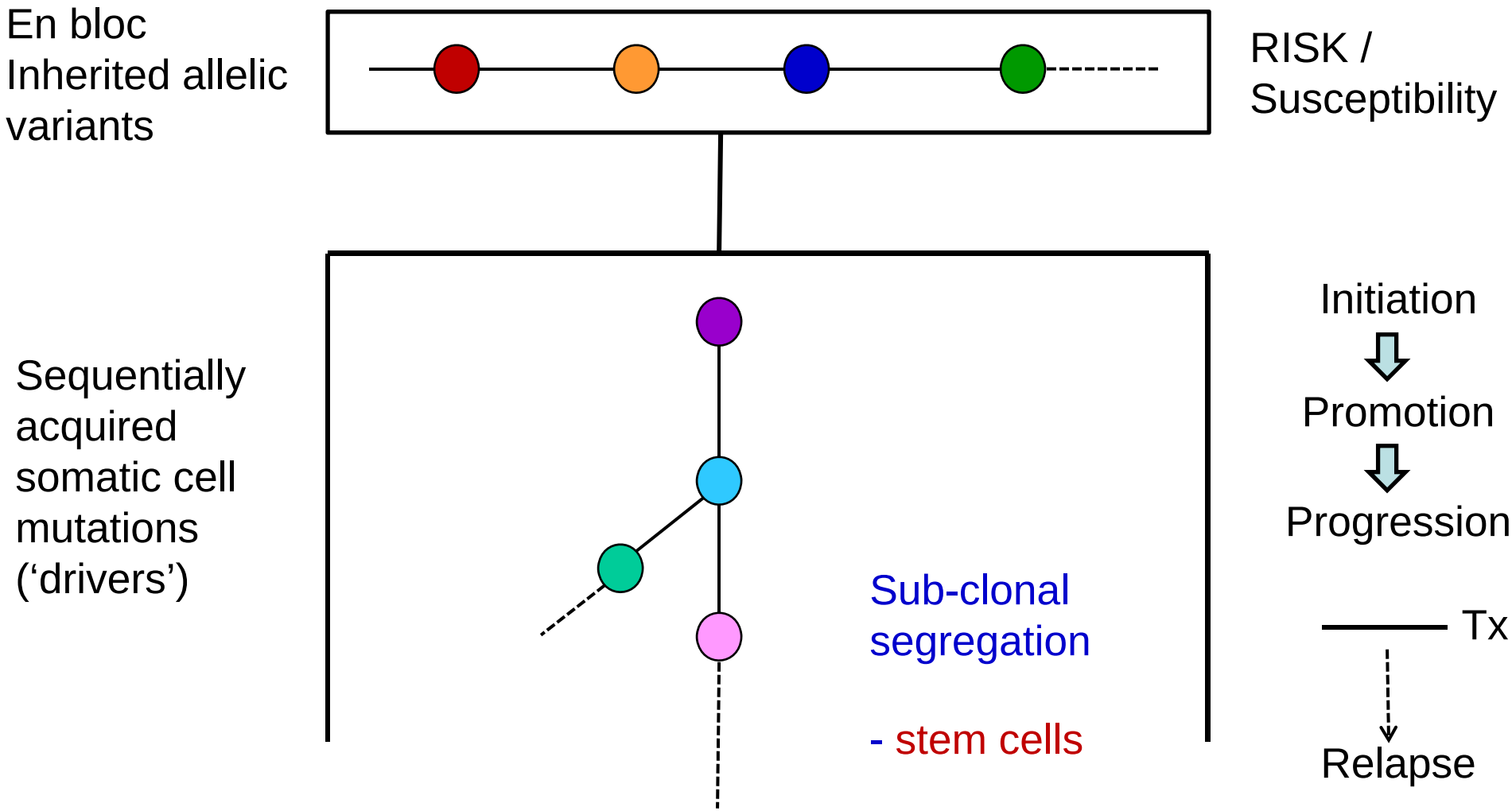
ICR
The Institute
of Cancer Research

GENETIC ARCHITECTURE OF LEUKAEMIA

Mel Greaves

Munich, 28 February 2011

GENETIC ARCHITECTURE OF CHILDHOOD LEUKAEMIA



VARIATION IN GENETIC SUSCEPTIBILITY TO CHILDHOOD ALL

- Candidate gene studies
  - Immune response genes : HLA, IL-12
  - Folate metabolism : MTHFR
  - Activation / detox : GSTs, NQ01

⊖ lack of reproducibility
(under-powered studies?)

VARIATION IN GENETIC SUSCEPTIBILITY TO CHILDHOOD ALL

- *G*enome-*W*ide *A*ssociated *S*tudies (GWAS)
  - SNP-associated normal genomes (Ca. vs control)
    - ⊕ agnostic, no prior assumptions
      precedents with other cancers
    - ⊖ incomplete genome coverage
      biased towards common alleles
      may not identify functional variant
      large case series required

VARIATION IN GENETIC SUSCEPTIBILITY TO CHILDHOOD ALL

- *G*enome-*W*ide *A*ssociated *S*tudies (GWAS)
  - SNP-associated normal genomes (Ca. vs control)

Richard Houlston *et al*

Childhood ALL

ILLUMINA CNV370-DUO

1° GWA 1 GWA 2

577 (1438 controls) 392 (960 controls)

Papaemmanuil E *et al*, Nature Genet, 2009; 41: 1006-1010

2° Validation 1 (for candidate genes)

1384 (1877 controls) Germany

Prasad R *et al*, Blood, 2010; 115: 1765-1767

3° Validation 2 (for candidate gene)

1428 (1516 controls) Germany

148 (137 controls) Spain

550 (450 controls) Hungary

260 (266 controls) Canada

Sherborne AL *et al*, Nature Genet, 2010; 42: 492-494

GWAS FOR CHILDHOOD ALL

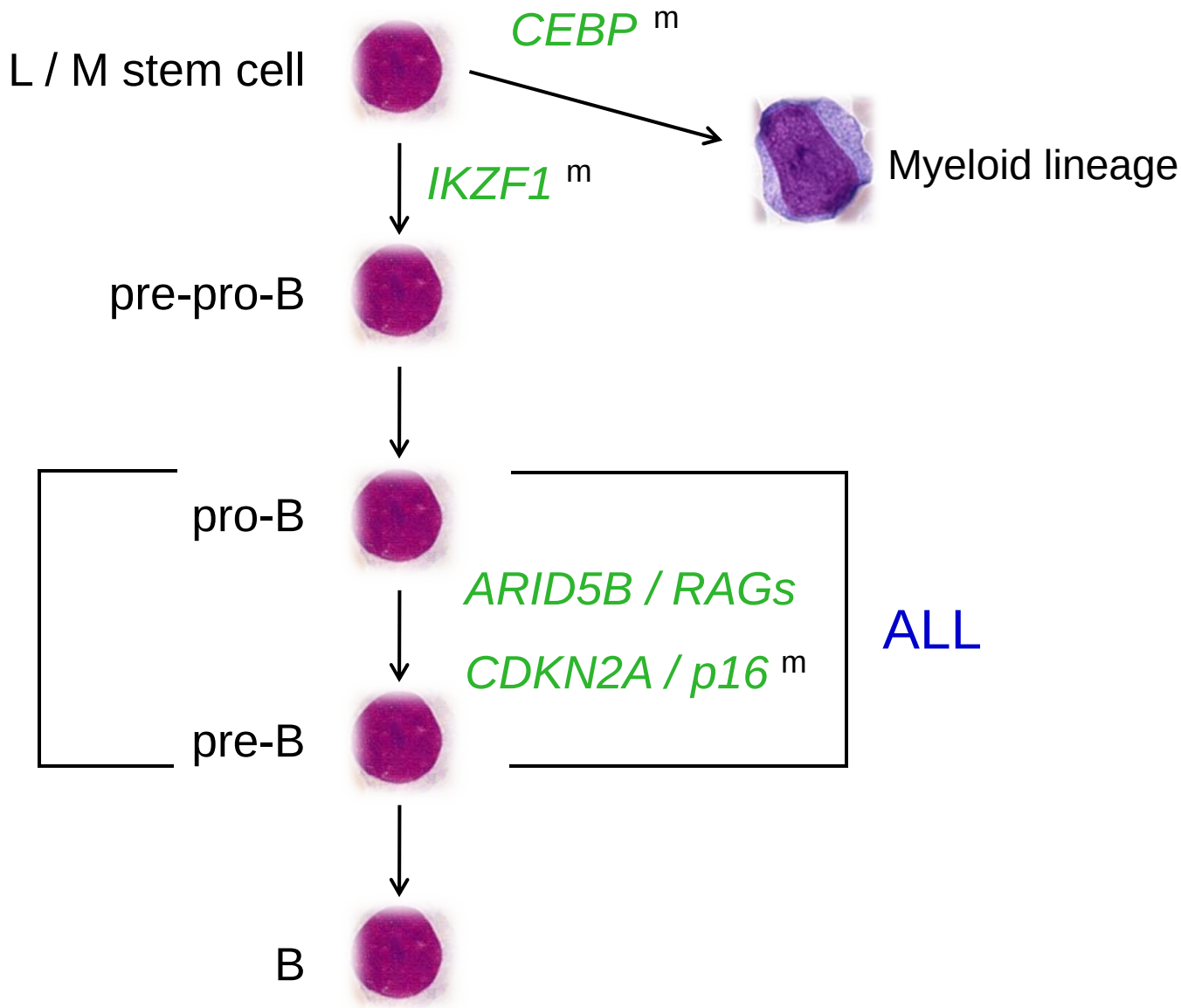
Common alleles of:

	OR	p
• *IKZF1*	1.69	1.2×10^{-19} *
• *ARID5B* (hyperdiploid)	1.65	6.7×10^{-19} *
• *CEBPE*	1.34	2.9×10^{-7}
• *CDKN2A / p16*	1.42	3.0×10^{-11}
Per allele	1.53	3.5×10^{42} (trend)

= Additive risk ~10x

* Trevino LR *et al*, Nature Genet, 2009; 41: 1001-1005

GENETIC SUSCEPTIBILITY AND MOLECULAR PATHOGENESIS OF ALL

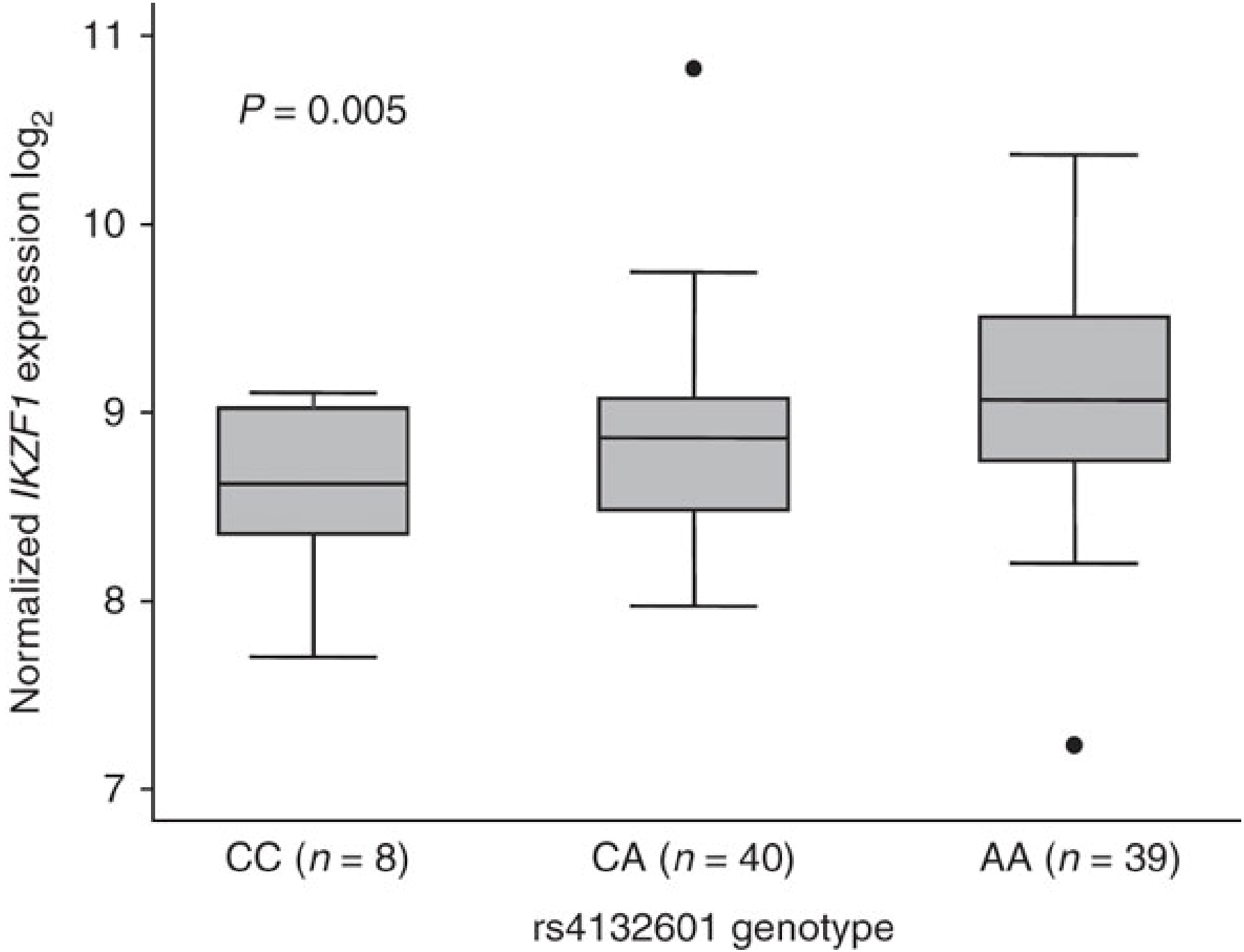


ALLELIC ARCHITECTURE of GENETIC SUSCEPTIBILITY to ALL

- Same genes involved as somatic mutants

- Gene variants that impact on intrinsic vulnerability of ‘target’ cells,
  i.e. component of molecular pathogenesis

    - via expression levels of key regulators of differentiation and cell cycle


11
10
9
8
7
P = 0.005
Normalized IKZF1 expression log2
CC (n = 8)
CA (n = 40)
AA (n = 39)
rs4132601 genotype

FUTURE:

1. Full GWAS on German series (1,500 cases)
2. International consortium (4-5,000 cases). Deep mining
3. Identification of functional variants (re-sequencing)
4. Functional validation
5. Weighted contribution to risk of ALL?

CAUSAL MECHANISMS for LEUKAEMIA / CANCER

Mutagenic Exposures

Risk 'Modifiers'

- genetics
- diet

Mutation / clonal selection

C

CAUSAL MECHANISMS for LEUKAEMIA / CANCER

Mutagenic Exposures

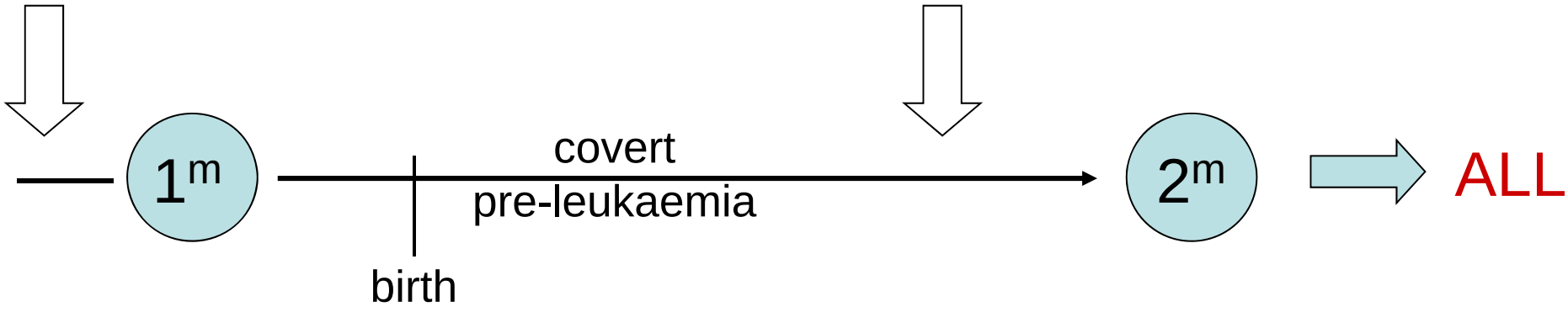
Risk 'Modifiers'

