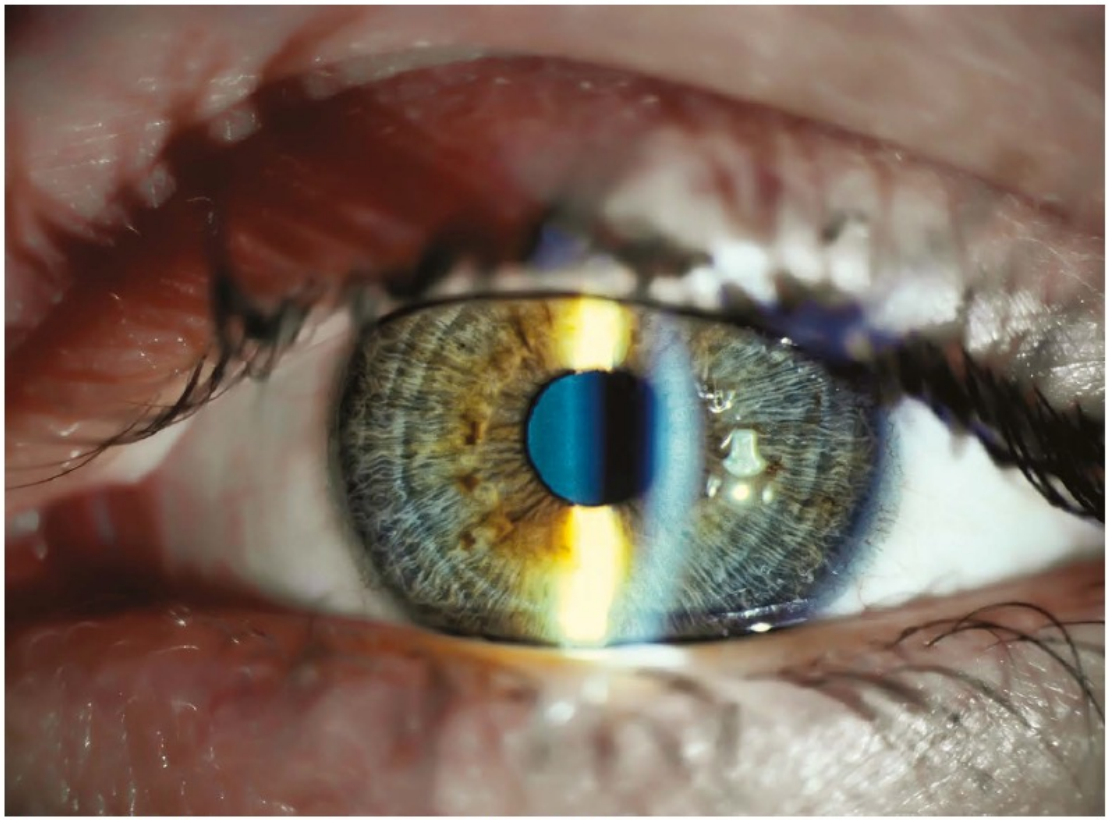
WORLD-FIRST STEM-CELL TREATMENT RESTORES VISION IN PEOPLE

The treatment, given to four people with damaged corneas, needs to be tested in larger trials.

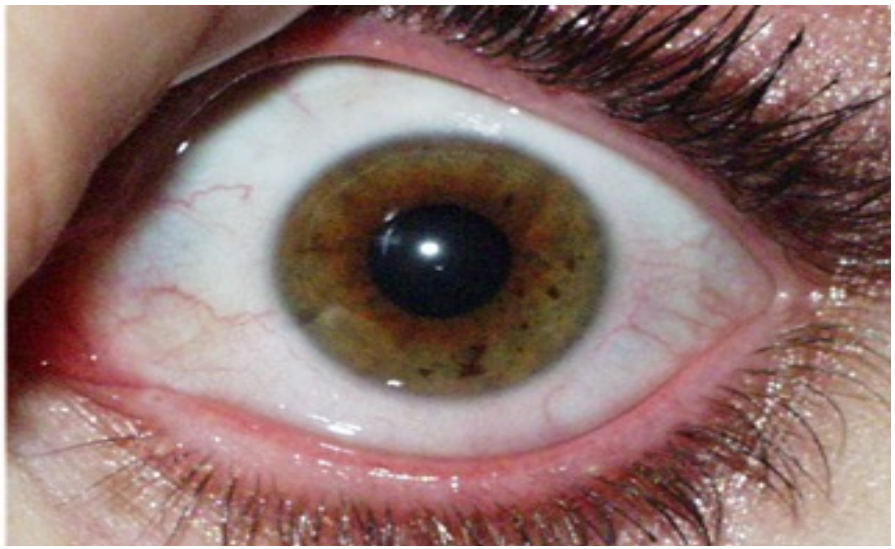


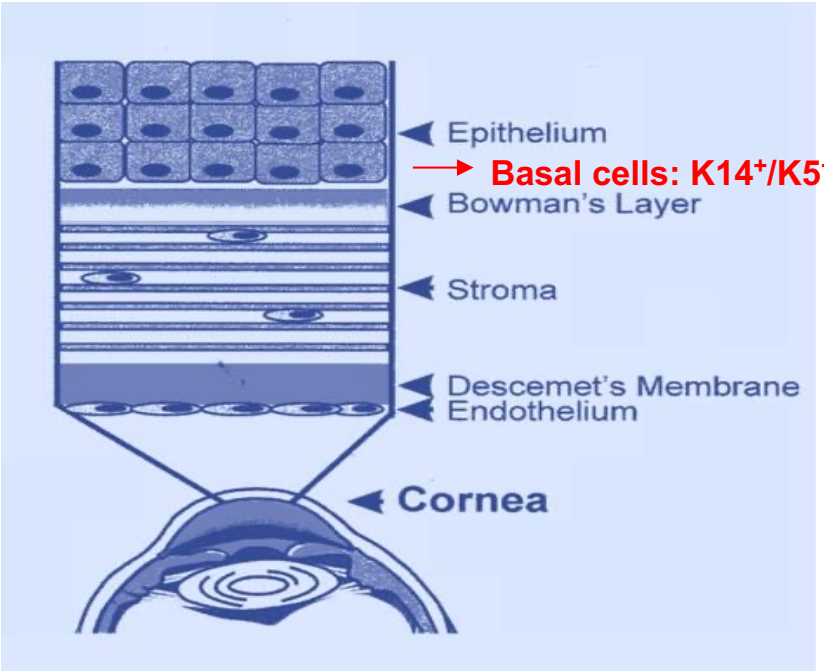
The cornea is the transparent, outermost layer of the eye.

Kohji Nishida, an ophthalmologist at Osaka University in Japan, and his colleagues used an alternative source of cells — induced pluripotent stem (iPS) cells — to make the corneal transplants. They took blood cells from a healthy donor and reprogrammed them into an embryonic-like state, then transformed them into a thin, transparent sheet of cobblestone-shaped corneal epithelial cells (T. Soma *et al. Lancet* **404**, 1929–1939; 2024).

Between June 2019 and November 2020, the team enrolled two women and two men aged between 39 and 72 years old with LSCD in both eyes. As part of the surgery, the team scraped off the layer of scar tissue covering the damaged cornea in only one eye, then stitched on epithelial sheets derived from a donor and placed a soft protective contact lens on top.

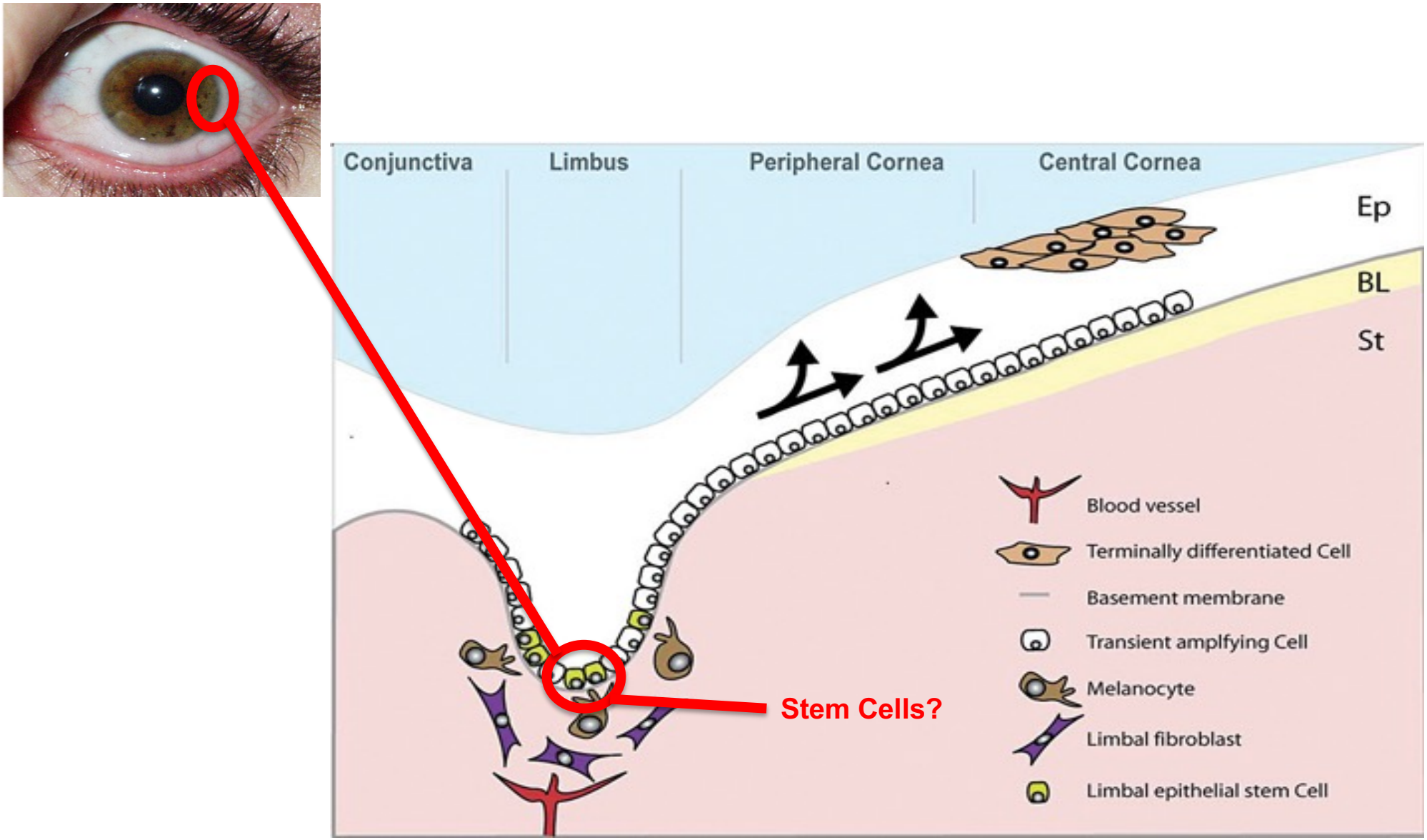
The Cornea



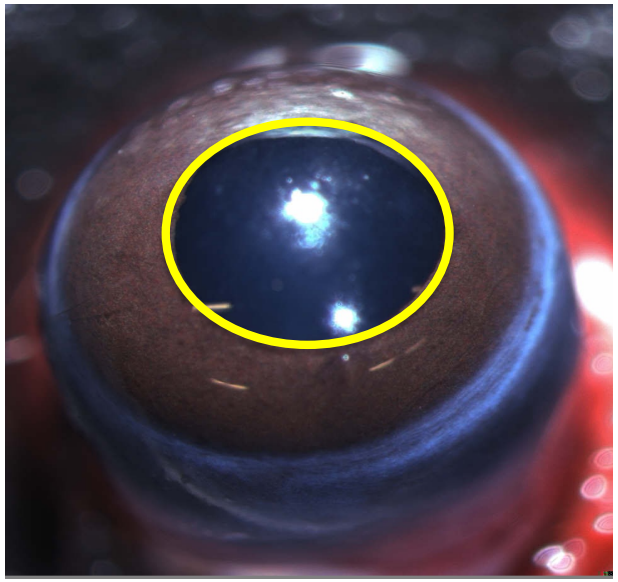


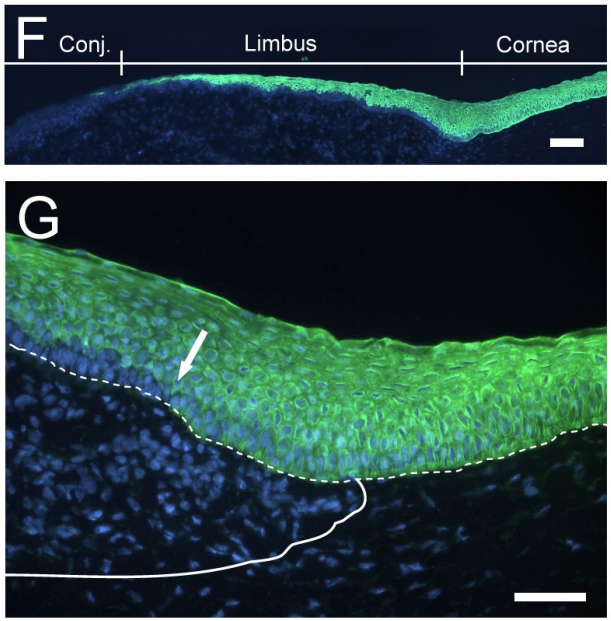
WHO: 25 x 10^6 people world wide are estimated to be affected by diseases or injuries causing corneal blindness with 1.5-2 x 10^6 new cases every year.

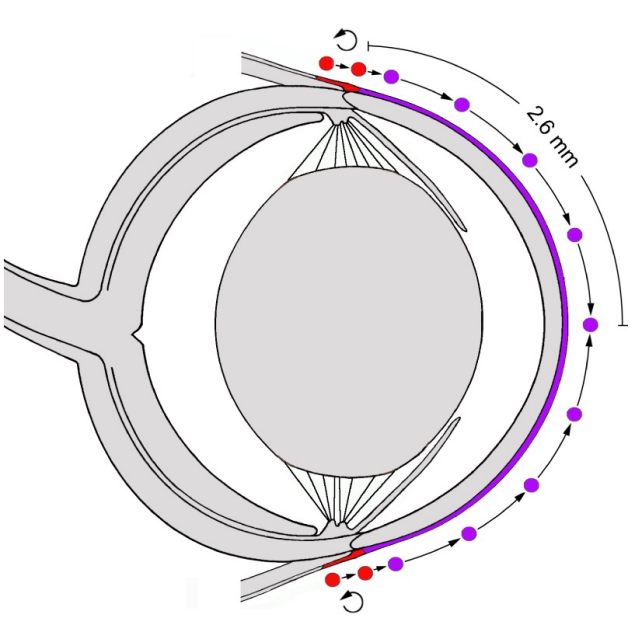
The corneal epithelium is maintained by stem cells



Corneal stem cells in the limbus are responsible for renewal of the cornea and wound healing







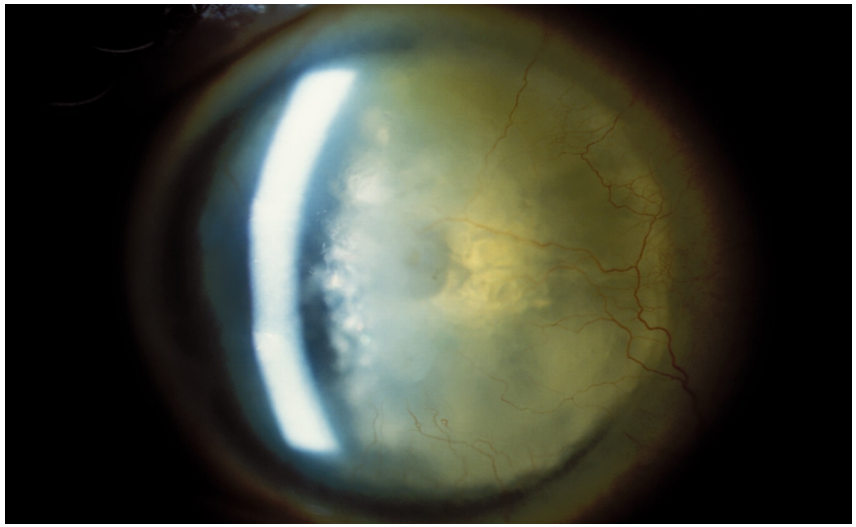
1. Do not express Keratin 3/12
2. Limbus cells are LRC
3. After severe injuries cells of the limbus migrate towards the wound to repair the injurie

Majo and Barrandon

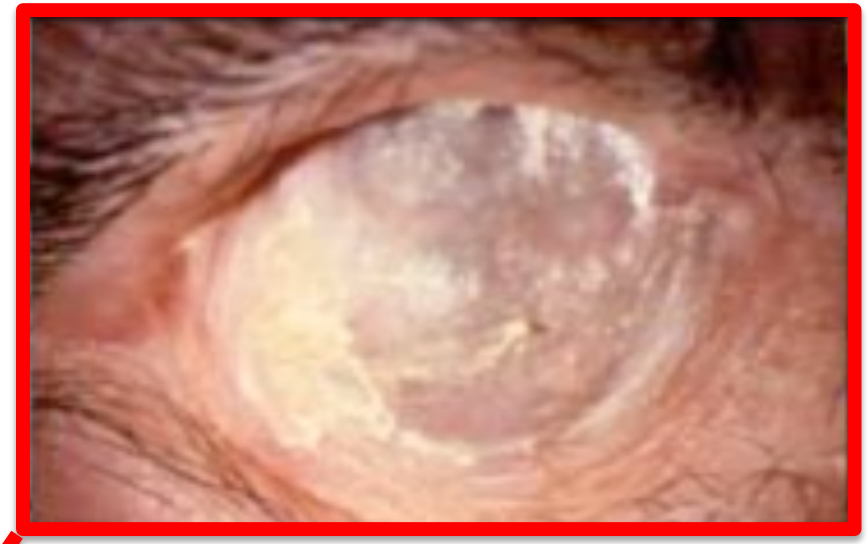
4. Cells can be cultured and transplanted to improve visison.

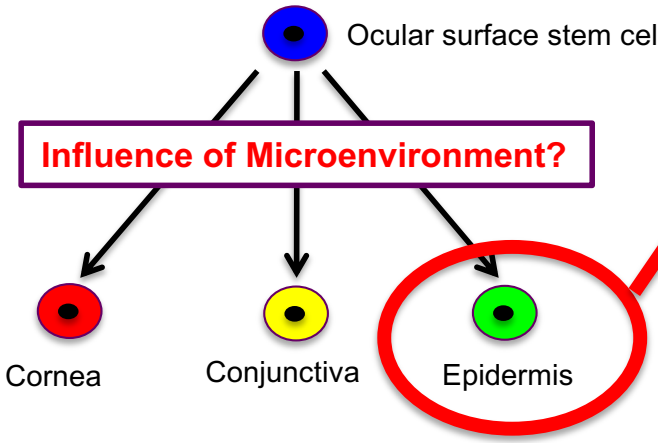
The corneal epithelium exhibits plasticity

Corneal conjunctivalisation

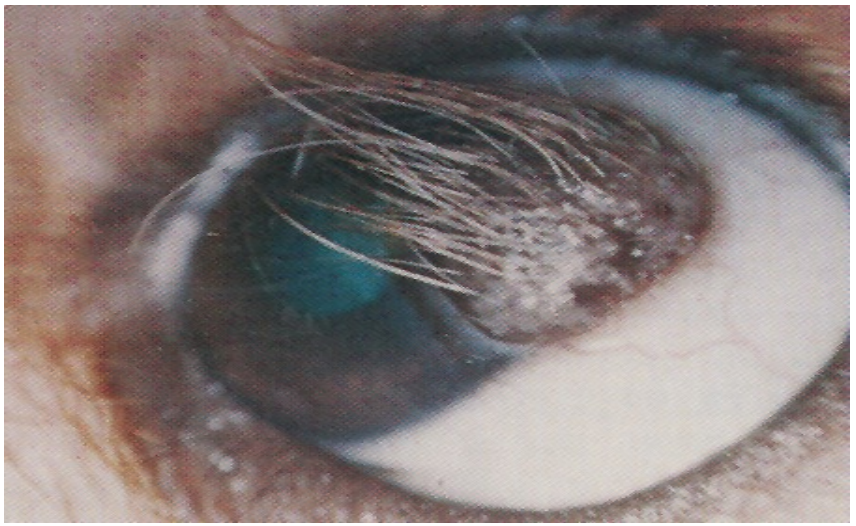


Squamous cell metaplasia/keratinisation





Limbal dermoide



Induced pluripotent stem-cell-derived corneal epithelium for transplant surgery: a single-arm, open-label, first-in-human interventional study in Japan

Takeshi Soma, Yoshinori Oie*, Hiroshi Takayanagi*, Shoko Matsubara, Tomomi Yamada, Masaki Nomura, Yu Yoshinaga, Kazuichi Maruyama, Atsushi Watanabe, Kayo Takashima, Zaixing Mao, Andrew J Quantock, Ryuhei Hayashi, Kohji Nishida*

Summary

Background The loss of corneal epithelial stem cells from the limbus at the edge of the cornea has severe consequences for vision, with the pathological manifestations of a limbal stem-cell deficiency (LSCD) difficult to treat. Here, to the best of our knowledge, we report the world's first use of corneal epithelial cell sheets derived from human induced pluripotent stem cells (iPSCs) to treat LSCD.

