INFECTION, IMMUNE RESPONSES AND THE AETIOLOGY OF CHILDHOOD LEUKAEMIA

Mel Greaves

POSTULATED EXPOSURES CAUSING CHILDHOOD LEUKAEMIA

Car exhaust fumes

Pesticides

Ionizing radiation

Non-ionizing electric magnetic fields

Electric fields

Vitamin K

Hot dogs or hamburgers

Domestic animals

POSTULATED EXPOSURES CAUSING CHILDHOOD LEUKAEMIA

Organic dust

Natural light deprivation

Artificial, fluorescent light exposure

Parental cigarette smoking

Maternal medicinal drug taking (during pregnancy)

Maternal alcohol consumption (during pregnancy)

Drinking water chemical contamination

Infections

CHILDHOOD LEUKAEMIA: THE BASICS

(80%) Acute Lymphoblastic Leukaemia (ALL)

- Infant, pro-B / monocyte (5%)
- Common, B cell precursor (80%) — peak incidence 2 - 5 years
- T cell precursor (15%)

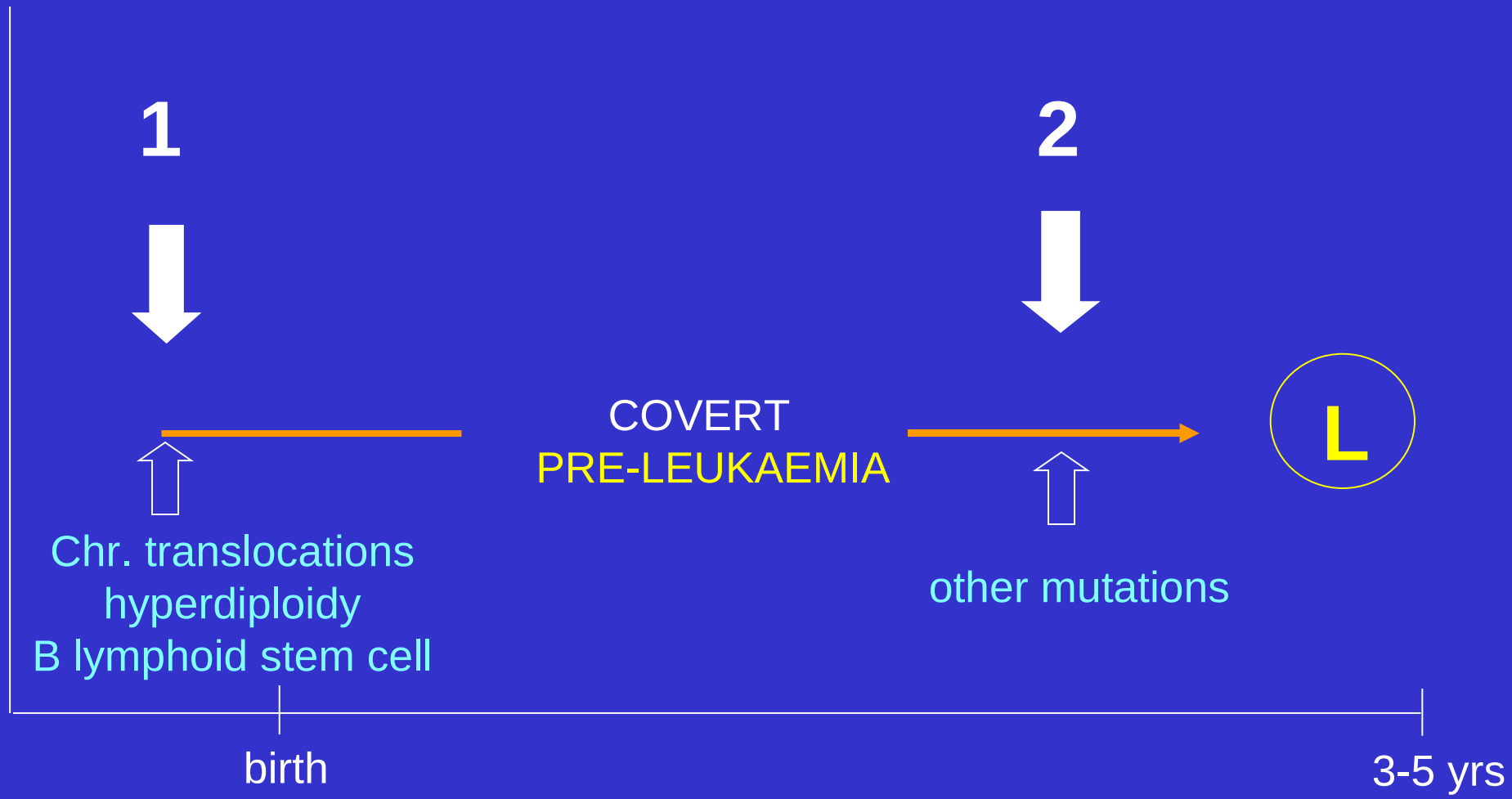
(20%) Acute Myeloid Leukaemia (AML)

Cumulative Risk 0 – 15 years = 1 in 2,000

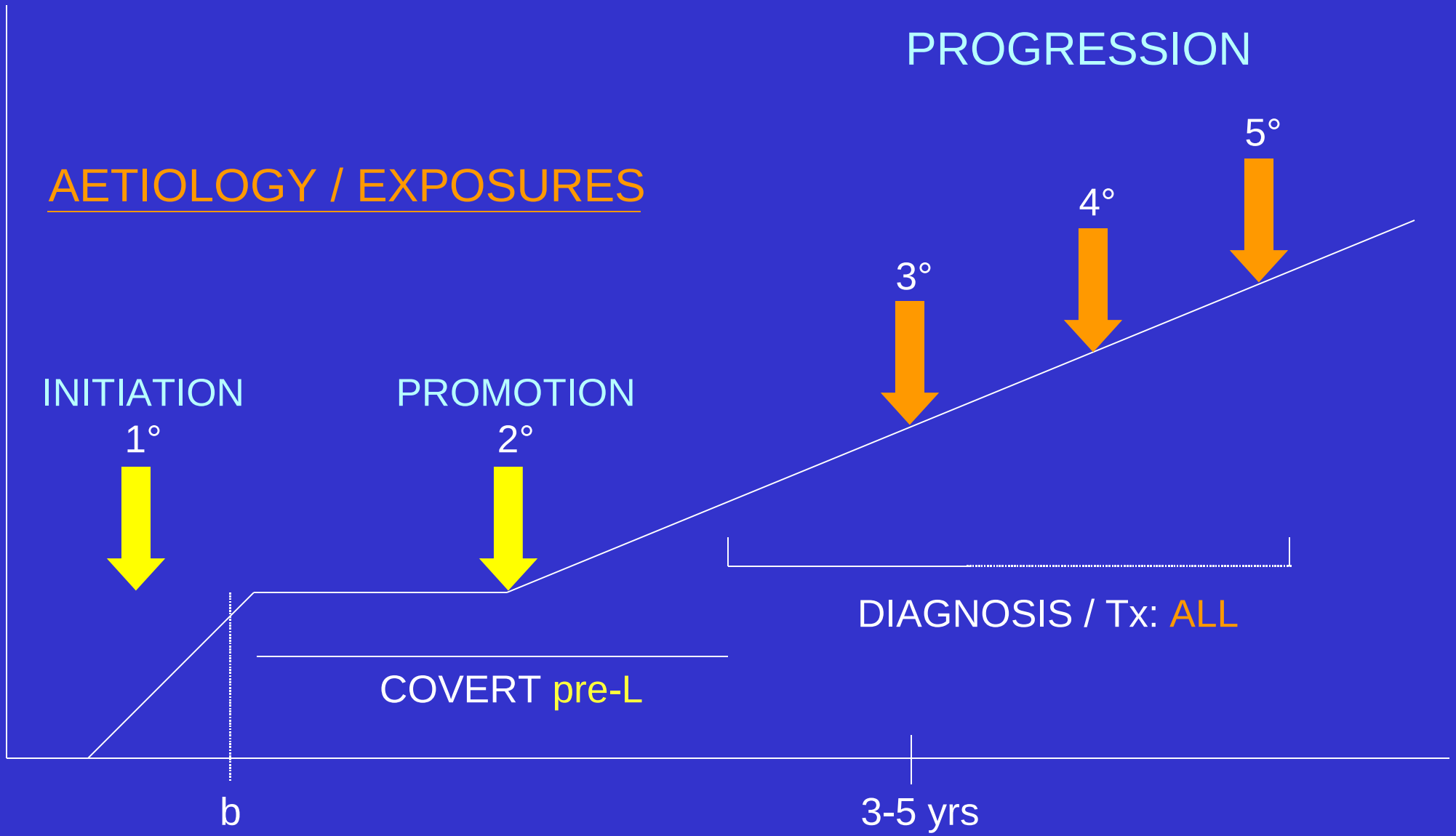
CLONOTYPIC MOLECULAR MARKERS OF PAEDIATRIC LEUKAEMIA SUBTYPES

- Infant ALL — *MLL-AF4* fusions / *FLT-3*m
- Common (pre-B) ALL — *TEL-AML1* fusions / *TEL*del
  Hyperdiploidy / *FLT-3*m
  (*IGH* rearrangements)
- T-ALL — *SIL-TAL* fusion / *NOTCH1*m
  (*TCR* rearrangements)
- AML — *AML1-ETO* fusions / *KIT*m

A MINIMAL 2 STEP MODEL FOR ACUTE LYMPHOBLASTIC LEUKAEMIA



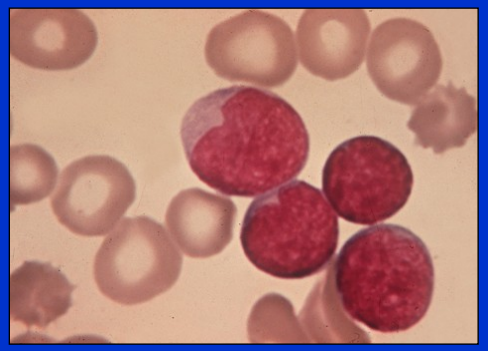
MULTI-STEP PATHOGENESIS



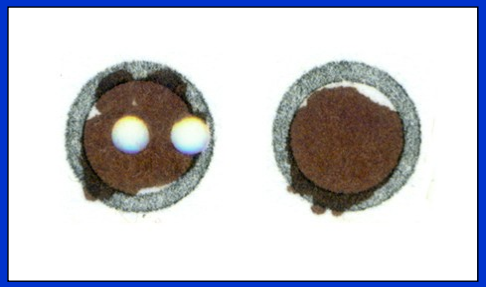
PRE-NATAL ORIGINS OF PAEDIATRIC LEUKAEMIA

