Applied Molecular Evolution

AME-133: A Next-Generation Anti-CD20 Engineered for Enhanced Killer Function

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Jim Breitmeyer

Lilly
Answers That Matter.

Antibody Therapy of Lymphoma

- **Rituximab as first-generation biotherapeutic**
  - **Efficacious in chemoresistant NHL**
  - **Less toxic than most chemotherapies**
  - **Multiple potential antitumor activities**
    - **CDC- Complement dependent cytotoxicity**
    - **ADCC- Antibody dependent cytotoxicity**
    - **Direct apoptotic effect (with cross linking)**
- **Opportunities for improvement**
  - **Chimeric mouse/human structure**
  - **Modest affinity for CD20**
  - **Role of host immune system in efficacy**
    - **FcR influences response**

Influence of FCγRIIIa Polymorphism on Rituximab Efficacy

- **Clinical and molecular response to rituximab in chemo-naive follicular NHL**
  - **Cartron, Blood. 2002; 99:754-758**
- **Clinical response to rituximab in relapsed follicular NHL**
  - **Weng, JCO 2003; 21:3940-47**
- **Clinical response to rituximab in Waldenstrom's macroglobulinemia**
  - **Treon, ASH 2002, Poster #2002**
- **B cell depletion in SLE**
  - **Anolik, Arth Rheum. 2003; 48:455-459**

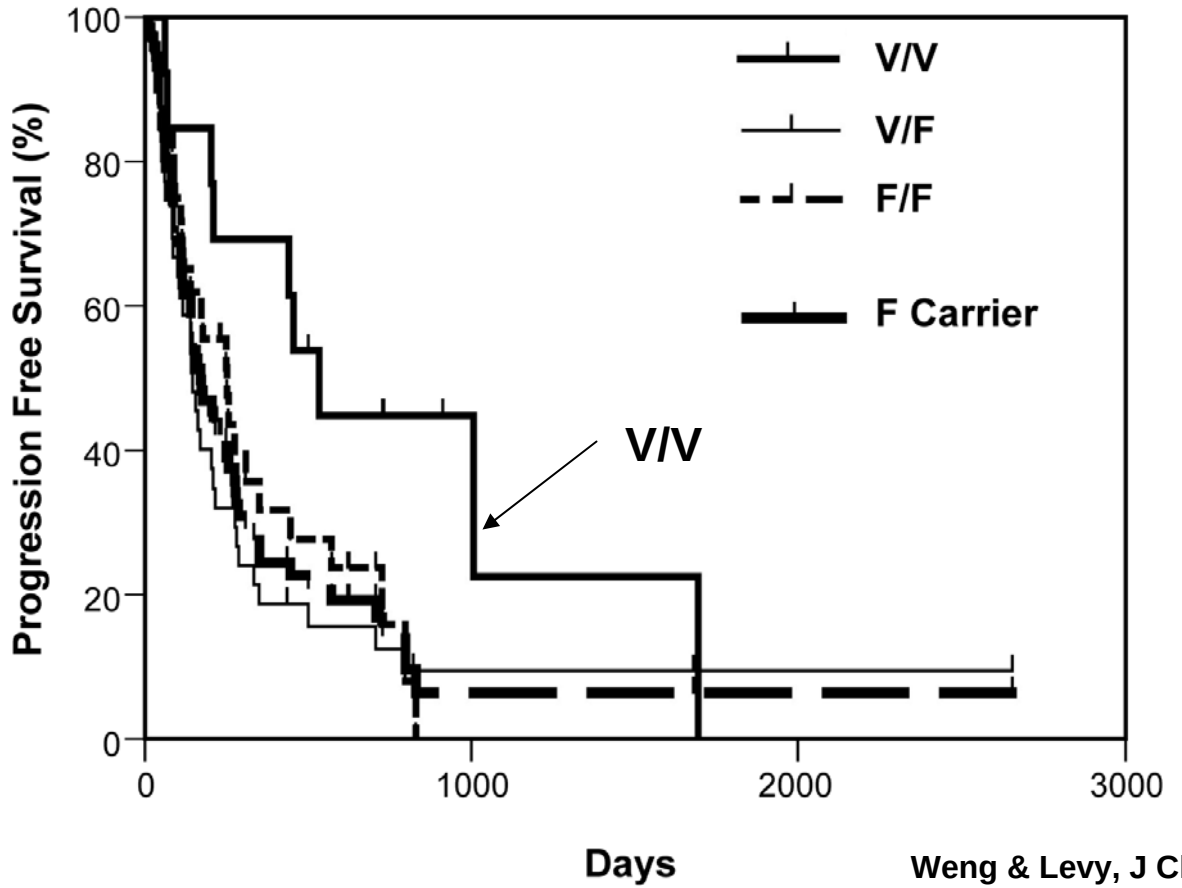
Effect of FCγRIIIa (CD16) Genotype on Rituximab Therapy of NHL

