基因工程抗体及其发展前景

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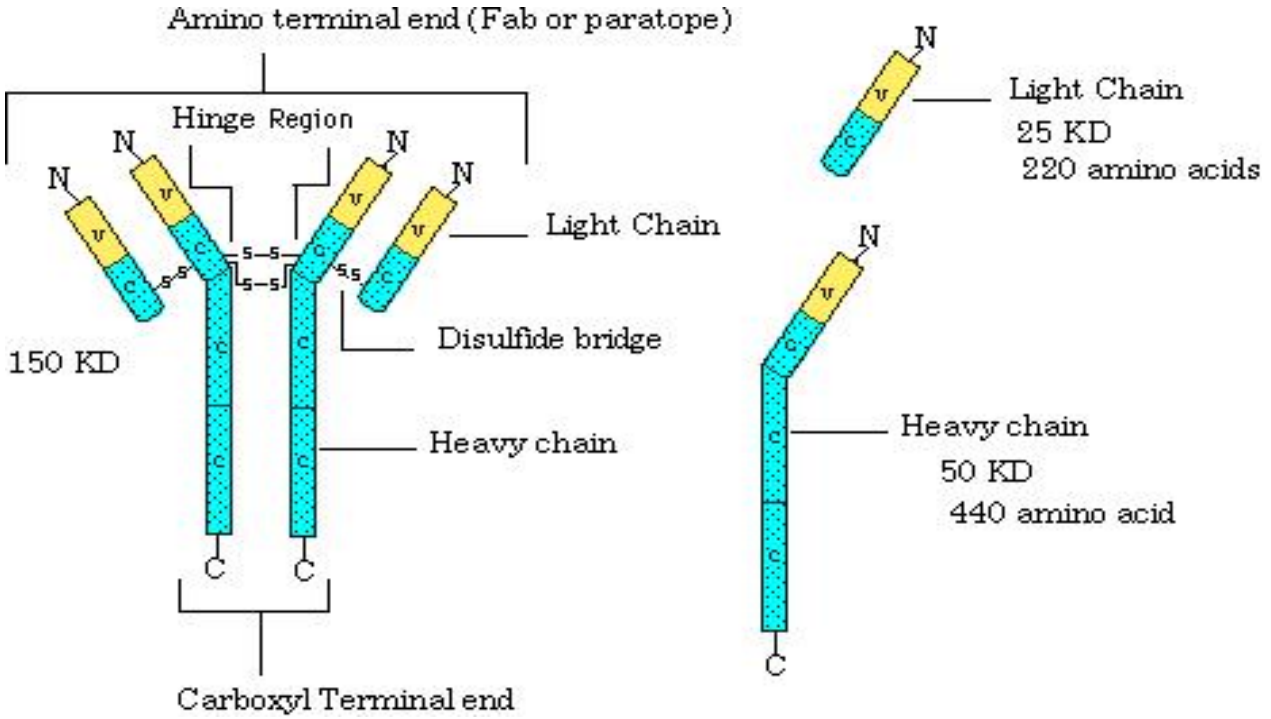
2020/11/17

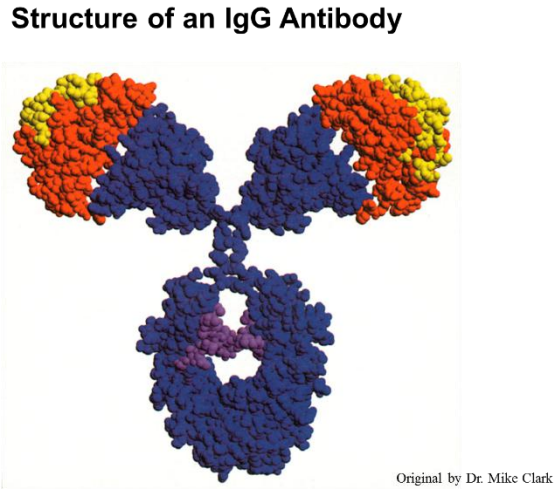
主要内容

- **1. 抗体的一些基本概念：**
- **2. 基因工程抗体的基本原理：**
- **3. 人源化抗体制备的主要方法：**
- **4. 单克隆抗体药物：**
- **5. 基因工程抗体的未来展望：**

第一节：抗体的一些基本概念

1、免疫球蛋白的基本结构





- 四肽链结构 ， 链间二硫键连接
- 两条重链（H）和两条轻链（L）
- 氨基端和羧基端。

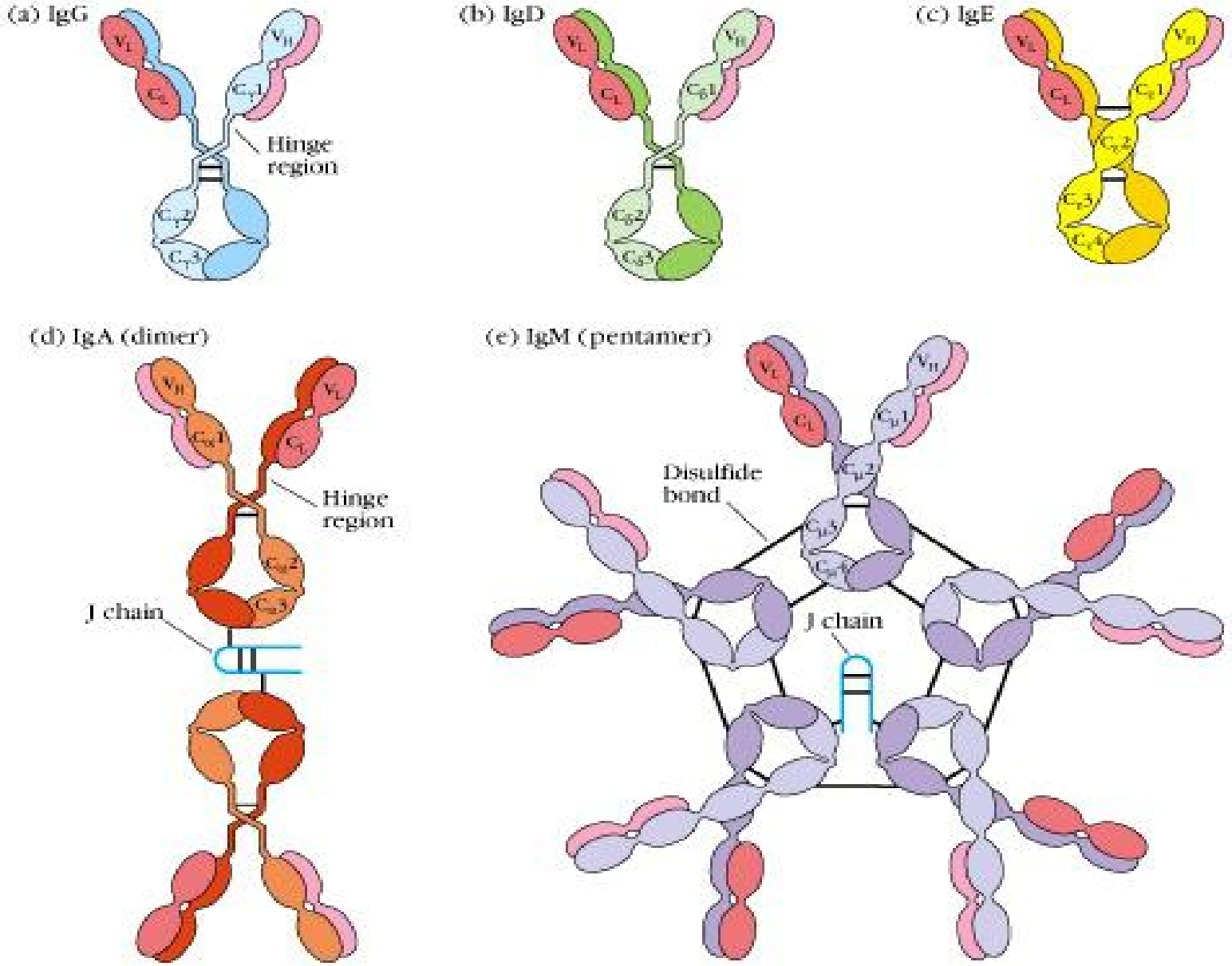
2、重链与轻链

(1)根据重链分类：

- 根据重链靠近羧基末端氨基酸组成及排列顺序不同，将重链分为 种：γ、α、μ、δ、ε
- 根据重链组成不同，将Ig分为五类：IgG、IgA、IgM、IgD、IgE

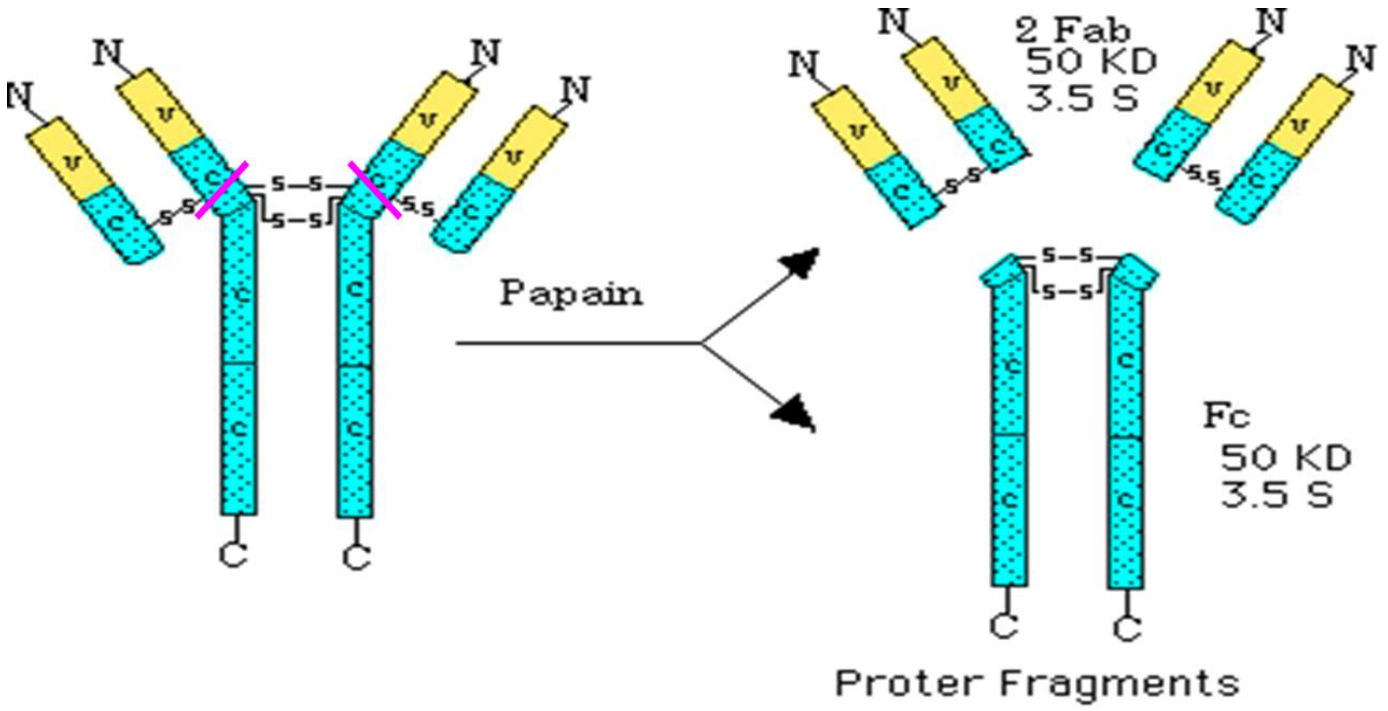
(2)根据轻链分型：κ、λ型

天然Ig分子中，重链同类，轻链同型

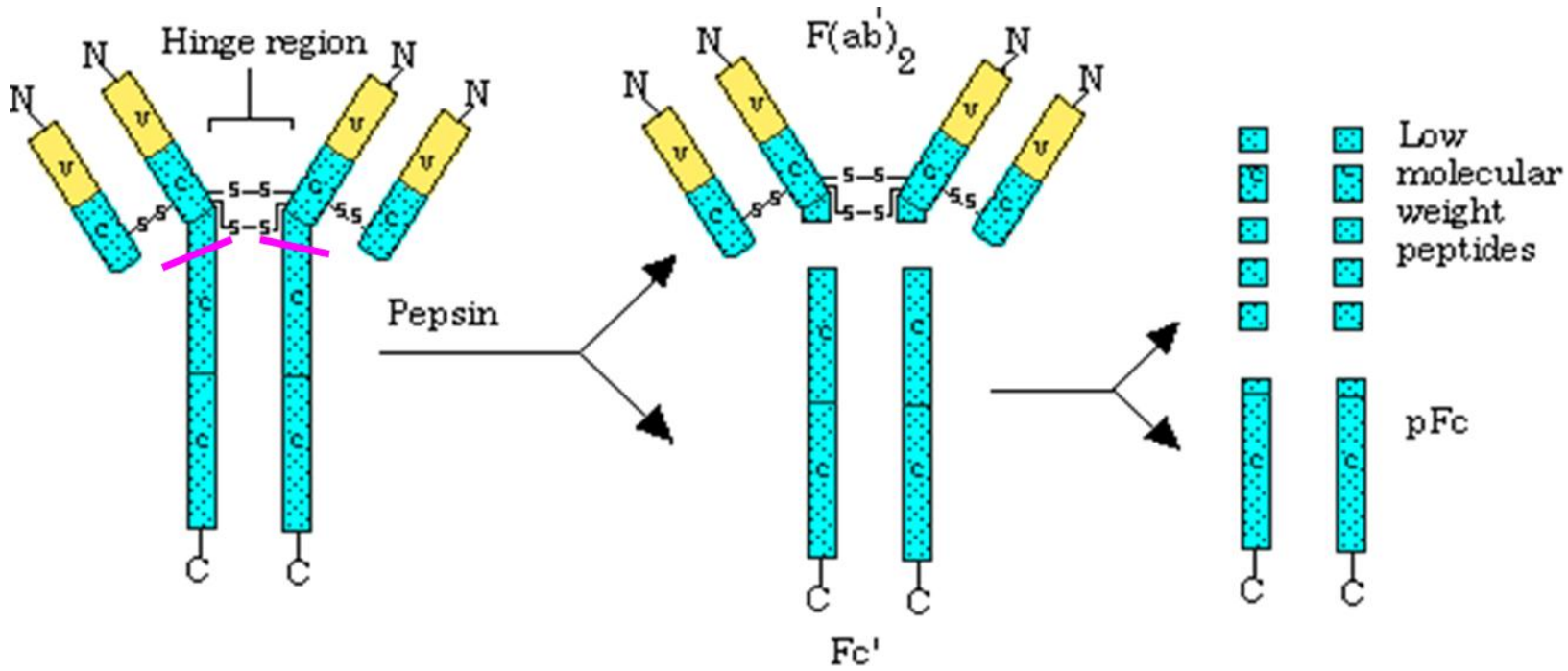

(a) IgG
Hinge region
(b) IgD
(c) IgE
(d) IgA (dimer)
Hinge region
J chain
(e) IgM (pentamer)
Disulfide bond
J chain

3、免疫球蛋白的水解片段

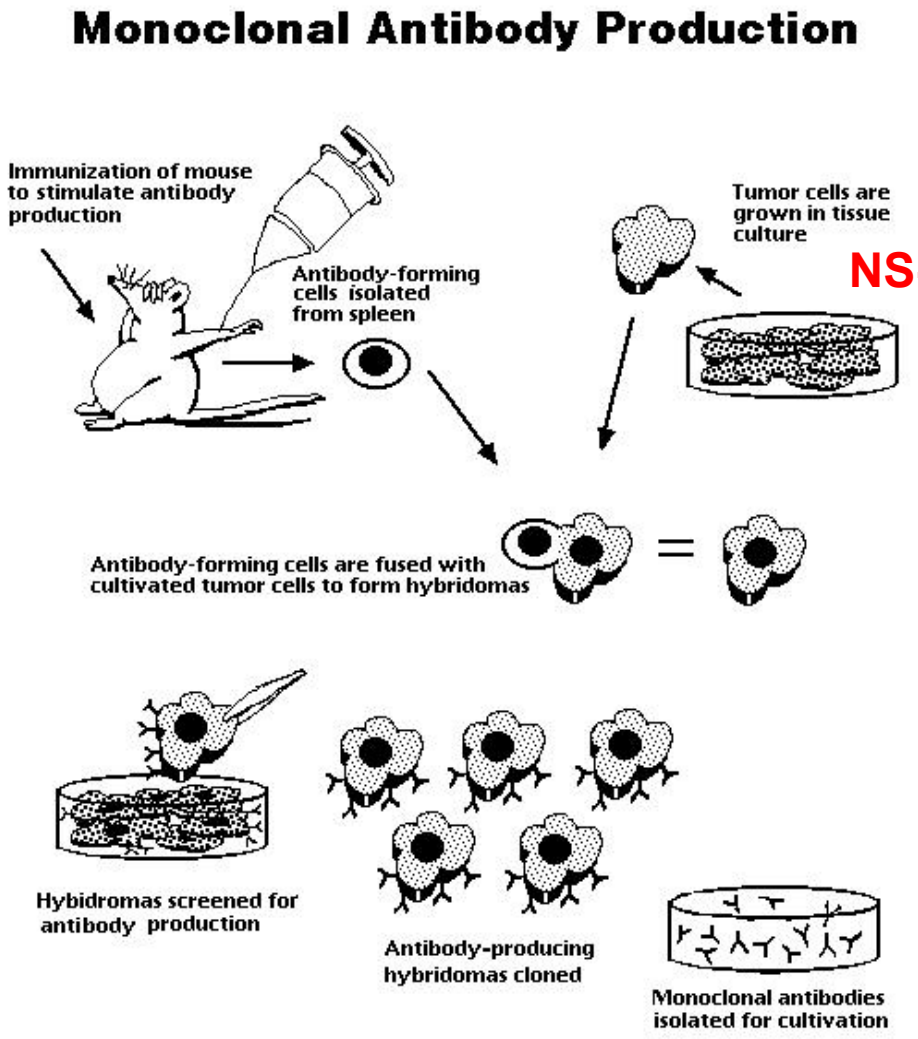
- 木瓜蛋白酶(papain)水解IgG：得到两个相同的Fab段和一个Fc段。



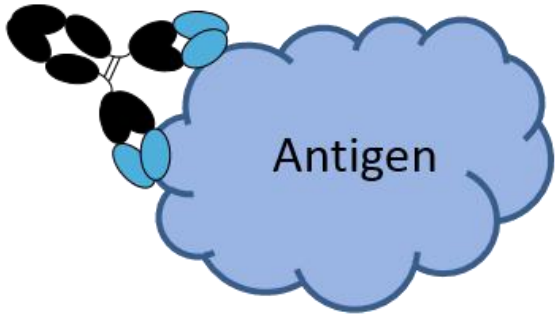
- 胃蛋白酶(pepsin)裂解IgG：得到一个具有双价活性的F（ab′)$_2$ 段和若干个小分子多肽碎片（pFc′）



4、 单克隆抗体

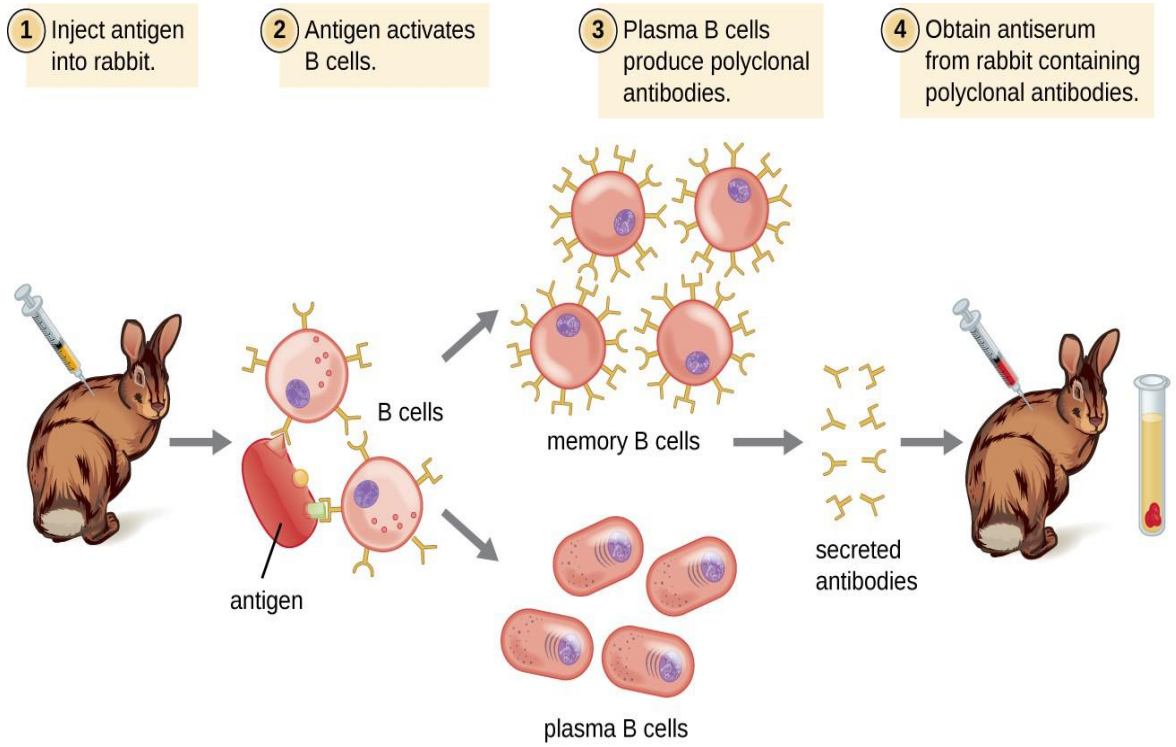


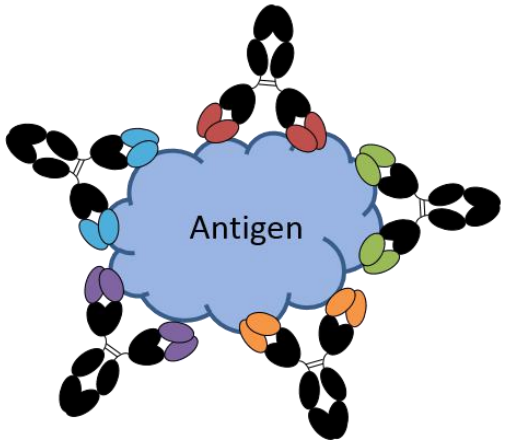
NS-1 and SP2/0



- 经典的杂交瘤技术
- 来源于一个**B**细胞
- 均一性，识别相同抗原表位

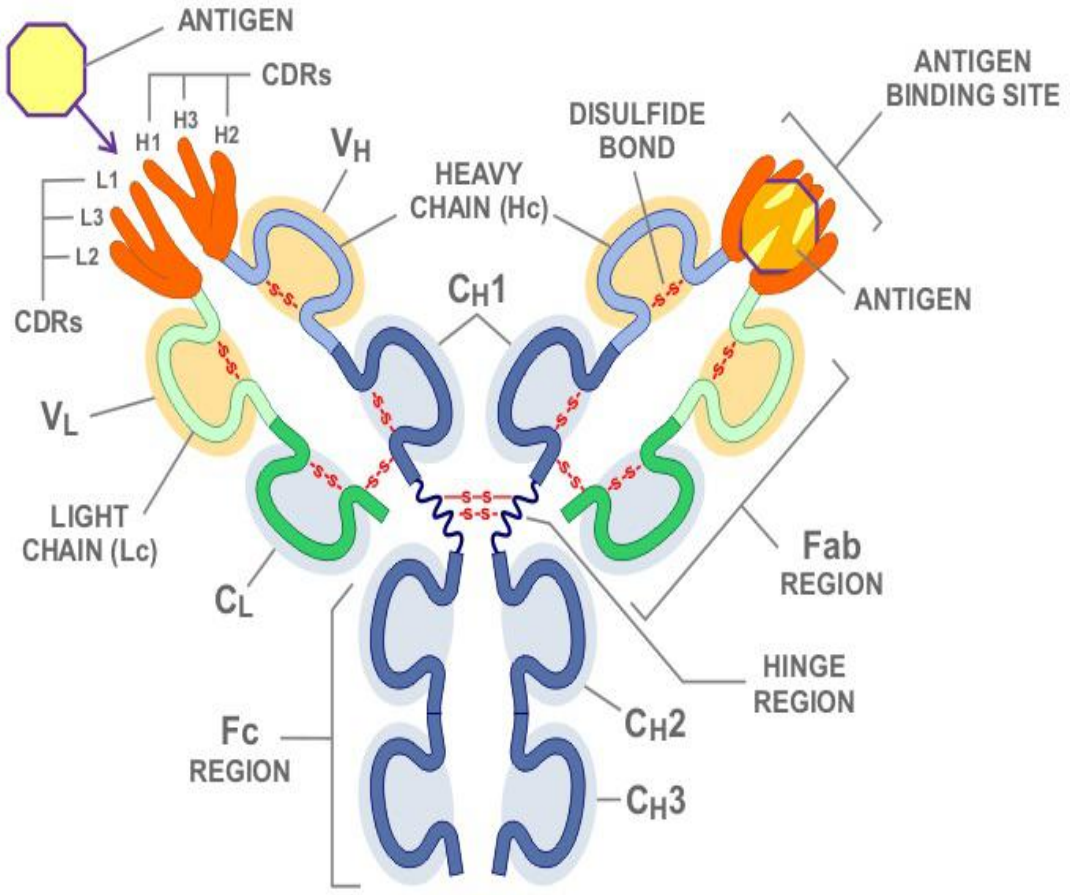
5、多克隆抗体



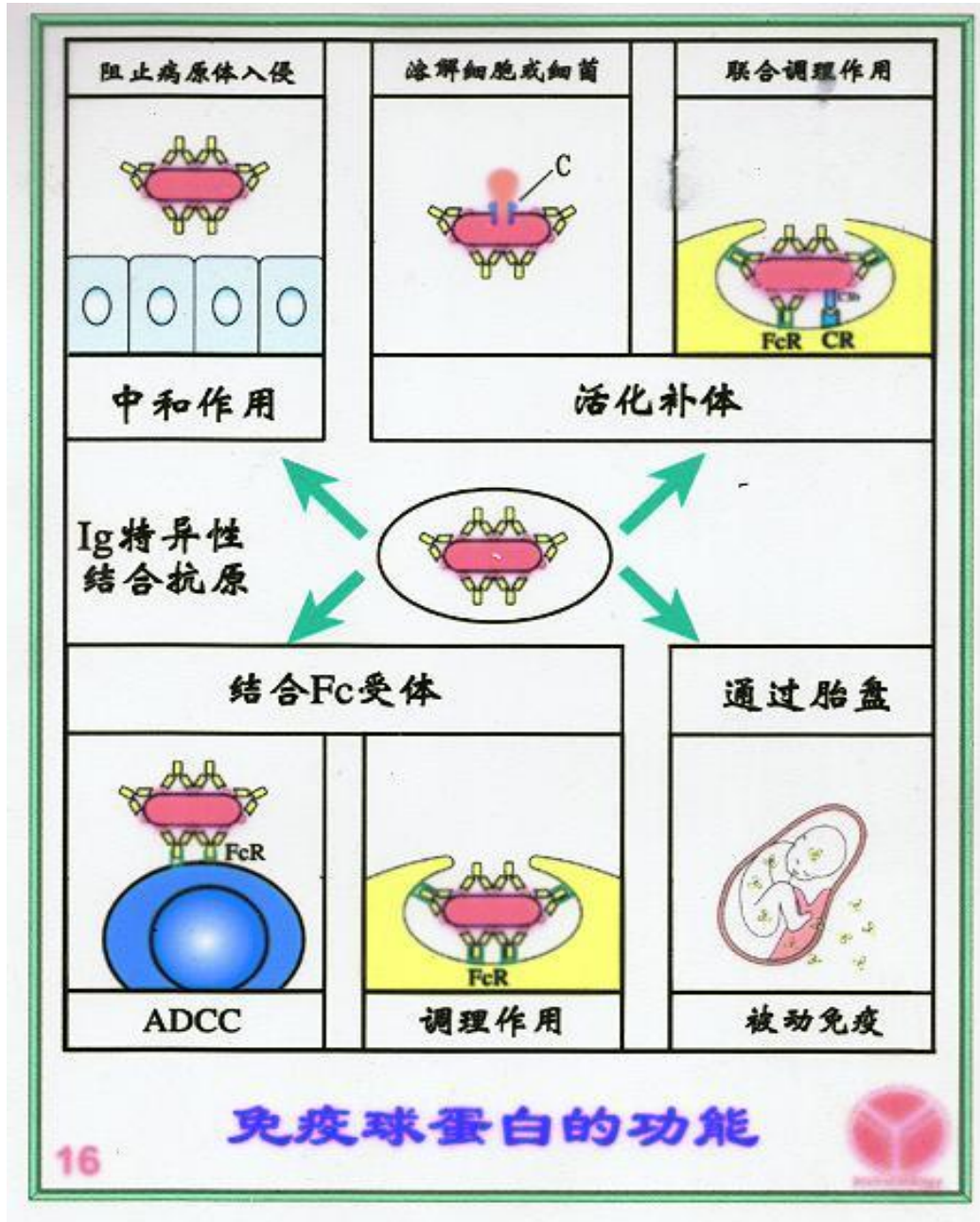


- 未经单克隆选择
- 识别不同抗原表位的抗体混合

6、抗体各功能区的作用



- – VH和VL:识别和结合抗原
- – CH和CL:同种异型的遗传标志
- – CH2:补体C1q结合位点， IgG可通过胎盘
- – CH3/CH4:与多种细胞表面的FcR结合(免疫调理,I型超敏反应)


阻止病原体入侵
溶解细胞或细菌
联合调理作用
C
FcR CR
中和作用
活化补体
Ig特异性
结合抗原
结合Fc受体
通过胎盘
FcR
FcR
ADCC
调理作用
被动免疫
免疫球蛋白的功能
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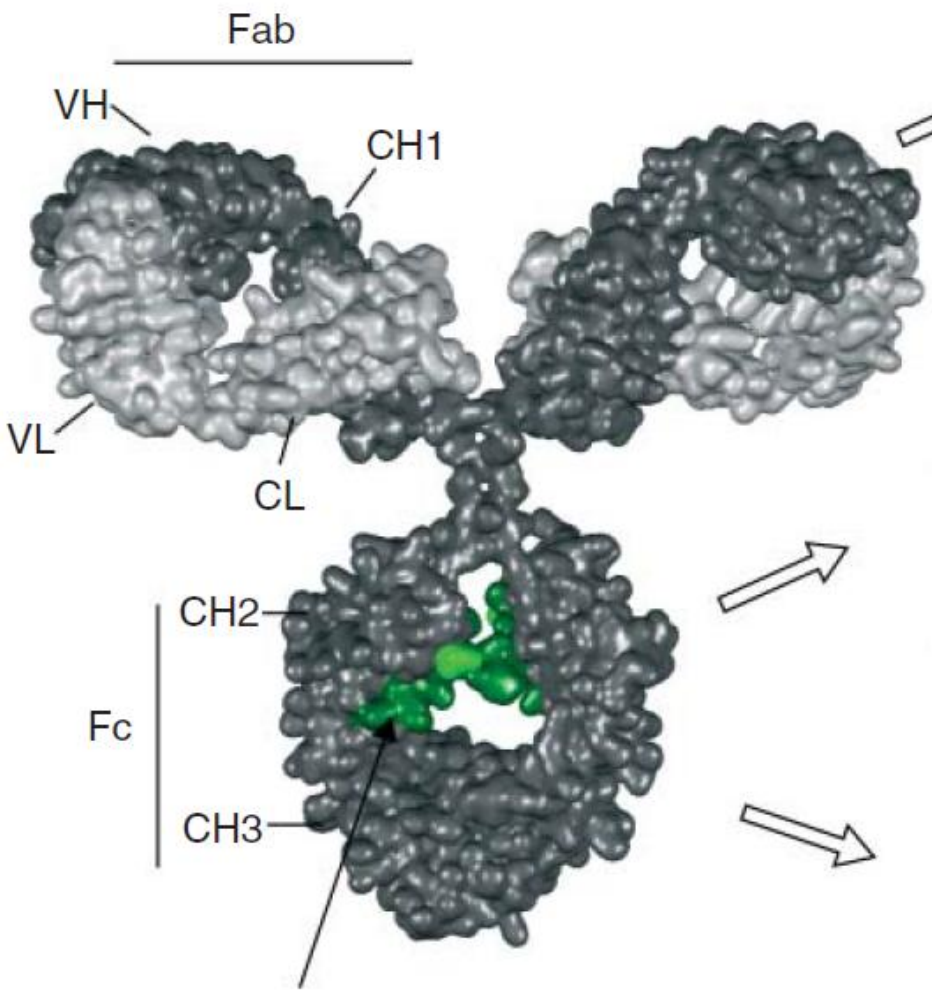
第二节：基因工程抗体的基本原理



Direct MoA

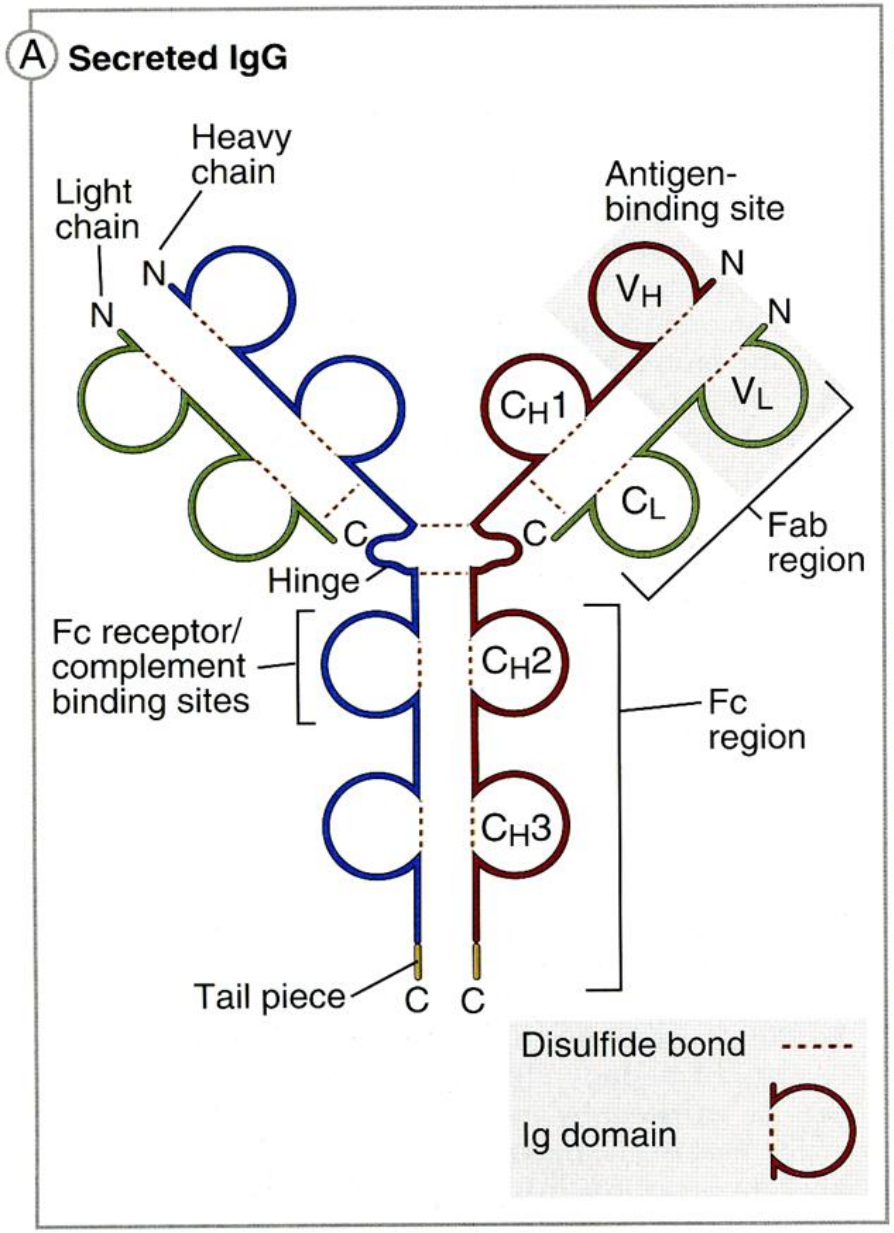
- Cross-linking of surface antigens
- Growth inhibition
- Induction of apoptosis
- Prevention of receptor dimerization
- Neutralization of soluble factors
- Blockade of receptors

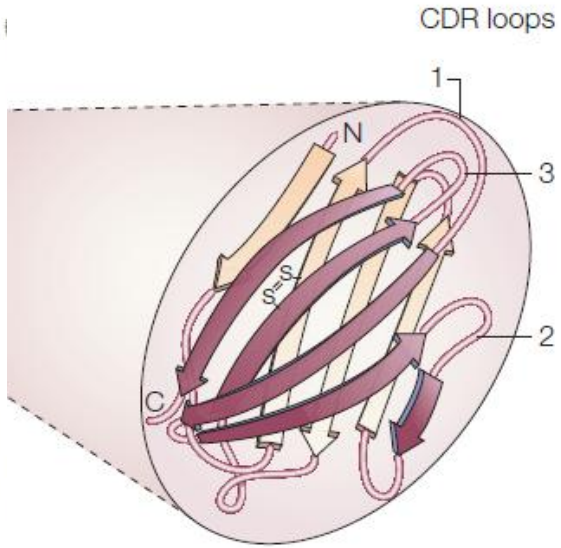
Indirect MoA

- Complement binding (CDC)
- Effector cell recruitment (ADCC, ADCP)

Pharmacokinetics

- FcRn binding site
- Plasma retention half-life





Nature Reviews Drug Discovery **2**, 52-62 (January 2003)

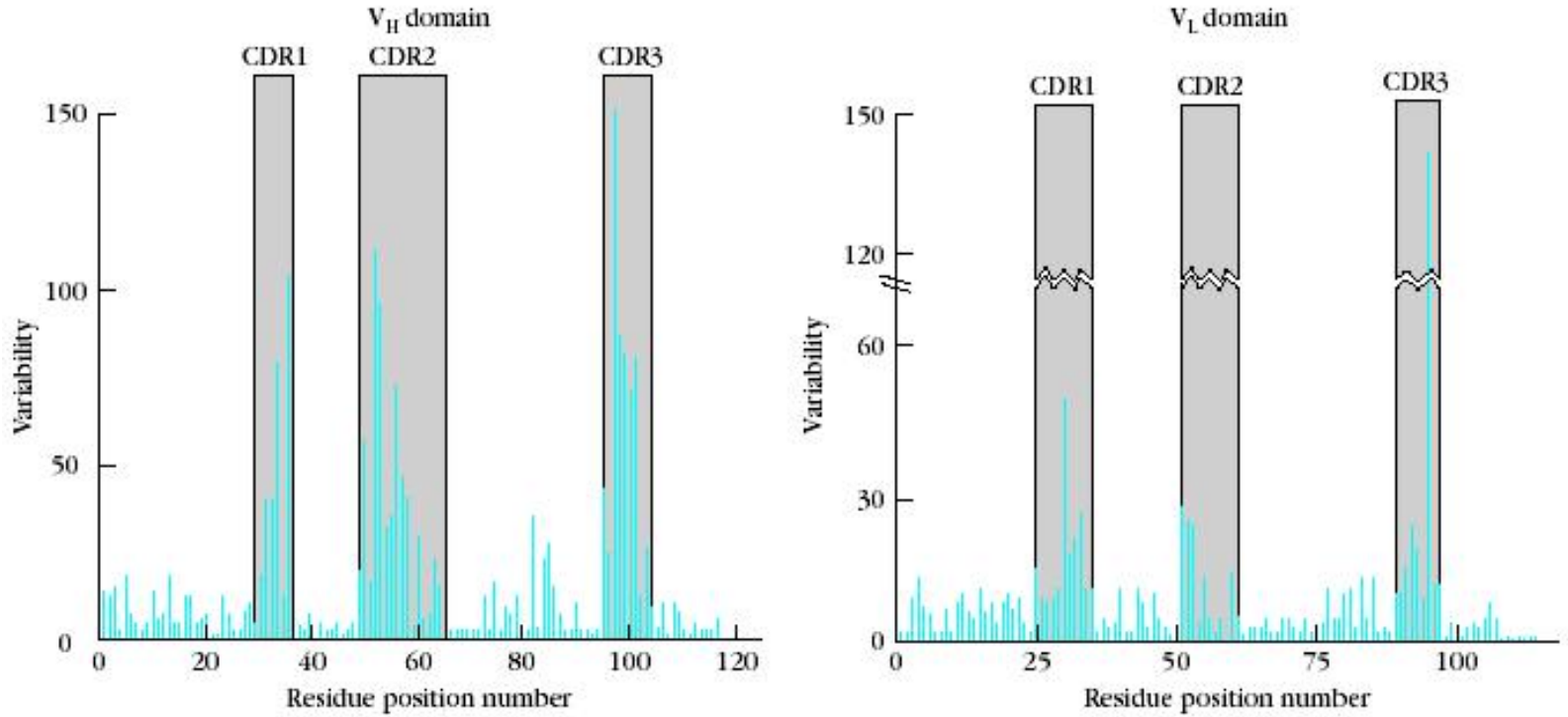
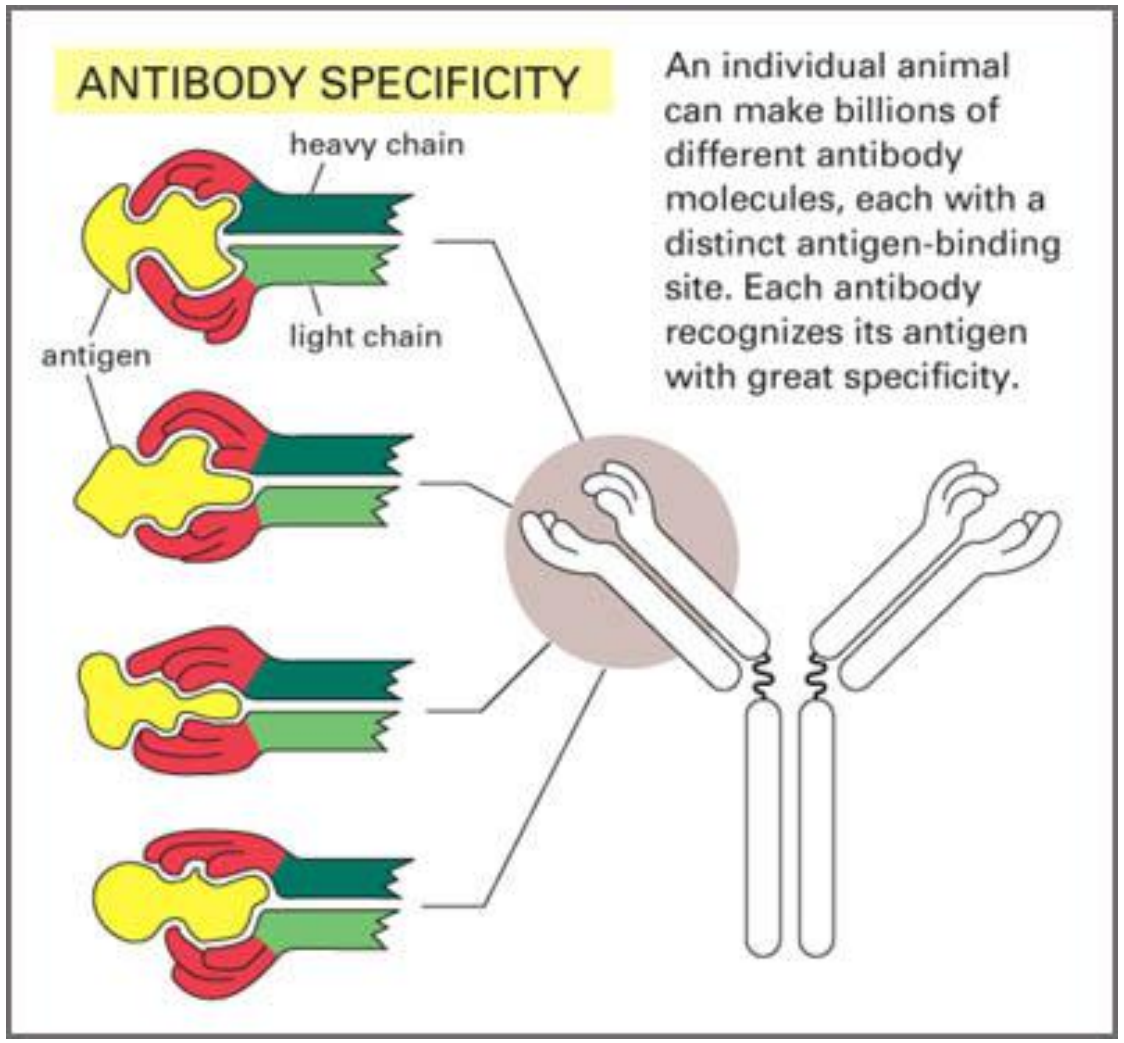


FIGURE 4-9 Variability of amino acid residues in the V_L and V_H domains of human antibodies with different specificities. Three hypervariable (HV) regions, also called complementarity-determining regions (CDRs), are present in both heavy- and light-chain V domains. As shown in Figure 4-7 (*right*), the three HV regions in the light-chain V domain are brought into proximity in the folded structure. The same is true of the heavy-chain V domain. *[Based on E. A. Kabat et al., 1977,* Sequence of Immunoglobulin Chains, *U.S. Dept. of Health, Education, and Welfare.]*

抗体的CDR(complementarity determining regions)突变频率较高，对抗体识别抗原有重要的意义。

Antibody has a higher specificity and affinity to bind to target protein.