- Clonal relationships of concordant leukaemia in monozygotic twins
- Retrospective molecular scrutiny of archived neonatal blood spots of children with leukaemia
- Molecular screening of cord blood of new borns

Leukaemic cells at diagnosis

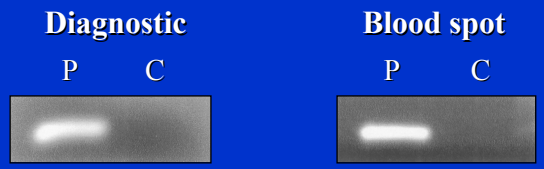


Neonatal blood spots (Guthrie card)



DNA

Amplification of the fusion gene



Fusion sequence

		TEL ↓	***AML1***
Diagnostic DNA	GGCTAAGCGAAAAC	*ATTT*	CAGCGACACTTCAGGAAG
Blood spot DNA	GGCTAAGCGAAAAC	*ATTT*	CAGCGACACTTCAGGAAG

PRENATAL ORIGINS OF LEUKAEMIA

Identical twins share same unique chromosomal/DNA breakpoints (but NOT inherited)
i.e. the leukaemia initiating event(s)

= Sharing of blood cells ('chimaeras')

= Leukaemia starts in one cell in one foetus and clonal progeny spread to the other twin via intraplacental anastomoses

EARLY OR INITIATING EVENTS IN LEUKAEMOGENESIS

- **Foetal haemopoiesis (liver / bone marrow?)**
- **Chromosome translocation / gene fusions**
  - ***MLL-AF4***
  - ***TEL-AML1***
  - ***AML1-ETO***
- **Chromosomal hyperdiploidy**
- **Chromosomal instability**
- **Mutations - *GATA1* in TMD / AML in Down's**

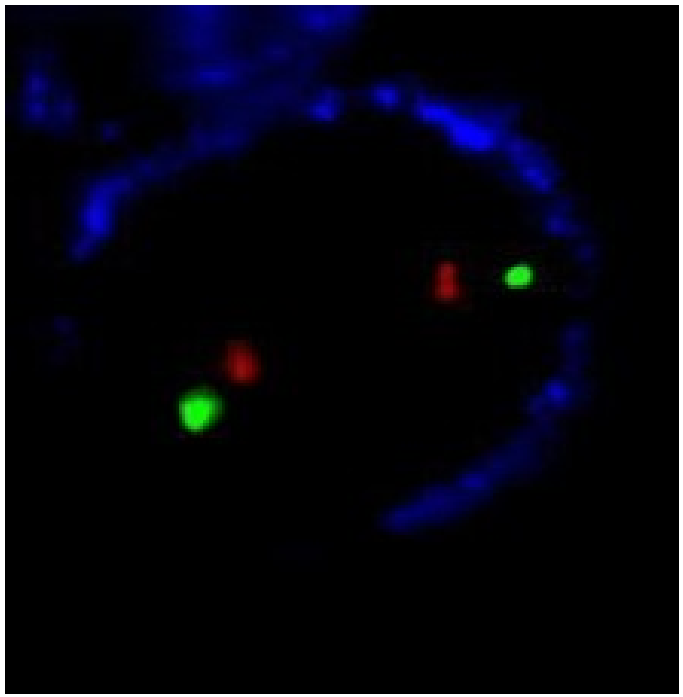
TEL-AML1 FUSION IS AN INITIATING EVENT BUT IS *INSUFFICIENT* FOR LEUKAEMOGENESIS

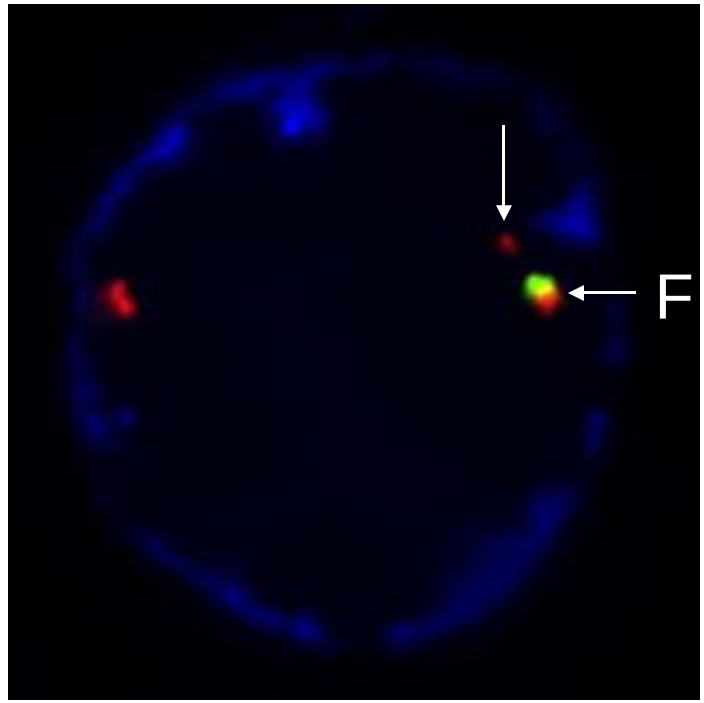
- Concordance rate in monozygotic twins is ~10%

  (Greaves et al, 2003, Blood, 102: 2321-2333)

