SUPPLEMENTARY DATA

STAT3/NF-κB signalling disruption in M2 tumour-associated macrophages is a major target of PLGA nanocarriers/PD-L1 antibody immunomodulatory therapy in breast cancer

Rômulo S. Cavalcante[a,b], Uta Ishikawa[b], Emanuell S. Silva[d,e], Arnóbio A. Silva Júnior[a,d,e], Aurigena A. Araújo[c], Luis J. Cruz[f], Alan B. Chan[f,g], Raimundo F. Araújo Júnior[1,2,7*]

[a]Postgraduate Program in Health Science, Federal University of Rio Grande do Norte, 59064 741, Natal, RN, Brazil.

[b]Cancer and Inflammation Research Laboratory, Department of Morphology, Federal University of Rio Grande do Norte, 59064 741, Natal, RN, Brazil.

[c]Postgraduate Program in Pharmaceutical Science, Department of Biophysics and Pharmacology, Federal University of Rio Grande do Norte, 59064 741, Natal, RN, Brazil.

[d]Postgraduate Program in Development and Technological Innovation in Medicines, Federal University of Rio Grande do Norte, Natal, RN, Brazil.

[e]Laboratory of Pharmaceutical Technology and Biotechnology, Department of Pharmacy, Federal University of Rio Grande do Norte, 59012 570, Natal, RN, Brazil.

[f]Translational Nanobiomaterials and Imaging, Department of Radiology, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands.

[g]Percuros B.V, Zernikedreef 8, 2333 CL, Leiden, The Netherlands.

Data available on request from the authors

Corresponding author: Raimundo Fernandes de Araújo Júnior, Radiology Department/ Leiden University Medical Center, Leiden, The Netherlands. Post code: 2333 ZA. +31655620247. **araujojr@cb.ufrn.br**

SUPPLEMENTARY TABLES

Table S1: Mouse primers sequence, amplicon size and annealing temperature used in this study.

Target	5' → 3' Forward	5' → 3' Reverse	Amplicon size (pb)	Annealing temperature (°C)	Access number
β-actin	AGGCCAACCTGTAAAAGATG	TGTGGTACGAGAGGCATAC	107	50.94	NM_007393.5
STAT3	GGGCCTGGTGTGAACTACTC	GGTATTGCTGCAGGTCGTTG	564	54.79	NM_213659.3
NFKB	CCGTCTGTCTGCTCTCTCT	CGTAGGGATCATCGTCTGCC	873	54.08	NM_001311126.1
SNAIL	GACTCCTTCCAGCCTTGGTC	CCAGTAACCACCCTGCTGAG	356	55.04	NM_011427.3
IL-10	GCTCTTGCACTACCAAAGCC	CTGCTGATCCTCATGCCAGT	112	54.65	NM_010548.2
CD-68	CGCCTAGTCCAAGGTCCAAG	GAAGTGTCCCTTGTCAGGCA	488	55.00	NM_001291058.1
CD-163	GGATCTCCGGGATGCTTCTG	CGCCTGCCAGACGAATATCT	878	54.97	NM_001170395.1
CCL22	GAGACAACAGTGGTCCCAGG	CTGGCACTGTCAATCCCTGT	185	54.96	NM_009137.2
FADD	AGAAGAAGAACGCCTCGGTG	GCTCACAGATTCCTGGGCTT	101	55.04	NM_010175.6
APAF-1	TTCCAGTGGCAAGGACACAG	CCACTCTCCACAGGGACAAC	567	55.07	NM_001042558.1
MDR1	TCAGCAACAGCAGTCTGGAG	ACTATGAGCACACCAGCACC	79	55.00	NM_011075.2
SURVIVIN	AGAACAAAATTGCAAAGGAGACCA	GGCATGTCACTCAGGTCCAA	138	54.76	NM_009689.2
CD8	GCTCAGTCATCAGCAACTCG	ATCACAGGCGAAGTCCAATC	197	53.48	NM_009857.1
CXCR4	CATGGAACCGATCAGTGTGAG	TGAAGGCCAGGATGAGAACG	395	54.24	NM_009911.3

Supplementary Table S2: Percentage of gene expression modulation by different treatments.

Target	MTX	PeiPLGA-MTX	HA-PeiPLGA-MTX	anti-PD-L1 + HA-PeiPLGA-MTX
FADD	Non regulation	Upregulation (67 %)	Upregulation (41 %)	Not applicable
APAF-1	Upregulation (59 %)	Upregulation (57 %)	Upregulation (141 %)	Not applicable
MDR1	Upregulation (9 %)	Downregulation (17 %)	Downregulation (33 %)	Not applicable
SURVIVIN	Non regulation	Downregulation (44 %)	Downregulation (66 %)	Not applicable
NF-κB	Downregulation (33 %)	Downregulation (63 %)	Downregulation (51 %)	Not applicable
SNAIL	Downregulation (70 %)	Downregulation (48 %)	Downregulation (45 %)	Not applicable
STAT3	Non regulation	Downregulation (54 %)	Downregulation (65 %)	Not applicable
CXCR4	Non regulation	Downregulation (66 %)	Downregulation (28 %)	Not applicable
CCL22	Downregulation (26 %)	Downregulation (56 %)	Downregulation (29 %)	Non regulation
CD8	Downregulation (49 %)	Upregulation (64 %)	Upregulation (48 %)	Upregulation (160 %)
CD68	Downregulation (40 %)	Upregulation (125 %)	Upregulation (81 %)	Downregulation (36 %)
CD163	Non regulation	Downregulation (76 %)	Downregulation (78 %)	Non regulation
IL-10	Non regulation	Downregulation (46 %)	Downregulation (79 %)	Upregulation (21 %)

Supplementary Table S3: Modulation of protein expression by different treatments expressed in IHC score units.

Target	MTX	PeiPLGA-MTX	HA-PeiPLGA-MTX	anti-PD-L1 + HA-PeiPLGA-MTX
FADD	Upregulation (15 un)[a]	Upregulation (216 un)	Upregulation (213 un)	Not applicable
APAF-1	Upregulation (69 un)	Upregulation (124 un)	Upregulation (162 un)	Not applicable
CASPASE-3	Upregulation (100 un)	Upregulation (180 un)	Upregulation (165 un)	Not applicable
BCL-2	Downregulation (96 un)	Downregulation (96 un)	Downregulation (159 un)	Not applicable
NF-κB	Downregulation (62 un)	Downregulation (142 un)	Downregulation (155 un)	Not applicable
STAT3	Downregulation (75 un)	Downregulation (184 un)	Downregulation (180 un)	Not applicable
E-CADHERIN	Upregulation (53 un)	Upregulation (104 un)	Upregulation (103 un)	Not applicable
CD163	Downregulation (4 un)[a]	Downregulation (93 un)	Downregulation (48 un)	Downregulation (53 un)
IL-10	Downregulation (6 un)[a]	Downregulation (176 un)	Downregulation (142 un)	Downregulation (153 un)
TGF-β	Upregulation (33 un)	Downregulation (19 un)	Downregulation (21 un)	Upregulation (17 un)
CD25	Upregulation (78 un)	Upregulation (185 un)	Upregulation (96 un)	Upregulation (59 un)
PD-L1	Downregulation (14 un)	Downregulation (40 un)	Downregulation (35 un)	Downregulation (166 un)

[a] Statistically not significant result; NA, not applicable