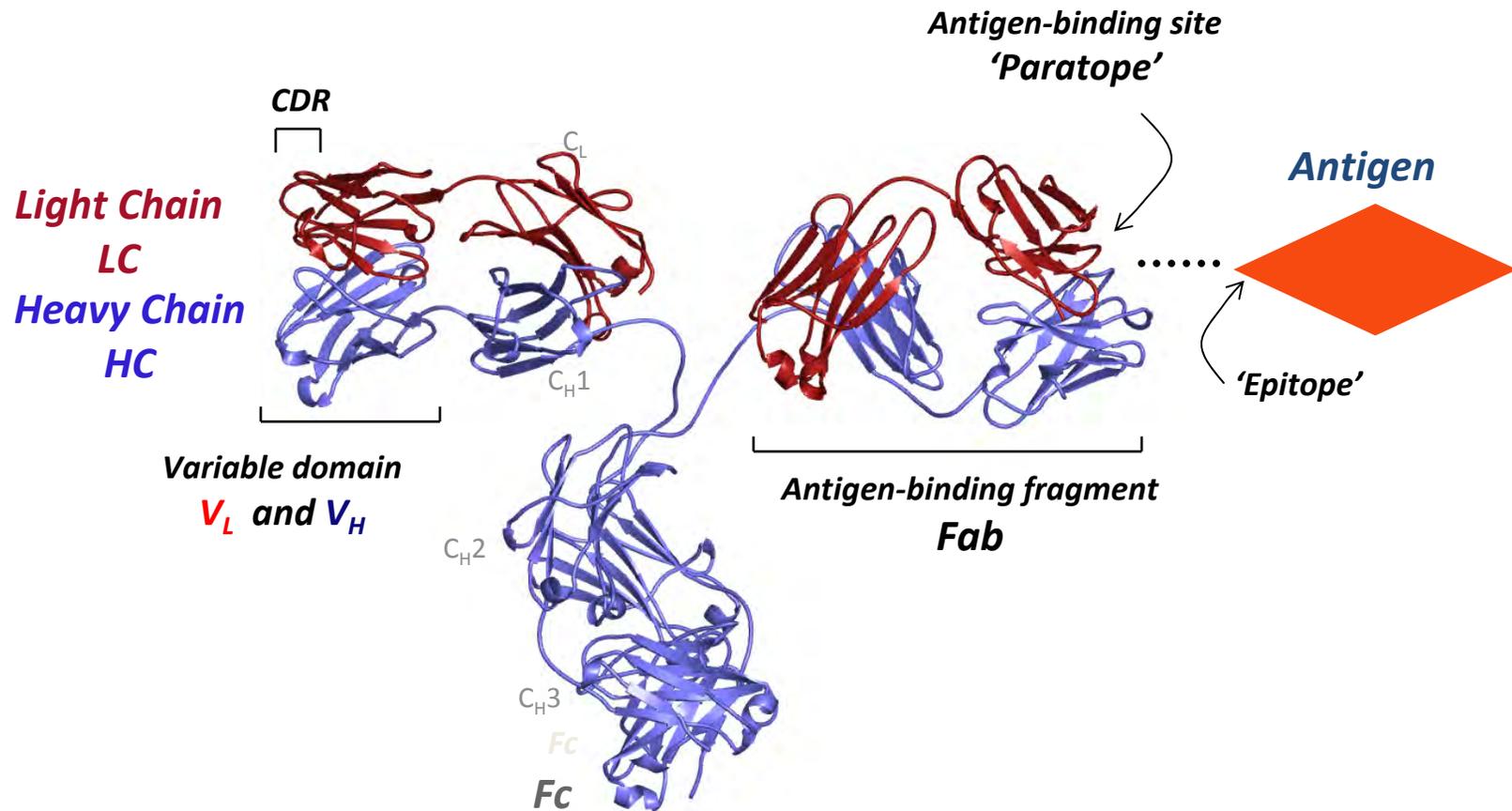


Two-in-One antibody:

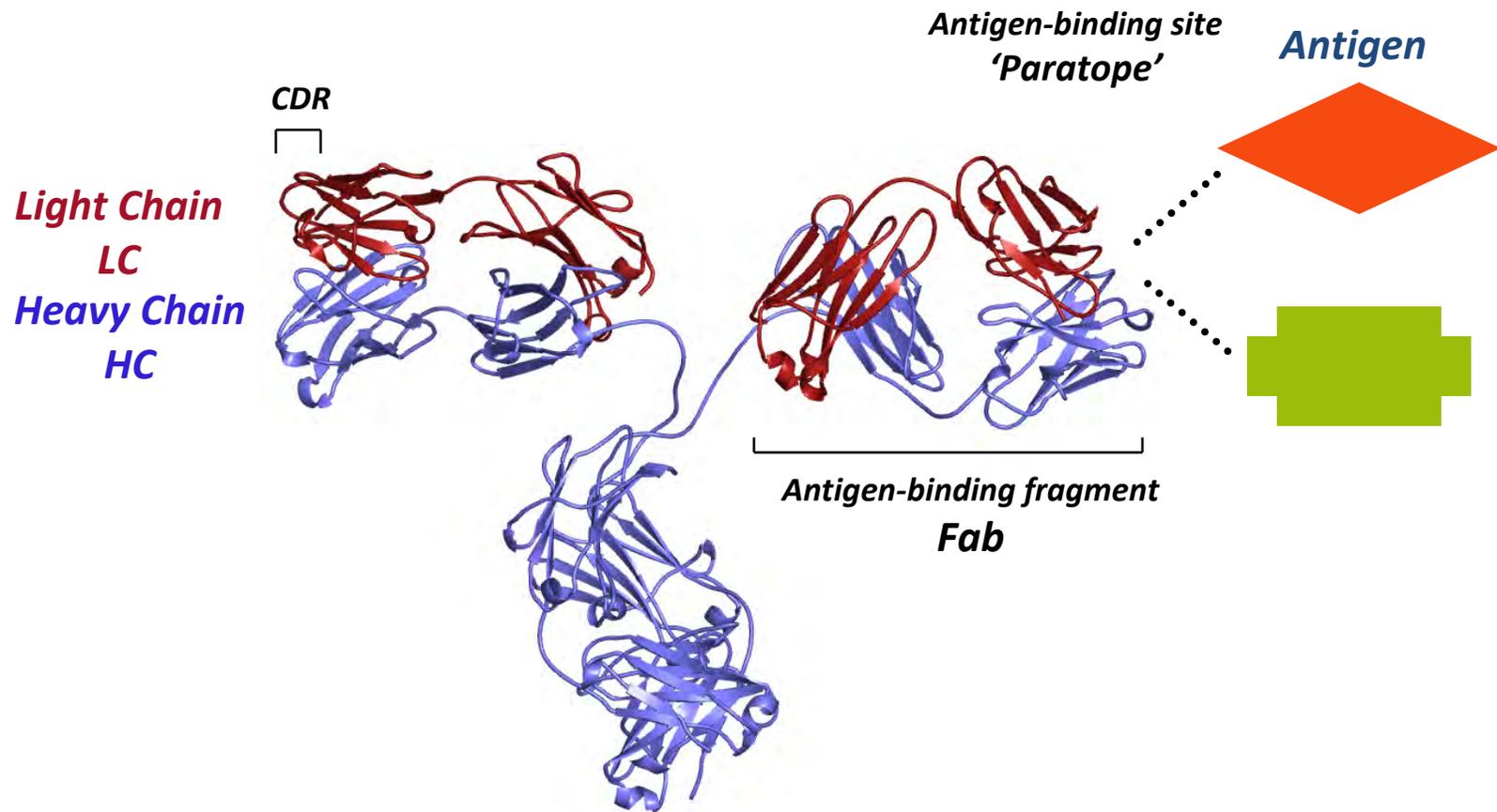
**From Proof-of-Concept to
Therapeutic Candidate**

Germaine Fuh
Antibody and Protein Engineering
Departments
Genentech

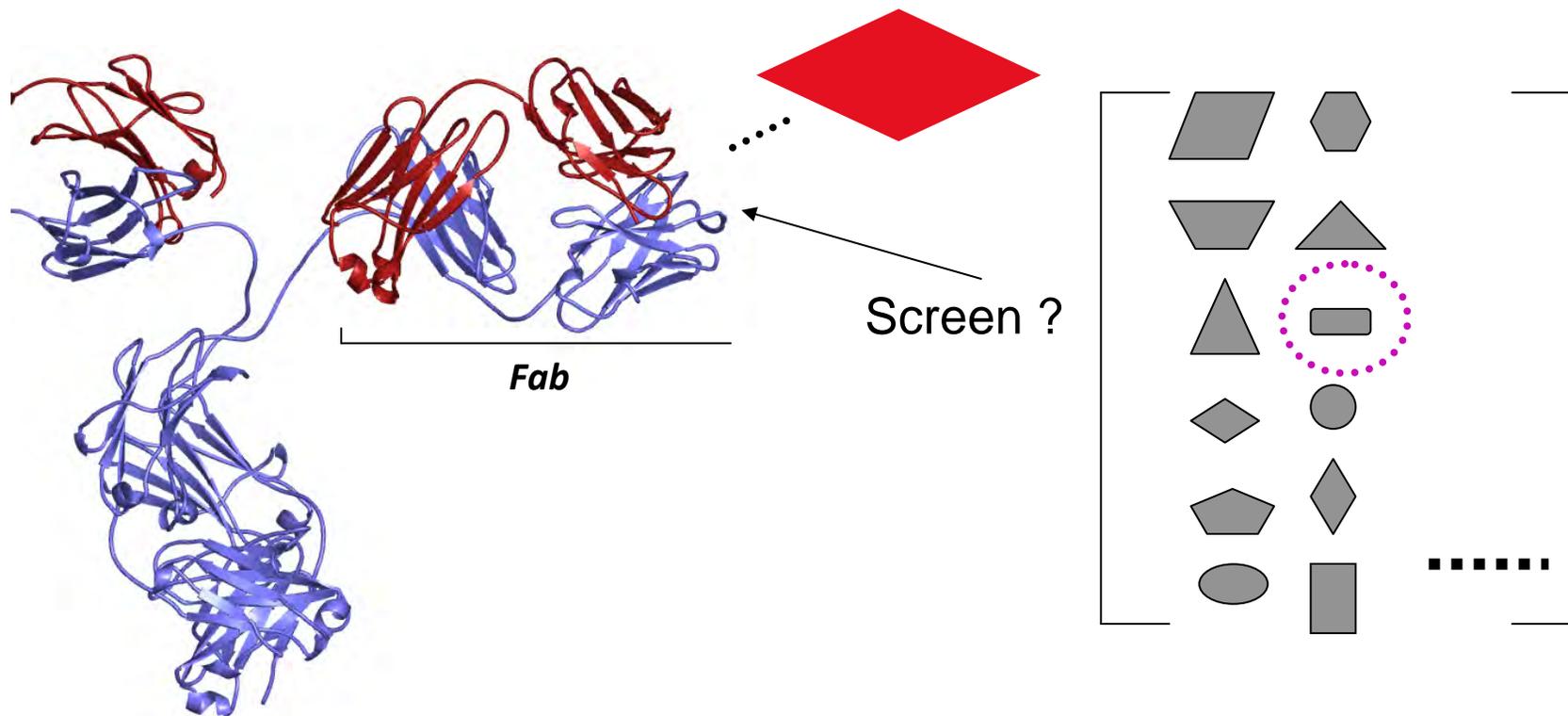
'One Antibody, One Antigen'



One Antibody, Two Antigens ?



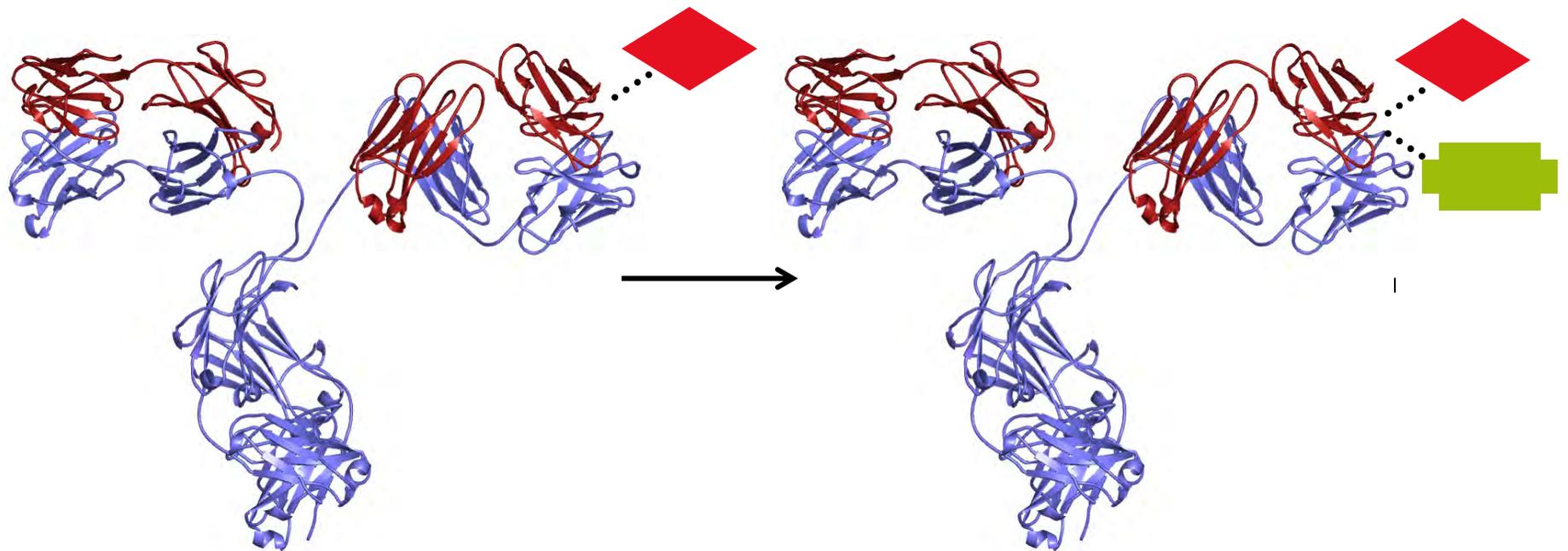
Previous studies on mechanisms of antibody multi-specificity



Kramer A et al. *Cell* (1997)
James L et al. *Science* (2003)
Sethi DK et al. *Immunity* (2006)

One antibody, Two antigens?

