

Supplemental Tables**Supplementary Table 1.** The clinical information of PCa patients in Control and ZA + Tα1 groups**Control group**

Patients	Age	Therapeutic regimens	Pre-treatment timepoints	Biomarker levels (ng/ml)			Post-treatment timepoints	Biomarker levels (ng/ml)			Gleason score	TNM stage
				PAP	PSA	Free PSA		PAP	PSA	Free PSA		
Patient 1	60	Bicalutamide	2010-6-30	100	254.18	27.592	2011-6-7	42.3	185.67	22.002	Unknown	TxN0M1b
Patient 2	61	Bicalutamide and chemotherapy (gemcitabine plus platinum)	2011-3-16	26.5	41.81	13.998	2012-8-16	6.25	34.733	11.441	Unknown	T4N1M1c
Patient 3	70	Bicalutamide, gemcitabine, and tripraline acetate	2013-4-27	100	207.23	27.486	2013-10-11	96.4	183.603	36.498	8	T4N1M1b
Patient 4	71	Bicalutamide, chemotherapy (docetaxel), pyramidal radiotherapy, and bilateral orchiectomy	2012-9-14	45.3	305.248	118.28	2013-9-25	0.71	0.033	0.019	9	TxN0M1c
Patient 5	63	Bicalutamide, goserrelin, and bilateral orchiectomy	2014-10-24	93.3	196.97	93.3	2015-8-5	7.31	462.59	38.366	Unknown	TxN0M1b
Patient 6	70	Bicalutamide and chemotherapy (docetaxel)	2017-4-6	1	0.49	0.14	2018-4-18	1.49	0.38	0.07	9	T4N1M1b
Patient 7	79	Bicalutamide	2018-9-14	1.18	2.26	0.36	2019-12-12	0.73	2	0.43	5	TxN0M1b
Patient 8	65	Bicalutamide, goserrelin, and abiraterone	2018-4-20	51	20.06	6.22	2019-5-14	49	18.24	9.14	9	T4N1M0
Patient 9	83	Bicalutamide, goserrelin, and radiotherapy	2019-6-11	10.41	279.09	55.23	2020-4-17	1.704	393.9	90.7	Unknown	TxN0M1b
Patient 10	75	Abiraterone and radiotherapy	2018-5-4	3.08	1.46	0.5	2019-5-29	0.864	2	0.81	8	T4N0M1b
Patient 11	68	Enzalutamide, abiraterone, and chemotherapy (paclitaxel liposome for injection), and radiotherapy	2019-12-17	3.187	295.1	41.6	2020-10-11	28.87	558.36	127.05	7	T2N1M1c
Patient 12	71	Bicalutamide, goserrelin, and bilateral orchiectomy	2021-3-26	0.204	0.28	0.03	2021-9-7	0.805	34.67	0.78	7	T2N1M0
Patient 13	65	Bicalutamide, chemotherapy (docetaxel and lobaplatin), and goserrelin	2020-5-5	2.128	0.91	0.29	2021-5-30	1.442	0.79	0.12	7	T4N1M1c
Patient 14	57	Abiraterone and zoledronic acid	2021-10-7	6.548	11.54	1.54	2022-8-20	2.543	2.77	0.43	Unknown	T4N1M1b
Patient 15	75	Bicalutamide, goserrelin, incadronnate disodium, and radiotherapy	2020-7-12	100	725.7	88.56	2020-8-4	89.02	328.4	47.1	7	TxN0M1c
Patient 16	77	Bicalutamide, chemotherapy (paclitaxel liposome for injection), and radiotherapy	2019-7-29	8.781	111.22	10.78	2020-1-2	33.38	316.35	36.85	Unknown	TxN0M1c
Patient 17	70	Enzalutamide, abiraterone, chemotherapy, and radiotherapy	2018-8-10	2.21	79.86	7.31	2018-10-27	5.14	116.46	7.2	Unknown	TxN1M1c
Patient 18	62	Bicalutamide and adjuvant pelvic radiation therapy	2019-2-17	1.31	0.24	0.07	2019-3-13	1.91	0.13	0.05	9	T4N0M0
Patient 19	73	Bicalutamide	2019-8-22	15.08	103.38	29.55	2020-7-25	8.182	184.8	54.23	7	T3N1M1b
Patient 20	65	Bicalutamide	2020-7-23	26.12	182.5	18.14	2021-5-18	23.95	36.12	11.81	8	T4N1M1b
Patient 21	71	Bicalutamide	2012-2-27	34.6	104.775	15.487	2012-11-20	50	559.998	49.374	Unknown	T4N0M1b
Patient 22	70	Bicalutamide	2012-3-20	1.45	52.791	14.621	2013-3-27	1.42	648.51	95.46	Unknown	T4N1M1c
Patient 23	58	Bicalutamide and chemotherapy (docetaxel)	2017-3-2	4.86	10.3	3.31	2018-5-23	53.3	252.15	44.25	9	T4N1M0

