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## **Antibodies and antibody fragments are therapeutic tools in the treatment of type-II diabetes mellitus**

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**Abstract**---Antibody fragments (FABs) are proteins that form part of the antigen recognition site. FABs are produced in genetically modified bacteriophages, bacteria, fungi, or plants and, consequently, can be produced in large quantities at a fraction of the cost of traditional antibodies. Antibody fragments are small and simple structure that today is highly regarded because of the many advantages they have over the use of whole antibodies. Single-domain antibodies are the smallest antigen-binding units of antibodies, consisting either only of one variable domain or one engineered constant domain that solely facilitates target binding. Fibroblast growth factor 21 (FGF21) is a promising drug candidate for the treatment of type 2 diabetes. Clinical use of recombinant fibroblast growth factor 21 (FGF21) for the treatment of type 2 diabetes and other disorders linked to obesity has been proposed; however, its clinical development has been challenging owing to its poor pharmacokinetics.

**Keywords**---antibody, antibody fragments, fibroblast, diabetes, monoclonal antibodies.

**Introduction**

Antibody fragments (FABs) are proteins that form part of the antigen recognition site. FABs are produced in genetically modified bacteriophages, bacteria, fungi, or plants and, consequently, can be produced in large quantities at a fraction of the cost of traditional antibodies. Antibody fragments are small and simple structure that today is highly regarded because of the many advantages they have over the use of whole antibodies. Antigen-binding fragments (Fab) and single chain variable fragments (scFV) are common antibody fragments that have been investigated and also another type known as “third generation” (3G) molecules. The Fab fragments are consisting of one constant and one inconstant domain of heavy and light chains, whereas in scFV fragments, the varying areas of heavy and light chains are merged and the constant heavy and light chain that was in the previous state is not here and in the case of the third type, it consists of only one variable heavy chain (Figure 1). A wide variety of antibody fragments has been developed as alternative platforms to IgGs. The most significant advantages to antibody fragments include size, manufacturing, tissue penetration, and ability to concatenate to generate multi-specificity. Thus far, only Fab fragments have been marketed, although several clinical and pre-clinical candidates have been generated using scFv, human VH or VL domains, humanized camelid VHH domains, and IgNAR single domains [1-4]. A new antibody fragment scaffold has recently also been constructed from the CH2 domain of a human IgG. Several strategies have been used to improve the naturally short half-life of antibody fragments, including PEGylation, the use of repeating peptide sequences, polysialylation, albumin or IgG binding or fusions, and other approaches. With genetic engineering, we can produce in the laboratory virtually any form of the

variable chains needed. Thus smaller recombinant antibody fragments, such as FAb (“fragment, antibody”), scFv (single chain variable chain fragments), and domain antibodies (dAbs) are credible alternatives to traditional IgG-based MAbs for certain functions. These fragments retain the targeting specificity of IgG-based MAbs but also possess other characteristics, such as smaller size, monovalency, ease of engineering and manufacture, improved tissue penetration, and broader biodistribution[5-7], as well as lack of potentially deleterious Fc effector function, that may be desired for certain applications (Figure 1).

