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

SUMMARY

In one aspect, provided herein is 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: i) the variant ADA2 protein, or catalytically active portion thereof, when in dimer form, exhibits reduced heparin binding 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; ii) modifications that confer reduced heparin binding are selected from among R20A, R20D, R20E, R366A, R366D, R366E, K371A, K371D, K371E, K372A, K372D, K372E, K452E, K11A/R20A, K11A/R20A/K371A, R20A/K371A, K11A/K371A, with reference to amino acid positions set forth in SEQ ID NO:5; iii) modifications comprise insertions, deletions, and replacements of amino acid residues; iv) the variant ADA2 protein or catalytically active portion thereof has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 10 amino acid modifications in the unmodified ADA2 protein; v) the unmodified ADA2 protein and catalytically active portion is selected from the group consisting of: a) a sequence of amino acids selected from the group consisting of SEQ ID NOS: 5 and 380-383; b) a sequence of amino acids selected from the group consisting of SEQ ID NOS: 5 and 380-383 with all or a part of the PRB domain deleted, wherein the PRB domain consists of residues corresponding to residues 98-156 of SEQ ID NO:5; c) a sequence of amino acids selected from the group consisting of SEQ ID NOS: 5 and 380-383 with all or a part of the PRB domain deleted and a linker in place of deleted residues, wherein: the PRB domain consists of residues corresponding to residues98-156 of SEQ ID NO:5; and the linker is (GGGGS)n, where n = 1 to 5, or is (Gly)n, where n is 2-20; d) a sequence of amino acids selected from the group consisting of the catalytic domain of the polypeptides of SEQ ID NOS: 5 and 380-383, wherein the catalytic domain consists of residues corresponding to residues 77-473 of the sequence of amino acids set forth in SEQ ID NO:5; e) a sequence of amino acids selected from the group consisting of the catalytic domain of SEQ ID NOS: 5 and 380-383 with all or a part of the PRB domain deleted, wherein: the catalytic domain consists of

residues corresponding to residues 77-473 of the sequence of amino acids set forth in SEQ ID NO:5; and the PRB domain consists of residues corresponding to residues 98-156 of SEQ ID NO:5; and f) a sequence of amino acids selected from the group consisting of the catalytic domain of SEQ ID NOS: 5 and 380-383 with all or a part of the PRB domain deleted, and a linker in place of deleted residues, wherein: the catalytic domain consists of residues corresponding to residues 77-473 of the sequence of amino acids set forth in SEQ ID NO:5; the PRB domain consists of residues corresponding to residues 98-156 of SEQ ID NO:5; and the linker is (GGGGS)n, where n = 1 to 5, or is (Gly)n, where n is 2-20; and vi) the variant ADA2 protein, when in dimer form, exhibits adenosine deaminase activity to convert adenosine to inosine.

In another aspect, provided herein is a variant ADA2 multimer, comprising a plurality of variant ADA2 proteins or the catalytically active portion thereof of the disclosure wherein the variant ADA2 proteins or catalytically active portion thereof are the same or different.

In another aspect, provided herein is a variant ADA2 dimer, comprising the variant ADA2 protein or the catalytically active portion thereof of the disclosure.

In another aspect, provided herein is a conjugate, comprising the variant ADA2 protein or the catalytically active portion thereof of the disclosure, or the multimer of the disclosure, or the dimer of the disclosure, linked directly or indirectly via a linker to a heterologous moiety.

In another aspect, provided herein is a nucleic acid molecule encoding the variant ADA2 protein or the catalytically active portion thereof of the disclosure, or the variant ADA2 multimer of the disclosure, or the variant ADA2 dimer of the disclosure.

In another aspect, provided herein is a vector, comprising the nucleic acid molecule of the disclosure.

In another aspect, provided herein is a isolated cell or a cell culture, comprising the vector of the disclosure.

In another aspect, provided herein is a method of producing a variant ADA2 protein or the catalytically active portion thereof, comprising culturing or expanding

the cell of the disclosure, under conditions whereby the variant ADA2 protein or the catalytically active portion thereof is expressed.

In another aspect, provided herein is a use of the expanded cells of the disclosure in the manufacture of a medicament for treating a tumor.

In another aspect, provided herein are expanded cells of the disclosure for use in therapy.

In another aspect, provided herein is a pharmaceutical composition, comprising the variant ADA2 protein or the catalytically active portion thereof, or the dimer or the multimer of the variant ADA2 protein or the catalytically active portion thereof of the disclosure, or the conjugate of the disclosure, in a pharmaceutically acceptable vehicle.

In another aspect, provided herein is a use of the variant ADA2 protein or the catalytically active portion thereof, or the dimer or the multimer of the variant ADA2 protein or the catalytically active portion thereof of the disclosure, or the conjugate of the disclosure, in the manufacture of a medicament for treating a tumor, a cancer, a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy, or an immunodeficiency disease.

In another aspect, provided herein is a use of the pharmaceutical composition of the disclosurein the manufacture of a medicament for treatinga tumor, a cancer, a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy, or an immunodeficiency disease in a subject.

In another aspect, provided herein is a pharmaceutical composition of the disclosure for use in therapy.

Provided 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 examples, 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 examples, the variant ADA2 protein or catalytically active portion thereof, when in dimer form, exhibits increased adenosine deaminase activity or increased adenosine deaminase activity and reduced heparin binding.

In some examples, the unmodified ADA2 protein is a homodimer, and the monomer form comprises the sequence of amino acid residues set forth in SEQ ID NO:5. In some examples, the variant ADA2 is a catalytically active portion of the variant ADA2 protein as provided herein, 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.

In some example, the ADA2 protein or catalytically active portion thereof 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.

In some examples, the unmodified ADA2 protein can include a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in SEQ ID NO:5 or is a catalytically active portion thereof. For example, the unmodified ADA2 protein has at least 95% sequence identity with the sequence of amino acids set forth in SEQ ID NO:5 or with the corresponding catalytically active portion thereof. For example, the unmodified ADA2 protein includes the sequence of amino acids set forth in any of SEQ ID NOS:5, 326-334, 340, 375 or 380-383 or is a catalytically active portion thereof, or the unmodified ADA2 protein has a 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. In particular examples, the unmodified ADA2 protein includes the sequence of amino acids set forth in SEQ ID NO:5 or is a catalytically active portion thereof.

In some examples, the catalytically active portion of the ADA2 protein can be an ADA2 protein that lacks all or a portion of the putative receptor binding (PRB) domain. For example, the catalytically active portion of the ADA2 protein can include the sequence of amino acids set forth in SEQ ID NOS:548-550. In some examples, the catalytically active portion of the unmodified ADA2 protein has the sequence set forth as residues 77-473 of the protein set forth in SEQ ID NO:5.

In some examples, the variant ADA2 protein can include up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70 or more amino

acid modifications compared to the unmodified ADA2 protein. In some examples the variant ADA2 protein includes up to 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid modifications. In some examples, the variant ADA2 protein does not contain the sequence of amino acids set forth in any of SEQ ID NOS:1, 5, 68, 286-302, 326-342 or 374-383 or catalytically active fragment thereof. In some examples, the primary amino acid sequence of the ADA2 protein variant is not the sequence of amino acids set forth in any of SEQ ID NOS:1, 5, 68, 286-302, 326-342 or 374-383.

In some examples, the variant ADA2 protein, when in dimer form, exhibits adenosine deaminase activity to convert adenosine to inosine. In some examples herein, the variant ADA2 protein, when in dimer form, can exhibit a catalytic efficiency (k_{cat}/K_M) that is at least or at least about 5 x 10^3 $M^{-1}s^{-1}$, 6 x 10^3 $M^{-1}s^{-1}$, 7 x 10^3 $M^{-1}s^{-1}$, 8 x 10^3 $M^{-1}s^{-1}$, 9 x 10^3 $M^{-1}s^{-1}$, 1 x 10^4 $M^{-1}s^{-1}$, 2 x 10^4 $M^{-1}s^{-1}$, 3 x 10^4 $M^{-1}s^{-1}$, 4 x 10^4 $M^{-1}s^{-1}$, 5 x 10^4 $M^{-1}s^{-1}$, 6 x 10^4 $M^{-1}s^{-1}$, 7 x 10^4 $M^{-1}s^{-1}$, 8 x 10^4 $M^{-1}s^{-1}$, 9 x 10^4 $M^{-1}s^{-1}$, 1 x 10^5 $M^{-1}s^{-1}$, 2 x 10^5 $M^{-1}s^{-1}$, 3 x 10^5 $M^{-1}s^{-1}$, 4 x 10^5 $M^{-1}s^{-1}$, 5 x 10^5 $M^{-1}s^{-1}$ or greater.

In some examples, the variant ADA2 protein, when in dimer form, can exhibit a thermal stability with a melting temperature (Tm) of at least 58°C. For example, the Tm of the ADA2 protein is at least 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 68°C, 69°C, 70°C, 71°C, 72°C or greater.

In some examples, the variant ADA2 protein can contain a modification(s) that is an amino acid replacement(s); and the variant ADA2 protein includes 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. For example, the amino acid replacement(s) are positions corresponding to amino acid residue 11, 20, 219, 221, 262, 264, 366, 371, 372 or 452, with reference to amino acid positions set forth in SEQ ID NO:5. In some examples, the variant ADA2 protein can include one or more amino acid replacement(s) selected from among K11A, K11D, K11E, K13A, K13D, K13E, R20A, R20D, R20E, R20N, V22S, K26A, K26D, K26E, D86A, D86C, D86E, D86F, D86G, D86H, D86I, D86K, D86L, D86M, D86N, D86P, D86Q, D86R, D86S, D86T,

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; and V296Y.

