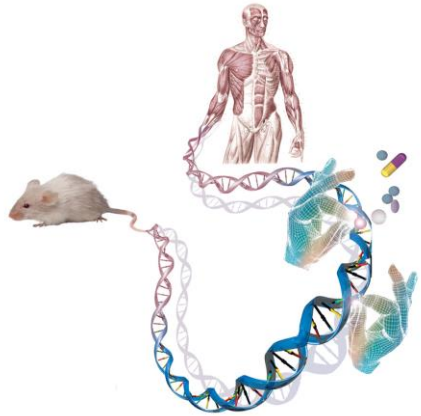
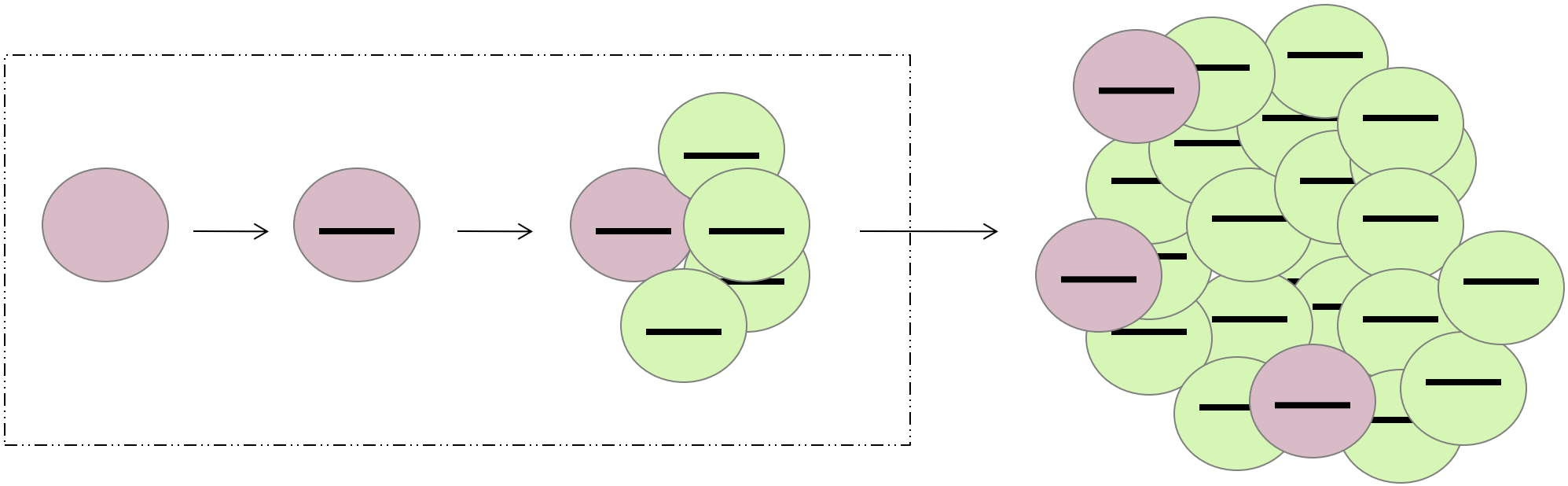


Stem cell reprogramming as a driver of cancer: Implications in its development and treatment

Isidro Sánchez-García (isg@usal.es)

VI Simposium de Bases Biológicas del Cáncer y Terapias Personalizadas
Salamanca, 22-23 de Mayo, 2014

Tumour cellular identity (tumour cell fate)

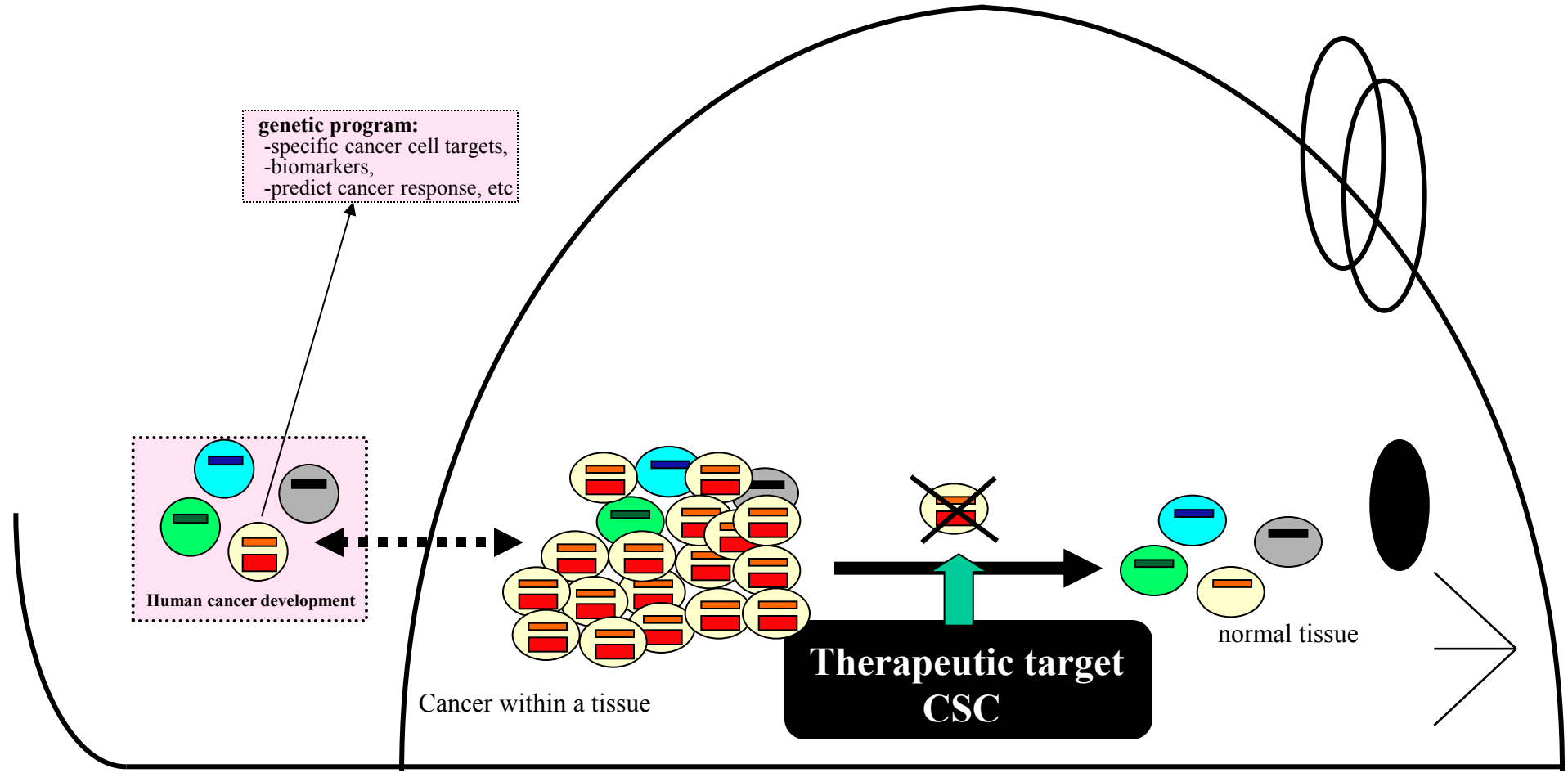


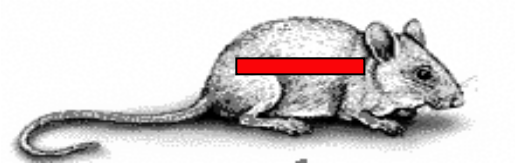
Clinical malignant tumor mass
"**billion-cell threshold**"
(Oncology remission means 0 ---- 10^9 cells)

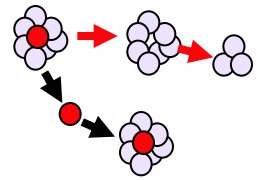
If cellular fate was immovable, cancer would not be possible, since no new lineages could be generated other than the normal, physiological one.

Which is the impact that oncogenes have in establishing the identity of the tumour cell?

Early decisions in cancer: reprogrammed tumor cell fates







mouse and human
normal stem cells
are similar

Outline

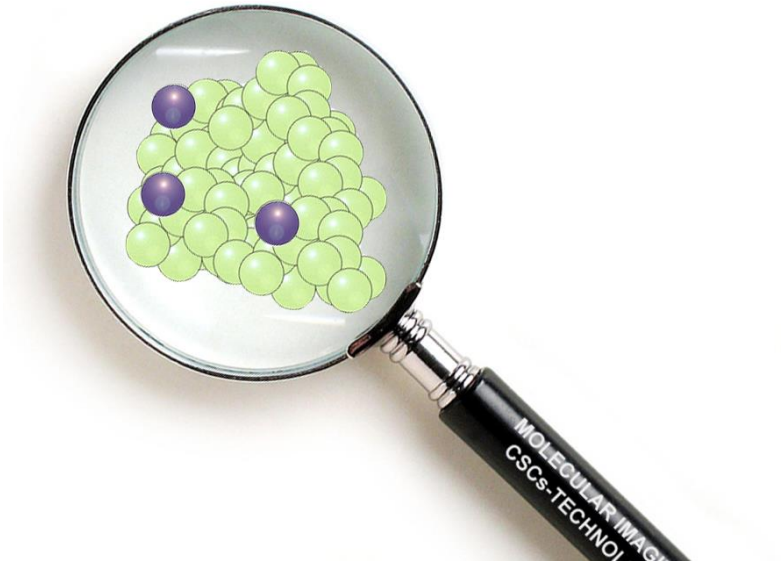
1- Current model of cancer

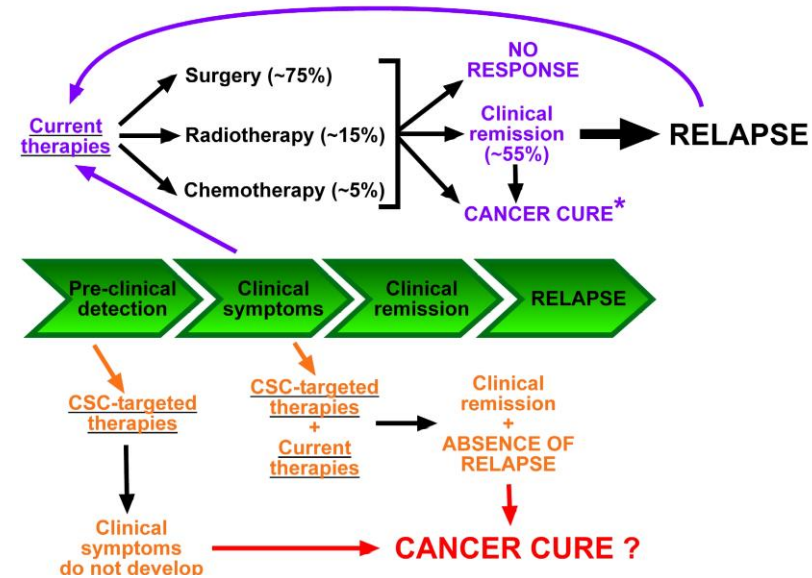
2- Tumoral epigenetic stem cell reprogrammimg hypothesis

3- Experimental validation and clinical application

4- Implications in the development and treatment of cancer

Current model of cancer





-Heterogenous tumor cell composition.

-Initiating genetic alteration is present in both CSC and differentiated tumor cells.

