

# CART细胞药物进展

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深圳普瑞金生物药业有限公司

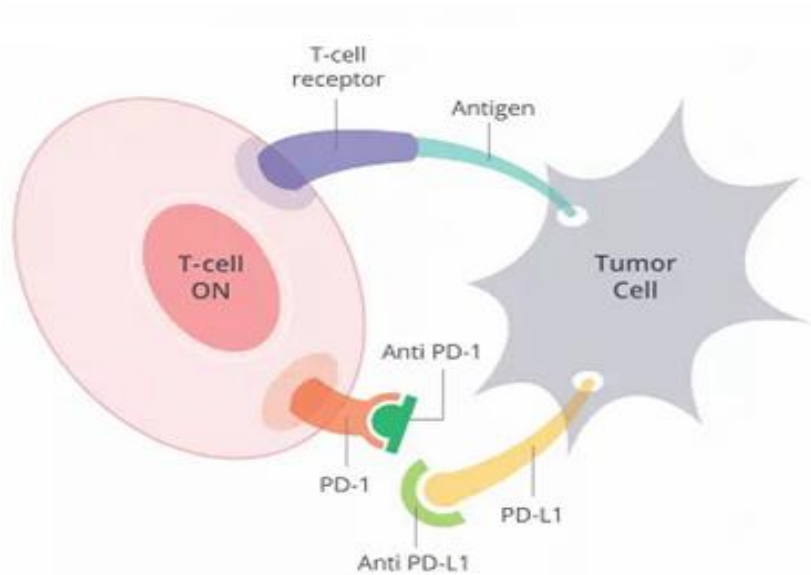
**1**

**CART细胞药物进展**

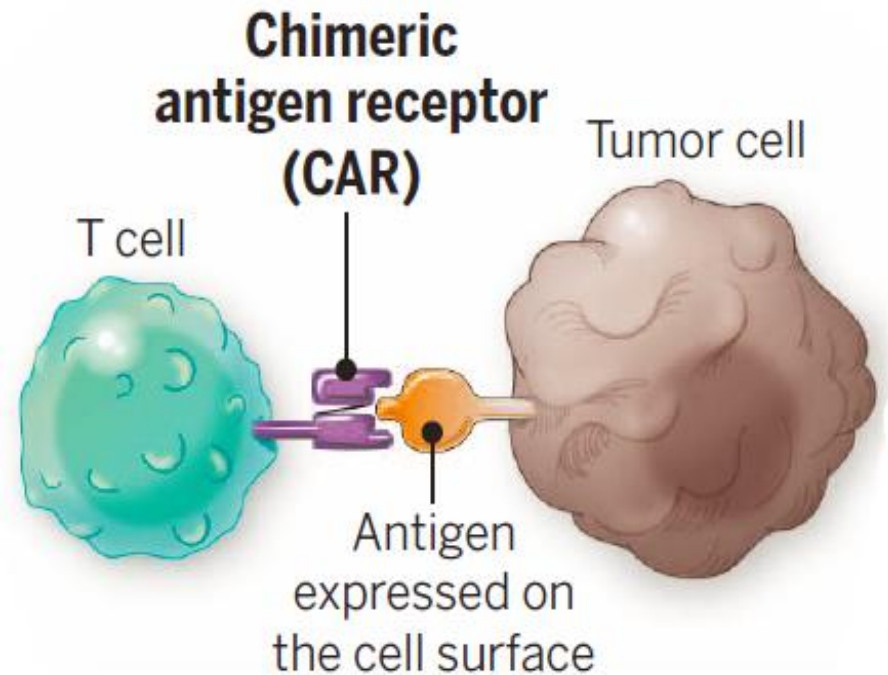
**2**

**单域抗体BCMA-CART研发**

# 免疫检查点抑制剂、CART

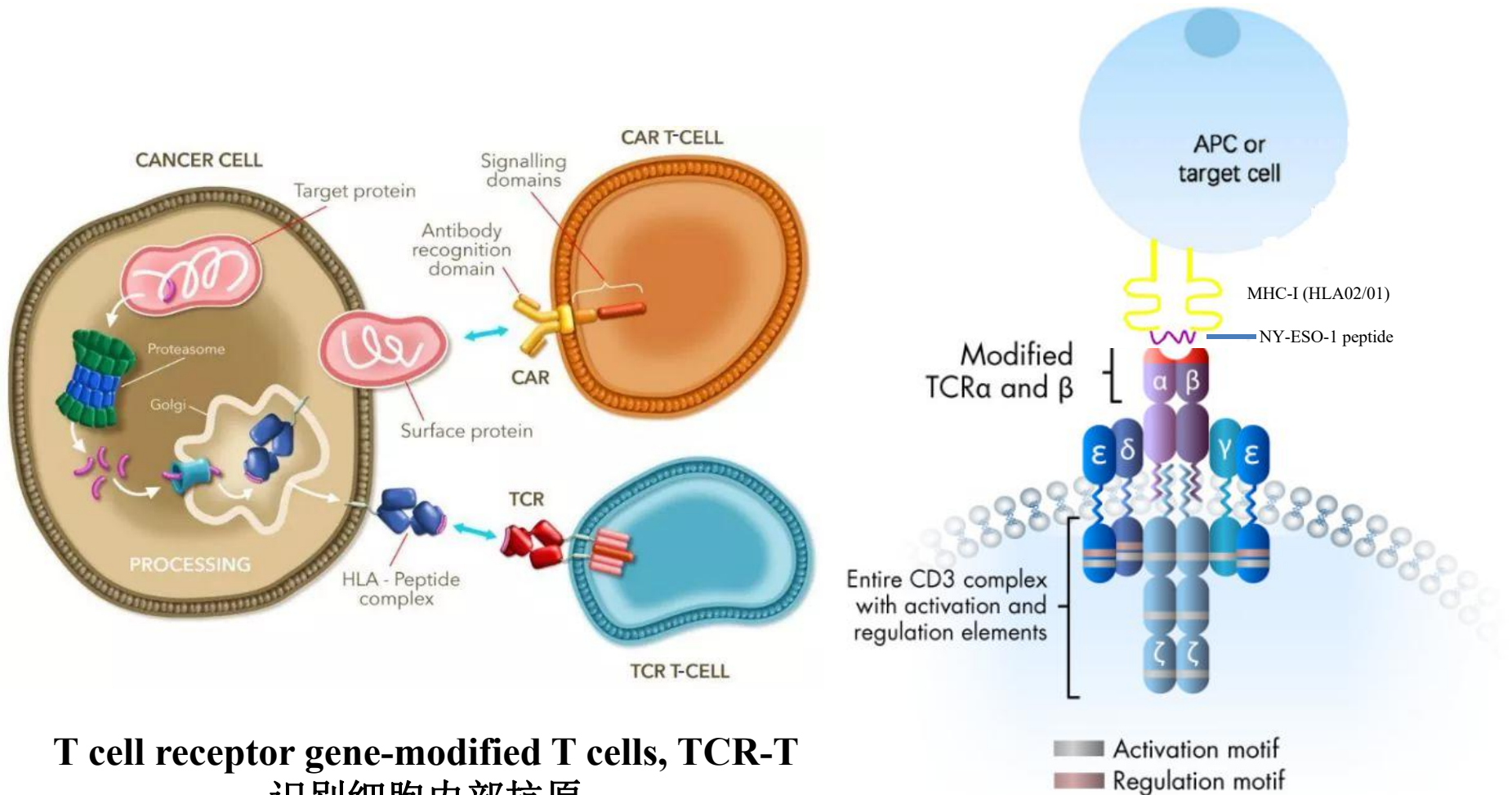


免疫检查点抑制剂



Chimeric antigen receptor T cells,  
CART  
识别细胞膜表面抗原

# T细胞受体T细胞 (TCR-T)



**T cell receptor gene-modified T cells, TCR-T**  
识别细胞内部抗原

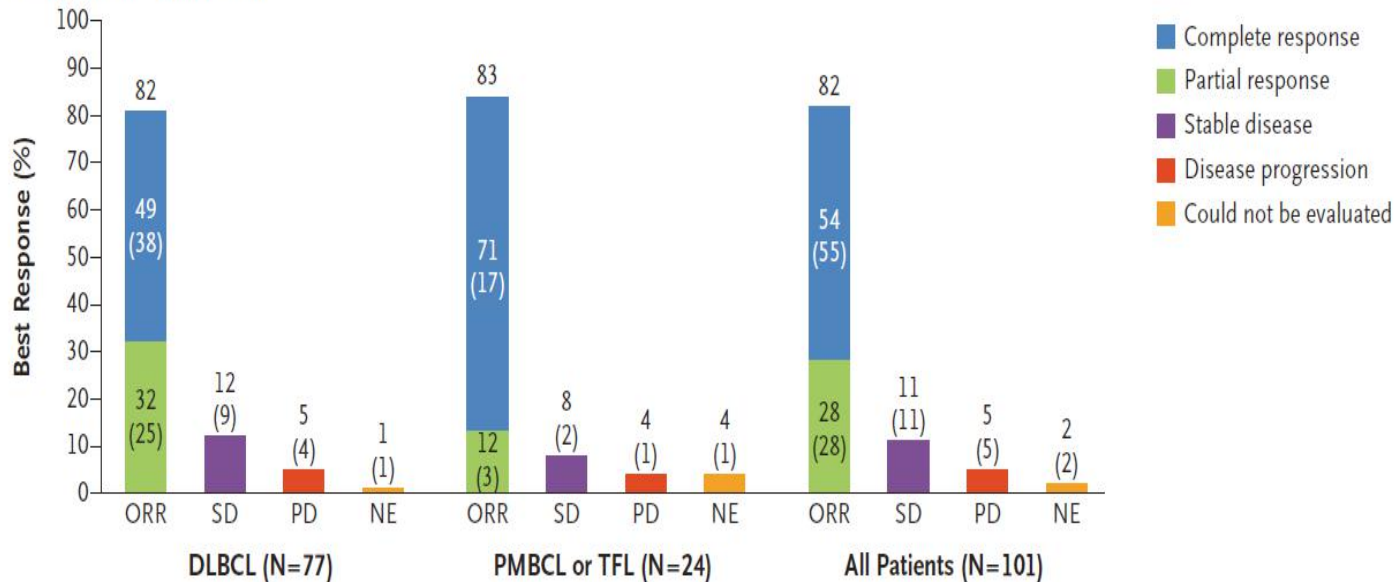
# CART疗效显著

公司	产品	临床效果	进度
Novartis	Kymriah	儿童ALL, CR 82%, 成人NHL, ORR 53%	2017年8月31日, ALL上市 2018年5月2日, NHL上市
Juno	JCAR017	成人NHL, CR 53%	2020年, 申报上市
Kite	Yescarta	成人NHL, CR 52%	2017年10月19日, 上市
Bluebird	bb2121	骨髓瘤, ORR (73.4%)	2019年12月, KarMMa 2期数据
强生/南京 传奇	JNJ-68284528	骨髓瘤, ORR (91%)	2019年12月, CARTITUDE-1 1b/2期数据

血液系统肿瘤：白血病、淋巴瘤、多发性骨髓瘤，效果显著

# CD19 CART长期疗效好

A Objective Response Rate



Yescarta治疗淋巴瘤Zuma-1注册临床试验中，中位随访27.1个月，仍有37%获得完全缓解。

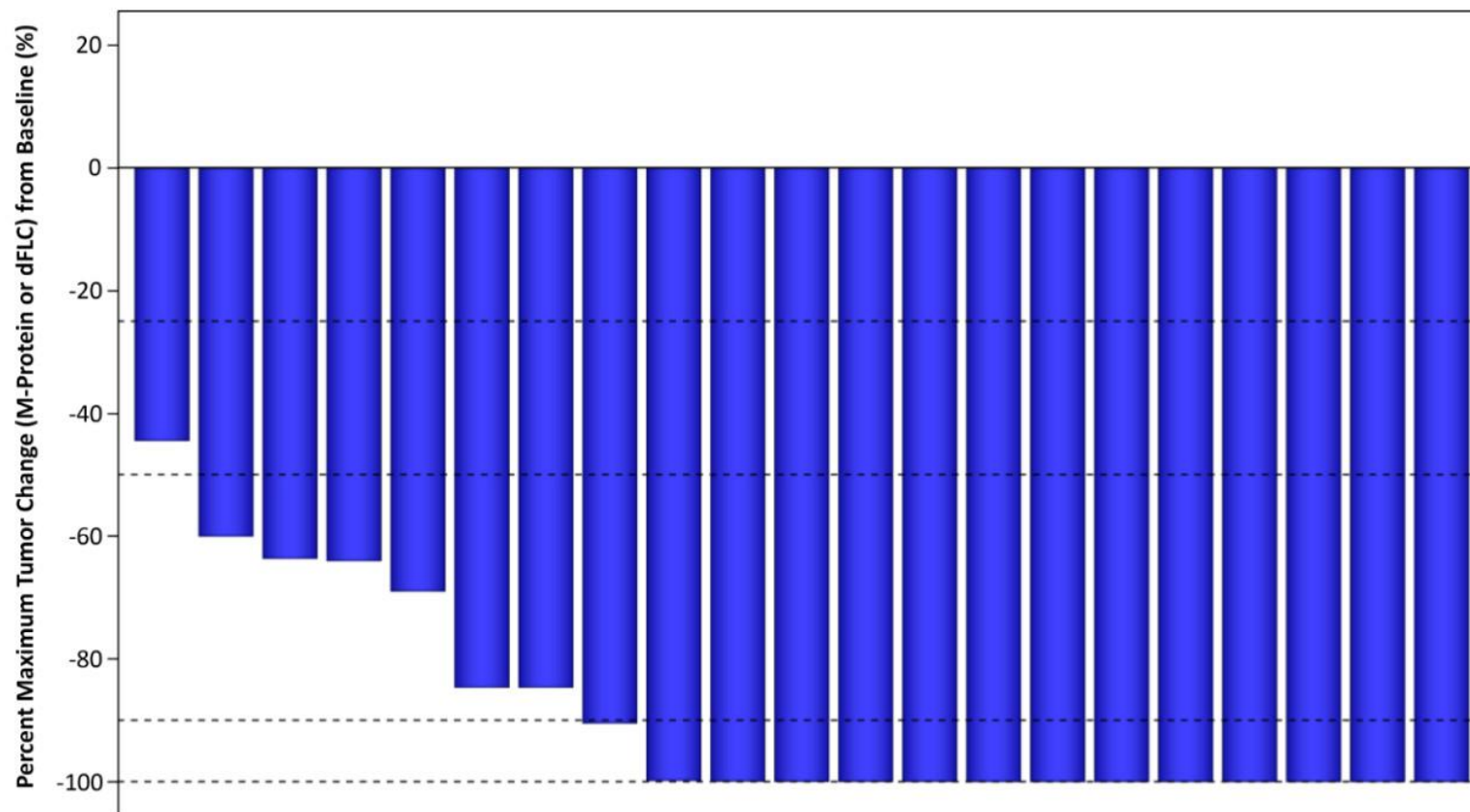
Kymriah治疗白血病，6个月，EFS 73%；12个月，50%。

# BCMA CART最新临床数据 (bb2121)

	Ide-cel Treated Population			
	150 x 10 <sup>6</sup>	300 x 10 <sup>6</sup>	450 x 10 <sup>6</sup>	150-450 x 10 <sup>6</sup>
	CAR+ T cells	CAR+ T cells	CAR+ T cells	CAR+ T cells
	(N=4)	(N=70)	(N=54)	(N=128)
ORR, n (%)	2 (50.0)	48 (68.6)	44 (81.5)	94 (73.4)
CR/sCR, n (%)	1 (25.0)	20 (28.6)	19 (35.2)	40 (31.3)
Median DoR, months	---	9.9	11.3	10.6
Median PFS, months	---	5.8	11.3	8.6

