Biobetters: Strategies to improve biologicals

"better than"

Definition: Biobetters or Biosuperiors are improved versions of a an existing biological drug that will improve its pharmacokinetics and/or efficacy and/or mode of administration and/or toxicity and/or immunogenicity.

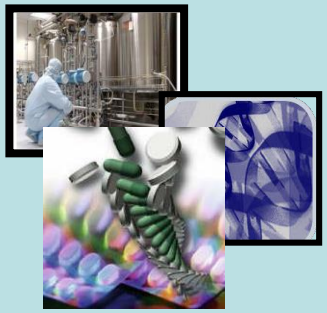
Refers to a recombinant protein drug that is in the same class as an existing biopharmaceutical but is not identical; it is improved over the original.

Reasons to improve therapeutic proteins

-**Stability**
-**Solubility**
-**Pharmacokinetics, pharmacodynamics** characteristics (half life, distribution, elimination …)
-**Efficacy**: more affinity for the receptor, more targeted to a tissue/organ/cell, less degradation
-**Patient compliance**: changeadministration route, frequency….
-Reduce production **costs**

A biobetter may provide one or more of the following advantages over the reference biologic

- Greater efficacy,
- greater purity,
- longer product half-life,
- less frequent dosing,
- Lower likelihood of aggregation,
- fewer adverse events,
- streamlined manufacturing,
- Longer shelf-life and greater stability
- Easier administration/ packaging improvements



Improvement of pharmacokinetic properties of a recombinant protein : interferon-alpha, a case study

Q1 human Interferon-alpha

1. What is human interferon-alpha (h-INF) ?
2. What is its mechanism of action ?
3. How recombinant h-INF a2 is administrated?
4. What is its half life time?

- *6 groups of 5 students*
- *One group selected to be the teachers afterwards, the rest of the class will answer to the teachers's questions*

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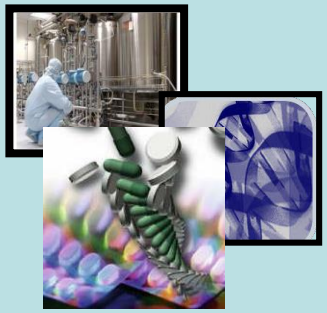
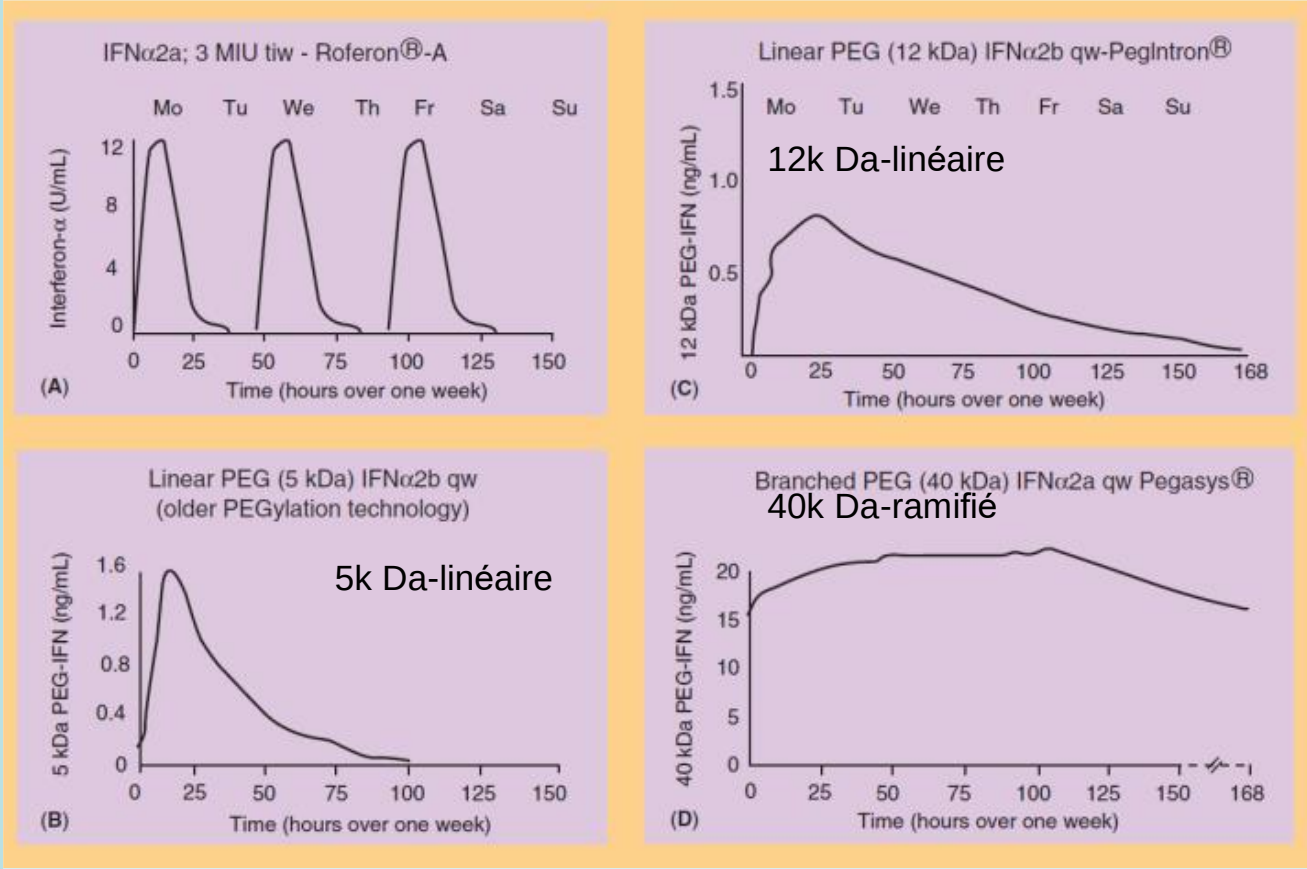


Improvement of pharmacokinetic properties of a recombinant protein : interferon-alpha, a case study

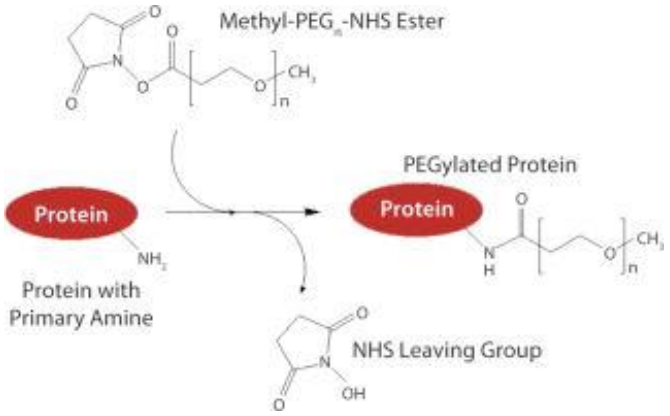
Q2 Pegylation of the interferon.