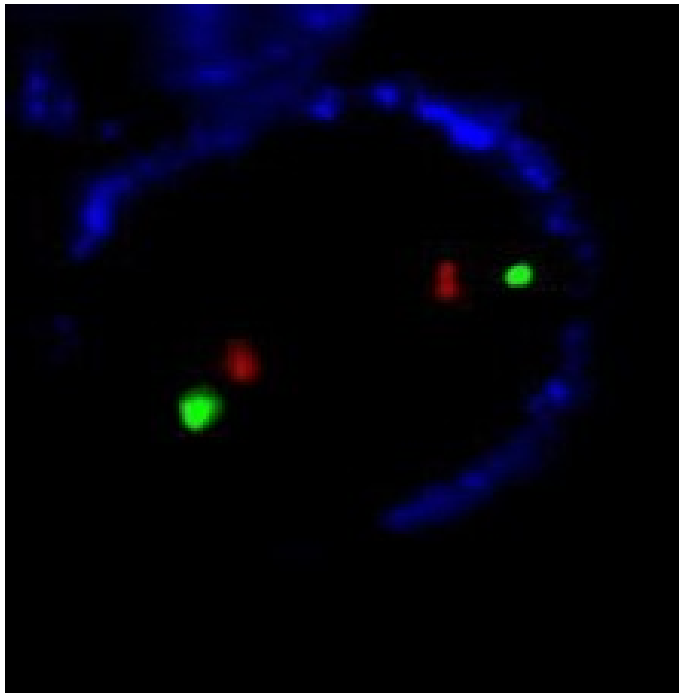
Normal cell

Leukaemic twin:
deleted normal *TEL*

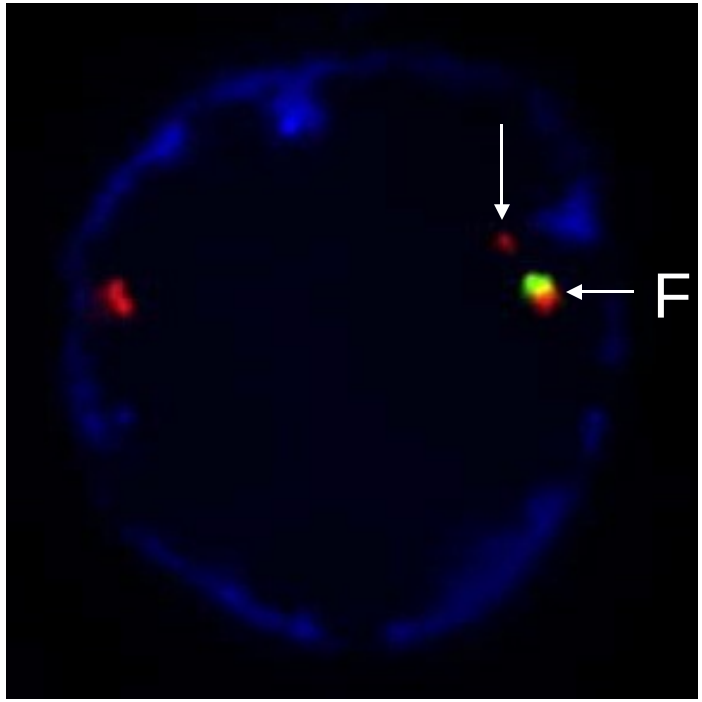




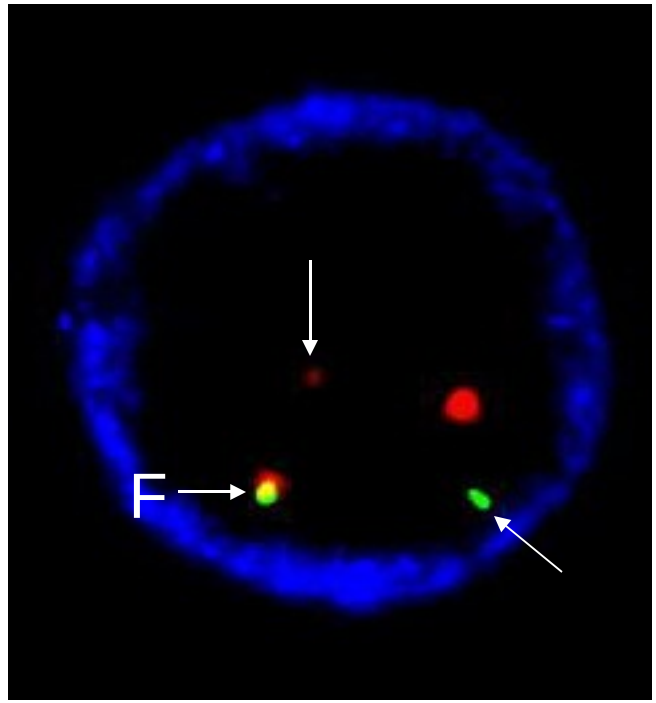
Normal cell



Leukaemic twin:
deleted normal *TEL*



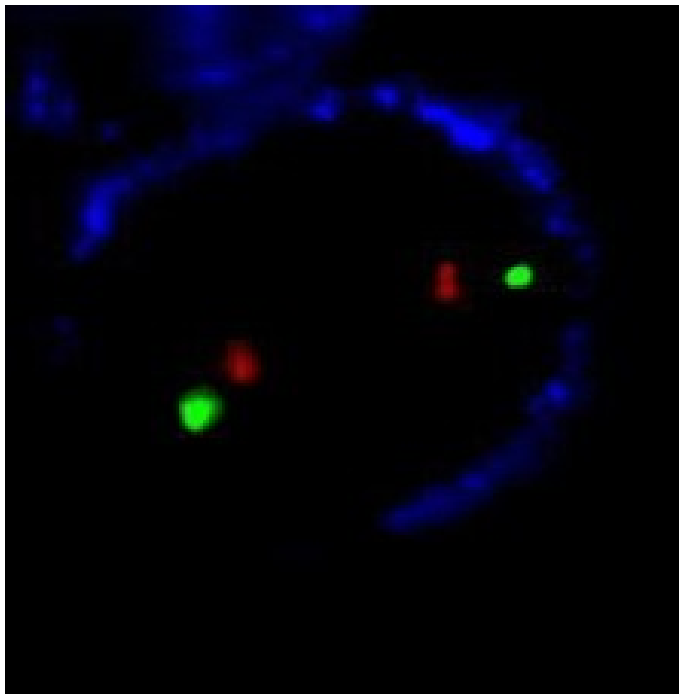
Non-leukaemic twin:
normal *TEL* present

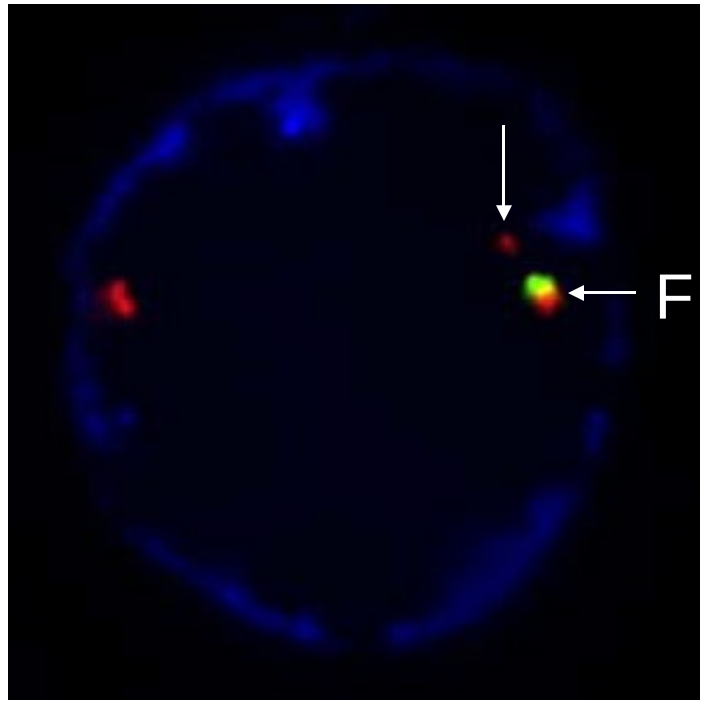


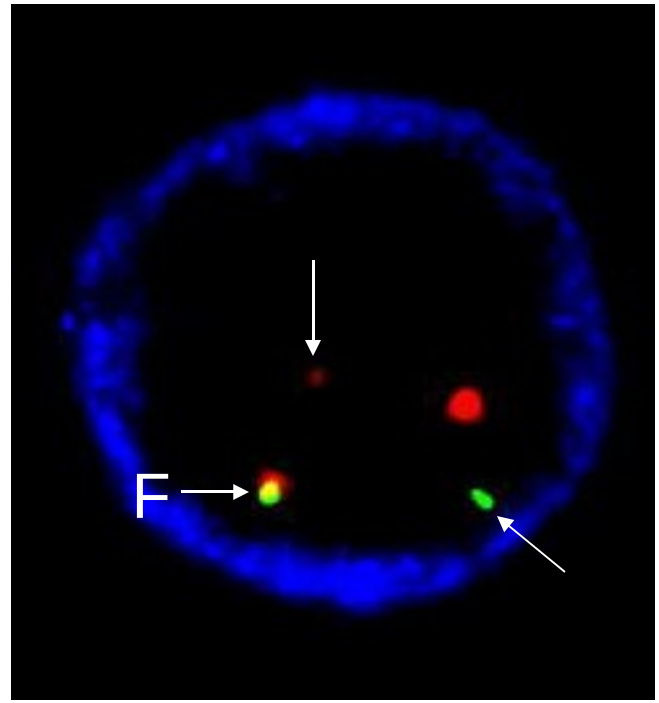
Normal cell

Leukaemic twin:
deleted normal *TEL*

Non-leukaemic twin:
normal *TEL* present







-0.14%
-0.12%
-0.46%
(10^{-3} – 10^{-4})

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- Concordance rate in monozygotic twins is ~10%

  (Greaves et al, 2003, Blood, 102: 2321-2333)

- Mice transgenic for *TEL-AML1* are pre-leukaemic

  (Tsuzuki et al, 2004, PNAS, 101: 8443-8448)

IN VIVO MODELS OF *TEL-AML1* 'PRE-LEUKAEMIA'/ALL

- Retroviral *TEL-AML1* into stem cells / transplant — Tsuzuki et al, Morrow et al, Fischer et al
- Transgenesis with *Eμ TEL-AML1* — Ford, Greaves et al, Bernadin et al
- Lentiviral *TEL-AML1* into cord blood stem cells $\rightarrow$ NOD/SCID — Hong, Enver et al

= Expanded pro-/pre-B cells (+ stem?): no leukaemia

TEL-AML1 FUSION IS AN INITIATING EVENT BUT IS *INSUFFICIENT* FOR LEUKAEMOGENESIS

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∴ secondary, post-natal events are critical

