Cancer stem cells in solid tumours.

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Cancer stem cells: definition

- A small subset of cancer cells which constitute a reservoir of self sustaining cells with the exclusive ability to self renew and maintain the tumor .
- Those cells within a tumor that possess the capacity to self renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor. (Clarke et al Can Res. 2006).

- This is simple and straightforward BUT
  - is it realistic to think of cancer stem cells as a uniform population?
  - can we actually identify such cells?

- An important implication of this definition is that it is (only) necessary to kill all the cancer stem cells to effect local control.

Normal tissue stem cells

- Many tissues appear to contain small populations of cells that have stem cell properties. Recent studies have implicated:
  - Signalling through the Notch, Hedgehog and Wnt pathways as involved in maintaining the phenotype.
  - Interactions with the local tissue environment as required to maintain the properties of such cells ("stem cell niche").
- Some authors have implicated escape from the niche as a factor that can promote tumorigenesis.

Cancer stem cells

- There are two possible explanations for the origin of cancer stem cells:
- 1) a normal stem cell that has lost growth regulation due to severe genetic damage or has been subjected to long-term aberrant activation;
- 2) a committed normal progenitor cell that is transformed by mutation and has acquired the properties of self renewal.

Cancer stem cell concept

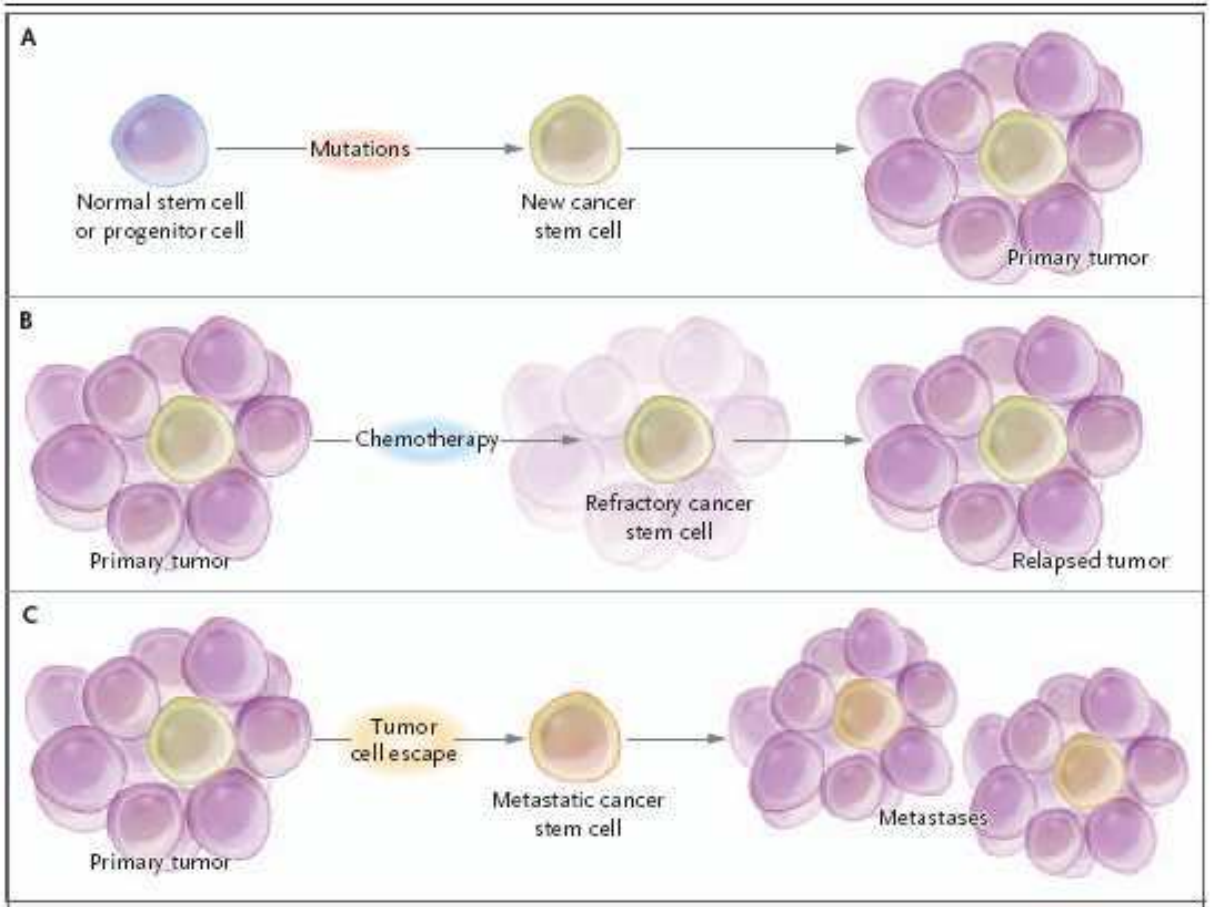


Figure 3. Scenarios Involving Cancer Stem Cells.

For tumors in which cancer stem cells play a role, at least three scenarios are possible. First, mutation of a normal stem cell or progenitor cell may create a cancer stem cell, which will then generate a primary tumor (Panel A). Second, during treatment with chemotherapy, the majority of cells in a primary tumor may be destroyed, but if the cancer stem cells are not eradicated, the tumor may regrow and cause a relapse (Panel B). Third, cancer stem cells arising from a primary tumor may emigrate to distal sites and create metastatic lesions (Panel C).

(From Jordan et al NEJM 2006)

Surface markers of cancer or normal stem cells in different tissues/cancers

Surface markers of normal stem cell and cancer stem cell in different tissues

Organ	Cancer type	Cancer stem cell markers	Normal stem cell markers
Hematopoietic	Leukemia	$CD34^+CD38^-Thy1^-Lin^-$	$CD34^+CD38^-Thy1^-Lin^-$
Hemangioblastic	CML	$Flk1^+CD31^-CD34^-$	$Flk1^+CD31^-CD34^-$
Breast	Mammary cancer	$CD44^+CD24^{-/low}ESA^+Lin^-$	$CD24^{med}$
Brain	Brain tumor	$CD133^+Nestin^+$	$CD133^+Lin^-$
Prostate	Prostate cancer	$CD44^+\alpha_2\beta_1^{hi}CD133^+$	$\alpha_2\beta_1^{hi}CD133^+$
Skin	Melanoma	$CD20^+CD166^+Nestin^+$	$K19^+\beta_1^+$ $CD166^+CD133^+Nestin^+$
Tongue, larynx, throat and sinus	Head and neck squamous cell carcinoma	$CD44^+Lin^-$	$CD44^+Lin^-$
Pancreas	Pancreatic cancer	$CD44^+CD24^+ESA^+$	$CXCR4^+Nestin^+$
Colon	Colorectal cancer	$ESA^{high}/CD44^+/CD166^+$ $CD133^+$	$CD133^+$
Liver	Liver cancer	$CD133^+$	$CD133^+ESA^+$
Lung	Lung cancer	$CD133^+$	$CD133^+$

