957**	1/2164	1/280030**	1/4170	1/43259*

Huang et al. PlosOne 2013

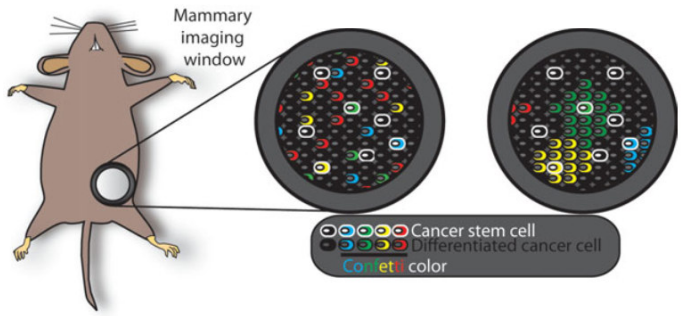
Co-transplantation of CSCs and non CSCs allows nonCSCs to participate in cancer growth

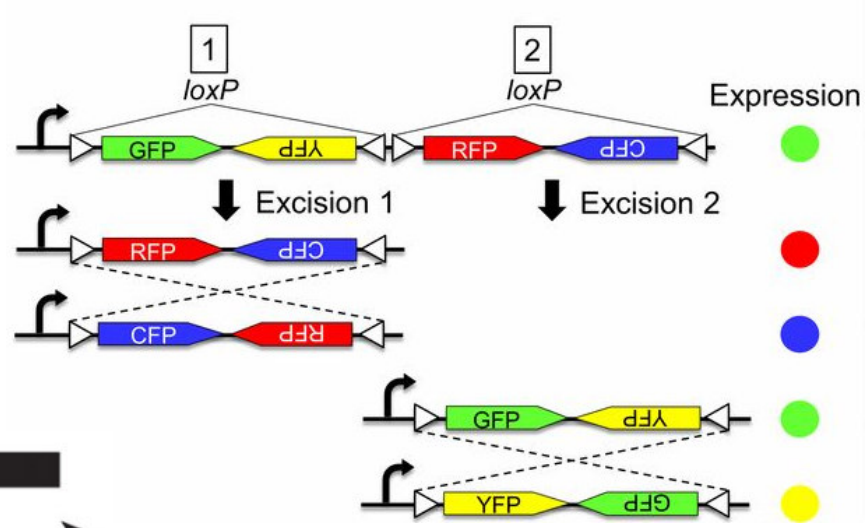


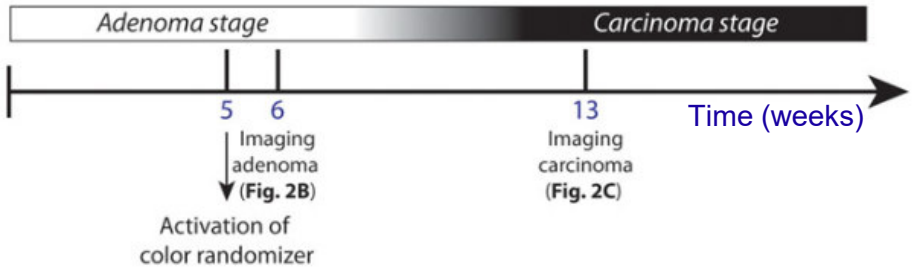
Huang et al. PlosOne 2013

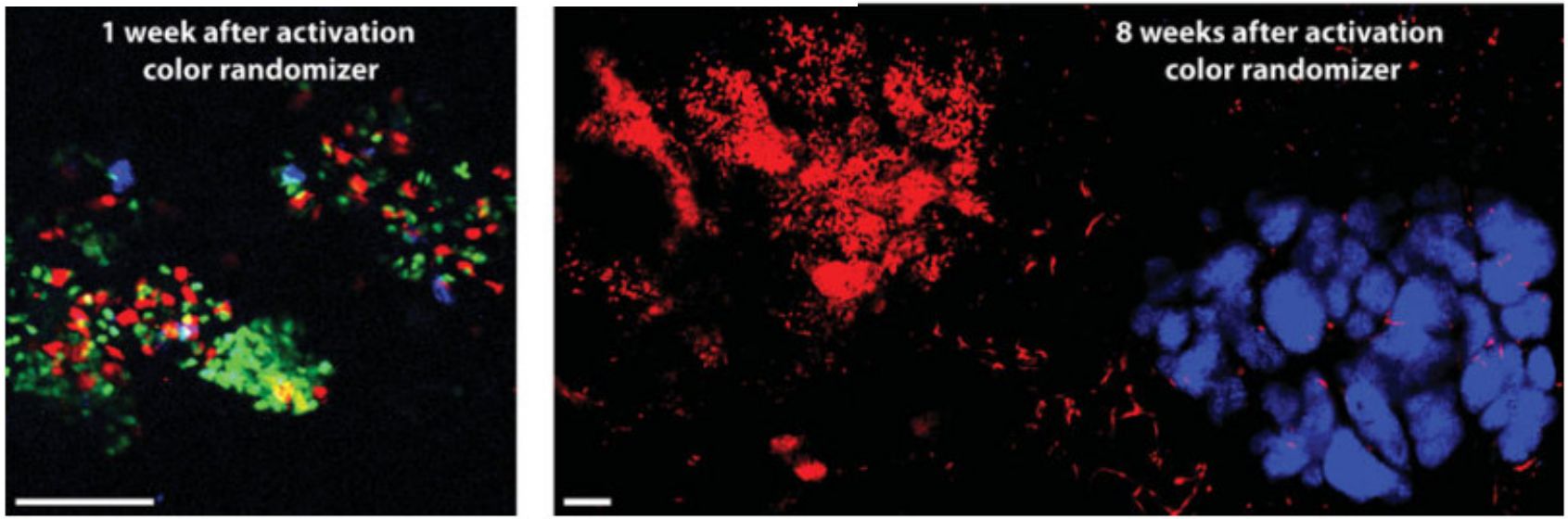
In vivo evidence for CSCs

Random labelling reveals CSC plasticity *in vivo*

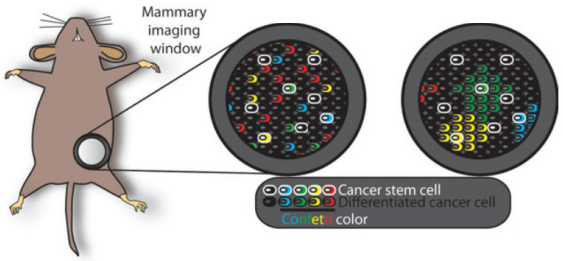


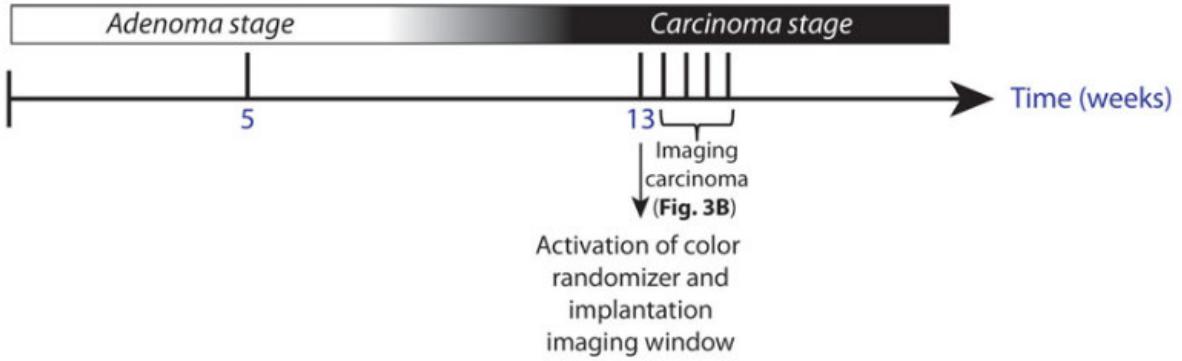


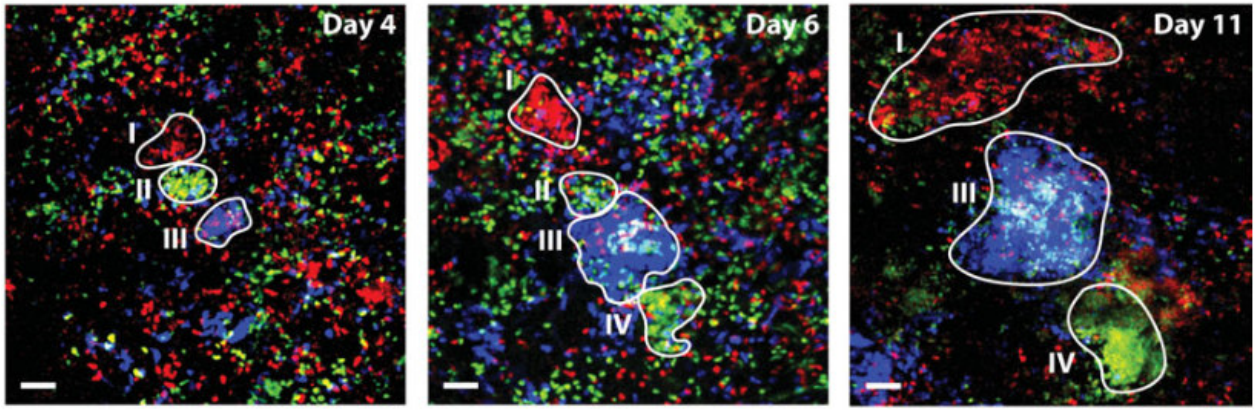


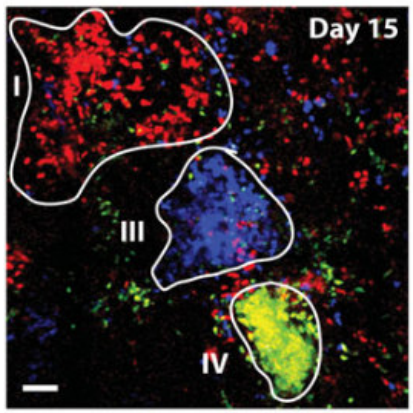


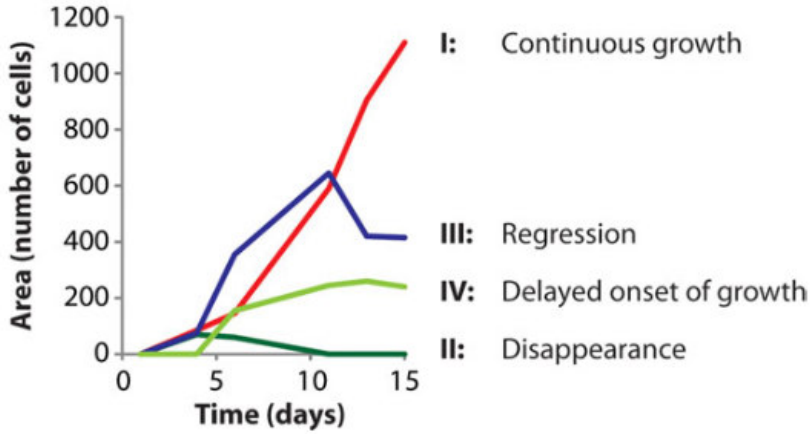
Random labelling reveals CSC plasticity *in vivo*





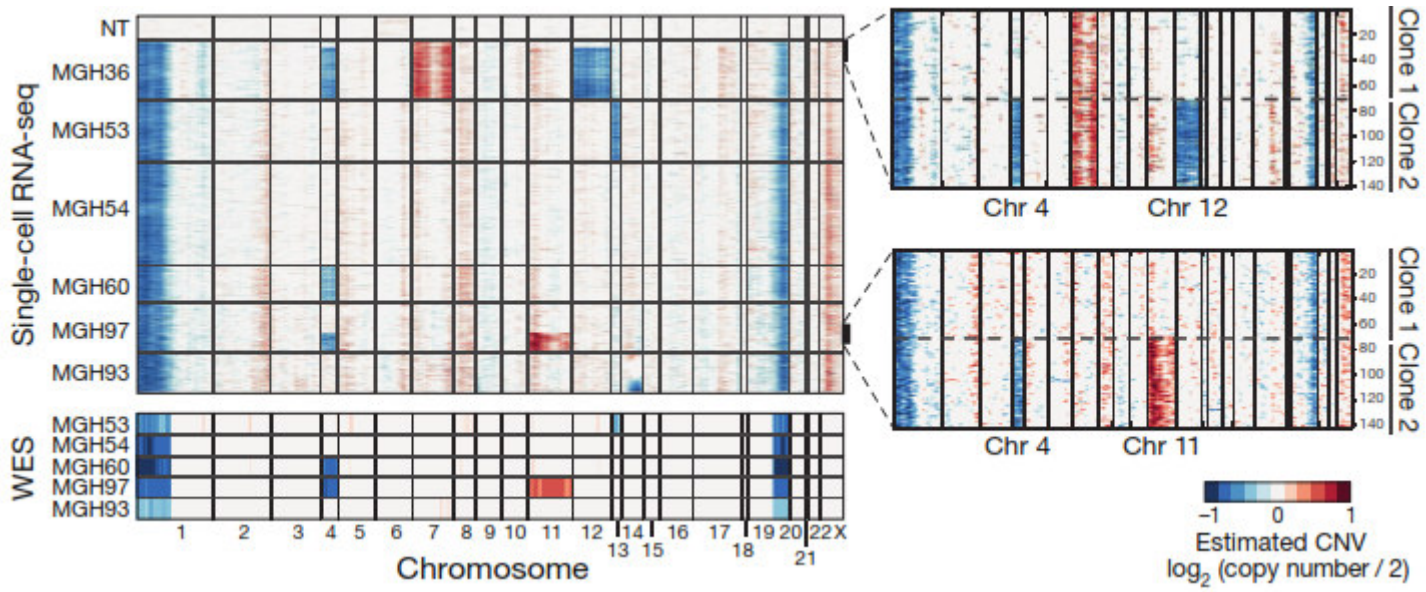


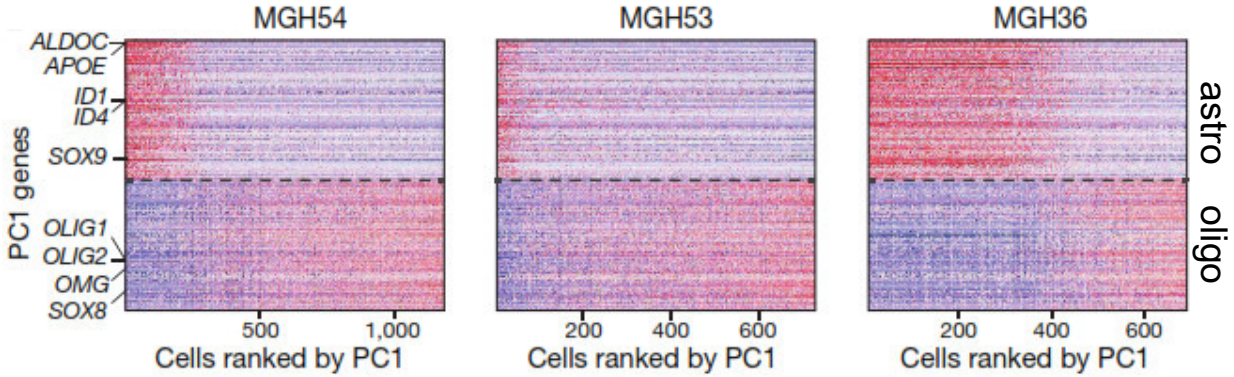




Zomer et al., Stem Cells 2013

Direct Evidence for Cancer Hierarchy in Patient Samples



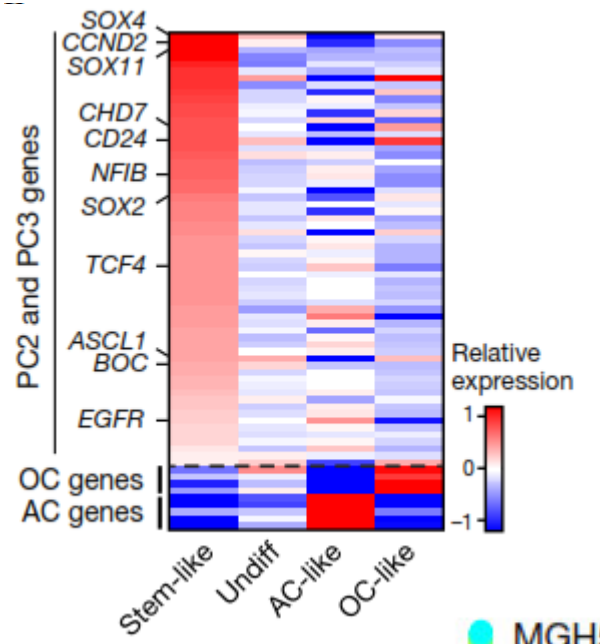


oligodendroglioma:
incurable glioma characterized by mutations in *IDH1 or IDH2* and 1p/19q co-deletion

single cell RNA sequencing reveals composition with two major populations of glial cells of the **astrocyte** or **oligodendrocyte** lineage

