Sentetik antikorlar
Peptidler
Gösterim sistemleri

14

chnikoff

Milstein

GENETECH

lls

Uretici - 2

Aracı

Poliklonal antikorlar/antiserum

- tanı
- tedavi

Paul Ehrlich

Elie Metchnikoff

Georges Köhler

César Milstein

'75

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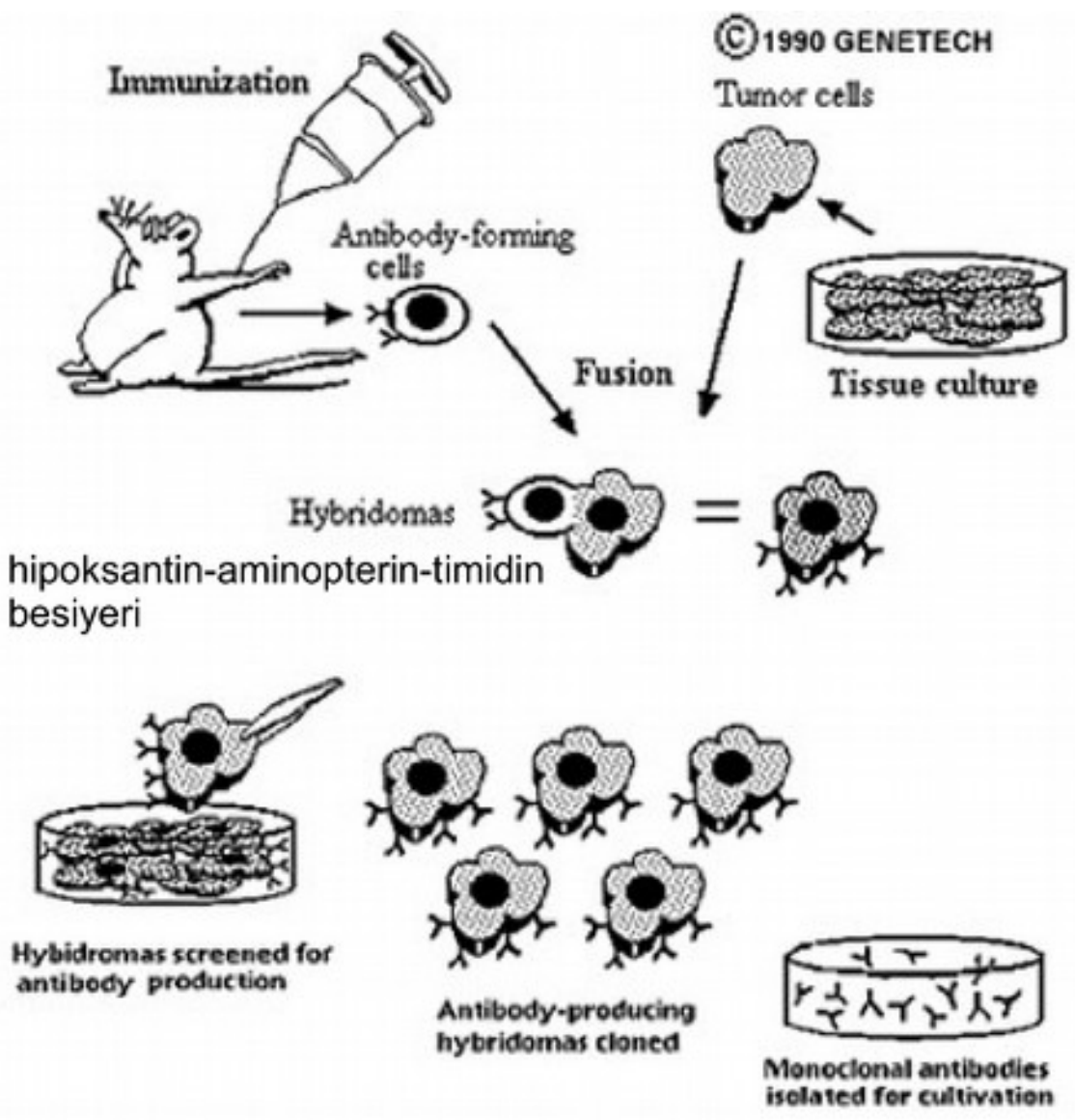
16

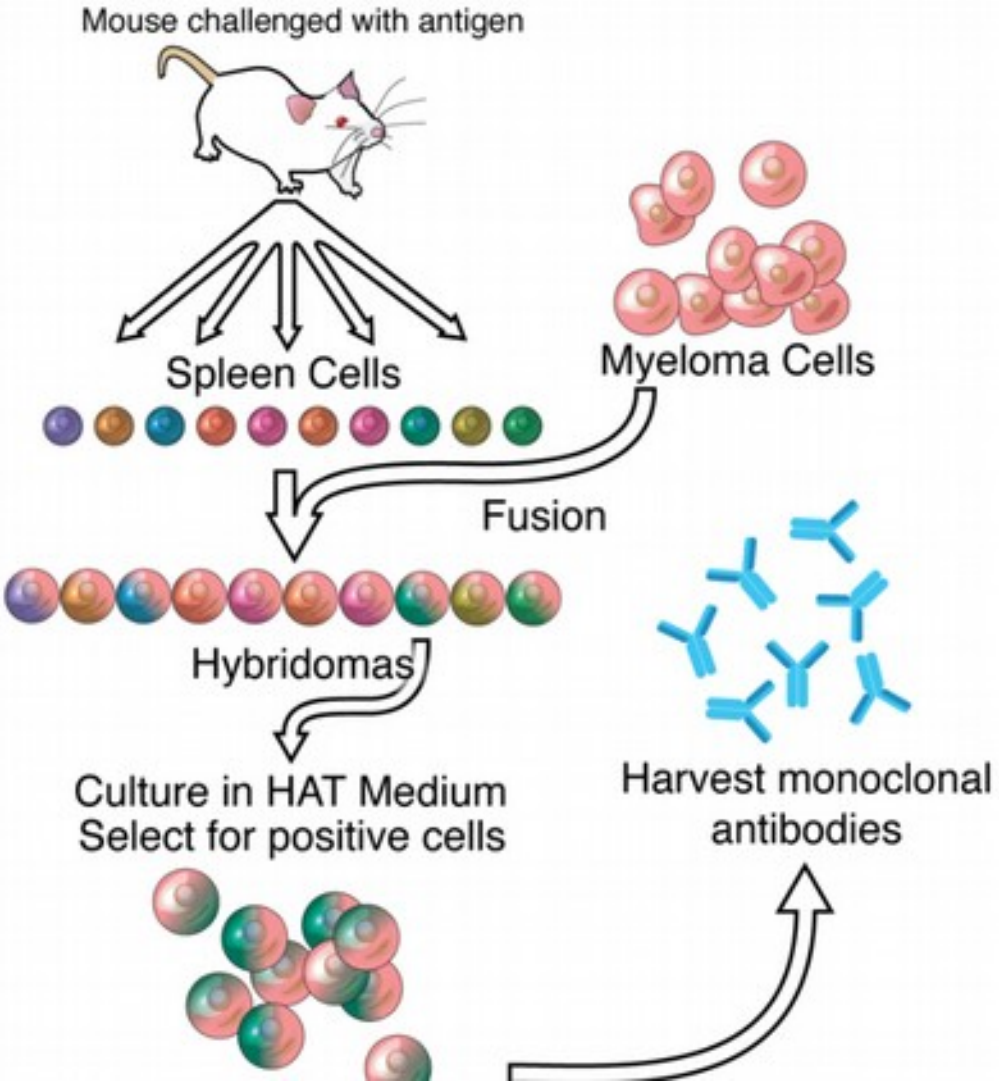
< biyoloji!

- tedavi



edüktaz inhibitörü
ezini inhibe eder

creleri HGPRT(-/-)



Hipoksantin: pürin türevi

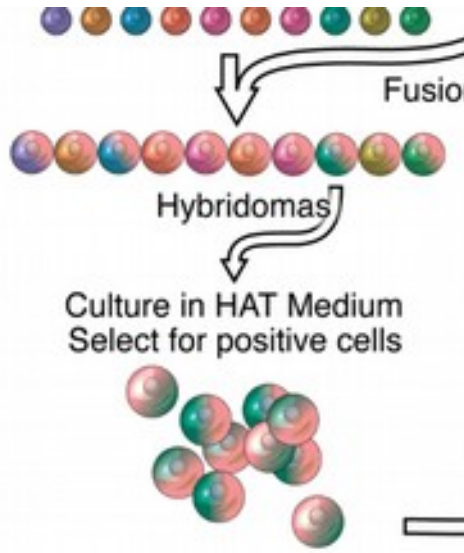
Aminopterin: dihidrofolat redüktaz inhibitörü
-> de novo DNA sentezini inhibe eder

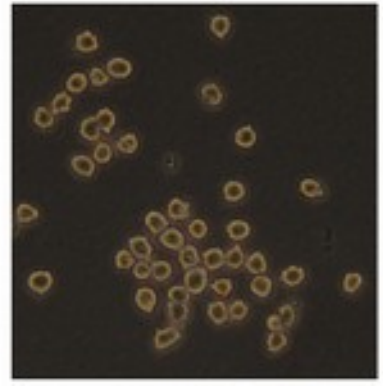
Timidin: deoksinükleosid

F0 myeloma hücreleri HGPRT(-/-)
- "salvage pathway"
ile nükleotid sentezleyemez
- *de novo* sentez blokajı

Dalaktan gelen **myelositler**
bu substraları kullarak
DNA sentezleyebilir

hipoksantin-guanin fosforiboziltransferaz (HGPRT)





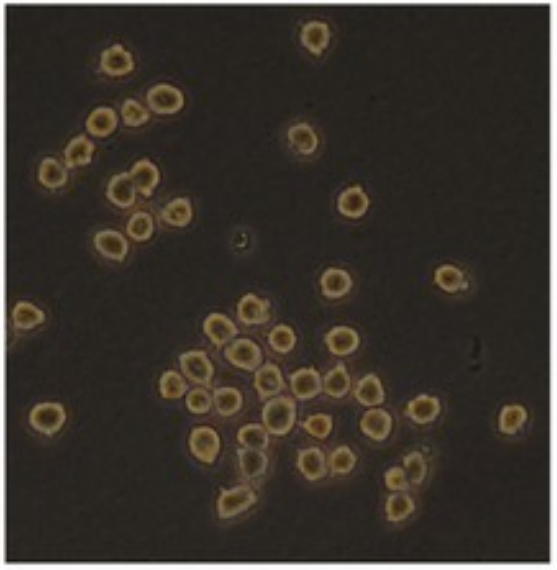
HAT besiyeri ile 14 gün seçim...

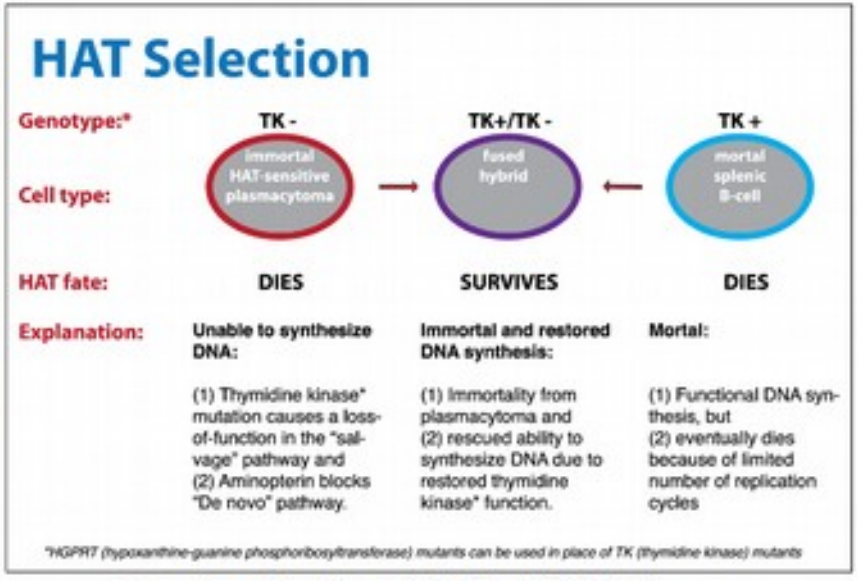
GPRT(-/-)

mez

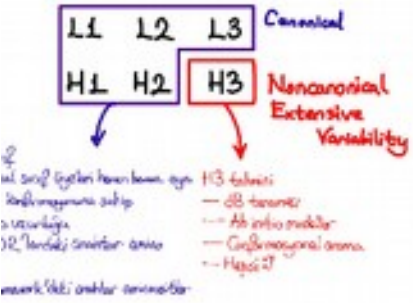
(HGPRT)

çim...



