adenosine-mediated immunosuppressive effects play a role. Hence, there is a need for treatments of such diseases and conditions.

SUMMARY

The present invention refers to subject matter as defined in the attached set of claims. Disclosed herein are variant Adenosine Deaminase 2 (ADA2) proteins or catalytically active portions thereof that contain a modification(s) in the sequence of amino acids of an unmodified ADA2 protein or a catalytically active portion thereof. In some embodiments, the unmodified ADA2 protein can include the sequence of amino acids set forth in SEQ ID NO:5, or a sequence of amino acids that can exhibit at least 85% sequence identity to the sequence of amino acids set forth in SEQ ID NO:5, or is a catalytically active portion thereof; the amino acid modification(s) are selected from among amino acid replacement(s), deletion(s) and insertion(s); and the variant ADA2, when in dimer form, can exhibit one or more properties selected from among increased adenosine deaminase activity, reduced heparin binding, longer serum half-life, altered pH optimum, increased thermal stability, altered receptor binding and hyperglycosylation compared to the corresponding dimer form of the unmodified ADA2 protein. A variety of amino acid modifications, including replacements, deletions and insertions are provided. It is understood that the discreet modifications that confer a particular activity or property can be combined; as in proteins effects of mutation or modifications generally are additive. Any of the variant ADA2 or catalytically active portion thereof provided herein that contains modifications, including replacements, deletions and insertions, and nucleic acids encoding the variant ADA2 or catalytically active portion thereof, can be used in any of the methods, compositions, conjugates, modified forms, vectors, cells, combinations, uses and compositions for use, and combinations for use, provided herein.

In some embodiments, there is provided a variant Adenosine Deaminase 2 (ADA2) protein or a catalytically active portion thereof, comprising a modification(s) in the sequence of amino acids of an unmodified ADA2 polypeptide or a catalytically active portion thereof, wherein: the unmodified ADA2 protein comprises the sequence of amino acids set forth in SEQ ID NO:5, or a sequence of amino acids that exhibits at least 95% sequence identity to the sequence of amino acids set forth in SEQ ID NO:5, or is a catalytically active portion thereof;

CLAIMS

1. A variant Adenosine Deaminase 2 (ADA2) protein or a catalytically active portion thereof, comprising a modification(s) in the sequence of amino acids of an unmodified ADA2 polypeptide or a catalytically active portion thereof, wherein:

the unmodified ADA2 protein comprises the sequence of amino acids set forth in SEQ ID NO:5, 326-334, 340, 375 and 380-383, or is a catalytically active portion thereof that lacks all or a portion of the PRB domain, which consists of all or part of residues corresponding to residues 98-156 of SEQ ID NO:5, and optionally contains a linker in place thereof;

the PRB domain is residues 98-156 with reference to SEQ ID NO:5;

the amino acid modification(s) are selected from among amino acid replacement(s), deletion(s) and insertion(s);

the variant ADA2 protein, when in dimer form, exhibits one or more properties selected from among increased adenosine deaminase activity, reduced heparin binding, longer serum half-life, altered pH optimum, increased thermal stability, reduced receptor binding, and hyperglycosylation compared to the corresponding dimer form of the unmodified ADA2 protein of SEQ ID NO:5 or dimer form of the corresponding catalytically activity portion thereof; and

the variant ADA2 protein, when in dimer form, exhibits adenosine deaminase activity to convert adenosine to inosine;

the variant ADA2 protein comprises one or more amino acid modifications selected from among: K11A; K11D; K11E; K13A; K13D; K13E; K371A; K371D; K371E; K372A; K372D; K372E; K452A; K452D; K452E; R20A; R20D; R20E; R366A; R366D; R366E; K26A; K26D; K26E; R217A; R217D; R217E; K258A; K258D; K258E; R277A; R277D; R277E; R283A; R283D; R283E; K309A; K309D; K309E; K317A; K317D; K317E; K321A; K321D; K321E; R352A; R352D; R352E; R441A; R441D; R441E; K444A; K444D; K444E; K461A; K461D; K461E; K469A; K469D; K469E; K470A; K470D; K470E; H264A; H264Q; H264N; H264G; R219K; R219Q; R219N; R219A; L221A; L221V; L221G; E179D; E179A; E179S; E179T; E179V; E179G; S262M; S262N; D86A; D86C; D86E; D86F; D86G; D86H; D86I; D86K; D86L; D86M; D86N; D86P; D86Q; D86R; D86S; D86T; D86V; D86W; D86Y; E179C; E179F; E179H; E179I; E179K; E179L; E179M; E179N; E179P; E179Q; E179R; E179W; E179Y; R219C; R219D; R219E; R219F; R219G; R219H; R219I; R219L; R219M; R219P; R219S; R219T; R219V; R219W; R219Y; L221C; L221D; L221E; L221F; L221H; L221I; L221K; L221M; L221N; L221P; L221Q; L221R; L221S; L221T; L221W; L221Y; S262C; S262D; S262E; S262F; S262G; S262H; S262I; S262K; S262L; S262P; S262Q; S262R; S262T; S262W; S262Y; H264C; H264D; H264E; H264F; H264I; H264K; H264L; H264M; H264P; H264R; H264S; H264T; H264V; H264W; H264Y; S266A; S266C; S266D; S266E; S266F; S266G; S266H; S266I; S266K; S266L; S266M; S266N; S266P; S266Q; S266R; S266T; S266V; S266W; S266Y; K267A; K267C; K267D; K267E; K267F; K267G; K267H; K267I; K267L; K267M; K267N; K267P; K267Q; K267R; K267S; K267T; K267V; K267W; K267Y; V296A; V296C; V296D; V296E; V296F; V296G; V296H; V296I; V296K; V296L; V296M; V296N; V296P; V296Q; V296R; V296S; V296T; V296W; V296Y; H7R, G18A, G18R, G18V, I64T, A80D, H83Q, V90A, C108G, A120V, H121R, W133G, R125C, R140Q, K141R, R142W, P164L, P222L, W235S, H306R, E330G, W333G, V365L, Y424C, F464S or a deletion corresponding to R8-K14del→--, with numbering with reference to amino acid residues set forth in SEQ ID NO:5; and

