

Supplemental Data

Combination of ultrasound-based mechanical disruption of tumor with immune checkpoint blockade modifies tumor microenvironment and augments systemic antitumor immunity

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Figure 1. Superior growth suppression of local and distant tumors and enhanced tumor antigen-specific cellular immune responses by M-HIFU compared to T-HIFU.

Figure 1A.

Leg Tumor	Tumor Volume on day 16 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
No treatment	1681.6	236.9	79.0	1330.0 – 2138.2	n = 9
T-HIFU	1375.5	339.0	94.0	910.3 – 1923.8	n = 13
M-HIFU	190.4	156.5	49.5	0.0 – 447.0	n = 10

Figure 1C.

Flank Tumor	Tumor Volume on day 18 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
No treatment	1015.2	773.8	214.6	0.0 – 2687.7	n = 13
T-HIFU	800.2	691.7	184.9	0.0 – 2146.9	n = 14
M-HIFU	410.1	464.8	124.2	0.0 – 1431.1	n = 14

Figure 5. M-HIFU and PD-L1 blockade synergize to reject local tumor.**Figure 5B.**

Flank Tumor Group	Tumor Volume on day 18 (mm ³)				Mouse number
	Average	SD	SEM	Range	
Isotype	1622.9	644.2	203.7	18.0 – 2401.7	n = 10
aPD-L1	980.1	749.2	236.9	0.0 – 1763.3	n = 10
M-HIFU	513.9	600.3	189.8	0.0 – 1494.7	n = 10
M-HIFU + aPD-L1	276.7	314.9	99.6	0.0 – 841.0	n = 10

Figure 5F.

Leg Tumor Group	Tumor Volume on day 19 (mm ³)				Mouse number
	Average	SD	SEM	Range	
Isotype	1987.2	453.8	202.9	1203.1 – 2378.5	n = 5
M-HIFU + aPD-L1	128.4	245.3	100.1	0.0 – 621.0	n = 6
M-HIFU + aPD-L1 + aCD4	28.0	38.4	17.2	0.0 – 72.0	n = 5
M-HIFU + aPD-L1 + aCD8a	1105.1	391.7	159.9	671.6 – 1737.3	n = 6
M-HIFU + aPD-L1 + aAsiaoGM1	969.9	404.7	165.2	328.7 – 1427.5	n = 6

Figure 7. The combination of M-HIFU and PD-1/PD-L1 blockades synergistically inhibited the distant tumor growth depending on CD8+ as well as NK cells.

Figure 7B.

Flank Tumor Group	Tumor Volume on day 18 (mm ³)				Mouse number
	Average	SD	SEM	Range	
Isotype	2074.8	389.4	123.1	1666.6 – 2250.0	n = 10
aPD-L1	1444.9	707.7	223.8	0.0 – 2393.7	n = 10
M-HIFU	1666.7	640.4	202.5	730.8 – 2420.6	n = 10
M-HIFU + aPD-L1	921.4	729.2	230.6	0.0 – 2025.3	n = 10

Figure 7F.

Leg Tumor Group	Tumor Volume on day 19 (mm ³)				Mouse number
	Average	SD	SEM	Range	
Isotype	1689.9	324.3	145.1	1298.4 – 1928.4	n = 5
M-HIFU + aPD-L1	150.9	337.4	150.9	0.0 – 754.4	n = 5
M-HIFU + aPD-L1 + aCD4	82.0	107.1	47.9	0.0 – 252.8	n = 5
M-HIFU + aPD-L1 + aCD8a	1049.5	717.8	321.0	0.0 – 2098.9	n = 5
M-HIFU + aPD-L1 + aAsiaoGM1	1184.9	400.4	179.1	0.0 – 1563.25	n = 5

Flank Tumor Group	Tumor Volume on day 19 (mm ³)				Mouse number
	Average	SD	SEM	Range	
Isotype	1323.5	270.7	121.1	991.9 – 1663.5	n = 5
M-HIFU + aPD-L1	732.6	292.0	130.6	0.0 – 1090.4	n = 5
M-HIFU + aPD-L1 + aCD4	256.6	227.9	101.9	0.0 – 523.3	n = 5
M-HIFU + aPD-L1 + aCD8a	1842.8	525.5	235.0	0.0 – 2573.2	n = 5
M-HIFU + aPD-L1 + aAsiaoGM1	1362.8	290.8	130.0	0.0 – 1561.6	n = 5

Supplementary Figure 1. Stronger tumor growth suppression and abscopal effect induced by M-HIFU against murine breast cancers.