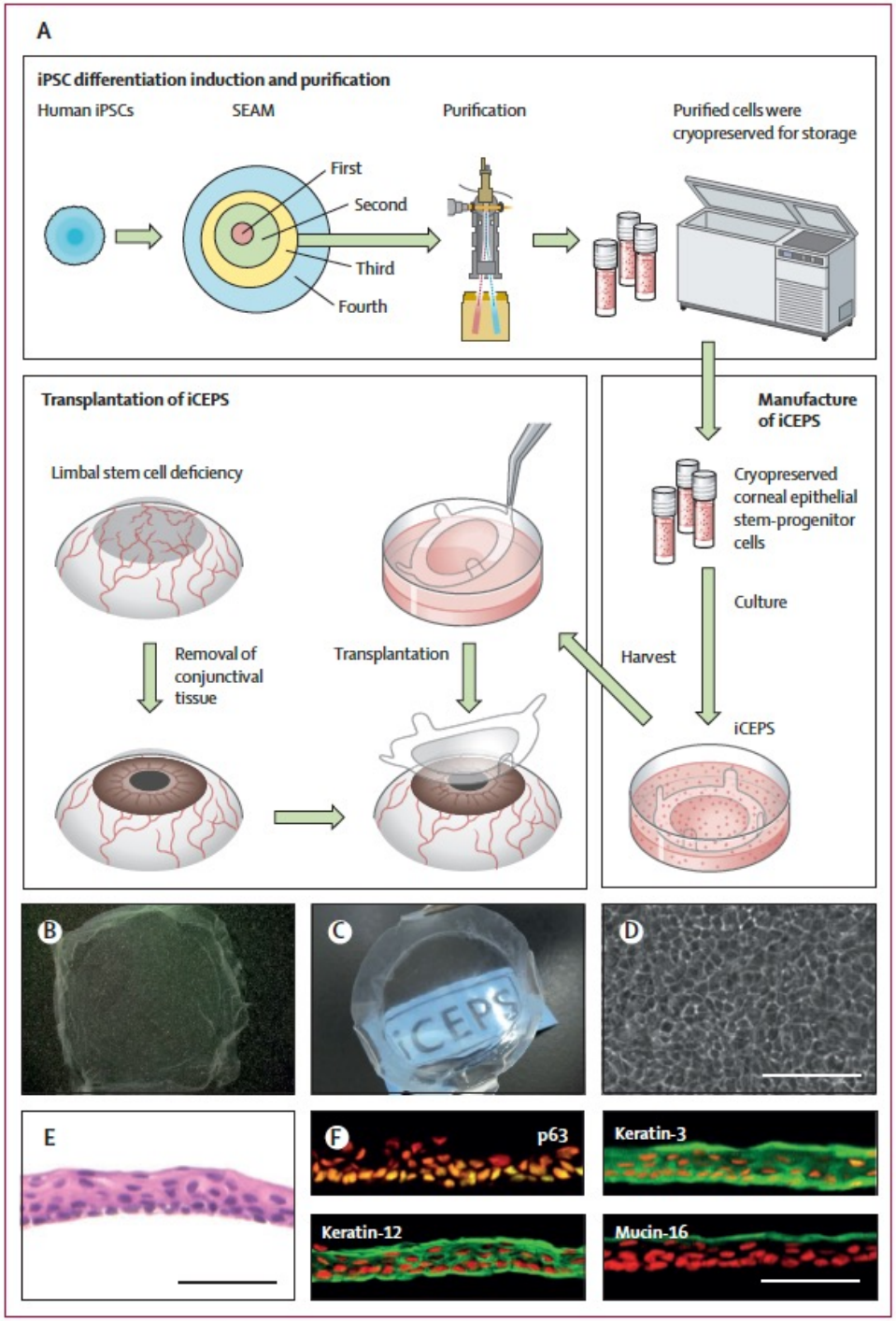


Figure 1: **Fabrication and transplantation of human iCEPSs**

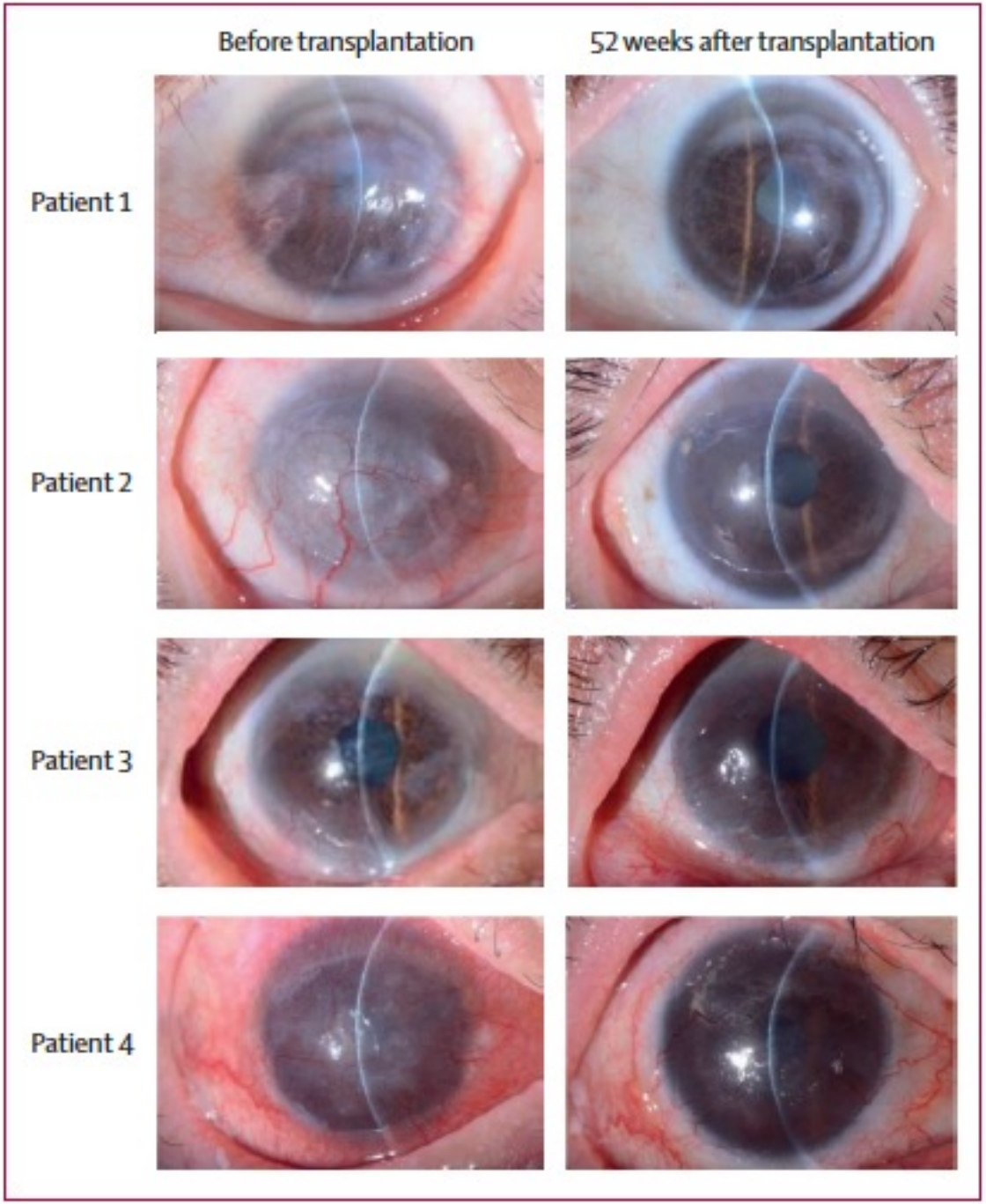
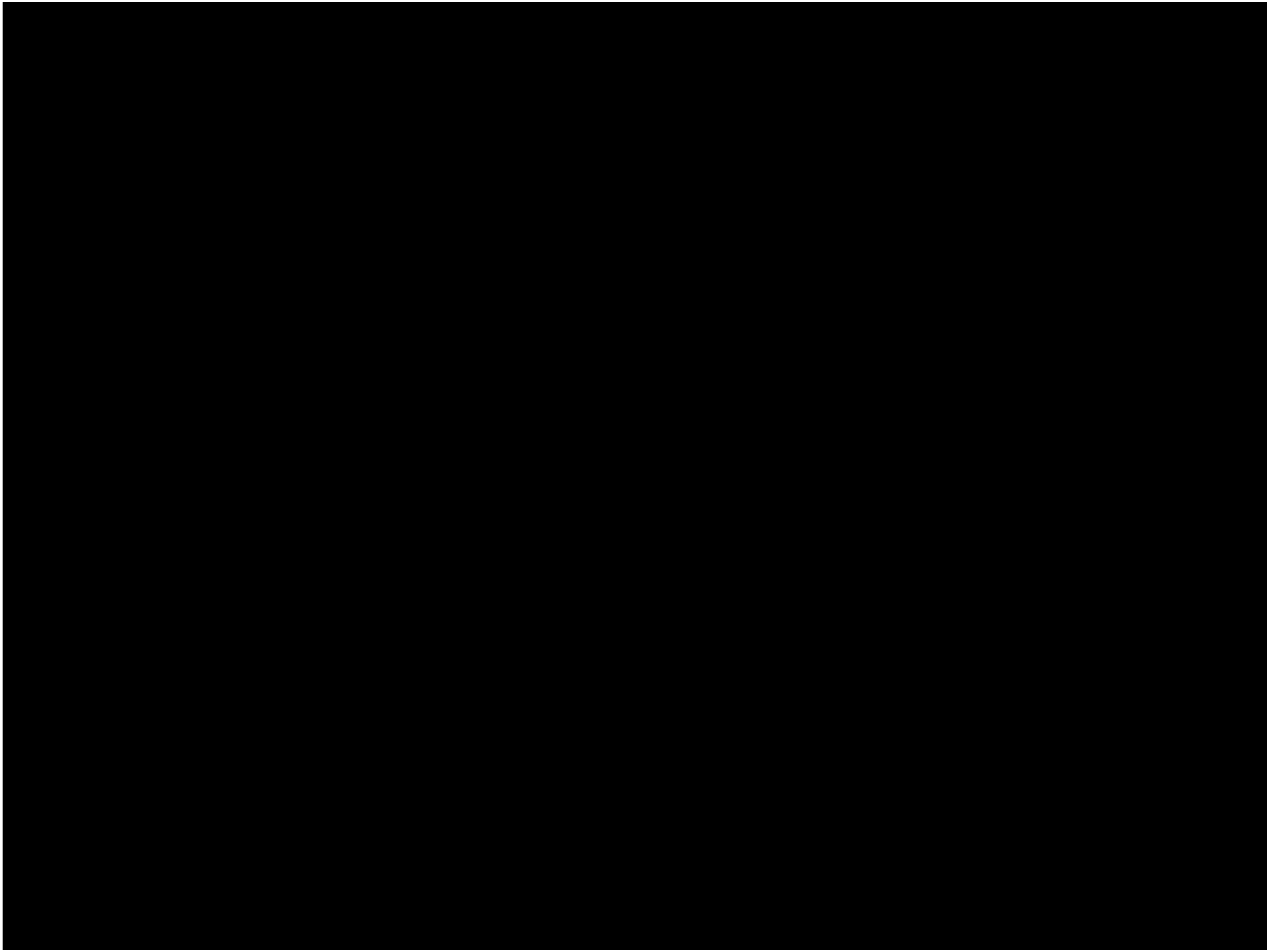
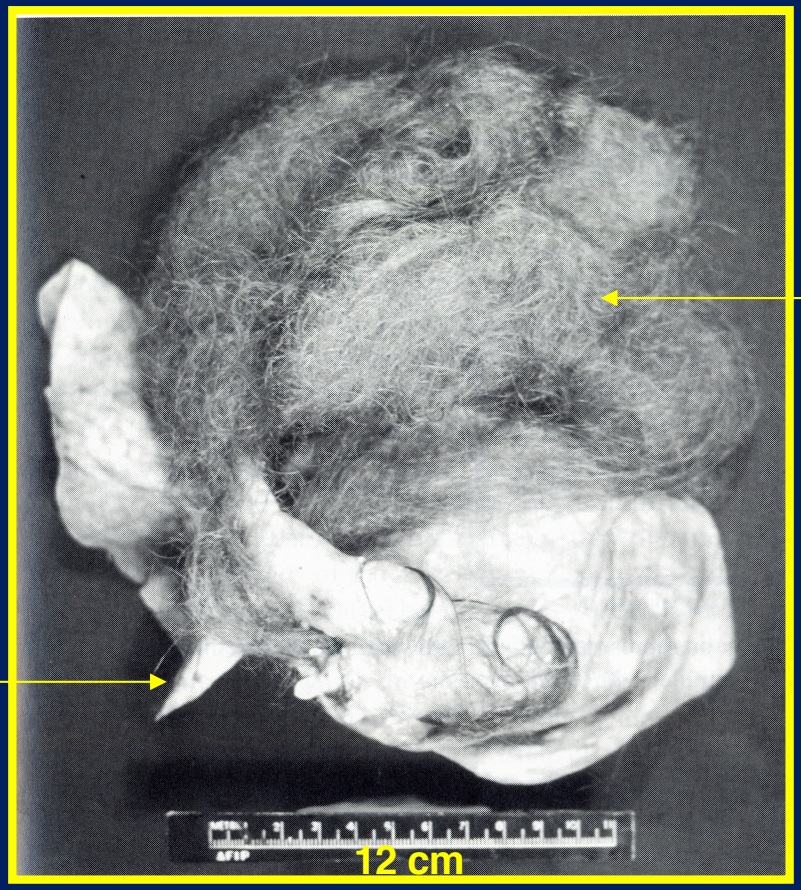


Figure 3: **Slit-lamp microscopy images of the treated eyes**



Stem cells and Cancer

1. Cancer stem cell is not a novel concept
2. Cancer
3. Characteristics of Stem Cells and Cancer Cells
4. Definition of Cancer Stem cells (CSC).
5. Examples for the existence of CSC
6. Experimental systems to study CSC(examples)
7. Possible mechanisms for drug resistance of CSC
8. CSC debated



Human Teratoma

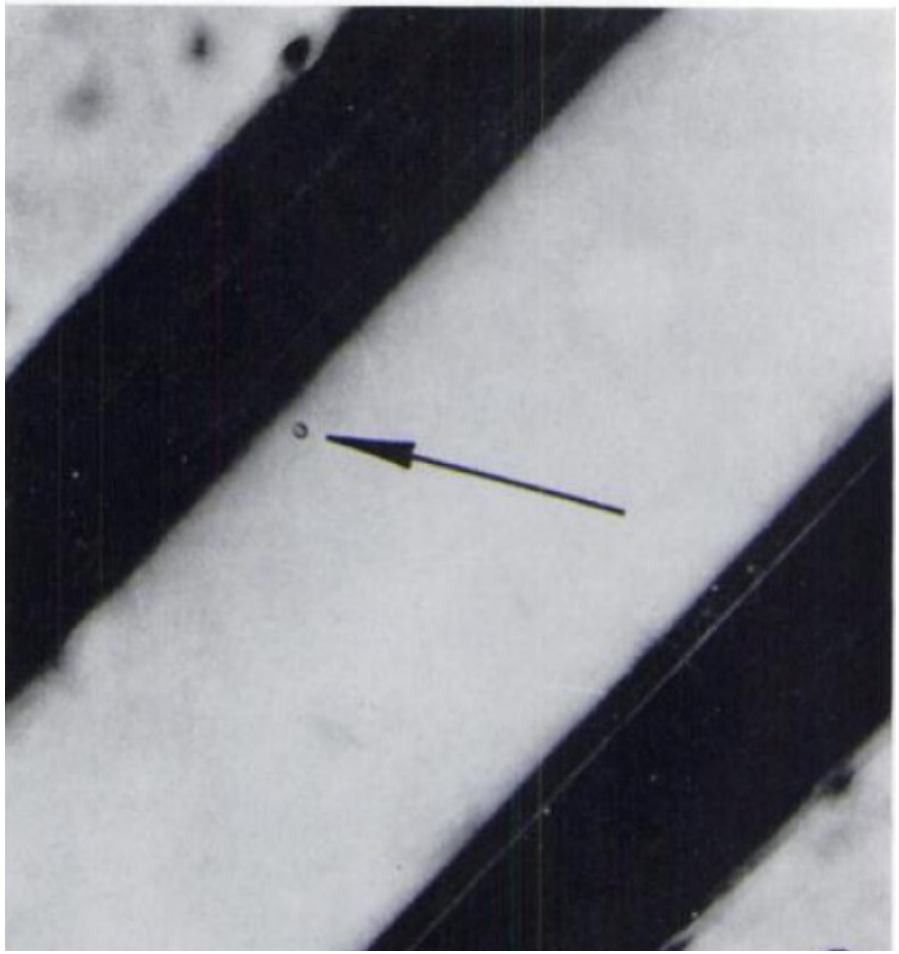
1907: Askanazy M. suggested that the well differentiated cystic structures from benign cystic ovarian teratomas develop from either a single multipotent type of cell or from a group of cells composed of representatives of each of the embryonic germinal layers

Multipotentiality of Single Embryonal Carcinoma Cells*

LEWIS J. KLEINSMITH† AND G. BARRY PIERCE, JR.‡

(Department of Pathology, The University of Michigan, Ann Arbor, Michigan)

Cancer Research 1964



From 1700 single cell grafts, 43 multilineage, transplantable, teratocarcinomas were obtained

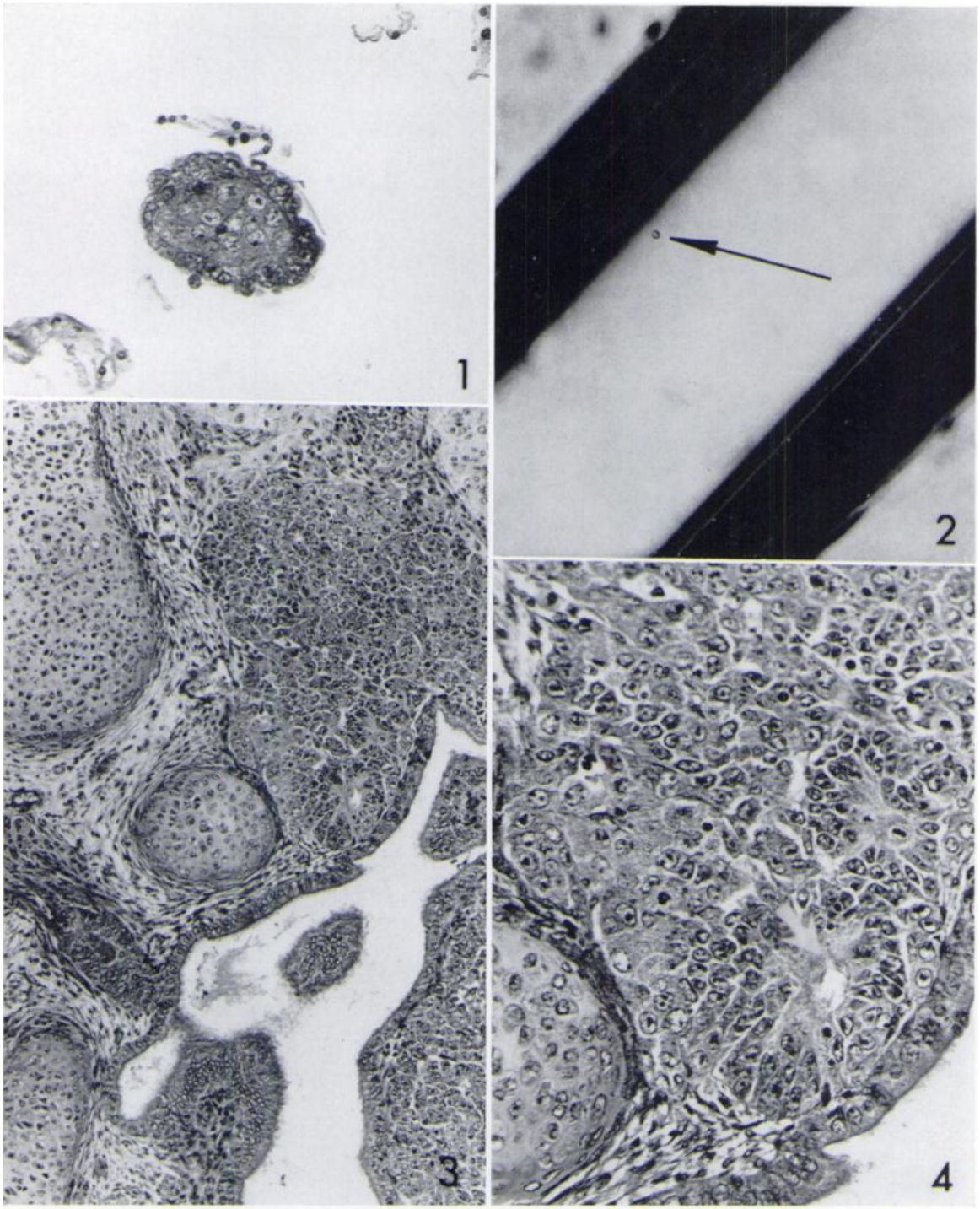


FIG. 1.—Small embryoid body consisting of a core of embryonal embryonal carcinoma invested with a layer of visceral yolk sac. × 300.

FIG. 2.—Single cell (*arrow*) isolated in a capillary tube. × 100.

FIG. 3.—Low-power view of an area from one of the well differentiated clones, showing cartilage, ciliated glands, mesenchyme, primitive neuroepithelium, and embryonal carcinoma. × 120.

FIG. 4.—Higher-power view of an area from Fig. 3, showing the embryonal carcinoma with primitive neuroepithelium (*arrow*) developing in it. × 300.

"These findings were interpreted as giving strong support to the stem cell theory of cancer."

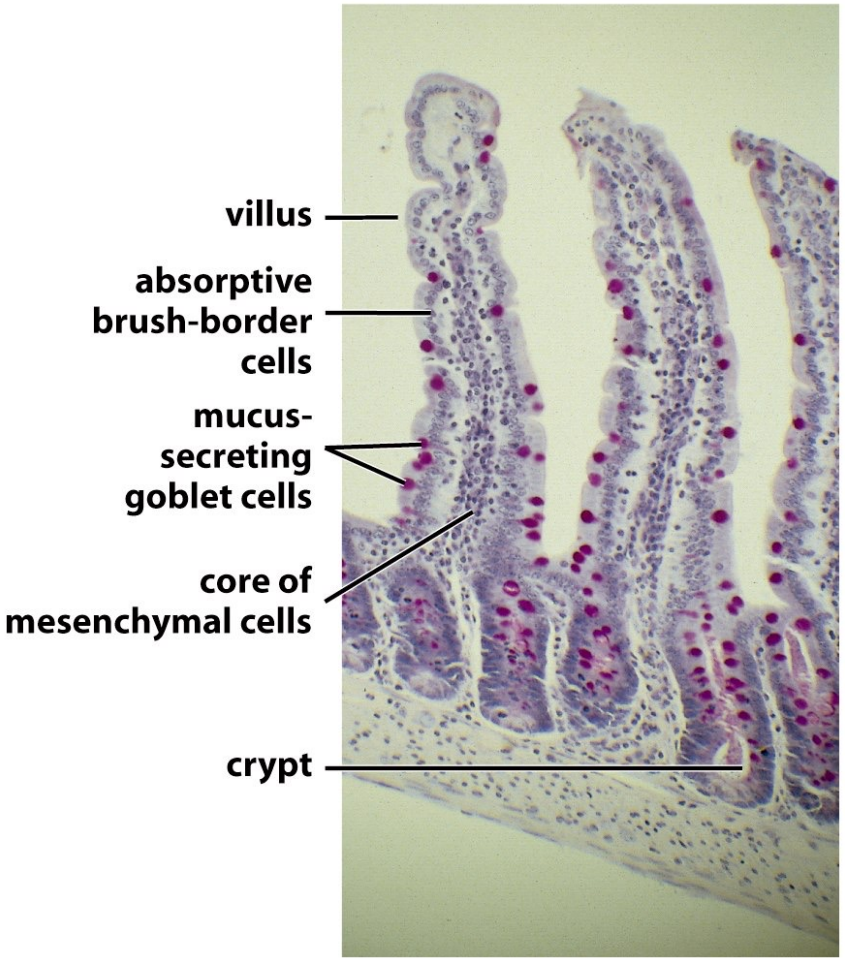
Multipotentiality of Single Embryonal Carcinoma Cells

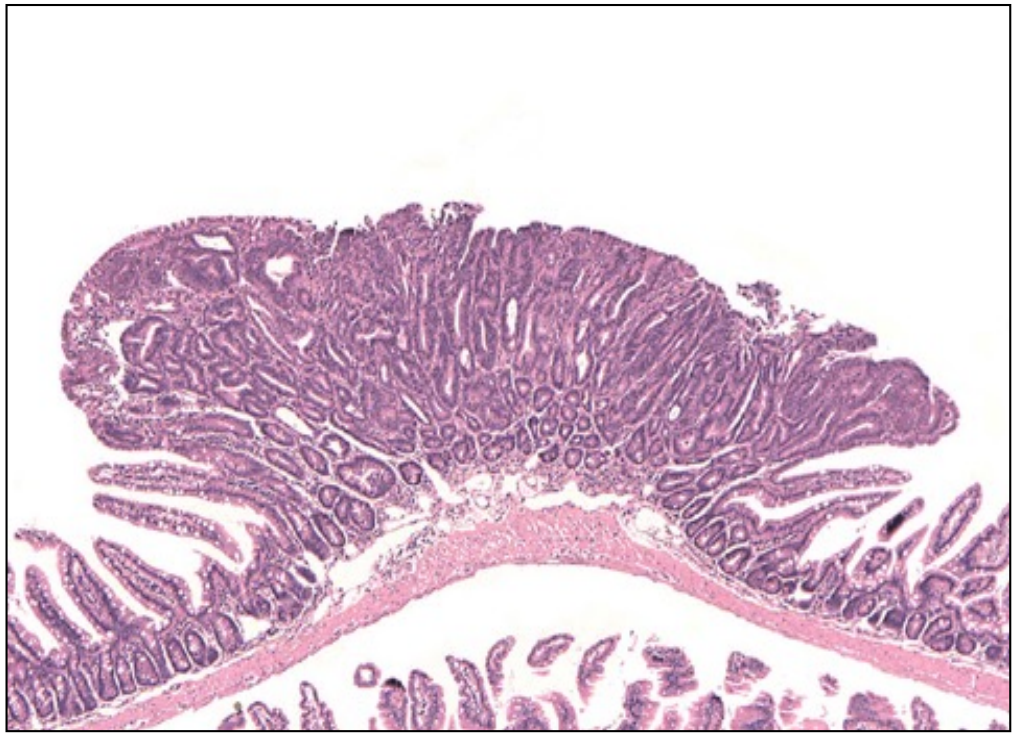
LEWIS J. KLEINSMITH AND G. BARRY PIERCE

Cancer Research, 1964

Proper tissue architecture depends on:

1. Maintenance of appropriate





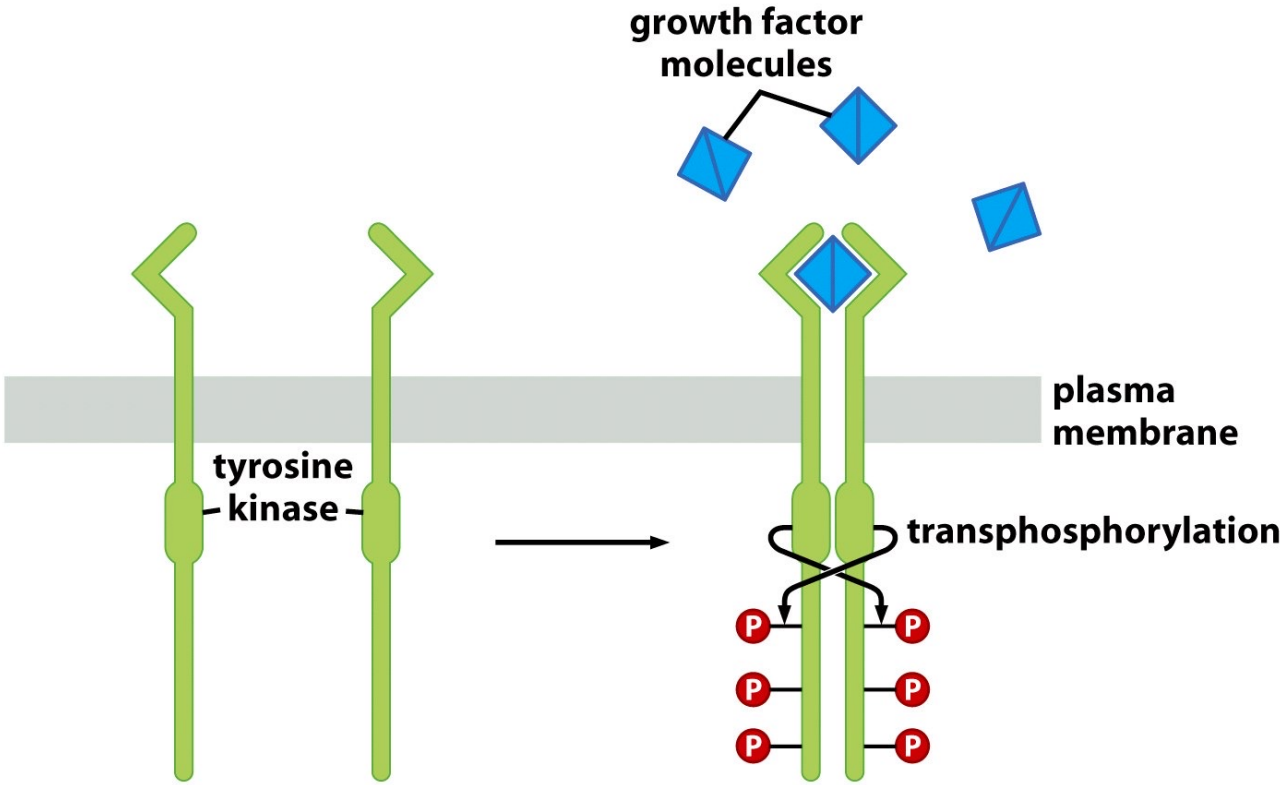
4 cell types: enterocytes, enteroendocrine cells, goblet cells and paneth cells, + stem and TA cells

Communication between cells is often done by
Growth factors

Growth factors are relatively small proteins that are released by some cells, make their way through the inter cellular space, bind to other cells and thereby induce a signal (e.g. growth versus no growth)

Decisions about growth versus no-growth must be made for the welfare of the entire tissue and whole organism, not for the benefit of an individual cell.

Free sliding of growth factor receptors is linked to cancer



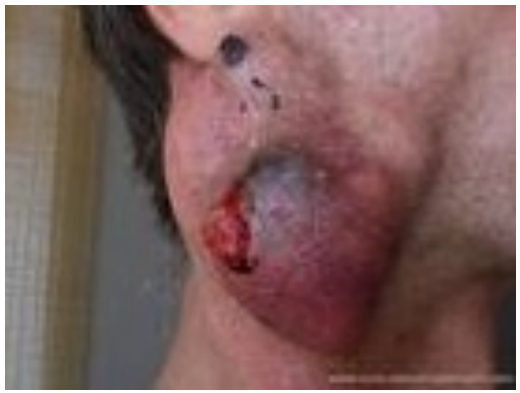
In the absence of ligand , receptor molecules (green) are free to move laterally in the plane of the plasma membrane. In the presence of growth factor ligands, two receptor molecules are brought together to form a dimer. Once the receptor is dimerized the tyrosine kinase domain of each subunit is able to phosphorylate the C-terminal cytoplasmic tail of the other subunit: "transphosphorylation"

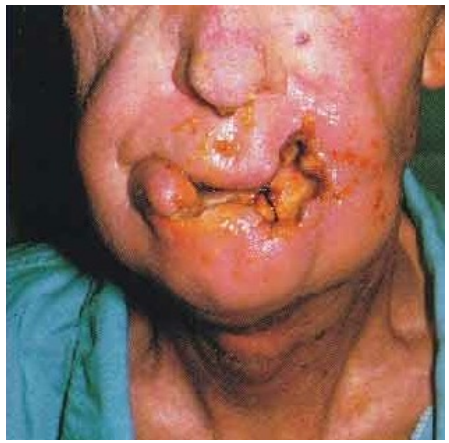
Tumors often overexpress growth factor receptors. Free sliding receptors collide, dimerize and subsequently transphosphorylate each others cytoplasmic tail, which may explain ligand independent signaling.

