CLAIMS

We claim:

1. A composition comprising:

mycobacterial RNA isolated from mycobacteria cells: and,

mycobacterial cell walls isolated from mycobacterial cells, wherein the composition does not contain phenol or pronase and the RNA is in the form of oligoribonucleotides and polyribonucleotides, wherein the length of the oligoribonucleotides and polyribonucleotides is 2 to 150 bases.

2. The composition of claim 1, further comprising intact mycobacterial cells.

3 The composition of claim 1, wherein the length of the oligoribonucleotides and polyribonucleotides is ~~about 2 to about 4000 bases, or about 2 to about 150 bases, or about~~ 20 to ~~about~~ 40 bases, or ~~about~~ 2 to ~~about~~ 40 bases.

4. The composition of claim 2, wherein the intact mycobacterial cells comprise from ~~about~~ 0. 05% to ~~about~~ 95% or ~~about~~ 0.19% to ~~about~~ 19.6% of the composition by weight (wt%).

5. The composition of any of the preceding claims, further comprising a pharmaceutically acceptable carrier or delivery system.

6. A method of making the composition of claim 1, comprising the sequential steps of:

A) disrupting a mycobacterial cell biomass to generate disrupted mycobacterial cells, intact mycobacterial cells, disrupted mycobacterial cell walls and RNA;

B) optionally separating intact mycobacterial cells from the disrupted mycobacterial cell walls and RNA; and,

C) separating the soluble, cytosolic contents of a portion of the disrupted mycobacterial cells from the disrupted mycobacterial cell walls and RNA to obtain a mycobacterial cell wall, ribonucleic acid composition; and

D) heating at a temperature sufficient to generate oligoribonucleotides and polyribonucleotides of 2 to 150 bases in length, wherein the intact mycobacterial cells are inactivated.

7. The method of claim 6, wherein the ~~RNA is in the form of~~ oligoribonucleotides and polyribonucleotides are~~of about 2 to about 4000 bases in length, about 2 to about 150 bases in length, or about~~ 20 to ~~about~~ 40 bases in length, or ~~about~~ 2 to ~~about~~ 40 bases in length.

~~8. The method of claim 6, further comprising heating after step C at a temperature sufficient to generate oligoribonucleotides and polyribonucleotides of about 2 to about 150 bases in length or about 20 to about 40 bases in length or about 2 to about 40 bases in length, wherein the intact mycobacterial cells are inactivated.~~

~~8~~9. The method of claim 6, wherein the disrupting step A comprises high pressure homogenization at ~~about~~ 10,000 psi or higher.

~~9~~10. The method of claim 6, wherein the disrupting step A is repeated 2 to 5 times before the separation steps B or C.

10~~11~~. The method of claim 6, further comprising repeating the disrupting step A 2 to 5 times after the separation step C.

11~~12~~. The method of claim 6, wherein the separating step B is low speed centrifugation, and the separating step C is high speed centrifugation.

12~~13~~. The method of claim 6, further comprising the sequential steps of:
repeating steps A) and B) to control the number of intact mycobacterial cells~~; and, heating the mycobacterial cell wall, ribonucleic acid composition to a temperature sufficient to obtain oligoribonucleotides and polyribonucleotides of about 2 to about 150 bases in length or about 20 to about 40 bases in length or about 2 to about 40 bases in length~~.

13~~14~~. Use of the composition of any of claims 1 to 5 in the manufacture of a medicament for the treatment of cancer.

14~~15~~. Use of the composition of any of claims 1 to 5 in the manufacture of a medicament for stimulating an immune system.

1516. The use of claim 1514, wherein stimulating the immune system comprises stimulating the differentiation of hematopoietic progenitor(s), increasing the secretion of cytokines or chemokines, increasing the synthesis of hematopoietic growth factors, activating the nucleotide-binding oligomerization domain 2 (NOD2) receptor, or activating the Toll-like receptor 2 (TLR2) receptor.

16. Use of the composition of any of claims 1 to 5 in the manufacture of a medicament for stimulating the synthesis of hematopoietic growth factors.

1717. The use of claim 15 or 16, wherein the mycobacteria is *M. phlei* and the hematopoietic growth factors are selected from the group consisting of Macrophage Colony Stimulating Factor (M-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), Granulocyte Colony Stimulating Factor (G-CSF), Stromal Cell Derived Factor-lalpha (SDF-la) and Leukemia Inhibitory Factor (LIF), and combinations thereof.

18. The composition of any of claims 1 to 5 for use as an adjuvant to cancer therapy or immune stimulant therapy.

~~19. A synthetic medium for the cultivation of mycobacteria comprising: Middlebrook 7H9 powdered medium; glucose; glycerol; additional iron in the form of ferric ammonium citrate; additional carbon in the form of ferric ammonium citrate, ammonium citrate or asparagine; additional nitrogen in the form of ferric ammonium citrate, ammonium citrate or asparagine; and, water.~~

~~20. A synthetic medium for the cultivation of mycobacteria comprising: ammonium citrate; L-glutamic acid; pyridoxine; biotin; disodium phosphate; monopotassium phosphate; ferric ammonium citrate; magnesium sulfate; calcium chloride; zinc sulfate; oleic acid; glycerol; glucose; and, water.~~

~~21. A composition comprising:~~

~~bacterial RNA isolated from bacterial cells; and,~~

~~bacterial cell walls isolated from bacterial cells, wherein the composition does not contain phenol or pronase and the RNA is in the form of oligoribonucleotides and polyribonucleotides.~~

~~22. The composition of claim 21, wherein the RNA and bacterial cell walls are~~

~~from gram positive bacteria or gram negative bacteria.~~

~~23. A method of making the composition of claim 21, comprising the sequential steps of:~~

~~(A) disrupting a bacterial cell biomass to generate disrupted bacterial cells, intact bacterial cells, disrupted bacterial cell walls and RNA;~~

~~(B) optionally separating intact bacterial cells from the disrupted bacterial cell walls and RNA; and,~~

~~(C) separating the soluble, cytosolic contents of a portion of the disrupted bacterial cells from the disrupted bacterial cell walls and RNA to obtain a bacterial cell wall, ribonucleic acid composition.~~

19~~24~~. The composition of any one of claims 1 to 5 ~~or 21 to 22~~, wherein the RNA and the cell walls are isolated in combination from the cells.