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多次跨膜蛋白靶点的抗体制备新策略：mRNA免疫技术

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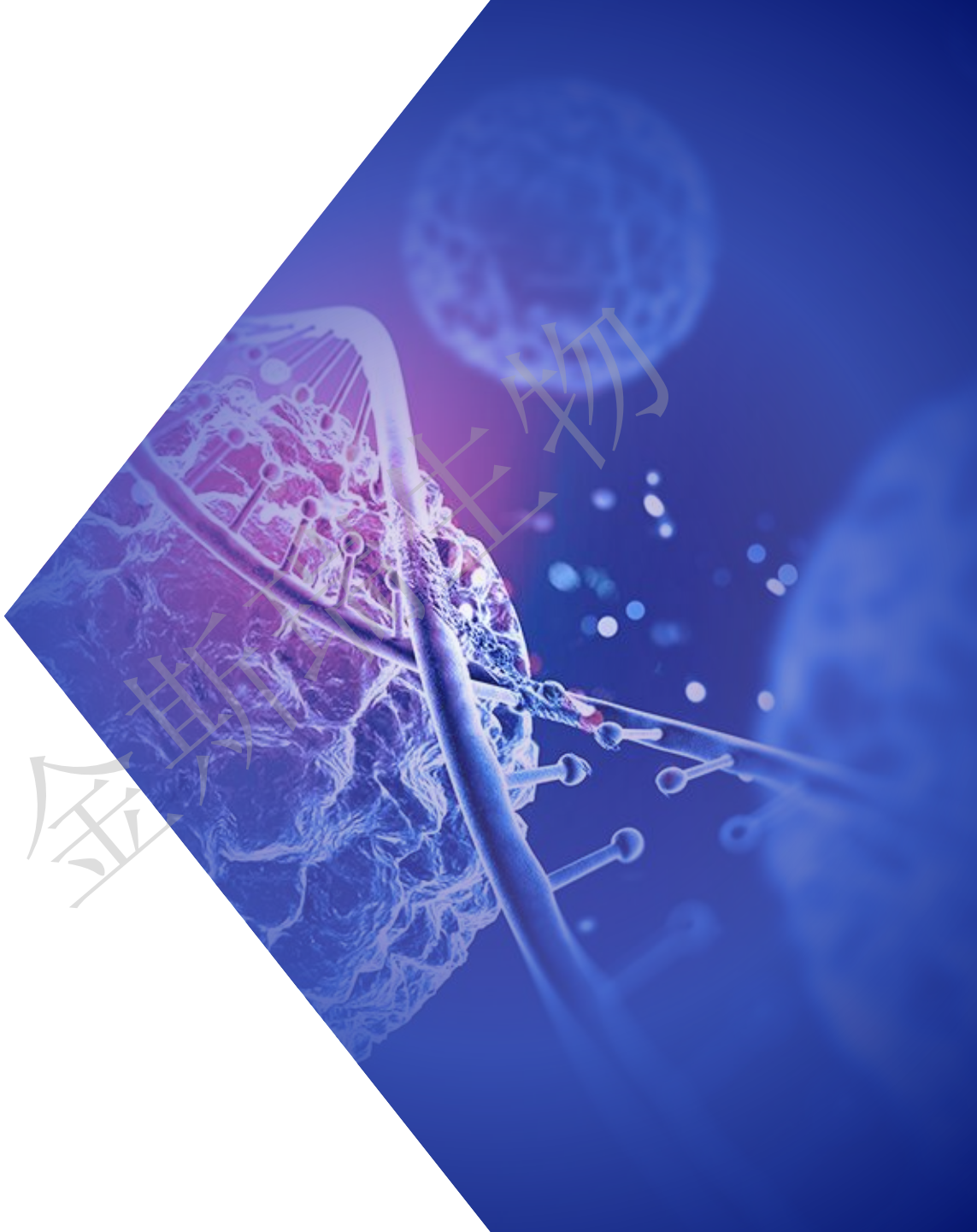
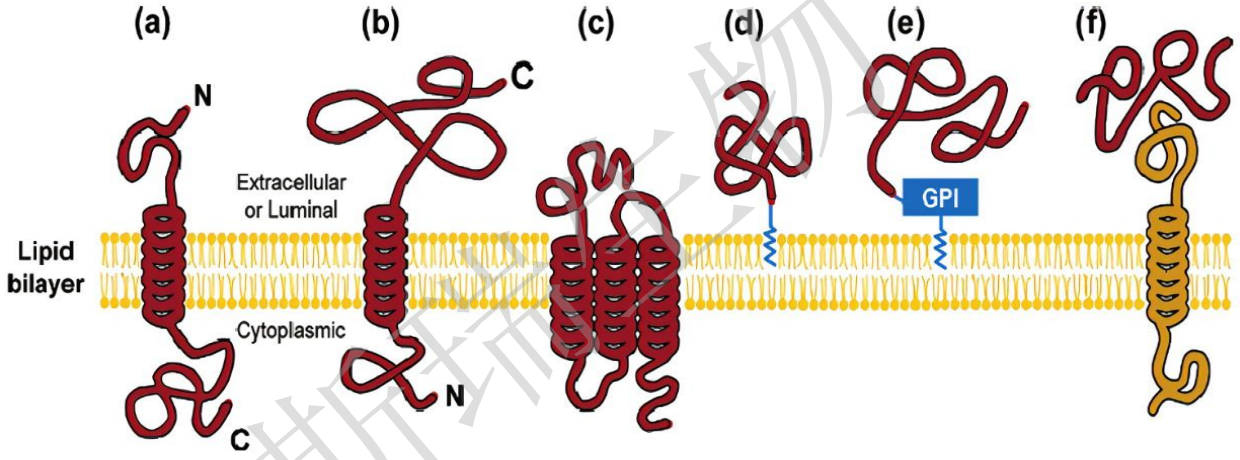
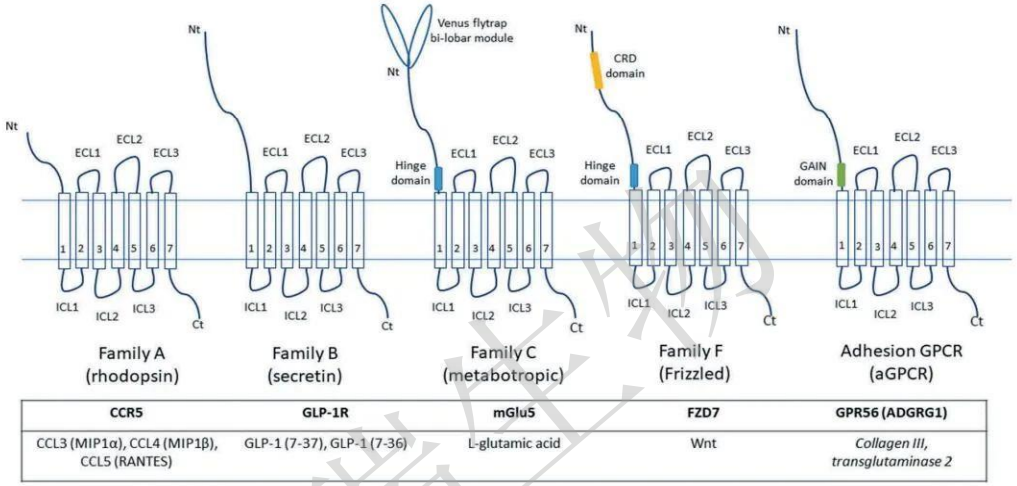
- GPCR 作为诊断/治疗靶点的全球市场现状
- 靶向GPCR的抗体研究进展