NATURAL HISTORY OF PAEDIATRIC ACUTE LEUKAEMIAS

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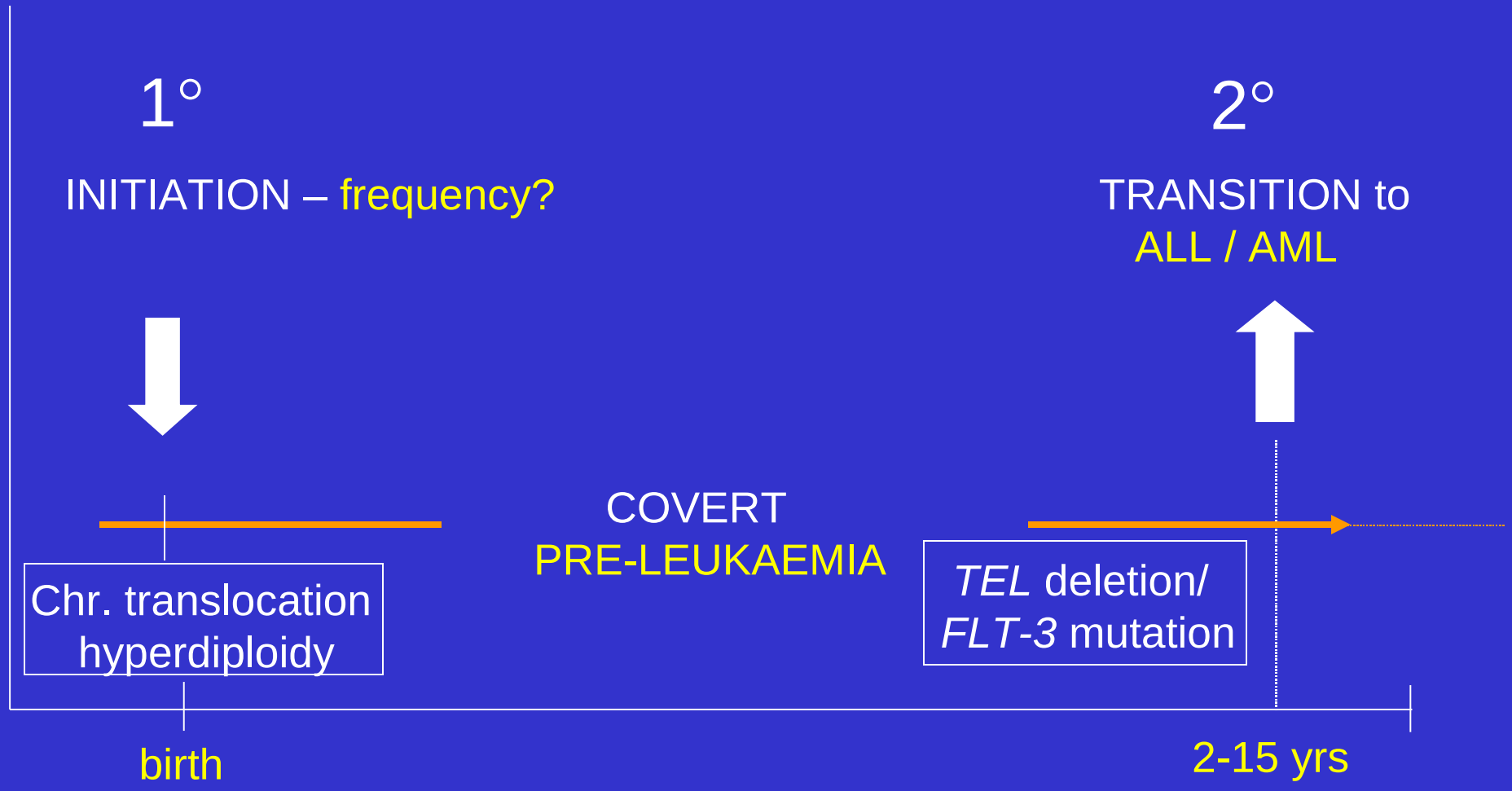
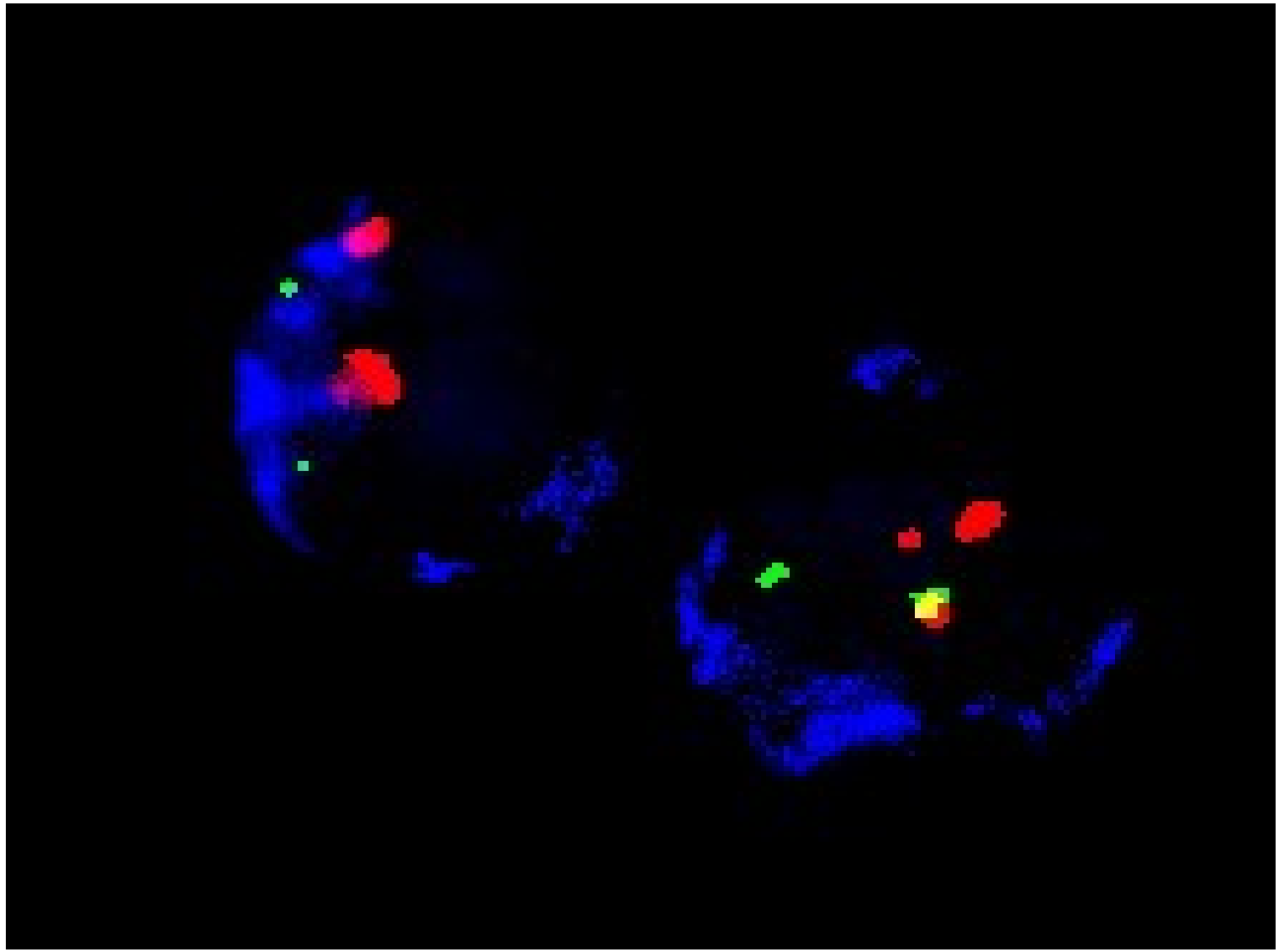


HOW OFTEN IS LEUKAEMIA INITIATED BEFORE BIRTH?

Compared with 1 in 2,000 risk of disease

⇨ Screen ~600 newborn umbilical cord blood samples for chromosome translocations

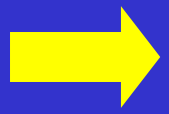
- RT/RQ-PCR assay for gene fusion
- immuno-FISH for gene fusion



FREQUENCY AND RISK OF ACUTE LYMPHOBLASTIC LEUKAEMIA?

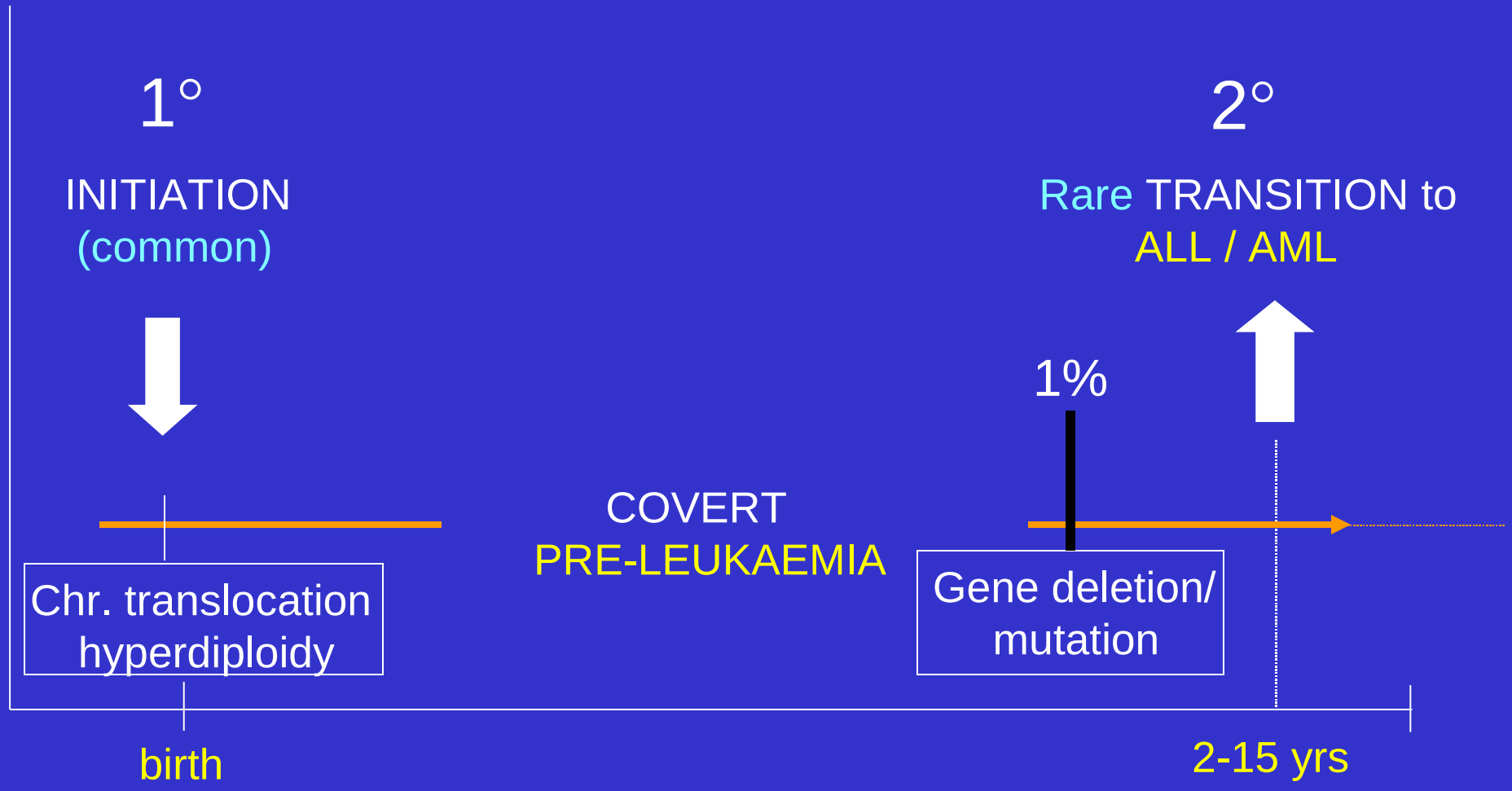
Risk of ALL	~ 1	in	2,000
Risk of ALL with *TEL-AML1*	~ 1	in	10,000
Risk of *TEL-AML1*$^{+}$ cord blood	~ 1	in	100

