

# Oral etoposide and trastuzumab in HER2-positive metastatic breast cancer: a retrospective study at Institut Curie Hospitals.

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## Research Article

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# Abstract

**Background:** The *TOP2A* and *ERBB2* genes are co-amplified in about 40% of HER2 positive (HER2+) breast cancers. Oral etoposide (VP16), an inhibitor of topoisomerase-II (encoded by *TOP2A*), has demonstrated clinical activity in metastatic breast cancer (MBC). However, the clinical benefit of oral VP16 combined with trastuzumab (VP16-T) in HER2+ MBC has not been evaluated.

**Methods:** Patients treated at Institut Curie Hospitals with VP16-T for HER2+ MBC were retrieved by an in-silico search. Trained medical oncologists retrospectively assessed progression-free survival (PFS), overall survival (OS), response rate, prolonged PFS (defined as a duration of at least 6 months), 6 months clinical benefit rate and toxicity. Co-amplification of *ERBB2* and *TOP2A* was assessed by shallow whole genome sequencing on tumor tissue whenever available.

**Results:** Forty-three patients received VP16-T after a median number of six prior treatment lines for HER2+ MBC. Median PFS and OS were 2.9 months (95% CI [2.4-4.7]) and 11.3 months (95% CI [8.3-25.0]), respectively. Three patients had a complete response while 12/40 (30%) had a clinical benefit. Only 3 patients stopped treatment for toxicity. Median PFS in the population with and without *TOP2A/ERBB2* co-amplification was respectively 4.7 months (95% CI [2.3-NA]) and 2.9 months (95% CI [1.2-NA]; p=0.36).

**Conclusion:** Our analysis suggests a favorable efficacy and toxicity profile for VP16-T in patients with heavily pretreated HER2+ MBC.

## Background

Approximately 15% of breast cancers display an amplification of *ERBB2*, which is encoding the human epidermal growth factor receptor 2 (HER2) and is associated with poorer prognosis (1–3). HER2-targeted cancer therapies such as trastuzumab, pertuzumab, trastuzumab emtansine (T-DM1), lapatinib and newer therapies (such as trastuzumab deruxtecan and tucatinib) have significantly improved the outcome of HER2 + metastatic breast cancer (HER2 + MBC) patients (4–9). Current treatment guidelines support the maintenance of anti-HER2 therapy throughout the different lines of treatment (10,11).

Oral etoposide (VP16) is an inhibitor of topoisomerase II. Oral VP16 has demonstrated clinical activity in heavily pre-treated patients with HER2-negative MBC compared to other active chemotherapies such as capecitabine, paclitaxel, eribulin or anthracyclines (17). Although not currently recommended in MBC guidelines, the use of oral VP16 could be relevant in heavily pre-treated MBC, with the advantage of an oral administration, a low cost and manageable toxicity. Moreover, while the cardiac toxicity of anthracyclins is overlapping that of anti-HER2 targeted agents (12), oral VP16 has no reported cardiac toxicity, thus allowing combination therapy.

*TOP2A*, the gene encoding topoisomerase II, is located on the long arm of chromosome 17 (17q21-22), close to *ERBB2* (18). It has been reported that up to 40% of HER2 + breast cancers display a co-

amplification of *TOP2A* and *ERBB2* genes (19), which has been investigated as a predictive marker of anthracycline efficacy (19–23) in HER2 + breast cancers, with controversial results (24–27).

To the best of our knowledge, there are no studies evaluating the efficacy of oral VP16 in combination with trastuzumab, a combination used in our institute as a palliative, late line therapy for HER2 + MBC patients. Here, we report a retrospective evaluation of the efficacy and safety of oral VP16 combined with trastuzumab in HER2 + MBC and the predictive value of *TOP2A/ERBB2* co-amplification.

## Methods

### Patients and clinical data

The research project was submitted and approved by the Internal Research Committee of the Institut Curie (No. DATA200187). A waiver of informed consent was obtained because of the retrospective nature of the study.

Patients treated with oral VP16 and trastuzumab were retrieved by an in-silico search in the database of Institut Curie Hospitals (Paris and Saint Cloud, France). Computerized medical files were then manually inspected by experienced medical oncologists. Inclusion criteria were: HER2 + MBC female patients treated with oral VP16 in combination with trastuzumab, regardless of the treatment line. HER2 + tumors were defined according to the 2018 American Society of Clinical Oncology/College of American Pathologists guidelines (3). Trastuzumab could have been received prior to oral VP16 and continued after VP16-T treatment. All oral VP16 administration regimens were included in the study. The dose of 50mg or 75mg per day for 10–14 days out of 21 was defined as the standard oral VP16 regimen (28).