G蛋白偶联受体（GPCR）

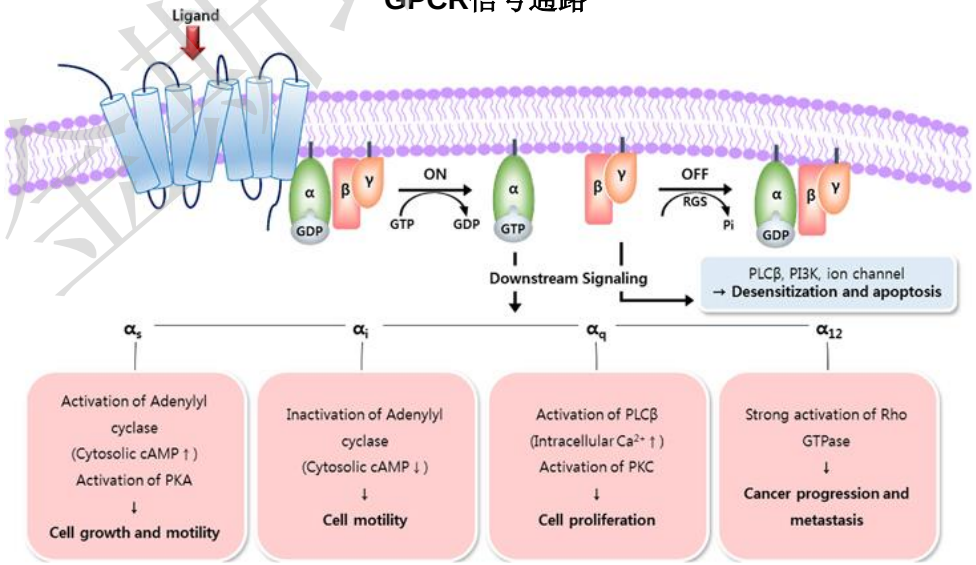


GPCR的不同类型



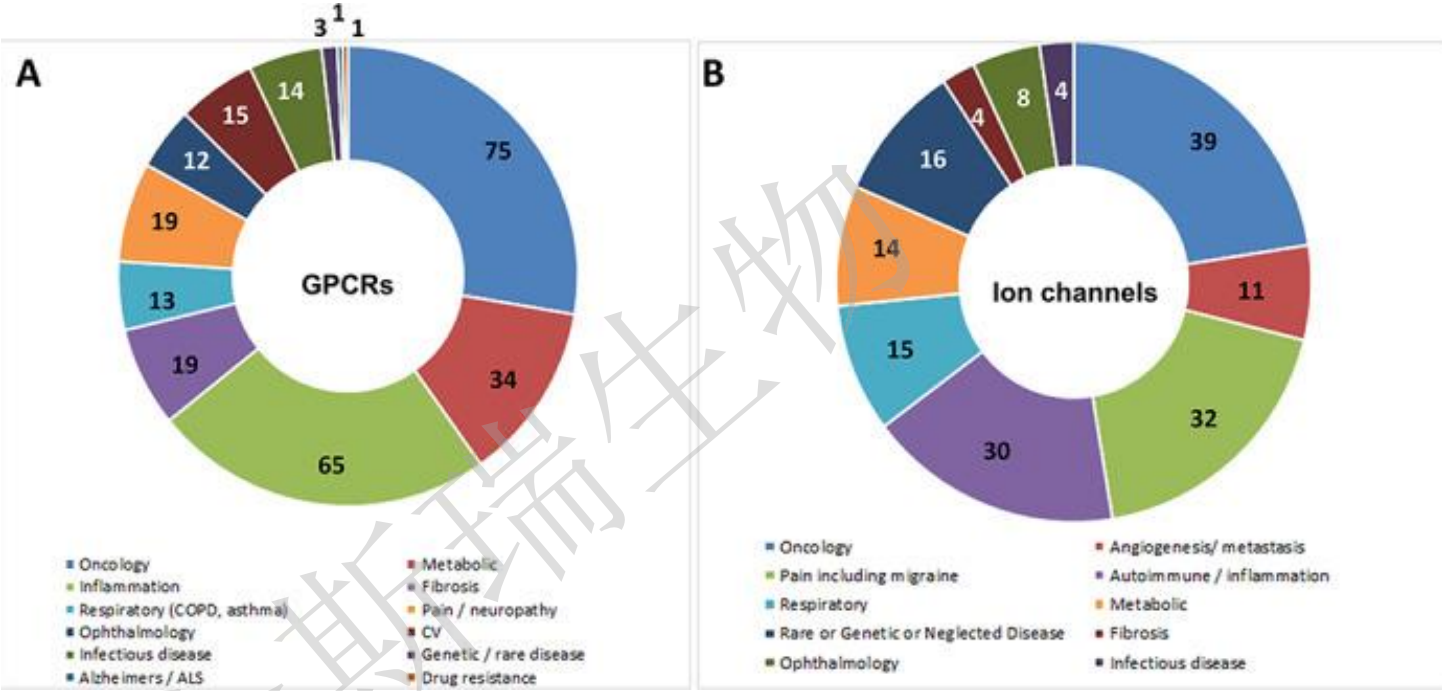
CCR5	GLP-1R	mGlu5	FZD7	GPR56 (ADGRG1)
CCL3 (MIP1α), CCL4 (MIP1β), CCL5 (RANTES)	GLP-1 (7-37), GLP-1 (7-36)	L-glutamic acid	Wnt	*Collagen III, transglutaminase 2*

GPCR信号通路



- 以GPCR为例，**跨膜蛋白参与调控多种细胞结构与功能变化**，如信号的识别转导、物质运输和代谢等
- 许多人类疾病都与异常的跨膜蛋白功能有关，因此**跨膜蛋白已成为重要的药物作用靶点**

