UNIVERSITY OF CAMBRIDGE

"Cancer stem cells, evolution and heterogeneity within tumours"

Brian Huntly,

Professor of Leukaemia Stem Cell Biology,
Stem Cell Institute, University of Cambridge
Co-Lead Haematological Malignancies Programme, Cambridge Cancer Centre
and Honorary Consultant Haematologist, CUH
Visiting Scientist WTSI

Lecture series in Cancer Biology

wellcome trust sanger institute

Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute

CANCER RESEARCH UK | CAMBRIDGE CENTRE

Talk outline

- **Context and definitions**

- Tumour hierarchy: the cancer stem cell hypothesis and its implications for tumour biology

- Pre-malignancy – overt and covert premalignant states and early tumour evolution

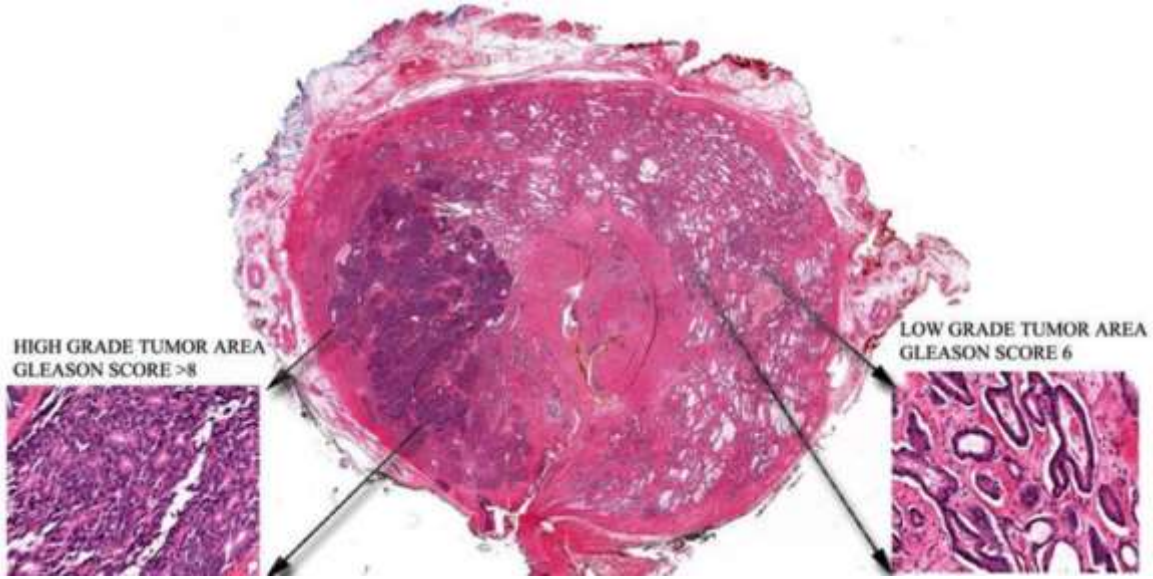
- Tumour evolution under selective pressures:
  - Relapse
  - Metastases

- Clinical implications: Cancer stem cells
  Targeting tumour heterogeneity

Levels of heterogeneity

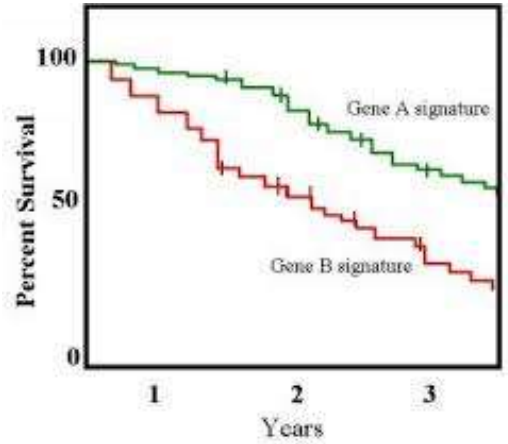
Heterogeneity of phenotype- Between tumours and within tumours

- Visual



- Behaviour – response to drugs

Between



Within

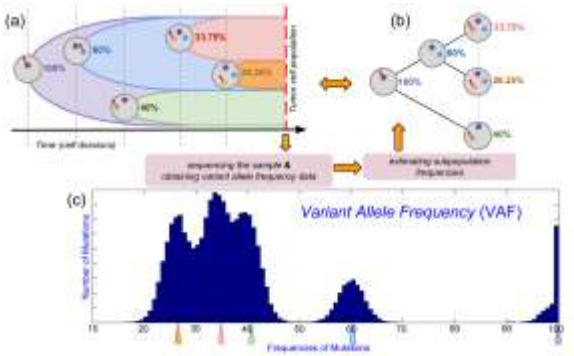
Chemo-resistant relapse

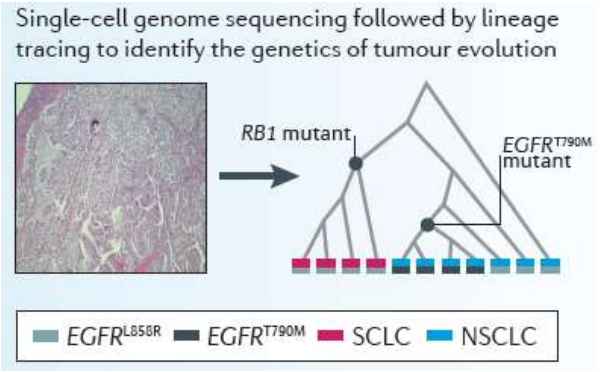
Levels of heterogeneity

Bulk tumour

Single cell analysis

- Genetic



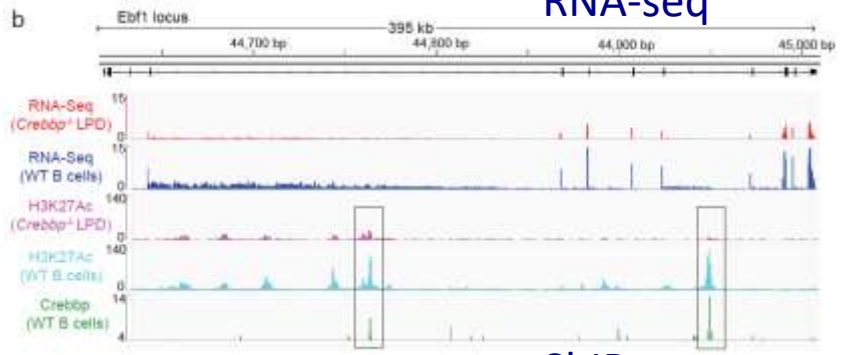


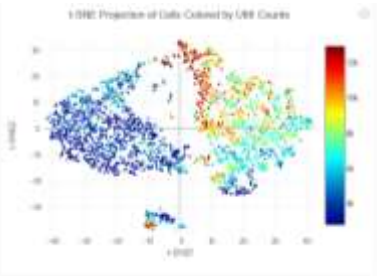
- Genomic

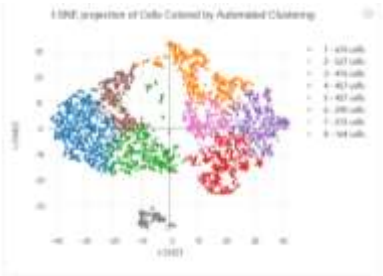
RNA-seq

10x scRNA-Seq

Transcription





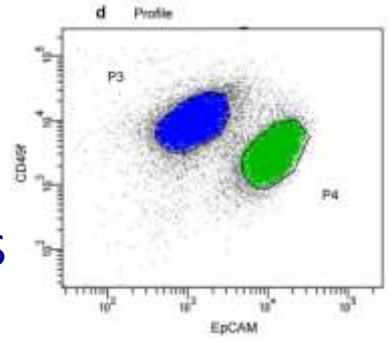


Epigenetic

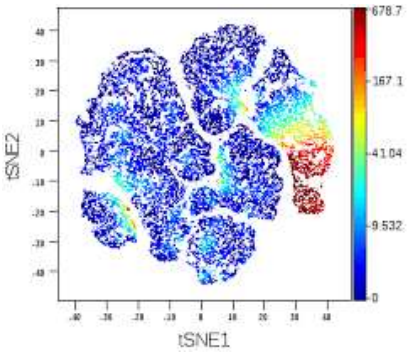
ChIP-seq

- Proteomic

FACS



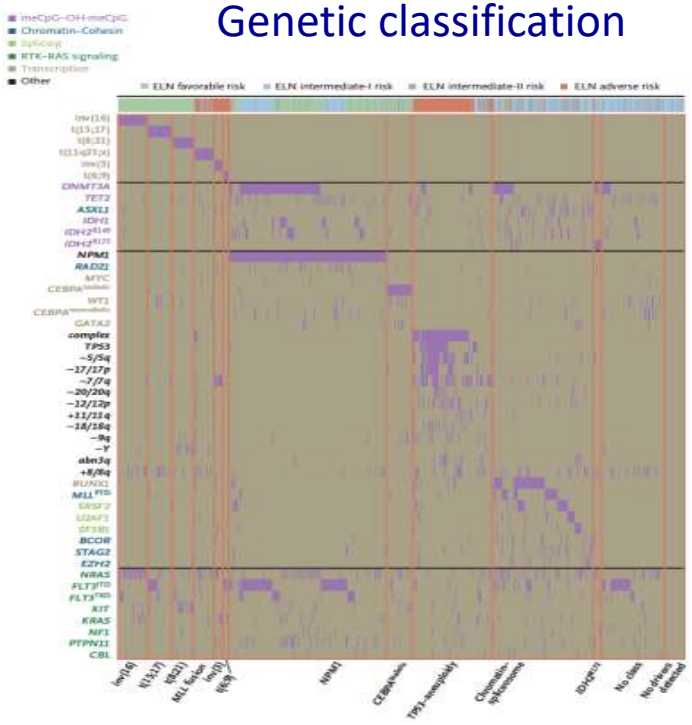
CyTOF Mass Cytometry



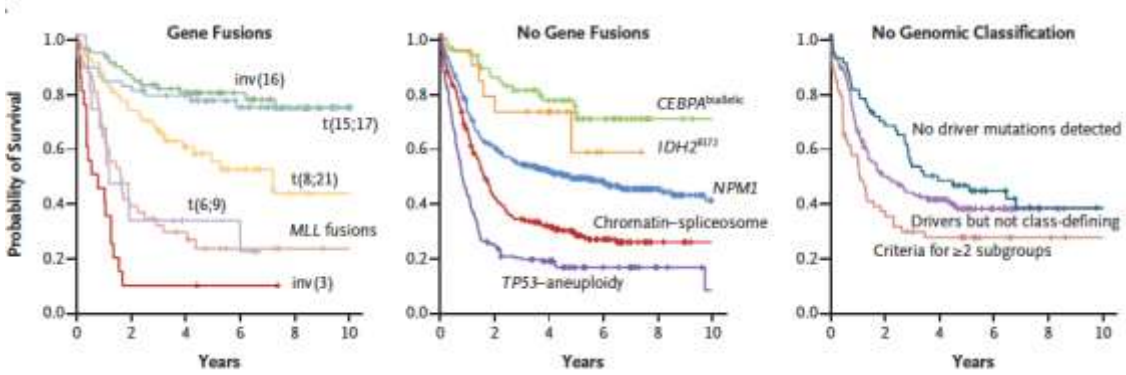
Heterogenetiy between tumours: the basis for tumour classiciation and disease prognostication

CLASSIFIERS

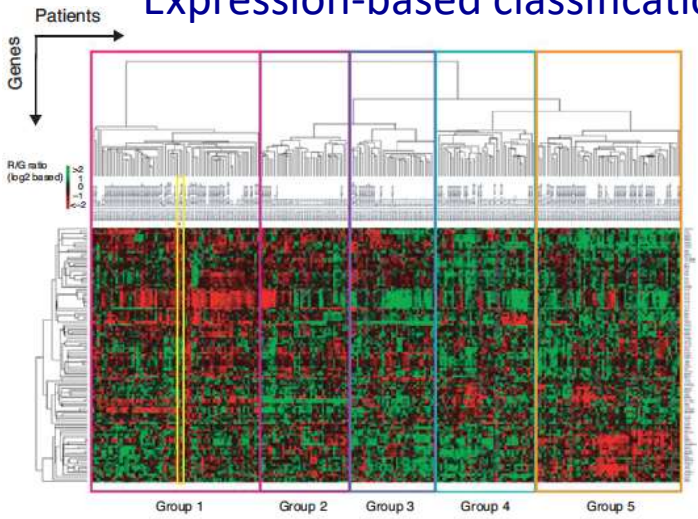
Genetic classification

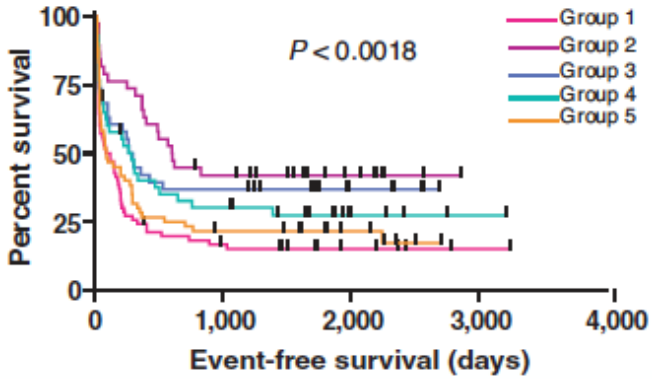


OUTCOME MEASURES

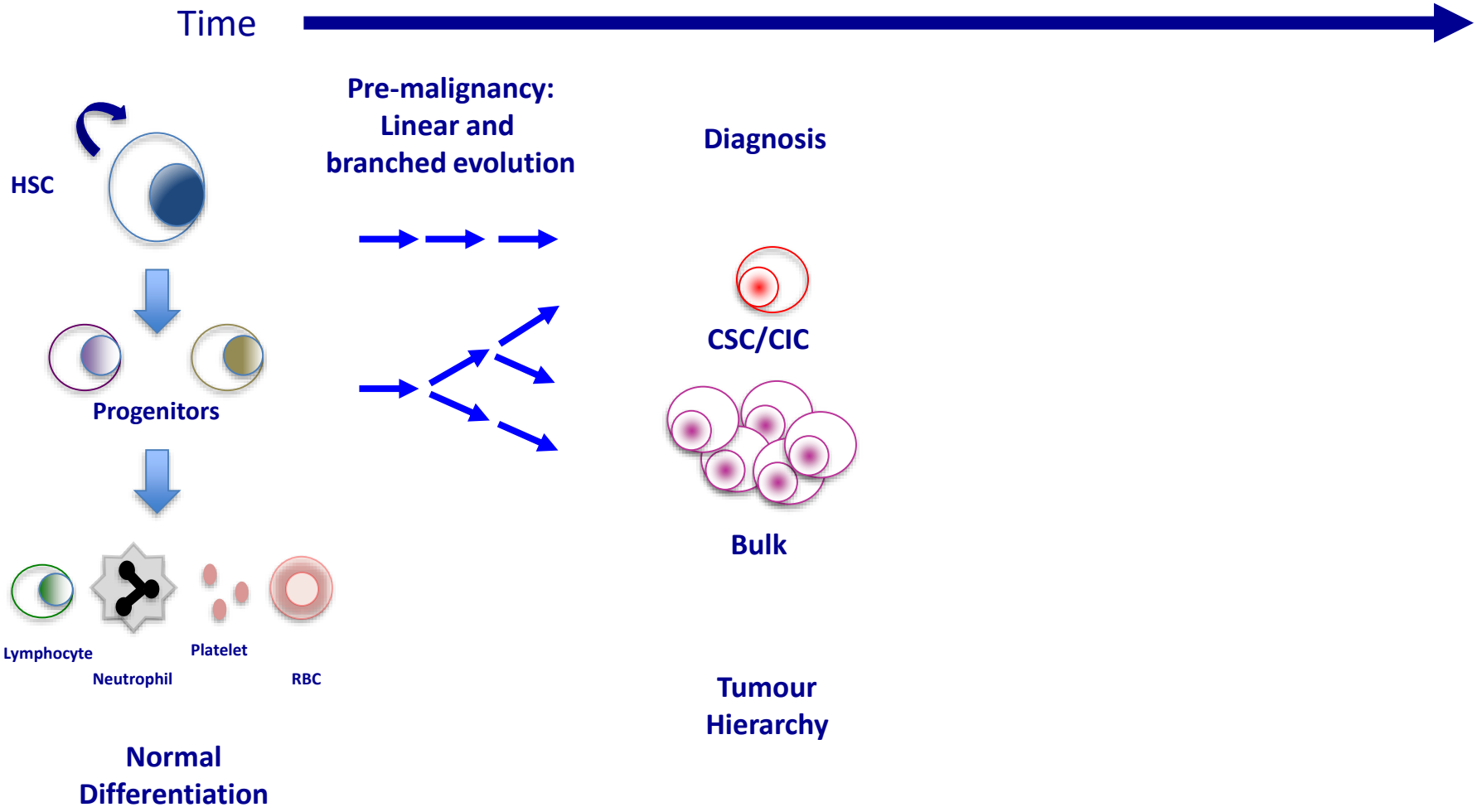


Expression-based classification

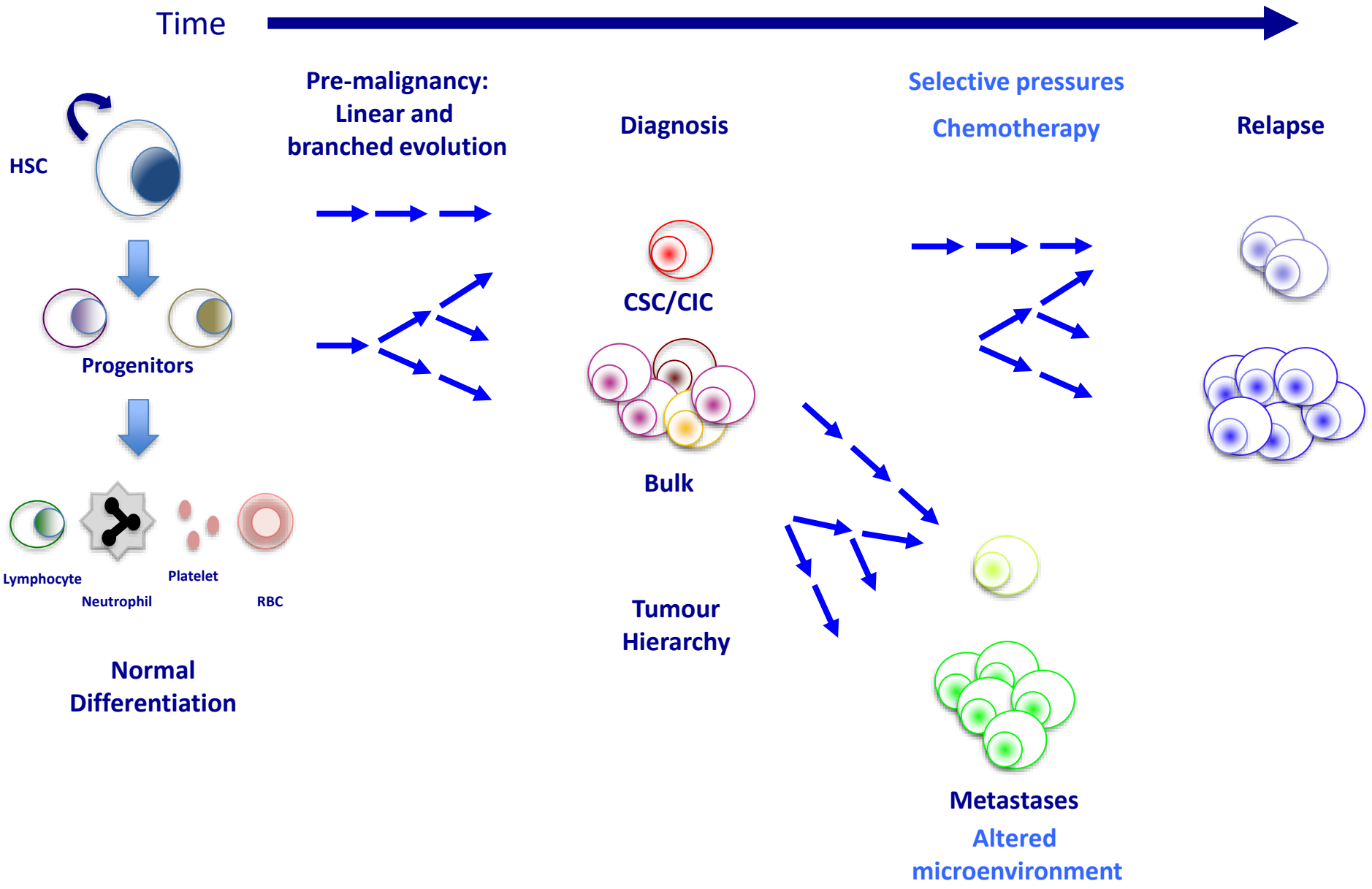




How is heterogeneity generated within tumours?



