ANTI-HtrA1 ANTIBODIES AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 61/547,649, filed October 14, 2011, which application is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing submitted in ASCII format via EFS-Web and hereby incorporated by reference in its entirety. Said ASCII copy, created on October 8, 2012, is named P4761R1WO_SequenceListing.TXT and is 47,273 bytes in size.

FIELD OF THE INVENTION

The present invention relates to anti-HtrA1 antibodies and methods of using the same.

BACKGROUND

The serine protease HtrA1 (PRSS11; Clan PA, family S1) belongs to an evolutionarily conserved family of HtrA proteins (Clausen, T., et al., *Nat Rev Mol Cell Biol* 12:152-62 (2011); Clausen, T., et al., *Mol Cell* 10:443-55 (2002)). In humans, HtrA1, 3, and 4 share the same domain architecture: an N-terminal IGFBP-like module and a Kazal-like module, a protease domain with trypsin-like fold, and a C-terminal PDZ domain. The physiological relevance of HtrA1 has been firmly established by the identification of human loss-of-function mutations causing familial ischemic cerebral small-vessel disease (Hara, K., et al., *N Engl J Med* 360:1729-39 (2009)). The molecular mechanism involves deficient TGF-β inhibition by HtrA1 resulting in increased TGF-β signaling (Hara et al., 2009). Dysregulated TGF-β signaling by aberrant HtrA1 expression may also contribute to arthritic disease (Oka, C., et al., *Development* 131:1041-53 (2004); Tsuchiya, A., et al., *Bone* 37:323-36 (2005)), perhaps in conjunction with HtrA1-mediated degradation of various extracellular matrix components (Chamberland et al., *J Biol Chem* 284:27352-9 (2009); Grau, S., et al., *J Biol Chem* 281:6124-9 (2006); Hadfield, K.D., et al., *J Biol Chem* 283:5928-38 (2008); Tocharus, J., et al., *Dev Growth Differ* 46:257-74 (2004); Tsuchiya et al., 2005)), or indirectly via up-regulation of matrix metalloproteases (Grau et al., 2006). In addition, human genetic studies identified a strong correlation between progression of age-related macular degeneration and a SNP in the

HtrA1 promoter region, which results in increased HtrA1 transcript levels (Dewan, A., et al., *Science* 314:989-92 (2006); Yang, Z., et al., *Science* 314:992-3 (2006)). Therefore, inhibition of HtrA1 enzymatic function is an attractive therapeutic approach, e.g. in age-related macular degeneration and in arthritic disease.

SUMMARY

The disclosure provides anti-HtrA1 antibodies and methods of using the same for diagnostic and therapeutic purposes.

Disclosed is an isolated monoclonal antibody that binds to HtrA1 competitively with an antibody comprising a VH sequence of SEQ ID NO:8 and a VL sequence of SEQ ID NO:7. In one aspect, there is provided an isolated monoclonal antibody comprising a VH and a VL that bind to HtrA1, wherein the antibody (i) binds to HtrA1 competitively with an antibody comprising a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7 and (ii) inhibits HtrA1 serine protease activity with a dissociation constant of ≤500 nM. Competitive binding may be determined, for example, using an ELISA assay.

In one embodiment, the disclosure provides an isolated antibody that bind to HtrA1 having one or more of the following properties: (i) an IC_{50} of less than 50 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 5 nM, 3 nM, 2.5 nM, 2 nM, 1 nM, or less for one or more HtrA1 substrates; (ii) binds to HtrA1 with a ratio of 1 variable domain to one subunit of an HtrA1 trimer (e.g., a Fab binds to an HtrA1 trimer with a ratio of 3 Fab to 1 HtrA1 trimer, and an IgG binds to an HtrA1 trimer with a ratio of 3 IgG to 2 HtrA1 trimers), (iii) for antibodies comprising two variable domains, binds to HtrA1 in a manner that results in the forming a "cage" similar to that shown in Fig. 9, (iv) does not prevent trimer formation of HtrA1, (v) binds to one or more residues in Loop C of the HtrA1 protein, (vi) binds to the protease domain of HtrA1, (vii) binds to an epitope comprising one or both of amino acids N224 or K248 of SEQ ID NO:13, or amino acids equivalent thereto in a different HtrA1 sequence (e.g., amino acids N224 and K248 of SEQ ID NO:14, see Figs. 10A and B); (viii) binds to an epitope comprising one or more of the following residues of N224, K248, V201, T223, K243, K225, E247 and H220 of SEQ ID NO:13, or amino acids equivalent thereto in a different HtrA1 sequence; (ix) cross-reacts with murine HtrA1; (x) does not cross-react with HtrA2, HtrA3 and/or HtrA4; (xi) binds to HtrA1 competitively with an antibody comprising a VH sequence of SEQ ID NO:8 and a VL sequence of SEQ ID NO:7, (xii) binds to HtrA1 with a dissociation constant of ≤500 nM, or (xiii) inhibits complex formation between HtrA1 and α1-antitrypsin (A1AT).

Also provided is an isolated antibody that binds to HtrA1, wherein the antibody (i) binds to an epitope that includes N224, K248, or both of HtrA1, and (ii) inhibits HtrA1 with an IC_{50} of ≤ 30 nM. In one aspect, the invention provides an isolated monoclonal antibody comprising a heavy chain variable domain (VH) and a light chain variable domain (VL) that bind to HtrA1, wherein the antibody (i) binds to an epitope that includes N224, K248, or both of HtrA1, and (ii) inhibits HtrA1 serine protease activity with a dissociation constant of ≤500 nM. In certain embodiments, the epitope further includes one or more of the following residues of HtrA1: V201, T223, K243, K225, E247 and H220. In certain embodiments, the antibody may further comprise one or more of the

following properties: (i) binds to HtrA1 with a ratio of 1 variable domain to one subunit of an HtrA1 trimer, or (ii) does not prevent trimer formation of HtrA1. In certain embodiments, the IC_{50} is determined using a serine protease assay with a substrate having SEQ ID NO:12, e.g., such as the FRET assay described herein.

In certain embodiments, an antibody described herein does not cross-react with one or more of HtrA2, HtrA3 and HtrA4.

In certain embodiments, an antibody described herein has a dissociation constant of ≤500 nM. The dissociation constant may be determined, for example, by BIAcore using a Fab.

In certain embodiments, an antibody described herein is a monoclonal antibody.

In certain embodiments, an antibody described herein is a human, humanized, or chimeric antibody.

In certain embodiments, an antibody described herein is an antibody fragment that binds HtrA1.

In certain embodiments, an antibody described herein comprises (a) HVR-H3 comprising the amino acid sequence $GTFLTX_pWGHYFDY$, wherein X_p is S or T (SEQ ID NO: 27); (b) HVR-L3 comprising the amino acid sequence $QQX_gX_hX_iX_jPX_kT$, wherein X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S (SEQ ID NO: 24); and (c) HVR-H2 comprising the amino acid sequence $WIDPYGGDTX_oYADSVKG$, wherein X_o is N or D (SEQ ID NO: 26).

In certain embodiments, an antibody described herein comprises (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3; and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5.

In certain embodiments, an antibody described herein comprises (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19; and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5.

In certain embodiments, an antibody described herein comprises (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22; and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5.

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising the amino acid sequence $GFX_lIX_mX_nYYIH$, wherein X_l is N, S or T; X_m is S, D, Y or A; and X_n is G or D (SEQ ID NO: 25); (b) HVR-H2 comprising the amino acid sequence

WIDPYGGDTX$_o$YADSVKG, wherein X$_o$ is N or D (SEQ ID NO:26); and (c) HVR-H3 comprising the amino acid sequence GTFLTX$_p$WGHYFDY, wherein X$_p$ is S or T (SEQ ID NO: 27).

In certain embodiments, an antibody described herein further comprises (a) HVR-L1 comprising the amino acid sequence RASQX$_a$X$_b$X$_c$X$_d$X$_e$X$_f$A, wherein X$_a$ is D, S or V; X$_b$ is V or I; X$_c$ is S, N or G; X$_d$ is T or N; X$_e$ is A or Y; and X$_f$ is V or L (SEQ ID NO: 23); (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence QQX$_g$X$_h$X$_i$X$_j$PX$_k$T, wherein X$_g$ is S, V or D; X$_h$ is Y, D or S; X$_i$ is T, S, A, D or N; X$_j$ is T, H, N, S, A, L or R; and X$_k$ is P, T, A or S (SEQ ID NO: 24).

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

In certain embodiments, an antibody described herein further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3.

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

In certain embodiments, an antibody described herein further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:18; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

In certain embodiments, an antibody described herein further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:21; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

In certain embodiments, an antibody described herein comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3.

In certain embodiments, an antibody described herein comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:18; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

In certain embodiments, an antibody described herein comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:21; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

In certain embodiments, an antibody described herein further comprises a heavy chain variable domain framework (FR2) sequence of SEQ ID NO:17.

In one embodiment, there is provided an antibody that binds to HtrA1 comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:8; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:7; or (c) a VH sequence as in (a) and a VL sequence as in (b).

In certain embodiments, an antibody described herein comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:29; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:7; or (c) a VH sequence as in (a) and a VL sequence as in (b).

In certain embodiments, an antibody described herein comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:29; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:28; or (c) a VH sequence as in (a) and a VL sequence as in (b).

In certain embodiments, an antibody described herein comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:29; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:30; or (c) a VH sequence as in (a) and a VL sequence as in (b).

In one embodiment, there is provided an antibody that binds to HtrA1 comprising (a) a VH sequence comprising SEQ ID NO: 32; (b) a VL sequence comprising SEQ ID NO: 31; or (c) a VH sequence comprising SEQ ID NO: 32 and a VL sequence comprising SEQ ID NO: 31.

In certain embodiments, an antibody described herein comprises a VH sequence of SEQ ID NO:8.

In certain embodiments, an antibody described herein comprises a VL sequence of SEQ ID NO:7.

In certain embodiments, an antibody described herein comprises a VH sequence of SEQ ID NO:30.

In certain embodiments, an antibody described herein comprises a VL sequence of SEQ ID NO:28.

In certain embodiments, an antibody described herein comprises a VL sequence of SEQ ID NO:29.

In one aspect, an antibody described herein comprises a VH sequence of SEQ ID NO:8 and a VL sequence of SEQ ID NO:7.

In one aspect, there is provided an antibody comprising a VH sequence of SEQ ID NO:29 and a VL sequence of SEQ ID NO:28.

In certain embodiments, an antibody described herein comprises a VH sequence of SEQ ID NO:30 and a VL sequence of SEQ ID NO:28.

In one aspect, an antibody described herein comprises a VH sequence of SEQ ID NO:30 and a VL sequence of SEQ ID NO:29.

In one embodiment, there is provided an antibody comprising (a) HVR-H1 comprising the amino acid sequence $GFX_lIX_mX_nYYIH$, wherein X_l is N, S or T; X_m is S, D, Y or A; and X_n is G or D (SEQ ID NO: 25); (b) HVR-H2 comprising the amino acid sequence $WIDPYGGDTX_oYADSVKG$, wherein X_o is N or D (SEQ ID NO:26); (c) HVR-H3 comprising the amino acid sequence $GTFLTX_pWGHYFDY$, wherein X_p is S or T (SEQ ID NO: 27); (d) HVR-L1 comprising the amino acid sequence $RASQX_aX_bX_cX_dX_eX_fA$, wherein X_a is D, S or V; X_b is V or I; X_c is S, N or G; X_d is T or N; X_e is A or Y; and X_f is V or L (SEQ ID NO: 23); (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence $QQX_gX_hX_iX_jPX_kT$, wherein X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S (SEQ ID NO: 24).

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising an amino acid sequence selected from: SEQ ID NO:4, 20,and 47-51; (b) HVR-H2 comprising an amino acid sequence selected from: SEQ ID NO:5 and 52; (c) HVR-H3 comprising an amino acid sequence selected from: SEQ ID NO:6 and 53; (d) HVR-L1 comprising an amino acid sequence selected from: SEQ ID NO:1, 18, 21 and 33; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising an amino acid sequence selected from: SEQ ID NO:3, 19, 22, and 34-46.

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:1; (e) HVR-L2 comprising

the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3.

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:18; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:21; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

In certain embodiments, an antibody described herein comprises (a) HVR-H3 comprising the amino acid sequence $GTFLTX_pWGHY$, wherein X_p is S or T (SEQ ID NO: 89); (b) HVR-L3 comprising the amino acid sequence $X_gX_hX_iX_jPX_k$, wherein X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S (SEQ ID NO: 86); and (c) HVR-H2 comprising the amino acid sequence $WIDPYGGDTX_o$, wherein X_o is N or D (SEQ ID NO:88).

In one embodiment, there is provided an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 87; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:88; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:89; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:85; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:58; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 86.

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising the amino acid sequence $GFX_lIX_mX_nYY$, wherein X_l is N, S or T; X_m is S, D, Y or A; and X_n is G or D (SEQ ID NO:87); (b) HVR-H2 comprising the amino acid sequence $WIDPYGGDTX_o$, wherein X_o is N or D (SEQ ID NO:88); and (c) HVR-H3 comprising the amino acid sequence $GTFLTX_pWGHY$, wherein X_p is S or T (SEQ ID NO: 89).

In certain embodiments, an antibody described herein further comprises (a) HVR-L1 comprising the amino acid sequence $X_aX_bX_cX_dX_eX_f$, wherein X_a is D, S or V; X_b is V or I; X_c is S, N or G; X_d is T or N; X_e is A or Y; and X_f is V or L (SEQ ID NO: 85); (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:58; and (c) HVR-L3 comprising the amino acid sequence $X_gX_hX_iX_jPX_k$, wherein X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S (SEQ ID NO: 86).

