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DOCTORAL THESIS

Improving the Management of Breast Cancer

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ABSTRACT

The clinical scenario for both early-stage breast cancer (EBC) and advanced breast cancer (ABC) is complex, multifaced and rapidly evolving. Overall, this work aimed at providing evidence to improve the management of EBC and ABC by:

1. Identifying or validating novel biomarkers predictive of response to treatments and novel pharmacological targets;
2. Improving treatment algorithms for hormone receptor positive/HER2-negative (HR+/HER2-neg.) ABC.

With respect to the first objective, the focus was put on breast cancer molecular subtypes and the validation of their use in neoadjuvant setting in HER2-positive (+) tumors, so to predict pCR and support escalated or de-escalated therapeutic approaches. Furthermore, following the discovery of the efficacy of novel anti-HER2-directed ADC in HER2-neg. tumors presenting some level of expression of HER2 (i.e. HER2-low tumors), the first extensive molecular and clinicopathological characterization of this potentially novel subgroup of breast tumors was conducted. Finally, the mTOR inhibitor everolimus, in combination with exemestane is approved for pretreated HR+/HER2-neg. ABC. However, a number of very effective therapeutic alternatives has emerged during the last few years and the identification of biomarkers of response would be particularly useful to identify patients that might benefit most from this drug.

With respect to the second aim, the focus was put on improving current treatment algorithms for advanced luminal tumors, due to the discrepancy observed between main international guidelines' recommendations and "real world" clinical practice. The studies conducted had the objective to provide a comprehensive assessment of the efficacy of current therapeutic options and novel pooled evidence to support current treatment guidelines and help clinician's with their therapeutic choices.

All the thesis objectives were addressed in 5 different studies, now published in different peer-reviewed international journals.

Overall, the articles that are part of this thesis provided evidence to:

1. Support the use of the PAM50 HER2-Enriched molecular subtype, if not in the clinical practice, at least in future clinical trials to assess neoadjuvant escalated or de-escalated therapeutic approaches in HER2+ tumors, irrespective of HR status;
2. Further explore circulating endothelial cells, neutrophil-to-lymphocyte ratio and lymphocytes subpopulations as biomarkers of response to select optimal candidates to everolimus in HR+ breast cancer patients;

3. Support main international treatment guidelines in recommending endocrine therapy + target therapy as the preferred 1st/2nd-line treatment of HR+/HER2-neg. postmenopausal metastatic breast tumors, especially CDK4/6-inhibitors-based regimens;
4. Support the use of CDK4/6-inhibitors-based regimens instead of single agent endocrine therapy, independently from age, menopausal status, endocrine sensitiveness and visceral involvement.

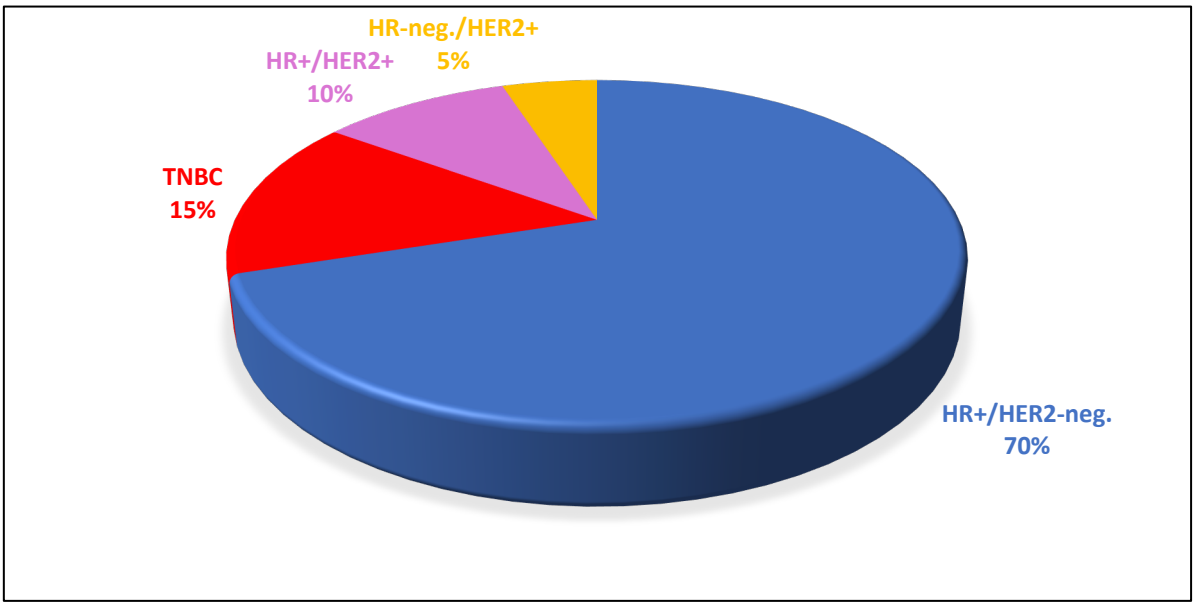
Finally, we dissected for the first time the clinicopathological and molecular characteristics of HER2-low breast tumors and find out that this category do not show the features of an independent breast cancer subtype. However, the detection of HER2 low protein levels as well as the assessment of *ERBB2* mRNA levels, might play an important role from a therapeutic perspective in the near future. Moreover, HR+/HER2-low showed distinct features from triple negative (TNBC)/HER2-low and HER2 0, as well as from HR+/HER2 0 tumors, while TNBC/HER2-low did not show substantial differences with TNBC/HER2 0.

INTRODUCTION

Breast cancer epidemiology and subtypes: an overview

Breast cancer is a major health care issue, being the most frequent malignant tumor in women (~15% of all new cancer cases) and the first cause of death for cancer in this gender[1]. Median age at diagnosis is 62 years, with half of the new cases diagnosed between 55 and 74 years[1]. More than 90% of breast tumors are diagnosed in early stage, with approximately 64% being diagnosed when localized and around 28% being diagnosed in locally advanced stage[1]. Conversely, metastatic tumors at diagnosis are rare, accounting for about 5% of all cases[1]. Taken together all stages, the current 5-year survival rate is around 90%[1], while, when metastatic, the rate decreases to ~27%, with significant differences according to tumor subtype[1,2]. In fact, breast cancer is not a single pathological entity. Four subgroups of breast tumors are routinely identified through immunohistochemistry (IHC) for clinical decision-making[3,4]. These groups are classified according to the presence of hormone receptors (HR) for estrogen and progesterone, and the amplification and/or overexpression of the human epidermal growth factor receptor 2 (HER2). Based on these parameters, we identify HR-positive (HR+)/HER2-negative (HER2-neg.), HR+/HER2-positive (HER2+), HR-negative (HR-neg.)/HER2+ tumors and triple negative breast cancer (TNBC), represented by tumors that do not express both HR and HER2 (**figure 1**)[5,6].

Figure 1. Breast cancer IHC-based subtypes



Legend. HR: hormone receptor positive; **TNBC:** triple negative breast cancer; **neg.:** negative; **+:** positive.

HR expression is detected through standardized IHC techniques[7]. Tumors are considered HR+ when estrogen receptor (ER) and/or progesterone receptor (PgR) show levels of expression of >1%[3,7].

HER2+ tumors are characterized by the overexpression and/or amplification of the HER2 gene, also known as *ERBB2*. HER2 protein overexpression is assessed through IHC techniques. An IHC score of 0 or 1+ identify HER2-neg. tumors, while a 2+ score identifies equivocal cases, for which ISH techniques have to be used to ultimately assess the HER2 status. HER2 2+ tumors are HER2-neg. if ISH-based techniques do not identify HER2 gene amplification, otherwise the tumors will be considered HER2+. An IHC score of 3+ identifies HER2+ tumors, without the need to rely to ISH techniques[8].

IHC-based subgroups are relevant from both prognostic and therapeutic perspectives[5]. HER2 positivity is the main predictor of response to anti-HER2 targeted agents. HR positivity confers sensitivity to endocrine therapy (ET), though tumors with 1-10% levels are considered low expressors, with doubtful endocrine sensitivity[3,9,10].

However, from a molecular perspective, at least four main subtypes have been identified in the last few years, which partially overlap with IHC-based subgroups[5,11]. These so-called intrinsic subtypes, namely Luminal A, Luminal B, HER2-Enriched (HER2-E) and Basal-like, have different biological features, natural history and response to treatments[11]. A Normal-Breast-like subgroup has been also identified, but nowadays it is mostly considered as an artifact and tumors identified within this subgroup share similar features of Luminal A cancer. In general, HR+ tumors mostly overlap with the Luminal subgroup, with Luminal A being the less proliferative, less chemo-sensitive and with better prognosis, and Luminal B being more proliferative and more chemo-sensitive with slightly worse prognosis. The main driver of growth for these tumors is represented by estrogens[11–14]. HER2+ tumors mostly overlap with the HER2-E subtype, whose main growth driver is represented by the HER2 pathway hyperactivation. This was originally considered as a negative prognostic factor, being associated with high proliferation rates and aggressiveness. At the same time, the introduction of very effective anti-HER2 target therapies (TT) has radically changed the natural history of this tumor subtype[11–14]. Finally, TNBC are usually Basal-like, from a molecular perspective. This is the molecular subtype at worse prognosis, considering the current limited therapeutic options available (only chemotherapy in early phase)[11–14].

It is important to highlight that each molecular subtype is represented within a specific IHC category, and *vice versa,* thus the two classifications are not completely interchangeable[11–14].

Early-stage breast cancer treatment strategies: introduction

Early-stage breast cancer (EBC) is represented by tumors without distant metastases and a disease localized in the breast and/or axillary lymph-nodes, potentially susceptible of locoregional definitive treatments.

In general, the mainstay of treatment for EBC is breast surgery (lumpectomy or mastectomy), with or without axillary nodes excision and post-surgical radiotherapy (RT)[3,9]. Surgery can be preceded or followed by several systemic treatments, namely chemotherapy (CT), ET and anti-HER2 TT in different sequential and/or combination strategies, according to the main clinicopathological prognostic factors, patient's age, menopausal status (pro- or post-menopausal), performance status and preferences.

In general, the main clinicopathological factors that guide the systemic therapeutic decision are represented by ER and PgR levels of expression, HER2 amplification/overexpression status, the levels of proliferation index Ki67, tumor grading (G), histologic subtype, primary lesion dimensions (T) and axillary nodal involvement (N).

In HR+/HER2-neg. tumors with a primary lesion of less than 5 cm of maximum diameter and without or with very limited nodal involvement (no more than 3 lymph-nodes), a number of genomic tests (e.g. Oncotype Dx®, Prosigna®, Mammaprint® etc.) can predict the risk of relapse and response to CT and can thus aid clinicians in deciding whether or not to prescribe adjuvant CT[3,9].

Adjuvant and neoadjuvant therapeutic approaches

A preoperative (neoadjuvant) systemic treatment approach is required when the disease is locally advanced and not immediately operable. In this case, the administration of CT (or ET in some HR+/HER2-neg. tumors) ± anti-HER2 TT has the aim to achieve an adequate tumor shrinkage and make radical surgery a viable therapeutic option. In case of operable disease *ab initio*, results from large clinical trials and retrospective reviews have indicated that breast conservation rates are significantly improved with preoperative systemic therapy[9]. Therefore, more conservative surgical approaches can be considered for patients undergoing neoadjuvant treatment instead of immediate surgery. Importantly, it has been demonstrated, especially for HER2+ tumors and TNBC, that the achievement of a pathologic complete response (pCR) after neoadjuvant therapy and surgery improves long-term outcomes[15]. The benefit is less clear for HR+/HER2-neg. tumors[15]. Several definitions for pCR have been

adopted in different clinical trials, including the absence of invasive tumor in both breast and axilla or only in breast. The major survival benefit has been observed when the tumor was absent from both breast (ypT0/is) and axilla (ypN0)[15].

Moreover, in both TNBC and HER2+ tumors, recent trials have shown that in case of administration of neoadjuvant standard CT ± anti-HER2 therapy (depending on the tumor subtype) and subsequent lack of achievement of pCR after surgery, novel post-surgical (adjuvant) therapeutic approaches can further reduce the risk of relapse and death for these high-risk patients[16,17]. More trials are exploring this post-neoadjuvant therapeutic scenario. In any case, when not strictly required, an adjuvant or neoadjuvant systemic approach can be considered interchangeable.

Early-stage breast cancer systemic treatment: chemotherapy

A number of CT regimens have activity in the preoperative (neoadjuvant) or postoperative (adjuvant) setting. In both cases, the underlying aim remains the eradication or control of undiscovered distant metastases, with a consequent reduction in the risk of relapse and death from breast cancer. The mainstay of treatment is currently represented by anthracyclines + taxanes-based 3rd generation regimens, although anthracycline-alone or other schemes might be useful in certain conditions[9]. Platinum agents have also demonstrated the capability of improving pCR rates in TNBC, therefore their incorporation in neoadjuvant schedules can be recommended in some cases[18]. Conversely, a direct benefit on long-term survival outcome in both neoadjuvant and adjuvant setting has not been clearly observed, therefore they cannot be considered an adjuvant therapeutic standard nor can be routinely administered in all neoadjuvant cases[3,9].

Overall, roughly a 36% breast cancer mortality rate reduction has been demonstrated for the most effective regimens versus no CT, with a 10-year risk of death from breast cancer reduced by about a third[19]. The choice of whether to adopt a neoadjuvant or adjuvant approach depends on several factors that will be further discussed. CT is not mandatory in all EBC. In general, CT is usually recommended for TNBC, since no other systemic treatments are available. Within this subset, only very small tumors (≤0.5 cm) without nodal involvement can be spared it. In HER2+ tumors CT is usually recommended in sequential and/or combined approaches with anti-HER2 TT (see **Table 1**). In HR+ disease, CT is recommended in cases at higher risk of relapse (big primary lesions and/or nodal involvement) and/or when a higher chemosensitivity and reduced endocrine sensitivity are expected due to clinicopathological

factors (e.g. low ER and PgR levels, high G, high Ki67). When available, the above mentioned genomic platforms are a useful tool for therapeutic decision-making.
Current viable option are reported in **Table 1**.

Table 1. Main chemotherapy and anti-HER2 regimens for the early setting

CHEMOTHERAPY REGIMENS + ANTI-HER2 TARGET THERAPY FOR HER2+ TUMORS IN (NEO)ADJUVANT SETTING		N. CYCLES	SCHEDULE TIMING
Preferred regimens	Dose-dense* (DD) Doxorubicin + cyclophosphamide (AC) followed by DD paclitaxel	4 and 4	every 2 weeks both
	DD AC followed by paclitaxel	4 and 12	every 2 weeks + weekly
	Docetaxel + cyclophosphamide (TC)	4	every 3 weeks
	AC or Epirubicin + cyclophosphamide (EC) followed by paclitaxel	4 and 12	every 3 weeks + weekly
	AC or EC followed by docetaxel	4 and 4	every 3 weeks both
	5-fluorouracil (5-FU) + epirubicin + cyclophosphamide (FEC) followed by paclitaxel	4 and 8	every 3 weeks + weekly
	FEC 100 followed by docetaxel	3 and 3	every 3 weeks + weekly
In selected cases	AC or EC	4	every 3 weeks
	FEC or 5-FU + doxorubicin + cyclophosphamide (FAC)	6	every 3 weeks
	Docetaxel + doxorubicin + cyclophosphamide (TAC)	6	every 3 weeks
	Cyclophosphamide + methotrexate + 5-FU (CMF)	6	every 4 weeks
	Only for HER2+ tumors: Paclitaxel + trastuzumab followed by trastuzumab to complete a year (see below)	12	weekly both
	Only for HER2+ tumors: Docetaxel + carboplatin + trastuzumab ± pertuzumab followed by the anti-HER2 therapy to complete a year (see below)	6	every 3 weeks all
	Only for TNBC in the neoadjuvant setting: Paclitaxel + carboplatin	12	every 3 weeks or weekly
	Only for TNBC in the neoadjuvant setting: Docetaxel + carboplatin	4-6	every 3 weeks
If TNBC, after neoadjuvant taxane/anthracycline-based CT and residual disease after surgery	Capecitabine	6-8	every 3 weeks
if HER2-positive, in the neoadjuvant setting	Trastuzumab°	4	every 3 weeks
	Pertuzumab + trastuzumab°	4	every 3 weeks both
If HER2-positive, in the adjuvant setting	Trastuzumab°	14-18#	every 3 weeks
	Pertuzumab + trastuzumab°	14-18#	every 3 weeks both
	After neoadjuvant antiHER2-based therapy and residual disease after surgery: T-DM1	14	every 3 weeks
	After 1 year of adjuvant trastuzumab in HR+ high-risk patients: Neratinib	Continuous for 1 year	every day

Legend. *: DD schedules have to be administered with granulocyte colony-stimulating factor (G-CSF); **°:** Trastuzumab or trastuzumab + pertuzumab are administered after or along with chemotherapy in the (neo)adjuvant setting, preferably in combination with taxanes, then are continued alone for a total of 1 year administration. Due to cardiotoxicity issues they cannot be administered in combination with anthracyclines. When the chemotherapeutic backbone is weekly paclitaxel, trastuzumab can be administered also in weekly schedule. In this case 12 weekly cycles are representative of 4 threeweekly cycles. One year of treatment is equal to 18 threeweekly

cycles, including a possible split in neoadjuvant and adjuvant treatment phases. **#:** 14 cycles, in order to complete a year of anti-HER2 therapy, if previously administered in the neoadjuvant setting; **HR+:** hormone receptor positive.

Early-stage breast cancer systemic treatment: endocrine therapy

For HR+ tumors, irrespective of HER2 status, ET represents another fundamental mainstay of treatment. The available therapeutic options are represented by the selective estrogen receptor modulator (SERM) tamoxifen, non-steroidal aromatase inhibitors (NSAI), namely anastrozole and letrozole, and steroidal AI (SAI), namely exemestane.

In premenopausal patients tamoxifen is the preferred treatment, but the combinations of tamoxifen or AI with a GnRH analogue are the recommended therapeutic choice for patients at higher risk of relapse[3,9].

In postmenopausal patients AI are the best treatment option. In this subset an all AI strategy or a sequential strategy of 2-3 years of tamoxifen followed by 3-2 years of AI are both viable[3,9]. Adjuvant ET has to last at least 5 years.

ET can be started before surgery in locally advanced HR+ tumors with high endocrine sensitivity instead of neoadjuvant CT. In this case, at least 3-4 months of continuous therapy are usually required to obtain significant reduction in tumor dimensions. The treatment will need to be continued after surgery to reach a minimum duration of 5 years. In adjuvant setting, ET has to be administered following adjuvant CT completion.

It has been demonstrated that adjuvant tamoxifen for 5 years is able to reduce the risk of recurrence by about half during the first 5 years and 1/3 during the following 5 years. It is also capable of reducing breast cancer mortality rate by about 30% throughout the first decade and beyond. At the same time, 5 years of an AI compared with no ET would reduce breast cancer recurrence by about two-thirds during the first 5 years and by about 1/3 during years 5–9, with a reduction in the breast cancer mortality rate by around 40% throughout the first decade, and perhaps beyond. These proportional reductions in risk are approximately independent of N, G, T, PR, and HER2 status[20,21].

An extended ET for a total of 7-10 years further reduces the risk of relapse and death. The available option are an extended treatment with tamoxifen in persistent premenopausal women, a switch to an AI or the prosecution of an ongoing AI for 2-5 additional years in postmenopausal women.

Several meta-analyses have demonstrated statistically significant improvement in the risk of recurrence with extended therapy, at the cost of higher toxicity. This strategy seems to confer

more benefit for patients with positive lymph-nodes, bigger tumors and previous CT use[22–24]. Thus a careful risk/benefit evaluation has to be conducted and previous tolerability and patient's preferences are also key factors to consider when making the therapeutic choice[3,9].

Early-stage breast cancer systemic treatment: HER2 targeted therapy

For patients with HER2-positive breast cancer, CT and trastuzumab-based therapy is recommended. In fact, since the introduction of the anti-HER2 monoclonal antibody trastuzumab as adjuvant treatment, only 3-18% of trastuzumab-treated patients currently relapse during the following 10 years [16,25,26]. The treatment usually starts in association with (neo)adjuvant CT and then continues for one year total. When given in association with CT, concomitant anthracyclines used has to be avoided, due to an excessive risk of cardiotoxicity. On the contrary, concomitant administration with taxanes is not prohibited and usually recommended. Combinatory schemes are reported in **Table 1**.

In patients at higher risk of relapse, mostly if node positive, the addition of the anti-HER2 monoclonal antibody pertuzumab to adjuvant trastuzumab further improves the invasive disease free survival of ~2%[27]. Thus a combination of pertuzumab and trastuzumab in adjuvant setting can be recommended for node positive tumors. Alternatively, a novel HER2 inhibitor, neratinib, slightly improved the risk of relapse for HR+/HER2+ tumors with one year of extended adjuvant treatment after previous (neo)adjuvant trastuzumab. A reduction in the risk of relapses translated in an absolute benefit of ~5% in invasive disease-free survival and ~2% in the 8-year survival rates. In patients who did not achieve a pCR after neoadjuvant treatment, an absolute 8-year survival benefit of ~9% was observed[28].

In the neoadjuvant setting, the NeoSphere trial demonstrated that CT with docetaxel associated to dual anti-HER2 blockade with trastuzumab and pertuzumab significantly improved pCR rates when compared to docetaxel and trastuzumab (45.8 vs 29.0%). This scheme rapidly became the preferrable neoadjuvant approach in HER2+ tumors, given also the prognostic implications of obtaining a pCR following neoadjuvant treatment[29]. Moreover, for patients not achieving pCR, the risk of recurrence of invasive breast cancer or death was demonstrated to be 50% lower with the administration of adjuvant T-DM1, instead of standard adjuvant trastuzumab[16]. T-DM1 is an antibody-drug conjugate (ADC) made of trastuzumab linked to DM1, a potent cytotoxic agent. In this peculiar post-neoadjuvant scenario, T-DM1 administered for 14 cycles to complete a year has become the preferred adjuvant regimen[9].

Advanced breast cancer systemic treatments: an overview

Despite numerous therapeutic advances, around 30% of early breast cancers still relapse. Unfortunately, when metastatic, breast cancer is still an incurable disease. In this setting, systemic treatments are mostly recommended for prolonging survival and palliation of symptoms[4,9]. Also in advanced setting, survival rates and therapeutic approaches differ according to tumor subtype. Median survivals for metastatic disease are ~36, 44, 13 and 34 months for HR+/HER2-neg., HR+/HER2+, TNBC and HR-/HER2+ tumors, respectively[2].

The therapeutic options currently available differ according to subtype. Differently form the early setting, where treatments have a curative purpose and are thus limited over time, in advanced disease, except for very limited cases, each treatment is administered until progression of the disease, occurrence of unacceptable toxicity or patient's death or will to stop[4,9,30].

For HR+ tumors numerous CT and ET, associated or not with a number of different TT, are available. International guidelines recommend the use of as much ET ± TT as possible, unless a condition of visceral crisis is present. Visceral crisis is defined as a severe organ dysfunction as assessed by signs and symptoms, laboratory studies, and rapid progression of disease which can rapidly lead to the permanent loss of the organ's function and/or patient's death. In this condition a rapid tumor shrinkage is required and CT is the recommended upfront treatment option[4,9]. This recommendation is mostly based on a consistent biological rationale and the fact that more limited toxicities in a palliative setting are preferable. Nevertheless, despite uniform indications from international guidelines, this recommendation is matter of debate and a high proportion (20-70%) of patients with metastatic HR+/HER2-neg. disease still receive CT as upfront treatment in the clinical practice all over the world, probably due to scarce high quality direct comparisons between ET and CT and the attitude to consider CT as a better therapeutic option than ET in case of high tumor burden or visceral disease, even in the absence of visceral crisis[31–33].

For TNBC the therapeutic approach is represented by standard CT, although the scenario is rapidly evolving, with the advent of PARP inhibitors in *BRCA*-mutant tumors and effective combinations with immune checkpoint inhibitors anti-PD-L1 (i.e. atezolizumab and pembrolizumab) and CT[34–37]. Another treatment, recently approved for advanced lines by the US Food and Drug Administration (FDA), is represented by the anti-TROP2 ADC sacituzumab govitecan, which showed impressive results in terms of survival improvements in late lines [38]. It is high likely that this treatment will become further approved also by the European Medicine Agency (EMA) and other regulatory agencies in the near future.

With respect to HER2+ tumors, the standard first-line is represented by the combination of trastuzumab + pertuzumab + a taxane, followed by T-DM1 in second-line. Subsequent lines can be represented by trastuzumab + CT combinations (e.g. capecitabine or vinorelbine), the anti-HER2 tyrosine kinase inhibitor (TKI) lapatinib + capecitabine or lapatinib + trastuzumab, or the more recent TKI anti-HER2 tucatinib combined with trastuzumab and capecitabine or neratinib + capecitabine, as well as combinations of ET + trastuzumab in case of HR+/HER2+ disease. Furthermore, due to impressive results observed in pre-treated HER2+ patients, the novel ADC trastuzumab deruxtecan has also been recently approved by the US FDA, with EMA recently granting accelerated assessment.

All main therapeutic options for advanced breast tumors are regrouped in **Table 2** and **Table 3** [4,9,30].

Table 2. Chemotherapy and target therapy for metastatic breast cancer

THERAPEUTIC REGIMENS FOR HER2-NEGATIVE ADVANCED BREAST CANCER			
MONOCHEMOTHERAPY	**POLICHEMOTHERAPY[c]**	**TARGET THERAPY**	**CHEMOTHERAPY + TARGET THERAPY**
Anthracyclines	Epirubicin + cyclophosphamide	**PARP inhibitors[d]**	**Antiangiogenic-based combinationsi**
Doxorubicin	Doxorubicin + cyclophosphamide	Olaparib	Paclitaxel + bevacizumab
Epirubicin	Non-peghylated liposomal doxorubicin + cyclophosphamide	Talazoparib	Capecitabine + bevacizumab
Peghylated liposomal doxorubicin	Carboplatin + paclitaxel	**TRAK inhibitors[e]**	**Immunotherapy-based combinations[j]**
Taxanes	Carboplatin + gemcitabine	Larotrectinib	Nab-paclitaxel + atezolizumab
Paclitaxel	Cyclophosphamide + methotrexate + 5-fluorouracil	Entrectinib	Nab-paclitaxel + pembrolizumab
Docetaxel	Docetaxel + capecitabine	**Anti-PD-L1[f]**	Paclitaxel + pembrolizumab
Nab-paclitaxel	Paclitaxel + gemcitabine	Pembrolizumab	Carboplatin + gemcitabine + pembrolizumab
Ixabepilone[a]		**Anti-TROP2**	**Anti-HER2-based combinations[h]**
Anti-metabolites		Sacituzumab govitecan-hzyi[g]	Lapatinib + capecitabine
Gemcitabine		**Anti-HER2[h]**	Trastuzumab + pertuzumab + paclitaxel/docetaxel
Capecitabine		Lapatinib + Trastuzumab	Trastuzumab + vinorelbine
Microtubule inhibitors		T-DM1	Trastuzumab + capecitabine
Vinorelbine		Trastuzumab Deruxtecan	Trastuzumab + paclitaxel +/- carboplatin
Eribuline			Tucatinib + trastuzumab + capecitabine[k]
Platinum agents[b]			Neratinib + capecitabine[k]
Carboplatin			Trastuzumab + docetaxel
			Trastuzumab + other CT effective for breast cancer, excluding Anthracyclines

Cisplatin			
Alkylating agents			
Chiclophosphamide			

Legend. a: not approved in Europe; **b:** especially for TNBC; **c:** to prefer only when rapid and substantial tumor shrinkage is required, otherwise mono-chemotherapies are preferrable; **d:** only for *BRCA1/2* germline mutant breast tumors pretreated with chemotherapy in (neo)adjuvant or metastatic setting and with at least one line of endocrine therapy in the metastatic setting, if HR-positive; **e:** approved for solid tumors with NTRAK fusions, including breast cancer; **f:** approved by US FDA in monotherapy for solid tumors with high microsatellite instability, including breast cancer. Not approved in Europe for this indication; **g:** only approved for TNBC; **h:** only for HER2+ tumors; **i:** only for first-line; **j:** only for first-line in TNBC with expression of PD-L1; **k**: US FDA approved combinations.