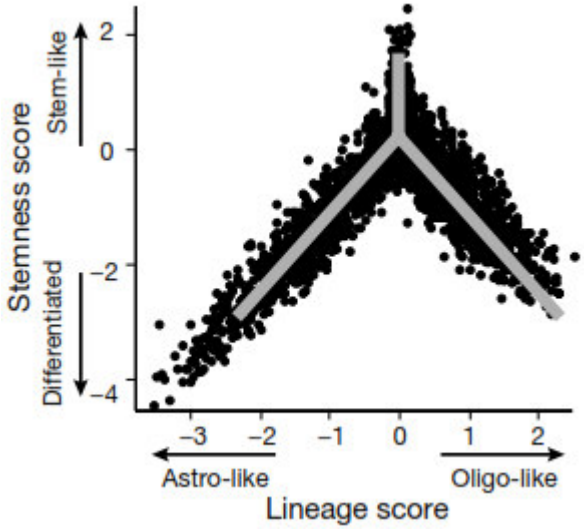
Tirosh et al., ***Nature 2016***

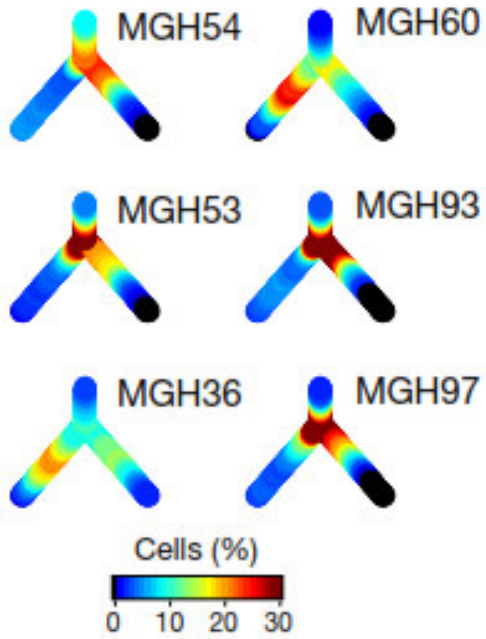
Direct Evidence for Cancer Hierarchy in Patient Samples



single cell analysis reveals **four main cell types** in every patient tumor:

- stem cell-like
- undifferentiated progenitors
- astrocyte-like
- oligodendrocyte-like



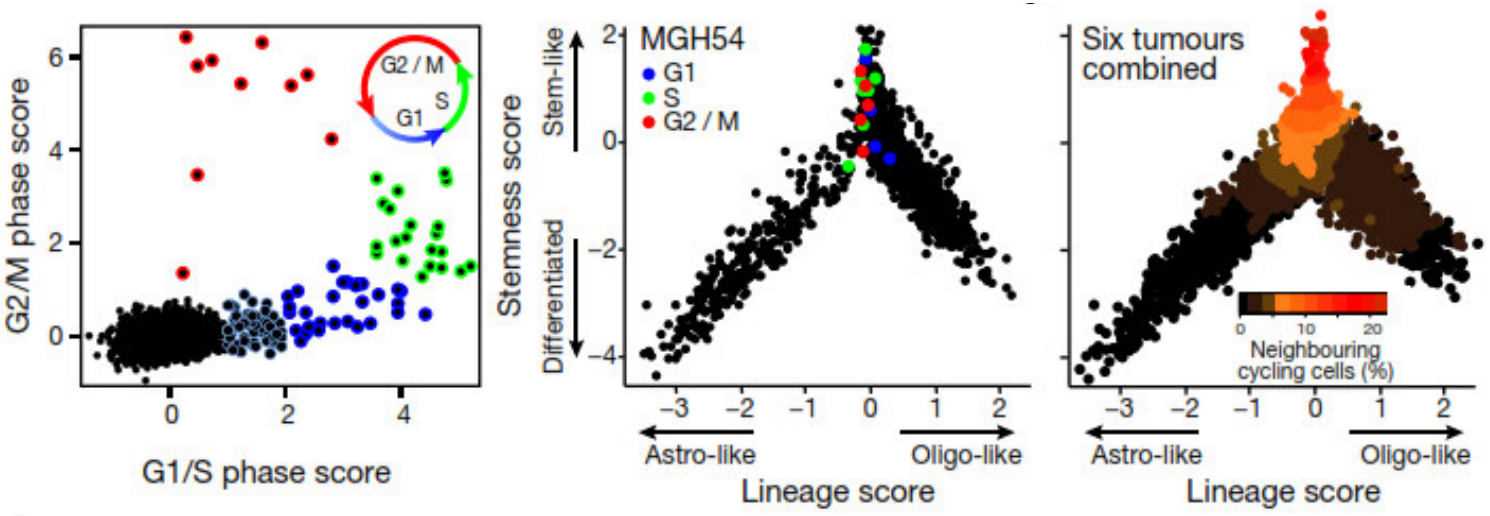


individual patients differ in the relative frequency of these four different cell types without loosing the general architecture

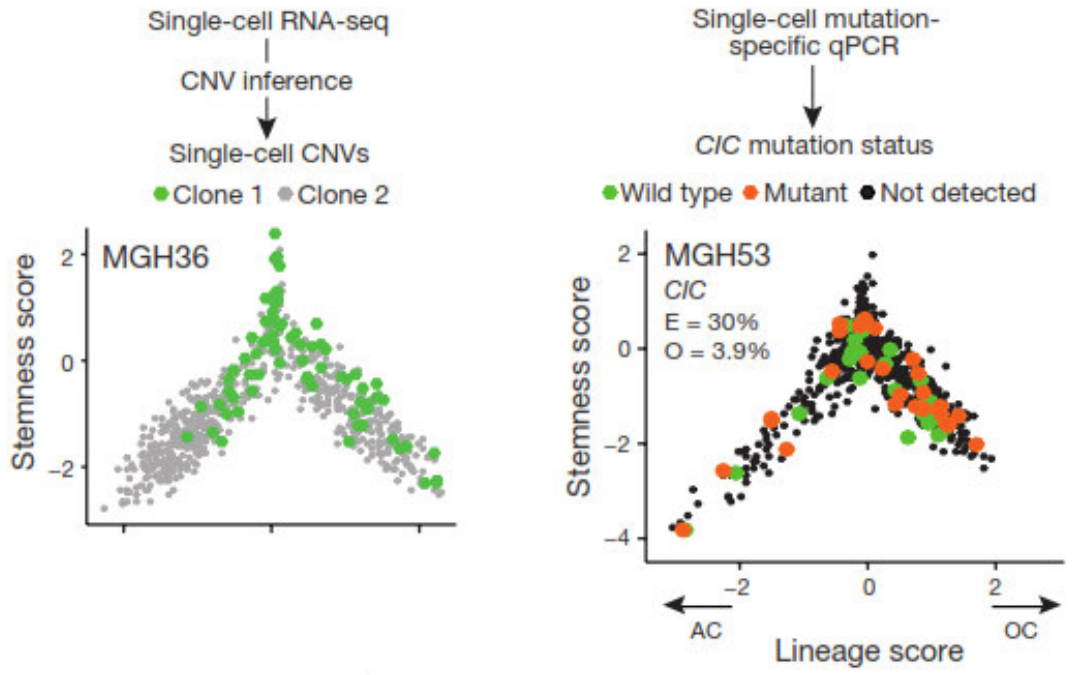
this architecture closely resembles the structure of the normal tissue

Tirosh et al., ***Nature 2016***

Direct Evidence for Cancer Hierarchy in Patient Samples



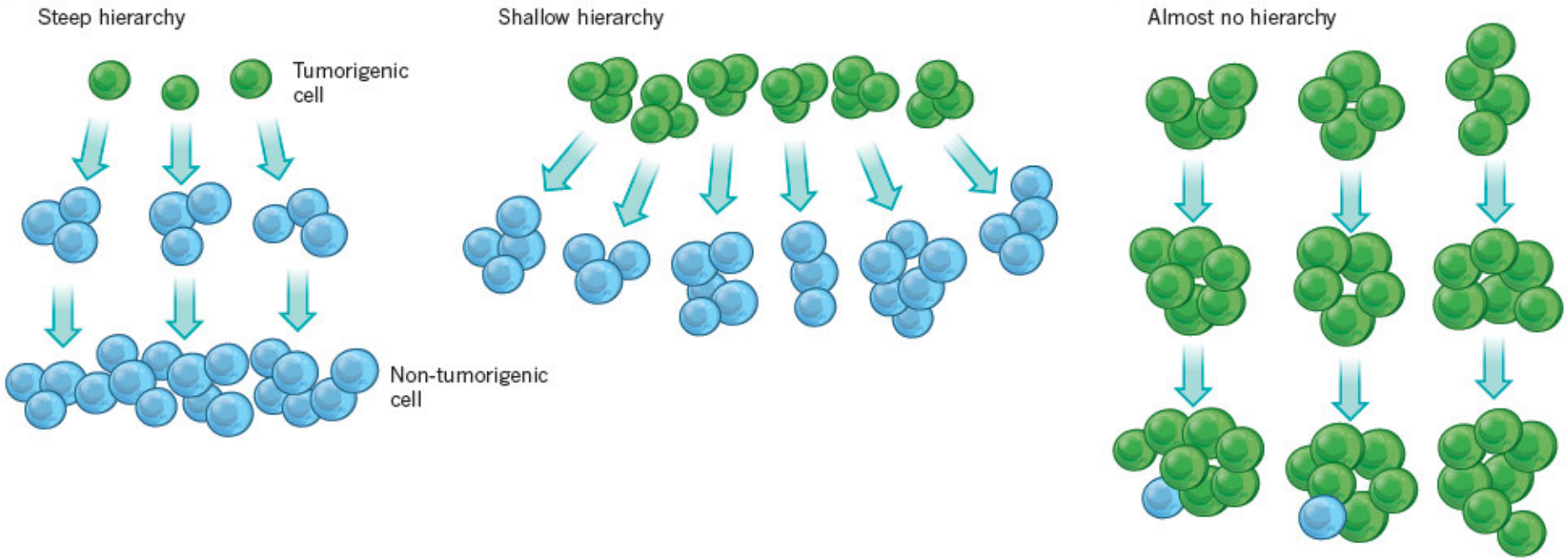
cell cycle activity is restricted to the stem and progenitor level



within the same patient genetically separate subclones co-exist which independently maintain the overall tissue architecture

Tirosh et al., ***Nature 2016***

Different levels of hierarchical organization

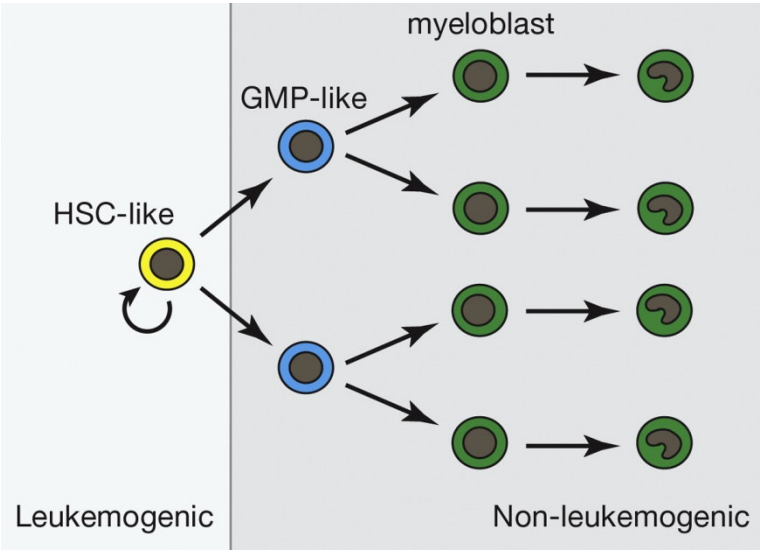


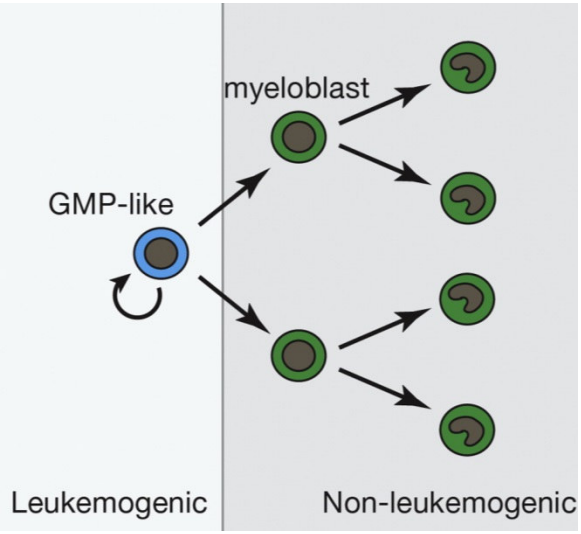
- Some hierarchies might be steep in which tumor-initiating cells are rare but give rise to numerous non-tumorigenic cells
- Other hierarchies might be shallow in which tumor-initiating cells are common but give rise to a small number of non-tumorigenic cells
- Some cancers may have almost no hierarchy, with very few non-tumorigenic cells.

The shallower the hierarchy, the lower the value of distinguishing between tumorigenic and non-tumorigenic cells in order to understand cancer biology and improve therapy.

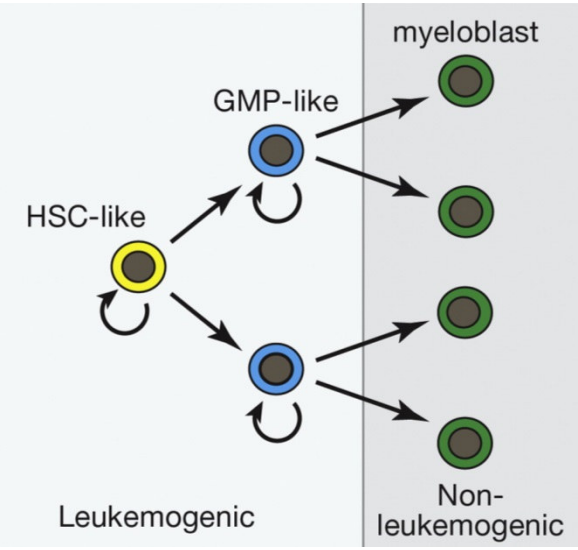
Meacham & Morrision, Nature 2013

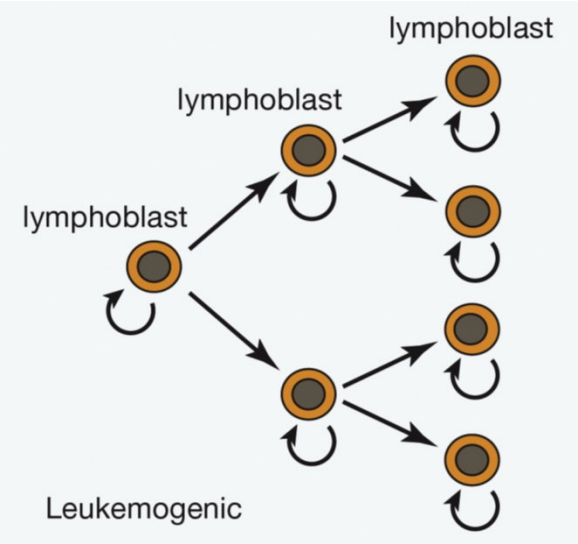
Hierarchical Organization in Cancer





MLL-AF9 driven acute myeloid leukemia (AML).

