

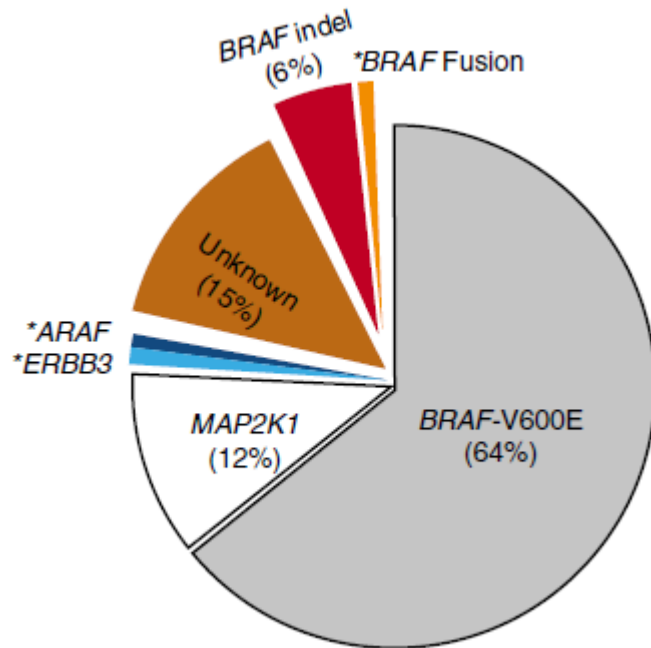
Prognostic value of *BRAF*^{V600E} in peripheral blood of children with LCH: a multicenter ECHO project

Elena Sieni

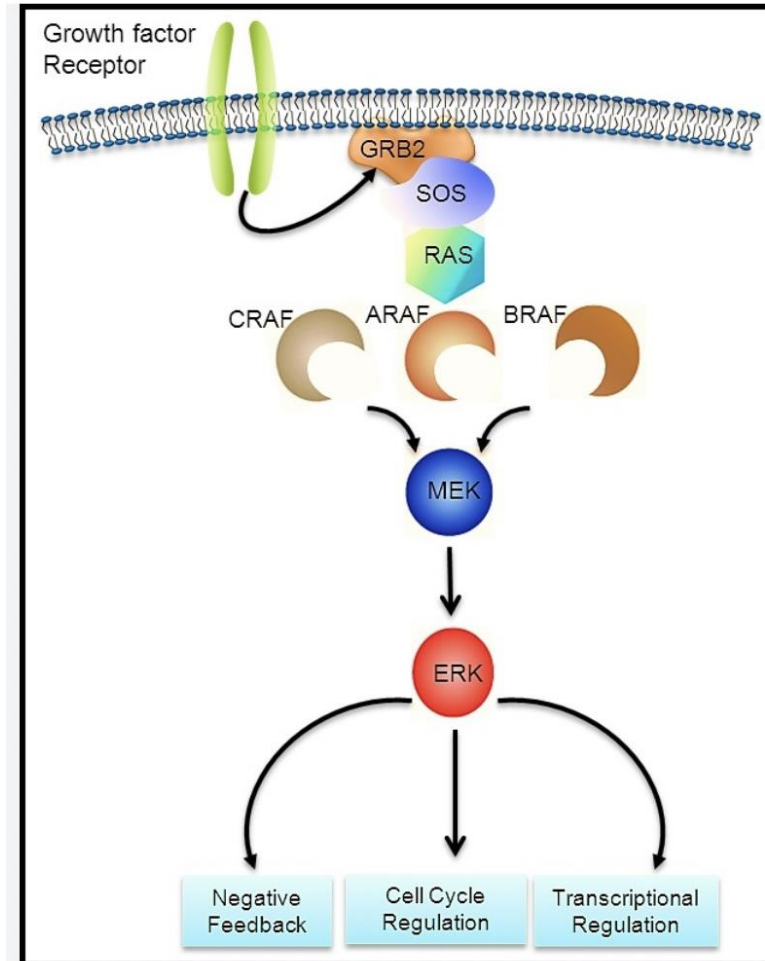
Oncoematologia AOU A. Meyer IRCCS

Recurrent *BRAF* mutations in Langerhans cell histiocytosis

Gayane Badalian-Very, Jo-Anne Vergilio, Barbara A. Degar, Laura E. MacConaill, Barbara Brandner, Monica L. Calicchio, Frank C. Kuo, Azra H. Ligon, Kristen E. Stevenson, Sarah M. Kehoe, Levi A. Garraway, William C. Hahn, Matthew Meyerson, Mark D. Fleming and Barrett J. Rollins



Chakraborty R et al. Blood 2016

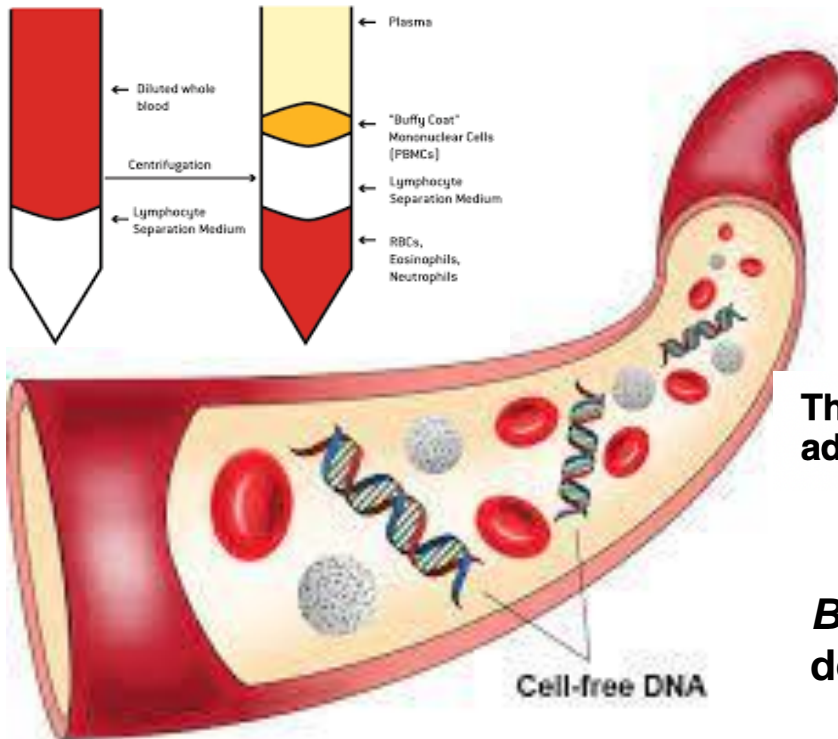


BRAF^{V600E} LCH:

- ✓ high-risk LCH
- ✓ reduced sensitivity to standard chemotherapy

Héritier S et al. J clin Onc 2016

***BRAF*^{V600E} can be detected in the peripheral blood of patients with LCH**



Prospective Blinded Study of *BRAF*V600E Mutation Detection in Cell-Free DNA of Patients with Systemic Histiocytic Disorders

Hyman DM et al. Cancer Discovery 2015

The *BRAF*-V600E mutation in circulating cell-free DNA is a promising biomarker of high-risk adult Langerhans cell histiocytosis

Kobayashi M, Tojo A. Blood 2014

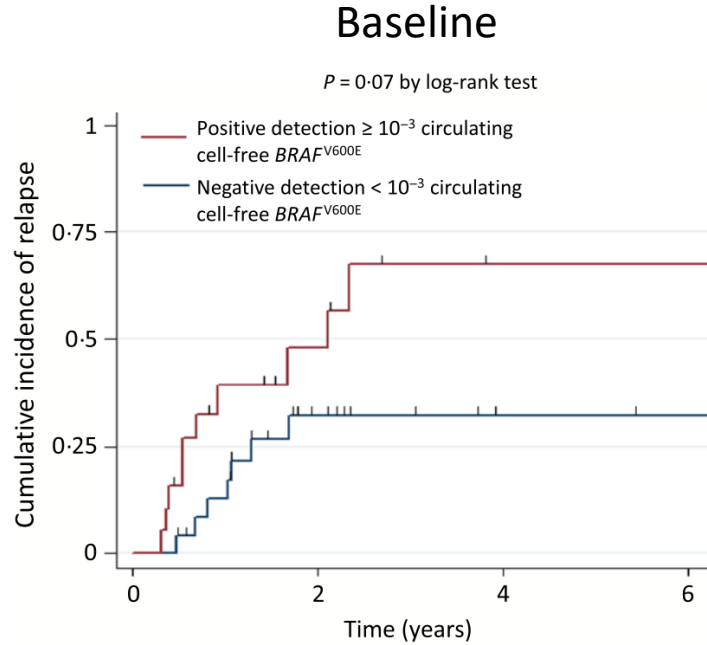
***BRAF*-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups.** Berres ML et al J Exp Med 2014

