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(71) Applicant (for all designated States except US): **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, California 94080-4990 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **WAGNER, Klaus W.** [DE/US]; 509 Burlingame Avenue, Burlingame, California 94010 (US).

(74) Agent: **MARSCHANG, Diane L.**; Genentech, Inc., MS 49, 1 DNA Way, South San Francisco, California 94080 (US).

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(54) Title: ASSAYS AND METHODS USING BIOMARKERS

(57) Abstract: Methods and assays examining expression of one or more biomarkers in a mammalian tissue or cell sample are provided. According to the disclosed methods and assays, detection of the expression of one or more such biomarkers is predictive or indicative that the tissue or cell sample will be sensitive to apoptosis-inducing agents such as Apo2L/TRAIL and anti-DR5 agonist antibodies. Certain biomarkers which may be examined include fucosyltransferases, in particular fucosyltransferase 3 (FUT3) and/or fucosyltransferase 6 (FUT6), as well as sialyl Lewis A and/or X antigens. Kits and articles of manufacture are also provided.



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ASSAYS AND METHODS USING BIOMARKERS

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RELATED APPLICATIONS

This application claims priority under Section 119(e) to U.S. provisional application number 60/599,425 filed August 6, 2004, the contents of which are incorporated herein by reference.

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FIELD OF THE INVENTION

The invention described herein relates to methods and assays to detect biomarkers predictive of sensitivity of mammalian cells to Apo2L/TRAIL and/or death receptor agonist antibodies.

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BACKGROUND OF THE INVENTION

Various ligands and receptors belonging to the tumor necrosis factor (TNF) superfamily have been identified in the art. Included among such ligands are tumor necrosis factor-alpha ("TNF-alpha"), tumor necrosis factor-beta ("TNF-beta" or "lymphotoxin-alpha"), lymphotoxin-beta ("LT-beta"), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, LIGHT, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), Apo-2 ligand (also referred to as Apo2L or TRAIL), Apo-3 ligand (also referred to as TWEAK), APRIL, OPG ligand (also referred to as RANK ligand, ODF, or TRANCE), and TALL-1 (also referred to as BlyS, BAFF or THANK) (See, e.g., Ashkenazi, *Nature Review*, 2:420-430 (2002); Ashkenazi and Dixit, *Science*, 281:1305-1308 (1998); Ashkenazi and Dixit, *Curr. Opin. Cell Biol.*, 11:255-260 (2000); Golstein, *Curr. Biol.*, 7:750-753 (1997) Wallach, *Cytokine Reference*, Academic Press, 2000, pages 377-411; Locksley et al., *Cell*, 104:487-501 (2001); Gruss and Dower, *Blood*, 85:3378-3404 (1995); Schmid et al., *Proc. Natl. Acad. Sci.*, 83:1881 (1986); Dealtry et al., *Eur. J. Immunol.*, 17:689 (1987); Pitti et al., *J. Biol. Chem.*, 271:12687-12690 (1996); Wiley et al., *Immunity*, 3:673-682 (1995); Browning et al., *Cell*, 72:847-856 (1993); Armitage et al. *Nature*, 357:80-82 (1992); WO 97/01633 published January 16, 1997; WO 97/25428 published July 17, 1997; Marsters et al., *Curr. Biol.*, 8:525-528 (1998); Chicheportiche et al., *Biol. Chem.*, 272:32401-32410 (1997); Hahne et al., *J. Exp. Med.*, 188:1185-1190 (1998); WO98/28426 published July 2, 1998; WO98/46751 published October 22, 1998; WO/98/18921 published May 7, 1998; Moore et al., *Science*, 285:260-263 (1999); Shu et al., *J. Leukocyte Biol.*, 65:680 (1999); Schneider et al., *J. Exp. Med.*, 189:1747-1756 (1999); Mukhopadhyay et al., *J. Biol. Chem.*, 274:15978-15981 (1999)).

Induction of various cellular responses mediated by such TNF family ligands is typically initiated by their binding to specific cell receptors. Some, but not all, TNF family ligands bind to, and induce various biological activity through, cell surface "death receptors" to activate caspases, or enzymes that carry out the cell death or apoptosis pathway (Salvesen et al., Cell, 91:443-446 (1997). Included among the members of the TNF receptor superfamily identified to date are TNFR1, TNFR2, TACI, GITR,, CD27, OX-40, CD30, CD40, HVEM, Fas (also referred to as Apo-1 or CD95), DR4 (also referred to as TRAIL-R1), DR5 (also referred to as Apo-2 or TRAIL-R2), DcR1, DcR2, osteoprotegerin (OPG), RANK and Apo-3 (also referred to as DR3 or TRAMP) (see, e.g., Ashkenazi, Nature Reviews, 2:420-430 (2002); Ashkenazi and Dixit, Science, 281:1305-1308 (1998); Ashkenazi and Dixit, Curr. Opin. Cell Biol., 11:255-260 (2000); Golstein, Curr. Biol., 7:750-753 (1997) Wallach, Cytokine Reference, Academic Press, 2000, pages 377-411; Locksley et al., Cell, 104:487-501 (2001); Gruss and Dower, Blood, 85:3378-3404 (1995); Hohman et al., J. Biol. Chem., 264:14927-14934 (1989); Brockhaus et al., Proc. Natl. Acad. Sci., 87:3127-3131 (1990); EP 417,563, published March 20, 1991; Loetscher et al., Cell, 61:351 (1990); Schall et al., Cell, 61:361 (1990); Smith et al., Science, 248:1019-1023 (1990); Lewis et al., Proc. Natl. Acad. Sci., 88:2830-2834 (1991); Goodwin et al., Mol. Cell. Biol., 11:3020-3026 (1991); Stamenkovic et al., EMBO J., 8:1403-1410 (1989); Mallett et al., EMBO J., 9:1063-1068 (1990); Anderson et al., Nature, 390:175-179 (1997); Chicheportiche et al., J. Biol. Chem., 272:32401-32410 (1997); Pan et al., Science, 276:111-113 (1997); Pan et al., Science, 277:815-818 (1997); Sheridan et al., Science, 277:818-821 (1997); Degli-Esposti et al., J. Exp. Med., 186:1165-1170 (1997); Marsters et al., Curr. Biol., 7:1003-1006 (1997); Tsuda et al., BBRC, 234:137-142 (1997); Nocentini et al., Proc. Natl. Acad. Sci., 94:6216-6221 (1997); vonBulow et al., Science, 278:138-141 (1997)).

Most of these TNF receptor family members share the typical structure of cell surface receptors including extracellular, transmembrane and intracellular regions, while others are found naturally as soluble proteins lacking a transmembrane and intracellular domain. The extracellular portion of typical TNFRs contains a repetitive amino acid sequence pattern of multiple cysteine-rich domains (CRDs), starting from the NH₂-terminus.

The ligand referred to as Apo-2L or TRAIL was identified several years ago as a member of the TNF family of cytokines. (see, e.g., Wiley et al., Immunity, 3:673-682 (1995); Pitti et al., J. Biol. Chem., 271:12697-12690 (1996); WO 97/01633; WO 97/25428; US Patent 5,763,223 issued June 9, 1998; US Patent 6,284,236 issued September 4, 2001). The full-length native sequence human Apo2L/TRAIL polypeptide is a 281 amino acid long,

Type II transmembrane protein. Some cells can produce a natural soluble form of the polypeptide, through enzymatic cleavage of the polypeptide's extracellular region (Mariani et al., J. Cell. Biol., 137:221-229 (1997)). Crystallographic studies of soluble forms of Apo2L/TRAIL reveal a homotrimeric structure similar to the structures of TNF and other related proteins (Hymowitz et al., Molec. Cell, 4:563-571 (1999); Cha et al., Immunity, 11:253-261 (1999); Mongkolsapaya et al., Nature Structural Biology, 6:1048 (1999); Hymowitz et al., Biochemistry, 39:633-644 (2000)). Apo2L/TRAIL, unlike other TNF family members however, was found to have a unique structural feature in that three cysteine residues (at position 230 of each subunit in the homotrimer) together coordinate a zinc atom, and that the zinc binding is important for trimer stability and biological activity. (Hymowitz et al., *supra*; Bodmer et al., J. Biol. Chem., 275:20632-20637 (2000)).

It has been reported in the literature that Apo2L/TRAIL may play a role in immune system modulation, including autoimmune diseases such as rheumatoid arthritis [see, e.g., Thomas et al., J. Immunol., 161:2195-2200 (1998); Johnsen et al., Cytokine, 11:664-672 (1999); Griffith et al., J. Exp. Med., 189:1343-1353 (1999); Song et al., J. Exp. Med., 191:1095-1103 (2000)].

Soluble forms of Apo2L/TRAIL have also been reported to induce apoptosis in a variety of cancer cells, including colon, lung, breast, prostate, bladder, kidney, ovarian and brain tumors, as well as melanoma, leukemia, and multiple myeloma (see, e.g., Wiley et al., *supra*; Pitti et al., *supra*; US Patent 6,030,945 issued February 29, 2000; US Patent 6,746,668 issued June 8, 2004; Rieger et al., FEBS Letters, 427:124-128 (1998); Ashkenazi et al., J. Clin. Invest., 104:155-162 (1999); Walczak et al., Nature Med., 5:157-163 (1999); Keane et al., Cancer Research, 59:734-741 (1999); Mizutani et al., Clin. Cancer Res., 5:2605-2612 (1999); Gazitt, Leukemia, 13:1817-1824 (1999); Yu et al., Cancer Res., 60:2384-2389 (2000); Chinnaiyan et al., Proc. Natl. Acad. Sci., 97:1754-1759 (2000)). *In vivo* studies in murine tumor models further suggest that Apo2L/TRAIL, alone or in combination with chemotherapy or radiation therapy, can exert substantial anti-tumor effects (see, e.g., Ashkenazi et al., *supra*; Walczak et al., *supra*; Gliniak et al., Cancer Res., 59:6153-6158 (1999); Chinnaiyan et al., *supra*; Roth et al., Biochem. Biophys. Res. Comm., 265:1999 (1999); PCT Application US/00/15512; PCT Application US/01/23691). In contrast to many types of cancer cells, most normal human cell types appear to be resistant to apoptosis induction by certain recombinant forms of Apo2L/TRAIL (Ashkenazi et al., *supra*; Walczak et al., *supra*). Jo et al. has reported that a polyhistidine-tagged soluble form of Apo2L/TRAIL induced apoptosis *in vitro* in normal isolated human, but not non-human, hepatocytes (Jo et al., Nature Med., 6:564-567 (2000); see

also, Nagata, *Nature Med.*, 6:502-503 (2000)). It is believed that certain recombinant Apo2L/TRAIL preparations may vary in terms of biochemical properties and biological activities on diseased versus normal cells, depending, for example, on the presence or absence of a tag molecule, zinc content, and % trimer content (See, Lawrence et al., *Nature Med.*, Letter to the Editor, 7:383-385 (2001); Qin et al., *Nature Med.*, Letter to the Editor, 7:385-386 (2001)).

Apo2L/TRAIL has been found to bind at least five different receptors. At least two of the receptors which bind Apo2L/TRAIL contain a functional, cytoplasmic death domain. One such receptor has been referred to as "DR4" (and alternatively as TR4 or TRAIL-R1) (Pan et al., *Science*, 276:111-113 (1997); see also WO98/32856 published July 30, 1998; WO99/37684 published July 29, 1999; WO 00/73349 published December 7, 2000; US 6,433,147 issued August 13, 2002; US 6,461,823 issued October 8, 2002, and US 6,342,383 issued January 29, 2002).

Another such receptor for Apo2L/TRAIL has been referred to as DR5 (it has also been alternatively referred to as Apo-2; TRAIL-R or TRAIL-R2, TR6, Tango-63, hAPO8, TRICK2 or KILLER) (see, e.g., Sheridan et al., *Science*, 277:818-821 (1997), Pan et al., *Science*, 277:815-818 (1997), WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998; Screaton et al., *Curr. Biol.*, 7:693-696 (1997); Walczak et al., *EMBO J.*, 16:5386-5387 (1997); Wu et al., *Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924 published August 2, 2001; US 2003/01255540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,342,369 issued February, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003; IS 6,743,625 issued June 1, 2004). Like DR4, DR5 is reported to contain a cytoplasmic death domain and be capable of signaling apoptosis upon ligand binding (or upon binding a molecule, such as an agonist antibody, which mimics the activity of the ligand). The crystal structure of the complex formed between Apo-2L/TRAIL and DR5 is described in Hymowitz et al., *Molecular Cell*, 4:563-571 (1999).

Upon ligand binding, both DR4 and DR5 can trigger apoptosis independently by recruiting and activating the apoptosis initiator, caspase-8, through the death-domain-containing adaptor molecule referred to as FADD/Mort1 [Kischkel et al., *Immunity*, 12:611-620 (2000); Sprick et al., *Immunity*, 12:599-609 (2000); Bodmer et al., *Nature Cell Biol.*, 2:241-243 (2000)].

Apo2L/TRAIL has been reported to also bind those receptors referred to as DcR1, DcR2 and OPG, which believed to function as inhibitors, rather than transducers of signaling (see., e.g., DCR1 (also referred to as TRID, LIT or TRAIL-R3) [Pan et al., Science, 276:111-113 (1997); Sheridan et al., Science, 277:818-821 (1997); McFarlane et al., J. Biol. Chem., 272:25417-25420 (1997); Schneider et al., FEBS Letters, 416:329-334 (1997); Degli-Esposti et al., J. Exp. Med., 186:1165-1170 (1997); and Mongkolsapaya et al., J. Immunol., 160:3-6 (1998); DCR2 (also called TRUNDD or TRAIL-R4) [Marsters et al., Curr. Biol., 7:1003-1006 (1997); Pan et al., FEBS Letters, 424:41-45 (1998); Degli-Esposti et al., Immunity, 7:813-820 (1997)], and OPG [Simonet et al., supra]. In contrast to DR4 and DR5, the DcR1 and DcR2 receptors do not signal apoptosis.

Certain antibodies which bind to the DR4 and/or DR5 receptors have been reported in the literature. For example, anti-DR4 antibodies directed to the DR4 receptor and having agonistic or apoptotic activity in certain mammalian cells are described in, e.g., WO 99/37684 published July 29, 1999; WO 00/73349 published July 12, 2000; WO 03/066661 published August 14, 2003. See, also, e.g., Griffith et al., J. Immunol., 162:2597-2605 (1999); Chuntharapai et al., J. Immunol., 166:4891-4898 (2001); WO 02/097033 published December 2, 2002; WO 03/042367 published May 22, 2003; WO 03/038043 published May 8, 2003; WO 03/037913 published May 8, 2003. Certain anti-DR5 antibodies have likewise been described, see, e.g., WO 98/51793 published November 8, 1998; Griffith et al., J. Immunol., 162:2597-2605 (1999); Ichikawa et al., Nature Med., 7:954-960 (2001); Hylander et al., "An Antibody to DR5 (TRAIL-Receptor 2) Suppresses the Growth of Patient Derived Gastrointestinal Tumors Grown in SCID mice", Abstract, 2d International Congress on Monoclonal Antibodies in Cancers, Aug. 29-Sept. 1, 2002, Banff, Alberta, Canada; WO 03/038043 published May 8, 2003; WO 03/037913 published May 8, 2003. In addition, certain antibodies having cross-reactivity to both DR4 and DR5 receptors have been described (see, e.g., US patent 6,252,050 issued June 26, 2001).

Neoplastic transformation of some mammalian cells has in certain instances, been associated with characteristic changes in the expression of sialyl Lewis A and sialyl Lewis X antigens. Relatively high amounts of sialyl Lewis A/X are present, for example, in some human adenocarcinomas of the colon, pancreas and stomach, and assays using antibodies directed to the carbohydrate structures on these antigens have been employed as a means to detect pancreatic and gastrointestinal cancers. (see, e.g., Ugorski et al., Acta Biochimica Polonica, 49:2:303-311 (2002). The level of expression of these carbohydrate tumor markers has also been correlated with clinical outcome, patient survival times and an indicator of metastatic disease.

Both sialyl Lewis A and sialyl Lewis X have been shown to bind to a family of carbohydrate-binding proteins involved in the extravasation of cells from the bloodstream, called the selectins. Some reports suggest that sialyl Lewis A and X are ligands for E-selectin, and may be responsible for the adhesion of human cancer cells to endothelium. Sialylated Lewis structures present on the surface of cancer cells are carried by the carbohydrate chains of glycoproteins and glycolipids and bind E-selectin present on endothelial cells. Selectins and their carbohydrate ligands may accordingly play an important role in the selective homing of tumor cells during metastasis.

The biosynthesis of sialyl Lewis A and X is believed to be dependent upon the final addition of fucose from guanosine diphosphate-fucose (GDP-Fuc) in alpha (1,3) and alpha (1,4) linkage to sialylated precursors by cell type-specific and developmental stage-specific enzymes, a step catalyzed by alpha-1,3/1,4-fucosyltransferases (alpha 1,3/1,4 Fuc-T, FUT).

Several human fucosyltransferase genes have been cloned and characterized to date. Expression of these genes (FUT 3-7) and their enzyme products (Fuc-TIII-VII) appears to be tissue specific. The enzymes encoded by the five genes are named FUTIII, FUTIV, FUTV, FUTVI and FUTVII. The three genes encoding FUTIII, FUTV and FUTVI are localized at close physical positions on chromosome 19p13.3. Biochemical and molecular cloning studies suggest that lineage-specific expression of the sialyl Lewis A/X moiety is determined by lineage-specific expression of alpha-1,3-fucosyltransferase genes, whose enzyme products operate on constitutively expressed oligosaccharide precursors to yield surface-localized sialyl Lewis A/X determinants. The human fucosyltransferases responsible for activity in epithelial tissues are FUT3 and FUT6. FUT3 [also called the Lewis alpha (1,3/1,4)fucosyltransferase gene] and FUT6 [the plasma alpha (1,3)fucosyltransferase gene] transcripts are present in both normal and transformed tissues. Fucosyltransferase transcripts are also prevalent in numerous adenocarcinoma cell lines, with notably high expression of FUT3 and 6 in colon carcinoma. (see, e.g, Ugorski et al., Acta Biochimica Polonica, 49:303-311 (2002); Nakamori et al., Dis. Colon Rectum., 40:420-431 (1997); Takada et al., Cancer Res., 53:354-361 (1993); Ichikawa et al., J. Surg. Oncol., 75:98-102 (2000)); Nakagoe et al., J Exp Clin Cancer Res., 2002 Mar;21(1):107-13; Matsumoto et al., Br J Cancer. 2002 Jan 21;86(2):161-7; Ito et al., J Gastroenterol. 2001 Dec;36(12):823-9; Nakagoe et al., Cancer Detect Prev. 2001;25(3):299-308; Kumamoto et al., Cancer Res. 2001 Jun 1;61(11):4620-7; Murata et al., Dis Colon Rectum. 2001 Apr;44(4):A2-A4; Nakagoe et al., J Exp Clin Cancer Res. 2001 Mar;20(1):85-90; Nakagoe et al., J Gastroenterol. 2001 Mar;36(3):166-72; Nakagoe et al., Tumour Biol. 2001 Mar-Apr;22(2):115-22; Nakagoe et al., Can J Gastroenterol. 2000 Oct;14(9):753-60; Izawa et al., Cancer Res. 2000

- Mar 1;60(5):1410-6; Tanaka et al., Hepatogastroenterology. 1999 Mar-Apr;46(26):875-82; Matsushita et al., Cancer Lett. 1998 Nov 27;133(2):151-60; Sato et al., Anticancer Res. 1997 Sep-Oct;17(5A):3505-11; Yamada et al., Br J Cancer. 1997;76(5):582-7; Nakamori et al., Dis Colon Rectum. 1997 Apr;40(4):420-31; Srinivas et al., Scand J Immunol. 1996 Sep;44(3):197-203; Matsushita et al., Lab Invest. 1990 Dec;63(6):780-91; Ashizawa et al., J Exp Clin Cancer Res. 2003 Mar;22(1):91-8; Nakagoe et al., J Exp Clin Cancer Res. 2002 Sep;21(3):363-9; Nakagoe et al., Anticancer Res. 2002 Jan-Feb;22(1A):451-8; Nakagoe et al., J Clin Gastroenterol. 2002 Apr;34(4):408-15; Nakagoe et al., Cancer Lett. 2002 Jan 25;175(2):213-21; Tatsumi et al., Clin Exp Metastasis. 1998 Nov;16(8):743-50; Ikeda et al., J Surg Oncol. 1996 Jul;62(3):171-6; Ikeda et al., Eur J Surg Oncol. 1995 Apr;21(2):168-75; Togayachi et al., Int J Cancer. 1999 Sep 24;83(1):70-9; Satoh et al., Clin Cancer Res. 1997 Apr;3(4):495-9; Satoh et al., Respiration. 1998;65(4):295-8; Satoh et al., Anticancer Res. 1998 Jul-Aug;18(4B):2865-8; Fukuoka et al., Lung Cancer. 1998 May;20(2):109-16; Fujiwara et al., Anticancer Res. 1998 Mar-Apr;18(2A):1043-6; Ogawa et al., Int J Cancer. 1997 Apr 22;74(2):189-92; Ogawa et al., J Thorac Cardiovasc Surg. 1994 Aug;108(2):329-36; Asao et al., Cancer. 1989 Dec 15;64(12):2541-5; Narita et al., Breast Cancer. 1996 Mar 29;3(1):19-23; Yamaguchi et al., Oncology. 1998 Jul-Aug;55(4):357-62; Sikut et al., Int J Cancer. 1996 May 29;66(5):617-23; Saito et al., Anticancer Res. 2003 Jul-Aug;23(4):3441-6; Fujii et al., Urol Int. 2000;64(3):129-33; Idikio et al., Glycoconj J. 1997 Nov;14(7):875-7; Inoue et al., Obstet Gynecol. 1992 Mar;79(3):434-40; Yamashita et al., Eur J Cancer. 2000 Jan;36(1):113-20; Hamanaka et al., Pancreas. 1996 Aug;13(2):160-5; Ho et al., Cancer Res. 1995 Aug 15;55(16):3659-63.

SUMMARY OF THE INVENTION

The invention disclosed herein provides methods and assays examining expression of one or more biomarkers in a mammalian tissue or cell sample, wherein the expression of one or more such biomarkers is predictive of whether the tissue or cell sample will be sensitive to apoptosis-inducing agents such as Apo2L/TRAIL and anti-DR5 agonist antibodies. In various embodiments of the invention, the methods and assays examine expression of biomarkers such as certain fucosyltransferases, in particular fucosyltransferase 3 (FUT3) and/or fucosyltransferase 6 (FUT6), as well as sialyl Lewis A and/or X antigens.

As discussed above, most normal human cell types appear to be resistant to apoptosis induction by certain recombinant forms of Apo2L/TRAIL (Ashkenazi et al., supra; Walzcak et al., supra). It has also been observed that some populations of diseased human cell types (such as certain populations of cancer cells) are resistant to apoptosis induction

by certain recombinant forms of Apo2L/TRAIL (Ashkenazi et al., J. Clin. Invest., 1999, supra; Walczak et al., Nature Med., 1999, supra). Consequently, by examining a mammalian tissue or cell sample for expression of certain biomarkers by way of an assay, one can conveniently and efficiently obtain information useful in assessing appropriate or effective therapies for treating patients. For example, information obtained from an assay to detect FUT3 or FUT6 expression in a mammalian tissue or cell sample can provide physicians with useful data that can be used to determine an optimal therapeutic regimen (using Apo2L/TRAIL or death receptor agonist antibodies) for patients suffering from a disorder such as cancer.

The invention provides methods for predicting the sensitivity of a mammalian tissue or cells sample (such as a cancer cell) to Apo2L/TRAIL or a death receptor agonist antibody. In certain embodiments, the methods comprise obtaining a mammalian tissue or cell sample and examining the tissue or cell for expression of fucosyltransferase 3 or fucosyltransferase 6. The methods may also comprise examining the tissue or cell for expression of another biomarker such as sialyl Lewis A and/or X antigen(s). The methods may be conducted in a variety of assay formats, including assays detecting mRNA expression, enzymatic assays detecting presence of enzymatic activity, immunohistochemistry assays, and others discussed herein. Determination of expression of such biomarkers in said tissues or cells will be predictive that such tissues or cells will be sensitive to the apoptosis-inducing activity of Apo2/TRAIL and/or death receptor antibody. In optional embodiments, the tissues or cells may also be examined for expression of DR4, DR5, Dcr1 or Dcr2 receptors.

Further methods of the invention include methods of inducing apoptosis in a mammalian tissue or cell sample, comprising steps of obtaining a mammalian tissue or cell sample, examining the tissue or cell for expression of one or more biomarkers, such as fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and upon determining said tissue or cell sample expresses said one or more biomarkers, exposing said tissue or cell sample to an effective amount of Apo2L/TRAIL or death receptor agonist antibody. The steps in the methods for examining expression of one or more biomarkers may be conducted in a variety of assay formats, including assays detecting mRNA expression, enzymatic assays detecting presence of enzymatic activity, and immunohistochemistry assays. In optional embodiments, the methods also comprise examining the tissue or cell sample for expression of DR4, DR5, Dcr1, or Dcr2 receptors. Optionally, the tissue or cell sample comprises cancer tissue or cells.

Still further methods of the invention include methods of treating a disorder in a mammal, such as an immune related disorder or cancer,

comprising steps of obtaining tissue or a cell sample from the mammal, examining the tissue or cells for expression of one or more biomarkers, such as fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and upon determining said tissue or cell sample expresses said one or more biomarkers, administering an effective amount of Apo2L/TRAIL or death receptor agonist antibody to said mammal. The steps in the methods for examining expression of one or more biomarkers may be conducted in a variety of assay formats, including assays detecting mRNA expression, enzymatic assays detecting presence of enzymatic activity, and immunohistochemistry assays. In optional embodiments, the methods also comprise examining the tissue or cell sample for expression of DR4, DR5, DcR1, or DcR2 receptors. Optionally, the methods comprise treating cancer in a mammal. Optionally, the methods comprise, in addition to administering an effective amount of Apo2L/TRAIL and/or death receptor agonist antibody, administering chemotherapeutic agent(s) or radiation therapy to said mammal.

