TARGETED IMMUNOCONJUGATES

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention generally relates to antigen-specific immunoconjugates for selectively delivering effector moieties that influence cellular activity. In addition, the present invention relates to nucleic acid molecules encoding such immunoconjugates, and vectors and host cells comprising such nucleic acid molecules. The invention further relates to methods for producing the immunoconjugates of the invention, and to methods of using these immunoconjugates in the treatment of disease.

Background Art

The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumor cells, while leaving healthy cells and tissues intact and undamaged. A multitude of signal transduction pathways in the cell are linked to the cell's survival and/or death. Accordingly, the direct delivery of a pathway factor involved in cell survival or death can be used to contribute to the cell's maintenance or destruction.

Cytokines are cell signaling molecules that participate in regulation of the immune system. When used in cancer therapy, cytokines can act as immunomodulatory agents that have anti-tumor effects and which can increase the immunogenicity of some types of tumors. However, rapid blood clearance and lack of tumor specificity require systemic administration of high doses of the cytokine in order to achieve a concentration of the cytokine at the tumor site sufficient to activate an immune response or have an anti-tumor effect. These high levels of systemic cytokine can lead to severe toxicity and adverse reactions.

One way to deliver a signal transduction pathway factor, such as a cytokine, to a specific site *in vivo* (e.g., a tumor or tumor microenvironment) is to conjugate the factor to an immunoglobulin specific for the site. Early strategies aimed at delivering signal transduction pathway factors, such as cytokines, to a specific site *in vivo* included immunoglobulin heavy chains conjugated to

various cytokines, including lymphotoxin, tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), and granulocyte macrophage-colony stimulating factor (GM-CSF). The immunoglobulin heavy chains were either chemically conjugated to a cytokine or the immunogobulin-cytokine conjugate was expressed as a fusion protein. *See* Nakamura K. and Kubo, A. *Cancer Supplement* 80:2650-2655 (1997); Jun, L. *et al., Chin. Med. J. 113*:151-153 (2000); and Becker J.C., *et al., Proc. Natl. Acad. Sci. USA 93*:7826-7831 (1996). Researchers observed that, not only were they able to target cytokines to specific sites *in vivo*, they were also able to take advantage of the fact that monoclonal antibodies have longer serum half-lives than most other proteins. Due to the systemic toxicity associated with high doses of certain unconjugated cytokines, *i.e.* IL-2, the ability of an immunoglobulin-cytokine fusion protein to maximize immunostimulatory activities at the site of a tumor whilst keeping systemic side effects to a minimum at a lower dose led researchers to believe that immunoglobulin-cytokine immunoconjugates were optimal therapeutic agents. However, one of the major limitations in the clinical utility of immunoglobulins as delivery agents is their inadequate uptake and poor distribution in tumors, partially due to the large size of the immunoglobulin molecule. *See* Xiang, J. *et al., Immunol. Cell Biol. 72*:275-285 (1994). Additionally, it has been suggested that immunoglobulin-cytokine immunoconjugates can activate complement and interact with Fc receptors. This inherent immunoglobulin feature has been viewed unfavorably because therapeutic immunoconjugates may be targeted to cells expressing Fc receptors rather than the preferred antigen-bearing cells. One approach to overcoming these problems is the use of engineered immunoglobulin fragments. Numerous studies have detailed the characteristics of immunoglobulin fragment-cytokine immunoconjugates. *See* Savage, P. *et al., Br. J. Cancer 67*:304-310 (1993); Harvill, E.T. and Morrison S.L., *Immunotechnol. 1*:95-105 (1995); and Yang J. *et al., Mol. Immunol. 32*:873-881 (1995). In general, there are two common immunoglobulin fragment-cytokine fusion protein constructs, the $F(ab')_2$-cytokine expressed in mammalian cells and the scFv-cytokine expressed in *Escherichia coli*. *See* Xiang, J. *Hum. Antibodies 9*:23-36 (1999). Both the tumor-binding reactivity of the immunoglobulin parent molecule and the functional activity of the cytokine are maintained in most of these types of immunoconjugates. Recent preclinical studies have shown that these fusion proteins are able to target cytokines to tumors expressing the tumor-associated antigen *in vivo*, and to inhibit both primary and metastatic tumors in an immune competent animal model.

Examples of immunoglobulin fragment-cytokine immunoconjugates include the scFv-IL-2 immunoconjugate as set forth in PCT publication WO 2001/062298 A2; the immunoglobulin

heavy chain fragment-GM-CSF immunoconjugate as set forth in U.S. Pat. No. 5,650,150; the immunoconjugate as set forth in PCT publication WO 2006/119897 A2, wherein scFv-IL-12 first subunit shares only disulfide bond(s) with IL-12 second subunit-scFv, and the immunoconjugate as described in PCT publication WO 99/29732 A2, wherein Ig heavy chain fragment-IL-12 first subunit shares only disulfide bond(s) with Ig heavy chain fragment-IL-12 second subunit. While these second generation immunoconjugates have some improved properties as compared to the first generation immunoglobulin-cytokine conjugates, development of more and even safer specific therapeutic agents is desirable for greater effectiveness against tumor cells and a decrease in the number and severity of the side effects of these products (*e.g.*, toxicity, destruction of non-tumor cells, *etc.*). Additionally, it is desirable to identify ways to further stabilize immunoconjugates while maintaining acceptable therapeutic activity levels.

The present invention provides immunoconjugates that exhibit improved efficacy, high specificity of action, reduced toxicity, and improved stability in blood relative to known immunoconjugates.

BRIEF SUMMARY OF THE INVENTION

One aspect of the present invention is directed to immunoconjugates that exhibit improved efficacy, high specificity of action, reduced toxicity and improved stability as compared to known immunoconjugates. The immunoconjugates of the present invention can be used to selectively deliver effector moieties to a target site *in vivo*. In another embodiment, the immunoconjugate delivers a cytokine to a target site, wherein the cytokine can exert a localized biological effect, such as a local inflammatory response, stimulation of T cell growth and activation, and/or activation of B and/or NK cells.

One aspect of the present invention relates to an immunoconjugate that comprises at least a first effector moiety and at least a first and a second antigen binding moiety independently selected from the group consisting of an Fv and an Fab, wherein a first effector moiety shares an amino- or carboxy-terminal peptide bond with a first antigen binding moiety and a second antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either i) the first effector moiety or ii) the first antigen binding moiety.

Another aspect of the present invention is an immunoconjugate that comprises at least a first single-chain effector moiety joined at its amino-terminal amino acid to one or more scFv molecules and wherein the first-single-chain effector moiety is joined at its carboxy-terminal amino acids to one or more scFv molecules.

Another aspect of the present invention is an immunoconjugate that comprises at least a first single-chain effector moiety and first and second antigen binding moieties, wherein each of the first and second antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising a immunoglobulin constant domain independently selected from the group consisting of: IgG CH1, IgG C_{kappa}, and IgE CH4, and wherein the first antigen binding moiety is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond.

Another aspect of the present invention is an immunoconjugate that comprises at least a first single-chain effector moiety and first and second antigen binding moieties, wherein each of the first and second antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to an IgG1 CH3 domain, and wherein the first antigen binding moiety is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond.

Another aspect of the present invention is directed to an immunoconjugate that comprises first and second single-chain effector moieties and first and second antigen binding moieties, wherein each of the antigen binding moieties comprises an Fab molecule joined at its heavy or light chain carboxy-terminal amino acid to an IgG1 CH3 domain, and wherein each of the IgG1 CH3 domains is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond.

In one embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 95. In another embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 104. In another embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 105. In another embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 106. In another embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 107. In another embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 96. In a further embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 96 and a polypeptide sequence selected from the group consisting of SEQ ID NOs: 95 and 104-107. In another embodiment, the immunoconjugate comprises a polypeptide having a sequence that is at least

about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of SEQ ID NOs: 95, 96, and 104-107.

In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 108. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of SEQ ID NO: 108. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 117. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of SEQ ID NO: 117. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 118. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of SEQ ID NO: 118. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 119. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of SEQ ID NO: 119. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 120. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of SEQ ID NO: 120. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 109. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of SEQ ID NO: 109.

In one embodiment, the immunoconjugate comprises a heavy chain variable region that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 13 or SEQ ID NO: 15. In another embodiment, the immunoconjugate comprises a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 9 or SEQ ID NO: 11. In a further embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%

identical to the sequence of either SEQ ID NO: 13 or SEQ ID NO: 15, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 9 or SEQ ID NO: 11.

In one embodiment, the immunoconjugate comprises a heavy chain variable region sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 14 or SEQ ID NO: 16. In another embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is encoded by the polynucleotide sequence of either SEQ ID NO: 14 or SEQ ID NO: 16. In one embodiment, the immunoconjugate comprises a light chain variable region sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 10 or SEQ ID NO: 12. In another embodiment, the immunoconjugate comprises a light chain variable region sequence that is encoded by the polynucleotide sequence of either SEQ ID NO: 10 or SEQ ID NO: 12.

In one embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 99. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 100 or SEQ ID NO: 215. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 101 or SEQ ID NO: 235. In a further embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 100, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 101. In a further embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 215, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 235.

In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 112. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of SEQ ID NO: 112. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a

polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 113 or SEQ ID NO: 216. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of either SEQ ID NO: 113 or SEQ ID NO: 216. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 114 or SEQ ID NO: 236. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of either SEQ ID NO: 114 or SEQ ID NO: 236.

In one embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 7, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 187, SEQ ID NO: 191, SEQ ID NO:195, SEQ ID NO: 199, SEQ ID NO: 203 and SEQ ID NO: 207. In another embodiment, the immunoconjugate comprises a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 177, SEQ ID NO: 181, SEQ ID NO:185, SEQ ID NO: 189, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 201 and SEQ ID NO: 205. In a further embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 7, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 187, SEQ ID NO: 191, SEQ ID NO:195, SEQ ID NO: 199, SEQ ID NO: 203 and SEQ ID NO: 207 and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 177, SEQ ID NO: 181, SEQ ID NO:185, SEQ ID NO: 189, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 201 and SEQ ID NO: 205.

In one embodiment, the immunoconjugate comprises a heavy chain variable region sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 8, SEQ ID NO: 180, SEQ ID NO: 184, SEQ ID NO: 188, SEQ ID NO: 192, SEQ ID NO: 196, SEQ ID NO: 200, SEQ ID NO: 204 and SEQ ID NO: 208. In another embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 8, SEQ ID NO: 180, SEQ ID NO: 184, SEQ ID NO:

188, SEQ ID NO: 192, SEQ ID NO: 196, SEQ ID NO: 200, SEQ ID NO: 204 and SEQ ID NO: 208. In one embodiment, the immunoconjugate comprises a light chain variable region sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 186, SEQ ID NO: 190, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 202 and SEQ ID NO: 206. In another embodiment, the immunoconjugate comprises a light chain variable region sequence that is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 186, SEQ ID NO: 190, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 202 and SEQ ID NO: 206.

In one embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 239, SEQ ID NO: 241 and SEQ ID NO: 243. In another embodiment, the conjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 245, SEQ ID NO: 247 and SEQ ID NO:249. In a further embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 239, SEQ ID NO: 241 and SEQ ID NO: 243, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 245, SEQ ID NO: 247 and SEQ ID NO:249.

In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 240, SEQ ID NO: 242 and SEQ ID NO: 244. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 240, SEQ ID NO: 242 and SEQ ID NO: 244. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 246, SEQ ID NO: 248 and SEQ ID NO: 250. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence selected from the group of SEQ ID NO: 246, SEQ ID NO: 248 and SEQ ID NO: 250.

In one embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 31, SEQ ID NO: 35, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151, SEQ ID NO: 155, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171 and SEQ ID NO: 175. In another embodiment, the immunoconjugate comprises a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 29, SEQ ID NO: 33, SEQ ID NO: 37, SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 49, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149, SEQ ID NO: 153, SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169 and SEQ ID NO: 173. In a further embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 31, SEQ ID NO: 35, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151, SEQ ID NO: 155, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171 and SEQ ID NO: 175, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 29, SEQ ID NO: 33, SEQ ID NO: 37, SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 49, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149, SEQ ID NO: 153, SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169 and SEQ ID NO: 173.

In one embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of: SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, SEQ ID NO: 156, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172 and SEQ ID NO: 176. In another embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, SEQ ID NO: 156, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172 and SEQ ID NO: 176. In one embodiment, the immunoconjugate comprises a light chain variable region sequence that is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 24, SEQ ID NO: 30, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170 and SEQ ID NO: 174. In another embodiment, the immunoconjugate comprises a light chain variable region sequence that is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 24, SEQ ID NO: 30, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170 and SEQ ID NO: 174.

In one embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225 and SEQ ID NO: 227. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233 and SEQ ID NO: 237. In a further embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 211, SEQ ID NO: 219, and SEQ ID NO: 221, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 231. In a further embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group of SEQ ID NO: 209, SEQ ID NO: 223, SEQ ID NO: 225 and SEQ ID NO: 227, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 229. In a further embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 213, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 233. In a further embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 217, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 237.

In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226 and SEQ ID NO: 228. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226 and SEQ ID NO: 228. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that

is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234 and SEQ ID NO: 238. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234 and SEQ ID NO: 238.

In one embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 257 or SEQ ID NO: 261. In another embodiment, the immunoconjugate comprises a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 259 or SEQ ID NO: 271. In a further embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to to the sequence of either SEQ ID NO: 257 or SEQ ID NO: 261, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 259 or SEQ ID NO: 271.

In one embodiment, the immunoconjugate comprises a heavy chain variable region sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of either SEQ ID NO: 258 or SEQ ID NO: 262. In another embodiment, the immunoconjugate comprises a heavy chain variable region sequence that is encoded by the polynucleotide sequence of either SEQ ID NO: 258 or SEQ ID NO: 262.

In one embodiment, the immunoconjugate comprises a light chain variable region sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of either SEQ ID NO: 260 or SEQ ID NO: 272. In another embodiment, the immunoconjugate comprises a light chain variable region sequence that is encoded by the polynucleotide sequence of either SEQ ID NO: 260 or SEQ ID NO: 272.

In one embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 251 or SEQ ID NO: 255. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 253 or SEQ ID NO: 265. In a further embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 251 or SEQ ID NO: 255, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%,

96%, 97%, 98%, 99%, or 100% identical to the sequence of either SEQ ID NO: 253 or SEQ ID NO: 265.

In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of either SEQ ID NO: 252 or SEQ ID NO: 256. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of either SEQ ID NO: 252 or SEQ ID NO: 256. In one embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 254 or SEQ ID NO: 266. In another embodiment, the immunoconjugate comprises a polypeptide sequence that is encoded by the polynucleotide sequence of either SEQ ID NO: 254 or SEQ ID NO: 266.

In another embodiment, the immunoconjugate comprises at least one effector moiety, wherein the effector moiety is a cytokine. In a specific embodiment, the effector moiety is a cytokine selected from the group consisting of: interleukin-2 (IL-2), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon-α (INF-α), interleukin-12 (IL-12), interleukin-8 (IL-8), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), and transforming growth factor-β (TGF-β). In another embodiment, at least one antigen binding moiety is specific for one of the following antigenic determinants: the Extra Domain B of fibronectin (EDB), the A1 domain of tenascin (TNC-A1), the A2 domain of tenascin (TNC-A2), the Fibroblast Activated Protein (FAP); and the Melanoma Chondroitin Sulfate Proteoglycan (MCSP).

In another embodiment, the immunoconjugate of the invention binds to an effector moiety receptor with a dissociation constant (K_D) that is at least about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 times greater than that for a control effector moiety. In another embodiment, the immunoconjugate inhibits an increase in tumor volume *in vivo* by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more at the end of an administration period. In another embodiment, the immunoconjugate prolongs the survival of mammals with malignant tumors by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% when administered to a mammal in need thereof, relative to a control effector moiety or an effector moiety in a "diabody" immunoconjugate molecule.

Another aspect of the present invention is directed to isolated polynucleotides encoding immunoconjugates of the invention or fragments thereof. Another aspect of the present

invention is directed to an expression vector comprising an expression cassette comprising the polynucleotide sequences of the invention.

Another aspect of the present invention is directed to host cells that express the immunoconjugates of the invention or fragments thereof.

Another aspect of the present invention is directed to methods for producing the immunoconjugates of the invention or fragments thereof, wherein the method comprises culturing host cells transformed with expression vectors encoding for the immunoconjugates of the invention or fragments thereof under conditions suitable for the expression of the same.

Another aspect of the present invention is directed to methods for promoting proliferation and differentiation in an activated T lymphocyte cell, comprising contacting an activated T lymphocyte cell with an effective amount of the immunoconjugates of the invention.

Another aspect of the present invention is directed to methods for promoting proliferation and differentiation in an activated B lymphocyte cell, comprising contacting an activated B lymphocyte cell with an effective amount of the immunoconjugates of the invention.

Another aspect of the present invention is directed to methods for promoting proliferation and differentiation in a natural killer (NK) cell, comprising contacting a NK cell with an effective amount of the immunoconjugates of the invention.

Another aspect of the present invention is directed to methods for promoting proliferation and differentiation in a granulocyte, a monocyte or a dendritic cell, comprising contacting a granulocyte, a monocyte or a dendritic cell with an effective amount of the immunoconjugates of the invention.

Another aspect of the present invention is directed to methods for promoting cytotoxic T lymphocyte (CTL) differentiation, comprising contacting a T lymphocyte cell with an effective amount of the immunoconjugates of the invention.

Another aspect of the present invention is directed to methods for inhibiting viral replication, comprising contacting a virus-infected cell with an effective amount of the immunoconjugates of the invention.

Another aspect of the present invention is directed to methods for upregulating the expression of major histocompatibility complex I (MHC I), comprising contacting a target cell with an effective amount of the immunoconjugates of the invention.

Another aspect of the present invention is directed to methods for inducing cell death, comprising administering to a target cell an effective amount of the immunoconjugates of the invention.

Another aspect of the present invention is directed to methods for inducing chemotaxis in a target cell, comprising administering to a target cell an effective amount of the immunoconjugate of the invention.

Another aspect of the present invention is directed to a method of treating a disease in an individual, comprising the steps of administering to an individual a therapeutically effective amount of a composition comprising the immunoconjugate of the invention and a pharmaceutical carrier.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

FIGURE 1. Schematic overview of the various immunoconjugate fusion formats. All constructs in FIGURE 1 comprise two antibody scFv fragments (in the antigen binding moiety), and one or two cytokine molecules (as the effector moieties) connected to it. Panels A to E show different connectivities and stoichiometries of the antigen binding moieties and effector moieties. Panel A) depicts a "diabody"-IL-2 fusion. The "diabody" assembles non-covalently from two identical polypeptide chains. Panel B shows an immunoconjugate comprising a heavy chain of an Fab molecule fused at its carboxy-terminus to a cytokine which, in turn, is fused at its carboxy-terminus to a second Fab heavy chain. A light chain is coexpressed with the heavy chain Fab-cytokine-heavy chain Fab polypeptide to form the immunoconjugate. Alternatively, the two light chains can be fused to the cytokine, and the heavy chains are coexpressed. In panel C, the two Fab heavy chains are fused directly to each other. The cytokine shares an amino-terminal peptide bond with the second antigen binding moiety heavy chain. The two molecular formats of panels B and C can be varied such that the Fab chain is replaced by an scFv fragment, as in panels D and E.

FIGURE 2. Schematic overview of additional immunoconjugates that comprise two antigen binding moieties and at least one or more effector moieties. Panel A shows a Fab molecule fused through its carboxy-terminus to an IgG CH3 domain. In order to achieve covalent antigen binding moiety homodimerization, an artificial disulfide bond can be introduced at the carboxy-terminus of the IgG CH3 domain (immunoconjugate on the right within panel A. The IgG1 CH3 domain shown in panel A can be substituted with an IgE CH4 domain. The Fab moieties in panel A are replaced by scFv fragments in panel B. For the immunoconjugate of panel C, the natural IgG hinge was fused C-terminal to the Fab molecules. Since the carboxy-terminus region of the hinge could impose some geometric constraints on the assembly of constant domains that are fused C-terminal to the IgG hinge region, an artificial linker can be introduced between the

carboxy-terminal region of the hinge and the amino-terminus of the IgG CH3 domain. The hinge region can also be introduced between a scFv fragment and an immunoglobulin constant domain, as shown in panel D. In panels A to D, an IgG1 CH3 or IgE CH4 domain is used to homodimerize the constructs. Panel E depicts an immunoconjugate in which dimerization is achieved via a CH1/C_{kappa} heterodimerization interaction. The immunoconjugate of panel D can have one or two cytokines per immunoconjugate.

FIGURE 3 presents the results of an efficacy experiment with two different interleukin-2 immunoconjugate molecular formats specific for tumor stroma. The F9 teratocarcinoma was subcutaneously injected into 129SvEv mice, and tumor size was measured using a caliper. The "diabody"-IL-2 molecule was compared at two different concentrations to the Fab-interleukin-2-Fab (Fab-IL2-Fab) immunoconjugate, wherein the concentrations reflected similar numbers of immunoconjugate molecules. The Fab-IL2-Fab immunoconjugate is labeled as "Fab-L19", the unconjugated interleukin-2 control is labeled as "Unconj rIL-2," the "diabody"-IL-2 molecule is labeled as "diabody" in FIGURE 3. The L19 antibody, directed against Extra Domain B of fibronectin (EDB), was used to generate the antigen binding moieties in both the diabody and the Fab-L19 immunoconjugates. The amount of immunoconjugate injected per mouse (in μg) is indicated in the figure legend.

FIGURE 4 presents the results of a survival experiment with two different interleukin-2 immunoconjugate molecular formats specific for tumor stroma. Human gastric tumor cell-line LS174T was intrasplenically injected into SCID-beige mice. The Fab-IL2-Fab immunoconjugate is labeled as "Fab-L19", the unconjugated interleukin-2 control is labeled as "Unconj rIL-2", the "diabody"-IL-2 molecule is labeled as "diabody" in FIGURE 4. The anti-EDB antibody, L19, was used to generate the antigen binding moieties in both the diabody and the Fab-L19 immunoconjugates. The amount of immunoconjugate injected per mouse (in μg) is indicated in the figure legend, and reflects same numbers of immunoconjugate molecules.

FIGURE 5 shows immunohistochemical images of human uterus tissue at 100X and 400X magnification. The 2B10 variable region generated by the methods described in Example 3 binds to the A2 domain of human tenascin (TNC-A2). The 2B10 variable region in a Fab fragment was fused to a FLAG fragment (SHD2B10-FLAG). Healthy and cancerous human uterine tissue samples were prepared for immunohistochemical staining. Subsequently, the samples were incubated with the SHD2B10-FLAG Fab fragment. The samples were then washed and incubated with a fluorescent antibody specific for the FLAG epitope. Cancerous

tissue samples exhibited higher expression levels of TNC-A2 as compared to the healthy tissue samples.

FIGURE 6 shows the expression levels of TNC-A2 in various human tissue samples in terms of % of immunofluorescence surface area. Various human tissue samples from healthy individuals and cancer patients were incubated with the SHD2B10-FLAG Fab fragment as described in FIGURE 5.

FIGURE 7 shows the expression levels of Fibroblast Activated Protein (FAP) in various human tissue samples in terms of % of immunofluorescence surface area. Various human tissue samples from healthy individuals and cancer patients were incubated with a commercial antibody against FAP (Abcam). The top portion of each bar on the graph represents tumor expression of FAP, while the bottom portion of each bar on the graph represents normal FAP expression.

FIGURE 8 presents BIACORE data showing the affinity of a known IgG antibody, L19, for EDB.

FIGURE 9 presents BIACORE data showing the affinity of an Fab-IL-2-Fab immunoconjugate specific for EDB. The Fab fragments in the immunoconjugate were derived from the L19 antibody.

FIGURE 10 presents BIACORE data showing the affinity of a "diabody"-IL2 fusion protein specific for EDB. The diabody scFv fragment was derived from the L19 antibody. The "diabody"-IL2 fusion protein includes an 8 amino acid linker located between the scFv fragment and the IL-2 molecule.

FIGURE 11 presents BIACORE data showing the affinity of a "diabody"-IL2 fusion protein specific for EDB. The diabody scFv fragment was derived from the L19 antibody. The "diabody"-IL2 fusion protein includes a 12 amino acid linker between the scFv fragment and the IL-2 molecule

FIGURE 12 presents BIACORE data showing the affinity of a known IgG antibody, F16, for immobilized domain A1 of tenascin (TNC-A1). FIGURE 12 also presents BIACORE data showing the affinity of an Fab fragment of the F16 antibody for TNC-A1. Dissociation constants (K_D) calculated for the F16 IgG and Fab molecules are indicated in the figure.

FIGURE 13 presents BIACORE data showing the affinity of IL-2 for immobilized IL-2 receptor. Heterodimerization of the β and γ chains of IL-2R was achieved by fusing the respective chains to the "knob-into-holes" variants of the Fc portion of a human IgG1 as described in Merchant,

A.M. *et al., Nat. Biotech. 16*:677-681 (1998). The K_D value calculated from the BIACORE data is indicated in the figure.

FIGURE 14 presents BIACORE data showing the affinity of a "diabody"-IL-2 fusion protein for TNC-A1 and IL-2 receptor. The scFv molecule in the diabody is derived from the F16 antibody. The K_D values calculated from the BIACORE data are indicated in the figure.

FIGURE 15 presents BIACORE data showing the affinity of an Fab-IL-2-Fab immunoconjugate for TNC-A1 and IL-2 receptor. The Fab molecules in the immunoconjugate were derived from the F16 antibody. The K_D values calculated from the BIACORE data are indicated in the figure.

FIGURE 16 presents BIACORE data showing the affinity of an scFv-IL-2-scFv immunoconjugate for TNC-A1 and IL-2 receptor. The scFv molecules in the immunoconjugate were derived from the F16 antibody. The K_D values calculated from the BIACORE data are indicated in the figure.

FIGURE 17 is a summary table of the K_D values obtained from the BIACORE studies presented in Figures 12-16.

FIGURE 18 presents the results of an efficacy experiment comparing the "diabody"-IL-2 molecule targeting the EDB domain of fibronectin to the Fab-interleukin-2-Fab immunoconjugate (labeled as "Fab-SH2B10", comprising the heavy and light chain variable regions of SEQ ID NOs: 3 and 7, respectively) targeting the A2 domain of tenascin C. The unconjugated interleukin-2 control is labeled as "Unconj rIL-2," the "diabody"-IL-2 molecule is labeled as "L19 diabody" in FIGURE 18. The anti-EDB antibody, L19, was used to generate the antigen binding moiety in the diabody immunoconjugate. The teratocarcinoma cell-line F9 was subcutaneously injected into immunocompetent mice of the 129 strain. The amount of immunoconjugate injected per mouse (in μg) is indicated in the figure legend. Treatment was started at day 6 and 5 injections were performed in total until day 11 of the experiment.

FIGURE 19 shows the induction of proliferation of NK-92 cells by anti-FAP, or anti-tenascin C, Fab-IL2-Fab immunoconjugates (generated using the V_H and V_L sequences of the 3F2, 3D9, 4B3 (anti-FAP), 2F11, and 2B10 constructs (anti-tenascin C)) compared to unconjugated human IL-2. Cell proliferation was measured using the CellTiter Glo system after two days of incubation.

FIGURE 20 presents the results of an ELISA assay measuring induction of IFN-γ production by various interleukin-12 containing immunoconjugates compared either to unconjugated cytokines, or to immunoconjugates that contain the p35 and p40 domains of IL-12 in separate molecules. Panel A shows the results on fibronectin coated plates. Panel B shows the results in solution.

FIGURE 21 shows Surface Plasmon Resonance (SPR)-based kinetic analyses of affinity-matured anti-FAP Fab fragments. Processed kinetic data sets are presented for clone 19G1 binding to human (hu) FAP (A) and murine (mu) FAP (B), for clone 20G8 binding to hu FAP (C), mu FAP (D) and for clone 4B9 binding to hu FAP (E) and mu FAP (F). Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 22 shows SPR-based kinetic analyses of affinity-matured anti-FAP Fab fragments. Processed kinetic data sets are presented for clone 5B8 binding to hu FAP (A) and mu FAP (B), for clone 5F1 binding to hu FAP (C), mu FAP (D) and for clone 14B3 binding to hu FAP (E) and mu FAP (F). Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 23 shows SPR-based kinetic analyses of affinity-matured anti-FAP Fab fragments. Processed kinetic data sets are presented for clone 16F1 binding to hu FAP (A) and mu FAP (B), for clone 16F8 binding to hu FAP (C), mu FAP (D) and for clone O3C9 binding to hu FAP (E) and mu FAP (F). Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 24 shows SPR-based kinetic analyses of affinity-matured anti-FAP Fab fragments. Processed kinetic data sets are presented for clone O2D7 binding to hu FAP (A) and mu FAP (B), for clone 28H1 binding to hu FAP (C), mu FAP (D), cyno FAP (E) and for clone 22A3 binding to hu FAP (F), mu FAP (G) and Cynomolgus (cyno) FAP (H). Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 25 shows SPR-based kinetic analyses of affinity-matured anti-FAP Fab fragments. Processed kinetic data sets are presented for clone 29B11 binding to hu FAP (A), mu FAP (B), cyno FAP (C) and for clone 23C10 binding to hu FAP (D), mu FAP (E) and cyno FAP (F). Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 26 shows SPR-based kinetic analyses of affinity-matured anti-TNC A2 Fab fragments binding to human (hu) TNC A2. Processed kinetic data sets are presented for clone 2B10_C3B6 (A), clone 2B10_6A12 (B), clone 2B10_C3A6 (C), clone 2B10_O7D8 (D), clone 2B10_O1F7 (E) and clone 2B10_6H10 (F). Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 27 gives an overview of the three purification steps performed for the purification of 3F2-based Fab-IL2-Fab.

FIGURE 28 shows results from the purification of 3F2-based Fab-IL2-Fab (A and B) and results from the purification of 4G8-based Fab-IL2-Fab (C and D). (A, C) 4-12% Bis-Tris and 3-8% Tris Acetate SDS-PAGE with fractions during the purification procedure and the end product. (B, D) Analytical size exclusion chromatography after the three applied purification steps.

FIGURE 29 shows results from the purification of the 2B10 Fab-IL2-Fab immunoconjugate. (A) 4-12% Bis-Tris SDS-PAGE with fractions during the purification procedure and the end product. B) Analytical size exclusion chromatography after the three applied purification steps.

FIGURE 30 shows the stability assessment of anti-fibronectin EDB L19-based Fab-IL2-Fab. L19 Fab-IL2-Fab was formulated in 20 mM histidine HCl, 140 mM NaCl, pH 6.0 at a concentration of 6.3 mg/ml and stored for 4 weeks at room temperature and at 4°C. Samples were analyzed every week for (A) concentration by UV spectroscopy (after centrifugation to pellet potential precipitated material) and (B) aggregate content by analytical size exclusion chromatography.

FIGURE 31 shows SPR-based kinetic analyses of FAP-targeted 3F2 Fab-IL2-Fab immunoconjugates for human, murine and Cynomolgus (cyno) FAP and human IL-2 receptor-β/γ (IL2R bg) as determined by Surface Plasmon Resonance. Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 32 shows SPR-based kinetic analyses of FAP-targeted 4G8 Fab-IL2-Fab immunoconjugates for human, murine and Cynomolgus (cyno) FAP as determined by Surface Plasmon Resonance. Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 33 shows SPR-based kinetic analyses of FAP-targeted 4G8 Fab-IL2-Fab constructs for human and murine IL-2 receptor β/γ and α chains, as determined by Surface Plasmon Resonance. Smooth lines represent a global fit of the data to a two-state-reaction model.

FIGURE 34 shows SPR-based kinetic analyses of FAP-targeted 3D9 Fab-IL2-Fab constructs for human, murine and Cynomolgus (cyno) FAP and human IL-2 receptor- β/γ (IL2R bg) as determined by Surface Plasmon Resonance. Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 35 shows SPR-based kinetic analyses of TNC A2-targeted 2B10 Fab-IL2-Fab constructs for human, murine and Cynomolgus (cyno) chimeric TNC A2 fusion proteins and human IL-2 receptor-β/γ (IL2R bg) as determined by Surface Plasmon Resonance. Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 36 illustrates the efficacy of targeted IL-2 Fab-IL2-Fab immunoconjugates recognizing TNC A2 (2B10) or FAP (3F2 and 4G8) in inducing proliferation of NK92 cells in comparison to IL-2 (Proleukin) and the L19 diabody recognizing fibronectin-EDB. The x-axis is normalized to the number of IL-2 molecules, as the diabody has two IL-2 effector moieties while the Fab-IL2-Fab constructs contain only one IL-2 effector moiety. Cell proliferation was measured using the CellTiter Glo system after two days of incubation.

FIGURE 37 shows the induction of STAT5 phosphorylation as a consequence of IL-2 mediated IL-2 receptor signaling following incubation with a FAP-targeted 4G8-based IL-2 Fab-IL2-Fab immunoconjugate recognizing FAP on different effector cell populations including (A) $CD56^+$ NK cells, (B) $CD4^+CD25^-CD127^+$ helper T cells, (C) $CD3^+$, $CD8^+$ cytotoxic T cells and (D) $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs) from human PBMCs in solution.

FIGURE 38 illustrates the efficacy of targeted IL-2 Fab-IL2-Fab immunoconjugates recognizing TNC A1 (2F11), TNC A2 (2B10) or FAP (3F2, 4B3 and 3D9) in inducing IFN-γ release and proliferation of NK92 cells in comparison to IL-2, when the immunoconjugates are either present in solution or immobilized via FAP or TNC A2 coated on microtiter plates.

FIGURE 39 presents the results of a survival experiment with two different IL-2 immunoconjugate molecular formats specific for tumor stroma. Human colon tumor cell line LS174T was intrasplenically injected into SCID mice. The TNC A2-targeted 2B10 Fab-IL2-Fab immunoconjugate is labeled as "SH2B10", the unconjugated IL-2 control is labeled as "proleukin", the fibronectin EDB-targeted diabody-IL-2 molecule is labeled as "diabody". The amount of immunoconjugate injected per mouse (in μg) is indicated in the figure legend, and reflects same numbers of immunoconjugate molecules.

FIGURE 40 presents the results of a survival experiment with two different IL-2 immunoconjugate molecular formats specific for tumor stroma. Human renal cell line ACHN was intrarenally injected into SCID mice. The FAP-targeted 3F2 or 4G8 Fab-IL2-Fab immunoconjugates are labeled as "FAP-3F2" and "FAP-4G8", the unconjugated IL-2 control is labeled as "proleukin", the fibronectin EDB-targeted diabody-IL-2 molecule is labeled as "diabody". The amount of immunoconjugates injected per mouse (in μg) is indicated in the figure legend, and reflects same numbers of immunoconjugate molecules.

FIGURE 41 presents the results of a survival experiment with two different IL-2 immunoconjugate molecular formats specific for tumor stroma. Human NSCLC cell line A549 was injected i.v. into SCID mice. The TNC A2-targeted 2B10 Fab-IL2-Fab immunoconjugate is labeled as "2B10", the fibronectin EDB-targeted diabody-IL-2 molecule is labeled as "diabody". The amount of immunoconjugate injected per mouse (in μg) is indicated in the figure legend, and reflects same numbers of immunoconjugate molecules.

FIGURE 42 presents (A) an overview of the purification procedure of the Fab-GM-CSF-Fab immunoconjugate with L19 (Fibronectin Ectodomain-B binder) as Fab, and (B) an SDS-PAGE (reduced, non-reduced) of the purified Fab-GM-CSF-Fab immunoconjugate.

FIGURE 43 presents the results of a GM-CSF-dependent proliferation assay comparing the effect of GM-CSF and the purified Fab-GM-CSF-Fab immunoconjugate with L19 (Fibronectin Ectodomain-B binder) as Fab on TF-1 cells.

FIGURE 44 presents (A) an overview of the purification procedure of the Fab-IL12-Fab immunoconjugate with 4G8 (FAP binder) as Fab, and (B) an SDS-PAGE (reduced, non-reduced) of the purified Fab-IL12-Fab immunoconjugate.

FIGURE 45 presents the results of an assay testing IL-12 induced IFN-γ release, comparing the effect of IL-12 and the purified Fab-IL12-Fab immunoconjugate with 4G8 (FAP binder) as Fab, using PBMCs isolated from fresh human blood of a healthy donor.

FIGURE 46 presents (A) an overview of the purification procedure of the Fab-IFNα2-Fab immunoconjugate with L19 (Fibronectin Ectodomain-B binder) as Fab, and (B) an SDS-PAGE (reduced, non-reduced) of the purified Fab-IFNα2-Fab immunoconjugate.

FIGURE 47 presents the results of an assay testing IFN-α-induced proliferation inhibition of (A) Jurkat T cells and (B) A549 tumor cells comparing the effect of IFN-α (Roferon A, Roche) and the purified Fab-IFNα2-Fab immunoconjugate with L19 (Fibronectin Ectodomain-B binder) as Fab.

FIGURE 48 shows (A) the elution profiles from the purification of the MCSP-targeted MHLG based Fab-IL2-Fab and (B) the results from the analytical characterization of the same Fab-IL2-Fab by SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer, reduced and non-reduced).

FIGURE 49 shows (A) the elution profiles from the purification of the MCSP-targeted MHLG1 based Fab-IL2-Fab and (B) the results from the analytical characterization of the same Fab-IL2-Fab by SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer, reduced and non-reduced).

FIGURE 50 presents the results of an assay testing IL-2 induced IFN-γ release comparing the effect of the purified Fab-IL2-Fab immunoconjugate with 4G8 (FAP binder) as Fab, and the purified Fab-IL2-Fab immunoconjugate with MHLG KV9 (MCSP binder) as Fab, using IL-2 starved NK92 cells.

FIGURE 51 presents the results of an assay testing IL-2 induced IFN-γ release comparing the effect of the purified Fab-IL2-Fab immunoconjugate with 4G8 (FAP binder) as Fab, and the purified Fab-IL12-Fab immunoconjugate with MHLG1 KV9 (MCSP binder) as Fab, using IL-2 starved NK92 cells.

FIGURE 52 shows the binding of MCSP-targeted MHLG1 KV9 Fab-IL2-Fab immunoconjugate to Colo38 cells, as determined by flow cytometry. Secondary antibody alone or cells only are shown as negative controls.

FIGURE 53 presents (A) an overview of the purification procedure of the 2B10 Fab-IL2-Fab immunoconjugate with 2B10 (TNC A2 binder) as Fab, and (B) an SDS-PAGE (reduced, non-reduced) of the purified 2B10 Fab-IL2-Fab immunoconjugate.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques and procedures are generally performed according to conventional methods in the art and various general references (*see* generally, Sambrook *et al.* Molecular Cloning: A Laboratory Manual, 2nd ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference), which are provided throughout this document.

As used herein, the term "immunoconjugate" refers to a polypeptide molecule that includes at least one effector moiety and at least one antigen binding moiety. In one embodiment, the immunoconjugate comprises at least one single-chain effector moiety, and at least two antigen binding moieties. The antigen binding molecule can be joined to the effector moiety by a variety of interactions and in a variety of configurations as described herein.

As used herein, the term "effector moiety" refers to a polypeptide, e.g., a protein or glycoprotein, that influences cellular activity, for example, through signal transduction or other cellular pathways. Accordingly, the effector moiety of the invention can be associated with receptor-mediated signaling that transmits a signal from outside the cell membrane to modulate a response in a cell bearing one or more receptors for the effector moiety. In one embodiment, an effector moiety can elicit a cytotoxic response in cells bearing one or more receptors for the effector moiety. In another embodiment, an effector moiety can elicit a proliferative response in cells bearing one or more receptors for the effector moiety. In another embodiment, an effector moiety can elicit differentiation in cells bearing receptors for the effector moiety. In another

embodiment, an effector moiety can alter expression (*i.e.,* upregulate or downregulate) of an endogenous cellular protein in cells bearing receptors for the effector moiety. Non-limiting examples of effector moieties include cytokines, growth factors, hormones, enzymes, substrates, and cofactors. The effector moiety can be associated with an antigen binding moiety in a variety of configurations to form an immunoconjugate.

As used herein, the term "cytokine" refers to a molecule that mediates and/or regulates a biological or cellular function or process (e.g., immunity, inflammation, and hematopoiesis). The term "cytokine" as used herein includes "lymphokines," "chemokines," "monokines," and "interleukins." Examples of useful cytokines include, but are not limited to, GM-CSF, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN-α, IFN-β, IFN-γ, MIP-1α, MIP-1β, TGF-β, TNF-α, and TNF-β.

As used herein, the term "single-chain" refers to a molecule comprising amino acid monomers linearly linked by peptide bonds. In one embodiment, the effector moiety is a single-chain effector moiety. Non-limiting examples of single-chain effector moieties include cytokines, growth factors, hormones, enzymes, substrates, and cofactors. When the effector moiety is a cytokine and the cytokine of interest is normally found as a multimer in nature, each subunit of the multimeric cytokine is sequentially encoded by the single-chain of the effector moiety. Accordingly, non-limiting examples of useful single-chain effector moieties include GM-CSF, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN-α, IFN-β, IFN-γ, MIP-1α, MIP-1β, TGF-β, TNF-α, and TNF-β.