# BCMA CART最新临床数据 (JNJ4528)

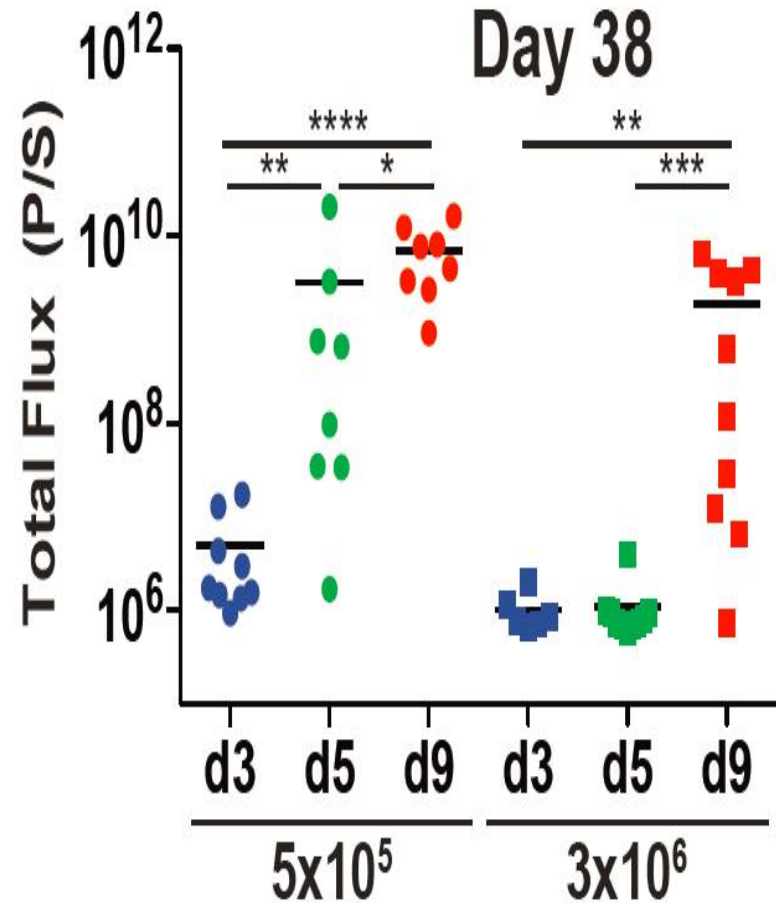
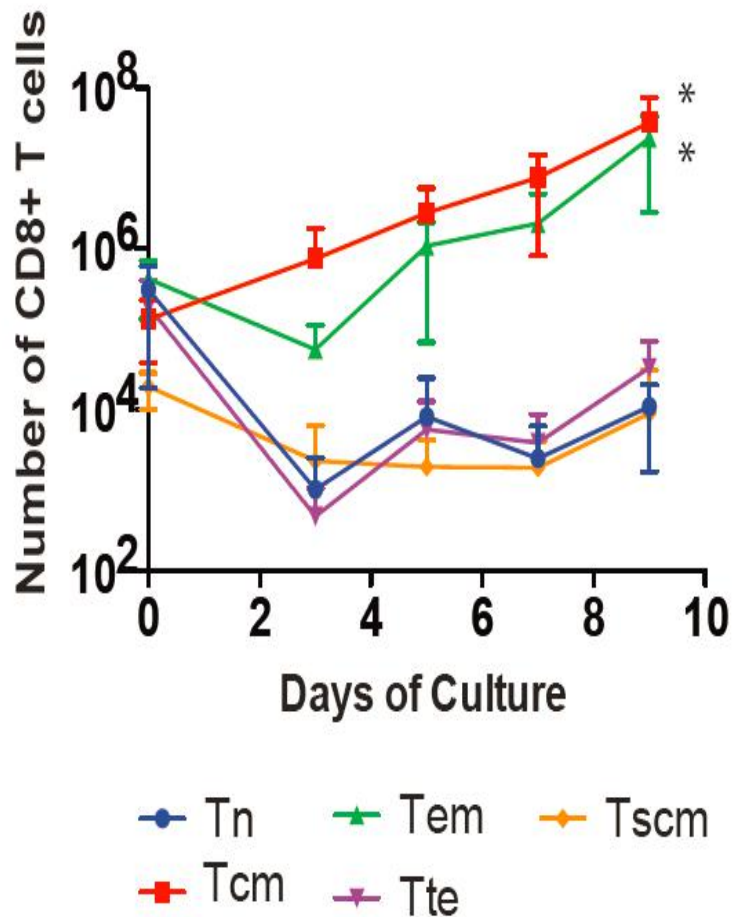
Response-Evaluable Patients (n=21)



21名随访满3个月的患者，ORR 91%，sCR/CR 28.6%，VGPR 33.3%，PR 28.6%。



# 缩短培养时间增加疗效



Reducing Ex Vivo Culture Improves the Antileukemic Activity of Chimeric Antigen Receptor (CAR) T Cells.  
Cancer Immunol Res. 2018 Sep;6(9):1100-1109.

# FasTCAR-19临床数据

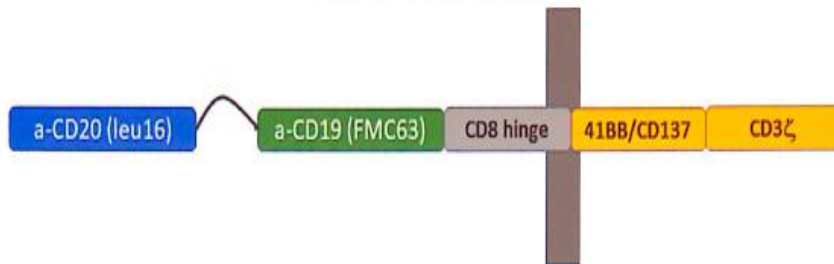
FasTCAR-19 或 GC007F 使用亘喜生物的专利FasTCAR™ 解决方案，FasTCAR-19细胞通过病毒转染的方式，仅用一天的时间即可完成制备。与目前已批准上市的其他同类产品相比，这些细胞杀伤能力更强、持续时间更长。共计入组 37 例年龄在14 至 70 岁间的复发或难治性B-ALL成人和青少年患者。这些患者此前均接受过多线治疗，但都未能获得持续临床缓解。所有患者均接受了FasTCAR-19单次输注治疗，试验设置了 3 个剂量组（低剂量组:  $0.6 \times 10^5/\text{Kg}$ ；中剂量组:  $1.0 \times 10^5/\text{kg}$ ，高剂量组:  $1.6 \times 10^5/\text{Kg}$ ）。CAR-T治疗前，所有患者均接受氟达拉滨联合环磷酰胺 (FC) 预处理治疗。

28 天随访期间，有 35 例患者达到评价时间点，其中：34 例 (97.1%) 达到了完全缓解，其中包括全血细胞计数已恢复或未恢复的患者(CR/CRi)；32 例 (91.4%) 达到最小残留病灶阴性完全缓解 (MRD- CR)。

# 抗CD19、CD20双CAR T细胞疗效较好

First-in-human bispecific, tandem CAR-T cell against two B-cell antigens CD19 and CD20 using a CD3 $\zeta$  and 4-1BB lentiviral

CAR-T construct



## Secondary Outcomes: Day 28 Clinical Responses

Day 28 Overall Response Rate: 82% (14/17 patients)

- CR 11/17 (65%)
- PR 3/17 (18%)
- 3 patients with PD at Day 28

## Response by Dose Level at Day 28

- $2.5 \times 10^5$  cells/kg: 1 CR, 1 PR, 1 PD
- $7.5 \times 10^5$  cells/kg: 1 CR, 1 PR, 1 PD
- $2.5 \times 10^6$  cells/kg: 9 CR, 1 PR, 1 PD (91% ORR)

Presented at 2019 ASCO Annual Meeting

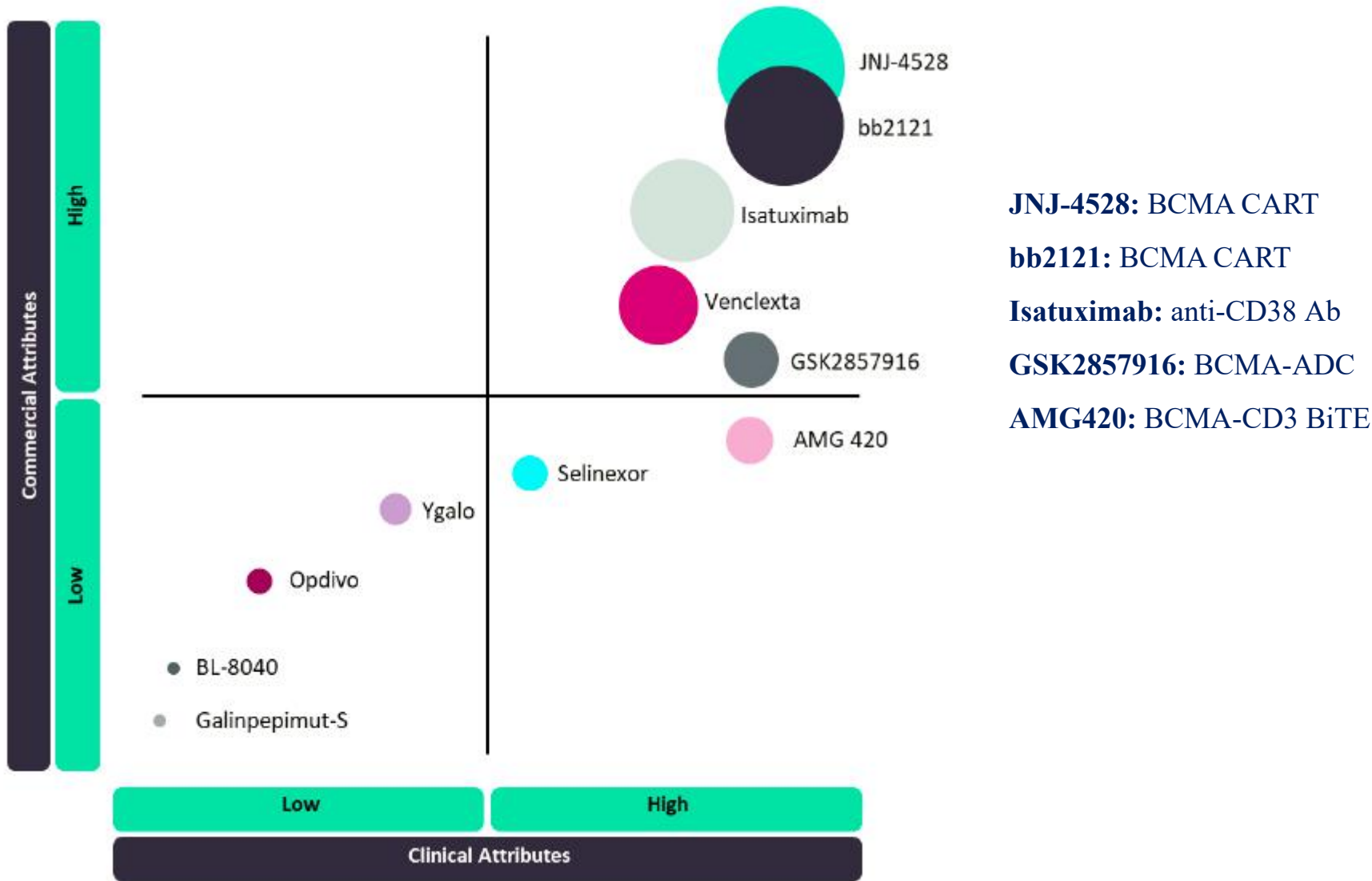
# 血液肿瘤常见CART靶点

类型	B细胞白血病、淋巴瘤	T细胞白血病、淋巴瘤	髓系白血病	多发性骨髓瘤
靶点	<b>CD19</b>	CD4	CD123	<b>BCMA</b>
	CD20	TCR	CD33	TACI
	CD22	CD5	Lewis Y	CD38
	CD30	CD7	NKG2D	CD138
	CS1		CLL-1	Kappa LC

# CAR-T的价值和研发特点

骨髓瘤治疗	抗体药 (裸抗体、ADC、BiTE)	CART
疗效	较好	更好，抗体治疗失败的也能显示疗效
持续用药	方便	一次性、待研发多次给药方式
研发周期	长	较短
成药潜力	开发已较充分	细胞药，具有巨大设计空间
CMC	成熟，平台化	工艺规模小，生产和质量控制成本高
商业化规模	规模放大	规模复制

# 多发性骨髓瘤新药竞争力分析



Source: GlobalData (2019)

