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THERAPY IN PRACTICE

c-MET as a Potential Resistance Mechanism to Everolimus in Breast Cancer: From a Case Report to Patient Cohort Analysis

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Abstract

Background We describe in a patient with breast cancer the change in c-MET expression during everolimus treatment, opening a better understanding of the resistance to everolimus and a role for cabozantinib.

Objective The objective of this study was to evaluate c-MET as a potential predictive biomarker for everolimus efficacy in breast cancer.

Methods We first selected a patient with breast cancer with a long-lasting response to everolimus and retrospectively profiled biopsies that were taken before everolimus initiation (Biopsy 1) and at progression on everolimus (Biopsy 2) using amplicon sequencing and immunohistochemistry. We then retrospectively evaluated c-MET expression in a cohort of patients with breast cancer treated with everolimus.

Results While not expressed in Biopsy 1, c-MET was highly expressed in Biopsy 2, suggesting a role for c-MET in breast cancer progression. Cabozantinib resulted in a rapid radiological response in this patient. Twenty-nine patients were included (12 c-MET-positive and 17 c-MET-negative patients) in the second part of the study. Baseline c-MET expression was associated with higher tumor grade, higher frequency of visceral metastases, and lower endocrine sensitivity. The c-MET-positive patients presented with a shorter progression-free survival (6.1 vs 10.5 months, respectively; $p = 0.002$) and a lower response rate (0% vs 12%) to everolimus, compared with c-MET-negative patients.

Conclusions c-MET could play a role in the resistance to everolimus and its inhibition should be evaluated in breast cancer.

1 Introduction

Breast cancer (BC) is the most common malignancy in women worldwide, representing a major threat to women's health [1]. Approximately 60–70% of BCs express estrogen and/or progesterone receptors and benefit from endocrine therapy (ET) including tamoxifen or aromatase inhibitors, both in the adjuvant and in the metastatic setting. However, resistance occurs frequently; almost 50% of patients with advanced disease do not respond to first-line ET (de novo resistance) and virtually all patients who presented with an initial positive response experience cancer progression (acquired resistance). Different mechanisms have been shown to play a role in the development of ET resistance, including activation of multiple signaling pathways [2–4].

The phosphatidylinositol 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) cascade plays a key role in many cellular functions. Activated mTOR leads to phosphorylation of ribosomal p70 S6 protein kinase 1 and 4E-binding protein 1 (4E-BP1), stimulating cell survival, proliferation, and angiogenesis. The PI3K-AKT-mTOR cascade is negatively regulated by the phosphatase and TENsin homolog (PTEN) protein, which prevents phosphorylation of AKT by PI3K. Excessive activation of the PI3K-AKT-mTOR cascade is common in cancer; up to 45% of BCs present with *PIK3CA* somatic mutations and around 15% present with a *PTEN* gene loss [5–7]. Preclinical studies showed that this pathway may be involved in the development of ET resistance in BC and that mTOR inhibitors are

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Key Points

Resistance to everolimus could be induced by primary or secondary activation of c-MET.

c-MET expression may appear as a predictive biomarker of resistance to everolimus and should be evaluated in prospective trials.

Cabozantinib may appear as a promising treatment option in selected patients with breast cancer harboring c-MET expression.

able to overcome this resistance in vitro and in vivo [8]. Different trials confirmed the efficacy of everolimus in metastatic BC (mBC); the randomized phase III BOLERO-2 trial showed that the addition of everolimus to exemestane improved progression-free survival (PFS) compared with exemestane alone (10.6 vs 4.1 months, respectively; $p < 0.001$) in patients with hormone-receptor-positive mBC who had recurrence or progression while receiving previous therapy with a nonsteroidal aromatase inhibitor in the adjuvant setting or in the advanced setting [9, 10]. Activation of the PI3K-AKT-mTOR cascade was correlated with higher sensitivity to everolimus in preclinical studies [11, 12].

However, in clinical trials, no clear link between *PIK3CA* mutation and/or PTEN status and clinical response to everolimus has been observed, reflecting the absence of efficient biomarkers in our clinical practice [13–15]. Furthermore, the development of resistance to everolimus could involve the activation of alternative regulating proteins such as c-MET, a tyrosine kinase protein that activates multiple survival cascades including the mitogen-activated protein kinase and extracellular-signal-regulated kinase (ERK) pathways [16, 17].