Table 3. Endocrine therapy for metastatic HR+/HER2-negative breast cancer

ENDOCRINE THERAPY FOR HR+/HER2-NEGATIVE ADVANCED BREAST CANCER		
MONOTHERAPY	**COMBINATIONS**	**ENDOCRINE THERAPY + TARGET THERAPY**
SERM	Fulvestrant + anastrozole/letrozole	**PI3K-inhibitors**
Tamoxifen		Alpelisib + fulvestrant
Toremifene		**CDK4/6-inhibitors**
NSAI		Palbociclib + AI
Anastrozole		Ribociclib + AI
Letrozole		Abemaciclib + AI
SAI		Palbociclib + fulvestrant
Exemestane		Ribociclib + fulvestrant
SERD		Abemaciclib + fulvestrant
Fulvestrant		**mTOR-inhibitors#**
Other*		Everolimus + exemestane
Medroxyprogesterone acetate		Everolimus + fulvestrant
Megestrol acetate		Everolimus + tamoxifen
Ethinyl estradiol		
Abemaciclib°		

Legend. SERM: selective estrogen receptor modulator; **SERD:** selective estrogen receptor degrader; **NSAI:** non-steroidal aromatase inhibitor; **SAI:** steroidal aromatase inhibitor; **AI:** aromatase inhibitor; ***:** useful in certain circumstances; **°:** only approved by the US FDA as monotherapy for pretreated metastatic disease; **#:** everolimus only approved in combination with exemestane in Europe. All endocrine agents have to be combined with a GnRH analogue to induce a iatrogenic postmenopausal status in case of premenopausal patients.

Treatment toxicity

One of the main issues related to the anticancer treatments, when compared to other therapeutics, is represented by a higher incidence of toxicities. When it comes to the definition and assessment of the severity of side effects, a common terminology criteria, along with a common grading system for adverse events (AEs) has been adopted [39]. Briefly, the following grading system is usually adopted:

G0 = no adverse event or within normal limits;

G1 = mild adverse event;

G2 = moderate adverse event;

G3 = severe and undesirable adverse event;

G4 = life-threatening or disabling adverse event;

G5 = death related to adverse event.

Despite AEs being a frequent event with the majority of anticancer agents, the incidence of more serious G3-5 toxicities differ. In general, treatments with the worse toxicity profile are CT agents, and combination schemes (poli-CT) increase the incidence of G3-5 toxicities. For this reason monochemotherapies (mono-CT) are usually recommended instead of combinations in the metastatic setting[4,9].

Typical and most frequent AEs are alopecia, stomatitis, febrile neutropenia, hand-foot syndrome and sensory/motor disorders. Biochemical disorders and hematologic toxicities (e.g. neutropenia and leukopenia) are frequent, as well (more with poli-CT than with mono-CT)[40]. Finally, cardiotoxicity is a peculiar side effect usually observed with anthracyclines. The pathognomonic manifestation of is a hypokinetic cardiomyopathy progressively leading to heart failure. The risk increases as the cumulative dose administered increases (e.g. minimal for doses $\leq$240 mg/m^2, 3–5% with 400 mg/m^2, up to 18–48% at 700 mg/m^2)[41]. Several concurrent factors can also increase the risk (e.g. patients<5 years old or >65 years old, prior or concurrent chest irradiation, pre-existing heart disease, cardiovascular risk factors, concomitant use of anti-HER2 TT)[41]. Finally, there is a small increased risk of developing second cancer, mostly leukemia and myelodysplastic syndrome, after receiving certain CT drugs for EBC. The most risky are considered to be anthracyclines and alkylating agents[42,43]. The longer the period of administration, the higher the dose and the dose intensity, the higher the risk for developing second tumors. In any case, CT-induced secondary cancers are a rare phenomenon in breast cancer patients[42,43].

Most frequent G3-5 AEs with ET are AST/ALT increase, hyperglicemia, pain, arthralgias (mostly with AI), fatigue, anemia, dyspnea and constipation. Osteoporosis is also a relatively

frequent side effect when AI are administered, mostly for prolonged periods. To note, tamoxifen slightly increases the risk for endometrial cancer. However, this risk is <1% *per* year. A prosecution of tamoxifen beyond 5 years induces an absolute increase in mortality due to endometrial cancer of ~0.2%[44–46].

Most frequent G3-5 AEs with currently approved ET+TT are diarrhea (especially with abemaciclib and alpelisib), rash and fatigue (mostly with alpelisib), stomatitis and pneumonitis (typically with everolimus), neutropenia and leukopenia (typically with CDK4/6-inhibitors but with higher frequency with palbociclib and ribociclib compared to abemaciclib), hyperglicemia (alpelisib) and AST/ALT increase (mostly with CDK4/6-inhibitors and everolimus) [40].

CT and ET are also frequently responsible for atrophic vulvovaginitis related to estrogen deprivation (for induction or worsening of a postmenopausal status)[47].

Anti-HER2 agents are characterized by cardiotoxicity as typical side-effect, although a systematic review of the literature showed that only 2% of cardiac events occurred in anti-HER2-based trials and these were not exclusive to trastuzumab-treated patients. The majority of side effects were also experienced by 1% or less of patients and were predominantly of G1-2 toxicity. Some other typical side effects are interstitial pneumonitis with trastuzumab deruxtecan, diarrhea and skin rash with lapatinib or neratinib, and liver toxicity for T-DM1[48–50]. Autoimmune effects (e.g. thyroiditis, colitis, hypophysitis, dermatitis etc.) with immune checkpoint inhibitors such as atezolizumab and pembrolizumab are also typical and usually manageable[51,52]. Sacituzumab govitecan has a chemo-like toxicity profile, with severe diarrhea, febrile neutropenia and anemia being among the most frequent high grade side effects[38]. Finally, PARP inhibitors are usually well tolerated, but myelosuppression and diarrhea are among the most frequent AEs to require the attention of the clinician[53].

Main objectives

The clinical scenario for both EBC and ABC is complex, multifaced and rapidly evolving. Overall, this work had the ambition to provide evidence to improve the management of EBC and ABC in different settings (**Figure 2**). More specifically, the two main purposes were the following:

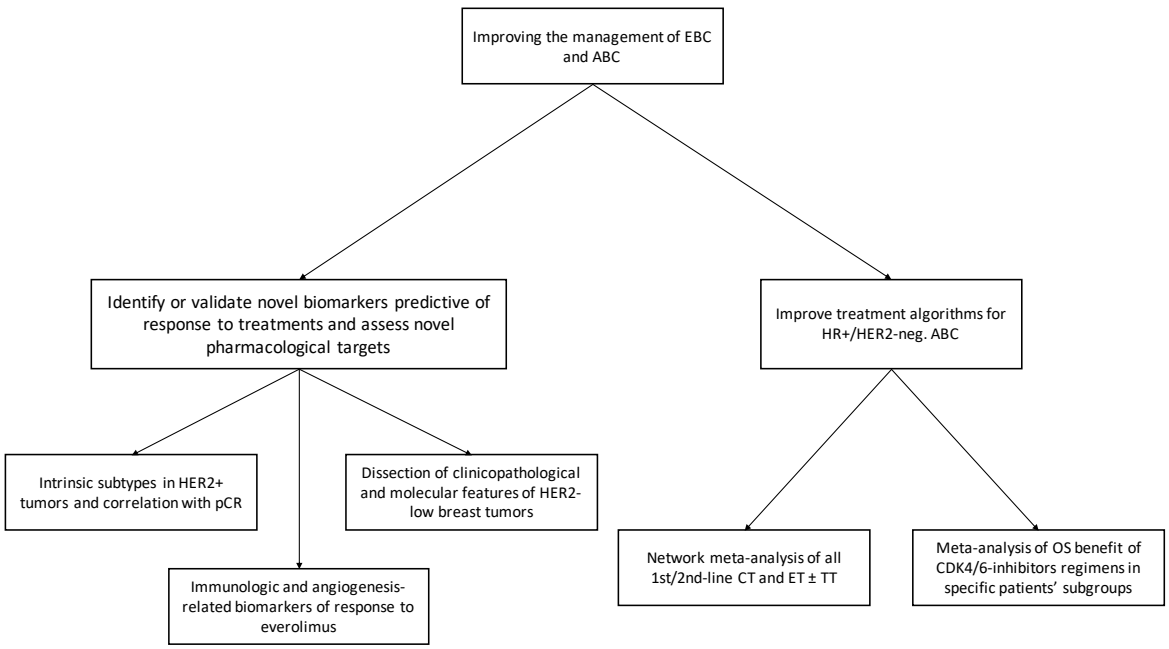
3. Identify or validate novel biomarkers predictive of response to treatments and assess novel pharmacological targets;
4. Improve treatment algorithms for HR+/HER2-neg. ABC.

With respect to the first objective, the focus was put on breast cancer molecular subtypes and the validation of their use in neoadjuvant setting in HER2+ tumors, so to predict pCR and

support escalated or de-escalated therapeutic approaches. Furthermore, following the discovery of the efficacy of novel anti-HER2-directed ADC in HER2-neg. tumors presenting some level of expression of HER2 (i.e. HER2-low tumors)[54,55], the first extensive molecular and clinicopathological characterization of this potentially novel subgroup of breast tumors was conducted. Finally, the mTOR inhibitor everolimus, in combination with the AI exemestane is approved for pretreated HR+/HER2-neg. ABC. However, a number of very effective therapeutic alternatives has emerged during the last few years and the identification of biomarkers of response would be particularly useful to identify patients that might benefit most from this drug.

With respect to the second aim, the focus was put on improving current treatment algorithms for advanced luminal tumors, due to the discrepancy observed between main international guidelines' recommendations and "real world" clinical practice[31–33]. The studies conducted had the objective to provide a comprehensive assessment of the efficacy of current therapeutic options and novel pooled evidence to support current treatment guidelines and help clinician's with their therapeutic choices.

Figure 2. Flow-chart resuming objectives and topics of the present doctoral thesis



Legend. EBC: early breast cancer; **ABC:** advanced breast cancer; **CT:** chemotherapy; **ET:** endocrine therapy; **TT:** target therapy; **pCR:** pathologic complete response; **OS:** overall survival.

Candidate's role in the development of the overall project and thesis structure

This thesis has been developed with the collaboration of several investigators from Italy, Spain, Belgium, Australia and America. For each objective, one or more studies were conducted and results have already been published in peer-reviewed international journals[13,40,56,57] or have been accepted but still not published (i.e. the HER2-low study). All articles have been regrouped in the present thesis, with each one representing a different chapter. Permissions to reproduce these original works have been obtained from the respective journals, whenever appropriate. All permission are collected in the **Appendix 1.** For all these articles, the candidate was involved in the study conception and writing of the first and subsequent drafts, as well as the final approved version. For the studies in chapters 1, 3, 4 and 5, the candidate was responsible for data collection, in total or in part. For the article in chapter 1, 3 and 5, the candidate performed all or part of the statistical and bioinformatical analyses. For all studies, the candidate participated in the interpretation of results. The candidate was first author (or co-first) in all articles and corresponding author, as well, for the article in chapter 5. This thesis' introduction ,discussion and conclusions have been conceived and written by the candidate. The final references are referred to main thesis' introduction, discussion and conclusions. For each article, the appropriate references are presented at the end of the respective chapter and the published supplementary materials are collected in the thesis' **Appendices 2-5**.

CHAPTER 1: HER2-enriched subtype and pathological complete response in HER2-positive breast cancer: A systematic review and meta-analysis

Original article reference

This article was originally published in the Cancer Treatment Reviews journal in 2020. The full reference is: Schettini F, Pascual T, Conte B, Chic N, Brasó-Maristany F, Galván P, Martínez O, Adamo B, Vidal M, Muñoz M, Fernández-Martinez A, Rognoni C, Griguolo G, Guarneri V, Conte PF, Locci M, Brase JC, Gonzalez-Farre B, Villagrasa P, De Placido S, Schiff R, Veeraraghavan J, Rimawi MF, Osborne CK, Pernas S, Perou CM, Carey LA, Prat A. HER2-enriched subtype and pathological complete response in HER2-positive breast cancer: A systematic review and meta-analysis. Cancer Treat Rev. 2020 Mar;84:101965. doi: 10.1016/j.ctrv.2020.101965. Epub 2020 Jan 17. PMID: 32000054; PMCID: PMC7230134.

Abstract

Background: HER2-positive (HER2+) breast cancer (BC) comprises all the four PAM50 molecular subtypes. Among these, the HER2-Enriched (HER2-E) appear to be associated with higher pathological complete response (pCR) rates following anti-HER2-based regimens. Here, we present a meta-analysis to validate the association of the HER2-E subtype with pCR following anti-HER2-based neoadjuvant treatments with or without chemotherapy (CT).

Methods: A systematic literature search was performed in February 2019. The primary objective was to compare the association between HER2-E subtype (versus others) and pCR. Selected secondary objectives were to compare the association between: 1) HER2-E subtype and pCR in CT-free studies, 2) HER2-E subtype within hormone receptor (HR)-negative and HR+ disease and 3) HR-negative disease (versus HR+) and pCR in all patients and within HER2-E subtype. A random-effect model was applied. The Higgins' I^2 was used to quantify heterogeneity.

Results: Sixteen studies were included, 5 of which tested CT-free regimens. HER2-E subtype was significantly associated with pCR in all patients (odds ratio [OR]: 3.50, $P<0.001$, $I^2=33\%$), in HR+ (OR: 3.61, $P<0.001$, $I^2=1\%$) and HR-negative tumors (OR: 2.28, $P=0.01$, $I^2=47\%$). In CT-free studies, HER2-E subtype was associated with pCR in all patients (OR: 5.52, $P<0.001$, $I^2=0\%$) and in HR + disease(OR: 4.08, $P=0.001$, $I^2=0\%$). HR-negative status was significantly associated with pCR compared to HR + status in all patients (OR: 2.41, $P<0.001$, $I^2=30\%$) and within the HER2-E subtype (OR: 1.76, $P<0.001$, $I^2=0\%$).

Conclusions: The HER2-E biomarker identifies patients with a higher likelihood of achieving a pCR following neoadjuvant anti-HER2-based therapy beyond HR status and CT use. Future trial designs to escalate or de-escalate systemic therapy in HER2+ disease should consider this genomic biomarker.

Introduction

Breast cancer (BC) with overexpression and/or amplification of the Human Epidermal Growth Factor Receptor 2 (HER2-positive) re- presents 11–30% of all breast tumors [1]. HER2 positivity is defined today by immunohistochemistry (IHC) as complete and strong mem- brane staining (i.e. score of 3+) in ≥10% of cancer cells, and/or by *in situ* immunofluorescence (ISH) techniques as amplified using a HER2/ CEP17 ratio cutoff of ≥2.0 and an average HER2 gene copy number ≥4.0 signals per cell [2]. This consensus definition is based on the methods and cutoffs used over the years in pivotal trials that led to the approval of trastuzumab [3], pertuzumab [4], neratinib [5], lapatinib [6] and T-DM1 [7] in HER2+ breast cancer.

The current HER2 definition do not sufficiently consider HER2+ disease′s clinical and biological heterogeneity. On one hand, high variability in patientś response and survival outcomes following anti-HER2-based therapy is common [8,9]. On the other hand, high biological variability exists within HER2+ disease [10–12]. For example, all the BC intrinsic subtypes [i.e. Luminal A, Luminal B, HER2- Enriched (HER2-E) and Basal-like] can be identified through gene ex- pression profiling [9,10,13]. Among them, the HER2-E subtype is the most frequent (31–76%), shows the highest levels of *ERBB2* mRNA and protein and appears to be the subtype with the highest activation of the EGFR-HER2 signaling pathway [11,14–31]. Importantly, these biological entities within HER2+ disease are not fully recapitulated by hormone receptor (HR) status since 40% of HER2+/HR+ tumors are HER2-E and 15% of HER2+/HR-negative tumors are Basal-like [10,11,32].

To date, no biomarker has demonstrated clinical utility in HER2+ early disease beyond HER2 and HR status [33]. However, accumulating evidence supports the clinical validity of two biomarkers: intrinsic subtyping and stromal tumor infiltrating-lymphocytes (TILs). In particular, either the HER2-E subtype or high TILs appears to be associated with high response to anti-HER2-based treatments in the neoadjuvant setting [14–31,34,35]. From a prognostic point of view, however, HER2-E subtype is associated with a worse prognosis [10,36] whereas TILs are associated with a better survival outcome [34,37,38]. Unfortunately, the majority of these data were derived from retrospective analyses from individual clinical trials using baseline tumor samples. In addition, most analyses were exploratory and unplanned, and limited by relatively small sample sizes.

To increase the level of evidence of the association of the HER2-E subtype with the response to anti-HER2–based neoadjuvant regimens, we decided to review the literature and perform a meta-analysis.

Materials and methods

Search strategy and selection criteria

A systematic literature search was performed on 12/February/2019 to identify published observational, phase II and phase III (randomized and non-randomized) neoadjuvant clinical studies involving anti-HER2-based treatments in HER2+ BC, where the association between pathological complete response (pCR) and BC molecular intrinsic subtypes was evaluated. The literature search had no time nor language restriction, however, only clinical studies involving anti-HER2-based neoadjuvant regimens were included, with or without chemotherapy.

Additional studies particularly relevant to the topic, for which molecular data had not been published but were available at the Translational Genomic and Targeted Therapeutics in Solid Tumors la- boratory of the IDIBAPS (Barcelona, Spain), were also included in the analysis. All pre-clinical studies, phase I trials, non-neoadjuvant trials and neoadjuvant trials without anti-HER2 agents were excluded. The recommendations of the Cochrane Collaboration [44] were followed to identify all relevant studies. For our query, we used a combination of disease characteristics, study design, treatment setting and strategies or drugs. The full query is reported in the **appendix 2**. Both full articles and studies published in the abstract form were included in the analysis, if odds ratios (OR) data were directly available or computable. The search was conducted on the electronic databases Pubmed and Web of Science®, as well as on San Antonio Breast Cancer Symposiums (SABCS)'s, American Society of Clinical Oncology (ASCO)'s and Eur- opean Society of Medical Oncology (ESMO)'s annual meetings online archives. Four reviewers (FS, TP, NC and CR) independently evaluated whether each selected randomized clinical trials (RCT) respected the predetermined criteria, and another reviewer (AP) was consulted in case of controversy.

Data extraction and objectives

Details on study design, patient/tumor characteristics, interventions and outcome were extracted from each paper. Only the most recent and complete reports were included when duplicate publications were identified. Crude odds ratio (OR) for pCR with their 95% confidence intervals (CI) were extracted or calculated, when necessary, from each published paper or internal datasets. The definition of pCR varied across studies. In 12/16 (75%) studies (2,176/2,703 patients with known PAM50 subtype), pCR was defined as the absence of invasive neoplastic cells at microscopic examination of the primary tumor at surgery in breast and axilla (pCR in-breast and axilla), with remaining in-situ lesions allowed. In 4/16 (25%) studies (527/2,703 patients with known molecular subtype), pCR was defined as the absence

of tumor cells only in breast, without considering tumor response in axillary lymph nodes (pCR in-breast).

The primary objective was to compare the association between HER2-E subtype (versus others) and pCR in all patients. Secondary objectives were to:

1. compare the association between HER2-E subtype (versus others) and pCR in CT-free studies;
2. compare the association between HR-negative disease (versus HR+) and pCR in all patients;
3. compare the association between HR-negative disease (versus HR+) and pCR within HER2-E subtype;
4. compare the association between HER2-E subtype (versus others) and pCR within HR+ and HR-negative disease;
5. compare the association between each intrinsic subtype (versus the others) and pCR.

Statistical analyses

Since a certain degree of heterogeneity was expected, analyses were performed under the Random-Effect Model of DerSimonian and Laird [45]. Heterogeneity was assessed with Higgin′s I^2 index [46]. Preplanned exploratory subgroup analyses for the primary endpoint were conducted, even if heterogeneity was not relevant. Subgroup analyses of interest were: (1) phase II vs phase III trials, (2) randomized vs. non- randomized trials (3) CT-containing vs. CT-free studies (4) pCR in-breast vs pCR in-breast and axilla.

For the primary endpoint, to assess whether the pooled OR estimates were stable or strongly dependent on one or few studies, sensitivity analyses were conducted by interactively recalculating the pooled OR estimates after exclusion of each single study. Publication bias was explored through funnel plot visual inspection and the Egger's linear regression test for funnel plot asymmetry [47,48]. All reported *P* values were two-sided. All statistical analyses and the generation of forest plots were conducted using R and RevMan [49,50]. The Cochrane risk of bias assessment tool was em- ployed to assess the quality of the data obtained and the risk of bias in each study. Significance was set at $P<0.05$, except for Egger's test, for which significance was set as $P<0.1$, as usual. The project was registered in the PROSPERO online database [51], with registration number: CRD42019140902.

Assessment of risk of bias

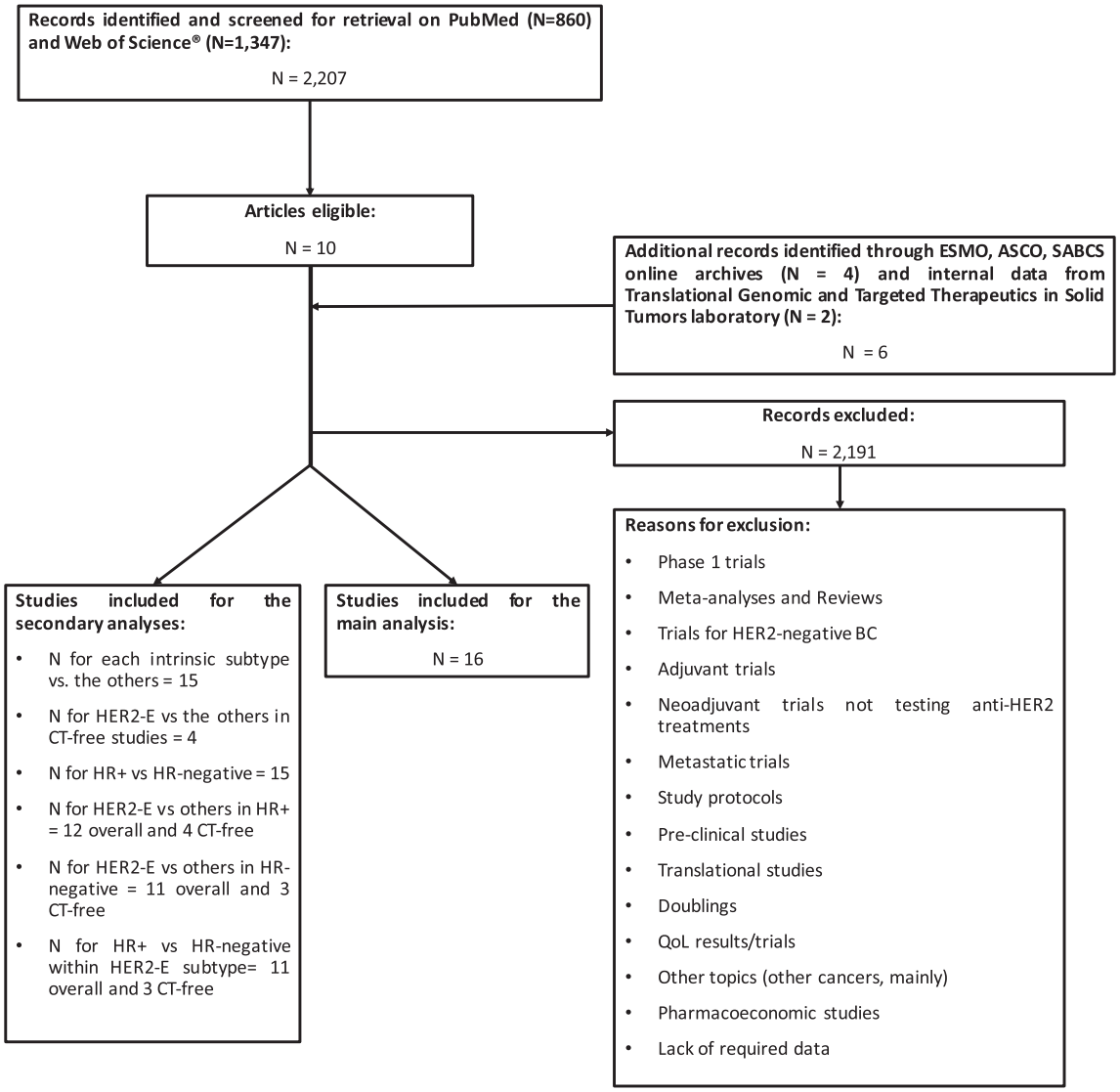
The risk of bias for each trial was assessed by using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions [44]. Each domain related to a risk of bias was assessed in each included trial, since there is evidence that these issues are asso- ciated with biased estimates of treatment effect. The domains were the following: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessment; (5) incomplete outcome data; (6) selective reporting; (7) other bias. Review authors' judgments were categorized as "low risk", "high risk" or "unclear risk" of bias. Internal validity of eligible studies was assessed according to the Cochrane Collaboration′s 'Risk of Bias' tool in Review Manager [50].

Results

Summary of studies and patient characteristics

A total of 16 studies were included (**Tables 1 and 2; Supplementary Tables 1 and 2**) [14–31]. From Pubmed and Web of Science® online databases, 2,207 studies were extracted and 10 were included [14–18,20–22,24,25,28]. From ASCO, ESMO and SABCS online abstracts books, 4 studies were included [19,26,27,30,31]. Finally, data from 2 studies (ICO-CLINIC, LPT109096) were available at the Translational Genomic and Targeted Therapeutics in Solid Tumors laboratory at IDIBAPS (Barcelona, Spain) [14,26,31]. Some data were also retrieved from later-published full articles [11,52]. The selection process is resumed in the PRISMA diagram (**Figure 1**). Overall 5 (31.25%) phase III RCT, 5 (31.25%) phase II RCT, 5 (31.25%) non-randomized phase II trials and 1 (6.25%) retrospective observational study were included. All the articles/abstracts containing molecular results have been published between 2014 and 2019. From a total of 3,733 patients, PAM50 intrinsic subtype was available for 2,703 (72.4%) patients, while HR status was known for 3,373 (90.3%) patients. Except for one trial (i.e. PerELISA) which enrolled HR+ tumors-only [28], the others included both HR+ and HR-negative tumors. All studies included evaluated anti-HER2-based neoadjuvant regimens with or without CT [14,23,28,29,53], and included tumor stages II or III, except for the PAMELA trial and the retrospective observational study from the Catalan Institute of Oncology and the Hospital Clinic of Barcelona (ICO-CLINIC), which allowed stage I disease [14,26]. Various methods for assessing the PAM50 BC intrinsic subtypes were used across all trials (**Tables 1 and 2**), but all were based upon gene expression data [14–31].

Figure 1. PRISMA diagram



Among the studies included, only the PAMELA single arm phase II trial was specifically designed to prospectively assess PAM50 intrinsic subtypes and test whether patients with the HER2-E subtype benefited more than the other subtypes from a neoadjuvant anti-HER2-based CT-free regimen [14]. The other studies evaluated PAM50 as an exploratory retrospective analysis; therefore, tumor samples were not always available for all patients included. However, samples were always available for at least half of the population enrolled within each study (**Tables 1 and 2**). pCR rates in HER2-E subtype were higher than non-HER2-E subtypes in each study, except in the LPT109096 trial. Individual trials′ results are reported in **Tables 1 and 2**.