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising the amino acid sequence $GFX_lIX_mX_nYY$, wherein X_l is N, S or T; X_m is S, D, Y or A; and X_n is G or D (SEQ ID NO:87); (b) HVR-H2 comprising the amino acid sequence

WIDPYGGDTX$_o$, wherein X$_o$ is N or D (SEQ ID NO:88); (c) HVR-H3 comprising the amino acid sequence GTFLTX$_p$WGHY, wherein X$_p$ is S or T (SEQ ID NO: 89); (d) HVR-L1 comprising the amino acid sequence X$_a$X$_b$X$_c$X$_d$X$_e$X$_f$, wherein X$_a$ is D, S or V; X$_b$ is V or I; X$_c$ is S, N or G; X$_d$ is T or N; X$_e$ is A or Y; and X$_f$ is V or L (SEQ ID NO: 85); (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:58; and (f) HVR-L3 comprising the amino acid sequence X$_g$X$_h$X$_i$X$_j$PX$_k$, wherein X$_g$ is S, V or D; X$_h$ is Y, D or S; X$_i$ is T, S, A, D or N; X$_j$ is T, H, N, S, A, L or R; and X$_k$ is P, T, A or S (SEQ ID NO:86).

In certain embodiments, an antibody described herein comprises (a) HVR-H1 comprising an amino acid sequence selected from: SEQ ID NO:4, 20,47-51, and 75-80; (b) HVR-H2 comprising an amino acid sequence selected from: SEQ ID NO:5, 52, and 81-82; (c) HVR-H3 comprising an amino acid sequence selected from: SEQ ID NO:6, 53 and 83-84; (d) HVR-L1 comprising an amino acid sequence selected from: SEQ ID NO:1, 18, 21, 33, and 54-57; (e) HVR-L2 comprising an amino acid sequence selected from: SEQ ID NO:2 and 58; and (f) HVR-L3 comprising an amino acid sequence selected from: SEQ ID NO:3, 19, 22, 34-46, and 59-74.

In certain embodiments, an antibody described herein is a full length IgG1 or IgG4 antibody.

In one aspect, there is provided an isolated nucleic acid encoding the antibody as defined herein.

In an embodiment, an isolated nucleic acid encoding an anti-HtrA1 antibody described herein is provided.

In one aspect, there is provided a host cell comprising the nucleic acid as defined herein.

In an embodiment, a host cell comprising an isolated nucleic acid encoding an anti-HtrA1 antibody described herein is provided.

In one aspect, there is provided a method of producing an antibody comprising culturing the host cell as defined herein under conditions suitable for expression of the nucleic acid encoding the anti-HtrA1 antibody.

In an embodiment, a method of producing an antibody is provided. The method may comprise culturing a host cell comprising an isolated nucleic acid encoding an anti-HtrA1 antibody under conditions suitable for expression of the nucleic acid encoding the anti-HtrA1 antibody. The method may further comprise recovering the anti-HtrA1 antibody from the host cell culture, purifying the anti-HtrA1 antibody, or formulating the anti-HtrA1 antibody with a pharmaceutically acceptable excipient.

In one aspect, there is provided an immunoconjugate comprising the antibody as defined herein and a cytotoxic agent.

In an embodiment, an immunoconjugate comprising an anti-HtrA1 antibody and a cytotoxic agent is provided.

In one aspect, there is provided a pharmaceutical formulation comprising the antibody as defined herein and a pharmaceutically acceptable carrier.

In an embodiment, a pharmaceutical formulation comprising an anti-HtrA1 antibody and a pharmaceutically acceptable carrier is provided.

In one aspect, there is provided the antibody as defined herein for use as a medicament.

In an embodiment, the application provides an anti-HtrA1 antibody for use as a medicament, e.g., for use in treating age-related macular degeneration (wet or dry), geographic atrophy, diabetic retinopathy, retinopathy of prematurity, or polypoidal choroidal vasculopathy,

for inhibiting degeneration of retinal or photoreceptor cells, or for inhibiting HtrA1 protease activity in an eye.

In one aspect, there is provided a use of the antibody as defined herein in the manufacture of a medicament.

In an embodiment, the application provides use of an anti-HtrA1 antibody for the manufacture of a medicament, e.g., a medicament for the treatment of age-related macular degeneration (AMD, wet or dry), geographic atrophy (GA), diabetic retinopathy (DR), retinopathy of prematurity (ROP), or polypoidal choroidal vasculopathy (PCV), for inhibiting degeneration of retinal or photoreceptor cells, or for inhibiting HtrA1 protease activity in an eye.

In another embodiment, the application provides a method of treating an individual having age-related macular degeneration (wet or dry), geographic atrophy, diabetic retinopathy, retinopathy of prematurity, or polypoidal choroidal vasculopathy, comprising administering to the individual an effective amount of an anti-HtrA1 antibody as described herein.

In another embodiment, the application provides a method for inhibiting retinal or photoreceptor cell degeneration in an individual comprising administering to the individual an effective amount of an anti-HtrA1 antibody as described herein to inhibit retinal or photoreceptor cell degeneration.

In another embodiment, the application provides a method of inhibiting HtrA1 serine protease activityin an eye of an individual comprising administering to the individual an effective amount of an anti-HtrA1 antibody as described herein to inhibit HtrA1 serine protease activity in the eye.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figs. 1A-B. Fig. 1A shows the light chain variable domain sequence of anti-HtrA1 antibody94 (YW505.94) (SEQ ID NO:7). Fig. 1B shows the heavy chain variable domain sequence of anti-HtrA1 antibody94 (YW505.94) (SEQ ID NO:8). The residues are numbered according to the Kabat numbering system (Kabat, E.A., et al., 1991, In: Sequences of proteins of immunological interest, fifth edition. National Institutes of Health, Bethesda, MD). The YW505.94 light chain variable domain sequence is aligned with human KappaI light chain consensus sequence (SEQ ID NO: 9) and the YW505.94 heavy chain variable domain sequence is aligned with the human subgroup III heavy chain sequence (SEQ ID NO: 10). The boxed

sequences are CDRs according to Kabat definitions. The sequence differences between anti-HtrA1 (YW505.94) and consensus sequences are shaded.

Fig. 2. Screening of panel of 13 phage derived antibodies (IgG). Single concentrations (0.08 – 0.28mg/ml final) of IgG were incubated with HuHtrA1 or HuHtrA1_PD and enzyme activity measured in the FRET assay. Of the 13 antibodies tested, antibodies YW503.57, YW504.57, YW504.61 and YW505.94 (also referred to as Ab94 or IgG94) strongly inhibited both HuHtrA1 and HuHtrA1_PD activities.

Fig. 3. Inhibition of HuHtrA1 by IgG94 and Fab94. HuHtrA1 was incubated with IgG94 and Fab94 for 20 min at 37 °C. Enzyme activity towards the peptide substrate H2-Opt was measured and fractional activities (v_i/v_o) calculated from the determined linear velocities. IgG94 at 300 nM did not inhibit H2-Opt hydrolysis by trypsin (1 nM) or elastase (1 nM).

Fig. 4. IgG94 inhibits hydrolysis of fluorescent dye-labeled casein (BODIPY FL) by HuHtrA1 and MuHtrA1. HuHtrA1 and MuHtrA1 were incubated with IgG94 for 15 min at 37 °C. Hydrolysis of casein BODIPY FL reagent was measured on a microplate reader at 37 °C and the linear rates of fluorescence increase determined and expressed as percent of uninhibited rates (% of control).

Fig. 5. Specificity of IgG94. MuHtrA1_PD, MuHtrA3_PD and MuHtrA4_PD were incubated with increasing concentrations of IgG94 and enzyme activities towards the peptide substrate H2-Opt (top panel) or casein BODIPY FL reagent (bottom panel) determined and expressed as percentage of uninhibited enzyme activities (% of control)

Fig. 6. Inhibition of HuHtrA1-mediated macromolecular substrate cleavage by IgG94. Increasing concentrations of IgG94 (2.3-150nM for β-casein; 2-125nM for decorin; 2.3-150nM for biglycan) were incubated with HuHtrA1 (10 nM for β-casein, 125 nM for decorin, 75 nM for biglycan) for 15min at 37 °C. The substrates β-casein, decorin and biglycan (50 μg/ml) were added and incubated for 2 – 14 h. After addition of SDS-sample buffer the digests were analyzed by SDS-PAGE (non-reducing conditions) and stained by SimplyBlue Safestain.

Figs. 7A-C. Mapping the functional epitope of IgG94 on HuHtrA1_PD. Fig. 7A shows the results of an ELISA measuring the binding of IgG94 to HuHtrA1_PD mutants with alanine substitutions of residues surrounding the active site. The shaded rows indicate alanine substitutions that resulted in a greater than 5-fold decrease in binding. Fig. 7B shows the structure of HuHtrA1_PD (PDB 3NWU) (Clausen, T., et al., Nat Rev Mol Cell Biol. 12:152-62 (2011)) with Mutated residues indicated. Medium gray shading shows mutations without loss of IgG94 binding in initial experiments; dark gray shading shows a subset of residues with >5-fold loss in binding affinity. The three monomers forming the protease domain trimer, shown

in surface representation, are shaded light gray. Catalytic triad residues D250 and S328 are underlined. Fig. 7C shows a close-up view of the loops harboring the epitope residues N224 and K248 on monomer (light gray, as cartoon) of HuHtrA1_PD (PDB 3NWU). Also included is the catalytic residue H220.

Figs. 8A-B. SEC-MALLS (size exclusion chromatography – multi-angle laser light scattering) of the Fab94:HuHtrA1_PD(S328A) complex (Fig. 8A) and the IgG94:HuHtrA1_PD(S328A) complex (Fig. 8B). Shown are the elution peaks by SEC (x-axis) and the masses of individual proteins and complexes (y-axis; dotted lines across the elution peaks).

Fig. 9. Hypothetical 'cage model' of the IgG:HuHtrA1-PD complex.

Figs. 10A-B. Alignment of human HtrA1 (SEQ ID NO:13), murine HtrA1 (SEQ ID NO:14), murine HtrA3 (SEQ ID NO:15), and murine HtrA4 (SEQ ID NO:16).

Fig. 11. HtrA1 mRNA expression increases in the retina in a mouse model of constant light exposure (top left panel). HtrA2 levels do not vary significantly in the same model (top right panel). HtrA1 expression is significantly higher than HtrA2, HtrA3 and HtrA4 expression levels in the mouse retina at baseline (bottom panel).

Fig. 12. Increased bi-polar cell/Muller cell responses in the absence of HtrA1 expression in a mouse model of constant light exposure.

Fig. 13. Sparing of retina in the absence of HtrA1 expression in a mouse model of constant light exposure.

Fig. 14. Sparing of outer nuclear layer (ONL) photoreceptor cells in the absence of HtrA1 expression in a mouse model of constant light exposure.

Fig. 15. Light chain HVR sequences for affinity-improved variants of anti-HtrA1 antibody YW505.94a.

Fig. 16. Heavy chain HVR sequences for affinity-improved variants of anti-HtrA1 antibody YW505.94a.

Fig. 17. Results of a phage competition assay demonstrating the binding of YW505.94a affinity-improved variants against HuHtrA1.

Fig. 18. Results of a phage competition assay demonstrating the binding of YW505.94a affinity-improved variants against MuHtrA1.

Figs. 19A-C. Results of ELISA assay showing levels of HtrA1 protein in rat tissues (Fig. 19A), mouse eye fluid (Fig. 19B) and mouse retina tissue (Fig. 19C).

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

The terms "anti-HtrA1 antibody" and "an antibody that binds to HtrA1" refer to an antibody that is capable of binding HtrA1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting HtrA1. In one embodiment, the extent of binding of an anti- HtrA1 antibody to an unrelated, non- HtrA1 protein is less than about 10% of the binding of the antibody to HtrA1 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to HtrA1 has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an

anti- HtrA1 antibody binds to an epitope of HtrA1 that is conserved among HtrA1 from different species.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal

origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

"Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

"Framework region" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. An HVR region as used herein comprise any number of residues located within positions 24-36 (for L1), 46-56 (for L2), 89-97 (for L3), 26-35B (for H1), 47-65 (for H2), and 93-102 (for H3). Therefore, an HVR includes residues in positions described previously:

A) 24-34 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987);

B) 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3 (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

C) 30-36 (L1), 46-55 (L2), 89-96 (L3), 30-35 (H1), 47-58 (H2), 93-100a-j (H3) (MacCallum et al. J. Mol. Biol. 262:732-745 (1996).

With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally, at a chromosomal location that is different from its natural chromosomal location, or contains only coding sequences.

"Isolated nucleic acid encoding an anti-HtrA1 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such

nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, 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 a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications,

usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide 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, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are

obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

The term "High-temperature requirement associated A" or "HtrA1," as used herein, refers to any native HtrA1 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed HtrA1 as well as any form of HtrA1 that results from processing in the cell. The term also encompasses naturally occurring variants of HtrA1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary HtrA1 is shown in SEQ ID NO:13. Exemplary fragments of human HtrA1 include fragments comprising, consisting essentially of, or consistingof amino acids Q23-P480 or P161-K379.

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may

be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

II. COMPOSITIONS AND METHODS

In one aspect, the invention is based, in part, on the discovery that a reduction of HtrA1 activity has protective effects on photoreceptor cells in the eye, the outer nuclear layer, and on elecroretinogram functionality. In certain embodiments, antibodies that bind to HtrA1 are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of various diseases associated with HtrA1 activity, including ocular disorders such as age-related macular degeneration or geographic atrophy.

