ANTI-TRANSGLUTAMINASE 2 ANTIBODIES

The present invention relates to inhibitors of TG2 and methods for providing and using such inhibitors.

Transglutaminase type 2 (TG2; also known as tissue transglutaminase, tTg) is part of the wider 9 member transglutaminase family that includes Factor XIIIa, which is critical for blood clotting, as well as Keratinocyte Transglutaminase (TG1) and Epidermal Transglutaminase (TG3) that are involved in terminal differentiation of the keratinocyte. In addition, there are other TG family members such as TG types 4 to 7 where no definitive role has been identified to date.

TG2 functions primarily as a protein cross-linking enzyme by catalysing the formation of ε(γ-glutamyl) lysine iso-dipeptide bonds. Elevated expression of TG2 leads to aberrant protein cross-linking which has been associated with several pathologies including various types of tissue scarring, the formation of neurofibrillary tangles in several brain disorders and resistance to chemotherapy in some cancers. TG2 is also able to deamidate proteins. TG2 deamidates gliadin and the TG2/Gliadin complex is the primary autoantigen in celiac disease. In addition, TG2 has a GTP binding function and can act as a GTPase, although this has not been linked to a pathological role.

Elevated TG2 activity is primarily associated with abnormal wound healing [1] leading to liver [2], pulmonary [3], heart [4] and kidney fibrosis, [5] as well as atherosclerosis [6]. The process of scarring and fibrosis is linked to the increased synthesis and, most importantly, raised export of TG2 to the interstitial space. Once outside the cell, TG2 is able to crosslink extracellular matrix (ECM) proteins such as fibronectin and collagen [7] by the incorporation of an ε(γ-glutamyl) lysine di-peptide bond [8]. Studies have shown that this can accelerate the deposition of available ECM components, while at the same time conferring resistance to proteolytic clearance by the matrix metalloproteinase (MMP) system [9, 10]. Taken together, this causes an accumulation of ECM proteins and thus scar tissue [9]. Further, TG2 has an emerging role in the activation of latent TGF-β1 in the scarring process [11] and has also been associated with interleukin-6 [12] and tumour necrosis factor-α activation pathways [13].

Inhibition of TG2 *in vitro* decreases extracellular matrix levels [14] while cells derived from TG2 knockout mice have lower levels of mature ECM [9]. *In vivo* application of pan TG inhibitors in models of chronic kidney disease reduce the development of

glomerulosclerosis and tubulointerstitial fibrosis preserving renal function [15, 16]. Similar benefits are seen in the TG2 knockout mouse subjected to unilateral ureteric obstruction [17].

There are several neurodegenerative diseases characterised by the presence of protein aggregates in the degenerative region of the brain that TG2 has been implicated in forming. The best characterised is in Huntington's chorea. Huntington protein (htt) contains expanded polyglutamine repeats in its N-terminal domain. Wild-type htt contains less than 35 consecutive glutamines while disease-related htt typically has over 40 consecutive glutamines which makes it an excellent TG2 substrate. Subsequently, insoluble aggregates are formed in the striatum and cortex of Huntington's disease patients. The frequency of aggregates correlates well with the severity of the disease.

Alzheimer's disease is typified by the presence of extracellular senile plaques composed of aggregated amyloid β-protein and intracellular neurofibrillary tangles consisting of a highly phosphorylated form of the protein tau. These plaques contain large amounts of ε(γ-glutamyl) lysine iso-dipeptide bonds.

Finally, a hallmark of Parkinson's disease is the presence of alpha-synuclein aggregates called Lewy bodies in the cytoplasm of affected neurons which again contain ε(γ-glutamyl) lysine iso-dipeptide bonds. All of the aforementioned proteins are good substrates of TG2 *in vitro*. Furthermore, the affected region of the brain contains higher levels of TG2 protein than non-affected regions of the brain in the same patients. The correlation between the TG2 substrate specificity for disease-relevant aggregated proteins and increased TG2 expression levels suggest a role for enzymatically active TG2 in each disease.

TG inhibitors have been shown to exert therapeutic effects in multiple biological models of neurodegenerative diseases. In a cell culture model of Parkinson's disease transfecting COS-7 cells with alpha-synuclein and TG2 simultaneously, covalent -synuclein aggregates, reminiscent of Lewy bodies in Parkinson's disease, form and are dependent upon enzymatically active TG2 since the C277S inactive TG2 mutant failed to induce aggregate formation. Treatment of these cotransfected cells with cystamine significantly reduced the quantity of alpha-synuclein aggregates as well as the percentage of cells containing the aggregates. There have been two other reports in which proteins with normal length and expanded polyglutamine repeat proteins, representative of expanded CAG diseases, such as Huntington's disease, have been

transfected into cell lines and shown to form aggregates. Treatment of these cell lines with the TG competitive inhibitor monodansylcadaverine led to a decrease in nuclear fragmentation, while treatment with cystamine lead to both a decrease in nuclear fragmentation, and a decrease in protein aggregate formation. An example of a pan TG inhibitor is 1,3-Dimethyl-2-[(2-oxo-propyl)thio]imidazolium chloride which is available from Zedira GmbH and referred to in several publications as NTU283 or r283.

Cystamine has a beneficial therapeutic effect *in vivo* when dosed in mouse models of Huntington's disease. Huntington R6/2 mice dosed with cystamine showed improved motor function, less severe weight loss, and increased survival compared to non-treated controls. Importantly, *ex vivo* TG2 activity in brain homogenates was lower after dosing with cystamine at least 60 min after injection. In a different mouse model of Huntington's disease, the YAC128 strain, cystamine was able to decrease the level of striatal atrophy but unable to improve animal weight or motor function, indicating a beneficial effect of cystamine at the cellular and tissue level but not in disease symptoms.

Probably the most convincing evidence that the beneficial therapeutic effect of cystamine on Huntington mice is independent of TG2 inhibition has come from crossing the R6/2 Huntington mouse with the TG2 knockout mouse to create a strain susceptible to neurodegeneration in the absence of TG2. When the R6/2 $TG2^{-/-}$ mice were treated with cystamine, the improved motor function and increased lifespan were not statistically different from the improvement seen in R6/2 $TG2^{+/+}$ mice treated with cystamine. Additionally, R6/1 and R6/2 $TG2^{-/-}$ mice had increased levels of neuronal protein aggregates compared to R6/1 and R6/2 $TG2^{+/+}$ mice suggesting a mechanism of protein aggregation independent of TG2 transamidation activity in these models. However, it is noteworthy that R6/2 $TG2^{-/-}$ mice showed a delay in the onset of motor dysfunction and improved survival compared to R6/2 $TG2^{+/+}$ mice implying a role for TG2 in the pathogenesis of neurodegeneration in the R6/2 model.

TG2 is also heavily implicated in celiac disease which affects 1 in 100 people in Western Europe. Celiac sprue is a T cell-mediated inflammatory disorder of the small intestine caused by a class of proteins called prolamins found in wheat, barley, and rye. The high proline and glutamine content of these proteins makes them resistant to natural gastric, pancreatic, and intestinal proteases, and peptidases during digestion. The resulting peptide fragments remain undigested well into the small intestine and gain access to the intestinal lamina propria where, after modification by TG2, they can stimulate a T cell-mediated immune response leading to inflammation and destruction of intestinal

architecture. Intestinal TG2 deamidates specific glutamine residues in the prolamin peptides to glutamate residues. In HLA-DQ2/8 individuals these modified peptides are presented to corresponding autoreactive T cells by class II MHC molecules. Although prolamins have a high glutamine content (around 30–35%), only a few of these glutamine residues are targeted by human TG2. An excellent correlation between TG2 substrate specificity, DQ2 binding affinity, and T cell stimulatory potential of TG2-treated prolamins strongly suggests that peptide deamidation is mediated by TG2 and plays a significant role in determining the severity of disease. Further, celiac patients generate an autoantibody response to TG2-gliadin complexes. These anti-TG2 antibodies are found in both the small intestine, where they have been shown to co-localize with extracellular TG2, and in the blood, where they are exploited as a diagnostic disease marker.

Despite the lack of animal models of celiac disease, *ex vivo* experiments indicate that TG2 inhibition has the potential to benefit patients with celiac sprue. Culturing celiac patient small intestinal biopsies with either TG2 treated (deamidated) or non-TG2 treated (non-deamidated) gluten digests both resulted in the generation of patient T-cell lines that preferentially recognised deamidated gluten peptides rather than non-deamidated gluten peptides. Also by blocking the activity of endogenous TG2 in the celiac biopsies with cystamine more than half of the resultant T-cell lines had reduced proliferative responses compared to non-cystamine-treated controls. Cell lines did not respond well to the non-deamidated digests. These results imply that the gluten responsive T-cell populations in celiac intestinal biopsies are naturally biased towards recognizing deamidated gluten peptides as opposed to non-deamidated peptides, that endogenous TG2 activity in these biopsies can result in gluten peptide deamidation *in situ* and that treatment of celiac biopsies with TG2 inhibitors can reduce the proliferative response of gluten-reactive T cells.

Another study showed that the pan-TG inhibitor 2-[(2-oxopropyl)thio]imidazolium inhibitor was able to prevent the *in situ* crosslinking of gluten peptides to endogenous proteins in thin tissue sections taken from both celiac sprue patients and controls. More importantly, the authors showed that incubation of intact celiac small intestinal biopsies with 2-[(2-oxopropyl)thio]imidazolium prevented T-cell activation induced by the non-deamidated form of an immunodominant gluten peptide. In contrast, TG inhibition was ineffective at controlling T-cell activation when the biopsies were incubated with the deamidated version of the same peptide. These results suggest that inhibition of endogenous TG2 in

celiac patient biopsies can prevent gluten peptide deamidation and, therefore, reduce T-cell activation.

Several observations support the hypothesis that TG2 plays a role in the development of certain types of cancer. Multiple studies have shown that TG2 protein is up-regulated in cancerous tissue relative to healthy tissue in cancers such as glioblastomas, malignant melanomas, and pancreatic ductal adenocarcinomas to name a few. A positive correlation between the chemotherapeutic resistance and metastatic potential of certain cancers with TG2 expression levels has been demonstrated, while in certain cell types TG2 has been shown to exert anti-apoptotic effects on cells while siRNA down-regulation of TG2 protein expression levels or treatment with TG2 inhibitors sensitizes these cells to apoptosis. On the other hand, there are also reports of the down-regulation of TG2 expression in certain types of cancer [18]. Recently, it has been shown that TG2 is a binding partner for GPR56, a protein down-regulated in highly metastatic cancer cells, suggesting that TG2 can act as a tumor suppressing protein through its interaction with GPR56 [18].

Current transglutaminase inhibitors fall into 3 main classes: 1) Competitive amine inhibitors (e.g. cystamine and spermine) that compete with natural amine substrates; 2) Reversible allosteric inhibitors such as GTP and a newly discovered class of compound with a thieno[2,3-d] pyrimidin-4-one acylhydrazide backbone; and 3) Irreversible inhibitors including 2-[(2-oxopropyl)thio]imidazolium derivatives (acetylate active site cysteine), 3-halo-4,5-dihydroisoxazoles (form a stable iminothioether in the active site) and carbobenzyloxy-L-glutaminyl glycine analogues with a variety of reactive moieties inserted.

Most have been used in the experimental systems above and shown beneficial outcomes. However, none of these inhibitors show TG isoform specificity as they all target the conserved catalytic triad within the transglutaminase family catalytic core. Consequently, all potentially have the disadvantage of co-inhibition of Factor XIIIa, TG1 and TG3, which effectively prevents their application in human disease due to the side effects that can be expected.

WO 2006/100679 describes a specific anti-TG2 antibody produced by recombinant technology from samples collected from three adult celiac patients with high anti-TG2 antibody titres.

Given the association of TG2 with numerous disease states and compelling data from non specific inhibitors, there is a need for highly selective and high efficacy TG2 inhibitors with minimal off target effects.

The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

In a first aspect the present invention provides an antibody or an antigen-binding fragment thereof that selectively binds to an epitope within the core region of transglutaminase type 2 (TG2).

In certain embodiments it is envisaged that the antibody or antigen-binding fragment thereof will selectively bind to an epitope within the core region of human TG2, rat TG2, and/or mouse TG2. In a particularly preferred embodiment, the TG2 is human TG2.

The full amino acid sequences of human, rat and mouse TG2 can be found under the Genbank Accession numbers NM_004613, NM_019386.2 and NM_009373.3. The coding part of these sequences are as follows:

Human TG2 nucleotide sequence:

atggccgaggagctggtcttagagaggtgtgatctggagctggagaccaatggccgagaccacca
cacggccgacctgtgccgggagaagctggtggtgcgacggggccagcccttctggctgaccctgc
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Human TG2 Amino Acid sequence:

MAEELVLERCDLELETNGRDHHTADLCREKLVVRRGQPFWLTLHFEGRNYEASVDSLTFS
VVTGPAPSQEAGTKARFPLRDAVEEGDWTATVVDQQDCTLSLQLTTPANAPIGLYRLSLE
ASTGYQGSSFVLGHFILLFNAWCPADAVYLDSEEERQEYVLTQQGFIYQGSAKFIKNIPW
NFGQFEDGILDICLILLDVNPKFLKNAGRDCSRRSSPVYVGRVVSGMVNCNDDQGVLLGR
WDNNYGDGVSPMSWIGSVDILRRWKNHGCQRVKYGQCWVFAAVACTVLRCLGIPTRVVTN
YNSAHDQNSNLLIEYFRNEFGEIQGDKSEMIWNFHCWVESWMTRPDLQPGYEGWQALDPT
PQEKSEGTYCCGPVPVRAIKEGDLSTKYDAPFVFAEVNADVVDWIQQDDGSVHKSINRSL
IVGLKISTKSVGRDEREDITHTYKYPEGSSEEREAFTRANHLNKLAEKEETGMAMRIRVG
QSMNMGSDFDVFAHITNNTAEEYVCRLLLCARTVSYNGILGPECGTKYLLNLNLEPFSEK
SVPLCILYEKYRDCLTESNLIKVRALLVEPVINSYLLAERDLYLENPEIKIRILGEPKQK
RKLVAEVSLQNPLPVALEGCTFTVEGAGLTEEQKTVEIPDPVEAGEEVKVRMDLLPLHMG
LHKLVVNFESDKLKAVKGFRNVIIGPA*

Rat TG2 Nucleotide sequence:

Atggccgaggagctgaacctggagaggtgcgatttggagatacaggccaatggccgtgatcacca
cacggccgacctgtgccaagagaaactggtgctgcggcgaggccagcgcttccggctgacactgt
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gaagtcggtcaagggttaccggaatatcatcatcggcccggcctaag

Rat TG2 Amino Acid sequence:

MAEELNLERCDLEIQANGRDHHTADLCQEKLVLRRGQRFRLTLYFEGRGYEASVDRLTFG
AVTGPDPSEEAGTKARFSLSDDVEEGSWSASVLDQQDNVLSLQLCTPANAPVGQYRLSLE
TSTGYQGSSFMLGHFILLFNAWCPADDVYLDSEAERREYVLTQQGFIYQGSVKFIKSVPW
NFGQFEDGILDACLMLLDVNPKFLKDRSRDCSRRSSPIYVGRVVSGMVNCNDDQGVLLGR
WDNNYGDGISPMAWIGSVDILRRWKEHGCQQVKYGQCWVFAAVACTVLRCLGIPTRVVTN
YNSAHDQNSNLLIEYFRNEYGELESNKSEMIWNFHCWVESWMTRPDLQPGYEGWQAIDPT
PQEKSEGTYCCGPVSVRAIKEGDLSTKYDASFVFAEVNADVVDWIRQSDGSVLKSINNSL
VVGQKISTKSVGRDDREDITYTYKYPEGSPEEREVFTRANHLNKLAEKEETGVAMRIRVG
DGMSLGNDFDVFAHIGNDTSESRECRLLLCARTVSYNGVLGPECGTEDINLTLDPYSENS
IPLRILYEKYSGCLTESNLIKVRGLLVEPAANSYLLAERDLYLENPEIKIRILGEPKQNR
KLVAEVSLKNPLSDSLYDCVFTVEGAGLTKEQKSVEVSDPVPAGDAVKVRVDLFPTDIGL
HKLVVNFQCDKLKSVKGYRNIIIGPA*X

Mouse TG2 Nucleotide sequence:

atggcagaggagctgctcctggagaggtgtgatttggagattcaggccaatggccgtgaccacca
cacggccgacctatgccaagagaaactggtgctgcgtcgtggtcagcgcttccggctgactctgt
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gtcgacctgttcccgactgatattggcctccacaagctggtggtgaacttccagtgtgacaagct
gaagtcggtgaagggttaccggaatgttatcatcggcccggcctaa

Mouse TG2 Amino Acid sequence:

MAEELLLERCDLEIQANGRDHHTADLCQEKLVLRRGQRFRLTLYFEGRGY
EASVDSLTFGAVTGPDPSEEAGTKARFSLSDNVEEGSWSASVLDQQDNVL
SLQLCTPANAPIGLYRLSLEASTGYQGSSFVLGHFILLYNAWCPADDVYL

DSEEERREYVLTQQGFIYQGSVKFIKSVPWNFGQFEDGILDTCLMLLDMN
PKFLKNRSRDCSRRSSPIYVGRVVSAMVNCNDDQGVLLGRWDNNYGDGIS
PMAWIGSVDILRRWKEHGCQQVKYGQCWVFAAVACTVLRCLGIPTRVVTN
YNSAHDQNSNLLIEYFRNEFGELESNKSEMIWNFHCWVESWMTRPDLQPG
YEGWQAIDPTPQEKSEGTYCCGPVSVRAIKEGDLSTKYDAPFVFAEVNAD
VVDWIRQEDGSVLKSINRSLVVGQKISTKSVGRDDREDITHTYKYPEGSP
EEREVFTKANHLNKLAEKEETGVAMRIRVGDSMSMGNDFDVFAHIGNDTS
ETRECRLLLCARTVSYNGVLGPECGTEDINLTLDPYSENSIPLRILYEKY
SGCLTESNLIKVRGLLIEPAANSYLLAERDLYLENPEIKIRVLGEPKQNR
KLVAEVSLKNPLSDPLYDCIFTVEGAGLTKEQKSVEVSDPVPAGDLVKAR
VDLFPTDIGLHKLVVNFQCDKLKSVKGYRNVIIGPA

The present inventors have raised antibodies to TG2 by immunising mice with a recombinant protein encompassing amino acids 143 to 473 of the human TG2 core. Hybridoma were screened for TG2 specificity and inhibition with any suitable candidates cloned. IgG was purified from these to calculate efficacy and the target epitope mapped by screening of a human TG2 library by phage display.

The present approach to producing antibodies against TG2 by using a recombinant TG2 core protein has not been tried before and it surprisingly led to the isolation and characterisation of antibodies to TG2 that were highly selective for TG2 and showed strong inhibitory characteristics. Prior attempts to raise antibodies to TG2 have led to the isolation of relatively unselective antibodies that cross-react with other members of the transglutaminase family and thus would not represent promising antibodies for clinical use. The antibodies of the present invention on the other hand are promising candidates for clinical trials for diseases exacerbated by or mediated by TG2 activity.

It is surprising that the approach in the present application has led to the production of much more effective antibodies than those previously produced. There was no guarantee that it would have been possible to raise antibodies that are effective inhibitors of TG2 by immunising with the core region. As indicated above, antibodies that are effective inhibitors may not be specific enough for TG2 to be used effectively in medicine. It is surprising that antibodies to the divergent regions (in particular, regions of the core that diverge slightly between different transglutaminase family members of TG2) are effective and selective inhibitors of TG2.

Without being bound by any theory we think that by raising antibodies to a smaller protein covering just the central core, we not only eliminate some of the favoured immunological epitopes present on the full length protein, but we also force core targeting. This appears to increase the variety of antibodies available for selection and provides wider coverage of the core.

Immunising with just the TG2 core removed much of the tertiary structure of the enzyme (in particular the two carboxy terminal beta barrel domains). It is possible that some of the epitopes that perhaps may be less available or immunogenic within a native full length TG2 molecule may be more attractive epitopes with the core in the format described herein. The antibodies described herein recognised linear epitopes (i.e. bound to TG2 on a reducing SDS PAGE gel), whereas 80 % of the antibodies we previously isolated using full length TG2 as an immunogen were conformation dependent. We were able to show that the recombinant core domain retained enzyme activity, and so the isolation of inhibitory antibodies was probably aided by the exposure of previously less favourable epitopes located in or near the active site. It is interesting and surprising that inhibitory antibodies to human TG2 were raised by immunising with the core given that the recombinant core protein may not have demonstrated the same folding characteristics as the full length protein.

The TG catalytic core is highly conserved between members of the TG family and across species. This suggests that development of not only specific small molecule inhibitors but also of antibody-based inhibitors may be technically challenging. Nevertheless, the present disclosure provides antibodies that are highly selective. That this is possible may reflect the fact that there are some regions within the TG2 catalytic domain where there is some heterogeneity. The present antibodies may therefore exploit these small differences. The surprising selectivity of the present antibodies may enable the development of therapeutics that can interfere efficiently with TG2 activity and thus provide potentially effective therapies for conditions exacerbated by or caused by TG2 activity where there is currently no satisfactory therapeutic option.

By way of comparison, the antibodies described in WO 2006/100679, which were produced by recombinant technology from samples from three adult celiac patients with high anti-TG2 antibody titres, proved to be of low efficacy when tested by the present inventors (Example 2). The antibodies of the present invention were far superior to those of WO 2006/100679 in terms of selectivity for TG2 and in terms of inhibitory activity. For example, the present inventors generated a Fab fragment of the antibody of WO 2006/100679, which was applied at the same concentration in TG2 inhibition assays as the antibodies of the present invention. The Fab fragment amount in the tests was twice molar wise that of the antibodies of the present invention, but they still failed to show any inhibition of TG2 activity. When the full length WO 2006/100679 antibody was tested for inhibition in our standard putrescine incorporation assay no inhibition of TG2

activity was found. Thus, the methods of the present invention and the antibodies produced by those methods are superior to those previously described.

By "antibody" we include substantially intact antibody molecules, as well as chimeric antibodies, humanised antibodies, human antibodies (wherein at least one amino acid is mutated relative to the naturally occurring human antibodies), single chain antibodies, bi-specific antibodies, antibody heavy chains, antibody light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen binding fragments and derivatives of the same. We also include variants, fusions and derivatives of the antibodies and antigen-binding fragments thereof within the meaning of the terms "antibody" and "antigen-binding fragments thereof".

The term "antibody" also includes all classes of antibodies, including IgG, IgA, IgM, IgD and IgE. Thus, the antibody may be an IgG molecule, such as an IgG1, IgG2, IgG3, or IgG4 molecule. Preferably, the antibody of the invention is an IgG molecule, or an antigen-binding fragment, or variant, fusion or derivative thereof. More preferably the antibody is an IgG2 molecule.

The antibodies, compositions, uses and methods of the invention encompass variants, fusions and derivatives of the defined antibodies and antigen-binding fragments thereof, as well as fusions of a said variants or derivatives, provided such variants, fusions and derivatives have binding specificity for TG2.

As antibodies and antigen-binding fragments thereof comprise one or more polypeptide component, variants, fusions and derivatives of the antibody and antigen-binding fragment thereof as defined herein may be made using the methods of protein engineering and site-directed mutagenesis well known in the art using the recombinant polynucleotides (see example, see *Molecular Cloning: a Laboratory Manual*, 3rd edition, Sambrook & Russell, 2001, Cold Spring Harbor Laboratory Press, which is incorporated herein by reference).

Thus, variants, fusions and derivatives of the antibody or antigen-binding fragment thereof as defined herein, may be made based on the polypeptide component of the antibody or antigen-binding fragment thereof.

By "fusion" we include said polypeptide fused to any other polypeptide. For example, the said polypeptide may be fused to a polypeptide such as glutathione-S-transferase (GST)

or protein A in order to facilitate purification of said polypeptide. Examples of such fusions are well known to those skilled in the art. Similarly, the said polypeptide may be fused to an oligo-histidine tag such as His6 or to an epitope recognised by an antibody such as the well-known Myc-tag epitope. Fusions to any variant or derivative of said polypeptide are also included in the scope of the invention. It will be appreciated that fusions (or variants or derivatives thereof) which retain desirable properties, such as have binding specificity for TG2, are preferred.

The fusion may comprise or consist of a further portion which confers a desirable feature on the said polypeptide; for example, the portion may be useful in detecting or isolating the polypeptide, or promoting cellular uptake of the polypeptide. The portion may be, for example, a biotin moiety, a radioactive moiety, a fluorescent moiety, for example a small fluorophore or a green fluorescent protein (GFP) fluorophore, as well known to those skilled in the art. The moiety may be an immunogenic tag, for example a Myc-tag, as known to those skilled in the art or may be a lipophilic molecule or polypeptide domain that is capable of promoting cellular uptake of the polypeptide, as known to those skilled in the art.

By "variants" of said polypeptide we refer to a polypeptide wherein at one or more positions there have been amino acid insertions, deletions, and/or substitutions, either conservative or non-conservative, provided that such changes result in a protein whose basic properties, for example binding properties, thermostability, activity in a certain pH-range (pH-stability) have not significantly been changed. "Significantly" in this context means that one skilled in the art would say that the properties of the variant may still be different but would not be unobvious over the ones of the original protein. Thus, we include variants of the polypeptide where such changes do not substantially alter the activity of the said polypeptide. In particular, we include variants of the polypeptide where such changes do not substantially alter the binding specificity for TG2.

By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Such variants may be made using the methods of protein engineering and site-directed mutagenesis.

The polypeptide variant may have an amino acid sequence which has at least 75% identity with one or more of the amino acid sequences given herein, for example at least

80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with one or more of the amino acid sequences specified herein.

The percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequences have been aligned optimally.

The alignment may alternatively be carried out using the Clustal W program (as described in Thompson *et al.,* 1994, *Nucl. Acid Res.* 22:4673-4680, which is incorporated herein by reference).

The parameters used may be as follows:

- Fast pair-wise alignment parameters: K-tuple(word) size; 1, window size; 5, gap penalty; 3, number of top diagonals; 5. Scoring method: x percent.
- Multiple alignment parameters: gap open penalty; 10, gap extension penalty; 0.05.
- Scoring matrix: BLOSUM.

Alternatively, the BESTFIT program may be used to determine local sequence alignments.

The antibody, antigen-binding fragment, variant, fusion or derivative used in the methods or uses of the invention may comprise or consist of one or more amino acids which have been modified or derivatised.

Chemical derivatives of one or more amino acids may be achieved by reaction with a functional side group. Such derivatised molecules include, for example, those molecules in which free amino groups have been derivatised to form amine hydrochlorides, *p*-toluene sulphonyl groups, carboxybenzoxy groups, *t*-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatised to form salts, methyl and ethyl esters or other types of esters and hydrazides. Free hydroxyl groups may be derivatised to form O-acyl or O-alkyl derivatives. Also included as chemical derivatives are those peptides which contain naturally occurring amino acid derivatives of the twenty standard amino acids. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine and ornithine

for lysine. Derivatives also include peptides containing one or more additions or deletions as long as the requisite activity is maintained. Other included modifications are amidation, amino terminal acylation (*e.g.* acetylation or thioglycolic acid amidation), terminal carboxylamidation (*e.g.* with ammonia or methylamine), and the like terminal modifications.

It will be further appreciated by persons skilled in the art that peptidomimetic compounds may also be useful. Thus, by 'polypeptide' we include peptidomimetic compounds which are capable of binding to an epitope within the TG2 core region. The term 'peptidomimetic' refers to a compound that mimics the conformation and desirable features of a particular peptide as a therapeutic agent.

For example, the said polypeptide includes not only molecules in which amino acid residues are joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere *et al.* (1997) *J. Immunol.* 159, 3230-3237, which is incorporated herein by reference. This approach involves making pseudo-peptides containing changes involving the backbone, and not the orientation of side chains. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis. Alternatively, the said polypeptide may be a peptidomimetic compound wherein one or more of the amino acid residues are linked by a -y(CH_2NH)- bond in place of the conventional amide linkage.

In a further alternative, the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the carbon atoms of the amino acid residues is used; it may be advantageous for the linker moiety to have substantially the same charge distribution and substantially the same planarity as a peptide bond.

It will be appreciated that the said polypeptide may conveniently be blocked at its N- or C-terminus so as to help reduce susceptibility to exo-proteolytic digestion.

A variety of un-coded or modified amino acids such as D-amino acids and N-methyl amino acids have also been used to modify mammalian peptides. In addition, a presumed bioactive conformation may be stabilised by a covalent modification, such as cyclisation or by incorporation of lactam or other types of bridges, for example see Veber

et al., 1978, *Proc. Natl. Acad. Sci. USA* 75:2636 and Thursell *et al.*, 1983, *Biochem. Biophys. Res. Comm.* 111:166, which are incorporated herein by reference.

A common theme among many of the synthetic strategies has been the introduction of some cyclic moiety into a peptide-based framework. The cyclic moiety restricts the conformational space of the peptide structure and this frequently results in an increased specificity of the peptide for a particular biological receptor. An added advantage of this strategy is that the introduction of a cyclic moiety into a peptide may also result in the peptide having a diminished sensitivity to cellular peptidases.

Thus, exemplary polypeptides useful in the methods and uses of the invention comprise or consist of terminal cysteine amino acids. Such a polypeptide may exist in a heterodetic cyclic form by disulphide bond formation of the mercaptide groups in the terminal cysteine amino acids or in a homodetic form by amide peptide bond formation between the terminal amino acids. As indicated above, cyclising small peptides through disulphide or amide bonds between the N- and C-terminus cysteines may circumvent problems of specificity and half-life sometime observed with linear peptides, by decreasing proteolysis and also increasing the rigidity of the structure, which may yield higher specificity compounds. Polypeptides cyclised by disulphide bonds have free amino and carboxy-termini which still may be susceptible to proteolytic degradation, while peptides cyclised by formation of an amide bond between the N-terminal amine and C-terminal carboxyl and hence no longer contain free amino or carboxy termini. Thus, the peptides can be linked either by a C-N linkage or a disulphide linkage.

The present invention is not limited in any way by the method of cyclisation of peptides, but encompasses peptides whose cyclic structure may be achieved by any suitable method of synthesis. Thus, heterodetic linkages may include, but are not limited to formation via disulphide, alkylene or sulphide bridges. Methods of synthesis of cyclic homodetic peptides and cyclic heterodetic peptides, including disulphide, sulphide and alkylene bridges, are disclosed in US 5,643,872, which is incorporated herein by reference. Other examples of cyclisation methods are discussed and disclosed in US 6,008,058, which is incorporated herein by reference.