抗体药物(Ab drug)：

可以针对人体内细胞生命过程中一些重要的生理和病理药物靶点，发挥特异性治疗作用。

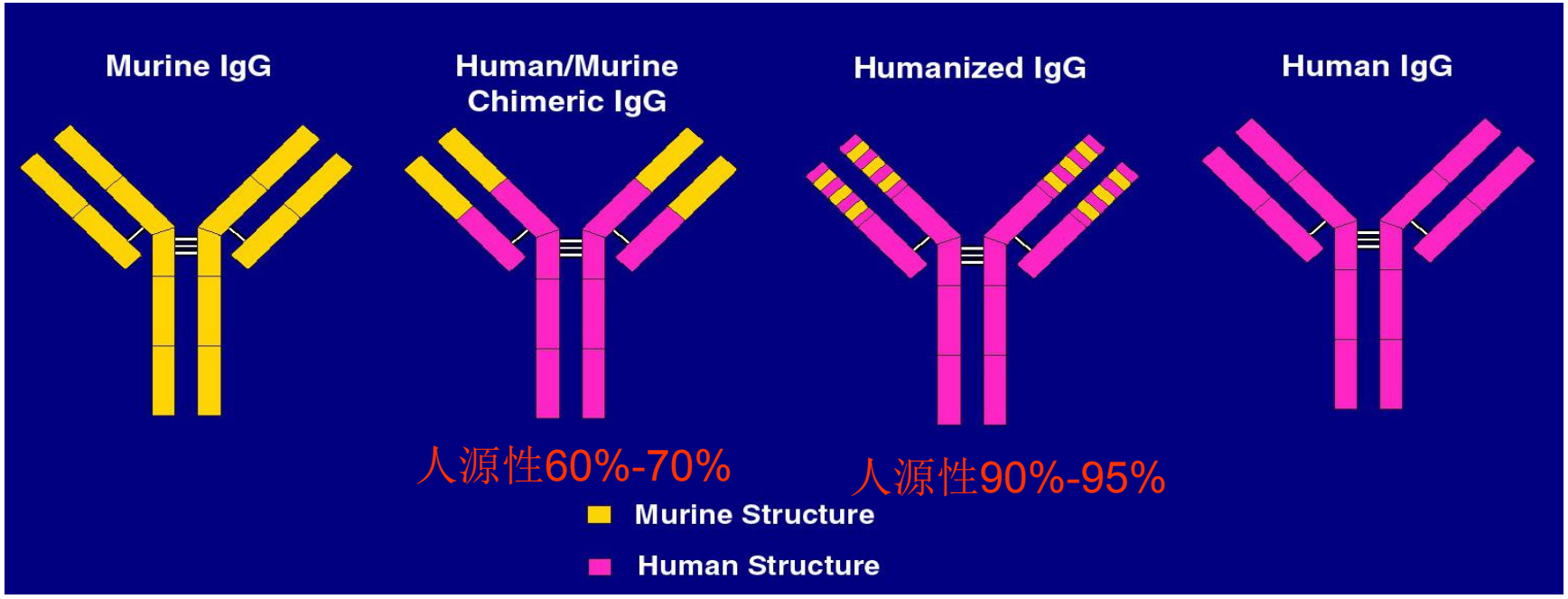
基因工程抗体的分类（1）

1.人源改造抗体

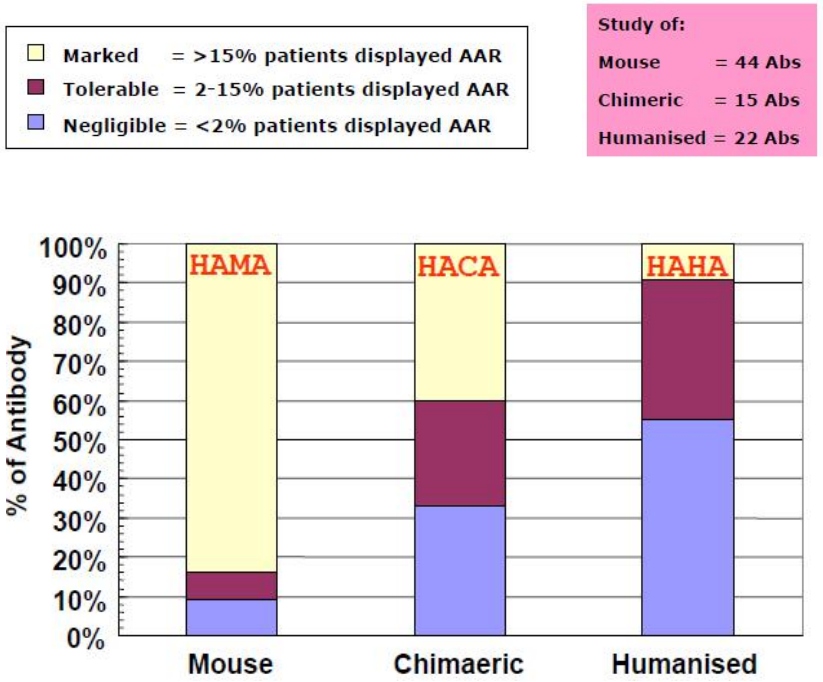
（1）**嵌合抗体（chimeric antibody）**

（2）人源化抗体（humanized antibody）

（3）**全人源抗体 （fully human antibody)**



抗体人源化改造



Hwang & Foote (2005)

To reduce HAMA(Human-anti-mouse antibody) or HACA(Human-anti-chimeric antiboy)reaction.

降低人抗小鼠抗体/ 人抗嵌合抗体反应

Types of mAbs

Murine	Entirely murine amino acids	'o' = mouse e.g. muromonab
Chimeric	Human constant (C) + murine variable (V) regions	'xi' = chimeric e.g. rituximab
Humanized	Murine complementarity determining regions (CDRs)	'zu' = humanized e.g. alemtuzumab
Human	Entirely human amino acids	'u' = human e.g. adalimumab

单克隆抗体药物命名规则

抗体药物中鼠源成分的问题

- 潜在的过敏反应风险
- 产生抗药抗体，再次使用抗体药物时被体内抗药抗体中和，影响疗效

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基因工程抗体的分类（2）

2. 小分子量抗体

（1）Fab片段

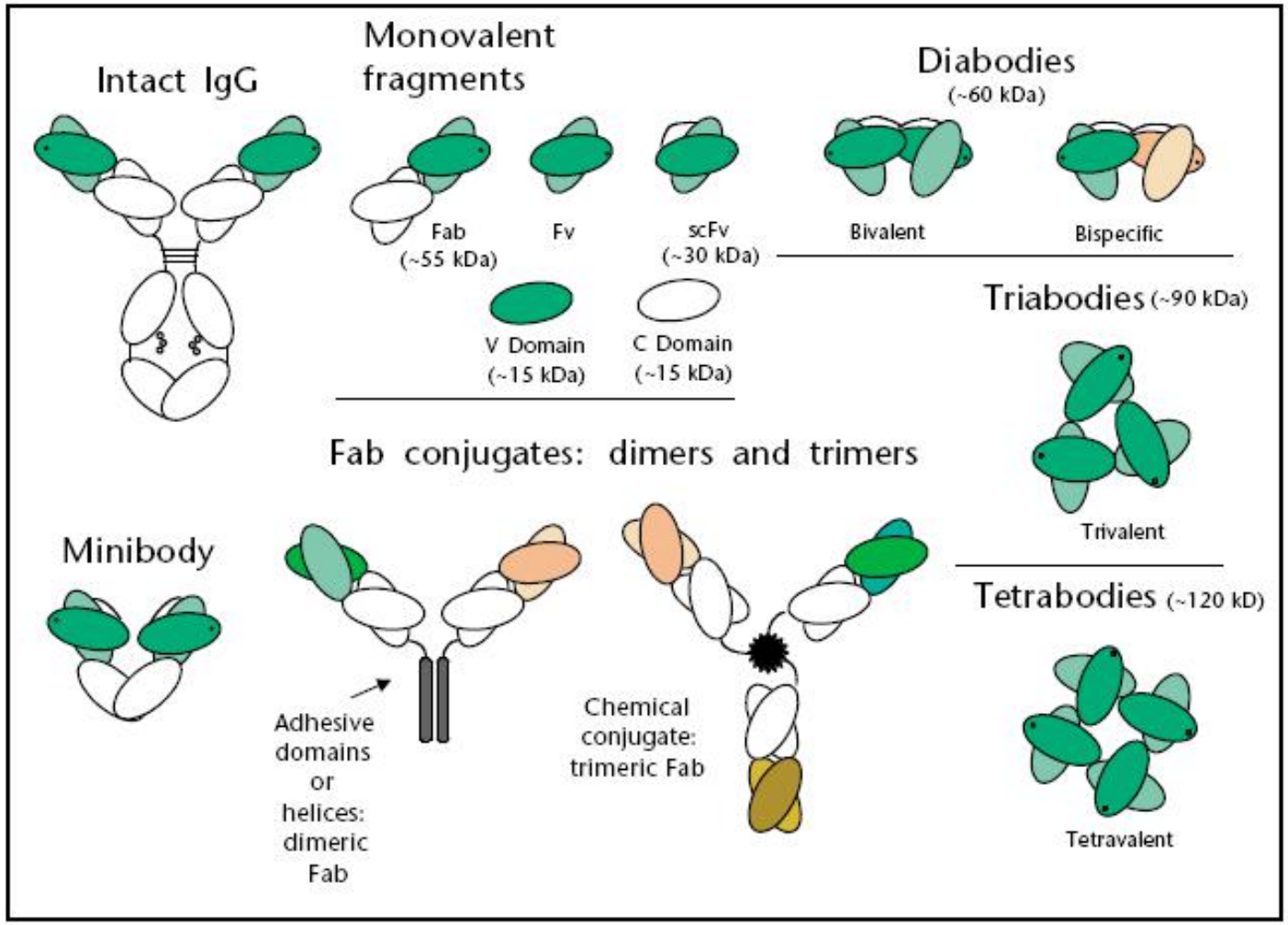
（2）单链抗体（single chain antibody，scFv）、双链抗体、三链抗体

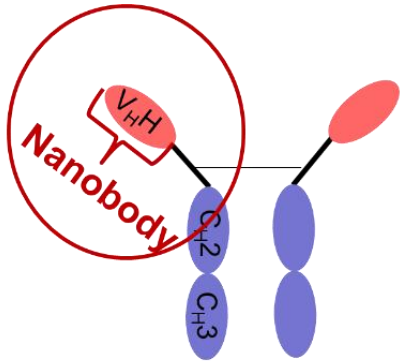
（3）微型抗体（minibody，两个scFv与抗体CH3区连接）

（4）其他形式抗体（细胞内抗体、催化抗体、免疫脂质体、最小结合单位等）

（5）Nanobody：驼类产生的单链抗体

小分子量抗体的分类

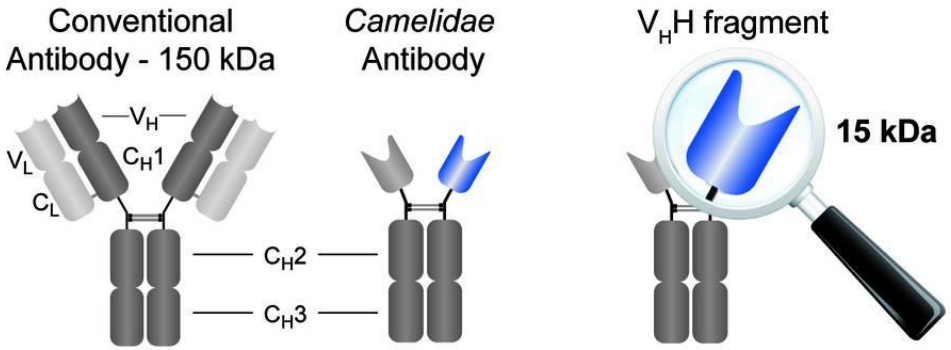




Single-chain IgG

Nanobody: Single-Chain IgG from Llama

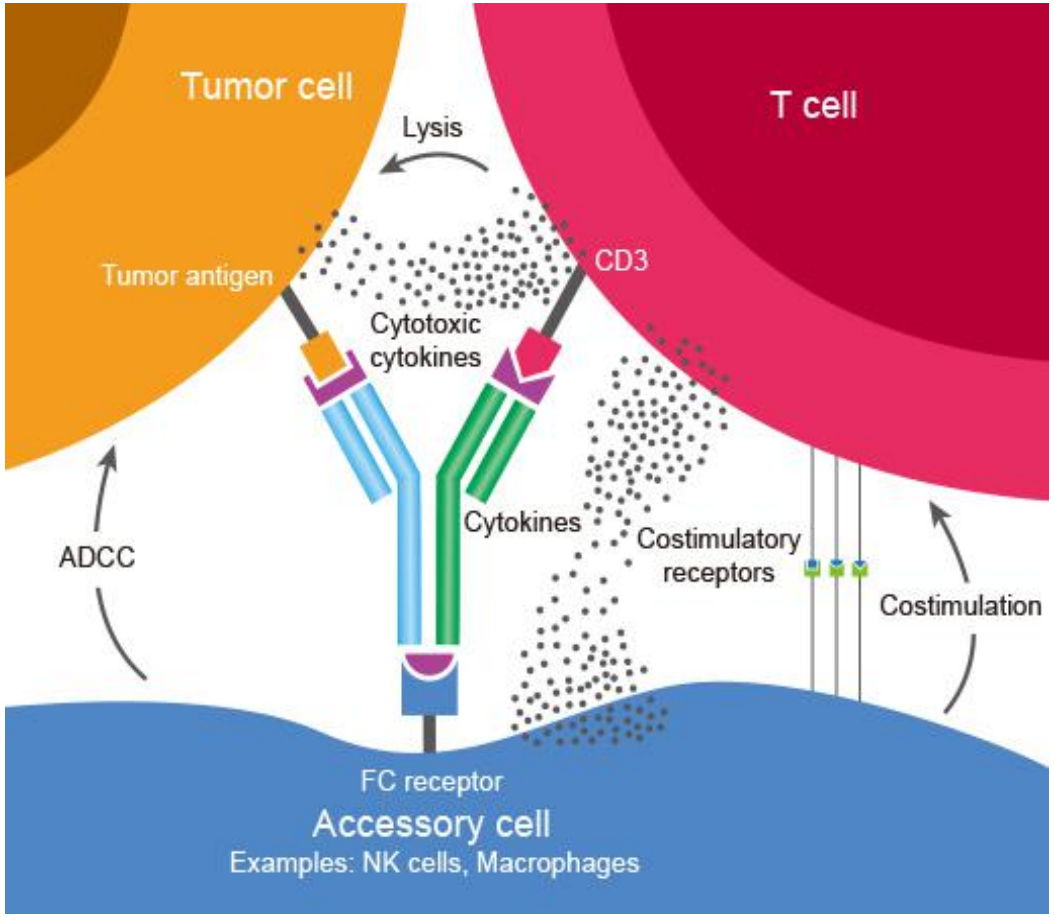
- A typical single-chain IgG antibody produced in camelid family (e.g. llama)



- Retain high affinity for antigen without light chain
- Antigen binding domain can be cloned and expressed to make “Nanobodies”:
  - Extremely Cheap & Unlimited Amounts
  - Tiny (~15 kDa) , Fold well & Stable in Solution
  - Easily Engineered for Special Needs: cross the blood-brain barrier, stable.

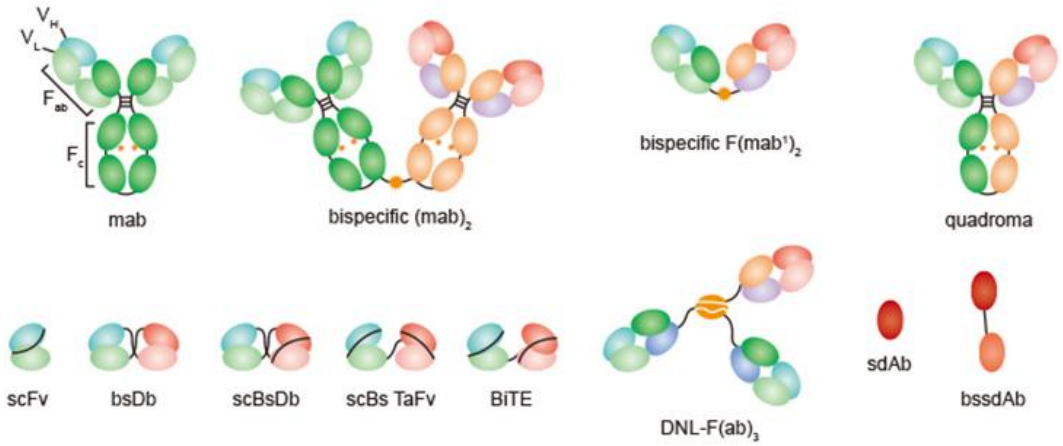
- CAR-T

3. 双特异性抗体（Bispecific antibody）



- 同时结合两种不同的抗原（靶点）

- 应用于肿瘤免疫治疗、感染性疾病治疗以及药物递送等

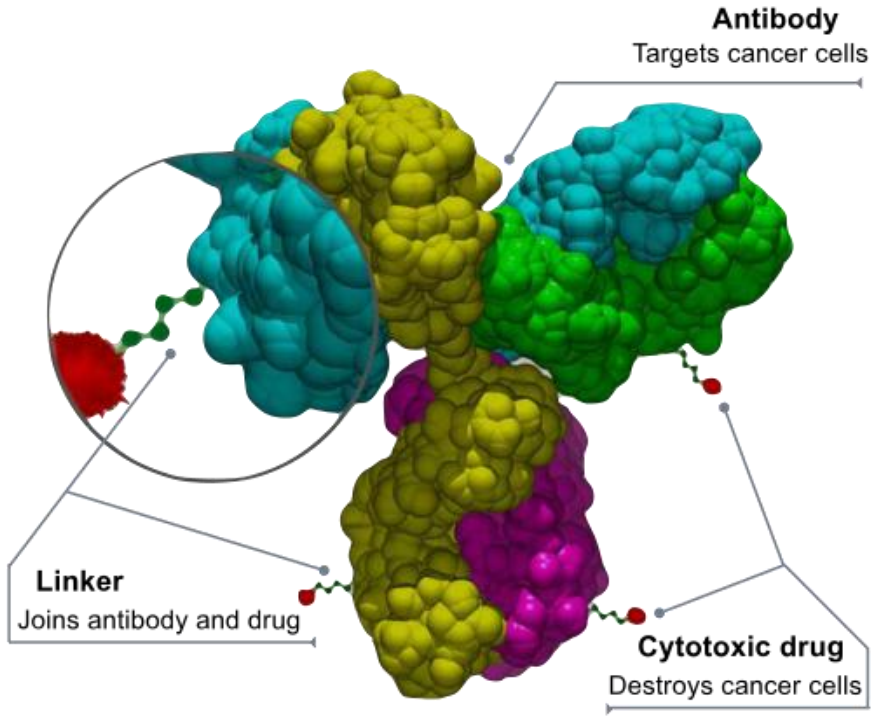


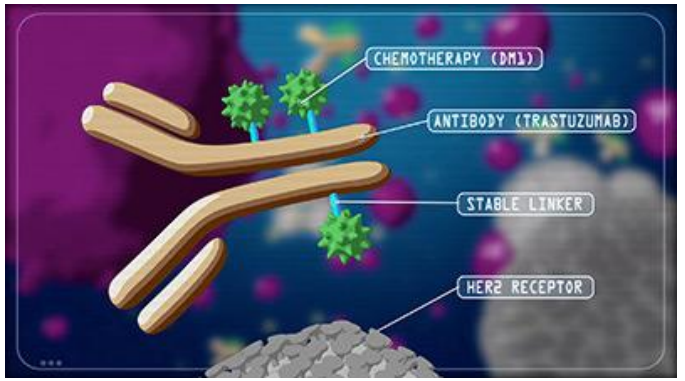
4. 抗体药物偶联（ADCs，Antibody-drug conjugates）

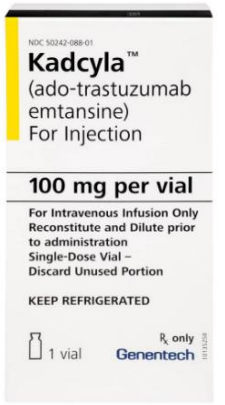
- ADC是由**抗体**与具有生物活性的**细胞毒(抗癌) 药物**相连接组成的复杂分子。

- ADC中抗体部分靶向癌细胞，细胞毒性药物被释放并杀死癌细胞。

- Kadcyla ： anti-HER2 +DM1







抗体药物在研发及应用中存在的问题：

- 使用杂交瘤技术制备单抗，故所得鼠源性单抗，必须人源化，在临床上可减少异源性蛋白所引起的过敏降低抗药抗体的产生以增加疗效。
- 鼠源抗体人源化后，抗体效价可能会有明显降低 。
- 全长抗体药物分子量较大，进入胞内、穿过生理屏障或进入肿瘤微环境发挥作用可能会被受限。
- 临床治疗需要大量的抗体(克级），故需要生物反应器制备抗体。由于抗体药物的研发及生产成本较高，造成抗体药物价格较高。

基因工程抗体目的：

- 人源化减小鼠/兔源性成份，保留抗体的抗原结合能力，降低HAMA反应（human anti mouse antibody reaction)。
- 小分子量抗体组织穿透性强，具有独特的理化性质
- 双特异性抗体发挥独特生理功能
- 工程化，易于大规模生产和临床应用。

基本原理：

- 借助基因工程手段，将编码抗体的重轻链可变区基因重组到表达载体上并进行抗体的重组表达。

基因工程抗体的表达

- 原核细胞和酵母可以用于表达小分子量抗体和抗体片段；
- 大部分完整抗体和双特异性抗体等需要在CHO等哺乳动物细胞中表达；
- 利用完整的动植物体通过转基因的方法表达外源蛋白：如利用转基因烟草生产抗狂犬病毒抗体，抗Ebola病毒抗体。

基因工程抗体的优点和缺点

- 优点：
  - 不受动物品系（species)和抗体类型（isotype)的限制。
  - 使动物来源抗体人源化，减少抗体药物本身潜在的T/B细胞抗原表位，降低抗体药物免疫原性，增强疗效。
  - 全人源化抗体，可以降低抗体的异源性和免疫源性，最大化提升抗体的的疗效。
- 缺点：
  - 不成熟的人源化技术活亲和力成熟技术可能带来更加严重的抗药体抗体反应。

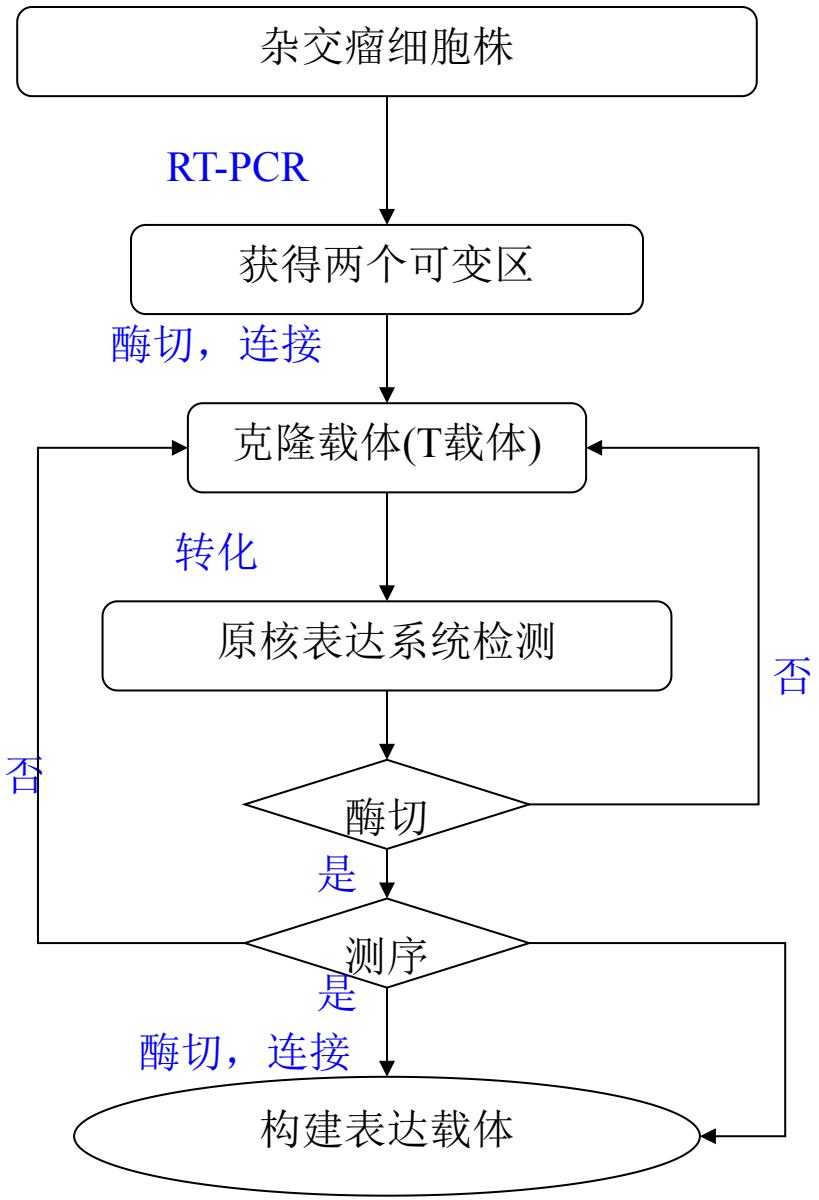
第三节： 基因工程抗体制备的主要方法

1.人鼠嵌合抗体（Chimeric Antibodies）

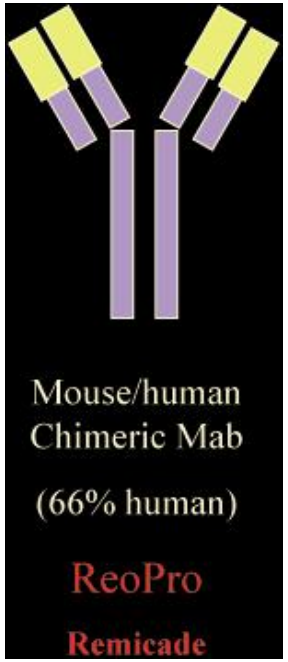
- 原理：利用基因重组技术，把鼠抗体的重轻链可变区部分与人抗体重轻链恒定区的进行重组，减少鼠源结构，增加人源结构，而保持抗体与原抗原的特异性结合。

- 缺点：
  - ✓鼠抗体部分亦能作为一种异种抗原，多次反复使用在人体产生抗体及过敏反应（HAMA反应，human against mouse antibody reaction)。
  - ✓嵌合抗体可保持特异性结合和外源性抗原降低，但亲和力可能下降。

Chimeric antibodies

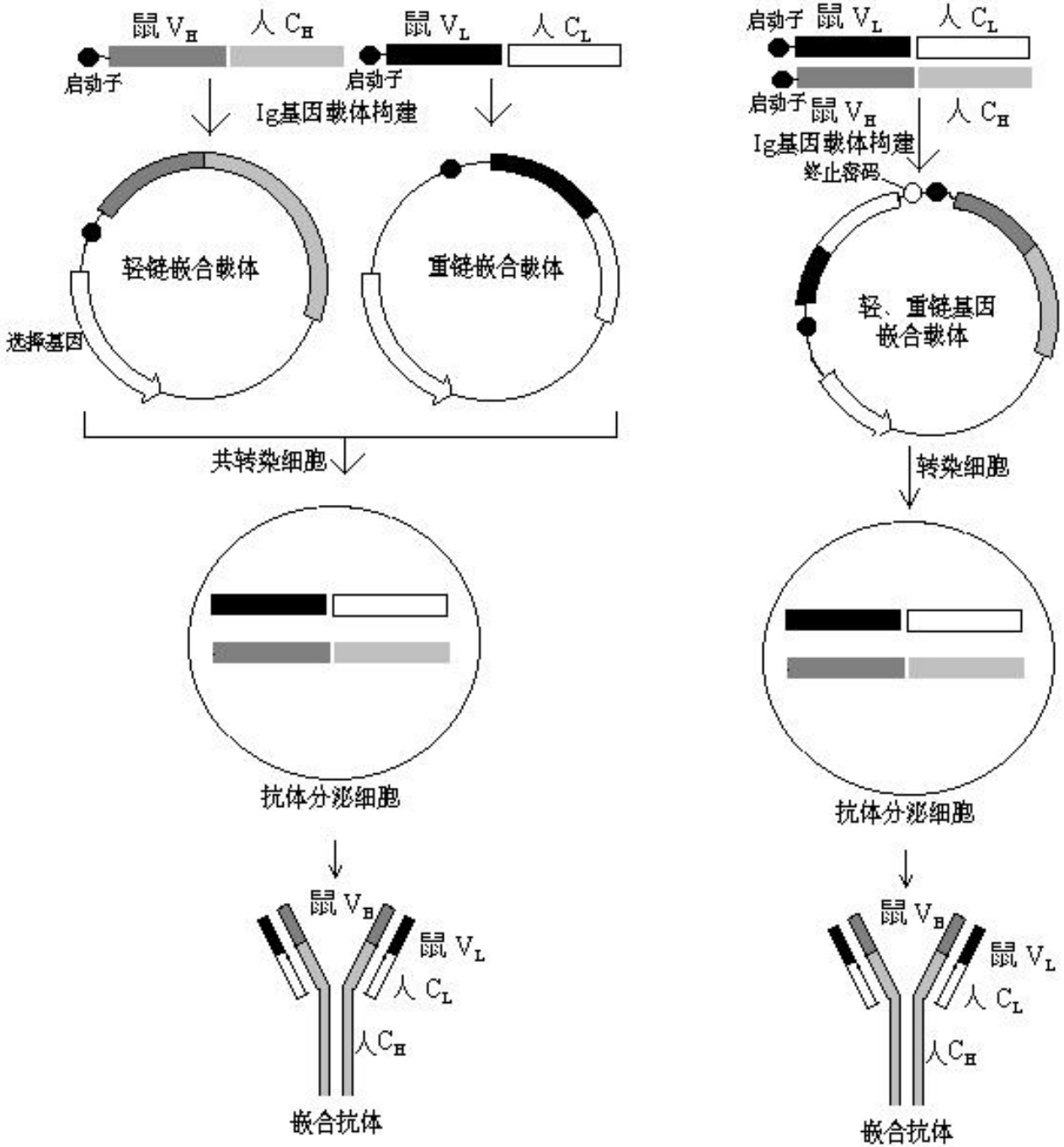


1. 获得鼠单抗重轻链可变区的基因片段。
2. 基因片段插入含有人IgG重轻链恒定区的表达载体。



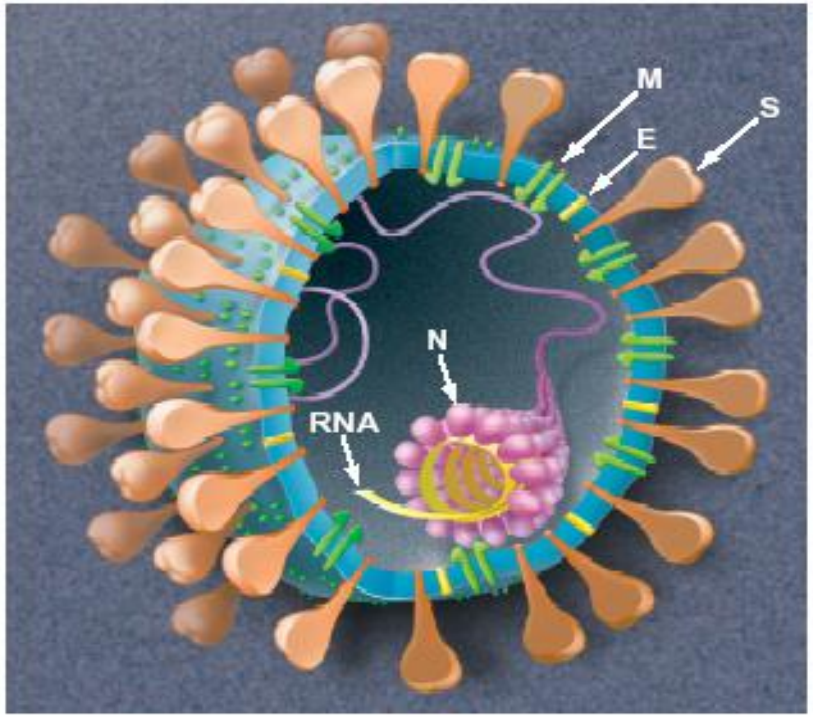
人鼠嵌合抗体的真核表达：CHO细胞

（共转染模式和单载体转染模式）



抗SARS 病毒中和性嵌合抗体（案例1）

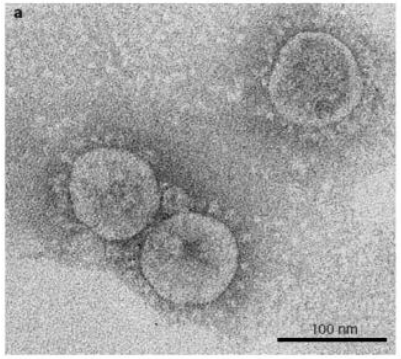
Until 2003,7， 8437 people have been infected with SARS over the 32 countries, in which 813 patients were died form disease. The disease incidence is about 10.5%. There are many unresolved questions about disease pathogenesis, treatment and *diagnosis.*

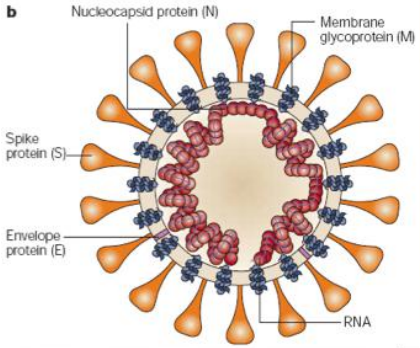


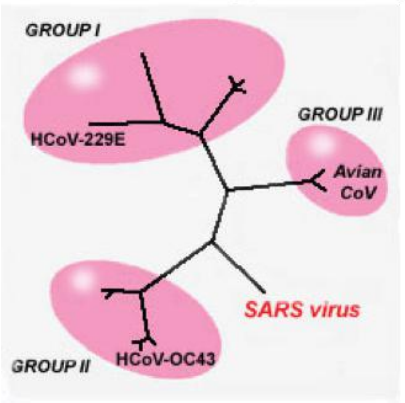
Coronavirus organization. A model of the coronavirus structure showing the organization of the spike (S), membrane (M), and envelope (E) glycoproteins. The RNA is protected by a helical capsid of N protein monomers.

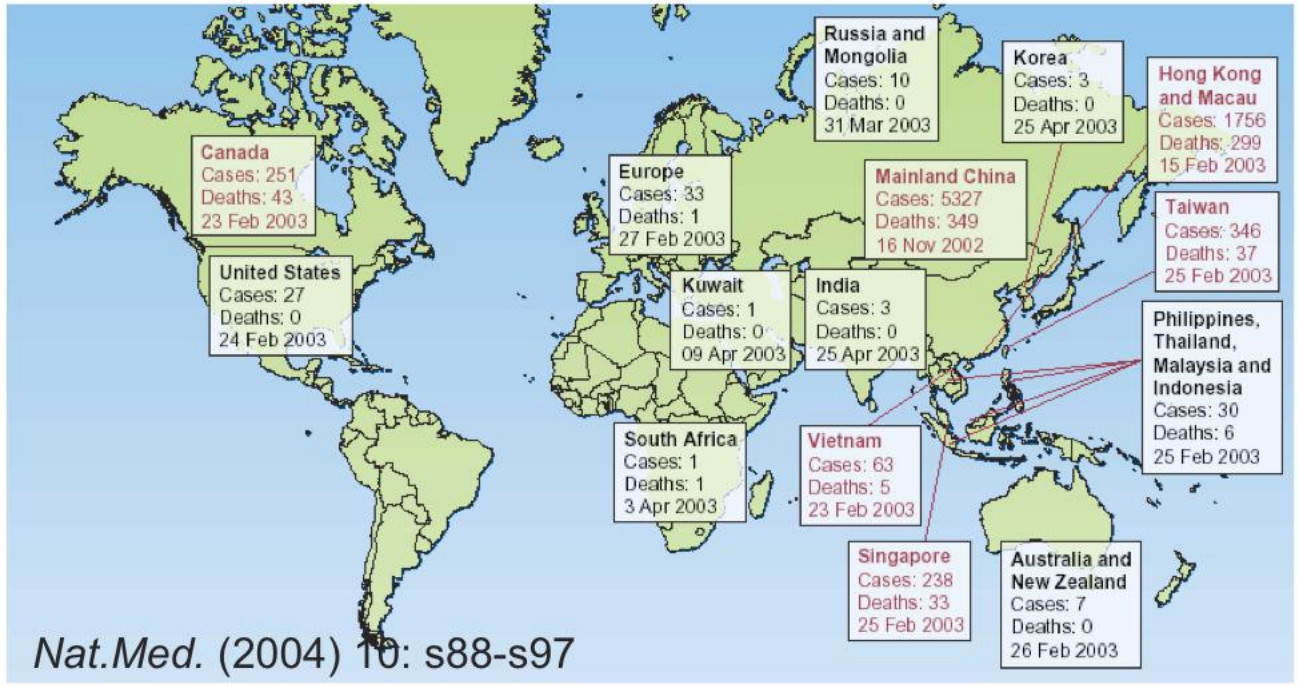
SARS

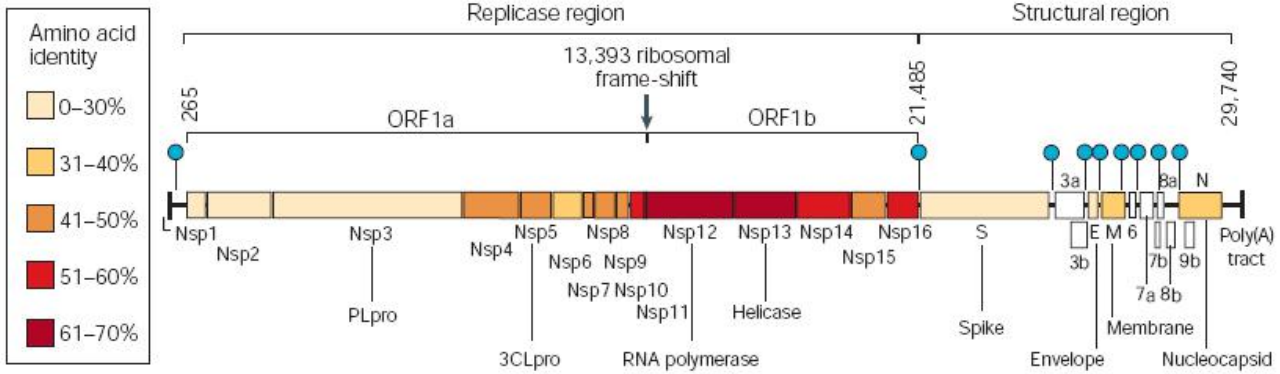
The global spread of SARS





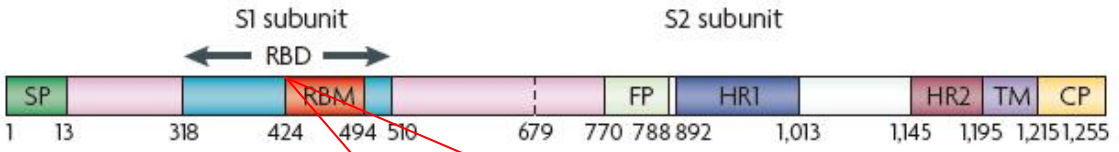


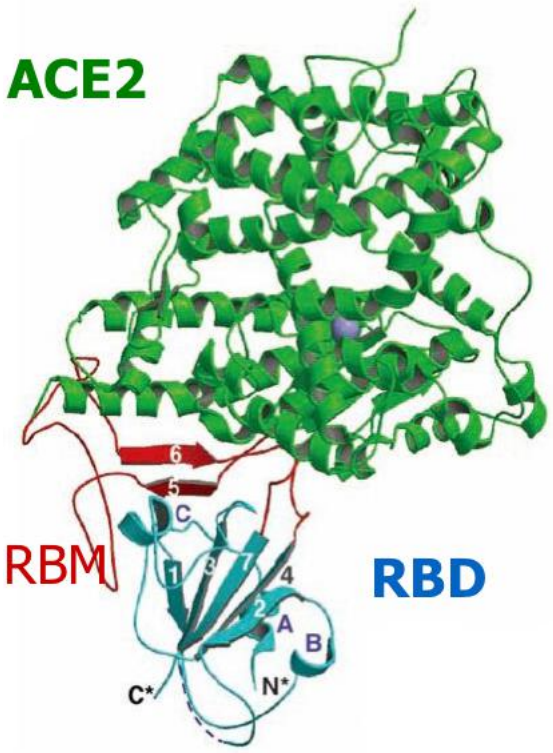




Stadler, K. et al. *Nat. Rev. Micro.* 2003 Vol 1 p210

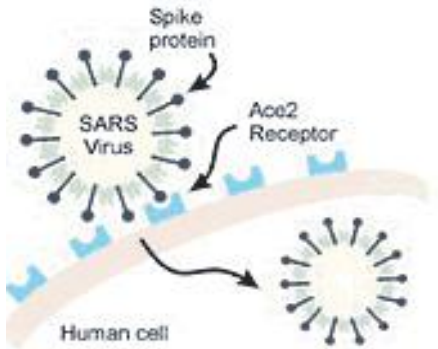
The structure of SARS Spike protein





Spike protein especially the receptor-binding domain (RBD) is the main target of neutralizing antibodies.