The primary objective was to evaluate the progression-free survival (PFS) in HER2 + MBC patients treated by VP16-T. PFS was defined as the period from initiation of combination therapy to disease progression or death for any cause, whichever came first.

Secondary objectives were to evaluate overall survival (OS), progression-free survival (PFS) under the prior treatment line, response rate, clinical benefit, toxicity and predictive value of *TOP2/ERBB2* co-amplification. OS was calculated from the start of treatment until death from any cause or until the last date the patient was known to be alive. The response rate was measured as the ratio between patients experiencing a partial or complete response as best response, using RECIST 1.1 criteria, and patients who had a measurable disease at treatment start (29). Clinical benefit at 24 weeks was defined as a PFS > 24 weeks and/or objective tumor response. Toxicities were retrospectively classified according to the National Cancer Institute's Common Criteria for Toxicity (version 5.0).

### TOP2A/ERBB2 co-amplification

*TOP2A/ERBB2* co-amplification was analyzed by shallow Whole Genome Sequencing (sWGS) using Formalin-Fixed Paraffin-Embedded (FFPE) tumor tissue (30–32) from an available tumor tissue (from metastasis or primary tumor). All slides have been reviewed by a pathologist, to ensure a minimum tumor

cellularity of 30%. Between 5 and 50 ng (when available) of tumor DNA were processed with the pre-capture kit XT-HS2 (Agilent) according to the manufacturing protocol. First, DNAs were fragmented with the ME220 sonicator, repaired, adenylated and ligated with the duplex molecular barcode and the Illumina paired-end sequencing elements during 1h. Then, unique dual sample indexes were added by 14 cycles of PCR amplification. The libraries were qualified and quantified by the HS Qubit kit and TapeStation 4200 (Agilent) with the D1000 DNA ScreenTape analysis kit prior to pooling in one single tube. The final pool was finally quantified by qPCR on the 7500 Real-Time PCR System (Thermo Fisher Scientific). 100 pb paired-end shallow sequencing was performed at Institut Curie core sequencing facility, using an Illumina Novaseq6000.

Sequencing files were pre-processed as indicated in Eeckhoutte et al, 2020 (33). Details are available upon request. Pre-processed alignment files were analyzed by counting and normalizing the number of aligned reads in fixed window of 50kb with QDNAseq (34). QDNAseq associates contiguous windows considered to be in the same copy number level in genomic segments. The middle of *TOP2A* and *ERBB2* loci were used to extract from QDNAseq their respective fixed window and genomic segment values. QDNAseq outputs were then processed with shallowHRD (33), which extracts a minimal copy number alteration (CNA) cut-off.

The *TOP2A/ERBB2* co-amplification status was defined when associated fixed window and segment values of both genes were over 4-fold of the CNA cut-off. The absence of *TOP2A/ERBB2* co-amplification status was defined when the fixed window and segment values of *ERBB2* were over 4-fold of the CNA cut-off and those of *TOP2A* less than 4-fold of the CNA cut-off. Samples were classified as “not interpretable” in case of discrepancy between window and segment values for one gene or if no amplification of *ERBB2* was retrieved by sWGS.

## Statistics

Quantitative variables are presented with their median, minimum and maximum. Qualitative variables are presented with the number and percentage. Missing data (not available = NA) are excluded from the denominator for the calculation of percentages. Median follow-up was determined by the inverted Kaplan-Meier method (35). Median values for PFS and OS (with their 95% confidence intervals [CI]) were estimated using the Kaplan-Meier method. All statistical analyses were performed using R 3.6 (36).

## Results

### *Patients and treatment*

2,003 patients treated for HER2+ MBC were retrieved by *in silico* screening of the Institut Curie electronic medical files. Among those patients, 43 met inclusion criteria and were analyzed as part of this retrospective study: their characteristics are shown in **Table 1**. The median age at diagnosis of primary breast cancer was 47 years (22-80 years). The median age at diagnosis of MBC was 51 years (22-83 years). Synchronous BC metastases were diagnosed in 14 (33%) patients (*de novo* stage IV). Patients

had received a median number of six prior treatment lines (range 0-12) at the time of receiving VP16-T regimen. Thirty-five patients (81%) had visceral metastases. Oral VP16 regimen was administered at the above-defined standard doses to 31 patients (72%). Median duration of VP16-T treatment was 2.9 months (0.2 - 14.6 months). VP16-T was stopped for disease-progression (n=35 patients, 81%), toxicity (n=3 patients, 7%), therapeutic break (n=3 patients, 7%) or unknown cause (n=2 patients, 5%).