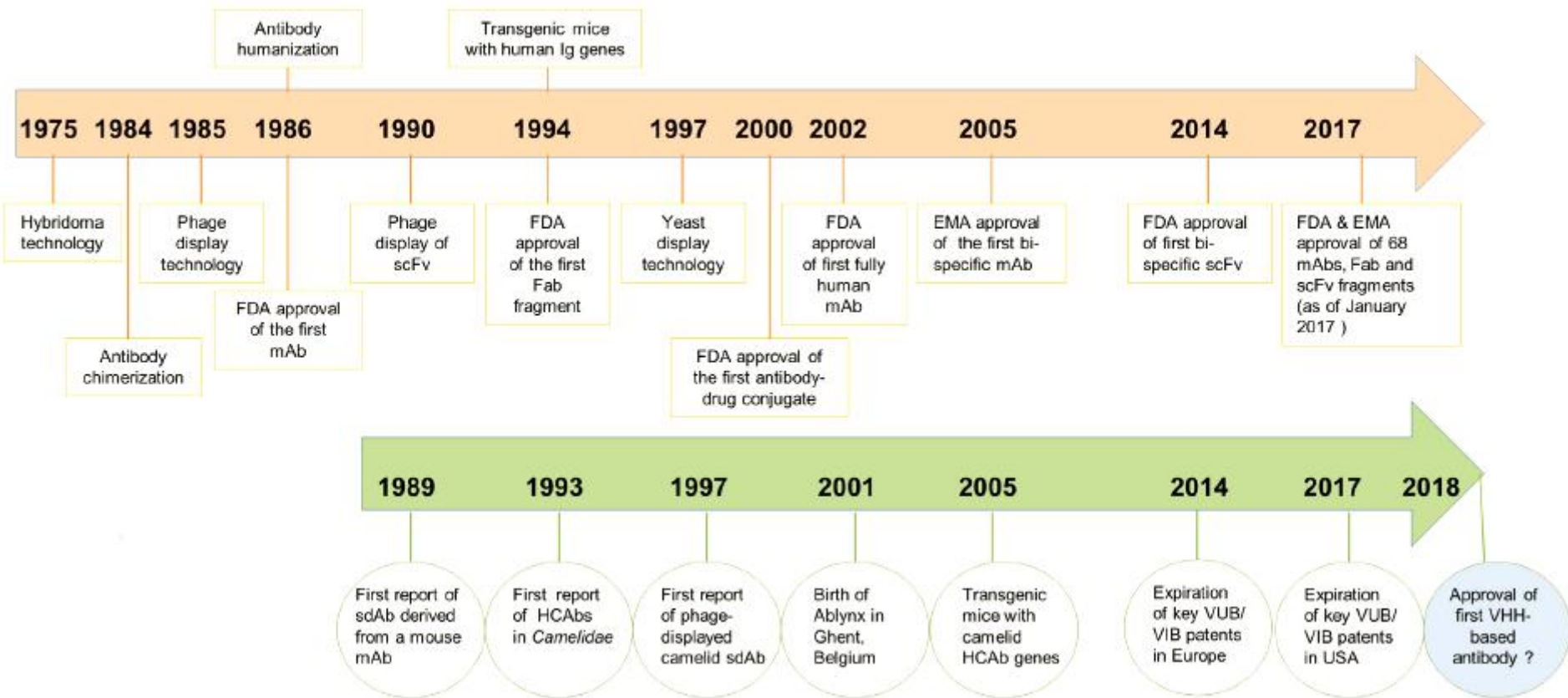
**1**

**CART细胞药物进展**

**2**

**单域抗体BCMA-CART研发**

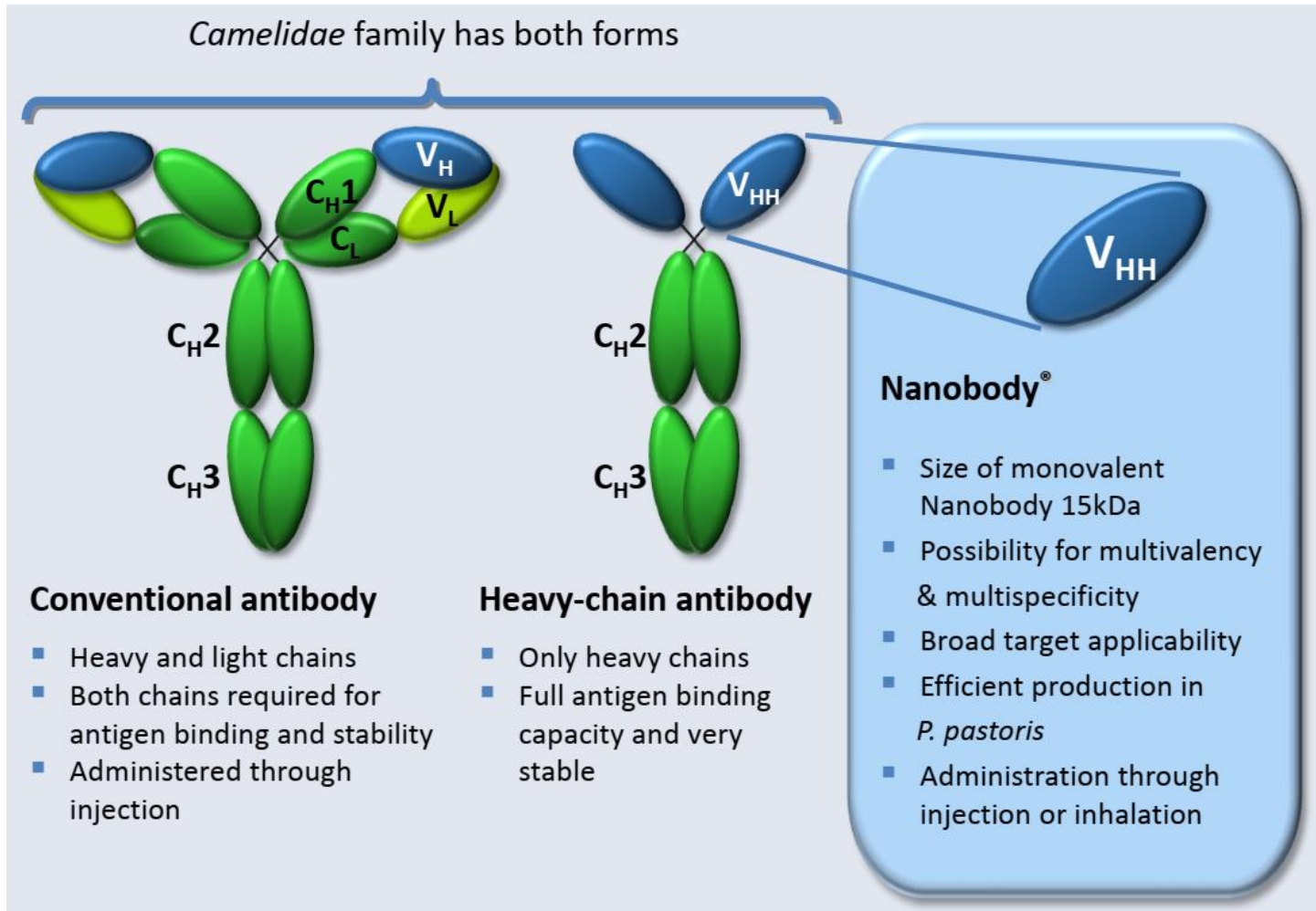
# 传统抗体和单域抗体技术发展



Camelid single-Domain Antibodies: Historical Perspective and Future Outlook, **Frontiers in Immunology**

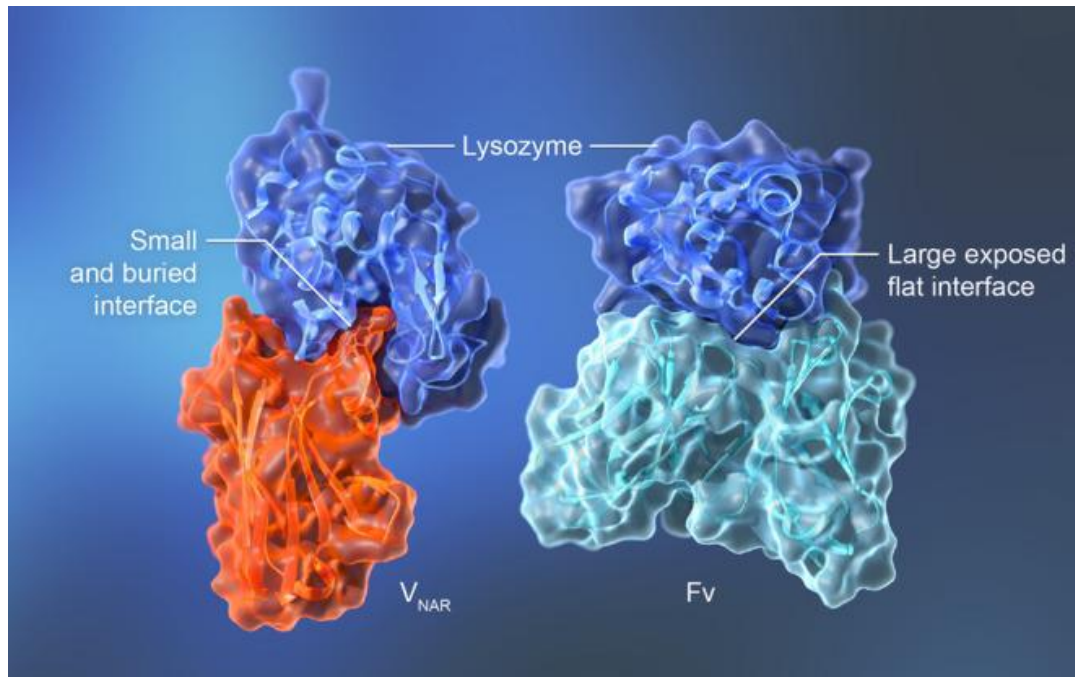
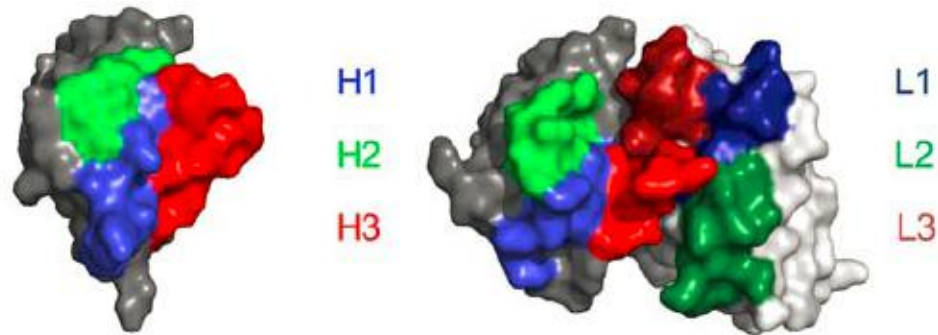


# 单域抗体的特点






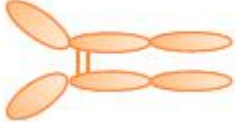
From website “<http://www.ablynx.com>”

# 单域抗体结合抗原的结构



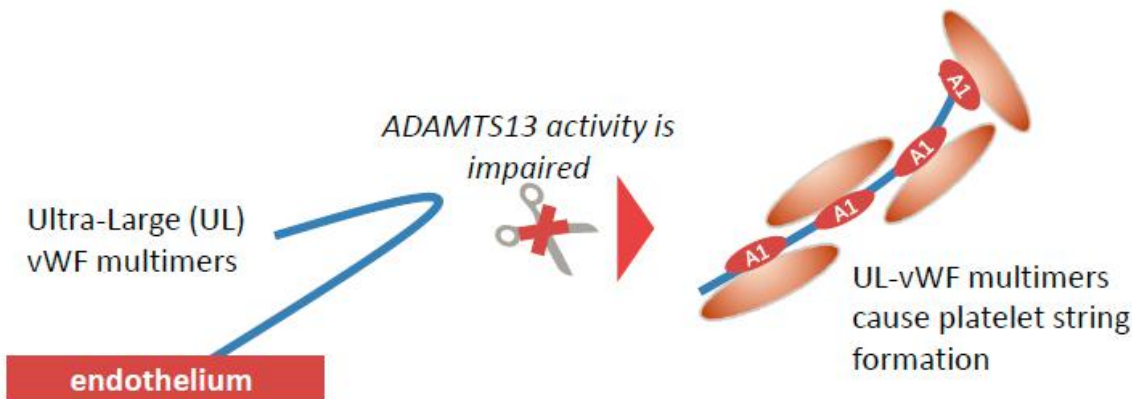
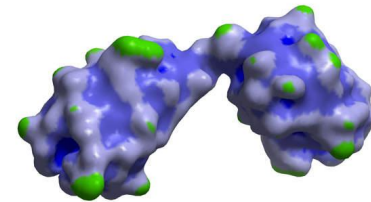
Comparative analysis of nanobody sequence and structure data, **Proteins**

# 单域抗体成药结构和表达系统

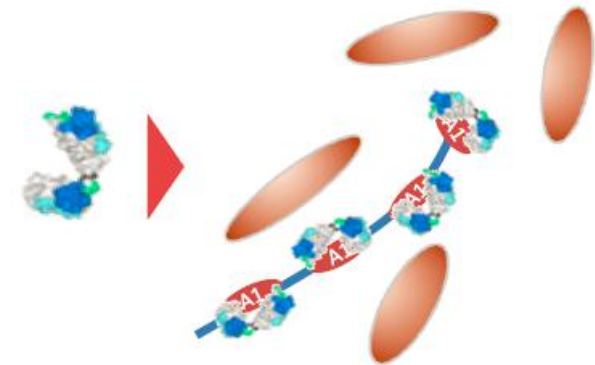
结构	图示	表达系统
单价纳米抗体		E.Coli
双价纳米抗体		E.Coli、酵母
三价纳米抗体		毕赤酵母
Fc融合蛋白		CHO细胞

# 第一个上市的单域抗体药物Caplacizumab

2018年9月，欧盟批准上市治疗aTTP；  
2019年2月，美国FDA批准上市。  
二价纳米抗体，细菌表达，皮下注射，  
但半衰期短，需要每天用药。



Caplacizumab (anti-vWF Nanobody) binds to A1 domain of vWF and inhibits platelet string formation



From Ablynx's Poster

# 三价单域抗体药物结构

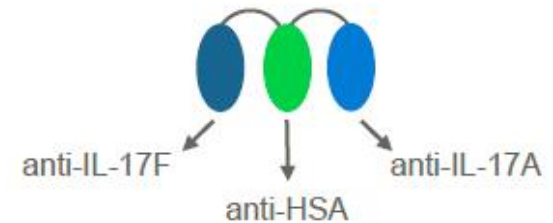
Multi-specific blocks two cytokines at once