A further approach to the synthesis of cyclic stabilised peptidomimetic compounds is ring-closing metathesis (RCM). This method involves steps of synthesising a peptide precursor and contacting it with an RCM catalyst to yield a conformationally restricted peptide. Suitable peptide precursors may contain two or more unsaturated C-C bonds.

The method may be carried out using solid-phase-peptide-synthesis techniques. In this embodiment, the precursor, which is anchored to a solid support, is contacted with a RCM catalyst and the product is then cleaved from the solid support to yield a conformationally restricted peptide.

Another approach, disclosed by D. H. Rich in *Protease Inhibitors*, Barrett and Selveson, eds., Elsevier (1986), which is incorporated herein by reference, has been to design peptide mimics through the application of the transition state analogue concept in enzyme inhibitor design. For example, it is known that the secondary alcohol of staline mimics the tetrahedral transition state of the scissile amide bond of the pepsin substrate.

In summary, terminal modifications are useful, as is well known, to reduce susceptibility by proteinase digestion and therefore to prolong the half-life of the peptides in solutions, particularly in biological fluids where proteases may be present. Polypeptide cyclisation is also a useful modification because of the stable structures formed by cyclisation and in view of the biological activities observed for cyclic peptides.

Thus, in one embodiment the said polypeptide is cyclic. However, in an alternative embodiment, the said polypeptide is linear.

By "selectively binds to an epitope within the core region of TG2" we mean an antibody or antigen-binding fragment thereof that is capable of binding to an epitope in the TG2 core region selectively. By "capable of binding selectively" we include such antibody-derived binding moieties which bind at least 10-fold more strongly to the TG2 core than to other proteins; for example at least 50-fold more strongly, or at least 100-fold more strongly. The binding moiety may be capable of binding selectively to an epitope in the TG2 core under physiological conditions, *e.g. in vivo*.

Such binding specificity may be determined by methods well known in the art, such as enzyme-linked immunosorbent assay (ELISA), immunohistochemistry, immunoprecipitation, western blot and flow cytometry using transfected cells expressing TG2 or the TG2 core, or a fragment thereof. Suitable methods for measuring relative binding strengths include immunoassays, for example where the binding moiety is an antibody (see Harlow & Lane, "Antibodies: A Laboratory Manual", Cold Spring Habor Laboratory Press, New York, which is incorporated herein by reference). Alternatively, binding may be assessed using competitive assays or using Biacore® analysis (Biacore International AB, Sweden).

It is preferred that the antibody or antigen-binding fragment of the invention binds exclusively to TG2.

It will be appreciated by persons skilled in the art that the binding specificity of an antibody or antigen binding fragment thereof is conferred by the presence of complementarity determining regions (CDRs) within the variable regions of the constituent heavy and light chains. As discussed below, in a particularly preferred embodiment of the antibodies and antigen-binding fragments thereof defined herein, binding specificity for TG2 is conferred by the presence of one or more of the CDRs identified. For example, sequences that may comprise or consist of the CDR sequences of AB-1 VL and VH include KASQDINSYLT, RTNRLFD, LQYDDFPYT, SSAMS, TISVGGGKTYYPDSVKG, and LISLY. In a further example, sequences that may comprise or consist of the CDR sequences of BB-7 VL and VH include KASQDINSYLT, LTNRLMD, LQYVDFPYT, SSAMS, TISSGGRSTYYPDSVKG, and LISPY. Sequences that may comprise or consist of the CDR sequences of DC-1 VL and VH include KASQDINSYLT, LVNRLVD, LQYDDFPYT , THAMS, TISSGGRSTYYPDSVKG, and LISTY. It is preferred that the antibodies and antigen-binding fragments thereof defined herein comprise or consist of CDR sequences, or CDR and flanking sequences, as defined in Table 24A. It is most preferred that the antibodies and antigen-binding fragments thereof defined herein comprise or consist of the CDR sequences, or CDR and flanking sequences, of the exemplary antibody AB-1, or BB-7, or DC-1 (as defined, for example, in Table 24A).

It is preferred that the antibody or antigen-binding fragment thereof retains the binding specificity for TG2 of the original antibody. By "retains the binding specificity" we mean that the antibody or antigen-binding fragment thereof is capable of competing for binding to TG2 with the exemplary antibodies of the invention, for example AB-1, AG-1, AG-9, AH-1, AH-3, BB-7, DC-1, EH-6, JE-12, IA-12, DF-4, DH-2, DD-6 and/or DD-9 (see accompanying Examples). For example, the antibody or antigen-binding fragment thereof may bind to the same epitope on TG2 as an antibody comprising the following sequences: KASQDINSYLT, RTNRLFD, LQYDDFPYT, SSAMS, TISVGGGKTYYPDSVKG, and LISLY.

By "epitope" it is herein intended to mean a site of a molecule to which an antibody binds, *i.e.* a molecular region of an antigen. An epitope may be a linear epitope, which is determined by *e.g.* the amino acid sequence, *i.e.* the primary structure, or a three-

dimensional epitope, defined by the secondary structure, *e.g.* folding of a peptide chain into beta sheet or alpha helical, or by the tertiary structure, *e.g.* way which helices or sheets are folded or arranged to give a three-dimensional structure, of an antigen.

Methods for determining whether a test antibody is capable of competing for binding with second antibody are well known in the art (such as, for example sandwich-ELISA or reverse-sandwich-ELISA techniques) and described, for example, in *Antibodies: A Laboratory Manual*, Harlow & Lane (1988, CSHL, NY, ISBN 0-87969-314-2), which is incorporated herein by reference.

The antibody or antigen-binding fragment thereof, with binding specificity for an epitope in the TG2 core region may also retain one or more of the same biological properties as the original antibody (such as the exemplary antibodies provided in the Examples).

As explained above, TG2 is a calcium-dependent multi-functional protein that catalyzes the formation of Nε-(γ -glutamyl)lysine isopeptide bonds between lysine and glutamine residues. TG2 comprises an N terminal beta sandwich domain that contains binding sites (e.g. fibronectin) and sequences required for enzyme export. This links to the catalytic core domain. Central to the domain is a catalytic triad consisting of Cys 277, Asp 358, and His 355, plus several putative calcium binding sites. This links to the third domain, beta barrel 1 where a GTP binding site resides conveying the enyme with GTPase activity. Beta barrel 1 also contains an integrin binding site used in cell adhesion. Beta Barrel 1 along with the fourth TG2 domain, Beta barrel 2, are involved in the conformational change in TG2 required for its activation. In a high calcium, low GTP environment Barrel 1 and 2 swing down from the closed and folded inactive form to convey TG2 with a linear structure opening up the catalytic core allowing activation (Pinkas *et al* (2007) *PLoS Biol.* Transglutaminase 2 undergoes a large conformational change upon activation. 5(12): e327).

By "the core region of transglutaminase type 2 (TG2)" we include a region of TG2 comprising the catalytic triad described above, excluding the beta sandwich domain and beta barrels 1 and 2. Preferably the core region comprises or consists of amino acids 143 to 473 of human TG2, or a fragment thereof.

Amino acids 143 to 473 of human TG2 consist of the following sequence:

CPADAVYLDSEEERQEYVLTQQGFIYQGSAKFIKNIPWNFGQFEDGILDICLILLDVNPK
FLKNAGRDCSRRSSPVYVGRVVSGMVNCNDDQGVLLGRWDNNYGDGVSPMSWIGSVDILR

RWKNHGCQRVKYGQCWVFAAVACTVLRCLGIPTRVVTNYNSAHDQNSNLLIEYFRNEFGE
IQGDKSEMIWNFHCWVESWMTRPDLQPGYEGWQALDPTPQEKSEGTYCCGPVPVRAIKEG
DLSTKYDAPFVFAEVNADVVDWIQQDDGSVHKSINRSLIVGLKISTKSVGRDEREDITHT
YKYPEGSSEEREAFTRANHLNKLAEKEETGM

Thus, in an embodiment of the invention, the core region may consist of amino acids 143 to 473 of human TG2. In this embodiment, the epitope of the antibody of the invention could thus be any epitope within the region defined by amino acids 143 to 473 of human TG2. Thus, the epitope may be a fragment of this sequence or it could be made up of various amino acid residues within this fragment that may not be adjacent one another in the primary amino acid structure but localise with one another in the secondary, tertiary or even quaternary structure of the protein, as would be understood by a person of skill in the art.

In an embodiment of the invention the antibody or antigen-binding fragment thereof may selectively bind in whole or in part to a region comprising amino acids 304 to 326 of human TG2. This region (amino acids 304 to 326 of human TG2) is referred to as Group 1 in Figure 5 and comprises the amino acid sequence AHDQNSNLLIEYFRNEFGEIQGD.

In a further embodiment, the antibody or antigen-binding fragment thereof may selectively bind in whole or in part to a region comprising amino acids 351 to 365 of human TG2. This region (amino acids 351 to 365 of human TG2) is referred to as Group 2 in Figure 5 and comprises the amino acid sequence YEGWQALDPTPQEKS.

In a yet further embodiment of the invention, the antibody or antigen-binding fragment thereof may selectively bind in whole or in part to a region comprising amino acids 450 to 467 of human TG2. This region (amino acids 450 to 467 of human TG2) is referred to as Group 3 in Figure 5 and comprises the amino acid sequence SEEREAFTRANHLNKLAE.

In a preferred embodiment of any aspect of the invention, the antibody or antigen-binding fragment thereof inhibits TG2 activity, for example human TG2 activity.

In an embodiment of the invention the antibody or antigen-binding fragment thereof may comprise one or more of the following amino acid sequences:

KASQDINSYLT; and/or

RTNRLFD; and/or

LQYDDFPYT; and/or
SSAMS; and/or
TISVGGGKTYYPDSVKG; and/or
LISLY.

The antibody or antigen-binding fragment thereof of the invention may comprise one or more of the following amino acid sequences:

TCKASQDINSYLTWF; and/or
TLIYRTNRLFDGVP or TLIYRTNRLFDGVPXXFSGSGSGQDFF; and/or
YCLQYDDFPYTFG; and/or
FTLSSSAMSWVR or CXAXXFTLSSSAMSWVR; and/or
WVATISVGGGKTYYPDSVKGRFTISR or WVATISVGGGKTYYPDSVKGRFTISRXNXXXXL; and/or
YCAKLISLYWG, wherein X is any amino acid.

The sequences of the immediately preceding embodiments are considered to comprise the complementarity determining regions of the light and heavy variable regions of the exemplary antibody AB-1 (see Example 1) and certain specified humanised variants of the AB-1 antibody. In a further embodiment, the antibody may comprise the amino acid sequence KASQDINSYLTXXXXXXXXXXXXXXXRTNRLFDXXXXXXXXXXXXXXXFXXXXXXXXXX XXXXXXLQYDDFPYT; or KASQDINSYLTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXF; or RTNRLFDXXXXXXXXXXXXXXXF; or FXXXXXXXXXXXXXXXXLQYDDFPYT, wherein X is any amino acid.

In a further embodiment, the antibody or antigen-binding fragment thereof may comprise the amino acid sequence TCKASQDINSYLTWF or TCKASQDINSYLTWY; and/or LLIYRTNRLFDGVP or SLIYRTNRLFDGVP or LLIYRTNRLFDGVPXXFSGSGSGQDFF or SLIYRTNRLFDGVPXXFSGSGSGQDFF; and/or
YCLQYDDFPYTFG; and/or
FTFSSSAMSWVR or CXAXXFTFSSSAMSWVR; and/or
WVSTISVGGGKTYYPDSVKGRFTISR or WVSTISVGGGKTYYPDSVKGRFTISRXNXXXXL; and/or
YCAKLISLYWG, wherein X is any amino acid.

Thus, in an embodiment, the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the following sequences: KASQDINSYLT; and RTNRLFD; and LQYDDFPYT.

In a further embodiment, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the following sequences: SSAMS; and TISVGGGKTYYPDSVKG; and LISLY.

In an embodiment of the invention the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the amino acid sequence DIQMTQTPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK or DIKMTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK. These light chain variable regions correspond with that found in exemplary antibody AB-1 (Figures 7 and 18).

Alternatively, the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the amino acid sequence: EIVLTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (AB-1_VK) or DIQMTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (AB-1_VK1).

In a particularly preferred embodiment the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the amino acid sequence: EIVLTQSPSSLSASVGDRVTITCKASQDINSYLTWYQQKPGKAPKLLIYRTNRLFDGVPS RFSGSGSGTDFFFTISSLQPEDFGTYYCLQYDDFPYTFGGGTKLEIK (hAB-1_RKE); or DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYRTNRLFDGVPS RFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK (hAB-1_RKJ). These sequences are humanised variants of the AB-1 light chain sequences provided above.

In an embodiment of the invention, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the amino acid sequence EVQLEESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY

PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS or EVQLVESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS. These heavy chain variable regions correspond with that found in exemplary antibody AB-1 (Figures 7 and 18).

Alternatively, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the amino acid sequence: EVQLQESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS (AB-1_VH).

In a particularly preferred embodiment, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the amino acid sequence: EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAPGKGLEWVSTISVGGGKTYY PDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISLYWGQGTLVTVSS (hAB-1_RHA). This sequence is a humanised variant of the AB-1 heavy chain sequence provided above.

Thus, it is envisaged that in an embodiment, the antibody or antigen-binding fragment thereof may have:

i) at least one light chain variable region comprising the amino acid sequence DIQMTQTPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK or DIKMTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK, or EIVLTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (AB-1_VK), or DIQMTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (AB-1_VK1), or EIVLTQSPSSLSASVGDRVTITCKASQDINSYLTWYQQKPGKAPKLLIYRTNRLFDGVPS RFSGSGSGTDFFFTISSLQPEDFGTYYCLQYDDFPYTFGGGTKLEIK (hAB-1_RKE), or DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYRTNRLFDGVPS RFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK (hAB-1_RKJ); and

ii) at least one heavy chain variable region comprising the amino acid sequence EVQLEESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS or EVQLVESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS, or EVQLQESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS (AB-1_VH), or EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAPGKGLEWVSTISVGGGKTYY PDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISLYWGQGTLVTVSS (hAB-1_RHA).

In an embodiment of any aspect of the present invention the antibody or antigen-binding fragment thereof may comprise one or more of the following amino acid sequences:

KASQDINSYLT; and/or

LTNRLMD; and/or

LQYVDFPYT; and/or

SSAMS; and/or

TISSGGRSTYYPDSVKG; and/or

LISPY.

The sequences of the immediately preceding embodiment are considered to comprise the complementarity determining regions of the light and heavy variable regions of the exemplary antibody BB-7 (see Figure 19). In a further embodiment, the antibody may comprise the amino acid sequence KASQDINSYLTXXXXXXXXXXXXXXLTNRLMDXXXXXXXXXXXXXXFXXXXXXXXXXX XXXXXLQYVDFPYT; or KASQDINSYLTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXF; or LTNRLMDXXXXXXXXXXXXXXF; or FXXXXXXXXXXXXXXXXXLQYVDFPYT, wherein X is any amino acid.

Thus, in an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the following sequences: KASQDINSYLT; and LTNRLMD; and LQYVDFPYT.

In a further embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the following sequences: SSAMS; and TISSGGRSTYYPDSVKG; and LISPY.

In an embodiment of any aspect of the present invention the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the amino acid sequence AIKMTQSPSSMYASLGERVIITCKASQDINSYLTWFQQKPGKSPKTLIYLTNRLMDGVPS RFSGSGSGQEFLLTISGLEHEDMGIYYCLQYVDFPYTFGGGTKLEIK. This light chain variable region corresponds with that found in exemplary antibody BB-7 (Figure 19).

In an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the amino acid sequence AVQLVESGGGLVKPGGSLKLSCAASGIIFSSSAMSWVRQTPEKRLEWVATISSGGRSTYY PDSVKGRFTVSRDSAKNTLYLQMDSLRSEDTAIYYCAKLISPYWGQGTTLTVSS. This heavy chain variable region corresponds with that found in exemplary antibody BB-7 (Figure 19).

Thus, it is envisaged that in an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have:

i) at least one light chain variable region comprising the amino acid sequence AIKMTQSPSSMYASLGERVIITCKASQDINSYLTWFQQKPGKSPKTLIYLTNRLMDGVPS RFSGSGSGQEFLLTISGLEHEDMGIYYCLQYVDFPYTFGGGTKLEIK; and

ii) at least one heavy chain variable region comprising the amino acid sequence AVQLVESGGGLVKPGGSLKLSCAASGIIFSSSAMSWVRQTPEKRLEWVATISSGGRSTYY PDSVKGRFTVSRDSAKNTLYLQMDSLRSEDTAIYYCAKLISPYWGQGTTLTVSS.

It is further envisaged that in an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have:

i) at least one light chain variable region comprising the amino acid sequence DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYLTNRLMDGVPS RFSGSGSGTDFFLTISSLQPEDFATYYCLQYVDFPYTFGQGTKVEIK or DIKMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKTLIYLTNRLMDGVPS RFSGSGSGQEFLLTISSLQPEDFATYYCLQYVDFPYTFGQGTKVEIK; and/or

ii) at least one heavy chain variable region comprising the amino acid sequence EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAPGKGLEWVSTISSGGRSTYY PDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISPYWGQGTLVTVSS or EVQLLESGGGLVQPGGSLRLSCAASGIIFSSSAMSWVRQAPGKGLEWVATISSGGRSTYY

PDSVKGRFTVSRDSSKNTLYLQMNSLRAEDTAVYYCAKLISPYWGQGTLVTVSS. These sequences correspond to humanised variants of antibody BB-7 (see Tables 23, 24 and 24A).

In an embodiment of any aspect of the present invention the antibody or antigen-binding fragment thereof may comprise one or more of the following amino acid sequences:

KASQDINSYLT; and/or
LVNRLVD; and/or
LQYDDFPYTand/or
THAMS; and/or
TISSGGRSTYYPDSVKG; and/or
LISTY.

The sequences of the immediately preceding embodiment are considered to comprise the complementarity determining regions of the light and heavy variable regions of the exemplary antibody DC-1 (see Figure 20). In a further embodiment, the antibody may comprise the amino acid sequence KASQDINSYLTXXXXXXXXXXXXXXXLVNRLVDXXXXXXXXXXXXXXXAXXXXXXXXXX XXXXXXLQYDDFPYT or KASQDINSYLTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXA; or LVNRLVDXXXXXXXXXXXXXXXA; or AXXXXXXXXXXXXXXXXLQYDDFPYT, wherein X is any amino acid.

Thus, in an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the following sequences: KASQDINSYLT; and LVNRLVD; and LQYDDFPYT
In a further embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the following sequences: THAMS; and TISSGGRSTYYPDSVKG; and LISTY.

In an embodiment of any aspect of the present invention the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the amino acid sequence DITMTQSPSSIYASLGERVTITCKASQDINSYLTWFQQKPGKSPKILIYLVNRLVDGVPS RFSGSGSGQDYALTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK. This light chain variable region corresponds with that found in exemplary antibody DC-1 (Figure 20).

In an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the amino acid sequence EVQLVESGGGLVKPGGSLKLSCAASGFTLSTHAMSWVRQTPEKRLEWVATISSGGRSTYY PDSVKGRFTISRDNVKNTLYLQLSSLRSEDTAVYFCARLISTYWGQGTTLTVSS. This heavy chain variable region corresponds with that found in exemplary antibody DC-1 (Figure 20).

Thus, it is envisaged that in an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have:

i) at least one light chain variable region comprising the amino acid sequence DITMTQSPSSIYASLGERVTITCKASQDINSYLTWFQQKPGKSPKILIYLVNRLVDGVPS RFSGSGSGQDYALTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK; and

ii) at least one heavy chain variable region comprising the amino acid sequence EVQLVESGGGLVKPGGSLKLSCAASGFTLSTHAMSWVRQTPEKRLEWVATISSGGRSTYY PDSVKGRFTISRDNVKNTLYLQLSSLRSEDTAVYFCARLISTYWGQGTTLTVS.

It is further envisaged that in an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have:

i) at least one light chain variable region comprising the amino acid sequence DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYLVNRLVDGVPS RFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK or DITMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKILIYLVNRLVDGVPS RFSGSGSGQDYALTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK; and/or

ii) at least one heavy chain variable region comprising the amino acid sequence EVQLLESGGGLVQPGGSLRLSCAASGFTFSTHAMSWVRQAPGKGLEWVSTISSGGRSTYY PDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISTYWGQGTLVTVSS or EVQLLESGGGLVQPGGSLRLSCAASGFTLSTHAMSWVRQAPGKGLEWVATISSGGRSTYY PDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCARLISTYWGQGTLVTVSS. These sequences correspond with the humanised variants of DC-1 provided in Tables 23, 24 and 24A.

In an embodiment of any aspect of the present invention the antibody or antigen-binding fragment thereof may comprise one or more of the following amino acid sequences:

KASQDINSYLT; and/or

XXNRLXD; and/or
LQYXDFPYT; and/or
XXAMS; and/or
TISXGGXXTYYPDSVKG; and/or
LISXY, wherein X is any amino acid.

In an embodiment of any aspect of the present invention the antibody or antigen-binding fragment thereof may comprise one or more of the following amino acid sequences:

(K/Q/R)ASQ(D/G)I(N/S/R)(S/N)YL(T/N/A); and/or
(R/L/V/D/A)(T/V/A)(N/S)(R/N)L(F/M/V/E/Q)(D/T/S); and/or
(L/Q)Q(Y/H)(D/V/N)(D/T)(F/Y)P(Y/L/W)T; and/or
(S/T)(S/H/Y)AMS; and/or
(T/A)IS(V/S/G)(G/S)G(G/R)(K/S)TYY(P/A)DSVKG; and/or
(L/D)(I/G)(S/G)(L/P/T/V)Y.

In a further embodiment of any aspect of the present invention the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the amino acid sequence QIVLTQSPAIMSASPGEKVTMTCSASSSVDYMYWYQQKPGSSPRLLIYDTSNLASGVPVR FSGSGSGTSYSLTISRMGAEDAATYYCQQWNSSPLTFGAGTKLELK. This light chain variable region corresponds with that found in exemplary antibody DD-9 (Figure 25).

In an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the amino acid sequence QVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVSWIRQSSGKGLEWLAHIYWDDDKR YNPSLKSRITISKDSSSNQVFLKITSVDTADTATYYCARSWTTAPFAFWGQGTLVTVSA. This heavy chain variable region corresponds with that found in exemplary antibody DD-9 (Figure 25).

Thus, it is envisaged that in an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have:

i) at least one light chain variable region comprising the amino acid sequence QIVLTQSPAIMSASPGEKVTMTCSASSSVDYMYWYQQKPGSSPRLLIYDTSNLASGVPVR FSGSGSGTSYSLTISRMGAEDAATYYCQQWNSSPLTFGAGTKLELK; and

ii) at least one heavy chain variable region comprising the amino acid sequence QVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVSWIRQSSGKGLEWLAHIYWDDDKR YNPSLKSRITISKDSSSNQVFLKITSVDTADTATYYCARSWTTAPFAFWGQGTLVTVSA.

In a further embodiment of any aspect of the present invention the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the amino acid sequence QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMYWYQQKPGSSPRLLIYDTSNLASGVPVR FSGSGSGTSYSLTISRMEAEDAATFYCQQWSSSPLTFGAGTKLELK. This light chain variable region corresponds with that found in exemplary antibody DH-2 (Figure 26).

In an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the amino acid sequence QVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVSWIRQPSGKGLEWLAHIYWDDDKR YNPSLKSRLTISKDTSSNQVFLKITSVDTADTATYYCARSGTTAPFAYWGQGTLVTVSA. This heavy chain variable region corresponds with that found in exemplary antibody DH-2 (Figure 26).

Thus, it is envisaged that in an embodiment of any aspect of the present invention, the antibody or antigen-binding fragment thereof may have:

i) at least one light chain variable region comprising the amino acid sequence QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMYWYQQKPGSSPRLLIYDTSNLASGVPVR FSGSGSGTSYSLTISRMEAEDAATFYCQQWSSSPLTFGAGTKLELK; and

ii) at least one heavy chain variable region comprising the amino acid sequence QVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVSWIRQPSGKGLEWLAHIYWDDDKR YNPSLKSRLTISKDTSSNQVFLKITSVDTADTATYYCARSGTTAPFAYWGQGTLVTVSA.

In an embodiment of any aspect of the invention, it is envisaged that, the antibody or antigen-binding fragment thereof may have:

i) at least one light chain variable region comprising an amino acid sequence corresponding with any of the VK sequences provided in any of Figures 18 to 28; and/or

ii) at least one heavy chain variable region comprising an amino acid sequence corresponding with any of the VH sequences provided in any of Figures 18 to 28 or a fragment, variant or derivative thereof.

As indicated above, it is envisaged that the antibody or antigen-binding fragment of the preceding embodiments may comprise an amino acid sequence having at least 75% identity with one or more of the amino acid sequences given above, for example at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with one or more of the amino acid sequences specified above. It is also envisaged that the antibody or antigen-binding fragment may comprise up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more insertions, deletions, conservative substitutions and/or non-conservative substitutions.

In a second aspect, the invention provides an antibody or antigen-binding fragment thereof comprising one or more of the following amino acid sequences:

KASQDINSYLT; and/or
XXNRLXD; and/or
LQYXDFPYT; and/or
XXAMS; and/or
TISXGGXXTYYPDSVKG; and/or
LISXY, wherein X is any amino acid.

In an embodiment, the invention provides an antibody or antigen-binding fragment thereof comprising one or more of the following amino acid sequences:

(K/Q/R)ASQ(D/G)I(N/S/R)(S/N)YL(T/N/A); and/or
(R/L/V/D/A)(T/V/A)(N/S)(R/N)L(F/M/V/E/Q)(D/T/S); and/or
(L/Q)Q(Y/H)(D/V/N)(D/T)(F/Y)P(Y/L/W)T; and/or
(S/T)(S/H/Y)AMS; and/or
(T/A)IS(V/S/G)(G/S)G(G/R)(K/S)TYY(P/A)DSVKG; and/or
(L/D)(I/G)(S/G)(L/P/T/V)Y.

In an embodiment, the invention provides an antibody or antigen-binding fragment thereof comprising one or more of the following amino acid sequences:

KASQDINSYLT; and/or
RTNRLFD; and/or
LQYDDFPYT; and/or
SSAMS; and/or
TISVGGGKTYYPDSVKG; and/or
LISLY.

In an embodiment, the antibody or antigen-binding fragment thereof of the second aspect may comprise one or more of the following amino acid sequences:

TCKASQDINSYLTWF; and/or

TLIYRTNRLFDGVP or TLIYRTNRLFDGVPXXFSGSGSGQDFF; and/or

YCLQYDDFPYTFG; and/or

FTLSSSAMSWVR or CXAXXFTLSSSAMSWVR; and/or

WVATISVGGGKTYYPDSVKGRFTISR or

WVATISVGGGKTYYPDSVKGRFTISRXNXXXXL; and/or

YCAKLISLYWG, wherein X is any amino acid.

The sequences of the preceding embodiment are considered to comprise the complementarity determining regions of the light and heavy variable regions of the exemplified antibody AB-1.

In a further embodiment, the antibody or antigen-binding fragment thereof may comprise the amino acid sequence KASQDINSYLTXXXXXXXXXXXXXXXRTNRLFDXXXXXXXXXXXXXXXFXXXXXXXXXX XXXXXXLQYDDFPYT; or

KASQDINSYLTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXF; or

RTNRLFDXXXXXXXXXXXXXXXF; or FXXXXXXXXXXXXXXXXLQYDDFPYT, wherein X is any amino acid.

In a further embodiment, the antibody or antigen-binding fragment thereof may comprise the amino acid sequence TCKASQDINSYLTWF or TCKASQDINSYLTWY; and/or

LLIYRTNRLFDGVP or SLIYRTNRLFDGVP or LLIYRTNRLFDGVPXXFSGSGSGQDFF or SLIYRTNRLFDGVPXXFSGSGSGQDFF; and/or

YCLQYDDFPYTFG; and/or

FTFSSSAMSWVR or CXAXXFTFSSSAMSWVR; and/or

WVSTISVGGGKTYYPDSVKGRFTISR or

WVSTISVGGGKTYYPDSVKGRFTISRXNXXXXL; and/or

YCAKLISLYWG, wherein X is any amino acid.

Thus, in an embodiment, the antibody or antigen-binding fragment thereof may have at least one light chain variable region comprising the following CDRs: KASQDINSYLT; and RTNRLFD; and LQYDDFPYT.

In a further embodiment, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the following CDRs: SSAMS; and TISVGGGKTYYPDSVKG; and LISLY.

In a further embodiment, the antibody or antigen-binding fragment thereof of the second aspect may have at least one light chain variable region comprising the amino acid sequence
DIQMTQTPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS
RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK or
DIKMTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS
RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK, or
EIVLTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS
RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (AB-1_VK), or
DIQMTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPS
RFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (AB-1_VK1), or
EIVLTQSPSSLSASVGDRVTITCKASQDINSYLTWYQQKPGKAPKLLIYRTNRLFDGVPS
RFSGSGSGTDFFFTISSLQPEDFGTYYCLQYDDFPYTFGGGTKLEIK (hAB-1_RKE), or
DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYRTNRLFDGVPS
RFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK (hAB-1_RKJ).

In a yet further embodiment of the second aspect, the antibody or antigen-binding fragment thereof may have at least one heavy chain variable region comprising the amino acid sequence
EVQLEESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY
PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS or
EVQLVESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY
PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS, or
EVQLQESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGGGKTYY
PDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTTLTVSS (AB-1_VH), or
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAPGKGLEWVSTISVGGGKTYY
PDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISLYWGQGTLVTVSS (hAB-1_RHA).

The invention also provides an antibody or antigen-binding fragment thereof having at least one light chain variable region as embodied in the second aspect and at least one heavy chain variable region as embodied in the second aspect.

For the avoidance of doubt, the antibody or antigen-binding fragment thereof of the second aspect may comprise any amino acid sequence provided in relation to the first aspect. Thus, the antibody or antigen-binding fragment thereof of the second aspect may include any VK and/or VK region as exemplified in any of Figures 18 to 28 or Tables 14 to 24 and 24A, or any variant, fragment or derivative thereof.