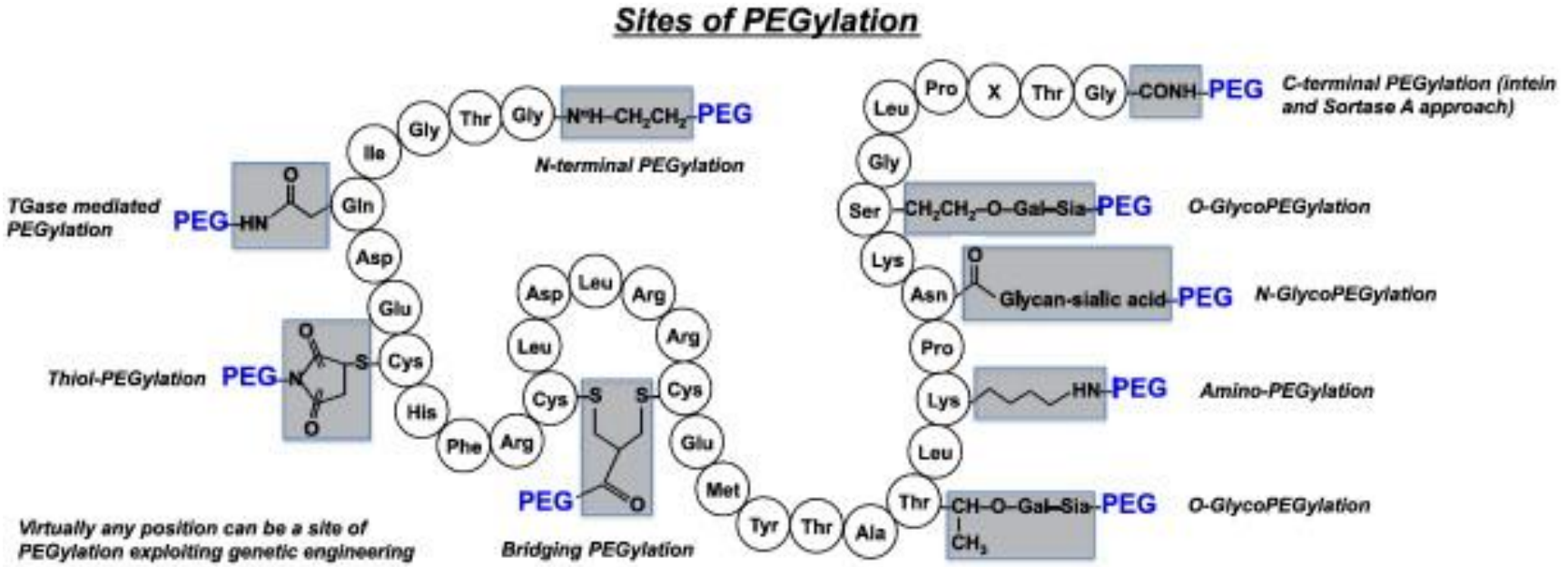
1. What does pegylation mean?
2. Analyze and comment the figure below
3. Search for other pegylated pharmaceutical products



Pegylation

- Non-toxic, hydrophilic, uncharged molecule
- Increase of ½ life in vivo (4-400x)
- Reduces the risk of immunogenicity
- Increases resistance to proteases
- Improves protein stability and solubility





Linear or branched PEGs, Variable size, Variable positions, Variable chemistry.

TABLE 1 | Approved PEGylated proteins.

International non-proprietary name	Brand name	Protein	Treatment	Company	CT	Stationary phase	Approval year	References
Pegademase bovine	Adagen®	ADA	ADA severe combined immunodeficiency	Enzon Pharmaceuticals Inc.	—	—	1990	Davis et al. (1981)
Pegaspargase	Oncaspar®	L-asparaginase	Acute lymphoblastic leukemia	Enzon Pharmaceuticals Inc.	AEX	NA	1994	Turecek et al. (2016)
Peginterferon alfa-2b	ViraferonPEG®	IFN alfa-2b	Chronic hepatitis C	Schering-Plough	CEX	TSKgel SP-5PW	2000	Gilbert and Cho, (1998)
Peginterferon alfa-2a	Pegasys®	IFN alfa-2a	Chronic hepatitis B, C	Hoffman-La Roche	CEX	Toyopearl CM-650S, TSKgel SP-5PW	2001	Karasiewicz et al. (1995)
Peginterferon alfa-2b	PEG-intron®	IFN alfa-2b	Chronic hepatitis C	Schering-Plough	CEX	TSKgel SP-5PW	2001	Gilbert and Cho (1998)
Pegfilgrastim	Neulasta®	G-CSF	Neutropenia	Amgen	CEX	SP Sepharose HP	2002	Molineux (2004); Bailon (2008)
Pegvisomant	Somavert®	GH receptor antagonist	Acromegaly	Pfizer	HIC-CEX	Phenyl Toyopearl 650M, SP Sepharose FF	2003	Clark et al. (1996)
PEG-epoetin beta	Mircera®	Erythropoietin (epoetin-beta)	Anemia in adults with chronic renal failure	Hoffman-La Roche	CEX	SP Sepharose FF	2007	Burg et al. (2011)
Certolizumab pegol	Cimzia®	Anti-TNF-alfa Fab	Inflammatory diseases	UCB Pharma	CEX	SP Sepharose HP	2008	Chapman et al. (1999)
Pegloticase	Krystexxa®	Uricase	Chronic gout	Savient Pharmaceuticals	AEX	Mono Q	2010	Sherman et al. (2004); Williams et al. (2003)
Peginterferon alfa-2b	Sylatron™	IFN alfa-2b	Melanoma (post-surgical resection)	Merck	CEX	NA	2011	Park et al. (2019)
Lipegfilgrastim	Lonquex®	G-CSF	Neutropenia	Teva	NA	NA	2013	Awwad et al., 2018
Peginterferon beta-1a	Plegridy®	IFN beta-1a	Relapsing forms of multiple sclerosis	Biogen	SEC-CEX	Superose 6, SP Sepharose FF	2014	Pepinsky et al. (2001); Pepinsky et al. (2005)
PEG-growth hormone	Jintrolong®	Human growth hormone	Growth hormone deficiency	GeneScience	AEX	Q Sepharose	2014	Jin et al., 2012
Rurioctocog alfa pegol	Adynovate®	Coagulation factor VIII	Hemophilia A	Shire	SEC	Superose 6 HR	2016	Bossard et al. (2012)
Nonacog beta	Rebinyn®	Coagulation	Hemophilia B	Novo Nordisk	AEX	POROS 50 HQ	2017	Wiendahl et al. (2020)