	FCγRIIIa 158 Genotype	
	VV	VF or FF
Prevalence n (%)	13(15)	74(85)
PR+CR M1-3 %	92*	59
PR+CR M6 %	85*	45
PR+CR M9 %	75*	36
PR+CR M12 %	75*	26

*p < 0.05 VV vs. VF+FF

Weng & Levy, J Clin Onc 21:3940-47, 2003

Progression Free Survival After Rituximab Correlates with FcγRIIIa Genotype



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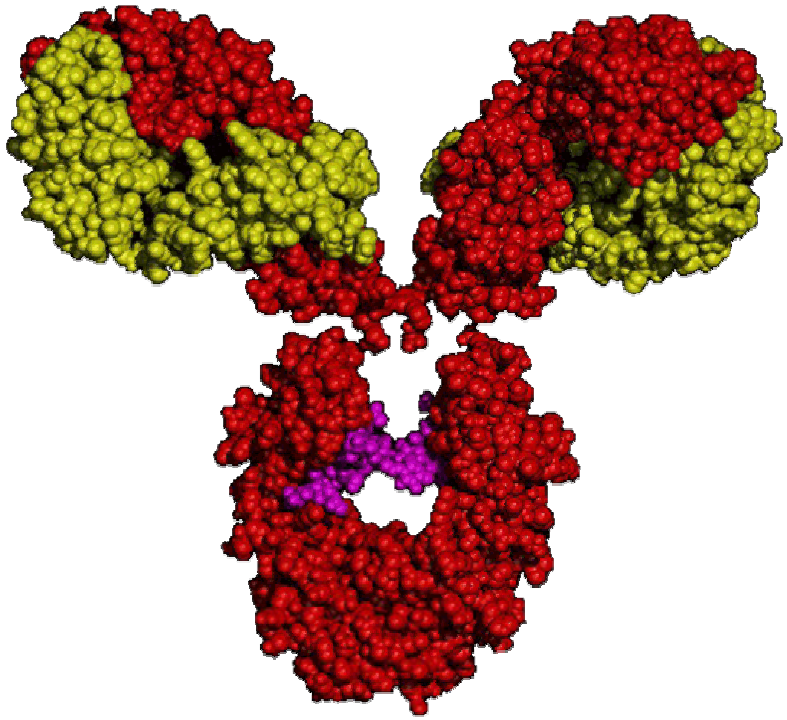
AME & Antibody Engineering Opportunities