Further embodiments are more particularly disclosed by the following claims:

1. A method for predicting the sensitivity of a mammalian tissue or cells sample to Apo2L/TRAIL, comprising the steps of:

obtaining a mammalian tissue or cell sample;

examining the tissue or cell sample to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), wherein

expression of said one or more biomarkers is predictive that said tissue or cell sample is sensitive to apoptosis-inducing activity of Apo2L/TRAIL.

2. The method of claim 1 wherein said expression of one or more biomarkers is examined by detecting mRNA expression of fucosyltransferase 3 or fucosyltransferase 6.

3. The method of claim 1 wherein said expression of one or more biomarkers is examined by immunohistochemistry to detect expression of sialyl Lewis A and/or X antigen(s).

4. The method of claim 1 further comprising the step of examining expression of DR4, DR5, DcR1, or DcR2 receptors in said tissue or cell sample.

5. The method of claim 1 wherein tissue or cell sample comprises cancer tissue or cells.

6. The method of claim 5 wherein said cancer cells are colon, colorectal, gastrointestinal, or pancreatic cancer cells or tissue.

7. A method for inducing apoptosis in a mammalian tissue or cell sample, comprising the steps of:

obtaining a mammalian tissue or cell sample;

- examining the tissue or cell sample to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and subsequent to detecting expression of said one or more biomarkers,
- 5 exposing said tissue or cell sample to an effective amount of Apo2L/TRAIL.
8. The method of claim 7 wherein said expression of one or more biomarkers is examined by testing for mRNA expression of fucosyltransferase 3 or fucosyltransferase 6.
9. The method of claim 7 wherein said expression of one or more biomarkers
- 10 is examined by immunohistochemistry to detect expression of sialyl Lewis A and/or X antigen(s).
10. The method of claim 7 further comprising the step of examining expression of DR4, DR5, DcR1 or DcR2 receptors in said tissue or cell sample.
- 15 11. The method of claim 7 wherein said tissue or cell sample comprises cancer tissue or cells.
12. The method of claim 11 wherein said cancer cells are colon, colorectal, gastrointestinal, or pancreatic cancer cells or tissue.
13. The method of claim 7 wherein said cells are exposed to an effective
- 20 amount of Apo2L/TRAIL polypeptide comprising amino acids 114-281 of Figure 1.
14. A method of treating a disorder in a mammal, such as an immune related disorder or cancer, comprising the steps of:
obtaining a tissue or cell sample from said mammal;
- 25 examining the tissue or cell sample to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and subsequent to detecting expression of said one or more biomarkers, administering to said mammal an effective amount of Apo2L/TRAIL.
- 30 15. The method of claim 14 wherein said expression of one or more biomarkers is examined by detecting mRNA expression of fucosyltransferase 3 or fucosyltransferase 6.
16. The method of claim 14 wherein said expression of one or more biomarkers is examined by immunohistochemistry to detect expression of
- 35 sialyl Lewis A and/or X antigen(s).
17. The method of claim 14 further comprising the step of examining expression of DR4, DR5, DcR1 or DcR2 receptors in said tissue or cell.
18. The method of claim 14 wherein tissue or cell sample comprises cancer tissue or cells.
- 40 19. The method of claim 18 wherein said cancer cells or tissue comprises colon, colorectal, gastrointestinal, or pancreatic cancer cells or tissue.

20. The method of claim 14 wherein an effective amount of Apo2L/TRAIL polypeptide comprising amino acids 114-281 of Figure 1 is administered to said mammal.

21. The method of claim 14 wherein a chemotherapeutic agent(s) or radiation therapy is also administered to said mammal.

22. The method of claim 14 wherein a cytokine, cytotoxic agent or growth inhibitory agent is also administered to said mammal.

23. The method of claim 7 wherein said Apo2L/TRAIL polypeptide is linked to a polyethylene glycol molecule.

24. The method of claim 14 wherein said Apo2L/TRAIL polypeptide is linked to a polyethylene glycol molecule.

25. The method of claim 6 wherein said cancer cells are colon or colorectal cancer cells.

26. The method of claim 1 wherein said Apo2L/TRAIL is a polypeptide comprising amino acids 41-281 of Figure 1 (SEQ ID NO:1) or a biologically active fragment thereof.

27. The method of claim 26 wherein said Apo2L/TRAIL is a polypeptide comprising amino acids 114-281 of Figure 1 (SEQ ID NO:1).

28. The method of claim 12 wherein said cancer cells are colon or colorectal cancer cells.

29. The method of claim 20 wherein said Apo2L/TRAIL polypeptide consists of amino acids 114-281 of Figure 1 (SEQ ID NO:1).

30. A method for predicting the sensitivity of a mammalian colon or colorectal cancer cells to Apo2L/TRAIL, comprising the steps of:
obtaining mammalian mammalian colon or colorectal cancer cells;
examining the cancer cells to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), wherein expression of said one or more biomarkers is predictive that said cancer cells are sensitive to apoptosis-inducing activity of Apo2L/TRAIL.

31. A method for inducing apoptosis in mammalian colon or colorectal cancer cells, comprising the steps of:
obtaining mammalian colon or colorectal cancer cells;
examining the cancer cells to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and
subsequent to detecting expression of said one or more biomarkers, exposing said cancer cells to an effective amount of Apo2L/TRAIL polypeptide.

32. The method of claim 31 wherein said Apo2L/TRAIL polypeptide comprises amino acids 41-281 of Figure 1 (SEQ ID NO:1) or a fragment thereof which has apoptotic activity.

33. The method of claim 32 wherein said Apo2L/TRAIL polypeptide is linked to a polyethylene glycol molecule.

34. The method of claim 32 wherein said Apo2L/TRAIL polypeptide comprises amino acids 114-281 of Figure 1 (SEQ ID NO:1).

5 35. A method of treating colon or colorectal cancer in a mammal, comprising the steps of:

obtaining a colon or colorectal cancer cell sample from said mammal;
examining the cancer cell sample to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3,

10 fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and subsequent to detecting expression of said one or more biomarkers, administering to said mammal an effective amount of Apo2L/TRAIL.

36. The method of claim 35 wherein said Apo2L/TRAIL polypeptide comprises amino acids 41-281 of Figure 1 (SEQ ID NO:1) or a fragment thereof which
15 has apoptotic activity.

37. The method of claim 36 wherein said Apo2L/TRAIL polypeptide is linked to a polyethylene glycol molecule.

38. The method of claim 36 wherein said Apo2L/TRAIL polypeptide comprises amino acids 114-281 of Figure 1 (SEQ ID NO:1).

20

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the nucleotide sequence of human Apo-2 ligand cDNA (SEQ ID NO:2) and its derived amino acid sequence (SEQ ID NO:1). The "N" at nucleotide position 447 is used to indicate the nucleotide base may be a "T" or "G".
25

Figures 2A and 2B show the nucleotide sequence of a cDNA (SEQ ID NO:4) for full length human DR4 and its derived amino acid sequence (SEQ ID NO:3). The respective nucleotide and amino acid sequences for human DR4 are also reported in Pan et al., *Science*, 276:111 (1997).
30

Figure 3A shows the 411 amino acid sequence (SEQ ID NO:5) of human DR5 as published in WO 98/51793 on November 19, 1998. A transcriptional splice variant of human DR5 is known in the art. This DR5 splice variant encodes the 440 amino acid sequence (SEQ ID NO:6) of human DR5 shown in Figures 3B and 3C as published in WO 98/35986 on August 20, 1998.
35

Figure 3D shows the nucleotide sequences of cDNA (SEQ IID NO:7) for full length human DcR1 and its derived amino acid sequence (SEQ ID NO:8). The respective nucleotide and amino acid sequences for human DcR1 (and particular domains thereof) are also shown and described in WO 98/58062.

40 Figure 3E shows the nucleotide sequences of cDNA (SEQ ID NO:9) for full length human DcR2 and its derived amino acid sequence (SEQ ID NO:10). The respective nucleotide and amino acid sequences for human DcR2 (and particular domains thereof) are shown in WO 99/10484.

Figure 4 shows the nucleotide sequence of a cDNA (SEQ ID NO:11) for full length human (1,3/1,4) fucosyltransferase (FUT3) and its derived amino acid sequence (SEQ ID NO:12). These sequences correspond to GenBank Accession Number HSU27328 and are described for example in Kukowska-Latallo et al., Genes Dev. 1990 Aug;4(8):1288-303.

Figure 5 shows the nucleotide sequence of a cDNA (SEQ ID NO:13) for full length human alpha (1,3) fucosyltransferase (FUT6) and its derived amino acid sequence (SEQ ID NO:14). These sequences correspond to GenBank Accession Number HSU27333 and are described for example in Koszdin and Bowen, Biochem Biophys Res Commun. 1992 Aug 31;187(1):152-7.

Figure 6 provides a summary chart of the data obtained in analyzing 28 colon or colorectal cancer cell lines for sensitivity or resistance to apoptotic activity of Apo2L (+ 0.5% fetal bovine serum "FBS" or 10% FBS) or DR5 monoclonal antibody "mab", cross-linked "XL" or not crosslinked, + 0.5% fetal bovine serum "FBS" or 10% FBS) and expression of FUT 3, FUT 6, Sialyl lewis A and Sialyl lewis X.

Figure 7 provides a comparison of sensitivity of various colon or colorectal cancer cell lines to DR5 antibody and the expression of FUT 3, as measured by quantitative PCR.

Figure 8 provides a comparison of various colon or colorectal cancer cell lines for sensitivity or resistance to DR5 antibody (plus cross-linker) and expression of sialyl lewis X or A, as determined by FACS.

Figure 9A shows a Spearman Rank Correlation test analyzing sensitivity or resistance of various colon or colorectal cancer cell lines and correlation to expression of FUT3.

Figure 9B shows the results of a Fisher's Exact test analyzing sensitivity ("sens") or resistance ("res") of the various colon or colorectal cancer cell lines and the statistical significance between FUT 3 and sialyl lewis A/X expression and sensitivity of the respective cell lines to DR5 antibody apoptotic activity.

Figure 10 provides a comparison of various colon or colorectal cancer cell lines for expression of DcR1 or DcR2 receptors (as determined by quantitative PCR) and the status (sensitive or resistant) of certain cell lines to Apo2L or DR5 antibody.

Figure 11 provides a comparison of various colon or colorectal cancer cell lines for expression of DcR1 or DcR2 receptors (as determined by FACS) and the status (sensitive or resistant) of certain cell lines to Apo2L or DR5 antibody.

Figure 12 show immunohistochemical staining for sialyl lewis A and X on four colorectal cancer cell lines, CaCo2, SW 1417, DLD-1, and Colo 205, and its correlation to expression of sialyl Lewis A and X as measured by FACS and its correlation to sensitivity to Apo2L.

Figure 13 shows a summary of IHC experiments demonstrating expression of sialyl Lewis A and X in tissue samples of normal colon mucosa, normal liver tissue, primary colon cancer, and colon cancer metastases.

5

DETAILED DESCRIPTION OF THE INVENTION

The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely
10 utilized molecular cloning methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd. edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined
15 protocols and/or parameters unless otherwise noted.

Before the present methods and assays are described, it is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, constructs, and reagents described as such may, of course, vary. It is also to be
20 understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the
25 singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a genetic alteration" includes a plurality of such alterations and reference to "a probe" includes reference to one or more probes and equivalents thereof known to those skilled in the art, and so forth.

All publications mentioned herein are incorporated herein by
30 reference to disclose and describe the methods and/or materials in connection with which the publications are cited. Publications cited herein are cited for their disclosure prior to the filing date of the present application. Nothing here is to be construed as an admission that
35 the inventors are not entitled to antedate the publications by virtue of an earlier priority date or prior date of invention. Further the actual publication dates may be different from those shown and require independent verification.

40 I. DEFINITIONS

The terms "Apo2L/TRAIL", "Apo-2L", and "TRAIL" are used herein to refer to a polypeptide sequence which includes amino acid residues 114-281, inclusive, 95-281, inclusive, residues 92-281, inclusive, residues

91-281, inclusive, residues 41-281, inclusive, residues 15-281, inclusive, or residues 1-281, inclusive, of the amino acid sequence shown in Figure 1, as well as biologically active fragments, deletional, insertional, or substitutional variants of the above sequences. In one embodiment, the polypeptide sequence comprises residues 114-281 of Figure 1), and optionally, consists of residues 114-281 of Figure 1. Optionally, the polypeptide sequence comprises residues 92-281 or residues 91-281 of Figure 1. The Apo-2L polypeptides may be encoded by the native nucleotide sequence shown in Figure 1. Optionally, the codon which encodes residue Pro119 (Figure 1 may be "CCT" or "CCG". In other embodiments, the fragments or variants are biologically active and have at least about 80% amino acid sequence identity, more preferably at least about 90% sequence identity, and even more preferably, at least 95%, 96%, 97%, 98%, or 99% sequence identity with any one of the above recited Apo2L/TRAIL sequences. Optionally, the Apo2L/TRAIL polypeptide is encoded by a nucleotide sequence which hybridizes under stringent conditions with the encoding polynucleotide sequence provided in Figure 1. The definition encompasses substitutional variants of Apo2L/TRAIL in which at least one of its native amino acids are substituted by an alanine residue. Particular substitutional variants of the Apo2L/TRAIL include those in which at least one amino acid is substituted by an alanine residue. These substitutional variants include those identified, for example, as "D203A"; "D218A" and "D269A." This nomenclature is used to identify Apo2L/TRAIL variants wherein the aspartic acid residues at positions 203, 218, and/or 269 (using the numbering shown in Figure 1) are substituted by alanine residues. Optionally, the Apo2L variants may comprise one or more of the alanine substitutions which are recited in Table I of published PCT application WO 01/00832. Substitutional variants include one or more of the residue substitutions identified in Table I of WO 01/00832 published January 4, 2001. The definition also encompasses a native sequence Apo2L/TRAIL isolated from an Apo2L/TRAIL source or prepared by recombinant or synthetic methods. The Apo2L/TRAIL of the invention includes the polypeptides referred to as Apo2L/TRAIL or TRAIL disclosed in PCT Publication Nos. WO97/01633 and WO97/25428. The terms "Apo2L/TRAIL" or "Apo2L" are used to refer generally to forms of the Apo2L/TRAIL which include monomer, dimer or trimer forms of the polypeptide. All numbering of amino acid residues referred to in the Apo2L sequence use the numbering according to Figure 1, unless specifically stated otherwise. For instance, "D203" or "Asp203" refers to the aspartic acid residue at position 203 in the sequence provided in Figure 1.

The term "Apo2L/TRAIL extracellular domain" or "Apo2L/TRAIL ECD" refers to a form of Apo2L/TRAIL which is essentially free of transmembrane and cytoplasmic domains. Ordinarily, the ECD will have less than 1% of

such transmembrane and cytoplasmic domains, and preferably, will have less than 0.5% of such domains. It will be understood that any transmembrane domain(s) identified for the polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified. In preferred embodiments, the ECD will consist of a soluble, extracellular domain sequence of the polypeptide which is free of the transmembrane and cytoplasmic or intracellular domains (and is not membrane bound). Particular extracellular domain sequences of Apo-2L/TRAIL are described in PCT Publication Nos. WO97/01633 and WO97/25428.

The term "Apo2L/TRAIL monomer" or "Apo2L monomer" refers to a covalent chain of an extracellular domain sequence of Apo2L.

The term "Apo2L/TRAIL dimer" or "Apo2L dimer" refers to two Apo-2L monomers joined in a covalent linkage via a disulfide bond. The term as used herein includes free standing Apo2L dimers and Apo2L dimers that are within trimeric forms of Apo2L (i.e., associated with another, third Apo2L monomer).

The term "Apo2L/TRAIL trimer" or "Apo2L trimer" refers to three Apo2L monomers that are non-covalently associated.

The term "Apo2L/TRAIL aggregate" is used to refer to self-associated higher oligomeric forms of Apo2L/TRAIL, such as Apo2L/TRAIL trimers, which form, for instance, hexameric and nanomeric forms of Apo2L/TRAIL. Determination of the presence and quantity of Apo2L/TRAIL monomer, dimer, or trimer (or other aggregates) may be made using methods and assays known in the art (and using commercially available materials), such as native size exclusion HPLC ("SEC"), denaturing size exclusion using sodium dodecyl sulphate ("SDS-SEC"), reverse phase HPLC and capillary electrophoresis.

"Apo-2 ligand receptor" includes the receptors referred to in the art as "DR4" and "DR5" whose polynucleotide and polypeptide sequences are shown in Figures 2 and 3 respectively. Pan et al. have described the TNF receptor family member referred to as "DR4" (Pan et al., *Science*, 276:111-113 (1997); see also WO98/32856 published July 30, 1998; WO 99/37684 published July 29, 1999; WO 00/73349 published December 7, 2000; US 6,433,147 issued August 13, 2002; US 6,461,823 issued October 8, 2002, and US 6,342,383 issued January 29, 2002). Sheridan et al., *Science*, 277:818-821 (1997) and Pan et al., *Science*, 277:815-818 (1997) described another receptor for Apo2L/TRAIL (see also, WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998). This receptor is referred to as DR5 (the receptor has also been alternatively referred to as Apo-2; TRAIL-R, TR6, Tango-63, hAPO8, TRICK2 or KILLER; Screaton et al., *Curr.*

Biol., 7:693-696 (1997); Walczak et al., EMBO J., 16:5386-5387 (1997); Wu et al., Nature Genetics, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924 published August 2, 2001; US 2003/01255540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003). As described above, other receptors for Apo-2L include DcR1, DcR2, and OPG (see, Sheridan et al., supra; Marsters et al., supra; and Simonet et al., supra). The term "Apo-2L receptor" when used herein encompasses native sequence receptor and receptor variants. These terms encompass Apo-2L receptor expressed in a variety of mammals, including humans. Apo-2L receptor may be endogenously expressed as occurs naturally in a variety of human tissue lineages, or may be expressed by recombinant or synthetic methods. A "native sequence Apo-2L receptor" comprises a polypeptide having the same amino acid sequence as an Apo-2L receptor derived from nature. Thus, a native sequence Apo-2L receptor can have the amino acid sequence of naturally-occurring Apo-2L receptor from any mammal. Such native sequence Apo-2L receptor can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence Apo-2L receptor" specifically encompasses naturally-occurring truncated or secreted forms of the receptor (e.g., a soluble form containing, for instance, an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants. Receptor variants may include fragments or deletion mutants of the native sequence Apo-2L receptor. Figure 3A shows the 411 amino acid sequence of human DR5 as published in WO 98/51793 on November 19, 1998. A transcriptional splice variant of human DR5 is known in the art. This DR5 splice variant encodes the 440 amino acid sequence of human DR5 shown in Figures 3B and 3C as published in WO 98/35986 on August 20, 1998.

"Death receptor antibody" is used herein to refer generally to antibody or antibodies directed to a receptor in the tumor necrosis factor receptor superfamily and containing a death domain capable of signalling apoptosis, and such antibodies include DR5 antibody and DR4 antibody.

"DR5 receptor antibody", "DR5 antibody", or "anti-DR5 antibody" is used in a broad sense to refer to antibodies that bind to at least one form of a DR5 receptor or extracellular domain thereof. Optionally the DR5 antibody is fused or linked to a heterologous sequence or molecule. Preferably the heterologous sequence allows or assists the antibody to

form higher order or oligomeric complexes. Optionally, the DR5 antibody binds to DR5 receptor but does not bind or cross-react with any additional Apo-2L receptor (e.g. DR4, DcR1, or DcR2). Optionally the antibody is an agonist of DR5 signalling activity.

5 Optionally, the DR5 antibody of the invention binds to a DR5 receptor at a concentration range of about 0.1 nM to about 20 mM as measured in a BIAcore binding assay. Optionally, the DR5 antibodies of the invention exhibit an $I_c 50$ value of about 0.6 nM to about 18 mM as measured in a BIAcore binding assay.

10 "DR4 receptor antibody", "DR4 antibody", or "anti-DR4 antibody" is used in a broad sense to refer to antibodies that bind to at least one form of a DR4 receptor or extracellular domain thereof. Optionally the DR4 antibody is fused or linked to a heterologous sequence or molecule. Preferably the heterologous sequence allows or assists the antibody to
15 form higher order or oligomeric complexes. Optionally, the DR4 antibody binds to DR4 receptor but does not bind or cross-react with any additional Apo-2L receptor (e.g. DR5, DcR1, or DcR2). Optionally the antibody is an agonist of DR4 signalling activity.

20 Optionally, the DR4 antibody of the invention binds to a DR4 receptor at a concentration range of about 0.1 nM to about 20 mM as measured in a BIAcore binding assay. Optionally, the DR5 antibodies of the invention exhibit an $I_c 50$ value of about 0.6 nM to about 18 mM as measured in a BIAcore binding assay.

25 The term "agonist" is used in the broadest sense, and includes any molecule that partially or fully enhances, stimulates or activates one or more biological activities of Apo2L/TRAIL, DR4 or DR5, *in vitro*, *in situ*, or *in vivo*. Examples of such biological activities binding of Apo2L/TRAIL to DR4 or DR5, include apoptosis as well as those further reported in the literature. An agonist may function in a direct or indirect manner. For
30 instance, the agonist may function to partially or fully enhance, stimulate or activate one or more biological activities of DR4 or DR5, *in vitro*, *in situ*, or *in vivo* as a result of its direct binding to DR4 or DR5, which causes receptor activation or signal transduction. The agonist may also function indirectly to partially or fully enhance, stimulate or
35 activate one or more biological activities of DR4 or DR5, *in vitro*, *in situ*, or *in vivo* as a result of, e.g., stimulating another effector molecule which then causes DR4 or DR5 activation or signal transduction. It is contemplated that an agonist may act as an enhancer molecule which functions indirectly to enhance or increase DR4 or DR5 activation or
40 activity. For instance, the agonist may enhance activity of endogenous Apo-2L in a mammal. This could be accomplished, for example, by pre-complexing DR4 or DR5 or by stabilizing complexes of the respective ligand

with the DR4 or DR5 receptor (such as stabilizing native complex formed between Apo-2L and DR4 or DR5).

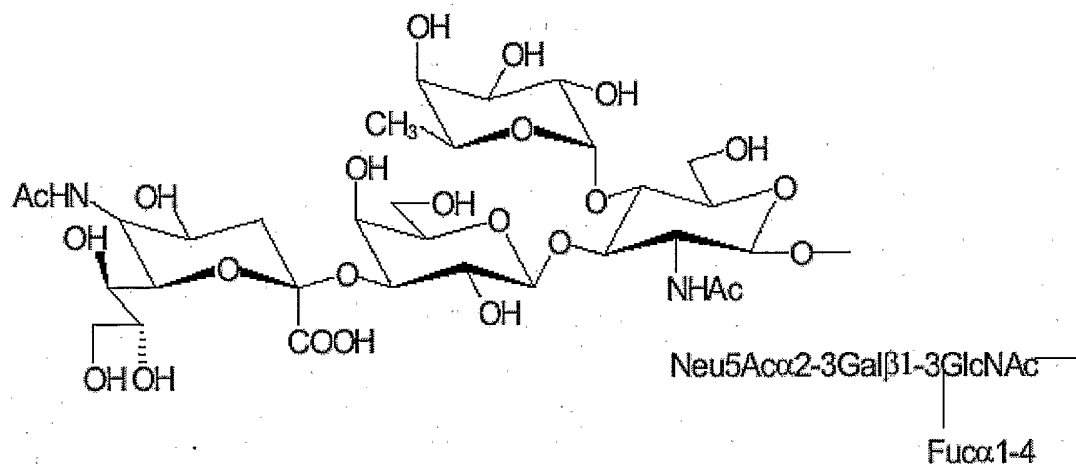
The term "biomarker" as used in the present application refers generally to a molecule, including a gene, protein, carbohydrate structure, or glycolipid, the expression of which in or on a mammalian tissue or cell can be detected by standard methods (or methods disclosed herein) and is predictive for a mammalian cell's or tissue's sensitivity to Apo2L/TRAIL or death receptor antibody. Such biomarkers contemplated by the present invention include but are not limited to "(1,3/1,4) fucosyltransferase" or "FUT3", "alpha (1,3) fucosyltransferase" or "FUT6", "Sialyl Lewis A", and "Sialyl Lewis X". Optionally, the expression of such a biomarker is determined to be higher than that observed for a control tissue or cell sample. Optionally, for example, the expression of such a biomarker will be determined in a PCR or FACS assay to be at least 50-fold, or preferably at least 100-fold higher in the test tissue or cell sample than that observed for a control tissue or cell sample. Optionally, the expression of such a biomarker will be determined in an IHC assay to score at least 2 or higher for staining intensity.

"(1,3/1,4) fucosyltransferase" or "FUT3" is used herein to refer to a molecule having structural features as described herein and optionally, catalyzing the transfer of a fucose residue from the donor substrate, GDP-fucose, to an acceptor substrate in an α 3- or α 4- linkage to GlcNAc (FUTs III-VII and IX). The DNA sequence and amino acid sequence for human FUT3 is provided in Figure 4. These sequences correspond to GenBank Accession Number HSU27328 and are described for example in Kukowska-Latallo et al., Genes Dev. 1990 Aug;4(8):1288-303. FUTs generally are type II transmembrane glycoproteins residing in the Golgi vacuoles, and typically composed of an N-terminal cytoplasmic tail, a membrane-spanning region, and a catalytic domain oriented lumenally in the Golgi apparatus. Between the membrane-spanning region and the catalytic domain is a region called the stem (Paulson and Colley, J. Biol. Chem., 264:17615-17618 (1989)).

"alpha (1,3) fucosyltransferase" or "FUT6" is used herein to refer to a molecule which structurally relates to, e.g, the DNA sequence and amino acid sequence for human FUT6 provided in Figure 5. These sequences correspond to GenBank Accession Number HSU27333 and are described for example in Koszdin and Bowen, Biochem Biophys Res Commun. 1992 Aug 31;187(1):152-7. FUT 6 is typically expressed in epithelial cells and in liver, kidney, and gastrointestinal tissues, specifically, stomach, jejunum and colon (and typically minimally expressed in spleen, lung and cervix uteri). FUT 6 is typically not detected in brain, adrenal cortex, or peripheral blood leukocytes.