corresponding residues are identified by alignment with SEQ ID NO:5.

2. The variant ADA2 protein or catalytically active portion thereof claim 1, wherein:

the modification(s) is on or more amino acid replacement(s); and

the variant ADA2 protein comprises one or more amino acid replacement(s) at an amino acid position corresponding to amino acid residue 11, 13, 20, 22, 26, 86, 179, 217, 219, 221, 258, 262, 264, 266, 267, 277, 283, 296, 309, 317, 321, 352, 366, 371, 372, 373, 374, 403, 404, 405, 406, 441, 444, 452, 461, 469 or 470, with reference to amino acid positions set forth in SEQ ID NO:5.

3. The variant ADA2 protein or catalytically active portion thereof of claim 1 or claim 2, wherein the ADA2 protein does not contain a modification selected from among an amino acid replacement corresponding to H7R, G18A, G18R, G18V, I64T, A80D, H83Q, V90A, C108G, A120V, H121R, W133G, R125C, R140Q, K141R, R142W, P164L, P222L, W235S, H306R, E330G, W333G, V365L, Y424C, F464S or a deletion corresponding to R8-K14del→--, with numbering with reference to amino acid residues set forth in SEQ ID NO:5.

4. The variant ADA2 protein or catalytically active portion thereof of any of claims 1-3, wherein the variant ADA2 protein comprises one or more amino acid replacement(s) selected from among R20A, R20D, R20E, S262N, R366A, R366D, R366E, K371A, K371D, K371E, K372A, K372D, K372E and K452E, with reference to amino acid positions set forth in SEQ ID NO:5.

5. The variant ADA2 protein or catalytically active portion thereof of any of claims 1-3, comprising one or more amino acid replacement(s) corresponding to K11A; K11D; K11E; K13A; K13D; K13E; K371A; K371D; K371E; K372A; K372D; K372E; K452A; K452D; K452E; R20A; R20D; R20E; R366A; R366D; R366E; K26A; K26D; K26E; R217A; R217D; R217E; K258A; K258D; K258E; R277A; R277D; R277E; R283A; R283D; R283E; K309A; K309D; K309E; K317A; K317D; K317E; K321A; K321D; K321E; R352A; R352D; R352E; R441A; R441D; R441E; K444A; K444D; K444E; K461A; K461D; K461E; K469A; K469D; K469E; K470A; K470D; and K470E.

6. A variant Adenosine Deaminase 2 (ADA2) protein or a catalytically active portion , comprising a modification(s) in the sequence of amino acids of an unmodified ADA2 polypeptide or a catalytically active portion thereof, wherein:

the unmodified ADA2 protein is selected from among an ADA protein that comprises the sequence of amino acids set forth in any of SEQ ID NOS: 5, 326-334, 340, 375 and 380-383 or is a catalytically active portion thereof that lacks all or a portion of the PRB domain, which consists of all or part of residues corresponding to residues 98-156 of SEQ ID NO:5, and optionally contains a linker in place thereof;

the modified ADA protein has up to 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid modifications compared to the unmodified ADA protein;

the amino acid modification(s) are selected from among amino acid replacement(s), deletion(s) and insertion(s);

the variant ADA2 protein comprises an amino acid replacement at one or both of positions corresponding to amino acid residue 219 and 262 with reference to amino acid positions set forth in SEQ ID NO:5,

the replacements at 219 and 262 are selected from among replacements corresponding to R219K, R219Q, R219N, S262M and S262N; and

corresponding residues are identified by alignment with the ADA2 of SEQ ID NO:5.

7. The variant ADA2 protein or catalytically active portion thereof of claim 6, wherein the unmodified ADA2 protein or catalytically active portion thereof lacks all or a portion of the PRB domain.

8. The variant ADA2 protein or catalytically active portion thereof of claim 6, wherein the unmodified ADA2 protein consists of the sequence of amino acids set forth in any of SEQ ID NOS:548-550 and 579.

9. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-8, wherein the unmodified ADA2 protein is a homodimer, and the monomer form comprises the sequence of amino acid residues set forth in any of SEQ ID NOS:5, 548-550 and 579.