PBMC

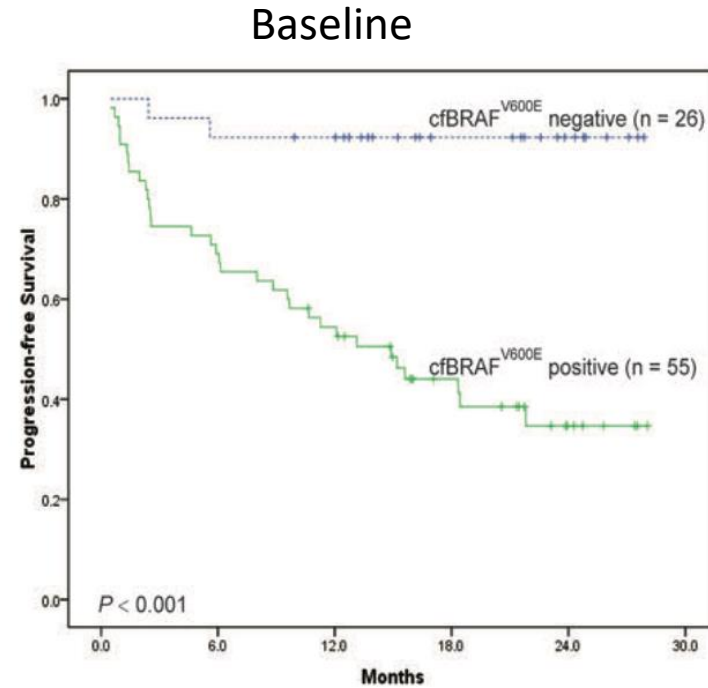
Longitudinal assessment of peripheral blood *BRAF*V600E levels in patients with Langerhans cell histiocytosis . Schwentner R et al Pediatr Res 2019

Whole blood

Circulating $BRAF^{V600E}$ is a promising biomarker in children with LCH



Héritier S et al. BJH 2017



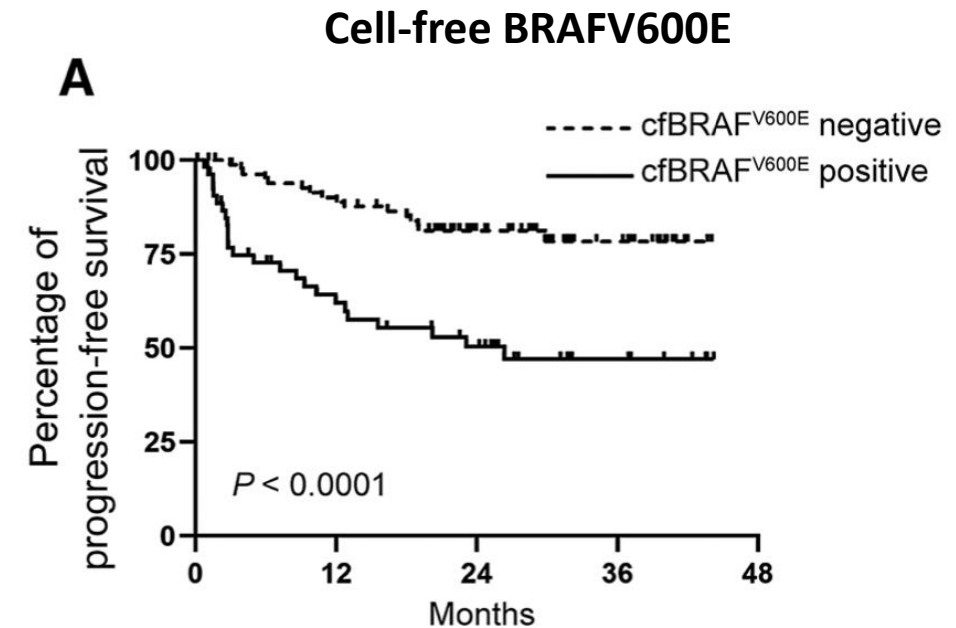
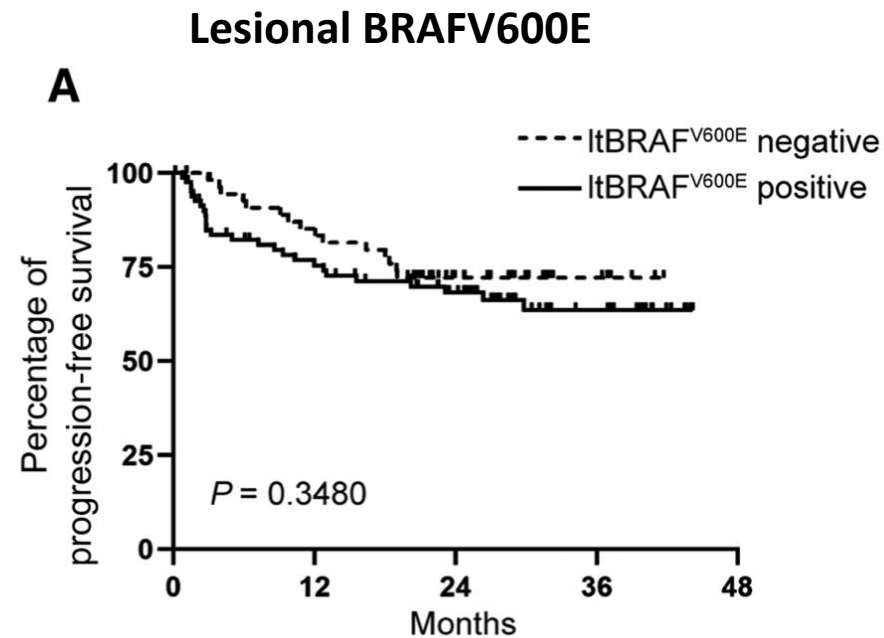
Cui L et al. Haematologica 2020

cfBRAF V600E is associated with increased incidence of reactivation in children with LCH

July 2021

***BRAF*^{V600E} Mutation in Cell-Free DNA, Rather than in Lesion Tissues, at Diagnosis Is An Independent Prognostic Factor in Children with Langerhans Cell Histiocytosis**

Chan-Juan Wang¹, Lei Cui², Hong-Hao Ma¹, Dong Wang¹, Li Zhang¹, Hong-Yun Lian¹, Wei-Jing Li², Qing Zhang², Tian-You Wang¹, Zhi-Gang Li², and Rui Zhang¹



Circulating *BRAF*^{V600E} : open questions

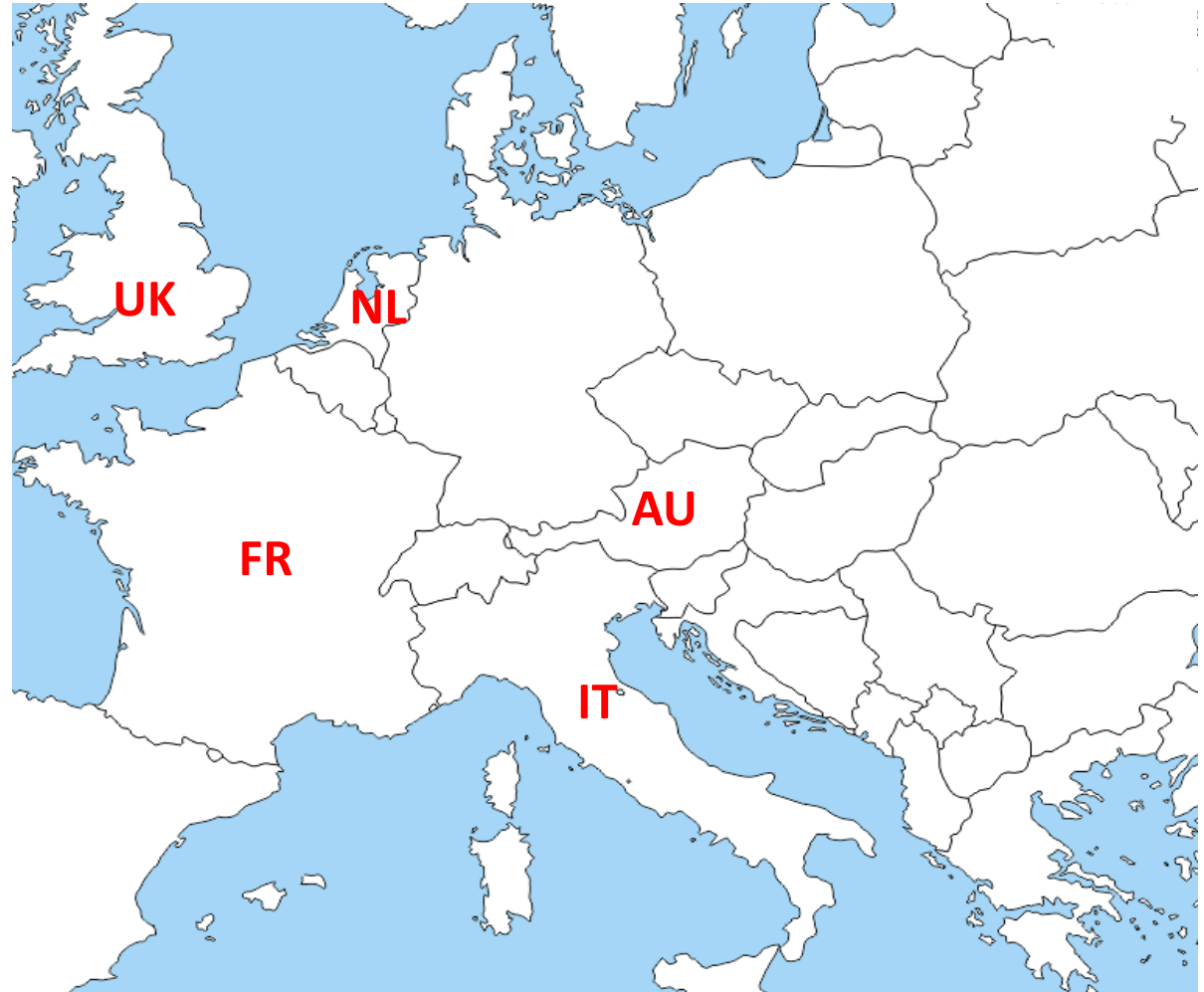
- Which DNA source ? Cell-free /cell-bound
- How the results could be interpreted for patient management?
- Is this method reproducible at a multi-center level?