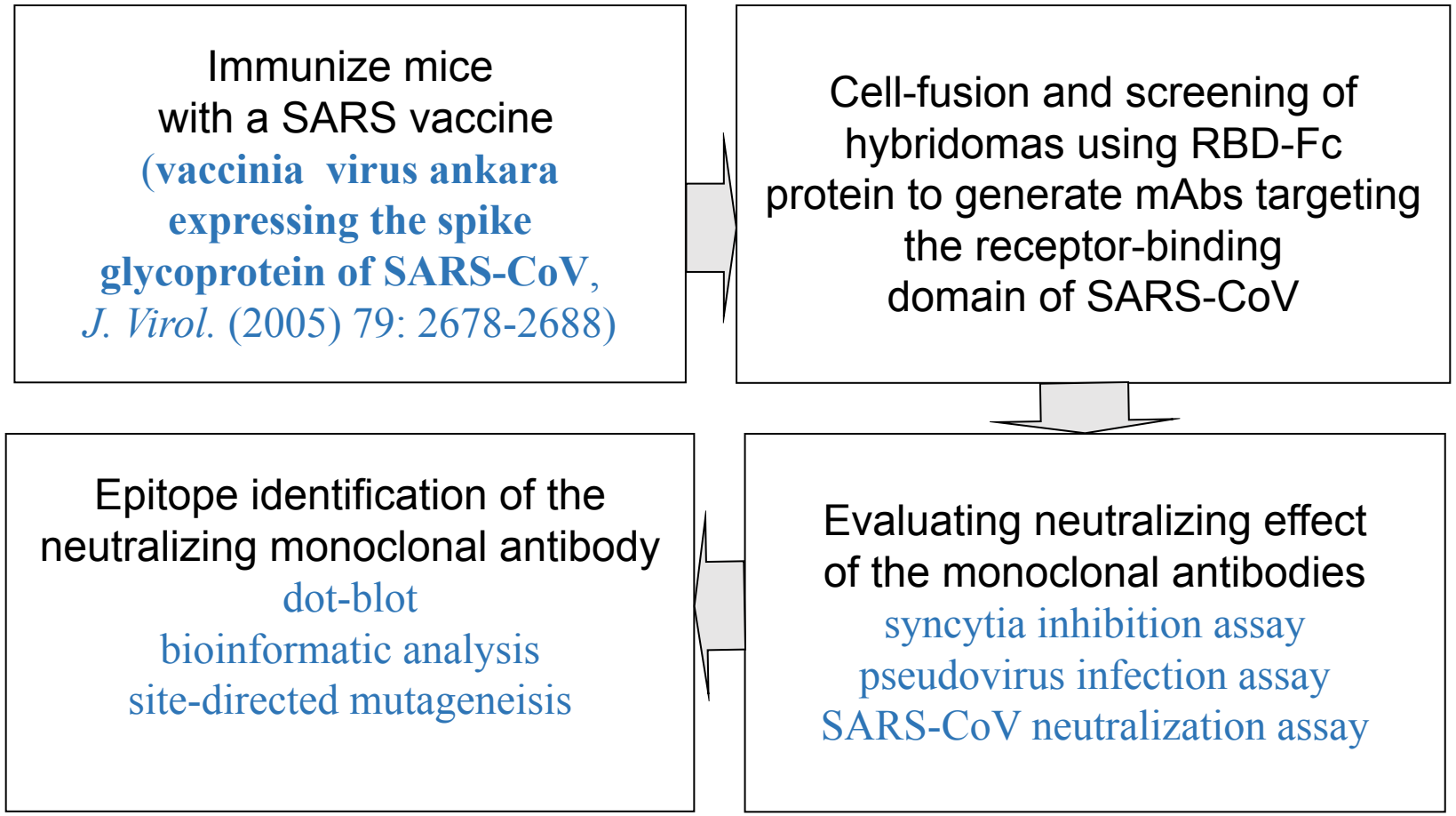
Nat. Rev. Microb. (2009) doi:10.1038



SARS virus enters cell via Ace2 receptor.

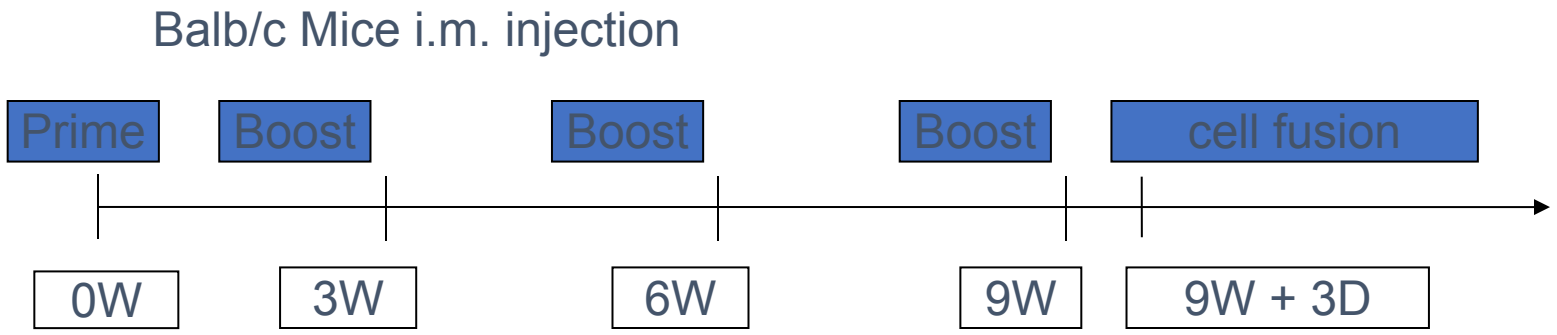
Science (2005) 309: 1864-1868

SARS 中和性抗体研究计划



制备抗SARS冠状病毒中和性鼠单克隆抗体

免疫程序



细胞融合

PEG Method

Immunized Mouse Spleen cells

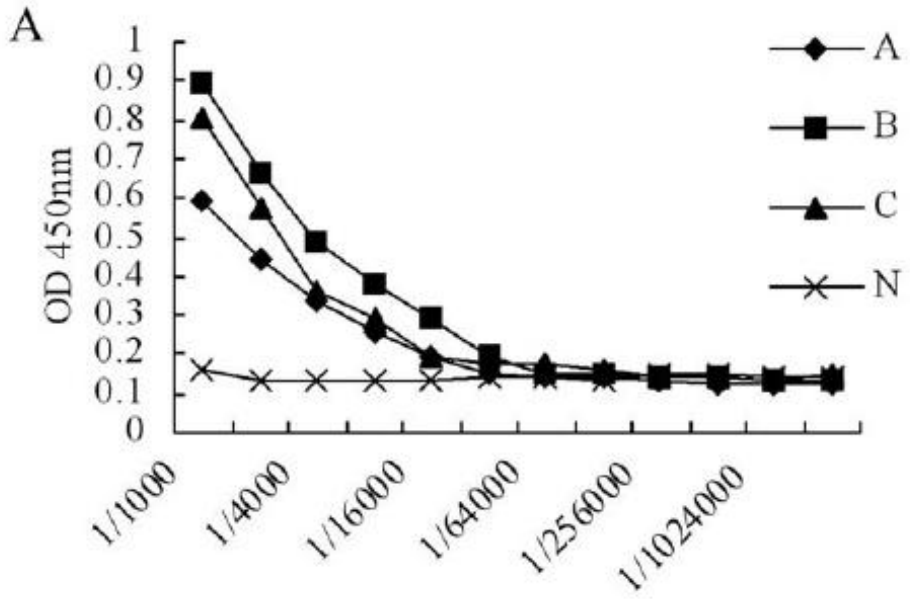
+

Fusion Partner Cell Lines: NS-1 and SP2/0

↓

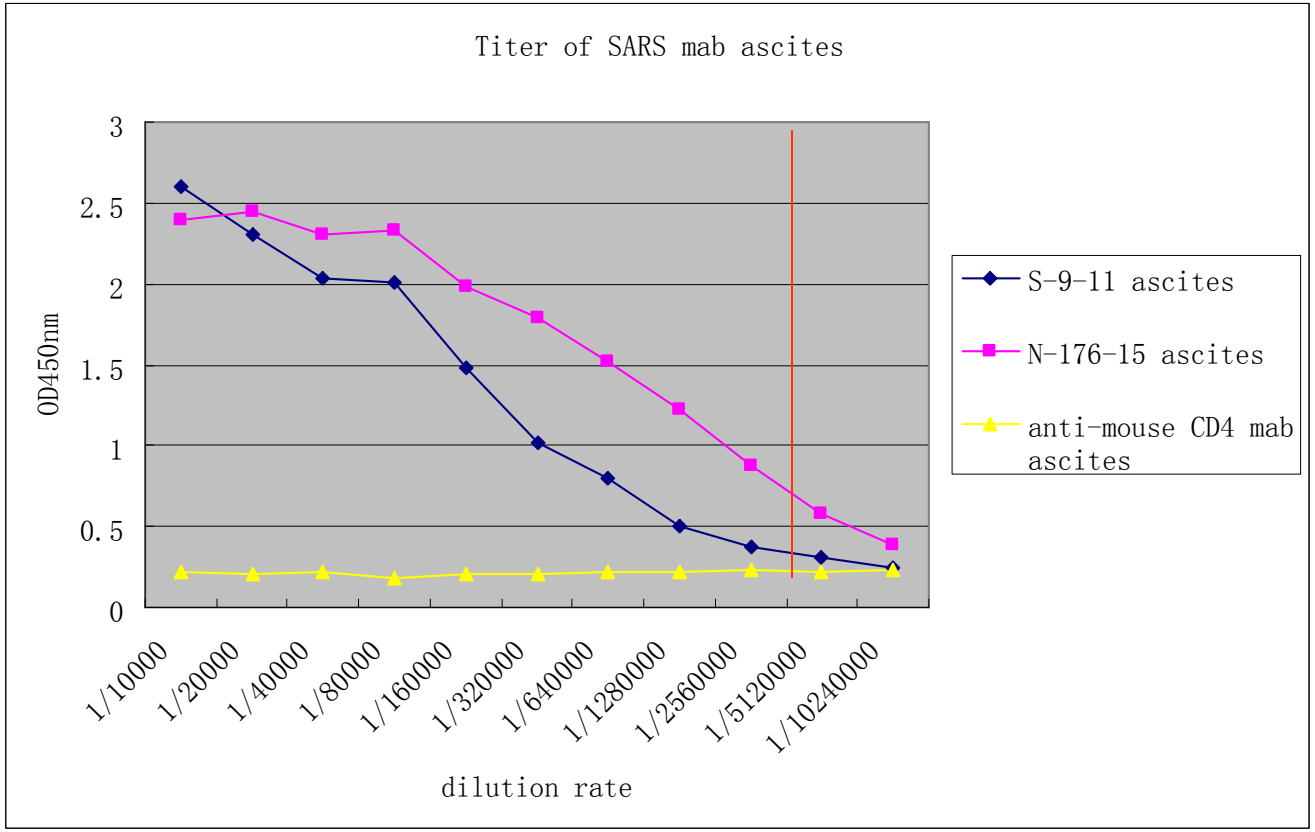
Hybridomas

RBD-specific Ab were tested using ELISA in sera collected after immunization of the SARS vaccine



Monoclonal antibody generation

Two hybridoma clones were selected and tested by ELISA



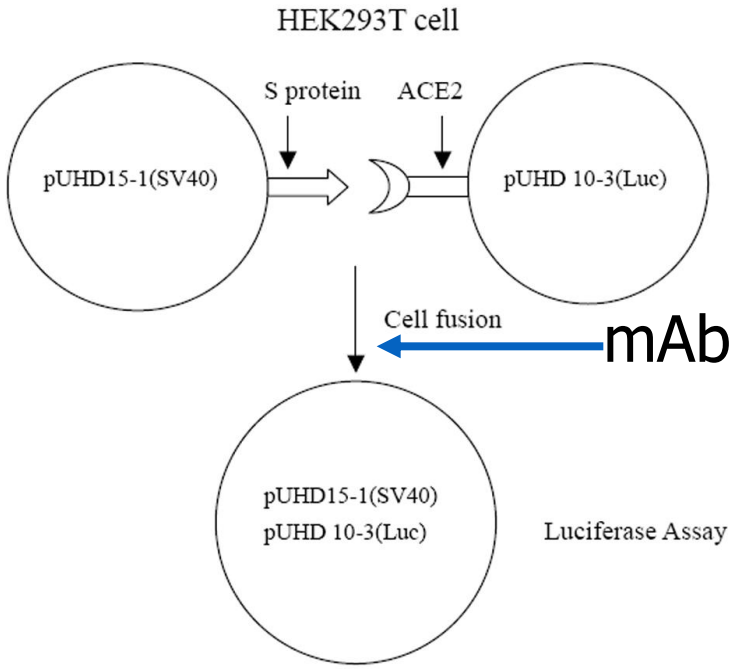
The titer of ascites can reach 1:512,000

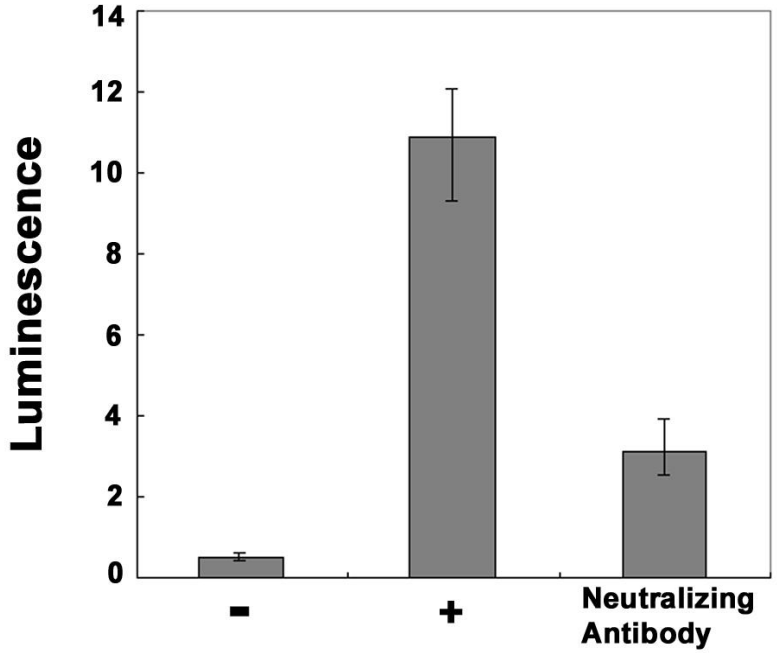
In vitro evaluation of SARS mAb’s neutralizing effect

1. **Syncytia Inhibition Assay**

2. **SARS Virus Neutralization Assay**

Syncytia Inhibition Assay.

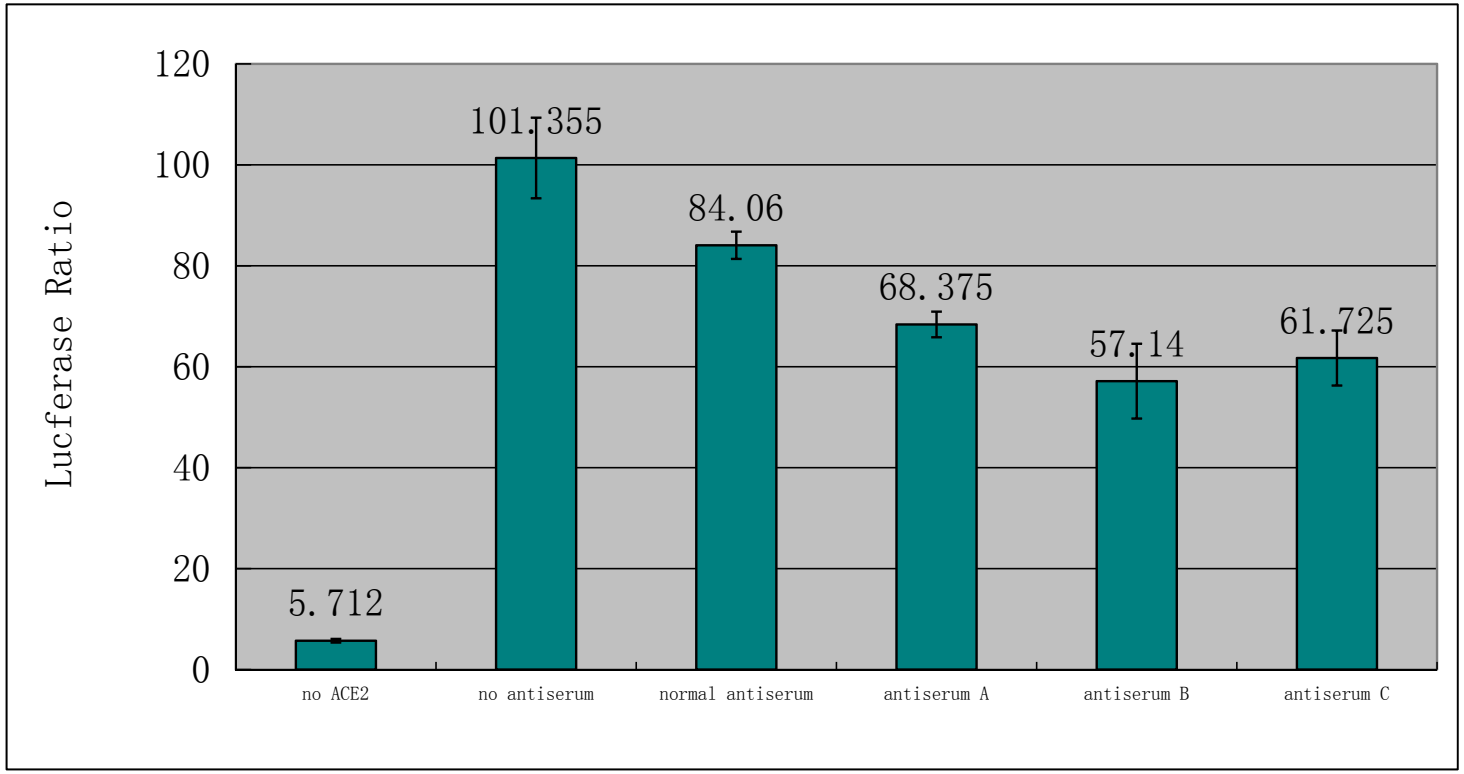




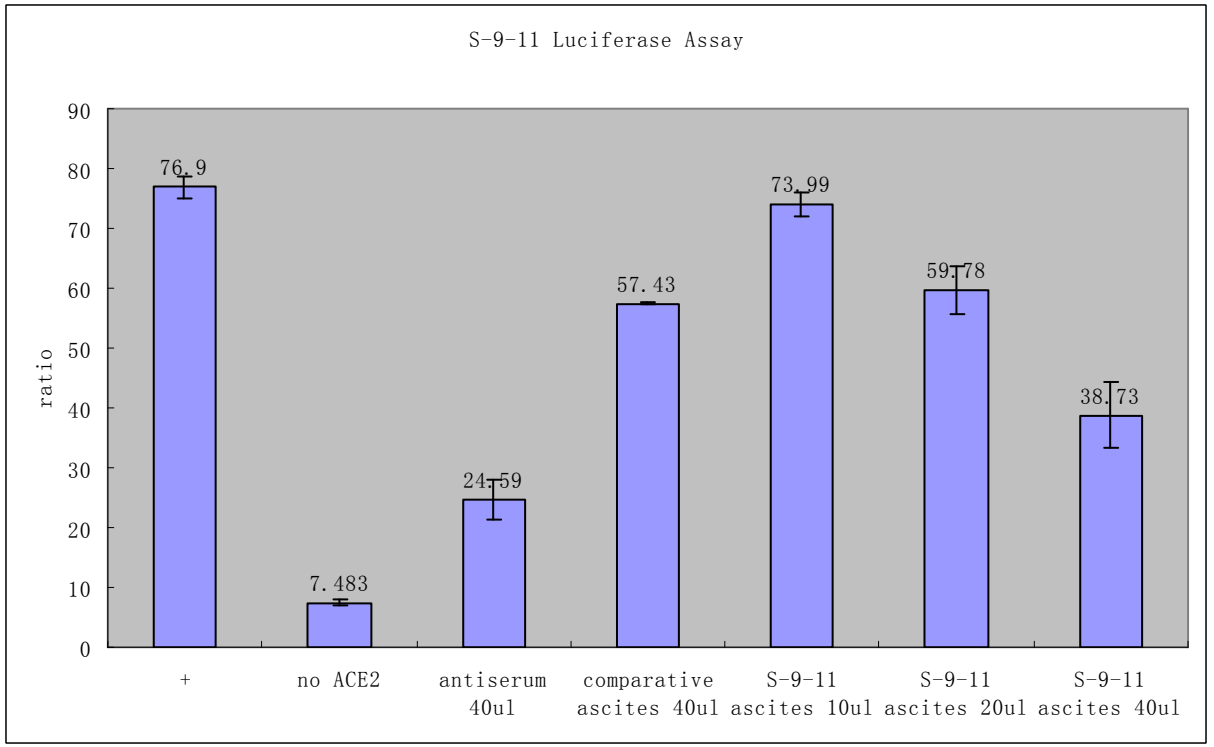
FEBS Letters 2005, 579: 2130-2136

Syncytia Formation assay has been developed in the Lab. The system is valuable in detecting the interaction between ACE2 receptor and spike protein of SARS. The anti-SARS neutralizing mouse mAb is being engineered into mouse-human chimeric mAb which has a therapeutic activity in human.

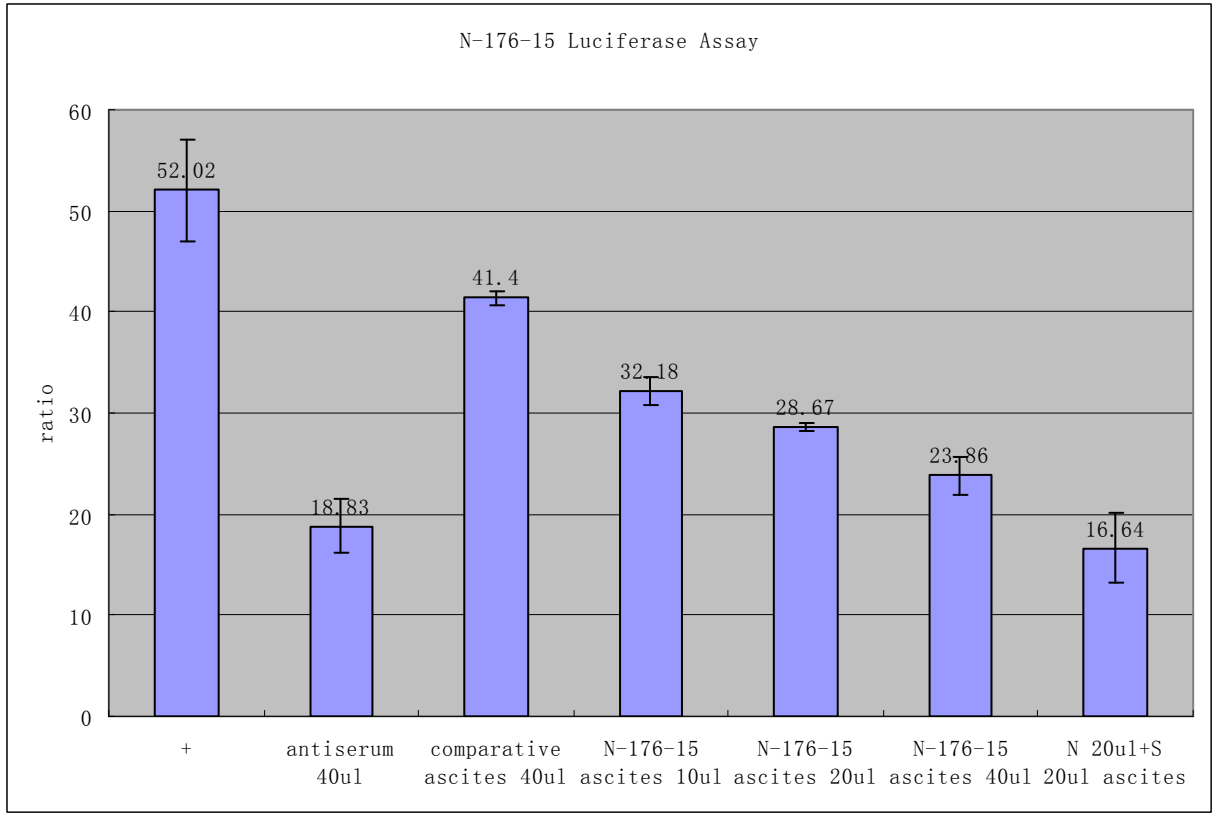
Syncytia Inhibition Assay: Mouse antiserum after the second immunization



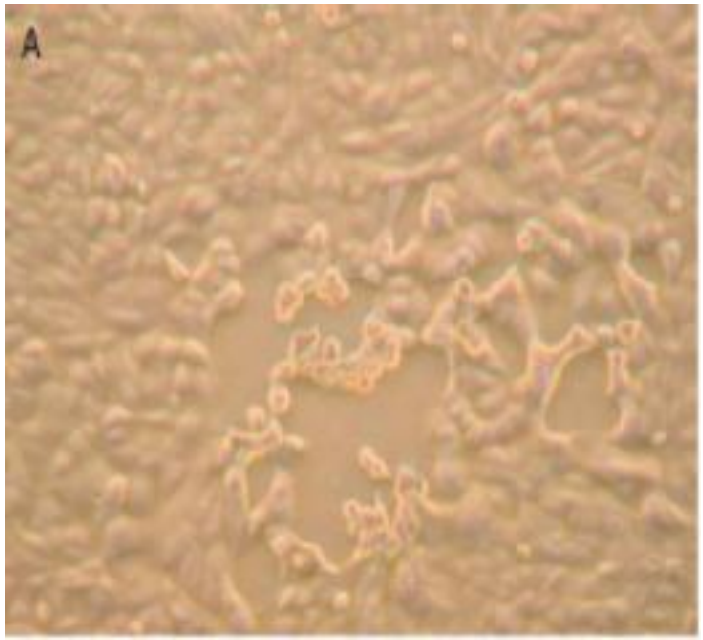
Syncytia Inhibition Assay:S-9-11 mab ascites

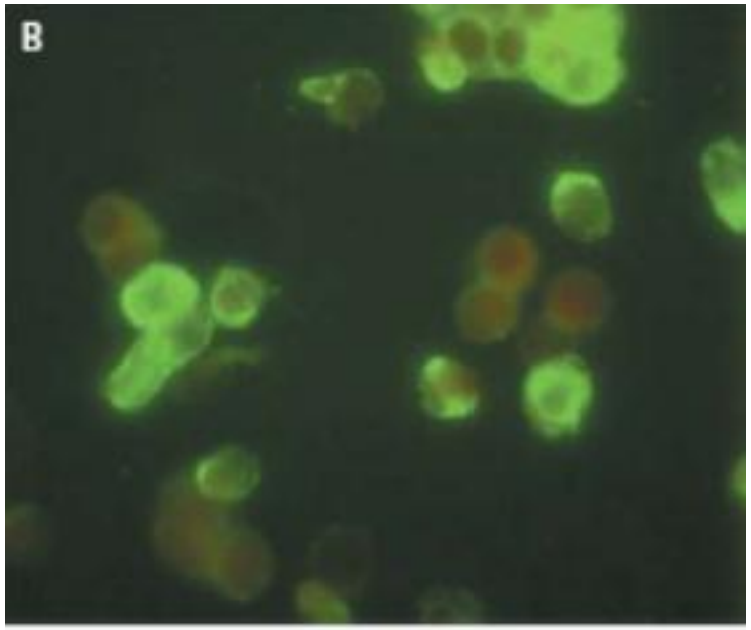


Syncytia Inhibition Assay: N-176-15 mab ascites



SARS Virus Neutralization Assay

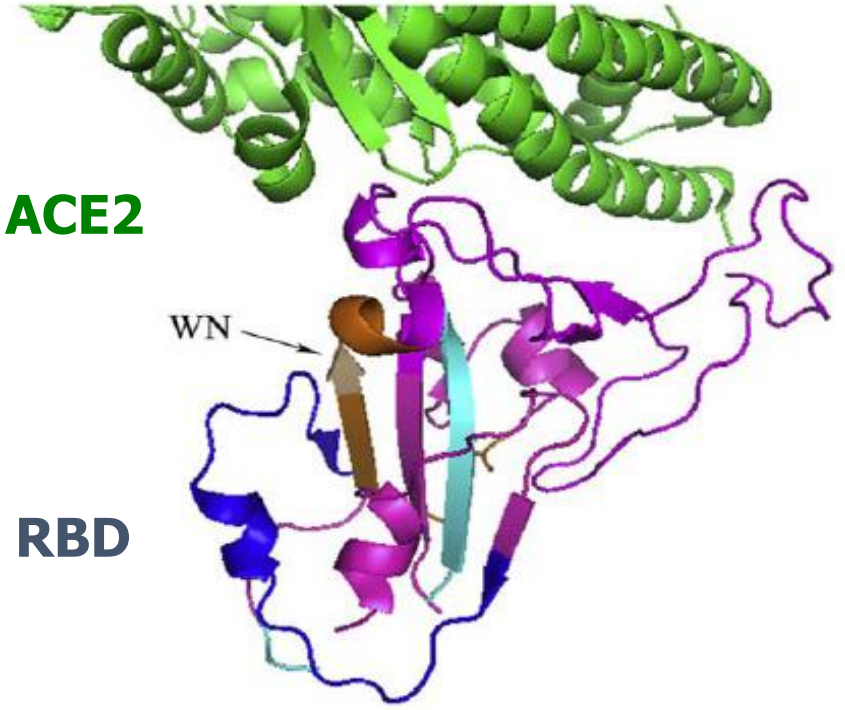




The typical early cytopathic effect was observed in SARS-CoV infected cultured-cells in P3 lab of Hong Kong University

Special amino acids (423W and 424 N) are essential to be recognized by the neutralizing mAbs. The two residues are conserved in all strains isolated from human. (**Virology, 2008**)

Spatial location of the epitope predicted using a computer-analysis model



epitope:
aa. 343-367,373-390,411-428
W423,N424: crucial site

SARS 中和性抗体相关专利及文章

证书号第751627号

发明专利证书

发明名称：一种SARS冠状病毒药物靶点及其应用

发明人：孙兵;郑伯健;吕伟;徐可

专利号：ZL 2006 1 0027476.X

专利申请日：2006年06月08日

专利权人：中国科学院上海生命科学研究院

授权公告日：2011年03月30日

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2011年03月30日

第1页（共1页）

Virology 383 (2009) 39–46

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Virology

journal homepage: www.elsevier.com/locate/yviro

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VIROLOGY

Conserved amino acids W423 and N424 in receptor-binding domain of SARS-CoV are potential targets for therapeutic monoclonal antibody

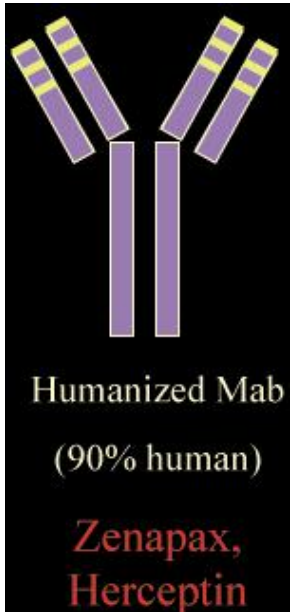
Chao Bian [a,1], Xiuqin Zhang [a,1], Xingfeng Cai [a,1], Linqi Zhang [c], Zhiwei Chen [c], Ye Zha [a], Ying Xu [a], Ke Xu [a], Wei Lu [a], Linchen Yan [a], Jianwei Yuan [a], Jiannan Feng [d], Pei Hao [e], Qidi Wang [f], Guoping Zhao [e], Gang Liu [f], Xueliang Zhu [a], Hao Shen [g,h], Bojian Zheng [i], Beifen Shen [d], Bing Sun [a,b,j,*]

[a] Laboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China
[b] Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai 200025, China
[c] Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY 10016, USA
[d] Beijing Institute of Basic Medical Sciences, Beijing 100850, China
[e] Chinese National Human Genome Center at Shanghai, Shanghai 201203, China
[f] Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
[g] Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA
[h] Shanghai Institute of Medical Genetics, Shanghai Children's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200040, China
[i] The University of Hong Kong, Hong Kong
[j] E-institutes of Shanghai Universities Immunology Division, Shanghai, China

临床应用价值：对**SARS**病毒有潜在的治疗作用。

2. 人源化抗体 Humanized antibodies

- "Humanization" or "reshaping" of murine antibodies is an attempt to transfer the full antigen specificity and binding avidity of murine monoclonal antibodies to a human antibody by grafting the murine complementarity determining regions (CDRs) onto a human variable region framework



保留鼠CDR
的人源化Ab

人源化策略 CDR-grafting

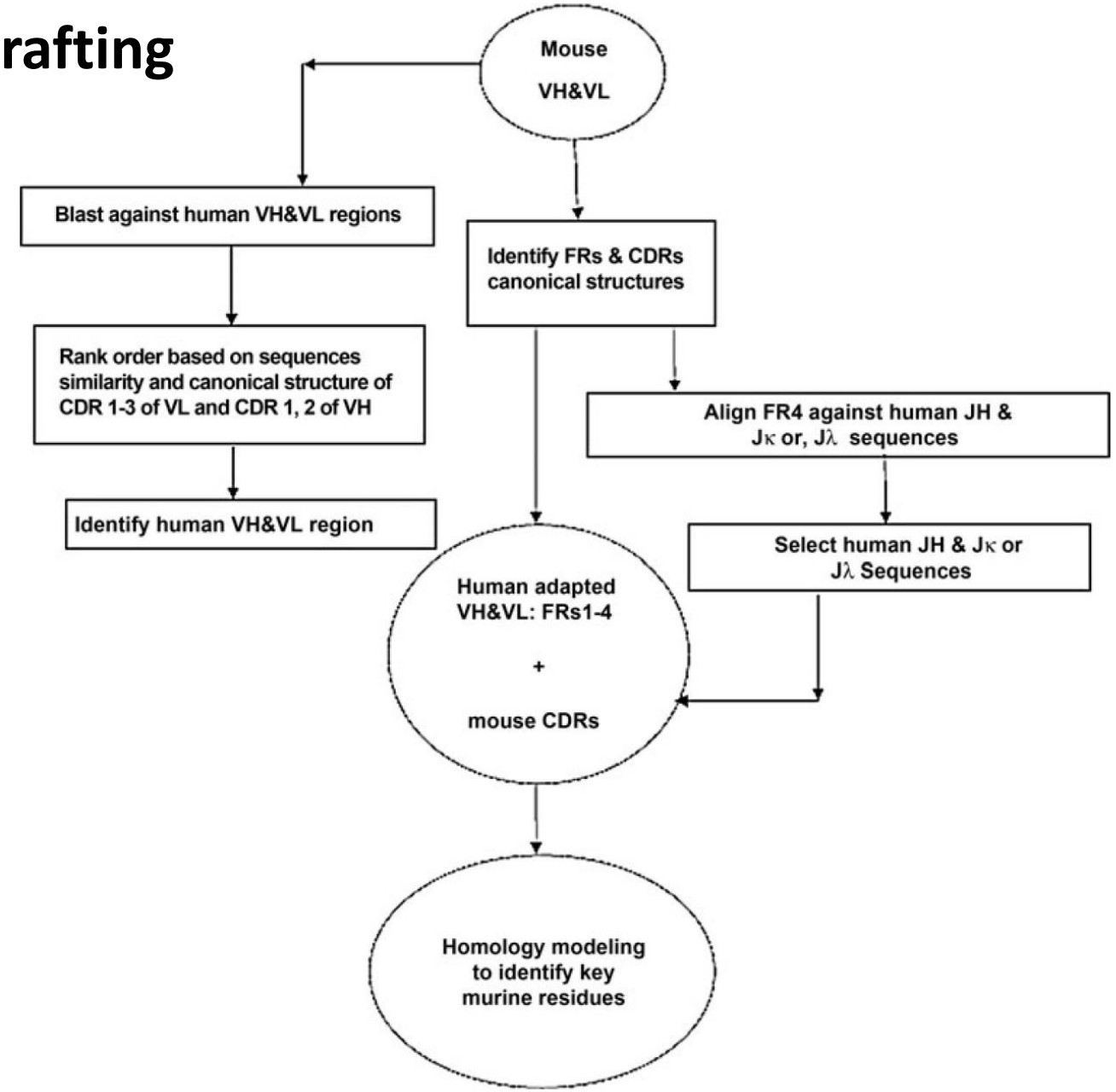
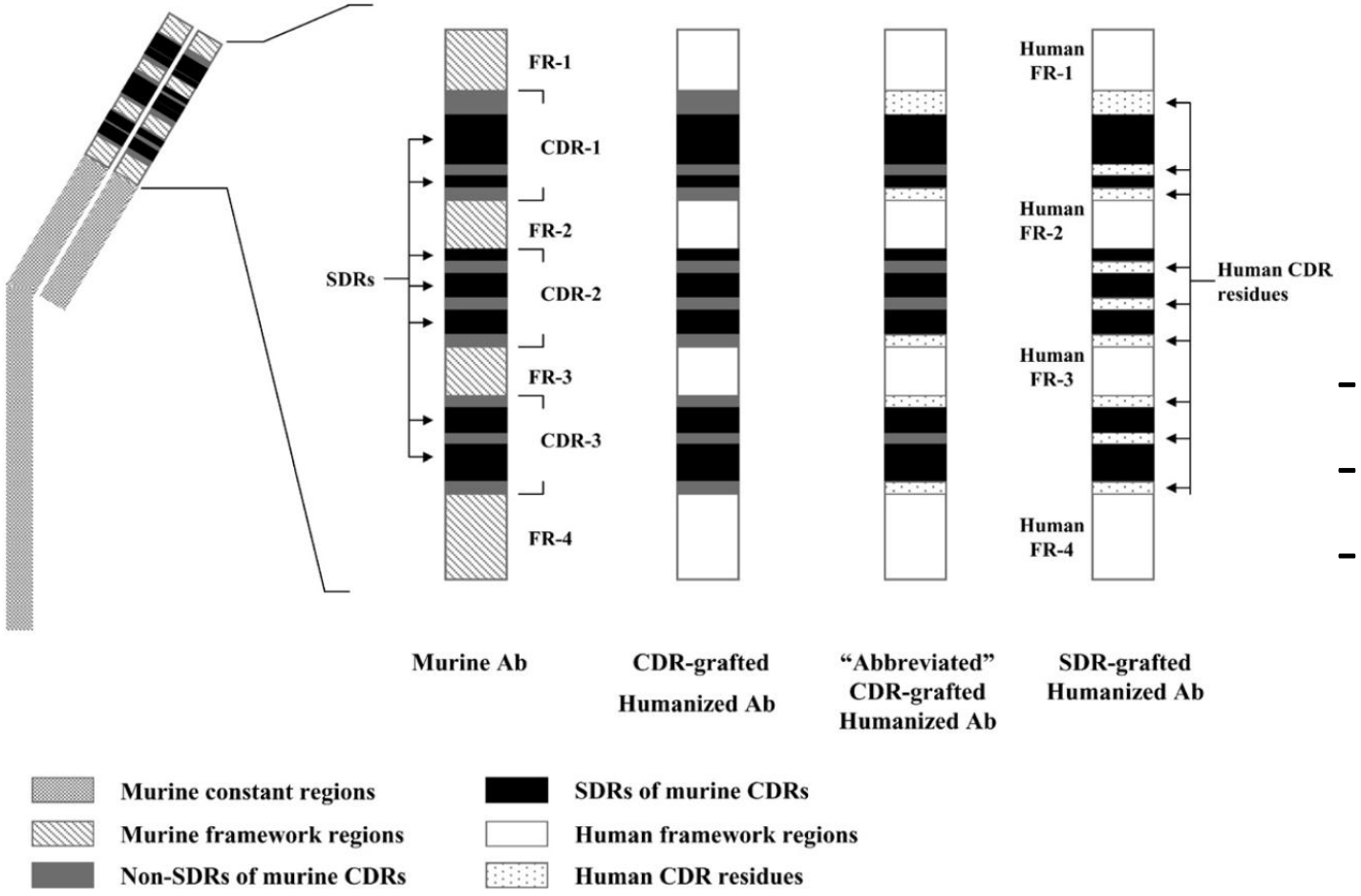


FIG. 1. Flowchart of antibody humanization using CDR-grafting method.