"Sialyl Lewis A" is used herein to refer to a tetrasaccharide carbohydrate structure or antigen having the following sequence or structure, which may be membrane bound or in soluble form, circulating, for instance, in serum:

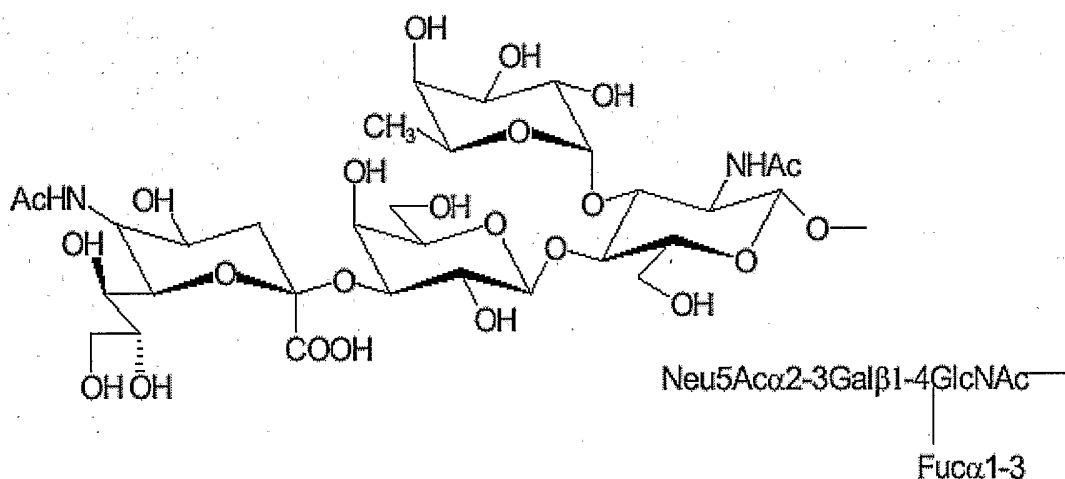
- 5 NeuAc α 2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 3[Fuc α 1 $\rightarrow$ 4]GlcNAc β 1 $\rightarrow$ R (NeuAc α 2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 3(Fucal α 1 $\rightarrow$ 4)GlcNAc β 1 $\rightarrow$ R)



- 10 "Sialyl Lewis X" is used herein to refer to tetrasaccharide carbohydrate structure or antigen having the following sequence or structure, which may be membrane bound or in soluble form, circulating, for instance, in serum:

NeuAc α 2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4[Fuc α 1 $\rightarrow$ 3]GlcNAc β 1 $\rightarrow$ R (NeuAc α 2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4(Fucal α 1 $\rightarrow$ 3)GlcNAc β 1 $\rightarrow$ R)

15



By "subject" or "patient" is meant any single subject for which therapy is desired, including humans, cattle, dogs, guinea pigs, rabbits, chickens, insects and so on. Also intended to be included as a subject are any subjects involved in clinical research trials not showing any clinical sign of disease, or subjects involved in epidemiological studies, or subjects used as controls.

The term "mammal" as used herein refers to any mammal classified as a mammal, including humans, cows, horses, dogs and cats. In a preferred embodiment of the invention, the mammal is a human.

By "tissue or cell sample" is meant a collection of similar cells obtained from a tissue of a subject or patient. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may also be primary or cultured cells or cell lines. Optionally, the tissue or cell sample is obtained from a primary or metastatic tumor. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

For the purposes herein a "section" of a tissue sample is meant a single part or piece of a tissue sample, e.g. a thin slice of tissue or cells cut from a tissue sample. It is understood that multiple sections of tissue samples may be taken and subjected to analysis according to the present invention, provided that it is understood that the present invention comprises a method whereby the same section of tissue sample is analyzed at both morphological and molecular levels, or is analyzed with respect to both protein and nucleic acid.

By "correlate" or "correlating" is meant comparing, in any way, the performance and/or results of a first analysis or protocol with the performance and/or results of a second analysis or protocol. For example, one may use the results of a first analysis or protocol in carrying out a second protocols and/or one may use the results of a first analysis or protocol to determine whether a second analysis or protocol should be performed. With respect to the embodiment of immunohistochemical analysis or protocol one may use the results of IHC to determine whether a specific therapeutic regimen should be performed.

By "nucleic acid" is meant to include any DNA or RNA. For example, chromosomal, mitochondrial, viral and/or bacterial nucleic acid present in tissue sample. The term "nucleic acid" encompasses either or both strands of a double stranded nucleic acid molecule and includes any fragment or portion of an intact nucleic acid molecule.

By "gene" is meant any nucleic acid sequence or portion thereof with a functional role in encoding or transcribing a protein or regulating other gene expression. The gene may consist of all the nucleic acids responsible for encoding a functional protein or only a portion of the nucleic acids responsible for encoding or expressing a protein. The nucleic acid sequence may contain a genetic abnormality within exons, introns, initiation or termination regions, promoter sequences, other regulatory sequences or unique adjacent regions to the gene.

The word "label" when used herein refers to a compound or composition which is conjugated or fused directly or indirectly to a reagent such as a nucleic acid probe or an antibody and facilitates detection of the reagent to which it is conjugated or fused. The label may itself be detectable (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed

throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable or complementary determining regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cell-mediated cytotoxicity (ADCC).

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H - V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

5 Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains
10 that correspond to the different classes of antibodies are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

"Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single
15 polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

20 The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain ($V_H - V_L$). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced
25 to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

The term "monoclonal antibody" as used herein refers to an antibody
30 obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional
35 (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture,
40 uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular

method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The
5 "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., *Nature*, 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light
10 chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or
15 subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g.
20 Old World Monkey, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780).

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human
25 immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues
30 of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at
35 least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region
40 (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

An antibody "which binds" an antigen of interest is one capable of binding that antigen with sufficient affinity and/or avidity such that the antibody is useful as a therapeutic or diagnostic agent for targeting a cell expressing the antigen.

For the purposes herein, "immunotherapy" will refer to a method of treating a mammal (preferably a human patient) with an antibody, wherein the antibody may be an unconjugated or "naked" antibody, or the antibody may be conjugated or fused with heterologous molecule(s) or agent(s), such as one or more cytotoxic agent(s), thereby generating an "immunoconjugate".

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antagonist or antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The expression "effective amount" refers to an amount of an agent (e.g. Apo2L/TRAIL, anti-DR4 or DR5 antibody etc.) which is effective for preventing, ameliorating or treating the disease or condition in question.

The terms "treating", "treatment" and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy. Consecutive treatment or administration refers to treatment on at least a daily basis without interruption in treatment by one or more days. Intermittent treatment or administration, or treatment or administration in an intermittent fashion, refers to treatment that is not consecutive, but rather cyclic in nature.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , - β , and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-13, IL-17; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., I^{131} , I^{125} , Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepea and cyclophosphamide (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine,

triethylenephosphoramidate, triethylenethiophosphoramidate and
 trimethylolomelamine; acetogenins (especially bullatacin and
 bullatacinone); a camptothecin (including the synthetic analogue
 topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin,
 5 carzelesin and bizelesin synthetic analogues); cryptophycins (particularly
 cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the
 synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a
 sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil,
 chlornaphazine, cholophosphamide, estramustine, ifosfamide,
 10 mechlorethamine, mechlorethamine oxide hydrochloride, melphalan,
 novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard;
 nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine,
 nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g.
 calicheamicin, especially calicheamicin gamma1I and calicheamicin phi1I,
 15 see, e.g., Agnew, Chem Intl. Ed. Engl., 33:183-186 (1994); dynemicin,
 including dynemicin A; bisphosphonates, such as clodronate; an esperamicin;
 as well as neocarzinostatin chromophore and related chromoprotein enediyne
 antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin,
 azaserine, bleomycins, cactinomycin, carabycin, carminomycin,
 20 carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-
 diazo-5-oxo-L-norleucine, doxorubicin (AdriamycinTM) (including morpholino-
 doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and
 deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin,
 mitomycins such as mitomycin C, mycophenolic acid, nogalamycin,
 25 olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin,
 streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin;
 anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid
 analogues such as denopterin, methotrexate, pteropterin, trimetrexate;
 purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine,
 30 thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-
 azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine,
 enocitabine, floxuridine; androgens such as calusterone, dromostanolone
 propionate, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as
 aminoglutethimide, mitotane, trilostane; folic acid replenisher such as
 35 frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid;
 eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine;
 demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone;
 etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine;
 maytansinoids such as maytansine and ansamitocins; mitoguazone;
 40 mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin;
 losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK[®];
 razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid;
 triaziquone; 2, 2',2''-trichlorotriethylamine; trichothecenes (especially

T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL[®], Bristol-Myers Squibb Oncology, Princeton, NJ) and doxetaxel (TAXOTERE[®], Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine (Gemzar[™]); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (Navelbine[™]); novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including Nolvadex[™]), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston[™]); aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate (Megace[™]), exemestane, formestane, fadrozole, vorozole (Rivisor[™]), letrozole (Femara[™]), and anastrozole (Arimidex[™]); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially cancer cell overexpressing any of the genes identified herein, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13.

The terms "apoptosis" and "apoptotic activity" are used in a broad sense and refer to the orderly or controlled form of cell death in mammals that is typically accompanied by one or more characteristic cell changes, including condensation of cytoplasm, loss of plasma membrane microvilli, segmentation of the nucleus, degradation of chromosomal DNA or loss of mitochondrial function. This activity can be determined and measured, for instance, by cell viability assays (such as Alamar blue assays or MTT assays), FACS analysis, caspase activation, DNA fragmentation (see, for example, Nicoletti et al., J. Immunol. Methods, 139:271-279 (1991), and poly-ADP ribose polymerase, "PARP", cleavage assays known in the art.

As used herein, the term "disorder" in general refers to any condition that would benefit from treatment with the compositions described herein, including any disease or disorder that can be treated by effective amounts of Apo2L/TRAIL, an anti-DR4 antibody, and/or an anti-DR5 antibody. This includes chronic and acute disorders, as well as those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant cancers; inflammatory, angiogenic, and immunologic disorders, autoimmune disorders, arthritis (including rheumatoid arthritis), multiple sclerosis, and HIV/AIDS.

The terms "cancer", "cancerous", or "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, leukemia, blastoma, and sarcoma. More particular examples of such cancers include squamous cell carcinoma, myeloma, small-cell lung cancer, non-small cell lung cancer, glioma, gastrointestinal (tract) cancer, renal cancer, ovarian cancer, liver cancer, lymphoblastic leukemia, lymphocytic leukemia, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, brain cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer.

The term "immune related disease" means a disease in which a component of the immune system of a mammal causes, mediates or otherwise contributes to morbidity in the mammal. Also included are diseases in which stimulation or intervention of the immune response has an ameliorative effect on progression of the disease. Included within this term are autoimmune diseases, immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, and immunodeficiency diseases. Examples of immune-related and inflammatory diseases, some of which are immune or T cell mediated, which can be treated according to the invention include systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies,

systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjogren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory and fibrotic lung diseases such as inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus-host-disease. Infectious diseases include AIDS (HIV infection), hepatitis A, B, C, D, and E, bacterial infections, fungal infections, protozoal infections and parasitic infections.

"Autoimmune disease" is used herein in a broad, general sense to refer to disorders or conditions in mammals in which destruction of normal or healthy tissue arises from humoral or cellular immune responses of the individual mammal to his or her own tissue constituents. Examples include, but are not limited to, lupus erythematosus, thyroiditis, rheumatoid arthritis, psoriasis, multiple sclerosis, autoimmune diabetes, and inflammatory bowel disease (IBD).

The term "tagged" when used herein refers to a chimeric molecule comprising an antibody or polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made or to provide some other function, such as the ability to oligomerize (e.g. as occurs with peptides having leucine zipper domains), yet is short enough such that it generally does not interfere with activity of the antibody or polypeptide. The tag polypeptide preferably also is fairly unique so that a tag-specific antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about

8 to about 50 amino acid residues (preferably, between about 10 to about 20 residues).

The term "divalent metal ion" refers to a metal ion having two positive charges. Examples of divalent metal ions include but are not limited to zinc, cobalt, nickel, cadmium, magnesium, and manganese. Particular forms of such metals that may be employed include salt forms (e.g., pharmaceutically acceptable salt forms), such as chloride, acetate, carbonate, citrate and sulfate forms of the above mentioned divalent metal ions. Optionally, a divalent metal ion for use in the present invention is zinc, and preferably, the salt form, zinc sulfate or zinc chloride.

"Isolated," when used to describe the various peptides or proteins disclosed herein, means peptide or protein that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the peptide or protein, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the peptide or protein will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain, or (3) to homogeneity by mass spectroscopic or peptide mapping techniques. Isolated material includes peptide or protein in situ within recombinant cells, since at least one component of its natural environment will not be present. Ordinarily, however, isolated peptide or protein will be prepared by at least one purification step.

"Percent (%) amino acid sequence identity" with respect to the sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art can determine appropriate parameters for measuring alignment, including assigning algorithms needed to achieve maximal alignment over the full-length sequences being compared. For purposes herein, percent amino acid identity values can be obtained using the sequence comparison computer program, ALIGN-2, which was authored by Genentech, Inc. and the source code of which has been filed with user documentation in the US Copyright Office, Washington, DC, 20559, registered under the US Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San

Francisco, CA. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to re-anneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired identity between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

"High stringency conditions", as defined herein, are identified by those that: (1) employ low ionic strength and high temperature for washing; 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent; 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The term "primer" or "primers" refers to oligonucleotide sequences that hybridize to a complementary RNA or DNA target polynucleotide and serve as the starting points for the stepwise synthesis of a polynucleotide from mononucleotides by the action of a

nucleotidyltransferase, as occurs for example in a polymerase chain reaction.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. *PNAS (USA)* 95:652-656 (1998).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and carry out ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred.

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel et al., *Immunomethods* 4:25-34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)). FcRs herein include polymorphisms such as the genetic dimorphism in the gene that encodes FcγRIIIa resulting in either a phenylalanine (F) or a valine (V) at amino acid position 158, located in the region of the receptor that binds to IgG1. The homozygous valine FcγRIIIa (FcγRIIIa-158V) has been shown to have a higher affinity for human IgG1 and mediate increased ADCC *in vitro* relative to homozygous phenylalanine FcγRIIIa (FcγRIIIa-158F) or heterozygous (FcγRIIIa-158F/V) receptors.

"Complement dependent cytotoxicity" or "CDC" refer to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996), may be performed.

II. TYPICAL METHODS AND MATERIALS OF THE INVENTION

The methods and assays disclosed herein are directed to the examination of expression of one or more biomarkers in a mammalian tissue or cell sample, wherein the determination of that expression of one or more such biomarkers is predictive or indicative of whether the tissue or cell sample will be sensitive to apoptosis-inducing agents such as Apo2L/TRAIL and anti-DR5 agonist antibodies. The methods and assays include those which examine expression of biomarkers such as certain

fucosyltransferases, in particular fucosyltransferase 3 (FUT3) and/or fucosyltransferase 6 (FUT6), as well as sialyl Lewis A and/or X antigens.

As discussed above, there are some populations of diseased human cell types (such as certain populations of cancer cells) which are resistant to apoptosis induction. It is therefore believed that the disclosed methods and assays can provide for convenient, efficient, and potentially cost-effective means to obtain data and information useful in assessing appropriate or effective therapies for treating patients. For example, a patient having been diagnosed with cancer or an immune related condition could have a biopsy performed to obtain a tissue or cell sample, and the sample could be examined by way of various *in vitro* assays to determine whether the patient's cells would be sensitive to a therapeutic agent such as Apo2L/TRAIL or death receptor antibody.

The invention provides methods for predicting the sensitivity of a mammalian tissue or cell sample (such as a cancer cell) to Apo2L/TRAIL or a death receptor agonist antibody. In the methods, a mammalian tissue or cell sample is obtained and examined for expression of one or more biomarkers. The methods may be conducted in a variety of assay formats, including assays detecting mRNA expression, enzymatic assays detecting presence of enzymatic activity, and immunohistochemistry assays. Determination of expression of such biomarkers in said tissues or cells will be predictive that such tissues or cells will be sensitive to the apoptosis-inducing activity of Apo2L/TRAIL and/or death receptor antibody. Applicants surprisingly found that the expression of such particular biomarkers correlates with the sensitivity of such tissues and cells to apoptosis-inducing agents such as Apo2L/TRAIL and death receptor agonist antibodies.

As discussed below, expression of various biomarkers in a sample can be analyzed by a number of methodologies, many of which are known in the art and understood by the skilled artisan, including but not limited to, immunohistochemical and/or Western analysis, quantitative blood based assays (as for example Serum ELISA) (to examine, for example, levels of protein expression), biochemical enzymatic activity assays, *in situ* hybridization, Northern analysis and/or PCR analysis of mRNAs, and genomic Southern analysis (to examine, for example, gene deletion or amplification), as well as any one of the wide variety of assays that can be performed by gene and/or tissue array analysis. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel et al. eds., 1995, Current Protocols In Molecular Biology, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis).

The protocols below relating to detection of particular biomarkers, such as fucosyltransferase 3 (FUT3), fucosyltransferase 6 (FUT6), Sialyl

Lewis A, and Sialyl Lewis X, in a sample are provided below for illustrative purposes.

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Optional methods of the invention include protocols which examine or test for presence of sialyl Lewis A and/or sialyl Lewis X proteins in a mammalian tissue or cell sample. A variety of methods for detecting sialyl Lewis A and/or sialyl Lewis X -related protein can be employed and include, for example, immunohistochemical analysis, immunoprecipitation, Western blot analysis, molecular binding assays, ELISA, ELIFA, fluorescence activated cell sorting (FACS) and the like. For example, an optional method of detecting the expression of sialyl Lewis A and/or sialyl Lewis X-related protein in a tissue or sample comprises contacting the sample with a sialyl Lewis A and/or sialyl Lewis X antibody, a sialyl Lewis A and/or sialyl Lewis X-reactive fragment thereof, or a recombinant protein containing an antigen binding region of a sialyl Lewis A and/or sialyl Lewis X antibody; and then detecting the binding of sialyl Lewis A and/or sialyl Lewis X-related protein in the sample.

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In particular embodiments of the invention, the expression of sialyl Lewis A and/or sialyl Lewis X proteins in a sample is examined using immunohistochemistry and staining protocols. Immunohistochemical staining of tissue sections has been shown to be a reliable method of assessing or detecting presence of proteins in a sample. Immunohistochemistry ("IHC") techniques utilize an antibody to probe and visualize cellular antigens *in situ*, generally by chromogenic or fluorescent methods.

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For sample preparation, a tissue or cell sample from a mammal (typically a human patient) may be used. Examples of samples include, but are not limited to, cancer cells such as colon, breast, prostate, ovary, lung, stomach, pancreas, lymphoma, and leukemia cancer cells. The sample can be obtained by a variety of procedures known in the art including, but not limited to surgical excision, aspiration or biopsy. The tissue may be fresh or frozen. In one embodiment, the sample is fixed and embedded in paraffin or the like.

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The tissue sample may be fixed (*i.e.* preserved) by conventional methodology (See *e.g.*, "Manual of Histological Staining Method of the Armed Forces Institute of Pathology," 3rd edition (1960) Lee G. Luna, HT (ASCP) Editor, The Blakston Division McGraw-Hill Book Company, New York; *The Armed Forces Institute of Pathology Advanced Laboratory Methods in Histology and Pathology* (1994) Ulreka V. Mikel, Editor, Armed Forces Institute of Pathology, American Registry of Pathology, Washington, D.C.). One of skill in the art will appreciate that the choice of a fixative is determined by the purpose for which the sample is to be histologically stained or otherwise analyzed. One of skill in the art will also

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appreciate that the length of fixation depends upon the size of the tissue sample and the fixative used. By way of example, neutral buffered formalin, Bouin's or paraformaldehyde, may be used to fix a sample.

Generally, the sample is first fixed and is then dehydrated through
5 an ascending series of alcohols, infiltrated and embedded with paraffin or other sectioning media so that the tissue sample may be sectioned. Alternatively, one may section the tissue and fix the sections obtained. By way of example, the tissue sample may be embedded and processed in paraffin by conventional methodology (See e.g., "Manual of Histological
10 Staining Method of the Armed Forces Institute of Pathology", *supra*). Examples of paraffin that may be used include, but are not limited to, Paraplast, Broloid, and Tissuemay. Once the tissue sample is embedded, the sample may be sectioned by a microtome or the like (See e.g., "Manual
15 of Histological Staining Method of the Armed Forces Institute of Pathology", *supra*). By way of example for this procedure, sections may range from about three microns to about five microns in thickness. Once sectioned, the sections may be attached to slides by several standard methods. Examples of slide adhesives include, but are not limited to, silane, gelatin, poly-L-lysine and the like. By way of example, the
20 paraffin embedded sections may be attached to positively charged slides and/or slides coated with poly-L-lysine.

If paraffin has been used as the embedding material, the tissue
sections are generally deparaffinized and rehydrated to water. The tissue
sections may be deparaffinized by several conventional standard
25 methodologies. For example, xylenes and a gradually descending series of alcohols may be used (See e.g., "Manual of Histological Staining Method of the Armed Forces Institute of Pathology", *supra*). Alternatively, commercially available deparaffinizing non-organic agents such as Hemo-De7 (CMS, Houston, Texas) may be used.

30 Optionally, subsequent to the sample preparation, a tissue section may be analyzed using IHC. IHC may be performed in combination with additional techniques such as morphological staining and/or fluorescence *in-situ* hybridization. Two general methods of IHC are available; direct and indirect assays. According to the first assay, binding of antibody to
35 the target antigen (e.g., sialyl Lewis A and/or sialyl Lewis X) is determined directly. This direct assay uses a labeled reagent, such as a fluorescent tag or an enzyme-labeled primary antibody, which can be visualized without further antibody interaction. In a typical indirect assay, unconjugated primary antibody binds to the antigen and then a
40 labeled secondary antibody binds to the primary antibody. Where the secondary antibody is conjugated to an enzymatic label, a chromogenic or fluorogenic substrate is added to provide visualization of the antigen.

Signal amplification occurs because several secondary antibodies may react with different epitopes on the primary antibody.

The primary and/or secondary antibody used for immunohistochemistry typically will be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories:

(a) Radioisotopes, such as ^{35}S , ^{14}C , ^{125}I , ^3H , and ^{131}I . The antibody can be labeled with the radioisotope using the techniques described in *Current Protocols in Immunology*, Volumes 1 and 2, Coligen et al., Ed. Wiley-Interscience, New York, New York, Pubs. (1991) for example and radioactivity can be measured using scintillation counting.

(b) Colloidal gold particles.

(c) Fluorescent labels including, but are not limited to, rare earth chelates (europium chelates), Texas Red, rhodamine, fluorescein, dansyl, Lissamine, umbelliferone, phycocrytherin, phycocyanin, or commercially available fluorophores such SPECTRUM ORANGE7 and SPECTRUM GREEN7 and/or derivatives of any one or more of the above. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in *Current Protocols in Immunology*, supra, for example. Fluorescence can be quantified using a fluorimeter.

(d) Various enzyme-substrate labels are available and U.S. Patent No. 4,275,149 provides a review of some of these. The enzyme generally catalyzes a chemical alteration of the chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al., *Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay*, in *Methods in Enzym.* (ed. J. Langone & H. Van Vunakis), Academic press, New York, 73:147-166 (1981).

Examples of enzyme-substrate combinations include, for example:

(i) Horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB));

5 (ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and

(iii) β -D-galactosidase (β -D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl- β -D-galactosidase) or fluorogenic substrate (e.g., 4-methylumbelliferyl- β -D-galactosidase).

10 Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see U.S. Patent Nos. 4,275,149 and 4,318,980. Sometimes, the label is indirectly conjugated with the antibody. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody can be
15 conjugated with biotin and any of the four broad categories of labels mentioned above can be conjugated with avidin, or *vice versa*. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated
20 with a small hapten and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody. Thus, indirect conjugation of the label with the antibody can be achieved.

Aside from the sample preparation procedures discussed above, further treatment of the tissue section prior to, during or following IHC
25 may be desired. For example, epitope retrieval methods, such as heating the tissue sample in citrate buffer may be carried out (see, e.g., Leong *et al. Appl. Immunohistochem.* 4(3):201 (1996)).

Following an optional blocking step, the tissue section is exposed to primary antibody for a sufficient period of time and under suitable
30 conditions such that the primary antibody binds to the target protein antigen in the tissue sample. Appropriate conditions for achieving this can be determined by routine experimentation. The extent of binding of antibody to the sample is determined by using any one of the detectable labels discussed above. Preferably, the label is an enzymatic label (e.g.
35 HRPO) which catalyzes a chemical alteration of the chromogenic substrate such as 3,3'-diaminobenzidine chromogen. Preferably the enzymatic label is conjugated to antibody which binds specifically to the primary antibody (e.g. the primary antibody is rabbit polyclonal antibody and secondary antibody is goat anti-rabbit antibody).

40 Optionally, the antibodies employed in the IHC analysis to detect expression of sialyl Lewis A or anti-sialyl Lewis X, are anti-sialyl Lewis A and anti-sialyl Lewis X antibody, respectively. Optionally, the

anti-sialyl Lewis A and the anti-sialyl Lewis X antibody is a monoclonal antibody. Anti-sialyl Lewis A and an anti-sialyl Lewis X antibodies are readily available in the art, including from various commercial sources.