In some examples, the variant ADA2 protein contains 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. For Example, the variant ADA2 protein or catalytically active portion thereof contains the replacement corresponding to S262N or S262Q. In some examples, the variant ADA2 contains the replacement corresponding to S262N. In some examples, the variant ADA2 contains the replacement corresponding to R219K, R219Q, R219N or R219A. In other examples, the variant ADA2 contains the replacement corresponding to R219Q or the replacements R219Q/R20E. In other examples, the variant ADA2 contains the replacement corresponding to R219Q/S262N. For example, the variant ADA2 protein or catalytically active portion thereof contains modification(s) selected from among any of R219Q/S262N/--→N1/--→A2/--→S3, 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/V296Y. In some examples, the variant ADA2 protein comprises the modifications selected from among R219Q/K11A/R20A, R219Q/K11A/R20A/K371A, R219Q/R20A/K371A, 219Q/K11A/K371A, S262N/K11A/R20A, S262N/K11A/R20A/K371A, S262N/R20A/K371A, S262N/K11A/K371A, R219Q/C105-T147del→(Gly)n, R219Q/V99-Q144del→(GGGGS)n, R219Q/C105-T147del→(GGGGS)n, R219Q/N98-N156del, R219Q/C105-E148del, R219Q/C105-T147del, R219Q/V99-Q144del, S262N/C105-T147del→(Gly)n, S262N/V99-Q144del→(GGGGS)n, S262N/C105-T147del→(GGGGS)n, S262N/N98-N156del, S262N/C105-E148del, S262N/C105-T147del and S262N/V99-Q144del.

In some examples, the variant ADA2 protein, when in dimer form, can exhibit increased adenosine deaminase activity. For example, the variant ADA2 protein, when in dimer form, can exhibit at least 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 225%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800% or more activity of the corresponding dimer form of the unmodified ADA2 protein, wherein adenosine deaminase activity is assessed under the same conditions. In some examples, the variant ADA2 protein, when in dimer form, can exhibit a catalytic efficiency (k_{cat}/K_M) that is at least or at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.2-fold, 2.5-fold, 3.0-fold, 3.5-fold, 4-fold, 4.5-fold, 5.0-fold, 6.0-fold, 7.0-fold, 8.0-fold, 9.0-fold, 10.0-fold or more compared to the catalytic efficiency (k_{cat}/K_M) of the corresponding dimer form of the unmodified ADA2 protein, wherein catalytic efficiency of adenosine deaminase activity is assessed under the same conditions. For example, the variant ADA2 protein, when in dimer form, can exhibit a catalytic efficiency (k_{cat}/K_M) that is at least or at least about 2×10^4 M^{-1} s^{-1}, 3×10^4 M^{-1} s^{-1}, 4×10^4 M^{-1} s^{-1}, 5×10^4 M^{-1} s^{-1}, 6×10^4 M^{-1} s^{-1}, 7×10^4 M^{-1} s^{-1}, 8×10^4 M^{-1} s^{-1}, 9×10^4 M^{-1} s^{-1}, 1×10^5 M^{-1} s^{-1}, 2×10^5 M^{-1} s^{-1}, 3×10^5 M^{-1} s^{-1}, 4×10^5 M^{-1} s^{-1}, 5×10^5 M^{-1} s^{-1} or greater.

In some examples, the variant ADA2 protein or catalytically active portion thereof contains the modifications selected from among K371D/V99-Q144del→$(GGGGS)_1$, K371D/V99-Q144del→$(GGGGS)_2$,

K371D/V99-Q144del→$(GGGGS)_3$, K371D/C105-T147del→$(GGGGS)_1$, K371D/C105-T147del→$(GGGGS)_2$, K371D/C105-T147del→$(GGGGS)_3$, R219Q/S262N/--→N1/--→A2/--→S3 , K371D/C105-T147del→(Gly)n, K371D/C105-T147del→$(Gly)_{15}$, K371D/C105-T147del→$(Gly)_{10}$, K371D/C105-T147del→$(Gly)_7$, K371D/C105-T147del→$(Gly)_5$, K371D/C105-T147del→$(Gly)_3$, K371D/V99-Q144del→(GGGGS)n, K371D/C105-T147del→(GGGGS)n, K371D/N98-N156del, K371D/C105-E148del, K371D/C105-T147del and K371D/V99-Q144del. In some examples, the variant ADA2 protein or catalytically active portion thereof contains modifications selected from among R125N/P126A, S127N/K129S, P126N/E128T, R112N/I114T, I134N/L135C/L136T, I134N/L135S/L136T, R142N/Q144S, E137N/Y139T, P111N/G113S, F119S, F119K, Y224R, Y224N, Y191S, Y191D, F183K, Y191D/Y224R, F109S, F109A, R118D, R118A, Y139T, Y139A, W133S, W133T, P124A, P124S, V99-Q144del, V99-Q144del→(GGGGS)n, C105-T147del→(GGGGS)n, V99-Q144del→$(GGGGS)_1$, V99-Q144del→$(GGGGS)_2$, V99-Q144del→$(GGGGS)_3$, C105-T147del→$(GGGGS)_1$, C105-T147del→$(GGGGS)_2$, and C105-T147del→$(GGGGS)_3$.

In some examples, the variant ADA2 protein or catalytically active portion thereof contains modifications selected from among R125N/P126A, S127N/K129S, P126N/E128T, R112N/I114T, I134N/L135C/L136T, I134N/L135S/L136T, R142N/Q144S, E137N/Y139T, P111N/G113S, F119S, F119K, Y224R, Y224N, Y191S, Y191D, F183K, Y191D/Y224R, F109S, F109A, R118D, R118A, Y139T, Y139A, W133S, W133T, P124A, P124S, V99-Q144del, V99-Q144del→(GGGGS)n, C105-T147del→(GGGGS)n, V99-Q144del→$(GGGGS)_1$, V99-Q144del→$(GGGGS)_2$, V99-Q144del→$(GGGGS)_3$, C105-T147del→$(GGGGS)_1$, C105-T147del→$(GGGGS)_2$ and C105-T147del→$(GGGGS)_3$.

For example, among such variant ADA2 proteins are any that include one or more amino acid replacement(s) at an amino acid position corresponding to amino acid residue 11, 20, 219, 221, 262, 264, 366, 371, 372 or 452, with reference to amino acid positions set forth in SEQ ID NO:5. For example, the variant ADA2 protein can include one or more amino acid replacement(s) selected from among K11A, K11E, R20A, R20E, R219K, R219Q, L221A, L221V, L221G, S262N, H264Q, H264G, R366E, K371A, K371D, K371E, K372D, K372E, K452D and K452E, with reference

to amino acid positions set forth in SEQ ID NO:5. In some examples, the variant ADA2 protein can include amino acid replacements selected from among K11A/R20A, K11A/R20A/K371A, R20A/K371A, K11A/K371A, S262N/K371D, S262N/K371E, S262N/R20E, S262N/R20E/K371D, S262N/R20E/K371E, R219Q/K371E, R219Q/K371D, R219Q/R20E, R219Q/K371E/R20E, R219Q/K371D/R20E, R219Q/S262N/K371E, R219Q/S262N/K371D, R219Q/S262N/R20E, R219Q/S262N/K371E/R20E, R219Q/S262N/K371D/R20E and R219Q/S262N, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples, the variant ADA2 protein, when in dimer form, can exhibit reduced heparin binding. For example, the variant ADA2 protein, when in dimer form, can exhibit no more than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% of the heparin binding of the corresponding dimer form of the unmodified ADA2 protein, wherein heparin binding is assessed under the same conditions.

For example, among such the variant ADA2 proteins are any that include one or more amino acid replacement(s) at an amino acid position corresponding to amino acid residue 20, 262, 366, 371, 372, or 452, with reference to amino acid positions set forth in SEQ ID NO:5. For example, the variant ADA2 protein can include 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. In some examples, the variant ADA2 protein can include amino acid replacements selected from among K11A/R20A, K11A/R20A/K371A, R20A/K371A, K11A/K371A, S262N/K371D, S262N/K371E, S262N/R20E, S262N/R20E/K371D and S262N/R20E/K371E, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples, the variant ADA2 protein or catalytically active portion thereof contains 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.

In some examples, the variant ADA2 protein, when in dimer form, can exhibit a longer serum half-life ($t_{1/2}$). For example, the variant ADA2, when in dimer form, can exhibit a half-life that is at least or at least about 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 225%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800% or more longer than the half-life of the corresponding dimer form of the unmodified ADA2 protein, wherein half-life is assessed under the same conditions.

In some examples, the variant ADA2 protein, when in dimer form, can exhibit increased thermal stability. For example, the variant ADA2 protein, when in dimer form, can exhibit thermal stability with a melting temperature (Tm) that is increased at least or at least about 0.5°C, 1.0°C, 2.0°C, 3.0°C, 4.0°C, 5.0°C, 6.0°C, 7.0°C, 8.0°C, 9.0°C, 10.0°C or more compared to the Tm of the corresponding dimer form of the unmodified ADA2 protein, wherein Tm is assessed under the same conditions. For example, the variant ADA2 protein can have a melting temperature (Tm) of at least or at least about 67.6°C, 67.8°C, 68.0°C, 68.2°C, 68.4°C, 68.6°C, 68.8°C, 69.0°C, 69.2°C, 69.4°C, 69.6°C, 69.8°C, 70.0°C, 70.2°C, 70.4°C, 70.6°C, 70.8°C, 71.0°C, 71.2°C, 71.4°C, 71.6°C, 71.8°C or higher.

In examples of any of the variant ADA2 proteins provided herein, the adenosine deaminase activity of the variant ADA2 protein can be assessed or exhibited at or about pH 6.5 ± 0.2. In some examples, the variant ADA2 protein, when in dimer form, can exhibit an altered pH optimum for adenosine deaminase activity. For example, the variant ADA2 protein, when in dimer form, can exhibit a pH optimum for adenosine deaminase activity that is at a higher pH compared to the pH optimum of the corresponding dimer form of the unmodified ADA2 protein. For example, the variant ADA2 protein, when in dimer form, can have a pH optimum with a pH that is at least or at least about 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5 or higher. In other examples, the variant ADA2 protein, when in dimer form, can exhibit a pH optimum for adenosine deaminase activity that is at a lower pH compared to the

pH optimum of the corresponding dimer form of the unmodified ADA2 protein. For example, the variant ADA2 protein, when in dimer form, can have a pH optimum with a pH that is less than or less than about 6.5, 6.4, 6.3, 6.3, 6.2, 6.1, 6.0 or less.

