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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	MPHSSLRHPSIPQPRGHGAQAALVLLSACLVTWGLGEPPEHTLRVVLH	50
Tasmanian	1	MPRSHLHPSIPQPRGWGAQAACVLLSLCMAAVYILGESAAFTLQWLLEY	50
Human	51	LASLQGLGILLGVCSLAEELRHHSRYRGSYWRVACLGCPLRGALLL	100
Tasmanian	51	FTTVQVGLLLGAYCLAEELYHIQSRHQSSYQAIQACHYPPQRIISLL	100
Human	101	LSIYFYSLPNAVGPPFTWMLALLGLSQALNILLGLKGLAPABISAVCEK	150
Tasmanian	101	LSGYFVTLNKNPNSLTWTTFALLGLSHALSFILGLQNLTSABISEVCEK	150
Human	151	GNFNVARGLAWSYYIGYLRLLPELQARIRTYNQHYNNLLRGAVSQRLYI	200
Tasmanian	151	RHLNVARGLAWSYYIGYLRLLPGLQTRIQIYNQFNKDVLSPEGHRLHI	200
Human	201	LPLDCGVFDNLSDADPNIRFLDKLPQQTGDHAGIKDRVYSNSIYELLEN	250
Tasmanian	201	LPLDCGVFDNLSDADPNIQFLQELPQHKLDRAIGKRIYTNISYKISED	250
Human	251	GQRAGTCVLEYATPLQTLFAMSQYSQAGFSREDRLQAKLFCRTLEDILA	300
Tasmanian	251	GQPAEVCVLEFATPLQTLFAMSQDARAGFSREDRLQAKLFCRTLEDILE	300
Human	301	DAPESQNNCRLLIAYQEPADD--SSFSLSQEVLRHLRQEEKEEVTGSLKT	348
Tasmanian	301	DAPEARDCCLVYQEPEDKAVSNFSLSKELRLRHLRQERQEEFAIGPKRA	350
Human	349	SAVSTSTMSQEPPELLISGMEKPLPLRTD-PS	379
Tasmanian	351	MTVTTSSTLSQEPQLLISGMEQPLSLRTDGF-	381

FIGURE 1

(57) Abstract: Provided are delivery immunostimulatory bacteria that have enhanced colonization of tumors, the tumor microenviron-
ment 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 adenine
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.