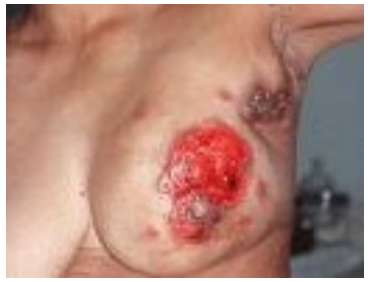
Table 5.2 Tyrosine kinase GF receptors altered in human tumors[a]

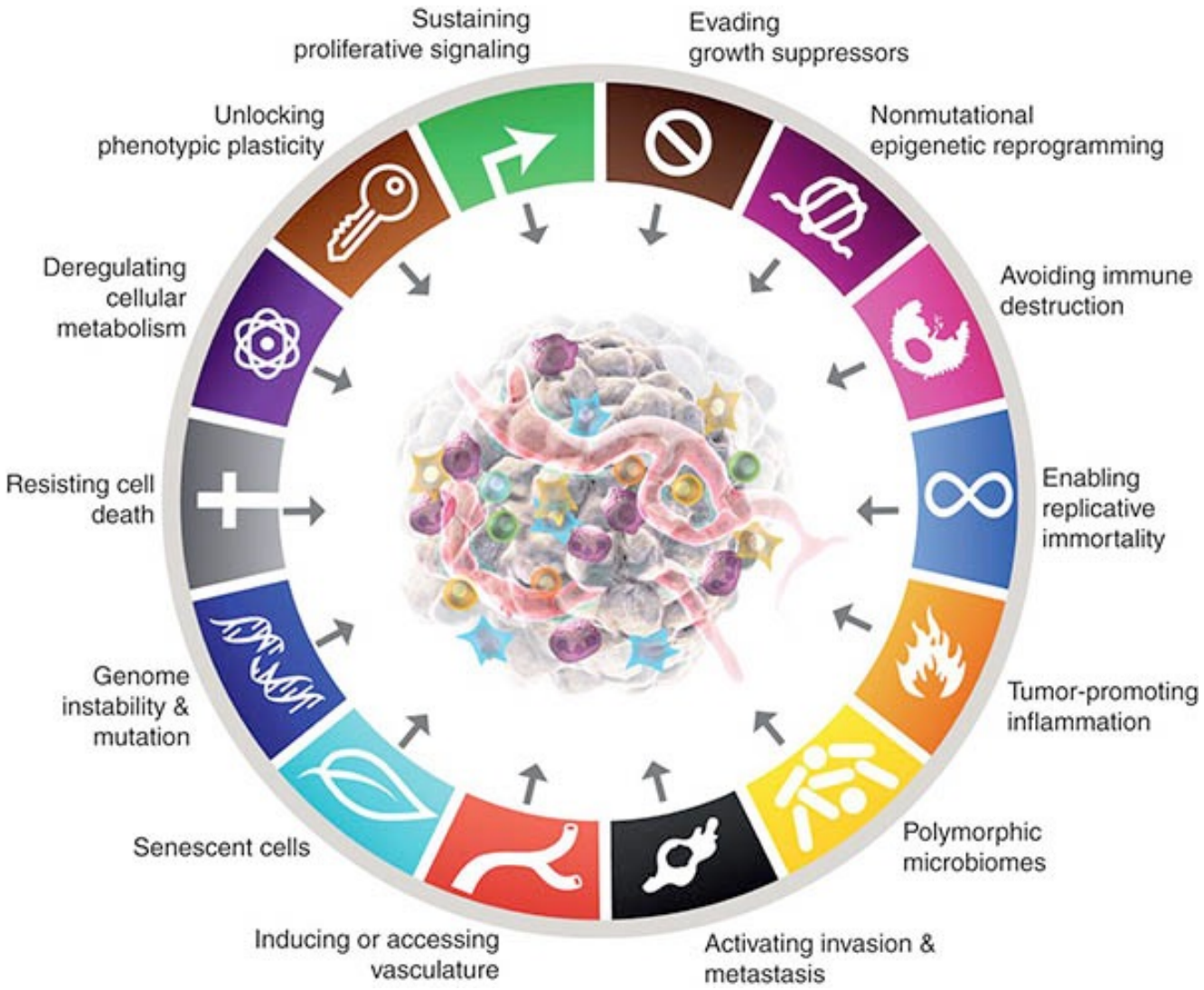
Name of receptor	Main ligand	Type of alteration	Types of tumor
EGF-R/ErbB1	EGF, TGF-α	overexpression	non-small cell lung cancer; breast, head and neck, stomach, colorectal, esophageal, prostate, bladder, renal, pancreatic, and ovarian carcinomas; glioblastoma
EGF-R/ErbB1		truncation of ectodomain	glioblastoma, lung and breast carcinomas
ErbB2/HER2/Neu	NRG, EGF	overexpression	30% of breast adenocarcinomas
ErbB3, 4	various	overexpression	oral squamous cell carcinoma
Flt-3	FL	tandem duplication	acute myelogenous leukemia
Kit	SCF	amino acid substitutions	gastrointestinal stromal tumor
Ret		fusion with other proteins, point mutations	papillary thyroid carcinomas, multiple endocrine neoplasias 2A and 2B
FGF-R3	FGF	overexpression; amino acid substitutions	multiple myeloma, bladder and cervical carcinomas

[a]See also Figure 5.17.





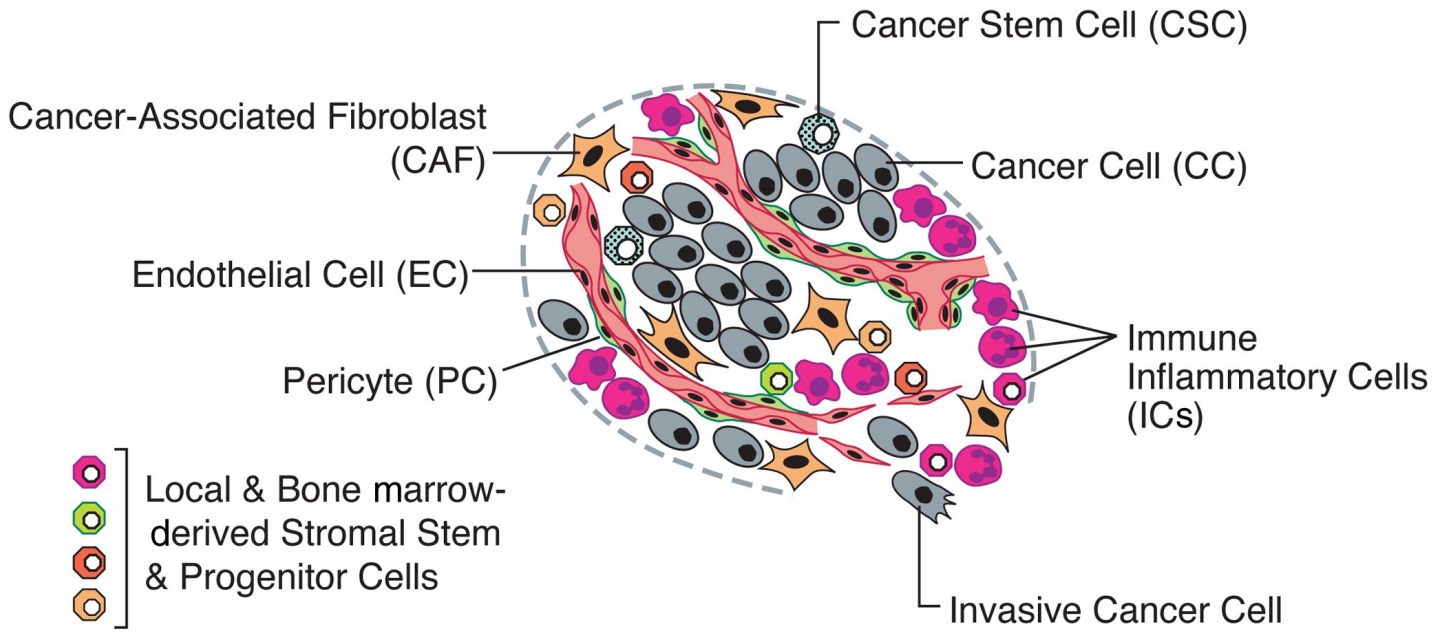


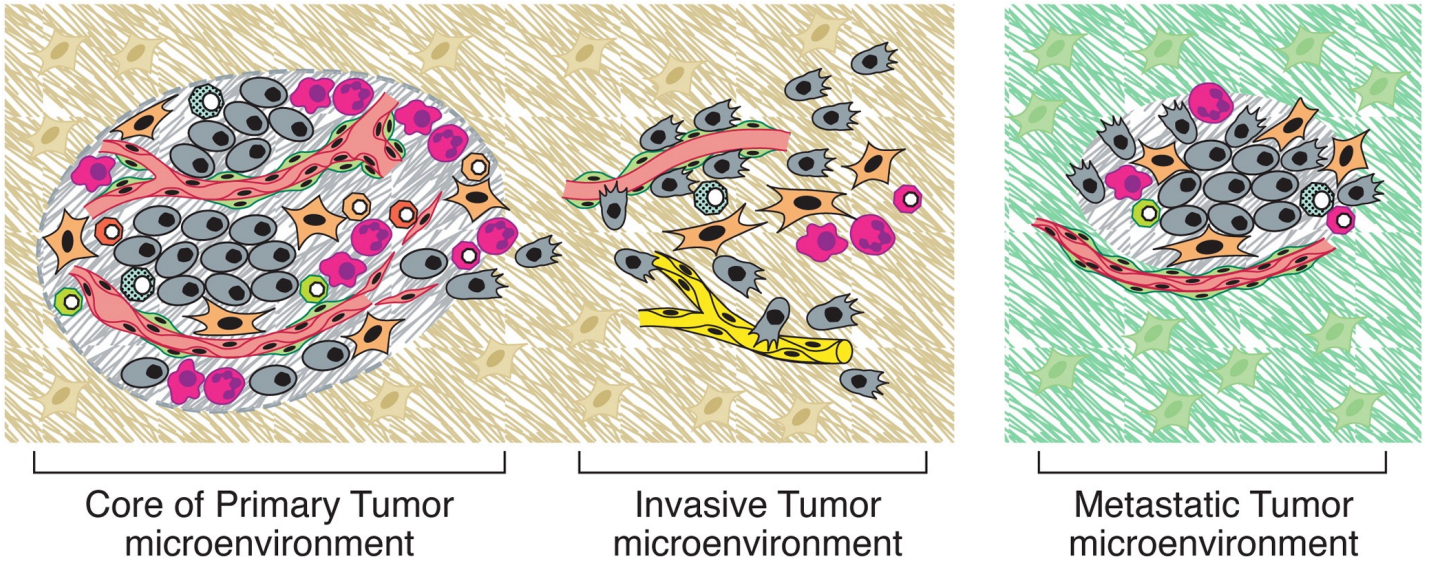


Breast
Colon
Lung
Prostate
Ovary
Constitute
approx. 80%
of all cancers

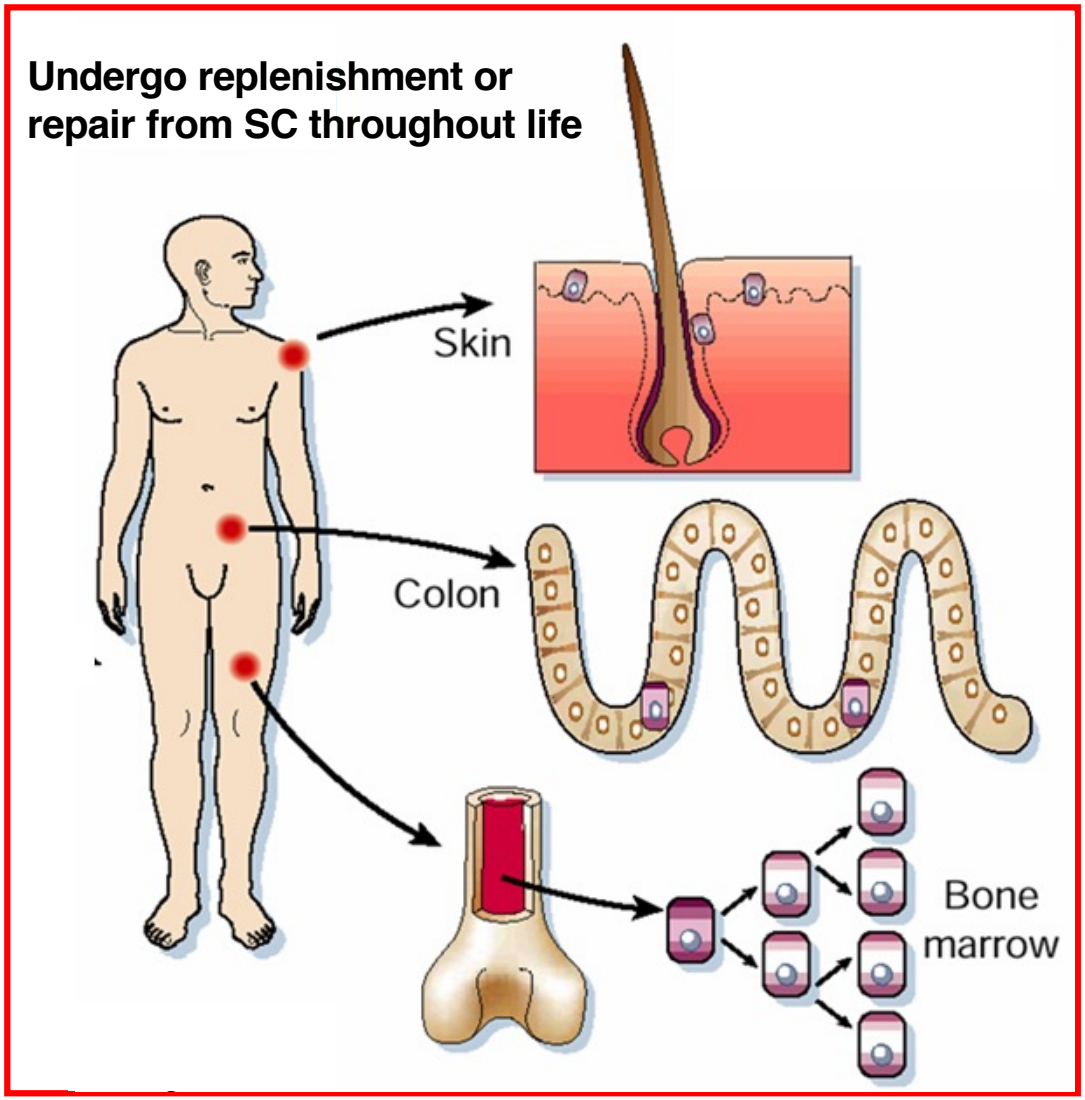
(Hanahan D. and Weinberg RA, (2000), Cell 100:57-70)

A tumor is a pathological mini organ

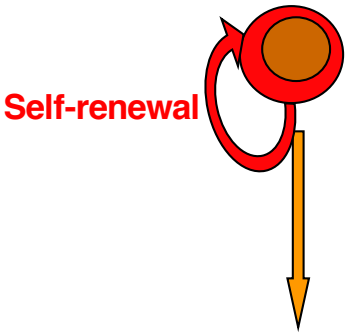




Adult tissue stem cells are essential for maintenance and repair of self-renewing tissues

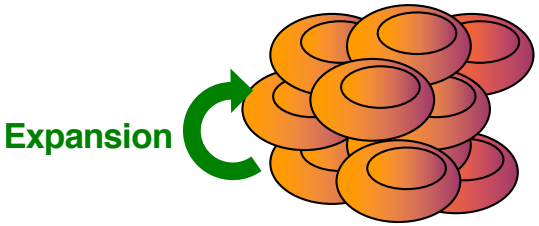


Self-renewing tissues are built by three basic cell types



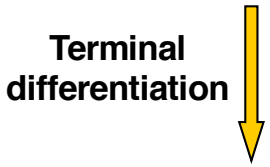
Stem cells

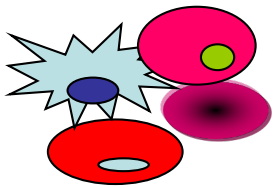
Self-renewal (life long)
Multipotent
Very rare
Long-lived
Infrequent divisions
Require interaction with the stem cell niche



Transit Amplifying cells

Rapidly dividing
Intermediate lifespan
Multipotent

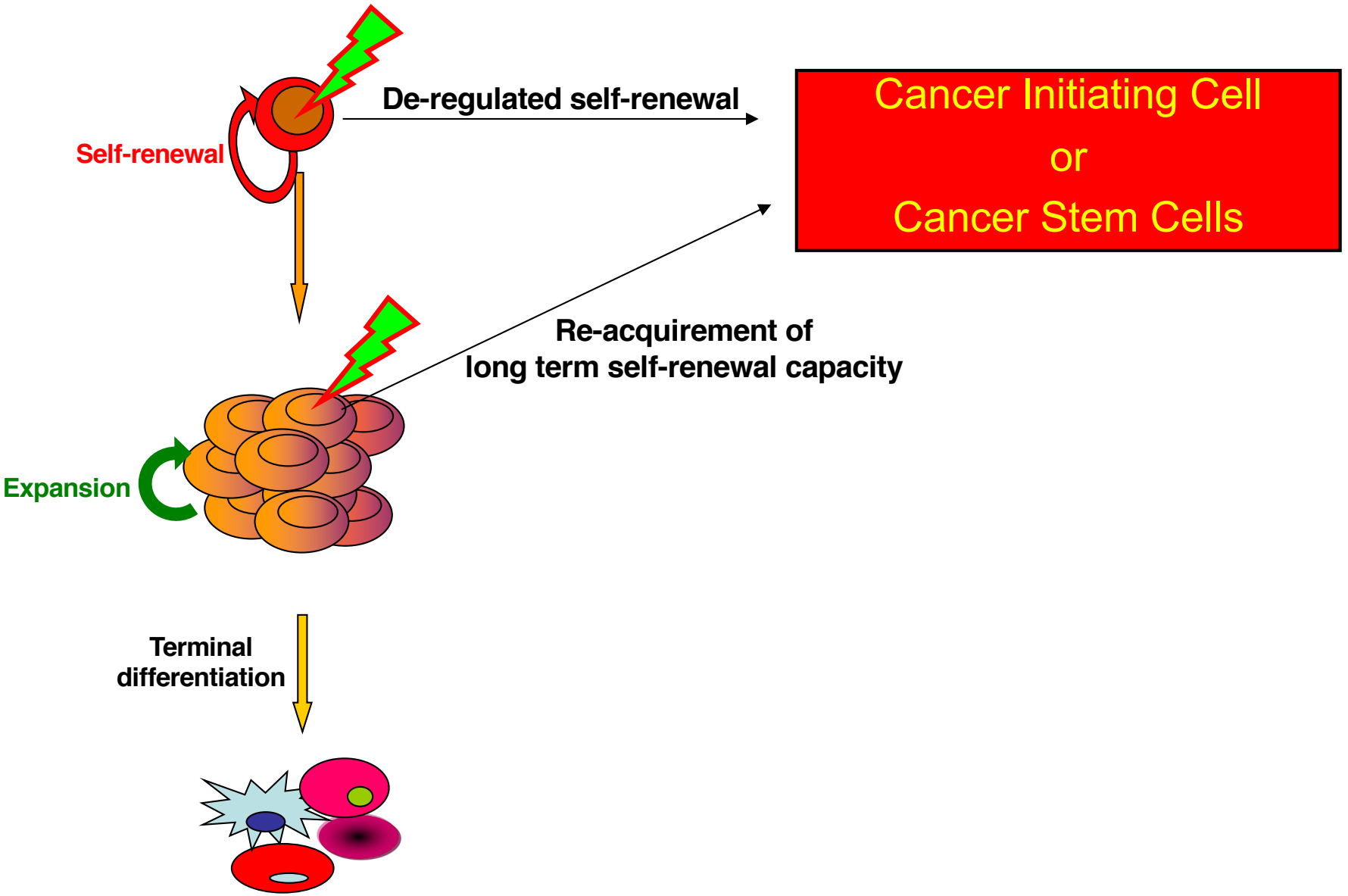




Mature cells

Non-dividing
Short lifespan
Terminally differentiated
Essential for the physiology of the tissue

Cancer is a disease of uncontrolled self-renewal



Stem cells are obvious (but not the only) targets for oncogenic transformation

Stem cells possess many characteristics of cancer cells:

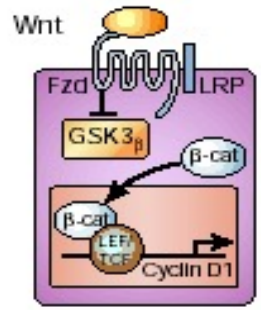
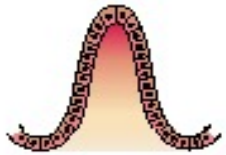
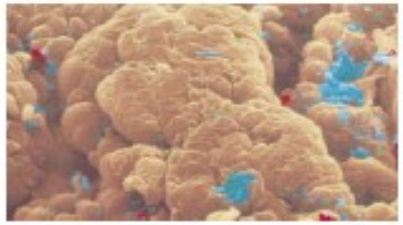
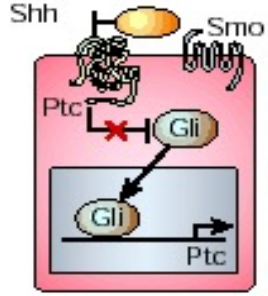
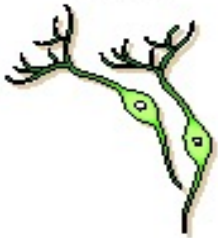
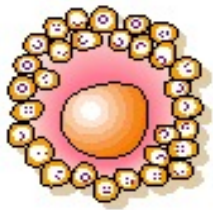
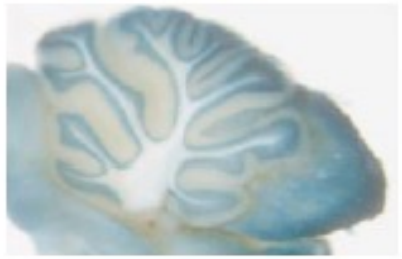
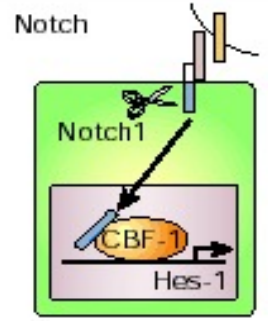
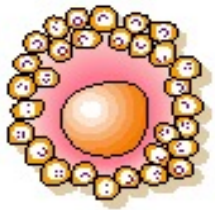
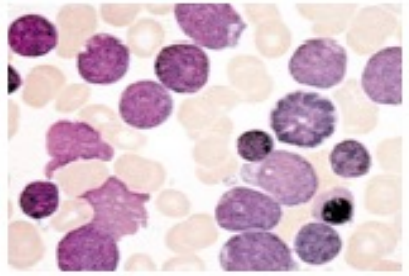
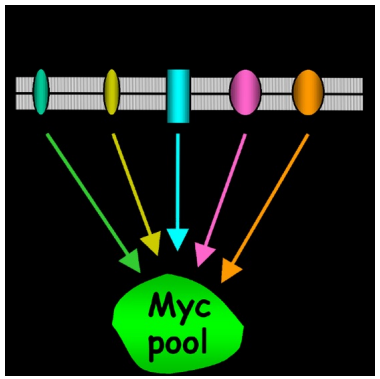
(1) Indefinite proliferation capacity through self-renewal

(2) Since stem cells are long lived - they have a higher chance to accumulate mutations

(3) Solid cancers often show a large degree of heterogeneity

(4) In carcinomas, epithelial tumor cells are intricately interwoven with a complex mixture of fibroblasts, endothelial cells, smooth muscle cells and inflammatory cells-termed "stroma".
Could the tumor stroma be an « evolved » stem cell niche!?

(5) Genetic pathways involved in cancer also regulate normal stem cell development (Wnt, Notch, Myc, PTEN, Shh, TGF-β)

	Stem/progenitor cell self-renewal	Tumorigenesis
	Haematopoietic  Epidermal  Gut 	Colon carcinoma Epidermal tumours 
	Haematopoietic  Neural  Germ line 	Medulloblastoma Basal cell carcinoma 
	Haematopoietic  Neural  Germ line 	Leukaemia Mammary tumours 
	Haematopoietic  Epidermal 	Leukemia Lymphoma Breast cancer Prostate cancer Colon cancer

Definition: Stem cell

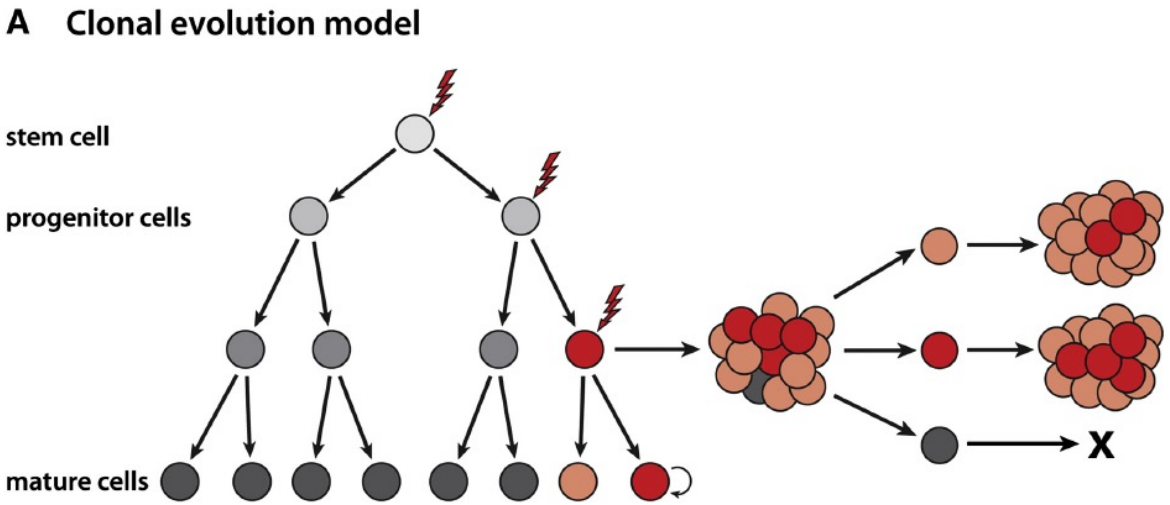
A stem cell is a cell that has self-renewal capacity and that can give rise to all cell types of a given tissue.

Definition: Cancer Stem cell

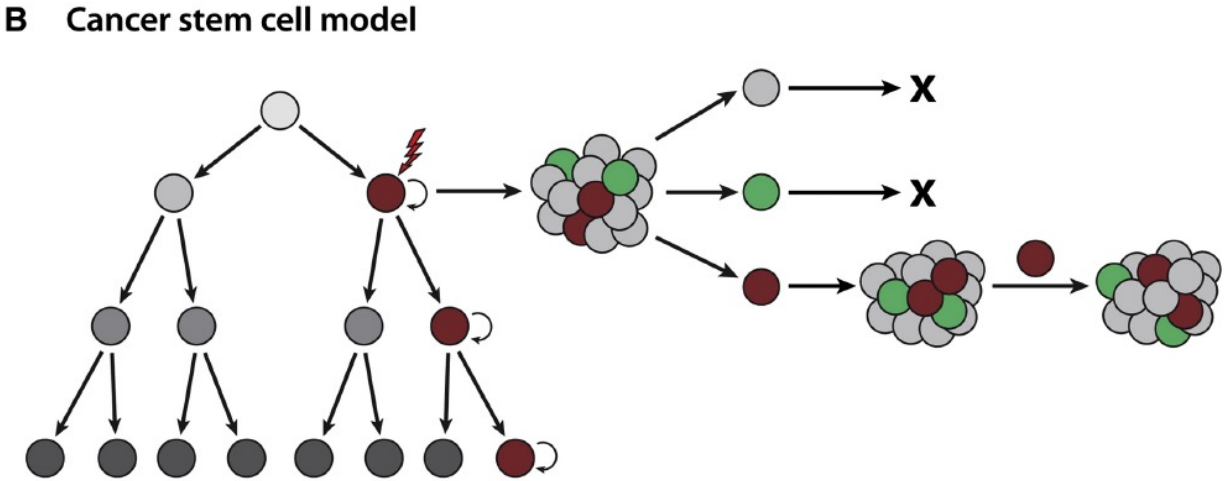
A Cancer Stem Cell is a cell within the tumor that posseses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor.

