HEMATOLOJİK ONKOLOJİDE KLONALİTE KAVRAMI

PROF. DR. SALİH AKSU
HACETTEPE ÜNİVERSİTESİ
ERİŞKİN HEMATOLOJİ BD


STAR WARS
THE CLONE WARS


X-MEN
LE COMMENCEMENT
TWENTIETH CENTURY FOX MARVEL ENTERTAINMENT A BAD HAT HARRY / DONNERS' COMPANY
MATTHEW VAUGHN 'X-MEN : LE COMMENCEMENT' [X-MEN: FIRST CLASS]
JAMES McAVOY MICHAEL FASSBENDER ROSE BYRNE
JANUARY JONES OLIVER PLATT KEVIN BACON
SAMMY SHELDON JOHN DYKSTRA HENRY JACKMAN LEE SMITH, A.C.E. EDDIE HAMILTON CHRIS SEAGERS JOHN MATHESON, BSC
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LAUREN SHULER DONNER SIMON KINBERG GREGORY GOODMAN BRYAN SINGER
SHELDON TURNER BRYAN SINGER ASHLEY EDWARD MILLER & ZACK STENTZ JANE GOLDMAN & MATTHEW VAUGHN MATTHEW VAUGHN
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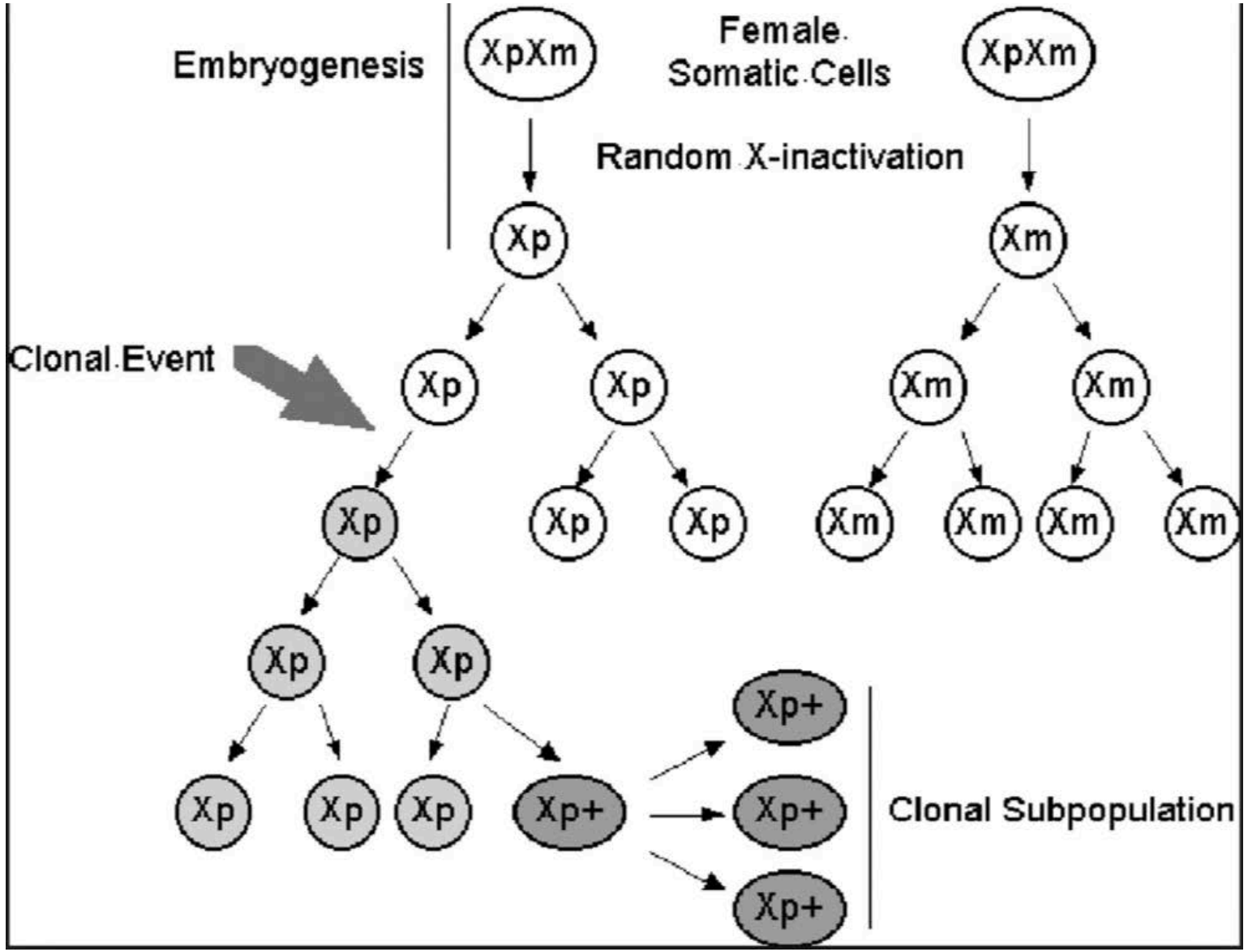
Kanserde Klonalite

- Kanserin temel özelliklerinden biri klonalite olmasıdır (tek hücreden çoğalma).
- Birçok tümörün tek hücreden türediği X kromozomu inaktivasyonu ile gösterilmiştir.
- Dişi hücrelerde X kromozomunun biri embriyo gelişimi sırasında rastlantısal olarak heterokromatine dönüşerek inaktifleşir.
- X kromozomundaki genlerden biri için heterozigot olan dişilerde farklı hücrelerde farklı alleler anlatım yapar.
- Normal doku farklı inaktif X kromozomlarını taşıyan hücrelerin karışımından oluştuğu için heterozigot dişilerin normal dokusunda iki allelin de anlatımı görülür.
- Tümör dokusunda ise heterozigot X kromozomu genlerinden sadece 1 tanesinin anlatım yaptığı görülür.
- Bu, X inaktivasyonunun tümör gelişmeden önce tamamlandığını ve söz konusu tümörü oluşturan hücrelerin hepsinin aynı hücreden türevlendiğini gösterir.