LEUKAEMIA IS INITIATED, PRE-NATALLY AT ~100 x THE DISEASE RATE

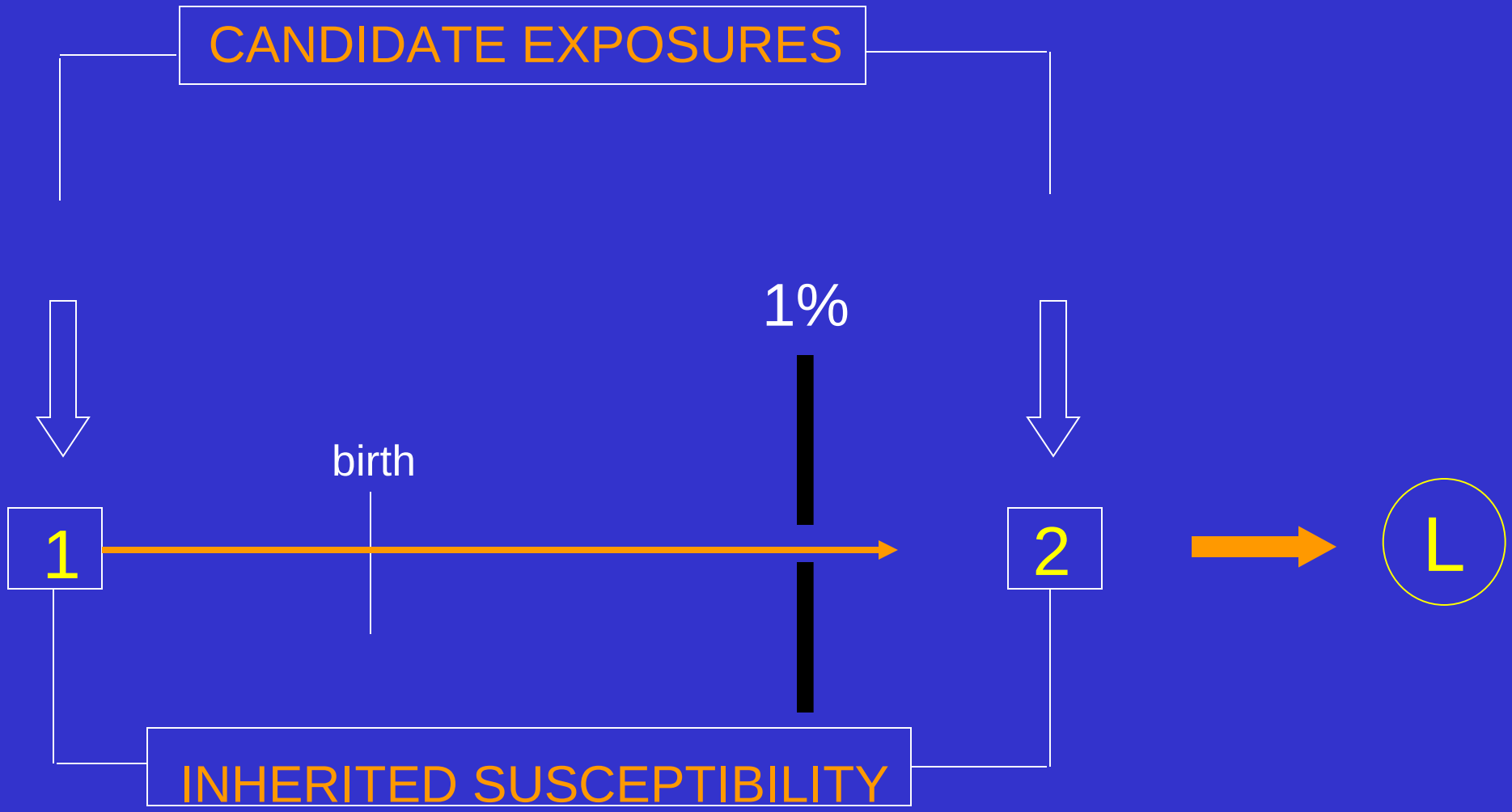


POST-NATAL SECONDARY EVENTS ARE THE BOTTLENECK FOR LEUKAEMIA AETIOLOGY

NATURAL HISTORY OF PAEDIATRIC ACUTE LEUKAEMIAS



A CAUSAL MECHANISM FOR CHILDHOOD LEUKAEMIA



INFECTIOUS AETIOLOGY OF CHILDHOOD ALL

- Direct - molecular virology

- Indirect - epidemiology / proxy measures
  - genetic / susceptibility alleles
  - functional / ‘immunological’

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MOLECULAR SCREENING FOR VIRAL SEQUENCES IN CHILDHOOD ALL

Virus Screened For	Screening Method
• Polyomaviruses JC and BK	Specific PCR*
• Parvovirus B19	Specific PCR
• Human herpesvirus family (HHV4, 5, 6, 7 and 8)	PCR using degenerate primers*
• Bovine leukaemia virus	Southern blotting
• TT virus	Specific PCR
• Exogenous microbial sequences	Representative difference analysis*

** MacKenzie J, Jarrett RF et al*

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INFECTION-BASED HYPOTHESES FOR THE AETIOLOGY OF CHILDHOOD LEUKAEMIA

Kinlen L (1988) *The Lancet*

The 'population mixing' hypothesis

Model : Transient increases in incidence of childhood leukaemia can be ascribed to rural / urban population mixing and transfer, from carriers to susceptibles, of a virus of low pathogenicity. Leukaemia would be a rare response.

? specific virus
? timing

CLUSTER BUSTING?

			RR
Then : ‘57-60	Niles, Chicago	8 cases / 3 years	4.3
Now : ’99-03	Fallon, Nevada	14 cases / 4 years	12.0

“We incline on our evidence to the belief that the solution of the problem of leukaemia lies rather in some peculiar reaction to infection than in the existence of some specific infective agent”

F J Poynton, H Thursfield and D Paterson

(Great Ormond Street Hospital for Sick Children)

Brit J Child Dis 1922 XIX 128-144

INFECTION-BASED HYPOTHESES FOR THE AETIOLOGY OF CHILDHOOD LEUKAEMIA

Greaves M (1988) *Leukemia*

The 'delayed infection' hypothesis

Model :

- Timing of common infections critical (- delay?)
  cf. hygiene hypothesis for allergies and type 1 diabetes
- Abnormal immune response facilitates expansion of pre-leukaemic clone
- Genetic susceptibility impacts on risk

THE ‘DELAYED INFECTION’ HYPOTHESIS - A GENETIC ADAPTATION – LIFESTYLE MISMATCH?

- **EVOLUTIONARY ADAPTATION**
  - The immune system has been evolutionarily programmed to anticipate infectious challenge after birth
  - The neonatal immune network is unstructured and requires modulation by infectious exposure
  - Selection of human genetic variants in immune response genes (strength of signal)
    - by past plagues / epidemics

THE ‘DELAYED INFECTION’ HYPOTHESIS - A GENETIC ADAPTATION – LIFESTYLE MISMATCH?

- **THE MISMATCHED LIFESTYLE FACTORS**

  Affluent societies / families provide insufficient opportunities for ‘natural’ infectious exposure in infancy

THE ‘DELAYED INFECTION’ HYPOTHESIS - A GENETIC ADAPTATION – LIFESTYLE MISMATCH?

- **THE CONSEQUENCES OF MISMATCH**

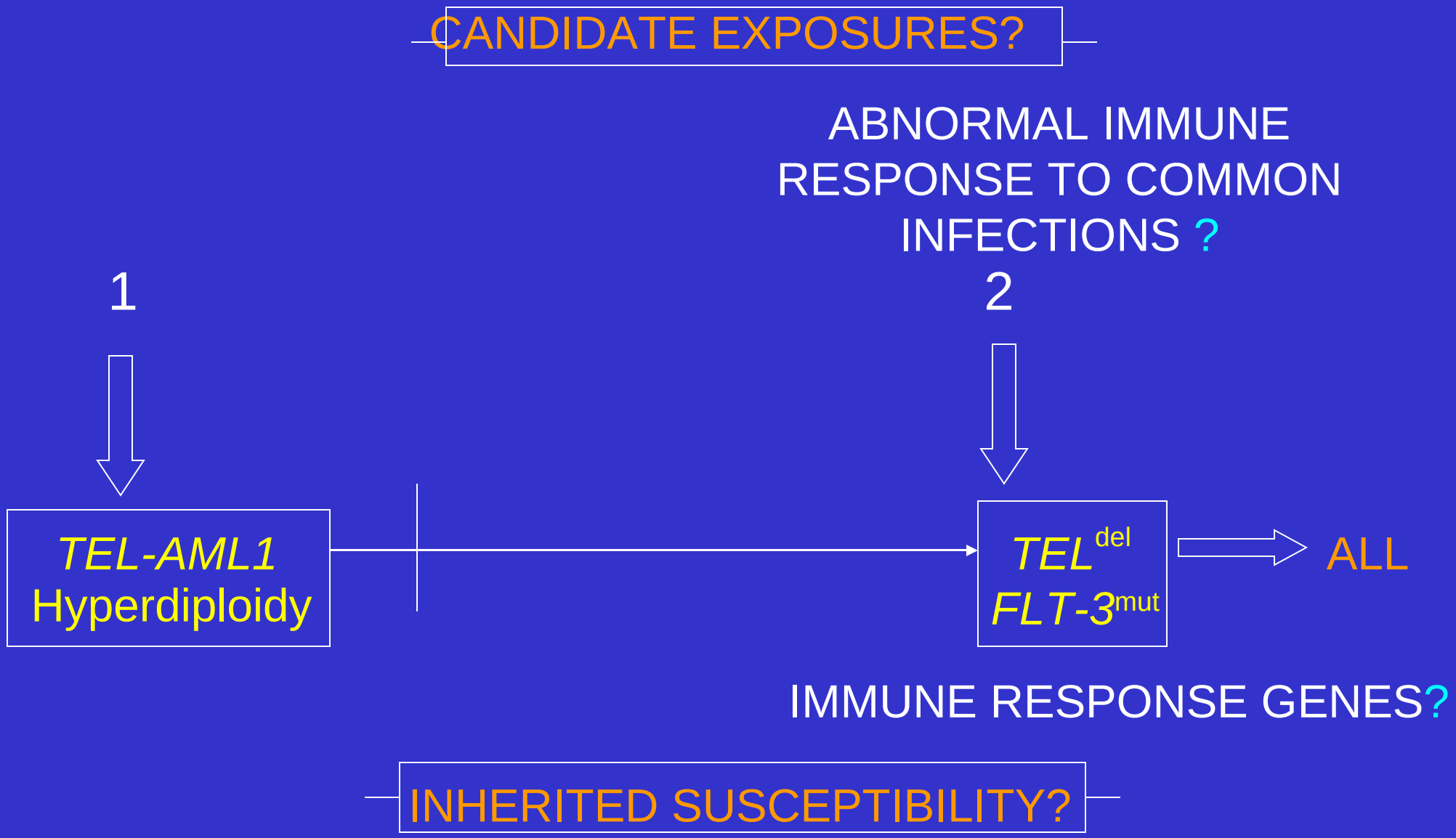
1. Later childhood infections precipitate highly dysregulated immune responses

2. Proliferative / apoptotic stress to bone marrow

THE ‘DELAYED INFECTION’ HYPOTHESIS: DEFINITION OF THOSE AT RISK

- Those with pre-existing pre-leukaemia (foetal) clone
  - developmental accident?
- Those who had deficient infectious exposure in infancy
  - social circumstances
- Those who have particular immune response gene alleles
  - historical contingency / adaptive selection?

