Molecular Hematopathology
Leukemias I

January 14, 2005

Chronic Myelogenous Leukemia

Diagnosis requires presence of Philadelphia chromosome

t(9;22)(q34;q11) translocation

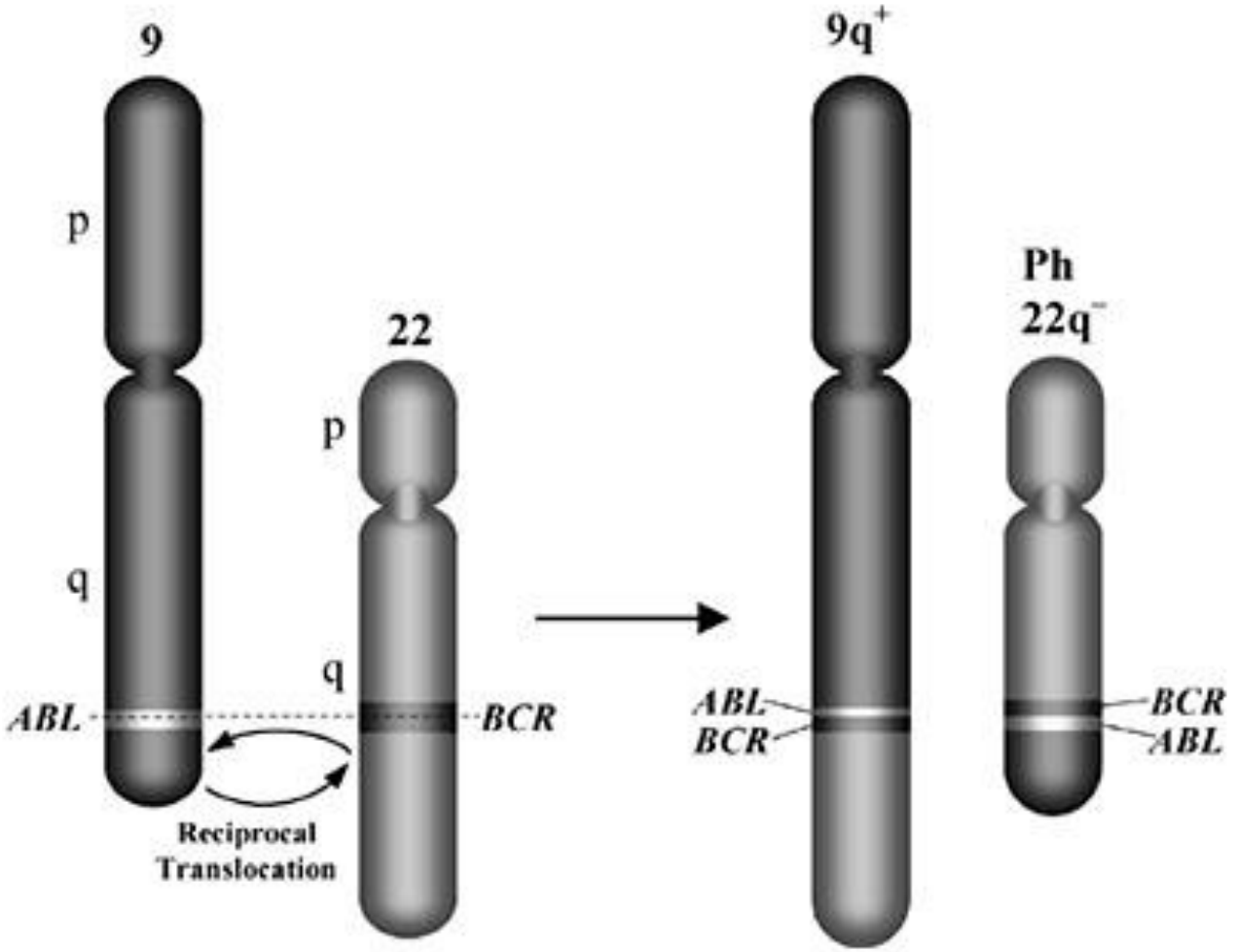
BCR-ABL is the result

BCR on chr 22 translocated to *ABL* on chr 9q

Normal *BCR* (breakpoint cluster region) is an unessential protein expressed in all cells; involved in G-protein signaling that involved in neutrophilic burst

Normal *ABL* (Abelson murine leukemia) is a tyrosine kinase expressed in all cells; involved in signal transduction from integrins, cell cycle arrest, response to genotoxic stress

t(9;22)(q34;q11) *BCR-ABL* Translocation



Chronic Myelogenous Leukemia

Mechanism of translocation- dsDNA breaks (? susceptible regions) with erroneous rejoining

Regions of translocation seem to co-localize during S-G2 phase

Translocation creates a growth advantage

BCR-ABL translocation occurs within introns

Exons are preserved

BCR-ABL gene is transcribed into *BCR-ABL* mRNA

BCR-ABL mRNA is translated into the BCR-ABL fusion protein

Chronic Myelogenous Leukemia

Breakpoints

Within introns of the 5' part of *ABL*	a2
Within introns of the 3' part of *BCR*	3 regions

***BCR* breakpoints** occur in 3 regions of the gene

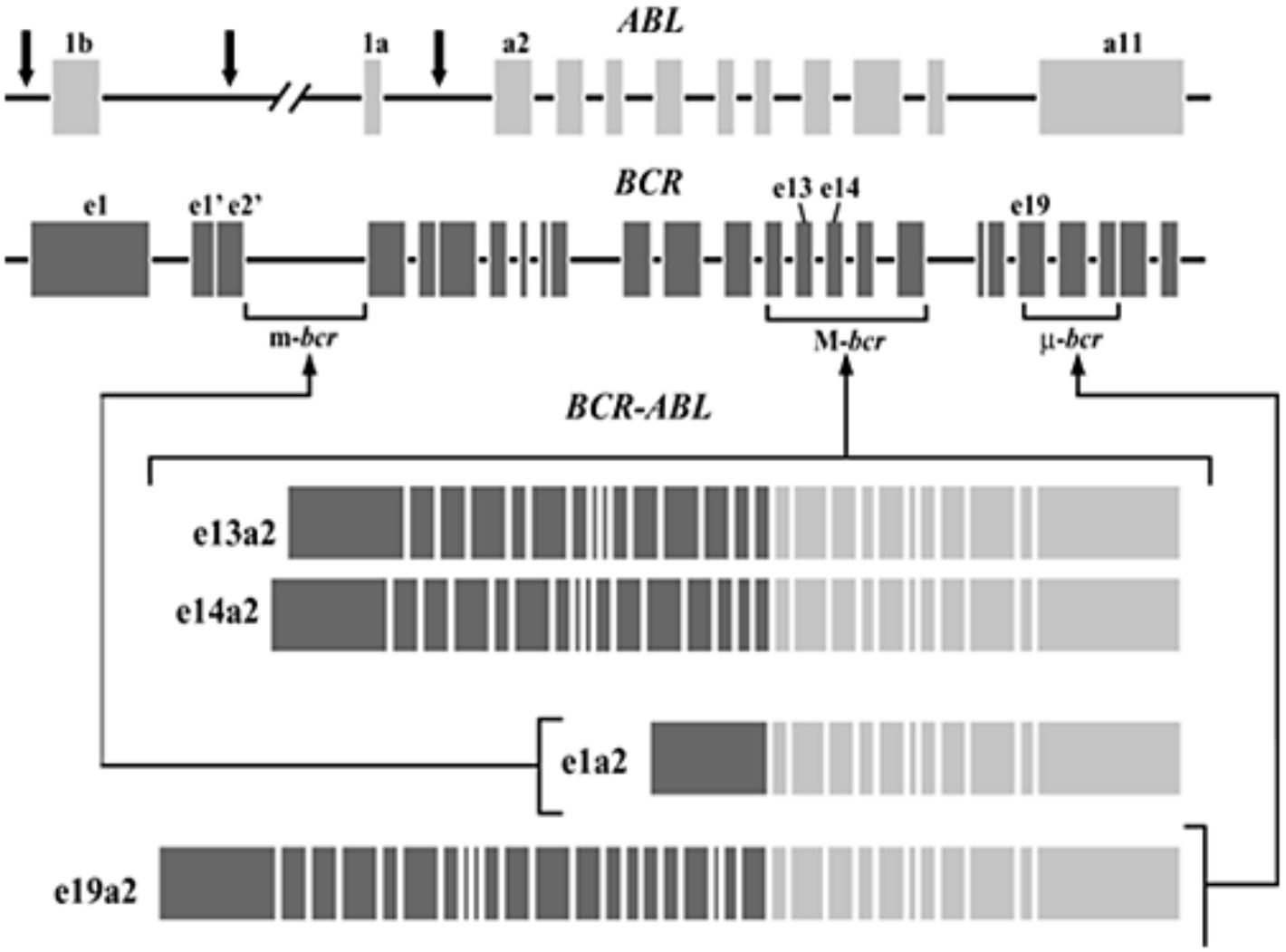
M-bcr – major breakpoint cluster region (~ exons 13-14)