GPCR 作为诊断/治疗靶点的全球市场现状



- **>270 GPCRs** 与疾病密切相关，可作为诊断/治疗的抗体靶点加以研究
- >170 ion channels同样与疾病的发生密切相关，亦可作为诊断/治疗的抗体靶点
- **体外表达量和溶解度低、纯化难、构象复杂等问题，一定程度上限制了以跨膜蛋白为靶点的药物开发进展**

GPCRs药物靶点

Target class	Modality	Organization	Indication	Stage
GPCRs				
FZD10	ADC (^{90}Y)	Oncotherapy Science	Synovial sarcoma	Phase 1
LGR5 × EGFR	BS (with ADCC)	Merus	Metastatic CRC	Phase 1
SSTR2 × CD3	BS	Xencor	NET/GIST	Phase 1
GPRC5D × CD3	BS (duobody)	Genmab/Janssen	Myeloma	Phase 1
CCR7	ADC	Novartis	CLL	Phase 1
GPR20	ADC	Daiichi Sankyo	GIST	Phase 1
CXCR4	Alternative scaffold—iBody	AdAlta	Fibrosis	Phase 1
CCR4	Diabody immunotoxin	MGH/Harvard University	Cancer	Preclinical
CXCR4	ADC	John Hopkins	AML	Preclinical
LGR5	ADC (^{89}Zr)	Texas Therapeutics Institute	CRC (and immunoPET)	Preclinical
PAC1 × CGRP-R	BS	Amgen	Migraine	Preclinical
CXCR5 × CD3	BS	Max-Delbrück-Center of Molecular Medicine	Cancer	Preclinical
Calcitonin R	ADC	Monash	GBM	Preclinical
GLP-1R × GCG	BS	Calibr	Metabolic diseases	Preclinical
SSTR4	Peptide–Ab conjugate	Peptide Logic	Pain	Preclinical
EMR1	CAR-T	Humanigen	Eosinophilic leukemia	Preclinical
GnRH	CAR-T	Protheragen	Ovarian, prostate, pancreatic cancer	Preclinical
GPRC5D	CAR-T	MSK/Juno Therapeutics	Myeloma	Preclinical
CB1 × AT1	BS	Ichan School of Medicine	Liver fibrosis	Discovery
CCR5	Biparatopic	Scripps Research Institute	HIV	Discovery
CCR5 × HIV envelope	BS/TS	Scripps Research Institute	HIV	Discovery
$GABA_B$ × TfR	BS	Denali	Alzheimer's disease	Discovery
Ion channels				
P2X7	Nanobody	Univ Med Centre Hamburg	Autoimmune	Preclinical
nfP2X7	CAR-T	Biosceptre	Hematological malignancies	Discovery
			Solid tumors	Discovery
K_v10.1	Nanobody–TRAIL fusion	Max Planck Institute	Cancer	Discovery
K_v2.1	Nanobody	Institut Pasteur Tunis	Cancer	Discovery
TRPV4	i-Body	AdAlta	Fibrosis	Discovery
K_v1.3	Knot-Body	Maxion Therapeutics	Autoimmune	Discovery
Na_v1.7	Ab–GPTx1 peptide conjugate	Amgen	Pain	Discovery
	Ab–JzTx-V peptide conjugate	Amgen	Pain	Discovery

Hutchings CJ. Mini-review: antibody therapeutics targeting G protein-coupled receptors and ion channels. Antib Ther. 2020 Dec 9;3(4):257-264.

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靶向GPCR的抗体研究进展

FDA批准的靶向GPCR的单克隆抗体药物

Drug	Erenumab (Genetical Recombination)	Mogamulizumab-KPKC	Leronlimab
Originator Organization	Amgen, Inc.	Kyowa Hakko Kirin Pharma, Inc.	CytoDyn, Inc.
Active Organization	Novartis Pharmaceuticals Corp.	Kyowa Kirin Co., Ltd. Kyowa Kirin Holdings BV Kyowa Kirin Pharmaceutical Developm… [+1]	CytoDyn, Inc.
Inactive Organization	-	Kyowa Hakko Kirin Pharma, Inc. Amgen, Inc.	-
Targets(Ranking)	No.1 CGRP receptor	No.1 CCR4	No.2 CCR5
Mechanism	CALCRL antagonists	CCR4 antagonists	CCR5 antagonists
Drug Types	Monoclonal antibody IgG2	Monoclonal antibody IgG1	Monoclonal antibody IgG4
Highest Phase(Global)	Phase 3	Approved	NDA/BLA
Highest Phase(CN)	Phase 3	Approved	-
First Approved Date(Global)	US (May 2018) Migraine Disorders	JP (Mar 2012) Peripheral T-Cell Lymphoma T-Cell Lymphoma Cutaneous T-Cell Lymphoma	-
First Approved Date(CN)	-	CN (Oct 2022) Mycosis Fungoides Sezary Syndrome	-
No. of Approved Indication	US(1) EU(1) JP(1)	US(2) EU(2) CN(2) JP(4)	-
Active Indication	Temporomandibular Joint Dysfunction Synd… Headache Rosacea [+1]	Solid Tumors Carcinoma Paraparesis, Tropical Spastic [+6]	Solid Tumors Metastatic Microsatellite Stable Colorectal … Nonalcoholic Steatohepatitis [+6]
Inactive Indication	Angina, Stable Hot Flashes	Non-Small Cell Lung Cancer Extranodal NK-T-Cell Lymphoma Asthma	HIV Infections
Regulation	-	Orphan Drug (JP)、 Orphan Drug (US)、 Breakthrough Therapy (US) [+1]	Fast Track (US)、 Emergency Use Authorization (CA)、 Orphan Drug (US)
No. of Clinical Trials	82	54	26
No. of Patents	28	42	32