Embryogenesis
XpXm
Female
Somatic Cells
XpXm
Random X-inactivation
Xp
Xm
Clonal Event
Xp
Xp
Xm
Xm
Xp
Xp
Xp
Xm
Xm
Xm
Xm
Xp
Xp
Xp
Xp
Xp
Xp+
Xp+
Xp+
Clonal Subpopulation

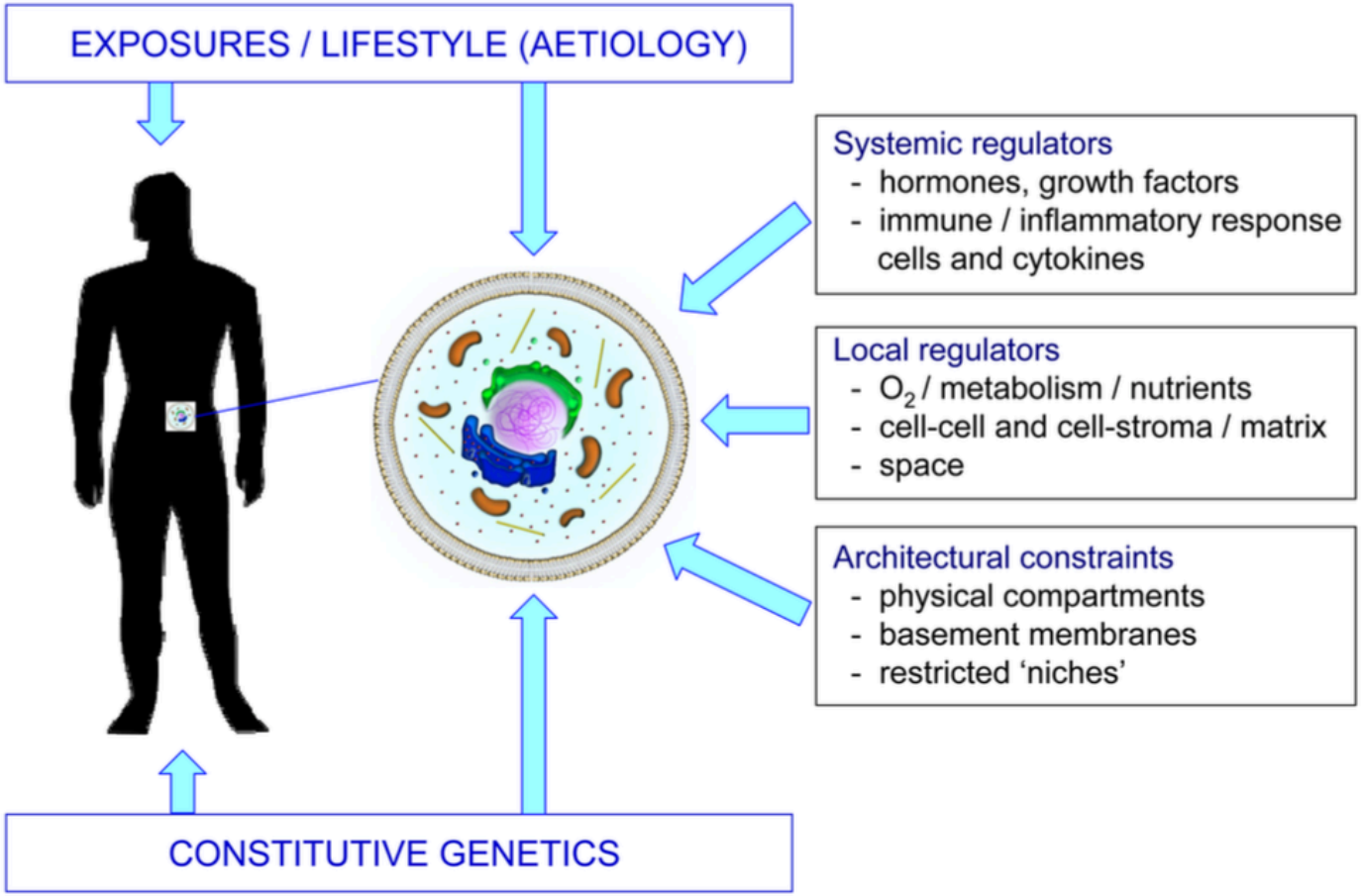
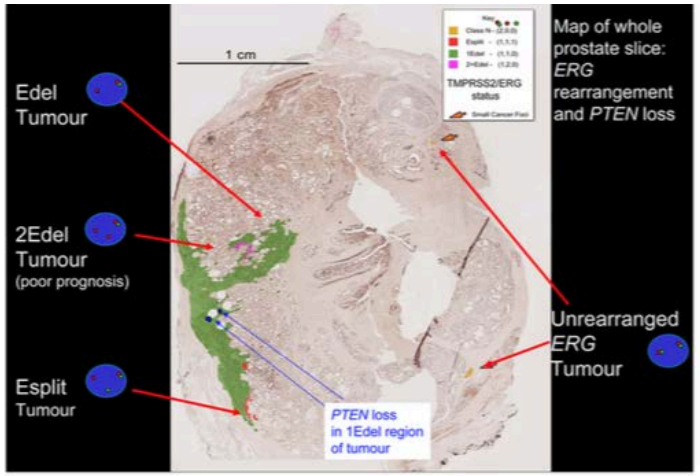
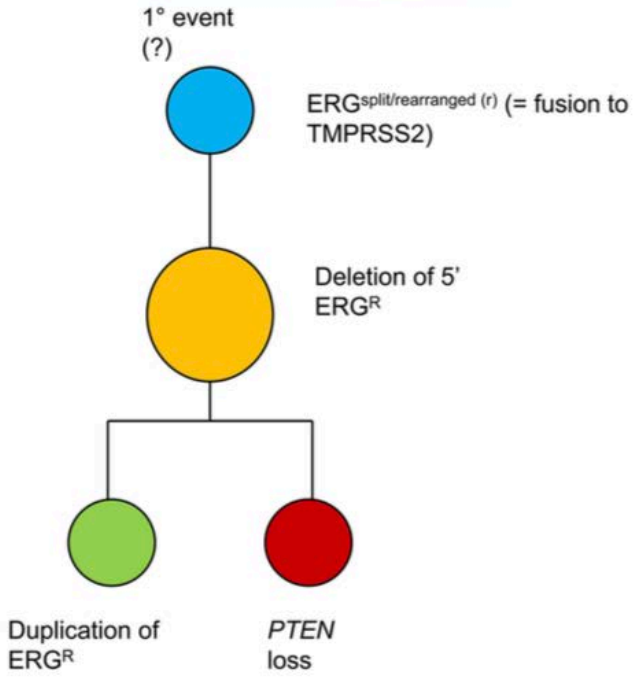