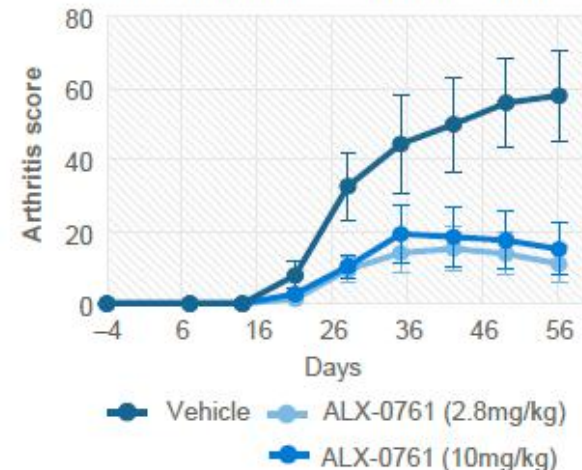
Bi-specific anti-IL-17A/IL-17F Nanobody ALX-0761



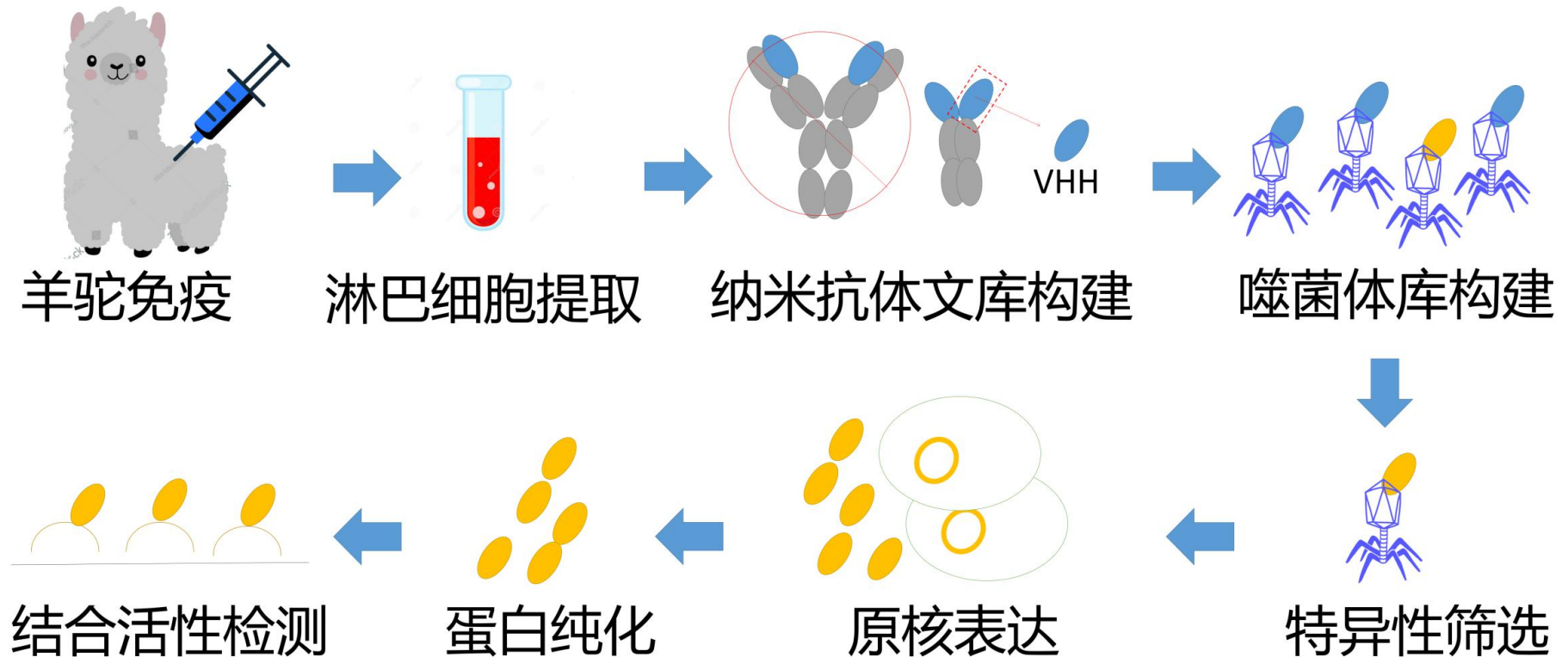
- ALX-0761 blocks both IL-17A and IL-17F for more effective blocking of the inflammatory response
- Binds human serum albumin for improved PK
- Proof of concept in primate CIA model
- ALX-0761 in development by Merck KGaA
  - completed Phase I SAD study in healthy volunteers
  - completed Phase Ib study in patients with psoriasis



Proof-of-concept achieved in primate collagen induced arthritis model<sup>1</sup>



# 单域抗体筛选流程



# 单域抗体筛选其他方案

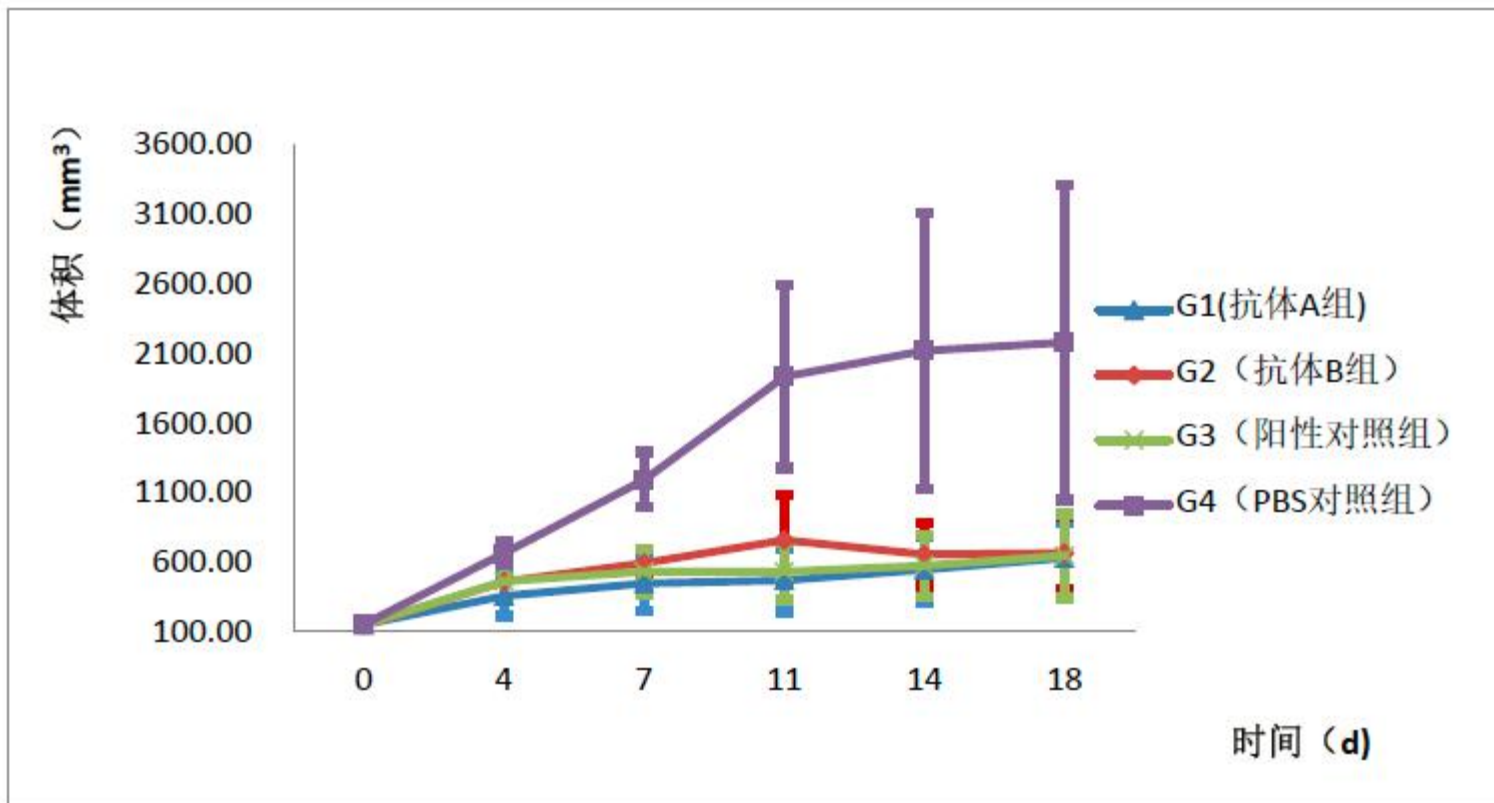
- SOURCES OF Nb LIBRARIES
- Immune Nb libraries (after immunizing a camelid)
  - Only for immunogenic, non-toxic targets
  - Affinity maturation and proliferation of target-specific B cells
  - Camel, dromedary, llama, alpaca, HCAb Xenomice, shark
  - Many small libraries ( $10^7$ ) to make
- Naïve or synthetic libraries
  - No need for immunization, less antigen is needed
  - One or a few large libraries ( $10^{10}$ )
  - More difficult panning, low affinity, low expression
- POSSIBLE SELECTIONS
  - Single cell (FACS)
  - deep sequencing + amino acid sequencing

# 单域抗体发现-VEGF抗体亲和力

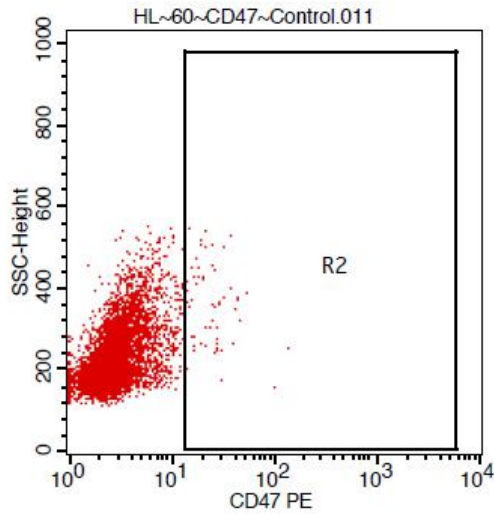
Sample ID	KD( nM)	Kon (1/Ms)	Koff(1/s)
V15 (#1)	9.77	1.18E+5	1.15E-3
V21 (#7)	4.83	1.41E+5	6.82E-4
V25 (#6)	1.36	1.36E+5	2.56E-5
V32 (#3)	1.61	6.7E+4	1.08E-4
V35 (#5)	1.16	3.31E+5	3.83E-4
V74 (#2)	8.08	2.91E+5	2.35E-3
V2-3 (#12)	0.11	8.82E+4	9.79E-6
V2-9 (#11)	2587	3.21E+4	8.31E-2
V2-11 (#10)	<0.001	1.42E+5	<1.00E-7
V22-4 (#14)	51.56	5.17E+4	2.67E-3
V22-17 (#15)	1.87	1.6E+5	3.00E-4
V22-28 (#16)	37.5	1.22E+5	4.56E-3
V22-37 (#17)	2.03	2.68E+5	5.44E-4
V22-46 (#18)	14.12	1.24E+5	1.74E-3
V22-60 (#13)	1.28	8.93E+4	1.14E-4



# 单域抗体发现-VEGF抗体功能



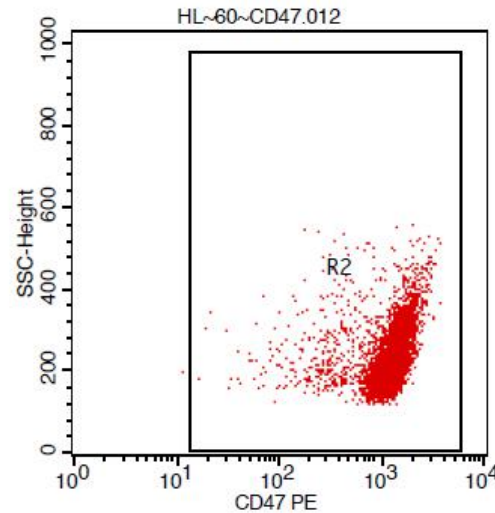
# 单域抗体发现-CD47抗体



Gate Statistics

File: HL~60~CD47~Control.011

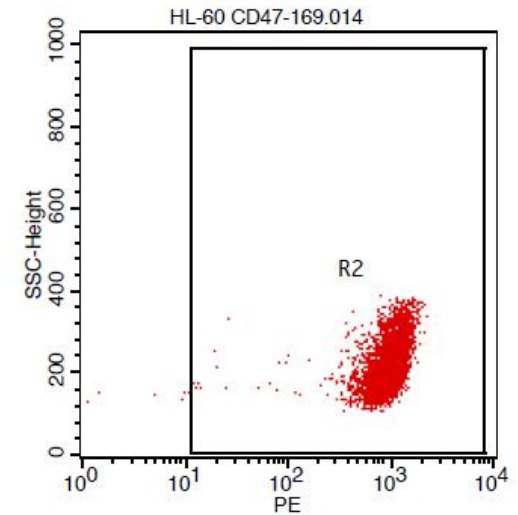
Gate	% Gated	% Total
G1	100.00	88.24
G2	1.22	1.08