ZA + Tα1 group

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				PAP	PSA	Free PSA		PAP	PSA	Free PSA		
Patient 1	72	Bicalutamide, zoledronic acid, thymalfasin, liver protection, nutritional supplement, and other symptomatic treatments	2018-8-13	51.8	272.56	14.26	2018-12-20	1.66	14.32	1.31	Unknown	T4N1M1c
Patient 2	73	Bicalutamide, zoledronic acid, thymalfasin, and vitamin C	2019-9-17	5.369	68.21	4.69	2020-9-15	1.706	5.06	0.49	Unknown	T3N1M1b

Patient 3	69	Abiraterone, bicalutamide, zoledronic acid, thymalfasin, liver protection, stomach protection, and invigorating qi and invigorating herbal medicines	2019-2-13	2.71	44.88	7.63	2020-2-19	0.575	8.37	1.06	10	T4N1M0
Patient 4	69	Bicalutamide, zoledronic acid, and thymalfasin	2018-8-23	8.77	49.07	2.4	2019-7-30	6.578	15.77	0.8	7	T4N1M0
Patient 5	66	Bicalutamide, zoledronic acid, thymalfasin, liver protection, and invigorating qi and invigorating herbal medicines	2019-11-6	23.8	196.74	14.83	2020-12-10	16.15	39.61	4.55	9	T4N0M0
Patient 6	81	Bicalutamide, Digiochemotherapy, zoledronic acid, and thymalfasin	2017-12-4	68.6	371.91	60.27	2020-7-21	46.17	162.8	23	Unknown	T3N1M1b
Patient 7	80	Bicalutamide, goserelin, zoledronic acid, thymalfasin, and liver protection treatment	2017-10-26	13.9	8.44	1.97	2018-10-29	0.839	0.02	0.01	8	T3N1M1b
Patient 8	62	Bicalutamide, docetaxel, zoledronic acid, and thymalfasin	2020-4-8	4.268	2.56	0.2	2020/05/23	4	2.21	0.15	10	T3N1M1b
Patient 9	75	Bicalutamide, zoledronic acid, thymalfasin, and dressing change, anemia correction, stomach protection, immunity enhancement, anti-infection, and other symptomatic support treatments	2017-6-6	2.53	11.74	1.22	2018-9-29	0.81	1.95	0.22	Unknown	T2N0M1b
Patient 10	70	Bicalutamide, zoledronic acid, and thymalfasin	2017-10-28	4.36	28.27	2.81	2019-11-21	0.199	0	0	Unknown	TxN0M1b
Patient 11	68	Bicalutamide, zoledronic acid, and thymalfasin	2021-10-28	100	37.67	3.42	2022-5-5	48.7	22.08	2.17	9	T4N1M1b
Patient 12	70	Bicalutamide, zoledronic acid, and thymalfasin	2021-10-17	40.93	36.87	15.55	2022-3-23	0.583	0.25	0.12	10	T4N1M0
Patient 13	62	Bicalutamide, zoledronic acid, and thymalfasin	2021-10-22	61.31	510	50	2022-4-29	20.59	110.2	4.02	Unknown	TxN1M1b
Patient 14	79	Abiraterone, enzalutamide, zoledronic acid, and thymalfasin	2022-3-3	17.4	493.9	40.1	2022-4-1	19.51	471.6	41.2	Unknown	T4N1M1c
Patient 15	51	Abiraterone, abiraterone, zoledronic acid, and thymalfasin	2021-12-23	5.516	1.82	0.35	2022-4-8	7.73	5.26	0.42	7	T2N1M1b
Patient 16	67	Bicalutamide, zoledronic acid, and thymalfasin	2021-6-8	7.424	27.63	3.3	2022-1-7	0.729	0.46	0.08	7	T3N1M1c
Patient 17	66	Bicalutamide, zoledronic acid, and thymalfasin	2018-10-23	12.3	23.56	1.47	2018-11-1	14.2	15.99	1.25	Unknown	T4N1M1c
Patient 18	70	Bicalutamide, zoledronic acid, thymalfasin, and vitamin C	2022-3-25	30.38	673.55	65.55	2022-3-31	23.91	687.2	62.4	Unknown	T2N1M1b
Patient 19	93	Bicalutamide, zoledronic acid, and thymalfasin	2021-5-26	52.6	240	19.35	2021-6-24	23.6	189.02	12.89	Unknown	T3N0M1c
Patient 20	73	Abiraterone, zoledronic acid, and thymalfasin	2020-3-10	31.55	701.47	33	2020-8-19	30.08	515.1	18.62	Unknown	T4N1M1c

Supplemental Table 2. Characteristics of 43 advanced or metastatic PCa patients enrolled in this retrospective analysis. Detailed information of PCa patients in Figure 1.