- genetics — ?
- diet

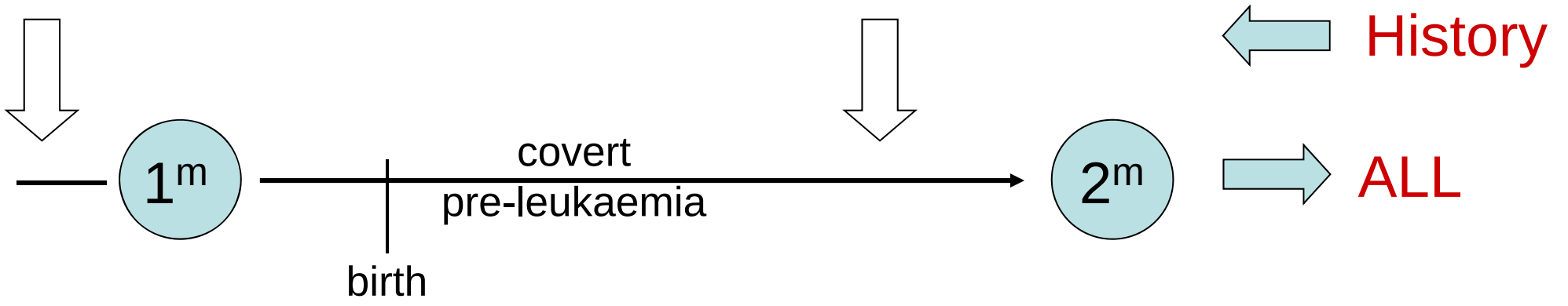
Mutation / clonal selection

C = ALL : 1 in 2000

Genetic architecture of cALL clones: a minimal natural history model



1988 Speculations on the cause of childhood acute lymphoblastic leukemia. *Leukemia*, 2: 120-125

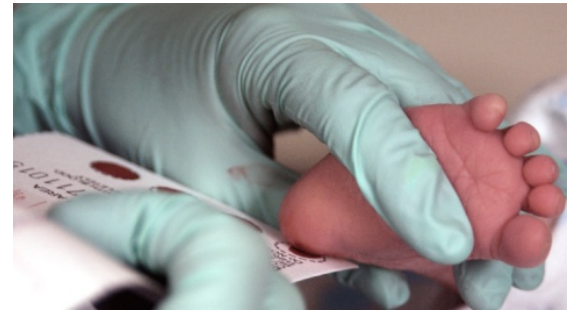

History !
1m
covert
pre-leukaemia
2m
ALL
birth

BACKTRACKING THE PRE-NATAL ORIGINS OF CHILDHOOD LEUKAEMIA

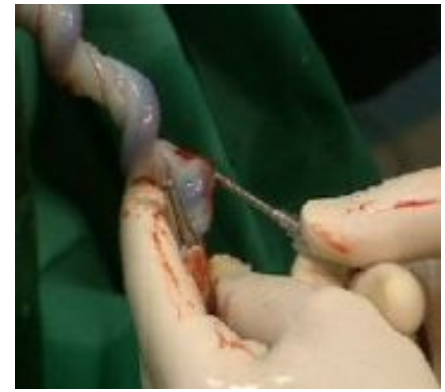
- Monozygotic (monochorionic) twins with concordant ALL

Courtesy of mother

- Archived neonatal blood spots (Guthrie cards) of patients with ALL / AML



- Frozen cord bloods
  - patients with ALL (rare)
  - unselected cohort



EARLY OR INITIATING EVENTS IN LEUKAEMOGENESIS

- Foetal haemopoiesis
- Chromosome translocation / gene fusions
  MLL-AF4
  ETV6-RUNX1 (TEL-AML1)
  AML1-ETO
- Chromosomal hyperdiploidy
- Chromosomal instability (rare)
- Mutations - *GATA1* in TMD / AML in Down's

Reviewed in Greaves & Wiemels, Nature Reviews Cancer, 2003

SECONDARY, POST-NATAL MUTATIONS ARE ESSENTIAL FOR CLINICAL LEUKAEMIA

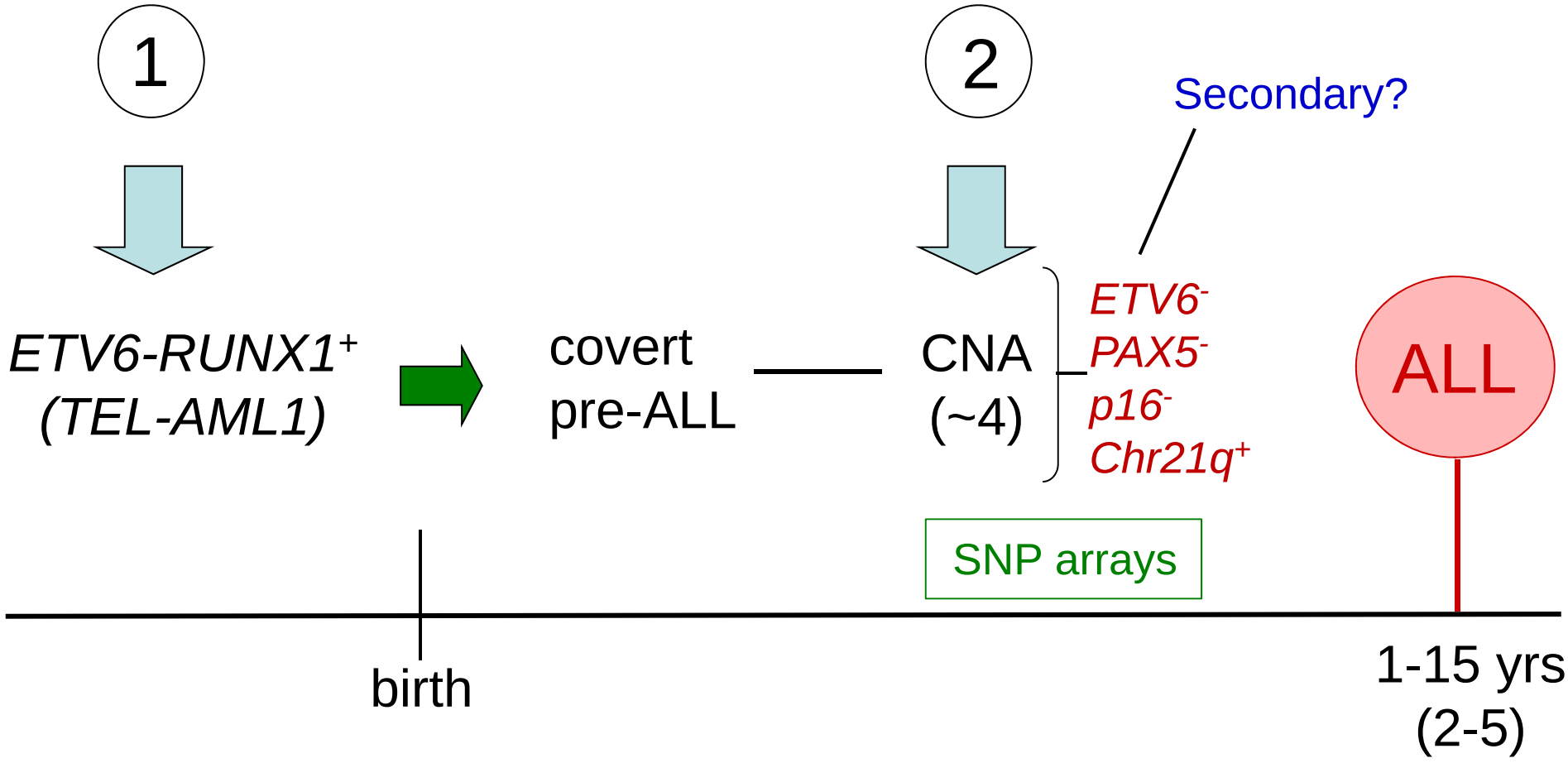
- Incidence of pre-leukaemic clones in cord blood (100x)
- Concordance rate in twins is 10-15%
- Other mutations are present and detectable at diagnosis
- Model systems:
  - *ETV6-RUNX1* transgenics (Ford et al, *J Clin Invest* 2009)
  - *ETV6-RUNX1* murine stem cell transplants (Tsuzuki et al, *PNAS* 2004)
  - *ETV6-RUNX1* in human stem cells (Hong et al, *Science* 2008)

RECURRENT CNA FROM SNP ARRAY STUDIES OF *ETV6-RUNX1*$^{+}$ ALL

Deletions	
12p13.2:	ETV6
9p13.2:	PAX5
9p21.3:	CDKN2A
3q13.2:	CD200, BTLA
6q16.2-q16.3:	16 genes, inc CCNC
6q21:	ARMC2, SESN1
3q26.32:	TBL1XR1
12q21.33:	BTG1
1q31.3:	TROVE2, GLRX2 etc
4q31.21:	telomeric to NR3C2
5q31.3:	NR3C1, LOC389335
5q33.3:	EBF1
3p14.2:	FHIT
8q12.1:	5' of TOX
9p21.3:	MLLT3 (AF9)
10q25.1:	ADD3
11q22-q23:	20 genes, incl ATM
13q14.2:	RB1
13q14.11:	ELF1
13q14.3:	DLEU etc
15q15.1:	18 genes incl LTK
17q11.2:	
19q13.11-q13.2:	GRLF1 etc
20p12.1:	c20orf94
3p22.3:	?gene
Gains	
Xq	
21q22.11-q22.12:	33 genes including RUNX1
1q23.3-q44	

'DRIVERS' (recurrency / function) vs. 'PASSENGERS'

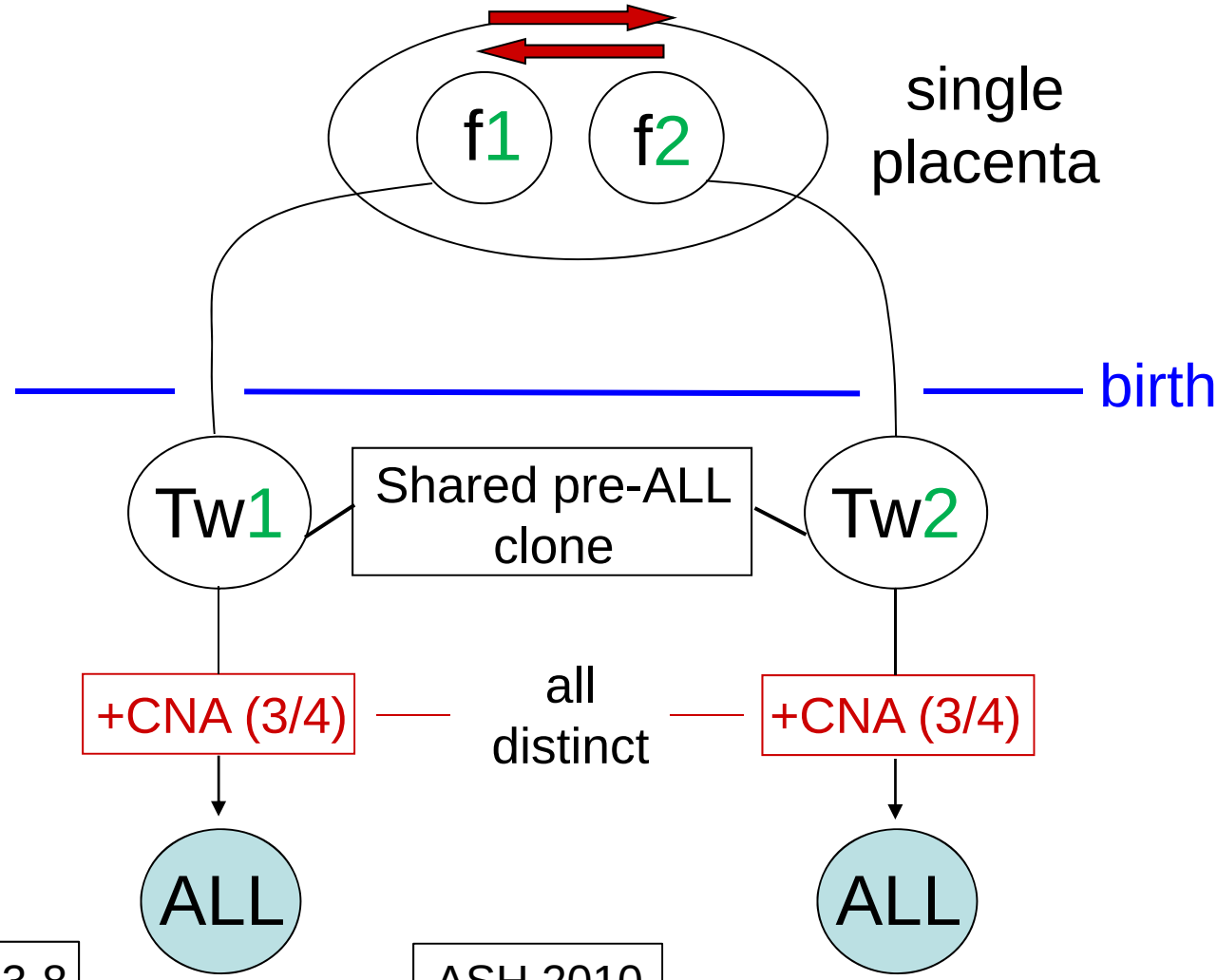
SEQUENTIAL MODEL FOR ALL



*CON*CORDANT ALL IN MONOZYGOTIC TWINS

(5x) *ETV6-RUNX1 / Hyperdiploidy* (3x)