A strategy to generate dual specific antibodies against two unrelated antigens with high affinity *on demand*



*To understand the propensity and mechanism of multispecificity
To provide dual targeting therapeutics*

Outline

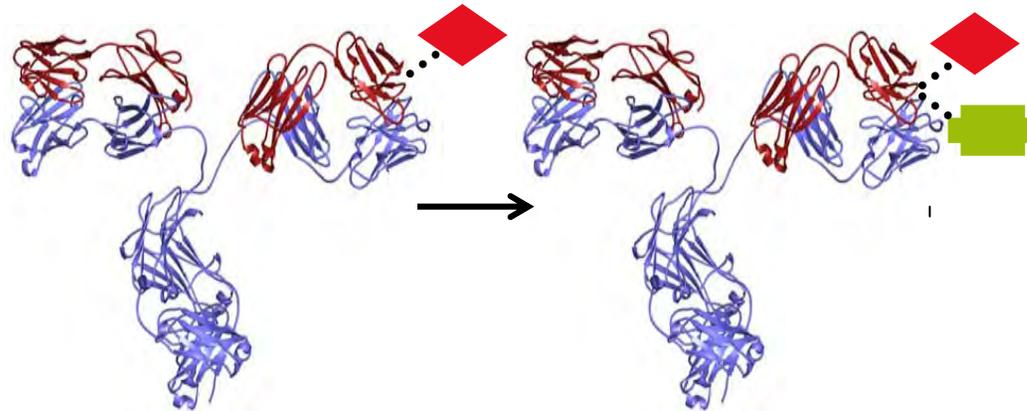
Two-in-One antibody

Rationale of the Engineering Approach:

- Mutate the LC CDRs of monospecific antibodies to derive dual specific antibodies

From POC to therapeutic candidate:

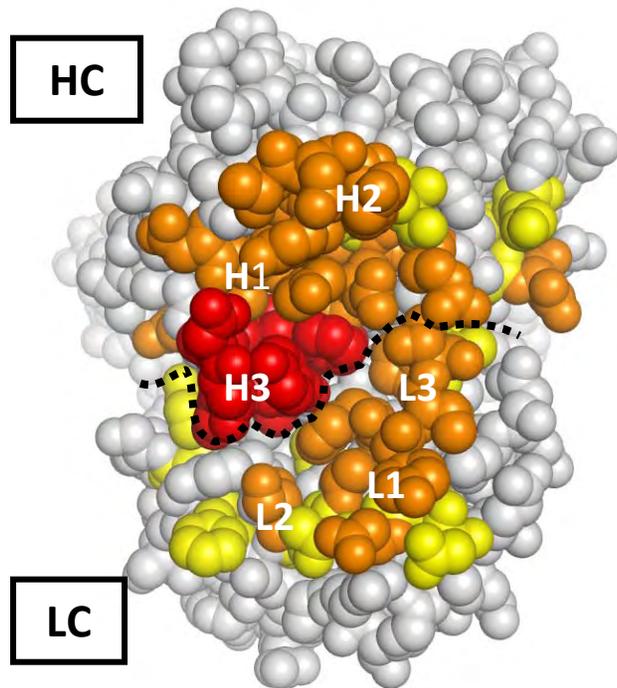
- Herceptin CDR variants that bind HER2 and VEGF with high affinity
- EGFR antibody evolved to EGFR/HER3 dual targeting antibody



Heavy chain CDRs more diverse

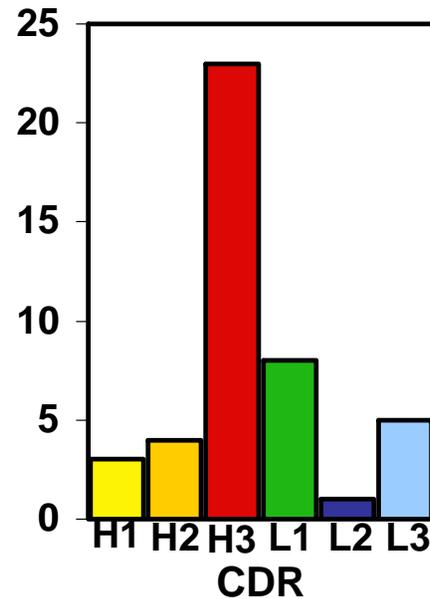
Often contain the main determinants of binding specificity

Amino acids

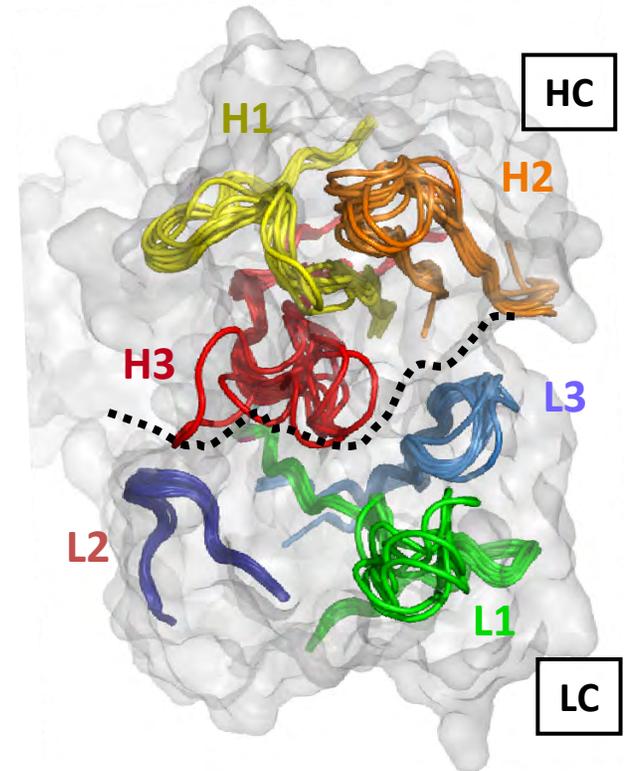


Top-down view of paratope

Length



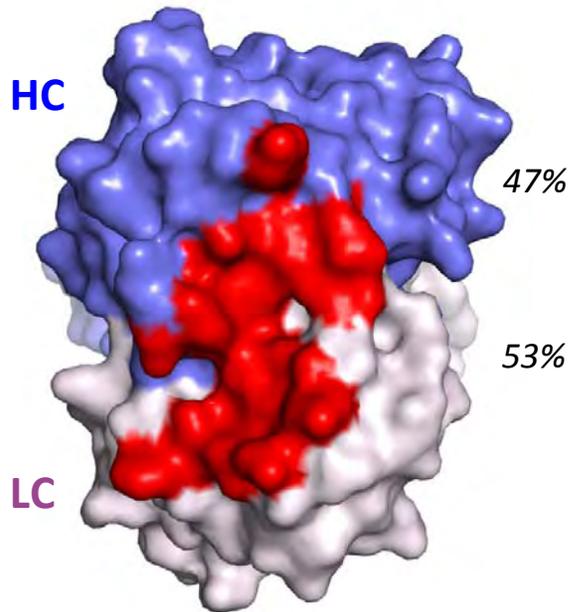
Conformation



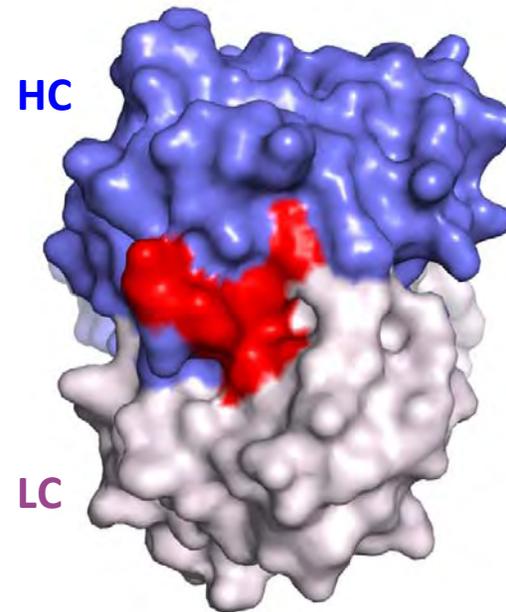
Overlay of the CDRs of several antibodies

Heavy chain CDRs are critical for Herceptin to bind HER2

Top down view of Herceptin Fab



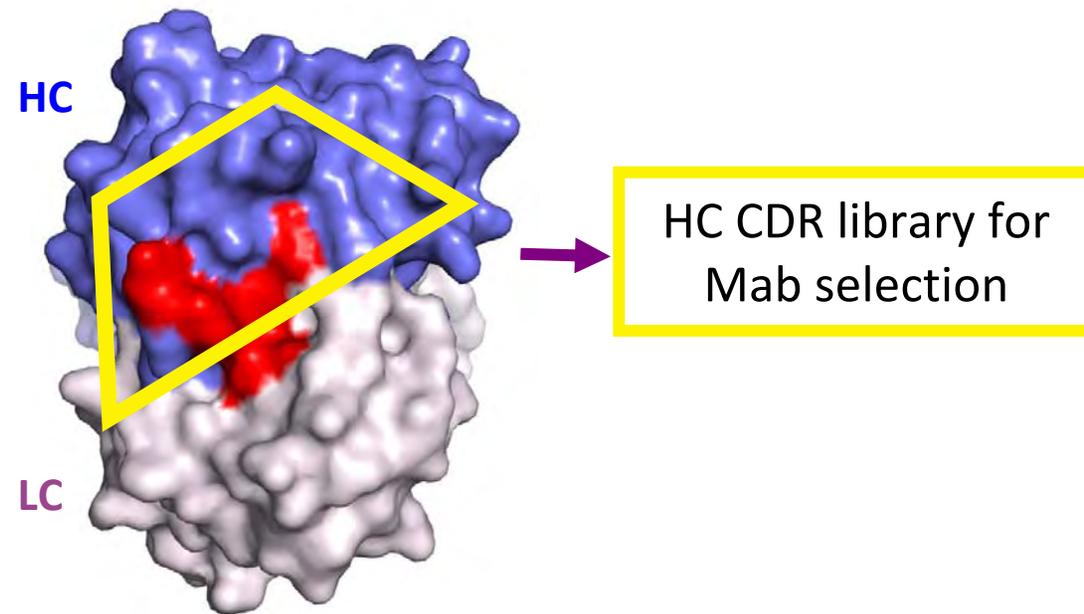
Structural paratopes
(Structural contacts)



Functional paratopes
(Alanine scanning hot spots)

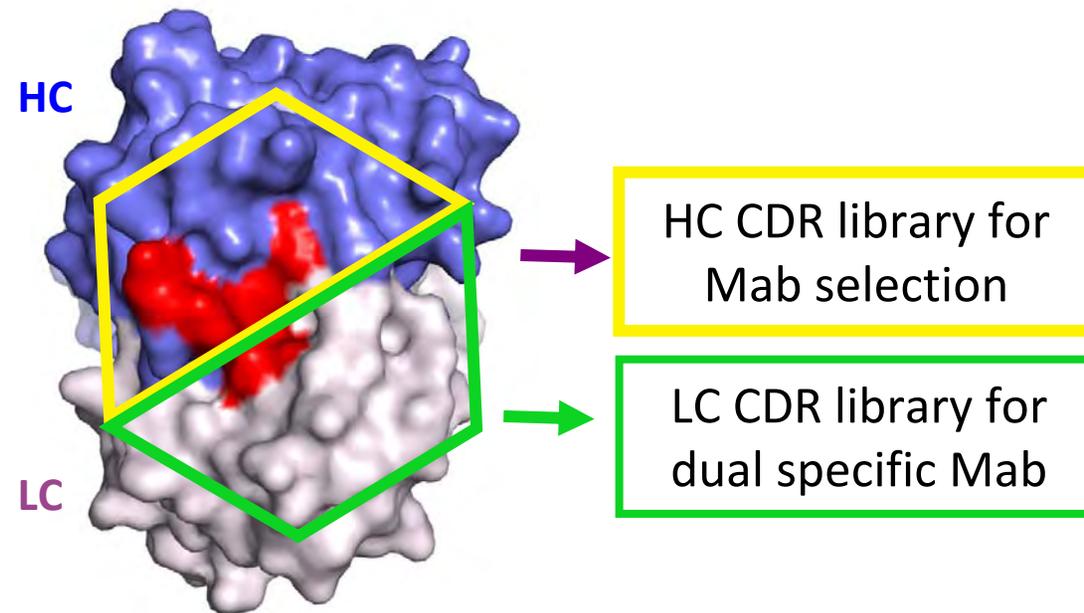
Heavy chain CDR phage libraries:

Currently a source of therapeutic antibodies



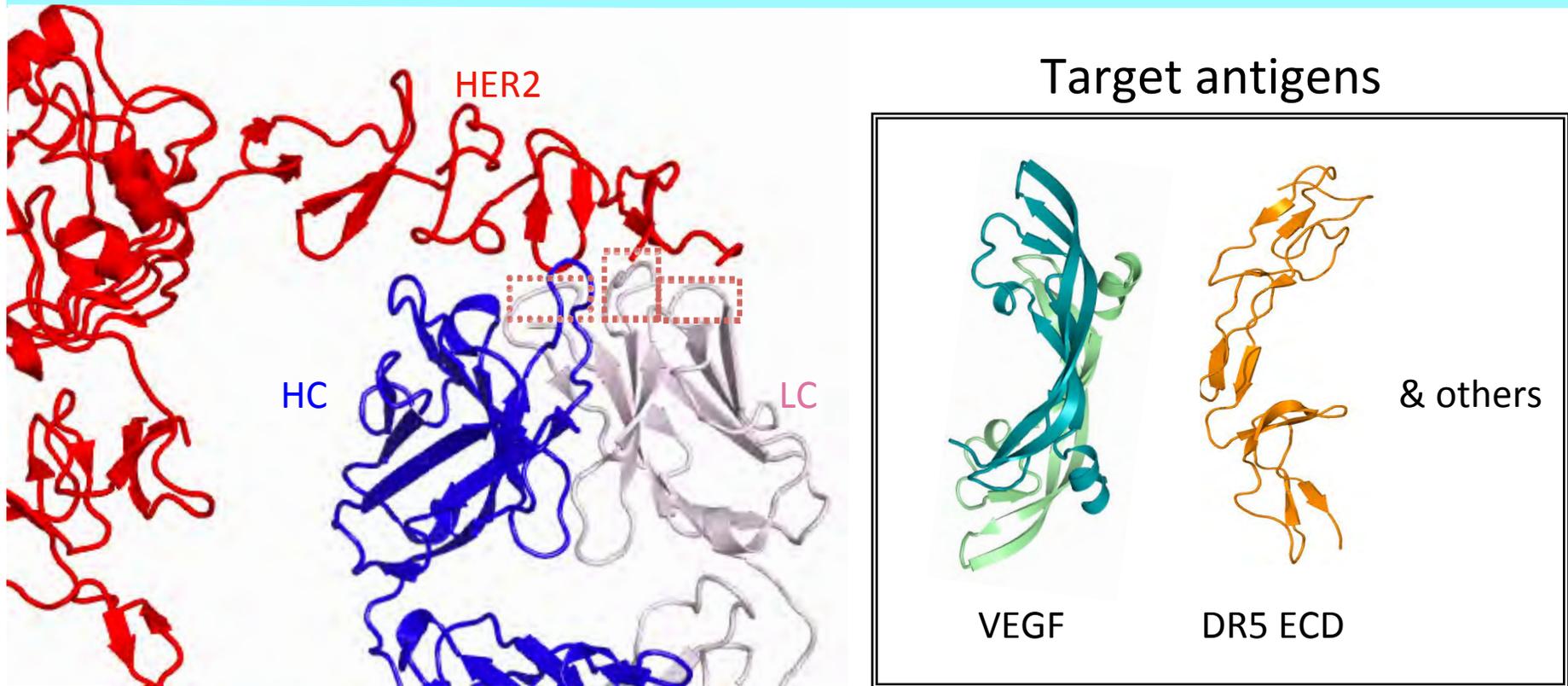
Functional paratopes
(Alanine scanning hot spots)

Light chain CDR phage libraries: To isolate dual specific antibodies



Functional paratopes
(Alanine scanning hot spots)

Herceptin light chain CDR libraries designed with restricted diversity



- Randomize 12 surface accessible LC CDR residues
- Mimic natural diversity
- Favor Herceptin CDR L3 sequence

Light chain CDR library success

bH1 focused as proof-of-concept

Many antibodies with new binding specificity were isolated

Some retained HER2 binding ability - average 8 mutations

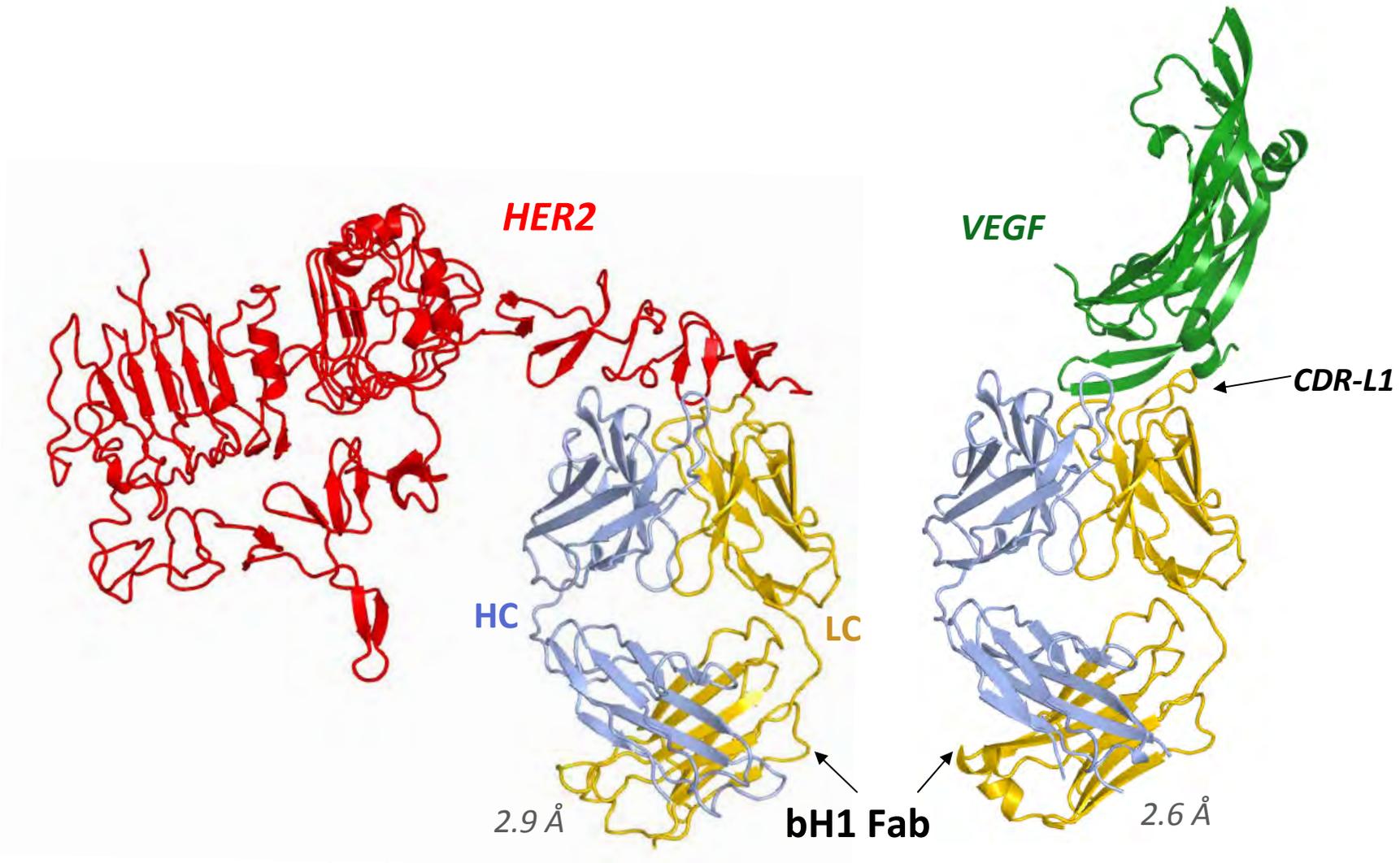
Some lost HER2 binding ability - average 12 mutations

	LC																Kd (nM)			
	CDR1										CDR2				CDR3				HER2	VEGF
	28	29	30	30a	30b	30c	30d	31	32	50	51	52	53	91	92	93	94			
Herceptin	D	V	S					T	A	S	A	S	F	H	Y	T	T	0.1	NB	
α-HER2/VEGF bH1	D	I	P	R	S	I	S	G	Y	W	G	S	Y	H	Y	T	T	20	300	
α-HER2/VEGF bH3	D	I	G	L				G	S	W	A	S	Y	H	Y	T	T	8	10,000	
α-VEGF 3-1	L	V	W					D	W	P	A	S	S	G	W	Y	I	A	NB	15

HC maintained as Herceptin

Crystal structure of bH1

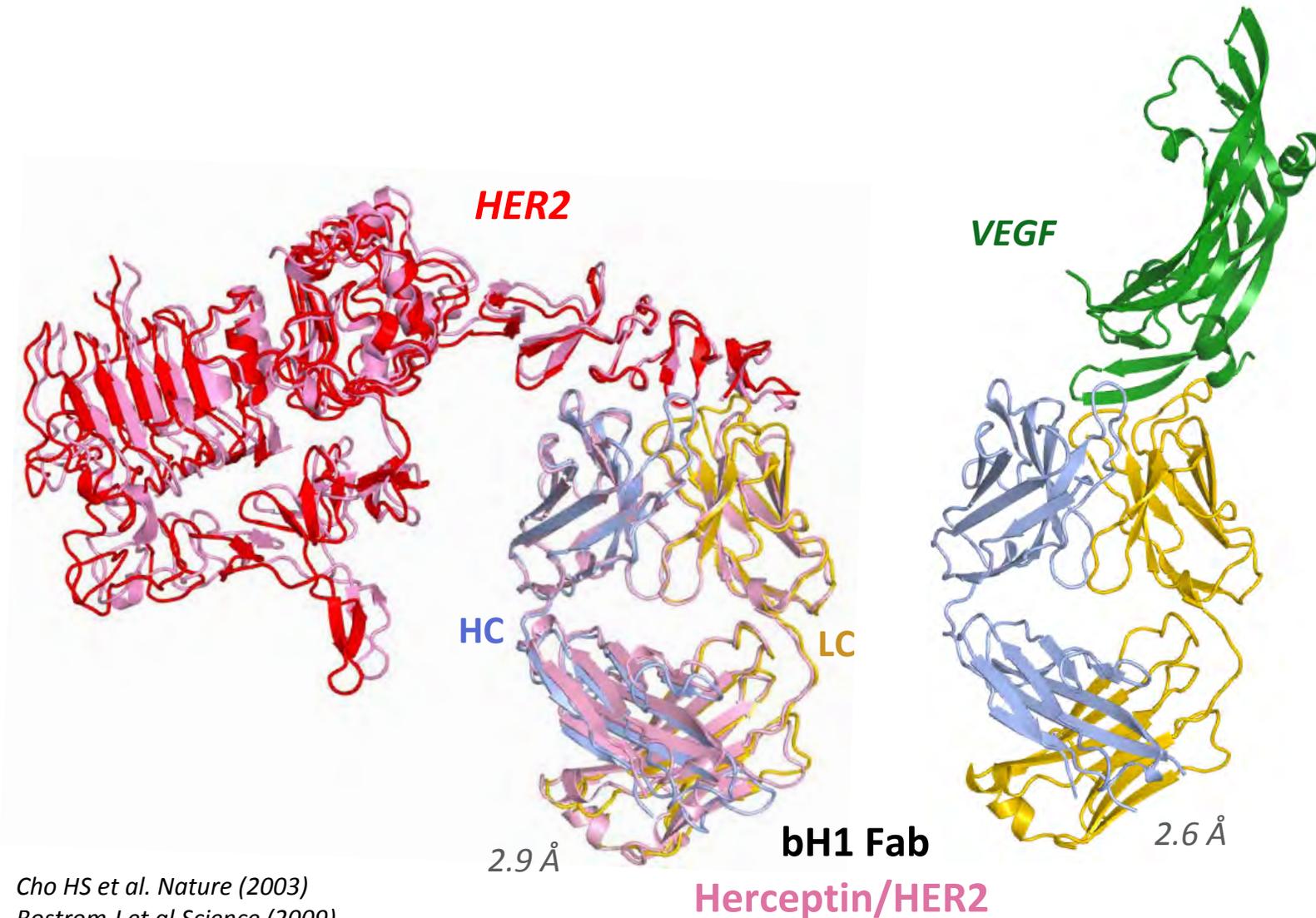
One antibody, Two antigens



Bostrom J et al. Science (2009)