For example, e13a2 or e14a2

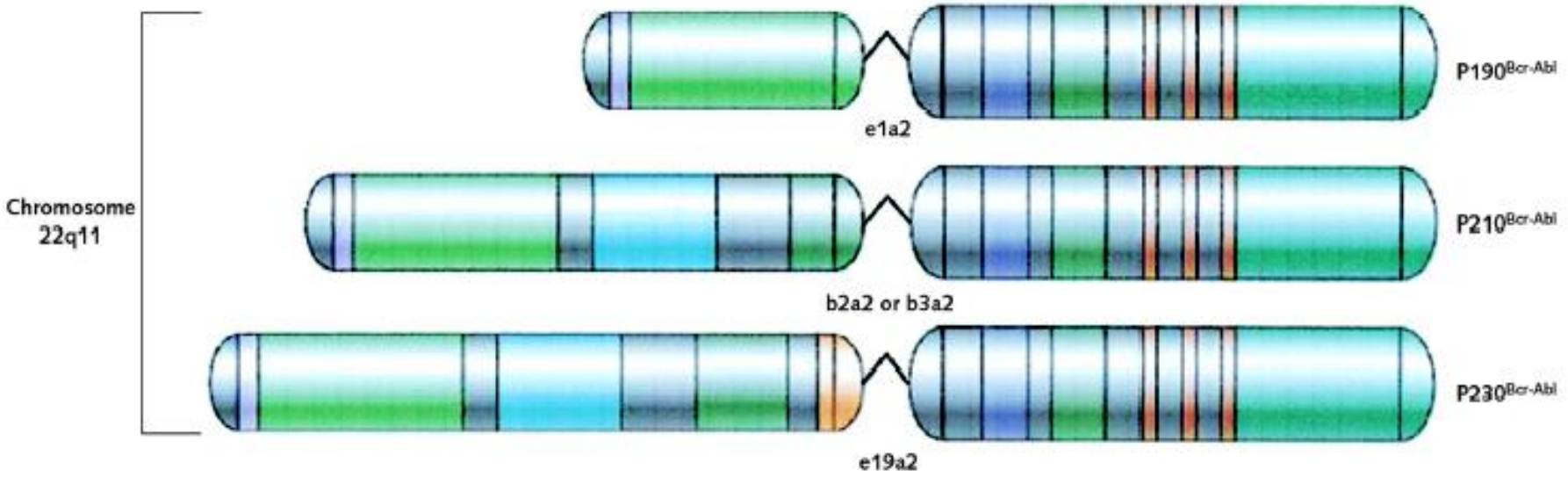
Forms p210 $^{\text{Bcr-Abl}}$ fusion protein

95% of CML cases, 30% of AML

BCR-ABL Translocations



BCR-ABL Translocations



Chronic Myelogenous Leukemia

m-bcr – minor breakpoint cluster region (btwn e2 and e2')

For example, e1a2

Forms p190 $^{Bcr\text{-}Abl}$ fusion protein

66% of Ph^{+} ALL cases, rare CML and AML

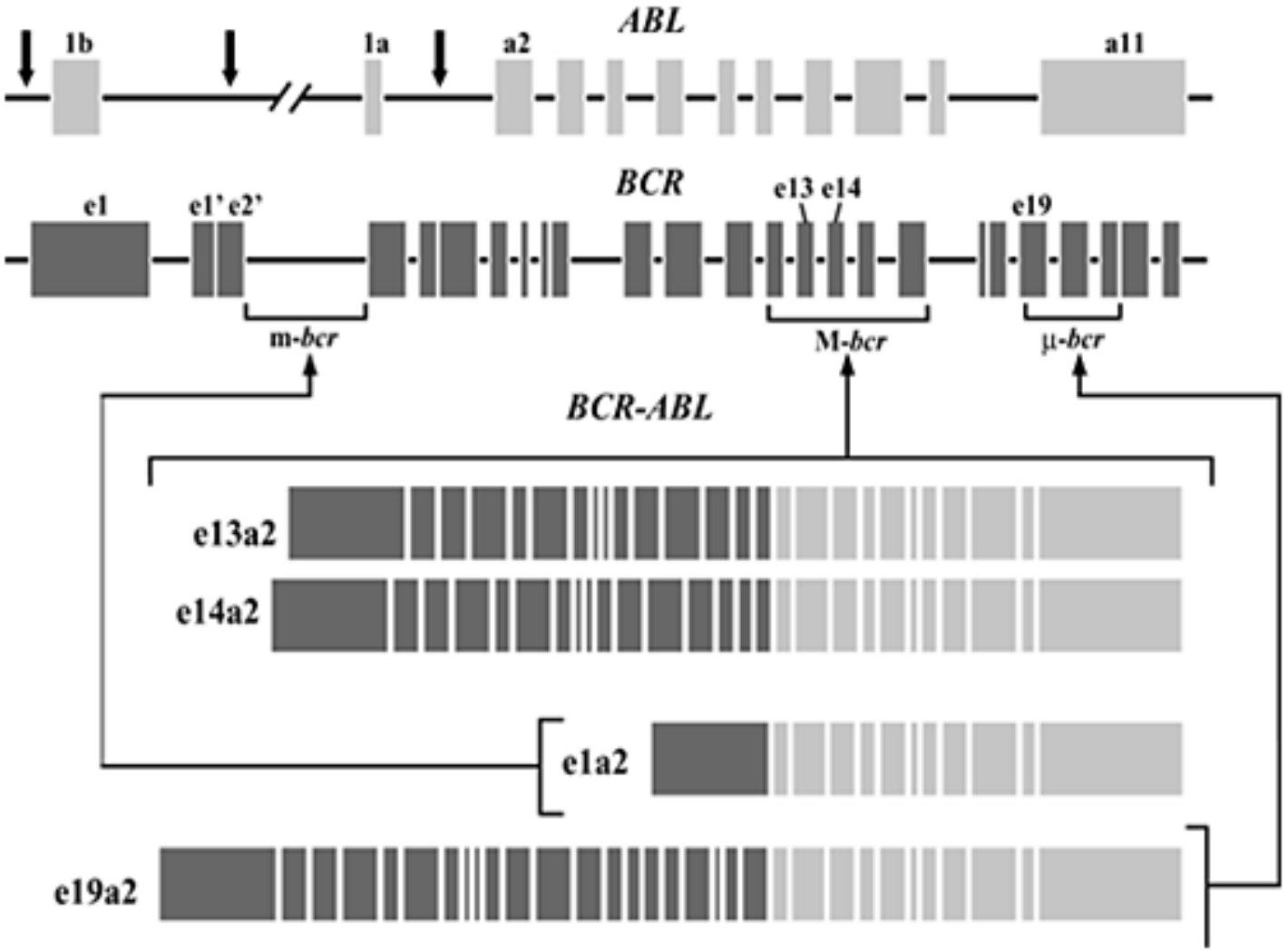
μ-bcr – micro breakpoint cluster region (btwn e19 and e20)