A. Exemplary Anti-HtrA1 Antibodies

In one aspect, the invention provides isolated antibodies that bind to HtrA1. In certain embodiments, an anti-HtrA1 antibody has one or more of the following properties: (i) has an IC_{50} of less than 50 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 5 nM, 3 nM, 2.5 nM, 2 nM, 1 nM, or less for one or more HtrA1 substrates; (ii) binds to HtrA1 with a ratio of 1 variable domain to one subunit of an HtrA1 trimer (e.g., a Fab binds to an HtrA1 trimer with a ratio of 3 Fab to 1 HtrA1 trimer, and an IgG binds to an HtrA1 trimer with a ratio of 3 IgG to 2 HtrA1 trimers), (iii) for antibodies comprising two variable domains, binds to HtrA1 in a manner that results in the forming a "cage" similar to that shown in Fig. 9, (iv) does not prevent trimer formation of HtrA1, (v) binds to one or more residues in Loop C of the HtrA1 protein, (vi) binds to the protease domain of HtrA1, (vii) binds to an epitope comprising one or both of amino acids N224 or K248 of SEQ ID NO:13, or amino acids equivalent thereto in a different HtrA1 sequence (e.g., amino acids N224 and K248 of SEQ ID NO:14, see Figs. 10A-B); (viii) binds to an epitope comprising one or more of residues N224, K248, V201, T223, K243, K225, E247 and H220 of SEQ ID NO:13, or amino acids equivalent thereto in a different HtrA1 sequence; (ix) cross-reacts with murine HtrA1; (x) does not cross-react with HtrA2, HtrA3

and/or HtrA4; (xi) binds to HtrA1 competitively with an antibody comprising a VH sequence of SEQ ID NO:8 and a VL sequence of SEQ ID NO:7, or (xii) binds to HtrA1 with a dissociation constant of ≤500 nM, or (xiii) inhibits complex formation between HtrA1 and α1-antitrypsin (A1AT).

In one aspect, the invention provides an anti-HtrA1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:87; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:88; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:89; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:85; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:58; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:86. In one embodiment, the invention provides an anti-HtrA1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24. In one embodiment, the invention provides an anti-HtrA1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence selected from: SEQ ID NO:4, 20, 47-51 and 75-80; (b) HVR-H2 comprising an amino acid sequence selected from: SEQ ID NO:5, 52 and 81-82; (c) HVR-H3 comprising an amino acid sequence selected from: SEQ ID NO:6, 53 and 83-84; (d) HVR-L1 comprising an amino acid sequence selected from: SEQ ID NO:1, 18, 21, 33 and 54-57; (e) HVR-L2 comprising an amino acid sequence selected from: SEQ ID NO:2 and 58; and (f) HVR-L3 comprising an amino acid sequence selected from: SEQ ID NO:3, 19, 22, 34-46 and 59-74. In one embodiment, the invention provides an anti-HtrA1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:1; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3. In one embodiment, the invention provides an anti-HtrA1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid

sequence of SEQ ID NO:18; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19. In one embodiment, the invention provides an anti-HtrA1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:21; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:87; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:88; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:89. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:89. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:89 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:86. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:89, HVR-L3 comprising the amino acid sequence of SEQ ID NO:86, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:88. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:87; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:88; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:89.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:27. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:27 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:24. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:27, HVR-L3 comprising the amino acid sequence of SEQ ID NO:24, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:26. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27.

In one embodiment, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising an amino acid sequence selected from: SEQ ID NO:4, 20, 47-51 and 75-80; (b) HVR-H2 comprising an amino acid sequence selected from SEQ ID NO:5, 52 and 81-82; and (c) HVR-H3 comprising an amino acid sequence selected from: SEQ ID NO:6, 53 and 83-84. In one embodiment, the antibody comprises HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6 and 53. In another embodiment, the antibody comprises HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6, 53 and 83-84 and HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:3, 19, 22, 34-46 and 59-74. In a further embodiment, the antibody comprises HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6, 53 and 83-84, HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 3, 19, 22, 34-46 and 59-74, and HVR-H2 comprising an amino acid sequence selected from SEQ ID NO:5, 52 and 81-82. In a further embodiment, the antibody comprises (a) HVR-H1 comprising an amino acid sequence selected from SEQ ID NO:4, 20, 47-51 and 75-80; (b) HVR-H2 comprising an amino acid sequence selected from SEQ ID NO:5, 52 and 81-82; and (c) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6, 53 and 83-84.

In one embodiment, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:6. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:6 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:3. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:6, HVR-L3 comprising the amino acid sequence of SEQ ID NO:3, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:5. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

In one embodiment, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID

NO:20. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:20 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:19. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:20, HVR-L3 comprising the amino acid sequence of SEQ ID NO:19, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:5. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:20 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:22. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:20, HVR-L3 comprising the amino acid sequence of SEQ ID NO:22, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:5. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:85; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:58; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:86. In one embodiment, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:85; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:58; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:86. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In another embodiment, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:1, 18, 21, 33 and 54-57; (b) HVR-L2 comprising an amino acid sequence selected from SEQ ID NO:2 and 58; and (c) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:3, 19, 22, 34-46 and 59-74. In one embodiment, the antibody comprises (a) HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:1, 18, 21, 33 and 54-57; (b) HVR-L2 comprising an amino acid sequence selected from

SEQ ID NO:2 and 58; and (c) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:3, 19, 22, 34-46 and 59-74.

In another embodiment, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3.

In another embodiment, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:18; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:18; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

In another embodiment, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:21; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:21; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:87, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:88, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:89; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:85, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:58, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:86. In one embodiment, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26, and (iii) HVR-H3 comprising the

amino acid sequence of SEQ ID NO:27; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In another embodiment, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising an amino acid sequence selected from SEQ ID NO:4, 20, 47-51 and 75-80, (ii) HVR-H2 comprising an amino acid sequence selected from SEQ ID NO:5, 52 and 81-82, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6, 53 and 83-84; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:1, 18, 21, 33 and 54-57, (ii) HVR-L2 comprising an amino acid sequence selected from SEQ ID NO:2 and 58, and (c) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:3, 19, 22, 34-46 and 59-74.

In another embodiment, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:4, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3.

In another embodiment, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:18, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; and

(b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:21, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:87; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:88; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:89; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:85; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:58; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:86. In one embodiment, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In another embodiment, the invention provides an antibody comprising (a) HVR-H1 comprising an amino acid sequence selected from SEQ ID NO:4, 20, 47-51 and 75-80; (b) HVR-H2 comprising an amino acid sequence selected from SEQ ID NO:5, 52 and 81-82; (c) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6, 53 and 83-84; (d) HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:1, 18, 21, 33 and 54-57; (e) HVR-L2 comprising an amino acid sequence selected from SEQ ID NO:2 and 58; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:3, 19, 22, 34-46 and 59-74.

In another embodiment the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:1; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:3.

In another embodiment the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:18; (e) HVR-L2

comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

In another embodiment the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:21; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22.

In any of the above embodiments, an anti-HtrA1 antibody is humanized. In one embodiment, an anti-HtrA1 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-HtrA1 antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising an FR2 sequence of SEQ ID NO:17.

In another aspect, an anti-HtrA1 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NO:8 or 29. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-HtrA1 antibody comprising that sequence retains the ability to bind to HtrA1. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:8 or 29. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-HtrA1 antibody comprises the VH sequence in SEQ ID NO:8, 29 or 32, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising an amino acid sequence selected from: SEQ ID NO:4, 20, 25 and 47-51, (b) HVR-H2 comprising an amino acid sequence selected from SEQ ID NO:5, 26 and 52, and (c) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6, 27 and 53.

In another aspect, an anti-HtrA1 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NO:7, 28 or 30. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions),

insertions, or deletions relative to the reference sequence, but an anti-HtrA1 antibody comprising that sequence retains the ability to bind to HtrA1. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:7, 28 or 30. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-HtrA1 antibody comprises the VL sequence in SEQ ID NO:7, 28, 30 or 31, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:1, 18, 21, 23 and 33; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:2; and (c) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:3, 19, 22, 24 and 34-36.

In another aspect, an anti-HtrA1 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:32 and SEQ ID NO:31, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:8 and SEQ ID NO:7, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:29 and SEQ ID NO:28, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:29 and SEQ ID NO:30, respectively, including post-translational modifications of those sequences.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-HtrA1 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-HtrA1 antibody comprising a VH sequence of SEQ ID NO:8 and a VL sequence of SEQ ID NO:7. In certain embodiments, an antibody is provided that binds to an epitope of HtrA1 containing residue N224, K248 or both of SEQ ID NO:13, or residues equivalent thereto in a different HtrA1 sequence. In certain embodiments, the epitope of HtrA1 further comprising one or more of the following residues V201, T223, K243, K225, E247 and H220 of SEQ ID NO:13, or amino acids equivalent thereto in a different HtrA1 sequence. In certain embodiments, an antibody is provided that binds to an epitope of HtrA1 containing one or more of residues N224, K248 and V201 or all of the foregoing of SEQ ID NO:13, or residues equivalent thereto in a different HtrA1 sequence. In certain embodiments, an antibody is provided that binds to an epitope of HtrA1 containing one or more of residues N224, K248, V201, T223 and K243 or all of the foregoing of SEQ ID

NO:13, or residues equivalent thereto in a different HtrA1 sequence. In certain embodiments, an antibody is provided that binds to an epitope of HtrA1 containing one or more of residues N224, K248, V201, T223, K243, K225, E247 and H22A or all of the foregoing of SEQ ID NO:13, or residues equivalent thereto in a different HtrA1 sequence. In certain embodiments, the epitope is a linear epitope. In other embodiments, the epitope is a conformational epitope.

In a further aspect of the invention, an anti-HtrA1 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-HtrA1 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG1 antibody or other antibody class or isotype as defined herein.

In certain embodiments, an anti-HtrA1 antibody according to any of the above embodiments is not an antibody having a VH sequence of SEQ ID NO:8 and a VL sequence of SEQ ID NO:7.

In a further aspect, an anti-HtrA1 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue

for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments,

see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, see U.S. Patent No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see,* e.g., U.S. Patent No. 6,248,516 B1).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are

substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S.

Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol.*

Biol. 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for HtrA1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of HtrA1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express HtrA1. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic

steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to HtrA1 as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the

resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a

neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297

(e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Antibody variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or

IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. 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.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assay methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). 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. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions

265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001)).

In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half lives and improved binding to the neonatal Fc 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)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region.

Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-HtrA1 antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the

antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-HtrA1 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an anti-HtrA1 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include

plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. *See,* e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

Anti-HtrA1 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an antibody that competes with Fab94 or IgG94 for binding to HtrA1. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by Fab94 or IgG94. Detailed exemplary methods for mapping an epitope to which an antibody

binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

In an exemplary competition assay, immobilized HtrA1 is incubated in a solution comprising a first labeled antibody that binds to HtrA1 (e.g., Fab94 or IgG94) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to HtrA1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized HtrA1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to HtrA1, excess unbound antibody is removed, and the amount of label associated with immobilized HtrA1 is measured. If the amount of label associated with immobilized HtrA1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to HtrA1. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

2. Activity assays

In one aspect, assays are provided for identifying anti-HtrA1 antibodies thereof having biological activity. Biological activity may include, e.g., blocking, antagonizing, suppressing, interfering, modulating and/or reducing one or more biological activities of HtrA1. Antibodies having such biological activity *in vivo* and/or *in vitro* are also provided.

In certain embodiments, an antibody of the invention is tested for such biological activity. In certain embodiments, an anti-HtrA1 antibody binds to HtrA1 and reduces or inhibits its serine protease activity for one or more HtrA1 substrates, including, for example, the H2-Opt peptide, β-casein or BODIPY FL casein substrates as described in the Examples below, or any other suitable HtrA1 substrate. In certain embodiments, an anti-HtrA1 antibody inhibits HtrA1 serine protease activity with an IC_{50} of less than 50 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 5 nM, 3 nM, 2.5 nM, 2 nM, 1 nM, or less for one or more HtrA1 substrates. In certain embodiments, an anti-HtrA1 antibody protects photoreceptor cells from degredation, protects the thickness of the outer nuclear layer, or protects electroretinogram functional activity in an ocular disease model, such as the constant light exposure mouse model descrbied in the Examples below.

D. Immunoconjugates

The invention also provides immunoconjugates comprising an anti-HtrA1 antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc^{99m} or I^{123}, or a spin label for nuclear magnetic resonance (NMR)

imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-HtrA1 antibodies provided herein is useful for detecting the presence of HtrA1 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as a sample comprising photoreceptor cells, retinal pigment epithelium cells, cells of the outer nuclear layer, the inner nuclear layer, Muller cells, ciliary epithelium, or retinal tissue.

In one embodiment, an anti-HtrA1 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of HtrA1 in a

biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-HtrA1 antibody as described herein under conditions permissive for binding of the anti-HtrA1 antibody to HtrA1, and detecting whether a complex is formed between the anti-HtrA1 antibody and HtrA1. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-HtrA1 antibody is used to select subjects eligible for therapy with an anti-HtrA1 antibody, e.g. where HtrA1 is a biomarker for selection of patients.

In certain embodiments, a patient suitable for treatment with an anti-HtrA1 antibody may be identified by detecting one or more polymorphisms in the HtrA1 gene or HtrA1 control sequence, such as the HtrA1 promoter polymorphism rs11200638(G/A) (see e.g., A. DeWan, et al., *Science* 314: 989-992 (2006)).

Exemplary disorders that may be diagnosed using an antibody of the invention include ocular disorders, such as, for example, wet age-related macular degeneration (AMD), dry age-related macular degeneration, geographic atrophy (GA), diabetic retinopathy (DA), retinopathy of prematurity (ROP), or polypoidal choroidal vasculopathy (PCV).