人源化策略 CDR&SDR-grafting



Specificity-determining residues (SDRs).

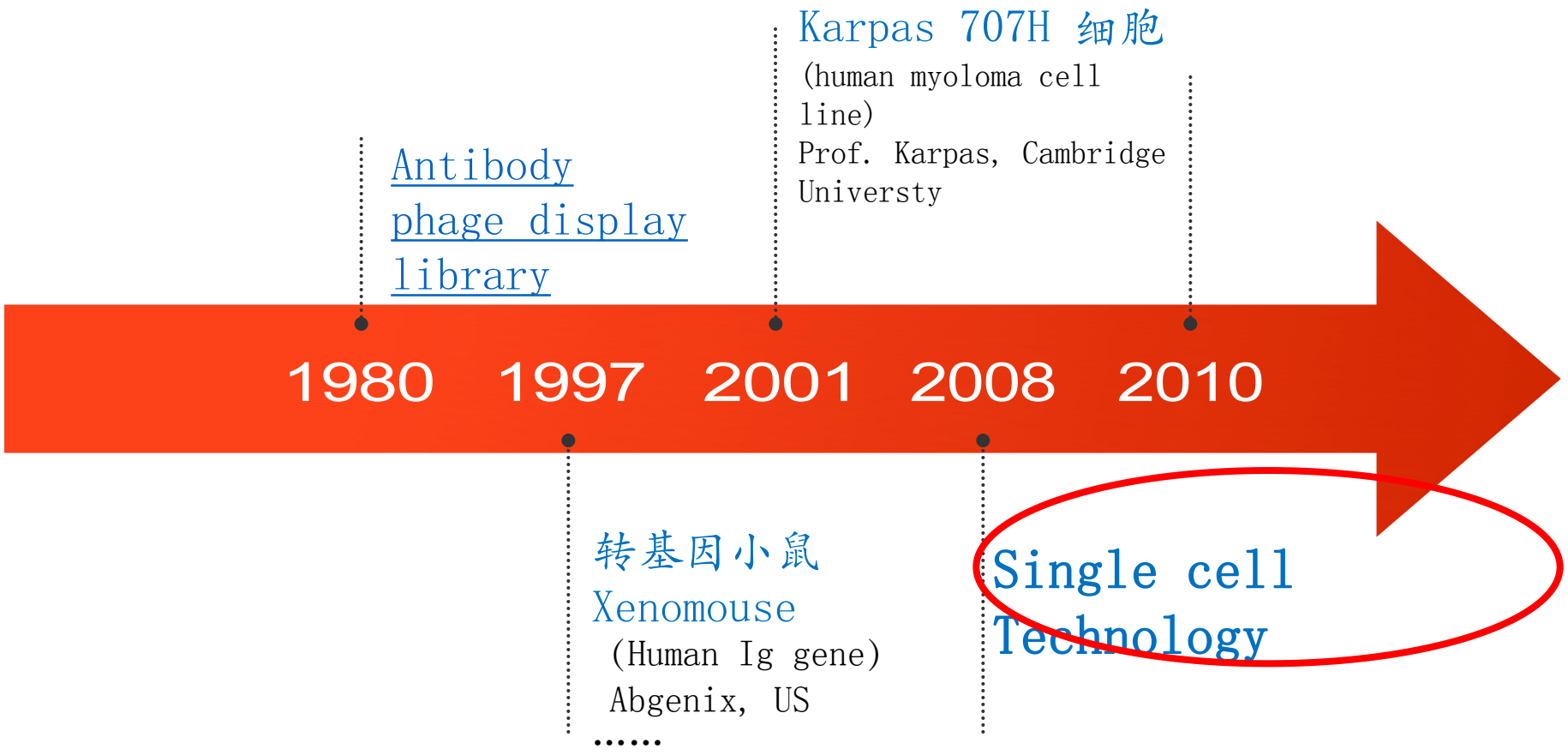
- 保留了更少的鼠源成分
- 最大限度接近人源成分
- 难度更高，挑战性

10个左右人源化序列

VS.

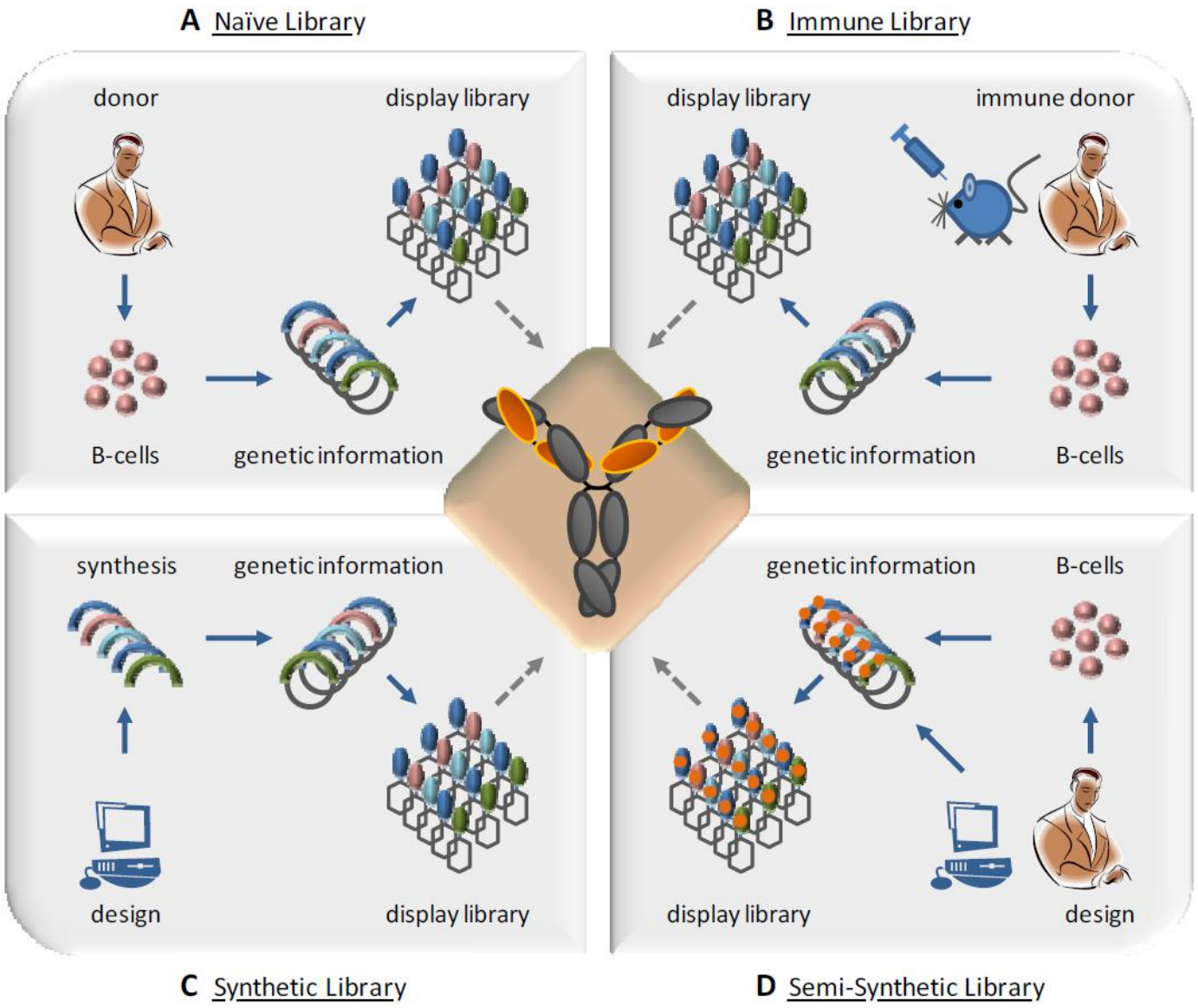
100-200 人源化序列

3. 全人抗体 Fully human mAbs



1）噬菌体抗体库技术
(Phage antibody library technique)

- 把人的Ig重轻链可变区基因片段展示在λ噬菌体表面,组成抗体库。
- 通过噬菌体把抗体的表型和基因型相偶联，易进行分子克隆和基因操作。
- 抗体库的来源影响筛选结果（免疫和正常人）。
- 高通量筛选与抗原结合的抗体，但亲和力低。



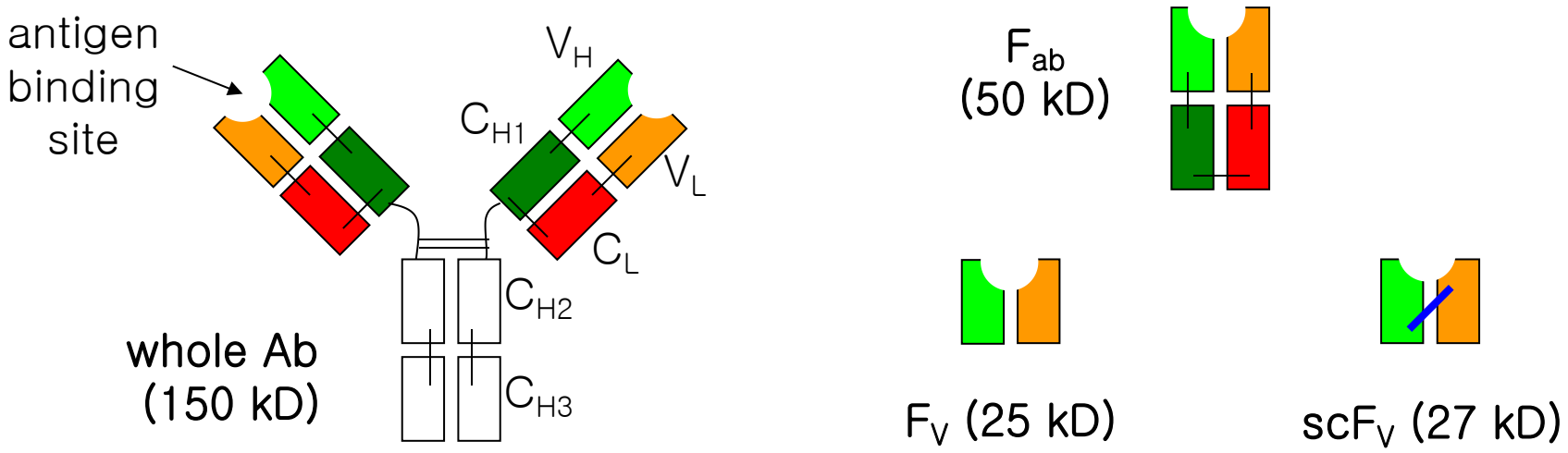
抗体展示：

抗体基因来源

- 天然库
- 免疫库
- 半合成库
- 合成库

- **Display of antibody fragments on bacteriophage**

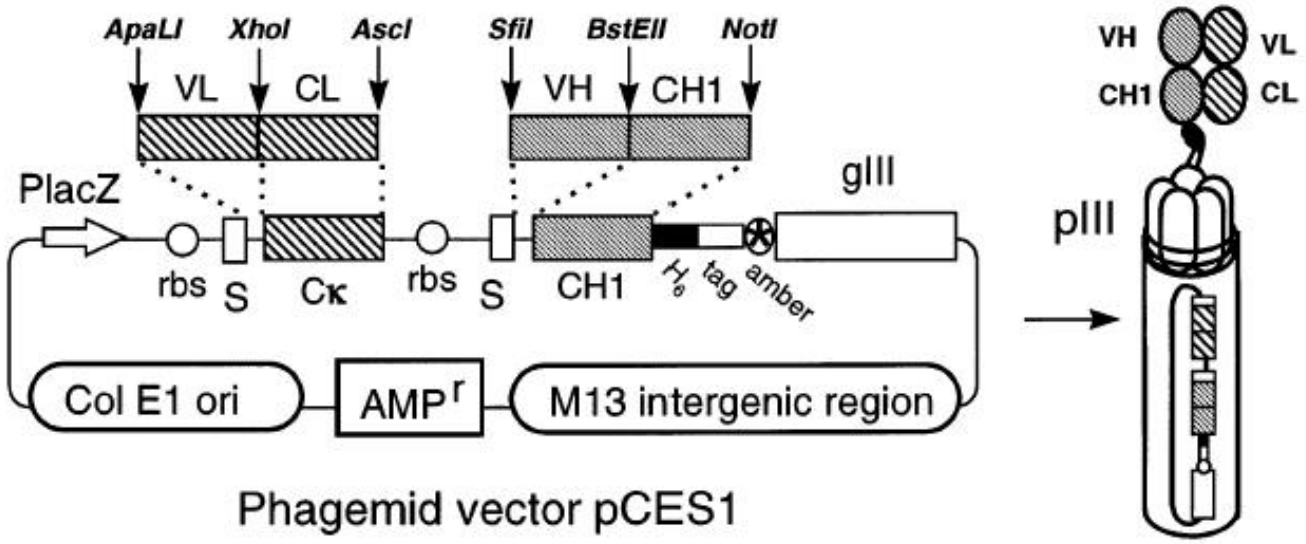
  - the favored format of antibody fragment is single-chain F_V (scF_V)



Schematic representation of different antibody formats

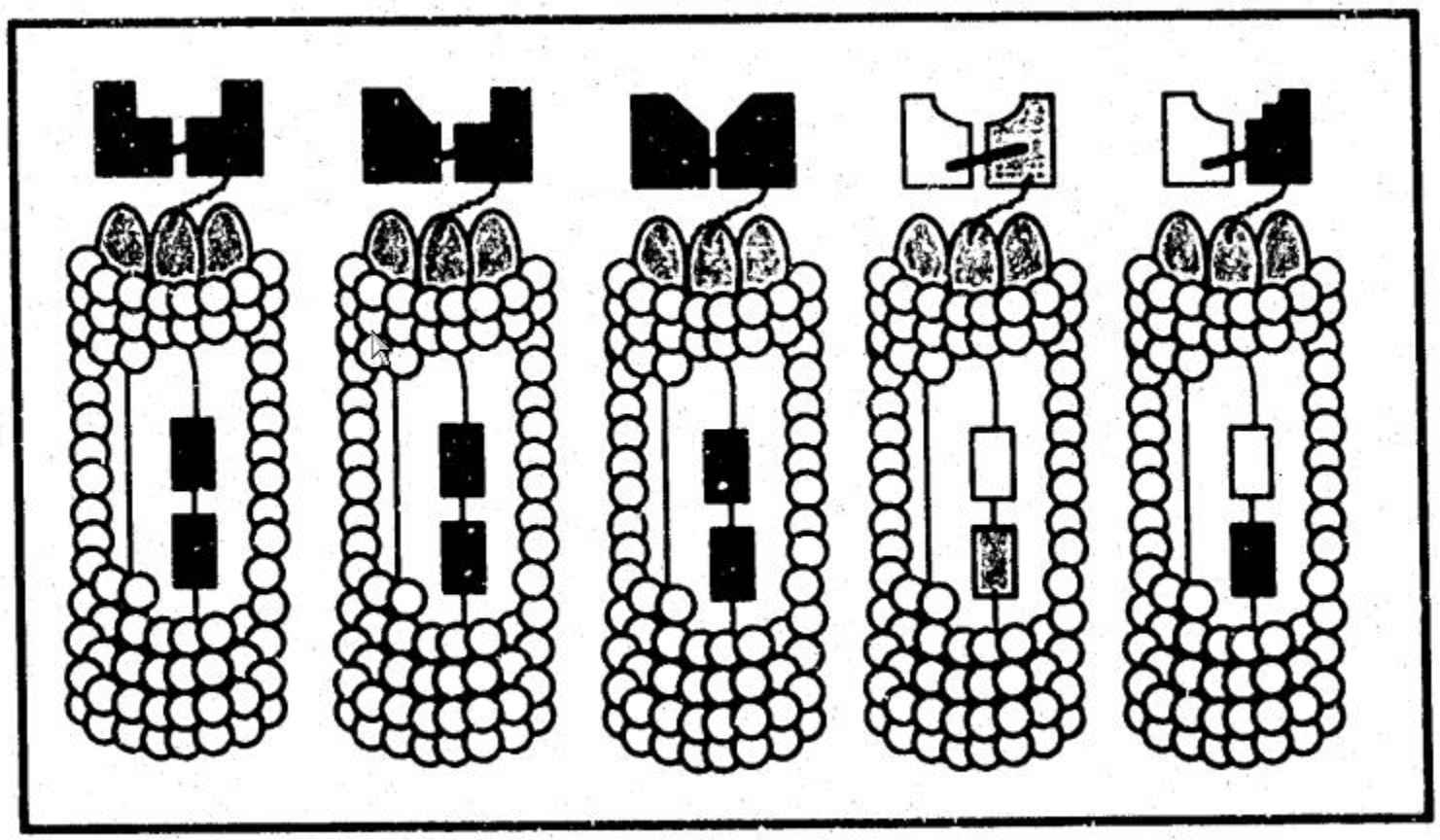
- **Construction of antibody libraries**
  - DNA sequences encoding V_H and V_L domains are amplified by PCR
  - V_H and V_L domains are paired randomly, increasing functional diversity in the library
  - The scF_V sequences are amplified by PCR using primers incorporating restriction sites
  - cloning into the phagemid vector

H.R. Hoogenboom et al. / Immunotechnology 4 (1998) 1–20



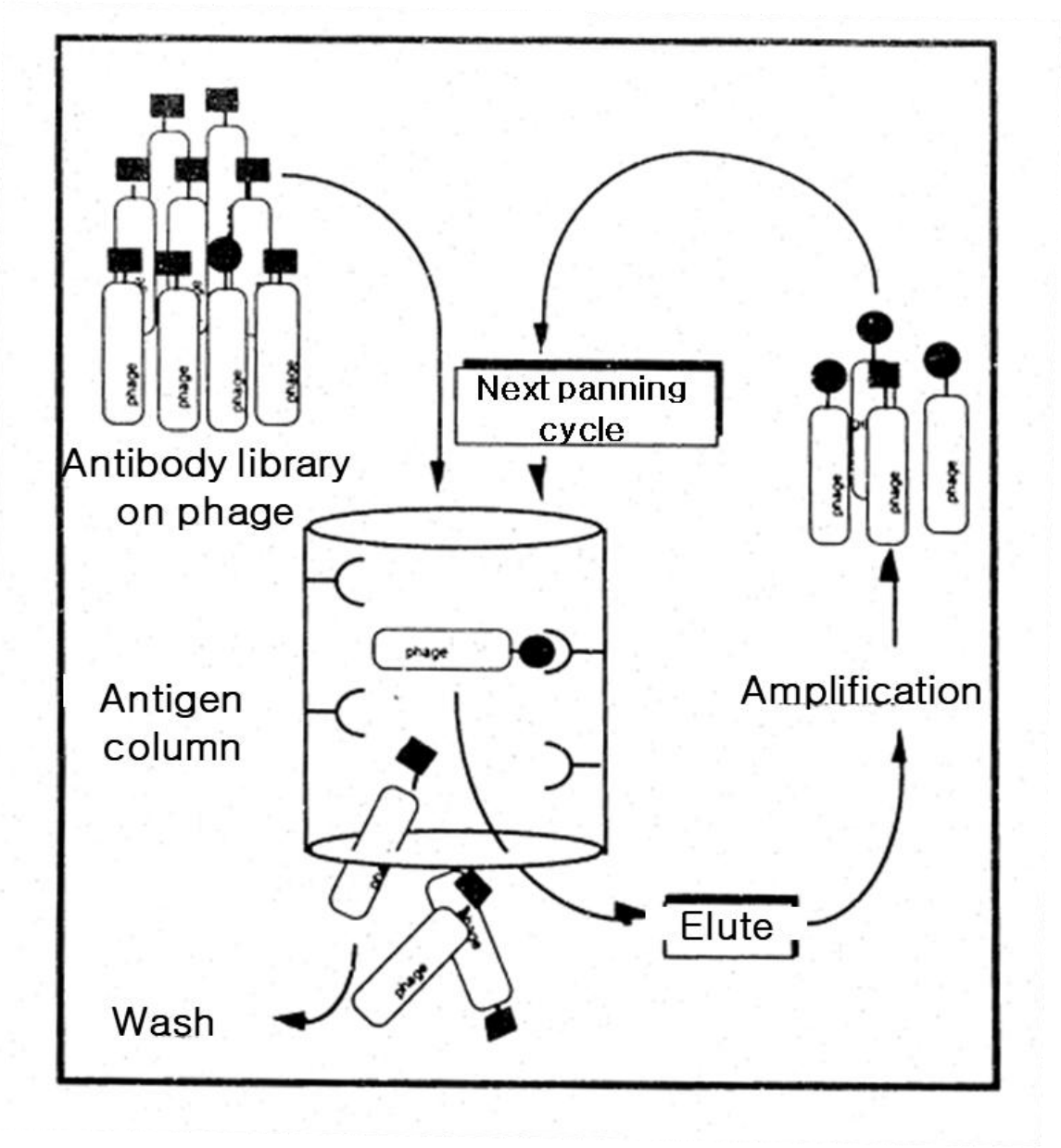
Display of Fab fragments o
n filamentous phage.

- transformed into *E. coli* by electroporation



Antibody display library

- **Selection of specific antibodies from a phage library**

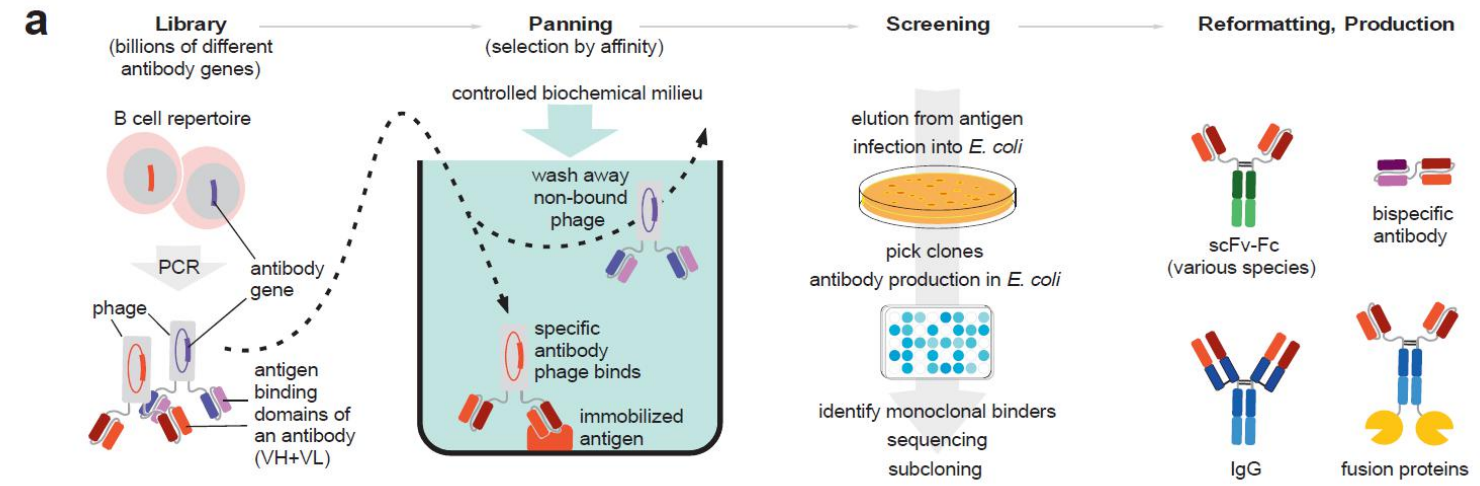


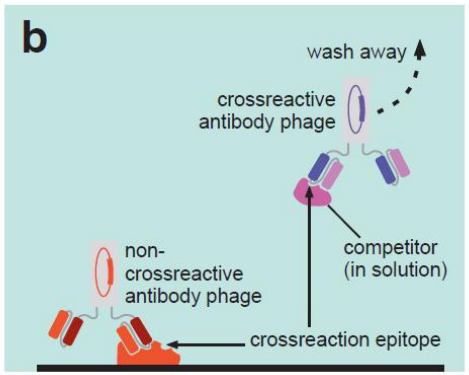
- **Library size and diversity**

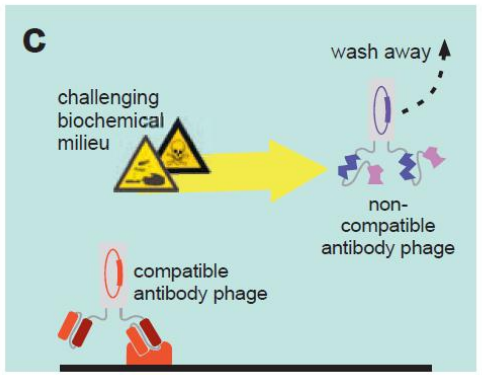
  - **Specificity and affinity are desirable qualities in any antibody** 特异性和亲和力

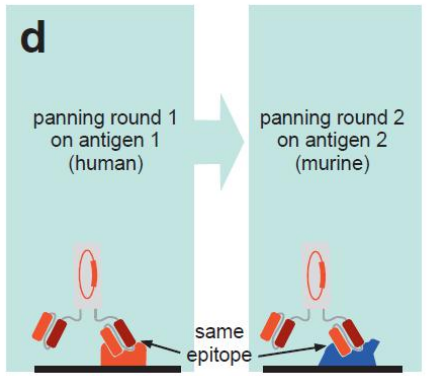
  - **The larger the library, the greater the likelihood that it will contain an antibody of very high affinity having the desired specificity** 库容量越大越有可能筛选到好的抗体

噬菌体展示优势

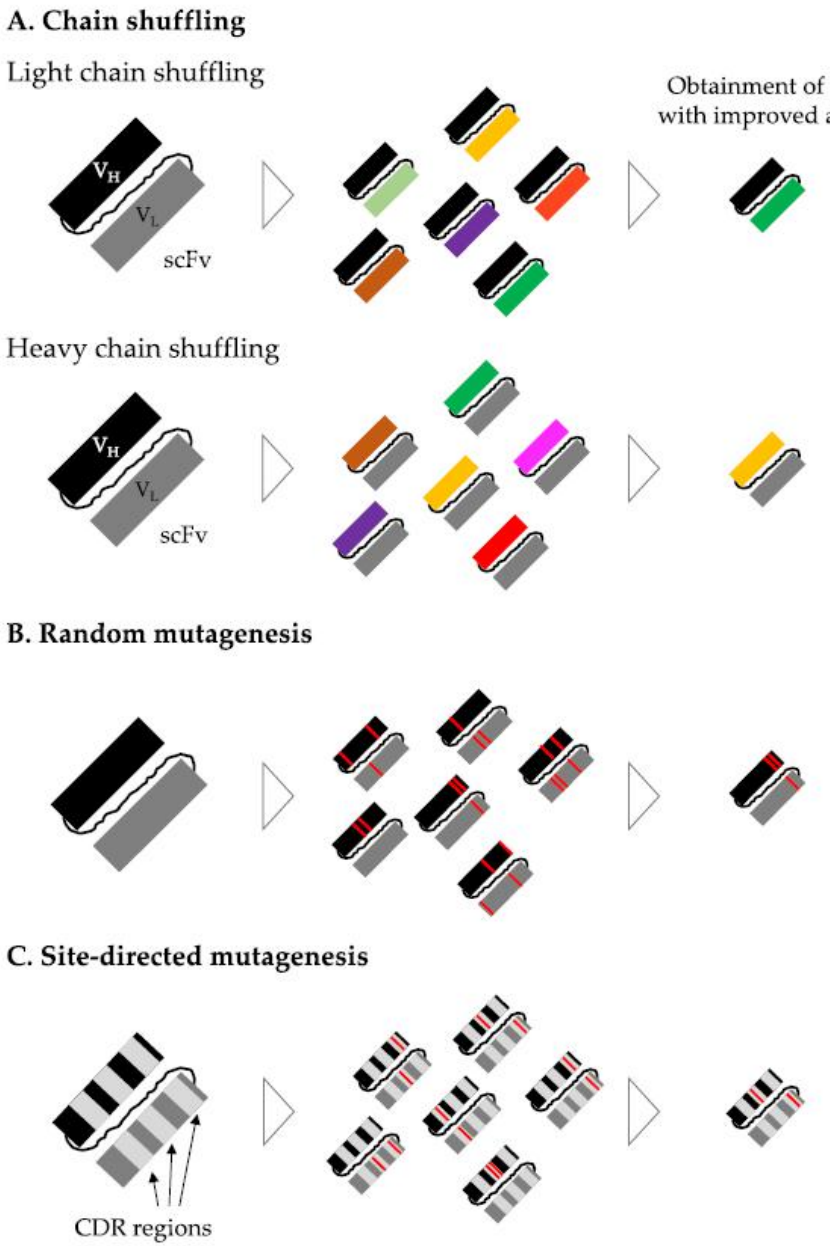








- 去除不希望有的交叉反应
- 筛选特殊条件下结合
- 筛选人-鼠交叉反应的抗体



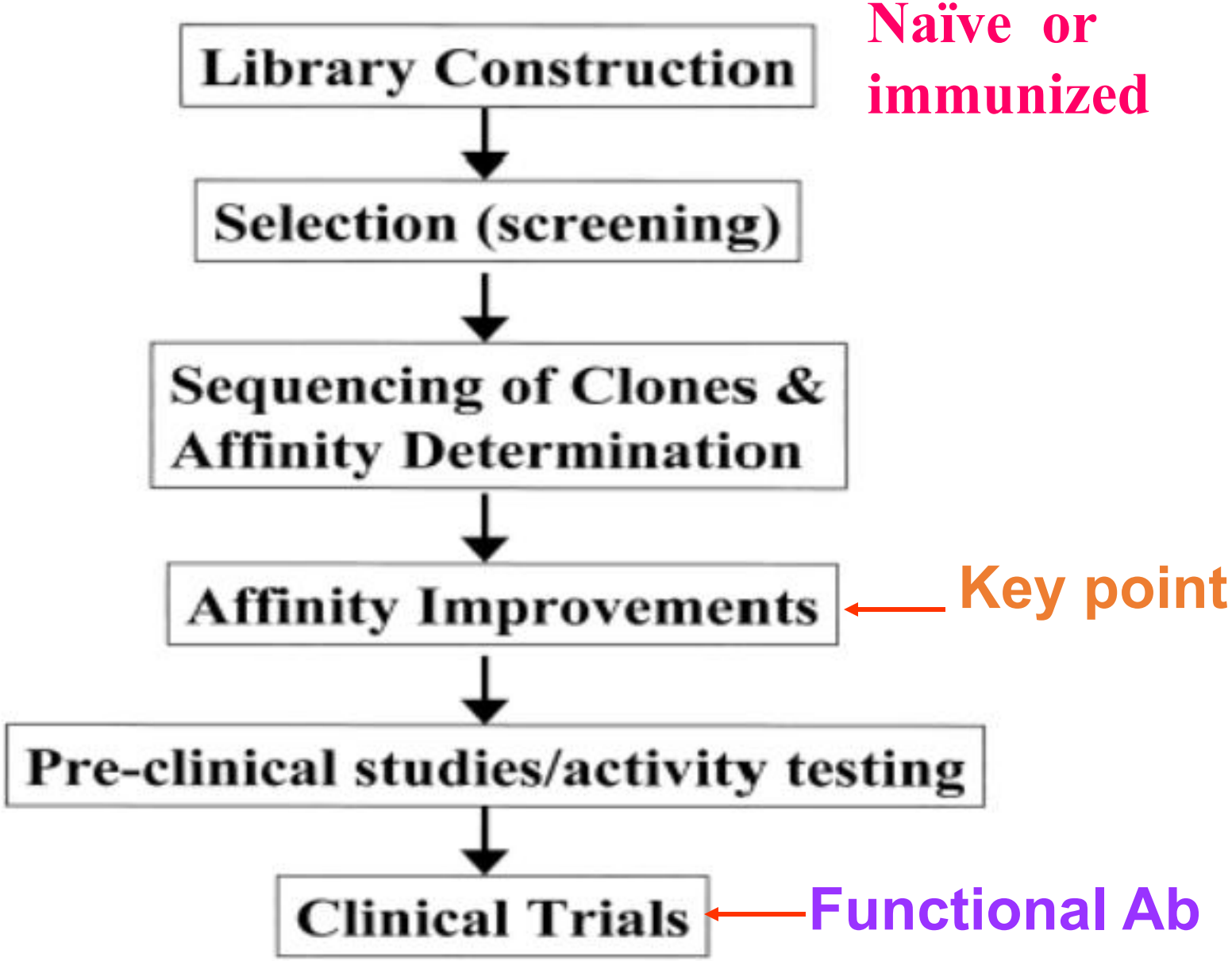
噬菌体展示的局限

- 亲和力问题：相对较低，需要做亲和力成熟；
- 理化性质：非自然组合，亲和力较低；
- 免疫原性：亲和力成熟引入一系列突变，有可能增加免疫原性。

亲和力成熟方法：

- 重链/轻链转换；
- 随机突变；
- 特异位点突变

Toxins **2018**, *10*, 236; doi:10.3390/toxins10060236


Library Construction
Naïve or immunized
Selection (screening)
Sequencing of Clones & Affinity Determination
Affinity Improvements
Key point
Pre-clinical studies/activity testing
Clinical Trials
Functional Ab

2） 转基因小鼠 Transgenic mice

1. 首先把小鼠编码Ig重轻链的基因剔除。

2. 制备表达人的Ig重轻链的转基因小鼠。

3. 上二种小鼠回交，获得只表达人Ig重轻链的基因的小鼠。当用抗原免疫后，小鼠可产生完全人源抗体。

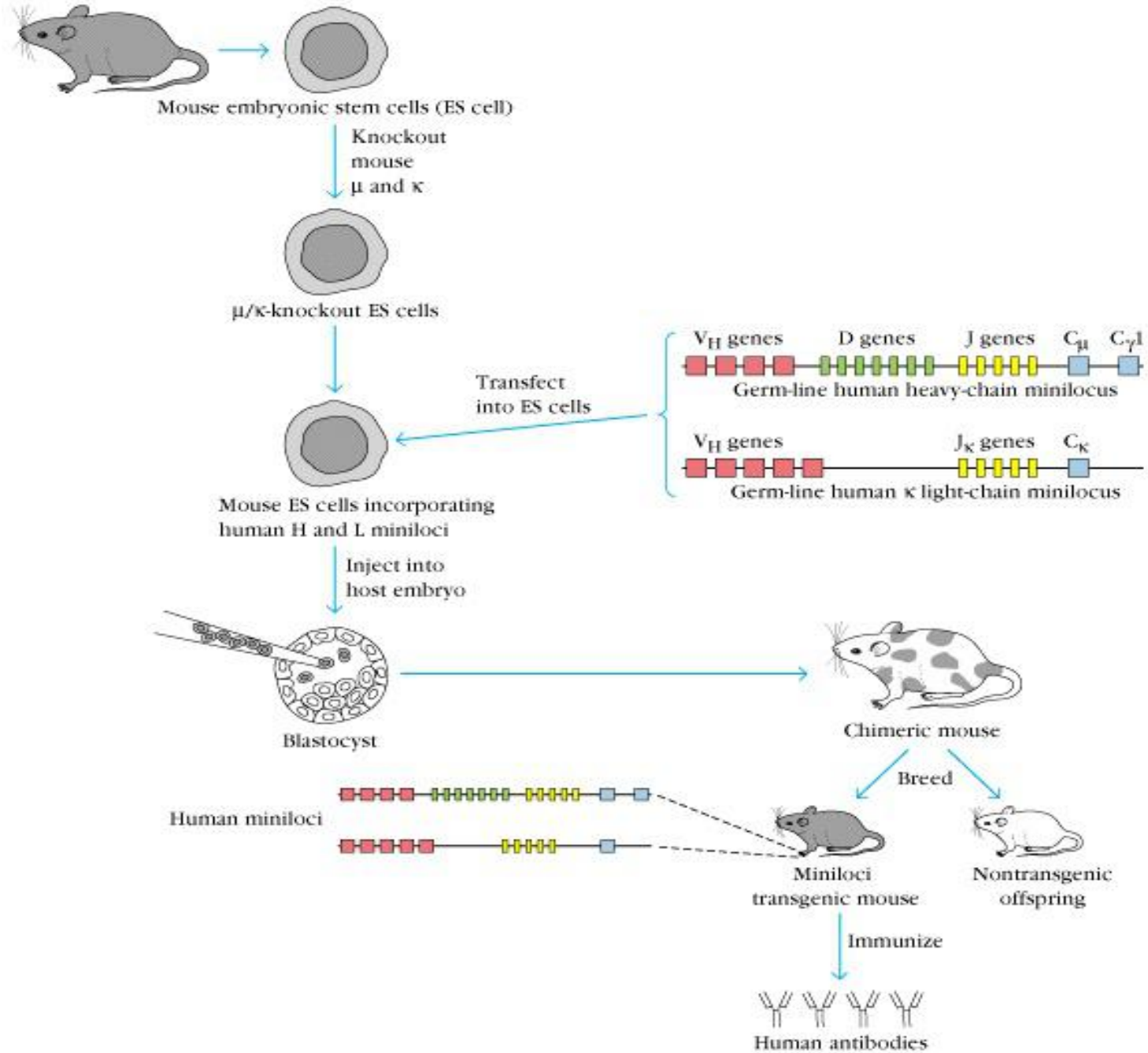

Mouse embryonic stem cells (ES cell)
Knockout mouse μ and κ
μ/κ-knockout ES cells
Transfect into ES cells
V_H genes
D genes
J genes
C_μ
C_γ1
Germ-line human heavy-chain minilocus
V_H genes
J_κ genes
C_κ
Germ-line human κ light-chain minilocus
Mouse ES cells incorporating human H and L miniloci
Inject into host embryo
Blastocyst
Chimeric mouse
Breed
Human miniloci
Miniloci transgenic mouse
Nontransgenic offspring
Immunize
Human antibodies

Table 2

Antigen-specific monoclonal antibodies from mice with human Ig transloci.

Translocus	Antigen	Human Ig chains*	Affinity	Reference
HuIgH	Phenyloxazolone	μ	60 and 160 nM	[10]
	Phycoerythrin			
	Lysozyme			
	Human lymphoma cells			
HuIgH^{Cos}	Phenyloxazolone	μ		[10]
	Lysozyme			
	Human lymphoma cells			
γH1/γK1	Tetanus toxin	μ + κ		[12]
HC2/KCo4	Human CD4	γ1 + κ	12 nM	[6]
HC2/KCo5	Human CD4	γ1 + κ	0.03–0.4 nM	[7•]
γH2/γK2	Human IL8	γ2 + κ	0.16–0.9 nM	[4••]
	Human EGFR		0.03–0.4 nM	
	Human TNFα		0.2–0.8 nM	

*The HuIgH and HuIgH^{Cos} transgenes were analysed in mice with disrupted endogenous IgH locus but capable of producing endogenous Igκ chains. The other mouse lines contained transloci encoding both human IgH and Igκ on mouse backgrounds disrupted for the corresponding endogenous loci. The mice that produce human IgG also produce human IgM but only the results for the specific IgG antibodies are shown. Cos, cosmid; EGFR, epidermal growth factor receptor; H, heavy chain; Hu, human; IL, interleukin.

