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Engineering therapeutic proteins

Content:

Improvement of protein drugs half-life:

PEGylation.

the removal of natural domains.

- conclusion:

Introduction:

Therapeutic proteins: are proteins that are engineered in the laboratory for pharmaceutical use, to become highly effective *in vivo* and have revolutionized treatment of disease.

Protein engineering techniques have been applied to circumvent some of the problems that hindered these earlier trials, resulting in clinical benefits from a range of engineered antibodies and other proteins.

Early drug limitations:

immunogenicity.

low affinity/specificity.

short half-lives .

Insulin journey:

1920

- People with diabetes didn’t live for long
- The most effective treatment was very strict diets with minimal carbohydrate intake.

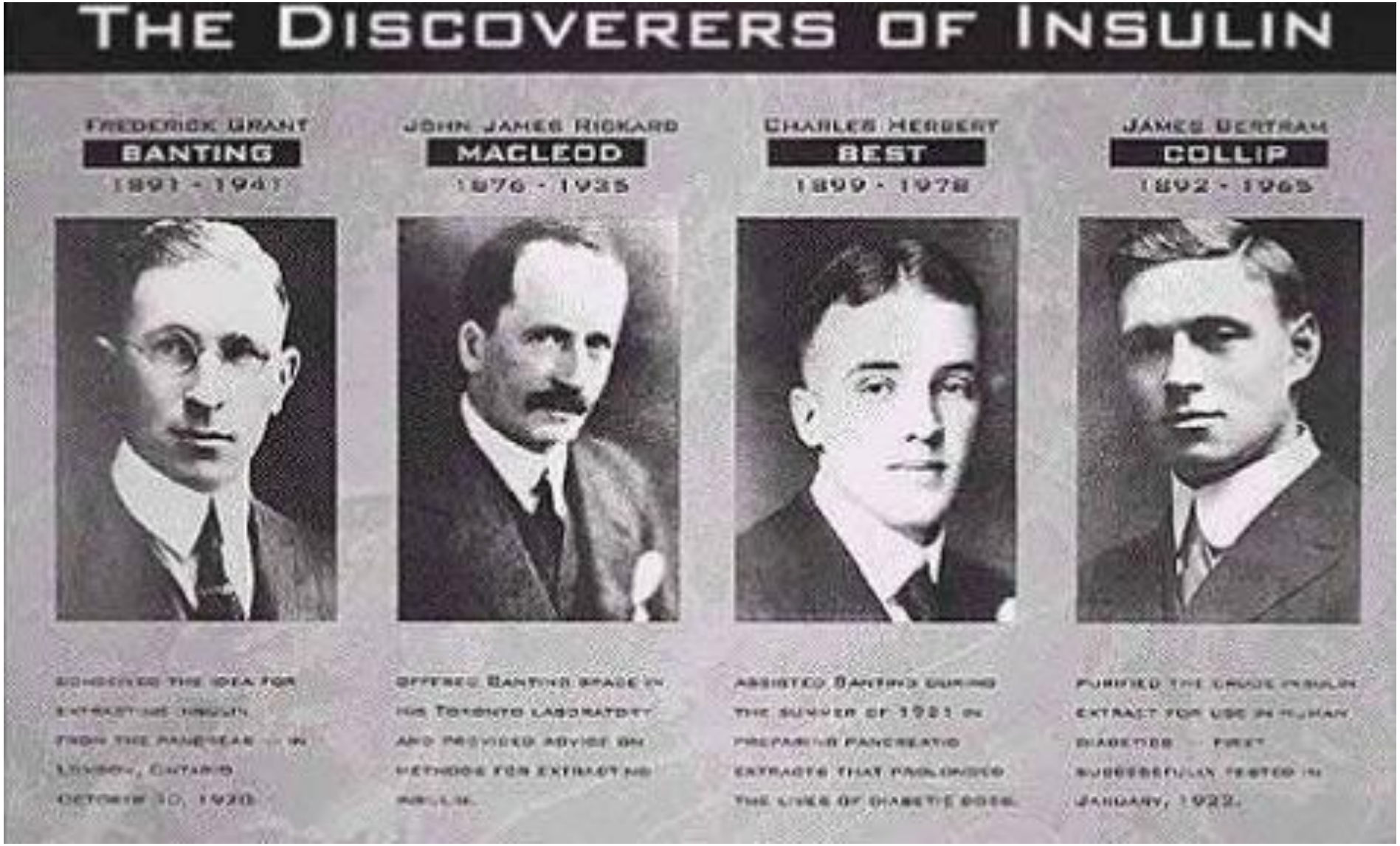
1921

- Banting and Best figured out how to remove insulin from a dog’s pancreas.
- kept dog with severe diabetes alive for 70 days—the dog died only when there was no more extract.
- Banting with Macleod refined and pure new form of insulin but this time from the pancreases of cattle.