Further, the antibody or antigen-binding fragment thereof of any aspect of the invention may be or comprise any humanised or chimeric antibody described herein, in particular, antibody or antigen-binding fragment thereof may comprise or consist of any of the sequences provided in Tables 14 to 24 and 24A.

It is envisaged that the antibody or antigen-binding fragment of the second aspect and related embodiments may comprise an amino acid sequence having at least 75% identity with one or more of the amino acid sequences given above, for example at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with one or more of the amino acid sequences specified above. It is also envisaged that the antibody or antigen-binding fragment may comprise up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more insertions, deletions, conservative substitutions and/or non-conservative substitutions.

In a preferred embodiment of any aspect of the invention, the antibody or antigen-binding fragment thereof may comprise or consist of an intact antibody. Alternatively, the antibody or antigen-binding fragment thereof may consist essentially of an intact antibody. By "consist essentially of" we mean that the antibody or antigen-binding fragment thereof consists of a portion of an intact antibody sufficient to display binding specificity for TG2.

The antibody or antigen-binding fragment of the invention may be a non-naturally occurring antibody. Of course, where the antibody is a naturally occurring antibody, it is provided in an isolated form (*i.e.* distinct from that in which it is found in nature).

In an embodiment of any aspect of the invention, the antibody or antigen-binding fragment thereof may comprise or consist of an antigen-binding fragment selected from

the group consisting of: an Fv fragment; an Fab fragment; and an Fab-like fragment. In a further embodiment, the Fv fragment may be a single chain Fv fragment or a disulphide-bonded Fv fragment. In a yet further embodiment, the Fab-like fragment may be an Fab' fragment or an $F(ab)_2$ fragment.

The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent-parented antibody (Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA* 81, 6851-6855).

Antigenic specificity is conferred by variable domains and is independent of the constant domains, as known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better *et al* (1988) *Science* 240, 1041); Fv molecules (Skerra *et al* (1988) *Science* 240, 1038); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird *et al* (1988) *Science* 242, 423; Huston *et al* (1988) *Proc. Natl. Acad. Sci. USA* 85, 5879) and single domain antibodies (dAbs) comprising or consisting of isolated V domains (Ward *et al* (1989) *Nature* 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) *Nature* 349, 293-299.

Thus, by "antigen-binding fragment" we include a functional fragment of an antibody that is capable of binding to TG2.

Exemplary antigen-binding fragments may be selected from the group consisting of Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv), and Fab-like fragments (*e.g.* Fab fragments, Fab' fragments and $F(ab)_2$ fragments).

In one embodiment of the invention, the antigen-binding fragment is an scFv.

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better penetration of solid tissue. Moreover, antigen-binding fragments such as Fab, Fv, ScFv and dAb antibody fragments can be expressed in and

secreted from *E. coli*, thus allowing the facile production of large amounts of the said fragments.

Also included within the scope of the invention are modified versions of antibodies and an antigen-binding fragments thereof, *e.g.* modified by the covalent attachment of polyethylene glycol or other suitable polymer.

It is particularly preferred that the antibody or antigen-binding fragment thereof is a recombinant molecule.

Methods of generating antibodies and antibody fragments are well known in the art. For example, antibodies may be generated via any one of several methods which employ induction of *in vivo* production of antibody molecules, screening of immunoglobulin libraries (Orlandi. *et al*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86:3833-3837; Winter *et al.*, 1991, *Nature* 349:293-299) or generation of monoclonal antibody molecules by cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the Epstein-Barr virus (EBV)-hybridoma technique (Kohler *et al.*, 1975. *Nature* 256:4950497; Kozbor *et al.*, 1985. *J. Immunol. Methods* 81:31-42; Cote *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80:2026-2030; Cole *et al.*, 1984. *Mol. Cell. Biol.* 62:109-120).

Conveniently, the invention provides an antibody or antigen-binding fragment thereof wherein the antibody is a recombinant antibody (*i.e.* wherein it is produced by recombinant means).

In a particularly preferred embodiment of any aspect of the invention, the antibody may be a monoclonal antibody.

Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in *"Monoclonal Antibodies: A manual of techniques"*, H Zola (CRC Press, 1988) and in *"Monoclonal Hybridoma Antibodies: Techniques and Applications"*, J G R Hurrell (CRC Press, 1982), which are incorporated herein by reference. Exemplary monoclonal antibodies of the invention and suitable methods for their manufacture are provided in the Examples below.

Antibody fragments can also be obtained using methods well known in the art (see, for example, Harlow & Lane, 1988, *"Antibodies: A Laboratory Manual"*, Cold Spring Harbor

Laboratory, New York, which is incorporated herein by reference). For example, antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (*e.g.* Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Alternatively, antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods.

In an embodiment of any aspect of the invention, the antibody or antigen-binding fragment thereof may be a human antibody or a humanised antibody.

It will be appreciated by persons skilled in the art that for human therapy or diagnostics, humanised antibodies may be used. Humanised forms of non-human (*e.g.* murine) antibodies are genetically engineered chimeric antibodies or antibody fragments having minimal-portions derived from non-human antibodies. Humanised antibodies include antibodies in which complementary determining regions of a human antibody (recipient antibody) are replaced by residues from a complementary determining region of a non human species (donor antibody) such as mouse, rat or rabbit having the desired functionality. In some instances, Fv framework residues of the human antibody are replaced by corresponding non-human residues. Humanised antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported complementarity determining region or framework sequences. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the complementarity determining regions correspond to those of a non-human antibody and all, or substantially all, of the framework regions correspond to those of a relevant human consensus sequence. Humanised antibodies optimally also include at least a portion of an antibody constant region, such as an Fc region, typically derived from a human antibody (see, for example, Jones *et al.*, 1986. *Nature* 321:522-525; Riechmann *et al.*, 1988, *Nature* 332:323-329; Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596, which are incorporated herein by reference).

Methods for humanising non-human antibodies are well known in the art. Generally, the humanised antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues, often referred to as imported residues, are typically taken from an imported variable domain. Humanisation can be essentially performed as described (see, for example, Jones *et al.*, 1986, *Nature* 321:522-525; Reichmann *et al.*, 1988. *Nature* 332:323-327; Verhoeyen *et al.*, 1988,

Science 239:1534-1536l; US 4,816,567, which are incorporated herein by reference) by substituting human complementarity determining regions with corresponding rodent complementarity determining regions. Accordingly, such humanised antibodies are chimeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanised antibodies may be typically human antibodies in which some complementarity determining region residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be identified using various techniques known in the art, including phage display libraries (see, for example, Hoogenboom & Winter, 1991, *J. Mol. Biol.* 227:381; Marks *et al.*, 1991, *J. Mol. Biol.* 222:581; Cole *et al.*, 1985, In: *Monoclonal antibodies and Cancer Therapy*, Alan R. Liss, pp. 77; Boerner *et al.*, 1991. *J. Immunol.* 147:86-95, Soderlind *et al.*, 2000, *Nat Biotechnol* 18:852-6 and WO 98/32845 which are incorporated herein by reference).

The term "amino acid" as used herein includes the standard twenty genetically-encoded amino acids and their corresponding stereoisomers in the 'D' form (as compared to the natural 'L' form), omega-amino acids other naturally-occurring amino acids, unconventional amino acids (*e.g.* α,α-disubstituted amino acids, N-alkyl amino acids, *etc.*) and chemically derivatised amino acids.

When an amino acid is being specifically enumerated, such as "alanine" or "Ala" or "A", the term refers to both L-alanine and D-alanine unless explicitly stated otherwise. Other unconventional amino acids may also be suitable components for polypeptides of the present invention, as long as the desired functional property is retained by the polypeptide. For the peptides shown, each encoded amino acid residue, where appropriate, is represented by a single letter designation, corresponding to the trivial name of the conventional amino acid.

In one embodiment, the polypeptides as defined herein comprise or consist of L-amino acids.

Once suitable antibodies are obtained, they may be tested for activity, such as binding specificity or a biological activity of the antibody, for example by ELISA, immunohistochemistry, flow cytometry, immunoprecipitation, Western blots, *etc.* The

biological activity may be tested in different assays with readouts for that particular feature.

In a further aspect, the present invention provides a polynucleotide encoding an antibody or an antigen-binding fragment thereof according to the second aspect and the related embodiments of the second aspect.

Accordingly, in an embodiment, the invention provides an isolated polynucleotide comprising or consisting of the nucleotide sequences:

i)

GACATCCAGATGACACAGACTCCATCTTCCATGTATGCATCTCTAGGAGAGAGAGTCACT
ATCACTTGCAAGGCGAGTCAGGACATTAATAGCTATTTAACCTGGTTCCAGCAGAAACCA
GGGAAATCTCCTAAGACCCTGATCTATCGTACAAATAGATTGTTTGATGGGGTCCCATCC
AGGTTCAGTGGCAGTGGATCTGGGCAAGATTTTTTTCTCACCATCAGCAGCCTGGAATAT
GAAGATATGGGAATTTATTATTGTCTACAGTATGATGACTTTCCGTACACGTTCGGAGGG
GGGACCAAACTGGAAATAAAA; and/or

ii)

GAAGTACAGCTGGAGGAGTCAGGGGGGGGCTTAGTGAAGCCTGGAGGGTCCCTGAAACTC
TCCTGTGCAGCCTCTGGATTCACTCTCAGTTCCTCTGCCATGTCTTGGGTTCGCCAGACT
CCGGACAGGAGGCTGGAGTGGGTCGCAACCATTAGTGTTGGTGGTGGTAAAACCTACTAT
CCAGACAGTGTGAAGGGTCGCTTCACCATCTCCAGAGACAATGCCAAGAACACCCTCTAT
CTGCAAATGAACAGTCTGAGGTCTGAGGACACGGCCATGTATTACTGTGCAAAACTAATC
AGTCTCTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA.

In a further embodiment, the invention provides an isolated polynucleotide comprising or consisting of any of the nucleic acid sequences listed in any of Figures 18 to 28.

Thus, in an embodiment, the invention provides an isolated polynucleotide comprising or consisting of the nucleotide sequences:

i)

GCCATCAAGATGACCCAGTCTCCATCTTCCATGTATGCATCTCTAGGAGAGAGAGTCATC
ATCACTTGCAAGGCGAGTCAGGACATAAATAGTTATTTAACCTGGTTCCAACAGAAACCA
GGAAAGTCTCCTAAGACCCTGATCTATCTTACAAATAGATTGATGGATGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGCAAGAATTTTTACTCACCATCAGCGGCCTGGAACAT

GAAGATATGGGCATTTATTATTGTCTCCAGTATGTTGACTTTCCGTACACGTTCGGAGGG
GGGACCAAGCTGGAAATAAAA; and/or

ii)

GCAGTGCAACTGGTAGAGTCTGGGGGAGGCTTGGTGAAGCCTGGAGGGTCCCTGAAACTC
TCCTGTGCAGCCTCTGGAATCATTTTCAGTTCCTCTGCCATGTCTTGGGTTCGCCAGACT
CCGGAAAAGAGACTGGAGTGGGTCGCAACTATTAGTAGTGGTGGTCGTTCCACCTACTAT
CCAGACAGTGTGAAGGGTCGATTCACCGTCTCCAGAGACAGTGCCAAGAACACCCTATAC
CTGCAAATGGACAGTCTGAGGTCTGAGGACACGGCCATTTATTACTGTGCAAAACTAATC
AGTCCCTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA.

In a further embodiment, the invention provides an isolated polynucleotide comprising or consisting of the nucleotide sequences:

i)

GACATCACGATGACCCAGTCTCCATCTTCCATATATGCATCTCTGGGAGAGAGAGTCACT
ATCACTTGCAAGGCGAGTCAGGACATTAATAGCTATTTAACCTGGTTCCAGCAGAAACCA
GGGAAATCTCCTAAGATCCTGATCTATCTTGTAAATAGATTGGTAGATGGGGTCCCATCA
AGGTTCAGTGGCAGTGGATCTGGGCAAGATTATGCTCTCACCATCAGCAGTCTGGAATAT
GAAGATATGGGAATTTATTATTGTCTACAATATGATGACTTTCCGTACACGTTCGGAGGG
GGGACCAAGCTGGAAATAAAA; and/or

ii)

GAAGTGCAGTTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAAACTC
TCCTGTGCAGCCTCTGGATTCACTCTCAGTACCCATGCCATGTCTTGGGTTCGCCAGACT
CCGGAGAAGAGGCTGGAGTGGGTCGCAACCATTAGTAGTGGTGGTCGTTCCACCTACTAT
CCAGACAGTGTGAAGGGTCGATTCACTATCTCCAGAGACAATGTCAAGAACACCCTATAT
CTGCAACTGAGCAGTCTGAGGTCTGAGGACACGGCCGTGTATTTCTGTGCAAGACTAATC
AGTACCTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA.

It is preferred that the antibody or antigen-binding fragment thereof of the invention inhibits TG2 activity. Thus, it is preferred that the antibody of the invention inhibits TG2 enzymatic activity and thus prevents it from functioning to cross-link lysine and glutamine residues with Nε-(γ -glutamyl)lysine isopeptide bonds. It is preferred that the enzymatic activity of TG2 is completely abrogated, but it is envisaged that the inhibition may be partial inhibition in instances where this partial inhibition is sufficient to provide a useful therapeutic or non-therapeutic outcome. The skilled person would be able to determine whether the extent of inhibition is effective to achieve the desired outcome.

It is preferred that the antibody or antigen-binding fragment thereof is specific for TG2 inhibition. Thus, it is preferred that the antibody or antigen-binding fragment thereof does not inhibit TG1, TG3, TG13 and/or TG7 activity. It is envisaged that an antibody that effectively inhibits TG2 activity but is sufficiently selective so that it does not significantly inhibit TG1, TG3, TG13 and/or TG7 activity may be particularly useful in medicine. Thus, it is preferred that the antibody exclusively inhibits TG2 activity.

In a further aspect, the invention provides an antibody or antigen-binding fragment thereof whose binding to TG2 (for example, human TG2) is inhibited or reduced when an antibody according to any preceding aspect is bound to TG2 (for example, human TG2).

Thus, the invention includes any antibody that selectively binds to an epitope within the region of TG2 such that it may compete with and disrupt binding of any antibody of the preceding aspects.

In a further aspect, the present invention provides a compound comprising an antibody or antigen-binding fragment thereof according to any preceding aspect and a further moiety.

In an embodiment, the further moiety may be a directly or indirectly cytotoxic moiety.

By "directly cytotoxic" we include the meaning that the moiety is one which on its own is cytotoxic. By "indirectly cytotoxic" we include the meaning that the moiety is one which, although is not itself cytotoxic, can induce cytotoxicity, for example by its action on a further molecule or by further action on it.

The cytotoxic moiety may be selected from, but is not limited to, the group comprising a directly cytotoxic chemotherapeutic agent, a directly cytotoxic polypeptide, a moiety which is able to convert a relatively non-toxic prodrug into a cytotoxic drug, a radiosensitizer, a directly cytotoxic nucleic acid, a nucleic acid molecule that encodes a directly or indirectly cytotoxic polypeptide, a nucleic acid molecule that encodes a therapeutic polypeptide, or a radioactive atom. It is envisaged that the radioactive atom may be any one of phosphorus-32, iodine-125, iodine-131, indium-111, rhenium-186, rhenium-188 or yttrium-90.

Cytotoxic chemotherapeutic agents, such as anticancer agents, include: alkylating agents including nitrogen mustards such as mechlorethamine (HN_2), cyclophosphamide, ifosfamide, melphalan (L-sarcolysin) and chlorambucil; ethylenimines and methylmelamines such as hexamethylmelamine, thiotepa; alkyl sulphonates such as busulfan; nitrosoureas such as carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU) and streptozocin (streptozotocin); and triazenes such as decarbazine (DTIC; dimethyltriazenoimidazole-carboxamide); Antimetabolites including folic acid analogues such as methotrexate (amethopterin); pyrimidine analogues such as fluorouracil (5-fluorouracil; 5-FU), floxuridine (fluorodeoxyuridine; FUdR) and cytarabine (cytosine arabinoside); and purine analogues and related inhibitors such as mercaptopurine (6-mercaptopurine; 6-MP), thioguanine (6-thioguanine; TG) and pentostatin (2'-deoxycoformycin). Natural Products including vinca alkaloids such as vinblastine (VLB) and vincristine; epipodophyllotoxins such as etoposide and teniposide; antibiotics such as dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin) and mitomycin (mitomycin C); enzymes such as L-asparaginase; and biological response modifiers such as interferon alphenomes. Miscellaneous agents including platinum coordination complexes such as cisplatin (*cis*-DDP) and carboplatin; anthracenedione such as mitoxantrone and anthracycline; substituted urea such as hydroxyurea; methyl hydrazine derivative such as procarbazine (N-methylhydrazine, MIH); and adrenocortical suppressant such as mitotane (*o,p'*-DDD) and aminoglutethimide; taxol and analogues/derivatives; and hormone agonists/antagonists such as flutamide and tamoxifen.

Various of these agents have previously been attached to antibodies and other target site-delivery agents, and so compounds of the invention comprising these agents may readily be made by the person skilled in the art. For example, carbodiimide conjugation (Bauminger & Wilchek (1980) *Methods Enzymol.* **70**, 151-159; incorporated herein by reference) may be used to conjugate a variety of agents, including doxorubicin, to antibodies.

Cytotoxic peptides or polypeptide moieties include any moiety which leads to cell death. Cytotoxic peptide and polypeptide moieties are well known in the art and include, for example, ricin, abrin, *Pseudomonas* exotoxin, tissue factor and the like. Methods for linking them to targeting moieties such as antibodies are also known in the art. The use of ricin as a cytotoxic agent is described in Burrows & Thorpe (1993) *Proc. Natl. Acad. Sci. USA* **90**, 8996-9000, incorporated herein by reference, and the use of tissue factor,

which leads to localised blood clotting and infarction of a tumour, has been described by Ran *et al* (1998) *Cancer Res.* **58**, 4646-4653 and Huang *et al* (1997) *Science* **275**, 547-550. Tsai *et al* (1995) *Dis. Colon Rectum* **38**, 1067-1074 describes the abrin A chain conjugated to a monoclonal antibody and is incorporated herein by reference. Other ribosome inactivating proteins are described as cytotoxic agents in WO 96/06641. Pseudomonas exotoxin may also be used as the cytotoxic polypeptide moiety (see, for example, Aiello *et al* (1995) *Proc. Natl. Acad. Sci. USA* **92**, 10457-10461; incorporated herein by reference).

Certain cytokines, such as TNFα and IL-2, may also be useful as cytotoxic agents.

Certain radioactive atoms may also be cytotoxic if delivered in sufficient doses. Thus, the cytotoxic moiety may comprise a radioactive atom which, in use, delivers a sufficient quantity of radioactivity to the target site so as to be cytotoxic. Suitable radioactive atoms include phosphorus-32, iodine-125, iodine-131, indium-111, rhenium-186, rhenium-188 or yttrium-90, or any other isotope which emits enough energy to destroy neighbouring cells, organelles or nucleic acid. Preferably, the isotopes and density of radioactive atoms in the compound of the invention are such that a dose of more than 4000 cGy (preferably at least 6000, 8000 or 10000 cGy) is delivered to the target site and, preferably, to the cells at the target site and their organelles, particularly the nucleus. The radioactive atom may be attached to the antibody in known ways. For example EDTA or another chelating agent may be attached to the antibody and used to attach ^{111}In or ^{90}Y. Tyrosine residues may be labelled with ^{125}I or ^{131}I.

The cytotoxic moiety may be a suitable indirectly cytotoxic polypeptide. In a particularly preferred embodiment, the indirectly cytotoxic polypeptide is a polypeptide which has enzymatic activity and can convert a relatively non-toxic prodrug into a cytotoxic drug. When the targeting moiety is an antibody this type of system is often referred to as ADEPT (Antibody-Directed Enzyme Prodrug Therapy). The system requires that the targeting moiety locates the enzymatic portion to the desired site in the body of the patient (ie the site expressing MR, such as new vascular tissue associated with a tumour) and after allowing time for the enzyme to localise at the site, administering a prodrug which is a substrate for the enzyme, the end product of the catalysis being a cytotoxic compound. The object of the approach is to maximise the concentration of drug at the desired site and to minimise the concentration of drug in normal tissues (see Senter, P.D. *et al* (1988) "Anti-tumor effects of antibody-alkaline phosphatase conjugates in combination with etoposide phosphate" *Proc. Natl. Acad. Sci. USA* **85**,

4842-4846; Bagshawe (1987) *Br. J. Cancer* **56**, 531-2; and Bagshawe, K.D. *et al* (1988) "A cytotoxic agent can be generated selectively at cancer sites" *Br. J. Cancer.* **58**, 700-703.)

The cytotoxic substance may be any existing anti-cancer drug such as an alkylating agent; an agent which intercalates in DNA; an agent which inhibits any key enzymes such as dihydrofolate reductase, thymidine synthetase, ribonucleotide reductase, nucleoside kinases or topoisomerase; or an agent which effects cell death by interacting with any other cellular constituent. Etoposide is an example of a topoisomerase inhibitor.

Reported prodrug systems include: a phenol mustard prodrug activated by an *E. coli* β-glucuronidase (Wang *et al*, 1992 and Roffler *et al*, 1991); a doxorubicin prodrug activated by a human β-glucuronidase (Bosslet *et al*, 1994); further doxorubicin prodrugs activated by coffee bean α-galactosidase (Azoulay *et al*, 1995); daunorubicin prodrugs, activated by coffee bean α-D-galactosidase (Gesson *et al*, 1994); a 5-fluorouridine prodrug activated by an *E. coli* β-D-galactosidase (Abraham *et al*, 1994); and methotrexate prodrugs (eg methotrexate-alanine) activated by carboxypeptidase A (Kuefner *et al*, 1990, Vitols *et al*, 1992 and Vitols *et al*, 1995). These and others are included in Table 1.

Table 1

Enzyme	**Prodrug**
Carboxypeptidase G2	Derivatives of L-glutamic acid and benzoic acid mustards, aniline mustards, phenol mustards and phenylenediamine mustards; fluorinated derivatives of these
Alkaline phosphatase	Etoposide phosphate
	Mitomycin phosphate
Beta-glucuronidase	*p*-Hydroxyaniline mustard-glucuronide
	Epirubicin-glucuronide
Penicillin-V-amidase	Adriamycin-N phenoxyacetyl
Penicillin-G-amidase	N-(4'-hydroxyphenyl acetyl) palytoxin
	Doxorubicin and melphalan
Beta-lactamase	Nitrogen mustard-cephalosporin *p*-phenylenediamine; doxorubicin derivatives; vinblastine derivative-cephalosporin, cephalosporin mustard; a taxol derivative

Beta-glucosidase	Cyanophenylmethyl-beta-D-gluco-pyranosiduronic acid
Nitroreductase	5-(Azaridin-1-yl-)-2,4-dinitrobenzamide
Cytosine deaminase	5-Fluorocytosine
Carboxypeptidase A	Methotrexate-alanine

(This table is adapted from Bagshawe (1995) *Drug Dev. Res.* **34**, 220-230, from which full references for these various systems may be obtained; the taxol derivative is described in Rodrigues, M.L. *et al* (1995) *Chemistry & Biology* **2**, 223).

Suitable enzymes for forming part of the enzymatic portion of the invention include: exopeptidases, such as carboxypeptidases G, G1 and G2 (for glutamylated mustard prodrugs), carboxypeptidases A and B (for MTX-based prodrugs) and aminopeptidases (for 2-α-aminocyl MTC prodrugs); endopeptidases, such as eg thrombolysin (for thrombin prodrugs); hydrolases, such as phosphatases (eg alkaline phosphatase) or sulphatases (eg aryl sulphatases) (for phosphylated or sulphated prodrugs); amidases, such as penicillin amidases and arylacyl amidase; lactamases, such as β-lactamases; glycosidases, such as β-glucuronidase (for β-glucuronomide anthracyclines), α-galactosidase (for amygdalin) and β-galactosidase (for β-galactose anthracycline); deaminases, such as cytosine deaminase (for 5FC); kinases, such as urokinase and thymidine kinase (for gancyclovir); reductases, such as nitroreductase (for CB1954 and analogues), azoreductase (for azobenzene mustards) and DT-diaphorase (for CB1954); oxidases, such as glucose oxidase (for glucose), xanthine oxidase (for xanthine) and lactoperoxidase; DL-racemases, catalytic antibodies and cyclodextrins.

The prodrug is relatively non-toxic compared to the cytotoxic drug. Typically, it has less than 10% of the toxicity, preferably less than 1% of the toxicity as measured in a suitable *in vitro* cytotoxicity test. It is likely that the moiety which is able to convert a prodrug to a cytotoxic drug will be active in isolation from the rest of the compound but it is necessary only for it to be active when (a) it is in combination with the rest of the compound and (b) the compound is attached to, adjacent to or internalised in target cells.

When each moiety of the compound is a polypeptide, the two portions may be linked together by any of the conventional ways of cross-linking polypeptides, such as those generally described in O'Sullivan *et al* (1979) *Anal. Biochem.* **100**, 100-108. Alternatively, the compound may be produced as a fusion compound by recombinant DNA techniques whereby a length of DNA comprises respective regions encoding the two moieties of the compound of the invention either adjacent one another or separated

by a region encoding a linker peptide which does not destroy the desired properties of the compound. Conceivably, the two portions of the compound may overlap wholly or partly.

The cytotoxic moiety may be a radiosensitizer. Radiosensitizers include fluoropyrimidines, thymidine analogues, hydroxyurea, gemcitabine, fludarabine, nicotinamide, halogenated pyrimidines, 3-aminobenzamide, 3-aminobenzodiamide, etanixadole, pimonidazole and misonidazole (see, for example, McGinn *et al* (1996) *J. Natl. Cancer Inst.* **88**, 1193-11203; Shewach & Lawrence (1996) *Invest. New Drugs* **14**, 257-263; Horsman (1995) *Acta Oncol.* **34**, 571-587; Shenoy & Singh (1992) *Clin. Invest.* **10**, 533-551; Mitchell *et al* (1989) *Int. J. Radiat. Biol.* **56**, 827-836; Iliakis & Kurtzman (1989) *Int. J. Radiat. Oncol. Biol. Phys.* **16**, 1235-1241; Brown (1989) *Int. J. Radiat. Oncol. Biol. Phys.* **16**, 987-993; Brown (1985) *Cancer* **55**, 2222-2228).

Also, delivery of genes into cells can radiosensitise them, for example delivery of the p53 gene or cyclin D (Lang *et al* (1998) *J. Neurosurg.* **89**, 125-132; Coco Martin *et al* (1999) *Cancer Res.* **59**, 1134-1140).

The further moiety may be one which becomes cytotoxic, or releases a cytotoxic moiety, upon irradiation. For example, the boron-10 isotope, when appropriately irradiated, releases α particles which are cytotoxic (see for example, US 4,348,376 to Goldenberg; Primus *et al* (1996) *Bioconjug. Chem.* **7**, 532-535).

Similarly, the cytotoxic moiety may be one which is useful in photodynamic therapy such as photofrin (see, for example, Dougherty *et al* (1998) *J. Natl. Cancer Inst.* **90**, 889-905).

The cytotoxic moiety may be a nucleic acid molecule which is directly or indirectly cytotoxic. For example, the nucleic acid molecule may be an antisense oligonucleotide which, upon localisation at the target site is able to enter cells and lead to their death. The oligonucleotide, therefore, may be one which prevents expression of an essential gene, or one which leads to a change in gene expression which causes apoptosis.

Examples of suitable oligonucleotides include those directed at bcl-2 (Ziegler *et al* (1997) *J. Natl. Cancer Inst.* **89**, 1027-1036), and DNA polymerase α and topoisomerase IIα (Lee *et al* (1996) *Anticancer Res.* **16**, 1805-1811.

Peptide nucleic acids may be useful in place of conventional nucleic acids (see Knudsen & Nielsen (1997) *Anticancer Drugs* **8**, 113-118).

In an embodiment of the compound of the invention, the antibody or antigen-binding fragment thereof and the cytotoxic moiety may be polypeptides which are fused. Thus, the invention further provides a polynucleotide encoding such a compound.

In a further embodiment, the further moiety may be a readily detectable moiety. It is envisaged that readily detectable moiety may comprise a suitable amount of any one of iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, technitium-99m, gadolinium, manganese or iron.

By a "readily detectable moiety" we include the meaning that the moiety is one which, when located at the target site following administration of the compound of the invention into a patient, may be detected, typically non-invasively from outside the body and the site of the target located. Thus, the compounds of this embodiment of the invention are useful in imaging and diagnosis.

Typically, the readily detectable moiety is or comprises a radioactive atom which is useful in imaging. Suitable radioactive atoms include technetium-99m or iodine-123 for scintigraphic studies. Other readily detectable moieties include, for example, spin labels for magnetic resonance imaging (MRI) such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Clearly, the compound of the invention must have sufficient of the appropriate atomic isotopes in order for the molecule to be readily detectable.

The radio- or other labels may be incorporated in the compound of the invention in known ways. For example, if the antibody is a polypeptide it may be biosynthesised or may be synthesised by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as ^{99m}Tc, ^{123}I, ^{186}Rh, ^{188}Rh and ^{111}In can, for example, be attached *via* cysteine residues in the antibody. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker *er al* (1978) *Biochem. Biophys. Res. Comm.* **80**, 49-57) can be used to incorporate iodine-123. Reference ("Monoclonal Antibodies in Immunoscintigraphy", J-F Chatal, CRC Press, 1989) describes other methods in detail.

In a further aspect, the present invention provides a vector comprising any polynucleotide of the invention.

Typical prokaryotic vector plasmids are: pUC18, pUC19, pBR322 and pBR329 available from Biorad Laboratories (Richmond, CA, USA); p*Trc*99A, pKK223-3, pKK233-3, pDR540 and pRIT5 available from Pharmacia (Piscataway, NJ, USA); pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16A, pNH18A, pNH46A available from Stratagene Cloning Systems (La Jolla, CA 92037, USA).

A typical mammalian cell vector plasmid is pSVL available from Pharmacia (Piscataway, NJ, USA). An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia (Piscataway, NJ, USA).

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems (La Jolla, CA 92037, USA). Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers *HIS3, TRP1, LEU2* and *URA3*. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

Methods well known to those skilled in the art can be used to construct expression vectors containing the coding sequence and, for example appropriate transcriptional or translational controls.

Yet further, the invention provides a host cell comprising any polynucleotide or vector of the invention.