C Bateman



Blood, 2010, 115: 3553-8

ASH 2010

Courtesy of mother

ETV6-RUNX1^{+}

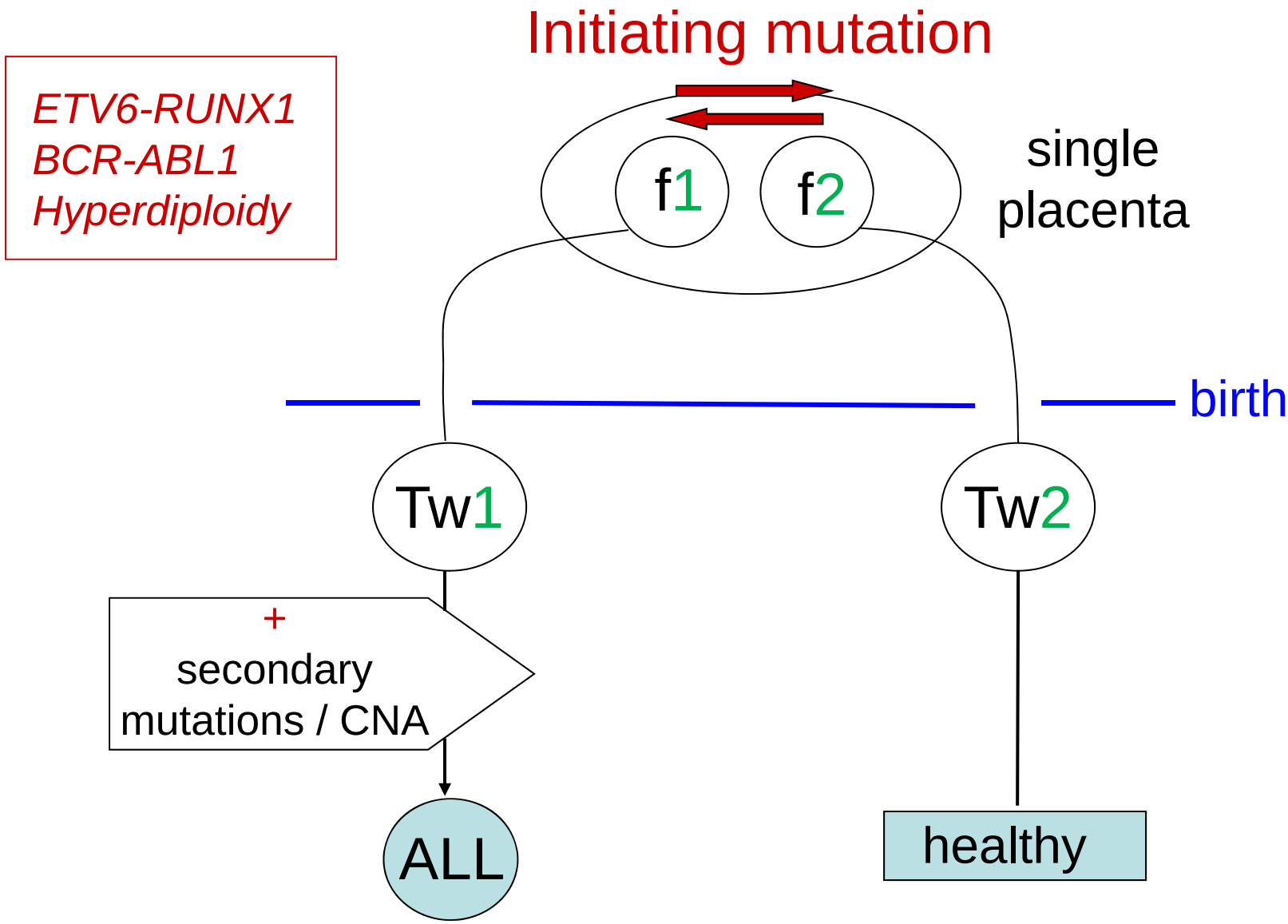
ALL

DISCORDANT

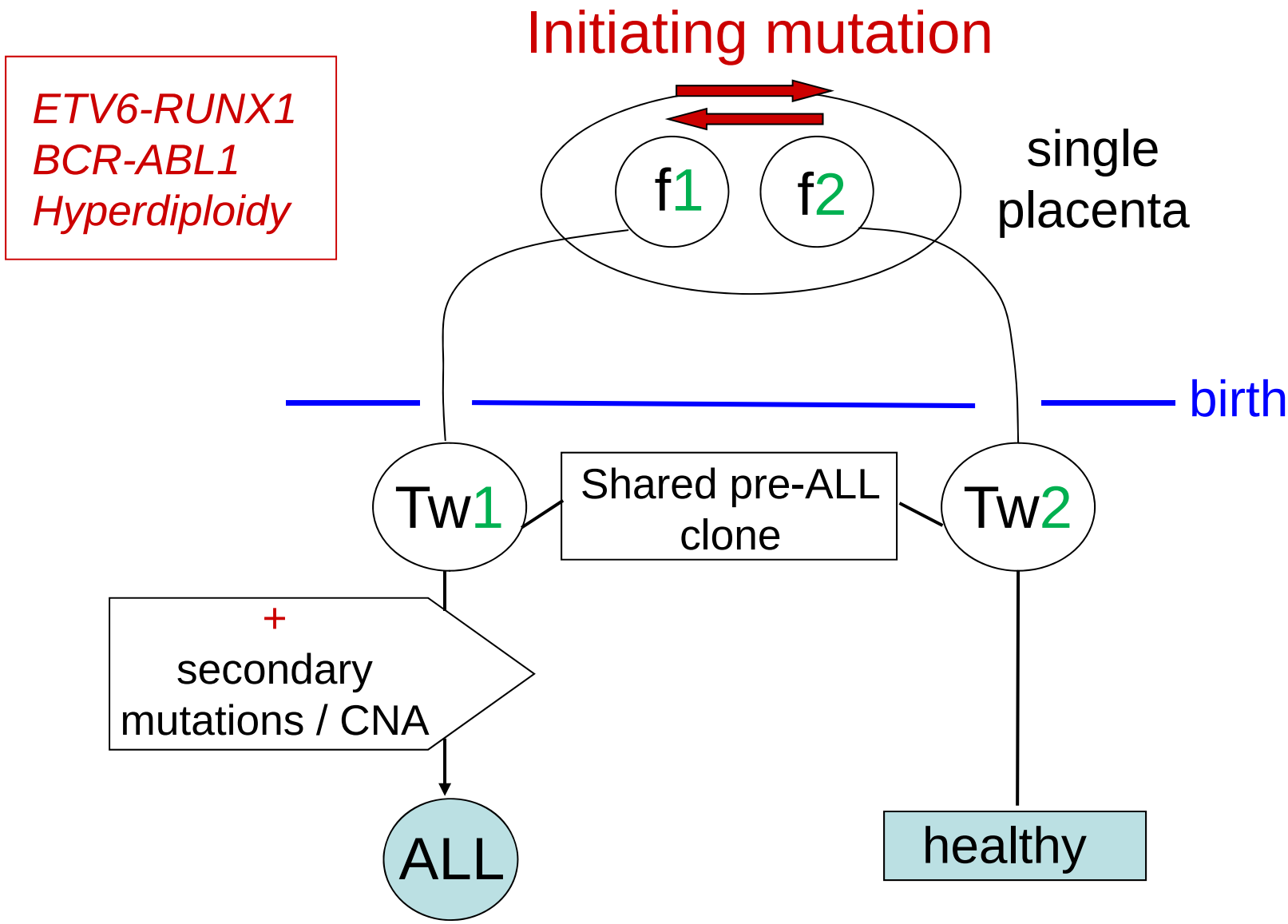
Risk = ~1 in 10

Hong D et al
Science, 2008

‘ARRESTED’ ANCESTRAL CLONES IN MONOZYGOTIC TWINS *DIS*CORDANT FOR ALL



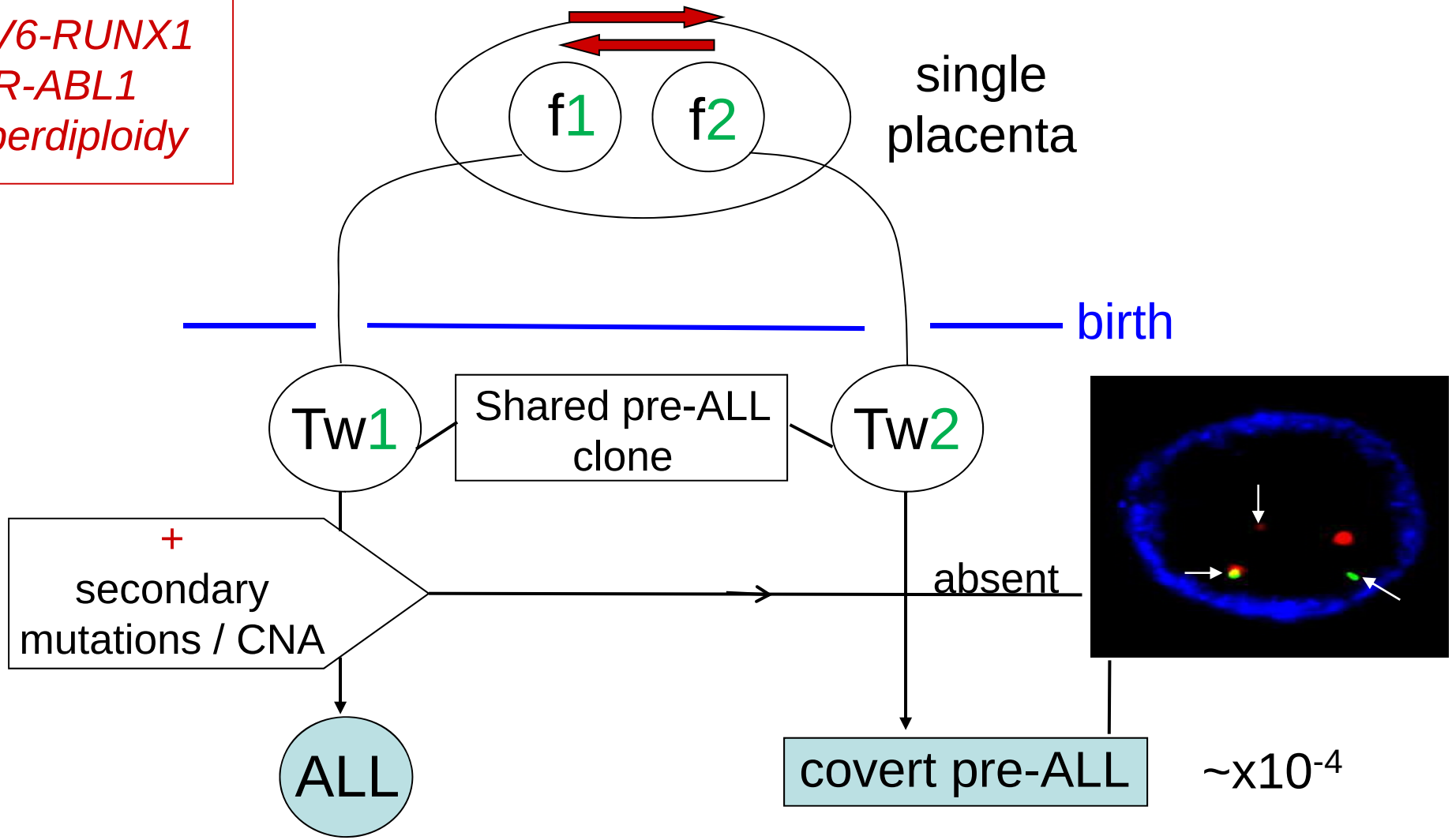
‘ARRESTED’ ANCESTRAL CLONES IN MONOZYGOTIC TWINS *DIS*CORDANT FOR ALL

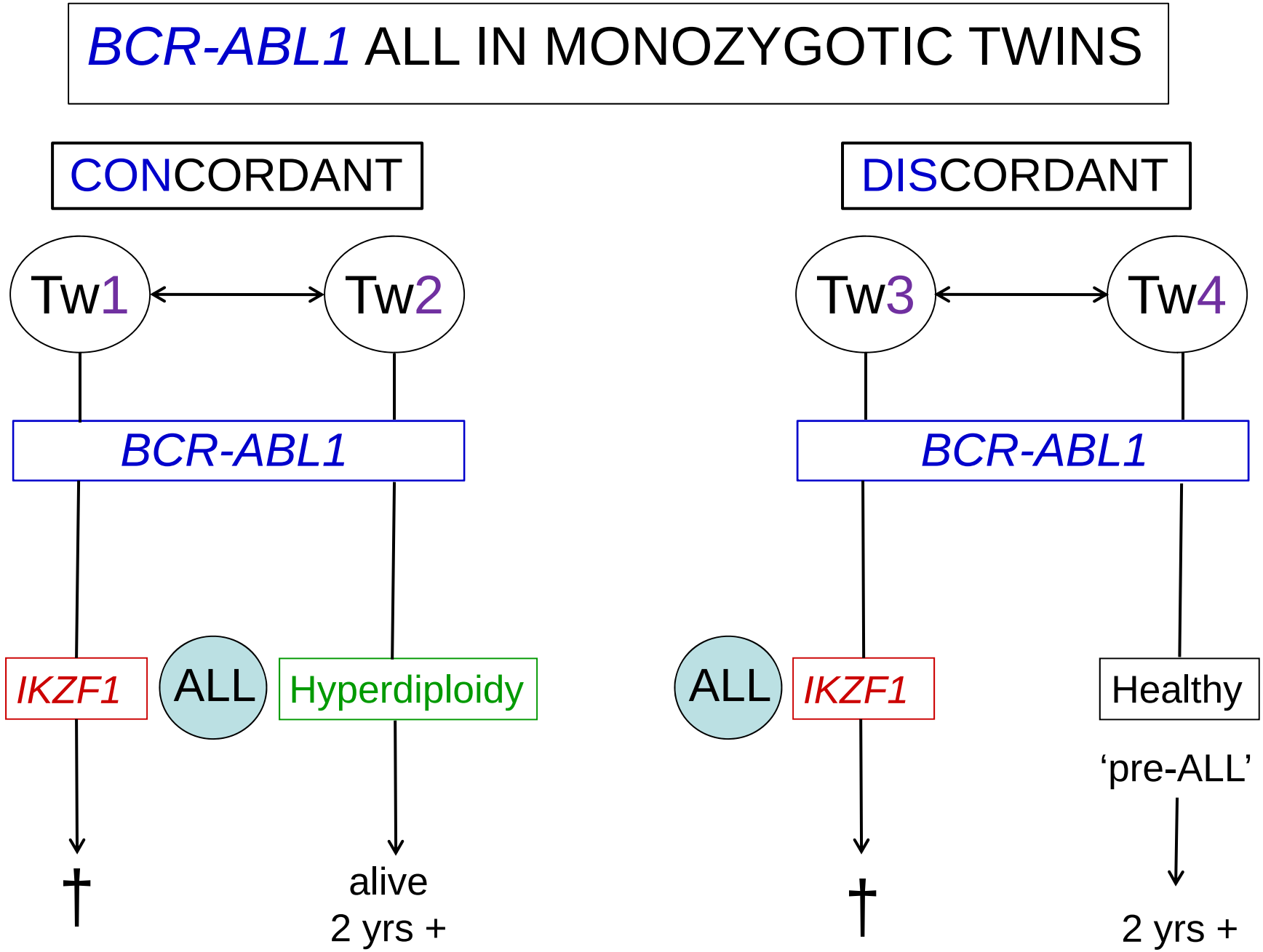


‘ARRESTED’ ANCESTRAL CLONES IN MONOZYGOTIC TWINS *DIS*CORDANT FOR ALL

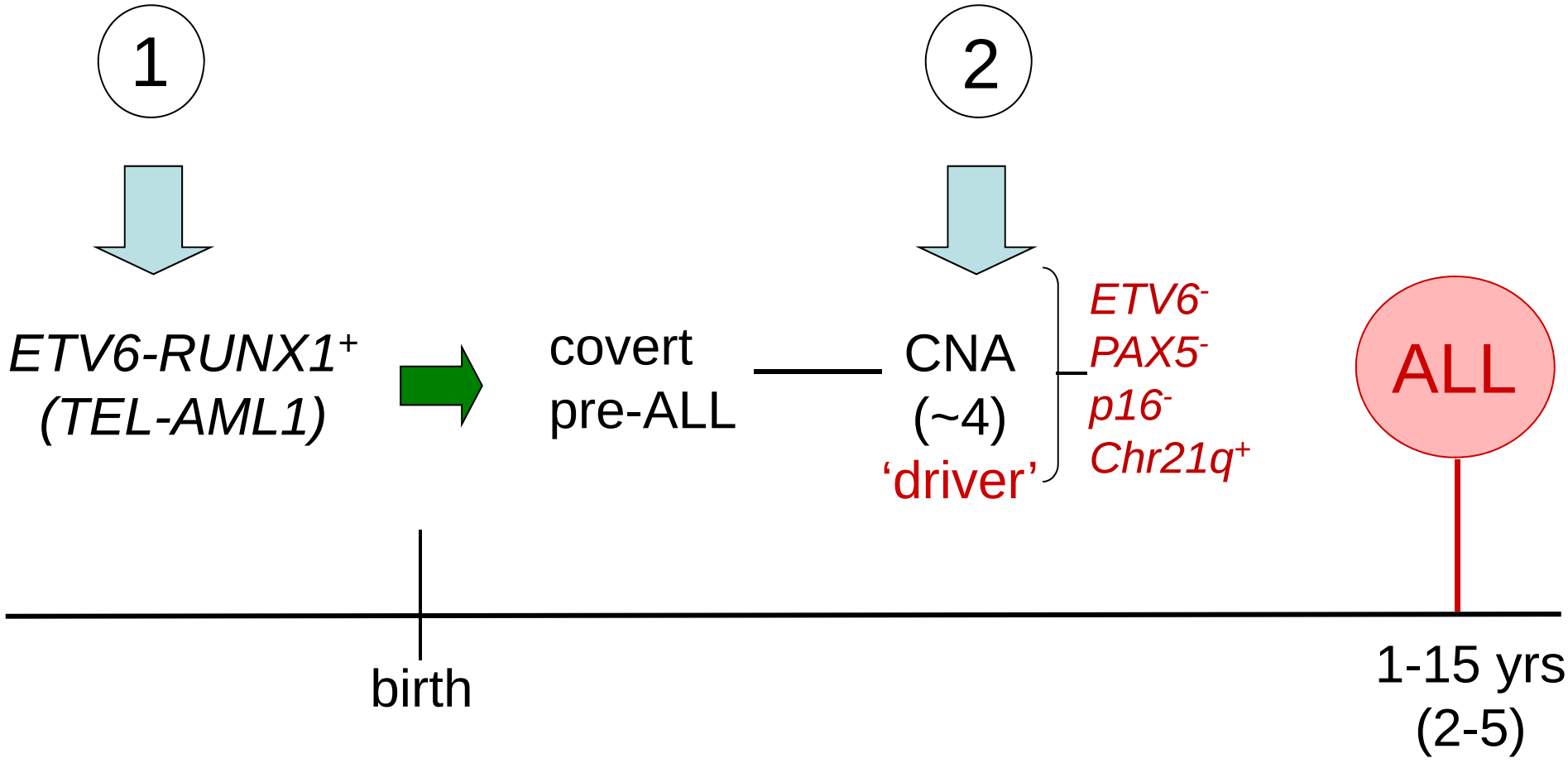
ETV6-RUNX1
BCR-ABL1
Hyperdiploidy

Initiating mutation

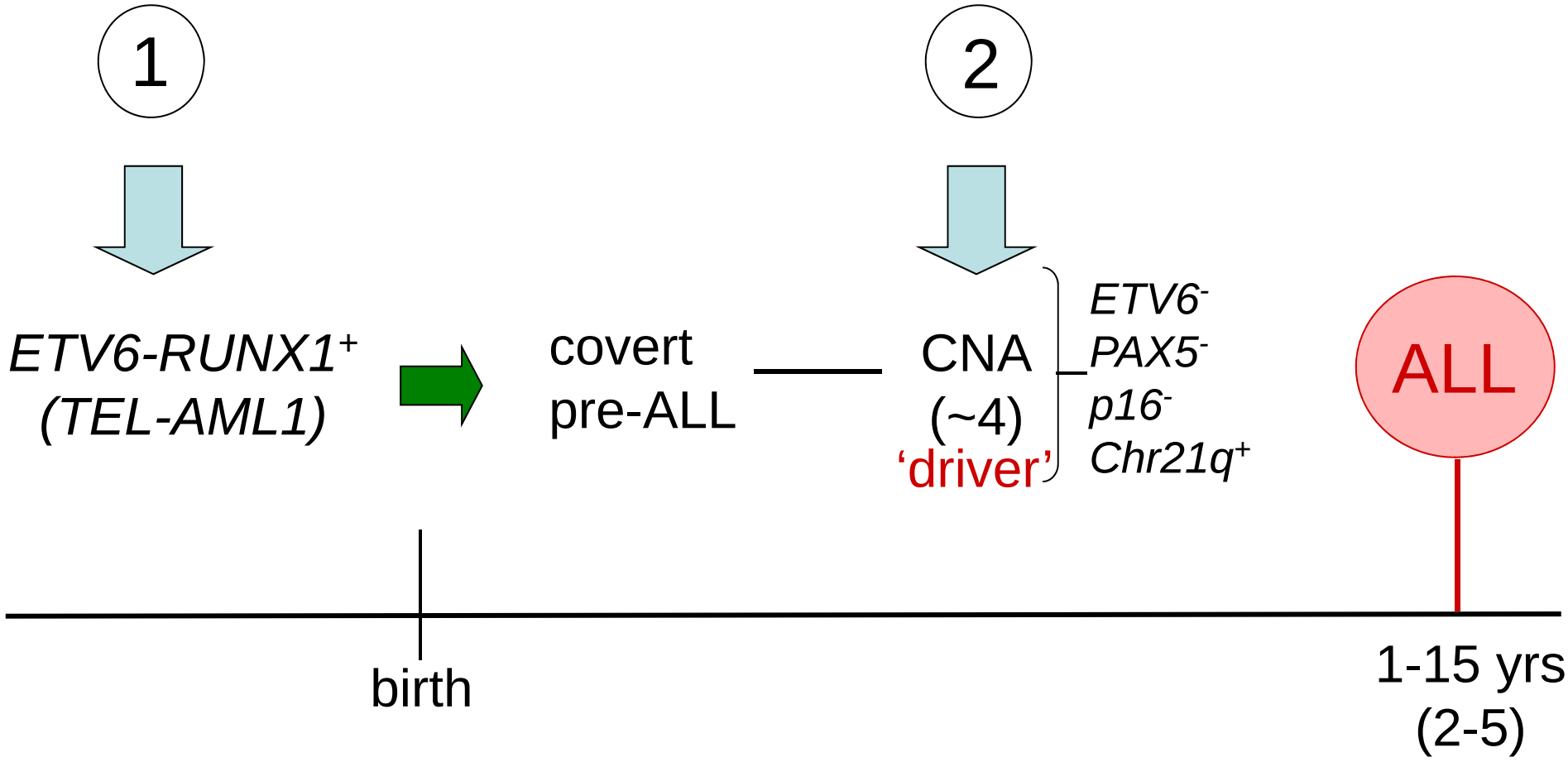



BCR-ABL1 ALL IN MONOZYGOTIC TWINS
CONCORDANT
DISCORDANT
Tw1
Tw2
Tw3
Tw4
BCR-ABL1
BCR-ABL1
IKZF1
ALL
Hyperdiploidy
ALL
IKZF1
Healthy
'pre-ALL'
†
alive
2 yrs +
†
2 yrs +

SEQUENTIAL MODEL FOR ALL



SEQUENTIAL MODEL FOR ALL: NUMBER OF MUTATIONS?



