Claims

1. A recombinant expression system that comprises
   a. a second nucleic acid construct comprising a sequence encoding at least one immunomodulatory gene or protein, said sequence is operatively linked to a STING-responsive promoter or a radiation inducible promoter.

2. The recombinant expression system of claim 1, wherein the at least one immunomodulatory gene or protein encoded by the sequence of the first nucleic acid construct is selected from the group consisting of interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-7, interleukin-8, interleukin-9, interleukin-11, single-chain interleukin-12, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-15-sushi, interleukin-16, interleukin-17, interleukin-18, interleukin-19, interleukin-20, interleukin-21, interleukin-22, interleukin-23, CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9, CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, CX3CL1, granulocyte-macrophage colony-stimulating factor, type I interferons, IFN-γ, TNF-α, FLT3-ligand, a blocking peptide targeting TGF-β, a blocking peptide targeting IL-10, a blocking peptide targeting FoxP3, a monoclonal antibody or single-chain variable fragment (scFv) or nanobody neutralizing PD1, PDL1, CTLA4, CD137, TIM3, LAG3, and a fragment or variant thereof;
   or
   a shRNA targeting TGF-β, a shRNA targeting IL-10, and a shRNA targeting FoxP3.

3. The recombinant expression system of claim 1, wherein the at least one immunomodulatory protein is selected from the group consisting of:
   a. the alpha subunit of IL-12;
   b. the β-subunit of IL-12;
   c. single chain IL-12 comprising the α– and β-subunit of IL-12;
   d. a single chain IL-12 comprising or consisting of the amino acid sequence of SEQ ID NO: 9 or 10;

e. a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with respect to SEQ ID NO: 9 or 10, wherein the polypeptide has the biological activity of interleukin-12;

f. an IFN-β comprising or consisting of the amino acid sequence of SEQ ID NO: 79;

g. a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with respect to SEQ ID NO: 79, wherein the polypeptide has the biological activity of IFN-β;

h. an IL-15-sushi, comprising or consisting of the amino acid sequence of SEQ ID NO: 80;

i. a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with respect to SEQ ID NO: 80, wherein the polypeptide has the biological activity of IL-15;

j. a CXCL10, comprising or consisting of the amino acid sequence of SEQ ID NO: 81; and

k. a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with respect to SEQ ID NO: 81, wherein the polypeptide has the biological activity of CXCL10.

4. The recombinant expression system of claim 1, wherein:
(i) the first nucleic acid construct comprises at least two sequences each encoding a different immunomodulatory protein, wherein:
the first immunomodulatory protein is a single chain IL-12 comprising or consisting of the amino acid sequence of SEQ ID NO: 9 or 10, or a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with respect to SEQ ID NO: 9 or 10, wherein the polypeptide has the biological activity of interleukin-12, and the second immunomodulatory protein is an IFN-β comprising or consisting of the amino acid sequence of SEQ ID NO: 79, or a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with respect to SEQ ID NO: 79, wherein the polypeptide has the biological activity of IFN-β; or
an IL-15-sushi, comprising or consisting of the amino acid sequence of SEQ ID NO: 80, or a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with respect to SEQ ID NO: 80, wherein the polypeptide has the biological activity of IL-15; or

a CXCL10, comprising or consisting of the amino acid sequence of SEQ ID NO: 81, or a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with respect to SEQ ID NO: 81, wherein the polypeptide has the biological activity of CXCL10; or

(ii) the second nucleic acid construct is a bi- or polycistronic expression cassette comprising two or more sequences each encoding a different immunomodulatory protein, or a protein or an RNA with anticancer properties, wherein the sequences are separated by a sequence encoding an IRES; and/or the sequences are separated by a sequence encoding a 2A peptide.

5. The recombinant expression system of claim 1, wherein the STING-responsive promoter of the second nucleic acid construct is selected from the group consisting of IFN-β-responsive promoter, IFN-β-minimal promoter, CXCL9-responsive promoter, CXCL10-responsive promoter, STAT-motif promoter, IRSF3-motif promoter, ISRE-motif promoter, NFκB-motif promoter, and STAT6-motif promoter, wherein the STING responsive promoter comprises at least one interferon-stimulated response element (ISRE) selected from the group consisting of:

a. a nucleotide sequence comprising one to ten copies of a sequence having at least 75%, at least 80%, at least 85%, at least 90% or at least 95% sequence identity with respect to SEQ ID NO: 11 or is identical to SEQ ID NO: 11;

b. a nucleotide sequence according to a., wherein the copies are interspaced by up to forty nucleotides;

c. a nucleotide sequence according to SEQ ID NO: 12; and

d. a nucleotide sequence according to SEQ ID NO: 13.