Many expression systems are known, including systems employing: bacteria (eg. *E. coli* and *Bacillus subtilis*) transformed with, for example, recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeasts (eg. *Saccharomyces cerevisiae*) transformed with, for example, yeast expression vectors; insect cell systems transformed with, for example, viral expression vectors (eg. baculovirus); plant cell systems transfected with, for example viral or bacterial expression vectors; animal cell systems transfected with, for example, adenovirus expression vectors.

The vectors can include a prokaryotic replicon, such as the Col E1 *ori*, for propagation in a prokaryote, even if the vector is to be used for expression in other, non-prokaryotic cell types. The vectors can also include an appropriate promoter such as a prokaryotic

promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

The polynucleotide in a suitable host cell may be expressed to produce the antibody or compound of the invention. Thus, the polynucleotide may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the antibody or compound of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859; 4,530,901; 4,582,800; 4,677,063; 4,678,751; 4,704,362; 4,710,463; 4,757,006; 4,766,075; and 4,810,648, all of which are incorporated herein by reference.

The polynucleotide may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

Generally, the polynucleotide is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. Thus, the DNA insert may be operatively linked to an appropriate promoter. Bacterial promoters include the *E. coli lacI* and *lacZ* promoters, the T3 and T7 promoters, the *gpt* promoter, the phage λ PR and PL promoters, the *phoA* promoter and the *trp* promoter. Eukaryotic promoters include the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters and the promoters of retroviral LTRs. Other suitable promoters will be known to the skilled artisan. The expression constructs will desirably also contain sites for transcription initiation and termination, and in the transcribed region, a ribosome binding site for translation. (Hastings *et al*, International Patent No. WO 98/16643, published 23 April 1998)

The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector and it will therefore be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a DNA sequence marker, with any necessary control elements, that codes for a selectable trait in the transformed cell. These markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture, and tetracyclin, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the recombinant DNA of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

The antibody or compound can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Yet further, the invention provides a stable host cell line producing an antibody or antigen-binding fragment thereof according to any preceding aspect or a compound of the invention resulting from incorporation in the cell line an exogenous polynucleotide or vector of the invention.

The host cell can be either prokaryotic or eukaryotic. Bacterial cells are preferred prokaryotic host cells and typically are a strain of *Escherichia coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Yeast host cells include YPH499, YPH500 and YPH501 which are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Preferred mammalian host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available

from the ATCC as CRL 1658, and monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650.

In addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium.

In a further aspect, the present invention provides a pharmaceutical composition/formulation comprising an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide according to the invention, or a compound according to the invention, in admixture with a pharmaceutically acceptable excipient, adjuvant, diluent or carrier.

By "pharmaceutically acceptable" is included that the formulation is sterile and pyrogen free. Suitable pharmaceutical carriers are well known in the art of pharmacy. The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free; however, other acceptable carriers may be used.

In an embodiment, the pharmaceutical composition/formulation of the invention may further comprise a further active ingredient, i.e. a therapeutically active agent other than the antibody or antigen-binding fragment thereof of the invention. It is envisaged that one or more additional active agents may increase the efficacy of the pharmaceutical composition/formulation against the targeted disease as appropriate. In an embodiment, the further active ingredient may be a therapeutic agent selected from an agent involved in reducing tissue scarring, reducing neurofibrilliary tangles, and/or reducing resistance to chemotherapy.

In a preferred embodiment, pharmaceutical composition/formulation may be formulated for intravenous, intramuscular, or subcutaneous delivery to a patient.

It is preferred that the pharmaceutical composition/formulation comprises an amount of the antibody or antigen-binding fragment of the invention effective to treat the various conditions described above and further below.

A further aspect of the invention provides a kit of parts comprising an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide according of the inveniton, or a compound of the invention; and one or more further agents. It is envisaged that the further agents may be any one of the further active ingredients described above, or any other suitable agent.

In a yet further aspect, the invention provides a therapeutically effective amount of an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide of the invention, or a compound, pharmaceutical composition/formulation, or kit of parts of the invention, for use in medicine.

TG2 clearly is a multifunction enzyme and has been linked to a range of cellular functions including nuclear stabilisation and transport [28, 29], endocytosis [30, 31], GTPase signalling [32-34], Apoptosis [35, 36], cell adhesion [37-39], cytoskeletal integrity [28, 29] and ECM stabilisation [9]. A small molecule inhibitor may impede on all of these functions as in general they have free access to the extracellular space and cell interior. An antibody cannot enter the cell and as such the intracellular roles of TG2 would not be affected by a TG2 specific antibody administered *in vivo*.

Importantly most of the pathological roles of TG2 appear to be extracellular such as its role in tissue scarring and fibrosis, celiac disease and cancer. Thus using an antibody which selectively binds TG2 in medicine would bring an additional degree of selectivity preventing undesired intracellular effects that could translate into undesired side effects of therapy.

Therefore the antibodies and antigen-binding fragments thereof of the invention would offer clinical advantages over previously available drugs, for example in blocking TG2 in fibrotic and scarring diseases where TG2 crosslinks ECM proteins, in celiac disease where gliadin is deamidated in the extracellular space and in chemo-resistance in cancer where cell adhesion appears to be the protective factor. Further, the small antibody fragments of the invention, for example the Fab fragments could cross the blood brain barrier and inhibit TG2 in the brain and potentially offer effective therapies for neurological pathologies with TG2 involvement.

Thus, a further aspect of the invention provides an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide of the invention, or a

compound, pharmaceutical composition/formulation, or kit of parts of the invention, for use in reducing or inhibiting TG2 enzyme activity in an individual in need thereof.

The invention further provides for the use of an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide of the invention, or a compound, pharmaceutical composition/formulation, or kit of parts of the invention, in the manufacture of a medicament for reducing or inhibiting TG2 enzyme activity in an individual in need thereof.

The invention also provides a method of reducing or inhibiting TG2 enzyme activity in an individual in need thereof, the method comprising the step of administering an antibody or antigen-binding fragment thereof, or a variant, fusion or derivative thereof according to any aspect of the invention, or a polynucleotide of the invention, or a compound, pharmaceutical composition/formulation, or kit of parts of the invention, to the individual.

A further aspect of the invention provides a therapeutically effective amount of an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide of the invention, or a compound, pharmaceutical composition/formulation, or kit of parts of the invention, for use in the treatment and/or diagnosis of Celiac disease, abnormal wound healing, scarring, scleroderma, keloids and hypertrophic scars, ocular scarring, inflammatory bowel disease, macular degeneration, Grave's opthalmopathy, drug-induced ergotism, psoriasis, fibrosis-related diseases (e.g. liver fibrosis, pulmonary fibrosis such as interstitial lung disease and fibrotic lung disease, cardiac fibrosis, skin fibrosis, myelofibrosis, kidney fibrosis such as glomerulosclerosis and tubulointerstitial fibrosis), atherosclerosis, restenosis, inflammatory diseases, autoimmune diseases, neurodegenerative/neurological diseases (e.g. Huntington's Disease, Alzheimer's disease, Parkinson's disease, polyglutamine disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, spinocerebellar ataxias 1, 2, 3, 6, 7 and 12, rubropallidal atrophy, spinocerebellar palsy), and/or cancer (e.g. glioblastomas such as glioblastoma in Li-Fraumeni syndrome and sporadic glioblastoma, malignant melanomas, pancreatic ductal adenocarcinomas, myeloid leukemia, acute myelogenous leukemia, myelodysplastic syndrome, myeloproliferative syndrome, gynaecological cancer, Kaposi's sarcoma, Hansen's disease, collagenous colitis).

The invention also provides for the use of a therapeutically effective amount of an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide of the invention, or a compound, pharmaceutical

composition/formulation, or kit of parts of the invention, in the manufacture of a medicament for treating and/or diagnosing Celiac disease, abnormal wound healing, scarring, scleroderma, keloids and hypertrophic scars, ocular scarring, inflammatory bowel disease, macular degeneration, Grave's opthalmopathy, drug-induced ergotism, psoriasis, fibrosis-related diseases (e.g. liver fibrosis, pulmonary fibrosis such as interstitial lung disease and fibrotic lung disease, cardiac fibrosis, skin fibrosis, myelofibrosis, kidney fibrosis such as glomerulosclerosis and tubulointerstitial fibrosis), atherosclerosis, restenosis, inflammatory diseases, autoimmune diseases, neurodegenerative/neurological diseases (e.g. Huntington's Disease, Alzheimer's disease, Parkinson's disease, polyglutamine disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, spinocerebellar ataxias 1, 2, 3, 6, 7 and 12, rubropallidal atrophy, spinocerebellar palsy), and/or cancer (e.g. glioblastomas such as glioblastoma in Li-Fraumeni syndrome and sporadic glioblastoma, malignant melanomas, pancreatic ductal adenocarcinomas, myeloid leukemia, acute myelogenous leukemia, myelodysplastic syndrome, myeloproliferative syndrome, gynaecological cancer, Kaposi's sarcoma, Hansen's disease, collagenous colitis).

The invention also provides a method of treating and/or diagnosing Celiac disease, abnormal wound healing, scarring, scleroderma, keloids and hypertrophic scars, ocular scarring, inflammatory bowel disease, macular degeneration, Grave's opthalmopathy, drug-induced ergotism, psoriasis, fibrosis-related diseases (e.g. liver fibrosis, pulmonary fibrosis such as interstitial lung disease and fibrotic lung disease, cardiac fibrosis, skin fibrosis, myelofibrosis, kidney fibrosis such as glomerulosclerosis and tubulointerstitial fibrosis), atherosclerosis, restenosis, inflammatory diseases, autoimmune diseases, neurodegenerative/neurological diseases (e.g. Huntington's Disease, Alzheimer's disease, Parkinson's disease, polyglutamine disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, spinocerebellar ataxias 1, 2, 3, 6, 7 and 12, rubropallidal atrophy, spinocerebellar palsy), and/or cancer (e.g. glioblastomas such as glioblastoma in Li-Fraumeni syndrome and sporadic glioblastoma, malignant melanomas, pancreatic ductal adenocarcinomas, myeloid leukemia, acute myelogenous leukemia, myelodysplastic syndrome, myeloproliferative syndrome, gynaecological cancer, Kaposi's sarcoma, Hansen's disease, collagenous colitis) in a patient, the method comprising administering a therapeutically effective amount of an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide of the invention, or a compound, pharmaceutical composition/formulation, or kit of parts of the invention, to the patient.

By "treatment" we include both therapeutic and prophylactic treatment of a subject/patient. The term "prophylactic" is used to encompass the use of an antibody, medicament, compound, composition, or kit described herein which either prevents or reduces the likelihood of the occurrence or development of a condition or disorder (such as a fibrosis-related disorder) in an individual.

It is preferred that the patient is a human but the patient may be any other mammal that may benefit from the treatment. For example, the patient may be a mouse, a rat, a hamster, a rabbit, a cat, a dog, a goat, a sheep, a monkey or an ape.

A "therapeutically effective amount", or "effective amount", or "therapeutically effective", as used herein, refers to that amount which provides a therapeutic effect for a given condition and administration regimen. This is a predetermined quantity of active material calculated to produce a desired therapeutic effect in association with the required additive and diluent, *i.e.* a carrier or administration vehicle. Further, it is intended to mean an amount sufficient to reduce or prevent a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host, for example a mammal.

The agents (*i.e.* antibody, antigen-binding fragment, variant, fusion or derivative thereof), medicaments, compounds, pharmaceutical compositions/formulations and kits of the invention may be delivered using an injectable sustained-release drug delivery system. These are designed specifically to reduce the frequency of injections. An example of such a system is Nutropin Depot which encapsulates recombinant human growth hormone (rhGH) in biodegradable microspheres that, once injected, release rhGH slowly over a sustained period. Preferably, delivery is performed intra-muscularly (i.m.) and/or sub-cutaneously (s.c.) and/or intravenously (i.v.).

The agents, medicaments, compounds, pharmaceutical compositions/formulations and kits of the invention can be administered by a surgically implanted device that releases the drug directly to the required site. For example, Vitrasert releases ganciclovir directly into the eye to treat CMV retinitis. The direct application of this toxic agent to the site of disease achieves effective therapy without the drug's significant systemic side-effects.

Preferably, the medicaments and/or pharmaceutical compositions/formulations of the present invention is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredient(s).

The agents, medicaments, compounds, pharmaceutical compositions and kits of the invention will normally be administered by any parenteral route, in the form of a pharmaceutical composition comprising the active ingredient(s), optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.

In human therapy, the agents, medicaments, compounds, pharmaceutical compositions/formulations, and kits of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient, diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

The agents, medicaments, compounds, pharmaceutical compositions/formulations and kits of the invention can be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intra-thecally, intra-muscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

Medicaments and pharmaceutical compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The medicaments and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions

may be prepared from sterile powders, granules and tablets of the kind previously described.

For parenteral administration to human patients, the daily dosage level of the agents, medicaments and pharmaceutical compositions of the invention will usually be from 1 μg to 10 mg per adult per day administered in single or divided doses. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

Typically, the medicaments, pharmaceutical compositions/formulations and kits of the invention will contain the agent of the invention at a concentration of between approximately 2 mg/ml and 150 mg/ml or between approximately 2 mg/ml and 200 mg/ml. In a preferred embodiment, the medicaments, pharmaceutical compositions/formulations and kits of the invention will contain the agent of the invention at a concentration of 10 mg/ml.

Generally, in humans, parenteral administration of the agents, medicaments, compounds, pharmaceutical compositions/formulations and kits of the invention is the preferred route, being the most convenient.

For veterinary use, the agents, medicaments, compounds, pharmaceutical compositions/formulations, and kits of the invention are administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

The present invention also includes pharmaceutical compositions/formulations comprising pharmaceutically acceptable acid or base addition salts of the polypeptide binding moieties of the present invention. The acids which are used to prepare the pharmaceutically acceptable acid addition salts of the aforementioned base compounds useful in this invention are those which form non-toxic acid addition salts, *i.e.* salts containing pharmacologically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulphate, bisulphate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, fumarate,

gluconate, saccharate, benzoate, methanesulphonate, ethanesulphonate, benzenesulphonate, *p*-toluenesulphonate and pamoate [*i.e.* 1,1'-methylene-bis-(2-hydroxy-3 naphthoate)] salts, among others.

Pharmaceutically acceptable base addition salts may also be used to produce pharmaceutically acceptable salt forms of the agents (*i.e.* antibody or antigen-binding fragment thereof) according to the present invention.

The chemical bases that may be used as reagents to prepare pharmaceutically acceptable base salts of the present agents that are acidic in nature are those that form non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to those derived from such pharmacologically acceptable cations such as alkali metal cations (*e.g.* potassium and sodium) and alkaline earth metal cations (*e.g.* calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines, among others.

The agents and/or polypeptide binding moieties of the invention may be lyophilised for storage and reconstituted in a suitable carrier prior to use. Any suitable lyophilisation method (*e.g.* spray drying, cake drying) and/or reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilisation and reconstitution can lead to varying degrees of antibody activity loss (*e.g.* with conventional immunoglobulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted upward to compensate. In one embodiment, the lyophilised (freeze dried) polypeptide binding moiety loses no more than about 20%, or no more than about 25%, or no more than about 30%, or no more than about 35%, or no more than about 40%, or no more than about 45%, or no more than about 50% of its activity (prior to lyophilisation) when re-hydrated.

Preferably, the invention provides an antibody, compound, pharmaceutical composition/formulation, kit, use or method wherein the effective amount of the antibody or antigen-binding fragment thereof is between about 0.0001 mg/kg to 50 mg/kg of the antibody or antigen-binding fragment thereof.

As is appreciated by those skilled in the art, the precise amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired

therapeutic effect in association with the required diluent. In the methods and use for manufacture of compositions of the invention, a therapeutically effective amount of the active component is provided. A therapeutically effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.*, as is well known in the art.

A further aspect of the invention provides an *in vitro* method of reducing or inhibiting TG2 enzyme activity, the method comprising administering an antibody or antigen-binding fragment thereof according to any aspect of the invention, or a polynucleotide according to the invention, or a compound or kit of the invention, to a sample comprising TG2.

The "sample" may be any sample obtained from an appropriate source, for example a mammalian source. For example, the sample may be a tissue or cell sample comprising TG2. Exemplary tissues include tissue obtained from a patient's brain, gastro-intestinal tract, lung, pancreas, liver, skin, kidney, eye, heart, blood vessels, lymph nodes, spine, and skeletal or smooth muscle.

The invention also provides a method of reducing or inhibiting TG2 enzyme activity in an individual in need thereof, the method comprising administering an effective amount of a polynucleotide encoding an antibody or antigen-binding fragment thereof according to any aspect of the invention, to the individual.

A further aspect provides the use of a polynucleotide encoding an antibody or antigen-binding fragment thereof according to any aspect of the invention, in the manufacture of a medicament for reducing or inhibiting TG2 enzyme activity in an individual in need thereof.

The invention also provides an *in vitro* method of reducing or inhibiting TG2 enzyme activity, the method comprising administering a polynucleotide encoding an antibody or antigen-binding fragment thereof according to any aspect of the invention, to a sample comprising TG2, for example a tissue or cell sample comprising TG2.

In a yet further aspect, the invention provides a method of producing an antibody or antigen-binding fragment according to the second aspect of the invention, or a compound of the invention comprising an antibody or antigen-binding fragment according

to the second aspect of the invention, the method comprising expressing a polynucleotide of the invention, or culturing a stable host cell line of the invention.

In a further aspect, the invention provides a method of selecting an antibody or antigen-binding fragment thereof that selectively binds a transglutaminase protein, the method comprising the step of selecting an antibody or antigen-binding fragment thereof that selectively binds a polypeptide comprising a transglutaminase core region/catalytic domain but not comprising a transglutaminase barrel or sandwich domain.

In a further aspect, the invention provides a method of selecting an antibody or antigen-binding fragment thereof according to the first or second aspects of the invention, or a compound of the invention comprising an antibody or antigen-binding fragment according to the first or second aspect of the invention, the method comprising the step of selecting an antibody or antigen-binding fragment thereof that selectively binds a polypeptide sequence consisting of the polypeptide sequence of amino acids 143 to 473 of human TG2 or a fragment thereof.

In an embodiment, the method may be carried out using antibody phage display. It is preferred that the antibody or antigen-binding fragment thereof is an inhibitory antibody that inhibits catalytic activity of the transglutaminase protein.

In a further aspect, the invention provides a method of producing an antibody or antigen-binding fragment thereof that selectively binds a transglutaminase protein, the method comprising administering to a non-human animal a compound comprising:

i) a polypeptide comprising a transglutaminase core region/catalytic domain but not comprising a transglutaminase barrel or sandwich domain, or a fragment thereof; and, optionally,

ii) an adjuvant.

It is envisaged that the polypeptide comprising a transglutaminase core region/catalytic domain but not comprising a transglutaminase barrel or sandwich domain will comprise the catalytic triad described above, and optionally, also the GTP binding site of the transglutaminase protein.

In an embodiment, the method may further comprise the step of selecting an antibody or antigen-binding fragment thereof on the basis of its selective binding to a transglutaminase protein.

In a further aspect, the invention provides a method of selecting an antibody or antigen-binding fragment thereof that selectively binds a transglutaminase protein, the method comprising the step of selecting an antibody or antigen-binding fragment thereof that selectively binds a polypeptide sequence consisting of the polypeptide sequence of amino acids 143 to 473 of human TG2 or a fragment thereof.

In a yet further aspect, the invention provides a method of producing an antibody or antigen-binding fragment according to any aspect of the invention comprising administering to a non-human animal a compound comprising:

i) a polypeptide sequence consisting of the polypeptide sequence of amino acids 143 to 473 of human TG2 or a fragment thereof; and optionally,

ii) an adjuvant.

In an embodiment, the method may further comprise the step of selecting an antibody or antigen-binding fragment thereof on the basis of its selective binding to TG2, for example human TG2.

In a further aspect, the invention provides an antibody or antigen-binding fragment thereof obtainable by any of the preceding methods of producing or selecting an antibody or antigen-binding fragment thereof.

By "adjuvant" we include any a pharmacological or immunological agent that enhances the recipient's immune response to the polypeptide of the invention. Immunologic adjuvants are added to vaccines to stimulate the immune system's response to the target antigen, but do not in themselves confer immunity. Examples of adjuvants include oil emulsions, inorganic compounds such as aluminium salts, for example aluminum hydroxide or aluminium phosphate, organic compounds such as Squalene, virosomes, or any other suitable compound or compounds as would be understood by a person of skill in the art.

In a further aspect, the invention provides an isolated polypeptide consisting of:

i) the polypeptide sequence of amino acids 143 to 473 of human TG2;

ii) the polypeptide sequence of amino acids 304 to 326 of human TG2;

iii) the polypeptide sequence of amino acids 351 to 365 of human TG2;

iv) the polypeptide sequence of amino acids 450 to 467 of human TG2; or

a fragment, derivative or polypeptide mimic thereof.

The invention also provides an isolated polynucleotide encoding the polypeptide of the immediately preceding aspect.

The invention provides an antibody or antigen-binding fragment thereof for use in treating a condition associated with TG2 activity substantially as described herein with reference to the description and figures.

The invention also provides the use of an antibody or antigen-binding fragment thereof substantially as described herein with reference to the description and figures.

As used herein, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes a plurality of such antibodies and reference to "the dosage" includes reference to one or more dosages and equivalents thereof known to those skilled in the art, and so forth.

All documents referred to herein are hereby incorporated by reference.

The invention is now described in more detail by reference to the following, non-limiting, Figures and Examples.

Figure 1: Generation of a human TG2 recombinant protein

A: The TG2 catalytic core cDNA was generated by PCR from the pClineo-hTG2 vector and inserted into the pET 21a plasmid. Following amplification in E.Coli this was digested with Nhe I and Hind III to release the TG2 core cDNA and run on a 1% Agarose gel (lane 3). Bands were sized by reference to a 100 bp ladder (lane 1) and λ DNA molecular weight marker (Lane 2).

B: The pET21a TG2 core vector was used to transform *E. coli* strain BL21-CodonPlus(DE3)-RIPL. Expression was induced using IPTG for 4 hours. TG2 core protein formed insoluble bodies that were recovered from lysates by centrifugation. These were re solubilised, and the 37 kDa His tagged TG2 core purified on a nickel column. 10 ng was separated by SDS-PAGE, western blotted and probed with CUB7402 anti TG2 antibody (lane 2) with reference to a precision plus molecular weight marker (lane 1).

Figure 2: Immunological Response in mice to rhTG2 core protein

A: Test bleeds were taken from 4 catalytic core immunised mice at day 45 after the first immunisation and 10 days after the second boost. Serum was serially diluted and reactivity checked by ELISA against immobilised TG2 core protein.

B Reactivity was further checked by screened against human rh TG2 and rh TG2 catalytic core domain. 20, 40, 80 ng of protein was fractionated by SDS PAGE and western blotted onto a PVDF membrane. This was immunoprobed with a 1:1000 dilution of serum. Antibody binding was revealed using anti-mouse γ-chain specific HRP. For size reference to a precision plus molecular weight marker was used.

Figure 3: Hybridoma Reactivity Against TG family Members

A: ELISA were carried out using plates coated with recombinant TGs (100 ng /well) to determine TG type specificity in 109 hybridoma supernatants that showed good reactivity to TG2. Antibody binding was revealed using anti-mouse γ-chain specific HRP. A random selection of those screened is shown including EF4, CG9 & FD8 that showed cross reactivity.

B: Nine selected hybridomas were double cloned. IgG was purified and tested for reactivity at 0.1ug/ml against recombinant human TG1, TG2, TG3, TG7 and Factor XIIIa using ELISA with plates coated with 100ng of each TG. Data represents mean OD value from 3 separate ELISA ±SEM.

Factor XIIIa is denoted on graphs as TG13.

Figure 4: Identification of Hybridoma with inhibitory activity against TG2

Conditioned media from 32 hybridoma wells with specificity to TG2 were screened for their effects on 100ng of rhTG2 activity using the ^{3}H putrescine incorporation assay. The chemical pan TG2 inhibitor 1,3-Dimethyl-2-[(2-oxo-propyl)thio]imidazolium chloride was used as a positive control for inhibition. RPMI (unconditioned medium) was used a negative control. 500 ng of a TG2 inhibitory antibody piloted by Quark biotechnology was included for comparison. Data represents mean CPM incorporated in 30 mins from at least three experiments done in duplicate ±SEM. Bars shown in grey show significant TG2 inhibition ($p<0.05$).

Figure 5: Mapping of Inhibitory Antibody Epitopes.

Each inhibitory monoclonal antibody was bound to an ELISA plate and panned against a human TG2 phage library. Phage binding to the antibody were rescued, amplified and subjected to 4 further rounds of panning. TG2 library fragments in the phage were then sequenced and overlapping sequences used to determine the epitope for each antibody. Common sequences between antibodies were then used to determine a consensus

sequence for a particular inhibitory epitope and antibodies grouped accordingly. 3 inhibitory epitopes were identified.

Figure 6: Structural location of Inhibitory Epitopes with the TG2 catalytic Core

The TG2 amino acid sequence was entered into Pymol and a 3D graphical representation of the structure generated in its open, Ca^{2+} activated state with putative calcium binding sites (turquoise) and the catalytic triad (grey) shown for reference (left panel). The consensus inhibitory epitopes were then added in blue (Antibody group 1 – AB1 site), red (antibody group 2 – DF4 site) and yellow (antibody group 3, DD9 site).

Figure 7: VL Sequence of Inhibitory antibodies

RNA from each inhibitory hybridoma was extracted, reverse transcribed and amplified by PCR using a degenerate FR1 primers, MH1 and MH2 primers and 3 constant region primers to amplify VH genes. The resulting VH and VK sequences are shown for AB1.

Figure 8. Efficacy of AB1 to inhibit TG2 activity in a cell homogenate

A: Hep2G cells were lysed and 45 ug of protein mixed with 750 ng of IgG from AB1, DH2, DD9, BB7, DC1 and EH6 for 20 minutes. This was subsequently assayed using the ^3H Putrescine incorporation TG activity assay with sampling over 1 hour. The rate of reaction was calculated and expressed as a percentage of the same lysate incubated with a random antibody (MAB002). Data represents the mean percentage inhibition ± SEM from 2 separate experiments done in duplicate. * $p<0.05$

B: HepG2 cells were exposed to increasing glucose concentrations for 96 hours to up regulate TG2 expression. Cells were harvested, lysed and 25 ug of lysate fractionated by SDS-PAGE, western blotted and then immunoprobed with a 1ng/ml solution of AB1 IgG using a chemiluminesant end point.

Figure 9. (Table 1): Comparative IC50 values for TG2 inhibitory antibodies

To determine an IC50 value for each antibody against human, rat and mouse the ^3H Putrescine assay was used. 100 ng of human TG2 or 25 ng of mouse and rat TG2 was used to generate a reaction where approximately 3000 cpm of Putrescine were incorporated per hour in 10ul of the reaction mixture. Serial dilutions of each antibody were then applied starting from adding 500ng (5ug/ml final concentration) to the reaction mixture and incubated with the TG2 for 20 minutes prior to activating the reaction. IC50 values were calculated by determining the concentration at which the enzymatic rate of reaction was reduced by 50% using an appropriate curve fit in graphpad prism. Values

are expressed as the amount of IgG in mg/ml in the reaction that would inhibit 1 ng of TG2.

Figure 10. Extracellular TG activity in HK2 cells in response to TG2 inhibition.

HK2 cells were plated onto fibronectin and incubated for 2 hours in the presence of 0.1M biotin cadaverine with either 4ng/µl of human anti-TG2 antibody (AB1) **(part A)**, 4ng/µl of human anti-TG2 antibody (DC1) **(part B)** or 400µM of the site-specific pan TG inhibitor 1,3-Dimethyl-2-[(2-oxo-propyl)thio]imidazolium chloride. Extracellular TG activity was measured by the incorporation of biotin cadaverine into fibronectin with incorporation revealed using extravadin-HRP and a TMB substrate. Changes in optical density were measured at 450nm in a 96 well plate reader. Data represents mean OD at 450nm corrected to 1 mg of cell protein. n = 6 wells per experimental group.

Figure 11. Comparison of TG2 inhibition by antibody AB1 to a fab fragment of Quark's TG2 inhibitory antibody using a ^{3}H putrescine incorporation assay

100ng of hTG2 was assayed for TG2 activity based on the incorporation of ^{3}H Putrescine into dimethylcasein over a 60 minute period with the addition of either 1µg of a fab fragment of an antibody described by Quark in WO2006/100679 and synthesised at Sheffield University or 500ng of AB1. Data represents mean TG activity as incorporation of ^{3}H putrescine (CPM) ± SEM from 3 independent experiments done in duplicate.

Figure 12. Percentage comparison of TG2 inhibition by antibody AB1 with a fab fragment of Quark's TG2 inhibitory antibody using a ^{3}H putrescine incorporation assay

Data from Figure 11 is alternatively expressed as a percentage of TG activity at each time point to display the relative comparative knockdown of TG2 activity by application of AB1 and the Quark antibody fab fragment.

Figure 13. Comparison of TG2 inhibition by antibody AB1 to a recombinant rat IgG of Quark's TG2 inhibitory antibody using a ^{3}H putrescine incorporation assay

100ng of hTG2 was assayed for TG2 activity based on the incorporation of ^{3}H Putrescine into dimethylcasein over a 60 minute period with the addition of either 500 ng of a recombinant rat version of a TG2 inhibitory antibody described by Quark in WO2006/100679 and synthesised at Medical Research Council Technology or 500ng of AB1. Data represents mean TG activity as incorporation of ^{3}H putrescine (CPM) ± SEM from 3 independent experiments done in duplicate.

Figure 14. Percentage comparison of TG2 inhibition by antibody AB1 to a recombinant rat IgG of Quark's TG2 inhibitory antibody using a ^{3}H putrescine incorporation assay

Data from Figure 13 is alternatively expressed as a percentage of TG activity at each time point to display the relative comparative knockdown of TG2 activity by application of AB1 and the Quark recombinant rat IgG.

Figure 15. Effect of AB1 on ECM levels in HK2 cells

Mature collagen levels in HK-2 cells were measured by the incorporation of ^{3}H proline into the ECM over a 76 hour period either with or without the addition of TG2 inhibitory antibody AB1. Data represents the incorporation of ^{3}H proline per mg of cellular protein expressed as a percentage of the mean level in untreated cells ± SEM. n=2.

