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REVIEW ARTICLE

PROTEIN ENGINEERING: EMERGING STRATEGY TO TACKLE HEMATOLOGICAL DISORDERS

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ABSTRACT:

Proteins are the coherent platform in the field of biotechnology since 1980's. The idea of designing and construction of proteins with novel or desired functions by modifying amino acid sequences paved the way for a new research discipline called protein engineering. Owing to the development of recombinant DNA technology and high throughput screening, protein engineering launched an era of protein therapeutics, a promising solution for molecular etiologies. Being the sister discipline of protein engineering, Protein therapeutics is on the doorsteps of a therapeutic revolution. This article overviews the current status and future prospective of protein engineering in tackling various hematological disorders.

INTRODUCTION: Although, the concept that gene sequences could be altered using synthetic nucleotides was put forwarded in early 1970's, it was only in the last years of the same decade that Michael Smith and colleagues could demonstrate that the approach could be successfully implemented. These findings have opened a new field of research that has become subsequently known as Protein Engineering. Protein engineering can be considered as a sub discipline of genetic engineering while the final product is a protein with modified amino acid sequences rather than a living organism. In this regard, engineered proteins closely resemble a new chemical compounds from non-biological sources[1]. Through new protein engineering technologies, the field of protein therapeutics is accelerating the rate of drug discovery offering promising solution for various hematological disorders too.

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Approaches of protein engineering: A number of different approaches have been developed to modify a protein by mutating the parent gene. These approaches fall into two categories mainly, rational approaches and random methods [2]. Rational design otherwise the rational approach is a strategy in protein engineering, which attempts to create improved protein molecules based on the three-dimensional structure and the relationship between structure and function. It is the classical method, which employ mainly the site directed mutagenesis of proteins, that involve manipulation of one or a few specific amino acid residue in the target gene so as to modify the natural protein in a predicted manner.

Therefore it is also useful for testing hypothesis about the structural and functional roles of specific amino acid residues in a protein. [1] On the other hand, many cases of protein engineering deals with the proteins, of limited structural and functional information. In such instances rational approach is limited and alternative approaches of random methods are adopted. The category of random methods include a variety of procedures for optimizing performance including evolutionary

techniques. Here the individual amino acids are randomly mutated to any of the other naturally occurring amino acids to generate a library of proteins, which is then screened to identify novel protein variant with desired phenotype. One of the approaches in random method, the Directed evolution employs recombinant DNA techniques to create thousands of possible variants, and then uses high through put screening methods to select the best variant [3]. It mimics Darwinian selection in vitro but operates at molecular level and offers promising strategies to enhance biological activity of native proteins especially enzymes[4]. DNA shuffling and error prone PCR methods are the most commonly chosen techniques, along with directed evolution in the random approach of protein engineering.

Protein engineering and therapeutic proteins: Therapeutic proteins have revolutionized the treatment of many diseases. Protein therapeutics aimed at developing clinically relevant of drug involves modifications derived from protein engineering. In protein therapeutics, proteins are purposefully modified by, glycol-engineering, Fc fusion, conjugation to polyethylene glycol, site directed mutagenesis or evolutionary approaches to enhance the clinical potential, which allows natural proteins to evolve as an utilizable efficient drug. Antibody-based drugs subsequently have evolved as the largest and fastest growing class of protein therapeutics. The rationale of protein engineering in protein therapeutics is to design drugs with enhanced efficacy, greater safety, longer half-life and reduced immunogenicity [5].

Even the classic drugs has been reported to induce almost the entire spectrum of hematological disorders, which may include conditions affecting white cells, red cells, platelets, and the coagulation system. Drug induced syndromes include hemolytic anemia, methemoglobinemia, red cell aplasia, sideroblastic anemia, megaloblastic anemia, polycythemia, aplastic anemia, leukocytosis, neutropenia, eosinophilia, immune thrombocytopenia, microangiopathic syndromes, hypercoagulability, hypothrombinemia, circulating anticoagulants, myelodysplasia and acute leukemia [6]. So, drugs with optimum affinity and specificity, with reduced side effects are mandatory and are being made possible through protein therapeutics by controlled manipulation of its physical chemical and biological properties. Since the field of protein therapeutics is advancing, sophisticated protein engineering efforts are also being undertaken for the development of new scaffolds, with unique properties.