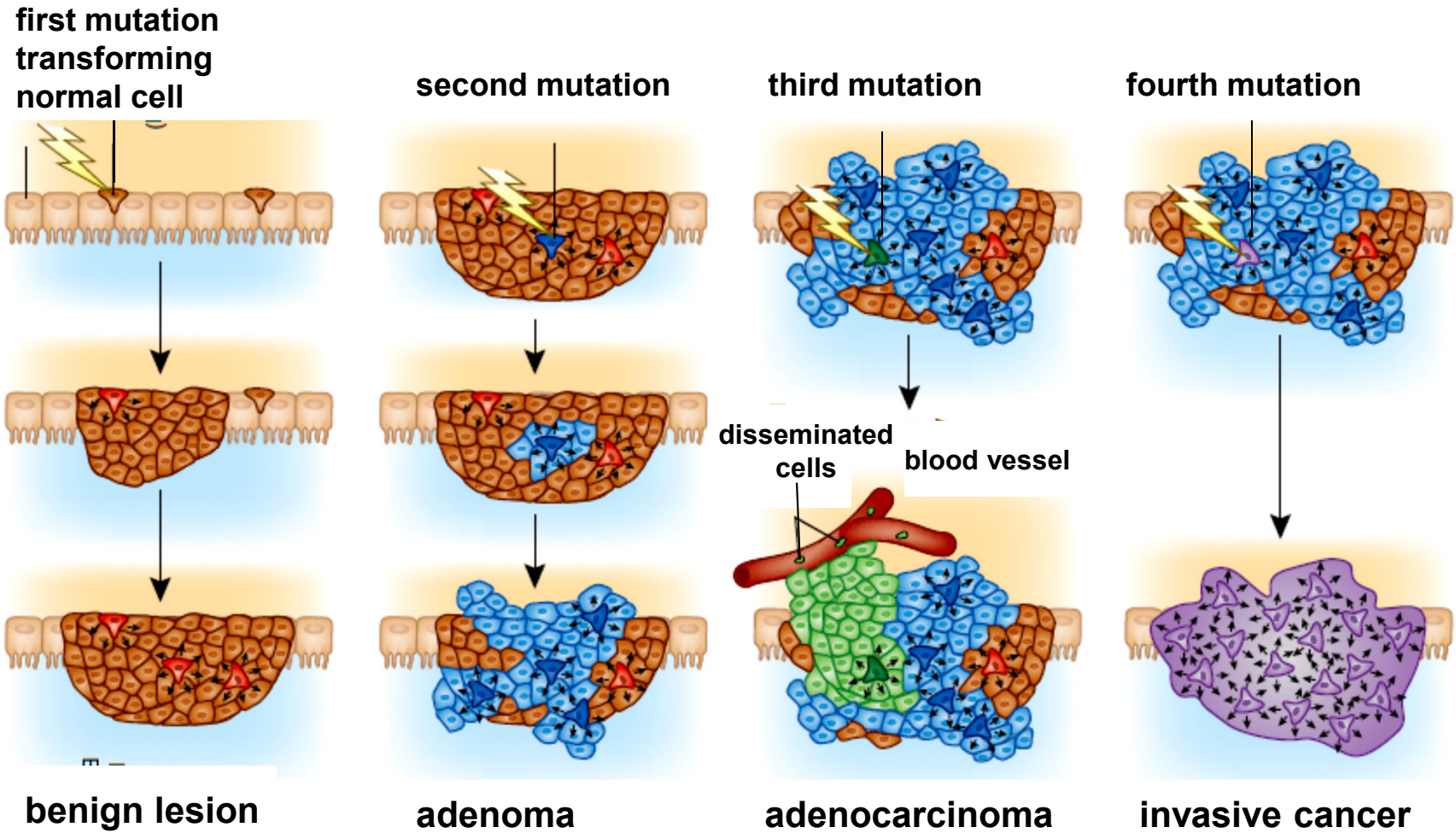




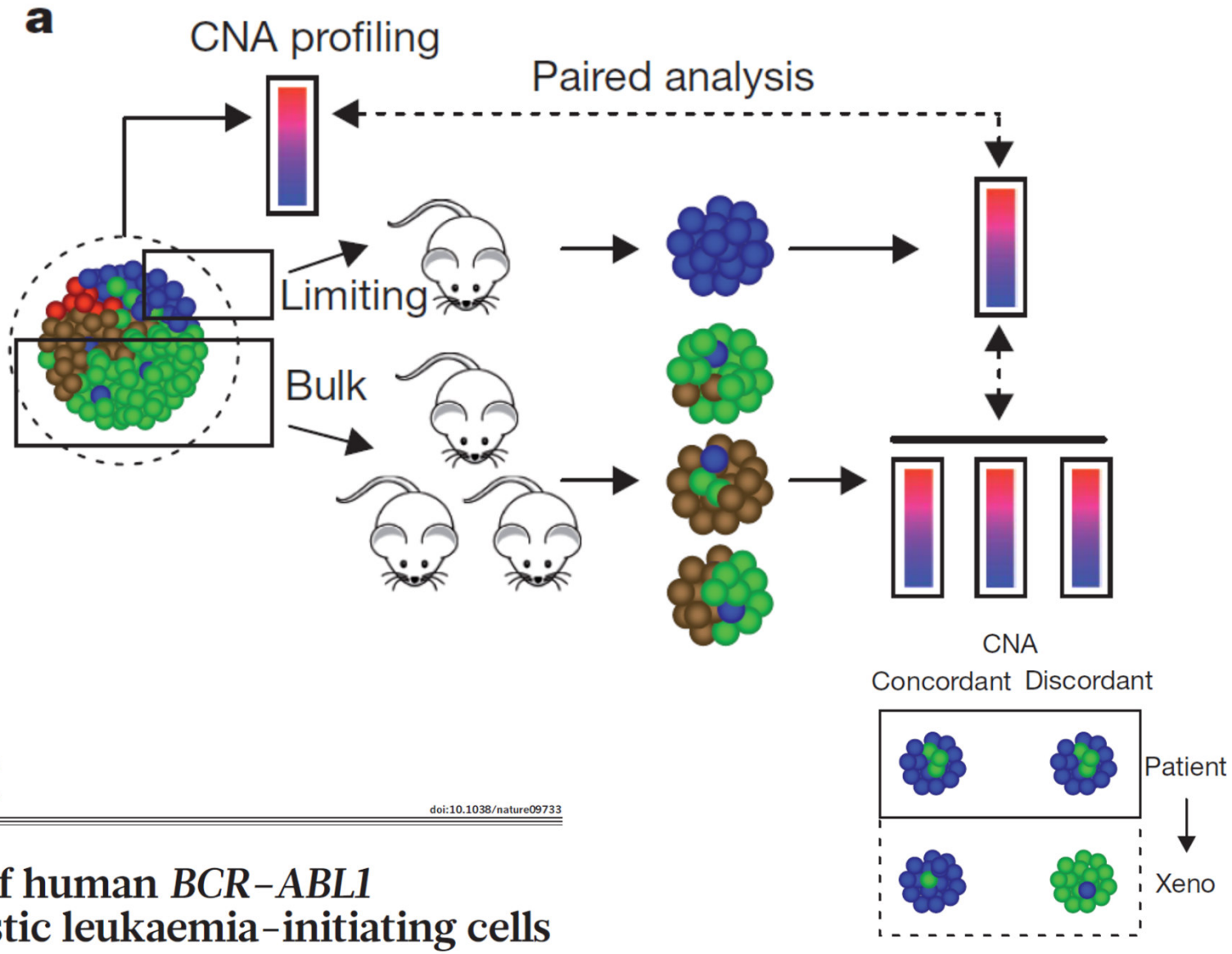
shallow/no hierarchy: childhood acute lymphoblastic leukemia

modified from Magee et al. ***Cancer Cell*** *2012*

Clonal evolution of Cancer



Clonal evolution of CSCs



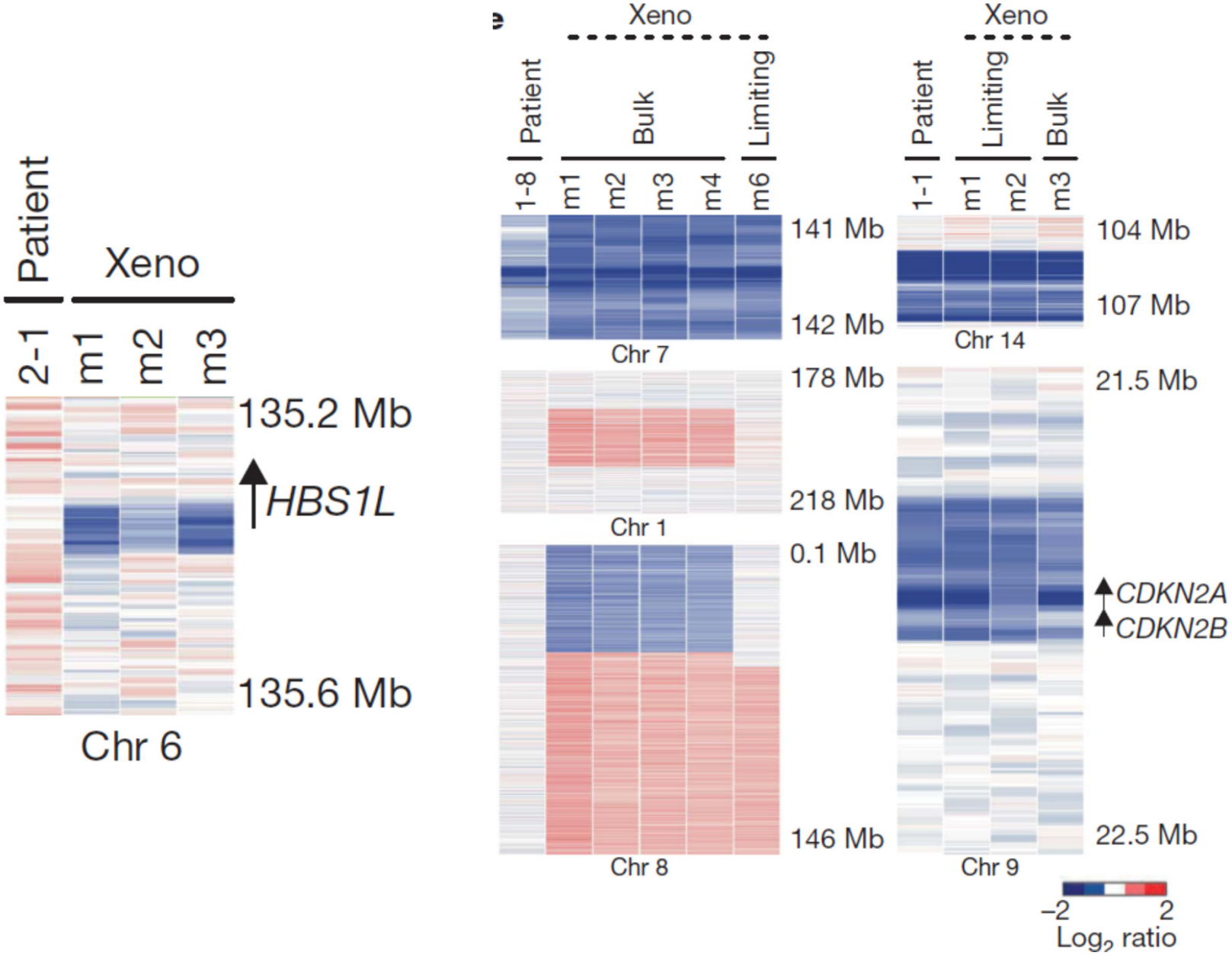
ARTICLE

doi:10.1038/nature09733

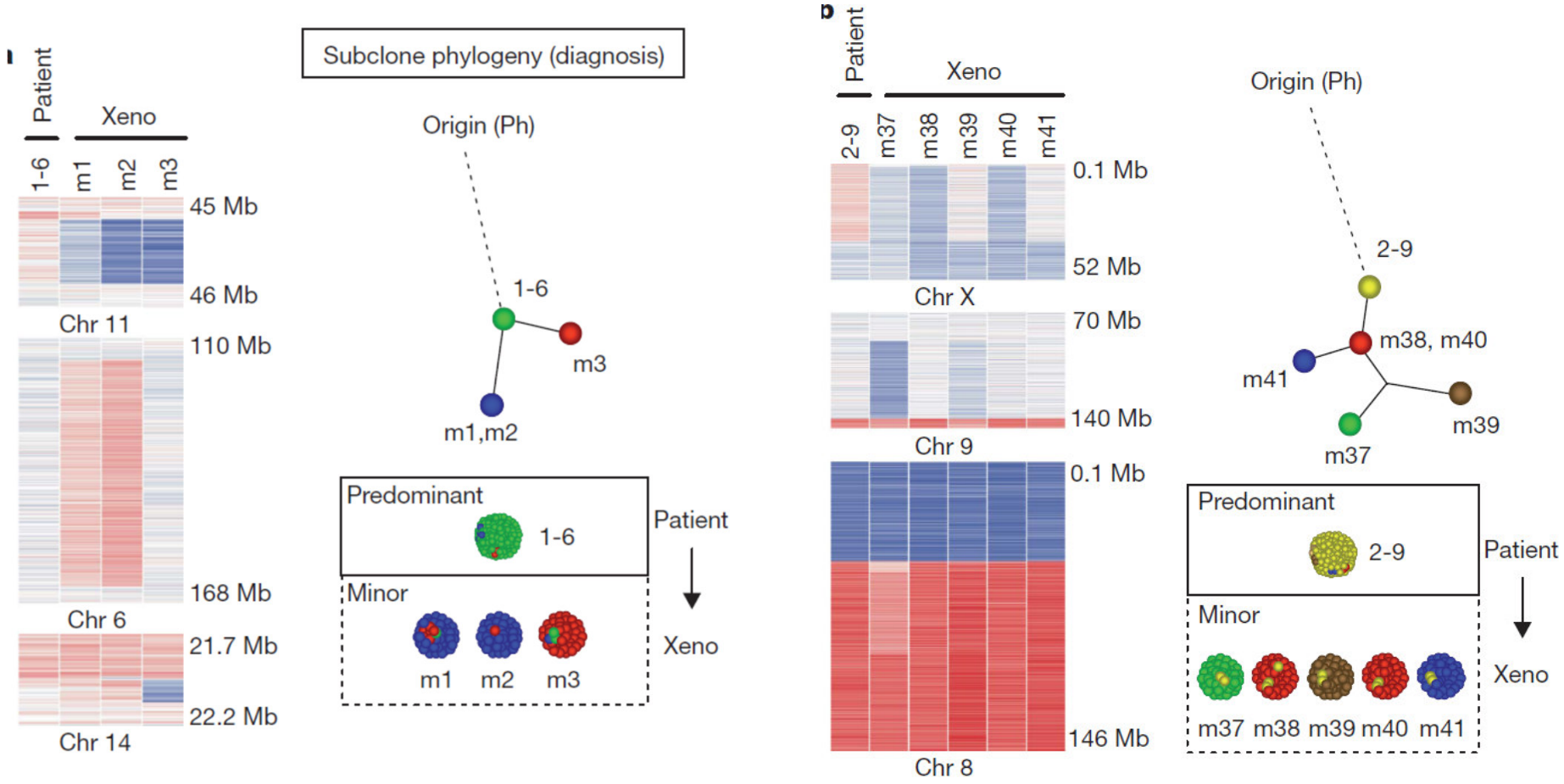
Evolution of human *BCR–ABL1* lymphoblastic leukaemia-initiating cells

Faiyaz Notta[1,2]*, Charles G. Mullighan[3]*, Jean C. Y. Wang[1,4], Armando Poeppl[1], Sergei Doulatov[1,2], Letha A. Phillips[3], Jing Ma[5], Mark D. Minden[4], James R. Downing[3] & John E. Dick[1,2]