name								
Pegademase bovine	Adagen®	ADA	ADA severe combined immunodeficiency	Enzon Pharmaceuticals Inc.	—	—	1990	Davis et al. (1981)
Pegaspargase	Oncaspar®	L-asparaginase	Acute lymphoblastic leukemia	Enzon Pharmaceuticals Inc.	AEX	NA	1994	Turecek et al. (2016)
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Certolizumab pegol	Cimzia®	Anti-TNF-alfa Fab	Inflammatory diseases	UCB Pharma	CEX	SP Sepharose HP	2008	Chapman et al. (1999)
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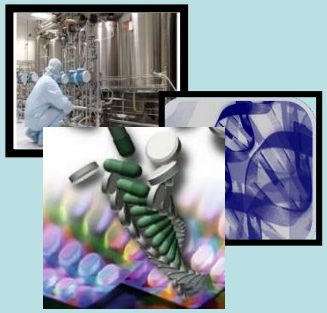
Lipegfilgrastim	Lonquex®	G-CSF	Neutropenia	Teva	NA	NA	2013	Awwad et al., 2018
Peginterferon beta-1a	Plegridy®	IFN beta-1a	Relapsing forms of multiple sclerosis	Biogen	SEC-CEX	Superose 6, SP Sepharose FF	2014	Pepinsky et al. (2001); Pepinsky et al. (2005)
PEG-growth hormone	Jintrolong®	Human growth hormone	Growth hormone deficiency	GeneScience	AEX	Q Sepharose	2014	Jin et al., 2012
Rurioctocog alfa pegol	Adynovate®	Coagulation factor VIII	Hemophilia A	Shire	SEC	Superose 6 HR	2016	Bossard et al. (2012)
Nonacog beta pegol	Rebinyn®	Coagulation factor IX	Hemophilia B	Novo Nordisk	AEX	POROS 50 HQ	2017	Wiendahl et al. (2020)
Calaspargase pegol	Asparlas™	L-asparaginase	Acute lymphoblastic leukemia	Servier Pharmaceuticals	NA	NA	2018	Marini et al., 2017
Elapegademase	Revcovi™	ADA	ADA severe combined immunodeficiency	Leadiant Biosciences	—	—	2018	Ramos-de-la-Peña and Aguilar, (2020)
Damoctocog alfa pegol	Jivi®	Coagulation factor VIII	Hemophilia A	Bayer	CEX	SP (Cytiva)	2018	Mei et al. (2010)
Pegvaliase	Palynziq®	Phenylalananine ammonia lyase	Phenylketonuria	BioMarin	—	—	2018	Park et al. (2019)
Rurioctocog alfa pegol	Adynovi®	Coagulation factor VIII	Hemophilia A	Baxalta Innovations	CEX	MacroCap SP	2018	Siekmann et al. (2011)
Pegfilgrastim jmdb	Fulphila™	G-CSF	Neutropenia	Mylan Pharmaceuticals	CEX	NA	2018	Hoy, (2019)
Pegfilgrastim cbqv	Udenyca™	G-CSF	Neutropenia	Coherus Bioscience	NA	NA	2018	Park et al. (2019)
Pegfilgrastim	Lapelga Pelgraz™	G-CSF	Neutropenia	Apotex Inc.	NA	NA	2018	Zalipsky and Pasut, 2020
Pegfilgrastim	Pelmeg™	G-CSF	Neutropenia	Mundipharma	NA	NA	2018	Zalipsky and Pasut, 2020
Pegfilgrastim bmez	Ziextenzo™	G-CSF	Neutropenia	Sandoz Inc.	NA	NA	2019	Zalipsky and Pasut, 2020
Turoctocog alfa pegol	Esperoct®	Coagulation factor VIII	Hemophilia A	Novo Nordisk	AEX	Source 15Q	2019	Stennicke et al. (2013)
Ropeginterferon alfa-2b	Besremi	IFN alfa-2b	Polycythemia vera	PharmaEssentia	CEX	SP Sepharose XL	2019	Lin and Widmann, (2013)
Pegfilgastrim apgf	Nyvepria	G-CSF	Neutropenia	Pfizer	CEX	NA	2020	Yang et al. (2021)

Sánchez-Trasviña 2021



Q3 Interferon-CTP (C-terminal peptide)

1. What is a fusion protein ?
2. What is the CTP peptide ? How IFN-CTP is obtained?
3. What properties of the therapeutic molecule are targeted by this modification.
4. Interpret the results of this preclinical studies.