10. A catalytically active portion of the variant ADA2 protein of any of claims 6-9, wherein the unmodified ADA2 protein is a homodimer of corresponding catalytically active portions of the polypeptide whose sequence is set forth in of SEQ ID NO:5, wherein corresponding portions are determined by alignment.

11. A variant ADA2 protein of any of claims 6-10, wherein the unmodified ADA2 protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 5, 326-330, 380-383, 548-55 and 579 or is a catalytically active portion thereof.

12. The variant ADA2 protein of any of claims 6-10, wherein the unmodified ADA2 protein consists of the sequence of amino acids set forth in any of SEQ ID NOS: 5, 326-330, 380-383, 548-55 and 579 or is a catalytically active portion thereof.

13. The variant ADA2 protein of any of claims 6-12, wherein the unmodified ADA2 protein comprises the sequence of amino acids set forth in SEQ ID NO: 5 or a catalytically active portion thereof.

14. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-13, comprising the replacement corresponding to S262N or S262Q.

15. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-13, comprising the replacement corresponding to R219K, R219Q, R219N or R219A.

16. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-13, comprising the replacement corresponding to R219Q/S262N.

17. The variant ADA2 protein or catalytically active portion thereof of claim 16, comprising the modifications selected from among any of R219Q/S262N/R20N/V22S, R219Q/S262N/K371N/D373S, R219Q/S262N/K372N/I374S, R219Q/S262N/T403N/H405S, R219Q/S262N/G404N/P406S, R219Q/S262N/C105-T147del→(Gly)15, R219Q/S262N/C105-T147del→(Gly)10, R219Q/S262N/C105-T147del→(Gly)7, R219Q/S262N/C105-T147del→(Gly)5, R219Q/S262N/C105-T147del→(Gly)3, R219Q/S262N/R125N/P126A, R219Q/S262N/S127N/K129S, R219Q/S262N/P126N/E128T, R219Q/S262N/R112N/I114T, R219Q/S262N/I134N/L135C/L136T, R219Q/S262N/I134N/L135S/L136T, R219Q/S262N/R142N/Q144S, R219Q/S262N/E137N/Y139T, R219Q/S262N/P111N/G113S, R219Q/S262N/F119S, R219Q/S262N/F119K, R219Q/S262N/Y224R, R219Q/S262N/Y224N, R219Q/S262N/Y191S, R219Q/S262N/Y191D, R219Q/S262N/F183K,