Strategies of protein engineering employed in protein therapeutics: ***Glycoengineering:*** Biological function of a protein is determined by the various modifications on it and carbohydrate modifications play a major role in molecular stability, solubility, in vivo activity, serum half life and immunogenicity[7][8]. The sialic acid component of carbohydrate is the key agent responsible for extending the serum half life of protein. Mainly two aspects of Glycoengineering are opted in protein therapeutics, first one is N-linked glycosylation and the other is O-linked glycosylation. N-linked glycosylation is particularly important because it deals with engineering protein by introducing N-linked glycosylation at consensus sequences into desirable positions of the peptide backbone. It results in modified proteins with increased sialic acid containing carbohydrate and thereby extends the serum half life of natural protein.

Basically it takes place by the transfer of a 14-residue oligosaccharide, to a nascent polypeptide at a Asn residue located within Asn-X-Ser/Thr consensus motif where X is any amino acid except Pro. O-linked glycosylation is by the attachment of single monosaccharide unit usually N-acetylgalactosamine, to a Ser or Thr residue [7]. Glycoengineering is serving a major contribution in enhancing the properties of protein intended for therapeutics.

Fc fusion: Fc fusion proteins or peptibodies are chimeric proteins engineered by fusing a biologically active peptide with the Fc domain of immunoglobin G. [9] One of the valuable features of Fc domain is that it can prolong the plasma half life of the protein of interest resulting a dramatic enhancement of its

therapeutic efficiency [10][11]. Fc protein can also improve the in vivo and in vitro solubility and stability of its binding partners. They are advantageous over the full-length monoclonal antibodies, since their production is efficient and less consuming. The affinity of these peptides towards their target can be further improved by concatamerization of its sequence or by addition of specifically designed linker sequences, spacers or flanking residues. The orientation of peptide sequences is of greater consideration, as it is capable of altering its activity e.g. peptides fused with carboxyl terminal Fc domain are found to be more active [12][13].

Pegylation: Pegylation is the process of modification of biological molecules by covalent conjugation with polyethylene glycol; which is highly soluble, non-toxic as well as non immunogenic and non antigenic polymer [14]. Davies and Abuchowsky introduced Pegylation in 1970 and has immense application in protein therapeutics. It aids in improving the drug solubility and reduces immunogenicity by altering the conformation, electrostatic binding and hydrophobicity which in particular reduces the dosage frequency of the drugs [15]. Efficacy of pegylation can be improved by and introducing a number of PEG binding sites through site-directed mutagenesis [16]. In some cases the pegylated proteins can be immunogenic too [17]. Pegylation of proteins has significantly improved the treatment of several chronic diseases including hematological malignancies.

Protein scaffolding: Scaffold is typically a structure made for support. Similarly protein scaffolds are nothing but a type of polypeptide fold. It is actually considered as an entity of protein engineering usually derived from a robust as well small soluble monomeric protein (Kunitz inhibitors or the lipocalins) or else from a stably folded extra membrane domain of cell surface receptor (e.g. protein A, fibronectin or the ankyrin repeat)[18]. An ideal protein scaffold should provide a rigid folding unit that spatially brings together the exposed loops, forming an wide interface which ensure tight binding of the target [19]. So through protein scaffolding, a combined characteristics of both the peptide and the scaffold protein are obtained which can ensure specificity, stability and sustained availability.

Antibody engineering: Antibody engineering can be considered as a sub discipline of protein engineering made possible with the development of hybridoma technology. It is one of the fastest growing disciplines under protein therapeutics for developing antibodies with intense specificity and affinity to the binding partner [20]. Fc region of the antibody mediates interaction with several receptors, which allows it to recruit the immune system. Since it is difficult to modify the epitope eventually binds to the antibody, Fc region is the major target for the enhancement of functional properties through rational protein engineering approaches [10][20][21].