Specimens thus prepared may be mounted and coverslipped. Slide evaluation is then determined, e.g. using a microscope, and staining intensity criteria, routinely used in the art, may be employed. Where the antigen is sialyl Lewis A and/or sialyl Lewis X protein, staining intensity criteria may be evaluated as follows:

TABLE 1

Staining Pattern	Score
No staining is observed in cells.	0
Faint/barely perceptible staining is detected in more than 10% of the cells.	1+
Weak to moderate staining is observed in more than 10% of the cells.	2+
Moderate to strong staining is observed in more than 10% of the cells.	3+

Typically, a staining pattern score of about 2+ or higher in such an IHC assay is believed to be predictive or indicative of sensitivity of a mammalian cell (such as a mammalian cancer cell) to Apo2L/TRAIL or a death receptor agonist antibody.

In alternative methods, the sample may be contacted with an antibody specific for said biomarker under conditions sufficient for an antibody-biomarker complex to form, and then detecting said complex. The presence of the biomarker may be accomplished in a number of ways, such as by Western blotting and ELISA procedures for assaying a wide variety of tissues and samples, including plasma or serum. A wide range of immunoassay techniques using such an assay format are available, see, e.g., U.S. Pat. Nos. 4,016,043, 4,424,279 and 4,018,653. These include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target biomarker.

Sandwich assays are among the most useful and commonly used assays. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate, and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time

sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of biomarker.

Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In a typical forward sandwich assay, a first antibody having specificity for the biomarker is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to 40°C such as between 25° C and 32° C inclusive) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the biomarker. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the molecular marker.

An alternative method involves immobilizing the target biomarkers in the sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule. By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the

detection of antigen-bound antibody. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

5 In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose
10 oxidase, -galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic
15 substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-molecular marker complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-
20 antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of biomarker which was present in the sample. Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be
25 chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in
30 the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-molecular marker complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the molecular marker of interest. Immunofluorescence and EIA
35 techniques are both very well established in the art. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

It is contemplated that the above described techniques may also be employed to detect expression of FUT3 or FUT 6 polypeptides.

40 Methods of the invention further include protocols which examine the presence and/or expression of mRNAs, such as FUT3 and/or FUT6 mRNAs, in a tissue or cell sample. Methods for the evaluation of mRNAs in cells are well known and include, for example, hybridization assays using

complementary DNA probes (such as *in situ* hybridization using labeled FUT3 and/or FUT6 riboprobes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for FUT3 and/or FUT6, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like).

Tissue or cell samples from mammals can be conveniently assayed for, e.g., FUT3 and/or FUT6 mRNAs using Northern, dot blot or PCR analysis. For example, RT-PCR assays such as quantitative PCR assays are well known in the art. In an illustrative embodiment of the invention, a method for detecting an FUT3 and/or FUT6 mRNA in a biological sample comprises producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using an FUT3 and/or FUT6 polynucleotide as sense and antisense primers to amplify FUT3 and/or FUT6 cDNAs therein; and detecting the presence of the amplified FUT3 and/or FUT6 cDNA. In addition, such methods can include one or more steps that allow one to determine the levels of FUT3 and/or FUT6 mRNA in a biological sample (e.g. by simultaneously examining the levels a comparative control mRNA sequence of a "housekeeping" gene such as an actin family member). Optionally, the sequence of the amplified FUT3 and/or FUT6 cDNA can be determined.

Material embodiments of this aspect of the invention include FUT3 and/or FUT6 primers and primer pairs, which allow the specific amplification of the polynucleotides of the invention or of any specific parts thereof, and probes that selectively or specifically hybridize to nucleic acid molecules of the invention or to any part thereof. Probes may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Such probes and primers can be used to detect the presence of FUT3 and/or FUT6 polynucleotides in a sample and as a means for detecting a cell expressing FUT3 and/or FUT6 proteins. As will be understood by the skilled artisan, a great many different primers and probes may be prepared based on the sequences provided in herein and used effectively to amplify, clone and/or determine the presence and/or levels of FUT3 and/or FUT6 mRNAs.

Optional methods of the invention include protocols which examine or detect mRNAs, such as FUT3 and FUT6 or other fucosyltransferase mRNAs, in a tissue or cell sample by microarray technologies. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes

that have potential to be expressed in certain disease states may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene. Differential gene expression analysis of disease tissue can provide valuable information. Microarray technology utilizes nucleic acid hybridization techniques and computing technology to evaluate the mRNA expression profile of thousands of genes within a single experiment. (see, e.g., WO 01/75166 published October 11, 2001; (See, for example, U.S. 5,700,637, U.S. Patent 5,445,934, and U.S. Patent 5,807,522, Lockart, *Nature Biotechnology*, 14:1675-1680 (1996); Cheung, V.G. et al., *Nature Genetics* 21(Suppl):15-19 (1999) for a discussion of array fabrication). DNA microarrays are miniature arrays containing gene fragments that are either synthesized directly onto or spotted onto glass or other substrates. Thousands of genes are usually represented in a single array. A typical microarray experiment involves the following steps: 1. preparation of fluorescently labeled target from RNA isolated from the sample, 2. hybridization of the labeled target to the microarray, 3. washing, staining, and scanning of the array, 4. analysis of the scanned image and 5. generation of gene expression profiles. Currently two main types of DNA microarrays are being used: oligonucleotide (usually 25 to 70 mers) arrays and gene expression arrays containing PCR products prepared from cDNAs. In forming an array, oligonucleotides can be either prefabricated and spotted to the surface or directly synthesized on to the surface (in situ).

The Affymetrix GeneChip® system is a commercially available microarray system which comprises arrays fabricated by direct synthesis of oligonucleotides on a glass surface. Probe/Gene Arrays: Oligonucleotides, usually 25 mers, are directly synthesized onto a glass wafer by a combination of semiconductor-based photolithography and solid phase chemical synthesis technologies. Each array contains up to 400,000 different oligos and each oligo is present in millions of copies. Since oligonucleotide probes are synthesized in known locations on the array, the hybridization patterns and signal intensities can be interpreted in terms of gene identity and relative expression levels by the Affymetrix Microarray Suite software. Each gene is represented on the array by a series of different oligonucleotide probes. Each probe pair consists of a perfect match oligonucleotide and a mismatch oligonucleotide. The perfect match probe has a sequence exactly complimentary to the particular gene and thus measures the expression of the gene. The mismatch probe differs from the perfect match probe by a single base substitution at the center base position, disturbing the binding of the target gene transcript. This helps to determine the background and nonspecific hybridization that contributes to the signal measured for the perfect match oligo. The

Microarray Suite software subtracts the hybridization intensities of the mismatch probes from those of the perfect match probes to determine the absolute or specific intensity value for each probe set. Probes are chosen based on current information from Genbank and other nucleotide repositories. The sequences are believed to recognize unique regions of the 3' end of the gene. A GeneChip Hybridization Oven ("rotisserie" oven) is used to carry out the hybridization of up to 64 arrays at one time. The fluidics station performs washing and staining of the probe arrays. It is completely automated and contains four modules, with each module holding one probe array. Each module is controlled independently through Microarray Suite software using preprogrammed fluidics protocols. The scanner is a confocal laser fluorescence scanner which measures fluorescence intensity emitted by the labeled cRNA bound to the probe arrays. The computer workstation with Microarray Suite software controls the fluidics station and the scanner. Microarray Suite software can control up to eight fluidics stations using preprogrammed hybridization, wash, and stain protocols for the probe array. The software also acquires and converts hybridization intensity data into a presence/absence call for each gene using appropriate algorithms. Finally, the software detects changes in gene expression between experiments by comparison analysis and formats the output into .txt files, which can be used with other software programs for further data analysis.

The expression of a selected biomarker may also be assessed by examining gene deletion or gene amplification. Gene deletion or amplification may be measured by any one of a wide variety of protocols known in the art, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization (e.g., FISH), using an appropriately labeled probe, cytogenetic methods or comparative genomic hybridization (CGH) using an appropriately labeled probe. By way of example, these methods may be employed to detect deletion of amplification of the FUT3 and/or FUT6 genes.

Additionally, one can examine the methylation status of the biomarker, such as the FUT3 and/or FUT6 gene, in a tissue or cell sample. Aberrant demethylation and/or hypermethylation of CpG islands in gene 5' regulatory regions frequently occurs in immortalized and transformed cells, and can result in altered expression of various genes. A variety of assays for examining methylation status of a gene are well known in the art. For example, one can utilize, in Southern hybridization approaches, methylation-sensitive restriction enzymes which cannot cleave sequences that contain methylated CpG sites to assess the methylation status of CpG islands. In addition, MSP (methylation specific PCR) can rapidly profile

the methylation status of all the CpG sites present in a CpG island of a given gene. This procedure involves initial modification of DNA by sodium bisulfite (which will convert all unmethylated cytosines to uracil) followed by amplification using primers specific for methylated versus unmethylated DNA. Protocols involving methylation interference can also be found for example in Current Protocols In Molecular Biology, Unit 12, Frederick M. Ausubel et al. eds., 1995; De Marzo et al., Am. J. Pathol. 155(6): 1985-1992 (1999); Brooks et al, Cancer Epidemiol. Biomarkers Prev., 1998, 7:531-536; and Lethe et al., Int. J. Cancer 76(6): 903-908 (1998).

Expression of a selected biomarker in a tissue or cell sample may also be examined by way of functional or activity-based assays. For instance, if the biomarker is an enzyme, one may conduct assays known in the art to determine or detect the presence of the given enzymatic activity in the tissue or cell sample.

In the methods of the present invention, it is contemplated that the tissue or cell sample may also be examined for the expression of Apo2L/TRAIL or receptors in the sample which bind Apo2L/TRAIL or death receptor antibody. As described above and in the art, it is presently believed Apo2L/TRAIL binds to at least five different receptors: DR4, DR5, DcR1, DcR2, and OPG. Using methods known in the art, including those described herein, the expression of Apo2L/TRAIL, DR4, DR5, DcR1, DcR2 and/or OPG can be detected on the mRNA level and on the protein level. As shown in Figures 10 and 11, data suggests that examining the tissue or cell sample for expression of DcR1 and/or DcR2 receptors may give further information as to whether the tissue or cell sample will be sensitive to either Apo2L/TRAIL or death receptor antibody. By way of example, the IHC techniques described above may be employed to detect the presence of one of more such molecules in the sample. It is contemplated that in methods in which a tissue or sample is being examined not only for the presence of a FUT or Lewis antigen marker, but also for the presence, e.g., DR4, DR5 or DcR1, separate slides may be prepared from the same tissue or sample, and each slide tested with a reagent specific for each specific biomarker or receptor. Alternatively, a single slide may be prepared from the tissue or cell sample, and antibodies directed to each biomarker or receptor may be used in connection with a multi-color staining protocol to allow visualization and detection of the respective biomarkers or receptors.

Subsequent to the determination that the tissue or cell sample expresses one or more of the biomarkers indicating the tissue or cell sample will be sensitive to the activity of Apo2L/TRAIL or death receptor antibody, it is contemplated that an effective amount of the Apo2L/TRAIL or death receptor antibody may be administered to the mammal to treat a

disorder, such as cancer or immune related disorder which is afflicting the mammal. Diagnosis in mammals of the various pathological conditions described herein can be made by the skilled practitioner. Diagnostic techniques are available in the art which allow, e.g., for the diagnosis or detection of cancer or immune related disease in a mammal. For instance, cancers may be identified through techniques, including but not limited to, palpation, blood analysis, x-ray, NMR and the like. Immune related diseases can also be readily identified.

The Apo2L/TRAIL or death receptor antibody can be administered in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Optionally, administration may be performed through mini-pump infusion using various commercially available devices.

Effective dosages and schedules for administering Apo2L/TRAIL or death receptor antibody may be determined empirically, and making such determinations is within the skill in the art. Single or multiple dosages may be employed. It is presently believed that an effective dosage or amount of Apo2L/TRAIL used alone may range from about 1 μ g/kg to about 100 mg/kg of body weight or more per day. Interspecies scaling of dosages can be performed in a manner known in the art, e.g., as disclosed in Mordenti et al., *Pharmaceut. Res.*, 8:1351 (1991).

When *in vivo* administration of Apo2L/TRAIL is employed, normal dosage amounts may vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 μ g/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

It is contemplated that yet additional therapies may be employed in the methods. The one or more other therapies may include but are not limited to, administration of radiation therapy, cytokine(s), growth inhibitory agent(s), chemotherapeutic agent(s), cytotoxic agent(s), tyrosine kinase inhibitors, ras farnesyl transferase inhibitors, angiogenesis inhibitors, and cyclin-dependent kinase inhibitors which are known in the art and defined further with particularity above. It is contemplated that such other therapies may be employed as an agent separate from the Apo2L/TRAIL or death receptor antibody. In addition, therapies

based on therapeutic antibodies that target tumor antigens such as Rituxan™ or Herceptin™ as well as anti-angiogenic antibodies such as anti-VEGF.

Preparation and dosing schedules for chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service Ed.*, M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of the Apo2L/TRAIL or death receptor antibody, or may be given simultaneously therewith.

It may be desirable to also administer antibodies against other antigens, such as antibodies which bind to CD20, CD11a, CD18, CD40, ErbB2, EGFR, ErbB3, ErbB4, vascular endothelial factor (VEGF), or other TNFR family members (such as OPG, TNFR1, TNFR2, GITR, Apo-3, TACI, BCMA, BR3). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be co-administered to the patient. Sometimes, it may be beneficial to also administer one or more cytokines to the patient. Following administration, treated cells *in vitro* can be analyzed. Where there has been *in vivo* treatment, a treated mammal can be monitored in various ways well known to the skilled practitioner. For instance, tumor cells can be examined pathologically to assay for necrosis or serum can be analyzed for immune system responses.

For use in the applications described or suggested above, kits or articles of manufacture are also provided by the invention. Such kits may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. For example, one of the container means may comprise a probe that is or can be detectably labeled. Such probe may be an antibody or polynucleotide specific for a FUT3 and/or FUT6 protein or a FUT3 and/or FUT6 gene or message, respectively. Where the kit utilizes nucleic acid hybridization to detect the target nucleic acid, the kit may also have containers containing nucleotide(s) for amplification of the target nucleic acid sequence and/or a container comprising a reporter-means, such as a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, florescent, or radioisotope label.

The kit of the invention will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. A label may be present on the container to indicate that the composition is used for a specific therapy or non-therapeutic application,

and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above.

The kits of the invention have a number of embodiments. A typical embodiment is a kit comprising a container, a label on said container, and a composition contained within said container; wherein the composition includes a primary antibody that binds to a FUT3 and/or FUT6 polypeptide sequence, the label on said container indicates that the composition can be used to evaluate the presence of FUT3 and/or FUT6 proteins in at least one type of mammalian cell, and instructions for using the FUT3 and/or FUT6 antibody for evaluating the presence of FUT3 and/or FUT6 proteins in at least one type of mammalian cell. The kit can further comprise a set of instructions and materials for preparing a tissue sample and applying antibody and probe to the same section of a tissue sample. The kit may include both a primary and secondary antibody, wherein the secondary antibody is conjugated to a label, e.g., an enzymatic label.

Another embodiment is a kit comprising a container, a label on said container, and a composition contained within said container; wherein the composition includes a polynucleotide that hybridizes to a complement of the FUT3 and/or FUT6 polynucleotide under stringent conditions, the label on said container indicates that the composition can be used to evaluate the presence of FUT3 and/or FUT6 in at least one type of mammalian cell, and instructions for using the FUT3 and/or FUT6 polynucleotide for evaluating the presence of FUT3 and/or FUT6 RNA or DNA in at least one type of mammalian cell.

Other optional components in the kit include one or more buffers (e.g., block buffer, wash buffer, substrate buffer, etc), other reagents such as substrate (e.g., chromogen) which is chemically altered by an enzymatic label, epitope retrieval solution, control samples (positive and/or negative controls), control slide(s) etc.

EXAMPLES

Various aspects of the invention are further described and illustrated by way of the examples that follow, none of which are intended to limit the scope of the invention.

METHODS AND MATERIALS:

Cell culture and cell lines.

The following human colorectal adenocarcinoma cell lines: HCT-8, COLO 205, HCT 116, SW403, LoVo, SW948, Caco-2, COLO 201, SW1417, DLD-1, CX-1, HCT-15, LS 180, RKO, RKO-AS45-1, SK-CO-1, SW480, SW620, SW837, CL-40, COLO-206F, COLO 320DM, COLO 320HSR, COLO-678, HT-29, KM12, LS1034, SW1116 were obtained from ATCC Depository (Manassas, Virginia), DSMZ (German Collection of Microorganisms and Cell Cultures), JCRB (Japanese

Cell Resources Bank) or ECACC (European Collection of Cell Cultures) and cultured in RPMI-1640 media supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine and 10 mM HEPES.

Cytotoxicity assays.

5 The MTT assay (CellTiter 96 ® Non-Radioactive Cell Proliferation Assay from Promega), which is a colorimetric assay based on the ability of viable cells to reduce a soluble yellow tetrazolium salt (MTT) to blue formazan crystals), was used to determine the amount of viable cells after treatment with Apo2L/TRAIL or DR5 antibody. The MTT assay was performed
10 by the addition of a premixed optimized dye solution to culture wells of a 96-well plate containing various concentrations (0 to 1000 ng/ml) of Apo2L/TRAIL or DR5 antibody. During a 4-hour incubation, living cells convert the tetrazolium component of the dye solution into a formazan product. The solubilization/stop solution was then added to the culture
15 wells to solubilize the formazan product, and the absorbance at 570nm was recorded using a 96-well plate reader (SpectraMax). The 570nm absorbance reading is directly proportional to the number of cells normally used in proliferation assays. Although the absorbance maximum for the formazan product is 570nm and pure solutions appear blue, the color at the end of
20 the assay may not be blue and depends on the quantity of formazan present relative to other components (including serum, acidified phenol red and unreduced MTT) in the culture medium.

Cell numbers were optimized by performing a cell titration to produce an assay signal near the high end of the linear range of the
25 assay. Since different cell types have different levels of metabolic activity, this was done for each cell line separately. For most tumor cells examined, 5,000 cells per well to 20,000 cells per well were used.

The following is a step by step description of the assays employed:

1. Cells used for bioassay were from stock cultures.
- 30 2. Determination of cell number and trypan blue viability, and suspension of the cells to a final number of 5,000 to 20,000 cells per well.
3. Dispensed 50µl of the cell suspension into 96-well plate.
4. Incubation of the plates at 37°C in a humidified 5% CO₂ atmosphere
35 over night.
5. Addition of 50µl culture medium containing various concentrations ranging from 0 to 1000 ng/ml of Apo2L/TRAIL or DR5 antibody to samples of the 96-well plate. The controls were 50µl of culture medium (without Apo2L/TRAIL or DR5 antibody) and 100µl culture medium (without cells).
- 40 Every experiment was performed in a triplicate set of wells and at three independent days. The total volume of the wells was 100µl/well.
6. Incubation of the plates at 37°C for 72 hours in a humidified 5% CO₂ atmosphere.

7. Addition of 15µl of dye solution to each well.
8. Incubation of the plates at 37°C for up to 4 hours in a humidified 5% CO₂ atmosphere.
9. Addition of 100µl of the solubilization/stop solution to each well.
- 5 10. Incubation of the plates overnight at 37°C overnight.
11. Record the absorbance at 570nm wavelength using a 96-well plate reader. A reference wavelength of 750 nm was used to reduce background contributed by cell debris, fingerprints and other nonspecific absorbance.
12. The average of the absorbance values for the negative control was used as a blank value and subtracted from all other readings. The average of the absorbance values for each concentration of Apo2L/TRAIL or DR5 antibody was divided by the average of the absorbance values of the positive control (100% viable cells - untreated) to calculate the amount of viable cells (in %).
- 10 13. Percent viable cells (Y axis) versus concentration of Apo2L/TRAIL or DR5 antibody (X axis, log scale) was plotted and the IC50 value was determined by locating the X-axis value (ng/ml) corresponding to 50 % viable cells.
- 15

20 Affymetrix Labeling Protocol

An OD260/280 reading was taken for all samples, and samples were run on the BioAnalyzer. 5 µg high quality Total RNA was used.

A. First Strand cDNA Synthesis:

1. Primer hybridization

- 25 DEPC-H₂O x µl Mix by vortexing. Quick spin.
- RNA (5 ug) y µl Incubate at 70°C for 10 minutes.
- Spike (1:4 dil of stock for 5 ug) 1 µl Quick spin and put on ice

T7-(dT)24 primer 1 µl

- 30 volume 12 µl

2. Temperature adjustment

- 5X-1st strand cDNA buffer 4 µl
- Add 7 µl (of the mix to the left) to each sample.
- 0.1 M DTT 2 µl Mix by vortexing. Quick spin.
- 35 10 mM dNTP mix 1 µl Incubate at 42°C for 2 minutes.
- volume 7 µl

3. First Strand Synthesis

- Add 1 µl SSII RT to each sample.
- SSII RT 1 µl Mix by pipetting up and down -OR- vortex
- 40 lightly.
- Quick spin.
- Total volume 20 µl Incubate at 42°C for 1 hour.

B. Second Strand cDNA Synthesis

1. Place First Strand reactions on ice. Centrifuge briefly to bring down condensation on sides of tube.

2. Make the following Second strand master-mix.

5 DEPC-treated H₂O 91 μ l
 5X-2nd Strand Reaction Buffer 30 μ l
 10 mM dNTP mix 3 μ l
 10 U/ μ l DNA Ligase 1 μ l
 10 U/ μ l DNA Polymerase I 4 μ l
 10 2 U/ μ l RNase H 1 μ l
 Total volume 130 μ l

3. Add 130 μ l Second strand master-mix to the 20 μ l First strand cDNA. (Final volume = 150 μ l)

4. Mix by pipetting up and down -OR- by vortexing lightly. Quick spin.

5. Incubate at 16°C for 2 hours in a cooling water bath.

6. Add 2 μ l [10 U] T4 DNA Polymerase. Mix by pipetting up and down -OR- vortex lightly. Quick spin.

7. Incubate for 5 minutes at 16°C.

8. Add 10 μ l 0.5 M EDTA. Vortex lightly. Quick spin.

9. Proceed to cleanup procedure for cDNA -OR- store at -20°C for later use.

Cleanup of Double-Stranded cDNA (GeneChip Sample Cleanup Module)

1. Add 600 μ l cDNA Binding Buffer to the 162 μ l final double-stranded cDNA synthesis preparation.

Mix by vortexing for 3 seconds.

2. Check that the color of the mixture is yellow (similar to cDNA Binding Buffer w/o the cDNA synthesis reaction).

30 If the color of the mixture is orange or violet, add 10 μ l of 3 M sodium acetate, pH5.0 and mix.

The color of the mixture will turn to yellow.

3. Apply 500 μ l of the sample to the cDNA Cleanup Spin Column sitting in a 2 ml Collection Tube, and centrifuge

35 for 1 minute at $\geq 8,000 \times g$ ($\geq 10,000$ rpm). Discard flow-through as hazardous waste.

4. Reload the spin column with the remaining mixture (262 μ l) and centrifuge as above.

Discard flow-through as hazardous waste and discard the Collection Tube.

5. Transfer spin column into a new 2 ml Collection Tube (supplied). Pipet 750 μ l cDNA Wash Buffer onto the spin column. Centrifuge for 1 minute at $\geq 8,000 \times g$ ($\geq 10,000$ rpm).

Discard flow-through.

6. Open the cap of the spin column and centrifuge for 5 minutes at maximum speed ($\leq 25,000 \times g$). Place

5 columns into the centrifuge using every second bucket. Position caps over the adjoining bucket so that

they are oriented in the opposite direction to the rotation, i.e., if rotation is clockwise, orient caps

in a counter-clockwise direction. This avoids damage to caps.

Discard flow-through and Collection Tube.

10 7. Transfer spin column into a 1.5 ml Collection Tube. Pipet 10 μ l of cDNA Elution Buffer directly onto the spin

column membrane. Ensure that the cDNA Elution buffer is dispensed directly onto the membrane.

15 Incubate for 1 minute at room temperature and centrifuge 1 minute at max. speed ($\leq 25,000 \times g$) to elute.

Setting Up and Running the IVT Reaction

Enzo: Bioarray HighYield RNA transcript Labeling Kit (Part No. 900182)

1. Use 10 μ l of the Cleaned-up Double-stranded cDNA

20 2. Make the following IVT master-mix:

Distilled or Deionized H₂O 12 μ l

10X HY Reaction buffer 4 μ l

10x Biotin labeled Ribonucleotides 4 μ l

10X DTT 4 μ l

25 10X RNase Inhibitor Mix 4 μ l

20X T7 RNA Polymerase 2 μ l

Total volume: 30 μ l

3. Add 30 μ l of the IVT master-mix to 10 μ l double-stranded cDNA. (Total volume = 40 μ l)

30 4. Mix by pipetting up and down -OR- by vortexing lightly. Quick spin.

5. Immediately place the tube in a 37°C water bath. Incubate for 5 hours.

6. Store at -20°C if not purifying RNA immediately.

35

Cleanup of Biotin-Labeled cRNA (GeneChip Sample Cleanup Module)

1. Add 60 μ l H₂O to the IVT reaction and mix by vortexing for 3 seconds.

2. Add 350 μ l IVT cRNA Binding Buffer to the sample, mix by vortexing for 3 seconds.

40 3. Add 250 μ l ethanol (96-100%) to the lysate, and mix well by pipetting. Do not centrifuge.

4. Apply sample (700 μ l) to the IVT cRNA Cleanup Spin Column sitting in a 2 ml collection tube.

Centrifuge for 15 seconds at $\geq 8,000 \times g$ ($\geq 10,000$ rpm).

5. Pass the eluate through the column once more.

Centrifuge for 15 seconds at $\geq 8,000 \times g$ ($\geq 10,000$ rpm).

- 5 Discard the flow-through as hazardous waste and discard the collection tube.

6. Transfer the spin column into a new 2-ml collection tube (supplied).

7. Add 500 μ l IVT cRNA Wash Buffer and centrifuge for 15 seconds at $\geq 8,000 \times g$ ($\geq 10,000$ rpm) to wash.

Discard the flow-through.

- 10 8. Pipet 500 μ l 80% (v/v) ethanol onto the spin column, and centrifuge for 15 seconds at

$\geq 8,000 \times g$ ($\geq 10,000$ rpm). Discard flow-through.