A multi-center ECHO project

PI: Elena Sieni

Co-Pis and participating centres:

- P. Milne, Newcastle, UK
- A.G.S. van Halteren, Rotterdam, NL
- C. Hutter, Wien, AU
- S. H  ritier, Paris, FR
- ML Coniglio, Florence, IT



Aim of the project

To assess longitudinal *BRAF*^{V600E} levels in blood
in relation to clinical outcome
in different DNA sources (cell-free / cell-bound)



Study design

- Pediatric patients with (new onset or reactivated) ***BRAF*^{V600E} mutated LCH**
- **Biobanked and prospectively collected blood samples**
 - pre-therapy (onset or react)
 - 6 weeks after tx (early monitoring)
 - 12 weeks
 - 6 months
 - Every 3 months
- **Clinical outcome:**
 - Event (tx intensification, disease progression, no response, reactivation) at any time
 - EFS is the clinical endpoint

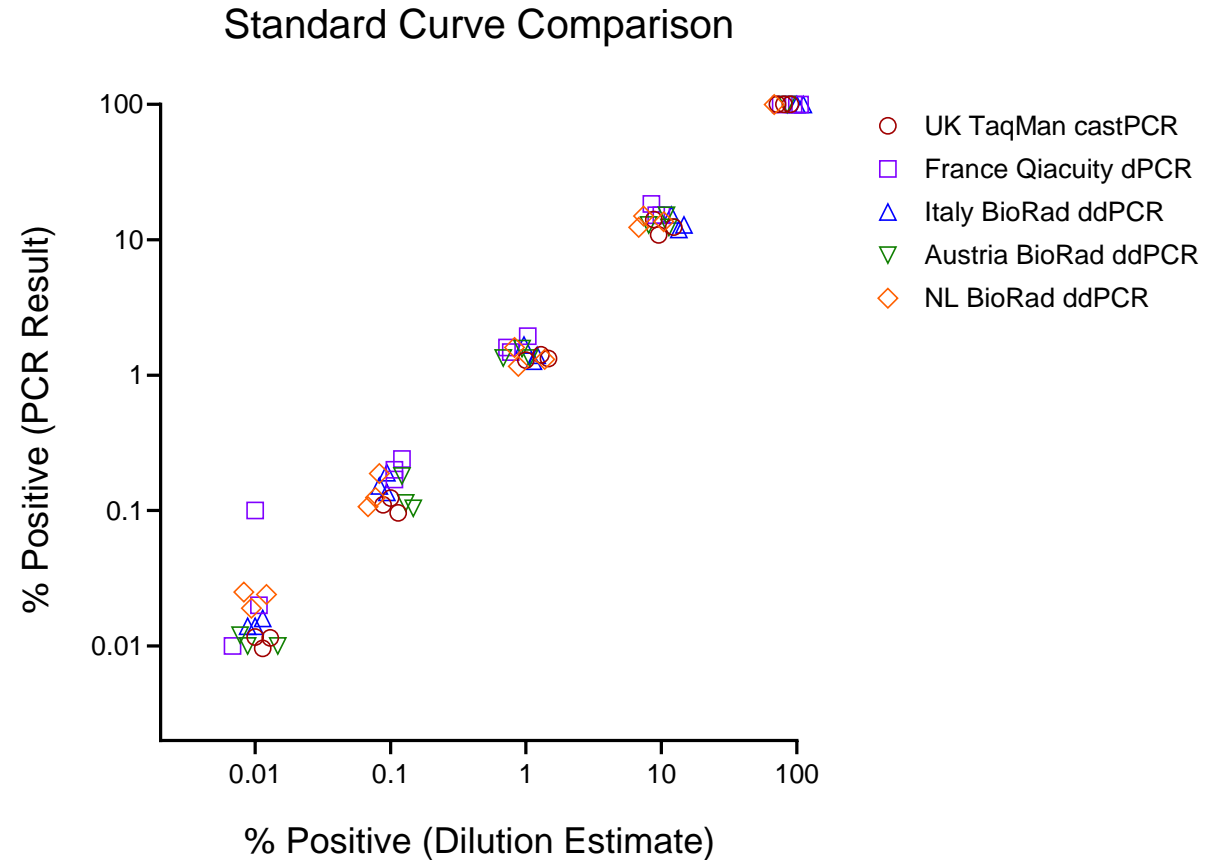
Methods

- Different DNA sources → separated analysis
 - Different PCR technologies
 - Local SOPs

} Quality controls

	Cell-bound	Cell-free	PCR platform	Plasma separation	DNA /cfDNA Extraction
AU	Whole blood DNA		BIORAD ddPCR	20min 1600g RT acceleration; 10min 16000g at RT	QIAGEN DNA: Blood Mini Kit
FR		x	QIAGEN Qiacuity dPCR	n.a.	Maxwell Promega
IT	PBMC	x	BIORAD ddPCR	QIAGEN protocol for Blood EDTA	QIAGEN: DNA Mini kit/ cfDNA Qiamp Circulating
NL	Total white cells	x (sent to Italy)	BIORAD ddPCR	2420 RPM, 10 min	QIAGEN QIAamp DNA Micro Kit
UK	PBMC	x	THERMOFISHER qPCR	QIAGEN protocol for Blood EDTA	QIAGEN: DNA Mini kit/ cfDNA Qiamp Circulating

Sensitivity and accuracy



Each lab recognizes positivity at 0.01% mutational burden

Included patients

Total	UK	Italy	France	Austria	NL
n=256	n=76	n=76	n=61	n=29	n=14

Median age at diagnosis, 27 months (0-205)

Sex: M: 151 (59%); F: 105 (41%)

Disease classification: 125 SS (49%), 70 MS RO- (27%) ; 61 MS RO+ (24%)
36/125 SS MFB (14%)
89/125 OTHER SS (35%)

Treatment at baseline:

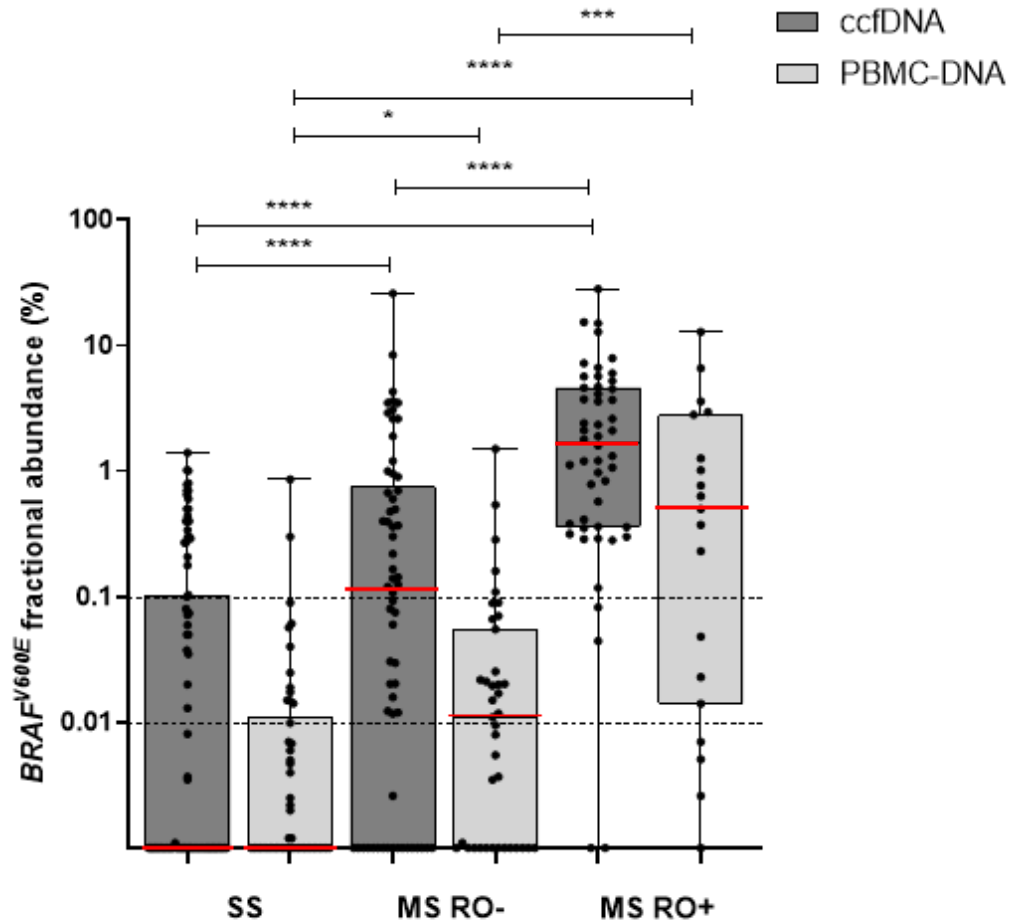
- Chemotherapy, n=172 (68%)
- BRAFi, n=14 (5%)
- Combined CT+BRAFi, n=2 (1%)
- None, n=62 (24%)
- Other (indomethacin, Ig), n=6 (2%)