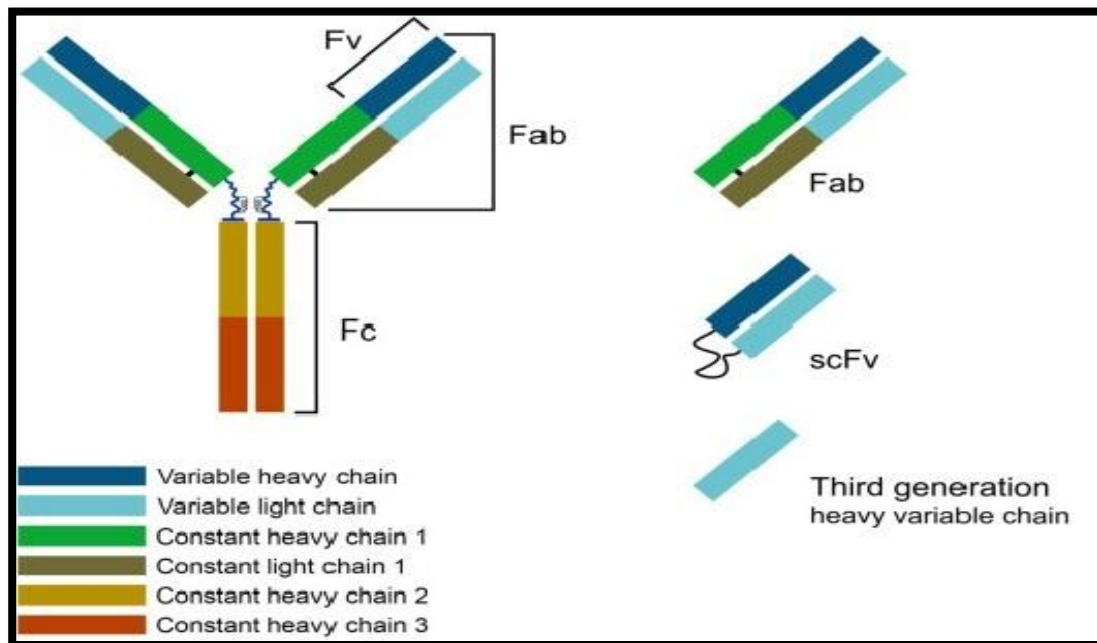


Figure 1 Diagrammatical Representation of Antibody Fragments

The Three technologies includes

- Antigen-binding fragments (Fab):
- Single chain variable fragments (scFv)
- Third generation(3G) molecules

### Antigen Binding Fragments

Fab agents are the oldest class of monoclonal antibody (mAb) fragment therapeutics, demonstrated by the fact that all eight fragment therapeutics that entered clinical development before 1995 were Fabs.<sup>1</sup> This class of fragments is also arguably the most successful, accounting for 49% of fragments to have entered clinical development and three FDA approved clinical applications. Abciximab (ReoPro, Centocor/Johnson & Johnson) is a Fab fragment of a chimeric antibody against platelet glycoprotein IIb/IIIa, approved in 1994 as an adjunct to prevent thrombosis during to coronary artery catheterization for ST-elevation myocardial infarction. Ranibizumab (Lucentis, Genentech) is a

humanized Fab directed against vascular endothelial growth factor A, approved in 2006 as a treatment for neovascular (wet) age-related macular degeneration. Certolizumab pegol (UCB) is a pegylated anti-TNF $\alpha$  Fab approved in 2008 for treatment of Crohn disease. Beyond these monoclonal fragments, polyclonal Fab agents are also marketed, including CroFab, DigiFab and DigiBind. The corollary to successful experiences with Fab technology is the large number of failed projects. Failure and success alike are learning experiences, and provide knowledge that can be applied in pharmacology, regulatory concerns and biomanufacturing, which may explain why numerous companies continue to test Fabs in clinical development.

### **Single-Chain Variable Fragments**

Single-chain variable fragments (scFvs) are recombinant molecules in which the variable regions of light and heavy immunoglobulin chains encoding antigen-binding domains are engineered into a single polypeptide. Generally, the VH and VL sequences are joined by a flexible linker sequence, and a series of variants are generated for optimizing binding affinity and stability.<sup>9</sup> Molecular engineers have continued to diversify the fundamental scFv molecule, resulting in paired scFvs that bind to one another through complementary regions to form bivalent molecules (diabodies), complementary scFvs themselves produced as a single chain (tandem scFvs or tascFvs), and bispecific tandem scFvs (bis-scFvs), among others. Nearly as many scFv therapeutics have entered clinical development as Fabs, accounting for 40% of clinically evaluated fragments.<sup>1</sup> These candidates include 12 active and nine discontinued agents, most in early development, with all having entered clinical study after 1995. With at least 11 scFvs publicly described in pre-clinical research,<sup>1</sup> interest in this technology remains strong. From these data, scFvs appear to be a promising technology that, while as yet unproven, will have many opportunities to achieve clinical success in the future. Five monovalent, monospecific scFvs were developed, only two of which remain active projects in early development. ESBA-105 is an anti-TNF $\alpha$  scFv in Phase 1 development by ESBA Tech for ophthalmic indications. Efungumab (Mycograb), an scFv that binds to the heat shock protein of *Candida albicans*, is in Phase 2 development by NeuTec, a wholly-owned subsidiary of Novartis. Novomab-G2 is an anti-cancer scFv discontinued in Phase 2 by Viventia after the company decided to pursue a formulation that included a cytotoxic conjugate. Pexelizumab is an anti-C5 scFv discontinued in 2007 by Alexion Pharmaceuticals and Proctor & Gamble after primary outcomes were not met in two Phase 3 trials. Aurograb, an scFv that binds to a surface protein of methicillin-resistant *Staphylococcus aureus*, was discontinued in 2008 after Phase 2 trials failed to show efficacy.