Protein engineering in hematological disorders: Anemia is a common hematological disorder predominant in patients with cancer. About 50% of cancer patients are anemic at diagnosis. Hematologic malignancies increase the likelihood of developing anemia; for example, 60–70% of patients with non-Hodgkin's lymphoma are anemic at the time of diagnosis [22]. It is primarily due to the erythropoietic aberrations associated with the chronic diseases either by a relative endogenous erythropoietin deficiency or a blunted erythropoietic response to EPO, caused by inflammatory cytokines [23]. Repeated cycles of chemotherapy may impair erythropoiesis cumulatively [24].

Treatment options for anemia in patients with cancer receiving chemotherapy include administration of red blood cell transfusions. Since there was no alternative to transfusion, treatment of mild-to-moderate anemia was generally avoided; intervention was withheld until hemoglobin concentrations declined to more severe levels (i.e., 7-8 g/dL) or the patient experienced signs and symptoms of severe anemia [25][26]. The human recombinant erythropoietin contribute an

alternative solution for the red blood cell transfusion. Epoetin alpha and beta are the two forms of chinese hamster ovary cell- derived recombinant DNA-derived erythropoietin (rhEPO) that are used clinically for anemia related to chronic kidney failure [27], but in 1990s, it was suggested to treat patients with multiple myeloma [28].

Alike native EPO, the recombinant HuEPO (Human erythropoietin) consists of a 165 amino acid single polypeptide chain that contains three N-linked glycosylation sites at asparagines residues (Asn24, Asn38, Asn83) and one O-linked site at serine residue Ser126, but differs in terminal N-acetylneuraminic acid (Neu5Ac) content, O-acetylation of the Neu5Ac residues, N-acetylactosamine extensions, and degree of branching [29][30]. Epoetin alpha and epoetin beta are isoforms containing 165 amino acids, but differs in their carbohydrate content, [31][32] due to which it has reported that both of them had a longer half life after sub cutaneous injection than the 8.5 hours of natural erythropoietin, with values ranging from 20.5 hours for epoetin beta to 24 hours for epoetin alpha [33][34][35][36].

Darbepoetin alpha, the later version introduced in 2003 posses an even longer half-life after subcutaneous injection (~49 hours) [38]. The engineering strategy applied behind this therapeutic invention is engineering protein with additional glycosylation sites that can eventually increase the in vivo serum half-life and thereby target exposure. A five amino acid changes (Ala30Asn, His32Thr, Pro87Val, Trp88Asn, Pro90Thr) were introduced resulting in two additional consensus sequences for N-linked carbohydrate addition, thereby increasing the carbohydrate content to 51% [8][38], which means eventually 2 additional glycosylation sites are added to the first generation drug epoetin alpha, to produce second generation drug Darbepoetin.

Hence glycoengineerd Darbepoetin alpha has higher specific activity in patients in increasing Hb response and decreasing transfusion rates.

Thrombocytopenia is common in critically ill patients in which platelet counts critically declines below 100,000/µL resulting in adverse outcomes. Idiopathic thrombocytopenic purpura (ITP), also known as immune thrombocytopenia is an autoantibody-mediated thrombocytopenic disorder characterized by the impairment of platelet production and accelerated destruction of platelets by the self-antibodies [39].

The initial treatment for ITP such as corticosteroids, intravenous immune globulin, or Rh_0 (D) immune globulin acts primarily by interfering with platelet destruction [40]. Steroids help to increase the platelet count by lowering the activity of immune system but it is not advantageous, as the disorder recurs when corticosteroids are tapered. Similarly other immunomodulatory agents suppress the production of antiplatelet antibodies, but relapse is common when these agents are discontinued. Splenectomy, in other respects has lasting effects and even cures the disease in some patients. Since the remedy is not expedient, instead of destroying the platelet by suppressing the immune system, an alternative strategy to increase the platelet production could be effective in managing the disorder [41][42]. Megakaryocyte Growth and Developmental Factor (MGDF) is a powerful inducer of megakaryopoiesis in vitro and thrombopoiesis in vivo [43].