Table 1. Characteristics of the included randomized phase II and III trials

RANDOMIZED TRIALS						
Study name	**NOAH***	**NSABP-B41**	**NeoALTTO**	**CALGB 40,601**	**KRISTINE**	**Cher-LOB**
Phase	III	III	III	III	III	II
Regimen	Dox + P → P → CMF → T (HER2 positive cohort)	AC → P + T / AC → P + L / AC → P + T + L	L + T → P / L → P / T → P	L + T + P / L + P / T + P	TCH + Pe / T-DM1 + Pe	L + T → P → FEC / L → P → FEC
Treatment category	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 + CT
N. of evaluable patients/ Total of the arm	63/117	271/529	254/455	262/305	354/444	84/121
TNM Stage	III	II and III	II and III	II and III	II and III	II and IIIA
HR status	Pos and neg	Pos and neg	Pos and neg	Pos and neg	Pos and neg	Pos and neg
HER2E (%)	34 (54.0)	197 (72.7)	110 (43.3)	82 (31.3)	194 (54.8)	22 (26.2)
Non-HER2E (%)	29 (46.0)	74 (27.3)	144 (56.7)	180 (68.7)	160 (45.2)	62 (73.8)
pCR in HER2E (%)	18 (62.1)	120 (60.9)	57 (51.8)	57 (69.5)	131 (67.5)	11 (50.0)
pCR in non-HER2E (%)	10 (34.5)	19 (25.7)	31 (21.5)	64 (35.6)	47 (29.4)	16 (25.8)
pCR definition	ypT0/is ypN0	ypT0/is ypN0	ypT0/is ypN0	ypT0/is	ypT0/is ypN0	ypT0/is ypN0
Gene expression platform	Microarray-based	nCounter	RNA seq.	RNA seq.	nCounter	Microarray-based
Data source	Published material	Published material	Published material	Published material	Published material	Published material
Year of publication	2014	2013/2019	2012/2016	2015	2017	2015/2016
First author	Prat A	Robidoux A/Swain SM	Baselga J/Fumagalli D	Carey L	Prat A	Guarneri V/Dieci MV
Publication form	Full article	Full article	Full article	Full article	Abstract	Full article
Publication site	Clin Can Res	Lancet Oncol/Breast Can Res Treat	Lancet/JAMA Oncol	J Clin Oncol	SABCS	The Oncologist/Ann Oncol

RANDOMIZED TRIALS						
Study name	**Cher-LOB**	**NeoSphere**	**TRYPHAENA**	**LPT109096**	**TBCRC023**	
Phase	II	II	II	II	II	
Regimen	T → P → FEC	T + D / Pe + T + D / Pe + T / Pe + D	FEC + T + Pe → T + Pe + D / FEC → T + Pe + D / TCH + Pe	T + FEC → T + P / L + FEC → L + P / T + L + FEC → T + L + P	L + T +/− Let +/− GnRHa 12 weeks	L + T +/− Let +/− GnRHa 24 weeks
Treatment category	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 w/o CT	
N. of evaluable patients/ Total of the arm	84/121	337/417	173/225	61/100[#]	85/97	
TNM Stage	II and IIIA	II and III	II and III	II and III	II and III	
HR status	Pos and neg	Pos and neg	Pos and neg	Pos and neg	Pos and neg	
HER2E (%)	22 (26.2)	135 (40.1)	82 (47.4)	41 (67.2)	51 (60.0)	
Non-HER2E (%)	62 (73.8)	202 (59.9)	91 (52.6)	20 (32.8)	34 (40.0)	
pCR in HER2E (%)	11 (50.0)	53 (39.3)	57 (69.5)	30 (73.1)	14 (27.5)	
pCR in non-HER2E (%)	16 (25.8)	52 (25.7)	46 (50.5)	15 (75.0)	3 (8.8)	
pCR definition	ypT0/is ypN0	ypT0/is ypN0	ypT0/is ypN0	ypT0/is ypN0	ypT0/is	
Gene expression platform	Microarray-based	nCounter	nCounter	nCounter	nCounter	
Data source	Published material	Published material	Published material	IDIBAPS lab	Published material	
Year of publication	2015/2016	2012/2018	2013	2011	2019/2019	
First author	Guarneri V/ Dieci MV	Gianni L/Bianchini G	Schneeweiss A	Holmes FA	Rimawi MF/Prat A	
Publication form	Full article	Full article/Abstract/Poster	Full article	Full article/internal data	Full article/Full article	
Publication site	The Oncologist/ Ann Oncol	Lancet Oncol/ESMO congress	Ann Oncol	BMC Research Notes/Internal data	Clin Cancer Res /JNCI	

Legend and footnotes: HER2E = HER2 enriched; non-HER2E = Basal-Like, Luminal A, Luminal B, Normal-like; Pos = positive; Neg = negative; HR = hormone receptors; CT = chemotherapy; N/A = not assessed; pCR = pathologic complete response; AC = doxorubicin + cyclophosphamide; Dox = doxorubicin; CMF = cyclophosphamide + methotrexate + 5-fluorouracil; FEC = 5-fluorouracil + epirubicin + cyclopho- sphamide; TCH = docetaxel + carboplatin + trastuzumab; P = paclitaxel; D = docetaxel; LD = liposomal doxorubicin; T = trastuzumab; Pe = pertuzumab; L = lapatinib; Let = letrozole; GnRHa = GnRH analogue → = followed by; *:HER2 positive cohort non-treated with trastuzumab and HER2 negative cohort not considered; #: pts with non-available information on pCR excluded; SABCS = San Antonio Breast Cancer Symposium; ASCO = American Society of Clinical Oncology; ESMO = European Society for Medical Oncology.

Table 2. Characteristics of the included non-randomized studies

NON-RANDOMIZED STUDIES							
Study name	**ICO-CLINIC**	**BERENICE**	**Opti-HER HEART**	**PerELISA**		**TBCRC006**	**PAMELA**
Study type	Retrospective Observational	Non-randomized Phase II	Single Arm Phase II	Non-Randomized Phase II		Single Arm Phase II	Single Arm Phase II
Regimen	Tax +/− Anthra + T	ddAC → P + T + Pe FEC → D + T + Pe	Pe + T + LD + P	Let + T + Pe	P + T + Pe	L + T +/− Let +/− GnRH	L + T +/− Let or Tam
Treatment category	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 + CT	Anti-HER2 w/o CT	Anti-HER2 + CT	Anti-HER2 w/o CT	Anti-HER2 w/o CT
N. of evaluable patients/Total of the arm	172/173	294/400	58/83	40/44	15/17	29/65	151/151
TNM Stage	I–III	II and III	II and III	II and III		II and III	I - III
HR status	Pos and neg	Pos and neg	Pos and neg	Pos		Pos and neg	Pos and neg
HER2E (%)	102 (59.3)	175 (59.5)	30 (51.7)	11 (27.5)	12 (75.0)	22 (75.9)	101 (66.9)
Non-HER2E (%)	70 (40.7)	119 (40.5)	28 (48.3)	29 (72.5)	3 (25.0)	7 (24.1)	50 (33.1)
pCR in HER2E (%)	63 (61.8)	130 (74.2)	25 (83.3)	5 (45.5)	10 (83.3)	6 (20.7)	41 (40.6)
pCR in non-HER2E (%)	19 (27.1)	52 (43.7)	13 (46.4)	4 (13.8)	2 (66.7)	1 (14.3)	5 (10.0)
pCR definition	ypT0/is ypN0	ypT0/is ypN0	ypT0/is ypN0	ypT0/is ypN0		ypT0/is	ypT0/is
Gene expression platform	nCounter	nCounter	nCounter	nCounter		nCounter	nCounter
Data source	Published material/Internal data	Published material	Published material	Published material		Published material	Published material
Year of publication	2017/2019	2017	2019	2019		2013/2019	2017
First author	Pernas S	Swain SM	Gavilà J	Guarneri V		Rimawi MF/Prat A	Llombart-Cussac A
Publication form	Abstract/Full article	Full article	Full article	Full article		Full article	Full article
Journal/Meeting	SABCS /Front Oncol	Ann Oncol	BMC Medicine	Ann Oncol		J Clin Oncol/JNCI	Lancet Oncol

Legend and footnotes: HER2E = HER2 enriched; non-HER2E = Basal-Like, Luminal A, Luminal B, Normal-like; Pos = positive; Neg = negative; HR = hormone receptors; CT = chemotherapy; N/A = not assessed; pCR = pathologic complete response; AC = doxorubicin + cyclophosphamide; Dox = doxorubicin; CMF = cyclophosphamide + methotrexate + 5-fluorouracil; FEC = 5-fluorouracil + epirubicin + cyclopho- sphamide; TCH = docetaxel + carboplatin + trastuzumab; Tax = taxanes; Anthra = anthracyclines; P = paclitaxel; D = docetaxel; LD = liposomal doxorubicin; T = trastuzumab; Pe = pertuzumab; L = lapatinib; Let = letrozole; Tam = tamoxifen; GnRHa = GnRH analogue; → = followed by; dd = dose dense.

pCR and HER2-E subtype

The HER2-E subtype was significantly associated with pCR compared to others (OR: 3.50, 95%CI: 2.79 – 4.39, *P*<0.001, I^2=33%, **Figure 2**). The funnel plot suggested the absence of publication bias (**Supplementary Figure 1**), confirmed by a non-significant Egger′s test (*P*=0.48**)**. The influential analysis showed consistent results when omitting a single trial with an I^2 range varying from 3.4% (omitting the NeoSphere trial) [20] to 37.7% (omitting the TBCRC023 trial) [31]. Full results of the influential analysis are reported in **Table 3**. Considering the absence of significant heterogeneity, an exploratory, non-preplanned analysis done with the fixed-effect model [54] was performed with a similar result (OR: 3.51, 95%CI: 2.96–4.16, *P*<0.001, I^2=33%).

Figure 2. Forest Plots comparing the association with pCR between the HER2-E and the other intrinsic subtypes in the overall population

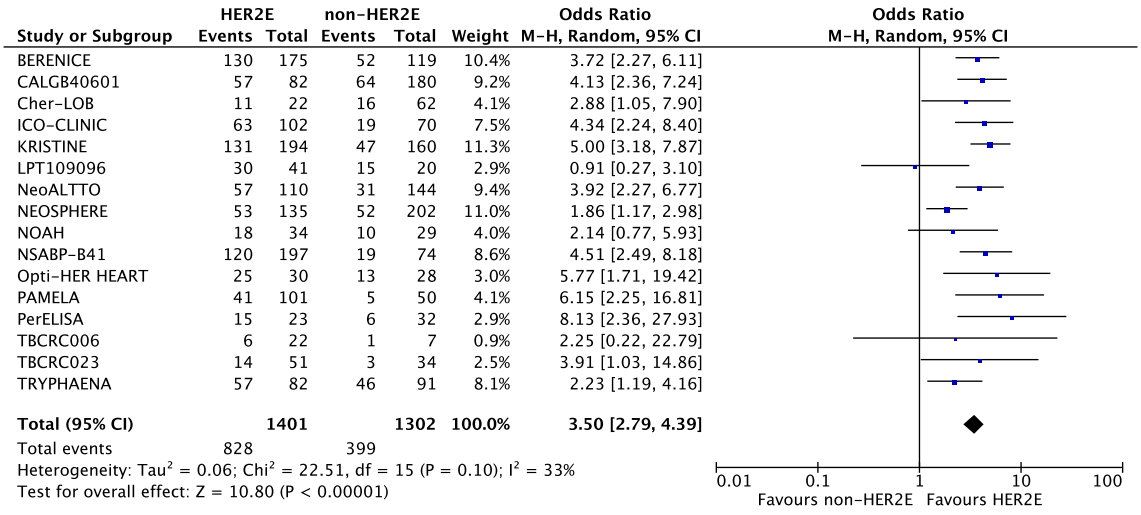


Table 3. Influenti al analyses concerning the primary end-point

Study omitted	OR	95% CI	*p*	I^2
BERENICE	3.47	2.70–4.47	< 0.0001	37.6%
CALGB40601	3.44	2.68–4.40	< 0.0001	36.7%
Cher-LOB	3.52	2.78–4.48	< 0.0001	37.4%
ICO-CLINIC	3.43	2.69–4.38	< 0.0001	36.6%
KRISTINE	3.34	2.63–4.23	< 0.0001	28.9%
LPT109096	3.62	2.95–4.45	< 0.0001	21.3%
NeoALTTO	3.45	2.69–4.43	< 0.0001	37.3%
NEOSPHERE	3.85	3.18–4.66	< 0.0001	3.4%
NOAH	3.57	2.82–4.50	< 0.0001	35.2%
NSABP-B41	3.41	2.67–4.35	< 0.0001	35.6%
Opti-HER-HEART	3.44	2.72–4.35	< 0.0001	35.9%
PAMELA	3.41	2.71–4.30	< 0.0001	34.1%
Per-ELISA	3.41	2.72–4.28	< 0.0001	32.3%
TBCRC006	3.51	2.78–4.43	< 0.0001	37.4%
TBCRC023	3.48	2.75–4.42	< 0.0001	37.7%
TRYPHAENA	3.64	2.88–4.59	< 0.0001	31.2%

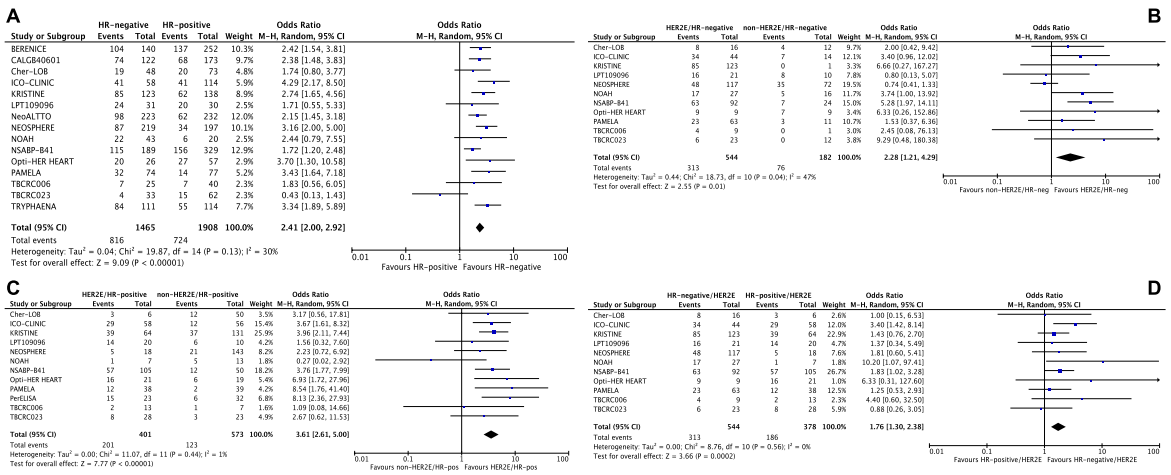
Legend. OR = odds ratio; CI = confidence intervals.

There were no statistically significant differences in terms of association with pCR for all the subgroups considered for the preplanned sensitivity analyses, namely randomized vs. non-randomized studies (*P*=0.46), phase II vs. phase III studies (*P*=0.13), CT-containing vs. CT-free studies (*P*= 0.30), pCR in-breast vs pCR in-breast + axilla (*P*=0.32). Compared to other intrinsic subtypes, the HER2-E subtype was significantly associated with pCR compared to Basal-like (OR: 2.50, 95%CI: 1.78–3.52, *P*<0.001, I^2=0%, **Supplementary Figure 2A**), Luminal A (OR: 4.81, 95%CI: 3.16 – 7.33, *P*<0.001, I^2=55%, **Supplementary Figure 2B**), Luminal B (OR: 3.82, 95%CI: 2.97–4.91, *P*<0.001, I^2=0%, **Supplementary Figure 2C**) and Luminal A/B (OR: 4.36, 95%CI: 3.17–6.00, *P*<0.001, I^2=52%, **Supplementary Figure 2D**) subtypes. Other comparisons among intrinsic subtypes can be found in the **appendix 2**.

pCR, HR status and HER2-E subtype

Fifteen of the 16 trials were used to assess the association between HR status and pCR. HR-negative disease was significantly associated with pCR compared to HR+ disease (OR: 2.41, 95%CI: 2.00 – 2.92, *P*<0.001, I^2=30%, **Figure 3A)**. The inspection of the funnel plot (**Supplementary Figure 3**), as well as the result of the Egger's test (*P*=0.68), did not reveal a significant publication bias. The HER2-E subtype was significantly associated with pCR within both HR-negative disease (OR=2.28, 95%CI: 1.21–4.29, *P*=0.01, I^2=47%, **Figure 3B**) and HR+ disease (OR: 3.61, 95%CI: 2.61 – 5.00, *P*<0.001, I^2=1%, **Figure 3C**). Similar to what was observed for the general population, HR- negative disease was significantly associated with pCR compared to HR + disease within the HER2-E subtype (OR: 1.76, 95%CI: 1.30-2.38, *P*<0.001, I^2=0%, **Figure 3D**).

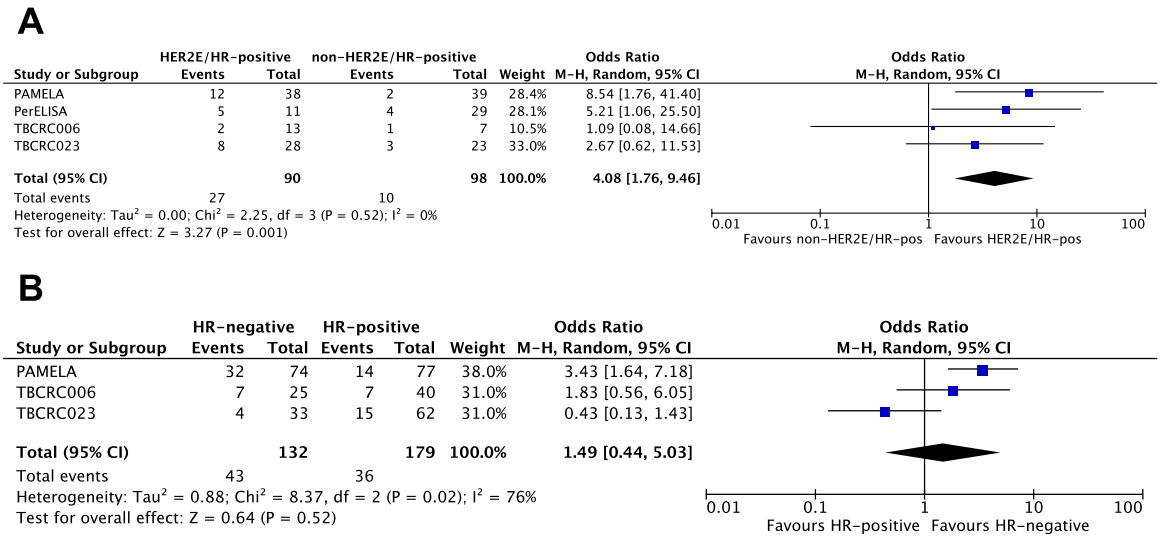
Figure 3. Forest Plots comparing the association with pCR between HR-positive and HR-negative tumors (A) in the overall population; the association with pCR between the HER2-E and the other intrinsic subtypes within the HR-negative (B) and HR-positive (C) disease, and the association of pCR between HR-positive and HR-negative tumors within the HER2-E subtype (D)



pCR, HR status and HER2-E subtype in the absence of CT

A total of 5 studies evaluated dual HER2 blockade in the absence of CT [14,20,28,29,31], although for one of these (i.e. NeoSphere), data for the CT-free arm were not available separately from the other CT-containing arms' data [20]. In CT-free regimens, HER2-E subtype was significantly associated with pCR compared to the other subtypes (OR: 5.52, 95%CI: 2.89–10.54, P<0.001, I^2=0%, **Figure 4A)**, while there was no apparent difference between HR-negative vs. HR+ disease (OR: 1.49, 95%CI: 0.44–5.03, P=0.52, I^2=76%, **Figure 4B**). When considering HR status, the HER2-E subtype was found to be significantly associated with pCR within HR+ disease (OR: 4.08, 95%CI: 1.76 – 9.46, P=0.001, I^2=0%, **Supplementary Figure 4A**), but not within HR-negative disease (OR: 2.18, 95%CI: 0.66–7.26, P=0.20, I^2=0%, **Supplementary Figure 4B**). Conversely, in patients with HER2-E subtype, HR status was not significantly associated with pCR (OR: 1.30, 95%CI: 0.67–2.52, P=0.44, I^2=0%, **Supplementary Figure 5**).

Figure 4. Forest Plots comparing the association with pCR between the HER2-E and the other subtypes (A), and between HR-negative and HR-positive tumors (B) in CT-free trials

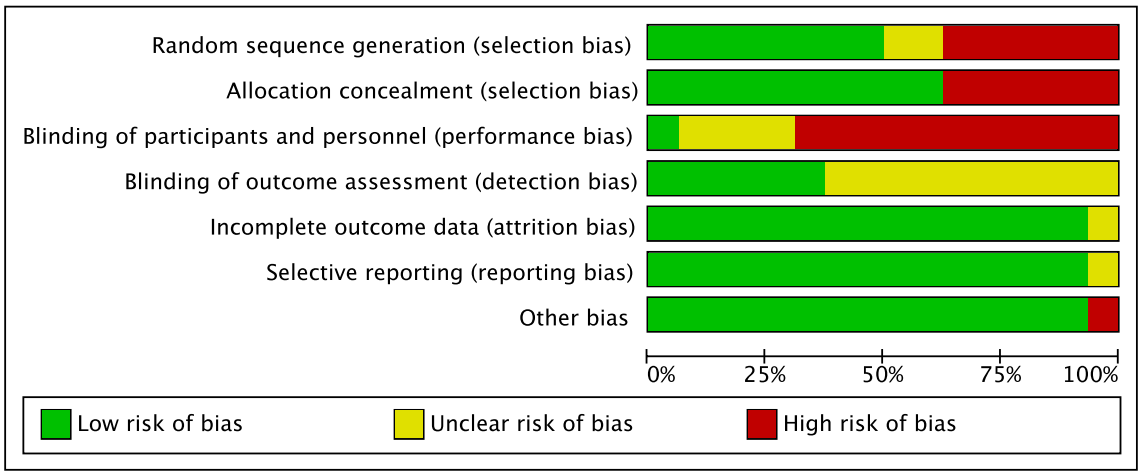


Risk of bias analysis

With respect to the risk of bias, as defined by the Cochrane's manual for systematic reviews [44], the risk of selection bias for random sequence generation and allocation concealments was present in the 6/16 (37.5%) of the studies in both cases, with an unclear risk in 1/16 (6.25%) studies included, concerning the random sequence generation selection bias (**Figure 5** and **Supplementary Figure 6**). The performance bias due to blinding of participants and personnel was present in 12/16 (75%) of cases, with an unclear risk in 3/16 (18.75%) of the studies included. No detection bias related to the blinding of outcome assessment, attrition bias due to incomplete outcome data and selective reporting

bias were observed. Concerning the last two, an unclear risk was present in 1/16 (6.25%) cases. Finally, we accounted for a 6.25% high risk of other bias related to the ICO-CLINIC study, due to its retrospective and non-trial design.

Figure 5. Risk of bias analysis



Discussion

The development of effective drugs against HER2+ BC has been particularly successful in the last few years [3–7]. Since the introduction of trastuzumab [3], other effective and tolerable anti-HER2 drugs (i.e. lapatinib, pertuzumab, neratinib and T-DM1) have been introduced in the metastatic and/or early disease settings, contributing to im- portant improvements in survival outcomes [8,55]. However, HER2+ disease is clinically and biologically heterogeneous and not all patients benefit to the same extend from current treatments. Thus, better identification of patients using biomarkers should allow the de- sign of prospective trials aiming to improve precision medicine in HER2+ BC. Among the different biomarkers evaluated in HER2+ disease over the last decade [10,14,21,31,34,35,37,39,40,56], the HER2-E subtype has been proposed to identify patients whose HER2+ tumors are HER2 “addicted” (meaning driven primarily by signaling via the HER2 pathway). Retrospective analysis of the HER2-E subtype, mostly exploratory and unplanned, using baseline tumor samples from individual clinical trials have linked this phenotype with high rates of pathological complete response following neoadjuvant anti-HER2-based therapies [14–31]. However, to date, no combined analysis or meta-analysis has been performed and analyses within all of those studies were limited by relatively small sample sizes. Here, we performed a trial-level meta-analysis of 16 neoadjuvant studies and 2,703 patients to evaluate the association of the HER2-E subtype with pCR. In particular, we confirmed that the HER2-E subtype is a consistent biomarker to identify patients with a higher likelihood of achieving a pCR following anti-HER2-based therapy

with or without cytotoxic therapy. Importantly, the association of the HER2-E subtype with pCR appeared to be independent of HR status, which is the only biomarker with clinical utility in HER2+ disease. Additionally, our results confirm the ability of HR status to predict pCR by itself and within the HER2-E subtype, although we could not demonstrate this in the CT-free setting, which had substantially fewer contributing trials.

We adopted pCR as our clinical endpoint for this *meta*-analysis. This is because numerous studies have demonstrated a favorable prognostic role in early stage HER2+ BC [56–60] so its use as primary endpoint in neoadjuvant trials has been increasing over the years and has also been endorsed for regulatory purposes by regulatory agencies such as US Food and Drug Administration (FDA), for accelerated approval of neoadjuvant treatments in high risk early-stage BC [61]. Furthermore, the FDA recently approved the use of adjuvant T-DM1 (in HER2+ BC) or capecitabine (in HER2-negative BC) in case of no achievement of pCR following standard neoadjuvant systemic therapy and surgery, making of pCR a fundamental tool in therapeutic decision-making in non-metastatic BC for escalating treatment strategies. At the same time, there is also an increasing use of pCR as a tool to identify potentially effective and safe de-escalating therapeutic approaches in HER2+ BC [14,28,29,62]. In fact, identification of effective de-escalating treatment strategies to spare toxicity and financial costs to patients is a main focus of the research community nowadays [63,64]. In adjuvant setting, several prospective trials of early stage HER2+ BC have attempted to demonstrate that de-escalating strategies based on a shorter duration of adjuvant trastuzumab provided similar benefits as the conventional 1 year; however, the results using non-inferiority trial designs were mixed [65]. On the contrary, a single-arm trial from a single institution (i.e. the APT trial) evaluating 12 doses of adjuvant weekly paclitaxel and 1-year of trastuzumab in largely HR+ stage I disease significantly impacted on daily clinical practice after showing extraordinary DFS and OS rates at 7-years [66]. In this scenario, at least 3 critical questions remain to be answered regarding de-escalation of systemic therapy in early HER2+ disease: (1) who can be treated with less or even no adjuvant trastuzumab after surgery? (2) who does not need (neo)adjuvant pertuzumab in stage II and III disease? (3) can we decrease the amount of chemotherapy? In fact, immunohistochemically HER2+/non-HER2-E tumors might be poorly dependent, if not totally independent, from the HER2-signaling pathway and not gain any benefit from adjuvant trastuzumab following previous neoadjuvant therapy and surgery. At the same time some HER2+ tumors might be “HER2 addicted” enough not to need chemotherapy at all or to require a shortened adjuvant trastuzumab duration and/or no adjuvant dual blockade therapy. To address these questions, well-designed clinical trials integrating clinical variables (such as tumor dimension and axillary nodes involvement), response

data and biomarkers such as the HER2-E subtype, TILs, intra-tumor heterogeneity [67] and PIK3CA status are needed.

This meta-analysis has several limitations. First, some secondary end-points were affected by discrete levels of heterogeneity ($I^2 \geq 75\%$ and $p_{heterogeneity} < 0.05$, results in **Figure 4B** and **Supplementary Materials**). This was mostly attributable to the paucity of molecular data from some trials and differences in the effects observed, preventing them from being fully reliable, regardless of the analytical model applied. However, this consideration doesn't apply to the main result of the study. Second, although several studies correlated pCR with long-term survival outcomes (EFS/DFS and OS) in the context of HER2+ BC [56–60], others failed to demonstrate its role as an efficient surrogate endpoint for survival [68,69]. Additionally, there is a specific lack of survival data related to intrinsic subtypes within the clinical trials included in this study. Therefore, no claims regarding the association of the HER2-E subtype with the patients' survival outcome can be made based on this meta-analysis. Moreover, 4/16 trials reported data regarding in-breast pCR, which has not been recognized by the FDA as a validated endpoint for drug approval in neoadjuvant setting [61]. Third, the methods used to apply the PAM50 algorithm varied across trials. For example, 13 studies used the nCounter platform [14,17,20,22–29,31], 2 studies used RNA-seq data [15,18] and 2 studies used microarray-based data [16,21]. Finally, we were only able to perform a study-level meta-analysis instead of a patient-level meta-analysis, which would have increased precision and homogeneity and enabled thorough exploration of potential effect moderators.

To conclude, our results demonstrate that the HER2-E subtype is a consistent biomarker of response following neoadjuvant anti-HER2-based regimens, with and without CT and beyond HR status. This biomarker, along with TILs and other biomarkers, such as *PIK3CA* mutations [39–42], either alone or in combination [43], should be routinely incorporated in future prospective clinical trials designed to implement strategies to escalate and/or de-escalate systemic therapies [11,14–31].