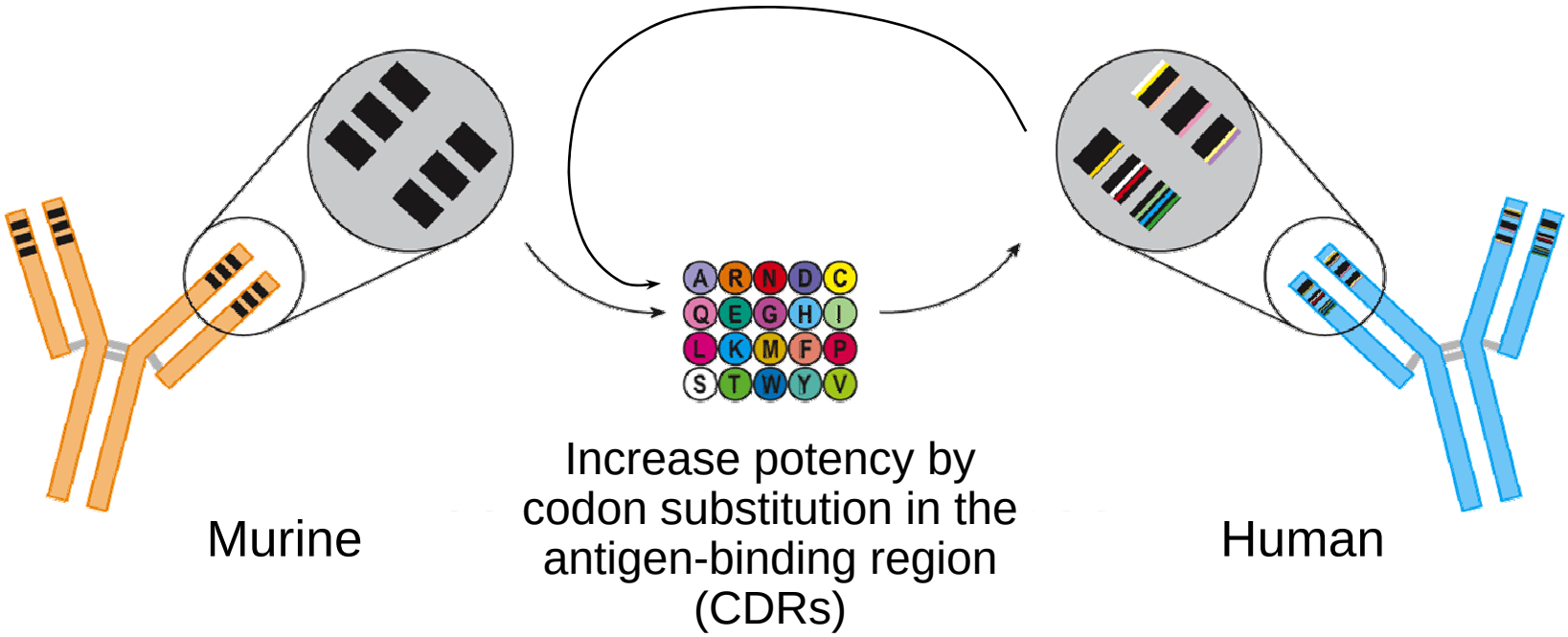


Addressed in anti-CD20 engineering

- Potency
  - Association rate
  - Dissociation rate
  - Complement dependent cytotoxicity
  - Cell mediated cytotoxicity (ADCC)
  - Half-life
- Immunogenicity
  - Framework chimeric residues
  - Somatic mutations (framework, V/C)
- Specificity (epitope, cross-reactions)
- Pharmaceutical properties
  - Oxidation sites
  - Deamidation sites
  - Glycosylation sites
  - Protease sites
  - Solubility
  - Production
  - Cost of facilities and goods