Disease extension and DNA sources at diagnosis and after 6 weeks

	Baseline				Week 6			
DNA source	Cell-free	PBMC	Total Leukocytes	Whole Blood	Cell-free	PBMC	Total Leukocytes	Whole Blood
LAB	IT, UK, FR, NL	UK, IT	NL	AT	IT, UK, FR, NL	UK, IT	NL	AT
SS MFB	22	12	n.a.	11	12	7	n.a.	4
other SS	70	38	2	9	28	22	n.a.	1
MS RO-	63	40	9	1	42	34	8	2
MS RO+	50	19	2	8	22	17	n.a.	2
TOTAL	205	109	13	29	106	80	8	9

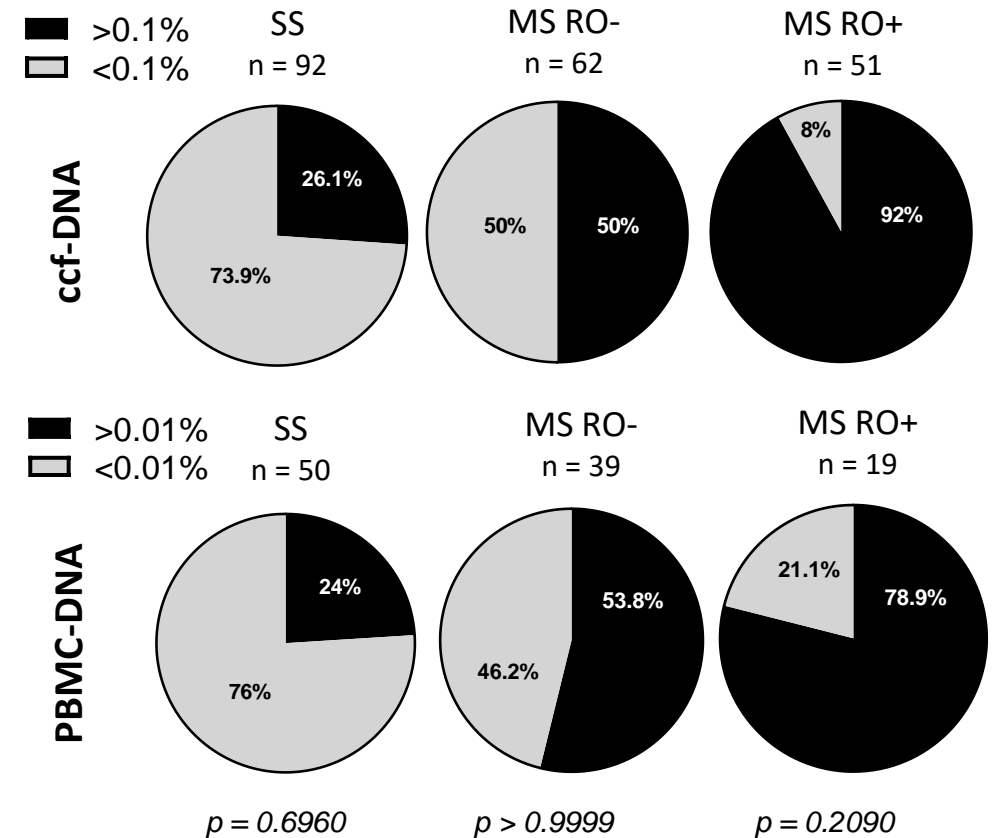
Results: circulating $BRAF^{V600E}$ at baseline