Declaration of Competing Interest

FS has declared travel and accommodation expenses paid by Roche, Pfizer and Celgene. SDP has declared honoraria from Roche, Pfizer, Astra-Zeneca, Novartis, Celgene, Eli Lilly, Amgen and Eisai. AP has declared an immediate family member being employed by Novartis, personal honoraria from Pfizer, Novartis, Roche, MSD Oncology, Lilly and Daiichi Sankyo, travel, accommodations and expenses paid by Daiichi Sankyo, research funding from Roche and Novartis, consulting/ advisory role for NanoString Technologies, Amgen, Roche, Novartis, Pfizer and Bristol-Myers Squibb and patent PCT/EP2016/080056: HER2 AS A PREDICTOR OF RESPONSE TO DUAL HER2 BLOCKADE IN THE ABSENCE OF CYTOTOXIC THERAPY. OTHER AUTHORS CoI. PFC had declared consultant role for Novartis, Eli Lilly, Astra Zeneca and Tesaro**,** honoraria from BMS, Roche, Eli Lilly, Novartis and AstraZeneca, research funding from Novartis, Roche, BMS, Merck-KGa, Italian Ministry of Health, Veneto Secretary of Health and University of Padova. CMP is an equity stock holder and consultant of BioClassifier LLC and is also listed an inventor on patent applications

on the Breast PAM50. LAC has declared that Companies who have provided funds to her institution in the past 1–2 years either for her service on advisory/ consultative programs or sponsored research were Genentech, Roche, Novartis, Seattle Genetics, G1 Therapeutics, Immunomedics and Innocrin. SP has received honoraria for talks and travel grants from Roche outside of the submitted work and serves as an advisor/consultant to Polyphor. RS has declared research funding from AstraZeneca, GlaxoSmithKline, Gilead Sciences, and PUMA Biotechnology, and consulting/advisory role with compensation for Macrogenics, and Eli Lilly. CKO has declared research funding from AstraZeneca and GlaxoSmithKline, advisory boards for Tolmar Pharmaceuticals, Genentech, and AstraZeneca, DMC for Eli Lilly and stockholder of GeneTex. MFR has declared research funding from GlaxoSmithKline and Genentech. JCB reports employment and stocks with Novartis. The other authors have nothing to declare.

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Authors contributions

FS, AP, LAC and CMP conceived the study. FS, TP, NC and CR performed the systematic review of the literature and AP was consulted for a final decision in case of controversy. FS performed the statistical analyses. FS, TP and AP wrote the first draft of the article. All authors contributed in interpreting the data, writing and correcting the manuscript drafts and approved the final version.

References

1. Cronin KA, Harlan LC, Dodd KW, Abrams JS, Ballard-Barbash R. Population-based estimate of the prevalence of HER-2 positive breast cancer tumors for early stage patients in the US. Cancer Invest 2010;28(9):963–8. https://doi.org/10.3109/ 07357907.2010.496759.
2. Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal growth factor receptor 2 testing in breast cancer: american society of clinical oncology/college of american pathologists clinical practice guideline focused update. J Clin Oncol Off J Am Soc Clin Oncol. 2018;36(20):2105–22. https://doi.org/10.1200/JCO.2018.77. 8738.
3. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344(11):783–92. https://doi.org/10.1056/ NEJM200103153441101.

4. Swain SM, Kim S-B, Cortés J, et al. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA study): overall survival results from a randomised, double-blind, placebo-controlled, phase 3 study. Lancet Oncol 2013;14(6):461–71. https://doi.org/10.1016/S1470-2045(13)70130-X.
5. Martin M, Holmes FA, Ejlertsen B, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2017;18(12):1688–700. https://doi.org/10.1016/S1470-2045(17)30717-9.
6. Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006;355(26):2733–43. https://doi.org/ 10.1056/NEJMoa064320.
7. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012;367(19):1783–91. https://doi.org/10. 1056/NEJMoa1209124.
8. Mounsey LA, Deal AM, Keith KC, et al. Changing natural history of HER2-positive breast cancer metastatic to the brain in the era of new targeted therapies. Clin Breast Cancer 2018;18(1):29–37. https://doi.org/10.1016/j.clbc.2017.07.017.
9. Prat A, Pineda E, Adamo B, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast Edinb Scotl 2015;24(Suppl 2):S26–35. https://doi. org/10.1016/j.breast.2015.07.008.
10. Prat A, Carey LA, Adamo B, et al. Molecular features and survival outcomes of the intrinsic subtypes within HER2-positive breast cancer. J Natl Cancer Inst 2014;106(8). https://doi.org/10.1093/jnci/dju152.
11. Prat A, Pascual T, De Angelis C, et al. HER2-enriched subtype and ERBB2 expression in HER2-positive breast cancer treated with dual HER2 blockade. J Natl Cancer Inst 2019;30. https://doi.org/10.1093/jnci/djz042.
12. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumors. Nature 2012;490(7418):61–70. https://doi.org/10.1038/nature11412.
13. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011;5(1):5–23. https://doi.org/10.1016/j.molonc.2010.11.003.
14. Llombart-Cussac A, Cortés J, Paré L, et al. HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer (PAMELA): an open-label, single-group, multicentre, phase 2 trial. Lancet Oncol 2017;18(4):545–54. https:// doi.org/10.1016/S1470-2045(17)30021-9.
15. Carey LA, Berry DA, Cirrincione CT, et al. Molecular heterogeneity and response to 10 neoadjuvant human epidermal growth factor receptor 2 targeting in CALGB 40601, a randomized phase III Trial of paclitaxel plus trastuzumab with or without lapatinib. J Clin Oncol 2016;34(6):542–9. https://doi.org/10. 1200/JCO.2015.62.1268.
16. Prat A, Bianchini G, Thomas M, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. Clin Cancer Res Off J Am Assoc Cancer Res 2014;20(2):511–21. https://doi.org/10.1158/1078-0432.CCR-13-0239.
17. Swain SM, Tang G, Lucas PC, et al. Intrinsic subtypes of HER2-positive breast cancer and their associations with pathologic complete response (pCR) and outcomes: Findings from NSABP B-41, a randomized neoadjuvant trial. J Clin Oncol. 2018;36(15_suppl):580-580. doi:10.1200/JCO.2018.36.15_suppl.580.
18. Fumagalli D, Venet D, Ignatiadis M, et al. RNA Sequencing to predict response to neoadjuvant anti-HER2 therapy: a secondary analysis of the NeoALTTO randomized clinical trial. JAMA Oncol 2017;3(2):227–34. https://doi.org/10.1001/jamaoncol. 2016.3824.

19. Bianchini G, Parker JS, Carey LA, et al. Research-based PAM50 predicts risk of relapse in residual disease after anti-HER2 therapies. Ann Oncol. 2018; 29(8_sup- pl):viii58–viii86.
20. Bianchini G, Pusztai L, Pienkowski T, et al. Immune modulation of pathologic complete response after neoadjuvant HER2-directed therapies in the NeoSphere trial. Ann Oncol 2015;26(12):2429–36. https://doi.org/ 10.1093/annonc/mdv395.
21. Dieci MV, Prat A, Tagliafico E, et al. Integrated evaluation of PAM50 subtypes and immune modulation of pCR in HER2-positive breast cancer patients treated with chemotherapy and HER2-targeted agents in the CherLOB trial. Ann Oncol 2016;27(10):1867–73. https://doi.org/10.1093/annonc/mdw262.
22. Ignatiadis M, Van den Eynden G, Roberto S, et al. Tumor-infiltratinglymphocytesin patients receiving trastuzumab/pertuzumab-based chemotherapy: a TRYPHAENA substudy. J Natl Cancer Inst 2019;111(1):69–77. https://doi.org/10.1093/jnci/ djy076.
23. Holmes FA, Espina V, Liotta LA, et al. Pathologic complete response after preoperative anti-HER2 therapy correlates with alterations in PTEN, FOXO, phosphorylated Stat5, and autophagy protein signaling. BMC Res Notes 2013;6:507. https://doi.org/10.1186/1756-0500-6-507.
24. Swain SM, Ewer MS, Viale G, et al. Pertuzumab, trastuzumab, and standard anthracycline- and taxane-based chemotherapy for the neoadjuvant treatment of patients with HER2-positive localized breast cancer (BERENICE): a phase II, open-label, multicenter, multinational cardiac safety study. Ann Oncol Off J Eur Soc Med Oncol 2018;29(3):646–53. https://doi.org/10.1093/annonc/mdx773.
25. Gavilá J, Oliveira M, Pascual T, et al. Safety, activity, and molecular heterogeneity following neoadjuvant non-pegylated liposomal doxorubicin, paclitaxel, trastuzumab, and pertuzumab in HER2-positive breast cancer (Opti-HER HEART): an open-label, single-group, multicenter, phase 2 trial. BMC Med 2019;17(1):8. https://doi.org/10.1186/s12916-018-1233-1.
26. Pernas S, Petit A, Climent F, et al. Abstract P2-09-11: PAM50 intrinsic subtyping as a predictor of pathological complete response to neoadjuvant trastuzumab-based chemotherapy in early HER2-positive breast cancer. Cancer Res. 2018;78(4 Supplement):P2-09-11. doi:10.1158/1538-7445.SABCS17-P2-09-11.
27. Prat A, Slamon D, Hurvitz S, et al. AbstractPD3-06:Associationofintrinsicsubtypes with pathological complete response (pCR) in the KRISTINE neoadjuvant phase 3 clinical trial in HER2-positive early breast cancer (EBC). Cancer Res. 2018; 78 (4 Supplement):PD3-06. doi:10.1158/1538-7445.SABCS17-PD3-06.
28. Guarneri V, Dieci MV, Bisagni G, et al. De-escalated therapy for HR+/HER2+ breast cancer patients with Ki67 response after 2 weeks letrozole: results of the PerELISA neoadjuvant study. Ann Oncol 2019;30(6):921–6. https://doi.org/10. 1093/annonc/mdz055.
29. Rimawi MF, Mayer IA, Forero A, et al. Multicenter phase II study of neoadjuvant lapatinib and trastuzumab with hormonal therapy and without chemotherapy in patients with human epidermal growth factor receptor 2-overexpressing breast cancer: TBCRC 006. J Clin Oncol 2013;31(14):1726–31. https://doi.org/10.1200/JCO.2012.44.8027.
30. Prat A, De Angelis C, Pascual T, et al. Abstract P2-09-12: Independent validation of the HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer. Cancer Res. 2018; 78(4 Supplement):P2-09-12. doi:10.1158/ 1538-7445.SABCS17-P2-09-12.
31. PratA, De Angelis C, Pascual T, et al. HER2-enriched subtype and ERBB2 mRNA as predictors of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast

cancer: A combined analysis of TBCRC006/023 and PAMELA trials. J Clin Oncol. 2018; 36(15_ suppl):509–509. doi:10.1200/JCO.2018.36.15_suppl.509.

32. Cejalvo JM, Pascual T, Fernández-Martínez A, et al. Clinical implications of the non-luminal intrinsic subtypes in hormone receptor-positive breast cancer. Cancer Treat Rev 2018;67:63–70. https://doi.org/10.1016/j.ctrv.2018.04.015.
33. Curigliano G, Burstein HJ, Winer EP, et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen international expert consensus conference on the primary therapy of early breast cancer 2017. Ann Oncol 2017;28(8):1700–12. https://doi. org/10.1093/annonc/mdx308.
34. Salgado R, Denkert C, Campbell C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO trial. JAMA Oncol 2015;1(4):448–54. https://doi. org/10.1001/jamaoncol.2015.0830.
35. Denkert C, von Minckwitz G, Brase JC, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol Off J Am Soc Clin Oncol 2015;33(9):983–91. https://doi.org/ 10.1200/JCO.2014.58.1967.
36. Conte PF, Griguolo G, Dieci MV, et al. PAM50 HER2-enriched subtype as an independent prognostic factor in early-stage HER2+ breast cancer following adjuvant chemotherapy plus trastuzumab in the ShortHER trial. J Clin Oncol 2019;37(suppl):544.
37. Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol Off J Eur Soc Med Oncol 2014;25(8):1544–50. https://doi.org/10.1093/annonc/mdu112.
38. Griguolo G, Pascual T, Dieci MV, Guarneri V, Prat A. Interaction of host immunity with HER2-targeted treatment and tumor heterogeneity in HER2-positive breast cancer. J ImmunoTher Cancer 2019;7. https://doi.org/10.1186/s40425-019-0548-6.
39. Loibl S, Majewski I, Guarneri V, et al. PIK3CA mutations are associated with reduced pathological complete response rates in primary HER2-positive breast cancer: pooled analysis of 967 patients from five prospective trials investigating lapatinib and trastuzumab. Ann Oncol Off J Eur Soc Med Oncol 2016;27(8):1519–25. https://doi.org/10.1093/annonc/mdw197.
40. Baselga J, Cortés J, Im S-A, et al. Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 2014;32(33):3753–61. https://doi.org/10.1200/JCO.2013.54.5384.
41. Kim S-B, Wildiers H, Krop IE, et al. Relationship between tumor biomarkers and efficacy in TH3RESA, a phase III study of trastuzumab emtansine (T-DM1) vs. treatment of physician's choice in previously treated HER2-positive advanced breast cancer. Int J Cancer 2016;139(10):2336–42. https://doi.org/10.1002/ijc. 30276.
42. Rimawi MF, De Angelis C, Contreras A, et al. Low PTEN levels and PIK3CA mutations predict resistance to neoadjuvant lapatinib and trastuzumab without che- motherapy in patients with HER2 over-expressing breast cancer. Breast Cancer Res Treat 2018;167(3):731–40. https://doi.org/10.1007/s10549-017-4533-9.
43. Veeraraghavan J, De Angelis C, Mao R, et al. A combinatorial biomarker predicts pathologic complete response to neoadjuvant lapatinib and trastuzumab without chemotherapy in patients with HER2+ breast cancer. Ann Oncol Off J Eur Soc Med Oncol 2019. https://doi.org/10.1093/annonc/mdz076.

44. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [Updated March 2011]. The Cochrane Collaboration http:// handbook.cochrane.org.
45. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7(3):177–88.
46. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21(11):1539–58. https://doi.org/10.1002/sim.1186.
47. Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. J Clin Epidemiol 2001;54(10):1046–55.
48. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315(7109):629–34. https://doi.org/10.1136/ bmj.315.7109.629.
49. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2017. https://www.R-project.org/.
50. The Cochrane Collaboration. Review Manager (RevMan). Copenhagen: The Nordic Cochrane Centre; 2014.
51. PROSPERO International prospective register of systematic reviews. https://www. crd.york.ac.uk/prospero/.
52. Pernas S, Petit A, Climent F, et al. PAM50 subtypes in baseline and residual tumors following neoadjuvant trastuzumab-based chemotherapy in HER2-positive breast cancer: a consecutive series from a single institution. Front Oncol 2019;9. https://doi.org/10.3389/fonc.2019.00707.
53. Gianni L, Pienkowski T, Im Y-H, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13(1):25–32. https://doi.org/10.1016/S1470-2045(11)70336-9.
54. Peto R. Why do we need systematic overviews of randomized trials? Stat Med 1987;6(3):233–44.
55. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235(4785):177–82. https://doi.org/10.1126/science. 3798106.
56. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014;384(9938):164–72. https://doi.org/10.1016/S0140-6736(13)62422-8.
57. Von Minckwitz G, Untch M, Blohmer J-U, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30(15):1796–804.
58. Nekljudova V, Loibl S, von Minckwitz G, et al. Trial-level prediction of long-term outcome based on pathologic complete response (pCR) after neoadjuvant chemotherapy for early-stage breast cancer (EBC). Contemp Clin Trials 2018;71:194–8.
59. Broglio KR, Quintana M, Foster M, et al. Association of pathologic complete response to neoadjuvant therapy in HER2-positive breast cancer with long-term outcomes: a meta-analysis. JAMA Oncol 2016;2(6):751–76.
60. Spring LM, Fell G, Arfe A, et al. Pathological complete response after neoadjuvant chemotherapy and impact on breast cancer recurrence and mortality, stratified by breast cancer subtypes and adjuvant chemotherapy usage: Individual patient-level meta-analyses of over 27,000 patients [abstract]. In: Proceedings of the 2018 San Antonio Breast Cancer Symposium; 2018 Dec 4-8; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2019;79(4 Suppl):Abstract nr GS2-03.
61. FDA Guidance for Industry. Pathological Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval. Available at: https://www.fda.gov/media/83507/download. Last accessed: 02/12/2020.

62. Rimawi MF, Niravath P, Wang T, et al. TBCRC023: A Randomized Phase II Neoadjuvant Trial of Lapatinib Plus Trastuzumab Without Chemotherapy for 12 vs. 24 Weeks in Patients with HER2-positive Breast Cancer. Clin Cancer Res. 2019; doi: 10.1158/1078-0432.CCR-19-0851 [Epub ahead of print].
63. Dieci MV, Vernaci G, Guarneri V. Escalation and de-escalation in HER2 positive early breast cancer. Curr Opin Oncol 2019;31(1):35–42. https://doi.org/10.1097/ CCO.0000000000000492.
64. Veeraraghavan J, De Angelis C, Reis-Filho JS, et al. De-escalation of treatment in HER2-positive breast cancer: determinants of response and mechanisms of resistance. Breast Edinb Scotl 2017;34(Suppl 1):S19–26. https://doi.org/10.1016/j. breast.2017.06.022.
65. Pondé N, Gelber RD, Piccart M. PERSEPHONE. NPJ Breast Cancer 2019;5:1. https://doi.org/10.1038/s41523-018-0098-y.
66. Tolaney SM, Guo H, Pernas S, et al. Seven-year follow-up analysis of adjuvant paclitaxel and trastuzumab trial for node-negative, human epidermal growth factor receptor 2-positive breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 2019;37(22):1868–75. https://doi.org/10.1200/JCO.19.00066.
67. Metzger Filho O, Viale G, Trippa L, et al. HER2 heterogeneity as a predictor of response to neoadjuvant T-DM1 plus pertuzumab: Results from a prospective clinical trial. J Clin Oncol. 2019;37(15_suppl):502-502. doi:10.1200/JCO.2019.37.15_ suppl.502.
68. Berruti A, Amoroso V, Gallo F, et al. Pathologic complete response as a potential surrogate for the clinical outcome in patients with breast cancer after neoadjuvant therapy: a meta-regression of 29 randomized prospective studies. J Clin Oncol 2014;32(34):3883–91. https://doi.org/10.1200/JCO.2014.55.2836.
69. Korn EL, Sachs MC, McShane LM. Statistical controversies in clinical research: assessing pathologic complete response as a trial-level surrogate end point for early-stage breast cancer. Ann Oncol 2016;27(1):10–5. https://doi.org/10.1093/annonc/ mdv507.

CHAPTER 2: Immune system and angiogenesis-related potential surrogate biomarkers of response to everolimus-based treatment in hormone receptor-positive breast cancer: an exploratory study

Original article reference

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Abstract

Purpose: mTOR-inhibitor everolimus is used for hormone receptor-positive (HR+)/HER2-negative metastatic breast cancer (mBC). No reliable predictive biomarker of response is available. Following evidences from other solid tumors, we aimed to assess the association between treatment-associated immune system features and everolimus activity.

Methods: We retrospectively explored a correlation with the therapeutic activity of everolimus and tumor-associated immune pathways with ingenuity pathway analysis (IPA), neutrophil-to-lymphocyte ratio (NLR), circulating lymphocytes and endothelial cells (CECs) in 3 different HR+ mBC studies, including the BALLET phase IIIb study.

Results: The circulating levels of $CD3^+/CD8^+$, $CD3^+/CD4^+$ and overall T-lymphocytes were higher in responders versus non-responders at baseline (P=0.017, P<0.001, P=0.034) and after treatment (P=0.01, P=0.003, P=0.023). Reduced CECs, a tumor neoangiogenesis marker, were observed in responders after treatment (P<0.001). Patients with low NLR (≤4.4) showed a better progression-free survival compared to patients with high NLR (>4.4) (P=0.01). IPA showed that the majority of immunity-related genes were found up-regulated in responders compared to non-responders before treatment, but not after.

Conclusions: Lymphocytes subpopulations, CECs and NLR could be interesting biomarkers predictive of response to everolimus-based regimens, potentially useful in daily clinical practice to select/monitor everolimus-based treatment in mBC. Further studies to confirm such hypotheses are warranted.

Introduction

Despite the demonstrated efficacy of anti-hormonal treatment in patients with hormone receptor positive (HR+) breast cancer (BC), intrinsic and acquired endocrine resistance occurs in a significant proportion of patients, leaving this tumor being still one of the most common causes of cancer-related death in women [1,2]. One mechanism of resistance relies on mTOR, a downstream effector of the phosphatidylinositol-3-kinase (PI3K) pathway, which is implicated in cell growth and survival, angiogenesis and immune regulation [3]. The PI3K/Akt/mTOR pathway frequently contributes to breast cancer progression playing a central role in multiple cellular functions and is a key mechanism of resistance to endocrine therapy [2,3]. The mTOR inhibitor everolimus is approved for HR+/HER2-negative (-) locally advanced or metastatic BC (mBC) treatment in combination with the aromatase inhibitor (AI) exemestane [4]. However, benefit from everolimus is variable and reliable biomarkers for the selection of patients who will most likely respond are urgently needed [5].

There has been accumulating evidence suggesting that the efficacy of conventional anticancer therapies might rely, at least in part, on eliciting an anti-tumor immune response [6,7]. In fact, several conventional chemotherapeutics, as well as targeted anticancer drugs, seem to modify the composition and activity of the tumor infiltrate, affecting treatment efficacy and ultimately outcome [6,7]. Moreover, the local or systemic immune system in patients with cancer appear to be of prognostic value and might be used to predict the therapeutic response to specific treatments [8]. Furthermore, recent evidence concerning the efficacy of immune-checkpoint inhibitors in PD-L1 positive triple negative (TN) BC has recently reignited the interests in BC immunotherapy and highlighted the potentially relevant role of immune modulation in BC treatment [9–11].

Everolimus acts by blocking cell-growth and metabolism; it is a powerful immune-suppressor used to avoid organ rejection in renal transplanted patients [12,13] by controlling homeostasis and the balance between effector T cells and regulatory T cells (Tregs) [14]. There is also emerging evidence highlighting the immunomodulatory role of everolimus in solid tumors such as renal cell [8,15,16] and hepatocellular carcinoma [17]. To the best of our knowledge, no data are available about the role of mTOR axis inhibitors on the immune system in BC treatment.

Based on preliminary evidence regarding everolimus immunomodulatory role in several solid tumors [8,15–17], we have investigated immune infiltrate and circulating immune cells in BC using several cohorts of patients treated with everolimus. Firstly, we obtained tumor biopsies and circulating lymphocytes populations in blood samples from patients with mBC to explore for potential differences among everolimus responders vs. non-responders. Secondly, we investigated a potential correlation between neutrophils-to-lymphocytes ratio (NLR) and progression-free survival (PFS) in the BALLET trial [18] and, thirdly, we performed differential gene immune expression analyses

between everolimus-responders and non-responders on tissue samples from a window of opportunity trial in locally advanced breast tumors. Finally, in blood samples from mBC patients we also investigated the potential presence of different levels of circulating endothelial cells (CECs) between everolimus responders and non-responders. The amount of circulating CECs correlates with angiogenesis in cancer and seem to correlate with plasma levels of angiogenic mediators VCAM-1 and VEGF [19,20], many of whose downstream pathways are also inhibited by everolimus, thus being a potential biomarker of its activity.

Overall, the aim of our study was to preliminarily find out potential easy-to-detect biomarkers of response related to immune-system and neoangiogenesis, to better selecting patients that may benefit from everolimus-based therapy.

Materials and methods

Case selection and studies descriptions

In our analysis, we retrospectively included postmenopausal patients affected by locally advanced or metastatic HR+ BC treated with everolimus-based regimens in 3 previous different clinical studies. Patients came from three separate cohorts pertaining to the MREC trial, the mTOR-Study and the BALLET trial.

The first one was a window-of-opportunity trial based on the administration of 5 mg everolimus in neoadjuvant locally advanced setting for 14 days prior to surgery. The study enrolled 32 women diagnosed with operable HR+ BC. Study details and population demographics have been previously reported [21].

The mTOR-Study was a prospective trial enrolling a total of 15 consecutive post-menopausal women diagnosed with relapsed HR+/HER2- mBC, treated in the first-line setting at the ASST-Cremona (Italy) with 10 mg of everolimus alone daily for 21 days, followed by the combination with exemestane (25 mg) until progression. Patients had relapsed after primary tumor surgery and adjuvant endocrine therapy with a non-steroidal aromatase inhibitor administered for 5 years. Pathologists from the ASST-Cremona performed all the histopathological diagnoses. Tissue samples were collected from the most accessible metastatic site in order to perform immunohistochemical (IHC) analysis before everolimus single agent administration and after 21 days, before the addition of exemestane; clinical data were retrieved from patients' charts in the Breast Unit of the ASST-Cremona. Blood samples were also obtained from patients enrolled before and after everolimus administration, for flow cytometry analysis. Responsiveness to everolimus was measured by ^{18}FDG-PET/CT after 21 days of everolimus-based treatment, at the 3rd month and every 3 months until progression. Patients were considered responsive to everolimus when a reduction of SUV_{max} was

present at first 21 days and maintained for the first 9 months at least; whereas with a detection of increase or stability in SUV_{max} during the 9^{th} months of treatment, the patients were classified as non-responsive.

The BALLET study was an expanded access European, phase IIIb, open-label, single-arm, multicenter clinical trial (EudraCT Number: 2012-000073-23), which has been previously described [18].

Immunohistochemistry

Tissue from tumor specimens was obtained through biopsy of the metastasis of 15 patients with mBC within the mTOR-Study, embedded in paraffin and fixed in formalin (FFPE) for IHC analysis. Regions with non-invasive carcinoma, normal tissue or necrosis were excluded from the evaluation. Standard IHC was performed on FFPE for HER2, estrogen receptor (ER), progesterone receptor (PgR), Ki67 and CD31 staining using standard protocols as described elsewhere [22–25]. Considering a demonstrated performance of circulating endothelial cells (CECs) and CD31 expression as a biomarker mirroring the occurrence of angiogenesis in the tumor [19], and given that PI3K/mTOR pathway is involved in angiogenesis, we also evaluated patients' CECs and CD31 modulation before/after treatment as a measure of everolimus' on-target activity.

Flow cytometry analysis

The study of circulating immune cells and CECs was performed on samples coming from the mTOR-Study. The whole blood samples before and after treatment allowed to analyse circulating cells and their changes under therapy. Flow cytometry analysis was performed with dual or triple-laser flow cytometers Becton Dickinson (BD) FACSCanto™ and BD FACSCanto II™, with BD™ Cytometer Setup and Tracking (CS&T) control, in order to make the signals reproducible and comparable regardless of the variation in environmental conditions. Acquisition of at least 1.5 x 10^6 events was assessed by BDFACSC Diva software. The lymphocytes subpopulations (B, NK, T with CD4 and CD8 subpopulation) were assessed with BD Multitest 6-Color TBNK kit (Becton Dickinson™). The kit contains FITC-labelled CD3 (SK7clone), PE-labelled CD16 (B73.1 clone) and CD56 (NCAM 16.2 clone), CD45 (2D1 clone) conjugated with the fluorochromes PerCP-Cy5.5, CD4 (SK3 clone) conjugated with PE-Cy7, CD19 (SJ2SC1 clone) conjugated with APC and CD8 (SK1 clone) conjugated with APC-Cy7. The BD FACSCanto clinical software was employed to carry out the analysis. Leucocytes were identified by CD45 expression and SSC/FCS morphological parameters. T lymphocytes were sorted by CD3 expression and then split into CD4 and CD8 populations. CD3 negative cells were split into B lymphocyte (expressing CD19) and NK cells (CD16 and CD56

positive). Subpopulations absolute count was done by the "trucount tube" (BD™) containing a known number of beads. The T-reg cells (CD4 positive, bright CD25 positive and CD127 negative) were sorted using single Becton Dickinson monoclonal antibodies: CD3 (SK7 clone) conjugated with the fluorochromes FITC, CD25 (2A3 clone) conjugated with PE, CD4 (SK3 clone) conjugated with PerCP-Cy5.5 and CD127 (HIL-7R-M21 clone) conjugated with V450, CD45 (HI30 clone) conjugated with V500.

The CECs are uncommon findings in the peripheral blood. They can be identified by CD45 negativity with CD31 and CD146 positivity. CECs sorting was assessed using a three colours panel: CD31 (WM59 clone) conjugated with the fluorochromes FITC, CD146 (P1H12 clone) conjugated with PE, CD45 (2D1 clone) conjugated PerCP-Cy 5.5.