**Table 1**

Patients' characteristics

		<b>N patients</b>	<b>%</b>
<b>Phenotype</b>	HER2+	43	100
	HER2+/HR+	21	49
	HER2+/HR-	22	51
<b>Age at primary BC (Years)</b>	< 50	27	63
	≥ 50	16	37
<b>Age at metastatic BC (Years)</b>	< 50	21	49
	≥ 50	22	51
<b>Stage at BC diagnosis</b>	0	1	2
	I	5	12
	II	10	23
	III	13	30
	IV	14	33
<b>Histological type</b>	Ductal	38	88
	Lobular	5	12
<b>Histological grade (EE)</b>	1	3	7
	2	19	44
	3	21	49
<b>Metastasis-Free Interval</b>	de novo	14	33
	[6-24] months	6	14
	]24-60] months	14	33
	>60 months	9	20
<b>Number of metastatic sites</b>	≤ 2	12	28
	> 2	31	72
<b>Visceral metastases</b>	No	8	19
	Yes	35	81
<b>Number of prior treatment lines</b>	< 2	3	7
	3	6	14
	4	8	19
	5	4	9

	6	7	16
	7	4	9
	> 8	11	26
<b>Median number of prior treatment lines</b>	6 (0-12)	-	-
<b>VP16 administration schedule</b>	Standard*	31	72
	- 50 mg	9	21
	- 75mg	22	51
	Other	9	21
	Not available	3	7
<p><i>*50-75 mg/D, 10 to 14D/21</i></p> <p><i>HR: Hormone Receptor</i></p> <p><i>EE: Elston and Ellis</i></p>			

## ***Efficacy***

Median follow-up was 56.8 months (range 3.8-82 months). Thirty-six PFS events were observed during VP16-T treatment. Median PFS was 2.9 months (95% CI [2.4-4.7]; **Figure 1A**). Median OS was 11.3 months (95% CI [8.3-25.0]) (**Figure 1B**). Forty patients were eligible for response rate assessment using RECIST 1.1 (**Suppl file 1**). Four patients (10%) had a partial or complete response to VP16-T. A complete response was observed in 3 patients who received VP16-T given as first, second and thirteenth line of treatment respectively. One patient had a partial response. Overall, 12 out of 40 evaluable patients (30%) had a clinical benefit at 24 weeks (24 weeks clinical benefit rate: 30%; **Figure 2**).

Clinical benefit is defined by either an objective tumor response (N=4 patients) and/or a PFS under VP16-T longer than 6 months (N=8 patients).

The different systemic treatments administered immediately prior to VP16-T are detailed in **Suppl file 2** (one patient received VP16-T as first line treatment). Progression-free survival on prior treatment with gemcitabine-trastuzumab, vinorelbine-trastuzumab and cyclophosphamide-trastuzumab were 2.3 months (95% CI [2.2-NA]), 1.9 months (95% CI [0.8-NA]), 3.4 months (95% CI [1.6-NA]), respectively. In six of the 12 patients with clinical benefit at 24 weeks, PFS with VP16-T was twice as long as the PFS under the prior line of treatment. Of note, the median number of prior treatment lines in these 6 patients was 5 (range 0-12), as for the overall study population. All patients had previously received taxanes and 63% had previously received anthracyclines. No significant difference in response rates, nor in PFS, was found between patients who had previously received or not anthracyclines (data not shown).



Brain metastases were observed in 22 of 40 evaluable patients and in 6 of 12 patients with prolonged PFS. Among these 6 patients with brain metastases and prolonged PFS, only one experienced a disease progression of her brain metastases while receiving VP16-T.

## ***Toxicity***

Toxicity has been retrospectively assessed for 42 patients (**Table 2**). Oral VP16 was discontinued due to toxicity in 3 patients: two for grade 3 nausea/vomiting, one for febrile neutropenia. Nauseas (grade 2 and 3) were observed in 14% of cases. Grade 1 alopecia was recorded in only 1 patient. No diarrhea, mucositis or allergies were observed.