9. Open the cap of the spin column and centrifuge for 5 minutes at max. speed ($\leq 25,000 \times g$).

- 15 Discard flow-through and Collection Tube.

10. Transfer the spin column into a new 1.5 ml collection tube.

11. Pipet 11 μ l RNase-free water directly onto the spin column membrane. Let stand for 1 minute.

Centrifuge for 1 minute at maximum speed ($\leq 25,000 \times g$) to elute.

- 20 12. Pipet 10 μ l RNase-free water directly onto the spin column membrane. Let stand for 1 minute.

Centrifuge for 1 minute at maximum speed ($\leq 25,000 \times g$) to elute.

Quantifying the cRNA (IVT Product)

- 25 Use spectrophotometric analysis to determine the RNA yield. Apply the convention that 1 OD at 260 nm equals 40 μ g/ml RNA.

Check the OD at 260 nm and 280 nm to determine sample concentration and purity.

- 30 Maintain the A260/A280 ratio close to 2.0 for pure RNA (ratios between 1.9 and 2.1 are acceptable).

For quantification of cRNA when using total RNA as starting material, an adjusted cRNA yield must be calculated to reflect carryover of unlabeled total RNA. Using an estimate of 100% carryover, use the formula below to determine adjusted cRNA yield:

$$\text{adjusted cRNA yield} = \text{RNAm} - (\text{total RNAi})(y)$$

RNAm = amount of cRNA measured after IVT (μ g)

total RNAi = starting amount of total RNA (μ g)

- 40 y = fraction of cDNA reaction used in IVT

Fragmenting the cRNA for Target Preparation

For fragmentation, use the adjusted cRNA concentration.

1. Add 2 μ l of 5x Fragmentation Buffer for every 8 μ l of RNA plus H₂O.
 20 μ g cRNA 1 to 32 μ l
 5X Fragmentation Buffer 8 μ l
 RNase-free water to 40 μ l
- 5 Total volume: 40 μ l
2. Incubate at 94°C for 30 minutes. Immediately, put on ice following the incubation.

Preparing the Hybridization Target

- 10 1. Heat the 20X Eukaryotic Hybridization Controls and the Oligo B2 for 5 minutes at 65°C.
 Affymetrix GeneChip Eukaryotic Hybridization Control Kit, Part #900362 (for 150 rxns)
 2. Lightly vortex, spin down.
 - 15 3. Master mix (Assuming the fragmented cRNA concentration is 0.5 μ g/ μ l):
- | | Standard Array (μ l) | Final Conc. |
|----|-------------------------------|-----------------------|
| | Fragmented cRNA 15 μ g 30 | 0.05 μ g/ μ l |
| | Oligo B2 (3 nM) 5 | 50 pM |
| 20 | 20x Control Spike 15 | 1.5, 5, 25, 100 pM |
| | (Bio B, C, D, Cre) | |
| | Herring Sperm DNA 3 | 0.1 mg/ml |
| | Acetylated BSA 3 | 0.5 mg/ml |
| | Hu cot-1 DNA (1 mg/ml) 30 | 0.1 mg/ml |
| 25 | 2X MES Hyb Buffer 150 | 1X |
| | H ₂ O 64 | |
| | Final Volume 300 | |
4. Aliquot 270 μ l master mix into tubes and add 30 μ l of fragmented cRNA to each. This is the Hybridization Mix.
 - 30 5. Equilibrate the probe arrays to room temperature immediately before use.
 6. Fill the probe array with 1x MES Hyb Buffer, and incubate in the rotisserie oven for 10 minutes at 45°C, 60 rpm.
 - 35 7. Heat the Hybridization Mix in a 99°C waterbath for 5 minutes.
 8. Transfer the Hybridization Mix to a 45°C waterbath for 5 minutes.
 9. Centrifuge the Hybridization Mix for 5 minutes at maximum speed.
 10. Remove the 1x MES Hyb Buffer from the probe arrays.
 11. Fill the probe array with the top 200 μ l of the Hybridization Mix.
 - 40 12. Seal the septa with Tough-Spots.
 13. Hybridize the probe array at 45°C, 60 RPM for 19 hours.
 14. Wash, stain and scan the probe array according to the Affymetrix protocols.

Affymetrix Materials

Item	Vendor	Catalog #		
	T7-(dT)24 primer	Biosearch Technologies	custom	
5	Control spikes	in-house	-	
	Superscript II/5X First Strand Buffer/0.1 M DTT	Invitrogen	18064-014	
	5X Second Strand Buffer	Invitrogen	10812-014	
	10 mM dNTP	Invitrogen	18427-088	
	10 U/ul E. coli DNA Ligase	Invitrogen	18052-019	
10	10 U/ul E. coli DNA Polymerase I	Invitrogen	18010-025	
	2 U/ul RNase H	Invitrogen	18021-071	
	10 U/ul T4 DNA Polymerase	Invitrogen	18005-025	
	0.5 M EDTA	Sigma E-7889		
	ENZO High Yield RNA Transcript labeling kit	Affymetrix	or	ENZO
15	900182 (ENZO)			
	GeneChip Sample Cleanup Module	Affymetrix	900371	
	Acetylated Bovine Serum Albumin	Invitrogen	15561-020	
	Goat IgG - Reagent Grade	Sigma I-5256		
	Anti-streptavidin antibody (goat), biotinylated	Vector Labs	BA-0500	
20	R-Phycoerythrin Streptavidin	Molecular Probes	S-866	
	20X SSPE	BioWhittaker	51214	
	Eukaryotic Control Kit	Affymetrix	900362	
	Water, Molecular Biology Grade	Ambion	9934	
	Human Cot-1 DNA	Roche	1-581-074	
25	5 M NaCl RNase-free, DNase-free	Ambion	9760	
	Antifoam 0-30	Sigma A-8082		
	10% Tween-20	Pierce Chemical	28320	
	MES Free Acid Monohydrate	Sigma M5287		
	MES Sodium Salt	Sigma M3885		
30	EDTA Disodium Salt, 0.5 M solution	Sigma E7889		
	Tough Spots, Label Dots USA	Scientific	9902	
	GeneChip Hybridization Oven 640	Affymetrix	800139	
	GeneChip Scanner 3000 w/Workstation	Affymetrix	00-0074	
	Fluidics Station	Affymetrix	00-0081	
35	Autoloader w/External Barcode Reader	Affymetrix	00-0129	

Quantitative PCR**cdNA Synthesis :**

Component	Volume (uL)
10X RT Buffer	10
25X dNTP mixture	4
10X Random Primers	10
MultiScribe RT (50U/uL)	5
RNase-free H ₂ O	21
RNA (100ng)	50
Final Volume	100

5 Incubation Conditions :

25° for 10 minutes

37° for 2 hours

TaqMan Reaction using the ABI Prism 7700 Sequencing Detector:

Component	Volume (uL)
TaqMan Universal PCR Master Mix (2X)	25
TaqMan probe (20X) (Assays-on-Demand™)	2.5
cdNA (100ng)	2
H ₂ O	20.5
Final Volume	50

10 Thermal Cycling Conditions :

95° for 10 minutes

40 cycles : 95° for 15 seconds

60° for 1 minute

- TaqMan probes : Assays-on-Demand™ (TaqMan® MGB probes, FAM™ dye-labeled)

15

- Amplification of the endogenous control, GAPDH (probe concentration 100nM, forward & reverse primer concentrations 200nM), was performed to standardize the amount of sample RNA (cdNA) added to each reaction.

20

Relative quantitation was performed using the standard curve method. For quantitation normalized to an endogenous control, standard curves were prepared for both the target and the endogenous reference. For each experimental sample, the amount of target and endogenous reference was determined from the appropriate standard curve. Then, the target amount

was divided by the endogenous reference amount to obtain a normalized target value. One of the experimental samples served as the calibrator, or 1x sample. Each of the normalized target values was then divided by the calibrator normalized target value to generate the relative expression levels.

FACS/Flow cytometry (2° Antibody Staining Protocol):

All incubations and spins were performed at 4°C and the tubes kept on ice while not in the refrigerator.

- 10 1. Determine the tube format by identifying the cell lines to be used, the antibodies of interest, and any special conditions or treatments.
 - a. controls.
 - i. Unstained, 2° Antibody, and compensation if the fluorochromes have overlapping emission spectra.

- 15 b. Example:

Tube	Cell Line	Time (min)	1° Antibody	2° Antibody
1	e.g., COLO-205	0	-	-
2	e.g., COLO-205	0	-	anti-Mouse-FITC
3	e.g., COLO-205	0	anti-Sialyl Lewis A	anti-Mouse-FITC
4	e.g., COLO-205	0	anti-CD15s (Sialyl Lewis X)	anti-Mouse-FITC

2. Label the FACS tubes.

- a. BD Falcon 12x75mm Polystyrene Round-Bottom. Catalog #: 352052

- 20 3. Prepare the cells for staining.

- a. Treat adherent cells with Accutase or Trypsin.

- i. Innovative Cell Technologies Inc, Accutase.

- ii. Gibco, Trypsin. Catalog #: 25200-106.

- b. Proceed with the remaining steps if the cells are suspension.

- 25 4. Aliquot the cells into a 15mL or 50 mL conical tube.

5. Spin the cells for 5 min, 1200rpm, 4°C.

6. Aspirate the supernatant.

7. Resuspend the cells in 5mLs FACS Buffer.

8. Spin the cells for 5 min, 1200rpm, 4°C.

- 30 9. Aspirate the supernatant.

10. Resuspend the cells in Blocking Buffer.

- a. Determine the volume of blocking buffer needed:

- i. Number of tubes per cell line/treatment X 100µl Blocking Buffer per tube.

- 35 ii. Want 1×10^6 cells per 100µl of Blocking Buffer.

11. Aliquot 100µl of the cells into the appropriate tube.

- a. Based on the pre-determined tube format.
12. Add the 1° antibody to the appropriate tube.
 - a. Lewis A:
 - i. Use 10µl of 0.2µg/µl stock per tube.
 - 5 1. Final concentration is 2µg.
 - ii. Chemicon: anti-Sialyl Lewis A. Catalog #: MAB2095.
 - b. Lewis X:
 - i. Use 5µl of 0.5µg/µl stock per tube.
 1. Final concentration is 2.5µg.
 - 10 ii. BD Pharmingen: CD15s (Sialyl Lewis X). Catalog #: 551344.
13. Incubate for 30min at 4°C.
14. Add 1mL of FACS Buffer to each tube.
15. Spin the cells for 5 min, 1200rpm, 4°C.
16. Aspirate the supernatant
- 15 17. Gently rack the tubes to dislodge the pellet.
 - a. "Rack"- Run the tubes across the surface of the 12x75mm tube rack.
18. Repeat steps 14-17.
19. Add 100µl of Blocking Buffer to each tube.
- 20 20. Add the 2° antibody into the appropriate tube.
 - a. Use 10µl per tube.
 - b. Jackson, Goat-anti-Mouse FITC. Catalog #: 115-096-068.
21. Incubate for 30min at 4°C.
22. Repeat steps 14-17 twice.
- 25 23. Resuspend cells in FACS Buffer/PI.
 - a. Determine volume needed:
 - i. Need 1mL of solution per tube.
 - ii. PI= 1µl per 1mL of Buffer.
 - b. Molecular Probes, Propidium Iodide. Catalog #: P3566.
- 30 24. Place tubes in an ice bucket or iced tube rack.
25. Cover with aluminum foil and take to the FACS lab for a qualified operator to acquire and analyze samples.
- 5% Blocking Buffer:
 1. FBS to 5% of total volume.
 - 35 2. FACS Buffer.
 3. Filter the solution through a 0.2µm filter.
- FACS Buffer:
 1. 980mLs PBS.
 2. 8mLs 0.25M EDTA.
 - 40 3. 20mLs FBS.
 4. Filter the solution through a 0.2µm filter.

Immunohistochemistry Procedure: Sialyl Lewis A

Antibody: Sialyl Lewis A AB-1

Clone: 121SLE

Supplier: NeoMarkers

Catalog No. MS-279-P

Ig Species: Mouse

IHC Method: Paraffin

Pretreatment: None

IHC Handling: Autostainer

Isotype: Mouse IgM

Procedure Species: Human

IgG Concentration: 200ug/ml

5 Normal Procedure:

Deparaffinize and hydrate to distilled water.

Block endogenous biotin with Vector Avidin Biotin Blocking System.

Rinse with TBS: 2 changes, 5 minutes each.

Block with 10% Normal Horse Serum for 30 minutes at RT.

- 10 Incubate sections with Mouse Monoclonal Sialyl Lewis A antibody diluted to 5ug/ml with 10% normal Horse Serum for 60 minutes at RT.

Use a mouse isotype IgM diluted at 5ug/ml in 10% Normal Horse Serum for the negative control.

Rinse with TBS: 2 changes, 5 minutes each.

- 15 Incubate sections with biotinylated horse anti-mouse antibody; 1:200 diluted in 10% Normal Horse Serum for 30 minutes at RT.

Rinse with TBS: 2 changes, 5 minutes each.

Incubate sections with diluted Vector ABC Elite System for 30 minutes at RT.

- 20 Rinse with TBS: 2 changes, 5 minutes each.

Incubate sections with Pierce Metal Enhanced DAB for 5 minutes

Rinse in Running Tap water for 5 minutes.

Counterstain with Mayers Hematoxylin for 1 minute.

Rinse in Running Tap water for 5 minutes.

- 25 Blue Hematoxylin with Richard-Allan Bluing Reagent for 1 minute.

Rinse in Running Tap water for 2 minutes.

Dehydrate, clear and mount in synthetic mounting media.

Immunohistochemistry Procedure: Sialyl Lewis X

Antibody: Mouse anti-Sialyl Lewis X

Clone: KM93

Supplier: Chemicon

Catalog No. MAB2096

Ig Species: Mouse

IHC Method: Paraffin

Pretreatment: DAKO Target Retrieval

IHC Handling: Autostainer

Isotype: Mouse IgM

Procedure Species: Human

IgG Concentration: 100ug/ml

Normal Procedure:

Deparaffinize and hydrate to distilled water.

- 5 Quench endogenous peroxidase activity with KPL Blocking Solution -- dilute concentrate 1:10 in dH₂O, RT for 4 minutes.

Rinse in Distilled water for 5 minutes.

- 10 Incubate in DAKO Target Retrieval (S1700) preheated to 99 degrees for 20 minutes in a boiling water bath. Remove from boiling bath and let cool for 20 minutes.

Block endogenous biotin with Vector Avidin Biotin Blocking System.

Block with 10% Normal Horse Serum for 30 minutes at RT.

Incubate sections with Mouse Monoclonal Sialyl Lewis X antibody diluted to 5ug/ml with 10% normal Horse Serum for 60 minutes at RT.

- 15 Use a mouse isotype IgM diluted at 5ug/ml in 10% Normal Horse Serum for the negative control.

Rinse with TBS: 2 changes, 5 minutes each.

Incubate sections with Vector biotinylated horse anti-mouse antibody; 1:200 diluted in 10% Normal Horse Serum for 30 minutes at RT.

- 20 Rinse with TBS: 2 changes, 5 minutes each.

Incubate sections with diluted Vector ABC Elite System for 30 minutes at RT.

Rinse with TBS: 2 changes, 5 minutes each.

Incubate sections with Pierce Metal Enhanced DAB for 5 minutes

- 25 Rinse in Running Tap water for 5 minutes.

Counterstain with Mayers Hematoxylin for 1 minute.

Rinse in Running Tap water for 5 minutes.

Blue Hematoxylin with Richard-Allan Bluing Reagent for 1 minute.

Rinse in Running Tap water for 2 minutes.

- 30 Dehydrate, clear and mount in synthetic mounting media.

Experimental Results:

- 35 Experiments were conducted using the methods and materials described above. Results of these experiments are illustrated in Figures 6-13, as discussed below.

- 40 Figure 6 provides a summary chart of the data obtained in analyzing 28 colon or colorectal cancer cell lines for sensitivity or resistance to apoptotic activity of Apo2L (+ 0.5% fetal bovine serum "FBS" or 10% FBS) or DR5 monoclonal antibody "mab", cross-linked "XL" or not crosslinked, + 0.5% fetal bovine serum "FBS" or 10% FBS) and expression of FUT 3, FUT 6, Sialyl lewis A and Sialyl lewis X.

Figure 7 provides a comparison of sensitivity of various colon or colorectal cancer cell lines to DR5 antibody and the expression of FUT 3, as measured by quantitative PCR).

Figure 8 provides a comparison of various colon or colorectal cancer cell lines for sensitivity or resistance to DR5 antibody (plus cross-linker) and expression of sialyl lewis X or A, as determined by FACS.

5 Figure 9A shows a Spearman Rank Correlation test analyzing sensitivity or resistance of various colon or colorectal cancer cell lines and correlation to expression of FUT3.

Figure 9B shows the results of a Fisher's Exact test analyzing sensitivity ("sens") or resistance ("res") of the various colon or colorectal cancer cell lines and the statistical significance between FUT
10 3 and sialyl lewis A/X expression and sensitivity of the respective cell lines to DR5 antibody apoptotic activity.

Figure 10 provides a comparison of various colon or colorectal cancer cell lines for expression of DcR1 or DcR2 receptors (as determined by quantitative PCR) and the status (sensitive or resistant) of certain
15 cell lines to Apo2L or DR5 antibody.

Figure 11 provides a comparison of various colon or colorectal cancer cell lines for expression of DcR1 or DcR2 receptors (as determined by FACS) and the status (sensitive or resistant) of certain cell lines to Apo2L or DR5 antibody.

20 Figure 12 shows immunohistochemical staining for sialyl lewis A and X on four colorectal cancer cell lines, CaCo 2 (Colo2), SW 1417, DLD-1, and Colo 205, and its correlation to expression of sialyl Lewis A and X as measured by FACS and its correlation to sensitivity to Apo2L/TRAIL. Colorectal cancer cell lines Colo 2 and SW1417 show no and weak staining,
25 respectively, or sialyl Lewis antigens, are negative and weakly positive, respectively, by FACS and are resistant to Apo2L/TRAIL. Colorectal cancer cells lines DLD-1 and Colo 205 show moderate and strong staining, respectively for sialyl Lewis antigens, are moderately and strongly positive, respectively, by FACS and are sensitive to Apo2L/TRAIL.

30 Figure 13 shows a summary of IHC experiments demonstrating expression of sialyl Lewis A and X in tissue samples of normal colon mucosa, normal liver tissue, primary colon cancer, and colon cancer metastases. Tissue samples of normal colon and primary colon cancer arrayed in a tissue microarray were tested in the IHC experiment, while
35 tissue samples of the normal liver and metastatic colon cancer were on individual glass slides. The prevalence of expression of sialyl Lewis A and X and the immunohistochemical staining intensity increases from normal colon tissue to primary colon cancer to metastatic colon cancer. The normal liver cells did not stain for for either sialyl Lewis A or X.

40

WHAT IS CLAIMED IS:

1. A method for predicting the sensitivity of a mammalian tissue or cells sample to Apo2L/TRAIL, comprising the steps of:
 - 5 obtaining a mammalian tissue or cell sample;
 - examining the tissue or cell sample to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), wherein expression of said one or more biomarkers is predictive that said tissue
 - 10 or cell sample is sensitive to apoptosis-inducing activity of Apo2L/TRAIL.
2. The method of claim 1 wherein said expression of one or more biomarkers is examined by detecting mRNA expression of fucosyltransferase 3 or fucosyltransferase 6.
- 15 3. The method of claim 1 wherein said expression of one or more biomarkers is examined by immunohistochemistry to detect expression of sialyl Lewis A and/or X antigen(s).
- 20 4. The method of claim 1 further comprising the step of examining expression of DR4, DR5, DcR1, or DcR2 receptors in said tissue or cell sample.
5. The method of claim 1 wherein tissue or cell sample comprises cancer
- 25 tissue or cells.
6. The method of claim 5 wherein said cancer cells are colon, colorectal, gastrointestinal, or pancreatic cancer cells or tissue.
- 30 7. A method for inducing apoptosis in a mammalian tissue or cell sample, comprising the steps of:
 - obtaining a mammalian tissue or cell sample;
 - examining the tissue or cell sample to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3,
 - 35 fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and
 - subsequent to detecting expression of said one or more biomarkers,
 - exposing said tissue or cell sample to an effective amount of Apo2L/TRAIL.
8. The method of claim 7 wherein said expression of one or more biomarkers
- 40 is examined by testing for mRNA expression of fucosyltransferase 3 or fucosyltransferase 6.

9. The method of claim 7 wherein said expression of one or more biomarkers is examined by immunohistochemistry to detect expression of sialyl Lewis A and/or X antigen(s).

5 10. The method of claim 7 further comprising the step of examining expression of DR4, DR5, DcR1 or DcR2 receptors in said tissue or cell sample.

10 11. The method of claim 7 wherein said tissue or cell sample comprises cancer tissue or cells.

12. The method of claim 11 wherein said cancer cells are colon, colorectal, gastrointestinal, or pancreatic cancer cells or tissue.

15 13. The method of claim 7 wherein said cells are exposed to an effective amount of Apo2L/TRAIL polypeptide comprising amino acids 114-281 of Figure 1 (SEQ ID NO:1).

20 14. A method of treating a disorder in a mammal, such as an immune related disorder or cancer, comprising the steps of:
obtaining a tissue or cell sample from said mammal;
examining the tissue or cell sample to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3,
fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and
25 subsequent to detecting expression of said one or more biomarkers, administering to said mammal an effective amount of Apo2L/TRAIL.

15. The method of claim 14 wherein said expression of one or more biomarkers is examined by detecting mRNA expression of fucosyltransferase
30 3 or fucosyltransferase 6.

16. The method of claim 14 wherein said expression of one or more biomarkers is examined by immunohistochemistry to detect expression of sialyl Lewis A and/or X antigen(s).
35

17. The method of claim 14 further comprising the step of examining expression of DR4, DR5, DcR1 or DcR2 receptors in said tissue or cell.

40 18. The method of claim 14 wherein tissue or cell sample comprises cancer tissue or cells.

19. The method of claim 18 wherein said cancer cells or tissue comprises colon, colorectal, gastrointestinal, or pancreatic cancer cells or tissue.

20. The method of claim 14 wherein an effective amount of Apo2L/TRAIL polypeptide comprising amino acids 114-281 of Figure 1 (SEQ ID NO:1) is administered to said mammal.

5

21. The method of claim 14 wherein a chemotherapeutic agent(s) or radiation therapy is also administered to said mammal.

10

22. The method of claim 14 wherein a cytokine, cytotoxic agent or growth inhibitory agent is also administered to said mammal.

23. The method of claim 7 wherein said Apo2L/TRAIL polypeptide is linked to a polyethylene glycol molecule.

15

24. The method of claim 14 wherein said Apo2L/TRAIL polypeptide is linked to a polyethylene glycol molecule.

25. The method of claim 6 wherein said cancer cells are colon or colorectal cancer cells.

20

26. The method of claim 1 wherein said Apo2L/TRAIL is a polypeptide comprising amino acids 41-281 of Figure 1 (SEQ ID NO:1) or a biologically active fragment thereof.

25

27. The method of claim 26 wherein said Apo2L/TRAIL is a polypeptide comprising amino acids 114-281 of Figure 1 (SEQ ID NO:1).

28. The method of claim 12 wherein said cancer cells are colon or colorectal cancer cells.

30

29. The method of claim 20 wherein said Apo2L/TRAIL polypeptide consists of amino acids 114-281 of Figure 1 (SEQ ID NO:1).

35

30. A method for predicting the sensitivity of a mammalian colon or colorectal cancer cells to Apo2L/TRAIL, comprising the steps of:
obtaining mammalian mammalian colon or colorectal cancer cells;
examining the cancer cells to detect expression of one or more biomarkers selected from the group of fucosyltransferase 3, fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), wherein expression of said one or more biomarkers is predictive that said cancer cells are sensitive to apoptosis-inducing activity of Apo2L/TRAIL.

40

31. A method for inducing apoptosis in mammalian colon or colorectal cancer cells, comprising the steps of:

obtaining mammalian colon or colorectal cancer cells;
examining the cancer cells to detect expression of one or more biomarkers
selected from the group of fucosyltransferase 3, fucosyltransferase 6,
sialyl Lewis A and/or X antigen(s), and

5 subsequent to detecting expression of said one or more biomarkers,
exposing said cancer cells to an effective amount of Apo2L/TRAIL
polypeptide.

10 32. The method of claim 31 wherein said Apo2L/TRAIL polypeptide comprises
amino acids 41-281 of Figure 1 (SEQ ID NO:1) or a fragment thereof which
has apoptotic activity.

33. The method of claim 32 wherein said Apo2L/TRAIL polypeptide is linked
to a polyethylene glycol molecule.

15 34. The method of claim 32 wherein said Apo2L/TRAIL polypeptide comprises
amino acids 114-281 of Figure 1 (SEQ ID NO:1).

35. A method of treating colon or colorectal cancer in a mammal,
comprising the steps of:

20 obtaining a colon or colorectal cancer cell sample from said mammal;
examining the cancer cell sample to detect expression of one or more
biomarkers selected from the group of fucosyltransferase 3,
fucosyltransferase 6, sialyl Lewis A and/or X antigen(s), and
subsequent to detecting expression of said one or more biomarkers,
25 administering to said mammal an effective amount of Apo2L/TRAIL.

30 36. The method of claim 35 wherein said Apo2L/TRAIL polypeptide comprises
amino acids 41-281 of Figure 1 (SEQ ID NO:1) or a fragment thereof which
has apoptotic activity.

37. The method of claim 36 wherein said Apo2L/TRAIL polypeptide is linked
to a polyethylene glycol molecule.

35 38. The method of claim 36 wherein said Apo2L/TRAIL polypeptide comprises
amino acids 114-281 of Figure 1 (SEQ ID NO:1).

