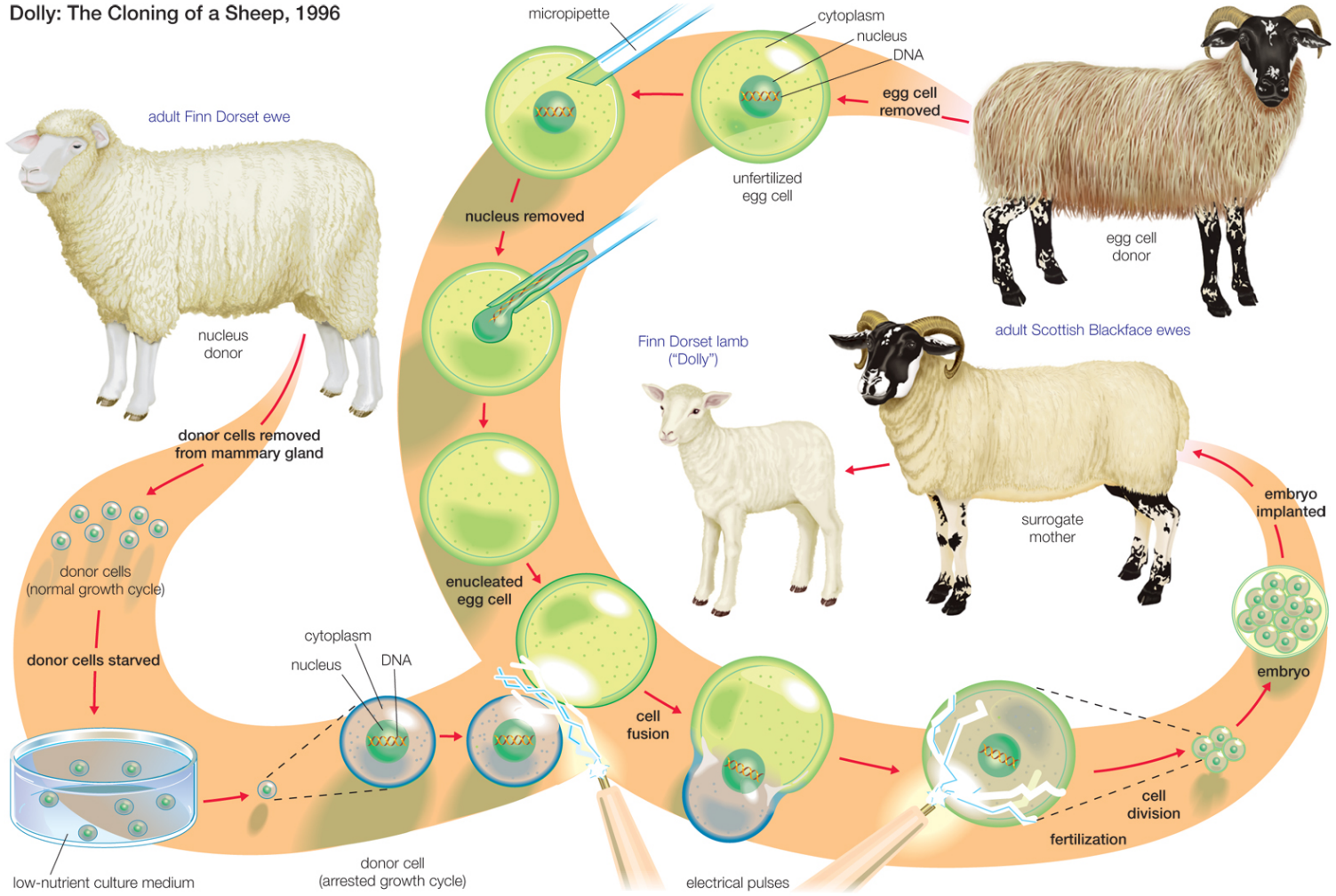
Oncology 520
Cancer Stem Cells
Lecturer: Michael J Hendzel, Ph.D.

Cancer Stem Cells

- What is a cancer stem cell? Is it a distinct entity or a potential state arising from epigenetic plasticity?
- What is the cell of origin and how does this relate to the cancer stem cell hypothesis?
- What are alternative explanations for tumour heterogeneity?

If every cell has the same DNA, do all cells have the same potential and if they have the same potential, do they have the same fate?

Differentiation is a Reversible Process Regulated Epigenetically



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Induced Pluripotent Stem Cells

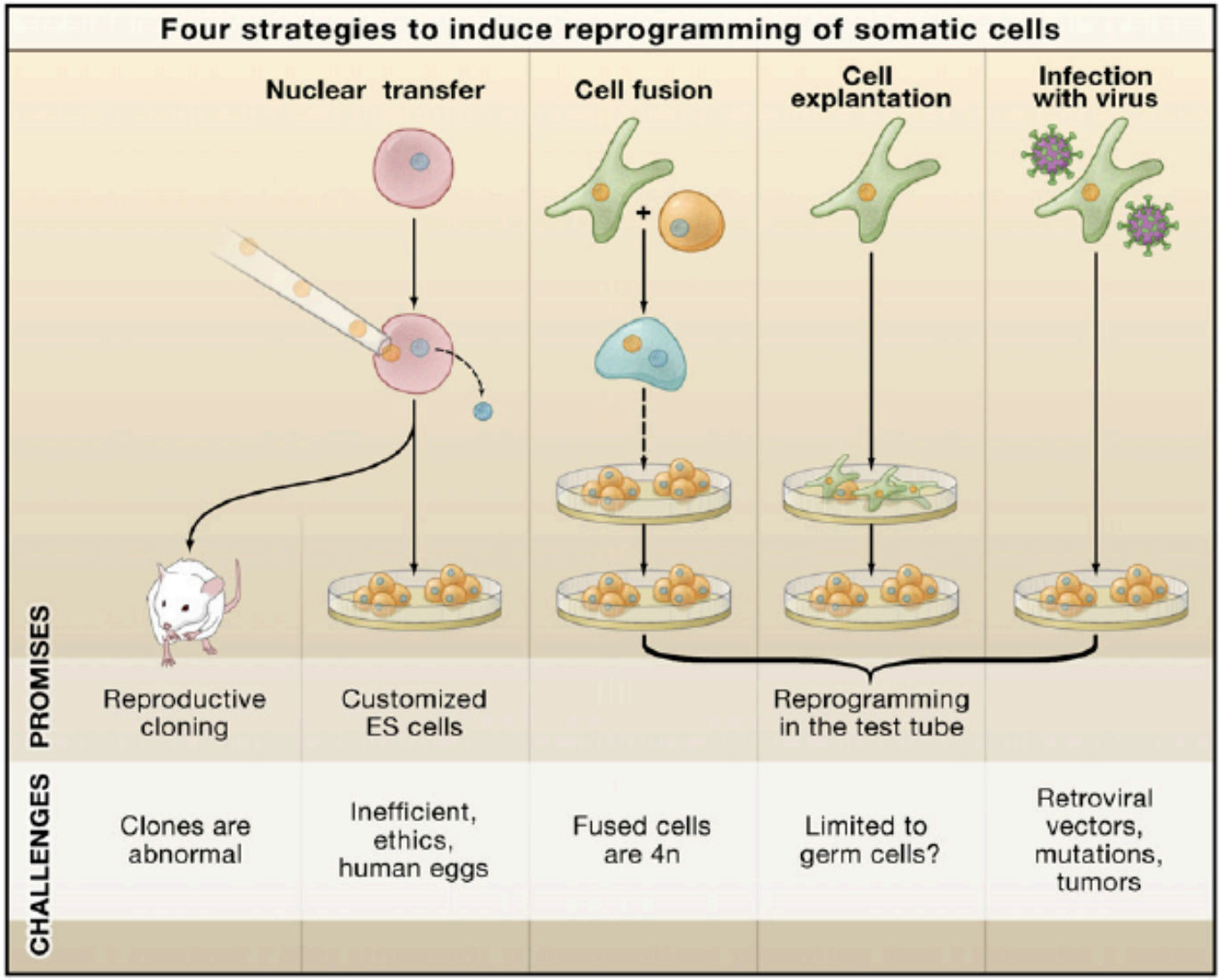

Figure 1. Four Strategies to Induce Reprogramming of Somatic Cells

(1) Nuclear transfer involves the injection of a somatic nucleus into an enucleated oocyte, which, upon transfer into a surrogate mother, can give rise to a clone ("reproductive cloning"), or, upon explanation in culture, can give rise to genetically matched embryonic stem (ES) cells ("somatic cell nuclear transfer," SCNT). (2) Cell fusion of somatic cells with ES cells results in the generation of hybrids that show all features of pluripotent ES cells. (3) Explantation of somatic cells in culture selects for immortal cell lines that may be pluripotent or multipotent. At present, spermatogonial stem cells are the only source of pluripotent cells that can be derived from postnatal animals. (4) Transduction of somatic cells with defined factors can initiate reprogramming to a pluripotent state.

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Stochastic Epigenetic Events in Cellular Reprogramming

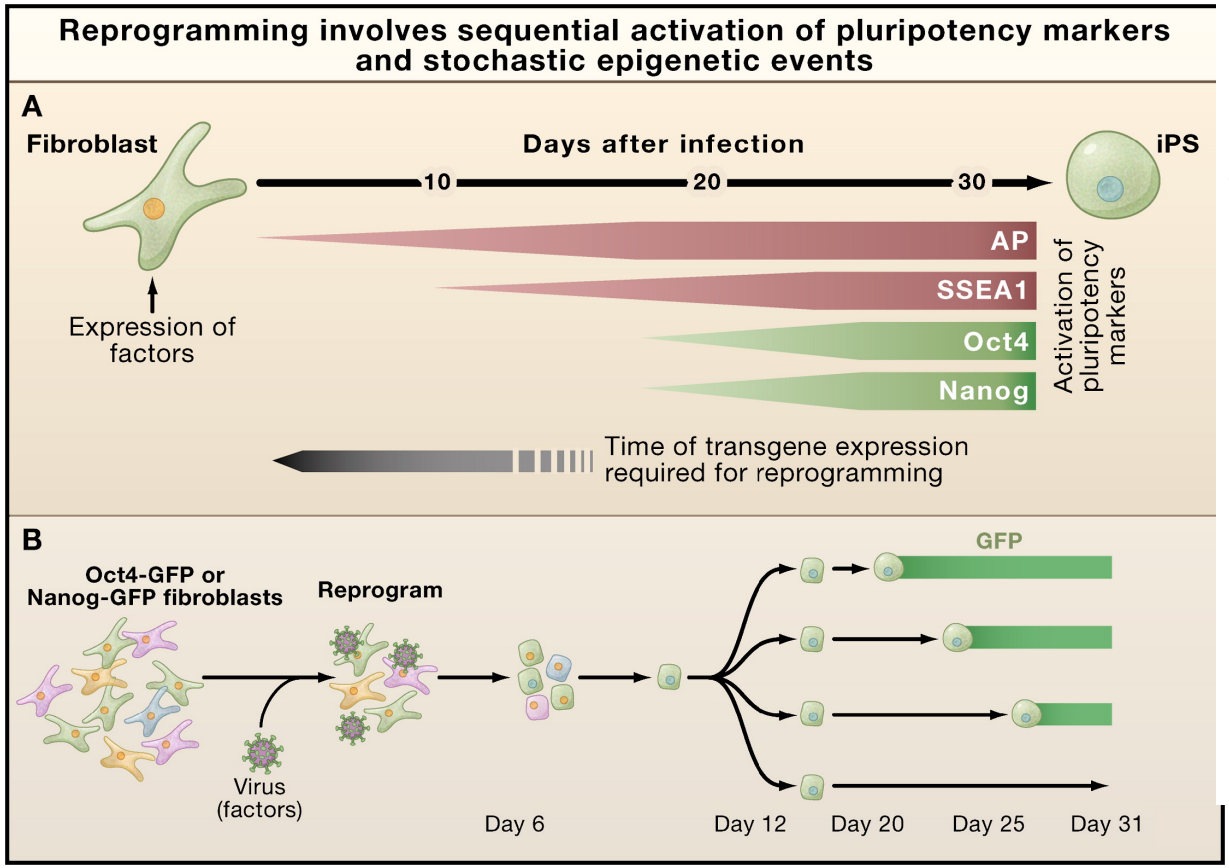

Figure 3. Reprogramming Involves Sequential Activation of Pluripotency Markers and Stochastic Epigenetic Events

(A) Kinetics of pluripotency-marker appearance. Alkaline phosphatase (AP) and SSEA1 positive cells are already detected 3 and 9 days, respectively, after factor transduction, whereas GFP expressed from the endogenous Oct4 or Nanog loci first appear only after 2 weeks. The virally transduced factors need to be expressed for about 2 weeks to initiate the reprogramming process (Brambrink et al., 2008).

(B) Oct4-GFP or Nanog-GFP fibroblasts were transduced with the four factors. Colonies displaying a transformed phenotype were GFP negative and were cloned a few days after infection. Further cloning yielded subclones that activated GFP at different times (Meissner et al., 2007). Because the subclones were derived from the same infected cell, stochastic epigenetic events must be important for reprogramming.

