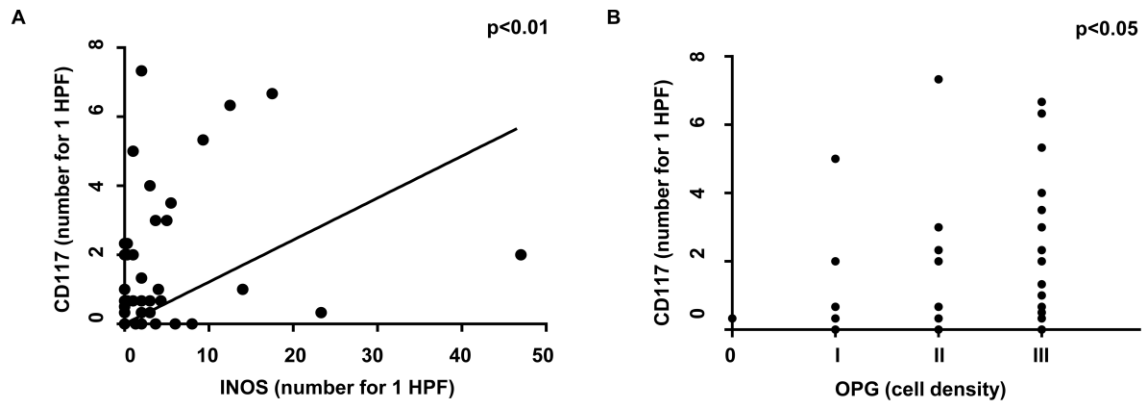


Dysregulation of macrophage polarization is associated with the metastatic process in osteosarcoma

Supplementary Material



Supplementary Figure 1: Correlation between CD117 expression and INOS (A) or OPG (B) expression in osteosarcoma samples

Supplementary Table 1: Primary antibodies used for the immunohistochemistry study

Human antigen	Antibody (host)	Clone	Source	Dilution
CD3	Polyclonal (rabbit)	A052	DAKO	1/100
CD20	Monoclonal (mouse)	M0755, L26	DAKO	1/250
CD4	Monoclonal (mouse)	NCL- L-CD4-368, 4B12	Novocastra	1/50
CD8	Monoclonal (mouse)	DM7103, C8/144B	DAKO	1/50
CD68	Monoclonal (mouse)	M0876, PG-M1	DAKO	1/200
INOS	Polyclonal (rabbit)	ab15323	Abcam	1/100
CD163	Monoclonal (mouse)	NCL-L-CD16, 10D6	Novocastra	1/100
CD117	Polyclonal (rabbit)	A4502, c-kit	DAKO	1/750
CD31	Monoclonal (mouse)	M082, JC70A	DAKO	1/50
SMA	Monoclonal (mouse)	F/MS-113, 1A4	MM France	1/400
CD146	Monoclonal (rabbit)	ab75769	Abcam	1/200
Ki-67	Monoclonal (mouse)	MIB-1	Dako	1/100
OPG	Polyclonal (rabbit)	ab9986	Abcam	1/50
RANKL	Polyclonal (goat)	AF626	R&D Systems	1/20

Supplementary Table 2: multivariate logistic regression analysis to study the relationship between different variables and the presence of metastases.

	Estimate	2.5 %	97.5 %	OR	IC 2.5%	IC 97.5%	Std. Error	z value	Pr(> z)
Male	2,517	0,0523	6,284256	12,39137	1,054394	536,0651	1,4917	1,687	0,0915
Age	0,08972	0,0112	0,210767	1,093868	1,011872	1,234625	0,0476	1,885	0,0594
Cd68	-0,19883	-0,512	0,022245	0,819689	0,599363	1,022495	0,12761	-1,558	0,1192
cd20	1,29536	-0,441	3,445052	3,652311	0,643191	31,34491	0,92546	1,4	0,1616
INOS	-0,80466	-1,732	-0,22411	0,44724	0,176863	0,799228	0,3704	-2,172	0,0298
CD117	0,44663	-0,172	1,207732	1,563036	0,84192	3,345886	0,32694	1,366	0,1719
OPG	-1,76788	-3,968	-0,38735	0,170694	0,018904	0,678856	0,84609	-2,089	0,0367
CD31 (density score)	1,44963	-0,091	3,256526	4,261537	0,912723	25,95919	0,8197	1,768	0,077
CD146 (density score)	1,55114	-0,458	4,542223	4,716844	0,63228	93,89932	1,25641	1,235	0,217

Supplementary Table 3: Cox model to test the relationship between different variables and survival time of patients

	Coef	Exp(coef)	Lower.95	Upper.95	p
Histological response to chemotherapy III	-1.5133	0.2202	0.07328	0.6617	0.00702
Metastasis	2.4825	11.9715	2.764	51.85	0.000903
Age	5.197 ^e -02	1.53 ^e +00	1.0271	1.0803	<0.0001
Sex	5.364 ^e -01	1.710 ^e +00	0.4807	6.0813	0.40738
CD3	-2.229 ^e -01	8.002 ^e -01	0.6122	1.0460	0.10287
CD4	1.030 ^e +00	2.801 ^e +00	1.3480	5.8190	0.00578
CD8	3.756 ^e -01	1.038 ^e +00	0.8835	1.2202	0.64840
CD68	-5.021 ^e -02	9.510 ^e -01	0.9052	0.9991	0.04609
INOS	-1.006 ^e -02	9.900 ^e -01	0.9310	1.0527	0.74837
CD163	-7.902 ^e -05	9.999 ^e -01	0.9055	1.1042	0.99875
CD117	1.642 ^e -01	1.178 ^e +00	0.7769	1.7875	0.43983
Ki-67	2.976e-01	1.030 ^e +00	0.9687	1.0957	0.34375