FACULTY OF MEDICINE

DEGREE IN MEDICINE
DEGREE FINAL PROJECT

# Retrospective evaluation of everolimus in endocrine therapy for metastatic breast cancer

## Evaluación retrospectiva del everolimus en la terapia endocrina para el cáncer de mama metastásico

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"No tendríamos la capacidad de soñar si no tuviésemos la posibilidad de hacer nuestros sueños realidad"

Anónimo

# INDEX

# ABSTRACT

Metastatic breast cancer admits only palliative treatment. Its commonest molecular subtype, the so-called 'luminal', does express hormone receptors, and allows a preference for endocrine therapy. Successive lines of hormone therapy both relieve symptoms and increase progression free intervals, without adverse events linked to cytotoxic chemotherapy.

As usual, 1st line consists of non-steroidal aromatase inhibitors (letrozole or anastrozole), while the steroidal one (exemestane) remains for 2nd line. It seems exemestane losses efficacy when PI3K/AKT/mTOR pathway is hyperactivated. The combination of exemestane with everolimus, a selective mTOR blocker, has been demonstrated to be synergistic (BOLERO-2 trial, Baselga et al, NEJM 2012).

We reviewed retrospectively 86 patients treated in University Hospitals of Santander and Oviedo during 2013-18, in order to check its efficacy and safety in current regional practice. The average time to treatment failure was 7.18 months, which is in agreement with BOLERO-2. In addition, the toxicity profile we observed (stomatitis, 38%, anaemia, 17%, asthenia, 13%; and pneumonitis, 10%) was similar or even more favourable.

In conclusion, the combination of exemestane plus everolimus is useful in current assistential practice, as reported in other European countries (BALLET study, Jerusalem G et al, Ann Oncol 2016).

**Key words:** everolimus, exemestane, luminal breast cancer, efficacy, toxicity

# RESUMEN

El cáncer de mama metastásico solo admite tratamiento paliativo. El subtipo molecular más común, el llamado 'luminal', expresa receptores hormonales y da pie a preferir la terapia endocrina. Sucesivas líneas de hormonoterapia alivian los síntomas y aumentan la supervivencia libre de progresión, sin la toxicidad propia de la quimioterapia.

En 1ª línea se usan más los inhibidores de la aromatasa no esteroideos (letrozol o anastrozol) y en 2ª se emplea uno esteroideo (exemestano). Se ha visto que el exemestano pierde eficacia al exacerbarse la ruta PI3K/AKT/mTOR, que se bloquea selectivamente con everolimus. El ensayo BOLERO-2 (Baselga y cols, NEJM 2012) constata la sinergia entre exemestano y everolimus, con aceptable toxicidad.

Hemos revisado retrospectivamente 86 pacientes tratadas en los Hospitales Universitarios de Santander y Oviedo en el período 2013-18, para verificar su eficacia y seguridad en la práctica asistencial regional. La media de tiempo al fallo del tratamiento fue de 7,18 meses, en consonancia con BOLERO-2. El perfil de toxicidad (estomatitis, 38%; anemia, 17%; astenia, 13%; y neumonitis, 10%) fue similar o incluso más favorable.

Concluimos que la combinación de exemestano y everolimus es útil en praxis cotidiana, como en otros centros europeos (Estudio BALLET, Jerusalem y cols, Ann Oncol 2016).

**Palabras clave:** everolimus, exemestano, cáncer de mama luminal, eficacia, toxicidad

# INTRODUCTION

## BREAST CANCER DIAGNOSIS

Breast cancer is the most common malignancy in women and it is one of the leadings causes of death among women in the whole world.

Initial diagnostic tests that are indicated in case of a casual finding of a suspicious nodule are different attending to our patient age and clinical symptoms and signs. However, the initial study of this pathology must include (1):

- Complete anamnesis and physical exploration
- Analytic tests that include hemogram, coagulation test and biochemistry and hepatic profile.

Once we complete this first part of the diagnosis, we must choose the most appropriate image test:

- If our patient is older than 35 years, we will start our exploration with a mammography and attending to the clinical data or the results of that mammography, we can complete the study with an echography.
- In young women (patient younger than 35), exploration must be initiated with an echography, and just in case this test shows possible malignancy of the nodule we are studying we will do a mammography.
- Patients with high percentage of having a benign pathology, pregnant women or patients with a non-pathological result of a mammography done in the previous 12 months are also subsidiary of starting the exploration with an echography.

When we find evidences of a suspicious radiological lesion, we must include a biopsy in our procedure in order to confirm the diagnose with the histological pattern. Years ago, the fine-needle aspiration was the gold standard technique to obtain the histological sample, but nowadays we are opting for vacuum-assisted biopsy or, in some cases, core-needle biopsy.

However, the most important part nowadays in diagnosis is the molecular classification. Biological characterization of our tumor is not plainly important for the diagnosis but also for the treatment and the prognosis of this disease (2).

## MOLECULAR CLASSIFICATION

There are 4 different subtypes of breast cancer attending to the expression of some receptors, and this classification is useful to discriminate if a tumor is subsidiary or not of a certain therapy (3):

- Luminal A (50-60%): Tumor with positive estrogen receptor (ER) and/or positive progesterone receptor (PR), with HER-2 negative and low levels of Ki67

(proliferation index). Those tumors have good evolution but lower response to chemotherapy.

- Luminal B (10-20%): Clinical behavior of these tumors is more aggressive than the previous subtype, with higher histological rate, worse prognosis and middle response to chemotherapy. Inside of this group we can divide:
  - ✓ Luminal B with HER-2 negative: ER and/or PR are positive, HER-2 is negative and high levels of Ki67 (higher than 20-30%).
  - ✓ Luminal B with HER-2 positive: ER and/or PR are positive and HER-2 is over-expressed.
- HER-2 positive (15%): Negative ER and/or PR with positive HER-2. HER-2 protein belongs to the epidermic growth factor receptors family and its expression in breast cancer is an independent prognosis factor related with more aggressiveness and lower overall survival. These tumors are very proliferative, with high sensitivity to chemotherapy but very bad prognosis. However, at the same time it is a predictive factor of response to anti-HER-2 therapies.
- +Basal like (10-20%): Negative ER and/or PR and negative HER-2. This group are characterized by its early debut age, its bigger tumor size and the higher proportion of nodes metastasis.

