

Prognostic and mifamurtide-related signatures for localized osteosarcoma: an ISG/OS2 trial correlative study of tumor immune microenvironment (TME)

Emanuela Palmerini, MD, PhD

Sylvester Comprehensive Cancer Center
Miller School of Medicine, University of Miami
Miami, FL, USA



Disclosures

- E. Palmerini has served on advisory boards for Daiichy Sankyo, Deciphera Pharmaceuticals, Eusa Pharma, SynOx Therapeutics , Ipsen Biopharmaceuticals and Servier, outside the submitted work
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Presented by:

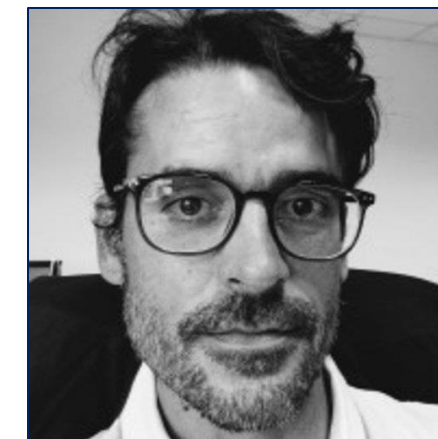
Emanuela Palmerini, MD, PhD

Background

GENE EXPRESSION SIGNATURE IN DIAGNOSTIC OSTEOSARCOMA SAMPLES OF 79 PATIENTS IN THE OS2006 PROTOCOL VALIDATED IN 82 PATIENTS IN THE TARGET DATASET

- Cohort pediatric and adult patients, diagnostic samples
- **Unsupervised classification stratified into favorable (G1) and unfavourable (G2) prognostic tumours**
- RNA seq – 15 gene set / Nanostring 37 gene set
- Gene involved in the **tumor micrenvironment**

Marchais A et al, Cancer Research 2022



Antonin Marchais



Nathalie Gaspar

79 pts
15 y (4.7–36.8 years)
80% nonmetastatic
76% good response

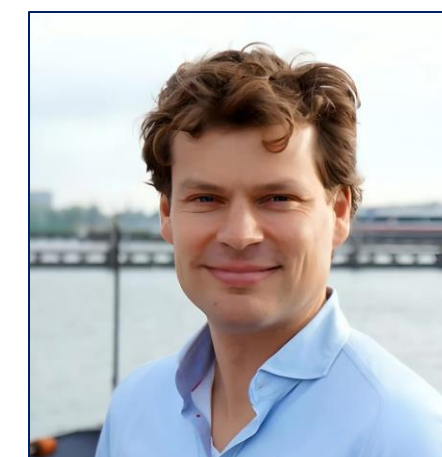
VALIDATION OF A FOCUSED GENE EXPRESSION SIGNATURE TO STRATIFY OSTEOSARCOMA IN AN INDEPENDENT PEDIATRIC AND ADOLESCENT COHORT: A WAY FORWARD FOR FUTURE TRIALS?

- Cohort pediatric and adult patients, diagnostic and relapse samples
- RNA seq – whole transcriptomics sequencing (KAPA) – **ribosome depleted**
- **With coefficient from the 15 gene signature**

van Ewijk R, J Prec Onc 2025



Lennart Kester



Roelof van Ewijk

41 pts
14 y (4-17 years)
56% nonmetastatic
34% good response

Background

PHASE 2 STUDY FOR NONMETASTATIC EXTREMITY HIGH-GRADE OSTEOSARCOMA IN PEDIATRIC AND AYA PATIENTS WITH A RISK-ADAPTED STRATEGY BASED ON P-GLYCOPROTEIN EXPRESSION: AN ITALIAN SARCOMA GROUP TRIAL (ISG/OS-2)

Pgp negative

M	P/A	M	P/A	
0	1	4	5	wks

M = methotrexate 12g/m2;
P = cisplatin 120 mg/m2;
A = adriamycin 75 mg/m2

Surgery

A°	MM	P	A	MM	P	A°	MM	P°	MM				
<hr/>													
9	12	13	14	17	20	21	22	25	28	29	30	33	34
weeks													

A° = adriamycin 90 mg/m2

Pgp positive

M	P/A	M	P/A	
0	1	4	5	wks

M = methotrexate 12g/m2;
P = cisplatin 120 mg/m2;
A = adriamycin 75 mg/m2

Good
Response
≥90%

Surgery

A°	MM	P	A	MM	P	A°	MM	P°	MM				
<hr/>													
9	12	13	14	17	20	21	22	25	28	29	30	33	34
weeks													

Poor
Response
<90%

A°	HDIFO	HDIFO	HDIFO	HDIFO
9	12	15	18	21
weeks				

HDIFO= ifosfamide 3 gr/m2/die, day 1-5

MIFAMURTIDE

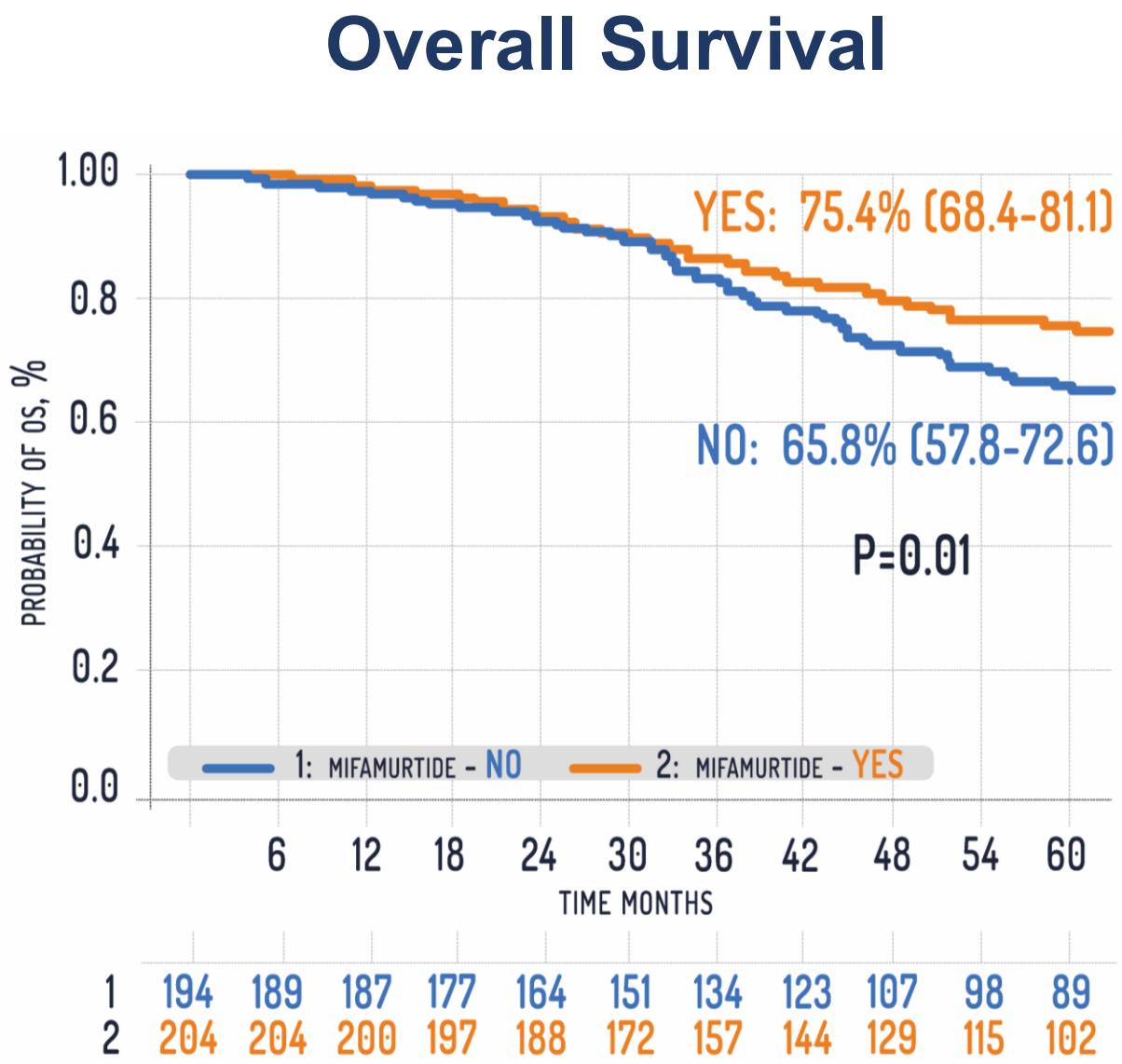
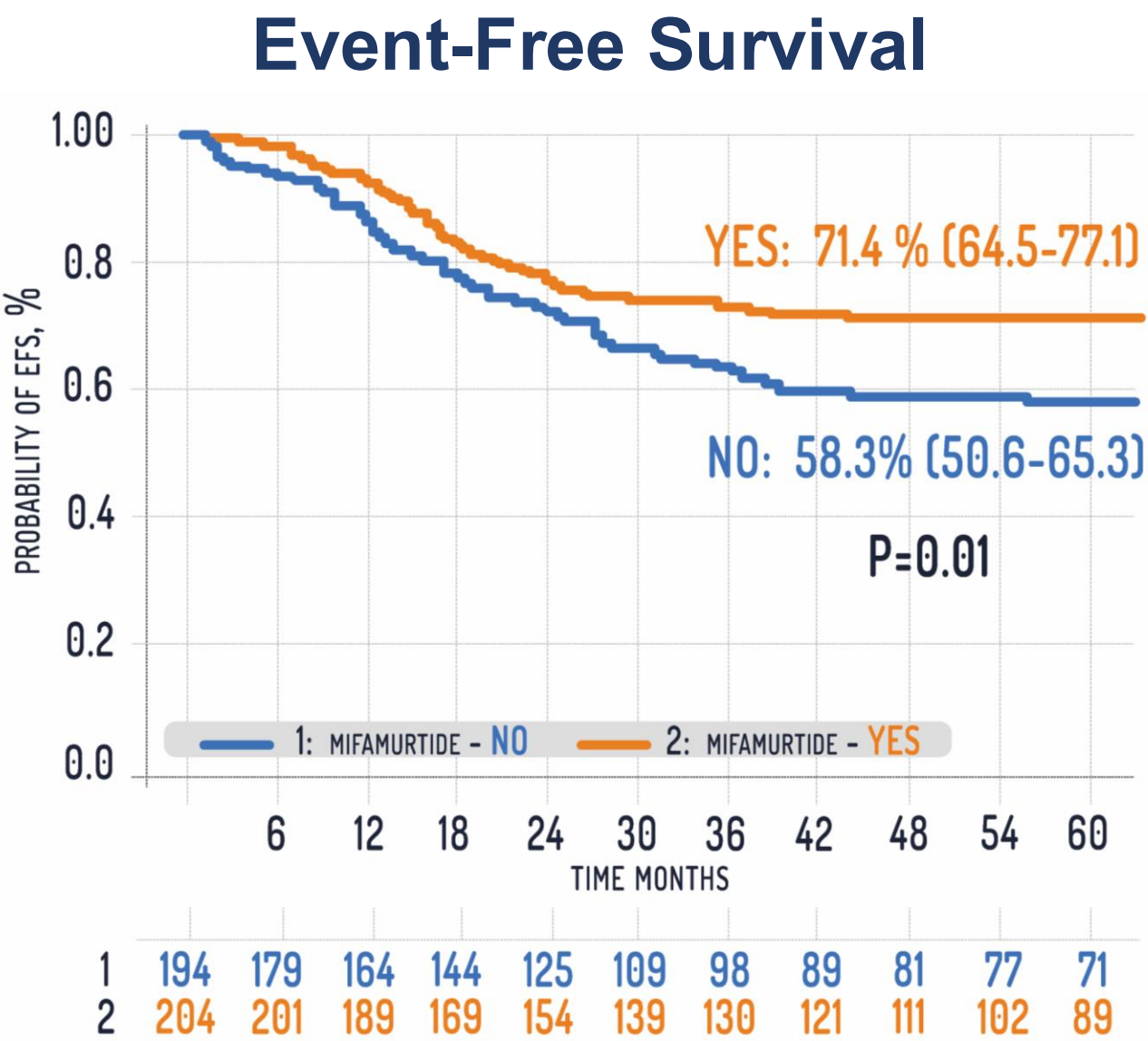
2 mg/m2 twice/week x 3 months,
than weekly for 6 months

