

# **The identification of TNFR5 as a therapeutic target in diabetes**

Running title: Anti-TNFR5 diabetes strategies

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## **Abbreviations**

CD40 – cluster of differentiation 40

CDR – complementarity determining region

Fab – fragment, antibody-binding region

Fc – fragment, crystallisable region

Fv – fragment, variable region

scFv – single chain variable region fragment

IgG – immunoglobulin G

NF- $\kappa$ B – nuclear factor kappa B

STAT1 – signal transducer and activator of transcription 1

T2D - type 2 diabetes

TNFR5 – tumour necrosis factor receptor 5

## **1. Type 2 diabetes: pancreatic $\beta$ -cell dysfunction due to glucolipototoxicity**

Latest global estimates indicate that there are over 422 million adults currently living with diabetes (<http://www.who.int/diabetes/global-report/en>), of which over 90% have type 2 diabetes (T2D). Furthermore, numbers continue to rise at an alarming rate, with the current figure almost four times the number reported back in 1980. This represents a staggering rise in prevalence from 4.7% to 8.5% of the adult population. With diabetes complications including heart attack, stroke, kidney failure, leg amputation, blindness, and nerve damage, the disease is a major global health challenge, both to individuals and their families, and to national economies. Given that current oral and injectable treatment options also often become less effective over time, there is therefore an urgent need both to better understand the causes of the disease, and to identify new targets for therapeutic intervention.

Along with failure of pancreatic  $\beta$ -cells to hypersecrete insulin in the face of ongoing insulin resistance, pancreatic  $\beta$ -cell death is a key component of T2D [1,2]. Obesity is a known causal link to the development of diabetes, and it is recognised that hyperglycaemia and hyperlipidaemia together contribute to drive glucolipotoxic loss of insulin-producing pancreatic  $\beta$ -cell mass. Obesity is also associated with increased visceral fat and central adiposity, Interestingly, whilst once thought to be cells whose only function was to serve as a general fat repository, it is now known that adipocytes play an important role in the release of pro-inflammatory cytokines that contribute to the pathogenesis of T2D [3].

With specific regard to the pancreas, detailed mechanistic understanding of precisely how glucolipotoxicity drives pancreatic  $\beta$ -cell death has until recently proven the subject of much debate. Crucially however, new findings [4] indicate that it is pancreatic upregulation of the TNF receptor superfamily member, TNFR5 (also known as CD40), that leads to activation of the transcription factors NF- $\kappa$ B and STAT1 that drive islet inflammation that ultimately induces  $\beta$ -cell death [5-7]. Furthermore, inhibition of TNFR5 signalling through selective RNA interference abrogates glucolipotoxic induction of NF- $\kappa$ B and STAT1 activation [4]. These findings have major therapeutic implications, as clinical intervention strategies that target TNFR5 could potentially preserve functional  $\beta$ -cell mass in patients with T2D. This in turn would slow down the progression of diabetes, and extend the period in which drugs such as sulphonylureas (that enhance insulin secretion) remain effective. This would also delay the onset of diabetes complications, thereby improving the quality of life, and potentially extending the lifespan, of affected individuals.

## **2. Expert Opinion**

TNFR5 is a type 1 transmembrane glycoprotein with a molecular mass of 48 kDa. It is also a member of the wider tumour necrosis factor receptor superfamily of death receptors. The identification of TNFR5 as the trigger that initiates islet cell death [4] opens the way for the development of a new class of diabetes therapeutic agents based upon TNFR5 antagonism. It should however be noted that antagonistic TNFR5 signalling strategies have previously been employed to aid immunosuppression following transplantation, and also to treat people suffering from lupus and several types of cancer.

Intervention has not always met with success though, and there are a number of reports where monoclonal antibodies targeted against the receptor ligand, CD40L, induced thromboembolic events [8-11]. Importantly, no such side-effects have been reported when the receptor itself, rather than ligand, was targeted. This fact notwithstanding, there are nevertheless still a number of potential pitfalls that could potentially arise through the use of full length anti-TNFR5 antibodies as T2D therapeutic agents. However, there are a number of molecular strategies that can be adopted to circumvent these dangers. These strategies are discussed below.

### ***2.1 Drug Repurposing***

In light of the aforementioned recent findings [4], existing antagonistic anti-TNFR5 antibodies would at first appear to offer promising potential for the treatment of diabetes. However the unmodified antibody structure carries with it potentially dangerous consequences if used to treat diabetes. Specifically, while TNFR5 would bind to the complementarity determining regions (CDRs) located between the variable region heavy and light chain domains of the Fab portion of the IgG structure (Fig. 1), the Fc region of the antibody poses a problem. In particular the possibility exists that following TNFR5 binding at the cell surface of  $\beta$ -cells, the Fc portion of the molecule might then bind to and activate immune cells. Should this occur there would be potentially catastrophic consequences for the pancreas, as this could for example trigger cell-mediated cytotoxicity through degranulation of natural killer cells, phagocytic opsonization of  $\beta$ -cells by neutrophils, or trigger immune cell production and release of reactive oxygen species that could also induce  $\beta$ -cell death. An alternative approach is therefore required.