For example, e19a2

Forms p230 $^{Bcr\text{-}Abl}$ fusion protein

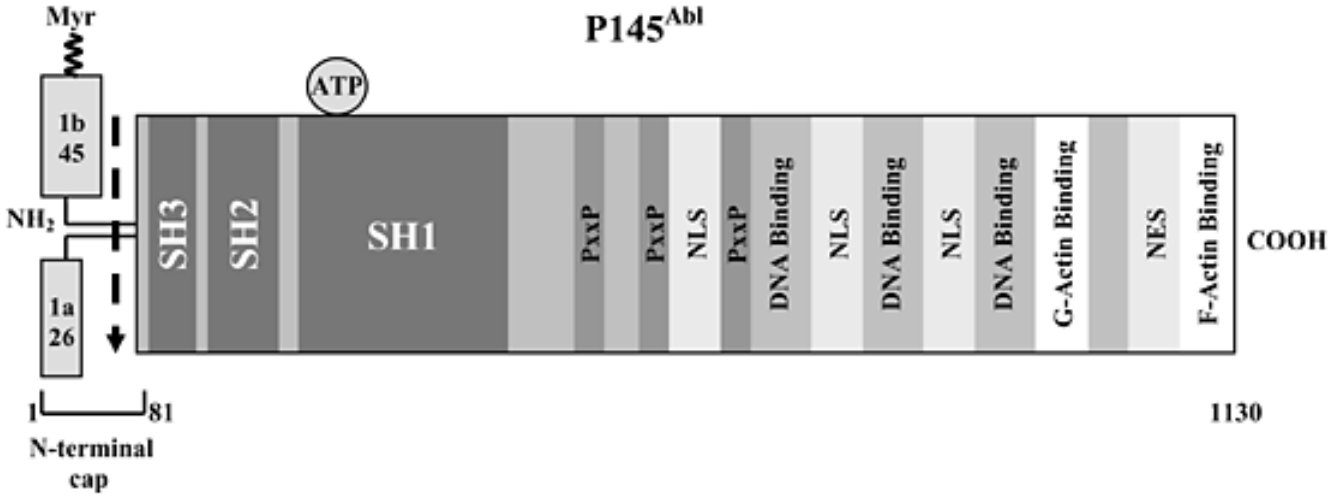
Some cases of chr neutrophilic leukemia

BCR and *ABL* Breakpoints

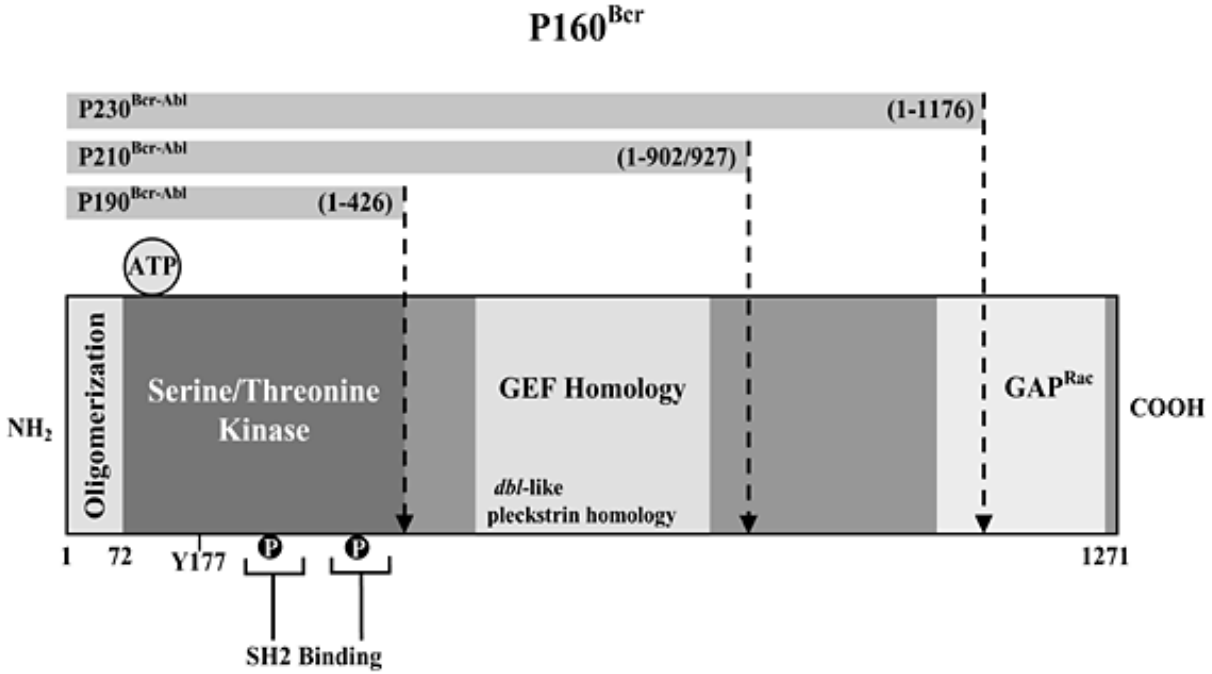


Bcr and Abl Proteins

Abl



Bcr



Chronic Myelogenous Leukemia

Pathogenesis

Bcr-Abl fusion protein results in defective, partial Abl protein which starts phosphorylating tyrosine residues on itself and other cellular proteins, interfering with:

1) mitogenic cell signaling pathways involved in granulocytic differentiation and maturation (via *ras*, Stat, PI-3, *myc)*
2) BM progenitor cell adhesion to stromal cells and normal cytokine exposure
3) inhibition of apoptosis (blocks cyt C, upregulates bcl-2)