https://synapse.zhihuiya.com/homepage

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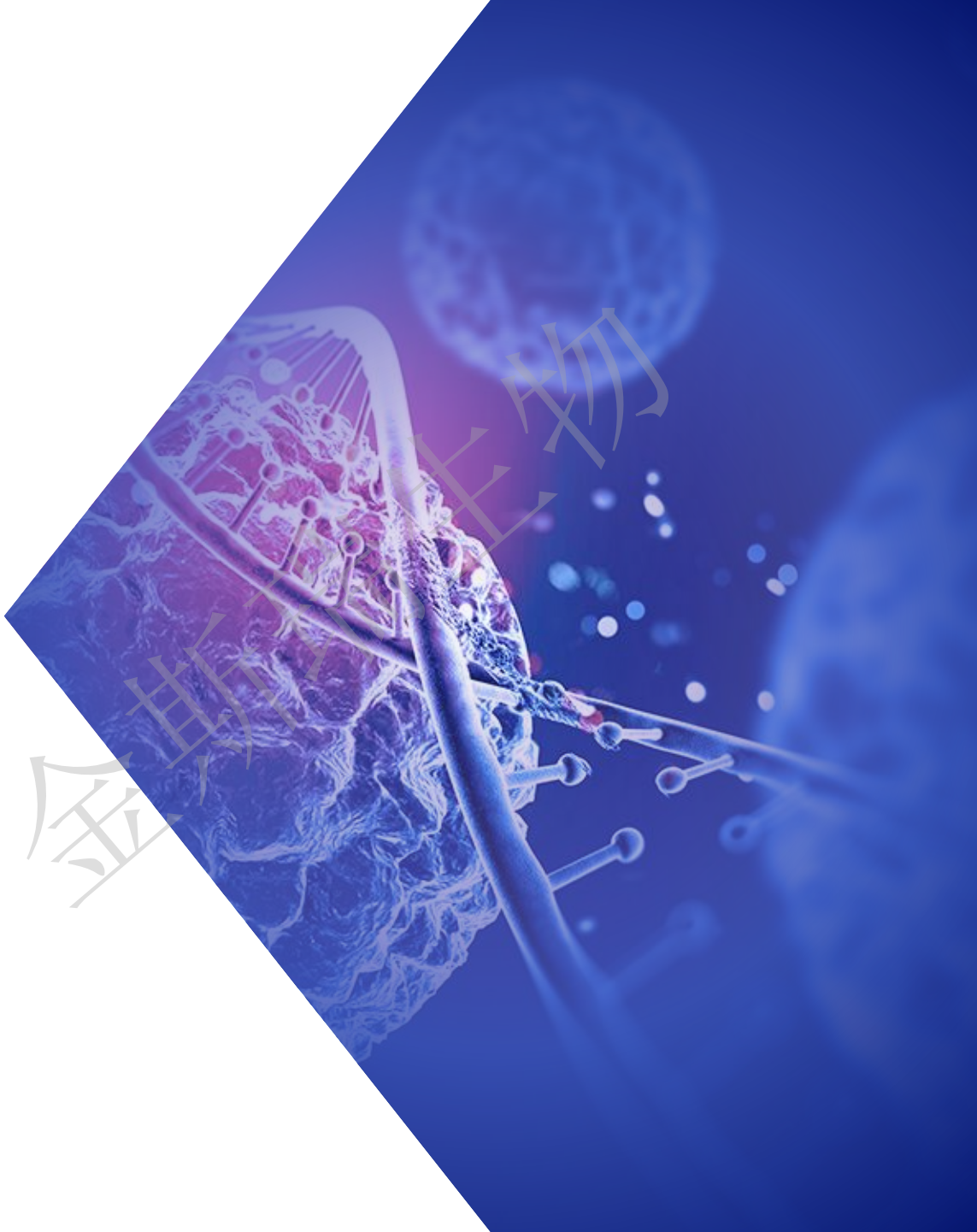
mRNA免疫技术用于多次跨膜蛋白抗体开发的应用优势

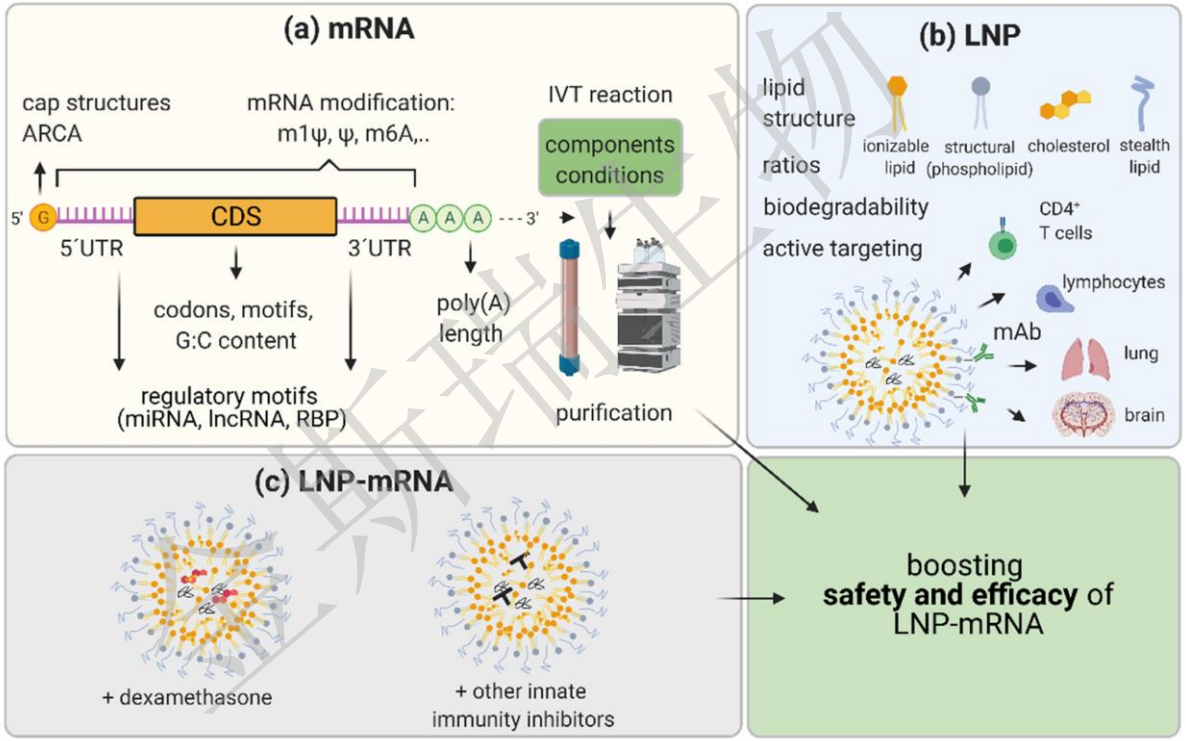
- mRNA免疫与其他免疫策略对比
- mRNA免疫技术如何加速GPCR的抗体开发进程