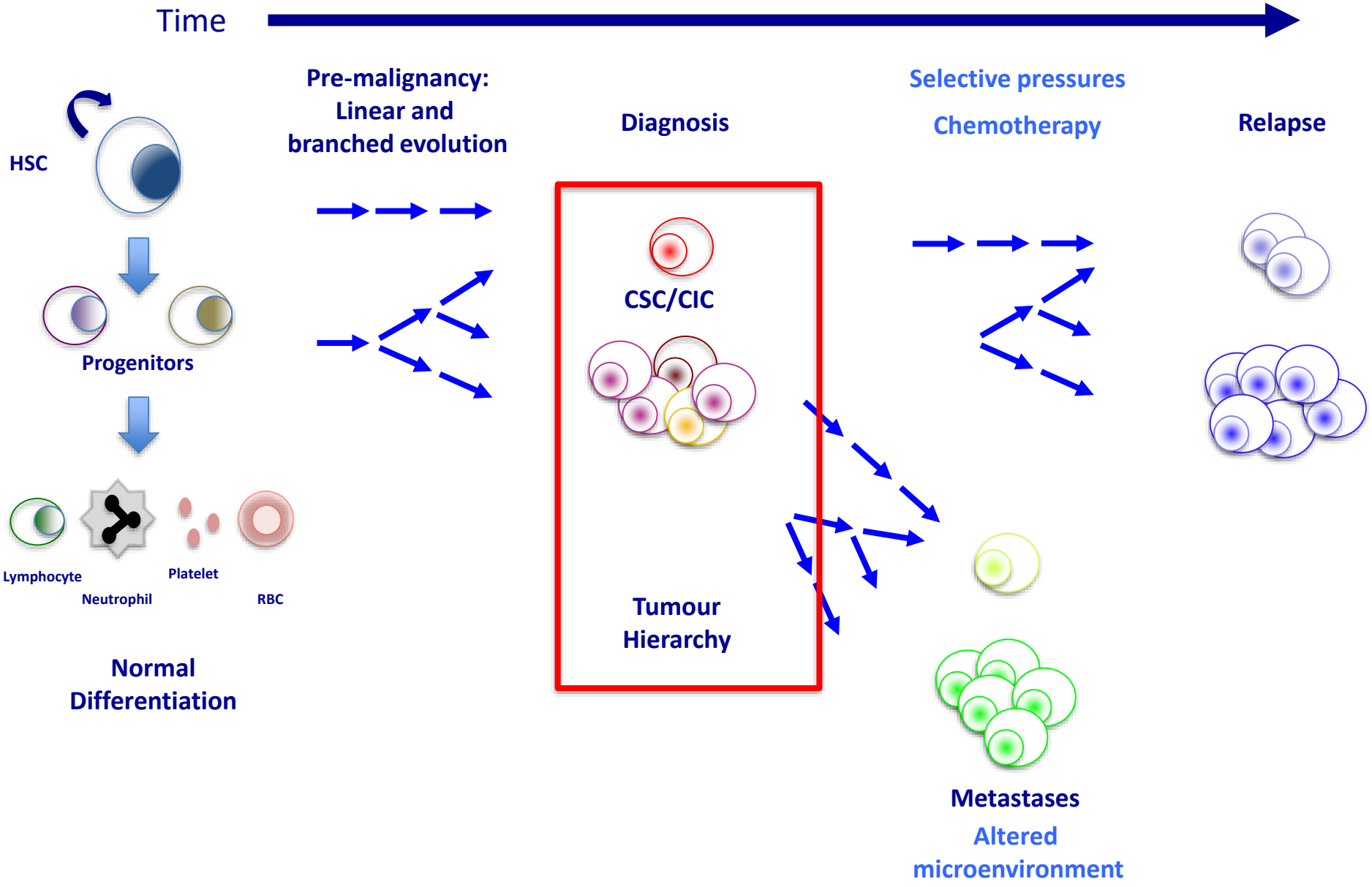
Heterogeneity in Cancer- definition of terms



Talk outline

- Context and definitions
- Tumour hierarchy: the cancer stem cell hypothesis and its implications for tumour biology
- Pre-malignancy – overt and covert premalignant states and early tumour evolution
- Tumour evolution under selective pressures:
  - Relapse
  - Metastases
- Clinical implications: Cancer stem cells
  Targeting tumour heterogeneity

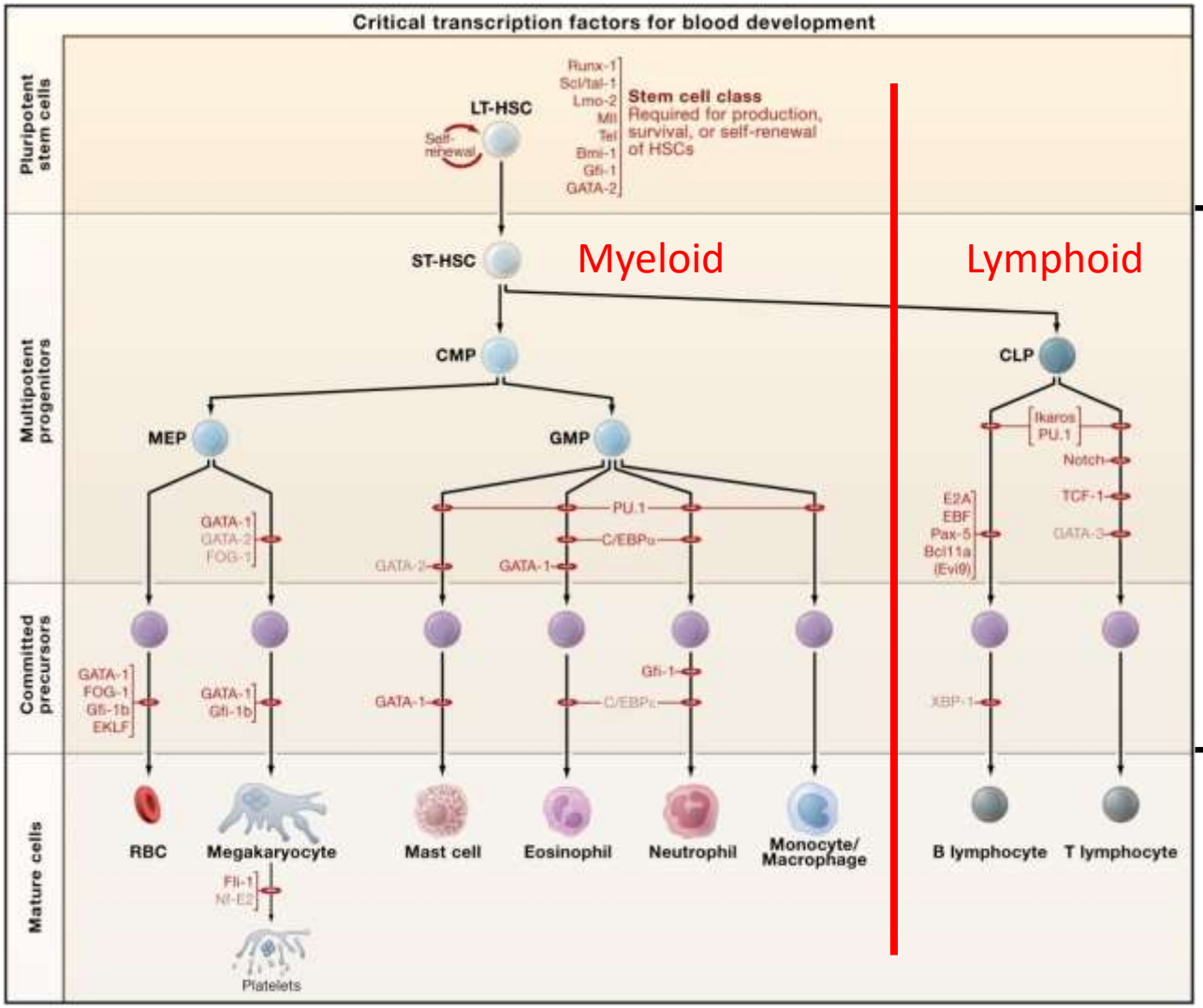
Heterogeneity in Cancer- definition of terms



The basis and implications of tumour heterogeneity

- Pathologist have noted visually for > century tumour heterogeneity
- Advent of tumour assays and model systems – similar functional heterogeneity
- Till and McCulloch – suggest existence of haematopoietic stem cell (HSC)
- 1963 - Only 1-4% of murine lymphoma cells transplant into recipients
- 1965 - Similar for solid organ tumours in soft agar
- Autologous tumour innoculations into forearms of human subjects (!) – need > 1 x 10^6 cells to initiate a tumour
- ? Existence of stem cells in cancer

Hierarchical organisation of adult mitotic organ systems



Stem cell compartment

Progenitor compartment

Mature effector compartment

O_2 carriage

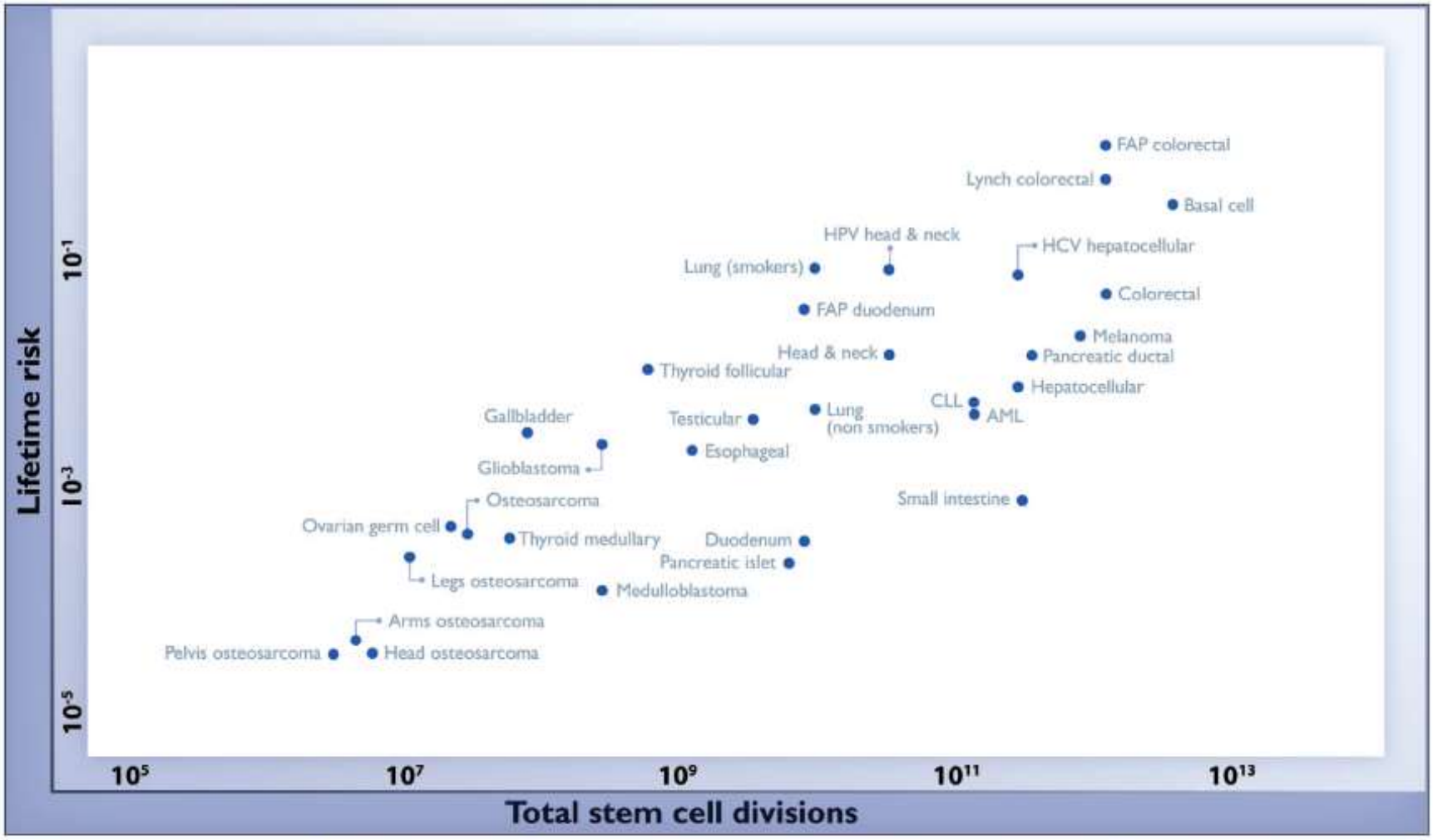
Clot formation

Innate immunity/allergy

Adaptive immunity

Cancers retain vestiges of normal tissue organisation

Tissue cancer risk relates to stem cell frequency and rate of division?



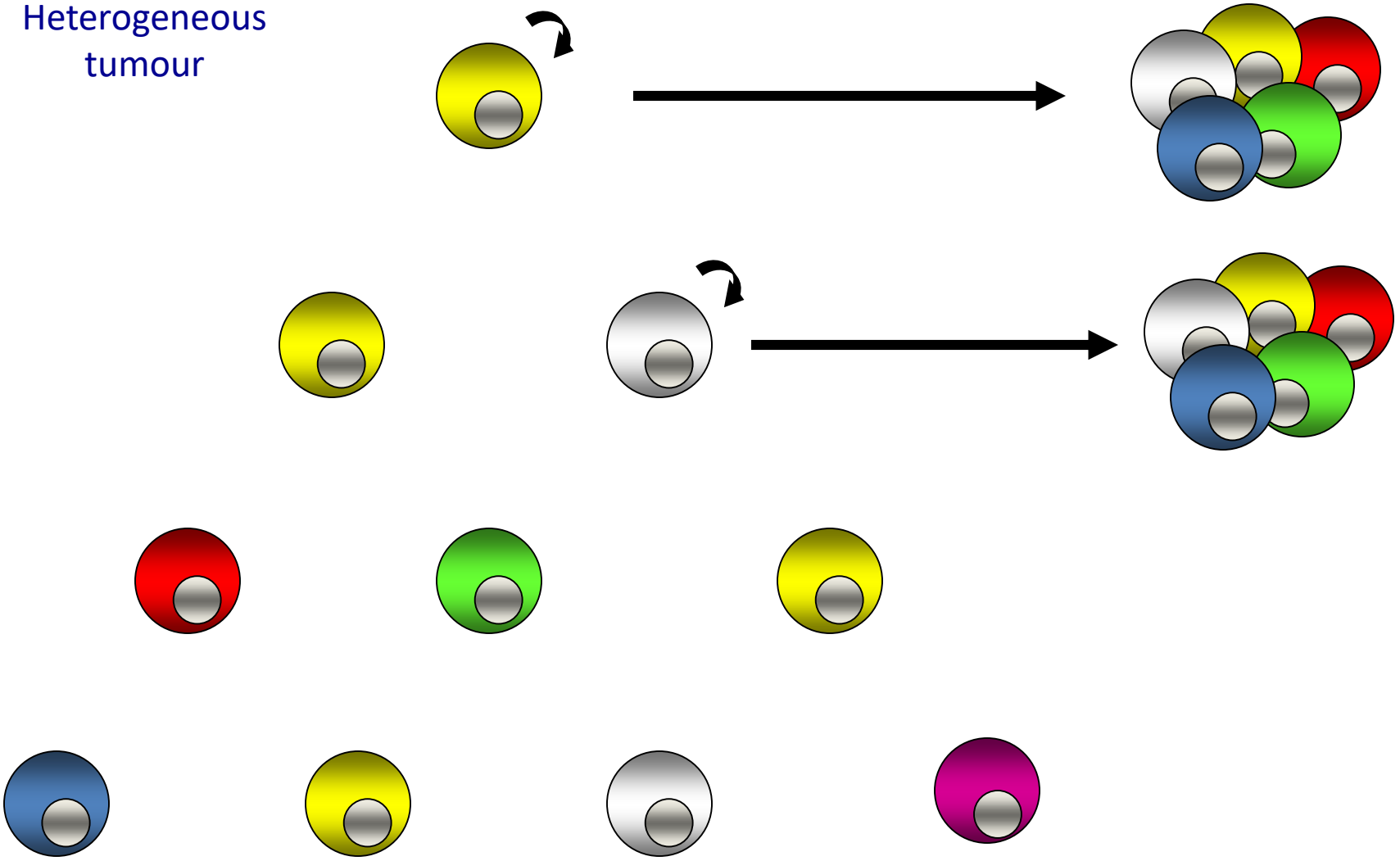
FAP = Familial Adenomatous Polyposis ◆ HCV = Hepatitis C virus ◆ HPV = Human papillomavirus ◆ CLL = Chronic lymphocytic leukemia ◆ AML = Acute myeloid leukemia

