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(54) Title: IMMUNOSTIMULATORY BACTERIA ENGINEERED TO COLONIZE TUMORS, TUMOR-RESIDENT IMMUNE CELLS, AND THE TUMOR MICROENVIRONMENT

Human and Tasmanian devil STING:

Human	1	MPHSSLHPSIFCPRGHGAQKAALVLLSACLVTWGLGEPPEHTLRYLVLH	50
Tasmanian	1	MFRSHLHPSIFQPRGWGAQKAACVLLSLCMAAVYILGESAAFTLQWLLEY	50
Human	51	IASLQLGLLLNGVCSLAELRHIHSRYRGSYWRVTRACLGCPPLRRGALLL	100
Tasmanian	51	FTTVQVGLLLKGAAYCLAELYHIQSRHQGSYQAIQACIHYPFORISLLL	100
Human	101	LSIFYFYSLPNAVGPFTWMLALLGLSOALNILLGLKGLAPAEISAVCEK	150
Tasmanian	101	LSGYFVVTLSNKNPNSLTWTFALLGLSHLSFILGLQNLTSAEISEVCEK	150
Human	151	GNFNVAGLAWSYIYGLRLILPELQARIRTYNQHYNNLLRGAVSQRLYI	200
Tasmanian	151	RHLNVAGLAWSYIYGLKLILPGLQTRIQIYNQFNKDVLSFEGHRLHI	200
Human	201	LLPLDCGVPDNLMSADPNIRFLDKLPQQTGDHAGIKDRVYSNSIYELLEN	250
Tasmanian	201	LIPLDGCVDPNLSQADPNIQFLQELPQHKLDRAKIGRIYTNISYKISED	250
Human	251	GQRAGTCVLEATPLQTLFAMSQYSQAGFSREDRLQAKLFCRTLEDILA	300
Tasmanian	251	GQPAEVCVLEFATPLQTLFAMSQDARAGFSREDRLQAKLFCRTLEDILE	300
Human	301	DAPESQNNCRLIAYQEPADD--SSFSLSQEVLRHLRQEEKEEVTGSLKT	348
Tasmanian	301	DAPEARDCCRLVYQEPEDKAVSNFSLSKELLRLRQERQEEFAIGPKRA	350
Human	349	SAVFSTSTMSQEPQLLISGMEKPLPLRTD-FS	379
Tasmanian	351	MTVTTSSSTLSQEPQLLISGMEQPLSLRTDGF-	381

FIGURE 1

(57) Abstract: Provided are delivery immunostimulatory bacteria that have enhanced colonization of tumors, the tumor microenvironment and/or tumor-resident immune cells, and enhanced anti-tumor activity. The immunostimulatory bacteria are modified by deletion of genes encoding the flagella or by modification of the genes so that functional flagella are not produced, and/or are modified by deletion of *pagP* or modification of *pagP* to produce inactive PagP product. As a result, the immunostimulatory bacteria are flagellin' and/or *pagP*'. The immunostimulatory bacteria optionally have additional genomic modifications so that the bacteria are adenosine and/or purine auxotrophs. The bacteria optionally are one or more of *ascT*, *purI*' and *msbB*'. The immunostimulatory bacteria, such as Salmonella species, are modified to encode proteins that induce type I interferon (IFN) expression, or that are variants thereof that

[Continued on next page]

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have increased activity to induce type I IFN expression, or that are variants thereof that result in constitutive expression of type I IFN. The bacteria can encode a modified Stimulator of Interferon Genes (STING) protein from a non-human species, that has lower NF- κ B signaling activity, and, optionally, higher type I IFN pathway signaling activity, compared to human STING. The bacteria preferentially infect immune cells in the tumor microenvironment, or tumor-resident immune cells, and/or induce less cell death in immune cells than in other cells. Also provided are methods of inhibiting the growth or reducing the volume of a solid tumor by administering the immunostimulatory bacteria.