**Table 2**

Toxicities

<b>Toxicity</b>	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>
	N (%)	N (%)	N (%)
<b>Nausea</b>	0	4 (10)	2 (5)
<b>Neutropenia</b>	3 (7)	1 (2)	1 (2)
<b>Alopecia</b>	1 (2)	0	0
<b>Asthenia</b>	17 (40)	10 (24)	8 (19)
<i>Toxicity data were available for 42 patients</i>			

## ***TOP2A/ERBB2 co-amplification***

FFPE tumor samples were available for DNA extraction for 23 patients. sWGS was not interpretable for 3 samples. Among the 20 patients included in sWGS analysis, seven (35%) displayed an *TOP2A/ERBB2* co-amplification (examples are shown in **Suppl file 3**). Three patients with *TOP2A/ERBB2* co-amplification had a clinical benefit at 24 weeks (including 2 patients with complete response). The median PFS was 3.4 months (95% CI [2.3-6.9]) in these 20 cases, which is comparable to the overall study population (2.9 months, 95% CI [2.4-4.7]).

No statistically significant correlation was found between outcome and *TOP2A/ERBB2* co-amplification: median PFS in the population with and without *TOP2A/ERBB2* co-amplification was respectively 4.7 months (95% CI [2.3-NA]) and 2.9 months (95% CI [1.2-NA]; p=0.36) (**Figure 3**). Three (43%) patients with clinical benefit had *TOP2A/ERBB2* co-amplification and four (31%) patients without clinical benefit had *TOP2A/ERBB2* co-amplification (Fisher p=0.65).

## Discussion

To our knowledge, no studies have evaluated the efficacy of oral VP16 and trastuzumab combination in HER2 + MBC. We show that this combination achieves clinically meaningful PFS with about one third of prolonged PFS (defined as PFS greater than or equal to 6 months), a clinical benefit in a third of patients, and 3 complete responses. PFS and OS were 2.9 months and 11.3 months, respectively. These results were obtained in a heavily pre-treated population with a median number of six prior treatment lines for MBC. Moreover, most of our patient population displayed unfavorable clinical features, such as visceral metastases. Limitations of our study are related to its limited size and retrospective nature. However, this study is the first specifically analyzing outcome and toxicity of oral VP16 associated with trastuzumab for HER2 + MBC.

Oral VP16 is a metronomic chemotherapy, defined as the regular administration of a minimally toxic dose of treatment over an extended period of time. In advanced breast cancer, metronomic chemotherapy has been shown to provide disease control with a lower incidence of adverse events compared to conventional chemotherapy at the maximum tolerated dose (37,38). In the years 1994 to 2000, oral VP16 showed interesting clinical activity in patients with MBC after multiple lines of treatment (39–43). More recently, a study by Cabel et al. (17) showed survival rates with oral VP16 comparable to other treatment lines including capecitabine, paclitaxel, eribulin or anthracycline (median PFS of 3.2 months) in patients with HER2-negative MBC.

Some studies reported the outcome of HER2 + MBC treated with oral VP16. In 2015, a retrospective study by Valaberga et al. (44) found a 4 months median PFS with oral VP16 in patients who received a median of 8 treatment lines (range 2–13). Twenty-one patients out of 66 had HER2 + MBC. The PFS did not differ between HER2-positive and HER2-negative status. Another retrospective study (45) included 110 pretreated patients with a median of 5 lines of treatment. Twenty-five of these patients had HER2 + MBC. The median duration of treatment was 4 months with, again, no significant difference according to HER2 status. In a prospective phase II study (46), a median PFS of 4.5 months as reported in 75 patients with MBC and a median number of 2 prior lines of therapy, of which 22 had an HER2 + disease. A review of twelve studies, including about a third of HER2 + MBC patients, reported an overall 18.5% response rate with oral VP16 (47). None of these studies specified the use of anti-HER2 therapy in combination with oral VP16. The low number of HER2 + MBC in these studies and the lack of specific subgroup analysis prevent any further comparison with our results.

