ESACT 2011 WORKSHOP C
Sunday 15 May 2011

Fc-Fusion Proteins: A Growing Class of Therapeutics

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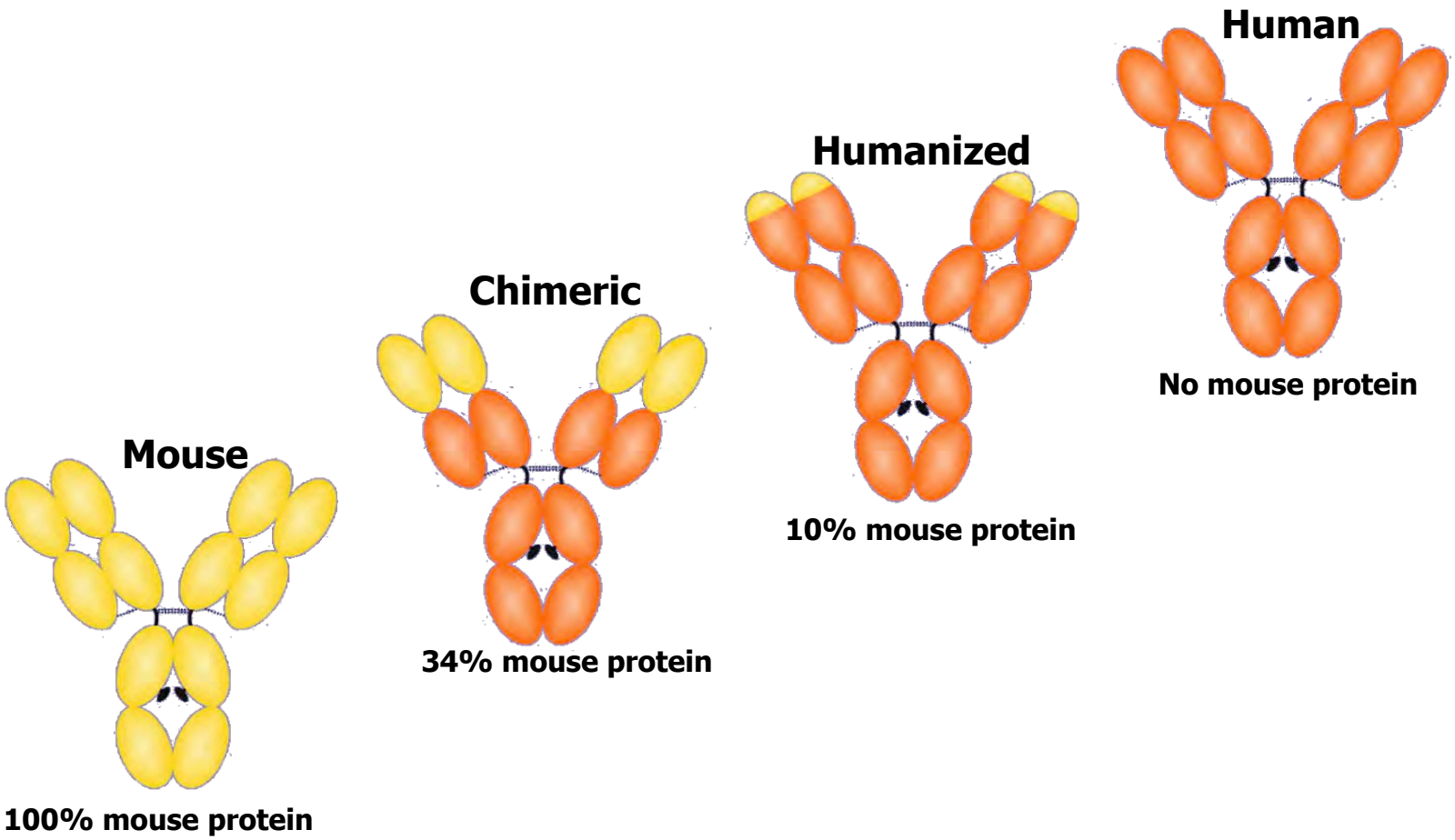
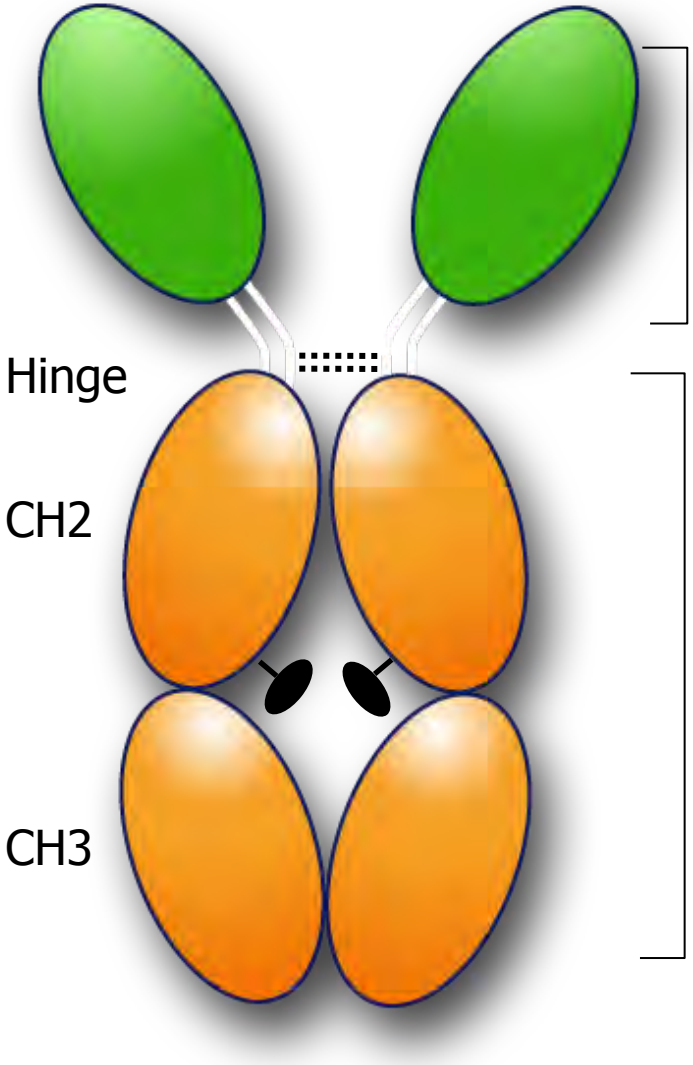
Workshop introduction