R219Q/S262N/Y191D/Y224R, R219Q/S262N/F109S, R219Q/S262N/F109A, R219Q/S262N/R118D, R219Q/S262N/R118A, R219Q/S262N/Y139T, R219Q/S262N/Y139A, R219Q/S262N/W133S, R219Q/S262N/W133T, R219Q/S262N/P124A, R219Q/S262N/P124S, R219Q/S262N/V99-Q144del→$(GGGGS)_1$, R219Q/S262N/V99-Q144del→$(GGGGS)_2$, R219Q/S262N/V99-Q144del→$(GGGGS)_3$, R219Q/S262N/C105-T147del→$(GGGGS)_1$, R219Q/S262N/C105-T147del→$(GGGGS)_2$, R219Q/S262N/C105-T147del→$(GGGGS)_3$, R219Q/S262N/K371D/V99-Q144del→$(GGGGS)_1$, R219Q/S262N/K371D/V99-Q144del→$(GGGGS)_2$, R219Q/S262N/K371D/V99-Q144del→$(GGGGS)_3$, R219Q/S262N/K371D/C105-T147del→$(GGGGS)_1$, R219Q/S262N/K371D/C105-T147del→$(GGGGS)_2$, R219Q/S262N/K371D/C105-T147del→$(GGGGS)_3$, R219Q/S262N/C105-T147del→$(Gly)_n$, wherein n is 2 to 20, R219Q/S262N/K11A, R219Q/S262N/K11D, R219Q/S262N/K11E, R219Q/S262N/K13A, R219Q/S262N/K13D, R219Q/S262N/V99-Q144del→$(GGGGS)_n$ wherein n = 1 to 5, R219Q/S262N/C105-T147del→$(GGGGS)_n$, wherein n = 1 to 5, R219Q/S262N/N98-N156del, R219Q/S262N/C105-E148del, R219Q/S262N/C105-T147del, R219Q/S262N/V99-Q144del, R219Q/S262N/K371D/C105-T147del→$(Gly)_n$, wherein n = 2 to 20, R219Q/S262N/K371D/C105-T147del→$(Gly)_{15}$, R219Q/S262N/K371D/C105-T147del→$(Gly)_{10}$, R219Q/S262N/K371D/C105-T147del→$(Gly)_7$, R219Q/S262N/K371D/C105-T147del→$(Gly)_5$, R219Q/S262N/K371D/C105-T147del→$(Gly)_3$, R219Q/S262N/K371D/V99-Q144del→$(GGGGS)_n$, wherein n = 1 to 5, R219Q/S262N/K371D/C105-T147del→$(GGGGS)_n$, , wherein n = 1 to 5, R219Q/S262N/K371D/N98-N156del, R219Q/S262N/K371D/C105-E148del, R219Q/S262N/K371D/C105-T147del, R219Q/S262N/K371D/V99-Q144del, R219Q/S262N/K13E, R219Q/S262N/K371A, R219Q/S262N/K372A, R219Q/S262N/K372D, R219Q/S262N/K372E, R219Q/S262N/K452A, R219Q/S262N/K452D, R219Q/S262N/K452E, R219Q/S262N/R20A, R219Q/S262N/R20D, R219Q/S262N/R366A, R219Q/S262N/R366D, R219Q/S262N/R366E, R219Q/S262N/H264A, R219Q/S262N/H264Q, R219Q/S262N/H264N, R219Q/S262N/H264G, R219K/S262N, R219N/S262N, R219A/S262N, R219Q/S262N/L221A, R219Q/S262N/L221V, R219Q/S262N/L221G, R219Q/S262N/E179D, R219Q/S262N/E179A, R219Q/S262N/E179S, R219Q/S262N/E179T, R219Q/S262N/E179V, R219Q/S262N/E179G, R219Q/S262A, R219Q/S262V, R219Q/S262M, R219Q/S262N/K11A/R20A, R219Q/S262N/K11A/R20A/K371A, R219Q/S262N/R20A/K371A, R219Q/S262N/K11A/K371A, R219Q/S262N/K26A, R219Q/S262N/K26D, R219Q/S262N/K26E, R219Q/S262N/R217A, R219Q/S262N/R217D, R219Q/S262N/R217E, R219Q/S262N/K258A, R219Q/S262N/K258D, R219Q/S262N/K258E, R219Q/S262N/R277A, R219Q/S262N/R277D, R219Q/S262N/R277E, R219Q/S262N/R283A, R219Q/S262N/R283D, R219Q/S262N/R283E, R219Q/S262N/K309A, R219Q/S262N/K309D, R219Q/S262N/K309E, R219Q/S262N/K317A, R219Q/S262N/K317D, R219Q/S262N/K317E, R219Q/S262N/K321A, R219Q/S262N/K321D, R219Q/S262N/K321E, R219Q/S262N/R352A, R219Q/S262N/R352D, R219Q/S262N/R352E, R219Q/S262N/R441A, R219Q/S262N/R441D, R219Q/S262N/R441E, R219Q/S262N/K444A, R219Q/S262N/K444D, R219Q/S262N/K444E, R219Q/S262N/K461A, R219Q/S262N/K461D, R219Q/S262N/K461E, R219Q/S262N/K469A, R219Q/S262N/K469D, R219Q/S262N/K469E, R219Q/S262N/K470A, R219Q/S262N/K470D, R219Q/S262N/K470E, R219Q/S262N/D86A, R219Q/S262N/D86C, R219Q/S262N/D86E, R219Q/S262N/D86F, R219Q/S262N/D86G, R219Q/S262N/D86H, R219Q/S262N/D86I, R219Q/S262N/D86K, R219Q/S262N/D86L, R219Q/S262N/D86M, R219Q/S262N/D86N, R219Q/S262N/D86P, R219Q/S262N/D86Q,

