Antibody Discovery for New CAR Constructs

Target Discovery and Immunogenicity Session
Cellicon Valley '21

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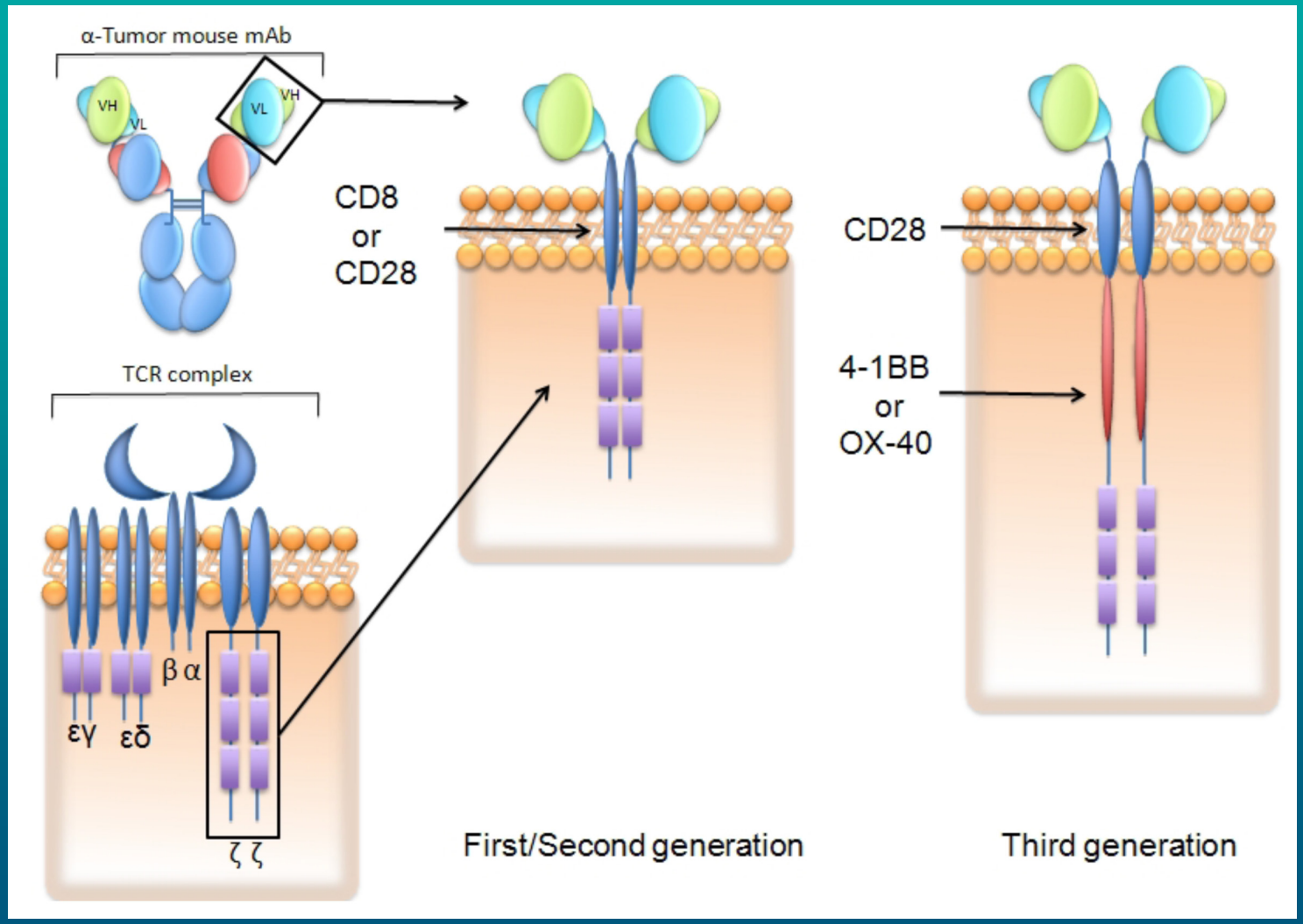
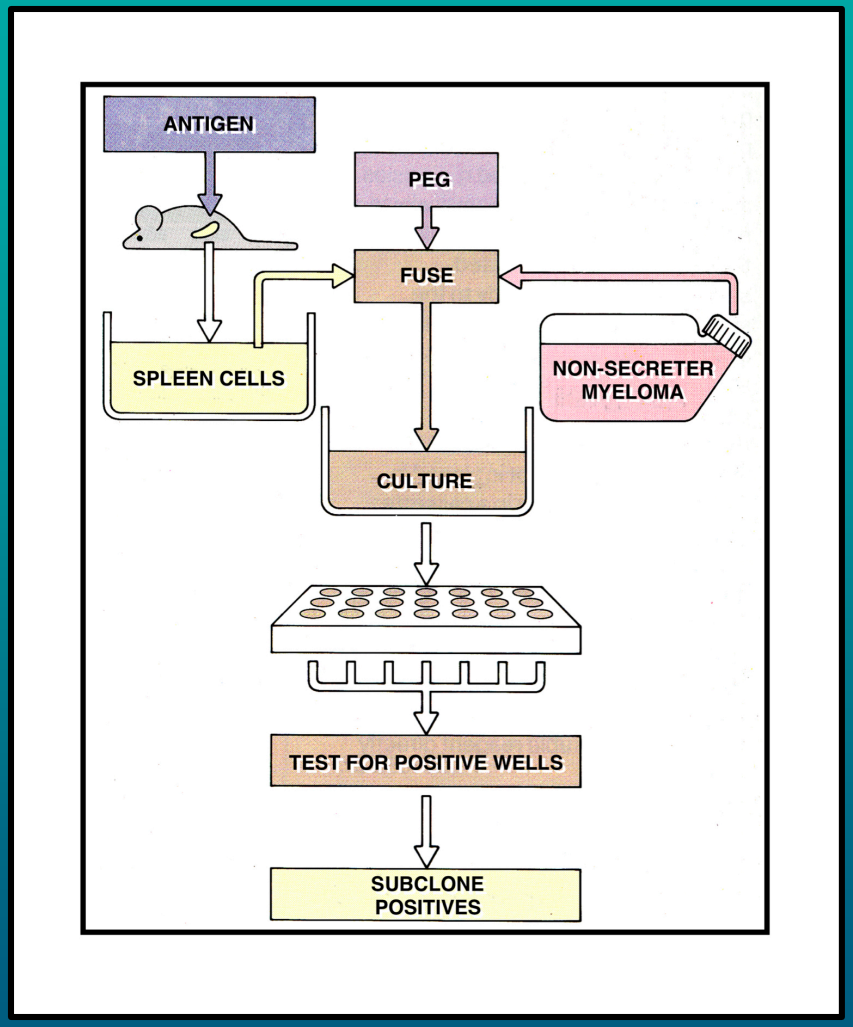
Target Recognition Domains of CAR-T Cells



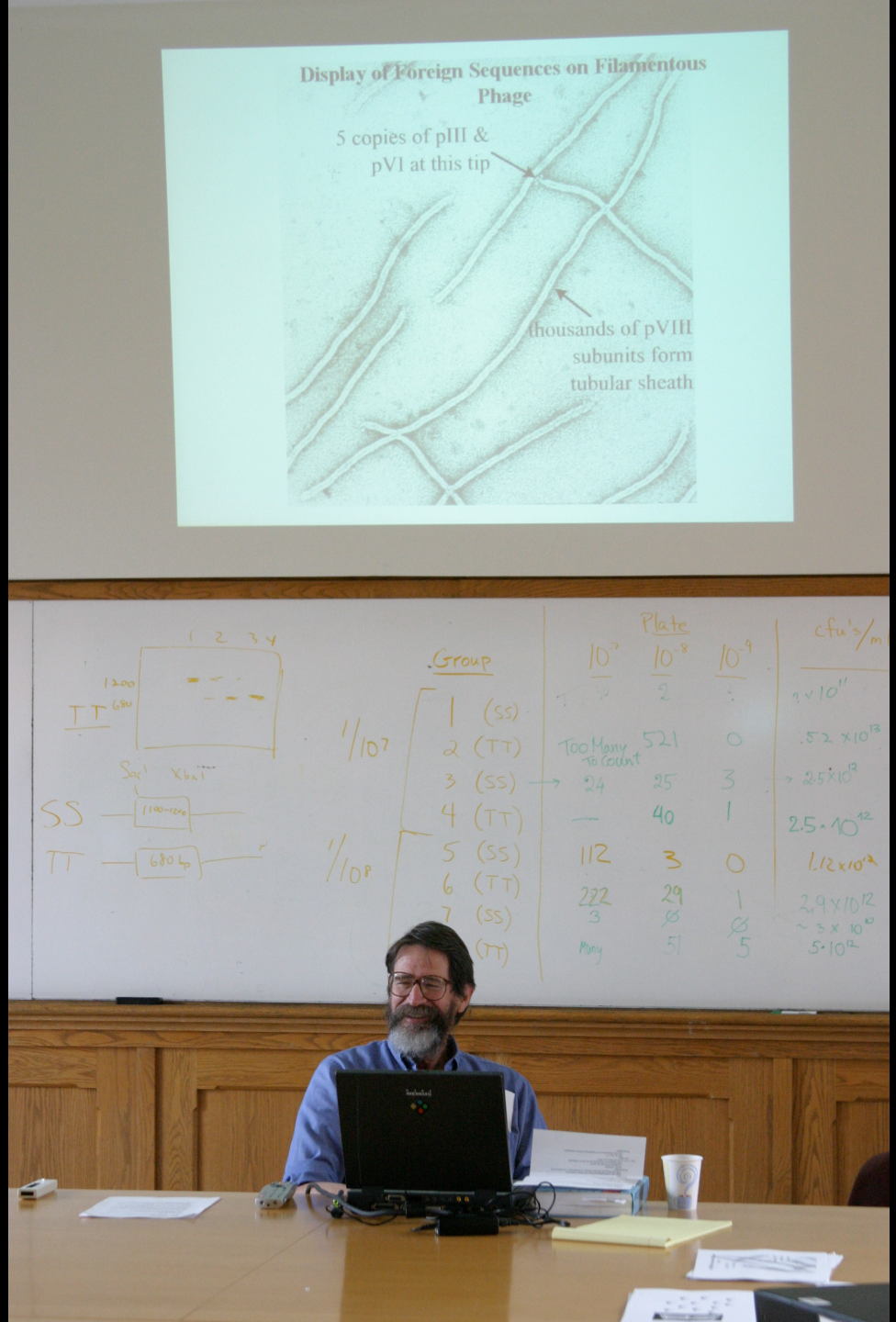
Conventional Hybridoma Technology



Potential Limitations

- Requires animal immunization/ sacrifice
- Limited by natural animal immune response
- Labor intensive and expensive
- Long turnaround time (~4-6 months)
- Low yield quantitatively and qualitatively
- Antibodies are not human