Controversial – but mainly due to modeling

Tomasetti and Vogelstein, Science, 2015

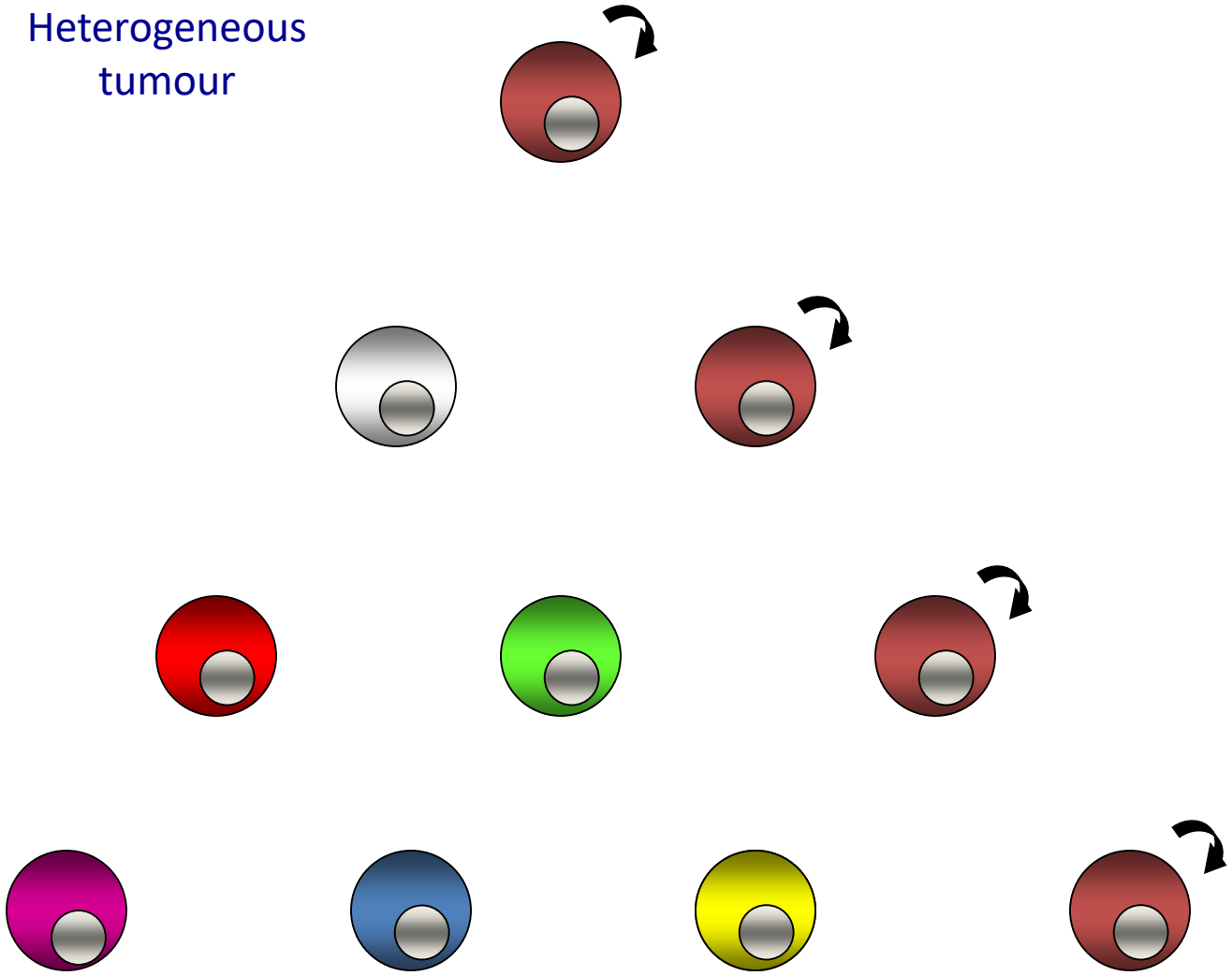
Models of Cancer organisation and Heterogeneity: Stochastic/deterministic model

Heterogeneous tumour

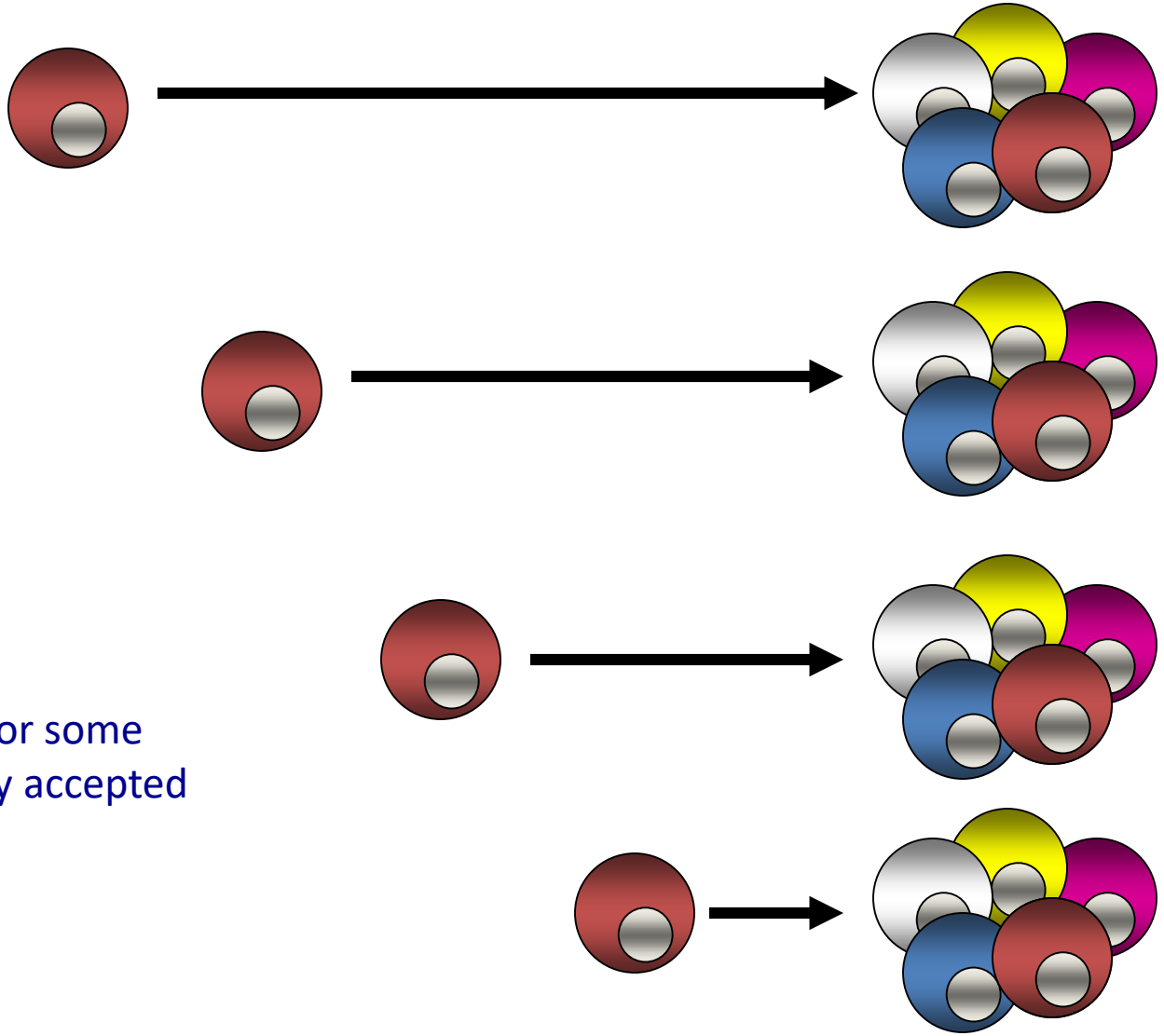


Cancer is also arranged as a hierarchy: Cancer stem cell model

Heterogeneous tumour



Cancer stem/initiating cell model



Concept is controversial for some malignancies but is widely accepted in most malignancies

Demonstration of cancer (leukaemia) stem cells in human acute myeloid leukaemia (AML)

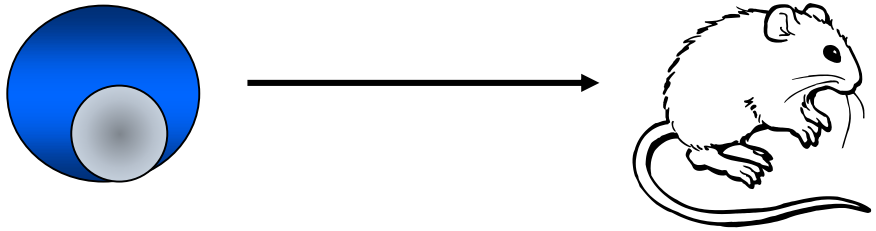
Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell

DOMINIQUE BONNET & JOHN E. DICK

Department of Genetics, Research Institute, Hospital for Sick Children and Department of Molecular and Medical Genetics, University of Toronto, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada
Correspondence should be addressed to J.E.D.
Department of Genetics, Research Institute, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8

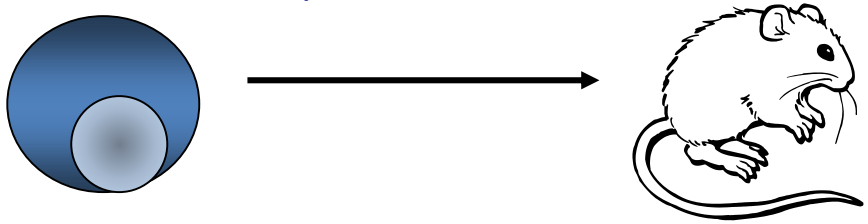
Leukaemia cells from patients

Immature CD34+/38-



LEUKAEMIA

More mature CD34+/38+



NO LEUKAEMIA

Dick and Bonnet, Nat Med 1997

Demonstration of cancer stem cells in other Solid organ malignancies

Prospective identification of tumorigenic breast cancer cells

PNAS, 2003

Muhammad Al-Hajj*, Max S. Wicha*, Adalberto Benito-Hernandez†, Sean J. Morrison*‡§, and Michael F. Clarke*‡¶

Departments of *Internal Medicine and †Pathology, Comprehensive Cancer Center, ‡Department of Developmental Biology, and §Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI 48109

Identification of human brain tumour initiating cells

Nature, 2004

Sheila K. Singh[1,2,3], Cynthia Hawkins[1,4], Ian D. Clarke[1,2], Jeremy A. Squire[6], Jane Bayani[6], Takuichiro Hide[1,2], R. Mark Henkelman[5], Michael D. Cusimano[3,7] & Peter B. Dirks[1,2,3]

A human colon cancer cell capable of initiating tumour growth in immunodeficient mice

Nature, 2006

Catherine A. O'Brien[1], Aaron Pollett[2], Steven Gallinger[3] & John E. Dick[1,4]

Identification and expansion of human colon-cancer-initiating cells

Nature, 2006

Lucia Ricci-Vitiani[1], Dario G. Lombardi[2], Emanuela Pilozzi[3], Mauro Biffoni[1], Matilde Todaro[4], Cesare Peschle[1] & Ruggero De Maria[1,2]

Identification of Pancreatic Cancer Stem Cells

Cancer Res, 2007

Chenwei Li,[1] David G. Heidt,[1] Piero Dalerba,[4] Charles F. Burant,[2,3] Lanjing Zhang,[3] Volkan Adsay,[4] Max Wicha,[3] Michael F. Clarke,[5] and Diane M. Simeone[1,2]

Departments of [1]Surgery, [2]Molecular and Integrative Physiology, and [3]Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan; [4]Department of Pathology, Karmanos Cancer Center, Detroit, Michigan; and [5]Department of Internal Medicine, Stanford University School of Medicine, Palo Alto, California

The Empire strikes back: evidence against Cancer Stem Cells

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]

Nature 2008

Tumor Growth Need Not Be Driven by Rare Cancer Stem Cells

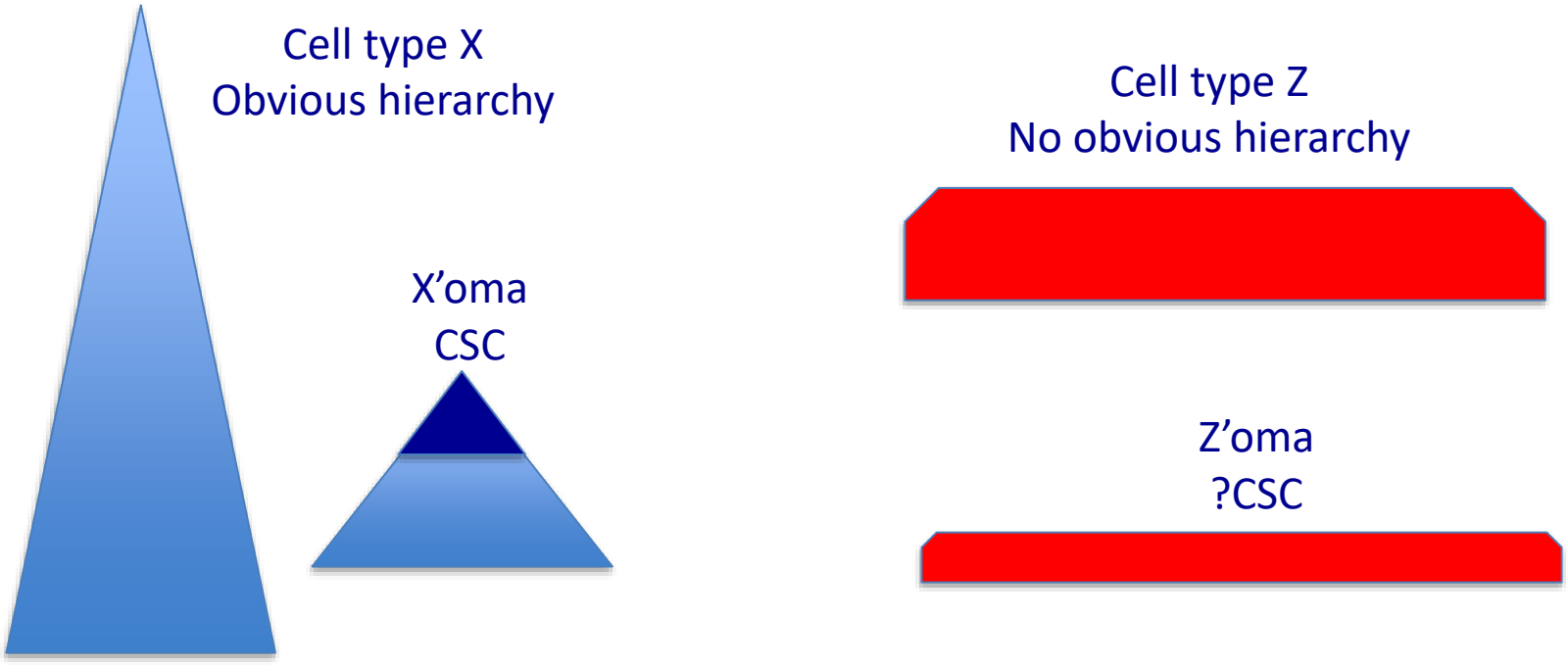
Priscilla N. Kelly,[1,2] Aleksandar Dakic,[1,2] Jerry M. Adams,[1]*
Stephen L. Nutt,[1]* Andreas Strasser[1]*†

Science 2007

- Similar assays
- High Frequency of cancer stem cells
- Lack of or shallow tissue Hierarchies

How do we reconcile the evidence?

- One size does not fit all?
- Cancer stem cells do not need to be RARE populations
- Related to the hierarchy of the tissue from which tumour derived?



Cell of origin and heterogeneity

- Stem cell vs Non-stem cell: Benefits and limitations for both

- Stem cell:

- Favourable – already has unlimited self-renewal, can accumulate the multiple mutations required for full malignant phenotype.

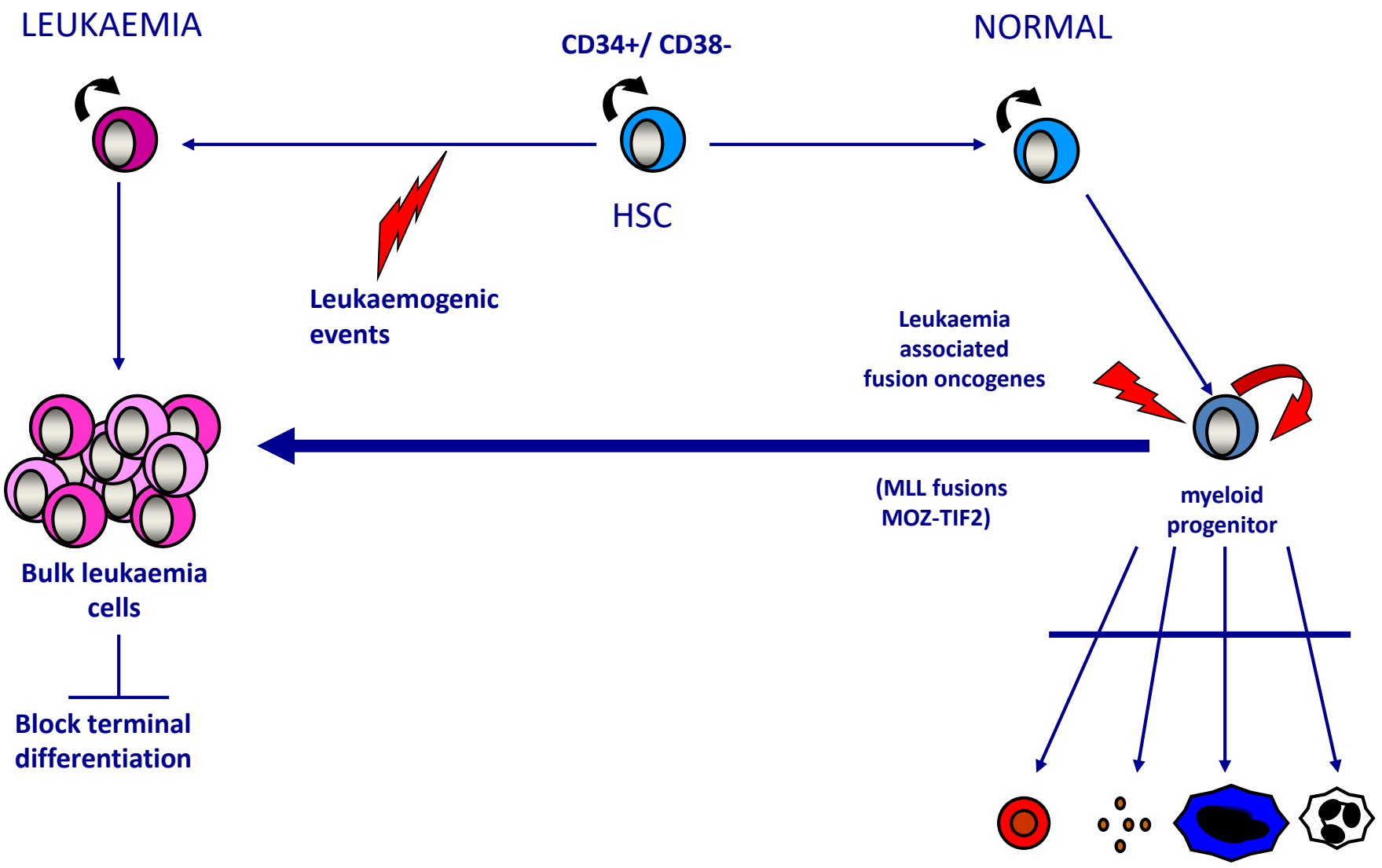
- Unfavourable – Most important cell in metazoan biology, highly evolved DNA repair and DDR mechanisms to prevent mutation acquisition.
  Metabolic state.
  Quiescence > proliferation

- Progenitor/other cell:

- Favourable- Genome less well protected.
  Metabolically more active
  Proliferation > Quiescence