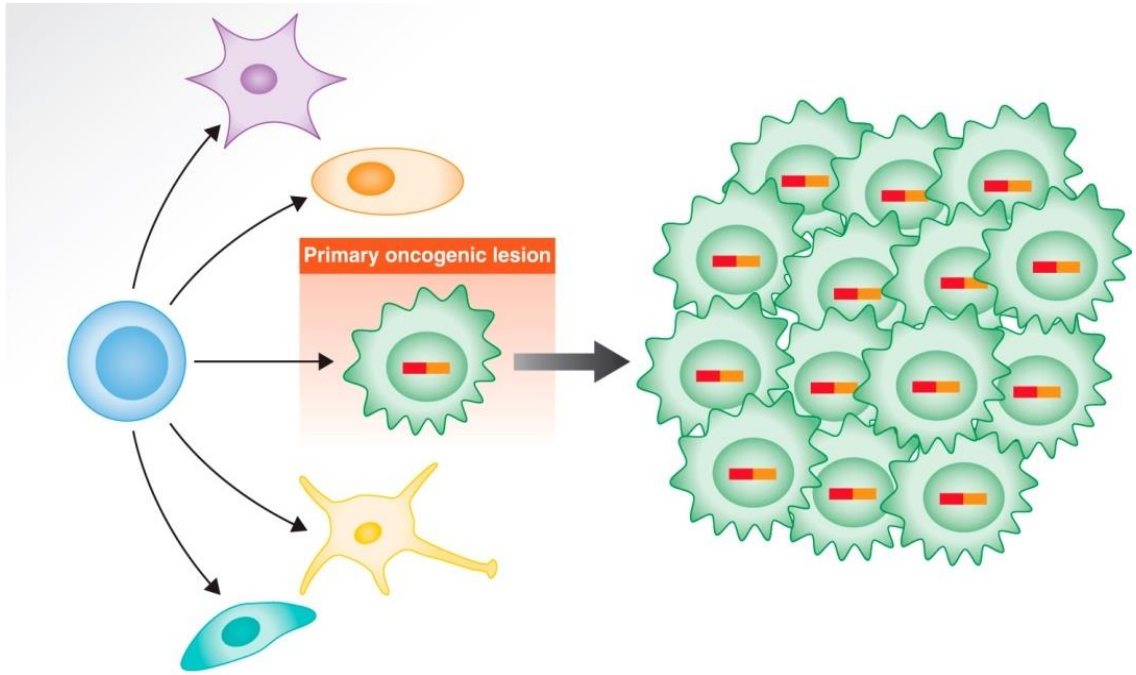
-Homogenous mode of action for oncogenes within cancer cells.

-Brief inactivation of oncogenes can cause cancer remission in model systems:oncogene addition

-However, unfortunately, the therapies based on this cancer model fail to eradicate tumours in humans.

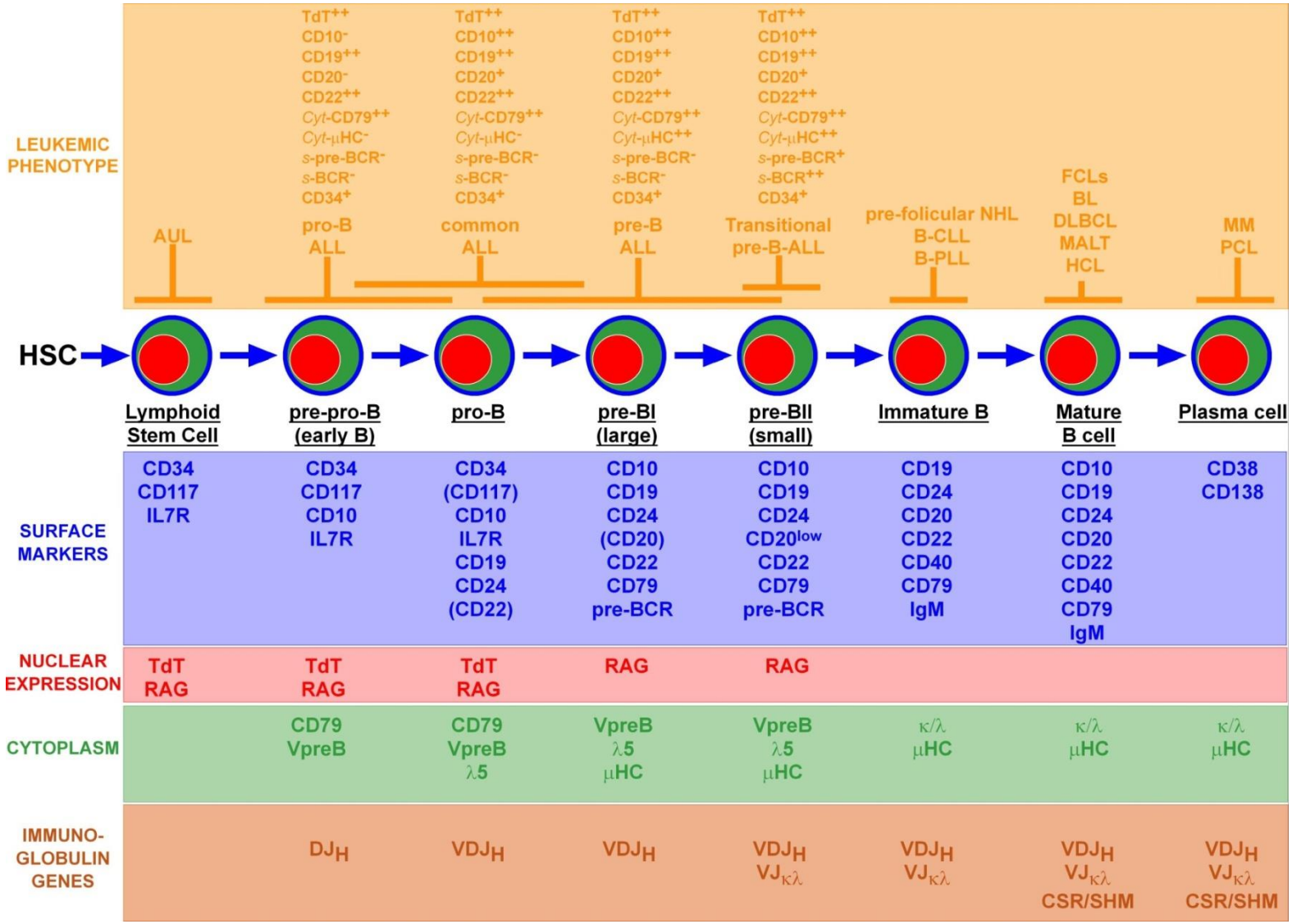
Do the oncogenes have a mode of action that is not homogeneous throughout the cancer cell population?

Classical model for the role of human cancer gene defects in tumour cell fate specification



Traditionally, the human cancer genetic defects have been thought to act on cells already committed to a differentiation program, in such a way that the tumoural phenotype is derived from that of the initial differentiated target cell

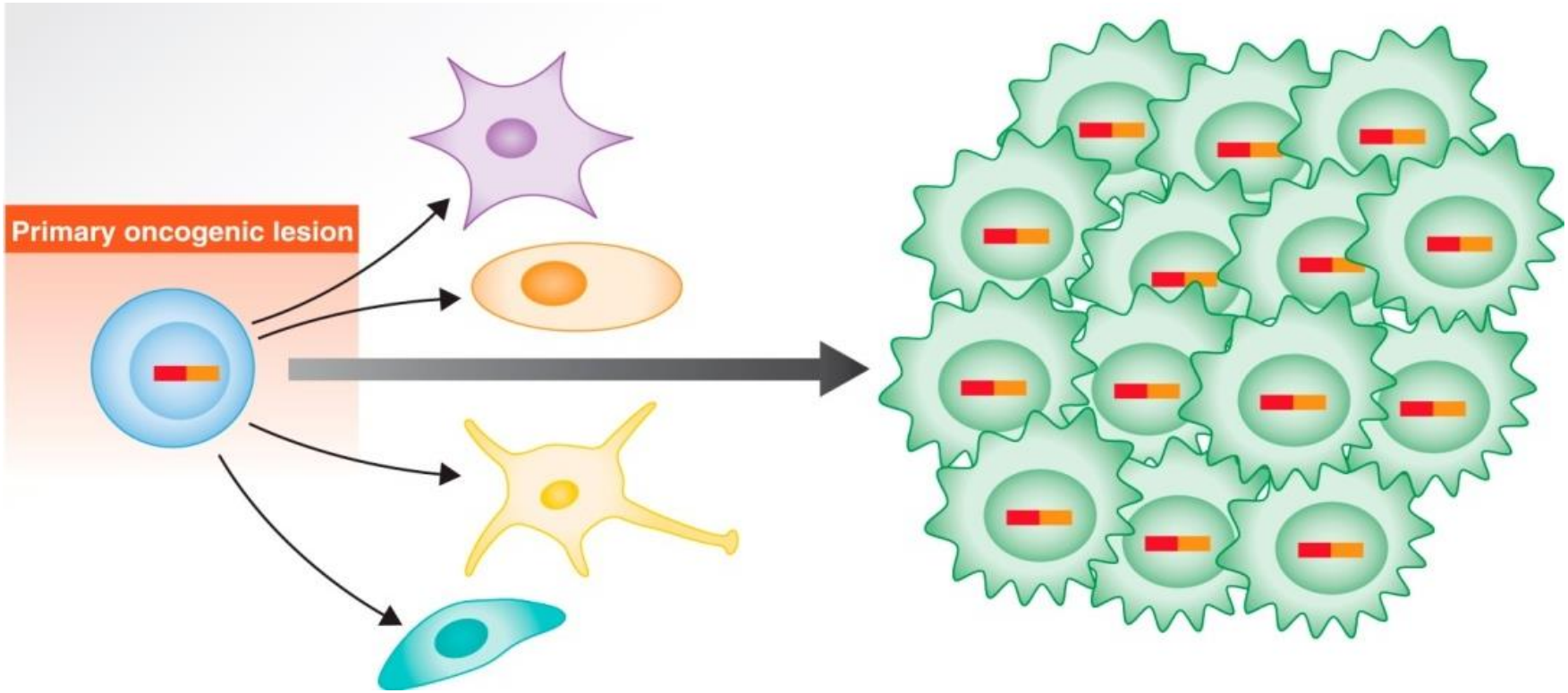
Presumptive Celular Origins of Chromosomal translocations in Human B-cell malignancies



Assignment of human B-cell malignancies to their normal B-cell counterparts

Cobaleda & Sanchez-García, BioEssays, 2009

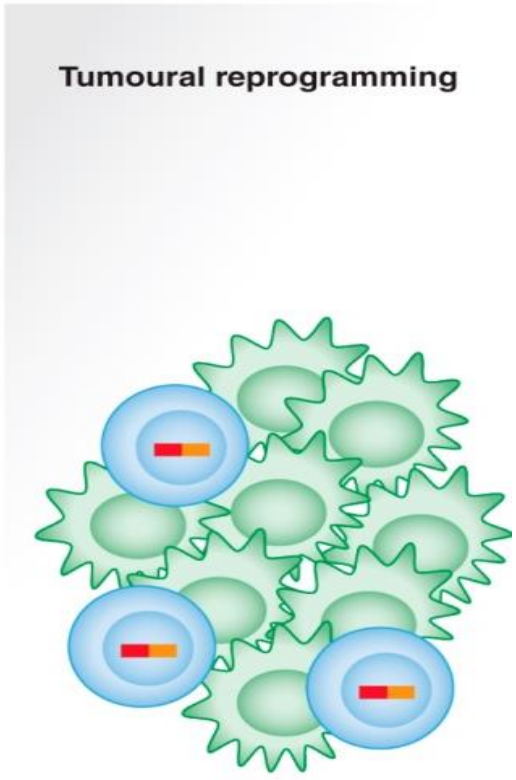
Alternative model for the role of human cancer gene defects in tumour cell fate specification



Alternative view in which the oncogenic lesion acts on stem/progenitor cells by imposing a given, oncogene-specific, tumour-differentiated cell fate.

The tumoural stem cell reprogramming hypothesis

Tumoural reprogramming: the process by which the initial oncogenic lesion(s) can 'reset' the epigenetic and/or transcriptome status of an initially healthy cell (the cancer cell-of-origin), therefore establishing a new, pathological differentiation program ultimately leading to cancer development, where the oncogenic lesion(s) does not need to be present anymore once the initial cancer fate-inducing change has taken place.