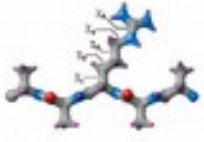


https://en.wikipedia.org/wiki/File:HAT_Selection.png



CDR-L3
Sınıf 1+2 → 9 üye
Sınıf 3 → 8 üye
Benzer hairpin loop'lardan oluşuyor
Farklılıklar Prolin pozisyonundan kaynaklanıyor
Sınıf 4 → 7 üye
Sınıf 5 → 8 üye

CDR-H3
Canonical sınıflar yerine konmuş yan zincirler bulunuyor



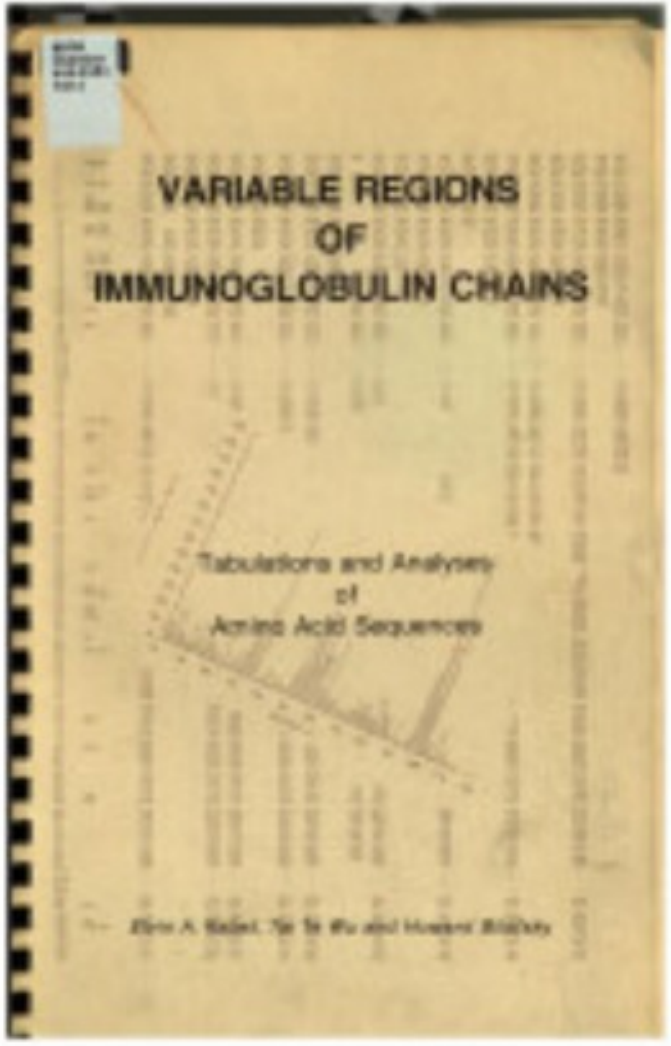
CR Variable Regions

Yaşamın fiziksel ve kimyasal esasları

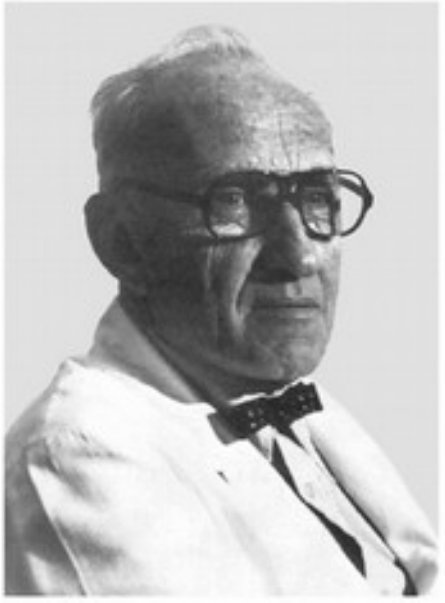
1912'ler için çok devrimsel...

'12 -> '80

Biyoteknoloji çağında antikorları da diğer peptidler gibi rekombinant DNA teknolojisi kullanarak üretebilir miyiz?

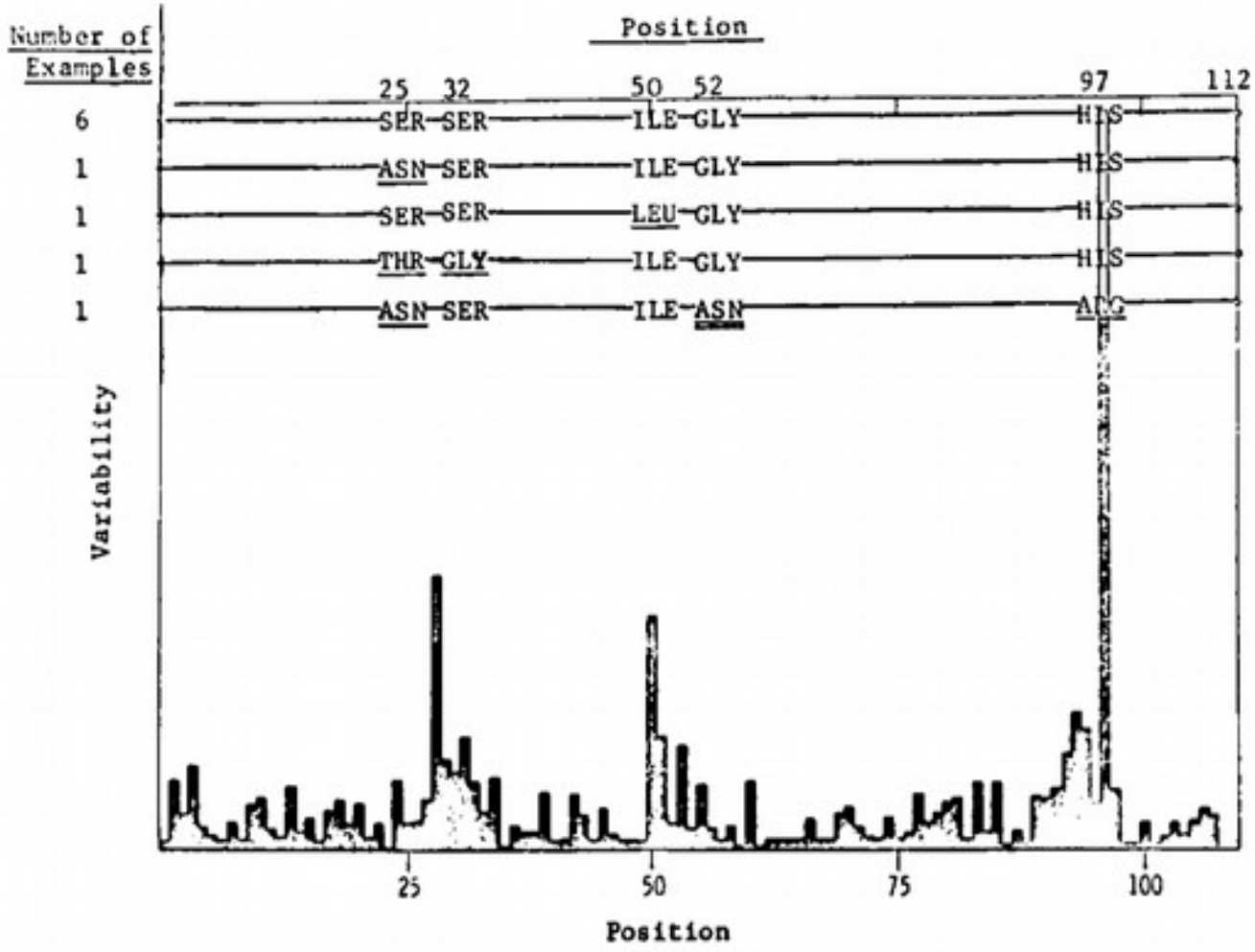


Variable Regions of Immunoglobulin Chains: Tabulations and Analyses of Amino Acid Sequences

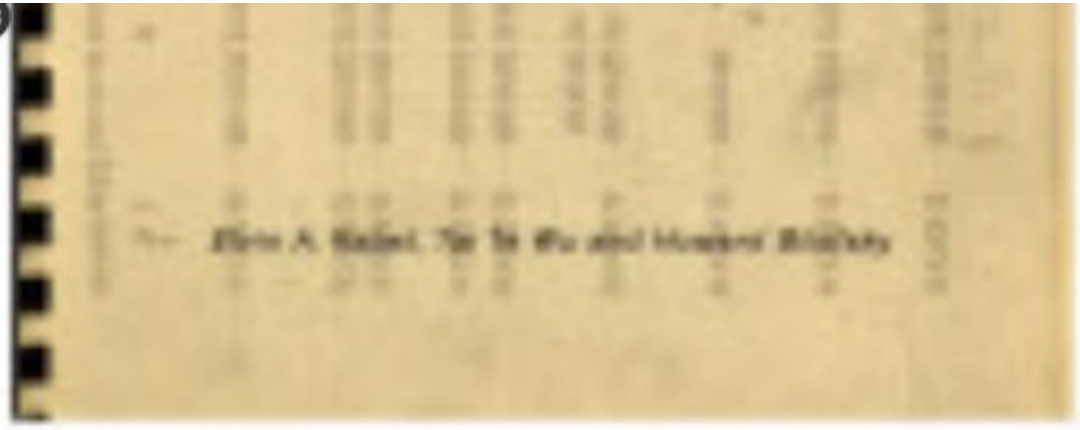


Elvin A. Kabat

ici - 1



Kabat EA, Wu TT. Attempts to Locate Complementarity-Determining Residues in the Variable Positions of Light and Heavy Chains *. Annals of the New York Academy of Sciences 1971;190(1):382–393.



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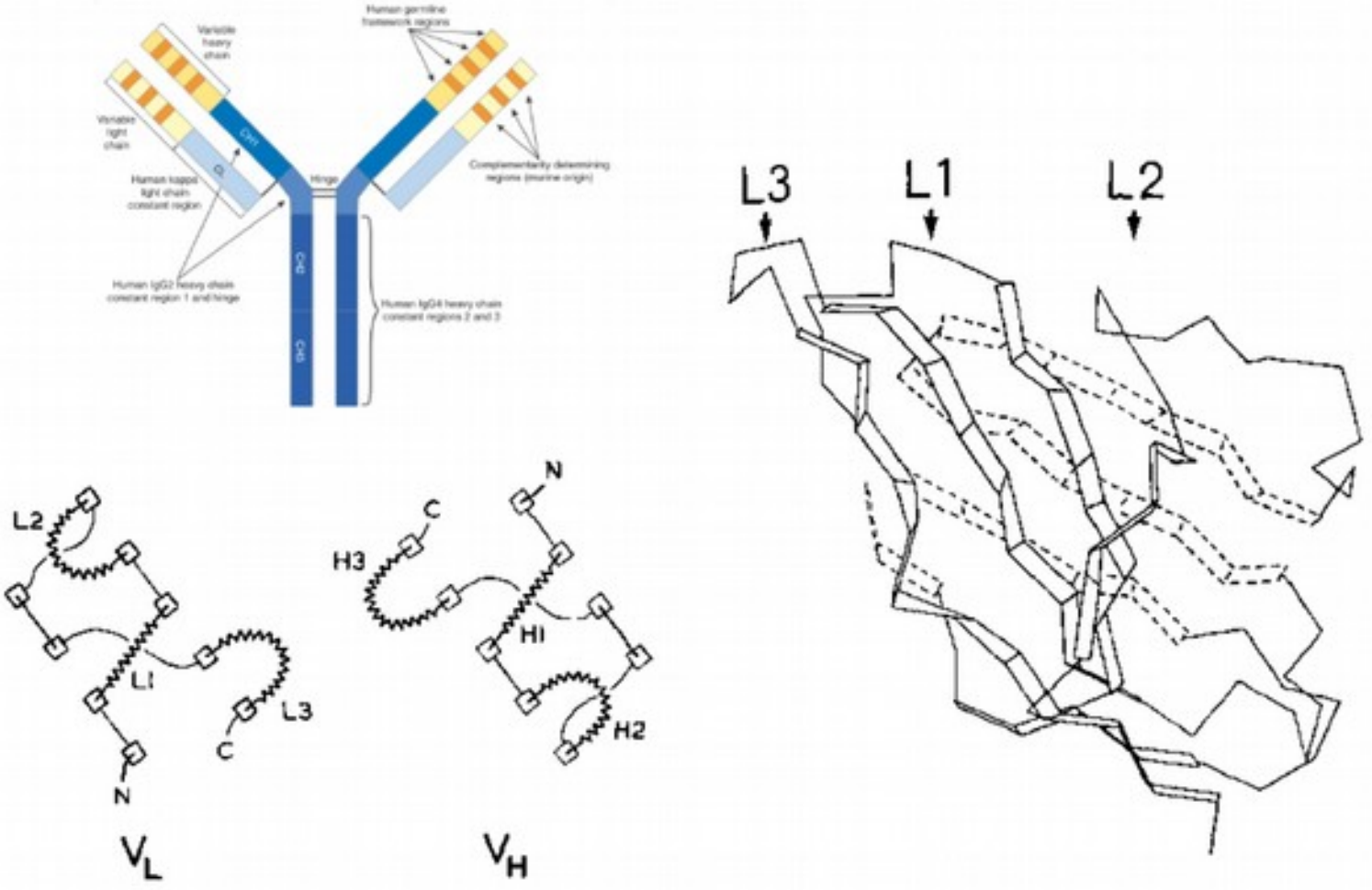
Annual Reviews