There is limited data available on the efficacy of other late line chemotherapies and trastuzumab in pretreated HER2 + MBC. The efficacy of vinorelbine and trastuzumab was assessed in two prospective studies. In 46 patients treated with vinorelbine in a second-line setting after progression on a first-line taxane-based regimen, Blancas et al. (52) reported a 7 months median PFS in seven HER2 + MBC patients. The phase II study of Lee et al. (53) showed a median PFS of 6.8 months in 33 HER2 + MBC patients with HER2 + MBC and a median of four prior lines of systemic treatment. Gemcitabine and trastuzumab has been investigated in two studies: Bartsch et al. (48) and Yardley et al. (49) included 23

and 37 patients respectively. These studies included patients who received a median of two prior lines of systemic therapy for HER2 + MBC and reported a median PFS of 3 and 4 months respectively. PFS of similar ranges were observed in the control arm of the TH3RESA trial (6). In this pivotal trial, 602 HER2 + MBC patients who received a median of four prior lines of therapy demonstrated a significantly improved median PFS with trastuzumab-emtansine compared with physician-selected therapy (6.2 months versus 3.3 months). In the treatment of physician's choice arm, 68% of patient received concomitant trastuzumab and chemotherapy (vinorelbine in 32% of patients, gemcitabine in 16% of patients). Interestingly, the median PFS in the control arm of TH3RESA is similar to that observed with VP16 and trastuzumab in our report.

Presence of a co-amplification of *TOP2A* and *ERBB2* on chromosome 17 suggests a biological interest to combine oral VP16 and trastuzumab in HER2 + MBC. In keeping with prior reports, our sWGS analysis retrieved a *TOP2A/ERBB2* co-amplification in 35% of cases. *TOP2A/ERBB2* co-amplification was numerically, but not statistically, more frequent in patients benefiting from VP16-T. The limited number of patients analyzed prevents any definitive conclusion about the predictive value of the co-amplification. Of note, other non-genetic mechanisms may also modulate the response to topoisomerase 2 inhibitors (20), such as epigenetic mechanisms modulating DNA accessibility (58).

## Conclusions

Finally, our retrospective study suggests oral VP16 and trastuzumab may be considered as a treatment option in heavily pre-treated HER2 + MBC patients. This combination yields to prolonged responses in some patients and has the advantage of an oral administration, limited cost and acceptable toxicity.

## Abbreviations

CNA: Copy Number Alteration

DNA: DeoxyriboNucleic Acid

EE: Elston and Ellis

FFPE: Formalin-Fixed Paraffin-Embedded

HER2: Human Epidermal growth Factor Receptor 2

HR: Hormone Receptor

MBC: Metastatic Breast Cancer

OS: Overall Survival

PCR: Polymerase Chain Reaction

PFS: Progression-Free Survival

QDNAseq: Quantitative DNA Sequencing

qPCR: quantitative Polymerase Chain Reaction

shallowHRD: shallow Homologous Recombination Deficiency

sWGS: shallow Whole Genome Sequencing

VP16: Oral etoposide

VP16-T: VP16 combined with trastuzumab

## Declarations

### - **Ethical Approval and Consent to participate:**

The research project was approved by the Internal Research Committee of the Institut Curie (No. DATA200187). A waiver of informed consent was obtained because of the retrospective nature of the study.

### - **Consent for publication:**

Not applicable.

### - **Availability of supporting data:**

The datasets used and analyzed during this study are available upon request from the corresponding author.

### - **Competing interests:**

The author declared no conflict of interest.

### - **Funding:**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## - Authors' contributions:

CC, MC, FCB and FL made substantial contributions to conception and design, and revising the manuscript, and gave final approval for publication. CC, MC, AE, SB, MHS, FCB and FL contributed to acquisition of data, analysis and interpretation of data. CC, MC, AE, FCB and FL drafted the manuscript. CC, MC, and AE performed data analysis for the study. CC, MC, FCB and FL participated in manuscript preparation and revision. All other authors made substantial contributions to the acquisition of data, revising the manuscript, and final approval.

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Not applicable.