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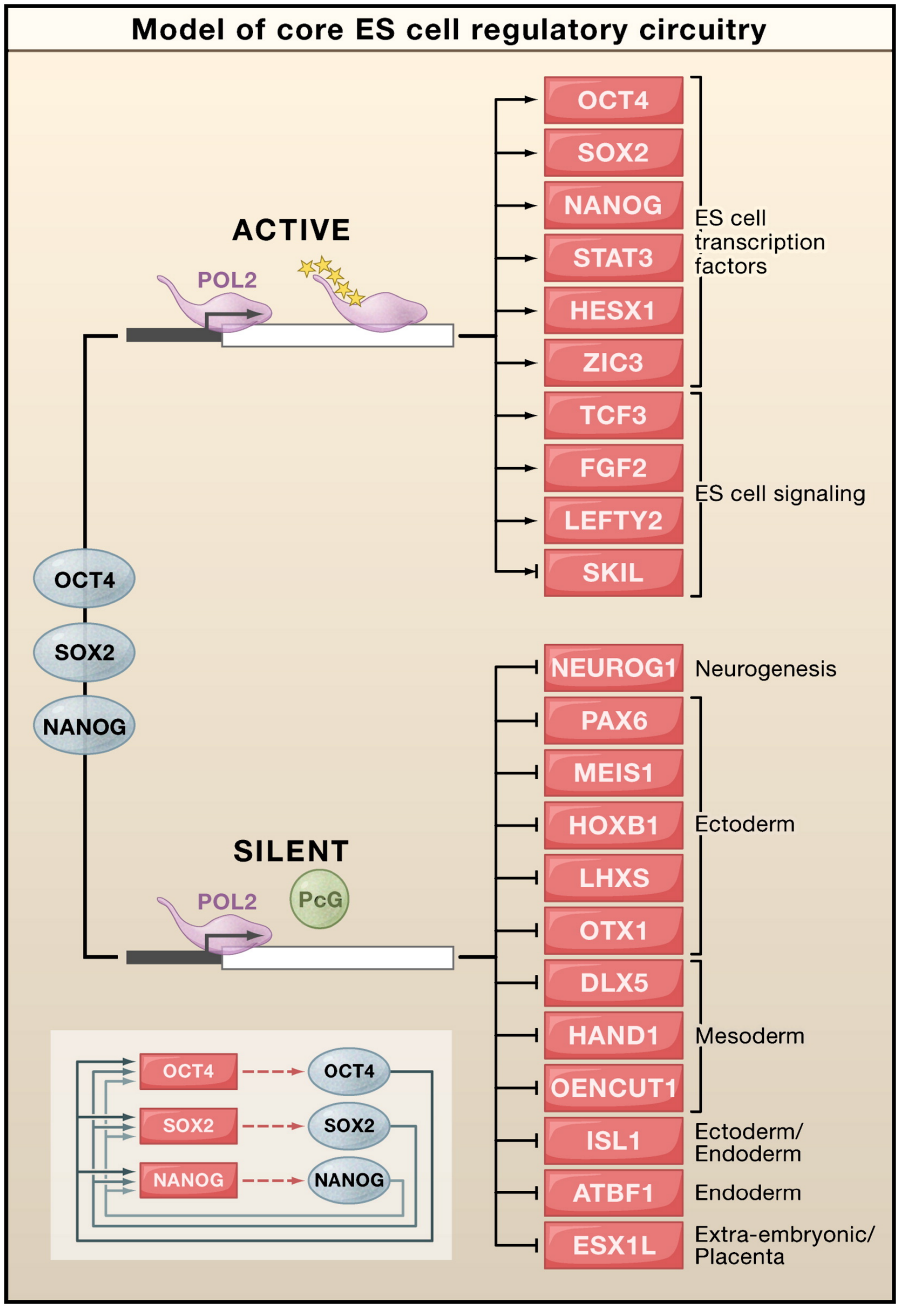

Figure 5. Model of Core ES Cell Regulatory Circuitry

The Oct4, Sox2, and Nanog transcription factors (blue) occupy actively transcribed genes, including transcription factors and signaling components necessary to maintain the ES cell state. The three regulators also occupy silent genes encoding transcription factors that, if expressed, would promote other more differentiated cell states. At this latter set of genes, RNA polymerase II (POL2) initiates transcription but does not produce complete transcripts due to the repressive action of PcG proteins. The PcG proteins prevent RNA polymerase from transitioning into a fully modified transcription elongation apparatus (represented by phosphorylated "stars" on the tail of the POL2 enzyme). The interconnected autoregulatory loop, where Oct4, Nanog, and Sox2 bind together at each of their own promoters, is shown (bottom left).

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Conclusion

Differentiation is regulated through epigenetic mechanisms and, due to the reversible nature of epigenetic changes, it is possible to reverse the differentiation process and restore a pluripotent state.

Epigenetic processes can contribute to the generation of human cancers by inactivating tumor suppressor genes and activating growth promoting genes.

Cancer Therapy

- Historically has targeted proliferation based on the assumption that cancer cells are engaged in uncontrolled proliferation.
- Assumes that a sensitive cancer is inherently sensitive in a homogeneous manner unless/ until resistance develops. This is true even with molecularly targeted therapies (e.g., Herceptin).

Historical Observations

- Solid and "liquid" cancers exhibit significant heterogeneity
- Although the first tumor grafts were generated by transplantation of a single tumour cell (mouse leukemia) (Am. J. Cancer, 1937, 31:276-282), subsequent studies showed that transplantation was often inefficient, requiring up to thousands of cells
- Cell proliferation studies in the 40s-70s revealed that only a small fraction of cells were proliferative and that there were two subpopulations of proliferative cells: one that cycled rapidly and was responsible for generating the bulk of the cells and one that cycled very slowly. Based on the similarity with hematopoietic stem cells, the slow cycling cells were proposed to be cancer stem cells (Clarkson, 1974. Control of Proliferation in Animal Cells. New York, NY: Cold Spring Harbor Laboratory; 1974 p. 945-972).
- Observed that Leukemic Stem Cells enter the cell cycle in response to cellular depletion of the tumor during treatment.
- Focus shifted to the proliferative fraction and AML-CFU cells were isolated and these were morphologically distinct from the bulk of the colony that they formed.
- Till and McCulloch (1961) (Radiation Research 14:213-222) demonstrated the existence of a single hematopoietic stem cell that was capable of reconstituting all hematopoietic lineages. This contrasted with the view at the time that there were separate stem cells for each lineage.
- The development of cells surface antigens and fluorescence activate cell sorting (FACS) to separate cells with different surface antigens was critical in establishing lineages in hematopoiesis.

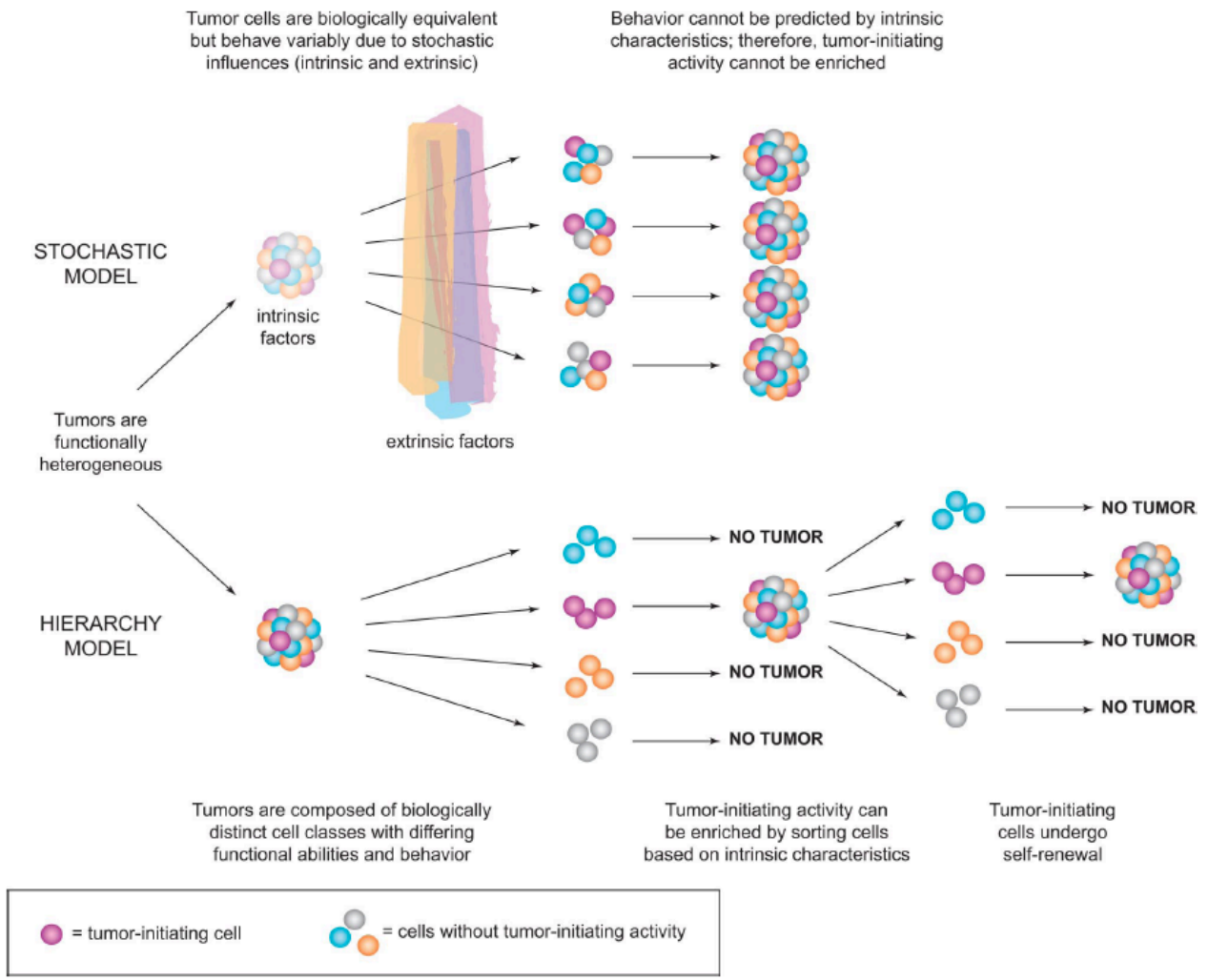

Figure 2. Models of tumor heterogeneity. Tumors are composed of phenotypically and functionally heterogeneous cells. There are 2 theories as to how this heterogeneity arises. According to the stochastic model, tumor cells are biologically equivalent, but their behavior is influenced by intrinsic and extrinsic factors and is therefore both variable and unpredictable. Thus, tumor-initiating activity cannot be enriched by sorting cells based on intrinsic characteristics. In contrast, the hierarchy model postulates the existence of biologically distinct classes of cells with differing functional abilities and behavior. Only a subset of cells can initiate tumor growth; these cancer stem cells possess self-renewal and give rise to nontumorigenic progeny that make up the bulk of the tumor. This model predicts that tumor-initiating cells can be identified and purified from the bulk nontumorigenic population based on intrinsic characteristics.

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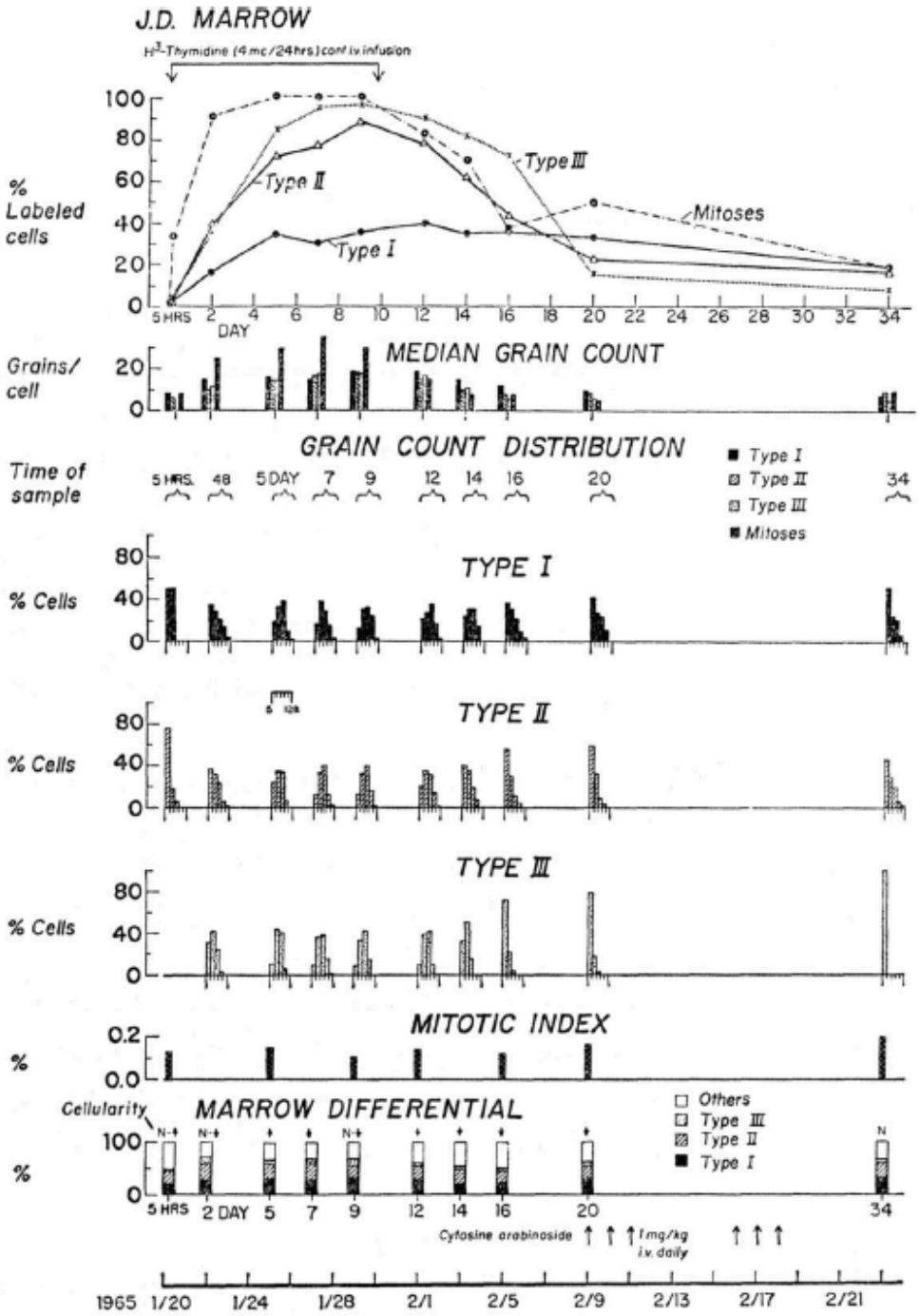
Basis of heterogeneity-Assumptions of Classical Models

- Intrinsic and extrinsic but largely stochastic events are responsible for creating heterogeneity
- Stem cell population that is biologically distinct and maintains itself through a self-renewal process. The distinct functional and phenotypic differences between cells is thought to be primarily epigenetic in nature. The Stem Cell Hypothesis, then, mirrors aspects of normal development.

Figure 1. Labeling pattern of leukemic cells in marrow of patient 1. Patient 1, a patient with acute myelomonocytic leukemia, received a continuous 10-day infusion of tritiated thymidine. Leukemic cells were arbitrarily divided into types I, II, and III based on increasing levels of morphologic maturity (type I indicates primitive blast forms; type III, most differentiated cells). At the end of the 10-day infusion, most type II and type III cells were labeled in both marrow (shown here) and blood (not shown), but only 40% of type I cells were labeled, reflecting their slow proliferative rate. Many of the type I cells remained highly labeled for over 3 weeks after the infusion. Reprinted from Clarkson[17] by permission.