Elvin A Kabat

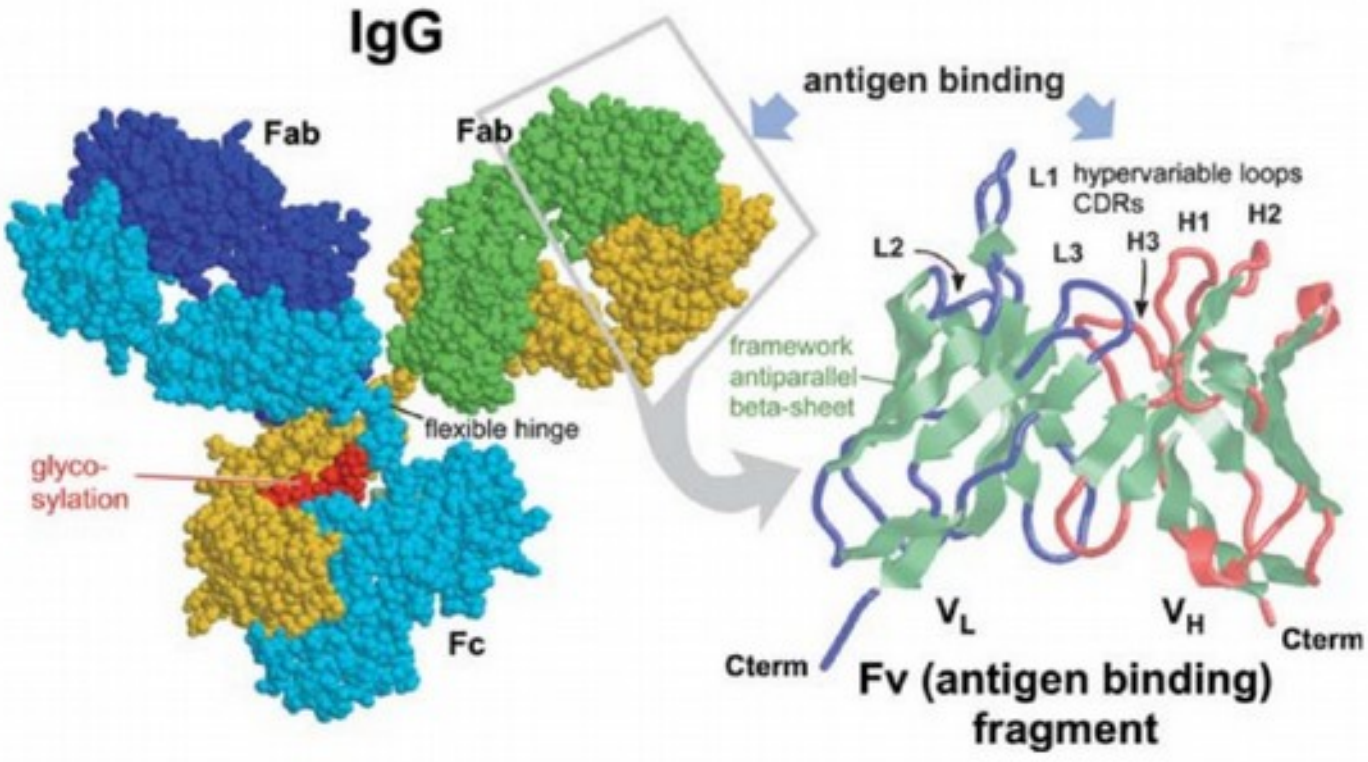
"desktop bioinformatics"

Number of Examples	Position 25	32	50	52	97	112
6	SER	SER	ILE	GLY	HIS	
1	ASN	SER	ILE	GLY	HIS	



Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. J. Mol. Biol. 1987 Aug;196(4):901–917.

Bağlanmanın anatomisi...

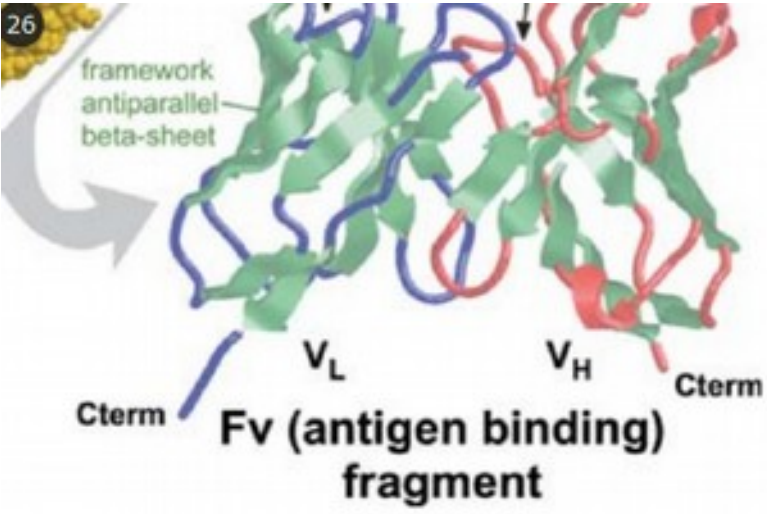


L1
H1

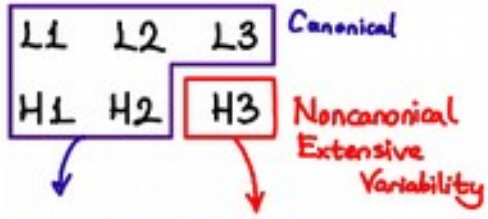
10 sınıf
Canonical sınıf üyeleri benzer
omurga konformasyonuna sahip
— loop uzunluğu
— CDR'lardaki önemli
asitler
— Framework'deki önemli

CDR-L1
5 belirgin κ
4 belirgin olmayan λ
1 belirgin olmayan κ

CDR-L2
çoğunluk bir yapıya uyuyor



Fv (antigen binding) fragment



10 sınıf
Canonical sınıf üyeleri hemen hemen aynı omurga konformasyonuna sahip
— loop uzunluğu
— CDR'lerdeki anahtar amino asitler
— Framework'deki anahtar aminoasitler

H3 tahmini
— dB taraması
— Ab initio modeller
— Conformasyonel arama
— Hepsi :)

Yaşamın
kimyasal

1912'ler için

CDR-L1
5 belirgin κ
4 belirgin olmayan λ
1 belirgin olmayan κ
Toplam 10 sınıf

CDR-L2
Çoğunluk bir yapıya uyuyor

CDR-L3
Sınıf 1+2 → 9 üye
Sınıf 3 → 8 üye
Benzer hairpin loop'lardan oluşuyor
Farklılıklar Prolinin pozisyonundan kaynaklanıyor
Sınıf 4 → 7 üye
Sınıf 5 → 8 üye

Recombination Signal Sequences

Heptamer sequence: Nonamer sequence:

5' CACAGTG 3' 5' ACAAAAACC 3'
3' GTGTCAC 5' 3' TGTTTTTTGG 5'

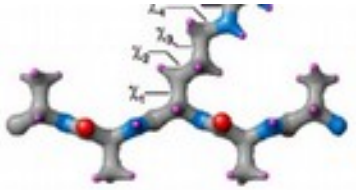
Highly conserved base pairs

CDR-H1
Sınıf 1 → 10 üye
Sınıf 2 → ?
Sınıf 3 → 11 üye
Sınıf 4 → 12 üye

CDR-H2
Sınıf 1 → 9 üye
Sınıf 2 → 10 üye
Sınıf 3 → 10 üye
Sınıf 4 → 12 üye

CDR-H3
Canonical sınıflar yerine korunmuş yan zincirler bulunuyor

Biyotek



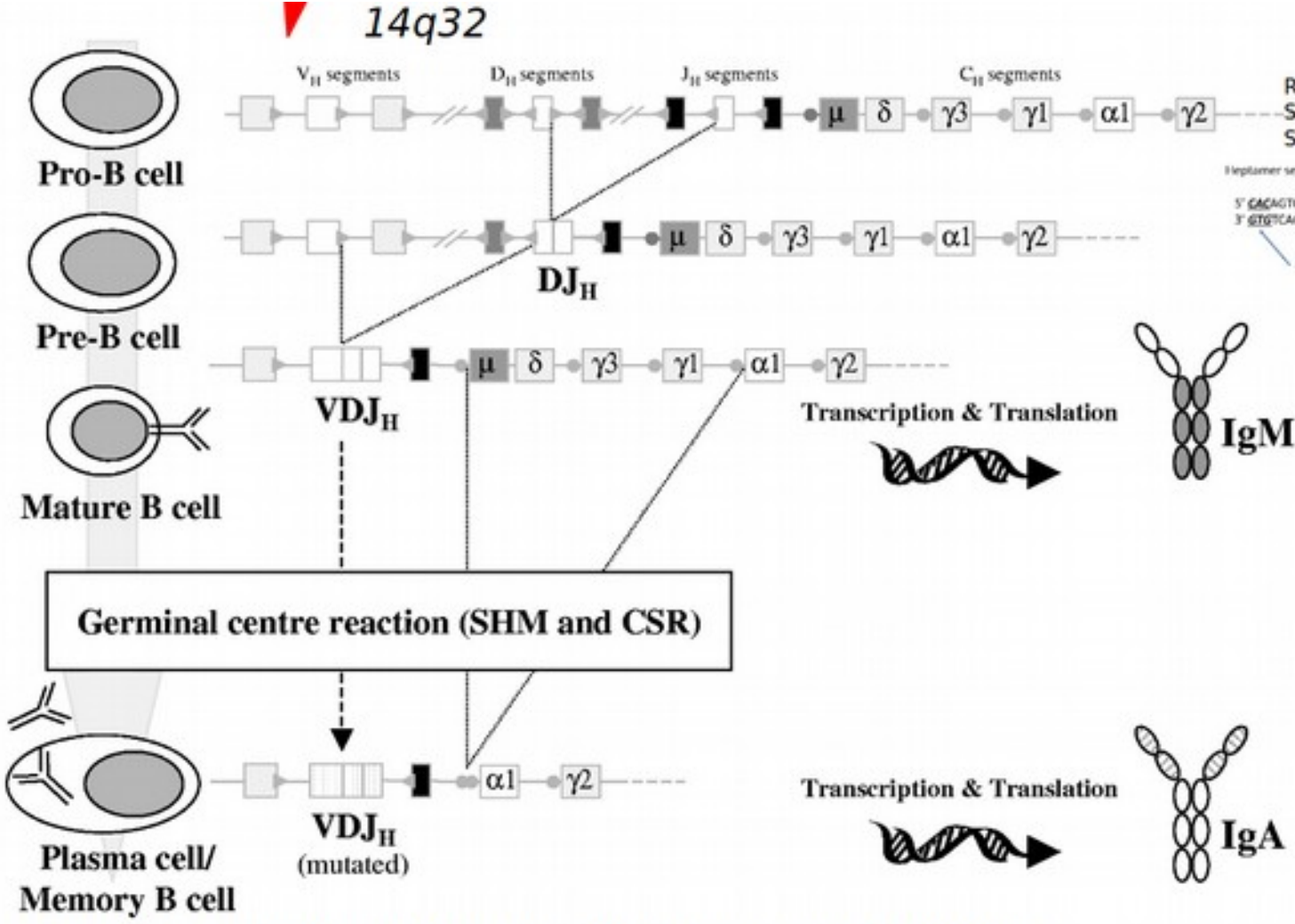
FRs and CDRs of Antibody and TCR Variable Regions

FR or CDR	V_L	V_H	V_α	V_β	V_γ	V_δ
FR1	1–23	1–22	1–22	1–23	1–21	1–22
CDR1	24–34	31–35B	23–33	24–33	22–34	23–34A
FR2	35–49	36–49	34–47	34–49	35–49	35–49
CDR2	50–56	50–65	48–56	50–56	50–59	50–57
FR3	57–88	66–91	57–92	57–94	60–95	58–89
CDR3	89–97	95–102	93–105	95–107	96–107	90–105
FR4	98–107	103–113	106–116	108–116A	108–116C	106–116

Soru: Bir memelide kaç farklı genom bulunur?