CRYPTIC MUTATIONS IN ALL?

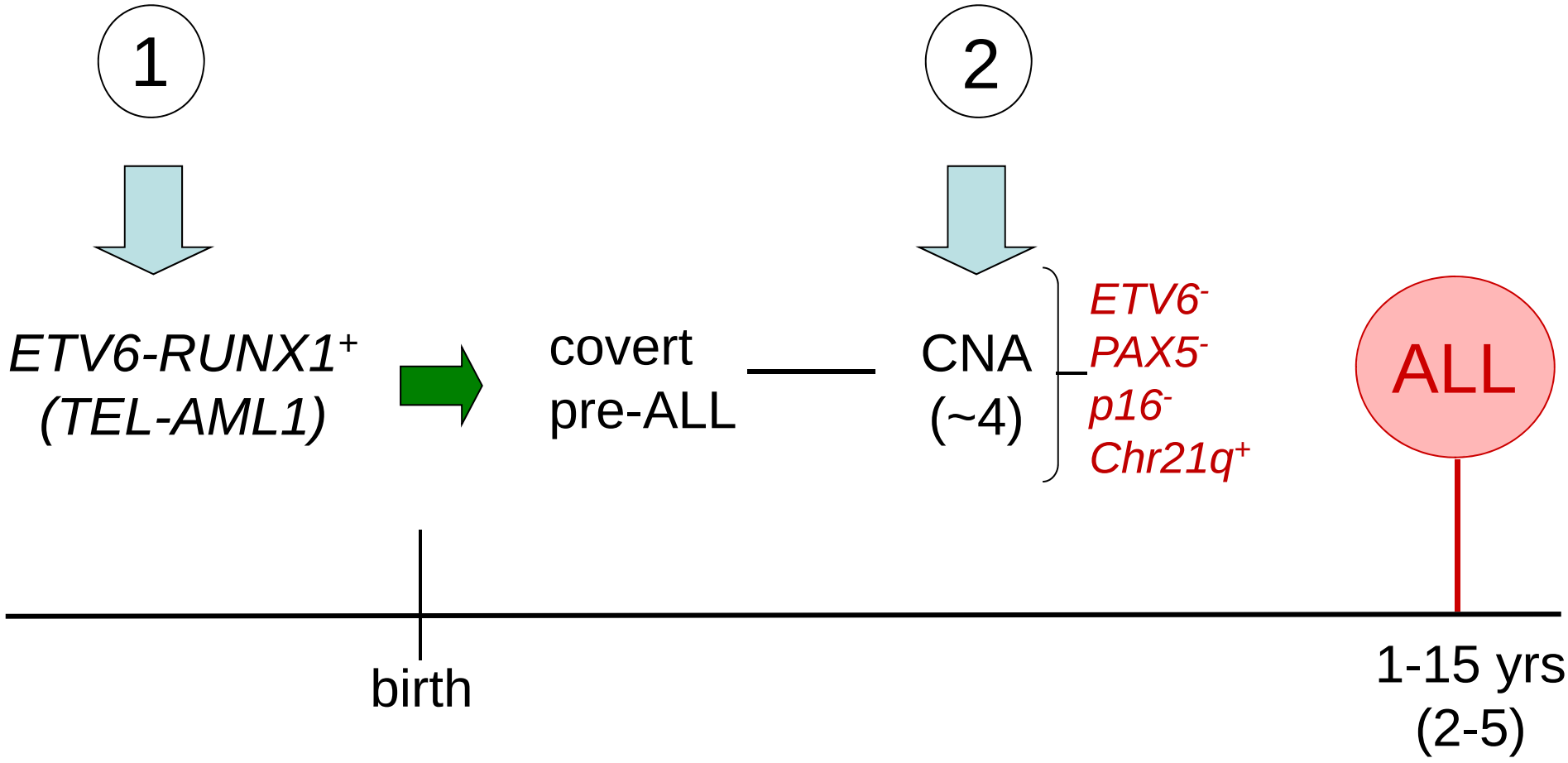
- 50 cases of *ETV6-RUNX1*$^{+}$ ALL (+ remission / normal DNA)
  - Paired end sequencing (- cryptic rearrangements)
  - Exon pull-down, solexa sequencing (- sequence based mutations)

(Collaboration with Sanger Centre; P Campbell *et al*)

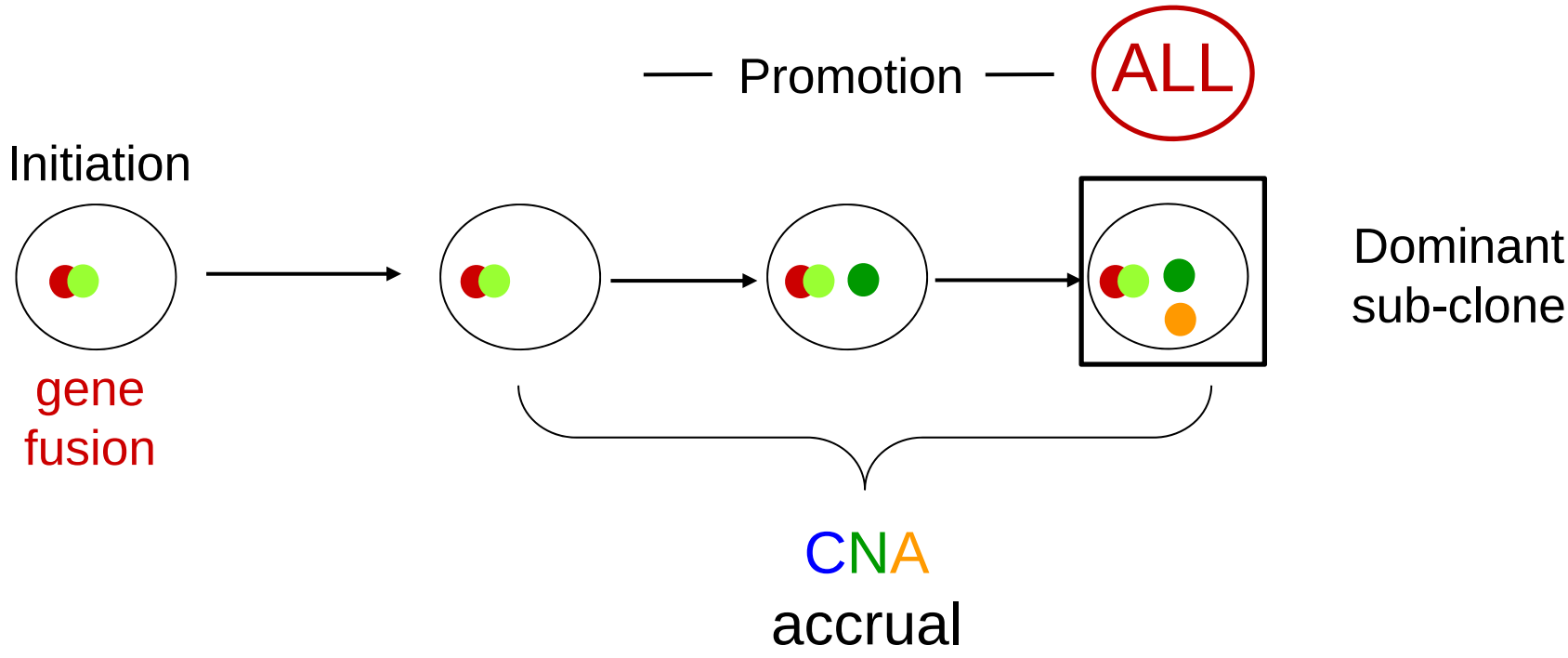
- 7 cases of ALL (+ remission / normal DNA)
  - Whole genome sequencing

(Collaboration with R Houlston, ICR and Complete Genomics)

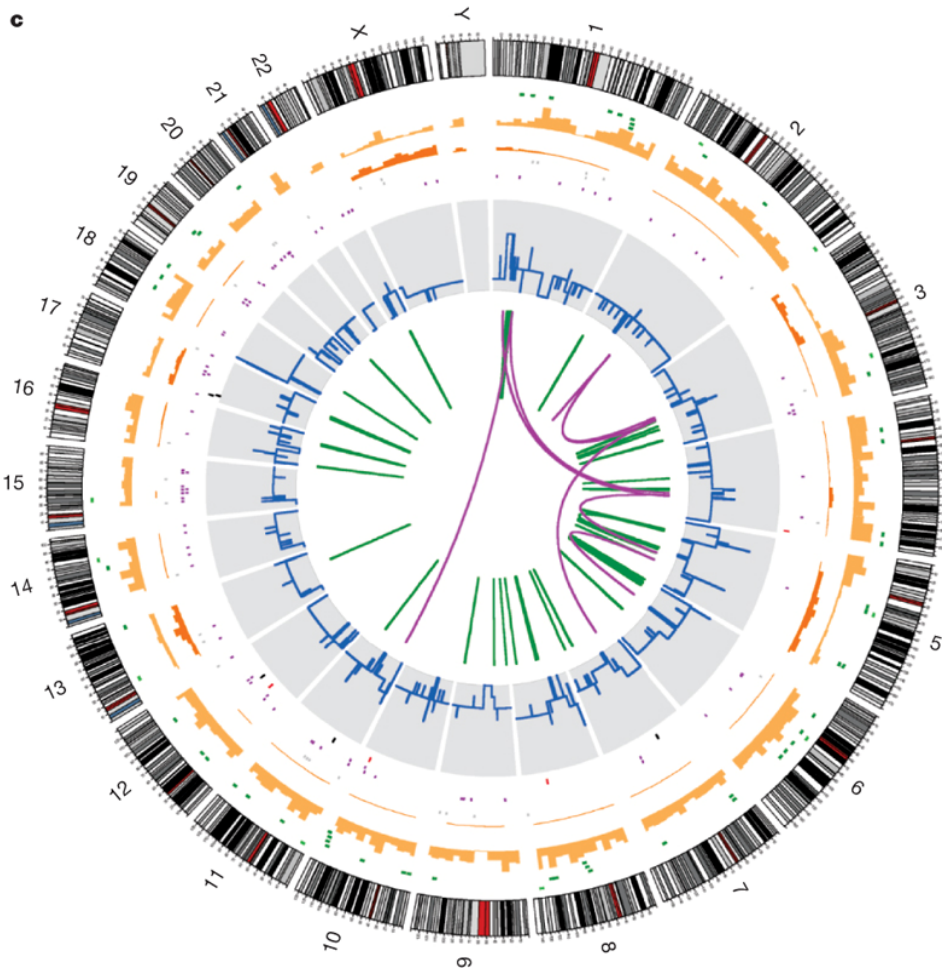
EVOLUTIONARY DYNAMICS & CLONAL ARCHITECTURE

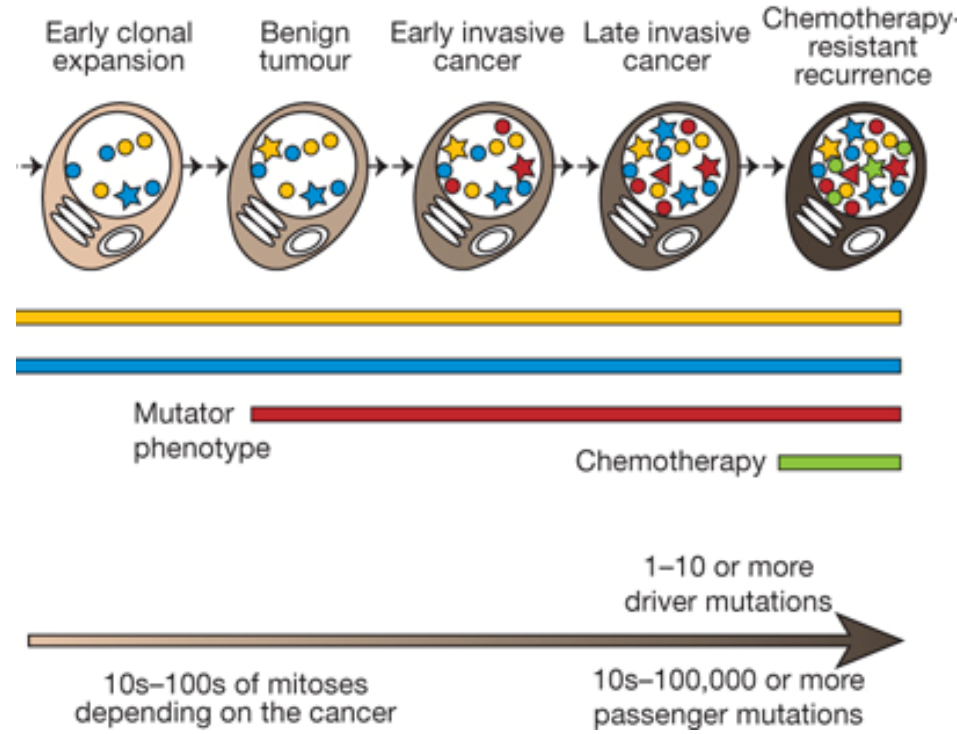


A LINEAR CLONAL ARCHITECTURE?



THE CANCER GENOME ‘LANDSCAPE’





ED Pleasance *et al. Nature* **463**, 184-190 (2010)

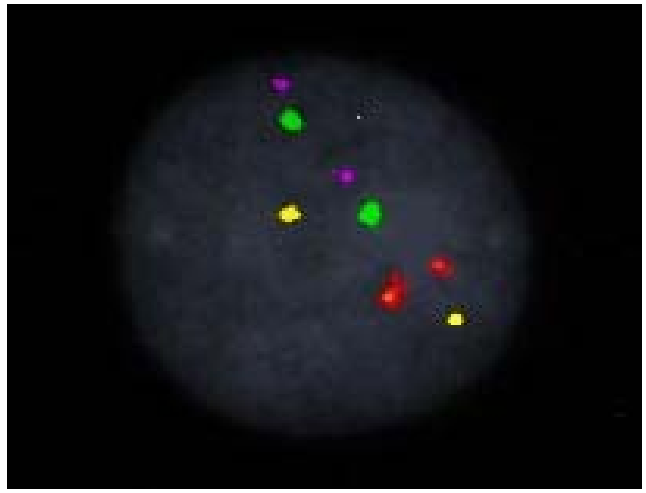
MR Stratton *et al. Nature* **458**, 719-724 (2009)

Genetic variegation of clonal architecture and propagating cells in leukaemia

Kristina Anderson, Christoph Lutz, Frederik van Delft,
Caroline Bateman, Yanping Guo, Susan Colman, Helena Kempski,
Anthony Moorman, Ian Titley, John Swansbury, Lyndal Kearney,
Tariq Enver, Mel Greaves

Anderson K et al
Nature, 2011, 469: 356-361

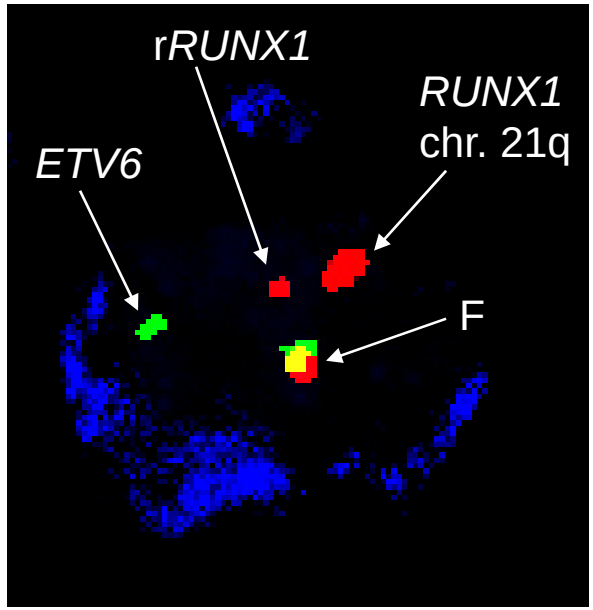
Kristina Anderson



Single cells x 200 / pt

Fusion gene

- *ETV6*
+ copies of fusion gene
+ copy of Chr 21q (*RUNX1*) / r+2
- copies of *PAX5 (-/+ or -/-)*
- copies of *CDKN2a/p16* (-/+ or -/-)