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1 TTTCTCACTGACTATAAAGAAATAGAGAAAGGAGGCTTCAGTGACCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC
 1 ATGGCTATGATGGAGGTCCAGGGGGACCCAGCCTGGCGTGCTGATCGTGATCTTACACAGTGCTCCTGCAGTCTCTCTGT
 1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys
 181 GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAAGTACTCCAAAAGTGGCATTTGTTCTTAAAGAA
 31 ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPheLeuLysGlu
 271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG
 61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys
 361 ATGATTTTGAGAACCTCTGAGGAAACCAATTTCTACAGTTCAAGAAAGCAACAAAATATTCTCCCTAGTGAGAGAAAGAGTCCNCAG
 91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln
 451 AGAGTAGCAGCTCACATAAAGTGGACAGAGGAAGCAACACATTTCTCTCCAAACTCCAAGAAATGAAAAGGCTCTGGGCCGCAAA
 121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys
 541 ATAAACTCCTGGGAATCATCAAGGAGTGGCATTTCTGAGCAACTTGCACCTTGAGGAATGGTGAACCTCCATCCATGAAAAAGGG
 151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly
 631 TTTTACTACATCTATTTCCCAACATACTTTTCGATTTCAGGAGGAAATAAAAGAAAAACACAAAGAACGACAAACAAATGGTCCCAATATTT
 181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle
 721 TACAAATACACAAGTTATCCTGACCCCTATATTGTTGATGAAAAGTGCTAGAAAATAGTTGTTGGTCTAAAGATGCAGAAATATGGACTCTAT
 211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr
 811 TCCATCTATCAAGGGGGAATATTGAGCCTTAAGGAAAATGACACAGAAATTTTGTGTTCTGTAACAAATGAGCCTTGTATAGACATGGACCAT
 241 SerIleTyrGlnGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis
 901 GAAGCCAGTTTTCGGGGCCCTTTTAGTTGGCTAACTGACCTGGAAAGAAAAGCAATAAACCTCAAAGTGACTATTTCAGTTTTCAGGAT
 271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp
 991 GATACACTATGAAGATGTTTCAAAAAAATCTGACCAAAACAAACACAGAAA

FIG. 1

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1 ATGGCGCCAC CACCAGCTAG AGTACATCTA GGTGCGTTCC TGGCAGTGAC
 TACCGCGGTG GTGGTCGATC TCATGTAGAT CCACGCAAGG ACCGTCAGTG
 1 MetAlaProP roProAlaAr gValHisLeu GlyAlaPheL euAlaValTh

 51 TCCGAATCCC GGGAGCGCAG CGAGTGGGAC AGAGGCAGCC GCGGCCACAC
 AGGCTTAGGG CCCTCGCGTC GCTCACCCTG TCTCCGTCGG CGCCGGTGTG
 rProAsnPro GlySerAlaA laSerGlyTh rGluAlaAla AlaAlaThrPro

 101 CCAGCAAAGT GTGGGGCTCT TCCGCGGGGA GGATTGAACC ACGAGGCGGG
 GGTCGTTTCA CACCCGAGA AGGCGCCCTT CCTAACTTGG TGCTCCGCCC
 35 SerLysVa lTrpGlySer SerAlaGlyA rgIleGluPr oArgGlyGly

 151 GGCCGAGGAG CGCTCCCTAC CTCCATGGGA CAGCACGGAC CCAGTGCCCCG
 CCGGCTCCTC GCGAGGGATG GAGGTACCCT GTCGTGCCTG GGTCACGGGC
 GlyArgGlyA laLeuProTh rSerMetGly GlnHisGlyP roSerAlaArg

 201 GGCCCGGGCA GGGCGCGCCC CAGGACCCAG GCCGGCGCGG GAAGCCAGCC
 CCGGGCCCGT CCCGCGCGGG GTCCTGGGTC CGGCCGCGCC CTCGGTTCGG
 68 AlaArgAla GlyArgAlaP roGlyProAr gProAlaArg GluAlaSerP

 251 CTCGGCTCCG GTCCACAAG ACCTTCAAGT TTGTCGTCGT CGGGGTCCTG
 GAGCCGAGGC CCAGGTGTTT TGGAAAGTTCA AACAGCAGCA GCCCCAGGAC
 roArgLeuAr gValHisLys ThrPheLysP heValValVa lGlyValLeu

 301 CTGCAGGTCG TACCTAGCTC AGCTGCAACC ATGATCAATC AATTGGCACA
 GACGTCCAGC ATGGATCGAG TCGACGTTGG TAGTTTGAAG TACTAGTTAG
 101 LeuGlnValV alProSerSe rAlaAlaThr IleLysLeuH isAspGlnSe

 351 AATTGGCACA CAGCAATGGG AACATAGCCC TTTGGGAGAG TTGTGTCCAC
 TTAACCGTGT GTCGTTACCC TTGTATCGGG AAACCCTCTC AACACAGGTG
 rIleGlyThr GlnGlnTrpG luHisSerPr oLeuGlyGlu LeuCysProPro

 401 CAGGATCTCA TAGATCAGAA CGTCCTGGAG CCTGTAACCG GTGCACAGAG
 GTCCTAGAGT ATCTAGTCTT GCAGGACCTC GGACATTGGC CACGTGTCTC
 135 GlySerHi sArgSerGlu ArgProGlyA laCysAsnAr gCysThrGlu

 451 GGTGTGGGTT ACACCAATGC TTCCAACAAT TTGTTTGCTT GCCTCCCATG
 CCACACCCAA TGTGGTTACG AAGGTTGTTA AACAAACGAA CGGAGGGTAC
 GlyValGlyT yrThrAsnAl aSerAsnAsn LeuPheAlaC ysLeuProCys

 501 TACAGCTTGT AAATCAGATG AAGAAGAGAG AAGTCCCTGC ACCACGACCA
 ATGTCGAACA TTTAGTCTAC TTCTTCTCTC TTCAGGGACG TGGTGTGTTG
 168 ThrAlaCys LysSerAspG luGluGluAr gSerProCys ThrThrThrA

 551 GGAACACAGC ATGTCAGTGC AAACCAGGAA CTTTCCGGAA TGACAATTCT
 CCTTGTGTCG TACAGTCACG TTTGGTCCTT GAAAGGCCTT ACTGTTAAGA
 rgAsnThrAl aCysGlnCys LysProGlyT hrPheArgAs nAspAsnSer

 601 GCTGAGATGT GCCGGAAGTG CAGCACAGGG TGCCCCAGAG GGATGGTCAA
 CGACTCTACA CGGCCTTCAC GTCGTGTCCC ACGGGGTCTC CCTACCAGTT
 201 AlaGluMetC ysArgLysCy sSerThrGly CysProArgG lyMetValLy

 651 GGTCAAGGAT TGTACGCCCT GGAGTGACAT CGAGTGTGTC CACAAAGAAT
 CCAGTTCCCTA ACATGCGGGA CCTCACTGTA GCTCACACAG GTGTTTCTTA
 sValLysAsp CysThrProT rpSerAspIl eGluCysVal HisLysGluSer

FIG. 2A

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701 CAGGCAATGG ACATAATATA TGGGTGATTT TGGTTGTGAC TTTGGTTGTT
 GTCCGTTACC TGTATTATAT ACCCACTAAA ACCAACACTG AAACCAACAA
 235 GlyAsnGly yHisAsnIle TrpValIleL euValValTh rLeuValVal

751 CCGTTGCTGT TGGTGGCTGT GCTGATTGTC TGTGTGTGCA TCGGCTCAGG
 GGCAACGACA ACCACCGACA CGACTAACAG ACAACAACGT AGCCGAGTCC
 ProLeuLeuL euValAlaVa lLeuIleVal CysCysCysI leGlySerGly

801 TTGTGGAGGG GACCCCAAGT GCATGGACAG GGTGTGTTTC TGGCGCTTGG
 AACACCTCCC CTGGGGTTCA CGTACCTGTC CCACACAAAG ACCCGGAACC
 268 CysGlyGly AspProLysC ysMetAspAr gValCysPhe TrpArgLeuG

851 GTCTCCTACG AGGGCCTGGG GCTGAGGACA ATGCTCACAA CGAGATTCTG
 CAGAGGATGC TCCCGGACCC CGACTCCTGT TACGAGTGTT GCTCTAAGAC
 lyLeuLeuAr gGlyProGly AlaGluAspA snAlaHisAs nGluIleLeu

901 AGCAACGCAG ACTCGCTGTC CACTTTCGTC TCTGAGCAGC AAATGGAAAG
 TCGTTGCGTC TGAGCGACAG GTGAAAGCAG AGACTCGTCG TTTACCTTTC
 301 SerAsnAlaA spSerLeuSe rThrPheVal SerGluGlnG lnMetGluSe

951 CCAGGAGCCG GCAGATTGGA CAGGTGTCAC TGTACAGTCC CCAGGGGAGG
 GGTCTCGGC CGTCTAAACT GTCCACATGT ACATGTCAGG GGTCCCCTCC
 rGlnGluPro AlaAspLeuT hrGlyValTh rValGlnSer ProGlyGluAla

1001 CACAGTGTCT GCTGGGACCG GCAGAAAGCTG AAGGGTCTCA GAGGAGGAGG
 GTGTCACAGA CGACCCTGGC CGTCTTCGAC TTCCAGAGT CTCCTCCTCC
 335 GlnCysLe uLeuGlyPro AlaGluAlaG luGlySerGl nArgArgArg

1051 CTGCTGGTTC CAGCAAATGG TGCTGACCCC ACTGAGACTC TGATGCTGTT
 GACGACCAAG GTCGTTTACC ACGACTGGGG TGA CTCTGAG ACTACGACAA
 LeuLeuValP roAlaAsnGly yAlaAspPro ThrGluThrL euMetLeuPhe

1101 CTTTGACAAG TTTGCAAACA TCGTGCCCTT TGACTCCTGG GACCAGCTCA
 GAAACTGTTC AAACGTTTGT AGCACGGGAA ACTGAGGACC CTGGTCGAGT
 368 PheAspLys PheAlaAsnI leValProPh eAspSerTrp AspGlnLeuM

1151 TGAGGCAGCT GGACCTCACG AAAAATGAGA TCGATGTGGT CAGAGCTGGT
 ACTCCGTCGA CCTGGAGTGC TTTTACTCT AGCTACACCA GTCTCGACCA
 etArgGlnLe uAspLeuThr LysAsnGluI leAspValVa lArgAlaGly

1201 ACAGCAGGCC CAGGGGATGC CTTGTATGCA ATGCTGATGA AATGGGTCAA
 TGTCGTCCGG GTCCCCTACG GAACATACGT TACGACTACT TTACCCAGTT
 401 ThrAlaGlyP roGlyAspAl aLeuTyrAla MetLeuMetL ysTrpValAs

1251 CAAAACCTGGA CGGAACGCCT CGATCCACAC CCTGCTGGAT GCCTTGGAGA
 GTTTTGACCT GCCTTGCGGA GCTAGGTGTG GGACGACCTA CGGAACCTCT
 nLysThrGly ArgAsnAlaS erIleHisTh rLeuLeuAsp AlaLeuGluArg

1301 GGATGGAAGA GAGACATGCA AAAGAGAAGA TTCAGGACCT CTTGGTGGAC
 CCTACCTTCT CTCTGTACGT TTTCTCTTCT AAGTCCTGGA GAACCACTG
 435 MetGluGl uArgHisAla LysGluLysI leGlnAspLe uLeuValAsp

1351 TCTGGAAAGT TCATCTACTT AGAAGATGGC ACAGGCTCTG CCGTGTCCCT
 AGACCTTTCA AGTAGATGAA TCTTCTACCG TGTCCGAGAC GGCACAGGAA
 SerGlyLysP heIleTyrLe uGluAspGly ThrGlySerA laValSerLeu

1401 GGAGTGA
 CCTCACT
 468 GluOP*

FIG. 2B

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1 MEQRGQNAPAASGARKRHGPPREARGARPGLRVPKTLVLVVAAVLLLVSAESALITQQD
61 LAPQQAAPQQKRSSPSEGLCPPGHHISEDGRDCISCKYQDYSTHWNDLLFCLRCTTRCD
121 SGEVELSPCTTTTRNTVCQCEEGTFREEDSPEMCRKCRTGCPRGMVKVGDCTPWSDIECVH
181 KESGIIIGVTVAAVVLIVAVFVCKSLLWKKVLPYLKGICSGGGDPERVDRSSQRPGAED
241 NVLNEIVSILQPTQVPEQEMEVQEPAEPTGVNMLSPGESEHLLEPAAEAERSQRRRLLVPA
301 NEGDPTETLRQCFDDFADLVPFDSWEPLMRKLGLMDNEIKVAKAEAAGHRDTLYTMLIKW
361 VNKTGRDASVHTLLDALETLGERLAKQKIEDHLLSSGKFMYLEGNADSSALS

FIG. 3A

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Met	Glu	Gln	Arg	Gly	Gln	Asn	Ala	Pro	Ala	Ala	Ser	Gly	Ala	Arg	Lys	1	5	10	15
Arg	His	Gly	Pro	Gly	Pro	Arg	Glu	Ala	Arg	Gly	Ala	Arg	Pro	Gly	Pro	20	25	30	
Arg	Val	Pro	Lys	Thr	Leu	Val	Leu	Val	Val	Ala	Ala	Val	Leu	Leu	Leu	35	40	45	
Val	Ser	Ala	Glu	Ser	Ala	Leu	Ile	Thr	Gln	Gln	Asp	Leu	Ala	Pro	Gln	50	55	60	
Gln	Arg	Ala	Ala	Pro	Gln	Gln	Lys	Arg	Ser	Ser	Pro	Ser	Glu	Gly	Leu	65	70	75	80
Cys	Pro	Pro	Gly	His	His	Ile	Ser	Glu	Asp	Gly	Arg	Asp	Cys	Ile	Ser	85	90	95	
Cys	Lys	Tyr	Gly	Gln	Asp	Tyr	Ser	Thr	His	Trp	Asn	Asp	Leu	Leu	Phe	100	105	110	
Cys	Leu	Arg	Cys	Thr	Arg	Cys	Asp	Ser	Gly	Glu	Val	Glu	Leu	Ser	Pro	115	120	125	
Cys	Thr	Thr	Thr	Arg	Asn	Thr	Val	Cys	Gln	Cys	Glu	Glu	Gly	Thr	Phe	130	135	140	
Arg	Glu	Glu	Asp	Ser	Pro	Glu	Met	Cys	Arg	Lys	Cys	Arg	Thr	Gly	Cys	145	150	155	160
Pro	Arg	Gly	Met	Val	Lys	Val	Gly	Asp	Cys	Thr	Pro	Trp	Ser	Asp	Ile	165	170	175	
Glu	Cys	Val	His	Lys	Glu	Ser	Gly	Thr	Lys	His	Ser	Gly	Glu	Ala	Pro	180	185	190	
Ala	Val	Glu	Glu	Thr	Val	Thr	Ser	Ser	Pro	Gly	Thr	Pro	Ala	Ser	Pro	195	200	205	
Cys	Ser	Leu	Ser	Gly	Ile	Ile	Ile	Gly	Val	Thr	Val	Ala	Ala	Val	Val	210	215	220	
Leu	Ile	Val	Ala	Val	Phe	Val	Cys	Lys	Ser	Leu	Leu	Trp	Lys	Lys	Val	225	230	235	240
Leu	Pro	Tyr	Leu	Lys	Gly	Ile	Cys	Ser	Gly	Gly	Gly	Gly	Asp	Pro	Glu	245	250	255	
Arg	Val	Asp	Arg	Ser	Ser	Gln	Arg	Pro	Gly	Ala	Glu	Asp	Asn	Val	Leu	260	265	270	
Asn	Glu	Ile	Val	Ser	Ile	Leu	Gln	Pro	Thr	Gln	Val	Pro	Glu	Gln	Glu	275	280	285	

FIG. 3B

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Met 290	Glu	Val	Gln	Glu	Pro	Ala 295	Glu	Pro	Thr	Gly	Val 300	Asn	Met	Leu	Ser
Pro 305	Gly	Glu	Ser	Glu	His 310	Leu	Leu	Glu	Pro	Ala 315	Glu	Ala	Glu	Arg	Ser 320
Gln	Arg	Arg	Arg	Leu 325	Leu	Val	Pro	Ala	Asn 330	Glu	Gly	Asp	Pro	Thr 335	Glu
Thr	Leu	Arg	Gln 340	Cys	Phe	Asp	Asp	Phe 345	Ala	Asp	Leu	Val	Pro 350	Phe	Asp
Ser	Trp	Glu 355	Pro	Leu	Met	Arg	Lys 360	Leu	Gly	Leu	Met	Asp 365	Asn	Glu	Ile
Lys	Val 370	Ala	Lys	Ala	Glu	Ala 375	Ala	Gly	His	Arg	Asp 380	Thr	Leu	Tyr	Thr
Met 385	Leu	Ile	Lys	Trp	Val 390	Asn	Lys	Thr	Gly	Arg 395	Asp	Ala	Ser	Val	His 400
Thr	Leu	Leu	Asp	Ala 405	Leu	Glu	Thr	Leu	Gly 410	Glu	Arg	Leu	Ala	Lys 415	Gln
Lys	Ile	Glu	Asp 420	His	Leu	Leu	Ser	Ser 425	Gly	Lys	Phe	Met	Tyr 430	Leu	Glu
Gly	Asn	Ala 435	Asp	Ser	Ala	Met	Ser 440	*							

FIG. 3C

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1 GCTGTGGGAA CCTCTCCACG CGCACGAACT CAGCCAAACGA TTTCTGATAG ATTTTTCGGA GTTTGACCAG AGATGCAAGG GGTGAAGGAG CGTTCCTTAC
 CGACACCCCTT GGAGAGGTGC GCGTGCTTGA GTGCGTTGCT TAAARACCTT CAAACTGGTC TCTACCTTCC CCACCTTCCT CCGAAGGATG
 101 CGTTAGGGAA CTCTGGGAC AGAGGGCCCC GCGCGCCTGA TGGCCGAGGC AGGGTGGAC CCAGGACCCA GGAGGGGCTC GGAACCATTA CCATGGGCCC
 GCAATCCCTT GAGACCCCTG TCTCGCGGGG CCGCGCGACT ACCGGCTCCG TCCCACGCTG GGTCTGGGT CCTTGGTAT GGTACCGGGC
 1 MetalaaArg
 201 GATCCCCAAG ACCCTAAAGT TCGTCGTGCT CATCGTCGCG GTCTGTCTGC CAGTCCTAGC TTACTCTGCC ACCCTGCC GGCAGGAGGA AGTTCCTCCAG
 CTAGGGGTTC TGGGATTCA AGCAGCAGCA GTAGCAGCGC CAGGACGACG GTACGGATCG AATGAGACGG TGGTGACGG CCGTCTCTCT TCAGGGGTC
 4 IleProLys ThrLeuLysp heValValVa lIleValAla ValLeuLeup roValLeuAl aTyrSerAla ThrThrAlaA rGlnGluGlu uValProGln
 301 CAGACAGTGG CCCCACAGCA ACAGAGGCAC AGCTTCAAGG GGGAGGAGTG TCCAGCAGGA TCTCATAGAT CAGAACATAC TGGAGCCTGT AACCCGTGCA
 GTCTGTCAAC GGGGTGTCGT TGTCTCCGTG TCGAAGTTCC CCTCTCTCAC AGGTCGTCTT AGAGTATCTA GTCTGTGTATG ACCTCGGACA TTGGGCACCT
 37 GlnThrVala laProGlnG1 nGlnArgHis serPheLysG lyGluGluCy sproAlaGly serHisArgS erGluHisTh rGlyAlaCys AsnProCysThr
 401 CAGAGGGTGT GGATTACACC AACGCTTCCA ACRATGAACC TTCTTGCTTC CCATGTACAG TTTGTAAATC AGATCAAAAA CATAAAAGTT CCTGCACCAT
 GTCTCCCA CA CCTAATGTGG TTCCGAAGGT TGTACTTGG AGAACGAAG GGTACATGTC AAACATTTAG TCTAGTTTTT GTATTTTCAA GGACGTGGTA
 71 GluGlyVa lasPtyrThr AsnAlaSerA snAsnGluPr oSerCysPhe ProCysThrV alyCysLysSe rAspGlnLys HisLysSerS erCysThrMet
 501 GACCAGAGAC ACAGTGTGTC AGGTAAAGA AGGCACCTTC CGGAATGAAA ACTCCCCAGA GATGTGCCGG AAGTGTAGCA GGTGCCCTAG TGGGGAAGTC
 CTGGTCTCTG TGTACACACG TCACATTTCT TCCGTGGAAG GCCTTACTTT TGAGGGGTCT CTACACGGCC TTCACATCGT CCACGGGATC ACCCTTCAG
 104 ThrArgasp ThrValCyB lncysLysG1 uGlyThrPhe ArgAsnGluA snserProG1 uMetCysArg LysCysSerA rGlyGluVal
 601 CAACTCAGTA ATTGTACGTC CTGGGATGAT ATCCAGTGTG TTGAAGAATT TGGTGCCAAT GCCACTGTGG AAACCCCGCC TGCTGAAGAG ACAATGAACA
 GTTCAGTCAT TAACATGCAG GACCTACTA TAGGTACACAC AACTCTTAA ACCACGGTTA CCGTGACACC TTTGGGGTGC ACACCTTCTC TGTACTTGT
 137 GlnValserA snCysThrse rTrpaspasp IleGlnCysV alGluGluPh eglyAlaAsn AlaThrValG luThrProAl aAlaGluGlu ThrMetAsnThr
 701 CAGCCCGGG GACTCTGCC CCAGCTGCTG AAGAGACAAT GAACACCAGC CCAGGGACTC CTGCCCCAGC TGCTGAAGAG ACAATGACCA CCAGCCCGGG
 GGTCCGGCCC CTGAGGACGG GGTCCAGCAC TTCTCTGTTA CTGTGGTCC GGTCCCTGAG GACGGGTGC ACGACTTCTC TGTACTTGT GGTCCGGCCC
 171 SerProG1 yThrProAla ProAlaAlaG luGluThrMe tAsnThrSer ProGlyThrP roAlaProAl aAlaGluGlu ThrMetThrT hrSerProGly

FIG. 3D-1

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801 GACTCCTGCC CCAGCTGCTG AAGAGACAAT GACCACCAGC CCGGGACTC CTGCCCCAGC TGCTGAAGAG ACAATGACCA CCAGCCCCGG GACTCCTGCC
CTGAGGACGG GGTGACGAC TTCTCTGTTA CTGGTGGTCG GGGCCCTGAG GACGGGGTCG ACGACTTCTC TGTACTGGT GGTGGGGCCC CTGAGGACGG
204 ThrProAla ProAlaAlaG luGluThrMe tThrThrSer ProGlyThrP roAlaProAl aAlaGluGlu ThrMetThrT hrSerProGl yThrProAla

901 TCTTCTCATT ACCTCTCATG CACCATCGTA GGGATCATAG TTCTAATTGT GCTTCTGATT GTGTTTGTGT GAAAGACTTC ACTGTGAAG AAATTCTTTC
AGAAGAGTAA TGGAGAGTAC GTGTAGCAT CCTAGTATC AAGATTAAACA CGAAGACTAA CACAACAATA CTTTCTGAAG TGACACCTTC TTTAAGGAAG
237 SerSerHisT yrLeuSerCy sThrIleVal GlyIleIleV alLeuIleVal ValPheVal

1001 CTTACTGAA AGGTTCAAGT AGGGCTGGC TGAGGGCGGG GGGCGCTGGA CACTCTCTGC CCTGCCCTCC TCTGCTGTGT TCCACAGAC AGAAACGCCCT
GAATGGACTT TCCAAGTCCA TCCGGGACCG ACTCCCGCCC CCGCGGACCT GTGAGAGACG GGACGGAGGG AGACGACACA AGGGTGCTG TCTTTGCGGA

1101 GCCCTGCC CAAAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA
CGGGACGG GTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT

1 GCTGTGGAA CCTCTCCAG CGCACGAAT CAGCCACGA TTTCTGATAG ATTTTGGGA GTTTGACCAG AGATGCAAGG GGTGAAGGAG CGCTTCTTAC
CGACACCCCT GGAGAGGTGC GCGTGTCTGA GTCCGTCTGT AAGACTATC TAAACCCCT CAAACTGGTC TCTACGTTC CCACCTCTC GCGAAGGATG
MetGlnG l yVallysGlu ArgPheLeuPro

101 CGTTAGGGAA CTCTGGGGAC AGAGCGCCCC GGCCGCCCTGA TGGCCGAGGC AGGCTGCGAC CCAGGACCCA GGACGGCGTC GGGAACCAT CCATGGCCCCG
GCAATCCCTT GACACCCCTG TCTCGCGGG TCTCGCGGACT CCGCGGACT ACCGGCTCCG TCCCACGCTG GGTCTCTGGT CCTGCCGAG CCCTTGGTAT GTTACCGGGC
-30 LeuGlyAs nserGlyAsp ArgAlaProA rgProProAs pGlyArgGly ArgValArgP roArgThrGl nAspGlyVal GlyAsnHisT hrMetAlaArg

201 GATCCCCAAG ACCCTAAAGT TCGTCTCGT CATCGTCCG GTCCCTGCTGC CAGTCTTAGC TTACTCTGCC ACCACTGCCC GGACGGAGGA AGTTCCTCCAG
CTAGGGGTTT TGGGATTCA AGCAGCAGCA GTAGCAGCG CAGGACGACG GTCAGGATCG AATGAGACGG TGGTACGGG CCGTCTCTCT TCAAGGGGTC
4 IleProLys ThrLeuLysP heValValva lIleValAla ValLeuLeup rovalLeuAl aTyrSerAla ThrThrAlaA rgGlnGluGl uValProGln

301 CAGACAGTGG CCCCACAGCA ACAGAGGCAC AGCTTCAAGG GGGAGGAGTG TCCAGCAGGA TCTCATAGAT CAGAACATAC TGGAGCCTGT AACCCGTGCA
GTCTGTCAAC GGGGTGCTGT TGTCTCCGTG TCGAAGTTCC CCCTCTCTAC AGGTCTCTCT ACAGTATCTA GTCCTGTATG ACCTCGGACA TTGGGCAAGT
37 GlnThrVala laProGlnG l nGlnArgHis SerPheLysG lyGluGluCy sProalaGly SerHisArgS erGluHisTh rGlyAlaCys AsnProCysThr