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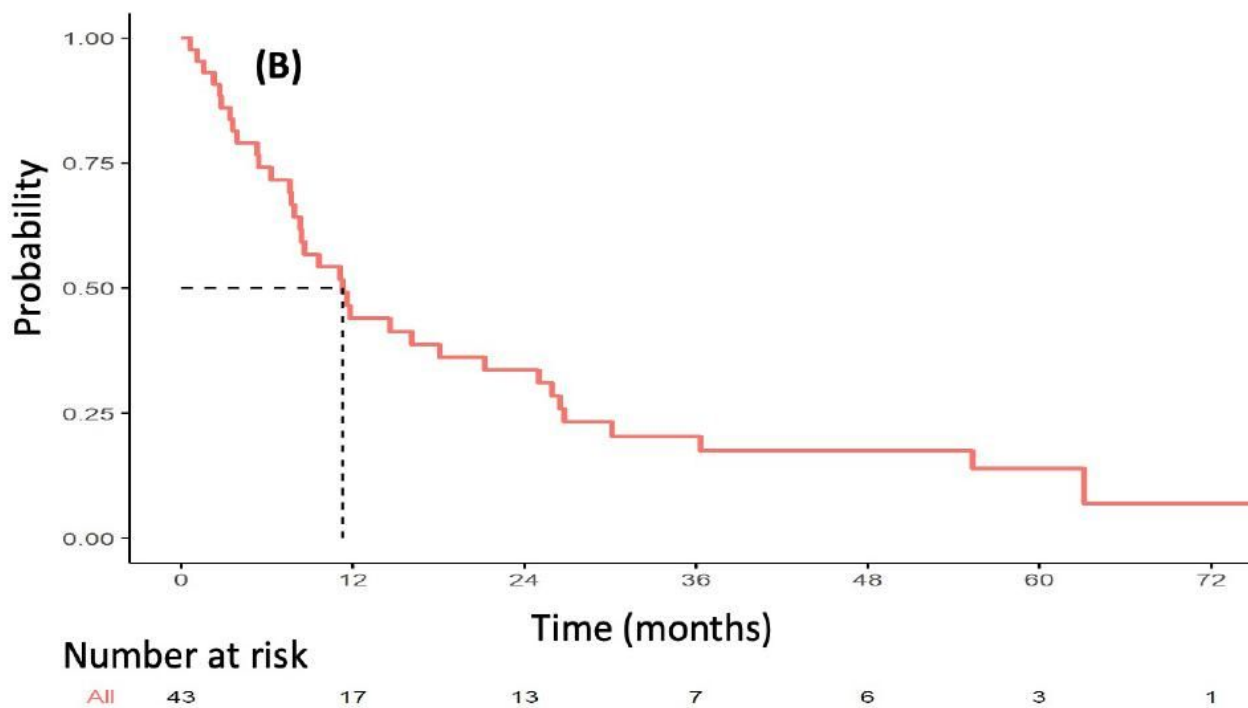
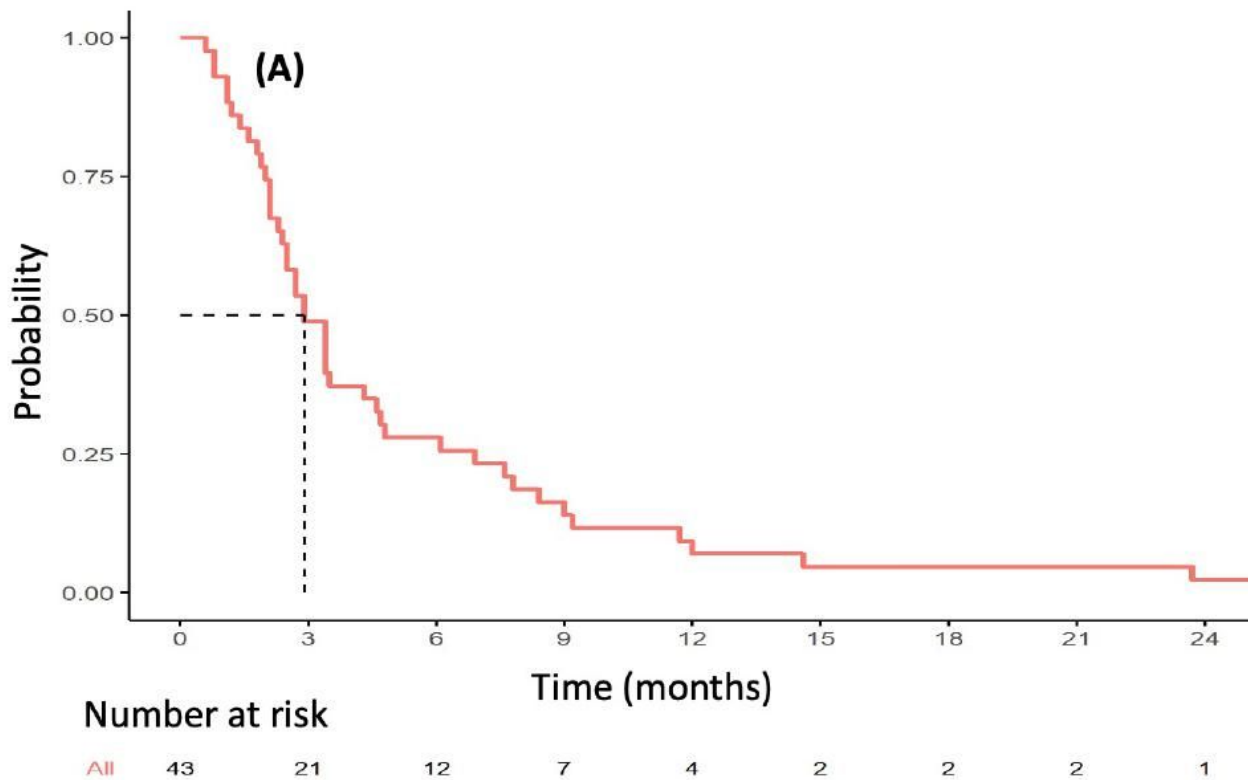