Design	Phase II, uncontrolled
Endpoint	EFS
Main inclusion	Non metastatic osteosarcoma
Age	4 (4-38)
N. of patients	279
Median follow up	51 months (IQR 36-65)

Palmerini E et al, Cancer 2022

Background

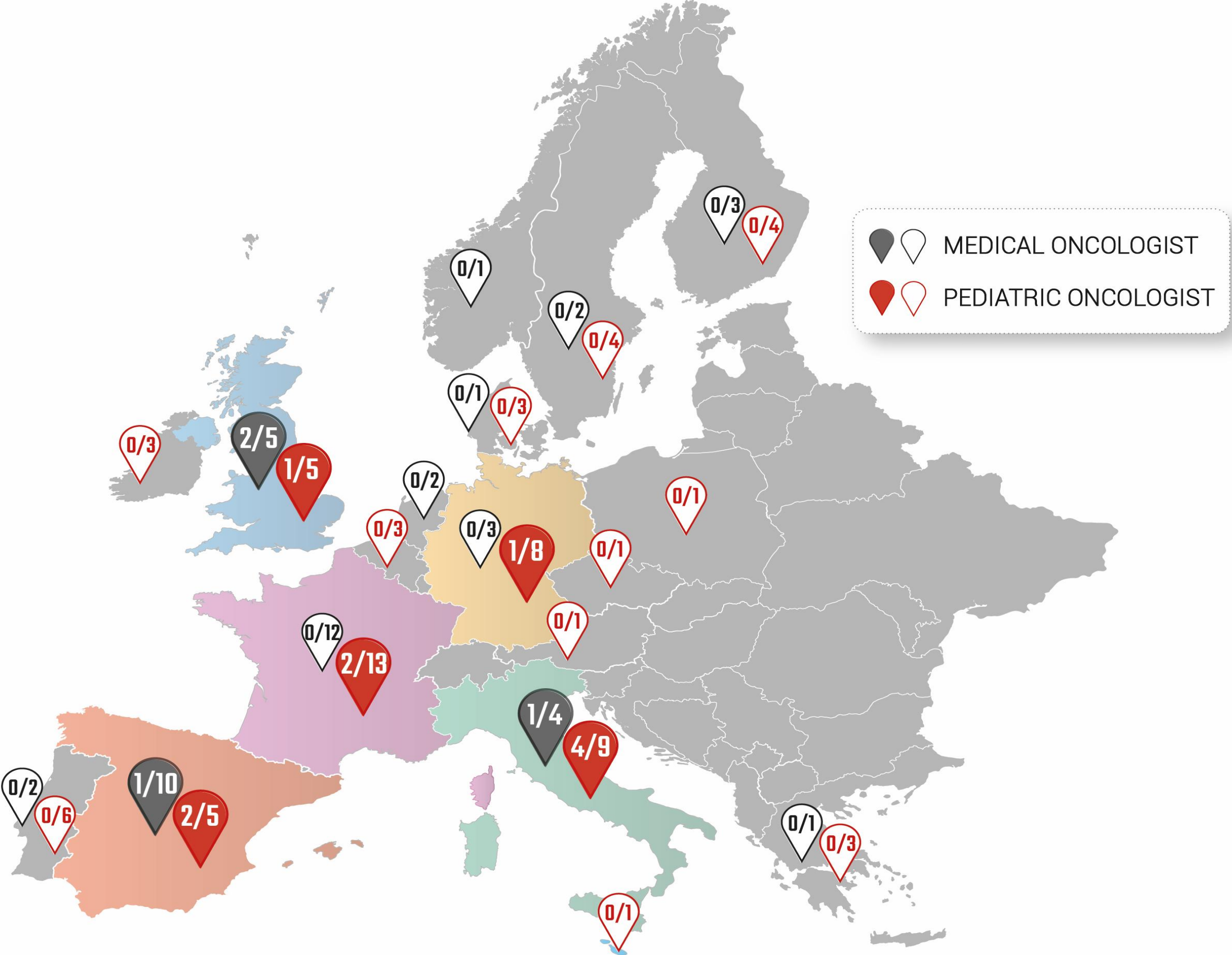
IS THERE A ROLE FOR MIFAMURTIDE IN NON-METASTATIC HIGH-GRADE OSTEOSARCOMA? RESULTS FROM THE ITALIAN SARCOMA GROUP (ISG/OS-2) AND SPANISH SARCOMA GROUP (GEIS-33) TRIALS



Design	Phase II, exploratory
Endpoint	EFS by mifamurtide
Main inclusion	Non metastatic osteosarcoma
Age	4 (4-40)
N. of patients	398
Median follow up	70 months (IQR 49-90)

Palmerini E et al, J Clin Onc 2025

Background



FOSTER Survey

Fight Osteosarcoma Through European Research

DO YOU PRESCRIBE MIFAMURTIDE?

Medical Oncologist	4/46	9%
Pediatric Oncologist	10/70	14%



Mifamurtide cost in Europe 142.999 euro/patient

Salt Lake City, Utah, 28 June 2028

PROGNOSTIC AND MIFAMURTIDE-RELATED SIGNATURES FOR LOCALIZED OSTEOSARCOMA: AN ISG/OS2 TRIAL CORRELATIVE STUDY OF TUMOR IMMUNE MICROENVIRONMENT (TME)



**University of Bologna,
Bologna, Italy**

**IRCCS Istituto Ortopedico Rizzoli,
Bologna, Italy**



**European Institute Of Oncology,
Milan Italy**

Presented by:

Emanuela Palmerini, MD, PhD



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PROGNOSTIC AND MIFAMURTIDE-RELATED SIGNATURES FOR LOCALIZED OSTEOSARCOMA:
AN ISG/OS2 TRIAL CORRELATIVE STUDY OF TUMOR IMMUNE MICROENVIRONMENT (TME)

Results

Demography

62 pts
14 y (5-40 years)
100% nonmetastatic
50% good response

	n	%
	62	100
Age		
median	14 years (5-40)	
0-14 years	32	51.6
> 14 years	30	48.4
Gender		
Male	43	69.4
Female	19	30.6
Serum alkaline phosphatase (ALP)		
High	16	25.8
Normal	46	74.2
Histologic Response		
Good response (GRs)	31	50
Poor response (PRs)	31	50
Mifamurtide		
Yes	33	53
No	29	47

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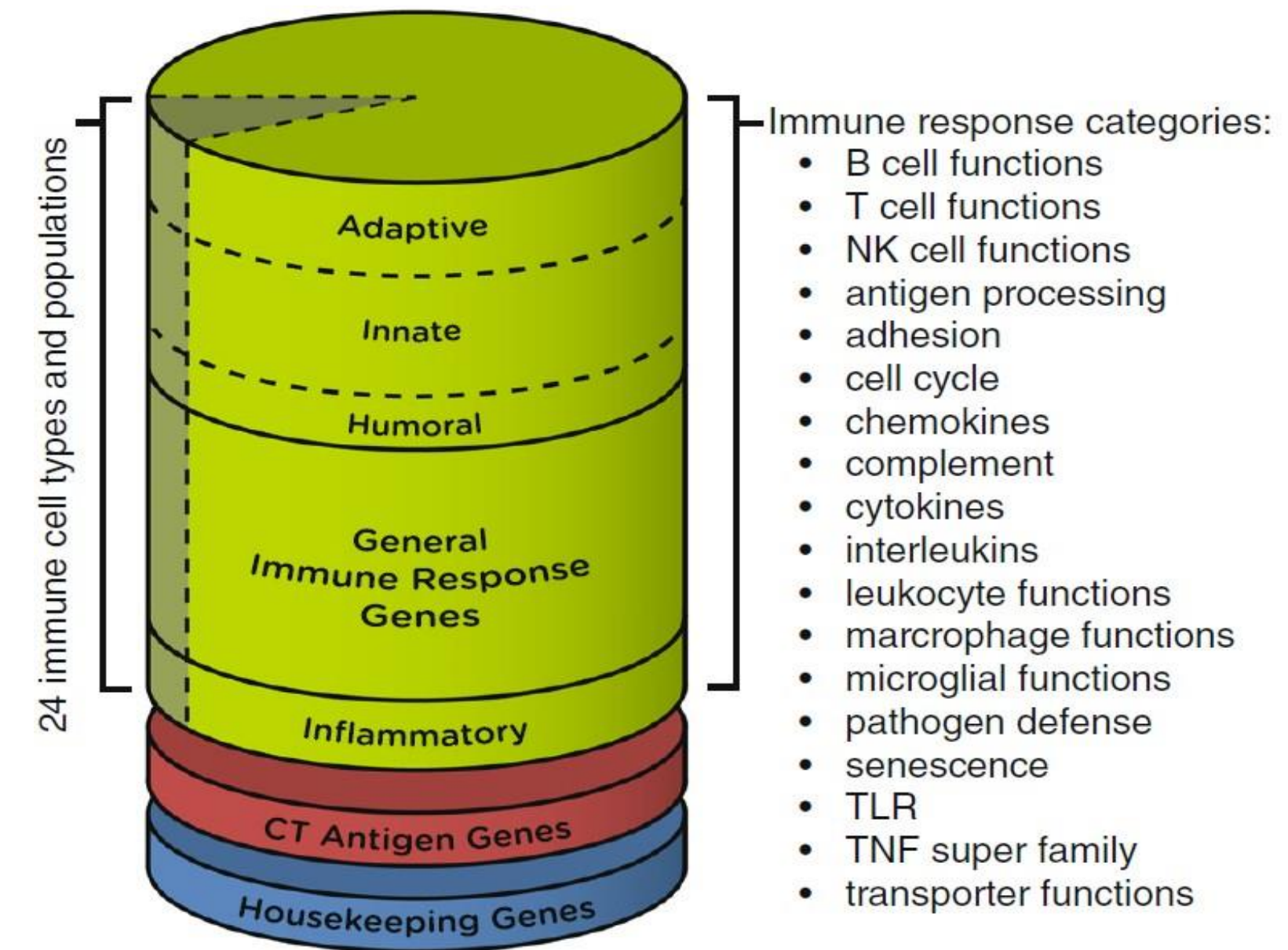
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Methods

PanCancer Immune Profiling Panel –

Nanostring

- **Input** non decalcified FFPE, bone, pre-treatment, biopsy
- **770 genes:** 730 TME + 40 House Keeping