R219Q/S262N/D86R, R219Q/S262N/D86S, R219Q/S262N/D86T, R219Q/S262N/D86V, R219Q/S262N/D86W, R219Q/S262N/D86Y, R219Q/S262N/E179C, R219Q/S262N/E179F, R219Q/S262N/E179H, R219Q/S262N/E179I, R219Q/S262N/E179K, R219Q/S262N/E179L, R219Q/S262N/E179M, R219Q/S262N/E179N, R219Q/S262N/E179P, R219Q/S262N/E179Q, R219Q/S262N/E179R, R219Q/S262N/E179W, R219Q/S262N/E179Y, R219C/S262N, R219D/S262N, R219E/S262N, R219F/S262N, R219G/S262N, R219H/S262N, R219I/S262N, R219L/S262N, R219M/S262N, R219P/S262N, R219S/S262N, R219T/S262N, R219V/S262N, R219W/S262N, R219Y/S262N, R219Q/S262N/L221C, R219Q/S262N/L221D, R219Q/S262N/L221E, R219Q/S262N/L221F,R219Q/S262N/L221H,R219Q/S262N/L221I, R219Q/S262N/L221K, R219Q/S262N/L221M, R219Q/S262N/L221N, R219Q/S262N/L221P, R219Q/S262N/L221Q, R219Q/S262N/L221R, R219Q/S262N/L221S, R219Q/S262N/L221T, R219Q/S262N/L221W, R219Q/S262N/L221Y, R219Q/S262C, R219Q/S262D, R219Q/S262E, R219Q/S262F, R219Q/S262G, R219Q/S262H, R219Q/S262I, R219Q/S262K, R219Q/S262L, R219Q/S262P, R219Q/S262Q, R219Q/S262R, R219Q/S262T, R219Q/S262W, R219Q/S262Y, R219Q/S262N/H264C, R219Q/S262N/H264D, R219Q/S262N/H264E, R219Q/S262N/H264F, R219Q/S262N/H264I, R219Q/S262N/H264K, R219Q/S262N/H264L, R219Q/S262N/H264M, R219Q/S262N/H264P, R219Q/S262N/H264R, R219Q/S262N/H264S, R219Q/S262N/H264T, R219Q/S262N/H264V, R219Q/S262N/H264W, R219Q/S262N/H264Y, R219Q/S262N/S266A, R219Q/S262N/S266C, R219Q/S262N/S266D, R219Q/S262N/S266E, R219Q/S262N/S266F, R219Q/S262N/S266G, R219Q/S262N/S266H, R219Q/S262N/S266I, R219Q/S262N/S266K, R219Q/S262N/S266L, R219Q/S262N/S266M, R219Q/S262N/S266N, R219Q/S262N/S266P, R219Q/S262N/S266Q, R219Q/S262N/S266R, R219Q/S262N/S266T, R219Q/S262N/S266V, R219Q/S262N/S266W, R219Q/S262N/S266Y, R219Q/S262N/K267A, R219Q/S262N/K267C, R219Q/S262N/K267D, R219Q/S262N/K267E, R219Q/S262N/K267F, R219Q/S262N/K267G, R219Q/S262N/K267H, R219Q/S262N/K267I, R219Q/S262N/K267L, R219Q/S262N/K267M, R219Q/S262N/K267N, R219Q/S262N/K267P, R219Q/S262N/K267Q, R219Q/S262N/K267R, R219Q/S262N/K267S, R219Q/S262N/K267T, R219Q/S262N/K267V, R219Q/S262N/K267W, R219Q/S262N/K267Y, R219Q/S262N/V296A, R219Q/S262N/V296C, R219Q/S262N/V296D, R219Q/S262N/V296E, R219Q/S262N/V296F, R219Q/S262N/V296G, R219Q/S262N/V296H, R219Q/S262N/V296I, R219Q/S262N/V296K, R219Q/S262N/V296L, R219Q/S262N/V296M, R219Q/S262N/V296N, R219Q/S262N/V296P, R219Q/S262N/V296Q, R219Q/S262N/V296R, R219Q/S262N/V296S, R219Q/S262N/V296T, R219Q/S262N/V296W and R219Q/S262N/V296Y.

18. The variant ADA2 protein of any of claims 6-17, wherein the variant ADA2 protein comprises amino acid replacements selected from among R219Q/S262N/K371E, R219Q/S262N/K371D, R219Q/S262N/R20E and R219Q/S262N/K371D/R20E.

19. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-17 that comprises the modifications corresponding to R219Q/S262N/--→N1/--→A2/--→S3; R219Q/S262N/R20N/V22S; R219Q/S262N/K371N/D373S; R219Q/S262N/K372N/I374S; R219Q/S262N/T403N/H405S; or R219Q/S262N/G404N/P406S.

20. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-17 that comprises amino acid replacements corresponding to R219Q/S262N/R125N/P126A; R219Q/S262N/S127N/K129S; R219Q/S262N/P126N/E128T; R219Q/S262N/R112N/I114T;

R219Q/S262N/I134N/L135C/L136T; R219Q/S262N/I134N/L135S/L136T; R219Q/S262N/R142N/Q144S; R219Q/S262N/E137N/Y139T; or R219Q/S262N/P111N/G113S.

21. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-20, further comprising a modification in the putative receptor binding domain (PRB) corresponding to one or more of the modifications selected from among: R125N/P126A; S127N/K129S; P126N/E128T; R112N/I114T; I134N/L135C/L136T; I134N/L135S/L136T; R142N/Q144S; E137N/Y139T; and P111N/G113S.

22. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-20, comprising amino acid replacements selected from among replacements corresponding to R219Q/S262N/F119S; R219Q/S262N/F119K; R219Q/S262N/Y224R; R219Q/S262N/Y224N; R219Q/S262N/Y191S; R219Q/S262N/Y191D; R219Q/S262N/F183K; R219Q/S262N/Y191D/Y224R; R219Q/S262N/F109S; R219Q/S262N/F109A; R219Q/S262N/R118D; R219Q/S262N/R118A; R219Q/S262N/Y139T; R219Q/S262N/Y139A; R219Q/S262N/W133S; R219Q/S262N/W133T; R219Q/S262N/P124A; and R219Q/S262N/P124S.