Figure 1.
The complexity of tissue ecosystems of cancer cells. Exposures, the constitutive genetics of the host cells, systemic regulators, local regulators and architectural constraints all impinge upon and constrain the evolution of somatic cells.





Nature. Author manuscript; available in PMC 2012 July 19.

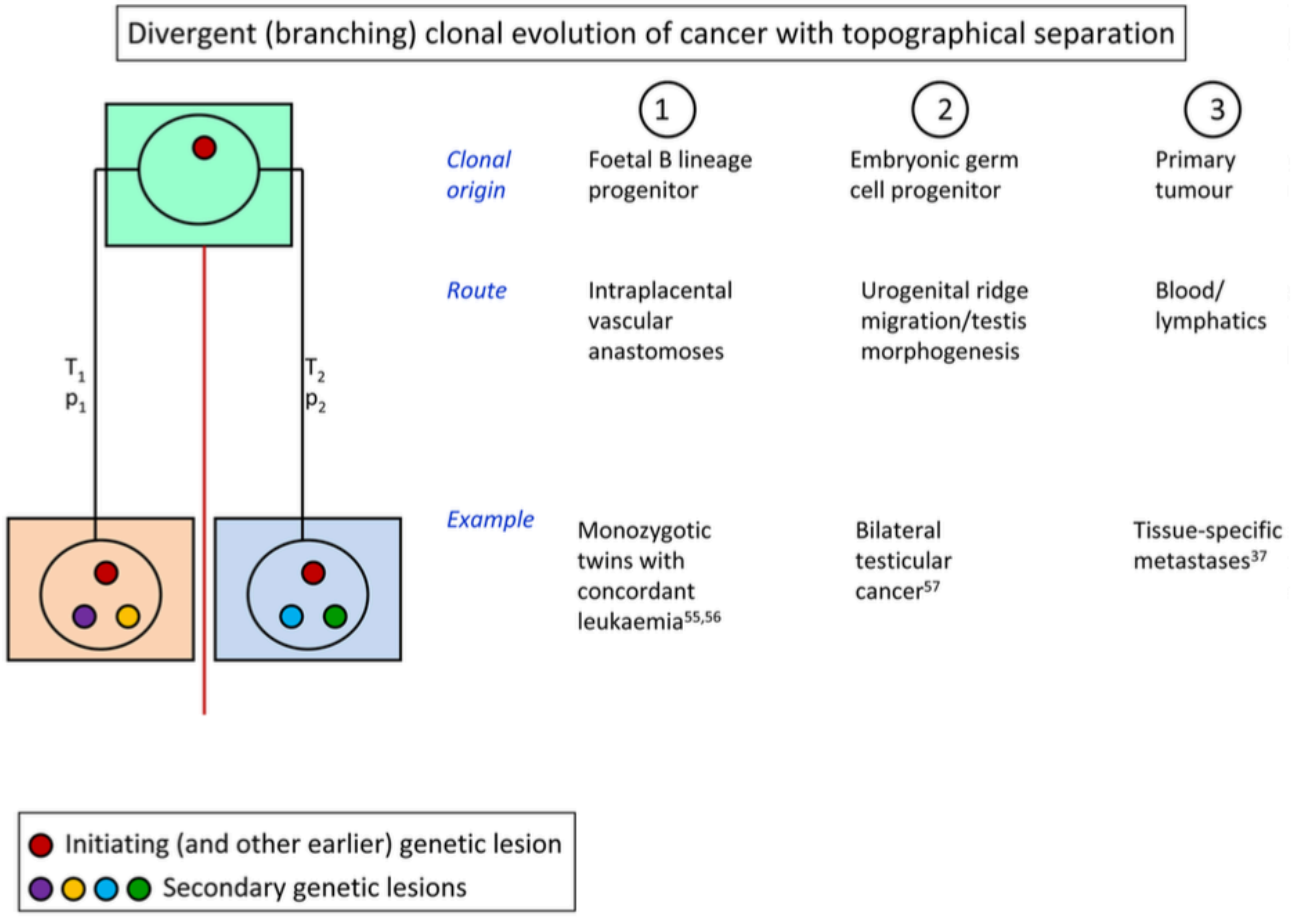


Figure 3.
Divergent (branching) clonal evolution of cancer with topographical separation.
In each example, a clonal (single cell) ancestry is indicated by shared acquired mutations, e.g. *ETV6-RUNX1* fusion