Figure 16. Binding ELISA of humanised versions of antibodies.

Supernatants from HEK293F cells co-transfected with different combinations of humanised light chains and heavy chain vectors were assayed in an anti-human IgG ELISA to determine concentration and in an anti huTG2 ELISA. Each supernatant was assayed in triplicate and IC_{50}'s determined. The most potent combination was selected for further studies and as the candidate humanised antibody.

Figure 17. Testing MRC Quark CTD190 on human Tg2 by ELISA.

96 well plates were plated with hTG2 (1µg/ml) in carbonate buffer overnight and ELISA detection performed using 100ng/ml primary antibody. Detection was performed using anti-mouse IgG (SIGMA 3673) for CUB and anti-rat IgG (SIGMA A5795) for the Quark (both 1:5000). The Quark antibody made by MRC T reacts with human TG2.

Figure 18: RNA from the AB1 hybridoma was extracted, reverse transcribed and amplified by PCR using the degenerate signal sequence primer MHV4 with heavy chain constant region primer MHCG1, or using the degenerate signal sequence primer MKV4 with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 19: RNA from the BB7 hybridoma was extracted, reverse transcribed and amplified by PCR using the degenerate signal sequence primer MHV4 with heavy chain constant region primer MHCG1, or using the degenerate signal sequence primer MKV4 with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 20: RNA from the DC1 hybridoma was extracted, reverse transcribed and amplified by PCR using the degenerate signal sequence primer MHV4 with heavy chain constant region primer MHCG1, or using the degenerate signal sequence primer MKV4 with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 21: RNA from the JE12 hybridoma was extracted, reverse transcribed and amplified by PCR using a 5' RACE PCR with heavy chain constant region primer MHCG1, or using the signal sequence primer MKV1 with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 22: RNA from the EH6 hybridoma was extracted, reverse transcribed and amplified by PCR using a 5' RACE PCR with heavy chain constant region primer MHCG2B, or using the signal sequence primer MKV with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 23: RNA from the AG9 hybridoma was extracted, reverse transcribed and amplified by PCR using the degenerate signal sequence primer MHV7 with heavy chain constant region primer MHCG1, or using a mix of degenerate signal sequence primers MKV1 - 11 with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 24: RNA from the AH3 hybridoma was extracted, reverse transcribed and amplified by PCR using the degenerate signal sequence primer MHV7 with heavy chain constant region primer MHCG2B, or using the signal sequence primer MKV1 with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 25: RNA from the DD9 hybridoma was extracted, reverse transcribed and amplified by PCR using a 5' RACE PCR with heavy chain constant region primer MHCG2A, or using the degenerate signal sequence primer MKV5 with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 26: RNA from the DH2 hybridoma was extracted, reverse transcribed and amplified by PCR using a 5' RACE PCR with heavy chain constant region primer

MHCG2B, or using the degenerate signal sequence primer MKV45with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 27: RNA from the DD6 hybridoma was extracted, reverse transcribed and amplified by PCR using a 5' RACE PCR with heavy chain constant region primer MHCG2B, or using a 5' RACE PCR with a lambda light chain constant region primer MLC. The resulting VH and VL sequences are shown.

Figure 28: RNA from the IA12 hybridoma was extracted, reverse transcribed and amplified by PCR using the degenerate signal sequence primer MHV9 with heavy chain constant region primer MHCG1, or using the degenerate signal sequence primer CL14 with a kappa light chain constant region primer MKC. The resulting VH and VK sequences are shown.

Figure 29. Dose response curves and IC50 values for enzymatic inhibition of recombinant human TG2 by chimeric anti-TG2 antibodies, (a) cAB003 , (b) cBB001, (c) cDC001, (d) cDD9001, (e) cDH001 and (f) the commercial TG2 antibody CUB7402. IC50 values are mean of 3 independent experiments.

Figure 30. Dose response curves and IC50 values for enzymatic inhibition of recombinant cynomogulus monkey TG2 by chimeric anti-TG2 antibodies (a) cDC001 and (b) the commercial TG2 antibody CUB7402.

Figure 31. Dose response curves and IC50 values for enzymatic inhibition of recombinant human TG2 by humanized anti-TG2 antibodies, (a) hBB001AA, (b) hBB001BB, (c) hAB005 and (d) hAB004.

Figure 32. Dose response curves and IC50 values for enzymatic inhibition of recombinant cynomogulus monkey TG2 by humanized anti-TG2 antibodies (a) hBB01AA and (b) hAB004.

Figure 33. Dose response curves and IC50 values for enzymatic inhibition of recombinant human TG2 by murine monoclonal anti-TG2 antibodies, (a) mAB003, (b) mBB001, (c) mDC001, (d) mDD9001, (e) mDH001 and (f) mDD6001.

Fig 34 – Binding ELISA of humanised versions of AB1 antibodies.

Supernatants from HEK293F cells co-transfected with different combinations of humanised AB1 light chains and AB1 heavy chain vectors were assayed in an anti-human IgG ELISA to determine concentration and in an anti huTG2 ELISA. Each supernatant was assayed in triplicate and IC_{50}'s determined. The most potent combination was selected for further studies and as the candidate humanised antibody.

Figure 35. Dose response ELISA binding curves and EC50 data for antibodies binding to human TG2 (a) chimeric antibodies cDD9001, cDH001, cDC001, commercial TG2 antibody CUB7402 and isotype-matched control, (b) chimeric antibody cBB001 and isotype-matched control and (c) chimeric antibody cAB003 and isotope-matched control.

Figure 36. Dose response ELISA binding curves and EC50 data for antibodies binding to cynomogulus monkey TG2 (a) chimeric antibodies cDD9001, cDH001, cDC001, commercial TG2 antibody CUB7402 and isotype-matched control, (b) chimeric antibody cBB001 and isotype-matched control and (c) chimeric antibody cAB003 and isotope-matched control.

Figure 37. Dose response ELISA binding curves and EC50 data for antibodies binding to human TG2 (a) humanized antibodies hBB001AA, HBB001BB, commercial TG2 antibody CUB7402 and isotype-matched control and (b) humanized antibody hAB004.

Figure 38. Dose response ELISA binding curves and EC50 data for antibodies binding to cynomogulus monkey TG2 (a) humanized antibodies hBB001AA, HBB001BB, commercial TG2 antibody CUB7402 and isotype-matched control and (b) humanized antibody hAB004 and isotope-matched control.

Figure 39: Humanised AB1 binding activity with extracellular TG2 Inhibition of Extracellular TG2 activity produced by HK2 cells was assayed using an ELISA measuring the incorporation of biotin cadaverine into fibronectin. An exemplar curve showing the inhibition of TG2 activity by humanised AB1 (hAB005) and the IC obtained is shown.

Figure 40: Humanised BB7 binding activity with extracellular TG2.
Inhibition of Extracellular TG2 activity produced by HK2 cells was assayed using an ELISA measuring the incorporation of biotin cadaverine into fibronectin. An exemplar

curve showing the inhibition of TG2 activity by versions of humanised BB7 (hBB001AA and hBB001BB) and the ICs obtained is shown.

Figure 41: Cytochalasin D, R281 and ZDON control screatch assay results and commercial antibody CUB7402 scratch assay results.

Scratch wound assays were performed using WI-38 cell, after plating and overnight growth, cells were washed in media without serum and a scratch wound generated using an Essen Wound Maker. Media was removed and replaces with 95 ul/well serum free media containing controls and test antibodies. The plate was placed in an Essen Incycte and the closure of the wound analysed using Incucyte software. Relative wound density was plotted against time for the controls cytochalasin D, R281 and Z –Don (panel A) and the commercial antibody CUB7402 and cytochalasin (panel B).

Figure 42: Humanised BB7 scratch assay results.

Scratch wound assays were performed using WI-38 cell, after plating and overnight growth, cells were washed in media without serum and a scratch wound generated using an Essen Wound Maker. Media was removed and replaces with 95 ul/well serum free media containing controls and test antibodies. The plate was placed in an Essen Incycte and the closure of the wound analysed using Incucyte software. Relative wound density was plotted against time for the humanised hBB001 AA and the control cytochalasin D (panel A) and hBB001BB and the control cytochalasin D (panel B).

Figure 43: Humanised AB1 scratch assay results.

Scratch wound assays were performed using WI-38 cell, after plating and overnight growth, cells were washed in media without serum and a scratch wound generated using an Essen Wound Maker. Media was removed and replaces with 95 ul/well serum free media containing controls and test antibodies. The plate was placed in an Essen Incycte and the closure of the wound analysed using Incucyte software. Relative wound density was plotted against time for the humanised hAB005 and the control cytochalasin D

Figure 44: Chimeric DC1 scratch assay results.

Scratch wound assays were performed using WI-38 cell, after plating and overnight growth, cells were washed in media without serum and a scratch wound generated using an Essen Wound Maker. Media was removed and replaces with 95 ul/well serum free media containing controls and test antibodies. The plate was placed in an Essen Incycte and the closure of the wound analysed using Incucyte software. Relative wound density

was plotted against time for the chimeric antibody cDC001 and the control cytochalasin D

Figure 45: Human TG2 binding to cAB003 immobilised antibody by Biacore. The association phases of human TG2 injections over the cAB003-coated biosensor at 25, 50, 100 and 200nM, including in duplicate at 50nM, are shown on the left. From the same experiment, two long dissociation phases were collected, as shown on the right. Fits are shown as solid black lines and the results are shown in Table 25.

Figure 46: Cynomolgus monkey TG2 binding to hAB004 immobilised antibody by Biacore. The association phases of cynomolgus monkey TG2 injections over the hAB004-coated biosensor at 25, 50, 100, 200 and 400nM, including in duplicate at 50nM, are shown on the left. From the same experiment, two long dissociation phases were collected, as shown on the right. Fits are shown as solid black lines and the results are shown in Table 26.

Figure 47: Human TG2 binding to cDH001 immobilised antibody by Biacore in the absence of calcium. The association phases of human TG2 injections over the cDH001-coated biosensor at 25, 50, 100 and 200nM, including in duplicate at 50nM, are shown on the left. From the same experiment, two long dissociation phases were collected, as shown on the right. Fits are shown as solid black lines and the results are shown in Table 25.

Example 1: Developing a TG2 inhibitory antibody suitable for therapeutic use in man with the identification of 3 specific inhibitory epitopes.

Transglutaminase type 2 (TG2) catalyses the formation of an ε-(γ-glutamyl)-lysine isopeptide bond between adjacent peptides or proteins including those of the extracellular matrix (ECM). Elevated extracellular TG2 leads to accelerated ECM deposition and reduced clearance that underlies tissue scarring and fibrosis. It also is linked to celiac disease, neurodegenerative disorders and some cancers. While numerous compounds have been developed that inhibit transglutaminases, none of these are specific to TG2, inhibiting all transglutaminases to some extent. While these have allowed proof of concept studies for TG2's role in these pathologies, the lack of isoform specificity has prevented their application in man. To address this, we set out to develop a high affinity TG2 specific antibody that would inhibit only TG2 activity.

A recombinant protein encompassing amino acids 143 to 473 of the human TG2 core was produced in *Escherichia coli*, re-folded and 100 µg injected into 4 mice with boosts at 2, 5, 7, and 10 weeks. Spleens were recovered 4 days after the final boost and splenocytes fused to Sp2/0-Ag-14 myeloma cells. Seventy-five hybridoma supernatants showed specificity to TG2. These hybridoma supernatants were screened for their ability to inhibit TG2 activity in a putrescine incorporation assay containing 100 µg of TG2. Ten TG2 specific supernatants were inhibitory. These were subsequently double cloned. Using phage display to screen a TG2 fragment library, each antibody was mapped to a precise epitope in the TG2 core domain and 3 distinct inhibitory epitopes determined. The amount of antibody to reduce the activity from 100 ng of TG2 by 50 % was determined.

The 2 most effective antibodies, AB1 and DC1 bound to amino acids 304 to 327 and had an IC_{50} of 1.1×10^{-5} mg/ml IgG per ng of recombinant TG2. Application of AB1 & DC1 was able to inhibit TG2 successfully in human Hep2G cells and extracellular TG2 in human HK-2 cells when applied to the culture media.

Thus, immunisation of mice with the TG2 core domain surprisingly enabled the generation of monoclonal antibodies that target previously unreported epitopes within the catalytic core. These antibodies are specific, inhibit TG2 activity effectively and are suitable for *in vivo* application.

Materials and Methods

Transglutaminase 2 catalytic core domain production

The catalytic core domain of human TG2 (residues Cys143 – Met 473 of TG2) was expressed, refolded and purified to permit immunisation in mice. The catalytic core domain (PCR sense primer GCG CGC GCT AGC TGC CCA GCG GAT GCT GTG TAC CTG GAC, anti-sense GCG CGC AAG CTT CAT CCC TGT CTC CTC CTT CTC GGC CAG) was cloned into the expression vector pET21a(+) and expressed as insoluble inclusion bodies in *E. coli* strain BL21-CodonPlus(DE3)-RIPL (Agilent Technologies). In brief, 50 µl of competent BL21 (DE3) pLysS cells were transformed with 1 µl of the expression plasmid (30 ng/µl) and plated onto LB agar plates containing the selective antibiotics (100 µg/ml ampicillin, 34 µg/ml chloramphenicol) and 1 % glucose and incubated overnight at 37°C. A single colony was picked to seed 10 ml of fresh LB medium containing 100 µg/ml ampicillin, 34 µg/ml chloramphenicol and 1 % glucose in shaking incubator at 37 °C and at 200 rpm. After overnight growth, cultures were

transferred in 100 ml 2xYT media with 1% glucose and grown to an OD_{600nm} of 0.8 and then transferred to 1L 2xYT medium until the OD_{600nm} reached 0.8 again.After 4 hours induction under 1mM IPTG to stimulate expression, pelleted and bacteria were lysed by sonication in buffer A (10 mM Tris; 1 mM EDTA; 10 mM DTT; 1 mM PMSF; 0.5 mg/ml lysozyme protease inhibitor tablets (Roche), pH 8.0). Inclusion bodies were harvested by centrifugation at 40,000 x g and washed three times in wash buffer B (50 mM Tris; 1 mM EDTA; 10 mM DTT; 2% sodium deoxycholate, pH 8.0) before a final wash in deionised water.

Inclusion bodies were solubilised in 3.5mls of resolubilisation buffer (40 mM Tris-HCl, 8 M urea, and 10 mM DTT pH12) and refolded over a period of 16 hours in refolding buffer (40 mM Tris HCl; 150 mM NaCl; 20% glycerol; 5 mM cysteine;0.5 mM cystine pH 8) at 4°C in the dark.

The resolubilised inclusion bodies were loaded onto a 1ml Nickel column. Briefly, the column was pre- equilibrated with binding buffer (40 mM Tris; 300 mM NaCl; 10 mM Imidazole) and the inclusion bodies applied. The column was extensively washed (40 mM Tris; 300 mM NaCl; 30 mM imidazole). The recombinant protein was eluted by high concentration imidazole buffer (40 mM Tris; 300 mM NaCl; 300 mM imidazole). Eluted protein containing fractions were pooled and dialysed overnight against an appropriate buffer (40 mM Tris; 300 mM NaCl pH 8). Protein was assessed using the Bradford protein assay

HepG2 cell culture & lysates

HepG2 cells were kindly supplied by Richard Ross (University of Sheffield). Cells were routinely grown at 37°C in a 95 % humidified atmosphere of 5 % CO_2 in DMEM/4.5g per litre glucose supplemented with 10 % foetal calf serum (FCS), 100 IU penicillin and 100 µg/ml streptomycin, 2 mM l-glutamine (all GIBCO). Two million cells were seeded on a 10cm dishes and grown for 48 hours. Cells were lysed in 250 µl of STE buffer (0.32M sucrose, 5mM Tris, 1 mM EDTA containing protease inhibitors Phenylmethylsulphonyl fluoride (1 mM), benzamidine (5 mM), and leupeptin (10 µg/ml) and sonicated on ice to produce a cell lysate usable in TG2 activity assay.

Human Kidney 2 (HK2) cells:

HK-2 cells (kidney proximal tubular epithelium) were purchased from the European cell culture collection at passage 3. Cells were routinely grown at 37°C in a 95 % humidified atmosphere of 5 % CO_2 in keratinocyte serum free medium (KSFM, Gibco 17005-042)

with L-glutamine supplemented with recombinant EGF (0.1-0.2ng/ml) and bovine pituitary extract (20-30ug/ml). For passage, media was removed and washed once with 1 x PBS before trypsinising with 1ml of 0.25% trypsin/EDTA (T75 flask) for 1 minute at 37°C. Cells were resuspended in 10 mls of KSFM and centrifuged at 400g for 1 minute. Media was removed and cells plated in KSFM (1:3 to 1:5 split is normal). Cells were used experimentally at passages 5-14. Cells typically grew well to 95% confluence.

Coomassie staining and western blotting

The purity of recombinant proteins was checked by running 5 µg of the recovered protein on a 10% (w/v) polyacrylamide denaturing gel and staining with Coomassie Brilliant Blue R staining solution (Sigma).

Confirmation of TG2 core protein synthesis as well as TG2 and TG2 core reactivity levels following immunisation were all measured by western blotting. Recombinant proteins (10 to 80 ng) were loaded on a 10 % (w/v) polyacrylamide denaturing or non-denaturing gel as required and transferred onto PVDF membranes (Transblot SD, Biorad, UK) for one hour at 100 V. Membranes were blocked overnight at 4°C with 3 % (w/v) BSA in TBS/0.1% (v/v) Tween 20. The membranes were then washed and probed with monoclonal mouse anti-transglutaminase antibodies in TBS/Tween containing 1 % BSA. For proof of recombinant TG2 core protein and as a positive control for antibody screening the commercial antibody Cub7402 (neomarkers) was used at a 1:1000 dilution. Binding of primary antibody was detected with the anti-mouse gamma-chain-HRP linked secondary antibody (Sigma, Poole, UK). Bands were visualised using ECL chemiluminescent detection system (Amersham, UK).

Mouse immunisation and fusion

Each mouse was immunised with a mixture of 50 µg of antigen (made up to a volume of 50 µl with sterile PBS) and 50 µl of complete Freund's adjuvant. Four (8-12 week old) BALB/C mice were injected. Two boost immunisations were carried out (day 14 and day 35) using the same procedure with the exception that incomplete Freund's adjuvant was used for these injections. At day 45, test bleeds were taken from all animals and assessed for reactivity to TG2 by ELISA.

The two best responders were further boosted by injection of 100 µg of core protein (in PBS) again mixed with incomplete Freuds Adjuvant at 10 weeks, and 4 days later the animals were sacrificed for splenocyte recovery and fusion with Sp2/0-Ag-14 myeloma

cells. From this fusion, approximately 1000 wells were screened for reactivity to TG2 protein by ELISA.

Screening for TG2 specificity

Conditioned medium or purified IgG were tested for reactivity to transglutaminase family members. The ability of each to bind to each transglutaminase (TG1, TG2, TG3, TG5, TG7 and Factor XIIIa; all Zedira) was determined using a plate binding assay. Microtiter plates (Costar, Cambridge, UK) were coated with recombinant TG (Zedira, Darmstadt, Germany) in 50 µl of 0.1 M bicarbonate/carbonate buffer (pH 9.6) overnight at 4°C. Plates were blocked for 2 h at 37°C with 200 µl PBS containing 3 % w/v BSA. Plates were washed three times with PBS containing 0.05 % Tween 20 (washing buffer) and 100 µl of diluted conditioned medium (dilution 1:5 to 1:20) or purified anti-TG2 catalytic core mAbs was added. Plates were incubated for a further 1 h at room temperature. The washing step was repeated and anti-mouse gamma chain-horseradish peroxidase (1:5000) in PBS containing 0.05 % Tween 20 (v:v) and 1 % BSA (w:v) (Sigma, Poole UK) was added for 1 h. After eight washes, binding was revealed with 50 µl of 3,3',5,5'-tetramethylbenzidine substrate. The reaction was stopped by adding 25 µl of 0.1 M H_2SO_4 and the absorbance at 450 nm was determined.

Screening for TG2 inhibition

TG activity is measured by the Ca^{2+} dependent incorporation of ^{3}H-putrescine into N',N'-dimethylcasein. Recombinant human TG2 (100 ng) was pre-incubated for twenty minutes at room temperature with the test sample (conditioned medium or purified IgG) before starting the reaction. Twenty-five µl of reaction mix (5 µl of 25 mM $CaCl_2$, 5 µl of 40 mM dithiothreitol, 5 µl ^{3}H-putrescine mix, and 10 µl 25 mg/ml of N,N' dimethylcasein (replace 25 mM $CaCl_2$ with 100 mM EDTA for a non-enzymatic control) was added to start the reaction and the samples incubated at 37°C for up to 1 hour. Aliquots of 10 µl were spotted onto a strip of 3 MM Whatman filter paper and plunged immediately into ice-cold 10 % trichloroacetic acid (TCA) in order to precipitate the cross-linked proteins typically at time 0, 10, 30 and 60 minutes into the reaction. After three extensive washes in ice-cold 5 % TCA followed by 3 rinses with ice-cold 95 % ethanol, the air dried filter was counted in 2 ml of scintillation fluid (Ultima Gold Packard, Perkin Elmer). The rate of reaction was calculated. 1 TG unit is equivalent to the incorporation of 1 nmol of putrescine per hour at 37°C.

The same protocol was used to assess TG inhibition in cell lysates by replacing the 25 µl of recombinant protein with 25 µl of cell lysate.

Hybridoma cloning & purification of antibodies from conditioned medium

Monoclonal antibody isolation was undertaken from the cloned inhibitory hybridomas. Initially identified hybridoma wells were doubly cloned by a limiting dilution process (to ensure stability and clonality) according to conventional methods (Loirat MJ et al, 1992) with sub-clones tested as described by ELISA and activity screens. Selected antibody producing clones were expanded in 25 and 75 cm^2 flasks and fed with serum free medium (Hyclone, Fisher Scientific, Loughborough, UK). As cells were expanded, conditioned medium was collected for IgG purification using affinity chromatography on protein G column (Amersham Life Sciences). The conditioned medium was diluted in an equal volume of 10 mM sodium phosphate, pH 7.25, and applied to the protein G column at a flow rate of 1.0 to 2.0 ml/min. The column was extensively washed with 10 column volumes of the same buffer. Bound antibody was eluted in glycine solution (0.1M; pH 2.7) and neutralised by 0.15% volumes of 1M Tris / HCl pH 9. Samples were dialysed against 1000 volumes of phosphate buffer saline solution for 24 hours with 2 buffer changes.

Phage display mapping of antibody epitopes

The full length coding sequence of human TG2 was amplified by polymerase chain reaction using the following primers; TG2-FL-1 5' ATGGCCGAGGAGCTGGTCTTAGAGA 3' and TG2-FL-2 5' GGCGGGGCCAATGATGACATTCCGGA 3'. The approximately 2 kb amplification product was purified using Quiagen PCR cleanup kit (Qiagen) and digested into random fragments using RQ DNAse I (Promega). The RQ DNAse reaction was treated with Klenow fragment of DNA polymerase I and T4 DNA polymerase to generate blunt-ended fragments. These were purified by gel electrophoresis, and fragments in the range of 50–150 bp extracted using Qiagen gel recovery kit (Qiagen, Crawley UK).

A phage display vector was digested with EcoRV, treated with alkaline phosphatase and purified by gel electrophoresis and the Qiagen gel recovery kit. 100 ng of purified vector was ligated to 15 ng of prepared blunt fragments of human TG2 cDNA. The resultant ligation was electroporated into XL1-Blue electrocompetent cells (Agilent Technologies) and the fragment library rescued with VCSM13 helper phage (Agilent). Phage particles were precipitated with 2 % glucose and 4 % PEG 6000 and resuspended in PBS 0.1 % Tween 20 (v:v) 1 % BSA (w:v).

Epitope mapping was carried out using the following procedure. ELISA wells were coated overnight at 4 °C with 30 µg of monoclonal antibody in 100 µl of coating buffer. The coated well was washed with PBS/Tween and blocked with 400 µl of 3 % BSA in PBS (w:v) for 1 h at room temperature. Approximately 10^{10} phage particles (100 µl) were added to the blocked well and incubated at room temperature for 1 h. The well was washed 8 times with 400 µl PBS/0.5 % Tween (v:v) and adherent phage eluted with 0.2 M glycine pH 2.2. Eluted phage were used to infect 1 ml of XL1-Blue host and samples plated onto LB agar (60 µg/ml ampicillin, 15 µg/ml tetracycline), the remaining host was added to 100 ml LB media (60 µg/ml ampicillin, 15 µg/ml tetracycline) and grown overnight at 37°C in a shaking incubator at 200 rpm to generate the enriched library of selected fragments. This enrichment process was repeated 5 times and random colonies from the final round were selected for sequencing.

Determining the sequence of the antibody VL region

Primers

Heavy chain sense primers – A pair of highly degenerate FR1 primers, MH1 and MH2 (Wang et al 2000), were combined with 3 constant region primers to amplify VH genes.

MH1 5' CGCGCGCTCGAGSARGTNMAGCTGSAGTC 3'

MH2 5'CGCGCGCTCGAGSARGTNMAGCTGSAGSAGTC 3'

Mouse-G1 5' AGGCGCAGTACTACAATCCCTGGGCACAATTTTCTTGTCCACC 3'

Mouse-G2a 5' AGGCGCAGTACTACAGGGCTTGATTGTGGGCCCTCTGGG 3'

Mouse-G2b 5' AGGCGCAGTACTACAGGGGTTGATTGTTGAAATGGGCCCG 3'

Kappa primers

VK1 5' CGCTGCGAGCTCGATATTGTGATGACBCAGDC 3'

VK2 5' CGCTGCGAGCTCGAGRTTKTGATGACCCARAC 3'

VK3 5' CGCTGCGAGCTCGAAAATGTGCTCACCCAGTC 3'

VK4 5' CGCTGCGAGCTCGAYATTGTGATGACACAGTC 3'

VK5 5' CGCTGCGAGCTCGACATCCAGATGACACAGAC 3'

VK6 5' CGCTGCGAGCTCGAYATTGTGCTSACYCARTC 3'

VK7 5' CGCTGCGAGCTCGACATCCAGATGACYCARTC 3'

VK8 5' CGCTGCGAGCTCCAAATTGTTCTCACCCAGTC 3'

K-CONST 5' GCGCCGTCTAGAATTAACACTCATTCCTGTTGAA 3'

Total RNA was extracted from monoclonal hybridoma cells (~ 10^5 cells) using Trizol (GIBCO) according to the manufacturer's protocol and quantified by A_{260nm}. cDNA was

synthesised using ImProm II reverse transcriptase (Promega) and random hexamer primers. The reaction mix was as follows; 1 µg total RNA, 0.1 µg oligo $(dN)_6$, 12 µl ImProm II buffer, 1 µl 10 mM dNTPs (Promega), 8 µl 25 mM $MgCl_2$, 4 µl ImProm II reverse transcriptase (Promega), DEPC-treated H_2O up to total reaction volume of 60 µl. The RNA and random primer mix was heated to 70 °C for 10 min and then placed on ice. The remaining reaction components were added and then incubated at 20 °C for 10 min, then at 40 °C for a further 40 min.

Amplification of VH and VK genes was carried out with GoTaq polymerase (Promega). Each 50 µl reaction contained the following; cDNA 2 µl, 20 pmol sense and antisense primers, 10 µl GoTaq reaction buffer, 1 µl 10 mM dNTPs, 5 µl 25 mM $MgCl_2$, 2.5 u GoTaq polymerase, H_2O to a final volume of 50 µl. Reactions were cycled 35 times using the following conditions: initial denature 95 °C 2 min; denature 94 °C 1 min, anneal 56 °C 1 min, extension 72 °C 1 min. PCR products were analysed by gel electrophoresis and cloned using the TOPO TA cloning kit (Invitrogen). Random minipreps of heavy and light chain PCR products were selected for sequencing.

Measurement of extracellular TG activity

Extracellular TG activity was measured by modified cell ELISA. HK-2 epithelial cells were harvested using 0.1M EDTA or 0.25% trypsin/EDTA and plated at a density of 8×10^4 cells/well in serum free medium onto a 96 well plate that had been coated overnight with 100 µl/well of fibronectin (5 µg/ml in 50 mM Tris–HCl pH 7.4) (Sigma, Poole UK). Cells were allowed to attach for 2.5 h at 37 °C in the presence of the 0.1 mM biotin cadaverine [N-(5 amino pentyl biotinamide) trifluoroacetic acid] (Molecular Probes, Eugene OR, USA). Plates were washed twice with 3 mM EDTA/PBS and cells removed with 0.1% (w/v) deoxycholate in 5 mM EDTA/PBS. The supernatant was collected and used for protein determination. Plates were washed with 50mMTris– HCl and incorporated biotin cadaverine revealed using 1:5000 extravidin HRP (Sigma, Poole, UK) for 1 h at room temperature followed by a TMB (3,3',5,5'-tetramethylbenzidine) substrate. The reaction was stopped with 50 µl 2.5 M H2SO4 and the absorbance read at 450 nm.

Measurement of Collagen levels by radiolabelling

Cells were seeded at a density of 3.75×10^6/10 cm^2 Petri dish or 1×10^6/well of a 6 well plate. ECM collagen was assessed by labelling with 20 iCi of $^{3,4}H$ proline (1.0 mCi/ml, ICN). Labelling was performed for 72 h under standard cell culture conditions. Following labelling, the media was removed, cells washed with PBS and removed with 2 ml of 0.25 M ammonium hydroxide in 50 mM Tris pH 7.4 at 37 °C for 10 min. The soluble fraction

was collected and protein concentration determined using the bicinchoninic acid (BCA) assay. The dishes were washed extensively with increasing volumes of PBS before the ECM was solubilised with 2 ml of 2.5% (w/v) SDS in 50 mM Tris pH 6.8. The dish was scraped to ensure complete removal of the ECM and 200 il was measured for radioactivity in a beta scintillation counter. Counts were corrected per mg of solubilised cell protein and expressed as a percentage of the mean control value.

Generation of recombinant ratified Quark IgG

For experimental purposes a human-rat chimeric antibody from the sequence of a 'human' single-chain Fv of an antibody against human type-II transglutaminase was generated. The antibody is called QPCDTGII (shortened to QCT), and the sequences of the variable regions are available in WO 2006/100679A2.