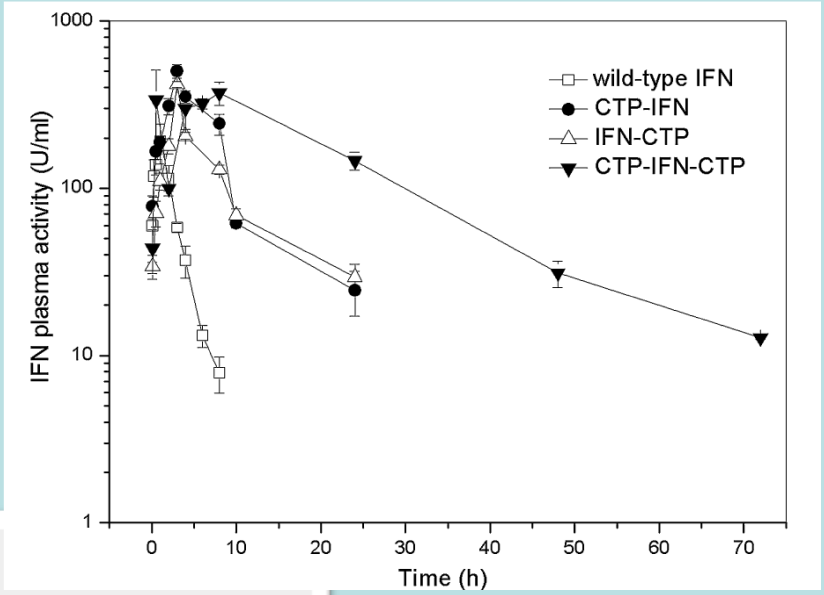


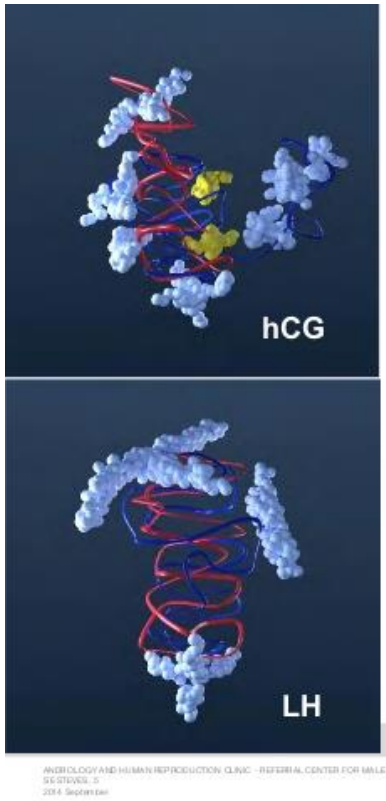
Fig. 2 Pharmacokinetic plasma profile of wild-type IFN, CTP-IFN, IFN-CTP and CTP-IFN-CTP after subcutaneous injection in rats. Data points are the average ± SEM of four animals in each group.

Table 1
In vitro specific bioactivities of purified CTP-IFN variants.

rhIFN-α2b variant	Specific antiviral bioactivity ($U ng^{-1}$)
Wild-type IFN	185 ± 30
CTP-IFN	65 ± 3
IFN-CTP	58 ± 6
CTP-IFN-CTP	44 ± 3

N. Ceaglio et al. / Journal of Biotechnology 221 (2016) 13–24

Technology of fusion with CTP

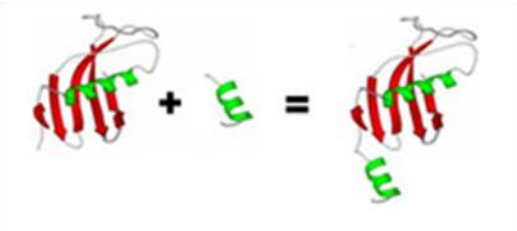


Amino acid sequence of hCG & hLH is almost identical

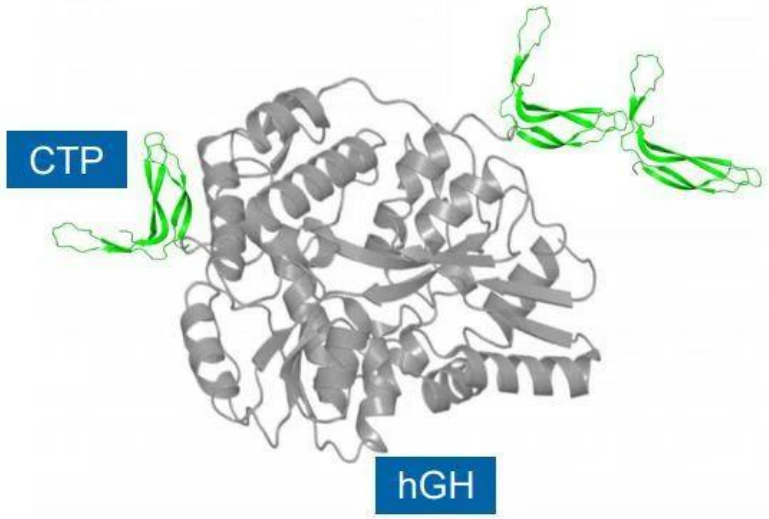
The 28 amino acid C-terminal peptide (CTP) of hCG with its 4 O-glycans does not exist in hLH

	LH	hCG
No. AA beta subunit	121	145
Receptor binding affinity	Low	High*
No. glycosylation sites[1]	1	6
Initial half-life (h)	0.6-1.3	3.9-5.5

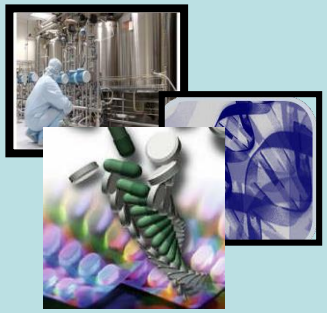
Ex : corifollitropin alfa (FSH-CTP) ELONVA® (2010 EMEA) .



Somatrogon© CTP Technology



hCG: human chorionic gonadotropin



Q4 Albuferon

Albuferon is a single polypeptide molecule that combining the sequence of the interferon alpha with the human serum albumin (HSA) as shown in figure 1**.**

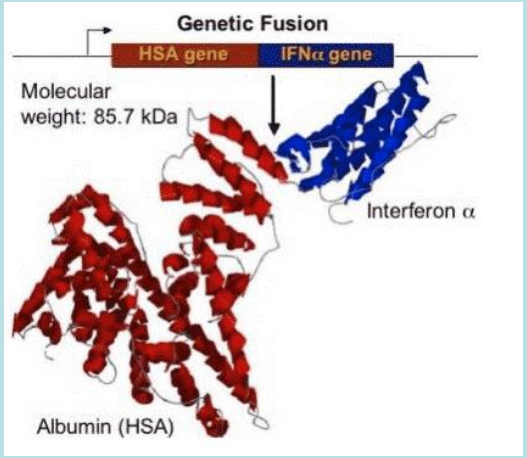


Fig. 1. Schematic representation of Albuferon.
From AASLD, American Association For The Study of Liver Diseases November 11-15, 2005

Protein	Nominal half-life (hours)
HSA	456
Transferrin	288
IgG_1, IgG_2, IgG_4	480
IgG_3	144
IgA monomer	120
Retinol-binding protein	12
Factor H	87
Factor XIII	168
C-reactive protein	48
Factor IX	22
Fibrinogen	100
IFN-α	5

Fig. 2 Nominal half-life values of human proteins in human serum
From Stohl, biodrugs, 2015