Thus these cells (stem cells) can only be defined experimentally by their ability to self-renew and to recapitulate the generation of all lineages of a given tissue or by the generation of a continuously growing tumor (CSC).

Two Main Cancer Models

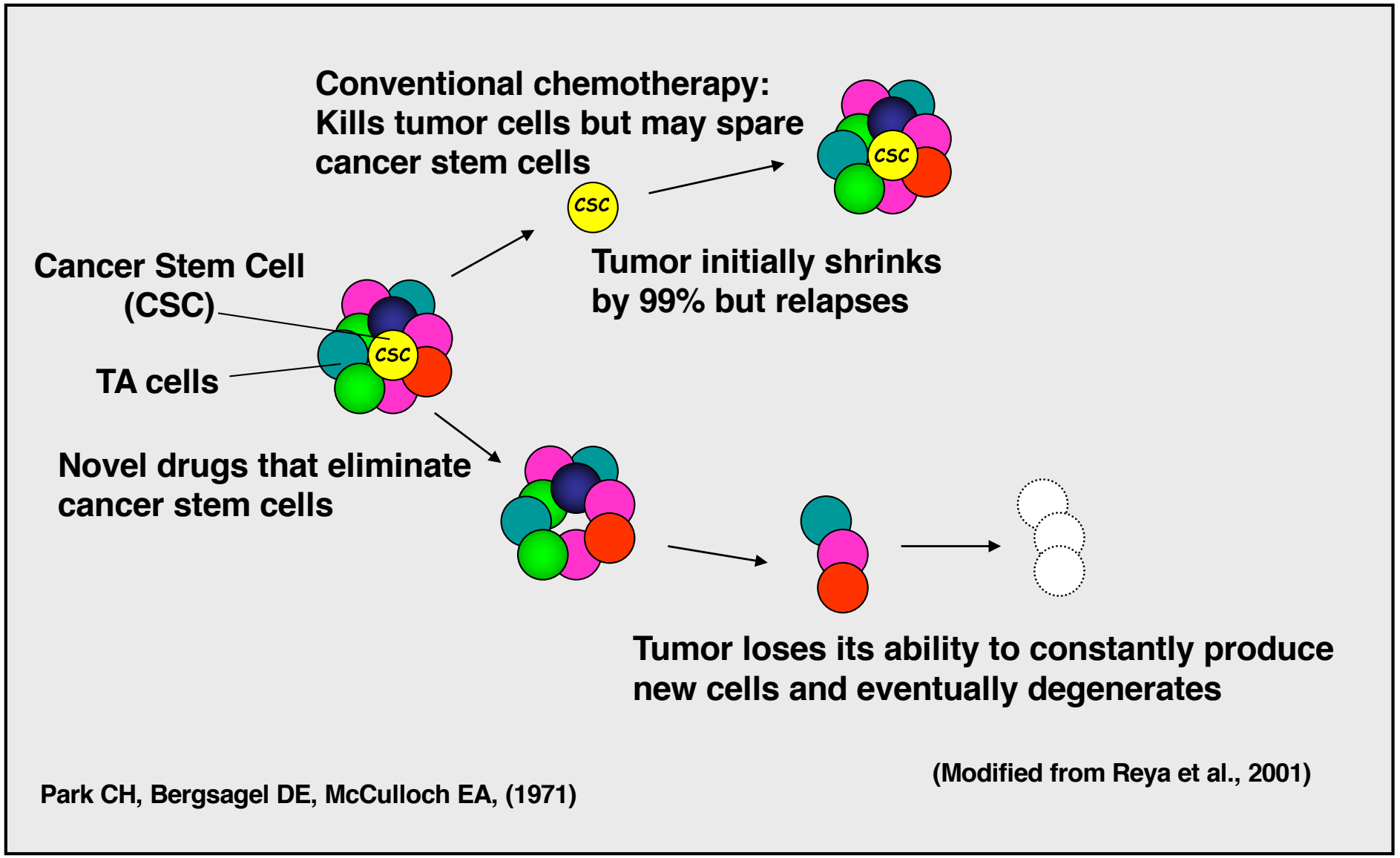


Genetic changes leading to development and progression of the malignancy are operative in all cells of the tumor!

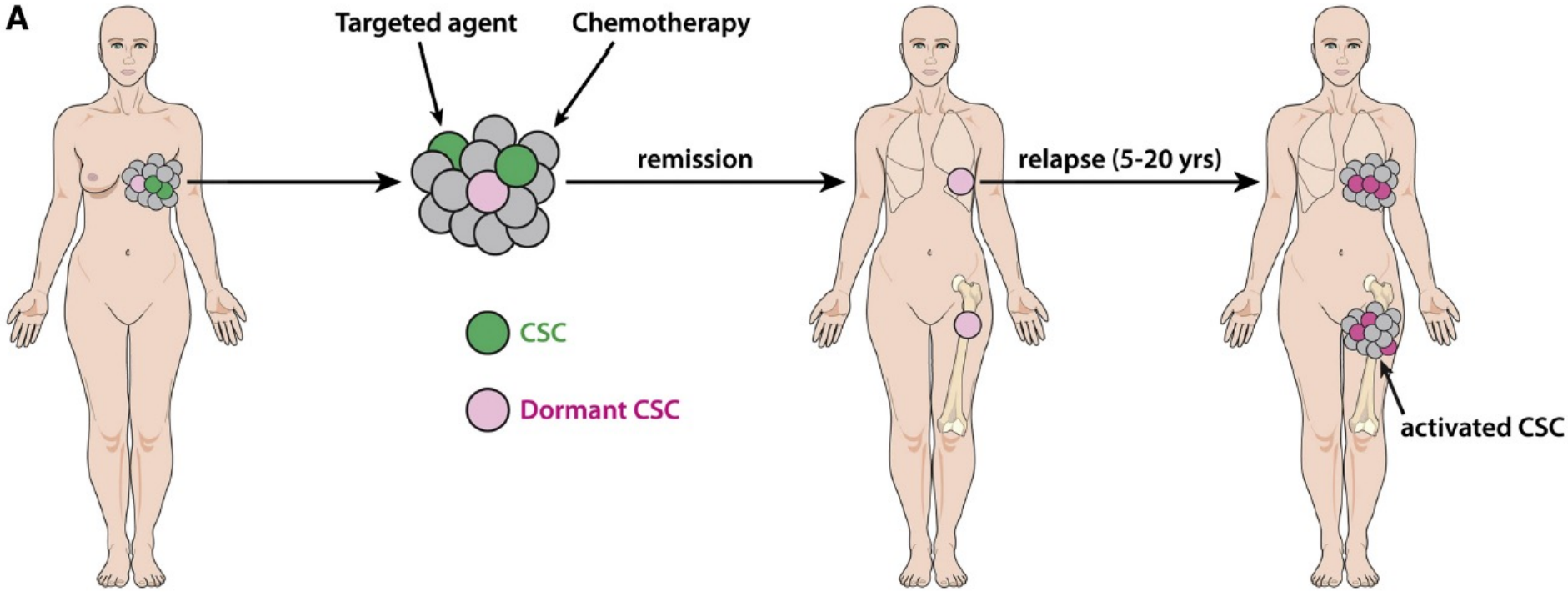


Only a small subset of cancer cells have the ability to initiate new cancer growth!

Current therapies succeed at eliminating bulky disease and rapidly proliferating cells but often miss a tumor reservoir (CSCs) that is the source of disease recurrence and metastasis.

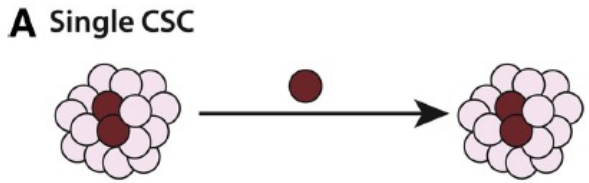


Examples for the existence of Cancer stem cells
Possible mechanisms of metastatic relapse after anticancer therapy

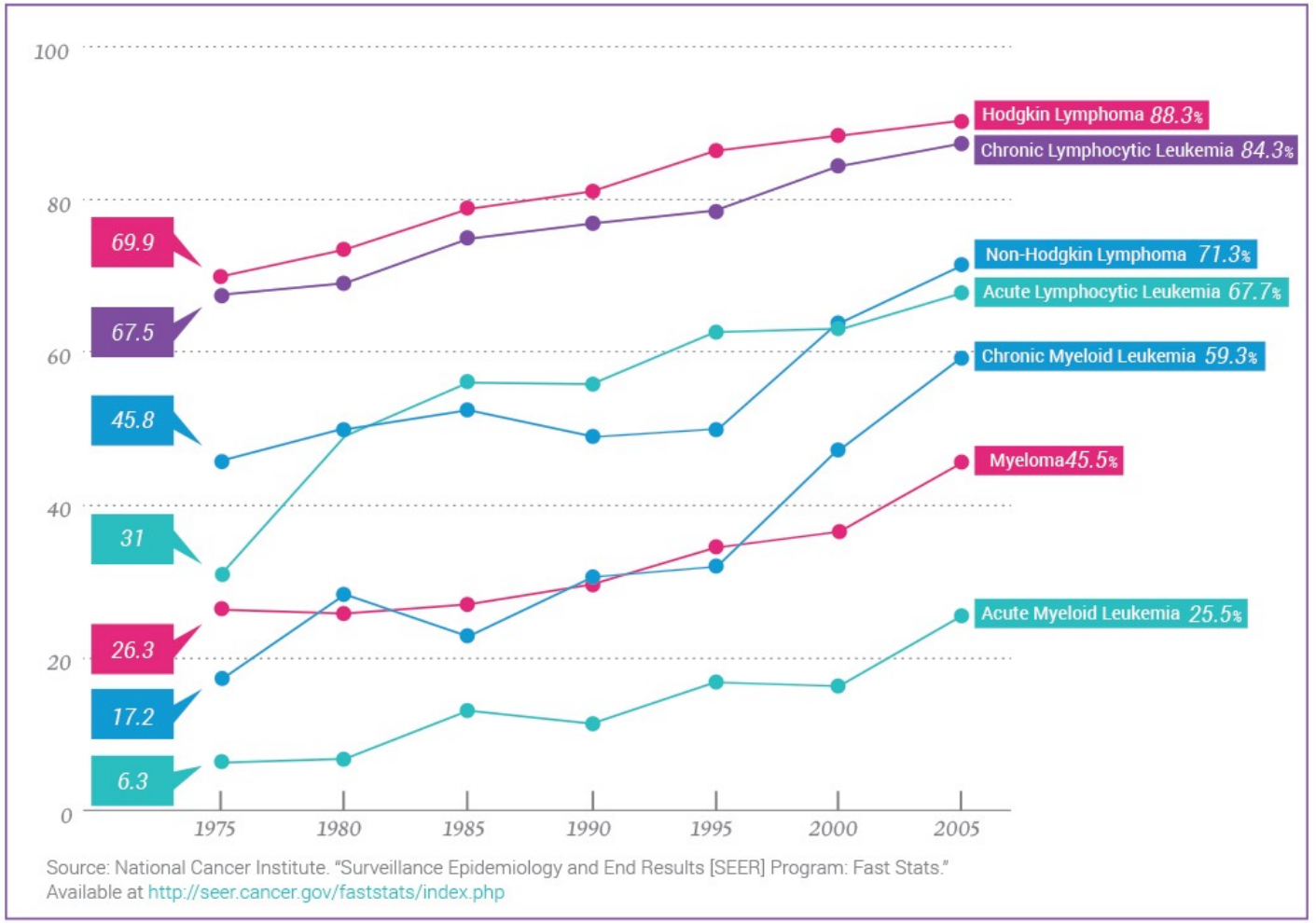


(A) Late relapse can be accounted for by CSC dormancy. Here a dormant CSC (pink) that is resistant to both chemotherapy and targeted therapy has seeded to distant organs. After a considerable latency period, reactivation of a CSC will result in tumor growth and clinical emergence of metastases. Intriguingly, the clinical appearance of metastases is often synchronous in breast cancer.

Schematic models of tumor propagation by Cancer stem cells



Blood Cancer Survival is on the Rise

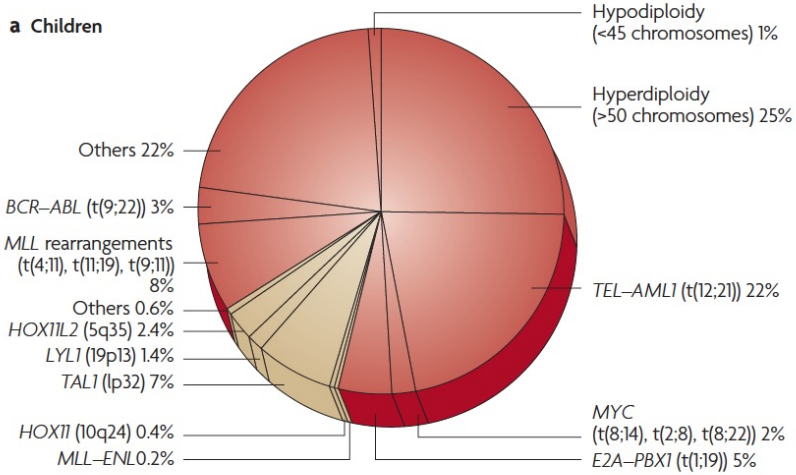


Source: National Cancer Institute. "Surveillance Epidemiology and End Results [SEER] Program: Fast Stats." Available at http://seer.cancer.gov/faststats/index.php

Retuximab
(anti-CD20)

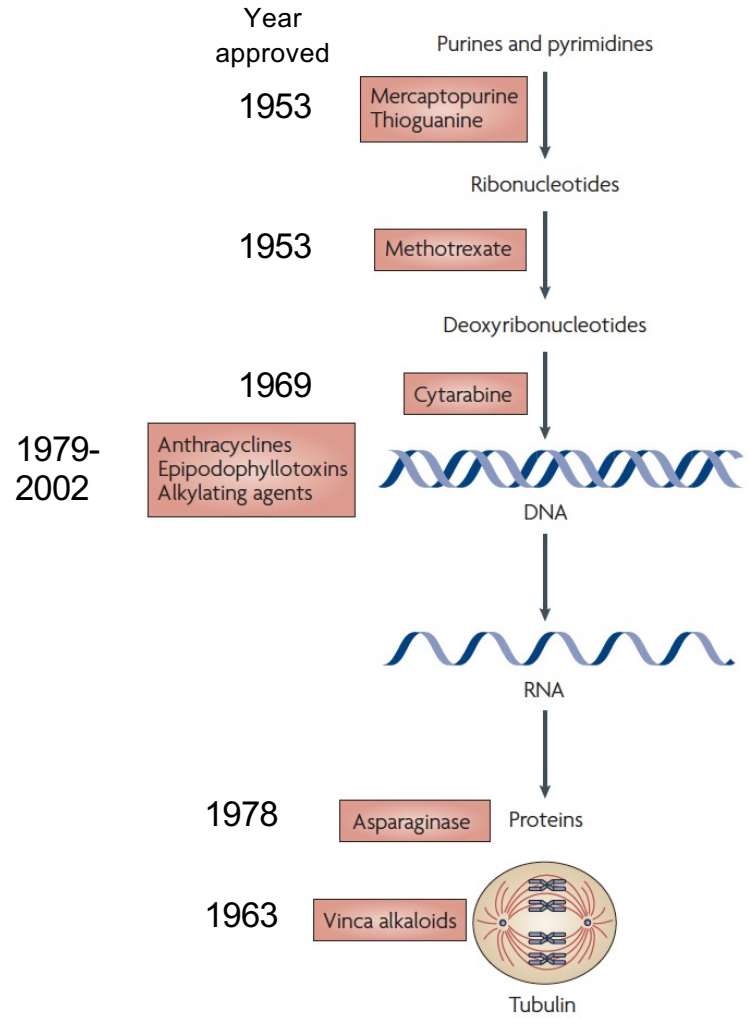
Gleevec

Cytogenetic and molecular genetic abnormalities of childhood and adult ALL

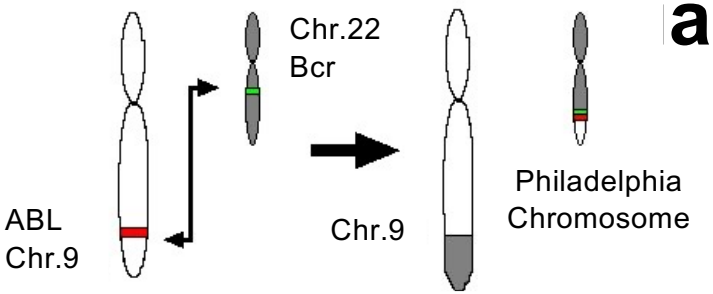


Pui and Jeha *Nature Reviews Drug Discovery* **6**, 149–165 (February 2007) | doi:10.1038/nrd2240

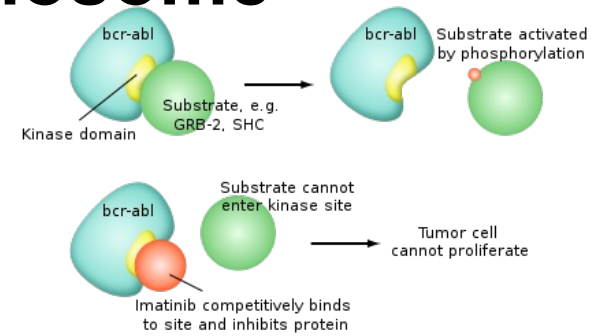
Agents that are conventionally used to treat ALL patients



CML – Chromosomal Translocation – a Chromosome



t(9;22)(q34;q11)



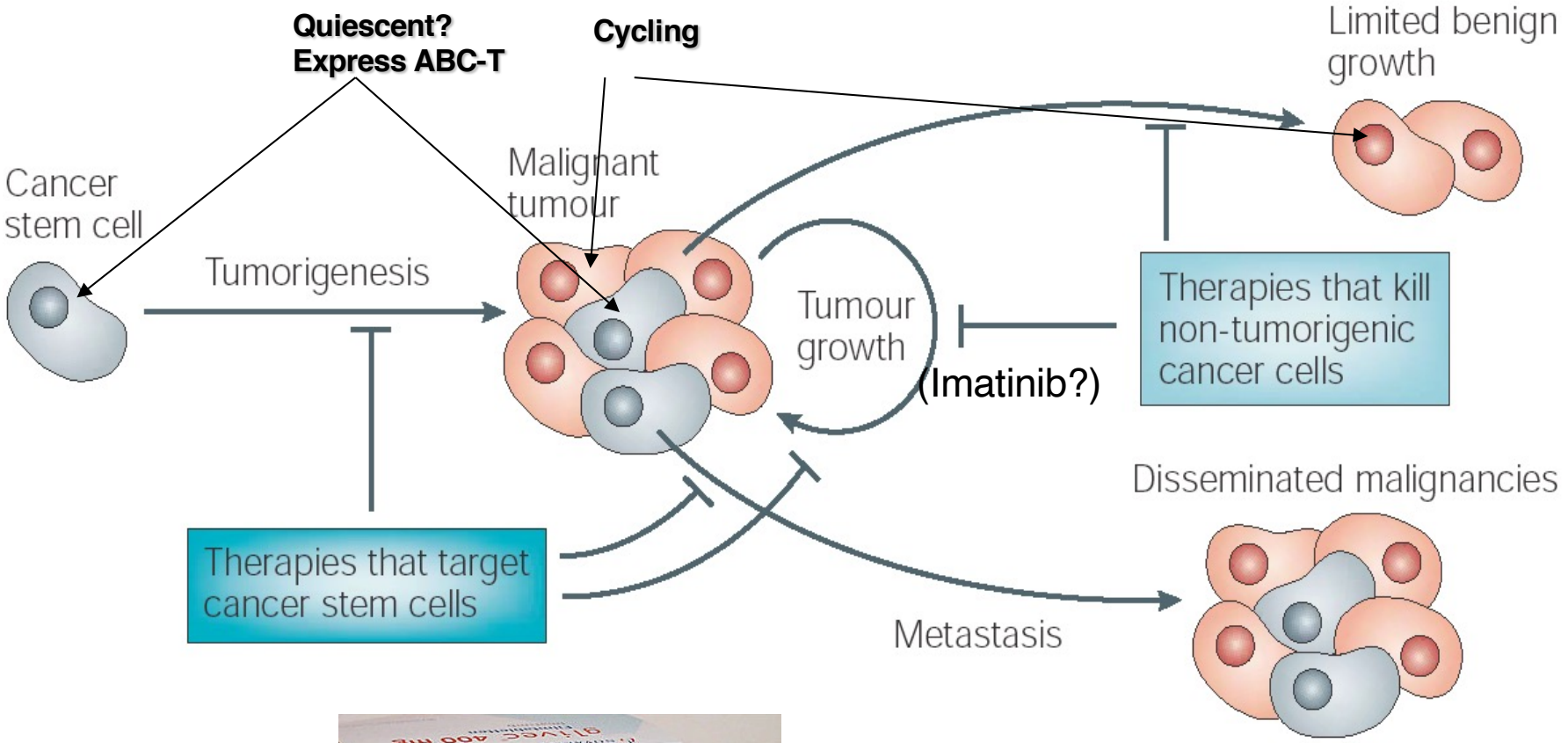
Some CML Facts:

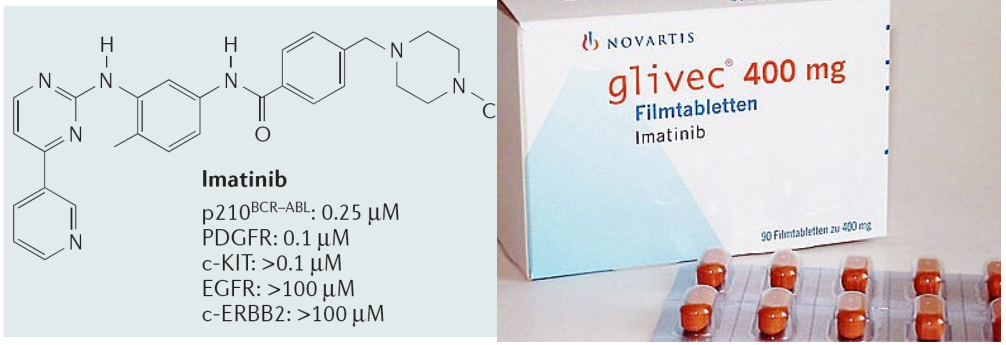
C-ABL: *Abelson Murine Leukemia Viral Oncogene Homolog 1 (Tyrosinekinase/G_protein-Ras).*

BCR: break cluster region

- CML occurs in all age groups, but most commonly in middle-aged to elderly (mean age at onset 53 yrs)
- Incidence of 1-2 cases per 100.000 per year & slightly more men than women are affected
- CML presents ≈ 15-20% of all cases of adult leukemia
- CML is treated with Bcr-Abl tyrosine kinase inhibitors (imatinib=Gleevec; desatinib; nilotinib)
- Patients treated with imatinib show 89% survival rate after 5 yrs follow-up

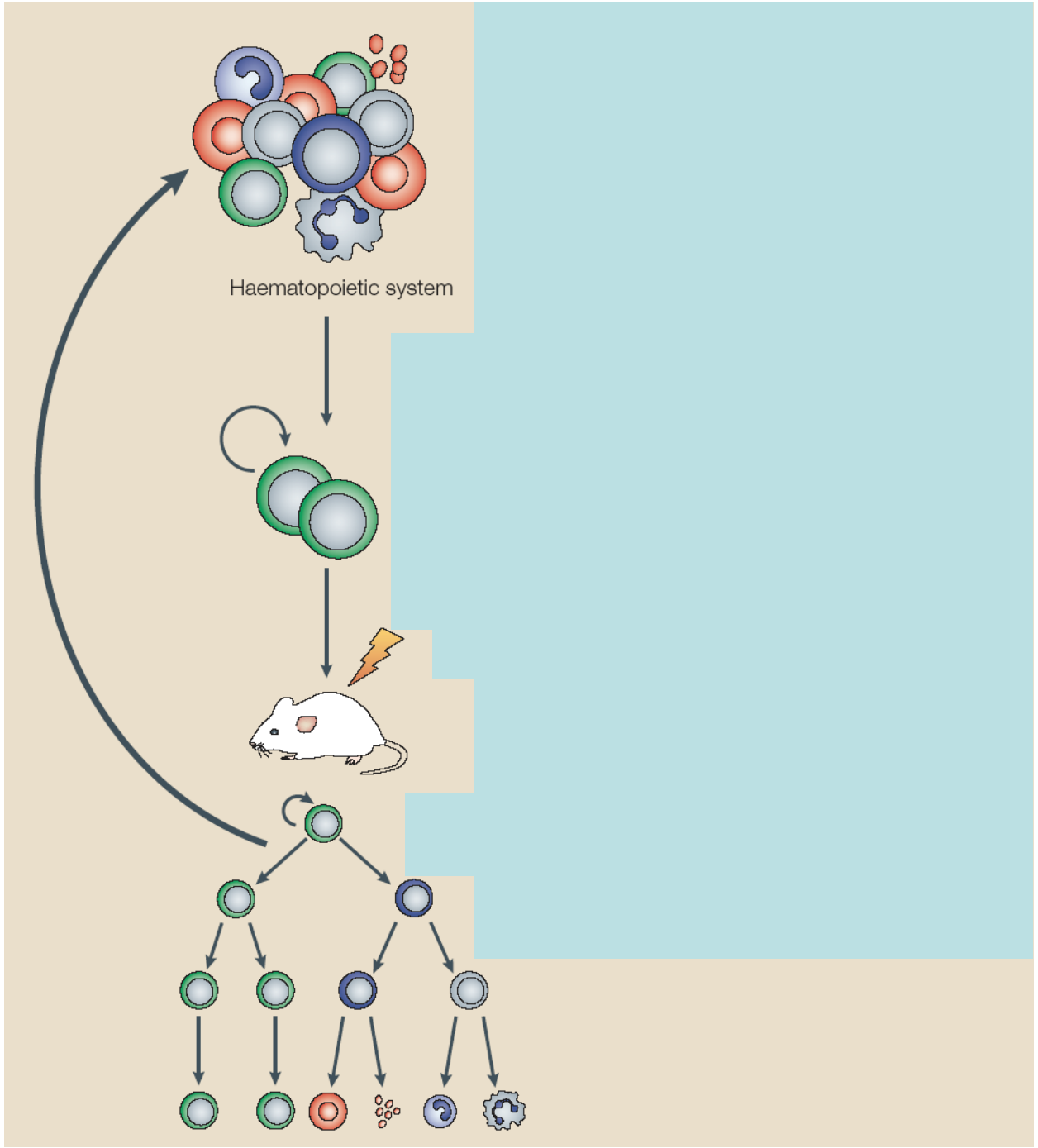
Imatinib targets (BCR-Abl carrying) leukemic progenitors but not the leukemic stem cell!