### **Third Generation Molecules**

Another approach to reducing the size of antigen-binding immunoglobulin-derived recombinant proteins has been to “miniaturize” full-sized mAbs by removing domains deemed non-essential for function.<sup>16</sup> Miniaturized mAbs were included in the “3G” fragment category due to the relatively recent advent of this approach, in contrast to scFvs, which were described as early as 1988. Only a handful of “miniaturized” antibodies have entered clinical development. Among the best examples of mAb miniaturization are the small modular immunopharmaceuticals

(SMIPs) from Trubion Pharmaceuticals. These molecules, which can be monovalent or bivalent, are recombinant single-chain molecules containing one VL, one VH antigen-binding domain, and one or two constant “effector” domains, all connected by linker domains. Presumably, such a molecule might offer the advantages of increased tissue or tumor penetration claimed by fragments while retaining the immune effector functions conferred by constant domains. At least three “miniaturized” SMIPs have entered clinical development. TRU-015, an anti-CD20 SMIP developed in collaboration with Wyeth, is the most advanced project, having progressed to Phase 2 for rheumatoid arthritis (RA). Earlier attempts in systemic lupus erythematosus (SLE) and B cell lymphomas were ultimately discontinued. Trubion and Facet Biotechnology are collaborating in the development of TRU-016, an anti-CD37 SMIP, for the treatment of CLL and other lymphoid neoplasias, a project that has reached Phase 2. Wyeth has licensed the anti-CD20 SMIP SBI-087 for the treatment of autoimmune diseases, including RA, SLE and possibly multiple sclerosis, although these projects remain in the earliest stages of clinical testing.

### **Advantages of anti-body fragments**

One advantage of fragments over full-size antibodies is that antibody fragments are smaller than conventional antibodies and generally lack glycosylation, allowing their production in prokaryotic expression systems, which provide time and cost savings. Because of their smaller size as functional components of the whole molecule, antibody fragments offer several advantages over intact antibodies for use in certain immunochemical techniques and experimental applications which includes, Reduced nonspecific binding from Fc interactions (many cells have receptors that bind the Fc region). Ability to control Fc-binding to Protein A or Protein G in experiments involving immunoprecipitation and Western blotting. More efficient penetration of tissue sections, resulting in improved staining in immunohistochemistry (IHC). Potentially higher sensitivity in antigen detection in solid phase applications as a result of reduced steric hindrance from large protein epitopes. Elimination of Fc-associated effector functions (e.g., complement fixation) in antigen-antibody binding studies. Simpler system for studying the structural basis for immune recognition using X-ray crystallography or NMR. Lower immunogenicity than intact antibody for experiments *in vivo*

### **Strategic reasons for selecting an antibody fragment as the drug candidate, instead of an intact IgG, for a particular application may include**

Desire or requirement for a short circulating half-life in serum, as was important for the first recombinant antibody ever made, Reopro. A molecule lacking an Fc effector functionality to eliminate both cellular responses against the target and potential for dimerization of receptors due to bivalency. A smaller biologic that would have broader tissue distribution or the ability to penetrate tumors. A molecule that can be manufactured in either yeast or *E. coli* to potentially reduce cost of goods or increase scale of manufacturing. A monovalent binding molecule that cannot activate receptors by dimerization, such as might be required for receptors such as c-met.