A rat γ2a subclass was selected for the heavy chain constant regions, removing the glycosylation site to reduce the chance of an ADCC reaction in the rat test animals. The selected rat constant region for the heavy chain was 013593 (Bruggemann, M. Gene **74:** 473-482 (1988); Bruggemann, M.,Free, J.,Diamond, A.,Howard, J.,Cobbold, S. and Waldmann, H. Proc. Natl. Acad. Sci USA **83:** 6075-6079 (1986)) from the Kabat database (Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S., and Foeller, C. Sequences of Proteins of Immunological Interest. (NIH National Technical Information Service, 1991)). That for the kappa light chain was 013718 (Sheppard, H. W. and Gutman, G. A. Proc. Natl. Acad. Sci. USA **78:** 7064-7068 (1981)) from Kabat.

In brief, heavy chain and kappa chain coding sequences were generated by DNA synthesis (codon usage was adapted to a mammalian codon bias).

The heavy chain gene synthesis product was amplified by PCR using the primers QCT_HindIII and QCT_H_rev. The PCR-product was cut with HindIII and NgoMIV and ligated into MRCT expression vector. Clones of competent DH5α bacteria chemically transformed by the ligation product were PCR-screened using the primers HCMVi and rat_gamma1. Three clones generating a PCR product of the predicted size were sequenced.

The kappa chain gene synthesis product was amplified by PCR using the primers QCT_HindIII and QCT_L_rev. The PCR-product was cut with HindIII and PpuMI and ligated into the expression vector pKN100. Clones of competent DH5α bacteria chemically transformed by the ligation product were PCR-screened using the primers

HCMVi and rat_kappa. Three clones generating a PCR product of the predicted size were sequenced.

A double insert expression vector coding for both Heavy and kappa chains was generated and transfected into HEK293T cells. Cell culture supernatant from two large scale HEK293T transfections was pooled and affinity purified on a 1ml Protein L-agarose column using an ÄKTA Explorer chromatography system, in accordance with the manufacturer's protocols. A single OD 280nm peak eluted with IgG Elution Buffer, and was dialysed against two changes of PBS. This was assayed both by UV absorption at 280nm, and by rat IgG_{2a} ELISA. The total yield was approximately 700 µg (by OD_{280nm}); 303.5 µg (by ELISA).

Humanisation of AB1 Antibody

Human VH and VK cDNA Databases

The protein sequences of human and mouse immunoglobulins from the International Immunogenetics Database 2009[101] and the Kabat Database Release 5 of Sequences of Proteins of Immunological Interest (last update 17-Nov-1999)[102] were used to compile a database of human immunoglobulin sequences in a Kabat alignment. Our database contains 10,606 VH and 2,910 VK sequences.

Molecular Model of AB1

A homology model of the mouse antibody AB1 variable regions has been calculated using the Modeller program[103] run in automatic mode. The atomic coordinates of 1 MQK.pdb, 3LIZ.pdb and 1MQK.pdb were the highest identity sequence templates for the Interface, VL and VH respectively as determined by Blast analysis of the Accelrys antibody pdb structures database. These templates were used to generate 20 initial models, the best of which was refined by modeling each CDR loop with its 3 best loop templates.

hAB1 Framework Selection

The sequence analysis program, gibsSR, was used to interrogate the human VH and VK databases with the AB1 VHc, VKc and VKc_1 protein sequences using various selection criteria. FW residues within 5Å of a CDR residue (Kabat definition) in the homology model of mouse antibody AB1, were identified, and designated as the "5Å Proximity" residues.

AF06220 was chosen as the FW on which to base the initial humanised AB1 VHc construct. Table 1 shows the alignment and residue identity of AF06220 to murine Ab1. Table 2 shows the 5Å proximity envelope of the sequences. AF062260 has only 1 somatic mutation away from its germline VH gene Z12347 (Table 3).

AY247656 was chosen as the FW on which to base the initial humanised AB1 VKc construct. The alignment and residue identity to murine AB1 are shown in Table 4; Table 5 shows the 5Å proximity envelope of the sequences. The sequence shows 5 somatic mutations from its germline VK gene X93620 (Table 6).

AF193851 was chosen as the FW on which to base the initial AB1 VKc_1 construct. The alignment and residue identity to murine AB1 are shown in Table 7. Table 8 shows the 5Å proximity envelope of the sequences. The sequence shows no somatic mutations from its germline VK gene J00248 (Table 9).

Binding ELISA

HEK 293F cells were co-transfected with combinations of different humanised light chain vectors in association with different humanised heavy chain vectors. Recombinant human TG2 was used to measure antibody binding by ELISA. The results indicated that the Heavy Chain version RHA (Table 10), in combination with either Light Chain versions RKE and RKJ (Table 11) (representing the different Light Chain versions humanised) showed optimal binding (Figure 16).

Heavy Chain version RHA is an un-modified graft of the mouse CDR regions of the AB1 antibody onto the Human donor sequence. However, both Light Chain versions RKE and RKJ, have the same single 5Å proximity reside backmutation, F72 (Kabat numbering – shown in green). This backmutation lies outside the Vernier[104], Canonical[105] or Interface[106] residues (see Table 11).

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TABLES

Table 1

Kabat Numbers[2]	1 10 20 30 40 50 60 70 80 90 100 110
	-|--------|---------|---------|-----AB----|---------|--ABC-------|---------|---------|--ABC-------|---------|ABCDEFGHIJK---------|-
Vernier[4]	-.*.....****.....***.....*.*.*.*....*.....**.....*.....
Canonical[5]	-.....1.11.1....1.....2...22.....2.....1.....
Interface[5]	-.....I...I.I.....I.I.....I.I.I.....I.....
5Å Proximity	**** * * **** *** *** ****** * * **** **
AB_VHc	-EVQLXESGGGLVK GGSLKLSCAASGFTLSSSAMS--WVRQT DRRLEWVATISV--GGGKTYYPDSVKGRFTISRD AKT TLYLQM SLRSEDTAMYYCAKLI----------SLYWGQGTTLTVSS
AF062260	C....L.....Q ....R.....F. Y ...-....A GKG....SA..G--S .S ..A ....S.S ....A....V.....DG----------GV .....LV....

Table 2

	5Å Proximity Residues
AB_VHc	EVQLCAFTLSWVRWVARFTISRNLYCAKWG
AF062260	.....F.....S.....

Table 3

```
                  ---------+---------+---------+---------+---------+---------+---------+---------+---------+--------
                           10        20        30        40        50        60        70        80        90
                  ---------+---------+---------+---------+---------+---------+---------+---------+---------+--------
Z12347.seq        EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
AF062260.seq      ..........................................................................................R     1
                                           <--->             <---------------->
```

Table 4

Kabat	1	10	20	30	40	50	60	70	80	90	100
Numbers[2]	-|--------|---------|-------ABCDEF--|---------|---------|---------|---------|---------|---------|-----ABCDEF----|------A-										
Vernier[4]	-.*.*.....				**.....		****.....		*.*.**.*		*.....
Canonical[5]	-.1.....		1.....	1111..1.....		2..22.....		2.....1.....		3....3.....	
Interface[6]	-.....			I.I.....I.....						I..I.....	II.....
5Å Proximity	******		**	**	****	***	**********		**		**
AB_VKc	-EIVLTQSPSSMYASLGERVTITCKASQ------DINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFP-------YTFGGGTKLEI-K										
AY247656	-.....|..LS..V.D.....Q..|-------..SN..N.Y...|..A|.L...DASN.RT..|.....T..TF.....Q|..F.T...Q..NTY.|-------L|.....-.										

Table 5

	5Å Proximity Residues
AB_VKc	EIVLTQTCWFTLIYGVPFSGSGSGQDFFYCFG
AY247656	.....YL.....T..T....

Table 6

```
                ---------+---------+---------+---------+---------+---------+---------+---------+---------+------
                         10        20        30        40        50        60        70        80        90
                ---------+---------+---------+---------+---------+---------+---------+---------+---------+------
X93620.seq      DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLPP
AY247656.seq    E.VL.....................................................................FG.......NTY.L     5
                                  <---------->        <----->                          <-------
```

Table 7

Kabat	1 10 20 30 40 50 60 70 80 90 100
Numbers[2]	-|--------|---------|-------ABCDEF--|---------|---------|---------|---------|---------|-----ABCDEF----|------A-
Vernier[4]	-.*.*.....**.....****.....*.*.**.*.....*.....
Canonical[5]	-.1.....1.....1111..1.....2..22.....2.....1.....3....3.....
Interface[6]	-.....I.I.....I.....I..I.....II.....
5Å Proximity	****** ** ** **** *** ********** ** **
AB_VKc	-DIQMTQS SSMYASLGERVTITC KASQ------DINSYLT WFQQK GKS KTLIY RTNRLFD GV SRFSGSGSGQDFFLTISSLEYEDMGIYYC LQYDDFP-----YTFGGGTKLEI-K
AF193851	-..... ..LS..V.D..... R ------G RN A..... ..A .S... AASN QS.. .....T..T.....Q ..FAT...Q HNTY-----W...Q...V..-.

Table 8

	5Å Proximity Residues
AB_VKc	DIQMTQTCWFTLIYGVPFSGSGSGQDFFYCFG
AF193851	.....S.....T..T....

Table 9

```
                    ---------+---------+---------+---------+---------+---------+---------+---------+---------+------
                             10        20        30        40        50        60        70        80        90
                    ---------+---------+---------+---------+---------+---------+---------+---------+---------+------
J00248.seq          DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPP
AF193851.seq        ..........................R.....................N.....................................H.T..W      0
                                 <---------->          <----->                              <-------
```

Table 10

Kabat	1	10	20	30	40	50	60	70	80	90	100	110
Numbers	-|--------|---------|---------|-----AB----|---------|--ABC-------|---------|---------|--ABC-------|---------|ABCDEFGHIJK---------|-											
Vernier[4]	-.*.....****.....***.....*.*.*.*....*.....**.....*.....											
Canonical[5]	-.....1.11.1....1.....2...22.....2.....1.....											
Interface[6]	-.....I...I.I.....I.I.....I.I.....I.I.I.....I.....											
5Å Proximity	**** * * **** *** *** ****** * * **** **											
AB_VHc	**-EVQLXESGGGLVKPGGSLKLSCAASGFTLSSSAMS--WVRQTPDRRLEWVATISV--GGGKTYYPDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLI----------SLYWGQGTTLTVSS**											
AB_RHA	-EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMS--WVRQAPGKGLEWVSTISV--GGGKTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLI----------SLYWGQGTLVTVSS											

TABLE 11

Kabat	1	10	20	30	40	50	60	70	80	90	100
Numbers	-|--------|---------|-------ABCDEF--|---------|---------|---------|---------|---------|---------|-----ABCDEF----|------A-										
Vernier[4]	-.*.*.....**.....****.....*.*.**.*.....*.....										
Canonical[5]	-.1.....1.....1111..1.....2..22.....2.....1.....3....3.....										
Interface[6]	-.....I.I.....I.....I..I.....II.....										
5Å Proximity	****** ** ** **** *** ********** ** **										
AB_VKc	**-EIVLTQSPSSMYASLGERVTITCKASQ------DINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFP------YTFGGGTKLEI-K**										
AB_RKE	-EIVLTQSPSSLSASVGDRVTITCKASQ------DINSYLTWYQQKPGKAPKLLIYRTNRLFDGVPSRFSGSGSGTDFFFTISSLQPEDFGTYYCLQYDDFP------YTFGGGTKLEI-K										
AB_RKJ	-DIQMTQSPSSLSASVGDRVTITCKASQ------DINSYLTWFQQKPGKAPKSLIYRTNRLFDGVPSRFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFP------YTFGQGTKVEI-K										

RESULTS

Generation of rh TG2 core protein.

To force the generation of antibodies that would be more likely to target epitopes critical for TG2 activity, rather than favoured sites on the TG2 molecule, we immunised mice with the TG2 catalytic core rather than the full-length TG2 molecule. To generate the recombinant TG2 domain, a PCR construct was generated running from bases 329 to 1419 and inserted into the Pet21+(a) vector (Figure 1A). Insertion and expression of this vector in BL21-CodonPlus (DE3)-RIPL bacteria resulted in the generation of an insoluble protein spanning amino acids 143 to 473 encompassing the entire catalytic core. This protein was solubilised and refolded in 40 mM Tris HCl; 150 mM NaCl; 20 % glycerol; 5 mM cysteine; 0.5 mM cystine pH 8. 10 ng of this was run on a non-reducing polyacrylamide gel, western blotted and immunoprobed with CUB7402. A clear band was visible at 37 kDa which is consistent with the predicted size of the TG2 core (Figure 1B). Larger bands were also immunoreactive to CUB7402 which most likely represent aggregates of the core protein as these were not present when a reducing gel was run (data not shown).

Immunisation and fusion.

Four mice were immunised with 50 µg of rhTG2 core. At approximately five and nine weeks post immunisation, a serum sample was taken from each mouse and tested for reactivity against rh TG2 by ELISA using a serial dilution of the serum. All mice showed a strong immune reaction to rhTG2 core, even at the highest dilution used (1: 51 000) (Figure 2A). To confirm the antibodies would also recognise the full-length TG2, rhTG2 and rhTG2 core protein were run on a non-denaturing gel, western blotted and immunoprobed with a 1 in 1000 dilution of mouse serum (Figure 2B). The mouse with the strongest reactivity (mouse C) against both proteins was boosted and splenocytes recovered for fusion using the University of Sheffield's Hybridoma service, Bioserv.

Selection of positive hybridoma and cloning.

Out of 400 hybridoma wells selected by Bioserv as highest positives, supernatants from 109 showed persistent reactivity to TG2, however only 34 did not react with other key

TG family members when tested in ELISA (representative examples shown in Figure 3A). Those that were specific to TG2 had the supernatant screened by ^{3}H-putrescine incorporation assay for the ability to inhibit TG2 activity resulting from 100 ng of TG2 (Figure 4). This initial screen indicated that 10 hybridoma supernatants were able to inhibit TG2 activity (AB1; DC1; BB7; EH6; DH2; DD9; JE12; AG9; AH3; DF4). Nine of the ten were successfully cloned by limited dilution. For the clone DF4, although clones were isolated post-cloning, they did not appear to be inhibitory. Post cloning, IgG was purified from each cloned hybridoma and retested for selective reactivity to TG2 (Figure 3B).

TG2 inhibitory potential.

Each cloned hybridoma had its IgG tested for TG2 inhibitory activity against human, rat and mouse TG2 and the IC_{50} calculated based on the amount of IgG required to inhibit 1 ng of TG2. There was approximately a 12 fold range in IC_{50} values against human TG2 ranging from the most effective AB-1 at $1.1x10^{-5}$ mg/ml of IgG to the least effective JE12 at 12.3 $1.1x10^{-5}$ mg/ml of IgG (Figure 9; Table 1). Interestingly we were only able to determine an IC_{50} for 4 antibodies (DH2, DD9, EH6 and BB7) against rat TG2 with the best, DH2 having a IC_{50} of 2.23×10^{-4} mg/ml of IgG being some 6 fold less active than against human TG2 and comparatively 38 fold less active that the best AB-1 inhibitor against TG2 (Figure 9; Table 1). None of the inhibitory antibodies were able to inhibit mouse TG2, probably due to immune tolerance.

Mapping the epitopes of inhibitory antibodies

To establish which epitopes in TG2 were immunologically unique to TG2 while inhibitory, as well as establishing if these 10 antibodies were targeting the same or different sites, each antibody was mapped using phage display. A TG2 phage library was constructed and panned against each mAb. The epitope was then determined by consensus sequencing of the binding phages.

AB1, AG1, AH1, BB7, DC1, EH6 and JE12 all appeared to bind in whole or part to a single epitope (Figure 5) which encompasses amino acids 304 to 326 and appears to sit in front of active site within a substrate binding pocket (Figure 6). This region we termed the AB-1 site and called antibodies targeting this site Group 1 antibodies.

DF4 uniquely targeted a sequence running from amino acid 351 to 365 (Figure 5) which runs from front to rear of core encompassing Asp 358 in the catalytic triad (Figure 6). This we termed Group 2.

DH2 and DD9 bound to a sequence spanning amino acids 450 to 467 (Figure 5). These Group 3 antibodies bind to a region at the rear of core near the junction with β barrel-1, that we termed the DH2 site. The epitope encompasses a putative calcium binding site (Figure 6).

Antibody Sequencing

In order to establish the variable light chain sequence for each antibody, RNA from each inhibitory hybridoma was extracted, reverse transcribed and amplified by PCR using a pair of highly degenerate FR1 primers, MH1 and MH2 primers being combined with 3 constant region primers to amplify VH genes.

The resulting VH and VK sequences are shown in Figure 7 for AB1.

The ability of AB1 to inhibit TG2 activity in a protein mixture *in vitro*.

The most potent inhibitory antibody against recombinant TG2 is AB1. To be of value therapeutically, it must be able to not only inhibit TG2 activity in a pure solution, but also in a complex protein solution and not associate in anyway with other proteins. To test this, a homogenate of the human hepatocyte cell line HepG2 was prepared. Application of 0.5 µg of AB1 was able to inhibit 70 % of the TG2 activity (Figure 8A). However BB7 produced a significantly better inhibition knocking down 90 % of the TG2 activity. Immunoprobing 25 µg of this lysate with AB1 showed no off target association with a single immunoreactive band at a size corresponding to TG2 (Figure 8B).

The ability of AB1 and DC1 to inhibit extracellular TG2 activity.

To asses if these antibodies could inhibit TG2 activity in a cell system. AB1 (Figure 10a) and DC1 (Figure 10b) were applied to human kidney 2 (HK-2) tubular epithelial cells in culture and extracellular TG activity assayed using the biotin cadaverine assay. AB1 was able to achieve a 60% inhibition and DC1 a 55% inhibition of activity when applied at 4ng/ul in the culture media which was comparable to the chemical pan TG inhibitor 1,3-Dimethyl-2-[(2-oxo-propyl)thio]imidazolium chloride applied at 400uM.

Comparison of antibody AB1 with other known inhibitory antibodies

To test the effectiveness of AB1 in comparison to other known TG2 inhibitory antibodies both fab fragments (Figures 11,12) and full IgG (Figures 13, 14) of an antibody as described by Quark biotechnology in patent application number WO2006/100679 were tested to inhibit TG2 activity in the ^{3}H Putrescine incorporation assay. The activity from 100ng of human TG2 could be inhibited by 60 to 80% by 500ng of AB1. In comparison neither the fab fragment or the full IgG of the Quark antibody could inhibit TG2 significantly in this assay.

Discussion

There is a clear need to validate TG2 as a therapeutic target in man across a range of diseases where experimental studies have suggested its involvement. These include tissue scarring, celiac disease, neurodegenerative diseases and chemo-resistance in some cancers. Limiting this has been the lack of truly TG2 specific compounds that can selectively inhibit TG2 activity in man.

In this study we have for the first time immunised mice with a fragment of TG2 with the aim of being able to isolate a wider range of anti TG2 antibodies against the enzyme's catalytic core in the search of an inhibitory epitope. This elicited a good immune response with antibodies recognising both the rhTG2 core and native TG2 but no other TG.

10 of the antibodies isolated showed inhibitory activity. These were subsequently mapped to 3 TG2 specific, yet inhibitory epitopes. These antibodies have been cloned, sequenced and IgG isolated with IC_{50} values calculated. Three antibodies (AB1, DC1 and BB7) targeting a substrate pocket proved particularly effective inhibitors. Most importantly these antibodies also worked well both in a cell lysate and in cell culture indicating that these antibodies have the potential to function in a protein rich environment which is critical for *in vivo* application.

We believe a key element in the successful generation of these inhibitory antibodies has been the decision to immunise with just the core protein. To our knowledge none of the commercial TG2 antibodies have inhibitory potential of any significance. Our own

attempts to use full length TG2 resulted in a large number of antibodies, few of which were specific to TG2 and none of which were inhibitory. This would appear to be due to a clear immunogenic preference for protein loops within full length TG2 many of which fall on the rear of the catalytic core in similar positions to the most widely used anti TG2 antibody, CUB7402 (aa447 aa478).

It is surprising that our approach has led to the production of much more effective antibodies. Without being bound by any theory we think that by simply raising antibodies to a smaller protein covering just the central core, we not only eliminate some of the favoured immunological epitopes, but we also force core targeting. This alone increases the variety of antibodies available for selection and thus wider coverage of the core. However, immunising with just the core means that much of the folding of the core is lost and thus some of the epitopes that perhaps may be less available within a whole TG2 molecule may be more attractive epitopes with the core in this format. Given that all 10 of the antibodies recognised linear epitopes (i.e. bound to TG2 on a reducing gel), while 80 % of the antibodies we previously isolated using full length TG2 as an immunogen were conformation dependent, does suggest this may be a major factor.

There have previously been other studies that have postulated the idea of a TG2 inhibitory antibody for human application. Esposito and colleagues developed recombinant antibodies from patients with celiac disease where it has been postulated that TG2 antibodies may have an inhibitory role [19]. One of these antibodies was developed for commercial application by Quark Biotechnology and a patent application filed (WO2006/100679). This antibody demonstrated some exciting early data in the prevention of kidney fibrosis in the rat UUO model. However, we produced a recombinant version of this antibody and while it reacted with TG2 in ELISA (Figure 17) and western blot, we achieved little inhibition up to 500 ng of IgG per ng of TG2 for this antibody at which all of the antibodies developed in this study block essentially all TG2 activity. Furthermore WO2006/100679 describes the generation of a mouse version of this human antibody, and as such, long application in recognised rat models of kidney disease would prove difficult.

Of note in the present study is the mapping of the 3 inhibitory epitopes within the TG2 core. The AB1 epitope is by far the most potent to target, which is perhaps surprising

given the position of the epitope. Examination of its position within the predicted TG2 active structure [20] suggests it binds in the entry port to the catalytic triad in what may be a substrate pocket. Given the substrates we used in our screening assay are relatively small (putrescine and dimethyl casein), it is perhaps surprising that this site is so effective. However the position of the epitope must be such that the large IgG (150 kDa) is positioned tightly into the catalytic site. From the epitope data one may have predicted that the DD9 site may be more effective as it is associated with a putative calcium binding site [21]. However examination of the literature suggests 5 or more putative Ca^{2+} binding sites [21] and while it clearly has a dramatic effect, is not critical for all TG2 activity.

The DF4 site would be hypothetically the most effective epitope as the antibody binds to 1 of the essential amino acids in the catalytic triad. However it has not been possible to successfully clone out DF4 producing this inhibitory antibody and as such the production of sufficient IgG to adequately perform IC_{50} tests has not been possible. It may in fact be very difficult to clone out antibodies that have too high efficacy given the work from Gunzler *et al* (1982) *FEBS Lett.* **150(2):** 390-6 that suggested that lymphocytes needed TG2 activity to proliferate and thus antibodies with better inhibitory potential may only be possible using recombinant approaches or a continual IgG extraction system.

One of the most frustrating problems in undertaking this work has been the apparent inability of all antibodies developed to efficiently block non-human TG2 activity, which is critical for preclinical testing. All antibodies reacted with rat and mouse TG2 in both western blot and ELISA, in some cases with little difference in intensity. However out of the 9 antibodies we produced IgG for, it was only possible to determine an IC_{50} for 4 in rat and none in mouse. The 4 where an IC_{50} was calculated against rat TG2 showed a 30 fold or lower IC_{50} against rat TG2 than AB1 against human TG2 meaning any *in vivo* dose would be prohibitively large. Further none would inhibit at all in a rat cell lysate. Given the reactivity in ELISA and western blots, plus there are just 5 mismatches between species for AB1 and 3 for DD9 the significant species specificity for inhibition was surprising and clearly demonstrates the critical importance of affinity for effective inhibition. Thus having identified these inhibitory epitopes for human TG2 it is now critical that analogue antibodies are developed for these sites in rat TG2 if their value is to be established in *in vivo* pre clinical models of disease.

There are a wide range of TG inhibitors available. Notably the thiomidazole based compounds originally developed by Merke Sharpe Dome [22] the CBZ-glutamyl analogues developed by Griffin and colleagues [23] which we have used very successfully to treat experimental kidney scarring [16] and the dihydroisoxazole type inhibitors developed by Khosla and collegues [24-27] used successfully in various cancer models. There has been hope that continual refinement of these compounds may yield a viable human TG2 inhibitor, but cross TG family reactivity or the potential toxic nature of the compounds seems to have prevented this. More recently Acylideneoxoindoles have been described as a new reversible class of TG2 inhibitors [24], but data regarding their cross reactivity to other TG family members is lacking. At the 2010 Gordon conference on TG2 in human disease Pasternack and collegues from Zedira presented details of a range of compounds that use side chain Michael acceptors as TG2 inhibitors with claims of suitability for *in vivo* application and TG2 selectivity, however a full publication on these has not materialised to date. At the same meeting early work from Macdonald *et al* demonstrated some interesting developments in designing a TG2 inhibitor for treatment of Huntington's Chorea, but again a full publication is still awaited. Undoubtedly a small molecule inhibitor of TG2 would be highly desirable should it be achievable. Tissue penetration, the ability to cross the blood brain barrier, production, cost and easy dosing are just some of the benefits. However, an antibody inhibitor as developed here may in some way be preferable.

TG2 clearly is a multifunction enzyme and has been linked to a range of cellular functions including nuclear stabilisation and transport [28, 29], endocytosis [30, 31], GTPase signalling [32-34], Apoptosis [35, 36], cell adhesion [37-39], cytoskeletal integrity [28, 29] and ECM stabilisation [9]. Clearly a small molecule inhibitor may impede on all of these functions as in general they have free access to the extracellular space and cell interior. An antibody cannot enter the cell and as such the intracellular roles of TG2 would not be affected. Importantly most of the pathological roles of TG2 appear to be extracellular such as its role in tissue scarring and fibrosis, celiac disease and cancer. Thus using an antibody would bring an additional degree of selectivity preventing undesired intracellular effects. Therefore an antibody would offer advantages in blocking TG2 in fibrotic and scarring diseases where TG2 crosslinks ECM proteins, in celiac disease where gliadin is deamidated in the extracellular space and in chemo-

resistance in cancer where cell adhesion appears to be the protective factor. However, unless a small Fab fragment could be designed that could cross the blood brain barrier a TG2 inhibiting antibody would be little use in treating neurological pathologies.

In conclusion, for the first time we have been able to develop TG2 inhibitory antibodies that selectively target TG2. We have also identified 3 novel inhibitory epitopes within the core domain of TG2. Humanisation of antibody AB1 will open up the possibility for the first time of targeted TG2 therapy in man.

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Example 2: Sequencing of novel TG2 inhibitory antibodies of the invention.

Antibody Sequencing

In order to establish the sequences of the variable regions of each antibody of the invention, a pellet of the hybridoma cells was processed using the Qiagen RNeasy Mini Kit to extract the RNA following the manufacturer's protocols. The extracted RNA was reverse transcribed to produce a cDNA using a 1st Strand cDNA Synthesis Kit (GE Healthcare), using a NotI-dT_{18} primer, in accordance with the manufacturer's protocols. The cDNA preparation was cleaned up using the Qiagen PCR Purification Kit, in accordance with the manufacturer's protocols.

To determine the heavy chain sequence, the mouse cDNA was amplified by PCR using a set of degenerate primers (MHV1-12) with a constant region primer (MHCG1, MHCG2A, MHCG2B, MHCG3, or a mixture of the four) as shown in Table 12. Similarly, to determine the light chain sequence, the mouse cDNA was amplified using a set of degenerate primers (MVK1-11) with a constant region primer MKC as shown in Table 13.

If no amplification products were seen using the initial set of Heavy Chain PCR a 5' RACE PCR (Invitrogen) was carried out, using the NotI-dT_{18} primer to generate cDNA; and the constant region primers (MHCG1, MHCG2A, MHCG2B, MHCG3, or a mixture of the four) and the 5' RACE Anchor Primer, GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG (where I is the base for deoxyinosine) for the PCR.

The resulting amplification bands were ligated into the pCR2.1®-TOPO® vector using the TOPO-TA Cloning® kit (Invitrogen) using the manufacturer's protocol and sent to GATC Biotech AG for sequencing.

Table 12: PCR Primers for Cloning Mouse Heavy Chain Variable Regions

Name	Sequence
MHV1	ATGAAATGCAGCTGGGGCATCTTCTTC
MHV2	ATGGGATGGAGCTRTATCATSYTCTT
MHV3	ATGAAGWTGTGGTTAAACTGGGTTTTT
MHV4	ATGRACTTTGGGYTCAGCTTGRTTT
MHV5	ATGGACTCCAGGCTCAATTTAGTTTTCCTT
MHV6	ATGGCTGTCYTRGSGCTRCTCTTCTGC
MHV7	ATGGRATGGAGCKGGRTCTTTMTCTT
MHV8	ATGAGAGTGCTGATTCTTTTGTG
MHV9	ATGGMTTGGGTGTGGAMCTTGCTATTCCTG
MHV10	ATGGGCAGACTTACATTCTCATTCCTG
MHV11	ATGGATTTTGGGCTGATTTTTTTTATTG
MHV12	ATGATGGTGTTAAGTCTTCTGTACCTG
MHCG1	CAGTGGATAGACAGATGGGGG
MHCG2A	CAGTGGATAGACCGATGGGGC
MHCG2b	CAGTGGATAGACTGATGGGGG
MHCG3	CAAGGGATAGACAGATGGGGC

Ambiguity codes: R = A or G; Y = C or T; M = A or C; K = G or T; S = G or C; W = A or T.

MHV indicates primers that hybridize to the leader sequences of mouse heavy chain variable region genes, MHCG indicates primers that hybridize to the mouse constant region genes.