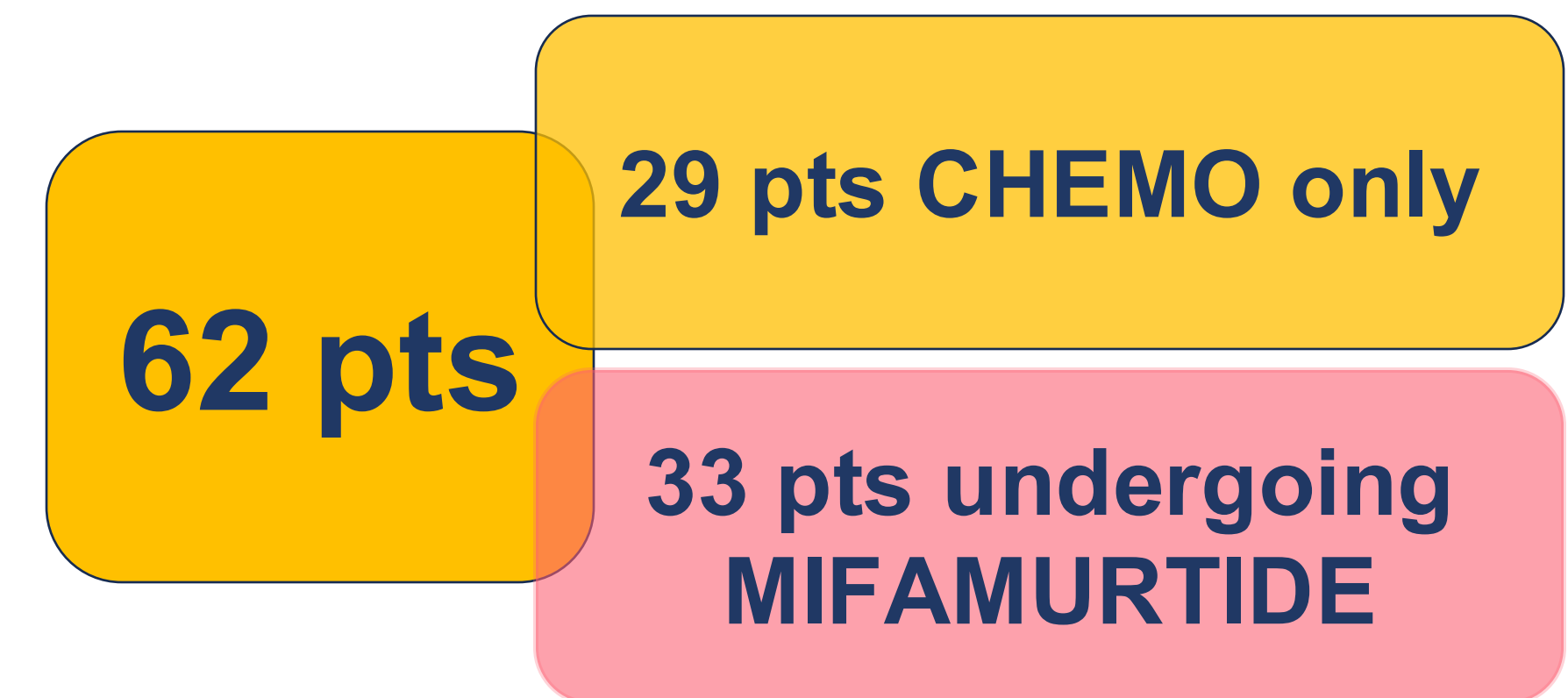
FFPE Formalin-fixed paraffin embadded
TME Tumor micro-environment

PROGNOSTIC AND MIFAMURTIDE-RELATED SIGNATURES FOR LOCALIZED OSTEOSARCOMA: AN ISG/OS2 TRIAL CORRELATIVE STUDY OF TUMOR IMMUNE MICROENVIRONMENT (TME)

Methods

Primary Objective

1 – identifying a prognostic signature
in all ISG/OS2 patients



2 – identifying a predictive signature in
the cohort of patients undergoing
mifamurtide



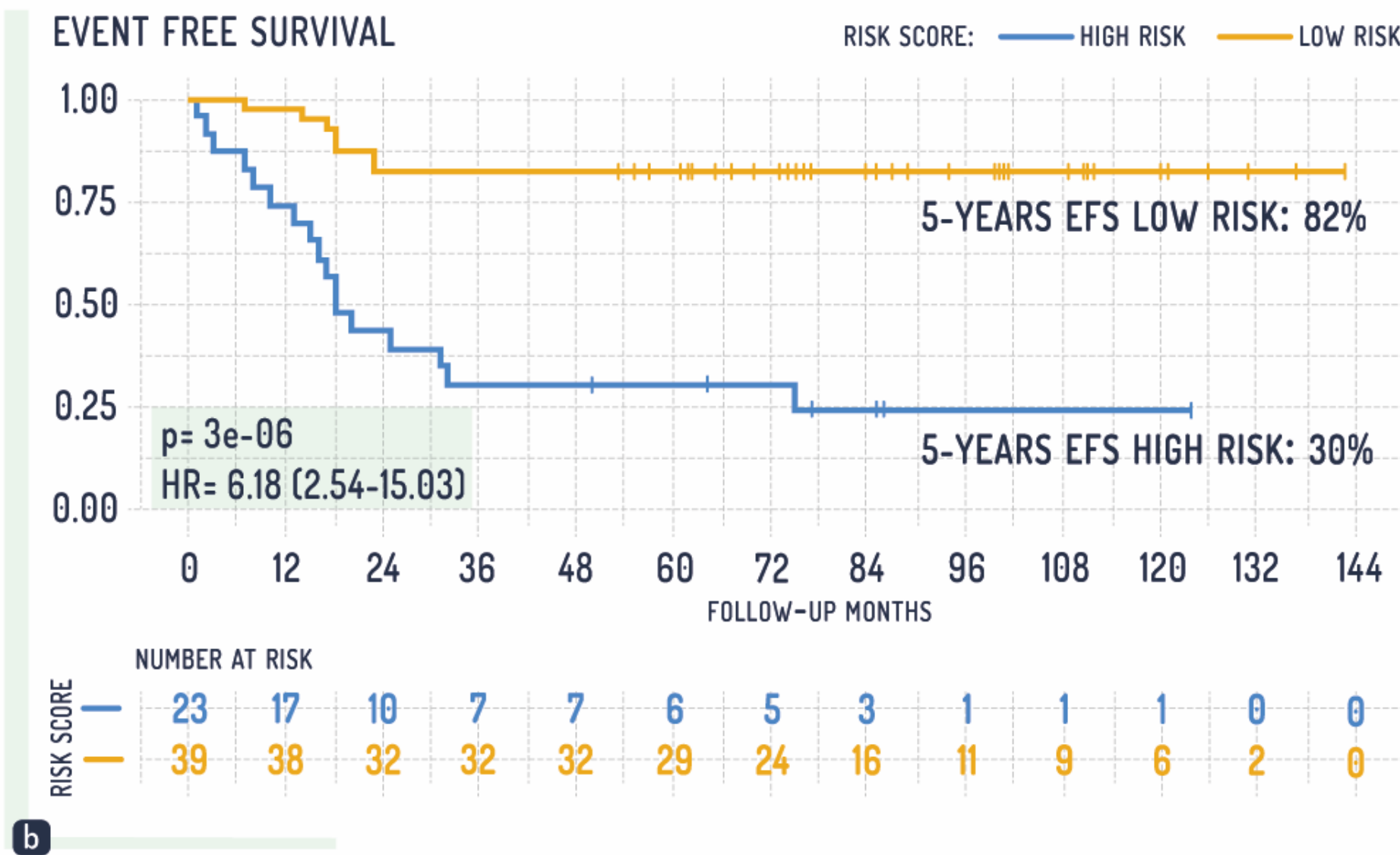
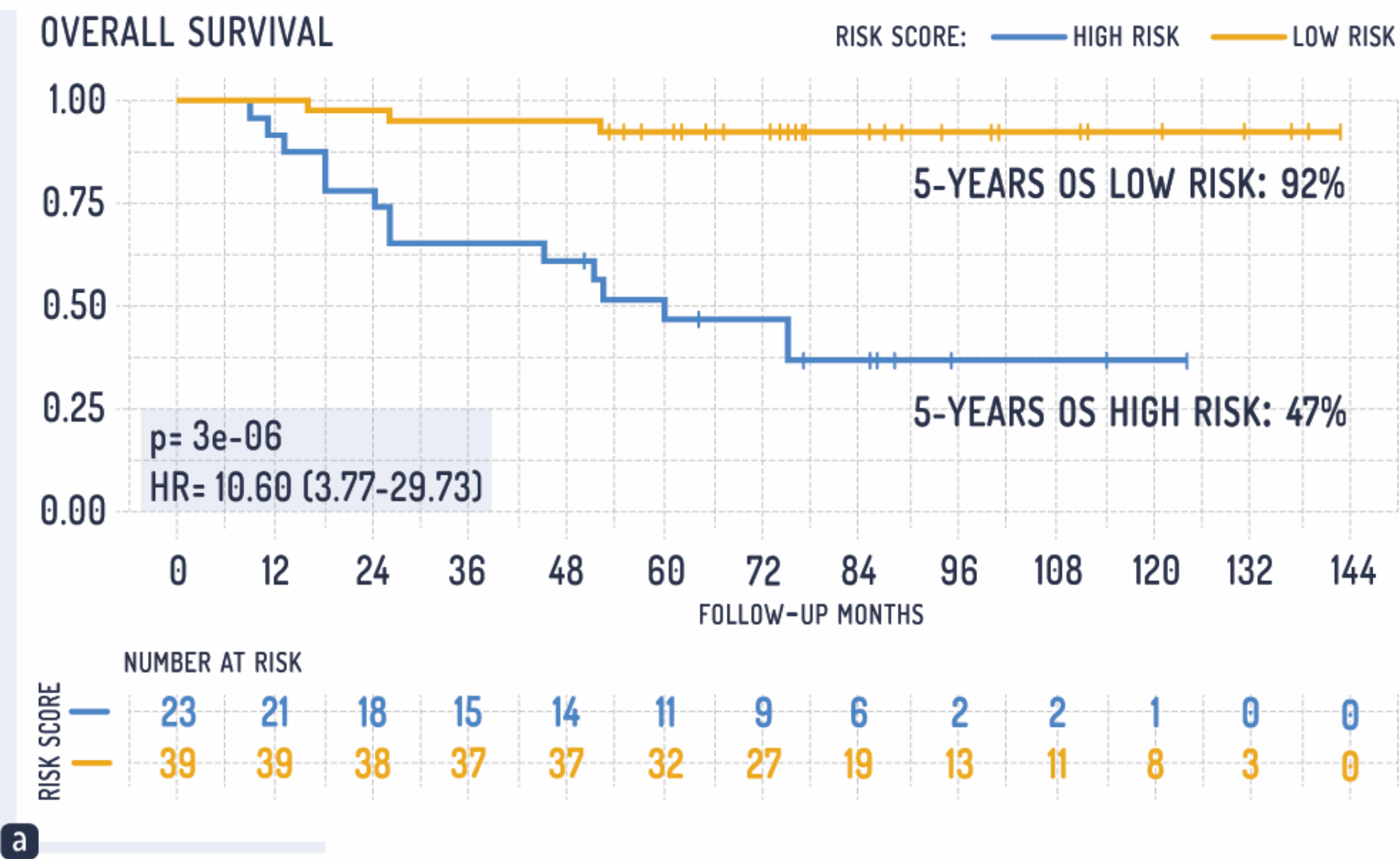
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Discovery Cohort
62 pts