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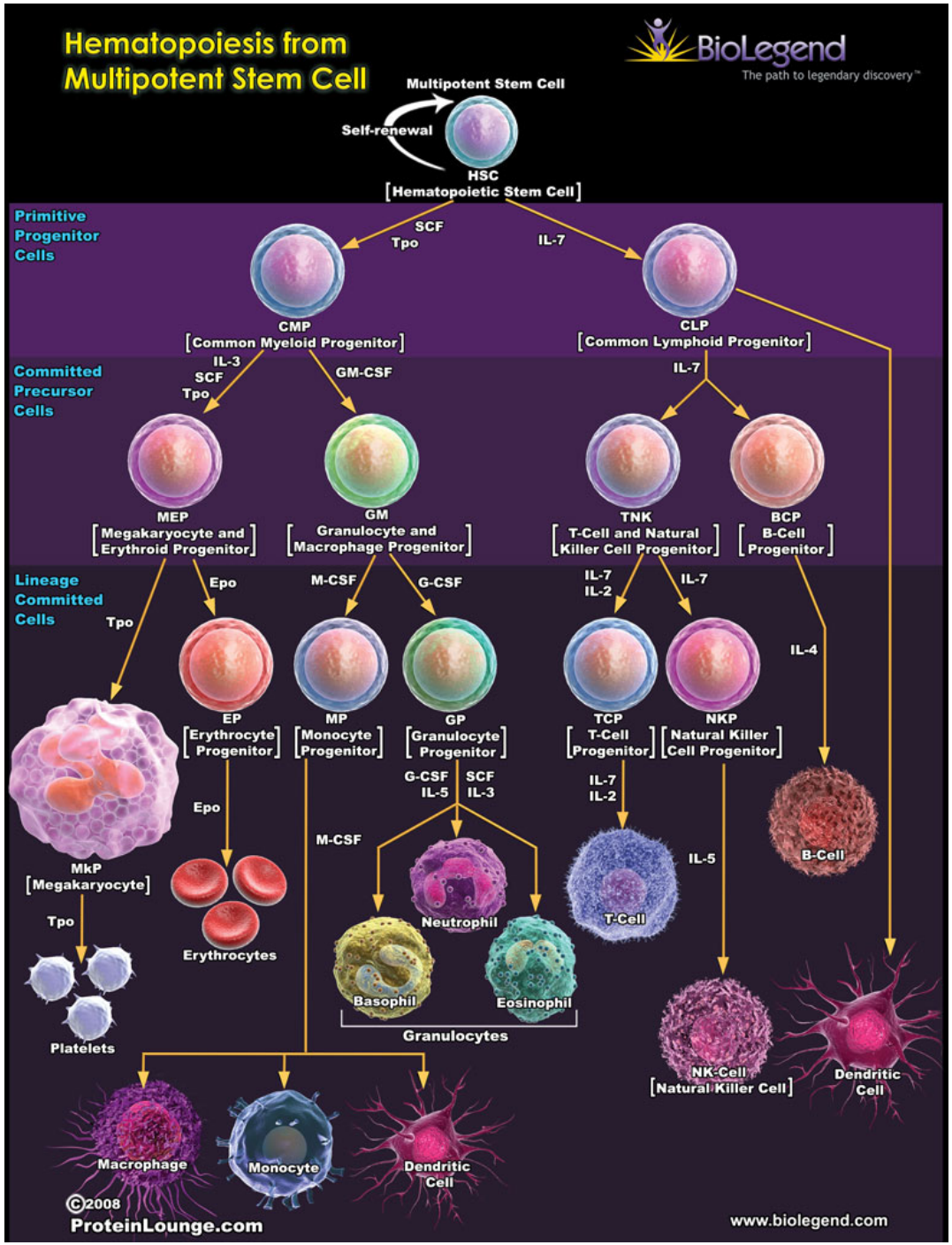
Hematopoiesis from Multipotent Stem Cell
BioLegend
The path to legendary discovery™
Multipotent Stem Cell
Self-renewal
HSC
[Hematopoietic Stem Cell]
Primitive Progenitor Cells
SCF
Tpo
IL-7
CMP
[Common Myeloid Progenitor]
CLP
[Common Lymphoid Progenitor]
Committed Precursor Cells
IL-3
SCF
Tpo
GM-CSF
IL-7
MEP
[Megakaryocyte and Erythroid Progenitor]
GM
[Granulocyte and Macrophage Progenitor]
TNK
[T-Cell and Natural Killer Cell Progenitor]
BCP
[B-Cell Progenitor]
Lineage Committed Cells
Tpo
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M-CSF
G-CSF
IL-7
IL-2
IL-7
IL-4
EP
[Erythrocyte Progenitor]
MP
[Monocyte Progenitor]
GP
[Granulocyte Progenitor]
TCP
[T-Cell Progenitor]
NKP
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G-CSF
IL-5
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Neutrophil
T-Cell
B-Cell
Basophil
Eosinophil
Granulocytes
Platelets
NK-Cell
[Natural Killer Cell]
Dendritic Cell
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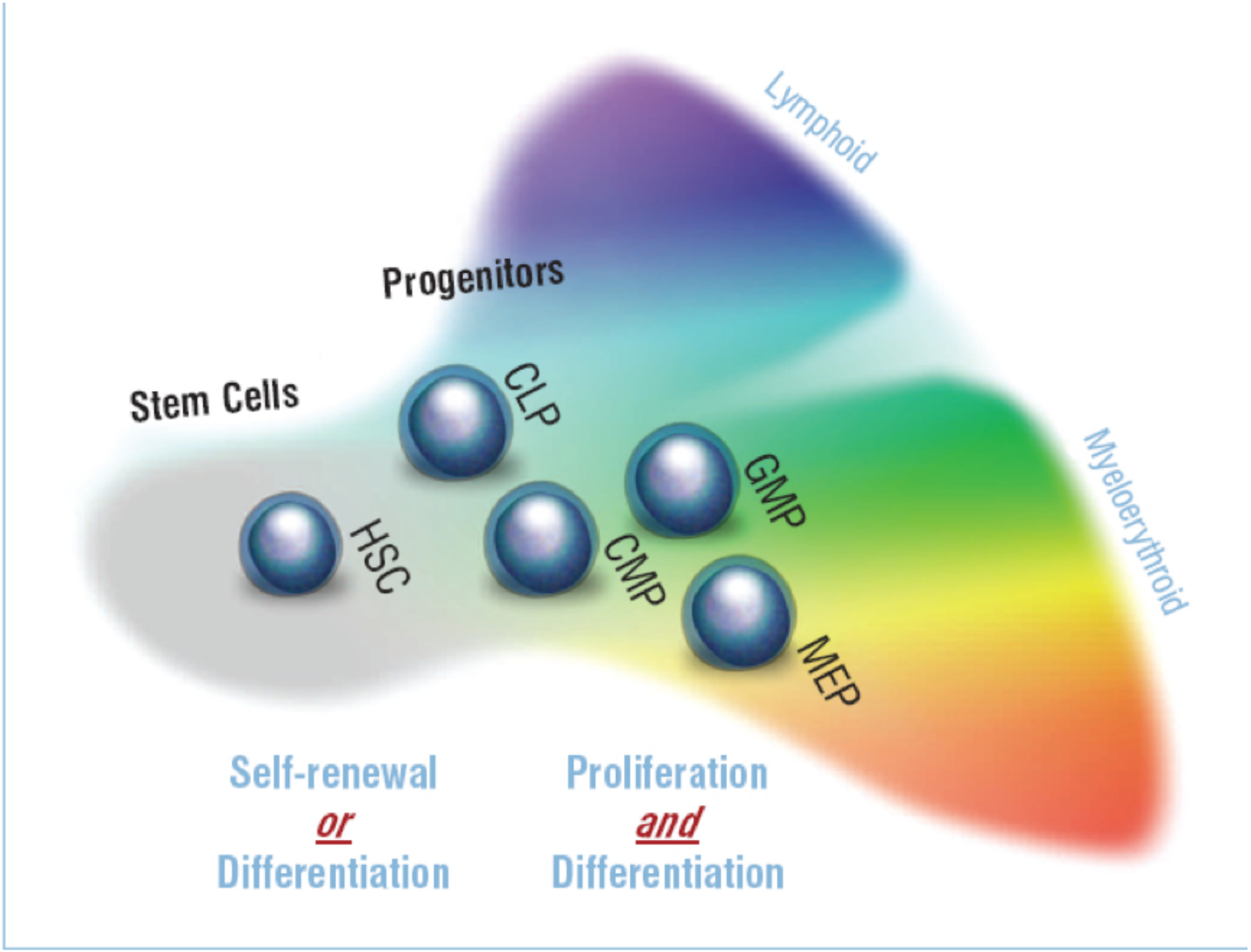

Figure 2.5. Relationship between several of the characterized hematopoietic stem cells and early progenitor cells. Differentiation is indicated by colors; the more intense the color, the more mature the cells. Surface marker distinctions are subtle between these early cell populations, yet they have clearly distinct potentials. Stem cells can choose between self-renewal and differentiation. Progenitors can expand temporarily but always continue to differentiate (other than in certain leukemias). The mature lymphoid (T-cells, B-cells, and Natural Killer cells) and myeloerythroid cells (granulocytes, macrophages, red blood cells, and platelets) that are produced by these stem and progenitor cells are shown in more detail in Figure 2.1.

Source: http://stemcells.nih.gov/info/2006report/2006chapter2.htm

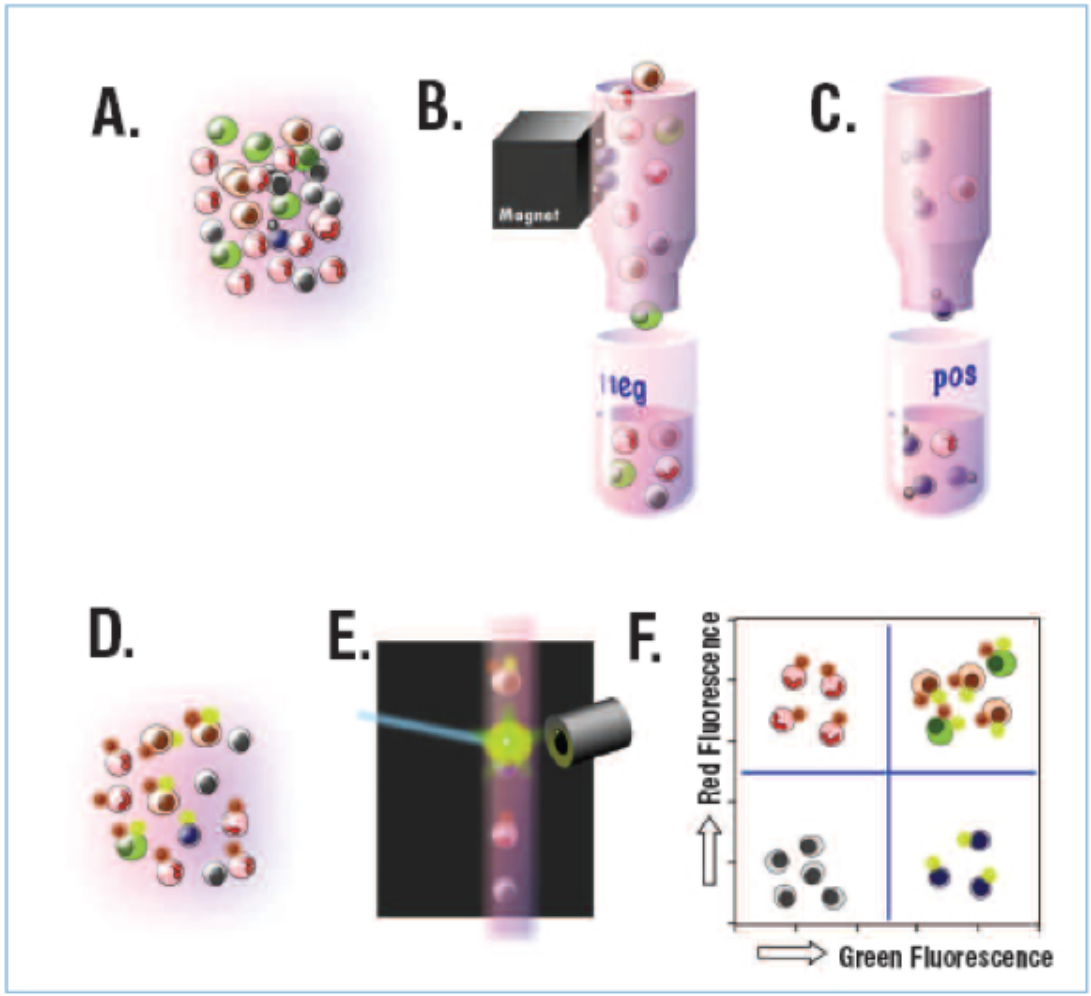

Figure 2.2. Enrichment and purification methods for hematopoietic stem cells. Upper panels illustrate column-based magnetic enrichment. In this method, the cells of interest are labeled with very small iron particles (A). These particles are bound to antibodies that only recognize specific cells. The cell suspension is then passed over a column through a strong magnetic field which retains the cells with the iron particles (B). Other cells flow through and are collected as the depleted negative fraction. The magnet is removed, and the retained cells are collected in a separate tube as the positive or enriched fraction (C). Magnetic enrichment devices exist both as small research instruments and large closed-system clinical instruments.

Source: http://stemcells.nih.gov/info/2006report/2006chapter2.htm

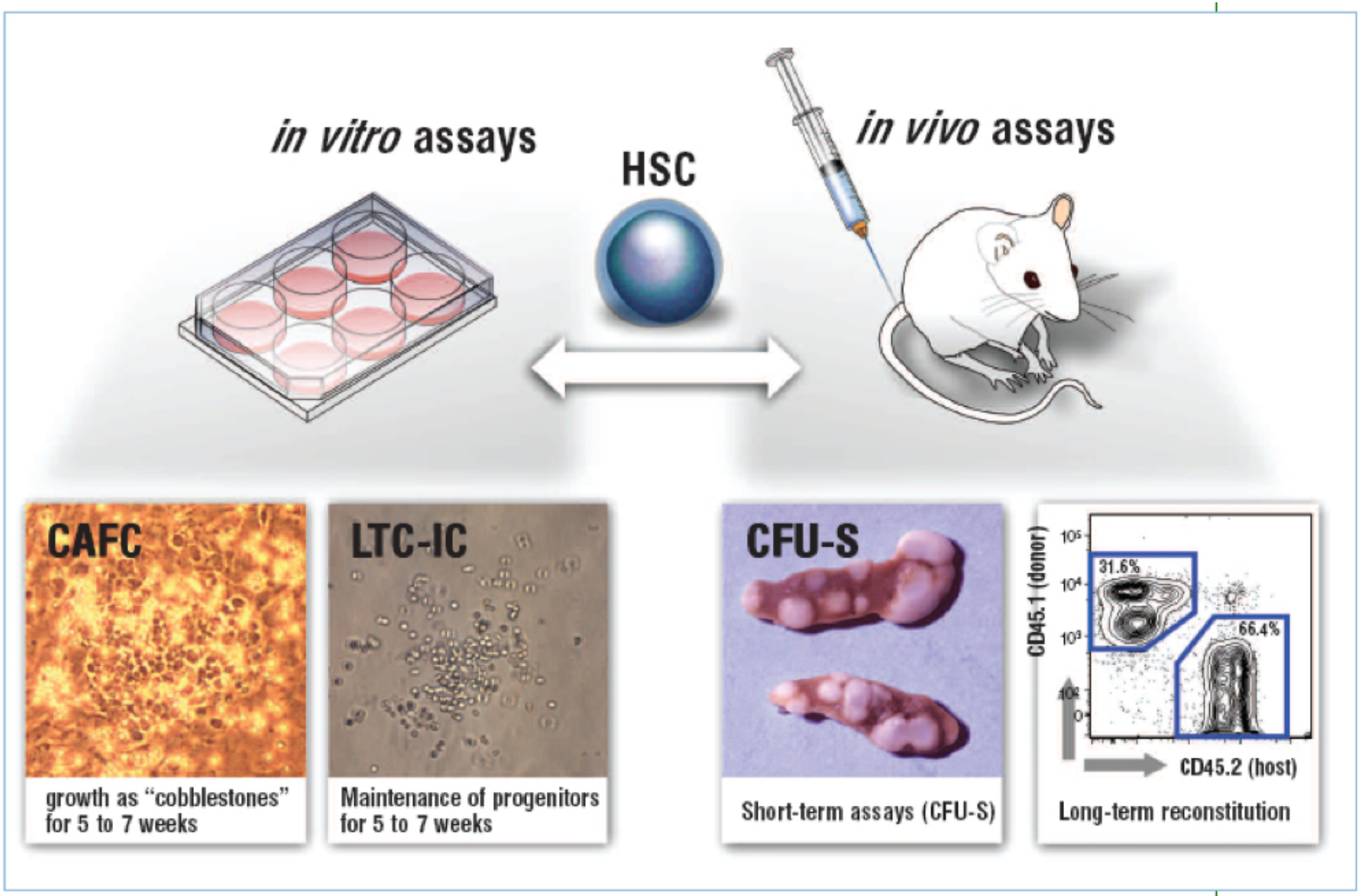

Figure 2.3. Assays used to detect hematopoietic stem cells. The tissue culture assays, which are used frequently to test human cells, include the ability of the cells to be tested to grow as quot;cobblestonesquot; (the dark cells in the picture) for 5 to 7 weeks in culture. The Long Term Culture-Initiating Cell assay measures whether hematopoietic progenitor cells (capable of forming colonies in secondary assays, as shown in the picture) are still present after 5 to 7 weeks of culture.