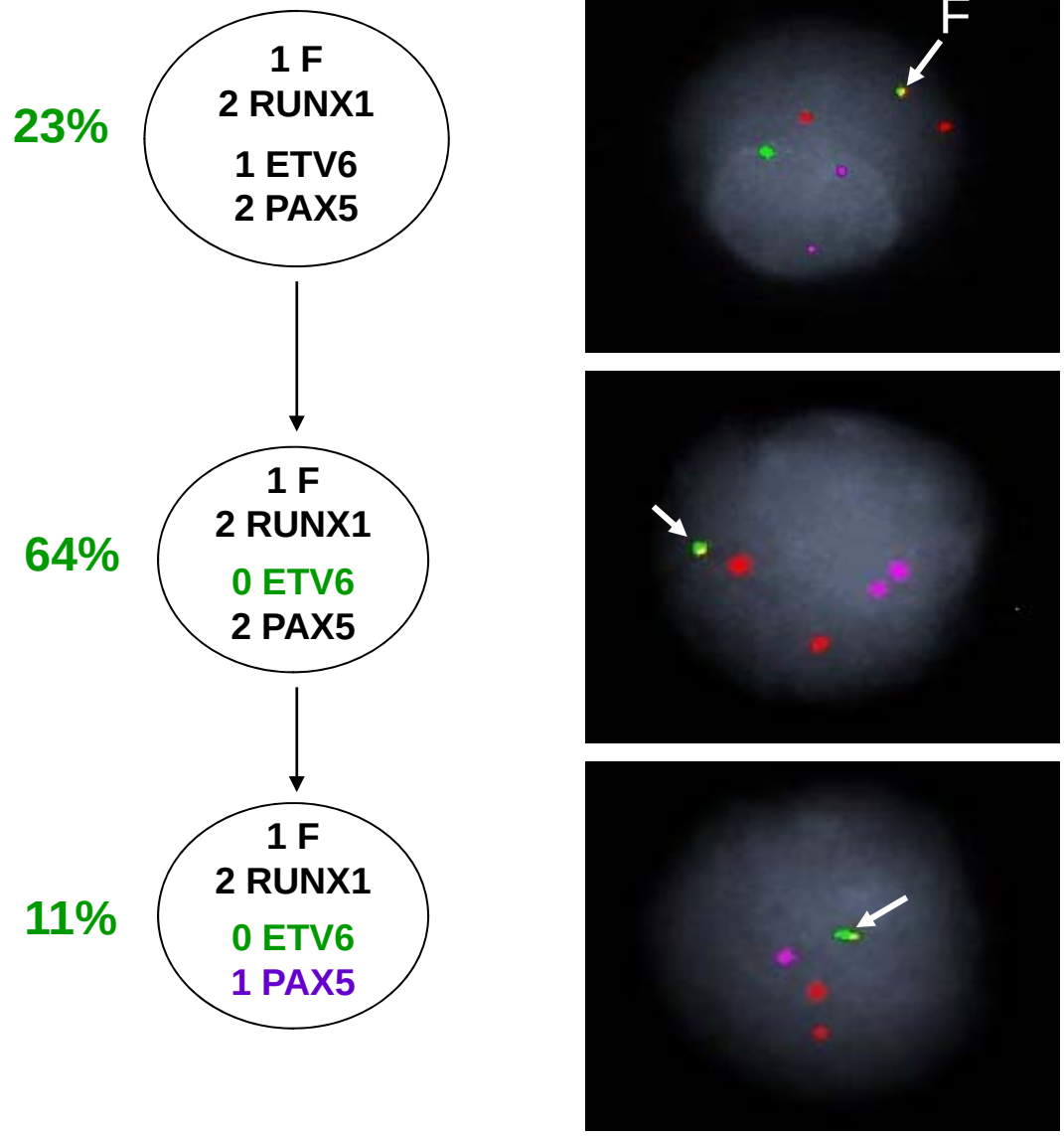


→ Genotype distinct sub-clones (%)

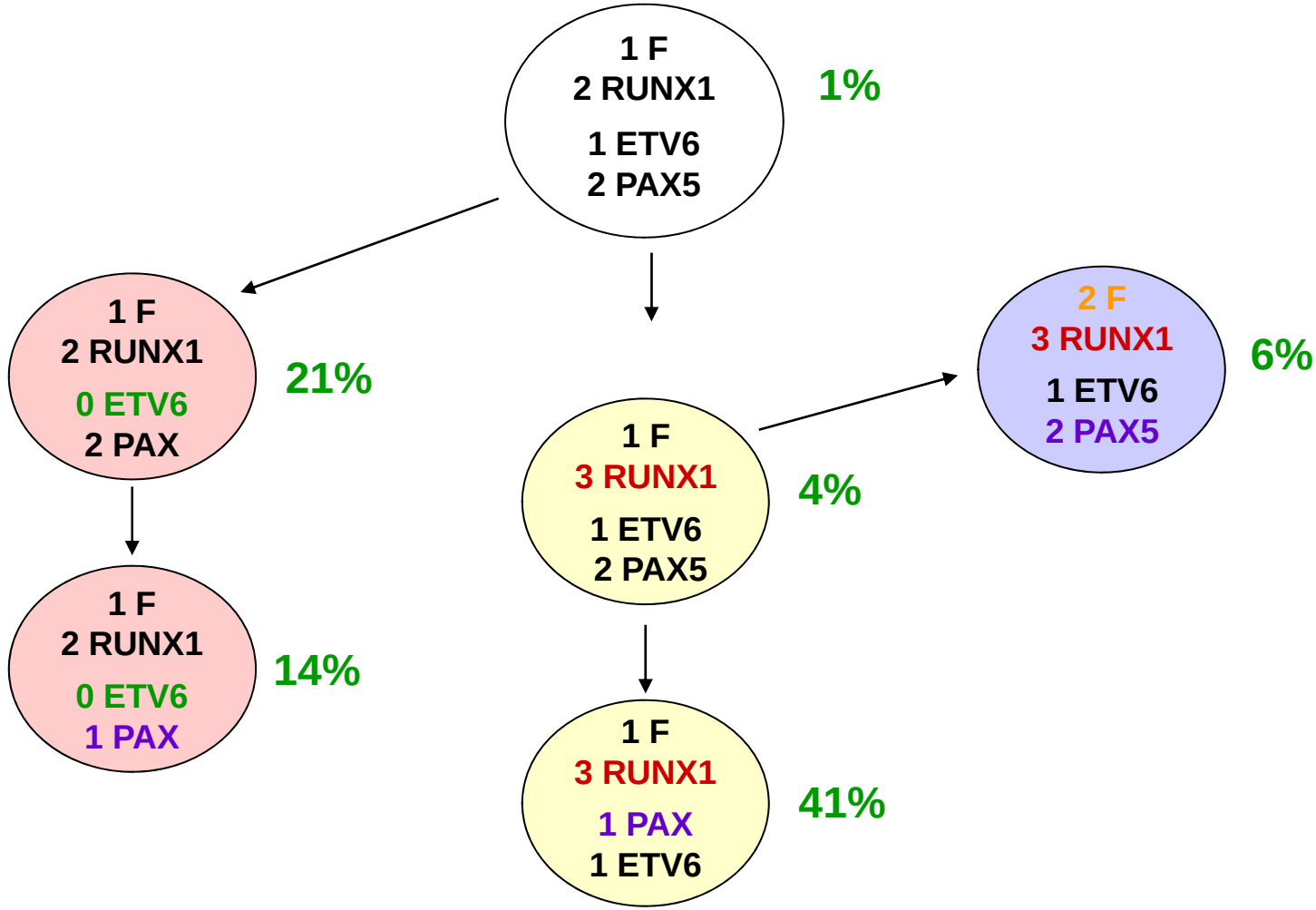
→ Clonal architecture / ancestral tree

~30 cases of *ETV6-RUNX1*$^{+}$ ALL

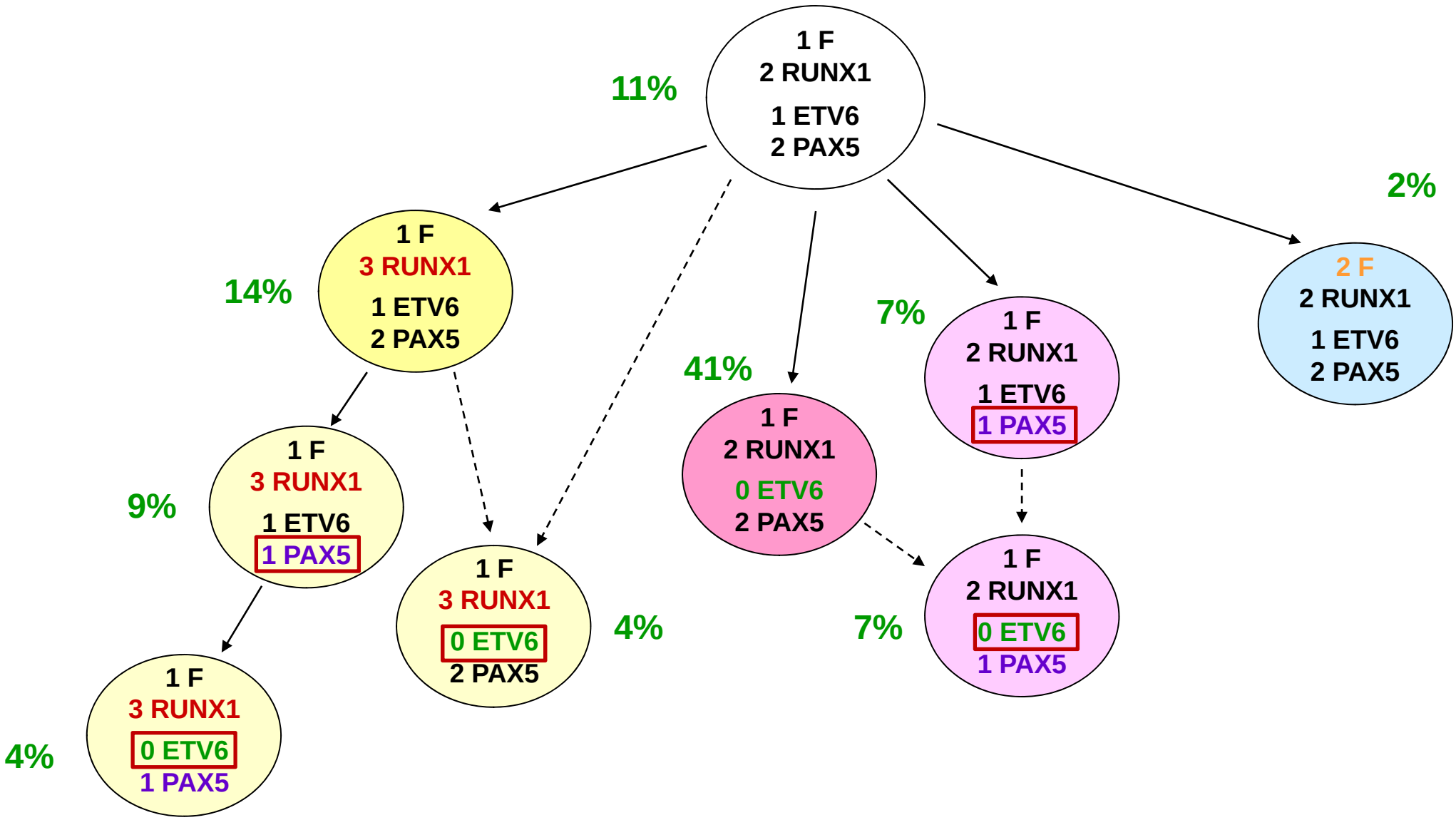
Pt #24: SNAPSHOT OF ANCESTRAL GENETIC TREES IN ALL