bH1 maintains HER2 binding site as Herceptin

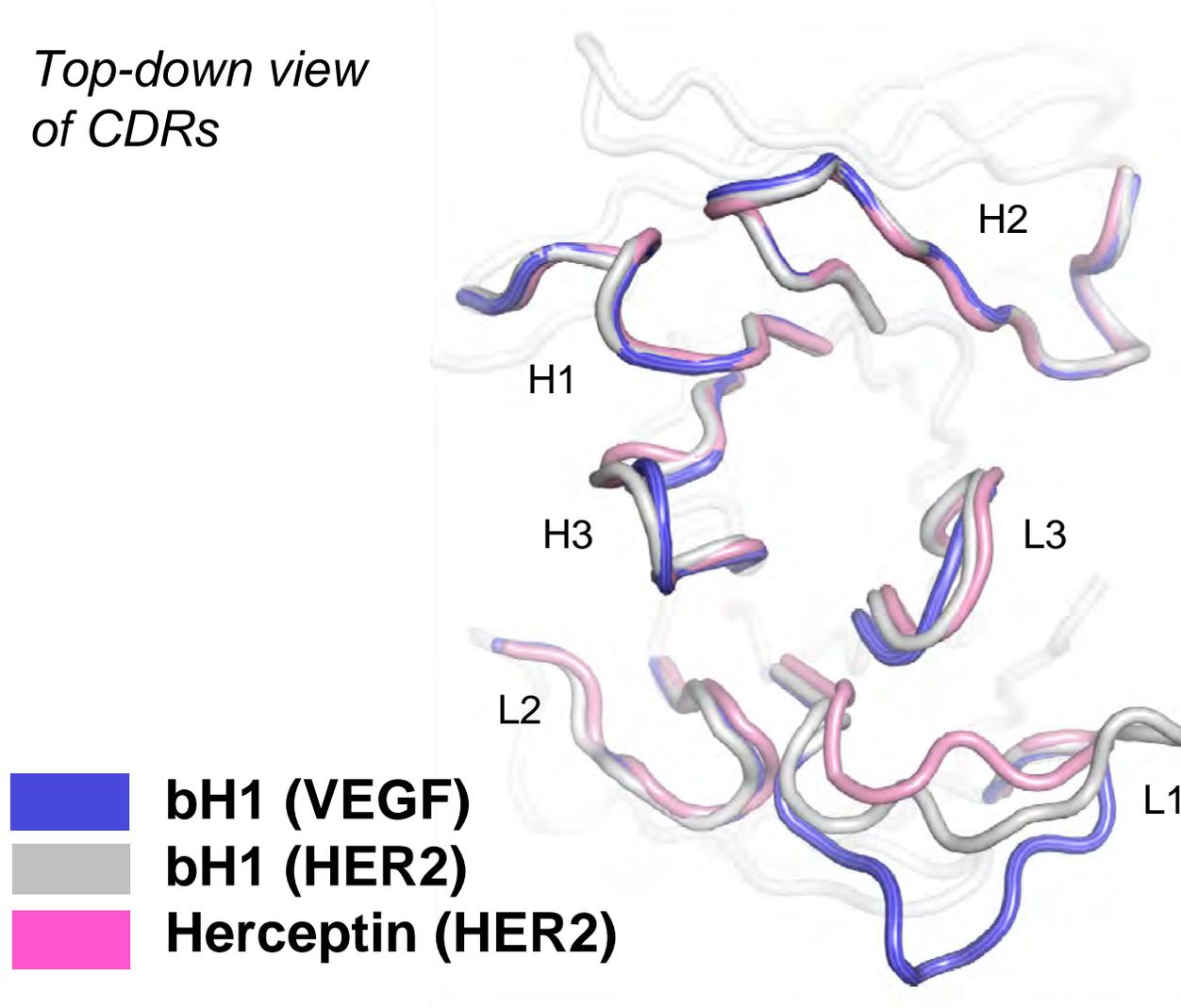
And it blocks VEGF receptor binding



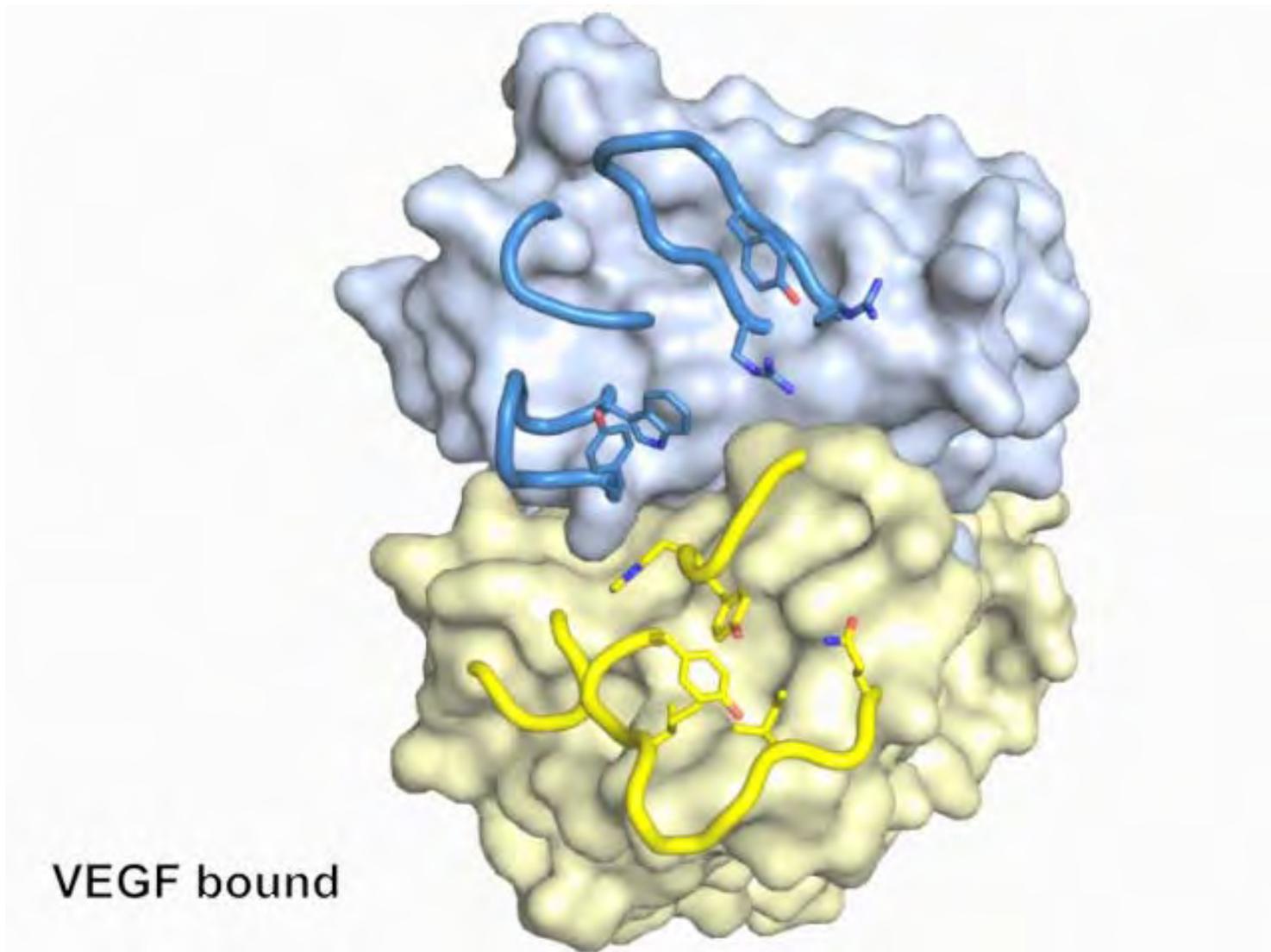
Cho HS et al. Nature (2003)
Bostrom J et al. Science (2009)