10^11 - 10^12

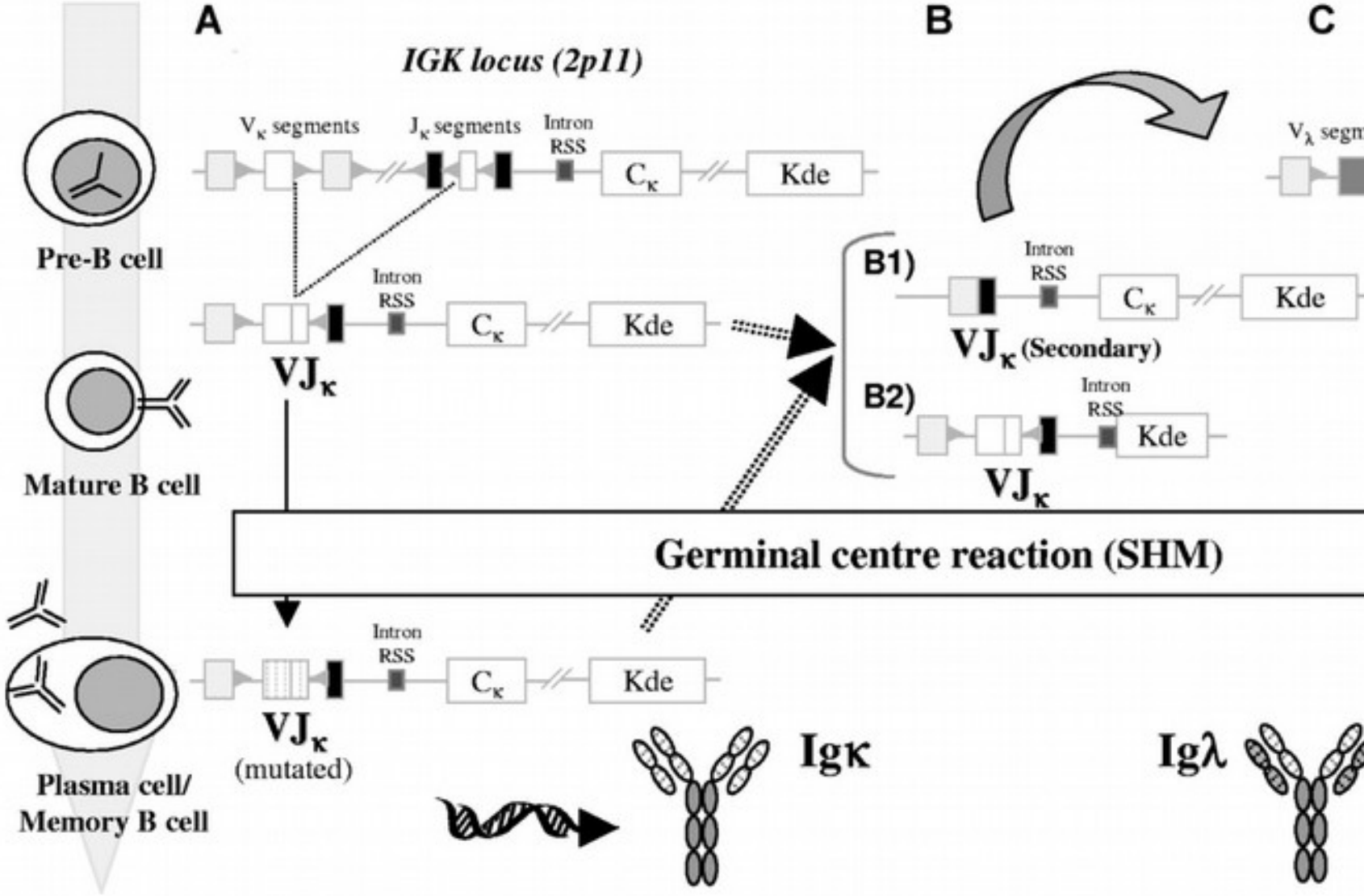
Ama nasıl?

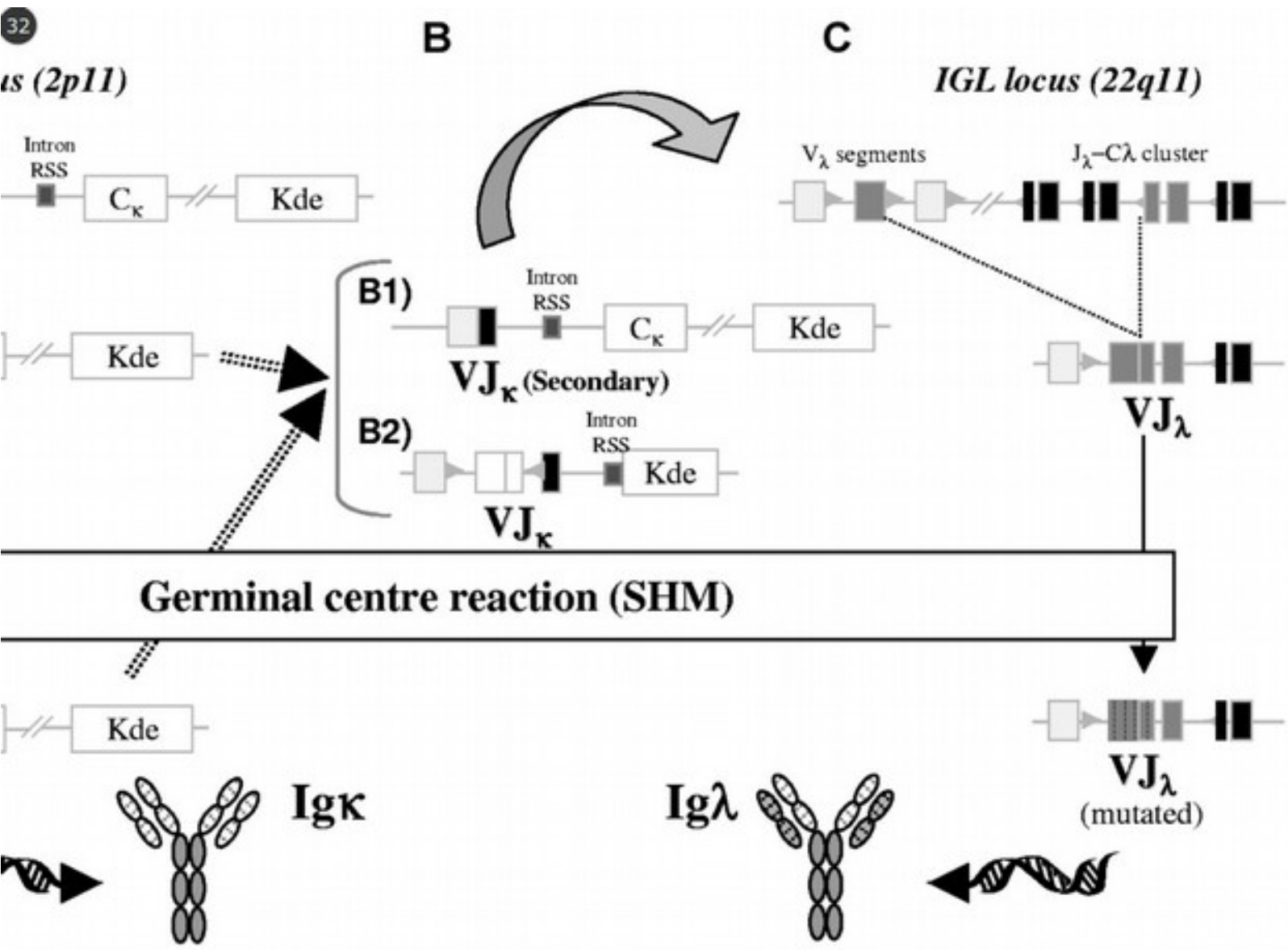


Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma
David González, Mirjam van der Burg, Ramón García-Sanz, James A. Fenton, Anton W. Langerak, Marcos González, Jacques J. M. van Dongen, Jesus F. San Miguel and Gareth J. Morgan, Blood 2007 110:3112-3121

FRs and CDRs of An

FR or CDR	V_L
FR1	1–23
CDR1	24–34
FR2	35–49
CDR2	50–56
FR3	57–88
CDR3	89–97
FR4	98–107


A
B
C
IGK locus (2p11)
Vκ segments
Jκ segments
Intron RSS
Cκ
Kde
Pre-B cell
VJκ
Mature B cell
B1)
VJκ (Secondary)
B2)
VJκ
Germinal centre reaction (SHM)
Plasma cell/ Memory B cell
VJκ (mutated)
Igκ
Igλ



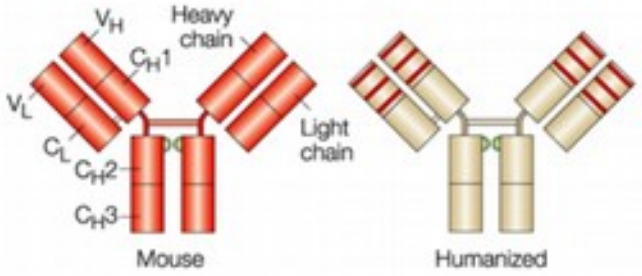
VDJ recombina

- recombination
- terminal deoxy
- Artemis nuclea
  oining (NHE
- DNA-depende
- X-ray repair cr
- DNA ligase IV
- Cernunnos or
  end-joining
- Paralog of XRC
- DNA polymera

VDJ recombinase

- recombination activating genes 1 and 2 (RAG)
- terminal deoxynucleotidyl transferase (TdT)
- Artemis nuclease (ubiquitous non-homologous end j oining (NHEJ) pathway for DNA repair)
- DNA-dependent protein kinase (DNA-PK)
- X-ray repair cross-complementing protein 4 (XRCC4)
- DNA ligase IV
- Cernunnos or XRCC4-like factor [XLF]: non-homologous end-joining factor 1
- Paralog of XRCC4 and XLF (PAXX)
- DNA polymerases λ and μ

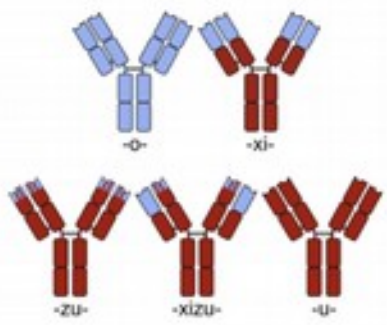
"Magic Bullet"



Nature Reviews | Cancer

İmmünojenisite

Biyolojik fonksiyon



Hümanizasyon derecelerine göre isimlendirme

ilk önceliğimiz: **HÜMANİZASYON**

Tüm bu
konfor

O hald
gereks

35

ɔjenisite

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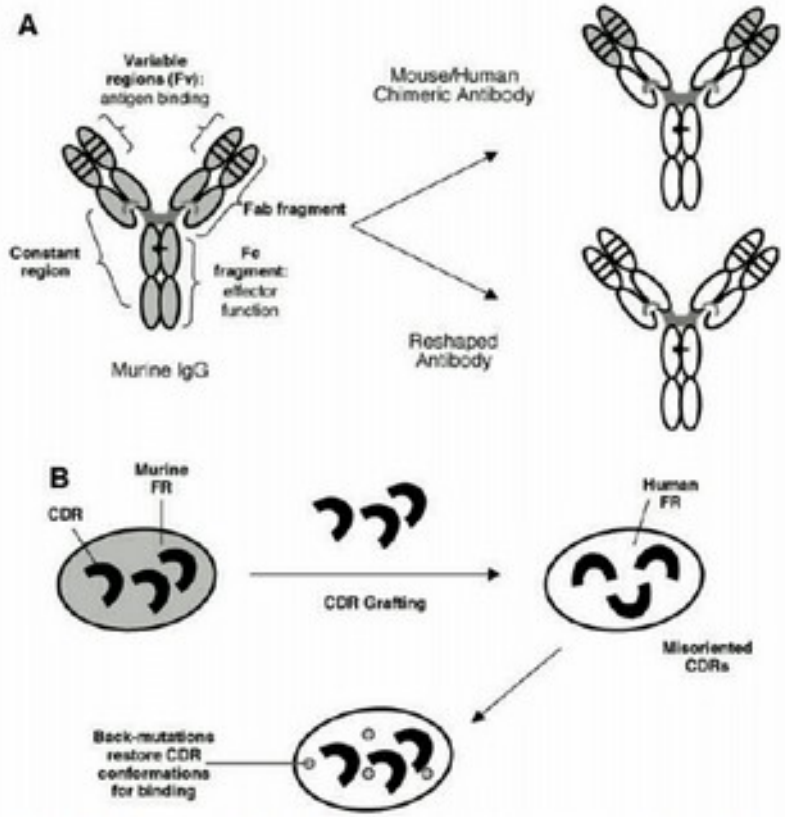
siyon