Gate Statistics

File: HL~60~CD47.012

Gate	% Gated	% Total
G1	100.00	87.14
G2	99.96	87.11

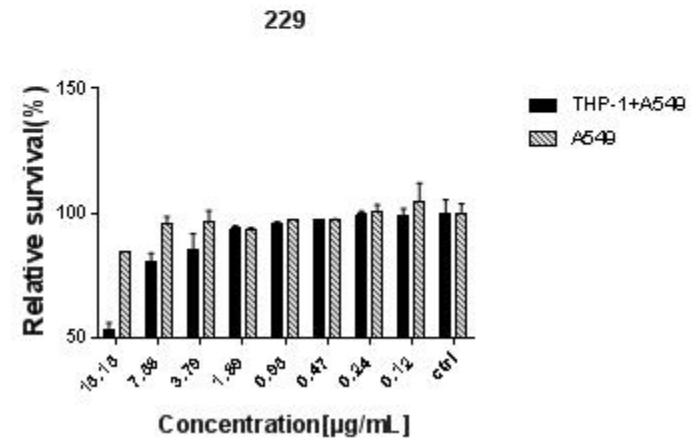
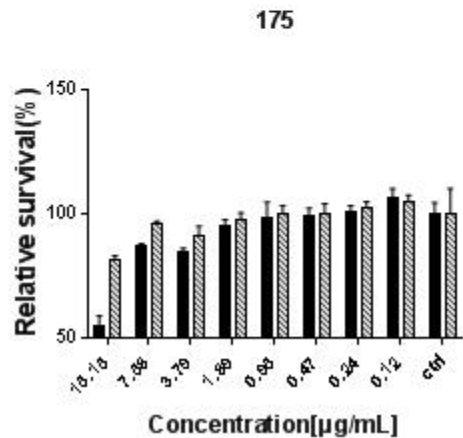
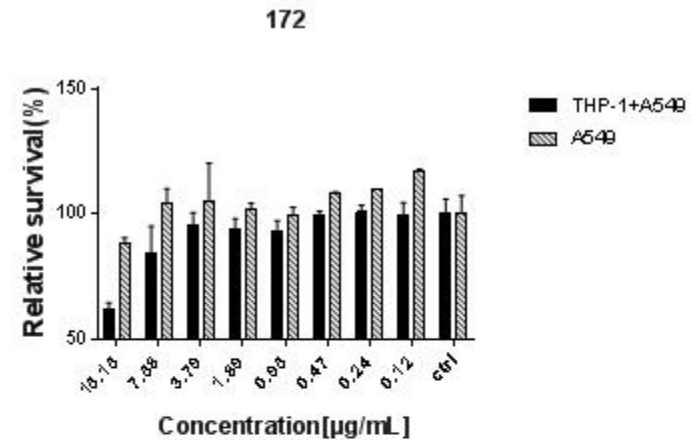
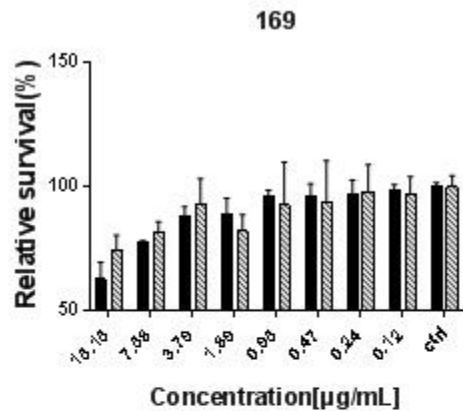


Gate Statistics

File: HL-60 CD47-169.014

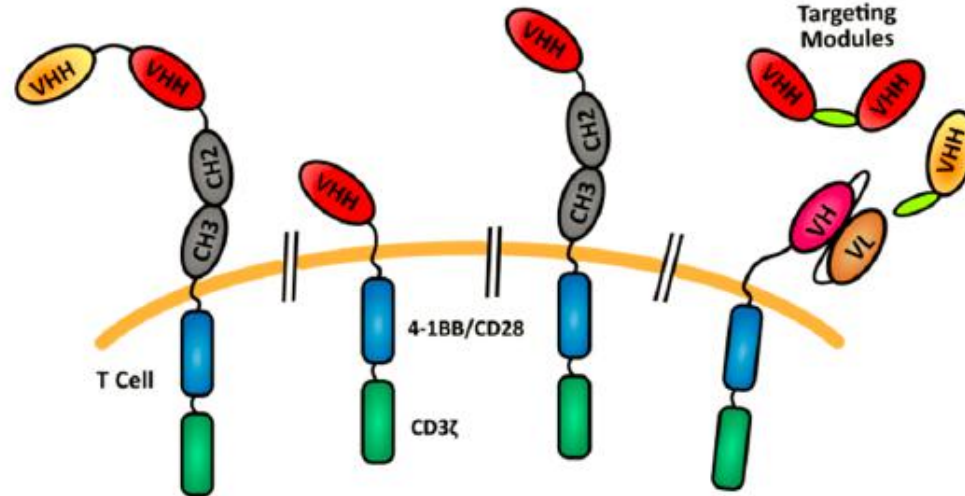
Gate	% Gated	% Total
G1	100.00	78.02
G2	99.90	77.94

# 单域抗体发现-CD47抗体功能



# 单域抗体在细胞治疗中的应用

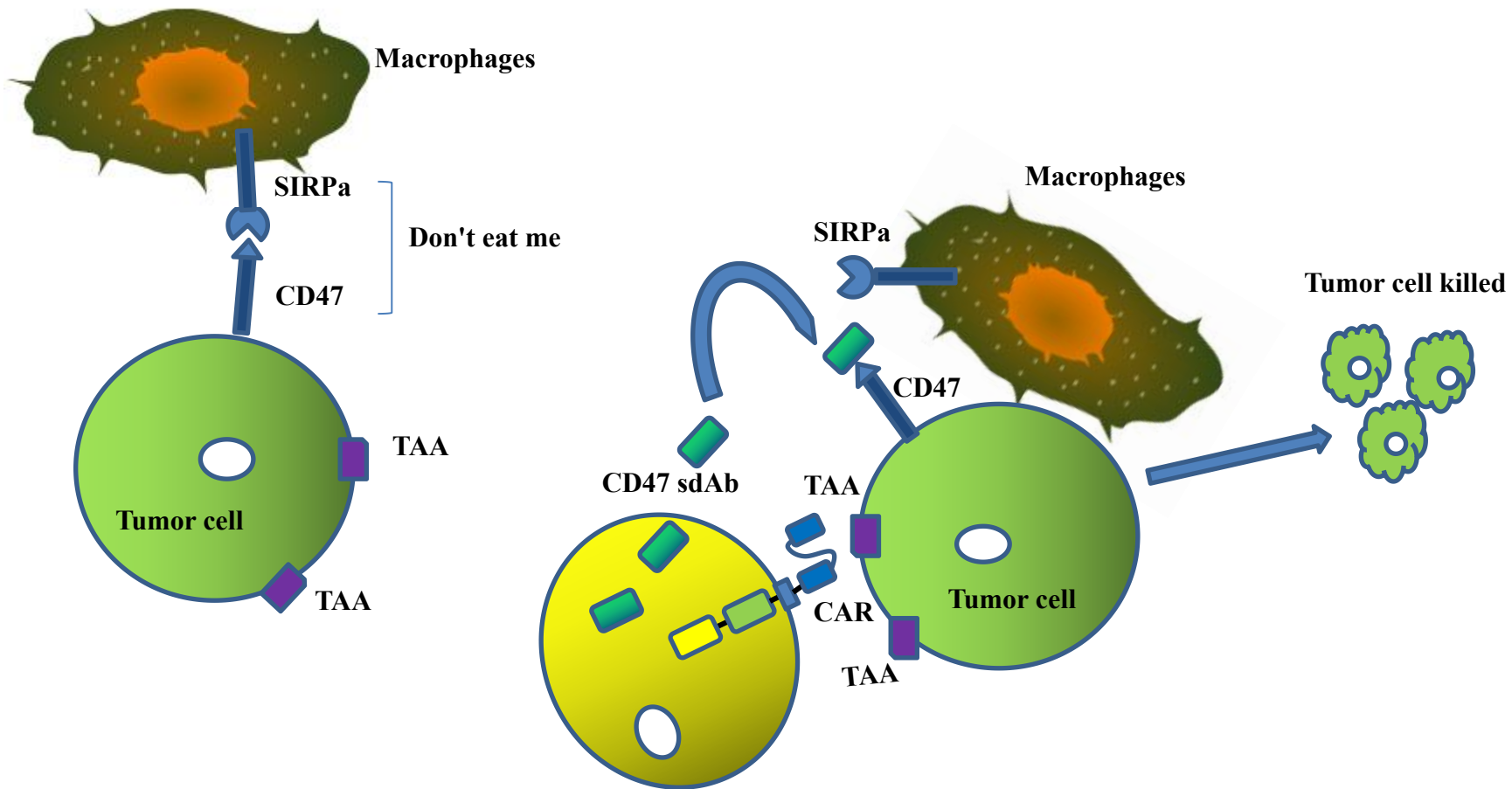
## sdAb (Nanobody, Nb) CART



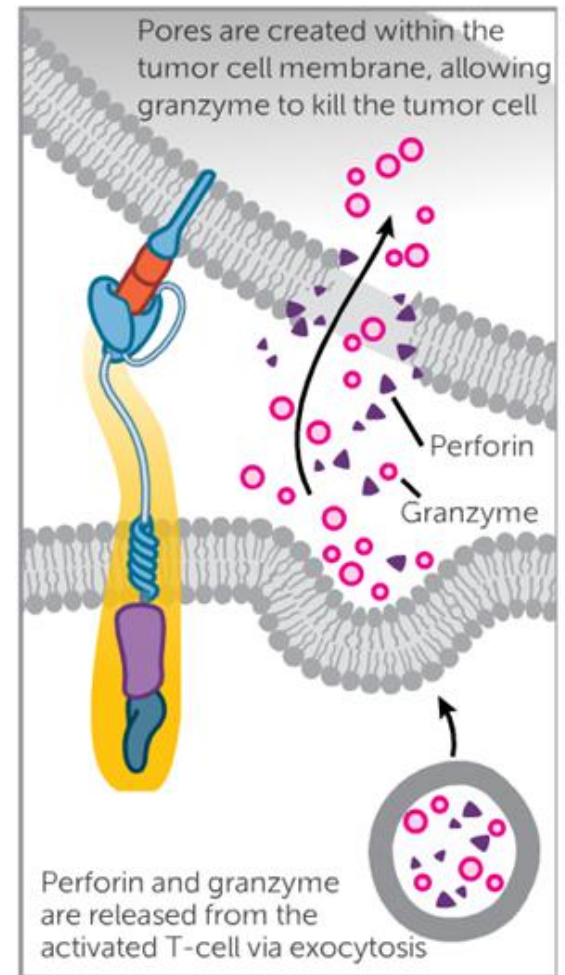
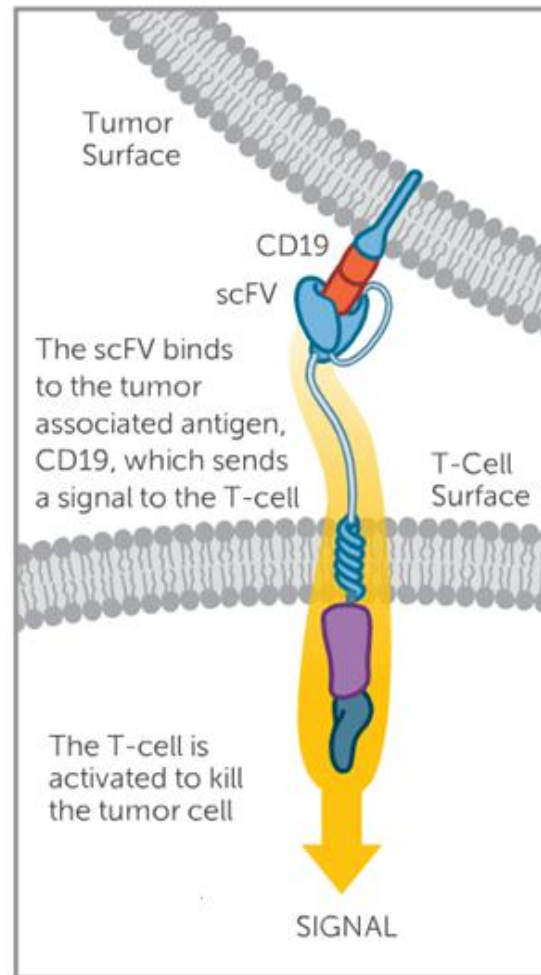
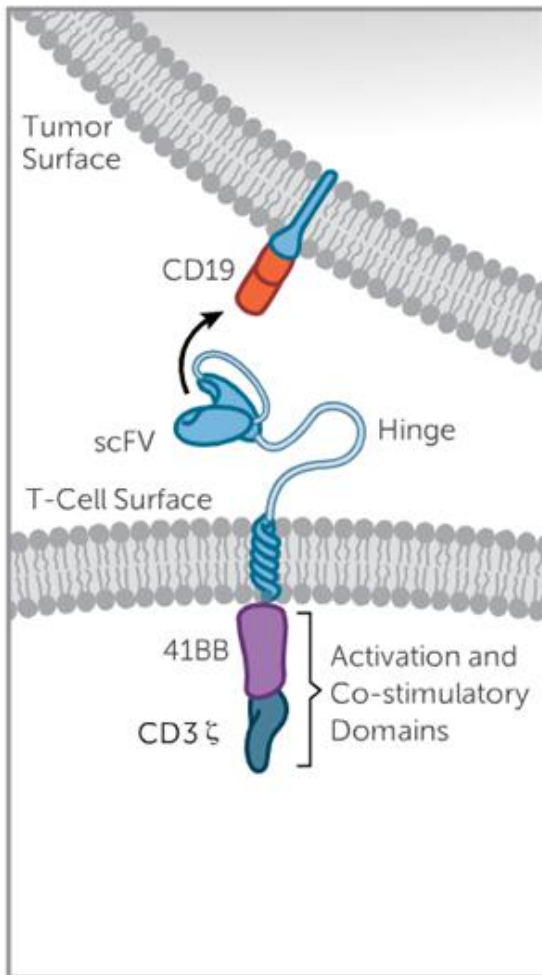
Target:

Muc1/PSMA/HER2/GPC2/CD7/VEGFR2/EGFR/CD38

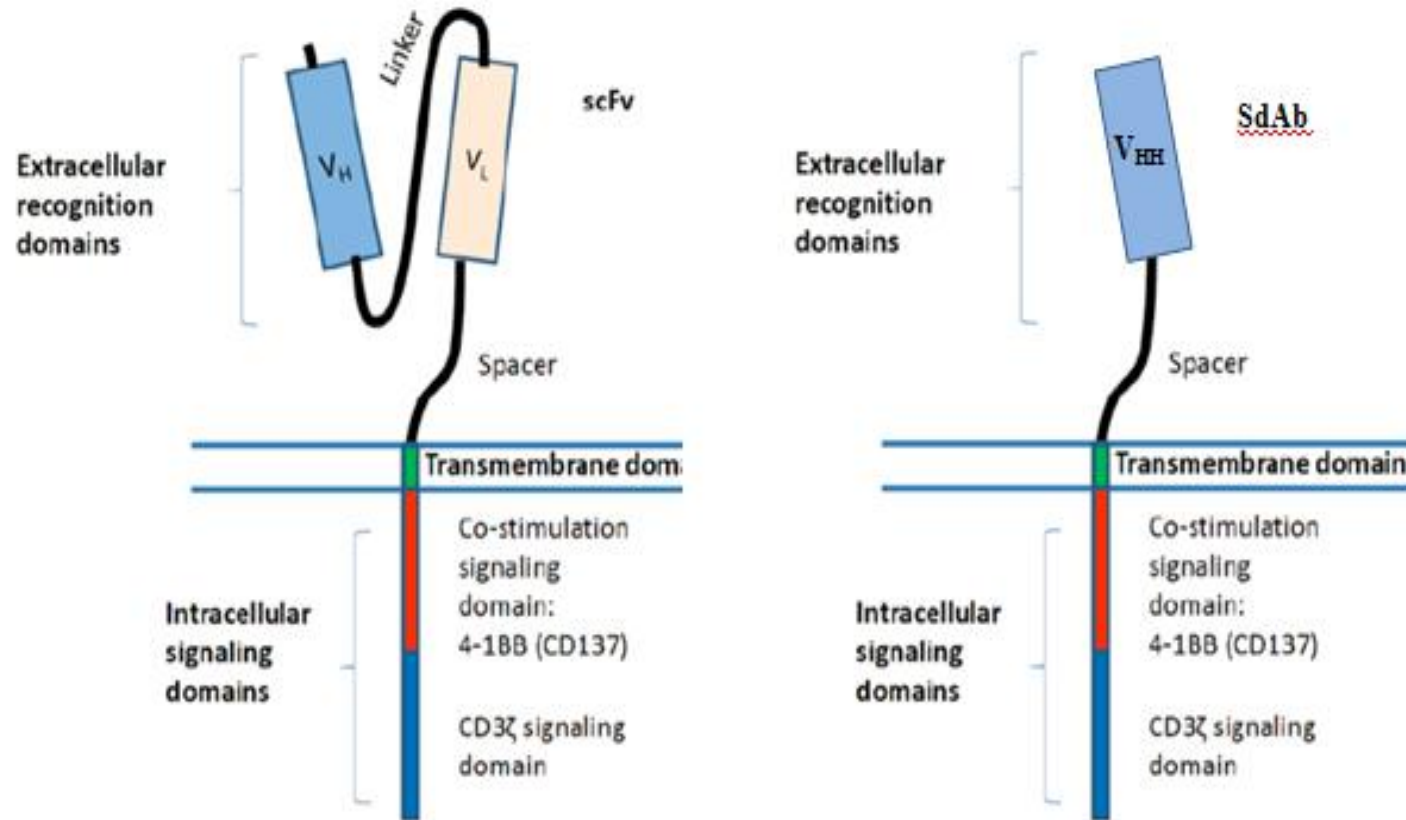
# CART分泌单域抗体



# CART治疗原理



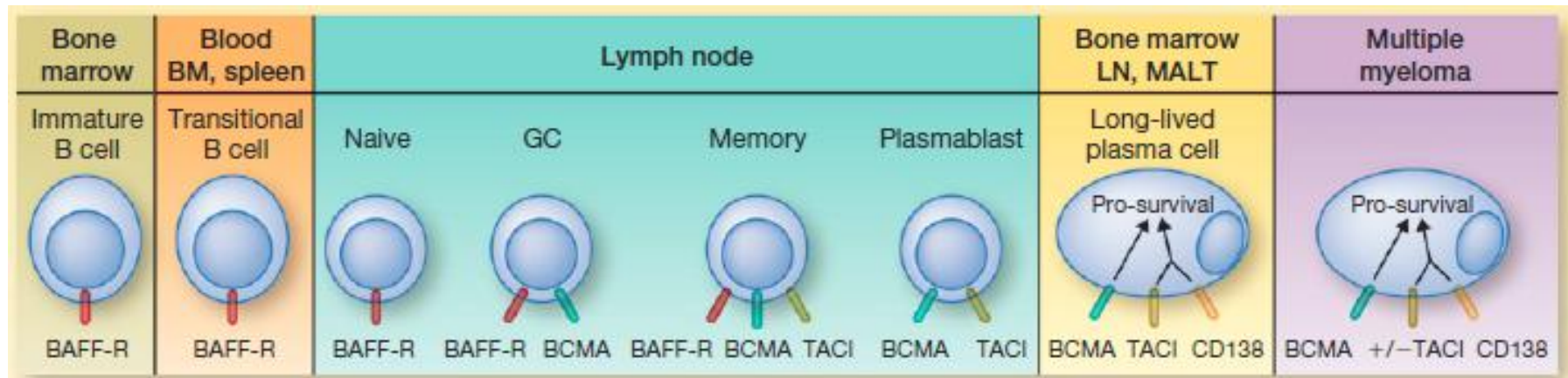
# 纳米抗体在CAR应用上的优势



- ◆ easy screening to get high affinity and specificity of sdAb to be used in CAR molecule.
- ◆ easy access to recognize the membrane proximal domain of tumor antigen.
- ◆ easy to construct the CAR structure to bind multiple tumor antigens using tandem sdAb.

# BCMA是多发性骨髓瘤治疗的较好靶点

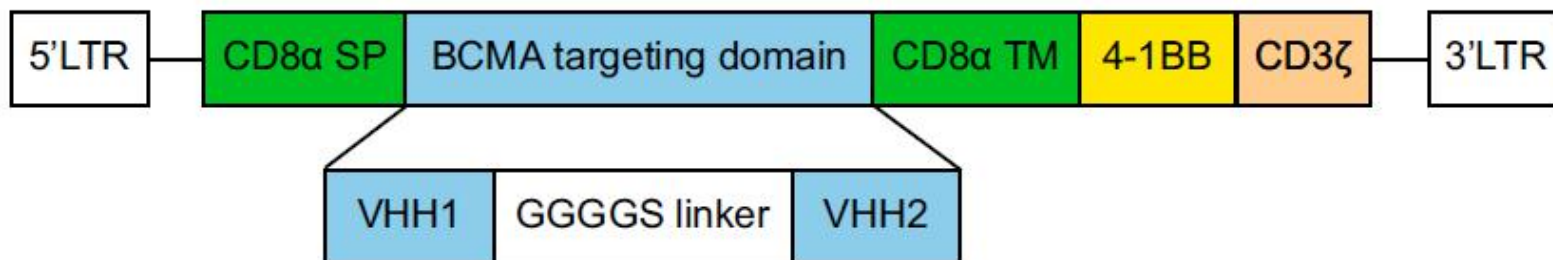
Multiple myeloma (MM) is a malignancy characterized by an accumulation of clonal plasma cells. B-cell maturation antigen (BCMA) is expressed only on mature B cells and plasma cells and promotes their survival. BCMA is a promising target for CAR-T cells in multiple myeloma.



Zoom Zoom: Racing CARs for Multiple Myeloma, Clin Cancer Res; 2013, 19(8); 1917-9.



# BCMA CART 在研产品: LCAR-B38M/JNJ-4528



*Legend Biotech specializes in developing cell therapy products.*

## LCAR-B38M

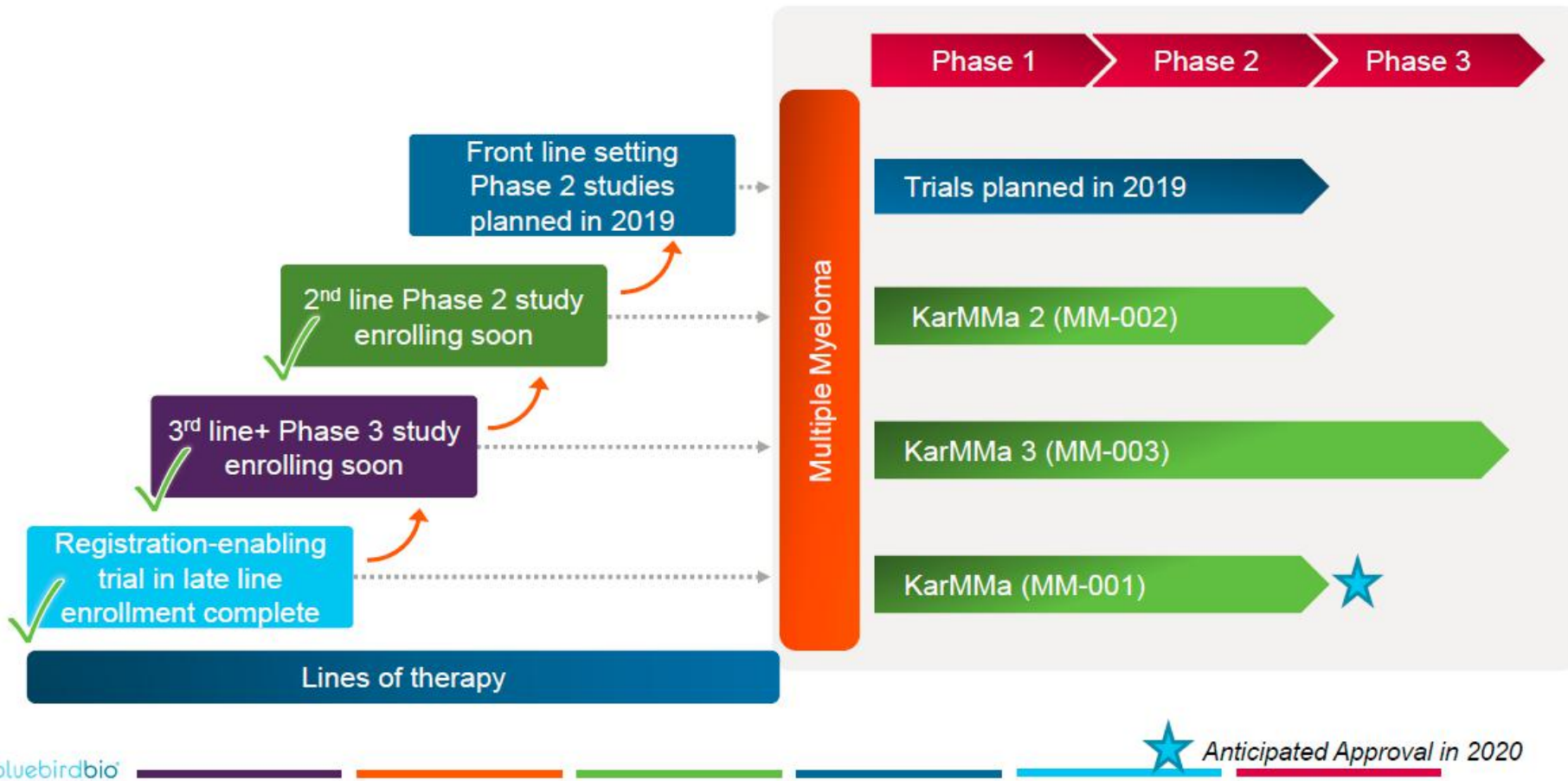
*in collaboration with*



Our first product candidate, LCAR-B38M, is a B cell maturation antigen (BCMA)-directed chimeric antigen receptor T (CAR-T) cell therapy. The novel dual-epitope design of the BCMA targeting domain facilitates tight binding to BCMA, which is highly expressed on primary myeloma cells and plasma cells.<sup>1</sup> Proof of concept has been achieved in an investigator-initiated, first-in-human study conducted in 74 patients with relapsed or refractory multiple myeloma in China. Legend Biotech has entered into a collaboration with Janssen Biotech, Inc., one of the Janssen Pharmaceutical Companies of Johnson & Johnson, to develop and commercialize LCAR-B38M (JNJ-68284528) worldwide. We received clearance from China National Medical Products Administration in March 2018 for a phase II registration study in China, and Janssen received US Food and Drug Administration clearance for an Investigational New Drug Application in May 2018, and has since initiated a phase Ib/II study intended for registration in the US and EU.

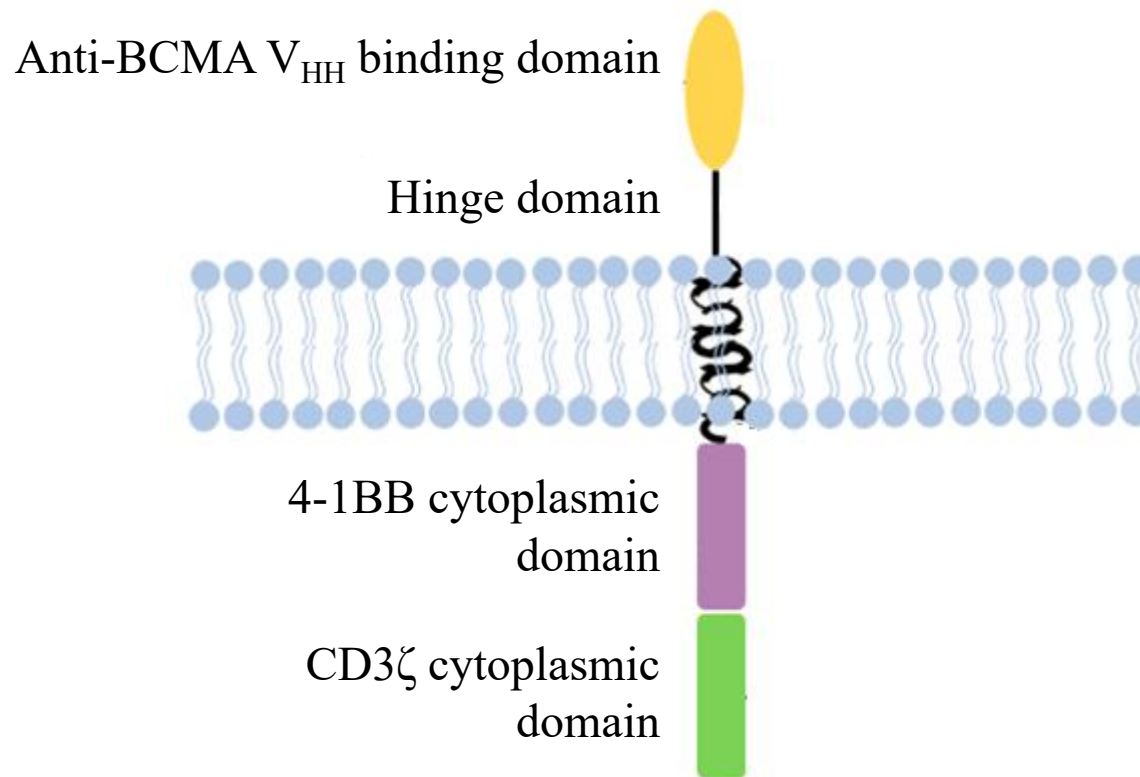
**Initiated the phase 1b/II study in China, US, and EU**