100% nonmetastatic

PROGNOSTIC SIGNATURE IN NON-METASTATIC OSTEOSARCOMA PATIENTS

FIG. 1A,B. 21-GENE SIGNATURE IN 62 PRIMARY OSTEOSARCOMA – DISCOVERY COHORT



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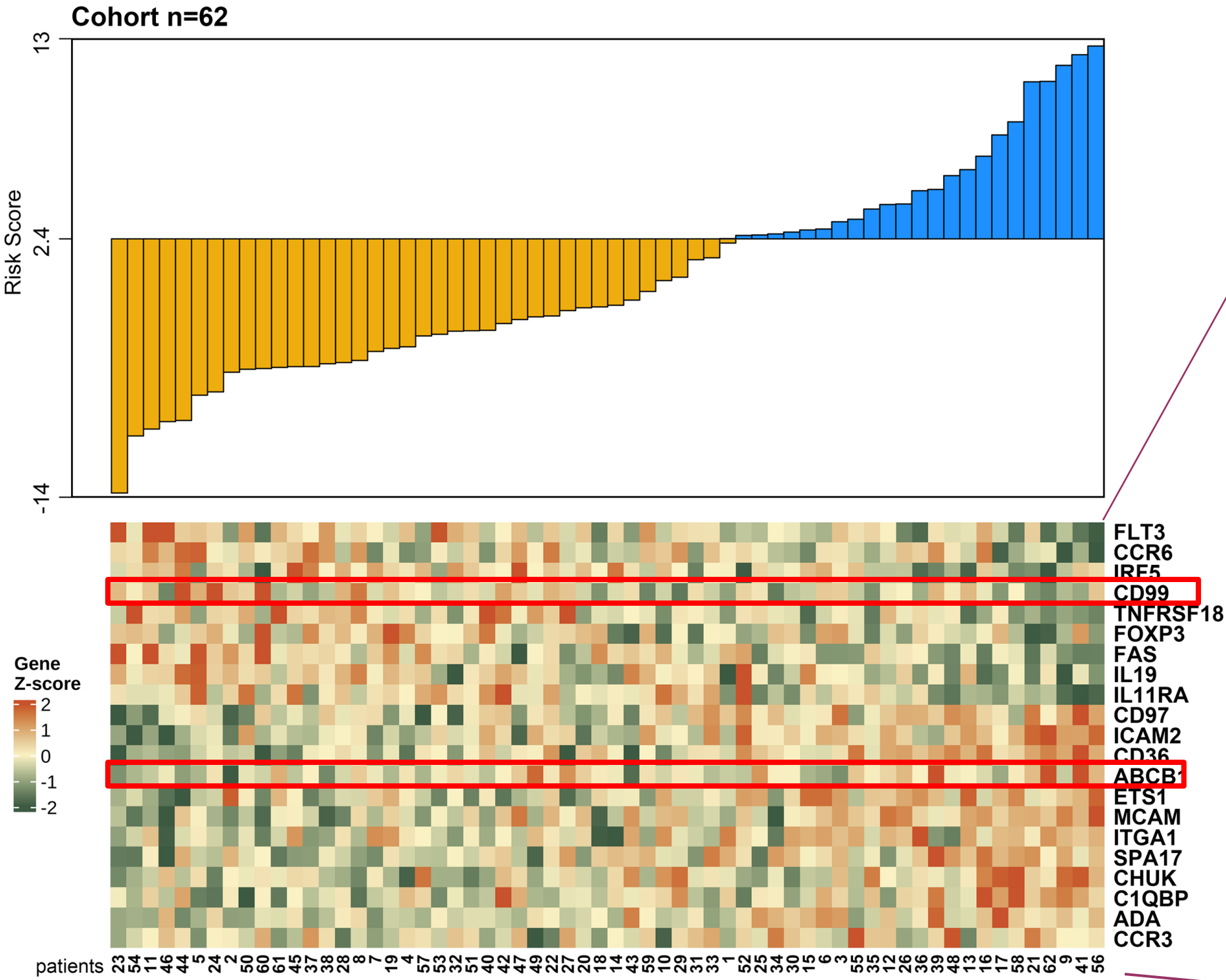
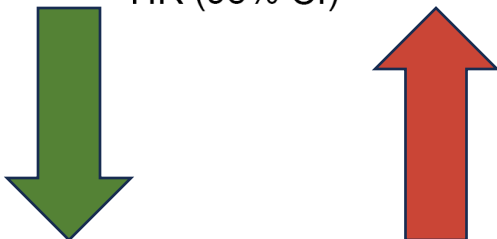
PROGNOSTIC AND MIFAMURTIDE-RELATED SIGNATURES FOR LOCALIZED OSTEOSARCOMA:
AN ISG/OS2 TRIAL CORRELATIVE STUDY OF TUMOR IMMUNE MICROENVIRONMENT (TME)

CARISBO
FOUNDATION

21- Genes Prognostic Signature

Cut Off
Risk Score 2.41

Gene	HR (95% CI)	p-value	
ABCB1	1.49 [1.04 - 2.14]	0.029	
ADA	1.65 [1.09 - 2.49]	0.017	
C1QBP	1.55 [1.01 - 2.40]	0.047	
CCR3	1.95 [1.24 - 3.09]	0.004	
CCR6	0.57 [0.37 - 0.90]	0.016	
CD36	2.46 [1.39 - 4.36]	0.002	
CD97	2.14 [1.24 - 3.72]	0.007	
CD99	0.53 [0.29 - 0.96]	0.035	
CHUK	1.63 [1.04 - 2.54]	0.032	
ETS1	1.76 [1.08 - 2.86]	0.023	
FAS	0.56 [0.33 - 0.97]	0.040	
FLT3	0.58 [0.35 - 0.98]	0.042	
FOXP3	0.59 [0.35 - 1.00]	0.051	
ICAM2	2.17 [1.22 - 3.84]	0.008	
IL11RA	0.55 [0.32 - 0.96]	0.034	
IL19	0.62 [0.40 - 0.96]	0.034	
IRF5	0.56 [0.36 - 0.86]	0.009	
ITGA1	1.69 [1.00 - 2.84]	0.048	
MCAM	1.89 [1.10 - 3.25]	0.021	
SPA17	1.73 [1.09 - 2.77]	0.021	
TNFRSF18	0.50 [0.28 - 0.88]	0.017	



FLT3
CCR6
IRF5
CD99
TNFRSF18
FOXP3
FAS
IL19
IL11RA
CD97
ICAM2
CD36
ABCB1
ETS1
MCAM
ITGA1
SPA17
CHUK
C1QBP
ADA
CCR3

Results

RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

Multivariate Analysis
For Prognostic Signature

Overall Survival Variable	Univariate Cox		Multivariate Cox	
	HR (95% CI)	P value	HR (95% CI)	P value
Discovery cohort (n = 62)				
Gene signature (high- vs low-risk)	10.60(3.77–29.73)	0.000003	9.83(2.74-35.2)	0.00044
Histologic Response (Prs vs Grs)	3.95(1.28-12.11)	0.016	4.21(1.24-14.21)	0.02
Age (0-14 vs > 14 years)	0.99(0.93-1.04)	0.63	0.97(0.90-1.04)	0.51
Serum alkaline phosphatase (normal vs high)	0.46(0.17-1.22)	0.12	0.55 (0.17-1.74)	0.32

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Results

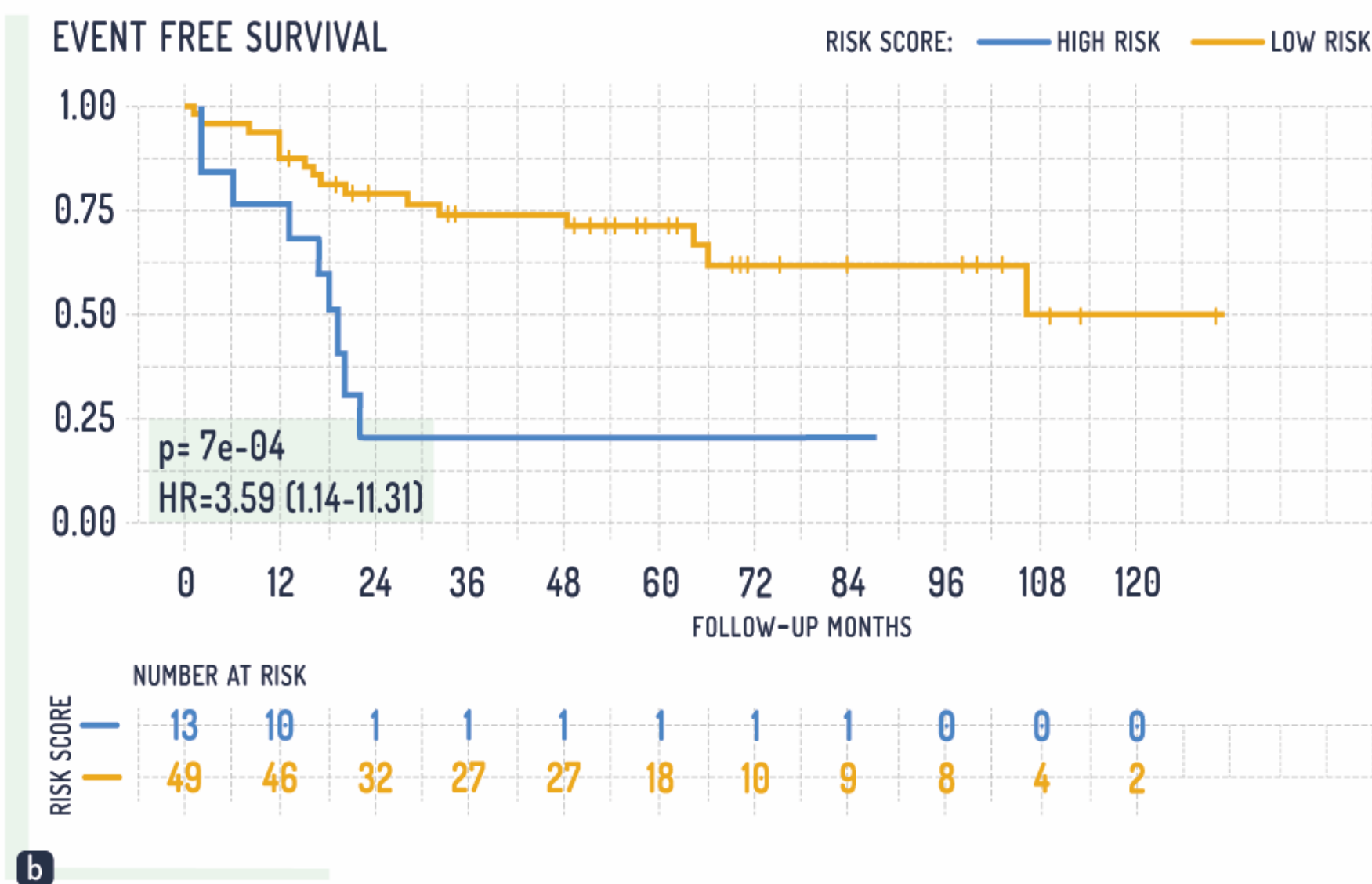
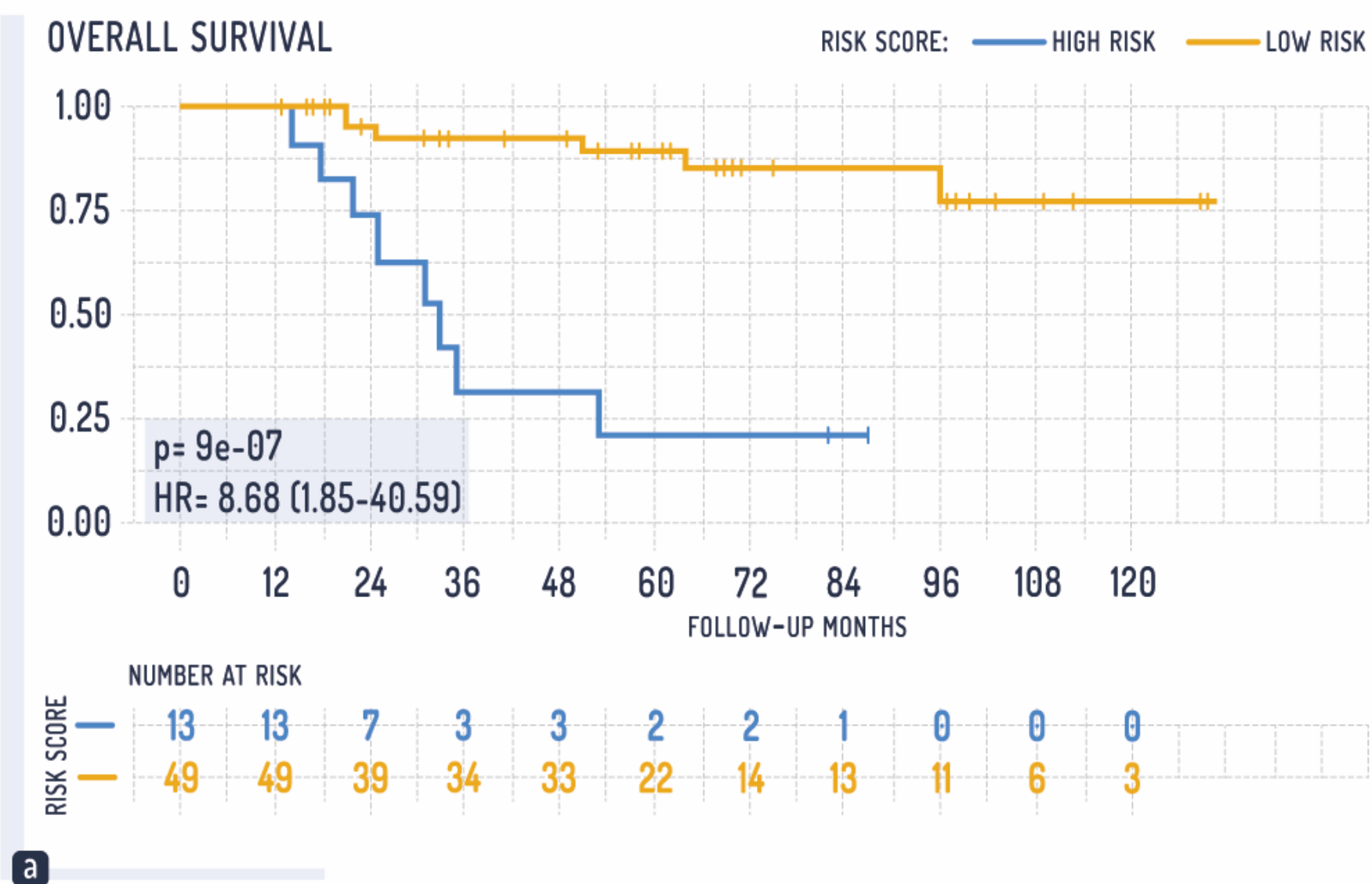
RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