In certain embodiments, labeled anti-HtrA1 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), 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, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

F. Pharmaceutical Formulations

Pharmaceutical formulations of an anti-HtrA1 antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically

acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredient as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, for treating an ocular disorder associated undesired neovascularization, such as wet AMD, it may be desirable to further provide an anti-angiogenic therapy, such as an anti-VEGF therapy like LUCENTIS™ (ranibizumab). Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. In other embodiments, treatment of a disease or disorder associated undesirable ocular neovascularization may involve a combination of an anti-HtrA1 antibody and photodynamic therapy (e.g., with MACUGEN™ or VISUDYNE™).

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in

colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

G. Therapeutic Methods and Compositions

Any of the anti-HtrA1antibodies provided herein may be used in therapeutic methods.

In certain embodiments, an anti-HtrA1 antibody is useful for inhibiting the degeneration of retinal cells (such as, for example, retinal pigment epithelium (RPE) cells) or photoreceptor cells in an eye of a patient suffering from or at risk for developing an ocular disorder (e.g., a patient having an HtrA1 polymorphism, increased HtrA1 expression in the eye, or drusen in at least one eye). In certain embodiments, an anti-HtrA1 antibody may be used for slowing the progression to late stage geographic atrophy or dry age-related macular degeneration in an eye of a patient having drusen in the eye to be treated. In certain embodiments, a patient suitable for treatment with an anti-HtrA1 antibody may be identified by detecting the onset of disease in at least one eye of the patient. For example, certain patients may be slected by detecting drusen, geographic atrophy, or choroidal neovascularization in one eye, while the other eye is symptom free. Such patients may be good candidates for preventative treatment in the symptom free eye, e.g., to delay the onset or reduce the severity of such symptoms (e.g., drusen, geographic atrophy, and/or choroidal neovascularization) in the symptom free eye. Alternatively, the symptomatic eye may be treated, or both the symptomatic and symptom free eye may be treated in accordance with the methods described herein. Accordingly, an anti-HtrA1 antibody may be used for preventing or inhibiting the progression of an ocular disorder in an eye of patient, wherein the patient has developed drusen, wet AMD, or geographic atrophy in the other eye, but the eye being treated is not yet symptomatic. Alternatively, the symptomatic eye is treated, or both the symptomatic and the symptom free eye are treated.

In another embodiment, an anti-HtrA1 antibody is useful for treating arthritis.

In one aspect, an anti-HtrA1 antibody for use as a medicament is provided. In further aspects, an anti-HtrA1 antibody for use in treating an ocular disease or disorder, such as, for

example, AMD (wet or dry), GA, DR, PCV or ROP, is provided. In certain embodiments, an anti-HtrA1 antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-HtrA1 antibody for use in a method of treating an individual having an ocular disease or disorder comprising administering to the individual an effective amount of the anti-HtrA1 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-HtrA1 antibody for use in inhibiting degeneration of retinal cells (such as, for example, RPE cells) or photoreceptor cells in an eye of a patient. In certain embodiments, the invention provides an anti-HtrA1 antibody for use in a method of inhibiting degeneration of retinal or photoreceptor cells in an individual comprising administering to the individual an effective amount of the anti-HtrA1 antibody to inhibit degeneration of retinal or photoreceptor cells. In further embodiments, the invention provides an anti-HtrA1 antibody for use in inhibiting HtrA1 serine protease activity in an eye of a patient. In certain embodiments, the invention provides an anti-HtrA1 antibody for use in a method of inhibiting HtrA1 serine protease activity in an eye of an individual comprising administering to the individual an effective amount of the anti-HtrA1 antibody to inhibit HtrA1 serine protease activity in the eye. An "individual" according to any of the above embodiments is preferably a human. In further embodiments, the invention provides an anti-HtrA1 antibody for use in inhibiting PCV in a patient in need thereof, e.g., a patient having choroidal vascular networks with polyp-like aneurysmal dilations.

In a further aspect, the invention provides for the use of an anti-HtrA1 antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of an ocular disorder, such as, for example, AMD (wet or dry), GA, DR, PCV or ROP. In a further embodiment, the medicament is for use in a method of treating an ocular disorder comprising administering to an individual having the ocular disorder an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for inhibiting the degredation of retinal cells or photoreceptor cells in an eye of an individual. In a further embodiment, the medicament is for use in a method of inhibiting degeneration of retinal cells or photoreceptor cells in an individual comprising administering to the individual an amount effective of the medicament to inhibit degeneration of retinal or photoreceptor cells in the individual. In a further embodiment, the medicament is for inhibiting HtrA1 serine protease activity in the eye of an individual. In a further embodiment, the medicament is for use in a

method of inhibiting HtrA1 serine protease activity in an eye of an individual comprising administering to the individual an amount effective of the medicament to inhibit HtrA1 serine protease activity in the eye. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating an ocular disorder, such as, for example, AMD (wet or dry), GA, DR, PCV or ROP. In one embodiment, the method comprises administering to an individual having such an ocular disorder an effective amount of an anti-HtrA1 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for inhibiting degeneration of retinal or photoreceptor cells in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-HtrA1 antibody to inhibit degeneration of retinal or photoreceptor cells in the individual. In one embodiment, an "individual" is a human.

In a further aspect, the invention provides a method for inhibiting HtrA1 serine protease activity in an eye of an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-HtrA1 antibody to inhibit HtrA1 serine protease activity in an eye of the individual. In one embodiment, an "individual" is a human.

The efficacy of the treatment of an ocular disorder using an anti-HtrA1 antibody, can be measured by various endpoints commonly used in evaluating intraocular diseases, such as, for example, performing an eye exam, measuring intraocular pressure, assessing visual acuity, measuring slitlamp pressure, assessing intraocular inflammation, measuring the size of CNV, measuring the leakage of CNV (e.g., by Fluorescein angiography), measuring the amount of drusen, measuring the location of drusen, etc. In certain embodiments, vision loss can be assessed, for example, by measuring the mean change in best correction visual acuity (BCVA) from baseline to a desired time point (e.g., where the BCVA is based on Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart and assessment at a test distance of 4 meters), measuring the proportion of subjects who lose fewer than 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at a desired tine point compared to baseline, measuring the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at a desired time point, or measuring the NEI Visual Functioning Questionnaire.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-HtrA1 antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-HtrA1 antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-HtrA1 antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a therapeutic agent suitable for treatment of an ocular disorder associated with undesirable neovascularization in the eye, such as, for example, wet AMD. Suitable therapeutic agents include, for example, anti-angiogenic therapies such as an anti-VEGF therapy like LUCENTIS™ (ranibizumab).

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies of the invention can also be used in combination with photdynamic therapy (e.g., with MACUGEN™ or VISUDYNE™).

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In an exemplary embodiment, an antibody of the invention (and any additional therapeutic agent) can be administered by intravitreal injection. Dosing can be by any suitable route, e.g. by injections, such as intravenous, intravitreal or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical

practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (*e.g.* such that the patient receives from about two to about twenty, or *e.g.* about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-HtrA1 antibody.

H. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated

with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-HtrA1 antibody.

SEQUENCE KEY

Light chain HVRs for Anti-HtrA1 Antibody YW505.94

HVR L1: RASQDVSTAVA (SEQ ID NO: 1)

HVR L2: SASFLYS (SEQ ID NO: 2)

HVR L3: QQSYTTPPT (SEQ ID NO: 3)

Heavy chain HVRs for Anti-HtrA1 Antibody YW505.94

HVR H1: GFNISGYYIH (SEQ ID NO: 4)

HVR H2: WIDPYGGDTNYADSVKG (SEQ ID NO: 5)

HVR H3: GTFLTSWGHYFDY (SEQ ID NO: 6)

Light chain VR for Anti-HtrA1 Antibody YW505.94
DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYTTPPTFGQGTKVEIKR (SEQ ID NO: 7)

Heavy chain VR for Anti-HtrA1 Antibody YW505.94
EVQLVESGGGLVQPGGSLRLSCAASGFNISGYYIHWVRQAPGKGLEWVGWIDPYGGD
TNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGTFLTSWGHYFDYWG
QGT (SEQ ID NO: 8)

Human HtrA1 (full length sequence in caps, protease domain is underlined, residues N224 and K248 are shaded)
mqipraallpllllllaapasaQLSRAGRSAPLAAGCPDRCEPARCPPQPEHCEGGRARDACGCCE
VCGAPEGAACGLQEGPCGEGLQCVVPFGVPASATVRRRAQAGLCVCASSEPVCGSDA
NTYANLCQLRAASRRSERLHRPPVIVLQRGACGQGQEDPNSLRHKYNFIADVVEKIAP
AVVHIELFRKLPFSKREVPVASGSGFIVSEDGLIVTNAHVVTNKHRVKVELKNGATYE
AKIKDVDEKADIALIKIDHQGKLPVLLLGRSSELRPGEFVVAIGSPFSLQNTVTTGIVSTT
QRGGKELGLRNSDMDYIQTDAIINYGNSGGPLVNLDGEVIGINTLKVTAGISFAIPSDKI
KKFLTESHDRQAKGKAITKKKYIGIRMMSLTSSKAKELKDRHRDFPDVISGAYIIEVIPD
TPAEAGGLKENDVIISINGQSVVSANDVSDVIKRESTLNMVVRRGNEDIMITVIPEEIDP
(SEQ ID NO:13)

Murine HtrA1 (full length sequence in caps, protease domain is underlined)
mqslrttllsllllllaapslalPSGTGRSAPAATVCPEHCDPTRCAPPPTDCEGGRVRDACGCCEVC
GALEGAACGLQEGPCGEGLQCVVPFGVPASATVRRRAQAGLCVCASSEPVCGSDAKT
YTNLCQLRAASRRSEKLRQPPVIVLQRGACGQGQEDPNSLRHKYNFIADVVEKIAPAV
VHIELYRKLPFSKREVPVASGSGFIVSEDGLIVTNAHVVTNKNRVKVELKNGATYEAKI
KDVDEKADIALIKIDHQGKLPVLLLGRSSELRPGEFVVAIGSPFSLQNTVTTGIVSTTQR
GGKELGLRNSDMDYIQTDAIINYGNSGGPLVNLDGEVIGINTLKVTAGISFAIPSDKIKK
FLTESHDRQAKGKAVTKKKYIGIRMMSLTSSKAKELKDRHRDFPDVLSGAYIIEVIPDT
PAEAGGLKENDVIISINGQSVVTANDVSDVIKKENTLNMVVRRGNEDIVITVIPEEIDP
(SEQ ID NO:14)

Murine HtrA3 (protease domain is underlined)
MQARALLPATLAILATLAVLALAREPPAAPCPARCDVSRCPSPRCPGGYVPDLCNCCL
VCAASEGEPCGRPLDSPCGDSLECVRGVCRCRWTHTVCGTDGHTYADVCALQAASR
RALQVSGTPVRQLQKGACPSGLHQLTSPRYKFNFIADVVEKIAPAVVHIELFLRHPLFG
RNVPLSSGSGFIMSEAGLIVTNAHVVSSSSTASGRQQLKVQLQNGDAYEATIQDIDKKS
DIATIVIHPKKKLPVLLLGHSADLRPGEFVVAIGSPFALQNTVTTGIVSTAQRDGKELGL
RDSDMDYIQTDAIINYGNSGGPLVNLDGEVIGINTLKVAAGISFAIPSDRITRFLSEFQNK
HVKDWKKRFIGIRMRTITPSLVEELKAANPDFPAVSSGIYVQEVVPNSPSQRGGIQDGDI
IVKVNGRPLADSSELQEAVLNESSLLLEVRRGNDDLLFSIIPEVVM (SEQ ID NO:15)

Murine HtrA4 (protease domain is underlined)
MSFQRLWAVRTQFLLLWLLLPAVPVPWAEARRSRVSLPCPDACDPTRCPTLPTCSAGL
APVPDRCGCCRVCAAAEGQECGGARGRPCAPRLRCGAPFSRDPSGGAWLGTCGCAE
GAEDAVVCGSDGRTYPSLCALRKENRAARQRGALPAVPVQKGACEEAGTTRAGRLR
RKYNFIAAVVEKVAPSVVHLQLFRRSPLTNQEIPSSSGSGFIVSEDGLIVTNAHVLTNQQ
KIQVELQSGARYEATVKDIDHKLDLALIKIEPDTELPVLLLGRSSDLRAGEFVVALGSPF
SLQNTVTAGIVSTTQRGGRELGLKNSDIDYIQTDAIINHGNSGGPLVNLDGDVIGINTLK
VTAGISFAIPSDRIRQFLEDYHERQLKGKAPLQKKYLGLRMLPLTLNLLQEMKRQDPEF
PDVSSGVFVYEVIQGSAAASSGLRDHDVIVSINGQPVTTTTDVIEAVKDNDFLSIIVLRG
SQTLFLTVTPEIIN (SEQ ID NO:16)

YW505.95 HC FR2
WVRQAPGKGLEWVG (SEQ ID NO:17)

Light chain HVRs for Anti-HtrA1 Antibody YW505.94a.28

HVR L1: RASQSINTYLA (SEQ ID NO: 18)

HVR L2: SASFLYS (SEQ ID NO: 2)

HVR L3: QQSDDTPPT (SEQ ID NO: 19)

Heavy chain HVRs for Anti-HtrA1 Antibody YW505.94a.28

HVR H1: GFSISGYYIH (SEQ ID NO: 20)

HVR H2: WIDPYGGDTNYADSVKG (SEQ ID NO: 5)

HVR H3: GTFLTSWGHYFDY (SEQ ID NO: 6)

Light chain VR for Anti-HtrA1 Antibody YW505.94a.28
DIQMTQSPSSLSASVGDRVTITCRASQSINTYLAWYQQKPGKAPKLLIYSASFLYSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQSDDTPPTFGQGTKVEIKR (SEQ ID NO: 28)