Table 13: PCR Primers for Cloning Mouse Kappa Light Chain Variable Regions

Name	Size	Sequence
MKV1	30-mer	ATGAAGTTGVVTGTTAGGCTGTTGGTGCTG
MKV2	29-mer	ATGGAGWCAGACACACTCCTGYTATGGGTG
MKV3	30-mer	ATGAGTGTGCTCACTCAGGTCCTGGSGTTG
MKV4	33-mer	ATGAGGRCCCCTGCTCAGWTTYTTGGMWTCTTG
MKV5	30-mer	ATGGATTTWAGGTGCAGATTWTCAGCTTC

MKV6	27-mer	ATGAGGTKCKKTGKTSAGSTSCTGRGG
MKV7	31-mer	ATGGGCWTCAAGATGGAGTCACAKWYYCWGG
MKV8	31-mer	ATGTGGGGAYCTKTTTYCMMTTTTTCAATTG
MKV9	25-mer	ATGGTRTCCWCASCTCAGTTCCTTG
MKV10	27-mer	ATGTATATATGTTTGTTGTCTATTTCT
MKV11	28-mer	ATGGAAGCCCCAGCTCAGCTTCTCTTCC
CL12A		ATGRAGTYWCAGACCCAGGTCTTYRT
CL12B		ATGGAGACACATTCTCAGGTCTTTGT
CL13		ATGGATTCACAGGCCCAGGTTCTTAT
CL14		ATGATGAGTCCTGCCCAGTTCCTGTT
CL15		ATGAATTTGCCTGTTCATCTCTTGGTGCT
CL16		ATGGATTTTCAATTGGTCCTCATCTCCTT
CL17A		ATGAGGTGCCTARCTSAGTTCCTGRG
CL17B		ATGAAGTACTCTGCTCAGTTTCTAGG
CL17C		ATGAGGCATTCTCTTCAATTCTTGGG
MKC	20-mer	ACTGGATGGTGGGAAGATGG

Ambiguity codes: R = A or G; Y = C or T; M = A or C; K = G or T; S = G or C; W = A or T.

MKV indicates primers that hybridise to leader sequences of the mouse kappa light chain variable region genes, MKC indicates the primer that hybridises to the mouse kappa constant region gene.

Sequence data

Antibody AB1 was sequenced in addition to Antibodies BB7, DC1, JE12, EH6, AG9, AH3, DD9, DH2, DD6 and IA12. The sequences are provided in Figures 18 to 28.

Example 3: Construction and characterisation of chimeric and humanised novel anti-TG2 antibodies of the invention.

To further characterise the antibodies of the invention and to enable ranking and prioritisation of antibodies for humanisation, a panel of chimeric TG2 antibodies were constructed (murine variable regions and human IgG1 and human kappa). The methodology used to produce the chimeric antibodies is set out below.

Methods

Human VH and VK cDNA Databases

The protein sequences of human and mouse immunoglobulins from the International Immunogenetics Database 2009[1] and the Kabat Database Release 5 of Sequences of Proteins of Immunological Interest (last update 17-Nov-1999)[2] were used to compile a database of human immunoglobulin sequences in a Kabat alignment. Our database contains 10,606 VH and 2,910 VK sequences.

Molecular Model of AB1

As a representative of the Group 1 antibodies (i.e. antibodies that bind the epitope spanning amino acids 304 to 326 of human TG2), a homology model of the mouse antibody AB1 variable regions has been calculated using the Modeller program[3] run in automatic mode. The atomic coordinates of 1 MQK.pdb, 3LIZ.pdb and 1MQK.pdb were the highest identity sequence templates for the Interface, VL and VH respectively as determined by Blast analysis of the Accelrys antibody pdb structures database. These templates were used to generate 20 initial models, the best of which was refined by modeling each CDR loop with its 3 best loop templates.

hAB1 Framework Selection

The sequence analysis program, gibsSR, was used to interrogate the human VH and VK databases with the AB1 VHc, VKc and VKc_1, the BB7 VHc and VKc and the DC1 VHc and VKc protein sequences using various selection criteria. FW residues within 5Å of a CDR residue (Kabat definition) in the homology model of mouse antibody AB1, were identified, and designated as the "5Å Proximity" residues.

AF06220 was chosen as the FW on which to base the initial humanised heavy chain versions. Table 14 shows the alignment and residue identity of AF06220 to murine antibodies. Table 15 shows the 5Å proximity envelope of the sequences. AF062260 has only 1 somatic mutation away from its germline VH gene Z12347 (Table 16).

AY247656 was chosen as the FW on which to base the initial AB1 humanised kappa light chain. The alignment and residue identity to murine AB1 antibody kappa light chain are shown in Table 17; Table 18 shows the 5Å proximity envelope of the sequences. The sequence shows 5 somatic mutations from its germline VK gene X93620 (Table 19).

AF193851 was chosen as the FW on which to base the other humanised kappa light chain constructs. The alignment and residue identity to the murine antibodies are shown in Table 20. Table 21 shows the 5Å proximity envelope of the sequences. The sequence shows no somatic mutations from its germline VK gene J00248 (Table 22).

Generation of Expression Vectors

Construction of chimeric expression vectors entails adding a suitable leader sequence to VH and VL, preceded by a Hind III restriction site and a Kozak sequence. The Kozak sequence ensures efficient translation of the variable region sequence. It defines the correct AUG codon from which a ribosome can commence translation, and the most critical base is the adenine at position -3, upstream of the AUG start.

For the heavy chain, the construction of the chimeric expression vectors entails introducing a 5′ fragment of the human γ1 constant region, up to a natural ApaI restriction site, contiguous with the 3′ end of the J region of the variable region. The CH is encoded in the expression vector downstream of the inserted VH sequence but lacks the V-C intron.

For the light chain, the natural splice donor site and a BamHI site is added downstream of the V region. The splice donor sequence facilitates splicing out the kappa V:C intron which is necessary for in-frame attachment of the VL to the constant region.

The DNA sequences of the variable regions were optimized and synthesized by GeneArt®. The leader sequence has been selected as one that gives good expression of antibody in cultured mammalian cells.

Heavy Chain variable region constructs were excised from the cloning vector using HindIII + ApaI digestion, purified and ligated into the similarly-cut and phosphatase-treated MRCT heavy chain expression vector, and were used to transform TOP10 bacteria.

Kappa chain variable region constructs were excised using HindIII + BamHI digestion, purified, ligated into the similarly-cut and phosphatase treated MRCT kappa light chain expression vector, and were used to transform TOP10 bacteria.

Antibody Expression

A double insert expression vector coding for both Heavy and kappa chains was generated and transfected into HEK293T cells. Cell culture supernatant was purified by affinity chromatography on Protein G-agarose in accordance with the manufacturer's protocols.

Binding ELISA

HEK 293F cells were co-transfected with combinations of different humanised light chain vectors in association with different humanised heavy chain vectors. Recombinant human TG2 was used to measure antibody binding by ELISA. The results indicated that the Heavy Chain version RHA (Table 23), in combination with either Light Chain versions RKE and RKJ (Table 24) (representing the different Light Chain versions humanised) showed optimal binding (Figure 34), and was therefore selected for further characterization. A similar approach was used to identify optimal pairs of humanized BB7 heavy and light chains, and humanised DC1 heavy and light chains.

Heavy Chain version RHA is an un-modified graft of the mouse CDR regions of the AB1 antibody onto the Human donor sequence. However, both Light Chain versions RKE and RKJ, have the same single 5Å proximity reside backmutation, F72 (Table 24). This back mutation lies outside the Vernier[4], Canonical[5] or Interface[6] residues.

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The following table A summarises the chimeric and humanised antibodies produced with a cross reference to the identifiers used in the figures.

Murine Antibody	Chimeric Antibody	Humanised Antibody
AB1	cAB001 cAB003	hAB004 (hAB001AE) hAB005 (hAB001AJ)
BB7	cBB001	hBB001AA hBB001BB
DC1	cDC001	hDC001AA hDC001BB
DD6	cDD6001	
DD9	cDD9001	
DH2	cDH001	

TABLES

Table 14

Kabat Numbers[2]	1 10 20 30 40 50 60 70 80 90 100 110
	-|--------|---------|---------|-----AB----|---------|--ABC-------|---------|---------|--ABC-------|---------|ABCDEFGHIJK---------|-
Vernier[4]	-.*.....****.....***.....*.*.*.*....*.....**.....*.....
Canonical[5]	-.....1.11.1....1.....2...22.....2.....1.....
Interface[6]	-.....I...I.I.....I.I.....I.I.I.....I.....
5Å Proximity	**** * * **** *** *** ****** * * **** **
CDR	<-----> <----------> <--------->
AB_VHc (mAB001VH)	-EVQLVESGGGLVKPGGSLKLSCAASGFTLSSSAMS--WVRQTPDRRLEWVATISV--GGGKTYYPDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLI----------SLYWGQGTTLTVSS
BB7_VHc (mBB7001VH)	-AVQLVESGGGLVKPGGSLKLSCAASGIIFSSSAMS--WVRQTPEKRLEWVATISS--GGRSTYYPDSVKGRFTVSRDSAKNTLYLQMDSLRSEDTAIYYCAKLI----------SPYWGQGTTLTVSS
DC1_VHc (mDC001VH)	-EVQLVESGGGLVKPGGSLKLSCAASGFTLSTHAMS--WVRQTPEKRLEWVATISS--GGRSTYYPDSVKGRFTISRDNVKNTLYLQLSSLRSEDTAVYFCARLI----------STYWGQGTTLTVSS
AF062260	C....L.....Q.....R.....F..Y...--....A.GKG....SA..G--S..S...A.....S.....A....V.....DG----------GV.....LV....

Table 14 showing the alignment and residue identity of AF062260 to the murine antibodies. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable. Gaps (-) are used to maintain Kabat numbering, and to show residue insertion or deletion where applicable.

Table 15

	5Å Proximity Residues
AB_VHc (mAB001VH)	EVQLCAFTLSWVRWVARFTISRNLYCAKWG
BB7_VHc (mBB7001VH)	AVQLCAIIFSWVRWVARFTVSRSLYCAKWG
DC1_VHc (mDC001VH	EVQLCAFTLSWVRWVARFTISRNLFCARWG
AF062260	.....F.....S.....

Table 15 showing the antibody heavy chain framework residues that lie within a 5Å envelope of the CDR's. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable.

Table 16

```
                  ---------+---------+---------+---------+---------+---------+---------+---------+---------+--------
                           10        20        30        40        50        60        70        80        90
                  ---------+---------+---------+---------+---------+---------+---------+---------+---------+--------
Z12347.seq        EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
AF062260.seq      ..........................................................................................R     1
                                          <---->            <---------------->
```

Table 16 showing AF062260 has 1 somatic mutation away from the germline VH gene Z12347. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable.

Table 17

```
Kabat Numbers[2]    1       10        20              30        40        50        60        70        80        90              100
                    -|--------|---------|-------ABCDEF--|---------|---------|---------|---------|---------|---------|-----ABCDEF----|------A-
Vernier[4]          -.*.*.....................................**.........****..............*.*.**.*.....................................*..........
Canonical[5]        -.1.....................1.......1111..1.............2..22...........2......1...................3....3.....................
Interface[6]        -.....................................I.I.....I.....................................................I..I.............II..........
5Å Proximity         ******              **              **         ****       ***  ***********              **              **
CDR                                      <---------------->            <----->                                  <------------->
AB_VKc (mAB001VK)   -EIVLTQSPSSMYASLGERVTITCKASQ------DINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFP------YTFGGGTKLEI-K
 AY247656           -.........LS..V.D.....Q...-------..SN..N.Y.....A..L...DASN.ET...........T..TF.....QP..F.T...Q..NTY.------L.........-.
```

Table 17 showing the alignment and residue identity of AY247656 to the murine AB1 antibody. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable. Gaps (-) are used to maintain Kabat numbering, and to show residue insertion or deletion where applicable.

Table 18

	5Å Proximity Residues
AB_VKc (mAB001VK)	EIVLTQTCWFTLIYGVPFSGSGSGQDFFYCFG
AY247656	.........YL.............T..T....

Table 18 showing the AB1 antibody kappa light chain framework residues that lie within a 5Å envelope of the CDR's. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable.

Table 19

```
                ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+------
                         10        20        30        40        50        60        70        80        90
                ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+------
X93620.seq      DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLPP
AY247656.seq    E.VL.....................................................................FG.......NTY.L     5
                                  <---------->              <------>                              <-------
```

Table 19 showing AY247656 has 5 somatic mutation away from the germline VK gene X93620. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable.

Table 20

```
Kabat Numbers[2]      1       10        20              30        40        50        60        70        80        90              100
                      -|--------|---------|-------ABCDEF--|---------|---------|---------|---------|---------|-----ABCDEF----|------A-
Vernier[4]            -.*.*.....................................**.........****.............*.*.**.*.....................................*..........
Canonical[5]          -.1.....................1.......1111..1.............2..22...........2......1.................3....3...................
Interface[6]          -.....................................I.I.....I.....................................I..I............II..........
5Å Proximity           ******               **                **         ****       ***  ***********              **                **
CDR                                          <---------------->              <----->                                <-------------->
AB_VKc1 (mAB002VK)    -DIQMTQSPSSMYASLGERVTITCKASQ------DINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFP------YTFGGGTKLEI-K
AB1_VKc2 (mAB003VK)   -DIQKTQSPSSMYASLGERVTITCKASQ------DINSYLTWFQQKPGKSPKTLIYRTNRLFDGVPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFP------YTFGGGTKLEI-K
BB7_VKc (mBB001VK)    -AIKMTQSPSSMYASLGERVIITCKASQ------DINSYLTWFQQKPGKSPKTLIYLTNRLMDGVPSRFSGSGSGQEFLLTISGLEHEDMGIYYCLQYVDFP------YTFGGGTKLEI-K
DC1_VKc (mDC001VK)    -DITMTQSPSSIYASLGERVTITCKASQ------DINSYLTWFQQKPGKSPKILIYLVNRLVDGVPSRFSGSGSGQDYALTISSLEYEDMGIYYCLQYDDFP------YTFGGGTKLEI-K
AF193851              -...M......LS..V.D......R...------G.RN..A........A..S...AASN.QS.............T..T......QP..FAT...Q.HNTY.------W...Q...V..-.
```

Table 20 showing the alignment and residue identity of AF193851 to the murine antibodies. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable. Gaps (-) are used to maintain Kabat numbering, and to show residue insertion or deletion where applicable.

Table 21

	5Å Proximity Residues
AB_VKc1 (mAB002VK)	DIQMTQTCWFTLIYGVPFSGSGSGQDFFYCFG
AB1_VKc2 (mAB003VK)	DIQKTQTCWFTLIYGVPFSGSGSGQDFFYCFG
BB7_VKc (mBB001VK)	AIKMTQTCWFTLIYGVPFSGSGSGQEFLYCFG
DC1_VKc (mDC001VK)	DITMTQTCWFILIYGVPFSGSGSGQDYAYCFG
AF193851	...M.....S.....T..T....

Table 21 showing the antibody kappa light chain framework residues that lie within a 5Å envelope of the CDR's. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable.

Table 22

```
                  ---------+---------+---------+---------+---------+---------+---------+---------+---------+------
                           10        20        30        40        50        60        70        80        90
                  ---------+---------+---------+---------+---------+---------+---------+---------+---------+------
J00248.seq        DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPP
AF193851.seq      ............................R.....................N.....................................H.T..W      0
                                 <---------->              <----->                                  <-------
```

Table 22 showing AF193851 has no somatic mutation away from the germline VK gene J00248. Residue identities are shown by a dot (.) character. Residue differences are shown where applicable.