A CAUSAL MECHANISM FOR CHILDHOOD LEUKAEMIA



GENETIC EPIDEMIOLOGY STUDIES

- US – CCG Case/Control Studies
- UK Children's Cancer Study (UKCCS)
- California Case/Control Studies

EPIDEMIOLOGICAL EVIDENCE SUPPORTING THE 'DELAYED INFECTION' HYPOTHESIS

- Increased common infections in *infancy* are *protective*

- Increased social contacts in *infancy* are *protective*
  - parity
  - attendance at playgroups

  (- proxies for infection)

BIRTH ORDER AND RISK OF cALL

# of older siblings		Odds Ratio for ALL (1 - 5 years)	
0	(890)	1.00	
1	(710)	0.85	(0.73 - 0.98)
2	(258)	0.74	(0.60 - 0.91)
3	(103)	0.64	(0.44 - 0.87)
4	(30)	0.61	(0.36 - 1.03)
5+	(27)	0.43	(0.26 - 0.73)

p for trend <0.001

Dockerty et al, *Int J Epidemiol* (2001)

SOCIAL CONTACT IN FIRST YEAR OF LIFE AND RISK OF ACUTE LYMPHOBLASTIC LEUKAEMIA

Cases	# 1277
Controls	6268

	OR (CI)
• None	1.00
• Social activity but no day care	0.73 (0.62 - 0.87)
• Informal day care	0.62 (0.51 – 0.75)
• Formal day care	0.48 (0.37 – 0.62)

p for trend = <0.001

UKCCS, 2005

OTHER EPI' DATA INDICATIVE OF AN 'INFECTIOUS' AETIOLOGY

- Relationship with allergies
- Seasonal diagnosis
- Vaccination (*Haemophilus influenzae*)

RECIPROCITY OF RISK FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA AND ALLERGY

UKCCS :	Eczema	0.68 (0.48 – 0.98)
	Hayfever	0.47 (0.26 – 0.85)

- not for asthma

- not for AML

INFECTIOUS AETIOLOGY OF CHILDHOOD ALL

- Direct - molecular virology

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GENETIC ASSOCIATION STUDIES

- MTHFR
  - ⊕ low function alleles MTHFR (CGTTT) (UKCCS)
- HLA
  - ⊕ HLA-DPB1*0201 (UKCCS)
  - Supertype $DP\beta_1$ 69E (UKCCS)
- Immune response genes
  - Cytokines, chemokines, receptors toll receptors
    - in progress (UKCCS)

INFECTIOUS AETIOLOGY OF CHILDHOOD ALL

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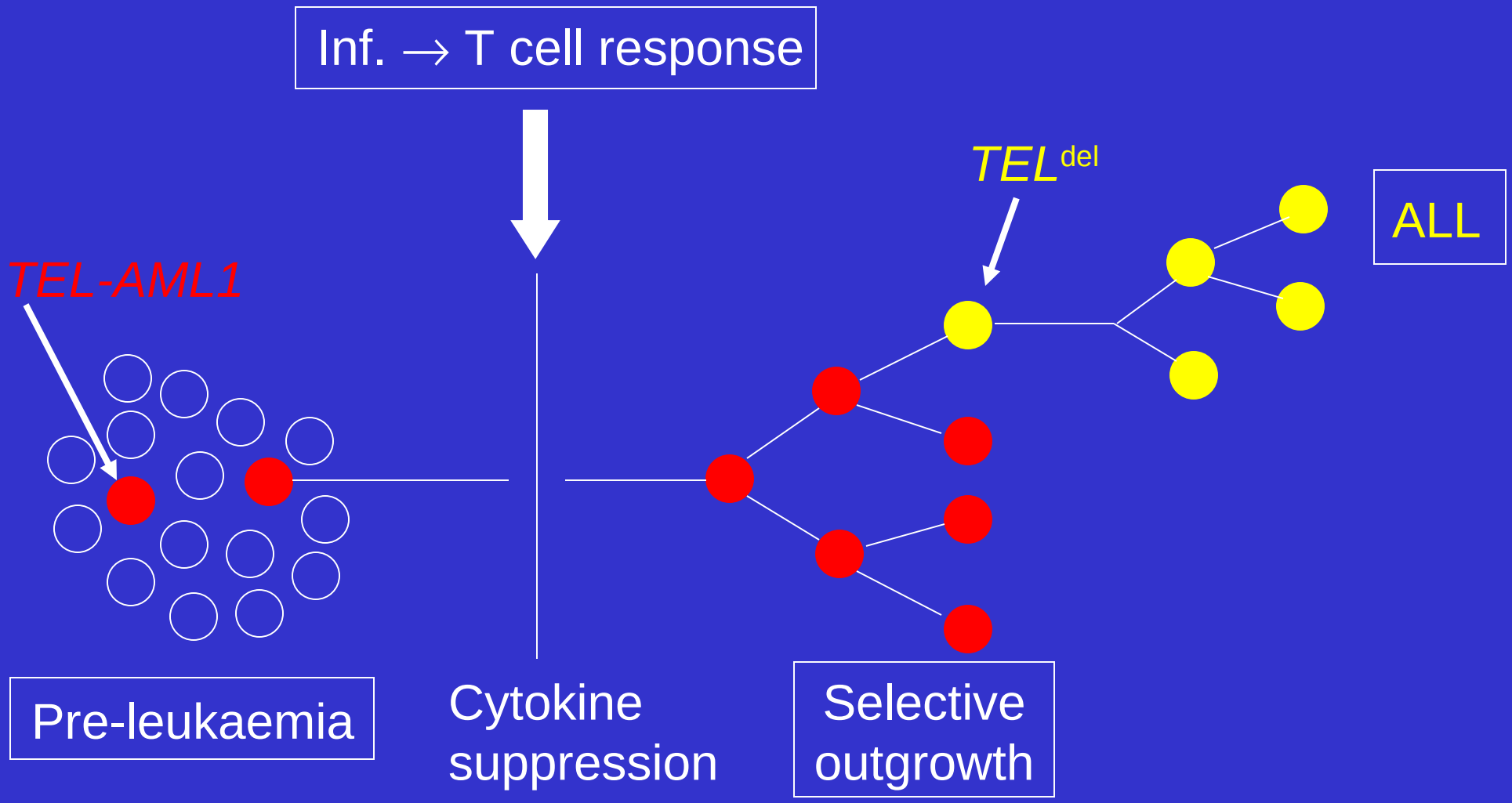
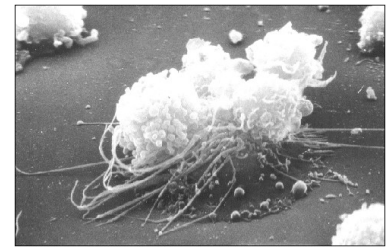
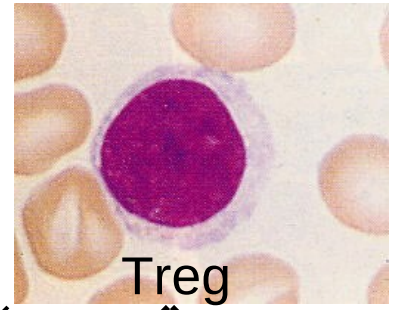
INFECTION, THE IMMUNE RESPONSE AND 'SELECTION' OF PRE-LEUKAEMIC CLONES