	Control (n = 23)	ZA+Tα1 (n = 20)
Age at enrollment (years old)		
≥ 60	21	19
< 60	2	1
ECOG		
0	6	6
1	10	7
≥ 2	7	7
Gleason score		
≤ 6	1	0
7	5	3
≥ 8	8	6
Unknown	9	11
Disease site at baseline		
Bone metastasis only	6	2
Lymph node metastasis only	3	3
Bone + lymph node metastases	5	8
Visceral + bone metastases	3	1
Visceral + bone + lymph node metastases	5	5
No/unknown	1	1

Supplemental Table 3. Adverse events of 43 advanced or metastatic PCa patients receiving ADT with/without ZA and Tα1. Detailed information of PCa patients in Figure 1.

Adverse events	Control (n = 23)		ZA+Tα1 (n = 20)	
	Any Grade	Grade ≥3	Any Grade	Grade ≥3
General disorders				
Fatigue	2 (8.7%)	0	1 (5.0%)	0
Fever	1 (4.3%)	0	2 (10.0%)	0
Gastrointestinal disorders				

Diarrhea	1 (4.3%)	1 (4.3%)	0	0
Nausea	3 (13.0%)	0	0	0
Anorexia	4 (17.4%)	0	3 (15.0%)	0
Vomiting	3 (13.0%)	0	0	0
Nervous system disorders				
Dizziness	1 (4.3%)	1 (5.0%)	0	0
Headache	1 (4.3%)	0	0	0
Laboratory abnormalities				
Renal insufficiency	2 (8.7%)	0	2 (10.0%)	0
Hypocalcemia	6 (26.1%)	0	4 (20.0%)	0
Hypophosphatemia	2 (8.7%)	0	2 (10.0%)	0
Hypomagnesemia	1 (4.3%)	0	0	0
Elevated ALT	2 (8.7%)	0	0	0
Elevated AST	1 (4.3%)	0	0	0
Blood and lymphatic system disorders				
Leukopenia	3 (13.0%)	1 (4.3%)	3 (15.0%)	0
Anemia	11 (47.8%)	0	4 (20.0%)	0
Thrombocytopenia	0	1 (4.3%)	2 (10.0%)	0

Supplementary Table 4. Primer sequences used for PCR.

Gene	Forward primer sequences	Reverse primer sequences
<i>m Cxcl9</i>	5'-TCCTTTTGGGCATCATCTTCC-3'	5'-TTTGTAGTGGATCGTGCCTCG-3'
<i>m Cxcl10</i>	5'-CCAAGTGCTGCCGTCATTTTC-3'	5'-GGCTCGCAGGGATGATTTCAA-3'
<i>m Cxcl11</i>	5'-GGCTTCCTTATGTTCAAACAGGG-3'	5'-GCCGTTACTCGGGTAAATTACA-3'
<i>m Myd88</i>	5'-TCATGTTCTCCATACCCTTGGT-3'	5'-AAACTGCGAGTGGGGTCAG-3'
<i>m Idol</i>	5'-GCTTTGCTCTACCACATCCAC-3'	5'-CAGGCGCTGTAACCTGTGT-3'
<i>m Ccl2</i>	5'-TTAAAAACCTGGATCGGAACCAA-3'	5'-GCATTAGCTTCAGATTTACGGGT-3'
<i>m Ccl5</i>	5'-GCTGCTTTGCCTACCTCTCC-3'	5'-TCGAGTGACAAACACGACTGC-3'
<i>m Tnf</i>	5'-CCCTCACACTCAGATCATCTTCT-3'	5'-GCTACGACGTGGGCTACAG-3'
<i>m Csf1</i>	5'-GGCTTGGCTTGGGATGATTCT-3'	5'-GAGGGTCTGGCAGGTACTC-3'
<i>m Csf2</i>	5'-GGCCTTGAAGCATGTAGAGG-3'	5'-GGAGAACTCGTTAGAGACGACTT-3'
<i>m Ifng</i>	5'-ATGAACGCTACACACTGCATC-3'	5'-CCATCCTTTTGCCAGTTCCTC-3'