for the leukaemias, *c-kit* mutation for the testicular cancers. The time at which the two subclones evolve (T_1, T_2) can be temporarily synchronous or develop several years apart[37,55–57]. The probabilities of sub-clones emerging as shown are independent and different (p_1, p_2). In most cases (90% for monozygotic twins), only one twin develops overt leukaemia. The penetrance of bilateral testicular cancer having a common origin[57] is unknown.

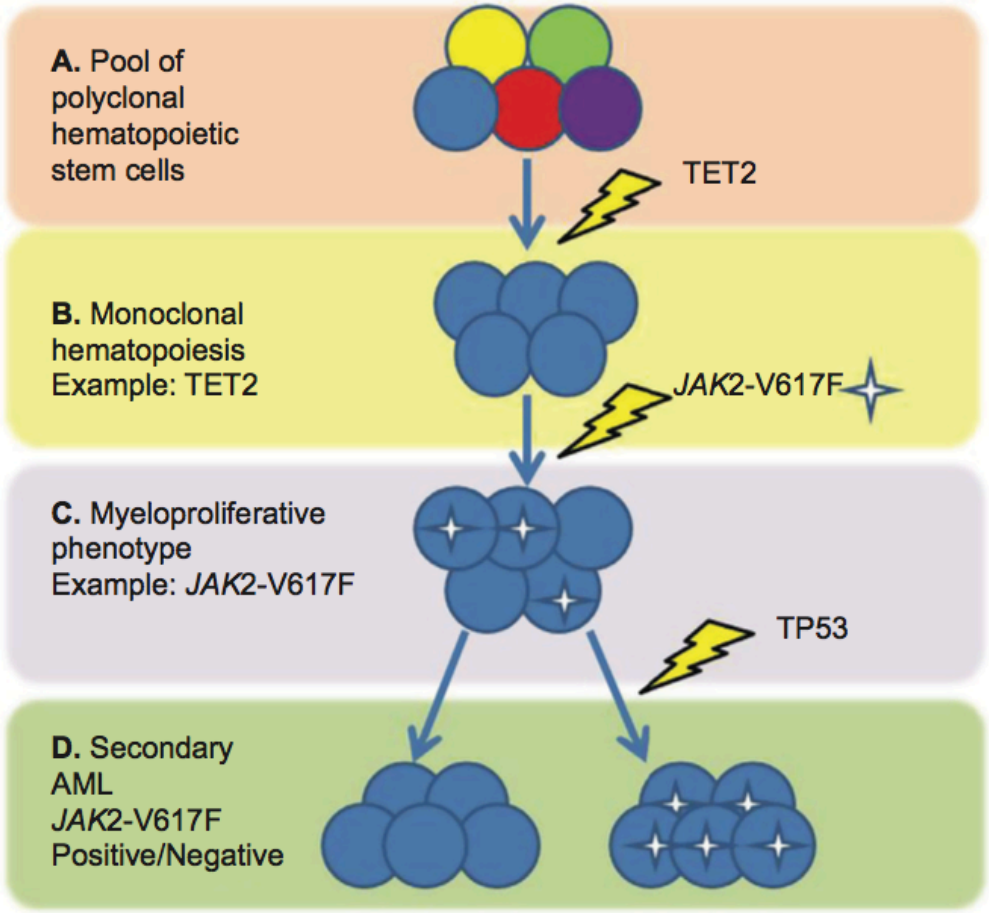
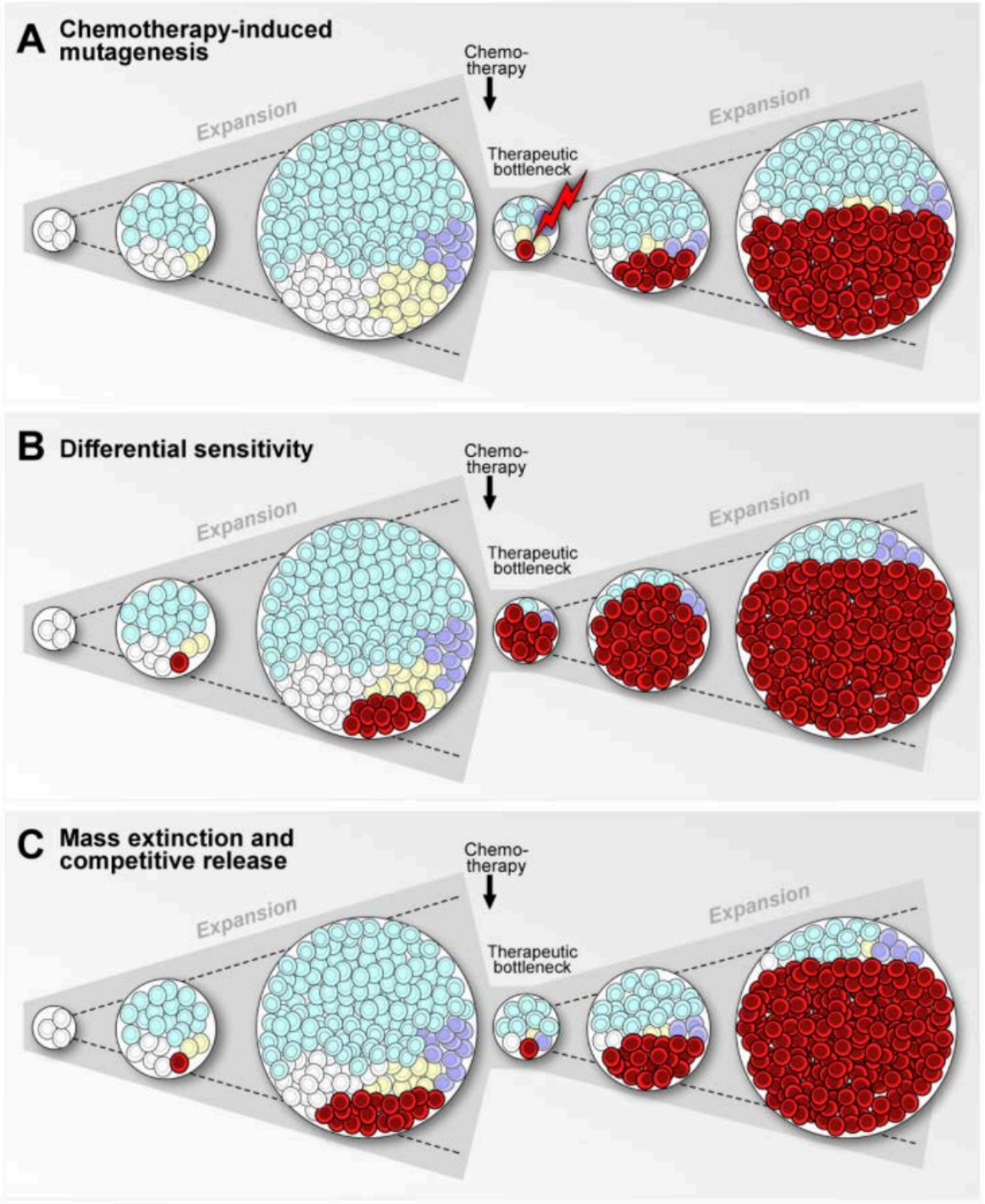