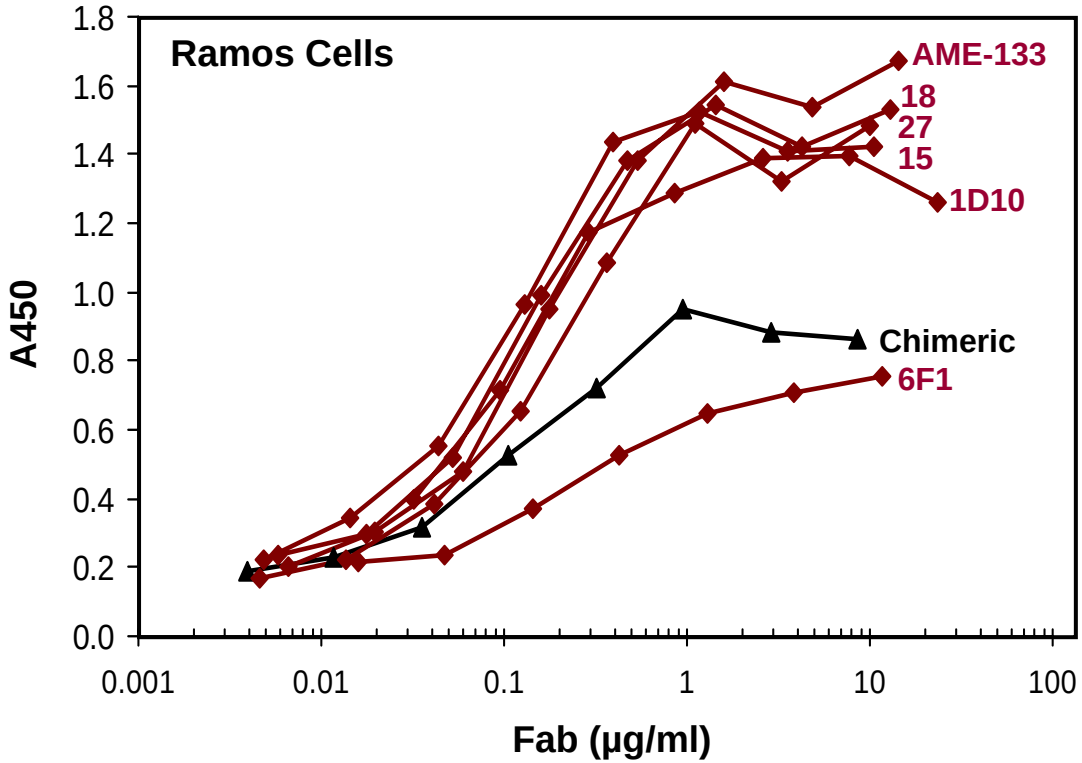
AME Antibody Optimization Technology

- **Generate DNA library with directed variability**
- **Express protein library**
- **Screen for desired activity**
- **Iterative cycles to optimize**



Anti-CD20 Antibodies with Fully Human Frameworks and Increased Affinity

- **Multiple high affinity variants identified**
  - **Variants provide tool for characterizing impact of affinity on ADCC, CDC, and apoptosis**
- **Fully human, common germline frameworks**



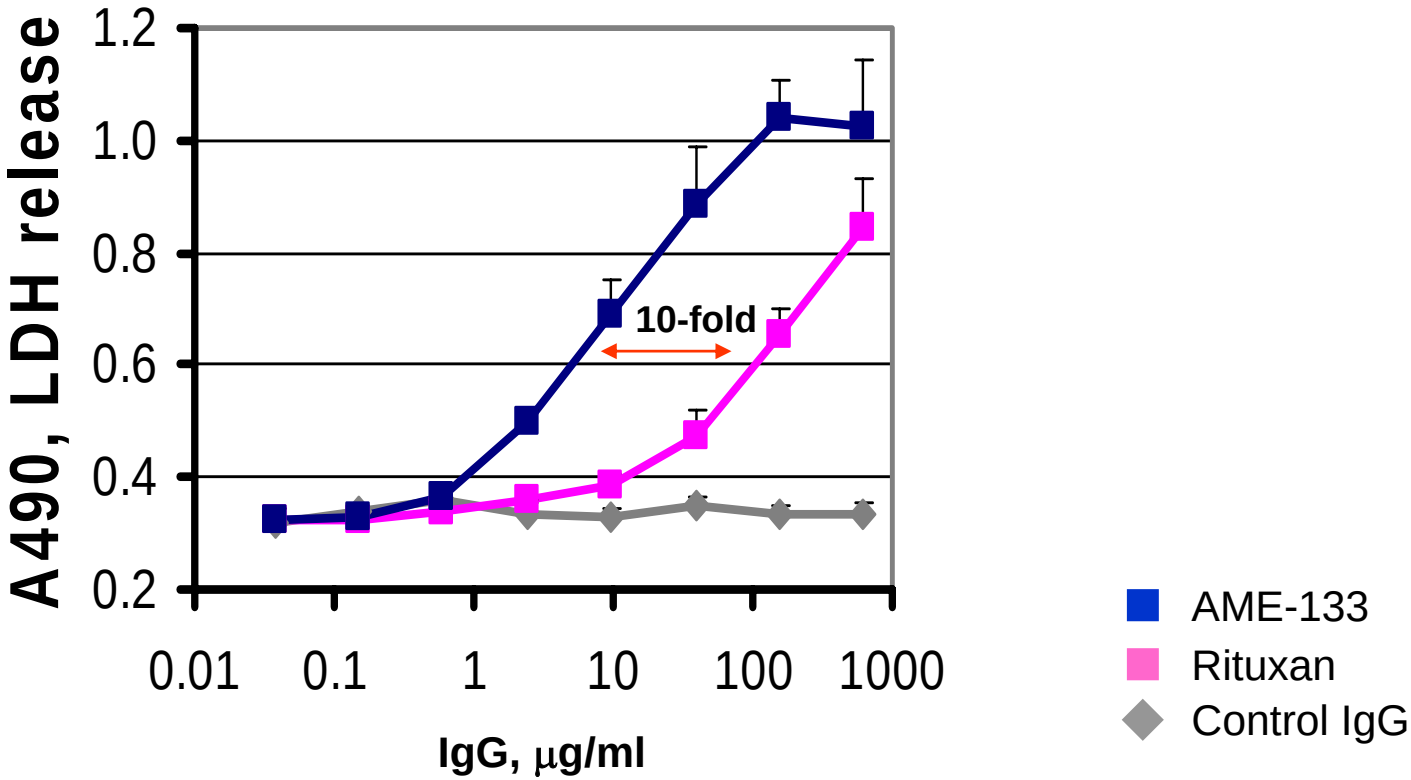
Fc Optimization - Cell Based Primary Screening with Human PBMC