Phage display technology


Display of Foreign Sequences on Filamentous Phage
5 copies of pIII & pVI at this tip
thousands of pVIII subunits form tubular sheath
Group
Plate
cfu's/ml
1/10^7
1/10^8
SS
TT
Too Many to Count
Many

Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Home Meetings Courses

Welcome | Travel & Location | Application | Sponsors & Stipends | Information | Payments | Policies

Antibody Engineering, Phage Display & Immune Repertoire Analysis

2018 Nobel Prize in Chemistry for Phage Display

DNA for Polypeptide

Expressed Polypeptide

FILAMENTOUS BACTERIOPHAGE (M13)

PHAGE-DISPLAYED POLYPEPTIDE

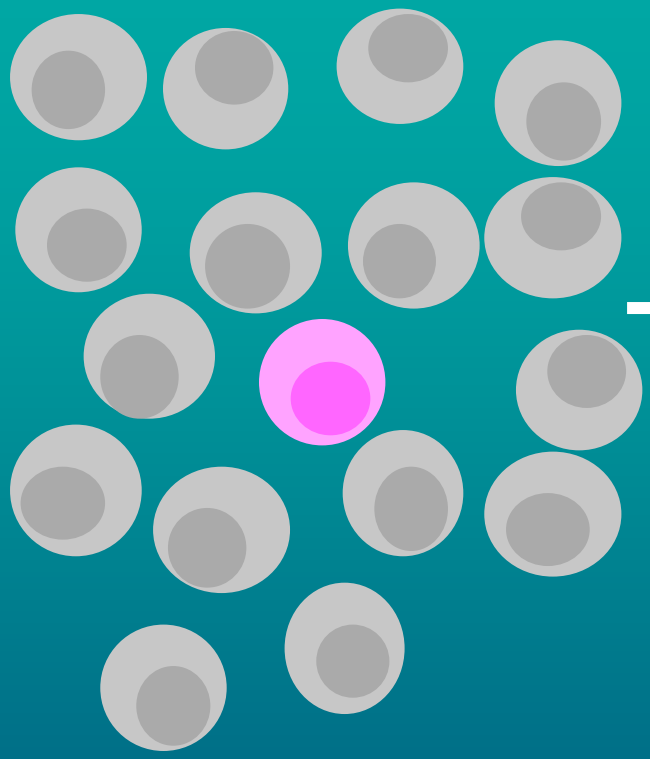
Science. 1985 Jun 14;228(4705):1315-7.

Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface.

Smith GP.

Abstract

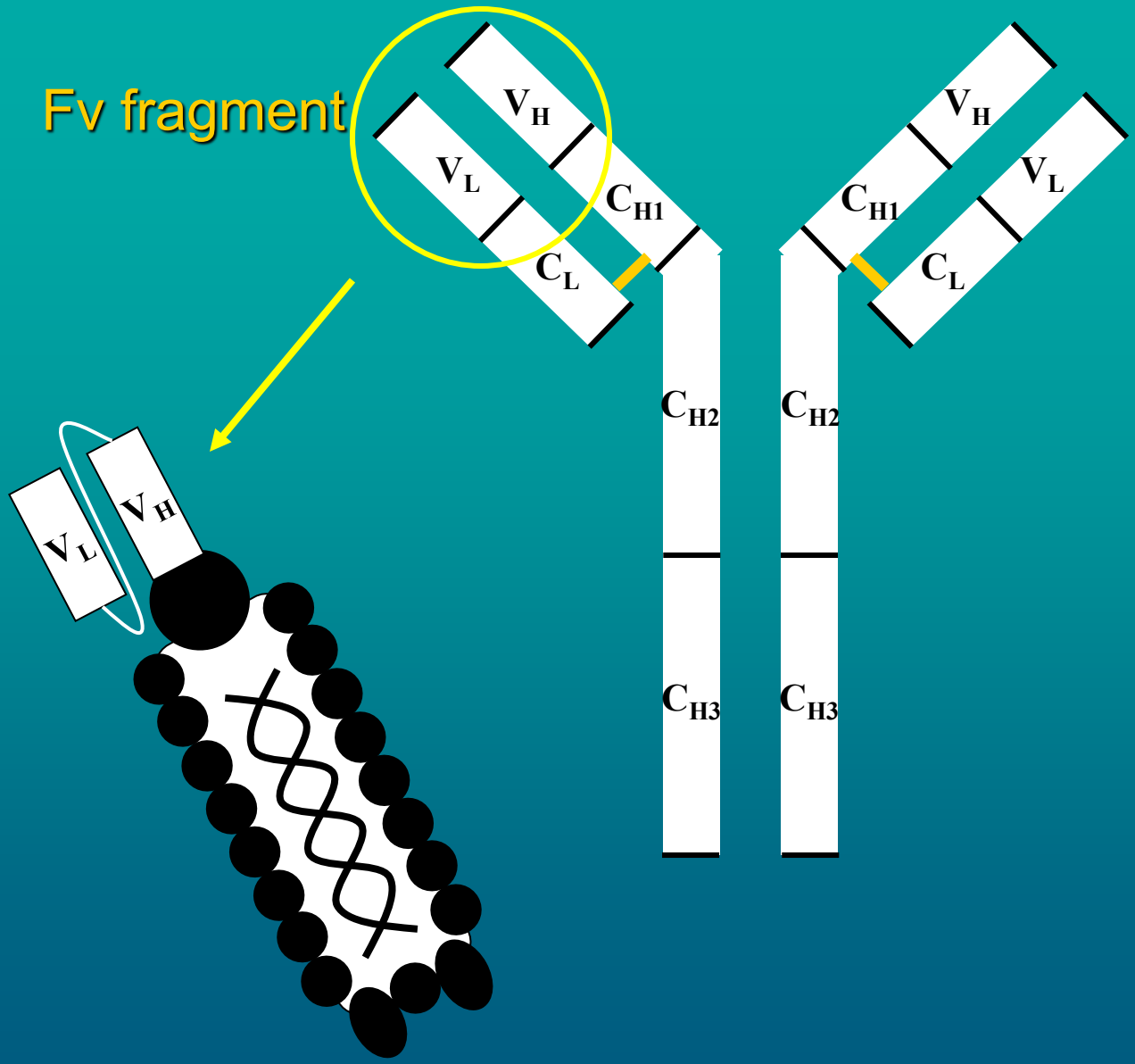
Foreign DNA fragments can be inserted into filamentous phage gene III to create a fusion protein with the foreign sequence in the middle. The fusion protein is incorporated into the virion, which retains infectivity and displays the foreign amino acids in immunologically accessible form. These "fusion phage" can be enriched more than 1000-fold over ordinary phage by affinity for antibody directed against the foreign sequence. Fusion phage may provide a simple way of cloning a gene when an antibody against the product of that gene is available.



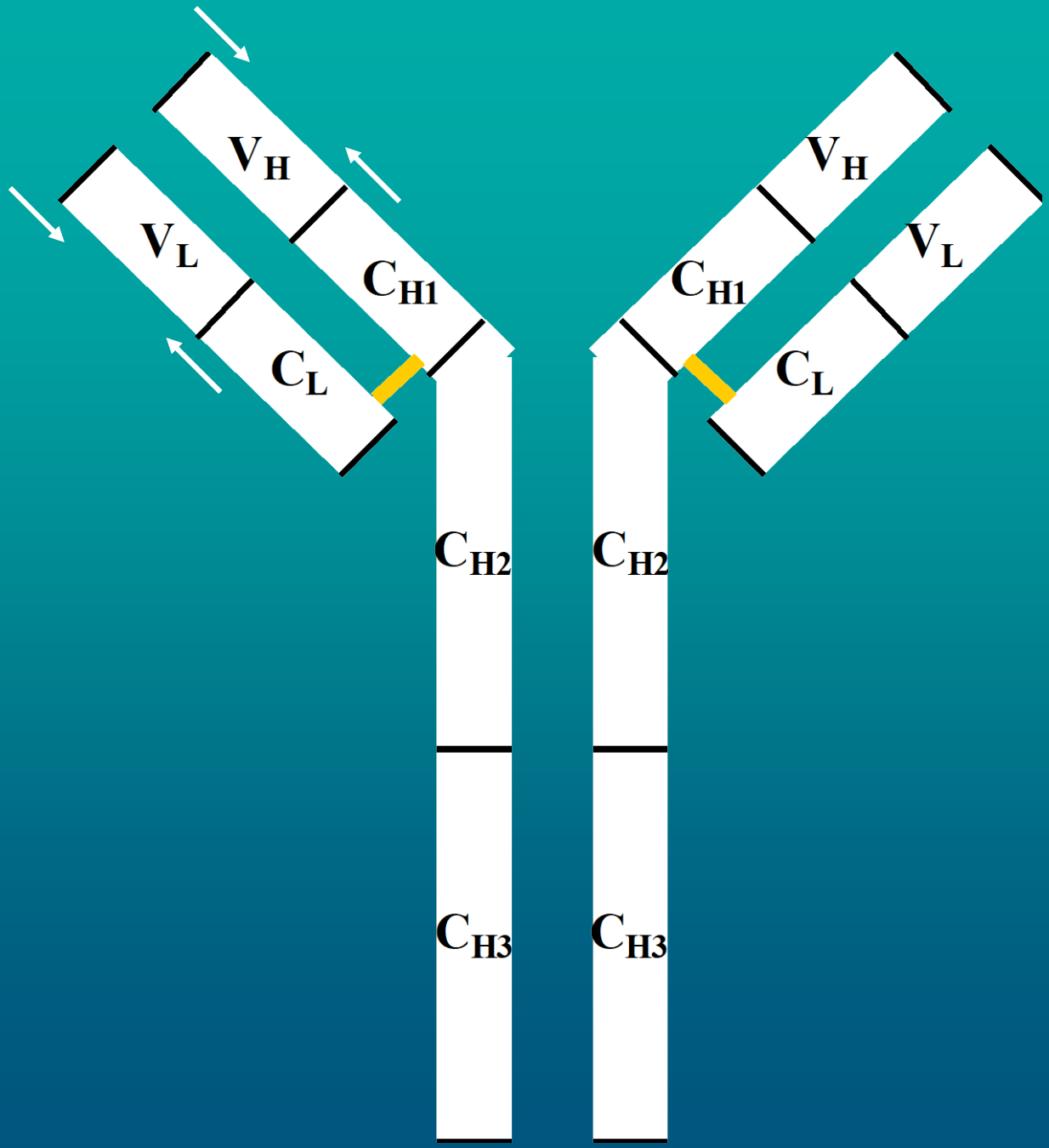
Clone Ig cDNA;
Express Antibody
on Phage Surface