As used herein, the term "control effector moiety" refers to an unconjugated effector moiety. For example, when comparing an IL-2 immunoconjugate of the present invention with a control effector moiety, the control effector moiety is free, unconjugated IL-2. Likewise, *e.g.,* when comparing an IL-12 immunoconjugate of the present invention with a control effector moiety, the control effector moiety is free, unconjugated IL-12 (*e.g.,* existing as a heterodimeric protein wherein the p40 and p35 subunits share only disulfide bond(s)).

As used herein, the term "effector moiety receptor" refers to a polypeptide molecule capable of binding specifically to an effector moiety. For example, where IL-2 is the effector moiety, the effector moiety receptor that binds to a IL-2 (e.g., an immunoconjugate comprising IL-2) is the IL-2 receptor. Similarly, *e.g.,* where IL-12 is the effector moiety of an immunoconjugate, the effector moiety receptor is the IL-12 receptor. Where an effector moiety specifically binds to more than one receptor, all receptors that specifically bind to the effector moiety are “effector moiety receptors” for that effector moiety.

As used herein, the term "antigen binding moiety" refers to a polypeptide molecule that specifically binds to an antigenic determinant. In one embodiment, an antigen binding moiety is able to direct the entity to which it is attached (*e.g.,* an effector moiety or a second antigen binding moiety) to a target site, for example to a specific type of tumor cell or tumor stroma bearing the antigenic determinant. Antigen binding moieties include antibodies and fragments thereof as further defined herein. By "specifically binds" is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties with constant regions as further defined herein and known in the art. Useful heavy chain constant regions include any of the five isotypes: α, δ, ε, γ, or μ. Useful light chain constant regions include any of the two isotypes: κ and λ.

As used herein, the term "antigenic determinant" is synonymous with "antigen" and "epitope," and refers to a site (e.g., a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex.

As used herein, the term "control antigen binding moiety" refers to an antigen binding moiety as it would exist free of other antigen binding moieties and effector moieties. For example, when comparing an Fab-IL2-Fab immunoconjugate of the invention with a control antigen binding moiety, the control antigen binding moiety is free Fab, wherein the Fab-IL2-Fab immunoconjugate and the free Fab molecule can both specifically bind to the same antigen determinant.

As used herein, the terms "first" and "second" with respect to antigen binding moieties, effector moieties, *etc.*, are used for convenience of distinguishing when there is more than one of each type of moiety. Use of these terms is not intended to confer a specific order or orientation of the immunoconjugate unless explicitly so stated.

In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light polypeptides. This particular region has been described by Kabat *et al.,* U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al., J. Mol. Biol. 196*:901-917 (1987), which are incorporated herein by reference, where the definitions include

overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table I as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1. CDR Definitions[1]

CDR	Kabat	Chothia	AbM[2]
V_H CDR1	31-35	26-32	26-35
V_H CDR2	50-65	52-58	50-58
V_H CDR3	95-102	95-102	95-102
V_L CDR1	24-34	26-32	24-34
V_L CDR2	50-56	50-52	50-56
V_L CDR3	89-97	91-96	89-97

[1]Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat et al. (see below).
[2] "AbM" with a lowercase "b" as used in Table 1 refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antigen binding moiety of the invention are according to the Kabat numbering system. The polypeptide sequences of the sequence listing (*i.e.,* SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 96, 97, etc.) are not numbered according to the Kabat numbering system. However, it is well within the ordinary skill of one in the art to convert the numbering of the sequences of the Sequence Listing to Kabat numbering.

Immunoconjugates

Immunoconjugates are polypeptide molecules that comprise at least one effector moiety and at least one antigen binding moiety. In one embodiment, the effector moiety is a single-chain

effector moiety. In one embodiment, the immunoconjugate comprises at least two antigen binding moieties. The antigen binding moieties and effector moieties of the immunoconjugate include those that are described in detail herein above and below and in the accompanying figures. The antigen binding moiety of the immunoconjugate can be directed againt a variety of target molecules (e.g., an antigenic determinant on a protein molecule expressed on a tumor cell or tumor stroma). Non-limiting examples of antigen binding moieties are described herein. In one embodiment, the at least one antigen binding moiety is directed to an antigenic determinant of one or more of the polypeptides represented in Table 5, herein below. Immunoconjugates of the invention typically exhibit one or more of the following properties: high specificity of action, reduced toxicity and/or improved stability, particularly as compared to known immunoconjugates of different configurations targeting the same antigenic determinants and carrying the same effector moities.

In one embodiment, the immunoconjugate comprises at least a first effector moiety and at least a first and a second antigen binding moiety. In a preferred embodiment, the first effector moiety is a single chain effector moiety. In a preferred embodiment, the first and second antigen binding moeity are independently selected from the group consisting of an Fv and an Fab. In a specific embodiment, the first effector moiety shares an amino- or carboxy-terminal peptide bond with a first antigen binding moiety and a second antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either i) the first effector moiety or ii) the first antigen binding moiety. In another embodiment, the immunoconjugate consists essentially of a first single-chain effector moiety and first and second antigen binding moieties.

In one embodiment, a first effector moiety, shares a carboxy-terminal peptide bond with a first antigen binding moiety and further shares an amino-terminal peptide bond with a second antigen binding moiety. In another embodiment, a first antigen binding moiety shares a carboxy-terminal peptide bond with a first effector moiety, preferably a single chain effector moiety, and further shares an amino-terminal peptide bond with a second antigen binding moiety. In another embodiment, a first antigen binding moiety shares an amino-terminal peptide bond with a first effector moiety, preferably a single chain effector moiety, and further shares a carboxy-terminal peptide with a second antigen binding moiety.

In one embodiment, an effector moiety, preferably a single chain effector moiety, shares a carboxy-terminal peptide bond with a first heavy chain variable region and further shares an amino-terminal peptide bond with a second heavy chain variable region. In another embodiment, an effector moiety, preferably a single chain effector moiety, shares a carboxy-

terminal peptide bond with a first light chain variable region and further shares an amino-terminal peptide with a second light chain variable region. In another embodiment, a first heavy or light chain variable region is joined by a carboxy-terminal peptide bond to a first effector moiety, preferably a single chain effector moiety, and is further joined by an amino-terminal peptide bond to a second heavy or light chain variable region. In another embodiment, a first heavy or light chain variable region is joined by an amino-terminal peptide bond to a first effector moiety preferably a single chain effector moiety, and is further joined by a carboxy-terminal peptide bond to a second heavy or light chain variable region.

In one embodiment, an effector moiety, preferably a single chain effector moiety, shares a carboxy-terminal peptide bond with a first Fab heavy or light chain and further shares an amino-terminal peptide bond with a second Fab heavy or light chain. In another embodiment, a first Fab heavy or light chain shares a carboxy-terminal peptide bond with a first single-chain effector moiety and further shares an amino-terminal peptide bond with a second Fab heavy or light chain. In other embodiments, a first Fab heavy or light chain shares an amino-terminal peptide bond with a first single-chain effector moiety and further shares a carboxy-terminal peptide bond with a second Fab heavy or light chain.

In one embodiment, the immunoconjugate comprises at least a first effector moiety sharing an amino-terminal peptide bond to one or more scFv molecules and wherein the first effector moiety further shares a carboxy-terminal peptide bond with one or more scFv molecules. In a preferred embodiment, the effector moiety is a single chain effector moiety.

In another embodiment, the immunoconjugate comprises at least a first effector moiety, preferably a single chain effector moiety, and first and second antigen binding moieties, wherein each of the antigen binding moieties includes an scFv molecule joined at its carboxy-terminal amino acid to a constant region that includes an immunoglobulin constant domain, and wherein the first antigen binding moiety is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of the first effector moiety, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond. In a preferred embodiment, the constant region is independently selected from the group consisting of IgG CH1, IgG CH2, IgG CH3, IgG C_{kappa}, IgG C_{lambda} and IgE CH_4 domains. In one embodiment, the immunoglobulin domain of the first antigen binding moiety is covalently linked to the immunoglobulin domain of the second antigen binding moiety through a disulfide bond. In one embodiment, at least one disulfide bond is located carboxy-terminal of the immunoglobulin domains of the first and second antigen binding moieties. In another embodiment, at least one

disulfide bond is located amino-terminal of the immunoglobulin domains of the first and second antigen binding moieties. In another embodiment, at least two disulfide bonds are located amino-terminal of the immunoglobulin domains of the first and second antigen binding moieties.

In a specific embodiment, the immunoconjugate comprises first and second antigen binding moieties, each comprising an scFv molecule joined at its carboxy-terminal amino acid to a constant region that comprises an IgG CH1 domain, wherein the first antigen binding moiety is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of the first effector moiety, preferably a single chain effector moiety, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond. The second antigen binding moiety of the immunoconjugate can be further joined at its carboxy-terminal amino acid to the amino-terminal amino acid of a second effector moiety. In one embodiment, the second effector moiety is a single chain effector moiety.

In a specific embodiment, the immunoconjugate comprises first and second antigen binding moieties each comprising an scFv molecule joined at its carboxy-terminal amino acid to a constant region that comprises an IgG C_{kappa} domain, wherein the first antigen binding moiety is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of the first effector moiety, preferably a single chain effector moiety, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond. The second antigen binding moiety of the immunoconjugate can be further joined at its carboxy-terminal amino acid to the amino-terminal amino acid of a second effector moiety. In one embodiment, the second effector moiety is a single chain effector moiety.

In another specific embodiment, the immunoconjugate comprises first and second antigen binding moieties, each comprising an scFv molecule joined at its carboxy-terminal amino acid to a constant region that comprises an IgE CH4 domain, wherein the first antigen binding moiety is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of the first effector moiety, preferably a single chain effector moiety, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond. The second antigen binding moiety of the immunoconjugate can be further joined at its carboxy-terminal amino acid to the amino-terminal amino acid of a second effector moiety. In one embodiment, the second effector moiety is a single chain effector moiety.

In another specific embodiment, the immunoconjugate comprises first and second antigen binding moieties each, comprising an scFv molecule joined at its carboxy-terminal amino acid to an IgE CH3 domain, wherein the first antigen binding moiety is joined at its carboxy-terminal

amino acid to the amino-terminal amino acid of the first effector moiety, preferably a single chain effector moiety, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond. The second antigen binding moiety of the immunoconjugate can be further joined at its carboxy-terminal amino acid to the amino-terminal amino acid of a second effector moiety. In one embodiment, the second effector moiety is a single chain effector moiety.

In another embodiment, the immunoconjugate comprises first and second effector moieties, and first and second antigen binding moieties, wherein each of the antigen binding moieties comprises an Fab molecule joined at its heavy or light chain carboxy-terminal amino acid to an IgG1 CH3 domain, and wherein each of the IgG1 CH3 domains is joined at its respective carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the first and second antigen binding moieties are covalently linked through at least one disulfide bond. In a preferred embodiment, the first and/or second effector moiety is a single chain effector moiety. In a further embodiment, the IgG1 CH3 domains of the antigen binding moieties may be joined by disulfide bond. In another embodiment, at least one disulfide bond is located carboxy-terminal of the IgG1 CH3 domains of the first and second antigen binding moieties. In another embodiment, at least one disulfide bond is located amino-terminal of the IgG1 CH3 domains of the first and second antigen binding moieties. In another embodiment, at least two disulfide bonds are located amino-terminal of the IgG1 CH3 domains of the first and second antigen binding moieties.

In another embodiment, the immunoconjugate comprises one or more proteolytic cleavage sites located between effector moieties and antigen binding moieties.

Components of the immunoconjugate (*e.g.,* antigen binding moieties and/or effector moieties) may be linked directly or through various linkers (*e.g.,* peptide linkers comprising one or more amino acids, typically about 2-10 amino acids) that are described herein or are known in the art.

In a particular embodiment, the immunoconjugate has improved stability in solution, particularly compared to known immunoconjugate preparations. In one embodiment, the immunoconjugate binds to an antigenic determinant with a dissociation constant (K_D) that is at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 times lower than that for a control antigen binding moiety. In a more specific embodiment, the immunoconjugate binds to an antigenic determinant with a K_D that is about 10 times lower than that for a control antigen binding moiety. In one embodiment the immunoconjugate binds to an antigenic determinant with a K_D that is lower than about 10 nM, lower than about 1 nM, or lower than about 0.1 nM.

In another embodiment, the immunoconjugate has a superior safety profile compared to known immunoconjugate preparations. The immunoconjugate preferably elicits fewer and less severe side effects, such as toxicity, destruction of non-tumor cells, *etc.* The decrease in side effects may be attributed to the reduced binding affinity of the immunoconjugates of the invention towards effector moiety receptors. In one embodiment, the immunoconjugate binds to an effector moiety receptor with a K_D that is at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 times greater than that for a control effector moiety. In a more specific embodiment, the immuonconjugate binds to an effector moiety receptor with a K_D that is about 2 times greater than that for a control effector moiety. In another embodiment, the immuonconjugate binds to an effector moiety receptor with a K_D that is about 10 times greater than that for a control effector moiety. In another embodiment, the immunoconjugate binds to an effector moiety receptor with a K_D that is at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 times greater than that for a corresponding effector moiety in a "diabody" immunoconjugate molecule. In another embodiment, the immunoconjugate binds to an effector moiety receptor with a dissociation constant K_D that is about 10 times greater than that for a corresponding effector moiety in a "diabody" immunoconjugate.

In another embodiment, the immunoconjugate has superior efficacy, particularly compared to known immunoconjugate preparations. In one embodiment, the immunoconjugate is better able to inhibit increases in tumor volume *in vivo* and/or better able to prolong survival in mammals with malignant tumors. In one embodiment, the immunoconjugate inhibits an increase in tumor volume *in vivo* by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% by the end of an administration period of about at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days. In one embodiment, the immunoconjugate inhibits an increase in tumor volume *in vivo* by at least 50%, 55%, 60%, 65%, 70%, or 75% by the end of a 13 day administration period. In another embodiment, the immunoconjugate prolongs the survival of mammals with malignant tumors by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% when administered to a mammal in need thereof, relative to a control effector moiety. In another embodiment, the immunoconjugate prolongs the survival of mammals with malignant tumors by at least 30%, 32% or 35% when administered to a mammal in need thereof, relative to a control effector moiety. In another embodiment, the immunoconjugate prolongs the survival of mammals with malignant tumors by about 30% when administered to a mammal in need thereof, relative to a control effector moiety.

In another embodiment, the immunoconjugate prolongs the survival of mammals with malignant tumors by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% when administered to a mammal in need thereof, relative to an effector moiety in a "diabody" immunoconjugate molecule. In another embodiment, the immunoconjugate prolongs the survival of mammals with malignant tumors by at least 30%, 32%, or 35% when administered to a mammal in need thereof, relative to an effector moiety in a "diabody" immunoconjugate molecule. In another embodiment, the immunoconjugate prolongs the survival of mammals with malignant tumors by about 30% when administered to a mammal in need thereof, relative to an effector moiety in a "diabody" immunoconjugate molecule. In another embodiment, the immunoconjugate prolongs the survival of mammals with malignant tumors by at least 5%, 10% or 15%, relative to a control effector moiety or an effector moiety in a “diabody” immunoconjugate molecule.

Antigen Binding Moieties

The antigen binding moiety of the immunoconjugate of the invention is generally a polypeptide molecule that binds to a specific antigenic determinant and is able to direct the entity to which it is attached (*e.g.,* an effector moiety or a second antigen binding moiety) to a target site, for example to a specific type of tumor cell or tumor stroma that bears the antigenic determinant. The immunoconjugate can bind to antigenic determinants found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, free in blood serum, and/or in the extracellular matrix (ECM).

Non-limiting examples of tumor antigens include MAGE, MART-1/Melan-A, gp100, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal associated antigen (CRC)-C017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (e.g., MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens (e.g., GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α-fetoprotein, E-cadherin, α-catenin, β-catenin and γ-

catenin, p120ctn, gp100 Pmel117, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, lmp-1, P1A, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, and c-erbB-2.

Non-limiting examples of viral antigens include influenza virus hemagglutinin, Epstein-Barr virus LMP-1, hepatitis C virus E2 glycoprotein, HIV gp160, and HIV gp120.

Non-limiting examples of ECM antigens include syndecan, heparanase, integrins, osteopontin, link, cadherins, laminin, laminin type EGF, lectin, fibronectin, notch, tenascin, and matrixin.

The immunoconjugates of the invention can bind to the following specific non-limiting examples of cell surface antigens: FAP, Her2, EGFR, CD2 (T-cell surface antigen), CD3 (heteromultimer associated with the TCR), CD22 (B-cell receptor), CD23 (low affinity IgE receptor), CD25 (IL-2 receptor α chain), CD30 (cytokine receptor), CD33 (myeloid cell surface antigen), CD40 (tumor necrosis factor receptor), IL-6R (IL6 receptor), CD20, MCSP, and PDGFβR (β platelet-derived growth factor receptor).

In one embodiment, the immunoconjugate of the invention comprises two or more antigen binding moieties, wherein each of these antigen binding moieties specifically bind to the same antigenic determinant. In another embodiment, the immunoconjugate of the invention comprises two or more antigen binding moieties, wherein each of these antigen binding moieties specifically bind to different antigenic determinants.

The antigen binding moiety can be any type of antibody or fragment thereof that retains specific binding to an antigenic determinant. Antibody fragments include, but are not limited to, V_H fragments, V_L fragments, Fab fragments, $F(ab')_2$ fragments, scFv fragments, Fv fragments, minibodies, diabodies, triabodies, and tetrabodies (*see, e.g.,* Hudson and Souriau, *Nature Med.* 9: 129-134 (2003)).

In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Extra Domain B of fibronectin (EDB). In another embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that can compete with monoclonal antibody L19 for binding to an epitope of EDB. See, e.g., PCT publication WO 2007/128563 A1 (incorporated herein by reference in its entirety). In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain derived from the L19 monoclonal antibody shares a carboxy-terminal peptide bond with an IL-2 molecule which in turn shares a carboxy-terminal peptide bond with a second

Fab heavy chain derived from the L19 monoclonal antibody. In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain derived from the L19 monoclonal antibody shares a carboxy-terminal peptide bond with an IL-12 molecule which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain derived from the L19 monoclonal antibody. In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain derived from the L19 monoclonal antibody shares a carboxy-terminal peptide bond with an IFN α molecule which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain derived from the L19 monoclonal antibody. In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain derived from the L19 monoclonal antibody shares a carboxy-terminal peptide bond with a GM-CSF molecule which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain derived from the L19 monoclonal antibody. In a further embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first scFv derived from the L19 monoclonal antibody shares a carboxy-terminal peptide bond with an IL-2 molecule which in turn shares a carboxy-terminal peptide bond with a second scFv derived from the L19 monoclonal antibody. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 95 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises an Fab light chain derived from the L19 monoclonal antibody. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 96 or a variant thereof that retains functionality. In yet another embodiment, the immunoconjugate comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 95 and SEQ ID NO: 96 or variants thereof that retain functionality. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 104 or a variant thereof that retains functionality. In yet another embodiment, the immunoconjugate comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 104 and SEQ ID NO: 96 or variants thereof that retain functionality. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 105 or a variant thereof that retains functionality. In yet another embodiment, the immunoconjugate comprises two polypeptide sequences that are at least about

80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 105 and SEQ ID NO: 96 or variants thereof that retain functionality. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 106 or a variant thereof that retains functionality. In yet another embodiment, the immunoconjugate comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 106 and SEQ ID NO: 96 or variants thereof that retain functionality. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 107 or a variant thereof that retains functionality. In yet another embodiment, the immunoconjugate comprises a polypeptide sequences that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 107 and SEQ ID NO: 96 or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond.

In one embodiment, the immunoconjugate of the invention comprises at least one, typically two or more antigen binding moieties that are specific for the A1 domain of Tenascin (TNC-A1). In another embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that can compete with monoclonal antibody F16 for binding to an epitope of TNC-A1. *See, e.g.,* PCT Publication WO 2007/128563 A1 (incorporated herein by reference in its entirety). In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the A1 and/or the A4 domain of Tenascin (TNC-A1 or TNC-A4 or TNC-A1/A4). In another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for the A1 domain of Tenascin shares a carboxy-terminal peptide bond with an IL-2 molecule, an IL-12 molecule, an IFN α molecule or a GM-CSF molecule, which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for the A1 domain of Tenascin. In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for the A1 domain of Tenascin shares a carboxy-terminal peptide bond with an IL-2 molecule which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for the A1 domain of Tenascin. In a further embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first scFv specific for the A1 domain of Tenascin shares a carboxy-terminal peptide bond with an IL-2 molecule which in turn shares a carboxy-terminal peptide bond with a second scFv specific for the A1 domain of Tenascin. In a specific embodiment, the antigen

binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 13 or SEQ ID NO: 15, or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 9 or SEQ ID NO: 11, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 13 or SEQ ID NO: 15 or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 9 or SEQ ID NO: 11 or variants thereof that retain functionality. In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 14 or SEQ ID NO: 16. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of either SEQ ID NO: 14 or SEQ ID NO: 16. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 10 or SEQ ID NO: 12. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of either SEQ ID NO: 10 or SEQ ID NO: 12. In a specific embodiment, the immunoconjugate comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 99 or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 100 or SEQ ID NO: 215, or variants thereof that retain functionality. In yet another specific embodiment, the immunoconjugate of the invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 101 or SEQ ID NO: 235 or variants thereof that retain functionality. In a more specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least

about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 100 and SEQ ID NO: 101 or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 215 and SEQ ID NO: 235 or variants thereof that retain functionality. In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 112. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 112. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 113 or SEQ ID NO: 216. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of either SEQ ID NO: 113 or SEQ ID NO: 216. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 114 or SEQ ID NO: 236. In yet another embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of either SEQ ID NO: 114 or SEQ ID NO: 236.

In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the A2 domain of Tenascin (TNC-A2). In another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for the A2 domain of Tenascin shares a carboxy-terminal peptide bond with an IL-2 molecule, an IL-12 molecule, an IFN α molecule or a GM-CSF molecule, which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for the A2 domain of Tenascin. In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for the A2 domain of Tenascin shares a carboxy-terminal peptide bond with an IL-2 molecule, which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for the A2 domain of Tenascin. In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 7, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 187, SEQ ID NO: 191, SEQ ID NO:195, SEQ ID NO: 199, SEQ ID NO: 203 and SEQ ID NO: 207, or variants thereof that retain functionality. In another specific

embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 5; SEQ ID NO: 177, SEQ ID NO: 181, SEQ ID NO:185, SEQ ID NO: 189, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 201 and SEQ ID NO: 205, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 7, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 187, SEQ ID NO: 191, SEQ ID NO:195, SEQ ID NO: 199, SEQ ID NO: 203 and SEQ ID NO: 207, or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 5; SEQ ID NO: 177, SEQ ID NO: 181, SEQ ID NO:185, SEQ ID NO: 189, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 201 and SEQ ID NO: 205, or variants thereof that retain functionality. In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 8, SEQ ID NO: 180, SEQ ID NO: 184, SEQ ID NO: 188, SEQ ID NO: 192, SEQ ID NO: 196, SEQ ID NO: 200, SEQ ID NO: 204 and SEQ ID NO: 208. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 8, SEQ ID NO: 180, SEQ ID NO: 184, SEQ ID NO: 188, SEQ ID NO: 192, SEQ ID NO: 196, SEQ ID NO: 200, SEQ ID NO: 204 and SEQ ID NO: 208. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 186, SEQ ID NO: 190, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 202 and SEQ ID NO: 206. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group of of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 186, SEQ ID NO: 190, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 202 and SEQ ID NO: 206. In a specific embodiment, the immunoconjugate of the invention comprises a polypeptide sequence that is at

least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 239, SEQ ID NO: 241 and SEQ ID NO: 243, or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 245, SEQ ID NO: 247 and SEQ ID NO:249, or variants thereof that retain functionality. In a more specific embodiment, the immunoconjugate of the present invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 239, SEQ ID NO: 241, and SEQ ID NO: 243 or variants thereof that retain functionality, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 245, SEQ ID NO: 247 and SEQ ID NO:249 or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 239 and either SEQ ID NO: 247 or SEQ ID NO: 249, or variants thereof that retain functionality. In yet another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 241 and either SEQ ID NO: 245 or SEQ ID NO: 247, or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 243 and SEQ ID NO: 245, or variants thereof that retain functionality. In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 240, SEQ ID NO: 242 and SEQ ID NO: 244. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of of SEQ ID NO: 240, SEQ ID NO: 242 and SEQ ID NO: 244. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 246, SEQ ID NO: 248 and SEQ ID NO: 250. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a

polynucleotide sequence selected from the group of of SEQ ID NO: 246, SEQ ID NO: 248 and SEQ ID NO: 250.

In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Fibroblast Activated Protein (FAP). In another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an IL-2 molecule, an IL-12 molecule, an IFN α molecule or a GM-CSF molecule, which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for FAP. In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an IL-2 molecule, which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for FAP. In another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an IL-12 molecule, which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for FAP.In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 31, SEQ ID NO: 35, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151, SEQ ID NO: 155, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171 and SEQ ID NO: 175, or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 29, SEQ ID NO: 33, SEQ ID NO: 37, SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 49, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149, SEQ ID NO: 153, SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169 and SEQ ID NO: 173, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain

variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 31, SEQ ID NO: 35, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151, SEQ ID NO: 155, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171, and SEQ ID NO: 175, or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 29, SEQ ID NO: 33, SEQ ID NO: 37, SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 49, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149, SEQ ID NO: 153, SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169, and SEQ ID NO: 173, or variants thereof that retain functionality. In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of: SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, SEQ ID NO: 156, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172, and SEQ ID NO: 176. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, SEQ ID NO: 156, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172, and SEQ ID NO: 176. In another specific

embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 24, SEQ ID NO: 30, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170, and SEQ ID NO: 174. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 24, SEQ ID NO: 30, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170, and SEQ ID NO: 174. In another specific embodiment, the immunoconjugate of the invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225 and SEQ ID NO: 227, or variants thereof that retain functionality. In yet another specific embodiment, the immunoconjugate of the invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233 and SEQ ID NO: 237 or variants thereof that retain functionality. In a more specific embodiment, the immunoconjugate of the present invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 211, SEQ ID NO: 219 and SEQ ID NO: 221 or variants thereof that retain functionality, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 231 or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the present invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected

from the group of SEQ ID NO: 209, SEQ ID NO: 223, SEQ ID NO: 225 and SEQ ID NO: 227 or variants thereof that retain functionality, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 229 or variants thereof that retain functionality. In a further specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 213 and SEQ ID NO: 233 or variants thereof that retain functionality. In yet another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 217 and SEQ ID NO: 237 or variants thereof that retain functionality. In yet another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 221 and SEQ ID NO: 231 or variants thereof that retain functionality. In yet another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 223 and SEQ ID NO: 229 or variants thereof that retain functionality. In yet another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 225 and SEQ ID NO: 229 or variants thereof that retain functionality. In yet another specific embodiment, the immunoconjugate of the present invention comprises two polypeptide sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 227 and SEQ ID NO: 229 or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, and SEQ ID NO: 228. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, and SEQ ID NO: 228. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID

NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, and SEQ ID NO: 238. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, and SEQ ID NO: 238.

In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Melanoma Chondroitin Sulfate Proteoglycan (MCSP). In another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for MCSP shares a carboxy-terminal peptide bond with an IL-2 molecule, an IL-12 molecule, an IFN α molecule or a GM-CSF molecule, which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for MCSP. In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a first Fab heavy chain specific for MCSP shares a carboxy-terminal peptide bond with an IL-2 molecule, which in turn shares a carboxy-terminal peptide bond with a second Fab heavy chain specific for MCSP. In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of either SEQ ID NO: 257 or SEQ ID NO: 261 or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of either SEQ ID NO: 259 or SEQ ID NO: 271 or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of either SEQ ID NO: 257 or SEQ ID NO: 261, or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of either SEQ ID NO: 259 or SEQ ID NO: 271, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 257, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 259. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or

100% identical to the sequence of SEQ ID NO: 261, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 259. In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of either SEQ ID NO: 258 or SEQ ID NO: 262. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of either SEQ ID NO: 258 or SEQ ID NO: 262. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of either SEQ ID NO: 260 or SEQ ID NO: 272. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of either SEQ ID NO: 260 or SEQ ID NO: 272. In a specific embodiment, the immunoconjugate of the invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 251 or SEQ ID NO: 255, or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 253 or SEQ ID NO: 265, or variants thereof that retain functionality. In a more specific embodiment, the immunoconjugate of the present invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 251 or SEQ ID NO: 255 or variants thereof that retain functionality, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 253 or SEQ ID NO: 265, or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the present invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 251 or variants thereof that retain functionality, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 253 or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate of the present invention comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 255 or variants thereof that retain functionality, and a polypeptide sequence that is at least

about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 253 or variants thereof that retain functionality. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of either SEQ ID NO: 252 or SEQ ID NO: 256. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of either SEQ ID NO: 252 or SEQ ID NO: 256. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of either SEQ ID NO: 254 or SEQ ID NO: 266. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of either SEQ ID NO: 254 or SEQ ID NO: 266.

In one embodiment the antigen binding moieties comprise at least a variable region capable of binding an antigenic determinant. Non-limiting variable regions useful in the present invention can be of murine, primate, or human origin. Human variable regions can be derived from human monoclonal antibodies made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor *et al., J Immunol. 133*:3001-3005 (1984) and Brodeur *et al.,* Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987). Human variable regions may also be produced by transgenic animals (*e.g.* mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigenic challenge. *See, e.g.,* Jakobovits *et al., Nature 362*:255-258 (1993).

Alternatively, phage display can be used to produce human antibodies and human variable regions *in vitro* from immunoglobulin variable (V) domain gene repertoires e.g., from unimmunized donors. (McCafferty *et al., Nature 348*:552-554 (1990).) In one example of this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle.

Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody/antibody fragments also result in selection of the gene encoding the antibody/antibody fragments exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats. For a review of phage display formats, *see* Hoogenboom *et al., Nucleic Acids Res. 19*:4133-4137 (1991). Several sources of V-gene segments can be used for phage display. Clackson *et al.,* isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. *See* Clackson *et al., Nature 352*:624-628 (1991). A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al., J. Mol. Bio. 222*:581-597 (1991). In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling." See Marks *et al., Biotech. 10*:779-783 (1992). In this method, the affinity of "primary" human antibodies or variable regions obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and variable regions with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse *et al., Nucl. Acids Res. 21:22*65-2266 (1993), and the isolation of a high affinity human antibody directly from such large phage library is reported by Griffith *et al., J. Cell. Bio. 120*:885-896 (1993). Gene shuffling can also be used to derive human antibodies and variable regions from rodent antibodies, where the human antibody or variable region has similar affinities and specificities to the starting rodent antibody or variable region. According to this method, which is also referred to as "epitope imprinting," the heavy or light chain V domain gene of rodent antibodies obtained by phage display techniques is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection with antigen results in the isolation of human variable regions capable of restoring a functional antigen binding site, *i.e.,* the epitope governs (imprints) the choice of partner. When the process is repeated to replace the remaining rodent V domain, a human antibody is obtained (see PCT publication WO 93/06213). Unlike traditional humanization of rodent antibodies, the epitope

imprinting technique provides completely human antibodies or variable regions, which have no framework or CDR residues of rodent origin.

Variable regions that can be used also include murine variable region sequences that have either been primatized or humanized or primate variable region sequences that have been humanized. As used herein, the term "humanized" refers to an antigen-binding moiety variable region sequence derived from a non-human antibody, for example, a murine antibody, that retains or substantially retains the antigen-binding properties of the parent molecule but which is less immunogenic in humans. This may be achieved by various methods including (a) grafting only the non-human CDRs onto human framework regions with or without retention of critical framework residues (*e.g.,* those that are important for retaining good antigen binding affinity or antibody functions) and (b) "cloaking" the non-human variable regions with a human-like section by replacement of surface residues. Such methods are disclosed by Jones *et al., Morrison et al,. Proc. Natl. Acad. Sci., 81*:6851-6855 (1984); Morrison and Oi, *Adv. Immunol., 44*:65-92 (1988); Verhoeyen *et al., Science, 239*:1534-1536 (1988); Padlan, *Molec. Immun., 28*:489-498 (1991); Padlan, *Molec. Immun., 31*(3):169-217 (1994), all of which are incorporated by reference in their entirety herein. There are generally 3 complementarity determining regions, or CDRs, (CDR1, CDR2, and CDR3) in each of the heavy and light chain variable regions of an antibody, which are flanked by four framework subregions (*i.e.,* FR1, FR2, FR3, and FR4) in each of the heavy and light chain variable domains of an antibody: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. A discussion of antibodies with humanized variable regions can be found, *inter alia*, in U.S. Patent No. 6,632,927, and in published U.S. Application No. 2003/0175269, both of which are incorporated herein by reference in their entirety.

Similarly, as used herein, the term "primatized" is used to refer to an antigen-binding moiety variable region derived from a non-primate antibody, for example, a murine antibody, that retains or substantially retains the antigen-binding properties of the parent molecule but which is less immunogenic in primates.

The choice of human variable domains, both heavy and light, in making humanized antigen binding moieties is very important to reduce antigenicity. According to the so-called "best fit" method, the sequence of the variable region of a rodent antigen binding moiety is screened against the entire library of known human variable-region sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antigen binding moiety (Sims *et al., J. Immunol., 151*:2296 (1993); Chothia *et al., J. Mol. Biol., 196*:901 (1987)). Another method of selecting the human framework sequence is

to compare the sequence of each individual subregion of the full rodent framework (*i.e.,* FR1, FR2, FR3, and FR4) or some combination of the individual subregions (*e.g.,* FR1 and FR2) against a library of known human variable region sequences that correspond to that framework subregion (*e.g.,* as determined by Kabat numbering), and choose the human sequence for each subregion or combination that is the closest to that of the rodent (U.S. Patent Application Publication No. 2003/0040606A1). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antigen binding moieties (Carter *et al., Proc. Natl. Acad. Sci. USA, 89*:4285 (1992); Presta *et al., J. Immunol., 151*:2623 (1993)).

Generally, the antigen binding moieties of the immunoconjugate of the invention retain high affinity for specific antigenic determinants and other favorable biological properties. Accordingly, humanized variable regions are prepared by analyzing the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformation structures of selected candidate immunoglobulin variable region sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin variable region sequence, *i.e.*, the analysis of residues that influence the ability of the candidate variable region sequence to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antigen binding moiety characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

In another embodiment, the antigen binding molecules of the present invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in U.S. Pat. Appl. Publ. No. 2004/0132066, the entire contents of which are hereby incorporated by reference. The ability of the immunoconjugate of the invention to bind to either an effector moiety receptor or to a specific antigenic determinant can be measured either through an enzyme linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g., surface plasmon resonance technique (analyzed on a BIACORE T100 system) (Liljeblad, *et al., Glyco. J. 17*:323-329 (2000)), and traditional binding assays (Heeley, R. P., *Endocr. Res. 28*:217-229 (2002)).

Effector Moieties

The effector moieties for use in the invention are generally polypeptides that influence cellular activity, for example, through signal transduction pathways. Accordingly, the effector moiety of the immunoconjugate of the invention can be associated with receptor-mediated signaling that transmits a signal from outside the cell membrane to modulate a response within the cell. For example, an effector moiety of the immunoconjugate can be a cytokine. In a particular embodiment, the effector moiety is a single-chain effector moiety as defined herein. In one embodiment, one or more effector moieties, typically single-chain effector moieties, of the immunoconjugates of the invention are cytokines selected from the group consisting of: IL-2, GM-CSF, IFN-α, and IL-12. In another embodiment, one or more single-chain effector moieties of the immunoconjugates are cytokines selected from the group consisting of: IL-8, MIP-1α, MIP-1β, and TGF-β.

In one embodiment, the effector moiety, preferably a single-chain effector moiety, of the immunoconjugate is IL-2. In a specific embodiment, the IL-2 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: proliferation in an activated T lymphocyte cell, differentiation in an activated T lymphocyte cell, cytotoxic T cell (CTL) activity, proliferation in an activated B cell, differentiation in an activated B cell, proliferation in a natural killer (NK) cell, differentiation in a NK cell, and NK/lymphocyte activated killer (LAK) antitumor cytotoxicity. In one embodiment, the effector moiety, preferably a single-chain effector moiety, of the immunoconjugate is GM-CSF. In a specific embodiment, the GM-CSF effector moiety can elicit proliferation and/or differentiation in a granulocyte, a monocyte or a dendritic cell. In one embodiment, the effector moiety, preferably a single-chain effector moiety, of the immunoconjugate is IFN-α. In a specific embodiment, the IFN-α effector moiety can elicit one or more of the cellular responses selected from the group consisting of: inhibiting viral replication in a virus-infected cell, and upregulating the expression of major histocompatibility complex I (MHC I). In another specific embodiment, the IFN α effector moiety can inhibit proliferation in a tumor cell. In one embodiment, the effector moiety, preferably a single-chain effector moiety, of the immunoconjugate is IL-12. In a specific embodiment, the IL-12 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: proliferation in a NK cell, differentiation in a NK cell, proliferation in a T cell, and differentiation in a T cell. In one embodiment, the effector moiety, preferably a single-chain effector moiety, of the immunoconjugate is IL-8. In a specific embodiment, the IL-8 effector

moiety can elicit chemotaxis in neutrophils. In one embodiment, the effector moiety, preferably a single-chain effector moiety, of the immunoconjugate, is MIP-1α. In a specific embodiment, the MIP-1α effector moiety can elicit chemotaxis in monocytes and T lymphocyte cells. In one embodiment, the effector moiety, preferably a single-chain effector moiety, of the immunoconjugate is MIP-1β. In a specific embodiment, the MIP-1β effector moiety can elicit chemotaxis in monocytes and T lymphocyte cells. In one embodiment, the effector moiety, preferably a single-chain effector moiety, of the immunoconjugate is TGF-β. In a specific embodiment, the TGF-β effector moiety can elicit one or more of the cellular responses selected from the group consisting of: chemotaxis in monocytes, chemotaxis in macrophages, upregulating the expression of IL-1 in activated macrophages, and upregulating the expression of IgA in activated B cells.

Immunoconjugate Polypeptides and Polynucleotides

The immunoconjugates of the invention comprise polypeptides and fragments thereof. As used herein, term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

A polypeptide of the invention may be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a

defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded.

By an "isolated" polypeptide or a variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for purposed of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

Also included as polypeptides of the present invention are derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "variant," "derivative" and "analog" when referring to polypeptides of the present invention include any polypeptides that retain at least some of the biological, antigenic, or immunogenic properties of the corresponding native polypeptide. Variants of polypeptides of the present invention include polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants may occur naturally or be non-naturally occurring. Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Variant polypeptides may comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives of polypeptides of the present invention, are polypeptides which have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins. Variant polypeptides may also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a polypeptide refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides which contain one or more 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 may be substituted for lysine.

Alternatively, recombinant variants encoding these same or similar polypeptides can be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of

any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, *i.e.,* conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are preferably in the range of about 1 to about 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the references sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96,%, 97%, 98%, or 99% identical to a reference polypeptide can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al., Comp. Appl. Biosci. 6*:237-245 (1990). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid

sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty-0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case, the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which

are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

Polypeptides of the invention include those that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequences set forth in Tables 3 and 4, below, including functional fragments or variants thereof. The invention also encompasses polypeptides comprising sequences of Tables 3 or 4 with conservative amino acid substitutions.

The polypeptides of the invention may be encoded by a single polynucleotide. Alternatively, the may be encoded by multiple (e.g., two or more) polynucleotides, so that the polypeptides are co-expressed. Polypeptides that are co-expressed from multiple polynucleotides may associate through, e.g., disulfide bonds or other means to form a functional immunoconjugate. For example, the heavy chain portion of an antigen binding moiety may be encoded by a separate polynucleotide from the portion of the immunoconjugate comprising the light chain portion of the antigen binding moiety and the effector moiety. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the antigen binding moiety. Alternatively, in another example, the light chain portion of the antigen binding moiety could be encoded by a separate polynucleotide from the portion of the immunoconjugate comprising the heavy chain portion of the antigen binding moiety and the effector moiety.

Immunoconjugates of the present invention and fragments thereof are generally encoded by polynucleotides. The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA), virally-derived RNA, or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refers to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a therapeutic polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the present invention, as well as positive and negative strand forms, and double-stranded forms, of pestivirus vectors disclosed herein.

Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' non-translated regions, and the like, are not part of a coding region. Two or more coding regions of the present invention can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, *e.g.*, a vector of the present invention may encode one or more polyproteins, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a first or second nucleic acid encoding the immunoconjugate of the invention, or variant or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid, which encodes a polypeptide normally may include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only

in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein.

A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (*e.g.*, the immediate early promoter, in conjunction with intron-A), simian virus 40 (*e.g.*, the early promoter), and retroviruses (such as, *e.g.*, Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). RNA of the present invention may be single stranded or double stranded.

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal

peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β-glucuronidase.