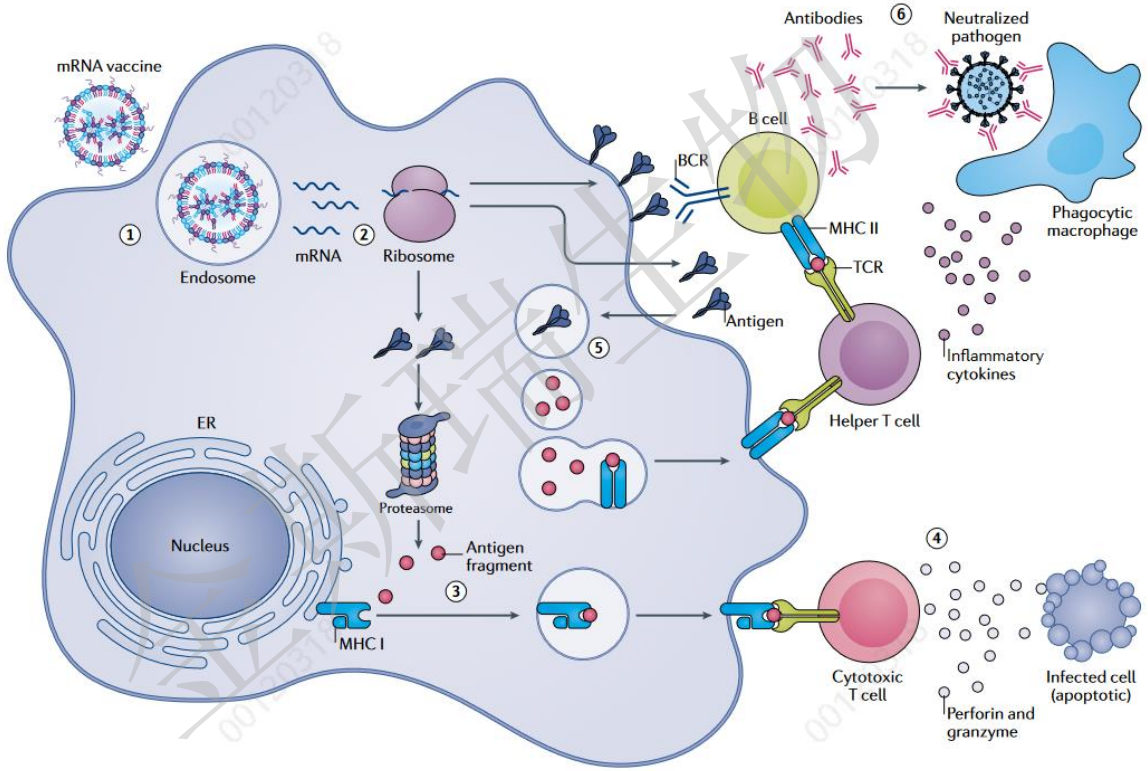
mRNA免疫与其他免疫策略对比

Immunogen format	Weaknesses	Strengths	Recommended index
DNA	low expression level since DNA must cross the nuclear membrane for transcription, high cost for DNA immunization (gene gun and complex adjuvants)		★
mRNA	**Instability of mRNA, mRNA delivery**	**✓ Full length GPCR expression ✓ Direct translation of mRNA in the cytoplasm ✓ spontaneous protein PTM in mammalian cells, natural conformation, no need for adjuvant**	★★★★★
Protein	High technical limitation of full length GPCRs expression and purification in vitro due to the presence of hydrophobic domains in the transmembrane zone	High immunogenicity	★★
Peptide	Only recognize linear epitopes, not conformational epitopes, epitope omission	Low synthesis cost	★★★★
VLP	Non-specific antibody generation, little difference between positive and negative screening	Higher abundance of target antigen compared with overexpressed cells	★★★
Overexpressed cell line	Low immunogenicity due to the very low percentage of GPCR of interest on the entire membrane		★★
Nanodisc	Low success rate of GPCRs assembly with membrane scaffold proteins and phospholipid to form Nanodisc, removal of detergent	Similar to natural cell membrane structure	★★★

mRNA-LNP免疫技术加速GPCR抗体的开发进程



mRNA-LNP的高表达与强免疫原性



mRNA-LNP诱导体液免疫应答途径

Chaudhary N, et al. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov. 2021 Nov;20(11):817-838.

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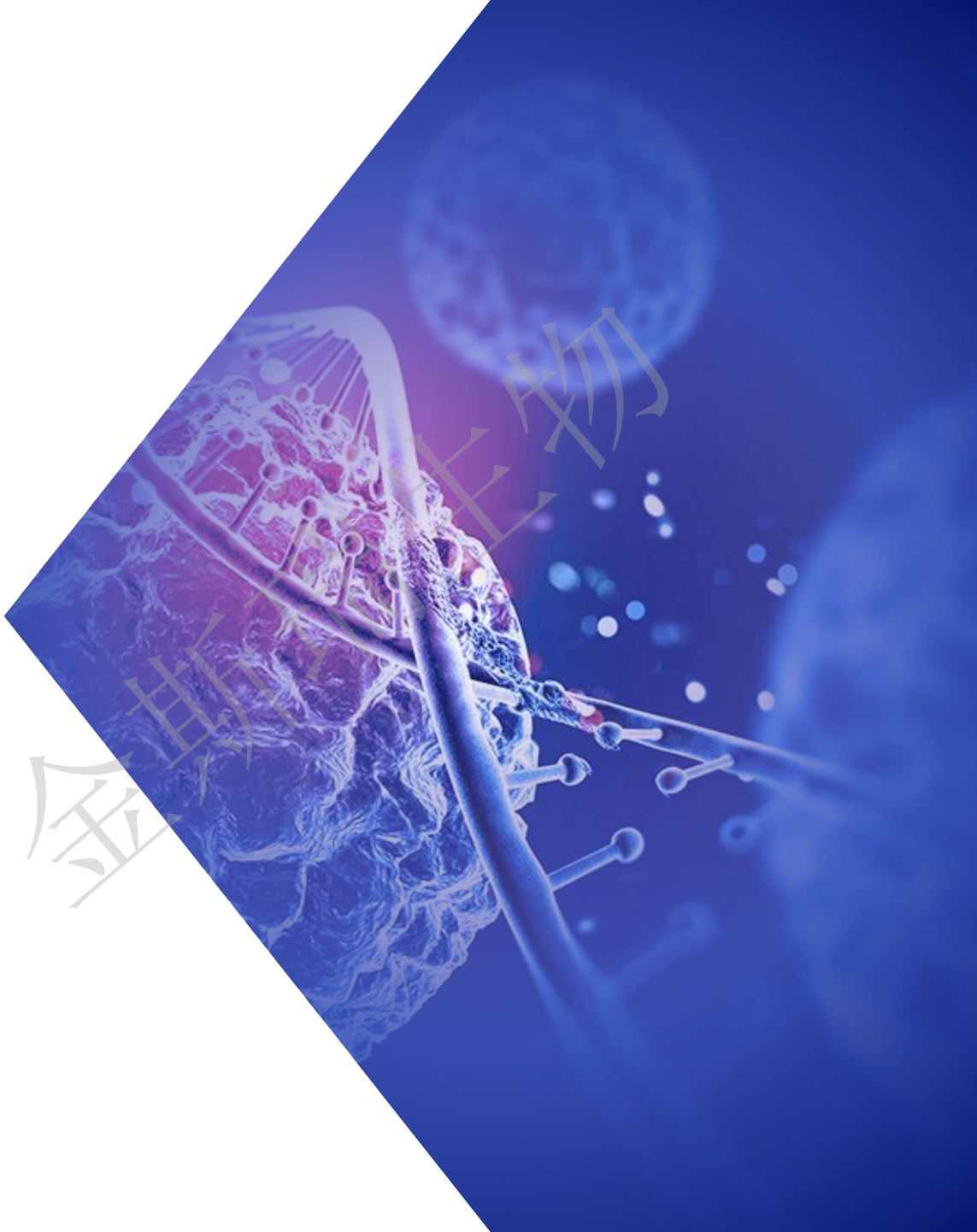
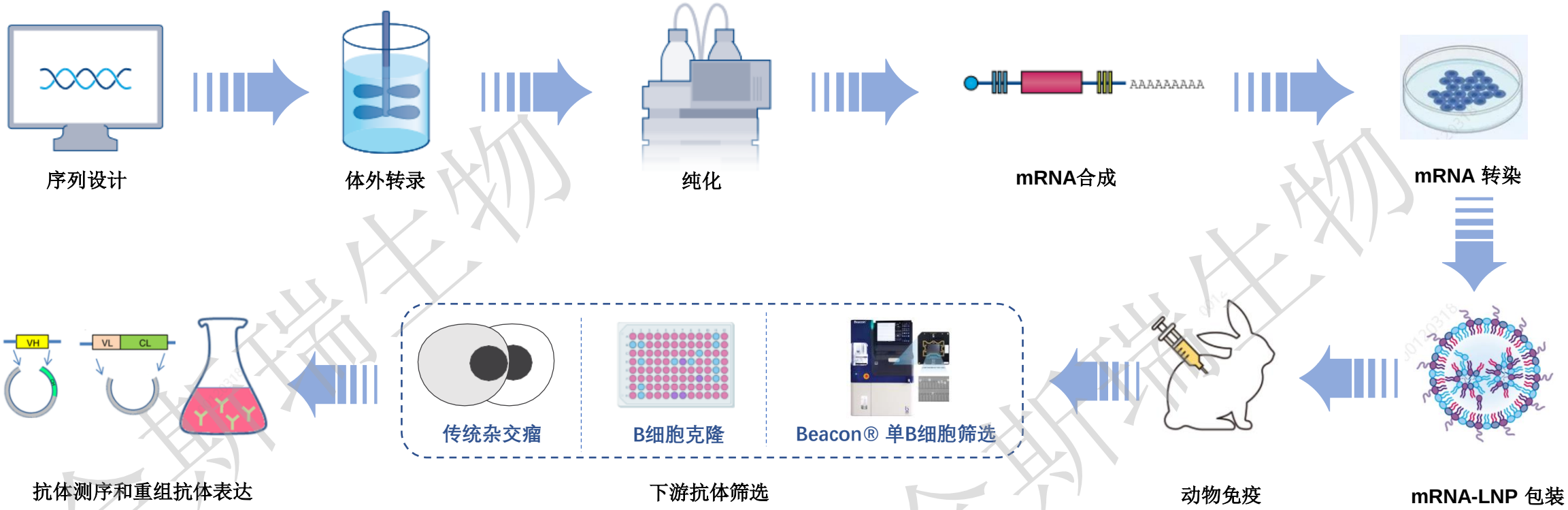
mRNA免疫用于抗体开发的流程及案例解析