Pt #36: SNAPSHOT OF ANCESTRAL GENETIC TREES IN ALL

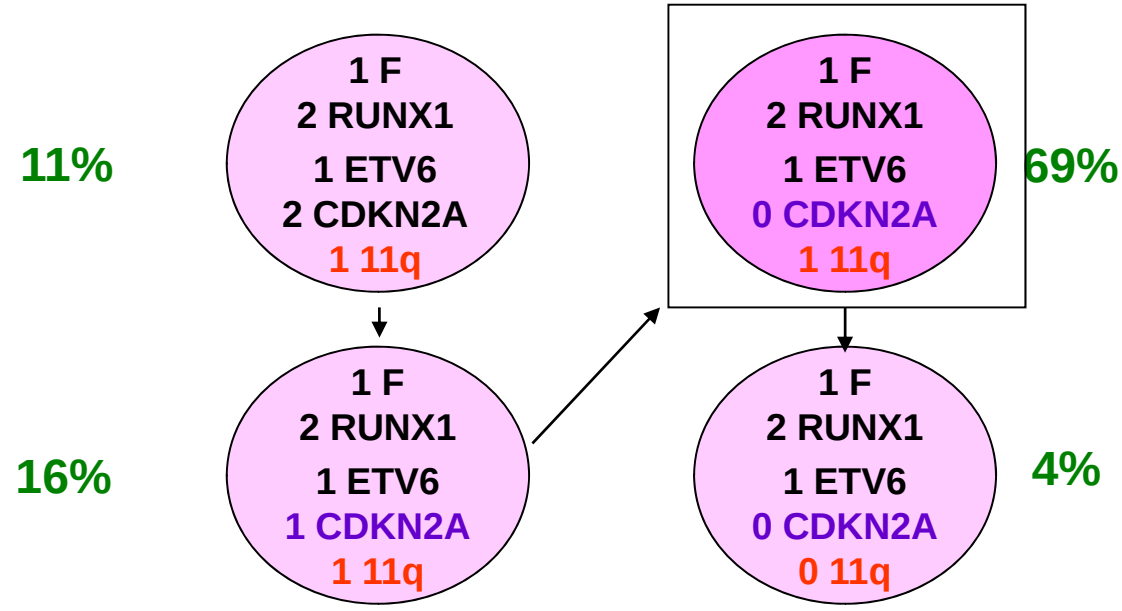


Pt #33: SNAPSHOT OF ANCESTRAL GENETIC TREES IN ALL

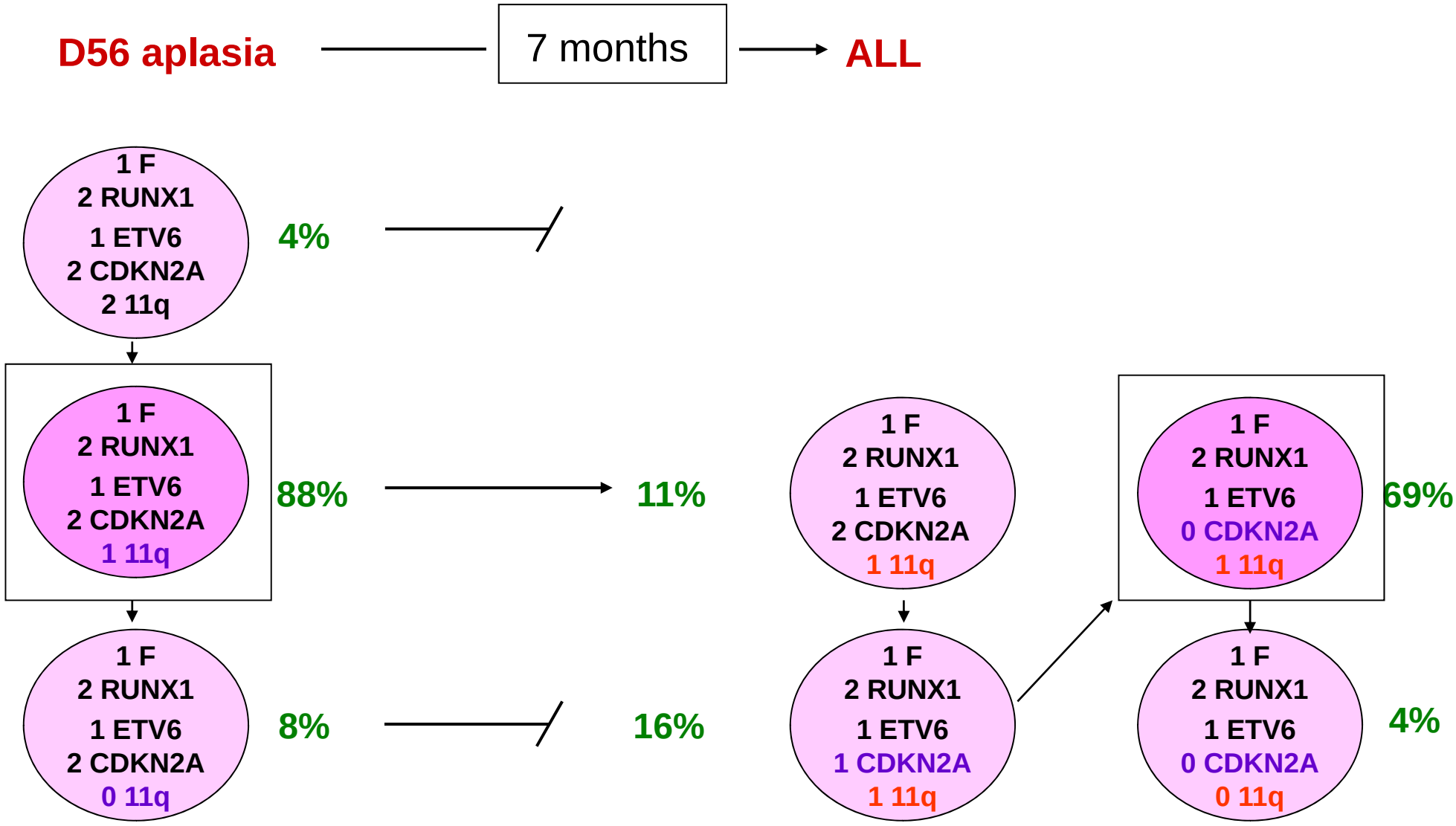


Clonal Dynamics

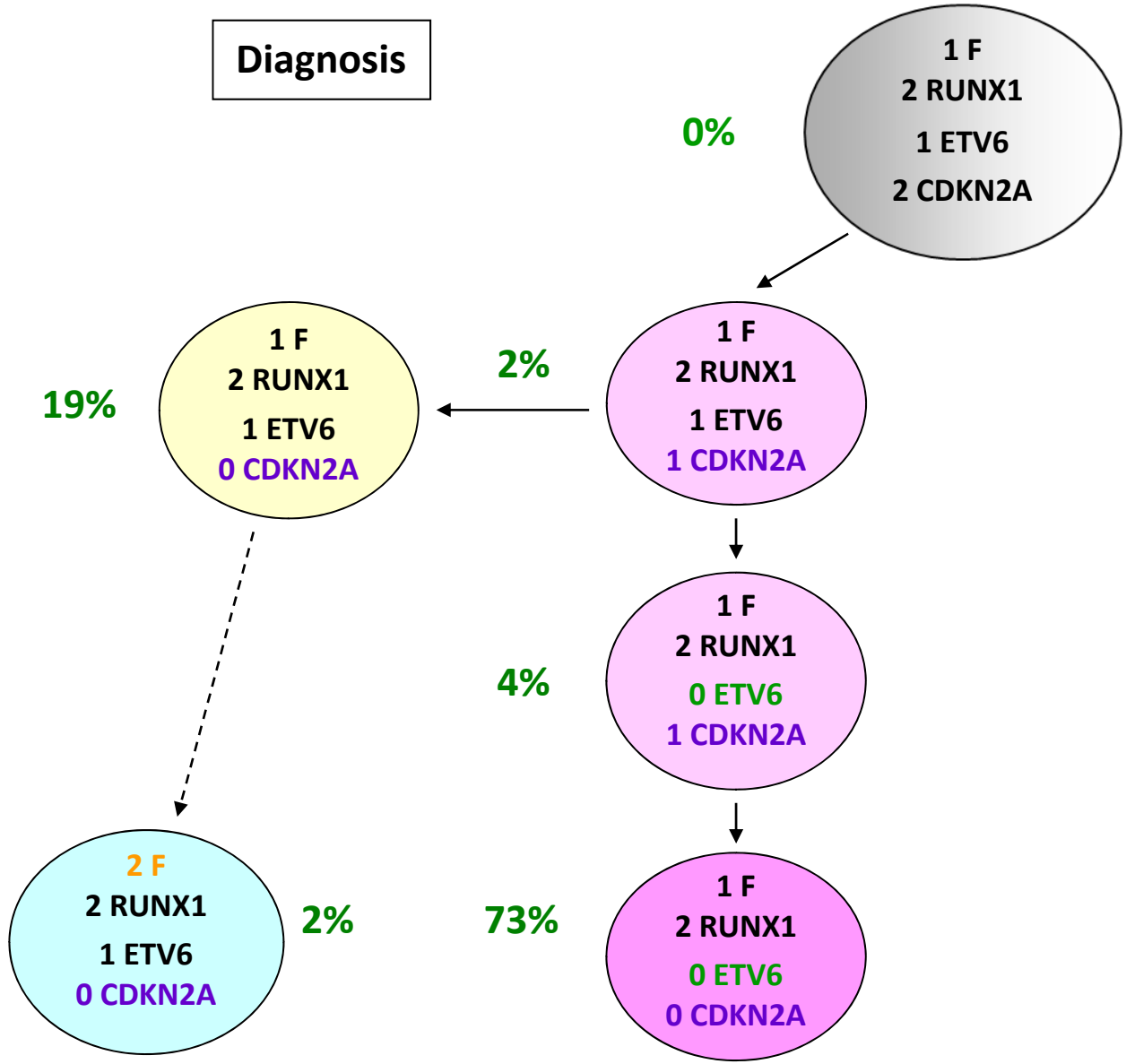
D56 aplasia —— 7 months ⟶ **ALL**



Clonal Dynamics



Pt #6: ANCESTRAL TREE RECONSTRUCTION IN RELAPSE



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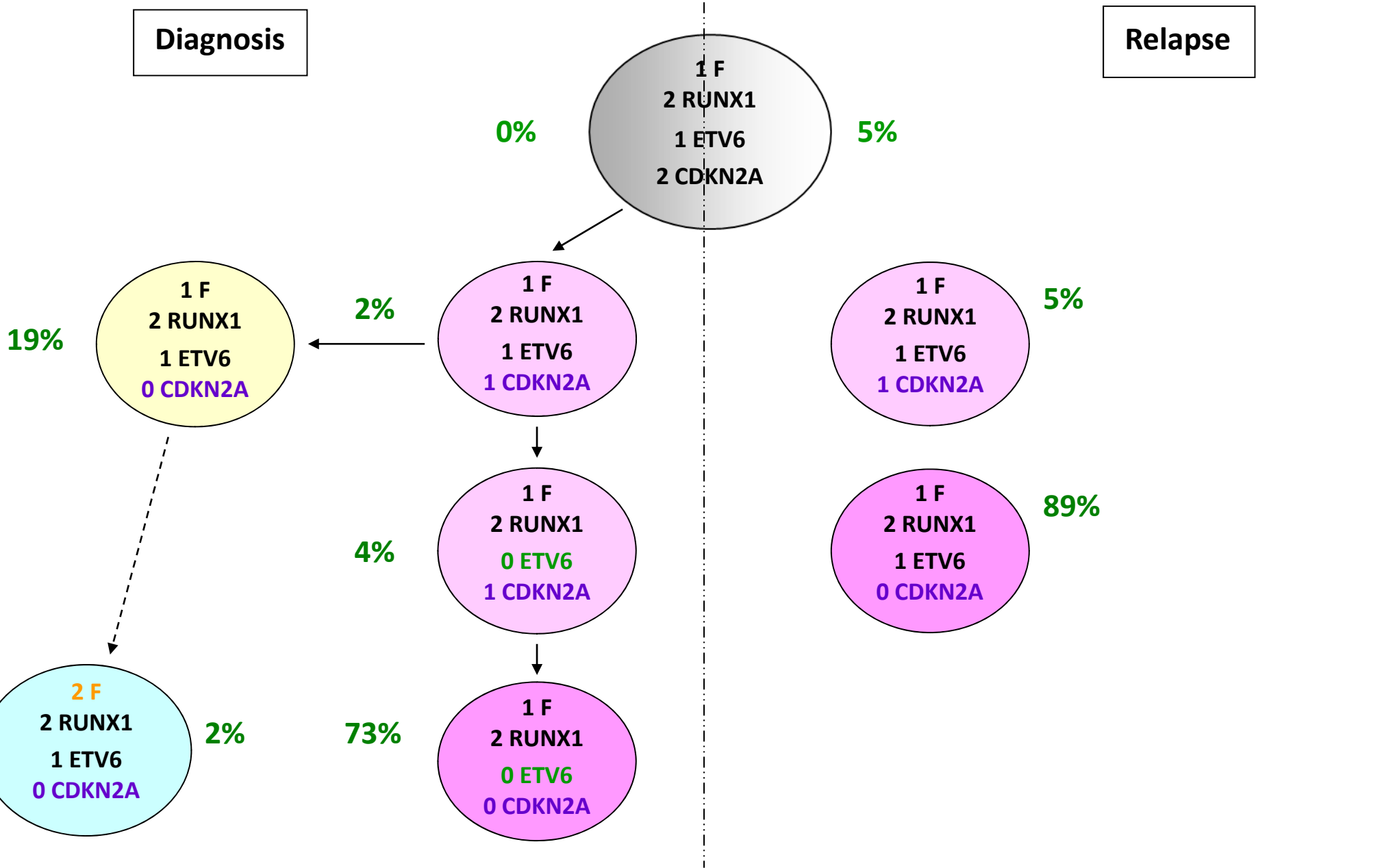

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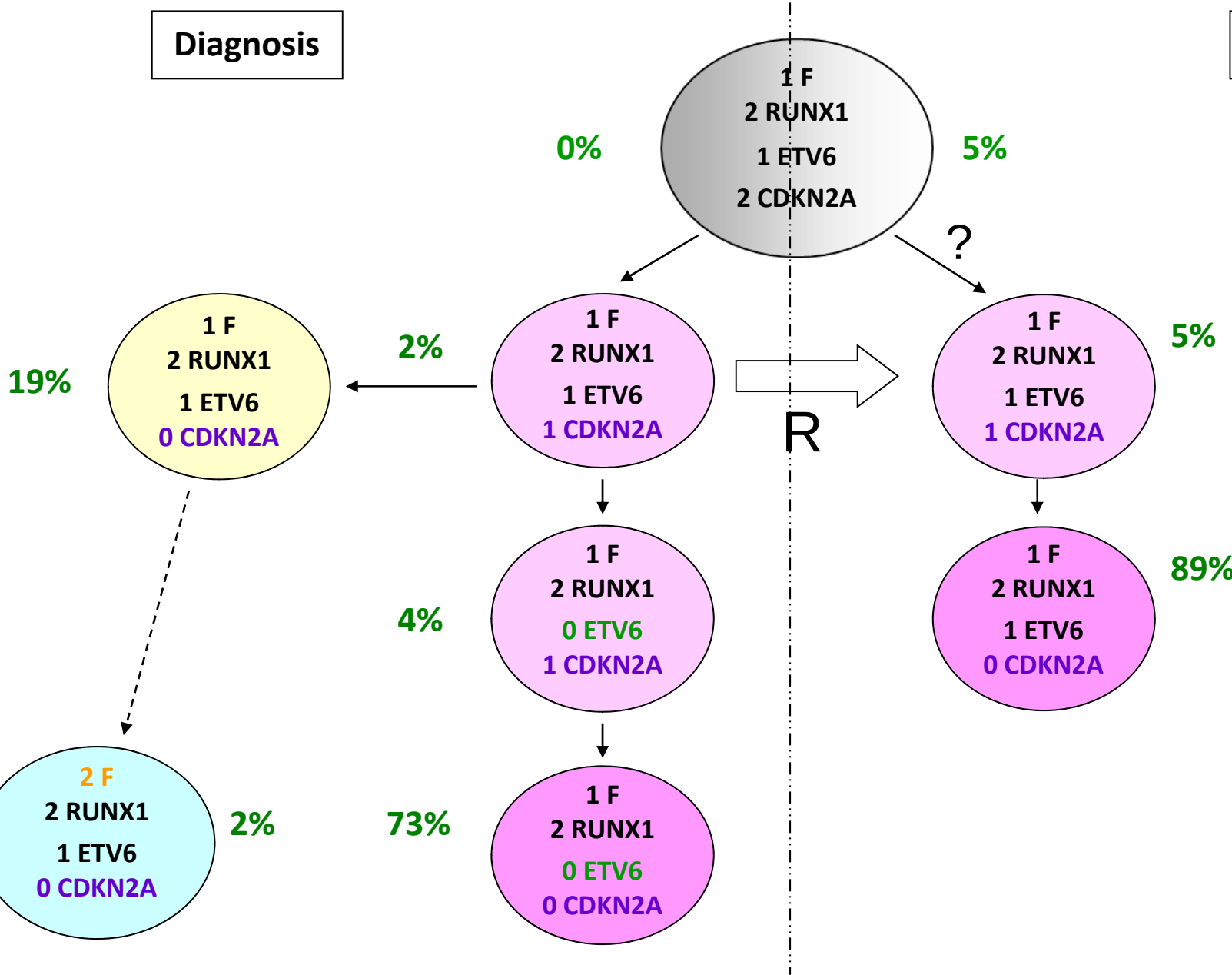
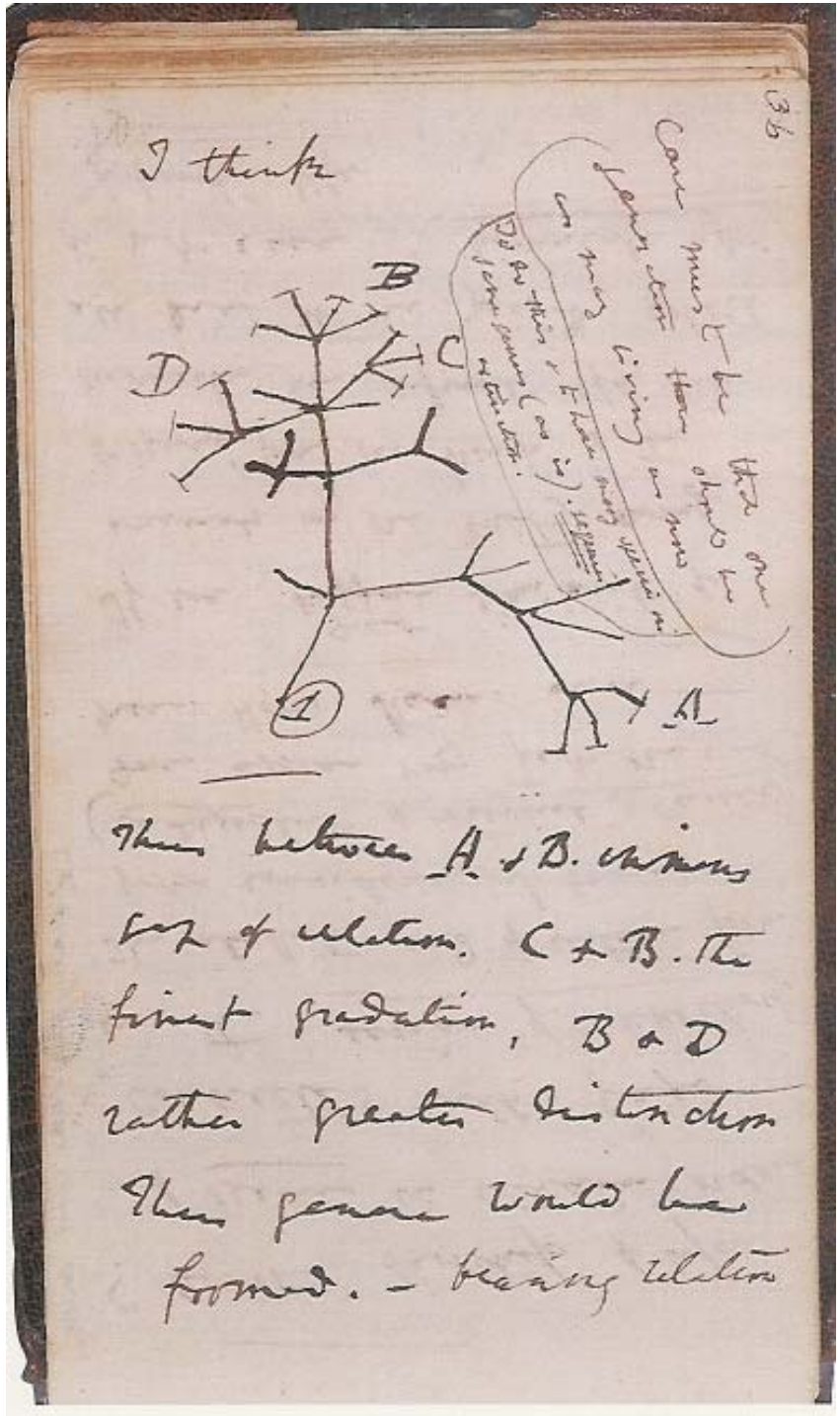


INTRA-CLONAL GENETIC HETEROGENEITY IN ALL

- Multiple sub-clones (3 - 14) (under-estimate)
- Independent / multiple acquisition of recurrent CNA (mechanistic issues)
- No preferential order of CNA
- Relapse originating from major or minor clones at diagnosis
- Relapse clone diversifies and may reiterate evolution of sub-clones at diagnosis
- Non-linear dynamic / branching clonal architecture



Charles Darwin

(Transmutation notebook B)

1837

‘Ancestral tree’

INTRA-CLONAL GENETIC COMPLEXITY IN CANCER

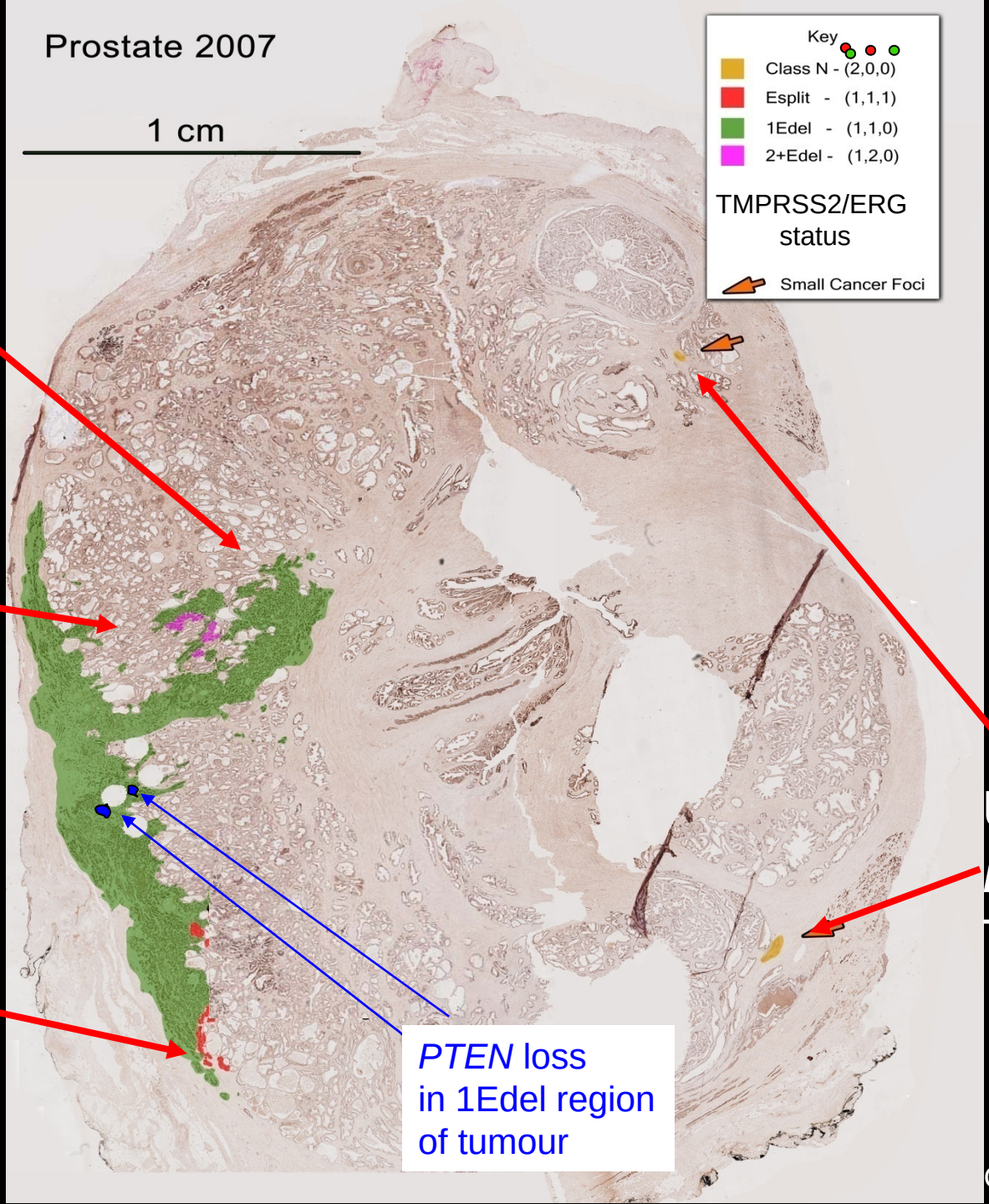
AML: $FLT3^{ITD}$ / RAS^{m} / sub-clonal

- Barrett's oesophagus / oesophageal ca.
- Multi-focal bladder ca.
- Colon adenoma / carcinoma transition
- Genetic diversification of clonal metastases in pancreatic ca.
- Topographical genetic variation in prostate ca.