The EMBO Journal (2013) 32, 1502–1513
www.embojournal.org

THE EMBO JOURNAL

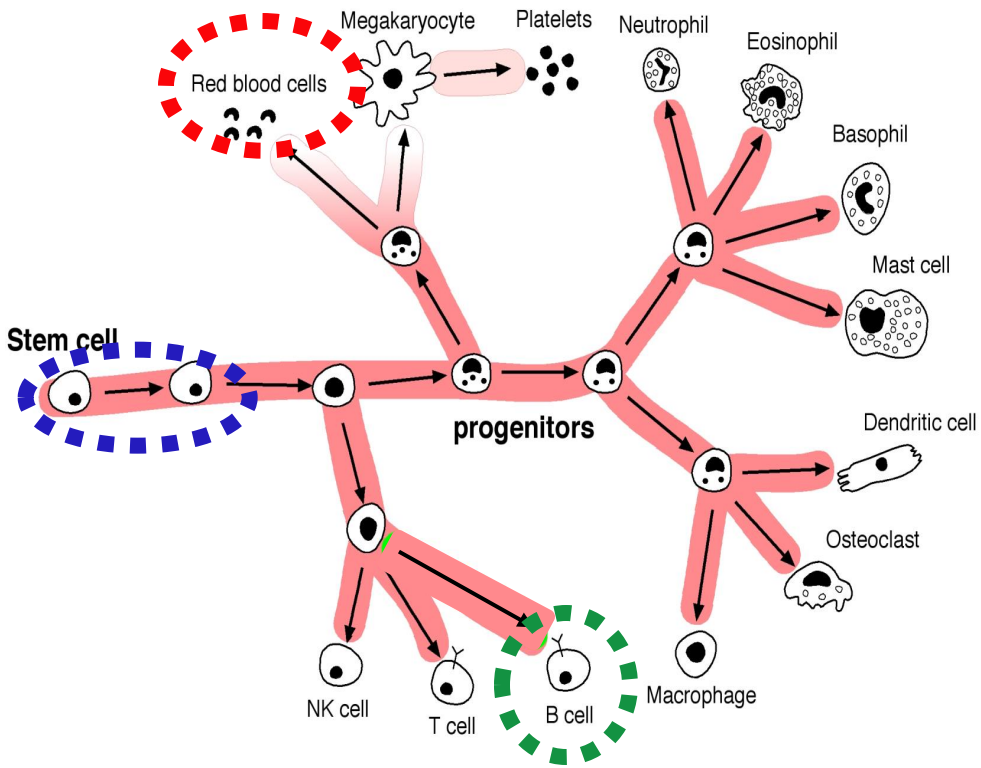
Review

Function of oncogenes in cancer development: a changing paradigm

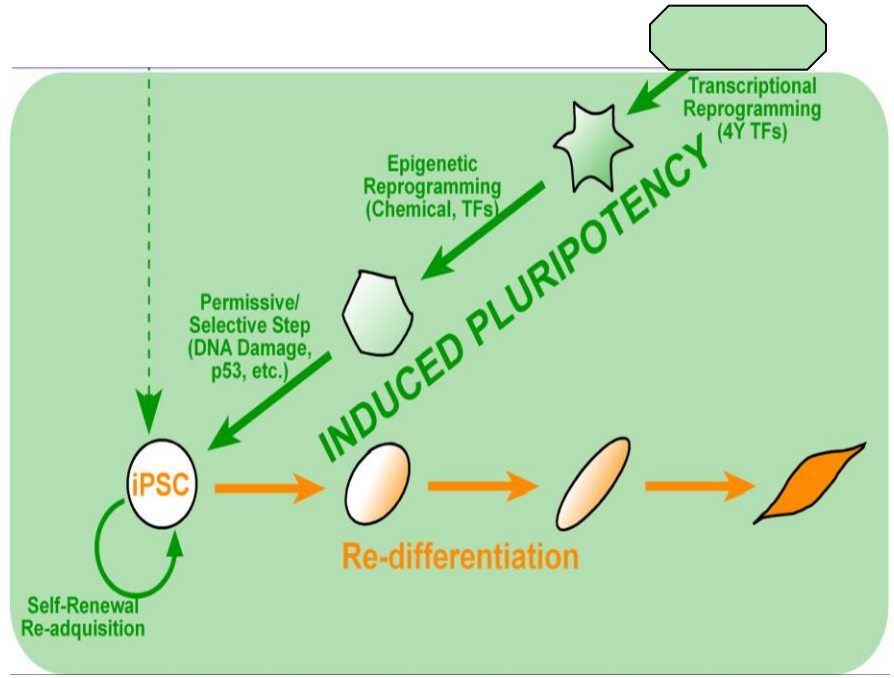
Carolina Vicente-Dueñas[1,2]
Isabel Romero-Camarero[1,2],
Cesar Cobaleda[3,*] and
Isidro Sánchez-García[1,2,*]

Vicente-Dueñas *et al. The EMBO Journal* 2013

Normal tissue (Blood system)



Reprogramming to plutipotency



We reasoned that a similar organization could be happening for cancer formation

(hypothesis-driven research project).

In vivo experimental model of tumoral stem cell reprogramming

To be able to demonstrate this lack of homogeneity in the mode of action of oncogenes throughout the biological history of the tumor, it would be necessary to dissect and isolate the function that the oncogene is playing at the earliest stages of the disease, at the level of the cell-of-origin

PNAS PNAS PNAS

Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance

Jean-Pierre Gillet[a], Anna Maria Calcagno[a], Sudhir Varma[b], Miguel Marino[a], Lisa J. Green[a], Meena I. Vora[c], Chirayu Patel[a], Josiah N. Orina[a], Tatiana A. Eliseeva[a], Vineet Singal[a], Raji Padmanabhan[a], Ben Davidson[d], Ram Ganapathi[e], Anil K. Sood[f], Bo R. Rueda[g], Suresh V. Ambudkar[a], and Michael M. Gottesman[a,1]

[a]Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, [b]Bioinformatics and Computational Biosciences Branch, Office of Cyber Infrastructure and Computational Biology, Office of Science Management and Operations, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; [c]Biophase Systems, Fremont, CA 94539; [d]Division of Pathology, Norwegian Radium Hospital, Oslo University Hospital, and The Medical Faculty, University of Oslo, 0310 Oslo, Norway; [e]Cleveland Clinic Taussig Cancer Institute, Cleveland, OH 44195; [f]Departments of Gynecologic Oncology and Cancer Biology, and Center for RNA Interference and Non-Coding RNA, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030; and [g]Vincent Center for Reproductive Biology, Vincent Department of Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA 02114

Edited by Ira Pastan, National Cancer Institute, National Institutes of Health, Bethesda, MD, and approved October 10, 2011 (received for review July 21, 2011)

Although in vitro models have been a cornerstone of anti-cancer drug development, their direct applicability to clinical cancer research has been uncertain. Using a state-of-the-art Taqman-based quantitative RT-PCR assay, we investigated the multidrug resistance (MDR) transcriptome of six cancer types, in established cancer cell lines (grown in monolayer, 3D scaffold, or in xenograft) and clinical samples, either containing >75% tumor cells or microdissected. The MDR transcriptome was determined a priori based on an extensive curation of the literature published during the last three decades, which led to the enumeration of 380 genes. No correlation was found between clinical samples and established cancer cell lines. As expected, we found up-regulation of genes that would facilitate survival across all cultured cancer cell lines evaluated. More troubling, however, were data showing that all of the cell lines, grown either in vitro or in vivo, bear more resemblance to each other, regardless of the tissue of origin, than to the clinical samples they are supposed to model. Although cultured cells can be used to study many aspects of cancer biology and response of cells to drugs, this study emphasizes the necessity for new in vitro cancer models and the use of primary tumor models in which gene expression can be manipulated and small molecules tested in a setting that more closely mimics the in vivo cancer microenvironment so as to avoid radical changes in gene expression profiles brought on by extended periods of cell culture.

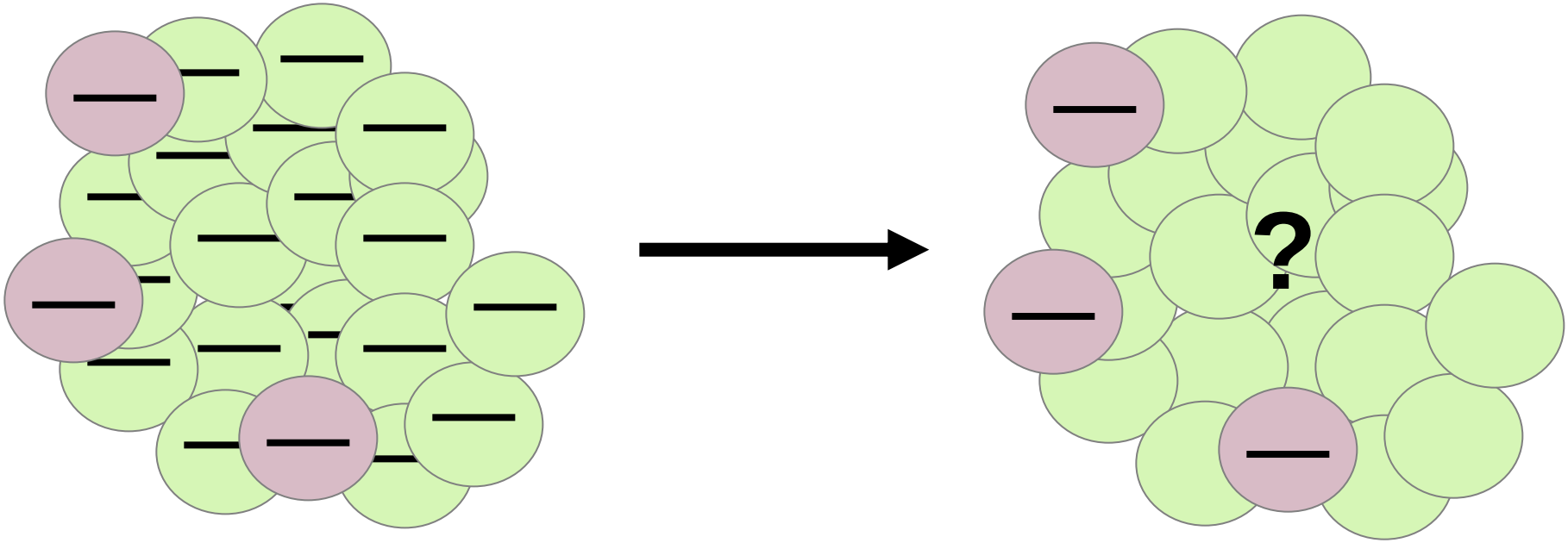
characterized, we chose to use them, and additional cancer cell lines, to assess the relevance of cultured cell lines in the study of clinical multidrug resistance (MDR) mechanisms (12).

Over the past 30 y, in vitro studies have led to the enumeration of close to 400 genes whose expression affects response to chemotherapy (13). Among those genes, ATP-binding cassette (ABC) transporters, a superfamily of 48 highly homologous members classified in seven subfamilies, have an important role in the pleiotropic mechanisms mediating MDR by exporting chemotherapeutic agents from the cell (14, 15). Although the roles of 13 ABC transporters in MDR have been fully characterized, recent studies suggest the involvement of up to 30 members of the 48 encoded in the human genome (16, 17). Moreover, besides classical drug efflux, it has also been demonstrated that some of these transporters may mediate the intracellular sequestration of chemotherapeutic drugs (18–20). This intracellular sequestration is the case for ABCA3, which was recently found to be overexpressed in clinical samples of childhood AML and correlated with poor response to treatment (21).