Supplementary Figure 1A.

E0771-OVA Leg Tumor	Tumor Volume on day 16 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
No treatment	2508.0	431.7	193.1	1847.3 – 2997.2	n = 6
T-HIFU	1600.4	189.6	84.8	1439.4 – 1934.3	n = 6
M-HIFU	1245.4	498.3	203.4	291.2 – 1628.3	n = 6

Supplementary Figure 1B.

JC-HER3 Leg Tumor	Tumor Volume on day 24 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
No treatment	2531.6	376.3	125.4	1858.4 – 2888.1	n = 9
M-HIFU	1182.5	767.9	256.0	262.4 – 2305.8	n = 9

Supplementary Figure 1C.

JC-HER3 Leg Tumor	Tumor Volume on day 24 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
No treatment	1762.9	399.9	133.3	1230.1 – 2271.6	n = 9
M-HIFU	788.7	373.3	124.4	228.5 – 1410.4	n = 9

JC-HER3 Flank Tumor	Tumor Volume on day 24 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
No treatment	933.1	185.5	61.8	645.0 – 1121.0	n = 9
M-HIFU	591.9	176.7	58.9	254.6 – 874.6	n = 9

Supplementary Figure 3. Cell death and immunogenicity of HIFU treated MM3MG-HER2 breast cancer cells.

Supplementary Figure 3F.

Flank Tumor	Tumor Volume on day 13 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
No treatment	1407.9	382.2	120.9	704.1 – 2160.9	n = 10
T-HIFU	553.8	537.3	169.9	0.0 – 1343.5	n = 10
M-HIFU	665.2	533.1	168.6	0.0 – 1409.5	n = 10

Supplementary Figure 8. Antitumor efficacy of combined M-HIFU and anti-PD-L1 antibody against JC-HER3 breast cancer model.

Supplementary Figure 8B.

JC-HER3 Leg Tumor	Tumor Volume on day 20 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
Isotype	1936.0	620.4	234.5	1250.0 – 2786.9	n = 8
M-HIFU	974.6	383.3	156.5	324.6 – 1433.4	n = 6
aPD-L1	1762.7	519.4	212.0	1023.4 – 2218.0	n = 7
M-HIFU + aPD-L1	356.7	461.1	174.3	0.0 – 1189.8	n = 8

Supplementary Figure 11. Enhanced abscopal effect induced by combined M-HIFU and anti-PD-L1 antibody.**Supplementary Figure 11B.**

JC-HER3 Leg Tumor	Tumor Volume on day 23 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
Isotype	2028.7	378.7	143.1	1661.6 – 2480.0	n = 7
M-HIFU	1009.5	554.4	209.6	129.6 – 1903.9	n = 7
aPD-L1	2123.3	549.9	207.8	1304.2 – 2645.4	n = 7
M-HIFU + aPD-L1	633.8	347.0	131.1	0.0 – 948.7	n = 7

JC-HER3 Flank Tumor	Tumor Volume on day 23 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
Isotype	774.9	108.8	41.1	667.0 – 984.2	n = 7
M-HIFU	637.6	173.2	65.5	450.4 – 914.4	n = 7
aPD-L1	677.3	188.6	71.3	488.7 – 1056.3	n = 7
M-HIFU + aPD-L1	426.9	218.7	82.7	71.7 – 778.2	n = 7

Supplementary Figure 11D.

JC-HER3 Leg Tumor	Tumor Volume on day 19 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
Isotype	1574.9	340.6	152.3	1238.3 – 2105.1	n = 5
M-HIFU + aPD-L1	588.1	328.7	147.0	62.4 – 877.3	n = 5
M-HIFU + aPD-L1 + aCD8a	923.0	317.3	141.9	502.3 – 1315.5	n = 5

JC-HER3 Flank Tumor	Tumor Volume on day 19 (mm ³)				
Group	Average	SD	SEM	Range	Mouse number
Isotype	1036.5	532.8	238.3	542.1 – 1739.6	n = 5
M-HIFU + aPD-L1	339.4	124.4	55.6	134.5 – 441.8	n = 5
M-HIFU + aPD-L1 + aCD8a	672.2	136.0	60.8	516.1 – 851.2	n = 5