Levels of circulating $BRAF^{V600E}$ in lesion-mutated patients



Mann-Whitney test

Proportion of circulating $BRAF^{V600E}$ in lesion-mutated patients



$p = 0.6960$

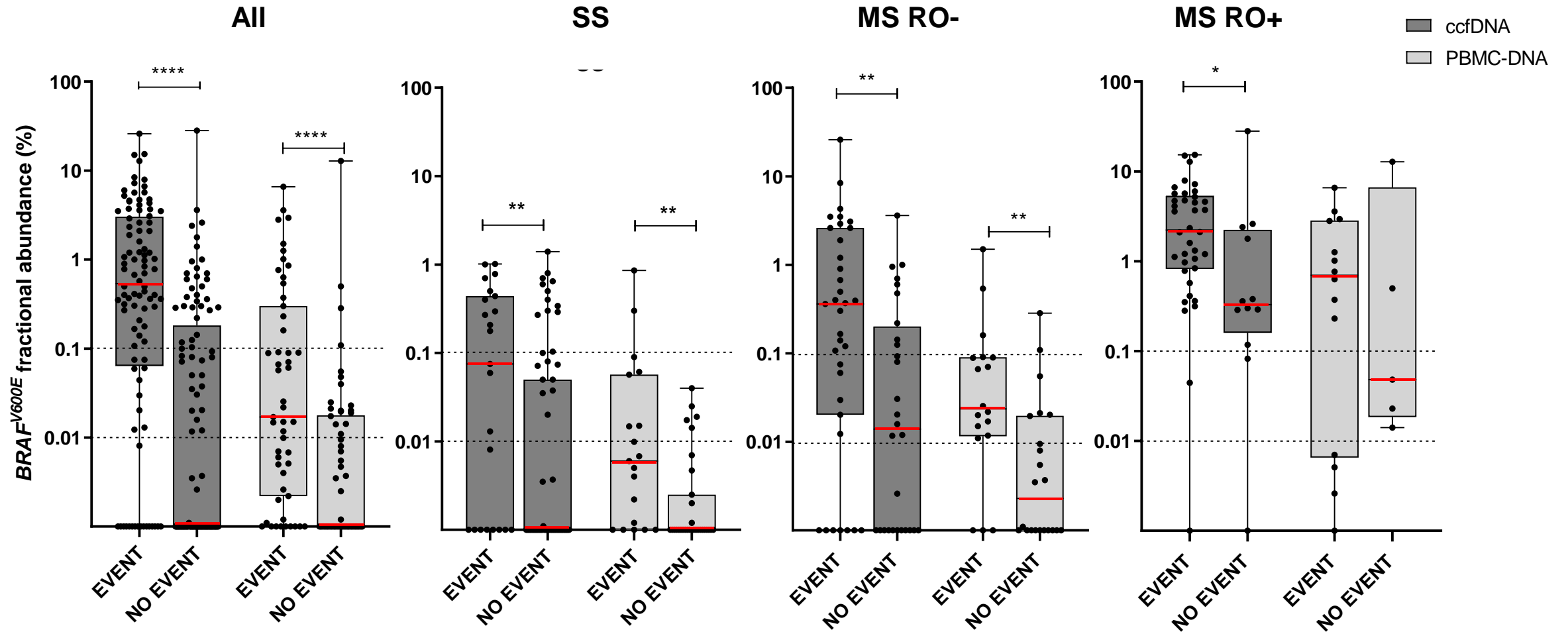
$p > 0.9999$

$p = 0.2090$

Fisher's exact test

Circulating $BRAF$ positivity correlates with disease extension in both sources

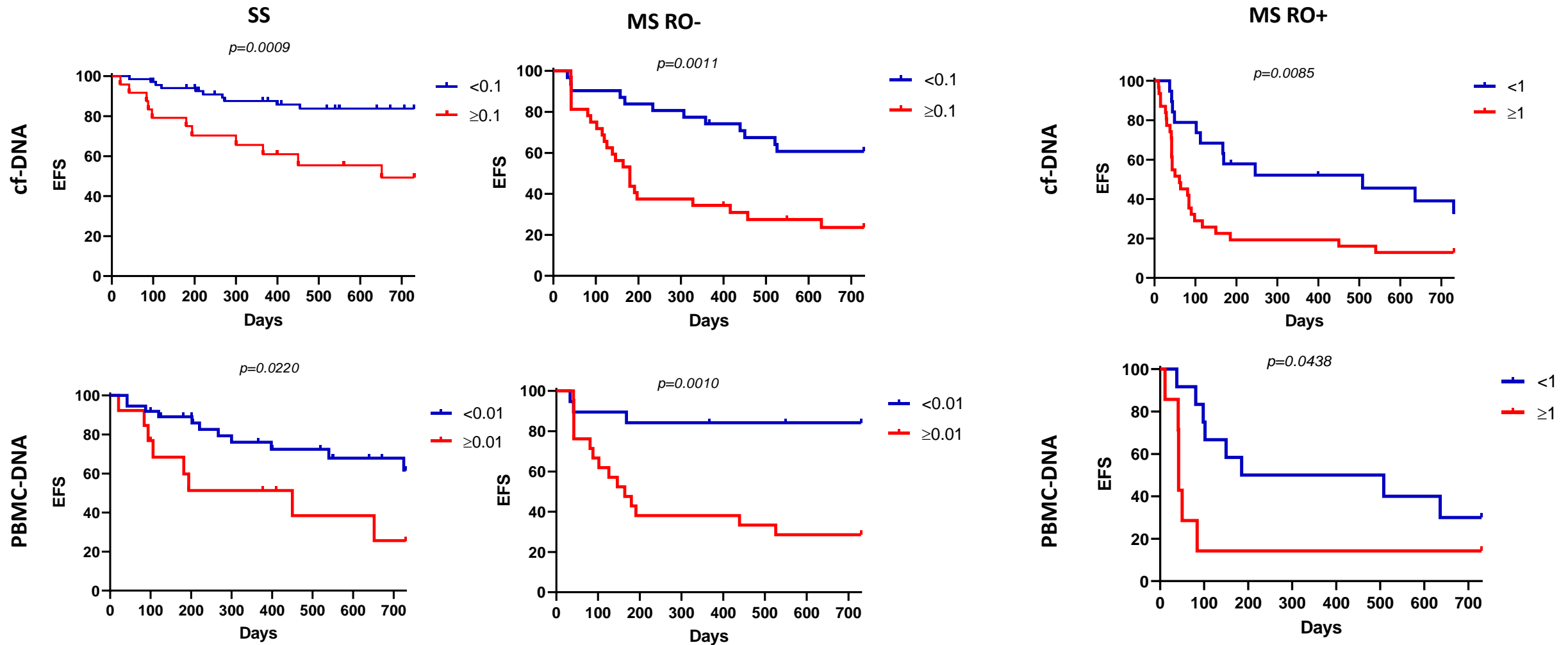
Results: circulating $BRAF^{V600E}$ at baseline in relation to clinical outcome



Mann-Whitney test

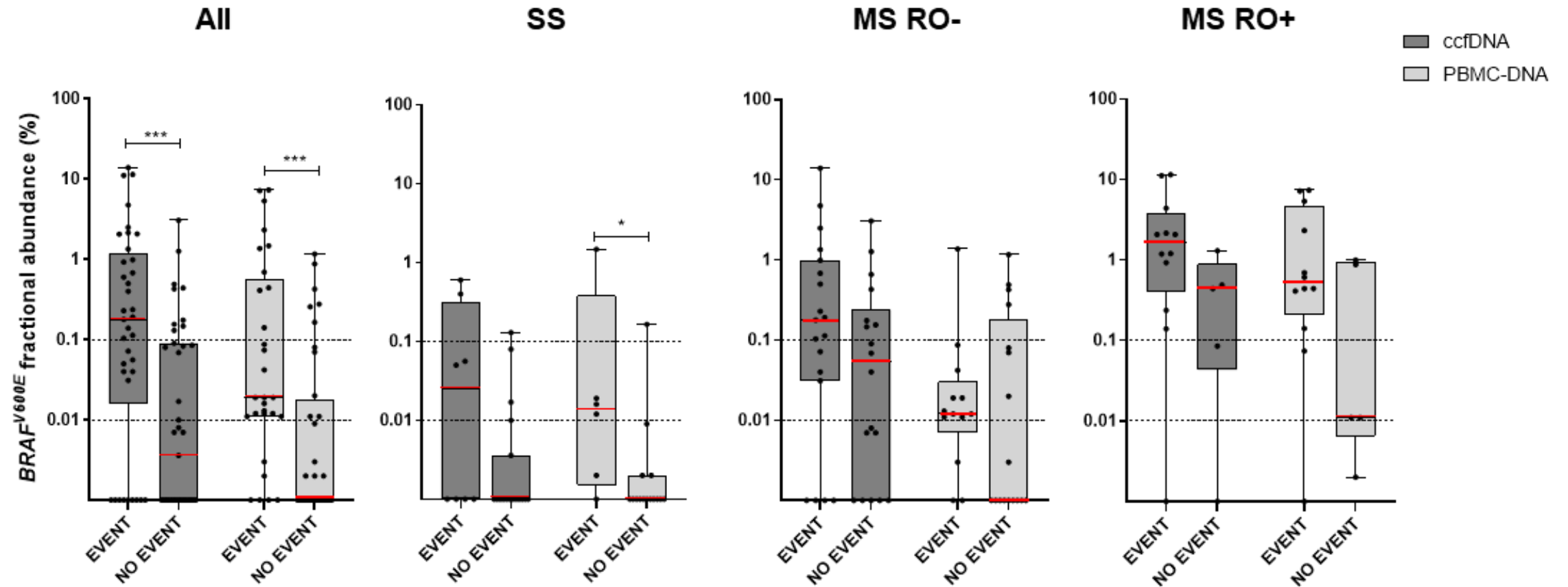
$BRAF^{V600E}$ levels are higher in patients with "event"

EFS according to circulating $BRAF^{V600E}$ at baseline in different disease groups