- Unfavourable – Very limited self-renewal – need to acquire this

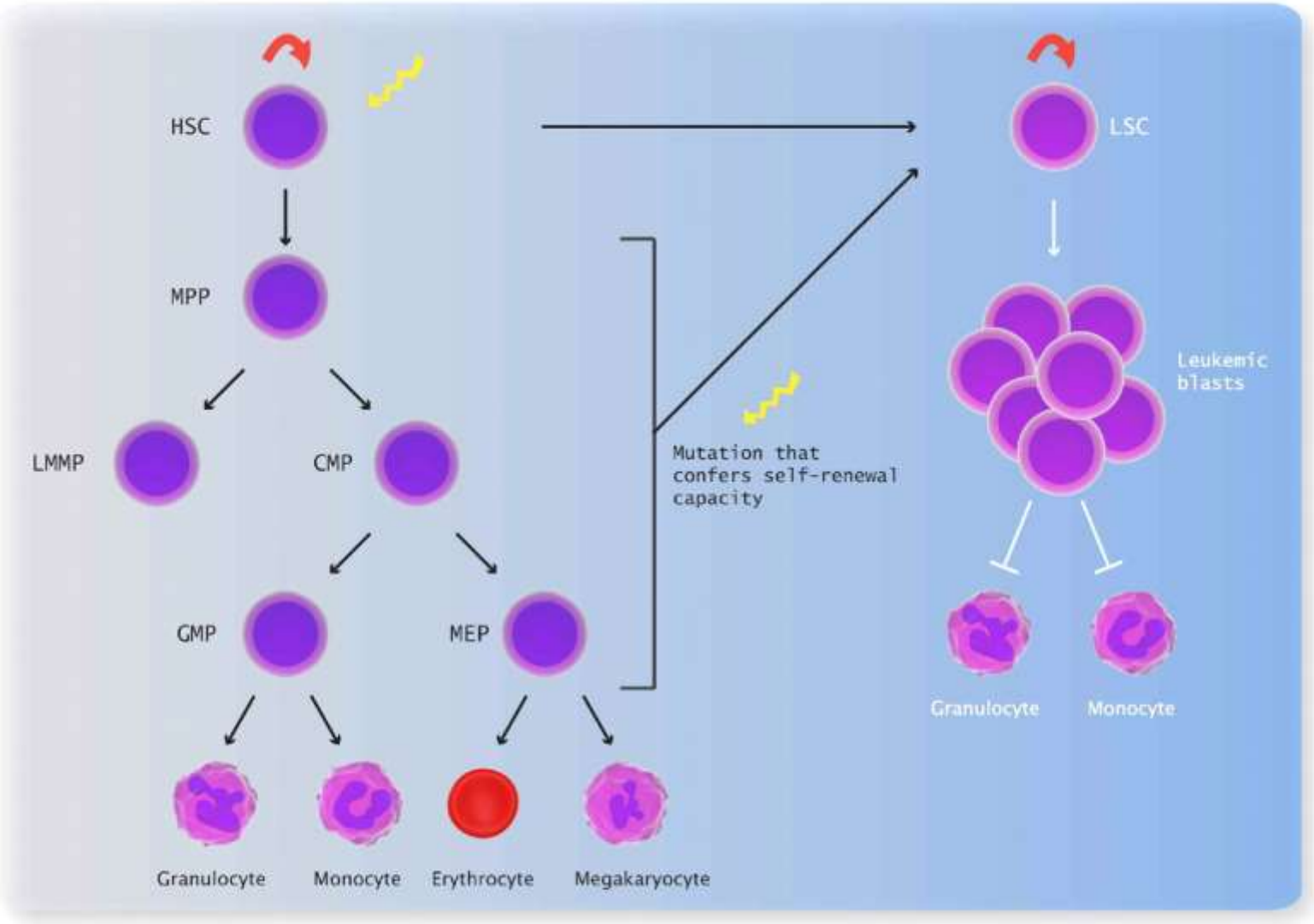
Committed progenitors may generate AML leukaemia stem cells



(Huntly et al Cancer Cell, Cozzio et al Genes and Development)

AML leukaemia stem cells have surface characteristics of either stem or progenitor cells

Myeloid malignancies

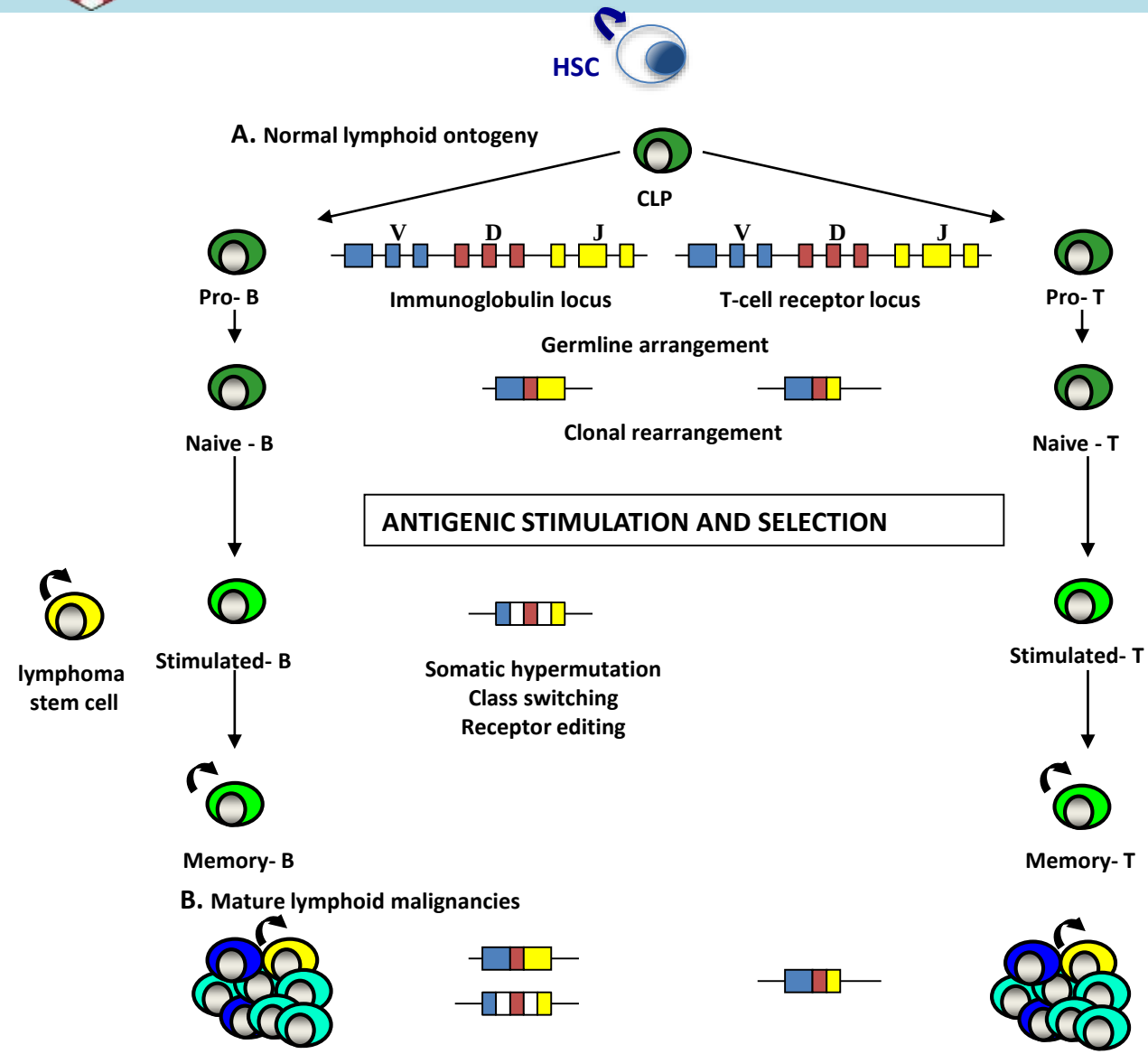


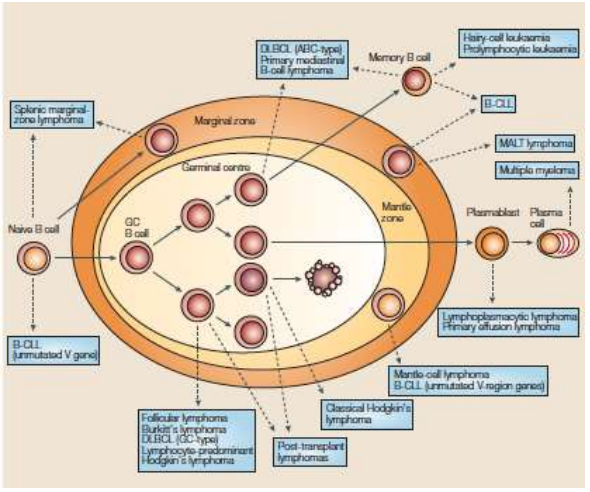
Horton and Huntly, Haematologica 20012

Cell of origin?

- Have to consider the stepwise acquisition of multiple mutations that is required to fully transform cells to the malignant state
- Some mutations as single events may alter progenitor cell function enough to generate a CSC
- Mutations may alter the frequency/probability of subsequent mutation acquisition
- Most likely that mutations are acquired in a stem cell but possible that subsequent downstream cells that acquire the full mutational complement are the tumour CSC

Lymphoma stem cell for mature lymphoid malignancies?





- Evidence suggests a cell post antigenic stimulation

Huntly and Gilliland, Nat Rev Cancer

Model for lymphoma evolution – early vs late

ARTICLES

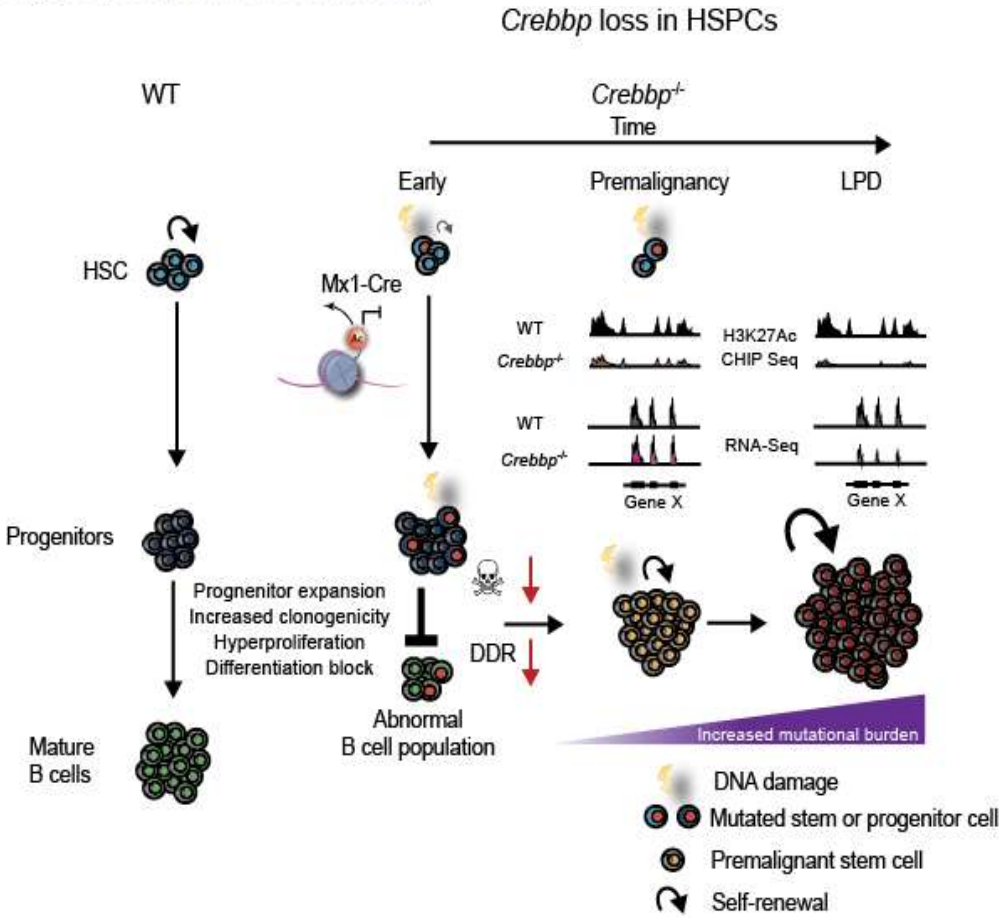
nature cell biology

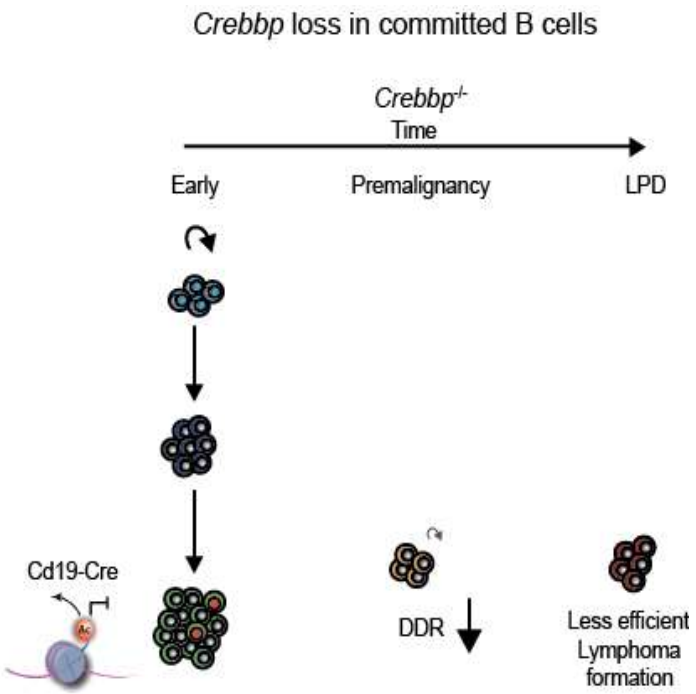
Horton et al, Nat Cell Biol, 2017

Early loss of *Crebbp* confers malignant stem cell properties on lymphoid progenitors

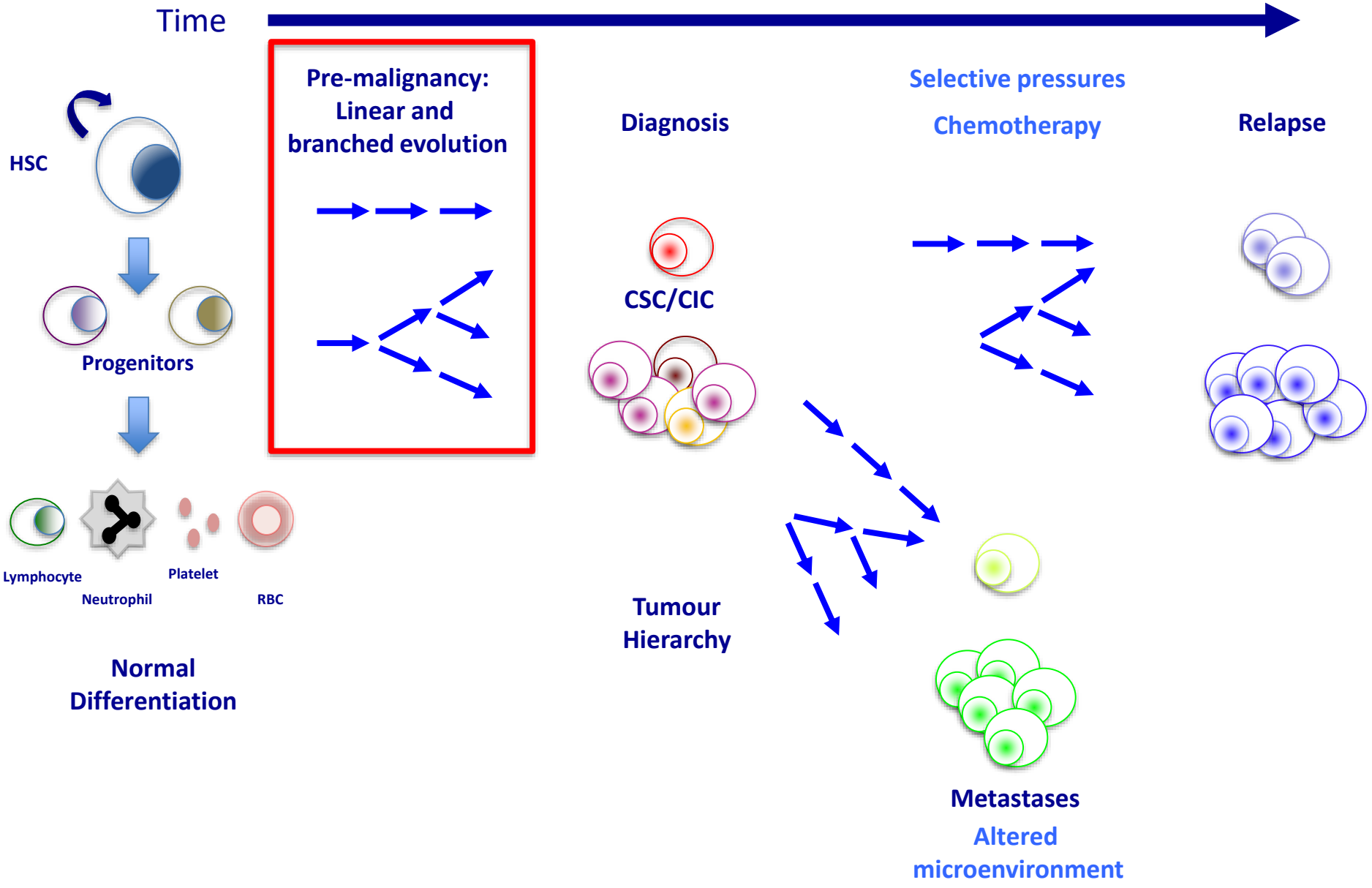
Sarah J. Horton[1,2,3], George Giotopoulos[1,2,3], Haiyang Yun[1,2,3], Shabana Vohra[1,2,3], Olivia Sheppard[1,2,3], Rachael Bashford-Rogers[3], Mamunur Rashid[4], Alexandra Clipson[3], Wai-In Chan[1,2,3], Daniel Sasca[1,2,3], Loukia Yiangou[1], Hikari Osaki[1,2,3], Faisal Basheer[1,2,3], Paolo Gallipoli[1,2,3], Natalie Burrows[3], Ayşegül Erdem[1,2,3], Anastasiya Sybirna[1], Sarah Foerster[1], Wanfeng Zhao[5], Tonci Sustic[1,2], Anna Petrunkina Harrison[7], Elisa Laurenti[1,2], Jessica Okosun[8], Daniel Hodson[1,2], Penny Wright[8], Ken G. Smith[3], Patrick Maxwell[3], Jude Fitzgibbon[8], Ming Q. Du[5], David J. Adams[4] and Brian J. P. Huntly[1,2,3,9,10]