6. The recombinant expression system of claim 1, wherein the second nucleic acid construct further comprises at least one sequence motif that inhibits transgenic expression of the immunomodulatory protein in tissues or cells not intended to express the proteins, the tissue being liver, wherein the sequence motif is a target sequence of a microRNA that is highly abundant in the liver, or the sequence motif is a miR-122 target sequence selected from the group consisting of:

a. a miR-122 target sequence having at least 90% or at least 95% sequence identity with respect to SEQ ID NO: 14 or is identical to SEQ ID NO: 14;

b. a nucleotide sequence comprising 1 to 12 copies of the miR-122 target sequence of a.;

c. a nucleotide sequence according to b., wherein the copies are interspaced by one to forty nucleotides; and

d. a nucleotide sequence comprising 5 copies of the miR-122 target sequence as depicted in SEQ ID NO: 15.

7. An expression vector encoding the first and/or the second nucleic acid construct according to claim 1, wherein the expression vector is a viral vector selected from the group consisting of adeno-associated virus (AAV) vector, adenoviral vector, lentiviral vector, vaccine virus vector, or herpes simplex virus vector, the adeno-associated virus vector has one or more of the following characteristics:

a. the first and/or the second nucleic acid construct sequence further comprise a 5'ITR and a 3'ITR sequence as depicted in SEQ ID NO: 17;

b. the AAV vector has the serotype AAV8, AAV1, AAV3, AAV6, AAV9, AAV2, AAV5, AAVrh.10, or any gain-of-function mutant of AAVrh.10.

8. A viral particle comprising the viral vector comprising the first and/or second nucleic acid construct of claim 7, wherein the viral particle is an AAV or an adenoviral particle.

9. A pharmaceutical composition that comprises a pharmaceutically acceptable excipient and: the recombinant expression system of claim 1.

10. Use of the pharmaceutical composition according to claim 9 in the manufacture of a medicament for treating a proliferative disorder.

11. A recombinant expression system that consists of a nucleic acid construct comprising a sequence encoding at least two immunomodulatory proteins, said sequence is operatively linked to a STING-responsive promoter or a radiation inducible promoter and at least one sequence motif that inhibits transgenic expression of the immunomodulatory proteins in tissues or cells not intended to express the proteins.

12. The recombinant expression system according to claim 11, wherein the at least two immunomodulatory proteins are selected from the group consisting of interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-7, interleukin-8, interleukin-9, interleukin-11, single-chain interleukin-12, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-15-sushi, interleukin-16, interleukin-17, interleukin-18, interleukin-19, interleukin-20, interleukin-21, interleukin-22, interleukin-23, CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9, CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19,

CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, CX3CL1, granulocyte-macrophage colony-stimulating factor, type I interferons, IFN-γ, TNF-α, FLT3-ligand, a blocking peptide targeting TGF-β, a blocking peptide targeting IL-10, a blocking peptide targeting FoxP3, a monoclonal antibody or single-chain variable fragment (scFv) or nanobody neutralizing PD1, PDL1, CTLA4, CD137, TIM3, LAG3, and a fragment or variant thereof.

13. An expression vector encoding the nucleic acid construct according to claim 11, wherein the expression vector is a viral vector selected from the list consisting of adeno-associated virus (AAV) vector, adenoviral vector, lentiviral vector, vaccine virus vector, or herpes simplex virus vector.

14. A viral particle comprising the viral vector comprising the nucleic acid construct of claim 13, wherein the viral particle is preferably an AAV or an adenoviral particle; more preferably an AAV viral particle.

15. The recombinant expression system of claim 1, wherein said expression system further comprises a second nucleic acid construct comprising a sequence encoding for a target STING, that is responsive to a STING agonist recognizing said target STING but not the human STING and natural variants of the human STING protein, said sequence encoding the target STING is operatively linked to elements allowing expression and/or translation of said target STING.

16. The recombinant expression system of claim 15, wherein the target STING is responsive to a human-non-reacting STING agonist selected from the group consisting of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), 7-bromo-DMXAA, 7-iodo-DMXAA, 7-hydroxy-DMXAA, 7-formyl-DMXAA, 7-hydroxymethyl-DMXAA, 7-(2-hydroxyethyl)-DMXAA, flavone-8-acetic acid (FAA), 2,7-bis(2-dimethylamino ethoxy)fluoren-9-one (Tilorone) and 10-carboxymethyl-9-acridanone (CMA).