### **Therapeutic tools for type-II diabetes mellitus**

Convenient generation of human dAbs with desired properties and specificities has been achieved using molecular evolution approaches (e.g. phage display) and repertoires of native or synthetic human VH or VL dAbs. However, most of the human dAb affinity reagents have been isolated from synthetic libraries that are usually constructed with engineered single scaffolds and complementarity determining regions (CDR)-based diversities. Although it has been proposed that dAbs have a low immunogenicity, a novel type of pre-existing anti-drug antibody was recently discovered during the development of anti-TNFR1 GSK199505799. Herein, autoantibody-binding to framework sequences of the fully human VH dAbs triggered activation of TNFR1 in *in-vitro* assays and in some drug-native subjects the release of cytokines *in vivo*. GSK2374697 was developed for treatment of type 2 diabetes and includes a human albumin targeting VH dAb for half-life extension thereby prolonging the agonistic effect of the adaptor expandin-4 on the glucagon-like peptide-1 (GLP-1) Receptor [8]. Human dAbs were achieved by molecular evolution approaches and with specificities and properties. dAb human affinity reagents were obtained from the artificial sources which are basically synthesized with one scaffold and complementarity determining regions. In *in-vitro* assays human VH dAbs triggered the activation of TNFR1 whereas cytokines were released *in-vivo*. In treatment of diabetes mellitus type-2, GSK2374697 was developed which contains a human albumin targeting VH dAb which prolonged the agonistic effect of the adaptor expandin-4 on the glucagon-like peptide-1 (GLP-1) Receptor [9-10]. The Fibroblast growth factor 21 was recognized as novel disease modifying agent in the treatment of metabolic disorder such as diabetes mellitus of type-2. FGF21 was considered as a suitable biological tool in the treatment of diabetes mellitus of type-2 which belongs to the endocrine FGF subfamily. Recombinant Fc-FGF21 fusion protein became constructed with the aid of fusing the Fc domain of human IgG1 to the Nterminus of human mature FGF21 through a linker peptide, described an engineering strategy to generate a mighty and efficacious Fc-fused FGF21 is a mighty and efficacious analog inside the treatment of human kind 2 diabetes. Single administration of Fc-FGF21 (RG) showed a sustained reduction in blood glucose levels and body weight gains. Two Fc-FGF21 variants significantly enhanced the *in-vitro*  $\beta$ -Klotho binding affinity and improved glucose lowering effect *in-vivo*. Dual agonists such as GLP-1-Fc-FGF21, provided the potent and sustained glucose lowering effect in diabetic patients. GLP-1-Fc-FGF21 D1 was improved the liver function and lipid profile. GLP-1/FGF21 is a potent dual agonist is worth in the therapy of diabetes type-2 [11-12]. In urine, more quantity of L-Carnitine is responsible for metabolic disorder like type-2 diabetes mellitus. The developed soluble antibody fragment has high specificity and affinity to L-Carnitine, which elucidates the role of L-Carnitine, is a new therapeutic tool for L-Carnitine metabolic mediated disease like type-2 diabetes mellitus [13]. Islets of langerhans (Beta cells) which are secreted by endocrine pancreas has significant role in maintenance in regulation of blood and glucose metabolisms. On other hand deregulation also takes place in diabetes mellitus type-2 by the failure of functioning of islets of langerhans or due to loss of insulin responsiveness. One of the finest and fastest growing human monoclonal antibodies in present clinical practice is the humanised monoclonal antibodies with a good therapeutic tool in the treatment of diabetes mellitus type-2. Monoclonal antibodies plays an vital