23. The variant ADA2 protein or catalytically active portion thereof of of any of claims 6-20 that comprises amino acid replacements selected from among replacements corresponding to R219Q/S262N/C105-T147del→$(Gly)_{15}$; R219Q/S262N/C105-T147del→$(Gly)_{10}$; R219Q/S262N/C105-T147del→$(Gly)_7$; R219Q/S262N/C105-T147del→$(Gly)_5$; R219Q/S262N/C105-T147del→$(Gly)_3$; R219Q/S262N/V99-Q144del→$(GGGGS)_1$; R219Q/S262N/V99-Q144del→$(GGGGS)_2$; R219Q/S262N/V99-Q144del→$(GGGGS)_3$; R219Q/S262N/C105-T147del→$(GGGGS)_1$; R219Q/S262N/C105-T147del→$(GGGGS)_2$; R219Q/S262N/C105-T147del→$(GGGGS)_3$; R219Q/S262N/K371D/V99-Q144del→$(GGGGS)_1$; R219Q/S262N/K371D/V99-Q144del→$(GGGGS)_2$; R219Q/S262N/K371D/V99-Q144del→$(GGGGS)_3$; R219Q/S262N/K371D/C105-T147del→$(GGGGS)_1$; R219Q/S262N/K371D/C105-T147del→$(GGGGS)_2$; R219Q/S262N/K371D/C105-T147del→$(GGGGS)_3$; R219Q/S262N/C105-T147del→(Gly)n, where n = 2 to 20; R219Q/S262N/V99-Q144del→(GGGGS)n, where n = 1 to 5; R219Q/S262N/C105-T147del→(GGGGS)n, where n = 1 to 5; R219Q/S262N/N98-N156del; R219Q/S262N/C105-E148del; R219Q/S262N/C105-T147del; R219Q/S262N/V99-Q144del; R219Q/S262N/K371D/C105-T147del→(Gly)n, where n = 2 to 20; R219Q/S262N/K371D/C105-T147del→$(Gly)_{15}$; R219Q/S262N/K371D/C105-T147del→$(Gly)_{10}$; R219Q/S262N /K371D/C105-T147del→$(Gly)_7$; R219Q/S262N/K371D/C105-T147del→$(Gly)_5$; R219Q/S262N/K371D/C105-T147del→$(Gly)_3$; R219Q/S262N/K371D/V99-Q144del→$(GGGGS)_n$, where n = 1 to 5; R219Q/S262N/K371D/C105-T147del→$(GGGGS)_n$, where n = 1 to 5; R219Q/S262N/K371D/N98-N156del; R219Q/S262N/K371D/C105-E148del; R219Q/S262N/K371D/C105-T147del; and R219Q/S262N/K371D/V99-Q144del, with reference to amino acid positions set forth in SEQ ID NO:5.

24. The variant ADA2 protein or catalytically active portion thereof of claim 6, wherein the variant ADA2 protein comprises the sequence of amino acids set forth in any of SEQ ID NOS:38-41, 51-54, 59-63, 145-159, 176-190 and 263-273 or a catalytically active portion thereof.

25. The variant ADA2 protein or catalytically active portion thereof of claim 6, wherein the variant ADA2 protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 596-645 and 658-931 or a catalytically active portion thereof

26. The variant ADA2 protein or catalytically active portion thereof of any of claims 6-20, comprising amino acid replacements selected from among replacements corresponding to R219Q/S262N/K371D; R219Q/S262N/R20E; R219Q/S262N/K371E/R20E; R219Q/S262N/K371D/R20E; R219Q/S262N; R219Q/S262N/K11A; R219Q/S262N/K11D; R219Q/S262N/K11E; R219Q/S262N/K13A; R219Q/S262N/K13D; R219Q/S262N/K13E; R219Q/S262N/K371A; R219Q/S262N/K372A; R219Q/S262N/K372D; R219Q/S262N/K372E; R219Q/S262N/K452A; R219Q/S262N/K452D; R219Q/S262N/K452E; R219Q/S262N/R20A; R219Q/S262N/R20D; R219Q/S262N/R366A; R219Q/S262N/R366D; R219Q/S262N/R366E; R219Q/S262N/H264A; R219Q/S262N/H264Q; R219Q/S262N/H264N; R219Q/S262N/H264G; R219K/S262N; R219N/S262N; R219A/S262N; R219Q/S262N/L221A; R219Q/S262N/L221V; R219Q/S262N/L221G; R219Q/S262N/E179D; R219Q/S262N/E179A; R219Q/S262N/E179S; R219Q/S262N/E179T; R219Q/S262N/E179V; R219Q/S262N/E179G; R219Q/S262A; R219Q/S262V; R219Q/S262M; R219Q/S262N/K11A/R20A; R219Q/S262N/K11A/R20A/K371A; R219Q/S262N/R20A/K371A; R219Q/S262N/K11A/K371A; R219Q/S262N/K26A; R219Q/S262N/K26D; R219Q/S262N/K26E; R219Q/S262N/R217A; R219Q/S262N/R217D; R219Q/S262N/R217E; R219Q/S262N/K258A; R219Q/S262N/K258D; R219Q/S262N/K258E; R219Q/S262N/R277A; R219Q/S262N/R277D; R219Q/S262N/R277E; R219Q/S262N/R283A; R219Q/S262N/R283D; R219Q/S262N/R283E; R219Q/S262N/K309A; R219Q/S262N/K309D; R219Q/S262N/K309E; R219Q/S262N/K317A; R219Q/S262N/K317D; R219Q/S262N/K317E; R219Q/S262N/K321A; R219Q/S262N/K321D; R219Q/S262N/K321E; R219Q/S262N/R352A; R219Q/S262N/R352D; R219Q/S262N/R352E; R219Q/S262N/R441A; R219Q/S262N/R441D; R219Q/S262N/R441E; R219Q/S262N/K444A; R219Q/S262N/K444D; R219Q/S262N/K444E; R219Q/S262N/K461A; R219Q/S262N/K461D; R219Q/S262N/K461E; R219Q/S262N/K469A; R219Q/S262N/K469D; R219Q/S262N/K469E; R219Q/S262N/K470A; R219Q/S262N/K470D; R219Q/S262N/K470E; R219Q/S262N/D86A; R219Q/S262N/D86C; R219Q/S262N/D86E; R219Q/S262N/D86F; R219Q/S262N/D86G; R219Q/S262N/D86H; R219Q/S262N/D86I; R219Q/S262N/D86K; R219Q/S262N/D86L; R219Q/S262N/D86M; R219Q/S262N/D86N; R219Q/S262N/D86P; R219Q/S262N/D86Q; R219Q/S262N/D86R; R219Q/S262N/D86S; R219Q/S262N/D86T; R219Q/S262N/D86V; R219Q/S262N/D86W; R219Q/S262N/D86Y; R219Q/S262N/E179C; R219Q/S262N/E179F; R219Q/S262N/E179H; R219Q/S262N/E179I; R219Q/S262N/E179K; R219Q/S262N/E179L; R219Q/S262N/E179M; R219Q/S262N/E179N; R219Q/S262N/E179P; R219Q/S262N/E179Q; R219Q/S262N/E179R; R219Q/S262N/E179W; R219Q/S262N/E179Y; R219C/S262N; R219D/S262N; R219E/S262N; R219F/S262N; R219G/S262N; R219H/S262N; R219I/S262N; R219L/S262N; R219M/S262N; R219P/S262N; R219S/S262N; R219T/S262N; R219V/S262N; R219W/S262N; R219Y/S262N; R219Q/S262N/L221C; R219Q/S262N/L221D; R219Q/S262N/L221E; R219Q/S262N/L221F; R219Q/S262N/L221H; R219Q/S262N/L221I; R219Q/S262N/L221K; R219Q/S262N/L221M; R219Q/S262N/L221N; R219Q/S262N/L221P; R219Q/S262N/L221Q; R219Q/S262N/L221R; R219Q/S262N/L221S; R219Q/S262N/L221T; R219Q/S262N/L221W; R219Q/S262N/L221Y; R219Q/S262C; R219Q/S262D; R219Q/S262E; R219Q/S262F; R219Q/S262G; R219Q/S262H; R219Q/S262I; R219Q/S262K; R219Q/S262L; R219Q/S262P; R219Q/S262Q; R219Q/S262R; R219Q/S262T; R219Q/S262W; R219Q/S262Y; R219Q/S262N/H264C; R219Q/S262N/H264D; R219Q/S262N/H264E; R219Q/S262N/H264F; R219Q/S262N/H264I; R219Q/S262N/H264K; R219Q/S262N/H264L;