Chronic Myelogenous Leukemia

Exact position of M-bcr breakpoint (eg, e13 vs e14) to make p210 does not predict disease severity, though e14a2 has a greater effect on thrombopoiesis

Note: 30-70% of normal population has mRNA p210 or p230 in 1 in 10^8 of their WBCs; "necessary but not sufficient"

Detection

Cannot do PCR on RNA

Do RT-PCR then gel electrophoresis

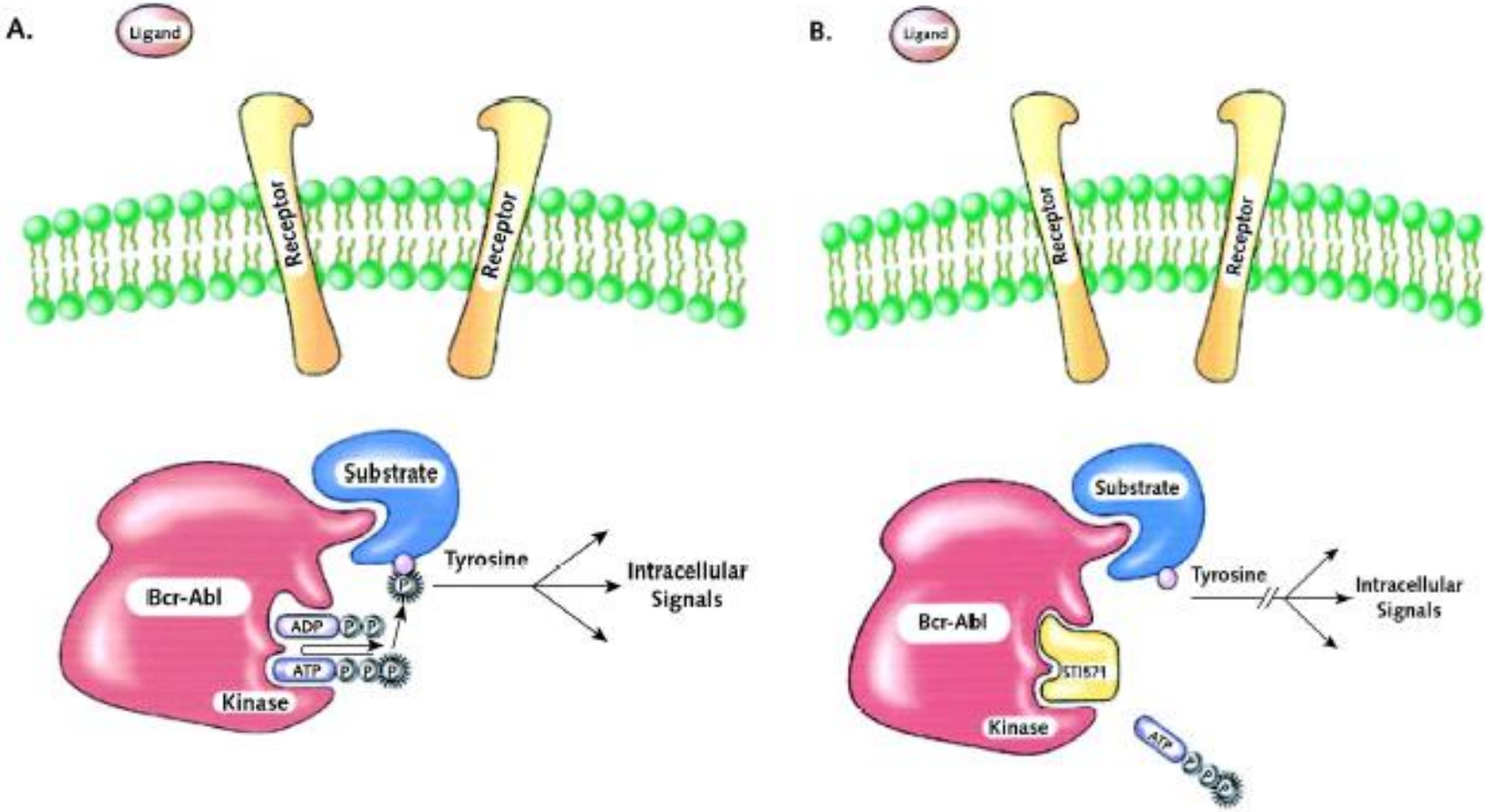
Treatment

Gleevec (imatinib mesylate, STI571, 2-phenylaminopyrimidine) inhibits the tyrosine kinase activity of Bcr-Abl protein

Competes with ATP for the kinase pocket on Abl

CML

Gleevec


A.
Ligand
Receptor
Receptor
Substrate
Tyrosine
Intracellular Signals
Bcr-Abl
ADP
ATP
Kinase
B.
Ligand
Receptor
Receptor
Substrate
Tyrosine
Intracellular Signals
Bcr-Abl
STI571
Kinase
ATP

Chronic Myelogenous Leukemia

Gleevec treated CML patients go into complete remission

Accelerated phase CML – 40% have complete response

Blast crisis – 7% get complete response

Acquired resistance now being reported; due to *BCR-ABL* gene amplification, MDR glycoprotein production, or point mutations in Abl's kinase domain

Acute Myelogenous Leukemia

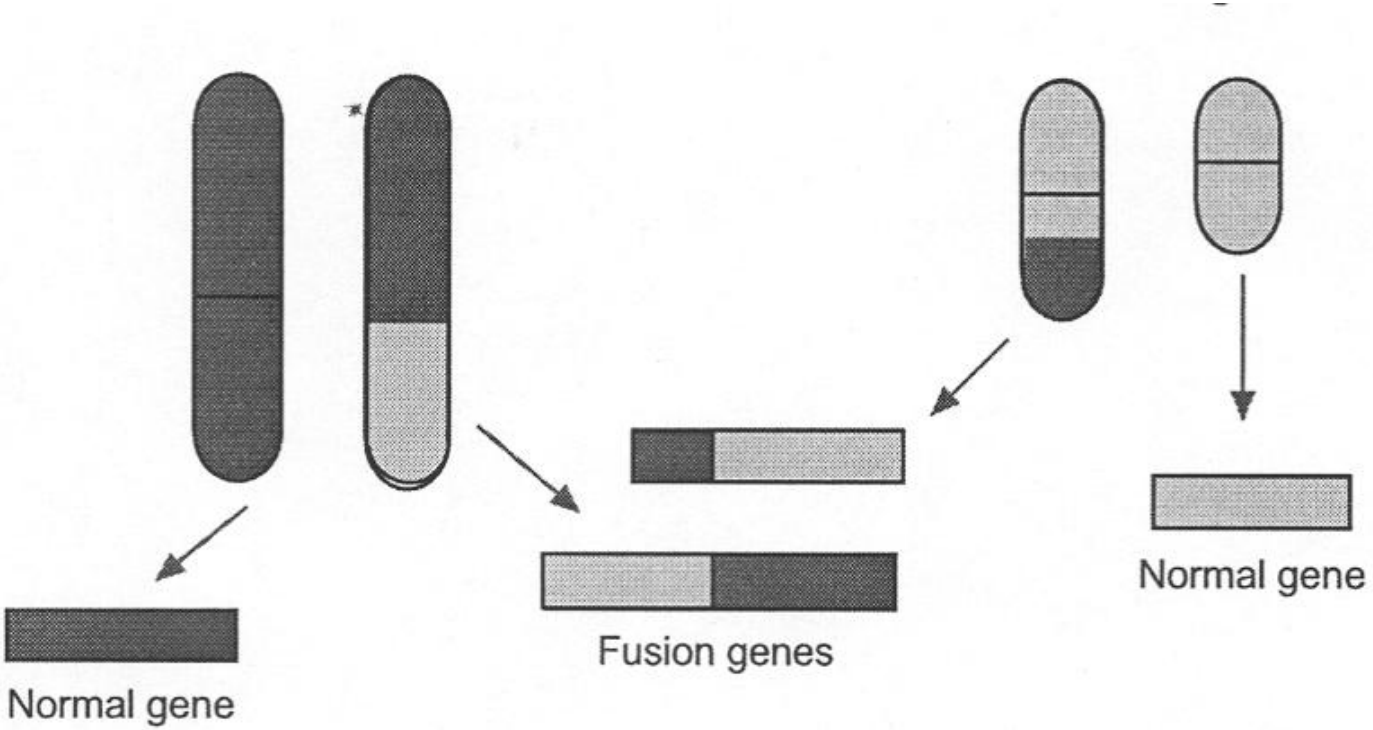
Molecular information has come from cytogenetic translocations