Source: http://stemcells.nih.gov/info/2006report/2006chapter2.htm

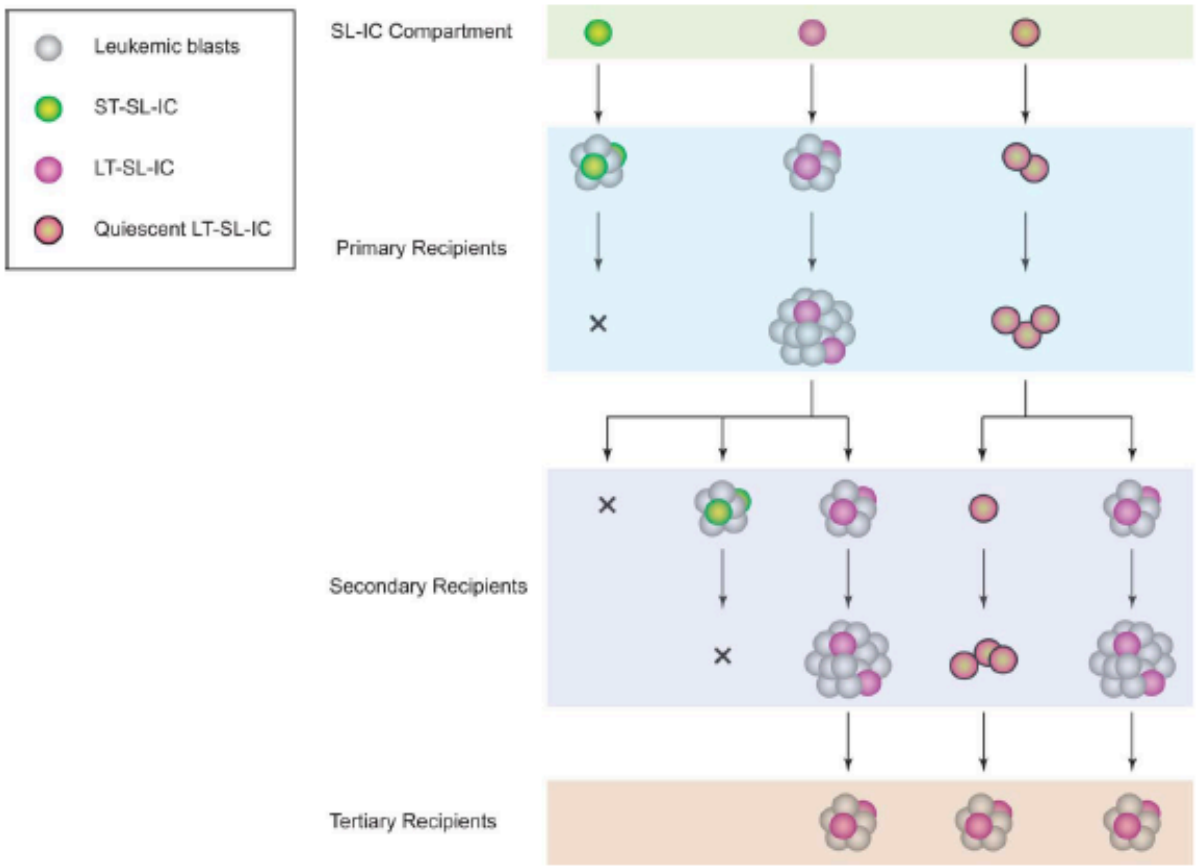

Figure 3. Hierarchy of leukemia stem cells in AML. Like the normal hematopoietic system, AML is organized as a hierarchy of distinct cell classes that is sustained by a subset of leukemia stem cells (or SCID-leukemia initiating cells [SL-ICs], as assayed in immunodeficient mice). Genetic tracking experiments have shown that SL-ICs are heterogeneous in their ability to repopulate secondary and tertiary recipients, pointing to the existence of distinct classes with differing self-renewal capacity, similar to what is seen in the normal hematopoietic stem cell compartment. Short-term (ST) SL-ICs are able to initiate leukemia in primary but not secondary recipients, whereas long-term (LT) SL-ICs can sustain leukemic growth for multiple passages. Quiescent LT SL-ICs may not initiate a substantial graft in primary recipients and may therefore only be detected on serial transplantation.

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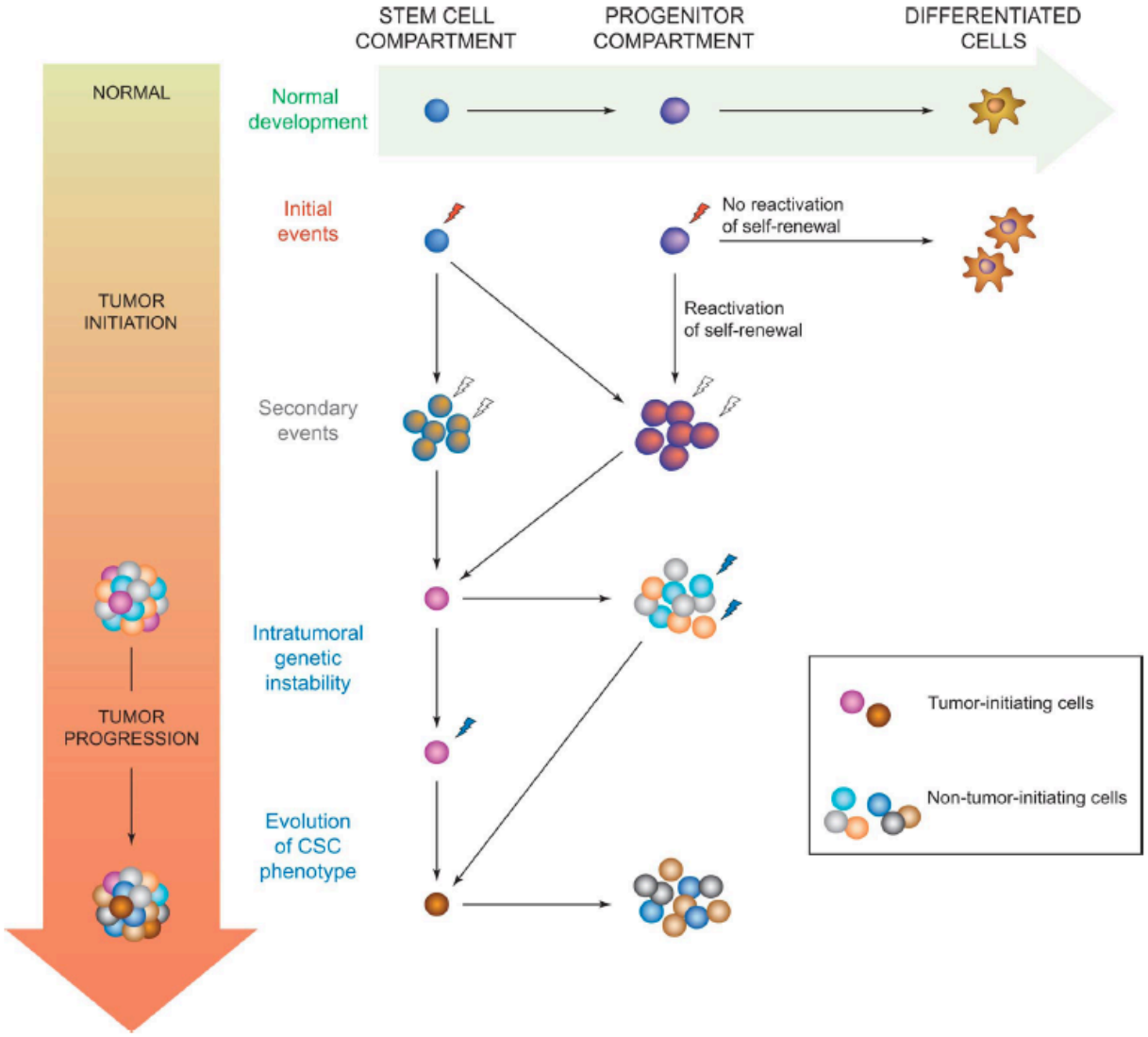

Figure 4. Models of tumor initiation and progression. Cancer stem cells may arise through neoplastic changes initiated in normal self-renewing stem cells or downstream progenitors, causing expansion of the stem cell and/or progenitor pool. Secondary events may occur in expanded pools of target cells. Oncogenic events acquired by short-lived progenitors may not persist if self-renewal is not reactivated, as these cells will probably die or undergo terminal differentiation before enough mutations occur for full neoplastic transformation. Tumor progression may be linked to ongoing genetic instability and acquisition of additional changes by cancer stem cells, or possibly by nontumorigenic bulk cells if such changes endow self-renewal. In both cases, evolution of tumor phenotype (including genetic and epigenetic signatures) may be observed.

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

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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.

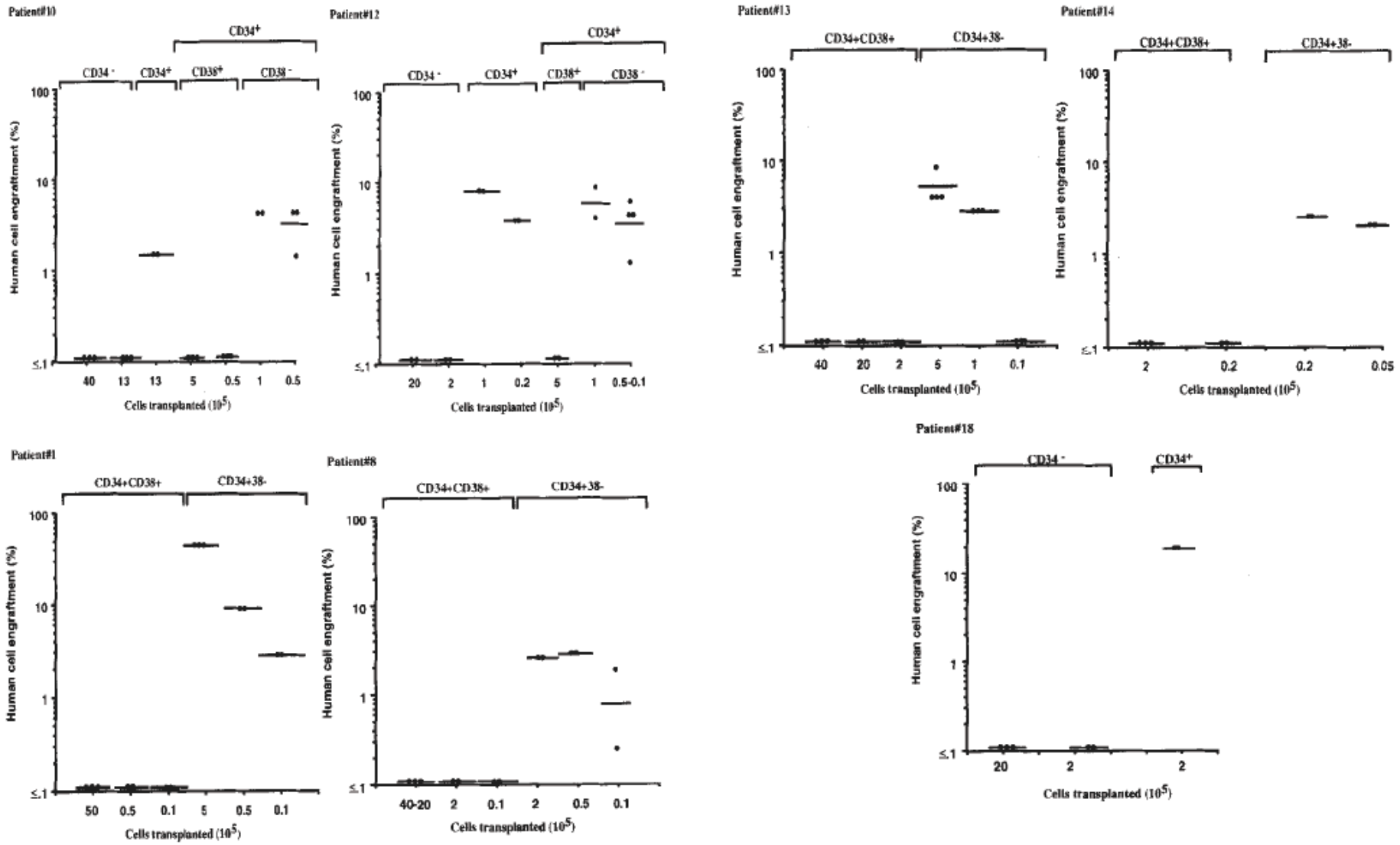


Fig. 3 Engraftment of NOD/SCID mice with AML cells fractionated according to CD34 and CD38 expression. Mice were transplanted with the indicated number of purified $CD34^+$, $CD34^-$, $CD34^{++}CD38^-$ or $CD34^+CD38^+$ cells. Cells from seven different AML patients have been studied (nos. 1, 8, 10, 12, 13, 14 and 18). Human cell engraftment was estimated by Southern blot 4 to 6 weeks after transplant. The different concentrations of sorted cells and the patient identification are indicated. Each dot represents a mouse and the horizontal line indicates the mean level of human cells engrafted in the murine bone marrow.