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- 获得专利使用权。

3）用人的骨髓瘤细胞直接制备全人抗体

- 关键点：建立好的人骨髓瘤细胞。
  - 稳定性高和融合率高。

Development of a fusion partner cell line for efficient production of human monoclonal antibodies from peripheral blood lymphocytes

(Human antibodies 2002, 11:85-96)

Isolation of Native Human Monoclonal Autoantibodies to Breast Cancer

HYBRIDOMA AND HYBRIDOMICS
Volume 21, Number 6, 2002

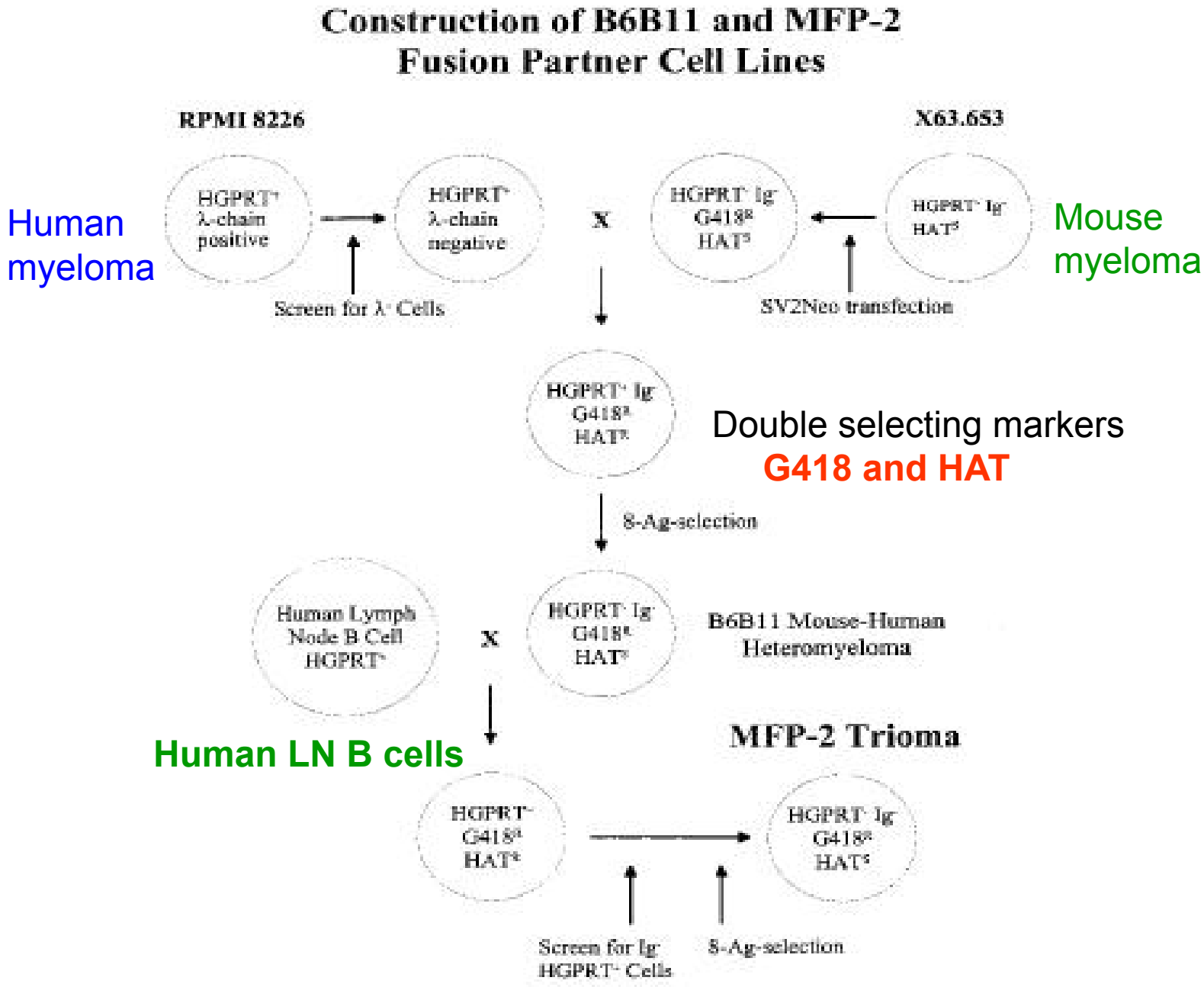
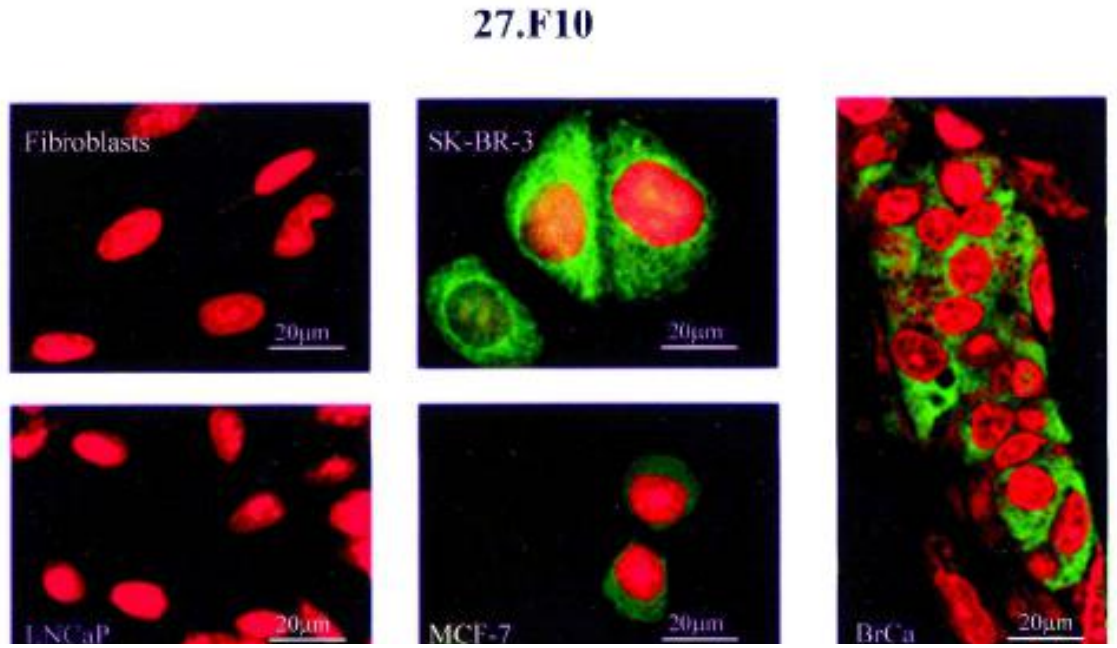
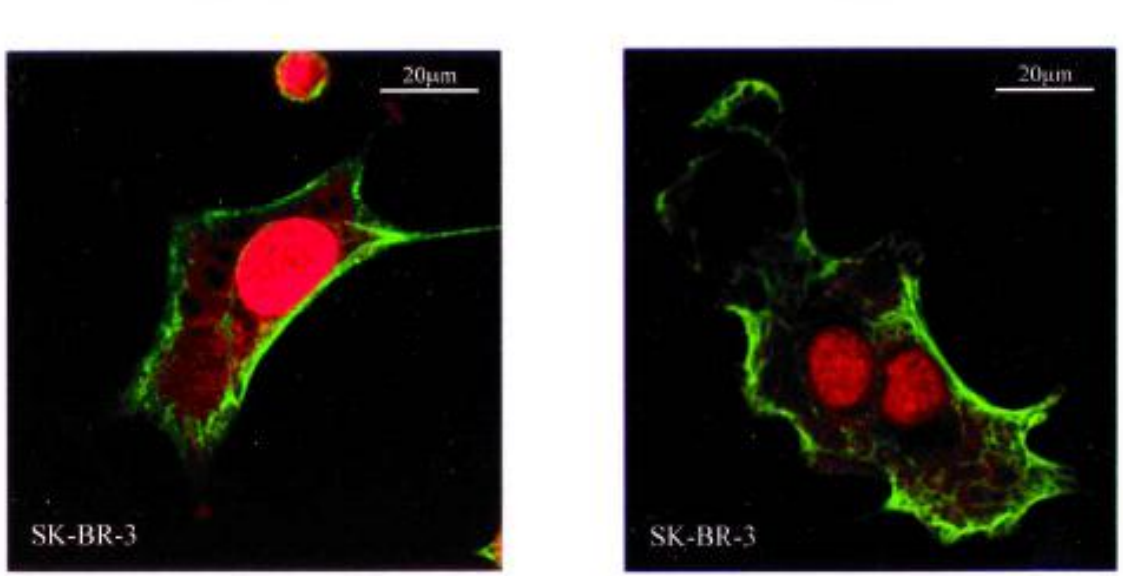


Fig. 1. Flow chart for the construction of the B6B11 and MFP-2 cell lines.

Breast cancer tissue



Breast cancer cells



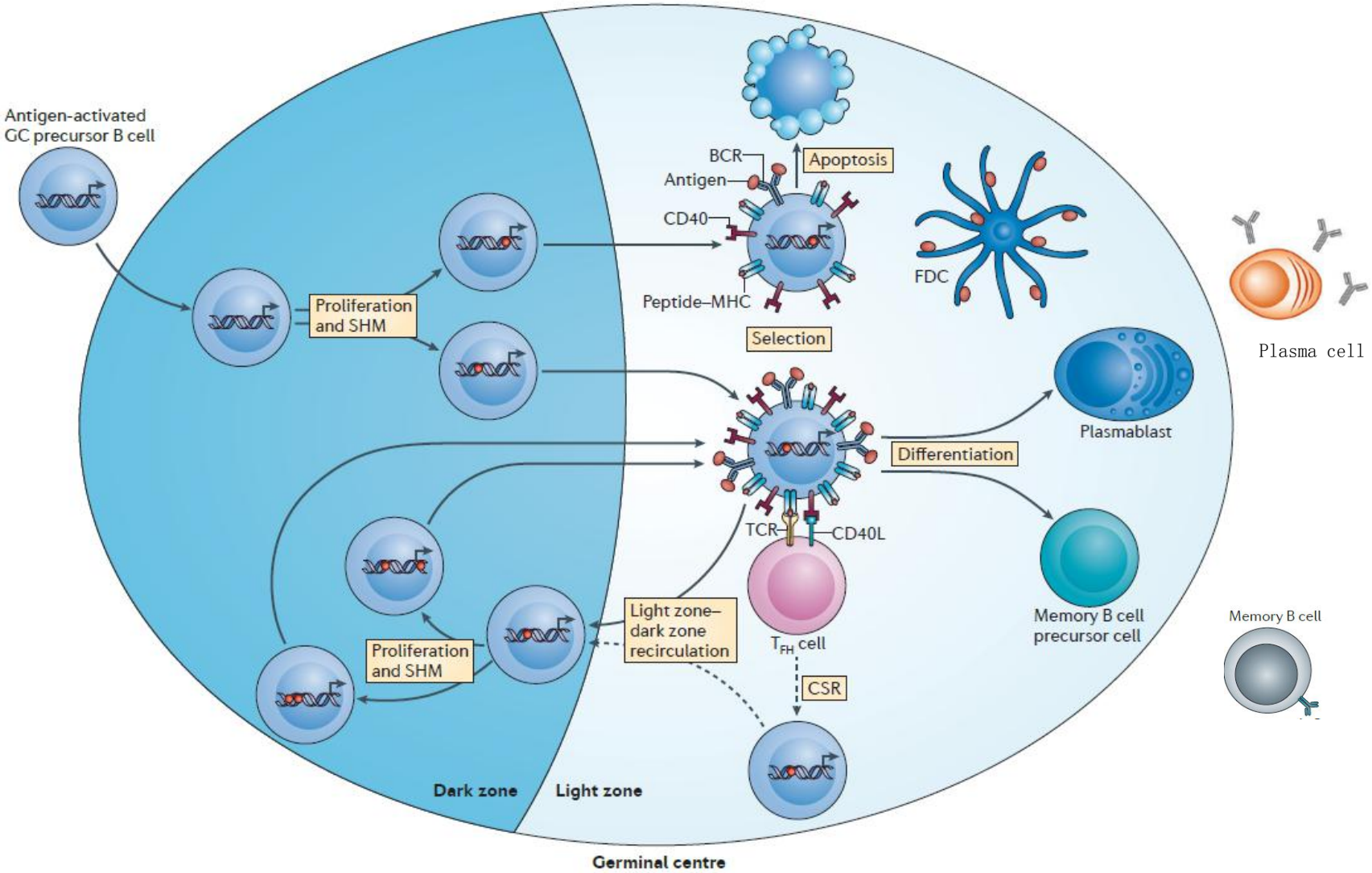
We work on HCV/E2 mAb and Rabbis virus S protein by using FMP2 cells.

4）B细胞永生化技术：

- 用EB病毒将人淋巴细胞永生化可产生分泌抗体的B细胞克隆；
- 这一技术较为成熟；
- 但是存在抗体分泌不稳定的缺点，限制了其应用。

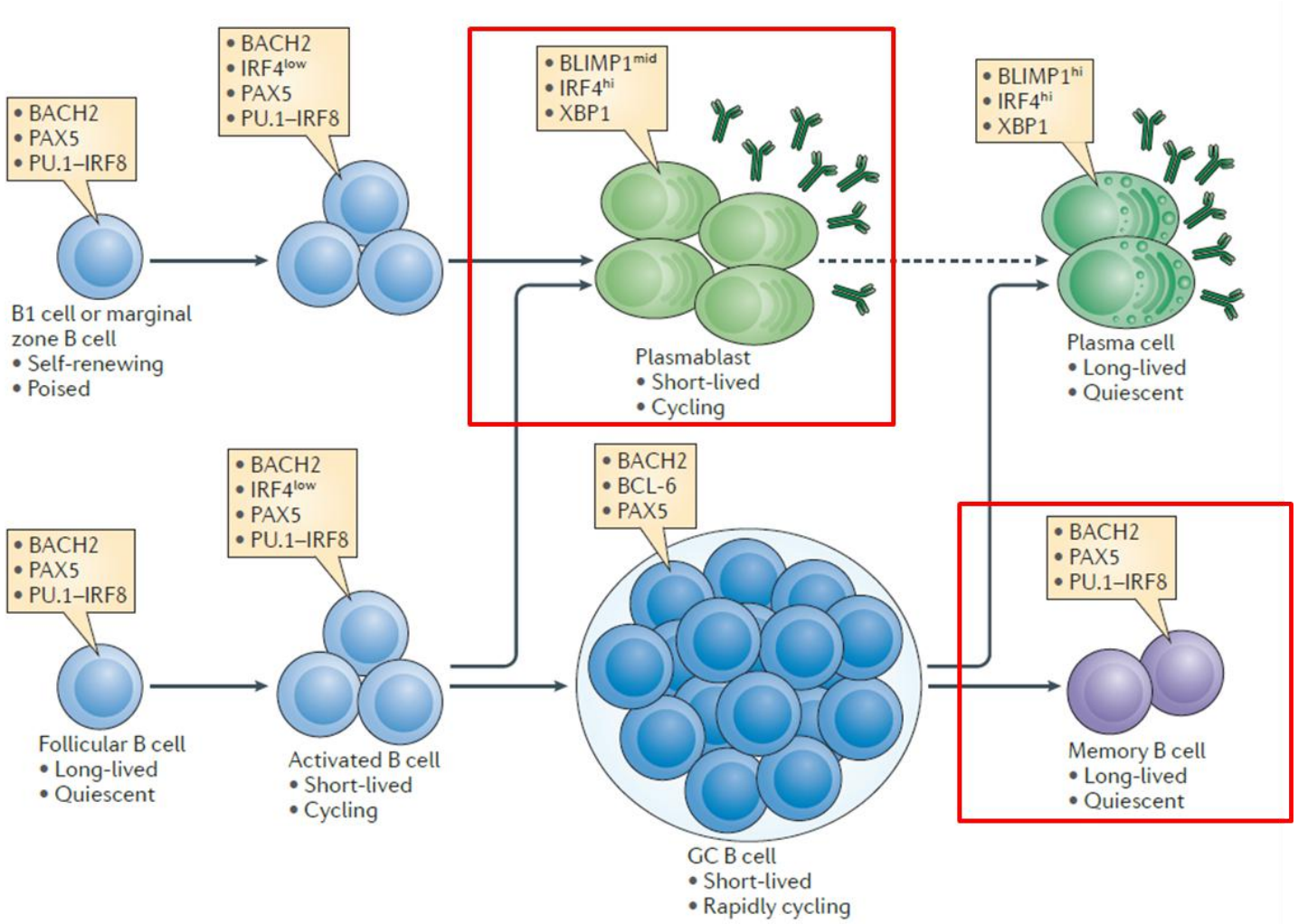
5) 单细胞技术制备全人单克隆抗体
Platform for the development of human mAbs using single-cell technology

Dynamics of the GC reaction and selection of high-affinity antibody mutants



Nature Reviews Immunology 15, 137–148 (2015)

Peripheral B cells for separating Ig genes



Nature Reviews Immunology 15, 137–148 (2015)

Phenotypic characterization of mouse and human peripheral B-cell subsets

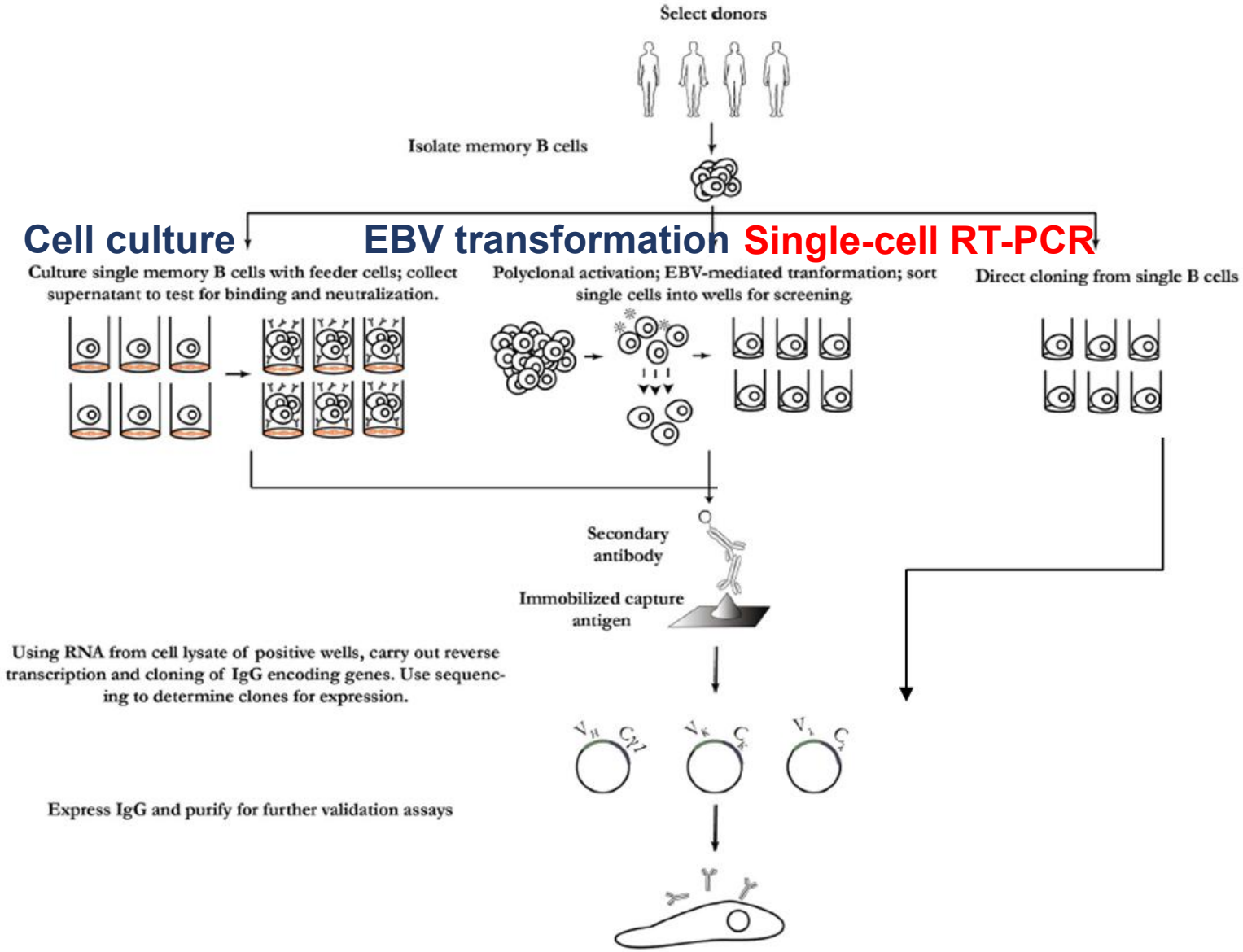
Table 1 Phenotypic characterization of mouse and human peripheral B-cell subsets

Subsets	*Mouse phenotype*	*Human phenotype*	*Ascribed function*
Transitional	CD19+B220+CD93+IgMhiAA4.1+ CD24hiHSAhighCD21-BR3+TACI+	CD19+CD20+CD27-CD10+CD24high CD38high IgD+CD5+BR3+	Precursor to mature naive
Mature naive	CD19+B220+IgDhiIgMlow CD21 +CD23hiCD1dlow HSAlowCD62L+(FO) CD19+B220+IgDlowIgMhi CD21hiC-D23lowCD1dhi HSA+(MZ)	CD19+CD20+CD27 CD24intCD38int CD21+CD10-IgD+IgM-	Precursor to germinal center, memory and antibody-secreting cells
Germinal Center	CD19+B220+CD38GL7+FAS+ PNA+IgD-IgM	CD19+CD10+CD20+CD27+CD38+ FAS+IgDBR3+	Rapidly proliferate and are precursors to memory B cells or antibody-secreting cells
Memory	CD19+B220+CD38+CD73+ CD80+PD-L2+	CD19+CD20+CD27+CD38	Recall responses and provide humoral immunity
Antibody-secreting cells	CD19low/−B220+CD38CD138+CD93 +MHCII+Ki67+(plasmablasts)	CD19+CD20^{-}CD27+CD38high IgD^{-}CD138- (plasmablasts)	Antibody secretion
	CD19lowB220−ID−TACI+CD38− CD138highCD93+ (Plasma cells)	CD19+CD20-IgDCD27highCD38highCD2 4loCD138high TACI+ (plasma cells)	

Major surface marker differences between pre-immune and antigen experienced B-cell subsets. Adapted from refs 4–11.

Cellular & Molecular Immunology advance online publication 19 June 2017

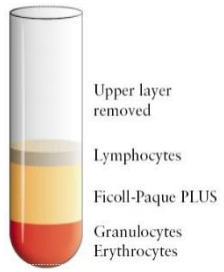
Single cell technology for generating human monoclonal antibodies



Single cell RT-PCR for generating human monoclonal antibodies

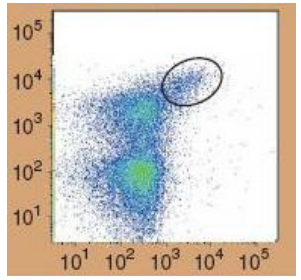
Screening of volunteers/patients

PBMC



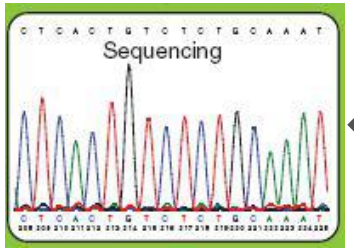
CD19+/IgG+/antigen+

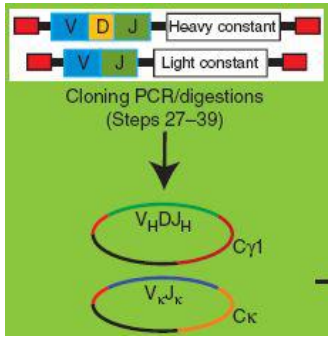
CD19+/ CD3-/CD20-/ CD27hi/CD38hi



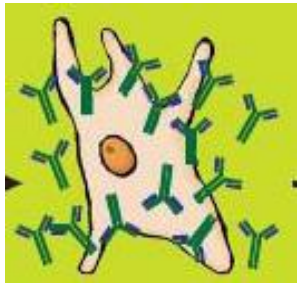
Antigen specific memory B cells/ Plasmablast

Single cell RT-PCR

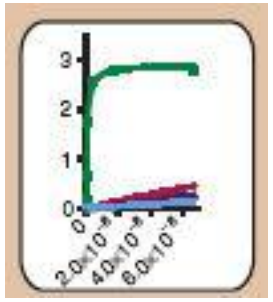




Expression



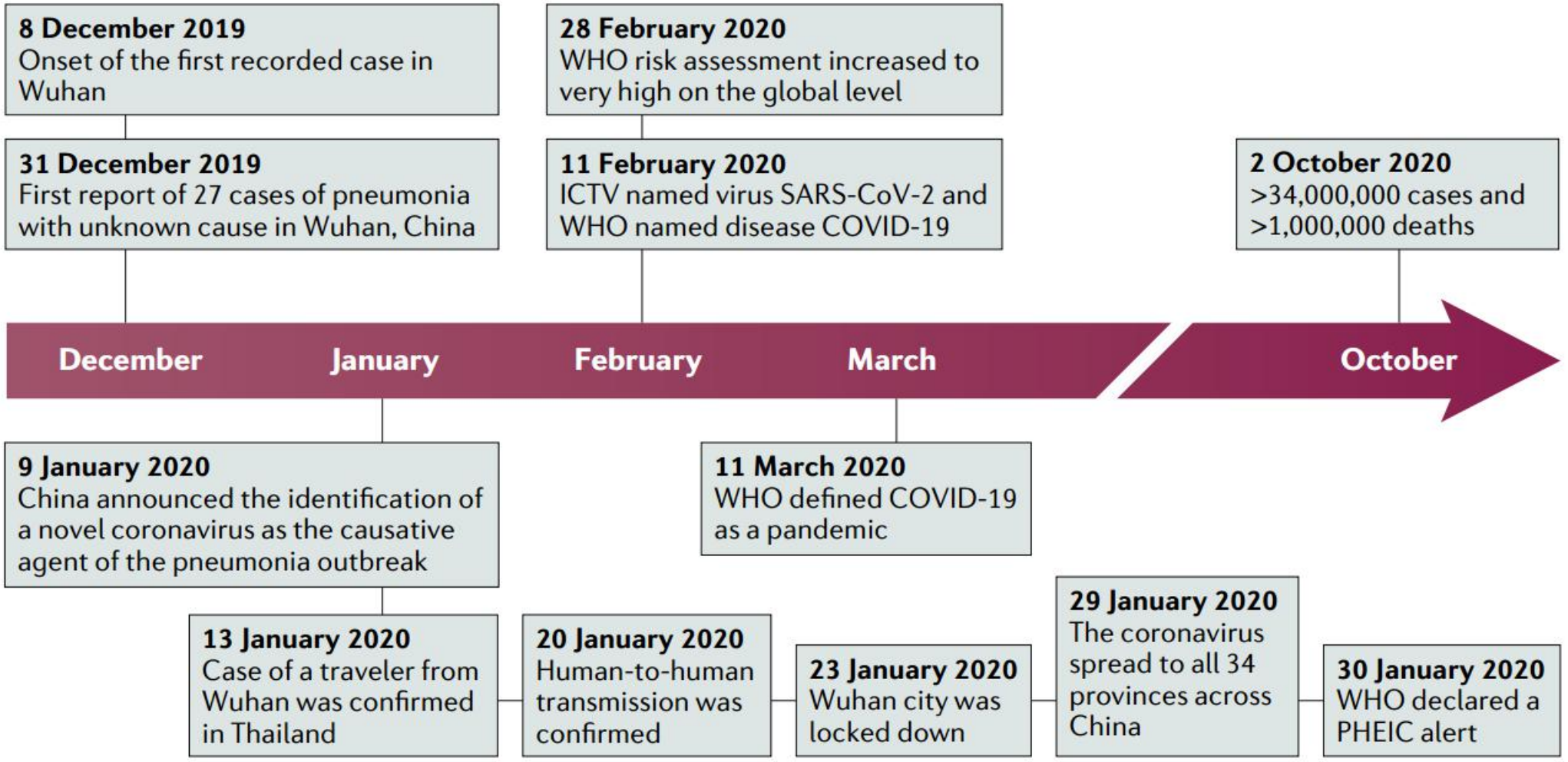
Eveluation



- Influenza
- HCV
- RSV
- Rabies
- HBV

SARS-CoV与新型冠状病毒免疫应答的差异和全人中和抗体的制备策略（案例2）

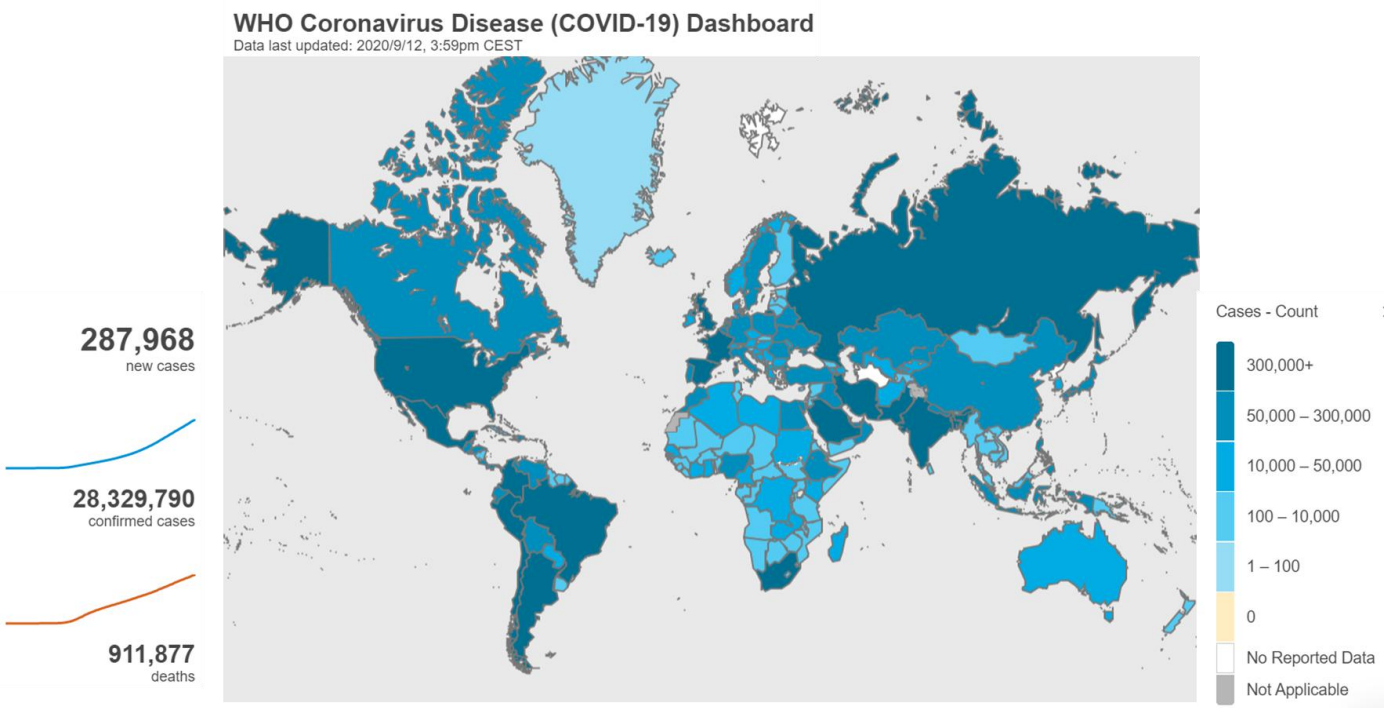
Timeline of the key events of the COVID-19 outbreak