R219Q/S262N/H264M; R219Q/S262N/H264P; R219Q/S262N/H264R; R219Q/S262N/H264S; R219Q/S262N/H264T; R219Q/S262N/H264V; R219Q/S262N/H264W; R219Q/S262N/H264Y; R219Q/S262N/S266A; R219Q/S262N/S266C; R219Q/S262N/S266D; R219Q/S262N/S266E; R219Q/S262N/S266F; R219Q/S262N/S266G; R219Q/S262N/S266H; R219Q/S262N/S266I; R219Q/S262N/S266K; R219Q/S262N/S266L; R219Q/S262N/S266M; R219Q/S262N/S266N; R219Q/S262N/S266P; R219Q/S262N/S266Q; R219Q/S262N/S266R; R219Q/S262N/S266T; R219Q/S262N/S266V; R219Q/S262N/S266W; R219Q/S262N/S266Y; R219Q/S262N/K267A; R219Q/S262N/K267C; R219Q/S262N/K267D; R219Q/S262N/K267E; R219Q/S262N/K267F; R219Q/S262N/K267G; R219Q/S262N/K267H; R219Q/S262N/K267I; R219Q/S262N/K267L; R219Q/S262N/K267M; R219Q/S262N/K267N; R219Q/S262N/K267P; R219Q/S262N/K267Q; R219Q/S262N/K267R; R219Q/S262N/K267S; R219Q/S262N/K267T; R219Q/S262N/K267V; R219Q/S262N/K267W; R219Q/S262N/K267Y; R219Q/S262N/V296A; R219Q/S262N/V296C; R219Q/S262N/V296D; R219Q/S262N/V296E; R219Q/S262N/V296F; R219Q/S262N/V296G; R219Q/S262N/V296H; R219Q/S262N/V296I; R219Q/S262N/V296K; R219Q/S262N/V296L; R219Q/S262N/V296M; R219Q/S262N/V296N; R219Q/S262N/V296P; R219Q/S262N/V296Q; R219Q/S262N/V296R; R219Q/S262N/V296S; R219Q/S262N/V296T; R219Q/S262N/V296W; R219Q/S262N/V296Y; R219Q/K11A/R20A; R219Q/K11A/R20A/K371A; R219Q/R20A/K371A; R219Q/K11A/K371A; S262N/K11A/R20A; S262N/K11A/R20A/K371A; S262N/R20A/K371A; and S262N/K11A/K371A, with reference to SEQ ID NO:5.