Zhao, R. C., et al., Pharmacol Ther (2008)

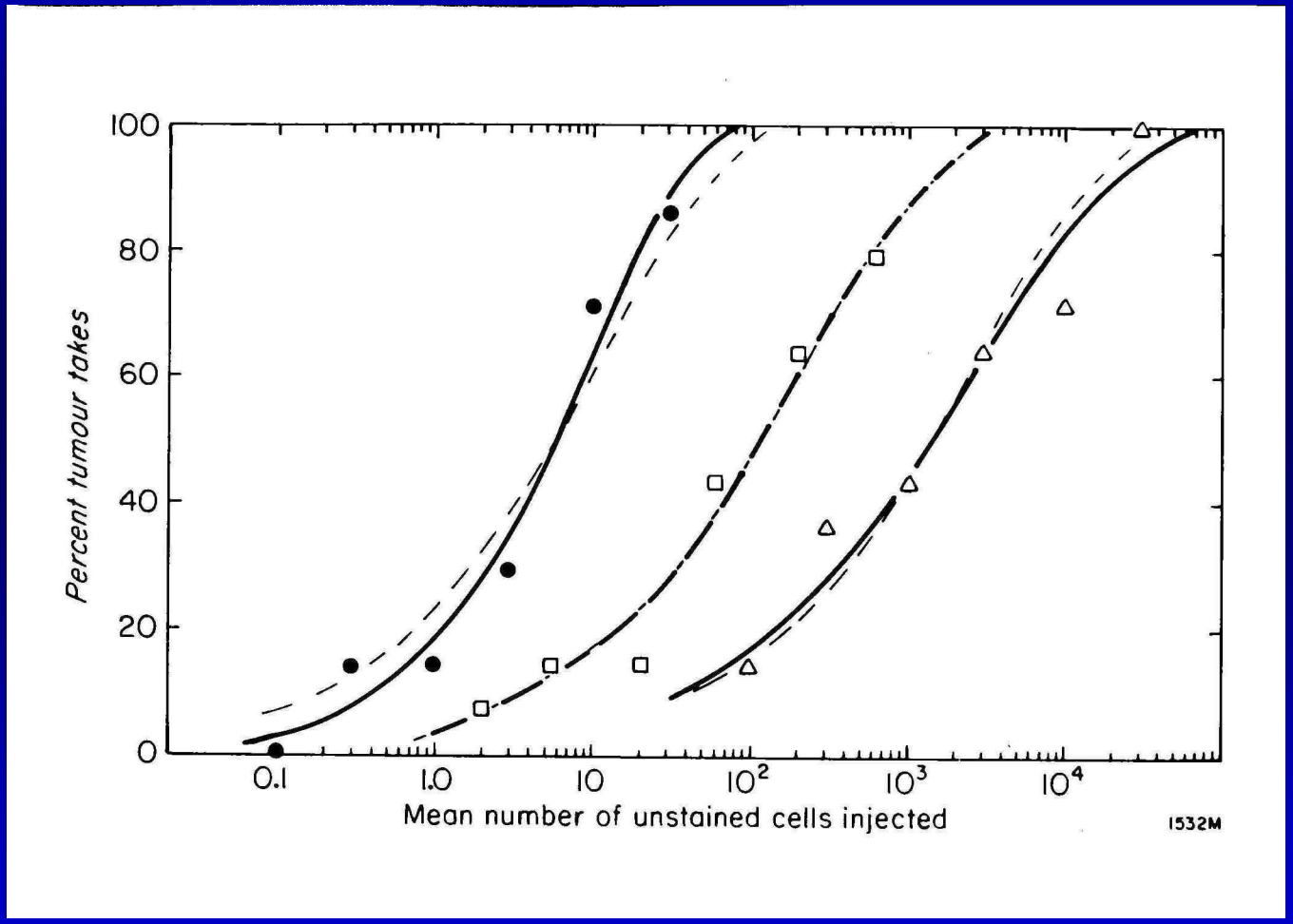
The Gold Standard

- Critical demonstration of the "stemness" of a cancer cell is its ability to transplant the tumour. In vivo assays are the gold standard for identifying stem cells.

YET

- we know that this assay can be highly heterogeneous depending on conditions.
- Eg (transplantation site, effects of feeder cells, hormones, matrigel, stromal fibroblasts from cancers.

TD_{50} analysis



TD50 values from spontaneous rodent tumour models

TABLE I.—*Results of Isogeneic Transplantation Assays of 27 Murine Tumours of Spontaneous Origin*