CREBBP- Lysine acetyltransferase
LOF mutations in up to 70% of lymphomas



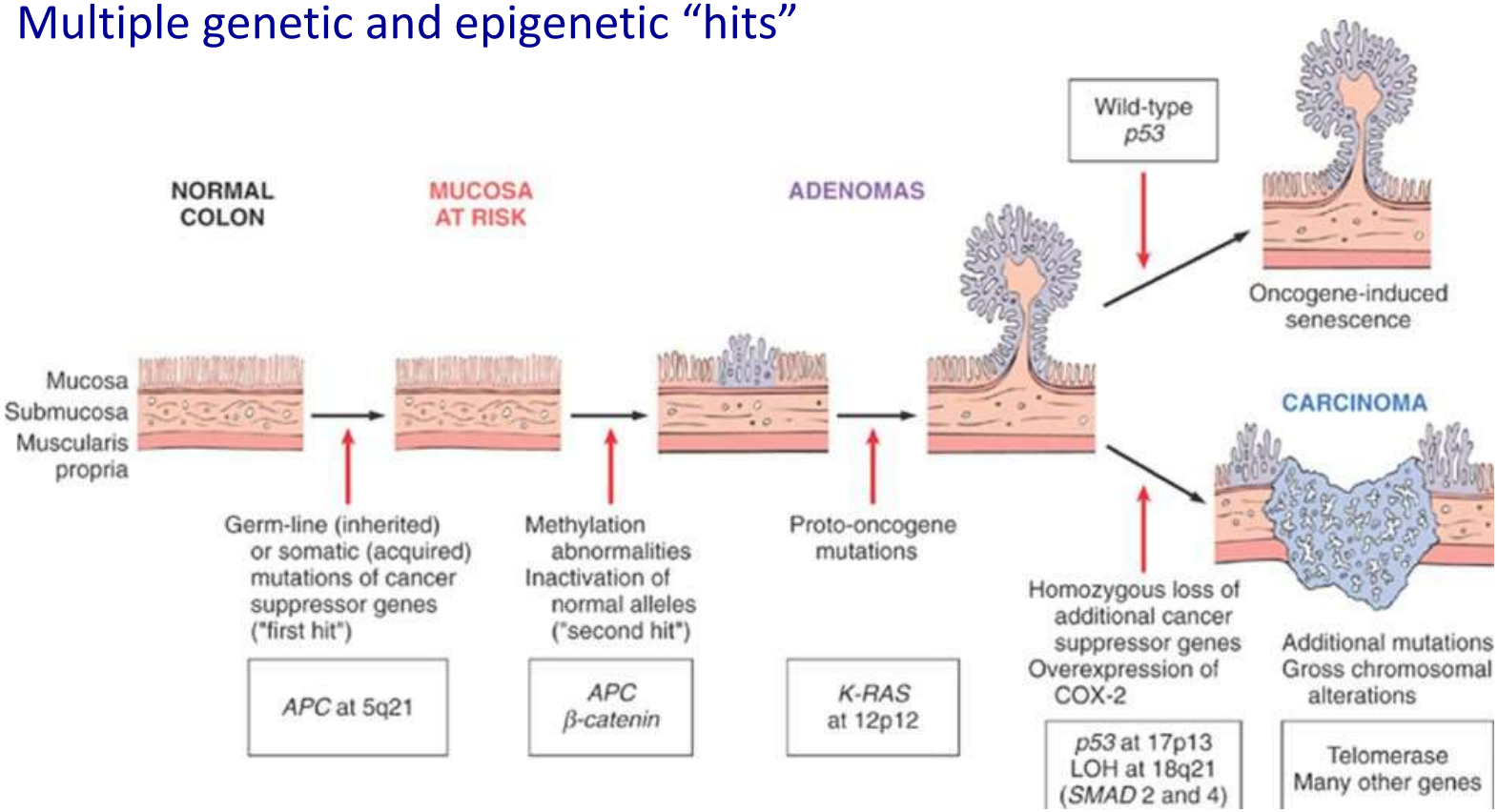


Heterogeneity in Cancer: Pre-malignancy



Premalignancy: Multi-step progression of cancer

Classical system – adenocarcinoma of the bowel
Multiple genetic and epigenetic "hits"

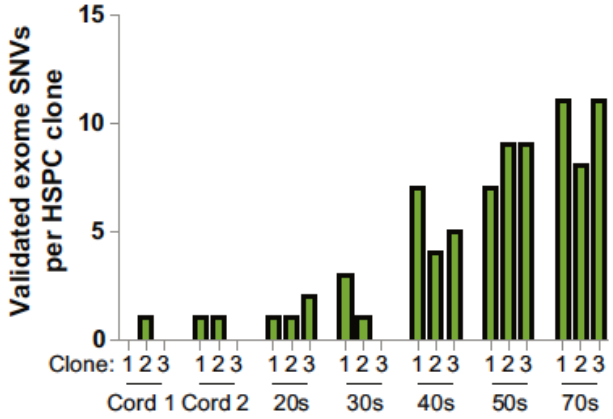


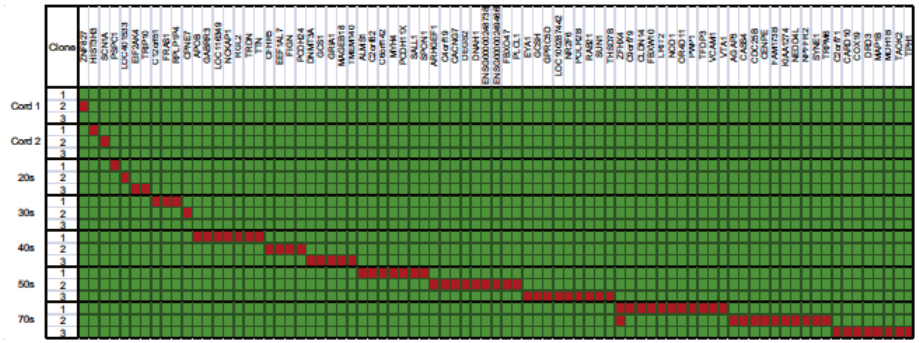
Overt phenotypes – dysplasia)
adenomatous polyps) *in situ*

Screening in some individuals (FAP, Lynch syndrome) – surveillance and eradication
Similar for many other epithelial malignancies – Prostate, Pancreas, etc.

Random mutation and Clonal Haematopoiesis

- Rate of mutation is one in every 50 milion nucleotides (but ~6 x 10^9 bases in human genome!)
- Mutations exist in haematopoietic stem cells (HSC) from entirely normal individuals
- Function of age – may help to explain the later onset of most myeloid malignancies





- Welch study - random mutations
- Suggests that normal HSC acquire random mutations as a function of time
- ?Stochastic whether they develop functional consequences/malignancy

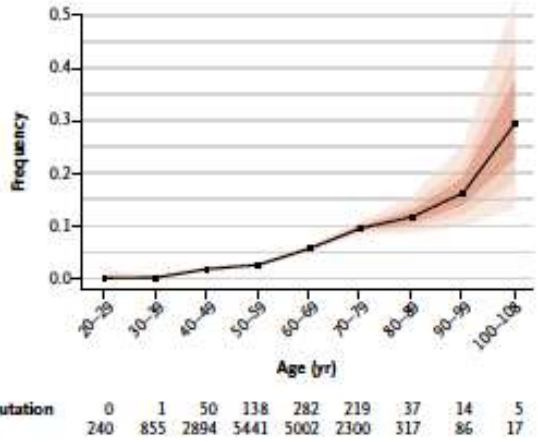
Age related clonal Haematopoiesis (ARCH)
Clonal Hameatopoiesis of indeterminate prognosis (CHIP)

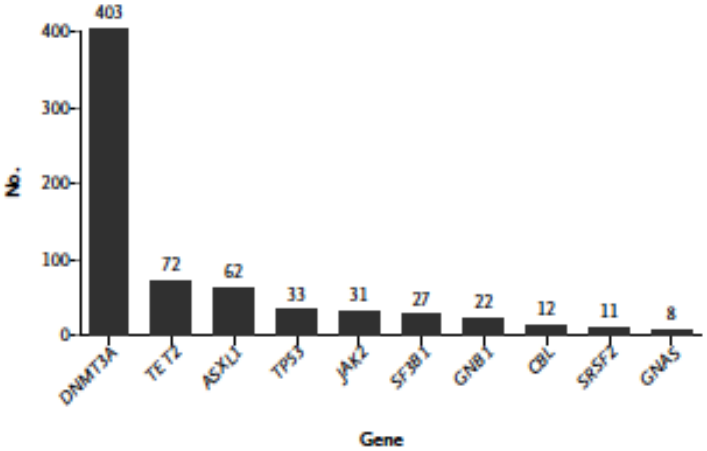
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes

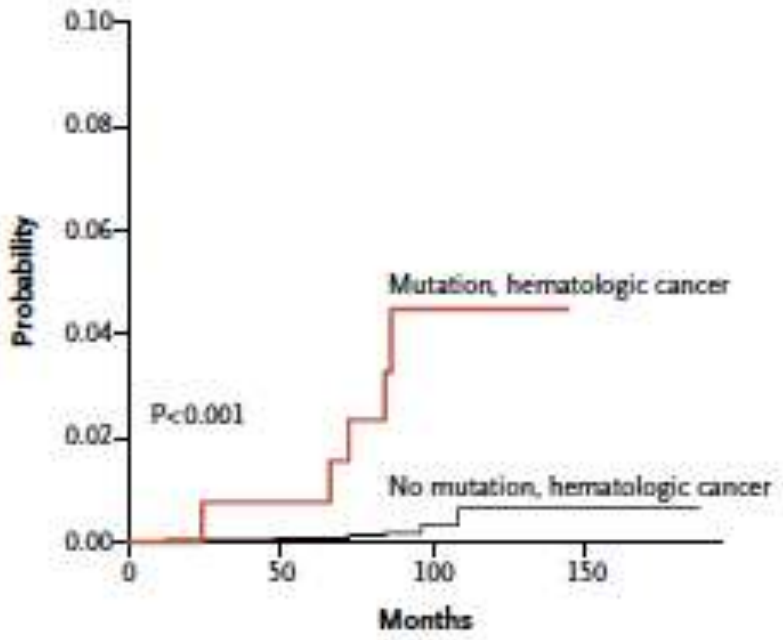
- WES data from 17,182 patients - mostly Type 2 diabetics for GWAS
- 805 somatic mutations in 73 genes from 746 individuals (4.5%) – age dependent phenomenon – rare under 40 but > 90 (18.4%).
- Majority in 3 genes - DNMT3A (403 VARIANTS) , TET2 (72) and ASXL1 (62)
- Median variant allelic frequency (VAF) 0.09 (i.e. 18%)





Age related clonal Haematopoiesis (ARCH)

- Median FU 8 years –
- Mutation associated with a HR of 11 of developing a haematological malignancy (95% CR 3.9-33)
- Assoc with an overall decreased survival (HR 1.4 and INDEPENDENT OF HAEM MALIGNANCY)
- NIDDM – OR 1.3
- IHD – OR 2.0
- CVA – OR 2.6



Age Related Clonal Haematopoiesis (ARCH)

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Age-related mutations associated with clonal hematopoietic expansion and malignancies

nature medicine

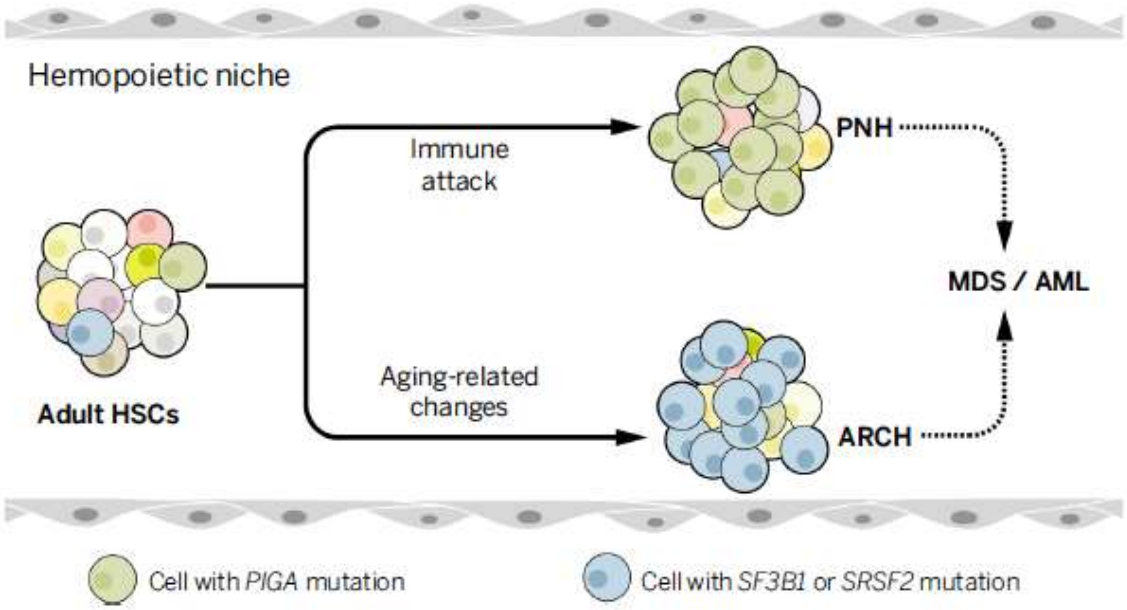
Report

Cell Reports

Leukemia-Associated Somatic Mutations Drive Distinct Patterns of Age-Related Clonal Hemopoiesis

EVIDENCE OF SIMILAR PREMALIGNANT PHENOTYPES THAT INCREASE WITH AGE IN OTHER TISSUES

Pre-malignant selective pressures



- Niche)
- Ageing)
- Immune) system)

Variable selective pressures

- Association with bone marrow failure aplastic anaemia
- Role of the niche and of competition between individual clones?
- Role of ageing and the immune system

Clonal evolution: Presence of pre-leukemia stem cells in AML

ARTICLE

doi:10.1038/nature13038

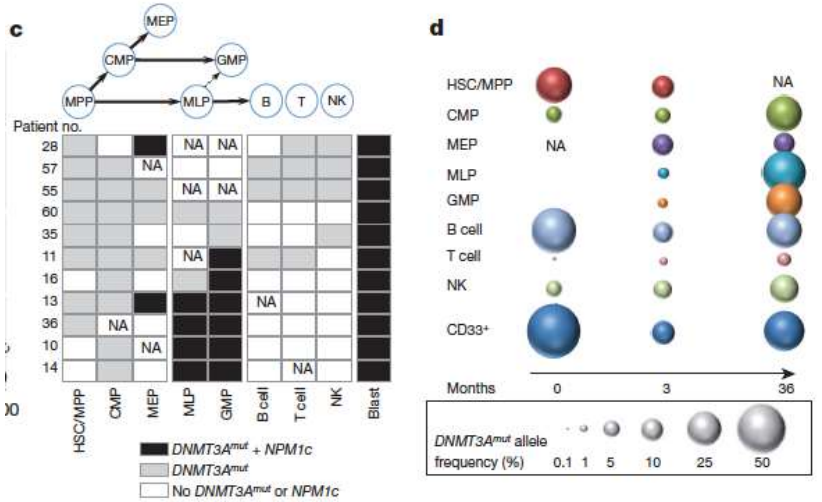
Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia

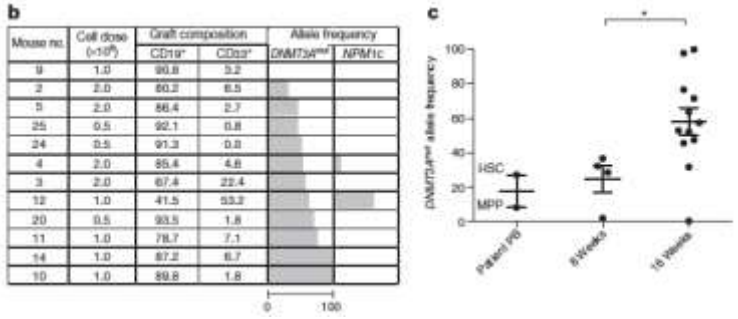
Liran I. Shlush[1]*, Sasan Zandi[1]*, Amanda Mitchell[1], Weihsu Claire Chen[1], Joseph M. Brandwein[1,2,3], Vikas Gupta[1,2,3], James A. Kennedy[1], Aaron D. Schimmer[1,2,3,4], Andre C. Schuh[1,2,3], Karen W. Yee[1,2,3], Jessica L. McLeod[1], Monica Doedens[1], Jessie J. F. Medeiros[1], Rene Marke[1,5], Hyeoung Joon Kim[6], Kwon Lee[6], John D. McPherson[4,7], Thomas J. Hudson[4,7,8], The HALT Pan-Leukemia Gene Panel Consortium†, Andrew M. K. Brown[7], Fouad Yousif[7], Quang M. Trinh[7], Lincoln D. Stein[7,8], Mark D. Minden[1,2,3,4], Jean C. Y. Wang[1,2,3] & John E. Dick[1,8]

Nature 2014

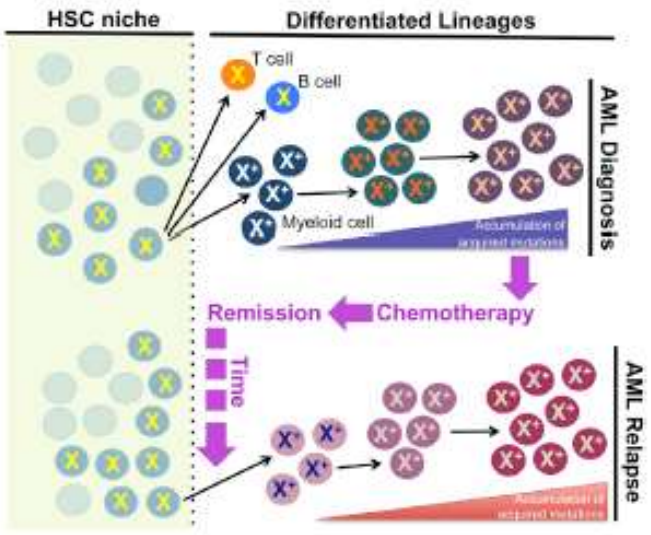
Existence of Pre-leukaemia stem cells: Resistance to therapy and biological properties

- AML patient samples vs cultured T-cells – targeted sequencing 103 commonly mutated genes - 12 samples, 4 with *DNMT3A* mutation- ALSO PRESENT in 3/4 T-CELLS
- NOT contamination as co-occurring mutations NOT present (*NPM1c*)
- Present in normal differentiating haem tissue
- Preleukaemic stem cell with transformation of a lower cell population
- *DNMT3A*^{mut} pre-LSC survive chemotherapy
- *DNMT3A*^{mut} pre-LSC outcompete normal HSC and undergo clonal expansion in NSG mice

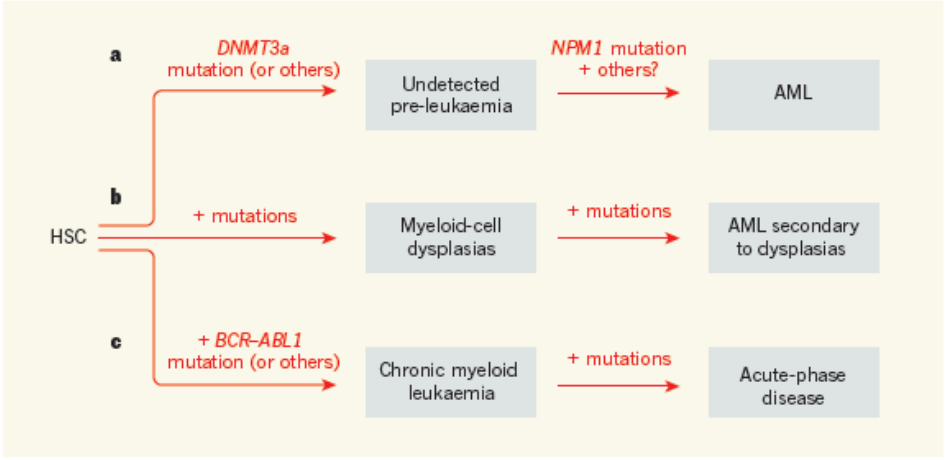




Existence of Pre-leukaemia stem cells: Resistance to therapy and biological properties