Pegylayed form of human recombinant MGDF is ten times more potent in vivo than the recombinant MGDF which shows positive results in cancer patients undergoing chemotherapy [42][43]. Recently a novel thrombopoiesis- stimulating protein, a peptide mimetic of thrombopoietin (TPO) AMG 531 has been developed by fusing with Fc domain of IgG, which functions as a TPO receptor agonist. AMG 531 was constructed by fusing two tandem repeats of the peptide sequences; Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala dimerized with an eight glycine linker; to carboxy terminus of human IgG Fc domain homodimer via a five glycine linkers [43][44]. It is clinically proven that AMG531 binds to TPO

receptor with high affinity and promotes the production and maturation of megakaryocytes [45][46]. Eltrombopag and AKR-501 are two drugs of this same type that have shown positive results in clinical trials. In addition, antibodies that can stimulate the c-Mpl receptor are being engineered to act as potent TPO agonists [47].

These and other drugs in pre-clinical / clinical development represent a new line of therapy for thrombocytopenic patients. Heparin induced thrombocytopenia (HIT) is a relatively common immune mediated disorder with a serious decrease in platelet count during or shortly after exposure to heparin [49]. Usually heparin prevents clotting but in HIT heparin triggers the immune system to destroy the platelets. Two distinct types of HIT are reported; Non immune and immune mediated HIT.

Non immune mediated HIT is not harmful since it causes mild decrease in the platelet count where as the type 2 HIT, theimmune mediated one is very dangerous. In those patients, heparin can form an immune complex with platelet factor 4 forming a heparin-PF4 complex. Immune system identifies this complex as foreign substance and as a result antibodies are raised against the complex followed by waves of platelet destruction. The disruption of platelets consequently leads to thrombosis [50]. Platelet transfusion is not suggested since it worsens the situation. So the recombinant hirudin, Lepirudin is chosen as an anticoagulant drug, which is very effective in deep venous thrombosis. It differs from normal hirudin by the substitution of leucine for isoleucine at the N-terminal end of the molecule and the absence of a sulfate group on the tyrosine at position 63 [51][52].

Acute lymphoblastic leukemia (ALL) / acute lymphocytic leukemia/ acute lymphoid leukemia is a type of blood cancer characterized by the overproduction and accumulation of immature white blood cells. It is most common in childhood with a peak incidence at 3-5 ages [53]. Asparagine is a non-essential amino acid, which leukemic cells and other tumor suspected cells are unable to synthesize. Leukemic cells require high amount of asparagine since it depends upon the circulating asparagine produced by the normal cells. Asparaginase is an enzyme that catalyzes the hydrolysis of asparagine to aspartic acid and thereby deprives the leukemic cells from circulating asparagine. *Erwinia* L-Asparaginase and *E. coli* L-asparaginase are the two Asparaginase used for the treatment of ALL in children. PEG-L asparaginse, a pegyalted modified version of native *E.coli* asparaginase has a much longer serum half life, producing a prolonged asparagine depletion [53]. Since it is an effective enzyme drug used for the treatment of acute lymphoblastic leukemia, its effective usage in clinical arena is complicated owing to the significant residual glutaminase activity. The Insilco mutagenesis at the vicinity of ligand binding site by the replacement of amino acid Asp96 at the enzymes active site with alanine decreased the glutaminase activity by 30% and more over increased the Asparaginase activity by 40%. The site directed mutagenesis of the Asparaginase in aforementioned way could develop a variant of enzyme drug with reduced side effect for treating acute lymphoblastic leukemia in children [55].

Using protein engineering approach, a new modified thrombin has been introduced which can act as an antithrombotic agent. Usually thrombin interacts with multiple procoagulants to mediate clotting. On the other hand the interaction of thrombin with the thrombomodulin present on the vascular endothelium activates the protein C, resulting the attenuation of blood clot. By omitting the property favoring coagulation by mutagenesis, a variant thrombin with only anticoagulation activity can be developed. This idea was practically exploited by protein engineers, where introduction of a single substitution E229A, resulted in single specificity towards anticoagulant substrate, protein C. This modified thrombin is successfully tested in monkeys, which show reversible anticoagulation without any pro-coagulant activity.[55]

A red blood substitute is now made possible by the use of recombinant hemoglobin, adopting recombinant DNA technology [56]. A recombinant factor VIIa (rFVIIa), novel drug

produced by recombinant technology approved for use in patients with congenital heamophilias [12]. Refacto, a recombinant factor VIII whose beta domain is deleted, is used for the treatment of hemophilia A [57]. CNTO-528 and CNTO-530 are peptibodies clinically exploited for RBC production [58][59]. Pegylation of RBCs at the level of membrane proteins, carbohydrates or lipid head groups has been used for transfusion purposes and also for the preparation of stealth RBCs for drug delivery system [60]. Ontak is an engineered IL-2 receptor used to treat persistant or recurrent cutaneous lymphoma [61]. Leukine is a GM-CSF (Granulocyte and Macrophage Colony Stimulating Factor) used to induce granulocyte and macrophage production in immunosuppressive conditions such as chemotherapy, transplantation and cancer [62].