Edel Tumour

2Edel Tumour (poor prognosis)

Esplit Tumour



Map of Whole prostate slice: *ERG* rearrangement and *PTEN* loss

Unrearranged *ERG* Tumour

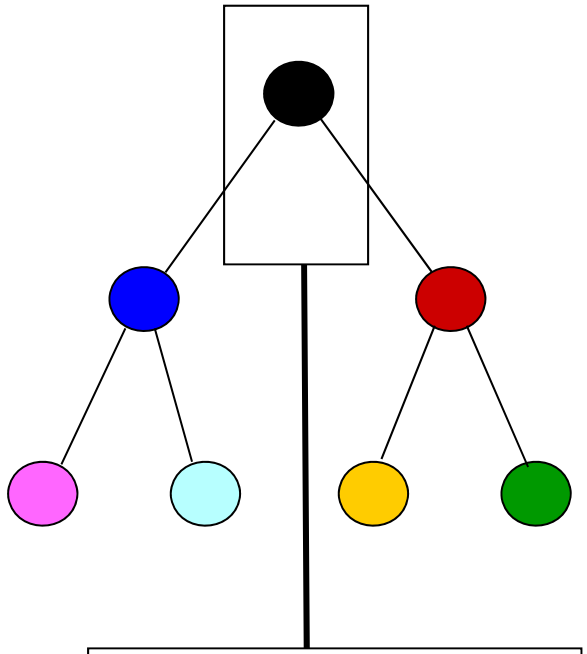
Clark *et al* Oncogene 2007

INTRA-CLONAL GENETIC COMPLEXITY IN CANCER

- Prognostic sample bias
- Substrate for drug resistance
- Identifying therapeutic targets
- Cancer stem cell heterogeneity ?

CANCER STEM CELLS

CANCER CLONE DIVERSITY AND PROPAGATION



Sustains cancer

Self-renews

Target for therapy

MODELS

- Stem cell
  (fixed; developmentally hierarchical)

- or -

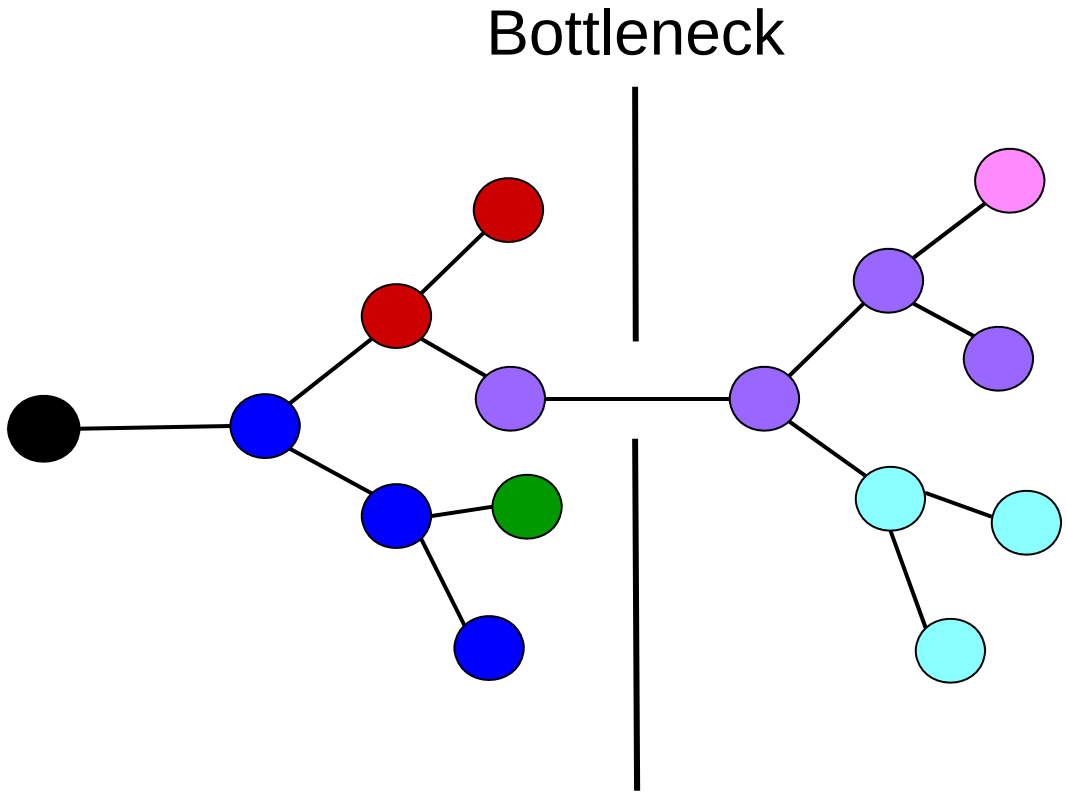
- Stochastic
  (random, variable)

- or -

- Evolution
  (dominant sub-clone)

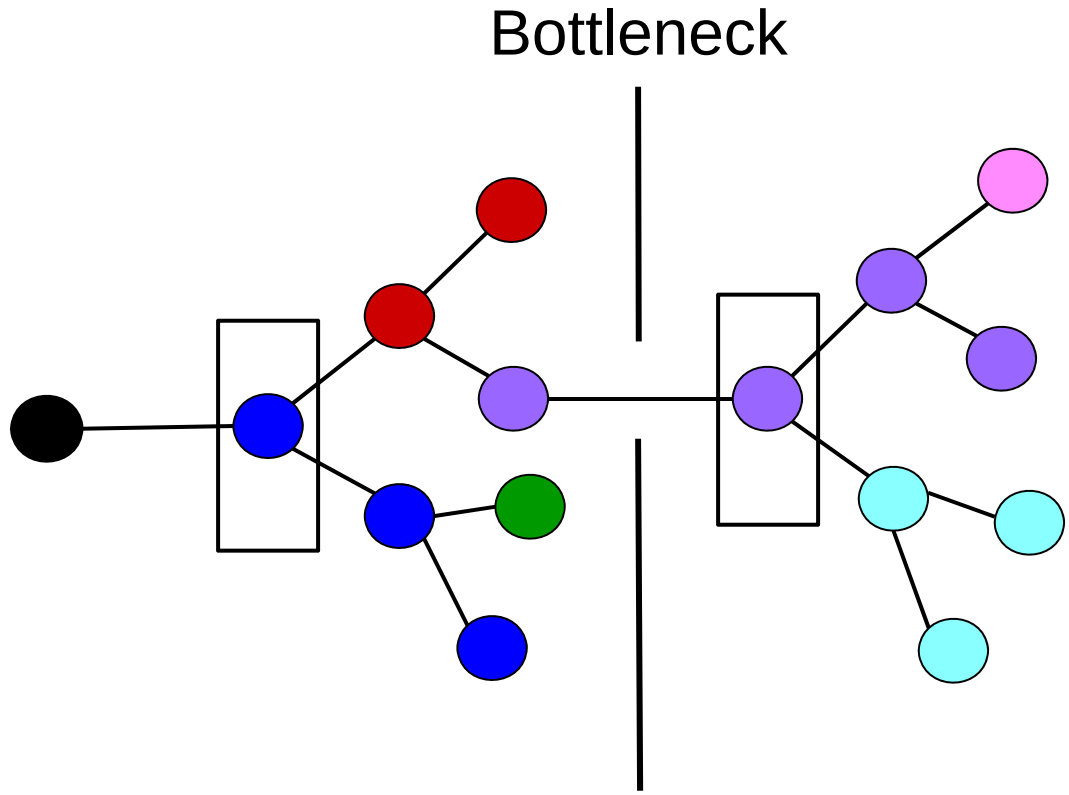
? Frequency / phenotype

EVOLUTIONARY PROGRESSION



- speciation
- antibiotic R.
- immune selection
- cancer

EVOLUTIONARY PROGRESSION



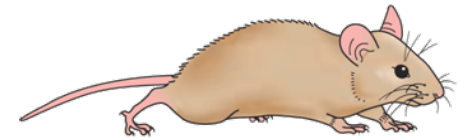
genetic diversity in
units of selection (= leukaemia ‘stem’ / propagating cell)

ARE LEUKAEMIC STEM CELLS GENETICALLY DIVERSE?

ALL cells of defined genetic, sub-clonal complexity

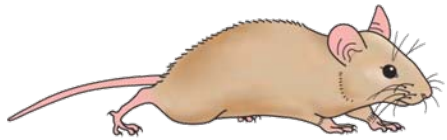
Inter-tibial injection

NOD/SCID/γ mice



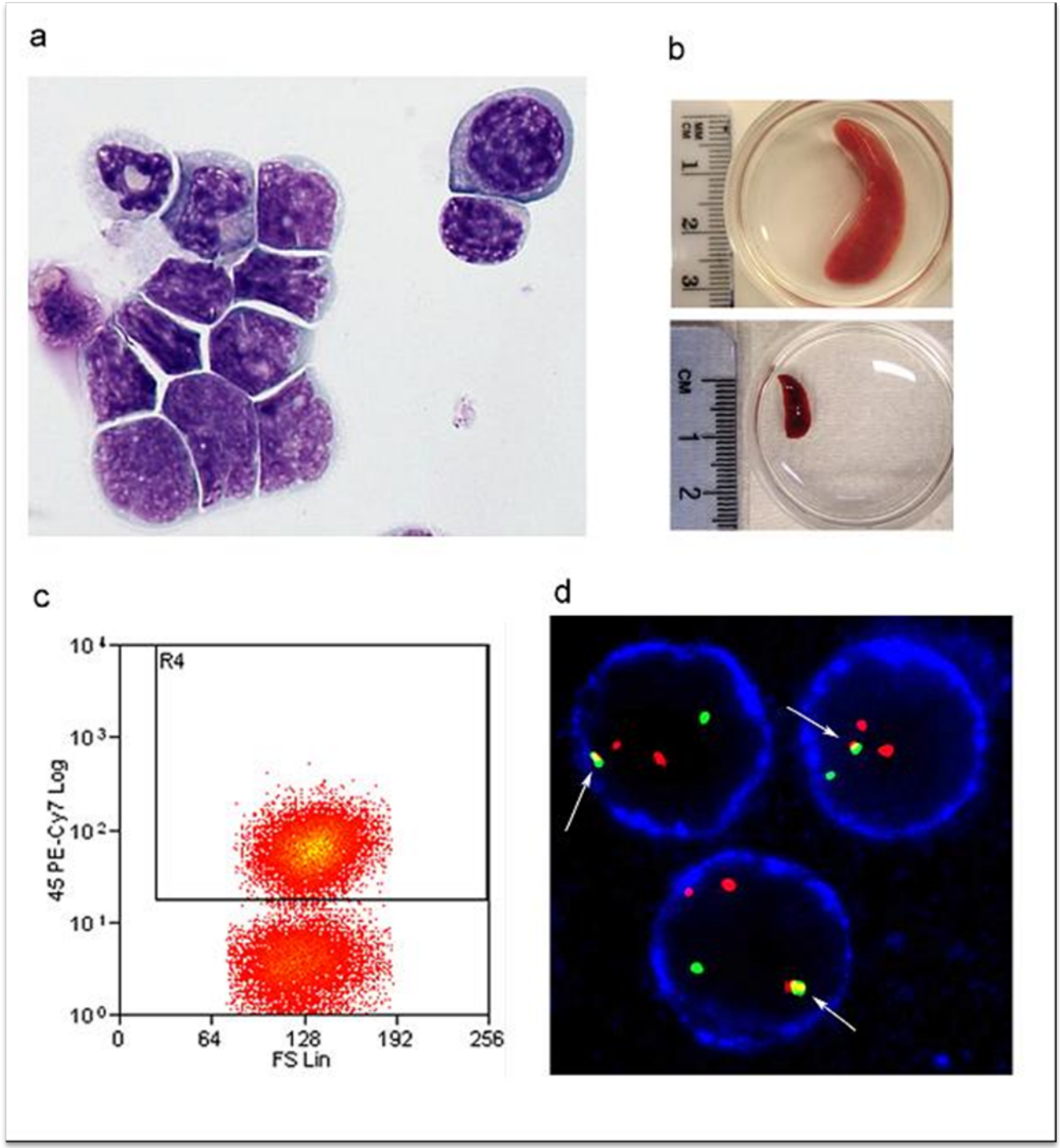
re-transplant

8-12 weeks



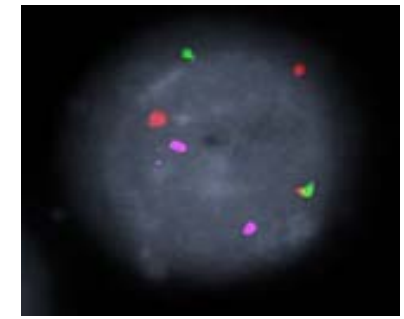
Stem cell dependent regeneration of ALL

Re-screen (M-FISH) for genetic complexity of leukaemic cells


a
b
c
d
R4
45 PE-Cy7 Log
FS Lin
0
64
128
192
256

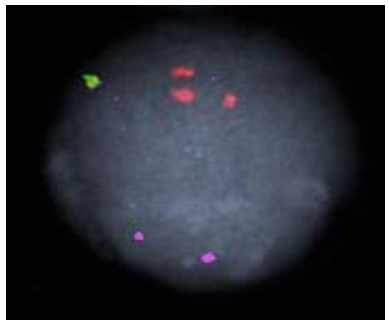
Pt #4

Diagnostic sample:



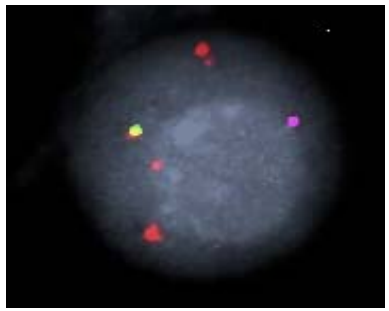
1 F
2 RUNX1
1 ETV6
2 CDKN2A

1%



1 F
3 RUNX1
0 ETV6
2 CDKN2A

33%

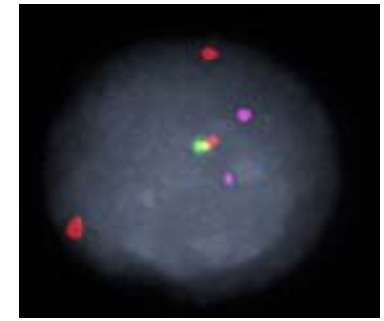


1 F
3 RUNX1
0 ETV6
1 CDKN2A

12%

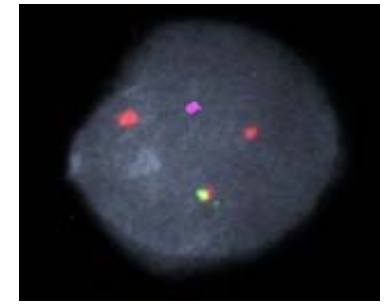
21%

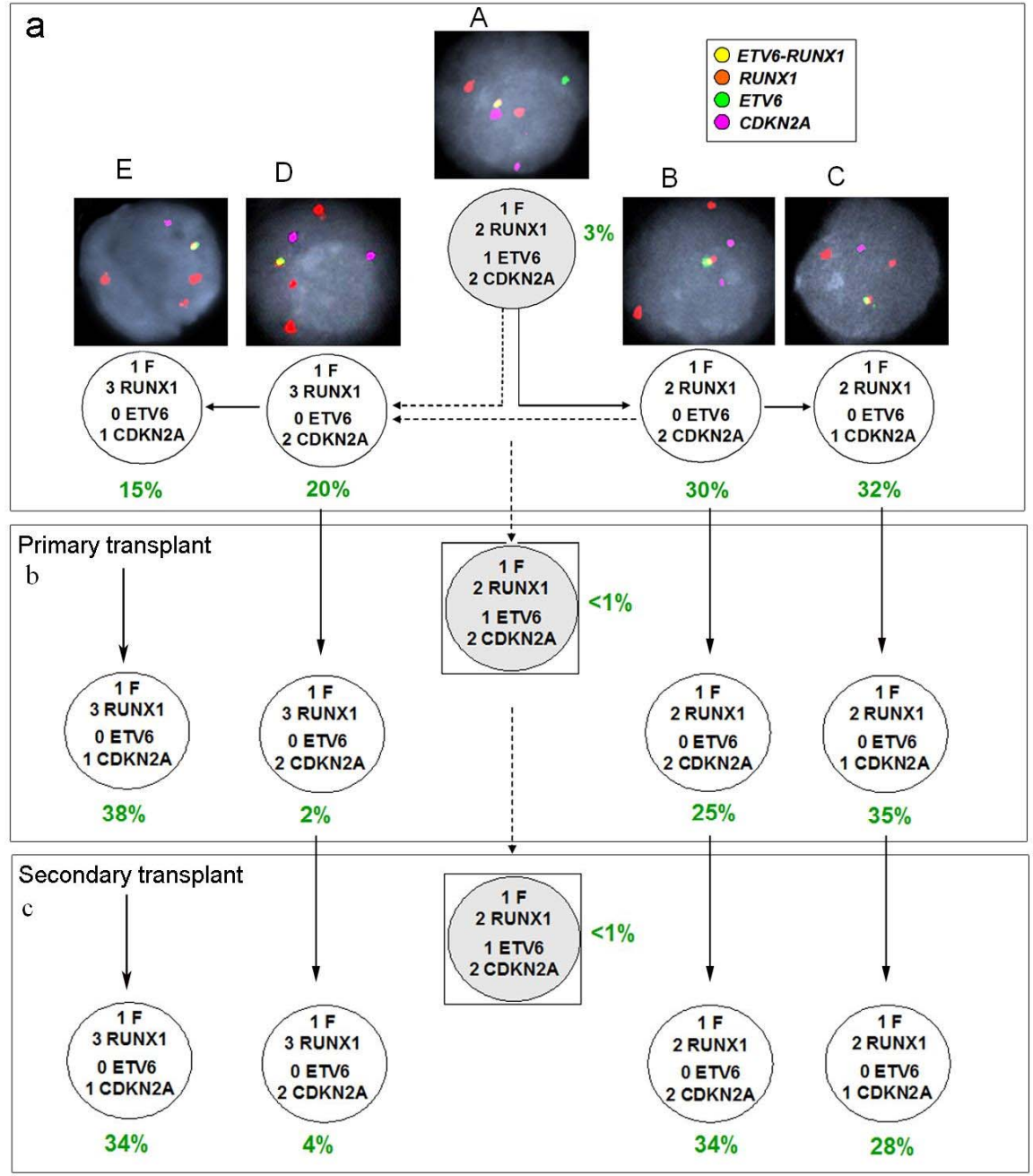
1 F
2 RUNX1
0 ETV6
2 CDKN2A

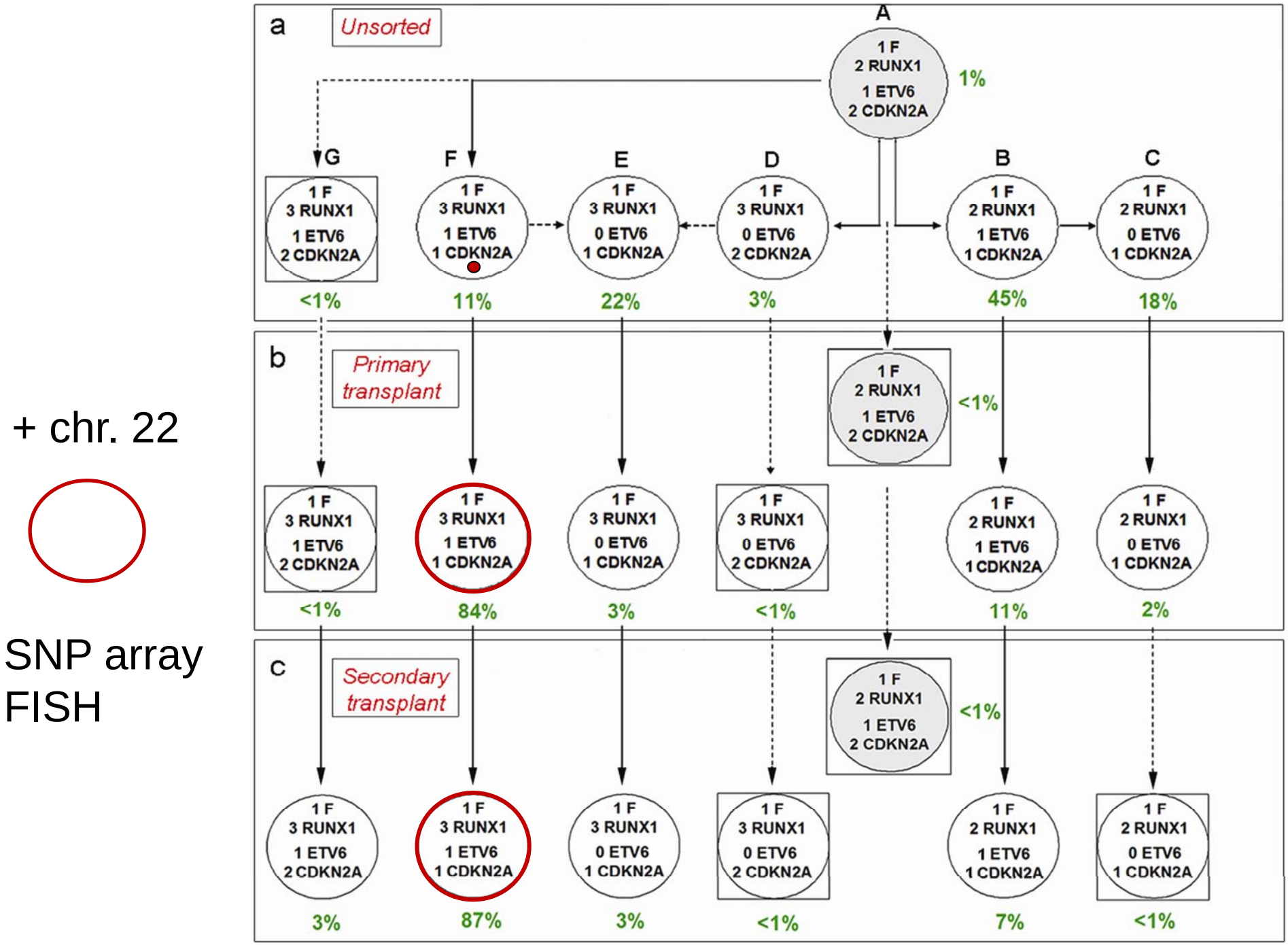


33%

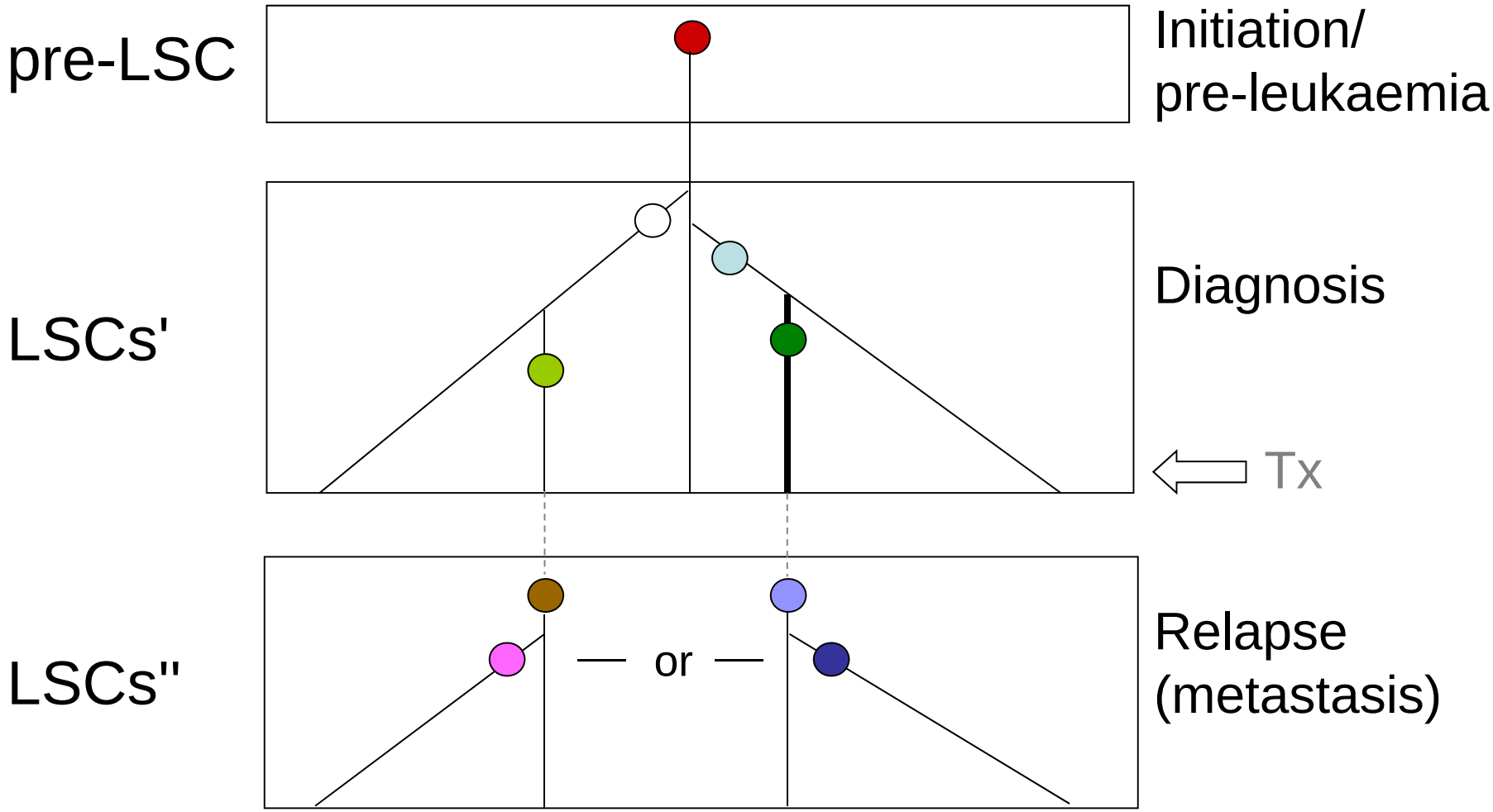
1 F
2 RUNX1
0 ETV6
1 CDKN2A




a
A
ETV6-RUNX1
RUNX1
ETV6
CDKN2A
E
D
B
C
1 F
2 RUNX1
1 ETV6
2 CDKN2A
3%
1 F
3 RUNX1
0 ETV6
1 CDKN2A
15%
1 F
3 RUNX1
0 ETV6
2 CDKN2A
20%
1 F
2 RUNX1
0 ETV6
2 CDKN2A
30%
1 F
2 RUNX1
0 ETV6
1 CDKN2A
32%
Primary transplant
b
1 F
2 RUNX1
1 ETV6
2 CDKN2A
<1%
1 F
3 RUNX1
0 ETV6
1 CDKN2A
38%
1 F
3 RUNX1
0 ETV6
2 CDKN2A
2%
1 F
2 RUNX1
0 ETV6
2 CDKN2A
25%
1 F
2 RUNX1
0 ETV6
1 CDKN2A
35%
Secondary transplant
c
1 F
2 RUNX1
1 ETV6
2 CDKN2A
<1%
1 F
3 RUNX1
0 ETV6
1 CDKN2A
34%
1 F
3 RUNX1
0 ETV6
2 CDKN2A
4%
1 F
2 RUNX1
0 ETV6
2 CDKN2A
34%
1 F
2 RUNX1
0 ETV6
1 CDKN2A
28%

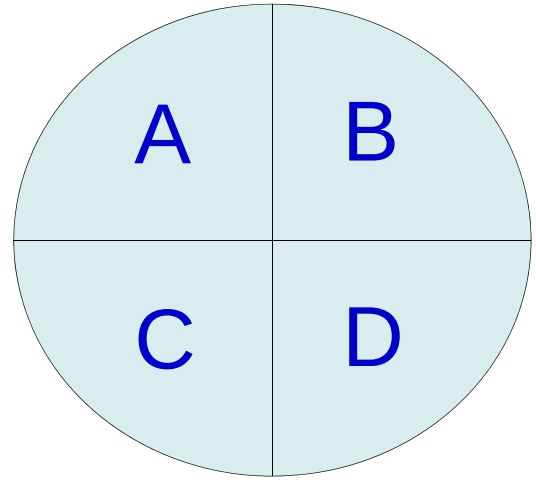

+ chr. 22
SNP array
FISH
a
Unsorted
A
1 F
2 RUNX1
1 ETV6
2 CDKN2A
1%
G
1 F
3 RUNX1
1 ETV6
2 CDKN2A
<1%
F
1 F
3 RUNX1
1 ETV6
1 CDKN2A
11%
E
1 F
3 RUNX1
0 ETV6
1 CDKN2A
22%
D
1 F
3 RUNX1
0 ETV6
2 CDKN2A
3%
B
1 F
2 RUNX1
1 ETV6
1 CDKN2A
45%
C
1 F
2 RUNX1
0 ETV6
1 CDKN2A
18%
b
Primary
transplant
1 F
2 RUNX1
1 ETV6
2 CDKN2A
<1%
1 F
3 RUNX1
1 ETV6
2 CDKN2A
<1%
1 F
3 RUNX1
1 ETV6
1 CDKN2A
84%
1 F
3 RUNX1
0 ETV6
1 CDKN2A
3%
1 F
3 RUNX1
0 ETV6
2 CDKN2A
<1%
1 F
2 RUNX1
1 ETV6
1 CDKN2A
11%
1 F
2 RUNX1
0 ETV6
1 CDKN2A
2%
c
Secondary
transplant
1 F
2 RUNX1
1 ETV6
2 CDKN2A
<1%
1 F
3 RUNX1
1 ETV6
2 CDKN2A
3%
1 F
3 RUNX1
1 ETV6
1 CDKN2A
87%
1 F
3 RUNX1
0 ETV6
1 CDKN2A
3%
1 F
3 RUNX1
0 ETV6
2 CDKN2A
<1%
1 F
2 RUNX1
1 ETV6
1 CDKN2A
7%
1 F
2 RUNX1
0 ETV6
1 CDKN2A
<1%

THE ‘BACK TO DARWIN’ MODEL: STEM CELL HIERARCHIES IN ALL

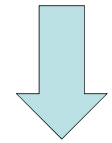


= genetically distinct stem cells

GLIOBLASTOMA MULTIFORM



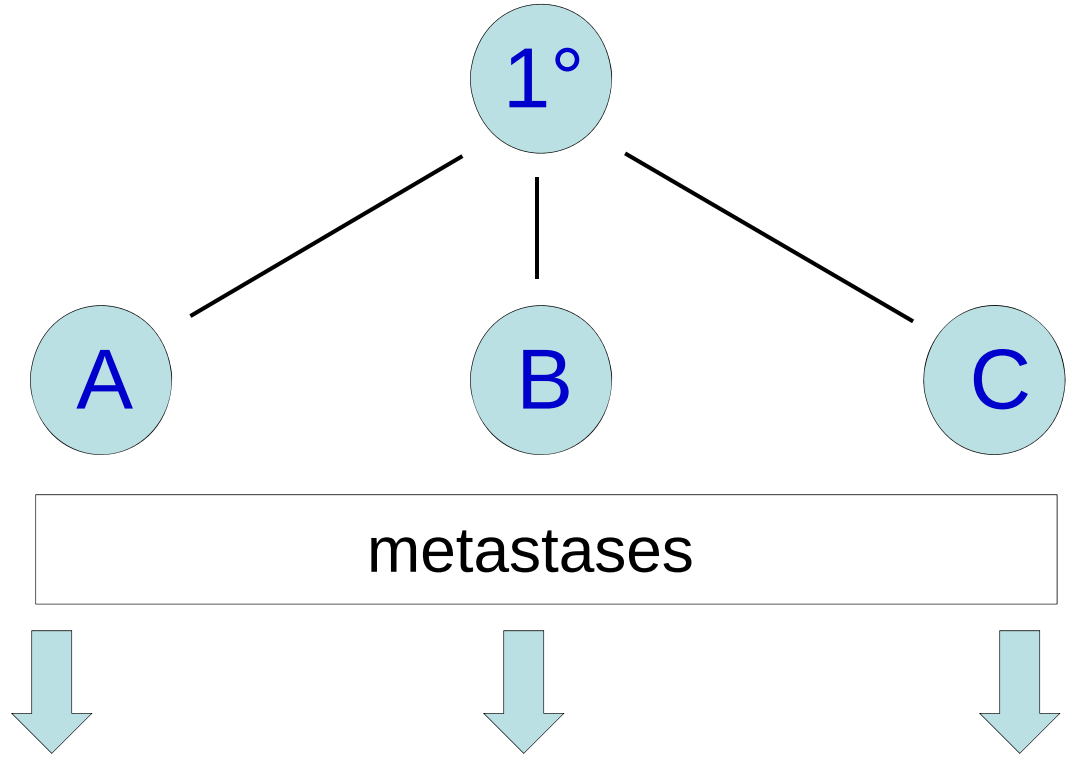
Clonally related but genetically karyotypically distinct



all 4 segments transplant (CNS) in NOD/SCID

Piccirillio *et al* (2009) Oncogene, 28: 1807-1811

PANCREATIC CANCER



Clonally related / genetically distinct

Campbell *et al* (2010) Nature, 467: 1109
Yachida *et al* (2010) Nature, 467: 1114

THE ‘BACK TO DARWIN’ MODEL: IMPLICATIONS

- Cancer stem cells (CSC) are genetically diverse –

  phenotypic diversity and frequency variation

- CSC are the units of selection in evolutionary progression of cancer and therapeutic resistance

- CSC are a diverse, moving and elusive therapeutic target

  – founder mutation is only universal target

ICR

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Ana Teresa Maia
Joe Wiemels
Sue Colman
Horishi Mori
Frederik van Delft
Lyndal Kearney
Tony Ford

Richard Houlston
Amy Sherborne
Elli Papaemmanuil

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Andrea Biondi
Conny Eckert
Philip Ancliff
Tim Eden
Shuki Mizutani

Leukaemia & Lymphoma Research
BEATING BLOOD CANCERS

THE KAY KENDALL LEUKAEMIA FUND
ESTABLISHED IN 1984 UNDER THE WILL OF THE LATE JAMES SAINSBURY

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The Institute of Cancer Research