27. The variant ADA2 protein or catalytically active portion thereof of any of claims 1-26, wherein the ADA2 protein is PEGylated.

28. The variant ADA2 protein or catalytically active portion thereof of claim 16, wherein the sequence of the ADA2 portion is set forth in SEQ ID NO:273.

29. The variant ADA2 protein or catalytically active portion thereof of claim 28, wherein the ADA2 protein is PEGylated.

30. The variant ADA2 protein or catalytically active portion thereof of any of claims 1-29 that lacks all or a portion of the PRB, wherein residues corresponding to 98-156 of SEQ ID NO.5 comprise the PRB.

31. A catalytically active portion of the variant ADA2 protein or catalytically active portion thereof of any of claims 1-30, wherein the catalytically active portion comprises the ADA domain.

32. A variant ADA2 multimer, comprising a plurality of variant ADA2 proteins or catalytically active portions thereof of any of claims 1-31, wherein the variant ADA2 protein or catalytically active portion thereof are the same or different.

33. A variant ADA2 dimer, comprising a variant ADA2 protein or catalytically active portion thereof of any of claims 1-32.

34. A variant ADA2 dimer, comprising a variant ADA2 protein or catalytically active portion thereof of claim 16.

35. A variant ADA2 dimer of claim 34, wherein the ADA2 protein comprises the polypeptide of SEQ ID NO:273.

36. The variant ADA2 dimer or catalytically active portion thereof of claim 32 or claim 35 that is a homodimer comprising two variant ADA2 protein or catalytically active portions thereof that are the same.

37. The variant ADA2 dimer or catalytically active portion thereof of claim 32 that is a heterodimer comprising two variant ADA2 protein or catalytically active portions thereof that are different from each other.

38. A conjugate, comprising an ADA2 protein or catalytically active portion thereof of claim 16, linked directly or indirectly via a linker to a heterologous moiety, wherein the heterologous moiety is a half-life extending moiety.

39. A conjugate, comprising an ADA2 protein or catalytically active portion thereof of any of claims 1-37, linked directly or indirectly via a linker to a heterologous moiety, wherein the heterologous moiety is a half-life extending moiety.

40. The conjugate of claim 38 or claim 39, wherein the a half-life extending moiety is a PEG and the ADA2 protein or catalytically active portion thereof is PEGylated.

41. A nucleic acid encoding a variant ADA2 protein or catalytically active portion thereof of any of claims 1-37.

42. A vector, comprising the nucleic acid molecule of claim 41.

43. An isolated cell or a cell culture, comprising the vector of claim 42.

44. The cell or cell culture of claim 43, wherein the cell is a selected from a tumor-infiltrating lymphocyte (TIL), a cytotoxic T lymphocyte (CTL), a natural killer (NK) cell or a lymphokine-activated killer (LAK) cell.

45. The cell or cell culture of claim 44, wherein the cell is a T cell that encodes and expresses chimeric antigen receptor (CAR) and the variant ADA2 protein or dimer, wherein the CAR is specific for a tumor cell antigen.

46. A method of producing an ADA2 variant or catalytically active portion thereof, comprising culturing a cell of claim 44 or claim45, under conditions whereby the ADA2 variant is expressed.

47. The method of claim 46, wherein the resulting ADA2 variant is a dimer.

48. A method, comprising, culturing or expanding the cells of any one of claims 46-48 to produce expanded cells.

49. The expanded cells produced by the method of claim 48.

50. Use of the expanded cells of claim 49 in the manufacture of a medicament for treating a tumor or cancer.

51. The expanded cells of claim 49 for use in the treatment of a tumor or cancer.

52. A pharmaceutical composition, comprising a variant ADA2 protein of any of claims 1-40 or a catalytically active portion thereof, or a dimer or multimer of the ADA2 protein or catalytically active portion thereof or a conjugate of any of claims 41-43.

53. The pharmaceutical composition of claim 52, wherein the ADA2 variant or catalytically active portion thereof is PEGylated.

54. The pharmaceutical composition of claim 52, wherein the ADA2 variant comprises the sequence of amino acid residues set forth in SEQ ID NO: 273 or a catalytically active portion thereof.

55. The pharmaceutical composition of claim 52, wherein the ADA2 protein or catalytically active portion thereof is PEGylated.

56. An ADA variant or multimer thereof of any of claims 1-40 or the conjugate of any of claims 41-43 for use in the treatment of a tumor.

57. The ADA variant or multimer of claim 56, wherein the ADA2 protein comprises the sequence of amino acid residues set forth in SEQ ID NO: 273 or a catalytically active portion thereof.

58. The ADA variant or multimer of claim 57, wherein the ADA2 protein or a catalytically active portion thereof is PEGylated.

59. Use of pharmaceutical composition of any of claims 52-61 in the manufacture of a medicament for treating a tumor.

60 The expanded cells of claim 51 and the use of claim 59, wherein the ADA2 protein comprises the sequence of amino acid residues set forth in SEQ ID NO: 273 or a catalytically active portion thereof.

61. The expanded cells and the use of claim 60 wherein the ADA2 protein or a catalytically active portion thereof is PEGylated.