The establishment of a specific and sensitive standard assay, capable of discriminating highly homologous genes, is critical to a better understanding of MDR mechanisms. We and others have shown that Taqman Low Density Arrays (TLDAs) provide the most sensitivity and specificity in measuring the expression patterns of ABC transporter genes (22, 23). Therefore, we chose to configure such a platform to study multidrug resistance

Human Cancer tissue

In vivo experimental model of tumoural stem cell reprogramming



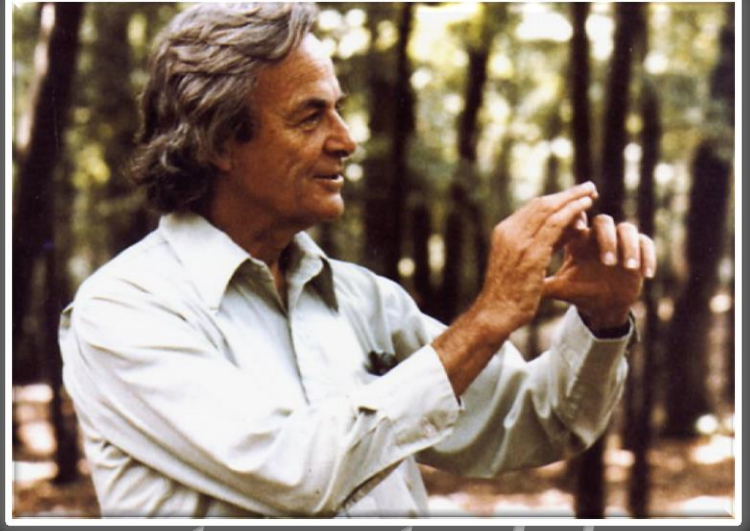
Genetic defect is present in both CSC and differentiated tumor cells

Genetic defect is only present in CSC

Might cancer stem cells initially arise thorugh a reprogramming-like mechanism?

To be able to demonstrate this lack of homogeneity in the mode of actions of oncogenes throughout the biological history of the tumour.

This still unexplored possibility would have major implications for our understanding of the genesis and treatment of cancer

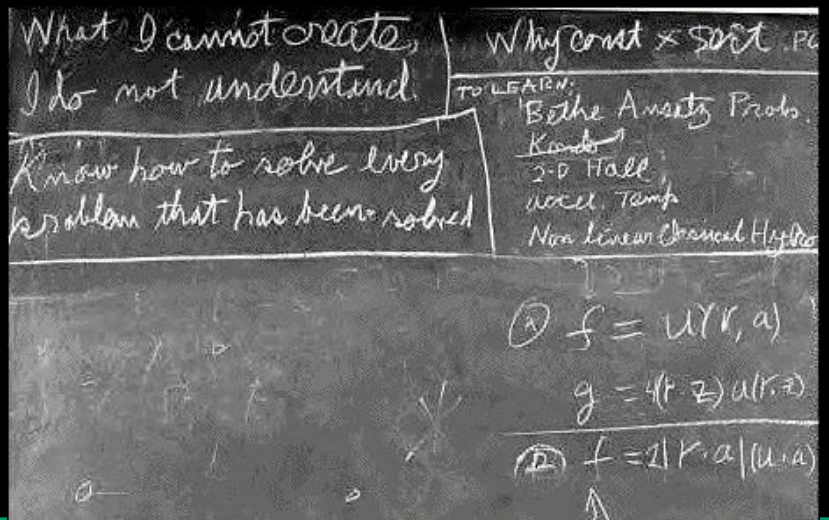


"What I cannot create, I do not understand"

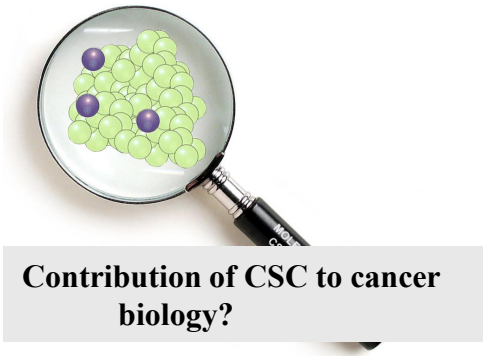
Richard P. Feynman

Nobel Prize in Physics 1965

Written on his blackboard at time of his death, in 1988



How to restrict oncogene expression to the stem cells

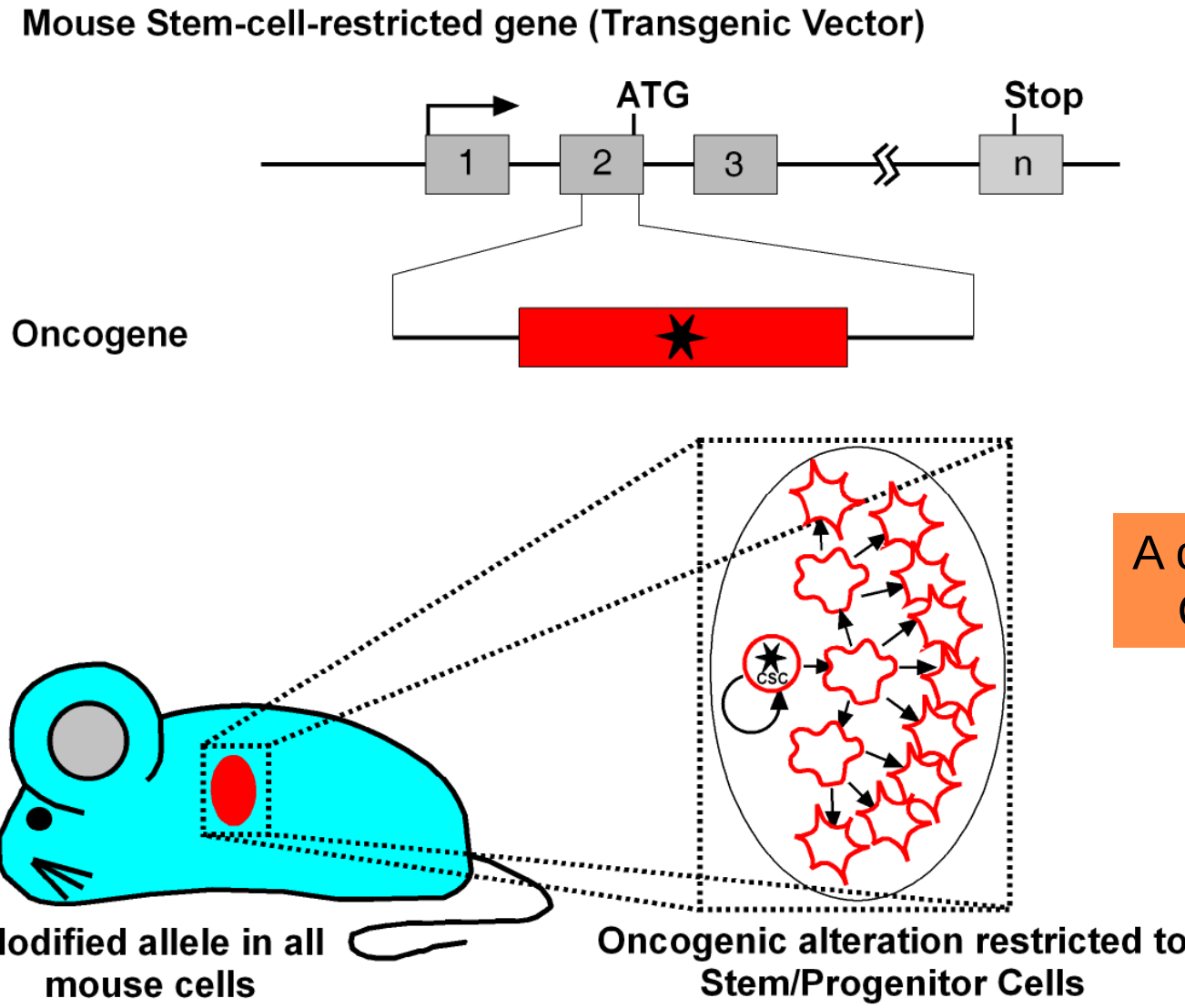


Contribution of CSC to cancer biology?

Sca1
(a gift of E. Dzierzak)

The key feature of these Sca1 mice is that they express an oncogene under the control of a promoter that is expressed in a population of stem/progenitor cells, but is switched off after lineage commitment.

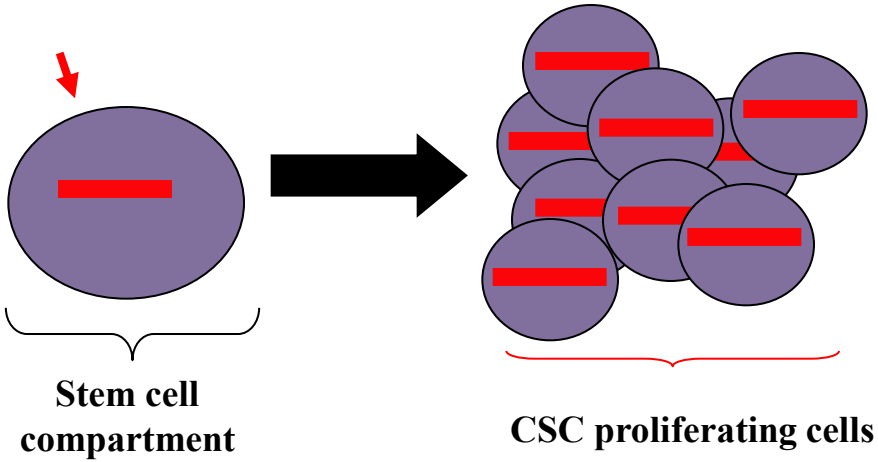
Constitutive Stem-Cell Restricted Oncogene Expression

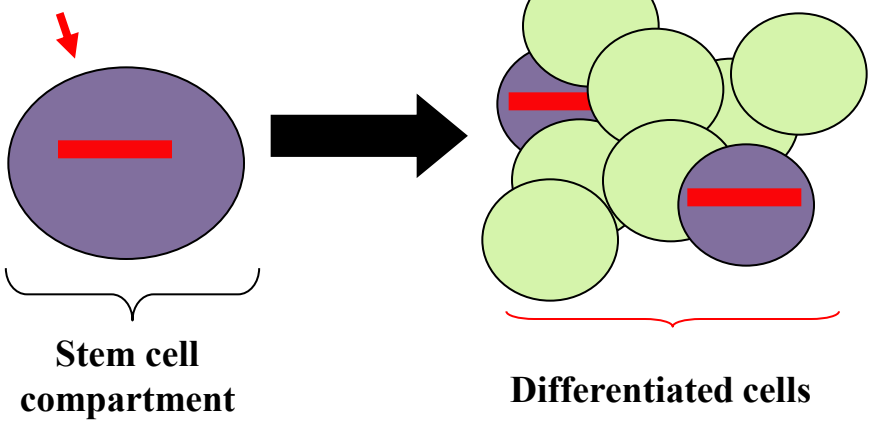


A cancer without Oncogene??