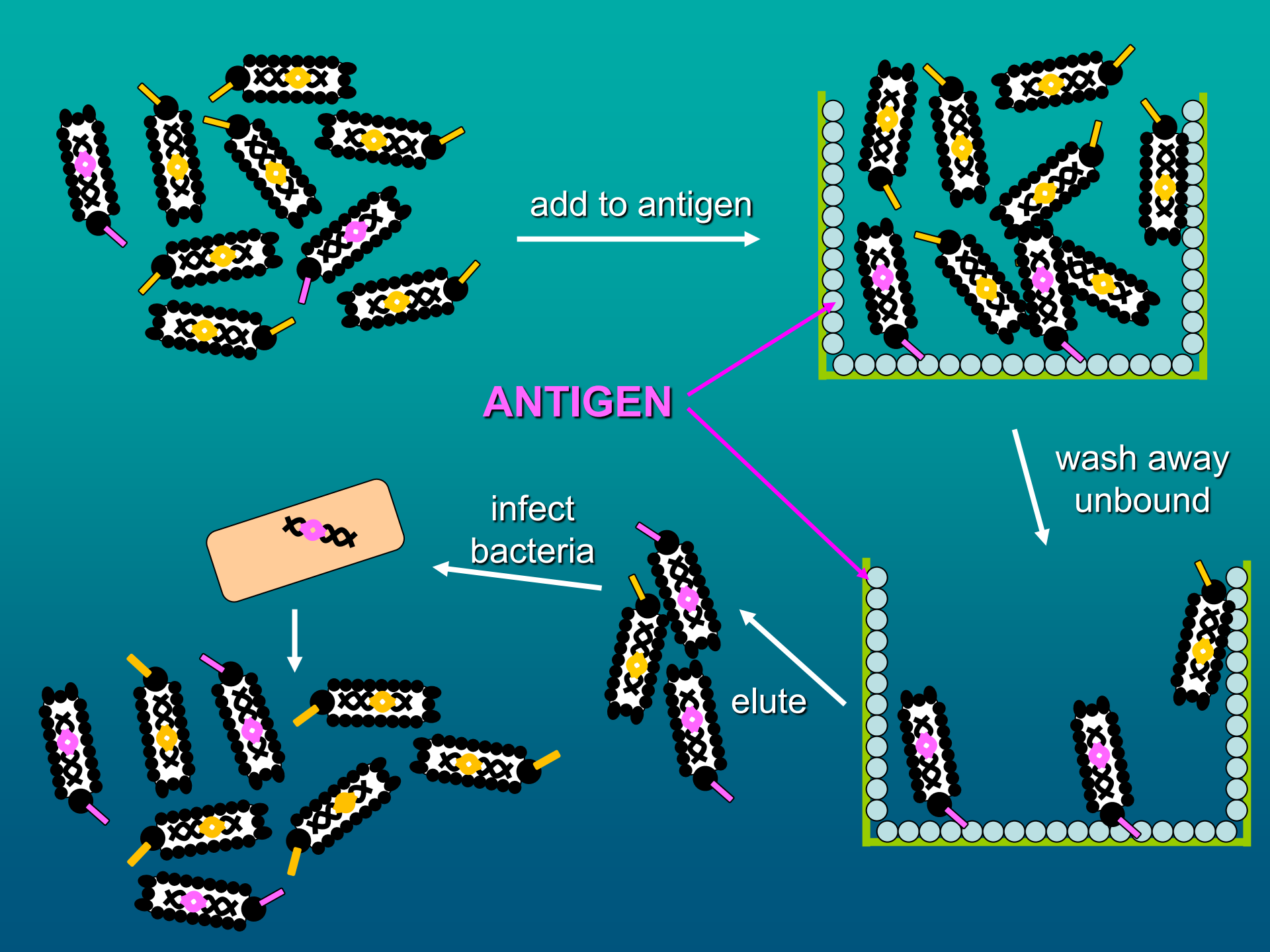
B Cells

Phage Display
Library

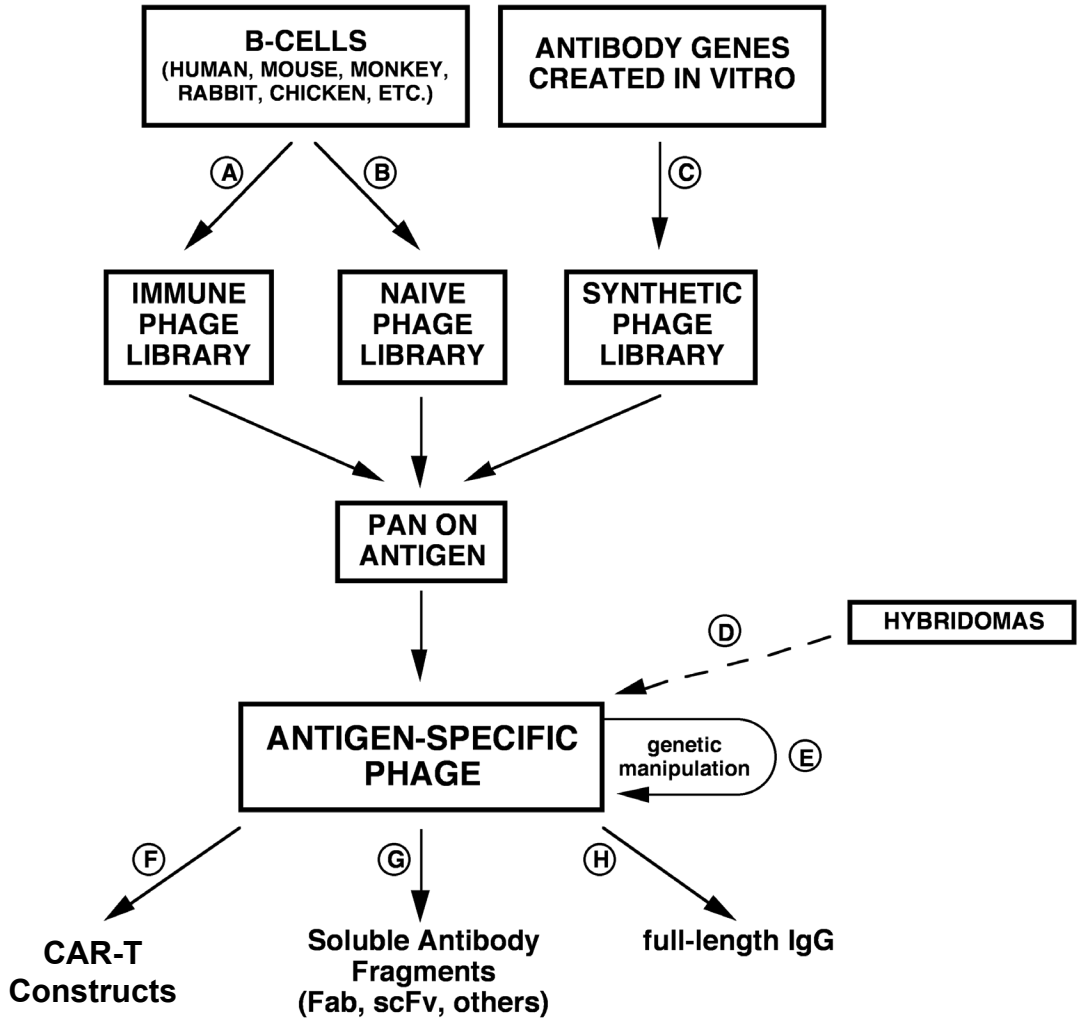


scFv = single polypeptide chain


V_H
V_L
C_{H1}
C_L
V_H
V_L
C_{H1}
C_L
C_{H2}
C_{H2}
C_{H3}
C_{H3}


add to antigen
ANTIGEN
wash away unbound
infect bacteria
elute

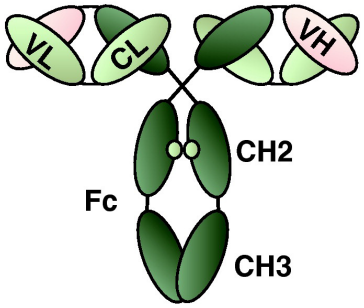
Phage Display Platform



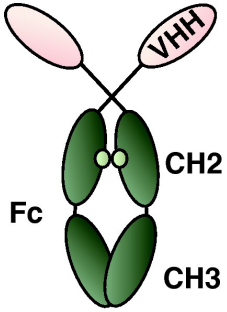
Advantages of Phage Display Methods

- does not rely on immortalization of lymphocytes
- streamlined screening and rapid production
- novel panning strategies can help select antibodies based on functional properties or desired affinity
- easily adapted to produce mAbs from virtually any species (human, rabbit, chicken, monkey, camelids, mouse, shark, cow)