Heavy chain VR for Anti-HtrA1 Antibody YW505.94a.28
EVQLVESGGGLVQPGGSLRLSCAASGFSISGYYIHWVRQAPGKGLEWVGWIDPYGGD
TNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGTFLTSWGHYFDYWG
QGT (SEQ ID NO: 29)

Light chain HVRs for Anti-HtrA1 Antibody YW505.94a.54

HVR L1: RASQVVGNYLA (SEQ ID NO: 21)

HVR L2: SASFLYS (SEQ ID NO: 2)

HVR L3: QQSDDHPPT (SEQ ID NO: 22)

Heavy chain HVRs for Anti-HtrA1 Antibody YW505.94a.54

HVR H1: GFSISGYYIH (SEQ ID NO: 20)

HVR H2: WIDPYGGDTNYADSVKG (SEQ ID NO: 5)

HVR H3: GTFLTSWGHYFDY (SEQ ID NO: 6)

Light chain VR for Anti-HtrA1 Antibody YW505.94a.54
DIQMTQSPSSLSASVGDRVTITCRASQVVGNYLAWYQQKPGKAPKLLIYSASFLYSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSDDHPPTFGQGTKVEIKR (SEQ ID NO:
30)

Heavy chain VR for Anti-HtrA1 Antibody YW505.94a.54
EVQLVESGGGLVQPGGSLRLSCAASGFSISGYYIHWVRQAPGKGLEWVGWIDPYGGD
TNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGTFLTSWGHYFDYWG
QGT (SEQ ID NO: 29)

Light chain HVR Consensus Sequences

HVR L1: RASQ$X_aX_bX_cX_dX_eX_f$A, wherein X_a is D, S or V; X_b is V or I; X_c is S, N or G; X_d is T or N; X_e is A or Y; and X_f is V or L (SEQ ID NO: 23);

HVR L2: SASFLYS (SEQ ID NO: 2)

HVR L3: QQ$X_gX_hX_iX_j$PX_kT, wherein X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S (SEQ ID NO: 24);

Heavy chain HVR Consensus Sequences

HVR H1: GFX_lI$X_m$$X_n$YYIH, wherein X_l is N, S or T; X_m is S, D, Y or A; and X_n is G or D (SEQ ID NO: 25);

HVR H2: WIDPYGGDTX_oYADSVKG, wherein X_o is N or D (SEQ ID NO: 26);

HVR H3: GTFLTX_pWGHYFDY, wherein X_p is S or T (SEQ ID NO: 27).

Consensus Light chain VR
DIQMTQSPSSLSASVGDRVTITCRASQ$X_aX_bX_cX_dX_eX_f$AWYQQKPGKAPKLLIYSASFLYS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ$X_gX_hX_iX_j$PX_kTFGQGTKVEIKR, wherein X_a is D, S or V; X_b is V or I; X_c is S, N or G; X_d is T or N; X_e is A or Y; X_f is V or L; X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S; (SEQ ID NO: 31)

Consensus Heavy chain VR
EVQLVESGGGLVQPGGSLRLSCAASGFX_lI$X_m$$X_n$YYIHWVRQAPGKGLEWVGWIDPYG GDTX_oYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGTFLTX_pWGHYFD YWGQGT, wherein X_l is N, S or T; X_m is S, D, Y or A; X_n is G or D; X_o is N or D; and X_p is S or T (SEQ ID NO: 32)

YW505.94a51 HVR L1: RASQDVGTYLA (SEQ ID NO:33)

YW505.94a.1 HVR L3: QQVYSHPPT (SEQ ID NO:34)

YW505.94a.7 HVR L3: QQSYTNPPT (SEQ ID NO:35)

YW505.94a.22 HVR L3: QQSYATPTT (SEQ ID NO:36)

YW505.94a.37 HVR L3: QQSYSSPAT (SEQ ID NO:37)

YW505.94a.39 HVR L3: QQVYTTPPT (SEQ ID NO:38)

YW505.94a.40 HVR L3: QQVYATPST (SEQ ID NO:39)

YW505.94a.42 HVR L3: QQSYNSPAT (SEQ ID NO:40)

YW505.94a.46 HVR L3: QQSYSTPAT (SEQ ID NO:41)

YW505.94a.50 HVR L3: QQSYTAPTT (SEQ ID NO:42)

YW505.94a.51 HVR L3: QQDSTLPPT (SEQ ID NO:43)

YW505.94a.52 HVR L3: QQSDAAPPT (SEQ ID NO:44)

YW505.94a.78 HVR L3: QQSYSTPPT (SEQ ID NO:45)

YW505.94a.89 HVR L3: QQSYTRPPT (SEQ ID NO:46)

YW505.94a.7 HVR H1: GFSISDYYIH (SEQ ID NO:47)

YW505.94a.26 HVR H1: GFSIDGYYIH (SEQ ID NO:48)

YW505.94a.38 HVR H1: GFTIYDYYIH (SEQ ID NO:49)

YW505.94a.78 HVR H1: GFSIAGYYIH (SEQ ID NO:50)

YW505.94a.82 HVR H1: GFTISDYYIH (SEQ ID NO:51)

YW505.94A.42 HVR H2: WIDPYGGDTDYADSVKG (SEQ ID NO: 52)

YW505.94A.46 HVR H3: GTFLTTWGHYFDY (SEQ ID NO:53)

Table A. HVR and variable domain sequences for anti-HtrA1 YW505.94 antibody and affinity-improved variants thereof.

	SEQ ID NOs							
	H1	H2	H3	L1	L2	L3	HC VR	LC VR
Consensus	25	26	27	23	2	24	32	31
YW505.94	4	5	6	1	2	3	8	7
YW505.94a	20	5	6	1	2	3	29	7
YW505.94a.28	20	5	6	18	2	19	29	28
YW505.94a.54	20	5	6	21	2	22	29	30
YW505.94a.1	20	5	6	1	2	34		
YW505.94a.7	47	5	6	1	2	35		
YW505.94a.22	20	5	6	1	2	36		
YW505.94a.26	48	5	6	1	2	3		
YW505.94a.37	47	5	6	1	2	37		
YW505.94a.38	49	5	6	1	2	3		
YW505.94a.39	20	5	6	1	2	38		
YW505.94a.40	20	5	6	1	2	39		
YW505.94a.42	20	52	6	1	2	40		
YW505.94a.46	20	5	53	1	2	41		
YW505.94a.47	20	52	6	1	2	3		
YW505.94a.50	47	5	6	1	2	42		
YW505.94a.51	20	5	6	33	2	43		
YW505.94a.52	20	5	6	1	2	44		
YW505.94a.77	20	5	53	1	2	3		
YW505.94a.78	50	5	6	1	2	45		
YW505.94a.82	51	5	6	1	2	3		
YW505.94a.89	20	52	6	1	2	46		

III. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

EXAMPLE 1: Generation of Anti-HtrA1 Antibodies

Phage Display. Synthetic antibody libraries displayed bivalent Fab fragments on the M13 phage and the diversity was generated by use of oligo-directed mutagenesis in three complementarity determining regions (CDRs) of the heavy chain. The details of the Fab libraries were described previously (Lee, C.V., et al., *J Mol Biol.* 340:1073-93 (2004); Lee, C.V., et al., *J Immunol Methods.* 284:119-32 (2004)). Nunc 96-well Maxisorp immunoplates were coated overnight at 4 °C with MuHtrA1_PD (10 μg/ml) and then blocked for 1 h at room temperature with phage blocking buffer (PBS, 1% (w/v) BSA, 0.05% (v/v) Tween 20). The antibody phage libraries were added to the MuHtrA1_PD-coated plates and incubated overnight at room temperature. The plates were washed with PBS, 0.05% (v/v) Tween-20 buffer and bound phage were eluted with 50 mM HCl - 500 mM NaCl for 30 min and neutralized with an equal volume of 1 M Tris-HCl, pH 7.5. Recovered phage was amplified in *E. coli* XL-1 blue cells. During subsequent selection rounds, incubation of antibody phage with the antigen-coated plates was reduced to 2-3 hours and the stringency of plate washing was gradually increased. 13 identified clones were reformatted to IgG (as described below for Fab94), expressed in 293 cells and purified by Protein A affinity chromatography. The 13 purified IgG bound to MuHtrA1_PD in a binding ELISA.

Anti-HtrA1 Fab94 and IgG94 expression and purification. The variable regions of both the heavy and light chains of Fab94 (YW505.94) were subcloned into a *E. coli* Fab expression vector (AEP1). The resulting plasmid was transformed into *E. coli.* strain 34B8. The single colony was grown overnight at 37 °C in 30 ml LB medium supplemented with 50 μg/ml of Carbenicilin. Five ml of the culture was inoculated into 500 ml of complete C.R.A.P. medium (Simmons, L.C., et al., *J Immunol Methods.* 263:133-47 (2002)) supplemented with Carbenicilin (50 μg/ml) and grown at 30 °C for 24 h. The Fab94 protein was purified using protein A agarose resin.

The variable domains of both the light chain and heavy chain of Fab94 were cloned into a pRK5-based plasmid with human light chain or heavy chain (human IgG1) constant domain for transient expression in Chinese hamster ovary (CHO) cells. The IgG94 protein was purified by use of protein A agarose chromatography.

The amino acid sequences for the light chain variable region and heavy chain variable region for Fab94 (YW505.94) are shown in Figs. 1A and 1B, respectively.

EXAMPLE 2: Production of HtrA1 Proteins

Protein Constructs. Full length human HtrA1 (HuHtrA1) (Q23-P480) and full length murine HtrA1 (MuHtrA1) (P24-P480) were cloned by PCR amplification from full length clones using Taq polymerase. The primers contained the nucleotides required for adding portions of the restriction sites BamHI and EcoRI to the resulting PCR products. The PCR fragments were ligated into a modified pAcGP67 baculovirus transfer vector (BD Pharmingen) containing a His_6 sequence (SEQ ID NO: 90) upstream of the BamHI restriction site resulting in an N-terminal His_6 tag (SEQ ID NO: 90) on the HtrA1 proteins.

The protease domain of human HtrA1 (HtrA1_PD) lacking the N-domain and the PDZ domain (e.g., containing residues D161-K379) was cloned by PCR amplification. The amplified DNA was inserted into a modified pET vector resulting in an N-terminal His_6 tag (SEQ ID NO: 90) and a thrombin cleavage site fused upstream of the D161 codon of HtrA1. HtrA1_PD Ala mutants were generated using the QuikChange XL site-directed mutagenesis kit according to the manufacturer's protocol (Agilent Technologies, Santa Clara CA). Constructs were verified by DNA sequencing. The protease domains of murine HtrA1 (MuHtrA1_PD) (G156-S367), murine HtrA3 (MuHtrA3_PD) (P133-F350) and murine HtrA4 (MuHtrA4_PD) (E159-Y371) were cloned from full length clones using Pfu (Agilent) polymerase. The primers contained the nucleotides required for adding the restriction sites Not I and Stu I to the resulting PCR products. The PCR fragments were ligated into pST23 of an expression vector with a phoA promoter. This expression vector contains the amino acid sequences $MK(HQ)_6MHQSTAA$ (SEQ ID NO: 11) upstream of the Not I restriction site resulting in N-terminal fusion of unizyme tag to the protease domains.

As described herein, amino acid residues of huHtrA1, muHtrA1, muHtrA3, and muHtrA4 are made with reference to SEQ ID NOs:13-16, respectively. Amino acids positions are specified by the one letter amino acid code followed by its position within one of SEQ ID NOs:13-16. For example, full length huHtrA1 comprises a sequence starting at glutamine at position 23 of SEQ ID NO:13 and ending at proline at position 480 of SEQ ID NO:13, e.g., Q23-P480. Similarly, mutations at a particular position within an HtrA protein are designated by the starting amino acid, followed by the position within one of SEQ ID NOs:13-16, followed by the substituted amino acid. For Example, a mutation of Lysine at position 248 of HuHtrA1 (SEQ ID NO:13) to alanine is referred to as K248A.

Protein Expression and Purification. HuHtrA1 and MuHtrA1 were expressed in *Trichoplusia ni* insect cells (Expression Systems LLC, Woodland, CA). Harvested insect cell

media (after pH was adjusted to 6.8) had $NiCl_2$, $CaCl_2$, and NaCl added to final concentrations of 1.0, 2.5 and 150 mM respectively.

HuHtrA1_PD constructs (wildtype and S328A mutant) were expressed in *E. coli* BL21 (Stratagene). *E. coli* cultures were grown at 37 °C in LB medium containing 50 μg/ml carbenicillin until A_{600} reached 0.8 to 1.0 and then induced with 0.4 mM IPTG and grown at 16 °C for 24 h. The bacterial cell pellets were resuspended in $1/10^{th}$ culture volume of 50 mM Tris pH 8.0, 500 mM NaCl and disrupted using a Microfluidizer. Lysates were centrifuged at 30,000 x g for 30 min.

HuHtrA1, MuHtrA1, HuHtrA1_PD and HuHtrA1_PD(S328A) were purified using nickel-nitrilo-triacetic acid resin (Qiagen). After loading insect cell media or *E. coli* lysates, columns were washed with 10 column volumes (CV) of 50 mM Tris pH 8.0, 500 mM NaCl followed by 10 CV of 50 mM Tris pH 8.0, 1 M NaCl, 20 mM imidazole. Proteins were eluted with 50 mM Tris pH 8.0, 200 mM NaCl, 10% glycerol, 0.25% CHAPS, 300 mM imidazole. Pooled fractions were further purified by size exclusion chromatography on a S-200 column (GE Healthcare) and peak fractions corresponding to the trimeric protein forms were collected. Protein purity was greater than 90% as assessed by SDS-PAGE and protein concentrations were determined using the BCA™ protein assay (Pierce, Rockford, IL).