Validation Cohort 1
62 pts – TARGET – OS TCGA

100% nonmetastatic

PROGNOSTIC SIGNATURE IN NON-METASTATIC OSTEOSARCOMA PATIENTS

FIG. 2A,B. 21-GENE SIGNATURE IN THE TARGET-OS TCGA – VALIDATION COHORT



TARGET Therapeutically Applicable
Research to Generate Effective Treatment

OS Osteosarcoma

TCGA The Cancer Genome Atlas

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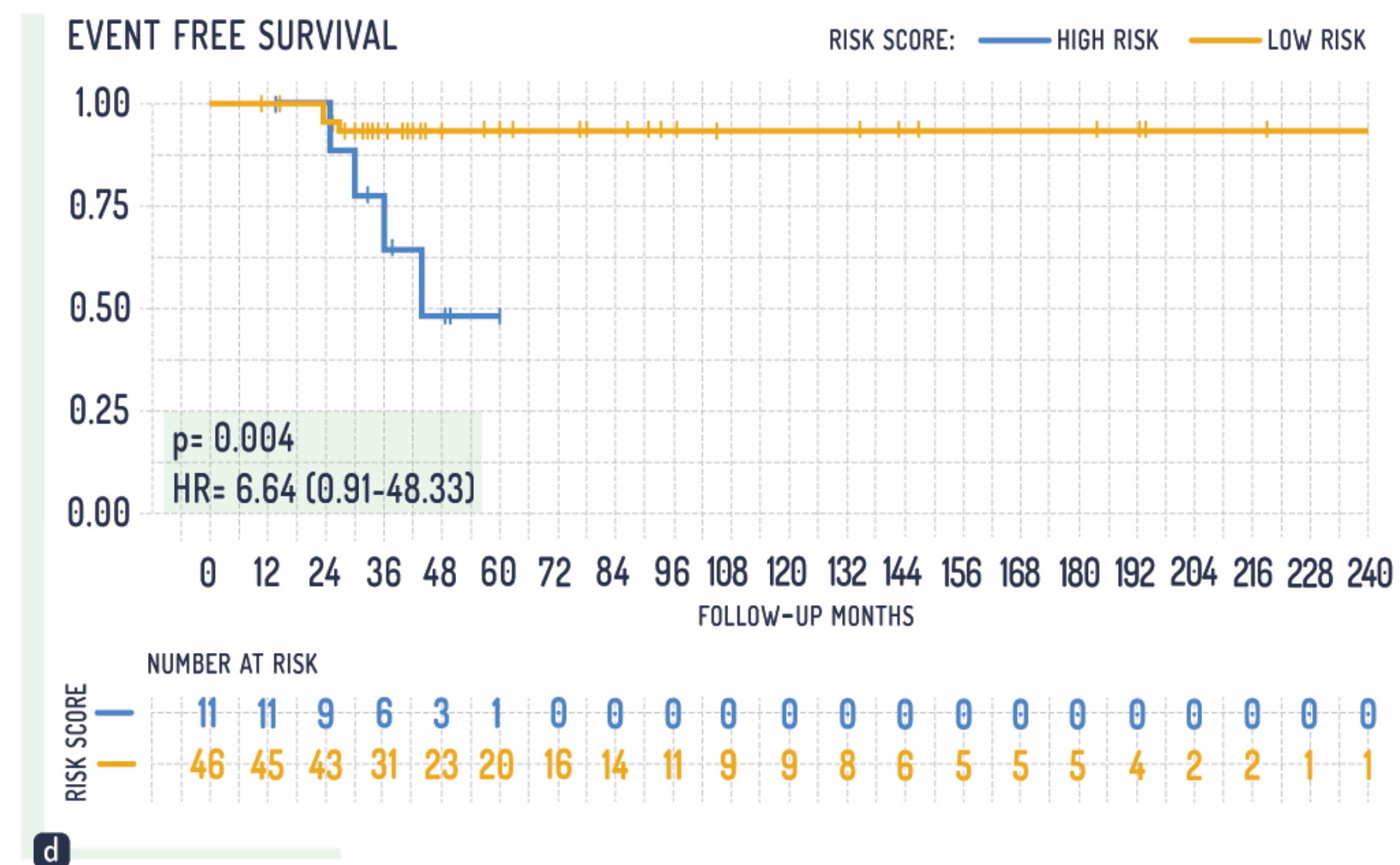
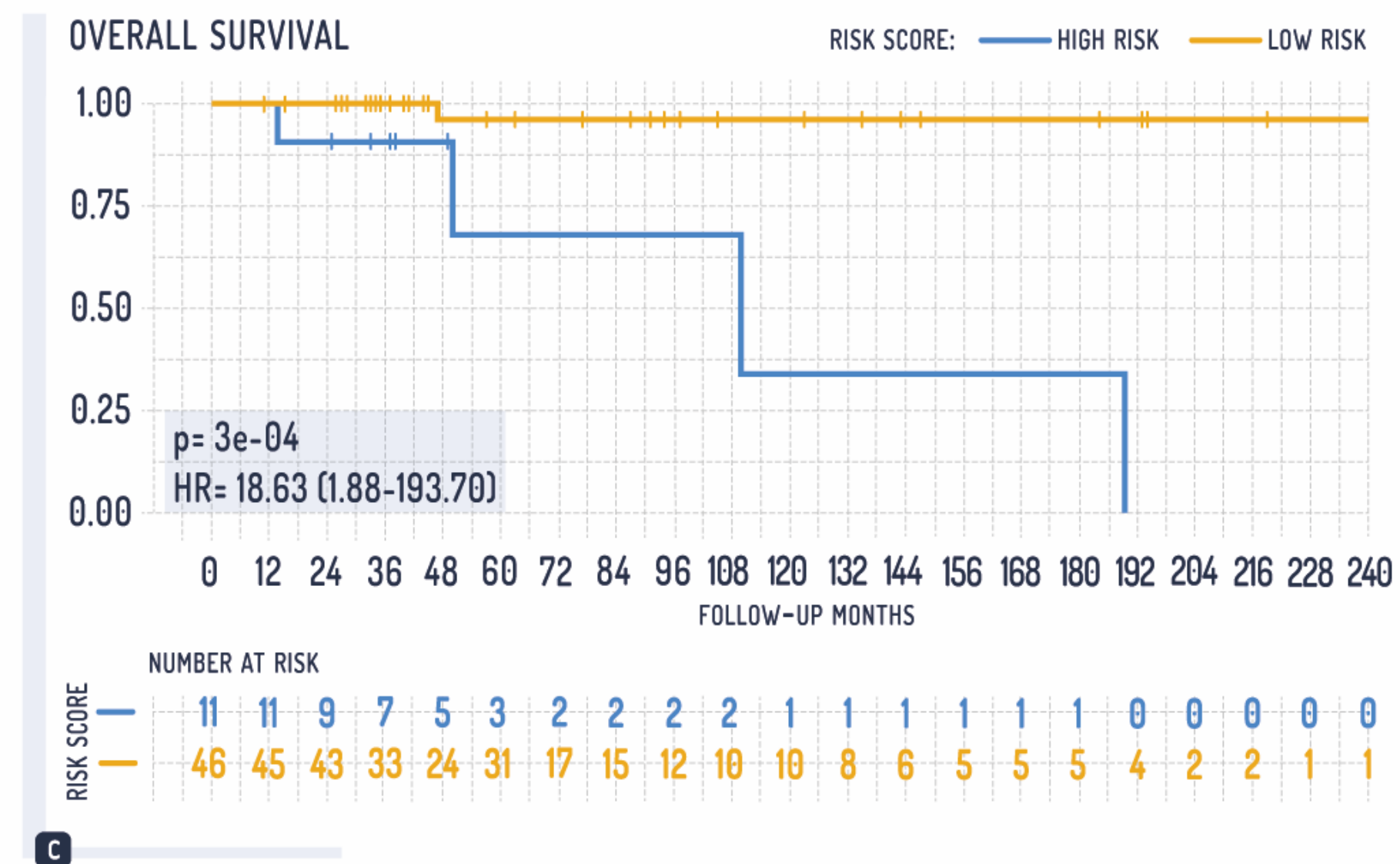
Results

RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

Validation Cohort 2
57 pts – GSE 33382

100% nonmetastatic

FIG. 2C,D. 21-GENE SIGNATURE IN THE GSE33382 – VALIDATION COHORT



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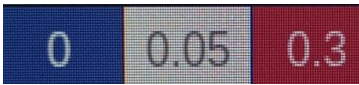
Results



RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

Cibersort X

LM-22 signature

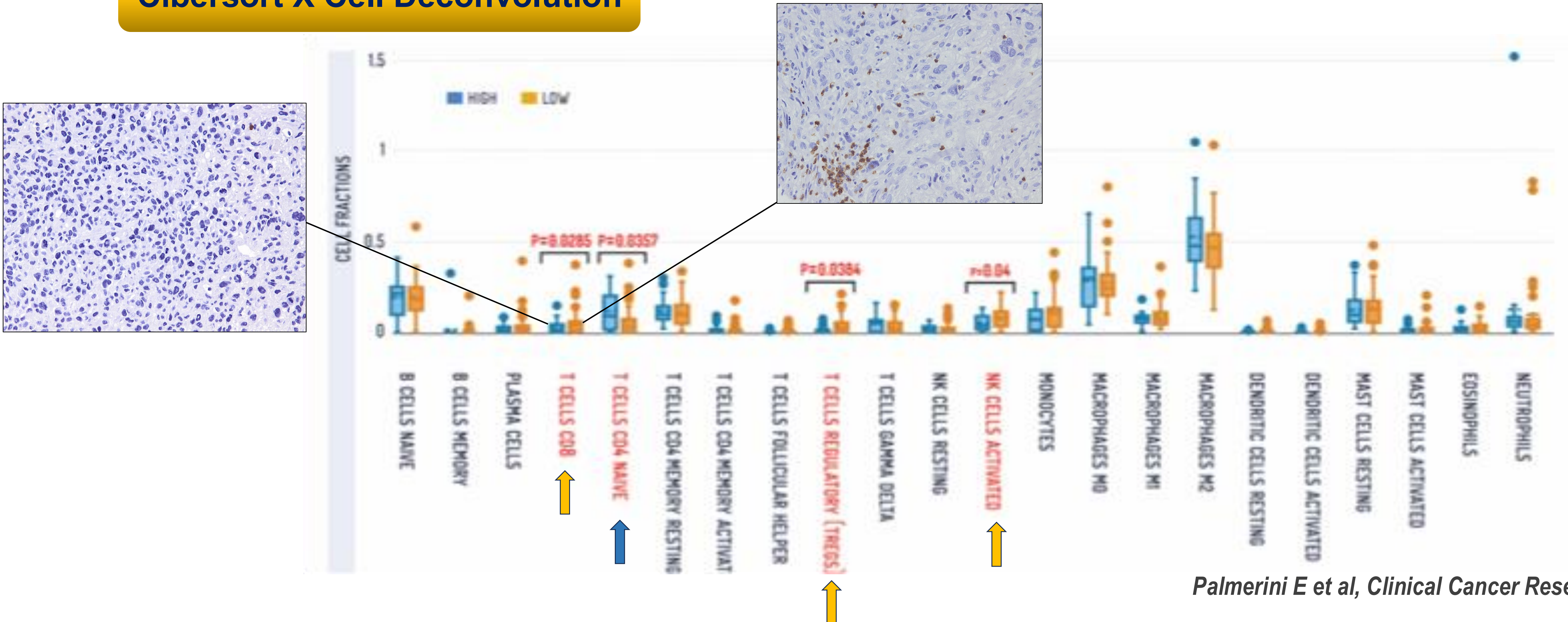


	B cells naive	B cells memory	Plasma cells	T cells CD8	T cells CD4 naive	T cells CD4 memory resting	T cells CD4 memory activated	T cells follicular helper	T cells regulatory (Tregs)	T cells gamma delta	NK cells resting	NK cells activated	Monocytes	Macrophages M0	Macrophages M1	Macrophages M2	Dendritic cells resting	Dendritic cells resting	Mast cells activated	Mast cells activated	Eosinophils	Neutrophils	P-value	Correlation	RMSE	
Pt_1	0.239	0	0.121	0.056	0.044	0.068	0	0	0	0.112	0	0.048	0.253	0.206	0.095	0.487	0	0	0.138	0.034	0	0.068	0.000	0.652	0.831	
Pt_2	0.106	0	0.006	0.036	0.018	0.048	0	0.024	0.005	0.005	0.019	0.022	0.282	0.237	0.04	0.752	0	0	0.082	0.055	0.022	0.007	0.000	0.675	0.815	
Pt_3	0.043	0	0	0	0.148	0.122	0	0	0	0.098	0	0.077	0	0.459	0.069	0.516	0	0	0.133	0	0	0.028	0.000	0.716	0.809	
Pt_4	0.184	0	0.02	0.024	0.087	0.054	0	0	0.093	0	0.055	0.038	0.248	0.172	0.192	0.47	0	0	0.011	0.025	0.028	0.048	0.000	0.760	0.758	
Pt_5	0.126	0	0.031	0.046	0	0.007	0	0.065	0.03	0	0.074	0.1	0.209	0.055	0.314	0.007	0	0	0.227	0.023	0	0.01	0.000	0.744	0.795	
Pt_6	0.128	0	0.007	0	0.024	0.115	0	0	0.014	0.059	0.001	0.006	0.112	0.361	0.086	0.75	0	0	0.06	0	0.005	0.049	0.000	0.700	0.807	
Pt_7	0.144	0	0	0.225	0	0.006	0	0	0.119	0.151	0	0.027	0.092	0.099	0.126	0.55	0.009	0	0.285	0	0.063	0.028	0.000	0.624	0.827	
Pt_8	0.289	0	0.071	0.022	0.1	0.147	0	0	0.011	0.031	0	0.095	0	0.211	0.077	0.66	0	0	0.127	0	0.056	0.000	0.785	0.764	0.764	
Pt_9	0.292	0	0	0	0.279	0.113	0.009	0	0	0.096	0.001	0.003	0	0.655	0.109	0.229	0	0	0.119	0	0.088	0.000	0.591	0.850		
Pt_10	0.256	0	0.003	0.022	0	0.038	0	0.003	0.03	0	0.031	0.018	0.051	0.257	0.058	0.314	0	0	0.159	0	0.021	0.000	0.714	0.804		
Pt_11	0.176	0	0.022	0.04	0	0.209	0	0	0.022	0	0	0.162	0.11	0.302	0.087	0.247	0.029	0	0	0.203	0.089	0.04	0.000	0.792	0.731	
Pt_12	0.311	0	0	0	0.029	0.088	0	0	0.043	0	0.056	0.012	0.018	0.426	0.106	0.358	0	0	0.198	0	0.078	0.000	0.720	0.800		
Pt_13	0.194	0	0.035	0	0.226	0.095	0.039	0	0	0.057	0.054	0	0.015	0.148	0.064	0.429	0.002	0	0.064	0	0.032	0.041	0.000	0.547	0.861	
Pt_14	0.162	0	0	0.001	0.03	0.099	0.035	0	0.01	0.019	0.045	0.026	0.04	0.339	0.093	0.487	0	0	0.099	0	0	0.08	0.000	0.782	0.782	
Pt_15	0.064	0	0	0.022	0	0.071	0	0	0.003	0.009	0	0.033	0.142	0.2	0.053	0.703	0	0	0.019	0.046	0.023	0.027	0.000	0.734	0.778	
Pt_16	0.262	0	0.083	0	0.293	0.138	0.091	0	0	0.053	0.033	0	0.183	0.324	0.074	0.544	0	0	0.02	0	0.06	0.029	0.002	0.466	0.897	
Pt_17	0.086	0	0	0.064	0.098	0.085	0	0	0.012	0	0.009	0.036	0	0.335	0.09	0.794	0.004	0	0.097	0.029	0.015	0.061	0.000	0.753	0.781	
Pt_18	0	0.039	0.058	0.111	0.184	0.066	0.014	0	0	0.014	0.072	0.058	0.08	0.414	0.213	0.769	0.015	0	0.133	0	0	0.021	0.000	0.746	0.777	
Pt_19	0.151	0	0	0.051	0.005	0	0	0.045	0.052	0.043	0	0.034	0.19	0.44	0.097	0.386	0	0	0.479	0	0.047	0	0.000	0.727	0.806	
Pt_20	0.212	0	0.018	0	0.209	0.272	0	0	0	0.094	0	0.147	0.137	0.802	0.036	0.416	0	0	0	0	0.031	0	0.000	0.693	0.810	
Pt_21	0.095	0	0.014	0	0.198	0.066	0	0	0	0.127	0	0.088	0.055	0.349	0.07	0.849	0	0	0.019	0.022	0	0.103	0.000	0.772	0.767	
Pt_22	0.18	0	0.001	0.028	0.04	0.181	0	0	0	0.053	0.024	0.01	0	0.137	0.018	1.034	0	0.004	0.364	0	0.018	0.013	0.000	0.714	0.790	
Pt_23	0.134	0	0.004	0.052	0	0.045	0.075	0	0.011	0	0.011	0.042	0.307	0.114	0.067	0.517	0.001	0.013	0.31	0	0	0.006	0.000	0.630	0.837	
Pt_24	0.159	0	0	0.05	0.059	0.183	0	0	0	0.002	0	0.148	0.043	0.378	0.075	0.533	0.003	0	0.129	0	0.03	0.05	0.000	0.808	0.759	
Pt_25	0.412	0	0.025	0.012	0.065	0.299	0	0	0	0.008	0.162	0	0.032	0.219	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817
Pt_26	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_27	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_28	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_29	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_30	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_31	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_32	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_33	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_34	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_35	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_36	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_37	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_38	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_39	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_40	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_41	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_42	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_43	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_44	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_45	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_46	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_47	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_48	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0	0.055	0.014	0.042	0.005	0.000	0.624	0.817	
Pt_49	0.238	0	0.003	0.018	0.038	0.238	0.061	0	0	0.033	0.012	0.003	0.238	0.064	0.113	0.38	0.015	0								

Results

RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

Cibersort X Cell Deconvolution



Methods

RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

Primary Objective

1 – identifying a prognostic signature in all ISG/OS2 patients

62 pts

29 pts CHEMO only

**33 pts undergoing
MIFAMURTIDE**

2 – identifying a predictive signature in the cohort of patients undergoing mifamurtide