In the first part of this work, we focused on a clinical case report; we characterize, through sequential biopsies, the change of c-MET expression in a patient who presented with progression on everolimus after an initial response. We showed that c-MET could be involved in the development of resistance to everolimus. Cabozantinib in this patient resulted in a rapid radiological response, confirming the role of c-MET in cancer progression. We then explored the association between c-MET and everolimus efficacy in a cohort of patients with advanced BC.

2 Materials and Methods

In the case report patient, tumor samples were analyzed using the OncoSTRAT&GO™ system from OncoDNA (Gosselies, Belgium). OncoSTRAT&GO™ is based on AmpliSeq technology, amplifying for next-generation sequencing whole exons and hotspot mutations of 313 genes (listed in Table 1 of the Electronic Supplementary Material [ESM]). Furthermore, it evaluates by immunohistochemistry the expression of multiple proteins that could be potentially targeted by current antitumoral agents, including c-MET, P-glycoprotein, phospho-4E-BP1, PTEN, phospho-ERK1/2, transducin-like enhancer protein 3, topoisomerase 2A, thymidylate synthetase, and retinoblastoma protein 1.

The patients included in the retrospective analysis were patients with mBC who were treated with everolimus 10 mg daily plus exemestane between January 2012 and December 2017. All these patients had mBC with estrogen receptor (ER) and/or progesterone receptor (PR) expression, been previously treated with ET in the metastatic setting (tamoxifen or aromatase inhibitors), not been treated with cyclin-dependent kinase (CDK) 4/6 inhibitors previously, and had an available tumor biopsy performed within 12 months before the initiation of everolimus treatment. The c-MET expression was analyzed by immunohistochemistry with a c-MET antibody (rabbit monoclonal antibody [SP44]; Ventana Medical Systems) and phospho-c-MET (Tyr1234 and 1235 rabbit monoclonal antibody; Cell Signaling). Both cytoplasmic and membranous staining were considered positive. Staining of c-MET and phospho-c-MET was scored as follows: 0 if no staining; 1 + if weak staining in any amount of tumor cells and moderate staining in < 50% of tumor cells; 2 + if ≥ 50% of tumor cells showed incomplete membranous and/or cytoplasmic staining with at least moderate intensity but < 50% cells with strong intensity; and 3 + if ≥ 50% of tumor cells with circumferential membranous and/or cytoplasmic staining with strong intensity. c-MET and phospho-c-MET were considered positive for expression if the staining was scored as 2 + or 3 + (at least ≥ 50% of tumor cells showed moderate or higher membranous staining). Endocrine sensitivity was defined as at least 24 months of ET before recurrence in the adjuvant setting or a response or disease stabilization for at least 24 weeks of ET for advanced disease.

Progression-free survival was defined as the time from everolimus initiation to clinical or radiological progression of the disease or death as a result of any cause. Overall survival (OS) was defined as the time from everolimus initiation to death. Progression-free survival and OS were summarized using Kaplan–Meier curves. The overall response rate was defined as the tumor response (partial response and complete response) according to RECIST criteria (version 1). The disease control rate was defined as the rate of patient with response (partial or complete) and stable disease, according to RECIST criteria.

Part 1 of this study describes the history of a specific patient. This patient provided consent for publication. The

Table 1 Differences in detected gene mutation and protein expression between paired biopsies in a patient treated with everolimus

	Biopsy 1 (before everolimus initiation)	Biopsy 2 (at progression on everolimus)
Gene mutations	*PIK3CA* E542K	*PIK3CA* E542K
	No other differences in evaluated genes (see list in Electronic Supplementary Material)	
Targetable protein expression	c-MET negative	c-MET positive Positive if more than 50% cells with medium or strong membranous or cytoplasmic staining
	p-ERK low expression	p-ERK high expression High expression if H-score > 100
	P-glycoprotein negative Positive if more than 10% of tumor cells with membranous staining 1 +	
	Phospho-4E-binding protein 1 high expression High expression if H-Score > 100	
	Retinoblastoma protein 1 negative Percentage score: 0 (negative), 1 (few nuclei), 2 (10%), 3 (10–50%), 4 (> 50%) Intensity staining: 1 (weak staining), 2 (moderate staining), 3 (strong staining); quick score: positive if ≥ 4	
	Phosphatase and TENsin homolog positive Positive if H-score > 10	
	Transducin-like enhancer protein 3 positive Positive if more than 30% of positive 2 + nuclear staining	
	Topoisomerase 2A positive Positive if more than 10% of positive nuclear 1 + staining	
	Thymidylate synthetase low expression Positive if more than 10% of tumor cells with 1 + cytoplasmic staining	
H-Score formula: (%1 + c + %1 + *n*)/2 + (%2 + c + %2 + *n*) + 3*(%3 + c + %3 + *n*)/2		

ERK extracellular-signal-regulated kinase, *H-score* Histo score

retrospective analysis was performed in accordance with the Declaration of Helsinki. The Ethics Committee of Hopital de Jolimont has given its approval for this retrospective study and analysis of patient data. This was a purely observational retrospective analysis of clinical data and there was no contact with patients (ethical committee approval is included in the ESM).