- **>2,400 variants screened**
- **Multiple novel variants identified**

% of Wild Type (Average)

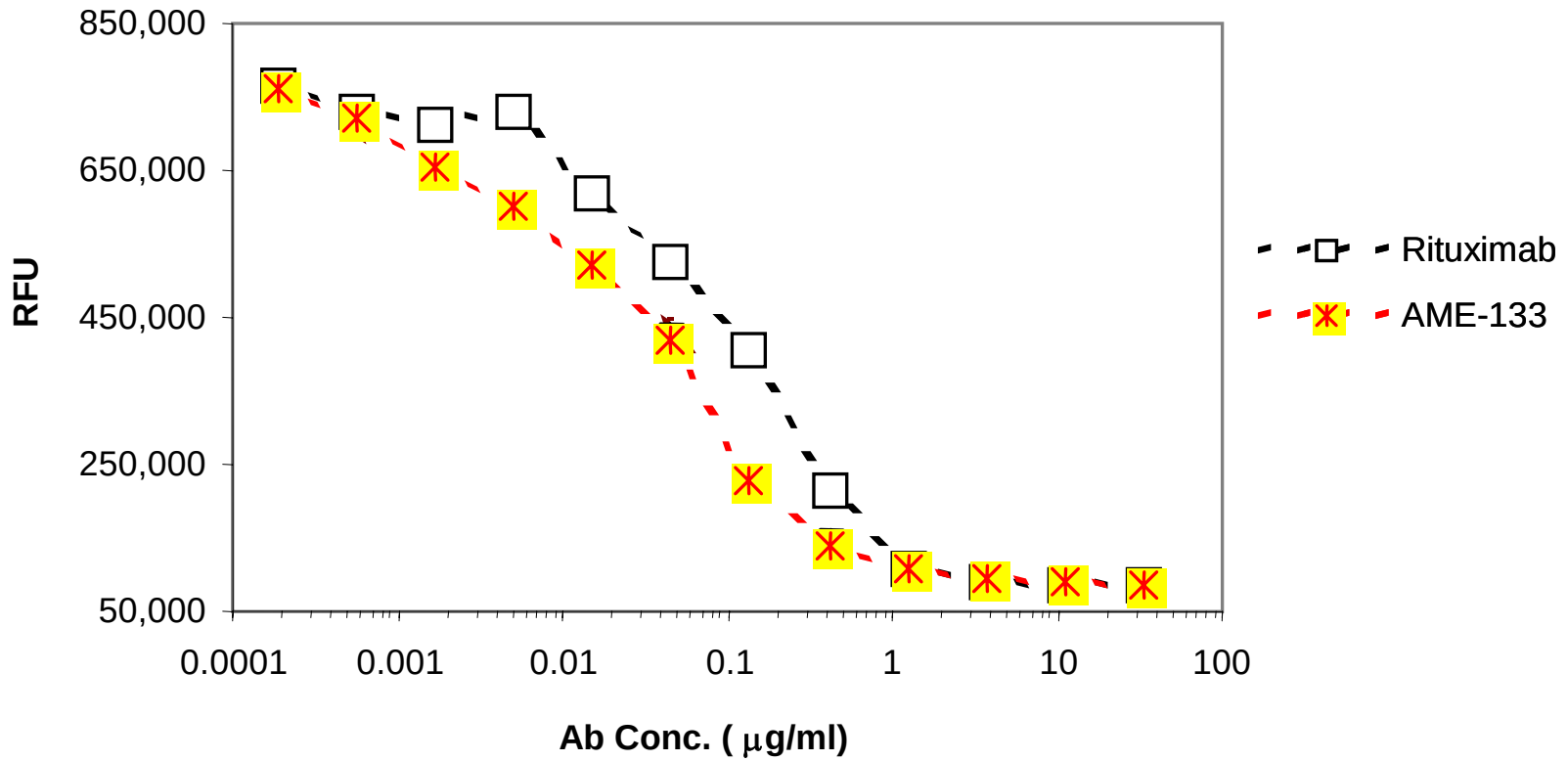
	1	2	3	4	5	6	7	8	9	10	11	12	
A			38.62	109.25	19.00	38.62	71.63	7.10	7.54	93.83	100.18		
B			-2.51	0.68	38.19	3.19	-6.19	50.48	102.02	99.37	72.03		
C			87.92	64.14	-4.60	73.30	77.68	77.89	70.41	93.29	93.95	93.76	1x wt
D			62.60	101.20	98.51	98.33	-9.62	91.19	114.40	83.05	101.06	97.76	1x wt
E			-0.48	-1.16	-4.58	-3.91	88.42	100.77	99.43	-3.91	-4.82	102.24	1x wt
F			-27.15	106.57	-0.27	113.76	-1.67	2.70	114.73	-4.14	107.90	155.00	2x wt
G			28.79	117.16	117.76	115.95	47.76	93.50	**137.20**	108.87	102.25	177.36	4x wt
H			118.66	112.14	118.67	**122.80**	113.87	108.37	49.71	70.45	114.88		