Tüm bu manipülasyonlar, "orijinal" konformasyonu bozuyor :/

1

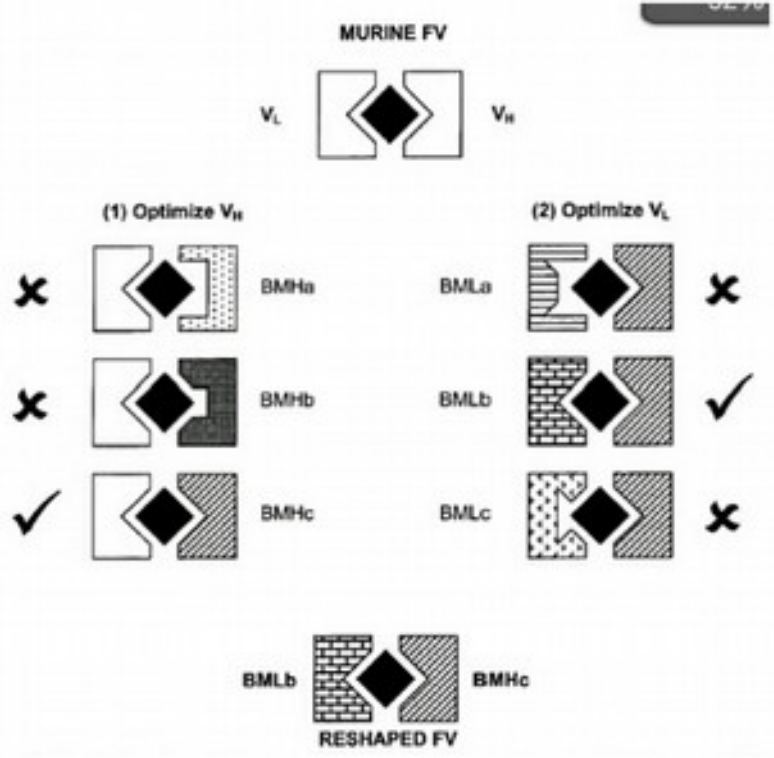
Brand
Humulin
Humatrope
Genotropin
Saizen
Nutropin/Protropin
Intron A &
Avonex
Betaseron/Betaferon
Procrit/Eprex
Epogen
NeoRecormon
Kogenate
NovoSeven
Benefix
Fabrazyme
Replagal
Pulmozyme
Activase/Actilyse

2

Brand

ine

O halde ciddi bir optimizasyona gereksinim var!



Yıllar içinde biyolojik peptid ilaçlar...

1

Brand	Generic	Company	Therapeutic category	Indications
Humulin	Insulin	Eli Lilly	Diabetes	Diabetes
Humatrope	Recombinant Somatropin	Eli Lilly	Hormones	Growth failure
Genotropin	Somatropin	Pfizer	Hormones	Growth failure
Saizen	Somatropin	Serono	Hormones	Growth failure
Nutropin/Protropin	Somatropin/Somatrem	Genentech	Hormones	Growth failure
Intron A &	Interferon alpha-2b/	Schering-Plough	Anti-infective	Viral infections
Avonex	Interferon beta-1a	Biogen Idec	Multiple sclerosis	Chronic inflammatory demyelinating polyneuropathy
Betaseron/Betaferon	Interferon beta-1b	Schering AG	Multiple sclerosis	Multiple sclerosis
Procrit/Eprex	Epoetin alpha	J&J	Blood modifier	Anaemia
Epogen	Epoetin alpha	Amgen	Blood modifier	Anaemia
NeoRecormon	Epoetin beta	Roche	Blood modifier	Anaemia
Kogenate	Factor VIII	Bayer	Blood modifier	Haemophilia
NovoSeven	Factor VIIa	Novo Nordisk	Blood modifier	Haemophilia
Benefix	Factor IX	Wyeth	Blood modifier	Haemophilia
Fabrazyme	Agalsidase beta	Genzyme	Enzymes	Fabry disease
Replagal	Agalsidase alfa	TKT Europe	Enzymes	Fabry disease
Pulmozyme	Dornase alpha	Genentech	Enzymes	Cystic fibrosis
Activase/Actilyse	Alteplase	Genentech	Blood factor	Myocardial infarction

http://laborant.pl/index.php/recombinant-protein-therapeutics-the-future-is-here

http://laborant.pl/index.php/recombinant-protein-therapeutics-the-future-is-here

2

Brand	Generic	Company	Therapeutic category	Indications
Humalog/Liprolog	insulin lispro,	Eli Lilly	Diabetes	Diabetes
Lantus	Glargine insulin	Sanofi-Aventis	Diabetes	Diabetes
Levemir	Datemir insulin	Novo Nordisk	Diabetes	Diabetes
Pegasys	Pegylated interferon alpha-2a	Roche	Interferon	Hepatitis C
Peg-Intron	Pegylated interferon alpha-2a	Schering Plough	Interferon	Hepatitis C
Aranesp	Darbepoetin alpha	Amgen	Blood modifier	Anaemia
Neulasta	PEG-Filgrastim	Amgen	Blood modifier	Neutropenia
ReFacto	Factor VIII	Wyeth	Blood modifier	Haemophilia
Amevive	alefacept	Biogen Idec.	Inflammation/Bone	Plaque psoriasis
Enbrel	Etanercept	Amgen	Anti-arthritic	Arthritis
Ontak	rIL2-diptheria toxin	Ligand Pharmaceuticals	Cancer	Cancer

http://laborant.pl/index.php/recombinant-protein-therapeutics-the-future-is-here

3

Brand	Generic	Company	Therapeutic category	Indications
ReoPro	Abciximab	Eli Lilly	Blood modifier	Acute coronary syndrome
Rituxan	rituximab	Genentech	Cancer	Non-Hodgkin's

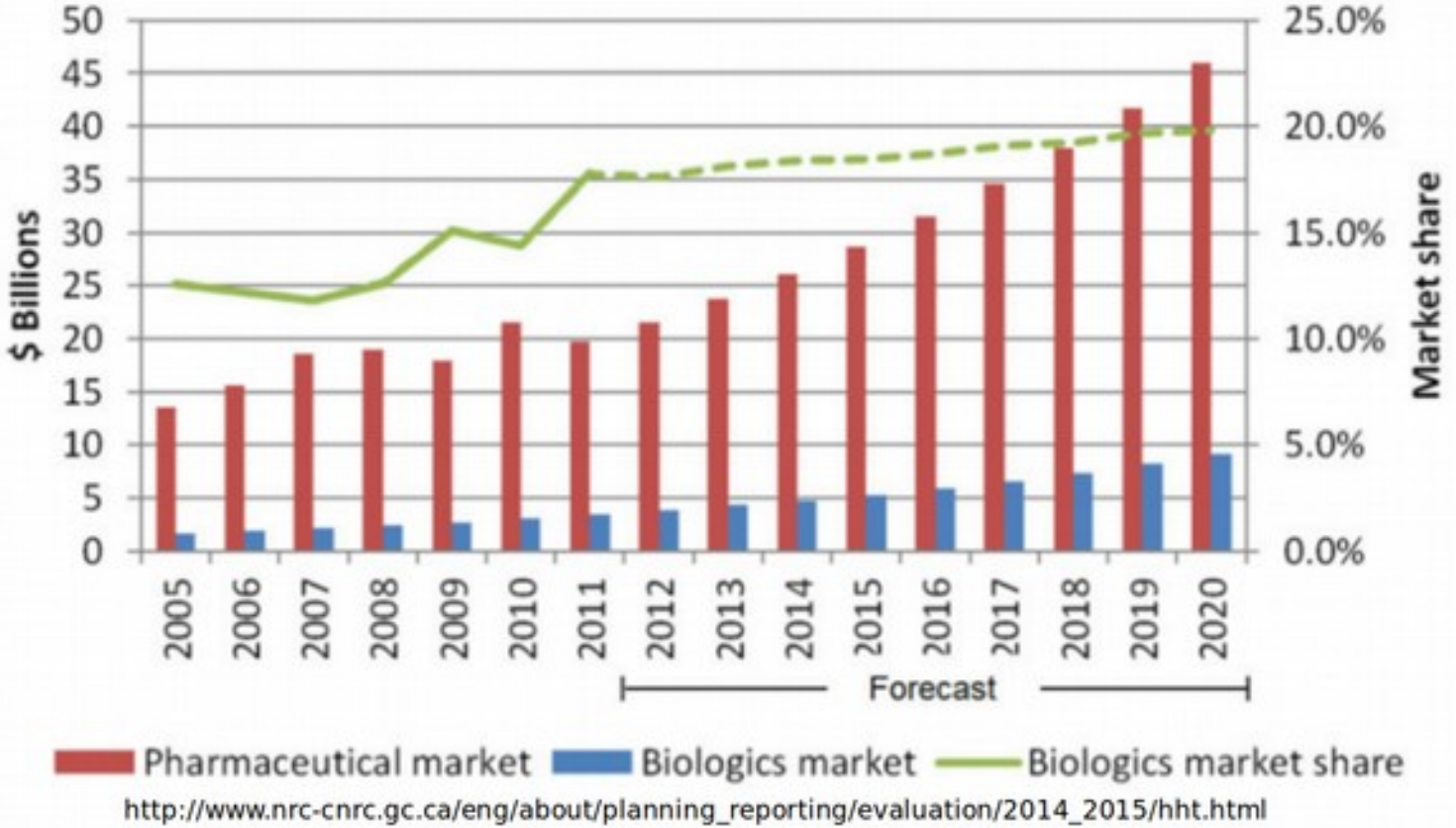
Enbrel	Etanercept	Amgen	Anti-arthritic	Arthritis
Ontak	rIL2-diptheria toxin	Ligand Pharmaceuticals	Cancer	Cancer

http://laborant.pl/index.php/recombinant-protein-therapeutics-the-future-is-here

3

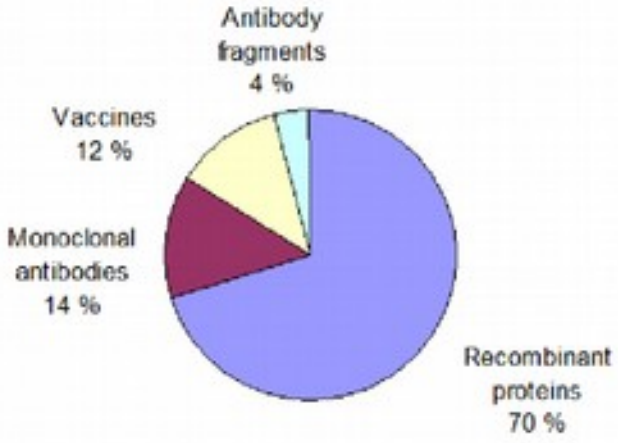
Brand	Generic	Company	Therapeutic category	Indications
ReoPro	Abciximab	Eli Lilly	Blood modifier	Acute coronary syndrome
Rituxan	rituxumab	Genentech	Cancer	Non-Hodgkin's lymphoma
Herceptin	Trastuzumab	Genentech	Cancer	Breast cancer
Synagis	Palivizumab	MedImmune	Respiratory	Respiratory syncytial virus
Campath	Alemtuzumab	Schering AG	Cancer	Non-Hodgkin's lymphoma
Humira	Adalimumab	Abbott Labs	Anti-arthritic	Rheumatoid arthritis
Xolair Omalizumab	Omalizumab	Genentech	Respiratory diseases	Paediatric asthma, peanut allergies
Erbitux	Cetuximab	Imclone Systems	Cancer	Colon cancer
Avastin	Bevacizumab	Genentech	Cancer	Colon cancer

http://laborant.pl/index.php/recombinant-protein-therapeutics-the-future-is-here



http://www.nrc-cnrc.gc.ca/eng/about/planning_reporting/evaluation/2014_2015/hht.html

Geldiğimiz noktada



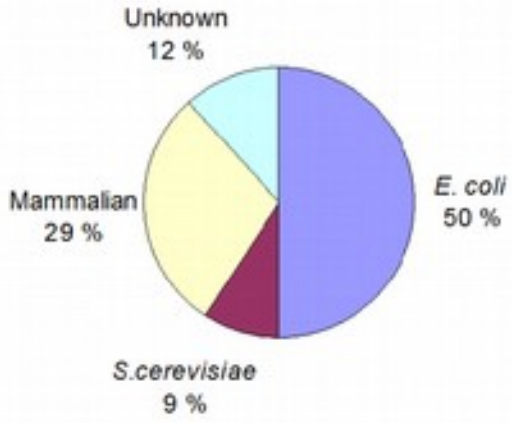


Table 1. The different biopharmaceutical products (Walsh, 2006).