Figure 1 Model of clonal evolution of MPNs.
(A) Pool of polyclonal hematopoietic cells in bone marrow. (B) These cells can acquire mutations involved in disease initiation such as *TET2*. (C) Acquisition of a driver mutation such as *JAK2*-V617F leads to a myeloproliferative phenotype. (D) This can then progress to secondary AML through the acquisition of various leukemic mutations such as TP53.


A Chemotherapy-induced mutagenesis
Chemo-therapy
Expansion
Therapeutic bottleneck
Expansion
B Differential sensitivity
Chemo-therapy
Expansion
Therapeutic bottleneck
Expansion
C Mass extinction and competitive release
Chemo-therapy
Expansion
Therapeutic bottleneck
Expansion

Tanı Açısından Klonalite

- Bildiğimiz mutasyonlar (philedelphia gibi)
- Minimal rezidüel hastalık bakılması
- IgV ve TCR ile klonalite bakılması
  - Southern Blot analizi
  - PCR
  - Kalitatif PCR
  - NGS (yeni jenerasyon sekanslama)

Southern Bot

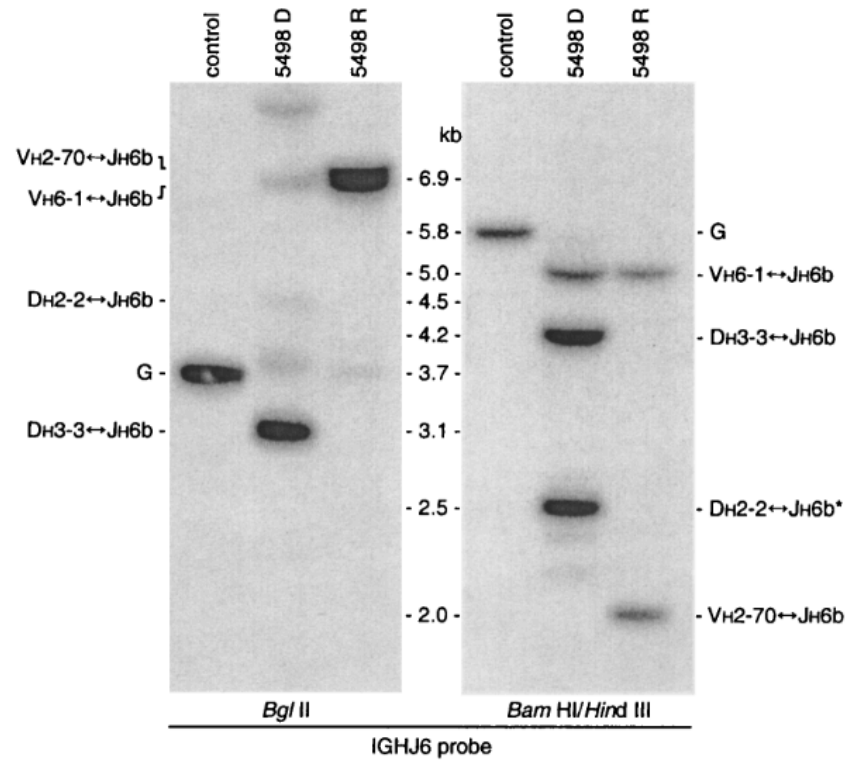
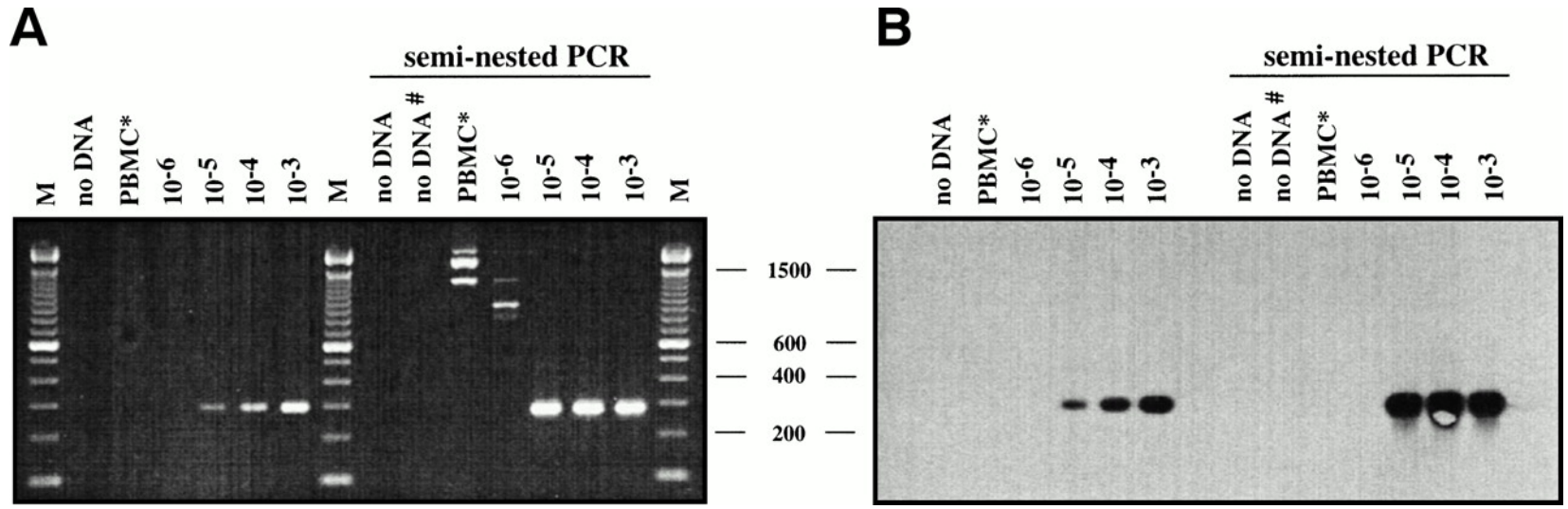