Dis Model Mech. 2010 Mar-Apr;3(3-4):149-55

Oncogene-induced plasticity and CSC

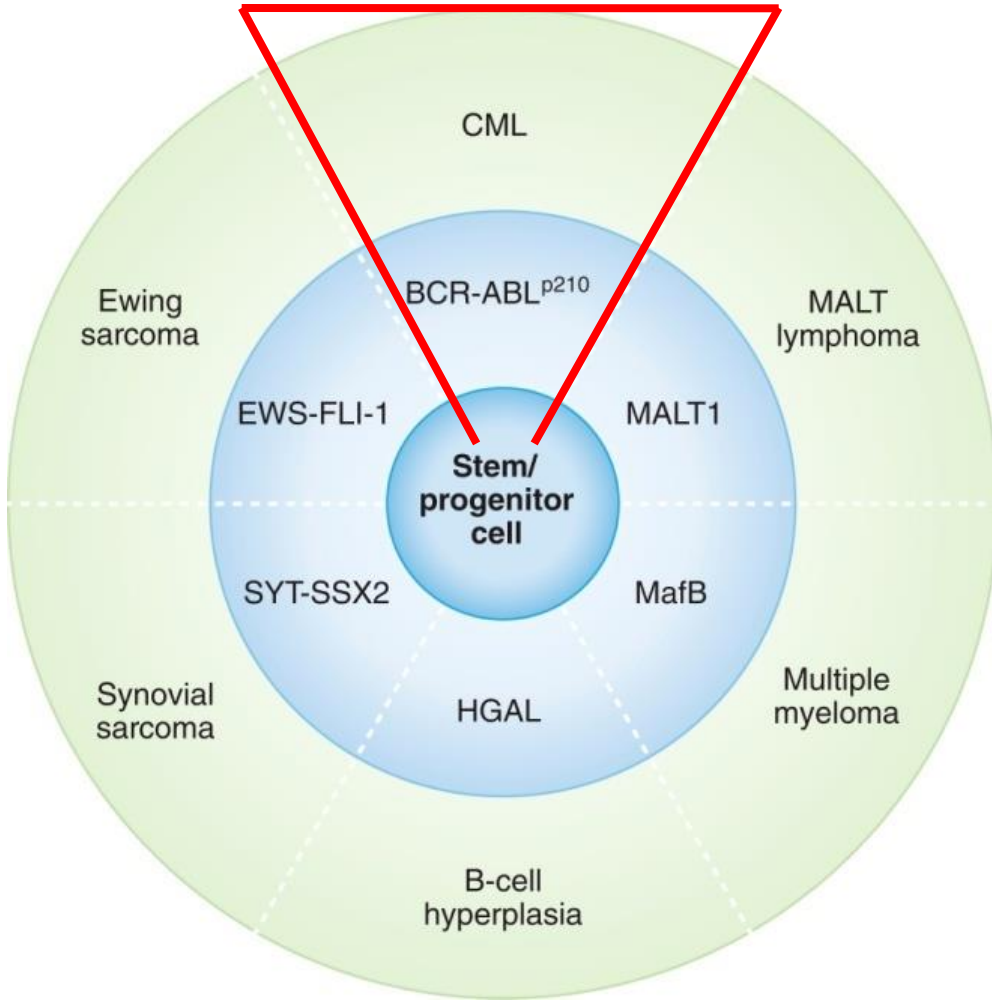




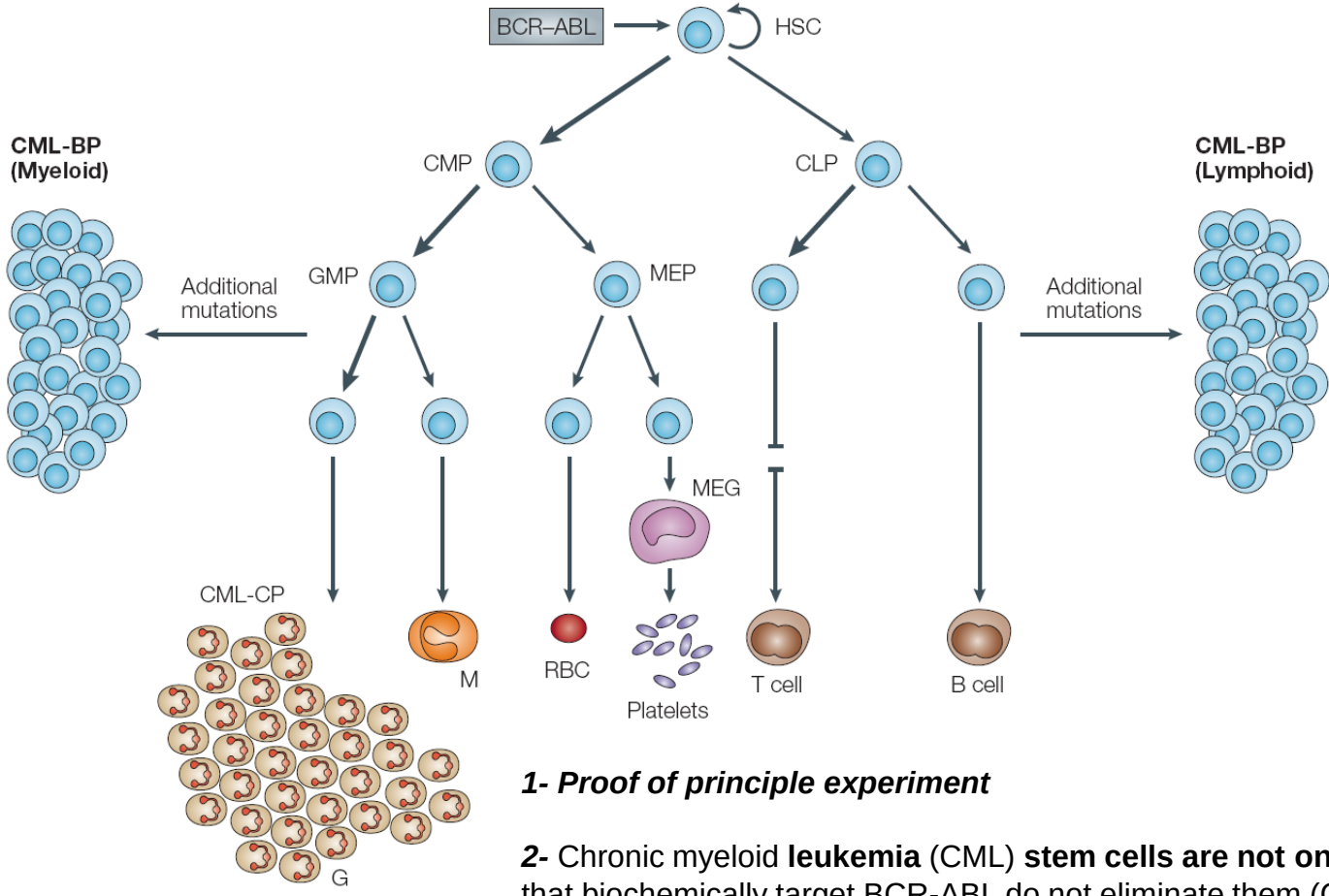
Genotype ⟷ **Phenotype**

Translocation	Genetic product	Tumour type
t(12;16)(q13;p11)	***FUS-DDIT3***	Myxoid Liposarcoma
t(16;21)(p11;q22)	***FUS-ERG***	Acute myeloid leukaemia
t(9;22)(q34;q11)	***BCR-ABLp190***	B acute lymphoblastic leukaemia
t(9;22)(q34;q11)	***BCR-ABLp210***	Chronic myeloid leukaemia
t(9;22)(q34;q11)	***BCR-ABLp230***	Chronic neutrophilic leukemia
t(**?**;3)(**?**;q27)	***?+ BCL6***	DLBCL/ Follicular lymphoma

Reprogramming in malignancies originated from stem cells



In vivo experimental model of tumoural stem cell reprogramming



1- Proof of principle experiment

2- Chronic myeloid **leukemia** (CML) **stem cells are not oncogene addicted** and the therapies that biochemically target BCR-ABL do not eliminate them (CML stem cells).

3-First animal model aniticipating human clinical results in the CSC field

4-Results were confirmed in human patients two years later

EMBO J. *28(1):8-20 (2009).*
Cell Cycle *8:1314-1318 (2009)*
N Engl J Med. *360(3):297-299 (2009)*

 Related Commentary, page 22

Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity

Amie S. Corbin,[1,2] Anupriya Agarwal,[1] Marc Loriaux,[1,3] Jorge Cortes,[4] Michael W. Deininger,[1] and Brian J. Druker[1,2]

[1]Division of Hematology and Medical Oncology, Oregon Health and Science University Cancer Institute, Portland, Oregon, USA. [2]Howard Hughes Medical Institute, Portland, Oregon, USA. [3]Department of Pathology, Oregon Health and Science University, Portland, Oregon, USA. [4]M.D. Anderson Cancer Center, University of Texas, Houston, Texas, USA.

Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival

*Ashley Hamilton,[1] *G. Vignir Helgason,[1] *Mirle Schemionek,[2] Bin Zhang,[3] Svetlana Myssina,[1] Elaine K. Allan,[1] Franck E. Nicolini,[4] Carsten Müller-Tidow,[2] Ravi Bhatia,[3] Valerie G. Brunton,[5] *Steffen Koschmieder,[2] and *Tessa L. Holyoake[1]

[1]Paul O'Gorman Leukemia Research Centre, Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom; [2]Department of Medicine A, Hematology, Oncology and Pneumology, University of Münster, Münster, Germany; [3]Department of Hematopoietic Stem Cell and Leukemia Research, City of Hope National Medical Center, Duarte, CA; [4]Hematology Department, Hôpital Edouard Herriot, Lyon, France; and [5]Institute of Genetics and Molecular Medicine, Edinburgh Cancer Research Centre, University of Edinburgh, Edinburgh, United Kingdom

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*A.H., G.V.H., M.S., S.K., and T.L.H. contributed equally to this study.

The online version of this article contains a data supplement.

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Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment

Su Chu,[1,2] Tinisha McDonald,[1,2] Allen Lin,[1,2] Sujata Chakraborty,[1,2] Qin Huang,[3] David S. Snyder,[2] and Ravi Bhatia[1,2]

[1]Division of Hematopoietic Stem Cell and Leukemia Research, [2]Department of Hematology and Hematopoietic Cell Transplantation, and [3]Department of Pathology, City of Hope National Medical Center, Duarte, CA

Submitted December 27, 2010; accepted September 6, 2011. Prepublished online as *Blood* First Edition paper, September 19, 2011; DOI 10.1182/blood-2010-12-327437.

The online version of this article contains a data supplement.

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Brief report

Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease

Jean-Claude Chomel,[1,2] Marie-Laure Bonnet,[2] Nathalie Sorel,[1,2] Angelina Bertrand,[2] Marie-Claude Meunier,[2] Serge Fichelson,[3] Michael Melkus,[4] *Annelise Bennaceur-Griscelli,[4] *François Guilhot,[2,5] and Ali G. Turhan[1,2]

[1]Service d'Hématologie et Oncologie Biologique, CHU de Poitiers, Poitiers, France; [2]Inserm U935, Université de Poitiers, Poitiers, France; [3]Institut Cochin, Inserm U1016, Université Paris Descartes, Paris, France; [4]Inserm U935, Université Paris-Sud 11, Paris, France; and [5]Inserm CIC 0802, CHU de Poitiers, Poitiers, France

Submitted February 7, 2011; accepted July 6, 2011. Prepublished online as *Blood* First Edition paper, July 25, 2011; DOI 10.1182/blood-2011-02-335497.

*A.B.-G. and F.G. contributed equally to this study.

The online version of this article contains a data supplement.