Application of antibody engineering is also amenable in hematological malignancies. Rituxan (rituximab) and Zevalin are monoclonal antibody with high affinity and specificity towards B cell specific antigen CDC20 . Both of them aids in the direct antiproliferative activity of B cells. It is clinically used as a potential drug for various cancers, including B cell non Hodgkins lymphoma [63]. Campath has been clinically utilized for the treatment of B cell chronic lymphocytic leukemia. It is an antibody engineered against CD52, an antigen present on the mature lymphocyte [64].

Zinc finger protein engineering is yet another approach that has been used in the task of gene regulation applications. Zinc finger proteins consist of repeated zinc binding motif containing conserved cysteine-histidine ligands which forms independently folded domains that are coordinated by one or more zinc ions. It performs diverse functions including DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly, and lipid binding [65]. This zinc finger design and principle is used to design DNA binding proteins to alter gene expression. E.g. three-fingered protein is found to block the expression of oncogene that was transformed into a mouse cell line. Fusion of zinc finger peptides to repression or activation domains allows selective gene switching [66]. Though the approach has not been employed in hematological disorders, it presents itself as a potent strategy to intervene hematological disorders.

CONCLUSION: Proteins are the established class of therapeutics which can be tuned in alternative ways to broadcast solution for various disorders. Protein engineering is a broad discipline with enormous possibilities to build up different strategies to develop better protein drugs. Protein therapeutics, along with the convenient strategies of protein engineering is on the urge to tackle hematological malignancies offering a next generation drugs with clinical and commercial safety.

References:

1. Aranesp® (darbepoetin alfa) prescribing information. Available from URL:http://www.aranesp.com/pdf/aranesp_PI.pdf
2. Baroletti S A, Goldhaber S Z. Heparin-induced thrombocytopenia. *Circulation* 2006; 114:355-356
3. Benjamin Wu, Yu- Nien Sun. Pharmacokinetics of peptide-Fc fusion proteins. *Journal of pharmaceutical sciences* 2013; 103: 53-64
4. Bohlius J, Weingart O, Trelle S, Engert A. Cancer-related anemia and recombinant human erythropoietin-an updated review. *Nature Clinical Practice Oncology* 2006; 3:152-164
5. Broudy VC, Lin NL. AMG531 stimulates megakaryopoiesis in vitro by binding to Mpl. *Cytokine* 2004; 25:52–60
6. Burcu T-Y, Ceren A, Petek Cakar Z .Protein Engineering Methods and Applications, Protein Engineering, Prof. Pravin Kaumaya (Ed.) (2012). ISBN: 978-953-51-0037-9, InTech, Available from URL :http://www.intechopen.com/books/protein-engineering/protein-engineering- methods-and-applications
7. Cancer Facts & Figures 2014. American Cancer Society. Available from URL: http://www.cancer.org/acs/groups/content/@research/documents/webcontent/a cspc-042151.pdf.
8. Caravella J, Lugovskoy A. Design of next generation protein therapeutics.*Current Opinion in Chemical Biology* 2010;14:520-528
9. Cheung E, Liebman H A. Anti-RhD immunoglobulin in the treatment of immune thrombocytopenia. *Biologics* 2009; 3:57-62
10. Cohn C S, Bussel J B. Romiplostim: a second-generation thrombopoietin agonist. *Drugs of Today* 2009; 45:175-188
11. Consensus conference. Perioperative red blood cell transfusion. *Journal of American Medical Association* 1988;260:2700–2703
12. Cwirla SE, Balasubramanian P, Duffin DJ, Wagstrom CR, Gates CM, Singer SC, Davis AM, Tansik RL, Mattheakis LC,