1975-1995: Combination of monoclonal antibody with genetic engineering technology



Fc-fusion protein (immunoadhesin)



Protein of interest

- Receptor
- Cytokine
- Enzyme
- Peptide

Fc region

FcγR binding	→	ADCC
C1q binding	→	CDC
FcRn binding	→	Half-life

1989 Report describing the first Fc-fusion protein CD4IgG as therapeutic

Designing CD4 immunoadhesins for AIDS therapy

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Capon *et al., Nature* **337**, 525-531 (1989)

Design of CD4IgG

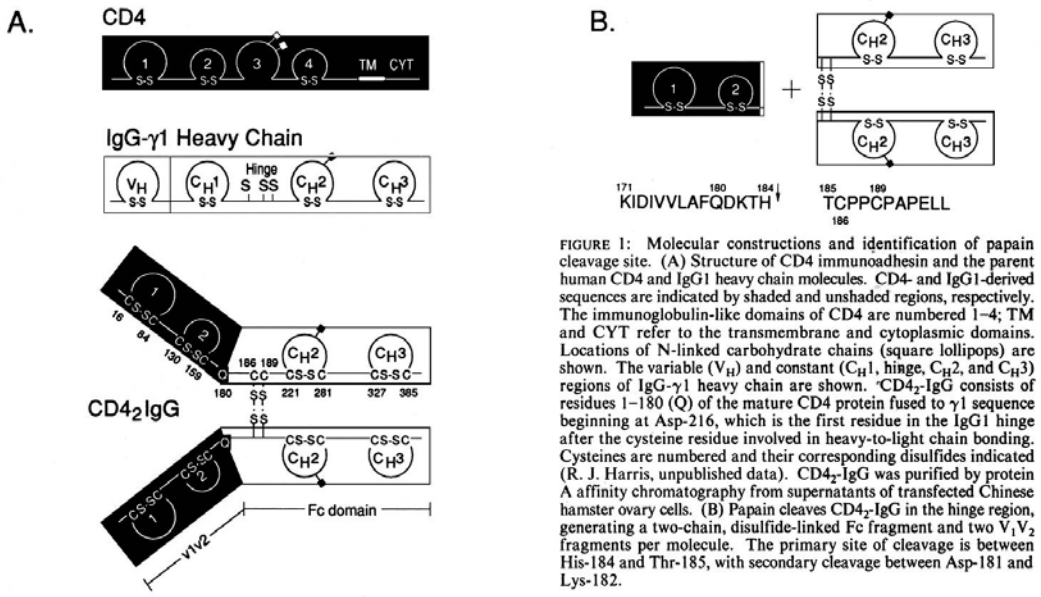
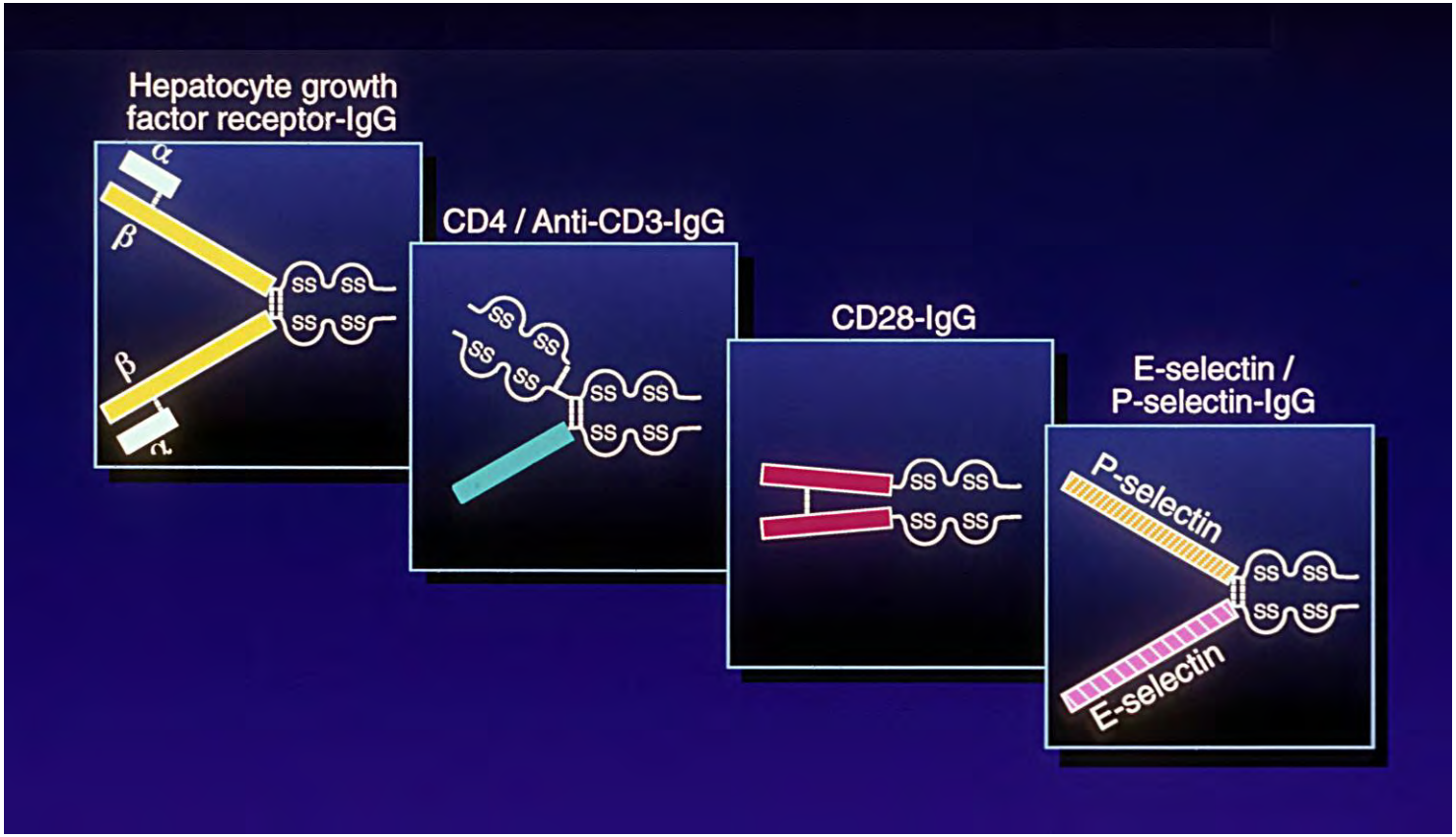