1923

- Banting and Macleod received the Nobel Prize in Medicine, which they shared with Best and Collip.

Noble price in1923



1936

- manufacturers developed a variety of slower-acting insulins from cattle and pigs.
- It wasn't perfect, as it caused allergic reactions in many patients.

1978

- The first genetically engineered, synthetic "human" insulin was produced using *E. coli* bacteria to produce the insulin.

1982

- The medical firm Eli Lilly sell the first commercially available biosynthetic human insulin under the brand name Humulin.

1936

now

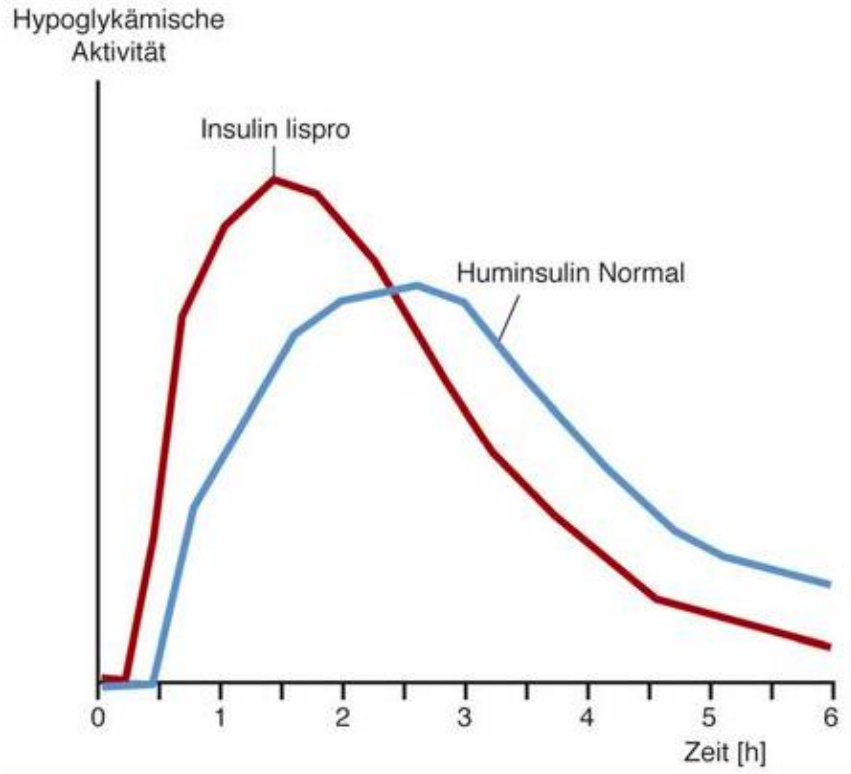
Now, insulin comes in many forms, from regular human insulin identical to what the body produces on its own, to ultra-rapid and ultra-long acting insulins. People with diabetes can choose from a variety of formulas and ways to take their insulin based on their personal needs and lifestyles, thanks to researchers and decades of research.

Liprolog/Humalog :

Engineered form of human insulin, proline–lysine sequence at positions 28 and 29 has been reversed.

This change affects the association between molecules and the product exists as a monomer at physiological concentrations.

As a consequence, the drug has a faster onset, but shorter duration of action because of a greater rate of absorption following subcutaneous administration, compared with other formulations of insulin.



The subject of review:

The engineered protein for therapeutics used in the clinic, where a primary protein molecule has been engineered to improve its potency or pharmacokinetic properties.

Engineering therapeutic proteins

John McCafferty* and David R Glover

Many early drug candidates derived from biotechnology failed in clinical trials because of their low affinity/specificity, short half-lives or immunogenicity. Protein engineering techniques have been applied to circumvent some of the problems that hindered these earlier trials, resulting in clinical benefits from a range of engineered antibodies and other proteins.