Advantages of Phage Display Methods

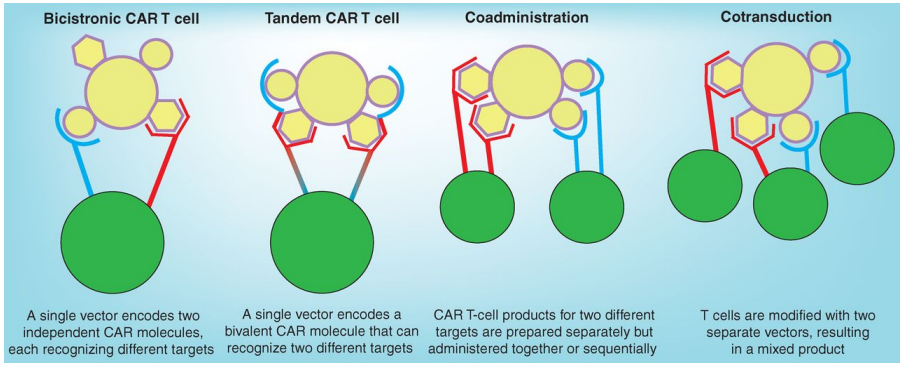


Conventional Camelid Abs



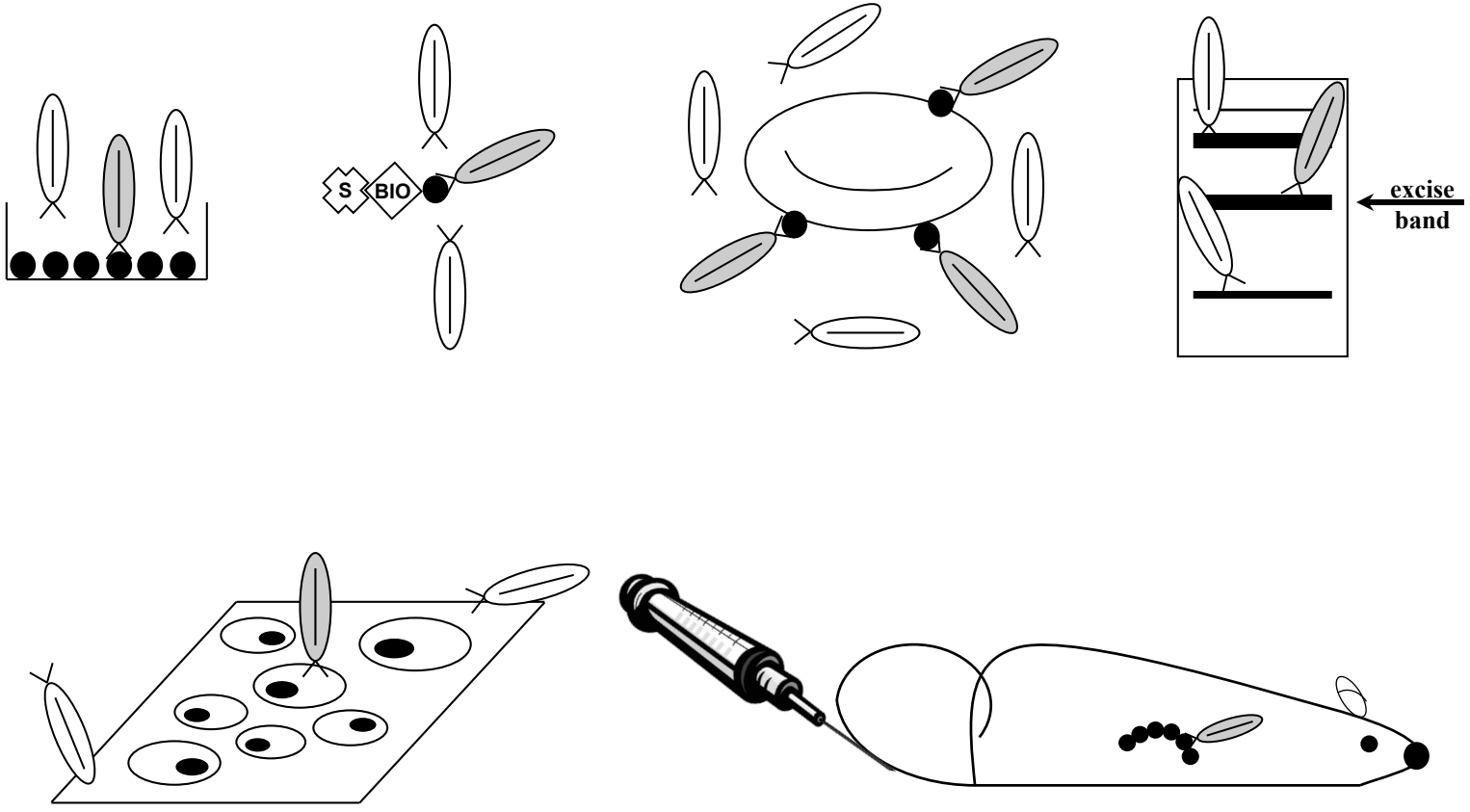
Heavy Chain Only Camelid Abs

- **Single chain simplifies library construction**
- **VHH shape and long CDR3 regions bind conformational epitopes, cavities, clefts**
- **Heavy chain only simplifies design of multi-specific CAR-Ts**



Majzner *Cancer Disc* 8:1219, 2018

Methods of Panning Phage Display Libraries

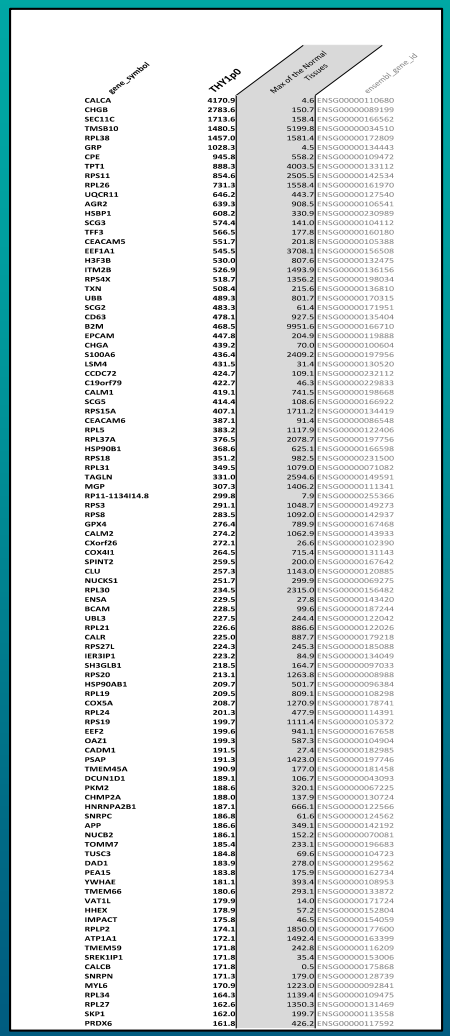


Case Study: CAR-T therapy for medullary thyroid cancer

- MTC is ~5% of all thyroid cancers (~3500 new cases/yr)
- Origin is parafollicular cells calcitonin-producing cells ("C-cells")
- ~50% have metastatic dz at diagnosis and eventually succumb to dz
- Prognosis associated with post-op calcitonin doubling time in blood
  - <25% alive in 5 years with CDT <6 months
- TKIs that inhibit RET can have activity for dz but median duration of response of ~15 months

Identify a target (patient-specific) (MTC-specific) (C cell-specific) → **Make an antibody** → **Make a CAR-T**

Bhoj et al. *Molec Ther: Oncolytics* 20:387, 2021



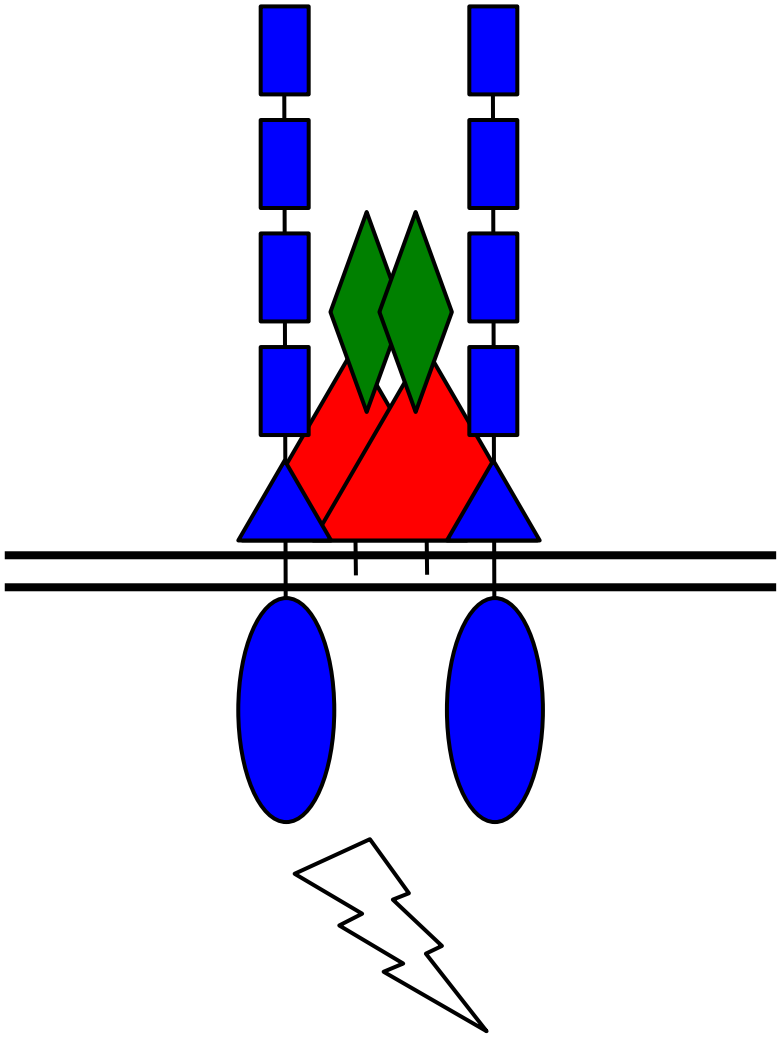
gene_symbol	THY1p0	Max of the Normal Tissues	ensembl_gene_id
CALCA	4170.9	4.6	ENSG00000110680
CHGB	2783.6	150.7	ENSG00000089199
SEC11C	1713.6	158.4	ENSG00000166562
TMSB10	1480.5	5199.8	ENSG00000034510
RPL38	1457.0	1581.4	ENSG00000172809
GRP	1028.3	4.5	ENSG00000134443
CPE	945.8	558.2	ENSG00000109472
TPT1	888.3	4003.5	ENSG00000133112
RPS11	854.6	2505.5	ENSG00000142534
RPL26	731.3	1558.4	ENSG00000161970
UQCR11	646.2	443.7	ENSG00000127540
AGR2	639.3	908.5	ENSG00000106541
HSBP1	608.2	330.9	ENSG00000230989
SCG3	574.4	141.0	ENSG00000104112
TFF3	566.5	177.8	ENSG00000160180
CEACAM5	551.7	201.8	ENSG00000105388
EEF1A1	545.5	3708.1	ENSG00000156508
H3F3B	530.0	807.6	ENSG00000132475
ITM2B	526.9	1493.9	ENSG00000136156
RPS4X	518.7	1356.2	ENSG00000198034
TXN	508.4	215.6	ENSG00000136810
UBB	489.3	801.7	ENSG00000170315
SCG2	483.3	61.4	ENSG00000171951
CD63	478.1	927.5	ENSG00000135404
B2M	468.5	9951.6	ENSG00000166710
EPCAM	447.8	204.9	ENSG00000119888
CHGA	439.2	70.0	ENSG00000100604
S100A6	436.4	2409.2	ENSG00000197956
LSM4	431.5	31.4	ENSG00000130520
CCDC72	424.7	109.1	ENSG00000232112
C19orf79	422.7	46.3	ENSG00000229833
CALM1	419.1	741.5	ENSG00000198668
SCG5	414.4	108.6	ENSG00000166922
RPS15A	407.1	1711.2	ENSG00000134419
CEACAM6	387.1	91.4	ENSG00000086548
RPL5	383.2	1117.9	ENSG00000122406
RPL37A	376.5	2078.7	ENSG00000197756
HSP90B1	368.6	625.1	ENSG00000166598
RPS18	351.2	982.5	ENSG00000231500
RPL31	349.5	1079.0	ENSG00000071082
TAGLN	331.0	2594.6	ENSG00000149591
MGP	307.3	1406.2	ENSG00000111341
RP11-1134I14.8	299.8	7.9	ENSG00000255366
RPS3	291.1	1048.7	ENSG00000149273
RPS8	283.5	1092.0	ENSG00000142937
GPX4	276.4	789.9	ENSG00000167468
CALM2	274.2	1062.9	ENSG00000143933
CXorf26	272.1	26.6	ENSG00000102390
COX4I1	264.5	715.4	ENSG00000131143
SPINT2	259.5	200.0	ENSG00000167642
CLU	257.3	1143.0	ENSG00000120885
NUCKS1	251.7	299.9	ENSG00000069275
RPL30	234.5	2315.0	ENSG00000156482
ENSA	229.5	27.8	ENSG00000143420
BCAM	228.5	99.6	ENSG00000187244
UBL3	227.5	244.4	ENSG00000122042
RPL21	226.6	886.6	ENSG00000122026
CALR	225.0	887.7	ENSG00000179218
RPS27L	224.3	245.3	ENSG00000185088
IER3IP1	223.2	84.9	ENSG00000134049
SH3GLB1	218.5	164.7	ENSG00000097033
RPS20	213.1	1263.8	ENSG00000008988
HSP90AB1	209.7	501.7	ENSG00000096384
RPL19	209.5	809.1	ENSG00000108298
COX5A	208.7	1270.9	ENSG00000178741
RPL24	201.3	477.9	ENSG00000114391
RPS19	199.7	1111.4	ENSG00000105372
EEF2	199.6	941.1	ENSG00000167658
OAZ1	199.3	587.3	ENSG00000104904
CADM1	191.5	27.4	ENSG00000182985
PSAP	191.3	1423.0	ENSG00000197746
TMEM45A	190.9	177.0	ENSG00000181458
DCUN1D1	189.1	106.7	ENSG00000043093
PKM2	188.6	320.1	ENSG00000067225
CHMP2A	188.0	137.9	ENSG00000130724
HNRNPA2B1	187.1	666.1	ENSG00000122566
SNRPC	186.8	61.6	ENSG00000124562
APP	186.6	349.1	ENSG00000142192
NUCB2	186.1	152.2	ENSG00000070081
TOMM7	185.4	233.1	ENSG00000196683
TUSC3	184.8	69.6	ENSG00000104723
DAD1	183.9	278.0	ENSG00000129562
PEA15	183.8	175.9	ENSG00000162734
YWHAE	181.1	393.4	ENSG00000108953
TMEM66	180.6	293.1	ENSG00000133872
VAT1L	179.9	14.0	ENSG00000171724
HHEX	178.9	57.2	ENSG00000152804
IMPACT	175.8	46.5	ENSG00000154059
RPLP2	174.1	1850.0	ENSG00000177600
ATP1A1	172.1	1492.4	ENSG00000163399
TMEM59	171.8	242.8	ENSG00000116209
SREK1IP1	171.8	35.4	ENSG00000153006
CALCB	171.8	0.5	ENSG00000175868
SNRPN	171.3	179.0	ENSG00000128739
MYL6	170.9	1223.0	ENSG00000092841
RPL34	164.3	1139.4	ENSG00000109475
RPL27	162.6	1350.3	ENSG00000131469
SKP1	162.0	199.7	ENSG00000113558
PRDX6	161.8	426.2	ENSG00000117592