- mRNA免疫策略的常规工作流程
- 金斯瑞通过mRNA免疫进行抗体开发的案例解析



金斯瑞一站式mRNA免疫策略的工作流程



金斯瑞mRNA免疫-抗体开发一站式服务：

- 仅需提供蛋白序列（single-pass, four-pass, GPCR, truncated protein）
- 采用mRNA-LNP免疫方式，抗原表达率高且动物免疫应答反应强
- 动物免疫周期极短（短至4周）
- 3种下游抗体筛选平台可选

从mRNA合成到重组抗体表达，助力多种跨膜蛋白靶点的抗体发现

案例1：mRNA免疫助力FACS阳性 hCCR9 抗体发现

Goal: Generate mouse monoclonal antibodies that are specific to 7-transmembrane protein hCCR9

Immunogen: hCCR9-mRNA-LNP

Screening Material: hCCR9-overexpressing CHO-K1

After one round of cell fusion

- ***19*** FACS positive parental clones
- ***9*** FACS positive stable hybridoma cell line & NGS results (with varies IgG1, IgG2a, IgG2b and IgG3 isotypes antibodies identified)
- ***7*** validated clones delivered
- ***7*** validated clones for FACS EC50

项目挑战：

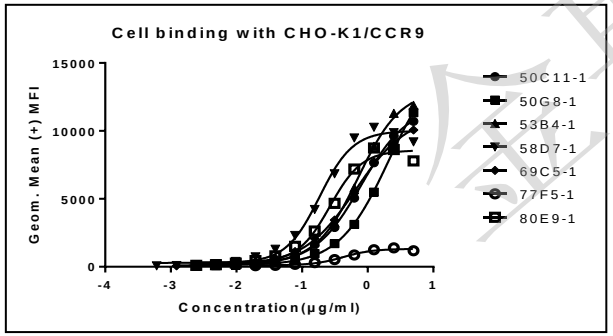
没有可用的纯化蛋白；DNA免疫失败

成功生成 9 个 hCCR9 阳性克隆，
最终客户选择了 7 个经过验证的克隆

Isotype identification of 9 hybridoma clones

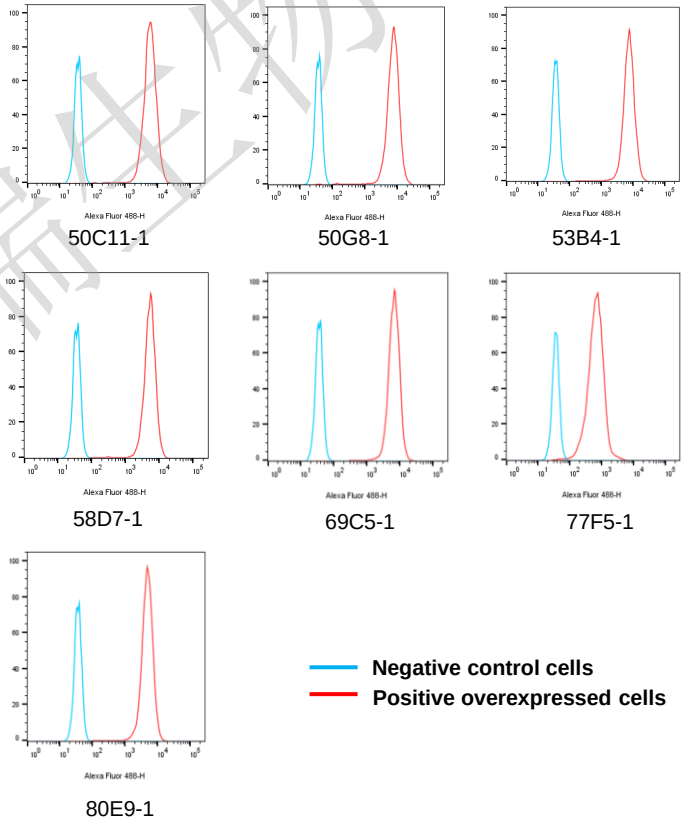
Isotype	IgG1, k	IgG2a, k	IgG2b, k	IgG3, k
Clone	58D7, 69D11, 80E9	69C5	50C11, 50G8, 53B4, 56B3	77F5