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FIG. 3D-2

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401 CAGAGGGTGT GGATTACACC AACGCTTCCA ACAATGAACC TTCTTGCTTC CCATGTACAG TTTGTAAATC AGATCAAAA CATAAAAGTT CTTGCACCAT
 GTCTCCACCA CCTAATGTGG TTGCGAAGGT TGTACITGG AAGAACGAAG GGTACATGTC AAACATTAG TCTAGTTTTT GTATTTTCAA GGACGTGGTA
 71 GluGlyVa LasPTyrThr AsnAlaSerA snAsnGluPr oSerCysPhe ProCysThrV alCysLysSe raspGlnLys HisLysSers erCysThrMet
 501 GACCAGAGAC ACAGTGTGTC AGTGTAAAGA AGGCACCTTC CGGAATGAAA ACTCCCCAGA GATGTGCCGG AAGTGTAGCA GGTGCCCTAG TGGGGAAGTC
 CTGGTCTCTG TGTACACACAG TCACATTCTT TCCGTGGAAG GCCTTACTTT TGAGGGGTCT CTACACGGCC TTCACATCGT CCACGGGATC ACCCTTTCAG
 104 ThrArgAsp ThrValCysG lncysLysG l uGlyThrPhe ArgAsnGluA snSerProG l uMetCysArg LysCysSerA rgCysProSe rGlyGluVal
 601 CAAAGTCAGTA ATTGTACGTC CTGGGATGAT ATCCAGTGTG TTGAAGAATT TGGTGCCAAT GCCACTGTGG AAACCCACG TGCTGAAGAG ACAATGAACA
 GTTCAGTCAT TAAACATGCAG GACCCCTACTA TAGGTCACAC AACTTCTTAA ACCACGGTTA CGGTGACACC TTTGGGGTGC ACGACTTCTC TGTACTTGT
 137 GlnValSerA snCysThrSe rTrpAspAsp IleGlnCysV alGluGluPh eGlyAlaAsn AlaThrValG luThrProAl aAlaGluGlu ThrMetAsnThr
 701 CCAGCCCCGG GACTCCTGCC CCAGCTGCTG AAGAGACAAT GAACACCAGC CCAGGGACTC CTGCCCCAGC TGCTGAAGAG ACAATGACCA CCAGCCCCGG
 GGTCCGGCCC CTGAGGACGG GGTCCGACGAC TTCTCTGTTA CTGTGTGTCG GGTCCCTGAG GACGGGTGCG ACGACTTCTC TGTACTTGGT GGTCCGGGCC
 171 SerProG l yThrProAla ProAlaAlaG luGluThrMe tAsnThrSer ProGlyThrP roAlaProAl aAlaGluGlu ThrMetThrT hrSerProGly
 801 GACTCCTGCC CCAGCTGCTG AAGAGACAAT GACCACCAGC CCGGGGACTC CTGCCCCAGC TGCTGAAGAG ACAATGACCA CCAGCCCCGG GACTCCTGCC
 CTGAGGACGG GGTGACGAC TTCTCTGTTA CTGGTGGTGG GGTCCCTGAG GACGGGTGCG ACGACTTCTC TGTACTTGGT GGTCCGGGCC CTGAGGACGG
 204 ThrProAla ProAlaAlaG luGluThrMe tThrThrSer ProGlyThrP roAlaProAl aAlaGluGlu ThrMetThrT hrSerProG l yThrProAla
 901 TCTTCTCATT ACCTCTCATG CACCATCGTA GGGATCATAG TTCTAATTGT GCTTCTGATT GTGTTTGTTC GAAAGACTTC ACTGTGGAAG AAATTCCTTC
 AGAAGAGTAA TGGAGAGTAC GTGGTAGCAT CCTAGTATC AAGATTAAAC CGAAGACTAA CACAAACAAA CTTTCTGAAG TGACACCTTC TTAAAGGAAG
 237 SerSerHisT yrLeuSerCy sThrIleVal GlyIleIleV alLeuIleVal ValPheVal
 1001 CTTACCTGAA AGGTTACAGT AGGCGCTGGC TGAGGGCGGG GGGCGCTGGA CACTCTCTGC CCTGCCCTCC TCTGCTGTGT TCCCACAGAC AGAAACGCCT
 GAATGGACTT TCCAAGTCCA TCCGGGACCG ACTCCCGCCC CCGCGGACCT GTGAGAGACG GGACGGAGG AGACGACACA AGGCTGTCTG TCTTTGCGGA
 1101 GCCCCTGCC CAAAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA
 CGGGGACGG GTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT TTTTTTTT

FIG. 3D-3

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1  CCAACTGCAC CTCGGTTCTA TCGATTGAAT TCCCCGGGGA TCCTCTAGAG ATCCCTCGAC
61  CTCGACCCAC GCGTCCGGAA CCTTGCACG CGCACAACT ACGGGGACGA TTTCTGATTG
121 ATTTTGGCG CTTTCGATCC ACCCTCCTCC CTTCTCATGG GACTTTGGGG ACAAAGCGTC
1  M G L W G Q S V
181 CCGACCGCCT CGAGCGCTCG AGCAGGGCGC TATCCAGGAG CCAGGACAGC GTCGGGAACC
9  P T A S S A R A G R Y P G A R T A S G T
241 AGACCATGGC TCCTGGACCC CAAGATCCTT AAGTTCGTG TCTTCATCGT CGCGGTTCTG
29 R P W L L D P K I L K F V V F I V A V L
301 CTGCCGGTCC GGGTTGACTC TGCCACCATC CCCC GG CAGG AC GAAGTTCC CCAGCAGACA
49 L P V R V D S A T I P R Q D E V P Q Q T
361 GTGGCCCCAC AGCAACAGAG GCGCAGCCTC AAGGAGGAGG AGTGTCCAGC AGGATCTCAT
69 V A P Q Q Q R R S L K E E C P A G S H
421 AGATCAGAAT ATACTGGAGC CTGTAACCCG TGCACAGAGG GTGTGGATTA CACCATTGCT
89 R S E Y T G A C N P C T E G V D Y T I A
481 TCCAACAATT TGCCTTCTTG CCTGCTATGT ACAGTTTGT AATCAGGTCA ACAAATAAAA
109 S N N L P S C L L C T V C K S G Q T N K
541 AGTTCCTGTA CCACGACCAG AGACACCGTG TGT CAGTGTG AAAAAGGAAG CTTCCAGGAT
129 S S C T T T R D T V C Q C E K G S F Q D
601 AAAA ACTCCC CTGAGATGTG CCGGACGTGT AGAACAGGGT GTCCCAGAGG GATGGTCAAG
149 K N S P E M C R T C R T G C P R G M V K
661 GTCAGTAATT GTACGCCCCG GAGTGACATC AAGTGCAAAA ATGAATCAGC TGCCAGTTCC
169 V S N C T P R S D I K C K N E S A A S S
721 ACTGGGAAA CCCAGCAGC GGAGGAGACA GTGACCACCA TCCTGGGGAT GCTTGCCTCT
189 T G K T P A A E E T V T T I L G M L A S
781 CCCTATCACT ACCTTATCAT CATAGTGGTT TTAGTCATCA TTTTAGCTGT GGTTGTGGTT
209 P Y H Y L I I I V V L V I I L A V V V V
841 GGCTTTTCAT GTCGGAAGAA ATTCATTCTT TACCTCAAAG GCATCTGCTC AGGTGGTGGG
229 G F S C R K K F I S Y L K G I C S G G G

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FIG. 3E-1

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901 GGAGGTCCCG AACGTGTGCA CAGAGTCCTT TTCCGGCGGC GTTCATGTCC TTCACGAGTT
 249 G G P E R V H R V L F R R R S C P S R V
 961 CCTGGGCGG AGGACAATGC CCGCAACGAG ACCCTGAGTA ACAGATACTT GCAGCCCACC
 269 P G A E D N A R N E T L S N R Y L Q P T
 1021 CAGGTCTCTG AGCAGGAAAT CCAAGGTCAG GAGCTGGCAG AGCTAACAGG TGTGACTGTA
 289 Q V S E Q E I Q G Q E L A E L T G V T V
 1081 GAGTYGCCAG AGGAGCCACA GCGTCTGCTG GAACAGGCAG AAGCTGAAGG GTGTCAGAGG
 309 E Xaa P E E P Q R L L E Q A E A E G C Q R
 1141 AGGAGGCTGC TGGTTCCAGT GAATGACGCT GACTCCGCTG ACATCAGCAC CTTGCTGGAT
 329 R R L L V P V N D A D S A D I S T L L D
 1201 GCCTCGGCAA CACTGGAAGA AGGACATGCA AAGGAAACAA TTCAGGACCA ACTGGTGGG
 349 A S A T L E E G H A K E T I Q D Q L V G
 1261 TCCGAAAGC TCTTTTATGA AGAAGATGAG GCAGGCTCTG CTACGTCCCTG CCTGTGAAAG
 369 S E K L F Y E E D E A G S A T S C L
 1321 AATCTCTTCA GGA AACCGA GCTTCCCTCA TTTACCTTTT CTCCTACAA GGAAGCAGC
 1381 CTGGAAGAAA CAGTCCAGTA CTTGACCCAT GCCCAACAA ACTCTACTAT CCAATATGGG
 1441 GCAGCTTACC AATGGTCCTA GAACTTTGT AACGCACTTG GAGTAATTTT TATGAAATAC
 1501 TCGGTGTGAT AAGCAAACGG GAGAAATTA TATCAGATTC TTGGCTGCAT AGTTATACGA
 1561 TTGTGTATTA AGGTCGTTT TAGGCCACAT GCGGTGGCTC ATGCCCTGTAA TCCCAGCACT
 1621 TTGATAGGCT GAGGCAGGTG GATTGCTTGA GCTCGGGAGT TTGAGACCAG CCTCATCAAC
 1681 ACAGTGAAAC TCCATCTCAA TTTAAAAAGA AAAAAAGTGG TTTTAGGATG TCATTCTTTG
 1741 CAGTTCTTCA TCATGAGACA AGTCTTTTTT TCTGCTTCTT ATATTGCAAG CTCCATCTCT
 1801 ACTGGTGTGT GCATTTAATG ACATCTAACT ACAGATGCCG CACAGCCACA ATGCTTTGCC
 1861 TTATAGTTTT TTAACCTTTAG AACGGGATTA TCTTGTTATT ACCTGTATTT TCAGTTTCGG
 1921 ATATTTTGA CTTAATGATG AGATTATCAA GACGTACCCC TATGCTAAGT CATGAGCATA
 1981 TGGACTTACG AGGTTTCGAC TTAGAGTTTT GAGCTTTAAG ATAGGATTAT TGGGGGCTTA
 2041 CCCCCACCTT AATTAGAAGA AACATTTTAT ATTGCTTTAC TA

FIG. 3E-2

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FUT3 Structure Fucosyltransferase III

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1      ATGGATCCCCTGGGTGCAGCCAAGCCACAATGGCCATGGCGCGGCTGTCTGGCCGCACTG
METAspProLeuGlyAlaAlaLysProGlnTrpProTrpArgArgCysLeuAlaAlaLeu
61     CTATTTTACGCTGCTGGTGGCTGTGTGTTTCTTCTCCTACCTGCGTGTGTCCCGAGACGAT
LeuPheGlnLeuLeuValAlaValCysPhePheSerTyrLeuArgValSerArgAspAsp
121    GCCACTGGATCCCCTAGGGCTCCCAGTGGGTCTCCCGACAGGACACCACTCCCACCCGC
AlaThrGlySerProArgAlaProSerGlySerSerArgGlnAspThrThrProThrArg
181    CCCACCTTCCTGATCCTGCTATGGACATGGCCTTTCACATCCCTGTGGCTCTGTCCCGC
ProThrLeuLeuIleLeuLeuTrpThrTrpProPheHisIleProValAlaLeuSerArg
241    TGITCAGAGATGGTGGCGGCACAGCCGACTGCCACATCACTGCCGACCGCAAGGTGTAC
CysSerGluMETValProGlyThrAlaAspCysHisIleThrAlaAspArgLysValTyr
301    CCACAGGCAGACACGGTCATCGTGCACCACTGGGATATCATGTCCAACCCTAAGTCACGC
ProGlnAlaAspThrValIleValHisHisTrpAspIleMETSerAsnProLysSerArg
361    CTCCACCTTCCCGAGGCGCAGGGGCAGCGCTGGATCTGGTTCAACTTGGAGCCACCC
LeuProProSerProArgProGlnGlyGlnArgTrpIleTrpPheAsnLeuGluProPro
421    CCTAACTGCCAGCACCTGGAAGCCCTGGACAGATACTTCAATCTCACCATGTCTACCGC
ProAsnCysGlnHisLeuGluAlaLeuAspArgTyrPheAsnLeuThrMETSerTyrArg
481    AGCGACTCCGACATCTTCAGGCCCTACGGCTGGCTGGAGCCGTGGTCCGGCCAGCCTGCC
SerAspSerAspIlePheThrProTyrGlyTrpLeuGluProTrpSerGlyGlnProAla
541    CACCCACCGCTCAACCTCTCGGCCAAGACCGAGCTGGTGGCCTGGGCGGTGTCCAACCTGG
HisProProLeuAsnLeuSerAlaLysThrGluLeuValAlaTrpAlaValSerAsnTrp
601    AAGCCGGACTCAGCCAGGGTGGCTACTACCAGAGCCTGCAGGCTCATCTCAAGGTGGAC
LysProAspSerAlaArgValArgTyrTyrGlnSerLeuGlnAlaHisLeuLysValAsp
661    GTGTACGGACGCTCCACAAAGCCCTGCCCAAGGGGACCATGATGGAGACGCTGTCCCGG
ValTyrGlyArgSerHisLysProLeuProLysGlyThrMETMETGluThrLeuSerArg
721    TACAAGTICTACCTGGCCTTCGAGAATCCTTGCACCCGACTACATCACCGAGAAGCTG
TyrLysPheTyrLeuAlaPheGluAsnSerLeuHisProAspTyrIleThrGluLysLeu
781    TGGAGGAACGCCCTGGAGGCCTGGGCCGTGCCCGTGGTGTGGGCCCCAGCAGAAGCAAC
TrpArgAsnAlaLeuGluAlaTrpAlaValProValValLeuGlyProSerArgSerAsn
841    TACGAGAGGTTCTGCCACCCGACGCCTTCATCCACGTGGACGACTTCCAGAGCCCCAAG
TyrGluArgPheLeuProProAspAlaPheIleHisValAspAspPheGlnSerProLys
901    GACCTGGCCCGGTACCTGCAGGAGCTGGACAAGGACCAGGCCCGCTACCTGAGCTACTTT
AspLeuAlaArgTyrLeuGlnGluLeuAspLysAspHisAlaArgTyrLeuSerTyrPhe
961    CGCTGGCGGGAGACGCTCGGCCTCGCTCCTTCAGCTGGGCACTGGATTTCTGCAAGGCC
ArgTrpArgGluThrLeuArgProArgSerPheSerTrpAlaLeuAspPheCysLysAla
1021   TGCTGGAAGTGCAGCAGGAATCCAGGTACCAGACGGTGGCAGCATAGCGGCTTGTTTC
CysTrpLysLeuGlnGlnGluSerArgTyrGlnThrValArgSerIleAlaAlaTrpPhe
1081   ACCTGA
ThrSTP

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FIG. 4

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FUT6
Structure Fucosyltransferase VI

1 ATGGATCCCCTGGGCCCCGCCAAGCCACAGTGGTTCGTGGCGCTGCTGTCTGACCACGCTG
METAspProLeuGlyProAlaLysProGlnTrpSerTrpArgCysCysLeuThrThrLeu
61 CTGTTTCAGCTGCTGATGGCTGTGTGTTTCTTCTCCTATCTGCGTGTGTCTCAAGACGAT
LeuPheGlnLeuLeuMETAlaValCysPhePheSerTyrLeuArgValSerGlnAspAsp
121 CCCAGTGTGTACCCTAATGGGTCCCGCTTCCCAGACAGCACAGGGACCCCCGCCCCACTCC
ProThrValTyrProAsnGlySerArgPheProAspSerThrGlyThrProAlaHisSer
181 ATCCCCCTGATCCTGCTGTGGACGTGGCCTTTTAACAAACCCATAGCTCTGCCCCGCTGC
IleProLeuIleLeuLeuTrpThrTrpProPheAsnLysProIleAlaLeuProArgCys
241 TCAGAGATGGTGCCTGGCAGCGCTGACTGCAACATCACTGCCGACCGCAAGGTGTATCCA
SerGluMETValProGlyThrAlaAspCysAsnIleThrAlaAspArgLysValTyrPro
301 CAGGCAGACGCGGTTCATCGTGACCACCGAGAGGTCACTGTACAACCCAGTGCCAGCTC
GlnAlaAspAlaValIleValHisHisArgGluValMETTyrAsnProSerAlaGlnLeu
361 CCACGCTCCCCGAGGCGGCAGGGGCAGCGATGGATCTGGTTCAGCATGGAGTCCCCAAGC
ProArgSerProArgArgGlnGlyGlnArgTrpIleTrpPheSerMETGluSerProSer
421 CACTGCTGGCAGCTGAAAGCCATGGACGGATACCTCAATCTCACCATGTCTACCGCAGC
HisCysTrpGlnLeuLysAlaMETAspGlyTyrPheAsnLeuThrMETSerTyrArgSer
481 GACTCCGACATCTTCACGCCCTACGGCTGGCTGGAGCCGTGGTCCGGCCAGCCTGCCAC
AspSerAspIlePheThrProTyrGlyTrpLeuGluProTrpSerGlyGlnProAlaHis
541 CCACCGCTCAACCTCTCGGCCAAGACCGAGCTGGTGGCCTGGGCAGTGTCCAACCTGGGGG
ProProLeuAsnLeuSerAlaLysThrGluLeuValAlaTrpAlaValSerAsnTrpGly
601 CCAAACTCCGCCAGGGTGGCCTACTACCAGAGCCTGCAGGCCCATCTCAAGGTGGACGTG
ProAsnSerAlaArgValArgTyrTyrGlnSerLeuGlnAlaHisLeuLysValAspVal
661 TACGGACGCTCCACAGAGCCCCTGCCCCAGGGAACCATGATGGAGACGCTGTCCCGGTAC
TyrGlyArgSerHisLysProLeuProGlnGlyThrMETMETGluThrLeuSerArgTyr
721 AAGTTCTATCTGGCCTTCGAGAACTCCTTGACCCCGACTACATCACCGAGAAGCTGTGG
LysPheTyrLeuAlaPheGluAsnSerLeuHisProAspTyrIleThrGluLysLeuTrp
781 AGGAACGCCCTGGAGGCCCTGGGCCGTGCCCGTGGTGGTGGGCCCCAGCAGAAGCAACTAC
ArgAsnAlaLeuGluAlaTrpAlaValProValValLeuGlyProSerArgSerAsnTyr
841 GAGAGGTTCCCTGCCACCCGACGCCTTCATCCACGTGGACGACTTCCAGAGCCCCAAGGAC
GluArgPheLeuProProAspAlaPheIleHisValAspAspPheGlnSerProLysAsp
901 CTGGCCCCGTACCTGCAGGAGCTGGACAAGGACCACGCCCGCTACCTGAGCTACTTTCGC
LeuAlaArgTyrLeuGlnGluLeuAspLysAspHisAlaArgTyrLeuSerTyrPheArg
961 TGGCGGGAGACGCTGCGGCCTCGCTCCTTCAGCTGGGCACTCGCTTCTCAGAGGCCTGC
TrpArgGluThrLeuArgProArgSerPheSerTrpAlaLeuAlaPheCysLysAlaCys
1021 TGGAAACTGCAGGAGGAATCCAGGTACCAGACACGCGGCATAGCGGCTTGGTTCACCTGA
TrpLysLeuGlnGluGluSerArgTyrGlnThrArgGlyIleAlaAlaTrpPheThrSTP

FIG. 5

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	Cell Line	Apo2L 0.5% FBS		Apo2L 10% FBS		DR5 mAb+XL 0.5% FBS		DR5 mAb+10% FBS				sialyl Lew is A	sialyl Lew is X
		MTT	Mean	STD	MTT	Mean	STD	MTT	Mean	STD	FUT3	FUT6	
1	COLO 205	10.4	3.3	47.5	7.5	11.6	3.0	122.0	140.7	3.8	3268.5	53507.2	783.6
2	COLO 201	125.7	47.5	4.2	11.6	11.6	3.0	156.2	108.6	37.0	1799.3	1994.9	1261.1
3	COLO-206F	40.2	4.2	21.0	17.9	17.9	0.3	166.4	192.2	0.3	417.5	195.3	1377.3
4	SW948	27.6	21.0	24.3	19.1	19.1	12.7	181.3	202.5	102.4	1256.9	1239.3	13.3
5	HCT-15	45.9	24.3	61.6	510.6	510.6	692.0	136.3	268.5	12.1	90.1	137.7	7.3
6	DLD-1	105.8	61.6	60.6	1000.0	1000.0		184.7	320.3	25.9	147.2	86.1	460.3
7	LS1034	97.7	60.6	215.3	1000.0	1000.0		59.1	338.4	19.0	361.4	16799.0	530.0
8	CL-40	847.8	215.3	0.0	1000.0	1000.0		379.0	435.8	74.5	1275.6	11073.8	506.4
9	COLO-678	1000.0	0.0	438.2	1000.0	1000.0		107.3	132.5	14.7	50.7	40.7	148.9
10	SW403	1000.0	0.0	371.6	1000.0	1000.0		201.0	300.0	62.2	2033.9	39360.7	251.2
11	LS 180	1000.0	0.0	347.6	1000.0	1000.0	0.0	343.7	376.8	34.9	2076.9	57960.7	26.8
12	SK-CO-1	814.2	371.6	497.0	1000.0	1000.0	0.0	311.6	699.9	103.4	443.6	333.2	7.3
13	HCT 116	347.6	438.2	361.6	1000.0	1000.0	0.0	999.7	1000.0	0.7	42.6	40.7	1.4
14	RKO-AS45-1	819.2	361.6	497.0	1000.0	1000.0	0.0	552.2	1000.0	517.1	0.3	3.5	3.3
15	SW480	569.6	497.0	1000.0	1000.0	1000.0	0.0	615.3	1000.0	392.4	0.3	6.3	6.6
16	LoVo	1000.0	0.0	1000.0	1000.0	1000.0		477.8	1000.0	356.2	4.4	5.8	5.6
17	SW620	1000.0	0.0	1000.0	1000.0	1000.0	0.0	651.5	1000.0	412.9	0.5	6.5	15.4
18	RKO	1000.0	0.0	1000.0	1000.0	1000.0	0.0	857.2	1000.0	285.7	0.1	0.6	4.5
19	CX-1	1000.0	0.0	1000.0	1000.0	1000.0	0.0	902.2	1000.0	195.6	30.7	162.1	18.3
20	HT-29	1000.0	0.0	1000.0	1000.0	1000.0	0.0	1000.0	1000.0	0.0	63.8	12.8	5.0
21	KM12	1000.0	0.0	1000.0	1000.0	1000.0		1000.0	1000.0	0.0	13.4	28.3	7.0
22	COLO 320HSR	1000.0	0.0	1000.0	1000.0	1000.0		1000.0	1000.0	0.0	0.9	0.7	3.8
23	COLO 320DM	1000.0	0.0	1000.0	1000.0	1000.0		1000.0	1000.0	0.0	1.1	0.6	5.2
24	SW1116	1000.0	0.0	1000.0	1000.0	1000.0		1000.0	1000.0	0.0	291.9	22176.9	386.0
25	SW837	1000.0	0.0	1000.0	1000.0	1000.0	0.0	1000.0	1000.0	0.0	65.2	9.1	7.2
26	Caco-2	1000.0	0.0	1000.0	1000.0	1000.0	0.0	1000.0	1000.0	0.0	3.0	149.7	5.4
27	HCT-8	1000.0	0.0	1000.0	1000.0	1000.0	0.0	1000.0	1000.0	0.0	3.2	6.8	1.3
28	SW1417	1000.0	0.0	1000.0	1000.0	1000.0	0.0	1000.0	1000.0	0.0	48.7	233.0	21.2