The term "expression cassette" refers to a polynucleotide generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter. In one embodiment, the expression cassette of the invention comprises polynucleotide sequences that encode immunoconjugates of the invention or fragments thereof.

The term "expression vector" is synonymous with "expression contsruct" and refers to a DNA molecule that is used to introduce and direct the expression of a specific gene to which it is operably associated into a target cell. The expression vector of the present invention comprises an expression cassette. Expression vectors allow transcription of large amounts of stable mRNA. Once the expression vector is inside the target cell, the ribonucleic acid molecule or protein that is encoded by the gene is produced by the cellular transcription and/or translation machinery. In one embodiment, the expression vector of the invention comprises an expression cassette comprises polynucleotide sequences that encode immunoconjugates of the invention or fragments thereof.

The term "artificial" refers to a synthetic, or non-host cell derived composition, *e.g.*, a chemically-synthesized oligonucleotide.

By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence or

polypeptide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., Comp. Appl. Biosci. 6:237-245 (1990). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty-30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject nucleotide sequences, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not

matached/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

Polynucleotides of the invention include those that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequences set forth in Tables 6 and 8, below, including functional fragments or variants thereof. The polynucleotides may be expressed as a single polynucleotide that encodes the entire immunoconjugate or as multiple (e.g., two or more) polynucleotides that are coexpressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional immunoconjugate. For example, the heavy chain portion of an antigen binding moiety may be encoded by a separate polynucleotide from the portion of the immunoconjugate comprising the light chain portion of the antigen binding moiety and the effector moiety. When coexpressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the antigen binding moiety. Alternatively, in another example, the light chain portion of the antigen binding moiety could be encoded by a separate polynucleotide from the portion of the immunoconjugate comprising the heavy chain portion of the antigen binding moiety and the effector moiety.

In a specific embodiment, an isolated polynucleotide of the invention encodes a fragment of an immunoconjugate comprising at least one effector moiety, preferably a single-chain effector moiety, and at least one, preferably two or more antigen binding moieties, wherein a first effector moiety shares an amino- or carboxy-terminal peptide bond with a first antigen binding moiety and a second antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either the first effector moiety or the first antigen binding moiety. In a preferred embodiment, the antigen binding moieties are independently selected from the group consisting of Fv and Fab. In another specific embodiment, the polynucleotide encodes the heavy chains of two of the antigen binding moieties and one of the effector moieties. In another specific embodiment, the polynucleotide encodes the light chains of two of the antigen binding moieties and one of the effector moieties. In another specific embodiment, the polynucleotide encodes one light chain from one of the antigen binding moieties, one heavy chain from a second antigen binding moiety and one of the effector moieties.

In another specific embodiment, an isolated polynucleotide of the invention encodes a fragment of an immunoconjugate, wherein the polynucleotide encodes the heavy chains of two Fab molecules and an effector moiety, preferably a single-chain effector moiety. In another specific

embodiment, an isolated polynucleotide of the invention encodes a fragment of an immunoconjugate, wherein the polynucleotide encodes the light chains of two Fab molecules and an effector moiety, preferably a single-chain effector moiety. In another specific embodiment an isolated polynucleotide of the invention encodes a fragment of an immunoconjugate, wherein the polynucleotide encodes the heavy chain of one Fab molecule, the light chain of second Fab molecule and an effector moiety, preferably a single-chain effector moiety.

In one embodiment, an isolated polynucleotide of the invention encodes an immunoconjugate comprising at least one effector moiety, preferably a single-chain effector moiety, joined at its amino- and carboxy-terminal amino acids to one or more scFv molecules.

In one embodiment, an isolated polynucleotide of the invention encodes an immunoconjugate fragment comprising at least one effector moiety, preferably a single-chain effector moiety and at least first and second antigen binding moieties, wherein each of the antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising an immunoglobulin constant domain independently selected from the group consisting of IgG1 CH1, IgG C_{kappa}, and IgE CH4, and wherein one of the antigen binding moieties is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the first and second antigen binding moieties are covalently linked through a disulfide bond. In a further embodiment, the polynucleotide of the invention encodes one of the antigen binding moieties and an effector moiety, preferably a single-chain effector moiety.

In one embodiment, an isolated polynucleotide of the invention encodes an immunoconjugate fragment comprising first and second effector moieties and two antigen binding moieties, wherein each of the antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising an immunoglobulin constant domain, and wherein one of the antigen binding moieties is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the second antigen binding moiety is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of the second effector moiety, and wherein the first and second antigen binding moieties are covalently linked through a disulfide bond. In a preferred embodiment, the first and/or second effector moieties are single chain effector moieties. In a preferred embodiment, the constant domain is independently selected from the group consisting

of IgG1 CH1, IgG C_{kappa}, and IgE CH4. In a further embodiment, the polynucleotide of the invention encodes one of the antigen binding moieties and one of the effector moieties.

In one embodiment, an isolated polynucleotide of the invention encodes an immunoconjugate fragment comprising at least one effector moiety, preferably a single-chain effector moiety, and at least first and second antigen binding moieties, wherein each of the antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to an IgG CH3 domain, and wherein one of the antigen binding moieties is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the first and second antigen binding moieties are covalently linked through a disulfide bond. In a further embodiment, the polynucleotide of the invention encodes one of the antigen binding moieties and an effector moiety, preferably a single chain effector moiety.

In one embodiment, an isolated polynucleotide of the invention encodes an immunoconjugate fragment comprising two effector moieties and two antigen binding moieties, wherein each of the antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to an IgG CH3 domain, and wherein one of the antigen binding moieties is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the second antigen binding moiety is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of the second effector moiety, and wherein the first and second antigen binding moieties are covalently linked through a disulfide bond. In a preferred embodiment, the first and/or second effector moieties are single chain effector moieties. In a further embodiment, the polynucleotide of the invention encodes one of the antigen binding moieties and one of the effector moieties, preferably a single chain effector moiety.

In one embodiment, an isolated polynucleotide of the invention encodes an immunoconugate fragment comprising two effector moieties and two antigen binding moieties, wherein each of the antigen binding moieties comprises an Fab molecule joined at its heavy or light chain carboxy-terminal amino acid to an IgG1 CH3 domain, and wherein each of the IgG1 CH3 domains is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of one of the effector moieties, and wherein the first and second antigen binding moieties are covalently linked through a disulfide bond. In a preferred embodiment, the first and/or second effector moieties are single chain effector moieties. In a further embodiment, the polynucleotide of the invention comprises a sequence encoding the heavy chain variable region of one of the antigen binding moieties and one of said effector moieties, preferably a single chain moiety. In yet another embodiment, the polynucleotide of the invention comprises a sequence encoding the

light chain variable region of one of the antigen binding moieties and one of the effector moieties, preferably a single chain effector moiety.

In another embodiment, the present invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable region sequence as shown in Table 3 below. In another embodiment, the present invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a polypeptide sequence as shown in Table 4. In another embodiment, the invention is further directed to an isolated nucleic acid encoding an immunoconjugate or fragment thereof, wherein the nucleic acid comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence shown in Tables 6 and 8 below. In another embodiment, the invention is directed to an isolated nucleic acid encoding an immunoconjugate or fragment thereof, wherein the nucleic acid comprises a nucleic acid sequence shown in Tables 6 and 8. In another embodiment, the invention is directed to an isolated nucleic acid encoding an immunoconjugate or fragment thereof, wherein the nucleic acid comprises a sequence that encodes a variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence in Table 3. In another embodiment, the invention is directed to an isolated nucleic acid encoding an immunoconjugate or fragment thereof, wherein the nucleic acid comprises a sequence that encodes a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence in Table 4. The invention encompasses an isolated nucleic acid encoding an immunoconjugate or fragment thereof, wherein the nucleic acid comprises a sequence that encodes the variable region sequences of Table 3 with conservative amino acid substitutions. The invention also encompasses an isolated nucleic acid encoding an immunoconjugate of the invention or fragment thereof, wherein the nucleic acid comprises a sequence that encodes the polypeptide sequences of Table 4 with conservative amino acid substitutions.

TABLE 2.

Construct	NUCLEOTIDE SEQUENCE	SEQ ID NO
Library Template for DP47-3 library; complete Fab coding region comprising PelB leader sequence + Vk3_20 kappa V-	ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTAT TACTCGCGGCCCAGCCGGCCATGGCCGAAATCGTGTTAAC GCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGA GCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCA GCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCC CAGGCTCCTCATCTATGGAGCATCCAGCAGGGCCACTGGC ATCCCAGACAGGTTCAGTGGCAGTGGATCCGGGACAGAC	1

Construct	NUCLEOTIDE SEQUENCE	SEQ ID NO
domain + CL constant domain for light chain and PelB + VH3_23 V-domain + CH1 constant domain for heavy chain; pMS25opt	TTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTG CAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGCTGAC GTTCGGCCAGGGGACCAAAGTGGAAATCAAACGTACGGT GGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAG CAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGA ATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGG TGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTG TCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCA GCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAAC ACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTG GAGCCGCAGAACAAAAACTCATCTCAGAAGAGGATCTGA ATGGAGCCGCAGACTACAAGGACGACGACGACAAGGGTG CCGCATAATAAGGCGCGCCAATTCTATTTCAAGGAGACA GTCATATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCT GCTGCTCCTCGCTGCCCAGCCGGCGATGGCCGAGGTGCA ATTGCTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGG GTCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTT AGCAGTTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGG AAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGT GGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTC ACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC AGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATT ACTGTGCGAAACCGTTTCCGTATTTTGACTACTGGGGCCA AGGAACCCTGGTCACCGTCTCGAGTGCTAGCACCAAAGG CCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCG CCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG TGAATCACAAGCCCAGCAACACCAAAGTGGACAAGAAAG TTGAGCCCAAATCTTGTGACGCGGCCGCAAGCACTAGTGC CCATCACCATCACCATCACGCCGCGGCATAG	
Library Template for DP88-3 library; complete Fab coding region comprising PelB leader sequence + Vk1_17 kappa V-domain + CL constant domain for light chain and PelB + VH1_69 V-domain + CH1 constant domain for heavy chain; pRJH32	ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTAT TACTCGCGGCCCAGCCGGCCATGGCCGATATCCAGATGA CCCAGTCTCCATCCTCCCTGTCTGCATCTGTCGGAGACCG GGTCACCATCACCTGCCGGGCAAGTCAGGGCATTAGAAA TGATTTAGGCTGGTACCAGCAGAAGCCAGGGAAAGCCCC TAAGCGCCTGATCTATGCTGCATCCAGTTTGCAGAGTGGC GTCCCATCAAGGTTCAGCGGCAGTGGATCCGGGACAGAG TTCACTCTCACCATCAGCAGCTTGCAGCCTGAAGATTTTG CCACCTATTACTGCTTGCAGCATAATAGTTACCCCACGTT TGGCCAGGGCACCAAAGTCGAGATCAAGCGTACGGTGGC TGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAG TTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATA ACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCA GCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACA AAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTGGAGC CGCAGAACAAAAACTCATCTCAGAAGAGGATCTGAATGG	2

Construct	NUCLEOTIDE SEQUENCE	SEQ ID NO
	AGCCGCAGACTACAAGGACGACGACGACAAGGGTGCCGC ATAATAAGGCGCGCCAATTCTATTTCAAGGAGACAGTCAT ATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGC TCCTCGCTGCCCAGCCGGCGATGGCCCAGGTGCAATTGGT GCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGT GAAGGTCTCCTGCAAGGCCTCCGGAGGCACATTCAGCAG CTACGCTATAAGCTGGGTGCGACAGGCCCCTGGACAAGG GCTCGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACA GCAAACTACGCACAGAAGTTCCAGGGCAGGGTCACCATT ACTGCAGACAAATCCACGAGCACAGCCTACATGGAGCTG AGCAGCCTGAGATCTGAGGACACCGCCGTGTATTACTGTG CGAGACTATCCCCAGGCGGTTACTATGTTATGGATGCCTG GGGCCAAGGGACCACCGTGACCGTCTCCTCAGCTAGCAC CAAAGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAG AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAAC TCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTG TCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC TGCAACGTGAATCACAAGCCCAGCAACACCAAAGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACGCGGCCGCAAGC ACTAGTGCCCATCACCATCACCATCACGCCGCGGCATAG	

TABLE 3.

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
2B10; V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPG KAPKRLIYAASSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFA TYYCLQNGLQPATFGQGTKVEIK	3
2B10(GS); V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPG KAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFAT YYCLQNGLQPATFGQGTKVEIK	5
2B10; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	7
2F11; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGVPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGQYTPPTFGQGTKVEIK	9
2F11(VI); V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQYTPPTFGQGTKVEIK	11
2F11; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDMAVYYCAKWRWMMFDYWGQGTLVTVSS	13
2F11(MT); V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKWRWMMFDYWGQGTLVTVSS	15
3F2; V_L	EIVLTQSPGTLSLYPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	17
3F2(YS); V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA	19

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	VYYCQQGIMLPPTFGQGTKVEIK	
3F2; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	21
3D9, V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQLIPPTFGQGTKVEIK	23
3D9, V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQTP GKGLEWVSAIGVSTGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGPFDYWGQGTLVTVSS	25
3D9(TA); V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIGVSTGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGPFDYWGQGTLVTVSS	27
4G8; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKPG QAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIK	29
4G8; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSS	31
4B3; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWYQQKPG QAPRLLIYGAYIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIK	33
4B3; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSS	35
4D6; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWYQQKPG QAPRLLIQGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIK	37
4D6; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSS	39
2C6; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQQIPPTFGQGTKVEIK	41
2C6; V_H	EVQLLESGGGLVQPGGSLRLSCAASGSTFSSYAMSWVRQAP GKGLEWVSAISGSAGYTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGNFDYWGQGTLVTVSS	43
5H5; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGNQIPPTFGQGTKVEIK	45
5H5; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMSWVRRSP GKGLEWVSAISGGGRTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKGWFTPFDYWGQGTLVTVSS	47
2C4; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWYQQKPG QAPRLLIYGASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGNQIPPTFGQGTKVEIK	49
2C4; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFTPFDYWGQGTLVTVSS	51
2D9; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGNQIPPTFGQGTKVEIK	67
2D9; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP	69

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFTPFDYWGQGTLVTVSS	
4B8; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIK	71
4B8; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSS	73
7A1; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQQIPPTFGQGTKVEIK	75
7A1; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGNFDYWGQGTLVTVSS	77
13C2; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQLIPPTFGQGTKVEIK	79
13C2; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGPFDYWGQGTLVTVSS	81
13E8; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGLNIPSTFGQGTKVEIK	83
13E8; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGPFDYWGQGTLVTVSS	85
14C10; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGHIIPPTFGQGTKVEIK	87
14C10; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKAWMGPFDYWGQGTLVTVSS	89
17A11; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGLNIPSTFGQGTKVEIK	91
17A11; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGPFDYWGQGTLVTVSS	93
19G1; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	121
19G1; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIISSGGLTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	123
20G8; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	125
20G8; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIIGSGSRTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	127
4B9; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	129

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
4B9; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIIGSGASTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	131
5B8; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	133
5B8; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIWGGGRSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	135
5F1; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	137
5F1; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIISSGASTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	139
14B3; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	141
14B3; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAILASGAITYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	143
16F1; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	145
16F1; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSGIIGSGGITYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	147
16F8; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	149
16F8; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAILGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	151
O3C9; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	153
O3C9; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFAMSWVRQSP GKGLEWVSAIIGSGSNTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	155
O2D7; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGTPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQAIMLPPTFGQGTKVEIK	157
O2D7; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	159
28H1; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKPG QAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIK	161
28H1; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVRQAP GKGLEWVSAIWASGEQYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSS	163
22A3; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA	165

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	VYYCQQGIMLPPTFGQGTKVEIK	
22A3; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIIGSGSITYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	167
29B11; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIK	169
29B11; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIIGSGGITYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS	171
23C10; V_L	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKPG QAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIK	173
23C10; V_H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAP GKGLEWVSAISTNGNYTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSS	175
2B10_C3B6; V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPG KAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFAT YYCLQNGLQPATFGQGTKVEIK	177
2B10_C3B6; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGAIIPILGIANYAQKFQGRVTITADKSTSTAYMEL SSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	179
2B10_6A12; V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPG KAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFAT YYCLQNGLQPATFGQGTKVEIK	181
2B10_6A12; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGVIIPILGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	183
2B10_C3A6; V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNVLGWYQQKPG KAPKRLIYDSSSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFAT YYCLQNGLQPATFGQGTKVEIK	185
2B10_C3A6; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	187
2B10_D1A2_wt; V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNVLGWYQQKPG KAPKRLIYDAYSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFA TYYCLQNGLQPATFGQGTKVEIK	189
2B10_D1A2_wt; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	191
2B10_D1A2_VD; V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPG KAPKRLIYDAYSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFA TYYCLQNGLQPATFGQGTKVEIK	193
2B10_D1A2_VD; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	195
2B10_O7D8; V_L	DIQMTQSPSSLSASVGDRVTITCRASQSIRNVLGWYQQKPGK APKRLIYDVSSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFAT YYCLQNGLQPATFGQGTKVEIK	197
2B10_O7D8; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	199
2B10_O1F7; V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNVLGWYQQKPG	201

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	KAPKRLIYDASSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFA TYYCLQNGLQPATFGQGTKVEIK	
2B10_O1F7; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	203
2B10_6H10; V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNVLGWYQQKPG KAPKRLIQAATSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFA TYYCLQNGLQPATFGQGTKVEIK	205
2B10_6H10; V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSS	207
MHLG1; V_H	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYWMNWVRQA PGKGLEWVAEIRLKSNNFGRYYAASVKGRFTISRDDSKNTL YLQMNSLKTEDTAVYYCTTYGNYVGHYFDHWGQGTTVTV SS	257
KV9; V_L	DIQLTQSPSFLSASVGDRVTITCKASQNVDTNVAWYQQKPG QAPRPLIYSASYRYTGVPSRFSGSGSGTEFTLTISSLQPEDFAT YYCQQYNSYPLTFGGGTKVEIKRT	259
MHLG; V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQA PGKGLEWVAEIRLKSNNFGRYYAASVKGRFTISRDDSKNTL YLQMNSLKTEDTAVYYCTTYGNYVGHYFDHWGQGTTVTV SS	261
KV1; V_L	DIQLTQSPSFLSASVGDRVTITCRASQNVDTNLAWYQQKPGKA PKLLIYSASYRYTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYC QQYNSYPLTFGGGTKVEIKRT	269
KV7; V_L	DIQLTQSPSFLSASVGDRVTITCKASQNVDTNVAWYQQKPGKA PKPLIYSASYRYTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYC QQYNSYPLTFGGGTKVEIKRT	271

TABLE 4.

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
Fab heavy chain derived from L19 monoclonal antibody-C125A variant of IL2-Fab heavy chain derived from L19 monoclonal antibody	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAP GKGLEWVSSISGSSGTTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKTQ LQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATE LKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLE LKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGGG SGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFSSF SMSWVRQAPGKGLEWVSSISGSSGTTYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCAKPFPYFDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKKVEPKSCD	95
Fab light chain derived from L19 monoclonal antibody	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQTGRIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF	96

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	NRGEC	
scFv derived from L19 monoclonal antibody-8 amino acid linker-C125A variant of IL2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAP GKGLEWVSSISGSSGTTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSSGGSGG ASEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQK PGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEISVLSSSSGSSSSGSSSSGAP TSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKF YMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIIS TLT	97
F16-diabody-IL2 protein	EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKAHNAFDYWGQGTLVTVSSASGGSS ELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAP VLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADY YCNSSVYTMPPVVFGGGTKLTVLGSSSSGSSSSGSSSSGAPTS SSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLIS NINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIIST LT	98
scFv-IL2-scFv (F16, protein)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKAHNAFDYWGQGTLVTVSRGGGGS GGGGSGGGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYA SWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTI TGAQAEDEADYYCNSSVYTMPPVVFGGGTKLTVLGSSSSGS SSSGSSSSGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL NRWITFAQSIISTLTSGGGGSGGGGSGGGGSSELTQDPAVSV ALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNN RPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSSVYTM PPVVFGGGTKLTVLGSGGGSGGGSGGGSGSEVQLLESGGGL VQPGGSLRLSCAASGFTFSRYGMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAKAHNAFDYWGQGTLVTVS	99
Fab-IL2-Fab (F16, heavy chain cytokine fusion construct, protein)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKAHNAFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDSSSSGSSSSGSSSSGAPTSSSTKKTQLQ LEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELK GSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGGGSG GGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFSRYG MSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKAHNAFDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKKVEPKSCD	100
F16, light chain,	SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQ	101

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
protein	APVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEAD YYCNSSVYTMPPVVFGGGTKLTVLGQPKAAPSVTLFPPSSEE LQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPS KQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTV APTECS	
IL2R-beta-Fc(hole) fusion protein, protein	MDMRVPAQLLGLLLLWFPGARCAVNGTSQFTCFYNSRANIS CVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFK PFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFE ARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVR VKPLQGEFTTWSPWSQPLAFRTKPAALGKDTGAQDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK	102
IL2R-gamma-Fc(knob), protein	MLKPSLPFTSLLFLQLPLLGVGLNTTILTPNGNEDTTADFFLT TMPTDSLSVSTLPLPEVQCFVFNVEYMNCTWNSSSEPQPTNL TLHYWYKNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHLYQ TFVVQLQDPREPRRQATQMLKLQNLVIPWAPENLTLHKLSE SQLELNWNNRFLNHCLEHLVQYRTDWDHSWTEQSVDYRH KFSLPSVDGQKRYTFRVRSRFNPLCGSAQHWSEWSHPIHWG SNTSKENPFLFALEAGAQDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	103
Fab-IL12-Fab L19 antibody, murine scIL12, protein	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAP GKGLEWVSSISGSSGTTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDSGGGGSGGGGSGGGGAMWELEKDVY VVEVDWTPDAPGETVNLTCDTPEEDDITWTSDQRHGVIGSG KTLTITVKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWS TEILKNFKNKTFLKCEAPNYSGRFTCSWLVQRNMDLKFNIK SSSSPPDSRAVTCGMASLSAEKVTLDQRDYEKYSVSCQEDV TCPTAEETLPIELALEARQQNKYENYSTSFFIRDIIKPDPPKNL QMKPLKNSQVEVSWEYPDSWSTPRSYFSLKFFVRIQRKKEK MKETEEGCNQKGAFFVEKTSTEVQCKGGNVCVQAQDRYY NSSCSKWACVPCRVRSGGDGSGGGGSGGGGSRVIPVSGPAR CLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRD QTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSLM MTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGML VAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHA FSTRVVTINRVMGYLSSAGGGGSGGGGSGGGGEVQLLESGG GLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSS ISGSSGTTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCAKPFPYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK	104

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	SCD	
Fab-IL12-Fab L19 antibody, human scIL12, protein	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAP GKGLEWVSSIRGSSGTTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDSGGGGSGGGGSGGGGIWELKKDVYV VELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGK TLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWST DILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSV KSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDS ACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKS KREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEW ASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQ NLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEA CLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELM QALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNASGGGGSGGGGSGGGGEVQLLESGGGLVQPGGS LRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSIRGSSGTTY YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPF PYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	105
Fab-GMCSF-Fab L19 antibody, human GM-CSF, protein	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAP GKGLEWVSSIRGSSGTTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPARSPSPSTQP WEHVNAIQEARRLLNLSRDTAAEMNETVEVISEMFDLQEPT CLQTRLELYKQGLRGSLTKLKGPLTMMASHYKQHCPPTPET SCATQIITFESFKENLKDFLLVIPFDCWEPVQESGGGGSGGGG SGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSW VRQAPGKGLEWVSSIRGSSGTTYYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCD	106
Fab-IFNα2-Fab, L19 antibody, protein	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAP GKGLEWVSSIRGSSGTTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDSGGGGSGGGGSGGGGCDLPQTHSLGN RRALILLAQMRRISPFSCLKDRHDFGFPQEEFDGNQFQKAQA ISVLHEMIQQTFNLFSTKDSSAAWDESLLEKFYTELYQQLND LEACVIQEVGVEETPLMNVDSILAVKKYFQRITLYLTEKKYS PCAWEVVRAEIMRSFSLSTNLQERLRRKESGGGGSGGGGSG GGGEVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVR QAPGKGLEWVSSIRGSSGTTYYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSASTK	107

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCD	
3F2 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKT QLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKAT ELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVL ELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGG GSGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	209
4G8 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKT QLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKAT ELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVL ELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGG GSGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	211
3D9 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQTP GKGLEWVSAIGVSTGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGPFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKT QLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKAT ELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVL ELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGG GSGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQTPGKGLEWVSAIGVSTGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGPFDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	213
2F11 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKWRWMMFDYWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN	215

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	HKPSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTK KTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKK ATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSG GGGSGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFT FSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCAKWRWMMFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCD	
4B3 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKT QLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKAT ELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVL ELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGG GSGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	217
4G8 Fab-IL12-Fab (murine IL-12; heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAMWELEKD VYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSDQRHGVIG SGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLHKKENGI WSTEILKNFKNKTFLKCEAPNYSGRFTCSWLVQRNMDLKFN IKSSSSPPDSRAVTCGMASLSAEKVTLDQRDYEKYSVSCQED VTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIIKPDPPKN LQMKPLKNSQVEVSWEYPDSWSTPRSYFSLKFFVRIQRKKE KMKETEEGCNQKGAFFVEKTSTEVQCKGGNVCVQAQDRY YNSSCSKWACVPCRVRSGGDGSGGGGSGGGGSRVIPVSGPA RCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSL MMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGM LVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLH AFSTRVVTINRVMGYLSSAGGGGSGGGGSGGGGEVQLLESG GGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWV SAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCAKGWLGNFDYWGQGTLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCD	219
28H1 Fab-IL2-Fab (heavy chain cytokine fusion	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVRQAP GKGLEWVSAIWASGEQYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASTK	221

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
construct)	GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKT QLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKAT ELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVL ELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGG GSGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFS SHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	
29B11 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIIGSGGITYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKTQ LQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATE LKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLE LKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGGG SGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFSS YAMSWVRQAPGKGLEWVSAIIGSGGITYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCD	223
19G1 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIISSGGLTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKT QLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKAT ELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVL ELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGG GSGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKGLEWVSAIISSGGLTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	225
20G8 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIIGSGSRTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTKKT QLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKAT ELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVL ELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSGGG GSGGGGSGGGGEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKGLEWVSAIIGSGSRTYYADSVKGRFTIS	227

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	RDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	
3F2 light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	229
4G8 light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKPG QAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	231
3D9 light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQLIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	233
2F11 light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQYTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	235
4B3 light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWYQQKPG QAPRLLIYGAYIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	237
2B10 Fab-IL2-Fab (heavy chain cytokine fusion construct)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSST KKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPK KATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNIN VIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTS GGGGSGGGGSGGGGQVQLVQSGAEVKKPGSSVKVSCKASG GTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGR VTITADKSTSTAYMELSSLRSEDTAVYYCARLYGYAYYGAF DYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	239
C3B6 Fab-IL2-Fab (heavy chain cytokine fusion	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGAIIPILGIANYAQKFQGRVTITADKSTSTAYMEL SSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG	241

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
construct)	ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSSTK KTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKK ATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTSG GGGSGGGGSGGGGQVQLVQSGAEVKKPGSSVKVSCKASGG TFSSYAISWVRQAPGQGLEWMGAIIPILGIANYAQKFQGRVT ITADKSTSTAYMELSSLRSEDTAVYYCARLYGYAYYGAFDY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	
6A12 Fab-IL2-Fab (heavy chain cytokine fusion construct)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGVIIPILGTANYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYCARLYGYAYYGAFDYWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPTSSST KKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPK KATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNIN VIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLTS GGGGSGGGGSGGGGQVQLVQSGAEVKKPGSSVKVSCKASG GTFSSYAISWVRQAPGQGLEWMGVIIPILGTANYAQKFQGR VTITADKSTSTAYMELSSLRSEDTAVYYCARLYGYAYYGAF DYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	243
2B10 light chain	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPG KAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFAT YYCLQNGLQPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	245
D1A2 light chain	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPG KAPKRLIYDAYSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFA TYYCLQNGLQPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	247
O7D8 light chain	DIQMTQSPSSLSASVGDRVTITCRASQSIRNVLGWYQQKPGK APKRLIYDVSSLQSGVPSRFSGGGSGTEFTLTISSLQPEDFAT YYCLQNGLQPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	249
MHLG1 Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYWMNWVRQA PGKGLEWVAEIRLKSNNFGRYYAASVKGRFTISRDDSKNTL YLQMNSLKTEDTAVYYCTTYGNYVGHYFDHWGQGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPT SSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLIS NINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIIST	251

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	LTSGGGGSGGGGSGGGGEVQLVESGGGLVKPGGSLRLSCA ASGFTFSNYWMNWVRQAPGKGLEWVAEIRLKSNNFGRYY AASVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTYGN YVGHYFDHWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	
KV9 light chain	DIQLTQSPSFLSASVGDRVTITCKASQNVDTNVAWYQQKPG QAPRPLIYSASYRYTGVPSRFSGSGSGTEFTLTISSLQPEDFAT YYCQQYNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	253
MHLG Fab-IL2-Fab (heavy chain cytokine fusion construct)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQA PGKGLEWVAEIRLKSNNFGRYYAASVKGRFTISRDDSKNTL YLQMNSLKTEDTAVYYCTTYGNYVGHYFDHWGQGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDSGGGGSGGGGSGGGGAPT SSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLIS NINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIIST LTSGGGGSGGGGSGGGGEVQLVESGGGLVQPGGSLRLSCA ASGFTFSNYWMNWVRQAPGKGLEWVAEIRLKSNNFGRYY AASVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTYGN YVGHYFDHWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	255
KV1 light chain	DIQLTQSPSFLSASVGDRVTITCRASQNVDTNLAWYQQKPGKA PKLLIYSASYRYTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYC QQYNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	263
KV7 light chain	DIQLTQSPSFLSASVGDRVTITCKASQNVDTNVAWYQQKPGKA PKPLIYSASYRYTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYC QQYNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	265

TABLE 5.

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
Human FAP ectodomain+poly-lys-tag+his_6-tag	RPSRVHNSEENTMRALTLKDILNGTFSYKTFFPNWISGQEYL HQSADNNIVLYNIETGQSYTILSNRTMKSVNASNYGLSPDRQ FVYLESDYSKLWRYSYTATYYIYDLSNGEFVRGNELPRPIQY LCWSPVGSKLAYVYQNNIYLKQRPGDPPFQITFNGRENKIFN GIPDWVYEEEMLATKYALWWSPNGKFLAYAEFNDTDIPVIA YSYYGDEQYPRTINIPYPKAGAKNPVVRIFIIDTTYPAYVGPQ EVPVPAMIASSDYYFSWLTWVTDERVCLQWLKRVQNVSVL SICDFREDWQTWDCPKTQEHIEESRTGWAGGFFVSTPVFSY DAISYYKIFSDKDGYKHIHYIKDTVENAIQITSGKWEAINIFR VTQDSLFYSSNEFEEYPGRRNIYRISIGSYPPSKKCVTCHLRK ERCQYYTASFSDYAKYYALVCYGPGIPISTLHDGRTDQEIKI LEENKELENALKNIQLPKEEIKKLEVDEITLWYKMILPPQFD	53

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	RSKKYPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVIAL VDGRGTAFQGDKLLYAVYRKLGVYEVEDQITAVRKFIEMG FIDEKRIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPVSSW EYYASVYTERFMGLPTKDDNLEHYKNSTVMARAEYFRNVD YLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYSDQ NHGLSGLSTNHLYTHMTHFLKQCFSLSDGKKKKKKGHHHH HH	
Murine FAP ectodomain+poly-lys-tag+his_6-tag	RPSRVYKPEGNTKRALTLKDILNGTFSYKTYFPNWISEQEYL HQSEDDNIVFYNIETRESYIILSNSTMKSVNATDYGLSPDRQF VYLESDYSKLWRYSYTATYYIYDLQNGEFVRGYELPRPIQY LCWSPVGSKLAYVYQNNIYLKQRPGDPPFQITYTGRENRIFN GIPDWVYEEEMLATKYALWWSPDGKFLAYVEFNDSDIPIIA YSYYGDGQYPRTINIPYPKAGAKNPVVRVFIVDTTYPHHVG PMEVPVPEMIASSDYYFSWLTWVSSERVCLQWLKRVQNVS VLSICDFREDWHAWECPKNQEHVEESRTGWAGGFFVSTPAF SQDATSYYKIFSDKDGYKHIHYIKDTVENAIQITSGKWEAIYI FRVTQDSLFYSSNEFEGYPGRRNIYRISIGNSPPSKKCVTCHL RKERCQYYTASFSYKAKYYALVCYGPGLPISTLHDGRTDQE IQVLEENKELENSLRNIQLPKVEIKKLKDGGLTFWYKMILPP QFDRSKKYPLLIQVYGGPCSQSVKSVFAVNWITYLASKEGIV IALVDGRGTAFQGDKFLHAVYRKLGVYEVEDQLTAVRKFIE MGFIDEERIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPVS SWEYYASIYSERFMGLPTKDDNLEHYKNSTVMARAEYFRN VDYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYS DQNHGILSGRSQNHLYTHMTHFLKQCFSLSDGKKKKKKGH HHHHH	55
Human TNC-A2+avi-tag +his_6-tag	ASTGETPNLGEVVVAEVGWDALKLNWTAPEGAYEYFFIQV QEADTVEAAQNLTVPGGLRSTDLPGLKAATHYTITIRGVTQ DFSTTPLSVEVLTASGLNDIFEAQKIEWHEGTHHHHHH	57
Human TNC-A1+avi-tag +his_6-tag	EQAPELENLTVTEVGWDGLRLNWTAADQAYEHFIIQVQEA NKVEAARNLTVPGSLRAVDIPGLKAATPYTVSIYGVIQGYRT PVLSAEASTASGLNDIFEAQKIEWHEGTHHHHHH	59
Murine TNC-A1+avi-tag +his_6-tag	ISEFGSSTEEVPSLENLTVTEAGWDGLRLNWTADDLAYEYF VIQVQEANNVETAHNFTVPGNLRAADIPGLKVATSYRVSIY GVARGYRTPVLSAETSTASGLNDIFEAQKIEWHEGTHHHHH H	61
Human TNC-A4+ avi-tag +his_6-tag	EDLPQLGDLAVSEVGWDGLRLNWTAADNAYEHFVIQVQEV NKVEAAQNLTLPGSLRAVDIPGLEAATPYRVSIYGVIRGYRT PVLSAEASTASGLNDIFEAQKIEWHEGTHHHHHH	63
Murine TNC-A4 +avi-tag +his_6-tag	ISEFGSLTEDLPQLGGLSVTEVSWDGLTLNWTTDDLAYKHF VVQVQEANNVEAAQNLTVPGSLRAVDIPGLKADTPYRVSIY GVIQGYRTPMLSTDVSTASGLNDIFEAQKIEWHEGTHHHHH H	65

TABLE 6.

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
2B10; V_L	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGCATTAGAAATGATTTAGGCTGGTACCAGCAGAAGC CAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCA GTTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTG	4

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCA GCCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGT CTGCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAG ATCAAG	
2B10(GS); V_L	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGCATTAGAAATGATTTAGGCTGGTACCAGCAGAAGC CAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCA GTTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCAGTG GATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCA GCCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGT CTGCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAG ATCAAG	6
2B10; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGGGATCATCC CTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCA	8
2F11; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCGTCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGTATACTCCCCCCACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	10
2F11(VI); V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGTATACTCCCCCCACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	12
2F11; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACATGGCC GTATATTACTGTGCGAAATGGAGATGGATGATGTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	14
2F11(MT); V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC	16

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCC GTATATTACTGTGCGAAATGGAGATGGATGATGTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	
3F2; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT ATCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	18
3F2(YS); V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	20
3F2; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	22
3D9, V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGCTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	24
3D9, V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGAC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTGGTGTT AGTACTGGTAGCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGTTGGCTGGGTCCTTTTGACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	26
3D9(TA); V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT	28

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTGGTGTT AGTACTGGTAGCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGTTGGCTGGGTCCTTTTGACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	
4G8; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCCGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCATTGGGGCCTC CACCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACGGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGGTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	30
4G8; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	32
4B3; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAATTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGCGCCTA CATCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGGTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	34
4B3; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	36
4D6; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAACTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCCAGGGCGCCTC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGGTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	38
4D6; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG	40

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	
2C6; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGGCTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGCAGATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	42
2C6; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT CCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG GAGTGCTGGTTATACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGTTGGTTTGGGAATTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	44
5H5; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA ATCAGATTCCCCCTACGTTCGGTCAGGGGACCAAAGTGG AAATCAAA	46
5H5; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATACCATGAGCTGGGTCCGCCGGTC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TGGTGGTAGGACATACTACGCAGACTCCGTGAAGGGCCG GTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT CTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTA TATTACTGTGCGAAAGGTTGGTTTACGCCTTTTGACTACT GGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	48
2C4; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGTAACTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCCTC CATTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA ATCAGATTCCCCCTACGTTCGGTCAGGGGACCAAAGTGG AAATCAAA	50

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
2C4; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGCGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGTTGGTTTACGCCTTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	52
2D9; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA ATCAGATTCCCCCTACGTTCGGTCAGGGGACCAAAGTGG AAATCAAA	68
2D9; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGCGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGTTGGTTTACGCCTTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	70
4B8; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGGTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	72
4B8; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	74
7A1; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGCAGATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG	76

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	AAATCAAA	
7A1; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGTTGGTTTGGGAATTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	78
13C2; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGCTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	80
13C2; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGTTGGCTGGGTCCTTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	82
13E8; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC TGAATATTCCCTCGACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	84
13E8; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGTTGGTTGGGTCCGTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	86
14C10; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC	88

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	ATATTATTCCCCCGACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	
14C10; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGCTTGGATGGGGCCTTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	90
17A11; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC TGAATATTCCCTCGACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	92
17A11; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGTTGGTTGGGTCCGTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	94
19G1; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	122
19G1; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCGATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCGATTATTAG TAGTGGTGGTCTCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	124
20G8; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG	126

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	
20G8; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCAATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTATTGG GAGTGGTAGTCGTACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	128
4B9; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	130
4B9; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTATTGGT AGTGGTGCTAGCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	132
5B8; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	134
5B8; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTTGGGG TGGTGGTCGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	136
5F1; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG	138

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	
5F1; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTATTAGT AGTGGGGCTAGCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	140
14B3; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	142
14B3; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTTTGGCT AGTGGTGCGATCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	144
16F1; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	146
16F1; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGGTATTATTGG TAGTGGTGGTATCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	148
16F8; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC	150

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	
16F8; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTCTTGGT AGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	152
O3C9; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	154
O3C9; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTTTGCCATGAGCTGGGTCCGTCAGTC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTATTGGT AGTGGTAGTAACACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	156
O2D7; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCACCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGCTA TTATGCTTCCTCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	158
O2D7; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGTCC	160
28H1; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCCGCAGCTACTTAGCCTGGTACCAGCAGA	162

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	AACCTGGCCAGGCTCCCAGGCTCCTCATCATTGGGGCCTC CACCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGGTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	
28H1; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTCATGCTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTTGGGC TAGTGGGGAGCAATACTACGCAGACTCCGTGAAGGGCCG GTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT CTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTA TATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACTACT GGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	164
22A3; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	166
22A3; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTATTGGT AGTGGTAGTATCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	168
29B11; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTACCAGTAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTC CCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTA TTATGCTTCCCCCGACGTTCGGCCAGGGGACCAAAGTGGA AATCAAA	170
29B11; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTATTGGT AGTGGTGGTATCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	172
23C10; V_L	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC	174

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	AGAGTGTTAGCCGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCATTGGGGCCTC CACCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGGTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG AAATCAAA	
23C10; V_H	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTCTGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTAC TAATGGTAATTATACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT	176
2B10_C3B6; V_L	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGCATTAGAAATGATTTAGGCTGGTACCAGCAGAAGC CAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCA GTTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCAGTG GATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCA GCCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGT CTGCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAG ATCAAG	178
2B10_C3B6; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGCTATCATCC CGATCCTTGGTATCGCAAACTACGCACAGAAGTTCCAGG GCAGGGTCACCATTACTGCAGACAAATCCACGAGCACAG CCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCG CCGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTA CGGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCA	180
2B10_6A12; V_L	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGCATTAGAAATGATTTAGGCTGGTACCAGCAGAAGC CAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCA GTTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCAGTG GATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCA GCCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGT CTGCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAG ATCAAG	182
2B10_6A12; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTATGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGTGATCATCC CTATCCTTGGTACCGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC	184

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GTCTCCTCA	
2B10_C3A6; V_L	GACATCCAGATGACCCAGTCTCCTTCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGCATTCGTAATGTTTTAGGCTGGTACCAGCAGAAGCC AGGGAAAGCCCCTAAGCGCCTGATCTATGATTCGTCCAGT TTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTGGA TCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCAGC CTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGTCT GCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAGAT CAAG	186
2B10_C3A6; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGGGATCATCC CTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCA	188
2B10_D1A2_wt; V_L	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGGATTCGTAATGTTTTAGGCTGGTACCAGCAGAAGC CAGGGAAAGCCCCTAAGCGCCTGATCTATGATGCTTACA GCTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTG GATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCA GCCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGT CTGCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAG ATCAAG	190
2B10_D1A2_wt; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGGGATCATCC CTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCA	192
2B10_D1A2_VD; V_L	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGGATTCGTAATGATTTAGGCTGGTACCAGCAGAAGC CAGGGAAAGCCCCTAAGCGCCTGATCTATGATGCTTACA GCTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTG GATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCA GCCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGT CTGCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAG ATCAAG	194
2B10_D1A2_VD; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGGGATCATCC CTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG	196

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCA	
2B10_O7D8; V_L	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGAGCATTCGTAATGTTTTAGGCTGGTACCAGCAGAAGCC AGGGAAAGCCCCTAAGCGCCTGATCTATGATGTGTCCAGT TTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTGGA TCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCAGC CTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGTCT GCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAGAT CAAG	198
2B10_O7D8; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGGGATCATCC CTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCA	200
2B10_O1F7; V_L	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGCATTCGTAATGTTTTAGGCTGGTACCAGCAGAAGCC AGGGAAAGCCCCTAAGCGCCTGATCTATGATGCGTCCAG TTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTGG ATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCAG CCTGAAGATTTTGCCACCTATTACTGCCTGCAGAATGGTC TGCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAGA TCAAG	202
2B10_O1F7; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGGGATCATCC CTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCA	204
2B10_6H10; V_L	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGCATTCGTAATGTTTTAGGCTGGTACCAGCAGAAGCC AGGGAAAGCCCCTAAGCGCCTGATCCAGGCTGCTACCAG TTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTGG ATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCAG CCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGTC TGCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAGA TCAAG	206
2B10_6H10; V_H	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG	208

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGGGATCATCC CTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCA	
MHLG1; V_H	GAAGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTCAAG CCTGGCGGGTCCCTGCGGCTCTCCTGTGCAGCCTCCGGAT TCACATTTAGCAACTATTGGATGAACTGGGTGCGGCAGGC TCCTGGAAAGGGCCTCGAGTGGGTGGCCGAGATCAGATT GAAATCCAATAACTTCGGAAGATATTACGCTGCAAGCGT GAAGGGCCGGTTCACCATCAGCAGAGATGATTCCAAGAA CACGCTGTACCTGCAGATGAACAGCCTGAAGACCGAGGA TACGGCCGTGTATTACTGTACCACATACGGCAACTACGTT GGGCACTACTTCGACCACTGGGGCCAAGGGACCACCGTC ACCGTCTCCAGT	258
KV9; V_L	GATATCCAGTTGACCCAGTCTCCATCCTTCCTGTCTGCATC TGTGGGCGACCGGGTCACCATCACCTGCAAGGCCAGTCA GAATGTGGATACTAACGTGGCTTGGTACCAGCAGAAGCC AGGGCAGGCACCTAGGCCTCTGATCTATTCGGCATCCTAC CGGTACACTGGCGTCCCATCAAGGTTCAGCGGCAGTGGA TCCGGGACAGAGTTCACTCTCACAATCTCAAGCCTGCAAC CTGAAGATTTCGCAACTTACTACTGTCAACAGTACAATAG TTACCCTCTGACGTTCGGCGGAGGTACCAAGGTGGAGATC AAGCGTACG	260
MHLG; V_H	GAAGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTCCAG CCTGGCGGGTCCCTGCGGCTCTCCTGTGCAGCCTCCGGAT TCACATTTAGCAACTATTGGATGAACTGGGTGCGGCAGGC TCCTGGAAAGGGCCTCGAGTGGGTGGCCGAGATCAGATT GAAATCCAATAACTTCGGAAGATATTACGCTGCAAGCGT GAAGGGCCGGTTCACCATCAGCAGAGATGATTCCAAGAA CACGCTGTACCTGCAGATGAACAGCCTGAAGACCGAGGA TACGGCCGTGTATTACTGTACCACATACGGCAACTACGTT GGGCACTACTTCGACCACTGGGGCCAAGGGACCACCGTC ACCGTCTCCAGT	262
KV1; V_L	GATATCCAGTTGACCCAGTCTCCATCCTTCCTGTCTGCATCT GTGGGCGACCGGGTCACCATCACCTGCAGGGCCAGTCAGAA TGTGGATACTAACTTAGCTTGGTACCAGCAGAAGCCAGGGA AAGCACCTAAGCTCCTGATCTATTCGGCATCCTACCGTTACA CTGGCGTCCCATCAAGGTTCAGCGGCAGTGGATCCGGGACA GAGTTCACTCTCACAATCTCAAGCCTGCAACCTGAAGATTTC GCAACTTACTACTGTCAACAGTACAATAGTTACCCTCTGACG TTCGGCGGAGGTACCAAGGTGGAGATCAAGCGTACGGTG	270
KV7; V_L	GATATCCAGTTGACCCAGTCTCCATCCTTCCTGTCTGCATC TGTGGGCGACCGGGTCACCATCACCTGCAAGGCCAGTCA GAATGTGGATACTAACGTGGCTTGGTACCAGCAGAAGCC AGGGAAAGCACCTAAGCCTCTGATCTATTCGGCATCCTAC CGGTACACTGGCGTCCCATCAAGGTTCAGCGGCAGTGGA TCCGGGACAGAGTTCACTCTCACAATCTCAAGCCTGCAAC CTGAAGATTTCGCAACTTACTACTGTCAACAGTACAATAG	272

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TTACCCTCTGACGTTCGGCGGAGGTACCAAGGTGGAGATC AAGCGTACGGTG	

TABLE 7.