FIGURE 1: Molecular constructions and identification of papain cleavage site. (A) Structure of CD4 immunoadhesin and the parent human CD4 and IgG1 heavy chain molecules. CD4- and IgG1-derived sequences are indicated by shaded and unshaded regions, respectively. The immunoglobulin-like domains of CD4 are numbered 1–4; TM and CYT refer to the transmembrane and cytoplasmic domains. Locations of N-linked carbohydrate chains (square lollipops) are shown. The variable (V_H) and constant (C_H1, hinge, C_H2, and C_H3) regions of IgG-γ1 heavy chain are shown. $CD4_2$-IgG consists of residues 1–180 (Q) of the mature CD4 protein fused to γ1 sequence beginning at Asp-216, which is the first residue in the IgG1 hinge after the cysteine residue involved in heavy-to-light chain bonding. Cysteines are numbered and their corresponding disulfides indicated (R. J. Harris, unpublished data). $CD4_2$-IgG was purified by protein A affinity chromatography from supernatants of transfected Chinese hamster ovary cells. (B) Papain cleaves $CD4_2$-IgG in the hinge region, generating a two-chain, disulfide-linked Fc fragment and two V_1V_2 fragments per molecule. The primary site of cleavage is between His-184 and Thr-185, with secondary cleavage between Asp-181 and Lys-182.

- Assembles into a homodimer
- Retains fidelity of cleavage site by papain in hinge
- Binds C1q
- Binds Protein A
- Is transported across placenta

Chamow *et al., Biochemistry* **29**, 9885-9891 (1990)