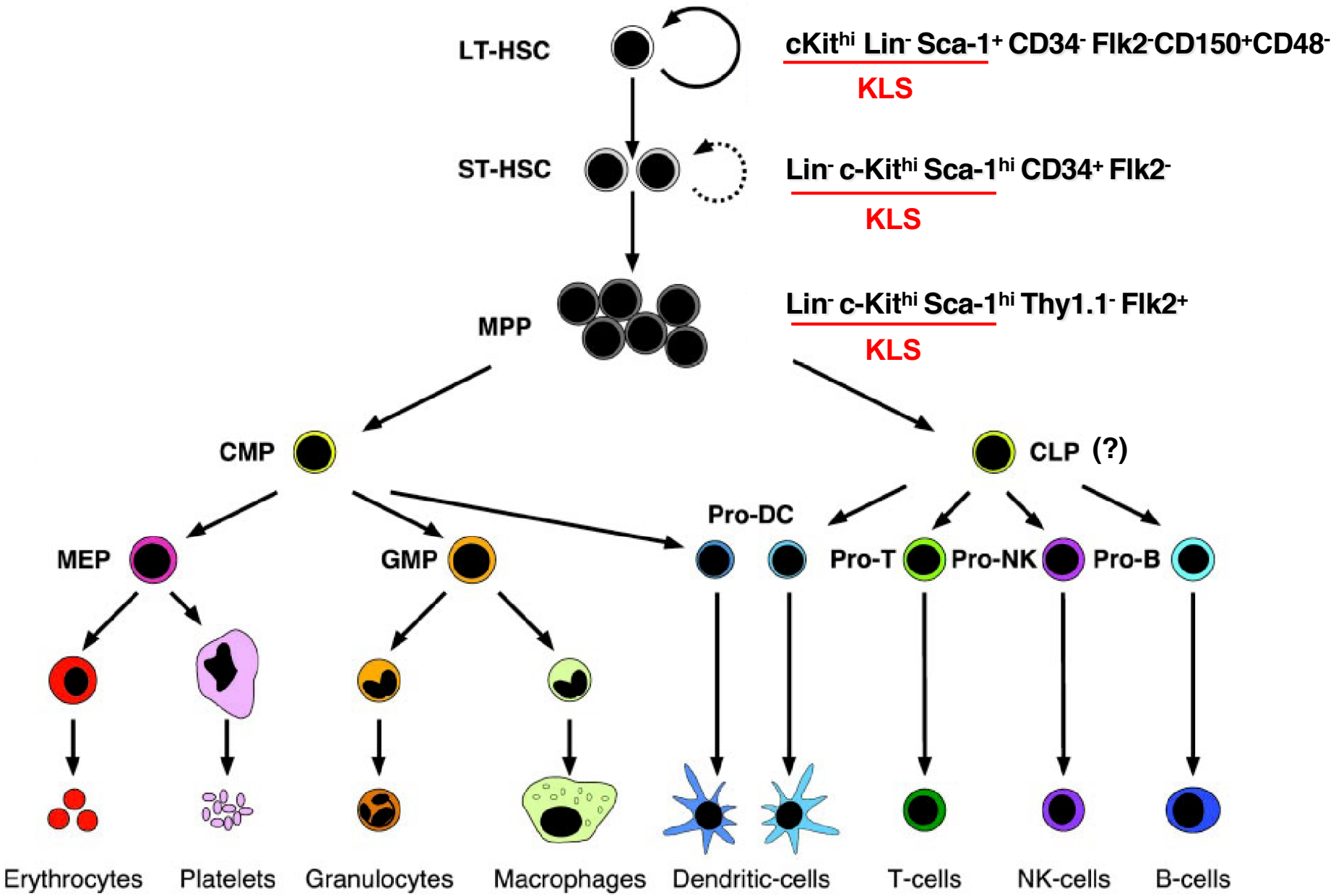


(modified from Pardal et al., 2003)

How are cancer stem cells studied?


Haematopoietic system

The adult murine hematopoietic system



Modified from Passegué E. et al., 2003, Yang et al., 2005, Kiel et al., 2005

The gold standard for identifying hematopoietic stem cells is Serial Bone Marrow Reconstitution

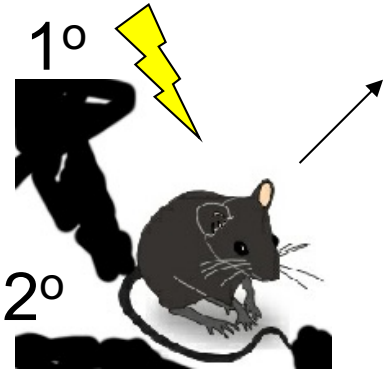
BM

1º

13 weeks

BM

1º



2º

13 weeks

BM

2º

3º

3º

The SCID-hu Mouse

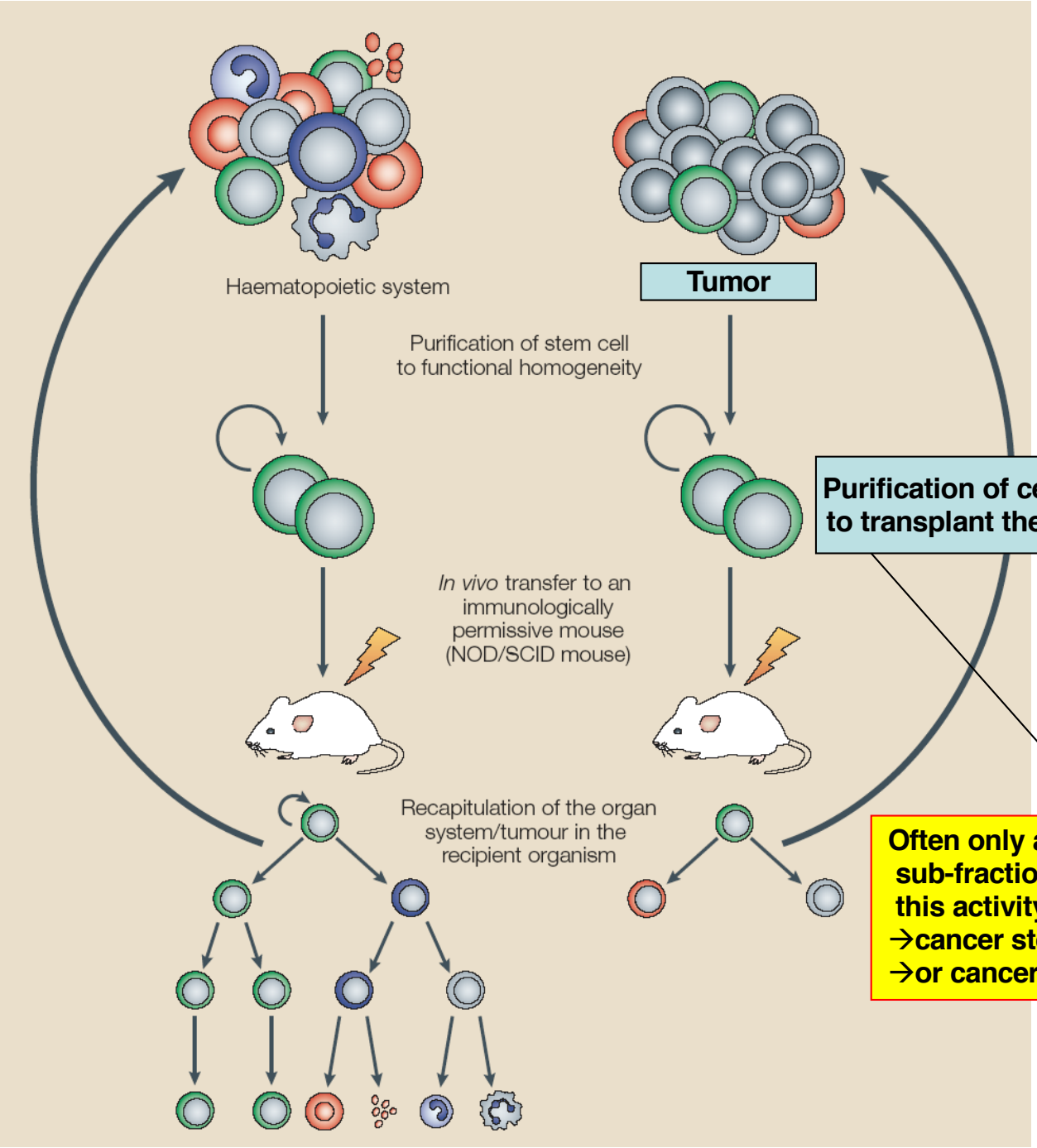
J. McCune and I.Weissman (1988)

Severe combined immunodeficiency (SCID).

The mice are defective in rearranging their antigen receptors in differentiating T and B cells.

By engrafting human fetal liver, lymph node or thymus onto the SCID mouse, human stem cell differentiation can be observed in a mouse model

Possible to construct SCID-hu mice with a variety of human lymphoid organs of defined genetic origin.

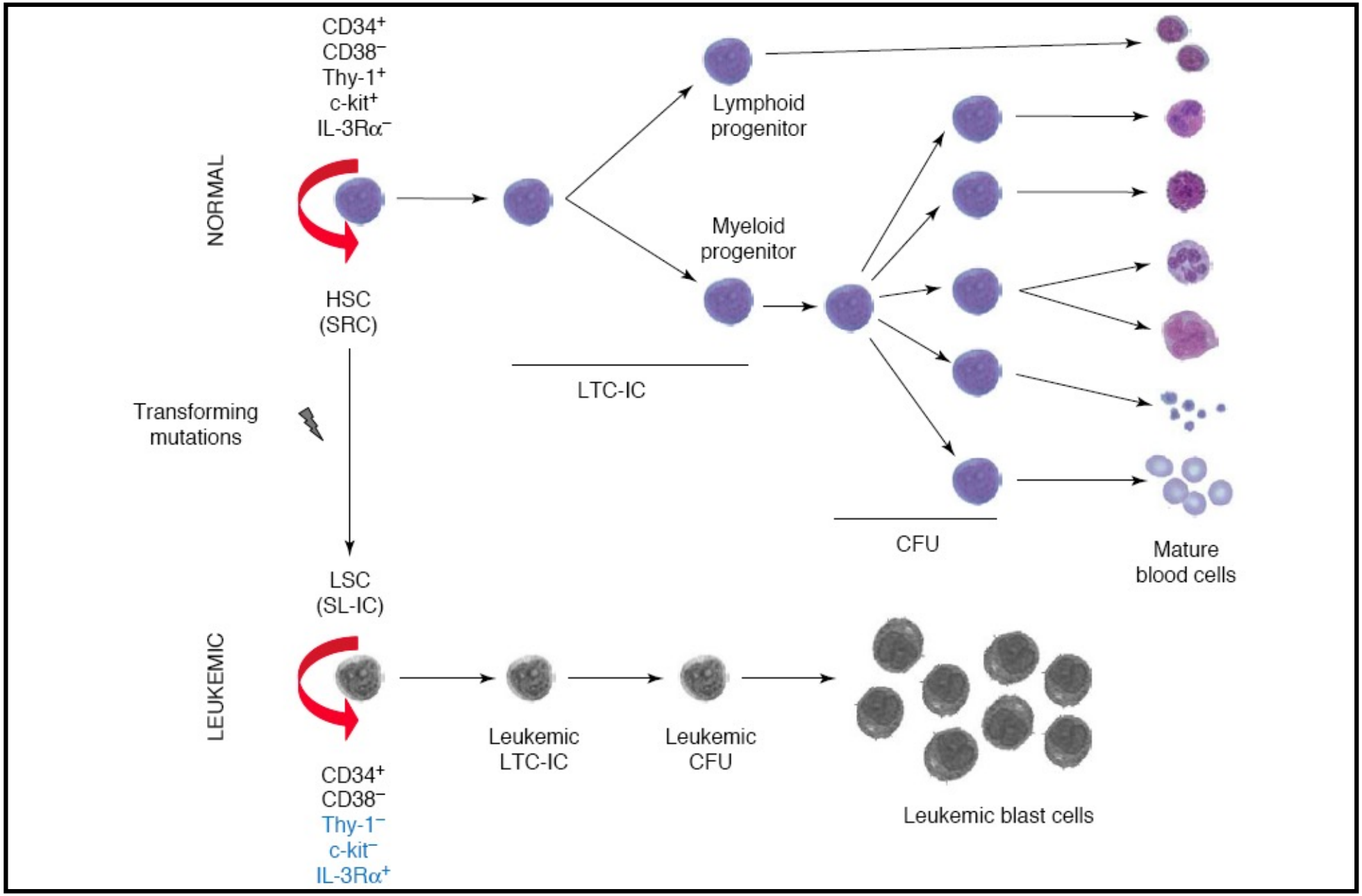

Haematopoietic system
Tumor
Purification of stem cell
to functional homogeneity
In vivo transfer to an
immunologically
permissive mouse
(NOD/SCID mouse)
Recapitulation of the organ
system/tumour in the
recipient organism
Purification of cells able
to transplant the tumor
Often only a very small
sub-fraction of the tumor has
this activity!
→cancer stem cell
→or cancer initiating cell

Leukemias

- In an AML patient only one small population 0.2% (CD34+CD38-) had transplantable activity (NOD/SCID)! Other AML cells were unable to induce leukemia
(Lapidot et al., 1994; Bonnet and Dick, 1997; Hope et al., 2004)

Similar studies:

ALL:	(George et al., 2001)
MDS:	(Nilsson et al., 2002)
Muliple Myeloma:	(Matsui et al., 2004)


CD34+
CD38−
Thy-1+
c-kit+
IL-3Rα−
NORMAL
HSC
(SRC)
Lymphoid
progenitor
Myeloid
progenitor
LTC-IC
CFU
Mature
blood cells
Transforming
mutations
LSC
(SL-IC)
LEUKEMIC
CD34+
CD38−
Thy-1−
c-kit−
IL-3Rα+
Leukemic
LTC-IC
Leukemic
CFU
Leukemic blast cells

Leukemias

- In an AML patient only one small population 0.2% (CD34+CD38-) had transplantable activity (NOD/SCID)! Other AML cells were unable to induce leukemia
(Lapidot et al., 1994; Bonnet and Dick, 1997; Hope et al., 2004)

Similar studies:

ALL:	(George et al., 2001)
MDS:	(Nilsson et al., 2002)
Muliple Myeloma:	(Matsui et al., 2004)

Solid tumors

Breast cancer: 200 $CD44^{+}$ $CD24^{neg}$ ESA^{+} cells transferred the tumor
(Al-Haij et al., and Clarke, PNAS 2003)

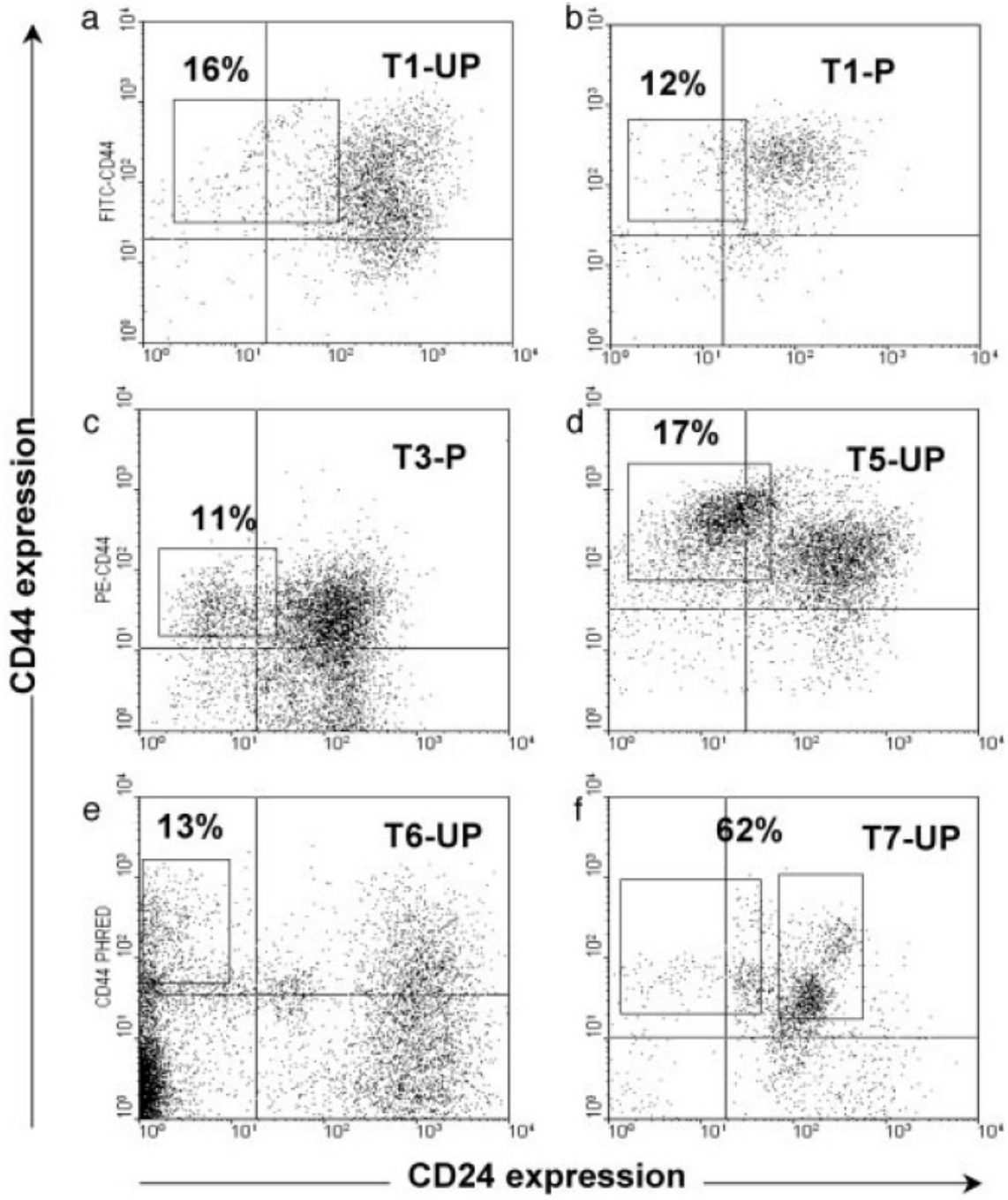
Glioblastoma: 100 $CD133^{+}$ tumor cells (Singh et al. and Dirks, Nature 2004)

Soli

- Brea
re-in
(orth
Howe
phen

tumor cells
CID mice

a different



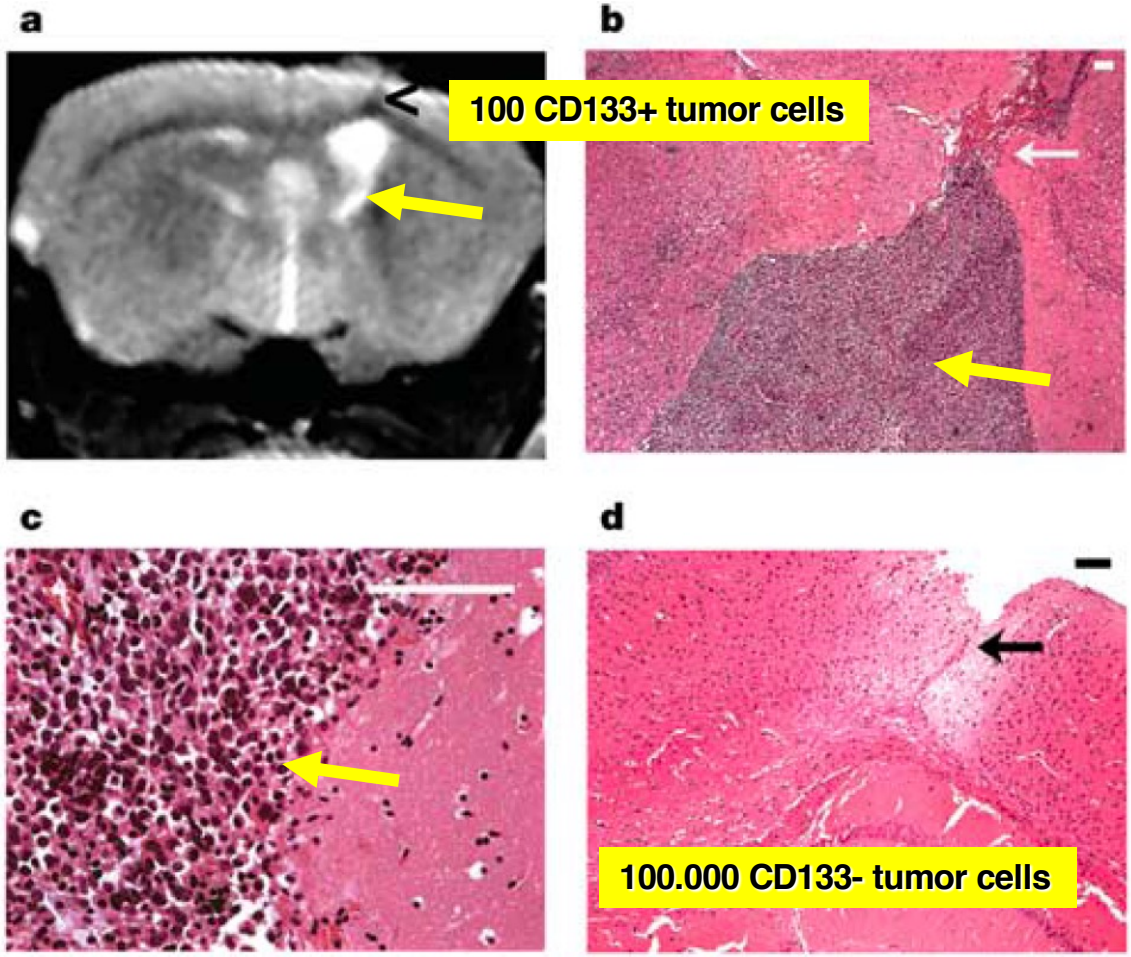
Solid Tumors 2

-Brain tumors: as few as 100 CD133+ tumor cells re-initiated (a phenotypic similar) tumor in NOD/SCID mice (orthotopic-frontal lobe). However, injecton of thousands of CD133- tumor cells did not.

(Singh et al. and Dirks, Nature 2004)

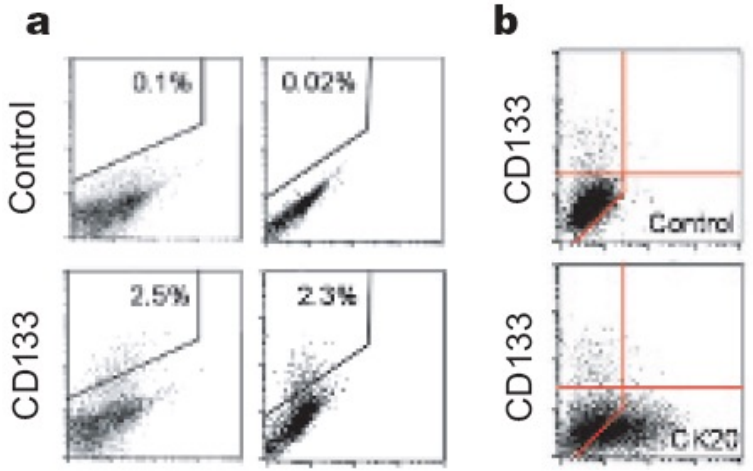
MRI

Magnetic resonance imaging



Identification and expansion of human colon-cancer-initiating cells

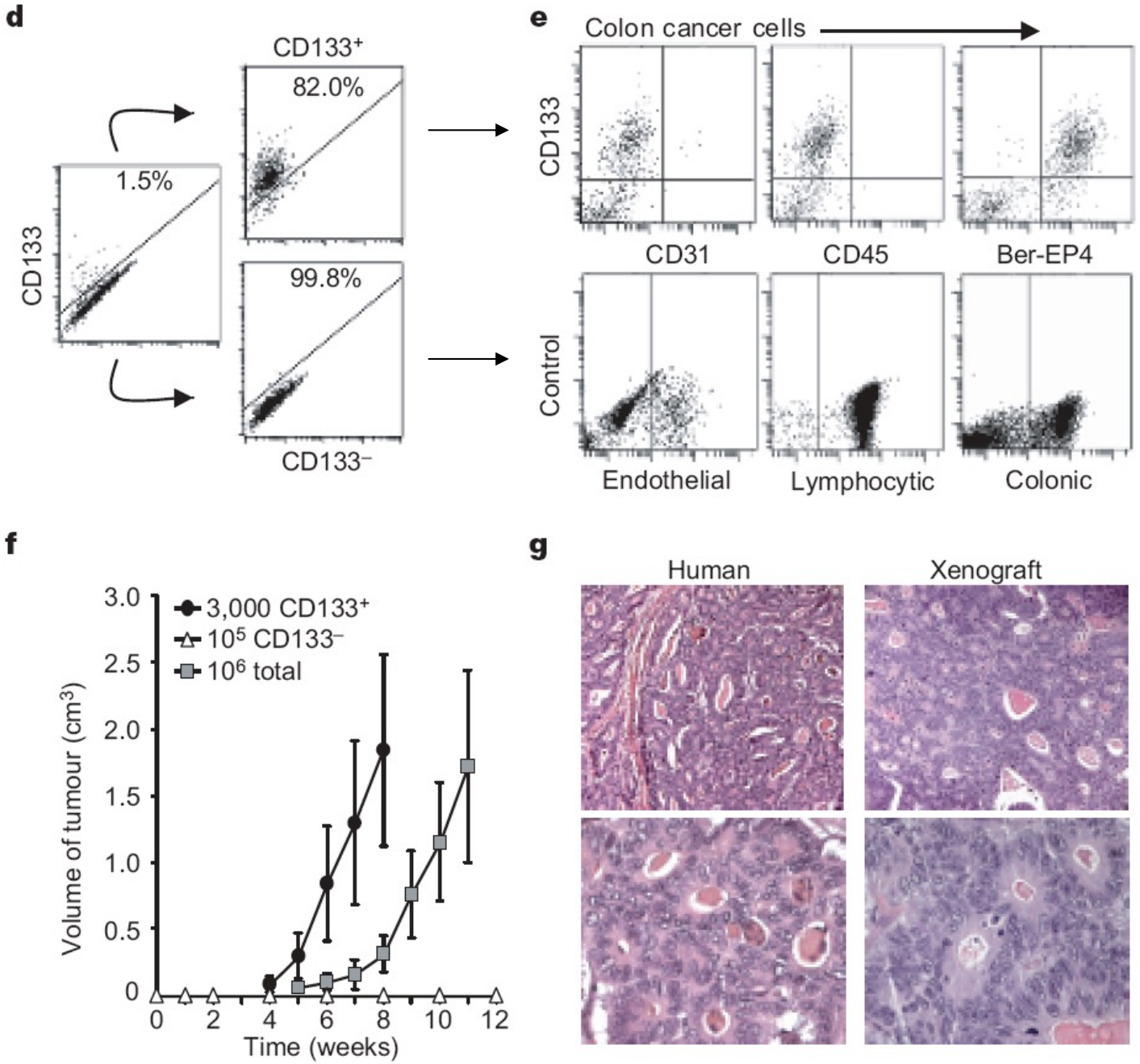
Lucia Ricci-Vitiani[1], Dario G. Lombardi[2], Emanuela Pilozzi
& Ruggero De Maria[1,2]