RNAseq of patient tumor

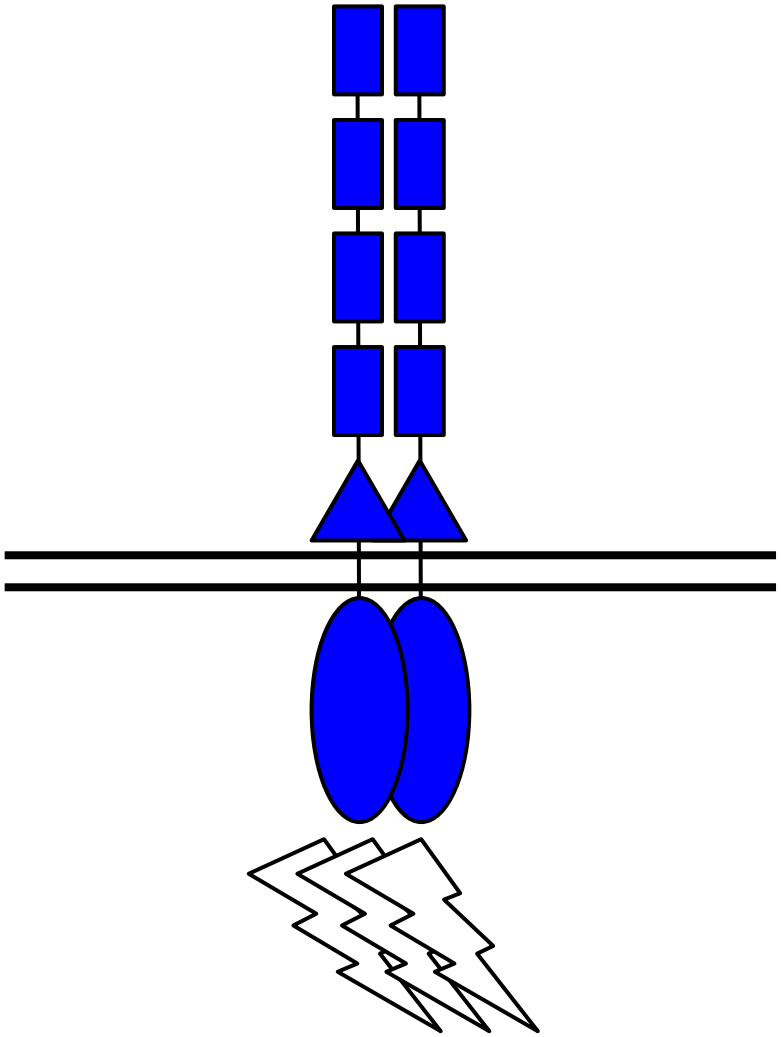
gene_symbol	THY1p0	Max of the Normal Tissues	ensembl_gene_id	
OR11H12	15.5	0.0	ENSG00000257115	#DIV/0!
CALCA	4170.9	4.6	ENSG00000110680	909.5
GFRA4	25.6	0.1	ENSG00000125861	336.5
CALCB	171.8	0.5	ENSG00000175868	314.4
OR4X2	4.3	0.0	ENSG00000172208	309.6
GRP	1028.3	4.5	ENSG00000134443	227.2
GABRR1	161.3	0.8	ENSG00000146276	214.5
OTOP3	9.8	0.1	ENSG00000182938	151.1

What is GFRα4?

RET signaling in normal vs. malignant parafollicular cells



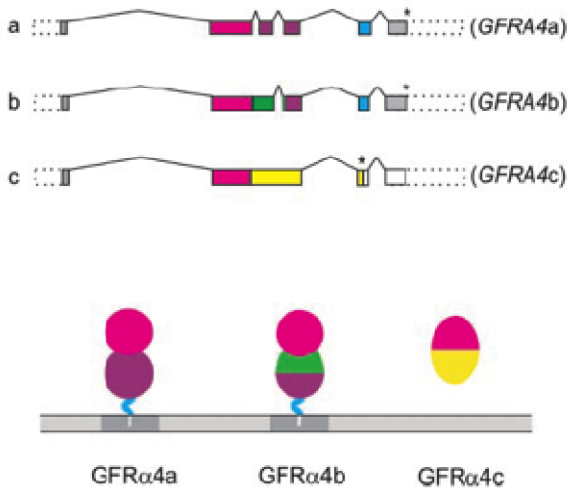
RET/GFRA4/PSPN heterohexamers

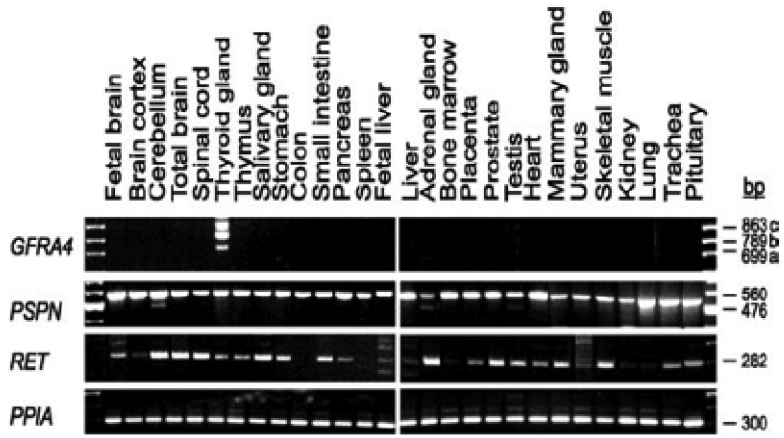


RET homodimers

What is GFRα4?