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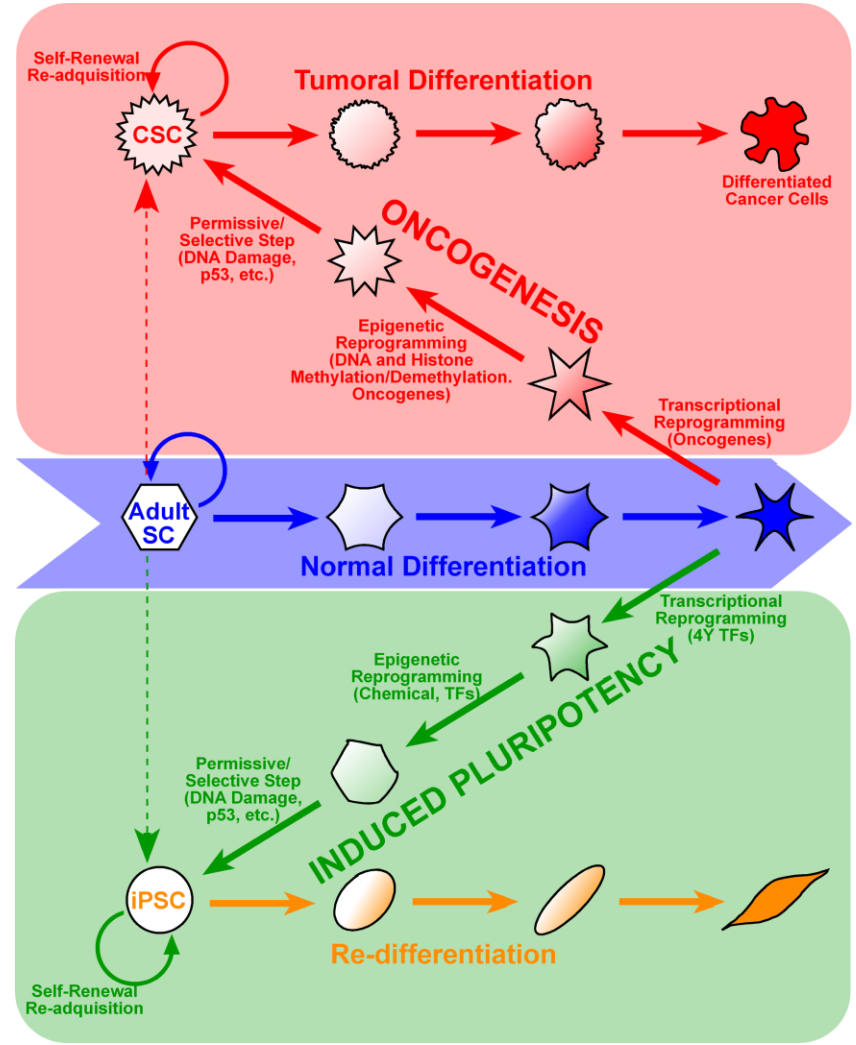
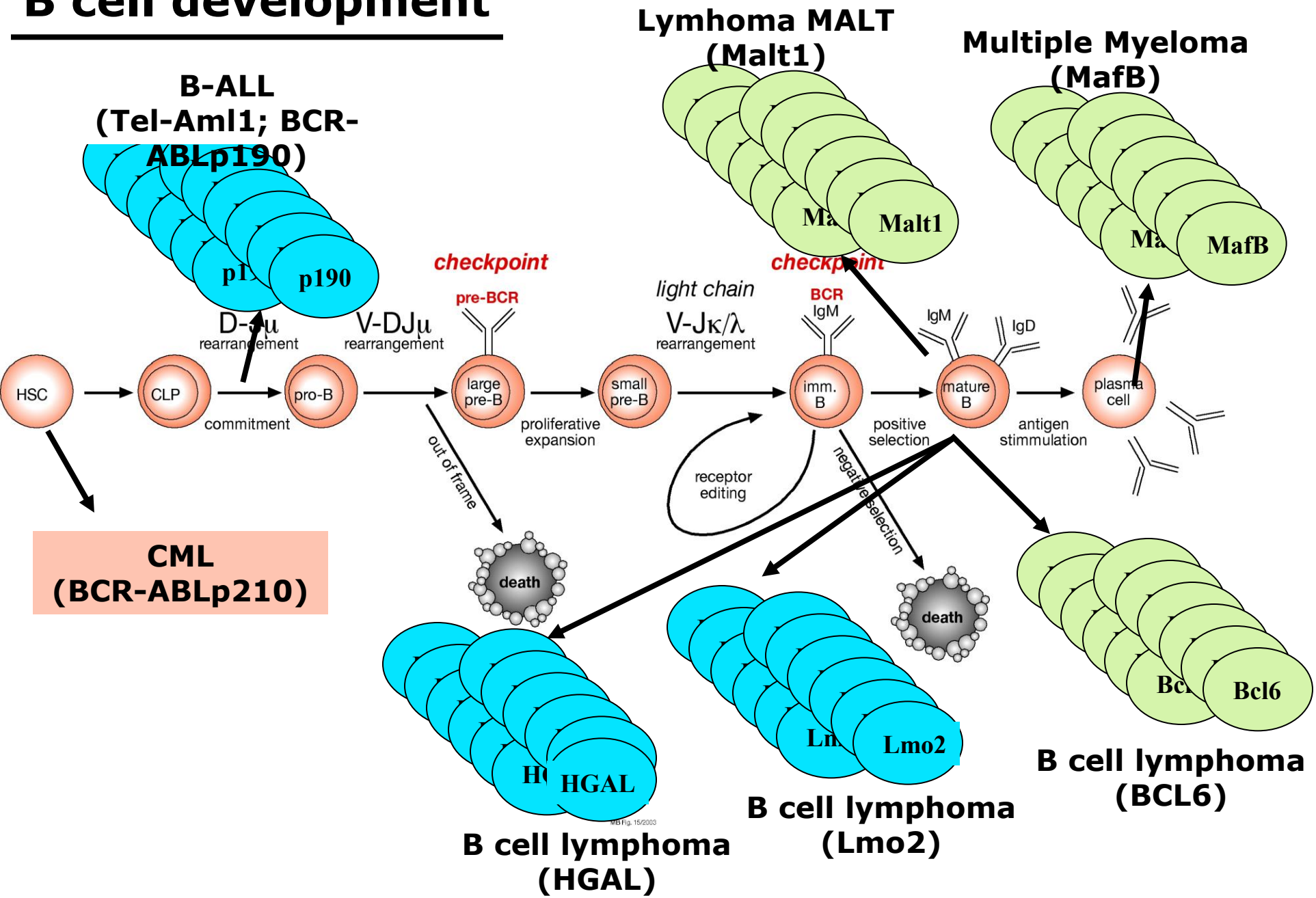
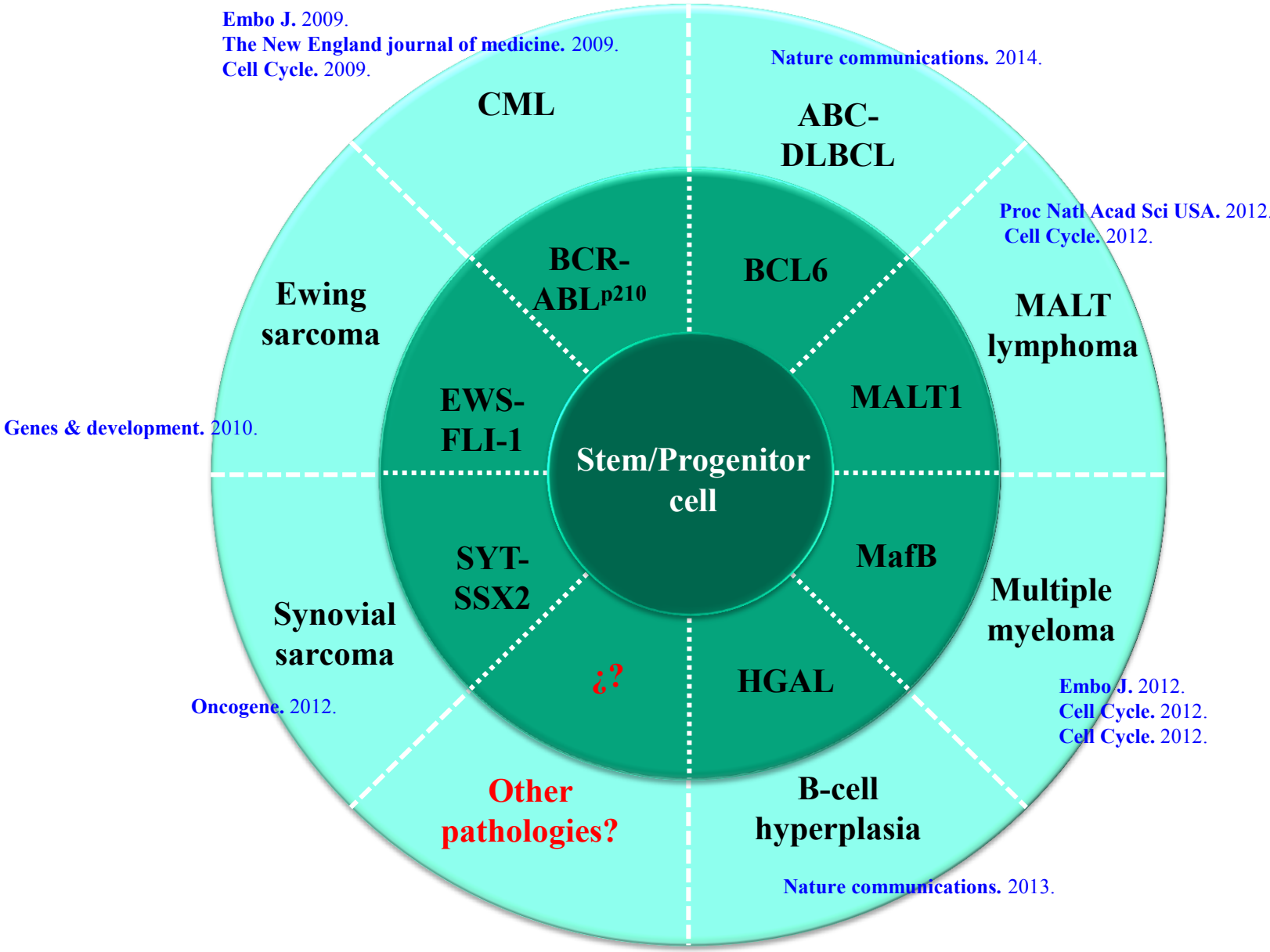
New concept of the human cancer as a Reprogramming-like Disease



Can this hypothesis be extrapolated to other malignancies?

B cell development



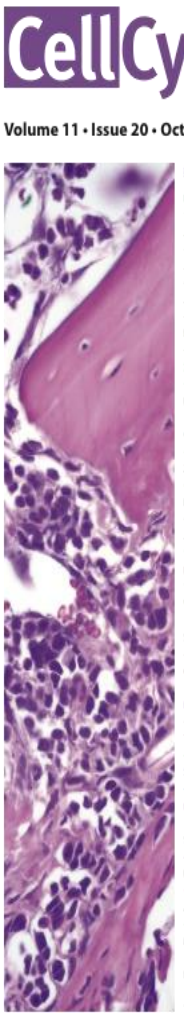

Embo J. 2009.
The New England journal of medicine. 2009.
Cell Cycle. 2009.
CML
Nature communications. 2014.
ABC-DLBCL
Proc Natl Acad Sci USA. 2012.
Cell Cycle. 2012.
MALT lymphoma
Multiple myeloma
Embo J. 2012.
Cell Cycle. 2012.
Cell Cycle. 2012.
B-cell hyperplasia
Nature communications. 2013.
Other pathologies?
Oncogene. 2012.
Synovial sarcoma
Genes & development. 2010.
Ewing sarcoma
BCR-ABLp210
BCL6
MALT1
MafB
HGAL
¿?
SYT-SSX2
EWS-FLI-1
Stem/Progenitor cell