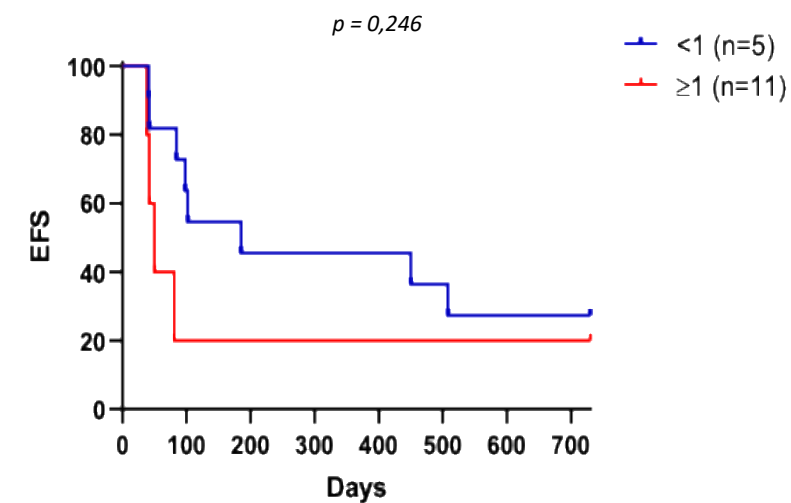
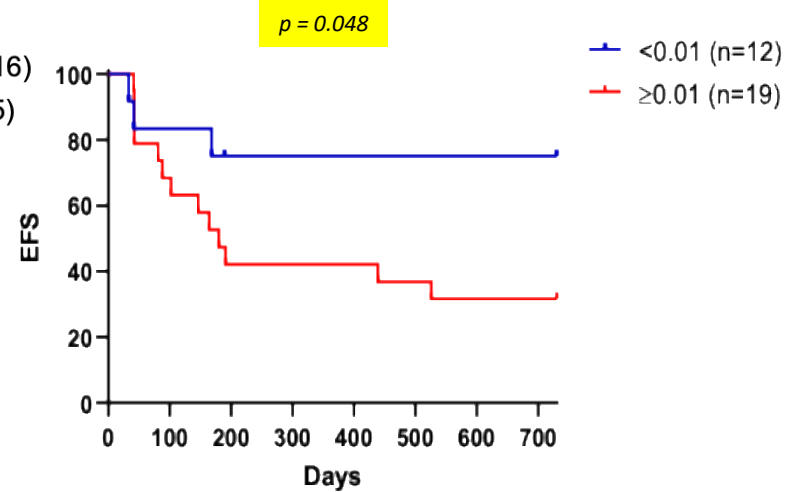
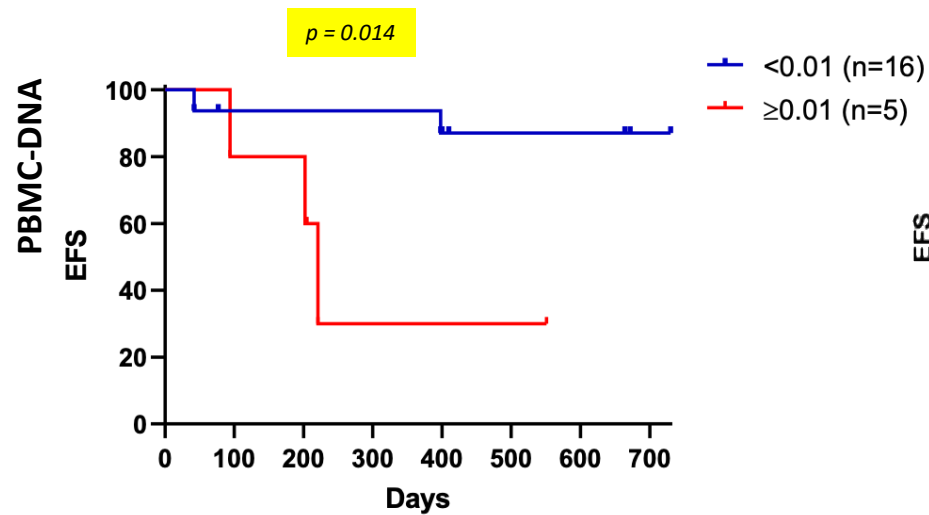
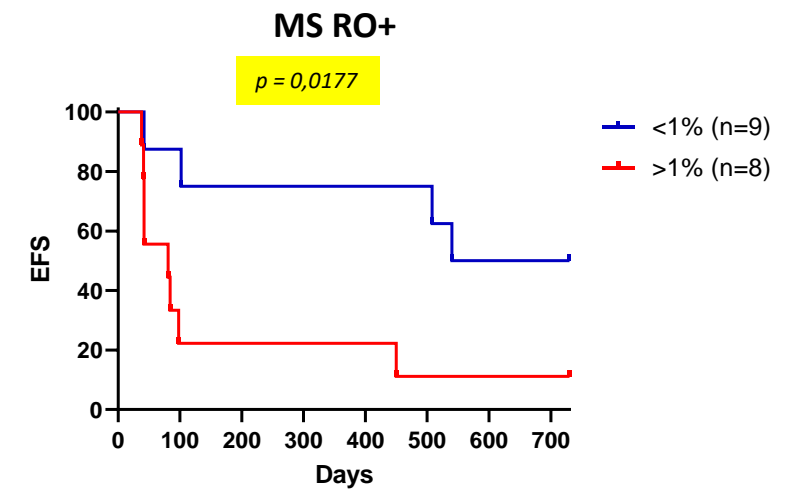
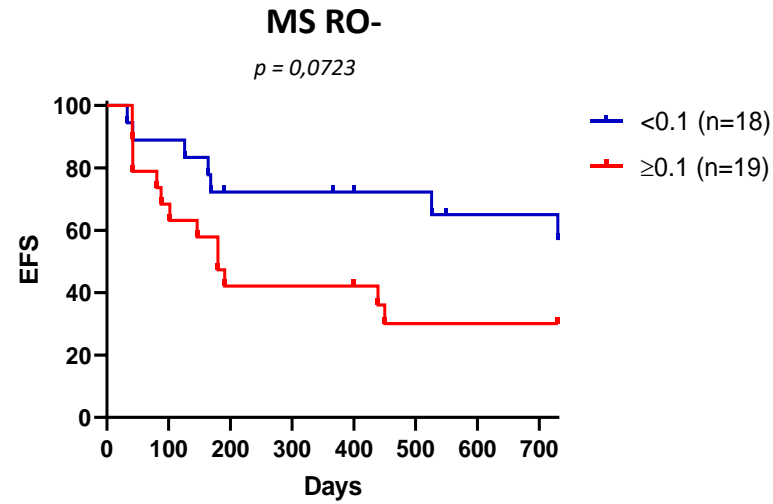
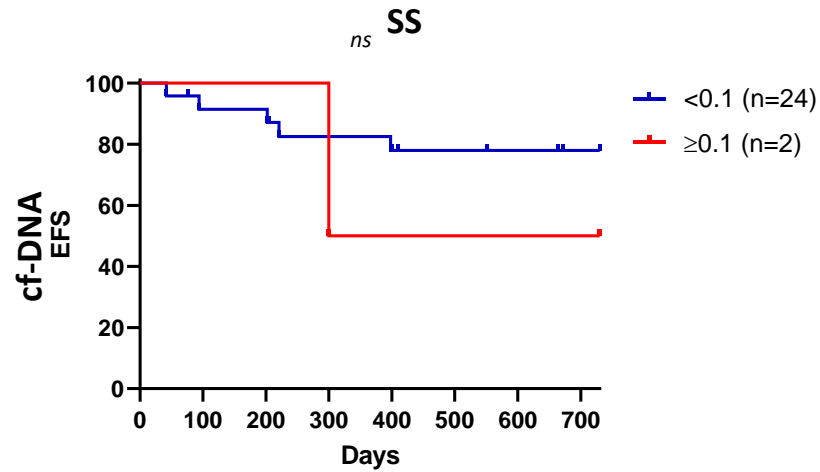
1. Positive BRAF levels predict shorter EFS in both sources in all sub-groups
2. Different thresholds were used for the two DNA sources and for MS-RO+ patients

Circulating $BRAF^{V600E}$ at week-6 after chemotherapy in relation to clinical outcome

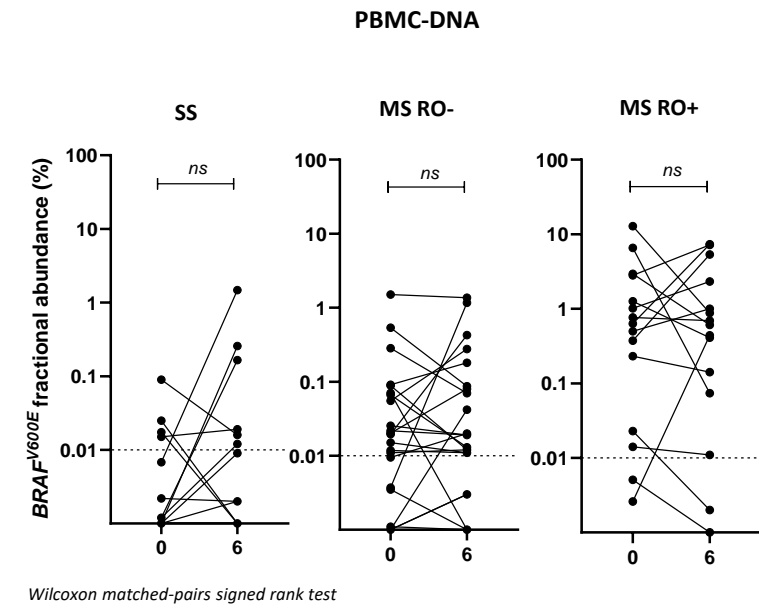
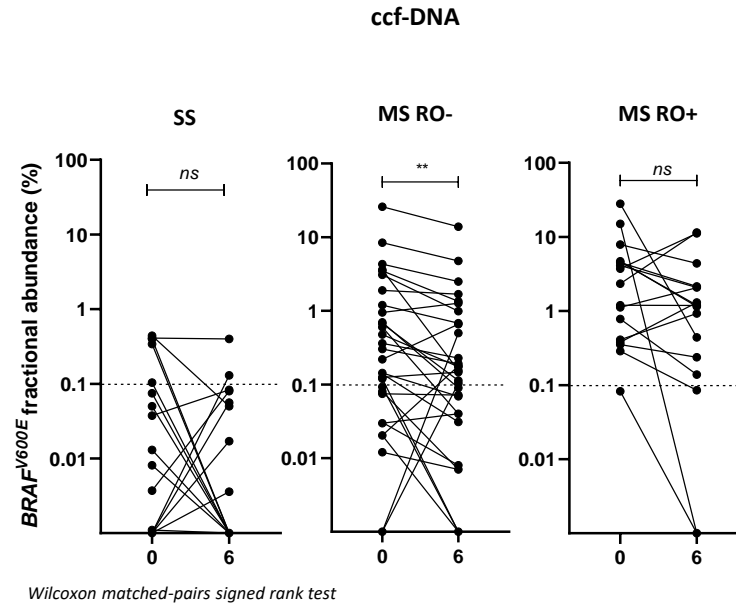
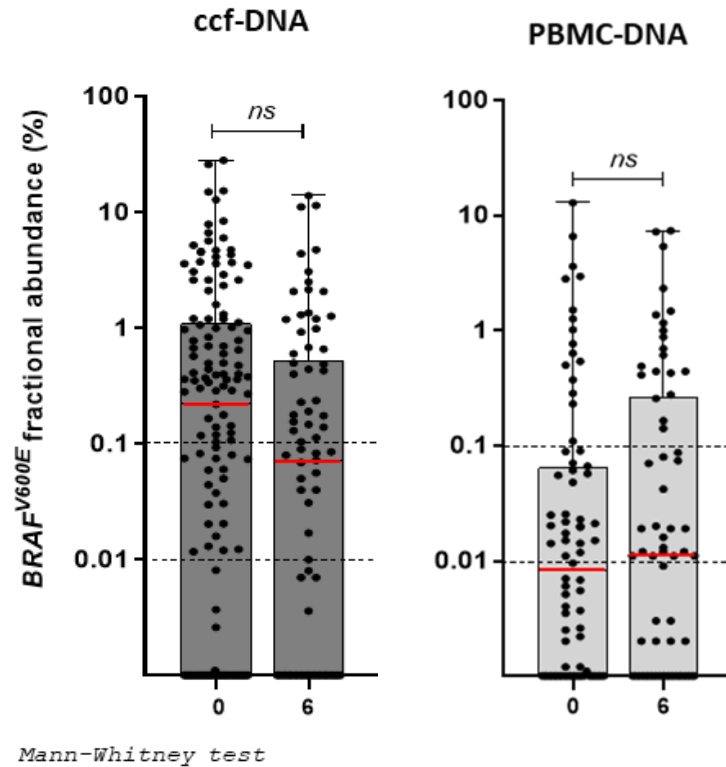