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Abbreviations

CDR	complementarity-determining region
IL	interleukin
PEG	polyethylene glycol
TGF	transforming growth factor
TNF	tumour necrosis factor
tPA	tissue plasminogen activator

Introduction

From a recent survey of the pharmaceutical/biotechnology sector, it was estimated that 100 drugs derived from biotechnology have been approved so far [1••]. These rep-

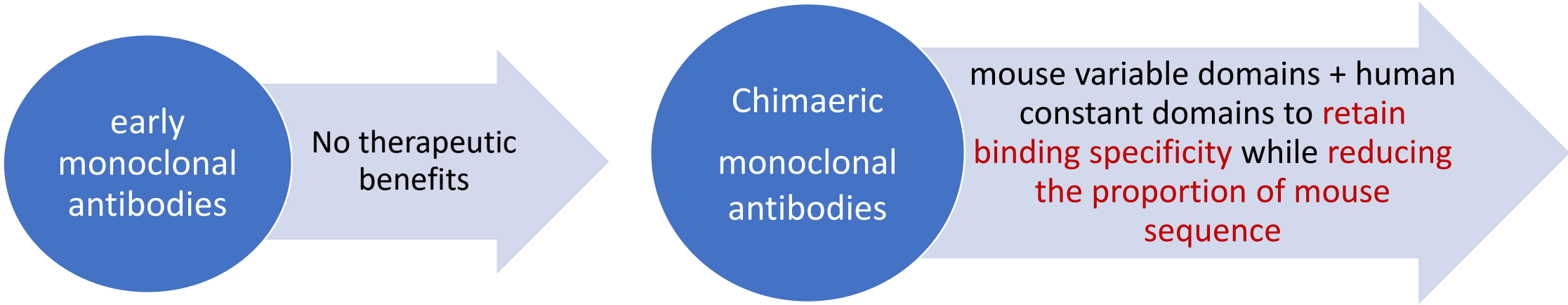
Reducing the immunogenicity of protein drug molecules

Many early attempts at introducing protein therapeutic molecules failed because the protein drug molecules were recognised as nonhuman and led to an immune response against the drug itself. As a result, most proteins used in clinical trials now are primarily human or are humanised, even if the original 'proof of concept' work was done with nonhuman proteins. For example, Pulmozyme (Genentech) is a drug based on human DNAse which was developed for use in managing cystic fibrosis [3], following successful 'proof of principle' studies with bovine pancreatic DNAse I [4]. The immunogenicity of mouse antibodies in humans was one of the major reasons why early monoclonal antibodies did not deliver the anticipated therapeutic benefits. This led to the development of chimaeric antibodies, created by fusing mouse variable domains to human constant domains to retain binding specificity while reducing the proportion of mouse sequence. A number of these antibodies have appeared in the clinic. Reopro (Centocor), an anticoagulant, was the first chimaeric antibody to be approved, at the end of 1994 [5]. Rituxan (Genentech/IDEC), which recognises CD20 on B lymphocytes, was approved in 1997 for the treatment of non-Hodgkin's lymphoma [3]. In 1998, Remicade (Centocor), a TNFα-neutralising chimaeric monoclonal

Reducing the immunogenicity of protein drug molecules:

- Many early protein therapeutic molecules failed because the protein drug led to introduce an immune response against the drug itself.
- So, most proteins used in clinical trials today are primarily human or are passing through humanized process.
- For example, Pulmozyme (Genentech) is a drug use in managing cystic fibrosis.

The immunogenicity of mouse antibodies:



- At the end of 1994, number of these antibodies have appeared in the clinic. Reopro (Centocor), an anticoagulant, was the first chimaeric antibody to be approved.

CDR-grafted monoclonal anti- bodies:

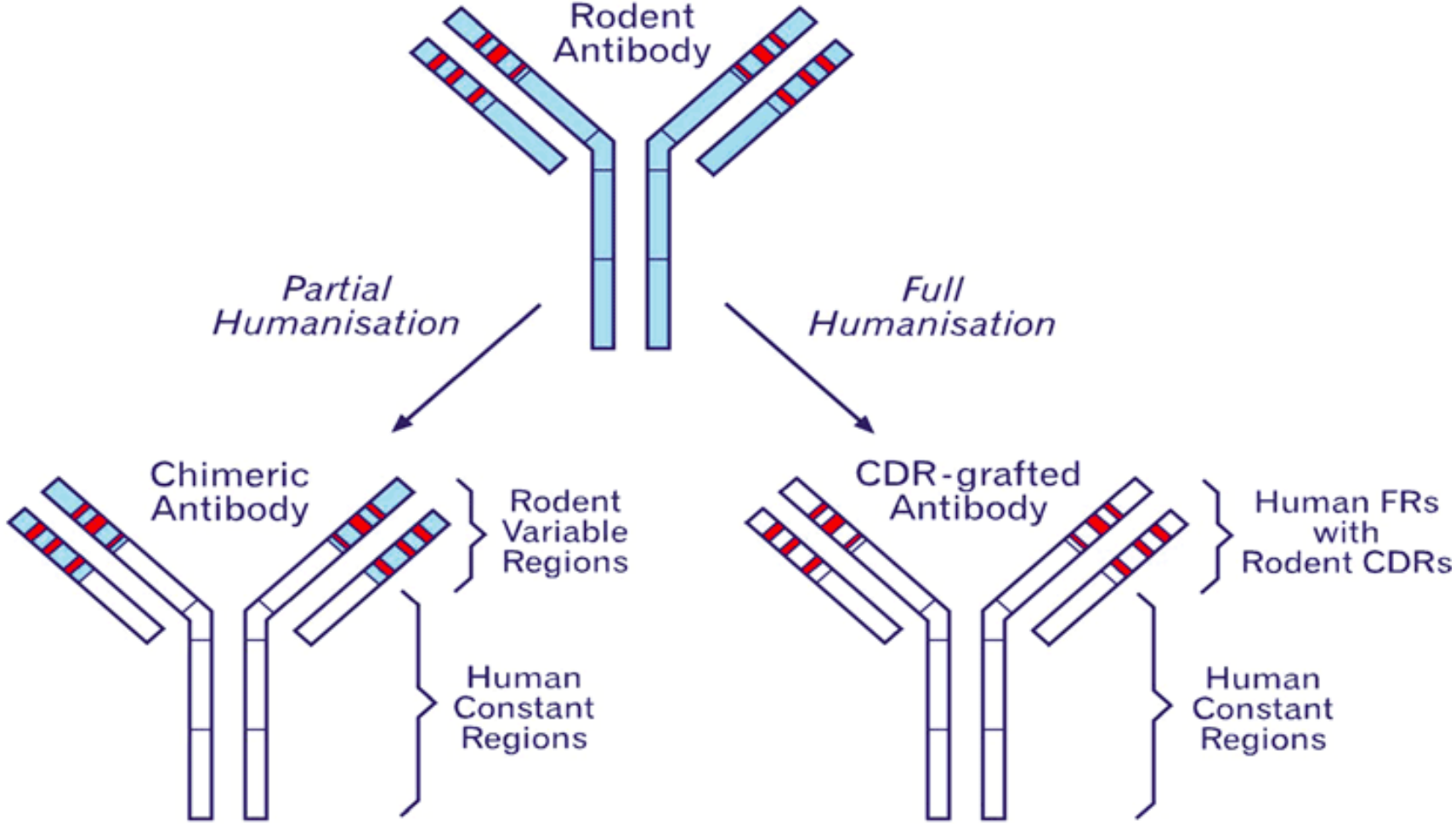
Reduction in monoclonal antibody immunogenicity achieved by grafting complementarity-determining region (CDR) of mouse anti-bodies onto human frameworks to reduce the proportion of mouse sequences in the drug, while retaining binding specificity.

Example: Herceptin, recognizes the erbB2 protein

and is used in treating refractory breast carcinoma

Complementarity-determining regions (**CDRs**) are part of the variable chains in immunoglobulins (antibodies) and T cell receptors, generated by B-cells and T-cells respectively, where these molecules bind to their specific antigen. A set of CDRs constitutes a paratope. As the most variable parts of the molecules, CDRs are crucial to the diversity of antigen specificities generated by lymphocytes.