In tumor mammary cells we can find estrogens receptors (ER) and progesterone receptors (PR), which are the hormones used by those cells to grow and develop. Positive expression or ER and/or PR is a prognosis factor because it means that this tumor will respond to an endocrine therapy. That is the reason why this determination by immunohistochemistry is very usual during the initial diagnosis of new breast cancer.

Over-expression or ER/PR is an important prognosis and predictive factor, and that is why its determination allows us to identify those patients that will have an important benefit if treated with endocrine therapy (4).

## THERAPEUTIC APPROACH TO METASTATIC BREAST CANCER

The presence of metastatic disease in patients with a previous diagnosis and treatment of breast cancer can be suspected by some symptoms or signs (like bone pain, dyspnea or anorexia). Although the initial diagnostic exploration is going to be guided by the possible metastatic location according to the clinical data, in order to complete the extension study, we must use a combination of different tests and biological markers.

There are lots of trials trying to demonstrate the superiority of an image test against the others in this field, but the truth is that de diagnostic performance of the different tests (MRI, PET, TC, SPECT) is very similar and its indication is going to be determined by the characteristics and the possible location of the metastasis. However, we must recognize that nowadays MRI and TC are the most used (5).

Just like we said before with the primary tumor, determination of biological markers is also important when metastasis have been confirmed:

- Expression of ER and / or PR in metastatic breast cancer is related with higher free-progression survival (6)
- Expression or ER and/ or PR is related with more benefit if we treat these patients with hormone therapies (6)
- In patients with metastatic breast cancer at the first diagnosis (non-previously treated), over-expression of HER-2 is related with worse behaviour and benefit if we treat them with specific inhibitors.
- Lower evidence for other biological markers like CEA or CA 15.3.
- Lower evidence that the presence of 5 or more tumoral cells in blood per 7.5 ml of blood is associated with higher risk of progression and death (7).

In conclusion, in patients with metastatic breast cancer, determination of ER, PR and HER-2 is strongly recommended to plan the new line of treatment.

Although metastatic breast cancer is unlikely to be cured (nowadays median overall survival approaches three and a half years), there have been meaningful improvements in survival due to the availability of more effective systemic therapies, which main goal is to improve not just the survival, but also the quality of patients' life. These new therapies are based in the great development of systemic therapies like the endocrine therapy or the adjuvant therapies, which are going to be the principal topic of this review.

The selection of a therapeutic strategy depends upon both biological and clinical factors, with the goal of being a tailored approach. Probably, the key point of this diagnosis that will help us to select the most appropriate treatment is the expression of the hormone receptors, the most important biologic feature of the luminal breast cancer. Most patients with oligometastatic luminal breast cancer receive systemic treatment which consist of endocrine therapy and/or biologic therapies, and of course other supportive cares measures.

## RATIONALE FOR ENDOCRINE THERAPY

When we diagnose a luminal cancer, we are getting sure that tumor cells are also sensible to those hormones. Estrogens promote the development and growth of this malign cells, so if we reduce their levels or inhibit their action, we are going to obtain a clinical benefit (8).

Classic parameters that reflect sensibility in breast cancer are the presence of hormone receptors, the tumor histology, the disease-free time and the places of metastasis. Recently we have included new parameters like HER-2 expression (bad response to endocrine treatments in tumor that over express this receptor) (9).

Previous clinical benefit with other endocrine therapies is the best efficacy predictor of a new line. Following the Athena trial, previous hormone sensitivity at the beginning of the process is the most important factor to know the prognosis of the disease (10).

There are different types of endocrine treatments and the selection of the most appropriate one must be individualized. This choice will depend on the main estrogen source.

Endocrine therapy is the first option in luminal cancers because it maintains quality of life in these patients. Nowadays, we are looking for introducing new lines of hormonal treatment, a better selection of patients and developing new drugs (8).

Estrogen receptor appears as positive in almost 70% of the total breast cancers, and 50-60% of those ER+ patients will have a positive answer to first line hormone treatment. However, 25% are going to be resistant since the beginning of the disease, and nearly the totality will develop some resistance mechanism during the process (9).

Patients with estrogen receptor positive metastatic breast cancer often respond to endocrine therapy, and this is the reason why it is the main tool in the initial treatment.

Endocrine therapy can reduce tumor burden and symptoms with fewer side effects than other possible treatments like chemotherapy. Furthermore, modern hormone therapies appear to prolong progression and possibly survival compared with the old ones. However, palliation is the goal of therapy because few patients with metastatic cancer will be cured (8).

Our goal as doctors must be to choose the therapy that is most likely to stabilize or reduce the burden of disease with the fewest side effects and maintain that therapy until either unacceptable toxicity is evident, or disease progression occurs.

## SEQUENTIAL ENDOCRINE THERAPIES

Cellular growth and differentiation in some tissues is controlled by the action of some hormones, which means that tumors located in those tissues could be sensible to hormone treatment. There are several types of hormone therapy, which can be divided attending to the point where our drug act as strategies to deplete estrogen (also called the anti-estrogens) and strategies to directly target the estrogen receptor (11).

While initial therapies to deplete estrogen were accomplished in premenopausal women by oophorectomy, estrogen can now be suppressed with the use of luteinizing hormone releasing hormone (LHRH) agonists and antagonists.

Although we know that ovarian estrogen production disappears with menopause, postmenopausal women continue to produce low levels of estrogen. This estrogen is derived from adrenal precursors, testosterone, and dehydroepiandrostenedione that are converted to estradiol and estrone by aromatase activity in peripheral cells and even in the cancers themselves.

To counter this extraovarian production, we have developed specific inhibitors of aromatase. Two of these, anastrozole and letrozole, are azole compounds, while the third is a 17-hydroxy steroid. Prospective randomized clinical trials in both the adjuvant

and metastatic setting have demonstrated that the clinical activity and toxicity of these three aromatase inhibitors (AIs) are almost identical (12).