Mann-Whitney test

EFS according to circulating $BRAF^{V600E}$ at week-6 after chemotherapy in different disease groups



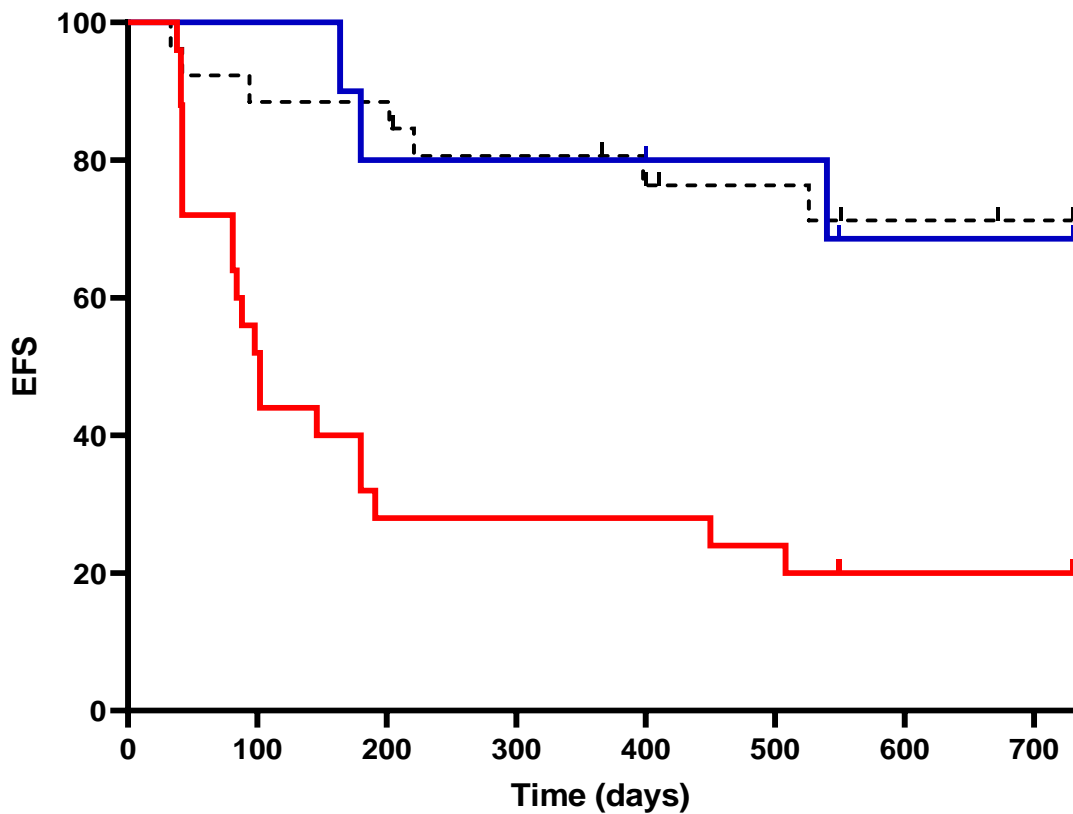
Circulating $BRAF^{V600E}$ at week-6 after chemotherapy in relation to clinical outcome



EFS according to W6-T0 variation in patients treated with chemotherapy

ccf-DNA (n=63)

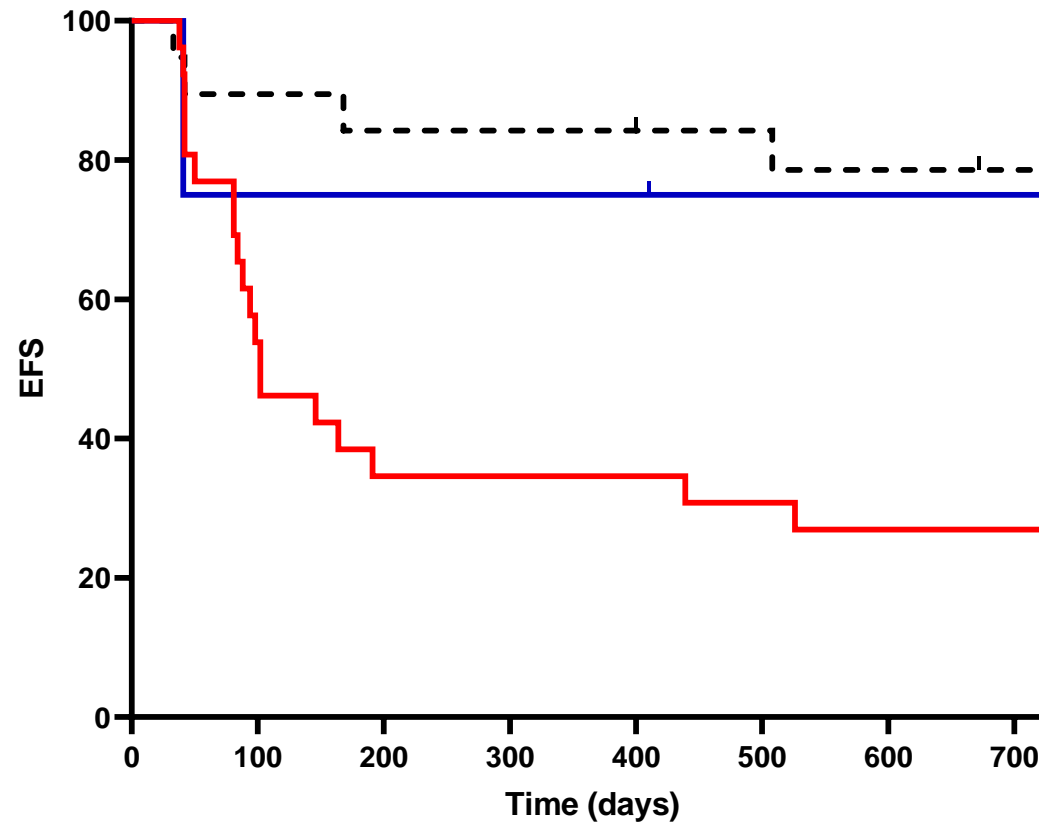
$p < 0.0001$



— neg to neg (n=26)
— pos to neg (n=10)
— pos to pos (n=27)