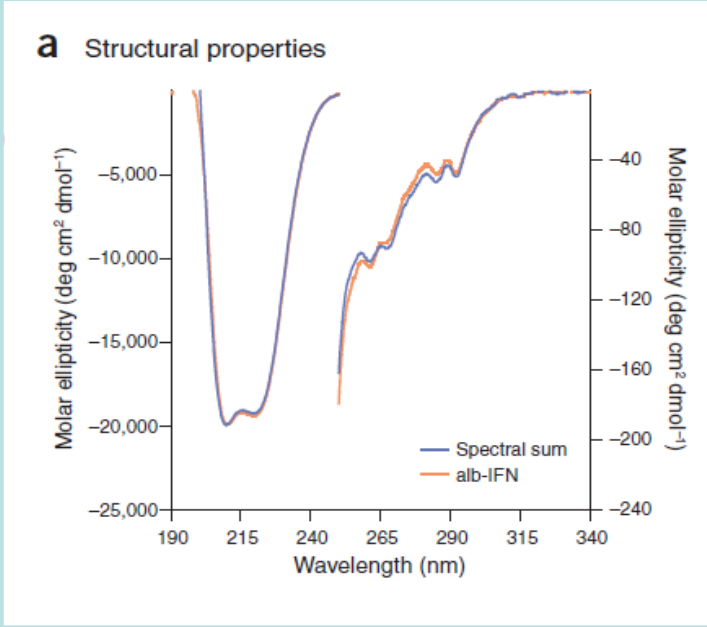


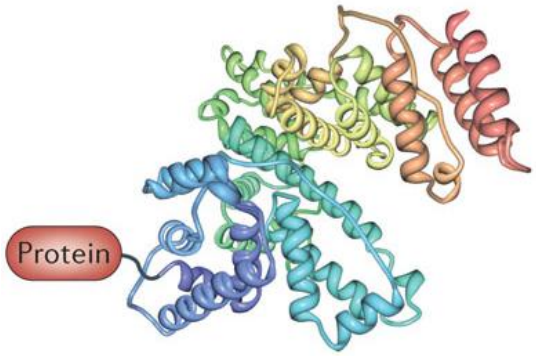
Fig. 3 Near- and far-ultraviolet circular dichroism (UV-CD) spectra of albuferon (alb-IFN). This panel shows the spectra of alb-IFN compared with the spectral sums of human albumin and IFN-alpha.
From Subramanian et al Nat Biotechnol. 2007 Dec;25(12):1411-9.doi:10.1038/nbt1364.

1. Based on figures 1 and 2, what is the strategy behind the development of Albuferon ?
2. Identify other therapeutic proteins that have the same characteristic than Albuferon
3. Analyze and comment figure 3

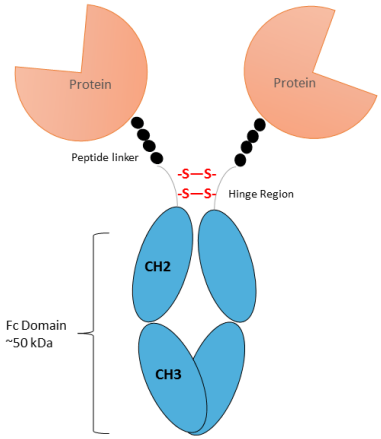
Fusion proteins and biobetters

Genetic constructs and fusion approaches

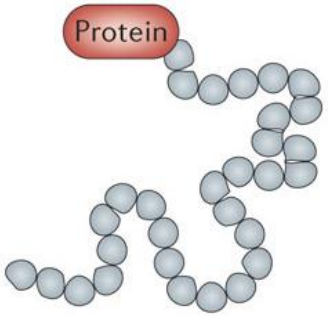
e Albumin

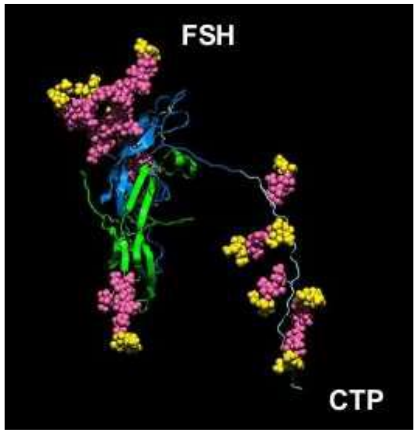


f Fc fusion



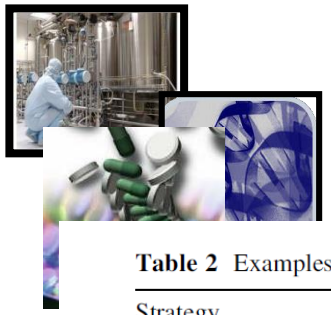
g Polyamino acid fusion protein





Fusion with CTP

- Increase in size and hydrodynamic radius
- Recycling via FcRn
- Increase of negative charges via sialylation
- Many biobetter in clinical trials



Half-life extension strategies employing polypeptide fusion

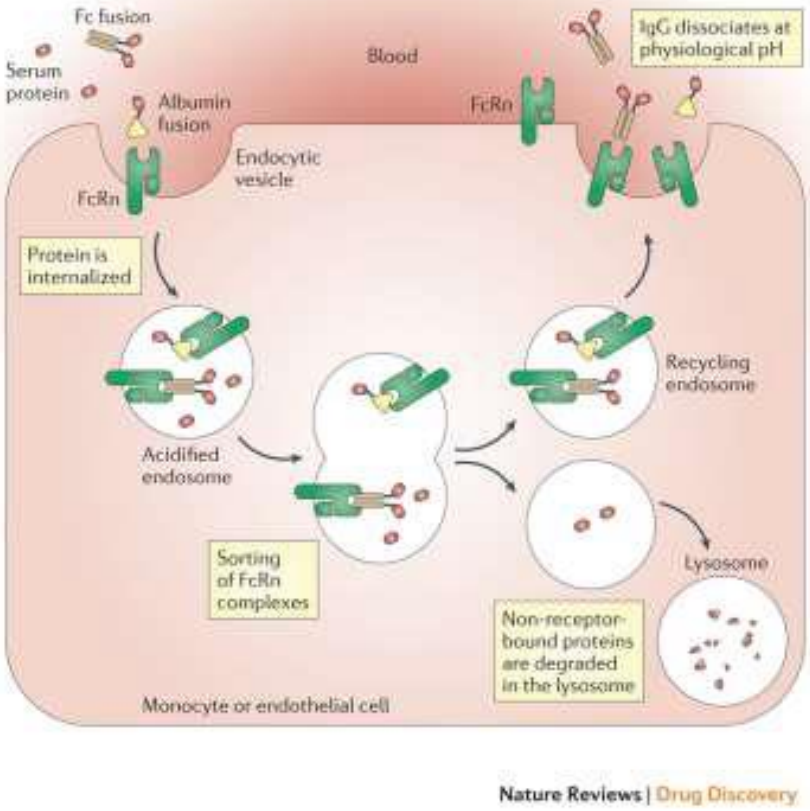
Table 2 Examples of half-life-extension strategies employing polypeptide fusions to small proteins and peptides to generate biobetters