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
Human FAP ectodomain+poly-lys-tag+his_6-tag	CGCCCTTCAAGAGTTCATAACTCTGAAGAAAATACAATG AGAGCACTCACACTGAAGGATATTTTAAATGGAACATTTT CTTATAAAACATTTTTTCCAAACTGGATTTCAGGACAAGA ATATCTTCATCAATCTGCAGATAACAATATAGTACTTTAT AATATTGAAACAGGACAATCATATACCATTTTGAGTAATA GAACCATGAAAAGTGTGAATGCTTCAAATTACGGCTTATC ACCTGATCGGCAATTTGTATATCTAGAAAGTGATTATTCA AAGCTTTGGAGATACTCTTACACAGCAACATATTACATCT ATGACCTTAGCAATGGAGAATTTGTAAGAGGAAATGAGC TTCCTCGTCCAATTCAGTATTTATGCTGGTCGCCTGTTGGG AGTAAATTAGCATATGTCTATCAAAACAATATCTATTTGA AACAAAGACCAGGAGATCCACCTTTTCAAATAACATTTA ATGGAAGAGAAAATAAAATATTTAATGGAATCCCAGACT GGGTTTATGAAGAGGAAATGCTTGCTACAAAATATGCTCT CTGGTGGTCTCCTAATGGAAAATTTTTGGCATATGCGGAA TTTAATGATACGGATATACCAGTTATTGCCTATTCCTATTA TGGCGATGAACAATATCCTAGAACAATAAATATTCCATAC CCAAAGGCTGGAGCTAAGAATCCCGTTGTTCGGATATTTA TTATCGATACCACTTACCCTGCGTATGTAGGTCCCCAGGA AGTGCCTGTTCCAGCAATGATAGCCTCAAGTGATTATTAT TTCAGTTGGCTCACGTGGGTTACTGATGAACGAGTATGTT TGCAGTGGCTAAAAAGAGTCCAGAATGTTTCGGTCCTGTC TATATGTGACTTCAGGGAAGACTGGCAGACATGGGATTG TCCAAAGACCCAGGAGCATATAGAAGAAAGCAGAACTGG ATGGGCTGGTGGATTCTTTGTTTCAACACCAGTTTTCAGC TATGATGCCATTTCGTACTACAAAATATTTAGTGACAAGG ATGGCTACAAACATATTCACTATATCAAAGACACTGTGGA AAATGCTATTCAAATTACAAGTGGCAAGTGGGAGGCCAT AAATATATTCAGAGTAACACAGGATTCACTGTTTTATTCT AGCAATGAATTTGAAGAATACCCTGGAAGAAGAAACATC TACAGAATTAGCATTGGAAGCTATCCTCCAAGCAAGAAG TGTGTTACTTGCCATCTAAGGAAAGAAAGGTGCCAATATT ACACAGCAAGTTTCAGCGACTACGCCAAGTACTATGCACT TGTCTGCTACGGCCCAGGCATCCCCATTTCCACCCTTCAT GATGGACGCACTGATCAAGAAATTAAAATCCTGGAAGAA AACAAGGAATTGGAAAATGCTTTGAAAAATATCCAGCTG CCTAAAGAGGAAATTAAGAAACTTGAAGTAGATGAAATT ACTTTATGGTACAAGATGATTCTTCCTCCTCAATTTGACA GATCAAAGAAGTATCCCTTGCTAATTCAAGTGTATGGTGG TCCCTGCAGTCAGAGTGTAAGGTCTGTATTTGCTGTTAAT TGGATATCTTATCTTGCAAGTAAGGAAGGGATGGTCATTG CCTTGGTGGATGGTCGAGGAACAGCTTTCCAAGGTGACA AACTCCTCTATGCAGTGTATCGAAAGCTGGGTGTTTATGA AGTTGAAGACCAGATTACAGCTGTCAGAAAATTCATAGA AATGGGTTTCATTGATGAAAAAAGAATAGCCATATGGGG CTGGTCCTATGGAGGATACGTTTCATCACTGGCCCTTGCA	54

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TCTGGAACTGGTCTTTTCAAATGTGGTATAGCAGTGGCTC CAGTCTCCAGCTGGGAATATTACGCGTCTGTCTACACAGA GAGATTCATGGGTCTCCCAACAAAGGATGATAATCTTGA GCACTATAAGAATTCAACTGTGATGGCAAGAGCAGAATA TTTCAGAAATGTAGACTATCTTCTCATCCACGGAACAGCA GATGATAATGTGCACTTTCAAAACTCAGCACAGATTGCTA AAGCTCTGGTTAATGCACAAGTGGATTTCCAGGCAATGTG GTACTCTGACCAGAACCACGGCTTATCCGGCCTGTCCACG AACCACTTATACACCCACATGACCCACTTCCTAAAGCAGT GTTTCTCTTTGTCAGACGGCAAAAAGAAAAAGAAAAAGG GCCACCACCATCACCATCAC	
Murine FAP ectodomain+poly-lys-tag+his_6-tag	CGTCCCTCAAGAGTTTACAAACCTGAAGGAAACACAAAG AGAGCTCTTACCTTGAAGGATATTTTAAATGGAACATTCT CATATAAAACATATTTTCCCAACTGGATTTCAGAACAAGA ATATCTTCATCAATCTGAGGATGATAACATAGTATTTTAT AATATTGAAACAAGAGAATCATATATCATTTTGAGTAATA GCACCATGAAAAGTGTGAATGCTACAGATTATGGTTTGTC ACCTGATCGGCAATTTGTGTATCTAGAAAGTGATTATTCA AAGCTCTGGCGATATTCATACACAGCGACATACTACATCT ACGACCTTCAGAATGGGGAATTTGTAAGAGGATACGAGC TCCCTCGTCCAATTCAGTATCTATGCTGGTCGCCTGTTGG GAGTAAATTAGCATATGTATATCAAAACAATATTTATTTG AAACAAAGACCAGGAGATCCACCTTTTCAAATAACTTAT ACTGGAAGAGAAAATAGAATATTTAATGGAATACCAGAC TGGGTTTATGAAGAGGAAATGCTTGCCACAAAATATGCTC TTTGGTGGTCTCCAGATGGAAAATTTTTGGCATATGTAGA ATTTAATGATTCAGATATACCAATTATTGCCTATTCTTATT ATGGTGATGGACAGTATCCTAGAACTATAAATATTCCATA TCCAAAGGCTGGGGCTAAGAATCCGGTTGTTCGTGTTTTT ATTGTTGACACCACCTACCCTCACCACGTGGGCCCAATGG AAGTGCCAGTTCCAGAAATGATAGCCTCAAGTGACTATTA TTTCAGCTGGCTCACATGGGTGTCCAGTGAACGAGTATGC TTGCAGTGGCTAAAAAGAGTGCAGAATGTCTCAGTCCTGT CTATATGTGATTTCAGGGAAGACTGGCATGCATGGGAAT GTCCAAAGAACCAGGAGCATGTAGAAGAAAGCAGAACA GGATGGGCTGGTGGATTCTTTGTTTCGACACCAGCTTTTA GCCAGGATGCCACTTCTTACTACAAAATATTTAGCGACAA GGATGGTTACAAACATATTCACTACATCAAAGACACTGTG GAAAATGCTATTCAAATTACAAGTGGCAAGTGGGAGGCC ATATATATATTCCGCGTAACACAGGATTCACTGTTTTATT CTAGCAATGAATTTGAAGGTTACCCTGGAAGAAGAAACA TCTACAGAATTAGCATTGGAAACTCTCCTCCGAGCAAGAA GTGTGTTACTTGCCATCTAAGGAAAGAAAGGTGCCAATAT TACACAGCAAGTTTCAGCTACAAAGCCAAGTACTATGCA CTCGTCTGCTATGGCCCTGGCCTCCCCATTTCCACCCTCCA TGATGGCCGCACAGACCAAGAAATACAAGTATTAGAAGA AAACAAAGAACTGGAAAATTCTCTGAGAAATATCCAGCT GCCTAAAGTGGAGATTAAGAAGCTCAAAGACGGGGGACT GACTTTCTGGTACAAGATGATTCTGCCTCCTCAGTTTGAC AGATCAAAGAAGTACCCTTTGCTAATTCAAGTGTATGGTG GTCCTTGTAGCCAGAGTGTTAAGTCTGTGTTTGCTGTTAA TTGGATAACTTATCTCGCAAGTAAGGAGGGGATAGTCATT GCCCTGGTAGATGGTCGGGGCACTGCTTTCCAAGGTGACA	56

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	AATTCCTGCATGCCGTGTATCGAAAACTGGGTGTATATGA AGTTGAGGACCAGCTCACAGCTGTCAGAAAATTCATAGA AATGGGTTTCATTGATGAAGAAAGAATAGCCATATGGGG CTGGTCCTACGGAGGTTATGTTTCATCCCTGGCCCTTGCA TCTGGAACTGGTCTTTTCAAATGTGGCATAGCAGTGGCTC CAGTCTCCAGCTGGGAATATTACGCATCTATCTACTCAGA GAGATTCATGGGCCTCCCAACAAAGGACGACAATCTCGA ACACTATAAAAATTCAACTGTGATGGCAAGAGCAGAATA TTTCAGAAATGTAGACTATCTTCTCATCCACGGAACAGCA GATGATAATGTGCACTTTCAGAACTCAGCACAGATTGCTA AAGCTTTGGTTAATGCACAAGTGGATTTCCAGGCGATGTG GTACTCTGACCAGAACCATGGTATATTATCTGGGCGCTCC CAGAATCATTTATATACCCACATGACGCACTTCCTCAAGC AATGCTTTTCTTTATCAGACGGCAAAAAGAAAAAGAAAA AGGGCCACCACCATCACCATCAC	
Human TNC-A2+avi-tag +his_6-tag	GCGTCCACCGGGGAAACCCCGAACCTGGGCGAAGTGGTG GTGGCGGAAGTGGGTTGGGATGCGCTGAAACTGAACTGG ACCGCGCCGGAAGGCGCGTATGAATATTTTTTCATCCAGG TGCAGGAAGCGGATACCGTTGAAGCGGCGCAGAACCTGA CCGTTCCGGGCGGTCTGCGTAGCACCGATCTGCCGGGCCT GAAAGCGGCGACCCATTATACCATTACCATCCGTGGGGT GACCCAGGATTTTAGCACCACCCCGCTGTCTGTGGAAGTG CTGACCGCTAGCGGCCTGAACGACATCTTCGAGGCTCAG AAAATCGAATGGCACGAAGGTACCCATCACCATCACCAC CAC	58
Human TNC-A1+avi-tag +his_6-tag	GAACAAGCCCCTGAGCTGGAAAACCTCACCGTGACTGAG GTTGGCTGGGATGGCCTCAGACTCAACTGGACCGCGGCT GACCAGGCCTATGAGCACTTTATCATTCAGGTGCAGGAG GCCAACAAGGTGGAGGCAGCTCGGAACCTCACCGTGCCT GGCAGCCTTCGGGCTGTGGACATACCGGGCCTCAAGGCT GCTACGCCTTATACAGTCTCCATCTATGGGGTGATCCAGG GCTATAGAACACCAGTGCTCTCTGCTGAGGCCTCCACAGC TAGCGGCCTGAACGACATCTTCGAGGCTCAGAAAATCGA ATGGCACGAAGGTACCCATCACCATCACCACCAC	60
Murine TNC-A1+avi-tag +his_6-tag	ATTTCAGAATTCGGATCCAGCACCGAAGAAGTGCCGAGC CTGGAAAACCTGACCGTGACCGAAGCGGGCTGGGATGGC CTGCGTCTGAACTGGACCGCGGATGATCTGGCCTATGAAT ATTTTGTGATCCAGGTGCAGGAAGCGAACAACGTTGAAA CCGCGCATAACTTTACCGTGCCGGGCAATCTGCGTGCGGC GGATATTCCGGGCCTGAAAGTGGCGACCAGCTATCGTGT GAGCATTTATGGCGTGGCGCGTGGCTATCGTACCCCGGTT CTGAGCGCGGAAACCAGCACCGCTAGCGGCCTGAACGAC ATCTTCGAGGCTCAGAAAATCGAATGGCACGAAGGTACC CATCACCATCACCACCAC	62
Human TNC-A4+avi-tag +his_6-tag	GAAGATCTGCCGCAGCTGGGCGATCTGGCCGTGAGCGAA GTGGGCTGGGATGGCCTGCGTCTGAACTGGACCGCGGCG GATAACGCGTATGAACATTTTGTGATTCAGGTGCAGGAA GTGAACAAAGTTGAAGCGGCGCAGAACCTGACCCTGCCG GGCAGCCTGCGTGCGGTGGATATTCCGGGCCTGGAAGCG GCGACCCCGTATCGTGTGAGCATCTATGGCGTGATTCGTG GCTATCGTACCCCGGTTCTGAGCGCGGAAGCGAGCACCG CTAGCGGCCTGAACGACATCTTCGAGGCTCAGAAAATCG AATGGCACGAAGGTACCCATCACCATCACCACCAC	64

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
Murine TNC-A4 +avi-tag +his_6-tag	ATTTCAGAATTCGGATCCCTGACCGAAGATCTGCCGCAGC TGGGCGGTCTGAGCGTGACCGAAGTGAGCTGGGATGGCC TGACCCTGAACTGGACCACCGATGATCTGGCCTATAAACA TTTTGTGGTGCAGGTGCAGGAAGCGAACAACGTTGAAGC GGCGCAGAACCTGACCGTTCCGGGTAGCCTGCGTGCGGT GGATATTCCGGGCCTGAAAGCGGATACCCCGTATCGTGTG AGCATTTATGGCGTGATTCAGGGCTATCGTACCCCGATGC TGTCTACCGATGTGAGCACCGCTAGCGGCCTGAACGACAT CTTCGAGGCTCAGAAAATCGAATGGCACGAAGGTACCCA TCACCATCACCACCAC	66

TABLE 8.

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
Fab heavy chain derived from L19 monoclonal antibody-C125A variant of IL2-Fab heavy chain derived from L19 monoclonal antibody	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCAGTTTTTCGATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCATCTATTTCCGGT AGTTCGGGTACCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAACCGTTTCCGTATTTTGACTACTG GGGCCAGGGAACCCTGGTCACCGTCTCGAGTGCTAGCAC CAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAG AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAAC TCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTG TCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAT AAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCGGAGGA GGGAGCGGCGGAGGTGGCTCCGGAGGTGGCGGAGCACCT ACTTCAAGTTCTACAAAGAAAACACAGCTACAACTGGAG CATTTACTGCTGGATTTACAGATGATTTTGAATGGAATTA ATAATTACAAGAATCCCAAACTCACCAGGATGCTCACATT TAAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACA TCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGA AGTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTTAAGA CCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGG AACTAAAGGGATCTGAAACAACATTCATGTGTGAATATG CTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGAT GGATTACCTTTGCCCAAAGCATCATCTCAACACTGACTTC CGGCGGAGGAGGATCCGGCGGAGGTGGCTCTGGCGGTGG CGGAGAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGT ACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCT GGATTCACCTTTAGCAGTTTTTCGATGAGCTGGGTCCGCC AGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCTATTTC CGGTAGTTCGGGTACCACATACTACGCAGACTCCGTGAA GGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACAC GCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACAC GGCCGTATATTACTGTGCGAAACCGTTTCCGTATTTTGAC TACTGGGGCCAGGGAACCCTGGTCACCGTCTCGAGTGCTA	108

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGATAAGAAAGTTGAGCCCAAATCTTGTGACTGA	
Fab light chain derived from L19 monoclonal antibody	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATTATGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGACGG GTCGTATTCCTCCGACGTTCGGCCAAGGGACCAAGGTGG AAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTT CCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCT GTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTA ACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAG CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCA CCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTTAG	109
scFv derived from L19 monoclonal antibody-8 amino acid linker-C125A variant of IL2	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCAGTTTTTCGATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCATCTATTTCCGGT AGTTCGGGTACCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAACCGTTTCCGTATTTTGACTACTG GGGCCAGGGAACCCTGGTCACCGTCTCGAGTAGCGGCGG GAGCGGCGGGGCTAGCGAAATTGTGTTGACGCAGTCTCC AGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTC TCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAG CCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCT CATCTATTATGCATCCAGCAGGGCCACTGGCATCCCAGAC AGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCA CCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTA CTGTCAGCAGACGGGTCGTATTCCTCCGACGTTCGGCCAA GGGACCAAGGTGGAAATCTCCGTGCTGTCTTCCTCATCGG GTAGTAGCTCTTCCGGCTCATCGTCCTCCGGAGCACCTAC TTCAAGTTCTACAAAGAAAACACAGCTACAACTGGAGCA TTTACTGCTGGATTTACAGATGATTTTGAATGGAATTAAT AATTACAAGAATCCCAAACTCACCAGGATGCTCACATTTA AGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACATC TTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAG TGCTAAATTTAGCTCAAAGCAAAAACTTTCACTTAAGACC CAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGA ACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCT GATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGG	110

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	ATTACCTTTGCCCAAAGCATCATCTCAACACTGACTTGA	
F16-diabody-IL2 DNA	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCCGGTATGGTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGCGCATAATGCTTTTGACTACT GGGGCCAGGGAACCCTGGTCACCGTGTCGAGTGCTAGCG GCGGATCGTCTGAGCTGACTCAGGACCCTGCTGTGTCTGT GGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGA CAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAA GCCAGGACAGGCCCCTGTACTTGTCATCTATGGTAAAAAC AACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCA GCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCA GGCGGAAGATGAGGCTGACTATTACTGTAACTCCTCTGTT TATACTATGCCGCCCGTGGTATTCGGCGGAGGGACCAAG CTGACCGTCCTAGGCTCTTCCTCATCGGGTAGTAGCTCTT CCGGCTCATCGTCCTCCGGAGCACCTACTTCAAGTTCTAC AAAGAAAACACAGCTACAACTGGAGCATTTACTGCTGGA TTTACAGATGATTTTGAATGGAATTAATAATTACAAGAAT CCCAAACTCACCAGGATGCTCACATTTAAGTTTTACATGC CCAAGAAGGCCACAGAACTGAAACATCTTCAGTGTCTAG AAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAG CTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAAT CAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATC TGAAACAACATTCATGTGTGAATATGCTGATGAGACAGC AACCATTGTAGAATTTCTGAACAGATGGATTACCTTTGCC CAAAGCATCATCTCAACACTGACTTGA	111
scFv-IL2-scFv (F16, DNA)	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCCGGTATGGTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGCGCATAATGCTTTTGACTACT GGGGCCAGGGAACCCTGGTCACCGTGTCGAGAGGTGGAG GCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGT CTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGG ACAGACAGTCAGGATCACATGCCAAGGAGACAGCCTCAG AAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACA GGCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCC TCAGGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGGAA ACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAG ATGAGGCTGACTATTACTGTAACTCCTCTGTTTATACTAT GCCGCCCGTGGTATTCGGCGGAGGGACCAAGCTGACCGT CCTAGGCTCTTCCTCATCGGGTAGTAGCTCTTCCGGCTCA TCGTCCTCCGGAGCACCTACTTCAAGTTCTACAAAGAAAA CACAGCTACAACTGGAGCATTTACTGCTGGATTTACAGAT GATTTTGAATGGAATTAATAATTACAAGAATCCCAAACTC ACCAGGATGCTCACATTTAAGTTTTACATGCCCAAGAAGG	112

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAAC TCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCA AAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATAT CAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAAC ATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTA GAATTTCTGAACAGATGGATTACCTTTGCCCAAAGCATCA TCTCAACACTGACTTCCGGCGGAGGAGGGAGCGGCGGAG GTGGCTCTGGCGGTGGCGGATCGTCTGAGCTCACTCAGGA CCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATC ACATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGC TGGTACCAGCAGAAGCCAGGACAGGCCCCTGTACTTGTC ATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGAC CGATTCTCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGA CCATCACTGGGGCTCAGGCGGAAGATGAGGCTGACTATT ACTGTAACTCCTCTGTTTATACTATGCCGCCCGTGGTATTC GGCGGAGGGACCAAGCTTACCGTACTAGGCTCAGGAGGC GGTTCAGGCGGAGGTTCTGGCGGCGGTAGCGGATCGGAG GTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCT GGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCA CCTTTAGCCGGTATGGTATGAGCTGGGTCCGCCAGGCTCC AGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAG TGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCG GTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTA TATTACTGTGCGAAAGCGCATAATGCTTTTGACTACTGGG GCCAGGGAACCCTGGTCACCGTGTCGTGA	
Fab-IL2-Fab (F16, heavy chain cytokine fusion construct, DNA)	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCCGGTATGGTATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGCGCATAATGCTTTTGACTACT GGGGCCAGGGAACCCTGGTCACCGTGTCGAGTGCTAGCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAA GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGT CAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGG TGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA TAAGAAAGTTGAGCCCAAATCTTGTGACTCTTCCTCATCG GGTAGTAGCTCTTCCGGCTCATCGTCCTCCGGAGCACCTA CTTCAAGTTCTACAAAGAAAACACAGCTACAACTGGAGC ATTTACTGCTGGATTTACAGATGATTTTGAATGGAATTAA TAATTACAAGAATCCCAAACTCACCAGGATGCTCACATTT AAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACAT CTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAA GTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTTAAGAC CCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGA ACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCT GATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGG	113

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	ATTACCTTTGCCCAAAGCATCATCTCAACACTGACTTCCG GCGGAGGAGGGAGCGGCGGAGGTGGCTCTGGCGGTGGCG GAGAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTAC AGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGG ATTCACCTTTAGCCGGTATGGTATGAGCTGGGTCCGCCAG GCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGT GGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAG GGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACG CTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACG GCCGTATATTACTGTGCGAAAGCGCATAATGCTTTTGACT ACTGGGGCCAGGGAACCCTGGTCACCGTGTCGAGTGCTA GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGACAAGAAAGTTGAGCCCAAATCTTGTGACTGA	
F16, light chain, DNA	TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCT TGGGACAGACAGTCAGGATCACATGCCAAGGAGACAGCC TCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAG GACAGGCCCCTGTACTTGTCATCTATGGTAAAAACAACCG GCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCAGCTCA GGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCG GAAGATGAGGCTGACTATTACTGTAACTCCTCTGTTTATA CTATGCCGCCCGTGGTATTCGGCGGAGGGACCAAGCTGA CCGTCCTAGGTCAACCCAAGGCTGCCCCCAGCGTGACCCT GTTCCCCCCCAGCAGCGAGGAACTGCAGGCCAACAAGGC CACCCTGGTCTGCCTGATCAGCGACTTCTACCCAGGCGCC GTGACCGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAG GCCGGCGTGGAGACCACCACCCCCAGCAAGCAGAGCAAC AACAAGTACGCCGCCAGCAGCTACCTGAGCCTGACCCCC GAGCAGTGGAAGAGCCACAGGTCCTACAGCTGCCAGGTG ACCCACGAGGGCAGCACCGTGGAGAAAACCGTGGCCCCC ACCGAGTGCAGCTGA	114
IL2R-beta-Fc(hole) fusion protein, DNA	ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGCCTCCTGC TGCTCTGGTTCCCAGGTGCCAGGTGTGCGGTGAATGGCAC TTCCCAGTTCACATGCTTCTACAACTCGAGAGCCAACATC TCCTGTGTCTGGAGCCAAGATGGGGCTCTGCAGGACACTT CCTGCCAAGTCCATGCCTGGCCGGACAGACGGCGGTGGA ACCAAACCTGTGAGCTGCTCCCCGTGAGTCAAGCATCCTG GGCCTGCAACCTGATCCTCGGAGCCCCAGATTCTCAGAAA CTGACCACAGTTGACATCGTCACCCTGAGGGTGCTGTGCC GTGAGGGGGTGCGATGGAGGGTGATGGCCATCCAGGACT TCAAGCCCTTTGAGAACCTTCGCCTGATGGCCCCCATCTC CCTCCAAGTTGTCCACGTGGAGACCCACAGATGCAACAT AAGCTGGGAAATCTCCCAAGCCTCCCACTACTTTGAAAGA CACCTGGAGTTCGAGGCCCGGACGCTGTCCCCAGGCCAC ACCTGGGAGGAGGCCCCCCTGCTGACTCTCAAGCAGAAG CAGGAATGGATCTGCCTGGAGACGCTCACCCCAGACACC CAGTATGAGTTTCAGGTGCGGGTCAAGCCTCTGCAAGGC GAGTTCACGACCTGGAGCCCCTGGAGCCAGCCCCTGGCCT	115

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TCAGAACAAAGCCTGCAGCCCTTGGGAAGGACACCGGAG CTCAGGACAAAACTCACACATGCCCACCGTGCCCAGCAC CTGAACTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCC TGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGT GCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA CAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCAC CAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCT CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTGCA CCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGG TCAGCCTCTCGTGCGCAGTCAAAGGCTTCTATCCCAGCGA CATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGG CTCCTTCTTCCTCGTGAGCAAGCTCACCGTGGACAAGAGC AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT CCCTGTCTCCGGGTAAATGA	
IL2R-gamma-Fc(knob), DNA	ATGTTGAAGCCATCATTACCATTCACATCCCTCTTATTCCT GCAGCTGCCCCTGCTGGGAGTGGGGCTGAACACGACAAT TCTGACGCCCAATGGGAATGAAGACACCACAGCTGATTT CTTCCTGACCACTATGCCCACTGACTCCCTCAGTGTTTCCA CTCTGCCCCTCCCAGAGGTTCAGTGTTTTGTGTTCAATGTC GAGTACATGAATTGCACTTGGAACAGCAGCTCTGAGCCC CAGCCTACCAACCTCACTCTGCATTATTGGTACAAGAACT CGGATAATGATAAAGTCCAGAAGTGCAGCCACTATCTATT CTCTGAAGAAATCACTTCTGGCTGTCAGTTGCAAAAAAAG GAGATCCACCTCTACCAAACATTTGTTGTTCAGCTCCAGG ACCCACGGGAACCCAGGAGACAGGCCACACAGATGCTAA AACTGCAGAATCTGGTGATCCCCTGGGCTCCAGAGAACCT AACACTTCACAAACTGAGTGAATCCCAGCTAGAACTGAA CTGGAACAACAGATTCTTGAACCACTGTTTGGAGCACTTG GTGCAGTACCGGACTGACTGGGACCACAGCTGGACTGAA CAATCAGTGGATTATAGACATAAGTTCTCCTTGCCTAGTG TGGATGGGCAGAAACGCTACACGTTTCGTGTTCGGAGCC GCTTTAACCCACTCTGTGGAAGTGCTCAGCATTGGAGTGA ATGGAGCCACCCAATCCACTGGGGGAGCAATACTTCAAA AGAGAATCCTTTCCTGTTTGCATTGGAAGCCGGAGCTCAG GACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAA CTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAAC CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGT CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAC GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGAC TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAA GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG CCCCCATGCCGGGATGAGCTGACCAAGAACCAGGTCAGC CTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACT ACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT	116

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTG GCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAG GCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT CTCCGGGTAAATGA	
Fab-IL12-Fab L19 antibody, murine scIL12, DNA	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCAGTTTTTCGATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCATCTATTTCCGGT AGTTCGGGTACCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAACCGTTTCCGTATTTTGACTACTG GGGCCAGGGAACCCTGGTCACCGTCTCGAGTGCTAGCAC CAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAG AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAAC TCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTG TCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAT AAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCGGAGGA GGGAGCGGCGGAGGTGGCTCCGGAGGGGGCGGAGCCAT GTGGGAGCTGGAAAAGGACGTGTACGTGGTGGAGGTGGA CTGGACCCCCGACGCCCCTGGCGAGACAGTGAACCTGAC CTGCGACACCCCCGAAGAGGACGACATCACCTGGACCAG CGACCAGCGGCACGGCGTGATCGGCAGCGGCAAGACCCT GACCATCACCGTGAAAGAGTTTCTGGACGCCGGCCAGTA CACCTGCCACAAGGGCGGCGAGACACTGAGCCACAGCCA CCTGCTGCTGCACAAGAAAGAGAACGGCATCTGGTCCAC CGAGATCCTGAAGAACTTCAAGAACAAGACCTTCCTGAA GTGCGAGGCCCCCAACTACAGCGGCCGGTTCACCTGCAG CTGGCTGGTGCAGCGGAACATGGACCTGAAGTTCAACAT CAAGAGCAGCAGCAGCCCCCCTGACAGCAGGGCCGTGAC CTGCGGCATGGCCAGCCTGAGCGCCGAGAAGGTGACCCT GGACCAGAGGGACTACGAGAAGTACAGCGTGAGCTGCCA GGAAGATGTCACCTGCCCCACCGCCGAGGAAACCCTGCC CATCGAGCTGGCCCTGGAAGCCCGGCAGCAGAACAAGTA CGAGAACTACTCTACCAGCTTCTTCATCCGGGACATCATC AAGCCCGACCCCCCCAAGAACCTGCAGATGAAGCCCCTG AAGAACAGCCAGGTGGAGGTGTCCTGGGAGTACCCTGAC AGCTGGTCCACCCCCAGAAGCTACTTCAGCCTGAAGTTCT TCGTGAGAATCCAGCGGAAGAAAGAAAAGATGAAAGAG ACAGAGGAAGGCTGCAACCAGAAGGGCGCCTTCTTCGTC GAGAAAACCAGCACCGAGGTGCAGTGCAAGGGCGGCAA CGTGTGCGTGCAGGCCCAGGACCGGTACTACAACAGCAG CTGCAGCAAGTGGGCCTGCGTGCCCTGCAGAGTGCGGTCT GGCGGCGACGGCTCTGGCGGCGGAGGAAGCGGCGGAGG GGGCAGCAGAGTGATCCCCGTGAGCGGCCCTGCCCGGTG CCTGAGCCAGAGCCGGAACCTGCTGAAAACCACCGACGA CATGGTGAAAACCGCCAGAGAGAAGCTGAAGCACTACAG CTGCACAGCCGAGGACATCGACCACGAGGACATCACCCG GGACCAGACCAGCACCCTGAAAACCTGCCTGCCCCTGGA ACTGCACAAAAACGAGAGCTGCCTGGCCACCCGGGAGAC	117

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	AAGCAGCACCACCCGGGGCAGCTGCCTGCCTCCCCAGAA AACCTCCCTGATGATGACCCTGTGCCTGGGCAGCATCTAC GAGGACCTGAAGATGTACCAGACCGAGTTCCAGGCCATC AACGCCGCCCTGCAGAACCACAATCACCAGCAGATCATC CTGGACAAGGGCATGCTGGTCGCCATCGACGAGCTGATG CAGAGCCTGAACCACAACGGCGAAACCCTGCGGCAGAAA CCCCCCGTGGGCGAGGCCGACCCCTACCGGGTGAAGATG AAGCTGTGCATCCTGCTGCACGCCTTCAGCACCCGGGTGG TGACCATCAACCGGGTGATGGGCTACCTGTCCTCTGCCGG GGGAGGGGGATCCGGCGGAGGTGGCTCTGGCGGTGGCGG AGAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACA GCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGA TTCACCTTTAGCAGTTTTTCGATGAGCTGGGTCCGCCAGG CTCCAGGGAAGGGGCTGGAGTGGGTCTCATCTATTTCCGG TAGTTCGGGTACCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAACCGTTTCCGTATTTTGACTACT GGGGCCAGGGAACCCTGGTCACCGTCTCGAGTGCTAGCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAA GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGT CAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGG TGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA TAAGAAAGTTGAGCCCAAATCTTGTGACTGA	
Fab-IL12-FabL19 antibody, human scIL12, DNA	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCAGTTTTTCGATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCATCTATTAGAGG TAGTTCGGGTACCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAACCGTTTCCGTATTTTGACTACT GGGGCCAGGGAACCCTGGTCACCGTCTCGAGTGCTAGCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAA GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGT CAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGG TGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA TAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCGGAGG AGGGAGCGGCGGAGGTGGCTCCGGAGGGGGCGGAATCTG GGAGCTGAAGAAAGACGTGTACGTGGTGGAGCTGGACTG GTATCCCGACGCCCCTGGCGAGATGGTGGTGCTGACCTGC GACACCCCCGAAGAGGACGGCATCACCTGGACCCTGGAC CAGAGCAGCGAGGTGCTGGGCAGCGGCAAGACCCTGACC ATCCAGGTGAAAGAGTTCGGCGACGCCGGCCAGTACACC TGCCACAAGGGCGGCGAAGTGCTGTCCCACAGCCTGCTG CTGCTGCACAAGAAAGAGGATGGCATCTGGTCCACCGAC ATCCTGAAGGACCAGAAAGAGCCCAAGAACAAGACCTTC	118

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CTGCGGTGCGAGGCCAAGAACTACAGCGGCCGGTTCACC TGTTGGTGGCTGACCACCATCAGCACCGACCTGACCTTCA GCGTGAAGAGCAGCCGGGGCAGCAGCGACCCTCAGGGCG TGACCTGCGGAGCCGCCACCCTGAGCGCCGAGAGAGTGC GGGGCGACAACAAAGAGTACGAGTACAGCGTCGAGTGCC AGGAAGATAGCGCCTGCCCTGCCGCCGAGGAAAGCCTGC CCATCGAGGTGATGGTGGACGCCGTGCACAAGCTGAAGT ACGAGAACTACACCAGCAGCTTTTTCATCCGGGACATCAT CAAGCCCGACCCCCCCAAGAACCTGCAGCTGAAGCCCCT GAAGAACAGCCGGCAGGTGGAGGTGTCCTGGGAGTACCC TGACACCTGGTCCACCCCCCACAGCTACTTCAGCCTGACA TTCTGTGTGCAGGTGCAGGGCAAGAGCAAGCGGGAGAAG AAAGACCGGGTGTTCACCGACAAGACCAGCGCCACCGTG ATCTGCCGGAAGAACGCCAGCATCAGCGTGCGGGCCCAG GACCGGTACTACAGCAGCTCCTGGTCCGAGTGGGCCAGC GTGCCTTGCAGCGGCGGAGGGGGCTCTGGCGGCGGAGGA TCTGGGGGAGGGGGCAGCCGGAACCTGCCCGTGGCCACC CCCGACCCCGGCATGTTCCCCTGCCTGCACCACAGCCAGA ACCTGCTGCGGGCCGTGAGCAACATGCTGCAGAAGGCCC GGCAGACCCTGGAATTCTACCCCTGCACCAGCGAGGAAA TCGACCACGAGGACATCACCAAGGATAAGACCAGCACCG TGGAGGCCTGCCTGCCCCTGGAACTGACCAAGAACGAGA GCTGCCTGAACAGCCGGGAGACAAGCTTCATCACCAACG GCAGCTGCCTGGCCAGCAGAAAGACCAGCTTCATGATGG CCCTGTGCCTGAGCAGCATCTACGAGGACCTGAAGATGT ACCAGGTGGAGTTCAAGACCATGAACGCCAAGCTGCTGA TGGACCCCAAGCGGCAGATCTTCCTGGATCAGAACATGCT GGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTCAA CAGCGAGACAGTGCCCCAGAAGTCCAGCCTGGAAGAGCC CGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTG CACGCCTTCAGAATCCGGGCCGTGACCATCGACCGGGTG ATGAGCTACCTGAACGCCAGCGGAGGGGGGGGATCCGGC GGAGGTGGCTCTGGCGGTGGCGGAGAGGTGCAGCTGTTG GAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTG AGACTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCAGTT TTTCGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGC TGGAGTGGGTCTCATCTATTAGAGGTAGTTCGGGTACCAC ATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCC AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC AGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCG AAACCGTTTCCGTATTTTGACTACTGGGGCCAGGGAACCC TGGTCACCGTCTCGAGTGCTAGCACCAAGGGCCCATCGGT CTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGC ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCA GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGG ACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCCAGCAACACCAAGGTGGATAAGAAAGTTGAGCCC AAATCTTGTGACTGA	
Fab-GMCSF-Fab L19 antibody, human GM-CSF,	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCAGTTTTTCGATGAGCTGGGTCCGCCAGGC	119