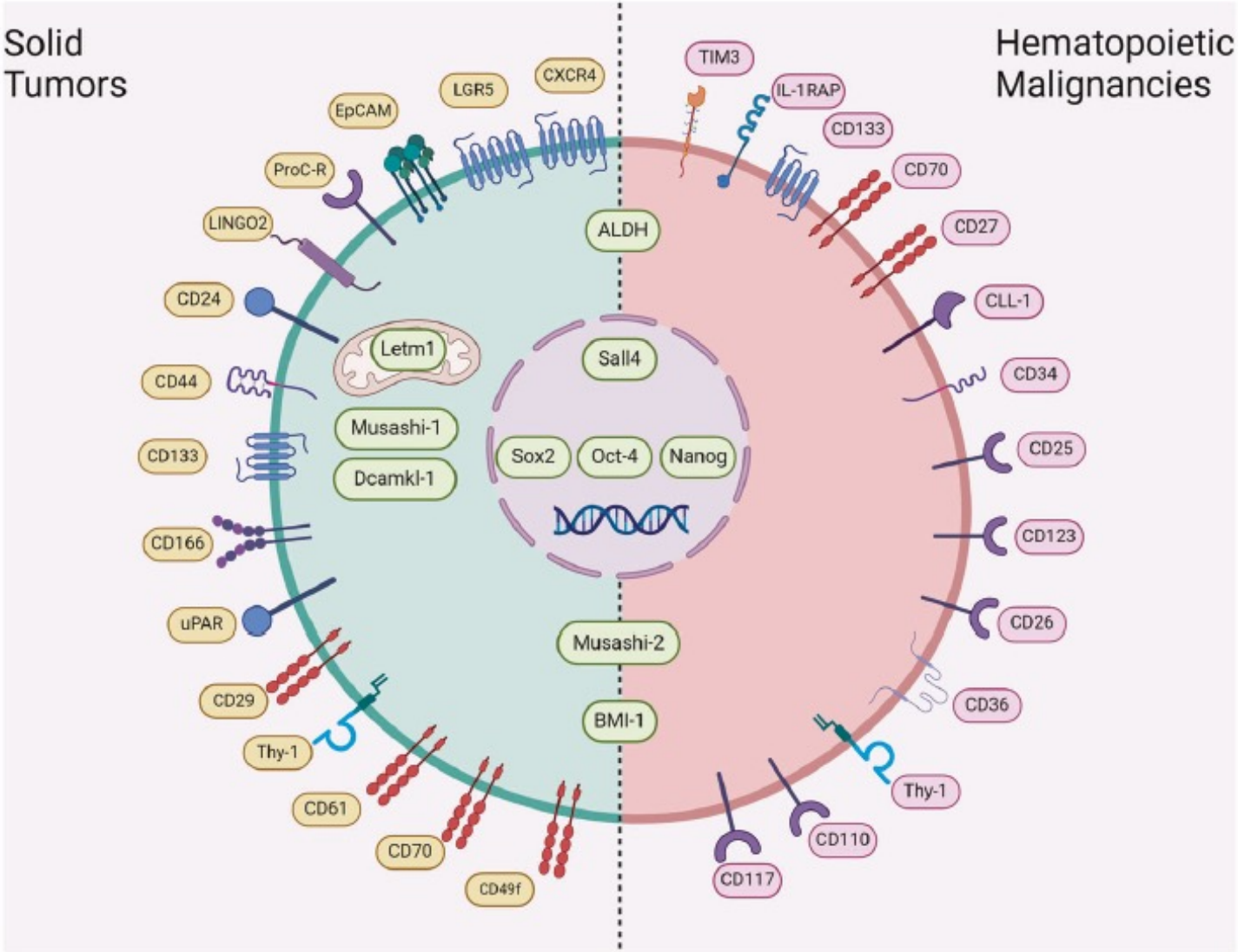
A rare CD133+ population of tumorig

CD133 antigen also known as **prominin-1** is a glycoprotein

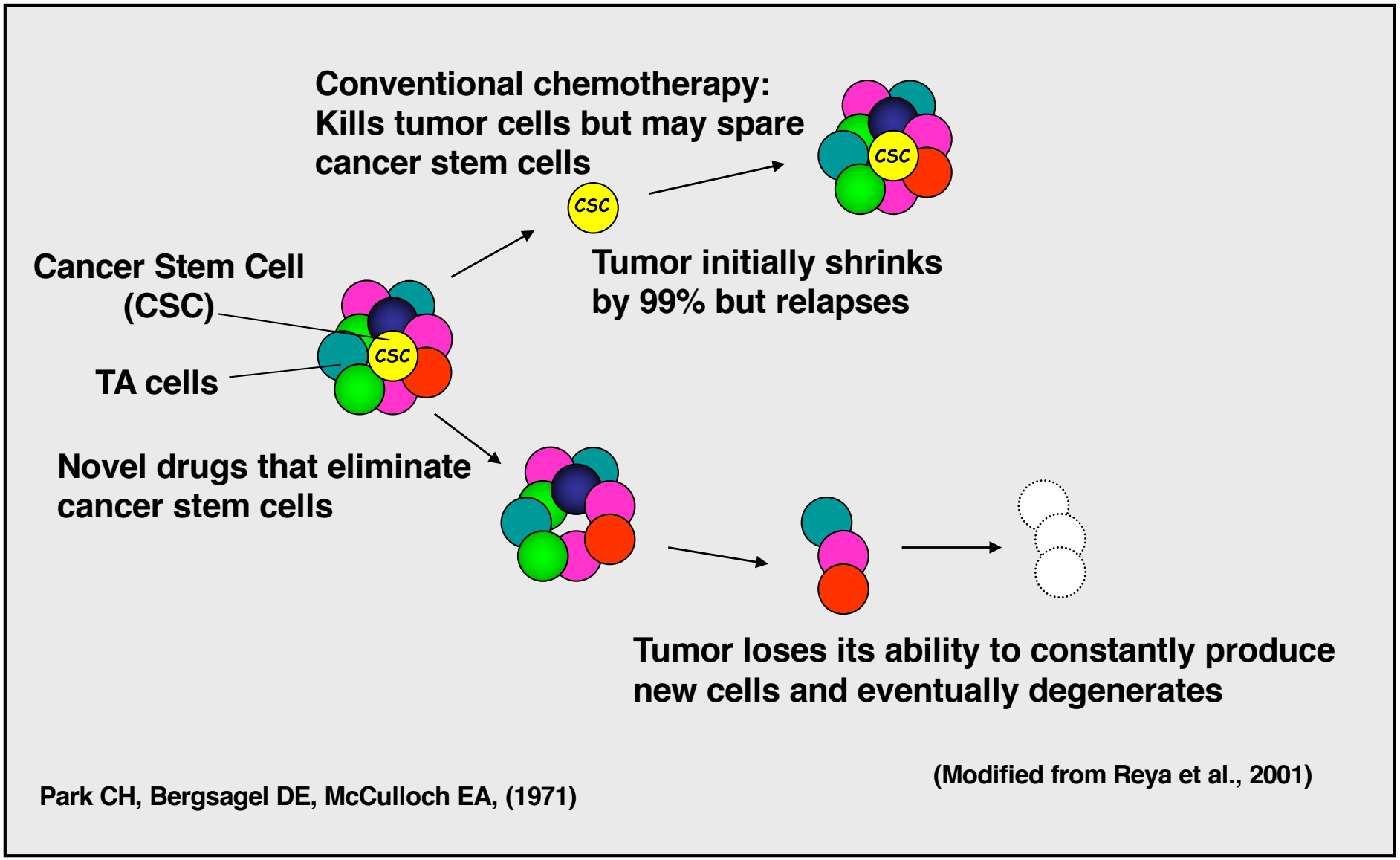
CD133 is expressed in hematopoietic stem cells,[4] endothelial progenitor cells,[5] glioblastoma, neuronal and glial stem cells,[6] various pediatric brain tumors,[7] as well as adult kidney, mammary glands, trachea, salivary glands, placenta, digestive tract, testes, and some other cell types.


d
CD133+
82.0%
1.5%
CD133
99.8%
CD133−
e
Colon cancer cells
CD133
CD31
CD45
Ber-EP4
Control
Endothelial
Lymphocytic
Colonic
f
3,000 CD133+
10^5 CD133−
10^6 total
Volume of tumour (cm3)
Time (weeks)
g
Human
Xenograft

Cancer stem cells can be identified by cell surface markers and isolated by fluorescence activated cell sorting (FACS)



Current therapies succeed at eliminating bulky disease and rapidly proliferating cells but often miss a tumor reservoir (CSCs) that is the source of disease recurrence and metastasis. What are these resistance mechanisms?



Possible mechanisms for drug resistance of Cancer Stem Cells

Stem Cells and probably also Cancer Stem cells:

….. are dividing rarely and are mainly quiescent

(refractory to drugs that target dividing cells…)

….. express ABC transporters, which could mediate resistance to chemotherapy!

(built-in drug resistance!)

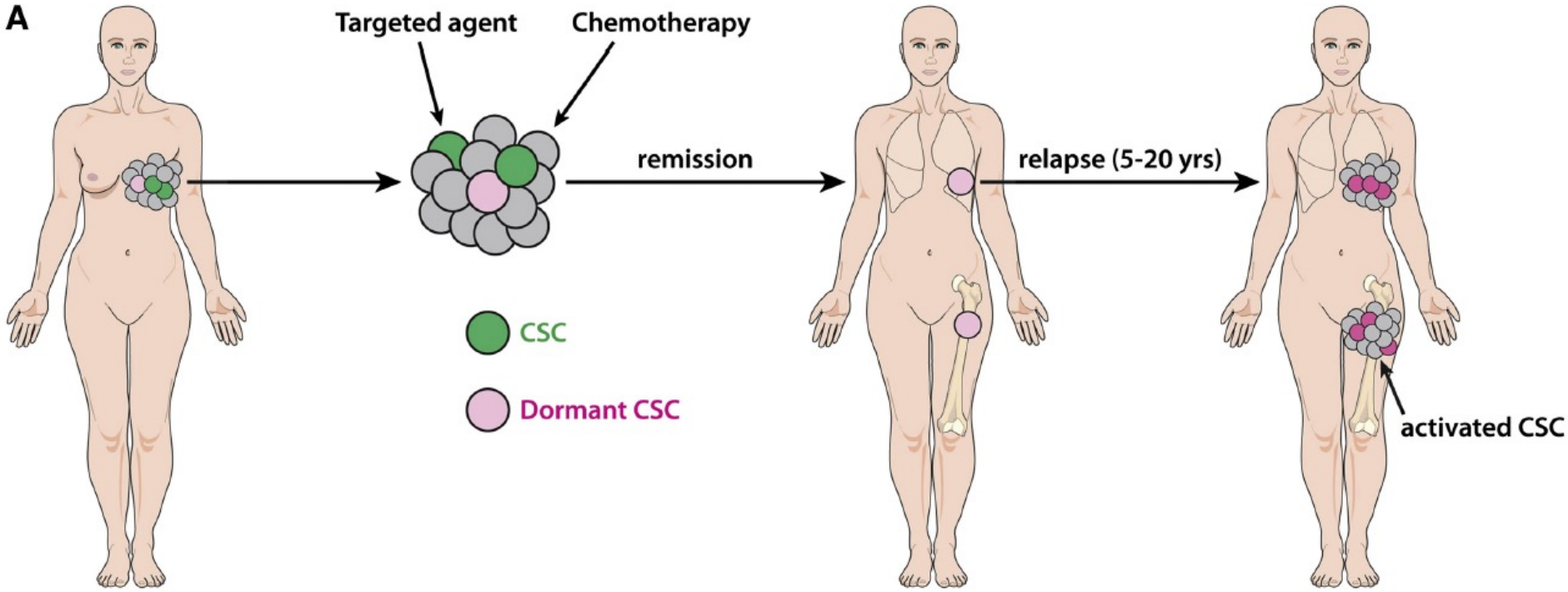
….. Activate the repaire machinerie more efficiently

(escape from irradiation induced apoptosis…)

! Even normal stem cells are resistant to chemotherapy

(recovery of blood and hair growth in patients after treatment)

Possible mechanisms of metastatic relapse after anticancer therapy



(A) Late relapse can be accounted for by CSC dormancy. Here a dormant CSC (pink) that is resistant to both chemotherapy and targeted therapy has seeded to distant organs. After a considerable latency period, reactivation of a CSC will result in tumor growth and clinical emergence of metastases. Intriguingly, the clinical appearance of metastases is often synchronous in breast cancer.

« Cancer Stem Cells » but not their progeny may be resistant to chemotherapy

Box 1 | **ABC transporters and multidrug resistance**

A major breakthrough in uncovering the mechanisms behind multidrug resistance (MDR) came in 1976, correlating drug resistance with the overexpression of a single protein encoded by the gene ATP binding cassette, subfamily B, member 1 (*ABCB1*; also known as *MDR1*). The encoded protein is an ATP-dependent efflux pump and a member of the ABC transporter family[151]. ABCB1 overexpression is now associated with treatment failure in many cancers, including those of the kidney, liver and colon, as well as lymphoma and leukaemia (reviewed in REF. 152). Some years later, ABCC1 (also known as multidrug resistance protein 1) was identified as having a role in MDR phenotypes of small-cell lung carcinoma cells[153]. ABCC1 overexpression has been correlated with drug resistance in prostate, lung and breast cancer, as well as in childhood neuroblastoma (reviewed in REFS 11, 154). ABCB1 and ABCC1 efflux overlapping but distinct sets of compounds (TABLE 1). The third major MDR pump to be discovered was ABCG2 (also known as BCRP), which has a remarkably wide range of substrates and has been associated with drug resistance in breast cancer and leukaemia[155–157]. A key physiological function of ABC transporters is the protection of cells from many toxic insults from either endogenous or exogenous molecules that can enter the cell by diffusion or active uptake (see the figure). The protective mechanism afforded by ABC transporter-mediated extrusion of such toxic substances, whether they are metabolic waste products, naturally occurring substances or drugs, can make tumour cells resistant to the toxic effects of various chemotherapeutic agents[155–157].

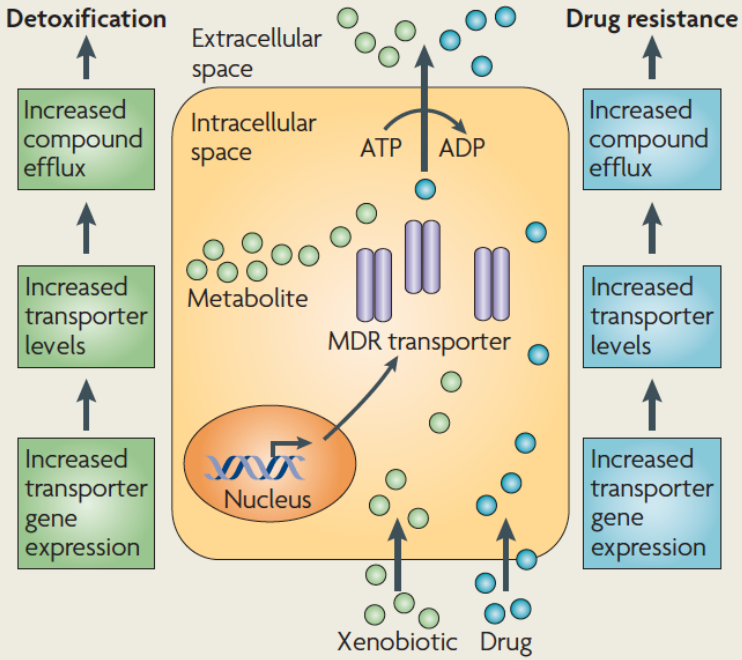


Table 1 | **Selected ABC transporters, cancer-related substrates and their expression in tumour stem cell-like populations***

ABC family	Chemotherapy substrates‡	Cancer-related cellular substrates	Expression in cancer stem cell-like populations	Refs
ABCA				
ABCA1	ND	S1P and cholesterol	ND	140,174
ABCA2	Estramustine and mitoxantrone	Cholesterol	Lung cancer cell lines and AML	149,175
ABCA3	Anthracyclines	Phospholipids	Neuroblastoma	176,177
ABCB				
ABCB1	Colchicine, anthracyclines, epipodophyllotoxins, vinca alkaloids, taxanes, camptothecins, bisantrene, imatinib, mitoxantrone, saquinivir, methotrexate and actinomycin D	PAF	AML and lung cancer cell lines	165,175,178
ABCB4	Anthracyclines, vinca alkaloids, taxanes, epipodophyllotoxins and mitoxantrone	ND	ND	–
ABCB5	Anthracyclines, camptothecins and thiopurines	ND	Melanoma	179
ABCB11	Taxanes	ND	ND	–
ABCC				
ABCC1§	Anthracyclines, mitoxantrone, vinca alkaloids, imatinib, epipodophyllotoxins, camptothecins, colchicine, saquinivir and methotrexate	LTC_4, PGA_2, 15d-PGJ_2, PGE_2 and S1P	Squamous cell carcinoma lines, lung cancer cell lines, glioma and AML	93,103,104, 121,122,141, 149,175,180, 181
ABCC2§	Vinca alkaloids, cisplatin, taxanes, anthracyclines, methotrexate, epipodophyllotoxins, camptothecins, mitoxantrone and saquinivir	LTC_4, PGD_2, PGA_1 and PGE_2	ND	93,120
ABCC3§	Methotrexate and epipodophyllotoxins	LTC_4 and 15d-PGJ_2	ND	104,124
ABCC4§	Thiopurines, PMEA, methotrexate, AZT and camptothecins	LTB_4, LTC_4, PGA_1, PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, TXB_2, cAMP and cGMP	ND	57,94–96, 123,159,160, 182,183
ABCC5	Thiopurines, methotrexate, cisplatin, PMEA and AZT	cAMP and cGMP	ND	161
ABCC6	Anthracyclines, cisplatin and epipodophyllotoxins	LTC_4	ND	117
ABCC10	Vinca alkaloids and taxanes	LTC_4	ND	119
ABCC11	Thiopurines	LTC_4, cAMP and cGMP	ND	118,162
ABCG				
ABCG2	Mitoxantrone, camptothecins, anthracyclins, bisantrene, imatinib, methotrexate, flavopiridol and epipodophyllotoxins	cGMP	Lung cancer, AML, oesophageal carcinoma, glioma, neuroblastoma, squamous cell carcinoma cell lines, melanoma, ovarian cancer and nasopharyngeal carcinoma cell lines	149,175,177, 179–181, 184,185

ABC, ATP binding cassette; cAMP, cyclic AMP; cGMP, cyclic GMP; LT, leukotriene; ND, not determined; PAF, platelet activation factor; PG, prostaglandin; S1P, sphingosine-1-phosphate; TX, thromboxane. *ABC transporters are major mediators of chemoresistance but also efflux endogenous substrates with well-established roles in tumour biology. ‡Reviewed in REFS 11,173. §Several substrates for ABCC1–4 are effluxed as either glutathione or glucuronide conjugates, or in a glutathione-dependent manner.

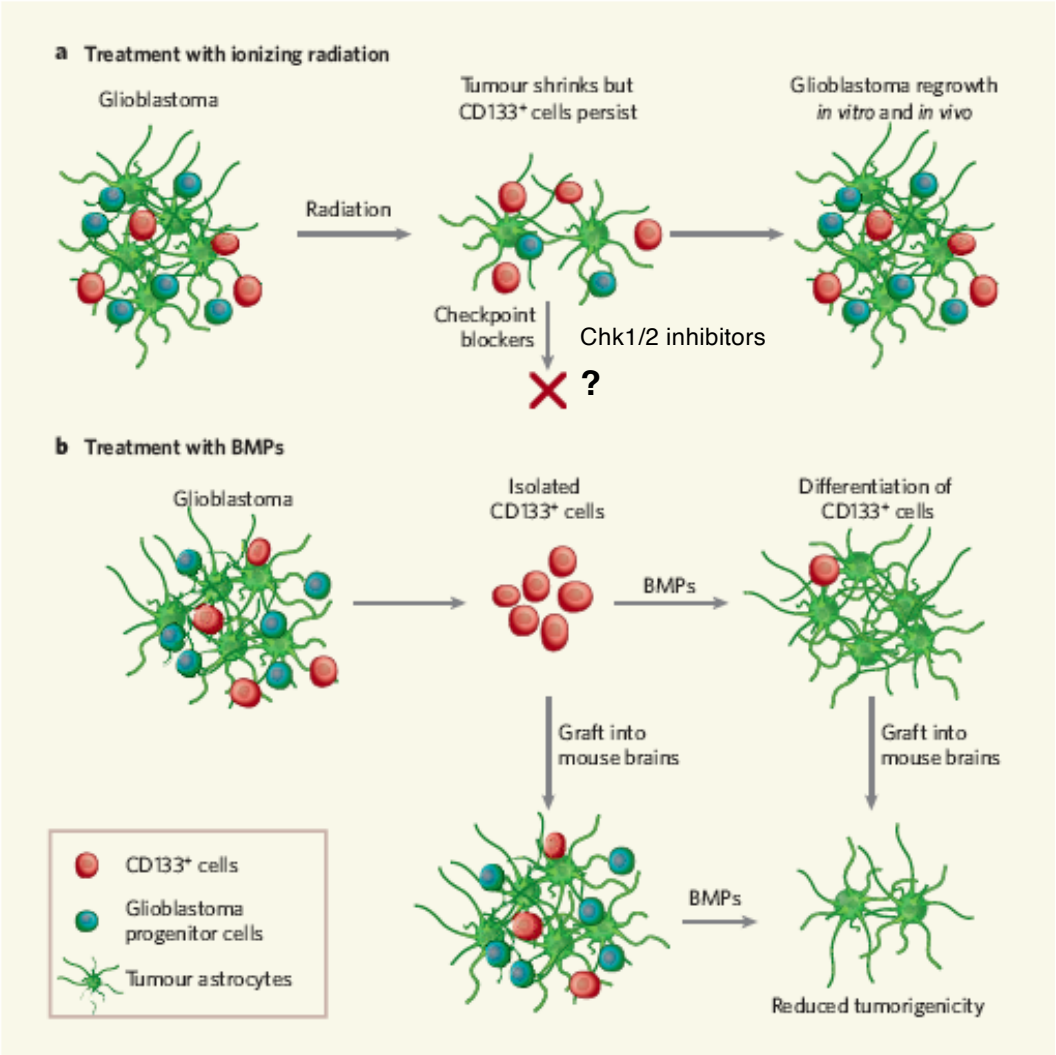


Figure 1 | Response of glioblastomas to ionizing radiation[3] and bone morphogenetic proteins (BMPs)[4]. Glioblastomas are heterogeneous tumours that contain a few tumour-initiating $CD133^+$ stem cells among other, more differentiated, $CD133^-$ cells, including glioblastoma progenitor cells. **a**, Following radiation, the bulk glioblastoma responds and the tumour shrinks. But $CD133^+$ cells activate checkpoint controls for DNA repair more strongly than $CD133^-$ cells, resist radiation and prompt the tumour to regrow. These cells could be targeted with DNA-checkpoint blockers to render them radiosensitive. **b**, BMPs normally cause neural stem cells to differentiate into astrocytes. When used to treat isolated glioblastoma $CD133^+$ cells, they weaken the cells' tumorigenicity both *in vitro* and, when engrafted into mice, *in vivo*. The knowledge that a tumour retains a developmental hierarchy suggests that targeting different cell populations is a promising therapeutic strategy.

NCSC can repair their DNA more efficient than other tumor cells.

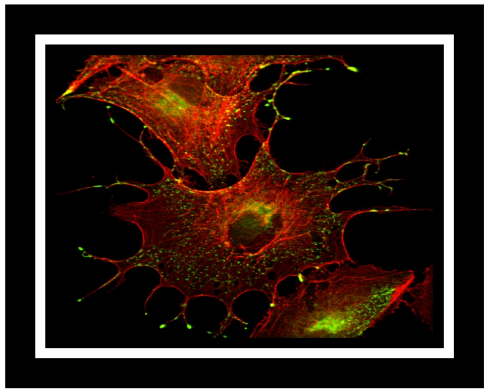
BMP induces differentiation of NSCs towards mature astrocytes!

(P. Dirks, Nature 2006)

The choice of your experimental model system will influence the outcome and also the interpretation of your data

Melanoma
Cancer stem cells
Yes or No?