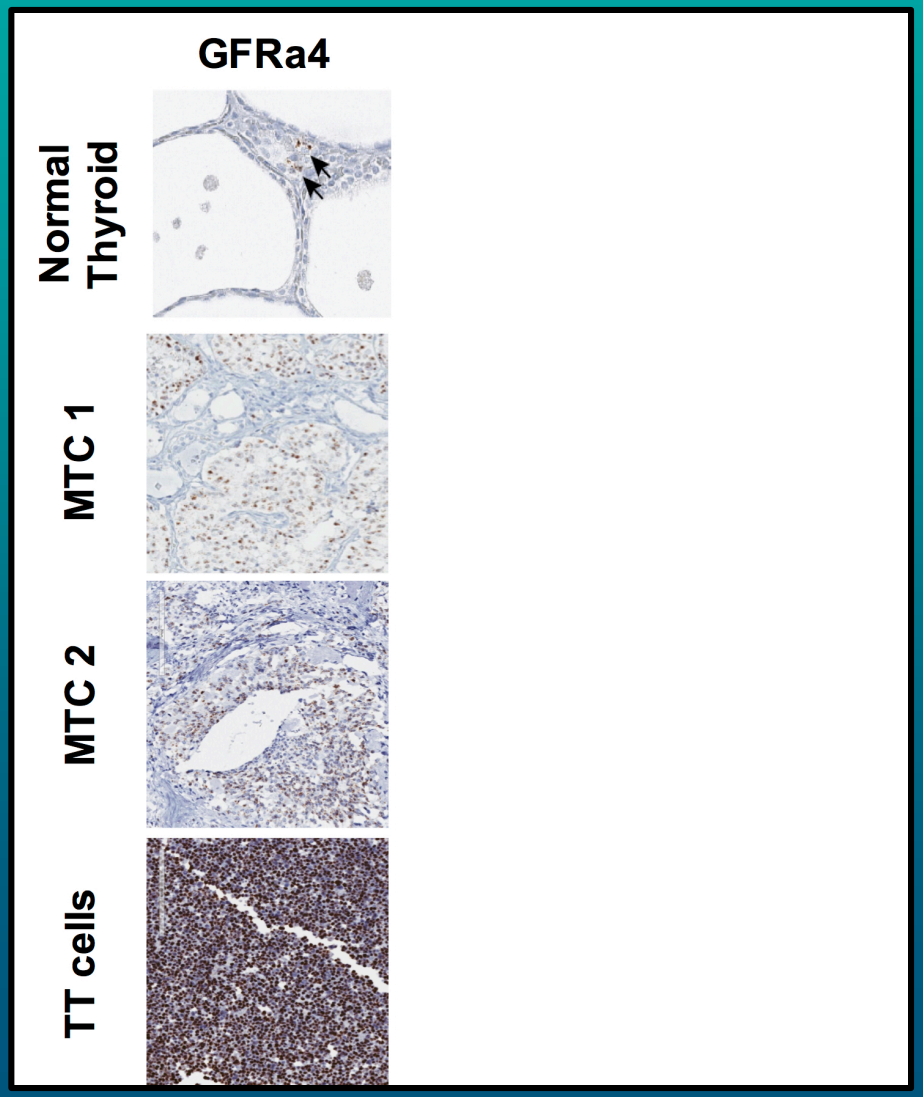
- GFRA4 is part of the family of GDNF receptors
- 3 splice variants exist
- Expression in humans appears restricted to normal C cells





Lindahl et al. J Biol Chem (2001) vol. 276 (12) pp. 9344-51

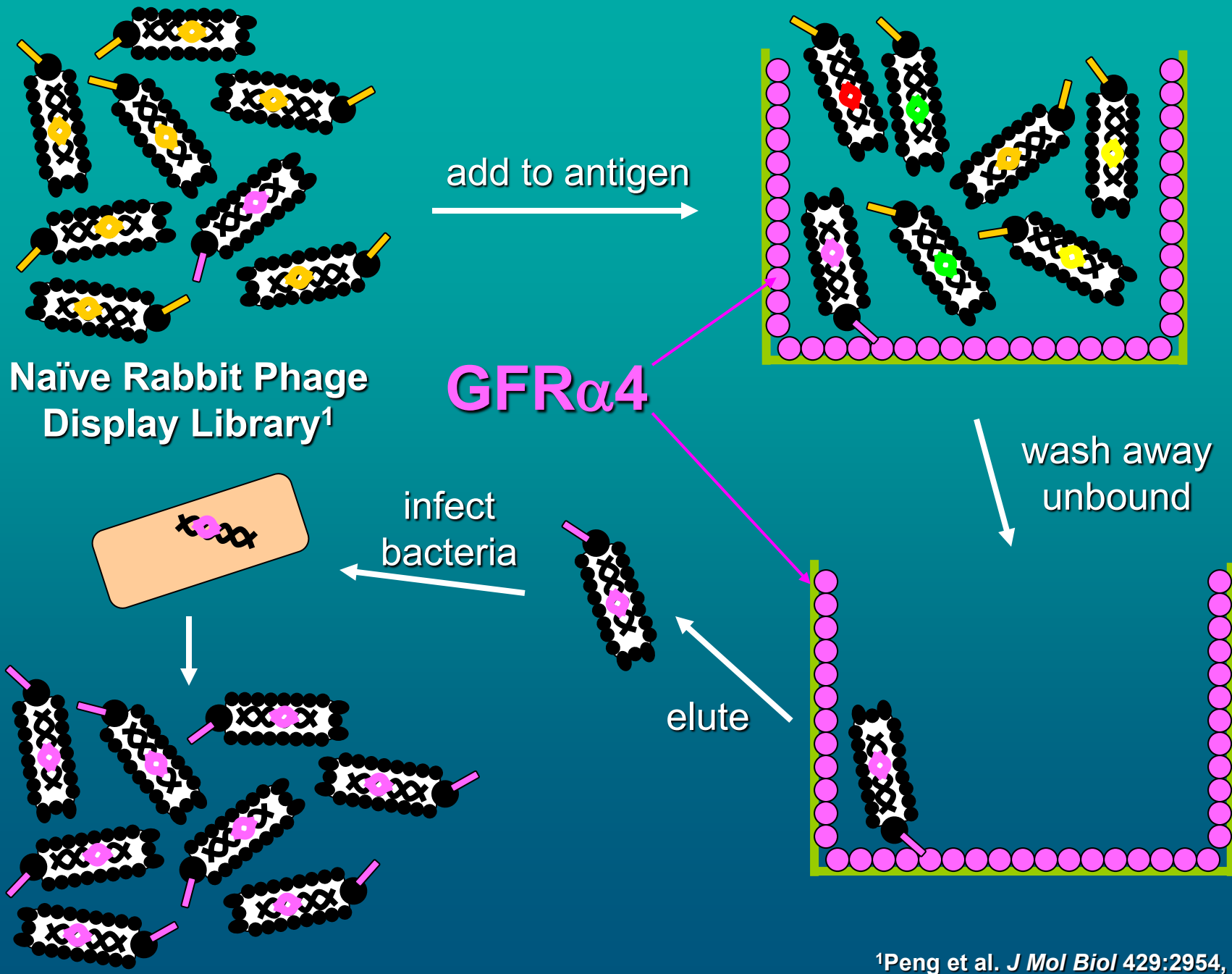
Expression of GFRα4



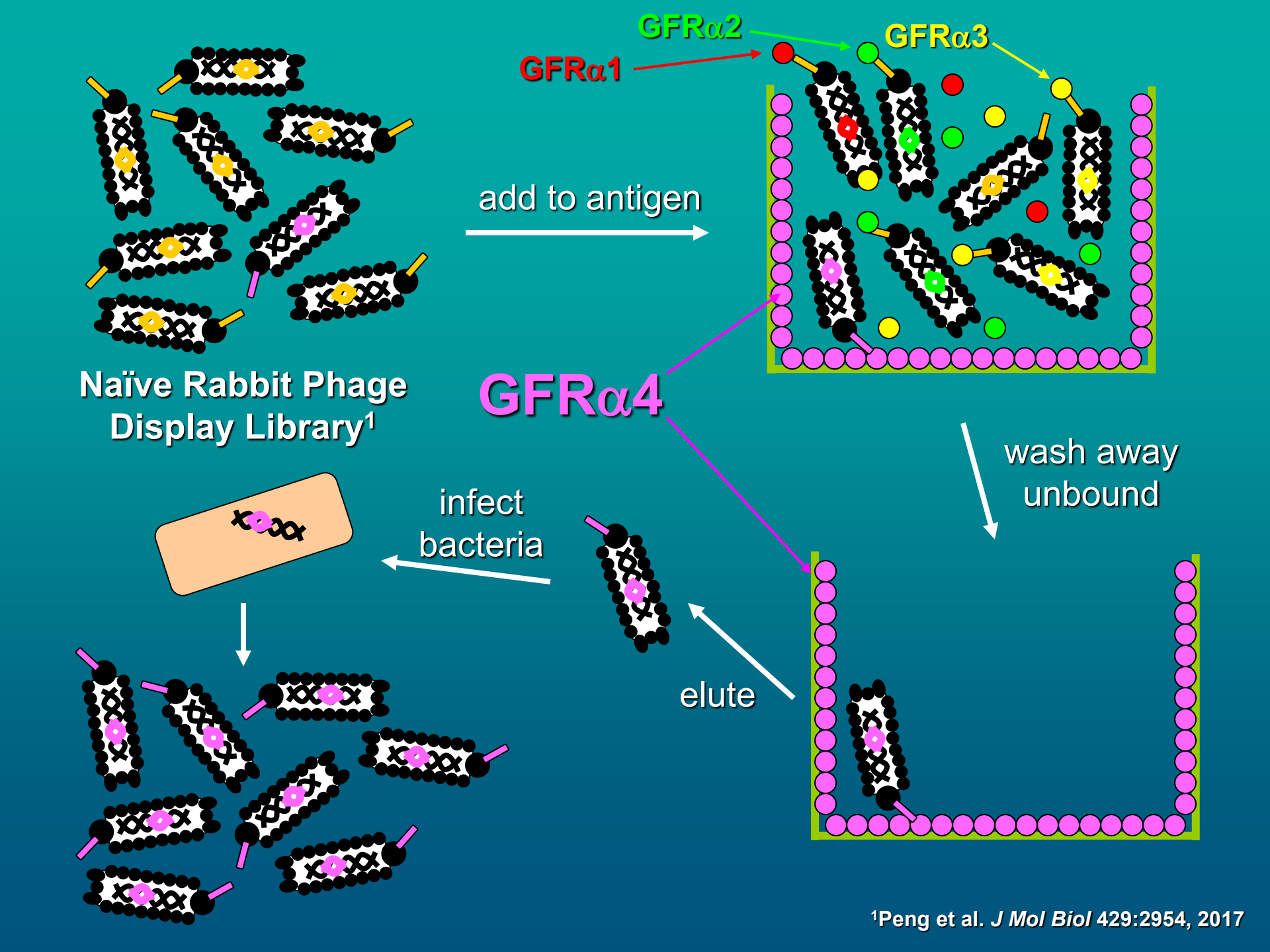
Tissue	Human	Cynomolgus Macaque
MTC	+	N/A
TT cell line	+	N/A
Thyroid	+	+
Parathyroid	-	-
Adrenal	-	-
Pituitary	-	-
Ovary	-	-
Thymus	-	-
Pancreas	-	N/T
Esophagus	-	N/T
Stomach	-	N/T
Liver	-	N/T
Small Bowel	-	N/T
Colon	-	N/T
Urinary Bladder	-	-
Testis	-	N/T
Cerebellum	-	-
Cerebral Cortex	-	-
Temporal lobe	-	
Frontal lobe	-	
Occipital lobe	-	
Insula	-	N/T
Pons	-	-
Hippocampus	-	N/T
Medulla	-	N/T
Spinal cord	-	-

Identify a target → **Make an antibody** → **Make a T-cell CAR**

(Patient-specific)
(MTC-specific)
(C cell-specific)