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
DNA	TCCAGGGAAGGGGCTGGAGTGGGTCTCATCTATTAGAGG TAGTTCGGGTACCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAACCGTTTCCGTATTTTGACTACT GGGGCCAGGGAACCCTGGTCACCGTCTCGAGTGCTAGCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAA GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGT CAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGG TGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA TAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCGGAGG AGGGAGCGGCGGAGGTGGCTCCGGAGGTGGCGGAGCACC CGCCCGCTCGCCCAGCCCCAGCACGCAGCCCTGGGAGCA TGTGAATGCCATCCAGGAGGCCCGGCGTCTCCTGAACCTG AGTAGAGACACTGCTGCTGAGATGAATGAAACAGTAGAA GTCATCTCAGAAATGTTTGACCTCCAGGAGCCGACCTGCC TACAGACCCGCCTGGAGCTGTACAAGCAGGGCCTGCGGG GCAGCCTCACCAAGCTCAAGGGCCCCTTGACCATGATGG CCAGCCACTACAAGCAGCACTGCCCTCCAACCCCGGAAA CTTCCTGTGCAACCCAGATTATCACCTTTGAAAGTTTCAA AGAGAACCTGAAGGACTTTCTGCTTGTCATCCCCTTTGAC TGCTGGGAGCCAGTCCAGGAGTCCGGCGGAGGAGGATCC GGCGGAGGTGGCTCTGGCGGTGGCGGAGAGGTGCAGCTG TTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCA GTTTTTCGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGG GGCTGGAGTGGGTCTCATCTATTAGAGGTAGTTCGGGTAC CACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATC TCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATG AACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGT GCGAAACCGTTTCCGTATTTTGACTACTGGGGCCAGGGAA CCCTGGTCACCGTCTCGAGTGCTAGCACCAAGGGCCCATC GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCC CCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGA CCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTC AGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT CACAAGCCCAGCAACACCAAGGTGGATAAGAAAGTTGAG CCCAAATCTTGTGACTGA	
Fab-IFNα2-Fab, L19 antibody, DNA	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTTAGCAGTTTTTCGATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCATCTATTAGAGG TAGTTCGGGTACCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAACCGTTTCCGTATTTTGACTACT GGGGCCAGGGAACCCTGGTCACCGTCTCGAGTGCTAGCA CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAA	120

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGT CAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGG TGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA TAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCGGAGG AGGGAGCGGCGGAGGTGGCTCCGGAGGGGGCGGATGCG ACCTGCCCCAGACCCACAGCCTGGGCAACAGACGGGCCC TGATCCTGCTGGCCCAGATGCGGCGGATCAGCCCCTTCAG CTGCCTGAAGGACCGGCACGACTTCGGCTTCCCCCAGGA AGAGTTCGACGGCAACCAGTTCCAGAAGGCCCAGGCCAT CAGCGTGCTGCACGAGATGATCCAGCAGACCTTCAACCT GTTCAGCACCAAGGACAGCAGCGCCGCCTGGGACGAGAG CCTGCTGGAAAAGTTCTACACCGAGCTGTACCAGCAGCTG AACGACCTGGAAGCCTGCGTGATCCAGGAAGTGGGCGTC GAGGAAACCCCCCTGATGAACGTGGACAGCATCCTGGCC GTGAAGAAGTACTTCCAGCGGATCACCCTGTACCTGACCG AGAAGAAGTATAGCCCCTGCGCCTGGGAGGTGGTGCGGG CCGAGATCATGCGGAGCTTCAGCCTGAGCACCAACCTGC AGGAACGGCTGCGGCGGAAAGAGAGCGGCGGAGGGGGA TCCGGCGGAGGTGGCTCTGGCGGTGGCGGAGAGGTGCAG CTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTA GCAGTTTTTCGATGAGCTGGGTCCGCCAGGCTCCAGGGAA GGGGCTGGAGTGGGTCTCATCTATTAGAGGTAGTTCGGGT ACCACATACTACGCAGACTCCGTGAAGGGCCGGTTCACC ATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA ATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTAC TGTGCGAAACCGTTTCCGTATTTTGACTACTGGGGCCAGG GAACCCTGGTCACCGTCTCGAGTGCTAGCACCAAGGGCC CATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTC TGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTA CTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGT CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCC CTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTG AATCACAAGCCCAGCAACACCAAGGTGGATAAGAAAGTT GAGCCCAAATCTTGTGACTGA	
3F2 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTA GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC	210

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGATAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCG GAGGAGGGAGCGGCGGAGGTGGCTCCGGAGGTGGCGGA GCACCTACTTCAAGTTCTACAAAGAAAACACAGCTACAA CTGGAGCATTTACTGCTGGATTTACAGATGATTTTGAATG GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGC TCACATTTAAGTTTTACATGCCCAAGAAGGCCACAGAACT GAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCT GGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAAT AGTTCTGGAACTAAAGGGATCTGAAACAACATTCATGTGT GAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGA ACAGATGGATTACCTTTGCCCAAAGCATCATCTCAACACT GACTTCCGGCGGAGGAGGATCCGGCGGAGGTGGCTCTGG CGGTGGCGGAGAGGTGCAATTGTTGGAGTCTGGGGGAGG CTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCA GCCTCCGGATTCACCTTTAGCAGTTATGCCATGAGCTGGG TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAG CTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTC CGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAA GAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGA GGACACGGCCGTATATTACTGTGCGAAAGGGTGGTTTGGT GGTTTTAACTACTGGGGCCAAGGAACCCTGGTCACCGTCT CGAGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGC ACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCT GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCA CCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA ACACCAAGGTGGATAAGAAAGTTGAGCCCAAATCTTGTG ACTGA	
4G8 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTA GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGATAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCG GAGGAGGGAGCGGCGGAGGTGGCTCCGGAGGTGGCGGA GCACCTACTTCAAGTTCTACAAAGAAAACACAGCTACAA CTGGAGCATTTACTGCTGGATTTACAGATGATTTTGAATG	212

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGC TCACATTTAAGTTTTACATGCCCAAGAAGGCCACAGAACT GAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCT GGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAAT AGTTCTGGAACTAAAGGGATCTGAAACAACATTCATGTGT GAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGA ACAGATGGATTACCTTTGCCCAAAGCATCATCTCAACACT GACTTCCGGCGGAGGAGGATCCGGCGGAGGTGGCTCTGG CGGTGGCGGAGAGGTGCAATTGTTGGAGTCTGGGGGAGG CTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCA GCCTCCGGATTCACCTTTAGCAGTTATGCCATGAGCTGGG TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAG CTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTC CGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAA GAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGA GGACACGGCCGTATATTACTGTGCGAAAGGGTGGCTGGG TAATTTTGACTACTGGGGCCAAGGAACCCTGGTCACCGTC TCGAGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGG CACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCT GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCA CCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA ACACCAAGGTGGATAAGAAAGTTGAGCCCAAATCTTGTG ACTGA	
3D9 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCTATGAGCTGGGTCCGCCAGAC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTGGTGTT AGTACTGGTAGCACATACTACGCAGACTCCGTGAAGGGC CGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCG TATATTACTGTGCGAAAGGTTGGCTGGGTCCTTTTGACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTAG CACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCC AAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG AACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCG GCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCG TGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTA CATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGT GGATAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCGG AGGAGGGAGCGGCGGAGGTGGCTCCGGAGGTGGCGGAG CACCTACTTCAAGTTCTACAAAGAAAACACAGCTACAACT GGAGCATTTACTGCTGGATTTACAGATGATTTTGAATGGA ATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCA CATTTAAGTTTTACATGCCCAAGAAGGCCACAGAACTGA AACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGG AGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTT AAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGT TCTGGAACTAAAGGGATCTGAAACAACATTCATGTGTGA	214

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	ATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAAC AGATGGATTACCTTTGCCCAAAGCATCATCTCAACACTGA CTTCCGGCGGAGGAGGATCCGGCGGAGGTGGCTCTGGCG GTGGCGGAGAGGTGCAATTGTTGGAGTCTGGGGGAGGCT TGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGC CTCCGGATTCACCTTTAGCAGTTATGCTATGAGCTGGGTC CGCCAGACTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCT ATTGGTGTTAGTACTGGTAGCACATACTACGCAGACTCCG TGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGA ACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGG ACACGGCCGTATATTACTGTGCGAAAGGTTGGCTGGGTCC TTTTGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCG AGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCAC CCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGG GCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGT GTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACAC CTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC ACCAAGGTGGATAAGAAAGTTGAGCCCAAATCTTGTGAC TGA	
2F11 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCC GTATATTACTGTGCGAAATGGAGATGGATGATGTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTA GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGATAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCG GAGGAGGGAGCGGCGGAGGTGGCTCCGGAGGTGGCGGA GCACCTACTTCAAGTTCTACAAAGAAAACACAGCTACAA CTGGAGCATTTACTGCTGGATTTACAGATGATTTTGAATG GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGC TCACATTTAAGTTTTACATGCCCAAGAAGGCCACAGAACT GAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCT GGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAAT AGTTCTGGAACTAAAGGGATCTGAAACAACATTCATGTGT GAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGA ACAGATGGATTACCTTTGCCCAAAGCATCATCTCAACACT GACTTCCGGCGGAGGAGGATCCGGCGGAGGTGGCTCTGG CGGTGGCGGAGAGGTGCAATTGTTGGAGTCTGGGGGAGG CTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCA GCCTCCGGATTCACCTTTAGCAGTTATGCCATGAGCTGGG	216

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAG CTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTC CGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAA GAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTATATTACTGTGCGAAATGGAGATGGAT GATGTTTGACTACTGGGGCCAAGGAACCCTGGTCACCGTC TCGAGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGG CACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCT GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCA CCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA ACACCAAGGTGGATAAGAAAGTTGAGCCCAAATCTTGTG ACTGA	
4B3 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGG TAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACT ACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTA GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGATAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCG GAGGAGGGAGCGGCGGAGGTGGCTCCGGAGGTGGCGGA GCACCTACTTCAAGTTCTACAAAGAAAACACAGCTACAA CTGGAGCATTTACTGCTGGATTTACAGATGATTTTGAATG GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGC TCACATTTAAGTTTTACATGCCCAAGAAGGCCACAGAACT GAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCT GGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAAT AGTTCTGGAACTAAAGGGATCTGAAACAACATTCATGTGT GAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGA ACAGATGGATTACCTTTGCCCAAAGCATCATCTCAACACT GACTTCCGGCGGAGGAGGATCCGGCGGAGGTGGCTCTGG CGGTGGCGGAGAGGTGCAATTGTTGGAGTCTGGGGGAGG CTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCA GCCTCCGGATTCACCTTTAGCAGTTATGCCATGAGCTGGG TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAG CTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTC CGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAA GAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGA GGACACGGCCGTATATTACTGTGCGAAAGGGTGGCTGGG TAATTTTGACTACTGGGGCCAAGGAACCCTGGTCACCGTC	218

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TCGAGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGG CACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCT GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCA CCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA ACACCAAGGTGGATAAGAAAGTTGAGCCCAAATCTTGTG ACTGA	
4G8 Fab-IL12-Fab (murine IL-12; heavy chain cytokine fusion construct)	GAGGTGCAATTGCTGGAAAGCGGCGGAGGACTGGTGCAG CCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGGC TTCACCTTCAGCAGCTACGCCATGTCTTGGGTCCGCCAGG CCCCTGGAAAGGGCCTGGAATGGGTGTCCGCCATCAGCG GCAGCGGCGGCAGCACCTACTACGCCGACAGCGTGAAGG GCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCC TGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCG CCGTGTACTACTGCGCCAAGGGCTGGCTGGGCAACTTCGA CTACTGGGGCCAGGGCACTCTGGTCACAGTGTCTAGCGCT AGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCT CCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGATAAGAAAGTTGAGCCCAAATCTTGTGACTCCGGCG GAGGAGGGAGCGGCGGAGGTGGCTCCGGAGGGGGCGGA GCCATGTGGGAGCTGGAAAAGGACGTGTACGTGGTGGAG GTGGACTGGACCCCCGACGCCCCTGGCGAGACAGTGAAC CTGACCTGCGACACCCCCGAAGAGGACGACATCACCTGG ACCAGCGACCAGCGGCACGGCGTGATCGGCAGCGGCAAG ACCCTGACCATCACCGTGAAAGAGTTTCTGGACGCCGGCC AGTACACCTGCCACAAGGGCGGCGAGACACTGAGCCACA GCCACCTGCTGCTGCACAAGAAAGAGAACGGCATCTGGT CCACCGAGATCCTGAAGAACTTCAAGAACAAGACCTTCC TGAAGTGCGAGGCCCCCAACTACAGCGGCCGGTTCACCT GCAGCTGGCTGGTGCAGCGGAACATGGACCTGAAGTTCA ACATCAAGAGCAGCAGCAGCCCCCCTGACAGCAGGGCCG TGACCTGCGGCATGGCCAGCCTGAGCGCCGAGAAGGTGA CCCTGGACCAGAGGGACTACGAGAAGTACAGCGTGAGCT GCCAGGAAGATGTCACCTGCCCCACCGCCGAGGAAACCC TGCCCATCGAGCTGGCCCTGGAAGCCCGGCAGCAGAACA AGTACGAGAACTACTCTACCAGCTTCTTCATCCGGGACAT CATCAAGCCCGACCCCCCCAAGAACCTGCAGATGAAGCC CCTGAAGAACAGCCAGGTGGAGGTGTCCTGGGAGTACCC TGACAGCTGGTCCACCCCCAGAAGCTACTTCAGCCTGAAG TTCTTCGTGAGAATCCAGCGGAAGAAAGAAAAGATGAAA GAGACAGAGGAAGGCTGCAACCAGAAGGGCGCCTTCTTC GTCGAGAAAACCAGCACCGAGGTGCAGTGCAAGGGCGGC AACGTGTGCGTGCAGGCCCAGGACCGGTACTACAACAGC AGCTGCAGCAAGTGGGCCTGCGTGCCCTGCAGAGTGCGG TCTGGCGGCGACGGCTCTGGCGGCGGAGGAAGCGGCGGA GGGGGCAGCAGAGTGATCCCCGTGAGCGGCCCTGCCCGG	220

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	TGCCTGAGCCAGAGCCGGAACCTGCTGAAAACCACCGAC GACATGGTGAAAACCGCCAGAGAGAAGCTGAAGCACTAC AGCTGCACAGCCGAGGACATCGACCACGAGGACATCACC CGGGACCAGACCAGCACCCTGAAAACCTGCCTGCCCCTG GAACTGCACAAAAACGAGAGCTGCCTGGCCACCCGGGAG ACAAGCAGCACCACCCGGGGCAGCTGCCTGCCTCCCCAG AAAACCTCCCTGATGATGACCCTGTGCCTGGGCAGCATCT ACGAGGACCTGAAGATGTACCAGACCGAGTTCCAGGCCA TCAACGCCGCCCTGCAGAACCACAATCACCAGCAGATCA TCCTGGACAAGGGCATGCTGGTCGCCATCGACGAGCTGA TGCAGAGCCTGAACCACAACGGCGAAACCCTGCGGCAGA AACCCCCCGTGGGCGAGGCCGACCCCTACCGGGTGAAGA TGAAGCTGTGCATCCTGCTGCACGCCTTCAGCACCCGGGT GGTGACCATCAACCGGGTGATGGGCTACCTGTCCTCTGCC GGGGGAGGGGGATCCGGCGGAGGTGGCTCTGGCGGTGGC GGAGAGGTGCAATTGCTGGAAAGCGGCGGAGGACTGGTG CAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGC GGCTTCACCTTCAGCAGCTACGCCATGTCTTGGGTCCGCC AGGCCCCTGGAAAGGGCCTGGAATGGGTGTCCGCCATCA GCGGCAGCGGCGGCAGCACCTACTACGCCGACAGCGTGA AGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACA CCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACA CCGCCGTGTACTACTGCGCCAAGGGCTGGCTGGGCAACTT CGACTACTGGGGCCAGGGCACTCTGGTCACAGTGTCTAGC GCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCT CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCT GCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTC GTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTT CCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC AAGGTGGATAAGAAAGTTGAGCCCAAATCTTGTGACTGA	
28H1 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAAGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGCAG CCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGCT TCACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACAGGC TCCTGGCAAAGGCCTGGAATGGGTGTCCGCCATCTGGGCC TCCGGCGAGCAGTACTACGCCGACTCTGTGAAGGGCCGG TTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTACC TGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGT ACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTG GGGACAGGGCACCCTGGTCACCGTGTCCAGCGCTAGCAC CAAGGGACCCTCCGTGTTCCCCCTGGCCCCCTCCAGCAAG TCTACCTCTGGCGGCACCGCCGCTCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAACTC TGGCGCCCTGACCAGCGGCGTCCACACCTTTCCAGCCGTG CTGCAGTCCTCCGGCCTGTACTCCCTGTCCTCCGTCGTGA CCGTGCCCTCCAGCTCTCTGGGCACCCAGACCTACATCTG CAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAA GAAGGTGGAACCCAAGTCCTGCGACAGTGGTGGGGGAGG ATCTGGTGGCGGAGGTTCTGGCGGAGGTGGCGCTCCTAC ATCCTCCAGCACCAAGAAAACCCAGCTCCAGCTGGAACA TCTCCTGCTGGATCTGCAGATGATCCTGAACGGCATCAAC AACTACAAGAACCCCAAGCTGACCCGGATGCTGACCTTC	222

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	AAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAACAT CTGCAGTGCCTGGAAGAGGAACTGAAGCCTCTGGAAGAG GTGCTGAACCTGGCCCAGTCCAAGAACTTCCACCTGAGGC CTCGGGACCTGATCTCCAACATCAACGTGATCGTGCTGGA ACTGAAGGGCTCCGAGACAACCTTCATGTGCGAGTACGC CGACGAGACAGCTACCATCGTGGAATTTCTGAACCGGTG GATCACCTTCGCCCAGTCCATCATCTCCACCCTGACCTCC GGTGGTGGCGGATCCGGGGGAGGGGGTTCTGGCGGAGGC GGAGAAGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTG CAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCG GCTTCACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACA GGCTCCAGGCAAGGGCCTGGAATGGGTGTCCGCCATCTG GGCCTCCGGCGAGCAGTACTACGCCGACTCTGTGAAGGG CCGGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTG TACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCC GTGTACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACT ACTGGGGCCAGGGCACCCTGGTCACCGTGTCCTCCGCCTC TACCAAGGGCCCCTCCGTGTTCCCTCTGGCCCCCTCCAGC AAGTCTACCTCTGGCGGCACCGCCGCTCTGGGCTGCCTGG TCAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAA CTCTGGCGCCCTGACCAGCGGCGTGCACACCTTTCCAGCC GTGCTGCAGTCCTCCGGCCTGTACTCCCTGTCCTCCGTCGT GACCGTGCCCTCCAGCTCTCTGGGCACCCAGACCTACATC TGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGAC AAGAAGGTGGAACCCAAGTCCTGCGACTGA	
29B11 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAAGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGCAG CCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGCT TCACCTTCTCCTCCTACGCCATGTCCTGGGTCCGACAGGC TCCTGGCAAAGGCCTGGAATGGGTGTCCGCCATCATCGGC TCCGGCGGCATCACCTACTACGCCGACTCTGTGAAGGGCC GGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTA CCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGT GTACTACTGTGCCAAGGGCTGGTTCGGAGGCTTCAACTAC TGGGGACAGGGCACCCTGGTCACCGTGTCCAGCGCTAGC ACCAAGGGACCCTCCGTGTTCCCCCTGGCCCCCTCCAGCA AGTCTACCTCTGGCGGCACCGCCGCTCTGGGCTGCCTGGT CAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAAC TCTGGCGCCCTGACCAGCGGCGTCCACACCTTTCCAGCCG TGCTGCAGTCCTCCGGCCTGTACTCCCTGTCCTCCGTCGTG ACCGTGCCCTCCAGCTCTCTGGGCACCCAGACCTACATCT GCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACA AGAAGGTGGAACCCAAGTCCTGCGACAGTGGTGGGGGAG GATCTGGTGGCGGAGGTTCTGGCGGAGGTGGCGCTCCTA CATCCTCCAGCACCAAGAAAACCCAGCTCCAGCTGGAAC ATCTCCTGCTGGATCTGCAGATGATCCTGAACGGCATCAA CAACTACAAGAACCCCAAGCTGACCCGGATGCTGACCTT CAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAACA TCTGCAGTGCCTGGAAGAGGAACTGAAGCCTCTGGAAGA GGTGCTGAACCTGGCCCAGTCCAAGAACTTCCACCTGAG GCCTCGGGACCTGATCTCCAACATCAACGTGATCGTGCTG GAACTGAAGGGCTCCGAGACAACCTTCATGTGCGAGTAC GCCGACGAGACAGCTACCATCGTGGAATTTCTGAACCGG TGGATCACCTTCGCCCAGTCCATCATCTCCACCCTGACCT	224

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CCGGTGGTGGCGGATCCGGGGGAGGGGGTTCTGGCGGAG GCGGAGAAGTGCAGCTGCTGGAATCCGGCGGAGGCCTGG TGCAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTC CGGCTTCACCTTCTCCTCCTATGCCATGTCCTGGGTCCGAC AGGCTCCAGGCAAGGGCCTGGAATGGGTGTCCGCCATCA TCGGCTCCGGCGGCATCACCTACTACGCCGACTCTGTGAA GGGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACAC CCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACAC CGCCGTGTACTACTGTGCCAAGGGCTGGTTCGGAGGCTTC AACTACTGGGGCCAGGGCACCCTGGTCACCGTGTCCTCCG CCTCTACCAAGGGCCCCTCCGTGTTCCCTCTGGCCCCCTC CAGCAAGTCTACCTCTGGCGGCACCGCCGCTCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCT GGAACTCTGGCGCCCTGACCAGCGGCGTGCACACCTTTCC AGCCGTGCTGCAGTCCTCCGGCCTGTACTCCCTGTCCTCC GTCGTGACCGTGCCCTCCAGCTCTCTGGGCACCCAGACCT ACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAGG TGGACAAGAAGGTGGAACCCAAGTCCTGCGACTGA	
19G1 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAGGTGCAGCTGCTCGAAAGCGGCGGAGGACTGGTGCAG CCTGGCGGCAGCCTGAGACTGTCTTGCGCCGCCAGCGGCT TCACCTTCAGCAGCTACGCCATGAGCTGGGTCCGCCAGGC CCCTGGCAAGGGACTGGAATGGGTGTCCGCCATCATCAG CTCTGGCGGCCTGACCTACTACGCCGACAGCGTGAAGGG CCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCT GTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGC CGTGTACTACTGCGCCAAGGGATGGTTCGGCGGCTTCAAC TACTGGGGACAGGGCACCCTGGTCACAGTGTCCAGCGCT AGCACCAAGGGACCCAGCGTGTTCCCCCTGGCCCCCAGC AGCAAGAGCACATCTGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAAGACTACTTCCCCGAGCCCGTGACCGTGTCCT GGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTTC CAGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCA GCGTGGTCACCGTGCCTAGCTCTAGCCTGGGCACCCAGAC CTACATCTGCAACGTGAACCACAAGCCCAGCAACACCAA GGTGGACAAGAAGGTGGAACCCAAGAGCTGCGACTCCGG CGGAGGCGGATCTGGCGGTGGAGGCTCCGGAGGCGGAGG CGCTCCTACTAGCAGCTCCACCAAGAAAACCCAGCTCCA GCTGGAACATCTGCTGCTGGATCTGCAGATGATCCTGAAC GGCATCAACAACTACAAGAACCCCAAGCTGACCCGGATG CTGACCTTCAAGTTCTACATGCCCAAGAAGGCCACCGAAC TGAAACATCTGCAGTGCCTGGAAGAGGAACTGAAGCCTC TGGAAGAGGTGCTGAACCTGGCCCAGAGCAAGAACTTCC ACCTGAGGCCCAGGGACCTGATCAGCAACATCAACGTGA TCGTGCTGGAACTGAAGGGCAGCGAGACAACCTTCATGT GCGAGTACGCCGACGAGACAGCCACCATCGTGGAATTTC TGAACCGGTGGATCACCTTCGCCCAGAGCATCATCAGCAC CCTGACAAGCGGAGGCGGCGGATCCGGCGGAGGCGGATC TGGCGGAGGAGGCGAGGTCCAGCTGCTCGAAAGCGGCGG AGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGTCTTG CGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAGC TGGGTCCGCCAGGCCCCTGGCAAGGGACTGGAATGGGTG TCCGCCATCATCAGCTCTGGCGGCCTGACCTACTACGCCG ACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA	226

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGG CCGAGGACACCGCCGTGTACTACTGCGCCAAGGGATGGT TCGGCGGCTTCAACTACTGGGGACAGGGCACCCTGGTCA CAGTGTCCAGCGCCAGCACCAAGGGCCCCAGCGTGTTCC CCCTGGCCCCCAGCAGCAAGAGCACATCTGGCGGAACAG CCGCCCTGGGCTGCCTGGTCAAAGACTACTTCCCCGAGCC CGTGACCGTGTCCTGGAACAGCGGAGCCCTGACCAGCGG CGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTG TACAGCCTGAGCAGCGTGGTCACCGTGCCTAGCTCTAGCC TGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGC CCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAG AGCTGCGACTGA	
20G8 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAGGTGCAGCTGCTCGAAAGCGGCGGAGGACTGGTGCAG CCTGGCGGCAGCCTGAGACTGTCTTGCGCCGCCAGCGGCT TCACCTTCAGCAGCTACGCCATGAGCTGGGTCCGCCAGGC CCCTGGCAAGGGACTGGAATGGGTGTCCGCCATCATCGG CTCTGGCAGCCGGACCTACTACGCCGACAGCGTGAAGGG CCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCT GTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGC CGTGTACTACTGCGCCAAGGGATGGTTCGGCGGCTTCAAC TACTGGGGACAGGGCACCCTGGTCACAGTGTCCAGCGCT AGCACCAAGGGACCCAGCGTGTTCCCCCTGGCCCCCAGC AGCAAGAGCACATCTGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAAGACTACTTCCCCGAGCCCGTGACCGTGTCCT GGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTTC CAGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCA GCGTGGTCACCGTGCCTAGCTCTAGCCTGGGCACCCAGAC CTACATCTGCAACGTGAACCACAAGCCCAGCAACACCAA GGTGGACAAGAAGGTGGAACCCAAGAGCTGCGACTCCGG CGGAGGCGGATCTGGCGGTGGAGGCTCCGGAGGCGGAGG CGCTCCTACTAGCAGCTCCACCAAGAAAACCCAGCTCCA GCTGGAACATCTGCTGCTGGATCTGCAGATGATCCTGAAC GGCATCAACAACTACAAGAACCCCAAGCTGACCCGGATG CTGACCTTCAAGTTCTACATGCCCAAGAAGGCCACCGAAC TGAAACATCTGCAGTGCCTGGAAGAGGAACTGAAGCCTC TGGAAGAGGTGCTGAACCTGGCCCAGAGCAAGAACTTCC ACCTGAGGCCCAGGGACCTGATCAGCAACATCAACGTGA TCGTGCTGGAACTGAAGGGCAGCGAGACAACCTTCATGT GCGAGTACGCCGACGAGACAGCCACCATCGTGGAATTTC TGAACCGGTGGATCACCTTCGCCCAGAGCATCATCAGCAC CCTGACAAGCGGAGGCGGCGGATCCGGCGGAGGCGGATC TGGCGGAGGAGGCGAGGTCCAGCTGCTCGAAAGCGGCGG AGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGTCTTG CGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAGC TGGGTCCGCCAGGCCCCTGGCAAGGGACTGGAATGGGTG TCCGCCATCATCGGCTCTGGCAGCCGGACCTACTACGCCG ACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACA GCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGG CCGAGGACACCGCCGTGTACTACTGCGCCAAGGGATGGT TCGGCGGCTTCAACTACTGGGGACAGGGCACCCTGGTCA CAGTGTCCAGCGCCAGCACCAAGGGCCCCAGCGTGTTCC CCCTGGCCCCCAGCAGCAAGAGCACATCTGGCGGAACAG CCGCCCTGGGCTGCCTGGTCAAAGACTACTTCCCCGAGCC	228

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CGTGACCGTGTCCTGGAACAGCGGAGCCCTGACCAGCGG CGTGCACACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTG TACAGCCTGAGCAGCGTGGTCACCGTGCCTAGCTCTAGCC TGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGC CCAGCAACACCAAGGTGGACAAGAAGGTGGAACCCAAG AGCTGCGACTGA	
3F2 light chain	GAGATCGTGCTGACCCAGTCCCCCGGCACCCTGTCTCTGA GCCCTGGCGAGAGAGCCACCCTGTCCTGCAGAGCCTCCC AGTCCGTGACCTCCTCCTACCTCGCCTGGTATCAGCAGAA GCCCGGCCAGGCCCCTCGGCTGCTGATCAACGTGGGCAG TCGGAGAGCCACCGGCATCCCTGACCGGTTCTCCGGCTCT GGCTCCGGCACCGACTTCACCCTGACCATCTCCCGGCTGG AACCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCA TCATGCTGCCCCCCACCTTTGGCCAGGGCACCAAGGTGGA AATCAAGCGTACGGTGGCCGCTCCCTCCGTGTTCATCTTC CCACCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCC GTCGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCA AGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCA ACTCCCAGGAATCCGTCACCGAGCAGGACTCCAAGGACA GCACCTACTCCCTGTCCTCCACCCTGACCCTGTCCAAGGC CGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGAC CCACCAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAAC CGGGGCGAGTGCTGATGA	230
4G8 light chain	GAGATCGTGCTGACCCAGTCCCCCGGCACCCTGTCTCTGA GCCCTGGCGAGAGAGCCACCCTGTCCTGCAGAGCCTCCC AGTCCGTGTCCCGGTCCTACCTCGCCTGGTATCAGCAGAA GCCCGGCCAGGCCCCTCGGCTGCTGATCATCGGCGCCTCT ACCAGAGCCACCGGCATCCCTGACCGGTTCTCCGGCTCTG GCTCCGGCACCGACTTCACCCTGACCATCTCCCGGCTGGA ACCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCCA GGTCATCCCTCCCACCTTTGGCCAGGGCACCAAGGTGGAA ATCAAGCGTACGGTGGCCGCTCCCTCCGTGTTCATCTTCC CACCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGT CGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAG GTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAAC TCCCAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGC ACCTACTCCCTGTCCTCCACCCTGACCCTGTCCAAGGCCG ACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCC ACCAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACCG GGGCGAGTGCTGATGA	232
3D9 light chain	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGCTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGGA AATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTG TTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA CTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAG	234

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGC AGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCAC CCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAA CAGGGGAGAGTGTTAG	
2F11 light chain	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGTATACTCCCCCCACGTTCGGCCAGGGGACCAAAGTGG AAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTT CCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCT GTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTA ACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAG CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCA CCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTTAG	236
4B3 light chain	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTC AGAGTGTTAGCAGCAATTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGCGCCTA CATCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTC AGGTTATTCCCCCTACGTTCGGCCAGGGGACCAAAGTGG AAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTT CCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCT GTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTA ACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAG CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCA CCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTTAG	238
2B10 Fab-IL2-Fab (heavy chain cytokine fusion construct)	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGGGATCATCC CTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTTCCCCC TGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGG CCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT GACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGT GCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTAC TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG	240

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA GCAACACCAAGGTGGATAAGAAAGTTGAGCCCAAATCTT GTGACTCCGGCGGAGGAGGGAGCGGCGGAGGTGGCTCCG GAGGTGGCGGAGCACCTACTTCAAGTTCTACAAAGAAAA CACAGCTACAACTGGAGCATTTACTGCTGGATTTACAGAT GATTTTGAATGGAATTAATAATTACAAGAATCCCAAACTC ACCAGGATGCTCACATTTAAGTTTTACATGCCCAAGAAGG CCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAAC TCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCA AAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATAT CAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAAC ATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTA GAATTTCTGAACAGATGGATTACCTTTGCCCAAAGCATCA TCTCAACACTGACTTCCGGCGGAGGAGGATCCGGCGGAG GTGGCTCTGGCGGTGGCGGACAGGTGCAATTGGTGCAGT CTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGG TCTCCTGCAAGGCCTCCGGAGGCACATTCAGCAGCTACGC TATAAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTCGA GTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAAC TACGCACAGAAGTTCCAGGGCAGGGTCACCATTACTGCA GACAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGC CTGAGATCTGAGGACACCGCCGTGTATTACTGTGCGAGAC TGTACGGTTACGCTTACTACGGTGCTTTTGACTACTGGGG CCAAGGGACCACCGTGACCGTCTCCTCAGCTAGCACCAA GGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAG GCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCA ACGTGAATCACAAGCCCAGCAACACCAAGGTGGATAAGA AAGTTGAGCCCAAATCTTGTGACTGA	
C3B6 Fab-IL2-Fab (heavy chain cytokine fusion construct)	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTACGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGCTATCATCC CGATCCTTGGTATCGCAAACTACGCACAGAAGTTCCAGG GCAGGGTCACCATTACTGCAGACAAATCCACGAGCACAG CCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCG CCGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTA CGGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTTCCCCC TGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGG CCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT GACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGT GCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTAC TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA GCAACACCAAGGTGGATAAGAAAGTTGAGCCCAAATCTT GTGACTCCGGCGGAGGAGGGAGCGGCGGAGGTGGCTCCG GAGGTGGCGGAGCACCTACTTCAAGTTCTACAAAGAAAA CACAGCTACAACTGGAGCATTTACTGCTGGATTTACAGAT GATTTTGAATGGAATTAATAATTACAAGAATCCCAAACTC	242

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	ACCAGGATGCTCACATTTAAGTTTTACATGCCCAAGAAGG CCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAAC TCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCA AAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATAT CAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAAC ATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTA GAATTTCTGAACAGATGGATTACCTTTGCCCAAAGCATCA TCTCAACACTGACTTCCGGCGGAGGAGGATCCGGCGGAG GTGGCTCTGGCGGTGGCGGACAGGTGCAATTGGTGCAGT CTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGG TCTCCTGCAAGGCCTCCGGAGGCACATTCAGCAGCTACGC TATAAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTCGA GTGGATGGGAGCTATCATCCCGATCCTTGGTATCGCAAAC TACGCACAGAAGTTCCAGGGCAGGGTCACCATTACTGCA GACAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGC CTGAGATCTGAGGACACCGCCGTGTATTACTGTGCGAGAC TGTACGGTTACGCTTACTACGGTGCTTTTGACTACTGGGG CCAAGGGACCACCGTGACCGTCTCCTCAGCTAGCACCAA GGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAG GCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCA ACGTGAATCACAAGCCCAGCAACACCAAGGTGGATAAGA AAGTTGAGCCCAAATCTTGTGACTGA	
6A12 Fab-IL2-Fab (heavy chain cytokine fusion construct)	CAGGTGCAATTGGTGCAGTCTGGGGCTGAGGTGAAGAAG CCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCCTCCGGAG GCACATTCAGCAGCTATGCTATAAGCTGGGTGCGACAGG CCCCTGGACAAGGGCTCGAGTGGATGGGAGTGATCATCC CTATCCTTGGTACCGCAAACTACGCACAGAAGTTCCAGGG CAGGGTCACCATTACTGCAGACAAATCCACGAGCACAGC CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACCGC CGTGTATTACTGTGCGAGACTGTACGGTTACGCTTACTAC GGTGCTTTTGACTACTGGGGCCAAGGGACCACCGTGACC GTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTTCCCCC TGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGG CCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT GACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGT GCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTAC TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA GCAACACCAAGGTGGATAAGAAAGTTGAGCCCAAATCTT GTGACTCCGGCGGAGGAGGGAGCGGCGGAGGTGGCTCCG GAGGTGGCGGAGCACCTACTTCAAGTTCTACAAAGAAAA CACAGCTACAACTGGAGCATTTACTGCTGGATTTACAGAT GATTTTGAATGGAATTAATAATTACAAGAATCCCAAACTC ACCAGGATGCTCACATTTAAGTTTTACATGCCCAAGAAGG CCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAAC TCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCA AAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATAT CAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAAC ATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTA	244

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GAATTTCTGAACAGATGGATTACCTTTGCCCAAAGCATCA TCTCAACACTGACTTCCGGCGGAGGAGGATCCGGCGGAG GTGGCTCTGGCGGTGGCGGACAGGTGCAATTGGTGCAGT CTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGG TCTCCTGCAAGGCCTCCGGAGGCACATTCAGCAGCTATGC TATAAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTCGA GTGGATGGGAGTGATCATCCCTATCCTTGGTACCGCAAAC TACGCACAGAAGTTCCAGGGCAGGGTCACCATTACTGCA GACAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGC CTGAGATCTGAGGACACCGCCGTGTATTACTGTGCGAGAC TGTACGGTTACGCTTACTACGGTGCTTTTGACTACTGGGG CCAAGGGACCACCGTGACCGTCTCCTCAGCTAGCACCAA GGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAG GCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCA ACGTGAATCACAAGCCCAGCAACACCAAGGTGGATAAGA AAGTTGAGCCCAAATCTTGTGACTGA	
2B10 light chain	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGCATTAGAAATGATTTAGGCTGGTACCAGCAGAAGC CAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCA GTTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCAGTG GATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCA GCCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGT CTGCAGCCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAG ATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCC CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGT TGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAA GTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAAC TCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC AGGGGAGAGTGTTAG	246
D1A2 light chain	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGGGGATTCGTAATGATTTAGGCTGGTACCAGCAGAAGC CAGGGAAAGCCCCTAAGCGCCTGATCTATGATGCTTACA GCTTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTG GATCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCA GCCTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGT CTGCAGCCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAG ATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCC CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGT TGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAA GTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAAC TCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC	248

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	AGGGGAGAGTGTTAG	
O7D8 light chain	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAT CTGTCGGAGACCGGGTCACCATCACCTGCCGGGCAAGTC AGAGCATTCGTAATGTTTTAGGCTGGTACCAGCAGAAGCC AGGGAAAGCCCCTAAGCGCCTGATCTATGATGTGTCCAGT TTGCAGAGTGGCGTCCCATCAAGGTTCAGCGGCGGTGGA TCCGGGACAGAGTTCACTCTCACCATCAGCAGCTTGCAGC CTGAAGATTTTGCCACCTATTACTGCTTGCAGAATGGTCT GCAGCCCGCGACGTTTGGCCAGGGCACCAAAGTCGAGAT CAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCG CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTG TGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGT ACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTC CCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAG ACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC ATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACA GGGGAGAGTGTTAG	250
MHLG1 Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAAGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTCAAG CCTGGCGGGTCCCTGCGGCTCTCCTGTGCAGCCTCCGGAT TCACATTTAGCAACTATTGGATGAACTGGGTGCGGCAGGC TCCTGGAAAGGGCCTCGAGTGGGTGGCCGAGATCAGATT GAAATCCAATAACTTCGGAAGATATTACGCTGCAAGCGT GAAGGGCCGGTTCACCATCAGCAGAGATGATTCCAAGAA CACGCTGTACCTGCAGATGAACAGCCTGAAGACCGAGGA TACGGCCGTGTATTACTGTACCACATACGGCAACTACGTT GGGCACTACTTCGACCACTGGGGCCAAGGGACCACCGTC ACCGTCTCCAGTGCTAGCACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGC GGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGC GTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTT GGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC CAGCAACACCAAGGTGGATAAGAAAGTTGAGCCCAAATC TTGTGACTCCGGCGGAGGAGGGAGCGGCGGAGGTGGCTC CGGAGGTGGCGGAGCACCTACTTCAAGTTCTACAAAGAA AACACAGCTACAACTGGAGCATTTACTGCTGGATTTACAG ATGATTTTGAATGGAATTAATAATTACAAGAATCCCAAAC TCACCAGGATGCTCACATTTAAGTTTTACATGCCCAAGAA GGCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGA ACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAG CAAAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAAT ATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACA ACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTG TAGAATTTCTGAACAGATGGATTACCTTTGCCCAAAGCAT CATCTCAACACTGACTTCCGGCGGAGGAGGATCCGGCGG AGGTGGCTCTGGCGGTGGCGGAGAAGTGCAGCTGGTGGA GTCTGGAGGAGGCTTGGTCAAGCCTGGCGGGTCCCTGCG GCTCTCCTGTGCAGCCTCCGGATTCACATTTAGCAACTAT TGGATGAACTGGGTGCGGCAGGCTCCTGGAAAGGGCCTC GAGTGGGTGGCCGAGATCAGATTGAAATCCAATAACTTC GGAAGATATTACGCTGCAAGCGTGAAGGGCCGGTTCACC	252

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	ATCAGCAGAGATGATTCCAAGAACACGCTGTACCTGCAG ATGAACAGCCTGAAGACCGAGGATACGGCCGTGTATTAC TGTACCACATACGGCAACTACGTTGGGCACTACTTCGACC ACTGGGGCCAAGGGACCACCGTCACCGTCTCCAGTGCTA GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGATAAGAAAGTTGAGCCCAAATCTTGTGACTGA	
KV9 light chain	GATATCCAGTTGACCCAGTCTCCATCCTTCCTGTCTGCATC TGTGGGCGACCGGGTCACCATCACCTGCAAGGCCAGTCA GAATGTGGATACTAACGTGGCTTGGTACCAGCAGAAGCC AGGGCAGGCACCTAGGCCTCTGATCTATTCGGCATCCTAC CGGTACACTGGCGTCCCATCAAGGTTCAGCGGCAGTGGA TCCGGGACAGAGTTCACTCTCACAATCTCAAGCCTGCAAC CTGAAGATTTCGCAACTTACTACTGTCAACAGTACAATAG TTACCCTCTGACGTTCGGCGGAGGTACCAAGGTGGAGATC AAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGC CATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGT GTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCAC CTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCA TCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAG GGGAGAGTGTTAG	254
MHLG Fab-IL2-Fab (heavy chain cytokine fusion construct)	GAAGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTCCAG CCTGGCGGGTCCCTGCGGCTCTCCTGTGCAGCCTCCGGAT TCACATTTAGCAACTATTGGATGAACTGGGTGCGGCAGGC TCCTGGAAAGGGCCTCGAGTGGGTGGCCGAGATCAGATT GAAATCCAATAACTTCGGAAGATATTACGCTGCAAGCGT GAAGGGCCGGTTCACCATCAGCAGAGATGATTCCAAGAA CACGCTGTACCTGCAGATGAACAGCCTGAAGACCGAGGA TACGGCCGTGTATTACTGTACCACATACGGCAACTACGTT GGGCACTACTTCGACCACTGGGGCCAAGGGACCACCGTC ACCGTCTCCAGTGCTAGCACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGC GGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGC GTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTT GGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC CAGCAACACCAAGGTGGATAAGAAAGTTGAGCCCAAATC TTGTGACTCCGGCGGAGGAGGGAGCGGCGGAGGTGGCTC CGGAGGTGGCGGAGCACCTACTTCAAGTTCTACAAAGAA AACACAGCTACAACTGGAGCATTTACTGCTGGATTTACAG ATGATTTTGAATGGAATTAATAATTACAAGAATCCCAAAC TCACCAGGATGCTCACATTTAAGTTTTACATGCCCAAGAA GGCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGA ACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAG	256

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	CAAAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAAT ATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACA ACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTG TAGAATTTCTGAACAGATGGATTACCTTTGCCCAAAGCAT CATCTCAACACTGACTTCCGGCGGAGGAGGATCCGGCGG AGGTGGCTCTGGCGGTGGCGGAGAAGTGCAGCTGGTGGA GTCTGGAGGAGGCTTGGTCCAGCCTGGCGGGTCCCTGCG GCTCTCCTGTGCAGCCTCCGGATTCACATTTAGCAACTAT TGGATGAACTGGGTGCGGCAGGCTCCTGGAAAGGGCCTC GAGTGGGTGGCCGAGATCAGATTGAAATCCAATAACTTC GGAAGATATTACGCTGCAAGCGTGAAGGGCCGGTTCACC ATCAGCAGAGATGATTCCAAGAACACGCTGTACCTGCAG ATGAACAGCCTGAAGACCGAGGATACGGCCGTGTATTAC TGTACCACATACGGCAACTACGTTGGGCACTACTTCGACC ACTGGGGCCAAGGGACCACCGTCACCGTCTCCAGTGCTA GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGATAAGAAAGTTGAGCCCAAATCTTGTGACTGA	
KV1 light chain	GATATCCAGTTGACCCAGTCTCCATCCTTCCTGTCTGCATCT GTGGGCGACCGGGTCACCATCACCTGCAGGGCCAGTCAGAA TGTGGATACTAACTTAGCTTGGTACCAGCAGAAGCCAGGGA AAGCACCTAAGCTCCTGATCTATTCGGCATCCTACCGTTACA CTGGCGTCCCATCAAGGTTCAGCGGCAGTGGATCCGGGACA GAGTTCACTCTCACAATCTCAAGCCTGCAACCTGAAGATTTC GCAACTTACTACTGTCAACAGTACAATAGTTACCCTCTGACG TTCGGCGGAGGTACCAAGGTGGAGATCAAGCGTACGGTGGC TGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTT GAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTT CTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACG CCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAG GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGAC GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCT GCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG AGCTTCAACAGGGGAGAGTGTTAG	264
KV7 light chain	GATATCCAGTTGACCCAGTCTCCATCCTTCCTGTCTGCATCT GTGGGCGACCGGGTCACCATCACCTGCAAGGCCAGTCAGAA TGTGGATACTAACGTGGCTTGGTACCAGCAGAAGCCAGGGA AAGCACCTAAGCCTCTGATCTATTCGGCATCCTACCGGTACA CTGGCGTCCCATCAAGGTTCAGCGGCAGTGGATCCGGGACA GAGTTCACTCTCACAATCTCAAGCCTGCAACCTGAAGATTTC GCAACTTACTACTGTCAACAGTACAATAGTTACCCTCTGACG TTCGGCGGAGGTACCAAGGTGGAGATCAAGCGTACGGTGGC TGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTT GAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTT CTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACG CCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAG GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGAC GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCT	266

Construct	POLYNUCLEOTIDE SEQUENCE	SEQ ID NO
	GCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG AGCTTCAACAGGGGAGAGTGTTAG	

Host Cells

As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to generate the immunoconjugates of the invention or fragments thereof. In one embodiment, the host cell is engineered to allow the production of an immunoconjugate fragment. Host cells include cultured cells, *e.g.,* mammalian cultured cells, such as CHO cells, HEK, BHK cells, NS0 cells, Sp2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell of the invention comprises an expression vector comprising polynucleotide sequences that encode immunoconjugates of the invention or fragments thereof. Host cells of the invention may be eukaryotic or prokaryotic.