# BCMA CART 在研产品: bb2121



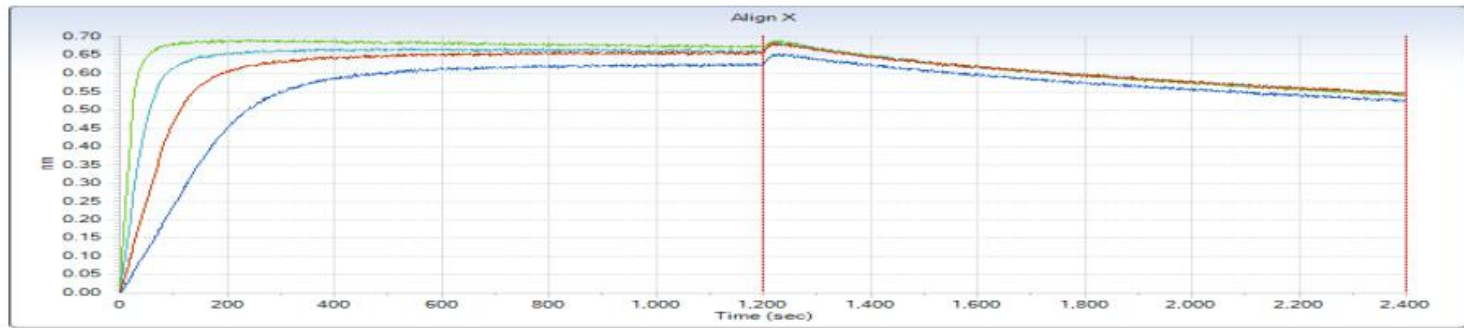
Co-development of bb2121 by Bluebird Bio and Celgene

# BCMA CART (PRG1801)的结构



# 普瑞金抗BCMA单域抗体亲和力测定

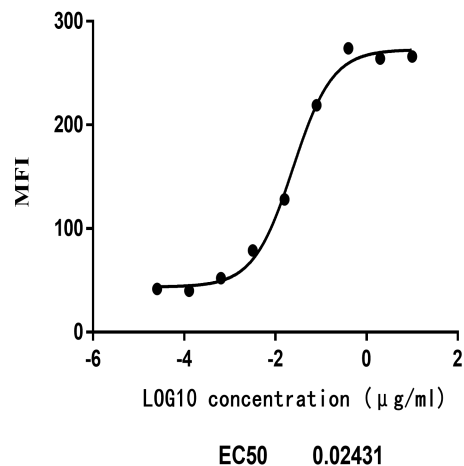
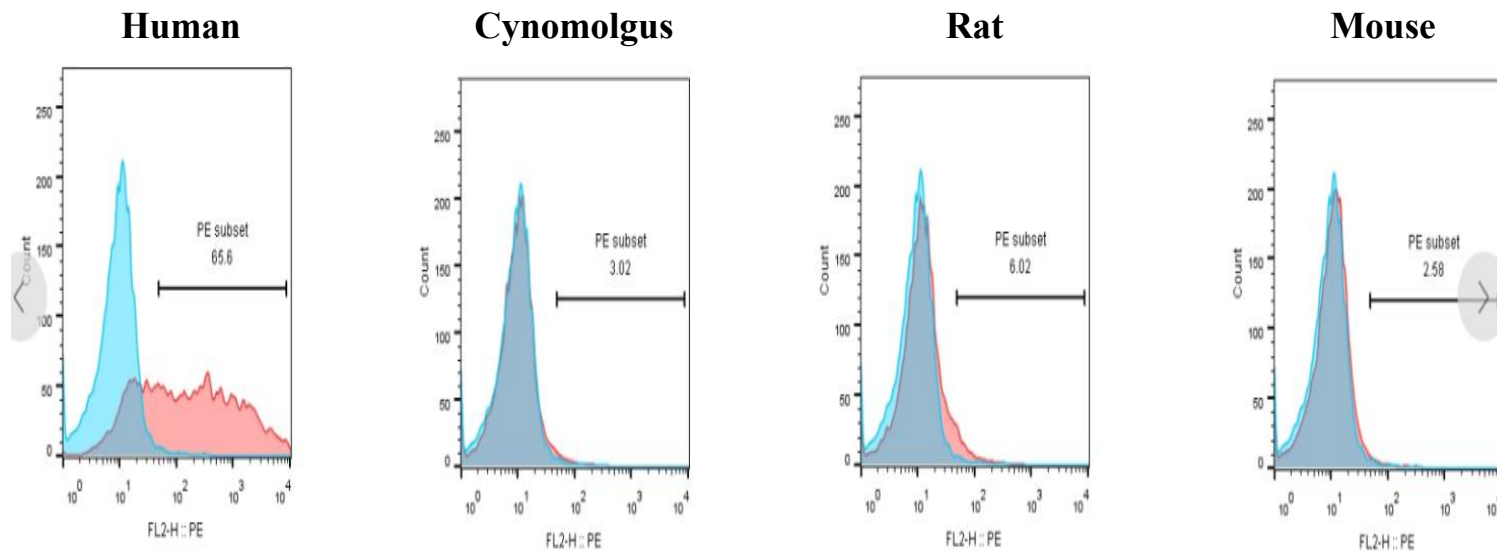
BCMA-Fch/Biotin(10620-H03H-B) vs B77



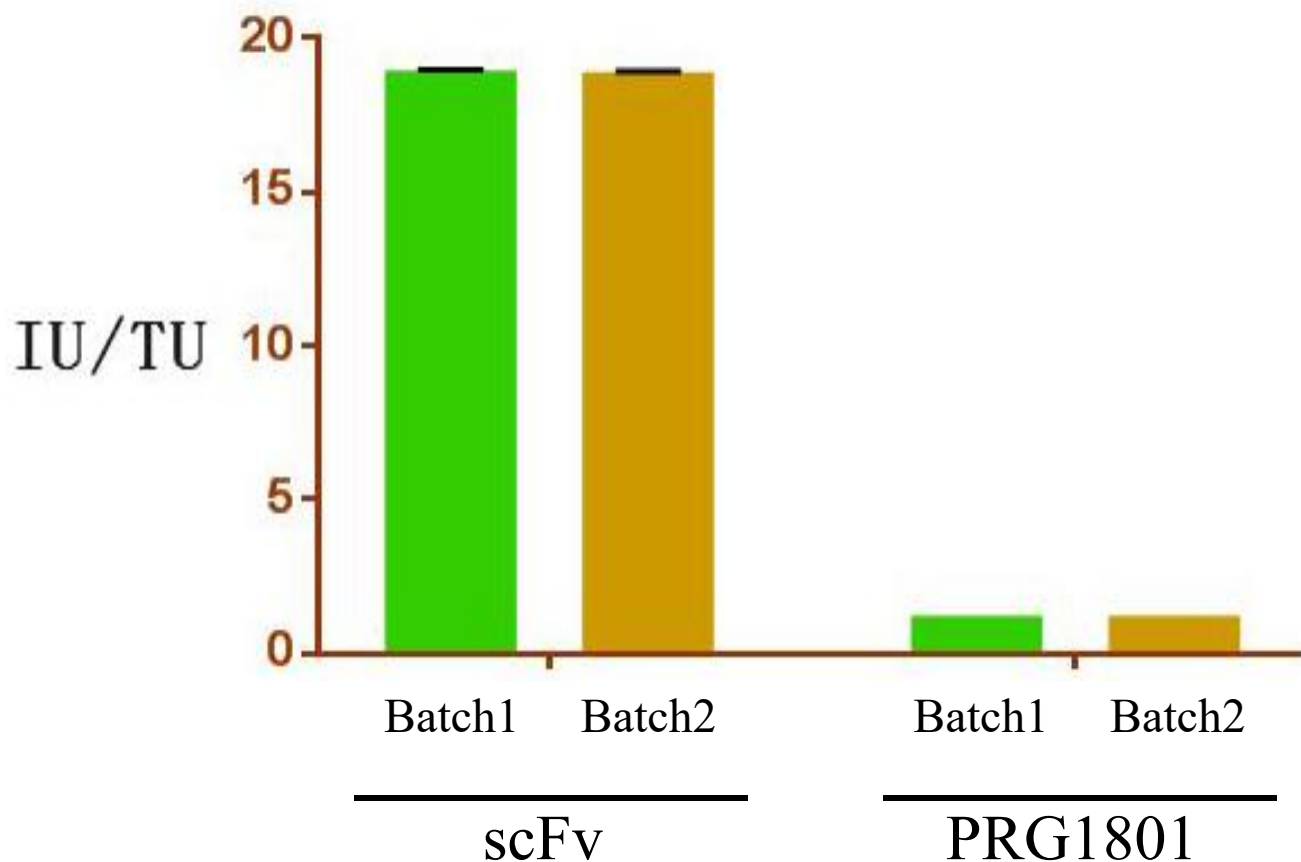
Loading Sample ID	Sample ID	Conc. (nM)	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Rmax	Full R^2
BCMA-Fch-Biotin(10620-H03H)-B	B77	36.8	0.623	1.14E-09	1.56E+05	1.77E-04	0.656	0.985
		73.5	0.655	1.14E-09	1.56E+05	1.77E-04	0.671	0.985
		147.1	0.659	1.14E-09	1.56E+05	1.77E-04	0.670	0.985
		294.1	0.673	1.14E-09	1.56E+05	1.77E-04	0.678	0.985

The affinity is 1.14 nM.

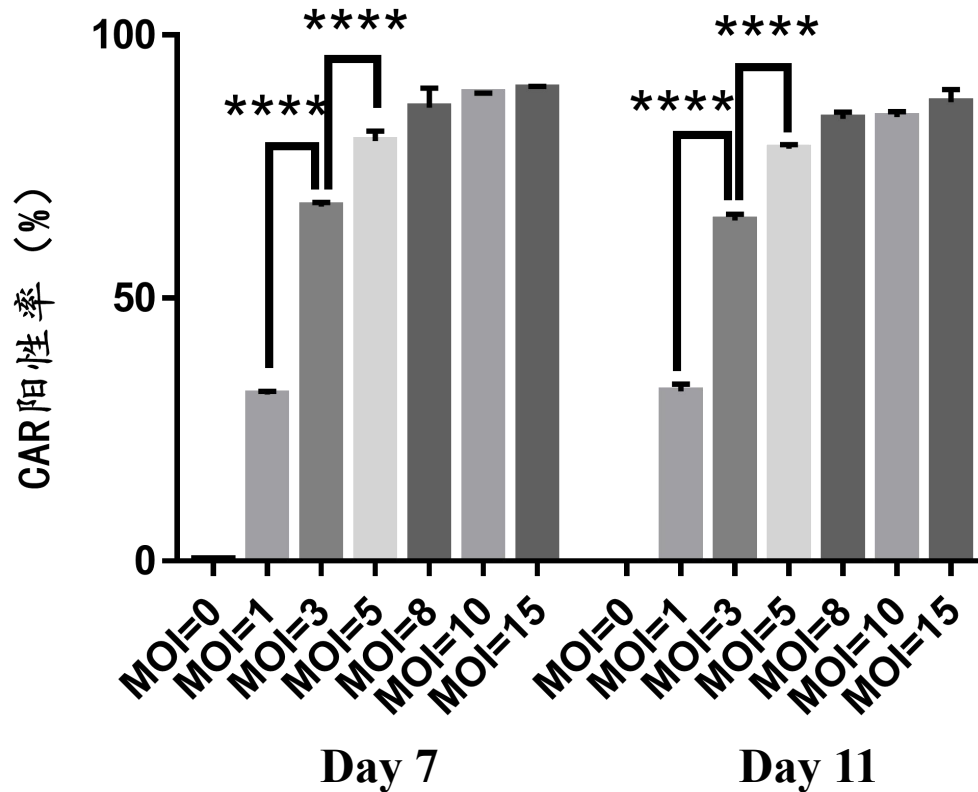
# 膜表面CAR分子与不同种属BCMA亲和力



# 单域抗体慢病毒具有较高的转导滴度



# 单域抗体CART制备时使用较少慢病毒

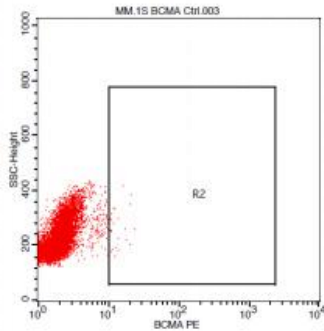


Day 7, MOI=1, 3, 5, CAR positive rate is  $31.63 \pm 0.48\%$ ,  $67.39 \pm 0.60\%$ ,  $79.89 \pm 1.52\%$  individually.

Day 11, MOI=1, 3, 5, CAR positive rate is  $32.29 \pm 1.08\%$ ,  $64.76 \pm 0.94\%$ ,  $78.39 \pm 0.65\%$  individually.

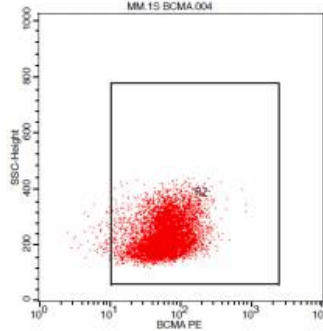
\*\*\*\* indicate P value  $< 0.0001$ , the difference is statistically significant.