Translocations usually result in creation of novel **fusion/chimeric protein**

Frequently it is the fusion of 2 transcription factors, eg

CBF	core binding factor
RARα	retinoic acid receptor
HOX	homeobox
ETS	E26 avian transforming virus-like

Translocation Results in Novel Fusion Proteins



Acute Myelogenous Leukemia

Translocations Involving Core Binding Factor

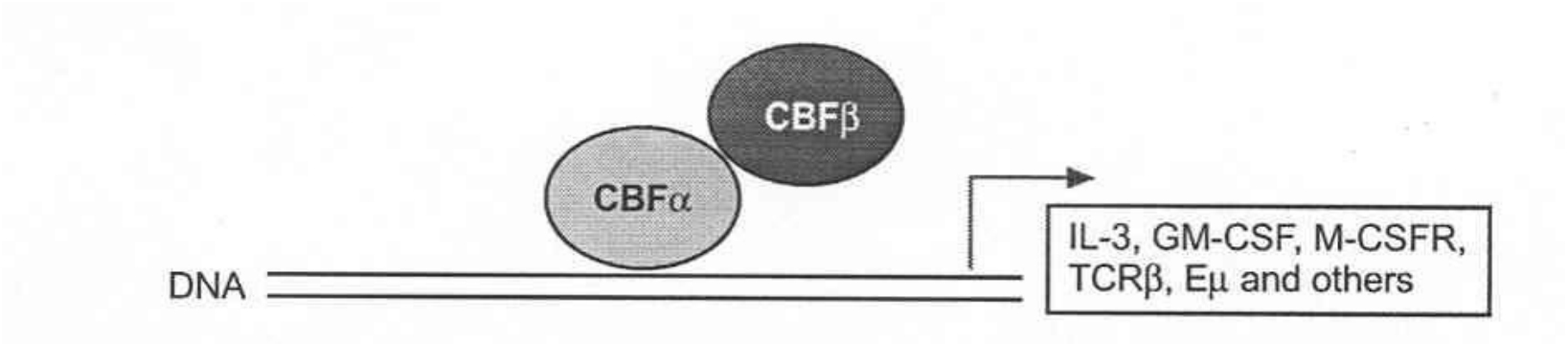
CBF has 2 subunits – CBFα (AML1) and CBFβ

CBF is a transcription factor that regulates expression of genes involved in hematopoietic differentiation, eg IL-3, GM-CSF, M-CSF, TCRβ, IgH enhacer

Four different translocations described:

t(8;21)(q22;q22)	**AML1/ETO**
t(12;21)(p13;q22)	**TEL/AML1**
inv 16(p11;q22)	**CBPβ/SMMHC**
t(3;21)(q26;q22)	**AML1/EVI1**

Core Binding Factor Protein is a Transcription Factor Involved in Hematopoiesis



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AML1/ETO Translocation

t(8;21)(q22;q22)

FAB M2 cases (myeloblastic with maturation)

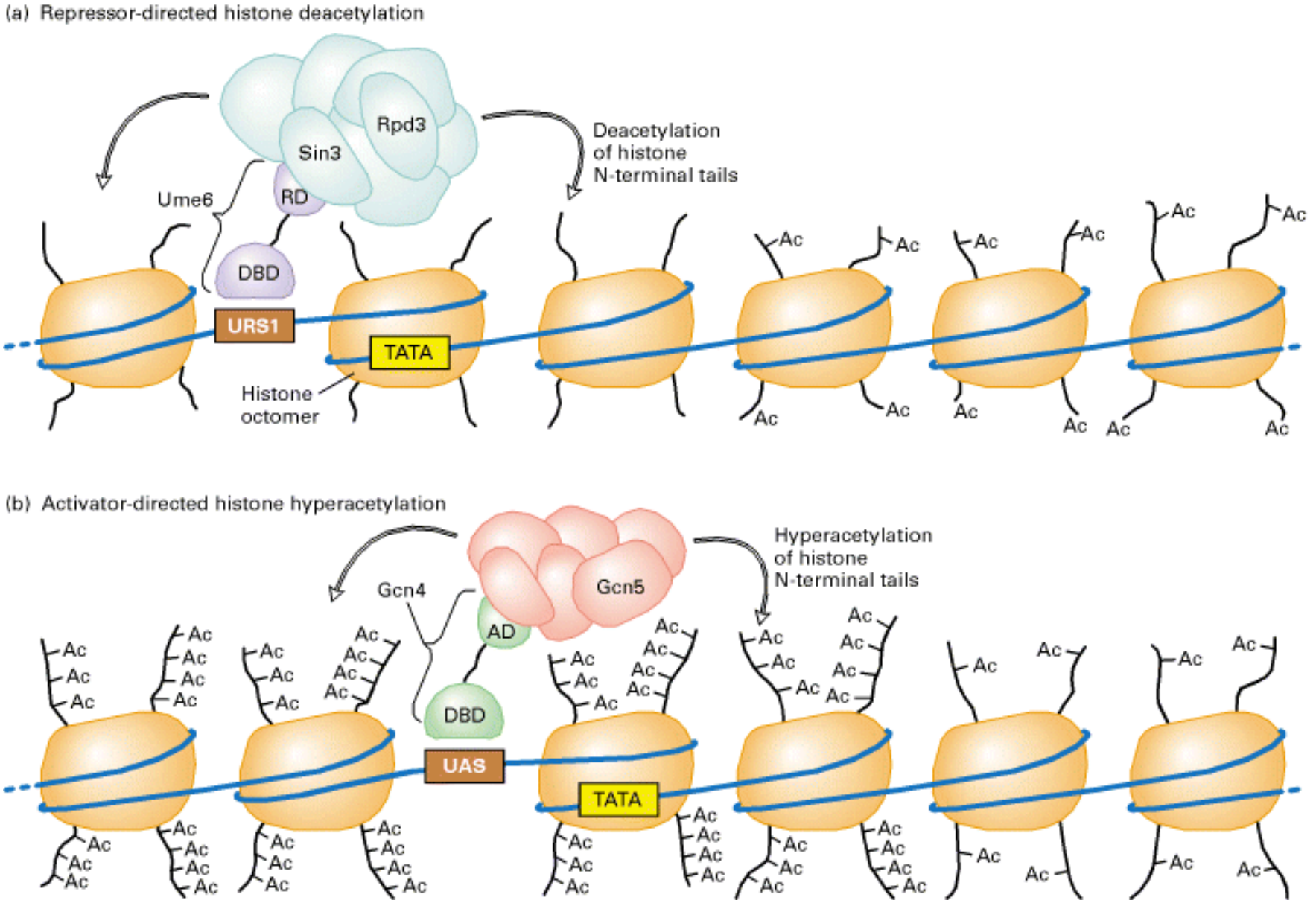
AML1 gene on 21q22 translocated to *ETO* (eight twenty-one) gene on 8q22