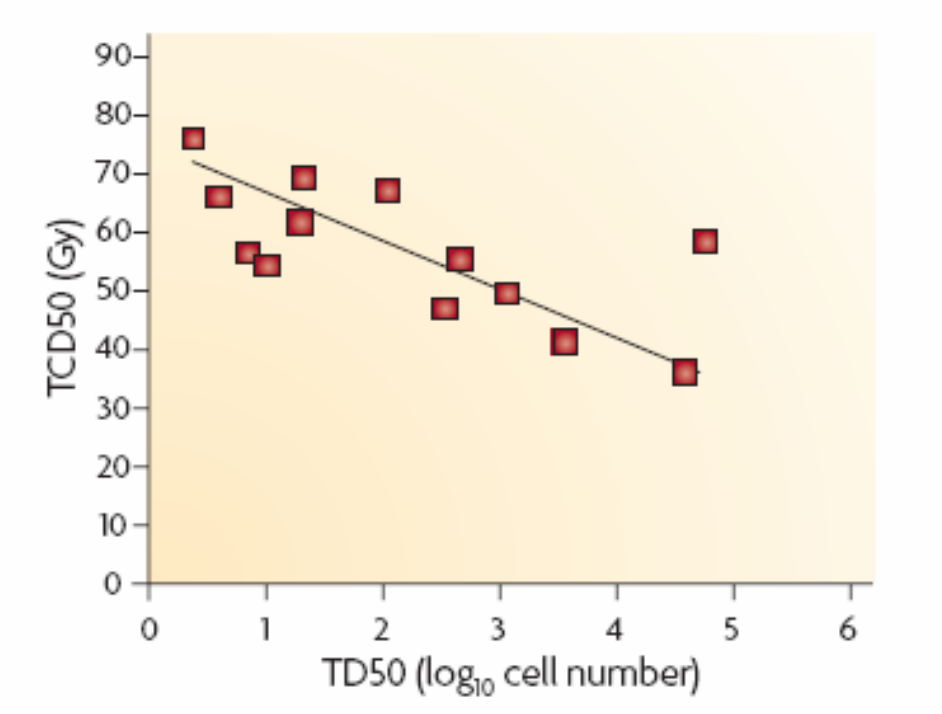
Serial* no.	Mouse strain	Tumour	Route	No. of assays	Serial passage(s)	TD_{50} (cells)†
1	WHT	Reticulum cell sarcoma	I.P.	1	1	1·2
2	CBA	Leukaemia " Th "	I.P.	14	35–231	1·4 (0·7–2·8)
3	WHT	Ascites Leukaemia I	I.P.	4	33–82	1·48 (0·7–3·4)
3	WHT	Ascites Leukaemia I	S.C.	1	144	195
4	CBA	Leukaemia " S1 " I	I.P.	9	76–325	2·0 (0·1–3·4)
5	WHT	Lymphosarcoma	I.P.	1	1	4·0
6	CBA	Leukaemia " Sp " II	I.P.	3	38–114	5·8 (2–16)
7	CBA	Leukaemia " S1 " II	I.P.	1	20	9
8	WHT	Carcinoma " M.T. "	S.C.	5	5–350	10·6 (4·8–23)
9	WHT	Sq. Carcinoma " D "	S.C.	11	14–289	14·4 (9·8–21)
10	WHT	Fibrosarcoma	S.C.	1	123	17
11	WHT	Sarcoma " Ax "	S.C.	3	10–26	25 (5·9–102)
12	WHT	Endothelioma II	S.C.	1	11	26
13	CBA	Sq. Carcinoma I	S.C.	1	61	32
14	CBA	Sarcoma " F "	S.C.	9	35–84	56 (31–103)
14	CBA	Sarcoma " F "	S.C.	12	170–488	263 (119–579)
15	WHT	Sarcoma " Ch "	S.C.	1	4	79
16	CBA	Sarcoma " S "	S.C.	1	51	100
17	WHT	Osteosarcoma I	S.C.	2	164–170	313 (146–671)
18	CBA	Fibrosarcoma	S.C.	2	138–208	416 (280–630)
19	WHT	Sq. Carcinoma " G "	S.C.	4	14–38	1000 (440–2400)
20	WHT	Carcinoma " N–C "	S.C.	1	19	1300
21	WHT	Endothelioma I	S.C.	1	3	1700
22	CBA	Carcinoma " Cr "	S.C.	1	1	1800
23	WHT	Carcinoma " Rh "	S.C.	1	1	2950
24	CBA	Carcinoma " N.T. "	S.C.	21	22–101	3900 (1940–7850)
25	WHT	Adenocarcinoma " NMT "	S.C.	1	9	10,000
26	CBA	Sq. Carcinoma II	S.C.	1	13	>11,000
27	WHT	Osteosarcoma II	S.C.	4	4–62	17,000 (11,000–27,000)

* In later references to the tumours, the serial number will be bracketed after the name.

† The value given for multiple assays is the log mean followed by limits representing ±1 standard deviation.

From Hewitt et al BJC 1976

TD50 vs TCD50 (animal models)

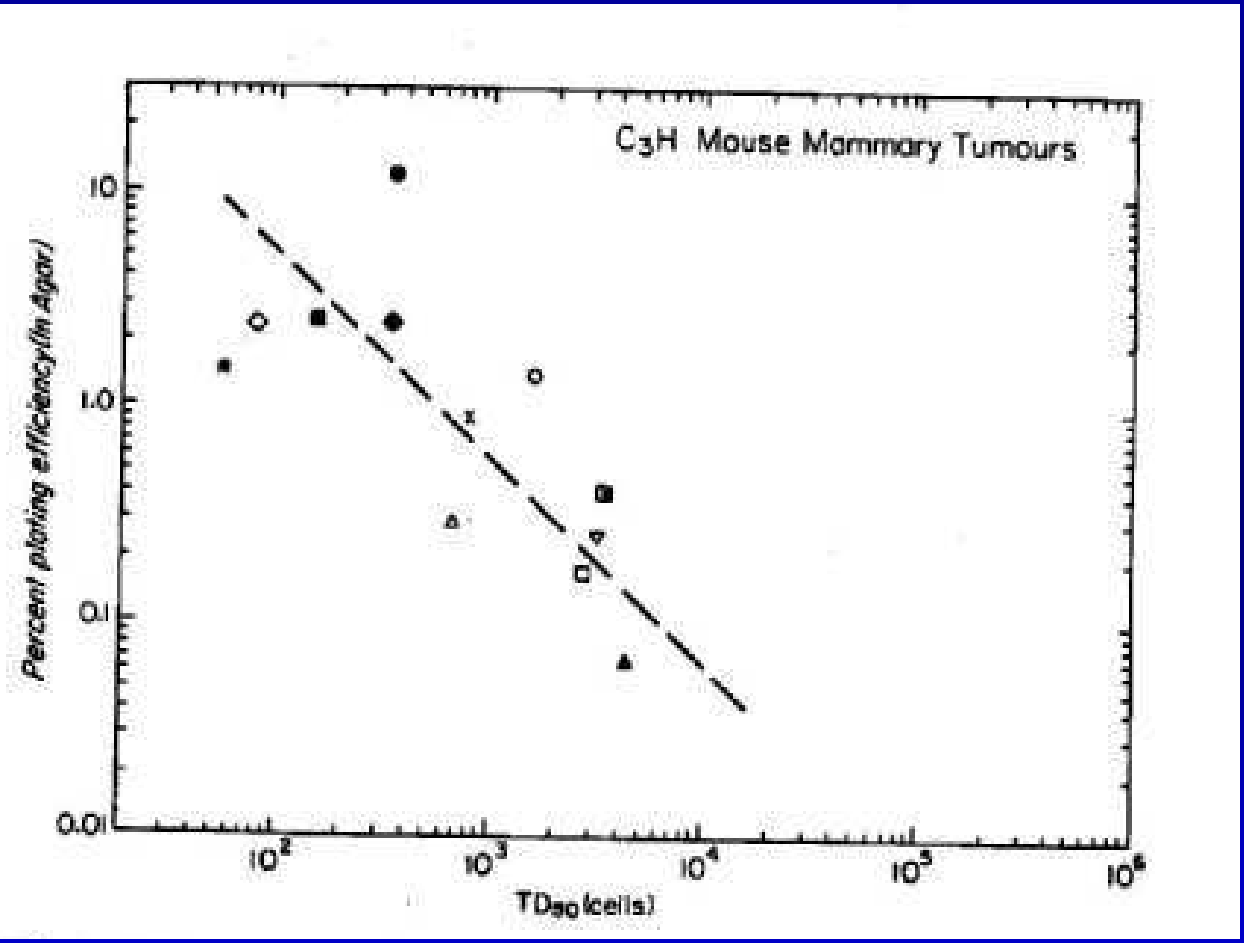


Correlation of transplantability and curability by irradiation of 13 different experimental tumour models.

Transplantability has been evaluated as the number of vital tumour cells that needs to be injected to induce a growing tumour in 50% of the animals (TD50). Curability has been assessed as the irradiation dose that needs to be applied to obtain permanent local control in 50% of the tumours (TCD50). Each data point on this graph represents data derived from ~100 animals.