3 Results

3.1 Identification of c-MET Expression as a Resistance Mechanism in an Everolimus-Treated Patient

A 69-year-old woman was treated with everolimus and exemestane for mBC. Her cancer had initially been diagnosed in 1999, as locally advanced with axillary lymph node involvement. Histology confirmed an invasive ductal carcinoma with high expression of ER and PR and absence of human epidermal receptor 2 (HER2) expression. Treatment included mammectomy followed by six cycles of anthracycline-based chemotherapy, external radiotherapy, and a 5-year duration of tamoxifen. In 2011, thoracic computed tomography showed an apparition of bilateral pulmonary lesions; a biopsy confirmed the resurgence of a mammary ductal carcinoma with high expression of ER, absence of PR, absence of HER2 receptor, and a high proliferation rate (Ki67 = 70%). Letrozole was initiated with a 2-year duration of disease control. However, in November 2013, the size and number of pulmonary metastases increased and cutaneous metastases appeared on the mammectomy scar; one of these lesions was biopsied (Biopsy 1), confirming the persistence of ER and the absence of PR and HER2 receptor. Everolimus was started in association with exemestane and resulted in a best response rate of 75% and a 17-month duration of disease control.

In April 2015, new cutaneous lesions appeared on the mammectomy scar and a computed tomography scan showed progression of pulmonary lesions; a new biopsy (Biopsy 2) of cutaneous metastases confirmed the previously described tumor characteristics (high expression of ER, absence of PR, absence of HER2 receptor, and a high proliferation rate). The paired analysis of these two biopsies revealed a *PIK3CA* mutation in Biopsies 1 and 2, as well as high expression of phospho-4EBP1, reflecting an activated PI3K-Akt-mTOR

pathway (Table 1). Upon progression on everolimus, the patient was treated with the CDK4/6 inhibitor palbociclib and fulvestrant, resulting in the rapid disappearance of cutaneous lesions within the first month, a best response rate of 70%, and in a 16-month response duration. At progression, in September 2017, weekly paclitaxel was started but had to be stopped after 6 weeks because of radiological progression. Capecitabine was then initiated but interrupted after 2 months because of progressive disease.

While not expressed in Biopsy 1, c-MET was highly expressed in Biopsy 2 (Fig. 1a, b), suggesting that c-MET could be implicated in disease progression and everolimus resistance. Phospho-ERK1/2 was also found to be negative in Biopsy 1 and positive in Biopsy 2 (Table 1). Based on the positive expression of c-MET, the patient was initiated on cabozantinib 40 mg daily; this c-MET inhibitor resulted in a rapid clinical improvement and radiological partial response, as shown on the computed tomography scan performed 4 weeks after the start of cabozantinib treatment (Fig. 2a, b). The response was maintained for 7 months, at the dose of 40 mg daily. Cabozantinib was well tolerated, without any significant adverse events except continuous fatigue. Unfortunately, the cancer progressed and the patient's general status deteriorated progressively; the patient died as a result of disease progression 10 months after cabozantinib initiation.

3.2 c-MET Expression and Sensitivity to Everolimus in a Cohort of Patients with Metastatic Breast Cancer

To better understand the influence of c-MET on everolimus sensitivity, we retrospectively analyzed a cohort of 29 women treated with exemestane plus everolimus in our center between 2012 and 2017. All these patients had mBC harboring ER and/or PR, were treated with previous ET in the metastatic setting, and had available tissue samples collected within 12 months before everolimus initiation. Immunostaining for c-MET was performed in all these patients and correlated with PFS on everolimus and OS. Tumor and patient characteristics are listed in Table 2. The c-MET expression was positive in 12 patients (including eight patients positive, zero patient negative, and two patients not evaluable for phospho-c-MET) and negative in 17 patients (including 14 patients negative, 0 patient positive, and 3 patients not evaluable for phospho-c-MET). Compared to c-MET-negative patients, the c-MET-positive patients presented with a lower rate of endocrine sensitivity (59% vs 82%), a higher rate of grade 3 tumors (41% vs 23%), a higher rate of visceral metastases (66% vs 53%), and a higher number of prior therapies (three or more prior treatments 50% vs 35%). Median PFS was significantly lower in c-MET-expressing tumors compared with c-MET-negative tumors (6.1 vs 10.5 months, respectively;