Boytos CM, Schatz PJ, Baccanari DP, Wrighton NC, Barrett RW, Dower WJ. Peptide agonist of the thrombopoietin receptor as potent as the natural cytokine. *Science* 1997; 276:1696–1699

13. D R Mould, A Baumann, J Kuhlmann, M J Keating, S Weitman, P Hillmen, L R Brettman, S Reif, P L Bonate. Population pharmacokinetics–pharmacodynamics of alemtuzumab (Campath®) in patients with chronic lymphocytic leukaemia and its link to treatment response. *British Journal of Clinical Pharmacology* 2007; 64:278-291
14. Egrie J C, Dwyer E, Browne J K, Hitz A, Lykos M A. Darbepoetin alpha has a longer circulating half-life and greater in vivo potency than recombinant human erythropoietin. *Experimental Hematology* 2003; 31:290-299
15. Elliot S, Lorenzini T, Asher S, Aoki K, Brankow D, Buck L, Busse L, Chang D, Fuller J, Grant J, Hernday N, Hokum M, Hu S, Knudten A, Levin N, Komorowski R, Martin F, Navarro R, Osslund T, Rogers G, Rogers N, Trail G, Egrie J. Enhancement of therapeutic protein in vivo activities through glycoengineering. *National Biotechnology* 2003; 21:414-421
16. Gebauer M, Skerra A. Engineered protein scaffolds as next-generation antibody therapeutics . *Current Opinion in Chemical Biology* 2009; 13:245–255
17. Gibbs C S, Coutre S E, Tsiang M, Li W-X, Jain A K, Dunn K E, Law V S, Mao C T, Matsumura S Y, Mejza S Y, Pabrorsky L R, Leung L L K. Conversion of thrombin into an anticoagulant by protein engineering. *Nature* 1995; 378:413-416
18. Gillis S, Urdal DL, Clevenger W, Klinke R, Sassenfeld H, Price V, Cosman D. Production of recombinant human colony stimulating factors in yeast. *Behring Institute Mitteilungen* 1988:1-7
19. Goldwasser E, Kung C K, Eliason J. On the mechanism of erythropoietin- induced differentiation: the role of sialic acid in erythropoietin action. *Journal of Biological Chemistry* 1974 ; 249: 4202–4206
20. Groopman J E, Itri L M. Chemotherapy-induced anemia in adults: Incidence and treatment. *Journal of National Cancer Institute* 1999; 19:1616-1634
21. Hall MP, Gegg C, Walker K, Spahr C, Ortiz R, Patel V, Yu S, Zhang L, Lu H, DeSilva B, Lee JW. Ligand-binding mass spectrometry to study biotransformation of fusion protein drugs and guide immunoassay development: Strategic approach and application to peptibodies targeting the thrombopoietin receptor. *AAPS Journal* 2010; 12:576–585
22. Harris J M, Martin N E, Modi M. Pegylation: a novel process for modifying pharmacokinetics. *Clinical Pharmacokinetics* 2001; 40:539-559
23. Hershfield M S, Chaffee S, Koro- Johnson L, Mary A, Smith A A, Short S A. Use of site-directed mutagenesis to enhance the epitope-shielding effect of covalent modification of proteins with polyethylene glycol. *Proceedings of the National Academy of Sciences U.S.A* 1991; 88:7185-7189
24. Hokom M M, Lacey D, Kinstler O B, Choi E, Kaufman S, Faust J, Rowan C, Dwyer E, Nichol J L, Grasel T. Pegylated megakaryocyte growth and development factor abrogates the lethal thrombocytopenia associated with carboplatin and irradiation in mice. *Blood* 1995; 86:12
25. Juan J P-R, Krzyzanski W, Bouman-Thio E, Miller B, Jang H, Bai S A, Zhou Honghui, Yohrling J, Cohen A, Buggraaf Jacobus, Franson Kari, Davis H M. Pharmacokinetics and pharmacodynamics of the erythropoeitn Mimetibody construct CNTO 528 in healthy subjects. *Clinical Pharmacokinetics* 2009; 48:601-613