AME-133 is More Potent than Rituxan in *ex vivo* ADCC



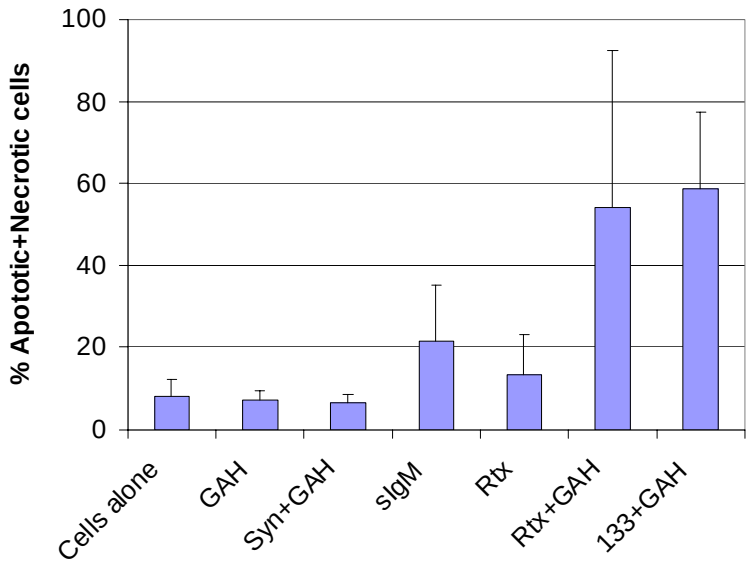
Human PBMCs and Wil-2S Target cells; mean +/- 1 SD

AME-133 Retains CDC Activity



Apoptosis

- **Rituxan induces apoptosis of Ramos cells (weakly) in the absence of antibody cross-linking**
- **Induction of apoptosis is enhanced significantly by secondary cross-linking reagents**
- **AME anti-CD20 variants induce apoptosis in the presence of secondary cross-linking reagents**



Summary

- **Multiple characteristics of proteins can be improved significantly through optimization**
  - **Improved efficacy, safety, and potency**
  - **Enhanced convenience**
  - **Decreased manufacturing costs**
  - **Broadened intellectual property**
  - **Increased understanding of biology**
- **AME-133, an optimized anti-CD20, will be tested for clinical activity in $CD20^+$ oncology indications**