Gene expression and statistical analyses

The gene expression data used in this study derived from the population of the MREC Study [21, 26]. Microarray data were processed starting from the authors' raw data. Class comparison analysis was performed using the Bioconductor package [27]. The probes from Illumina profile expression data were normalized using quantile normalization within the beadarray package and batch processing effects were corrected using the combat tool [28,29]. Pairwise Significance Analysis of Microarrays (SAM) implemented with siggenes package was used to identify the differentially expressed genes and to predict false discovery rate (FDR) [30]. To define significantly differentially expressed genes an FDR<5% was applied as cut-off. The data on the reduction in the percentage of Ki67 positive cells after treatment was used to separate responders from non-responders. Analyses were performed using R, version 3.4.2 and BioConductor, release 3.6 [27,31]. We used the list of differentially expressed genes to analyze our patients' cohorts for enrichment in canonical signalling pathways, in order to evaluate potential enrichment in immune pathways through ingenuity pathway analysis (IPA) [32]. The web-based pathway analysis tool QIAGEN IPA (QIAGEN Digital Insights, https://digitalinsights.qiagen.com) was used. Patients were separated into 2 groups according to response to everolimus neoadjuvant treatment as illustrated in a previously published work [21] and IPA on differentially expressed genes between these 2 groups was performed at two different time points (i.e. before and after therapy completion).

Circulating immune cells and CECs median levels in blood were calculated with standard non-parametric statistical methods (Mann–Whitney test for unpaired data, Wilcoxon's matched-pairs signed-rank test for paired data, Spearman Rho for simple correlation analysis). Statistical analyses were performed using the Statistica software (Statsoft, Tulsa, OK, USA) for Windows (Microsoft, Redmond, WA, USA) software.

A post-hoc analysis was conducted from the neutrophils and lymphocytes values derived from the BALLET study in order to investigate a correlation with survivals of patients. Information about the neutrophil and lymphocyte status was collected at basal and at the time of progression from the combination of everolimus/exemestane, when available. NLRs were calculated based on four cut-off values and patients discriminated based on four quartiles accordingly to Santoni et al. [15]. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. Pre-treatment percentage of neutrophils and NLRs were considered. The Kaplan-Meier method was used to assess PFS differences according to NLRs, and the Long-rank test was used to evaluate the significance of each comparison. PFS was defined as the time from the first day of study treatment until disease progression or death, whichever occurred first.

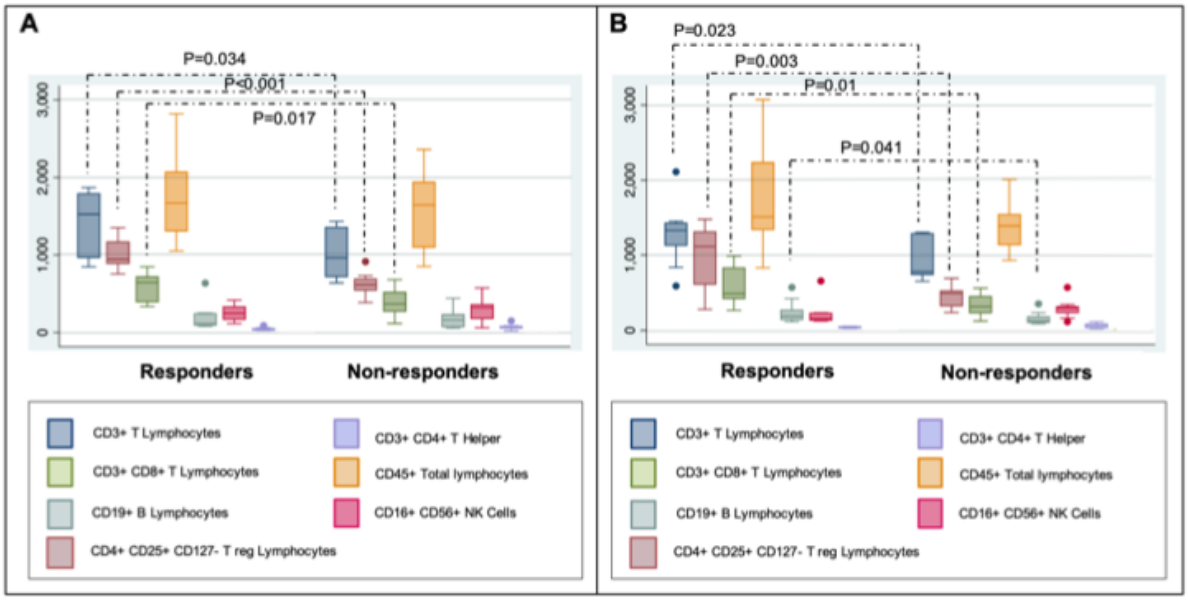
The analyses were conducted on SPSS (15.0 version; SPSS Inc., Chicago, IL, USA). All analyses were two-sided and statistical significance was established at the $P<0.05$ level. REMARK criteria were followed to report data [33].

Results

Circulating immune-related cells and CECs in patients according to response to everolimus in the metastatic setting

Based on the association between expression of immune–related genes in tumors responsive to short term everolimus in neoadjuvant setting, we investigated whether the number of circulating immune cells could predict response to 10 mg everolimus administered alone in a cohort of 15 patients with mBC **(Figure 1 A-B)**. While no difference in the number of $CD45^+$ total lymphocytes at baseline or after treatment was found between responders and non-responders, the levels of $CD3^+$ T-lymphocyte were higher in responders versus non-responders at both baseline ($P=0.034$) and after treatment ($P=0.023$). Likewise, the levels of T-lymphocytes $CD3^+/CD8^+$ and $CD3^+/CD4^+$ were higher in responders compared to non-responders at baseline ($P=0.017$, $P<0.001$, respectively) and after treatment ($P=0.01$, $P=0.003$ respectively). In contrast, there was no statistically significant difference in the number of $CD19^+$ B-lymphocytes between responders and non-responders at both baseline and final stages of treatment. There was a trend of a reduced number of T-regulatory lymphocytes $CD4^+/CD25^+/CD127^-$ in responders compared with non-responders at baseline ($P=0.075$) and post-treatment ($P=0.059$), although not statistically significant. $CD16^+/CD56^+$ NK cells showed no difference in number at baseline, but responsive tumors post-treatment showed slightly lower circulating NK cells compared with non-responders ($P=0.041$).

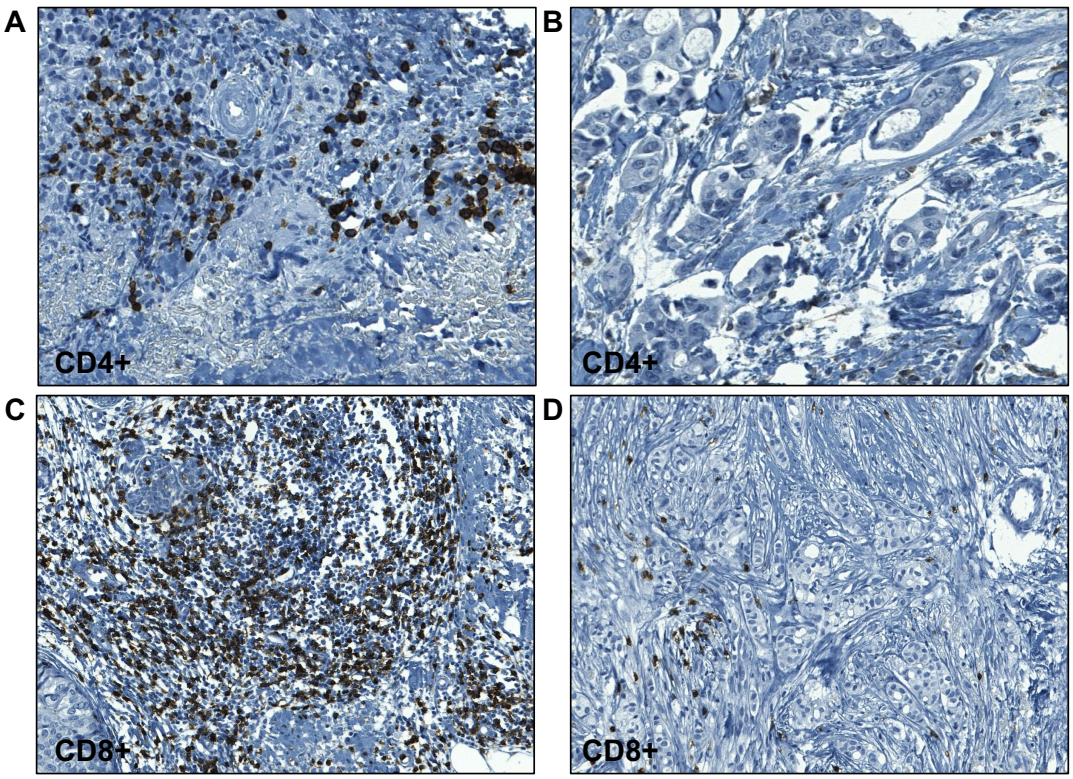
Figure 1. Quantification of lymphocytes populations in the blood of responders and non-responders at basal (A) or after (B) everolimus therapy



Legend. Only significant p-values from unpaired t-test are reported

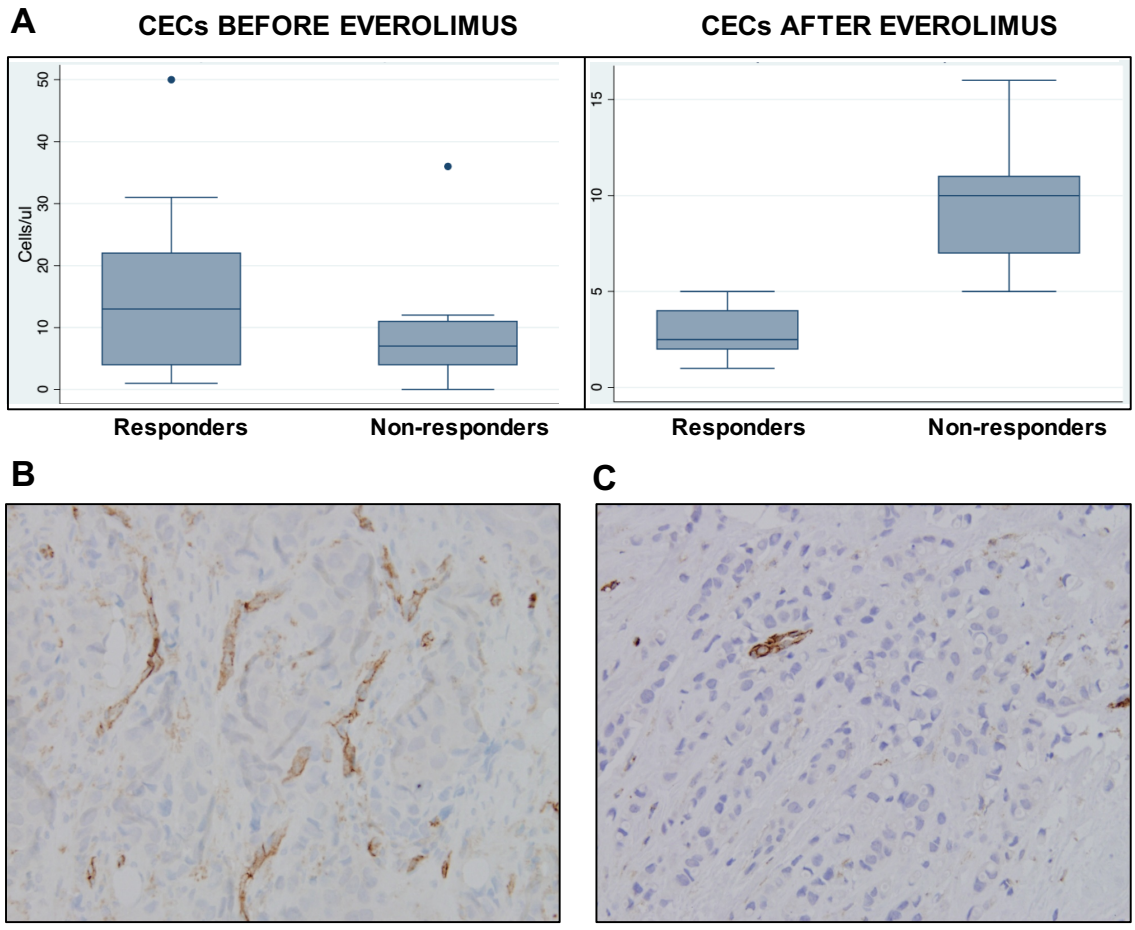
Interestingly, the higher number of circulating CD4+ and CD8+ T cells was associated with higher pre-treatment infiltration of these cells in the tumor microenvironment of responsive patients (**Figure 2A-C**) compared to non-responders (**Figure 2B-D**), as evaluated by IHC in both primary and metastatic lesions.

Figure 2. Representative images of $CD3^+/CD4^+$ T cells (A-B) and $CD3^+/CD8^+$ T cells (C-D) infiltrating tumor tissues of responsive (A-C) and non-responsive (B-D) patients



CECs were found in all 15 patients. No significant differences were observed between responders and non-responders before treatment (**Figure 3A**). However, after everolimus treatment there was a significant reduction in CECs number only in responders, resulting in a highly significant different numbers between responders and non-responders (P<0.001), demonstrating the biological activity of everolimus. Notably, responders showed a higher tumor vascularisation at baseline using CD31+ vascular density (**Figure 3B**), compared with non-responders (**Figure 3C**).

Figure 3. Quantification of CEC in blood of responsive and non-responsive patients before and after treatment with everolimus (A) and representative images of CD31+ vessels in tumor tissues of responsive (B) and non-responsive (C) patients



Prognostic significance of the NLR in the BALLET Study

Blood cells counts were obtained from 114 patients. The following NLR-based quartiles were generated: quartile 1 (NLR ≤ 2.3), quartile 2 (2.3 < NLR ≤ 3.2), quartile 3 (3.2 < NLR ≤ 4.4), quartile 4 (NLR > 4.4). As shown in **Table 1**, the median lymphocyte and neutrophil counts differed

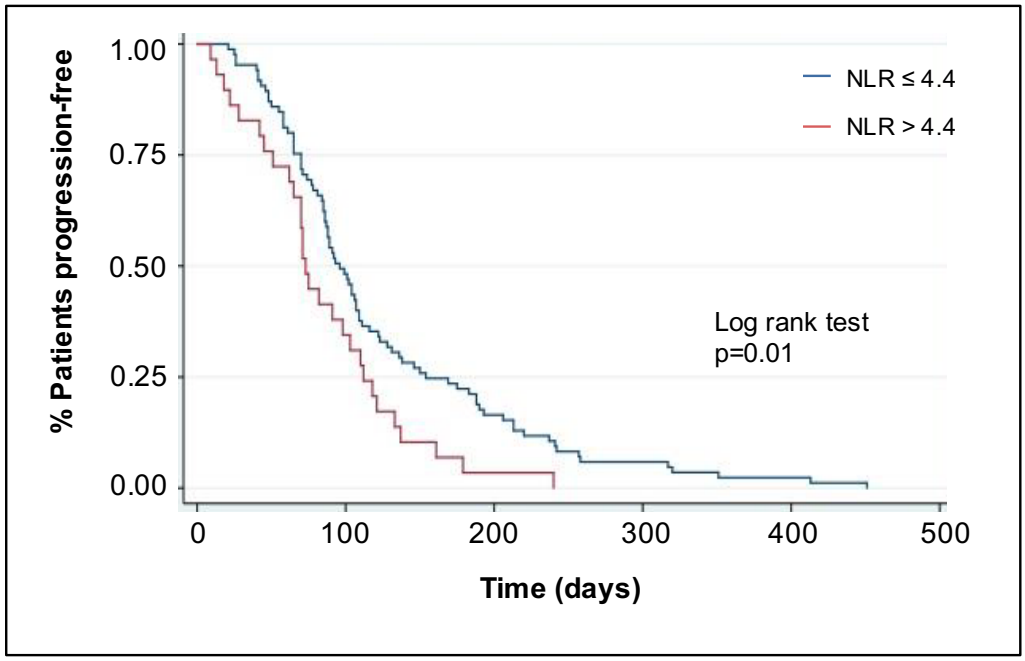
significantly among the 4 groups (P<0.001 for both), without differences in basophils (P=0.82), eosinophils (P=0.63), monocytes (P=0.21) and platelets (P=0.32). The differences in PFS were analyzed through Kaplan Meier curves and Log-rank test. Overall, a statistically significant difference was observed when comparing all the 4 patient groups (P=0.01). When comparing NLR≤2.3 vs. NLR>2.3 (P=0.19), NLR≤ 3.2 vs. NLR>3.2 (P=0.12) and NLR≤4.4 vs. NLR>4.4 (P=0.01), the lower quartile was always apparently favoured in terms of PFS, compared to the higher, however a statistically significant difference was only observed when comparing NLR≤4.4 vs. NLR>4.4 (P=0.01; **Figure 4**). From each comparison it was possible to evince that lower NLR corresponds to better survival outcomes in mBC treated with everolimus.

Table 1. Blood cells count according to NLR quartiles

Blood cell line[a]	Quartile 1 NLR ≤ 2.3	Quartile 2 2.3 < NLR ≤ 3.2	Quartile 3 3.2 < NLR ≤ 4.4	Quartile 4 NLR > 4.4	No. of patients	$p^{\#}$
Monocytes					114	0.21
Median	0.44	0.58	0.53	0.65		
Min–max range	0.14–1.31	0.2–1.76	0.2–1.22	0.15–6.6		
Lymphocytes					114	**< 0.01**
Median	2	1.51	1.36	0.82		
Min–max range	0.76–4.74	0.83–4.69	0.74–1.93	0.36–16.7		
Neutrophils					114	**< 0.01**
Median	3	4.23	4.89	5.73		
Min–max range	1.24–8.91	1.9–12.79	2.68–8.43	3.12–74		
Basophils					113	0.82
Median	0.02	0.02	0.03	0.02		
Min–max range	0–0.2	0–0.19	0–0.11	0–0.6		
Eosinophils					113	0.63
Median	0.1	0.08	0.07	0.09		
Min–max range	0–0.58	0–0.35	0–0.32	0–2		
Platelets					114	0.32
Median	213	240	267	261		
Min–max range	56–442	160–445	92–517	118–636		

Legend and footnotes. *: cells x 10^3/mL; #: Kruskal-Wallis test for continues variables; NLR: neutrophil-to-lymphocyte ratio.

Figure 4. Kaplan-Meier curves of progression-free survival of patients with NLR≤4.4 vs. NLR>4.4 from the BALLET trial

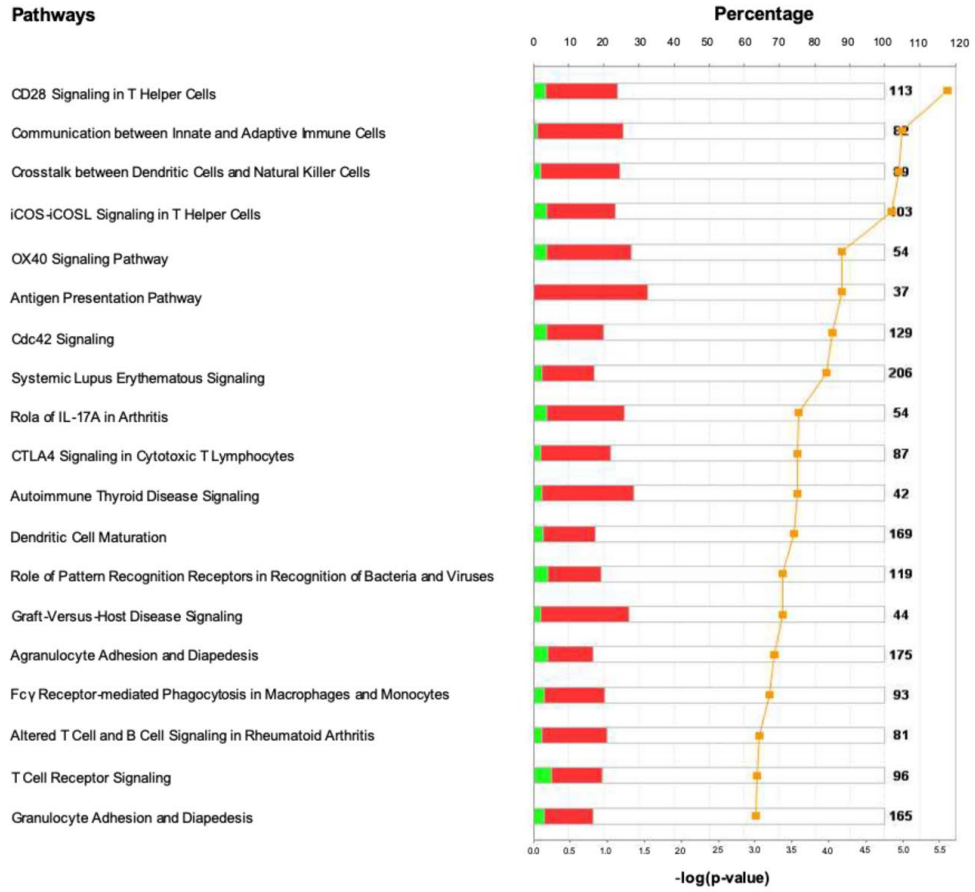


Ingenuity pathway analysis according to response to everolimus in neoadjuvant setting

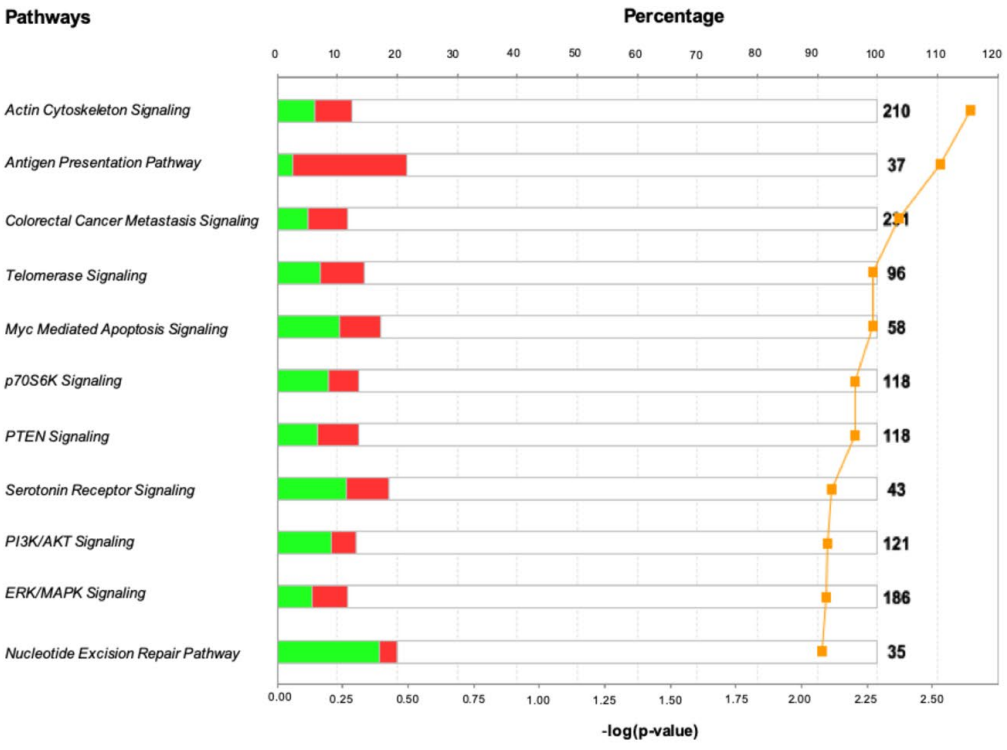
Overall, 2,063 genes were differentially expressed between everolimus "responders" and "non-responders" before treatment, as observed elsewhere [26]. Between the two groups several pathways were found to be associated with the immune system, as top scoring (P<0.001) (**Figure 5A**), with the majority of innate and adaptive immunity related genes up-modulated in everolimus-responsive compared with everolimus-unresponsive tumors before treatment. Post-treatment, the majority of pathways that were differentially enriched in responders compared with non-responders were those typically represented in epithelial cells and associated with response to everolimus, such as PI3K, actin cytoskeleton and ERK, with the majority of genes down-regulated in responsive tumors **(Figure 5B**). The only immune-related pathway that remained significantly positively enriched in responsive tumors was the one related to antigen presentation **(Figure 5B**).

Figure 5. Gene classification according to canonical signalling pathways using Ingenuity Pathway Analysis (IPA), before (A) and after (B) everolimus treatment

A


Pathways
Percentage
0 10 20 30 40 50 60 70 80 90 100 110 120
CD28 Signaling in T Helper Cells 113
Communication between Innate and Adaptive Immune Cells 82
Crosstalk between Dendritic Cells and Natural Killer Cells 89
iCOS-iCOSL Signaling in T Helper Cells 103
OX40 Signaling Pathway 54
Antigen Presentation Pathway 37
Cdc42 Signaling 129
Systemic Lupus Erythematous Signaling 206
Rola of IL-17A in Arthritis 54
CTLA4 Signaling in Cytotoxic T Lymphocytes 87
Autoimmune Thyroid Disease Signaling 42
Dendritic Cell Maturation 169
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses 119
Graft-Versus-Host Disease Signaling 44
Agranulocyte Adhesion and Diapedesis 175
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes 93
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis 81
T Cell Receptor Signaling 96
Granulocyte Adhesion and Diapedesis 165
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
-log(p-value)

B


Pathways
Percentage
0 10 20 30 40 50 60 70 80 90 100 110 120
Actin Cytoskeleton Signaling 210
Antigen Presentation Pathway 37
Colorectal Cancer Metastasis Signaling 231
Telomerase Signaling 96
Myc Mediated Apoptosis Signaling 58
p70S6K Signaling 118
PTEN Signaling 118
Serotonin Receptor Signaling 43
PI3K/AKT Signaling 121
ERK/MAPK Signaling 186
Nucleotide Excision Repair Pathway 35
0.00 0.25 0.50 0.75 1.00 1.25 1.50 1.75 2.00 2.25 2.50
-log(p-value)

Legend. The bars denote the percentage of downregulated (green) and upregulated (red) differentially expressed genes in responsive compared to non-responsive tumors out of the total number of genes present in the IPA database (shown in black to farthest right) within each pathway. Orange squares represent −log (*p* value).

Discussion

Everolimus with exemestane has been approved for the treatment of post-menopausal HR+/HER2- mBC following a significant PFS improvement observed in the BOLERO-2 trial [34]. After that, newer effective treatment strategies based on CDK4/6 inhibitors combined with aromatase inhibitors or fulvestrant and the PI3K inhibitor alpelisib combined with fulvestrant for PIK3CA-mutant patients have also been added to the therapeutic armamentarium in the last few years [35–37]. A recent comprehensive network meta-analysis highlighted comparable therapeutic performances between such therapies and chemotherapy [38]. However, at present, the optimal treatment sequence is not known, as there is a lack of direct comparisons and no effective biomarkers of response for all these treatment strategies.

This study was designed to identify potential biomarkers of response to everolimus, with the aim of better recognizing patients with a higher probability of benefiting from everolimus, and tend towards a more personalized treatment approach for HR+/HER2- mBC. Our study supports the notion that HR+ breast cancer patient's responsiveness to everolimus, as described for other targeted therapies [6], might be mediated by an interplay with the immune system. Thus, an immune system biomarker could be a valuable tool to identify patients most likely to benefit from this drug.

The IPA showed that, before treatment, several pathways associated with active immune response were up-regulated in everolimus responders compared to non-responders. Interestingly, everolimus treatment induced the loss of enriched immune pathways in responders, apart from those related to antigen presentation. Furthermore, NLRs in blood samples derived from the BALLET study showed that lower basal NLRs were associated with better PFS. More specifically, our analysis pointed out a significant difference only when comparing all the lowest quartiles to the highest. Of note, a recently published study, among various results, confirmed an unfavorable prognostic role for high levels of NLR in MBC, by using propensity score-matched MBC patients and healthy women [39].