Microbial Exposure



Dendritic cells

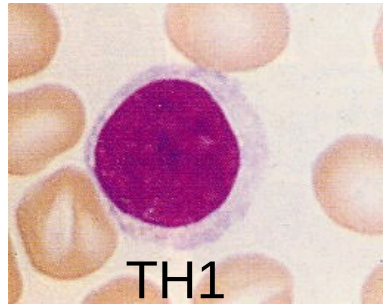


Treg

IL-12

IL-10
TGFβ

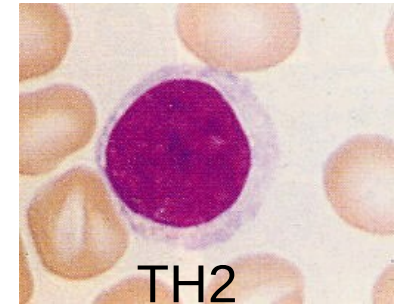
IL-4



TH1

IFNγ

IL-4

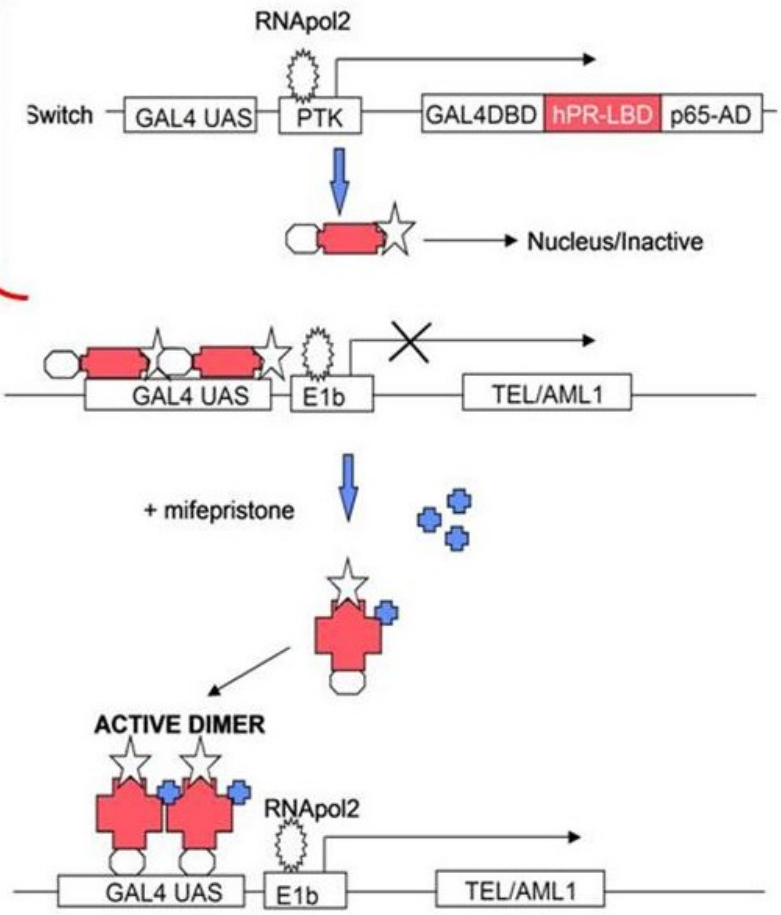


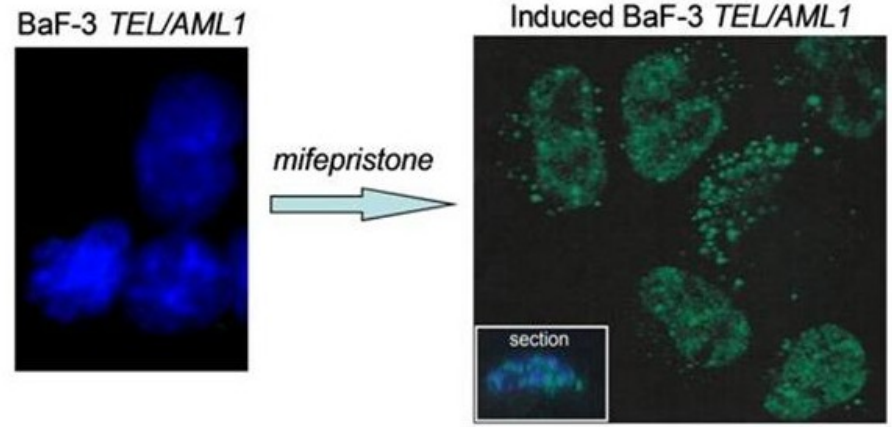
TH2

Induction of TEL-AML1 in BaF-3 cells

Clone 1/27

Clone Cl1

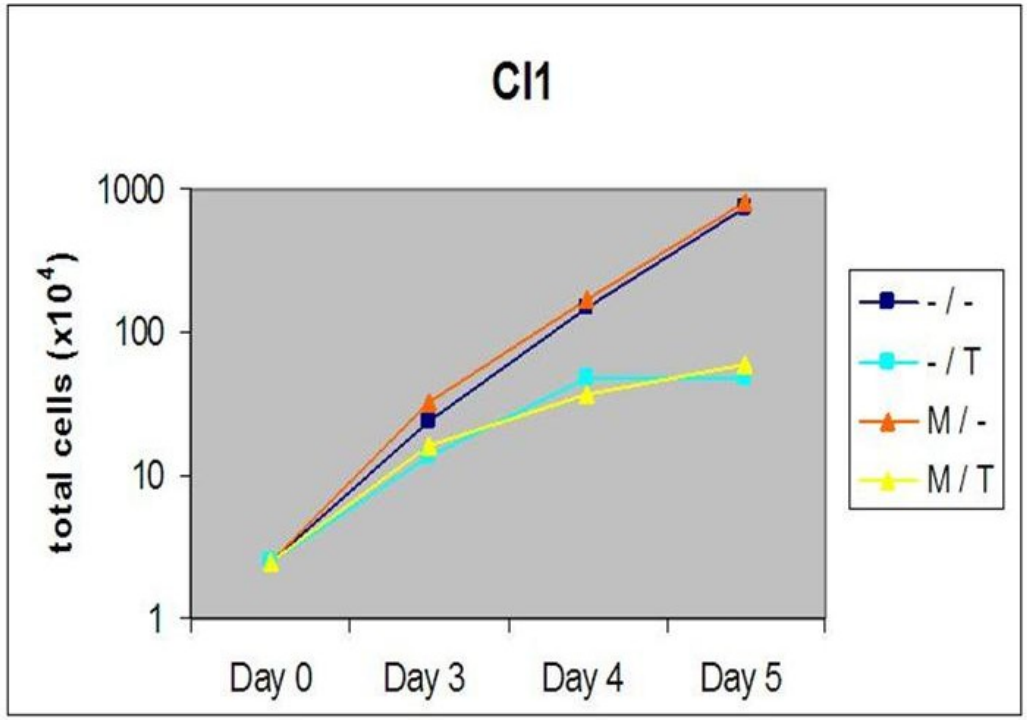


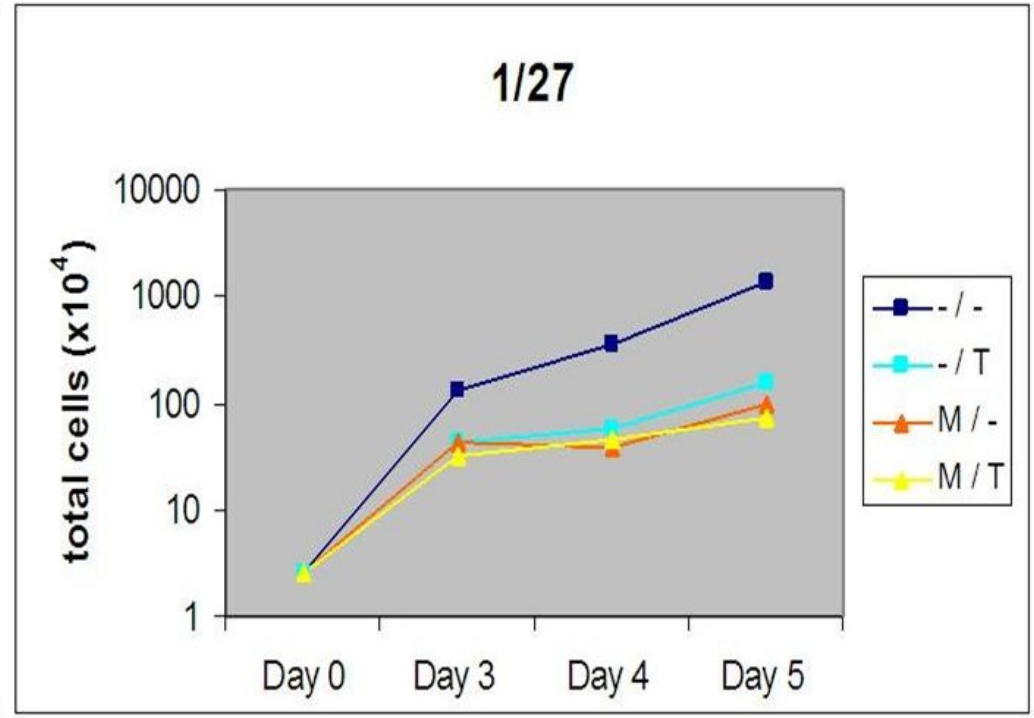


Blue= nucleus (DAPI)

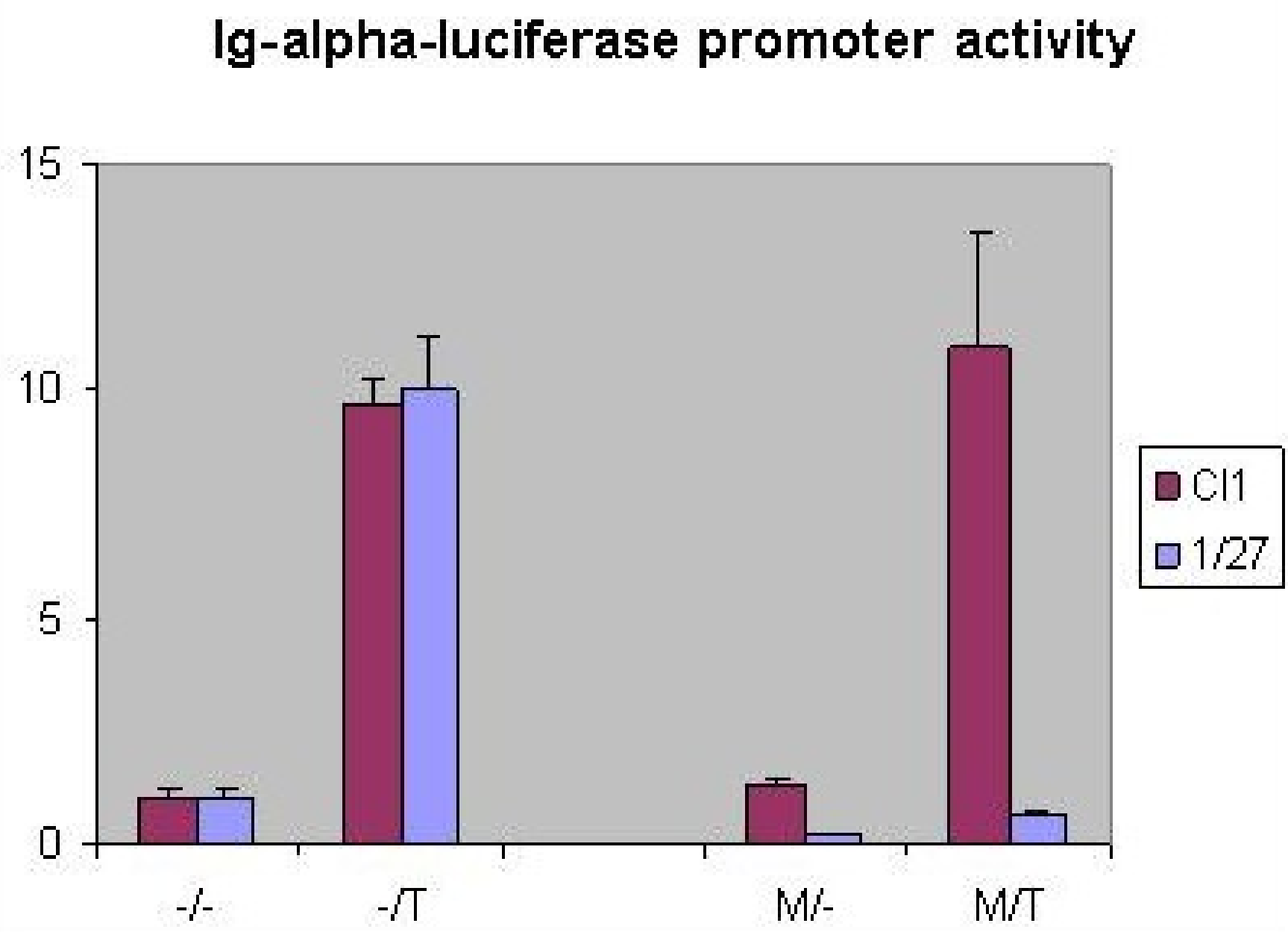
Green= TEL-AML1

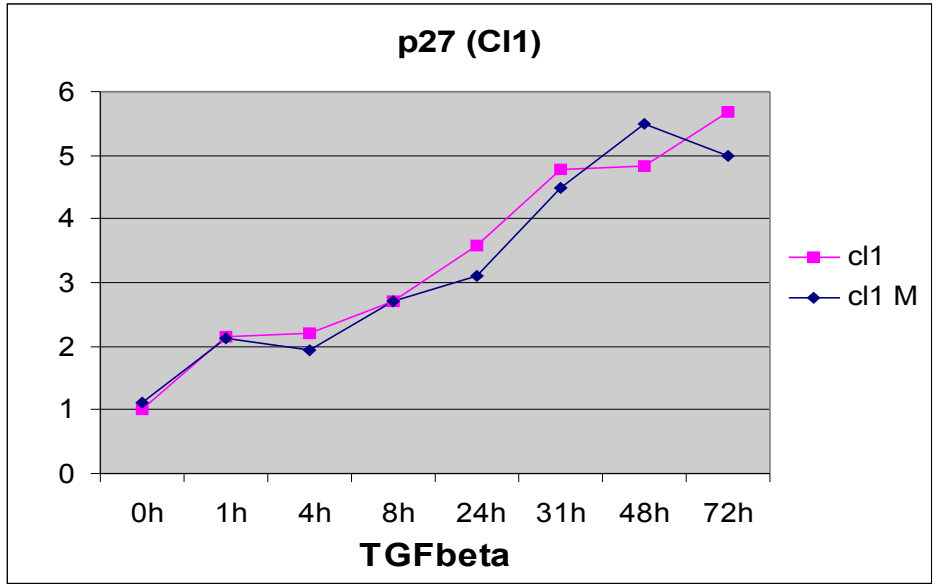
Effects of TGF-beta1 on cell growth profiles