Melanocyte biology

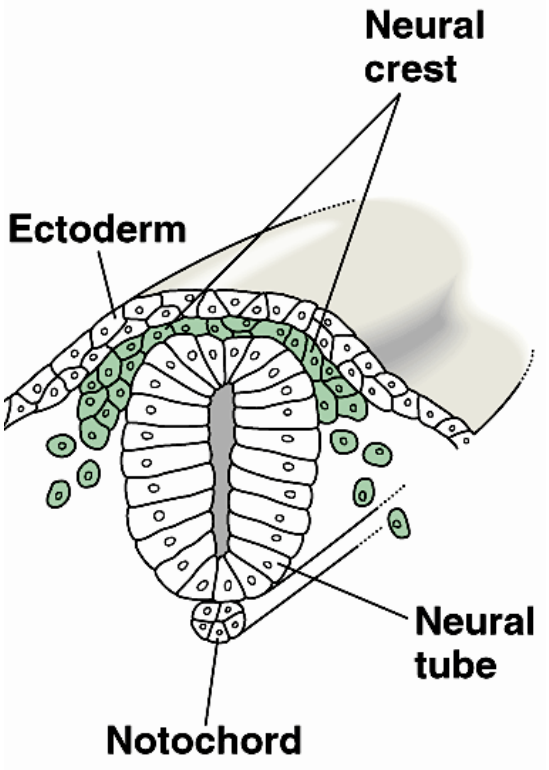


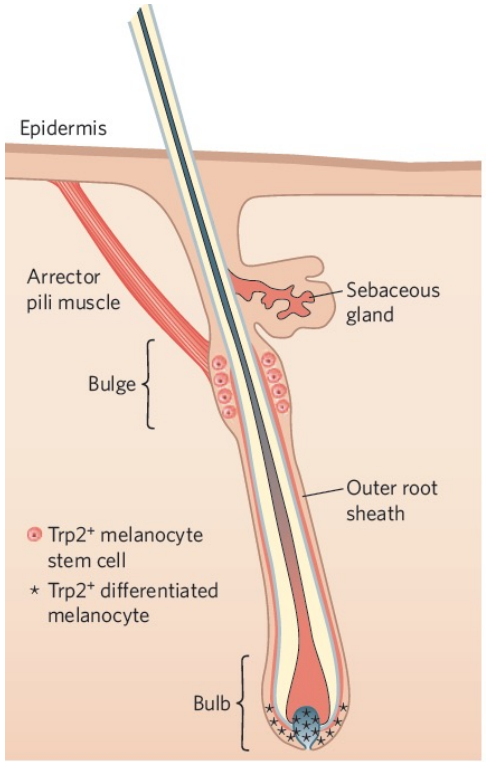
❖Melanocytes in skin are low in number, but lead to prominent phenotype

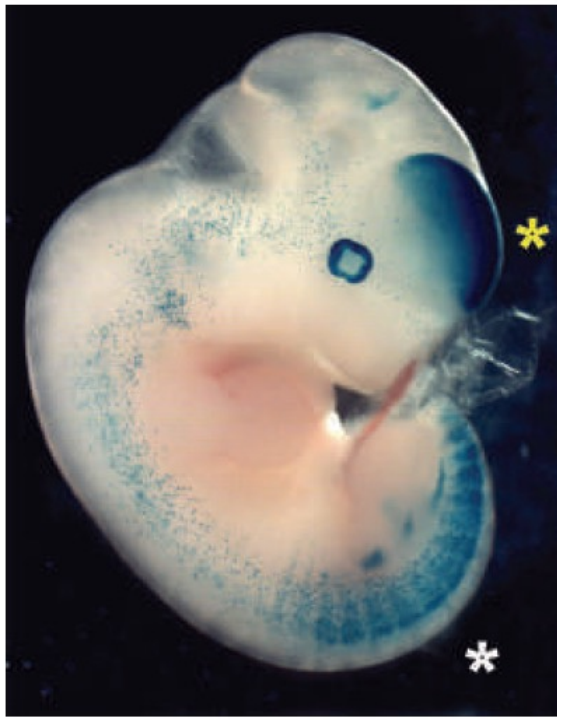
❖Melanin - Pigment
Eumelanin & Pheomelanin

❖Melanosome - A lysosome-related intracellular membrane-coated organelle that originates from endosomes

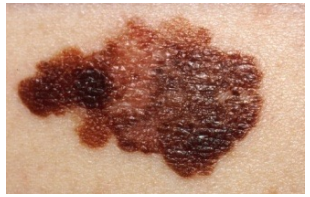
Skin and melanocytes







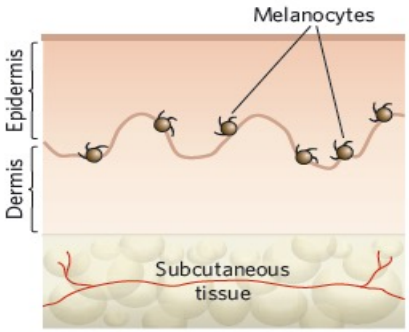
Mackenzie , 1997



Melanoma formation

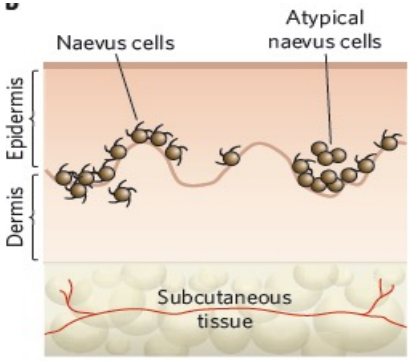
Malignant melanoma is one of the most aggressive types of human cancers

normal skin



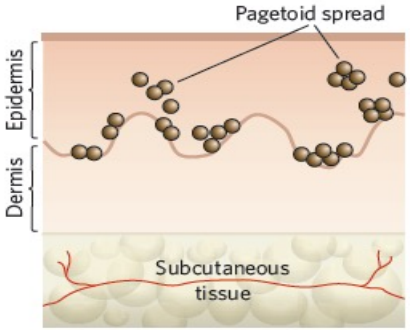
Normal proliferation

naevi



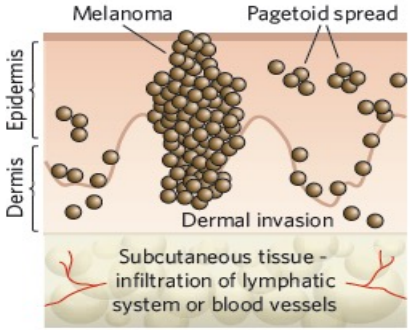
Melanocyte proliferation

RGP melanoma



Local microinvasion (intra-epidermal)

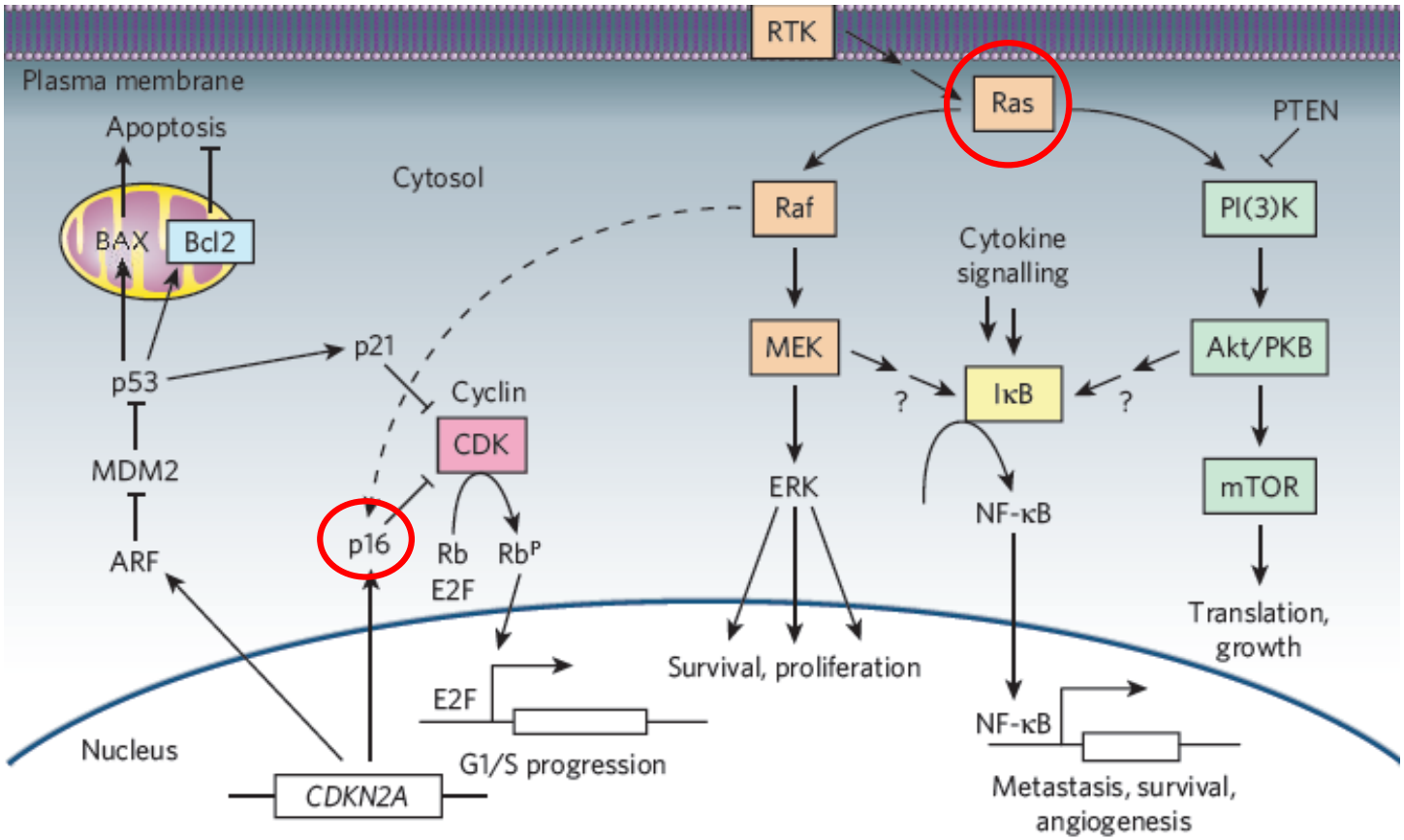
VGP melanoma



Metastasis (invasion of dermis)

(Chin et al,2006)

Genetics of melanoma



Adapted from Gray-Schopfer et al., Nature, 2008

Genetic alterations

Genotype	Gene	Alteration frequency/type(s) in melanoma (%)
Oncogenes	BRAF	50-70% mutated
	NRAS	15-30% mutated
Tumour suppressors	CDKN2A	30-70% deleted, mutated or silenced
	p53	10% lost or mutated

Adapted from Gray-Schopfer et al, Nature, 2008

Vol 451|17 January 2008|doi:10.1038/nature06489

nature

LETTERS

Identification of cells initiating human melanomas

Tobias Schatton[1], George F. Murphy[2], Natasha Y. Frank[1,3], Kazuhiro Yamaura[1], Ana Maria Waaga-Gasser[4], Martin Gasser[4], Qian Zhan[2], Stefan Jordan[1], Lyn M. Duncan[5], Carsten Weishaupt[6], Robert C. Fuhlbrigge[6], Thomas S. Kupper[6], Mohamed H. Sayegh[1] & Markus H. Frank[1]

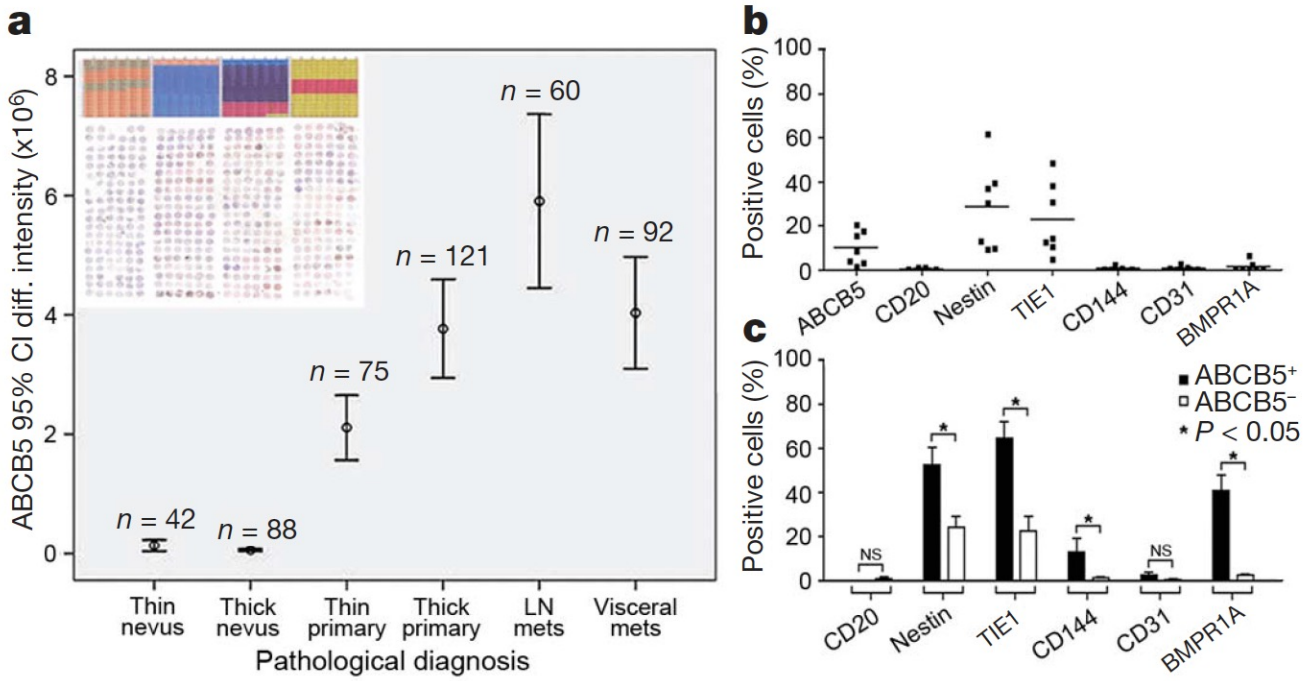


Figure 1 | ABCB5 expression analyses. **a**, Melanoma progression tissue microarray analysis for ABCB5, showing significant differences in ABCB5-staining intensities (mean ± 95% confidence interval (CI); thin or thick nevi versus thin or thick primary melanomas, or versus lymph node or visceral metastases, P values < 0.001; thin versus thick primary melanomas, $P = 0.004$; thin and thick primary melanomas versus lymph node metastases, $P = 0.001$; lymph node versus visceral metastases, $P = 0.025$; n, provided in figure). The picture colour map corresponds to sample types represented in the core array: green, thin nevi; orange, thick nevi; violet, thin primary melanoma; blue, thick primary melanoma; pink, lymph node metastases; yellow, visceral metastases. The scanning view of ABCB5 staining of the entire array corresponds to the colour key. **b**, Flow cytometry analysis of ABCB5, CD20, nestin, TIE1, CD144, CD31 or BMPR1a expression in $n = 7$ melanoma patients. **c**, Marker expression by ABCB5$^+$ or ABCB5$^-$ melanoma cells determined by flow cytometry (mean ± s.e.m., $n = 4$–7 patients).

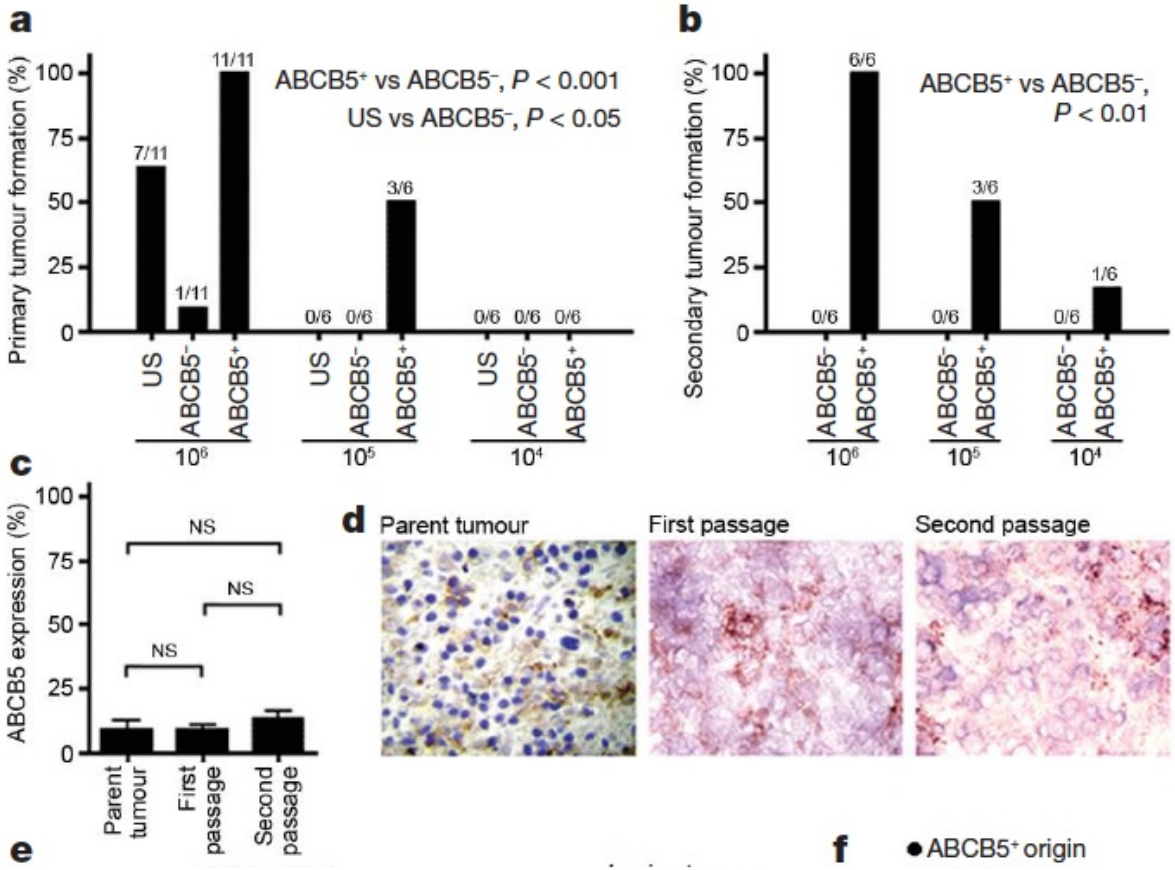


Figure 2 | Tumorigenicity, self-renewal and differentiation of ABCB5$^+$ MMIC. **a**, Primary tumour formation of unsegregated (US), ABCB5$^-$ or ABCB5$^+$ cells. **b**, Secondary tumour formation of ABCB5$^-$ or ABCB5$^+$ cells. **c**, ABCB5 expression (mean ± s.e.m.) in parent tumours ($n = 3$) and respective ABCB5$^+$-derived primary ($n = 11$) and secondary ($n = 7$) xenografts. **d**, ABCB5 immunohistochemistry (patient P3). **e**–**h**, *In vivo* genetic lineage tracking of human ABCB5$^+$ melanoma cells. **e**, EYFP versus DsRed plots of a genetically labelled inoculum (left) and a corresponding 6-week-old tumour (right). Controls (small panels): non-transfected cells (top), DsRed$^+$ cells (middle) and EYFP$^+$ cells (bottom). **f**, Percentage of DsRed$^+$ or EYFP$^+$ cells (mean ± s.e.m.) in inocula ($n = 6$) and respective tumour xenografts ($n = 3$) as a function of time. **g**, Fluorescence microscopy

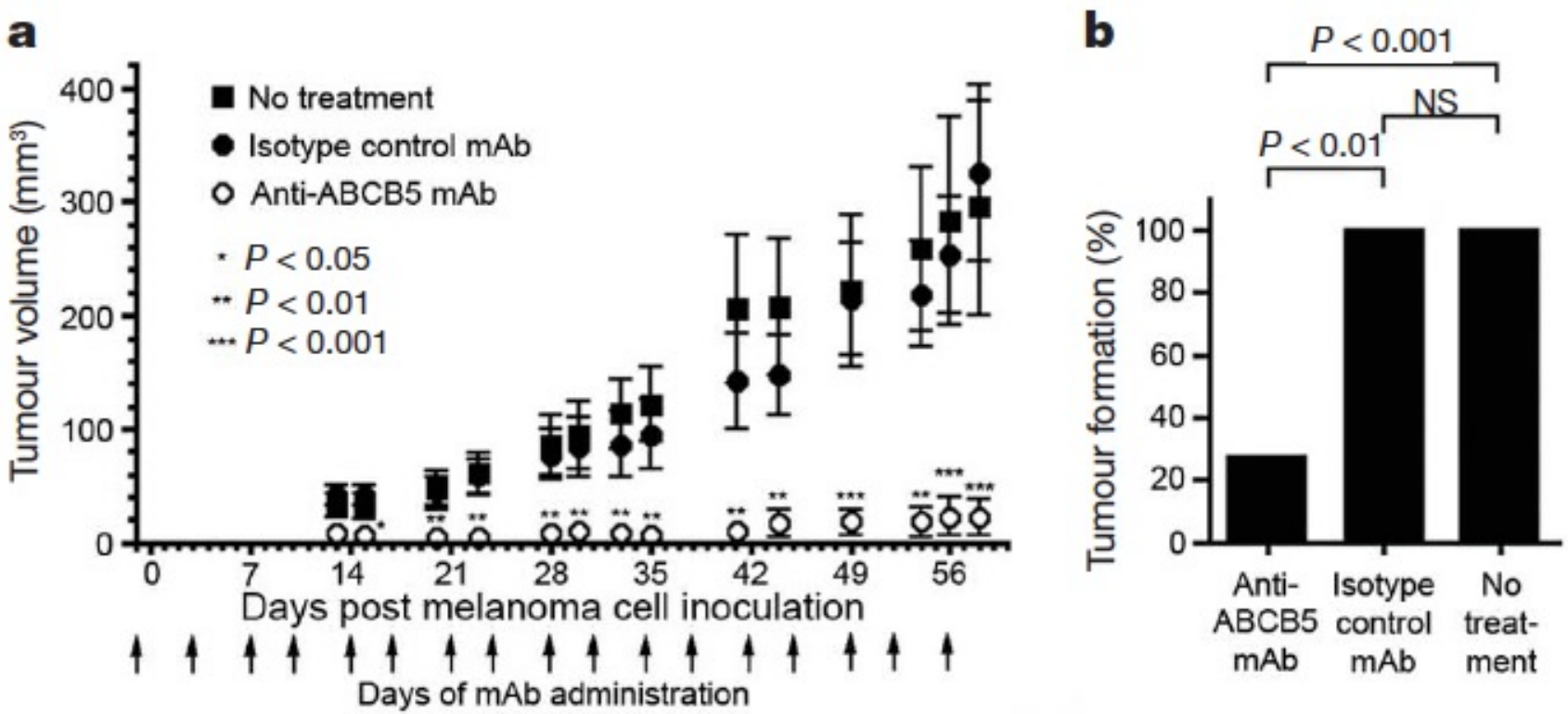


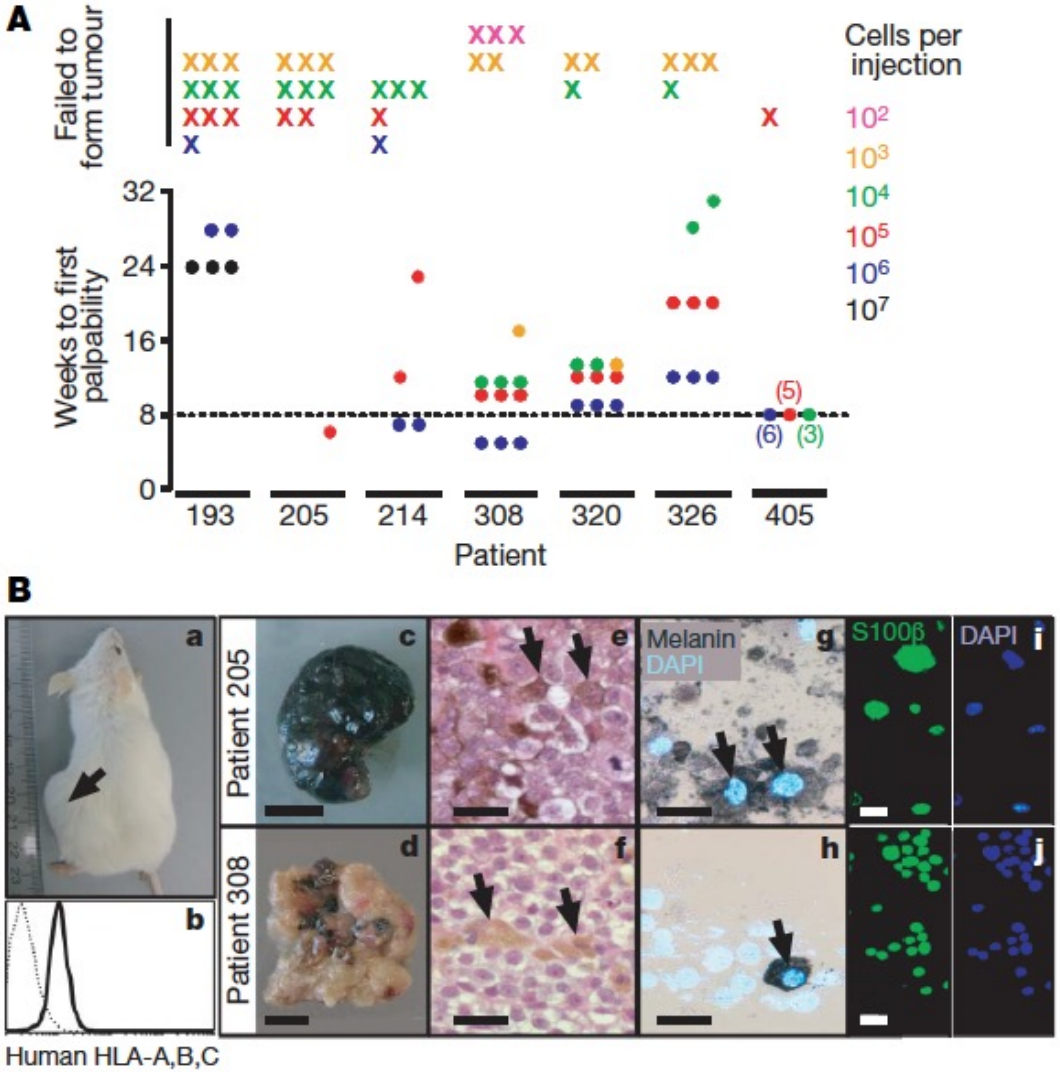
Figure 3 | ABCB5 targeting. **a**, Tumour volumes (mean ± s.e.m.) plotted against time. **b**, tumour formation rate in untreated ($n = 18$), control-monoclonal-antibody (mAb)-treated ($n = 10$), or anti-ABCB5 mAb-treated ($n = 11$) animals. **c**, Flow cytometric assessment of ADCC in anti-ABCB5

Vol 456|4 December 2008|doi:10.1038/nature07567 nature

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]



C

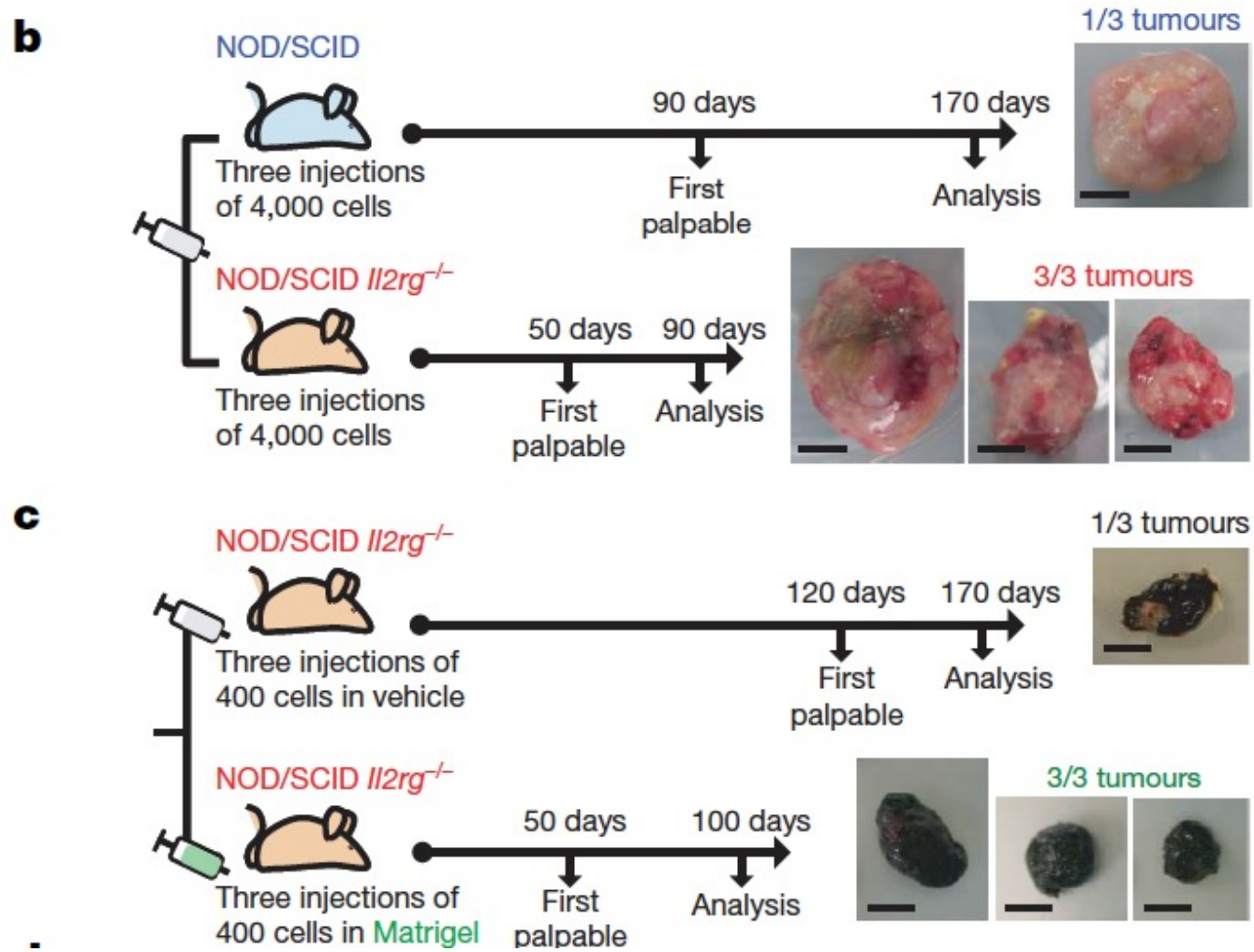
Time after injection	Melanoma-initiating cell frequency (95% confidence interval)	
8 weeks	1/837,000	(1/512,000–1/1,370,000)
32 weeks	1/111,000 *	(1/67,000–1/185,000)

Figure 1 | Only rare human melanoma cells form tumours in NOD/SCID mice. **A**, Tumour development after subcutaneous injection of unfractionated primary melanoma cells directly from seven patients into NOD/SCID mice. Dots represent the times after injection at which individual tumours were first palpable and are coloured according to cell dose. Crosses are injections that failed to form tumours. Dotted line indicates 8 weeks after injection. **B**, All tumours were diagnosed as metastatic melanoma by clinical pathology (see Supplementary Table 1 for more information). The tumours that formed in mice (**a**, arrow) became large, grew quickly once they were palpable and were histologically similar to the patient tumours from which they were derived. Flow cytometry demonstrated that most tumour cells expressed human HLA (**b**; dotted line represents unstained control). Some tumours were highly pigmented (**c**) whereas others contained variable pigmentation (**d**) or were amelanotic (scale bar, 1 cm). Sections stained with haematoxylin and eosin through the same tumours showed pigmented cells (**e**, **f**, see arrows; bars, 25 μm). Cytospun cells contained melanin, as indicated by Fontana-Masson staining (**g**, **h**, arrows; bars, 25 μm), and showed widespread S100β staining (**i**, **j**), a marker used to diagnose melanoma[40]. **C**, Limiting dilution analyses of the frequency of tumorigenic melanoma cells in Fig. 1A at 8 weeks or 32 weeks after transplantation (*$P < 0.0001$).