There are two strategies to interfere with endocrine receptor signaling: the use of selective estrogen receptor modulators (SERMs) or selective estrogen receptor down-regulators (SERDs).

- Tamoxifen is a SERM with mixed antagonistic and agonistic properties. It is principally antagonistic in breast tissue, and that's why we use it in this treatment, whereas it has agonistic effects in bone, liver, and uterus (12). Raloxifene appears to be a weaker SERM, and it is only indicated in the prevention setting.
- Fulvestrant is the only available agent that downregulates the estrogen receptor. It is a highly insoluble compound that must be given intramuscularly. Fulvestrant is very dose-dependent, with studies showing improved efficacy at 500 mg rather than 250 mg intramuscularly (13).

Endocrine therapy is the right and appropriate selection for those patients who have the kind of tumor we are talking about in this project. Anywhere, we must mention that it is not the first option if our patient has rapidly progressive metastases or end-organ dysfunction.

In postmenopausal women or those with non-functional ovaries, levels of estrogens are going to be lower than the previous group but anyway there is a little production in other tissues by the conversion of androgens produced in adrenal glands.

In the 90s, tamoxifen was the first line treatment in these patients. However, due to three trials that compared new generation aromatase inhibitors (anastrozole, letrozole and exemestane) against tamoxifen in first line treatment for luminal metastatic breast cancer and showed more effectiveness and less toxicity, nowadays we can consider this kind on endocrine therapies as the gold standard for postmenopausal patients.

Anastrozole: It is a competitive, non-steroidal aromatase inhibitor that is active in oral administration. In some trials it has shown an increase of 1.5 months in time until progression and less toxicity (vaginal bleeding and embolism) than tamoxifen.

Letrozole: Another selective aromatase inhibitor with better response (especially in bone metastasis) and less toxicity than tamoxifen.

Exemestane: Irreversible inhibitor of the aromatase with high oral bioavailability. Different mechanism of inhibition than anastrozole or letrozole, but it inhibits the enzyme in a similar grade. Some trials had obtained positive results in favor of exemestane against tamoxifen. (9)

As a summary, aromatase inhibitors have shown to be more effective than tamoxifen as first line treatment in postmenopausal women and, attending to their good tolerance, they are now the new gold standard of treatment (14).

At the moment that the metastatic disease progress during the first line endocrine treatment, we have to answer an important question: should we continue with a second endocrine line or start chemotherapy?

The question is going to be answered by setting some points like clinical benefit with previous endocrine therapy, duration of that response or HER-2 expression (9,14).

In patients that progress even they were treated with tamoxifen as first line, aromatase inhibitors are the first option.

In more recent diagnosed cases, we are using aromatase inhibitors as first line. Some trials have shown that tamoxifen or some SEDRs like fulvestrant increase the clinical benefit, so they can be used as second or third line treatments.

Third line treatment can be fulvestrant or progestogens in postmenopausal women after resistance development to aromatase inhibitors and anti-estrogens. In premenopausal women, if castration fails, aromatase inhibitors could be an option.

## MECHANISMS OF RESISTANCE: PI3K/AKT/mTOR PATHWAY

Endocrine therapy is a crucial treatment for estrogen receptor-positive breast cancer. However, adaptative resistance mechanism emerge in the tumour, causing misfunction of this therapy. A better understanding of what is happening at molecular levels is needed to overcome this problem and to develop new lines of treatment.

Resistance to endocrine therapies in ER+, HER2- disease is common. Even though endocrine therapy is initially the best option of treatment, most patients will inevitably face disease progression (15). In the last years, significant advances have been made to uncover several important signalling pathways that promote estrogen-independent activation of the estrogen receptor.

Studies indicate a predominant role for the phosphatidyl-inositol 3-kinases (PI3K/AKT/mTOR), and cyclin-dependent kinase (CDK 4/6) and retinoblastoma protein (RB) pathways in cancer cells growth and survival. Those studies have culminated in the development of new and specific inhibitors for each pathway.

### COMPONENTS OF THE PATHWAY

1. PI3KS

Phosphoinositides are one of the most important components of the membrane phospholipids, and their phosphorylation has been recognized as an important signal transduction mechanism of oncogenic receptor tyrosine kinases (RTKs) for decades (16). There are three classes of PI3Ks, with different substrates. It is important to remark that class IA is the most widely concerned class in cancer, and its main function is to generate PIP3 by phosphorylating PIP2 on the plasma membrane. Class IA PI3Ks are most often activated by RTK signalling.

Accumulation of PIP3 on the cell membrane leads to colocalization of signalling proteins and to the activation of some proteins and the propagation of PI3K signalling (17).

### 2. AKT AND mTOR

AKT and PDK1 directly bind to PIP3. Phosphorylation of AKT by PDK1 and mTOR complex 2 results in complete activation of this protein kinase. In turn, phosphorylated Akt will activate many proteins related with cell survival and cell cycle entry (18).

## PATHWAY ACTIVATION IN ONCOLOGY

The PI3Ks/AKT signalling pathway is aberrantly activated in various cancers, including breast cancer. The two most widely observed mechanism of PI3Ks/AKT activation include activations by RTKs (however, translation to this scientific understanding into a clinical improvement has not been achieved yet) and somatic mutations (17). Activating alterations in PI3K signalling is one of the most frequent events in cancer and a major focus for drug development.

PI3KCA is frequently mutated in ER+, HER2- breast cancer. These mutations lead to increased downstream signalling and oncogenesis by the upregulation of ER transcription and expression (19). Additionally, somatic genetic events occurring in luminal breast cancer, representing potential new therapeutic targets have described, including mutations in AKT1, and amplification of FGFR1.

## ABERRATIONS IN CANCER

### 1. PTEN

One of the genetic mechanisms discovered for breast cancer was the loss of the PTEN tumour suppressor, which encodes a PIP3-phosphatase that inhibits PI3K signalling pathway (20). Although this suppression is tumorigenic, it is unclear if PTEN loss alone is sufficient to activate PI3K (17).