In some examples, the variant ADA2 protein can include a modification of one or more amino acids in the putative receptor binding (PRB) domain, wherein the modification is an amino acid deletion, insertion or replacement. In any of such examples, the variant ADA2 protein does not contain a modification that is an amino acid replacement corresponding to amino acid replacement C108G, A120V, H121R, R125C, R140Q, K141R or R142W, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples, the variant ADA2 protein or catalytically active portion thereof lacks all or a portion of the putative receptor binding (PRB) domain or has a modification of the PRB, whereby any receptor binding or growth factor activity is reduced or eliminated or other activity of ADA2 other than deaminase activity is reduced or eliminated or interaction with the ADA domain is reduced or eliminated and the PRB domain corresponds to residues 98 to 156 set forth in SEQ ID NO:5. In some examples, the variant ADA2 lacks residues 105-148 or 105 to 147 or 99 to 144, with reference to amino acid positions set forth in SEQ ID NO:5. In some examples, the variant ADA2 protein or catalytically active portion thereof contains the sequence of amino acids set forth in any of SEQ ID NOS:548-550 and 579. In some examples, variant ADA2 protein or catalytically active portion thereof contains a deletion of all or a portion of the PRB domain and optionally an insertion of peptide linker.

In some examples, the variant ADA2 protein can have a deletion of one or more contiguous amino acid residues corresponding to any one or more contiguous amino acid residues between or between about amino acid residues 98 and 156 or amino acid residues 105 and 148, inclusive, with reference to amino acid positions set forth in SEQ ID NO:5. In any of such examples, the variant of the ADA2 polypeptide can further include substitution of the deleted region with a peptide linker. For example, the peptide linker can be selected from among (Gly)n (SEQ ID NO:368), where n is 2 to 20; (GGGGS)n (SEQ ID NO:343), where n is 1 to 6; (SSSSG)n (SEQ ID NO:344), where n is 1 to 6; (AlaAlaProAla)n (SEQ ID NO:350), where n is 1 to 6;

GKSSGSGSESKS (SEQ ID NO:345); GGSTSGSGKSSEGKG (SEQ ID NO:346); GSTSGSGKSSSEGSGSTKG (SEQ ID NO:347); GSTSGSGKPGSGEGSTKG (SEQ ID NO:348); and EGKSSGSGSESKEF (SEQ ID NO:349). For example, the peptide linker can be selected from among GGG (SEQ ID NO:369); GGGGG (SEQ ID NO:360); GGGGGGG (SEQ ID NO:370); GGGGGGGGGG (SEQ ID NO:371); and GGGGGGGGGGGGGGG (SEQ ID NO:372).

In some examples, the variant ADA2 can contain the variant ADA2 polypeptide that can include a modification in the PRB domain that corresponds to C105-T147del→$(Gly)_n$, where n is 2 to 20, such as C105-T147del→$(Gly)_{15}$, C105-T147del→$(Gly)_{10}$, C105-T147del→$(Gly)_7$, C105-T147del→$(Gly)_5$ or C105-T147del→$(Gly)_3$, with reference to amino acid positions set forth in SEQ ID NO:5. In some examples, the variant ADA2 can include modification in the PRB domain that corresponds to C105-T147del→(Gly)n, where n = 2 to 20; C105-T147del→$(Gly)_{15}$; C105-T147del→$(Gly)_{10}$; C105-T147del→$(Gly)_7$; C105-T147del→$(Gly)_5$; C105-T147del→$(Gly)_3$; N98-N156del; C105-E148del; C105-T147del; V99-Q144del; V99-Q144del→(GGGGS)n, where n = 1 to 5; C105-T147del→(GGGGS)n, where n = 1 to 5; V99-Q144del→$(GGGGS)_1$; V99-Q144del→$(GGGGS)_2$; V99-Q144del→$(GGGGS)_3$; C105-T147del→$(GGGGS)_1$; C105-T147del→$(GGGGS)_2$; and C105-T147del→$(GGGGS)_3$, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples, the variant ADA2 protein or catalytically active portion thereof contains one or more amino acid replacement(s) selected from among replacements corresponding to F119S; F119K; Y224R; Y224N; Y191S; Y191D; F183K; Y191D/Y224R; F109S; F109A; R118D; R118A; Y139T; Y139A; W133S; W133T; P124A; and P124S, with reference to amino acid positions set forth in SEQ ID NO:5. In some examples, the variant ADA2 protein or catalytically active portion thereof contains 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. In some examples, the variant ADA2 contains modifications selected from among K371D/V99-Q144del→$(GGGGS)_1$; K371D/V99-Q144del→$(GGGGS)_2$; K371D/V99-Q144del→$(GGGGS)_3$; K371D/C105-T147del→$(GGGGS)_1$; K371D/C105-T147del→$(GGGGS)_2$; K371D/C105-T147del→$(GGGGS)_3$; 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→$(GGGGS)_3$; K371D/C105-T147del→(Gly)n, where n = 2 to 20; K371D/C105-T147del→$(Gly)_{15}$; K371D/C105-T147del→$(Gly)_{10}$; K371D/C105-T147del→$(Gly)_7$; K371D/C105-T147del→$(Gly)_5$; K371D/C105-T147del→$(Gly)_3$; K371D/V99-Q144del→(GGGGS)n, where n = 1 to 5; K371D/C105-T147del→(GGGGS)n, where n = 1 to 5; K371D/N98-N156del; K371D/C105-E148del; K371D/C105-T147del; K371D/V99-Q144del; 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;

R219Q/S262N/K371D/V99-Q144del; R219Q/C105-T147del→(Gly)n, where n = 2 to 20; R219Q/V99-Q144del→(GGGGS)n, where n = 1 to 5; R219Q/C105-T147del→(GGGGS)n, where n = 1 to 5; R219Q/N98-N156del; R219Q/C105-E148del; R219Q/C105-T147del; R219Q/V99-Q144del; S262N/C105-T147del→(Gly)n, where n = 2 to 20; S262N/V99-Q144del→(GGGGS)n, where n = 1 to 5; S262N/C105-T147del→(GGGGS)n, where n = 1 to 5; S262N/N98-N156del; and S262N/C105-E148del; S262N/C105-T147del; and S262N/V99-Q144del.

In some examples of a variant ADA2 protein, including examples containing a modified PRB domain, the variant ADA2 protein, when in dimer form, can exhibit reduced binding to any one or more adenosine receptor (ADR) selected from among A_1, A_{2A}, A_{2B} and A_3 compared to binding of the unmodified ADA2 protein to the same receptor when assessed under the same conditions. For example, the variant ADA2 protein has a binding that is reduced at least or at least about 0.5-fold, 1-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more.

In some examples of the variant ADA2 provided herein, the variant ADA2 can be glycosylated, for example, at a native or a non-native glycosylation site. In some examples, the variant ADA2 protein can include a modification(s) that alters glycosylation by introduction of a non-native glycosylation site, whereby, when expressed in a cell capable of glycosylation, the variant ADA2 protein is hyperglycosylated compared to the unmodified ADA2 polypeptide. For example, the non-native glycosylation site is introduced by amino acid replacement(s) or insertion of one, two or three amino acids. For example, the modifications are selected from among modifications corresponding to --→N1/--→A2/--→S3, R20N/V22S, K371N/D373S, K372N/I374S, T403N/H405S and G404N/P406S, with reference to amino acid positions set forth in SEQ ID NO:5. In some examples, the variant ADA2 or catalytically active portion thereof contains 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. In some examples, the variant ADA2 protein or catalytically active portion thereof contains 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. In some examples, the variant ADA2 protein or catalytically active portion thereof contains 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.

In some examples, the variant ADA2 protein can be a human ADA2. In some examples, the variant ADA2 protein can be isolated or purified.

In some examples, the variant ADA2 protein can contain a polypeptide that exhibits at least 65% sequence identity to SEQ ID NO:5 or a catalytically active portion thereof. For example, the variant ADA2 protein can contain a polypeptide that exhibits at least 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:5 or a catalytically active portion thereof. For example, the variant ADA2 protein contains a polypeptide that has the sequence of amino acids set forth in any of SEQ ID NOS:13-63 or 71-285 or a catalytically active portion thereof. In some examples, the variant ADA2 protein or catalytically active portion thereof contains the sequence of amino acids set forth in any of SEQ ID NOS: 551-579 or 581-993 or a catalytically active portion thereof.

In some examples, the variant ADA2 protein or a catalytically active portion thereof can contain amino acid replacements selected from among replacements corresponding to K11A/R20A; K11A/R20A/K371A; R20A/K371A; K11A/K371A; S262N/K371D; S262N/K371E; S262N/R20E; S262N/R20E/K371D; S262N/R20E/K371E; R219Q/K371E; R219Q/K371D; R219Q/R20E; R219Q/K371E/R20E; R219Q/K371D/R20E; R219Q/S262N/K371E; 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/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.

The variant ADA2 protein can be a monomer or a dimer. In particular, among variant ADA2 proteins provided herein are dimers of a variant ADA2 protein, that can include any of the variant ADA2 proteins provided. In some examples, the dimer can be a homodimer. In other examples, the dimer can be a heterodimer.