FIG. 6

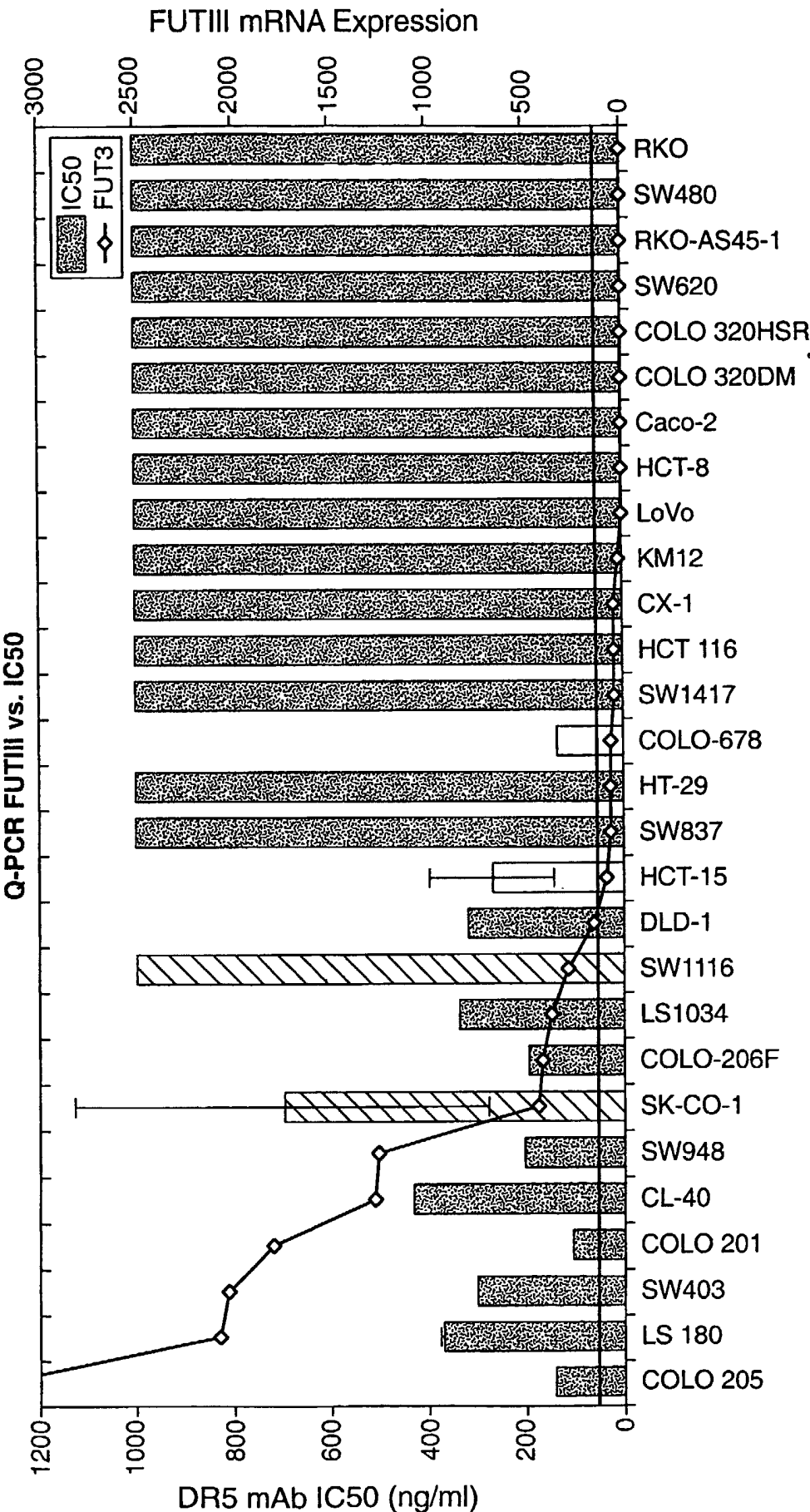


FIG. 7

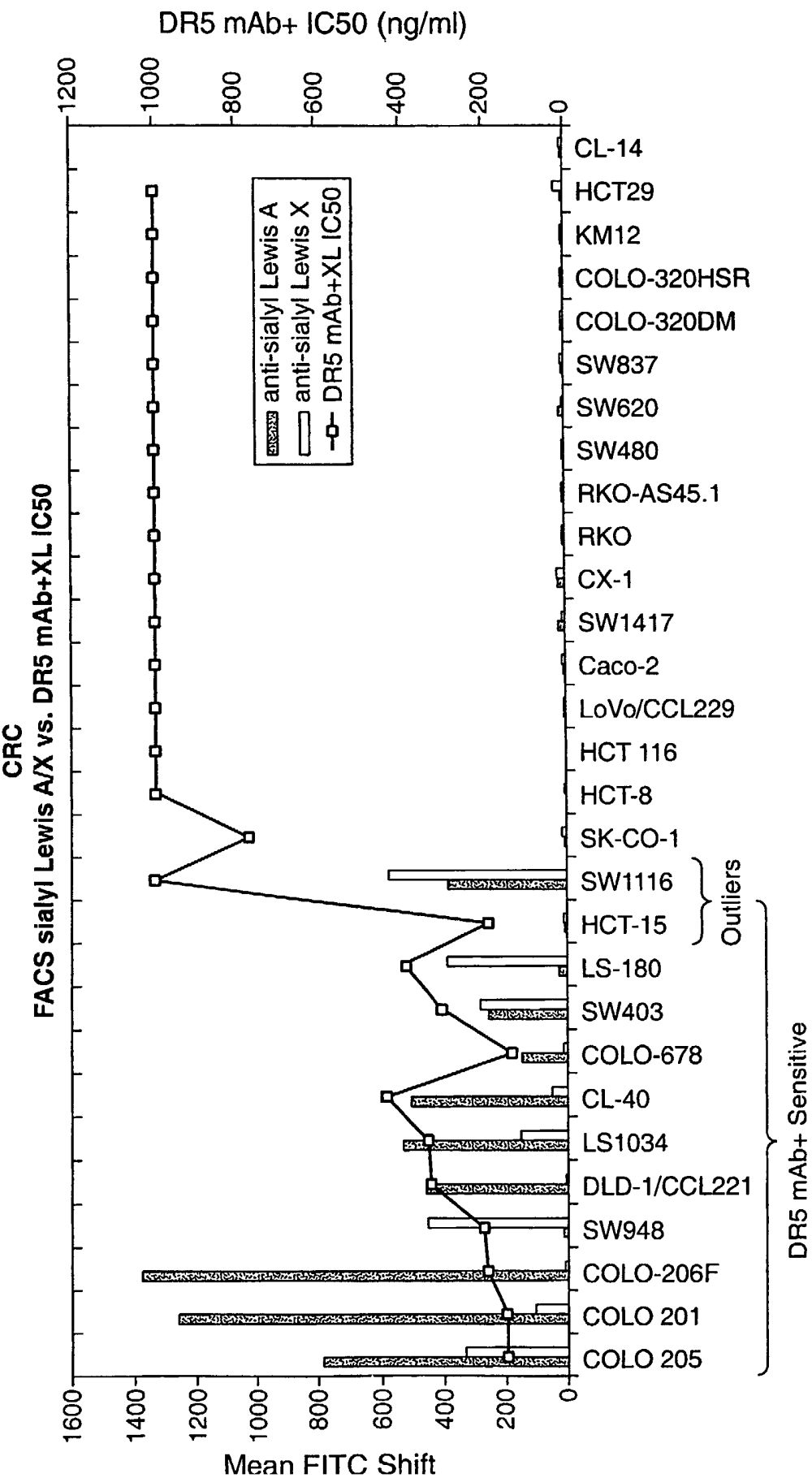


FIG. 8

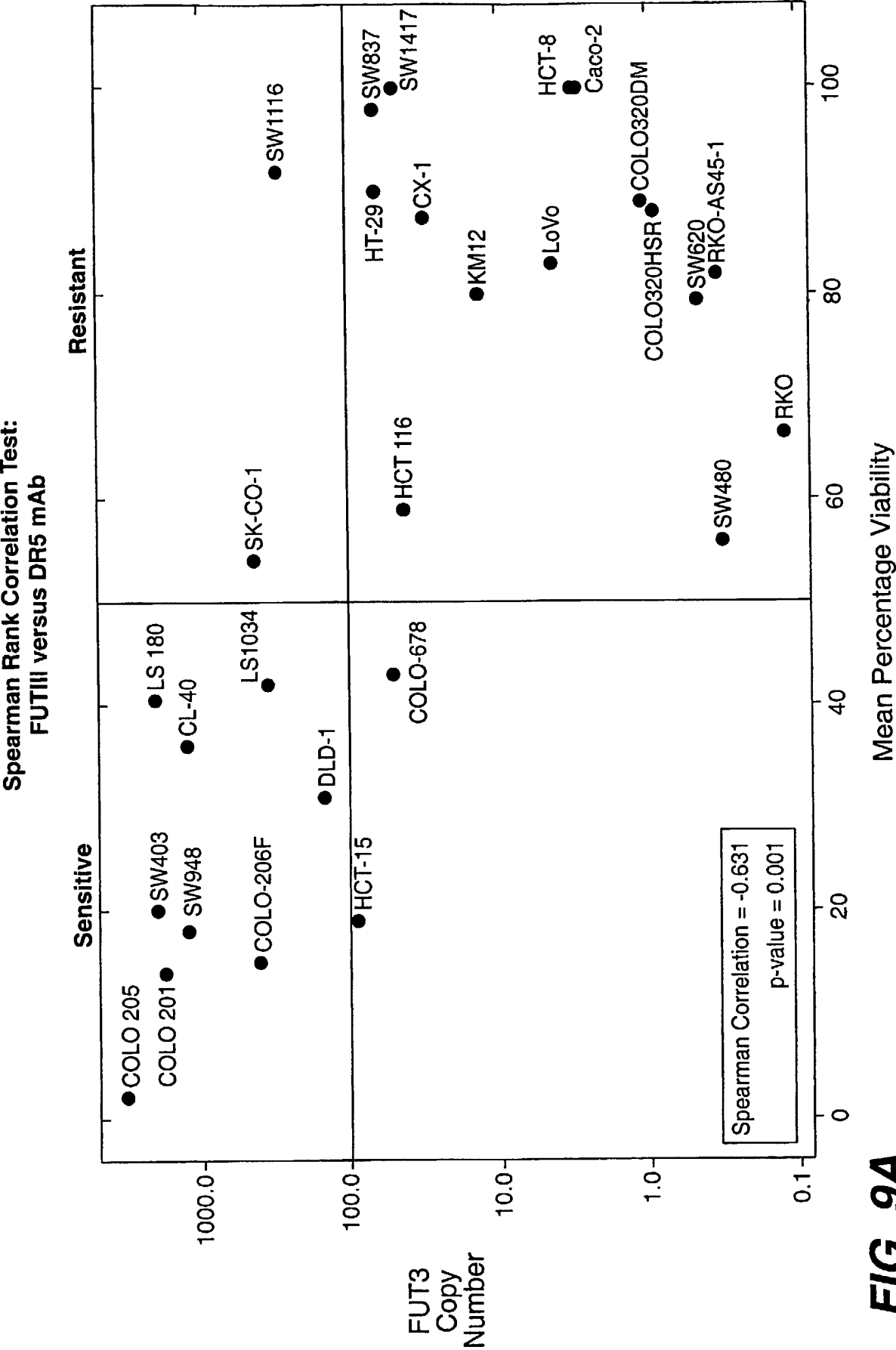


FIG. 9A

Fisher's Exact Test
FUTIII/sialyl-Lewis A/X versus DR5 mAb

Fisher's Exact Test	FUT3		Fisher's Exact Test	sLA/X	
	YES	NO		YES	NO
	9	2		10	1
DR5 mAb	2	15	SENS	1	16
	11	17	RES	11	17
		28			28

p-value = 0.00035707

p-value = 0.00000875

Statistically significant association between FUTIII and sialyl Lewis A/X expression and DR5 mAb sensitivity.

Test Set:

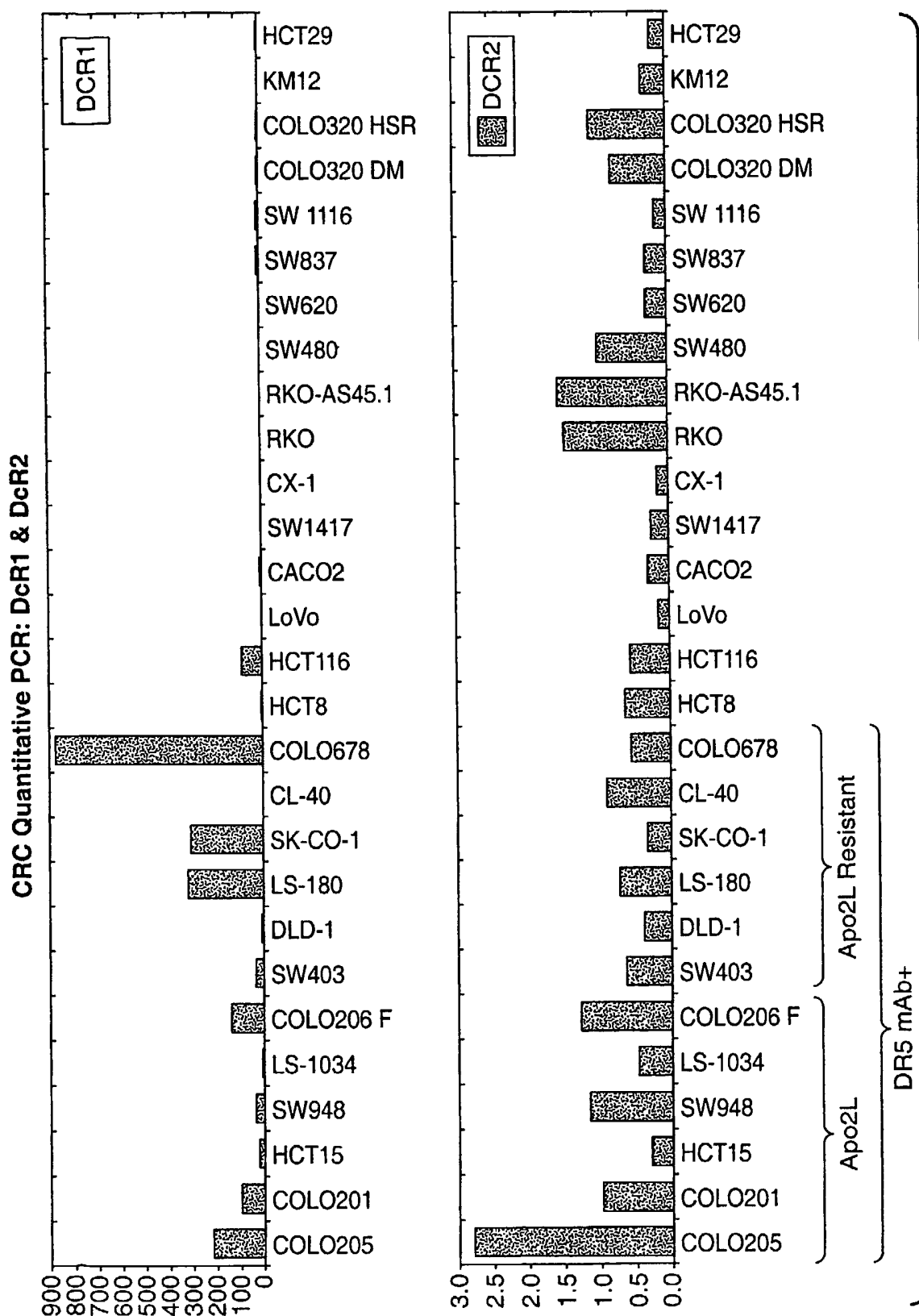
Fisher's Exact Test	FUT3		Fisher's Exact Test	sLA/X	
	YES	NO		YES	NO
	4	2		5	1
DR5 mAb	2	10	SENS	1	11
	6	12	RES	6	12
		18			18

p-value = 0.05726136

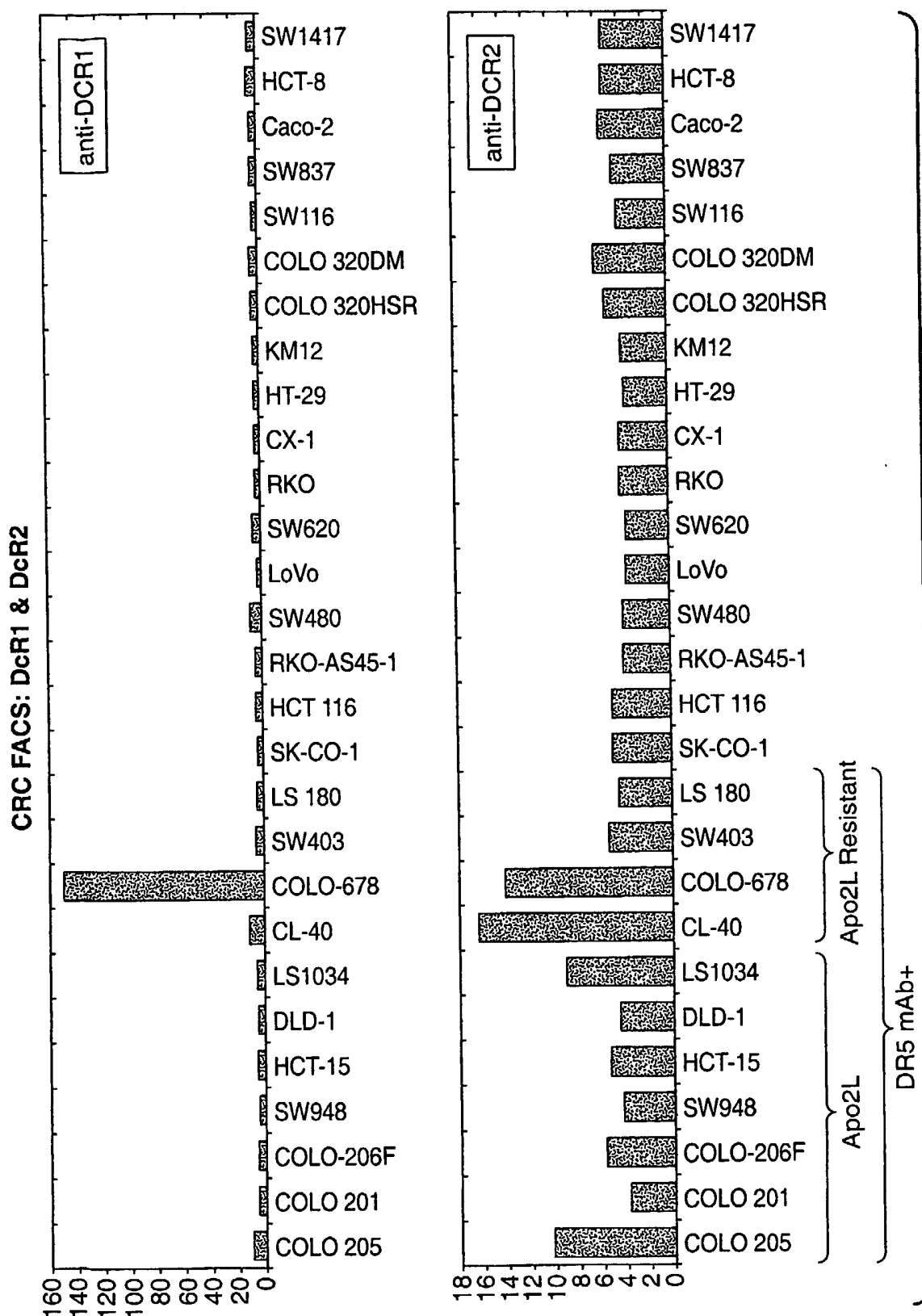
p-value = 0.00393234

FIG. 9B

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IHC Data on CRC Cell Lines

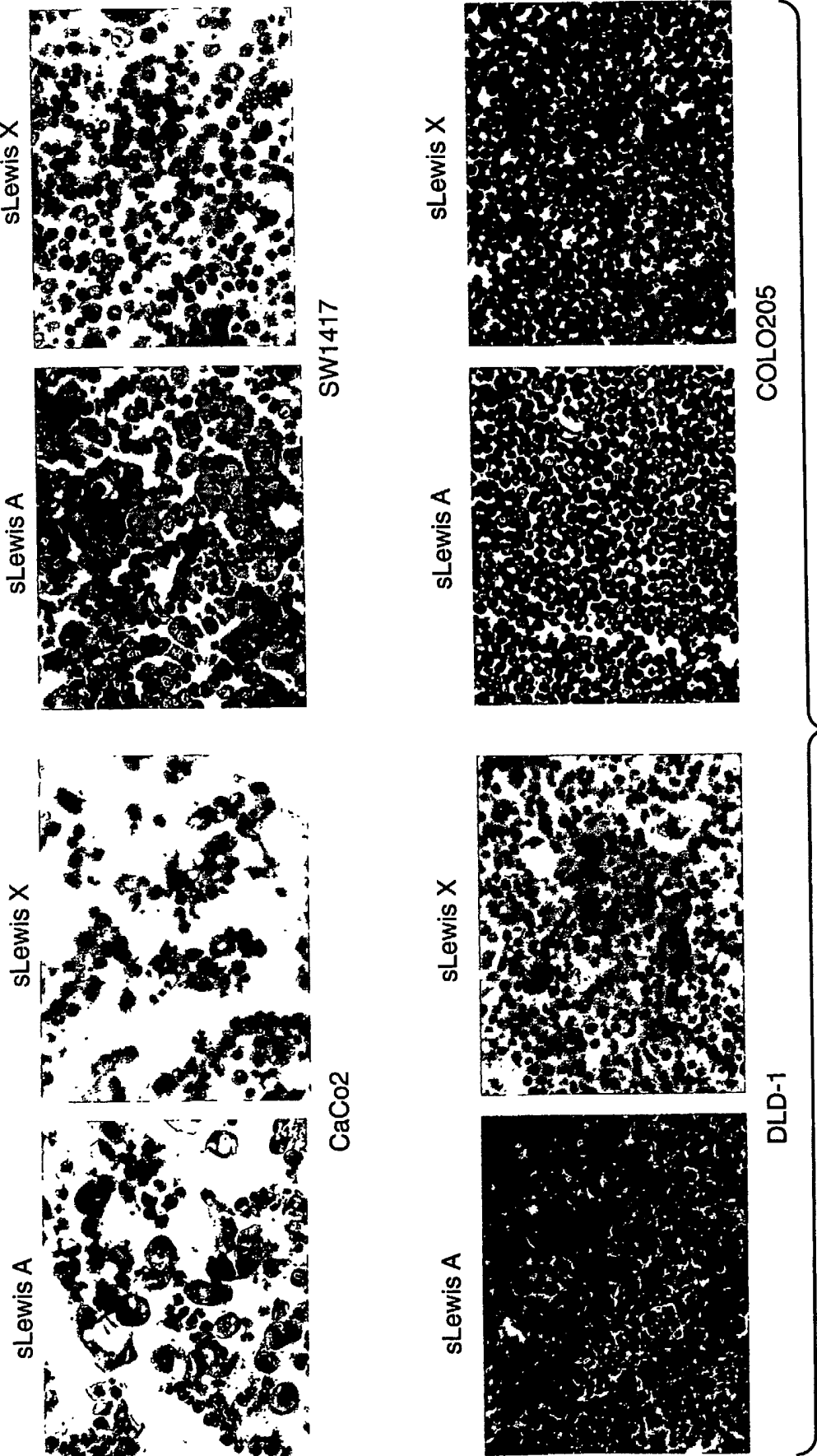
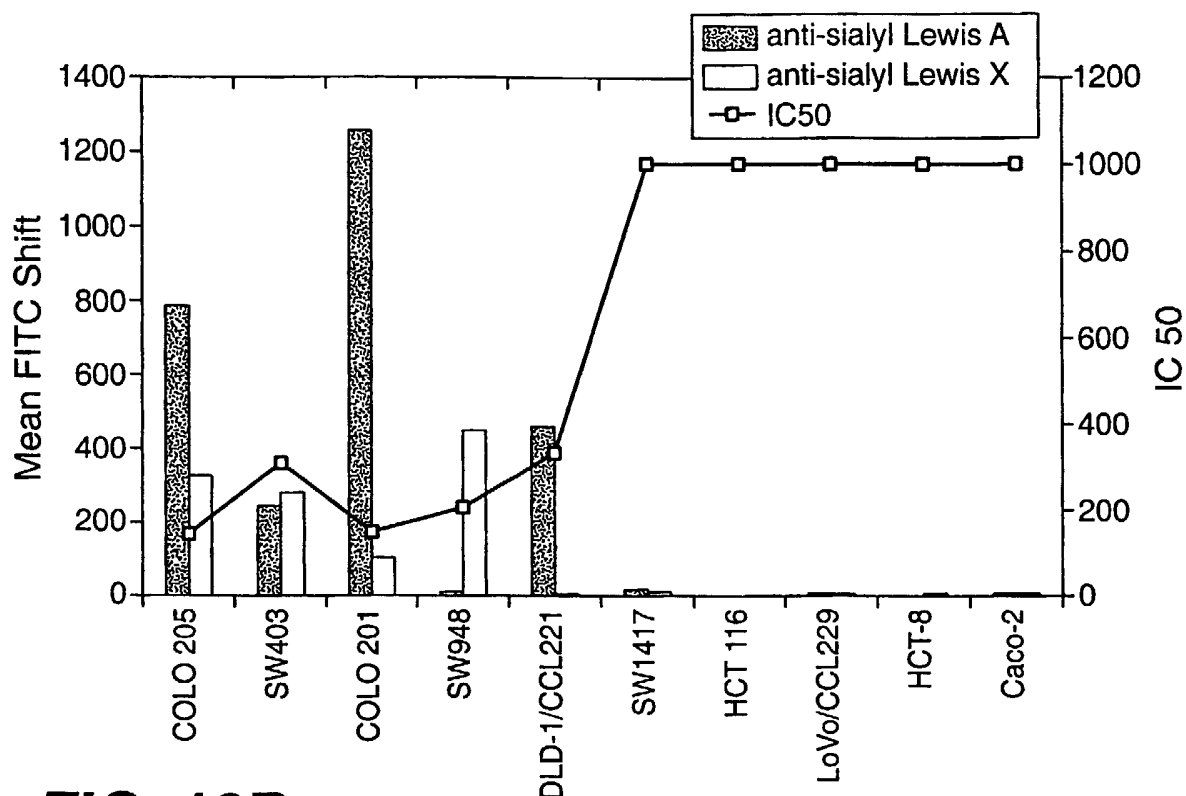


FIG. 12A

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**FIG. 12B****IHC Data on CRC Samples****Sialyl-Lewis A staining of primary colorectal adenocarcinomas (Colorectal TMAs):**

37 of 74 (50%) are positive; of these 37 cases

19 are 1+

10 are 2+

8 are 3+ in staining intensity

2 of 33 (6%) cases of benign mucosa show positive staining, both cases are 1+

Sialyl-Lewis X staining of primary colorectal adenocarcinomas (Colorectal TMAs):

47 of 75 (63%) are positive; of these 47 cases

25 are 1+

19 are 2+

3 are 3+ in staining intensity

21 of 32 (66%) cases of benign mucosa show positive staining (19 cases are 1+, 2 cases are 2+).

☐ Benign liver tissue shows positive staining on **biliary epithelium** lining small and intermediate intrahepatic bile ducts; **hepatocytes** do not show positive staining.

☐ **8 of 9 (88%) metastatic colorectal adenocarcinomas are positive for sialyl-Lewis A and X**
7 of the 8 positive cases show strong staining. There is a higher prevalence of sialyl-Lewis A and X expression and stronger reactivity in metastatic compared to primary colorectal adenocarcinomas.

FIG. 13