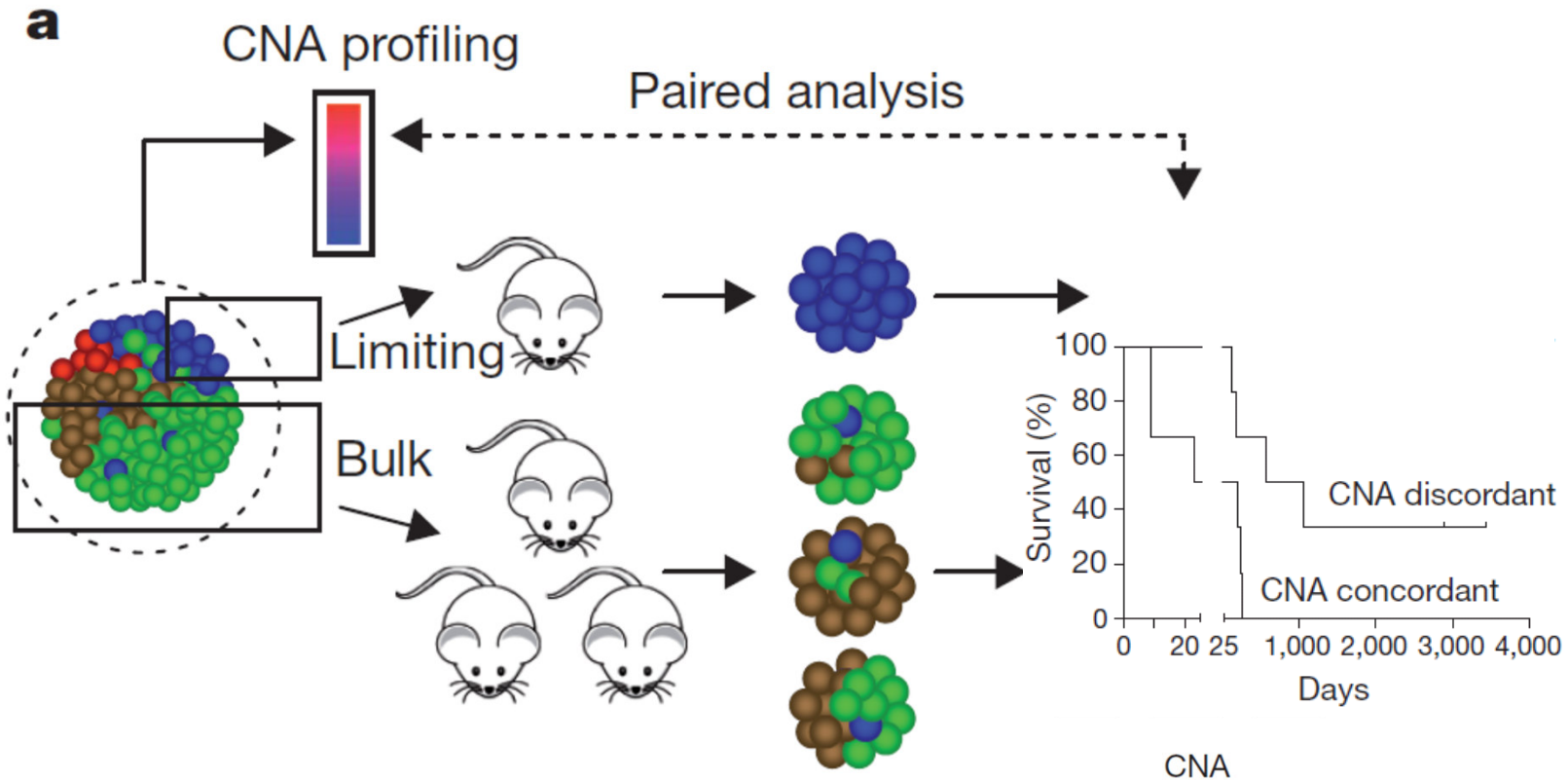
Clonal evolution of CSCs: CML

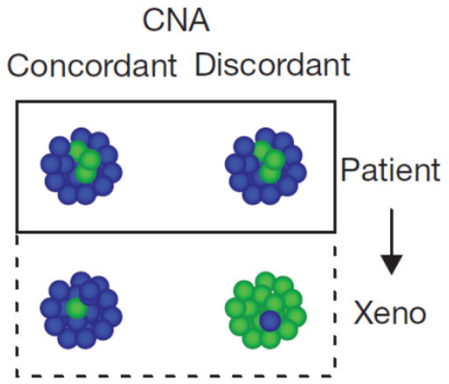


Clonal evolution of CSCs: CML



Clonal evolution of CSCs





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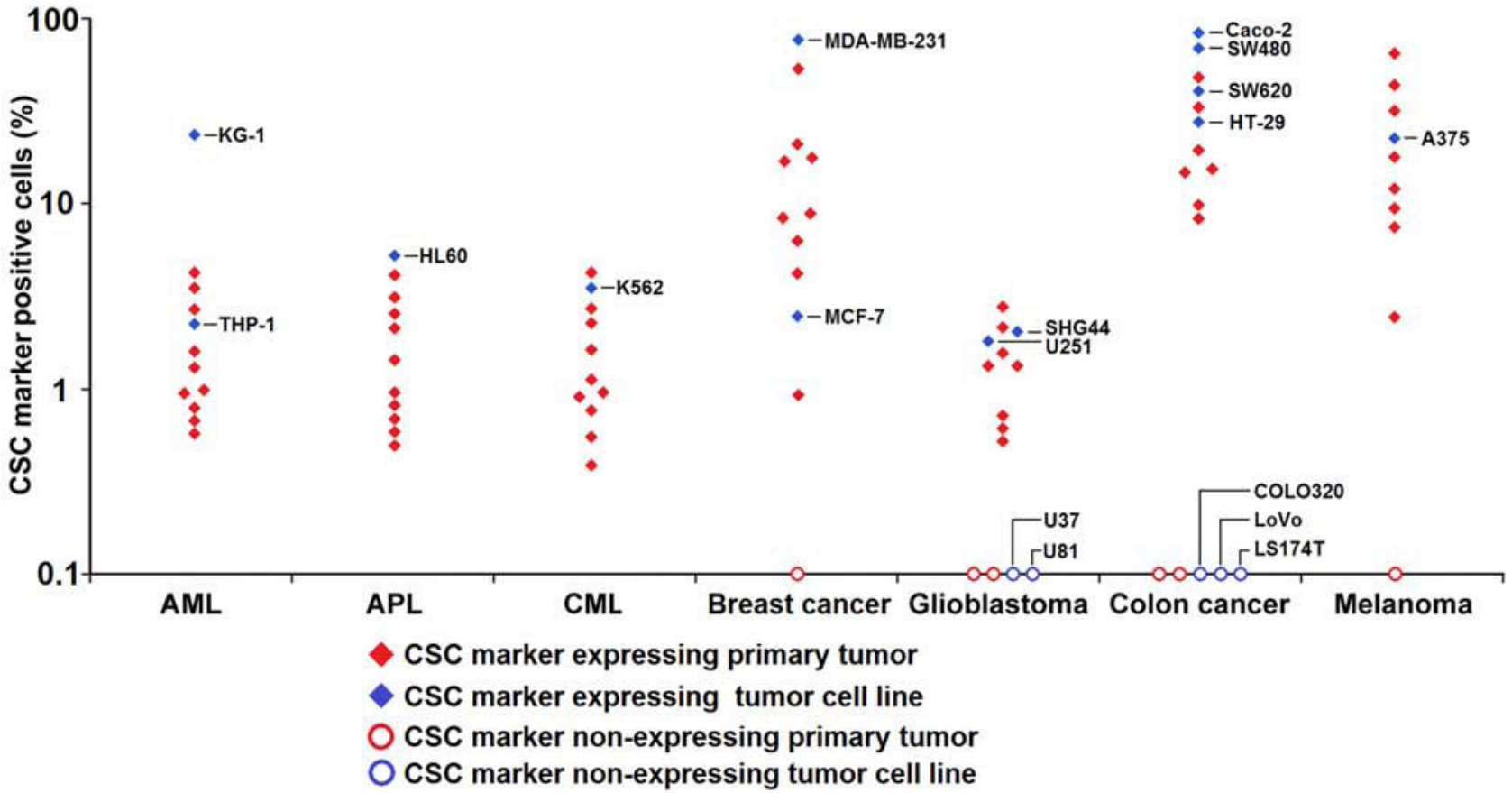
Markers to identify CSCs

Markers to isolate CSCs

Breast	Colon	Glioma	Liver	Lung	Melanoma	Ovarian	Pancreatic	Prostate
ALDH1 CD24 CD44 CD90 CD133 Hedgehog-Gli activity α6-integrin	ABCB5 ALDH1 β-catenin activity CD24 CD26 CD29 CD44 CD133 CD166 LGR5	CD15 CD90 CD133 α6-integrin nestin	CD13 CD24 CD44 CD90 CD133	ABCG2 ALDH1 CD90 CD117 CD133	ABCB5 ALDH1 CD20 CD133 CD271	CD24 CD44 CD117 CD133	ABCG2 ALDH1 CD24 CD44 CD133 c-Met CXCR4 Nestin Nodal-Activin	ALDH1 CD44 CD133 CD166 α2β1-integrin α6-integrin Trop2

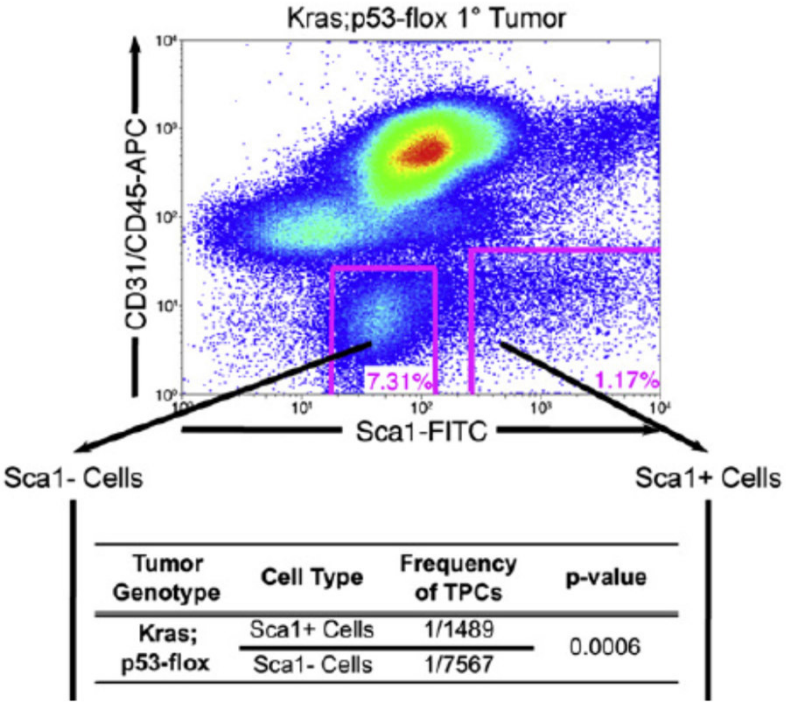
- all current markers are not specific for CSCs, but are expressed by many other cells outside of the tumor
- this makes direct targeting of the CSC population using these markers unlikely
- most current markers are not stem cell markers for the normal tissue stem cells where the tumor derives from
  - -exceptions: CD34/CD38 for human leukemia/HSC
    CD133 for human glioblastoma/neuronal stem cells
    Sca1/c-kit for mouse leukemia/HSC
    CD34 for mouse SCC/skin stem cells
- nearly all current markers play no functional role in CSCs or normal stem cells
  - -exceptions: CD44 for glioblastoma and colon cancer
    Sca1/c-kit for mouse leukemia/HSC
    CD34 for mouse SCC
- markers that were originally characterized in a limited number of tumours have often been assumed to be generalizable. Such markers have frequently been used in other tumours, or even in cell lines, without independent confirmation that the markers were informative in these contexts
- in some cancers CSCs express different markers depending on the causative mutations

CSC marker expression can be heterogeneous

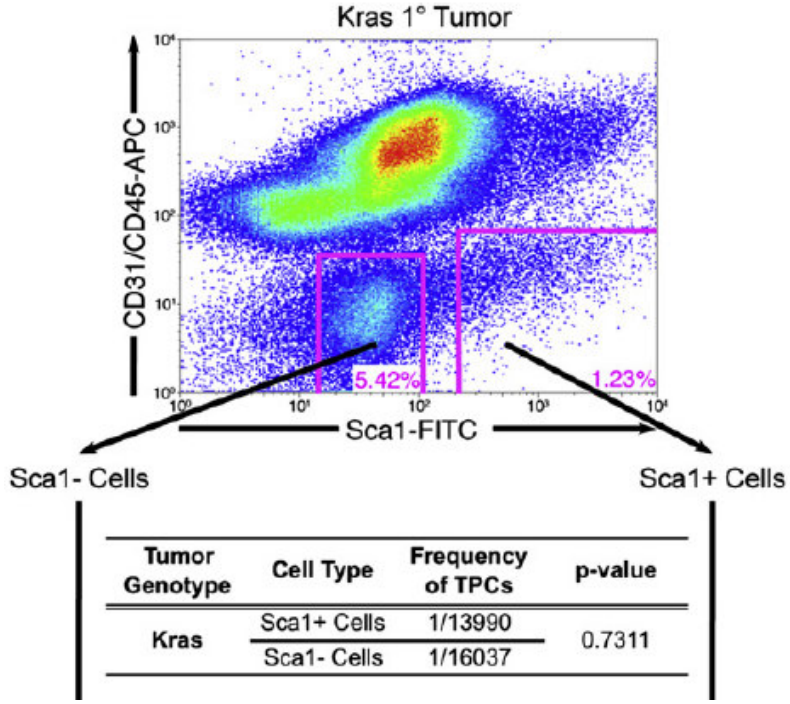


Huang et al. PlosOne 2013

Within one tissue, CSC markers may depend on the genetic cause of tumorigenesis



Tumor Genotype	Cell Type	Frequency of TPCs	p-value
Kras; p53-flox	Sca1+ Cells	1/1489	0.0006
	Sca1- Cells	1/7567	



Tumor Genotype	Cell Type	Frequency of TPCs	p-value
Kras	Sca1+ Cells	1/13990	0.7311
	Sca1- Cells	1/16037	

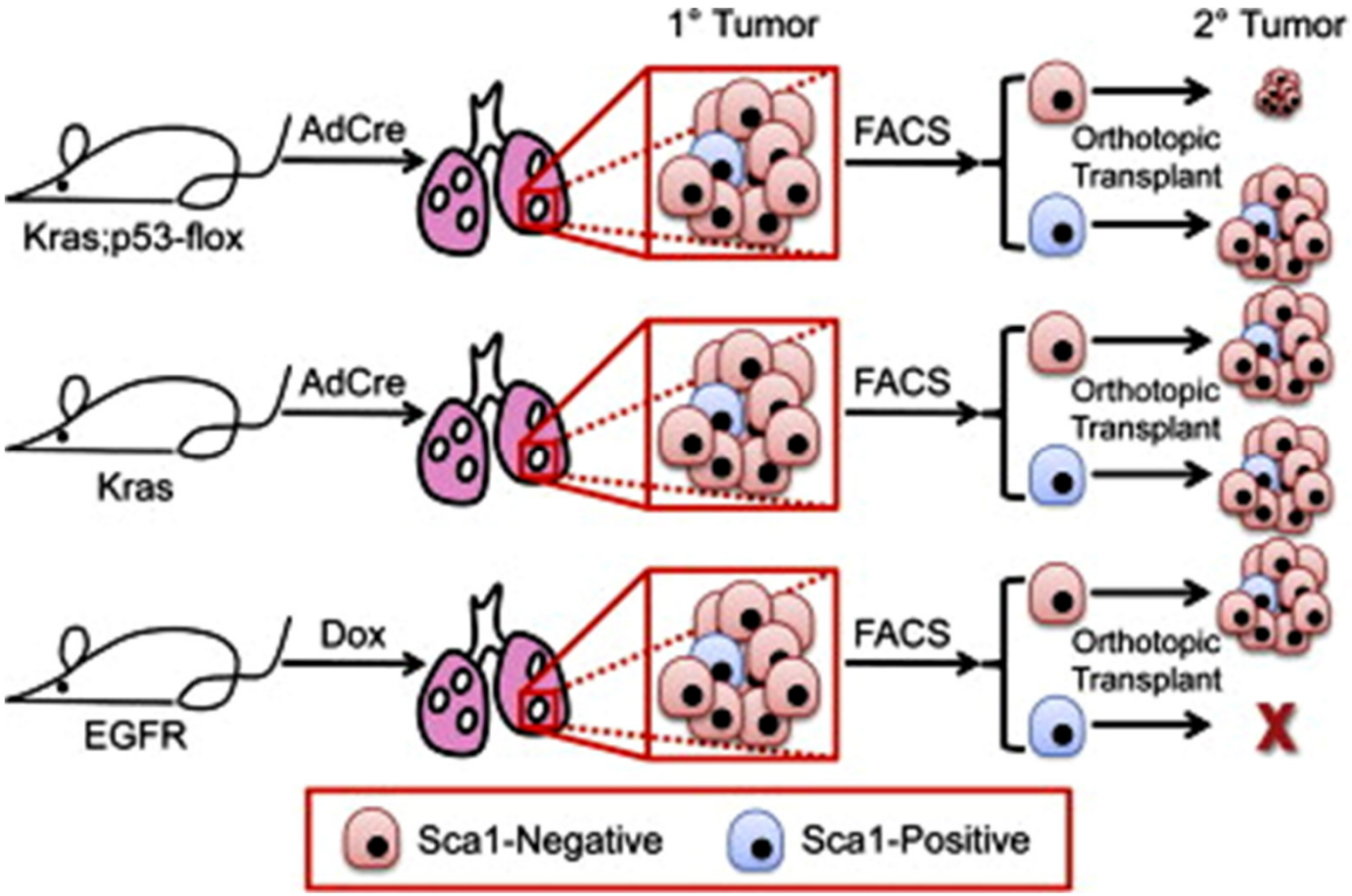
Tumor Genotype	Cell Type	Tumor formation
EGFR	Sca1+ Cells	0/4
	Sca1- Cells	7/7