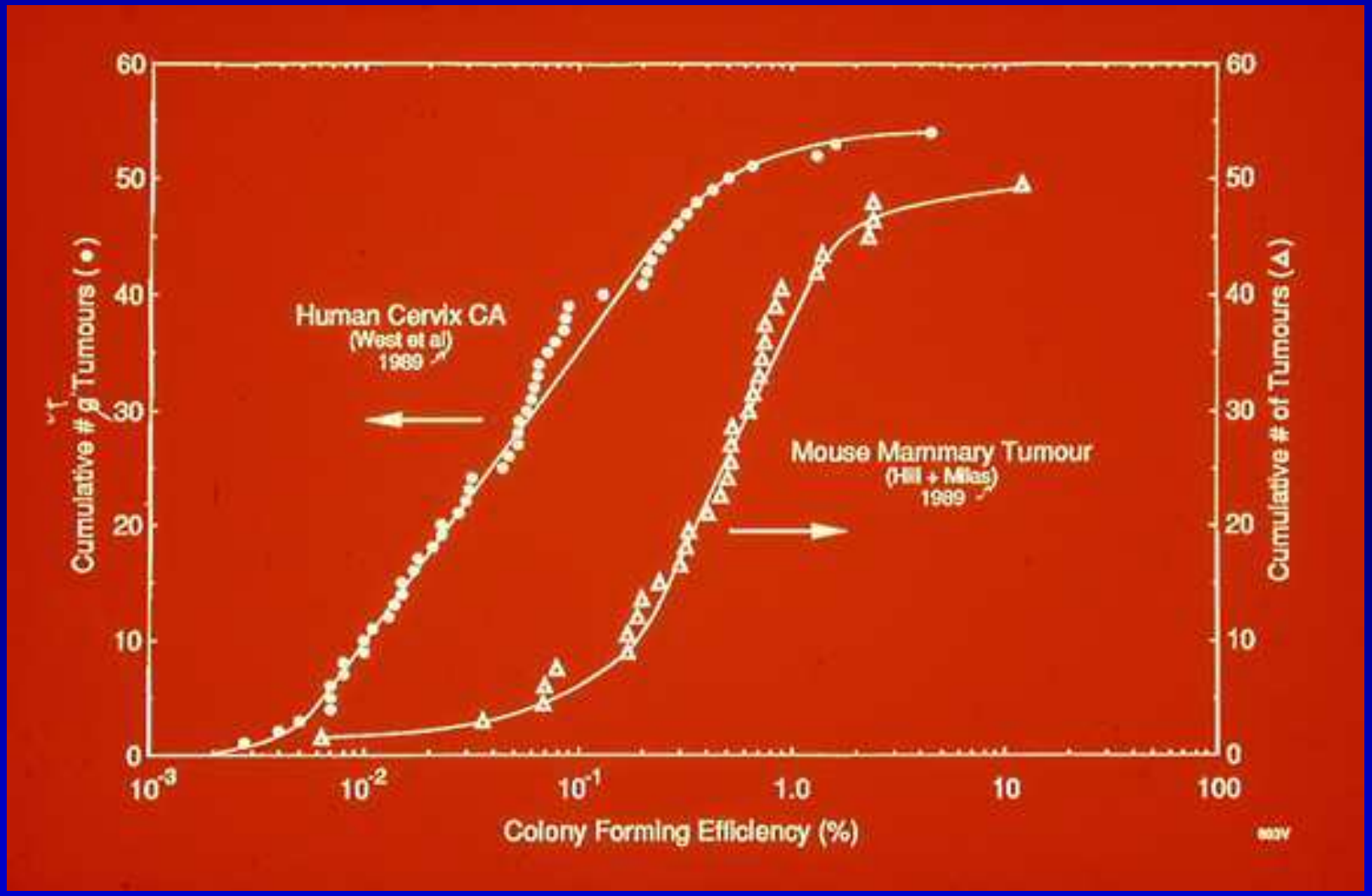
From Hill and Milas 1989

In vivo transplantation efficiency vs in vitro colony formation

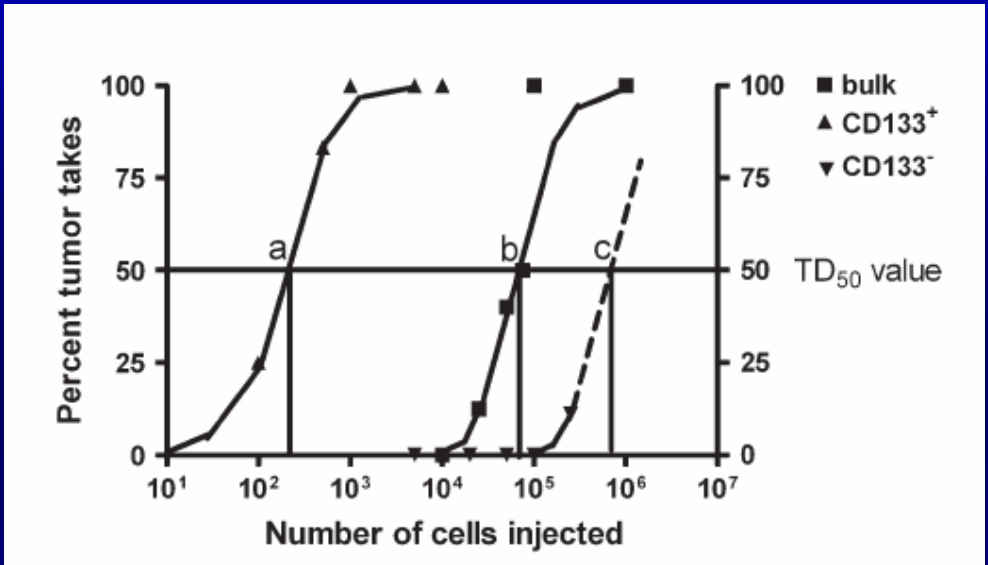


From Hill and Milas 1989

Plating efficiency in vitro cervix Ca



Analysis of colon cancers



Data from O'brien et al 2006

The percentage of injection sites (kidney capsule of immune-deprived mice) in which tumors were established is expressed as percent tumor takes. Values were obtained from 17 primary or metastatic human colon carcinoma cells that were either unsorted (bulk) or sorted by the expression of the surface marker CD133. The numbers of cells necessary for a tumor to form in 50% of the injection sites (TD_{50} value) are as follows:

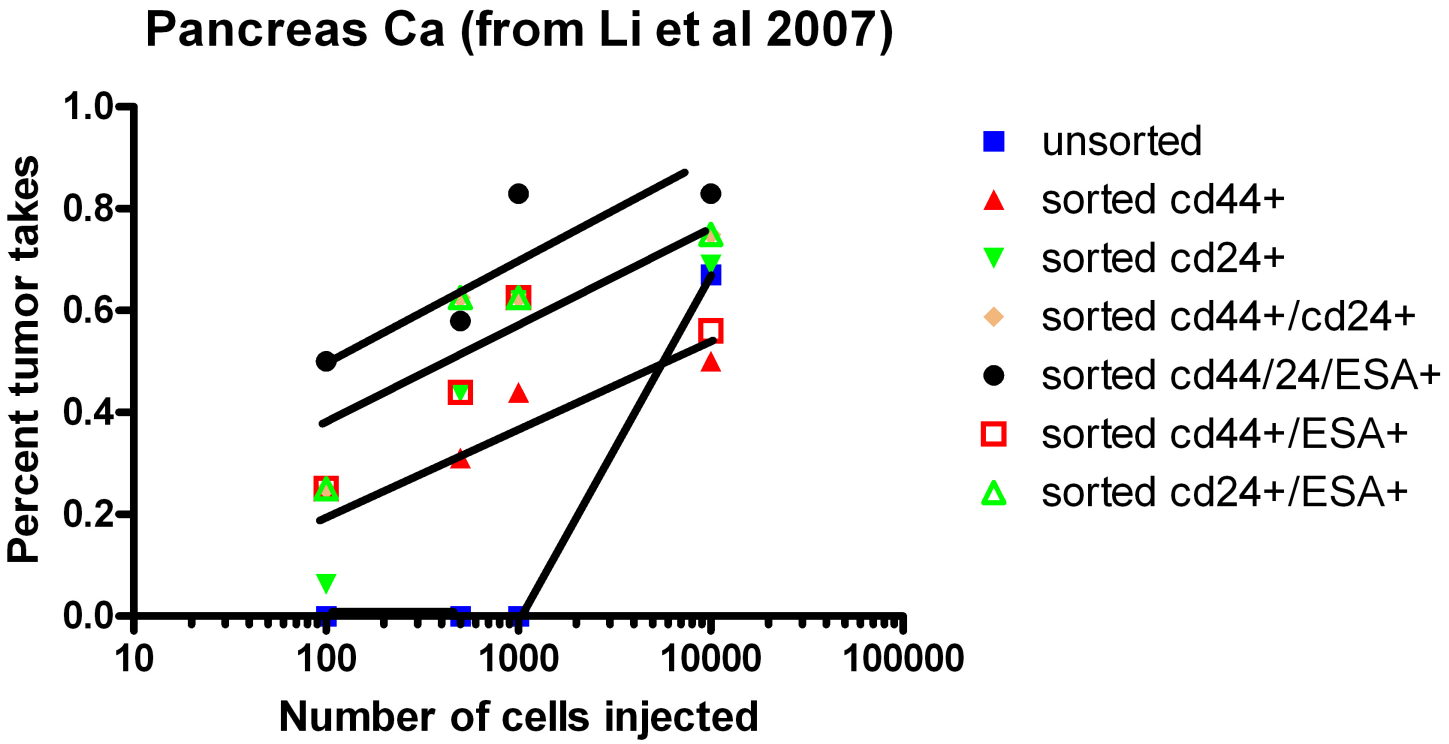
a = approximately 200 cells;
b = approximately 60,000 cells,
c = approximately 700,000 cells.

The mean percentage of CD133-positive cells in the tumors was 11.8% (range = 1.8% – 24.5%).

The numbers problem

Tumor type	Percent of tumor cells expressing CIC markers	No. of cells for tumor take: unsorted	No. of cells for tumor take: sorted cells expressing CIC markers	No. of cells expressing CIC markers in unsorted population	Inoculation site	Ref
Breast	CD44+/CD24+/- 11-35%	$>10^4$	200	$\sim10^3$	Local	(30)
Brain	CD133+ 6-29%	($>10^5$)	100	$\sim10^4$	Local	(31)
Brain	CD133+ (2-3%)	$2\text{-}4x10^4$	500-1000	$\sim10^3$	Local	(23)
Colon	CD133+ 0.7-6.1%	$>10^6$	$<3x10^3$	$\sim2x10^4$	Subcutaneous	(32)
Colon	CD133+ 2-25%	$8x10^4$	200	$\sim9x10^3$	Kidney capsule	(18)
Pancreas	CD133/CD24/ESA+ 0.2-0.8%	$\sim6 \times 10^3$	100	~30	Pancreatic tail	(24)

Data from Pancreas Ca



The numbers problem (2)

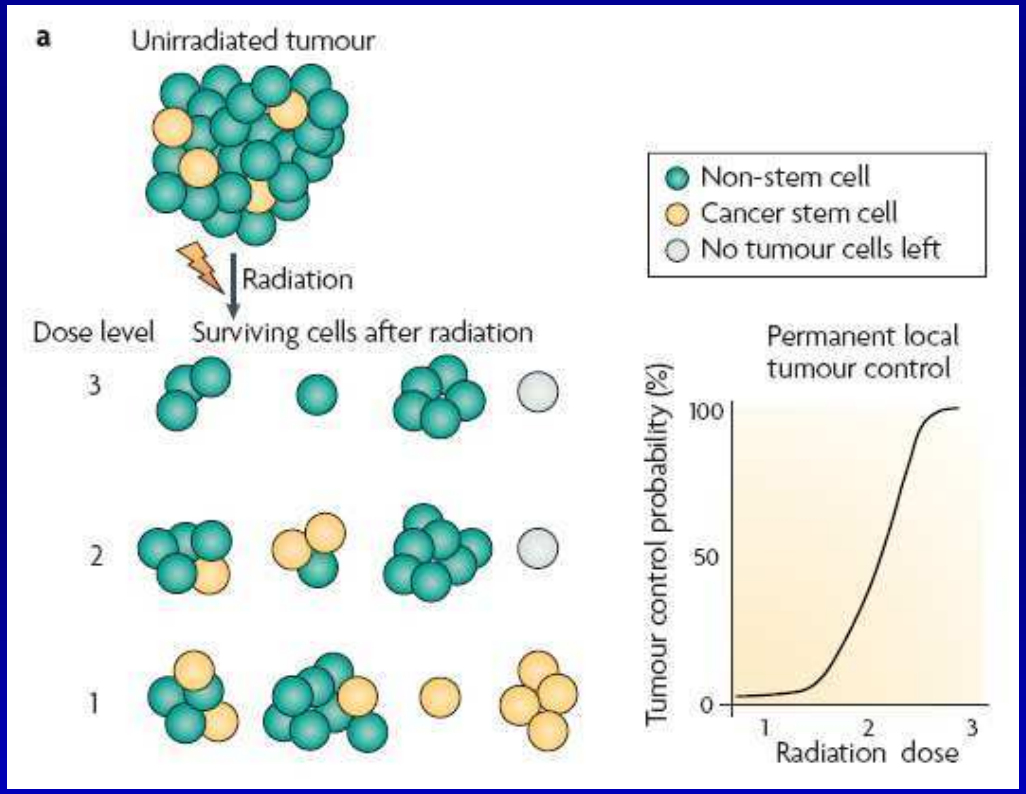
- The numbers problem leaves us with the conclusion that the markers currently used to sort putative cancer stem cells are NOT specific for stem cells. i.e. the sorting only enriched for CSC but did not produce a pure population.