role in the present clinical setting as a major diversifying and in diagnosis and identification of radiographic images in cancer therapy like tracing the various tumors as a therapeutic tools in the drug delivery systems for toxins and various cells and tissues. Humanised, recombinant antibody fragments have lesser danger of eliciting detrimental responses from the affected person's immune response and accountable for progressed scientific ability [14]. Additional accumulation and deposition of insulin in lipid and peripheral tissues is responsible for impairment and uptake of glucose and are proposed to make a contribution to the pathophysiology of diabetes mellitus type-2. Vascular endothelial growth factor B (VEGF-B) acts on the vascular endothelium, regulates trans-endothelial transport fatty acids in to skeletal and cardiac muscles. VEGF-B has high metabolic activity with tissue cells such as skeletal myocytes, cardiac muscles and skeletal myocytes and pancreatic b-cells which signals to the endothelium (Figure 2). VEGF-B overexpression of cardiac muscle causes accumulation of ceramide in heart, which leads to dysfunction of mitochondria. VEGF-B plays a vital role in mediating the lipid transport and metabolism, it has been proposed as a novel therapeutic molecule for the Type-2 diabetes mellitus through the gene slicing, gene deletion and neutralizing monoclonal antibodies (mAbs). Vascular endothelium can characteristic as an efficient barrier to extra muscle lipid uptake even under conditions of diabetes mellitus type-2 and such barrier may be maintained by way of inhibiting the VEGF-B signalling. VEGF-B antagonism as a novel pharmacological method for diabetes mellitus type-2 targeting the transportation of lipid to the endothelium to improve muscle insulin sensitivity and glucose disposal [15].

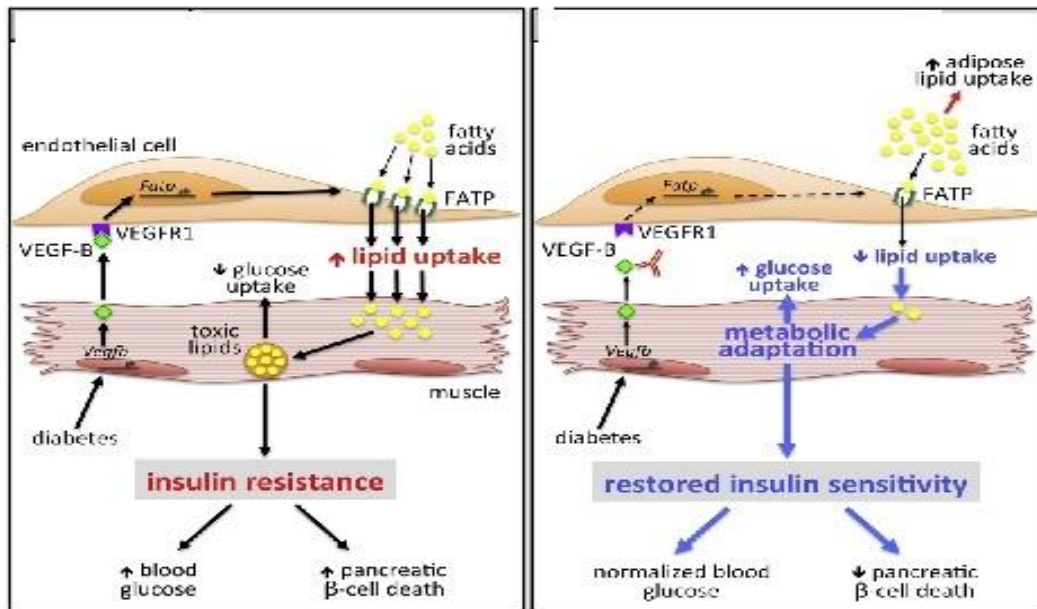


Figure 2. VEGF Promotes Diabetes by Facilitating Lipid Uptake by Muscle

## Conclusion

Current scenario in the fields of antibody and antibody fragments for a huge form of applications. Engineered antibody fragments effectively retained their binding

traits specially with high-affinity, small size, and stepped forward in the field of tissue penetration. Humanised, recombinant antibody fragments have a lower danger of eliciting damaging responses from a patient's immune gadget and, therefore, have enormously stepped forward scientific capability. Antibodies and Antibody fragments are the novel therapeutic approach for the treatment of type-2 diabetes mellitus.

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### **Conflicts of Interest**

Authors have declared no conflict of interest

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