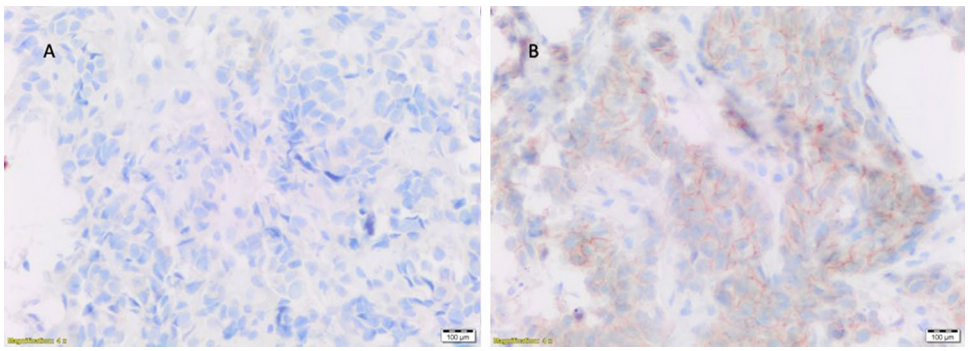

Fig. 1 c-MET expression in the tumor samples obtained before everolimus initiation (**a**) and obtained at progression on everolimus (**b**)

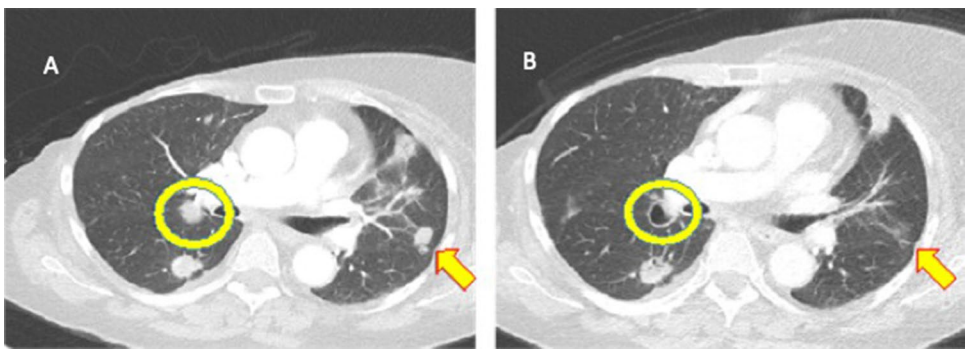

Fig. 2 Tumor response to cabozantinib. Before (**a**) and 1 month after (**b**) initiation of cabozantanib: necrotizing node (circle) and lung node decrease (arrow)

Table 2 Demographic and tumor characteristics of the retrospective cohort

Characteristics	c-MET positive ($n=12$)	c-MET negative ($n=17$)
Median age, years (range)	59 (33–78)	60 (40–76)
Ethnicity, Caucasian, *n* (%)	12 (100)	17 (100)
Ductal carcinoma, *n* (%)	9 (75)	11 (65)
Lobular carcinoma, *n* (%)	3 (25)	6 (35)
Grade in metastatic setting, *n* (%)		
1	0 (0)	2 (12)
2	7 (59)	11 (65)
3	5 (41)	4 (23)
De novo metastatic, *n* (%)	3 (25)	3 (17)
Previous chemotherapy, *n* (%)		
Adjuvant/neoadjuvant only	5 (42)	6 (35)
Treatment of metastatic disease (with or without adjuvant/neoadjuvant), *n* (%)	3 (25)	3 (17)
Previous endocrine therapy, *n* (%)		
Adjuvant	9 (75)	14 (82)
Metastatic disease	12 (100)	17 (100)
Previous therapies (including those used in adjuvant or metastatic setting), *n* (%)		
1	1 (8)	2 (12)
2	5 (42)	9 (53)
3 or more	6 (50)	6 (35)
Endocrine therapy sensitivity, *n* (%)	7 (59)	14 (82)
Metastases location at initiation of everolimus, *n* (%)		
Visceral metastasis only	4 (33)	4 (24)
Bone metastases/lymph nodes	4 (33)	8 (48)
Visceral + bone metastases/lymph nodes	4 (33)	5 (29)
Measurable disease, *n* (%)	10 (83)	15 (88)
Phospho-c-MET positive, *n* (%)	8/10 (80) 2 pts not evaluable	0/14 (0) 3 pts not evaluable