Recombinant proteins
Blood factors (e.g. Factor VIII)
Thrombolytic agents (e.g. tissue plasminogen activator)
Hormones (e.g. insulin, growth hormones)
Growth factors (e.g. erythropoietin)
Interferons (e.g. interferon-α)
Interleukin-based products
Monoclonal antibodies and antibody fragments
Vaccines
Nucleic-acid based products
Therapeutic enzymes

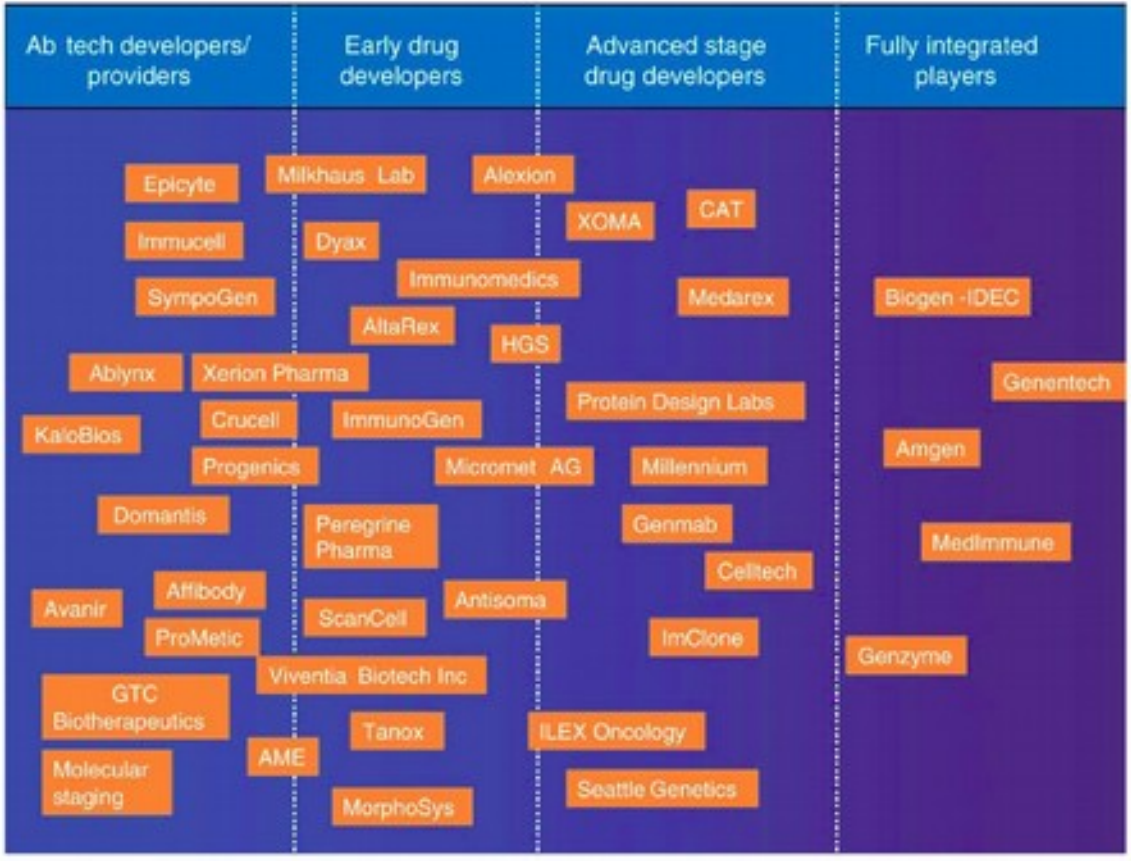
s-here

Advanced stage | Fully integrated

)

ein 4 (XRCC4)

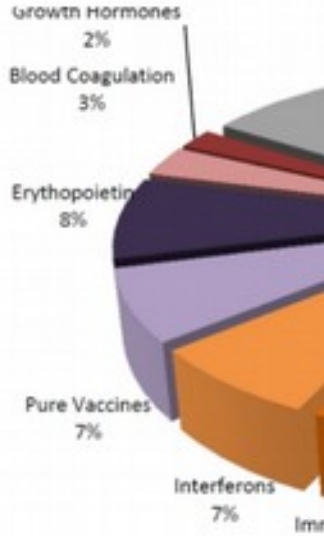
non-homologous

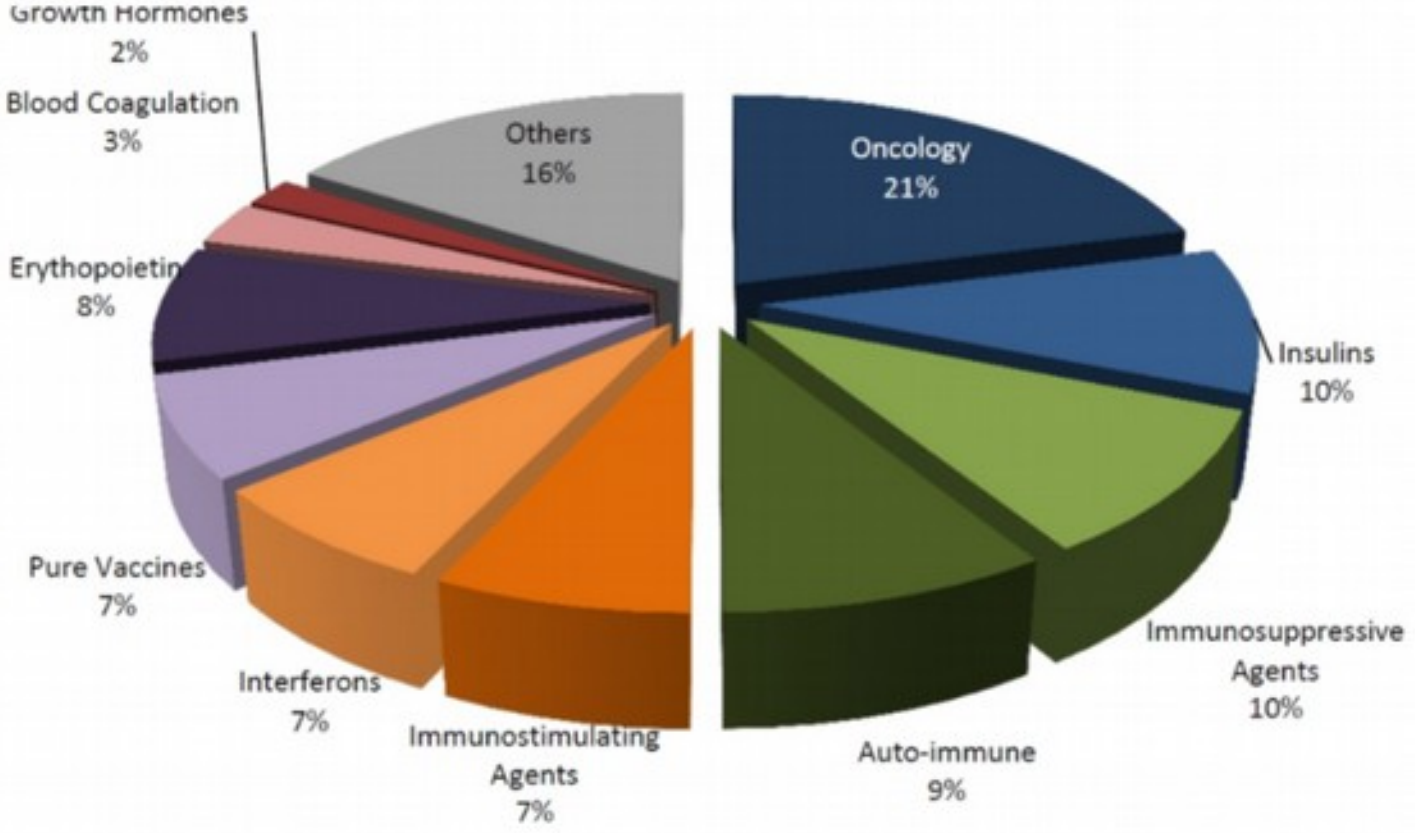


Vaccines

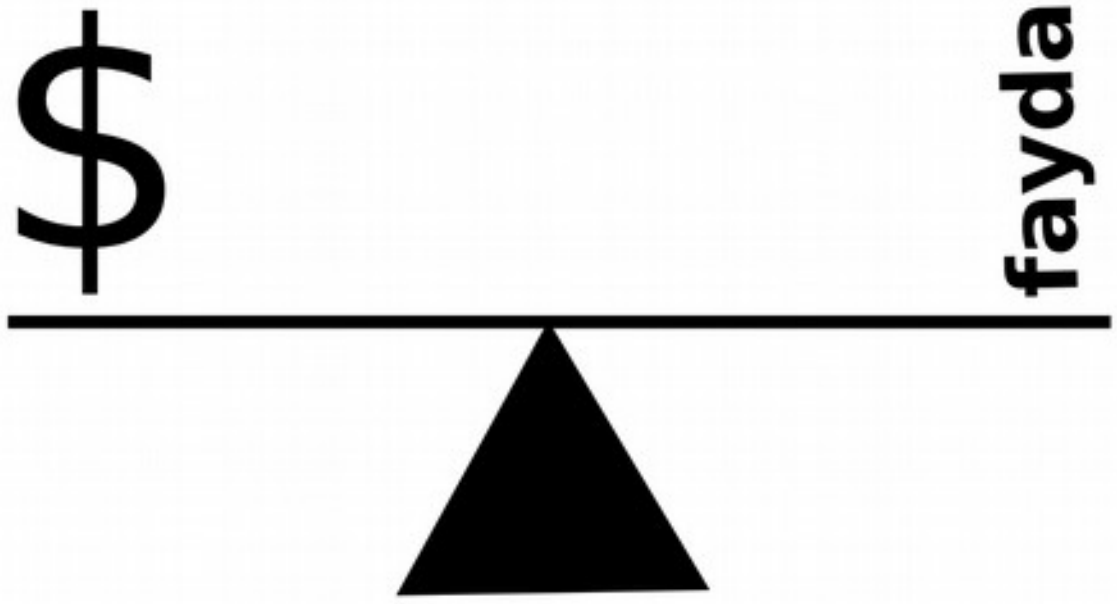
Nucleic-acid based pro

Therapeutic enzymes




Growth Hormones
2%
Blood Coagulation
3%
Others
16%
Oncology
21%
Erythopoietin
8%
Insulins
10%
Pure Vaccines
7%
Interferons
7%
Immunostimulating
Agents
7%
Immunosuppressive
Agents
10%
Auto-immune
9%

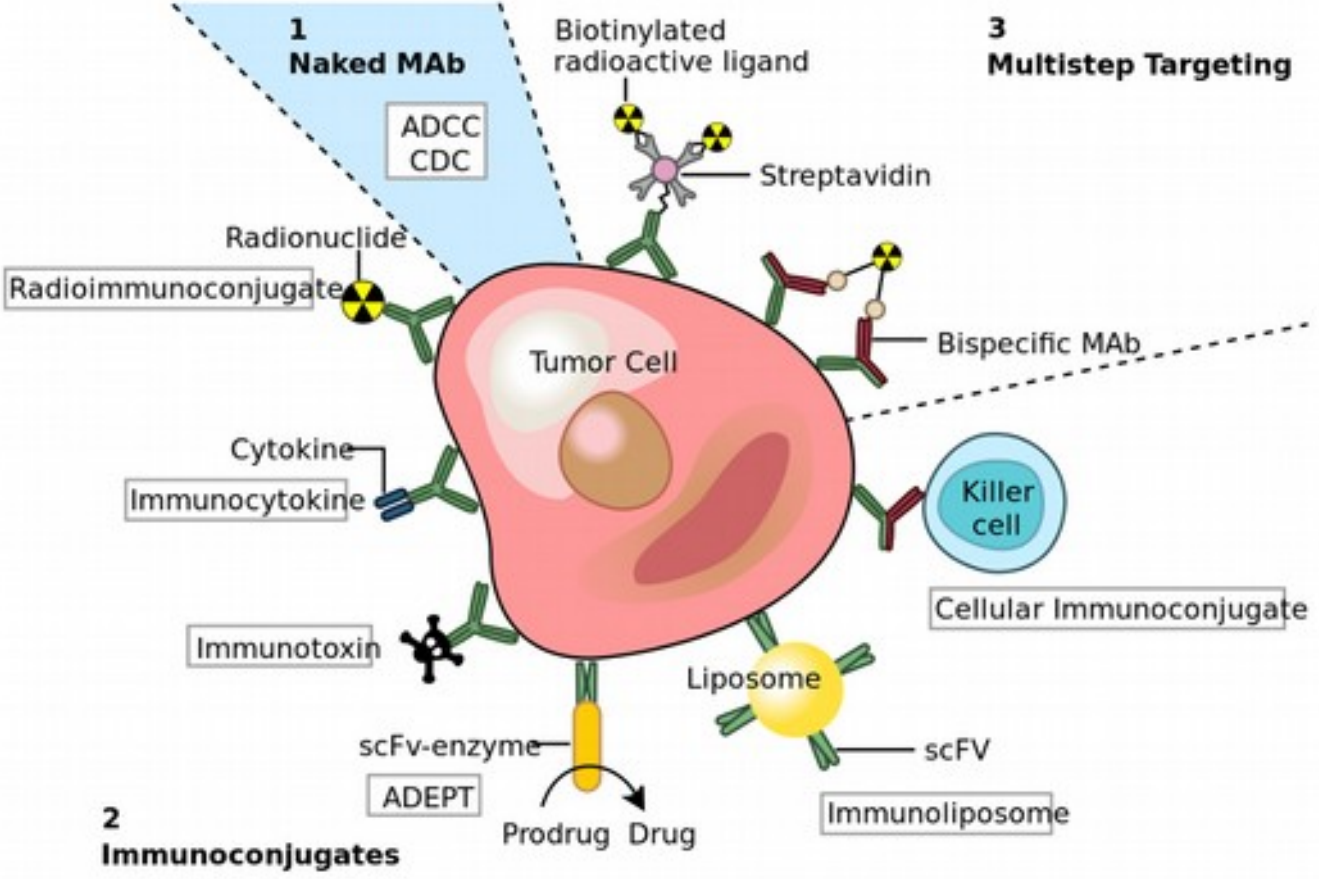
Ne kadar "sihirli"?!?