Cancer as a result of tumoral epigenetic stem cell reprogramming

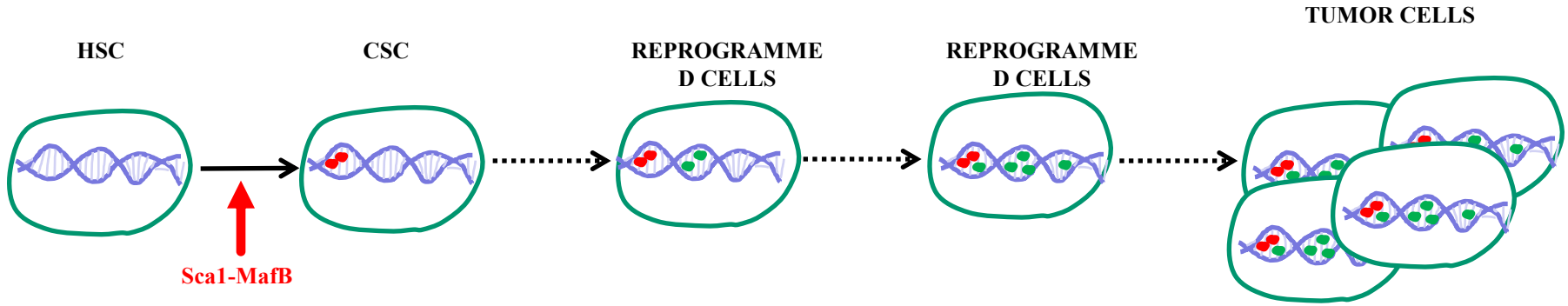
DNA damage

p53 loss should accelerate the tumor reprogramming process

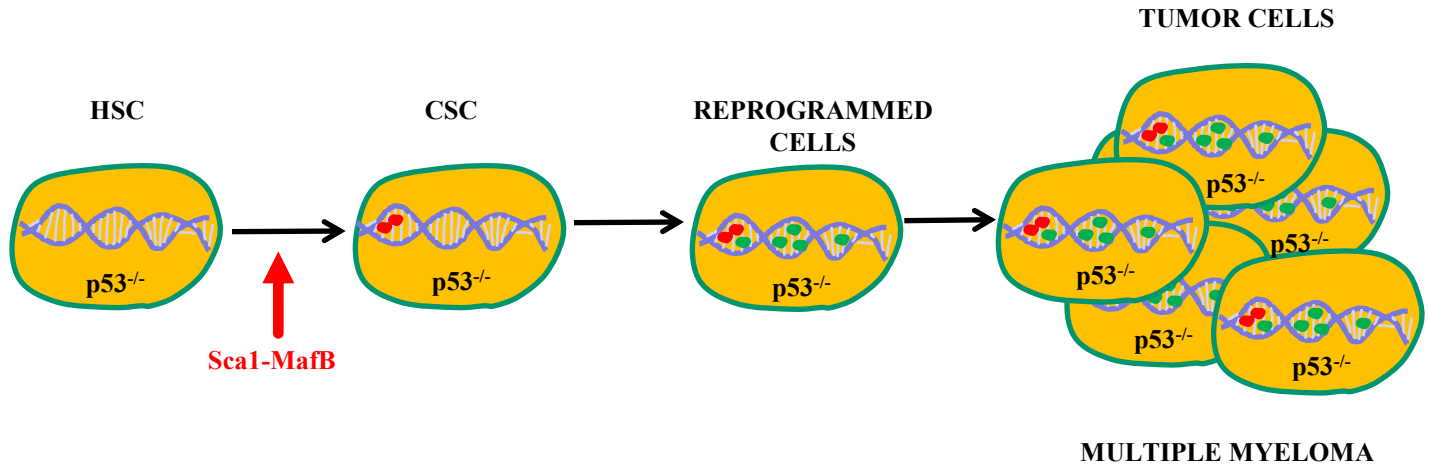
Tumour suppressors can act as barriers for tumoural stem cell reprogramming



Sca1-MafB MM development

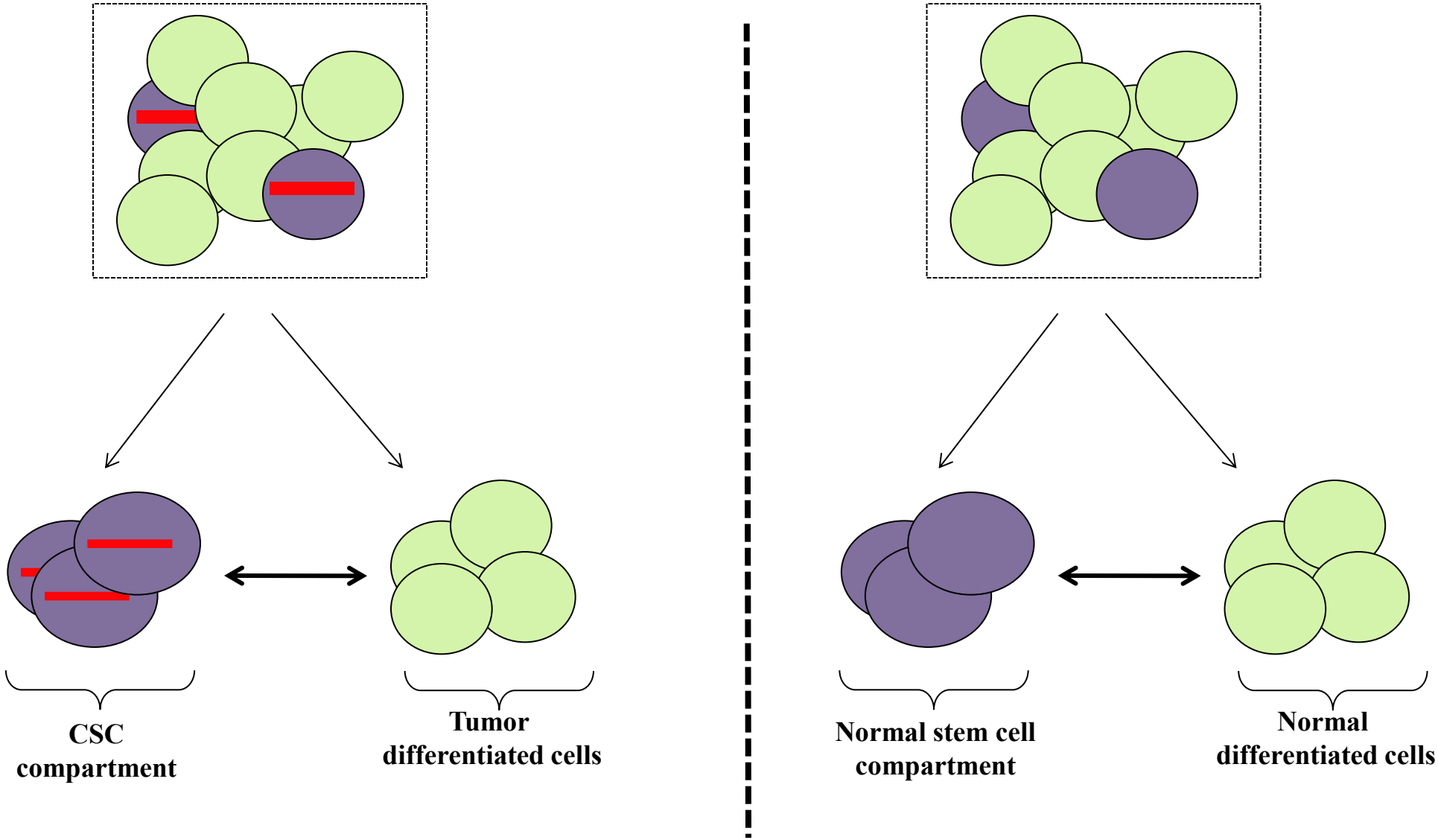


Sca1-MafB MM development in the absence of p53

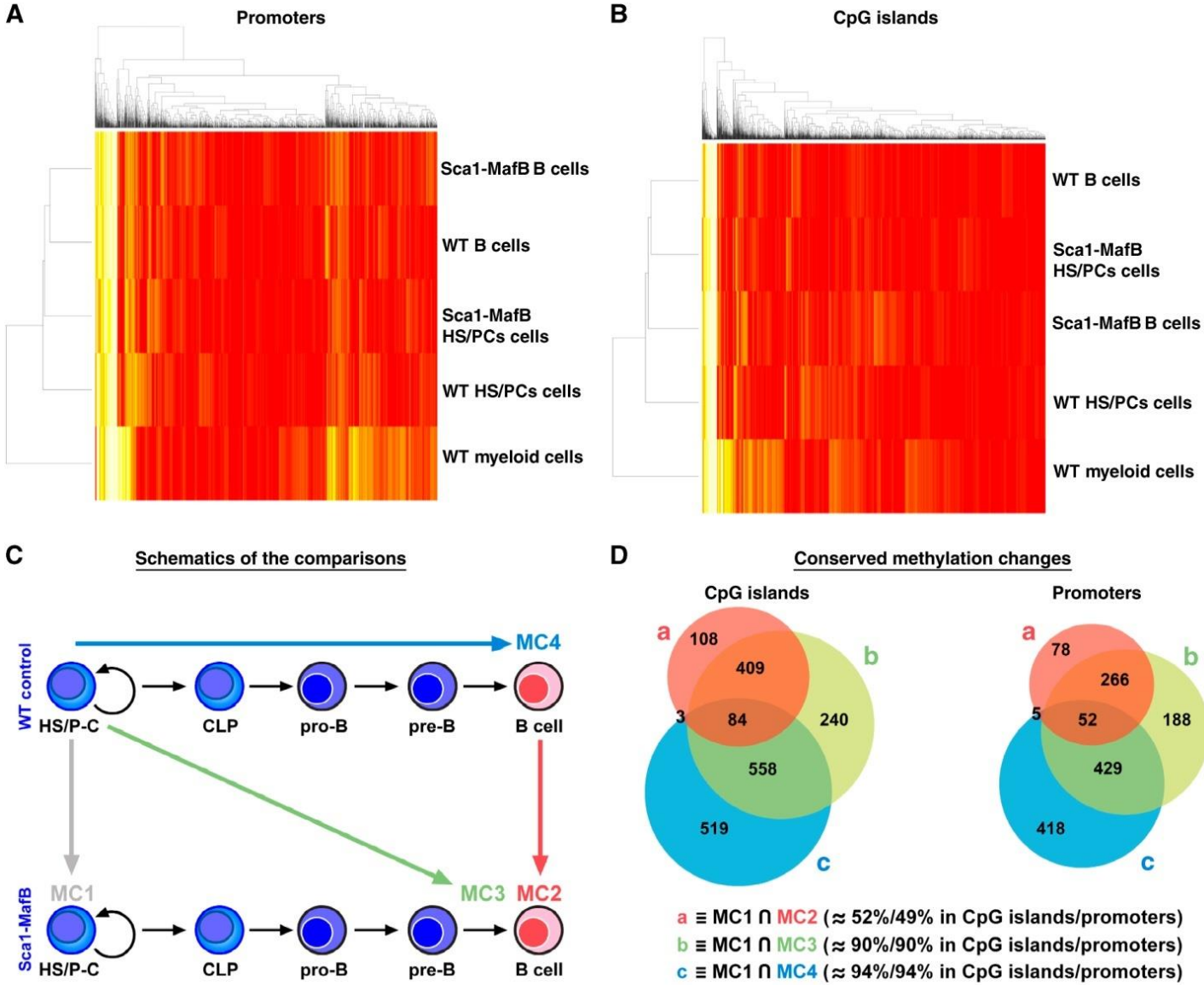


Cell Cycle 2012, 11(20): 3896-3900 (issue cover)

Are there evidences of tumoral epigenetic stem cell reprogramming??



Genome-scale DNA methylation maps of stem cells and mature B cells in mice predicts human cancer organization



Whether this mechanism is involved in the genesis of human cancers was presently not known, but recent results confirmed similar cellular hierarchy in human MM patients

Cancer Cell
Article

Xbp1s-Negative Tumor B Cells and Pre-Plasmablasts Mediate Therapeutic Proteasome Inhibitor Resistance in Multiple Myeloma

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Significance

PIs, including bortezomib, are a mainstay of treatment for MM but fail to cure. Previously reported in vitro resistance mechanisms have not been validated in the clinic and reflect an artifact of cell culture. An alternative PI resistance mechanism is described here that occurs in patients with MM; because this differs from in vitro resistance reports, the need for clinical confirmation of in vitro drug resistance models is highlighted. Our results reveal that MM cells tolerate *XBP1* inactivation, which contributes to therapeutic resistance, suggesting that *IRE1* inhibitors may prove ineffectual in MM. Furthermore, an extensive progenitor organization is revealed in primary MM. Our results suggest that to achieve cure, treatment strategies must better address early MM progenitors.

blood
Prepublished online February 22, 2013;
doi:10.1182/blood-2012-12-471888

Characterization Of IgH breakpoints in multiple myeloma indicates a subset of translocations appear to occur In pre-germinal center B cells

Brian A. Walker, Christopher P. Wardell, David C. Johnson, Martin F. Kaiser, Dil B. Begum, Nasrin B. Dahir, Fiona M. Ross, Faith E. Davies, David Gonzalez and Gareth J. Morgan

blood
2013 122: 1437-1447
Prepublished online July 11, 2013;
doi:10.1182/blood-2013-02-482919