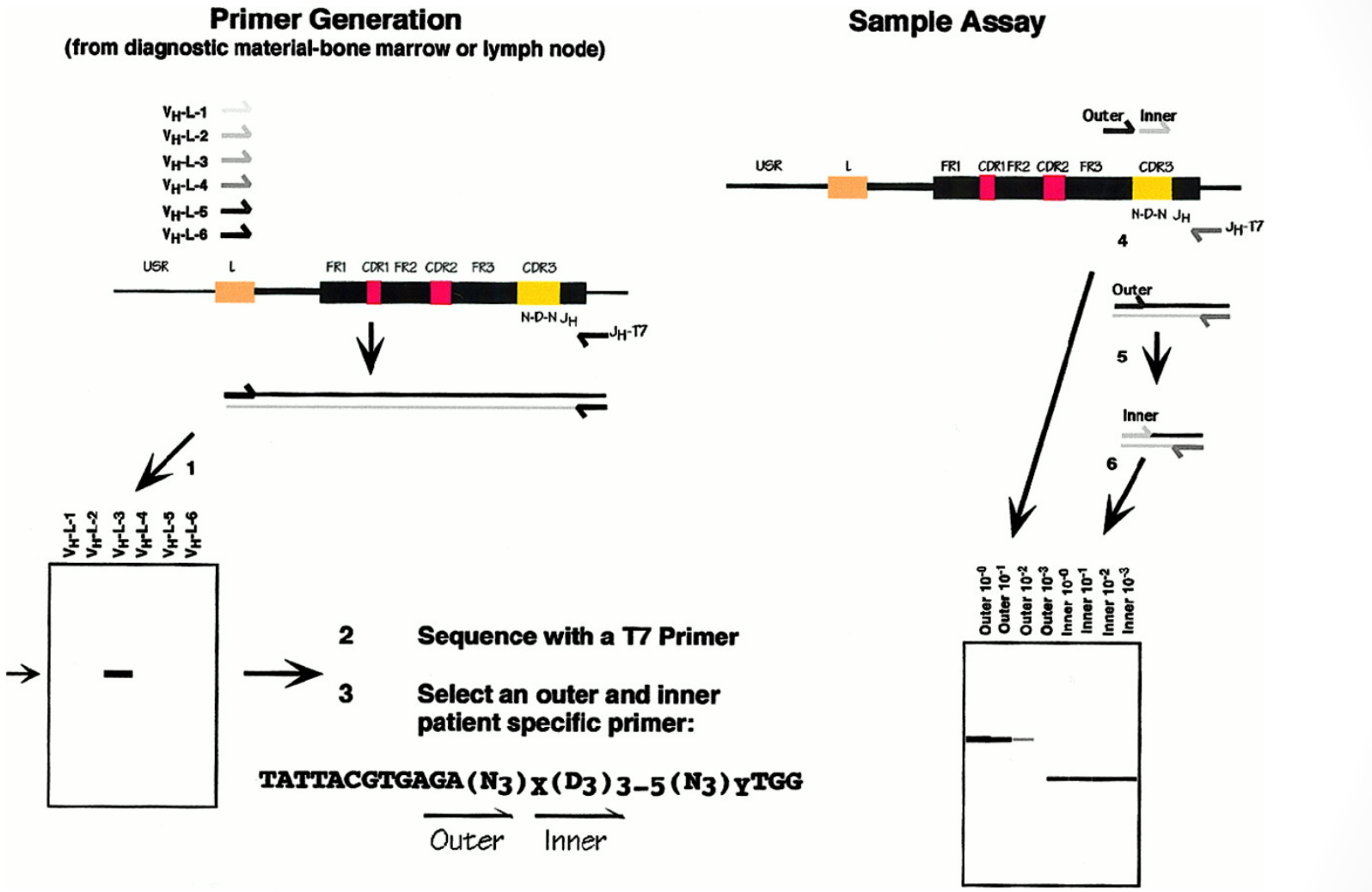
Fig. 1. Southern blot analysis of the *IGH* gene in patient 5498. Control DNA and patient's DNA isolated from diagnosis (D) and relapse (R) BM samples were digested with *Bgl*II and *Bam*HI/*Hin*dIII restriction enzymes, size separated, and blotted onto nylon membrane filters, which were hybridized with the ^{32}P-labeled IGHJ6 probe [17]. At diagnosis multiple bands of different densities were found, reflecting *IGH* oligoclonality. In contrast, the relapse sample displayed a monoclonal pattern with biallelic *IGH* gene rearrangements. Based on the germline sequence and the restriction map of the *IGH* locus [30], rearrangements found by direct sequencing were assigned