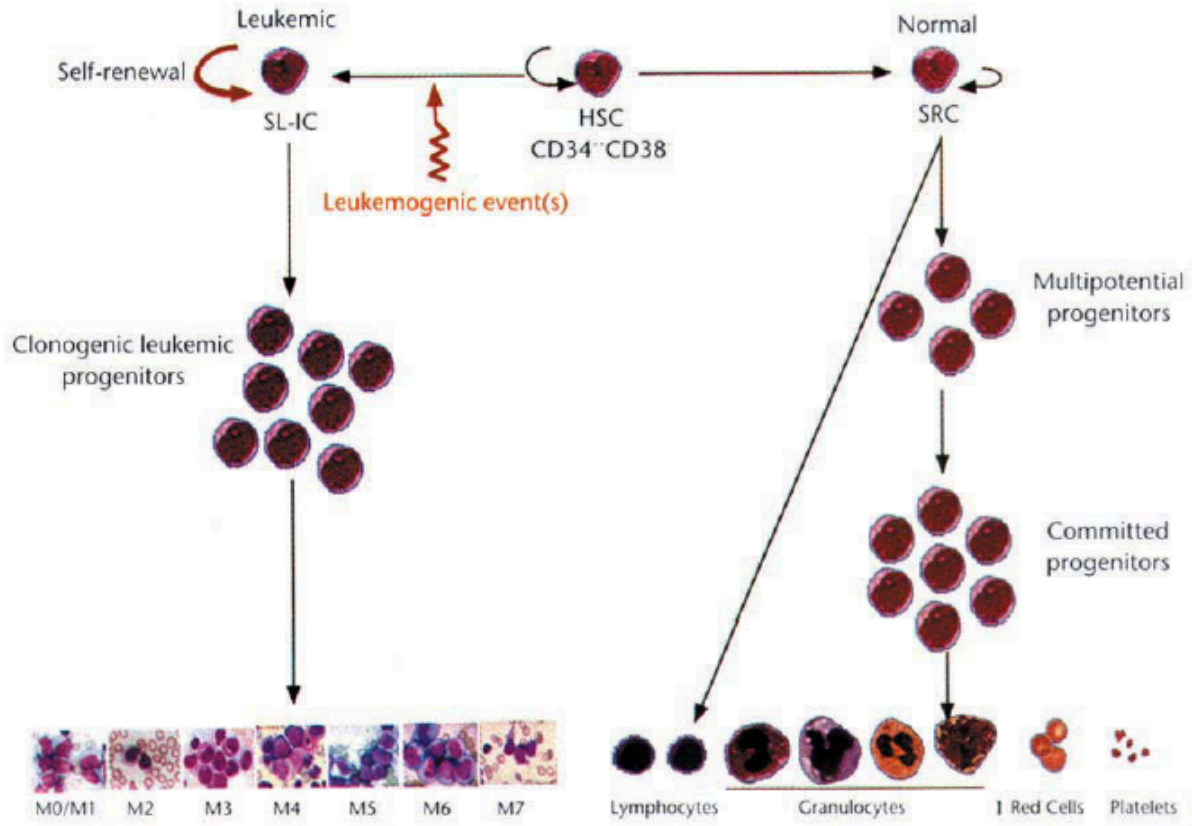
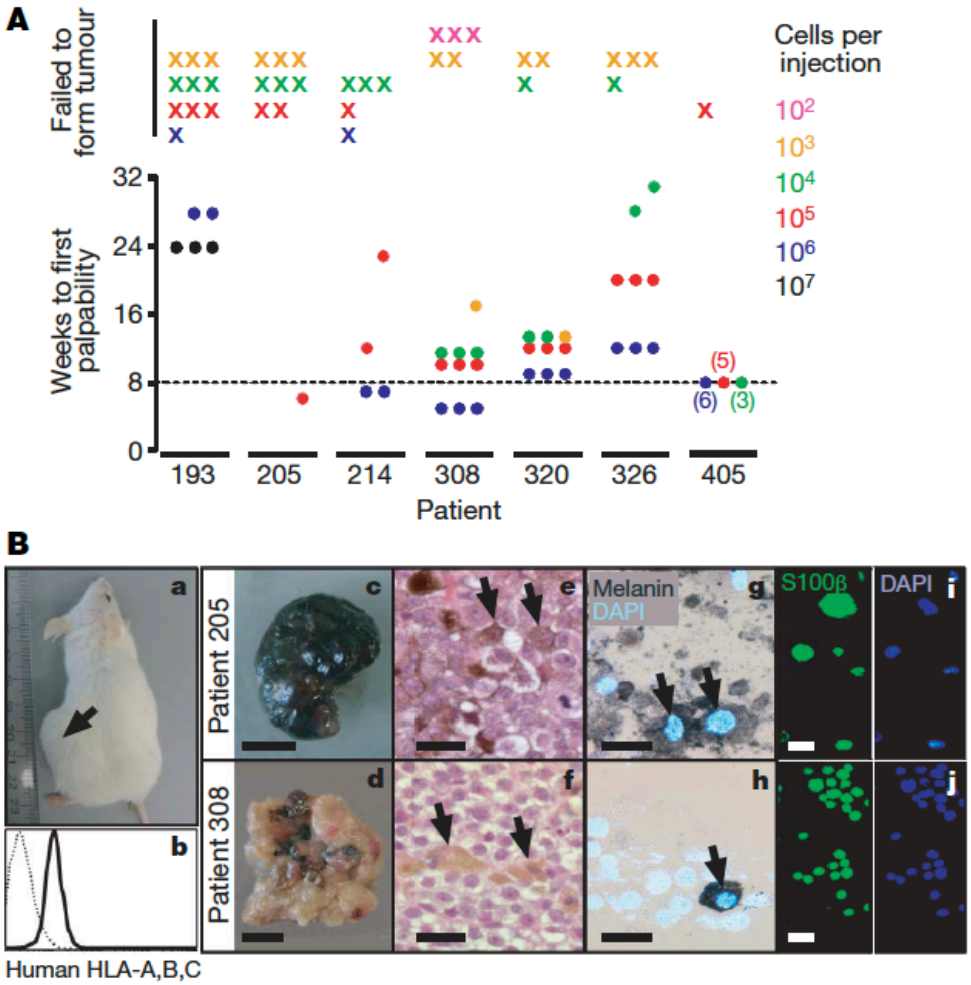


Fig. 6 A model comparing the organization of the normal and AML human hematopoietic system. This model postulates that the SRC assay detects a primitive human stem cell on the basis of repopulation of NOD/SCID mice. Although some overlaps undoubtedly exist, the SRCs are more immature than most multipotential progenitors (for example, 5 week-LTC-IC, colony-forming unit-granulocyte/erythrocyte/megakaryocyte/macrophage) and committed clonogenic progenitors[18]. The committed progenitor pool produces all the mature myelo-erythroid cells found in the peripheral blood. On the basis of the data presented here, this model postulates that a leukemogenic event occurs in a primitive stem cell. As a consequence, the cell has increased self-renewal and some impairment of the normal developmental program, so normal lineages cannot develop. This leukemic stem cell is detected in the SL-IC assay on the basis of the initiation of AML after transplantation into NOD/SCID mice. The SL-ICs produce clonogenic leukemic progenitors (AML-CFU), which in turn produce leukemic blasts. The nature of the leukemogenic event(s), and not the lineage commitment of the leukemia-initiating cell, determines the differentiation program of the leukemic blasts. Hence, this model suggests that the AML clone is organized as a hierarchy with many similarities to the normal system.

Critical to the development of the Cancer Stem Cell Hypothesis is the identification of cell surface markers that can distinguish cells with tumour initiating potential from those that do not. Without distinct features identifying these cancer stem cells, there is no way of distinguishing between the clonal selection hypothesis and the stem cell hypothesis.

How important is the model system (NOD/SCID mice) in the identification of a subset of cells as the tumour initiating population?



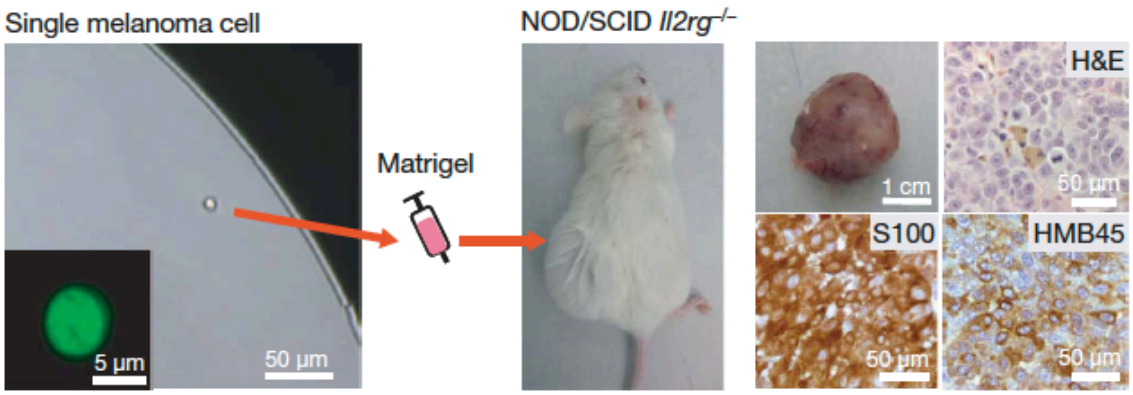
C

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 (*$P < 0.0001$).

Quintana et al. (2008). Nature 456:593-598

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.

Quintana et al. (2008). Nature 456:593-598

Table 1. Models to Explain Cancer Cell Heterogeneity

	Cancer Stem Cell Model	(Stochastic) Clonal Evolution Model[a]
Frequency of cancer cells with tumorigenic potential	Rare to moderate	High
Phenotype of cancer cells	Heterogeneous	Heterogeneous or homogeneous
Tumor organization	Hierarchical	Not necessarily hierarchical
Intrinsic differences between tumorigenic and nontumorigenic cells	Stable, epigenetic	Unstable, epigenetic or genetic
Rational approach to therapy	Possible to target only tumorigenic cells	Target most or all cells
Compelling clinical evidence	Germ lineage cancers	High-grade B cell lymphoblastic leukemia[b]

[a]The clonal evolution model holds that genetic and epigenetic changes occur over time in individual cancer cells, and that if such changes confer a selective advantage they will allow individual clones of cancer cells to out-compete other clones. Clonal evolution can lead to genetic heterogeneity, conferring phenotypic and functional differences among the cancer cells within a single patient. Note that the clonal evolution and cancer stem cell models are not mutually exclusive in cancers that follow a stem cell model, as cancer stem cells would be expected to evolve by clonal evolution. However, heterogeneity in cancers that do not follow a cancer stem cell model (not hierarchically organized into epigenetically distinct tumorigenic and nontumorigenic populations) could be determined entirely by clonal evolution.

[b]B cell lymphoblastic leukemias have extraordinarily high frequencies of leukemogenic cells that are not hierarchically organized in a mouse model (Williams et al., 2007) and appear homogeneous by histopathology in patients, yet heterogeneity can arise in sensitivity to therapy through clonal genetic changes.

Shackleton et al. (2009) Cell 138:822-829

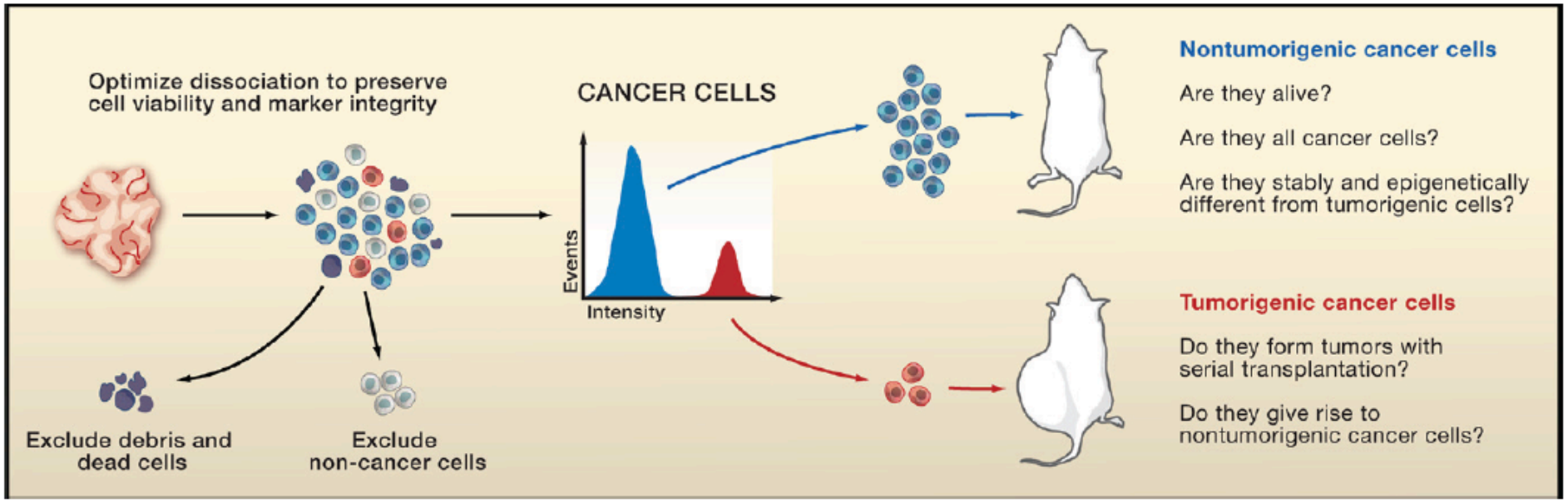
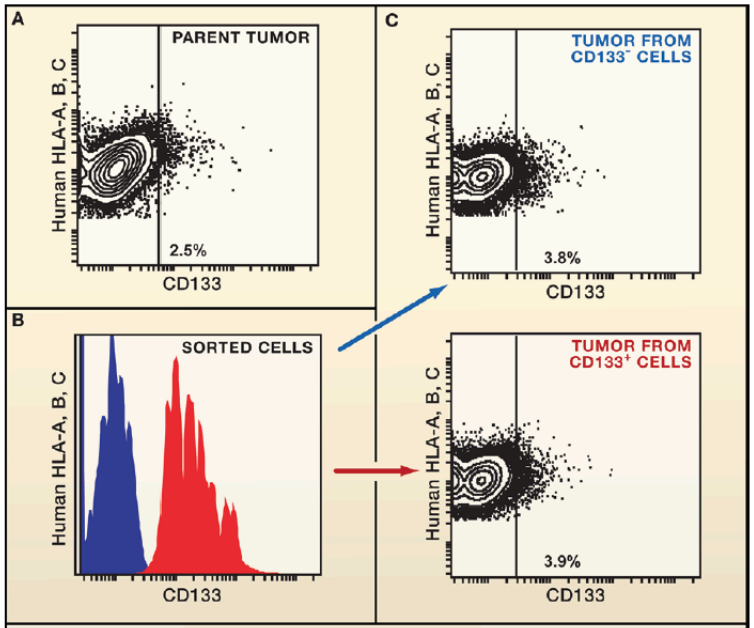


Figure 1. Testing the Cancer Stem Cell Model

During the dissociation of solid tumors (left), conditions must be optimized to maximize the preservation of cell viability and surface marker expression. During cell separation (middle), care must be taken to use viability dyes and markers to exclude dead cells, hematopoietic cells, endothelial cells, and stromal cells (if possible) by flow cytometry from the cancer cell preparation. The tumorigenicity of all cells must be tested in assays optimized for the engraftment of human cancer cells (right). For nontumorigenic cell populations, it is critical to confirm that they contain live cancer cells, rather than normal cells or debris. If markers can be identified that distinguish tumorigenic from nontumorigenic cells, an important question is whether these cancer cell populations are distinguished by epigenetic rather than genetic differences.