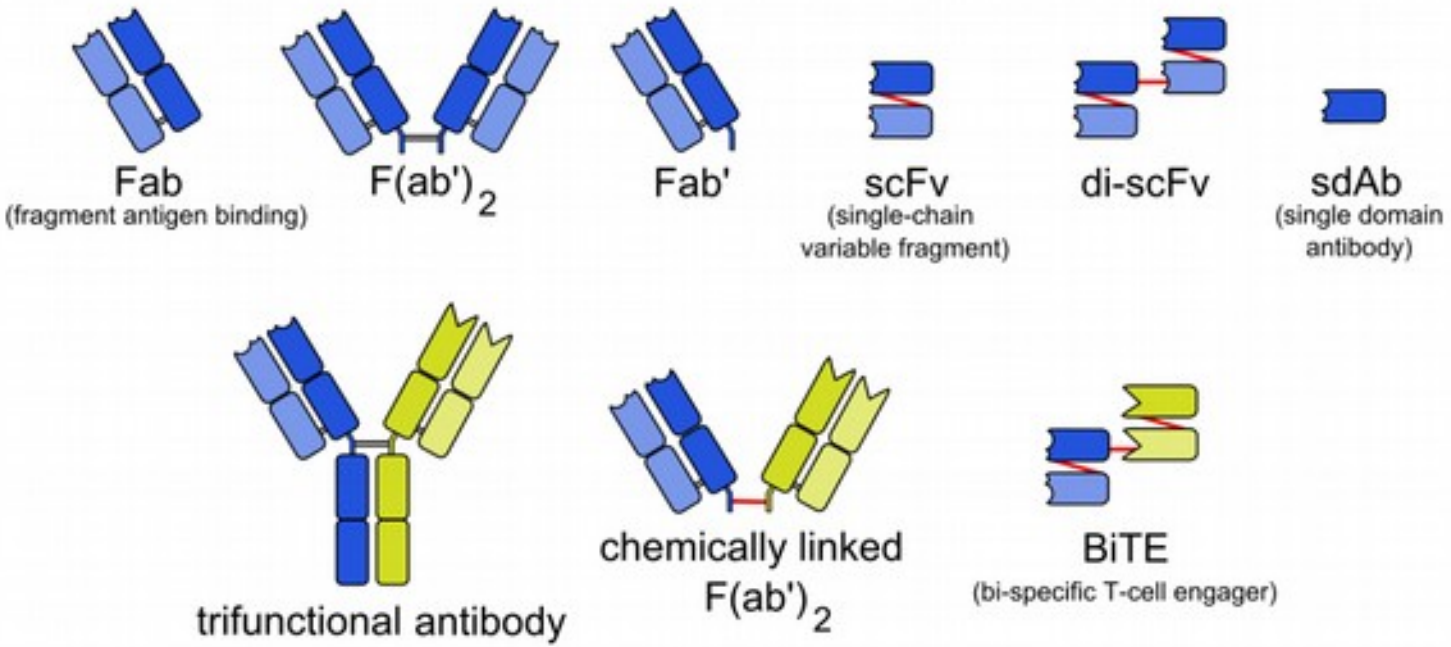


sulins
10%

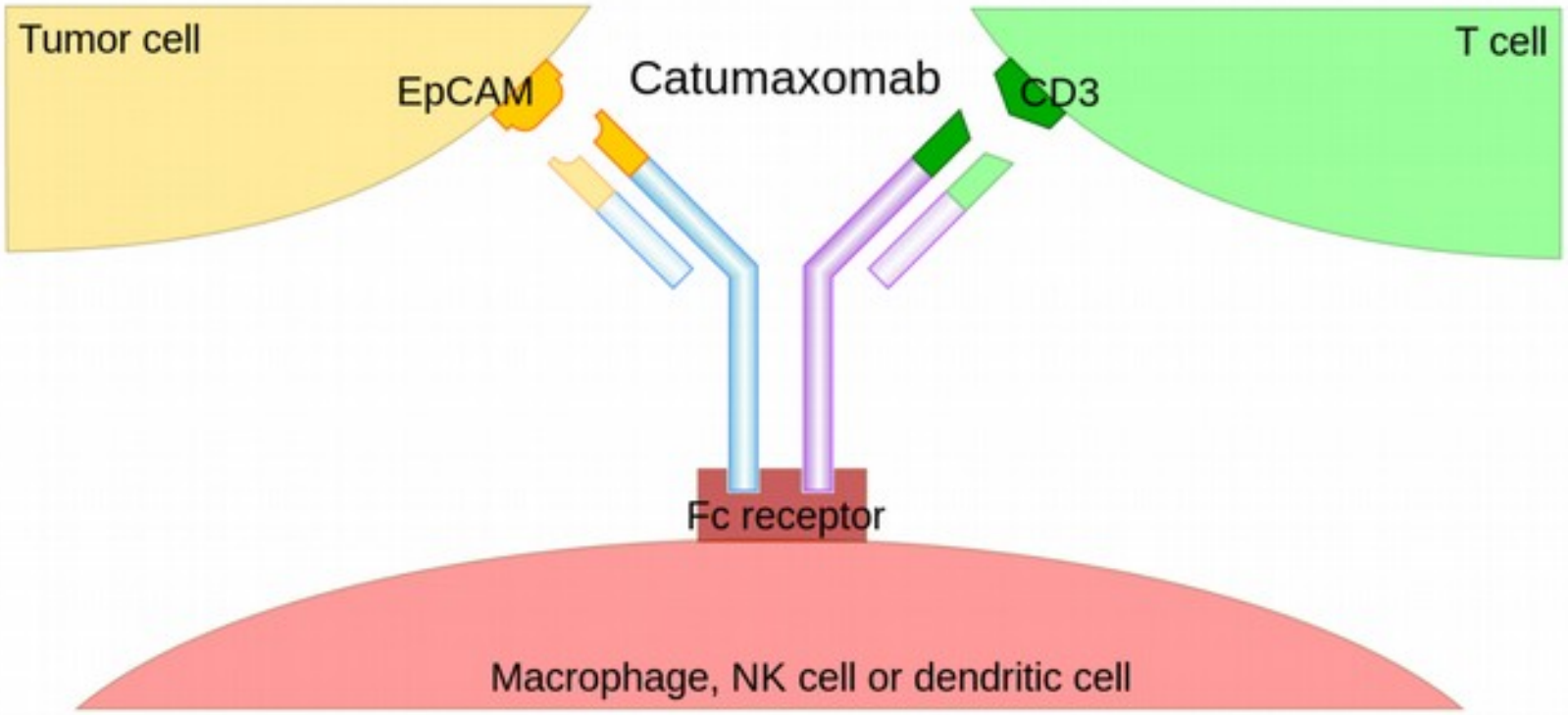
pressive
ts

Daha yaratıcı kombinasyonlar...



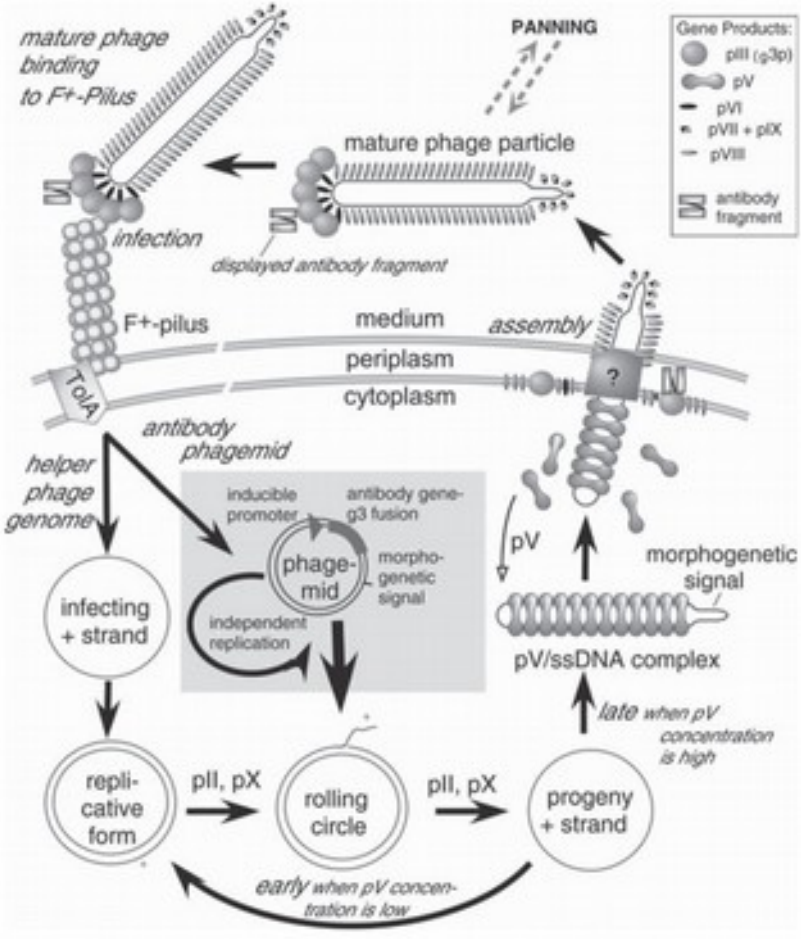


daha küçük... daha kompakt... hücre içi hedefler?
çoklu hedefleme...

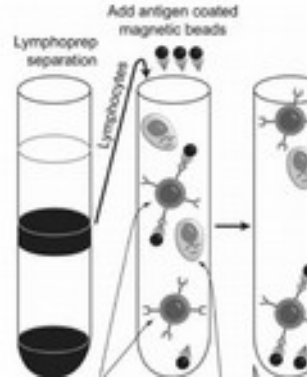


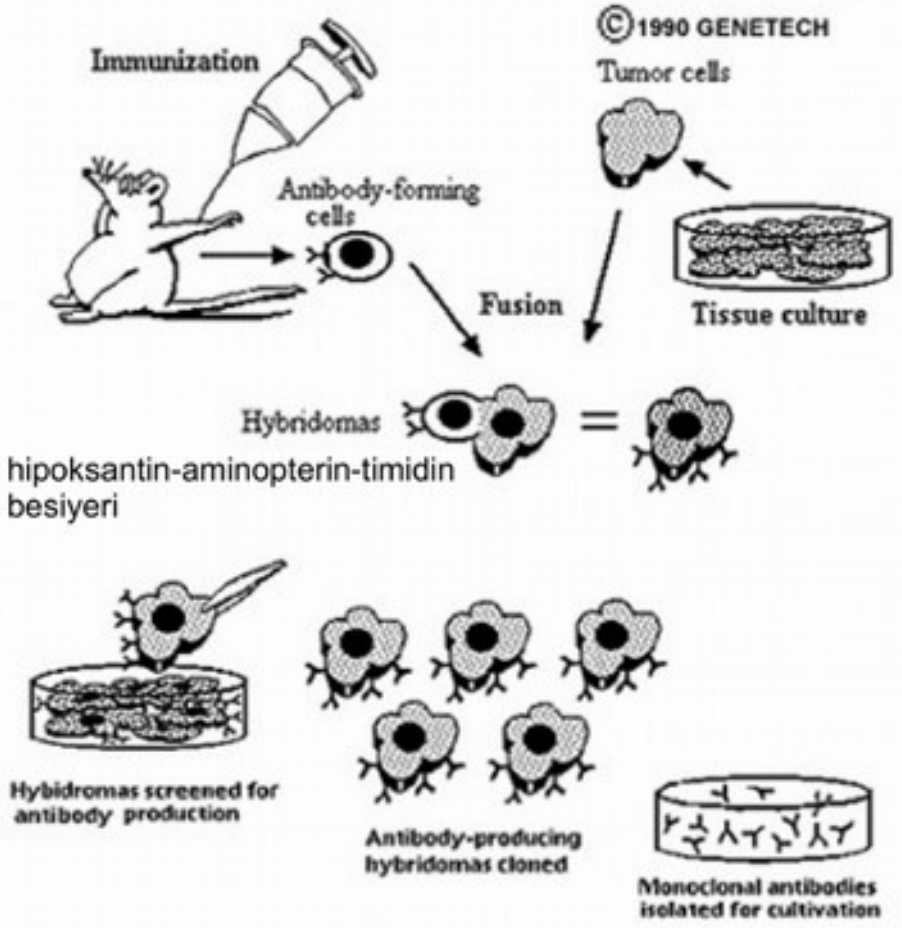
çoklu hedeleme...