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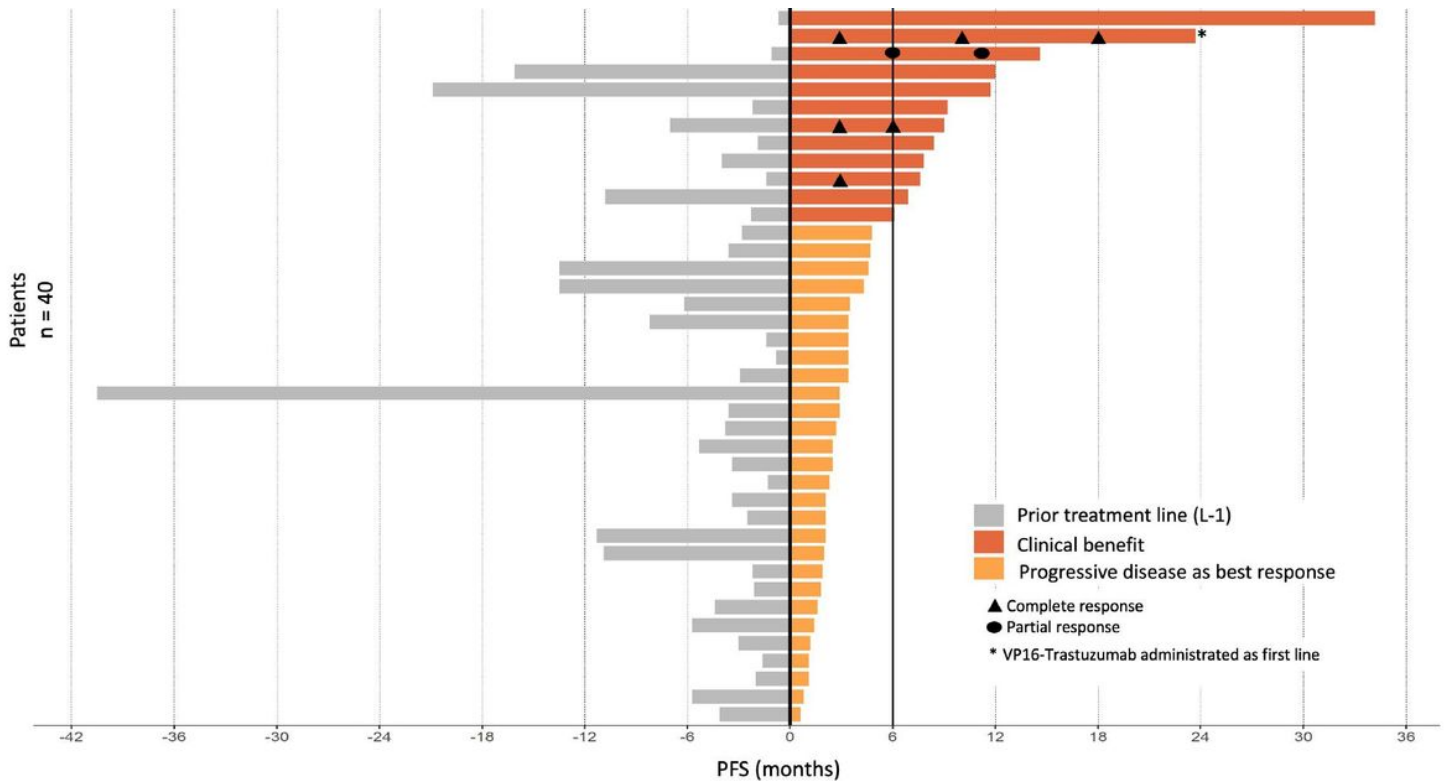
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## Figures