**33 pts undergoing
MIFAMURTIDE**

Results

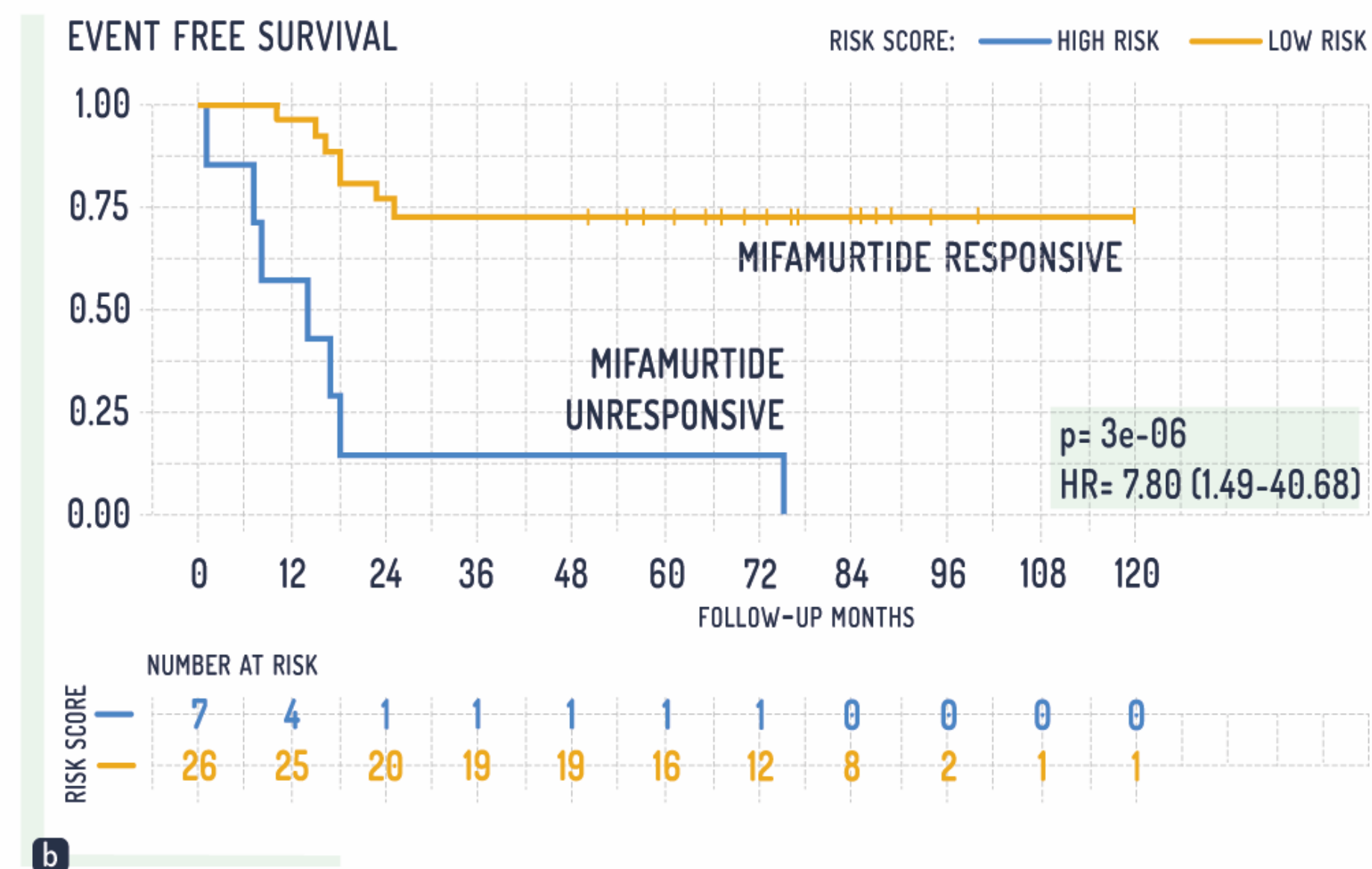
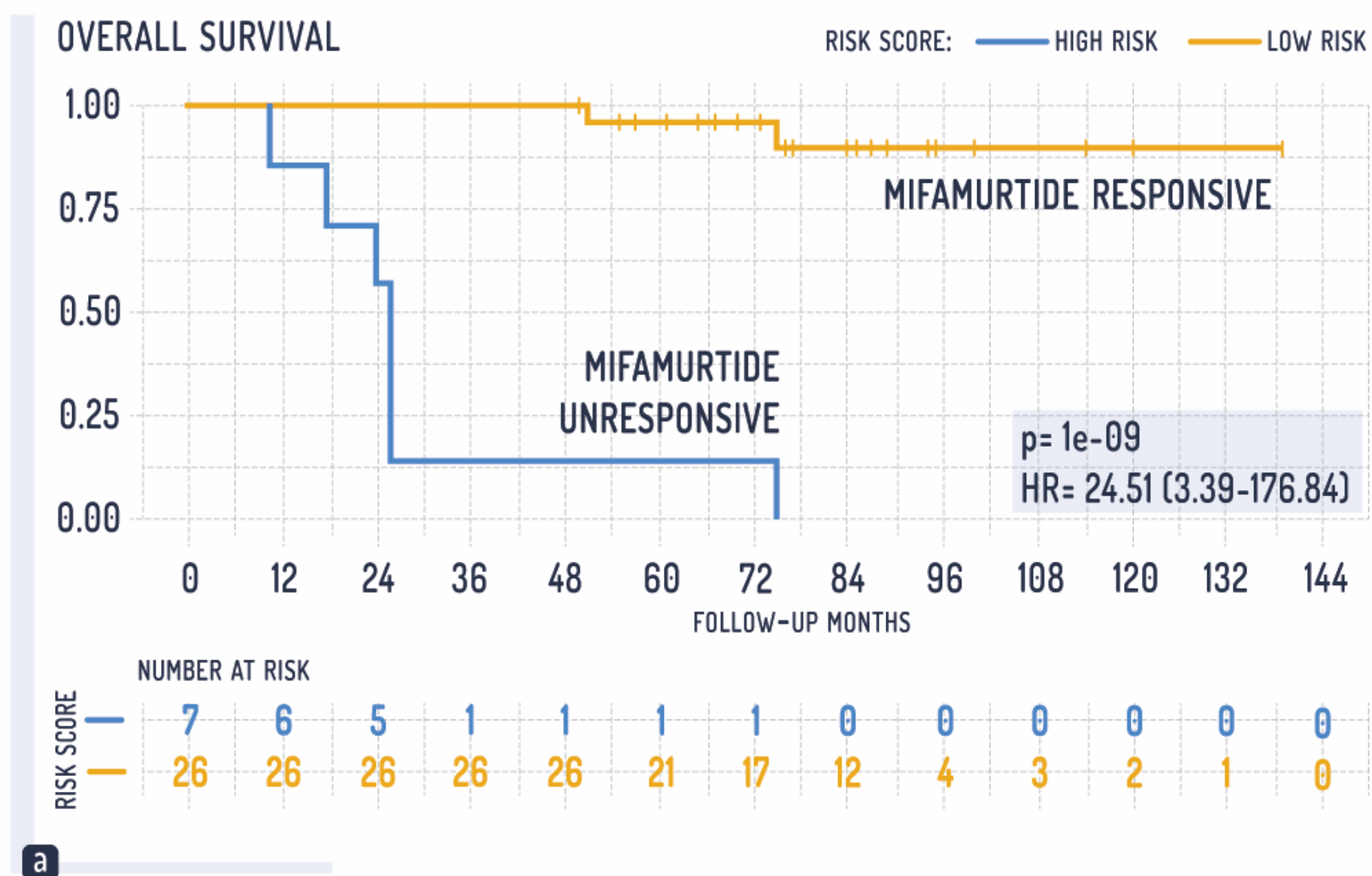
RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

31-Genes Mifamurtide Signature

33 pts undergoing
MIFAMURTIDE

MIFAMURTIDE SENSITIVITY SIGNATURE IN PGP POSITIVE PATIENTS

FIG. 4. 31-GENE SIGNATURE IN 33 PGP-POSITIVE PRIMARY OSTEOSARCOMA UNDERGOING MIFAMURTIDE



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Results

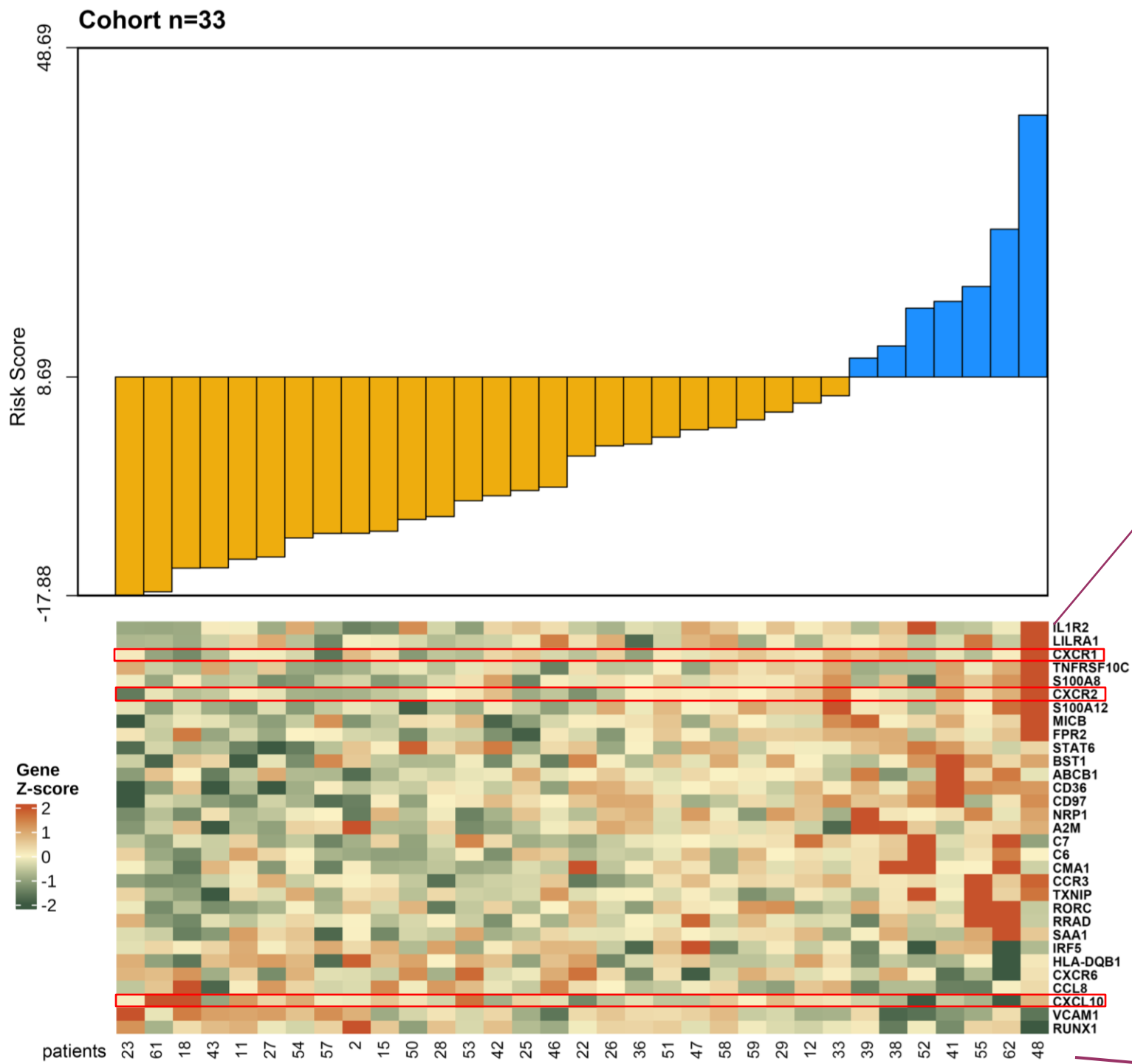
RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

31-Genes Mifamurtide Signature

33 pts undergoing MIFAMURTIDE

Cut Off
Risk Score 8.69

Gene	HR (95% CI)	p-value
A2M	2.11 [1.14 - 3.92]	0.018
ABCB1	1.64 [0.99 - 2.70]	0.053
BST1	2.59 [1.23 - 5.43]	0.012
C6	1.62 [1.01 - 2.59]	0.046
C7	1.83 [1.05 - 3.20]	0.033
CCL8	0.45 [0.20 - 0.98]	0.045
CCR3	2.89 [1.42 - 5.87]	0.003
CD36	5.43 [2.12 - 13.90]	0.000
CD97	2.33 [1.14 - 4.78]	0.021
CMA1	2.20 [1.18 - 4.11]	0.013
CXCL10	0.42 [0.19 - 0.91]	0.027
CXCR1	2.24 [1.05 - 4.76]	0.037
CXCR2	3.00 [1.45 - 6.20]	0.003
CXCR6	0.48 [0.24 - 0.99]	0.046
FPR2	2.73 [1.29 - 5.79]	0.009
HLA-DQB1	0.44 [0.21 - 0.93]	0.032
IL1R2	2.24 [1.10 - 4.56]	0.026
IRF5	0.38 [0.19 - 0.75]	0.005
LILRA1	2.08 [1.01 - 4.29]	0.047
MICB	2.99 [1.40 - 6.37]	0.005
NRP1	1.71 [1.07 - 2.73]	0.024
RORC	2.20 [1.09 - 4.43]	0.028
RRAD	2.13 [1.10 - 4.12]	0.025
RUNX1	0.41 [0.18 - 0.96]	0.040
S100A12	2.13 [1.12 - 4.07]	0.021
S100A8	2.82 [1.31 - 6.10]	0.008
SAA1	2.04 [1.14 - 3.63]	0.016
STAT6	2.24 [1.03 - 4.87]	0.042
TNFRSF10C	2.22 [1.07 - 4.60]	0.033
TXNIP	2.89 [1.49 - 5.62]	0.002
VCAM1	0.44 [0.21 - 0.95]	0.036



IL1R2
LILRA1
CXCR1
TNFRSF10C
S100A8
CXCR2
S100A12
MICB
FPR2
STAT6
BST1
ABCB1
CD36
CD97
NRP1
A2M
C7
C6
CMA1
CCR3
TXNIP
RORC
RRAD
SAA1
IRF5
HLA-DQB1
CXCR6
CCL8
CXCL10
VCAM1
RUNX1