Shackleton et al. (2009) Cell 138:822-829



D

Sorted fraction	# of tumors/# of injections Cells/injections 50	10	Melanoma-initiating cell frequency (95%CI)	
Unfractionated	18/18	14/18	1/7	(1/4-1/12)
CD133⁻	17/17	12/18	1/9	(1/5-1/15)
CD133⁺	14/18	9/18	1/25	(1/15-1/40)

Figure 2. Cancers Need Not Be Hierarchically Organized to Be Heterogeneous

CD133 expression distinguishes tumorigenic from nontumorigenic cancer cells in some brain tumors and some colon cancers (Singh et al., 2004; O'Brien et al., 2007; Ricci-Vitiani et al., 2007). However, the expression of CD133 (or other stem cell markers) by small subpopulations of cells in other cancers does not necessarily mean that these cells are cancer stem cells. CD133 expression was heterogeneous in melanomas from 6 of 12 patients (Quintana et al., 2008).

(A) Representative CD133 staining in one of these melanomas (positive staining was defined using an isotype control).

(B) A reanalysis of the $CD133^-$ (blue) and $CD133^+$ (red) fractions after separation using magnetic beads.

(C) When these cells were transplanted into NOD/SCID $IL2R\gamma^{null}$ mice, both the $CD133^-$ and $CD133^+$ fractions of cells contained high frequencies of tumorigenic cells (D) (Quintana et al., 2008). The tumors that arose from $CD133^-$ cells and from $CD133^+$ cells contained similar proportions of $CD133^-$ and $CD133^+$ cells. This indicates that individual cancer cells can recapitulate the heterogeneity of the tumors from which they derive, even when there is no evidence that the cancer follows a cancer stem cell model or that tumorigenic cells are hierarchically organized.

Shackleton et al. (2009) Cell 138:822-829

Phenotypic Heterogeneity among Tumorigenic Melanoma Cells from Patients that Is Reversible and Not Hierarchically Organized

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Table 1. Summary of Tumor Formation by Single Cells or by Limit Dilution Analyses of Melanoma Cells from Sixteen Stage II, III, and IV Patients

Tumor Origin	Patient	AJCC Clinical Stage (Tumor Site)	Tumors/Injections: Cells Per Injection 1000	100	10	1	Engraftment Rate (%) or Tumorigenic Cell Frequency (95% CI)
Single cell injections							
Directly from patients	526	III (regional LN metastasis)				10/32	31%
	528	III (regional LN metastasis)				4/27	15%
	530	III (regional LN metastasis)				6/36	17%
	534	III (regional LN metastasis)				15/30	50%
	600	III (regional subcutaneous metastasis)				9/30	30%
Xenograft (up to 2 passages)	405	III (regional LN metastasis)				6/15	40%
	501	III (regional LN metastasis)				7/27	26%
	491	III (regional subcutaneous metastasis)				5/13	38%
ALL	n = 8					62/210	30%
Limit dilution analysis							
Directly from patients	610	II (cutaneous primary)		6/6	5/6		1/6 (1/2–1/15)
	486	III (cutaneous primary)	6/6	6/6	2/3		1/9 (1/2–1/39)
	597	III (cutaneous primary)	6/6	6/6	2/6		1/22 (1/8–1/62)
	495	III (cutaneous metastasis)	6/6	6/6	1/3		1/20 (1/5–1/76)
	510	III (regional LN metastasis)		6/6	3/3		>1/21
	514	III (regional LN metastasis)	6/6	6/6	6/6		>1/11
	631	III (regional LN metastasis)		6/6	3/3		>1/21
	632	III (regional LN metastasis)		6/6	6/6		>1/11
	633	III (regional LN metastasis)		6/6	6/6		>1/11
	641	III (regional LN metastasis)		6/6	4/6		1/9 (1/3–1/25)
	608	IV (distant subcutaneous metastasis)		6/6	6/6		>1/11
ALL	n = 11		24/24	66/66	44/54		1/6 (1/4–1/8)

AJCC: American Joint Committee on Cancer, CI: confidence interval, LN: lymph node. Melanoma cells were mixed with Matrigel and injected into NSG mice. Twenty-eight percent (44 of 155) of single cells obtained directly from patients formed tumors. AJCC is the clinical stage of the patient at the time of melanoma removal. See also Figure S1.

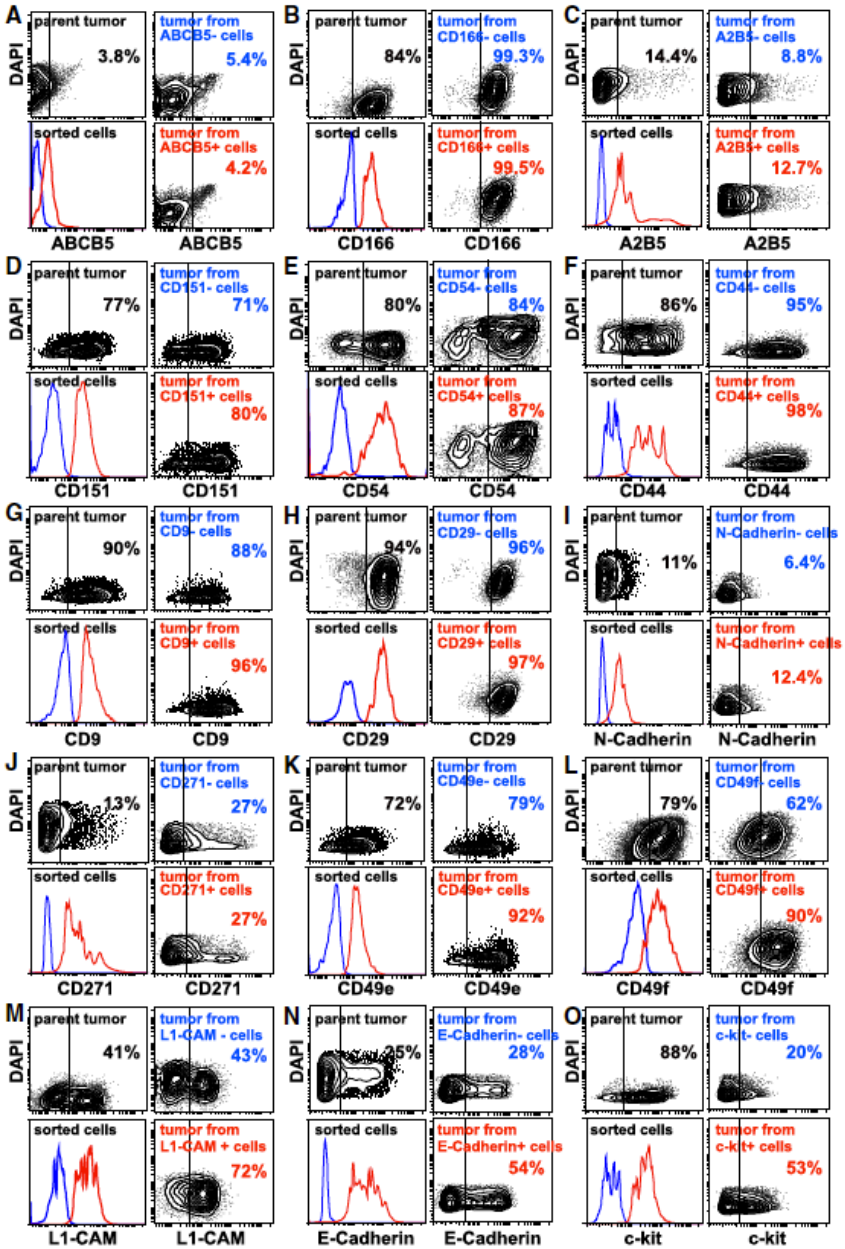

Figure 7. Many Phenotypically Distinct Fractions of Melanoma Cells Can Recapitulate the Heterogeneity of the Tumors from which They Derive

Expression of ABCB5 (A), CD166 (B), A2B5 (C), CD151 (D), CD54 (E), CD44 (F), CD9 (G), CD29 (H), N-Cadherin (I), CD271 (J), CD49e (K), CD49f (L), L1-CAM (M), E-Cadherin (N), and c-kit (O) in parent tumors (upper left) compared with expression in secondary tumors derived from marker$^{-/low}$ and marker$^{+/high}$ fractions (top right and bottom right, respectively). Bottom left panels show reanalyses of the sorted cell fractions used to generate secondary tumors. See also Figure S3. Every marker was tested in two to four separate melanomas, except for CD44, CD49f, E-Cadherin, and c-kit, which were tested in one.

Epithelial to Mesenchymal Transition

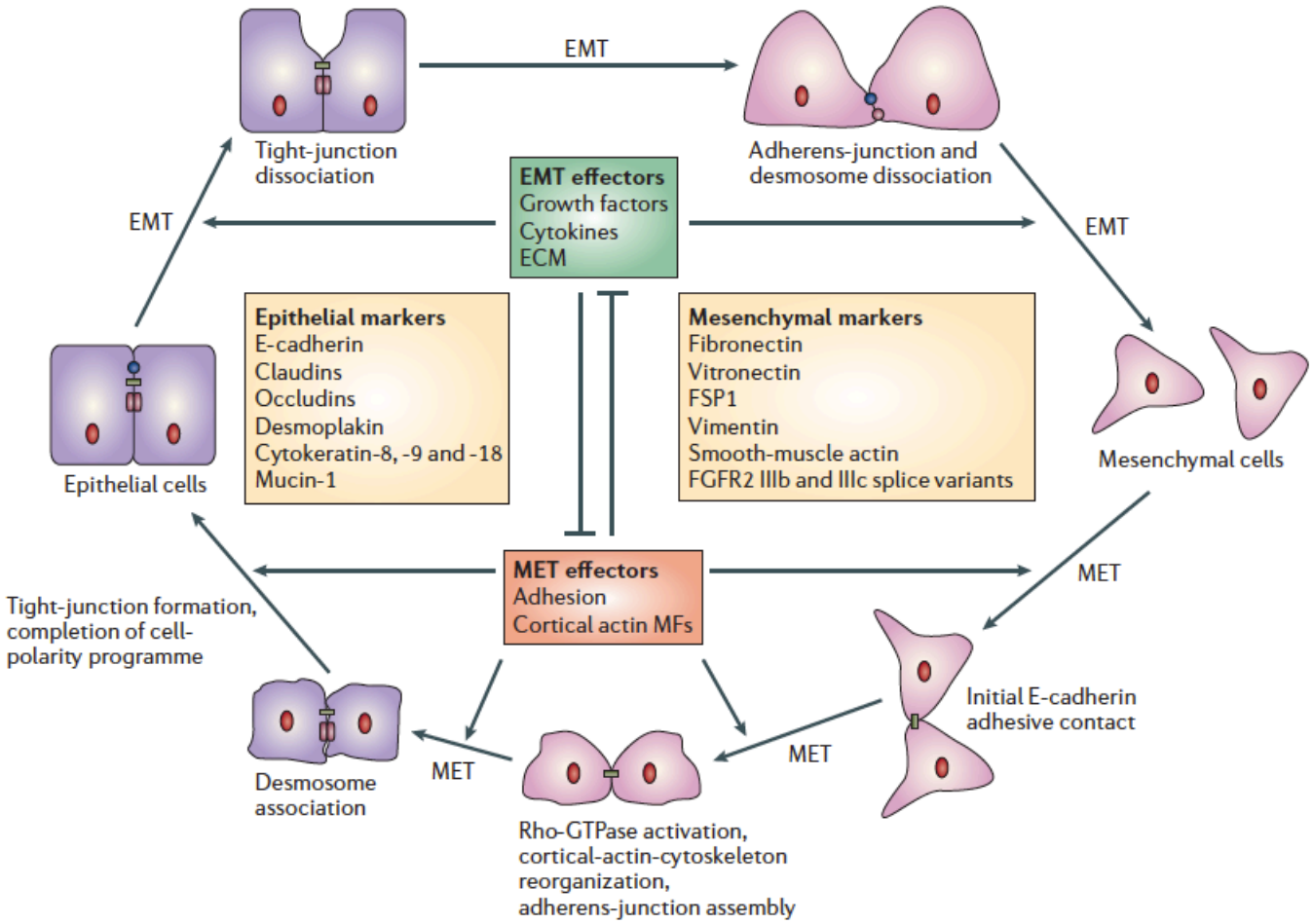

Figure 2 | The cycle of epithelial-cell plasticity. The diagram shows the cycle of events during which epithelial cells are transformed into mesenchymal cells and vice versa. The different stages during EMT (epithelial–mesenchymal transition) and the reverse process MET (mesenchymal–epithelial transition) are regulated by effectors of EMT and MET, which influence each other. Important events during the progression of EMT and MET, including the regulation of the tight junctions and the adherens junctions, are indicated. A number of markers have been identified that are characteristic of either epithelial or mesenchymal cells and these markers are listed in BOX 1 and BOX 2. E-cadherin, epithelial cadherin; ECM, extracellular matrix; FGFR2, fibroblast-growth-factor receptor-2; FSP1, fibroblast-specific protein-1; MFs, microfilaments.