Cell Stem Cell
Short Article

Primary Tumor Genotype Is an Important Determinant in Identification of Lung Cancer Propagating Cells

Stephen J. Curtis,[1,2,3] Kerstin W. Sinkevicius,[1,2,3] Danan Li,[4,5,6] Allison N. Lau,[1,2,3] Rebecca R. Roach,[1,2,3] Raffaella Zamponi,[1,2,3] Amber E. Woolfenden,[7] David G. Kirsch,[8] Kwok-Kin Wong,[4,5,6] and Carla F. Kim[1,2,3,*]
[1]Stem Cell Program, Children's Hospital Boston, Boston, MA 02115, USA
[2]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
[3]Harvard Stem Cell Institute, Cambridge, MA 02138, USA
[4]Department of Medical Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA
[5]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[6]Ludwig Center at Dana-Farber/Harvard Cancer Center, 44 Binney Street, Boston, MA 02115, USA
[7]Novartis Institutes for BioMedical Research, Inc., Cambridge, MA 02139
[8]Departments of Radiation Oncology and Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC 27708, USA
*Correspondence: carla.kim@childrens.harvard.edu
DOI 10.1016/j.stem.2010.05.021

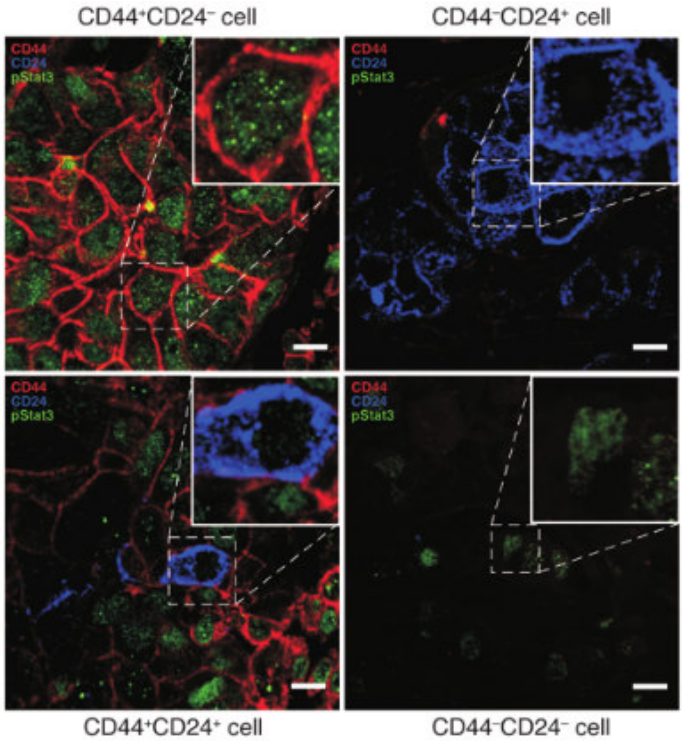
Within one tissue, CSC markers may depend on the genetic cause of tumorigenesis

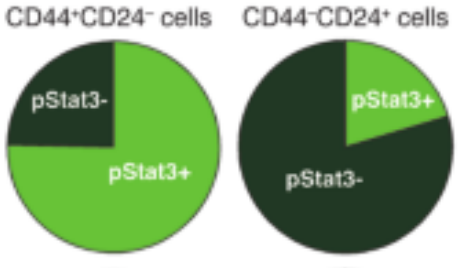


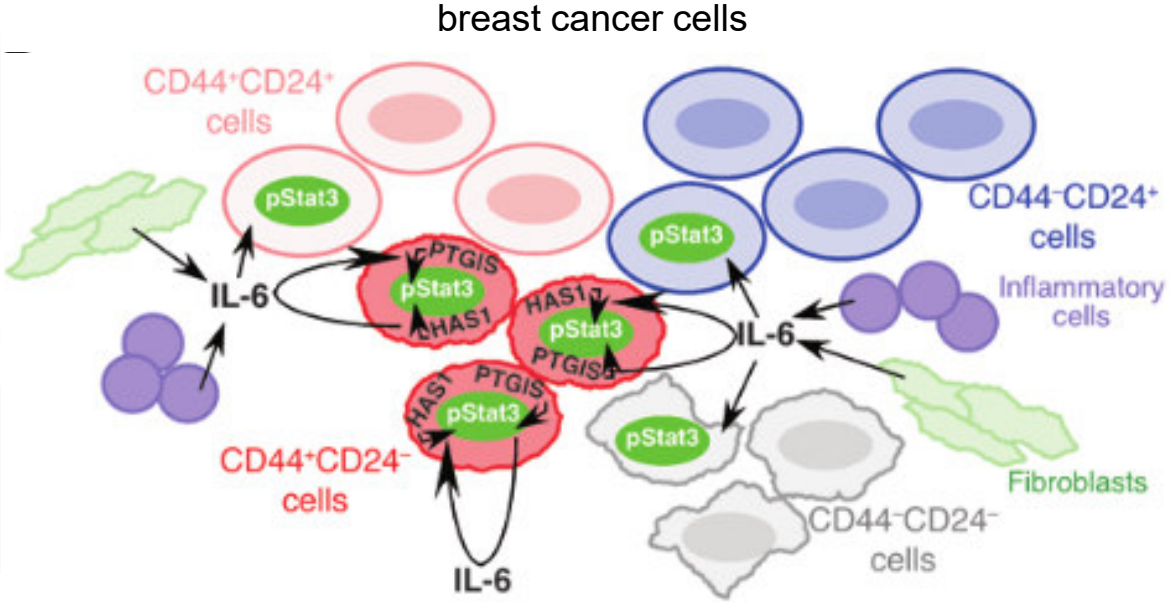
Stability of the CSC phenotype

Stability and Induction of CSCs

any experiment only represents a snapshot in time: can nonCSCs generate CSCs ?