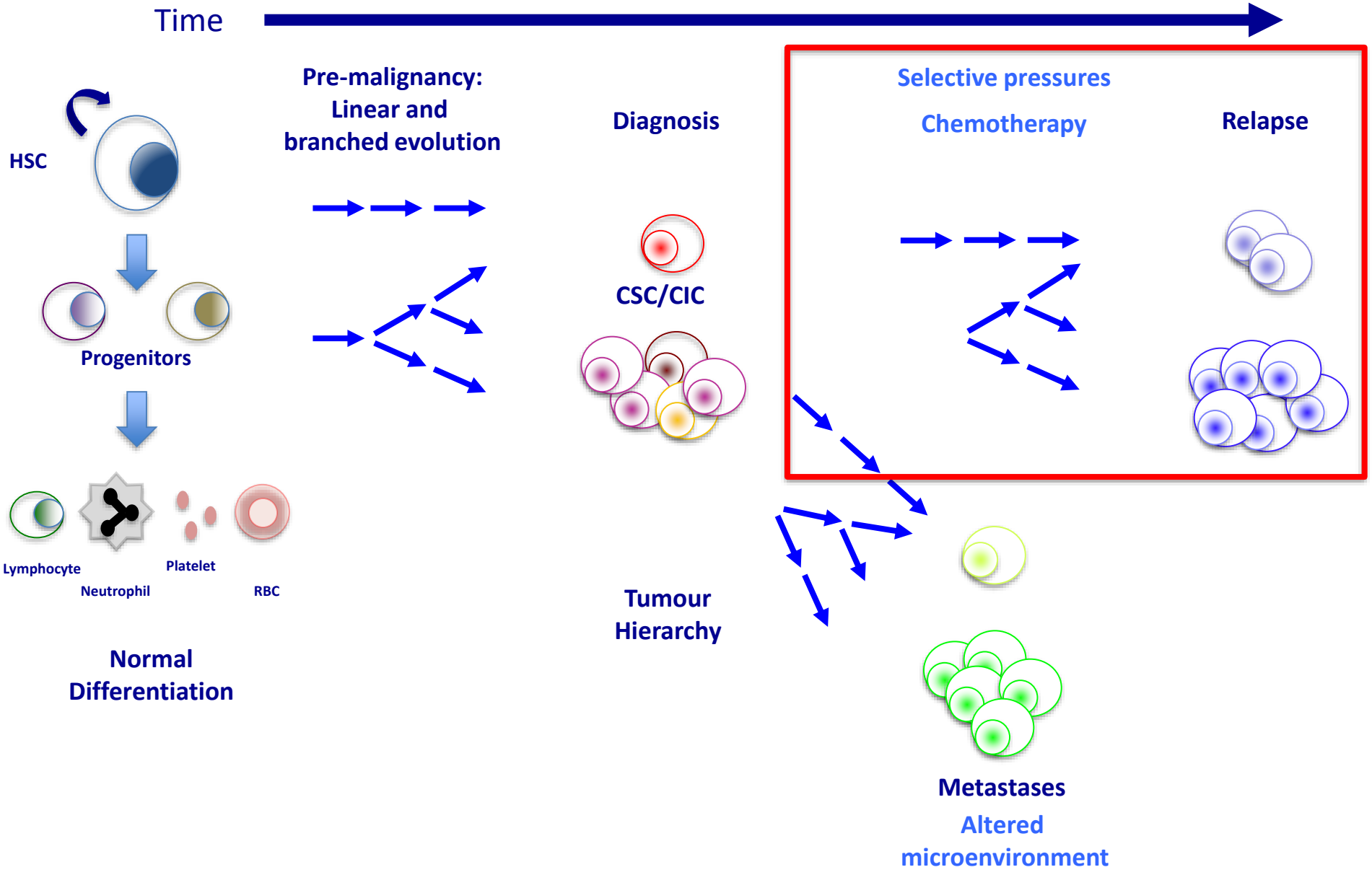


- DNMT3A pre-leukaemic stem cells (P-LSC):
- Not eradicated (therefore chemoresistant)
- Form reservoir from which relapse occurs



- Precedent where accumulation of mutations is cumulative
- Subsequent mutations may transform a cell later in differentiation
- P-LSC vs LSC diff phenotypes
- Moving target

Heterogeneity in Cancer- definition of terms



Fatal consequences of cancer: Relapse

- We are actually pretty successful at treating primary cancers

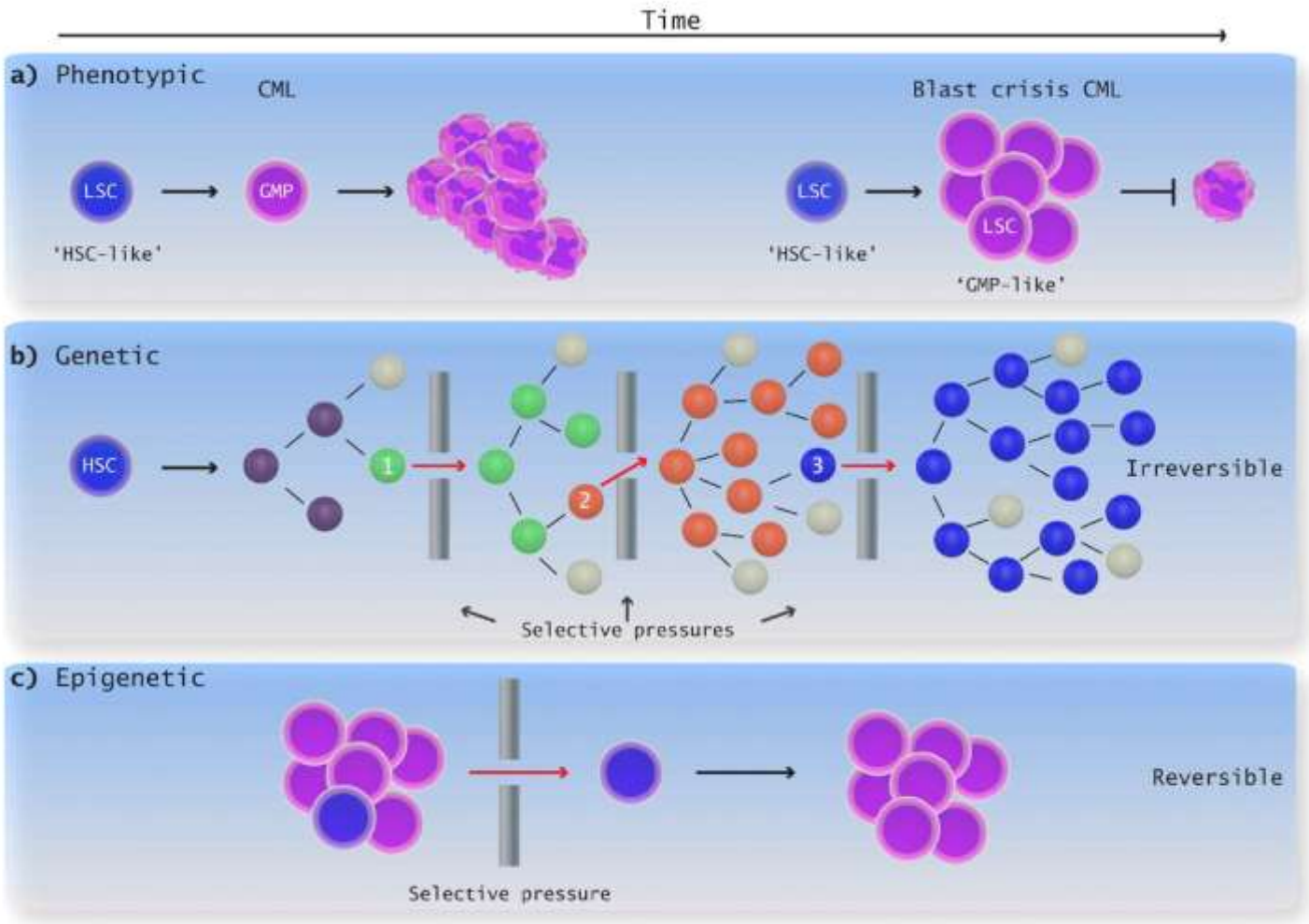
- Surgery
- Radiotherapy
- Chemotherapy
- Immunotherapy

- NOT at preventing spread – Metastasis

- NOT at preventing recurrence – **Relapse**

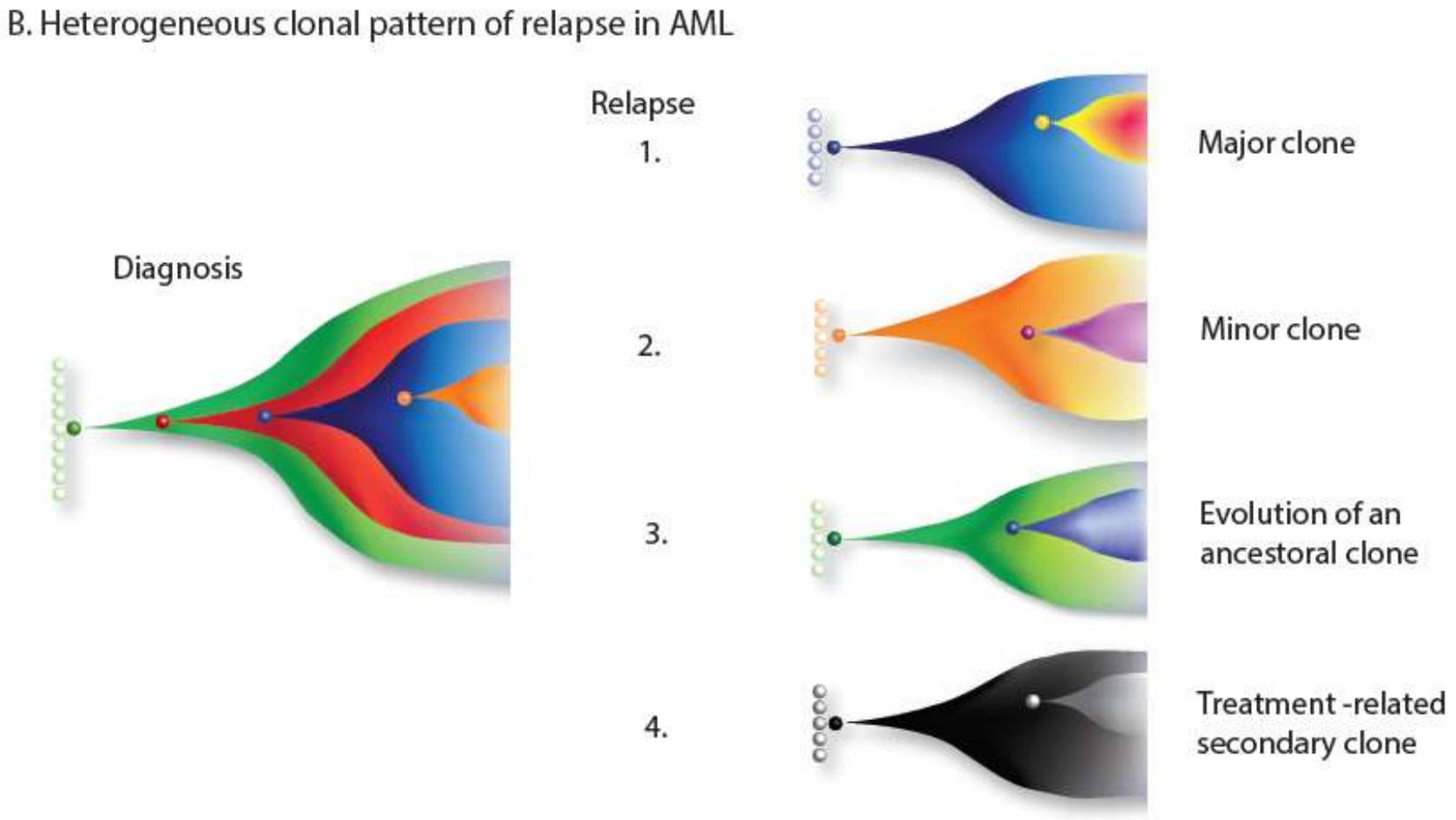
- **Most patients die from relapse and/or spread of disease**

Heterogeneity of Cancer Stem Cells: Evolution under selective pressures



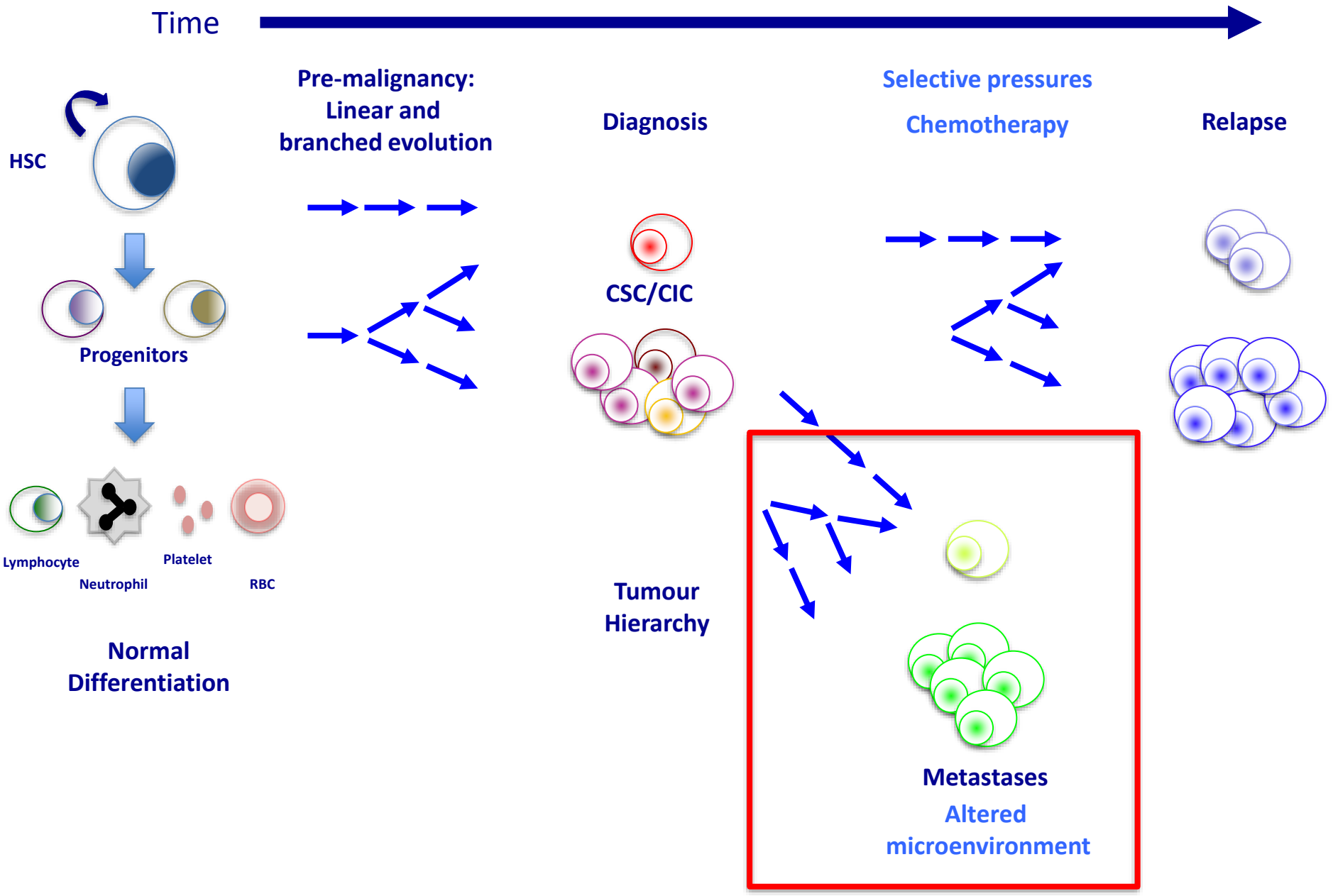
- Tumours are constantly evolving under fixed and mobile selective pressures
- Therapy: standard chemo, DRT, immunotherapy and targeted therapy

Patterns of relapse disease also demonstrates temporal intratumoural hetreogeneity



- Pattern of relapse reflects significant intratumoural heterogeneity at diagnosis
- Provides further temporal heterogeneity
- Multiple patterns of relapse – major clone, minor clone, evolution of an ancestral clone

Heterogeneity in Cancer- definition of terms



Fatal consequences of cancer: Metastases and relapse

- We are actually pretty successful at treating primary cancers

- Surgery
- Radiotherapy
- Chemotherapy
- Immunotherapy

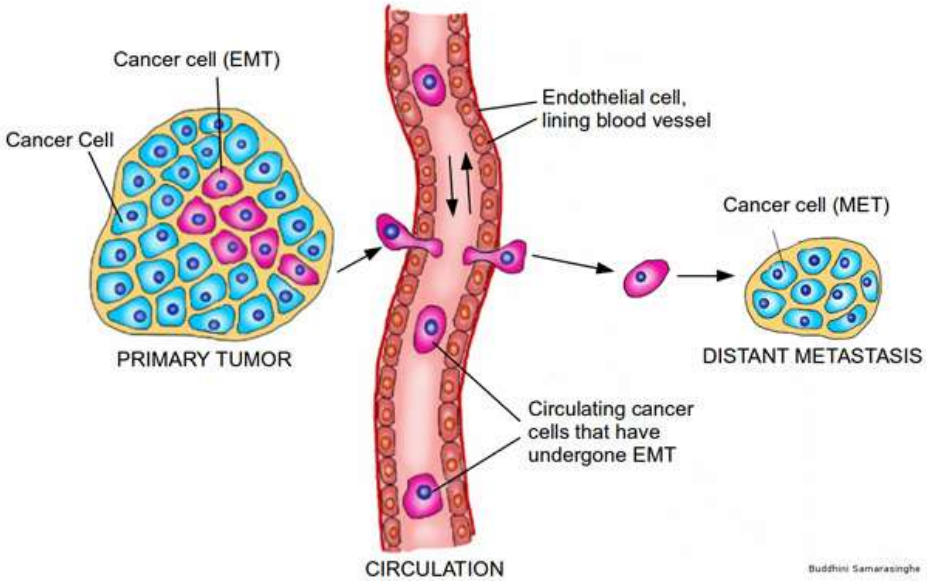
- NOT at preventing spread – **Metastasis**

- NOT at preventing recurrence – Relapse

- Most patients die from relapse and/or spread of disease

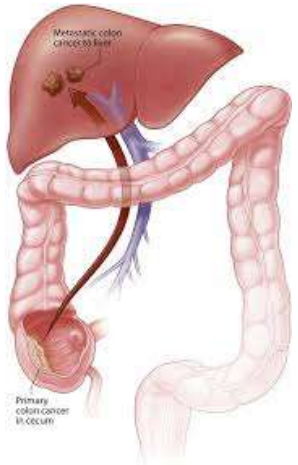
Fatal consequences of cancer: Metastasis