Strategy	Specific approach	Construct	Mechanism for half-life extension
Fusion to human protein with inherently long serum half-life	Fusion to human IgG Fc domain	Genetic fusion to C-terminus or N terminus of human IgG Fc, which has a half-life of about 14 days in human serum	Recycling via FcRn [43–46]
	Fusion to HSA	Genetic fusion to C-terminus or N terminus of HSA, which has ~19-day half-life in human serum	Recycling via FcRn [45, 47, 48]
	Fusion to human transferrin	Genetic fusion to C terminus or N terminus of human transferrin, which has a ~12-day half-life in human serum	Recycling via transferrin receptor [49]
Fusion to non-structured polypeptide to increase overall size and hydrodynamic radius	XTENylation (also known as rPEG)	Genetic fusion of non-exact repeat peptide sequence (Amunix, Versartis) to therapeutic peptide	Increase in size and hydrodynamic radius [17]
	PASylation	Genetic fusion of polypeptide sequences composed of PAS (XL-Protein GmbH) forms uncharged random coil structures with large hydrodynamic volume	Increase in size and hydrodynamic radius [28]
	ELPylation	Genetic fusion to ELP repeat sequence (PhaseBio) can extend half-life	Increase in size and hydrodynamic radius [29, 30, 50]
	HAPylation	HAP (e.g., homopolymer of glycine residues)	Increase in size and hydrodynamic radius [27]
	GLK fusion	Fusion with artificial GLK	Increase in size and hydrodynamic radius [51]
Fusion to highly anionic polypeptide to increase negative charge	CTP fusion	Genetic fusion of CTP peptide from human CG β-subunit to antibody fragment (Prolor Biotech)	Increase in negative charge via sialylation of CTP [34, 35]

CG chorionic gonadotropin, *CTP* carboxy-terminal peptide, *ELP* elastin-like peptide, *Fc* constant fragment, *FcRn* neonatal Fc receptor, *GLK* gelatin-like protein, *HAP* homo-amino acid polymer, *HSA* human serum albumin, *Ig* immunoglobulin, *PAS* proline-alanine-serine polymer, *rPEG* recombinant poly-ethylene glycol, *XTEN* genetic fusion of non-exact repeat peptide sequence

BioDrugs (2015) 29:215–239
DOI 10.1007/s40259-015-0133-6

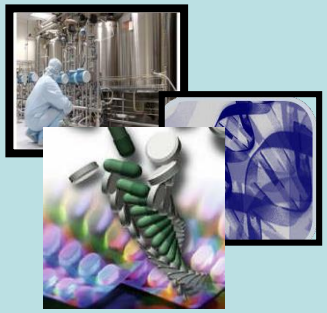
Fusion with albumin or Fc



Nominal Half life of human proteins in serum

Protein	Nominal half-life (hours)	Molecular mass (kDa)	Ratio of half-life to molecular mass
HSA	456	67	6.8
Transferrin	288	80	3.6
IgG_1, IgG_2, IgG_4	480	146	3.3
IgG_3	144	165	0.87
IgA monomer	120	160	0.75
Retinol-binding protein	12	21	0.57
Factor H	87	155	0.56
Factor XIII	168	320	0.5
C-reactive protein	48	125	0.38
Factor IX	22	57	0.38
Fibrinogen	100	340	0.29
IFN-α	5	19	0.26
IgE	48	188	0.25
Pentameric IgM	144	970	0.15
IL-2	1.7	15	0.11
Thyroglobulin	65	660	0.1
G-CSF	2	20	0.1
Factor VIIa	3	50	0.06
PYY3–36	0.13	4	0.03
IGF-1	0.17	8	0.02
hGH	0.3	22	0.014
GLP-1	0.03	4	0.008

G-CSF granulocyte colony-stimulating factor, *GLP* glucagon-like peptide, *hGH* human growth hormone, *HSA* human serum albumin, *IFN* interferon, *Ig* immunoglobulin, *IGF* insulin-like growth factor, *IL* interleukin, *PYY* peptide tyrosine tyrosine



Q5 Albuferon b Analyze and comment figure 4

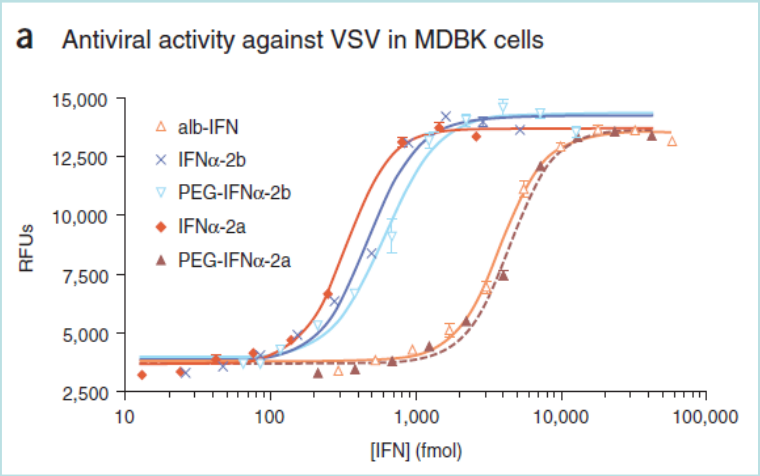


Fig. 4. Antiviral activity against vesicular stomatitis virus (VSV) in Madin-Darby bovine kidney (MDBK) cells. The relative antiviral potency on a molar basis (given the differences in molecular weight) of several IFN formulations were compared in MDBK epithelial cells infected with VSV.
From Subramanian et al Nat Biotechnol. 2007 Dec;25(12):1411-9.doi:10.1038/nbt1364

Different strategies to improve Therapeutic proteins

- **Modifications of the coding sequence:**
  - Mutations, deletions
  - Fusion proteins,
  - Humanization (antibodies)
  - Modification of glycosylation sites
- **Non-translational engineering**
  - Conjugation with hydrophilic polymers
  - Conjugation to an active molecule (cytotoxic)
  - Modification of already formed glycan chains
  - Lipid binding
- **New production technologies**

➢ Host cell engineering: glycosylation improvement (glycoengineering)

- **New formulations and new routes of administration**

Example of Commercially available biobetters

Table 3 Examples of biobetters and the improvement offered by them

Reference Biologic	Biobetter	Improved Characteristic Compared to the Original
Erythropoietin-alpha	ARANESP® (Amgen) FDA approval in 2001.	Reduced dosing frequency to once every fortnight.
	MIRCERA® (Roche) FDA approval in 2007.	Reduced dosing frequency to once monthly.
Filgrastim	NEULASTA® (Amgen) FDA approval in 2002.	Once in a 21-day chemotherapycycle versus once daily.
Follicle Stimulating Hormone	ELONVA® (Merck) [Corifollitropin - alpha]Sustained follicle stimulant EC approval in 2010.	Single subcutaneous injectioninstead of first seven injections of daily FSH preparation.
Trastuzumab	KADCYLA® (Genentech) [Trastuzumab emtansine or T-DM1: Anantibody–drug conjugate, combining theHER2 inhibition of trastuzumab and the microtubule inhibition of DM1] FDA approval in 2013.	Combination with improved efficacy over current standard of care, Trastuzumab emtansine is indicated as a single agent for thetreatment of HER2-positive, unresectable, locallyadvanced or metastatic breast cancer who previously receivedtrastuzumab and a taxane, separately or in combination.
Rituximab	GAZVYA® (Roche) [Obinutuzumab] FDA approval in 2013.	Improved pharmacokinetics.
Recombinant Anti-hemophilicFactor	ELOCTATE™ (Biogen Idec) [B-domain deleted recombinant Factor VIII,Fc fusion protein (BDD-rFVIIIFc)] FDA approval in 2014.	Reduced dosing frequency.