Structural Plasticity of CDRs enables dual specificity

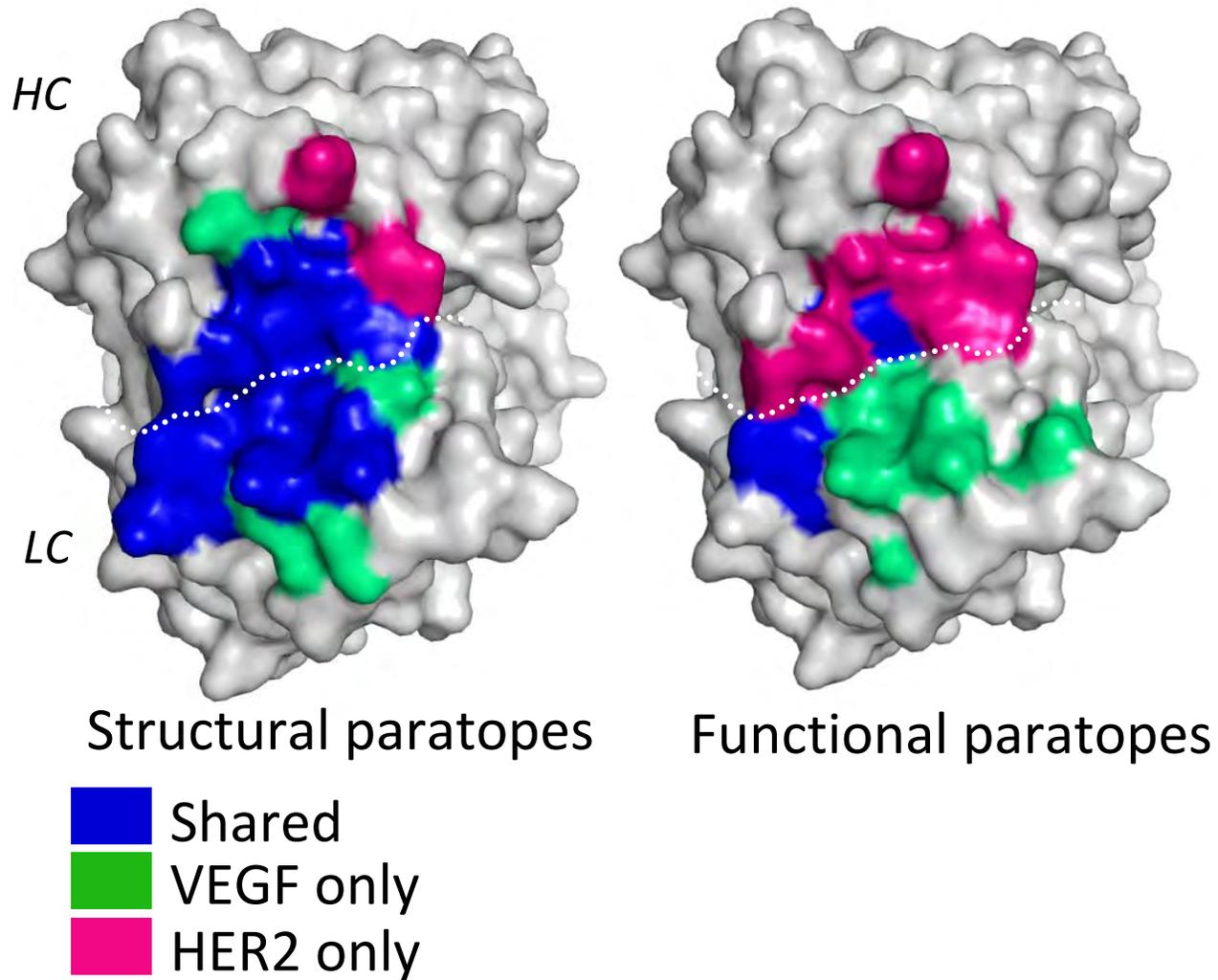
*Top-down view
of CDRs*



Structural Plasticity of CDRs enables dual specificity



A shared contact surface: LC CDRs and HC CDRs engage differently



Improved two-in-one anti-HER2/VEGF active and potent *in vitro*

Anti-HER2 affinity

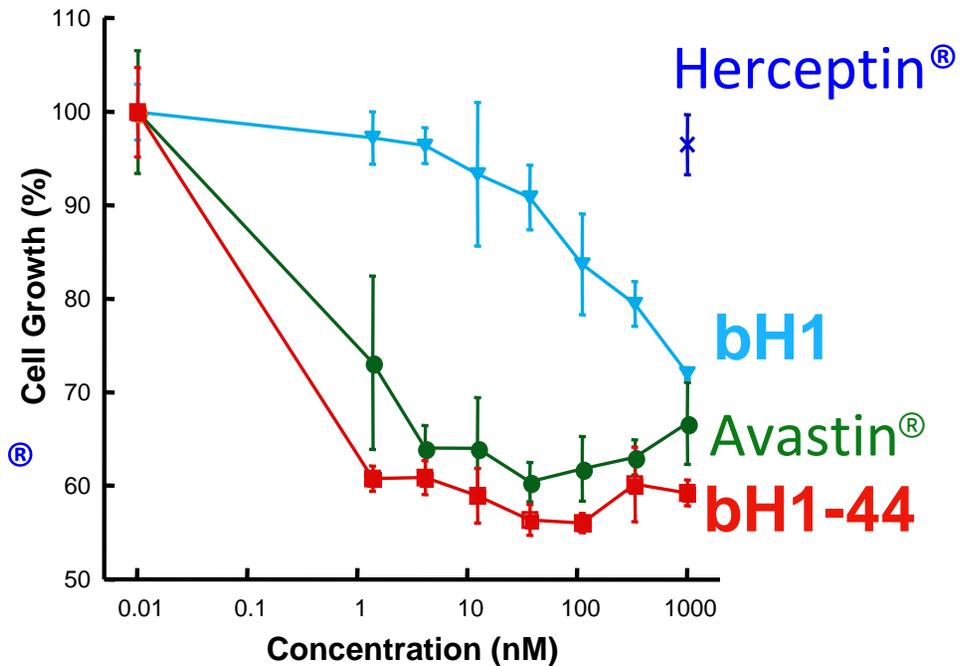
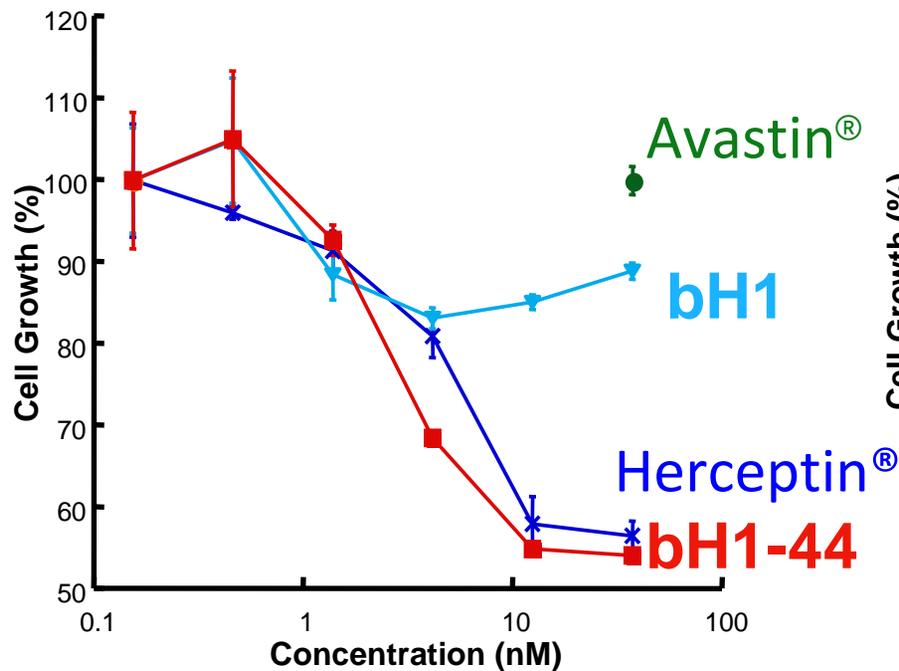
Kd = 20 → 0.2 nM

VEGF binding

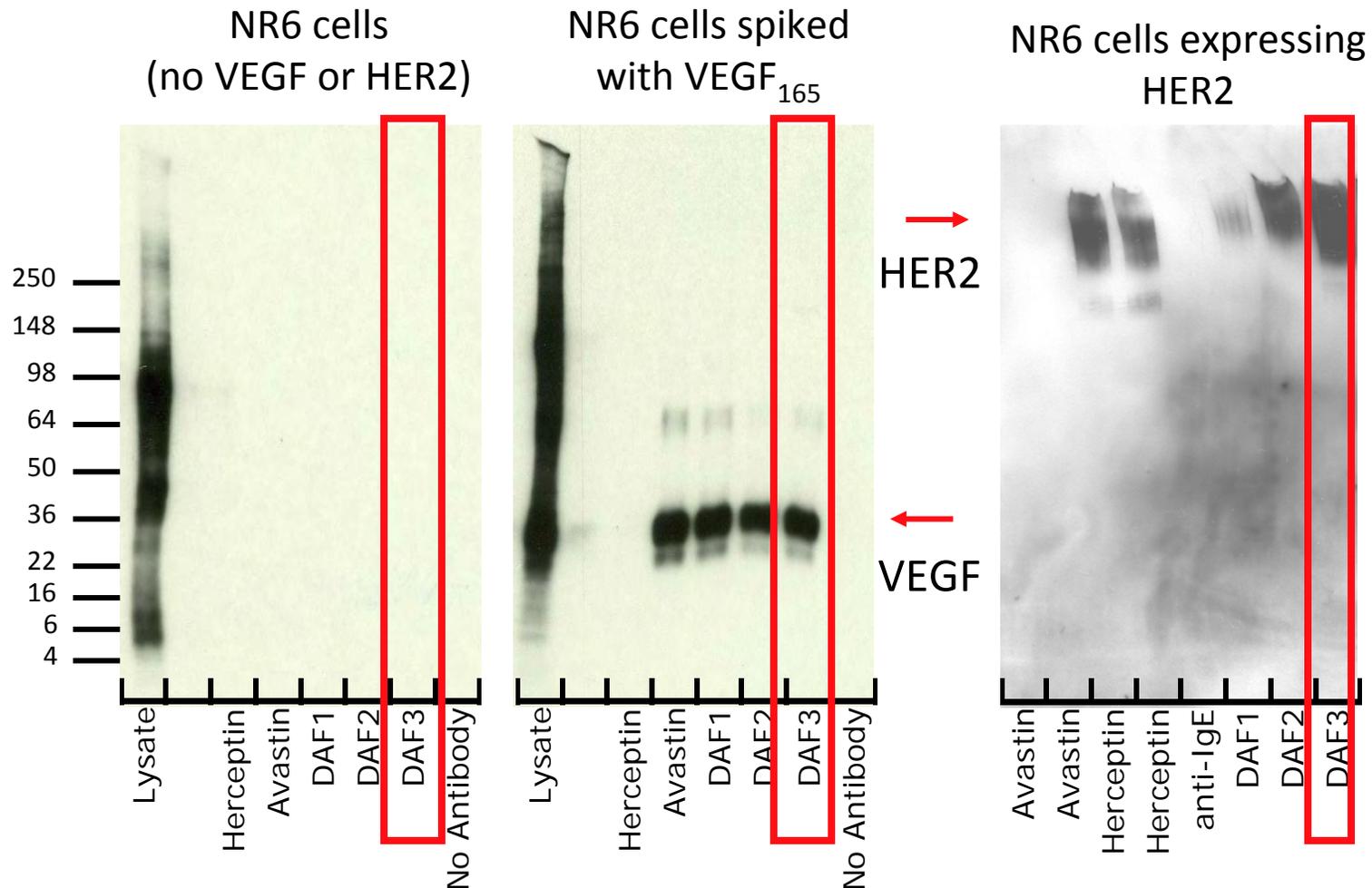
Kd = 300 → 3 nM

HER2-expressing BT474 cells

VEGF-stimulated HUVEC cells



Improved two-in-one anti-HER2/VEGF highly specific



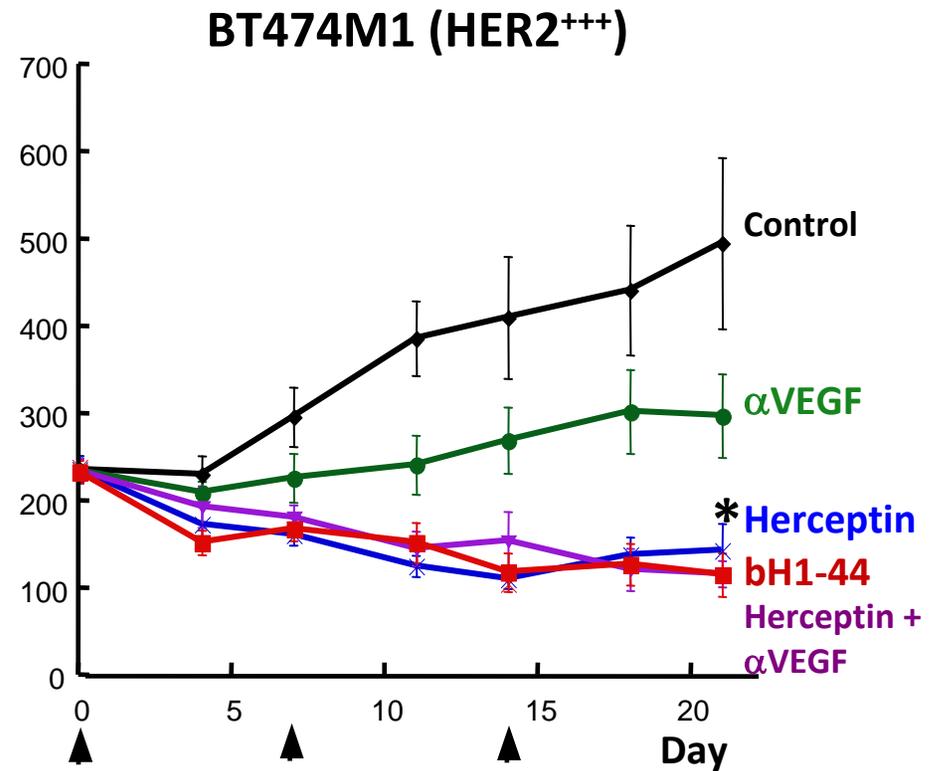
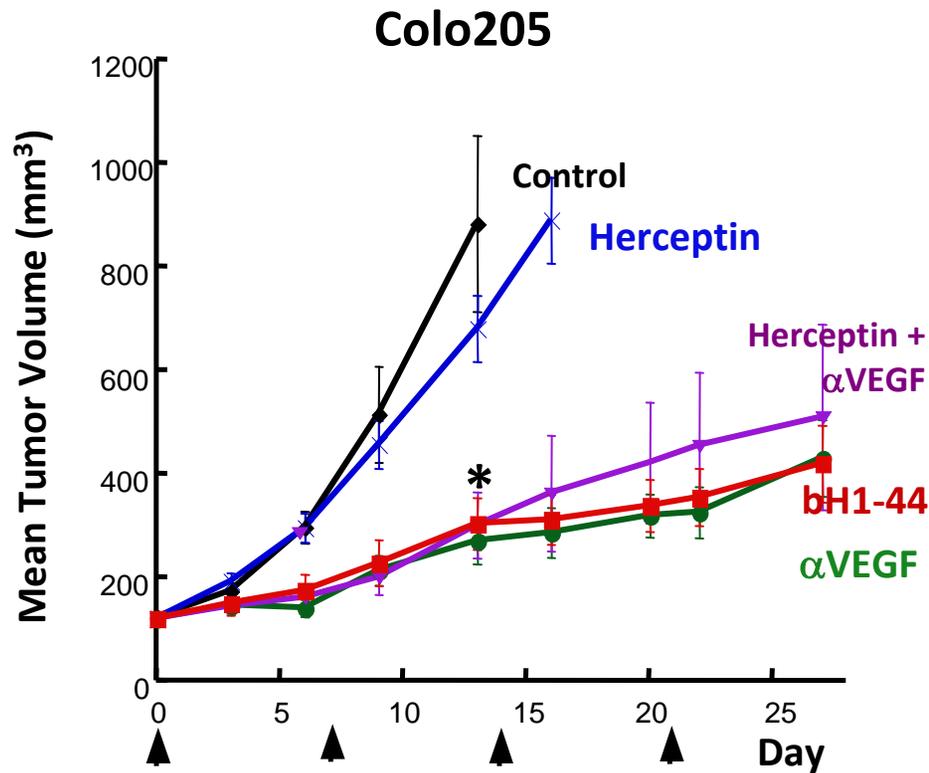
Specificity also confirmed with FACS on cells, proteomic array and the expected behavior in cell assays and rodent PK

High affinity bH1-44 *in vivo*

Inhibits VEGF and HER2-mediated xenograft growth

Anti-VEGF activity

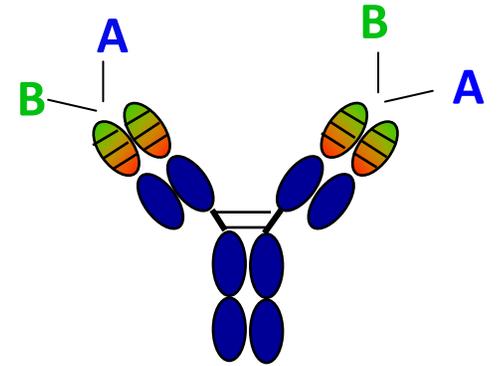
Anti-HER2 activity



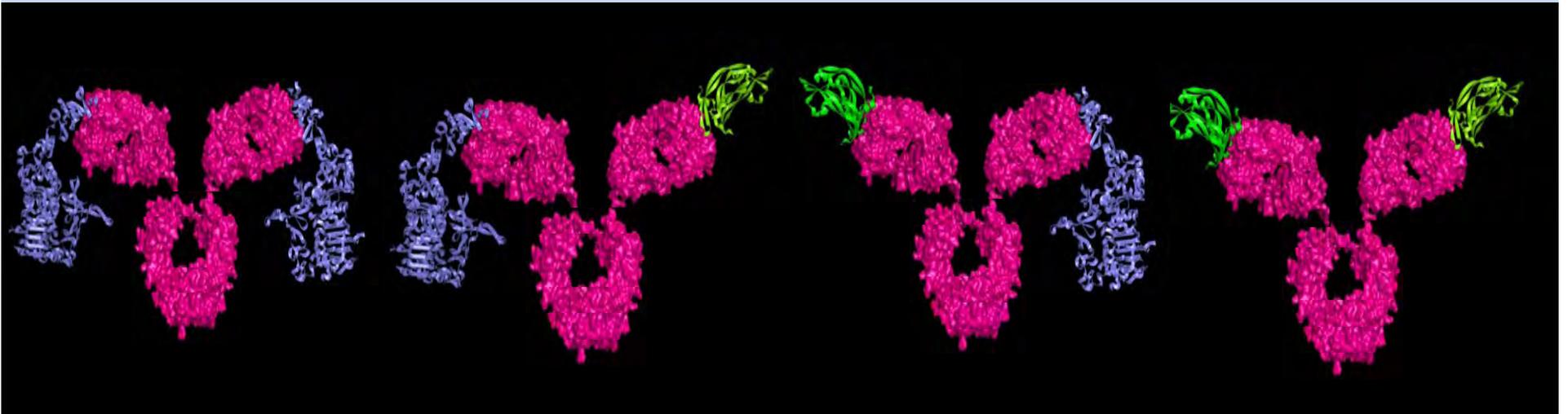
Each antibody 10mg/Kg weekly
 bH1-44 PK similar to anti-VEGF, B20-4.1