### 2. PI3K

Two classes of PI3KCA mutations promote constitutive PI3K signalling through different mechanisms.

Somatic activating mutations occur in up to 30% of some epithelial cancers including breast cancer (17), being this one the most frequently affected cancer. The majority of these mutations occur in two main locations: exon 9 and exon 20. These mutations were shown to be gain-of-function and have transforming capacity.

It is not clear yet in which moment of cancer progression these mutations occur. Anyway, the question of whether these alterations produce resistance is of great interest.

### 3. PI3KR1 LOW EXPRESSION

Some recent studies suggest that some cancers harbour activating mutations in the PI3K regulatory subunit (p85α). This PI3KRI product appears to play a tumour-suppressive role. Mutations in this regulatory subunit have been found in breast cancer, although with much lower incidence than PI3KCA (21).

### 4. AKT1 MUTATION

AKT1 mutations have been found in 1.5-8% of breast cancers (21). Although this low frequency, some studies revealed that AKT1 mutations were just observed in ER and progesterone receptor positive tumours.
AKT activation is associated with poor outcome in patients with breast cancer who are receiving endocrine therapy.

### 5. mTOR

mTOR was discovered in the early 90s in studies of the mechanism of rapamycin, which is a macrolide originally discovered as an antifungal agent, but later recognized to have anticancer properties.
mTOR is a serine/threonine protein kinase that plays an integral role in signal transduction pathways that control cell growth, autophagy, cell survival and cell metabolism (22). It consists of two protein complexes (mTORC1 and mTORC2):

- mTORC1 is regulated by PI3K activation, which allows mTORC1 to promote cell growth and proliferation. It is the biological target of rapamycin and other mTOR suppressors. Constitutive activation of PI3K/mTORC1 signalling in cancer cells strongly inhibits autophagy (22).
- mTORC2 is also a multi-protein complex that regulates and organizes the cellular actin cytoskeleton and also regulates AKT phosphorylation.

## MTOR INHIBITORS IN BREAST CANCER

As we have explained before, mTOR signalling pathways can mediate resistance to endocrine therapy. That´s why several studies have postulated that the effects of mTOR inhibitors in breast cancer may be twofold (23,24):

- In patients with acquired resistance they could be used to restore sensitivity and provide an additional treatment period for endocrine therapy.
- In patients that have not been previously treated with endocrine lines, they could be used in combination with these drugs to delay the onset of resistance.

Addition of the MTOR inhibitor everolimus to endocrine therapy can reverse endocrine resistance in ER + metastatic breast cancer, as shown in the BOLERO-2 randomized trial (patients previously treated with aromatase inhibitors, with progression after an initial response). This led to the approval in 2012 by the FDA and the EMA to use everolimus in combination with endocrine therapy after AI failure (25).

# BACKGROUND

## THE BOLERO – 2 PIVOTAL CLINICAL TRIAL

In postmenopausal patients with advanced breast cancer, initial gold standard therapy consists of an aromatase inhibitor. Unfortunately, in nearly 50% of these patients this hormonal therapy is no longer affective because of the development of "de novo" resistance. Subsequent options after the appearance of this resistances include changing the class of AI or substitute it for an ER antagonist or an ER modulator (26).

The study of resistance to endocrine therapies in ER+ breast cancer has aimed at identifying new therapeutic strategies. Activation of the PI3K/AKT/mTOR pathway has been implicated in resistance to endocrine therapy in breast cancer. This is the reason why some preclinical and clinical researches like BOLERO-2 have been performed to examine the molecular bases of this resistance mechanism and to use some inhibitors of the key points of this pathway to overcome this resistance (23).

Everolimus is a rapamycin analogue that is orally active and has a better profile than other members of his family. It is an allosteric mTOR antagonist, so it is going to block just only the mTORC1 function. Everolimus was approved by the FDA for the treatment of renal cell cancer and, due to clinical studies in breast cancer, it is also now available for this disease (26). The use of Everolimus in combination with an AI results in a synergistic inhibition of the proliferation and induction of apoptosis. BOLERO-2 evaluated the efficacy and safety of this combination (27).

### STUDY DESIGN

Bolero-2 is a phase 3, international, double-blinded, randomized trial, where everolimus plus exemestane were compared to exemestane plus placebo in 724 menopausal patients with ER+ and HER-2 negative advanced breast cancer, with a ratio 2:1 in favour of the everolimus-exemestane group. Recruited patients had measurable disease and were refractory to non-steroidal aromatase inhibitors but a single prior chemotherapy regimen was allowed (27,28)

The primary end point was progression-free survival determined by the investigator by radiographic studies.
Secondary end points included overall survival, overall response rate, clinical benefit rate, time to deterioration of ECOG performance status, safety and quality of life (27,28).
Treatment continued until disease progression or unacceptable toxicity, or withdrawal of consent.

### EFFICACY

The trial met its primary end point of progression-free survival. The median duration of exposure to everolimus was 14.6 weeks, compared to 12.0 weeks of exposure to placebo.
According to central assessment, the median progression-free survival was 10.6 months for the combination exemestane-everolimus group versus 4.1 months for the placebo plus exemestane arm (28).
The most frequent primary reason for discontinuation was disease progression (37% in the combination-therapy group and 66% in the exemestane-alone group) (27).

### SAFETY/TOXICITY

Serious adverse events were defined in the study protocol as grade ¾ stomatitis, anaemia, dyspnoea, hyperglycaemia, fatigue and pneumonitis.
In the combination arm, 23% of the patients had serious adverse events, with approximately half of those (11%) though to be related to the study treatment. There were 7 deaths in this group, two of them because of sepsis. In the placebo arm, 12% of the patients has serious adverse effects, although only 1% were attributed to the treatment, and one death occurred from pneumonia (27,28).
There was also an increased withdrawal of consent (5 vs 2%) in the combination group, and a higher proportion of patients who discontinued everolimus compared to placebo (19% vs 4%) because of adverse events (28).
The most common grade 3 or 4 adverse events were stomatitis, anaemia, fatigue and pneumonitis. The time to deterioration of ECOG status and time to deterioration of quality of life were not statistically different between the two arms of the study (28).