Provided are nucleic acid molecules encoding the variant ADA2 proteins provided herein. Also provided herein is a vector that includes the nucleic acid encoding any of the variant ADA2 proteins provided herein. The vector can be a eukaryotic or a prokaryotic vector, such as a mammalian vector or a viral vector. For example, the vector is an adenovirus vector, an adeno-associated-virus vector, a retrovirus vector, a herpes virus vector, a lentivirus vector, a poxvirus vector, or a cytomegalovirus vector. In some examples, the vector is an oncolytic vector. In some examples, the vector can also encode a soluble hyaluronidase. Also provided herein are cells containing any of the vectors provided herein. The cell can be a eukaryotic cell, such as a mammalian cell. If human, the cells are isolated or are provided as a cell culture. For example, the cell is a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell. In some examples, the cell can expresses the variant ADA2 protein, such as a dimer. Also provided herein are variant ADA2 proteins, such as variant ADA2 dimers, that are produced by the cell provided herein. In some examples, the cell is an isolated cell or a cell culture, such as a eukaryotic cell, a non-human cell, a mammalian cell, or a cell that is not a human stem cell. In some examples, the cell is an immune cell, such as a T cell, a tumor-infiltrating

lymphocyte (TIL), a cytotoxic T lymphocyte (CTL), a natural killer (NK) cell or a lymphokine-activated killer (LAK) cell. In some examples, the cell is a T cell that encodes and expresses chimeric antigen receptor (CAR) and the variant ADA2 protein or dimer. In some examples, the CAR is specific for a tumor cell antigen, and the tumor antigen is selected from among HER2, CD19, HERV-K, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY- ESO-1 TCR, MAGE A3 TCR and GD2 and combinations thereof.

Provided herein are conjugates include a variant ADA2 protein or a catalytically active portion of any ADA2 protein provided herein, such as a variant ADA2 dimer provided in any examples herein, linked directly or indirectly via a linker to a heterologous moiety, such as a toxin or therapeutic drug.

Also provided herein are conjugates that include an ADA2 protein linked directly or indirectly via a linker to a heterologous moiety. In any of the conjugates, the ADA2 protein can be a monomer or a dimer. In some examples, the dimer is a homodimer; in other it is a heterodimer. In any of the conjugates in the examples herein, the heterologous moiety is conjugated to one or both subunits in the dimer. The heterologous moiety, for example, can be selected from among a peptide, small molecule, nucleic acid, carbohydrate and polymer.

In some examples of the conjugates provided herein, the heterologous moiety is a half-life extending moiety. For example, the half-life extending moiety is selected from among biocompatible fatty acids and derivatives thereof, hydroxy alkyl starch (HAS), polyethylene glycol (PEG), Poly $(Gly_x\text{- }Ser_y)_n$, homo-amino-acid polymers (HAP), hyaluronic acid (HA), heparosan polymers (HEP), phosphorylcholine-based polymers (PC polymer), Fleximers, dextran, polysialic acids (PSA), Fc domain, Transferrin, Albumin, elastin-like peptides, XTEN sequences, albumin binding peptides, a CTP peptide, and any combination thereof.

In some examples, the half-life extending moiety is a PEG and the ADA2 protein is PEGylated. For example, the PEG can be selected from among methoxy-polyethylene glycols (mPEG), PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG), branched PEGs and polyethylene oxide (PEO). In some examples, the PEG has a molecular weight of from or from about 1 kDa to about 100 kDa. The PEG can be linear or branched. In some examples of

conjugates provided herein, the PEG moieties result from reaction with a PEG reagent selected from among mPEG-Succinimidyl Propionates (mPEG-SPA), mPEG Succinimidyl Carboxymethyl Ester (mPEG-SCM), mPEG-Succinimidyl Butanoates (mPEG-SBA), mPEG2-N-Hydroxylsuccinimide, mPEG-succinimidyl butanoate (mPEG-SBA), mPEG-succinimidyl α-methylbutanoate (mPEG-SMB), mPEG-butyrldehyde, PEG-p-nitrophenyl-carbonate and PEG-propionaldehyde. For example, the PEG moieities result from reaction with a PEG reagent selected from among mPEG-SCM (20kDa), mPEG-SCM (30kDa), mPEG-SBA (5kDa), mPEG-SBA (20kDa), mPEG-SBA (30kDa), mPEG-SMB (20kDa), mPEG-SMB (30kDa), mPEG-butyrldehyde (30kDa), mPEG-SPA (20kDa), mPEG-SPA (30kDa), mPEG2-NHS (10kDa branched), mPEG2-NHS (20kDa branched), mPEG2-NHS (40kDa branched), mPEG2-NHS (60kDa branched), PEG-NHS-biotin (5kDa biotinylated), PEG-p-nitrophenyl-carbonate (30kDa) and PEG-propionaldehyde (30kDa).

In examples of the conjugates provided herein, the ADA2 protein can contain the sequence of amino acids set forth in any of SEQ ID NOS:5, 326-334, 340, 375 or 380-383, a sequence that exhibits at least 85% sequence identity to the sequence of amino acids set forth in any of SEQ ID NOS:5, 326-334, 340, 375 or 380-383 or a catalytically form thereof. For example, the ADA2 protein can contain a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in any of SEQ ID NOS:5, 326-334, 340, 375 or 380-383 or a catalytically active portion thereof. For example, the ADA2 protein can contain a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in SEQ ID NOS:5 or a catalytically active portion thereof. In another example, the ADA2 protein can include a polypeptide having the sequence of amino acids set forth in SEQ ID NO:5 or a catalytically active portion thereof.

In examples of conjugates provided herein, the ADA2 protein is a variant ADA2 protein that contains a sequence of amino acids that includes a modification(s) in the sequence of amino acids of an unmodified ADA2 protein or a catalytically active portion thereof, where the unmodified ADA2 protein contains the sequence of

amino acids set forth in SEQ ID NO:5, or a sequence of amino acids that exhibits 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. In any of such examples, the amino acid modification(s) are selected from among amino acid replacement(s), deletion(s) and insertion(s); and the variant ADA2 protein, when in dimer form, can exhibit adenosine deaminase activity to convert adenosine to inosine. In any of the conjugates provided herein, the ADA2 protein, when in dimer form, can exhibit a catalytic efficiency (k_{cat}/K_M) that is at least or at least about 5 x 10^3 M^{-1} s^{-1}, 6 x 10^3 M^{-1} s^{-1}, 7 x 10^3 M^{-1} s^{-1}, 8 x 10^3 M^{-1} s^{-1}, 9 x 10^3 M^{-1} s^{-1}, 1 x 10^4 M^{-1} s^{-1}, 2 x 10^4 M^{-1} s^{-1}, 3 x 10^4 M^{-1} s^{-1}, 4 x 10^4 M^{-1} s^{-1}, 5 x 10^4 M^{-1} s^{-1}, 6 x 10^4 M^{-1} s^{-1}, 7 x 10^4 M^{-1} s^{-1}, 8 x 10^4 M^{-1} s^{-1}, 9 x 10^4 M^{-1} s^{-1}, 1 x 10^5 M^{-1} s^{-1}, 2 x 10^5 M^{-1} s^{-1}, 3 x 10^5 M^{-1} s^{-1}, 4 x 10^5 M^{-1} s^{-1}, 5 x 10^5 M^{-1} s^{-1} or greater.

In any of the examples of conjugates provided herein, the modification(s) of the ADA2 protein can be an amino acid replacement(s); and the variant ADA2 protein can include 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. For example, in some examples of conjugates provided herein, the variant ADA2 protein can include one or more amino acid replacement(s) selected from among K11A, K11D, K11E, K13A, K13D, K13E, R20A, R20D, R20E, R20N, V22S, K26A, K26D, K26E, D86A, D86C, D86E, D86F, D86G, D86H, D86I, D86K, D86L, D86M, D86N, D86P, D86Q, D86R, D86S, D86T, D86V, D86W, D86Y, E179A, E179C, E179D, E179F, E179G, E179H, E179I, E179K, E179L, E179M, E179N, E179P, E179Q, E179R, E179S, E179T, E179V, E179W, E179Y, R217A, R217D, R217E, R219A, R219C, R219D, R219E, R219F, R219G, R219H, R219I, R219K, R219L, R219M, R219N, R219P, R219Q, R219S, R219T, R219V, R219W, R219Y, L221A, L221C, L221D, L221E, L221F, L221G, L221H, L221I, L221K, L221M, L221N, L221P, L221Q, L221R, L221S, L221T, L221V, L221W, L221Y, K258A, K258D, K258E, S262A, S262C, S262D, S262E, S262F, S262G, S262H, S262I, S262K, S262L, S262M, S262N, S262P, S262Q, S262R, S262T, S262V, S262W, S262Y, H264A, H264C, H264D, H264E, H264F, H264G, H264I, H264K,

H264L, H264M, H264N, H264P, H264Q, 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, R277A, R277D, R277E, R283A, R283D, R283E, V296A, V296C, V296D, V296E, V296F, V296G, V296H, V296I, V296K, V296L, V296M, V296N, V296P, V296Q, V296R, V296S, V296T, V296W, V296Y, K309A, K309D, K309E, K317A, K317D, K317E, K321A, K321D, K321E, R352A, R352D, R352E, R366A, R366D, R366E, K371A, K371D, K371E, K371N, K372A, K372D, K372E, K372N, D373S, I374S, T403N, G404N, H405S, P406S, R441A, R441D, R441E, K444A, K444D, K444E, K452A, K452D, K452E, K461A, K461D, K461E, K469A, K469D, K469E, K470A, K470D, K470E, with reference to amino acid positions set forth in SEQ ID NO:5. For example, the variant ADA2 protien can include one or more amino acid replacement(s) selected from among K11A, K11E, R20A, R20D, R20E, R219K, R219Q, L221A, L221V, L221G, S262N, H264Q, H264G, R366A, R366D, R366E, K371A, K371D, K371E, K372A, K372D, K372E, K452D and K452E, with reference to amino acid positions set forth in SEQ ID NO:5. In some examples, the variant ADA2 protein can include amino acid replacements selected from among K11A/R20A, K11A/R20A/K371A, R20A/K371A, K11A/K371A, S262N/K371D, S262N/K371E, S262N/R20E, S262N/R20E/K371D, S262N/R20E/K371E, R219Q/K371E, R219Q/K371D, R219Q/R20E, R219Q/K371E/R20E, R219Q/K371D/R20E, R219Q/S262N/K371E, R219Q/S262N/K371D, R219Q/S262N/R20E, R219Q/S262N/K371E/R20E, R219Q/S262N/K371D/R20E and R219Q/S262N, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of conjugates provided herein, the variant ADA2 protein can include a modification of one or more amino acids in the putative receptor binding (PRB) domain that is an amino acid deletion, insertion or replacement. For example, in some examples of conjugates provided herein, the variant ADA2 protein can include deletion of one or more contiguous amino acid residues corresponding to any one or more contiguous amino acid residues between or between about amino acid residues 98 and 156 or amino acid residues 105 and 148, inclusive, with reference to