Purification of Immunoconjugate Polypeptides and Fragments Thereof

The immunoconjugates of the invention or fragments thereof can be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity, *etc.,* and will be apparent to those having skill in the art. For affinity chromatography purification, a matrix with protein A or protein G may be used. Alternatively, for affinity chromatography purification, any antibody which specifically binds the single-chain effector moiety of the immunoconjugate may be used. For the production of antibodies, various host animals, including, but not limited to rabbits, mice, rats, etc., may be immunized by injection with a immunoconjugate of the invention or a fragment thereof. The immunoconjugate may be attached to a suitable carrier, such as bovine serum albumin (BSA), by means of a side chain functional group or linkers attached to a side chain functional group. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhold limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacilli Calmette-Guerin) and *Cornyebacterium parvum.*

Accordingly, one embodiment includes a method for producing the immunoconjugates of the invention by culturing a host cell comprising an expression vector comprising polynucleotide sequences that encode immunoconjugates of the invention or fragments thereof under conditions suitable for the expression of the same.

Methods of Using Immunoconjugates

The immunoconjugates of the invention are useful for targeting specific antigenic determinants and eliciting various cellular responses in target and recruited cells. The immunoconjugate of the invention is also useful as a diagnostic reagent. The binding of an immunoconjugate to an antigenic determinant can be readily detected by using a secondary antibody specific for the effector moiety. In one embodiment, the secondary antibody and the immunoconjugate facilitate the detection of binding of the immunoconjugate to an antigenic determinant located on a cell or tissue surface.

In some embodiments, an effective amount of the immunoconjugates of the invention are administered to a cell. In other embodiments, a therapeutically effective amount of the immunoconjugate of the invention is administered to an individual for the treatment of disease. The term "effective amount" as used herein is defined as the amount of the immunoconjugate of the invention that is necessary to result in a physiological change in the cell or tissue to which it is administered. The term "therapeutically effective amount" as used herein is defined as the amount of the immunoconjugate of the invention that eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

The immunoconjugates of the invention may be administered to a subject *per se* or in the form of a pharmaceutical composition. In one embodiment, the disease is a proliferative disorder, such as cancer. Non-limiting examples of proliferative disorders such as cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer. Other cell proliferation disorders that can be treated using an immunoconjugate of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and

cancer metastases. Similarly, other cell proliferation disorders can also be treated by the immunoconjugates of the present invention. Examples of such cell proliferation disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other cell proliferation disease, besides neoplasia, located in an organ system listed above. In another embodiment, the disease is related to autoimmunity, transplantation rejection, post-traumatic immune responses and infectious diseases (e.g. HIV). More specifically, the immunoconjugates may be used in eliminating cells involved in immune cell-mediated disorders, including lymphoma; autoimmunity, transplantation rejection, graft-versus-host disease, ischemia and stroke. A skilled artisan readily recognizes that in many cases the immunoconjugates may not provide a cure but may only provide partial benefit. In some embodiments, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some embodiments, an amount of immunoconjugate that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount."

The subject, patient, or individual in need of treatment is typically a mammal, more specifically a human.

Compositions, Formulations, Dosages, and Routes of Administration

Pharmaceutical compositions of the present invention comprise an effective amount of one or more immunoconjugates dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one immunoconjugate and optionally an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (*e.g.,* human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards or corresponding authorities in other countires.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.,* antibacterial agents,

antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (*see, for example,* Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The immunoconjugates may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. The present invention can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrasplenically, intrarenally, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctivally, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularally, orally, topically, locally, inhalation (*e.g.* aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (*e.g.,* liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (*see, for example,* Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference). Parenteral administration, in particular intravenous injection, is most commonly used for administering polypeptide molecules such as the immunoconjugates of the invention.

The actual dosage amount of a composition of the present invention administered to a subject can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of the immunoconjugate of the invention. In other embodiments, the immunoconjugates may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body

weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above.

In any case, the composition may comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (e.g., methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

The immunoconjugates may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine.

In embodiments where the composition is in a liquid form, a carrier can be a solvent or dispersion medium comprising but not limited to, water, ethanol, polyol (*e.g.,* glycerol, propylene glycol, liquid polyethylene glycol, *etc*), lipids (*e.g.,* triglycerides, vegetable oils, liposomes) and combinations thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin; by the maintenance of the required particle size by dispersion in carriers such as, for example liquid polyol or lipids; by the use of surfactants such as, for example hydroxypropylcellulose; or combinations thereof such methods. In many cases, it will be preferable to include isotonic agents, such as, for example, sugars, sodium chloride or combinations thereof, and/or buffering agents to maintain physiologically acceptable pH values.

In other embodiments, one may use eye drops, nasal solutions or sprays, aerosols or inhalants in the present invention. Such compositions are generally designed to be compatible with the target tissue type. In a non-limiting example, nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, in some embodiments the aqueous nasal solutions usually are isotonic or slightly buffered to maintain a pH of about 5.5 to about 6.5. In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations, drugs, or appropriate drug stabilizers, if required, may be included in the formulation. For example, various commercial nasal preparations are known and include drugs such as antibiotics or antihistamines.

In certain embodiments, the immunoconjugate is prepared for administration by such routes as oral ingestion. In these embodiments, the solid composition may comprise, for example, solutions, suspensions, emulsions, tablets, pills, capsules (*e.g.,* hard or soft shelled gelatin capsules), sustained release formulations, buccal compositions, troches, elixirs, suspensions, syrups, wafers, or combinations thereof. Oral compositions may be incorporated directly with the food of the diet. Preferred carriers for oral administration comprise inert diluents, assimilable edible carriers or combinations thereof. In other aspects of the invention, the oral composition may be prepared as a syrup or elixir. A syrup or elixir, and may comprise, for example, at least one active agent, a sweetening agent, a preservative, a flavoring agent, a dye, a preservative, or combinations thereof.

In certain embodiments, an oral composition may comprise one or more binders, excipients, disintegration agents, lubricants, flavoring agents, and combinations thereof. In certain embodiments, a composition may comprise one or more of the following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof; an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or combinations thereof; a lubricant, such as, for example, magnesium stearate; a sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc.; or combinations thereof the foregoing. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Various other materials may be present as coatings or to

otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both.

Additional formulations which are suitable for other modes of administration include suppositories. Suppositories are solid dosage forms of various weights and shapes, usually medicated, for insertion into the rectum, vagina or urethra. After insertion, suppositories soften, melt or dissolve in the cavity fluids. In general, for suppositories, traditional carriers may include, for example, polyalkylene glycols, triglycerides or combinations thereof. In certain embodiments, suppositories may be formed from mixtures containing, for example, the active ingredient in the range of about 0.5% to about 10%, and preferably about 1% to about 2%.

Sterile injectable solutions are prepared by incorporating the immunoconjugates of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The preparation of highly concentrated compositions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small area.

The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less that 0.5 ng/mg protein.

In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

Pharmaceutical compositions comprising the immunoconjugates of the invention may be manufactured by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically

acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For topical administration the immunoconjugates of the invention may be formulated as solutions, gels, ointments, creams, suspensions, *etc.* as are well-known in the art.

Systemic formulations include those designed for administration by injection, *e.g.* subcutaneous, intravenous, intramuscular, intrathecal or intraperitoneal injection, as well as those designed for transdermal, transmucosal, inhalation, oral or pulmonary administration.

For injection, the immunoconjugates of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Alternatively, the immunoconjugates may be in powder form for constitution with a suitable vehicle, *e.g.,* sterile pyrogen-free water, before use.

For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the immunoconjugates can be readily formulated by combining the immunoconjugates with pharmaceutically acceptable carriers well known in the art. Such carriers enable the immunoconjugates of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. For oral solid formulations such as, for example, powders, capsules and tablets, suitable excipients include fillers such as sugars, *e.g.* lactose, sucrose, mannitol and sorbitol; cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP); granulating agents; and binding agents. If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

If desired, solid dosage forms may be sugar-coated or enteric-coated using standard techniques.

For oral liquid preparations such as, for example, suspensions, elixirs and solutions, suitable carriers, excipients or diluents include water, glycols, oils, alcohols, *etc.* Additionally, flavoring agents, preservatives, coloring agents and the like may be added.

For buccal administration, the immunoconjugates may take the form of tablets, lozenges, *etc.* formulated in conventional manner.

For administration by inhalation, the immunoconjugates for use according to the invention are conveniently delivered in the form of an aerosol spray from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.,* dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the immunoconjugate and a suitable powder base such as lactose or starch.

The immunoconjugates may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, *e.g.,* containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the immunoconjugates may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the immunoconjugates may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Alternatively, other pharmaceutical delivery systems may be employed. Liposomes and emulsions are well known examples of delivery vehicles that may be used to deliver immunoconjugates of the invention. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the immunoconjugates may be delivered using a sustained-release system, such as semipermeable matrices of solid polymers containing the therapeutic agent. Various of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the immunoconjugates for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the immunoconjugates, additional strategies for immunoconjugates stabilization may be employed.

As the immunoconjugates of the invention may contain charged side chains or termini, they may be included in any of the above-described formulations as the free acids or bases or as pharmaceutically acceptable salts. Pharmaceutically acceptable salts are those salts which substantially retain the biologic activity of the free bases and which are prepared by reaction with inorganic acids. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

The immunoconjugates of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the immunoconjugates of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. A therapeutically effective amount is an amount effective to ameliorate or prevent the symptoms, or prolong the survival of, the patient being treated. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from *in vivo* data, *e.g.,* animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the immunoconjugates which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. Therapeutically effective serum levels may be achieved by administering multiple doses each day.

In cases of local administration or selective uptake, the effective local concentration of the immunoconjugates may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

The amount of immunoconjugate administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

The therapy may be repeated intermittently while symptoms detectable or even when they are not detectable. The therapy may be provided alone or in combination with other drugs. In the case of autoimmune disorders, the drugs that may be used in combination with immunoconjugates of the invention include, but are not limited to, steroid and non-steroid anti-inflammatory agents.

Toxicity

A therapeutically effective dose of the immunoconjugates described herein will generally provide therapeutic benefit without causing substantial toxicity. Toxicity of the immunoconjugates can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.,* by determining the LD_{50} (the dose lethal to 50% of the population) or the LD_{100} (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. In one embodiment, the immunoconjugate exhibits a high therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic, for example, for use in human. The dosage of the immunoconjugates described herein lies preferably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (*See, e.g.,* Fingl *et al.,* 1975, In: The Pharmacological Basis of Therapeutics, Ch. 1, p. 1) (incorporated herein by reference in its entirety.

Other Agents and Treatments

It is contemplated that other agents may be used in combination with the present invention to improve the therapeutic efficacy of treatment. These additional agents include immunomodulatory agents, agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, or agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers. Immunomodulatory agents include tumor necrosis factor; interferon alpha, beta, and gamma; IL-2 and other cytokines; F42K and other cytokine analogs; or MIP-1, MIP-1β, MCP-1, RANTES, and other chemokines. It is further contemplated that the upregulation of cell surface receptors or their ligands such as Fas/Fas ligand, DR4 or DR5 /TRAIL would potentiate the apoptotic inducing abililties of the present invention by establishment of an autocrine or paracrine effect on hyperproliferative cells. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with the present invention to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present invention. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further

contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody C225, could be used in combination with the present invention to improve the treatment efficacy.

Hormonal therapy may also be used in conjunction with the present invention or in combination with any other cancer therapy previously described. The use of hormones may be employed in the treatment of certain cancers such as breast, prostate, ovarian, or cervical cancer to lower the level or block the effects of certain hormones such as testosterone or estrogen. This treatment is often used in combination with at least one other cancer therapy as a treatment option or to reduce the risk of metastases.

The immunonconjugates of the invention may also be administred in conjunction with chemotherapy, radiation therapy or other immunotherapies. Anti-cancer agents for such combination therapy may, *e.g.*, be selected from the groups of microtubule disruptors (e.g. vinca alkaloids such as vinblastine or vincristine, taxanes such as docetaxel or paclitaxel, epothilones such as ixabepilone), antimetabolites (e.g. anti-folates such as methotrexate or aminopterin, anti-purines such as fludarabine, 6-mercaptopurine or 6-thioguanine, anti-pyrimidines such as 5-fluorouracil, capecitabine or gemcitabine, hydroxyurea), topoisomerase inhibitors (e.g. camptothecin, irinotecan, topotecan, or podophyllotoxins such as etoposide), DNA intercalators (e.g. doxorubicin, daunorubicin, actinomycin, bleomycin), alkylating agents (e.g. cyclophosphamide, chlorambucil, nitrosureas such as carmustine or nimustine, streptozocin, busulfan, cisplatin, oxaliplatin, triethylenemelamine, dacarbazine), hormonal therapies (e.g. glucocorticoids, aromatase inhibitors such as tamoxifene, antiandrogens such as flutamide, gonadotropin-releasing hormone (GnRH) analogs such as leuprolide), antibiotics, kinase inhibitors (e.g. erlotinib, gefitinib, imatinib), receptor antagonists (e.g. antibodies targeting cell surface receptors known to promote carcinogenesis and tumor growth), enzyme inhibitors (e.g. cyclin-dependent kinase (CDK) inhibitors), amino acid-depleting enzymes (e.g. asparaginase), leucovorin, retinoids, activators of tumor cell apoptosis, and antiangiogenic agents.

EXAMPLES

Example 1

When antibody-mediated delivery of cytokines was first performed by Harvill E.T., and Morrison S.L. *Immunotech. 1(2)*:95-105 (1995), the constructs had two antigen binding moieties for tumor antigen targeting and two cytokine moieties for inducing lymphocyte activation (an IL-

2 molecule is fused to each of the heavy chain C-termini of IgG3). The affinity of the cytokine is generally high towards its receptor. A molecule carrying two cytokine units could have an even higher affinity towards cytokine receptors because of avidity (or multivalency) effects. Such a molecule could therefore easily activate lymphocytes in the blood stream, even before the targeting to the tumor takes place. This effect would not be desired for the patient. In contrast, an immunoconjugate molecule carrying only one cytokine moiety and two or more targeting domains would be less likely to activate lymphocytes in the circulation and could direct the entire immunoconjugate molecule to the tumor, where lymphocyte activation can take place at a lower speed. Therefore, molecules as depicted in Figures 1 and 2, were constructed with interleukin-2 (IL-2) as a model cytokine. All of the generated molecules are bivalent for the tumor antigen, and are either bivalent or monovalent for the IL-2 cytokine as indicated in the drawings. Affinities towards the antigen were compared for two different immunoconjugate formats using the L19 antibody as an example. As a reference, this antibody was cloned into the human IgG1 format, the diabody format (Figure 1A), and the Fab-IL2-Fab fusion (Figure 1B). One variable was tested within the diabody format such that the linker peptide between the V_H and the V_L was either eight or twelve amino acids in length. The purified antigen Extra Domain B of fibronectin (EDB) was immobilized on a BIACORE chip, and the antibody fusion construct was used as the soluble analyte for affinity determination. Figures 8 to 11 show the results of this experiment. The IgG was considered to be the ideal case of a bivalent binding event. Here, an affinity constant of 260 pM was observed. The Fab-IL2-Fab fusion construct gave an affinity of 310 pM, which is essentially identical to the IgG. The two variants of the diabody (shown in Figures 10 and 11) had measured affinities of 270 pM and 360 pM, respectively. Therefore, all of these constructs have similar affinities towards the antigen. The affinity towards the antigen and the IL-2 receptor was addressed using similar constructs based on the F16 antibody sequence (Brack, S.S. *et al., Clin. Canc. Res. 12(10)*:3200-3208 (2006)). Figure 12 shows the BIACORE sensograms of the F16 IgG and its corresponding monovalent Fab fragment, when the TNC-A1 domain was immobilized on the BIACORE chip. Under these particular experimental conditions, the IgG molecule showed an affinity constant of 2.6 nM, and the Fab molecules showed an affinity constant of 50 nM. Here, the increase of affinity attributed to bivalency is a factor of 20. The diabody, the Fab-IL2-Fab and the scFv-IL2-scFv immunoconjugates showed affinities toward the antigen of 5 nM, 4.8 nM, and 12 nM, respectively. All of these constructs therefore have affinities toward the antigen that more closely resemble the bivalent character of an IgG molecule than the monovalent behavior of the Fab fragment.

The differences were more pronounced when looking at affinities toward the IL-2 receptor. To study IL-2 receptor binding affinity, a tool was generated that allowed for the expression of a heterodimeric IL-2 receptor; the β chain of the IL-2 receptor was fused to an Fc molecule that was engineered to heterodimerize (Fc(knob)) using the "knobs-into-holes" technology (Merchant, A.M. *et al., Nat. Biotech. 16*:677-681 (1998)). The gamma chain of the IL-2 receptor was then fused to the Fc(hole) variant, which heterodimerized with Fc(knob). This heterodimeric Fc-fusion protein was then used as a substrate for analyzing the IL-2/IL-2 receptor interaction. Figure 13 shows the BIACORE sensogram of commercially available IL-2 (Proleukin), as the analyte, with the immobilized IL-2 receptor. The measured affinity of ~0.5 nM is in accordance with previously published values. The affinities of the various constructs towards the IL-2 receptor are summarized in Figure 17. An important result that was observed was the difference between the diabody (F16 dia IL2), which is bivalent with respect to the cytokine, and the Fab-IL2-Fab molecule which carries only one IL-2 moiety. The IL-2 receptor binding affinity of the diabody (0.8 nM) was similar to that of unconjugated IL-2 (Proleukin) (0.5 nM), despite the diabody being bivalent and the IL-2 being monovalent. The Fab-IL2-Fab fusion had an IL-2 receptor binding affinity almost reduced by a factor of 10 compared to the diabody, which was reflected in a reduced capacity to induce proliferation of NK92 cells as shown in Figure 19.

Example 2

Construction of Generic Fab-Libraries

Generic antibody libraries in the Fab-format were constructed on the basis of human germline genes using the following V-domain pairings: Vk3_20 kappa light chain with VH3_23 heavy chain for the DP47-3 library and Vk1_17 kappa light chain with VH1_69 heavy chain for the DP88-3 library. See Table 2. Both libraries were randomized in CDR3 of the light chain (L3) and CDR3 of the heavy chain (H3) and were assembled from 3 fragments per library by splicing by overlapping extension (SOE) PCR. Fragment 1 comprises the 5' end of the antibody gene including randomized L3, fragment 2 is a central constant fragment spanning from L3 to H3, whereas fragment 3 comprises randomized H3 and the 3' portion of the antibody gene.

The following primer combinations were used to generate library fragments for DP47-3 library: fragment 1 (LMB3 – LibL1b_new), fragment 2 (MS63 – MS64), fragment 3 (Lib2H - fdseqlong). See Table 9. The following primer combinations were used to generate library

fragments for the DP88-3 library: fragment 1 (LMB3 – RJH_LIB3), fragment 2 (RJH31 – RJH32) and fragment 3 (LIB88_2 - fdseqlong). See Table 10.

TABLE 9.

Primers Used In the DP47-3 Library	
LMB3	CAGGAAACAGCTATGACCATGATTAC
LibL1b_new	CACTTTGGTCCCCTGGCCGAACGTMNNGGGMNNMNNMNNACCCTGCTGAC AGTAATACACTGC
MS63	TTTCGCACAGTAATATACGGCCGTGTCC
MS64	ACGTTCGGCCAGGGGACCAAAGTGG
Lib2H	GGCCGTATATTACTGTGCGAAANNKNNKNNKNNKNNKTTTGACTACTGGGG CCAAGGAAC
fdseqlong	GACGTTAGTAAATGAATTTTCTGTATGAGG

TABLE 10.

Primers Used in DP88-3 Library	
LMB3	CAGGAAACAGCTATGACCATGATTAC
RJH_LIB3	GACTTTGGTGCCCTGGCCAAACGT MNN GGG MNN MNN ACC MNN CTGCAAGCAGTAATAGGTGGCAAAATC
RJH31	ACGTTTGGCCAGGGCACCAAAGTCGAG
RJH32	TCTCGCACAGTAATACACGGCGGTGTCC
LIB88_2	GGACACCGCCGTGTATTACTGTGCGAGA –[(33% GAC Asp; 26% GGT Gly; 10% GAA Glu; 9% CGT Arg; 7% Lys; 6% GTT Val; 5% TCT Ser; 4% CTG Leu)1 - (23% GGT Gly; 17% TAC Tyr; 16% TCT Ser; 11% GCT Ala; 9% CGT Arg; 7% AAC Asn; 6% ACT Thr; 6% GTT Val; 5% CCG Pro)8]- TTTGACTACTGGGGCCAAGGGACCACCGTGACCGTCTCC
fdseqlong	GACGTTAGTAAATGAATTTTCTGTATGAGG

The PCR protocol for the production of library fragments included: 5 minutes of initial denaturation at 94°C; 25 cycles of 1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C; and terminal elongation for 10 minutes at 72 °C. For assembly PCR, equimolar ratios of the 3 fragments were used as template. The assembly PCR protocol included: 3 minutes of initial denaturation at 94°C; and 5 cycles of 30 seconds at 94°C, 1 minute at 58°C, and 2 minutes at 72°C. At this stage, primers complementary to sequence outside fragments 1-3 were added and an additional 20 cycles were performed prior to a terminal elongation for 10 min at 72 °C.

After assembly of sufficient amounts of full length randomized Fab constructs, the Fab constructs were digested with *Nco*I / *Not*I for the DP47-3 library and with *Nco*I / *Nhe*I for the DP88-3 library alongside with similarly treated acceptor phagemid vector. For the DP47-3 library, 22.8 µg of Fab library was ligated with 16.2 µg of phagemid vector. For the DP88-3 library, 30.6 µg of Fab library was ligated with 30.6 µg of phagemid vector.

Purified ligations were used for 68 transformations for the DP47-3 library and 64 transformations for the DP88-3 library, respectively, to obtain final library sizes of 4.2×10^{10} for DP47-3 and 3.3×10^{9} for DP88-3.

Phagemid particles displaying the Fab libraries were rescued and purified by PEG/NaCl purification to be used for selections.

Example 3

Selection of Anti-TNC A2 Clone 2B10

Selections were carried out against *E. coli*-expressed human TNC-A2 which was subcloned 5' of an avi-tag and 6×his-tag. See SEQ ID NO: 57 in Table 5. The antigen was biotinylated *in vivo* upon expression. Selections have been carried out in solution according to the following protocol: (i) binding of ~ 10^{12} phagemid particles of library DP88-3 and 100nM biotinylated human TNC A2 for 0.5 hours in a total volume of 1ml; (ii) capture of biotinylated human TNC-A2 and attached phage by the addition of 5.4×10^{7} streptavidin-coated magnetic beads for 10 minutes; (iii) washing of beads using 5x 1ml PBS/Tween20 and 5x 1ml PBS; (iv) elution of phage particles by the addition of 1 mL 100mM TEA (triethylamine) for 10 minutes and neutralization by the addition of 500 µL 1M Tris/HCl pH 7.4; and (v) re-infection of log-phase *E. coli* TG1 cells, infection with helperphage VCSM13 and subsequent PEG/NaCl precipitation of phagemid particles to be used in subsequent selection rounds.

Selections were carried out over 3 rounds using constant antigen concentrations at 100 nM. In round 2, capture of antigen:phage complexes was performed on neutravidin plates instead of streptavidin beads. Specific binders were identified by ELISA as follows using: 100 µl of 100 nM biotinylated human TNC-A2 was coated in each well of neutravidin plates.

Fab-containing bacterial supernatants were added and binding Fabs were detected via their Flag-tags by using an anti-Flag/HRP secondary antibody. Once identified, clone 2B10 was bacterially expressed in a 0.5 litre culture volume, affinity purified and further characterized by SPR-analysis using BIACORE T100. See SEQ ID NOs: 3 and 7 of Table 3.

Example 4

Selection of Anti-TNC A1/A4 Clone 2F11

Selections were carried out against *E. coli* expressed human TNC A1 which was subcloned 5' of an avi-tag and 6×his-tag. See SEQ ID NO: 59 of Table 5. The antigen was biotinylated *in vivo*

upon expression. Selections were carried out in solution according to the following protocol: (i) binding of ~ 10^{12} phagemid particles of library DP47-3 and 100 nM biotinylated human TNC-A1 for 0.5 hours in a total volume of 1 mL; (ii) capture of biotinylated human TNC-A1 and attached phage by the addition of 5.4 × 10^7 streptavidin-coated magnetic beads for 10 minutes; (iii) washing of beads using 5x 1 mL PBS/Tween20 and 5x 1ml PBS; (iv) elution of phage particles by the addition of 1 mL 100 mM TEA (triethylamine) for 10 minutes and neutralization by the addition of 500 µl 1M Tris/HCl pH 7.4; and (v) re-infection of log-phase *E. coli* TG1 cells, infection with helperphage VCSM13 and subsequent PEG/NaCl precipitation of phagemid particles to be used in subsequent selection round.

Selections were carried out over 3 rounds using constant antigen concentrations at 100 nM. In round 2, capture of antigen:phage complexes was performed on neutravidin plates instead of streptavidin beads.

All binding reactions were supplemented with 100 nM non-biotinylated human IgG CH3 constant domain comprising a carboxy-terminal avi-tag and 6×his-tag in order to compete for unwanted clones recognizing the tags of the antigen.

In a first screening step, specific binders were identified by ELISA as follows: 100 µl of 100 nM biotinylated human TNC-A1 was coated in each of neutravidin plates. Fab-containing bacterial supernatants were added and Fabs that specifically bound to human TNC-A1 were detected via their Flag-tags by using an anti-Flag/HRP secondary antibody.

In a second screening step, the above ELISA was repeated using also murine TNC-A1, human TNC A4 and mu TNC A4 as antigens in order to determine cross-reactivity. All antigens comprised the same avi-tag and 6×his-tag at their C-terminus and were *in vivo* biotinylated. See SEQ ID NOs: 50 and 61 of Table 5.

Once identified, clone 2F11 was bacterially expressed in a 0.5 litre culture volume, affinity purified and further characterized by SPR-analysis using BIACORE T100. See SEQ ID NOs: 9 and 13 of Table 3.

Example 5

Selection of anti-FAP clones (primary selections)

Selections were carried out against the ectodomain of human or murine fibroblast activating protein (FAP) which were cloned upstream a poly-lysine and a 6×his-tag. See SEQ ID NOs: 53 and 55 of Table 5. Prior to selections, the antigens were coated into immunotubes at a concentration of either 10 µg/ml or 5 µg/ml, depending on round of selection. Selections were

carried out according to the following protocol: (i) binding of ~ 10^{12} phagemid particles of library DP47-3 to immobilized human or murine FAP for 2 hours; (ii) washing of immunotubes using 5 × 5mL PBS/Tween20 and 5 × 5ml PBS; (iii) elution of phage particles by addition of 1mL 100mM TEA (triethylamine) for 10 minutes and neutralization by the addition of 500 µL 1M Tris/HCl pH 7.4; and (iv) re-infection of log-phase *E. coli* TG1 cells, infection with helperphage VCSM13 and subsequent PEG/NaCl precipitation of phagemid particles to be used in subsequent selection rounds.

Selections have been carried out over three or four rounds using decreasing antigen concentrations of human FAP and in some cases using murine FAP at 5ug/ml in the final selection round. Specific binders were defined as signals 5 × higher than background and were identified by ELISA. NUNC maxisorp plates were coated with 10ug/ml of human or murine FAP followed by addition of Fab-containing bacterial supernatants and detection of specifically binding Fabs via their Flag-tags by using an anti-Flag/HRP secondary antibody.

ELISA-positive clones were bacterially expressed as 1 mL cultures in 96-well format and supernatants were subjected to a kinetic screening experiment using BIACORE T100.

Example 6

Construction of Anti-FAP Affinity Maturation Libraries

Three affinity maturation libraries were constructed on basis of pre-selected antibodies from the primary anti-FAP selections. More precisely, they were based on (i) anti-human FAP clone 2D9 (library a.m.FAP2D9) (see SEQ ID NOs: 67 and 69 of Table 3), (ii) anti-murine FAP clone 4B8 (library a.m.FAP4B8) (see SEQ ID NOs: 71 and 73 of Table 3) and (iii) cross-reactive clones 7A1, 13B2, 13C2, 13E8, 14C10 and 17A11 (library a.m.FAPpool) (see SEQ ID NOs: 75 and 77 of Table 3 corresponding to the variable region sequences of 7A1; SEQ ID NOs: 79 and 81 of Table 3 corresponding to the variable region sequences of 13C2; SEQ ID NOs: 83 and 85 corresponding to the variable region sequences of 13E8; SEQ ID NOs: 87 and 89 corresponding to the variable region sequences of 14C10; and SEQ ID NOs: 91 and 93 corresponding to the variable region sequences of 17A11).

Each of these libraries consists of two sublibraries, randomized in either CDR1 and CDR2 of the light chain (L1/L2) or CDR1 and CDR2 of the heavy chain (H1/H2), respectively. These sublibraries were pooled upon transformation. Each of these sublibraries was constructed by four subsequent steps of amplification and assembly.

For L1/L2 libraries, the amplification and assembly protocol included: (i) amplification of fragment 1 (LMB3 – DPK22_CDR1_rand_ba_opt) and fragment 2 (DPK22_CDR1_fo – DPK22_Ck_*Bsi*WI_ba); (ii) assembly of fragments 1 and 2 using outer primers LMB3 and DPK22_Ck_*Bsi*WI_ba to create the template for fragment 3; (iii) amplification of fragment 3 (LMB3 – DPK22_CDR2_rand_ba) and fragment 4 (DPK22_CDR2_fo – DPK22_Ck_*Bsi*WI_ba); and (iv) final assembly of fragments 3 and 4 using the same outer primers as above. See Table 11 for primer sequences.

TABLE 11.

Primers Used in L1/L2 Affinity Maturation Libraries for anti-FAP Affinity Maturation	
LMB3	CAGGAAACAGCTATGACCATGATTAC
DPK22_CDR1_rand_ba_opt	CAGGTTTCTGCTGGTACCAGGCTAA**G**TA**G**CT**G**CT**G**CTAACA CTCTGACTGGCCCTGCAAG
DPK22_CDR1_fo	TTAGCCTGGTACCAGCAGAAACCTG
DPK22_Ck_*Bsi*WI_ba	GGTGCAGCCACCGTACGTTTGATTTCC
DPK22_CDR2_rand_ba	CTGTCTGGGATGCCAGTGGCCCT**G**CT**G**GA**G**GC**G**CCATAGAT GAGGAGCCTGGGAGCCTG
DPK22_CDR2_fo	AGGGCCACTGGCATCCCAGACAG

Bold: 60% original base and 40% randomization as M
Underline: 60% original base and 40% randomization as N

For H1/H2 libraries, the amplification and assembly protocol included: (i) amplification of fragment 1 (RJH53 – DP47_CDR1_rand_ba_opt) and fragment 2 (DP47_CDR1_fo – MS52); (ii) assembly of fragments 1 and 2 using outer primers RJH53 and MS52 to create the template for fragment 3; (iii) amplification of fragment 3 (RJH53 – DP47_CDR2_rand_ba) and fragment 4 (DP47_CDR2_fo – MS52); and (iv) final assembly of fragments 3 and 4 using the same outer primers as above. See Table 12 for primer sequences.

TABLE 12.

Primers Used in H1/H2 Affinity Maturation Libraries for anti-FAP Affinity Maturation	
RJH53	CATCAGGGCCTGAGCTCGCCCGTCAC
DP47_CDR1_rand_ba_opt	GAGCCTGGCGGACCCAGCTCAT**G**GC**A**TA**A**CTGCTAAAGGTG AATCCGGAGGC
DP47_CDR1_fo	ATGAGCTGGGTCCGCCAGGCTC
MS52	GAAGACCGATGGGCCTTTGGTGCTAG
DP47_CDR2_rand_ba	CCTTCACGGAGTCTGCGTAGTATGT**G**CTA**C**CA**C**CA**C**TA**C**CA CTAATA**G**CTGAGACCCACTCCAGCCCCTTCCC
DP47_CDR2_fo	ACATACTACGCAGACTCCGTGAAGG

Bold: 60% original base and 40% randomization as M
Underline: 60% original base and 40% randomization as N

Final assembly products have been digested with *Nco*I / *Bsi*WI for L1/L2 sublibraries of a.m.FAP2D9 and a.m.FAP4B8, with *Mun*I and *Nhe*I for H1/H2 sublibraries of a.m.FAP2D9 and a.m.FAP4B8 as well as with *Nco*I / *Bam*HI for L1/L2 library of a.m.FAPpool and with *Bsp*EI/*Pst*I for H1/H2 libraries of a.m.FAPpool, respectively, alongside with similarly treated acceptor vectors based on plasmid preparations of clones 2D9, 4B8 or an equimolar mixture of clones 7A1, 13B2, 13C2, 13E8, 14C10 and 17A11, respectively. The following amounts of digested randomized (partial) V-domains and digested acceptor vector(s) were ligated for the respective libraries (µg V-domain/µg vector): a.m.FAP2D9 L1/L2 sublibrary (5.7/21.5), a.m.FAP2D9 H1/H2 sublibrary (4.1/15.5), a.m.FAP4B8 L1/L2 sublibrary (6.5/24.5), a.m.FAP4B8 H1/H2 sublibrary (5.7/21.5), a.m.FAPpool L1/L2 sublibrary (4.4/20), a.m.FAPpool H1/H2 sublibrary (3.4/15.5).

Purified ligations of L1/L2 and H1/H2 sublibraries were pooled and used for 60 transformations for each of the 3 affinity maturation libraries, to obtain final library sizes of 6.2×10^9 for a.m.FAP2D9, 9.9×10^9 for a.m.FAP4B8 and 2.2×10^9 for a.m.FAPpool.

Phagemid particles displaying these Fab libraries were rescued and purified by PEG/NaCl purification to be used for secondary selections.

Example 7

Selection of Affinity-Matured Anti-FAP Clones

Selections were carried out against the ectodomain of human or murine fibroblast activating protein (FAP) which were cloned 5' of a poly-lysine and a 6×his-tag. See SEQ ID NOs: 53 and 55 of Table 5. Prior to selections, the antigens were coated into immunotubes at a concentration of either 10 µg/mL, 5 µg/mL or 0.2 µg/mL, depending on the library and round of selection. Selections were carried out according to the following protocol: (i) binding of ~ 10^{12} phagemid particles of library a.m.FAP2D9, a.m.FAP4B8 or a.m.FAPpool to immobilized human or murine FAP for 2 hours; (ii) washing of immuno tubes using 10 – 20 × 5 mL PBS/Tween20 and 10 – 20 × 5 mL PBS (depending on library and selection round); (iii) elution of phage particles by addition of 1 mL 100m M TEA (triethylamine) for 10 minutes and neutralization by addition of 500 µL 1M Tris/HCl pH 7.4; and (iv) re-infection of log-phase *E. coli* TG1 cells, infection with helperphage VCSM13 and subsequent PEG/NaCl precipitation of phagemid particles to be used in subsequent selection rounds.