## MAIN HYPOTHESIS

BOLERO-2 findings are very interesting by they come from the strict context of a randomised clinical trial. Our purpose is to check if it is applicable to current clinical practice, following the approach of so-called real data world (29,30).

# OBJECTIVES

1. To review the efficacy and safety of the combination everolimus plus exemestane in second line therapy for luminal metastatic breast cancer.

2. To check if these observations are in agreement with the pivotal trial, BOLERO-2.

3. To define if this combination is useful in real clinical practice.

# PATIENTS AND METHODS

We did a retrospective study on patients treated in two near academic institutions of northern Spain, the Hospital 'Marqués de Valdecilla' (HUMV, Santander) and the Asturias Central Hospital (HUCA, Oviedo), with the combination of exemestane and everolimus as endocrine therapy for breast cancer. Patients were collected from data-management systems of Oncology departments of both centers. Inclusion criteria were: 1) Metastatic breast cancer, 2) Menopausal status, 3) Expression of ER and/or PR by tumor cells; and 4) Previous progression to a first line endocrine therapy with an antiestrogen or an aromatase inhibitor, regardless the use of cytotoxic chemotherapy.

Clinical records were reviewed from January to May 2018. Electronically recorded data were the following: age at the first diagnosis, menopausal status, tobacco habit, diabetes, histology of the tumour, number of metastatic sites, involved organs, number of chemotherapy lines, number of endocrine therapy lines, use of aromatase inhibitors, use of antiestrogens, time to failure with the first treatment, time to failure with everolimus plus exemestane, initial everolimus dose, modifications in everolimus dose, maximum objective response observed to everolimus and exemestane, and toxicities.

Treatment consisted of oral exemestane 25 mg/day, and oral everolimus 10 mg/day. Therapy was maintained until progression or unacceptable toxicity.

Evaluations were:

1. Time to failure on treatment (TTF), measured in months from the date of beginning of both drugs on study, until any of these: tumor progression -according image studies-, unacceptable adverse events, or medical convenience due to an intercurrent medical illness.
2. Objective response rate.
3. Adherence to therapy.

4. Toxicity, with special focus on frequently seen effects in the pivotal BOLERO-2 trial: myelosupression, stomatitis, diarrhoea, asthenia, hyperglucemia, liver dysfunction, hypertriglyceridemia, and penumonitis. Adverse events were graduated as 1 (mild), 2 (moderate), and 3 (severe).

# RESULTS

## CLINICAL SERIES

A total of 86 women were included (22 from HUMV, and 64 from HUCA). Median age at the diagnosis of breast cancer was 57 years. Main metastatic sites were: bone, 77,9%; liver 45,3%; and lung/pleura, 40,7%. Twenty-three patients had metastases in 3 organs simultaneously, and 9 in 4 or more locations.

All tumors were ER-positive, and 81.4% also expressed PR. Previous hormone therapy with tamoxifen, letrozole, anastrozole, or exemestane was registered in 80 patients, which were fully evaluated. Six patients receiving everolimus as first endocrine line were considered not for TTF, but only for toxicity. Previous cytotoxic chemotherapy was used in 67 patients.

The average TTF to first-line therapy was 63 months. Smoking was observed in forty patients (46,5%), either quitting sometime before or being active under study treatment.

## EFFICACY

Median TTF was 5 months, being average 7.2 months. The better objective responses were: partial, 15 patients (17.4 %); and stable disease, 37 (43%).

## ADHERENCE

Among eighty patients, starting dose of everolimus was 10 mg/day, as per protocol. In 6 cases, starting dose was 5 mg/day, due to medical limitations. A dose adaptation by toxicity reasons did occur in 38 patients (44.2%), being the most frequent an alternating use of 10/5 mg per day *(See Appendix, Figure 1)*.

## SAFETY

Grade 1 (mild) or 2 (moderate) toxicities included diarrhea, peripheral edema, skin reactions, arthralgia, hyperesthesia, conjunctivitis, decreased appetite, nausea, vomiting, anxiety, depression, hemorrhoids, hyperglycemia and epistaxis.

A severe adverse event (grade 3) was described in 18 patients (20.9%). We observed stomatitis (5), anaemia (4), pneumonitis (4) and asthenia (4). Less frequent were cough (without radiographic expression), thrombocytopenia, hyperglycemia, hypertriglyceridemia, and liver dysfunction. *(See Appendix, Table 1)* No toxic deaths were reported. Cognitive impairment, leading to therapy interruption, was seen in 1

patient (1.16%). The 10 most common adverse events registered in our study are shown in table 2.

| MOST COMMON ADVERSE EFFECTS (NUMBER OF CASES) | ANY EVENT | GRADE 3-4 EVENTS |
|---|---|---|
| STOMATITIS | 33 | 5 |
| ANEMIA | 15 | 4 |
| ASTHENIA | 11 | 4 |
| PNEUMONITIS | 9 | 4 |
| HYPERTRIGLYCERIDEMIA | 8 | 2 |
| THROMBOCYTOPENIA | 7 | 1 |
| HYPERGLYCEMIA | 7 | 1 |
| NEUTROPENIA | 6 | 0 |
| DIARRHOEA | 5 | 0 |
| LIVER TEST ABNORMALITY | 5 | 1 |

Table 2. Safety: 10 most common adverse events registered in HUMV and HUCA patients (number of cases)

# DISCUSSION

## EFFICACY

Focusing on objective response rate, less 'progressive diseases' and more 'stable diseases' have been reported in randomized trial than in our series *(See Appendix, Table 3)*. Differences can be explained by inequalities in measurable lesions, radiologic methods, and intervals between examinations. At the end, BOLERO-2 investigators gave a progression free survival of 6.9 months with the addition of everolimus and 2.8 months for exemestane alone. Although we have tabulated another parameter, time to treatment failure, our observed 7 months seem to be in good agreement with them. Both results are similar and acceptable as well, before the appearance of palbociclib, a cyclin-D inhibitor which has been recently incorporated to 2$^{nd}$ line endocrine options (31). Anyway, there are not randomized trials comparing everolimus with palbociclib, so choosing a rescue agent remains optional.