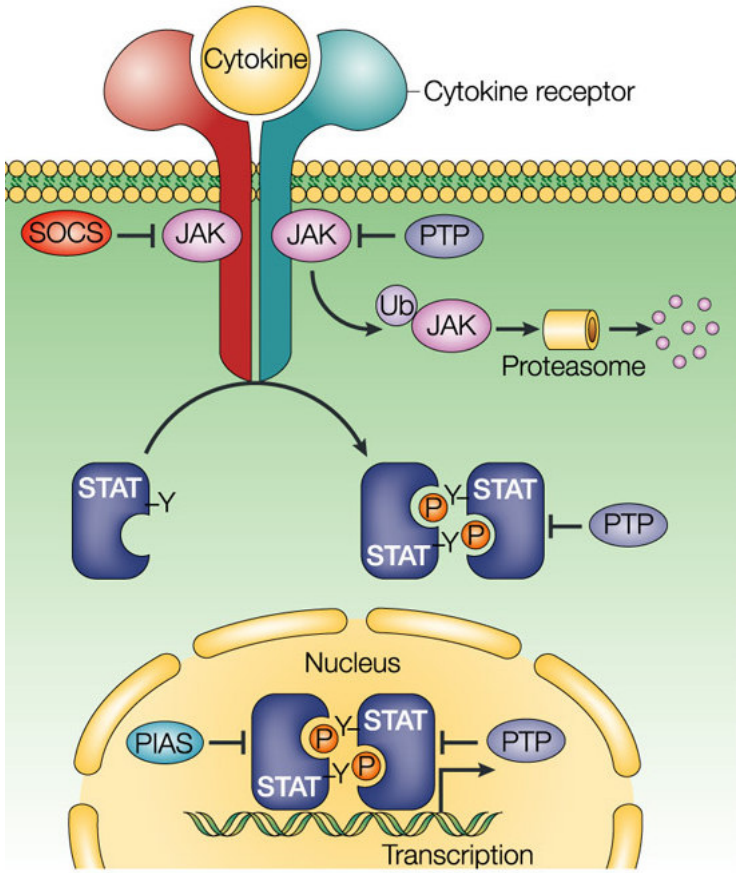


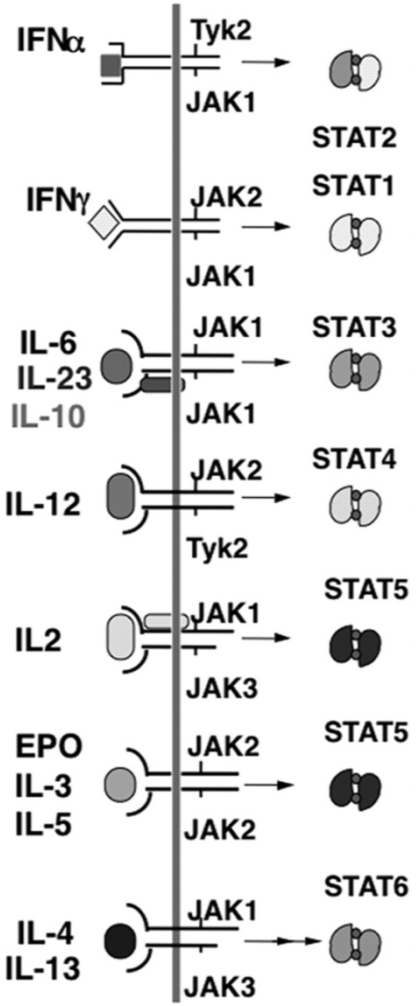




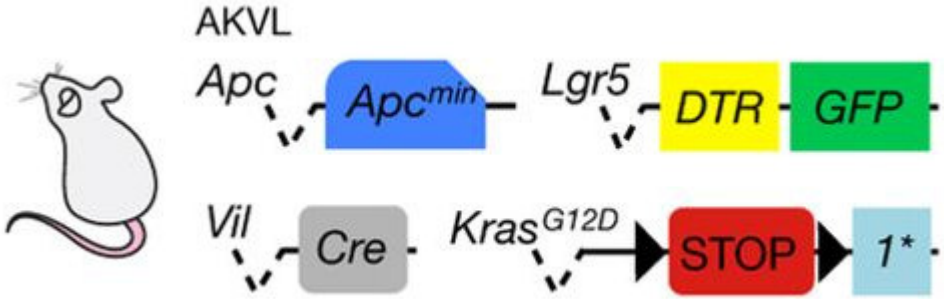
*Marotta et al., **JCI 2011***

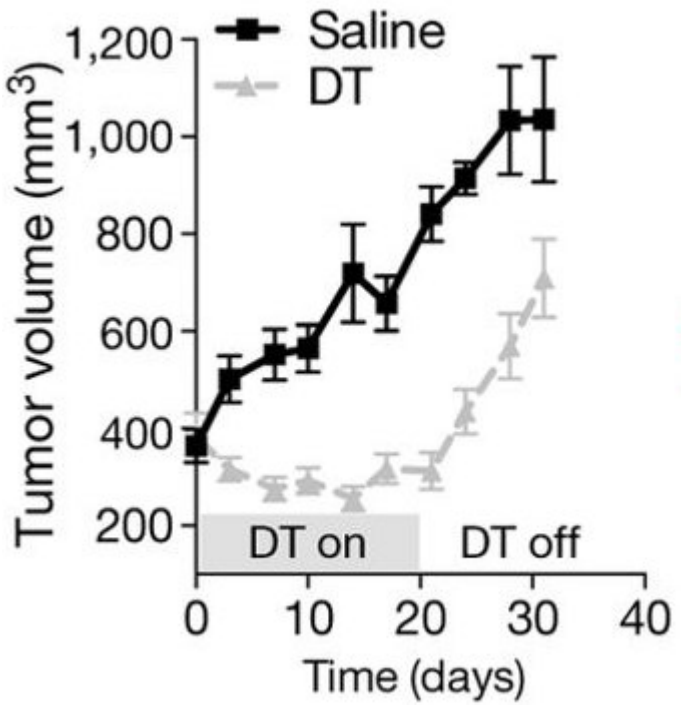
Stability and Induction of CSCs

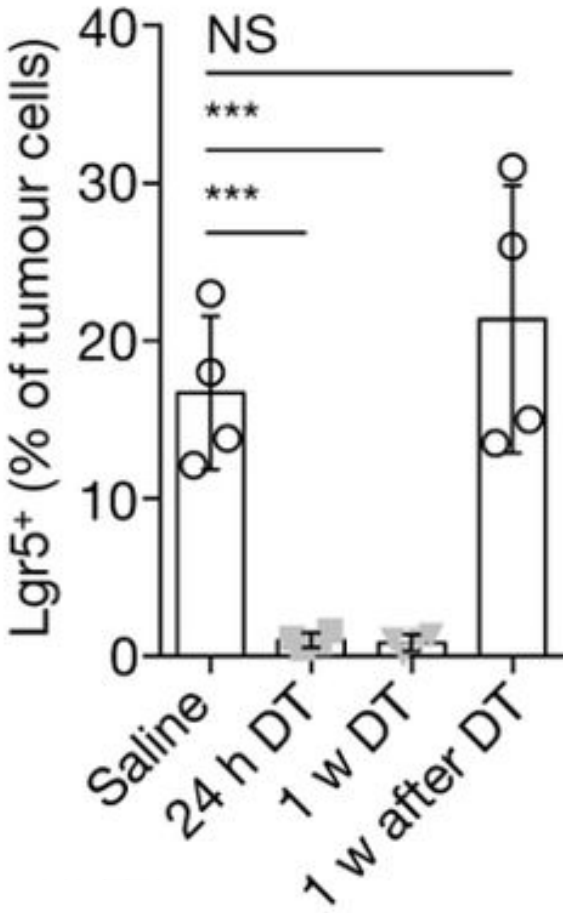




In vivo ablation of CSC is only transient

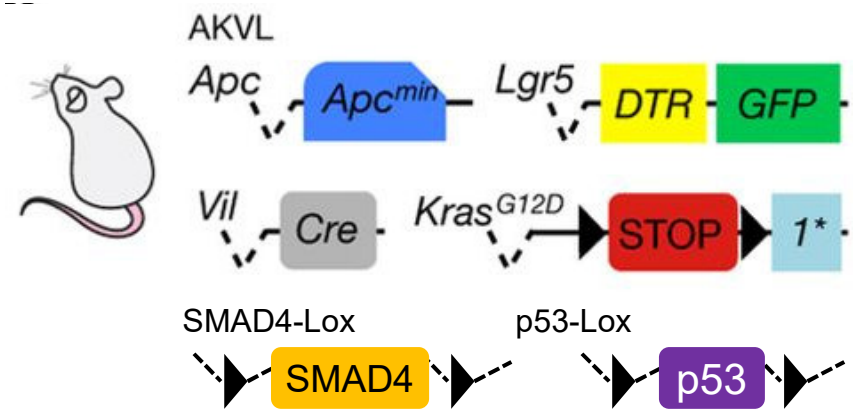


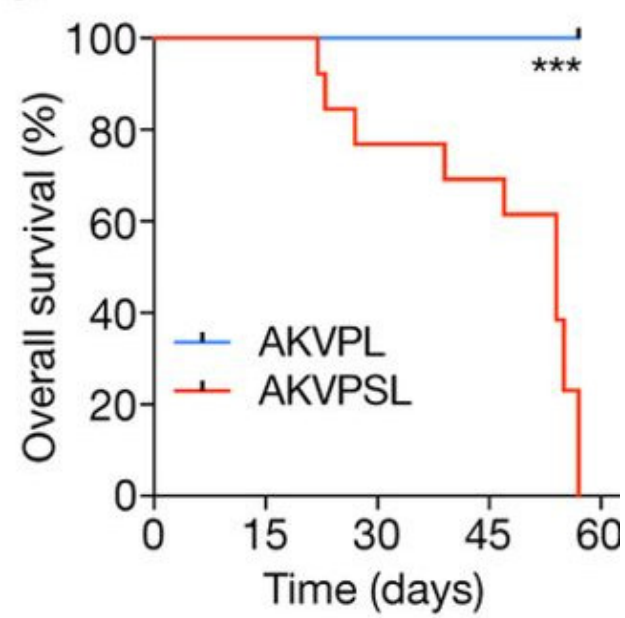


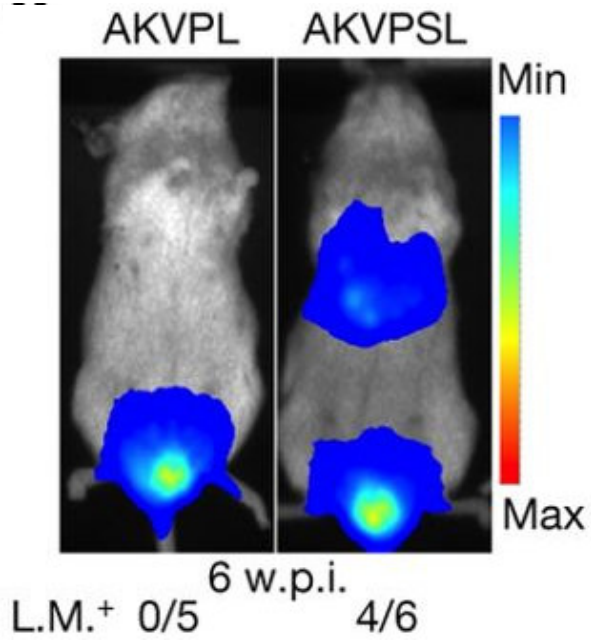


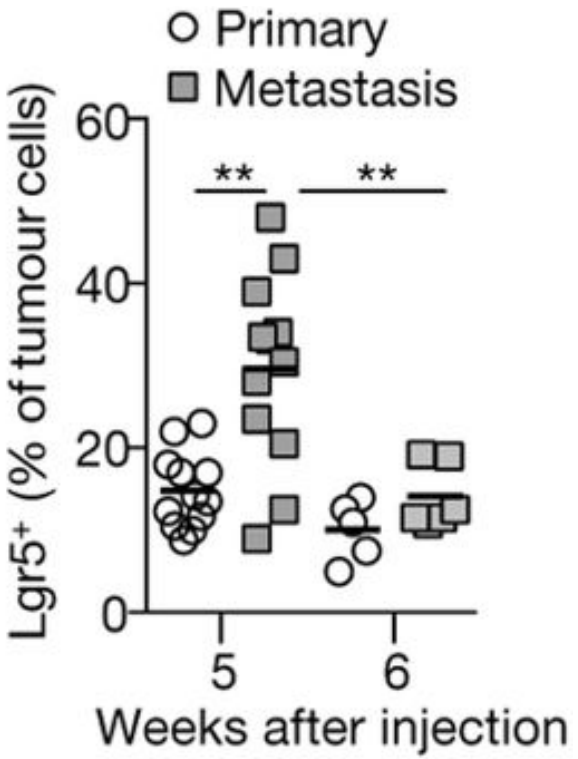
de Sousa e Melo, Nature 2017

CSC frequency during metastasis formation



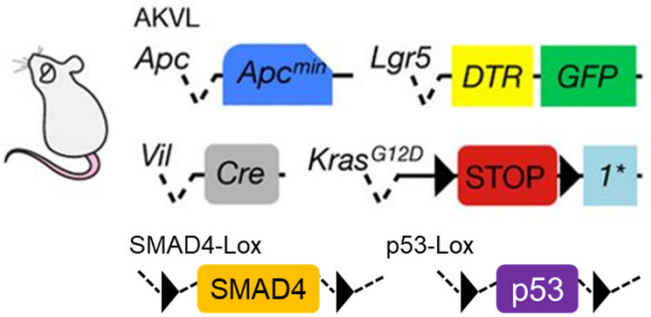


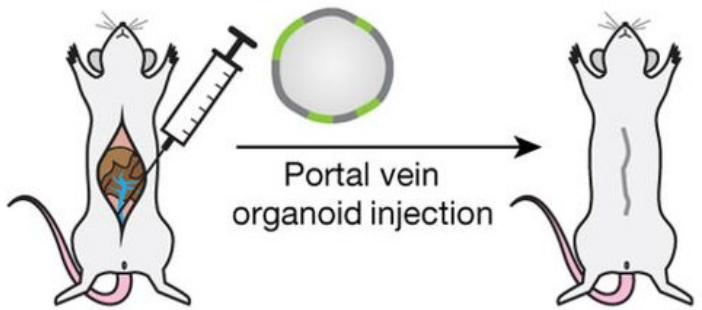


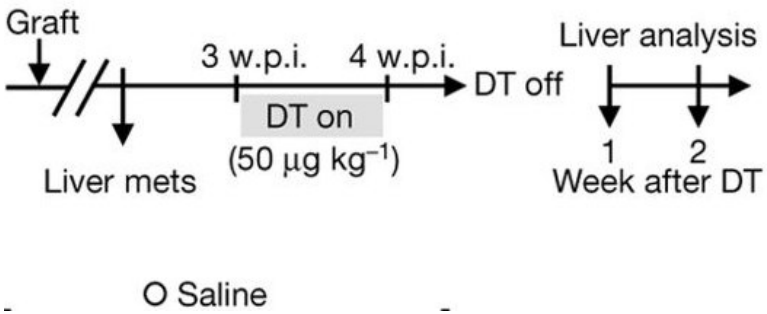


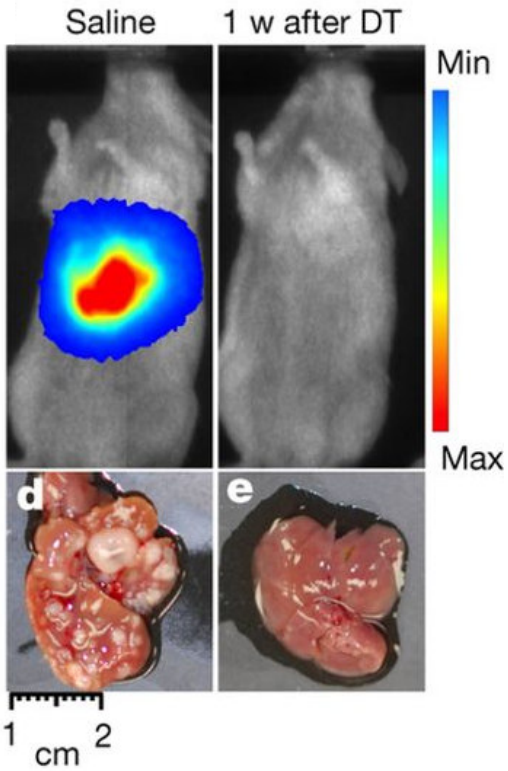
de Sousa e Melo, Nature 2017

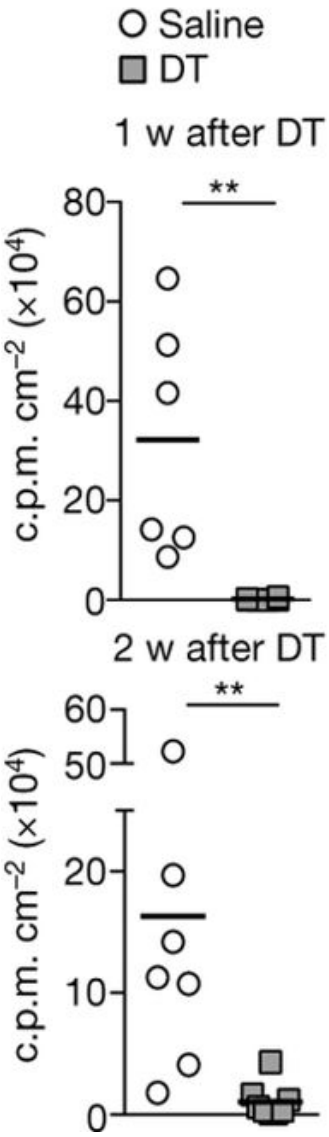
In vivo ablation of CSC can prevent metastasis formation









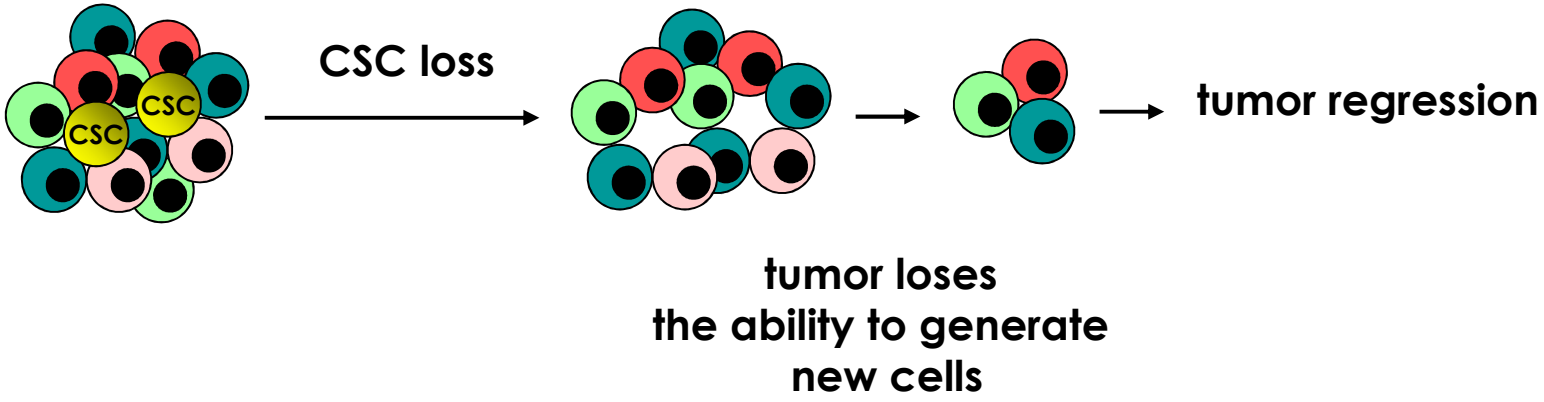


de Sousa e Melo, Nature 2017

Targeting CSCs

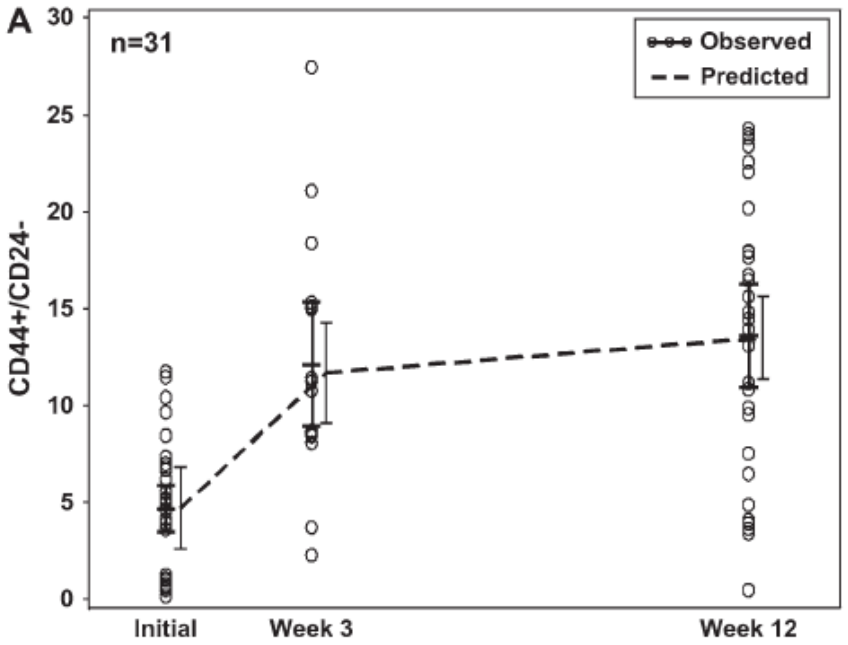
Promises and challenges of the cancer stem cell concept

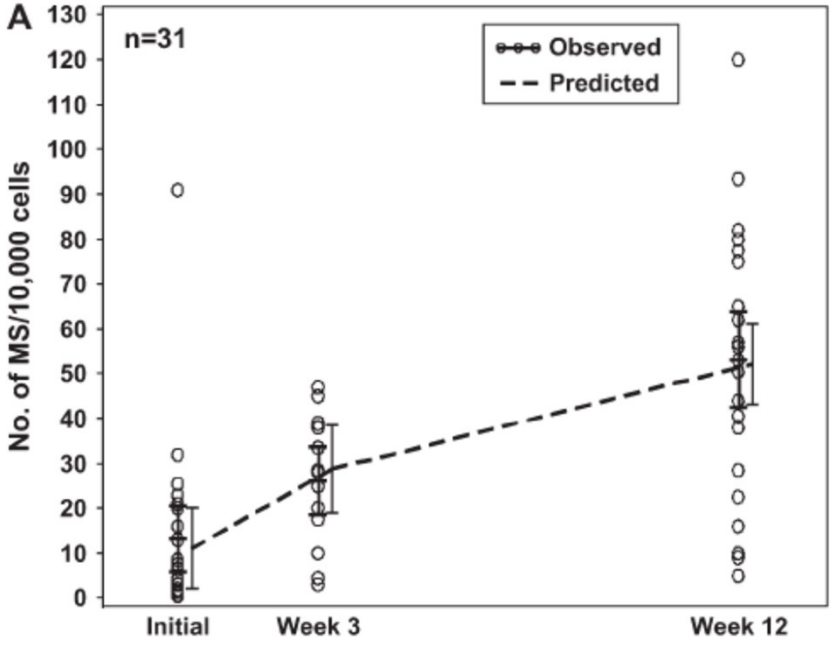
Targeted elimination of CSC will induce complete tumor regression and prevent recurrence



It is central to identify signaling pathways which are critical for cancer stem cell maintenance, however are not essential for normal tissue homeostasis

Resistance to therapy
breast cancer chemotherapy



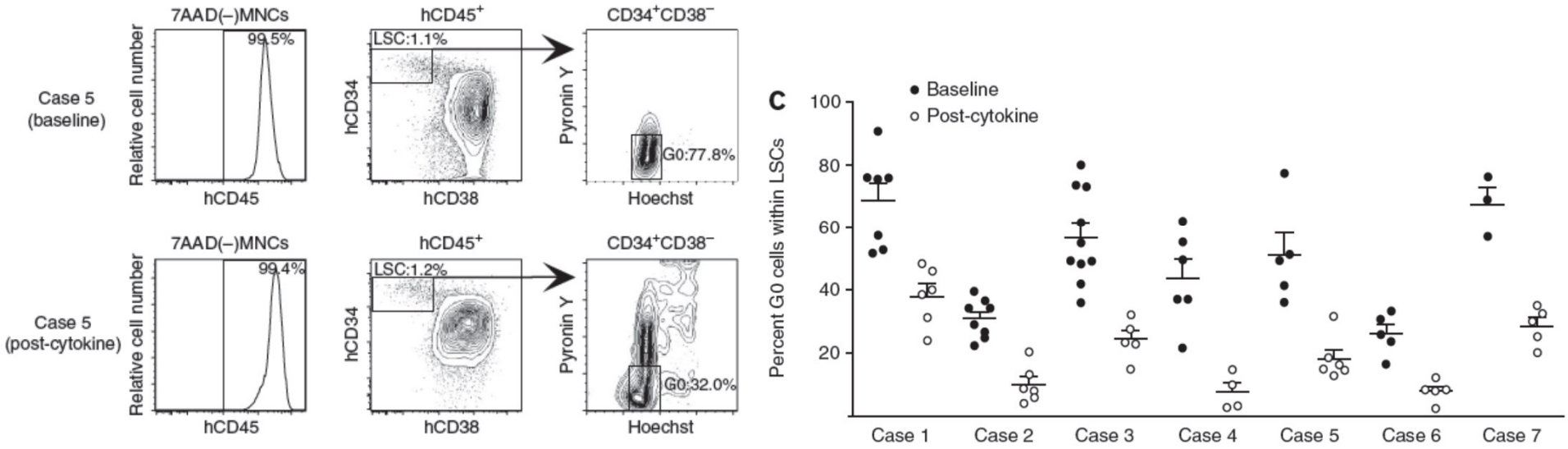


Intrinsic Resistance of Tumorigenic Breast Cancer Cells to Chemotherapy

Xiaoxian Li, Michael T. Lewis, Jian Huang, Carolina Gutierrez, C. Kent Osborne, Meng-Fen Wu, Susan G. Hilsenbeck, Anne Pavlick, Xiaomei Zhang, Gary C. Chamness, Helen Wong, Jeffrey Rosen, Jenny C. Chang

moreover: CSC show increased resistance to DNA damaging agents (e.g. radiation)