Normal AML1 is a transcription factor involved in the development of all hematopoietic lineages

Normal ETO is a co-repressor, binds to histone deacetylases

Transcription and translation results in novel AML1/ETO fusion protein

Acetylation Activates…..De-Acetylation Deactivates Transcription



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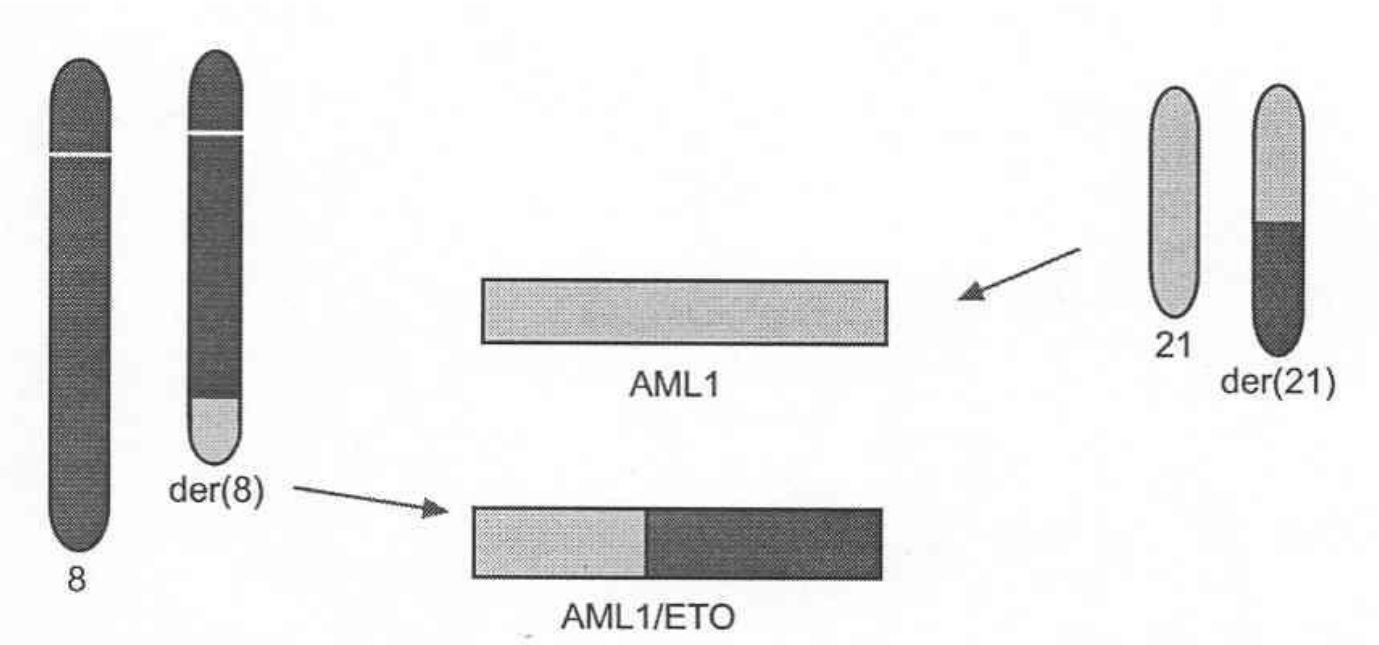
AML1/ETO Translocation Creates Fusion Protein



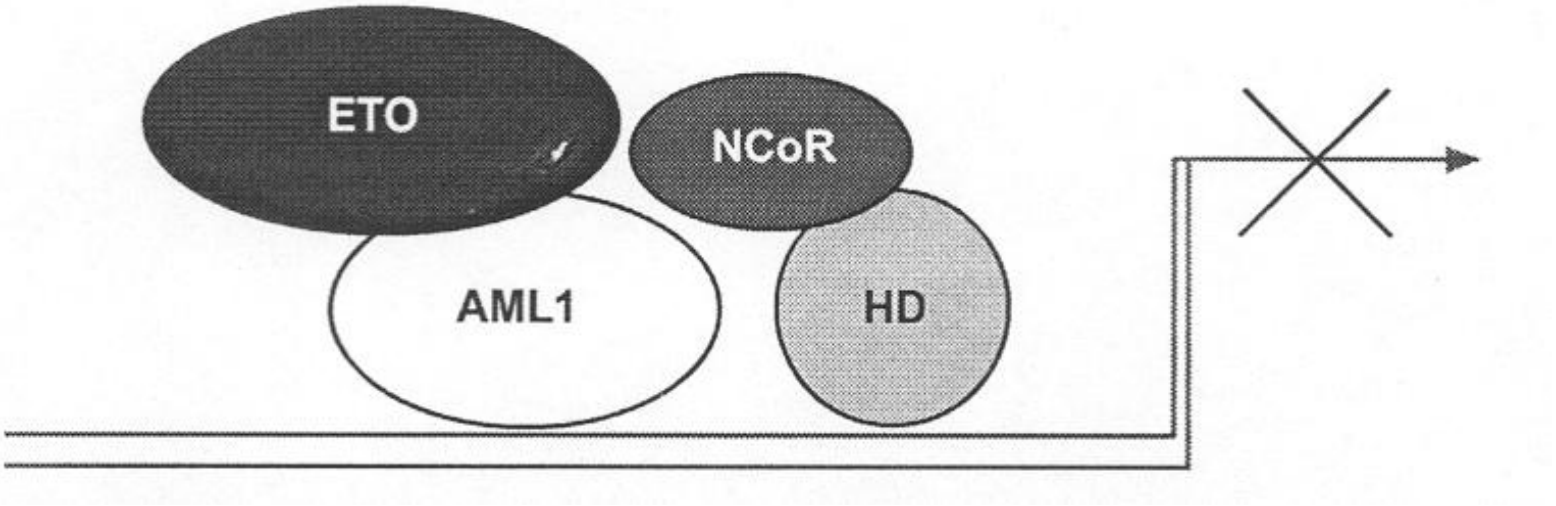
AML1/ETO Translocation

Chimeric protein inhibits normal AML1 function - “dominant negative inhibition”