26. Karien van der P, Braam B, Jie K E, Gaillard C AJM. Mechanisms of disease: erythropoietin resisitance in patients with both heart and kidney failure. *Nature Clinical Practice Nephrology* 2008; 4:47-57
27. Karpatkin S. Autoimmune thrombocytopenic purpura. Blood 1980Idiopathic Thrombocytopenic Purpura. MedicineNet.com. Available from URL: http://www.medicinenet.com/idiopathic_thrombocytopenic_purpura_itp/page5.htm#what_are_the_treatments_for_idiopathic_thrombocytopenic_purpura_itp
28. Kelley B. Industrialization of mAb production technology: The bioprocessing industry at a crossroads. *mAbs* 2009; 1:443–452
29. Klug A. The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annual Review of Biochemistry* 2010; 79:213-31
30. Kurtzman L A, Govindarajan S, Vahle K, Johnes T J, Heinrich V, Patten P A. Advances in directed protein evolution by recursive genetic recombination: applications to therapeutic proteins. *Current Opinion in Biotechnology* 2001; 12:361-370
31. Laity J H, Lee B M, Wright P E. Zinc finger proteins: new insights into structural and functional diversity. *Current Opinion in Structural Biology* 2001; 11:39-46
32. Leemhuis H, Kelly R M, Dijkhuizen L. Directed evolution of enzymes: library screening strategies. *IUBMB Life* 2009;61:222–228
33. Lepirudin. Drug Bank. Available form URL: http://www.drugbank.ca/drugs/db00001#references
34. Li J, Yang C, Xia Y, Bertino A, Glaspy J, Roberts M, Kuter D J. Thrombocytopenia caused by development of antibodies to thrombopoietin. *Blood* 2001; 98:3241-3248
35. Ludwig H., Fritz E, Kotzmann H, et al. Erythropoietin treatment of anemia associated with multiple myeloma. *New England Journal of Medicine* 1990; 322: 1693-9.
36. Maynard J, Georgiou G. Antibody engineering. *Annual Review of Biomedical Engineering* 2000; 2:339-376
37. Mintzer D M, Billet S N, Chmiielewski L. Drug-Induced hematologic syndromes. *Advances in hematology* 2009
38. Molineux G, Newland A. Development of romiplostim for the treatment of patients with chronic immune thrombocytopenia: From bench to bedside. *British Journal of Haematology* 2010; 150:9–20
39. Murad K L, Mahany K L, Brugnara C, Kuypers F A, Eaton J W, Scott M D. Structural and functional consequences of antigenic modulation of red cells with methoxypoly(ethylene glycol). *Blood* 1999; 93:2121
40. Neumann T A, Foote M. Megakaryocyte growth and development factor (MGDF): an MpI ligand and cytokine that regulates thrombopoiesis. *Cytokines* 2000; 6: 47-56
41. Nina M, Antikainen, Stephan F. Martin. Altering protein specificity:techniques and applications: *Bioorganic and Medicinal Chemistry*. 2005; 13:2701-2716
42. Ogden J E. Recombinant haemoglobin in the development of red-blood- substitute. *Trends in Biotechnology* 1992; 10:91-96
43. Panetta J C, Gajjar A, Hijjya N, Hak L J, Cheng C, Liu W, Pui C H, Relling M V. Comparative pharmacokinetic studies of three asparaginase preparations. *Journal of Clinical Oncology* 1993;11:1780-1786
44. Paul J C. Introduction to current and future protein therapeutics: A protein engineering perspective. *Experimental*