HuHtrA1_PD Ala mutants (panel of 15 mutants except S328A) were expressed as described for the wildtype form of HuHtrA1_PD (see above). Lysis buffer (PopCulture, EMD Chemical, San Diego CA) was added to bacterial cell pellets at 50 ml lysis buffer/500 ml pellet. The pellet was suspended completely in lysis buffer with a polytron and stirred at room temperature for 30 min. The cell lysate was centrifuged (Beckman, LA-16.250 rotor) at 33,700 x g for 30 min at 4 °C and the resulting supernatant was filtered through a 0.22μm filter unit (Nalgene). The filtered supernatant was loaded onto a Ni-NTA column (3 ml, Qiagen) pre-equilibrated with 50 mM Tris, 300 mM NaCl, 10 mM imidazole, 10% glycerol pH 8.0 (buffer A). Once loaded the column was washed with 12 CV of buffer A followed by 20 CV of buffer A+ 0.1% Triton X-114. The column was washed again with 15 CV of buffer A to remove detergent. Proteins were eluted with buffer A + 200 mM imidazole, 0.25% CHAPS. Pooled Fractions were further purified with Superdex 200 (Hi load 16/60, 120 ml, GE Healthcare) equilibrated with 50 mM Tris pH 8.0, 200 mM NaCl, 10% glycerol, 0.25% CHAPS. The fractions corresponding to the HtrA1_PD trimer peak were pooled. Protein purity was greater than 90% as assessed by SDS-PAGE and protein concentrations were determined by use of the BCA™ protein assay (Pierce, Rockford, IL).

MuHtrA1_PD, MuHtrA3_PD and MuHtrA4_PD were expressed in *E. coli* 58F3 (Szeto, W., et al., *Cancer Res.* 61:4197-205 (2001)). Overnight *E. coli* cultures, grown at 30 °C in LB medium containing 50 μg/ml carbenicillin, were diluted (1/100 vol) into a larger culture containing C.R.A.P. medium (Simmons, L.C., et al., *J Immunol Methods.* 263:133-47 (2002)) supplemented with 50 μg/ml carbenicillin. After inoculation *E. coli* were grown for approximately 20 h at 30 °C. MuHtrA1_PD was purified as described for HuHtrA1_PD (see above). For purification of MuHtrA3_PD and MuHtrA4_PD, lysis buffer (50 mM Tris pH 8.5, 500 mM NaCl, 10 mM imidazole, 10% glycerol) was added to the bacterial cell pellet. The pellet was homogenized in lysis buffer with a polytron and cells disrupted with a mircofludizer. The cell lysate was centrifuged and the resulting supernatant filtered through a 0.22μm filter unit (Nalgene). The filtered supernatant was loaded onto a 3 ml Ni-NTA column (Qiagen) pre-equilibrated with 25 mM Tris pH 8.5, 500 mM NaCl, 20 mM imidazole, 10% glycerol (buffer B). After loading, the column was washed with 12 CV of buffer B followed by 20 CV of buffer B + 0.1% Triton X-114. The column was washed again with 15 CV of buffer B to remove detergent. Proteins were eluted with 25 mM Tris pH 8.5, 500 mM NaCl, 250 mM imidazole, 10% glycerol, 0.25% CHAPS. Pooled fractions were further purified on a 120 ml Superdex 75 (GE Healthcare) equilibrated with 25 mM Tris pH 8.5, 10% glycerol, 0.5 mM TCEP, 0.25% CHAPS. The fractions corresponding to the protease trimer peak were pooled. Protein purity was greater than 90% as assessed by SDS-PAGE.

EXAMPLE 3: Determination of Inhibitory Activity of Anti-HtrA1 Antibodies Using a FRET Assay

Synthesis of peptide substrate. The peptide H2-Opt (Mca-IRRVSYSF(Dnp)KK) (SEQ ID NO:12), originally described as a substrate for HtrA2 (Martins, L.M., et al., *J Biol Chem.* 278:49417-27 (2003)), was synthesized on Fmoc-Lys(Boc)-wang resin using standard coupling procedures with HBTU. Fmoc-Lys(DNP)-OH (Anaspec) was incorporated in the P5' position. The peptide was synthesized up to P5 (Mca, 7-Methoxy-coumarin, Aldrich) and then cleaved from the solid support using trifluoroacetic acid, triisoproplysilane and water for 2 hours at room temperature. Peptide was precipitated from ethyl ether, extracted with acetic acid, acetonitrile, water and lyophilized. Crude labeled peptide was dissolved and purified on preparative reverse phase C18 column using acetonitrile/water. Purified fractions were pooled, lyophilized and analyzed by liquid chromatography/mass spectrometry (PE/Sciex) and found to be consistent with their calculated masses.

Enzymatic assays with peptide substrate. HtrA1 was incubated in 96-well black optical bottom plates (Nalge Nunc Int., Rochester, NY) with IgG94 or Fab94 serially diluted in 50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 0.25% CHAPS (assay buffer) for 20 min at 37 °C. For initial testing a panel of 13 phage derived anti-HtrA1 antibodies, single concentrations of IgGs (final concentrations 0.16 mg/ml – 0.28 mg/ml) were incubated with HuHtrA1 or HuHtrA1_PD. A 10 mM stock solution of the peptide substrate Mca-IRRVSYSF(Dnp)KK (SEQ ID NO:12) (H2-Opt) in DMSO was diluted in water to 12.5 μM, pre-warmed at 37 °C and then added to the reaction mixture. The final concentrations of the reactants were: 5 nM HuHtrA1 or MuHtrA1, 0.005 – 300 nM IgG94, 0.02 – 900 nM Fab94, 2.5 μM H2-Opt. The increase of fluorescence signal (excitation 328 nm, emission 393 nm) was measured on a SPECTRAmax M5 microplate reader (Molecular Devices, Sunnyvale, CA) and the linear rates of H2-Opt cleavage (mRFU/min) determined. Experiments with IgG94 inhibition of trypsin (Roche) and elastase (MP Biomedicals) were carried out identically, except that the final enzyme concentrations were 1 nM, the IgG94 concentration was 300 nM and the incubation time was 15 min.

As shown in Fig. 2, a panel of 13 phage derived antibodies (IgG) was incubated with HuHtrA1 or HuHtrA1_PD and enzyme activity measured. Of the 13 antibodies tested, antibodies YW503.57, YW504.57, YW504.61 and YW505.94 (also referred to as Fab94, antibody 94, Ab94 or IgG94) strongly inhibited both HuHtrA1 and HuHtrA1_PD activities.

As shown in Fig. 3, IgG94 inhibited the enzymatic activity of HuHtrA1 towards the H2-Opt substrate in a concentration-dependent fashion with an IC_{50} value of 1.8 nM. Complete inhibition was achieved above 30 nM IgG94. In contrast, two other members of the trypsin-like serine protease family, trypsin and elastase, were not inhibited by IgG94 at 300 nM, indicating that IgG94 has specificity towards HuHtrA1. Fab94 also inhibited HuHtrA1 in a concentration-dependent manner, but less potently than IgG94 as indicated by the IC_{50} value of 29.1 nM. These results are in excellent agreement with the K_D value of 31.1 nM determined by surface plasmon resonance experiments (see Table 1 below). The results show that IgG94 and Fab94 are able to completely neutralize the enzymatic activity of HuHtrA1 towards a small synthetic peptide substrate and that this activity is specific. Moreover, the about 16-fold increase in potency of IgG94 vs. Fab94 is consistent with the avidity effects predicted from the 'cage-like' IgG94:HtrA1 complex based on mass analysis (see e.g., Table 2 and Figure 9).

The inhibitory potenciesof affinity-improved variant antibody YW505.94a.28 was also determined using the assay described above with the fluorescence quenched peptide substrate H2-Opt and 1.0 nM of HuHtrA1 or 1.5 nM of HuHtrA1_PD. The results are shown below.

YW505.94a.28 format	HuHtrA1_PD IC_{50} nM	HuHtrA1 IC_{50} nM
IgG	0.229	0.335
Fab	2.03	1.95

EXAMPLE 4: Determination of Inhibitory Activity of Anti-HtrA1 Antibodies Using Macromolecular Substrates

Bovine β-casein (Sigma-Aldrich) was repurified on a MonoQ ion exchange column to yield highly purified material. HuHtrA1 was incubated in Eppendorf tubes together with increasing concentrations of IgG94 in assay buffer for 15 min at 37 °C after which the macromolecular substrates were added. For β-casein digestion, the final concentration of reactants were: 10 nM HuHtrA1, 50 μg/ml β-casein, 2.3-150 nM IgG94. For decorin the final concentrations were: 125 nM HuHtrA1, 50 μg/ml decorin (R & D Systems), 2 – 125 nM IgG94. For biglycan the final concentrations were: 75 nM HuHtrA1, 50 μg/ml biglycan (R & D Systems), 2.3 – 150 nM IgG94. After incubation at 37 °C (2 h for β-casein, 14 h for decorin, 6 h for biglycan), SDS sample buffer was added and samples boiled and analyzed by SDS-PAGE (non-reducing).

Hydrolysis of fluorescent dye-labeled casein, BODIPY FL casein (Invitrogen), was carried out in 96-well black optical bottom plates (Nalge Nunc Int., Rochester, NY). HuHtrA1 or MuHtrA1 were incubated with IgG94 serially diluted in assay buffer for 15 min at 37 °C. After addition of BODIPY FL in assay buffer, the reactant concentrations were as follows: 30 nM HuHtrA1, 30 nM MuHtrA1, 5μg/ml BODIPY FL, 0.24-250 nM IgG94. The increase of fluorescence signal (excitation 484 nm, emission 535 nm) was measured on a SPECTRAmax M5 microplate reader (Molecular Devices, Sunnyvale, CA) and the linear rates of BODIPY FL cleavage (mRFU/min) determined and expressed as percentage of uninhibited rates (control).

As shown in Fig. 6, HuHtrA1 degraded the macromolecular substrates β-casein, decorin and biglycan (lane 2). In the absence of HuHtrA1, the substrates remained intact during the experimental period (Fig. 6, lane 1). Preincubation of HuHtrA1 with increasing concentrations of IgG94 (Fig. 6, lane 9 – lane 3) resulted in a concentration-dependent inhibition of substrate degradation. At the highest IgG94 concentrations tested, degradation of β-casein, decorin and biglycan was completely prevented (Fig. 6, lane 3). The results show that IgG94 potently and effectively inhibited HuHtrA1 activity towards three macromolecular substrates.

As shown in Fig. 4, IgG94 concentration-dependently inhibited BODIPY FL hydrolysis by both HuHtrA1 and MuHtrA1 with IC_{50} values of 8.3 nM and 5.4 nM, respectively. The

results show that IgG94 recognizes and completely neutralizes the enzymatic activities of both HuHtrA1 and MuHtrA1 towards the macromolecular substrate BODIPY FL.

EXAMPLE 5: Determination of the Specificity of Anti-HtrA1 Antibodies to HtrA1

The specificity of IgG94 was assessed by measuring its activity for inhibiting muHtrA1_PD as compared to its ability to inhibit the structurally related proteases MuHtrA3_PD and MuHtrA4_PD (see Fig. 5). The specificity was determined using the FRET assay as described above in Example 3, except that the concentrations of MuHtrA1_PD, MuHtrA3_PD and MuHtrA4_PD 6nM, 20nM and 20nM, respectively, and the concentration range of IgG94 was 0.2 – 400 nM. The specificity was also analyzed using the BODIPY FL casein substrate as described above in Example 4, except that the concentration of MuHtrA1_PD, MuHtrA3_PD and MuHtrA4_PD was 50 nM and the concentration range of IgG94 was 1.4 – 1000 nM. IgG94 inhibited MuHtrA1_PD cleavage of H2-Opt (Fig. 5, top panel) and BODIPY FL (Fig. 5, bottom panel), but did not inhibit cleavage of the same substrates by MuHtrA3_PD and MuHtrA4_PD up to concentrations of 400 nM and 1000 nM for H2-Opt and BODIPY FL substrates, respectively. The results suggest that IgG94 has excellent specificity and does not impair activities of related proteases.

EXAMPLE 6: Determination of Antibody Affinity by BIAcore

To determine the binding affinity of Fab94 by single-cycle kinetics, Surface Plasmon Resonance (SRP) measurement with a BIAcore™ T100 instrument was used. Briefly, series S sensor chip CM5 was activated with EDC and NHS reagents according to the supplier's instructions, and streptavidin (Pierce) was coupled to achieve approximately 2000 response units (RU), followed by blocking unreacted groups with 1M ethanolamine.

For kinetics measurements, biotinylated HuHtrA1_PD or MuHtrA1_PD were first injected at 10μl/min flow rate to capture approximately 100 RU at 3 different flow cells (FC), except for FC1 (reference cell), and then 5-fold serial dilutions of Fab94 (0.48 nM – 300 nM) in 0.01M HEPES pH 7.4, 0.15M NaCl, 0.005% surfactant P20 were injected (flow rate: 30μl/min) with no regeneration between injections. The sensorgrams were recorded and evaluated by BIAcore™ T100 Evaluation Software (version 2.0) after subtraction of reference cell signal. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model. The equilibrium dissociation constant (K_D) was calculated as the ratio k_{off}/k_{on} (see Table 1 below). The binding kinetics of Fab94 to

HuHtrA1_PD and to MuHtrA1_PD were very similar. The K_D values were 31.1 nM and 28.9 nM, respectively.