erbB2 protein has major role in cell signaling



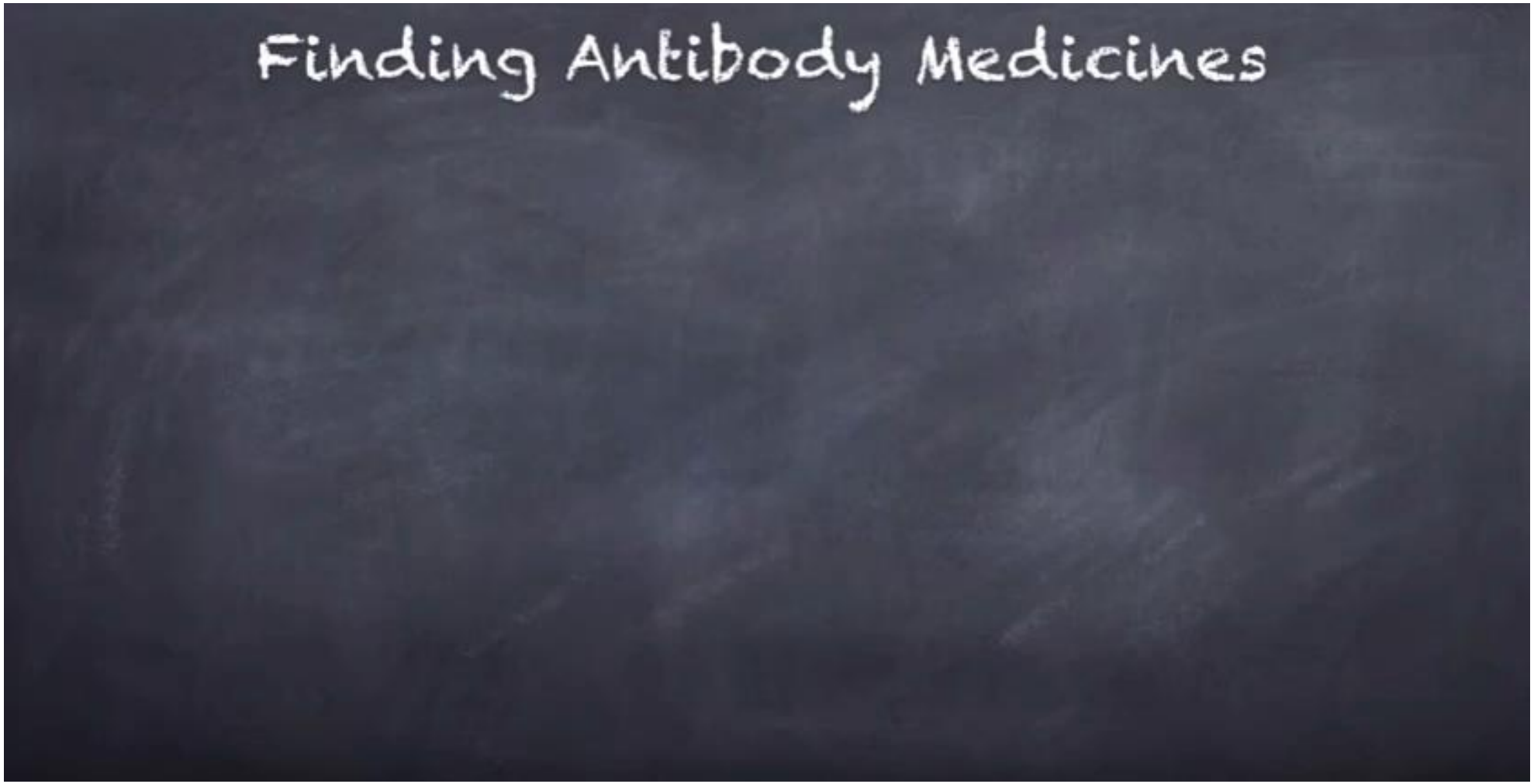
CDR-grafted monoclonal anti- bodies

Engineering affinity improvements:

Humanized antibodies often reduced affinity relative to the original murine antibody and need additional changes restore the activity of CDR- grafted molecules.

Altered residues do not necessarily participate directly in binding, but affect the conformation of antibody that are involved in the interaction with the target molecule. With the advent of phage display, it became possible to directly create human antibody drug candidates with high affinity.

Phage Display Technique:



In phage display, antibody genes are fused to a coat protein of the phage to create viral particles that directly link antibody proteins to the genes which encode them.

Whole libraries of human antibody genes are created and the antibody genes selected on the basis of the binding characteristics which they encode by panning the phage libraries on immobilized antigen.