RARα2 expression confers myeloma stem cell features

Ye Yang, Jumei Shi, Giulia Tolomelli, Hongwei Xu, Jiliang Xia, He Wang, Wen Zhou, Yi Zhou, Satyabrata Das, Zhimin Gu, Dana Levasseur, Fenghuang Zhan and Guido Tricot

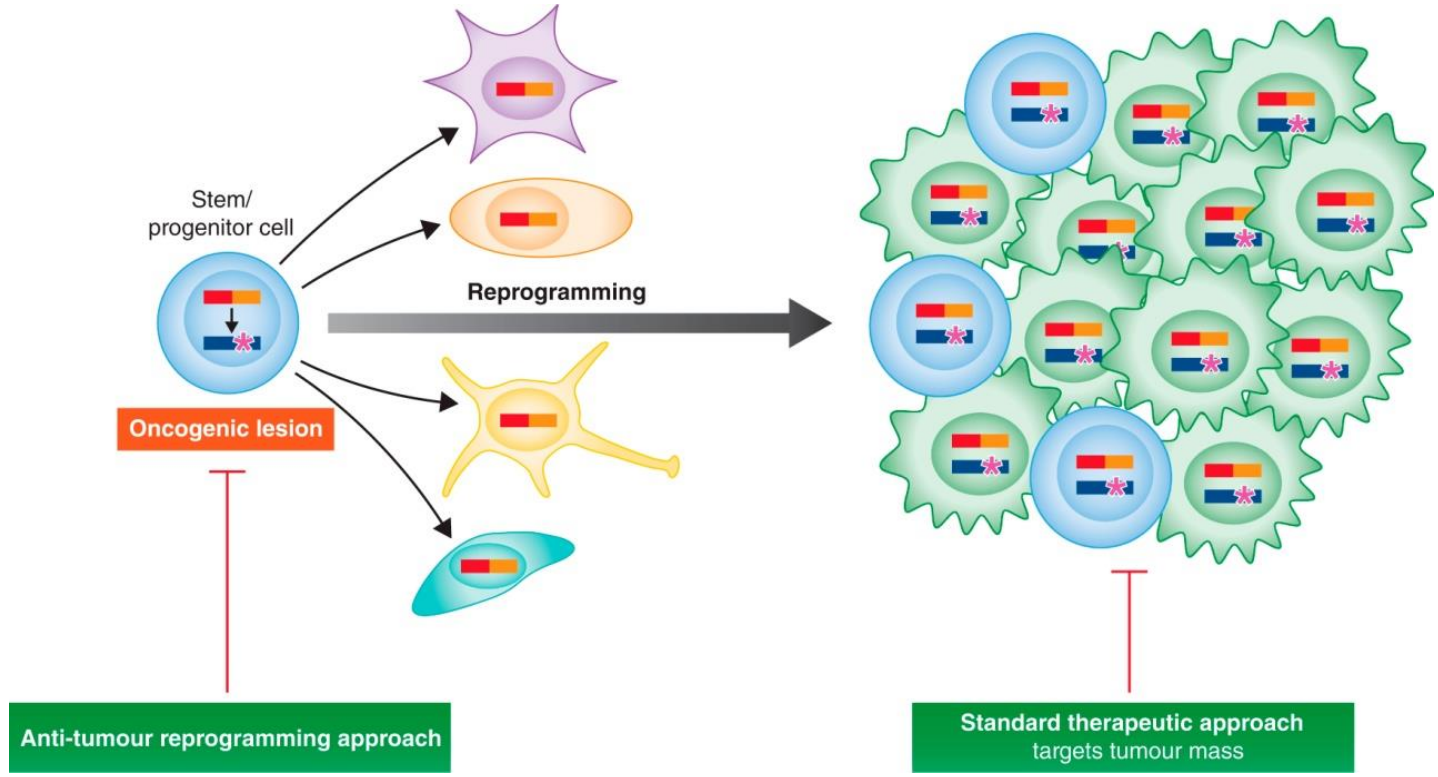
Clinical Cancer Research

Stemness of B cell progenitors in multiple myeloma bone marrow

Kelly Boucher, Nancy Parquet, Raymond Widen, et al.

Clin Cancer Res Published OnlineFirst September 17, 2012.

Tumour stem cell reprogramming and therapeutic implications



Reprogramming the cancer epigenome to an alternative lineage cell fate, non-tumoral fate, losing their malignancy?

Tumour stem cell reprogramming largely relies on epigenetic modifications. These, unlike genetic changes, can be erased, manipulated, and reinitiated, therefore implying that anti-tumour reprogramming strategies can provide a new window of opportunity to interfere with the cancer fate-inducing change.

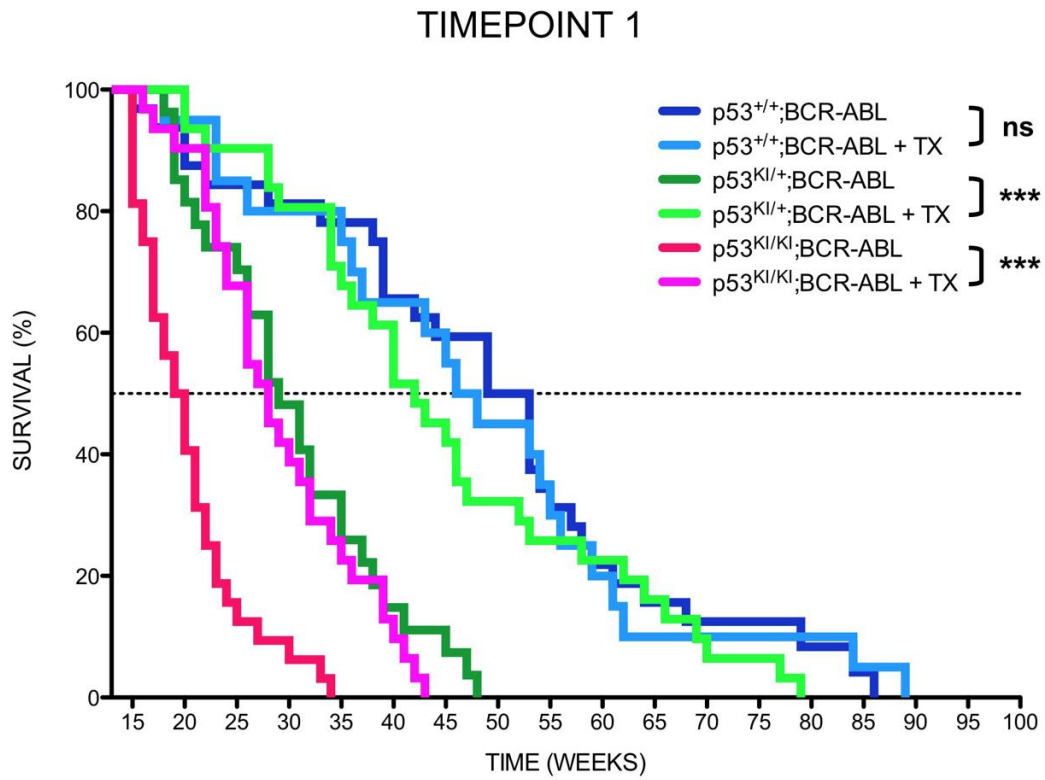
CSCs do not have oncogene addition

Oncogenes cannot be used as a target to kill CSCs

BUT, Tumour stem cell reprogramming is a specific CSC target

Could we use it to prevent/kill CSCs?

p53 RESTORATION KILLS PRIMITIVE LEUKEMIA CELLS *IN VIVO* AND INCREASES OVERALL SURVIVAL OF LEUKEMIC MICE

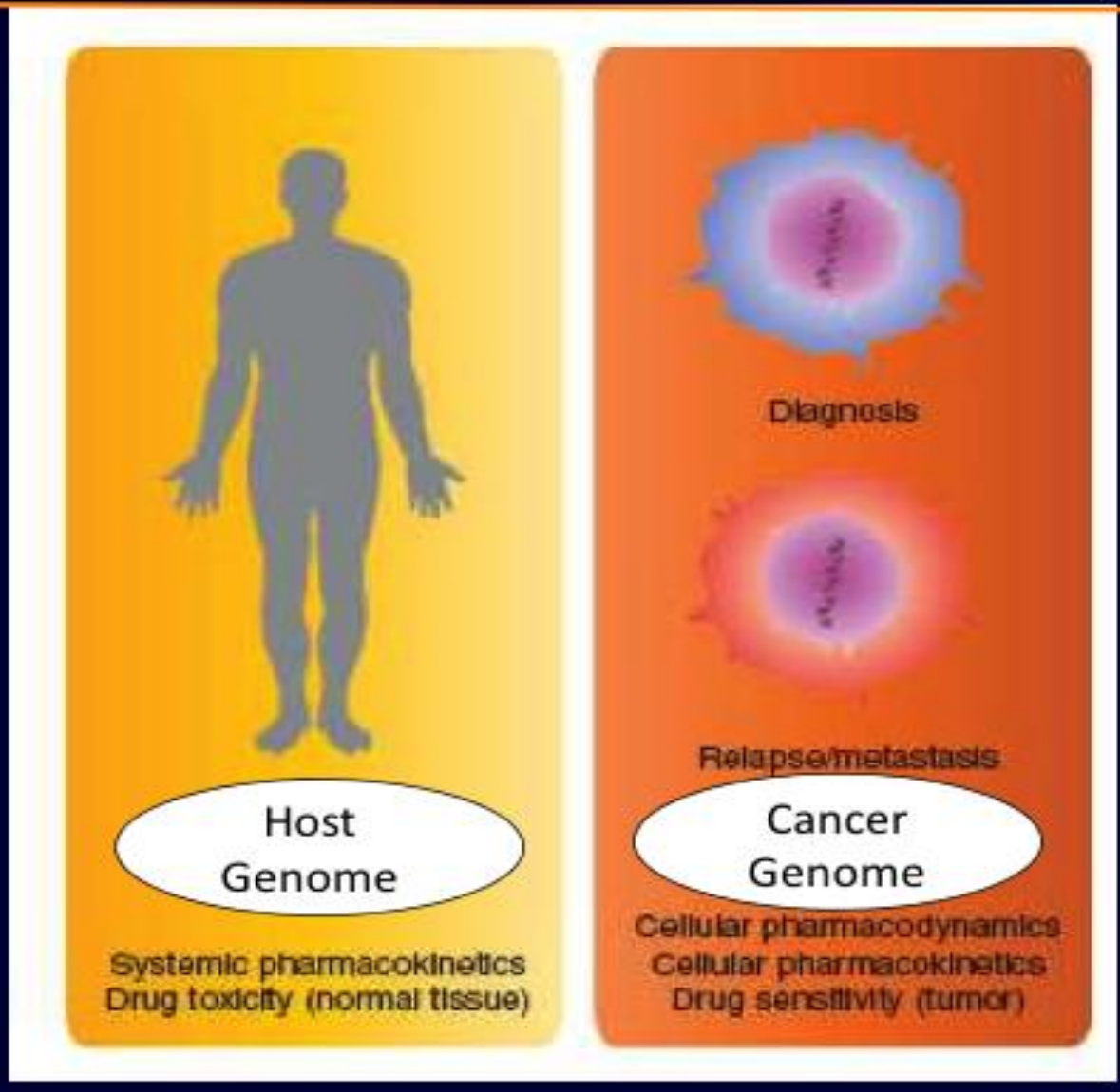


15 weeks (timepoint 1)

Cell Cycle. 2013;12(1):122-32.

Two Genomes Influence Every Cancer Patient

Germline



Somatic

Genetic background affects susceptibility to tumoral stem cell reprogramming

Genetic background affects stem cell reprogramming in Sca1-BCRABLp210 mice

Strain	No. of mice	No. with CML(%)	No.with B-cell leukemia	No. with T-cell lymphoma	NO TUMORS
B6	23	23(100)	0	0	0
B6/FVB	35	10(28,5)	6(17,2)	0	19(54,3)
FVB	11	0	0	11(100)	0

These results demonstrate for the first time that tumoral stem cell reprogramming fate is subject to polymorphic genetic control

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Thank you for your attention!!!!