amino acid positions set forth in SEQ ID NO:5. In some examples, the variant ADA2 protein in the conjugate can further include substitution of the deleted region with a peptide linker. For example, the peptide linker can be selected from among (Gly)n (SEQ ID NO:368), where n is 2 to 20; (GGGGS)n (SEQ ID NO:343), where n is 1 to 6; (SSSSG)n (SEQ ID NO:344), where n is 1 to 6; (AlaAlaProAla)n (SEQ ID NO:350), where n is 1 to 6; GKSSGSGSESKS (SEQ ID NO:345); GGSTSGSGKSSEGKG (SEQ ID NO:346); GSTSGSGKSSSEGSGSTKG (SEQ ID NO:347); GSTSGSGKPGSGEGSTKG (SEQ ID NO:348); and EGKSSGSGSESKEF (SEQ ID NO:349). In some examples, the peptide linker is selected from among GGG (SEQ ID NO:369); GGGGG (SEQ ID NO:360); GGGGGGG (SEQ ID NO:370); GGGGGGGGGG (SEQ ID NO:371); and GGGGGGGGGGGGGGG (SEQ ID NO:372). For example, the modification in the PRB domain can correspond to C105-T147del→$(Gly)_{n,}$ where n is 2 to 20, such as, C105-T147del→$(Gly)_{15,}$ C105-T148del→$(Gly)_{10,}$ C105-T147del→$(Gly)_7$, C105-T147del→$(Gly)_5$ or C105-T147del→$(Gly)_{3,}$ with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of conjugates provided herein, the ADA2 protein in the conjugate can be glycosylated at one or more native or non-native glycosylation site. For example, in some examples of conjugates provided herein containing a variant ADA2 protein, the variant ADA2 protein in the conjugate can include a modification(s) that alters glycosylation by introduction of a non-native glycosylation site. The non-native glycosylation site can be introduced by creating the canonical glycosylation sequence (NXT/S, where X is not Pro for N-linked carbohydrates, S/T for O-linked) by introducing amino acid replacement(s), insertions or deletions of one, two or three amino acids. For example, the modifications that alter glycosylation are selected from among modifications corresponding to --→N1/--→A2/--→S3, R20N/V22S, K371N/D373S, K372N/I374S, T403N/H405S and G404N/P406S, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of conjugates provided herein, the variant ADA2 protein in the conjugate can have the sequence of amino acid set forth in any of SEQ ID NOS:13-63 or 71-285 or a catalytically active portion thereof.

In some examples of conjugates containing an ADA2 or variant ADA2 protein provided herein, the conjugate retains the adenosine deaminase activity

compared to the ADA2 protein that is not conjugated. For example, the conjugate can exhibit from or from about 50% to 500%, 50% to 200%, 50% to 100%, 50% to 80%, 80% to 500%, 80% to 200%, 80% to 100%, 100% to 500% or 100% to 200%, each inclusive, of the adenosine deaminase activity of the ADA2 protein that is not conjugated, such as at least 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500% or more the adenosine deaminase activity of the ADA2 protein that is not conjugated. In some examples of conjugates provided herein, the ADA2 in the conjugate can exhibit a catalytic efficiency (k_{cat}/K_M) that is at least or at least about 5 x 10^3 M^{-1} s^{-1}, 6 x 10^3 M^{-1} s^{-1}, 7 x 10^3 M^{-1} s^{-1}, 8 x 10^3 M^{-1} s^{-1}, 9 x 10^3 M^{-1} s^{-1}, 1 x 10^4 M^{-1} s^{-1}, 2 x 10^4 M^{-1} s^{-1}, 3 x 10^4 M^{-1} s^{-1}, 4 x 10^4 M^{-1} s^{-1}, 5 x 10^4 M^{-1} s^{-1}, 6 x 10^4 M^{-1} s^{-1}, 7 x 10^4 M^{-1} s^{-1}, 8 x 10^4 M^{-1} s^{-1}, 9 x 10^4 M^{-1} s^{-1}, 1 x 10^5 M^{-1} s^{-1}, 2 x 10^5 M^{-1} s^{-1}, 3 x 10^5 M^{-1} s^{-1}, 4 x 10^5 M^{-1} s^{-1}, 5 x 10^5 M^{-1} s^{-1} or greater.

Provided herein are combinations containing any of the variant ADA2 proteins or a catalytically active portion thereof provided herein, any variant ADA2 dimer provided herein or any conjugate of any of the examples provided herein, and a therapeutic agent. Also provided herein are combinations containing any ADA2 protein; and a therapeutic agent. In any examples of the combination provided herein, the ADA2 protein can be a monomer or a dimer. For example, the ADA2 protein can be a dimer, such as a homodimer.

In some examples of combinations provided herein, the therapeutic agent can be selected from among an antibody, cytotoxic agent, chemotherapeutic agents, cytokine, growth inhibitory agent, anti-hormonal agent, kinase inhibitor, anti-angiogenic agent, cardioprotectant, immunostimulatory agent, immunosuppressive agent, immune checkpoint inhibitor, antibiotic and angiogenesis inhibitor. For example, the therapeutic agent can be an anti-cancer agent. In some examples of combinations provided herein, the anti-cancer agent can be an anti-cancer antibody, a chemotherapeutic agent, a radioimmunotherapeutic, an anti-angiogenic agent or an immune checkpoint inhibitor.

For example, the anti-cancer agent can be an immune checkpoint inhibitor; and the target of the immune checkpoint inhibitor can be selected from among CTLA4, PD-1, and PD-L1.In some examples of combinations provided herein,

the immune checkpoint inhibitor can be an antibody, a fusion protein, an aptamer, or an immune checkpoint protein-binding fragment thereof. For example, the immune checkpoint inhibitor is an anti-immune checkpoint protein antibody or antigen-binding fragment thereof. In particular examples, the immune checkpoint inhibitor is selected from among: an anti-CTLA4 antibody, derivative thereof, or antigen-binding fragment thereof; an anti-PD-L1 antibody, derivative thereof, or antigen-binding fragment thereof; and an anti-PD-1 antibody, derivative thereof, or antigen-binding fragment thereof. For example, the immune checkpoint inhibitor can be selected from among: Ipilimumab, a derivative thereof, or an antigen-binding fragment thereof; Tremelimumab, a derivative thereof, or an antigen-binding fragment thereof; Nivolumab, a derivative thereof, or an antigen-binding fragment thereof; and Pidilizumab, a derivative thereof, or an antigen-binding fragment thereof.

In some examples of combinations provided herein, the therapeutic agent can be an anti-hyaluronan agent. For example, the anti-hyaluronan agent can be a soluble hyaluronidase. In some examples of combinations provided herein, the soluble hyaluronidase can exhibit hyaluronidase activity at neutral pH. In particular, the soluble hyaluronidase can be selected from among bovine, ovine or a C-terminal truncated human PH20 that lacks all or a portion of the glycosylphosphatidylinositol (GPI) anchor attachment sequence. For example, the soluble hyaluronidase is a C-terminally truncated human PH20 that lacks all or a portion of the GPI anchor attachment sequence, such as those set forth in any of SEQ ID NOS:481-488, 493-514, or 526-532, or that has a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to a sequence of amino acids set forth in any of SEQ ID NOS:481-488, 493-514, or 526-532 and is soluble and retains hyaluronidase activity. In some examples of combinations provided herein, the anti-hyaluronan agent or soluble hyaluronidase can be conjugated to a polymer, such as a PEG moiety.

In some examples of combinations provided herein, the ADA2 protein can include a polypeptide having the sequence of amino acids set forth in any of SEQ ID NOS:5, 326-334, 340, 375 or 380-383, a sequence that can exhibit at least 85% sequence identity to the sequence of amino acids set forth in SEQ ID NOS:5, 326-334, 340, 375 or 380-383 or a catalytically active form thereof. For example, the ADA2

protein can include a protein having a sequence of amino acids that can exhibit at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in SEQ ID NOS:5, 326-334, 340, 375 or 380-383 or a catalytically active portion thereof. In particular examples, the ADA2 protein can contain a sequence of amino acids that can exhibit at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in SEQ ID NO:5. For example, the ADA2 protein can contain the sequence of amino acids set forth in SEQ ID NO:5.

In some examples of combinations provided herein, the ADA2 protein is a variant ADA2 protein having a sequence of amino acids that includes a modification(s) in the sequence of amino acids of an unmodified ADA2 polypeptide or a catalytically active portion thereof. In any of such examples, 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 protein, when in dimer form, can exhibit adenosine deaminase activity to convert adenosine to inosine.

In some examples of combinations provided herein, the ADA2 protein, when in dimer form, can exhibit a catalytic efficiency (k_{cat}/K_M) that is at least or at least about 5 x 10^3 M^{-1} s^{-1}, 6 x 10^3 M^{-1} s^{-1}, 7 x 10^3 M^{-1} s^{-1}, 8 x 10^3 M^{-1} s^{-1}, 9 x 10^3 M^{-1} s^{-1}, 1 x 10^4 M^{-1} s^{-1}, 2 x 10^4 M^{-1} s^{-1}, 3 x 10^4 M^{-1} s^{-1}, 4 x 10^4 M^{-1} s^{-1}, 5 x 10^4 M^{-1} s^{-1}, 6 x 10^4 M^{-1} s^{-1}, 7 x 10^4 M^{-1} s^{-1}, 8 x 10^4 M^{-1} s^{-1}, 9 x 10^4 M^{-1} s^{-1}, 1 x 10^5 M^{-1} s^{-1}, 2 x 10^5 M^{-1} s^{-1}, 3 x 10^5 M^{-1} s^{-1}, 4 x 10^5 M^{-1} s^{-1}, 5 x 10^5 M^{-1} s^{-1} or greater.