- Metastasis – process of spread of cancer



Metastases have differing genetics and functional properties to primary tumours

Seed and soil – different Tumours have varying predilictions for spread



NEED CSC FUNCTION

MAY DEVELOP HETEROGENEITY FROM PRIMARY TUMOUR
DUE TO DIFFERENT SELECTIVE PRESSURES OF MICROENVIRONMENT

Therapeutic implications

- Targeting cancer stem cells

- Targeting tumour heterogeneity

Therapeutic implications

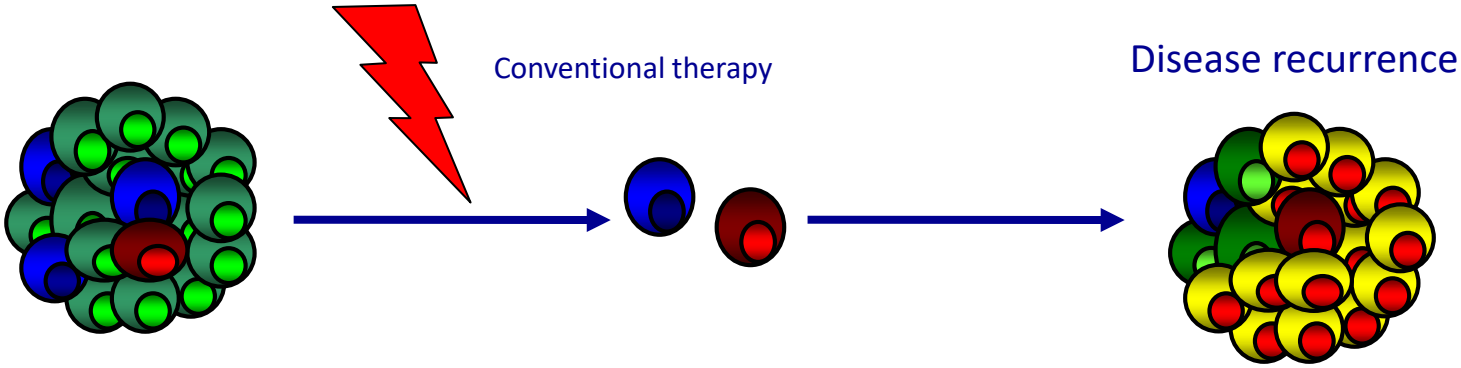
- Targeting cancer stem cells

- Targeting tumour heterogeneity

TARGETING CSC

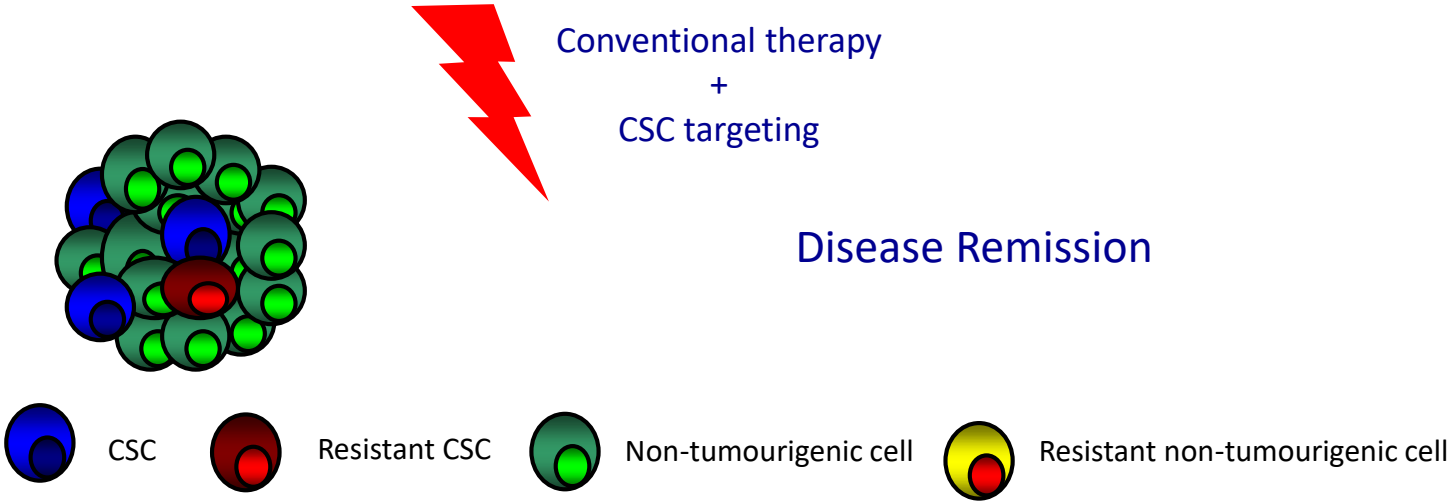
Clinical implications of Cancer Stem Cell model

A



Targeted elimination of Cancer stem cells (CSC)

B



SIMILAR FOR TARGETING HETEROGENEITY

Targeting cancer stem cells

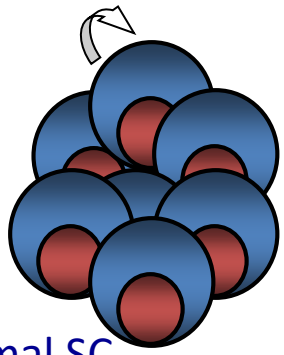
Considerations:

- Selectivity/Specificity for CSC
- Limited toxicity against normal stem cells from same (or other) organs
- Darwinian selection pressures inherent in CSCs– “MOVING TARGET”

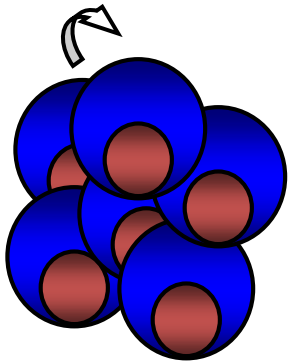
- Need to have an extensive knowledge of the CSC AND its normal counterpart

- Signalling
- Transcriptional/epigenetic
- Metabolic
- Surface proteome
- Immunogenetics

Identifying differences between normal and cancer stem cells

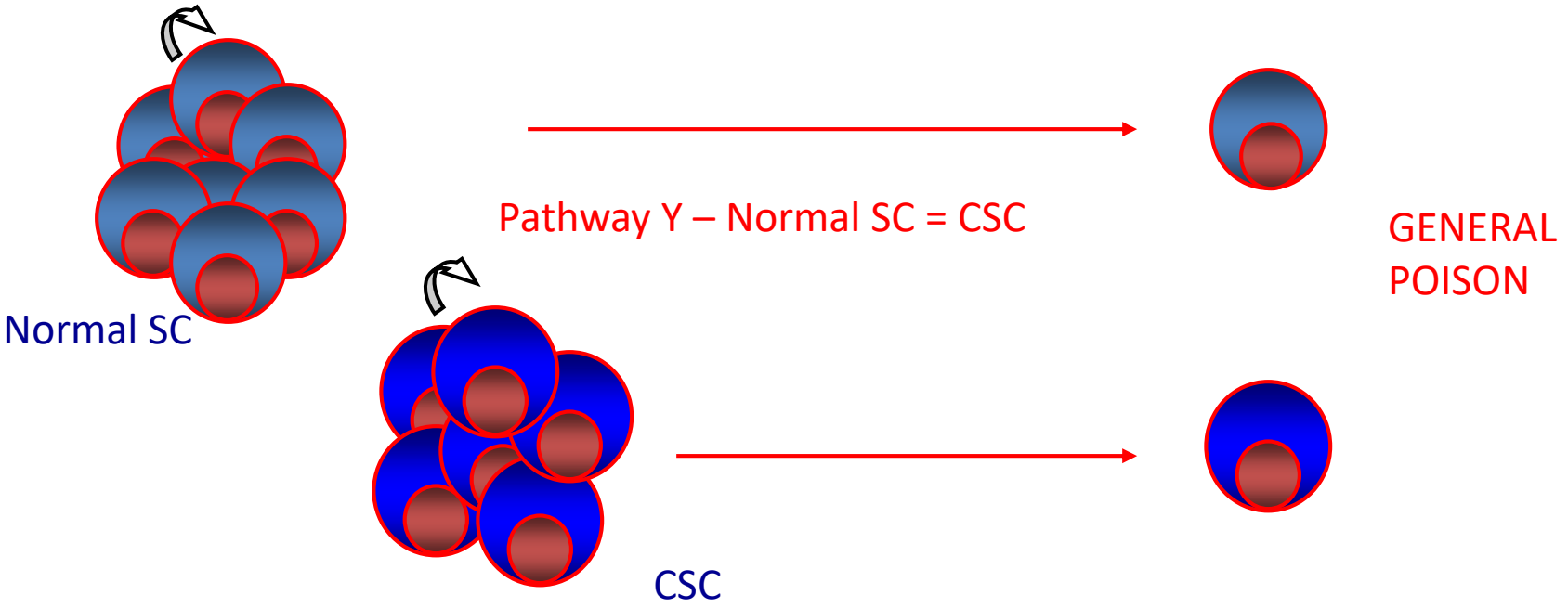


Normal SC

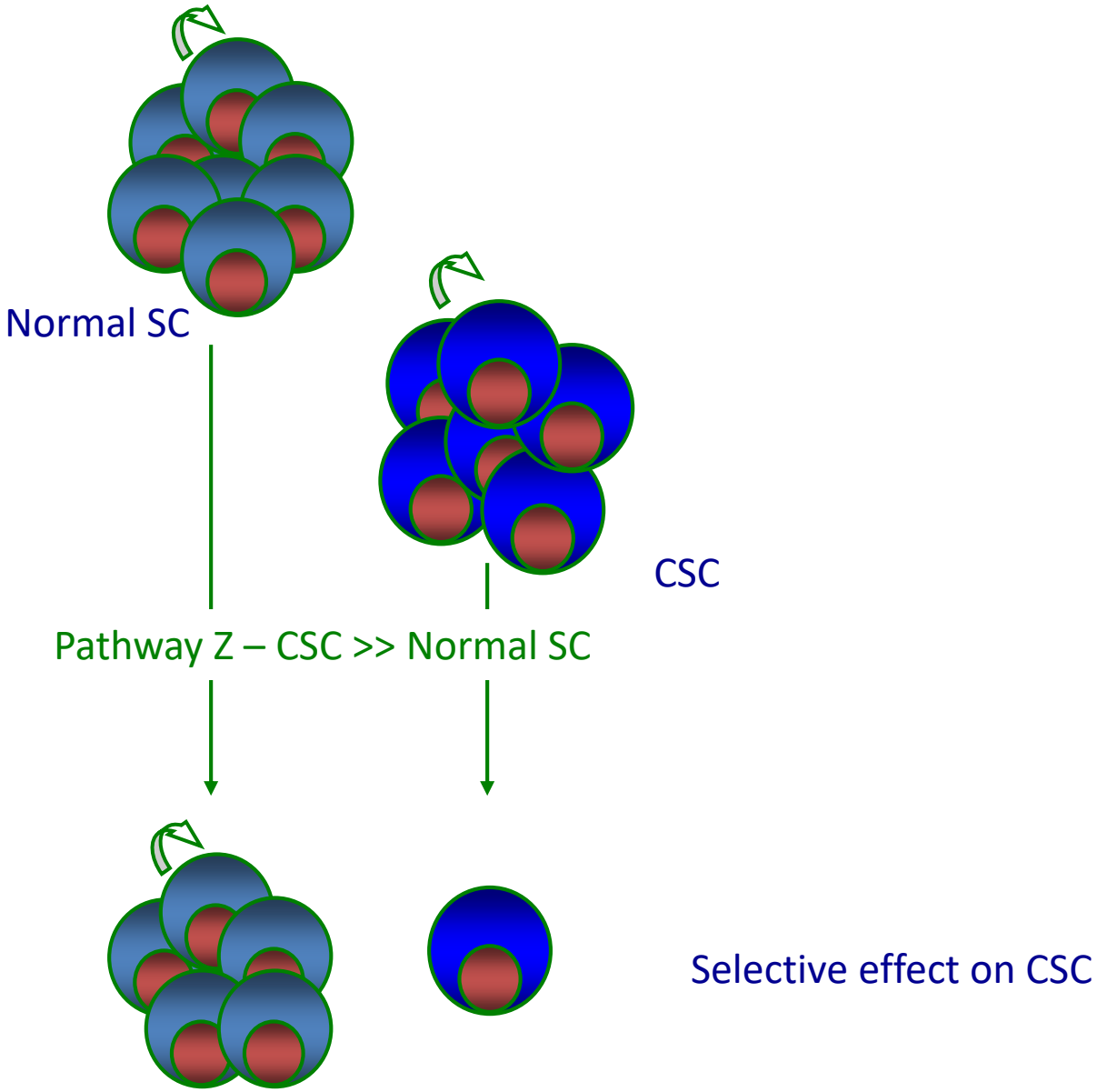


CSC

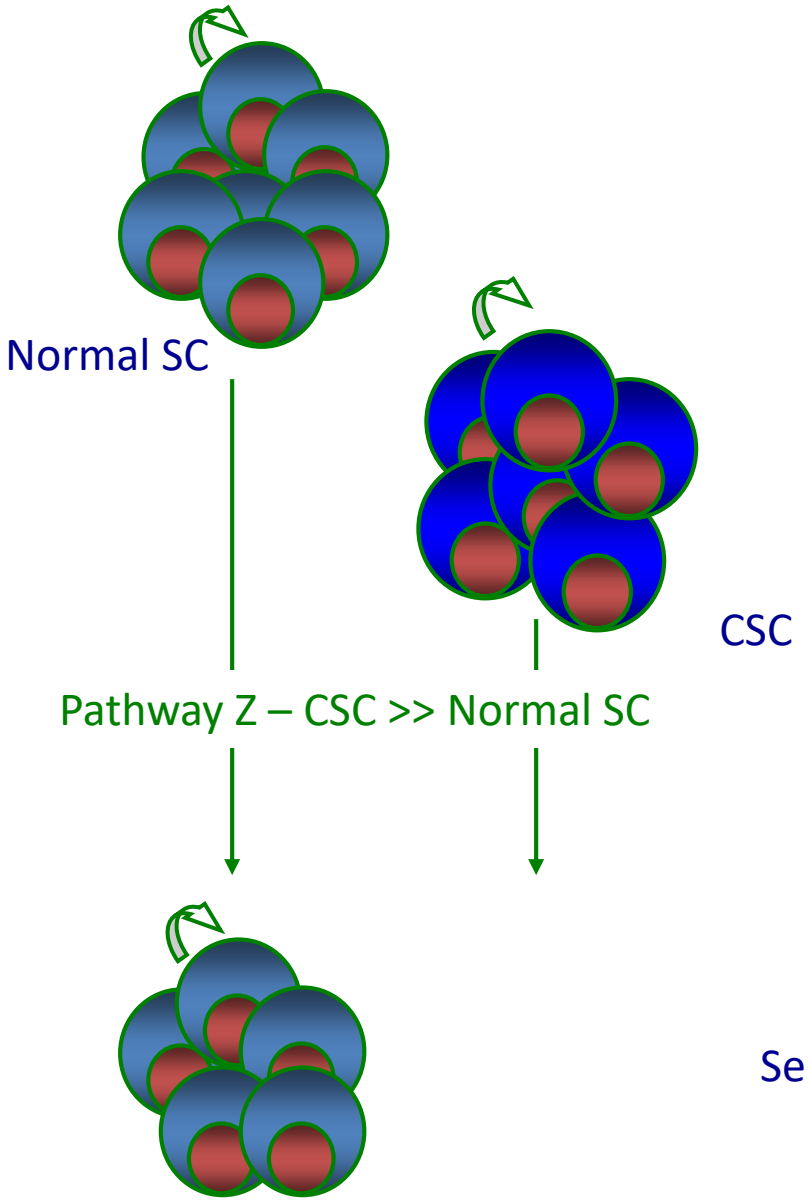
Identifying differences between normal and leukemic stem cells



Identifying differences between normal and leukemic stem cells



Identifying differences between normal and leukemic stem cells



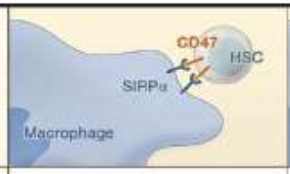
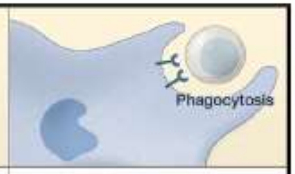
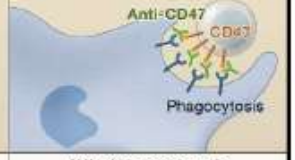
Targeting LSC surface phenotype: CD47

Cell | Cell

CD47 Is Upregulated on Circulating Hematopoietic Stem Cells and Leukemia Cells to Avoid Phagocytosis

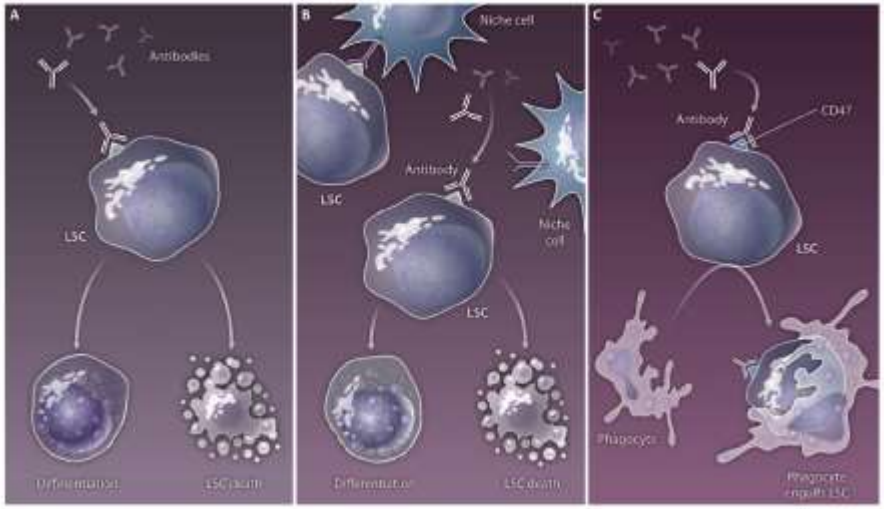
CD47 Is an Adverse Prognostic Factor and Therapeutic Antibody Target on Human Acute Myeloid Leukemia Stem Cells