Table 1. Binding Affinities of Fab94 to HuHtrA1_PD and MuHtrA1_PD by Surface Plasmon Resonance.

	k_{on} (10^5 $M^{-1}s^{-1}$)	k_{off} (10^4 s^{-1})	K_D (nM)
HuHtrA1_PD	3.5	109.0	31.1
MuHtrA1_PD	4.5	129.0	28.9

EXAMPLE 7: Epitope Mapping of IgG94 on HuHtrA1_PD

To identify the functional binding epitope of IgG94, a panel of HuHtrA1_PD mutants with individual alanine mutations was generated for binding experiments. Most of the mutated residues are located on surface loops surrounding the active site and also included the catalytic serine (S328) and aspartate (D250). The mutants were expressed in *E. coli* and the trimers purified by size exclusion chromatography as described in Example 2. Binding was determined using an ELISA assay, which was conducted using a MAXISORP™ microtiter plate coated with HuHtrA1_PD mutants at 2 μg/ml in PBS for 1 h, followed by blocking with PBST buffer (0.5% BSA and 0.05% Tween 20 in PBS) for 1 h at room temperature. Five-fold serially diluted Ig94 (50 nM to 0.0006 nM) in PBST buffer was added and incubated for 30 min. The plates were washed with PBT buffer (0.05% Tween 20 in PBS), and HRP-conjugated goat anti-human IgG (H+L) (Invitrogen) was added (1:5000 in PBST buffer) and incubated for 1h. The plates were washed with PBT buffer and developed by adding tetramethylbenzidine substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The absorbance at 450 nm was plotted as a function of antibody concentration in solution to determine EC_{50} values. The location of mutants effecting binding were then mapped onto the structure of HtrA1.

In the binding ELISA, IgG94 showed the greatest reduction in binding to the mutants HuHtrA1_PD(N224A) and HuHtrA1_PD(K248A) (see Fig. 7A). Upon repetition of the experiment using a lower concentration of HtrA1 to coat the plates (1 μg/ml) and a shortened incubation time (reduced from 1 hour to 20 minutes incubation), IgG94 also showed at least a 5-fold reduction in binding to HuHtrA1 mutants V201A, H220A, T223A, K225A, K243A and E247A (see Fig. 7A). The results indicate that IgG94 binds to an epitope that includes residues N224 and K248, which are located on Loop B and Loop C (see Figs. 7B and 7C). In trypsin-like serine proteases these loops are known to be part of the specificity determining region that interacts with substrates. K248 (Loop C) is very close to the catalytic D250 and N224 to the

catalytic H220. Therefore, binding of IgG94 to these residues may impair substrate access to the catalytic cleft either by direct steric hindrance or by an indirect allosteric mechanism as found for neutralizing anti-HGFA antibodies that also recognize residues in Loop C (Ganesan, R., et al., *Structure* 17:1614-1624 (2009); Wu, Y., et al., *Proc Natl Acad Sci USA* 104:19784-9 (2007)). Alternatively, antibody binding may inhibit catalysis by directly influencing the catalytic triad residues D250 and/or H220.

EXAMPLE 8: Stoichiometry of Complexes of HtrA1_PD with Fab94 and IgG94 by Size Exclusion Chromatography – Multi-angle Light Scattering (SEC-MALLS)

A catalytically inactive form HuHtrA1_PD(S328A) was used for complex formation. The Fab94:HuHtrA1_PD(S328A) and IgG94:HuHtrA1_PD(S328A) complexes were formed in Tris buffer (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10% glycerol, 0.25% CHAPS). The complexes and the individual components alone were incubated at 4 °C overnight and then injected and resolved on a S200 Superdex 10/300 GL column (GE Healthcare) in Tris buffer with a flow rate of 0.5 mL/min. Data was collected with an 18-angle light scatter detector (Dawn Helios II with QELS) and a refractive index detector (Optilab reX) from Wyatt Technologies. Analysis was done with Astra 5 software to yield molar mass independently of elution time. Normalization was performed with a control IgG.

SEC-MALLS experiments show that Fab94 and IgG94 form complexes with HuHtrA1_PD(S328A) that have distinct masses. The determined masses and deduced stoichiometry of the complexes are shown in Table 2.

Table 2. Stoichiometry of HuHtrA1_PD(S328A) in complex with Fab94 and IgG94 determined by SEC-MALLS.

Components*	Mass by MALLS (Da)**	Best-fit stoichiometry&	Expected mass (Da)	Mass difference (%)
HtrA1_PD	76,755	--	--	--
Fab94	47,400	--	--	--
IgG94	151,200	--	--	--
Fab94:HtrA1_PD	210,100	3:1	218,955	4.2
IgG94:HtrA1_PD	618,600	3:2	607,110	1.9

* HuHtrA1_PD was HuHtrA1_PD with the catalytic serine mutated to alanine (HuHtrA1_PD(S328A)); the mass represents the trimer

** Average of two independent experiments

& Ratio of antibody:protease trimer

The determined mass of the Fab94:HtrA1_PD(S328A) complex of 210,100 Da is consistent with a complex of 3 Fabs binding to one HuHtrA1_PD(S328A) trimer (3:1 complex). The difference between the experimental and the theoretical masses of such a 3:1 complex is only 4.2%. Therefore, one Fab is able to bind to each HuHtrA1_PD(S328A) monomer within one HtrA1 homo-trimer.

The determined mass of the IgG94:HtrA1_PD(S328A) complex of 618,600 Da fits well to a 3:2 stoichiometry, in that 3 IgG molecules bind to two HuHtrA1_PD(S328A) trimers. The difference between the experimental and the theoretical mass of such a 3:2 complex is only 1.9%.

The elucidated stoichiometries are in agreement with the findings that Fab94 and IgG94 are able to completely inhibit HtrA1 activity, in that each monomer within an HtrA1 trimer is binding to one Fab or to one Fab arm of an IgG. Thus, in these complexes there is no 'free' HtrA1 monomer available, in agreement with the complete inhibition of HtrA1 enzyme activity by Fab94 and IgG94. We propose a 'cage' model for the IgG94:HtrA1_PD(S328A) complex in which the Fab arms of the 3 IgGs are bridging two trimers, each Fab arm binding to one monomer (see Fig. 9). The model also accounts for the about 16-fold increased potency of IgG94 over Fab94 in enzymatic assays (see e.g., Fig. 3) in that IgG94, strongly benefits from avidity effects in its binding to HtrA1_PD trimers.

EXAMPLE 9: Affinity Maturation of YW505.94

Construct libraries for anti-HtrA1 affinity Maturation. Clone YW505.94a was derived from YW505.94 [Ab94] by changing Kabat residue N28 to serine within CDR H1 in order to remove a potential N-linked glycosylation site. Phagemid pW0703, derived from phagemid pV0350-2b (Lee et al., *J. Mol. Biol* **340**, 1073-1093 (2004)), which contains a stop codon (TAA) in all CDR-L3 positions and displays monovalent Fab on the surface of M13 bacteriophage, served as a library template for grafting the heavy chain variable domain (V_H) of clone YW505.94a for affinity maturation. Both hard and soft randomization strategies were used for affinity maturation. For hard randomization, one light chain library (L1/L2/L3hard) with selected positions at three light chain CDRs was randomized using amino acids designed to mimick a natural human antibody and the designed DNA degeneracy was as described (Lee et al., 2004). For soft randomization, selected residues at Kabat positions 91, 92, 93, 94 and 96 of CDR-L3, 28–35 of CDR-H1, 50–58 of CDR-H2, and 95–100 of CDR-H3 with two different combinations of CDR loops, L3/H1soft, L3/H2soft and L3/H3soft, were targeted for randomization. To achieve the soft randomization conditions, which introduce a mutation rate

of approximately 50% at the selected positions, the mutagenic DNA was synthesized with 70-10-10-10 mixtures of bases favoring the wild type nucleotides (Gallop et al., *J. of Med. Chem.* **37**, 1233-1251 (1994)).

Phage sorting strategy to isolate affinity-improved variants. For affinity improvement selection, phage libraries were subjected to plate sorting for the first round, followed by four rounds of solution sorting. For the first round of plate sorting, four libraries (L1/L2/L3hard, L3/H1soft, L3/H2soft and L3/H3soft) were sorted against a human HtrA1 (HuHtrA1) coated plate (NUNC Maxisorp plate) separately with phage input at about 3 O.D./ml in 1% BSA and 0.05% Tween 20 for 1 hour at room temperature (RT). After the first round of plate sorting, four rounds of solution sorting were performed to increase the stringency of selection. For solution sorting, 1 O.D./ml of phage propagated from first round of plate sorting were incubated with 500nM of biotinylated HuHtrA1 in 100ul buffer containing 1% Superblock (Pierce biotechnology) and 0.05% Tween20 for at least 1 hour at room termperature (RT). The mixture was further diluted 10X with 1% Superblock and applied 100ul/well to neutravidin-coated wells (10ug/ml) for 15 min at RT with gentle shaking so that biotinylated HuHtrA1 could bind phage. The wells were washed with PBS-0.05% Tween20 ten times. To determine background binding, control wells containing phage without biotinylated HuHtrA1 selection were captured on neutravidin-coated plates. Bound phage was eluted with 0.1N HCl for 20 min, neutralized by 1/10 volume of 1M Tris pH11, titered, and propagated for the next round. Next, three more rounds of solution sorting were carried out using two methods of increasing selection stringency simultaneously. The first method, which is for on-rate selection, decreases biotinylated target protein concentration from 10nM to 0.1nM. The second method, which is for off-rate selection, adds excess amounts of non-biotinylated HuHtrA1 protein (100~1000 fold more) to compete off weaker binders. Also, the phage input was decreased (0.1~0.5 O.D/ml) to lower the background phage binding.

High throughput affinity screening ELISA (single spot competition). Colonies were picked from the fifth round screening and grown overnight at 37 °C in 350ul/well of 2YT media with 50ug/ml carbenicillin and 1e 10/ml KO7 in 96-well block (QIAgene). From the same plate, a colony of XL-1 infected parental phage was picked as a control. 96-well Nunc Maxisorp plates were coated with 100ul/well of HuHtrA1 protein (2ug/ml) in PBS at RT for 2 hours. The plates were blocked with 100ul of 0.5% BSA and 0.05% Tween in PBS (PBST buffer) for one hour.

The phage supernatant was diluted 1: 5 in PBST buffer with or without 5nM HuHtrA1 in 100ul total volume and incubated at least 1 hour at RT. Then, 75ul of mixture were

transferred to the HuHtrA1 coated plates. The plate was gently shacken for 15 min to allow the capture of unbound phage to the HuHtrA1 coated plate. The plate was washed five times with 0.05% Tween20 in PBS (PBT buffer). The binding was quantified by adding horse radish peroxidase (HRP)-conjugated anti-M13 antibody in ELISA buffer (1:5000) and incubated for 30 min at RT. The plates were washed with PBT buffer five times. Next, 100ul/well of a 1:1 ratio of 3,3',5,5'-tetramethylbenzidine (TMB) Peroxidase substrate and Peroxidase Solution B (H_2O_2) (Kirkegaard-Perry Laboratories (Gaithersburg, MD)) was added to the well and incubated for 5 minutes at RT. The reaction was stopped by adding 100ul 1M Phosphoric Acid (H_3PO_4) to each well and allowed to incubate for 5 minutes at RT. The OD of each well was determined using a standard ELISA plate reader at 450 nm. The OD reduction (%) was calculated by the following equation:

OD_{450nm} reduction (%) = [(OD_{450nm} of wells with competitor) / (OD_{450nm} of well with no competitor)]*100

In comparison to the OD_{450nm} reduction (%) of the well of parental phage (100%), clones that had the OD_{450nm} reduction (%) lower than 50% were picked for sequence analysis (see Figs. 15 and 16). Unique clones were selected for phage preparation to determine binding affinity (phage IC_{50}) by phage competition ELISA (see Figs. 17 and 18). Then, the most affinity-improved clones (YW505.94a.28 & YW505.94a.54) were reformatted into human IgG1 for antibody production and further BIAcore binding kinetic analysis.

Phage competition ELISA to determine IC_{50}. MAXISORP™ microtiter plates were coated with HuHtrA1 at 2 μg/ml in PBS for 2 hr at RT and then blocked with PBST buffer for another hour at RT. Purified phage from culture supernatants were incubated with serially diluted HuHtrA1 or MuHtrA1 in PBST buffer in a tissue-culture microtiter plate for an hour, after which 80 μl of the mixture was transferred to the HuHtrA1-coated wells for 15 minutes to capture unbound phage. The plate was washed with PBT buffer, and HRP-conjugated anti-M13 (Amersham Pharmacia Biotech) was added (1:5000 in PBST buffer) for one hour. The plate was washed with PBT buffer and developed by adding tetramethylbenzidine substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The absorbance at 450 nm was plotted as a function of HuHtrA1 or MuHtrA1 concentration in solution to determine phage IC_{50}. This was used as an affinity estimate for the Fab clone displayed on the surface of the phage. Fig. 17 shows results from a phage competition assay demonstrating the binding of YW505.94a affinity-improved variants against HuHtrA1. Fig. 18 shows results from a phage competition assay demonstrating the binding of YW505.94a affinity-improved variants against MuHtrA1.

Antibody affinity determinations by BIAcore. To determine the binding affinity of HtrA1 antibodies by single cycle kinetics, Surface Plasmon Resonance (SRP) measurement with a BIAcore™ T100 instrument was used. Briefly, a series S sensor chip CM5 was activated with EDC and NHS reagents according to the supplier's instructions, and streptavidin (Pierce) was coupled to achieve approximately 2000 response units (RU), followed by blocking un-reacted groups with 1M ethanolamine.

For kinetics measurements, biotinylated human or murine HtrA1 was first injected at 10ul/min flow rate to capture approximately 100 RU at 3 different flow cells (FC), except for FC1 (reference), and then 5-fold serial dilutions of anti-HtrA1 Fab in HBS-P buffer (0.01M HEPES pH 7.4, 0.15M NaCl, 0.005% surfactant P20) from low (0.48nM) to high (300nM) [5 points] were injected (flow rate: 30µl/min) one after the other in the same cycle with no regeneration between injections. The sensorgram was recorded and subject to reference and buffer subtraction before evaluation by BIAcore™ T100 Evaluation Software (version 2.0). Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model. The equilibrium dissociation constant (Kd) was calculated as the ratio k_{off}/k_{on}. The results are shown in Table 3 below.