Sahil Kumar et al: Biobetters J Rational Pharmacother Res 2018 Volume 4

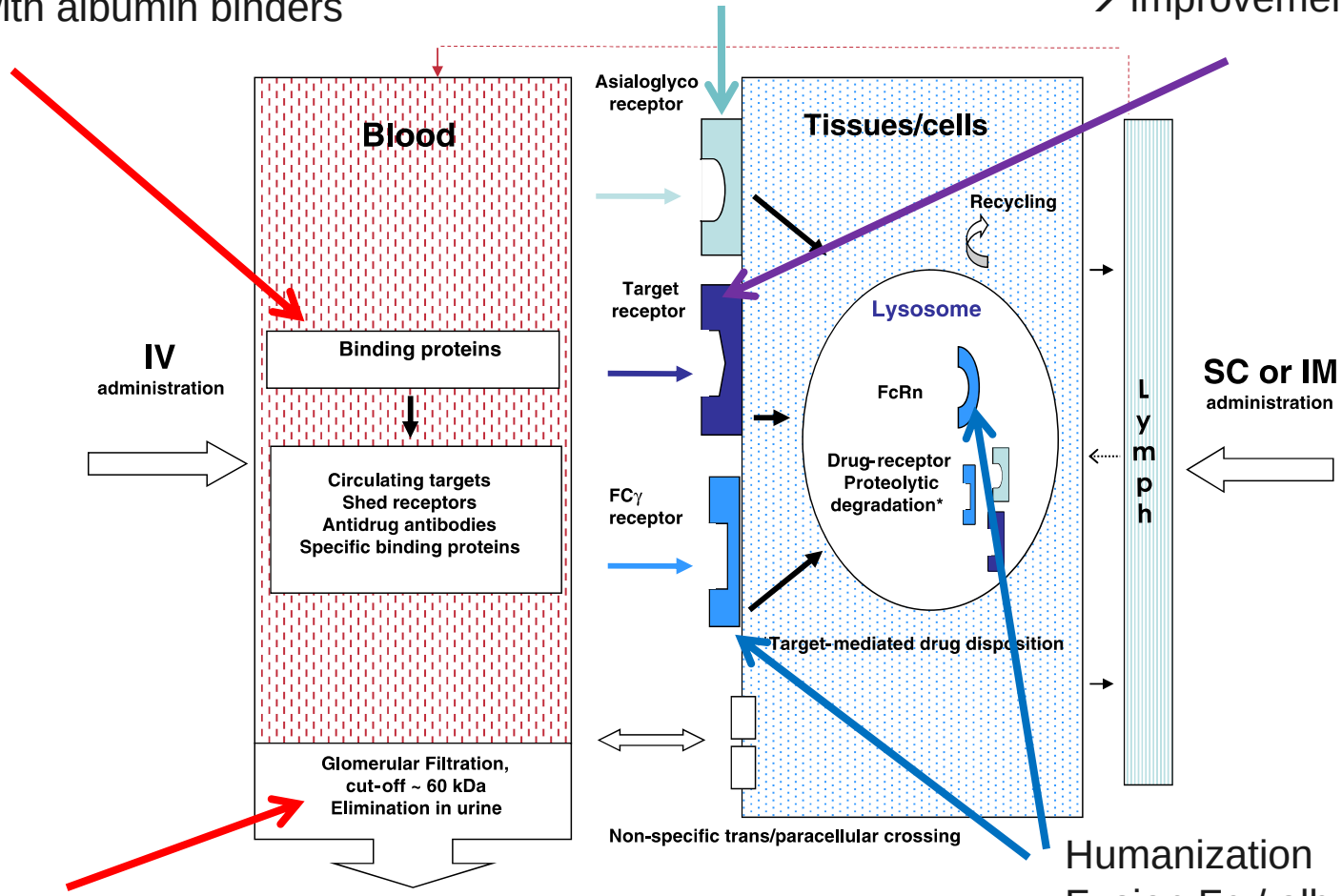
BIOBETTER SUMMARY

Ezan , Adv Drug Deliv, 2013

Glycoengineering:
addition of sialic acids

Molecular engineering
(Mutation/deletion on DNA sequence)
→ improvement of the Kd

Fusion with albumin binders



Glycoengineering : Addition of complex type glycans on the protein
Conjugaison to a hydrophilic polymer (PEG)