In some examples of combinations provided herein, the modification(s) in the sequence of amino acids of an unmodified ADA2 protein can include an amino acid replacement(s); and the variant ADA2 protein can include 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. For example,

replacements selected from among K11A/R20A, K11A/R20A/K371A, R20A/K371A, K11A/K371A, S262N/K371D, S262N/K371E, S262N/R20E, S262N/R20E/K371D, S262N/R20E/K371E, R219Q/K371E, R219Q/K371D, R219Q/R20E, R219Q/K371E/R20E, R219Q/K371D/R20E, R219Q/S262N/K371E, R219Q/S262N/K371D, R219Q/S262N/R20E, R219Q/S262N/K371E/R20E, R219Q/S262N/K371D/R20E and R219Q/S262N, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of combinations provided herein, the variant ADA2 protein can include a modification of one or more amino acids in the putative receptor binding (PRB) domain, wherein the modification is an amino acid deletion, insertion or replacement. For example, the variant ADA2 can include deletion of one or more contiguous amino acid residues corresponding to any one or more contiguous amino acid residues between or between about amino acid residues 98 and 156 or amino acid residues 105 and 148, inclusive, with reference to amino acid positions set forth in SEQ ID NO:5. In some examples, the variant of ADA2 polypeptide can further include substitution of the deleted region with a peptide linker. For example, the peptide linker can be selected from among (Gly)n (SEQ ID NO:368), where n is 2 to 20; (GGGGS)n (SEQ ID NO:343), where n is 1 to 6; (SSSSG)n (SEQ ID NO:344), where n is 1 to 6; (AlaAlaProAla)n (SEQ ID NO:350), where n is 1 to 6; GKSSGSGSESKS (SEQ ID NO:345); GGSTSGSGKSSEGKG (SEQ ID NO:346); GSTSGSGKSSSEGSGSTKG (SEQ ID NO:347); GSTSGSGKPGSGEGSTKG (SEQ ID NO:348); and EGKSSGSGSESKEF (SEQ ID NO:349). In particular examples, the peptide linker is selected from among GGG (SEQ ID NO:369); GGGGG (SEQ ID NO:360); GGGGGGG (SEQ ID NO:370); GGGGGGGGGG (SEQ ID NO:371); and GGGGGGGGGGGGGGG (SEQ ID NO:372). In some examples of combinations provided herein, the modification in the PRB domain of the variant ADA2 polypeptide corresponds to C105-T147del→$(Gly)_{n,}$ where n is 2 to 20, such as C105-T147del→$(Gly)_{15,}$ C105-T147del→$(Gly)_{10,}$ C105-T147del→$(Gly)_7$, C105-T147del→$(Gly)_5$ or C105-T147del→$(Gly)_{3,}$ with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of combinations provided herein, the ADA2 protein in the combination can be glycosylated at one or more native or non-native glycosylation

site. For example, in some examples of combinations provided herein containing a variant ADA2 protein, the variant ADA2 protein in the combination include a modification(s) that alters glycosylation by introduction of a non-native glycosylation site. The non-native glycosylation site is introduced by amino acid replacement(s) or insertion of one, two or three amino acids. For example, the modifications that alter hyperglycosylation can be selected from among modifications corresponding to --→N1/--→A2/--→S3, R20N/V22S, K371N/D373S, K372N/I374S, T403N/H405S and G404N/P406S, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of combinations provided herein, the variant ADA2 polypeptide has the sequence of amino acids set forth in any of SEQ ID NOS:13-63 or 71-285 or a catalytically active portion thereof.

Provided herein are pharmaceutical compositions that can include any of the variant ADA2 proteins or a catalytically active portion thereof provided herein, any variant ADA2 dimer provided herein or any conjugate provided herein, in a pharmaceutically acceptable vehicle. In some examples, the pharmaceutical composition can be formulated for local or systemic administration. For example, the pharmaceutical composition is formulated for intravenous administration.

Provided herein are methods of treating a tumor or cancer in a subject, that can include administering to the subject any of the variant ADA2 proteins or a catalytically active portion thereof provided herein, any variant ADA2 dimer provided herein, any conjugate provided herein, or any combination provided herein. Also provided are medical uses or pharmaceutical compositions for use of any of the variant ADA2 proteins or a catalytically active portion thereof provided herein, any variant ADA2 dimer provided herein or any conjugate provided herein for treating a tumor or a cancer in a subject. Also provided are combinations for use of any of the combinations provided herein for use in treating a tumor or cancer.

Also provided herein are methods of treating a tumor or cancer in a subject that can include administering to the subject any ADA2 protein. Also provided are medical uses of an ADA2 protein or pharmaceutical composition for use containing an ADA2 protein for treating a tumor or a cancer. Also provided are combinations for use containing an ADA2 protein and a therapeutic agent for treating a tumor or cancer.

In some examples of methods, uses, pharmaceutical compositions for use or uses provided herein, the tumor can be a solid tumor or a metastatic tumor. In particular examples, the tumor can be a carcinoma, gliomas, sarcoma, adenocarcinoma, adenosarcoma, or adenoma. In some examples, the tumor can be a tumor of the breast, heart, lung, small intestine, colon, spleen, kidney, bladder, head and neck, ovary, prostate, brain, pancreas, skin, bone, bone marrow, blood, thymus, uterus, testicles, cervix or liver.

In some examples of methods provided herein, the subject can be selected for treatment based on elevated levels of plasma adenosine, tumor-associated expression of adenosine receptor (ADR) or tumor-associated expression of a nucleotidase. In particular examples, the ADR is A2A or A2B. In particular examples, the nucleotidase is CD39 or CD73. In some examples of methods provided herein, the elevated level is at least 0.5-fold, 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold, 500-fold, 1000-fold or more, compared to the predetermined level or predetermined amount or control sample.

In some examples of methods provided herein, the method of treating a tumor or cancer in a subject can further include administration of one or more anticancer agents or treatments. For example, the anti-cancer agent can be selected from among an anti-cancer antibody, a chemotherapeutic agent, a radioimmunotherapeutic, an anti-angiogenic agent and an immune checkpoint inhibitor.

Provided herein are methods of treating a disease or condition in a subject, that can include administering to the subject any of the variant ADA2 proteins or a catalytically active portion thereof provided herein, any variant ADA2 dimer provided herein, any conjugate provided herein, or any combination provided herein for treating a disease or condition that is a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy or Severe Combined Immunodeficiency (SCID). Also provided are medical uses or pharmaceutical compositions for use of any of the variant ADA2 proteins or a catalytically active portion thereof provided herein, any variant ADA2 dimer provided herein or any

conjugate provided herein for treating a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy or Severe Combined Immunodeficiency (SCID) in a subject. Also provided are combinations for use of any of the combinations provided herein for use in treating a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy or Severe Combined Immunodeficiency (SCID). Also provided herein are methods of treating a disease or condition in a subject, that can include administering to the subject any ADA2 protein, for treating a disease or condition that is a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy or Severe Combined Immunodeficiency (SCID). Also provided are medical uses of an ADA2 protein or pharmaceutical compositions for use containing an ADA2 protein for treating a disease or condition that is a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy or Severe Combined Immunodeficiency (SCID). Also provided are combinations for use containing an ADA2 protein and a therapeutic agent for treating a disease or condition that is a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy or Severe Combined Immunodeficiency (SCID)

In some examples of methods, uses, pharmaceutical compositions for use or uses provided herein, the ADA2 protein can be a monomer or a dimer. For example, the ADA2 protein can be a dimer, in particular, a homodimer. In some examples of methods, uses, pharmaceutical compositions for use or combinations for use provided herein, the ADA2 protein can contain the sequence of amino acids set forth in any of SEQ ID NOS:5, 326-334, 340, 375 or 380-383, a sequence that can exhibit at least 85% sequence identity to the sequence of amino acids set forth in SEQ ID NOS:5, 326-334, 340, 375 or 380-383 or a catalytically active form thereof. For example, the ADA2 protein can contain a sequence of amino acids that can exhibit at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in SEQ ID NOS:5, 326-334, 340, 375 or 380-383 or a catalytically active portion thereof. In particular examples, the ADA2 protein can contain a sequence of amino acids that can exhibit at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence of amino acids set forth in SEQ ID

NOS:5 or a catalytically active portion thereof. For example, the ADA2 protein can contain the sequence of amino acids set forth in SEQ ID NO:5.

In some examples of methods, uses, pharmaceutical compositions for use, or combinations for use provided herein, the ADA2 protein is a variant ADA2 protein that includes modification(s) in the sequence of amino acids of an unmodified ADA2 polypeptide or a catalytically active portion thereof. In any of such examples, 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 protein, when in dimer form, can exhibit adenosine deaminase activity to convert adenosine to inosine.

In some examples of methods, uses, pharmaceutical compositions for use or combinations for use provided herein, the ADA2 protein, when in dimer form, can exhibit a catalytic efficiency (k_{cat}/K_M) that is at least or at least about 5 x 10^3 M^{-1} s^{-1}, 6 x 10^3 M^{-1} s^{-1}, 7 x 10^3 M^{-1} s^{-1}, 8 x 10^3 M^{-1} s^{-1}, 9 x 10^3 M^{-1} s^{-1}, 1 x 10^4 M^{-1} s^{-1}, 2 x 10^4 M^{-1} s^{-1}, 3 x 10^4 M^{-1} s^{-1}, 4 x 10^4 M^{-1} s^{-1}, 5 x 10^4 M^{-1} s^{-1}, 6 x 10^4 M^{-1} s^{-1}, 7 x 10^4 M^{-1} s^{-1}, 8 x 10^4 M^{-1} s^{-1}, 9 x 10^4 M^{-1} s^{-1}, 1 x 10^5 M^{-1} s^{-1}, 2 x 10^5 M^{-1} s^{-1}, 3 x 10^5 M^{-1} s^{-1}, 4 x 10^5 M^{-1} s^{-1}, 5 x 10^5 M^{-1} s^{-1} or greater.