Eski bir dosttan yardım...



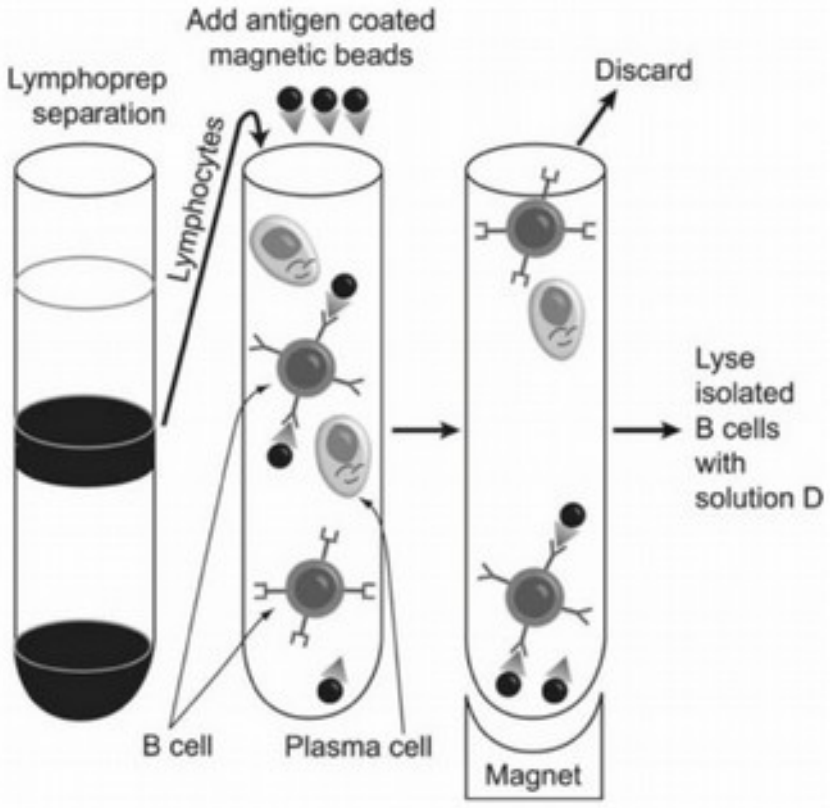
hibridoma teknoloj
ya da
dolaşımdan B-hücr

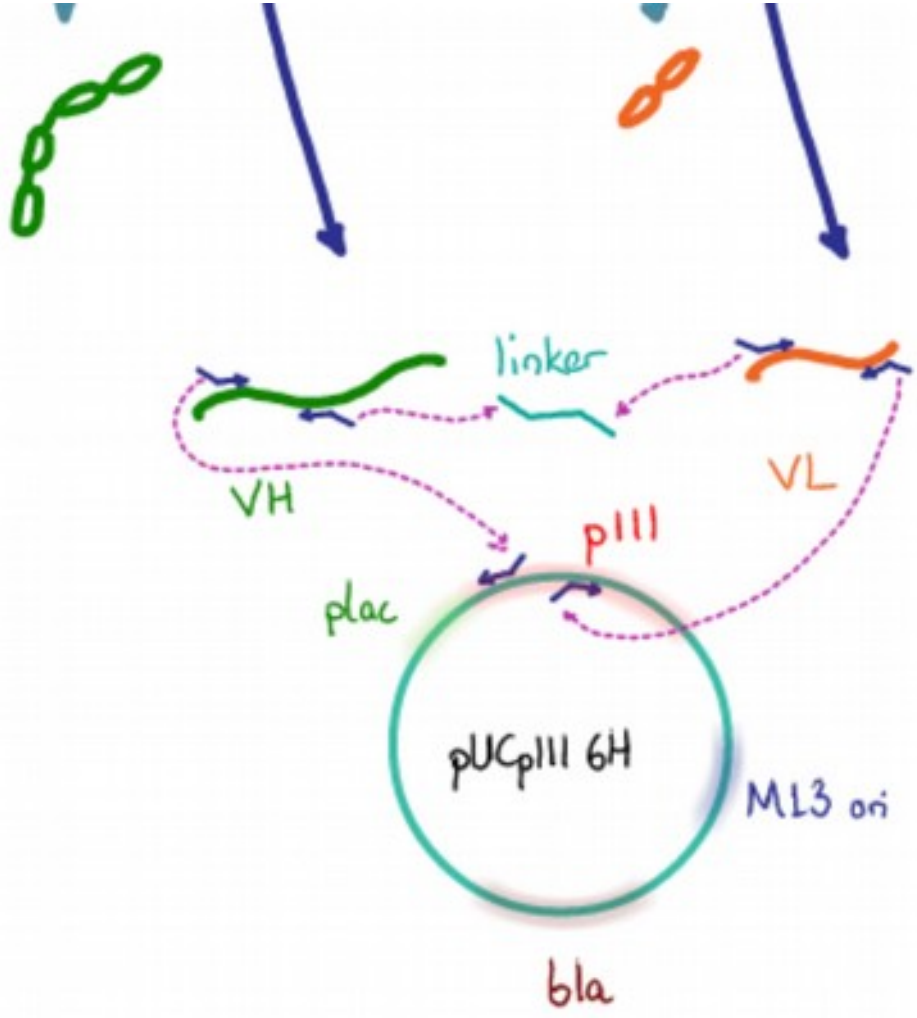


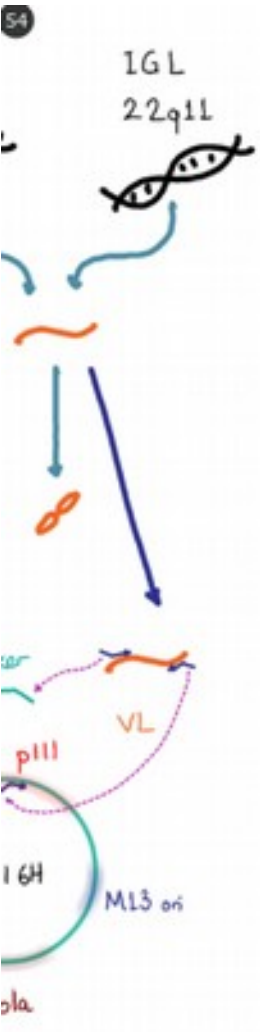


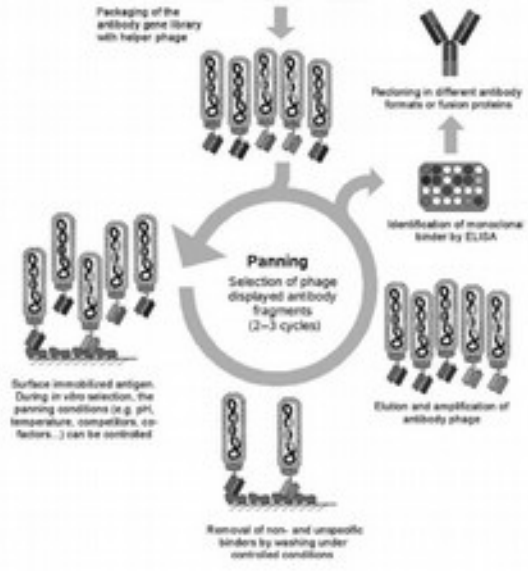
hibridoma teknolojisi

ya da
dolaşımdan B-hücresi hasatı...

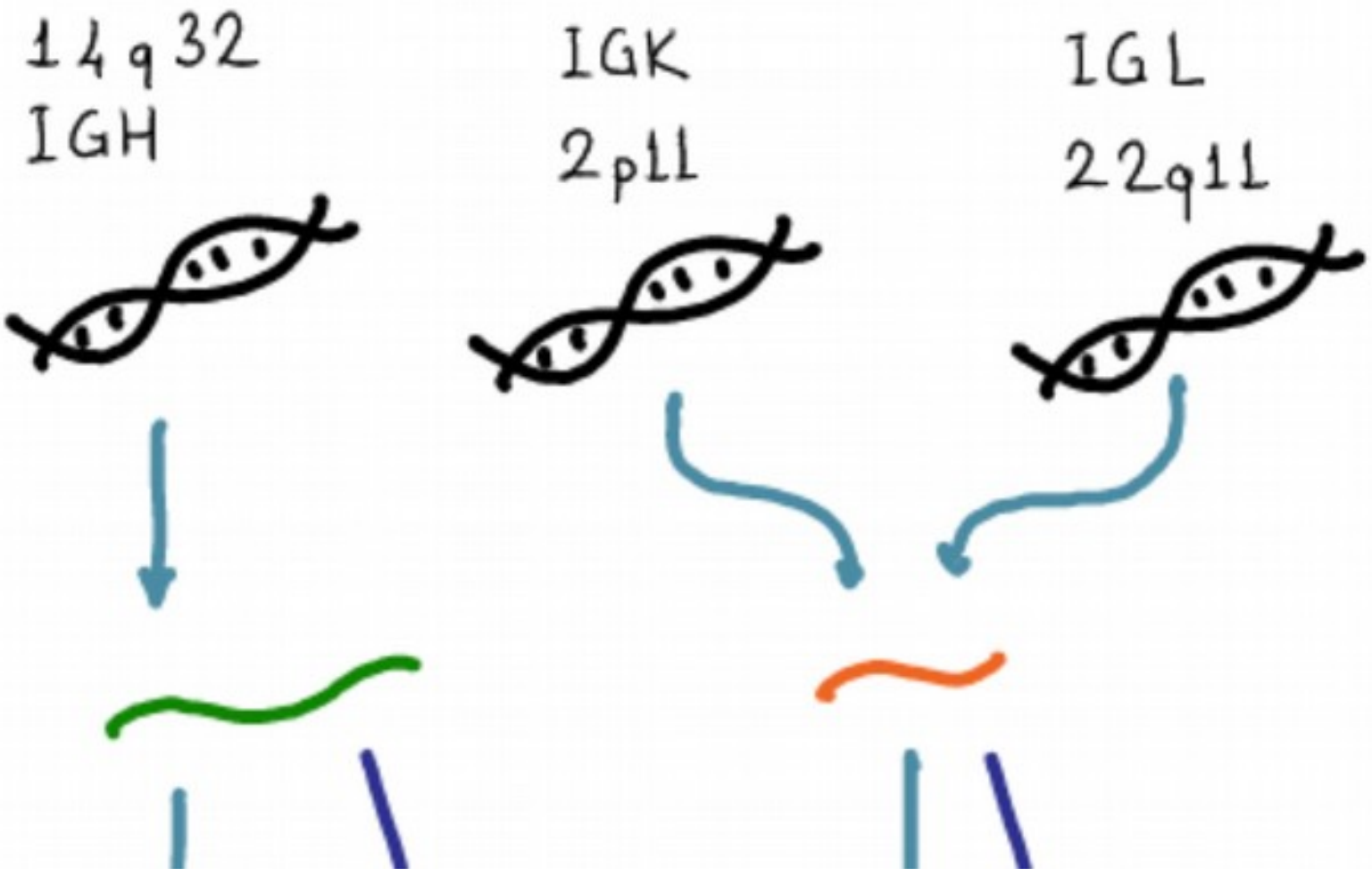



linker
VH
VL
pIII
plac
pUCpIII 6H
M13 ori
bla





```
Heavy Forward          _N
         GAGTCATTC Tgc
mHF1: 5'-GAGTCATTCT gc


(-c) konstraktı için u
Heavy Forward
            GAGTCATTCTg
mHF1-c: 5'-GAGTCATTCTg
mHF2-c: 5'-GAGTCATTCTg
mHF3-c: 5'-GAGTCATTCTg
mHF4-c: 5'-GAGTCATTCTg


Sadece Light Chain var
Light Forward
          ______Linker_
            S   G   G
mLF1: 5'-AGA GCC GCC T

pdLF:
          __NotI____^
    TTCT gcg gcc gcA A
           R   G   C
pdLF: 5'-TTCTgcggccgcA

Sadece Heavy Chain var

          ______Linker__
            G   G   G
mHR1: 5'-GGC GGC GGT G

pdHR:
       __SalI__     G   G
TTCT gt cga cTG GGC GG
     R   R   L^
pdHR: 5'-TTCTgtcgacTGG
```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2p11
IGL
22q11