FACS EC50 of 7 recombinant antibody



	50C11-1	50G8-1	53B4-1	58D7-1	69C5-1	77F5-1	80E9-1
EC50	0.7974	2.121	0.7245	0.1847	0.6506	0.4246	0.266
R square	0.9997	0.9996	0.9987	0.9922	0.9997	0.9808	0.9899

FACS Results of 7 validated hybridoma clones



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案例2：mRNA免疫助力 FACS 阳性 hCLD18.2 抗体发现

Goal: Generate mouse monoclonal antibodies that are specific to 4-transmembrane protein hCLD-18.2 and suitable for IHC application

Immunogen: hCLD18.2-mRNA-LNP

Screening Material: hCLD18.2-overexpressing CT26

After one round of cell fusion

386 FACS positive parental clones

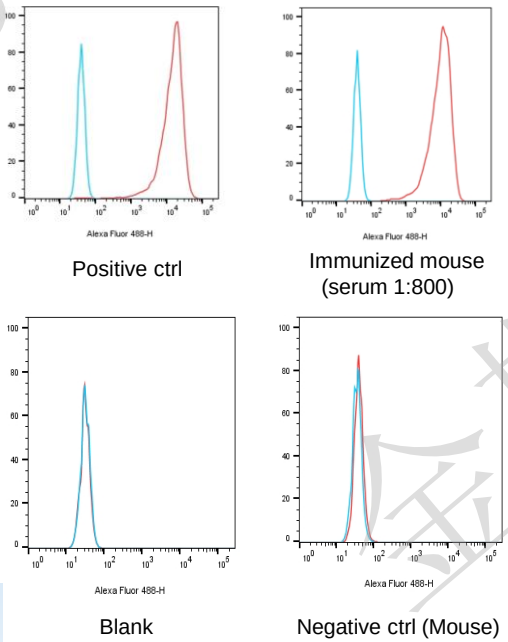
90 Positive subclones for NGS sequencing and ***84*** subclones showing sequence-specific

83/84 Antibody recombinant expression for FACS-positive

项目挑战：能通过蛋白免疫策略筛选出的能用于FACS应用的抗体很少

成功生成 386 个 hCLD18.2 阳性亲本克隆

Serum titer after CLD18.2-LNP immunization



FACS results of top 20 positive parental clones

Clone #	Median (+) MFI	Median (-) MFI	Median (+) MFI/ Median (-) MFI
39G6	11621	34	341.8
40C8	9621	34	283.0
40C4	9418	34	277.0
39D5	8701	34	255.9
38D9	8491	35	242.6
21E6	8727	37	235.9
11F8	7965	36	221.3
38A1	7314	34	215.1
37E3	7404	35	211.5
38F6	7051	34	207.4
40C11	7051	34	207.4
40B1	6903	34	203.0
40H8	6716	35	191.9
36E12	6635	35	189.6
38H3	6358	34	187.0
40C12	6534	35	186.7
39C1	6000	34	176.5
40H5	6055	35	173.0
36C2	5856	34	172.2
26C2	6000	36	166.7
Positive control	7893	35	225.5

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案例3：mRNA 免疫助力 FACS 阳性 hPTX 抗体发现

Goal: Generate mouse monoclonal antibodies that are specific to 7-transmembrane protein hPTX （9 phosphorylation sites, 1 glycosylation, 1 disulfide bond）

Immunogen: hPTX-mRNA-LNP

Screening Material: hPTX-overexpressing 293FT

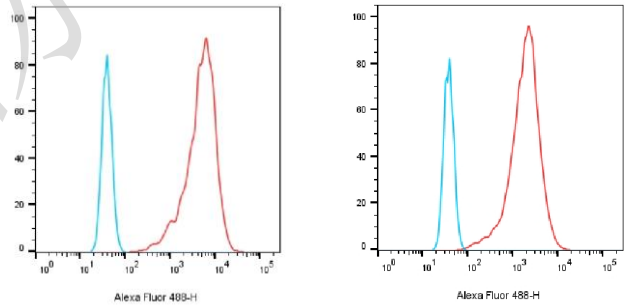
After one round of cell fusion

- ***21*** FACS positive parental clones
- ***21*** positive parental clones for subclone
- ***19/21*** positive subclone

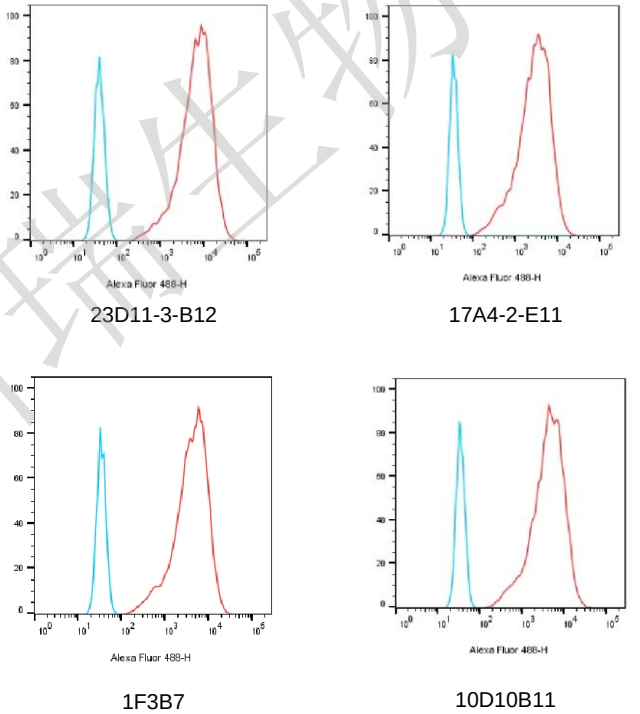
项目挑战：没有可用的纯化蛋白；此跨膜蛋白具有高度PTM

成功生成 21 个 hPTX 阳性亲本克隆

Serum titer after hPTX-LNP immunization



FACS results of representative positive subclones



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案例4：mRNA 免疫助力 FACS 阳性 hCCR9 兔抗发现

Goal: Generate rabbit monoclonal antibodies that are specific to 7-transmembrane protein hCCR-9