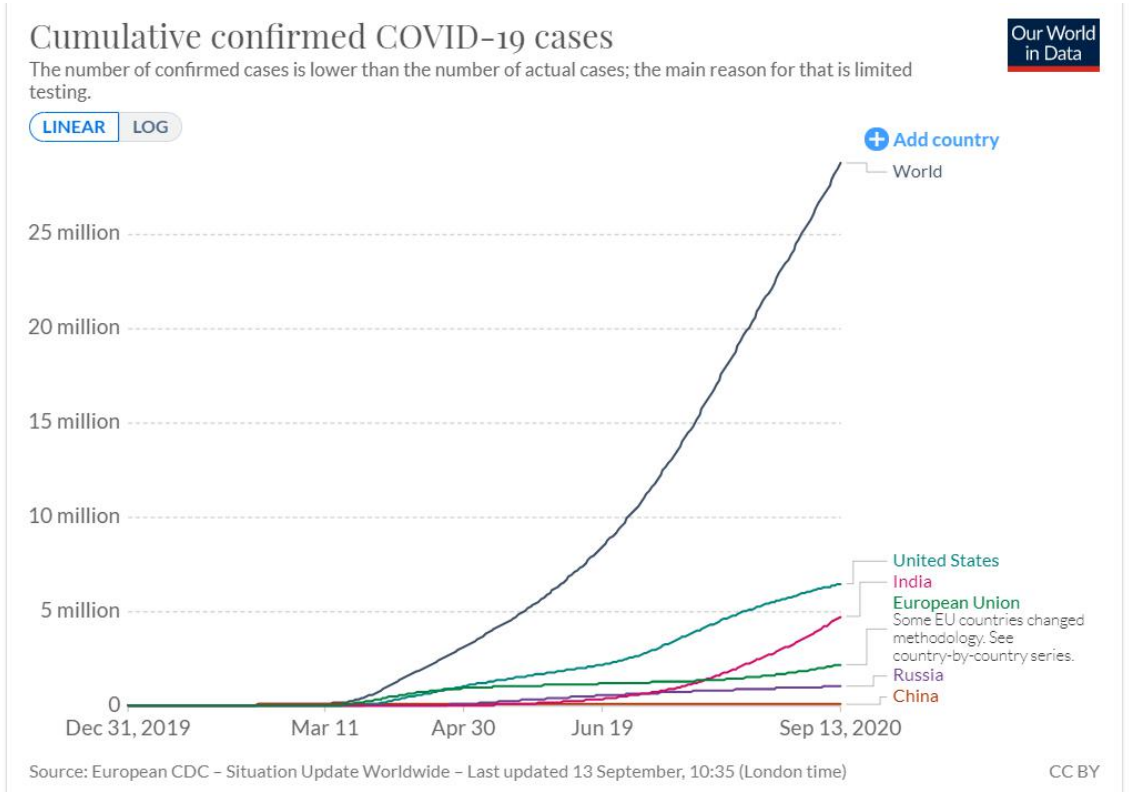


Nature Reviews | Microbiology

新冠病毒感染数字

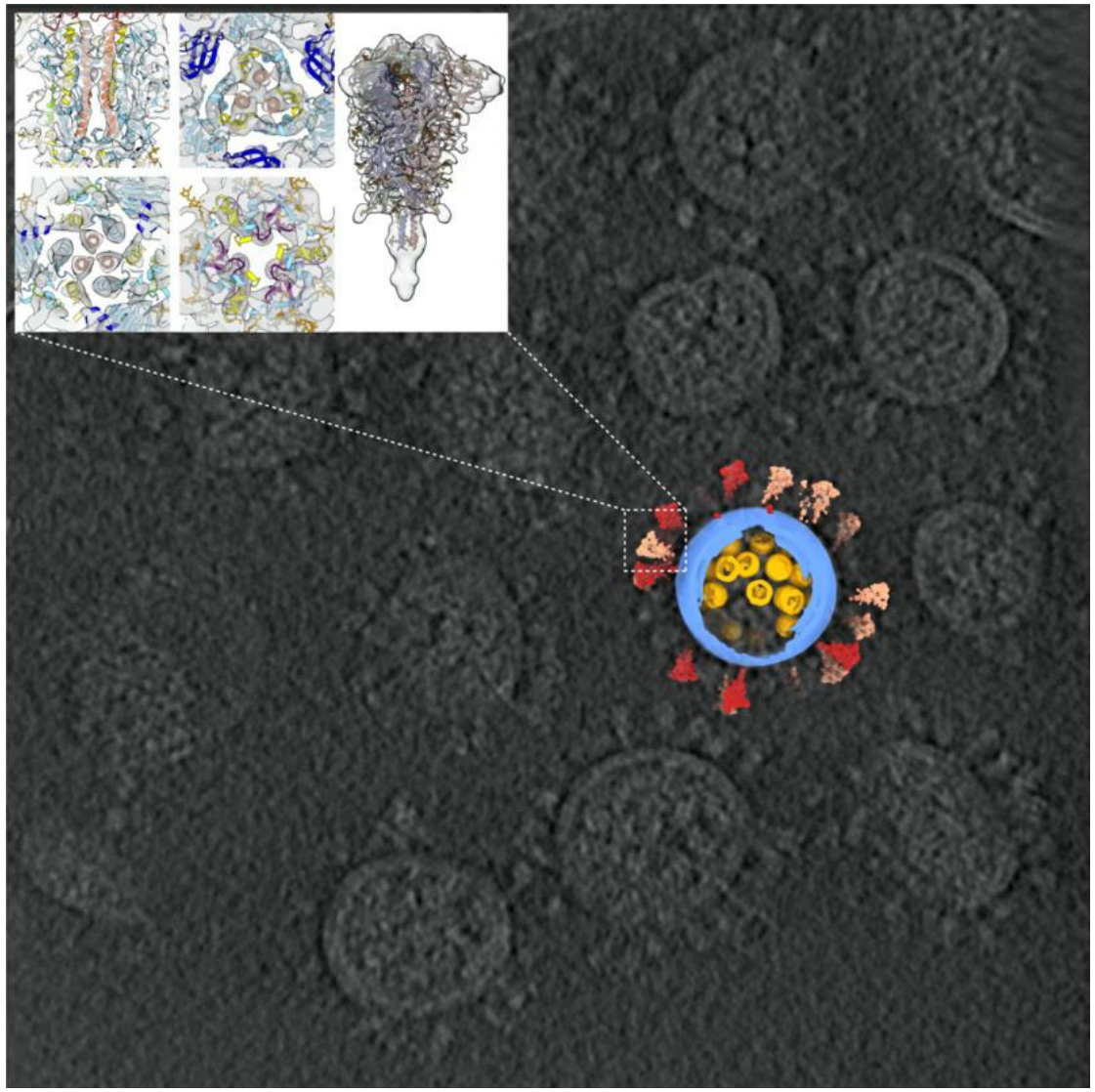
感染5000万
死亡120万

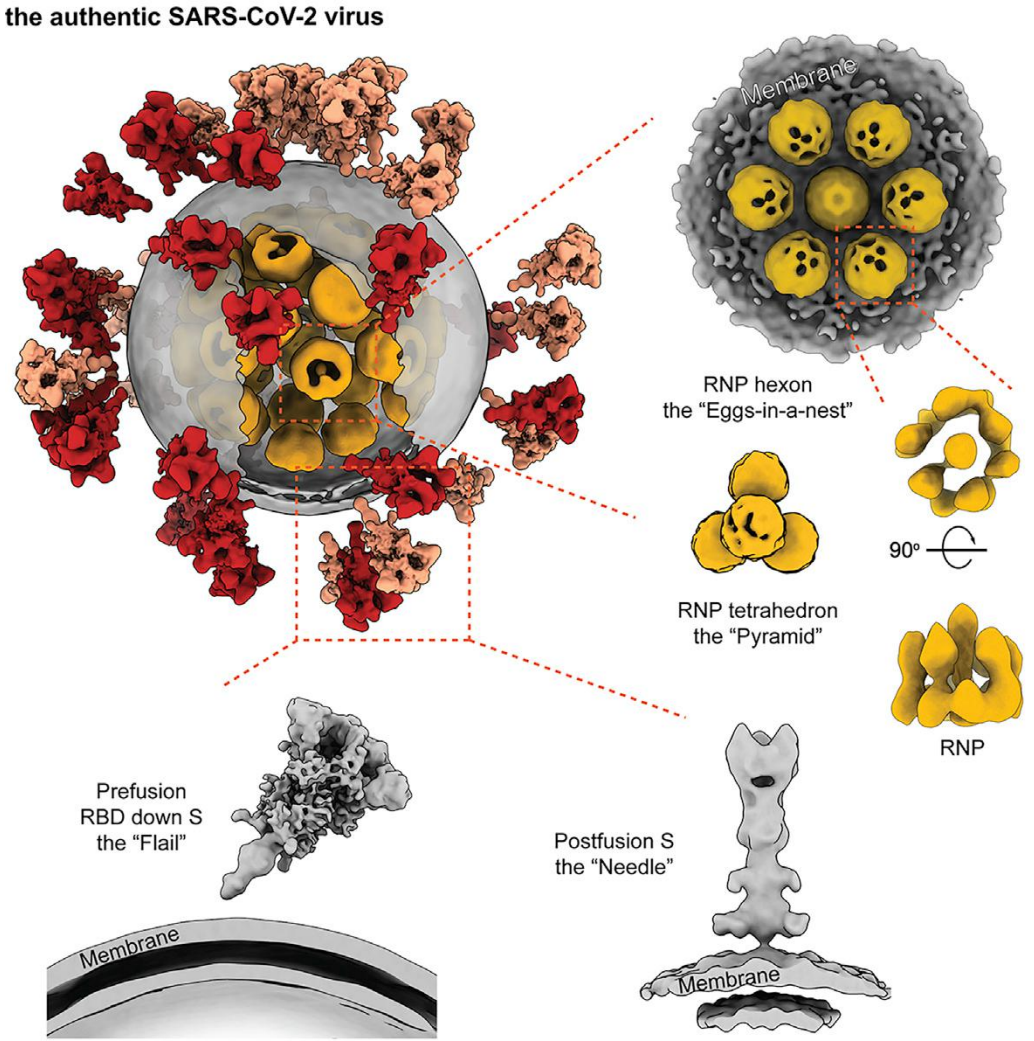




中国疫情防御有成效

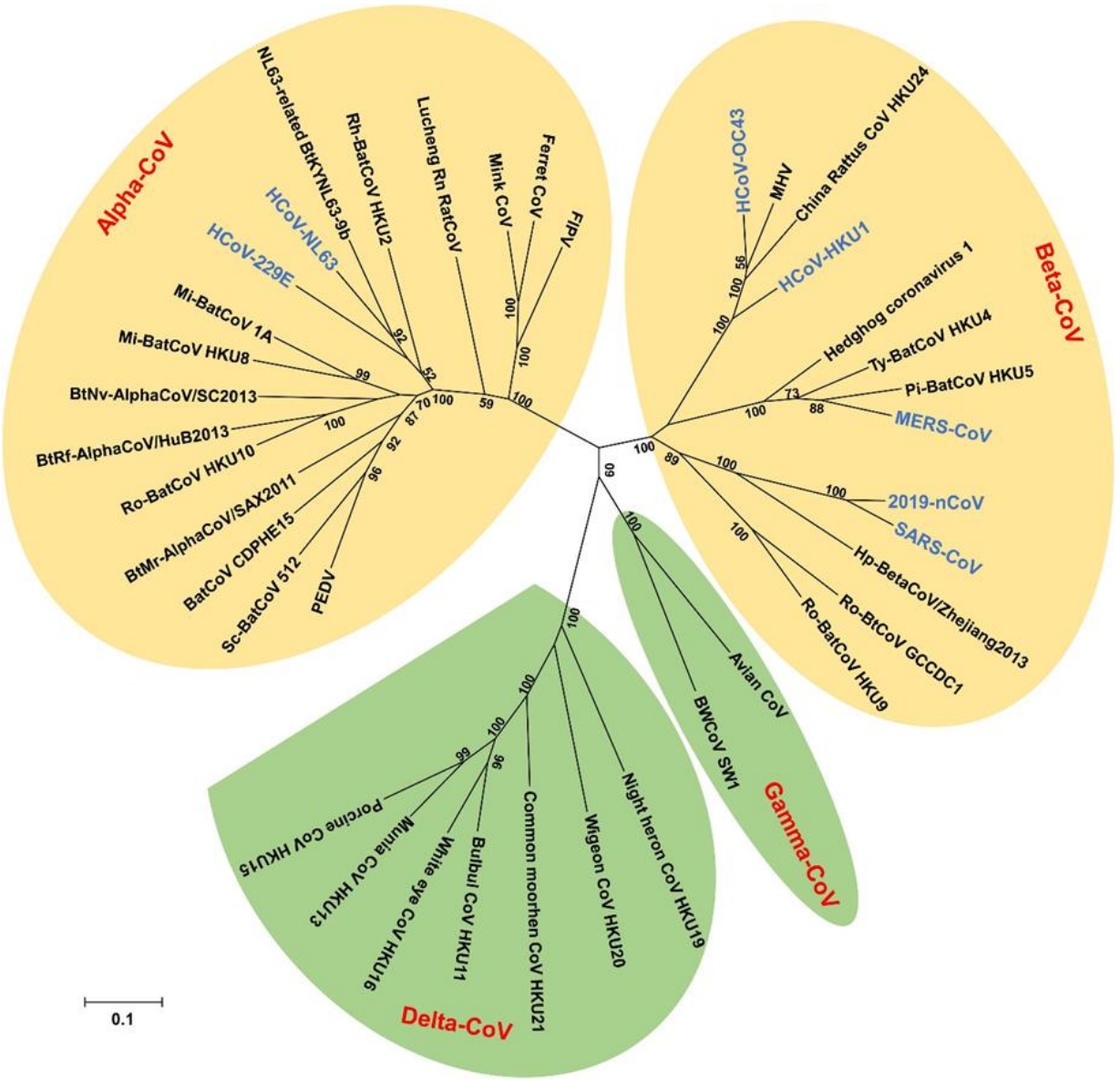
SARS-CoV-2 病毒的分子结构

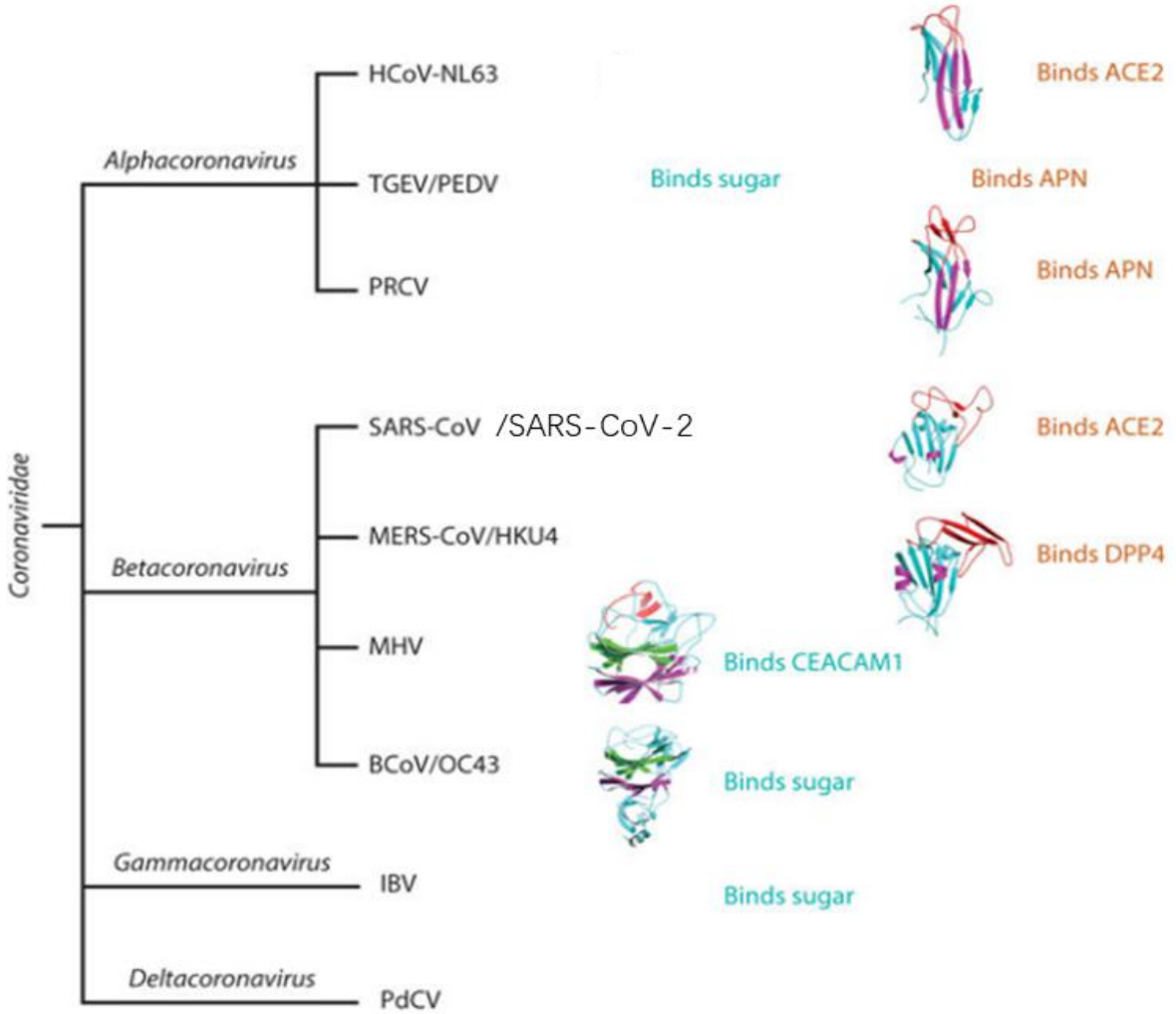




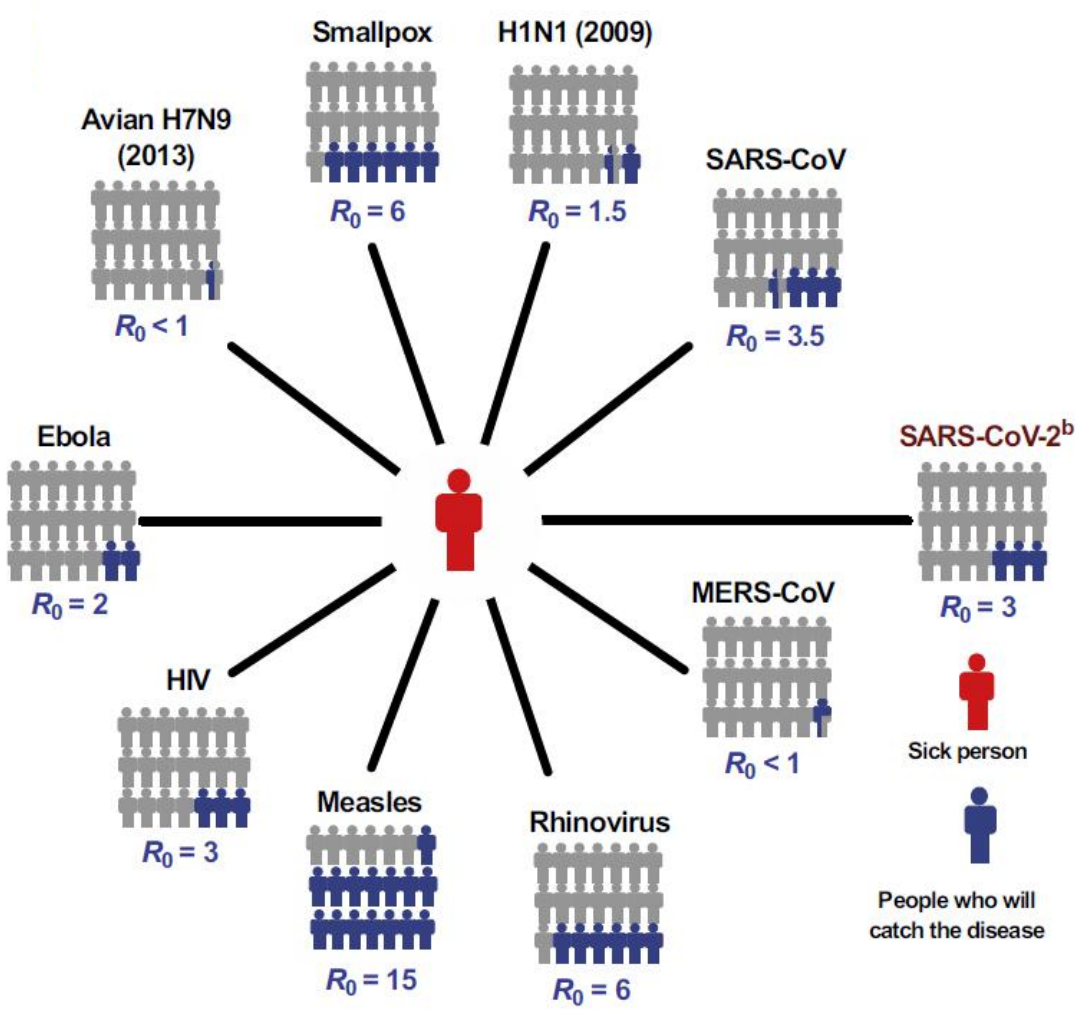
Yao et al., 2020, Cell 183, 1–9
October 29, 2020 ª 2020 Elsevier Inc.

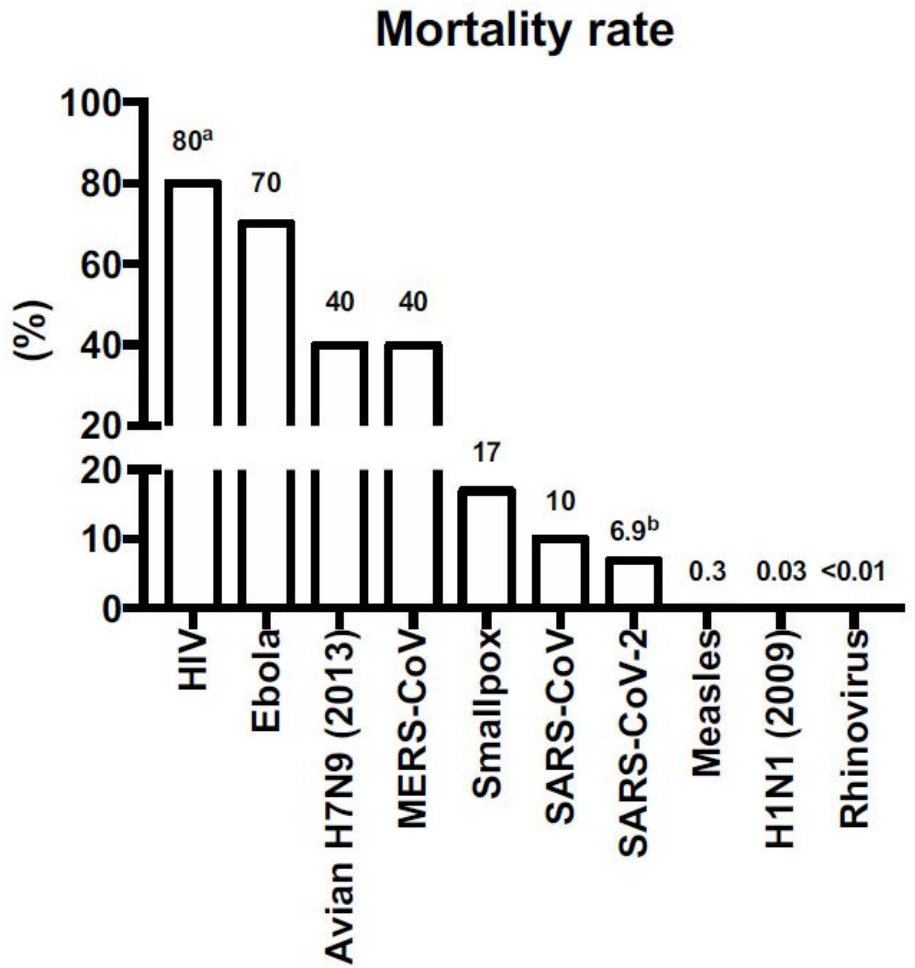
冠状病毒分类


Alpha-CoV
NL63-related BtKYNL63-9b
Rh-BatCoV HKU2
Lucheng Rn RatCoV
Mink CoV
Ferret CoV
FIPV
HCoV-NL63
HCoV-229E
Mi-BatCoV 1A
Mi-BatCoV HKU8
BtNv-AlphaCoV/SC2013
BtRf-AlphaCoV/HuB2013
Ro-BatCoV HKU10
BtMr-AlphaCoV/SAX2011
BatCoV CDPHE15
Sc-BatCoV 512
PEDV
Beta-CoV
HCoV-OC43
MHV
China Rattus CoV HKU24
HCoV-HKU1
Hedghog coronavirus 1
Ty-BatCoV HKU4
Pi-BatCoV HKU5
MERS-CoV
2019-nCoV
SARS-CoV
Hp-BetaCoV/Zhejiang2013
Ro-BtCoV GCCDC1
Ro-BatCoV HKU9
Gamma-CoV
Avian CoV
BWCoV SW1
Night heron CoV HKU19
Wigeon CoV HKU20
Common moorhen CoV HKU21
Bulbul CoV HKU11
White eye CoV HKU16
Munia CoV HKU13
Porcine CoV HKU15
Delta-CoV
0.1


Coronaviridae
Alphacoronavirus
HCoV-NL63
TGEV/PEDV
PRCV
Betacoronavirus
SARS-CoV /SARS-CoV-2
MERS-CoV/HKU4
MHV
BCoV/OC43
Gammacoronavirus
IBV
Deltacoronavirus
PdCV
Binds ACE2
Binds sugar
Binds APN
Binds APN
Binds ACE2
Binds DPP4
Binds CEACAM1
Binds sugar
Binds sugar

新冠的R_0及致死率





Trends in Microbiology, August 2020, Vol. 28, No. 8

新冠及其他冠状病毒感染症状

Table 1. Clinical Features of COVID-19, SARS, and MERS

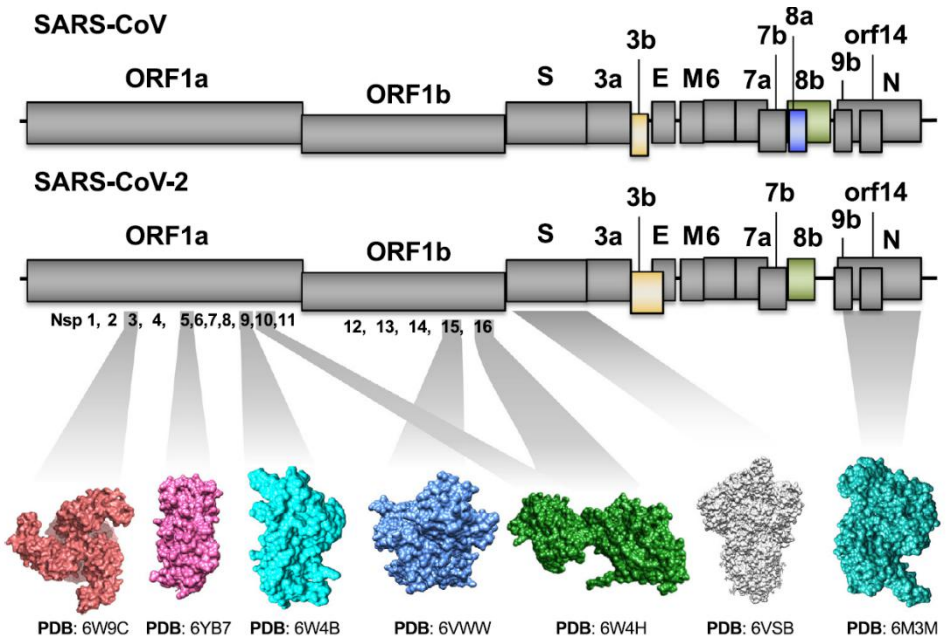
Symptom	COVID-19							SARS	MERS
	Study[a]								
	1 (n = 62) [91]	2 (n = 1099) [8]	3 (n = 138) [92]	4 (n = 140) [9]	5 (n = 41) [7]	6 (n = 99) [10]	Range (average)	7 Range [93]	8 Range [94]
Fever	77.4	88.7	98.6	91.7	97.6	82.8	77.4–98.6 (89.5)	99–100	98
Cough	80.6	67.8	59.4	75.0	75.6	81.8	67.8–86.2 (73.4)	62–100	83
Dyspnea	–	–	31.2	36.7	55.0	31.3	31.2–55.0 (38.5)	–	–
Myalgia	51.6	14.9	34.8	–	43.9	11.1	11.1–51.6 (31.3)	45–61	32
Panting	3.2	–	–	–	29.3	–	3.2–29.3 (16.2)	–	–
Headache	34.4	13.6	6.5	–	7.9	8.1	6.5–34.4 (14.1)	20–56	11
Sore throat	–	13.9	17.4	–	–	5.1	5.1–17.4 (12.1)	13–25	14
Nausea/vomiting	–	5.0	6.3-10	22.3	–	1.0	2.0–22.3 (9.4)	20–35	21
Diarrhea	4.8	3.8	10.1	12.9	2.6	2.0	2.0–12.9 (6.1)	20–25	26
Rhinorrhea	–	–	–	–	–	4.0	4.0 (4.0)	2–24	6
Abdominal pain	–	–	2.2	5.8	–	–	2.2–5.8 (4.0)	–	–

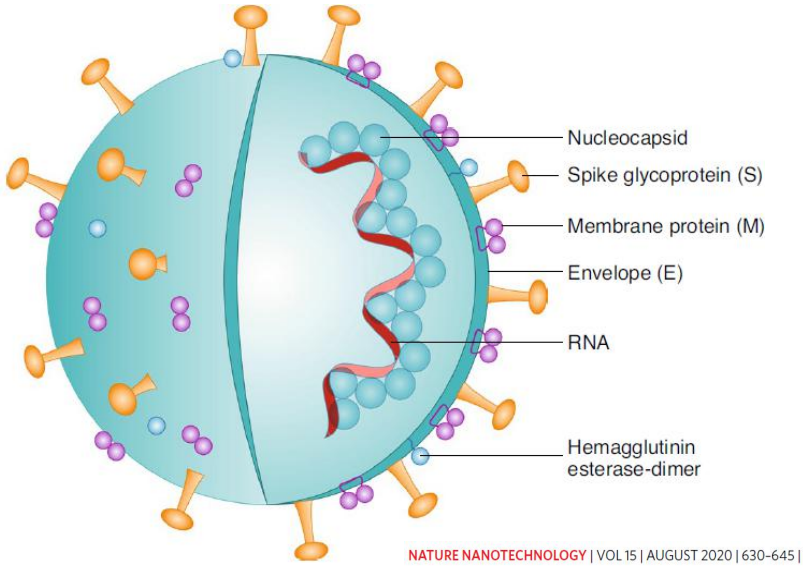
[a]Percentage of study subjects that experienced the indicated symptom; for fields without values, this symptom was not evaluated.

Trends in Microbiology, August 2020, Vol. 28, No. 8

并非所有新冠病毒感染都有发热/咳嗽症状：隐性感染

新冠和SARS基因

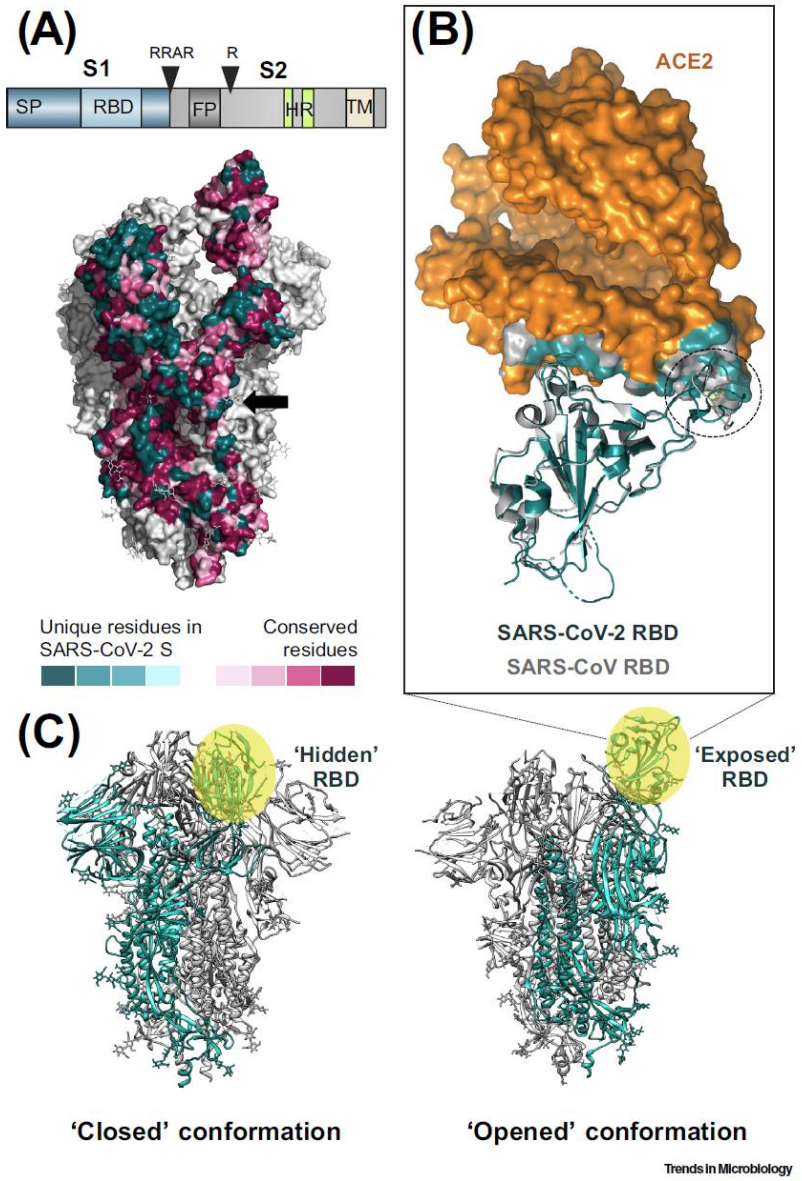




Virus strain	ORF1ab	S	E	M	N
SARS-CoV Urbani Protein ID	AAP13442.1	AAP13441.1	QHD43418.1	QHD43419.1	QHD43423.2
SARS-CoV-2 Wuhan-Hu-1 Protein ID	QHD43415.1	QHD43416.1	AAP13443.1	AAP13444.1	AAP13445.1
% Identity (a.a.)	86.46	77.46	96.00	89.59	89.74

Trends in Microbiology

新冠和SARS-CoV的受体均为ACE2



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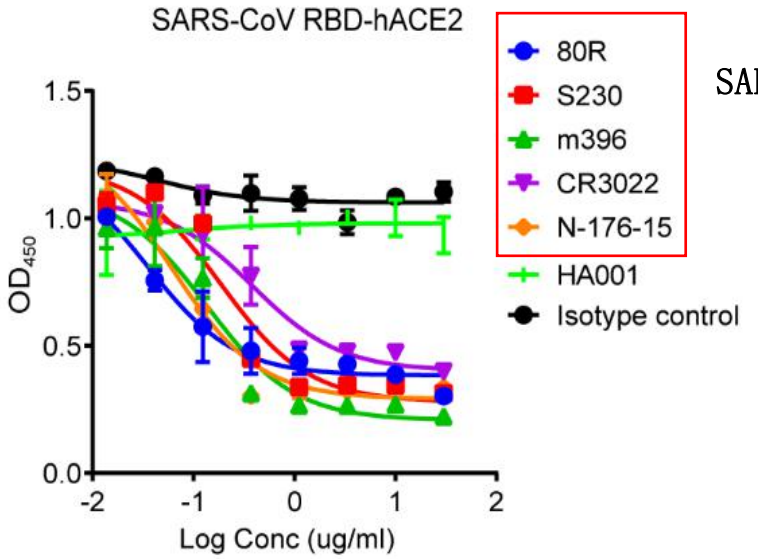
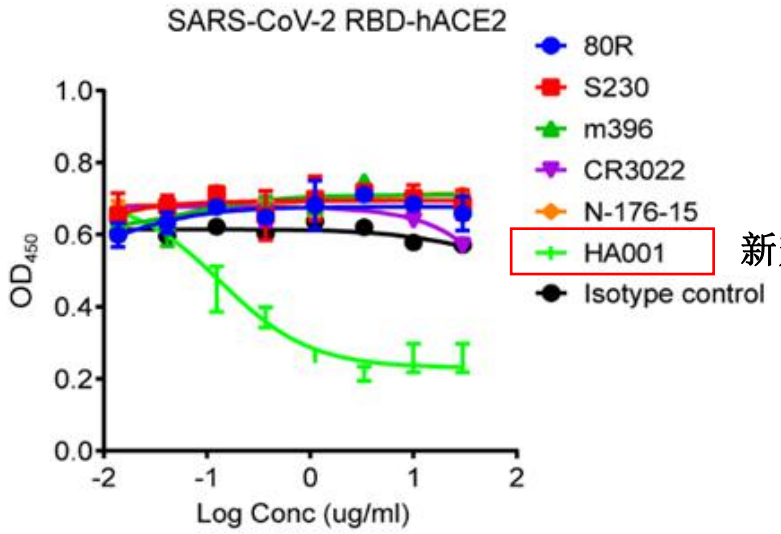
SARS-CoV中和抗体无法中和新冠病毒



SARS 中和抗体

新冠中和抗体无法阻断SARS-CoV与其受体的结合



新冠中和抗体

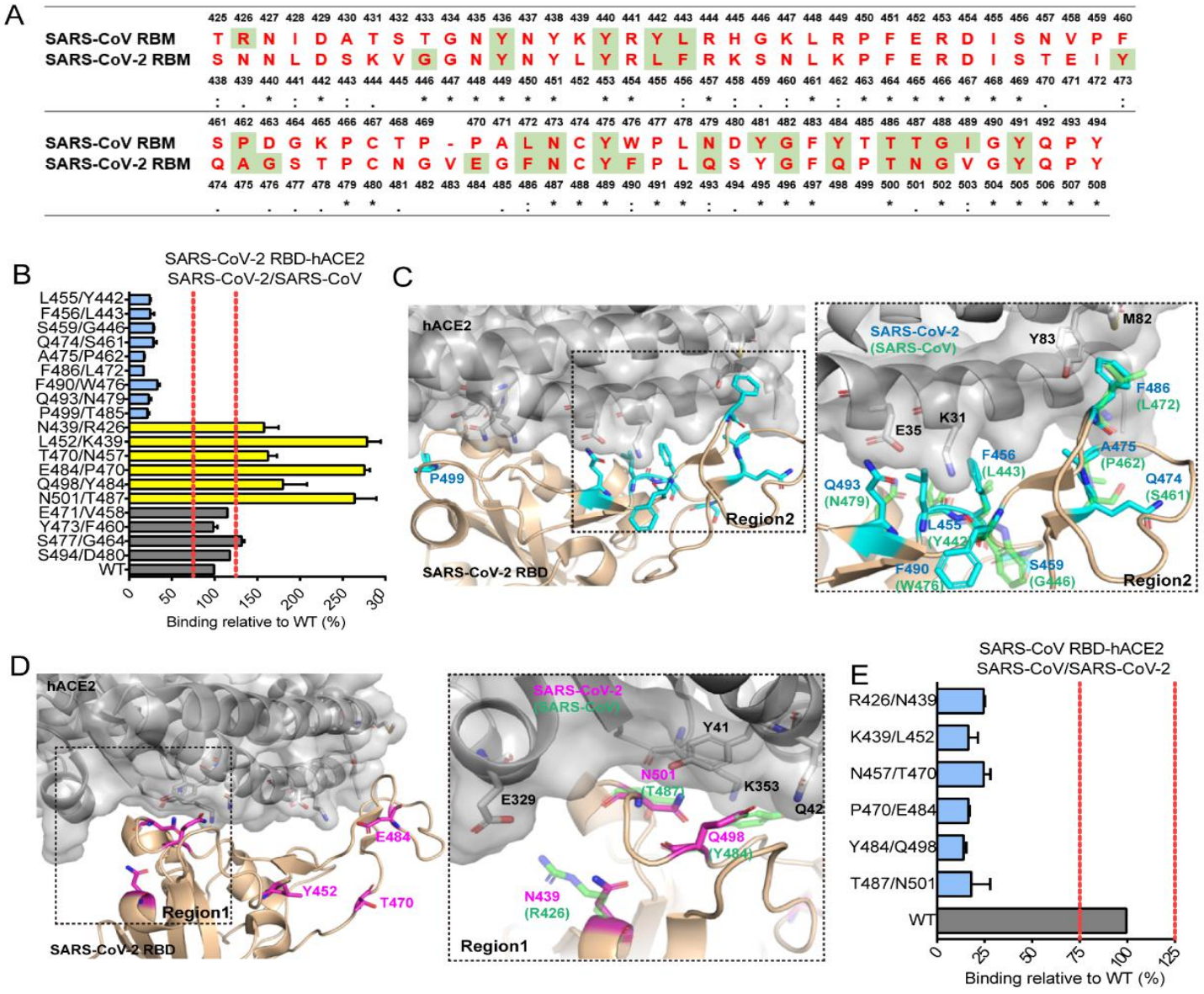
SARS-CoV中和抗体无法阻断新冠病毒与其受体的结合

mAbs	SARS-CoV IC_{50} (ug/ml)	SARS-CoV-2 IC_{50} (ug/ml)
80R	0.4	>50
S230	0.016	>50
m396	0.08	>50
N-176-15	0.07	>50
CR3022	2.0	>50
HA001	>50	0.016

SARS-CoV中和抗体无法中和新冠病毒

Cellular & Molecular Immunology (2020) 17:621-630;

新冠病毒S蛋白与受体ACE2 结合的模式发生变化

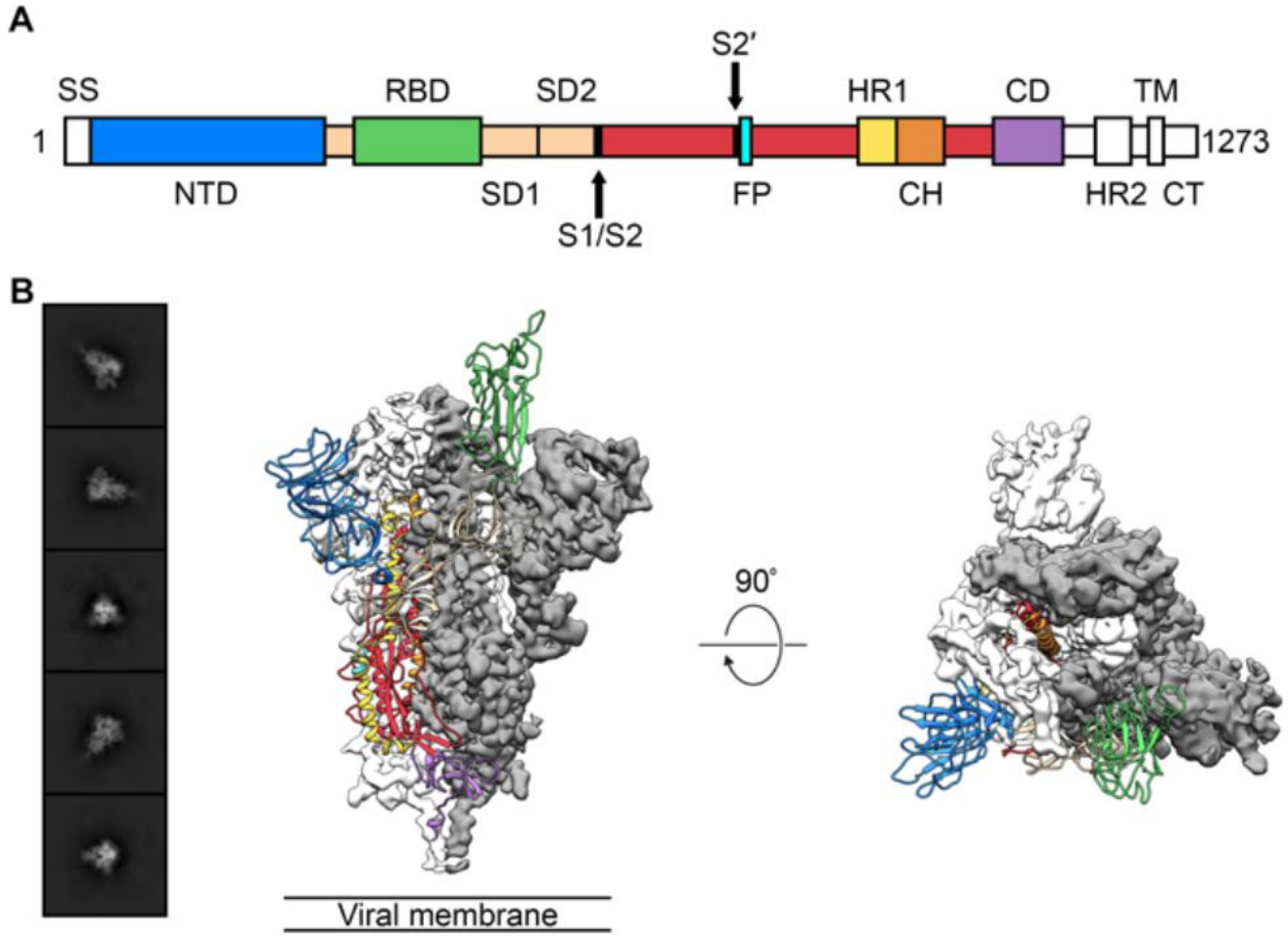


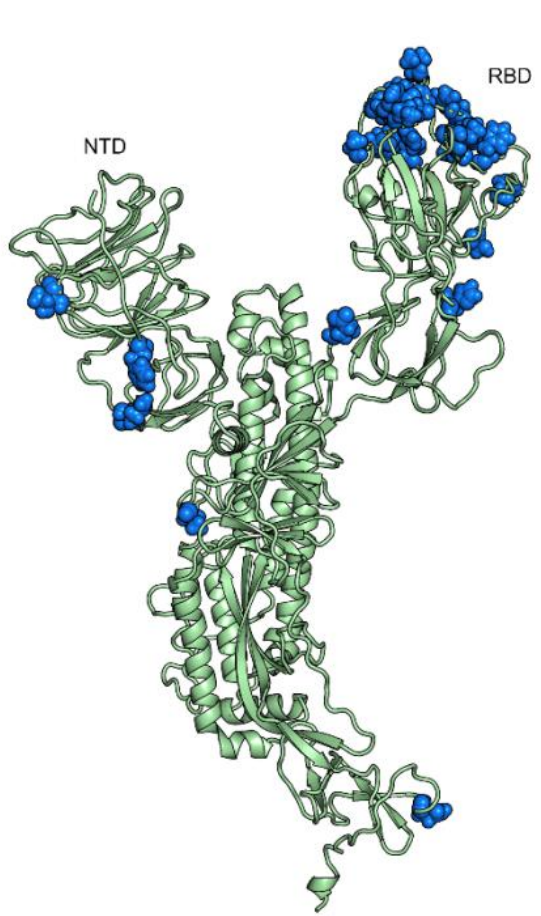
Cellular & Molecular Immunology (2020) 17:621–630;

Antibody Therapy 治疗性抗体 VS 疫苗 Vaccine

Antibody Therapy 治疗性抗体		Vaccine 疫苗
A molecule that binds and neutralizes virus 结合并中和病毒的分子	What is it?	A non-infectious virus and/or piece of virus your immune system will recognize 非致病性病毒和/或可被免疫系统识别的成分
Directly treat someone infected with SARS-CoV-2 直接治疗/预防病毒感染	What is the goal?	Train a healthy immune system to protect from a future SARS-CoV-2 infection 诱导产生保护性免疫应答以应对潜在的感染
Immediately 即时性	How quickly does it work?	1-2 weeks after vaccination 免疫后1-2周
Weeks to Months 几周到几个月	How long will protection last?	Years to lifetime (sometimes need boosters) 几年到终生（常需要加强免疫）

SARS-CoV-2 spike 蛋白是中和抗体的重要靶标

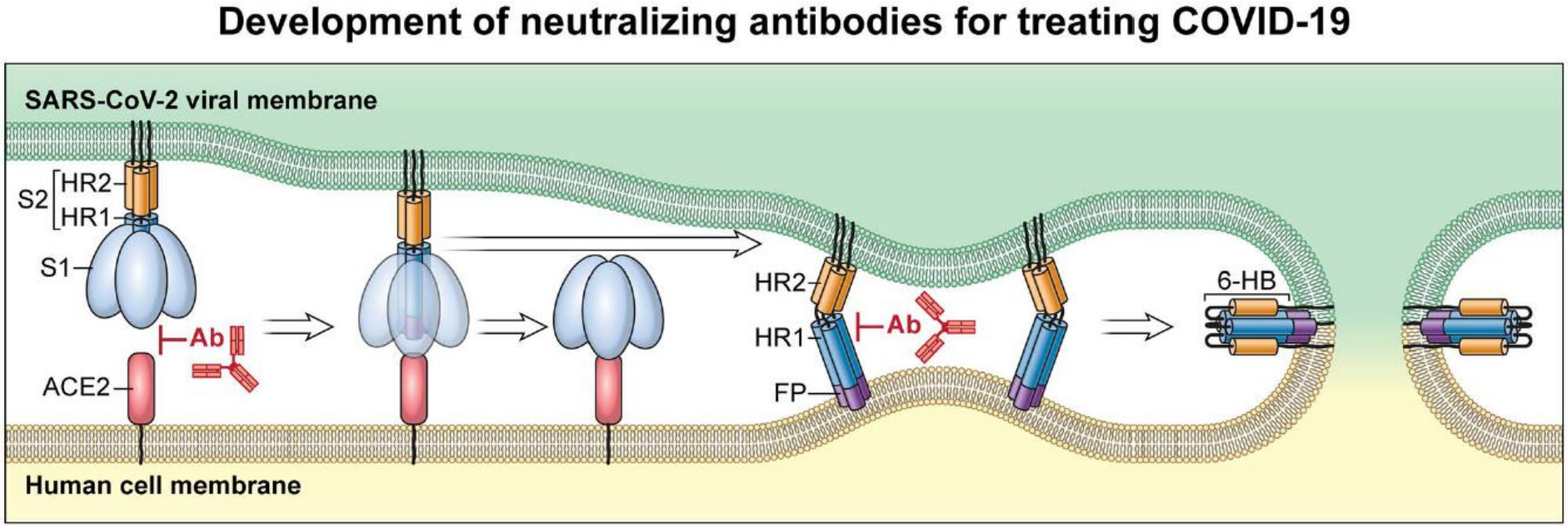




A,B: Structure of 2019-nCoV S in the prefusion conformation.
C: A single monomer of the 2019-nCoV S.

Daniel Wrapp, 2020

SARS-CoV2 抗体药物作用机制



- Receptor binding inhibition
- Fusion inhibation

Antibody Therapeutics, 2020, Vol. 3, No. 2

临床新冠抗体进展

临床分期	实验主办方	候选中和抗体	国家	试验目的/适应症	入组人群	入组人数	开始时间
I 期	礼来	LY-CoV555	美国	重症治疗	重症住院患者	40	2020/05/28
	君实	JS016	中国	安全性，耐受性，药代动力学	健康受试者	40	2020/06/05
	Tychan	TY027	新加坡	安全性，耐受性，药代动力学	健康受试者	25	2020/06/09
	礼来	LY-CoV016（JS016）	美国	安全性，耐受性，药代动力学	健康受试者	24	2020/06/19
	腾盛博药	BRII-196	中国	安全性，耐受性，药代动力学	健康受试者	12	2020/07/12
	腾盛博药	BRII-198	中国	安全性，耐受性，药代动力学	健康受试者	12	2020/07/13
	Celltrion	CT-P59	韩国	安全性，耐受性，药代动力学	健康受试者	32	2020/07/18
	阿斯利康	AZD7442	英国	安全性，耐受性，药代动力学	健康受试者	48	2020/07/25
	迈威生物	MW33	中国	安全性，耐受性，药代动力学	健康受试者	42	2020/08/07
	神州细胞	SCTA01	中国	安全性，耐受性，药代动力学	健康受试者	33	2020/08/17
II期	礼来	LY-CoV555，LY-CoV555+LY-CoV016	美国	轻症治疗	轻至中度患者	550	2020/06/17
II/III期	VIR x GSK	VIR-7831	美国	轻症治疗	轻症患者	1300	2020/08/31
III期	再生元	REGN-COV2（鸡尾酒）	美国	高风险感染者及密切接触者预防	阳性患者的家庭密切接触者/医护人员或急救人员	2000	2020/07/13
	礼来	LY-CoV555	美国	高风险感染者预防	疗养院和辅助生活社区居住者和工作人员	2400	2020/08/02
I/II/III期	再生元	REGN-COV2（鸡尾酒）	美国	重症治疗	重症住院患者	2970	2020/06/10
	再生元	REGN-COV2（鸡尾酒）	美国	轻症治疗	非住院患者	2104	2020/06/16

临床新冠抗体进展

1，君实生物：JS016（CB6）

君实生物新冠中和抗体JS016完成I期临床试验受试者入组

07-13
2020

Article

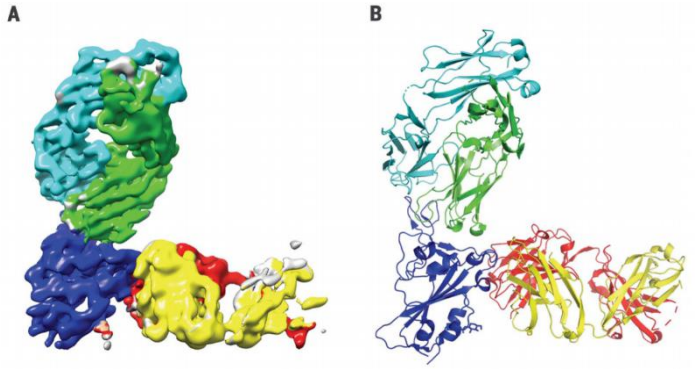
A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2

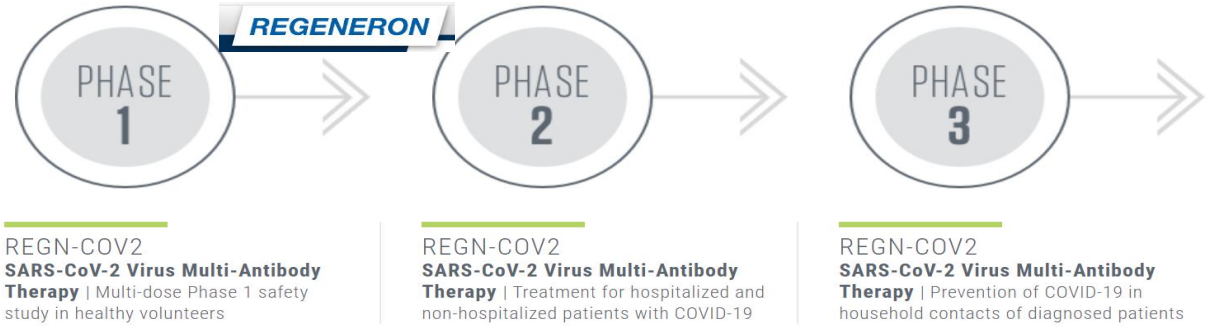
https://doi.org/10.1038/s41586-020-2381-y
Received: 2 April 2020
Accepted: 19 May 2020
Published online: 26 May 2020

Rui Shi[1,2,14], Chao Shan[3,14], Xiaomin Duan[1,2,14], Zhihai Chen[4,14], Peipei Liu[5,14], Jinwen Song[6,14], Tao Song[1,7,8], Xiaoshan Bi[1,9], Chao Han[1,2], Lianao Wu[9,10], Ge Gao[3], Xue Hu[3], Yanan Zhang[3], Zhou Tong[1,10], Weijin Huang[11], William Jun Liu[5], Guizhen Wu[5], Bo Zhang[3], Lan Wang[11], Jianxun Qi[10,12], Hui Feng[13], Fu-Sheng Wang[6]✉, Qihui Wang[1,10,12]✉, George Fu Gao[10]✉, Zhiming Yuan[3]✉ & Jinghua Yan[1,10,12]✉

君实生物计划尽快开展针对轻型/普通型新冠肺炎患者的Ib期临床研究，以及针对重型及危重型新冠肺炎患者的II/III期临床研究。同时，公司也将后续开展针对新冠病毒高危人群的研究，以评价JS016对新冠病毒感染的预防作用。

2，再生元：cocktail 双抗





2020/06

CORONAVIRUS

Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies

Alina Baum, Benjamin O. Fulton, Elzbieta Wloga, Richard Copin, Kristen E. Pascal, Vincenzo Russo, Stephanie Giordano, Kathryn Lanza, Nicole Negron, Min Ni, Yi Wei, Gurinder S. Atwal, Andrew J. Murphy, Neil Stahl, George D. Yancopoulos, Christos A. Kyratsous*

CORONAVIRUS

Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail

Johanna Hansen[1]*, Alina Baum[1]*, Kristen E. Pascal[1], Vincenzo Russo[1], Stephanie Giordano[1], Elzbieta Wloga[1], Benjamin O. Fulton[1], Ying Yan[1], Katrina Koon[1], Krunal Patel[1], Kyung Min Chung[1], Aynur Hermann[1], Erica Ullman[1], Jonathan Cruz[1], Ashique Rafique[1], Tammy Huang[1], Jeanette Fairhurst[1], Christen Libertiny[1], Marine Malbec[1], Wen-yi Lee[1], Richard Welsh[1], Glen Farr[1], Seth Pennington[1], Dipali Deshpande[1], Jemmie Cheng[1], Anke Watty[1], Pascal Bouffard[1], Robert Babb[1], Natasha Levenkova[1], Calvin Chen[1], Bojie Zhang[1], Annabel Romero Hernandez[1], Kei Saotome[1], Yi Zhou[1], Matthew Franklin[1], Sumathi Sivapalasingam[1], David Chien Lye[2], Stuart Weston[3], James Logue[3], Robert Haupt[3], Matthew Frieman[3], Gang Chen[1], William Olson[1], Andrew J. Murphy[1], Neil Stahl[1], George D. Yancopoulos[1], Christos A. Kyratsous[1]†

2020/10

Science

REPORTS

Cite as: A. Baum *et al.*, *Science* 10.1126/science.abe2402 (2020).

REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters

Alina Baum[1], Dharani Ajithdoss[1], Richard Copin[1], Anbo Zhou[1], Kathryn Lanza[1], Nicole Negron[1], Min Ni[1], Yi Wei[1], Kusha Mohammadi[1], Bret Musser[1], Gurinder S. Atwal[1], Adelekan Oyejide[1], Yenny Goez-Gazi[2], John Dutton[2], Elizabeth Clemmons[2], Hilary M. Staples[2], Carmen Bartley[2], Benjamin Klaffke[2], Kendra Alfson[2], Michal Gazi[2], Olga Gonzalez[2], Edward Dick Jr.[2], Ricardo Carrion Jr.[2], Laurent Pessaint[3], Maciel Porto[3], Anthony Cook[3], Renita Brown[3], Vaneesha Ali[3], Jack Greenhouse[3], Tammy Taylor[3], Hanne Andersen[3], Mark G. Lewis[3], Neil Stahl[1], Andrew J. Murphy[1], George D. Yancopoulos[1], Christos A. Kyratsous[1]*

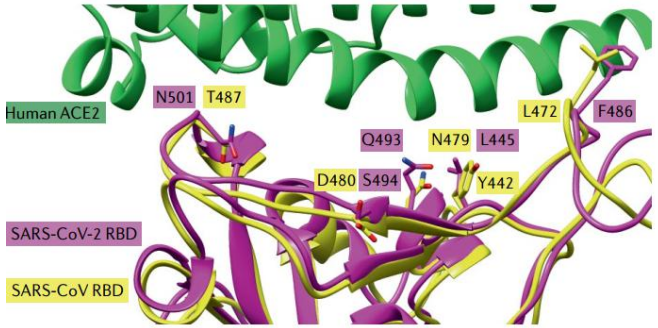
今年6月，两篇发表在Science期刊上的论文报道了一种针对冠状病毒SARS-CoV-2的抗体鸡尾酒（也称为抗体混合物），这种抗体鸡尾酒是通过探究人源化小鼠和康复患者开发出来的（Science, 2020, doi:10.1126/science.abd0827; Science, 2020, doi:10.1126/science.abd0831）。所开发出的这种由两种抗体REGN10987和REGN10933组成的抗体鸡尾酒可结合这种冠状病毒，从而降低出现它出现耐药性的风险。

如今，在一项新的研究中，通过在这两篇论文的基础上进行扩展，研究人员发现这种抗体鸡尾酒无论是预防性还是治疗性施用，都给模拟SARS-CoV-2感染的恒河猴和金仓鼠的动物模型带来了益处。

新冠spike蛋白高度突变

SARS_COV_2的Spike序列突变分析（截止2020年9月）

在S蛋白共有**2080个位点**发生突变，其中引起**氨基酸变化的突变共1131个**，
位于蛋白**受体结合区（RBD）的氨基酸134个**，
其中在已报导**蛋白互作中关键的17个氨基酸中发生非同义突变的氨基酸13个**。

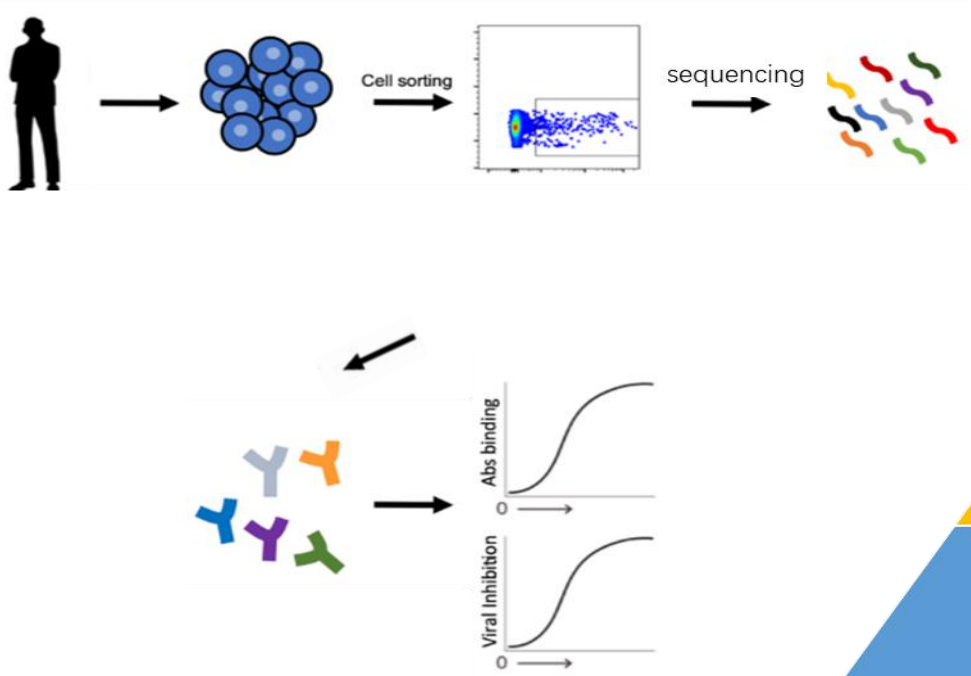


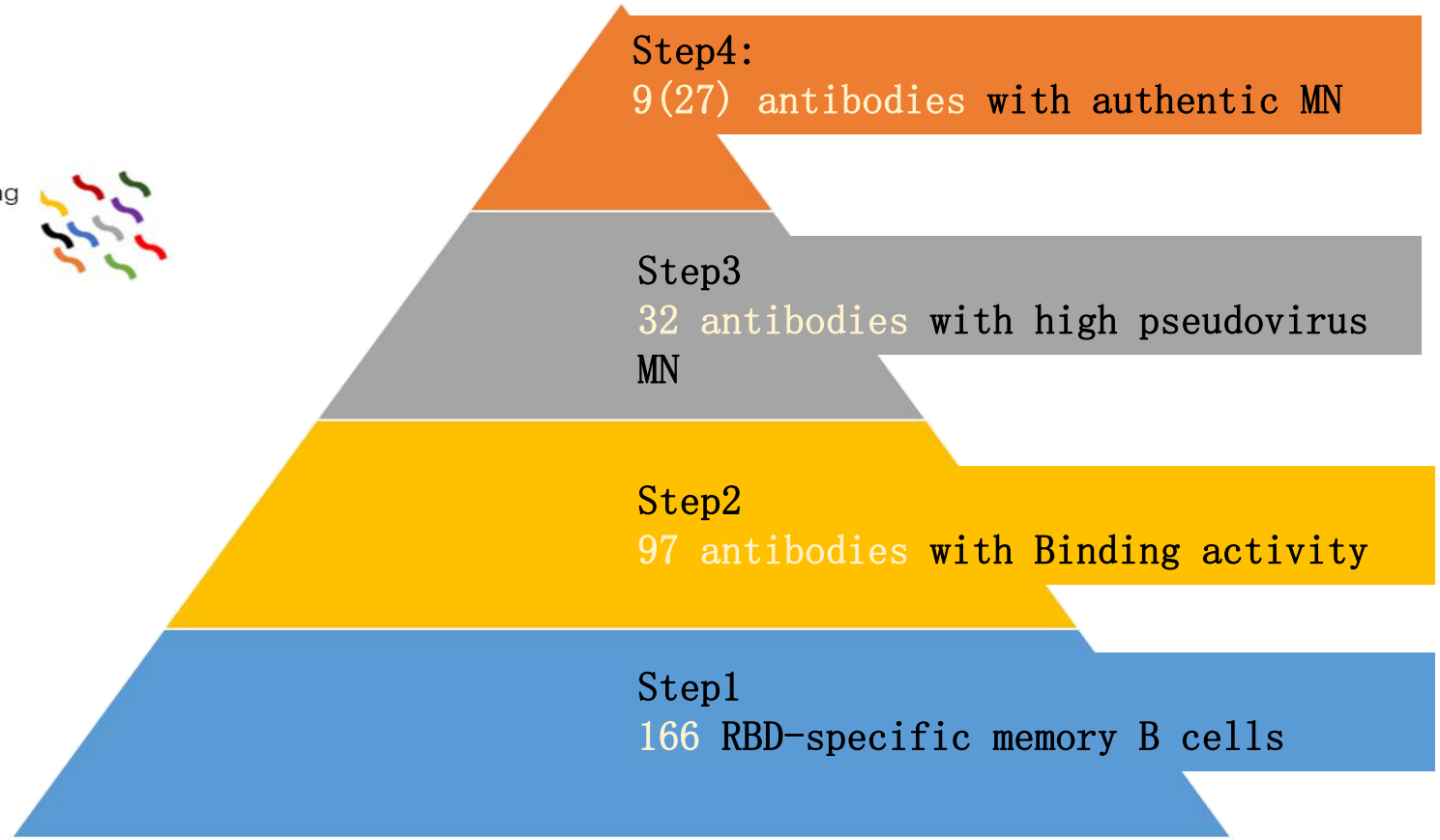
部分RBD突变使单抗失去中和活性

目前新冠病毒spike蛋白已经发生突变，可能增强了传播性，并造成某一类抗体失去中和活性。

SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies, *2020, nature*

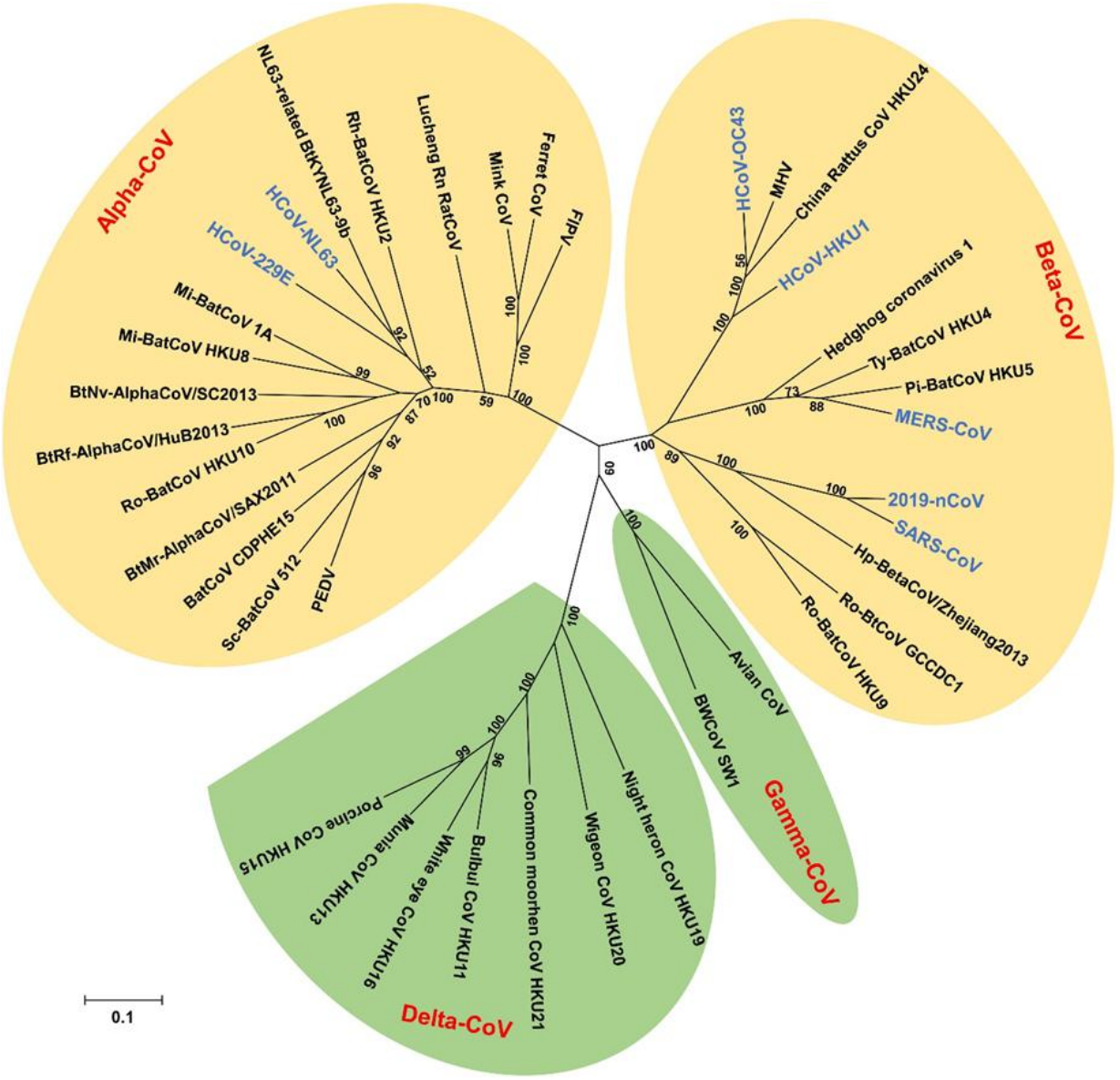
单细胞技术筛选全人源RBD新冠抗体

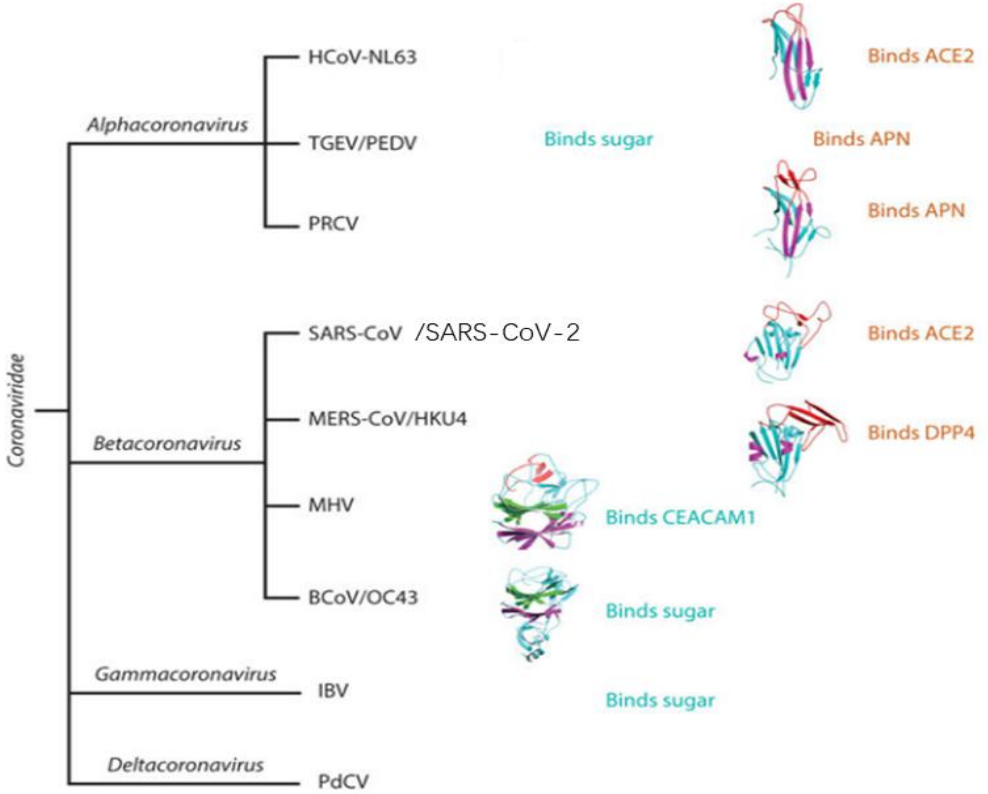




冠状病毒广谱全人中和抗体

Phylogenetic tree of coronaviruses





Questions

- Neutralizing antibodies against S2?
- Structure, function and mechanism?

1. Aid vaccine design;
2. Resistant to RBD antibody-resistant strains;
3. Broad spectrum (mutated SARS-CoV-2 and other coronaviruses).

- 是否有靶向S2表位的抗体和广谱中和作用？
- 其功能，结构及作用机制如何？

1，为疫苗设计提供指导

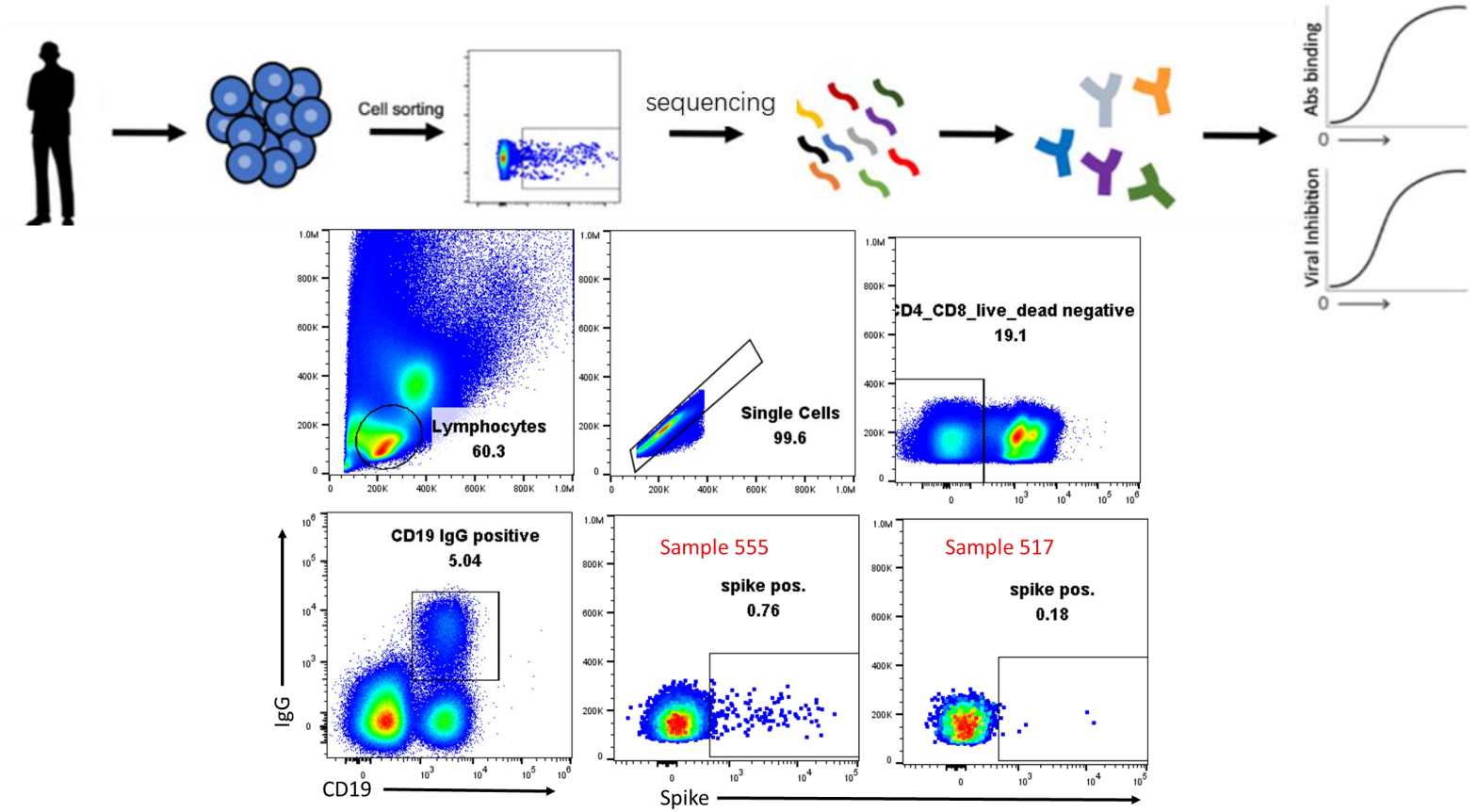
2，弥补RBD抗体的不足，**抵抗耐药毒株**， 广谱性（突变新冠病毒和其他冠状病毒），

3，与RBD抗体联合疗法，不同作用机制产生协同效应，提高中和活性

1. Isolation of 76E1 from convalescent patients of SARS-CoV-2

A 5例康复患者外周血中获得识别SARS-CoV-2 Spike（S）全长蛋白的全人抗体

Human antibodies against full-length SARS-CoV-2 spike (S) were obtained from 5 convalescent patients

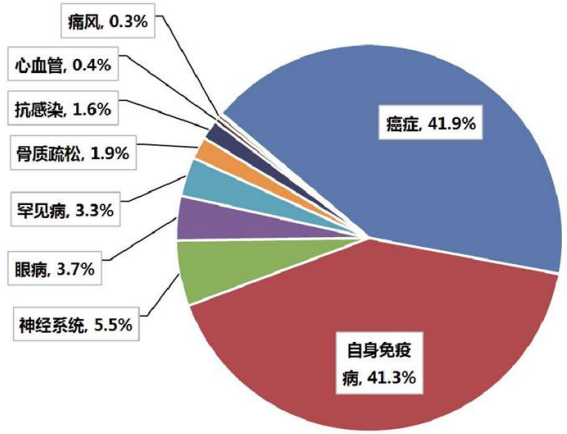


截至目前，全球已有200余种生物技术药物获准进入市场，1000多种处于临床试验的不同阶段。其中接近一半为抗体药物。

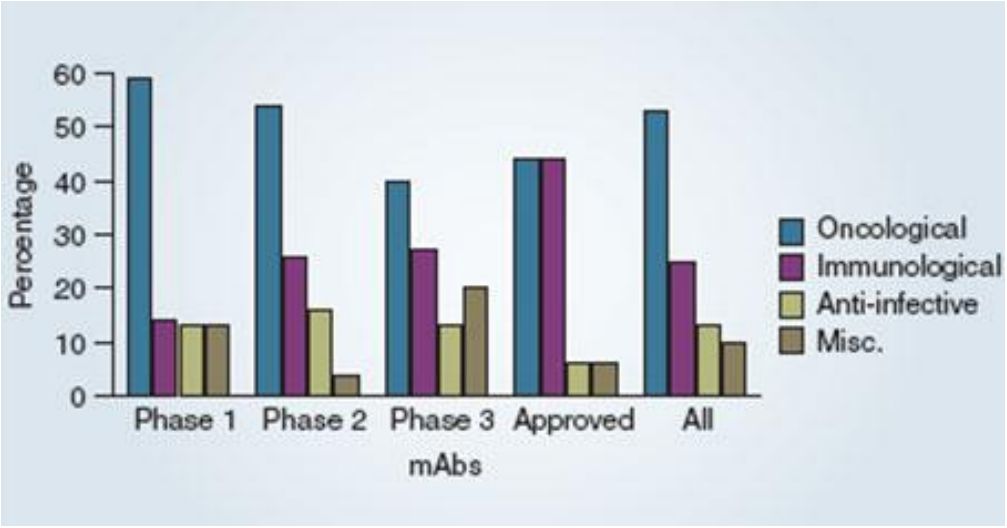
目前全球销售额最高的6大类生物技术药物：

抗肿瘤药物
anti-TNFα药物
EPO
胰岛素
β- 干扰素
凝血因子

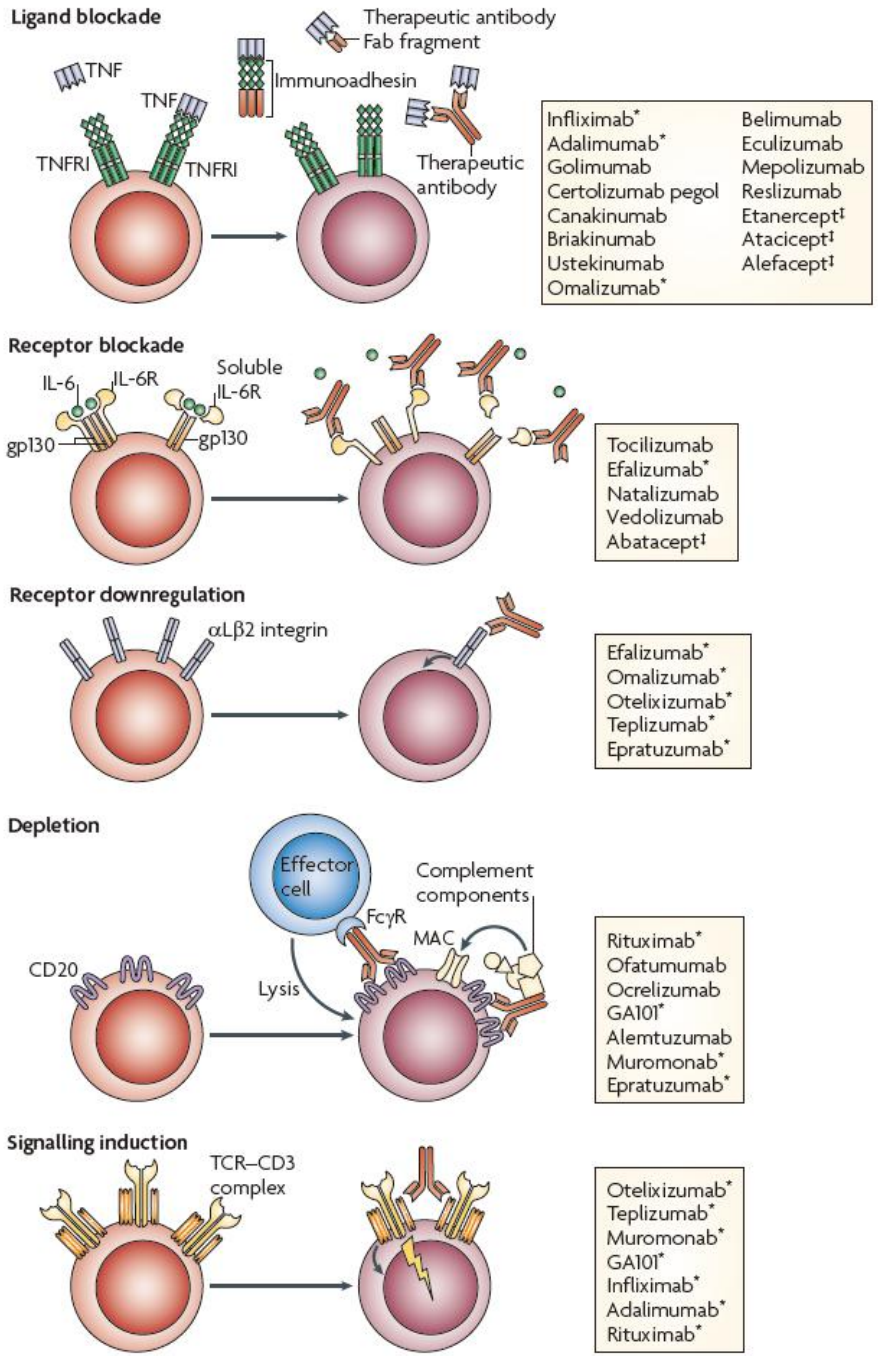
抗体药物的应用领域



- 抗器官移植排斥反应；
- 肿瘤靶向治疗，肿瘤免疫治疗；
- 哮喘、银屑病、类风湿性关节炎、红斑狼疮、急性心梗、脓毒症、多发性硬化症及其他自身免疫性疾病；
- 心脑血管疾病；
- 感染性疾病；
- 其他



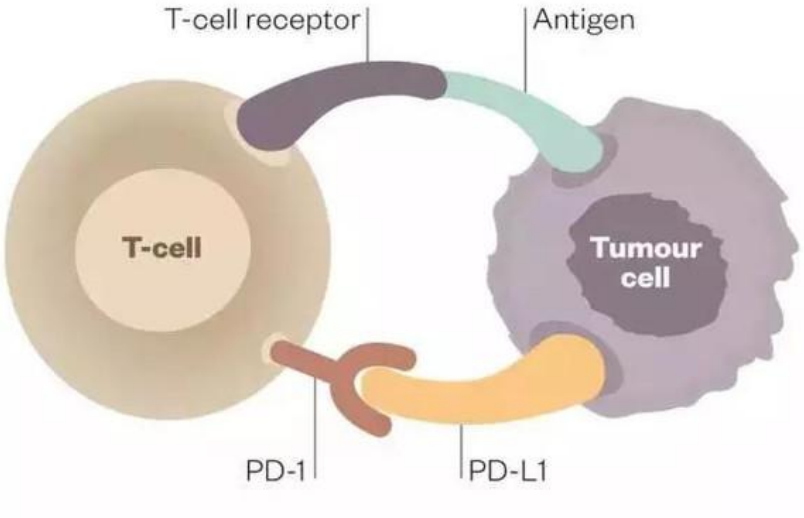
抗体药物的作用机制

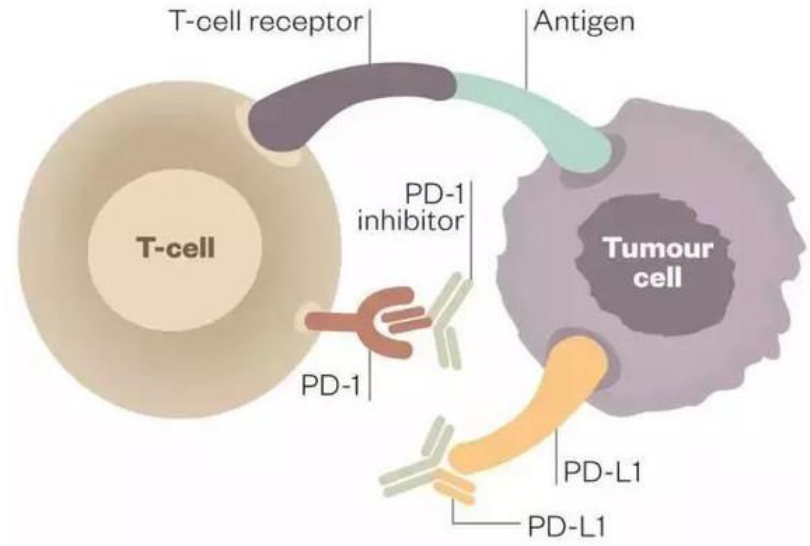


Nat. Rev. Immunol. 2010. Vol. 10. 301-316

第五节：工程抗体的未来展望

肿瘤的抗体免疫治疗是一个重要方向
PD-1/PD-L1 抗体药物成为癌症治疗的明星





- Nivolumab (Opdivo);
- Fully Human antibody;
- anti-PD-1;
- Bristol-Myers Squibb, 2014.
- Pembrolizumab (Keytruda);
- Humanized antibody,/humanized by MRCT;
- anti-PD-1;
- MSD, 2014.

黑色素瘤，非小细胞肺癌，三阴乳腺癌等不同癌症都有较好疗效。非小细胞肺癌（NSCLC）患者中，nivolumab表现出了持续的疗效，一年存活率达42%，两年存活率达24%。

Nobelförsamlingen

The Nobel Assembly at Karolinska Institutet

has today decided to award

the 2018 Nobel Prize in Physiology or Medicine

jointly to

James P. Allison and Tasuku Honjo

for their discovery of cancer therapy by inhibition of negative immune regulation

Tasuku Honjo was born in 1942 in Kyoto, Japan. In 1966 he became an MD, and from 1971-1974 he was a research fellow in USA at Carnegie Institution of Washington, Baltimore and at the National Institutes of Health, Bethesda, Maryland. He received his PhD in 1975 at Kyoto University. From 1974-1979 he was a faculty member at Tokyo University and from 1979-1984 at Osaka University. Since 1984 he has been Professor at Kyoto University. He was a Faculty Dean from 1996-2000 and from 2002-2004 at Kyoto University.

James P. Allison was born 1948 in Alice, Texas, USA. He received his PhD in 1973 at the University of Texas, Austin. From 1974-1977 he was a postdoctoral fellow at the Scripps Clinic and Research Foundation, La Jolla, California. From 1977-1984 he was a faculty member at University of Texas System Cancer Center, Smithville, Texas; from 1985-2004 at University of California, Berkeley and from 2004-2012 at Memorial Sloan-Kettering Cancer Center, New York. From 1997-2012 he was an Investigator at the Howard Hughes Medical Institute. Since 2012 he has been Professor at University of Texas MD Anderson Cancer Center, Houston, Texas and is affiliated with the Parker Institute for Cancer Immunotherapy.

- *Can our immune defense be engaged for cancer treatment?*
- **Accelerators and brakes in our immune system**
- **A new principle for immune therapy: CTLA-4, 1990s; PD-1, 1992.**
- **Immunotherapy for cancer today and in the future**

- Can our immune defense be engaged for cancer treatment?

Tumor Immunotherapy started 129 years ago !

Coley — pioneer of vaccine therapy.

Nature Reviews Cancer **9** , 361–371

A patient with round cell sarcoma of the jaw and abdominal metastases seen by Coley in 1899

Coley's toxins：a mixture consisting of killed bacteria of species *Streptococcus pyogenes* and *Serratia marcescens*, named after William Coley, a surgical oncologist at the Hospital for Special Surgery who developed the mixture in the late 19th century as a treatment for cancer.

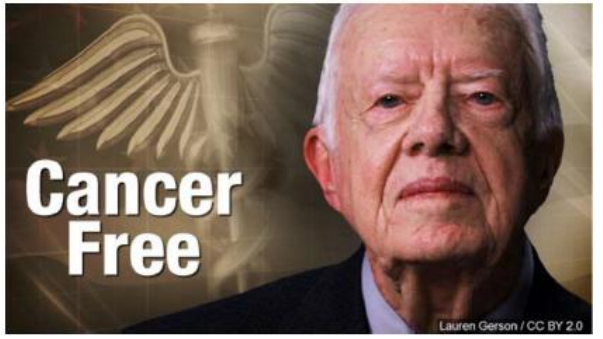
Endotoxins and TNF

- Can our immune defense be engaged for cancer treatment?

Significant Events in the History of Cancer Immunotherapy[3,5–9]

1893	Dr. William B. Coley invents Coley's toxins
1899	Parke-Davis & Company manufactures Coley's toxins
1915	Memorial Hospital (now Memorial Sloan Kettering) bans Coley's toxins, preferring radiation treatments
1943	Chemotherapy use begins for cancer treatment
1953	Helen Coley Nauts (Dr. Coley's daughter) founds the Cancer Research Institute in New York City
1957	Dr. E. Donnall Thomas has success treating patients with advanced leukemia with allo-HSCT
1971	President Richard Nixon declares a war on cancer
1986	Recombinant IFN-α approved by FDA for hairy cell leukemia
1988	Dr. Steven A. Rosenberg publishes work regarding curative treatment of melanoma patients with activated immune cells and cytokines
1992	rhIL-2 approved by FDA for RCC
1996	First mAB approved by FDA for select B-cell malignancies
2010	First DC-based cancer vaccine approved by FDA for prostate cancer
2011	First CTLA-4 ICB FDA approval for melanoma
2014	First PD-1 ICBs FDA approved for metastatic melanoma

allo-HSCT = allogeneic hematopoietic stem cell transplantation; CTLA-4 = cytotoxic T-lymphocyte–associated antigen 4; DC = dendritic cell; FDA = Food and Drug Administration; ICB = immune checkpoint blocker; IFN-α = interferon alpha; mAb = monoclonal antibody; PD-1 = programmed death-1; RCC = renal cell carcinoma; rhIL-2 = recombinant interleukin-2



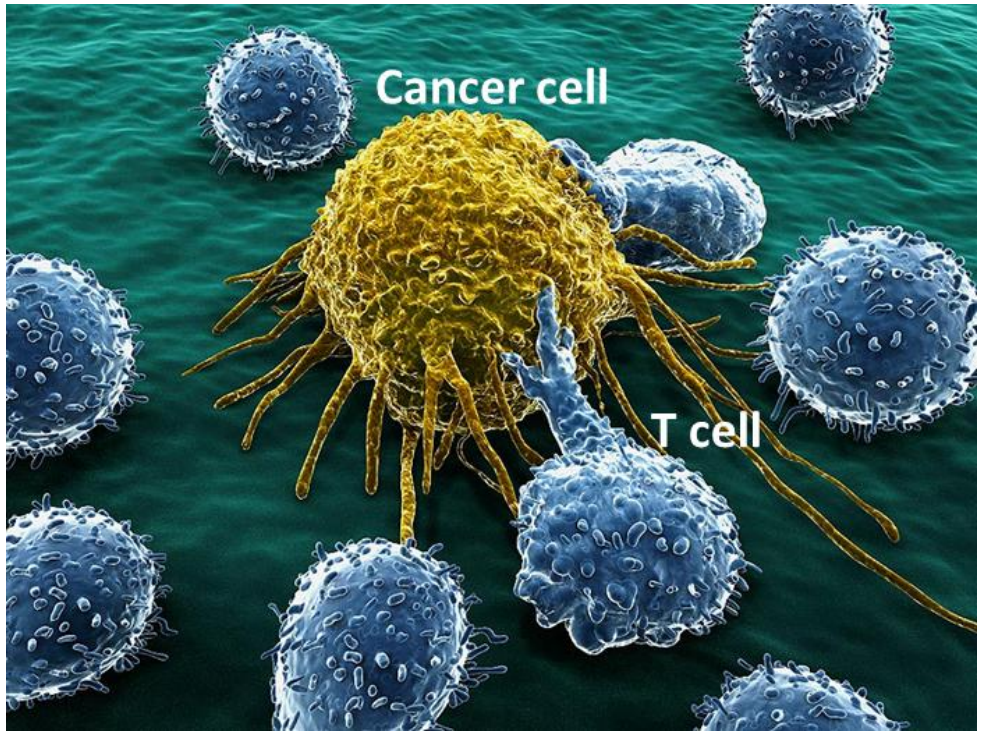
AACR 2016

5 yr survival on anti-PD1 therapy = 34%, double the historical rate

Hodi FS, et al. Abstract CT001. Presented at: American Association for Cancer Research Annual Meeting; April 16-20, 2016; New Orleans.