Endocrine sensitivity was defined as at least 24 months of endocrine therapy before recurrence in the adjuvant setting or a response or stabilization for at least 24 weeks of endocrine therapy for advanced disease

pts patients

$p=0.002$) [Fig. 3a]. In c-MET-positive patients, there was no partial response and five stable diseases (42%), resulting in a disease control rate of 42%, whereas in c-MET-negative patients, there were two partial responses (12%) and 12 stable diseases (70%), resulting in a disease control rate of 82%. Overall survival reached 37 months in c-MET-negative patients and 24.5 months in c-MET-positive patients ($p=0.061$) [Fig. 3b].

4 Discussion

In this article, we first focused on a patient with interesting sensitivity to everolimus, with a PFS of 17 months [8, 9]. A broad spectrum of genes and proteins were analyzed on metastatic lesion samples that were collected before everolimus initiation and at progression on everolimus. The initial sensitivity to everolimus could be explained by an excessive activation of the PI3K-AKT-mTOR pathway, which was reflected by both a *PIK3CA* mutation and high expression of phospho-4E-BP1. Although this finding is concordant with preclinical studies, this was never confirmed in clinical trials, possibly owing to the heterogeneity of the tumors, the small number of patients included in the biomarker studies, and the molecular discordance between primary tumors and metastatic sites [10, 12]. The most interesting finding in our patient was the change observed in c-MET expression during everolimus treatment: while absent on the pre-everolimus tumor sample, c-MET appeared highly expressed in the biopsy collected at everolimus progression, suggesting a role of c-MET in the development of resistance to everolimus and in the progression of cancer. Interestingly,

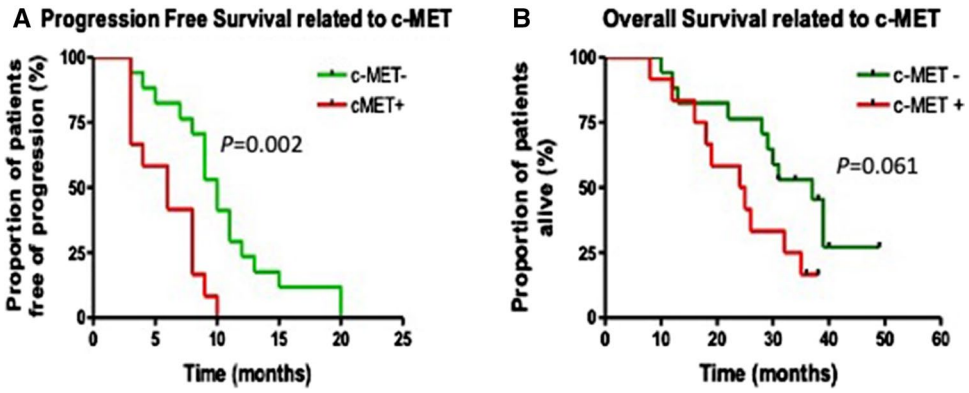

Fig. 3 **a** Progression-free survival based on c-MET expression. **b** Overall survival based on c-MET expression

the expression of phospho-ERK1/2 also became positive during everolimus treatment, suggesting an activation of the c-MET-ERK1/2 axis. Furthermore, the rapid radiological response induced by cabozantinib in this patient may corroborate this hypothesis, as well as the 7-month duration of response, which is particularly significant in such heavily pretreated and chemorefractory patients.

The tyrosine kinase c-MET is a key regulator of organ development and cancer progression [18–20]. c-MET has previously been implicated in other models of acquired resistance to treatments such as chemotherapy, radiotherapy, and targeted therapies [21, 22]. Activation of important survival signaling pathways could be implicated in the c-MET-induced aggressiveness of cancer cells. In BC, c-MET was shown to increase the risk of resistance to trastuzumab by enhancing the mitogen-activated protein kinase signaling pathway and ERK1/2 phosphorylation, leading to a loss of epithelial integrity and cell invasion [23]. Mitogen-activated protein kinase/ERK activation has also been shown to be involved in primary resistance to BRAF inhibition in melanoma cell lines [24]. In lung cancer, c-MET activation is associated with resistance to epidermal growth factor receptor inhibitors via the recruitment of other tyrosine kinase receptors involved in cell survival such as Axl and EphA2 [25]. The c-MET receptor may also interact with vascular endothelial growth factor receptor 2 to promote angiogenesis via activation of the ERK pathways [26]. The role of c-MET in the development of resistance to everolimus is, however, unknown.