Equivalence between PFS and TTF in BOLERO-2 and our series, respectively, is strengthened by the fact metastatic sites and number of metastasis are very similar, as depicted in Figure 2 and Figure 3 *(See Appendix: Tables 4 and 5)*.

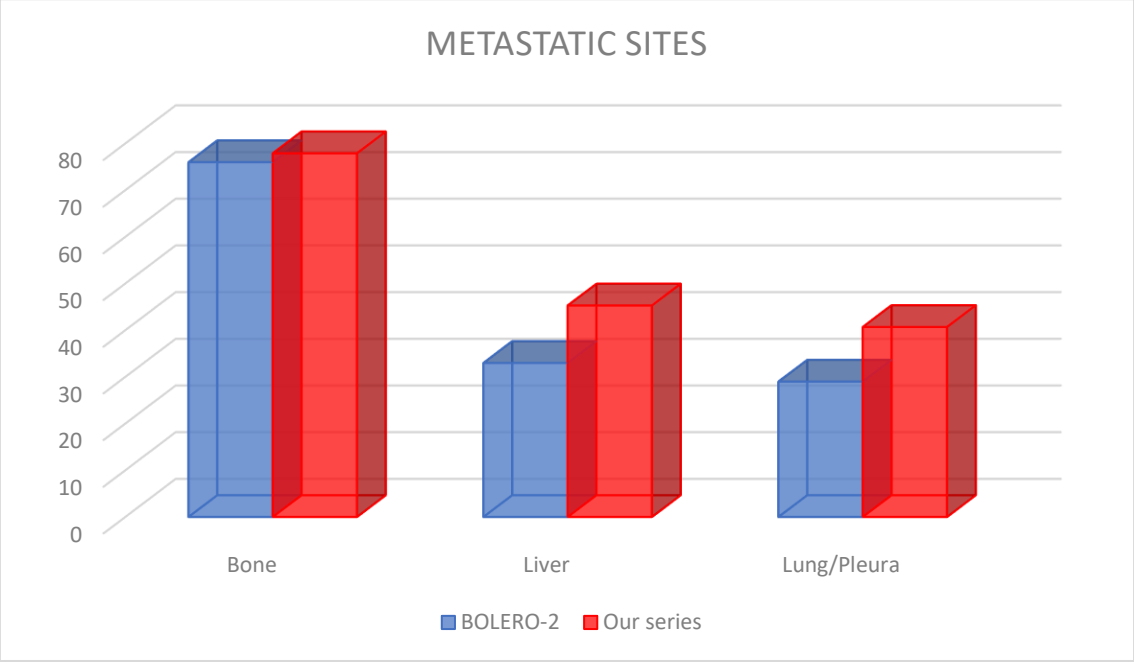


Figure 2. Most common metastatic sites: Comparison between the 3 most common metastatic sites in BOLERO-2 and HUCA/HUMV patients (%)

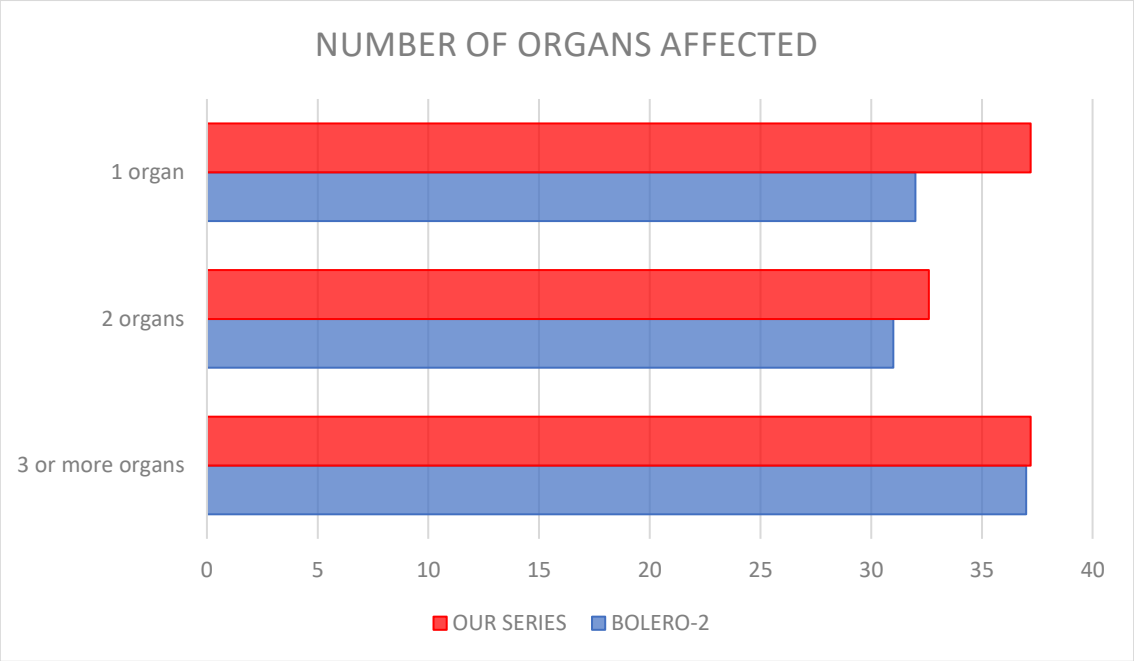


Figure 3. Number of organs affected: Comparison between the number of organs with metastasis in BOLERO-2 patients and HUCA/HUMV patients (%)

## TOXICITY

Actual guidelines in treatment of luminal metastatic breast cancer recommend a daily dose of 10 mg of everolimus, unless toxicity appears (30, 32). In our series, near 90% of patients did start at 10 mg/day, but some patients did at a level of 5 mg/day, due to liver dysfunction.