Humanization
Fusion Fc / albumin
engineering of Fc fragment

BIOBETTER SUMMARY

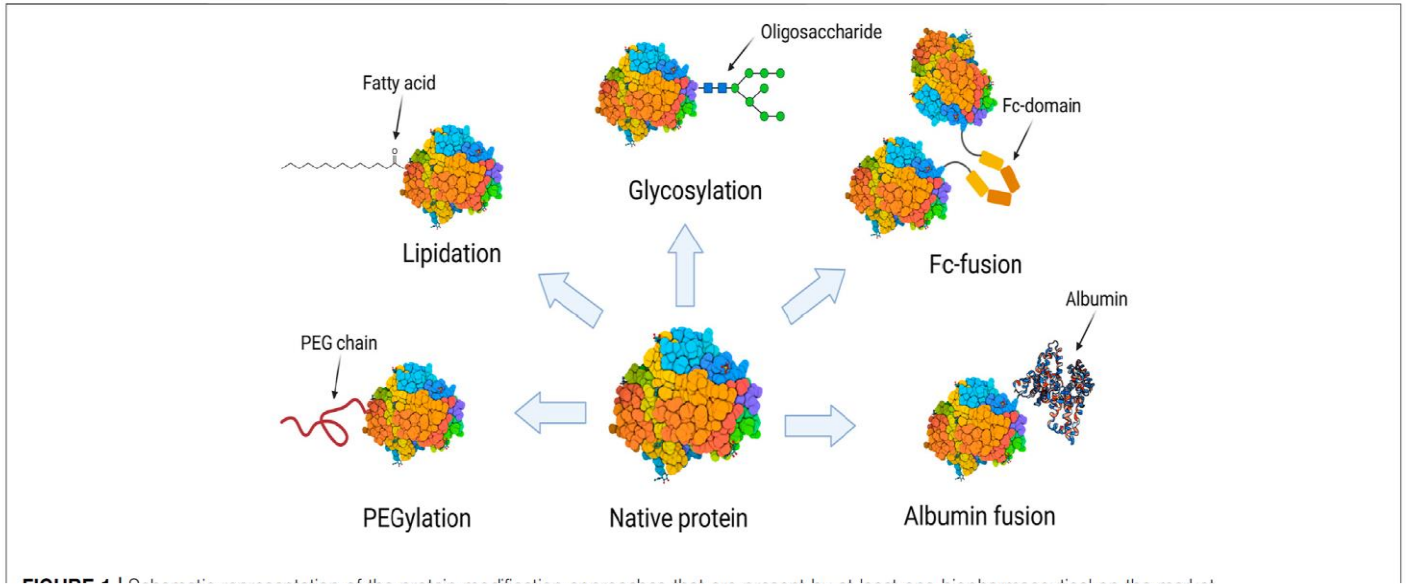
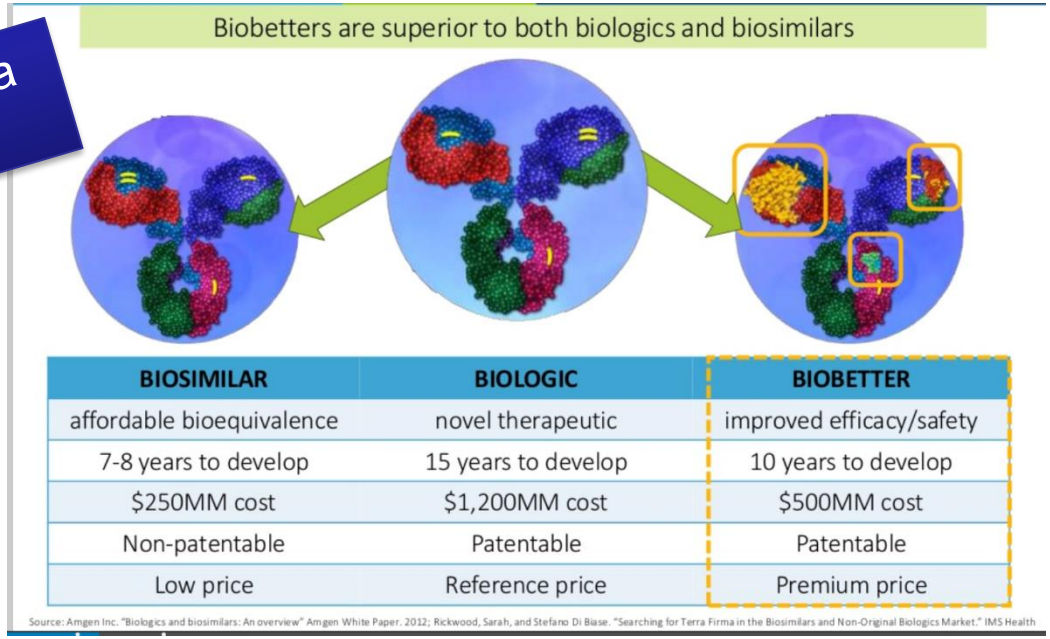


Table 1 Currently available approaches to enhance polypeptide pharmacokinetics

Approach	Advantages	Challenges	Examples
Chemical/covalent modification	• Increased *in vivo* half-life • Protection from degradation • Reduced renal clearance • Increased solubility/stability	• Decreased tissue uptake • Immunogenicity • Functional heterogeneity • Maintenance of functional activity; depends on chemistry used	PEGylation (PEG-hGH[7], PEG-IFN-β[18] and PEG-IFN-α-2b[9]), albumin and fatty acid acylated insulin[13]
Microsphere/nanoparticle delivery	• Sustained/targeted drug release • Protection from degradation	• Encapsulation efficiency • Efficiency of drug release from microspheres • Functional heterogeneity • Maintenance of functional activity	Poly L-Glu nanoparticle, IFN-α-XL[6], poly(lactic-co-glycolic acid) (PLGA) microsphere, IFN-α[19], human growth hormone[5] and PEG-insulin[12]
Protease-resistant variants	• Improved stability • Protection from degradation	• Maintenance of functional activity • Immunogenicity	Ala20Pro-RNaseA[14], MART-1 with β amino acid substitution[11], T-cell mimotopes[22] and G15A growth hormone releasing hormone[20]
Albumin fusion	• Increased *in vivo* half-life • No modification required • Design flexibility • Reduced renal clearance • Increased solubility/stability	• Maintenance of functional activity • Immunogenicity	alb-IFN-α[4], alb-GLP-1, alb-insulin[8], alb-GH[16], alb-IL-2[15] and alb-BNP[21]

Biosimilaires, biobetters et nouvelles générations de Mabs

biobetter is not a biosimilar



BIOSIMILAR	BIOLOGIC	BIOBETTER
affordable bioequivalence	novel therapeutic	improved efficacy/safety
7-8 years to develop	15 years to develop	10 years to develop
$250MM cost	$1,200MM cost	$500MM cost
Non-patentable	Patentable	Patentable
Low price	Reference price	Premium price

Source: Amgen Inc. "Biologics and biosimilars: An overview" Amgen White Paper. 2012; Rickwood, Sarah, and Stefano Di Biase. "Searching for Terra Firma in the Biosimilars and Non-Original Biologics Market." IMS Health

- -Commercial development is considered less risky than for a novel protein.
- - The product will be considered as a new active substance, but reduced R&D costs
- - Market exclusivity in case of EMEA approval

Table 1. Selected examples of first generation, Biosimilars, Biobetter, second and third generation monoclonal antibodies and alternatives formats

1st generation mAbs	Biosimilars	Biobetters	2nd generation	3nd generation	Alternative formats
CD20					
Rituximab (1997) chIgG1 (CHO) (Rituxan/Mabthera)	Reditux (2007, Dr. Reddy) chIgG1 (CHO)	"Rituximab" GS4:0 aFuc hzIgG1 (*Pichia pastoris*) Same epitope	Ofatumumab (2009) hIgG1 (CHO) Different epitope and mechanism of action (MOA) (Arzerra)	Obinutuzumab (PhIII) aFuc hIgG1 (CHO) Different epitope and MOA	TRU-015 (PhIIb) SMIP

Beck A. mAbs 3:2, 107-110; March/April 2011