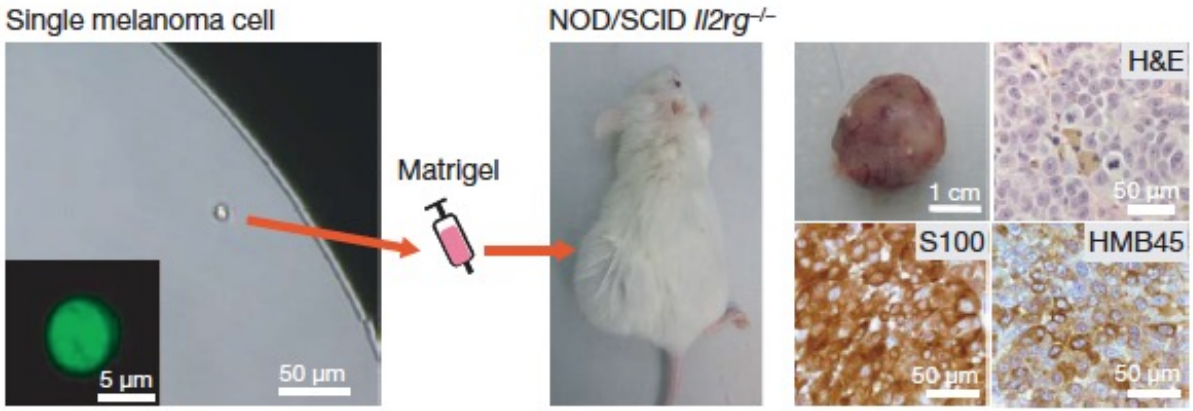
d

400 cells in Matrigel palpable

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)



a



b

Patient	Engraftment rate tumours/injections (%)		Melanoma-initiating cell frequency (95% confidence interval)		Weeks to first palpability
205	11/89	(12%)	1/8	(1/5–1/14)	7 ± 2
214	12/73	(16%)	1/6	(1/4–1/10)	10 ± 4
481	40/62	(65%)	1/2	(1/1–1/2)	12 ± 3
487	6/30	(20%)	1/5	(1/3–1/11)	10 ± 1
All	69/254	(27%)	1/4	(1/3–1/5)	11 ± 3

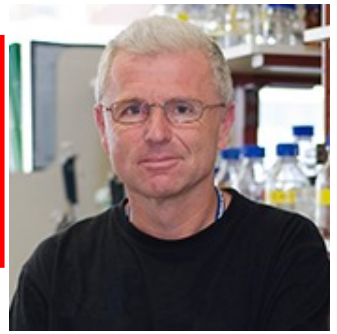
Figure 4 | Efficient tumour development from the xenotransplantation of single human melanoma cells. **a**, Flow-cytometrically isolated human melanoma cells derived from xenografts from four patients were diluted into Terasaki microwells such that wells containing single cells could be identified by phase contrast microscopy. In control experiments, the presence of single cells was confirmed by the observation of single nuclei with Acridine Orange staining (inset) in 90 out of 90 cases. The single cells were mixed with Matrigel and injected into NOD/SCID *Il2rg*$^{-/-}$ mice. Tumours arising from the injection of single cells were confirmed to be melanoma by haematoxylin and eosin, S100 and HMB45 staining (right panels show sections from a tumour that arose from a single cell obtained from patient 214). **b**, The percentage of single-cell injections (69/254 = 27%) that formed tumours within 20 weeks of transplantation. Weeks to first palpability (mean ± s.d.) are indicated for each set of tumours.

Xeno-transplantation assays of human tumor derived cells into NOD/SCID mice for the identification and determination CSC and their frequencies was recently heavily criticized.

- Growth of tumor cells is not independent from its microenvironment.
- Tumor needs soluble and membrane bound factors (tumor cell niche?)
- Many of the soluble factors secreted by murine cells cannot bind the cognate human receptors and vise versa.

Thus the low frequency of human cancer derived cells might reflect (at least in part) cells that more easily adapt to growth in a foreign (mouse) environment.

Cancer stem cell hypothesis can best be tested by transfer of titrated numbers of mouse tumor cells into non-irradiated histocompatible recipient mice.



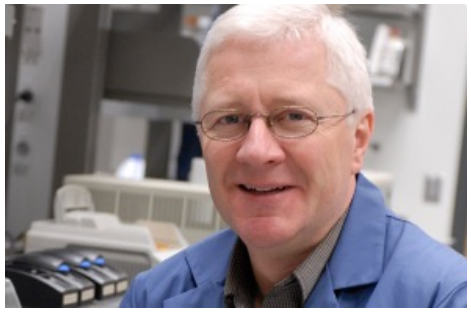
Tumor Growth Need Not Be Driven by Rare Cancer Stem Cells

Priscilla N. Kelly,[1,2] Aleksandar Dakic,[1,2] Jerry M. Adams,[1*] Stephen L. Nutt,[1*] Andreas Strasser[1*†]

www.sciencemag.org SCIENCE VOL 317 20 JULY 2007

	Recipients that developed tumors (days to kill)			
	10^5 cells	10^3 cells	10^2 cells	10 cells
	Eμ-myc B lymphoma			
Case 1	3/3 (25)	3/3 (25)	3/3 (32)	2/2 (35)
Case 2	3/3 (21)	3/3 (23)	3/3 (24)	3/3 (24)
Case 3 Sca-1^+ AA4.1^{hi}	3/3 (21)	3/3 (21)	ND	3/3 (17)
Sca-1^+ AA4.1^{lo}	2/2 (17)	2/2 (28)	2/2 (28)	2/2 (40)
	Eμ-N-RAS T lymphoma			
Case 1	3/3 (28)	3/3 (42)	3/3 (28)	3/3 (28)
	PU.$1^{-/-}$ *AML*			
Case 1	1/1 (54)	2/2 (168)	1/2 (192)	0/2
Case 2	2/2 (84)	2/2 (85)	2/2 (224)	1/2 (114)
Case 3	1/1 (85)	2/2 (62)	2/2 (69)	2/2 (90)
Case 4	1/1 (30)	1/1 (37)	2/2 (79)	2/2 (88)

Leukemia stem cell signature and HSC stem cell signature in patient AML samples correlate with clinical outcome



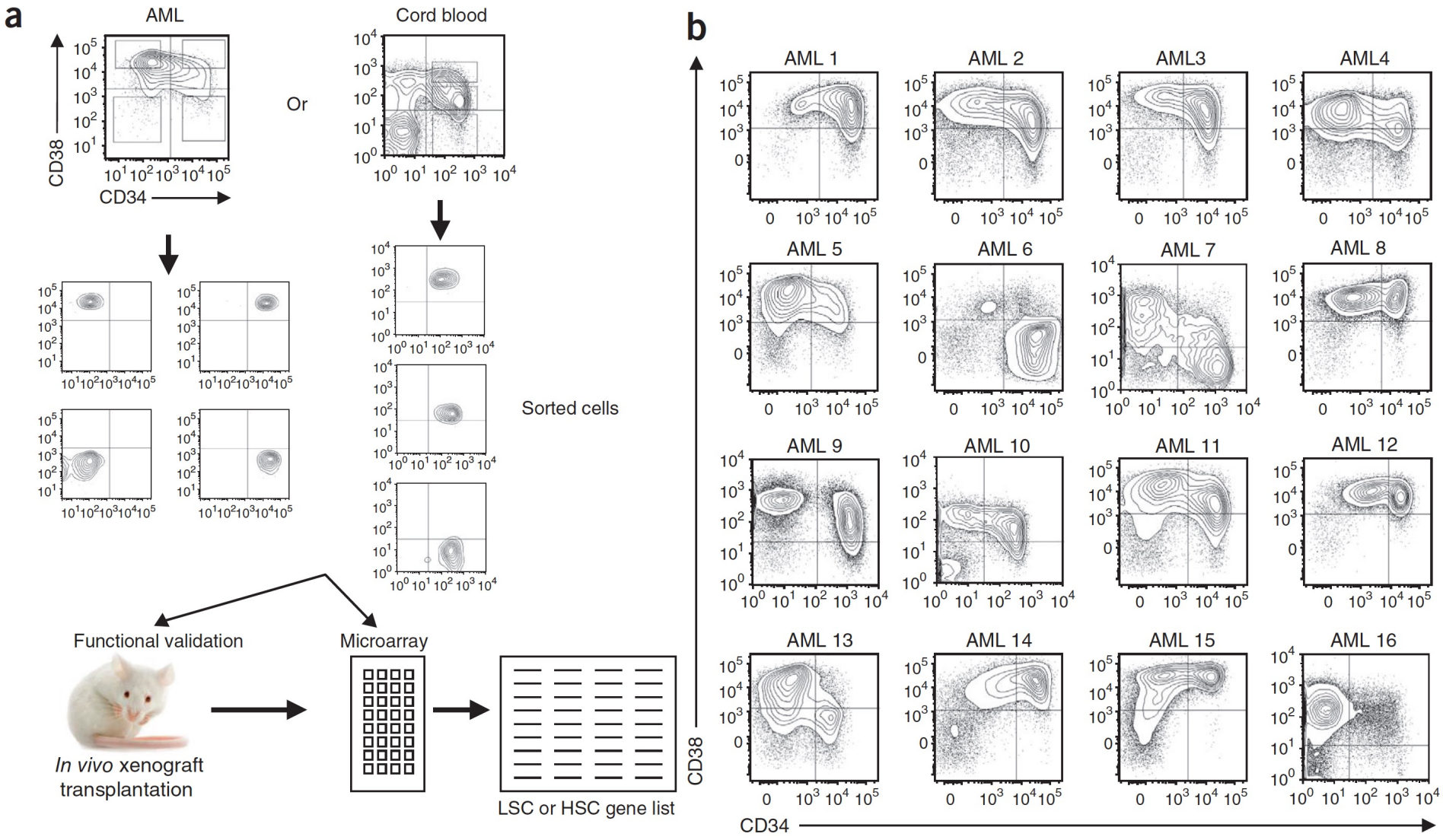
nature medicine

Stem cell gene expression programs influence clinical outcome in human leukemia

Kolja Eppert[1], Katsuto Takenaka[2,12], Eric R Lechman[1,12], Levi Waldron[3,12], Björn Nilsson[4,12], Peter van Galen[1], Klaus H Metzeler[5], Armando Poeppl[1], Vicki Ling[6], Joseph Beyene[6], Angelo J Canty[7], Jayne S Danska[8], Stefan K Bohlander[5], Christian Buske[9], Mark D Minden[10], Todd R Golub[11], Igor Jurisica[3], Benjamin L Ebert[4] & John E Dick[1]

VOLUME 17 | NUMBER 9 | SEPTEMBER 2011

Experimental strategy of transcriptional profiling of stem cell fractions identified by function



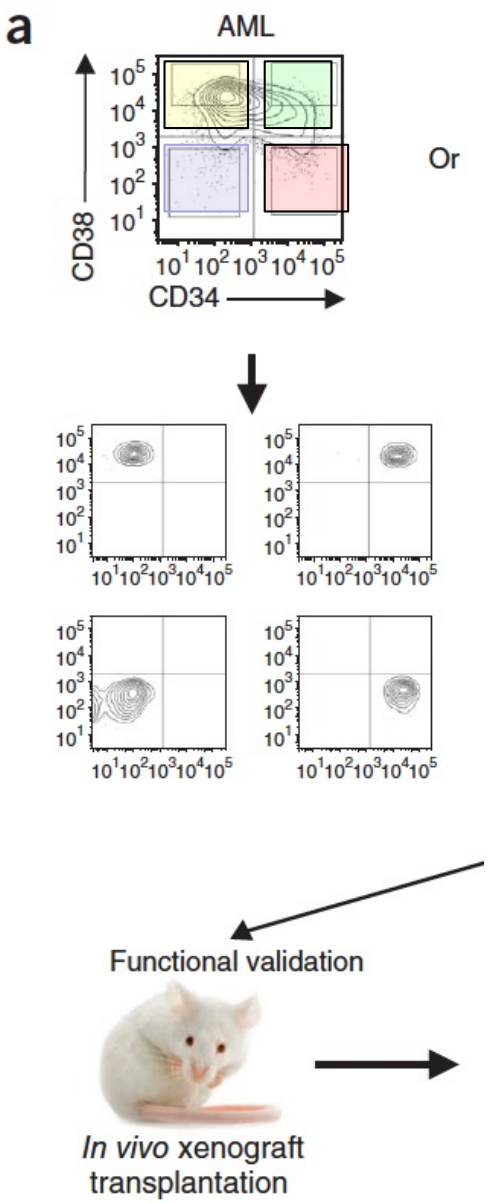
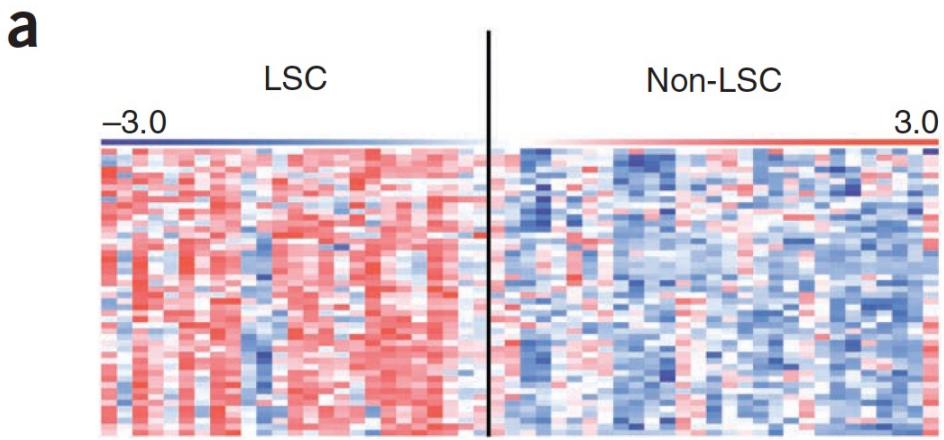


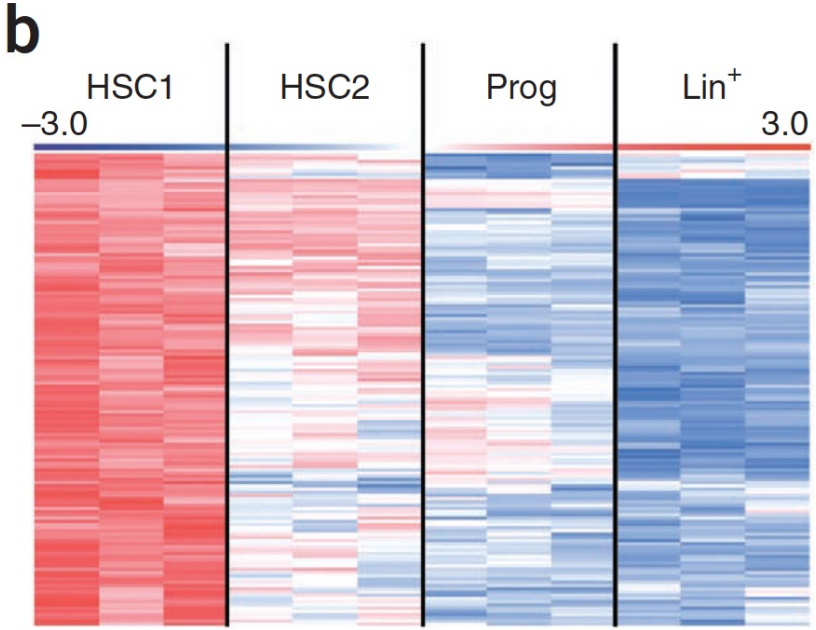
Table 1 LSC frequency in sorted AML samples

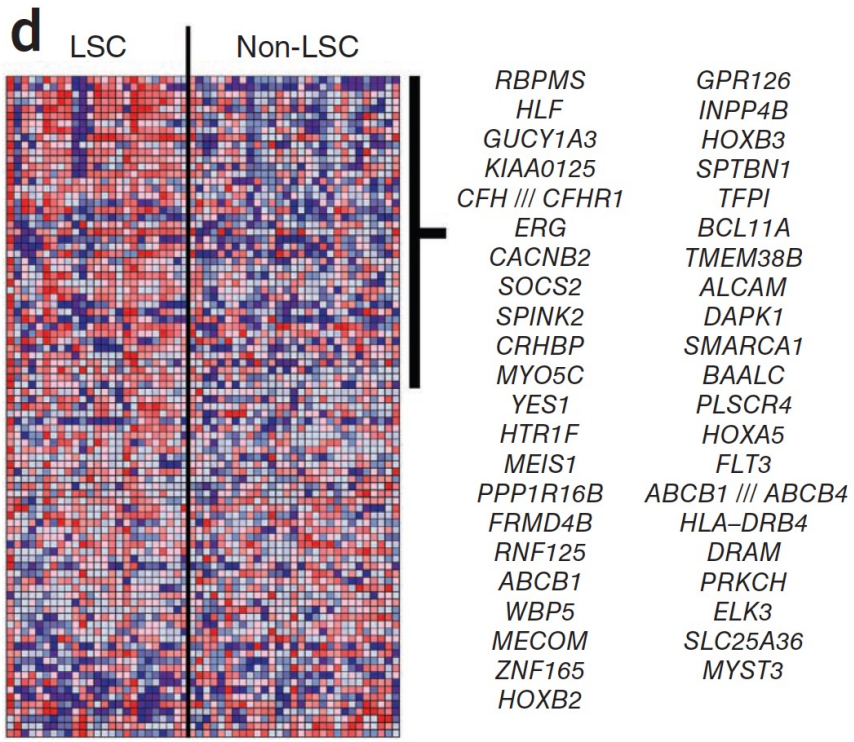
AML	$CD34^+CD38^-$ Frequency 1 LSC per *n* cells (95% CI)	$CD34^+CD38^+$ Frequency 1 LSC per *n* cells (95% CI)	$CD34^-CD38^+$ Frequency 1 LSC per *n* cells (95% CI)	$CD34^-CD38^-$ Frequency 1 LSC per *n* cells (95% CI)
1	1.6×10^3 (2.7×10^2–9.9×10^3)	1.3×10^5 (4.6×10^4–3.7×10^5)	0	0
2	5.8×10^3 (1.8×10^3–1.8×10^4)	4.2×10^3 (1.4×10^3–1.3×10^4)	0	0
3	6.2×10^{3a} (1–6.2×10^3)	7.6×10^{3a} (1–7.6×10^3)	9.6×10^3 (2.5×10^3–3.7×10^4)	7.7×10^{3a} (1–7.7×10^3)
4	7.1×10^3 (1.1×10^3–4.6×10^4)	9.2×10^4 (2.7×10^4–3.1×10^5)	0	4.4×10^{5a} (1–4.4×10^5)
5	1.1×10^4 (3.7×10^3–3.4×10^4)	4.5×10^4 (1.8×10^4–1.2×10^5)	0	0
6	1.7×10^5 (6.9×10^4–4.2×10^5)	1.5×10^5 (5.8×10^4–4.1×10^5)	0	0
7	1.7×10^{5a} (1–1.7×10^5)	NT	NT	9.1×10^{5a} (1–9.1×10^5)
8	2.1×10^5 (9.3×10^4–4.9×10^5)	0	0	0
9	2.6×10^{5a} (1–2.6×10^5)	NT	NT	NT
10	2.5×10^5 (6.0×10^4–1.0×10^6)	NT	NT	NT
11	4.5×10^5 (6.4×10^4–3.1×10^6)	4.9×10^4 (1.9×10^4–1.3×10^5)	0	0
12	4.9×10^5 (6.9×10^4–3.5×10^6)	0	0	0
13	1.1×10^6 (2.7×10^5–4.3×10^6)	2.4×10^5 (9.0×10^4–6.3×10^5)	0	0
14	*	0	0	0
15	**	0	0	0
16	**	0	0	0
Total	13/14 (93%)	8/13 (62%)	1/13 (8%)	3/14 (21%)

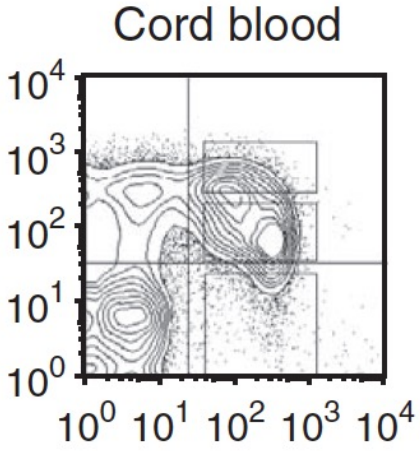
NT, no antibody to CD122 used.
[a]Estimate from lower 95% interval. *No LSC detected at this sensitivity (see Online Methods). **Normal engraftment detected.

LSCs express HSC gene expression profile









LSCs and HSC gene signatures predict patient survival

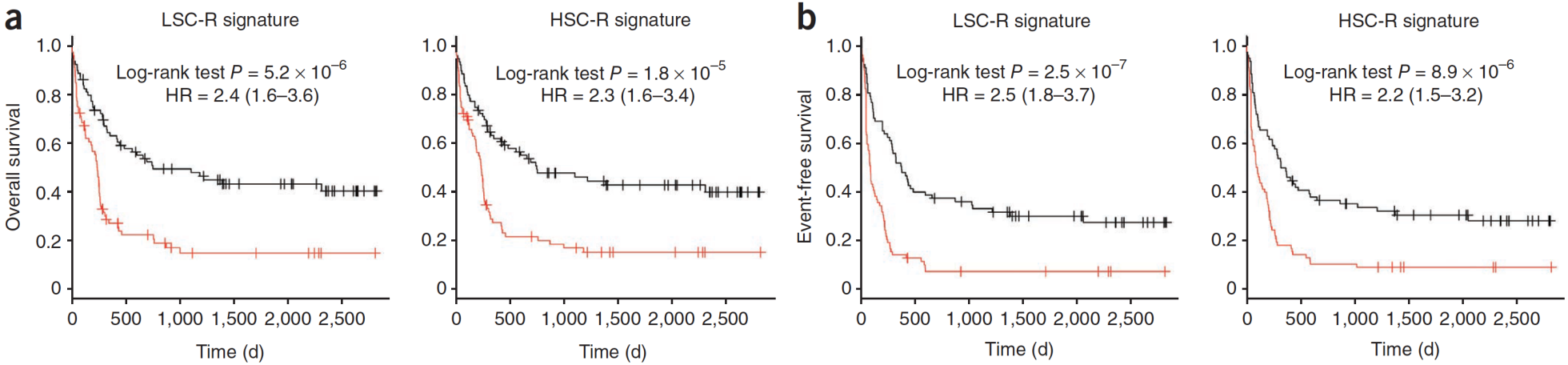
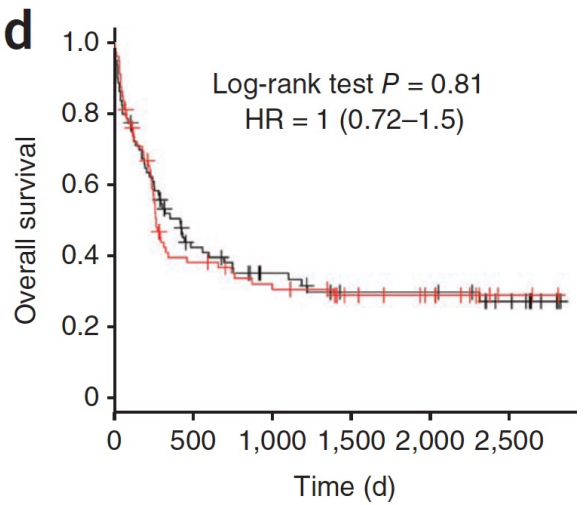


Figure 3 LSC-R and HSC-R gene signatures are correlated with disease outcome. Unsorted cytogenetically normal AML samples (160) were divided into two populations of 80 AML samples by expression of stem cell gene signatures. (**a**) Correlation of LSC-R and HSC-R signatures and overall survival. Red line, subjects whose AML cells expressed LSC-R (left) or HSC-R (right) signatures greater than the median; black line, those whose AML cells expressed respective stem cell signature less than the median. (**b**) Event-free survival of subjects stratified by expression of the LSC-R and HSC-R, as in **a**. (**c**) Additive



(d) Correlation of an AML signature based on phenotypic markers (CD34+CD38-, stem cells versus CD34+CD38+, progenitor; 23 AML samples) and overall survival. Red line, subjects whose AML expressed the CD34+CD38- gene list greater than the median; black line, those who expressed the CD34+CD38- gene list less then median

Lines of evidence of whether human tumors contain CSCs

- Fractionating primary tumors into populations that can generate xenografts and those that cannot.
- Transplanting tumors into secondary recipient mice to demonstrate self-renewal capacity.
- Biological properties specific to CSC (e.g. stem cell gene signature, tumor initiation potential in xenografts), but not to non-CSCs predict clinical important parameters such as patient survival.

Exam

- Wednesday 22. January 2025
- 09h15 to 12h15
- BCH 2201