Thiery and Sleeman (2006). Nat. Rev. Mol. Cell Biol. 7:131-142

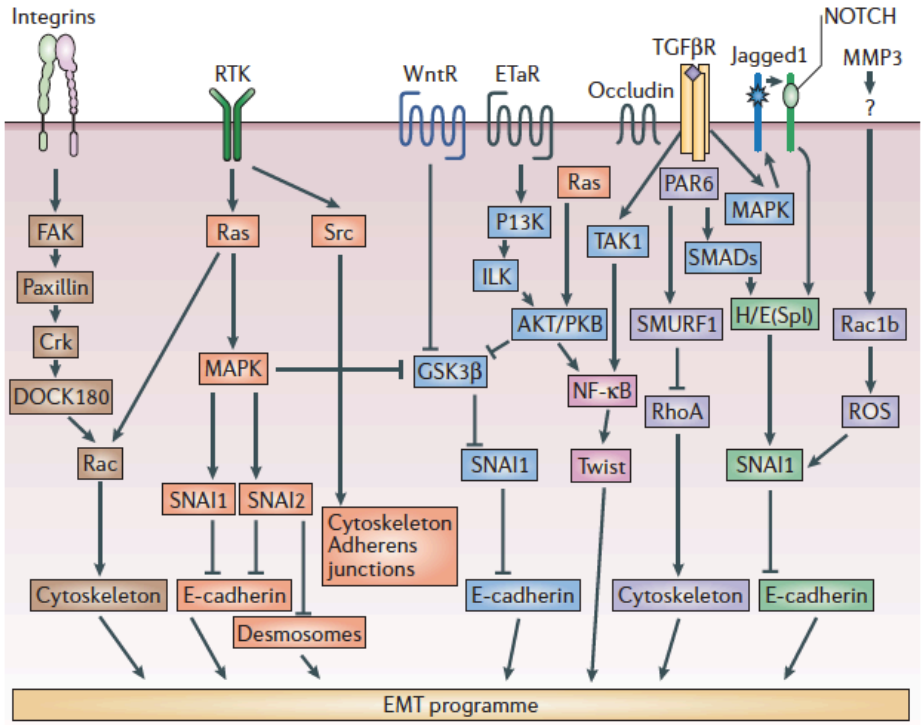

Figure 3 | **Overview of the molecular networks that regulate EMT.** A selection of the signalling pathways that are activated by regulators of EMT and a limited representation of their crosstalk is illustrated. Activation of receptor tyrosine kinases (RTKs) is known to induce EMT in several epithelial cell types and *in vivo*, but it is now clear that the EMT process often requires co-activation of integrin receptors. The role of transforming growth factor-β (TGFβ) signalling in EMT is established for a limited number of normal and transformed cell lines, whereas *in vivo* data has indicated a mutual regulation of the TGFβ and NOTCH pathways during EMT. There is now increasing evidence that other signalling pathways could have an important role in EMT, including G-protein-coupled receptors. Matrix metalloproteinases (MMPs) can also trigger EMT through as-yet-undefined receptors. ETaR, endothelin-A receptor; FAK, focal adhesion kinase; GSK3β, glycogen-synthase kinase-3β; H/E(Spl), hairy/enhancer of split; ILK, integrin-linked kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PAR6, partitioning-defective protein-6; PI3K, phosphatidyl-inositol 3-kinase; PKB, protein kinase-B; ROS, reactive oxygen species; TAK1, TGFβ-activated kinase-1; TGFβR, TGFβ receptor; WntR, Wnt receptor.

Thiery and Sleeman (2006). Nat. Rev. Mol. Cell Biol. 7:131-142

Cell

The Epithelial-Mesenchymal Transition Generates Cells with Properties of Stem Cells

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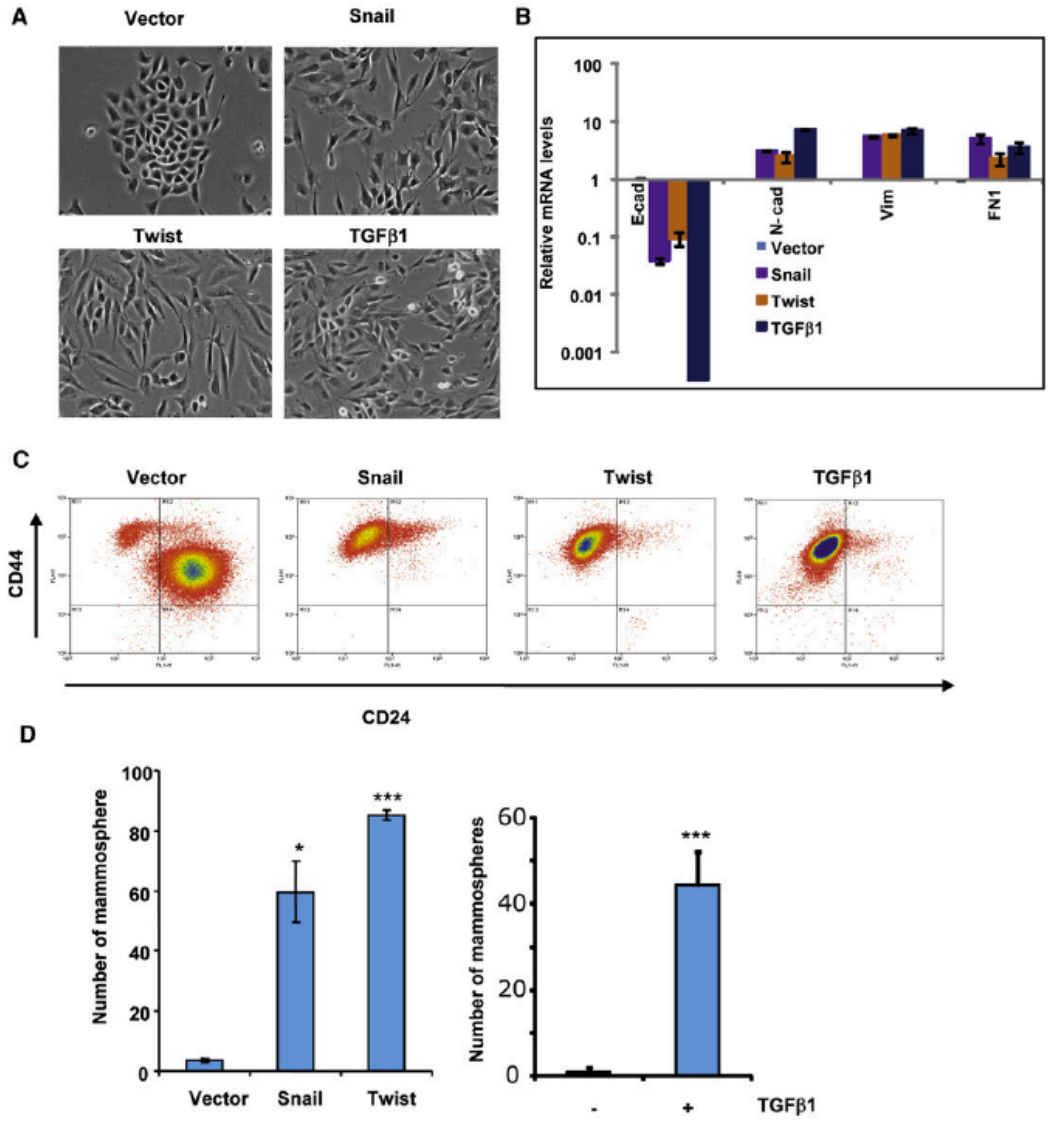

Figure 1. The Epithelial-Mesenchymal Transition (EMT) Generates Cells with Properties of Stem Cells

(A) Phase-contrast images of HMLE cells expressing Snail, Twist, or the control vector, as well as HMLE cells treated with recombinant TGFβ1 (2.5 ng/ml) for 12 days (bottom right).

(B) Relative expression of the mRNAs encoding E-cadherin, N-cadherin, vimentin, and fibronectin in HMLE cells induced to undergo EMT by the methods outlined in (A), as determined by real-time RT-PCR. GAPDH mRNA was used to normalize the variability in template loading. The data are reported as mean ± SEM.

(C) FACS analysis of cell-surface markers, CD44 and CD24, in the cells described in (A).

(D) In vitro quantification of mammospheres formed by cells described in (A). The data are reported as the number of mammospheres formed/1000 seeded cells ± SEM, (* - $p < 0.05$; *** - $p < 0.001$ compared to the control).

Table 2. Tumor Incidence of Transformed HMLEs Induced to Undergo EMT by Ectopic Expression of Snail or Twist and Then Injected into Host Mice in Limiting Dilutions

	Tumors Incidence/Number of Injections			
Cells Injected	1×10^6	1×10^5	1×10^4	1×10^3
HMLE-Vector-Ras	2/6	3/9	0/9	0/9
HMLE-Snail-Ras	6/6	9/9	9/9	6/9
HMLE-Twist-Ras	6/6	9/9	9/9	7/9

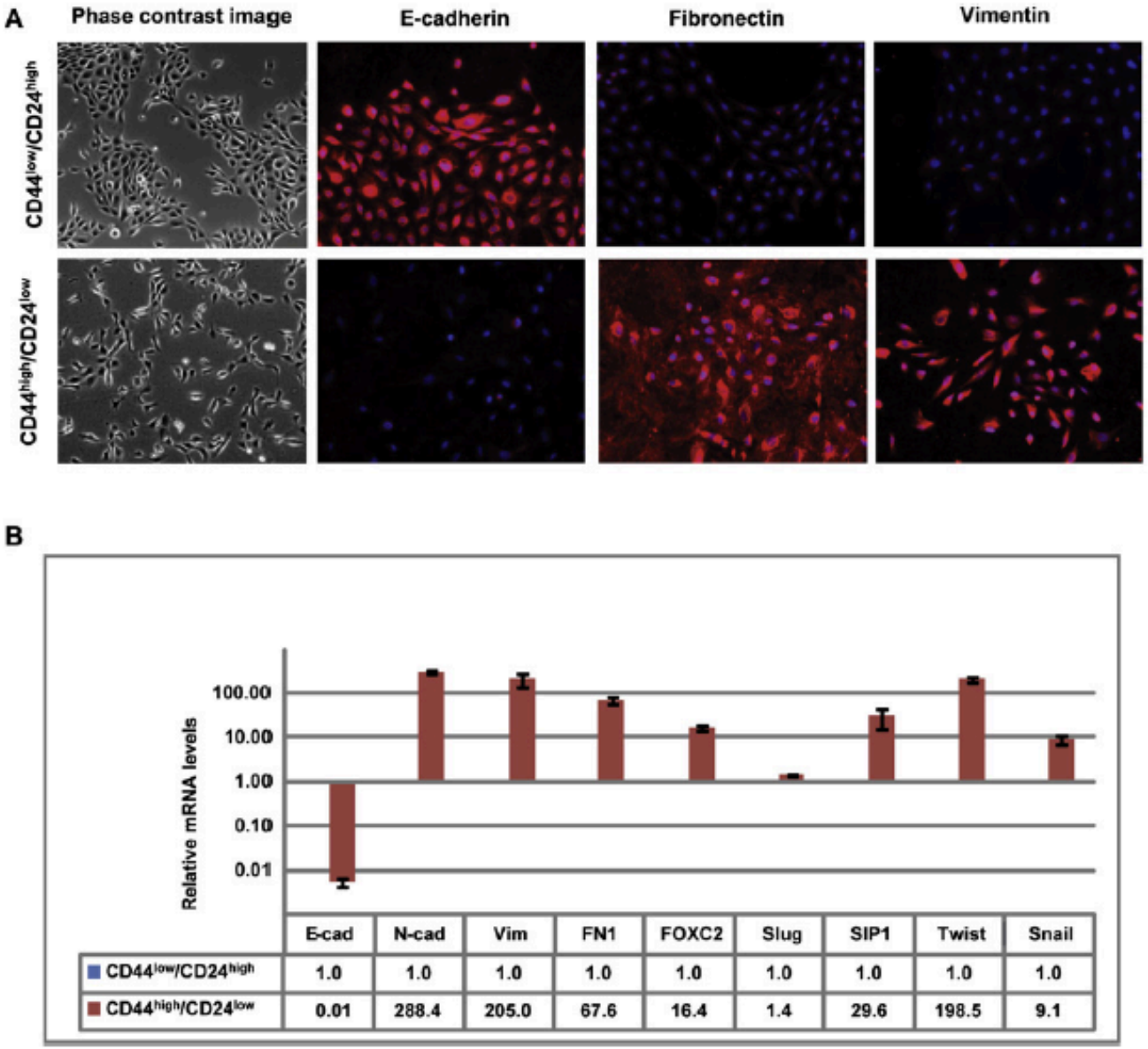

Figure 3. Stem-like CD44^{high}/CD24^{low} Cells Isolated from HMLE Cells Exhibit Attributes of Cells that Have Undergone an EMT

(A) Phase-contrast images (left) and immunofluorescence images of CD44^{high}/CD24^{low} and CD44^{low}/CD24^{high} cells stained using antibodies against E-cadherin, fibronectin, or vimentin (right panels). (B) The expression levels of the mRNAs encoding E-cadherin, N-cadherin, vimentin, fibronectin, FOXC2, Slug, SIP1, Twist, and Snail in CD44^{high}/CD24^{low} cells relative to CD44^{low}/CD24^{high} as determined by real-time RT-PCR. GAPDH mRNA was used to normalize the variability in template loading. The data are reported as mean + SEM.

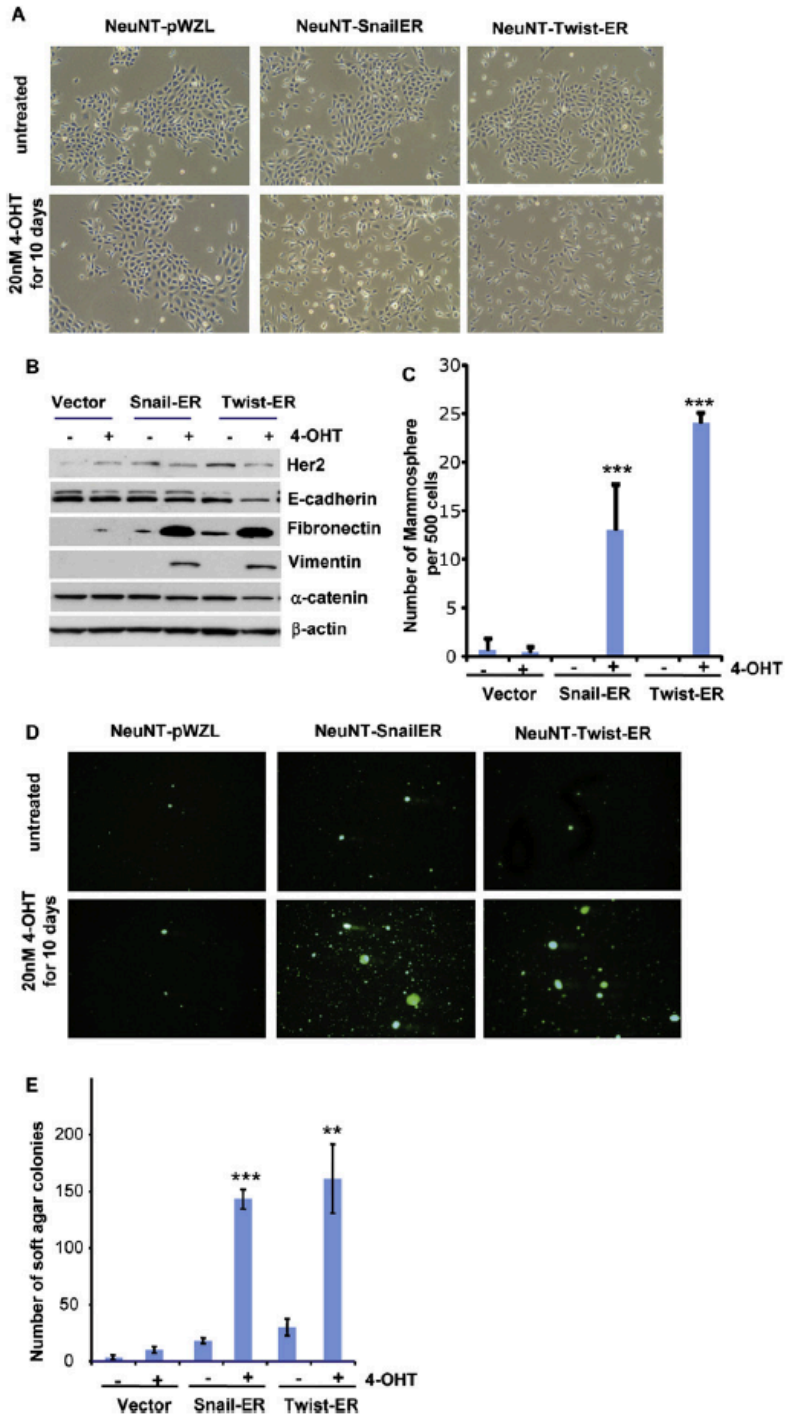

Figure 5. EMT Induces Phenotypes Associated with Cancer Stem Cells

(A) Phase-contrast images of NeuNT-Snail-ER, NeuNT-Twist-ER, and NeuNT-control vector cells treated with tamoxifen for a period of 10 days as well as images of untreated cells.