to corresponding bands on the Southern blot. The asterisk indicates the comigration of a faint rearranged band corresponding to the incomplete Dh2-2↔Dh6-13↔Jh6b rearrangement with a major non-identified rearranged band in the *Bam*HI/*Hin*dIII digest.

Estimation of PCR and Southern blot sensitivity for detection of L428 cells admixed with normal PBMCs. (A and B) DNAs isolated from serial dilutions of L428 cells in normal PBMCs were amplified using VH5FRI and L428rev1 (296 bp)/rev2 primers (287 bp).



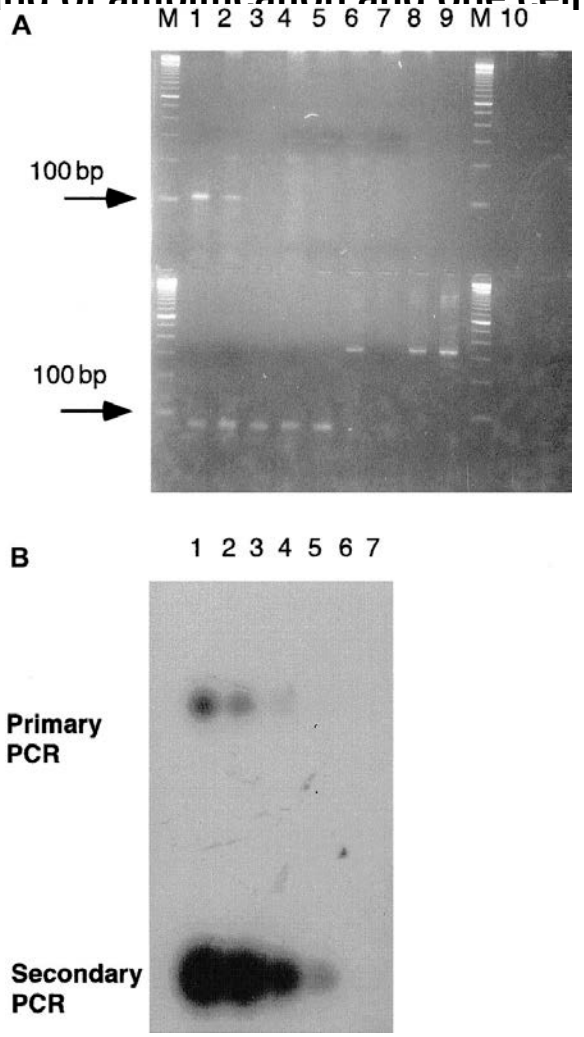
Martina Vockerodt et al. Blood 1998;92:2899-2907

Clonotypic PCR technique.The CDR-III region is formed by the sequential D-JH and V-JH rearrangements in the pre-B cell.