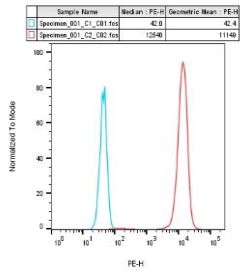
Immunogen: hCCR9-mRNA-LNP

Screening Material: hCCR9-overexpressing CHO-K1

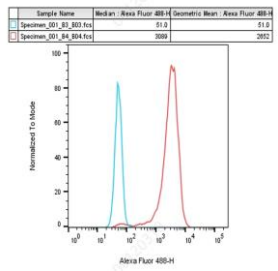
After one round of B cell cloning

- ***394*** FACS positive clones
- ***Top 20*** NGS sequencing
- ***10*** validated recombinant clones
- ***9*** recombinant clones for FACS EC50

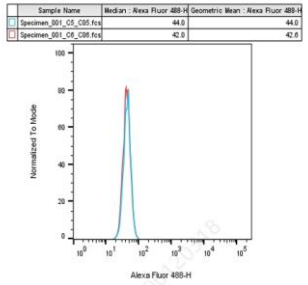
Serum titer after 2nd immunization of hCCR9-LNP



Positive ctrl



Immunized rabbit (serum 1:100)



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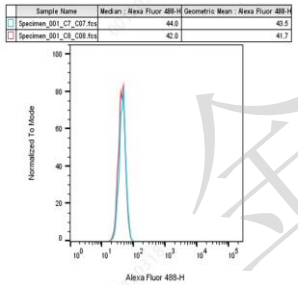


Negative ctrl (Rabbit)

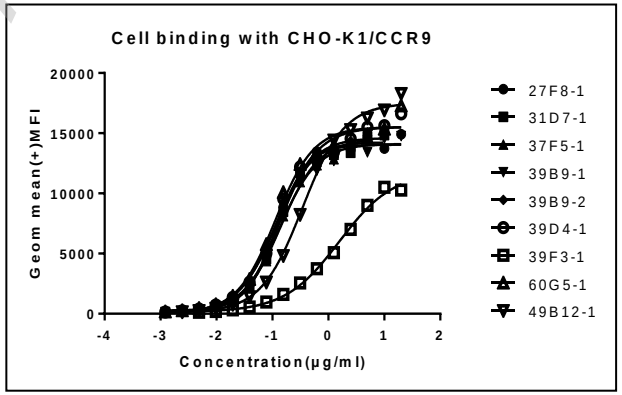
项目挑战：没有可用的纯化蛋白；DNA免疫失败

成功生成 394 个 hCCR9 阳性克隆

FACS results of recombinant antibody

Clone #	Median (+) MFI	Median (-) MFI	Median (+) MFI/ Median (-) MFI
27F8-1	22098	35	631.4
39D4-1	22031	35	629.5
31D7-1	21240	35	606.9
37F5-1	21964	36	610.1
39B9-1	20046	36	556.8
39B9-2	21698	34	638.2
20G5-1	3776	36	104.9
39F3-1	4674	41	114.0
60G5-1	14559	38	383.1
49B12-1	10867	38	286.0
Positive control	11763	42	280.1

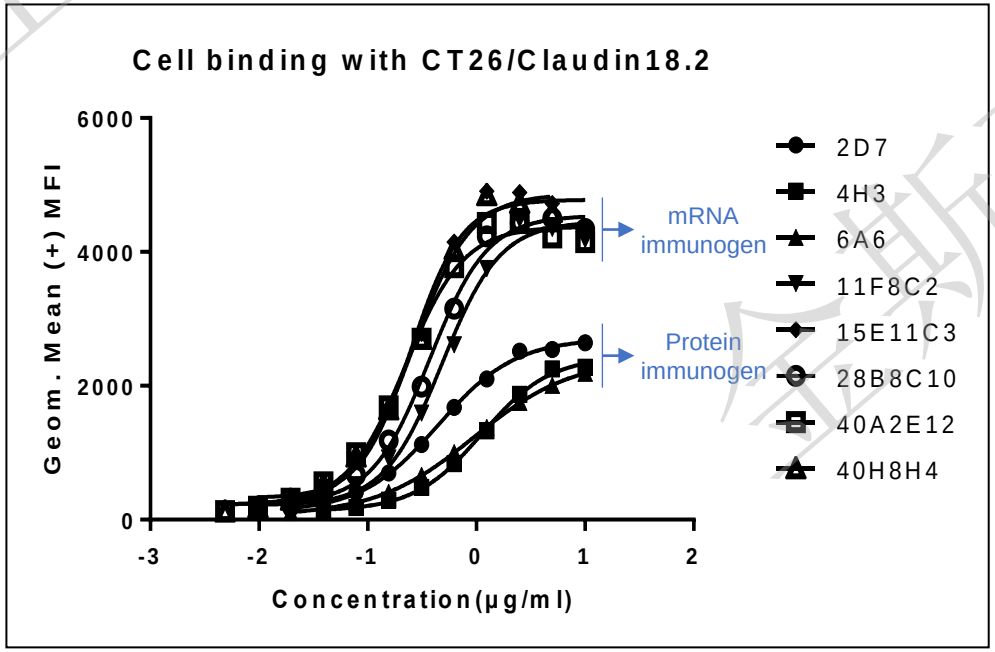
FACS EC50 of 9 recombinant antibody



	27F8-1	31D7-1	37F5-1	39B9-1	39B9-2	39D4-1	39F3-1	60G5-1	49B12-1
EC50	0.118	0.126	0.143	0.133	0.114	0.128	1.561	0.113	0.345
R square	0.997	0.996	0.996	0.995	0.998	0.992	0.996	0.990	0.997

案例5：mRNA免疫获得的抗体FACS亲和力 > 蛋白质免疫

Immunogen	ELISA-Positive	FACS-Positive	FACS EC50
18.2-Protein	111	36 (only 3 showed strong signal)	Shown in figure (6A6, 2D7, 4H3)
18.2-mRNA-LNP	N/A	386	Shown in figure (11F8C2, 40A2E12, 40H8H4, 15E11C3, 28B8C10)



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- Mutagenesis
- GenTitan™ Oligo Pool

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- DNA Oligo
- RNA Oligo
- qPCR Oligo
- NGS Oligo

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- Customized Peptide Synthesis
- Peptide Library

Virus & Cell line
- Lentivirus Packaging
- Assay Cell Line Engineering
- CRISPR Cell Line Engineering

Antibody
- Rabbit Monoclonal Antibody
- Mouse Monoclonal Antibody
- Beacon® Single B cell Sorting
- Polyclonal Antibody
- Antibody Sequencing and Production
- Customized ELISA Kit Development
- Anti-Idiotype Antibody
- Antibody Engineering

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