

1

## COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF CD45 GENE

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Ser. No. 61/028,162, filed Feb. 12, 2008. The entire contents of the provisional application are hereby incorporated by reference in the present application.

### Sequence Listing

The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Aug. 5, 2010, is named 15491US CRF sequencelisting.txt and is 126,484 bytes in size.

### Government Support

This invention was made with government support under HDTRA1-07-C-0082 awarded by the Defense/Defense Threat Reduction Agency, and HHSN266200600012C awarded by the Department of Health and Human Services/NIH/NAIAD. The government has certain rights in the invention.

### FIELD OF THE INVENTION

This invention relates to double-stranded ribonucleic acid (dsRNA), and its use in mediating RNA interference to inhibit the expression of the CD45 gene and the use of the dsRNA to treat infectious diseases and autoimmune disease.

### BACKGROUND OF THE INVENTION

CD45 is a hematopoietic cell-specific transmembrane protein tyrosine phosphatase essential for T and B cell antigen receptor-mediated signaling and also plays an important role in cytokine receptor signaling, chemokine and cytokine response and apoptosis regulation in multiple different leukocyte cell subsets (T cells, B cells, NK cells, myeloid cells, granulocytes, and dendritic cells). CD45 constitutes nearly 10% of T and B cell surface protein. The protein includes a large extracellular domain, and a phosphatase containing cytosolic domain. CD45 may act as both a positive and negative regulator depending on the nature of the stimulus and the cell type involved. CD45 RNA transcripts are alternatively spliced at the N-terminus, which results in extracellular domains of various sizes. The protein controls the activity of Src-family kinases, which if left unregulated, can cause cancer and autoimmunity. Mice and humans lacking CD45 expression have been shown to be immunodeficient.

Multiple human or rodent mutations that result in altered CD45 expression or functional activity are associated with distinct malignancies, including autoimmunity, immunodeficiency, overt activation of T cells, susceptibility to infection, type I or type II associated immune disorders, and haematologic malignancies (reviewed in Tchilian and Beverly, Trends in Immunology, 2006).

Double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of the unc-22 gene in *C. elegans*. dsRNA has also been shown to degrade

2

target RNA in other organisms, including plants (see, e.g., WO 99/53050, Waterhouse et al.; and WO 99/61631, Heifetz et al.), *Drosophila* (see, e.g., Yang, D., et al., *Curr. Biol.* (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.).

### SUMMARY OF THE INVENTION

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of the CD45 gene in a cell or mammal using such dsRNA. The invention also provides compositions and methods for treating pathological conditions and diseases caused by the expression of the CD45 gene, such as infectious disease and autoimmune disease. The dsRNA featured in the invention includes an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and which is substantially complementary or fully complementary to the corresponding region of an mRNA transcript of the CD45 gene.

In one aspect, the invention features, double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of the CD45 gene. The dsRNA includes at least two sequences that are complementary, e.g., substantially or fully complementary, to each other. The dsRNA includes a sense strand including a first sequence and an antisense strand including a second sequence. The antisense strand includes a nucleotide sequence which is substantially or fully complementary to the corresponding region of an mRNA encoding CD45, and the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length, e.g., 19 to 21 nucleotides in length. In some embodiments, the dsRNA is from about 10 to about 15 nucleotides, and in other embodiments the dsRNA is from about 25 to about 30 nucleotides in length. In another embodiment, the dsRNA is at least 15 nucleotides in length. The dsRNA, upon contacting with a cell expressing the CD45, e.g., in an assay described herein, e.g., in a P388D1 cell assay as described herein (or an assay based on a cell with similar properties), inhibits the expression of the CD45 gene by at least 20% or 25%, and preferably by at least 35%, or preferably by at least 40%. In one embodiment, the CD45 dsRNA is formulated in a stable nucleic acid particle (SNALP).

The dsRNA molecules featured in the invention include dsRNAs that cleave a CD45 mRNA in a target sequence selected from the group consisting of SEQ ID NOs:97-144. The dsRNAs featured herein also include dsRNAs having a first sequence selected from the group consisting of the sense sequences of Tables 2, 4 and 5, and a second sequence selected from the group consisting of the antisense sequences of Tables 2, 4 and 5. The dsRNA molecules featured in the invention can include naturally occurring nucleotides or can include at least one modified nucleotide, such as a 2'-O-methyl modified nucleotide, a nucleotide including a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. Alternatively, the modified nucleotide may be chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide. Generally, the first sequence of the dsRNA is selected from the group consisting of the sense sequences of Tables 2, 4 and 5, and the second sequence is selected from the group consisting of the antisense sequences of Tables 2, 4 and 5.