<i>m Il4</i>	5'-GGTCTCAACCCCCAGCTAGT-3'	5'-GCCGATGATCTCTCTCAAGTGAT-3'
<i>m Cd86</i>	5'-TGTTTCCGTGGAGACGCAAG-3'	5'-TTGAGCCTTTGTAAATGGGCA-3'
<i>m Tgfb1</i>	5'-CTCCCGTGGCTTCTAGTGC-3'	5'-GCCTTAGTTTGGACAGGATCTG-3'
<i>m Nos2</i>	5'-GTTCTCAGCCCAACAATACAAGA-3'	5'-GTGGACGGGTCGATGTCAC-3'
<i>m Arg1</i>	5'-CTCCAAGCCAAAGTCCTTAGAG-3'	5'-AGGAGCTGTCATTAGGGACATC-3'
<i>m Cd206</i>	5'-CTCTGTTTCAGCTATTGGACGC-3'	5'-CGGAATTTCTGGGATTCAGCTTC-3'
<i>m Gzmb</i>	5'-CCACTCTCGACCCTACATGG-3'	5'-GGCCCCAAAGTGACATTTATT-3'
<i>m Il10</i>	5'-GCTCTTACTGACTGGCATGAG-3'	5'-CGCAGCTCTAGGAGCATGTG-3'
<i>m Il12</i>	5'-CCCAGCACTGCATAAACTAAGTATG-3'	5'-ATTCCAAAAGCTTCTGTTCTTCCAG-3'
<i>m Actb</i>	5'-GGCTGTATTCCCCTCCATCG-3'	5'-CCAGTTGGTAACAATGCCATGT-3'
<i>h CD86</i>	5'-CTGCTCATCTATAACCGTTACC-3'	5'-GGAAACGTCGTACAGTTCTGTG-3'
<i>h NOS2</i>	5'-GATCAAAAAGTGGGGCAGCG-3'	5'-CTCATCTGGAGGGTAGGCT-3'
<i>h ARG1</i>	5'-GTCTGTGGGAAAAGCAAGCG-3'	5'-CACCAGGCTGATTCTTCCGT-3'
<i>h CD206</i>	5'-GGGTTGCTATCACTCTCTATGC-3'	5'-TTTCTGTCTGTTGCCGTAGTT-3'
<i>h CCL2</i>	5'-GATCTCAGTGCAGAGGCTCG-3'	5'-TTTGCTTGTCAGGTGGTCC-3'
<i>h CCL5</i>	5'-CCCCATATTCTCGGACACC-3'	5'-TCCTTGACCTGTGGACGACT-3'
<i>h CXCL9</i>	5'-ATTGGAGTGCAAGGAACCCC-3'	5'-ATTTTCTCGCAGGAAGGGCT-3'
<i>h CXCL10</i>	5'-CTGAGCCTACAGCAGAGGAAC-3'	5'-GATGCAGGTACAGCGTACAGT-3'
<i>h CXCL11</i>	5'-TGCCCAAAGGAGTCCAACAA-3'	5'-TCCGATGGTAACCAGCCTTT-3'
<i>h TNF</i>	5'-CTCAAAAAGAGAATTGGGGGCTTAG-3'	5'-AGTTTGAATTCTTAGTGGTTGCCAG-3'
<i>h IL4</i>	5'-CTTTGCTGCCTCCAAGAACAC-3'	5'-GCGAGTGCCTTCTCATGGT-3'
<i>h CSF1</i>	5'-CCGGGGAAAGTGAAAGTTTGC-3'	5'-CAGACCAACAACAGCAGGGA-3'
<i>h CSF2</i>	5'-AATGTTTGACCTCCAGGAGCC-3'	5'-TCTGGGTTGCACAGGAAGTTT-3'
<i>h TGFBI</i>	5'-GGAGAGAGGACTGCGGATCT-3'	5'-CAGTGCCCAAGGTGCTCAATA-3'
<i>h IL12</i>	5'-TGCCCATGAGGTCATGGTG-3'	5'-CTTGGGTGGGTCAGGTTTGA-3'
<i>h IL10</i>	5'-AAAAGAAGGCATGCACAGCTCA-3'	5'-ACATGCGCCTTGATGTCTGG-3'
<i>h GZNB</i>	5'-GGCAGCCTTCTGAGAAGAT-3'	5'-GGAAAAGTCTGCATCTGCCCT-3'
<i>h IFNG</i>	5'-TGTCGCCAGCAGCTAAAACA-3'	5'-TGCAGGCAGGACAACCATTA-3'
<i>h PRF1</i>	5'-GTGGGACAATAACAACCCCAT-3'	5'-TGGCATGATAGCGGAATTTAGG-3'

<i>h ACTB</i>	5'-AGCCTCGCCTTTGCCGATCC-3'	5'-ACATGCCGGAGCCGTTGTCG-3'
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Supplementary Table 5. siRNA sequences used for cell transfection.

Gene	Sense sequences	Anti-sense sequences
siMyD88 #1	5'-GGCACCUGUGUCUGGUCUATT-3'	5'-UAGACCAGACACAGGUGCCTT-3'
siMyD88 #2	5'-CUGGAACAGACAAACUAUUCTT-3'	5'-GAUAGUUUGUCUGUCCAGTT-3'