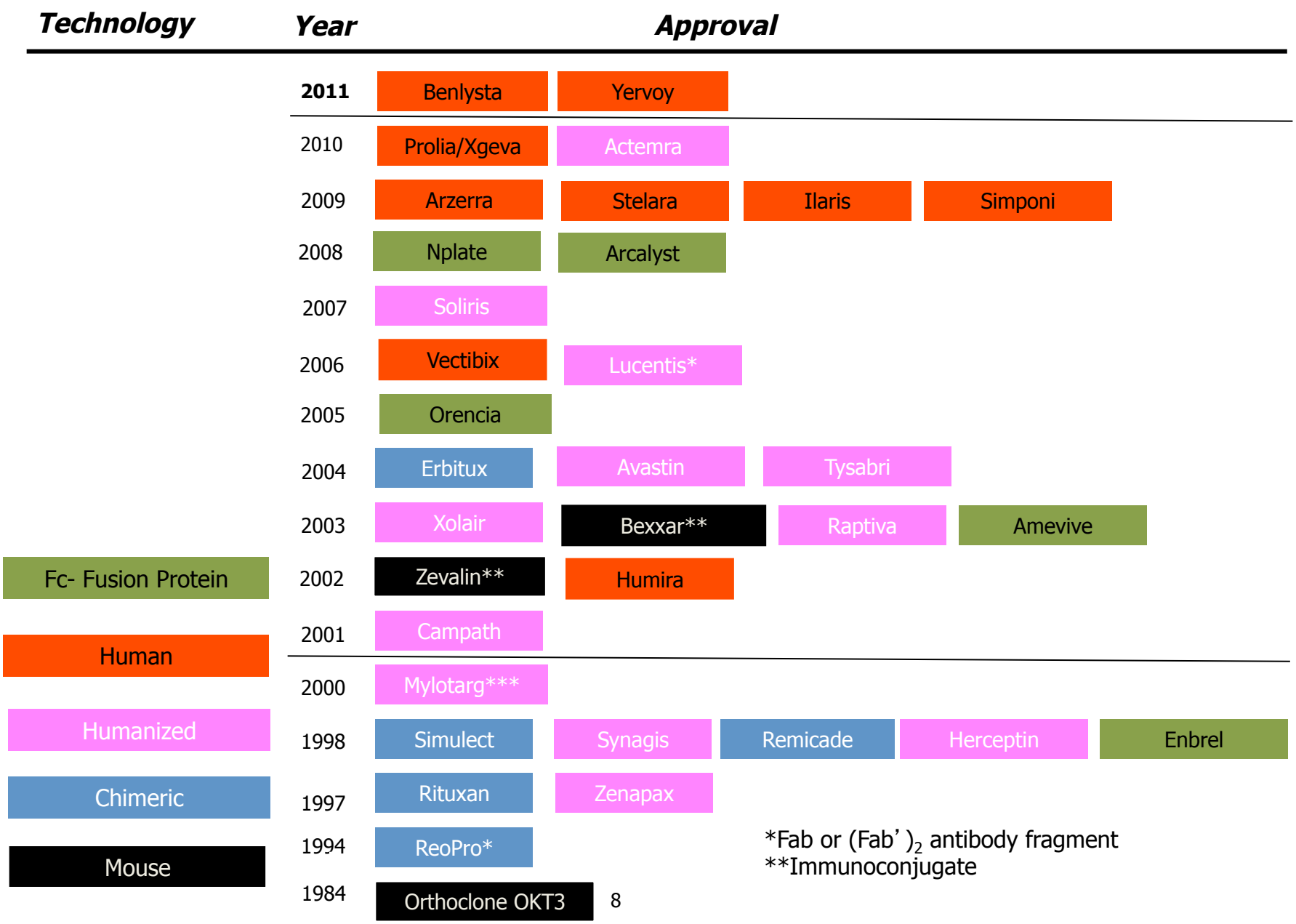
Structural variety of Fc-fusion proteins



2008--At least 40 Fc-fusion cytokines described

Jazayeri and Carroll, *Biodrugs* **22**, 11-26 (2008)