ETO portion of the chimeric protein recruits nuclear co-repressors and histone deacetylases to CBF promoters, inhibiting transcription of CBF target genes

M2 cases w/ this transcription respond to Tx, good prognosis

AML1/ETO Fusion Protein Recruits HD/Co-repressor Complex to CBF Promoters



TEL/AML1 Translocation

t(12;21)(q13;q22)

Childhood B-ALL

TEL (translocation ets leukemia) gene on 12q13 translocated to *AML1* gene on 21q22

TEL protein normally inhibits megakaryocytic development

TEL/AML1 fusion protein is a dominant negative inhibitor of normal AML1

Inhibition occurs due to transcription co-repression by TEL

B-ALL cases with this translocation have favorable Px

CBFβ/SMMHC Translocation

inv 16(p11;q22)

M4Eo cases (acute myelomonocytic with eosinophilia)

CBFβ at 16q22 flips and fuses to *SMMHC* at 16p13

CBFβ/SMMHC fusion protein is dominant negative inhibitor of normal CBF protein

Acute Myelogenous Leukemia

Translocations Involving RARα

All assoc with APL (acute promyelocytic leukemia) FAB M3

Normal RARα on 17q11 is a non-essential gene involved in modifying/regulating various genes that control myelo-proliferative progenitor/stem cells; done through interaction with transcription factors and co-repressors

Three main translocations:

t(15;17)(q22;q11)	**PML/RARα**
t(5;17)(q31;q11)	**NPM/RARα**
t(11;17)(p13;q11)	**PLZF/RARα**

PML/RARα Translocation

t(15;17)(q22;q11)

PML on 15q22 translocated to *RARα* on 17q11

Translocation results in novel PML/RARα fusion protein

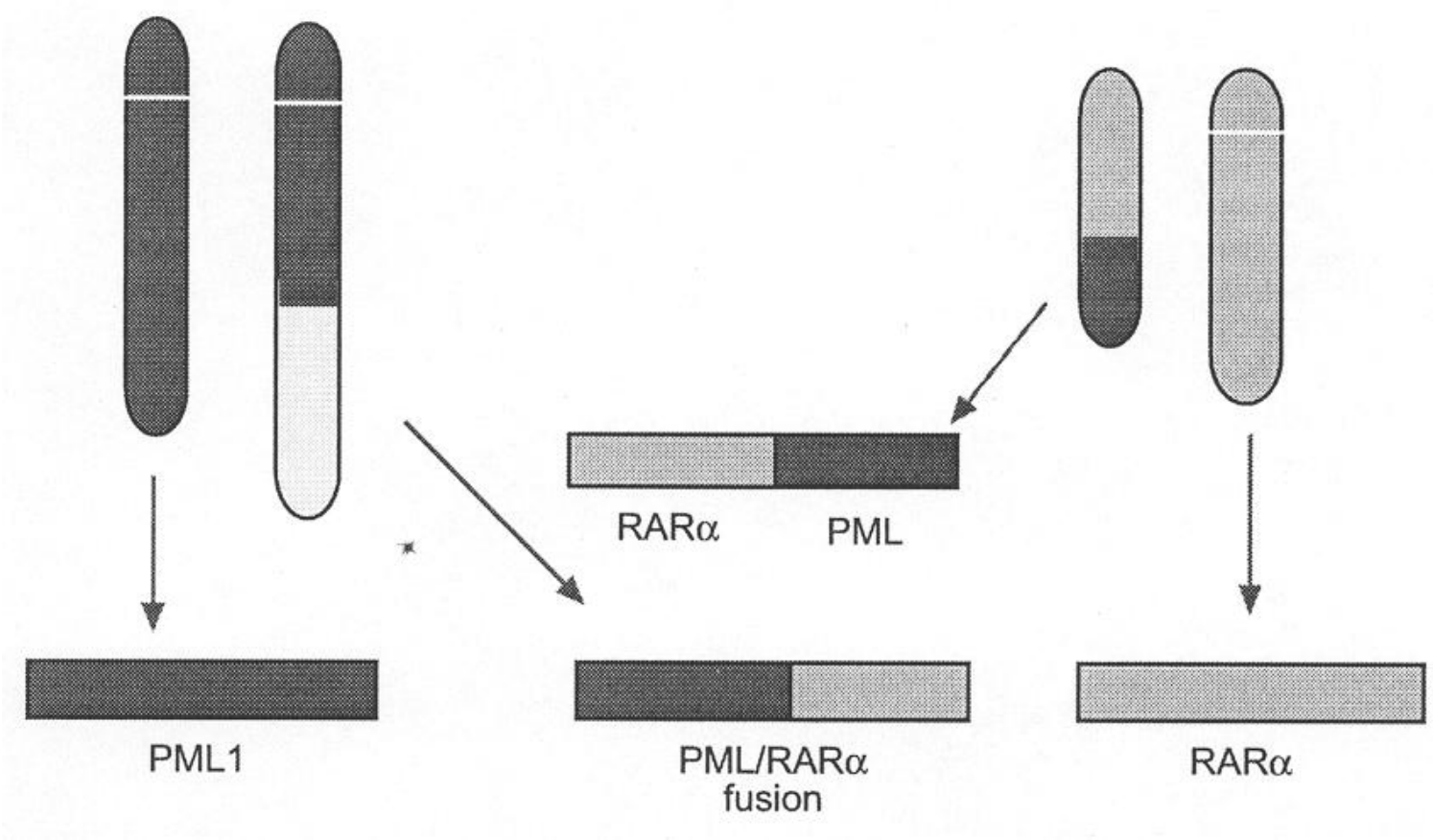
Normal PML function is associated with early hematopoiesis, and erythroid, but not myeloid, maturation; apoptosis