Selections were carried out over 2 rounds and conditions were adjusted for each of the 3 libraries individually. In detail, selection parameters were: a.m.FAP2D9 (5 µg /mL human FAP and 20

washes in total for round 1, 1 μg/mL human FAP and 30 washes in total for round 2), a.m.FAP4B8 (1 μg/mL murine FAP and 30 washes in total for round 1, 0.2 μg/mL human FAP and 40 washes in total for round 2) and a.m.FAPpool (5 μg/mL human FAP and 30 washes in total for round 1, 5 μg/mL murine FAP and 30 washes in total for round 2). Specific binders were defined as signals 5 × higher than background and were identified by ELISA. NUNC maxisorp plates were coated with 1 μg/mL or 0.2 μg/mL of human or murine FAP followed by addition of Fab-containing bacterial supernatants and detection of specifically binding Fabs via their Flag-tags by using an anti-Flag/HRP secondary antibody.

ELISA-positive clones were bacterially expressed as 1ml cultures in 96-well format and supernatants were subjected to a kinetic screening experiment using BIACORE T100.

Example 8

Efficacy Studies of Different Formats of Targeted IL-2

An efficacy experiment was performed using two different Interleukin-2 immunoconjugate molecular formats specific for tumor stroma. The F9 teratocarcinoma was subcutaneously injected into 129SvEv mice, and tumor size was measured using a caliper. The "diabody"-IL-2 molecule was compared at two different concentrations to the Fab-interleukin-2-Fab (Fab-IL2-Fab) immunoconjugate, wherein the concentrations reflected similar numbers of immunoconjugate molecules. Results are shown in Figure 3. The Fab-IL2-Fab immunoconjugate shows a significant tumor growth inhibition and is better than the diabody format at two different concentrations and better than controls.

Survival of mice treated with two different Interleukin-2 immunoconjugate molecular formats specific for tumor stroma was also examined. Human gastric tumor cell-line LS174T was intrasplenically injected into SCID-beige mice. The "diabody"-IL-2 molecule was compared at two different concentrations to the Fab-IL-2-Fab immunoconjugate, wherein the concentrations reflected similar numbers of immunoconjugate molecules. Results are shown in Figure 4. The Fab-IL-2-ab format resulted in a higher percent survival compared to the diabody format and controls.

Example 9

Recombinant DNA Techniques

Standard methods were used to manipulate DNA as described in Sambrook, J. etal., Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer's instructions.

General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A. et al., (1991) Sequences of Proteins of Immunological Interest, Fifth Ed., NIH Publication No 91-3242.

DNA Sequencing

DNA sequences were determined by double strand sequencing.

Gene Synthesis

Desired gene segments were prepared by Geneart AG (Regensburg, Germany) from synthetic oligonucleotides and PCR products by automated gene synthesis. The gene segments which are flanked by singular restriction endonuclease cleavage sites were cloned into pGA18 (ampR) plasmids. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The DNA sequence of the subcloned gene fragments was confirmed by DNA sequencing. Gene Segments were designed with suitable restriction sites to allow sub-cloning into the respective expression vectors. All constructs were designed with a 5'-end DNA sequence coding for a leader peptide which targets proteins for secretion in eukaryotic cells. Table 13 and 14 give exemplary leader peptides and polynucleotide sequences encoding them, respectively.

TABLE 13.

Leader Sequences for Secretion: Polypeptide Sequences.

Polypeptide Sequence	SEQ ID NO
MDWTWRILFLVAAATGAHS	273
MDMRVPAQLLGLLLLWFPGARC	276
MGWSCIILFLVATATGVHS	278

TABLE 14.

Leader Sequences for Secretion: Polynucleotide Sequences.

Polynucleotide Sequence	SEQ ID NO
ATGGACTGGACCTGGAGAATCCTCTTCTTGGTGGCAGCAGCC ACAGGAGCCCACTCC	274
ATGGACTGGACCTGGAGGATCCTCTTCTTGGTGGCAGCAGCC ACAGGAGCCCACTCC	275
ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGCCTCCTGCTGC TCTGGTTCCCAGGTGCCAGGTGT	277
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTA CCGGTGTGCATTCC	279
ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTGGCTACCGCCA CTGGAGTGCATTCC	280
ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTCGCCACAGCCA CCGGCGTGCACTCT	281

Preparation of Immunoconjugates

The resulting DNA sequences were subcloned into mammalian expression vectors (one for the light chain and one for the heavy chain/fusion protein) under the control of the MPSV promoter and upstream of a synthetic polyA site, each vector carrying an EBV OriP sequence.

Immunoconjugates as applied in the examples below were produced by co-transfecting exponentially growing HEK293-EBNA cells with the mammalian expression vectors using a calcium phosphate-transfection. Alternatively, HEK293 cells growing in suspension were transfected by polyethylenimine (PEI) with the expression vectors, or stably transfected CHO cell pools were used. While 3F2 and 4G8 based FAP-targeted Fab-IL2-Fab constructs can be purified by affinity chromatography using a protein A matrix, 2B10 based TNC A2-targeted Fab-IL2-Fab constructs have to be purified by affinity chromatography on a protein G matrix.

Briefly, TNC A2-targeted 2B10 Fab-IL2-Fab was purified from supernatants by one affinity step (protein G) followed by size exclusion chromatography (Superdex 200, GE Healthcare). The protein G column was equilibrated in 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5, supernatant was loaded, and the column was washed with 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. Fab-IL2-Fab was eluted with 8.8 mM formic acid pH 3. The eluted fractions were pooled and polished by size exclusion chromatography in the final formulation buffer: 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine pH 6.7. Figure 53 shows the exemplary results from purification and analytics.

FAP-targeted 3F2 Fab-IL2-Fab or 4G8 Fab-IL2-Fab were purified by a similar method composed of one affinity step (protein A) followed by size exclusion chromatography (Superdex 200, GE Healthcare). The protein A column was equilibrated in 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5, supernatant was loaded, and the column was washed with 20 mM

sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, pH 7.5, followed by a wash with 13.3 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, pH 5.45. A third wash with 10 mM MES, 50 mM sodium chloride pH 5 was optionally performed. Fab-IL2-Fab was eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine, pH 3. The eluted fractions were pooled and polished by size exclusion chromatography in the final formulation buffer: 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine pH 6.7.

Example 10

Construction of Additional Anti-FAP Affinity Maturation Libraries (based on clones 3F2, 3D9, 4G8, 4B3 and 2C6)

Four additional affinity maturation libraries were constructed on the basis of pre-selected cross-reactive antibodies from the first affinity-maturation campaign of anti-FAP antibodies, namely clones 3F2, 3D9, 4G8, 4B3 and 2C6 (see SEQ ID NOs: 17 and 21 of Table 3 corresponding to the variable region sequences of 3F2; SEQ ID NOs: 23 and 25 of Table 3 corresponding to the variable region sequences of 3D9; SEQ ID NOs: 33 and 35 of Table 3 corresponding to the variable region sequences of 4B3; SEQ ID NOs: 41 and 43 of Table 3 corresponding to the variable region sequences of 2C6). More precisely, the four libraries were based on 1) anti-FAP clones 3F2, 4G8 and 4B3 (V_H library, randomized in CDRs 1 and 2 of variable heavy chain, i.e. H1/H2 library), 2) anti-FAP clones 3D9 and 2C6 (V_L library, randomized in CDRs 1 and 2 of variable light chain, i.e. L1/L2 library), 3) anti-FAP clone 3F2 (L3 library with soft randomization in CDR3 of light chain, i.e. L3 library) and 4) anti-FAP clone 3F2 (H3 library with soft randomization in CDR3 of heavy chain, i.e. H3 library). The first two libraries were constructed exactly the same way as outlined for the first affinity-maturation campaign of anti-FAP antibodies, for the L1/L2 and H1/H2 libraries, respectively. In contrast, for the L3 and H3 affinity-maturation libraries based on clone 3F2, two new primers were used to introduce soft randomization in L3 (AM_3F2_DPK22_L3_ba: CACTTTGGTCCCCTGGCCGAACGT CGGGGGAAGCA TAATACCCTGCTGACAGTAATACACTGC with underlined bases being 60% given base and 40% mixture N (mixture of the four nucleotides A, C, G, and T)) and H3 (AM_3F2_DP47_H3_fo: GGCCGTATATTACTGTGCG A*A*A GGG TGG TT*T* GGT GGT TTT *A*AC TACTGGGGCCAAGGAAC with underlined bases being 60% given base and 40% mixture N, bases in italics being 60% given base and 40% G, as well as underlined bases in

italics being 60% given base and 40% mixture K (mixture of the two nucleotides G and T)) of the parental clone. Library sizes were as follows: H1/H2 library (1.13×10^{10}), L1/L2 library (5.6×10^{9}), L3 library (2.3×10^{10}) and H3 library (2.64×10^{10}).

Example 11

Selection of Affinity-Matured Anti-FAP Clones

Selections were carried out against the ectodomain of human and murine fibroblast activating protein (FAP) which were cloned upstream a 6x-lysine and a 6x-his tag (see SEQ ID NOs: 53 and 55 of Table 5). Prior to selections, the antigens were coated into immunotubes at a concentration of either 1 μg/ml, 0.2 μg/ml or 0.02 μg/ml, depending on the library and round of selection. Selections and ELISA-based screenings were carried out as described for the first affinity-maturation campaign of anti-FAP antibodies. Secondary screenings were carried out using a ProteOn XPR36 biosensor (Biorad), and kinetic rate constants and affinities were determined analyzing affinity-purified Fab preparations on the same instrument. The following affinity-matured clones were identified: 19G1 (see SEQ ID NOs: 121 and 123 of Table 3), 20G8 (see SEQ ID NOs: 125 and 127 of Table 3), 4B9 (see SEQ ID NOs: 129 and 131 of Table 3), 5B8 (see SEQ ID NOs: 133 and 135 of Table 3), 5F1 (see SEQ ID NOs: 137 and 139 of Table 3), 14B3 (see SEQ ID NOs: 141 and 143 of Table 3), 16F1 (see SEQ ID NOs: 145 and 147 of Table 3), 16F8 (see SEQ ID NOs: 149 and 151 of Table 3), O3C9 (see SEQ ID NOs: 153 and 155 of Table 3), 22A3 (see SEQ ID NOs: 165 and 167 of Table 3) and 29B11 (see SEQ ID NOs: 169 and 171 of Table 3) (all these clones were selected from the H1/H2 library and are derived from parental clone 3F2), O2D7 (see SEQ ID NOs: 157 and 159 of Table 3) (selected from the L3 library based on parental clone 3F2), and 28H1 (see SEQ ID NOs: 161 and 163 of Table 3) and 23C10 (see SEQ ID NOs: 173 and 175 of Table 3) (these two clones were selected from the H1/H2 library and are derived from parental clone 4G8).

Figures 21-25 show the Surface Plasmon Resonance sensorgrams of the selected affinity matured Fabs against FAP and Table 15 gives the respective affinities. The selected Fabs span a high affinity range in the pM to nM range and are cross-reactive for human (hu) and murine (mu) FAP, as well as Cynomolgus (cyno) FAP as determined for selected clones. The affinity matured anti-FAP Fabs were converted into the Fab-IL2-Fab format. Specificity of binding was shown by lack of binding to DPPIV as close homologue of FAP, expressed on HEK293 or CHO cells.

TABLE 15.

Summary of kinetic equilibrium constants (K_D) of affinity-matured anti-FAP antibodies as Fab fragments (monovalent binding).

antibody	affinity (K_D) to hu FAP [pM]	affinity (K_D) to mu FAP [pM]	affinity (K_D) to cyno FAP [pM]
19G1	76	2600	n.d.
20G8	69	2800	n.d.
4B9	157	3300	n.d.
5B8	690	3200	n.d.
5F1	243	4100	n.d.
14B3	377	3800	n.d.
16F1	193	3400	n.d.
16F8	301	3800	n.d.
O3C9	160	3700	n.d.
O2D7	619	8300	n.d.
28H1	200	9	3600
22A3	34	655	522
29B11	35	436	23
23C10	1600	125	990

Example 12

Construction of Anti-TNC A2 Affinity Maturation Libraries (based on clone 2B10)

An affinity maturation library was constructed on the basis of a pre-selected antibody from the primary TNC A2 selections. More precisely, it was based on parental clone 2B10 and consisted of two sub-libraries: 1) V_L sub-library, randomized in CDR1 and CDR2 of the light chain (L1/L2) and 2) V_H sub-library, randomized in CDR1 and CDR2 of the heavy chain (H1/H2). These sub-libraries were pooled upon transformation. Each of these sub-libraries was constructed by four subsequent steps of amplification and assembly. For L1/L2 libraries: 1) amplification of fragment 1 (LMB3 – AM_Vk1A30_L1_ba) and fragment 2 (RJH50 (Vk1A30_L1/L2_fo) – RJH51 (Vk1A30_BsiWI_ba)), 2) assembly of fragments 1 and 2 using outer primers LMB3 and RJH51 (Vk1A30_BsiWI_ba) to create the template for 3) amplification of fragment 3 (LMB3 – AM_Vk1A30_L2_ba) and fragment 4 (RJH52 (Vk1A30_L2/L3) – RJH51 (Vk1A30_BsiWI_ba)) and 4) final assembly of fragments 3 and 4 using the same outer primers as above. For H1/H2 libraries: 1) amplification of fragment 1 (RJH53 – AM_DP88_H1_ba_opt) and fragment 2 (RJH54(DP88_H1/H2_fo) – MS52), 2) assembly of fragments 1 and 2 using outer primers RJH53 and MS52 to create the template for 3)

amplification of fragment 3 (RJH53 – AM_DP88_H2_ba) and fragment 4 (RJH55 (DP88_H2H3_fo) – MS52) and 4) final assembly of fragments 3 and 4 using the same outer primers as above. Final assembly products have been digested *Nco*I / *Bsi*WI for V_L sub-libraries and *Mun*I and *Nhe*I for V_H sub-libraries and were cloned in similarly digested acceptor vectors. Library size resulted in 1.16×10^{10} independent clones.

TABLE 16.

Primers used in L1/L2 Affinity Maturation Libraries for Anti-TNC A2 binder 2B10	
LMB3	CAGGAAACAGCTATGACCATGATTAC
AM_Vk1A30_L1_ba	CCTGGCTTCTGCTGGTACCAGCCTAAA**T**C**A**T**T**A**C**G**C**AAT GCCCTGACTTGCCCGGCAGGTGATG
RJH50(Vk1A30_L1/L2_fo)	GCTGGTACCAGCAGAAGCCAGGGAAAG
RJH51(Vk1A30_BsiWI_ba)	GGTGCAGCCACCGTACGCTTGATCTC
AM_Vk1A30_L2_ba	CTTGATGGGACGCCACTCTGCAAA**C**T**G**G**A**C**G**C**A**G**C**A**T** AGATCAGGCGCTTAGGGGCTTTCC
RJH52(Vk1A30_L2/L3)	TTGCAGAGTGGCGTCCCATCAAGGTTC

Underline: 60% original base and 40% randomization as V
Bold: 60% original base and 40% randomization as N

TABLE 17.

Primers used in H1/H2 Affinity Maturation Libraries for Anti-TNC A2 binder 2B10	
RJH53	CATCAGGGCCTGAGCTCGCCCGTCAC
AM_DP88_H1_ba_opt	GTCCAGGGGCCTGTCGCACCCAGCTTAT**A**G**C**G**T**A**G**C**T** GCTGAATGTGCCTCCGGAGGCCTTG
RJH54(DP88_H1/H2_fo)	ATAAGCTGGGTGCGACAGGCCCCTGGAC
MS52	GAAGACCGATGGGCCTTTGGTGCTAG
AM_DP88_H2_ba	GACCCTGCCCTGGAACTTCTGTGCGTAGTTTGC**G**G**T**AC C**A**A**G**A**T**A**G**G**G**A**T**GAT**C**C**C**TCCCATCCACTCGAGCCC TTGTCCAG
RJH55 (DP88_H2H3_fo)	TACGCACAGAAGTTCCAGGGCAGGGTCAC

Underligned: 60% original base and 40% randomization as V
Bold: 60% original base and 40% randomization as N

Example 13

Selection of Affinity-Matured Anti-TNC A2 Clones

Selections were carried out against *E. coli* expressed human TNC A2 which was cloned upstream an avi-tag and 6×his-tag (see SEQ ID NO: 57 of Table 5). The antigen was biotinylated *in vivo* upon expression. Selections have been carried out in solution as described for the primary TNC A2 selections using decreasing concentrations of human TNC A2 ranging from 100 to 2

nM. After identification of affinity-matured clones by ELISA, secondary screenings were carried out using a ProteOn XPR36 biosensor (Biorad) and kinetic rate constants and affinities were determined analyzing affinity-purified Fab preparations on the same instrument. The following affinity-matured clones were identified: 2B10_O1F7 (see SEQ ID NOs: 201 and 203 of Table 3), 2B10_6H10 (see SEQ ID NOs: 205 and 207 of Table 3), 2B10_C3A6 (see SEQ ID NOs: 185 and 187 of Table 3), 2B10_D1A2 (see SEQ ID NOs: 189 and 191 of Table 3), and 2B10_O7D8 (see SEQ ID NOs: 197 and 199 of Table 3) (all of these are derived from the V_L sub-library), as well as 2B10_C3B6 (see SEQ ID NOs: 177 and 179 of Table 3) and 2B10_6A12 (see SEQ ID NOs: 181 and 183 of Table 3) (these two clones are derived from the V_H sub-library). Moreover, for clone 2B10_D1A2, a V32D mutant was generated (see SEQ ID NOs: 193 and 195 of Table 3) (numbering according to Kabat).

Figure 26 shows the Surface Plasmon Resonance sensorgrams of the selected affinity matured Fabs against TNC A2 and Table 18 gives the respective affinities. The selected Fabs span a high affinity range in the pM range.

TABLE 18.

Summary of kinetic equilibrium constants (K_D) of affinity-matured anti-TNC A2 antibodies as Fab fragments (monovalent binding).

antibody	affinity (KD) to hu TNC A2 [pM]
2B10_C3B6	191
2B10_6A12	290
2B10_C3A6	497
2B10_O7D8	147
2B10_O1F7	56
2B10_6H10	810

Example 14

Purification of 2B10, 3F2 and 4G8-based Fab-IL2-Fab Constructs

Another purification method (in addition to the one described in Example 9) was developed for 2B10, 3F2 and 4G8 based Fab-IL2-Fab constructs. While 3F2 and 4G8 based Fab-IL2-Fab constructs can be purified by affinity chromatography using a protein A matrix (e.g. MabSelect Sure), 2B10 based Fab-IL2-Fab constructs have to be purified by affinity chromatography on a protein G matrix. The purification procedure is based on the following four steps:

1. Affinity chromatography with MabSelect Sure or protein G
2. Low pH hold for retroviral inactivation
3. Anion exchange chromatography – CaptoQ chromatography, to remove DNA
4. Cation exchange chromatography – SP Sepharose FF chromatography, to remove aggregates

For removal of aggregates in small scale, size exclusion chromatography on a Superdex 200 column (GE Healthcare) can be alternatively used.

An example of the purification procedure is given subsequently for 3F2-based Fab-IL2-Fab. In a first step supernatant from transiently PEI-transfected HEK293 cells in Freestyle medium (Invitrogen) was adjusted to pH 7 and applied to a MabSelect protein A column (GE Healthcare), washed with 100 mM $NaPO_4$, 250 mM NaCl pH 7 and eluted with 8.8 mM sodium formiate pH 3. Selected fractions were exchanged in wash buffer and applied to a CaptoQ column (GE Healthcare), washed with 10 mM $NaPO_4$, 40 mM NaCl pH 6.5 and eluted with 2 M NaCl. The flowthrough was adjusted to pH 5 and applied to a SP Sepharose FF column (GE Healthcare), washed with 25 mM sodium acetate, 25 mM NaCl, pH 5 and eluted with 25 mM sodium acetate, 300 mM NaCl pH 5. Fractions were exchanged into final formulation buffer. Figure 27 shows an overview of the purification procedure.

The purified 3F2-based Fab-IL2-Fab was pure after purification (Figure 28A) and contained no aggregates (Figure 28B). The described purification procedure was applied to 4G8-based Fab-IL2-Fab. 4G8-based Fab-IL2-Fab behaved similarly to the 3F2-based Fab-IL2-Fab. The purified material was pure after purification and contained no aggregates (Figure 28, C-D).

Purification was performed for the Fab-IL2-Fab format with 2B10 (TNC A2 binder) as Fab fragment. In a first step supernatant from transiently PEI-transfected HEK293 cells in Freestyle medium (Invitrogen) was adjusted to pH 7 and applied to a protein G column (GE Healthcare), washed with 100 mM $NaPO_4$, 250 mM NaCl pH 7, and eluted with 8.8 mM Na formiate pH 3. Selected fractions were exchanged in wash buffer and applied to a CaptoQ column (GE Healthcare), washed with 10 mM $NaPO_4$, 40 mM NaCl pH 6.5 and eluted with 2 M NaCl. The flowthrough was adjusted to pH 5 and applied to a SP Sepharose FF column (GE Healthcare), washed with 25 mM sodium acetate, 25 mM NaCl, pH 5 and eluted with 25 mM sodium acetate, 300 mM NaCl pH 5. Fractions were exchanged into final formulation buffer.

Figure 29 shows the results from (A) the analytical characterization of the product by SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer, reduced and non-

reduced) and (B) analytical size exclusion chromatography of the product after each of the three purification steps. 2.3% aggregates were detected in the final product.

Example 15

Stability Testing

Stability testing was performed for the Fab-IL2-Fab format with the fibronectin Ectodomain-B binder L19 as Fab fragment. For stability tests the Fab-IL2-Fab construct was purified by protein A affinity chromatography with an elution step at pH 3, followed by size exclusion chromatography on a Superdex 200 column (GE Healthcare) as described. Three different buffers were tested and 20 mM histidine HCl, 140 mM NaCl, pH 6.0 was identified as a suitable buffer. Subsequently, L19 Fab-IL2-Fab was formulated in 20 mM HistidineHCl, 140 mM NaCl, pH 6.0 at a concentration of 6.3 mg/ml and stored for four weeks at room temperature and at 4°C. Figure 30 shows exemplary stability data: Probes were analyzed every week for concentration by UV spectroscopy (Figure 30A) (after centrifugation to pellet potential precipitated material) and for aggregate content by analytical size exclusion chromatography on a Superdex 200 column (Figure 30B). The results show that no aggregation and no degradation occured, when the construct was stored at 4°C or at room temperature for 28 days as well as after a freeze/thaw cycle at 6 mg/ml concentration. These data show that the Fab-IL2-Fab format is highly stable and behaves comparably to IgG antibodies.

Example 16

FAP-targeted Fab-IL2-Fab Binding Affinities by Surface Plasmon Resonance (Biacore)

Binding affinities of the three FAP-targeted Fab-IL2-Fab constructs, 3F2 Fab-IL2-Fab, 4G8 Fab-IL2-Fab and 3D9 Fab-IL2-Fab, were determined by Surface Plasmon Resonance.

For determination of FAP binding, FAP was captured by an immobilized anti-His antibody (Penta His, Qiagen #34660) and the constructs were used as analytes. Temperature of analysis was 25°C and Fab-IL2-Fab constructs were diluted 1:5 from 10 nM to 3.2 pM. The following measurement parameters were applied: Association time 180 s, dissociation 900 s, flow 90

µl/min. The chip was regenerated with 10 mM glycine pH 2 for 60 s. The curves were fitted with the 1:1 model to get the K_D values (Rmax local, RI=0).

To determine the affinity for IL-2 receptor (IL-2R) chains, the β and γ chains (b/g; knob-into-hole construct) or the α chain (a) of IL-2R were immoblized on the chip and the Fab-IL2-Fab constructs were used as analytes. Temperature of analysis was 25°C. Fab-IL2-Fab constructs 3F2 and 3D9 were diluted 1:2 from 25 nM to 0.78 nM and the following measurement parameters were applied: Association time 100 s, dissociation 180 s, flow 90 µl/min. Regeneration was done with 10 mM glycine pH 1.5 for 20 s. Fab-IL2-Fab constructs 4G8 were diluted from 100 nM to 3.125 nM and the following measurement parameters were applied: Association time 180 s, dissociation 180 s, flow 40 µl/min. Regeneration with 3 M $MgCl_2$ for 30 s.

Table 19 gives a summary of binding affinities of the 3F2 Fab-IL2-Fab, 4G8 Fab-IL2-Fab and 3D9 Fab-IL2-Fab immunoconjugates. Picomolar values of affinity reach the limit of detection of the Biacore. Figures 31-34 show the respective Biacore sensorgrams and affinities.

TABLE 19.

Summary of kinetic equilibrium constants (K_D) of FAP-targeted Fab-IL2-Fab constructs for FAP from different species and IL-2 receptor as determined by Surface Plasmon Resonance

Construct	Human FAP	Murine FAP	Cynomolgus FAP	Affinity to IL-2 receptor (b/g) Receptor immobilized	Affinity to IL-2 receptor (a) Receptor immobilized
Fab-IL2-Fab 3F2	Avidity: 25 pM	Avidity: 49 pM	Avidity: 24 pM	2.7 nM hu IL-2R bg	ND
Fab-IL2-Fab 4G8	Avidity: 83 pM	Avidity: 2.3 pM	Avidity: 74 pM	3.8 nM hu IL-2R bg 45.6 nM mu IL-2R bg	4.5 nM hu IL-2R a 29 nM mu IL-2R a
Fab-IL2-Fab 3D9	Avidity: 96 pM	Avidity: 63 pM	Avidity: 105 pM	2.7 nM hu IL-2R bg	ND

Example 17

TNC A2-targeted Fab-IL2-Fab Binding Affinities by Surface Plasmon Resonance (Biacore)

For determination of TNC A2 binding the biotinylated antigens (TNC fn5-A1-A2-A3 domains, fused together, expressed in *E.coli*. While the fn5 and the A3 domains are always of human

origin, A1 and A2 domains are human, murine or cynomolgus) were immobilized on a streptavidin chip and the immunoconjugate constructs were used as analytes. Temperature of analysis was 25°C. Fab-IL2-Fab were diluted 1:2 from 25 nM to 0.39 nM and the following measurement parameters were applied: Association time 180 s, dissociation 180 s, flow 50 µl/min. Regeneration with 10 mM glycine pH 1.5 for 60 s. The curves were fitted with the 1:1 model to get the K_D values. As a negative control, TNC domains 1 to 8 produced in HEK cells were applied (TNC 1-8 HEK).

To determine the affinity for IL-2 receptor (IL-2R) β and γ chains (b/g; knob-into-hole construct) the IL-2R construct was immoblized on the chip and the Fab-IL2-Fab immunoconjugates were used as analytes. Temperature of analysis was 25°C. Fab-IL2-Fab immunoconjugates were diluted 1:2 from 25 nM to 0.78 nM and the following measurement parameters were applied: Association time 100 s, dissociation 180 s, flow 90 µl/min. Regeneration was done with 10 mM glycine pH 1.5 for 20 s. Fab-IL2-Fab constructs were diluted from 100 nM to 3.125 nM and the following measurement parameters were applied: Association time 180 s, dissociation 180 s, flow 40 µl/min. Regeneration with 3 M $MgCl_2$ for 30 s. Table 20 gives a summary of binding affinities for the 2B10 Fab-IL2-Fab immunoconjugate, Figure 35 shows the respective Biacore sensorgrams and affinities.

TABLE 20.

Summary of kinetic equilibrium constants (K_D) of TNC A2-targeted Fab-IL2-Fab constructs for TNC A2 from different species and IL-2 receptor-β/γ as determined by Surface Plasmon Resonance

Construct	Human TNC	Murine TNC	Cynomolgus TNC	Affinity to hu IL-2 receptor (β/γ) Receptor immobilized
Fab-IL2-Fab 2B10 Immob. *E. coli* Antigen	Avidity: 0.12 nM	Avidity: 0.77 nM	Avidity: 0.12 nM	2.7 nM
TNC 1-8 HEK (neg. cont)	no binding			

Example 18

Biological Activity Assays with Targeted IL-2 Fab-IL2-Fab Immunoconjugates

The biological activity of targeted IL-2 Fab-IL2-Fab immunoconjugates was investigated in several cellular assays in comparison to IL-2 (Proleukin).

Induction of Proliferation of NK92 Cells

Targeted IL-2 Fab-IL2-Fab molecules recognizing TNC A2 (2B10) or FAP (3F2 and 4G8) were investigated for their potential to induce proliferation of NK92 cells in comparison to IL-2 (Proleukin) and the IL-2 L19 diabody recognizing Fibronectin-EDB.

2 µg/ml of human Tenascin, FAP or Fibronectin were coated over night. at 4°C in a 96-well flat bottom ELISA plate. After blocking the plate, the Fab-IL2-Fab constructs or the diabody were titrated into the plate and incubated for 90 min at room temperature (RT) for binding. After intensive washing to remove the unbound constructs, IL-2 starved NK92 cells (10000 cells/well) were added. As positive control, Proleukin was added in solution to some of the wells. The cells were incubated for 2 days at 37°C in a humidified incubator (with 5 % CO_2) before lysing the cells to determine proliferation by ATP measurement using the CellTiter Glo Kit (Promega).

The results in Figure 36 show that all Fab-IL2-Fab constructs were able to activate IL-2R signaling on NK92 cells and stimulate their proliferation. Due to the reduced binding affinity for the IL-2R β/γ heterodimer required for signaling, the potency of induction of cell growth was reduced by a factor of approximately 10 or more compared to IL-2 (Proleukin). However, the overall efficacy at higher doses was retained and comparable to IL-2 (Proleukin).

Induction of STAT5 Phosphorylation

In another experiment we tested the induction of STAT5 phosphorylation as a consequence of IL-2 mediated IL-2R signaling following incubation with an IL-2 Fab-IL2-Fab molecule recognizing FAP (based on 4G8) on different effector cell populations, including A) $CD56^+$ NK cells, B) $CD4^+CD25^-CD127^+$ helper T cells, C) $CD3^+$, $CD8^+$ cytotoxic T cells and D) $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs) from human PBMCs in solution.

PBMCs isolated from blood of healthy donors were treated for 20 min with different concentrations of Proleukin or Fab-IL2-Fab before fixing/permeabilizing them and staining them with anti-PhosphoSTAT5 antibody (Becton Dickinson) according to the instructions of the supplier. After intracellular staining of phosphorylated STAT5 as well as FOXP3, surface markers (CD3, CD4, CD8, CD56 and CD127) were stained for determination of the different subpopulations by flow cytometry (FACS Canto II).

The results in Figure 37 confirm the findings from Figure 36 and show that the 4G8 Fab-IL2-Fab construct was able to activate IL-2R signaling on various IL-2R positive effector cells and induce IL-2R downstream signaling and STAT5 phosphorylation. Due to the reduced binding affinity for the IL-2R β/γ heterodimer required for signaling, the potency of induction of STAT5 phosphorylation was reduced by a factor of approximately 10 or more compared to IL-2 (Proleukin). However, the overall efficacy at higher doses was retained and comparable to IL-2 (Proleukin).

IFN-γ Release and Proliferation Induction in Solution and upon Immobilization

In another experiment we aimed to mimic the situation as it will occur in a tumor where the targeted IL-2 immunoconjugate is bound and immobilized on the tumor cells or tumor stroma and can activate effector cells. In order to do this, we performed the IFN-γ release assay with NK92 cells as well as a proliferation assay with the immunoconjugates in solution or we coated microtiter plates with TNC or FAP antigen so that the targeted IL-2 immunoconjugates are immobilized upon binding to TNC or FAP.

2 µg/ml of human Tenascin or FAP were coated over night at 4°C in a 96-well flat bottom ELISA plate. After blocking the plate, the Fab-IL2-Fab constructs were titrated into the plate and incubated for 90 min at RT for binding. After intensive washing to remove the unbound constructs, IL-2 starved NK92 cells (10000 cells/well) were added. As positive control, Proleukin was added in solution to some of the wells. For determination of proliferation the cells were incubated for 2 days at 37°C in a humidified incubator (with 5 % CO_2) before lysing the cells to determine proliferation by ATP measurement using the CellTiter Glo Kit (Promega). The release of IFN-γ was measured in a separate approach after 24 h of incubation with Fab-IL-2-Fab in the supernatant of the cells with the human IFN-γ ELISA Kit from Becton Dickinson.

The results confirmed that all Fab-IL2-Fab constructs targeting FAP, TNC A1 or TNC A2 were able to activate IL-2R signaling on NK92 cells and induce proliferation (Figure 38A) of the cells as well as IFN-γ secretion (Figure 38C) when present in solution. Due to the reduced binding affinity for the IL-2R β/γ heterodimer required for signaling the potency of induction of IFN-γ release was reduced by a factor of approximately 10 or more compared to IL-2 (Proleukin). However, the overall efficacy at higher doses was retained and comparable to IL-2 (Proleukin).

If the microtiter plates were coated with FAP or TNC and the constructs immobilized on the plate, all Fab-IL2-Fab constructs targeting FAP, TNC A1 or TNC A2 were still able to activate IL-2R signaling on NK92 cells and induce cell growth (Figure 38B) and IFN-γ release (Figure

38D). In comparison to the assay performed in solution the difference of efficacy between un-coated IL-2 (Proleukin) and the immobilized Fab-IL2-Fab constructs was an order of magnitude higher, however, the overall efficacy at higher doses was retained and comparable to IL-2 (Proleukin).

These data strongly support the concept of generating targeted immunoconjugates with low systemic exposure, but accumulation at the site of disease where they mediate their function.

In the following example we investigated whether these *in vitro* properties translate into superior efficacy *in vivo* in xenograft models.

Example 19

In vivo Efficacy of Targeted Fab-IL2-Fab Immunoconjugates Against FAP and TNC A2 in Xenografts of Human Tumor Cell Lines

Targeted Fab-IL2-Fab immunoconjugates against FAP and TNC A2 were tested for their anti-tumoral efficacy in several xenograft models.

LS174T Xenograft Model

The TNC A2-targeted 2B10 Fab-IL2-Fab immunoconjugate was tested in the human colorectal LS174T cell line, intrasplenically injected into SCID mice.

LS174T cells (human colon carcinoma cells) were originally obtained from ECACC (European Collection of Cell Culture) and after expansion deposited in the Glycart internal cell bank. LS174T were cultured in MEM Eagle`s medium containing 10% FCS (PAA Laboratories, Austria), 1% Glutamax and 1% MEM Non-Essential Amino Acids (Sigma). The cells were cultured at 37 °C in a water-saturated atmosphere at 5 % CO_2. *In vitro* passage 15 was used for intrasplenic injection, at a viability of 92.8%. A small incision was made at the left abdominal site of anesthetized SCID/beige mice. Fifty microliters ($3x10^6$ LS174T cells in AimV medium) cell suspension was injected through the abdominal wall just under the capsule of the spleen. Skin wounds were closed using clamps.

Female SCID mice; aged 8-9 weeks at the start of the experiment (purchased from Taconics, Denmark) were maintained under specific-pathogen-free conditions with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (P 2008016). After

arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis.

Mice were injected intrasplenically on study day 0 with $3x10^6$ LS174T cells, randomized and weighed. One week after the tumor cell injection mice were injected i.v. TNC A2-targeted 2B10 Fab-IL2-Fab (Fab-IL2-Fab-SH2B10), Fibronectin-EDB targeted L19 IL-2-diabody, or Proleukin twice weekly for three weeks.

All mice were injected i.v. with 200 µl of the appropriate solution. The mice in the vehicle group were injected with PBS and the treatment groups with the Fab-IL2-Fab construct, the diabody, or Proleukin. To obtain the proper amount of immunoconjugate per 200 µl, the stock solutions were diluted with PBS when necessary.

Figure 39 shows that the TNC A2 targeted 2B10 Fab-IL2-Fab immunoconjugate mediated superior efficacy in terms of enhanced median survival compared to naked IL-2 (Proleukin) and the IL-2 diabody molecule targeting fibronectin-EDB.

TABLE 21.

Compound	**Dose**	**Formulation buffer**	**Concentration (mg/mL)**
L19 IL-2 diabody	12 µg	20 mM sodium citrate, 190 mM sucrose, 20 mM arginine, pH 6.5	2.00 (= stock solution)
Proleukin (IL-2)	4 µg	mannitol sodium laurylsulphate sodium phosphate	1.0 (= stock solution)
huTNC A2 2B10 (G65S) Fab-IL2-Fab = SH2B10	16 µg	25 mM potassium phosphate, 125 mM NaCl, 100 mM glycine, pH 6.7	1.86 (= stock solution)

ACHN Xenograft Model

The FAP-targeted 3F2 or 4G8 Fab-IL2-Fab immunoconjugates were tested in the human renal cell line ACHN, intrarenally injected into SCID mice.

ACHN cells (human renal adenocarcinoma cells) were originally obtained from ATCC (American Type Culture Collection) and after expansion deposited in the Glycart internal cell bank. ACHN were cultured in DMEM containing 10% FCS. The cells were cultured at 37°C in a

water-saturated atmosphere at 5 % CO_2. *In vitro* passage 9 was used for intrarenal injection, at a viability of 97.7%. A small incision (2 cm) was made at the right flank and peritoneal wall of anesthetized SCID mice. Fifty µl ($1x10^6$ ACHN cells in AimV medium) cell suspension was injected 2 mm subcapsularly in the kidney. Skin wounds and peritoneal wall were closed using clamps.

Female SCID mice; aged 8-9 weeks at the start of the experiment (purchased from Charles River, Sulzfeld, Germany) were maintained under specific-pathogen-free conditions with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (P 2008016). After arrival, animals were maintained for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on a regular basis.

Mice were injected intrarenally on study day 0 with $1x10^6$ ACHN cells, randomized and weighed. One week after the tumor cell injection, mice were injected i.v. with Proleukin, L19 IL-2 diabody or FAP-targeted 4G8 or 3F2 Fab-IL2-Fab immunoconjugates twice weekly for three weeks.

All mice were injected i.v. with 200 µl of the appropriate solution. The mice in the vehicle group were injected with PBS and the treatment groups with Proleukin, L19 IL-2 diabody or FAP-targeted 4G8 or 3F2 Fab-IL2-Fab immunoconjugates. To obtain the proper amount of immunoconjugate per 200 µl, the stock solutions were diluted with PBS when necessary.

Figure 40 shows that the FAP targeted 3F2 and 4G8 Fab-IL2-Fab immunoconjugates mediated superior efficacy in terms of enhanced median survival compared to naked IL-2 (Proleukin) and the IL-2 diabody molecule targeting fibronectin-EDB. The 4G8-based Fab-IL2-Fab, which has a higher affinity for murine FAP, mediated superior efficacy than 3F2-based Fab-IL2-Fab.

TABLE 22.

Compound	**Dose**	**Formulation buffer**	**Concentration (mg/mL)**
L19 IL-2 diabody	12 µg	20 mM sodium citrate, 190 mM sucrose, 20 mM arginine, pH 6.5	2.00 (= stock solution)
Proleukin (IL2)	4 µg	mannitol sodium laurylsulphate sodium phosphate	1.0 (= stock solution)
FAP 3F2 Fab-IL2-	16 µg	25 mM potassium phosphate, 125 mM NaCl,	2.46 (= stock solution)

Fab = FAP 3F2		100 mM glycine, pH 6.7	
FAP 4G8 Fab-IL2-Fab = FAP 4G8	16 µg	25 mM potassium phosphate, 125 mM NaCl, 100 mM glycine, pH 6.7	11.8 (= stock solution)

A549 Xenograft Model

The TNC A2-targeted 2B10 Fab-IL2-Fab immunoconjugate was tested in the human NSCLC cell line A549, injected i.v. into SCID-human FcγRIII transgenic mice.

The A549 non-small cell lung carcinoma cells were originally obtained from ATCC (CCL-185) and after expansion deposited in the Glycart internal cell bank. The tumor cell line was routinely cultured in DMEM containing 10 % FCS (Gibco) at 37 °C in a water-saturated atmosphere at 5% CO2. Passage 2 was used for transplantation, at a viability of 98%. $2x10^6$ cells per animal were injected i.v. into the tail vein in 200 µl of Aim V cell culture medium (Gibco).

Female SCID-FcγRIII mice (GLYCART-RCC), aged 8-9 weeks at the start of the experiment (bred at RCC, Switzerland) were maintained under specific-pathogen-free condition with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (P 2008016). After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis.

Mice were injected i.v. on study day 0 with $5x10^6$ of A549 cells, randomized and weighed. One week after the tumor cell injection, mice were injected i.v. with 2B10 Fab-IL2-Fab or L19 IL-2 diabody twice weekly for three weeks.

All mice were injected i.v. with 200 µl of the appropriate solution. The mice in the vehicle group were injected with PBS and the treatment group with the 2B10 Fab-IL2-Fab construct or the L19 IL-2 diabody. To obtain the proper amount of immunoconjugate per 200 µl, the stock solutions were diluted with PBS when necessary.

Figure 41 shows that the TNC A2 targeted 2B10 Fab-IL2-Fab immunoconjugate mediated superior efficacy in terms of enhanced median survival compared to the IL-2 diabody molecule targeting fibronectin-EDB.

TABLE 23.