## ***2.2 Enzymatic Antibody Remodelling***

The simplest and least costly strategy to generate new therapeutic tools would be to start with well characterised antagonistic TNFR5 antibodies, then remove the Fc region through use of enzymatic IgG cleavage sites first demonstrated more than half a century ago using either papain [12] or pepsin [13]. Pepsin cleavage (Fig. 1a, yellow arrows) would generate one bivalent anti-TNFR5 F(ab')<sub>2</sub> molecule, which if required could then be further processed through addition of disulphide reducing agent to generate two monovalent Fab molecules. Papain cleavage (Fig 1b, light blue arrows) by contrast would directly result in the generation of two monovalent Fab molecules. In both cases removal of the Fc portion of the antibody would produce antibody fragments still capable of antagonizing TNFR5 signalling, but with the Fc portion of the original antibody molecule no longer present the remaining fragments would not now be able to promote agglutination, precipitation, opsonization, or cell lysis.

## ***2.3 Phage Display***

Phage display was first documented in 1985, molecular biology strategies being employed to generate peptides fused to bacteriophage coat protein III [14]. This technique was subsequently refined for display and selection of human antibodies on phage [15]. Further technological advances mean that it is now possible to use phage display to generate soluble F(ab')<sub>2</sub> or Fab molecules, or the smaller scFv or Fv variable region fragments (Fig. 2). The use of phage display to construct scFv fragments has in particular proven to be highly successful, both for diagnostic and therapeutic applications.

A key consideration when designing scFv constructs is the need to allow sufficient steric flexibility for the CDR to form between the respective variable heavy and light chain domains. This necessitates incorporating a linker region (shown in red, Fig. 2b lower left hand panel) that is suitably flexible. As a consequence the linker region is typically rich in glycine and approximately 15 amino acids long [16]. However if the linker region were to be reduced to 5 amino acids, then the two variable domains would no longer be able to fold together. Instead adjacent scFvs would likely dimerize to form diabodies. This could significantly reduce the antigen dissociation constant, thereby leading to an increased antibody affinity of as much as 40-fold [17]. Were such an approach adopted to produce an anti-TNFR5 diabody, this could result in a drug able to be dosed at a much lower concentrations than therapeutic antibody fragments generated through either enzymatic cleavage or conventional scFv phage display strategies. Moreover, were still shorter linkers employed (one or two amino acids), then this would likely lead to the formation of triabodies or tetrabodies of even higher affinity for TNFR5.

In conclusion there is still some way to go in the development of this new class of therapeutic drug but, based on the latest developments [4], neutralization of TNFR5 should prevent, or at least delay, islet cell death that is driven by glucolipototoxicity. Anti-TNFR5 biologics are likely to prove the most effective way to achieve this goal. Only once these novel candidate molecules have been generated though will we be able to assess each for efficacy and potential toxicity. The challenge now is for academia and

industry to come together to successfully develop such drugs, and by so doing help address the currently unmet clinical need to find effective new diabetes treatments.

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### **Declaration of Interest**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.



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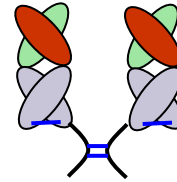
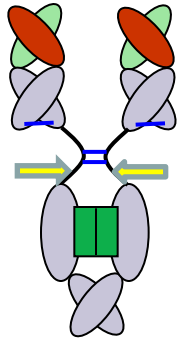
## Figure Legends

**Figure 1. Enzymatic generation of anti-TNFR5 antibody fragments.** Utilising existing known antagonistic anti-TNFR5 antibodies it is possible to separate the TNFR5-binding complementarity determining regions located between the variable heavy chain (light green) and light chain (brown) domains of the IgG structure, from the immune cell-interacting Fc portion of the antibody. This can be accomplished using either, a) papain (yellow arrow), or b) pepsin (light blue arrow). This generates either bivalent  $F(ab')_2$ , or monovalent Fab antagonistic anti-TNFR5 antibody fragments.

**Figure 2. Phage display generation of anti-TNFR5 antibody fragments.** Utilising phage display technology it is possible to generate a number of different possible antibody fragments, including bivalent  $F(ab')_2$ , or monovalent Fab, scFv, or Fv domains. This could be accomplished for TNFR5 by amplifying the immunoglobulin heavy and light chain region genes using PCR technology, ligating with a linker region where necessary, then subcloning into a phagemid vector to generate a recombinant phage capable of expressing the desired antibody fragment.

**a)**

**Pepsin Cleavage**

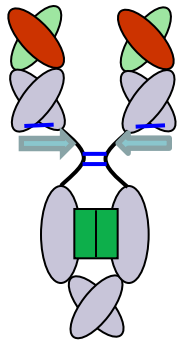


**F(ab')<sub>2</sub>**

**Anti-TNFR5 IgG**

**b)**

**Papain Cleavage**

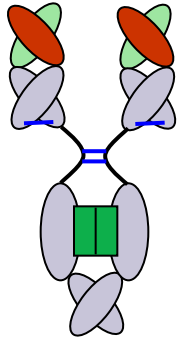


**Fab**

**Fab**

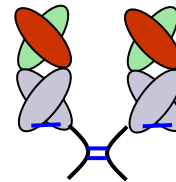
**Anti-TNFR5 IgG**

**a) Anti-TNFR5 IgG**



**b) Phage Display Fragments**

**F(ab')<sub>2</sub>**



**Fab**



**scFv**



**Fv**