# BCMA单域抗体CART有效杀死肿瘤细胞



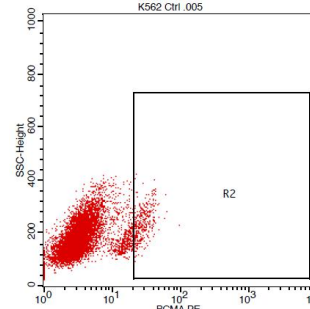
Gate Statistics  
File: MM.1S BCMA Ctrl.003

Gate	% Gated	% Total
G1	100.00	80.17
G2	0.28	0.22



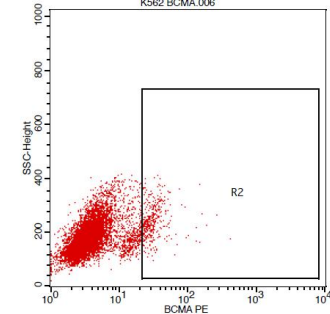
Gate Statistics  
File: MM.1S BCMA.004

Gate	% Gated	% Total
G1	100.00	79.45
G2	99.18	78.80



Gate Statistics  
File: K562 Ctrl.005

Gate	% Gated	% Total
G1	100.00	88.41
G2	3.88	3.43

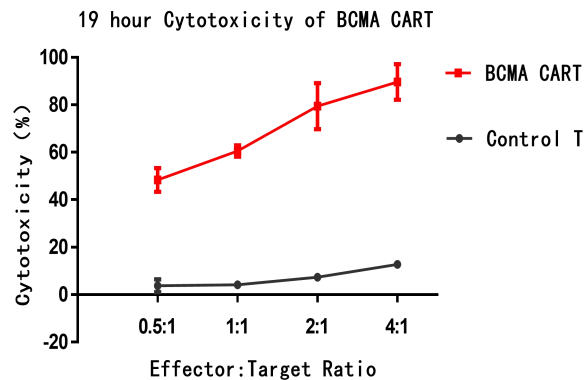


Gate Statistics  
File: K562 BCMA.006

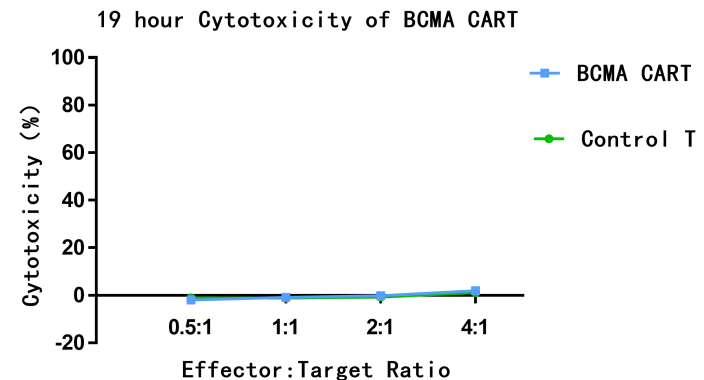
Gate	% Gated	% Total
G1	100.00	88.16
G2	4.66	4.11

MM.1S

K562



MM.1S

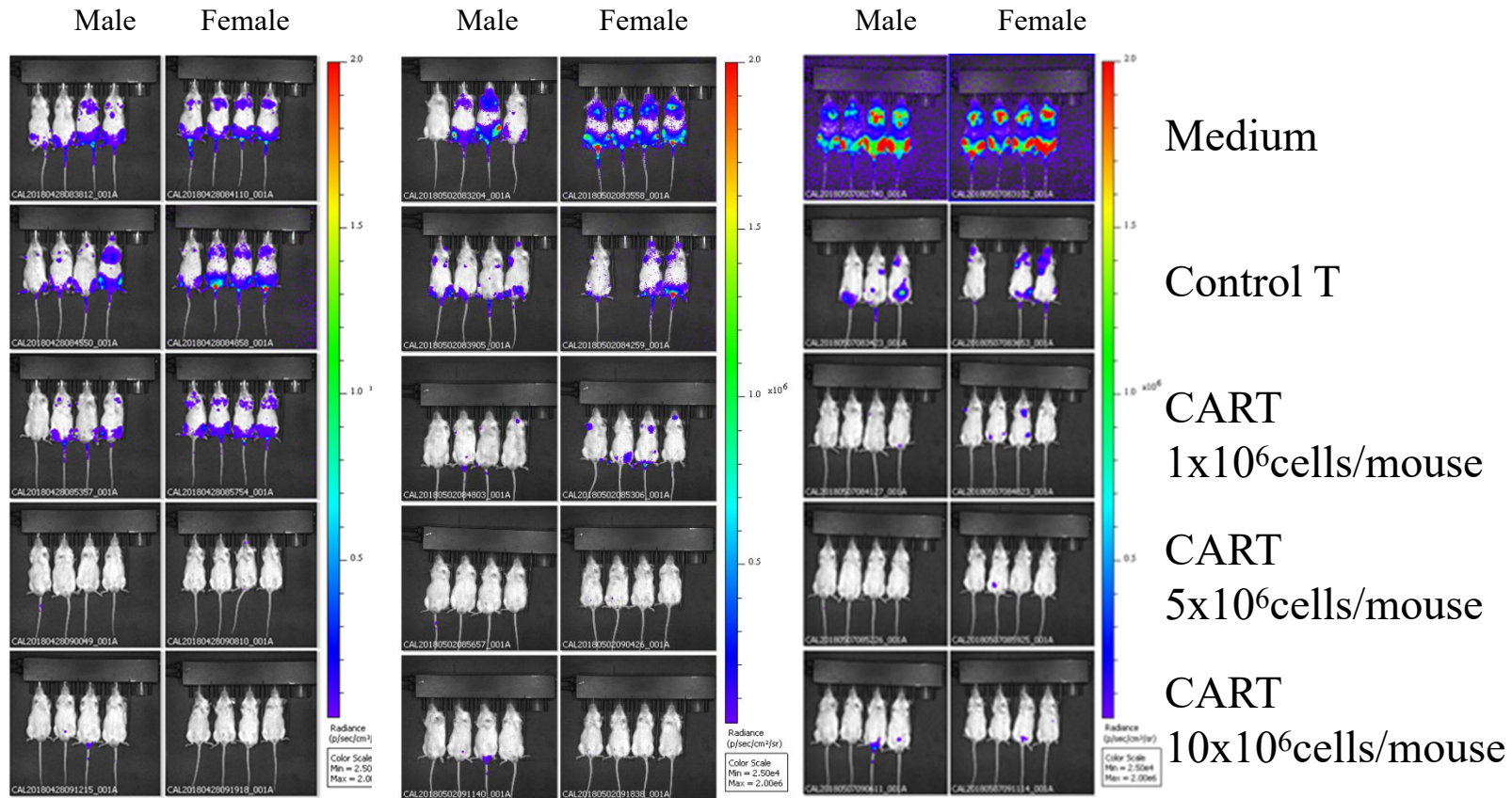


K562



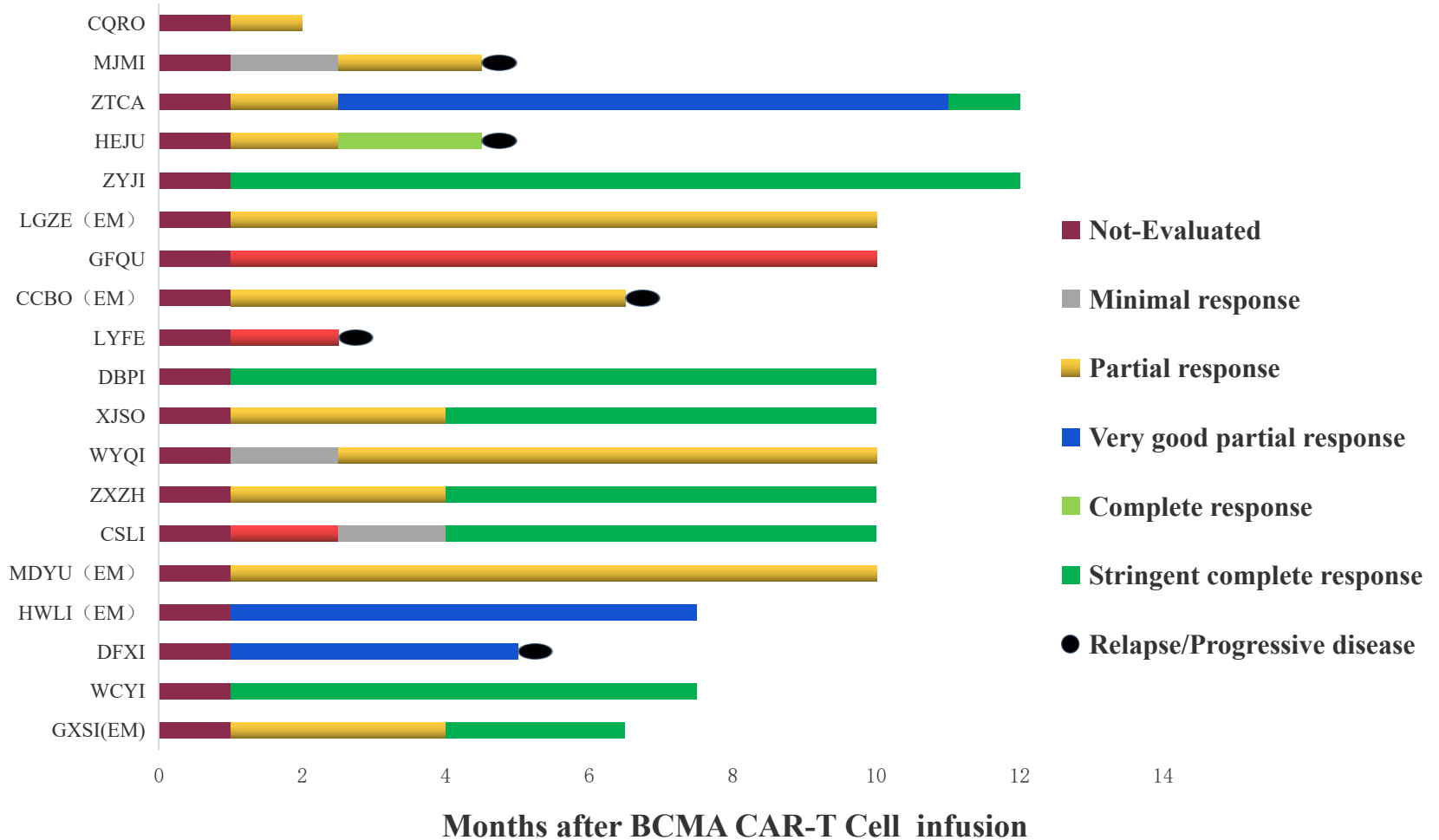
# BCMA单域抗体CART动物体内有效清除肿瘤

Postinoculation (days)	21	25	30
Postinfusion (days)	3	7	12

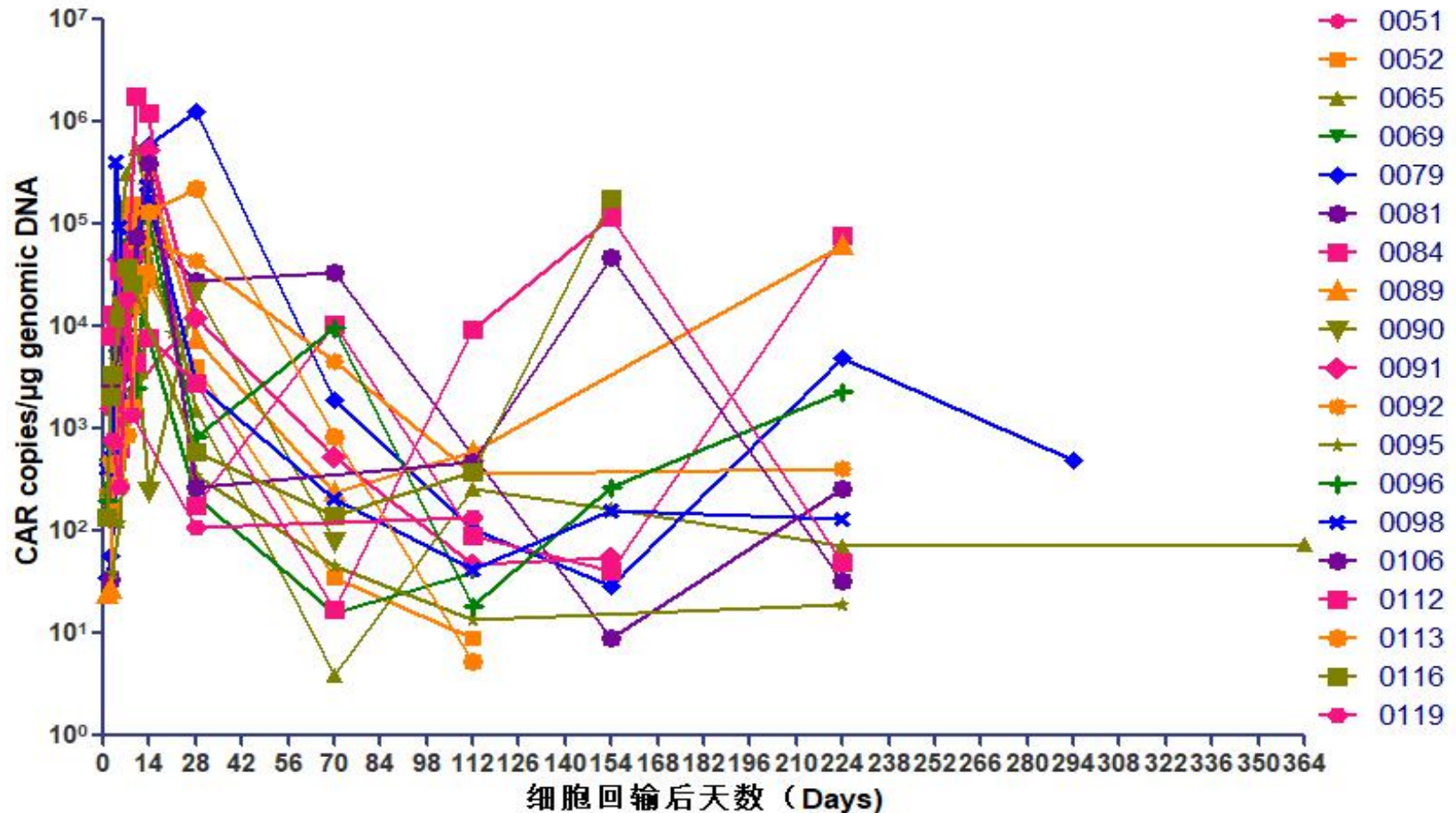


Tumor cells: MM.1S-Luc, inoculation number: 1.5x10<sup>6</sup>/mouse

# 临床疗效总结



# CART细胞存续时间长



# BCMA CART临床疗效对比

候选品种	公司	CAR抗原识别结构	临床效果
bb2121	BMS/ Bluebird	scFv	N=128, ORR 73.4%, CR 31.3%
JNJ-4528	Legend Biotech/J&J	2个单域抗体	N=21, ORR 91%, CR 28.6%
PRG1801	Pregene	1个单域抗体	N=19, ORR 89.5%, CR 47%

# 总结

- ◆ 单域抗体作为一种新的抗体发现平台，在抗体药物研发和细胞治疗药物研发中具有独特优势。
- ◆ 使用单域抗体制备CAR分子，单域抗体尺寸较小，包装病毒出毒多、感染T细胞效率高。
- ◆ JNJ-4528和PRG1801的显著疗效表明，单域抗体在CART研发中具有巨大价值。

## 致谢!

1. 参加临床研究的患者.
2. 河南省肿瘤医院：宋永平院长、高全力主任、韩露、周可树主任、周健主任、尹青松主任、房佰俊主任、朱兴虎主任、周虎主任.



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