Therapeutic approaches sensitizing CSCs to standard therapy

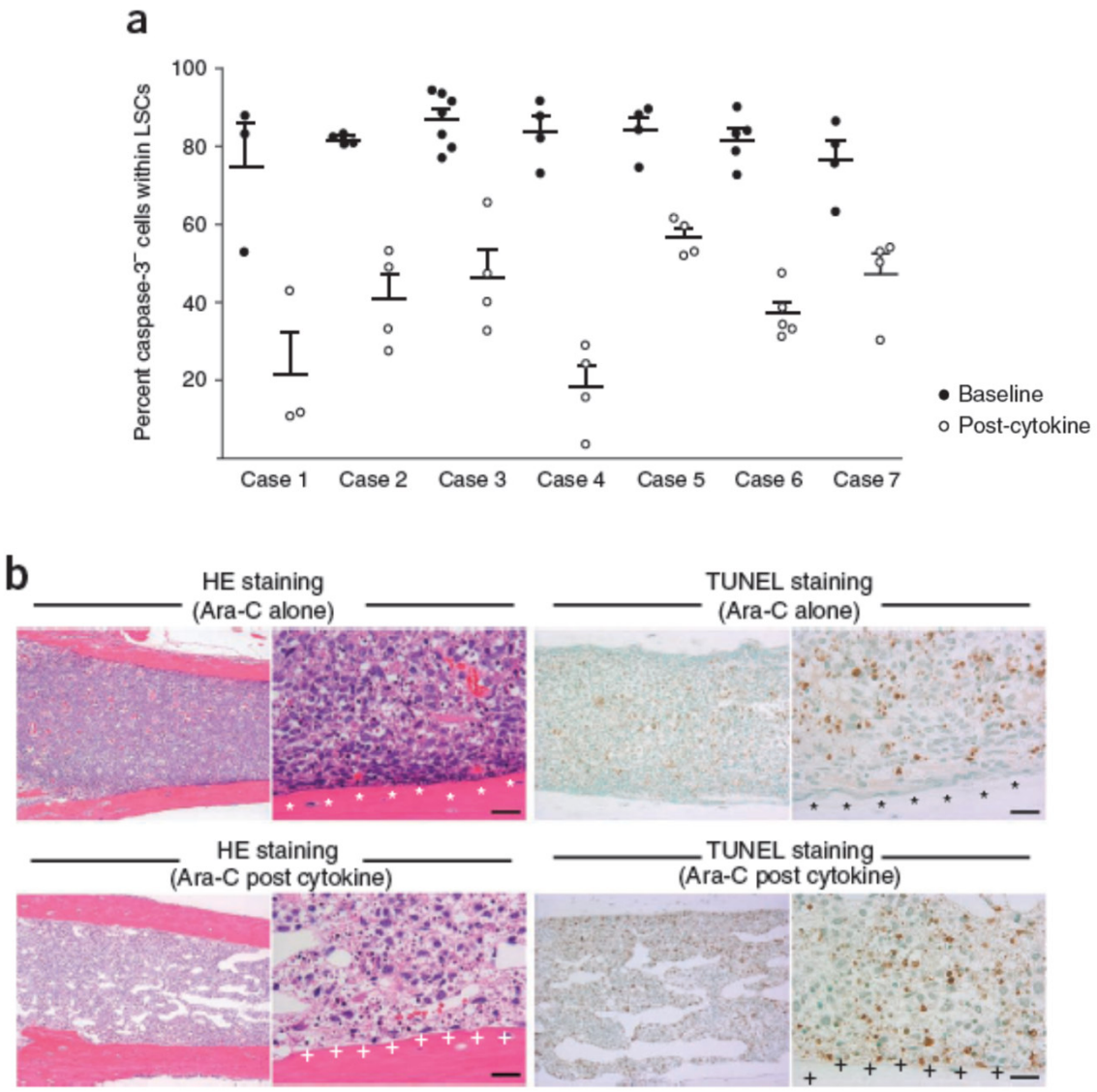


nature biotechnology

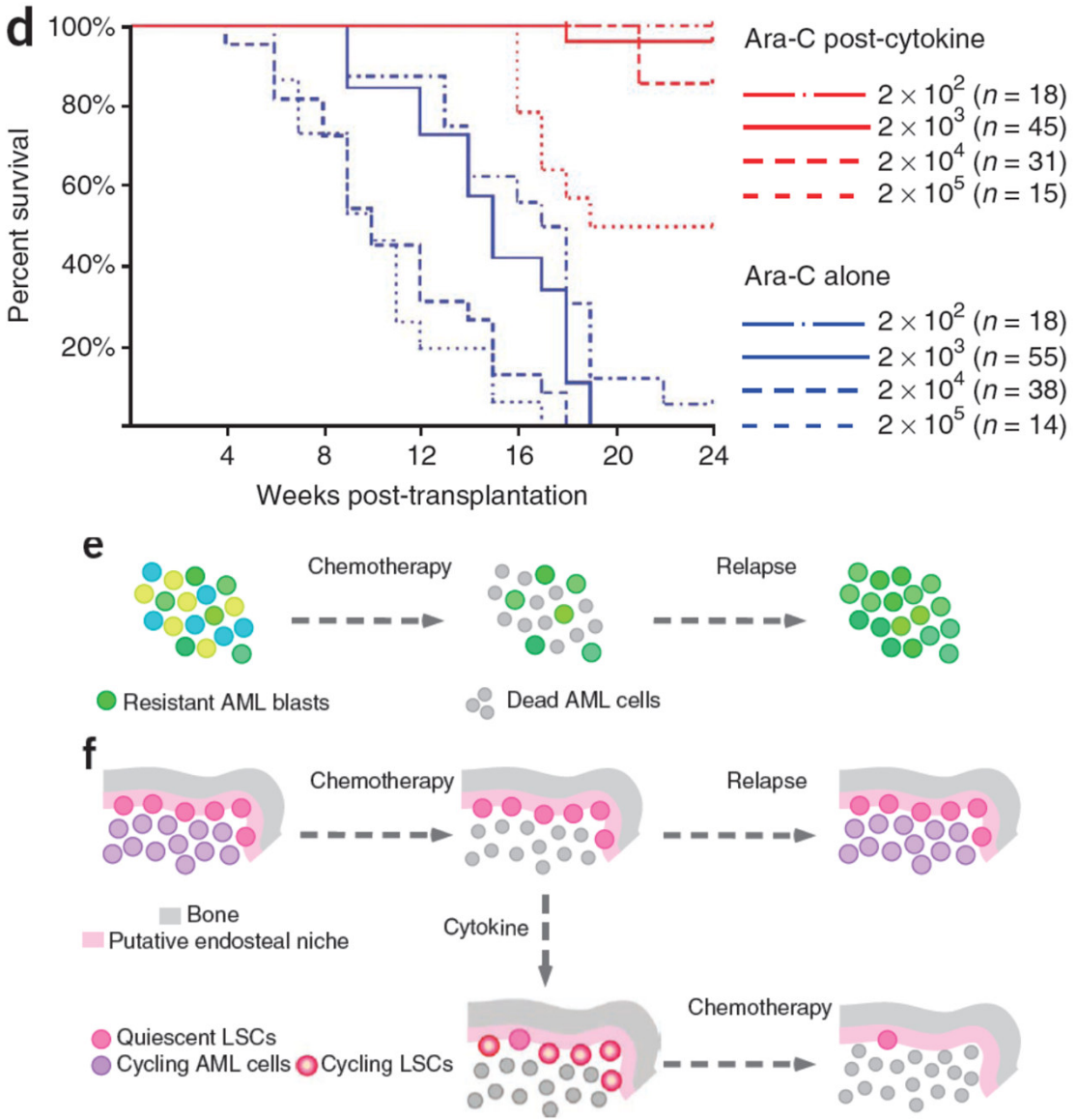
Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML

Yoriko Saito[1], Naoyuki Uchida[2], Satoshi Tanaka[3], Nahoko Suzuki[1], Mariko Tomizawa-Murasawa[1], Akiko Sone[1], Yuho Najima[1], Shinsuke Takagi[1,2], Yuki Aoki[1], Atsushi Wake[2], Shuichi Taniguchi[2], Leonard D Shultz[4] & Fumihiko Ishikawa[1]

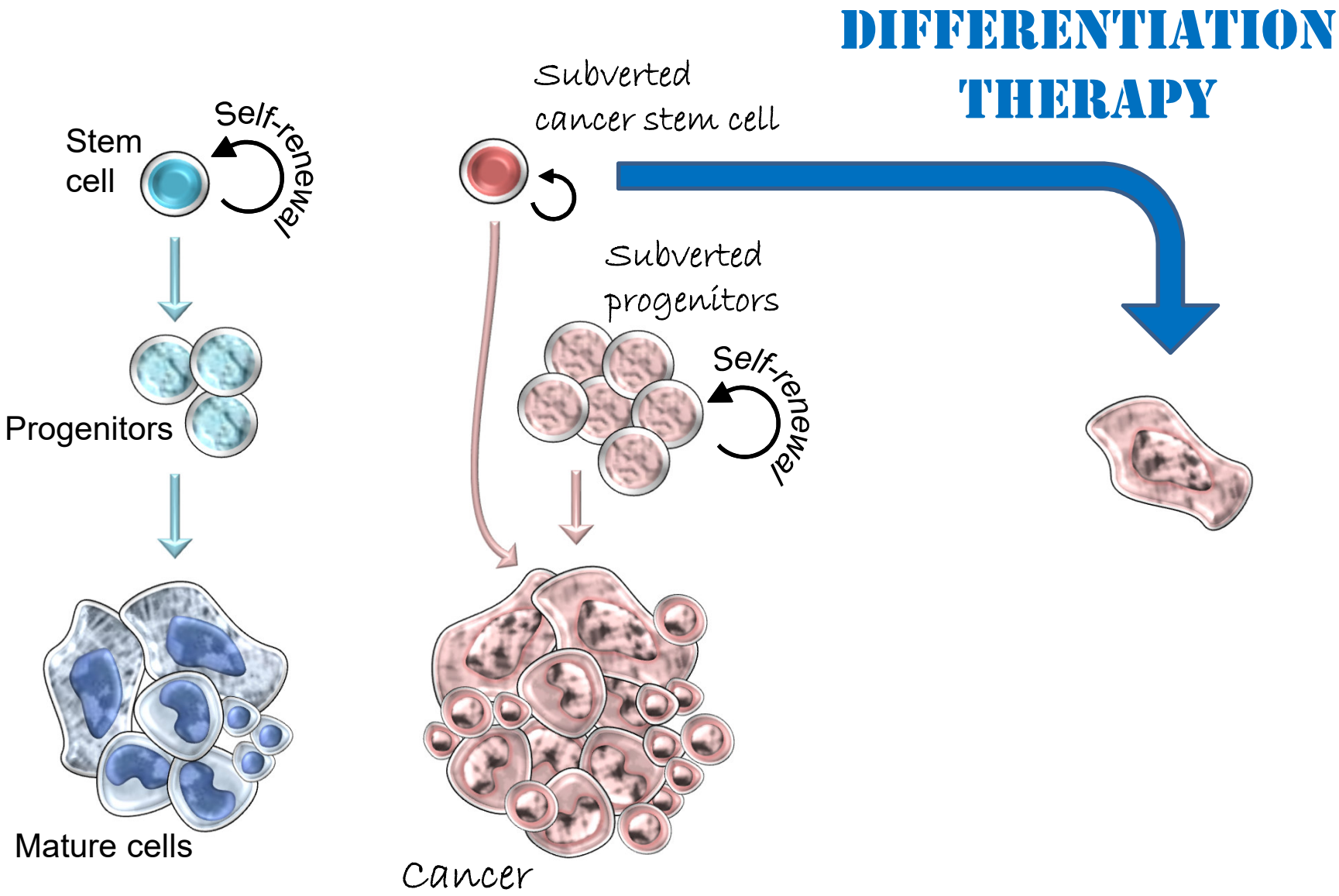
Therapeutic approaches sensitizing CSCs to standard therapy



Therapeutic approaches sensitizing CSCs to standard therapy

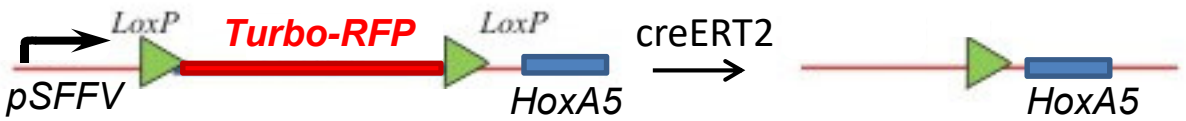


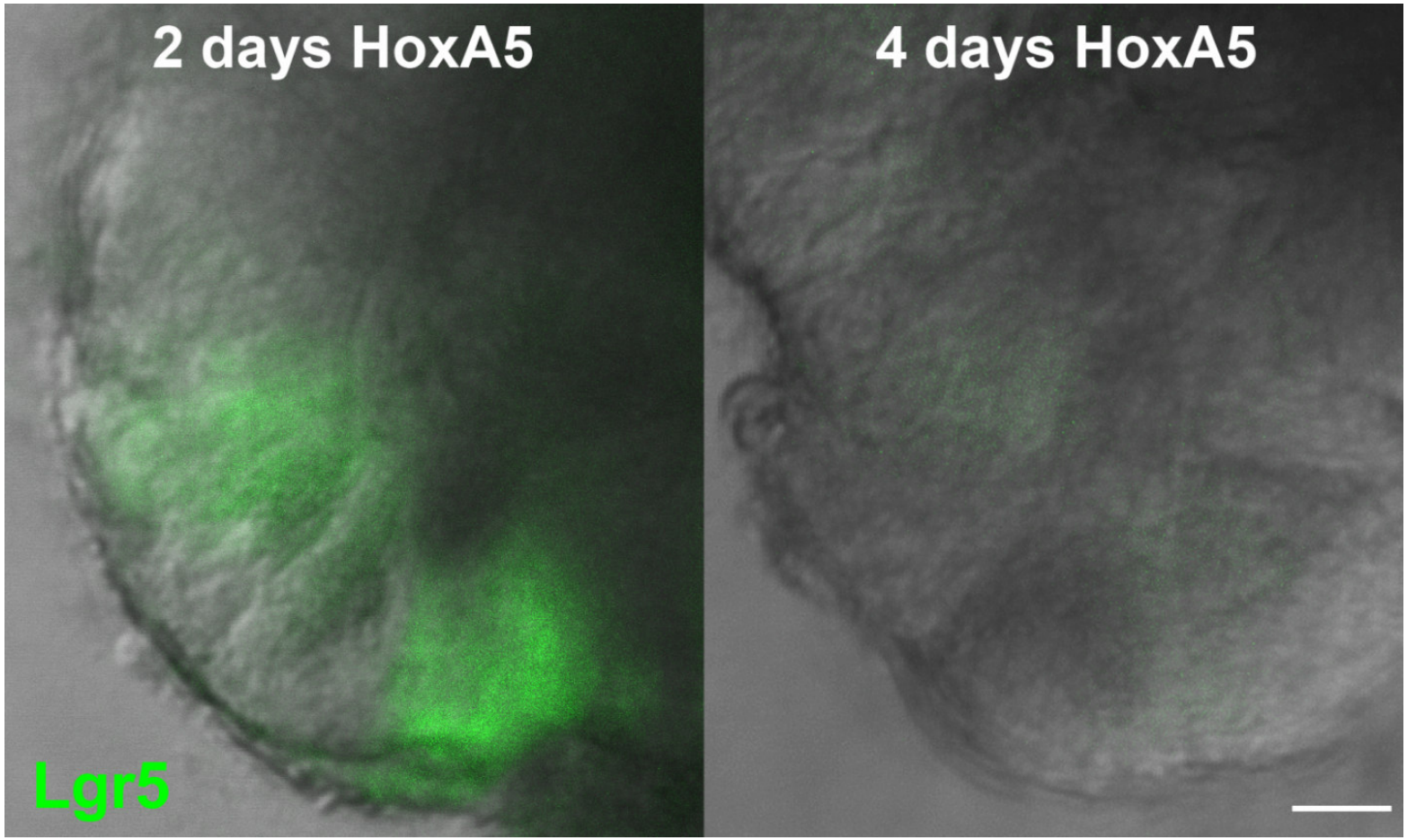
Stem cells in Cancer



Ordonez et al., **Cancer Cell 2015**

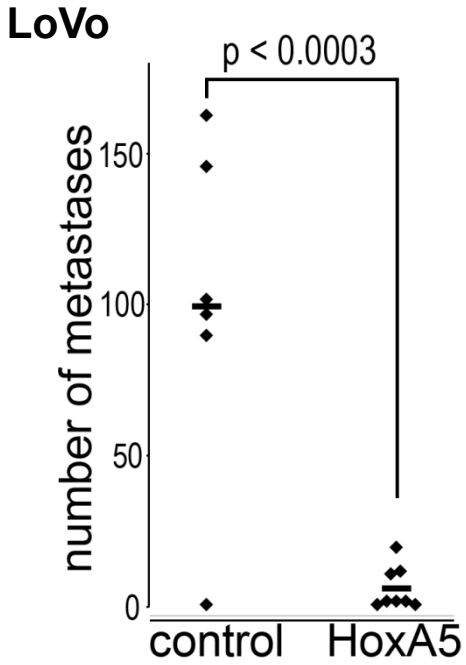
HoxA5 expression reduces intestinal Lgr5^{+} stem cells