Table 3. Binding Affinities of Affinity Matured Fabs as Compared to the Parent Fab.

	HuHtrA1			MuHtrA1		
Fab	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (nM)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (nM)
YW505.94	3.51e5	109e-4	31.1	4.47e5	129e-4	28.9
YW505.94a	8.13e5	4.48e-2	55	1.48e6	8.56e-2	58
YW505.94a.28	1.69e6	5.74e-3	3.4	2.02e6	9.3e-3	4.6
YW505.94a.54	2.97e6	7.84e-3	2.6	2.82e6	4.55e-3	1.6

EXAMPLE 10: Sparing of Photoreceptors, Outer Nuclear Layer (ONL), and Functional Responses in the Absence of HtrA1 Following Constant Light Exposure

Construction of HtrA1 Knockout Mouse. To generate HtrA1**(+/-)** embryonic stem cells, linearized targeting vector DNA was electroporated into ES cells of Balb/c background to introduce Flox sites in the region 5' of exon 1 and 3' of exon 1 and an introduced neomycin-resistance gene (Neo). ES clones resistant to neomycin were selected, and exon 1 plus the neomycin cassette was excised by electroporating the ES cells with a cre-recombinase-expressing expression plasmid. Homologous recombination was confirmed by sequencing of the entire HtrA1 locus and upstream promoter region in the targeted ES cell. Targeted clones

were injected into C57BL/6 blastocysts to generate male chimeric mice of germline transmission. Interbreeding of +/- female and +/- male mice was performed to generate -/- female or -/- male HtrA1 mice on a Balb/c background. Successful deletion of HtrA1 was confirmed by RT-PCR of ovaries from HtrA1 wt and ko mice.

Constant light exposure model. Male Balb/c Htra1 wt/wt or ko/ko mice, 8-13 weeks old, are in normal housing (<100 lux in cage) until start of constant light exposure (CLE). To start CLE, mice are housed singly in normal cages covered with a wire rack only, without a filter top. Food pellets and Hydrogels are placed on the bottom of the cage, and not on the wire top, for nourishment so as to not impede light entering the cage. Ten cages are placed on a Metro rack outfitted with fluorescent lights and enclosed in white panels to deliver ~1200 lux to mice for up to 14 days. Cages are rotated counterclockwise around the rack daily during CLE to ensure equal light exposure. If multiple shelves are used, cages are also rotated between shelves to ensure equal light exposure.

Optical Coherence Tomography. Optical coherence tomography (OCT) is performed 4-7 days before light exposure to provide a retinal thickness baseline measurement. OCT is performed using a Heidelberg Spectralis HRA+OCT camera. Animals are anesthetized with 70-80 mg/kg ketamine/15 mg/kg xylazine in 150-300 ul sterile saline, eyes are dilated with 1% tropicamide drops and retina thickness is measured by OCT. Artificial tears are used to keep eyes moist to prevent cataracts. After OCT, animals are allowed to recover from anesthesia and returned to their cages and light rack.

Electroretinogram. Electroretinograms (ERG) ERGs are performed 7 days before light exposure and at 15 days post CLE. ERGs are performed and recorded using a Diagnosys LLC Espion 2 visual electrophysiology system and a Colordome desktop Ganzfeld as a light source. Mice are dark adapted overnight in a dark room to equilibrate photoreceptors. Once dark adapted, all subsequent procedures are performed in the dark with only a red light for illumination. Animals are anesthetized with Ketamine, 75-80 mg/kg, & Xylazine, 7.5-15 mg/kg, IP in 200-300 ul PBS. Mouse body temperature is maintained at 37 °C using a homeothermic plate connected to its control unit. Pupils are dilated with 1% tropicamide. ERGs from both eyes will be recorded simultaneously with Burian-Allen silver or platinum wire loop electrodes. Mice are placed on a platform, a reference electrode is inserted subcutaneously through the forehead, and a ground electrode is inserted subcutaneously at the base of the tail. Gonak hypermellose solution is placed on the cornea to establish an electrical contact between the cornea and the electrode, and protect eyes from drying during the experiment. Electrodes are placed on the left and right eyes and mouse inserted into a

ColorDome light stimulator. Eyes will be stimulated with white light (7 flash intensities 4e-5, 2e-5, 0.5, 2, 5, 10, 20 cd.s/m^2) and signals bandpass-filtered at 0.15-1000 Hz and sampled at 2 kHz.

Determination of HtrA mRNA levels in the mouse retina and ovary. Whole eyes were enucleated, the retina removed and placed in RLT Buffer (Qiagen) and frozen at -80 ^{0}C until homoginization. Retina's were homogenized and shredded by gentleMACS M tubes (Miltinyi Biotec). Similar to the retina, ovarys were isolated from female HtrA1 wt, het and ko mice and homogenized as described for the retina. RNA was isolated using Qiagen Plus RNeasy kit; cDNA was generated with High Capacity cDNA kit (Applied Biosystem); Taqman qPCR was performed using 20 ug cDNA, Taqman Gene Expression Master Mix (Applied Biosystem) and Taqman Gene Expression Assay primers (Applied Biosystem) to exon 1-2 (retina and ovary), exon 3-4 (ovary) and exon 5-6 (ovary) and ran on an ABI 7500 Real-time PCR System (Applied Biosystem). HtrA expression was normalized to 18s expression (primer from Applied Biosystem).

As shown in Figure 11, HtrA1 mRNA expression increases in a mouse model of constant light exposure. However, in the same model, HtrA2 levels do not increase in the retina. In addition, HtrA1 levels are are significantly higher than HtrA2, HtrA3 and HtrA4 levels in the retina of a non-exposed mouse.

In addition, we found that mice lacking HtrA1 expression show a sparing of photoreceptors (Fig. 13), the outer nuclear layer (ONL) (Fig. 14), and functional responses (ERG) (Fig. 12) in a mouse model of light-induced degeneration.

EXAMPLE 11: Effects of an Anti-HtrA1 Antibody in a Rat Model of Constant Light Exposure

Rats will be subjected to constant light exposure as described above for mice. The efficacy of an anti-HtrA1 antibody to protect the rat eye from degeneration caused by the light exposure will be evaluated. Rat eyes will be subject to intravitreal injections of an anti-HtrA1 antibody at a dose and frequency suitable to maintain an effective concentration of the anti-HtrA1 antibody in the eye during the course of the experiment. The rat eyes will be monitored following light exposure to determine retinal thickness by OCT and functionality by ERG, as described above for the mouse model.

EXAMPLE 12: HtrA1 Expression is Abundant in Rat Vitreous and Accumulates in Mouse Eye Fluid and Eye Tissue Upon Light Stress

ELISA Assay. For analysis of rat tissues, an anti–muHtrA1 rabbit polyclonal antibody (Genentech) was diluted to 125 ng/mL, while for analysis of mouse eye fluid and retina tissue, an anti–huHtrA1 mouse monoclonal antibody (Genentech) was diluted to 250 ng/mL, both in PBS, and coated onto 384-well ELISA plates (Nunc; Neptune, NJ) during an overnight incubation at 4˚C. Plates were washed with PBS plus 0.05% Tween-20 and blocked during a two hour incubation with PBS plus 0.5% bovine serum albumin (BSA). This and all subsequent incubations were performed at room temperature with gentle agitation. Recombinant murine HtrA1 standard (Genentech) and samples from rat or murine tissues were diluted in sample/standard dilution buffer (PBS, 0.5% BSA, 15 ppm Proclin, 0.05% Tween 20, 0.25% CHAPS, 0.2% BgG, 5 mM EDTA, 0.35M NaCl, (pH 7.4)), added to washed plates, and incubated for 1.5 - 2 hours. Plate-bound HtrA1 was detected during a 1-hour incubation with a biotinylated anti–muHtrA1 rabbit polyclonal antibody (Genentech) diluted to 100 ng/mL for rat tissues, and for mouse eye fluid and retina tissue, biotinylated anti–huHtrA1 mouse monoclonal antibody (Genentech) was diluted to 200 ng/mL, both in assay buffer (PBS, 0.5% BSA, 15 ppm Proclin, 0.05% Tween 20), followed by a wash step and a 30-minute incubation with streptavidin-HRP (GE Healthcare; Piscataway, NJ), also diluted in assay buffer (1:20,000). After a final wash, tetramethyl benzidine (KPL, Gaithersburg, MD) was added, color was developed for 10-15 minutes, and the reaction was stopped with 1 M phosphoric acid. The plates were read at 450 nm with a 620 nm reference using a microplate reader. The concentrations of rat or murine HtrA1 were calculated from a four-parameter fit of the muHtrA1 standard curve.

As shown in Fig. 19A, expression of HtrA1 was low in rat ovary, brain, spleen, and total eye tissue as compared to the levels of expression of HtrA1 in rat vitreous. As shown in Fig. 19B, the level of HtrA1 in mouse eye fluid increased in a model of constant light exposure as compared to a control. As shown in Fig. 19C, the level of HtrA1 in mouse retinal tissue increased in a model of contant light exposure as compared to a control.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1. An isolated monoclonal antibody comprising a heavy chain variable domain (VH) and a light chain variable domain (VL) that bind to HtrA1, wherein the antibody (i) binds to an epitope that includes N224, K248, or both of HtrA1, and (ii) inhibits HtrA1 serine protease activity with a dissociation constant of ≤500 nM.

2. The antibody of claim 1, wherein the antibody further comprises one or more of the following properties: (i) binds to HtrA1 with a ratio of one variable domain to one subunit of an HtrA1 trimer, or (ii) does not prevent trimer formation of HtrA1.

3. The antibody of claim 1 or 2, wherein the antibody does not cross-react with one or more of HtrA2, HtrA3 and HtrA4.

4. The antibody of claim 1, wherein the dissociation constant is determined by BIAcore using a Fab at 25 °C.

5. An isolated monoclonal antibody comprising a VH and a VL that bind to HtrA1, wherein the antibody (i) binds to HtrA1 competitively with an antibody comprising a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7 and (ii) inhibits HtrA1 serine protease activity with a dissociation constant of ≤500 nM.

6. The antibody of claim 5, wherein competitive binding is determined using an ELISA assay.

7. The antibody of claim 5 or 6, wherein the antibody binds to an epitope that includes N224, K248, or both of HtrA1.

8. The antibody of any one of claims 1-7, which is:

(i) an antibody which is a human, humanized, or chimeric antibody, or

(ii) an antibody which is an antibody fragment comprising a VH and a VL that bind HtrA1.

9. The antibody of any one of claims 1-8, wherein the VH sequence comprises the amino acid sequence of SEQ ID NO: 32 and the VL sequence comprises the amino acid sequence of SEQ ID NO: 31.

10. An antibody comprising a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7.

11. An antibody comprising a VH sequence of SEQ ID NO: 29 and a VL sequence of SEQ ID NO: 28.

12. An antibody comprising a VL sequence of SEQ ID NO: 30 and a VH sequence of SEQ ID NO: 29.

13. The antibody of any one of claims 1-8, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence $GFX_lIX_mX_nYYIH$, wherein X_l is N, S or T; X_m is S, D, Y or A; and X_n is G or D (SEQ ID NO: 25); (b) HVR-H2 comprising the amino acid sequence $WIDPYGGDTX_0YADSVKG$, wherein X_0 is N or D (SEQ ID NO: 26); (c) HVR-H3 comprising the amino acid sequence $GTFLTX_pWGHYFDY$, wherein X_p is S or T (SEQ ID NO: 27); (d) HVR-L1 comprising the amino acid sequence $RASQX_aX_bX_cX_dX_eX_fA$, wherein X_a is D, S or V; X_b is V or I; X_c is S, N or G; X_d is T or N; X_e is A or Y; and X_f is V or L (SEQ ID NO: 23); (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising the amino acid sequence $QQX_gX_hX_iX_jPX_kT$, wherein X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S (SEQ ID NO: 24).

14. The antibody of claim 13, selected from a group consisting of:

(i) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 1; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 3,

(ii) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 18; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19, and

(iii) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 22.

15. The antibody of any one of claims 1-14, which is a full-length IgG1 antibody.

16. The antibody of any one of claims 1-14, which is a full-length IgG4 antibody.

17. An isolated nucleic acid encoding the antibody of any one of claims 1-16.

18. A host cell comprising the nucleic acid of claim 17.

19. A method of producing an antibody comprising culturing the host cell of claim 18 under conditions suitable for expression of the nucleic acid encoding the anti-HtrA1 antibody.

20. The method of claim 19, further comprising recovering the anti-HtrA1 antibody from the host cell culture.

21. An immunoconjugate comprising the antibody of any one of claims 1-16 and a cytotoxic agent.

22. A pharmaceutical formulation comprising the antibody of any one of claims 1-16 and a pharmaceutically acceptable carrier.

23. The antibody of any one of claims 1-16 for use as a medicament.

24. Use of the antibody of any one of claims 1-16 in the manufacture of a medicament.

25. The use of claim 24, selected from a group consisting of:

(i) a use wherein the medicament is for treatment of treating age-related macular degeneration, geographic atrophy, diabetic retinopathy, retinopathy of prematurity, or polypoidal choroidal vasculopathy,

(ii) a use wherein the medicament is for inhibiting degeneration of retinal or photoreceptor cells, and

(iii) a use wherein the medicament is for inhibiting HtrA1 protease activity in an eye.

26. The use of claim 25(i), wherein the age-related macular degeneration is dry age-related macular degeneration.