- An alternative explanation may be that stem cell growth is controlled (limited) by large numbers of non-stem cells (this seems unlikely based on the animal data using LI cells)

Difficulties inherent in designing trials for new therapies based upon the "cancer stem cell concept".

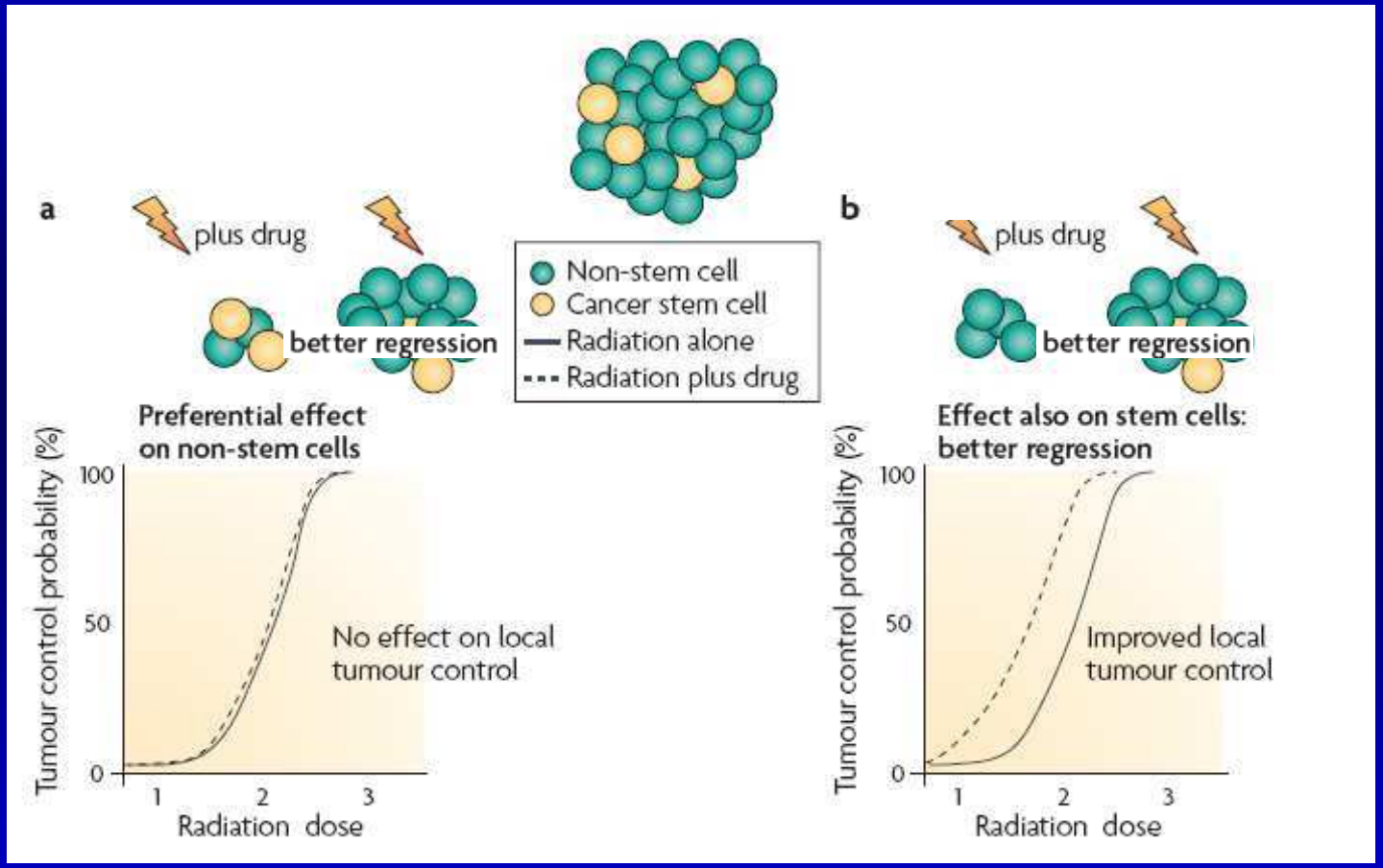
- The efficacy of targeting a small fraction of "stem cells" in a tumor is unlikely to be revealed by the current (short-term) approach of testing new drugs for their ability to cause tumor regression.
- Longer term tumor control or survival measures using combination treatments will likely be required.
- Similarly, the possibility of long term toxicities associated with depletion of normal tissue stem cell pools needs to be considered.
- The importance of depletion of the stem cell pool is well recognised for "renewal tissues" such as the bone marrow but our knowledge of the importance of the stem cell pool in maintenance of "non-renewal" tissues is currently very limited.

Cancer stem cell survival for local tumour control after irradiation.



Tumours are irradiated using different dose levels. In this example, in all tumours at least one cancer stem cell survives at dose level 1. With increasing dose, more and more cancer stem cells are inactivated, leading to half of the tumours being completely sterilized at dose level 2 and all tumours being sterilized at level 3.

Potential disassociation of endpoints after treatment with radiation with drugs.



In this example the drug was selected on the basis of its effect on tumour regression after irradiation.