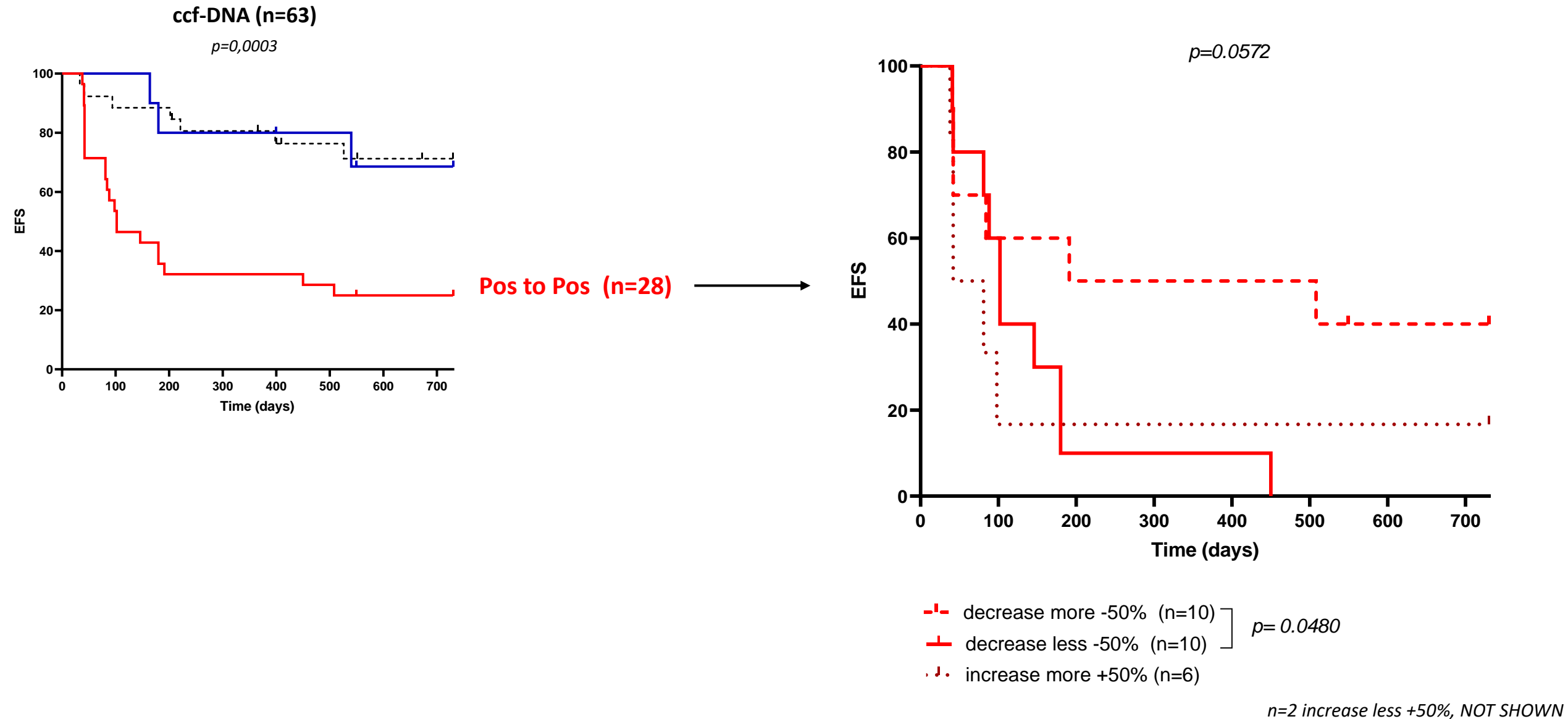
PBMC-DNA (n=48)

$p = 0.0038$



— neg to neg (n=19)
— pos to neg (n=4)
— pos to pos (n=27)

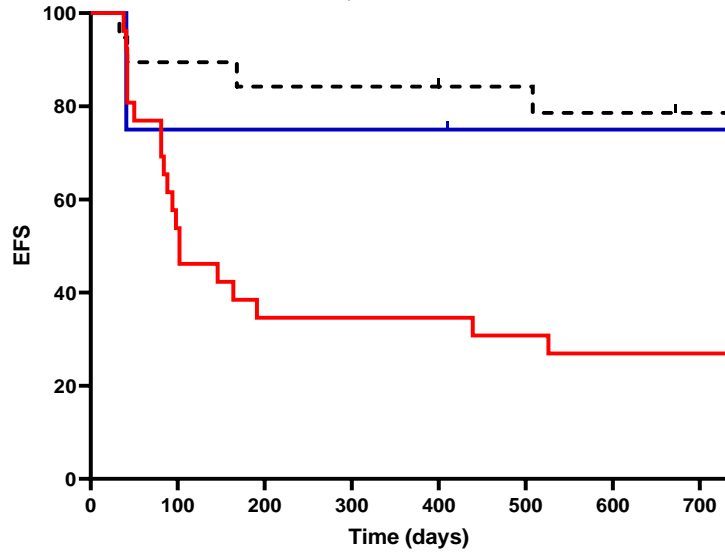
EFS according to W6-T0 variation in patients treated with chemotherapy



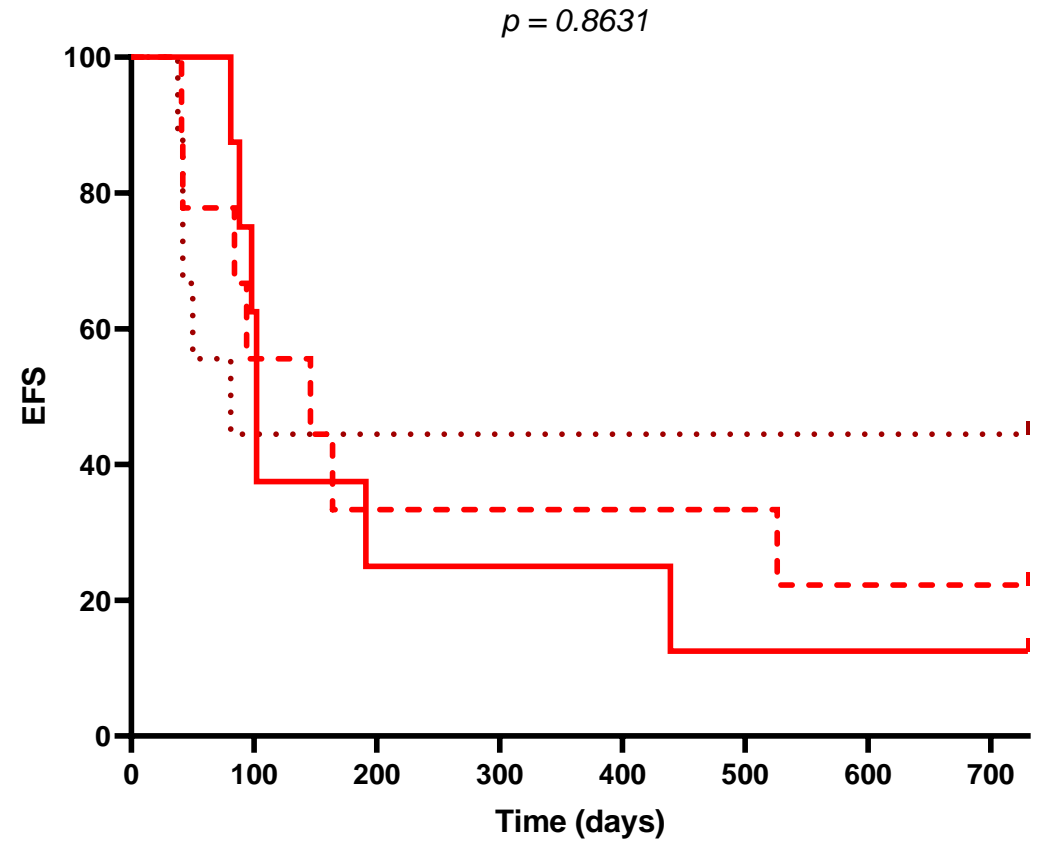
EFS according to W6-T0 variation in patients treated with chemotherapy

PBMC-DNA (n=48)

$p=0.0038$



Pos to Pos (n=28)



- - decrease over -50% (n=9)
- decrease under -50% (n=8)
- . . increase more +50% (n=9)

n=1 increase less +50%, NOT SHOWN

Conclusions

- This study shows the feasibility of measuring *BRAF*^{V600E} in the peripheral blood of children with LCH at multi-center international level
- Both DNA sources are informative for Braf status and prognosis
- We propose a **molecular-based risk stratification at baseline** in different disease groups in both sources with a new cut-off of 1% for MS-RO+ patients
- **Treatment-induced Braf changes at W6** identify patients with worst prognosis that may benefit from treatment intensification
- Incorporation of this tool in the next prospective therapeutical trials is suggested in order to confirm its role in guiding treatment decisions

Thank you...



Paul Milne



Sebastien Héreitier



Astrid van Halteren



Caroline Hutter

European Consortium for
HistiOcytosis



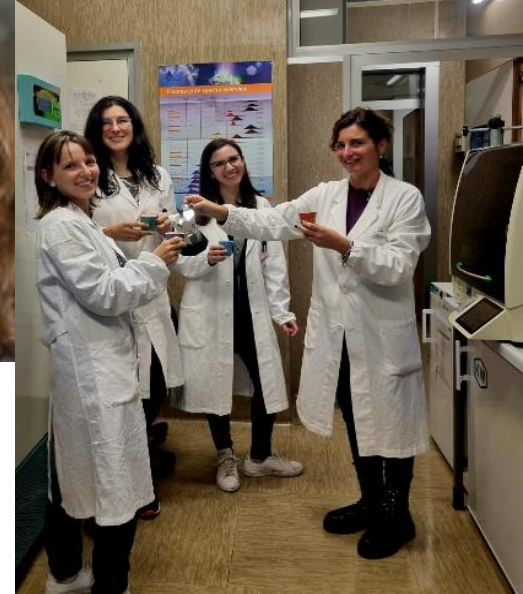
Italian referral centres:

Bari, Bologna, Cagliari, Catania,
Genova, Monza, Napoli, Padova,
Pavia, Roma OBG, San Giovanni
Rotondo, Torino

E tutti i membri del GdL istiocitosi



**Michele
Tanturli,
statistician**



Lab:
Maria Luisa Coniglio
Aurora Chinnici
Linda Beneforti
Daniela Balasco



Pediatric Hem-Onc:
Irene Trambusti
Francesco Pegoraro