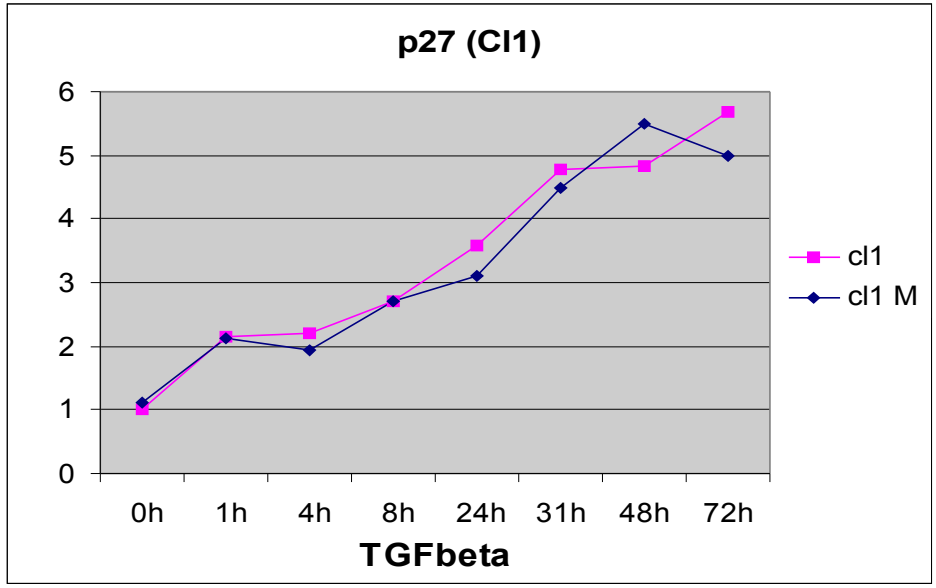




No difference due to *Mifepristone*!


Ig-alpha-luciferase promoter activity
15
10
5
0
-/-
-/T
M/-
M/T
Cl1
1/27


p27 (Cl1)
6
5
4
3
2
1
0
0h
1h
4h
8h
24h
31h
48h
72h
TGFbeta
cl1
cl1 M


p27 (Cl1)
6
5
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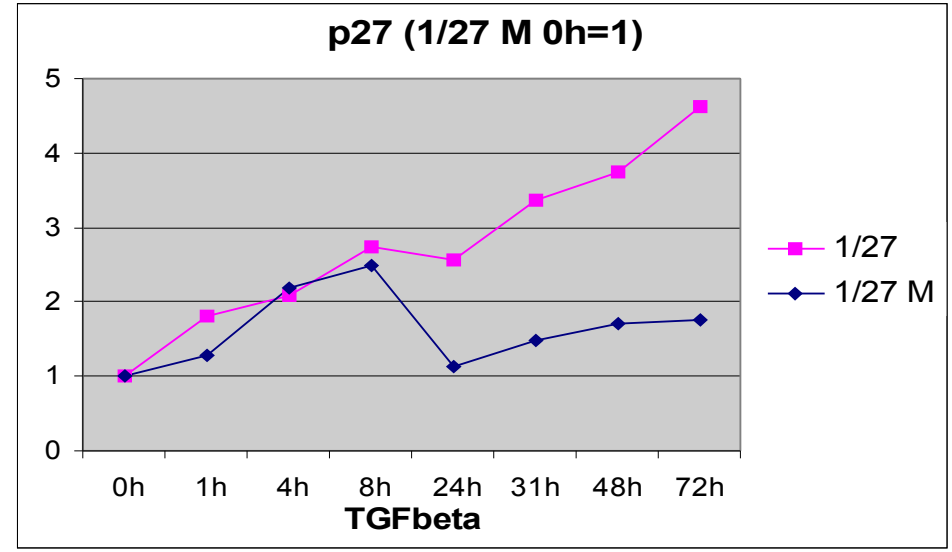
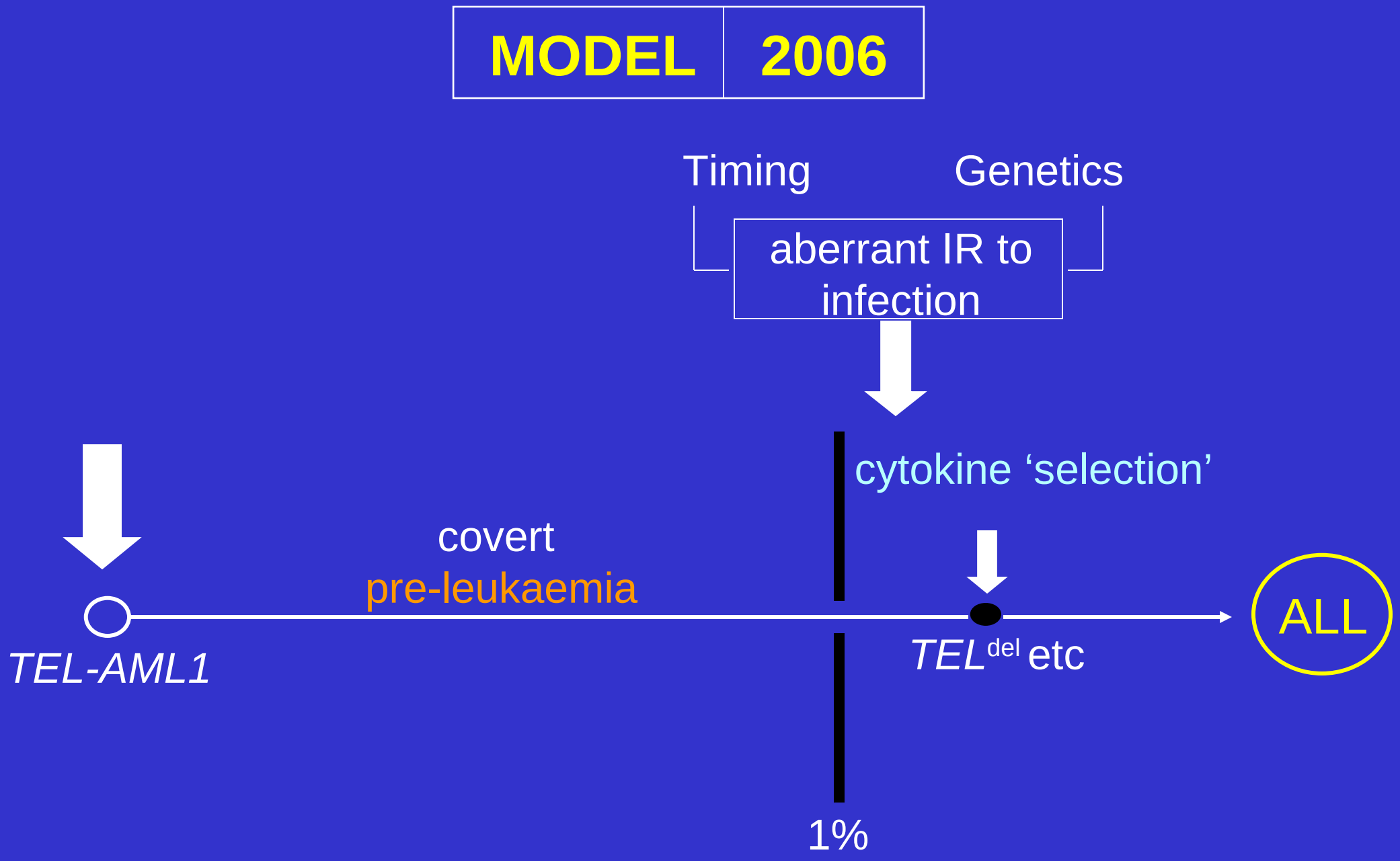
1h
4h
8h
24h
31h
48h
72h
TGFbeta
cl1
cl1 M


p27 (1/27 M 0h=1)
5
4
3
2
1
0
0h
1h
4h
8h
24h
31h
48h
72h
TGFbeta
1/27
1/27 M


MODEL 2006
Timing
Genetics
aberrant IR to infection
cytokine 'selection'
covert
pre-leukaemia
TEL-AML1
TEL del etc
ALL
1%

MOLECULAR GENETICS AND NATURAL HISTORY OF PAEDIATRIC LEUKAEMIA

LRF CENTRE
Tony Ford
Joseph Wiemels
Ana Teresa Maia
Hiroshi Mori
Jan Zuna
Zhijian Xiao
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Lyndal Kearney
Mel Greaves

CLINICAL LINKS (UK)
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GENETICS
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MODELLING
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Dengli Hong

INTERNATIONAL LINKS

S Mizutani	Japan
M-E Cabrera	Chile
M Pombo de Oliveira	Brazil
A Biondi	Italy
G Cazzaniga	Italy
E Van Wering	The Netherlands
A Borkhardt	Germany
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