Table 23

```
Kabat Numbers          1       10        20        30        40        50        60        70        80        90       100                   110
                       -|--------|---------|---------|-----AB----|---------|--ABC-------|---------|---------|--ABC-------|---------|ABCDEFGHIJK---------|-
Vernier[4]             -.*.....................****.................***.....................*.*.*.*....*.................**.................*..........
Canonical[5]           -.....................1.11.1....1.....................2...22.............2.........................1.........................
Interface[6]           -.................................I...I.I.....I.I.....................................................I.I.I.............I..........
5Å Proximity           ****                 * *  ****      ***       ***                 ****** *    *                ****                    **
CDR                                          <----->          <------------------>                                    <---------------->
AB_RHA (hAB001HA)      -EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMS--WVRQAPGKGLEWVSTISV--GGGKTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLI-----------SLYWGQGTLVTVSS
BB7_RHA (hBB001HA)     -EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMS--WVRQAPGKGLEWVSTISS--GGRSTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLI-----------SPYWGQGTLVTVSS
BB7_RHB (hBB001HB)     -EVQLLESGGGLVQPGGSLRLSCAASGIIFSSSAMS--WVRQAPGKGLEWVATISS--GGRSTYYPDSVKGRFTVSRDSSKNTLYLQMNSLRAEDTAVYYCAKLI-----------SPYWGQGTLVTVSS
DC1_RHA (hDC001HA)     -EVQLLESGGGLVQPGGSLRLSCAASGFTFSTHAMS--WVRQAPGKGLEWVSTISS--GGRSTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLI-----------STYWGQGTLVTVSS
DC1_RHB (hDC001HB)     -EVQLLESGGGLVQPGGSLRLSCAASGFTLSTHAMS--WVRQAPGKGLEWVATISS--GGRSTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCARLI-----------STYWGQGTLVTVSS
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Table 23 showing the sequence alignments of the final humanised versions of AB1, BB7 and DC1 heavy chains. Gaps (-) are used to maintain Kabat numbering, and to show residue insertion or deletion where applicable.

5

TABLE 24

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Kabat Numbers          1       10        20              30        40        50        60        70        80        90              100
                       -|--------|---------|-------ABCDEF--|---------|---------|---------|---------|---------|---------|-----ABCDEF----|------A-
Vernier[4]             -.*.*.....................................**.........****.............*.*.**.*.....................................*..........
Canonical[5]           -.1.....................1.......1111..1.............2..22...........2......1...................3....3.....................
Interface[6]           -.....................................I.I.....I.....................................................I..I.............II..........
5Å Proximity           ******               **                **          ****       ***  ***********                **                **
CDR                                          <---------------->          <------>                                              <-------------->
AB_RKE (hAB001KE)      -EIVLTQSPSSLSASVGDRVTITCKASQ------DINSYLTWYQQKPGKAPKLLIYRTNRLFDGVPSRFSGSGSGTDFFFTISSLQPEDFGTYYCLQYDDFP------YTFGGGTKLEI-K
AB_RKJ (hAB001KJ)      -DIQMTQSPSSLSASVGDRVTITCKASQ------DINSYLTWFQQKPGKAPKSLIYRTNRLFDGVPSRFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFP------YTFGQGTKVEI-K
BB7_RKA (hBB001KA)     -DIQMTQSPSSLSASVGDRVTITCKASQ------DINSYLTWFQQKPGKAPKSLIYLTNRLMDGVPSRFSGSGSGTDFFLTISSLQPEDFATYYCLQYVDFP------YTFGQGTKVEI-K
BB7_RKB (hBB001KB)     -DIKMTQSPSSLSASVGDRVTITCKASQ------DINSYLTWFQQKPGKAPKTLIYLTNRLMDGVPSRFSGSGSGQEFLLTISSLQPEDFATYYCLQYVDFP------YTFGQGTKVEI-K
DC1_RKA (hDC001KA)     -DIQMTQSPSSLSASVGDRVTITCKASQ------DINSYLTWFQQKPGKAPKSLIYLVNRLVDGVPSRFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFP------YTFGQGTKVEI-K
DC1_RKB (hDC001KB)     -DITMTQSPSSLSASVGDRVTITCKASQ------DINSYLTWFQQKPGKAPKILIYLVNRLVDGVPSRFSGSGSGQDYALTISSLQPEDFATYYCLQYDDFP------YTFGQGTKVEI-K
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Table 24 showing the sequence alignments of the final humanised versions of AB1, BB7 and DC1 kappa light chains. Gaps (-) are used to maintain Kabat numbering, and to show residue insertion or deletion where applicable.

TABLE 24A

Table 24a summarises the sequence information presented in Tables 23 and 24, in particular showing the sequence of the CDRs, and the CDRs with flanking regions, in the heavy- and light-chains of the AB, BB-7 and DC-1 antibodies.

Heavy chain - CDR1	
AB	CAASGFTFSSSAMSWVR or FTFSSSAMSWVR or SSAMS.
BB7 RHB	CAASGFTFSSSAMSWVR or FTFSSSAMSWVR or SSAMS.
BB7 RHA	CAASGIIFSSSAMSWVR or IIFSSSAMSWVR or SSAMS.
DC1 RHA	CAASGFTFSTHAMSWVR or FTFSTHAMSWVR or THAMS.
DC1 RHB	CAASGFTLSTHAMSWVR or FTLSTHAMSWVR or THAMS.
Heavy chain – CDR2	
AB	WVSTISVGGGKTYYPDSVKGRFTISRDNSKNTL or WVSTISVGGGKTYYPDSVKGRFTISRDN or WVSTISVGGGKTYYPDSVKGRFTISR or TISVGGGKTYYPDSVKG.
BB7 RHB	WVSTISSGGRSTYYPDSVKGRFTISRDNSKNTL or WVSTISSGGRSTYYPDSVKGRFTISRDN or WVSTISSGGRSTYYPDSVKGRFTISR or TISSGGRSTYYPDSVKG.
BB7 RHA	WVATISSGGRSTYYPDSVKGRFTVSRDSSKNTL or WVATISSGGRSTYYPDSVKGRFTVSRDS or WVATISSGGRSTYYPDSVKGRFTVSR or TISSGGRSTYYPDSVKG.
DC1 RHA	WVSTISSGGRSTYYPDSVKGRFTISRDNSKNTL or WVSTISSGGRSTYYPDSVKGRFTISRDN or WVSTISSGGRSTYYPDSVKGRFTISR or TISSGGRSTYYPDSVKG.
DC1 RHB	WVATISSGGRSTYYPDSVKGRFTISRDNSKNTL or WVATISSGGRSTYYPDSVKGRFTISRDN or WVATISSGGRSTYYPDSVKGRFTISR or TISSGGRSTYYPDSVKG.
Heavy chain – CDR3	
AB	YCAKLISLYWG or LISLY.
BB7 RHB	YCAKLISPYWG or LISPY.
BB7 RHA	YCAKLISPYWG or LISPY.
DC1 RHA	YCAKLISTYWG or LISTY.
DC1 RHB	FCARLISTYWG or LISTY.

Light chain - CDR1	
AB RKE	TCKASQDINSYLTWY or KASQDINSYLT.
AB RKJ	TCKASQDINSYLTWF or KASQDINSYLT.
BB7 RKB	TCKASQDINSYLTWF or KASQDINSYLT.
BB7 RKA	TCKASQDINSYLTWF or KASQDINSYLT.
DC1 RKA	TCKASQDINSYLTWF or KASQDINSYLT.
DC1 RKB	TCKASQDINSYLTWF or KASQDINSYLT.
Light chain – CDR2	
AB RKE	LLIYRTNRLFDGVPSRFSGSGSGTDFF or LLIYRTNRLFDGVP or RTNRLFD
AB RKJ	SLIYRTNRLFDGVPSRFSGSGSGTDFF or SLIYRTNRLFDGVP or RTNRLFD
BB7 RKB	SLIYLTNRLMDGVPSRFSGSGSGTDFF or SLIYLTNRLMDGVP or LTNRLMD
BB7 RKA	TLIYLTNRLMDGVPSRFSGSGSGQEFL or TLIYLTNRLMDGVP or LTNRLMD
DC1 RKA	SLIYLVNRLVDGVPSRFSGSGSGTDFF or SLIYLVNRLVDGVP or LVNRLVD
DC1 RKB	ILIYLVNRLVDGVPSRFSGSGSGQDYA or ILIYLVNRLVDGVP or LVNRLVD
Light chain – CDR3	
AB RKE	YCLQYDDFPYTFG or LQYDDFPYT.
AB RKJ	YCLQYDDFPYTFG or LQYDDFPYT.
BB7 RKB	YCLQYVDFPYTFG or LQYVDFPYT.
BB7 RKA	YCLQYVDFPYTFG or LQYVDFPYT.
DC1 RKA	YCLQYDDFPYTFG or LQYDDFPYT.
DC1 RKB	YCLQYDDFPYTFG or LQYDDFPYT.

Characterization of chimeric and humanised antibodies

Chimeric and humanised Abs were assayed for binding to human and cynomolgus monkey TG2 and for enzymatic inhibition of these enzymes according to the methodology described below.

METHODS

ELISA assay for TG2 binding

Antibody binding to human and cynomolgus monkey TG2 was determined in an ELISA assay. Clear polystyrene "Maxisorp" 96-well plates (Nunc) were coated with 50ng purified human or cynomogulus monkey TG2 in 50µl 0.05 M carbonate-bicarbonate buffer pH 9.6 at 4°C overnight. Control wells were coated with 50µl 100µg/ml bovine serum albumin (BSA). Plates were washed 3x with 300 µl phosphate-buffered saline pH7.4 (PBS) containing 0.1% Tween 20 (PBST) and blocked with 300 µl 3% w/v Marvel skimmed milk in PBS for 1 hour at room temperature. After 3x wash with PBST, 50µl protein-A purified chimeric or humanised anti-TG2 antibodies or human IgG1 kappa isotope control antibody or CUB7402 (Abcam) were serially diluted 4-fold from a top concentration of 50nM in PBS, and added to the plate in duplicate. After 1 hour at room temperature, the plates were washed 3x in PBST and incubated with 50µl peroxidase-conjugated goat anti-human IgG (Fc) (Serotec) diluted 1/5,000 in 3% w/v Marvel skimmed milk in PBS or for wells containing CUB7402 peroxidase-conjugated 1/5,000 goat anti-mouse IgG (Fc) (Sigma) for 1 hour at room temperature. After 3x washes with PBST, the plates were developed with 50µl TMB substrate (Sigma) for 5 min at room temperature before stopping the reaction with 25µl 0.5M H_2SO_4 and reading absorbance at 450nM in a microtiter plate reader (BioTek EL808). Dose response curves were analysed and EC50 values and other statistical parameters determined using a 4-parameter logistical fit of the data (GraphPad Prism).

Fluorescence-based transglutaminase assay of TG2 inhibition by antibodies of the invention.

Transglutaminase activities of purified human (Zedira) or cynomogolus monkey TG2 enzymes (Trenzyme) were measured by incorporation of dansylated lysine KxD (Zedira)

into *N,N*-dimethylated casein (DMC, Sigma). Human or cynomogolus monkey TG2 were diluted in transamidation buffer (25mM HEPES pH 7.4 containing 250mM NaCl, 2mM $MgCl_2$, 5mM $CaCl_2$, 0.2mM DTT and 0.05% v/v Pluronic F-127) to 1nM and 10nM respectively and mixed with various concentrations of protein-A purified murine, chimeric or humanised TG2 antibodies for 180 min at room temperature in 384-well black microtiter plates (Corning). Reactions were initiated by addition of DMC and KxD to a final concentration of 10uM and 20uM respectively and a final reaction volume of 30ul, and allowed to proceed at RT for 180min and the increase in fluorescence (RFU) (excitation at 280nm, emission 550nm) monitored using a Tecan Safire^2 plate reader. Data were normalised to percentage activity where %activity=(RFU test antibody –RFU low controls)/ RFU high controls –RFU low controls) x 100, where low controls contained all components except enzyme and high controls contained all components except antibody.

Antibody dose response curves were plotted using GraphPad prism software and fitted using a 4-parameter logistical model to return IC50 and other statistical parameters. The results are illustrated in Figures 29 to 33.

Results and discussion of enzyme inhibition and ELISA binding experiments by humanized and murine anti-TG2 antibodies

The ability of chimeric and humanized TG2 antibodies to inhibit transamidation by human TG2 was determined by dose-dependent inhibition of TG2-dependent incorporation of dansylated lysine into *N,N*-dimethylated casein (exemplified in figures 29 and 31). Both chimeric and humanized antibodies from group 1 (e.g. cAB003, cBB001, cDC001, hBB001AA, hAB001BB, hAB005 and hAB004) show potent inhibition of TG2 activity in the low nanomolar range, consistent with ELISA data that shows binding to immobilized human TG2 in the same range (figures 35 and 37). In contrast, the commercial antibody CUB7402 failed to inhibit human or cynomogulus monkey TG2 enzymatic activities (figures 29F and 30B), despite comparable binding to group 1 antibodies in the ELISA assays (figures 35A, 36A, 37A and 38A) consistent with recognition by CUB7402 of an epitope that does not interfere with the transamidation function of the enzyme. Therefore group 1 antibodies can be distinguished by their ability to inhibit enzyme function from other antibodies such as CUB7402, which bind but have no effect on enzymatic activity. Similarly, murine and chimeric antibodies representative of group 3 (e.g. mDD9001, mDH001, cDD9001 and cDH001) consistently inhibited human TG2 transamidation, but with lower potency than group 1 antibodies (figures 29 and 33). Inhibition of human TG2

by exemplified parent murine monoclonal antibodies from group 1 and group 3 (figure 33) show comparable potencies to their chimeric and humanized versions, indicating that the functional potency of the murine antibodies has been retained in the humanized versions. Similarly the human TG2 ELISA binding data for the exemplified humanized antibodies hBB001AA, hBB001BB and hAB004 (figure 37) show comparable EC50 values with those obtained for the chimeric versions cBB001 and cAB003, indicating that binding affinity has also been preserved in the humanized versions. Chimeric and humanized antibodies also demonstrate potent inhibition of cynomogulus monkey TG2 (figures 30 and 32) and comparable ELISA EC50's (figures 36 and 38) across the species, consistent with the conservation of the cognate epitope in cynomogulus monkey TG2, which has overall 99% sequence identity. In contrast CUB7402 shows comparable binding to TG2 of both species as the group 1 antibodies, but inhibits neither enzyme activity (figures 35-38 and 29-30).

Cell based assays

Binding of antibodies of the invention to extracellular TG2 from HK-2 epithelial cells was assayed using the following protocol.

Measurement of extracellular TG activity

Extracellular TG activity was measured by modified cell ELISA. HK-2 epithelial cells were harvested using Accutase and plated at a density of 2×10^4 cells/well in serum free medium onto a 96 well plate that had been coated overnight with 100 µl/well of fibronectin (5 µg/ml in 50 mM Tris–HCl pH 7.4) (Sigma, Poole UK). Cells were allowed to attach for O/N at 37 °C. Media was replaced with DMEM (Life Technologies) and compounds, antibodies or controls were added and allowed to bind at 37°C. 0.1 mM biotin cadaverine [N-(5 amino pentyl biotinamide) trifluoroacetic acid] (Zedira) was added to wells and the plate returned to 37 °C for 2 hours. Plates were washed twice with 3 mM EDTA/PBS and cells removed with 0.1% (w/v) deoxycholate in 5 mM EDTA/PBS. The supernatant was collected and used for protein determination. Plates were washed with PBS/Tween and incorporated biotin cadaverine revealed using 1:5000 extravidin HRP (Sigma, Poole, UK) for 1 h at room temperature followed by K Blue substrate (SkyBio). The reaction was stopped with Red Stop (SkyBio) and the absorbance read at 4
650 nm. Each antibody was tested on at least three separate occasions.

Results are provided in Figures 39 and 40 and show an exemplar curve and table of IC50 values obtained for the antibodies tested. Figure 39 displays the results with Humanised AB1 and Figure 40 displays the results with Humanised BB7.

hAB005 inhibited the extracellular TG2 of the HK2 cells with an IC50 of 71.85nM and a maximal inhibition of about 30% control activity. hBB001AA inhibited the activity with an IC50 of 19.8nM and a maximal inhibition of 40% control activity. hBB001BB had a better IC50 of 4.9nM but a maximal inhibition of about 55% control.

Scratch assays

Scratch wound assays were also performed to assess binding activity of humanised and or chimeric anti-TG2 antibodies of the invention.

TG2 has been shown to have an important role in lung fibrosis and TG2 knockout mice show reduced scarring and fibrosis in the bleomycin model. (Keith C. Olsen, Ramil E. Sapinoro, R. M. Kottmann, Ajit A. Kulkarni, Siiri E. Iismaa, Gail V. W. Johnson, Thomas H. Thatcher, Richard P. Phipps, and Patricia J. Sime. (2011) Transglutaminase 2 and Its Role in Pulmonary Fibrosis. Am.J of Respiratory & Critical Care Med. **184** 0699-707) Migration of cells from TG2 knockout mice on wounding was reduced compared to wild type. Scratch wound assay were performed to assess the effect of humanised and/or chimeric anti-TG2 antibodies of the invention on the rate of wound closure in a layer of normal lung fibroblasts (WI-38 cells).

Scratch assay protocol:

WI-38 cells (normal human lung fibroblasts ATCC cat#CCL-75) were plated in a 96 well Image Lock plate (Essen cat#4379) at 2 x 10^4/well in αMEM media (Life Technologies cat#32561) with 10% FBS and grown O/N to >97% confluence. Cells were washed 2X with αMEM media without serum and a scratch wound was generated using an Essen Wound Maker and the manufacturers protocol. The media was removed and replaced with 95µl/well serum free media. Controls and test antibodies were added to the wells. The plate was placed in an Essen Incucyte and the closure of the wound was analysed using the scratch wound protocol.

Cytochalasin D was used as an assay control at 0.1µM. R281, a small molecule non-specific transglutaminase inhibitor, was tested at 100µM. Z DON, a peptide non reversible transglutaminase inhibitor was tested at 10µM and 100µM. The commercially available TG2 antibody Cub7402 (ABcam cat#ab2386) was tested at 5µg/ml. Antibodies of the invention were tested on at least three occasions at various concentrations as indicated. In all experiments controls were Cytochalasin D at 0.1uM and ZDON at two concentrations to show a dose dependant effect.

Exemplar results of the scratch assays are shown in Figures 41 to 44. As can be seen in Figure 41, Cytochalasin D, R281 and ZDON all inhibited wound closure (ZDON was shown to inhibit in a dose dependant manner) but the antibody Cub7402 did not inhibit wound closure. Humanised BB7, Humanised AB1 and Chimeric DC1 all inhibited wound closure.

Affinities of chimeric and humanised anti TG2 Abs

The binding affinities (Kds and off rates) for a panel of chimeric and humanised Abs of the invention against human TG2 and cyano TG2 were assessed using Biacore techniques. The protocols and results are described below and shown in Figures 45 to 47.

Biacore methods

Recombinant human TG2 was obtained from Zedira GmbH (cat. no.: T002). Recombinant cynomolgus monkey TG2 was obtained from Trenzyme. Surface plasmon resonance (SPR) was measured on a Biacore T200 instrument (GE Healthcare). CM5 chips (GE Healthcare cat. no.: BR-1006-68) were coated with monoclonal mouse anti-human IgG1 (Fc) (MAH) antibody (GE Healthcare cat. no.: BR-1008-39) by amine-coupling as described in the manufacturers instructions. HBS-EP+ buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20) and HBS-P+ buffer (0.01 M HEPES, 0.15 M NaCl, 0.05 % Surfactant P20) were purchased from GE Healthcare as 10x stocks (cat. nos.: BR-1006-69 and BR-1006-71). Calcium Chloride solution was obtained from Sigma Aldrich (cat. no.:21115).

The method employed to determine the affinity of the anti-TG2 antibodies involved the capture of the chimeric or humanised antibodies on a MAH coated CM5 chip, followed by the injection of a series of TG2 samples in running buffer. The running buffer was 1x HBS-P+ containing 1 mM $CaCl_2$, or 1x HBS-EP+ for calcium-free experiments. Antibody capture was carried out for a contact time of 120 seconds at a flow rate of 10 µl/min resulting in the capture of approximately 40-80 RU. TG2 was injected over the immobilised antibody at concentrations ranging from 25 nM to 400 nM with a contact time up to 600 seconds at a flow rate of 30 µl/min. Dissociation of TG2 was typically measured for up to 5400 seconds (1.5 hours). Regeneration of the chip was then performed using 3 M $MgCl_2$, for a contact time of 60 seconds at a flow rate of 30µl/ml, followed by a 300s stabilisation period before the next sample. For each of human and

cynomolgus monkey TG2, at least 5 injections at a variety of concentrations were performed in at least two separate experiments.

Kinetic data were exported from the Biacore T200 Evaluation Software and analysed using GraphPad Prism, where the association phases and dissociation phases were analysed separately using a one phase association model and one-phase exponential decay model respectively. Association rates (k_{on}) were calculated for each curve individually, and dissociation rates (k_{off}) values from the long dissociation phase data collected. Where k_{off} values were calculated to be < $1x10^{-5}$ s^{-1}, values were set at $1x10^{-5}$ for analysis, as rates slower than this could not be estimated accurately. Values for k_{on} and k_{off} are presented in the tables below as the mean of the individual calculated values for each antibody for each TG2 species from multiple concentrations +/- 1 standard deviation. K_D values are calculated as mean k_{off} / mean k_{on}.

Results of Biacore experiments

Table 25

Antibody		Human TG2				
		k_{off} (s^{-1})	st.dev	k_{on} ($M^{-1}s^{-1}$)	st.dev	K_D (M)
cAB003	+ Ca^{2+}	$< 10^{-5}$	-	$1.7x10^{5}$	$3.2x10^{4}$	$< 6x10^{-11}$
	- Ca^{2+}	$< 10^{-5}$	-	$8.6x10^{4}$	$2.1x10^{4}$	$< 1x10^{-10}$
cBB001	+ Ca^{2+}	$< 10^{-5}$	-	$2.1x10^{5}$	$6.9x10^{4}$	$< 5x10^{-11}$
	- Ca^{2+}	$< 10^{-5}$	-	$1.5x10^{5}$	$1.8x10^{4}$	$< 7x10^{-11}$
hAB004 (hAB001AE)		$2.4x10^{-5}$	$1.0x10^{-5}$	$2.0x10^{5}$	$1.4x10^{5}$	$1.2x10^{-10}$
hAB005 (hAB001AJ)		$< 10^{-5}$	-	$1.9x10^{5}$	$6.8x10^{4}$	$< 5x10^{-11}$
hBB001AA		$< 10^{-5}$	-	$2.7x10^{5}$	$1.1x10^{5}$	$< 4x10^{-11}$
hBB001BB		$< 10^{-5}$	-	$2.4x10^{5}$	$1.2x10^{5}$	$< 4x10^{-11}$
cDC001		$< 10^{-5}$	-	$3.2x10^{5}$	$5.7x10^{4}$	$< 3x10^{-11}$
cDH001	+ Ca^{2+}	$1.8x10^{-5}$	$4.9x10^{-6}$	$2.8x10^{4}$	$1.3x10^{4}$	$6.4x10^{-10}$
	- Ca^{2+}	$4.9x10^{-4}$	$1.6x10^{-5}$	$2.1x10^{4}$	$1.2x10^{4}$	$2.3x10^{-8}$
cDD9001	+ Ca^{2+}	$1.3x10^{-5}$	$1.8x10^{-6}$	$3.0x10^{4}$	$2.3x10^{4}$	$4.3x10^{-10}$
	- Ca^{2+}	$7.1x10^{-5}$	$1.6x10^{-5}$	$2.3x10^{4}$	$7.6x10^{3}$	$3.1x10^{-9}$

Table 25 shows the kinetic data obtained against human TG2. Where k_{off} rates were calculated to be less than 10^{-5} s^{-1}, values were set to 10^{-5} s^{-1} for analysis, as rates slower than this could not be accurately determined.

Table 26

Antibody		Cynomolgus TG2				
		k_{off} (s^{-1})	st.dev	k_{on} ($M^{-1}s^{-1}$)	st.dev	K_D (M)
cAB003	+ Ca^{2+}	$1.2x10^{-5}$	$8.1x10^{-6}$	$2.4x10^{5}$	$1.1x10^{5}$	$5.0x10^{-11}$

	- Ca^{2+}	$1.3x10^{-5}$	$1.5x10^{-6}$	$1.4x10^{5}$	$1.9x10^{4}$	$9.3x10^{-11}$
cBB001	+ Ca^{2+}	$< 10^{-5}$	-	$2.9x10^{5}$	$9.0x10^{4}$	$< 3x10^{-11}$
	- Ca^{2+}	$< 10^{-5}$	-	$1.9x10^{5}$	$2.1x10^{4}$	$< 5x10^{-11}$
hAB004 (hAB001AE)		$1.8x10^{-5}$	$2.3x10^{-6}$	$1.6x10^{5}$	$7.3x10^{4}$	$1.1x10^{-11}$
hAB005 (hAB001AJ)		$3.4x10^{-5}$	$4.6x10^{-6}$	$1.4x10^{5}$	$4.1x10^{4}$	$2.4x10^{-10}$
hBB001AA		$< 10^{-5}$	-	$3.0x10^{5}$	$1.4x10^{5}$	$< 3x10^{-11}$
hBB001BB		$3.7x10^{-5}$	$3.4x10^{-6}$	$2.7x10^{5}$	$1.3x10^{5}$	$1.4x10^{-10}$
cDC001		$< 10^{-5}$	-	$4.2x10^{5}$	$3.3x10^{5}$	$2.4x10^{-11}$
cDH001	+ Ca^{2+}	$< 10^{-5}$	-	$1.5x10^{4}$	$9.5x10^{3}$	$< 7x10^{-10}$
	- Ca^{2+}	$6.3x10^{-5}$	$1.2x10^{-5}$	$2.6x10^{4}$	$1.0x10^{4}$	$2.4x10^{-9}$
cDD9001	+ Ca^{2+}	$1.6x10^{-5}$	$1.4x10^{-5}$	$3.1x10^{4}$	$1.6x10^{4}$	$5.2x10^{-10}$
	- Ca^{2+}	$4.1x10^{-5}$	$1.6x10^{-5}$	$4.6x10^{4}$	$1.3x10^{4}$	$8.9x10^{-10}$

Table 26 shows the kinetic data obtained against cynomolgus TG2. Where k_{off} rates were calculated to be less than 10^{-5} s^{-1}, values were set to 10^{-5} s^{-1} for analysis, as rates slower than this could not be accurately determined.

Figures 45 to 47 provide example Biacore data sets. As can be seen, the humanised and chimeric antibodies cAB003, cBB001, hAB004, hAB005, hBB001AA, hBB001BB, cDC001 for TG2 have excellent affinity for human and cynomolgus TG2, with K_D values of 120pM or better. The chimeric antibodies cDH001 and cDD9001 exhibit slower association rates to human and cynomolgus TG2 and weaker overall affinity. Examination of a selection of antibodies in the absence of calcium shows that there is little or no effect, except in the case of cDH001 and cDD9001, where binding is weaker due to faster dissociation rates (k_{off}).

CLAIMS

1. An antibody or an antigen-binding fragment thereof that selectively binds to an epitope within the core region of transglutaminase type 2 (TG2) and that inhibits human TG2 cross-linking lysine and glutamine with Nε(γ-glutamyl)lysine isopeptide bonds, wherein the antibody or antigen-binding fragment thereof comprises the following complementarity determining region (CDR) sequences or CDR and flanking region sequences, or CDR or CDR and flanking region sequences having at least 90% identity with the following:

(a) a LCDR1 sequence, or LCDR1 and flanking region sequence, selected from the group consisting of KASQDINSYLT, TCKASQDINSYLIWF and TCKASQDINSYLIWY;

(b) a LCDR2 sequence, or LCDR2 and flanking region sequence, selected from the group consisting of RTNRLFD, TLIYRTNRLFDGVP, LLIYRTNRLFDGVP, SLIYRTNRLFDGVP, LTNRLMD, LVNRLVD, LLIYRTNRLFDGVPSRFSGSGSGTDFF, SLIYRTNRLFDGVPSRFSGSGSGTDFF, SLIYLTNRLMDGVPSRFSGSGSGTDFF, TLIYLTNRLMDGVPSRFSGSGSGQEFL, SLIYLVNRLVDGVPSRFSGSGSGTDFF, ILIYLVNRLVDGVPSRFSGSGSGQDYA, TLIYLTNRLMDGVP, SLIYLTNRLMDGVP, SLIYLVNRLVDGVP, LVNRLVD and ILIYLVNRLVDGVP;

(c) a LCDR3 sequence, or LCDR3 and flanking region sequence, selected from the group consisting of LQYDDFPYT, YCLQYDDFPYTFG, LQYVDFPYT, LQYVDFPYT and YCLQYVDFPYTFG;

(d) an HCDR1 sequence, or HCDR1 and flanking region sequence, selected from the group consisting of SSAMS, FTLSSSAMSWVR, FTFSSSAMSWVR, THAMS, CAASGFTFSSSAMSWVR, CAASGIIFSSSAMSWVR, IIFSSSAMSWVR, CAASGFTFSTHAMSWVR, FTFSTHAMSWVR, CAASGFTLSTHAMSWVR and FTLSTHAMSWVR;

(e) an HCDR2 sequence, or HCDR2 and flanking region sequence, selected from the group consisting of TISVGGGKTYYPDSVKG, VATISVGGGKTYYPDSVKGRFTISR, WVSTISVGGGKTYYPDSVKGRFTISR, TISSGGRSTYYPDSVKG, TISSGGRSTYYPDSVKG, WVSTISVGGGKTYYPDSVKGRFTISRDNSKNTL, WVSTISVGGGKTYYPDSVKGRFTISRDN, WVSTISSGGRSTYYPDSVKGRFTISRDNSKNTL, WVSTISSGGRSTYYPDSVKGRFTISRDN, WVSTISSGGRSTYYPDSVKGRFTISR, WVATISSGGRSTYYPDSVKGRFTVSRDSSKNTL, WVATISSGGRSTYYPDSVKGRFTVSRDS, WVATISSGGRSTYYPDSVKGRFTVSR, WVATISSGGRSTYYPDSVKGRFTISRDNSKNTL, WVATISSGGRSTYYPDSVKGRFTISRDN and WVATISSGGRSTYYPDSVKGRFTISR; and

(f) an HCDR3 sequence, or HCDR3 and flanking region sequence, selected from the group consisting of LISLY, YCAKLISLYWG, LISPY, LISTY, YCAKLISPYWG, YCAKLISTYWG and FCARLISTYWG.

2. The antibody or antigen-binding fragment thereof of Claim 1, wherein the core region consists of amino acids 143 to 473 of human TG2.

3. The antibody or antigen-binding fragment thereof of Claim 1 or 2, wherein the antibody or antigen-binding fragment thereof selectively binds in whole or in part to a region comprising:

i) amino acids 304 to 326 of human TG2; or

ii) amino acids 351 to 365 of human TG2; or

iii) amino acids 450 to 467 of human TG2.

4. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment thereof comprises the following amino acid

sequences, or amino acid sequences having at least 90% identity with the following amino acid sequences:

(a) KASQDINSYLT (LCDR1);
LVNRLVD (LCDR2);
LQYDDFPYT (LCDR3);
THAMS (HCDR1);
TISSGGRSTYYPDSVKG (HCDR2); and
LISTY (HCDR3); or

(b) KASQDINSYLT (LCDR1);
LTNRLMD (LCDR2);
LQYVDFPYT (LCDR3);
SSAMS (HCDR1);
TISSGGRSTYYPDSVKG (HCDR2); and
LISPY (HCDR3); or

(c) KASQDINSYLT (LCDR1);
RTNRLFD (LCDR2);
LQYDDFPYT (LCDR3);
SSAMS (HCDR1);
TISVGGGKTYYPDSVKG (HCDR2); and
LISLY (HCDR3).

5. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment thereof has at least one light chain variable region comprising the amino acid sequence DIKMTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFD GVPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK, or DIQMTQTPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFD GVPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK, or

EIVLTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFDG
VPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (AB-
1_VK), or
DIQMTQSPSSMYASLGERVTITCKASQDINSYLTWFQQKPGKSPKTLIYRTNRLFD
GVPSRFSGSGSGQDFFLTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (AB-
1_VK1), or
EIVLTQSPSSLSASVGDRVTITCKASQDINSYLTWYQQKPGKAPKLLIYRTNRLFDG
VPSRFSGSGSGTDFFFTISSLQPEDFGTYYCLQYDDFPYTFGGGTKLEIK (hAB-
1_RKE), or
DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYRTNRLFD
GVPSRFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK (hAB-
1_RKJ), or
AIKMTQSPSSMYASLGERVIITCKASQDINSYLTWFQQKPGKSPKTLIYLTNRLMD
GVPSRFSGSGSGQEFLLTISGLEHEDMGIYYCLQYVDFPYTFGGGTKLEIK (BB-7
VK), or
DITMTQSPSSIYASLGERVTITCKASQDINSYLTWFQQKPGKSPKILIYLVNRLVDG
VPSRFSGSGSGQDYALTISSLEYEDMGIYYCLQYDDFPYTFGGGTKLEIK (DC-1
VK), or
QIVLTQSPAIMSASPGEKVTMTCSASSSVDYMYWYQQKPGSSPRLLIYDTSNLASG
VPVRFSGSGSGTSYSLTISRMGAEDAATYYCQQWNSSPLTFGAGTKLELK (DD-9
VK), or
QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMYWYQQKPGSSPRLLIYDTSNLASG
VPVRFSGSGSGTSYSLTISRMEAEDAATFYCQQWSSSPLTFGAGTKLELK (DH-2
VK), or
DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYLTNRLMD
GVPSRFSGSGSGTDFFLTISSLQPEDFATYYCLQYVDFPYTFGQGTKVEIK, or
DIKMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKTLIYLTNRLMD
GVPSRFSGSGSGQEFLLTISSLQPEDFATYYCLQYVDFPYTFGQGTKVEIK, or
DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYLVNRLVD
GVPSRFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK, or

DITMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKILIYLVNRLVDG VPSRFSGSGSGQDYALTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK; and at least one heavy chain variable region comprising the amino acid sequence EVQLVESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGG GKTYYPDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTT LTVSS, or EVQLEESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGG GKTYYPDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTT LTVSS, or EVQLQESGGGLVKPGGSLKLSCAASGFTLSSSAMSWVRQTPDRRLEWVATISVGG GKTYYPDSVKGRFTISRDNAKNTLYLQMNSLRSEDTAMYYCAKLISLYWGQGTT LTVSS (AB1_VH), or EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAPGKGLEWVSTISVGG GKTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISLYWGQGTLV TVSS (hAB-1_RHA), or AVQLVESGGGLVKPGGSLKLSCAASGIIFSSSAMSWVRQTPEKRLEWVATISSGGR STYYPDSVKGRFTVSRDSAKNTLYLQMDSLRSEDTAIYYCAKLISPYWGQGTTLT VSS (BB-7VH), or EVQLVESGGGLVKPGGSLKLSCAASGFTLSTHAMSWVRQTPEKRLEWVATISSGG RSTYYPDSVKGRFTISRDNVKNTLYLQLSSLRSEDTAVYFCARLISTYWGQGTTLT VSS (DC-1VH), or QVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVSWIRQSSGKGLEWLAHIYWD DDKRYNPSLKSRITISKDSSSNQVFLKITSVDTADTATYYCARSWTTAPFAFWGQG TLVTVSA (DD-9 VH), or QVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVSWIRQPSGKGLEWLAHIYWD DDKRYNPSLKSRLTISKDTSSNQVFLKITSVDTADTATYYCARSGTTAPFAYWGQ GTLVTVSA (DH-2 VH), or EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAPGKGLEWVSTISSGG RSTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISPYWGQGTLV TVSS, or

EVQLLESGGGLVQPGGSLRLSCAASGIIFSSSAMSWVRQAPGKGLEWVATISSGGR
STYYPDSVKGRFTVSRDSSKNTLYLQMNSLRAEDTAVYYCAKLISPYWGQGTLVT
VSS, or
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTHAMSWVRQAPGKGLEWVSTISSGG
RSTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISTYWGQGTLV
TVSS, or
EVQLLESGGGLVQPGGSLRLSCAASGFTLSTHAMSWVRQAPGKGLEWVATISSGG
RSTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCARLISTYWGQGTLV
TVSS, or an amino acid sequence having at least 90% identity with one or more of the amino acid sequences specified above.

6. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or fragment thereof has:

(a) a light chain variable region comprising the sequence
DITMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKILIYLVNRLVDG
VPSRFSGSGSGQDYALTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK, and a heavy chain variable region comprising the sequence
EVQLLESGGGLVQPGGSLRLSCAASGFTLSTHAMSWVRQAPGKGLEWVATISSGG
RSTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCARLISTYWGQGTLV
TVSS,
or amino acid sequences having at least 90% identity therewith; or

(b) a light chain variable region comprising the sequence
DIKMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKTLIYLTNRLMD
GVPS RFSGSGSGQEFLLTISSLQPEDFATYYCLQYVDFPYTFGQGTKVEIK, and a heavy chain variable region comprising the sequence
EVQLLESGGGLVQPGGSLRLSCAASGIIFSSSAMSWVRQAPGKGLEWVATISSGGR
STYYPDSVKGRFTVSRDSSKNTLYLQMNSLRAEDTAVYYCAKLISPYWGQGTLVT
VSS,
or amino acid sequences having at least 90% identity therewith; or

(c) a light chain variable region comprising the sequence

EIVLTQSPSSLSASVGDRVTITCKASQDINSYLTWYQQKPGKAPKLLIYRTNRLFDG
VPSRFSGSGSGTDFFFTISSLQPEDFGTYYCLQYDDFPYTFGGGTKLEIK, or
DIQMTQSPSSLSASVGDRVTITCKASQDINSYLTWFQQKPGKAPKSLIYRTNRLFDG
VPSRFSGSGSGTDFFLTISSLQPEDFATYYCLQYDDFPYTFGQGTKVEIK,
and a heavy chain variable region comprising the sequence
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAPGKGLEWVSTISVGG
GKTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLISLYWGQGTLV
TVSS,
or amino acid sequences having at least 90% identity therewith.

7. An anti-TG2 antibody or an antigen-binding fragment thereof, which comprises:

(a) a light chain variable region comprising the sequence
DVLMTQNPLSLPVSLGDQASISCRSSQSIEHINGNTYLEWYLQKPGQSPKFLIYKVS
NRFSGVPDRFSGSGSGTDFTLRISRVEAEDLGIYYCFQGSHVPFTFGGGTKLEIK,
and a heavy chain variable region comprising the sequence
EVQLQQSGPELVKPGASVKMSCKASGYRFTSYVMHWVKQKSGQGLEWIGYINPY
NDGAKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARLSSDYWGQGT
TLTVSS; or

(b) a light chain variable region comprising the sequence
DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKV
SNRFSGVPDRFSGSGSGTDFTLKISRVGAEDLGVYYCLQVSHVPFTFGSGTKLEIK,
and a heavy chain variable region comprising the sequence
EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGFINPY
NDGTKYNEKFKGKATLTSDKASTTAYMELSSLTSEDSAVYYCARFSSGYWGQGT
TLTVSS; or

(c) a light chain variable region comprising the sequence
DVLMTQNPLSLPVSLGDQASISCRSSRSIEHSNGNTYLEWYLQKPGQSPKFLIYKVS
NRFSGVPDRFSGSGSGTDFTLRISSVEAEDLGVYYCFQGSHVPFTFGGGTKLEIK,
and a heavy chain variable region comprising the sequence
EVQLQQSGPELVKPGASVKMSCRASGYTFTTYVIHWVKQKPGQGLEWIGYINPYN

DGARYNEKFKGKATLTSDKSSTTAYMELSSLTSEDSAVYYCARLSSDYWGQGTTL TVSS; or

(d) a light chain variable region comprising the sequence QIVLTQSPAIMSASPGEKVTMTCSASSSVDYMYWYQQKPGSSPRLLIYDTSNLASG VPVRFSGSGSGTSYSLTISRMGAEDAATYYCQQWNSSPLTFGAGTKLELK, and a heavy chain variable region comprising the sequence QVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVSWIRQSSGKGLEWLAHIYWD DDKRYNPSLKSRITISKDSSSNQVFLKITSVDTADTATYYCARSWTTAPFAFWGQG TLVTVSA; or

(e) a light chain variable region comprising the sequence QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMYWYQQKPGSSPRLLIYDTSNLASG VPVRFSGSGSGTSYSLTISRMEAEDAATFYCQQWSSSPLTFGAGTKLELK, and a heavy chain variable region comprising the sequence QVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVSWIRQPSGKGLEWLAHIYWD DDKRYNPSLKSRLTISKDTSSNQVFLKITSVDTADTATYYCARSGTTAPFAYWGQ GTLVTVSA; or

(f) a light chain variable region comprising the sequence QAVVTQESALTTSPGETVTLTCRSSTGAVAANNYANWIQEKPDHLFTGLIAGTNK RAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNYWVFGGGTKVTVLG, and a heavy chain variable region comprising the sequence EVQLQQSGPELVKPGASVKMSCKASGYRFTDYNMHWVKQNLGKSLEWIGYINPK NGVIYYNQKFKGKATLTVNRSSNTAYMEIRSLTSEDSAVYYCATALTYWGQGTL VTVSA.

8. The antibody of any preceding claim, which comprises or consists of an intact antibody.

9. The antigen-binding fragment of any preceding claim, which comprises or consists of an antigen-binding fragment selected from the group consisting of: an Fv fragment, for example a single chain Fv fragment or a disulphide-bonded Fv fragment; an Fab fragment; and an Fab-like fragment, for example an Fab' fragment or an $F(ab)_2$ fragment.

10. The antibody or antigen-binding fragment thereof of any preceding claim, which is an IgG1, IgG2, IgG3 or IgG4.

11. A polynucleotide encoding an antibody or an antigen-binding fragment thereof according to any of Claims 1 to 10.

12. An antibody according to any of Claims 1 to 10, wherein the antibody or antigen-binding fragment thereof does not inhibit TG1, TG3, TG13 and/or TG7 activity.

13. An antibody or antigen-binding fragment thereof that selectively binds to an epitope within the core region of transglutaminase type 2 (TG2) and that inhibits human TG2 cross-linking lysine and glutamine with Nε(γ-glutamyl)lysine isopeptide bonds whose binding to TG2, for example human TG2, is inhibited or reduced when an antibody according to any of Claims 1 to 10 or 12 is bound to TG2, for example human TG2.

14. A compound comprising an antibody or antigen-binding fragment thereof according to any of Claims 1 to 10, 12 or 13 and a further moiety, for example a readily detectable moiety, and/or a directly or indirectly cytotoxic moiety.

15. A pharmaceutical composition/formulation comprising an antibody or antigen-binding fragment thereof according to any of Claims 1 to 10, 12 or 13, or a compound according to Claim 14, in admixture with a pharmaceutically acceptable excipient, adjuvant, diluent or carrier.

16. The pharmaceutical composition/formulation according to Claim 15, wherein the composition/formulation is formulated for intravenous, intramuscular, or subcutaneous delivery to a patient.

17. An antibody or antigen-binding fragment thereof according to any of Claims 1 to 10, 12 or 13, or a compound according to Claim 14, for use in medicine.

18. Use of an antibody or antigen-binding fragment thereof according to any of Claims 1 to 10, 12 or 13, or a compound according to Claim 14, in the manufacture of a medicament for treating and/or diagnosing Celiac disease, abnormal wound healing, scarring, hypertrophic scars, ocular scarring, inflammatory bowel disease, macular degeneration, psoriasis, fibrosis-related diseases (e.g. liver fibrosis, pulmonary fibrosis such as interstitial lung disease and fibrotic lung disease, cardiac fibrosis, myelofibrosis, kidney fibrosis such as glomerulosclerosis and tubulointerstitial fibrosis), atherosclerosis, restenosis, inflammatory diseases, autoimmune diseases, neurodegenerative/neurological diseases (e.g. Huntington's Disease, Alzheimer's disease, Parkinson's disease, polyglutamine disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, spinocerebellar ataxias 1, 2, 3, 6, 7 and 12, rubropallidal atrophy, spinocerebellar palsy), glioblastomas such as glioblastoma in Li-Fraumeni syndrome and sporadic glioblastoma, malignant melanomas, pancreatic ductal adenocarcinomas, myeloid leukemia, acute myelogenous leukemia, myelodysplastic syndrome, myeloproliferative syndrome, gynaecological cancer, Kaposi's sarcoma, Hansen's disease, collagenous colitis.

19. An *in vitro* method of reducing or inhibiting TG2 enzyme activity, the method comprising administering an antibody or antigen-binding fragment thereof according to any of Claims 1 to 10, 12 or 13 or a polynucleotide according to Claim 11, or a compound according to Claim 14, to a sample comprising TG2, for example a tissue or cell sample comprising TG2.

20. A method of producing an antibody or antigen-binding fragment thereof according to any of Claims 1 to 10, 12 or 13, or a compound according to Claim 14, the method comprising expressing a polynucleotide according to Claim 11.

21. A method of producing an antibody or antigen-binding fragment thereof that selectively binds a human TG2 and that inhibits human TG2 cross-linking lysine and glutamine with Nε(γ-glutamyl)lysine isopeptide bonds, the method comprising providing a non-human animal that had been administered with a compound comprising:

i) a polypeptide comprising a human TG2 core region/catalytic domain but not comprising a transglutaminase barrel or sandwich domain, or a fragment thereof; and, optionally,

ii) an adjuvant.

22. The method of Claim 21 further comprising the step of selecting an antibody or antigen-binding fragment thereof that selectively binds a transglutaminase protein, for example a TG2 such as human TG2.

23. An antibody or antigen-binding fragment thereof obtainable by the method of any of Claims 20 to 22, wherein the antibody or antigen-binding fragment thereof selectively binds to an epitope within the core region of TG2 and inhibits human TG2 cross-linking lysine and glutamine with Nε(γ-glutamyl)lysine isopeptide bonds.