Tumour regrowth from small stem cell numbers

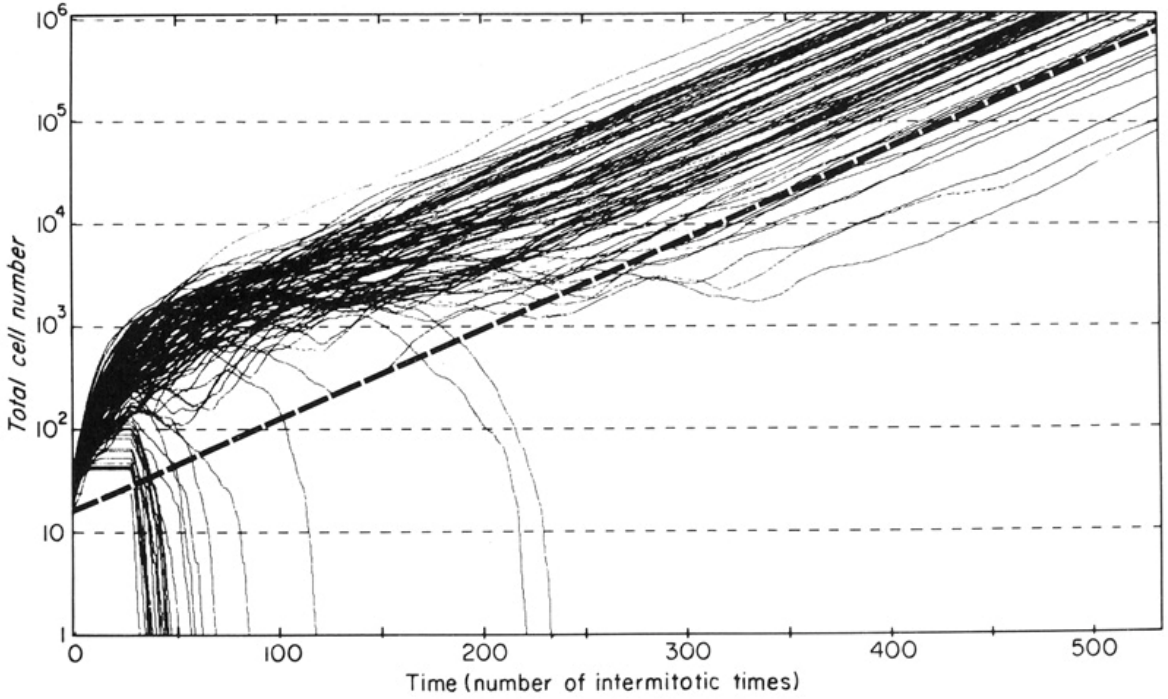


Figure 20 Computer simulation of the growth of 100 different tumours from 15 clonogenic cells where each cell has a probability of 0.51 that two clonogenic cells result from cellular division, and 0.49 that two sterile cells are produced. See text for discussion.

Three subpopulations

1) Cancer stem cells
2) Non stem cells which may undergo up to 10 divisions before becoming end cells.
3) End cells which cannot proliferate but can survive up to 10 intermitotic times in the tumour before being lost.

From Bush, de Boer , Hill

Prolonged Arrest of Cancer Ed B.A. Stoll Pub Wiley and Sons 1982

Tumour regrowth from small stem cell numbers (Caveats)

- It is assumed that the probability of producing new stem cells is fixed for all stem cells.
  - In particular that it is not affected by the tumour microenvironment or treatment.
- Stem cells are assumed to be proliferating at the same rate as the non-stem cells.
  - If cancer stem cells are similar to normal stem cells this may be incorrect.

Questions and Answers (1):

- Do such cells exist in tumours? **YES**, must do so if tumour is to continue growing. Thus the critical issue is proportions and stability of phenotype.
- How many such cells exist in tumours? **Assumed to be a small minority** (<1% from animal studies) but this is not well established in human tumours. Initial sorting studies give quite large percentages but markers are probably not specific. **They enrich but do not purify the CSC.**
- Current sorting and transplantation assays are problematic: **in most studies they have not been optimized**.
- Is the molecular phenotype that allows a tumor cell to manifest as a cancer stem cell on transplantation consistent? **Possibly, multiple transplantation using same markers can work but it may vary depending on the procedure adopted for testing stemness**.

Questions and Answers (2):

- Are all cancer stem cells the same and do they retain the same features throughout tumour growth. **Unknown but I think it is unlikely, i.e such cells are likely to change properties as the tumour progresses and as a result of genetic instability.** (~10^3 genetic or epigenetic changes per day.)

- What is the role of the ECM/tumor microenvironment. Certain environments/niches (e.g hypoxia) may enhance stem cell properties (or expression of surface markers – eg CD133).

- Can we grow cancer stem cells in culture. **Yes most successful way to maintain phenotype is by growing them as spheroids - but**

Neurospheres

- Neurosphere cells are most accurately described as cultures of neural precursors.
- The term “precursor” refers to a mix of stem and progenitor cells.
- Progenitors are distinct from stem cells because they have limited self-renewal and an increased proliferative ability.

From Dirks JCO 2008

Early stem cell modelling for solid tumours in Toronto

- Mackillop WJ, Ciampi A, Till JE, Buick RN.
  A stem cell model of human tumor growth: implications for tumor cell clonogenic assays. J Natl Cancer Inst. 1983 Jan;70(1):9-16.

- Ciampi A, Kates L, Buick R, Kriukov Y, Till JE.
  Multi-type Galton-Watson process as a model for proliferating human tumour cell populations derived from stem cells: estimation of stem cell self-renewal probabilities in human ovarian carcinomas. Cell Tissue Kinet. 1986 Mar;19(2):129-40.

Thank you

Matrigel and Tumorigenicity

TABLE I – TAKE RATE OF BREAST AND OVARIAN CANCER CELL LINES INOCULATED IN THE PRESENCE/ABSENCE OF MATRIGEL

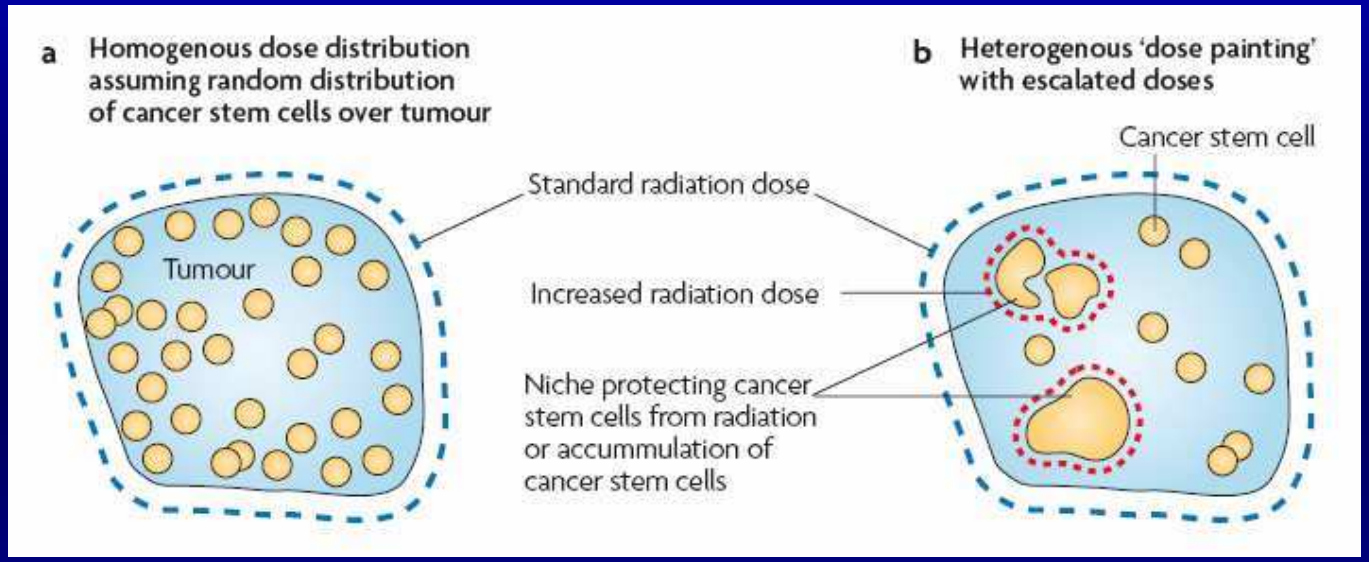
Cell line	Total experience		Paired studies	
	Take rate – Matrigel	Take rate + Matrigel	Take rate – Matrigel	Take rate + Matrigel
MCF-7	1/14 (7.1%)	25/25 (100%)	1/10 (10%)	10/10 (100%)
T47D	1/10 (10%)	25/25 (100%)	1/10 (10%)	10/10 (100%)
MDA.MB.231	6/12 (50%)	12/12 (100%)	6/12 (50%)	12/12 (100%)
PEO1	0/17 (0%)	10/10 (100%)	0/6 (0%)	6/6 (100%)
PEO1 cDDPr	0/11 (0%)	10/10 (100%)	0/6 (0%)	6/6 (100%)
PEO4	0/6 (0%)	6/6 (100%)	0/6 (0%)	6/6 (100%)
PEO14	3/10 (30%)	10/10 (100%)	3/6 (50%)	6/6 (100%)
OV(hyg)CAR3	0/6 (0%)	2/6 (33%)	0/6 (0%)	2/6 (33%)