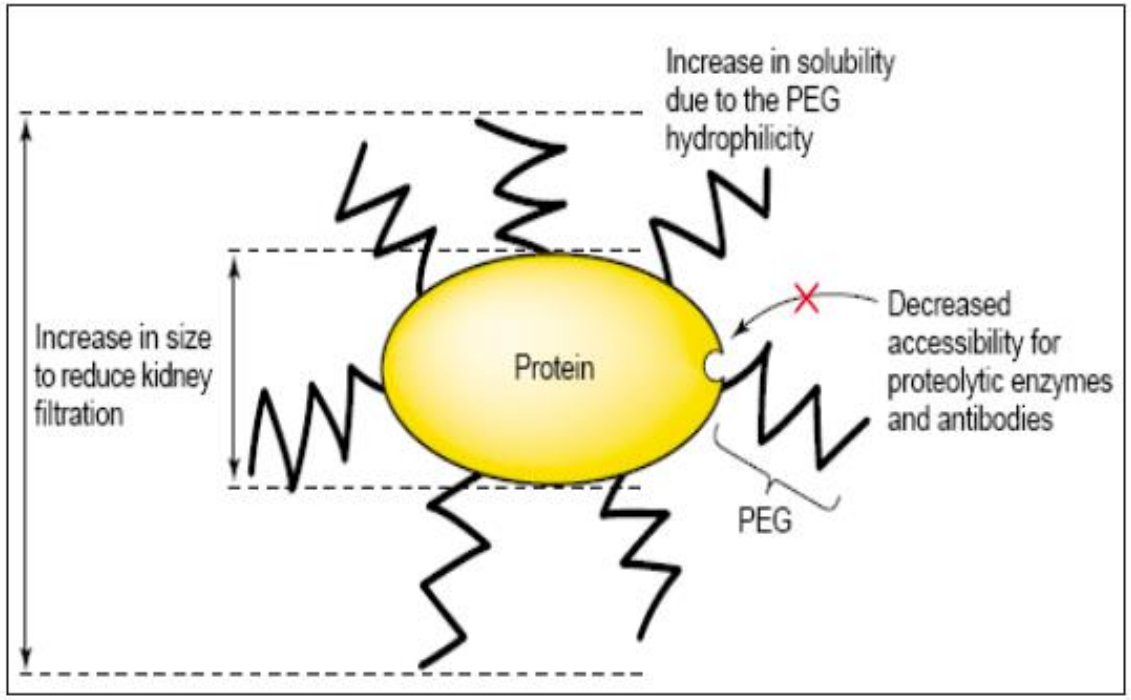
This approach also permits affinity maturation of lead clone(s) by mutagenesis, followed by selection of improved variants from the resulting repertoire. Thus, drug candidates with predefined properties in terms of affinity and specificity can be produced.

Improvement of protein drugs half-life:

1- PEGylation: covalent coupling of non-toxic, hydrophilic polyethylene glycol (PEG) to active pharmaceutical ingredients such as proteins, peptides, antibodies, colloids etc..

(example: PEG–IL-2),

The addition of PEG to proteins such as IL-2 has been used to extend half-life in serum.



2- the removal of natural domains :

Natural domains can be removed to improve the pharmacokinetics and specificity of protein drugs.

(example: Retaplase),

the N-terminal finger domain, the epidermal growth factor domain and the kringle 1 domains have been removed, leaving the catalytic domain and the kringle 2 domain, which drives the association with fibrin. This modification extends the half-life of the truncated form.

Conclusion:

Engineered proteins have been successful in both clinical and financial terms.

Since the approval of recombinant insulin, the first biotechnology drug, in 1982, an increasing number of engineered protein products are appearing in the pharmacy, with many more in clinical trials, heralding a ‘golden age’ of protein therapeutics.

In 1999, total sales of all chimaeric monoclonal antibodies was just under one billion US dollars. CDR-grafted antibodies are now beginning to generate significant sales. Herceptin generated US$188 million in its first year, making it the most successful anticancer drug launch to date.

References:

[1] J. McCafferty and D. R. Glover, “Engineering therapeutic proteins,” *Curr. Opin. Struct. Biol.*, vol. 10, no. 4, pp. 417–420, 2000.

[2] F. C. Breedveld, “Therapeutic monoclonal antibodies,” Lancet, vol. 355, no. 9205, pp. 735–740, 2000.

[3] https://www.drugtargetreview.com/article/4540/developing-antibody-therapeutics/

[4]https://www.youtube.com/watch?v=AqQDZxoCGqE