Cell Research 2011; 317:1261-1269
45. Procrit® epoetin alfa full prescribing information. Available from URL: http://www.orthobiotech.com/common/prescribing_ information/PROCRIT/PDF/ProcritBooklet.pdf
46. Psaila B, Bussel J B. Immune thrombocytopenic purpura. *Hematology/ Oncology Clinics of North America* 2007; 21: 743-759
47. Roche UK package leafeat. Available from URL: http://www.rocheuk.com/ ProductDB/Documents/rx/pil/NeoRecormon_PFS_ PIL.pdf
48. Rubingh D N, Grayling R A. Protein engineering. 3 volume Biotechnology; EOLSS. Available from URL: http://www.eolss.net/sample-chapters/c17/E6-58-03-06.pdf
49. Rush RS, Derby PL, Smith DM, Merry C, Rogers G, Rohde M F, Katta V. Microheterogeneity of erythropoietin carbohydrate structure. *Analytical Chemistry* 1995;67: 1442–1452
50. Sandberg H, Almstedt A, Brandt J, Gray E, Holmquist L, Oswaldsson U, Sebring S, Mikaelsson M. Structural and functional characteristics of the B- domain-deleted recombinant factor VIII protein, r-VIII SQ. *Thrombosis and Haemostasis* 2001;85:93-100
51. Sasaki H, Bothner B, Dell A, Fukuda M. Carbohydrate structure of erythropoietin expressed in Chinese hamster ovary cells by a human erythropoietin cDNA. *Journal of Biological Chemistry* 1987;262:12059–12076
52. Sathyanarayana P, Houde E, Marshall D, Volk A, Makropoulos D, Emerson C, Pradeep A, Bugelski PJ, Wojchowski DM. CNTO 530 functions as a potent EPO mimetic via unique sustained effects on bone marrow proerythroblast pools. *Blood* 209; 113:4955–4962
53. Schiele F, Vuillemenot A, Kramarz P, Kieffer Y, Angyenot T, Bernard Y, Bassand J P. Use of recombinant hirudin as a antithrombotic treatment in patients with heparin-induced thrombocytopenia. *American Journal of Hematology* 1995; 50:20-25
54. Schmidt C A, Codevilla C F, Fronza M, Casali R G, Dalmora S L. Physico- chemical characterisation and biological evaluation of recombinant human erythropoietin in pharmaceutical products. *Latin American Journal of Pharmacy* 2003; 22:343-350
55. Shannon A M, Greg A L, Arthur J C, John R D. Rational design and engineering of therapeutic proteins. *Drug Discovery Today* 2003; 8:212-221
56. Shimamoto G, Gegg C, Boone T, Queva C. Peptibodies: A flexible alternative format to antibodies. *mAbs* 2012; 4:586–591
57. Silberstein LE, Kruskall MS, Stehling LC, Johnston MF, Rutman RC, Samian CT, et al. Strategies for the review of transfusion practices. *Journal of American Medical Association* 1990;263:1993-1997
58. Sinclair A M, Elliot S. Glycoengineering: the effect of glycosylation on properties of therapeutic proteins. *Journal of Pharamaceutical Sciences* 2005; 94:1626-1635
59. Skerra A. Engineered protein scaffolds for molecular recognition. *Journal of Molecular Recognition* 2000; 13: 167-187
60. Skibeli V, Nissen-Lie G, Torjesen P. Sugar profiling proves that human serum erythropoietin differs from recombinant human erythropoietin. *Blood* 2001; 98(13)
61. Smith R E, Jaiyesmi A, Meza L A, Tchekmedyian N S, Chan D, Griffith H, Brosman S, Bukowski R, M Murdoch, Rarick M, Saven A, Colowick A B, Fleishman A, Gayko U, Glaspy J. Novel erythropoiesis stimulating protein (NESP) for the treatment of anaemia of chronic disease associated with cancer. *British Journal of Cancer* 2001; 84:24-30
62. Storring P L, Tiplady R J, Gaines Das R E, Stenning B E, Lamikanra A, Rafferty B, Lee J. Epoetin alpha and beta differ in their erythropoietin isoform composition and biological properties. *British Journal of Hematology* 1998; 100:79-89
63. Strohl W R. Optimization of Fc-mediated effector functions of monoclonal antibodies. *Current Opinion in Biotechnology* 2009; 20:685-691
64. Veronese F M, Pasut G. PEGylation, successful approach to drug delivery. *Drug Discovery Today* 2005; 10:1451-1458
65. Warkentin TE. An overview of the heparin-induced thrombocytopenia syndrome. *Seminars in Thrombosis and Hemostasis* 2004; 30:273-283
66. White C A. Rituxan immunotherapy and Zevalin radioimmunotherapy in the treatment of non-Hodgkin's lymphoma. *Current Pharmaceutical Biotechnology* 2003; 4: 221-238.

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