4×10^6 cells injected

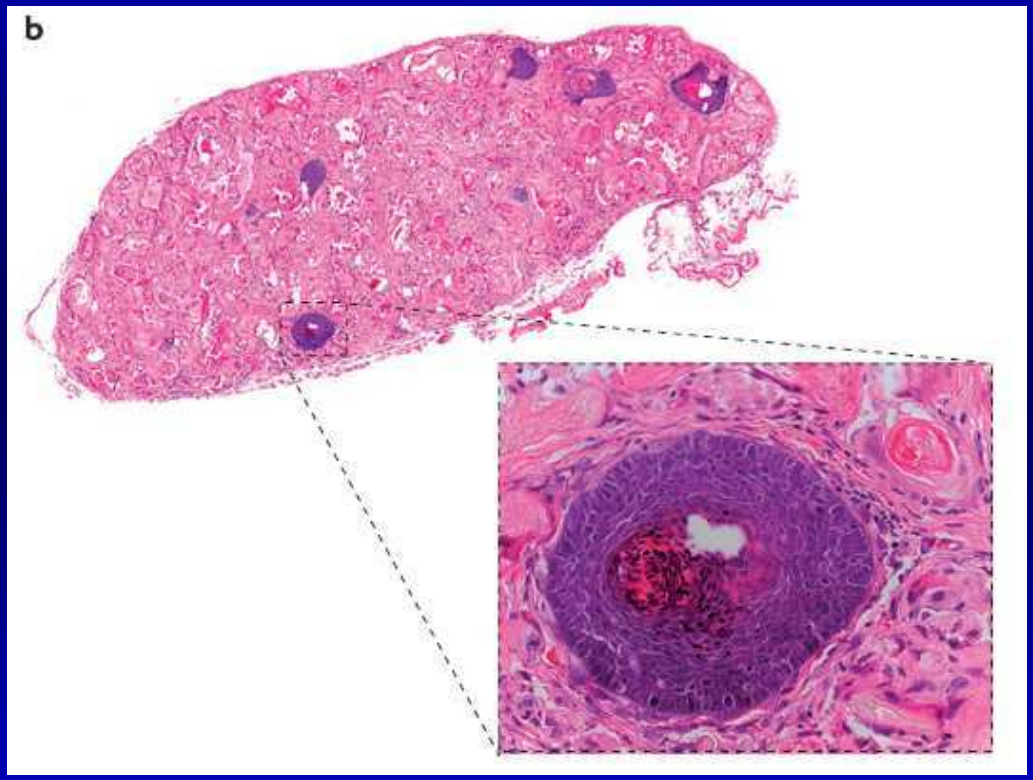
(From Mullen et al 1996)

We studied the development of human breast carcinoma xenografts in athymic mice with and without coinjection of Matrigel. Tumors developed in only 7.3% of enzyme-dispersed tumors injected subcutaneously in saline solution alone. None of these tumors metastasized to distant sites. On the other hand, 50% of enzyme-dispersed tumors coinjected with Matrigel developed xenografts; four out of five of these tumors metastasized to distant sites.

(From Mehta et al 1993)



The potential importance of stem cell niches for radiotherapy treatment planning. a | Standard treatment plans would deliver the irradiation dose with a safety margin homogeneously over the tumour. This is optimal if cancer stem cells are distributed randomly over the tumour. **b** | If niches exist where cancer stem cells accumulate or where they are protected from the effects of radiation, delivery of higher radiation doses to the niche is expected to improve local tumour control.



A haematoxylin–eosin-stained section of the AT17/7 tumour. The tumour has been irradiated *in vivo* with a single dose of 32 Gy under homogeneous hypoxia and excised 17 days later. As a unique feature of this model, radiation-killed tumour cells are rapidly cleared from this tumour, leaving within 7–9 days a tumour mass that, after high doses, is
largely depleted of parenchymal cells. Surviving cancer stem cells expand rapidly in number and within 2–3 weeks form distinct multicellular colonies (insert) that can easily be counted by histology. The stemness of the colony forming cells has been demonstrated by correlation with permanent local tumour control. With increasing irradiation dose, permanent local tumour control increases and the number of colonies decreases.

The Promise and the Problem

- Investigations of cancer stem cells offer the possibility of generating novel targets that could overcome issues of resistance, improve therapeutic efficacy and make cancer treatment more successful and perhaps even curative, while obviating systemic toxicity. (from Clarke et al Can Res 66, 9339-9344, 2006)

BUT

- There are many unanswered questions about cancer stem cells and about the stability of their phenotype which need to be addressed before such a promise can be realistically assessed.

Stem cell issues

- Are cancer–initiating cells (CIC) true stem cells?
  - Can tumors arise from progenitor cells?
  - How many such cells exist in tumours?
  - How heterogeneous is this number?
- Are there specific surface (or other) markers that can be used to identify such cells?
  - How do these markers relate to markers on normal tissue stem cells?
  - Do cancer stem cells retain the same molecular phenotype (markers) during tumour growth and progression OR are they a moving target ?

    For example
  - Hypoxic exposure has also been reported recently has been reported to maintain expression of stem/precursor cell genes in preadipocytes[(13)] and to upregulate expression of CD133, a putative stem cell marker, in a medulloblastoma cell line

TD_{50} assays: effect of transplant conditions

- TD_{50} values can be influenced by where the cells are transplanted.
  - Inflammatory site
  - Irradiated site
  - Kidney capsule
- Decreased TD_{50} values are observed in the presence of:
  - Lethally irradiated tumour cells
  - Brain extract
  - Matrigel
  - Tumour-associated fibroblast (human vs mouse)

Outstanding issues