Translocation necessary but not sufficient for tumorigenesis

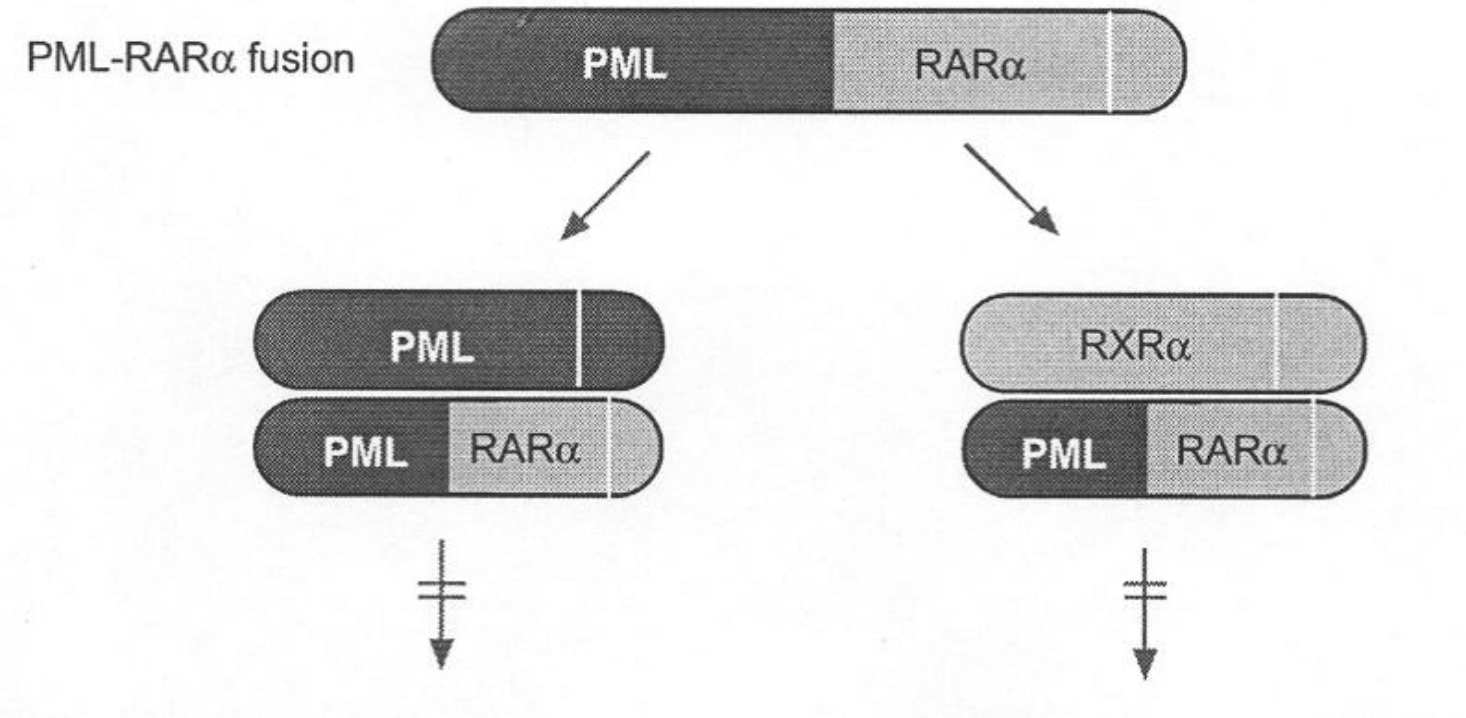
PML/RARα acts as a dominant negative inhibitor of normal PML and normal RXRα

RXRa (retinoic X receptor) normally binds RARα

Fusion of *PML* and *RARα* to form PML/RARα



PML/RARa Is a Dominant Negative Inhibitor of PML and RXRa



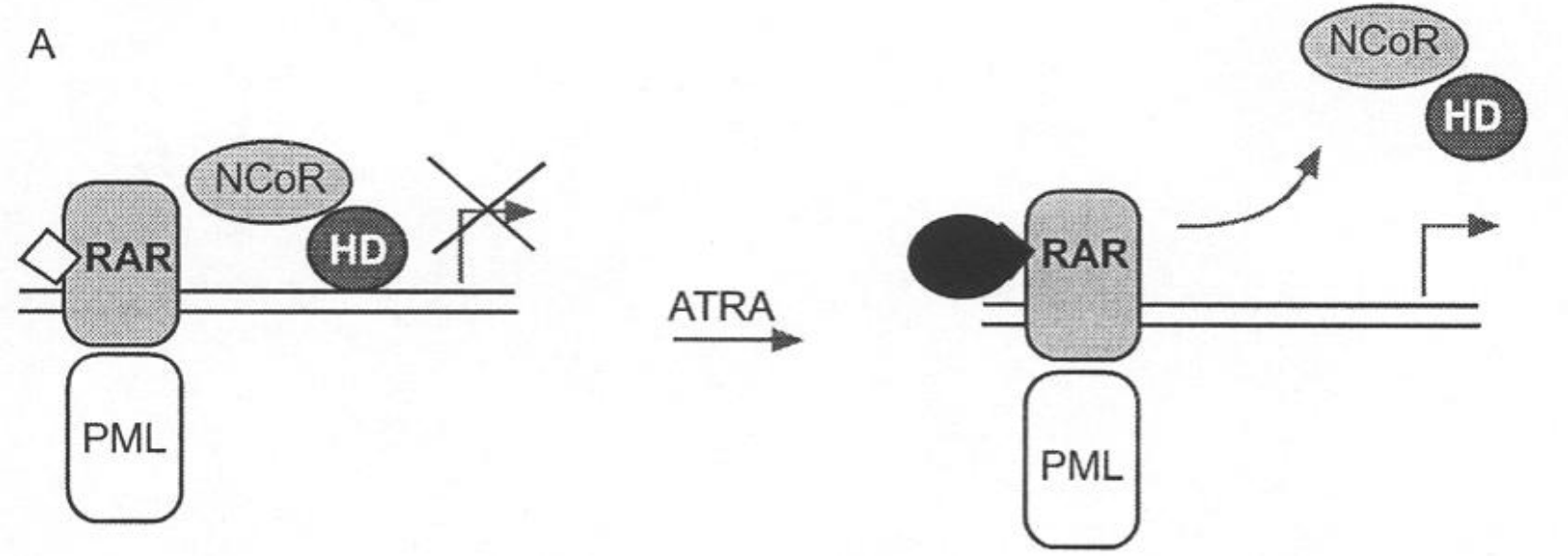
PML/RARα Translocation

PML/RARα binds normal PML and relocates it away from DNA

PML/RARα also recruits HD/co-repressor complex (histone de-acetylase), which represses transcription of RARα target genes, preventing normal hematopoietic differentiation/ maturation

ATRA (all-trans retinoic acid) is the Tx for APL – it binds the RARα part of PML/RARα and causes the release of the HD/ co-repressor complex, allowing expression of those genes required for promyelocyte maturation to PMNs

ATRA Causes Dissociation of HD/Co-Repressor Complex



Acute Myelogenous Leukemia

Translocations Involving HOX Genes

Seen in cases of AML, T-ALL, MDS

HOX genes are a family of genes that regulate embryonic development, including hematopoietic

t(7;11)(p15;p15)	**NUP98/HOXA9**
t(2;11)(q31;p15)	**NUP98/HOXD13**

Mechanism of oncogenesis not understood –perhaps related to inhibition of differentiation of hematopoietic progenitors due to HOX over-expression

NUP98 – nuclear pore protein; ? recruits CBP/p300 activators

Acute Myelogenous Leukemia

Miscellaneous translocations

MLL – mixed lineage leukemia; embryonic dev and h'poiesis

CBP – cAMP response element binding protein binding prot

p300 – transcriptional co-activator, like CBP

Examples:

MLL/CBP

MLL/p300

Mechanism of oncogenesis unknown

Acute Myelogenous Leukemia

Blast Crisis CML → AML

Probably associated with acquisition of additional mutations

CML → AML/EVI1 translocation → AML

CMML → AML/ETO translocation → AML

(TEL/PDGFβR)

Acute Myelogenous Leukemia

Detection of Translocation in Leukemias

Fused translocated gens are transcribed in the tumor cells

Isolate the mRNA and do reverse transcription to obtain the cDNA (complementary DNA)

Do PCR on the cDNA using primers specific for the translocation, on either side of the fusion point

Detect the PCR amplified product, if it is present, on a polyacrylamide gel or by capillary electrophoresis