17. The recombinant expression system of claim 15, wherein the target STING encoded by the sequence of the second nucleic acid construct is:

l. a mutated form of a natural variant of human STING that further comprises at least one, at least two, or at least three amino acid substitutions that renders the human STING variant sensitive to the human-non-reacting STING agonist as determined in an *in vitro*-agonist determination assay; or

a non-human STING that is sensitive to the human-non-reacting STING agonist as determined in an *in vitro*-agonist determination assay.

18. The recombinant expression system of claim 17, wherein the target STING encoded by the sequence of the second nucleic acid construct is a DMXAA-sensitive human STING variant that is selected from the group consisting of:

m. a human STING[R] variant further comprising at least 1, at least 2, or at least 3 amino acid substitutions selected from the group consisting of S162A, G230I, and Q266I;

n. a human STING[AQ] variant further comprising at least 1, at least 2, or at least 3 amino acid substitutions selected from the group consisting of S162A, A230I, Q266I, E260I and E260V;

o. a human STING[H] variant further comprising at least 1, at least 2, or at least 3 amino acid substitutions selected from the group consisting of S162A, G230I, and Q266I; and

a truncated human STING[H] variant further comprising at least 1, or at least 2 amino acid substitutions selected from the group consisting of S162A and G230I.

19. The recombinant expression system of claim 18, wherein the DMXAA-sensitive human STING variant encoded by the sequence of the second nucleic acid construct is selected from the group consisting of hSTING[R]-S162A/G230I/Q266I, hSTING[R]-S162A/Q266I, hSTING[R]-G230I, hSTING[R]-S162A, hSTING[R]-Q266I, hSTING[R]-S162A/G230I, hSTING[AQ]-S162A/A230I/Q266I, hSTING[AQ]-S162A, hSTING[AQ]-S162A/R169A, hSTING[AQ]-S162A/T267A, hSTING[AQ]-E260I, hSTING[AQ]-E260V, hSTING[H]-S162A/G2301/Q266I, hSTING[H]-S162A/Q266I, hSTING[H]-G230I, hSTING[H]-S162A, hSTING[H]-S162A/G230I and hSTING[H]-Q266I.

20. The recombinant expression system of claim 18, wherein the sequence of the second nucleic acid construct encoding for the DMXAA-sensitive human STING variant is selected from the group consisting of:

p. SEQ ID NO: 5;

q. a polynucleotide sequence having at least 75%, at least 80%, at least 85%, at least 90% or at least 95% sequence identity with respect to SEQ ID NO: 5;

r. a polynucleotide sequence encoding a polypeptide comprising SEQ ID NO: 6;

s. a polynucleotide sequence encoding a polypeptide having at least 75%, at least 80%, at least 85%, at least 90% or at least 95% sequence identity with respect to SEQ ID NO: 6;

t. a polynucleotide sequence encoding a polypeptide to c) or d) with at least one modification of the polypeptide that is an amino acid substitution, amino acid insertion, amino acid deletion, C-terminal truncation or N-terminal truncation;

u. a polynucleotide sequence that hybridizes under at least stringent conditions to the complement of the polynucleotide sequence of any of a) to e); and

v. a region of the polynucleotide sequence of a) to f) encoding a fragment of at least 50 amino acid residues of the DMXAA-sensitive human STING variant;

wherein the polypeptide encoded by the polynucleotide of any of p) to v) has the biological activity of being responsive to the agonist DMXAA as determined in an *in vitro*-DMXAA determination assay.

21. The recombinant expression system of claim 15, wherein the promoter of the second nucleic acid construct is a constitutive promoter selected from the group consisting of SV40, CMV0, UBC, EF1a, PGK, CAG, EFS, CBh, CBA human β actin, Ac5, Polyhedrin, TEF1, GDS, CaMV35S, Ubi, H1 and U6;

or

the promoter of the second nucleic acid construct is an inducible promoter selected from the group consisting of Telomerase-responsive promoter, IL-6 responsive promoter, TGF-β-inducible promoter, VEGFR-inducible promoter, Epidermal Growth factor receptor-

inducible promoter, HER2/Neu-inducible promoter, MUC1 promoter, BRCA1 promoter, BRCA2 promoter, LALBA promoter, PSA promoter, ERBB2 promoter, CEA promoter, uPAR promoter and Thyroid transcription factor1 (TTF1).

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