**Figure 1**

Kaplan-Meier Estimates of Progression-Free Survival (A), Overall Survival (B) in patients treated with VP16-T



**Figure 2**

PFS by patient, under prior treatment line and under VP16-T

Clinical benefit is defined by either an objective tumor response (N=4 patients) and/or a PFS under VP16-T longer than 6 months (N=8 patients).

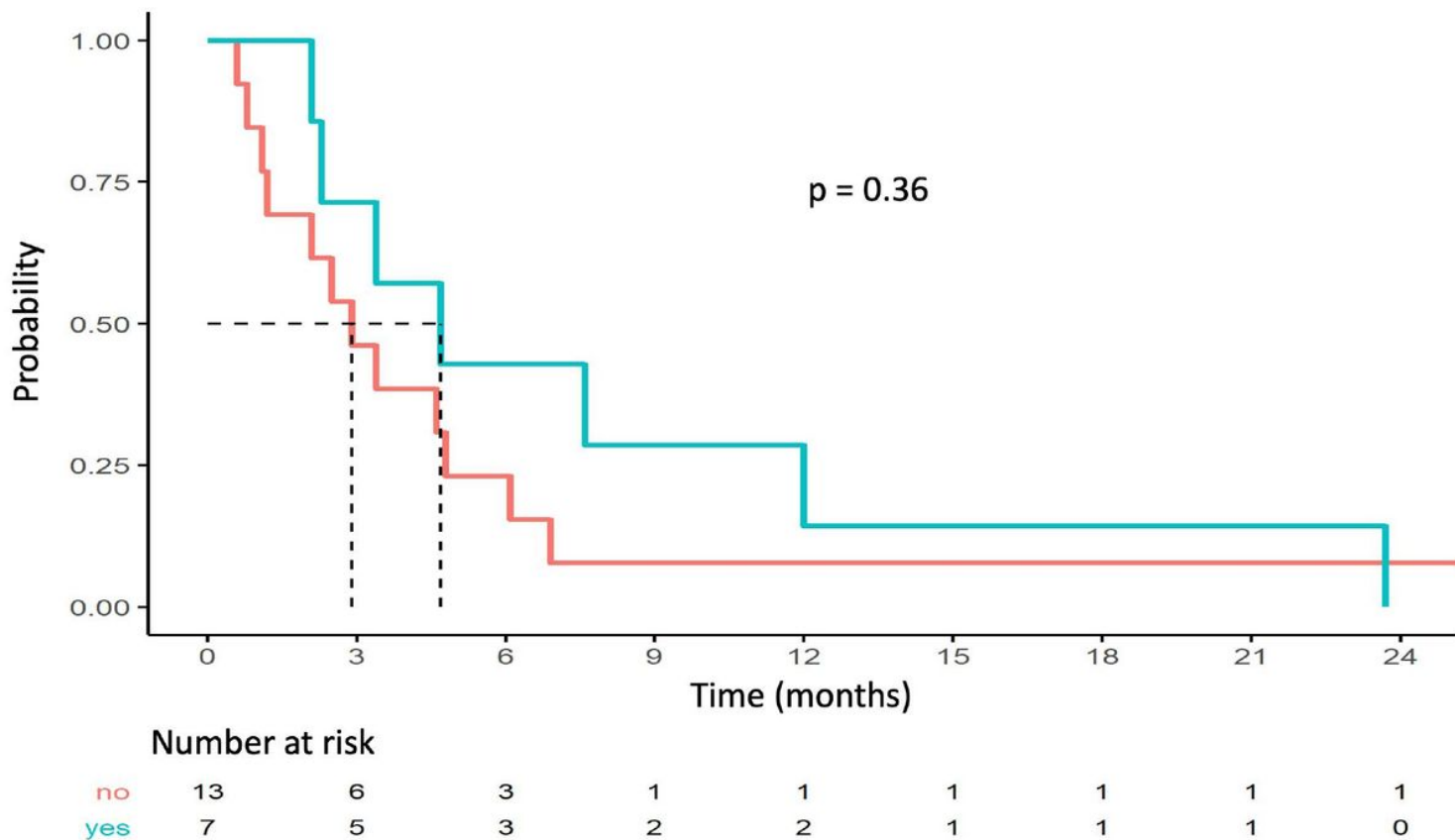


Figure 3

PFS depending of *TOP2A/ERBB2* co-amplification status

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