We thus retrospectively evaluated c-MET expression in a cohort of patients treated with everolimus for mBC. We observed that patients with c-MET-positive tumors presented with lower endocrine sensitivity, higher tumor grade, and a higher frequency of visceral metastases than patients with c-MET-negative tumors, which is concordant with other studies [18–20]. Furthermore, we describe for the first time that tumor c-MET expression could be associated with a low efficacy of everolimus in BC: patients with c-MET-positive tumors presented with shorter PFS and a lower response rate with everolimus compared with patients with c-MET-negative tumors. The median OS was lower in c-MET-positive patients than in c-MET-negative patients, indirectly confirming the poor prognosis of this group; however, the absence of a statistical difference could be explained by the fact that c-MET-positive patients could be sensitive to a further regimen of cytotoxic agents [9, 10].

Our results are consistent with two preclinical studies showing that c-MET could be associated with resistance to everolimus. Aristizabal Prada et al. observed in pancreatic neuroendocrine tumors that everolimus-resistant cells displayed baseline c-MET overactivation compared with control cells [27]. Raimondo et al. showed in multiple cancer cell types, including BC cell lines, that high levels of phosphorylated/activated MET were detectable in the absence of its ligand hepatocyte growth factor in everolimus-resistant cell lines while in everolimus-sensitive cell lines, phospho-MET was not detectable without hepatocyte growth factor stimulation; the authors also showed that the combination of everolimus with a MET inhibitor significantly inhibited cell growth of everolimus-resistant cells [28]. It is interesting to observe in our patient with sequential biopsies that ERK became positive with everolimus treatment, in parallel to the c-MET positivity, suggesting a potential c-MET-induced activation of the ERK cascade as a mechanism of resistance to everolimus, which is consistent with previous reports [23, 24, 26]. This requires further investigations in preclinical models and in clinical trials with sequential biopsies.

There are of course many limitations in this study, including the retrospective analysis, the limited number of patients, and the imbalance in their baseline characteristics. It remains unknown whether the low sensitivity to everolimus of c-MET-positive patients is secondary to the poor prognosis factors of the patients (grade 3, higher number of prior treatments, visceral metastases, low endocrine sensitivity) or to molecular mechanisms that decrease the efficacy of mTOR inhibitors. Another important limitation is the fact that our retrospective analysis concerned patients who were treated before the CDK4/6 inhibitors era and we do not know whether the predictive role of c-MET for everolimus resistance is still relevant in patients with prior progression on

CDK4/6, which is currently the standard of care in the first-line metastatic setting. Another limitation is the absence of a validated assay for c-MET and phosphor-c-MET expression. A strength of our study is that we included in our analysis only patients with available tumor biopsies performed within the 12 months before everolimus initiation.

5 Conclusions

We have demonstrated that c-MET-positive patients have a lower sensitivity to everolimus than c-MET-negative patients. c-MET could play a role in BC progression and targeting this protein could be beneficial in selected patients. The role of c-MET as a biomarker of everolimus efficacy needs to be evaluated in further large prospective trials.

6 Patient Perspective

The patient declared after the first radiological evaluation: "Personalized treatment remains important for patient, as it allows to administer directly the most efficient treatment and prevent an inefficient therapy". The patient signed an informed consent to receive cabozantinib and to allow publication of her medical history.

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Author Contributions VV, JG, BP, and ES collected the data and wrote the manuscript. GG, JP, and NH helped to write and correct the manuscript. SS and JFL analyzed the tumor sample and performed the OncoDNA analysis. DA analyzed tumor samples with immunohistochemistry.

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Data Availability Information concerning the results can be found in a database (Excel sheet), which can be made available upon reasonable request.

Compliance with Ethical Standards

Conflict of Interest Valentin Van den Bossche, Gaspard Jadot, Guillaume Grisay, Julien Pierrard, Natasha Honoré, Bénédicte Petit, David Augusto, Sébastien Sauvage, Jean-François Laes, and Emmanuel Seront have no conflicts of interest that are directly relevant to the content of this article.

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