Two-in-One HER2/VEGF Antibody Summary

- *Two antigens bind to nearly identical site*
- *Distinct interactions, HC and LC work together*
- *Potent dual action in vitro and in vivo*
- *One antibody is capable of binding two unrelated antigens with high affinity*

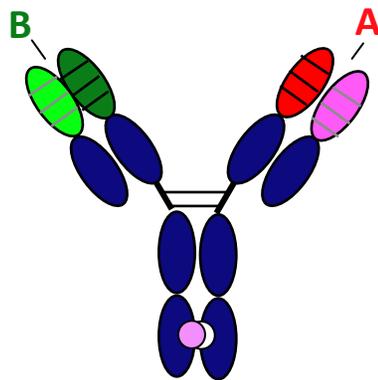


- *A general approach to make other dual action antibodies?*



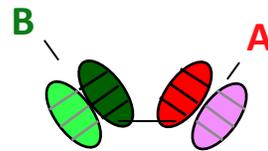
Options to target more than one molecule

'Two-in-One' is conventional IgG



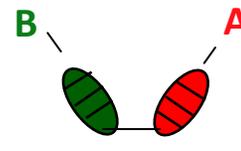
Knob-Hole Ig

Genentech



BiTE

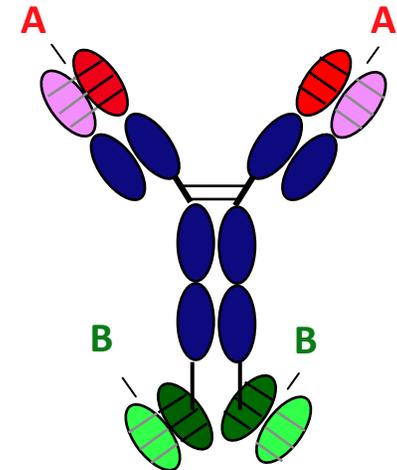
Micromet



dAB

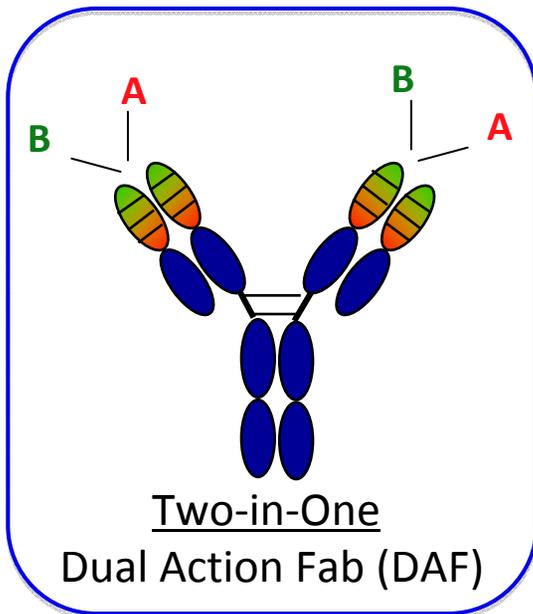
Domantis

GSK



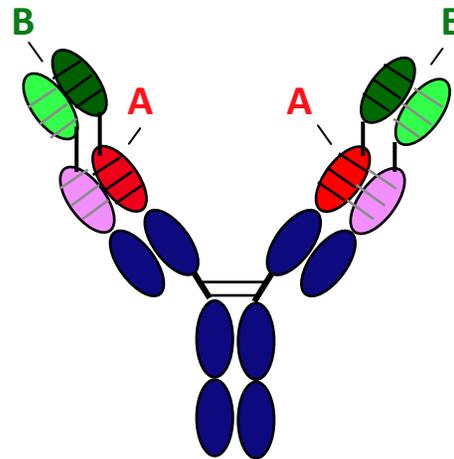
scFv-IgG

Biogen-Idec



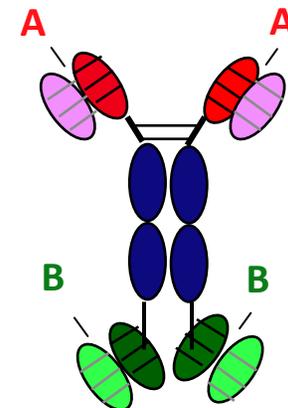
Two-in-One

Dual Action Fab (DAF)



DVD

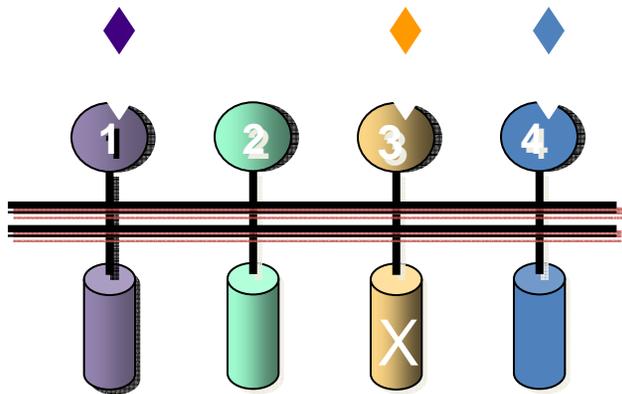
Abbott



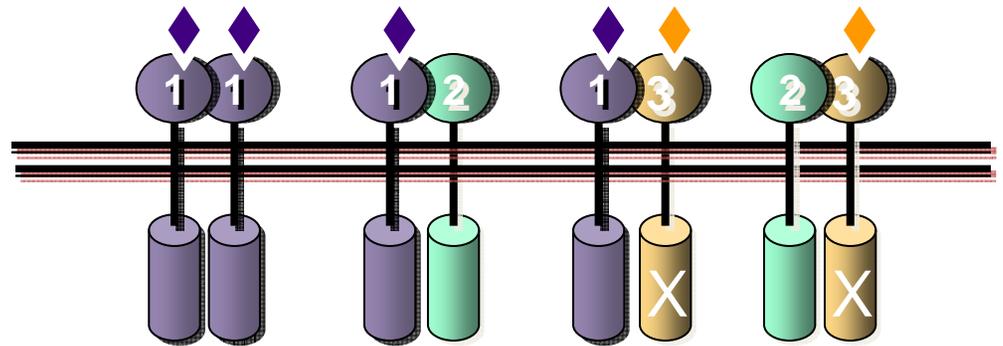
Scorpion

Trubion

Targeting erbB/HER family with Two-in-One antibody



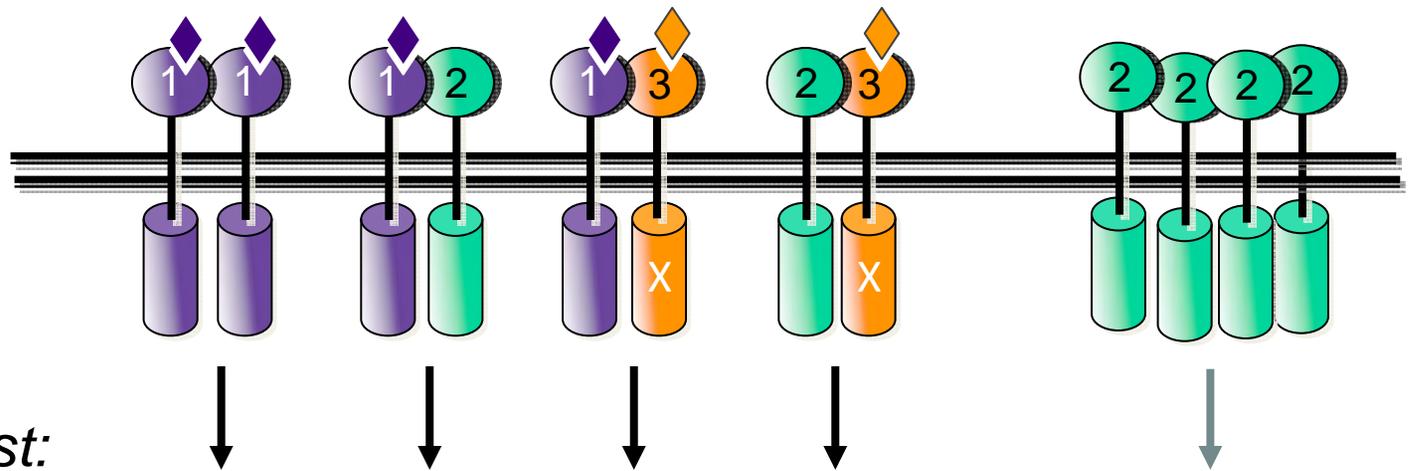
HER1 HER2 HER3 HER4
(EGFR)



Major signaling pairs in cancer

Combined blockade of EGFR and HER3 inhibits all major HER family signaling pairs

Ligand dependent dimers



Drugs against:

EGFR, Mab, SMi	✓	✓	✓	
HER2, Mab, SMi		✓		✓
HER3, Mab			✓	✓

✓ ✓ ✓ ✓

Trastuzumab

EGFR/HER3 Two-in-One antibody generated

HC CDR phage libraries

EGFR Ab

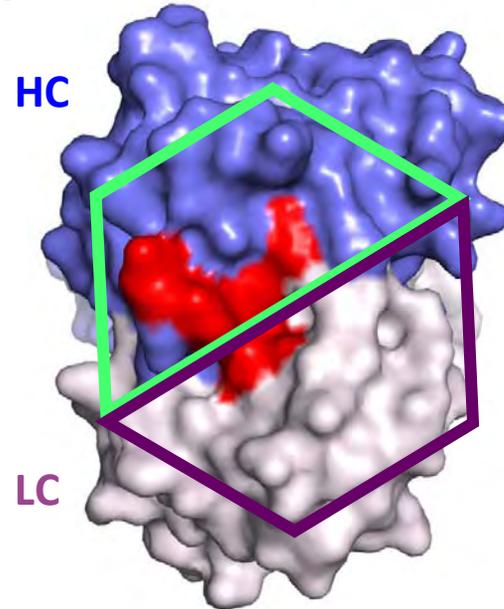
LC CDR libraries

EGFR/HER3 Ab1

Affinity improvement

EGFR/HER3 Ab2

DL11f or MEHD7950A



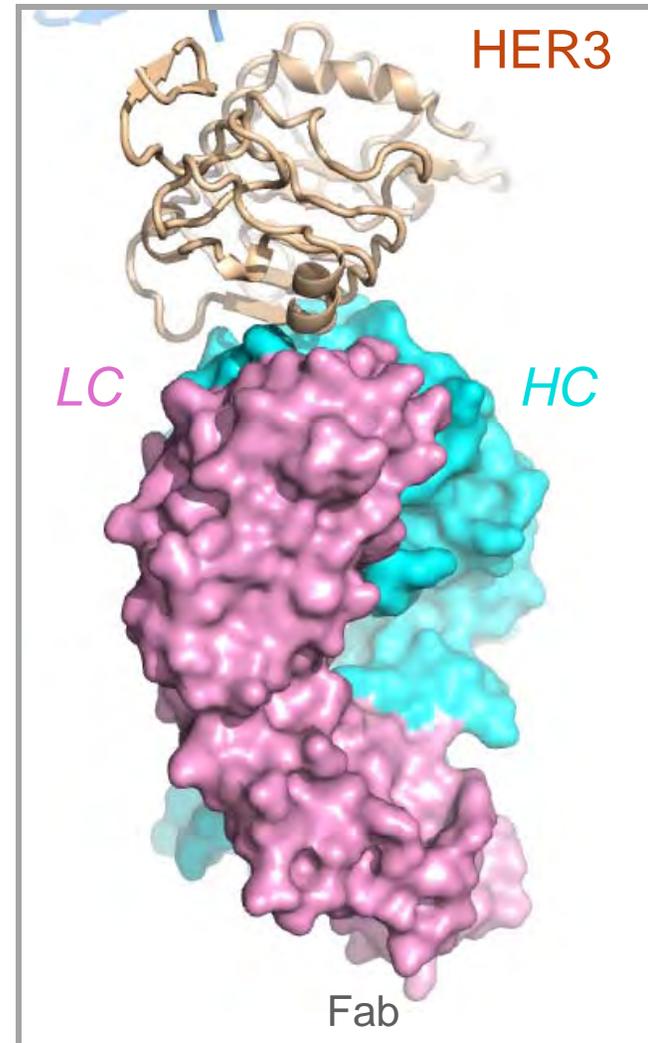
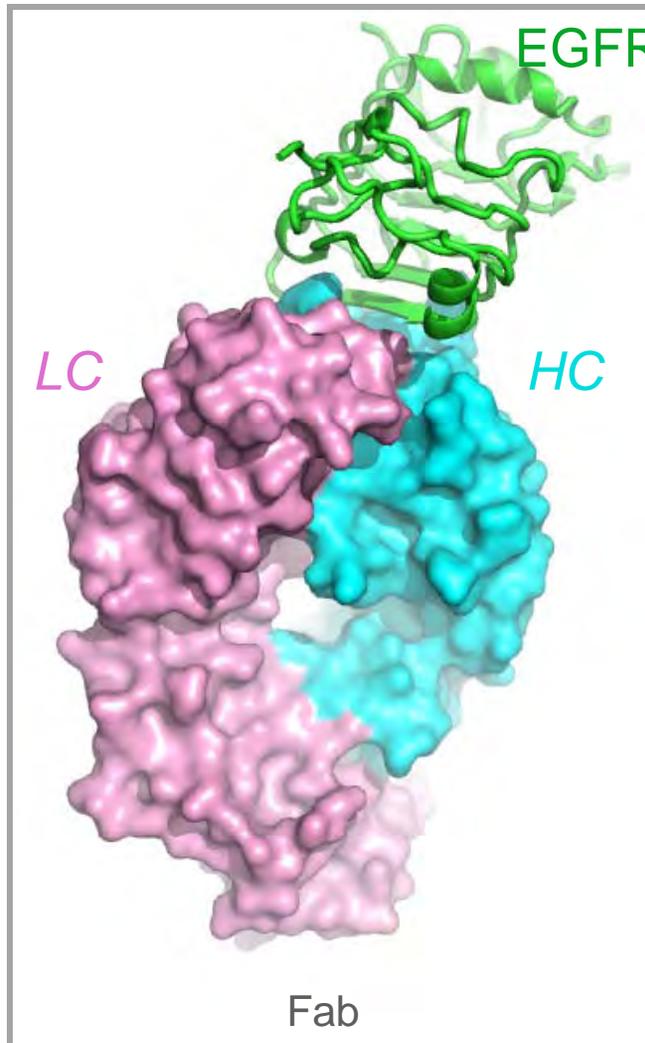
Blocks ligand binding to HER3

Blocks ligand binding to EGFR

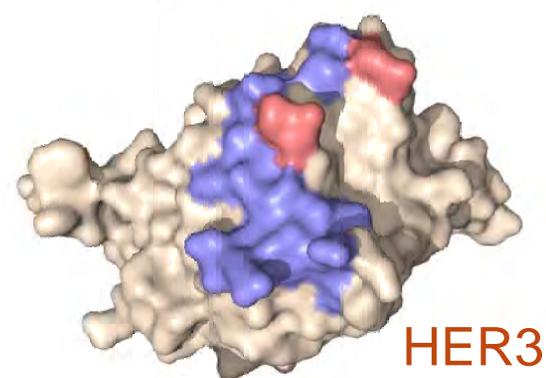
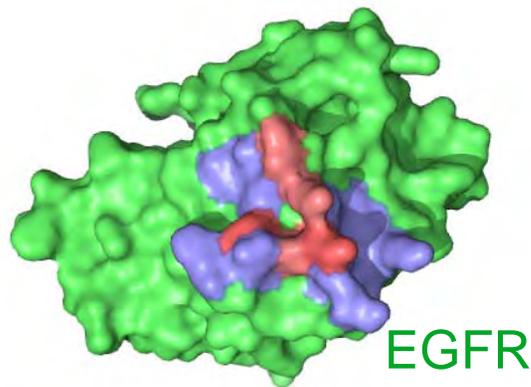
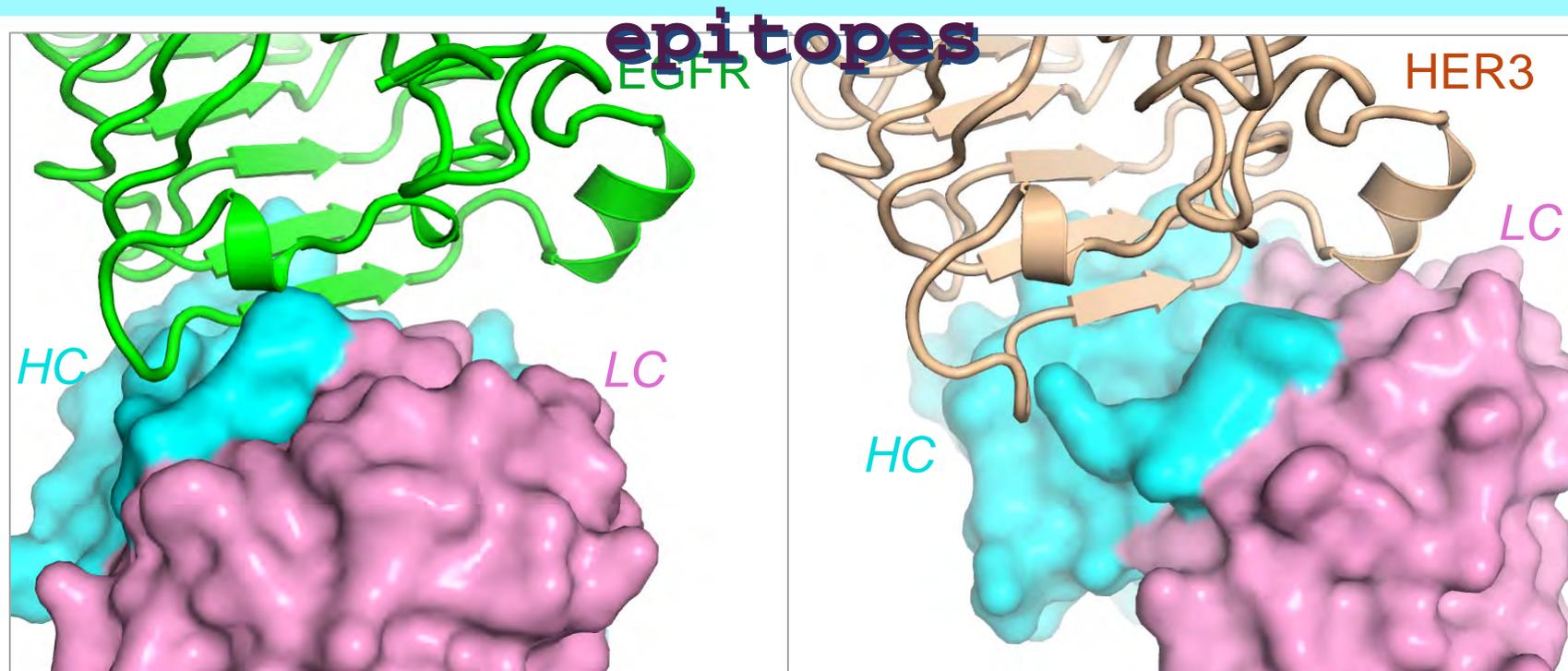
➤ K_d (hu HER3) = 0.39 nM

➤ K_d (hu EGFR) = 1.9 nM

Crystal structures of DL11f with EGFR or HER3 indicate distinct epitopes

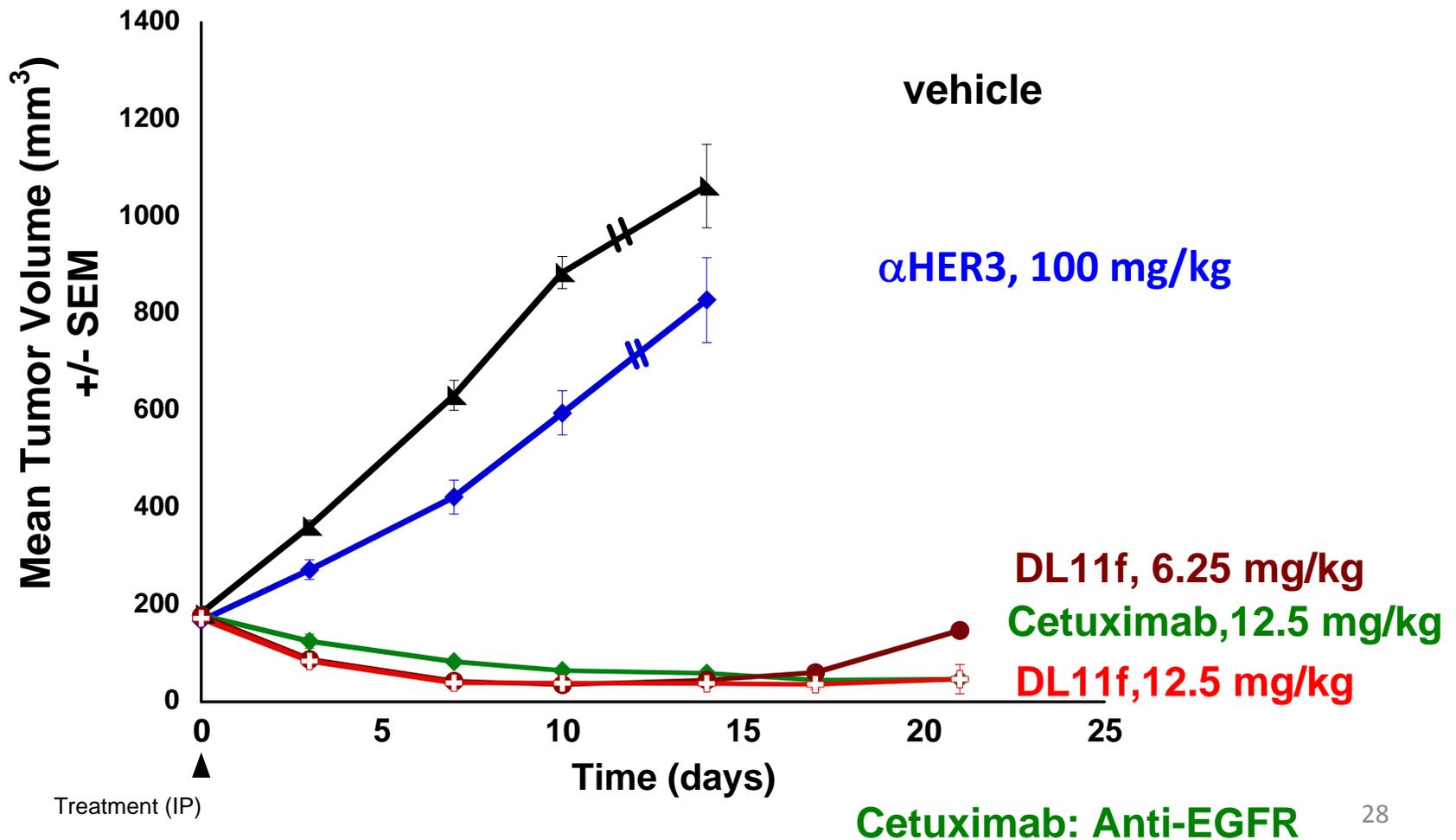


Crystal structures of DL11f with EGFR or HER3 indicate distinct



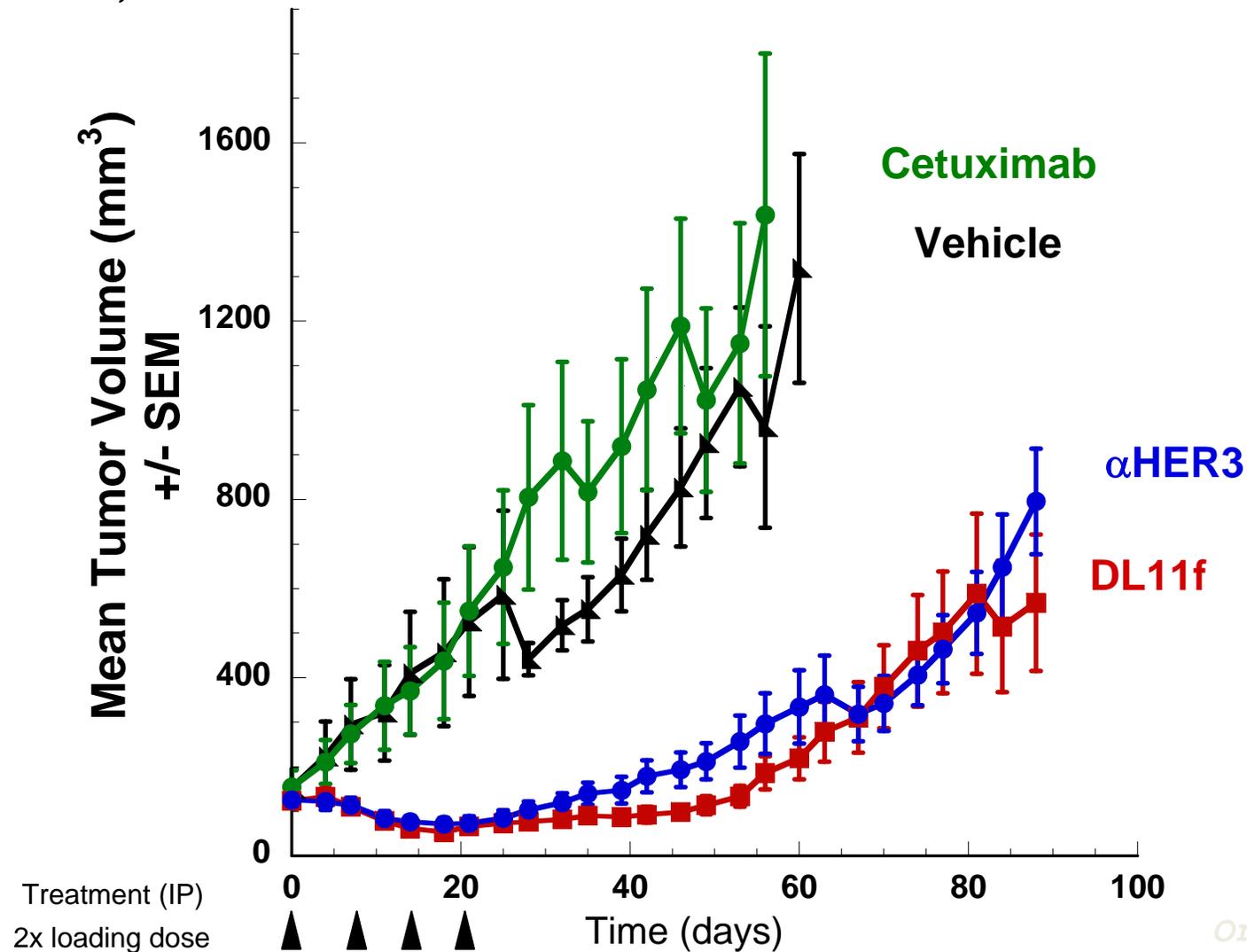
Potent Inhibition of Tumor Growth in EGFR Dependent NSCLC Model