In some examples of methods, uses, pharmaceutical compositions for use, or combinations for use provided herein, the modification(s) in the sequence of amino acids of an unmodified ADA2 polypeptide can include an amino acid replacement(s); and the variant ADA2 protein can include 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. For example, the variant ADA2 protein can include one or more amino acid replacement selected from among K11A, K11D, K11E, K13A, K13D, K13E, R20A, R20D, R20E, R20N, V22S, K26A, K26D, K26E, D86A, D86C, D86E, D86F, D86G, D86H, D86I, D86K, D86L, D86M, D86N, D86P, D86Q, D86R, D86S, D86T, D86V, D86W, D86Y, E179A, E179C, E179D, E179F, E179G, E179H, E179I, E179K, E179L, E179M, E179N, E179P, E179Q,

E179R, E179S, E179T, E179V, E179W, E179Y, R217A, R217D, R217E, R219A, R219C, R219D, R219E, R219F, R219G, R219H, R219I, R219K, R219L, R219M, R219N, R219P, R219Q, R219S, R219T, R219V, R219W, R219Y, L221A, L221C, L221D, L221E, L221F, L221G, L221H, L221I, L221K, L221M, L221N, L221P, L221Q, L221R, L221S, L221T, L221V, L221W, L221Y, K258A, K258D, K258E, S262A, S262C, S262D, S262E, S262F, S262G, S262H, S262I, S262K, S262L, S262M, S262N, S262P, S262Q, S262R, S262T, S262V, S262W, S262Y, H264A, H264C, H264D, H264E, H264F, H264G, H264I, H264K, H264L, H264M, H264N, H264P, H264Q, H264R, H654S, 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, R277A, R277D, R277E, R283A, R283D, R283E, V296A, V296C, V296D, V296E, V296F, V296G, V296H, V296I, V296K, V296L, V296M, V296N, V296P, V296Q, V296R, V296S, V296T, V296W, V296Y, K309A, K309D, K309E, K317A, K317D, K317E, K321A, K321D, K321E, R352A, R352D, R352E, R366A, R366D, R366E, K371A, K371D, K371E, K371N, K372A, K372D, K372E, K372N, D373S, I374S, T403N, G404N, H405S, P406S, R441A, R441D, R441E, K444A, K444D, K444E, K452A, K452D, K452E, K461A, K461D, K461E, K469A, K469D, K469E, K470A, K470D, and K470E, with reference to amino acid positions set forth in SEQ ID NO:5.

In particular examples, the variant ADA2 protein can include one or more amino acid replacement(s) selected from among K11A, K11E, R20A, R20D, R20E, R219K, R219Q, L221A, L221V, L221G, S262N, H264Q, H264G, R366A, R366D, R366E, K371A, K371D, K371E, K372A, K372D, K372E, K452D and K452E, with reference to amino acid positions set forth in SEQ ID NO:5. In some examples of methods, uses, pharmaceutical compositions for use or combinations for use provided herein, the variant ADA2 protein can include amino acid replacements selected from among K11A/R20A, K11A/R20A/K371A, R20A/K371A, K11A/K371A, S262N/K371D, S262N/K371E, S262N/R20E, S262N/R20E/K371D, S262N/R20E/K371E, R219Q/K371E, R219Q/K371D, R219Q/R20E, R219Q/K371E/R20E, R219Q/K371D/R20E, R219Q/S262N/K371E,

R219Q/S262N/K371D, R219Q/S262N/R20E, R219Q/S262N/K371E/R20E, R219Q/S262N/K371D/R20E and R219Q/S262N, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of methods, uses, pharmaceutical compositions for use or combinations for use provided herein, the variant ADA2 protein can include a modification of one or more amino acids in the putative receptor binding (PRB) domain, such as an amino acid deletion, insertion or replacement. For example, the variant ADA2 can include deletion of one or more contiguous amino acid residues corresponding to any one or more contiguous amino acid residues between or between about amino acid residues 98 and 156 or amino acid residues 105 and 148, inclusive, with reference to amino acid positions set forth in SEQ ID NO:5. In any of such examples of methods, uses, pharmaceutical compositions for use or combinations for use provided herein, the variant of ADA2 protein can further include substitution of the deleted region with a peptide linker. For example, the peptide linker is selected from among (Gly)n (SEQ ID NO:368), where n is 2 to 20; (GGGGS)n (SEQ ID NO:343), where n is 1 to 6; (SSSSG)n (SEQ ID NO:344), where n is 1 to 6; (AlaAlaProAla)n (SEQ ID NO:350), where n is 1 to 6; GKSSGSGSESKS (SEQ ID NO:345); GGSTSGSGKSSEGKG (SEQ ID NO:346); GSTSGSGKSSSEGSGSTKG (SEQ ID NO:347); GSTSGSGKPGSGEGSTKG (SEQ ID NO:348); and EGKSSGSGSESKEF (SEQ ID NO:349). In particular examples the peptide linker is selected from among GGG (SEQ ID NO:369); GGGGG (SEQ ID NO:360); GGGGGGG (SEQ ID NO:370); GGGGGGGGGG (SEQ ID NO:371); and GGGGGGGGGGGGGGG (SEQ ID NO:372).

For example, the modification in the PRB domain of the ADA2 protein corresponds to C105-T147del→$(Gly)_{n,}$ where n is 2 to 20, such as C105-T147del→$(Gly)_{15,}$ C105-T147del→$(Gly)_{10,}$ C105-T147del→$(Gly)_7$, C105-T147del→$(Gly)_5$ or C105-T147del→$(Gly)_{3,}$ with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of methods, uses, pharmaceutical compositions for use or combinations for use provided herein, the ADA2 protein can be glycosylated at one or more native or non-native glycosylation sites. For example, in some examples provided herein containing a variant ADA2 protein, the variant ADA2 protein includes a modification(s) that alters glycosylation by introduction of a non-native

glycosylation site. For example, the non-native glycosylation site is introduced by amino acid replacement(s) or insertion of one, two or three amino acids. In particular examples, the modifications that alter glycosylation are selected from among modifications corresponding to --→N1/--→A2/--→S3, R20N/V22S, K371N/D373S, K372N/I374S, T403N/H405S and G404N/P406S, with reference to amino acid positions set forth in SEQ ID NO:5.

In some examples of methods, uses, pharmaceutical compositions for use or combinations for use provided herein, the variant ADA2 can include a polypeptide that has the sequence of amino acids set forth in any of SEQ ID NOS:13-63 or 71-285 or a catalytically active portion thereof.

In some examples of methods, uses, pharmaceutical compositions for use or uses provided herein, the subject can be a mammal, in particular a human. In some examples of methods provided herein, the pharmaceutical composition can be administered parenterally, locally, or systemically. For example, the pharmaceutical composition can be administered intranasally, intramuscularly, intradermally, intraperitoneally, intravenously, subcutaneously, orally, or by pulmonary administration.

In some examples, in the variant ADA2 protein or catalytically active portion thereof provided herein, the variant ADA2 protein in the methods, compositions, conjugates, modified forms, vectors, cells, combinations, uses and compositions for use, and the nucleic acids encoding the variant ADA2 provided herein and vectors that include the nucleic acids, the modifications can be from any one or more of the following amino acid replacement(s), insertion(s), deletion(s), and any combination thereof. The modification(s) listed below are with reference to mature numbering, as set forth in the amino acid positions set forth in SEQ ID NO:5.

Exemplary of ADA2 variants provided herein are the following; it is understood that the different types of mutants (amino acid modifications) can be combined to exploit the properties of each type of mutation. It is understood by those of skill in the art that, in general, the effects of mutations in proteins are at least additive, and can be synergistic.

DETAILED DESCRIPTION

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:

i) the variant ADA2 protein, or catalytically active portion thereof, when in dimer form, exhibits reduced heparin binding 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;

ii) modifications that confer reduced heparin binding are selected from among R20A, R20D, R20E, R366A, R366D, R366E, K371A, K371D, K371E, K372A, K372D, K372E, K452E, K11A/R20A, K11A/R20A/K371A, R20A/K371A, K11A/K371A, with reference to amino acid positions set forth in SEQ ID NO:5;

iii) modifications comprise insertions, deletions, and replacements of amino acid residues;

iv) the variant ADA2 protein or catalytically active portion thereof has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 10 amino acid modifications in the unmodified ADA2 protein;

v) the unmodified ADA2 protein and catalytically active portion is selected from the group consisting of:

a) a sequence of amino acids selected from the group consisting of SEQ ID NOS: 5 and 380-383;

b) a sequence of amino acids selected from the group consisting of SEQ ID NOS: 5 and 380-383 with all or a part of the PRB domain deleted, wherein the PRB domain consists of residues corresponding to residues 98-156 of SEQ ID NO:5;

c) a sequence of amino acids selected from the group consisting of SEQ ID NOS: 5 and 380-383 with all or a part of the PRB domain deleted and a linker in place of deleted residues, wherein:

the PRB domain consists of residues corresponding to residues98-156 of SEQ ID NO:5; and

the linker is (GGGGS)n, where n = 1 to 5, or is (Gly)n, where n is 2-20;

d) a sequence of amino acids selected from the group consisting of the catalytic domain of the polypeptides of SEQ ID NOS: 5 and 380-383, wherein the catalytic domain consists of residues corresponding to residues 77-473 of the sequence of amino acids set forth in SEQ ID NO:5;

e) a sequence of amino acids selected from the group consisting of the catalytic domain of SEQ ID NOS: 5 and 380-383 with all or a part of the PRB domain deleted, wherein:
the catalytic domain consists of residues corresponding to residues 77-473 of the sequence of amino acids set forth in SEQ ID NO:5; and
the PRB domain consists of residues corresponding to residues 98-156 of SEQ ID NO:5; and

f) a sequence of amino acids selected from the group consisting of the catalytic domain of SEQ ID NOS: 5 and 380-383 with all or a part of the PRB domain deleted, and a linker in place of deleted residues, wherein:
the catalytic domain consists of residues corresponding to residues 77-473 of the sequence of amino acids set forth in SEQ ID NO:5;
the PRB domain consists of residues corresponding to residues 98-156 of SEQ ID NO:5; and
the linker is (GGGGS)n, where n = 1 to 5, or is (Gly)n, where n is 2-20; and