- *Can our immune defense be engaged for cancer treatment?*

The adaptive immune system: an “ideal” anti-cancer agent



Diversity:
T cells - 10^{18}
Antibodies - 10^{22}

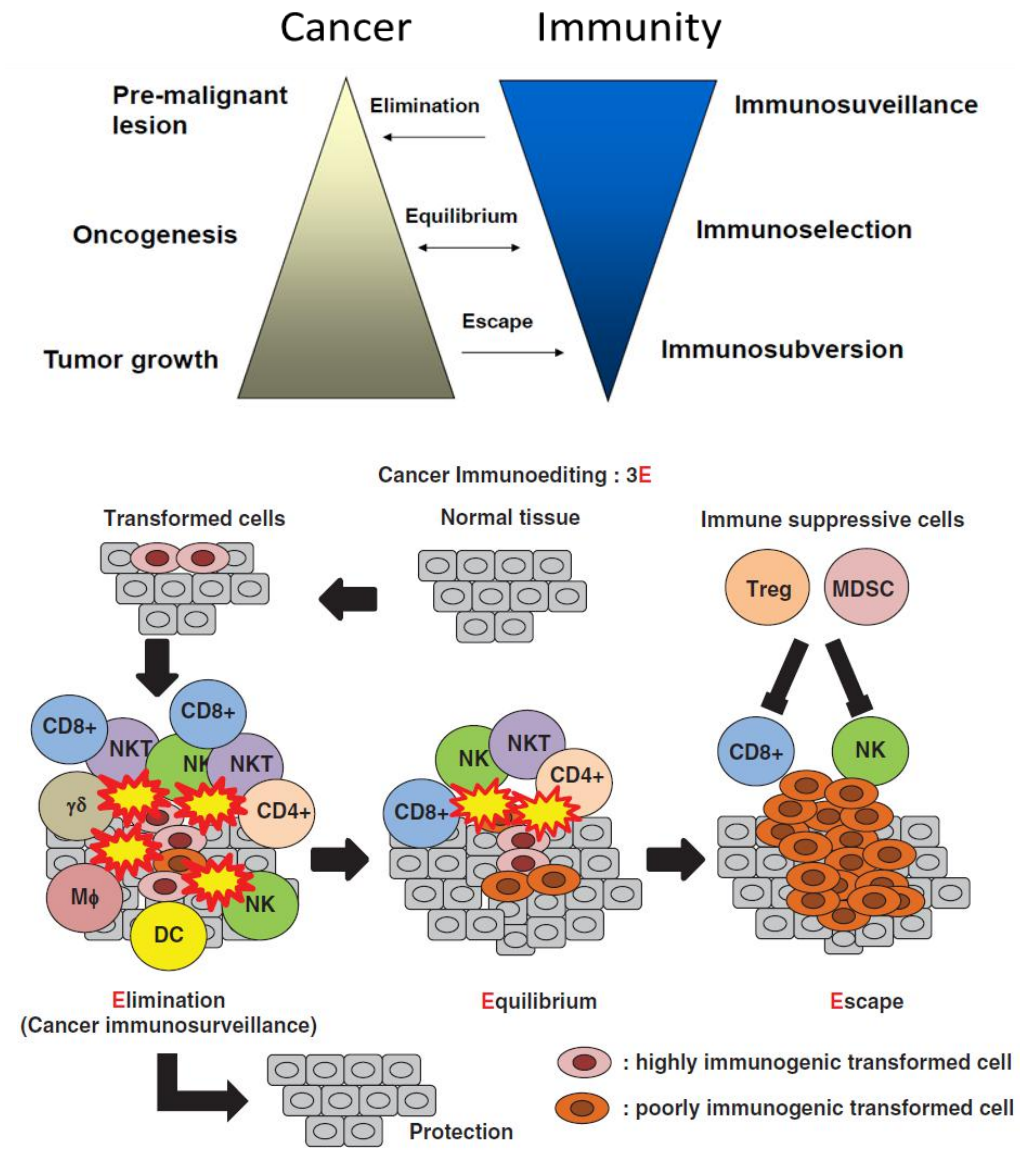
Specificity:

Can distinguish minute alterations

Memory:

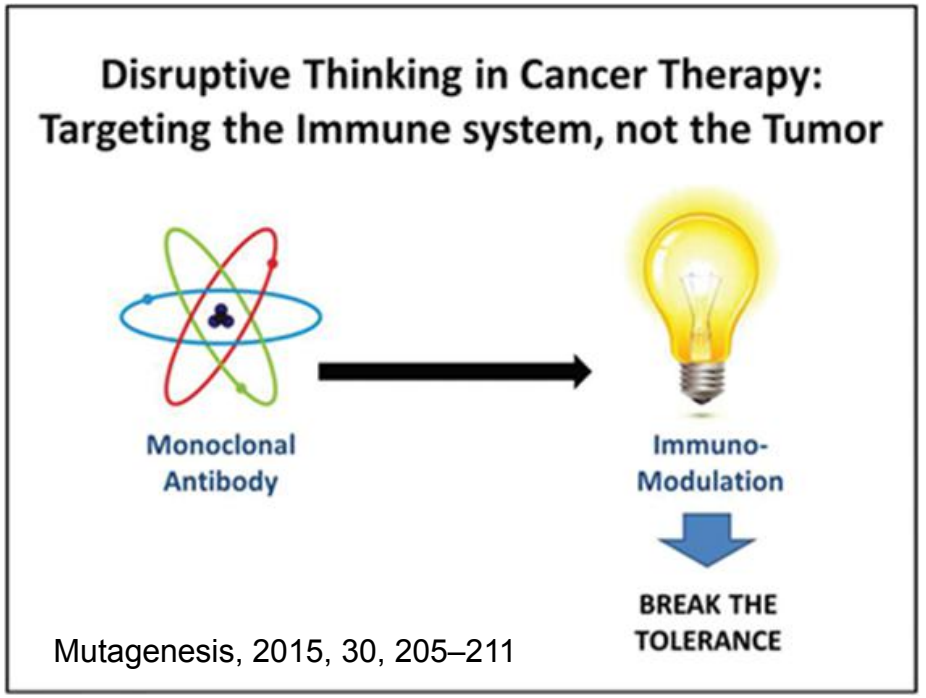
After effective antigen priming, immunity can last for decades

- Can our immune defense be engaged for cancer treatment?

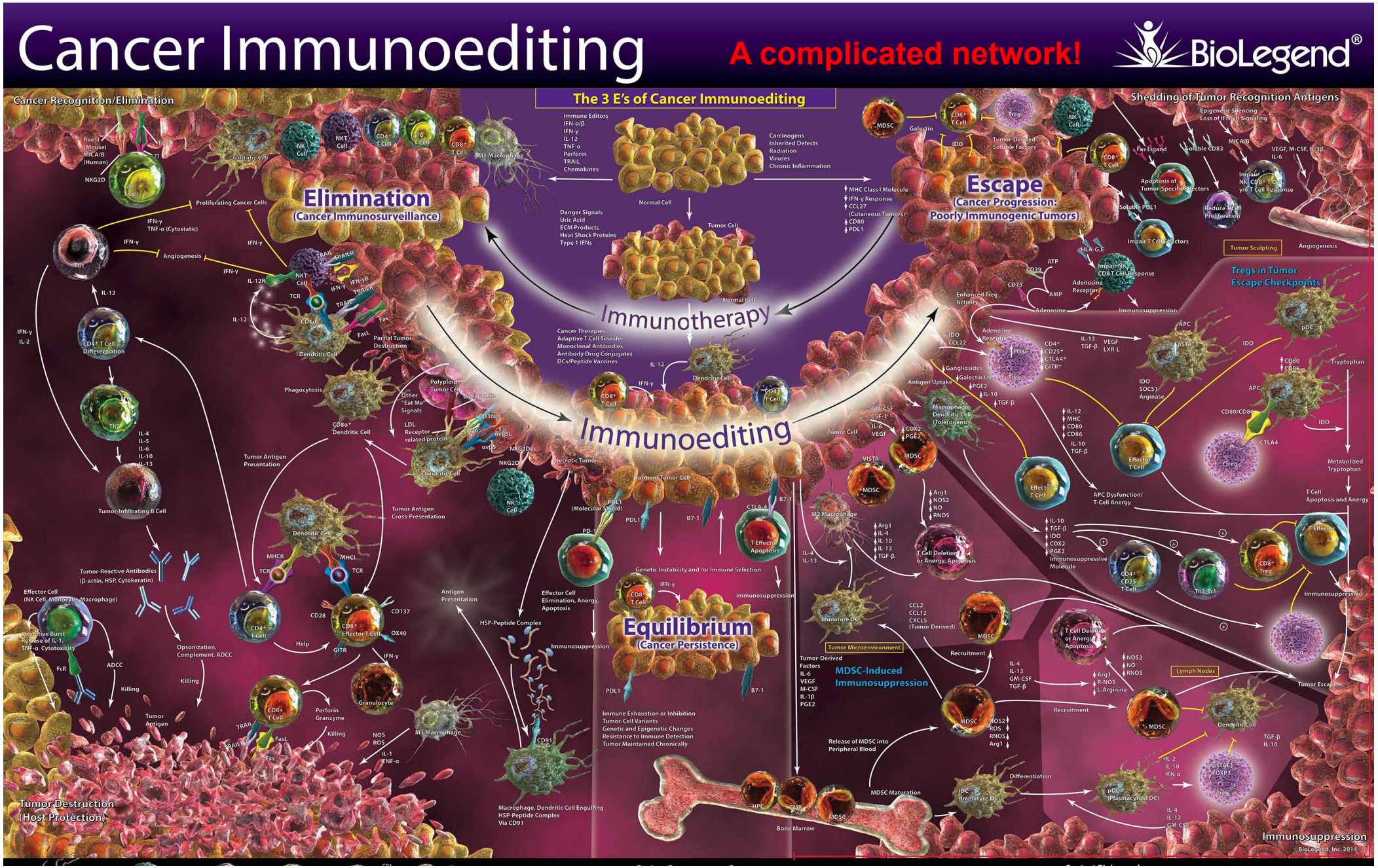


2013 Koichi Sakakura and Kazuaki

Immunity and Tumor Progression



- **Can our immune defense be engaged for cancer treatment?**

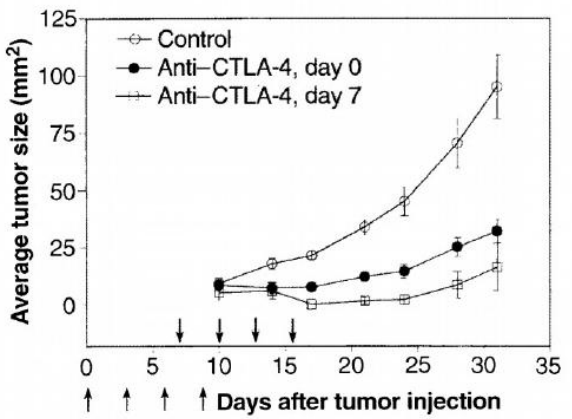


- **A new principle for immune therapy：anti-PD-1/anti-CTLA-4**

1996 Science 271:1734

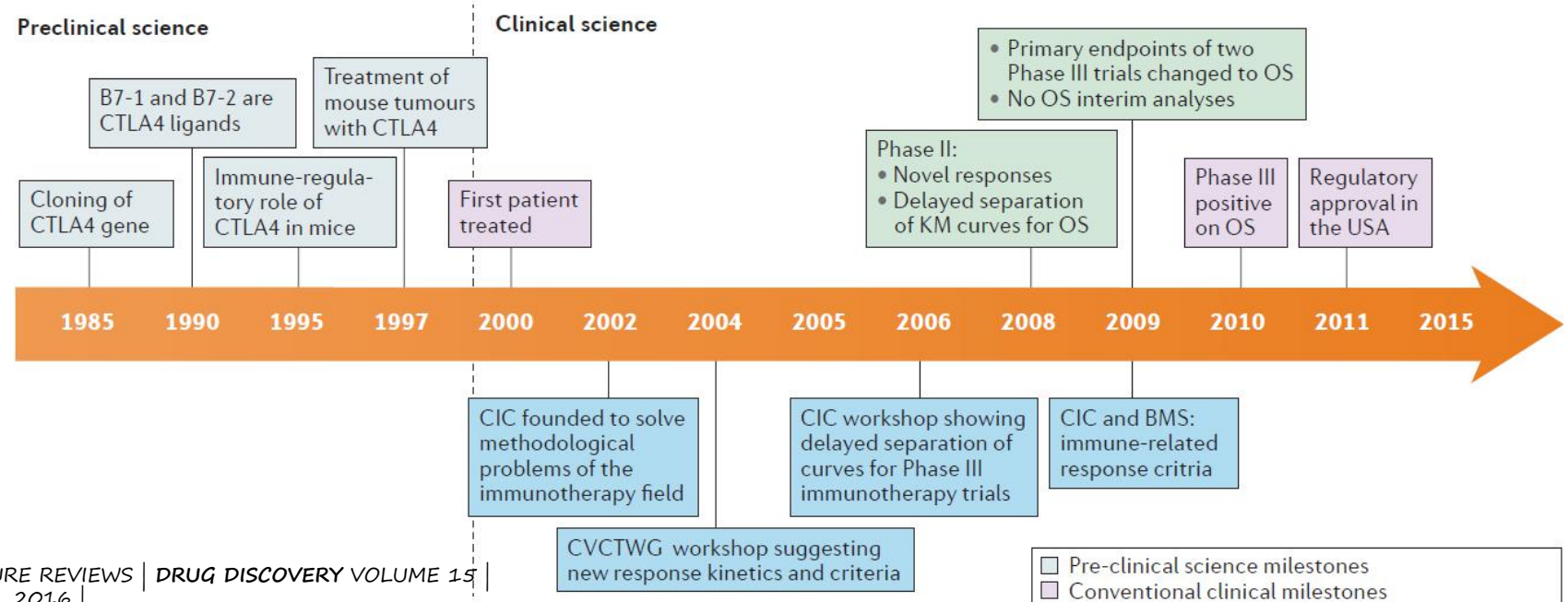
Enhancement of Antitumor Immunity by CTLA-4 Blockade

Dana R. Leach, Matthew F. Krummel, James P. Allison*



Dr. James Allison

2018



30 years of efforts！

NATURE REVIEWS | DRUG DISCOVERY VOLUME 15 | APRIL 2016 |

- **A new principle for immune therapy：anti-PD-1/anti-CTLA-4**

PD-1/PD-L1 (programed cell death -1) – another key co-inhibitory pathway of immune checkpoints

2018

Tasuku Honjo, MD Ph.D

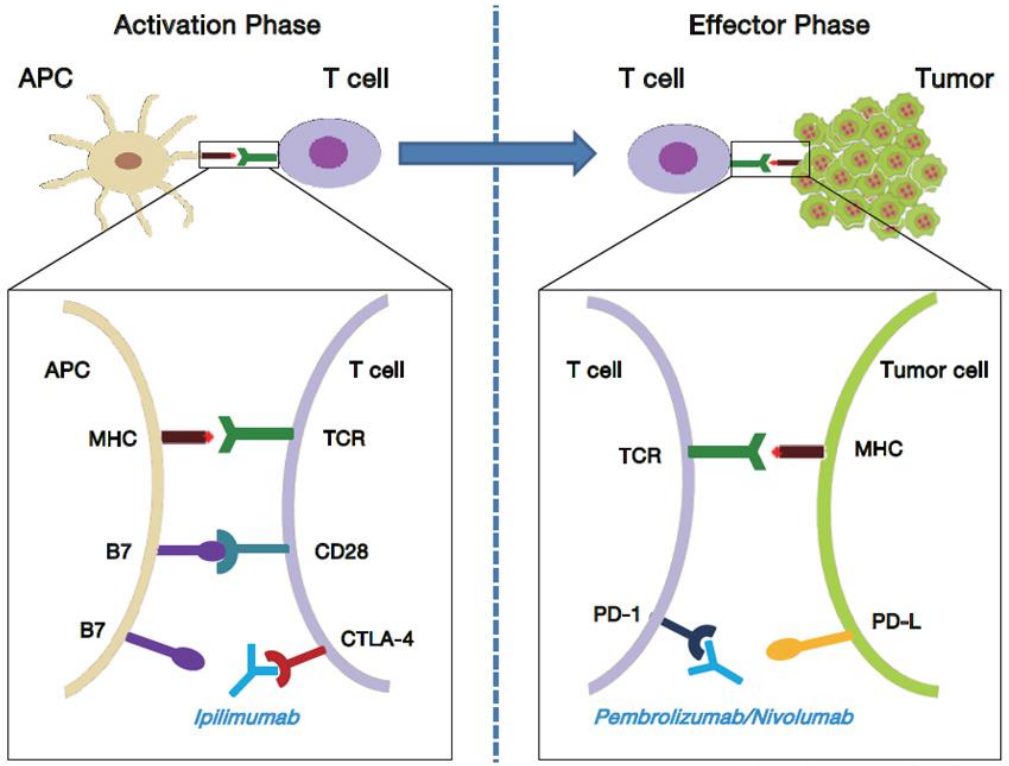
Gordon Freeman, PhD

Arlene Sharpe, MD PhD

Lieping Chen, MD PhD

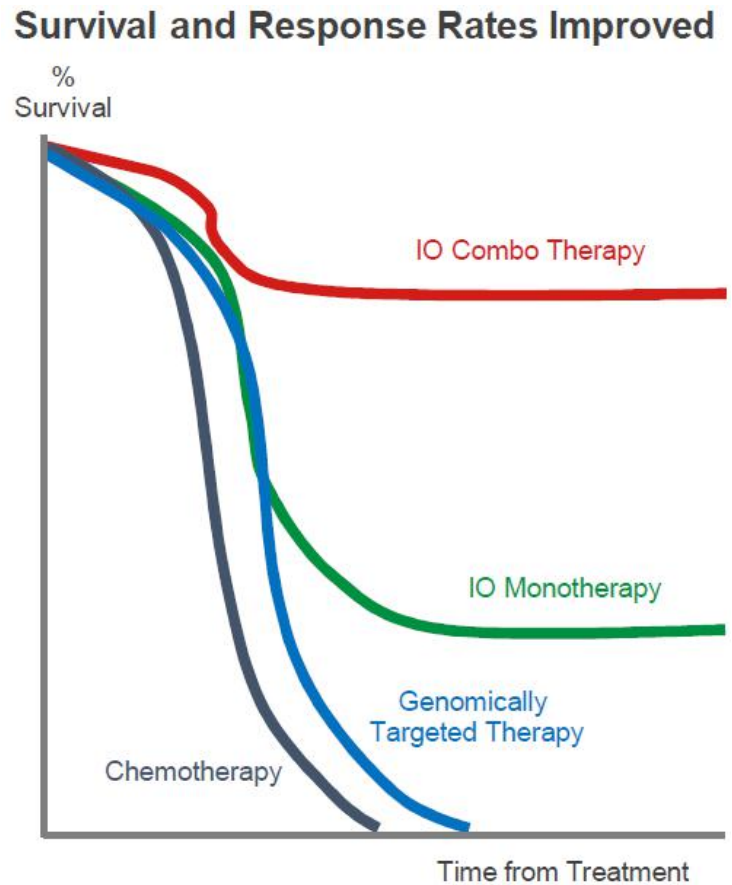
Discovering the PD-1 Checkpoint: Winners of the 2014 William B. Coley Award for Tumor Immunology

- **Immunotherapy for cancer today and in the future**



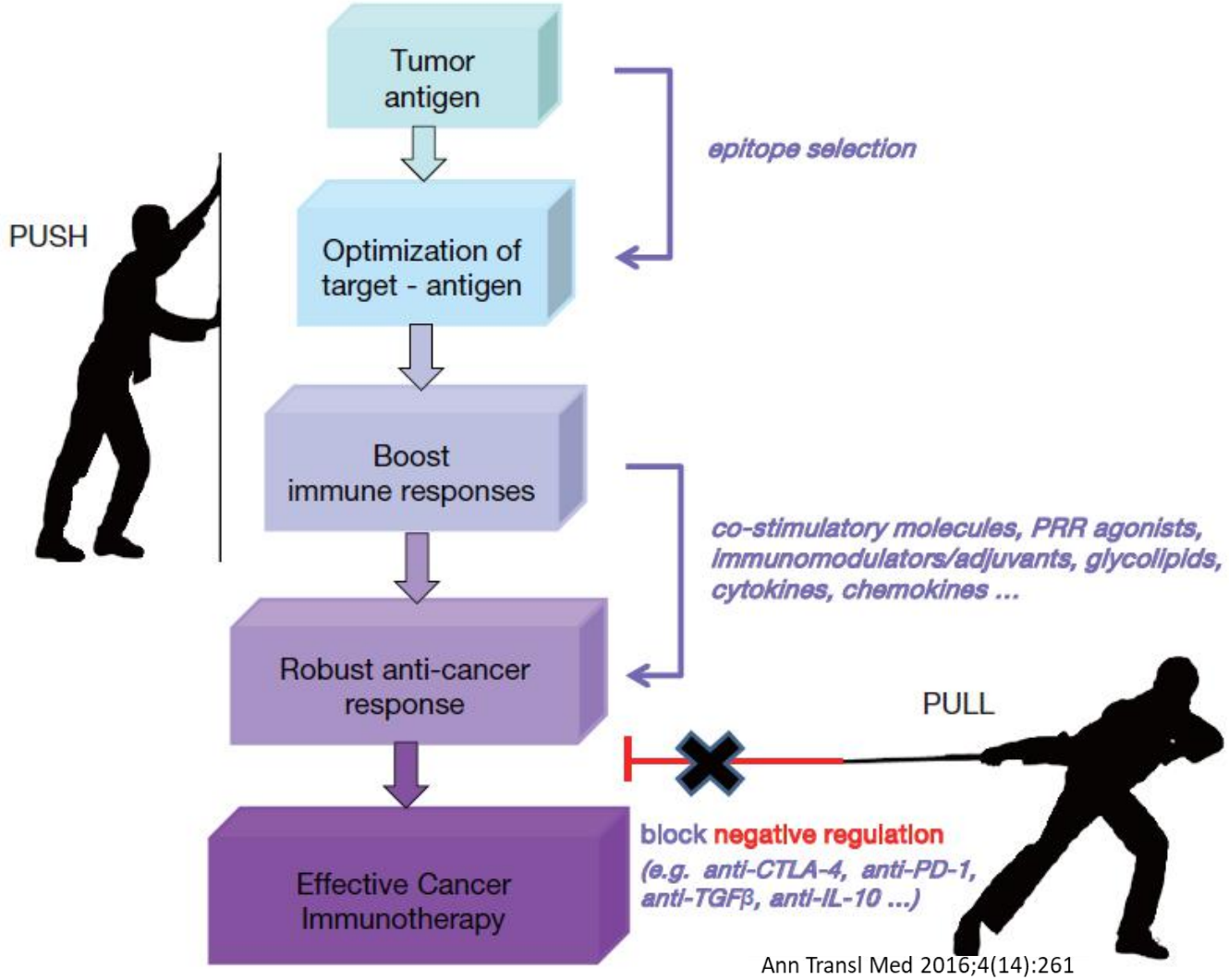
Ann Transl Med 2016;4(14):261

Schematic representation of important signals mediating T cell, APC and tumor cell interactions.



Immunoregulatory Therapy in Oncology: Bending the Survival Curve?

- **Immunotherapy for cancer today and in the future**



Ann Transl Med 2016;4(14):261

The PUSH and PULL approach could optimize anti-cancer immunity.

近期值得关注的研究进展

- **Antibody-drug conjugates （ADC）**
- 2013年上半年，美国FDA先后批准两种ADC上市，分别为Seattle Genetics公司的Adcetris TM和罗氏公司的Kadcyla。
- 目前，在世界范围内进入临床研究的ADCs药物累计已达三十余种。ADCs候选药物在数量上已经超过同为“改型抗体”的双特性抗体、抗体片段等类别，成为单克隆抗体药物，尤其是肿瘤治疗用单抗的研究热点与发展方向。

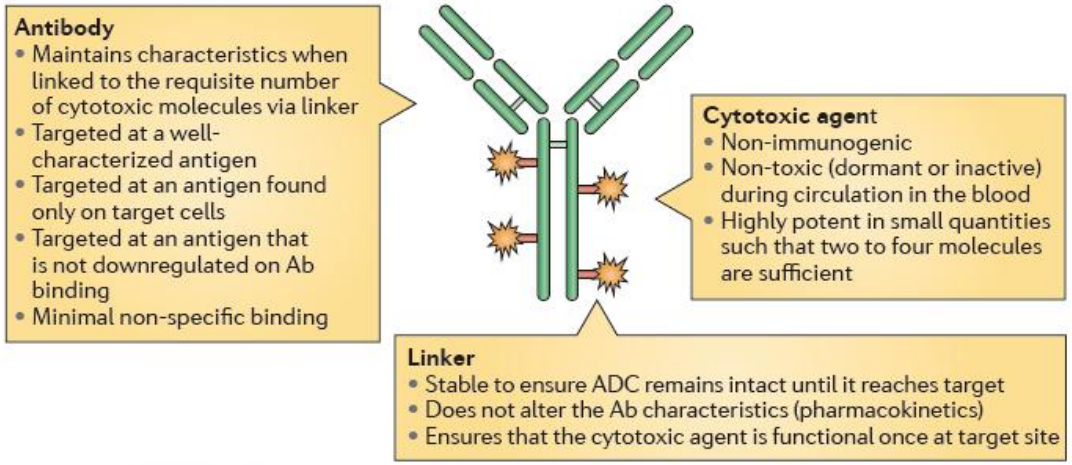


Figure 1 | **Antibody–drug conjugate structure and desired characteristics.** An antibody–drug conjugate (ADC) comprises three main structural units: the antibody (Ab) targeting a particular cell, the linker and the cytotoxic drug that will be used to kill the targeted cell.

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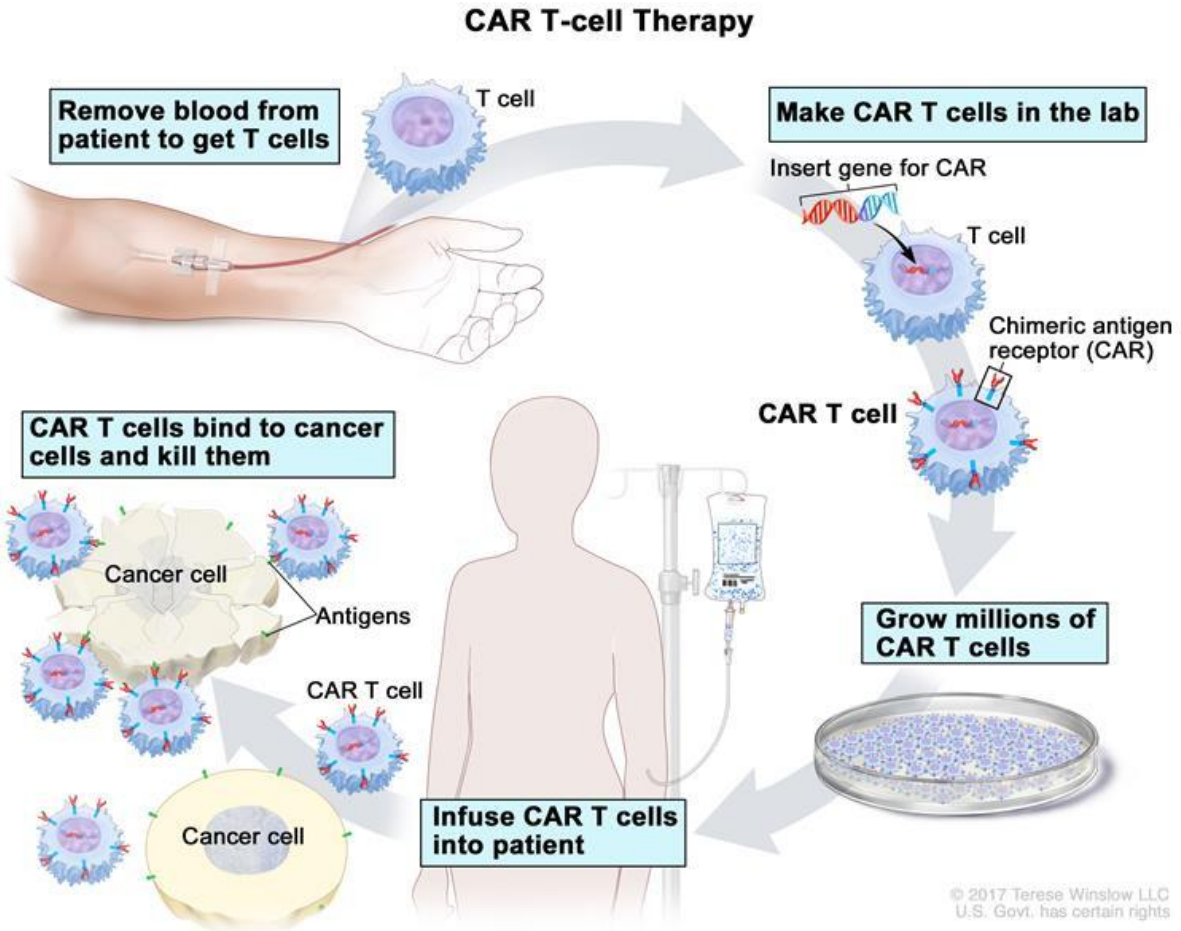
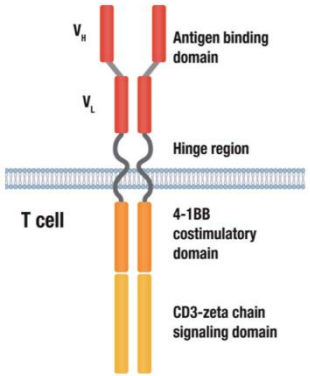
Chimeric Antigen Receptor (CAR)-T cells

- Blood from a vein in the patient's arm flows through a tube to an apheresis machine (not shown), which removes the white blood cells, including the T cells, and sends the rest of the blood back to the patient. **采集患者细胞：单采机，分离白细胞，其他血液成分回输；**
- Then, the gene for a special receptor called a chimeric antigen receptor (CAR) is inserted into the T cells in the laboratory. **实验室中将嵌合抗原受体 (CAR)转入T细胞，成为CAR-T 细胞；**
- Millions of the CAR-T cells are grown in the laboratory and then given to the patient by infusion. **CAR-T 细胞在实验室中进行扩增并回输病人**
- The CAR T cells are able to bind to an antigen on the cancer cells and kill them**. CAR-T 细胞能够结合肿瘤细胞并对其杀伤。**





Aducanumab（Biogen/Eisai 公司研发）

- 靶向淀粉样蛋白，治疗阿兹海默症
- 健康老年志愿者体内分离的全人单克隆抗体
- 2002年发表抗体发现的文章

Generation of antibodies specific for β-amyloid by vaccination of patients with Alzheimer disease

Christoph Hock, Uwe Konietzko, Andreas Papassotiropoulos, Axel Wollmer, Johannes Streffer, Ruth C. von Rotz, Gabriela Davey, Eva Moritz & Roger M. Nitsch

Division of Psychiatry Research, University of Zurich, Zurich, Switzerland
C.H. and U.K. contributed equally to this study.
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NATURE MEDICINE • VOLUME 8 • NUMBER 11 • NOVEMBER 2002

- 2016年发表Ib临床试验结果文章

The antibody aducanumab reduces Aβ plaques in Alzheimer's disease

Jeff Sevigny[1]*, Ping Chiao[1]*, Thierry Bussière[1]*, Paul H. Weinreb[1]*, Leslie Williams[1], Marcel Maier[2], Robert Dunstan[1], Stephen Salloway[3], Tianle Chen[1], Yan Ling[1], John O'Gorman[1], Fang Qian[1], Mahin Arastu[1], Mingwei Li[1], Sowmya Chollate[1], Melanie S. Brennan[1], Omar Quintero-Monzon[1], Robert H. Scannevin[1], H. Moore Arnold[1], Thomas Engber[1], Kenneth Rhodes[1], James Ferrero[1], Yaming Hang[1], Alvydas Mikulskis[1], Jan Grimm[2], Christoph Hock[2,4], Roger M. Nitsch[2,4]§ & Alfred Sandrock[1]§

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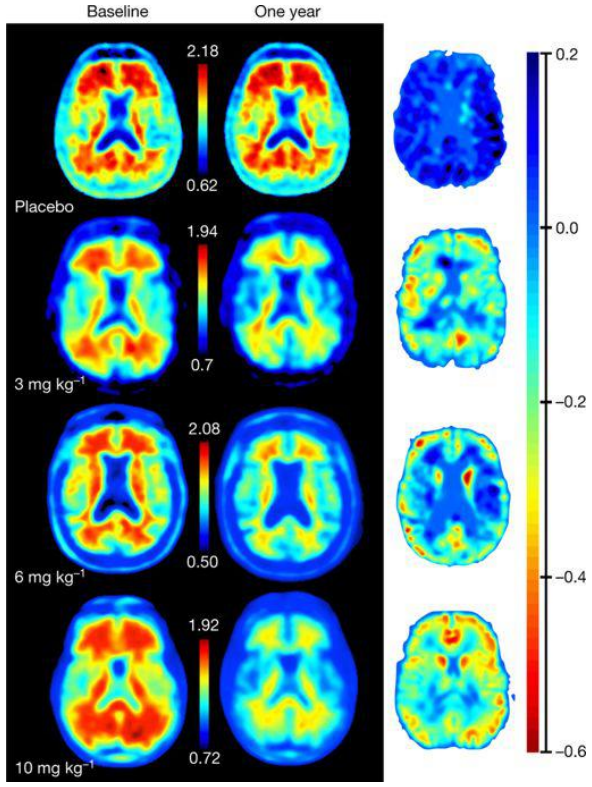
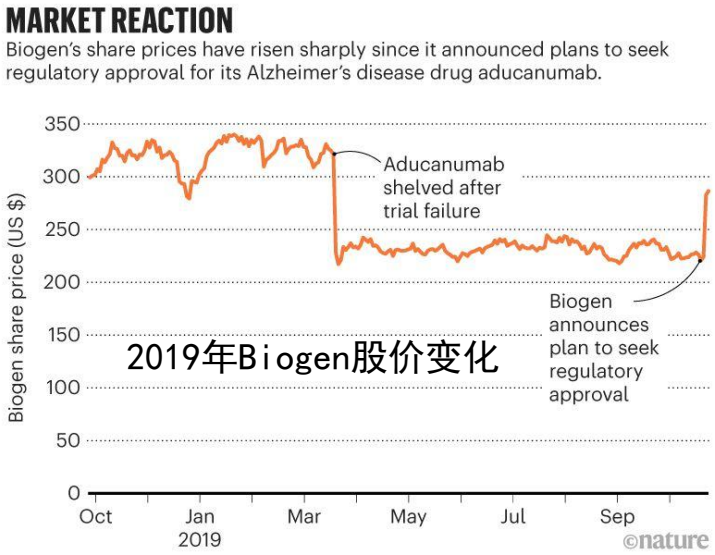


Ib 临床试验取得了阳性结果



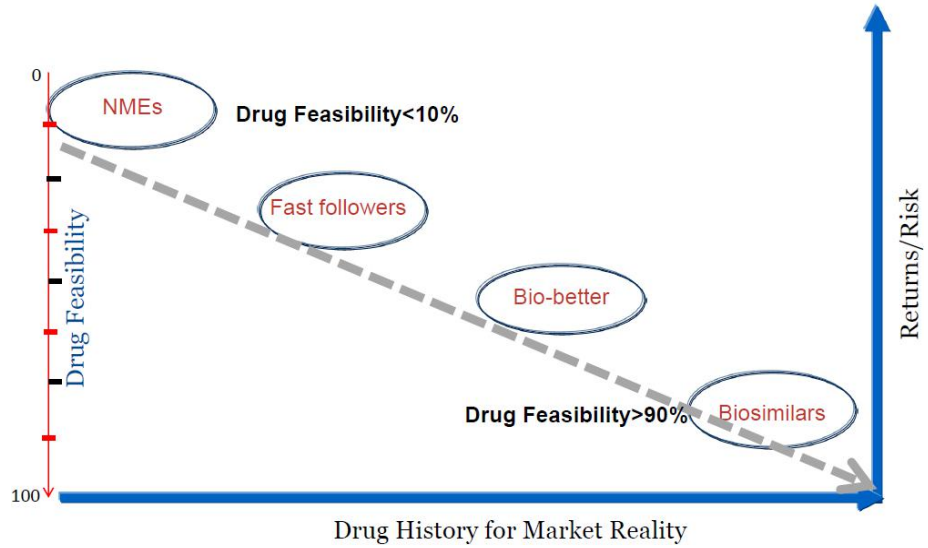
- 2019年3月宣布终止III期临床试验；
- 2019年10月，重新分析III期临床试验数据后，发现其中一项指标达到临床试验终点；
- 预计FDA将于2020年批准Aducanumab上市

High light：

- **人体可产生针对某些致病性自身抗原的抗体，可以使用抗体工程技术进行分离，作为抗体药物候选；**
- **抗体药物研发是漫长的，充满风险的过程！**

抗体药物中国市场—新药与仿制药

- 生物类似药是目前中国抗体药物领域的主流.
- 2012-2014年中国新药临床申报 (IND), 抗体药物领域约70% 的 靶点为TNFα, Her2, VEGF, EGFR, CD20等, 创新靶点很少.



我国批准上市的国产单克隆抗体类生物治疗药物(2013 年)

序号	药物名称	生产单位	靶点	抗体类型	适应证
1	抗 CD3 单抗	武汉生研所	CD3	Mouse	异体移植
2	抗人白介素-8 鼠单抗乳膏(恩博克)	宏远逸士	IL-8	Mouse	牛皮癣
3	碘 [31] 美妥昔单抗(利卡汀)	成都华神	CD147	Mouse, [31] I Labeled	肝癌
4	碘 [31] 肿瘤细胞和人鼠嵌合单抗(唯美生)	上海美恩	Tumor cells Nucleus	Chimeric, [31] I Labeled	肺癌
5	尼妥珠单抗(泰欣生)	百泰药业	EGFR	Humanized	鼻咽癌
6	注射用重组人II型肿瘤坏死因子受体-抗体融合蛋白(益赛普)	中信国健	TNFa	Fusion Protein	类风湿性关节炎
7	注射用重组人II型肿瘤坏死因子受体-抗体融合蛋白(强克)	上海赛金	TNFa	Fusion Protein	类风湿性关节炎
8	重组抗 CD25 人源化单抗(健尼哌)	中信国健	CD25	Humanized	肾脏移植
9	抗血管内皮生长因子-抗体融合蛋白(康柏西普)	成都康弘	VEGF	Fusion Protein	湿性年龄相关性黄斑变性

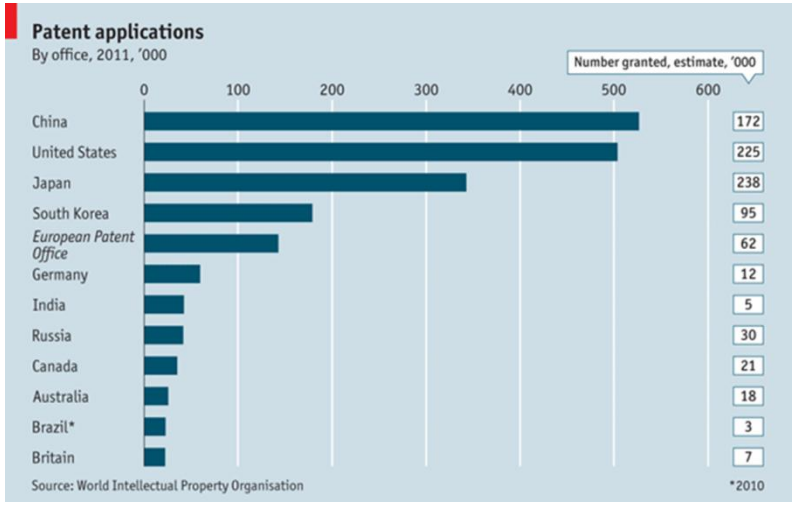
- 研究所/大学/医院，基础研究领域的持续投入，有新的抗体药物靶点发现，但是产业化的经验不足;
- 中国生物制药工业拥有一流的生产设备，对于创新的抗体药物的研发态度保守;

2018-2019年我国批准上市药物（4个国产）

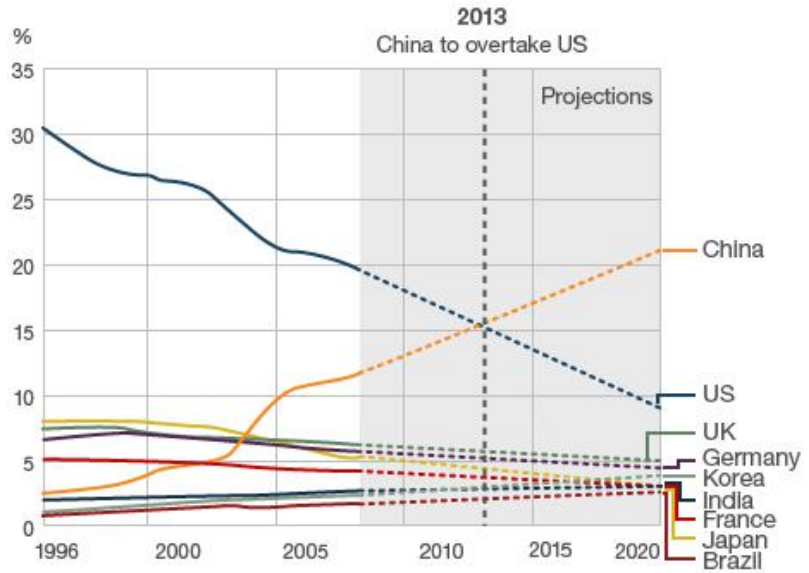
上市时间	通用名	商品名	公司	靶点	适应症
2018.6.15	纳武利尤单抗 (Nivolumab)	欧狄沃 (Opdivo)	百事美施贵宝BMS	PD-1	EGFR阴性、ALK阴性的局部晚期或转移性非小细胞肺癌（NSCLC）
2018.6.20	依达赛珠单抗 (Idarucizumab)	泰毕安 (Praxbind)	勃林格殷格翰BI	-	需要迅速逆转泰毕全（达比加群酯）的抗凝效应时使用
2018.7.26	帕博利珠单抗 (Pembrolizumab)	可瑞达 (Keytruda)	默沙东MSD	PD-1	经过系统治疗的晚期黑色素瘤
2018.7.31	依洛尤单抗 (Evolocumab)	瑞百安 (Repatha)	安进Amgen	PCSK9	纯合子家族性高胆固醇血症
2019.1.24					成人动脉粥样硬化性心血管疾病（ASCVD）
2018.9.5	依库珠单抗 (Eculizumab)	舒立瑞 (Soliris)	亚力兄弟Alexion	补体蛋白C5	阵发性睡眠性血红蛋白尿症（PNH）和非典型溶血性尿毒症综
2018.11.30	艾美赛珠单抗 (Emicizumab)	舒友立乐 (Hemlibra)	罗氏Roche	-	存在凝血因子Ⅷ抑制物的A型血友病
2018.12.17	帕妥珠单抗 (Pertuzumab)	帕捷特 (Perjeta)	罗氏Roche	HER2	高复发风险的HER2阳性早期乳腺癌的辅助治疗
2018.12.17	特瑞普利单抗	拓益	君实生物	PD-1	既往标准治疗失败后的局部进展或转移性黑色素瘤
2018.12.27	信迪利单抗	达伯舒	信达生物	PD-1	至少经过二线系统化疗的复发或难治性经典型霍奇金淋巴瘤
2019.2.22	利妥昔单抗生物类似药	汉利康	复宏汉霖	CD20	非霍奇金淋巴瘤
2019.5.28	地舒单抗 (Denosumab)	安加维 (XGEVA)	安进Amgen	RANKL	不可手术切除或者手术切除可能导致严重功能障碍的骨巨细胞瘤
2019.5.31	卡瑞利珠单抗	艾立妥	恒瑞	PD-1	至少经过二线[illegible]难治性经典型霍奇金淋巴瘤

抗体药物产业在中国发展基础

- 国家对**医疗领域投入增加**，推进医改，并实施“重大新药创制”等项目，对生物技术尤其是抗体药物大力支持
- 抗体药物产业化发展目前仍滞后于欧美国家，主管部门已经意识**创新抗体药物研发的重要性，抗体药物**原始创新能力能力正逐步改善。
- 中国对高质量的生物技术药物（尤其是**治疗性单抗药物）的需求日益强劲**：经济发展、人口老龄化、医疗支出能力的增加等驱动。
- 中国对基础研究的巨额投入已经产出大量国际一流水平研究，**创新抗体药物靶点的“中国发现”成为现实**。



2011年中国专利申请的数量已经超过美国



Royal Society 2011

中国科研论文的引用量也将超过美国 位于第一位

寄语

- 1. 科学研究和产品开发需要激情和热情。
- 2. 学无止境!
- 3. 敢于挑战和勇于创新。
  - **热情，好学。**
  - **创新和享受过程，而不仅是结果。**


谢 谢！