NLRs have long been observed to be correlated with prognosis upon everolimus treatment also in other types of solid tumors (e.g. in patients with renal cell carcinoma treated with everolimus) [15]. Although the precise immune system's microenvironment of RC is likely to be different from that in BC, our results from the BALLET study seem to support a common mechanism at the basis of everolimus anti-tumor activity, at least in patients with very high NLR values. Our results from the first large study of mBC patients treated with everolimus, although preliminary, suggest that a simple NLR might be a useful clinical tool without additional costs to determine everolimus responders *a*

priori. Moreover, another study showed a better overall survival for patients with MBC and stable low NLR through time and treatment change [39]. This suggests that the evaluation of the dynamics of NLR might also be studied to understand its relevance in monitoring treatment efficacy.

Another potential biomarker are T-lymphocyte subpopulations. Our analysis of everolimus treated mBC patients within the mTOR-Study showed that both at baseline and after everolimus treatment, overall T-lymphocytes, including both CD8+ (T-killers) and T-helpers CD4+ were significantly higher in everolimus responders vs. non-responders with a trend for a Tregs CD4+ reduction, in keeping with the prognostically favorable role of lower NLR basal values observed in the BALLET patients. A higher number of T-helper might explain the higher number of CD8+ in everolimus responders, being the first particularly involved in recruiting and activating the effectors T-killers in immune adaptive responses. At the same time, a reduction of Tregs might be responsible for the increase in both T-helpers and killers, due to Tregs immunosuppressive function [40,41] supported by preclinical studies of murine tumor models [42]. Albeit speculative, it is possible that patients with higher infiltration of these cells in the tumor tissue before treatment are those that better benefit from treatment, due to the presence of the cell targets of everolimus. In fact, mTOR is active in immune cells, where it regulates important and diverse functions in all T-cell lineages [43]. Nevertheless, the Tregs reduction was not statistically significant and the number of patients was too small to draw any definitive conclusion.

The high pre-treatment infiltration of immune cells in responsive tumors might mirror their high intrinsic basal mTOR activation, reported to be involved in the recruitment of immune cell in the tumor microenvironment [44]. Everolimus on-target activity in these tumors could thus explain the downregulation of immune pathways after treatment in everolimus responders and consequent lack of differences in immune pathways with non-responders observed after treatment. In accordance with this hypothesis is also the association between low number of CECs and response in patients on treatment with everolimus. Indeed, the levels of CECs, a potential neoangiogenesis marker [20], correlate with plasma levels of VCAM-1 and VEGF [19], whose downstream pathways include PI3K/Akt/mTOR signaling and are also inhibited by everolimus [45]. In this context, the higher basal vascularity in tumor tissues in responders, compared to non-responders, might reflect the higher activation of the mTOR pathway in tumors from patients who will benefit the most from everolimus treatment. Thus, the reduction in circulating CECs in patients on treatment with everolimus, might represent a potential midcourse biomarker for guiding patients toward the ideal regimen after brief exposure to everolimus.

We are aware that this work has several limitations. First of all, the retrospective nature of the three studies limits the statistical power and the number of variables analyzed, such as time-to-drug

exposure, at the decision of the investigators. Secondly, the total number of patients analyzed in the local study (15 patients) and the neoadjuvant study (23 patients) is relatively small and different kind of analyses were conducted on the different cohorts of patients. Moreover, the cohorts of the studies differ in terms of clinical setting (neoadjuvant vs metastatic) and none of the studies included a control arm, needed to clearly distinguish between a prognostic vs predictive role.

However, the importance of our study relies in the facts that, to our knowledge, for the first time the potential relevance of lymphocytes subpopulations, CECs and NLR as easily-detectable biomarkers of response to everolimus-based regimens in HR+ BC is reported.

Despite not being conclusive, our data, corroborated by an increasing body of evidence [39,46], might provide the rationale for larger, prospective and more homogeneous trials, which could pave the way to the development of a new tool capable of easily predicting and monitoring everolimus response in HR+/HER2- BC.

Ethics approval and consent to participate

The MREC study received the ethical approval from the UK Northern and Yorkshire MREC (MREC reference 04/MRE03/89) and all patients gave their informed consent to participate. The mTOR study was approved by the Ethical Committee Val Padana-Cremona (IRB code: 12063/2015) and patients provided informed written consent to participate. In the BALLET trial informed consent was formally obtained from all patients. The protocol was independently approved by the Ethical Committee review board at each site [18]. All these studies were conducted accordingly to Good Clinical Practice guidelines and in conformity with the 1964 Declaration of Helsinki and its later amendments.

Data availability

Data are available upon reasonable request.

Conflict of interest

The funders had no role in the design of the study, nor in the collection, analysis, and interpretation of the data, writing of the manuscript and the decision to submit the manuscript for publication. Francesco Schettini has declared travel and accommodation expenses paid by Pfizer and Celgene. Guy Jerusalem has reported grants, personal fees and non-financial support from Novartis, grants, personal fees and non-financial support from Roche, grants, personal fees and non-financial support from Pfizer, personal fees and non-financial support from Lilly, personal fees from Celgene, personal fees and non-financial support from Amgen, personal fees and non-financial support from BMS, personal fees from Puma Technology, personal fees and non-financial support from Astra-Zeneca, personal fees from Daiichi Sankyo, personal fees from Abbvie, outside the submitted work. DG has declared consulting fees from Novartis, Lilly and Pfizer, research funding from LILT, Novartis Astra-Zeneca and University of Trieste. Sherine Loi.'s and Stephen B Fox's institution receives research funding from Bristol-Myers Squibb, Eli Lilly, Genentech, Merck, Novartis, Pfizer, Puma Biotechnology and Roche. Sherine Loi has acted as a non-compensated consultant of AstraZeneca, Bristol-Meyers Squibb, Merck, Novartis, Pfizer, Roche-Genentech and Seattle Genetics. All other authors declared no conflict of interest.

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Authors' contributions

All authors conceived the study. FG performed the statistical analyses. FS, NS, TT and DG interpreted the data and wrote the first manuscript draft. All authors reviewed and/or edited and approved the manuscript prior to submission.

References

1. SEER statistics for breast cancer [Internet]. Available from: https://seer.cancer.gov
2. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med. 2011;62:233–47.
3. Conciatori F, Bazzichetto C, Falcone I, Pilotto S, Bria E, Cognetti F, et al. Role of mTOR Signaling in Tumor Microenvironment: An Overview. Int J Mol Sci. 2018 Aug 19;19(8).
4. Sobhani N, Generali D, Zanconati F, Bortul M, Scaggiante B. Current status of PI3K-mTOR inhibition in hormone-receptor positive, HER2-negative breast cancer. World J Clin Oncol. 2018 Dec 20;9(8):172–9.
5. Yi Z, Ma F. Biomarkers of Everolimus Sensitivity in Hormone Receptor-Positive Breast Cancer. J Breast Cancer. 2017 Dec;20(4):321–6.
6. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. Cancer Cell. 2015 Dec 14;28(6):690–714.
7. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov. 2012 Feb 3;11(3):215–33.
8. Santoni M, Buti S, Conti A, Porta C, Procopio G, Sternberg CN, et al. Prognostic significance of host immune status in patients with late relapsing renal cell carcinoma treated with targeted therapy. Target Oncol. 2015 Dec;10(4):517–22.
9. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2018 29;379(22):2108–21.
10. Schmid P, Salgado R, Park YH, Muñoz-Couselo E, Kim SB, Sohn J, et al. Pembrolizumab plus chemotherapy as neoadjuvant treatment for high-risk, early-stage triple-negative breast cancer: results from the phase 1b open-label, multicohort KEYNOTE-173 study. Ann Oncol [Internet]. 2020 Feb 14 [cited 2020 Feb 19];0(0). Available from: https://www.annalsofoncology.org/article/S0923-7534(20)36032-4/abstract
11. Adams S, Loi S, Toppmeyer D, Cescon DW, De Laurentiis M, Nanda R, et al. Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: cohort B of the phase II KEYNOTE-086 study. Ann Oncol Off J Eur Soc Med Oncol. 2019 01;30(3):405–11.
12. Lin CPM 2016By TLTLA, PharmD, BCPS, Center B is an oncology pharmacist at C-SM, Blvd 8700 Beverly, Angeles L, et al. Mammalian target of rapamycin (mTOR) inhibitors in solid tumours [Internet]. Pharmaceutical Journal. [cited 2020 Feb 27]. Available from: https://www.pharmaceutical-journal.com/research/review-article/mammalian-target-of-rapamycin-mtor-inhibitors-in-solid-tumours/20200813.article
13. Pascual J. The use of everolimus in renal-transplant patients. Int J Nephrol Renov Dis. 2009 Jun 2;2:9–21.
14. Sabbatini M, Ruggiero G, Palatucci AT, Rubino V, Federico S, Giovazzino A, et al. Oscillatory mTOR inhibition and Treg increase in kidney transplantation. Clin Exp Immunol. 2015 Nov;182(2):230–40.

15. Santoni M, De Giorgi U, Iacovelli R, Conti A, Burattini L, Rossi L, et al. Pre-treatment neutrophil-to-lymphocyte ratio may be associated with the outcome in patients treated with everolimus for metastatic renal cell carcinoma. Br J Cancer. 2013 Oct;109(7):1755–9.
16. Kobayashi M, Kubo T, Komatsu K, Fujisaki A, Terauchi F, Natsui S, et al. Changes in peripheral blood immune cells: their prognostic significance in metastatic renal cell carcinoma patients treated with molecular targeted therapy. Med Oncol Northwood Lond Engl. 2013 Jun;30(2):556.
17. Personeni N, Giordano L, Abbadessa G, Porta C, Borbath I, Daniele B, et al. Prognostic value of the neutrophil-to-lymphocyte ratio in the ARQ 197-215 second-line study for advanced hepatocellular carcinoma. Oncotarget. 2017 Feb 28;8(9):14408–15.
18. Jerusalem G, Mariani G, Ciruelos EM, Martin M, Tjan-Heijnen VCG, Neven P, et al. Safety of everolimus plus exemestane in patients with hormone-receptor-positive, HER2-negative locally advanced or metastatic breast cancer progressing on prior non-steroidal aromatase inhibitors: primary results of a phase IIIb, open-label, single-arm, expanded-access multicenter trial (BALLET). Ann Oncol Off J Eur Soc Med Oncol. 2016;27(9):1719–25.
19. Mancuso P, Calleri A, Cassi C, Gobbi A, Capillo M, Pruneri G, et al. Circulating endothelial cells as a novel marker of angiogenesis. Adv Exp Med Biol. 2003;522:83–97.
20. Martin-Padura I, Bertolini F. Circulating endothelial cells as biomarkers for angiogenesis in tumor progression. Front Biosci Sch Ed. 2009 Jun 1;1:304–18.
21. Macaskill EJ, Bartlett JMS, Sabine VS, Faratian D, Renshaw L, White S, et al. The mammalian target of rapamycin inhibitor everolimus (RAD001) in early breast cancer: results of a pre-operative study. Breast Cancer Res Treat. 2011 Aug;128(3):725–34.
22. Malmström P, Bendahl PO, Boiesen P, Brünner N, Idvall I, Fernö M, et al. S-phase fraction and urokinase plasminogen activator are better markers for distant recurrences than Nottingham Prognostic Index and histologic grade in a prospective study of premenopausal lymph node-negative breast cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2001 Apr 1;19(7):2010–9.
23. Balaton AJ, Baviera EE, Galet B, Vaury P, Vuong PN. [Immunohistochemical evaluation of estrogen and progesterone receptors on paraffin sections of breast carcinomas. Practical thoughts based on the study of 368 cases]. Arch Anat Cytol Pathol. 1995;43(1–2):93–100.
24. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol Off J Am Soc Clin Oncol. 2018 Jul 10;36(20):2105–22.
25. Koukourakis MI, Giatromanolaki A, Sivridis E, Simopoulos C, Gatter KC, Harris AL, et al. LYVE-1 immunohistochemical assessment of lymphangiogenesis in endometrial and lung cancer. J Clin Pathol. 2005 Feb;58(2):202–6.
26. Sabine VS, Sims AH, Macaskill EJ, Renshaw L, Thomas JS, Dixon JM, et al. Gene expression profiling of response to mTOR inhibitor everolimus in pre-operatively treated post-menopausal women with oestrogen receptor-positive breast cancer. Breast Cancer Res Treat. 2010 Jul;122(2):419–28.
27. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004;5(10):R80.
28. Dunning MJ, Smith ML, Ritchie ME, Tavaré S. beadarray: R classes and methods for Illumina bead-based data. Bioinforma Oxf Engl. 2007 Aug 15;23(16):2183–4.

29. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostat Oxf Engl. 2007 Jan;8(1):118–27.
30. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A. 2001 Apr 24;98(9):5116–21.
31. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2017. Available from: https://www.R-project.org/
32. Krämer A, Green J, Pollard J, Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinforma Oxf Engl. 2014 Feb 15;30(4):523–30.
33. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer. 2005; 93(4):387-391.
34. Baselga J, Campone M, Piccart M, Burris HA, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med. 2012 Feb 9;366(6):520–9.
35. NCCN Guidelines for Breast Cancer, vers.3.2019 [Internet]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf
36. Rugo HS, Rumble RB, Macrae E, Barton DL, Connolly HK, Dickler MN, et al. Endocrine Therapy for Hormone Receptor–Positive Metastatic Breast Cancer: American Society of Clinical Oncology Guideline. J Clin Oncol. 2016 May 23;34(25):3069–103.
37. Cardoso F, Senkus E, Costa A, Papadopoulos E, Aapro M, André F, et al. 4th ESO–ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4)†. Ann Oncol. 2018 Jul 19;29(8):1634–57.
38. Giuliano M, Schettini F, Rognoni C, Milani M, Jerusalem G, Bachelot T, et al. Endocrine treatment versus chemotherapy in postmenopausal women with hormone receptor-positive, HER2-negative, metastatic breast cancer: a systematic review and network meta-analysis. Lancet Oncol. 2019; 20(10):1360-1369.
39. Gerratana L, Basile D, Toffoletto B, Bulfoni M, Zago S et al. Biologically driven cut-off definition of lymphocyte ratios in metastatic breast cancer and association with exosomal subpopulations and prognosis. Sci Rep. (2020) 10:7010.
40. Corthay A. How do Regulatory T Cells Work? Scand J Immunol. 2009 Oct;70(4):326–36.
41. Patton DT, Garden OA, Pearce WP, Clough LE, Monk CR, Leung E, et al. Cutting edge: the phosphoinositide 3-kinase p110 delta is critical for the function of CD4+CD25+Foxp3+ regulatory T cells. J Immunol Baltim Md 1950. 2006 Nov 15;177(10):6598–602.
42. Zeng H. mTOR signaling in immune cells and its implications for cancer immunotherapy. Cancer Lett. 2017 01;408:182–9.
43. Beziaud L, Mansi L, Ravel P, Marie-Joseph EL, Laheurte C, Rangan L, et al. Rapalogs Efficacy Relies on the Modulation of Antitumor T-cell Immunity. Cancer Res. 2016 15;76(14):4100–12.
44. Wellenstein MD, de Visser KE. Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. Immunity. 2018 20;48(3):399–416.
45. Abhinand CS, Raju R, Soumya SJ, Arya PS, Sudhakaran PR. VEGF-A/VEGFR2 signaling network in endothelial cells relevant to angiogenesis. J Cell Commun Signal. 2016 Dec;10(4):347–54.
46. Ethier J-L, Desautels D, Templeton A, Shah PS & Amir E. Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis. Breast Cancer Res. 2017; 19:2.

CHAPTER 3: Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer

Original article reference

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Abstract

Novel antibody-drug conjugates against HER2 are showing high activity in HER2-negative breast cancer (BC) with low HER2 expression (i.e. 1+ or 2+ and lack of *ERBB2* amplification). However, the clinical and molecular features of HER2-low BC are yet to be elucidated. Here, we collected retrospective clinicopathological and PAM50 data from 3,689 patients with HER2-negative disease and made the following observations. First, the proportion of HER2-low was higher in HR+ disease (65.4%) than triple-negative BC (TNBC, 36.6%). Second, within HR+ disease, *ERBB2* and luminal-related genes were more expressed in HER2-low than HER2 0. In contrast, no gene was found differentially expressed in TNBC according to HER2 expression. Third, within HER2-low, *ERBB2* levels were higher in HR+ disease than TNBC. Fourth, HER2-low was not associated with overall survival in HR+ disease and TNBC. Finally, the reproducibility of HER2-low among pathologists was suboptimal. This study emphasizes the large biological heterogeneity of HER2-low BC, and the need to implement reproducible and sensitive assays to measure low HER2 expression.

Introduction

HER2-positive breast cancer is currently defined according to the ASCO/CAP guidelines using immunohistochemistry (IHC) and/or *in situ* hybridization (ISH)-based techniques [1,2]. These guidelines identify a tumor as HER2-positive when there is a complete and intense circumferential HER2 IHC staining in ≥10% of cells (score 3+) and/or the gene is amplified with an HER2/CEP17 ratio ≥ 2.0 and an average HER2 gene (*ERBB2*) copy number ≥ 4.0 signals/cell using ISH-based techniques [1]. In breast cancer, 10-20% of tumors are HER2-positive and 80-90% are HER2-negative [3,4].

Within HER2-negative disease, substantial heterogeneity exists regarding the expression of hormone receptors (HR) and HER2. For example, HER2-negative tumors can express some protein level of HER2 by IHC [5] (i.e. 1+ or 2+ and lack of *ERBB2* amplification by in-situ hybridization techniques) and are identified as HER2-low. Traditionally, patients with HER2-low-expressing tumors do not seem to benefit from HER2-targeted therapies, such as 1-year of adjuvant trastuzumab [6]. However, two HER2-directed antibody-drug conjugates (ADC) with chemotherapeutics, namely trastuzumab deruxtecan (T-DXd) and trastuzumab duocarmazine (SYD985) have shown very promising therapeutic activity in patients with HER2-low breast cancer [7-9]. A large pivotal randomized phase III trial of T-DXd in patients with pre-treated HER2-low metastatic breast cancer is underway (i.e. NCT03734029/DESTINY-Breast04).

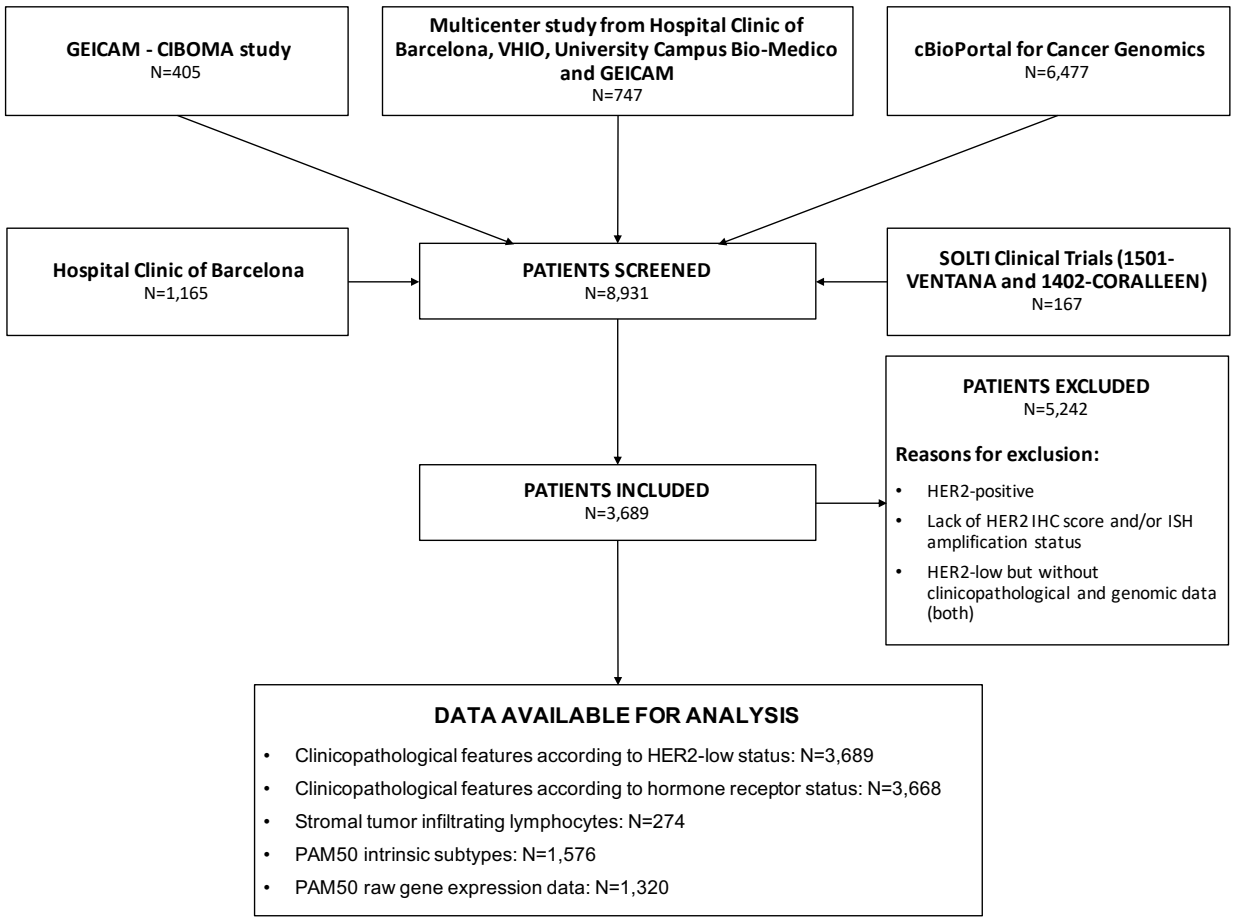
Due to the recent and increased interest in the HER2-low group, there is an urgent need to better understand its clinicopathological and molecular features. Thus, we decided to collect clinicopathological and PAM50 gene expression data from multiple datasets [10-17] of HER2-negative disease and compare many features between HER2-low and HER2 0. Analyses were focused on the overall population and according to hormone receptor (HR) status and HER2 IHC expression.

Results

Clinicopathological characteristics of HER2-low disease

Thirteen independent datasets for a total of 3,689 patients with HER2-negative breast cancer were explored (**Figure 1**). Overall, 1,486 (40.3%) patients had HER2 0 tumors, 1,489 (40.4%) had HER2 1+ tumors and 714 (19.3%) had HER2 2+ tumors. Clinicopathological and gene expression data (when available) were largely obtained from primary disease (71.1% in HER2-low and 73.7% in HER2 0). According to HR status, 2,962 (80.8%) patients had HR-positive disease and 706 (19.2%) had triple-negative breast cancer (TNBC).

Figure 1. STROBE flow-chart



Legend and captions. Flow-chart resuming the patient selection process, showing causes for exclusion and the number of patients with available data for the main analyses presented in the study. **GEICAM:** Grupo Español de Investigación en Cáncer de Mama; **CIBOMA:** Coalición Iberoamericana de Investigación en Oncología Mamaria; VHIO: Vall d'Hebron Institute of Oncology; **SOLTI:** Solid Tumor Intensification Group; **IHC:** immunohistochemistry; **ISH:** in-situ hybridization, **HR:** hormone receptors.

HER2-low tumors were more frequently found within HR-positive disease compared to TNBC (65.4% vs. 36.5%, *P*<0.001; **Figure 2**). More specifically, HR-positive disease was characterized by higher rates of IHC 1+ and 2+ tumors, compared to TNBC (43.8% vs. 26.8% and 21.6% vs. 9.8%, respectively, *P*<0.001; **Figure 2**). In terms of other clinicopathological variables, HER2-low tumors presented larger primary tumor sizes (*P*=0.007) and more nodal involvement (*P*=0.010) compared to HER2 0 tumors (**Table 1 and Supplementary table 1** in **appendix 3**). No male patient was observed within the HER2 0 cohort, compared to the 15 cases observed in the HER2-low subset (*P*=0.001). The median age at diagnosis was higher for the HER2-low tumors compared to HER2 0 (59 vs. 55 years, *P*=0.003). No statistically significant differences were observed in terms of menopausal status (*P*=0.898), histological grade (*P*=0.175), Ki67 IHC scores (*P*=0.092 using a 14% cut-off) and

percentage of stromal tumor infiltrating lymphocytes (TILs) (*P*=0.218), although TILs' levels were differently distributed according to HER2 IHC levels (*P*=0.033) and were higher in HER2 2+ (median: 5; interquartile range [IQR] 1-5) compared to 1+ (median: 1; IQR 1-5; p=0.035) and 0 (median: 1; IQR 1-5; *P*=0.035).

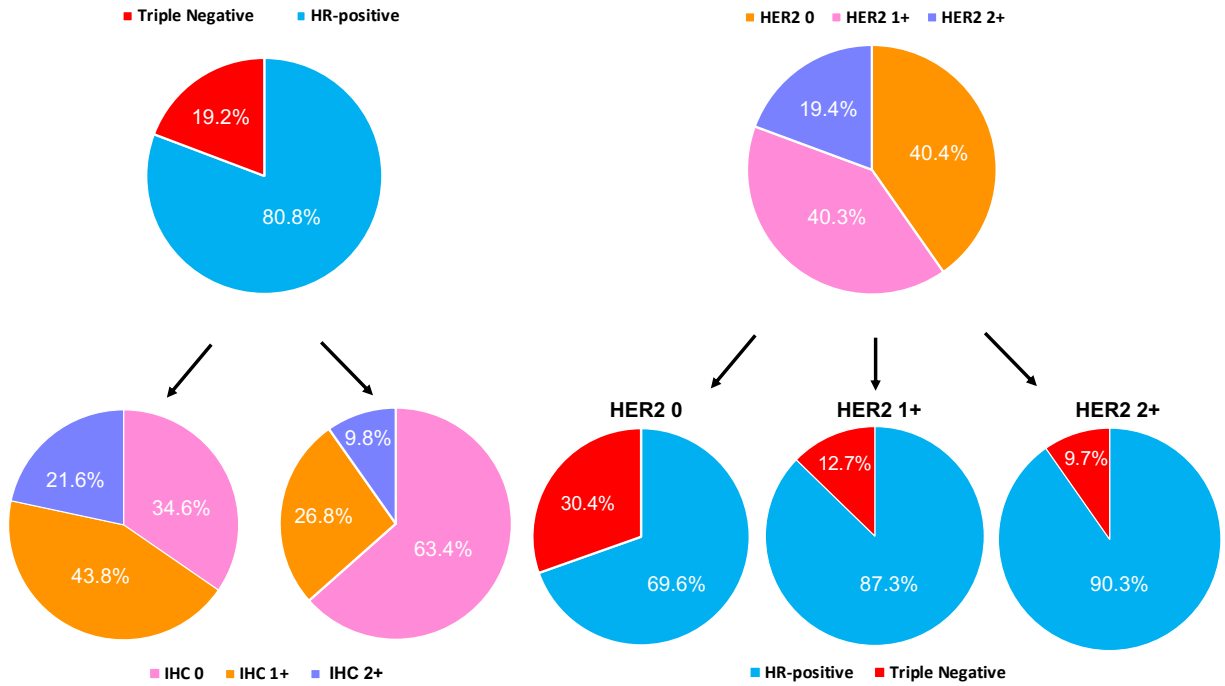
Table 1. Population characteristics according to HER2 status

DEMOGRAPHICS	HER2-NEGATIVE						P*
	HER2 0		HER2-LOW		OVERALL POPULATION		
	N	%	N	%	N	%	
	1486	40.3	2203	59.7	3689	100	
Age at diagnosis (years)							
Median	55		59		58		**0.003**
IQR	46 - 65		49 - 67		48 - 67		
Min - max	24 - 93		26 - 96		24 - 96		
Pts with available data	259	27.4	685	72.6	944	100	
Sex							
Male	0	0	15	0.7	15	0.4	**0.001**
Female	1486	100	2187	99.3	3673	99.6	
Total	1486	40.3	2202	59.7	3688	100	
Menopaual status							
Pre/perimenopausal	385	37.3	660	37.1	1045	37.2	0.898
Postmenopausal	646	62.7	1119	62.9	1765	62.8	
Total	1031	36.7	1779	63.3	2810	100	
Biospecimen							
Primary lesion	1000	73.7	1382	71.1	2382	72.1	0.096
Other lesion	357	34.6	563	28.9	920	27.9	
Total	1357	41.1	1945	58.9	3302	100	
Histotype							
Ductal	639	70.8	1214	74.3	1853	73	0.175
Lobular	194	21.5	314	19.2	508	20	
Other	69	7.6	107	6.5	176	6.9	
Total	902	35.6	1635	64.4	2537	100	
T							
1	509	55.8	807	48.7	1316	51.2	**0.007**
2	294	32.2	618	37.3	912	35.5	
3	71	7.8	142	8.6	213	8.3	
4	38	4.2	89	5.4	127	4.9	
Total	912	35.5	1656	64.5	2568	100	
N							
0	556	58.8	937	55.6	1493	56.8	**0.01**
1	272	28.8	464	27.6	736	28	

2	71	7.5	148	8.8	219	8.3	
3	46	4.9	135	8	181	6.9	
Total	945	35.9	1684	64.1	2629	100	
ER							
Positive	983	67	1894	87.1	2877	79	
Negative	484	33	280	12.9	764	21	**<0.001**
Total	1467	40.3	2174	59.7	3641	100	
PgR							
Positive	789	54.7	1542	71.8	2331	64.9	
Negative	654	45.3	606	28.2	1260	35.1	**<0.001**
Total	1443	40.2	2148	59.8	3591	100	
G							
1	67	8.8	139	10.6	206	9.9	
2	272	35.6	514	39.1	786	37.8	**0.0499**
3	426	55.7	660	50.3	1086	52.3	
Total	765	36.8	1313	63.2	2078	100	
Ki67							
Median	16		18		18		
IQR	9 - 30		10 - 27		10 - 27		0.892
Min - max	0.5 - 95		0.5 - 95		0.5 - 95		
Pts with available data	433	36.4	756	63.6	1189	100	
≤14%	190	43.9	294	38.9	484	40.7	0.092
>14%	243	56.1	462	61.1	705	59.3	
<20%	236	54.5	411	54.4	647	54.4	0.963
≥20%	197	45.5	345	45.6	542	45.6	
TILs							
Median	1		1		1		
IQR	0 - 5		1 - 5		1 - 5		0.218
Min - max	0 - 80		0 - 80		0 - 80		
Pts with available data	102	37.2	172	62.8	274	100	
PAM50 subtypes							
Luminal A	193	28.7	459	50.8	652	41.4	
Luminal B	127	18.9	260	28.8	387	24.6	
HER2-enriched	40	5.9	32	3.5	72	4.6	**<0.001**
Basal-like	294	43.7	120	13.3	414	26.3	
Normal-like	19	2.8	32	3.5	51	3.1	
Total	673	42.7	903	57.3	1576	100	
HR status							
HR-positive	1025	69.6	1937	88.2	2962	80.8	
TNBC	448	30.4	258	11.8	706	19.2	**<0.001**
Total	1473	40.2	2195	59.8	3668	100	

Legend and footnotes. Pts: patients; HR: hormone receptors; IQR: interquartile range; IHC: immunohistochemical; TILs: tumor-infiltrating lymphocytes; *: Chi square test for differences in proportions, Kruskalis-Wallis and Wilcoxon rank sum test with continuity correction, where appropriate, for continuous variables (median comparisons).