34 Therapeutic monoclonal antibody/Fc-fusion protein approvals—USA



USA-approved Fc-fusion proteins

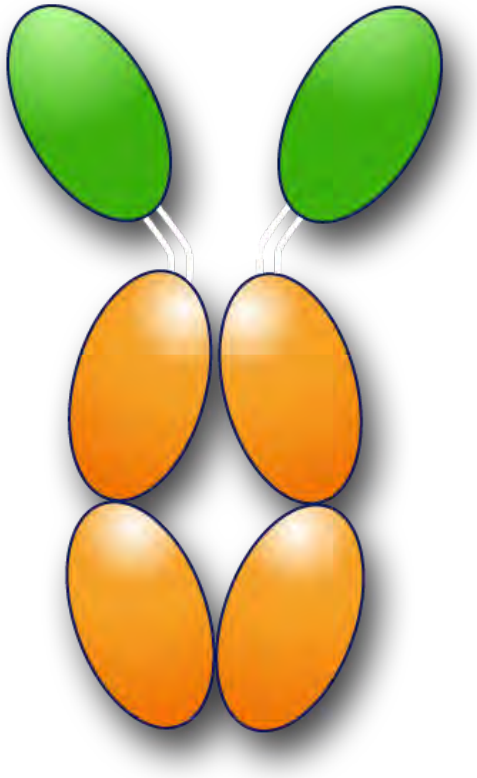
Brand name	Generic name	Sponsor	Year approved	Construct		MW (kDa)	Expression system	Target	Clinical indication
				Target binding domain	Ig domain				
Enbrel	etanercept	Immunex/ Amgen	1998	TNFR2	ϒ1 Fc	150	CHO	TNF α	Rheumatoid arthritis; juvenile idiopathic arthritis; psoriasis
Amevive	alefacept	Biogen-Idec	2003	LFA3	ϒ1 Fc	92	CHO	CD2	Psoriasis
Orencia	abatacept	BMS	2005	CTLA4	ϒ1 Fc	92	CHO	CD28 (indirect)	Rheumatoid arthritis; juvenile idiopathic arthritis
Arcalyst	rilonacept	Regeneron	2008	IL1-RI➔IL 1RAcP	ϒ1 Fc	251	CHO	IL1	Cryopyrin-associated periodic syndromes
Nplate	romiplostim	Amgen	2008	Peptide mimetic of TPO	ϒ1 Fc, fusion at C term	59	*E. coli*	TPOR	Chronic idiopathic thrombocytopenic purpura

Potential production issues with Fc-fusion proteins

- Upstream
  - Folding and secretion
  - Disulfide bond formation
  - Glycosylation
    - Non-Ig domains bring addition glycosylation
- Downstream
  - Acid lability
    - Use of Protein A---High pH for elution
      - Rea *et al., BioPharm Int'l*, Mar supplement (2008)
    - Virus inactivation
      - Solvent/detergent
  - Reduced Protein A chromatographic capacity
    - Ghose *et al., Biotechnol. Bioeng.* **96**, 768-779 (2006)
  - Increased proteolysis
  - Aggregation
    - Hydrophobic interaction chromatography
  - Glycoform heterogeneity
    - Highly sialylated forms often desirable

Fc-fusion proteins in development

- Receptor Fc-fusion
  - VEGF Trap
    - VEGFR:Fc, binds to VEGF-A, VEGF-B and PIGF, aflibercept, Ph 3, Regeneron/Sanofi-Aventis
    - Economides *et al., Nat. Med.* **9**, 47-52 (2003)
  - Atacicept
    - Receptor for BLyS and APRIL, Merck-Serono
  - Belatacept
    - CTLA4 binds to B7, Bristol Myers Squibb
    - Differs from abatacept by 2 amino acids
- Peptide Fc-fusion ("peptibody")
  - AMG 386
    - peptide inhibitor of TIE2/ANG2:Fc, I-SPY 2 TRIAL adaptive design in breast cancer, Amgen
- Enzyme Fc-fusion
  - Factor VIII-Fc
    - Biogen-Idec
  - Factor IX-Fc
    - Biogen-Idec



See JM Reichert, *Mabs* **3**, 76-99 (2011)

Presentations

- Engineering a CHO cell line for enhanced production of Fc fusion proteins and blood clotting factors
  - Pierre-Alain Girod, CSO, Selexis SA, Geneva, Switzerland
- Assessment of Regeneron's cytokine trap technology
  - Kevin Bailey, VP, Regeneron, Tarrytown, NY, USA
- Product quality challenges during process improvements for an Fc fusion protein
  - Barbara Woppmann, Sr. Engineer, Biogen-Idec, Cambridge, MA, USA
- Challenges in upstream and downstream processing for Fc fusion proteins
  - Michiel Ultee, CSO, Laureate Bioservices, Princeton, NJ, USA