Ariela Noy et al. Blood 2001;97:1929-1936

Sensitivity of PCR versus hybridization of an internal probe to the first round product.(A) Primary and semi-nested PCR demonstrating a sensitivity of one cell in 102 polyclonal cells after the primary round of amplification and one cell in 105 after the se...



Ariela Noy et al. Blood 2001;97:1929-1936

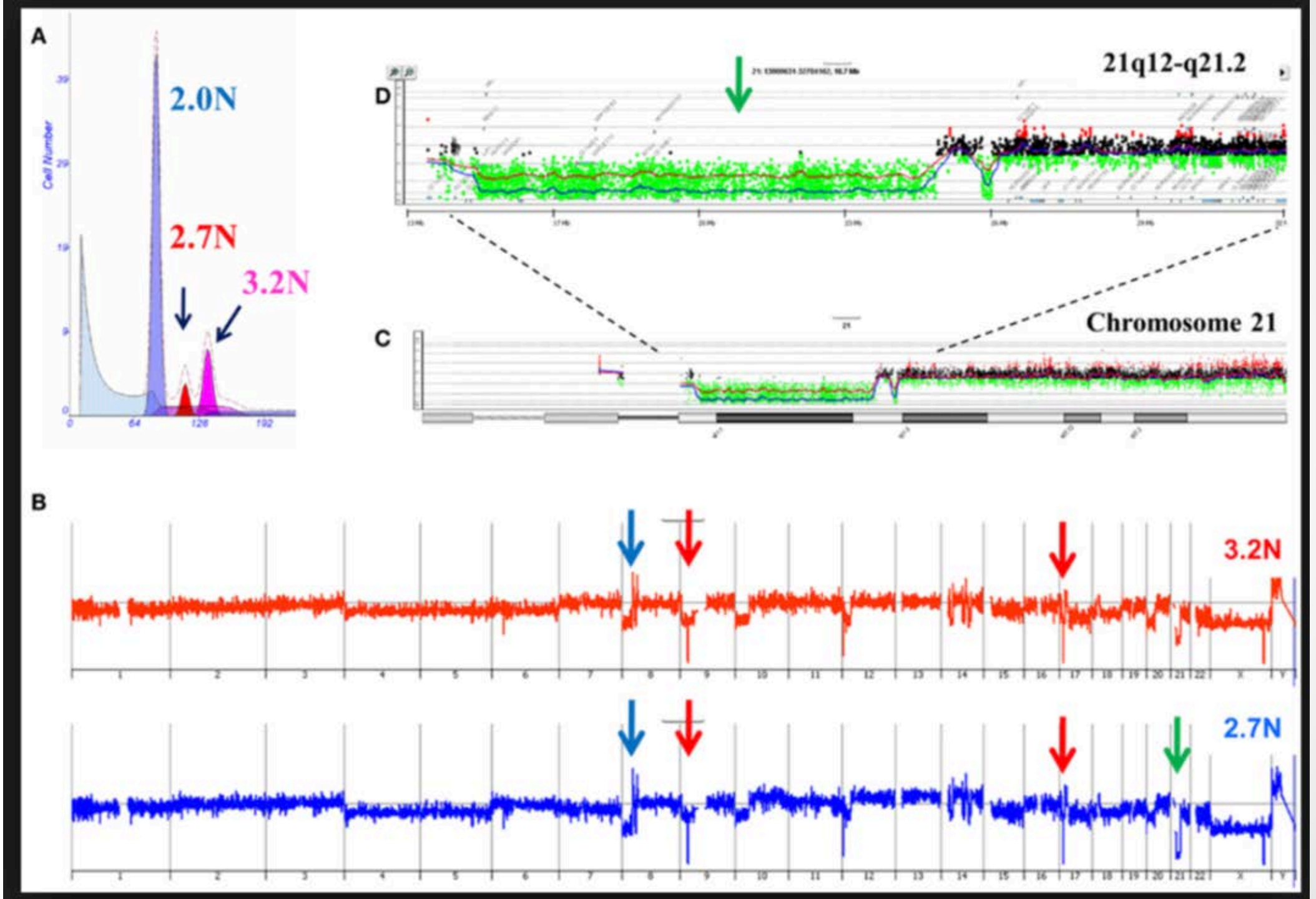

A
2.0N
2.7N
3.2N
Cell Number
0
64
128
192
D
21q12-q21.2
C
Chromosome 21
B
3.2N
2.7N

Table 1. A comparison of different techniques for clonality detection.

	Time	Material load	Sensitivity	Specificity
Southern blotting	Days	10,000–20,000 ng	Low/ intermediate	Very high
Polymerase chain reaction	Hours	100–500 ng	Very high	high
Next-generation sequencing	Hours	20 ng	High?*	High?*

*To be validated.

Tedavide Önemi

- *AML'de FLT3* (*FLT3*-ITD) tandem duplikasyonu veya, *MLL* (*MLL*-PTD) parsiyel duplikasyonu ya da*ASXL1* ve *PHF6* mutasyounu kötü prognozla
- *AML'de CEBPA* and *IDH2 mutasyonları kötü prognozla ilgili*
- *AML'de DNMT3A, NPM1* mutasyonları ya da *MLL translokasyonunda Daunorubicin yüksek doz olumlu etkisi var iken bunların olmadığı hastalarda aynı şekilde etkili değil*
- *Gelecekte daha iyi ve geniş popülasyona uygulanabilecek sekanslama teknikleri ile belki de hastaya özel tedaviler belirlenecek*