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

2. The variant ADA2 protein or catalytically active portion thereof of claim 1, further comprising amino acid modifications that confer, to the dimer form, one or more properties selected from among longer serum half-life, altered pH optimum, increased thermal stability, altered receptor binding, and addition of one or more glycosylation sites, compared to the corresponding dimer form of the unmodified ADA2 protein of SEQ ID NO:5 or dimer form of the corresponding catalytically active portion thereof, wherein:
replacements that confer longer serum half-life are selected from among R20E and K371D, with reference to amino acid positions set forth in SEQ ID NO:5;

replacements that confer altered pH optimum are selected from among R20E, K371D, and K371E, with reference to amino acid positions set forth in SEQ ID NO:5;

the replacement that confers increased thermal stability is K371E, with reference to amino acid positions set forth in SEQ ID NO:5;

replacements that confer altered receptor binding are selected from among F119S, F119K, Y224R, Y224N, Y191S, Y191D, F183K, Y191D/Y224R, F109S, F109A, R118D, R118A, Y139T, Y139A, W133S, W133T, P124A, and P124S, with reference to amino acid positions set forth in SEQ ID NO:5; and

amino acid modifications that result in one or more additional glycosylation sites are selected from among --→N1/--→A2/--→S3, R20N/V22S, K371N/D373S, K372N/I374S, T403N/H405S, G404N/P406S, R125N/P126A, S127N/K129S, P126N/E128T, R112N/I114T, I134N/L135C/L136T, I134N/L135S/L136T, R142N/Q144S, E137N/Y139T, and P111N/G113S, 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 unmodified ADA2 protein or catalytically active portion thereof has a deletion of all or a portion of the PRB domain.

4. The variant ADA2 protein or catalytically active portion thereof of claim 3, wherein:

the unmodified ADA2 protein or catalytically active portion thereof contains a linker in place of the deleted portion of the PRB domain; and

the linker is (GGGGS)n, where n = 1 to 5, or is (Gly)n, where n is 2-20.

5. The variant ADA2 protein or the catalytically active portion thereof of any of claims 1-4 wherein the variant ADA2 protein or the catalytically active portion thereof, when in dimer form, exhibits increased adenosine deaminase activity and reduced heparin binding compared to the dimer form of the unmodified ADA2 protein of SEQ ID NO:5 or the dimer form of the catalytically active portion thereof.

6. The variant ADA2 protein or the catalytically active portion thereof of any of claims 1-5, wherein the variant ADA2 protein or catalytically active portion thereof comprises one or more amino acid replacement(s) selected from the group consisting of R20A, R20D, R20E, R366A, R366D, R366E, K371A, K371D, K371E,

K372A, K372D, K372E and K452E, with reference to amino acid positions set forth in SEQ ID NO:5.

7. The variant ADA2 protein or catalytically active portion thereof of any of claims 1-6, wherein the variant ADA2 protein or catalytically active portion thereof lacks residues N98-N156, C105-E148, C105-T147, or V99-Q144, and optionally, includes a linker in place thereof, wherein the linker is (GGGGS)n, where n = 1 to 5, or is (Gly)n, where n is 2-20.

8. The variant ADA2 protein or catalytically active portion thereof of any of claims 1-5, comprising the sequence of amino acids set forth in any of SEQ ID NOS: 19-24, 27-33, 55-58, 589-594, and 646-657.

9. The variant ADA2 protein or catalytically active portion thereof of any of claims 2-8, wherein, when in dimer form, the variant ADA2 protein or catalytically active portion thereof exhibits one or more properties selected from among longer serum half-life, altered pH optimum, increased thermal stability, altered receptor binding, and addition of one or more glycosylation sites, and has increased adenosine deaminase activity and reduced heparin binding, compared to the dimer form of the unmodified ADA2 protein of SEQ ID NO:5 or dimer form of the catalytically active portion thereof.

10. The variant ADA2 protein or catalytically active portion thereof of any of claims 1-9, wherein the unmodified ADA2 protein is a homodimer, and the monomer form of the unmodified ADA2 protein comprises the sequence of amino acid residues set forth in SEQ ID NO:5.

11. The catalytically active portion of the variant ADA2 protein of any of claims 1-10, wherein the unmodified ADA2 protein is a homodimer of the corresponding catalytically active portions of the polypeptide whose sequence is set forth in of SEQ ID NO:5.

12. The variant ADA2 protein or the catalytically active portion thereof of any of claims 1-11, wherein the unmodified ADA2 protein comprises the sequence of amino acids selected from the group consisting of SEQ ID NOS: 5 and 380-383, or is the catalytically active portion thereof.

13. The variant ADA2 protein or the catalytically active portion thereof of any of claims 1-12, wherein the unmodified ADA2 protein consists of the sequence of

amino acids selected from the group consisting of SEQ ID NOS: 5 and 380-383 or is the catalytically active portion thereof.

14. The variant ADA2 protein or the catalytically active portion thereof of any of claims 1-13, wherein the unmodified ADA2 protein comprises the sequence of amino acids set forth in SEQ ID NO: 5 or is the catalytically active portion thereof.

15. The variant ADA2 protein or the catalytically active portion thereof of any of claims 1-14, wherein the variant ADA2 protein or the catalytically active portion thereof is PEGylated.

16. The variant ADA2 protein or the catalytically active portion thereof of any of claims 1-15, wherein the catalytically active portion comprises the ADA domain that corresponds to residues 77-473 of the sequence of amino acids set forth in SEQ ID NO:5.

17. A variant ADA2 multimer, comprising a plurality of variant ADA2 proteins or the catalytically active portion thereof of any of claims 1-16, wherein the variant ADA2 proteins or catalytically active portion thereof are the same or different.

18. A variant ADA2 dimer, comprising the variant ADA2 protein or the catalytically active portion thereof of any of claims 1-16.

19. A conjugate, comprising the variant ADA2 protein or the catalytically active portion thereof of any of claims 1-16, or the multimer of claim 17, or the dimer of claim 18, linked directly or indirectly via a linker to a heterologous moiety.

20. A nucleic acid molecule encoding the variant ADA2 protein or the catalytically active portion thereof of any of claims 1-16, or the variant ADA2 multimer of claim 17, or the variant ADA2 dimer of claim 18.

21. A vector, comprising the nucleic acid molecule of claim 20.

22. An isolated cell or a cell culture, comprising the vector of claim 21.

23. The cell or cell culture of claim 22, wherein the cell is a tumor-infiltrating lymphocyte (TIL), a cytotoxic T lymphocyte (CTL), a natural killer (NK) cell, or a lymphokine-activated killer (LAK) cell.

24. The cell or cell culture of claim 23, wherein the cell is a T cell that encodes and expresses:

a chimeric antigen receptor (CAR) that is specific for a tumor cell antigen; and

the variant ADA2 protein or the catalytically active portion thereof, or the variant ADA2 multimer, or the variant ADA2 dimer.

25. A method of producing a variant ADA2 protein or the catalytically active portion thereof, comprising culturing or expanding the cell of any of claims 22-24, under conditions whereby the variant ADA2 protein or the catalytically active portion thereof is expressed.

26. The method of claim 25, wherein the variant ADA2 protein is a dimer.

27. The method of claim 25 or claim 26, comprising expanding the cells to produce expanded cells.

28. The expanded cells produced by the method of claim 27.

29. Use of the expanded cells of claim 28 in the manufacture of a medicament for treating a tumor.

30. The expanded cells of claim 28 for use in therapy.

31. A pharmaceutical composition, comprising the variant ADA2 protein or the catalytically active portion thereof, or the dimer or the multimer of the variant ADA2 protein or the catalytically active portion thereof of any of claims 1-18, or the conjugate of claim 19, in a pharmaceutically acceptable vehicle.

32. The pharmaceutical composition of claim 31, wherein the variant ADA2 protein or the catalytically active portion thereof is PEGylated.

33. The pharmaceutical composition of claim 31 or claim 32, wherein the variant ADA2 protein comprises the sequence of amino acid residues set forth in any of SEQ ID NOs: 19-24, 27-33, 55-58, 589-594, and 646-657, or the catalytically active portion thereof.

34. Use of the variant ADA2 protein or the catalytically active portion thereof, or the dimer or the multimer of the variant ADA2 protein or the catalytically active portion thereof of any of claims 1-18, or the conjugate of claim 19, in the manufacture of a medicament for treating a tumor, a cancer, a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy, or an immunodeficiency disease.

35. The variant ADA2 protein or the catalytically active portion thereof, or the dimer or the multimer of the variant ADA2 protein or the catalytically active portion thereof of any of claims 1-18, or the conjugate of claim 19, for use in therapy.

36. The variant ADA2 protein or the catalytically active portion thereof, or the dimer or the multimer of the variant ADA2 protein or the catalytically active portion thereof, or the conjugate of claim 34 to 35, wherein the variant ADA2 protein or the catalytically active portion thereof is PEGylated.

37. Use of the pharmaceutical composition of any of claims 31-33 in the manufacture of a medicament for treatinga tumor, a cancer, a non-cancer hyperproliferative disease, a fibrotic disease, an infectious disease, a vasculopathy, or an immunodeficiency disease in a subject.

38. The pharmaceutical composition of any of claims 31-33 for use in therapy.

39. The pharmaceutical composition of claims 37 to 38, wherein the variant ADA2 protein or the catalytically active portion thereof is PEGylated.