Figure 2. Hormone receptor status, HER2-low status and IHC scores distributions within the HER2-negative population



Legend. HR: hormone receptors; **IHC:** immunohistochemistry; **ISH:** *in-situ* hybridization (including either FISH, SISH and CISH).

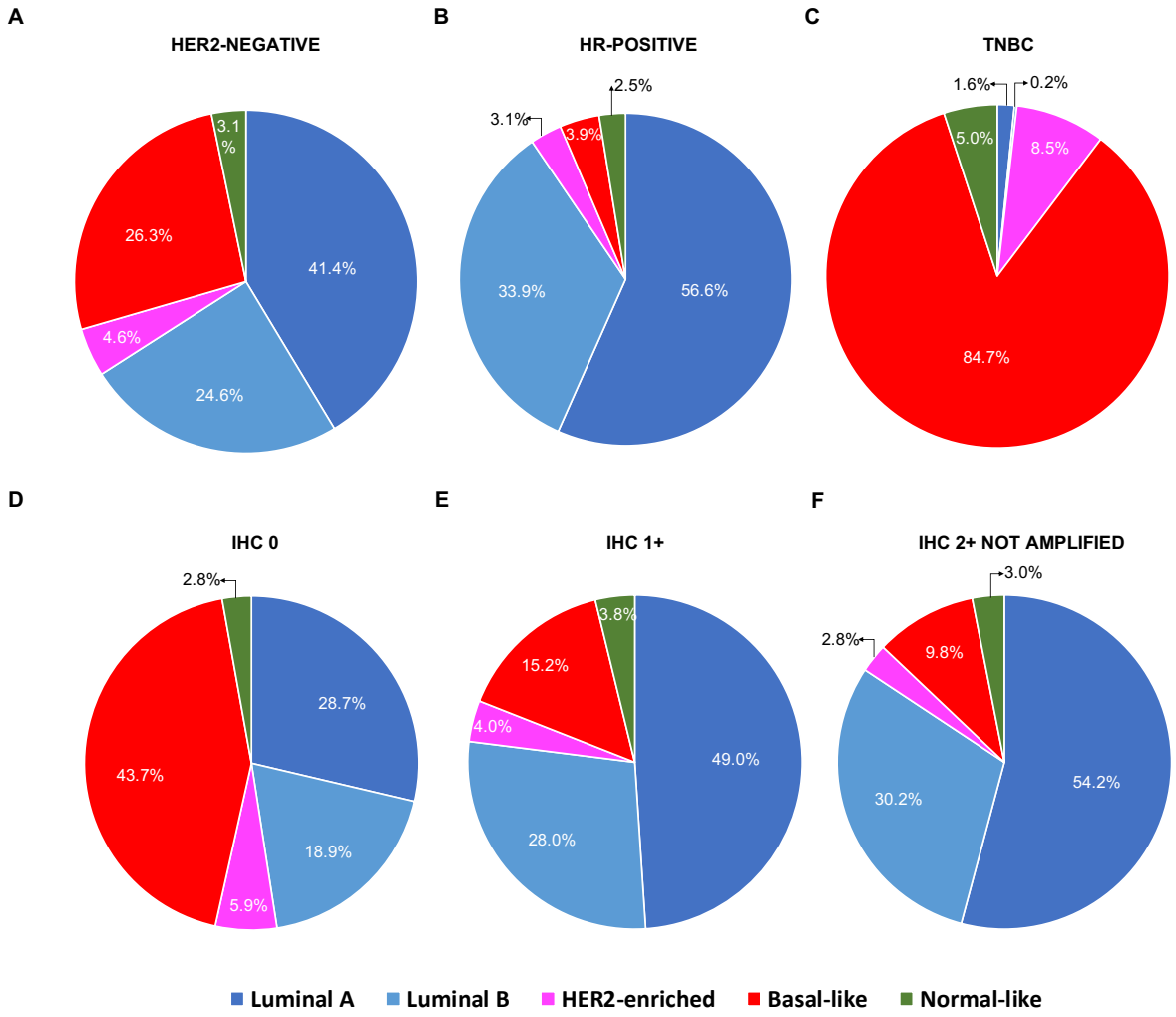
Reproducibility of the HER2-low classification

To evaluate the reproducibility of HER2 IHC scoring among pathologists, we scanned 200 HER2 IHC stained slides from 100 independent cases of the Hospital Clinic case series. The images were representative of the 4 HER2 IHC categories (i.e. 0, 1+, 2+ and 3+). Five breast cancer-specialized pathologists (BG, ES, RF, GP and VP), coming from 4 different institutions (Clinic, VHIO, VHV and Campus Bio-Medico), revised and scored the 100 cases in a blinded fashion. Overall, 35 discordant cases (35%) were observed. The discordances were between IHC 1+ vs. 0 (n=15), 1+ vs. 2+ (n=12), 2+ vs. 0 (n=1), 3+ vs. 1+ (n=1) and 3+ vs. 2+ (n=6) scores. In most cases (25 of 35, 71.4%), only 1 pathologist was discordant with the others. The multi-rater overall kappa concordance score was 0.79 (P<0.001), which is considered a substantial agreement. The kappa scores according to the HER2 IHC categories 0, 1+, 2+ and 3+ were 0.82 (almost perfect agreement), 0.67 (substantial agreement), 0.74 (substantial agreement) and 0.92 (almost perfect agreement), respectively (P<0.001). Similar results were obtained when the HER2 3+ cases were removed (data not shown).

Distribution of the PAM50 intrinsic subtypes

PAM50 intrinsic subtypes were available from 1,576 (42.7%) patients. Intrinsic subtypes were differentially distributed among the three IHC-based groups, as well as between HER2-low and HER2 0 tumors (P<0.001 for both) (**Figure 3, Table 1** and **Supplementary table 1** in **appendix 3**). Intrinsic subtypes distribution varied also between HR-positive and TNBC (P<0.001) (**Figure 3** and **Supplementary table 2** in **appendix 3**). Specifically, Luminal A tumors were more frequent within the IHC 2+ (54.2%), HER2-low (50.8%) and HR-positive (56.6%) groups compared to IHC 1+ (49.0%), IHC 0 (28.7%) and TNBC (1.6%). Similarly, Luminal B were more frequent within the IHC 2+ (30.2%), HER2-low (28.8%) and HR-positive (33.9%) groups compared to IHC 1+ (28.0%), IHC 0 (18.9%) and TNBC (0.2%); HER2-enriched (HER2-E) were more frequent within the IHC 0 (5.9%) and TNBC (8.5%) groups compared to IHC 2+ (2.8%), IHC 1+ (4.0%), HER2-low (3.5%) and HR-positive tumors (3.1%); Basal-like tumors were mostly concentrated within the IHC 0 (43.7%) and TNBC (84.7%) groups compared to IHC 2+ (9.8%), IHC 1+ (15.2%), HER2-low (13.4%) and HR-positive tumors (3.9%).

Figure 3. Intrinsic subtype distribution according to HER2 status and HR status



Legend and caption. HR: hormone receptors; **TNBC:** triple negative breast cancer; **IHC:** immunohistochemistry; **ISH:** *in-situ* hybridization (including either FISH, SISH and CISH). Number of patients in **A** (n=1,576), **B** (n=1,137); **C** (n=437); **D** (n=673); **E** (n=701); **F** (n=325).

Within HR-positive disease, intrinsic subtypes were differentially distributed between HER2-low and HER2 0 tumors, as well as according to IHC score (P<0.001 in both cases; **Table 2** and **Supplementary table 3** in **appendix 3**). Specifically, Luminal B and Basal-like subtypes were less frequent in HER2-low compared to HER2 0 (Luminal B: 8.0% vs. 34.9%; Basal-like: 1.9% vs. 33.4%), while Luminal A subtype was more frequent in HER2-low compared to HER2 0 (58.9% vs. 2.8%). There was no significant difference in subtype distribution in TNBC according to HER2-low status and IHC score (P=0.438 and P=0.284, respectively; **Table 2** and **Supplementary table 3** in **appendix 3**). When comparing HR-positive and TNBC according to the same HER2 IHC score, intrinsic subtypes were significantly differentially distributed, with Basal-like tumors being the predominant subtype in each TNBC/HER2 subset (85.2% in HER2 0, 85.4% in HER2 1+, 78.4% in HER2 2+). As expected, Luminal A (51.8% in HER2 0, 57.9% in HER2 1+, 60.6% in HER2 2+), followed by Luminal B subtype (34.9% in HER2 0, 33.1% in HER2 1+, 33.8% in HER2 2+), were the most frequent in each HR-positive/HER2 subset **(Supplementary table 4** in **appendix 3).**

Table 2. PAM50 intrinsic subtypes distribution within HR-positive and TN tumors according to HER2 status

HR-POSITIVE							
PAM50 subtypes	HER2 0+		HER2-LOW		Overall		P*
	N	%	N	%	N	%	
Luminal A	187	51.8	457	58.9	644	56.6	<0.001
Luminal B	126	34.9	259	33.4	385	33.9	
HER2-enriched	12	3.3	23	3.0	35	3.1	
Basal-like	29	8.0	15	1.9	44	3.9	
Normal-like	7	1.9	22	2.8	29	2.6	
Total	361	31.8	776	100.0	1137	100.0	
TNBC							
PAM50 subtypes	HER2 0+		HER2-LOW		Overall		P*
	N	%	N	%	N	%	
Luminal A	5	1.6	2	1.6	7	1.6	0.438
Luminal B	1	0.3	0	0.0	1	0.2	
HER2-enriched	28	9.0	9	7.1	37	8.5	
Basal-like	265	85.2	105	83.3	370	84.7	
Normal-like	12	3.9	10	7.9	22	5.0	
Total	311	71.2	126	100.0	437	100.0	

Legend and footnotes. *: Chi square test for differences in proportions; **HR:** hormone receptor; **TNBC:** triple negative breast cancer.

Finally, we investigated if the distribution of PAM50 subtypes within HER2-low breast cancer differed according to *ERBB2* mRNA levels. To approach it, we divided all patients with HER2-negative disease into tertiles (i.e. from low to high: T1, T2 and T3) based on *ERBB2* expression (**Table 3**). As expected, subtype distribution differed in HER2-low breast cancer according to *ERBB2* levels (P<0.001) with the T2-3 group being more enriched with Luminal A, Luminal B and HER2-E subtypes (51.5%, 34.9% and 6.3%) compared to the T1 group (31.7%, 15.8% and 3.6%). On the contrary, the Basal-like subtype was more frequent in the T1 group compared to the T2-3 group (44.6% vs 2.9%). The results were similar when comparing either *ERBB2* high/HER2-low and *ERBB2* low/HER2-low tumors with the whole HER2-low population (P<0.001 both) (**Table 3**).

Table 3. Intrinsic subtypes distribution in HER2-low tumors according to *ERBB2* mRNA levels

INTRINSIC SUBTYPE	*ERBB2* high (T3-T2)		*ERBB2* low (T1)		HER2-low		P*	P°	P#
	N	%	N	%	N	%			
Luminal A	140	51.5	44	31.7	184	44.8			
Luminal B	95	34.9	22	15.8	117	28.5			
HER2-Enriched	17	6.3	5	3.6	22	5.4			
Basal-like	8	2.9	62	44.6	70	17.0	**<0.001**	**<0.001**	**<0.001**
Normal-like	12	4.4	6	4.3	18	4.4			
Total	272	66.2	139	33.8	411	100.0			

Legend and footnotes. *ERBB2* is italicized, as per standard gene ID formatting guidelines. **T1:** tertile one; **T2:** tertile two; **T3:** tertile 3; *: referred to the comparison between ERB*B2* high vs low; °: referred to *ERBB2* high vs the overall HER2-low population; #: referred to *ERBB2* low vs the overall HER2-low population.

PAM50 and individual gene expression analyses

PAM50 and individual gene expression data was available in 1,320 (35.8%) patients. The full list of genes and subtypes' signatures evaluated for differential expression analyses in the overall HER2-negative population and according to HR status are reported in **Supplementary table 5** (**appendix 3**).

In the overall population, 34 of 55 genes (61.8%) were found differentially expressed between HER2-low and HER2 0 (false discovery rate [FDR]<5%) (**Table 4, Supplementary table 6** and **Supplementary figure 1** in **appendix 3**). Specifically, 14 genes (41.2%) were found significantly down-regulated in HER2-low compared to HER2 0, including proliferation-related genes (e.g. *CCNB1, CCNE1, MELK, MKI67, MYBL2* etc.), Basal-like-related genes (e.g. *KRT14, KRT17, KRT5,*

FOXC1, MYC etc.), tyrosine-kinase receptors (i.e. *EGFR, FGFR4*) and 3 PAM50 signatures (i.e. HER2-E, Basal-like and Normal-like). Conversely, 20 genes (58.8%) were found significantly up-regulated in HER2-low compared to HER2 0, including luminal-related genes (e.g. *BCL2, BAG1, FOXA1, ESR1, PGR, GPR160* and *AR*) and 2 PAM50 signatures (i.e. Luminal A and B). According to HR status, similar findings were observed in HR-positive disease as in the general population (**Table 4, Supplementary table 6** and **Supplementary figure 2** in **appendix 3**). In TNBC, however, no individual gene, or PAM50 signature, was found differentially expressed between HER2-low and HER2 0. Similar findings were observed when HER2-low disease was subdivided into 1+ and 2+ **(Table 4, Supplementary table 6** and **Supplementary figure 3** in **appendix 3).**

Table 4. Top 20 differentially expressed genes between HER2-low and HER2 0 disease

GENE SYMBOL	Association	OVERALL		HR-POSITIVE		TNBC	
		Score(d) (strength of relationship)	FDR*	Score(d) (strength of relationship)	FDR*	Score(d) (strength of relationship)	FDR*
ESR1	Higher in HER2-low	14.3	**0**	5.0	**0**	1.0	100
FOXA1	Higher in HER2-low	13.3	**0**	4.9	**0**	1.0	100
NAT1	Higher in HER2-low	12.3	**0**	4.1	**0**	-0.3	68.3
SLC39A6	Higher in HER2-low	11.6	**0**	4.0	**0**	-0.4	64.3
PGR	Higher in HER2-low	11.2	**0**	3.2	**0**	0.5	100
AR	Higher in HER2-low	10.6	**0**	-	-	0.4	100
ERBB2	Higher in HER2-low	10.0	**0**	5.2	**0**	1.7	100
MAPT	Higher in HER2-low	9.9	**0**	2.9	**0**	-0.2	68.3
MLPH	Higher in HER2-low	8.8	**0**	2.6	**0**	0.0	68.3
BCL2	Higher in HER2-low	8.2	**0**	2.5	**0**	0.0	68.3
CENPF	Lower in HER2-low	-7.0	**0**	-1.8	**0**	-1.0	64.3
EXO1	Lower in HER2-low	-7.1	**0**	-2.4	**0**	-1.2	64.3
ANLN	Lower in HER2-low	-7.4	**0**	-2.3	**0**	-0.4	64.3
ORC6L	Lower in HER2-low	-7.6	**0**	-2.3	**0**	-0.8	64.3
KNTC2	Lower in HER2-low	-7.8	**0**	-2.3	**0**	-0.7	64.3

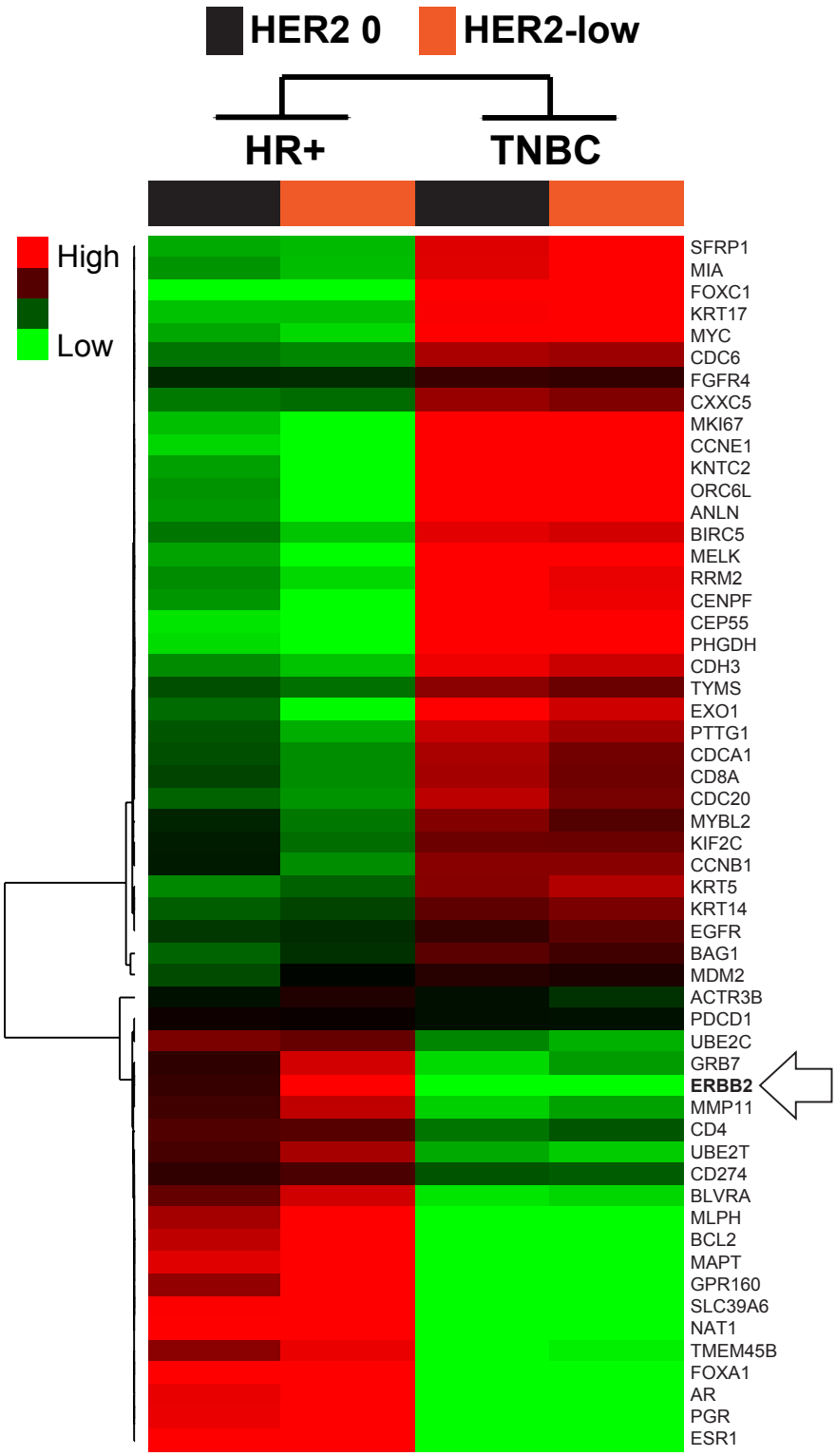
CEP55	Lower in HER2-low	-7.8	**0**	-1.3	**3.2**	-1.1	64.3
PHGDH	Lower in HER2-low	-8.4	**0**	-1.7	**0**	-1.2	64.3
FOXC1	Lower in HER2-low	-8.4	**0**	-0.7	9.7	0.2	100
MKI67	Lower in HER2-low	-8.7	**0**	-2.4	**0**	-1.0	64.3
CCNE1	Lower in HER2-low	-9.6	**0**	-3.0	**0**	-0.9	64.3

Legend, caption and footnotes. In the table only significantly subtype signatures, top-10 up-regulated and top-10 down-regulated genes for the overall population are reported, along with their corresponding result in the HR+ and TNBC populations. Genes are italicized, as per standard formatting guidelines. HR: hormone receptors; TNBC: triple negative breast cancer; FDR: false discovery rate; *: significant if FDR<5.0; Score(d): a T-statistic value that reflects a standardized change in expression and measures the strength of the relationship between gene expression and the HER2-low category (versus HER2 0).

Gene expression profiles according to HER2 expression and HR status

The previous results suggested that HR status is a key determinant of the underlying biology of HER2-low breast cancer. To further explore this, we evaluated the overall gene expression profile of HER2-negative breast cancer according to HER2 expression (i.e. HER2 0, 1+ and 2+) and HR status (i.e. positive and negative). The result clearly shows that HR status is the main driver of the underlying biology (**Figure 4** and **Supplementary table 7, appendix 3**). As expected, proliferation-related genes (e.g. *CCNE1, MKI67* and *EXO1*) were found more expressed in TNBC compared to HR-positive, regardless of HER2 IHC status (i.e. HER2-low versus HER2 0). On the contrary, luminal-related genes (e.g. *ESR1, AR* and *BCL2*) and *ERBB2* were found more expressed in HR-positive compared to TNBC, regardless of HER2 IHC status. Of note, the highest *ERBB2* expression was found in the HR-positive /HER2-low group. Finally, concordant with the previous results, HER2-low tumors within HR-positive disease showed a relatively lower expression of proliferation-related genes and higher expression of luminal-related genes compared to the HER2 0 group (**Supplementary figure 4** and **Supplementary table 8, appendix 3**).

Figure 4. Gene expression profiles of HER2-negative breast cancer according to HER2 expression and HR status

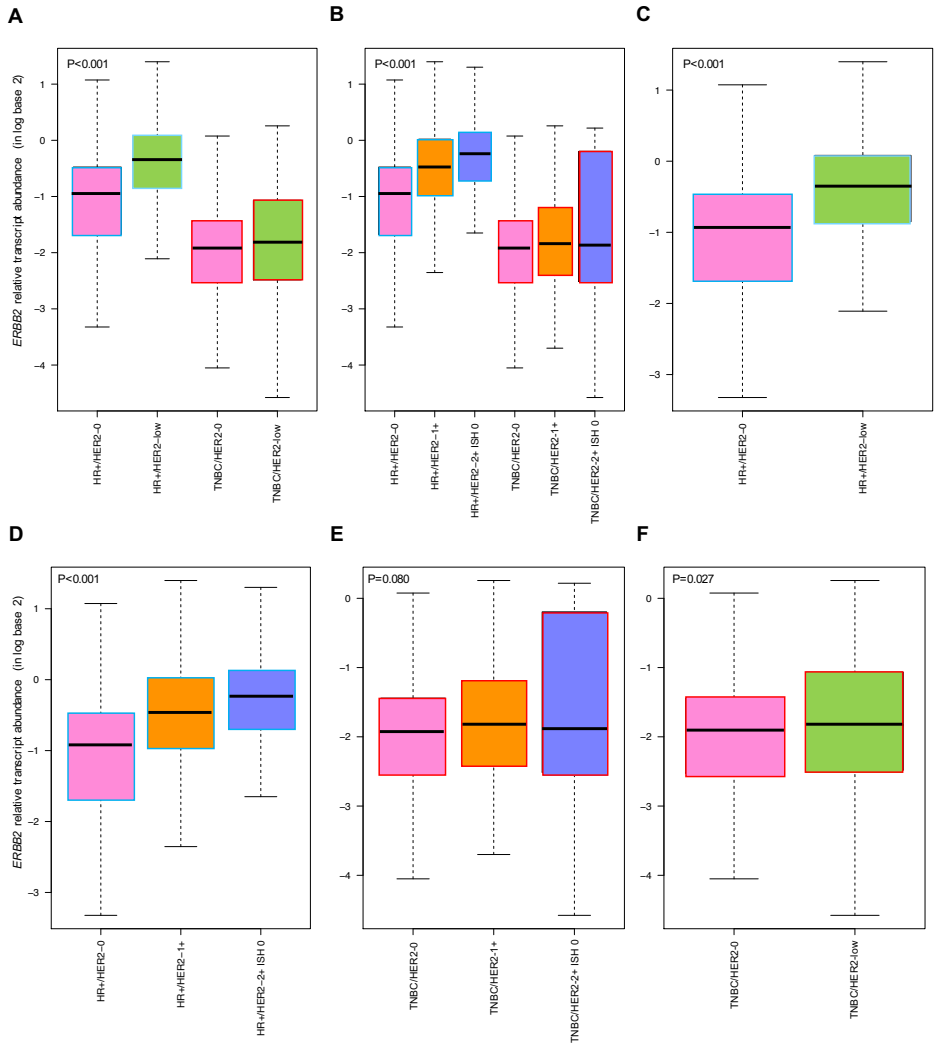


Legend. Supervised clustering of 55 genes across 4 tumor classes defined according to HER2 IHC expression and HR status. All samples and gene expression data in each category have been combined into a single group. For each gene in a group, we calculated the standardized mean difference between the gene's expression in that class vs. its overall mean expression in the dataset using a 4-class Significance Analyses of Microarrays. The red color represents relative high gene score, green represents relative low gene score, and black represents median gene score. **HR+:** hormone receptor positive; **TNBC:** triple negative breast cancer.

ERBB2 expression analysis

The previous observation that *ERBB2* levels differ according to HER2 IHC expression (HER2 0, 1+ and 2+) and HR status was somewhat unexpected. To further explore this finding, we formally compared the abundance of *ERBB2* in HR-positive disease and TNBC based on HER2 IHC expression. *ERBB2* levels were statistically significantly higher in HR-positive tumors compared to TNBC regardless of HER2 IHC expression (P<0.001; **Figure 5A-B**). Within HR-positive disease, *ERBB2* levels were significantly higher in HER2-low tumors compared to HER2 0 (1.4-fold mean difference, P<0.001, **Figure 5C**), with the highest amount observed in HER2 IHC 2+ tumors, followed by 1+ and 0 (**Figure 5D**), in decreasing order (1.7-fold mean difference between HER2 2+ vs. HER2 0). Within TNBC, there was no statistically significantly difference in *ERBB2* levels across the three HER2 IHC groups (P=0.080, **Figure 5E**); however, TNBC/HER2-low tumors showed statistically significantly higher levels of *ERBB2* compared to HER2 0 tumors (P=0.027), although the absolute mean difference was very small (**Figure 5F**).