Results

RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

Multivariate Analysis
For Mifamurtide

33 pts undergoing
MIFAMURTIDE

Mifamurtide sensitivity signature of the 31-gene

Overall Survival Variable	Univariate Cox		Multivariate Cox	
	HR (95% CI)	P value	HR (95% CI)	P value
PgP positive primary Osteosarcoma (n = 33)				
Gene signature (high- vs low-risk)	24.51(3.39-176.84)	0.00000001	75.17(8.79-642.42)	0.000079
Histologic Response (Prs vs Grs)	3.06(0.76-12.36)	0.097	4.20(0.46-38.15)	0.25
Age (0-14 vs > 14 years)	0.96(0.85-1.08)	0.47	0.93(0.80-1.08)	0.36
Serum alkaline phosphatase (normal vs high)	0.52(0.14-1.93)	0.31	2.16(0.29-16.30)	0.45

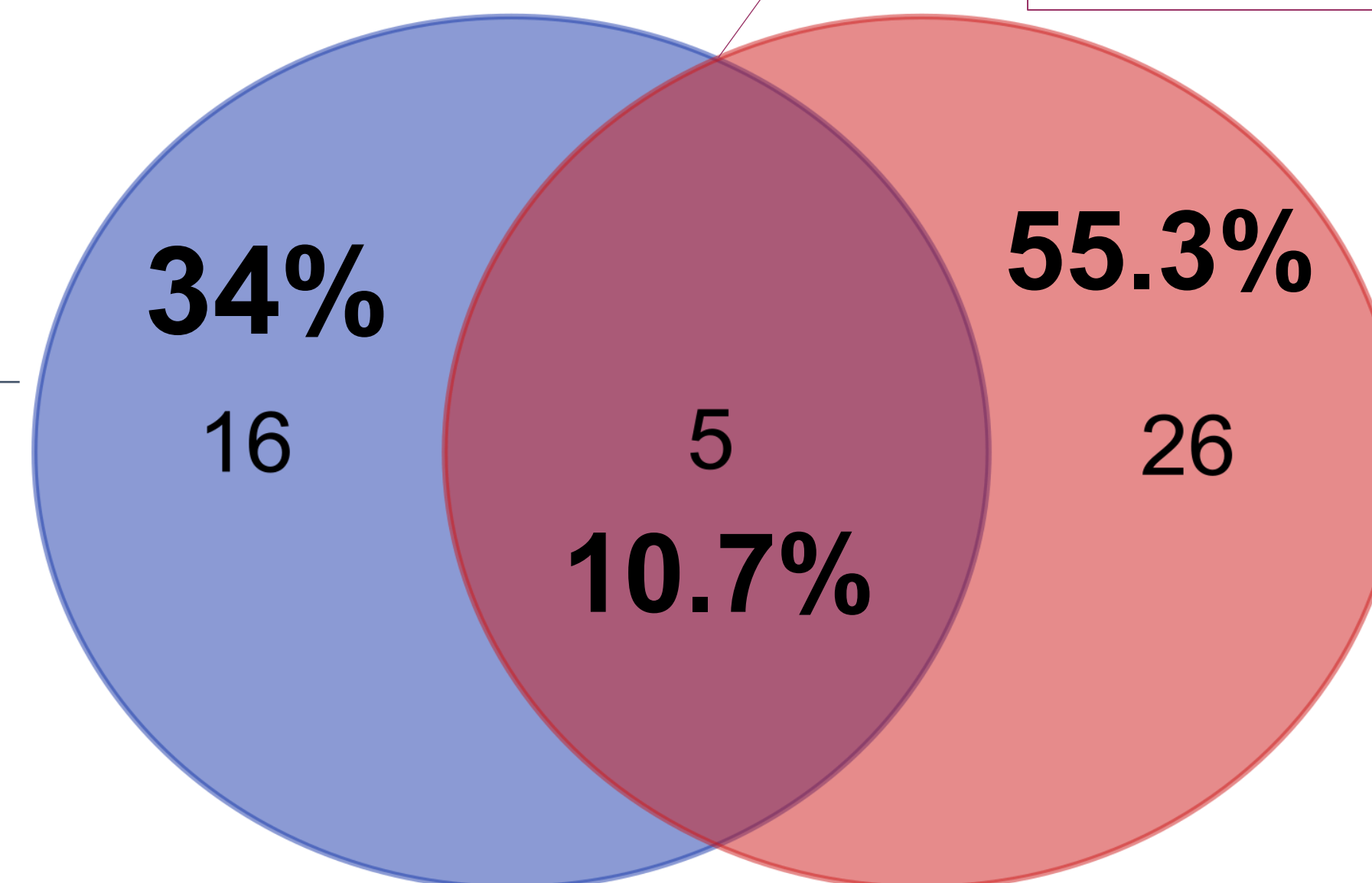
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Results

RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

Genes Only Partially Overlapping

**21-gene *prognostic*
*signature***



1. CD36
2. CCR3
3. IRF5
4. CD97
5. ABCB1

**31-gene *mifamurtide*-
*sensitivity signature***

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Conclusions

RESPONSE TO MIFAMURTIDE AND TUMOR MICRO-ENVIRONMENT: A DECONVOLUTION NANOSTRING ANALYSIS IN LOCALIZED OSTEOSARCOMA PATIENTS TREATED WITHIN THE ISG/OS2 TRIAL

- A validated osteosarcoma TME prognostic gene signature has been identified, independent of mifamurtide treatment.
- Importantly, a mifamurtide-specific signature was developed.
- Tumor-immune interactions possibly implicated in disease progression and treatment response were shown.
- If the mifamurtide-sensitive signature will be validated, a NanoString Customized Test, which might cost about 170 USD per patient, will represent a new tool to assign mifamurtide treatment.

Thanks to the ISG/OS2 Investigators, Patients and their Families!

Emanuela Palmerinia*, Maria Rosaria Sapienza*, Stefano A Pileri, Alberto Righi, Antonina Parafioriti, Alessandro Franchi, Claudio Agostinelli, Cristina Meazza, Virginia Ferraresi, Sebastian Dorin Asaftei, Luca Coccoli, Angela Tamburini, Marco Gambarotti, Massimo Serra, Davide Maria Donati, Franca Fagioli, Marilena Cesari, Giorgio Frega, Katia Scotlandi, Maria Antonella Laginestra‡, Toni Ibrahim‡;

Hemolymphopathology, IEO - Istituto Europeo di Oncologia IRCCS, Milan, Italy; European Institute of Oncology, Milan, Italy; Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy; Istituto Ortopedico Gaetano Pini, Milan, Italy; University of Pisa, Pisa, Italy; University of Bologna, Bologna, Italy; Pediatric Oncology Unit, Fondazione IRCCS, Istituto Nazionale dei Tumori, Milano, Italy; Sarcomas and Rare Tumors Departmental Unit - IRCCS Regina Elena National Cancer Institute, Roma, Italy; Pediatric Department, Regina Margherita Children's Hospital, AOU Città della Salute e della Scienza, Turin, Italy; Pediatric Oncology-Hematology Unit, Stem Cell Transplantation, S. Chiara Hospital, AOUP, Pisa, Italy; Department of Paediatric Haematology-Oncology, Meyer Children's Hospital IRCCS, Florence, Italy; IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy; Regina Margherita Childrens Hospital and University of Turin, Turin, Italy; Osteoncology, Bone and Soft Tissue Sarcomas and Innovative Therapies, Orthopaedic Institute Rizzoli, Bologna, Italy

* equally contributed

‡ equally contributed



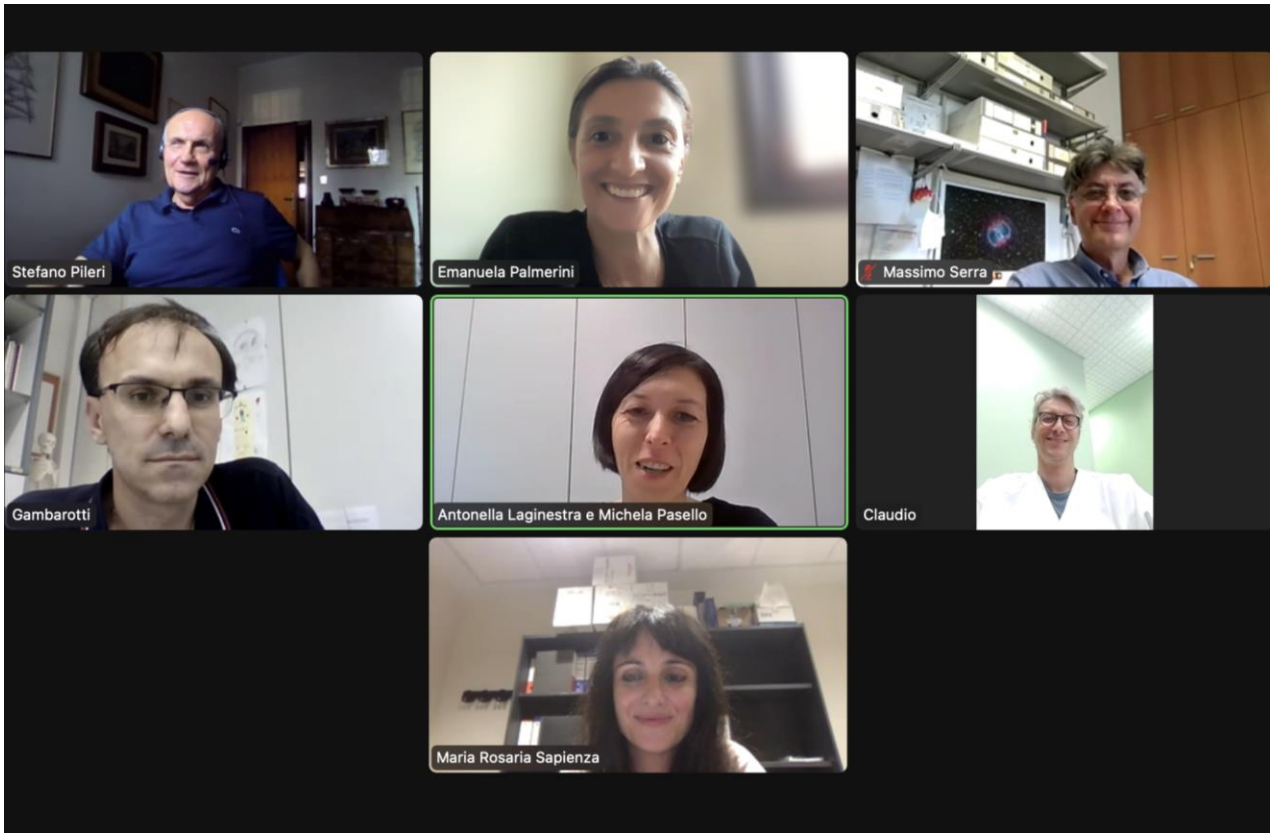
*.... for Mentorship
Dr Stefano Ferrari
Dr Piero Picci*






Presented by:
Emanuela Palmerini, MD, PhD

Thank you for your attention!

epalmerini@miami.edu



-  University of Bologna, Bologna, Italy
-  IEO - Istituto Europeo di Oncologia IRCCS, Milan
-  IOR – IRCCS Istituto Ortopedico Rizzoli, Bologna



Prof Stefano Pileri



Dr Maria Rosaria Sapienza



Dr Maria Antonietta Laginestra



Prof Claudio Agostinelli



Dr Toni Ibrahim



Prof Katia Scotlandi



Dr Massimo Serra



Dr Marco Gamberotti



Dr Alberto Righi



Prof Davide Donati

- For Graphical Support
- Dr Maria Pia Cumani
 - Dr Cristina Ghinelli



Osteoncology, Bone and Soft Tissue
Sarcomas and Innovative Therapies
IRCCS Istituto Ortopedico Rizzoli, Bologna,
Italy



Thiago Motta
Former Bologna FC coach
visiting our patients