- *H292, NSCLC model*



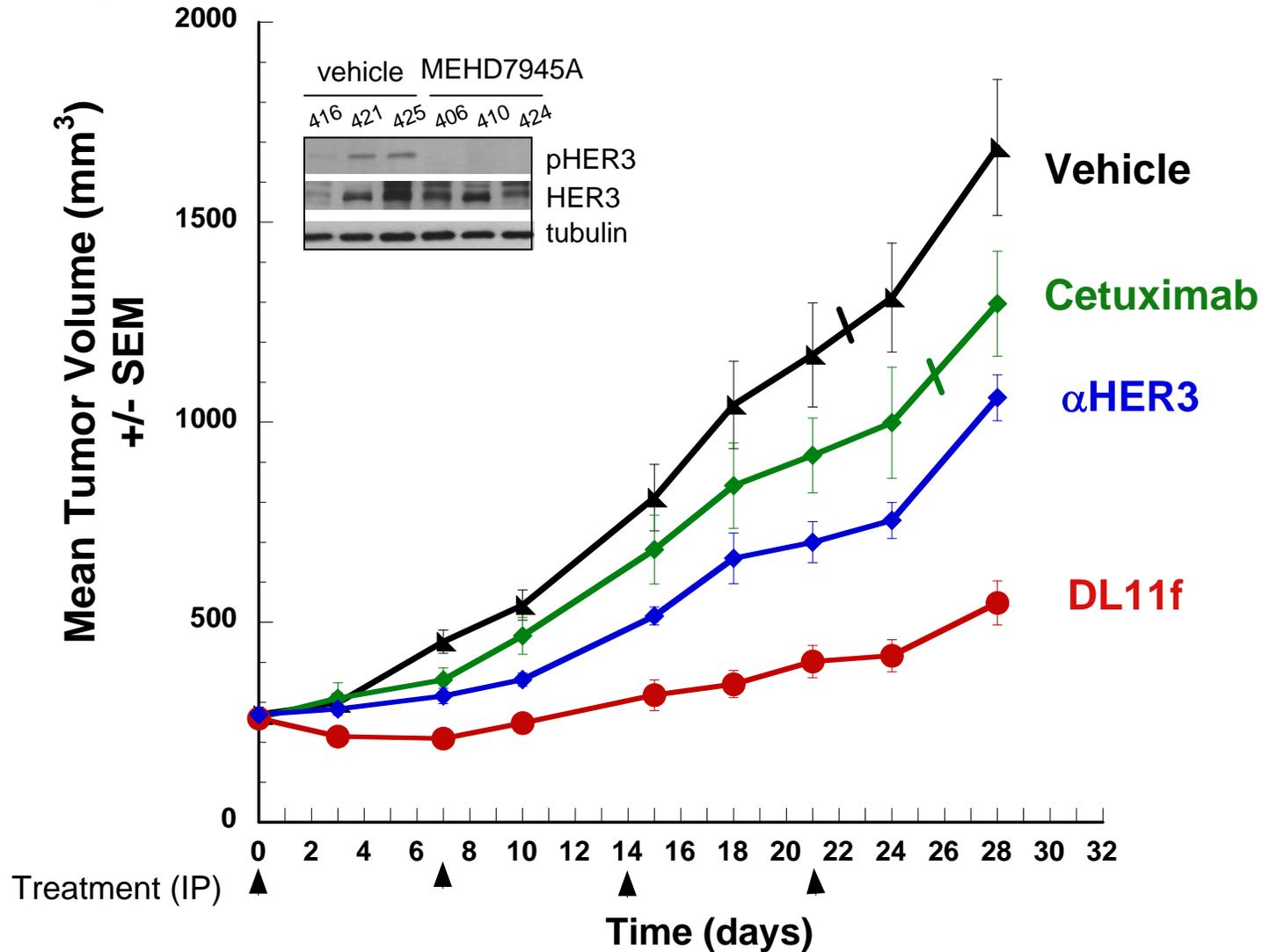
Potent Inhibition of Tumor Growth in HER3 Dependent Breast Cancer Model

- MAXF449, breast model



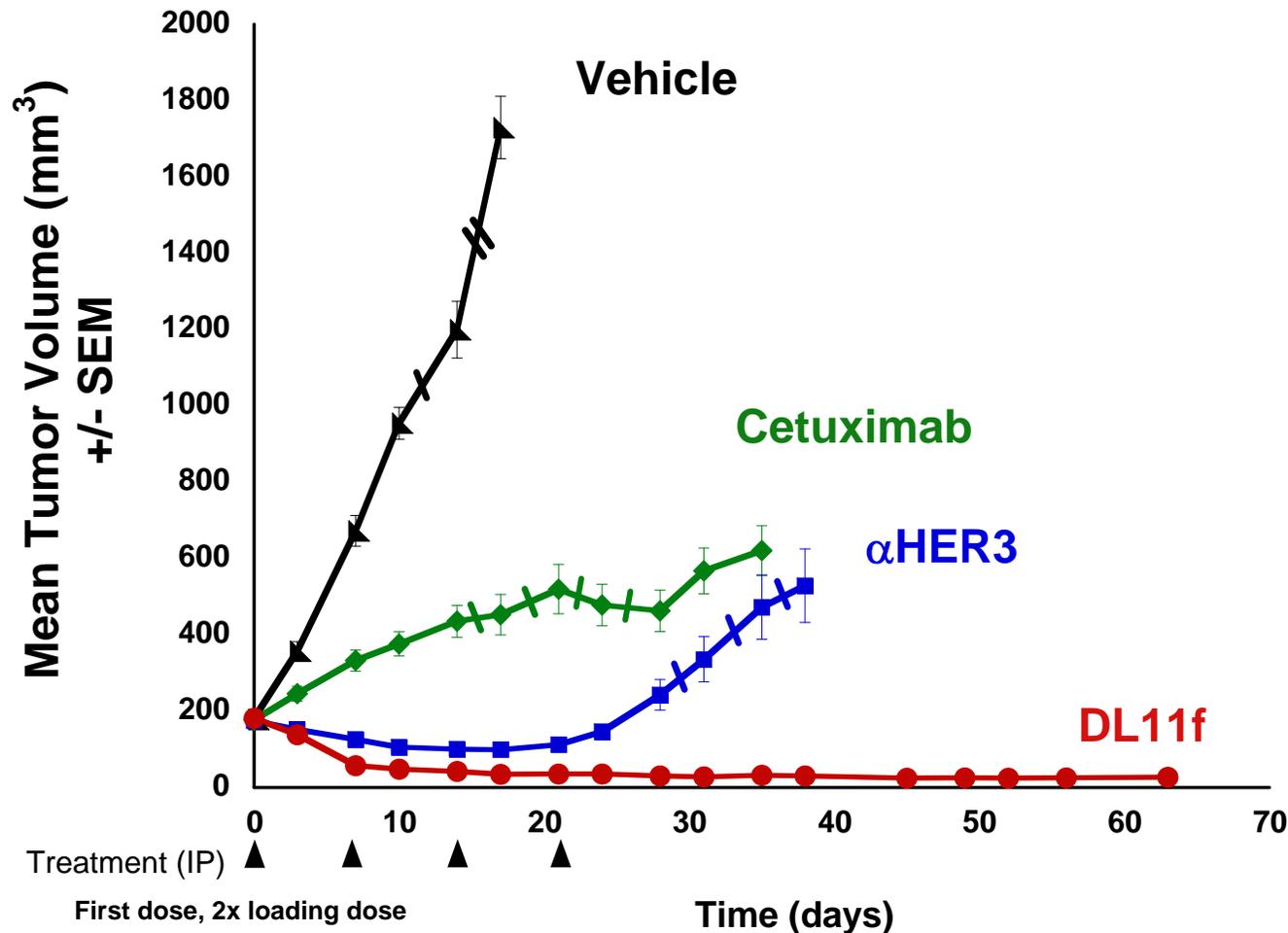
Increased Efficacy Compared to Mono-specific Antibodies

- *BxPC3, pancreatic model*



Increased and Prolonged Efficacy in FaDu model

- *FaDu, HNSCC model*



Does Dual Function of DL11f Increase Toxicity?

Skin rash is a major toxicity for EGFR inhibitors

- Cetuximab
- Panitumumab
- Erlotinib
- Gefitinib

Does combined inhibition of EGFR and HER3
elevate adverse reactions?

- no reported skin toxicities with anti-HER3 to date

Pilot Toxicity Study in Cynomolgus Monkeys: DL11f Compared to Cetuximab

- Study Objectives:

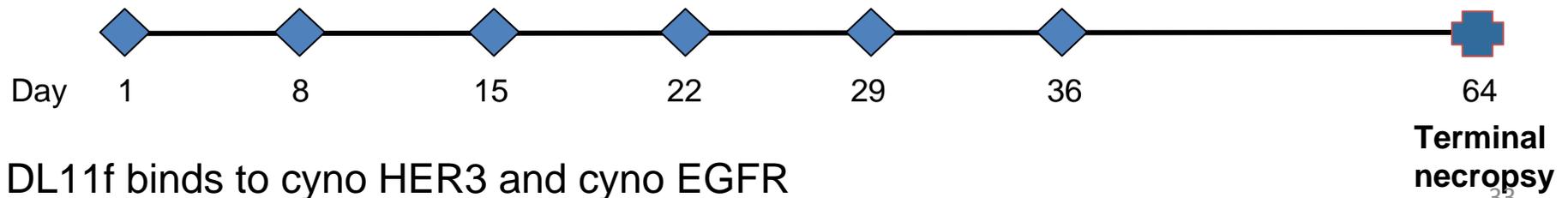
- To determine the initial safety profile of DL11f
- To compare the relative toxicity of Cetuximab with DL11f

Dose Selection:

- ❖ Group 1: Cetuximab 25 mg/kg
- ❖ Group 2: MEHD7945A 25 mg/kg
- ❖ Group 3: MEHD7945A 12.5 mg/kg

Schedule: qwx6; iv

Followed by a 4 wk recovery



Reduced Skin Toxicity with DL11f Relative to Cetuximab in Cynomolgus Monkeys

- DL11f is well-tolerated dosed up to 25mg/kg weekly (X6)
- All cynos dosed with cetuximab developed skin lesions between the 3rd and 4th dose administration
- Only one animal dosed with DL11f (25 mg/kg) developed skin lesions one week after the last (6th) dose

In conclusion:

DL11f had delayed onset, lower incidence and reduced severity of skin rash compared to cetuximab at equal dose and exposure

EGFR/HER3 two-in-one antibody summary

- Novel, first-in-class antibody, binds to HER3 or EGFR with either Fab
- Blocks ligand binding to HER3 and EGFR and signaling downstream of receptors
- Shows broader activity *in vitro* and *in vivo* compared to monospecific antibodies
- Shows significant activity in colon, lung, pancreatic, HNSCC, breast and ovarian xenograft models
- Pilot study shows reduced skin toxicity with MEHD relative to cetuximab in cynomolgus monkeys, and no new toxicities were identified
- MEHD7945A (= DL11f) IND approved

Acknowledgements

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Jeffery Lau

Karen Billeci

Frank Peale

Gail Phillips

Sarajane Ross

Gabriele

Schaefer

Lauric Haber

Charlie

Eigenbrot

Lily Shao

Lisa Crocker

Don Dowbenko

Rob Akita

Steven Shia

Robyn Clark

Leanne Berry

Klara Totpal

Michelle Butler

Rod Prell

Dimitri

Danilenko

Amrita Kamath

Anne Wong

Karen Billeci

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Sumati Murlidhar