Jeffry Watkins
Mary-Ann Campbell
Barrett Allan
David Marquis
Brian Ondek

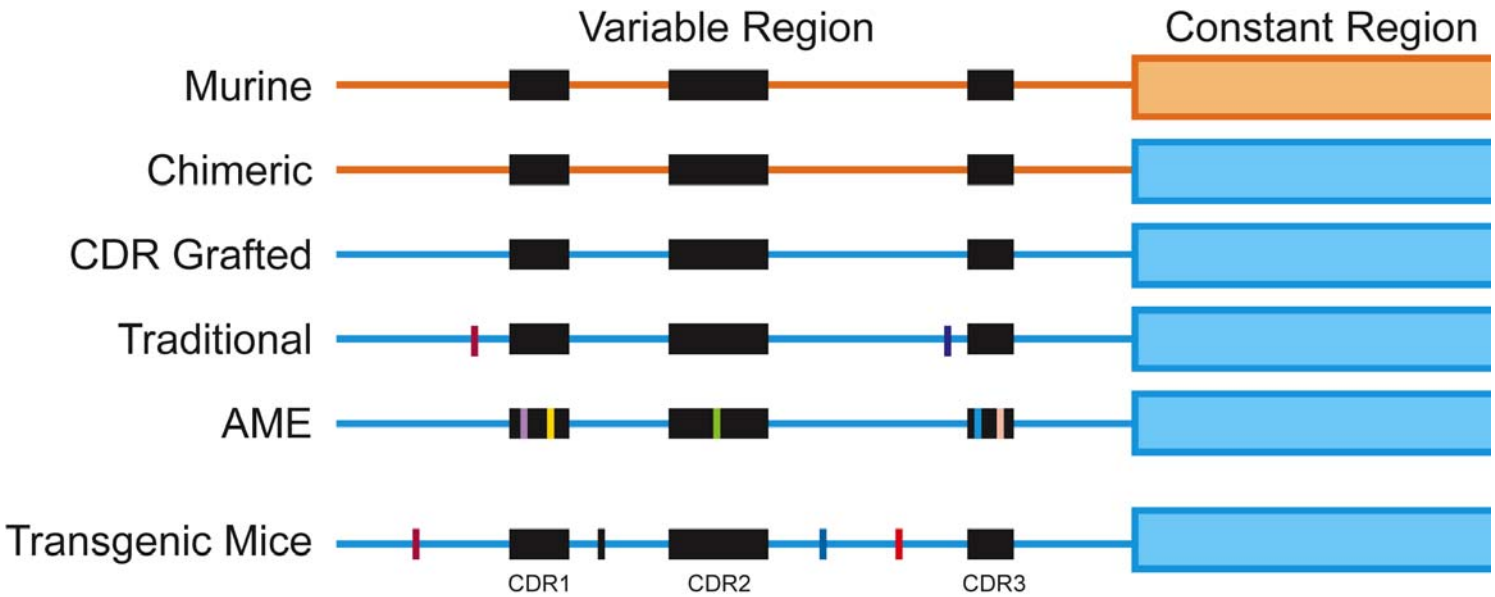
Christopher Slapak
Aaron Weitzman

Jim Breitmeyer
Chief Medical Officer
and Vice President, Pharmaceutical Operations
Applied Molecular Evolution / Eli Lilly
(858) 638-8511
jbreitmeyer@lilly.com

Answers That Matter.

Humanization of Antibodies

- **Reduces immunogenicity associated with murine Igs**
  - **CDR grafting typically diminishes affinity**
  - **Structural modeling is used to predict framework residues key for maintaining affinity**



AME- Applied Molecular Evolution

- San Diego directed evolution company founded in 1989
  - All classes of protein therapeutics engineered
- Partnerships with MedImmune, Centocor, Eli Lilly, Bristol-Myers Squibb, Chiron, Seattle Genetics, CancerVax, Biosynexus
- Application of the protein engineering to in-house projects
  - A development function was added to go from gene to clinic
  - AME-527 (TNF, inflammation) & AME-133 (CD-20, oncology)
- AME and Eli Lilly and Company performed multiple collaborative projects
  - Successes led to discussion on broadened collaboration
  - AME was acquired by Lilly in 2004