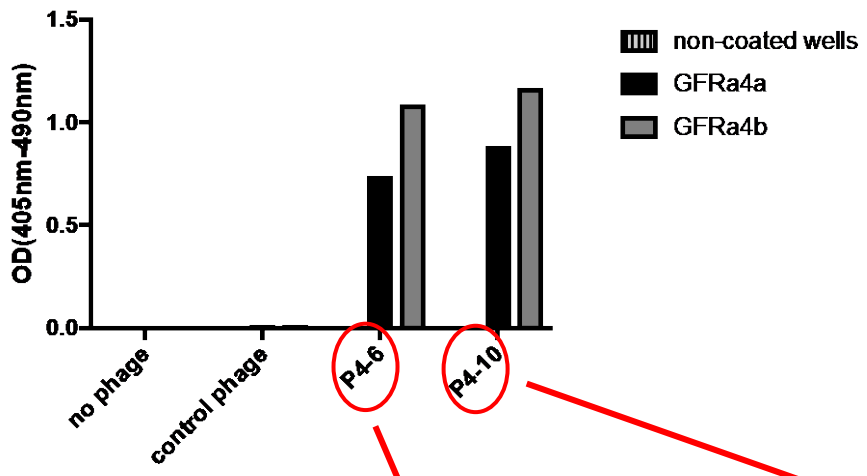


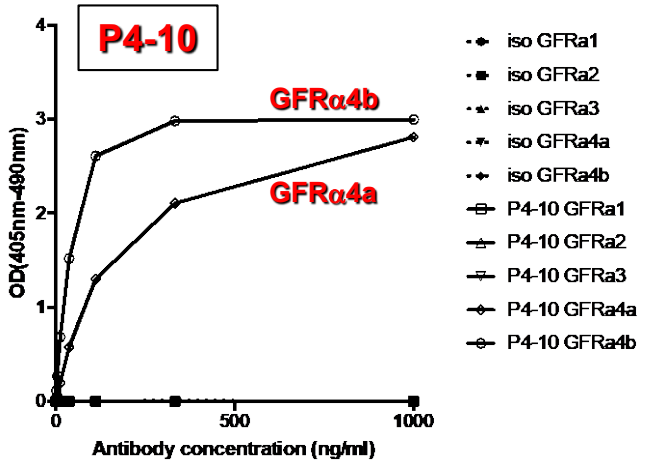
[1]Peng et al. *J Mol Biol* 429:2954, 2017

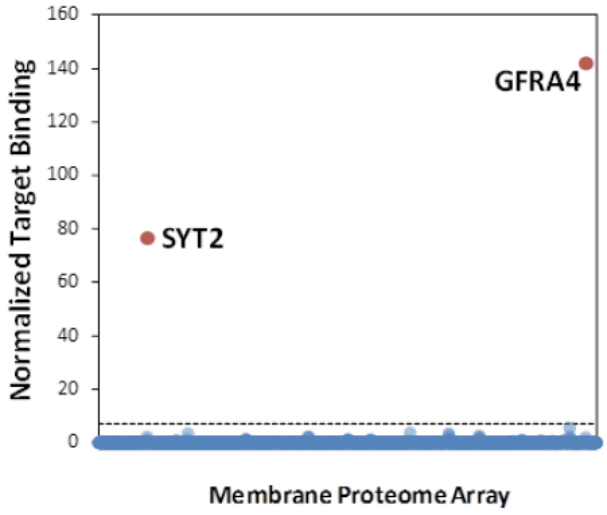


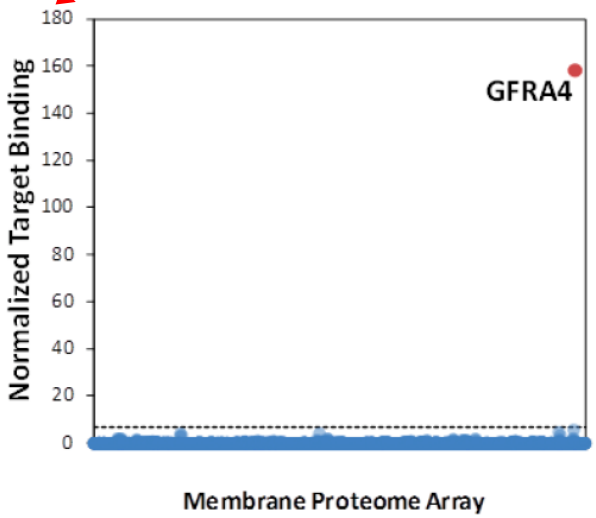
[1]Peng et al. *J Mol Biol* 429:2954, 2017

Binding Specificity of 2 Unique anti-GFRα4 Abs





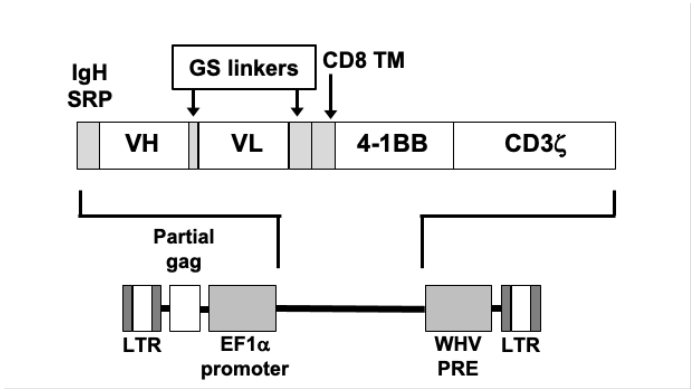


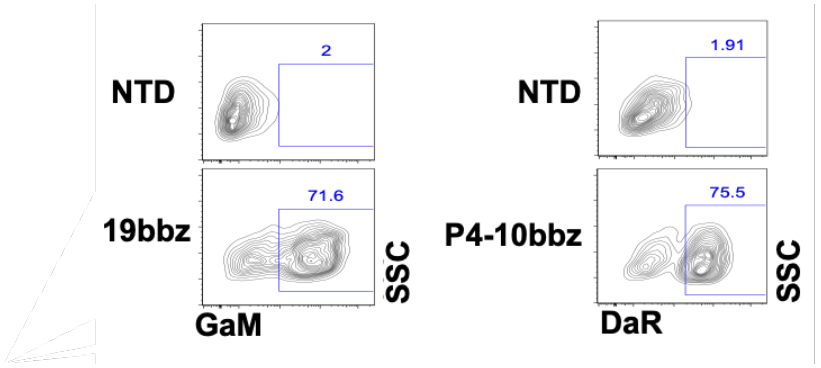


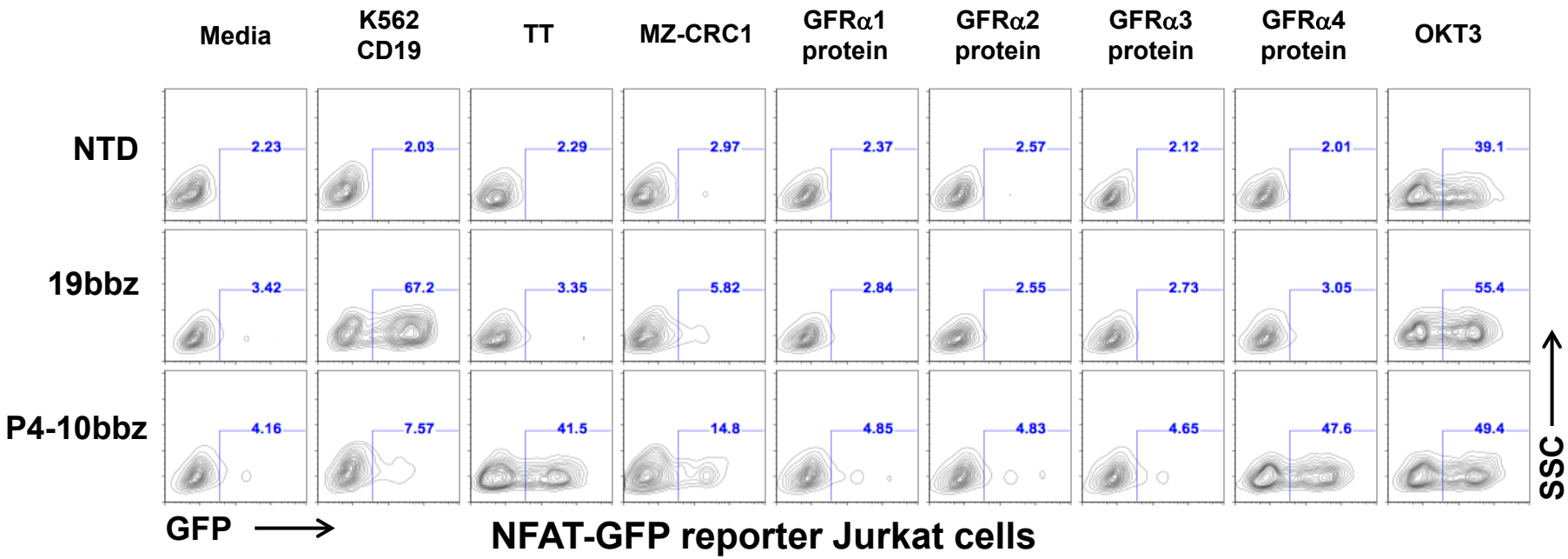
Identify a target → Make an antibody → Make a T-cell CAR

(patient-specific)
(MTC-specific)
(C cell-specific)

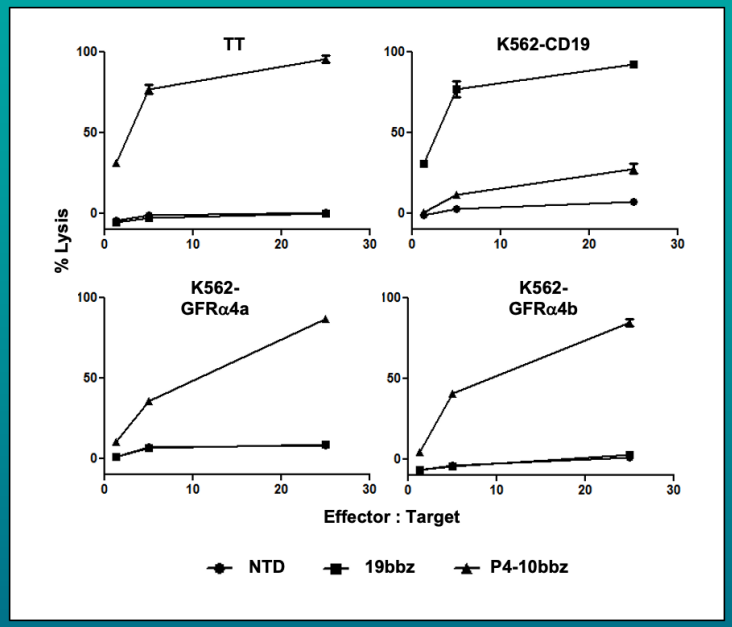
P4-10 CAR-T Cells Specifically Respond to GFRα4 *In Vitro*