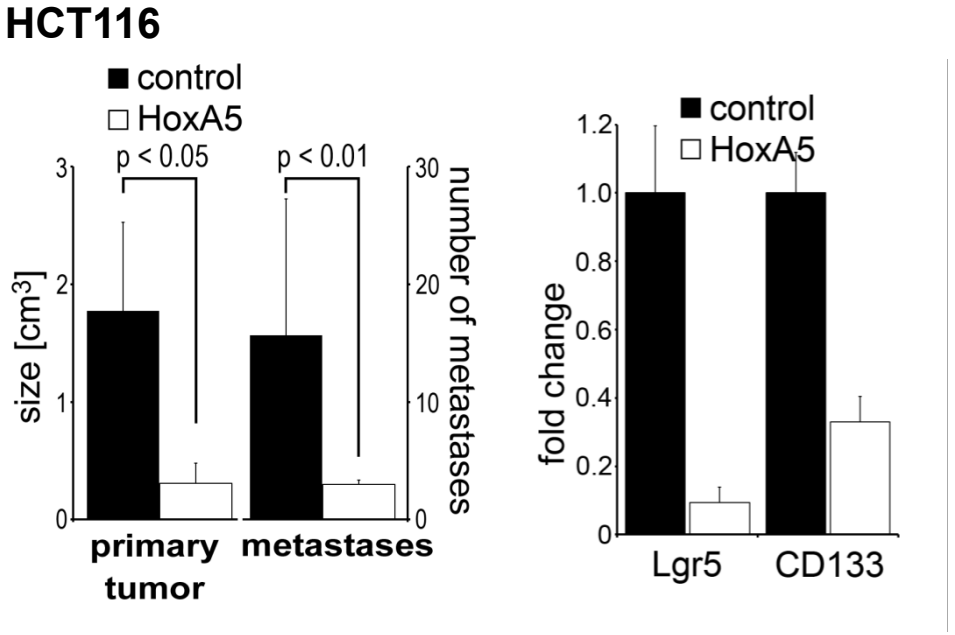


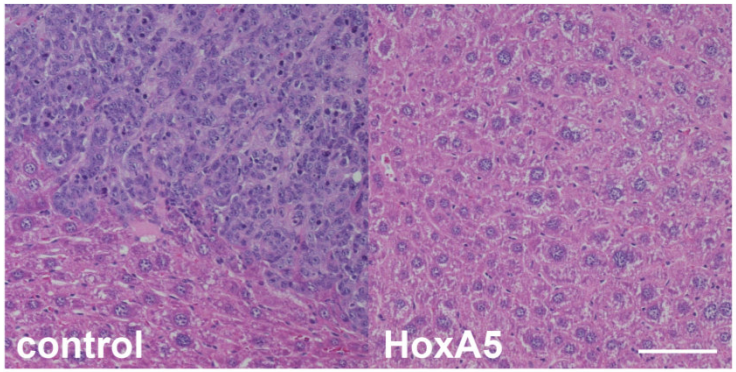


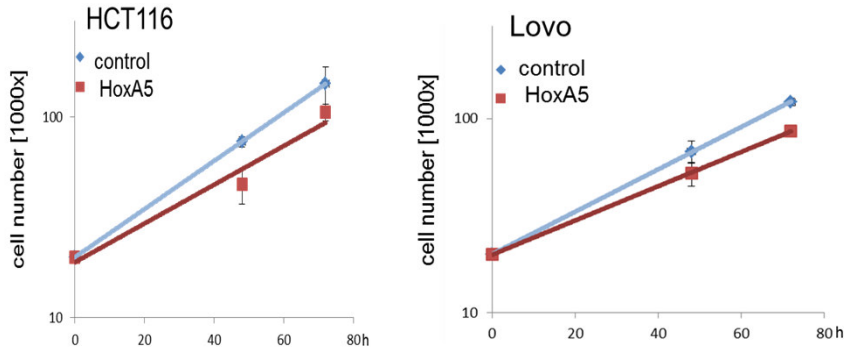
Ordonez et al., ***Cancer Cell 2015***

HoxA5 expression reduces Cancer Stem Cell properties *in vivo*





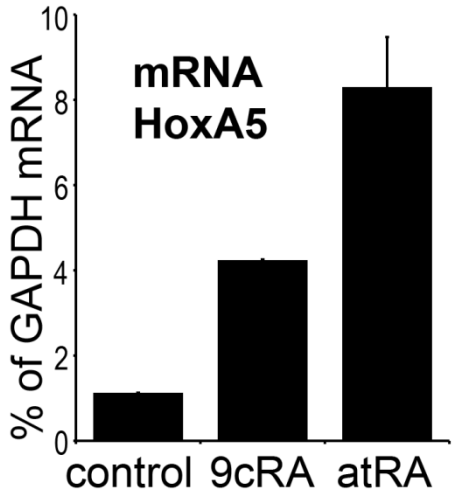


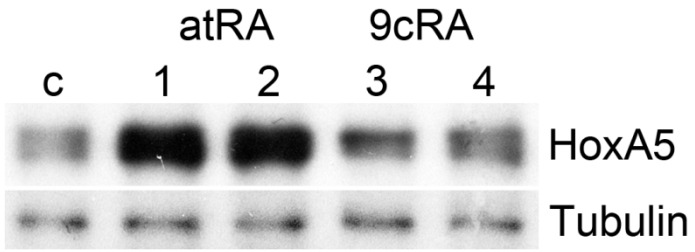


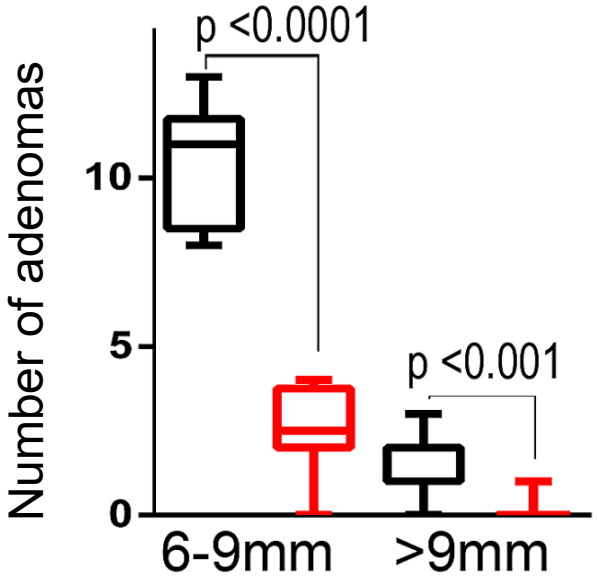
***in vitro* growth**

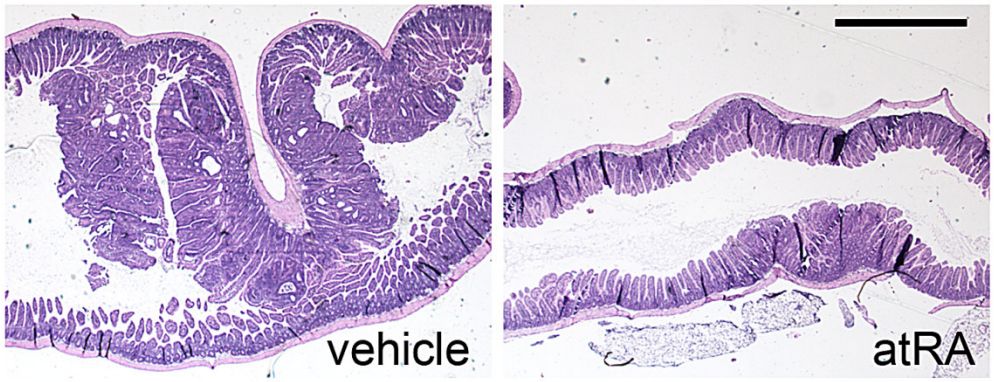
Ordonez et al., ***Cancer Cell 2015***

Retinoic Acid counteracts tumour initiation driven by Wnt *in vivo*



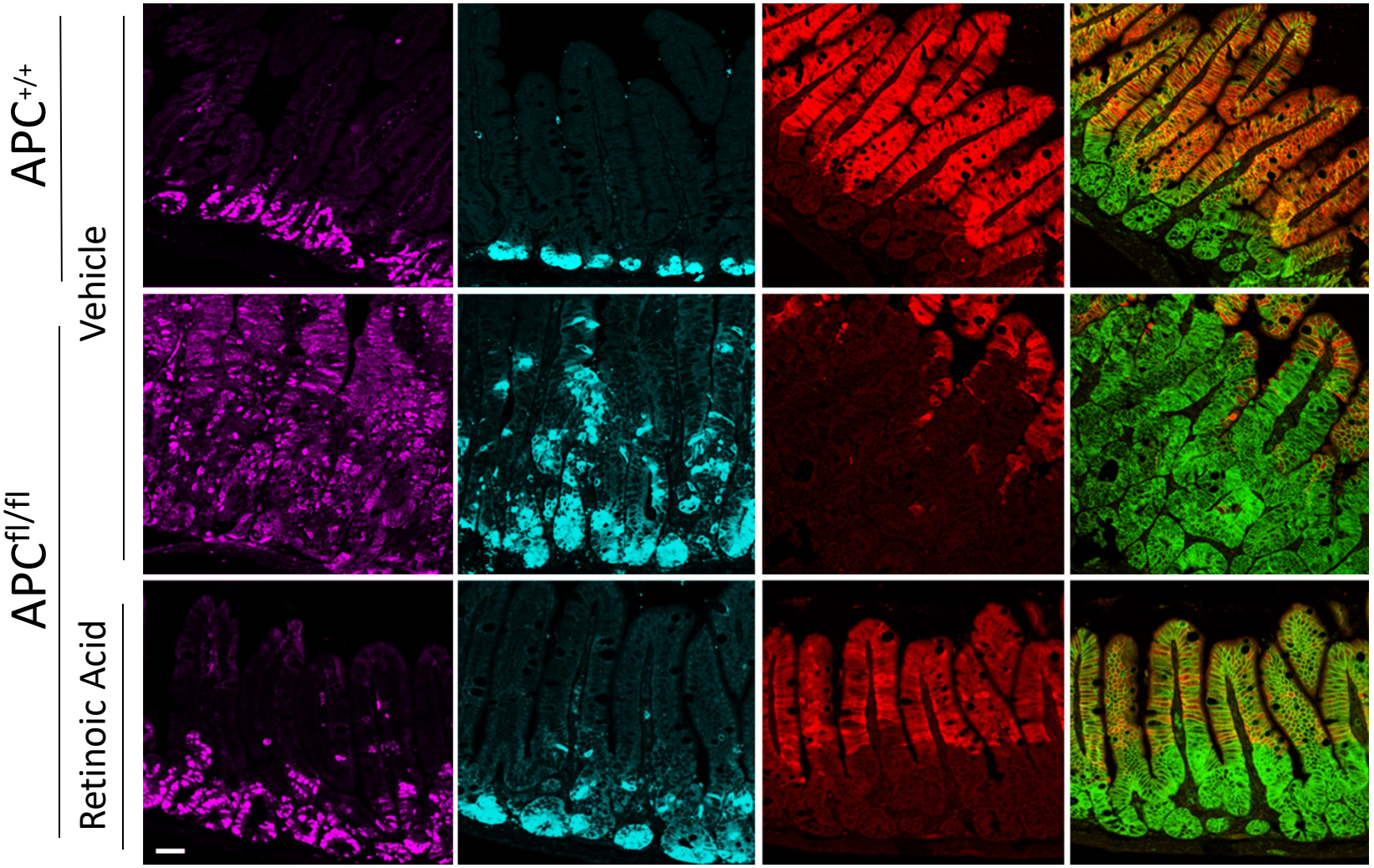






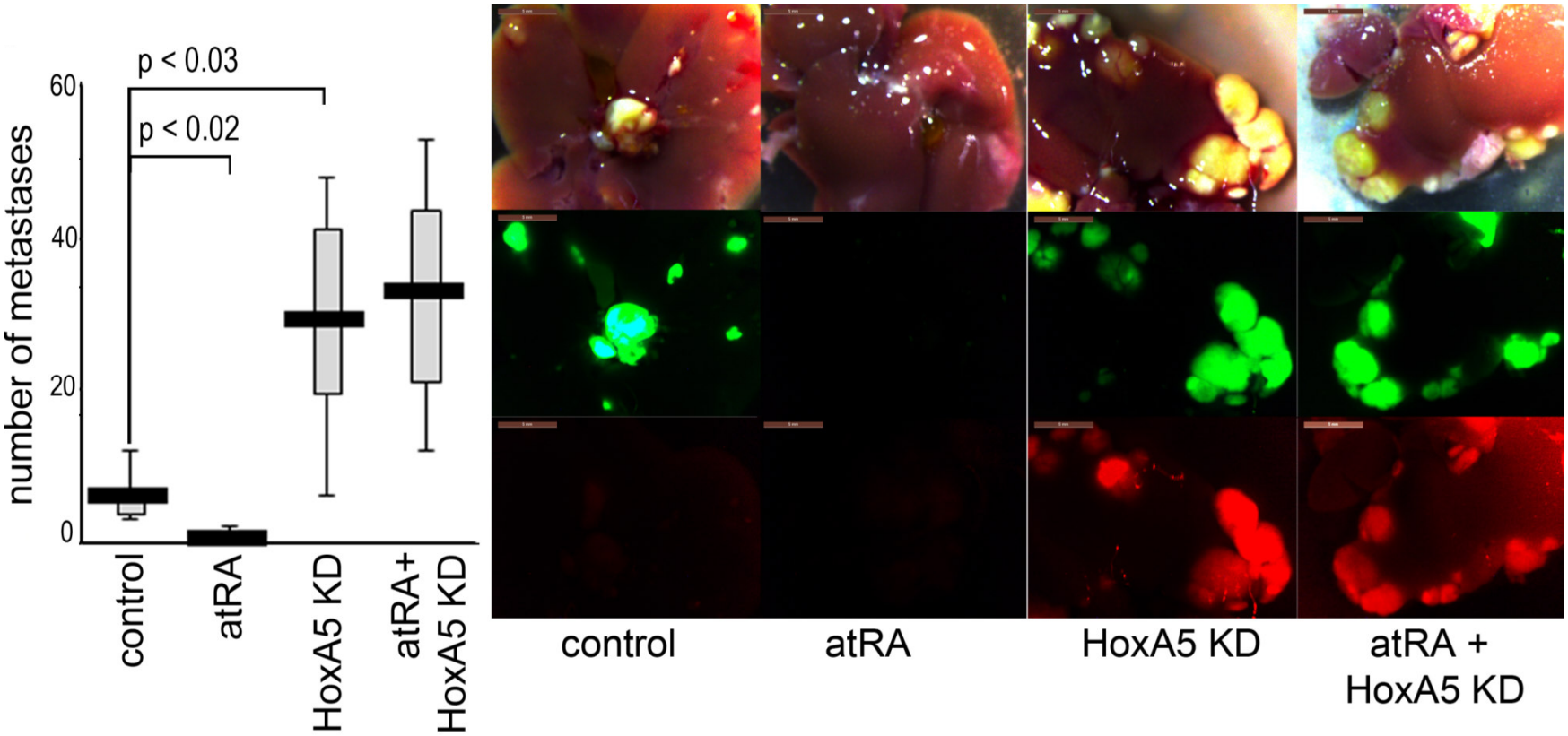
Ordonez et al., ***Cancer Cell 2015***

atRA Counteracts Tumour Initiation Driven by Wnt *in vivo*



Ordonez et al., ***Cancer Cell 2015***

Retinoid induced differentiation therapy requires HoxA5



Ordonez et al., ***Cancer Cell 2015***