(B) Western blot analysis of expression of HER2/*neu*, E-cadherin, fibronectin, and vimentin proteins in the cells shown in (A). β-actin was used as a loading control.

(C) Quantification of the mammospheres seeded by NeuNT-Snail-ER, NeuNT-Twist-ER, or NeuNT-control vector cells treated or not treated with tamoxifen for 10 days. The data are reported as mean ± SD.

(D) Images of the colonies formed during soft agar culture of NeuNT-Snail-ER, NeuNT-Twist-ER, and NeuNT-control vector cells after being treated with tamoxifen for 10 days. The soft agar assays were performed in the absence of tamoxifen.

(E) Quantification of the soft agar colonies shown in (D). The data are reported as mean ± SD (** - $p < 0.01$; *** - $p < 0.001$ compared to the control).

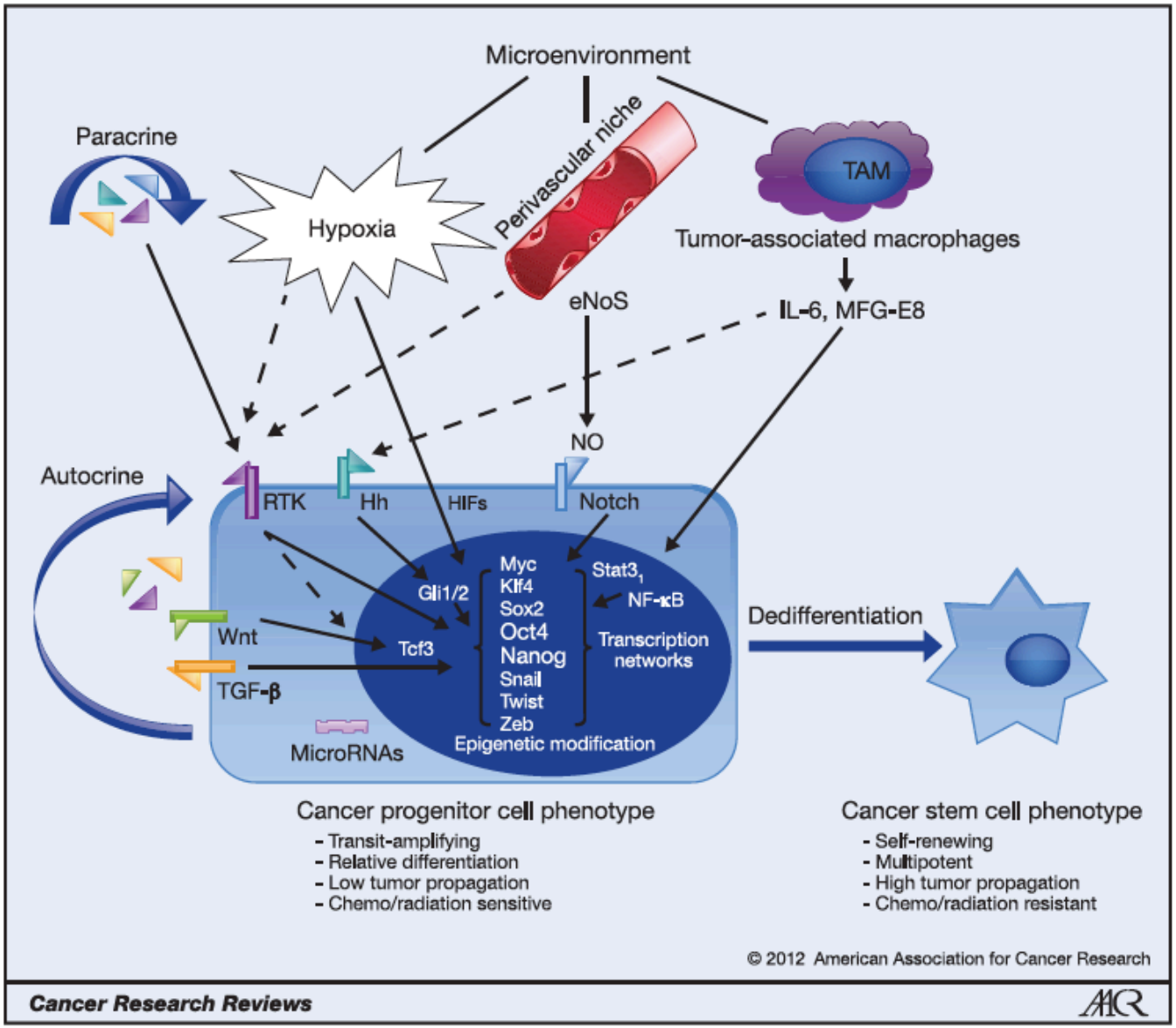

Figure 1. Transit-amplifying cancer progenitor cells acquire stem-like phenotypes by dedifferentiating mechanisms. This schematic model depicts the functional connections among microenvironmental signals, signal transduction pathways, and molecular circuitries, including transcriptional networks, miRNAs, and epigenetic modifications that induce dedifferentiation of cancer progenitor cells into CSC phenotypes. Transcriptional networks involving Oct4, Nanog, and other TFs act as key inducers of dedifferentiation mechanisms. eNOS, endothelial nitric oxide synthase; Hh, hedgehog; NO, nitric oxide; RTK, receptor tyrosine kinase.

Yunqing Li and John Laterra

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The epigenetic progenitor origin of human cancer

Andrew P. Feinberg, Rolf Ohlsson‡ and Steven Henikoff§*

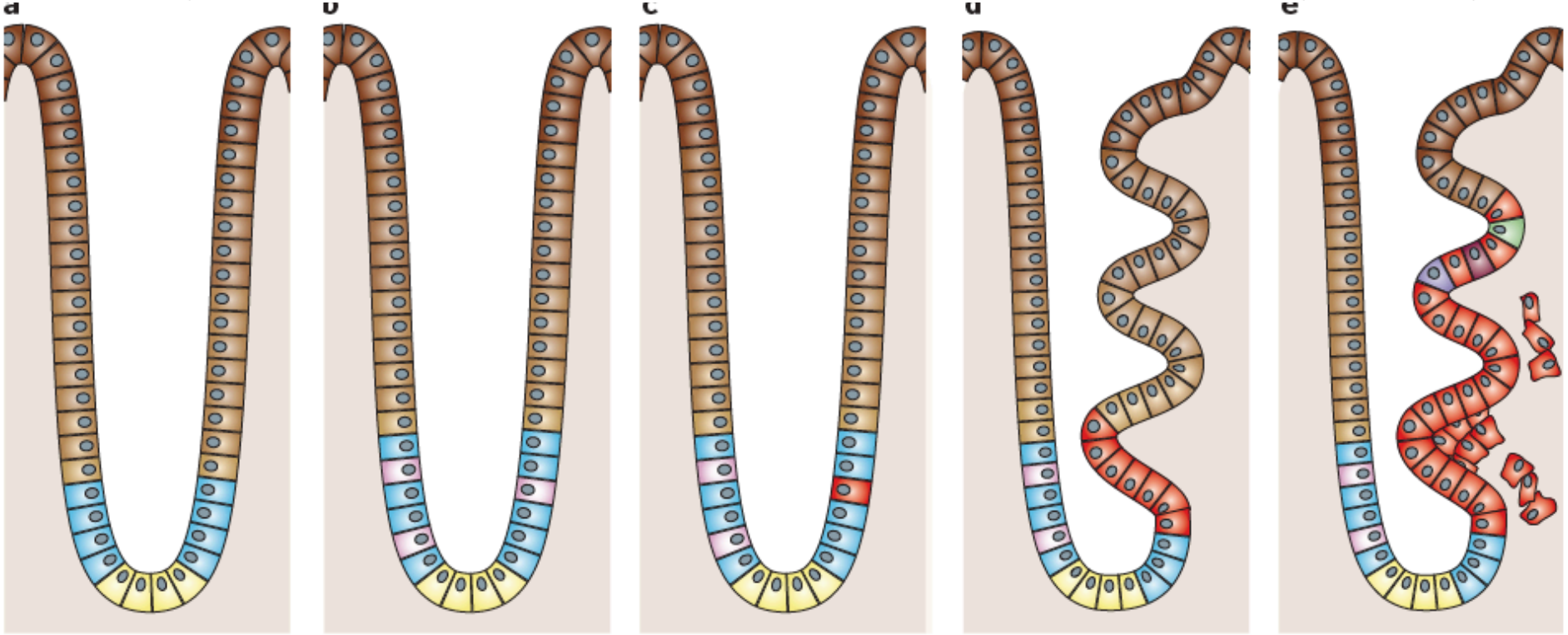

Figure 3 | **The epigenetic progenitor model in the context of a stem cell niche.** Normal colonic epithelium (first panel) includes a proliferative zone that contains stem cells (blue), which give rise to differentiated cells further up the crypt (shades of brown represent differentiation stages) (**a**). The epigenetic progenitor model suggests that the stem cell compartment is altered epigenetically (**b**), which can involve an expansion of the progenitor compartment or other epigenetic changes in gene expression (pink), followed by genetic mutation (**c**, red). Subsequent evolution of the tumour involves genetic and epigenetic plasticity; the latter allows expression of phenotypic features (invasion, metastasis and drug resistance, the last of which is denoted by altered colour) that are inherent properties of the stem cell progenitor (**d** and **e**).

The epigenetic progenitor origin of human cancer

Andrew P. Feinberg, Rolf Ohlsson‡ and Steven Henikoff§*

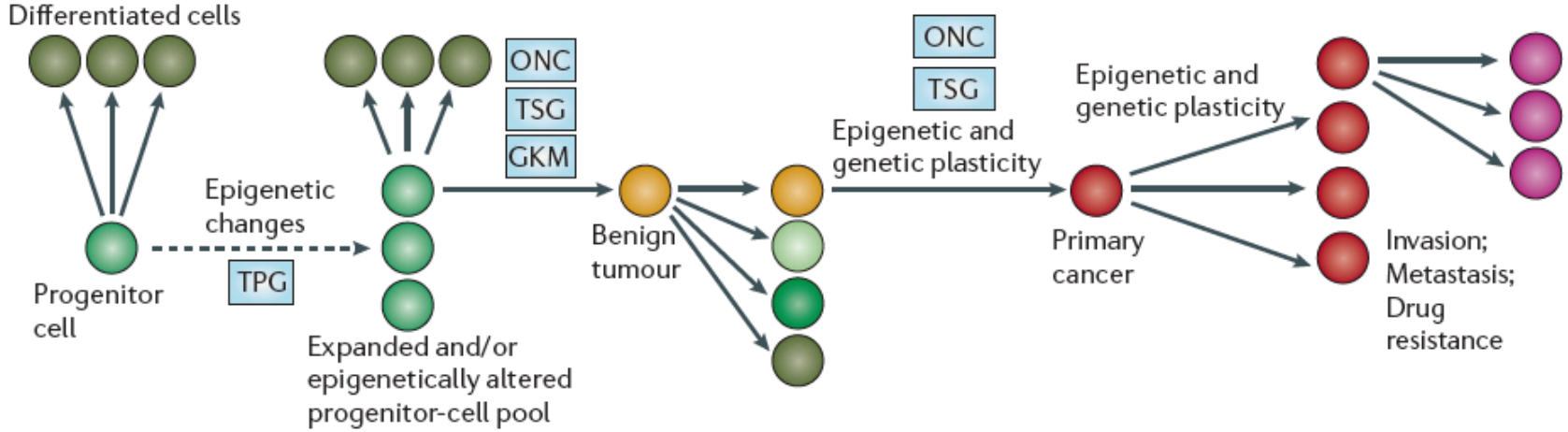

Figure 2 | **The epigenetic progenitor model of cancer.** According to this model, cancer arises in three steps. First is an epigenetic alteration of stem/progenitor cells within a given tissue, which is mediated by aberrant regulation of tumour-progenitor genes (TPG). This alteration can be due to events within the stem cells themselves, the influence of the stromal compartment, or environmental damage or injury. Second is a gatekeeper mutation (GKM) (tumour-suppressor gene (TSG) in solid tumours, and rearrangement of oncogene (ONC) in leukaemia and lymphoma). Although these GKMs are themselves monoclonal, the expanded or altered progenitor compartment increases the risk of cancer when such a mutation occurs and the frequency of subsequent primary tumours (shown as separately arising tumours). Third is genetic and epigenetic instability, which leads to increased tumour evolution. Note that many of the properties of advanced tumours (invasion, metastasis and drug resistance) are inherent properties of the progenitor cells that give rise to the primary tumour and do not require other mutations (highlighting the importance of epigenetic factors in tumour progression).

Take Home Messages

- The cancer stem cell hypothesis was developed as an alternative explanation for heterogeneity within tumours with respect to phenotypic or functional markers (e.g., morphology, cell surface markers, proliferation rate and resistance to therapy)
- The cell of origin and the cancer stem cell are not interchangeable. In some cases, there is compelling evidence for normal multipotent stem cells being the cell of origin.
- The epigenetic state of the cell may be far more plastic than previously recognized.
- EMT is a normal developmental process that provides one pathway for de-differentiation that may be exploited in solid tumours.
- Clonal evolution and cancer stem cells are not mutually exclusive hypotheses. The plasticity of the epigenetic state further obscures distinctions between the two models to explain heterogeneity in response to therapy.