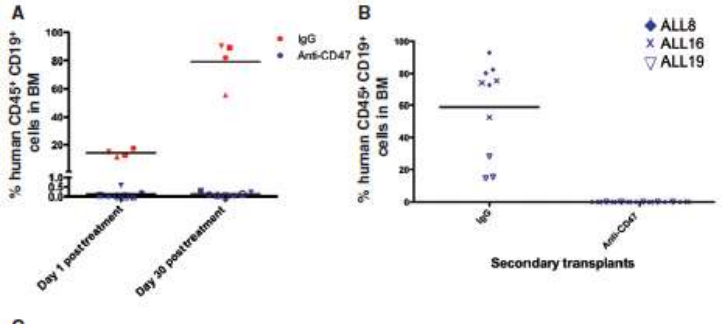
Cell 2009

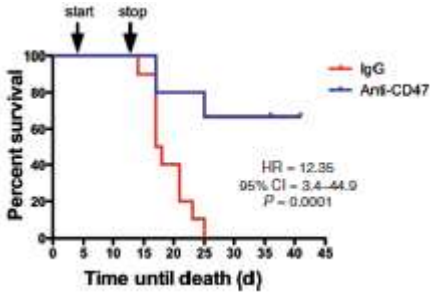
Normal hematopoietic stem cell			
Setting	Homeostatic maintenance	Inflammation/mobilization	HSC senescence
CD47 expression	Physiological	Increased	Reduced/absent
Macrophage activity	Physiological	Increased	Physiological
Net result	No phagocytosis	No phagocytosis	Increased phagocytosis
Experimental transplantability	Normal	High	Low
Leukemic stem cell			
Setting	AML with good prognosis	AML with poor prognosis	AML with poor prognosis treated with anti-CD47
CD47 expression	Low	High	High (but blocked)
Macrophage activity	Physiological	Suppressed	Physiological or increased
Net result	Enhanced phagocytosis	No phagocytosis	Enhanced phagocytosis
Experimental transplantability	Low	Very high	Low
Clinical prognosis	Good	Very poor	Improved

- CD47 a “don’t eat me” signal
- Binds to SIRPα on Macrophages and prevents phagocytosis
- During physiology –protective to circulating HSC
- Upregulated in AML vs HSC – selectively protective to LSC
- Potential target

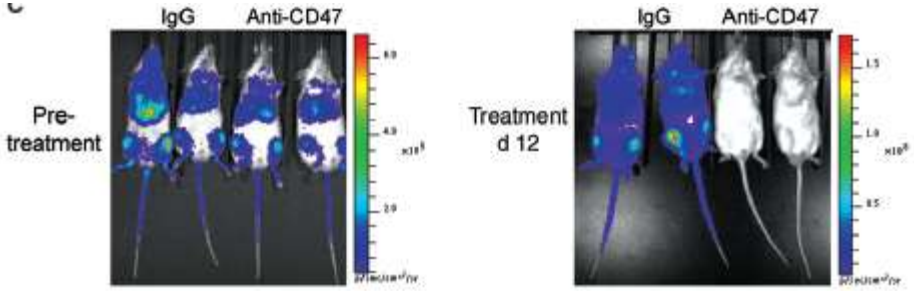
Targeting LSC surface phenotype: Anti-CD47 preclinical data in ALL







- Preclinical effects vs Acute Lymphoblastic leukaemia (ALL)
- Decreased human engraftment (flow)
- Decreased human engraftment (IVIS- bioluminescence)
- Increased survival



Therapeutic implications

- Targeting cancer stem cells

- Targeting tumour heterogeneity

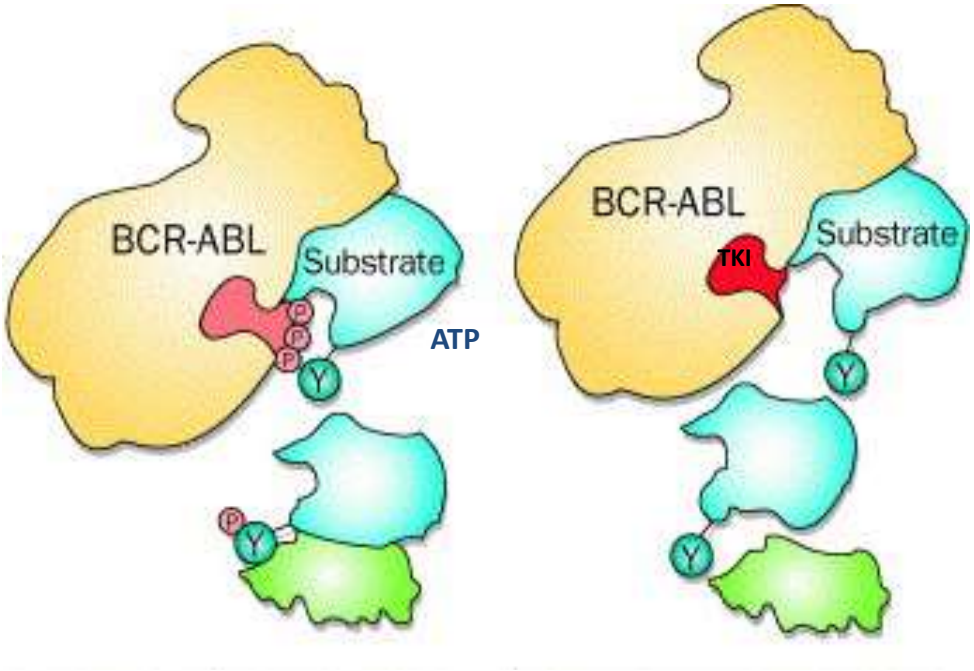
Targeted therapy: Glivec for Chronic Myeloid Leukaemia (CML)

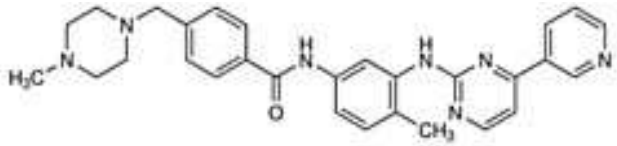
• BCR-ABL gene product, from t (9;22) chromosomal translocation – causal in ALL CASES of CML – little genetic heterogeneity

• Constitutively active Tyrosine kinase

• Competitive inhibition of ATP-binding pocket possible

Prototype compound – Imatinib Mesylate

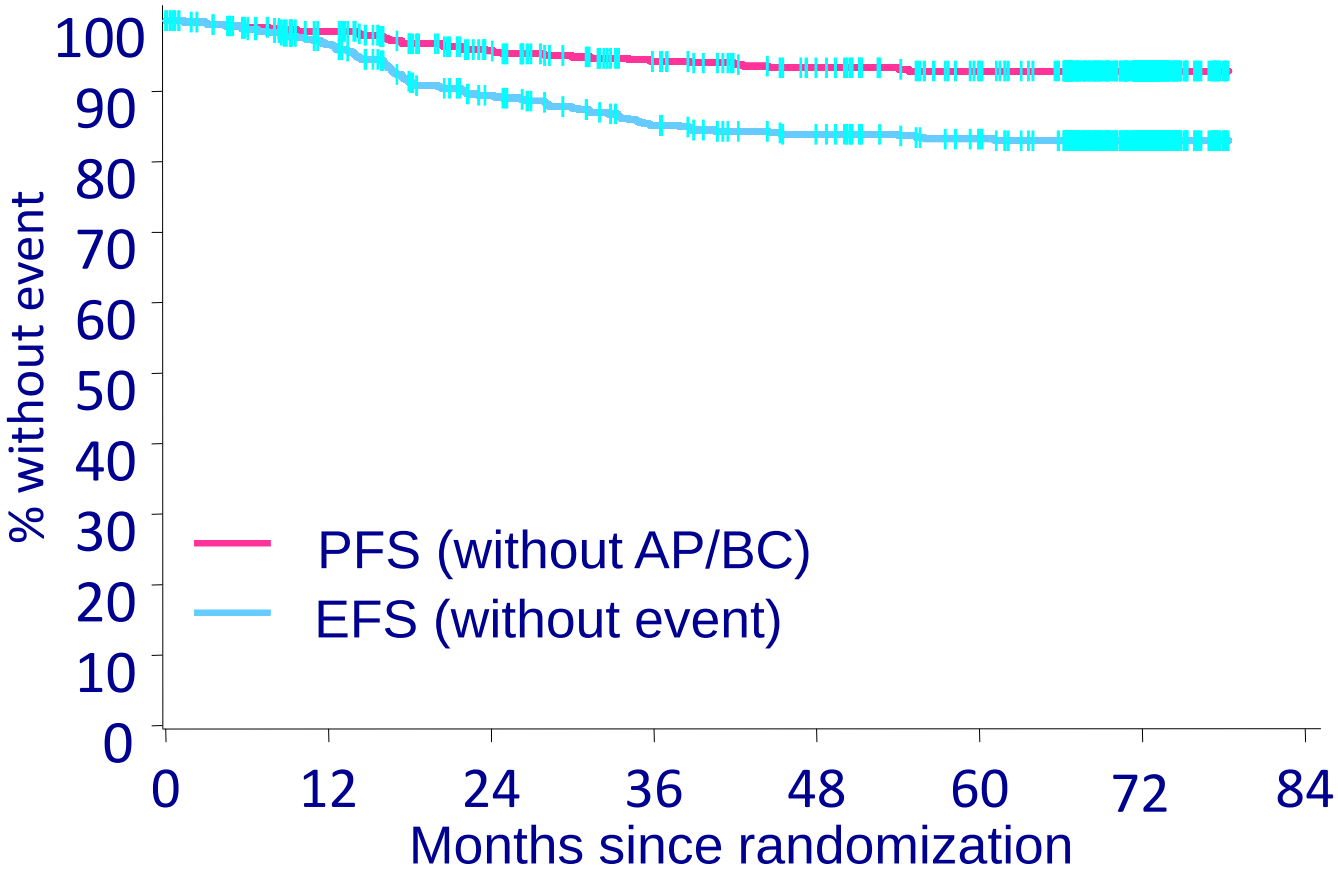




2-phenylaminopyridine derivative

Targeted therapy: Glivec for Chronic Myeloid Leukaemia (CML)

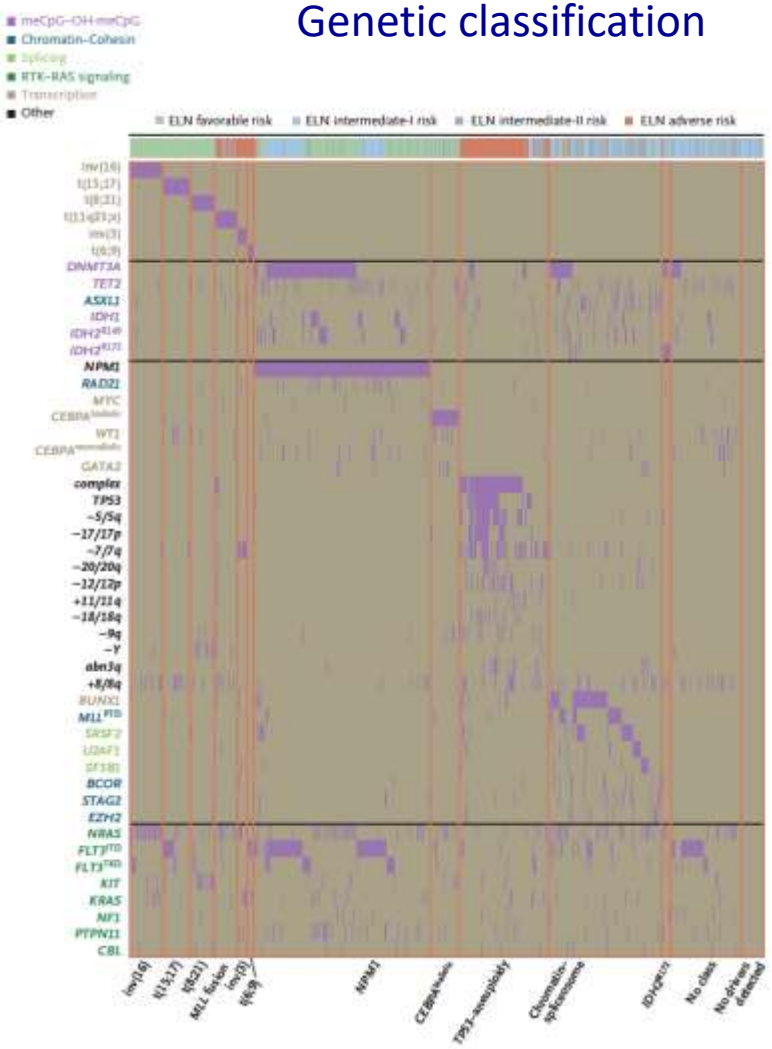
- Previous median survival for CML ~4-5 years
- Was the commonest indication for BMT

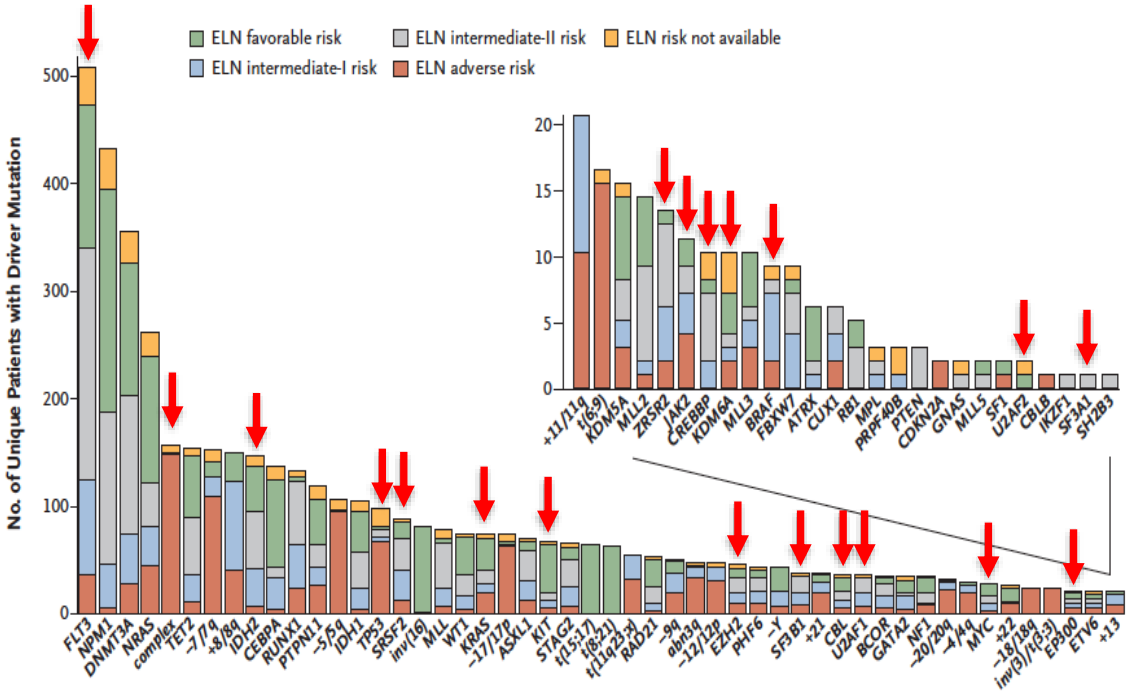


Poor targeting of CML CSC – most patients controlled rather than cured

Heterogenetiy between tumours: the basis of personalised medicine

Genetic classification



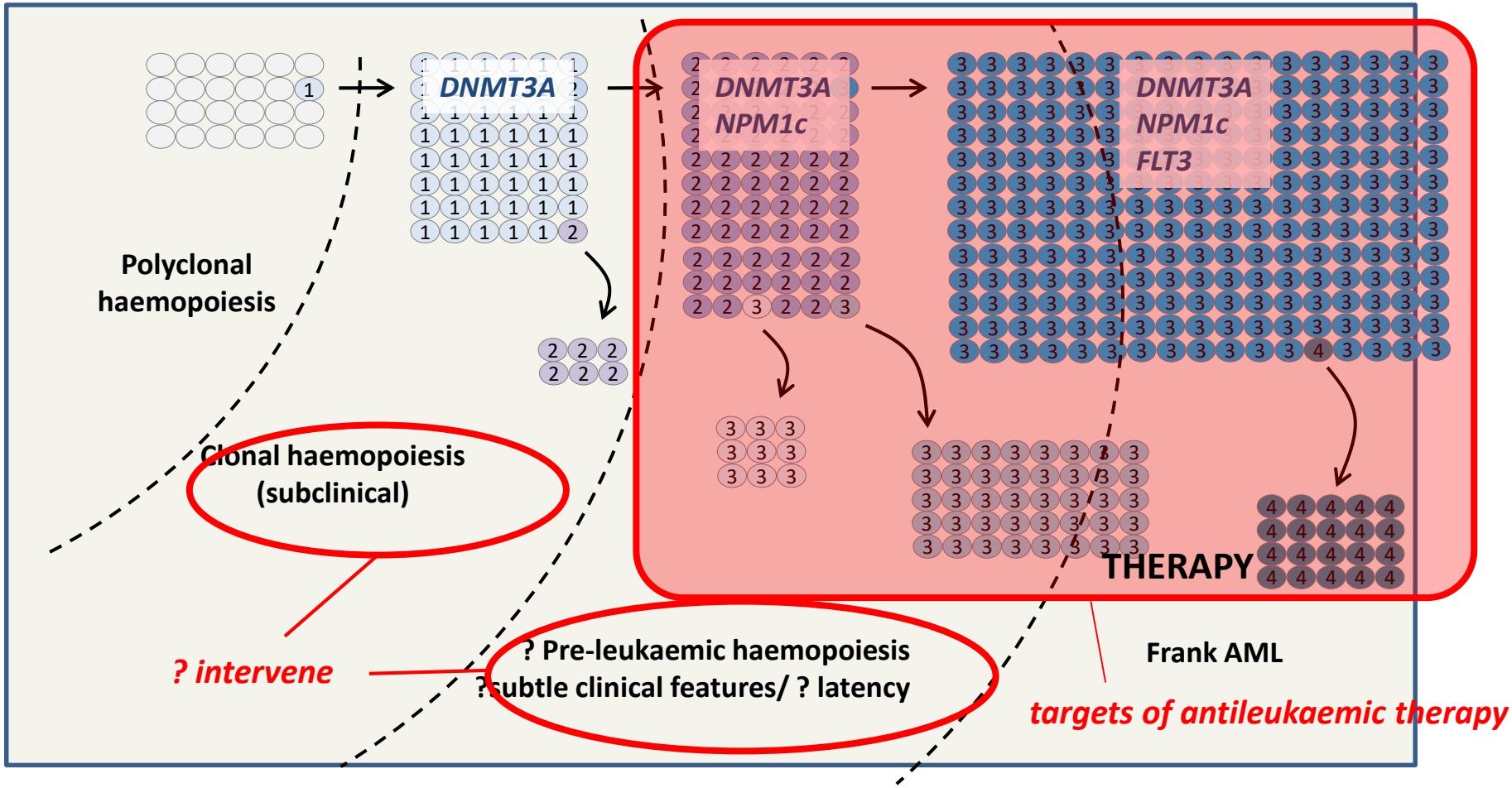


Multiple intuitive targets identified from mutational analysis:

- *FLT3*
- *IDH2*
- *SRSF2/U2AF1/U2AF2*
- *KIT*
- *JAK2*
- *BRAF*

Many mutations SUBCLONAL
Overt disease
Occur late during tumour devolution

To obviate heterogeneity: target initiating lesions



Summary of salient points

- Intra-tumoural vs Inter-tumoural heterogeneity
- Sources of heterogeneity – cell of origin, multiple selective pressures
- Phases during cancer evolution – premalignancy, diagnostic disease, relapsed disease, metastases
- Constant Darwinian nature of tumour evolution – MOVING TARGET
- To target CSC we need to understand them better
- Need to target early/initiating mutations
- A better understanding of early tumour development should allow for earlier (and more successful) therapeutic intervention