Compound	Dose	Formulation buffer	Concentration (mg/mL)
L19 IL-2 diabody	12 µg	20 mM sodium citrate, 190 mM sucrose, 20 mM arginine, pH 6.5	2.00 (= stock solution)
huTNC A2 2B10 (G65S) Fab-IL2-Fab = 2B10	16 µg	25 mM potassium phosphate, 125 mM NaCl, 100 mM glycine, pH 6.7	1.86 (= stock solution)

Example 20

Purification of Targeted GM-CSF Fab-GM-CSF-Fab Immunoconjugate

Initial purification of the Fab-GM-CSF-Fab immunoconjugate with L19 (Fibronectin Ectodomain-B binder) as Fab fragment was performed from supernatants of transiently transfected HEK 293 EBNA cells. Briefly, Fab-GM-CSF-Fab was purified by protein A followed by size exclusion chromatography. The Protein A column was equilibrated in 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5. The supernatant was loaded and the column washed first with 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5, followed by 20 mM sodium phosphate, 20 mM sodium citrate, 100 mM sodium chloride, pH 7.5. Targeted GM-CSF was eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine pH 3 and subsequently neutralized. For formulation the following buffer was applied: 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine pH 6.7.

Figure 42 shows the elution profiles from the purification and the results from the analytical characterization of the product by SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer, reduced and non-reduced). The yield was 4.8 mg/L.

Example 21

Biological Activity Assay with Targeted GM-CSF Fab-GM-CSF-Fab Immunoconjugate

The purified Fab-GM-CSF-Fab immunoconjugate with L19 (Fibronectin Ectodomain-B binder) as Fab was subsequently analyzed in a GM-CSF-dependent proliferation assay. Briefly, TF-1 cells, which grow GM-CSF-dependent, were seeded after over night starvation at 10000

cells/well into a 96-well flat bottom plate. Human recombinant GM-CSF (Miltenyi #130-093-862) or Fab-GM-CSF-Fab immunoconjugate were titrated onto the cells in solution. After 2 days of proliferation at 37°C in a humidified incubator with 5 % CO_2, the cells were lysed and ATP content was measured with the CellTiter Glo assay from Promega. GM-CSF-untreated cells were set as 0% growth for calculation. Results in Figure 43 show that the Fab-GM-CSF-Fab immunoconjugate induced strong proliferation of TF-1 cells.

Example 22

Purification of Targeted-IL-12 Fab-IL12-Fab Immunoconjugate

Initial purification of the Fab-IL12-Fab immunoconjugate with 4G8 (FAP binder) as Fab fragment was performed from supernatants of transiently transfected HEK 293 EBNA cells. Briefly, Fab-IL12-Fab was purified by protein A followed by size exclusion chromatography. The protein A column was equilibrated with 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5. Supernatant was loaded and the column washed with 20 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride pH 7.5. A second wash was performed with 13.3 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, pH 5.45. After a third wash with 10 mM MES, 50 mM sodium chloride pH 5, targeted IL-12 was eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine, pH 3 and subsequently neutralized. For formulation the following buffer was applied: 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine pH 6.7.

Figure 44 shows the elution profiles from the purification and the results from the analytical characterization of the product by SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer, reduced and non-reduced). The yield was 43 mg/L.

Example 23

Biological Activity Assay with Targeted-IL-12 Fab-IL12-Fab Immunoconjugate

The purified Fab-IL12-Fab immunoconjugate with 4G8 (FAP binder) as Fab was subsequently analyzed for IL-12 induced IFN-γ release, comparing the effect of IL-12 and the purified 4G8 Fab-IL12-Fab immunoconjugate, using PBMCs isolated from fresh human blood of a healthy donor.

Briefly, PBMCs were isolated from fresh human blood of a healthy donor and seeded in a 96-well U-bottom plate ($1.5x10^5$ cells/well) in AIM V medium. A constant concentration of 10 ng/ml hu IL-2 (Peprotech) was added to all wells. The Fab-IL12-Fab construct was diluted in medium and titrated onto the PBMCs. Supernatants were collected after approximately 20 hours to determine the IFN-γ concentrations using the hu IFN-γ ELISA Kit II from Becton Dickinson (#550612).

Results in Figure 45 show that A) the chosen amount of human (hu) IL-2 alone as well as IL-12 alone were not able to induce significant IFN-γ release by human PBMCs whereas the combination of both cytokines led to significant IFN-γ release by PBMCs. B) The Fab-IL-12-Fab construct induced IFN-γ release by human PBMCs in a concentration-dependent manner in the presence of 10 ng/ml human IL-2.

Example 24

Purification of Targeted IFN-α Fab-IFNα2-Fab Immunoconjugate

Initial purification of the Fab-IFNα2-Fab immunoconjugate with L19 (Fibronectin Ectodomain-B binder) as Fab fragment was performed from supernatants of transiently transfected HEK 293 EBNA cells. Briefly, Fab-IFNα2-Fab was purified by protein A followed by size exclusion chromatography. The protein A column was equilibrated in 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5. The supernatant was loaded and the column washed first with 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5, followed by 20 mM sodium phosphate, 20 mM sodium citrate, 100 mM sodium chloride, pH 7.5. Fab-IFNα2-Fab was eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine pH 3 and subsequently neutralized. For formulation the following buffer was applied: 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine pH 6.7.

Figure 46 shows the elution profiles from the purification and the results from the analytical characterization of the product by SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer, reduced and non-reduced). The yield was 8.4 mg/L.

Example 25

Biological Activity Assay with IFN-α Fab-IFNα2-Fab Immunoconjugate

The purified Fab-IFNα2-Fab immunoconjugate with L19 (Fibronectin Ectodomain-B binder) as Fab was subsequently analyzed for IFN-α-induced proliferation inhibition of Jurkat T cells and A549 tumor cells, comparing the effect of IFN-α (Roferon A, Roche) and the purified L19 Fab-IFNα2-Fab immunoconjugate. Briefly, A549 and Jurkat T cells which are susceptible for IFN-α-induced proliferation inhibition were seeded at 5000 cells/well (A549) or 10000 cells/well (Jurkat) into 96-well flat bottom plates. Dilutions of Roferon A (Roche) or Fab-IFNα2-Fab in the appropriate cell culture medium were titrated onto the cells in solution. After two days of proliferation at 37°C in a humidified incubator with 5 % CO_2, the cells were lysed and ATP content was measured with the CellTiter Glo assay from Promega. IFN-α-untreated cells were set as 0% growth for calculation.

Results in Figure 47 show that Fab-IFNα2-Fab constructs inhibited proliferation of A) Jurkat T cells and B) A549 cells in a concentration-dependent manner comparable to IFN-α (Roferon A).

Example 26

Preparation of MCSP targeted Fab-IL2-Fab Immunoconjugates

The humanized anti-MCSP MHLG antibody was generated as described in WO 2006/100582 (see in particular Example 1 therein), the entire contents of which are incorporated herein by reference, and converted into the Fab-IL2-Fab format (see SEQ ID NOs: 255, 256, 261, 262).

The humanized anti-MCSP MHLG1 antibody was generated as follows: The murine amino acid sequence of anti-MCSP antibody 225.28 (light chain, and heavy chain, see below) was aligned to a collection of human germ-line antibody V-genes, and sorted according to sequence identity and homology.

225.28 light chain; GenBank Acc. No. CAA65007 (SEQ ID 267):

DIELTQSPKFMSTSVGDRVSVTCKASQNVDTNVAWYQQKPGQSPEPLLFSASYRYTGVP
DRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYNSYPLTFGGGTKLEIK

225.28 heavy chain; no GenBank Acc. No. available (SEQ ID 268):

QVKLQQSGGGLVQPGGSMKLSCVVSGFTFSNYWMNWVRQSPEKGLEWIAEIRLKSNNF
GRYYAESVKGRFTISRDDSKSSAYLQMINLRAEDTGIYYCTSYGNYVGHYFDHWGQGT
TVTVSS

The potential acceptor sequence was selected based on high overall homology, and the presence of the right canonical residues already in the acceptor sequence. The human germ-line sequence IGHV3-15 (IMGT Acc. No. X92216) was chosen as the acceptor for the heavy chain and sequence IGKV1-9 (IMGT Acc. No. Z00013) was chosen for the light chain. The humanized constructs were denoted M-KV1 (see SEQ ID NOs: 263, 264, 269, 270), 7 (SEQ ID NOs: 265, 266, 271, 272), and 9 (SEQ ID NOs: 253, 254, 259, 260) for the light chain, and MHLG1 (see SEQ ID NOs: 251, 252, 257, 258), for the heavy chain.

The genes for those designed antibody sequences were generated by conventional PCR techniques and fused to human IgG1 and kappa constant domains for the construction of the expression plasmids.

Antibodies were expressed either as IgG or as Fab-IL2-Fab fusion proteins in mammalian cell culture systems like HEK or CHO, and purified via protein A and size exclusion chromatography. Comparison of the binding data of light chain variants M-KV1, and M-KV7 revealed that a proline residue at Kabat position 46 is essential for functional binding to the antigen. Two different approaches were taken to ensure the presence of this amino acid: A) A so-called back-mutation was introduced into the human framework of IGKV1-9. And B) To avoid the presence of back-mutations, the entire framework 2 region (Kabat positions 35 to 49) was exchanged by the corresponding region of the human antibody with GenBank entry AAA17574. This antibody has naturally a proline residue at position 46.

MCSP-targeted MHLG or MHLG1 KV9 Fab-IL2-Fab was purified by the method described above (Example 9) composed of one affinity step (protein A) followed by size exclusion chromatography (Superdex 200, GE Healthcare). The protein A column was equilibrated in 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5, supernatant was loaded and the column was washed with 20 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, pH 7.5, followed by a wash with 13.3 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, pH 5.45. A third wash with 10 mM MES, 50 mM sodium chloride, pH 5 was optionally included. Fab-IL2-Fab was eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine, pH 3. The eluted fractions were pooled and polished by size exclusion chromatography in the final formulation buffer: 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine pH 6.7.

Figure 48 (for MHLG Fab-IL2-Fab) and Figure 49 (for MHLG1 Fab-IL2-Fab) show (A) the elution profiles from the purification and (B) the results from the analytical characterization of

the MCSP-targeted MHLG or MHLG1 Fab-IL2-Fab by SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer, reduced and non-reduced).

Example 27

Biological Activity Assay with MCSP targeted Fab-IL2-Fab Immunoconjugates

The purified Fab-IL2-Fab immunoconjugates with MHLG KV9 or MHLG1 KV9 (MCSP binders) as Fab were subsequently analyzed for IL-2 induced IFN-γ release, comparing the effect of purified 4G8 Fab-IL2-Fab (FAP binder) and MHLG or MHLG1 Fab-IL2-Fab on NK92 cells. IL-2 starved NK92 cells (pre-incubated for 2 hours without IL-2) were seeded in 96-well U bottom plates (10^5 cells/well) in NK cell medium (MEMa + 10 % FCS + 10 % horse serum + 0.1 mM 2-mercaptoethanol + 0.2 mM inositol + 0.02 mM folic acid). The MCSP-targeted Fab-IL-2-Fab immunoconjugates were diluted in NK cell medium and titrated onto the NK92 cells in direct comparison to the FAP-targeted 4G8-based Fab-IL2-Fab immunoconjugate. Supernatants were collected after 22 to 24 hours to determine the IFN-γ concentrations using the human IFN-γ ELISA Kit II from Becton Dickinson (#550612).

The results in Figure 50 (for MHLG KV9 Fab-IL2-Fab) and Figure 51 (for MHLG1 KV9 Fab-IL2-Fab) show that all Fab-IL2-Fab immunoconjugates, targeted against MCSP or FAP, induced comparable IFN-γ secretion in NK92 cells in a concentration dependent manner, independent of the antigen binding moiety used.

Example 28

Cellular Binding Assay with the MCSP-targeted MHLG1 KV9 Fab-IL2-Fab Immunoconjugate

The purified MCSP-targeted MHLG1-KV9 Fab-IL2-Fab immunoconjugate was tested by flow cytometry for binding to human MCSP-expressing Colo38 melanoma cells. Briefly, cells were harvested, counted and checked for viability. Cells were adjusted to 1.112 x 10^6 (viable) cells/ml in PBS/0.1 % BSA and aliquoted 180 µl/well (200'000 cells/well) in a round-bottom 96-well plate. 20 µl MHLG1 KV9 Fab-IL2-Fab immunocytokine (in different dilutions) was added to the cell containing wells and incubated for 30 min at 4°C. Cells were subsequently collected by centrifugation (4 min, 400 x g), washed with 150 µl/well PBS/0.1 % BSA, resuspended and

incubated for 30 min at 4°C with 12 µl/well secondary antibody (FITC-conjugated AffiniPure F(ab')2 Fragment goat anti-human F(ab')2 (Jackson Immuno Research Lab #109-096-097), dissolved in 1.5 ml of a 1:1 mixture of water and glycerol = stock solution), diluted 1:20 in PBS/0.1% BSA. Cells were subsequently washed in 150 µl/well PBS/0.1% BSA, followed by a washing step in PBS, collected by centrifugation (4 min, 400 x g), and resuspended with 200 µl/well PBS/0.1% BSA containing propidium iodide (PI). Measurements were performed using a FACSCantoII machine (Software FACS Diva). Results are presented in Figure 52, which shows that the MCSP-targeted MHLG1 KV9 Fab-IL2-Fab immunoconjugate bound very well, in a dose-dependent manner, to Colo38 cells.

* * *

It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections may set forth one or more but not all exemplary embodiments of the present invention as contemplated by the inventor(s), and thus, are not intended to limit the present invention and the appended claims in any way.

The present invention has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

Claims

1. An immunoconjugate comprising:

(a) at least a first single-chain effector moiety; and

(b) a first and a second antigen binding moiety independently selected from the group consisting of an Fv and an Fab,

wherein said first effector moiety shares an amino- or carboxy-terminal peptide bond with said first antigen binding moiety; and

wherein said second antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either i) said first effector moiety or ii) said first antigen binding moiety.

2. The immunoconjugate of claim 1, wherein said immunoconjugate consists essentially of a first effector moiety and first and second antigen binding moieties joined by one or more linker sequences.

3. The immunoconjugate of claim 1 or 2, wherein each of said first and second antigen binding moieties is a Fab molecule.

4. The immunoconjugate of claim 3, wherein said first Fab molecule is joined at its heavy or light chain carboxy-terminal amino acid to the amino-terminal amino acid of the heavy or light chain of the second Fab molecule.

5. The immunoconjugate of claim 4, wherein said second Fab molecule is joined at its heavy or light chain carboxy-terminal amino acid to the amino-terminal amino acid of said effector moiety.

6. The immunoconjugate of claim 4, wherein said effector moiety is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of said first Fab molecule.

7. The immunoconjugate of claim 3, wherein said first Fab molecule is joined at its heavy or light chain carboxy-terminus to the amino-terminal amino acid of said effector moiety.

8. The immunoconjugate of claim 7, wherein said effector moiety is joined at its carboxy-terminal amino acid to the terminal amino acid of the heavy or light chain of the second Fab molecule.

9. An immunoconjugate comprising a first single-chain effector moiety joined at its amino-terminal amino acid to one or more scFv molecules; and

wherein said first single-chain effector moiety is joined at its carboxy-terminal amino acid to one or more scFv molecules.

10. An immunoconjugate comprising:

a) at least a first single-chain effector moiety; and

b) first and second antigen binding moieties, wherein each of said antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising an immunoglobulin constant domain independently selected from the group consisting of: IgG CH1, IgG C_{kappa}, and IgE CH4;

wherein said first antigen binding moiety is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of said first effector moiety; and

wherein said first and second antigen binding moieties are covalently linked through at least one disulfide bond.

11. The immunoconjugate of claim 10, wherein each of said antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising an IgG CH1 domain.

12. The immunoconjugate of claim 10, wherein each of said antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising an IgE CH4 domain.

13. The immunoconjugate of claim 10, wherein each of said antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising an IgG C_{kappa} domain.

14. The immunoconjugate of claim 10, wherein said first antigen binding moiety comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising an IgG CH1 domain, and wherein said second antigen binding moiety comprises an scFv molecule joined at its carboxy-terminal amino acid to a constant region comprising an IgG C_{kappa} domain.

15. An immunoconjugate comprising:

a) at least a first single-chain effector moiety; and

b) first and second antigen binding moieties, wherein each of said antigen binding moieties comprises an scFv molecule joined at its carboxy-terminal amino acid to an IgG CH3 domain;

wherein said first antigen binding moiety is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of said first effector moiety; and

wherein said first and second antigen binding moieties are covalently linked through at least one disulfide bond.

16. The immunoconjugate of any one of claims 10-15, wherein the constant region of said first antigen binding moiety is covalently linked to the constant region of said second antigen binding moiety through at least one disulfide bond.

17. The immunoconjugate of claim 16, wherein the immunoglobulin domain of said first antigen binding moiety is covalently linked to the immunoglobulin domain of said second antigen binding moiety through a disulfide bond.

18. The immunoconjugate of claim 16, wherein at least one disulfide bond is located carboxy-terminal of the immunoglobulin domains of said first and second antigen binding moieties.

19. The immunoconjugate of claim 16, wherein at least one disulfide bond is located amino-terminal of the immunoglobulin domains of said first and second antigen binding moieties.

20. The immunoconjugate of claim 19, wherein at least two disulfide bonds are located amino-terminal of the immunoglobulin domains of said first and second antigen binding moieties.

21. The immunoconjugate of claim 10, wherein said immunoconjugate further comprises a second effector moiety and wherein said second antigen binding moiety is joined at its constant region carboxy-terminal amino acid to the amino-terminal amino acid of said second effector moiety.

22. An immunoconjugate comprising:

a) first and second single-chain effector moieties; and

b) first and second antigen binding moieties, wherein each of said antigen binding moieties comprises an Fab molecule joined at its heavy or light chain carboxy-terminal amino acid to an IgG1 CH3 domain;

wherein each of said IgG1 CH3 domains is joined at its carboxy-terminal amino acid to the amino-terminal amino acid of one of said effector moieties; and

wherein said first and second antigen binding moieties are covalently linked through at least one disulfide bond.

23. The immunoconjugate of claim 22, wherein said IgG1 CH3 domains are joined by a single disulfide bond.

24. The immunoconjugate of claim 22 or claim 23, wherein at least one disulfide bond is located carboxy-terminal of the IgG1 CH3 domains of said first and second antigen binding moieties.

25. The immunoconjugate of claim 22 or claim 23, wherein at least one disulfide bond is located amino-terminal of the IgG1 CH3 domains of said first and second antigen binding moieties.

26. The immunoconjugate of claim 25, wherein at least two disulfide bonds are located amino-terminal of the IgG1 CH3 domains of said first and second antigen binding moieties.

27. The immunoconjugate of any one of claims 1-26, wherein proteolytic cleavage sites are located between said antigen binding moieties and said effector moieties.

28. The immunoconjugate of any one of claims 1-26, wherein the variable regions of said first and second antigen binding moieties are specific for the same antigen.

29. The immunoconjugate of any one of claims 1-26, wherein the variable region of said first and second antigen binding moieties are specific for different antigens.

30. The immunoconjugate of claim 28, wherein said first and second antigen binding moieties are specific for the Extra Domain B of fibronectin (EDB).

31. The immunoconjugate of claim 29, wherein said first or said second antigen binding moiety is specific for the Extra Domain B of fibronectin (EDB).

32. The immunoconjugate of claim 30 or claim 31, wherein said one or two antigen binding moieties which are specific for EDB compete with the monoclonal antibody L19 for binding to an epitope of EDB.

33. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 95.

34. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 108.

35. The immunoconjugate of claim 34, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 108.

36. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 104.

37. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 117.

38. The immunoconjugate of claim 37, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 117.

39. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 105.

40. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 118.

41. The immunoconjugate of claim 40, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 118.

42. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 106.

43. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 119.

44. The immunoconjugate of claim 43, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 119.

45. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 107.

46. The immunoconjugate of claim 30 or claim 32, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 120.

47. The immunoconjugate of claim 46, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 120.

48. The immunoconjugate of any one of claims 30 to 32, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 96.

49. The immunoconjugate of any one of claims 30 to 32, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 109.

50. The immunoconjugate of claim 49, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 109.

51. The immunoconjugate of any one of claims 33-47, wherein said immunoconjugate further comprises the polypeptide sequence of SEQ ID NO: 96.

52. The immunoconjugate of claim 28, wherein said first and second antigen binding moieties are specific for the A1 domain of Tenascin (TNC-A1).

53. The immunoconjugate of claim 29, wherein said first or said second antigen binding moiety is specific for the A1 domain of Tenascin (TNC-A1).

54. The immunoconjugate of claim 52 or 53, wherein said one or two antigen binding moieties which are specific for TNC-A1 compete with the monoclonal antibody F16 for binding to an epitope of TNC-A1.

55. The immunoconjugate of claim 52 or claim 54, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 99.

56. The immunoconjugate of claim 52 or claim 54, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 112.

57. The immunoconjugate of claim 56, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 112.

58. The immunoconjugate of claim 52 or claim 54, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 100.

59. The immunoconjugate of claim 52 or claim 54, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide having at least 80% identity to SEQ ID NO: 113.

60. The immunoconjugate of claim 59, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 113.

61. The immunoconjugate of any one of claims 52 to 54, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 101.

62. The immunoconjugate of any one of claims 52 to 54, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 114.

63. The immunoconjugate of claim 62, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 114.

64. The immunoconjugate of any one of claims 58 to 60, wherein said immunoconjugate further comprises the polypeptide sequence of SEQ ID NO: 101.

65. The immunoconjugate of claim 52, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 215.

66. The immunoconjugate of claim 52, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide having at least 80% identity to SEQ ID NO: 216.

67. The immunoconjugate of claim 66, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 216.

68. The immunoconjugate of claim 52 or 53, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 235.

69. The immunoconjugate of claim 52 or 53, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 236.

70. The immunoconjugate of claim 69, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 236.

71. The immunoconjugate of any one of claims 65 to 67, wherein said immunoconjugate further comprises the polypeptide sequence of SEQ ID NO: 235.

72. The immunoconjugate of claim 52 or claim 53, wherein said immunoconjugate comprises a heavy chain variable region sequence of SEQ ID NO: 13 or SEQ ID NO: 15.

73. The immunoconjugate of claim 52 or claim 53, wherein said immunoconjugate comprises a light chain variable region sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

74. The immunoconjugate of claim 72, wherein said immunoconjugate further comprises a light chain variable region sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

75. The immunoconjugate of claim 72, wherein said heavy chain variable region sequence is encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 14 or SEQ ID NO: 16.

76. The immunoconjugate of claim 75, wherein said heavy chain variable region sequence is encoded by the polynucleotide sequence of SEQ ID NO: 14 or SEQ ID NO: 16.

77. The immunoconjugate of claim 73, wherein said light chain variable region sequence is encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 10 or SEQ ID NO: 12.

78. The immunoconjugate of claim 77, wherein said light chain variable region sequence is encoded by the polynucleotide sequence of SEQ ID NO: 10 or SEQ ID NO: 12.

79. The immunoconjugate of claim 28, wherein said first and second antigen binding moieties are specific for the A2 domain of Tenascin (TNC-A2).

80. The immunoconjugate of claim 29, wherein said first or said second antigen binding moiety is specific for the A2 domain of Tenascin (TNC-A2).

81. The immunoconjugate of claim 79 or 80, wherein said immunoconjugate comprises a heavy chain variable region sequence selected from the group of SEQ ID NO: 7, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 187, SEQ ID NO: 191, SEQ ID NO:195, SEQ ID NO: 199, SEQ ID NO: 203, and SEQ ID NO: 207.

82. The immunoconjugate of claim 79 or 80, wherein said immunoconjugate comprises a light chain variable region sequence selected from the group of SEQ ID NO: 3, SEQ

ID NO: 5; SEQ ID NO: 177, SEQ ID NO: 181, SEQ ID NO:185, SEQ ID NO: 189, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 201, and SEQ ID NO: 205.

83. The immunoconjugate of claim 81, wherein said immunoconjugate further comprises a light chain variable region sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 5; SEQ ID NO: 177, SEQ ID NO: 181, SEQ ID NO:185, SEQ ID NO: 189, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 201, SEQ ID NO: 205.

84. The immunoconjugate of claim 81, wherein said heavy chain variable region sequence is encoded by a polynucleotide sequence having at least 80% identity to a sequence selected from the group of SEQ ID NO: 8, SEQ ID NO: 180, SEQ ID NO: 184, SEQ ID NO: 188, SEQ ID NO: 192, SEQ ID NO: 196, SEQ ID NO: 200, SEQ ID NO: 204, and SEQ ID NO: 208.

85. The immunoconjugate of claim 84, wherein said heavy chain variable region sequence is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 8, SEQ ID NO: 180, SEQ ID NO: 184, SEQ ID NO: 188, SEQ ID NO: 192, SEQ ID NO: 196, SEQ ID NO: 200, SEQ ID NO: 204, and SEQ ID NO: 208.

86. The immunoconjugate of claim 82, wherein said light chain variable region sequence is encoded by a polynucleotide sequence having at least 80% identity to a sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 186, SEQ ID NO: 190, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 202, and SEQ ID NO: 206.

87. The immunoconjugate of claim 86, wherein said light chain variable region sequence is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 186, SEQ ID NO: 190, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 202, and SEQ ID NO: 206.

88. The immunoconjugate of claim 79, wherein said immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: SEQ ID NO: 239, SEQ ID NO: 241, and SEQ ID NO: 243.

89. The immunoconjugate of claim 79, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide having at least 80% identity to a sequence selected from the group of SEQ ID NO: 240, SEQ ID NO: 242, and SEQ ID NO: 244.

90. The immunoconjugate of claim 89, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 240, SEQ ID NO: 242, and SEQ ID NO: 244.

91. The immunoconjugate of claim 79 or 80, wherein said immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 245, SEQ ID NO: 247, and SEQ ID NO:249.

92. The immunoconjugate of claim 79 or 80, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to a sequence selected from the group of SEQ ID NO: 246, SEQ ID NO: 248, and SEQ ID NO: 250.

93. The immunoconjugate of claim 92, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 246, SEQ ID NO: 248, and SEQ ID NO: 250.

94. The immunoconjugate of any one of claims 88 to 90, wherein said immunoconjugate further comprises a polypeptide sequence selected from the group of SEQ ID NO: 245, SEQ ID NO: 247, and SEQ ID NO:249.

95. The immunoconjugate of claim 28, wherein said first and second antigen binding moieties are specific for Fibroblast Activated Protein (FAP).

96. The immunoconjugate of claim 29, wherein said first or said second antigen binding moiety is specific for the Fibroblast Activated Protein (FAP).

97. The immunoconjugate of claim 95 or 96, wherein said immunoconjugate comprises a heavy chain variable region sequence selected from the group consisting of: SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 31, SEQ ID NO: 35, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151, SEQ ID NO: 155, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171, and SEQ ID NO: 175.

98. The immunoconjugate of claim 95 or 96, wherein said immunoconjugate comprises a light chain variable region sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 29, SEQ ID NO: 33, SEQ ID NO: 37,

SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 49, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149, SEQ ID NO: 153, SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169, and SEQ ID NO: 173.

99. The immunoconjugate of claim 97, wherein said immunoconjugate further comprises a light chain variable region sequence selected from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, SEQ ID NO: 29, SEQ ID NO: 33, SEQ ID NO: 37, SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 49, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149, SEQ ID NO: 153, SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169, and SEQ ID NO: 173.

100. The immunoconjugate of claim 97, wherein said heavy chain variable region sequence is encoded by a polynucleotide sequence having at least 80% identity to a sequence selected from the group consisting of: SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, SEQ ID NO: 156, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172, and SEQ ID NO: 176.

101. The immunoconjugate of claim 100, wherein said heavy chain variable region sequence is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, SEQ ID NO: 156, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172, and SEQ ID NO: 176.

102. The immunoconjugate of claim 98, wherein said light chain variable region sequence is encoded by a polynucleotide sequence having at least 80% identity to a sequence

selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 24, SEQ ID NO: 30, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170, and SEQ ID NO: 174.

103. The immunoconjugate of claim 102, wherein said light chain variable region sequence is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 24, SEQ ID NO: 30, SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170, and SEQ ID NO: 174.

104. The immunoconjugate of claim 95, wherein said immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, and SEQ ID NO: 227.

105. The immunoconjugate of claim 95, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide having at least 80% identity to a sequence selected from the group of SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, and SEQ ID NO: 228.

106. The immunoconjugate of claim 105, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, and SEQ ID NO: 228.

107. The immunoconjugate of claim 95 or 96, wherein said immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, and SEQ ID NO: 237.

108. The immunoconjugate of claim 95 or 96, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to a sequence selected from the group of SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, and SEQ ID NO: 238.

109. The immunoconjugate of claim 108, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, and SEQ ID NO: 238.

110. The immunoconjugate of claim 95, wherein said immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 211, SEQ ID NO: 219, and SEQ ID NO:221, and a polypeptide sequence of SEQ ID NO: 231.

111. The immunoconjugate of claim 95, wherein said immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 209, SEQ ID NO: 223, SEQ ID NO: 225 and SEQ ID NO:227, and a polypeptide sequence of SEQ ID NO: 229.

112. The immunoconjugate of claim 95, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 213 and the polypeptide sequence of SEQ ID NO: 233.

113. The immunoconjugate of claim 95, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 217 and the polypeptide sequence of SEQ ID NO: 237.

114. The immunoconjugate of claim 28, wherein said first and second antigen binding moieties are specific for Melanoma Chondroitin Sulfate Proteoglycan (MCSP).

115. The immunoconjugate of claim 29, wherein said first or said second antigen binding moiety is specific for Melanoma Chondroitin Sulfate Proteoglycan (MCSP).

116. The immunoconjugate of claim 114 or 115, wherein said immunoconjugate comprises a heavy chain variable region sequence of SEQ ID NO: 257 or SEQ ID NO: 261.

117. The immunoconjugate of claim 114 or 115, wherein said immunoconjugate comprises a light chain variable region sequence of SEQ ID NO: 259 or SEQ ID NO: 271.

118. The immunoconjugate of claim 116, wherein said immunoconjugate further comprises a light chain variable region sequence of SEQ ID NO: 259 or SEQ ID NO: 271.

119. The immunoconjugate of claim 116, wherein said heavy chain variable region sequence is encoded by a polynucleotide sequence having at least 80% identity to the sequence of SEQ ID NO: 258 or SEQ ID NO: 262.

120. The immunoconjugate of claim 119, wherein said heavy chain variable region sequence is encoded by the polynucleotide sequence of SEQ ID NO: 258 or SEQ ID NO: 262.

121. The immunoconjugate of claim 117, wherein said light chain variable region sequence is encoded by a polynucleotide sequence having at least 80% identity to the sequence of SEQ ID NO: 260 or SEQ ID NO: 272.

122. The immunoconjugate of claim 121, wherein said light chain variable region sequence is encoded by the polynucleotide sequence of SEQ ID NO: 260 or SEQ ID NO: 272.

123. The immunoconjugate of claim 114, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 251 or SEQ ID NO: 255.

124. The immunoconjugate of claim 114, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide having at least 80% identity to SEQ ID NO: 252 or SEQ ID NO: 256.

125. The immunoconjugate of claim 124, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 252 or SEQ ID NO: 256.

126. The immunoconjugate of claim 114 or 115, wherein said immunoconjugate comprises the polypeptide sequence of SEQ ID NO:253 or SEQ ID NO: 265.

127. The immunoconjugate of claim 114 or 115, wherein said immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence having at least 80% identity to SEQ ID NO: 254 or SEQ ID NO: 266.

128. The immunoconjugate of claim 127, wherein said immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 254 or SEQ ID NO: 266.

129. The immunoconjugate of any one of claims 123-125, wherein said immunoconjugate further comprises the polypeptide sequence of SEQ ID NO:253 or SEQ ID NO: 265.

130. The immunoconjugate of any one of claims 1-28, wherein the variable regions of said first and second antigen binding moieties are specific for a cell surface antigen of a cancer cell or a virus-infected cell or for an ECM molecule expressed in a tumor.

131. The immunoconjugate of claim 130, wherein said cell surface antigen is a tumor associated antigen or a viral antigen.

132. The immunoconjugate of any one of claims 1-20, wherein said immunoconjugate has only one effector moiety; and wherein said effector moiety is a cytokine.

133. The immunoconjugate of claim 132, wherein said cytokine is selected from the group consisting of: interleukin-2 (IL-2), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon-α (INF-α), and interleukin-12 (IL-12).

134. The immunoconjugate of claim 133, wherein said cytokine is IL-2.

135. The immunoconjugate of claim 133, wherein said cytokine is IL-12.

136. The immunoconjugate of any one of claims 132-135, wherein said cytokine elicits a cytotoxic response by cells bearing a receptor for said cytokine.

137. The immunoconjugate of any one of claims 132-135 wherein said cytokine elicits a proliferative response by cells bearing a receptor for said cytokine.

138. The immunoconjugate of any one of claims 1 or 10-26, wherein said immunoconjugate has two effector moieties; and wherein said effector moieties are cytokines.

139. The immunoconjugate of claim 138, wherein each of said cytokines are independently selected from the group consisting of: IL-2, GM-CSF, INF-α, and IL-12.

140. The immunoconjugate of claim 139, wherein both of said cytokines are IL-2.

141. The immunoconjugate of claim 139, wherein both of said cytokines are IL-12.

142. The immunoconjugate of any one of claims 138-141, wherein said cytokines elicit a cytotoxic response by cells bearing receptors for said cytokines.

143. The immunoconjugate of any one of claims 138-141, wherein said cytokines elicit a proliferative response by cells bearing receptors for said cytokines.

144. The immunoconjugate of any one of claims 1-143, wherein said immunoconjugate binds to an effector moiety receptor with a dissociation constant (K_D) that is at least 2 times greater than that of a control effector moiety.

145. The immunoconjugate of any one of claims 1-144, wherein said immunoconjugate inhibits an increase in tumor volume *in vivo* by at least 30% at the end of an administration period.

146. An isolated polynucleotide encoding a fragment of the immunoconjugate of claim 1 or 2, wherein said polynucleotide encodes the heavy chains of said first and second antigen binding moieties and said first effector moiety.

147. An isolated polynucleotide encoding a fragment of the immunoconjugate of claim 1 or 2, wherein said polynucleotide encodes the light chains of said first and second antigen binding moieties and said first effector moiety.

148. An isolated polynucleotide encoding a fragment of the immunoconjugate of claim 1 or 2, wherein said polynucleotide encodes one light chain from said first antigen binding moiety, one heavy chain from said second antigen binding moiety and said first effector moiety.

149. An isolated polynucleotide encoding a fragment of the immunoconjugate of any one of claims 3-8, wherein said polynucleotide encodes the heavy chains of both of said first and second Fab molecules and said effector moiety.

150. An isolated polynucleotide encoding a fragment of the immunoconjugate of any one of claims 3-8, wherein said polynucleotide encodes the light chains of both of said first and second Fab molecules and said effector moiety.

151. An isolated polynucleotide encoding a fragment of the immunoconjugate of any one of claims 3-8, wherein said polynucleotide encodes one light chain from said first Fab molecules, one heavy chain from said second Fab molecule, and said effector moiety.

152. An isolated polynucleotide encoding the immunoconjugate of claim 9.

153. An isolated polynucleotide encoding a fragment of the immunoconjugate of any one of claims 10-20, wherein the immunoconjugate has only one effector moiety; and wherein said polynucleotide encodes one of said antigen binding moieties and said effector moiety.

154. An isolated polynucleotide encoding a fragment of the immunoconjugate of any one of claims 10-21, wherein the immunoconjugate has two effector moieties; and wherein said polynucleotide encodes one of said antigen binding moieties and one of said effector moieties.

155. An isolated polynucleotide encoding a fragment of the immunoconjugate of any one of claims 22-26, wherein said polynucleotide comprises a sequence encoding the heavy chain variable region of said first antigen binding moiety and said first effector moiety.

156. An isolated polynucleotide encoding a fragment of the immunoconjugate of any one of claims 22-26, wherein said polynucleotide comprises a sequence encoding the light chain variable region of said first antigen binding moiety and said first effector moiety.

157. An expression cassette comprising the polynucleotide sequence of any one of claims 146-156.

158. An expression vector which comprises the expression cassette of claim 157.

159. A host cell comprising the expression vector of claim 158.

160. The host cell of claim 159, further defined as a prokaryotic host cell.

161. The host cell of claim 159, further defined as an eukaryotic host cell.

162. A method of producing the immunoconjugate of any one of claims 1-145, wherein the method comprises culturing the host cell of any one of claims 159-161 under conditions suitable for the expression of the immunoconjugate.

163. A method for promoting proliferation and differentiation in an activated T lymphocyte cell, comprising contacting an activated T lymphocyte cell with an effective amount of the immunoconjugate of any one of claims 1-145.

164. The method of claim 163, wherein said immunoconjugate induces cytotoxic T cell (CTL) activity.

165. A method for promoting proliferation and differentiation in an activated B lymphocyte cell, comprising contacting an activated B lymphocyte cell with an effective amount of the immunoconjugate of any one of claims 1-134, 136-140, or 142-145.

166. A method for promoting proliferation and differentiation in a natural killer (NK) cell, comprising contacting an NK cell with an effective amount of the immunoconjugate of any one of claims 1-145.

167. The method of claim 166, wherein said immunoconjugate induces NK/lymphocyte activated killer (LAK) antitumor cytotoxicity.

168. The method of any one of claims 163-167, wherein at least one effector moiety of said immunoconjugate is IL-2.

169. A method for promoting proliferation and differentiation in a granulocyte, a monocyte or a dendritic cell, comprising contacting a granulocyte, a monocyte or a dendritic cell

with an effective amount of the immunoconjugate of any one of claims 1-133, 137-139, or 143-145.

170. The method of claim 169, wherein at least one effector moiety of said immunoconjugate is GM-CSF.

171. A method for promoting T lymphocyte differentiation, comprising contacting a T lymphocyte cell with an effective amount of the immunoconjugate of any one of claims 1-145.

172. The method of any one of claims 163, 166 or 171, wherein at least one effector moiety of said immunoconjugate is IL-12.

173. A method of inhibiting viral replication, comprising contacting a virus-infected cell with an effective amount of the immunoconjugate of any one of claims 1-133, 138, 139, 144, or 145.

174. A method of upregulating the expression of major histocompatibility complex I (MHC I), comprising contacting a cell with an effective amount of the immunoconjugate of any one of claims 1-133, 138, 139, 144, or 145.

175. The method of any one of claims 173 or 174, wherein at least one effector moiety of said immunoconjugate is IFN-α.

176. A method of inducing cell death, comprising administering to a cell an effective amount of the immunoconjugate of any one of claims 1-132, 136, 138, 142, 144, or 145.

177. The method of claim 176, wherein said immunoconjugate is specific for EDB.

178. The method of claim 176, wherein said immunoconjugate is specific for TNC-A1.

179. The method of claim 176, wherein said immunoconjugate is specific for TNC-A2.

180. The method of claim 176, wherein said immunoconjugate is specific for MCSP.

181. The method of claim 176, wherein the effector moiety of said immunoconjugate is selected from the group consisting of: IL-2, tumor necrosis factor-α (TNF-α) and tumor necrosis factor-β (TNF-β).

182. The method of claim 181, wherein said effector moiety is IL-2.

183. A method of inducing chemotaxis in a target cell, comprising administering to a target cell an effective amount of the immunoconjugate of any one of claims 1-132, 138, 144, or 145.

184. The method of claim 183, wherein the effector moiety of said immunoconjugate is selected from the group consisting of: interleukin-8 (IL-8), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), and transforming growth factor-β (TGF-β).

185. The method of any one of claims 163-184, wherein said cell is *in vivo*.

186. A method of treating a disease in an individual, comprising the steps of administering to said individual a therapeutically effective amount of a composition comprising the immunoconjugate of any one of claims 1-145.

187. The method of claim 186, wherein said disease is cancer.

188. The method of claim 186 or 187, wherein said immunoconjugate is specific for EDB.

189. The method of claim 186 or 187, wherein said immunoconjugate is specific for TNC-A1.

190. The method of claim 186 or 187, wherein said immunoconjugate is specific for TNC-A2.

191. The method of claim 186 or 187, wherein said immunoconjugate is specific for FAP.

192. The method of claim 186 or 187, wherein said immunoconjugate is specific for MCSP.

193. The method of any one of claims 186 to 192, wherein said immunoconjugate comprises IL-2.

194. The method of any one of claims 186 to 193, wherein said composition is administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraprostatically, intrasplenically, intrarenally intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctivally, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularally, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, via a catheter, via a lavage, in a cream, or in a lipid composition.

195. A composition comprising the immunoconjugate of any one of claims 1-145 and a pharmaceutical carrier.

196. The immunoconjugate of any one of claims 1-145 or the composition of claim 195 for use in the manufacture of a medicament for treating disease in a patient in need thereof.

197. The immunoconjugate of claim 196, wherein said disease is cancer.

198. Use of the immunoconjugate of any one of claims 1-145 or the composition of claim 195 for the manufacture of a medicament for treating disease in a patient in need thereof.

199. The use of claim 198, wherein said disease is cancer.

200. The immunoconjugate of any one of claims 1-145 for the treatment of a disease in a patient in need thereof.

201. The immunoconjuate of claim 200, wherein said disease is cancer.

202. The invention as described hereinbefore.