Current recommendations include dose reductions or even temporary interruptions of everolimus in case of toxicity. When reduction is needed, the usual new recommended dose is 5 mg per day (32). In our series, nevertheless, most reductions were to a different pattern (10mg/5mg once a day, in alternating days), when a moderate adverse reaction did occur, perhaps trying to reduce toxicity without compromising efficacy.

Whatever, the main reason for discontinuation of everolimus in our study was lack of tolerability, like in BOLERO-2 and BALLET (30). Toxicity comparisons are always difficult, because different doctors can explain symptoms in a different 'toxic' category, and grades can be attributed in a different way. Thus, a weakness of our study consists of different reporting criteria between HUCA and HUMV. In the latest, we have tabulated all the observed events, while in HUCA reports are limited to more frequent ones. Nevertheless, taking into account all these considerations, the adverse events profile and safety concerns observed in our hospitals are consistent with those reported in BOLERO-2 and other studies with mTOR inhibitors (30). Moreover, 'frequent' events and

'severe' ones are virtually the same in BOLERO-2 and our series *(See Appendix, tables 6 and 7)*.

## LOCAL REMARKS ON TOLERANCE

Some adverse events described in BOLERO-2 study have been barely observed outside the trial. For example, insomnia, pruritus, back pain, pyrexia or headache have not observed in our series. In the opposite, our clinical records include some effects not reported by BOLERO-2 authors., such as cognitive impairment, urinary tract infection, conjunctivitis or psychiatric pathologies, i.e. anxiety and depression.

In an attempt to explain these discrepancies, we have reviewed some articles related with toxicity of rapamycin analogues.

### INFECTIONS

Everolimus has immunosuppressive properties, so it can predispose to bacterial infections including pneumonia and urinary tract infection (32). We have seen one case of *E.coli* urinary infection, which did not reach severity described by others (33).

### PSYCHIATRIC DISORDERS

Insomnia is the most frequent psychiatric problem described in patients treated with everolimus. For example, in BOLERO-2, 11% of patients developed any grade of insomnia. However, we have not registered insomnia, but some patients suffered from other psychiatric pictures like anxiety or depression. Moreover, one out of our patients (70 years old) developed cognitive impairment. No bibliographic references have been found to establish a direct relationship between everolimus and those disorders (32). In a nutshell it is hard to attribute neurological complaints to everolimus.

### INFLUENCE OF TOBACCO USE

Due to pneumonitis is one of the most frequent and severe side effect related to everolimus, we explored a potential relationship with smoking.

Among smokers (40 patients, 46.5%), TTF was 6.2 months on average, whilst in the non-smoking group, it was 8 months, but we could not link this difference to a differential toxic pattern. Nine out of 86 patients developed pneumonitis, but only 3 of them were smokers. Severe pneumonia was registered in 4 cases and just one was smoker. Therefore, we think tobacco has no role in everolimus lung toxicity.

# CONCLUSIONS

1. Efficacy of second line endocrine therapy with everolimus and exemestane in real clinical world is comparable to previously seen in pivotal BOLERO-2 trial.
2. The safety pattern is also similar, although the commonest adverse events previously described have been less frequent in our hospitals.

   2b. In contrast with previous experiences, we have observed some neurological effects that could not be linked to everolimus therapy.

   2c. The risk of pneumonitis is around 10% and it seems not be associated with tobacco use.
3. The combination of everolimus and exemestane behaves useful in current clinical practice, although some authors do suggest a preference for palbocicib.

# APPENDIX

| ADVERSE EFFECTS IN HUMV | ANY EVENT (G1+2+3) | SEVERE EVENTS (G 3) |
|---|---|---|
| STOMATITIS | 9 | 2 |
| ASTHENIA | 8 | 2 |
| DYSPNEA | 6 | 1 |
| ANEMIA | 6 | 3 |
| RASH | 5 | 0 |
| COUGH | 5 | 1 |
| DIARRHEA | 3 | 0 |
| PERIPHERAL EDEMA | 3 | 0 |
| SGOT INCREASE | 3 | 1 |
| HYPERGLYCEMIA | 3 | 0 |
| PNEUMONITIS | 3 | 1 |
| SGPT INCREASE | 3 | 1 |
| NAUSEA | 2 | 0 |
| ARTHRALGIA | 2 | 0 |
| EPISTAXIS | 2 | 0 |
| VOMITING | 2 | 0 |
| THROMBOCYTOPENIA | 2 | 1 |
| DECREASED APPETITE | 1 | 0 |
| CONSTIPATION | 1 | 0 |
| ASCITES | 1 | 0 |
| HYPERSTHESIA | 1 | 0 |
| CONJUNCTIVITIS | 1 | 0 |
| DEPRESSION | 1 | 0 |
| HAEMORRHOIDS | 1 | 0 |
| ANXIETY | 1 | 0 |
| URINARY INFECTION | 1 | 0 |
| COGNITIVE IMPAIRMENT | 1 | 0 |

Table 1: Safety: adverse events registered in HUMV patients treated with everolimus plus exemestane (number of cases).

| MAXIMUM RESPONSE | BOLERO-2 (%) | OUR SERIES (%) |
|---|---|---|
| PROGRESSION | 9.9 | 37.2 |
| STABLE DISEASE | 70.1 | 43 |
| PARTIAL RESPONSE | 9.1 | 17.5 |
| COMPLETE RESPONSE | 0.4 | 0 |
| UNKNOWN RESPONSE | 10.5 | 2.3 |

Table 3. Maximum response: Comparation of the maximum responses obtained in patients treated with everolimus plus exemestane in BOLERO-2 study against those obtained in HUMV and HUCA patients (%).

| METASTATIC SITES | OUR LOCAL SERIES (%) | BOLERO-2 (%) |
|---|---|---|
| BONE | 77.9 | 76 |
| LIVER | 45.3 | 33 |
| LUNG/PLEURA | 40.7 | 29 |

Table 4. Metastatic sites: comparation of the three most frequent metastatic sites between the results of BOLERO-2 and patients from HUMV and HUCA (%).

| NUMBER OF METASTATIC SITES | OUR LOCAL SERIES (%) | BOLERO-2 (%) |
|---|---|---|
| 1 | 30.2 | 32 |
| 2 | 32.6 | 31 |
| ≥3 | 37.2 | 37 |