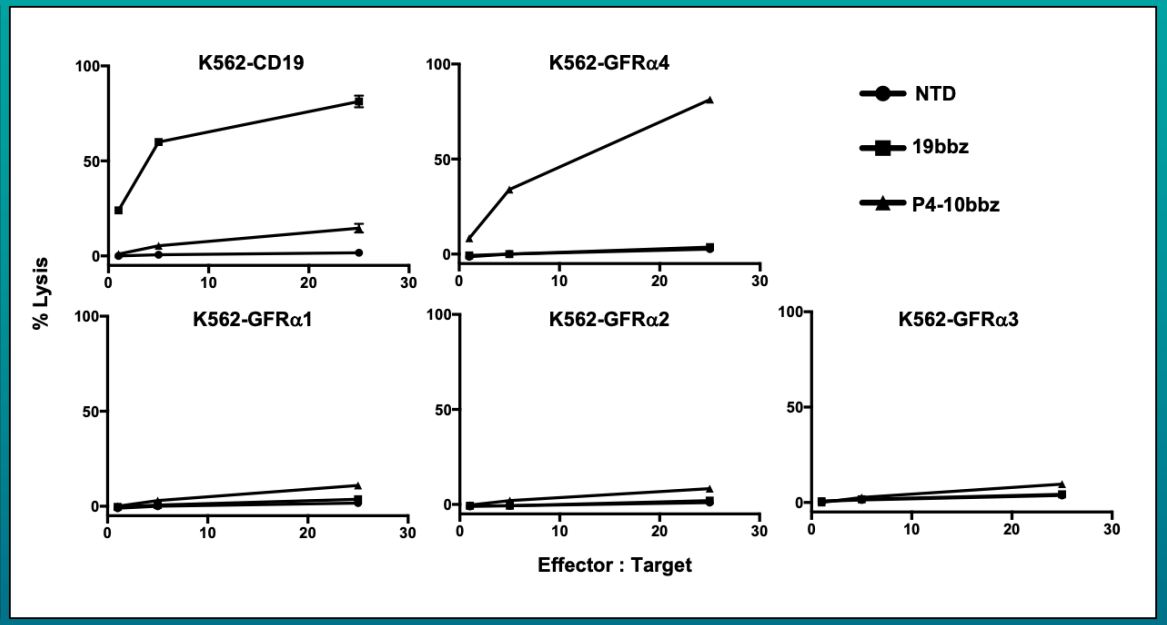






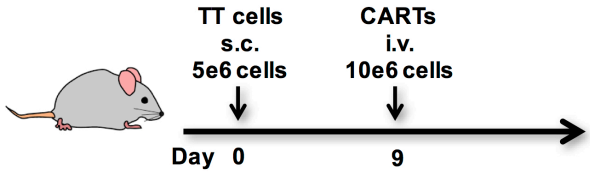
Anti-GFRα4 CARs Kill MTC *in vitro*

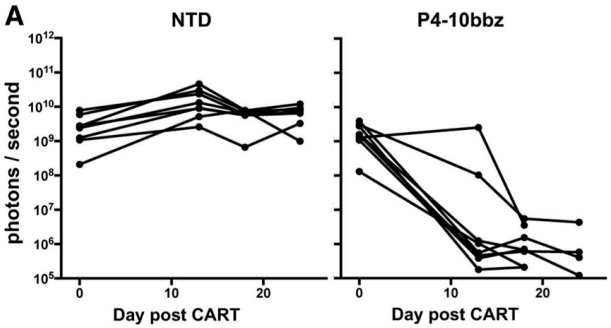


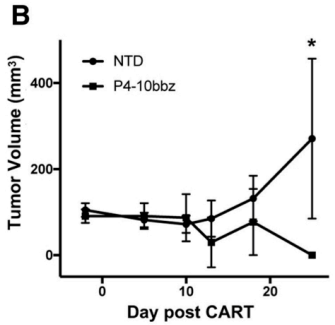


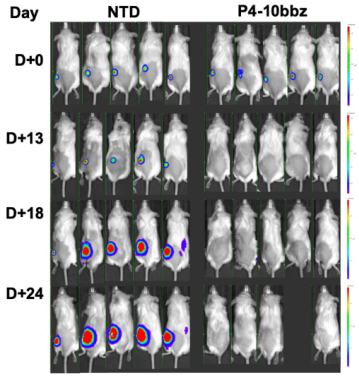
Activity *in vivo*?

Anti-GFRα4 CARs Kill MTC in Murine Model



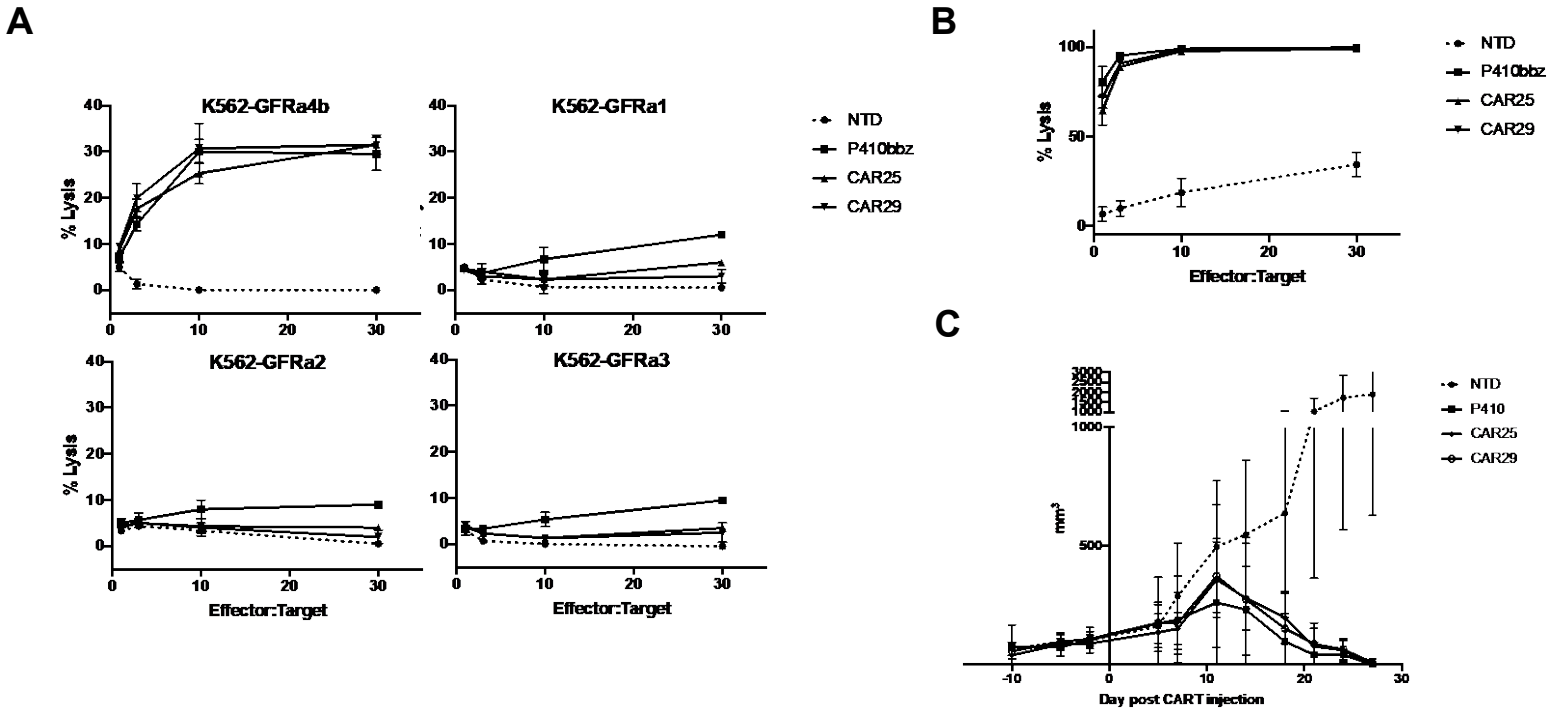






Final Step: Humanization of P4-10

Heavy chains	V region germline gene	% Human homology	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
P410	Rabbit IGHV1S34*01	54%	QSVKESEGGLFKPTDTLTLTCTVSGFSLS	RHALT	WVRQAPGNGLEWIG	AIDNAGTTYYASWAKS	RSTITRNTDLHTVTLKMTSLTASDTATYFCAR	VFYDINSGYYLDGMDL	WGPGTLVTVSS
CAR25	Human IGHV4-38-2*02	85%	QVQLQESGPGLVKPSETLSLTCAVSGYSIS	RHALT	WIRQPPGKGLEWIG	AIDNAGTTYYASWAKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	VFYDINSGYYLDGMDL	WGPGTLVTVSS
CAR29	Human IGHV3-48*03	85%	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	RHALT	WVRQAPGKGLEWVS	AIDNAGTTYYASWAKS	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	VFYDINSGYYLDGMDL	WGPGTLVTVSS
Light chains									
			FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
P410	Rabbit IGLV4S4*01	70%	QFVLTQSPSVSAALGASAKLTC	TLSSAHKTYTID	WYQQQQGEAPRYLMQ	VKSDGSYTKGT	GVPDRFSGSSSGADRYLIIPSVQADDEAGYVC	GADDNGGYV	FGGGTQLTVT
CAR25/CAR29	Human IGLV4-69*01	85%	QLVLTQSPSASASLGASVKLTC	TLSSAHKTYTID	WYQQQPEKGPRYLMQ	VKSDGSYTKGT	GVPDRFSGSSSGADRYLTISSLQSEDEADYYC	GADDNGGYV	FGGGTQLTVL



Summary

- Antibody phage display can offer a number of advantages over cellular methods for antibody discovery depending on particular application

- Use of "single-pot" pre-constructed naïve or non-immune libraries obviate the need for animal or human immunization and can provide potentially useful antibody fragments to virtually any antigen very rapidly

  e.g., total time from GFRα4 target identification to anti-GFRα4 scFv's to construction of GFRα4 CAR-T cells to completion of initial *in vitro* target cell killing assays – 2½ months

- Use of non-conventional heavy chain-only camelid antibody fragments may offer unique epitope specificities and may better facilitate multi-specific CAR-T cell design

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