Table 5. Metastatic sites: Comparation of the number of metastatic sites between BOLERO-2 and the patients from HUMV and HUCA (%)

| MOST COMMON ADVERSE EVENTS | ALL GRADES, OUR SERIES (%) | ALL GRADES, BOLERO-2 (%) | GRADE 3, OUR SERIES (%) | GRADE 3-4, BOLERO-2 (%) |
|---|---|---|---|---|
| STOMATITIS | 38 | 56 | 6 | 8 |
| ANEMIA | 17 | 16 | 5 | 6 |
| ASTHENIA | 13 | 12 | 5 | 2 |
| PNEUMONITIS | 10 | 12 | 5 | 3 |
| HYPERTRIGLYCERIDEMIA | 9 | - | 2 | - |
| THROMBOCYTOPENIA | 8 | 12 | 1 | 3 |
| HYPERGLYCEMIA | 8 | 13 | 1 | 5 |
| NEUTROPENIA | 7 | - | 0 | - |
| DIARRHEA | 6 | 30 | 0 | 3 |
| HEPATIC FUNCTION ALT. | 6 | 24 | 1 | 8 |

Table 6. 10 most common adverse events in HUMV-HUCA patients. Comparation of the percentage of patients that present those toxicities in BOLERO-2 and our study (%).

| ADVERSE EVENTS | ALL GRADES, BOLERO-2 (%) | ALL GRADES, OUR SERIES (%) | GRADE 3, BOLERO-2 (%) | GRADE 3-4, OUR SERIES (%) |
|---|---|---|---|---|
| STOMATITIS | 56 | 38 | 8 | 6 |
| RASH | 36 | 6 | 1 | 0 |
| FATIGUE | 33 | 0 | 4 | 0 |
| DECREASED APPETITE | 29 | 0 | 1 | 0 |
| NAUSEA | 27 | 2 | 2 | 0 |
| COUGH | 22 | 6 | 1 | 1 |
| DYSGEUSIA | 21 | 0 | 1 | 0 |
| HEADACHE | 19 | 0 | 1 | 0 |
| DYSPNEA | 18 | 7 | 4 | 1 |
| ANEMIA | 17 | 16 | 5 | 6 |
| ARTHRALGIA | 16 | 2 | 1 | 0 |
| EPIXTAXIS | 15 | 2 | 0 | 0 |
| VOMITING | 14 | 2 | 2 | 0 |
| PERIPHERAL EDEMA | 14 | 3 | 1 | 0 |
| PYREXIA | 14 | 0 | 1 | 0 |
| ASTHENIA | 13 | 12 | 5 | 2 |
| CONSTIPATION | 13 | 1 | 1 | 0 |
| PRURITUS | 11 | 0 | 1 | 0 |
| INSOMNIA | 11 | 0 | 1 | 0 |
| BACK PAIN | 11 | 0 | 0 | 0 |
| PNEUMONITIS | 10 | 12 | 5 | 3 |
| HYPERTRIGLYCERIDEMIA | 9 | - | 2 | - |
| THROMBOCYTOPENIA | 8 | 12 | 1 | 3 |
| HYPERGLYCEMIA | 8 | 13 | 1 | 5 |
| NEUTROPENIA | 7 | - | 0 | - |
| DIARRHEA | 6 | 30 | 0 | 3 |
| HEPATIC FUNCTION ALT. | 6 | 24 | 1 | 8 |

Table 7. Adverse events in BOLERO-2: Comparation of the adverse events registered in BOLERO-2 with their percentages in patients from HUMV and HUCA (%).

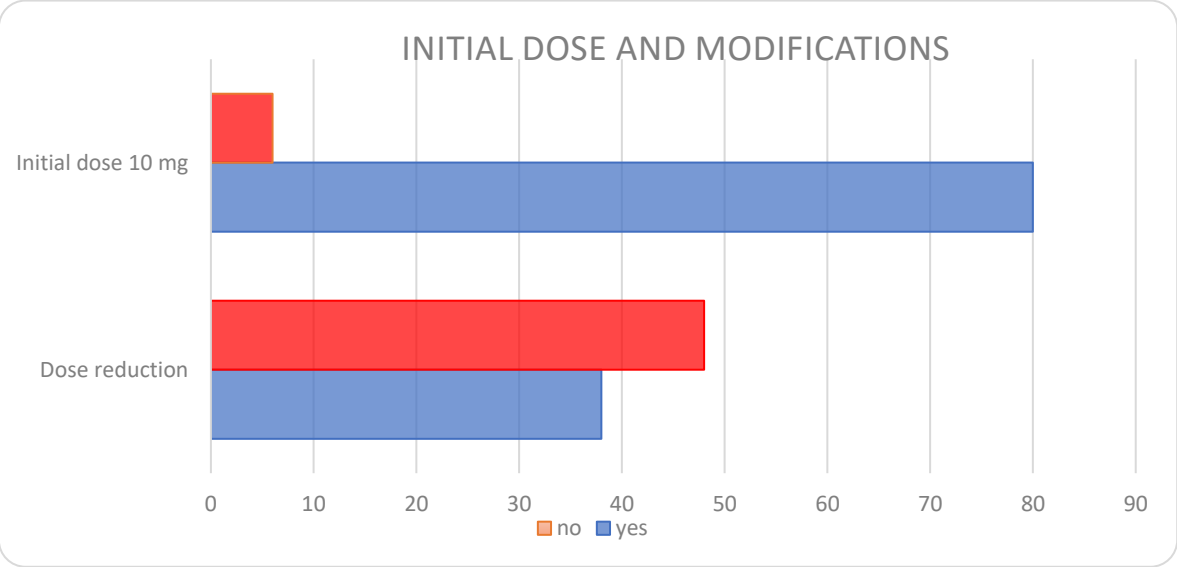


Figure 1. Initial dose of everolimus and dose modifications in HUMV and HUCA patients (number of patients).

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Contributions to the interpretation of data and the subsequent writing of this section were also made by the authors.

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