Figure 5. *ERBB2* mRNA levels within the overall, HR-positive and TNBC populations according to HER2-low expression

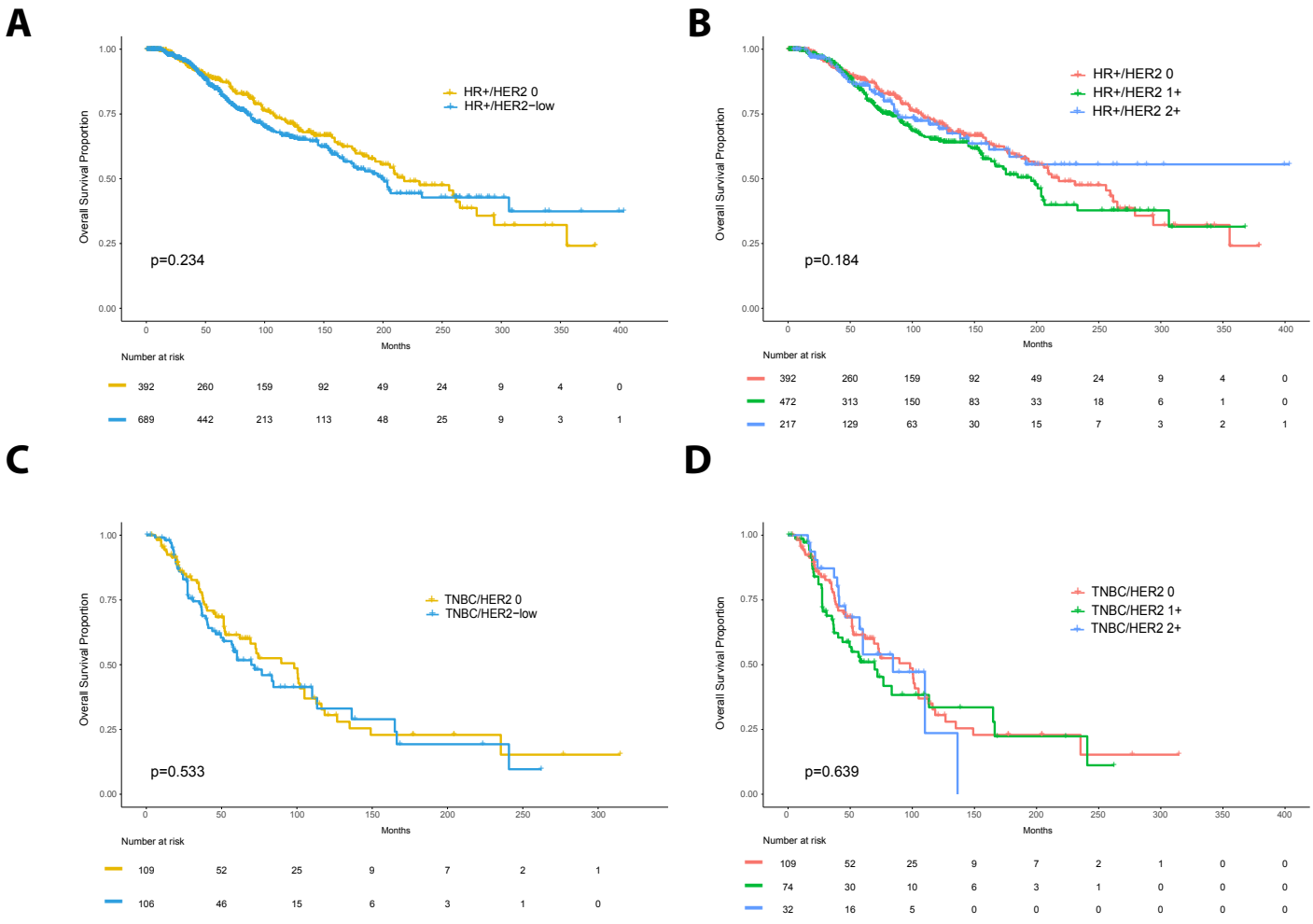


Legend. Relative transcript abundance of *ERBB2* (HER2 gene) within the overall population (n=871) and within HR-positive disease (n=494) and TNBC (n=377) according to HER2 IHC-based expression. The boxes represent the interquartile range (25th and 75th percentiles), and the horizontal line in the box represents the median value. The whiskers show the range of largest and smallest values. **HR+:** hormone receptor-positive; **TNBC:** triple-negative breast cancer.

Prognosis of HER2-low in advanced HER2-negative breast cancer

We conducted an exploratory overall survival (OS) analysis in 1,304 patients with advanced breast cancer across 2 datasets (i.e. Memorial Sloan Kettering Cancer Center database[18] and Hospital Clinic internal database). OS was defined from the date of the first diagnosis of breast cancer. The median follow-up for the overall population was 90.3 months (95% confidence interval [CI]: 84.6 – 99.4). In all patients, no statistically significantly differences in OS were observed between the HER2-low and HER2 0 groups (*P*=0.787). Similar results were obtained according to HR status and HER2 IHC levels (**Figure 6**).

Figure 6. Overall survival in patients with advanced HER2-negative breast cancer according to HER2 expression



Legend and captions: The figure shows Kaplan-Meier curves of overall survival for HER2-low vs HER2 0 tumors in the HR+ (A) and TNBC (C) populations, as well as OS curves for HER2 2+ vs. HER2 1+ vs. HER2 0 tumors for the HR+ (B) and TNBC (D) populations with number at risk shown at the bottom of each box. P values for log-rank tests are also reported; HR+: hormone receptor positive; TNBC: triple-negative.

Discussion

Our results provide preliminary insights of the clinical and molecular characteristics of HER2-low breast cancer. According to our results, patients with HER2-low disease represent the vast majority (59.7%) of patients with HER2-negative tumors. Clinically, HER2-low breast cancer is apparently more frequent in older and male patients and shows more axillary lymph-node involvement compared to HER2 0 disease. Importantly, we observed that HR status has an important role in HER2-low disease. For example, the frequency of HER2-low disease is higher in HR-positive breast cancer than TNBC (65.4% vs. 36.6%) and most HER2-low tumors are HR-positive (88.2%) or Luminal A or B (79.6%). Another important result of our study is that the vast majority (67.6%) of HER2-low tumors have an IHC 1+ score, regardless of HR status. Interestingly, when HR-positive disease and TNBC are divided according to the HER2 IHC score, no significant difference in subtype distribution is observed in TNBC, which was characterized by a high prevalence of the Basal-like subtype (84.7%), followed by the HER2-E (8.5%) subtype. On the contrary, HR-positive/HER2-low tumors appeared to be characterized by a higher proportion of luminal subtypes compared to HER2 0 tumors. Of note, the HER2-E subtype was infrequent and similarly distributed in HER2-low and HER2 0 breast cancer.

As expected, the differences in subtype distribution according to HER2 IHC expression and HR status are consistent with the observed changes in expression of individual genes. For example, the vast majority of proliferation-related genes and tyrosine-kinase receptor genes are found more expressed in HER2 0 tumors compared to HER2-low tumors, while HER2-low tumors have more expression of luminal-related genes. This finding is especially relevant in HR-positive disease. On the contrary, no clear biological differences are observed in TNBC according to HER2 IHC expression. Overall, these findings suggest that HR-positive/HER2-low tumors are a more distinct biological entity compared to TNBC/HER2-low tumors.

The lack of enrichment of the HER2-E subtype within HER2-low disease is intriguing and somewhat unexpected. However, previous studies have shown that the HER2-E phenotype is not defined by the expression of a single gene such as *ERBB2*. In fact, we and others have previously shown that the two variables (i.e. HER2-E subtype and *ERBB2* levels) provide independent predictive and prognostic information [19]. Overall, this finding clearly highlights the need to separate expression of single genes or receptors from the underlying tumor phenotype.

Recent studies have opened up a new therapeutic scenario by showing potent activity of HER2-targeted novel ADCs in HER2-low breast cancer [8]. To date, T-DXd, a trastuzumab conjugated to 8 molecules of deruxtecan, a topoisomerase I inhibitor, is at the most advanced in clinical development. A recently published phase Ib study enrolling highly pretreated patients with advanced HER2-expressing/mutated solid tumors, including HER2-low breast cancer, revealed a remarkable overall response rate (ORR) of 37.0% (95% CI: 24.3% - 51.3%) in HER2-low breast cancer and an impressive median duration of response of 10.4 months (95% CI: 8.8 month - not evaluable), with no apparent differences in ORR between 1+ and 2+ IHC tumors (35.7% vs 38.5%) [9]. Interestingly, the ORR did seem to differ according to HR status (40.4% in HR-positive disease and 14.3% in TNBC). This result is concordant with our findings that *ERBB2* levels are more expresses in HR-positive /HER2-low tumors than in TNBC/HER2-low tumors. A phase III trial specifically enrolling patients with HER2-low metastatic breast cancer (i.e. NCT03734029/DESTINY-Breast04) is ongoing. Importantly, we previously demonstrated in HER2-positive disease that *ERBB2* mRNA levels might provide a better selection of patients that benefit to the ADC T-DM1 [20]. This might also be the case for HER2-low tumors and might be worth focusing on this aspect in further studies.

SYD985 is another ADC comprised of trastuzumab covalently bound to a linker drug containing duocarmycin. This drug also showed a promising ORR of 28% and 40% in HR-positive/HER2-low and TNBC/HER2-low, respectively [21]. In addition, other anti-HER2 ADCs (i.e. PF-06804103, MEDI4276 and XMT-1522) have shown promising activity in HER2-low tumors in the preclinical setting [8,22], and phase 1 clinical trials are ongoing (clinicaltrials.gov identifier: NCT03284723, NCT02564900 and NCT02952729, respectively).

Tumors with high *ERBB2* mRNA levels, but overall HER2-negative, might also benefit from novel tumor vaccines targeted against the HER2 protein, as shown by a recent randomized phase II trial of HER2-targeted vaccine nelipepimut-S combined with trastuzumab as adjuvant treatment in HER2-low high-risk breast cancer [23]. In this direction, we observed higher levels of TILs in the HER2 2+ group compared to the HER2 0 and 1+ groups, although this analysis was based on a very restricted number of cases. Further studies are needed to study the immune compartment of HER2-low breast cancer.

Our study presents limitations that need attention. First, we retrospectively combined patients from databases pertaining to different studies, with different original purposes and inclusion/exclusion criteria; therefore, patients were not consecutively enrolled and a large proportion of them had metastatic disease. These might explain some of the imbalances that we observed between groups. Additionally, HER2 IHC status was not evaluated centrally; thus, inter-pathologist variability might have affected the results. Moreover, criteria for defining negative or equivocal *ERBB2* amplification

have changed over time [1,2] and most *ERBB2* amplification results were only available in qualitative form (i.e. amplified, not amplified or equivocal). Another limitation is that we did not address intra-tumor HER2 heterogeneity, which represents 1%-34% of all breast tumors [24] and has clinical and prognostic implications, with poor response to anti-HER2-based regimens and worse prognosis, compared to HER2-positive tumors [24]. However, this feature is more common in HER2 equivocal disease [24], a condition that was an exclusion criteria in our study, somewhat mitigating this issue. Finally, we limited our genomic analysis to the PAM50 genes and 5 additional genes. Thus, broader genomic analyses are likely to shed more light on this topic.

To our knowledge, this is the first comprehensive study focused specifically on HER2-low breast tumors. We provided extensive comparisons among the three different IHC-based classes of HER2-negative breast cancer and according to HR status. We found that HER2-low breast tumors are complex and heterogeneous, with no specific prognostic implications and HR-positive/HER2-low emerge as a more distinct biological entity compared to the other groups. In addition, the evidence of *ERBB2* levels being higher in HER2-low/HER2 2+ tumors (especially in the HR-positive) compared to HER2 1+/0 is in line with some previous findings from single institutions-based studies, and contributes to reassure about the reliability of our results [25,26]. Similarly, the high prevalence of luminal disease in HER2-low disease has also been observed in other studies [24]. Finally, the concordance analysis of HER2 scoring by different pathologists showed an almost perfect agreement for HER2 0 and 3+ scores; however, the agreement for the HER2 1+ and 2+ categories was only substantial, according to Landis and Koch interpretation [27]. This result clearly suggests that more efforts are needed to standardize the scoring of HER2-low disease and potentially implement new and more sensitive assays that can help better discriminate HER2 levels within HER2-negative breast cancer.

Methods

Patients datasets

All non-overlapping publicly available breast datasets (i.e. 12 studies and 6,477 patients) were interrogated from the cBio Cancer Genomics Portal (http://cbioportal.org). From these databases, HER2-negative tumors with known IHC and HER2 amplification status were extracted [10-13]. Other patients were extracted from internal databases from the Hospital Clinic (Barcelona, Spain), from two SOLTI clinical trials (SOLTI 1501-VENTANA and SOLTI 1402-CORALEEN) [14,15], from the Spanish Cancer Research Group (GEICAM)/CIBOMA study [16] and from a previously published collaboration between Hospital Clinic (Barcelona, Spain), Hospital Vall d'Hebron (Barcelona, Spain), University Campus Bio-Medico (Roma, Italy) and GEICAM [17] (see **Supplementary table 9** in **appendix 3** for study details). All studies had received proper ethical

approval by the local institutional research ethics committee of all participating institutions and patients had given their consent to participate.

Inclusion criteria

Patients were included if they were HER2-negative with known IHC and HER2 amplification status and if they had at least one of the following information available: 1) clinicopathological features, 2) PAM50 gene expression data and 3) PAM50 intrinsic subtype identified. The following clinical-pathological features were evaluated, when available: Ki67 IHC, histological grade, estrogen receptor and progesterone receptor status, age at diagnosis, menopausal status, tumor sample origin (primary versus metastatic), histological subtype and TILs.

IHC-based classification

Tumors were divided into HR-positive (i.e. ER and/or PgR ≥1%) or TNBC, defined as ER<1% and PgR<1%. In addition, tumors were classified into HER2 0, in case of an IHC score of 0, and HER2-low, defined as HER2 IHC of 1+ or 2+ with an HER2 amplification negative result by in-situ hybridization (ISH) techniques. HER2 IHC 0 and 1+ were considered HER2 0 and HER2-low, respectively, unless ISH-based data was available and reported as HER2-amplified. HER2 status in each cohort had been previously determined using standard FDA-approved antibodies and ISH-techniques and classified according to the ASCO/CAP guidelines [1,2]. Whenever available, we interpreted ISH-derived HER2/CEP17 ratio value and *ERBB2* copy number results jointly with HER2 IHC score, according to last ASCO/CAP guidelines [1]. More specifically, tumors with an average HER2 copy number <4.0 signals/cell, were considered HER2-negative, and also HER2-low in case of an IHC score of 1+ or 2+, irrespective of the HER2/CEP17 ratio. However, if the HER2/CEP17 ratio was ≥2.0 and HER2 IHC 3+, tumors were considered HER2-positive and excluded [1].

In case of available average HER2 copy number ≥4.0 and <6.0 signals/cell without HER2/CEP ratio and an IHC 3+, the tumor was considered positive and excluded. In case of IHC 0 or 1+, the tumor was considered HER2-negative, and also HER2-low in the latter case [1]. In case of IHC 2+, considering the unfeasibility of a retesting, in our case, if the categorization HER2-positive/negative was available from the original dataset, it was adopted and the tumor was considered HER2-negative and HER2-low. If the categorization was not provided, the sample was excluded.

In case of IHC score 0, 1+ or 2+ and a concurrent average HER2 copy number ≥4.0 and <6.0 signals/cell, with HER2/CEP17 ratio <2.0, the tumor was considered HER2-negative, and HER2-low in the last 2 cases. On the contrary, if the HER2/CEP17 watio was ≥2.0, the tumor was considered HER2-positive and excluded [1].

In case of HER2 copy number ≥6.0 signals/cell, the tumor was considered HER2-positive and excluded in case of IHC of 2+ or 3+, regardless of the HER2/CEP17 ratio result, but in case of HER2/CEP17 ratio <2.0 and IHC 0 or 1+, the tumor was considered negative, and also HER2-low in the second case [1].

Patients with a persistent HER2 equivocal result were excluded [1].

To evaluate the concordance of the HER2 IHC categories among pathologists, we performed an inter-pathologist concordance analysis across 100 independent cases of HER2 staining (HER2 0, 1+, 2+ and 3+). Five independent breast cancer specialized pathologists (i.e. BG, ES, RF, VP and GP) from 4 institutions (i.e. Clinic, VHIO, HVH and Campus Bio-Medico) were involved. Blinded scores were provided to FS and AP, who performed the concordance analysis.

PAM50 subtypes and gene expression data

We obtained PAM50 subtype information and individual gene expression data from 9 of the 13 retrospective cohorts (Hospital Clinic internal series, SOLTI and GEICAM trials reported in the **appendix 3, Supplementary Table 9**). An nCounter-based research version of PAM50 had been previously used [28,29]. Intrinsic subtypes and raw gene expression data had been obtained from formalin-fixed paraffin-embedded (FFPE) tumor samples. For RNA purification (Roche High Pure FFPET RNA isolation kit), at least 1 to 3 10-µm FFPE slides had been used for each tumor specimen, and macrodissection performed, when needed, to avoid normal breast tissue contamination. A minimum of approximately 150 ng of total RNA had been used to measure the expression of 50 breast cancer–related genes, 4 immune-related genes, androgen receptor gene (full gene list included in the **appendix 3**, **Supplementary table 5**) and 5 housekeeping genes (*ACTB*, *MRPL19*, *PSMC4*, *RPLP0* and *SF3A1*) using the nCounter platform (NanoString Technologies, Seattle WA) [28,30]. Data had been log base 2 transformed and normalized using the 5 housekeeping genes. Intrinsic subtyping (Luminal A, Luminal B, HER2-E, Basal-like and Normal-like) had been previously performed using the research-based PAM50 intrinsic subtype predictor [29]. We also retrieved intrinsic subtypes from the publicly available TCGA database (see *Data Availability* section for further information).

Statistical analysis

Patient and tumor characteristics were analyzed using chi square (χ^2) test, Fisher's exact test, Kruskalis-Wallis and Wilcoxon rank sum test with continuity correction, where appropriate. The concordance analysis among pathologists was performed using the Fleiss' Kappa. The agreement

among pathologists was considered poor for k<0, low for k=0.01-0.20, fair for k=0.21-0.40, moderate for k=0.41-0.60, substantial for k=0.61-0.80 and almost perfect for k=0.81-1.00 [27].

All differences were considered significant at $P<0.05$. Bonferroni–Holm method was used to control the family-wise error rate in case of multiple comparisons.

OS was evaluated for patients with homogeneous follow-up with available or computable survival data. Such patients pertained to the Memorial Sloan Kettering Cancer Center (MSKCC)'s subset of the cBio Cancer Genomics Portal group and to the Hospital Clinic of Barcelona subset. All patients were affected by metastatic disease and presented available information regarding primary tumor diagnosis.

The OS distributions were estimated using the Kaplan-Meier method and the log-rank test was used to assess the difference in survival distribution between the groups [31]. Censoring was done at the date of last available follow-up. Significance Analysis of Microarray (SAM) for unpaired samples (multiclass and 2 class) was used to compare gene expression profiles between groups [32]. Differences were considered significant at an FDR<5%. All analyses were performed with R version 3.6.1 [33], Cluster 3.0, Javatreeview 1.1.6r4 [34] and Microsoft Excel.

Code availability

R codes are available from the corresponding author on reasonable request.

Data availability

This study involved the collection and analysis of clinicopathological and PAM50 gene expression data from multiple publicly available datasets[35-46]. The following cBioPortal datasets were used:

https://identifiers.org/cbioportal:breast_msk_2018;

https://identifiers.org/cbioportal:bfn_duke_nus_2015;

https://identifiers.org/cbioportal:brca_mskcc_2019;

https://identifiers.org/cbioportal:brca_bccrc_xenograft_2014;

https://identifiers.org/cbioportal:brca_bccrc;

https://identifiers.org/cbioportal:brca_broad;

https://identifiers.org/cbioportal:brca_sanger;

https://identifiers.org/cbioportal:brca_tcga;

https://identifiers.org/cbioportal:brca_igr_2015;

https://identifiers.org/cbioportal:brca_metabric;

https://identifiers.org/cbioportal:brca_mbcproject_wagle_2017;

https://identifiers.org/cbioportal:acbc_mskcc_2015.

Data from the internal studies of the Hospital Clinic of Barcelona, and data from patients involved in the SOLTI and GEICAM trials included, are not publicly available to protect patient privacy, but will be made available on reasonable request from the corresponding author, Prof. Aleix Prat (email address: alprat@clinic.cat). An anonymized data file containing all PAM50 normalized gene expression data used for the genomic analyses of this study, is publicly available

in the figshare repository[47], with doi: https://doi.org/10.6084/m9.figshare.13171655. The complete version of the data file used and/or analyzed during the current study, is available upon reasonable request from the corresponding author, as described in the figshare data record above.

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Competing interests

Aleix Prat has declared an immediate family member being employed by Novartis, personal honoraria from Pfizer, Novartis, Roche, MSD Oncology, Lilly and Daiichi Sankyo, travel, accommodations and expenses paid by Daiichi Sankyo, research funding from Roche and Novartis, consulting/advisory role for NanoString Technologies, Amgen, Roche, Novartis, Pfizer and Bristol-Myers Squibb and patent PCT/EP2016/080056: HER2 AS A PREDICTOR OF RESPONSE TO DUAL HER2 BLOCKADE IN THE ABSENCE OF CYTOTOXIC THERAPY. Carlos Barrios declares Research Funding, Consulting and Honoraria form Astra Zeneca, Novartis, Roche, GSK, Pfizer, Libbs, Daiichi Sankyo and MSD. Ana Lluch declares clinical research fundings from Amgen, Astra Zeneca, Boehringer-Ingelheim, GSK, Novartis, Pfizer, Roche/Genentech, Eisai, Celgene, Pierre Fabre and advisory boards and consulting for Novartis, Pfizer, Roche/Genentech, Eisai, Celgene. Miguel Martín declares research grants from Roche, PUMA and Novartis, consulting/advisory fees from AstraZeneca, Amgen, Taiho Oncology, Roche/Genentech, Novartis, PharmaMar, Eli Lilly, PUMA, Taiho Oncology, Daiichi Sankyo and Pfizer and speakers' honoraria from AstraZeneca, Amgen, Roche/Genentech, Novartis and Pfizer. Joaquín Gavilá has declared speakers' honoraria and participation in advisory boards from Pfizer, Roche and Novartis. Sabino De Placido has declared honoraria from Roche, Pfizer, Astra-Zeneca, Novartis, Celgene, Eli Lilly, Amgen and Eisai. The other authors have nothing to declare.

Author contributions

FS and AP conceived the study. FS and LP performed the statistical analyses. All authors contributed to the interpretation of results, writing and/or critical revision of the manuscript and approved the final version.

References

1. Wolff, A. C. et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 36, 2105–2122 (2018).
2. Wolff, A. C. et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 31, 3997–4013 (2013).
3. Cronin, K. A., Harlan, L. C., Dodd, K. W., Abrams, J. S. & Ballard-Barbash, R. Population-based estimate of the prevalence of HER-2 positive breast cancer tumors for early stage patients in the US. Cancer Invest. 28, 963–968

(2010).
4. Slamon, D. J. et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235, 177–182 (1987).
5. Schalper, K. A., Kumar, S., Hui, P., Rimm, D. L. & Gershkovich, P. A retrospective population-based comparison of HER2 immunohistochemistry and fluorescence in situ hybridization in breast carcinomas: impact of 2007 American Society of Clinical Oncology/College of American Pathologists criteria. Arch. Pathol. Lab. Med. 138, 213–219 (2014).
6. Fehrenbacher, L. et al. NSABP B-47/NRG Oncology Phase III Randomized Trial Comparing Adjuvant Chemotherapy With or Without Trastuzumab in High-Risk Invasive Breast Cancer Negative for HER2 by FISH and With IHC 1+ or 2. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 38, 444–453 (2020).
7. Iwata, H. et al. Trastuzumab deruxtecan (DS-8201a) in subjects with HER2-expressing solid tumors: Long-term results of a large phase 1 study with multiple expansion cohorts. J. Clin. Oncol. 36, 2501–2501 (2018).
8. Rinnerthaler, G., Gampenrieder, S. P. & Greil, R. HER2 Directed Antibody-Drug-Conjugates beyond T-DM1 in Breast Cancer. Int. J. Mol. Sci. 20(5):1115, (2019).
9. Modi, S. et al. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low-Expressing Advanced Breast Cancer: Results From a Phase Ib Study. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. JCO1902318–JCO1902318 (2020) doi:10.1200/JCO.19.02318.
10. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 490, 61–70 (2012).
11. Ciriello, G. et al. Comprehensive molecular portraits of invasive lobular breast cancer. Cell 163, 506–519 (2015).
12. The Metastatic Breast Cancer Project. https://www.mbcproject.org/.
13. Razavi, P. et al. The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers. Cancer Cell 34, 427-438.e6 (2018).
14. Adamo, B. et al. Oral metronomic vinorelbine combined with endocrine therapy in hormone receptor-positive HER2-negative breast cancer: SOLTI-1501 VENTANA window of opportunity trial. Breast Cancer Res. BCR 21, 108 (2019).
15. Prat, A. et al. Ribociclib plus letrozole versus chemotherapy for postmenopausal women with hormone receptor-positive, HER2-negative, luminal B breast cancer (CORALLEEN): an open-label, multicentre, randomised, phase 2 trial. Lancet Oncol. 21, 33–43 (2020).
16. Lluch, A. et al. Phase III Trial of Adjuvant Capecitabine After Standard Neo-/Adjuvant Chemotherapy in Patients With Early Triple-Negative Breast Cancer (GEICAM/2003-11_CIBOMA/2004-01). J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 38, 203–213 (2020).
17. Fernandez-Martinez, A. et al. Limitations in predicting PAM50 intrinsic subtype and risk of relapse score with Ki67 in estrogen receptor-positive HER2-negative breast cancer. Oncotarget 8, 21930–21937 (2017).
18. Cerami, E. et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2, 401–404 (2012).
19. Prat, A. et al. HER2-enriched subtype and ERBB2 expression in HER2-positive breast cancer treated with dual HER2 blockade. J. Natl. Cancer Inst. (2019) doi:10.1093/jnci/djz042.
20. Griguolo G. et al. ERBB2 mRNA Expression and Response to Ado-Trastuzumab Emtansine (T-DM1) in HER2-Positive Breast Cancer. Cancers (Basel) 2020; 12(7):1902. doi: 10.3390/cancers1207190.
21. Banerji, U. et al. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing

breast cancer: a phase 1 dose-escalation and dose-expansion study. Lancet Oncol. 20, 1124–1135 (2019).

22. Graziani, E. I. et al. PF-06804103, a site-specific anti-HER2 antibody-drug conjugate for the treatment of HER2-expressing breast, gastric, and lung cancers. Mol. Cancer Ther. molcanther.0237.2020 (2020) doi:10.1158/1535-7163.MCT-20-0237.
23. Clifton, G. T. et al. Results of a randomized phase IIb trial of nelipepimut-S + trastuzumab vs trastuzumab to prevent recurrences in high-risk HER2 low-expressing breast cancer patients. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. (2020) doi:10.1158/1078-0432.CCR-19-2741.
24. Marchiò, C. et al. Evolving concepts in HER2 evaluation in breast cancer: Heterogeneity, HER2-low carcinomas and beyond. Semin. Cancer Biol. (2020) doi:10.1016/j.semcancer.2020.02.016.
25. Marchiò, C. et al. The Dilemma of HER2 Double-equivocal Breast Carcinomas: Genomic Profiling and Implications for Treatment. Am. J. Surg. Pathol. 42, 1190–1200 (2018).
26. Gupta, S. et al. Quantitative assessments and clinical outcomes in HER2 equivocal 2018 ASCO/CAP ISH group 4 breast cancer. NPJ Breast Cancer 5, 28–28 (2019).
27. Landis, J. R. & Koch, G. G. The measurement of observer agreement for categorical data. Biometrics 33, 159–174 (1977).
28. Geiss, G. K. et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat. Biotechnol. 26, 317–325 (2008).
29. Prat, A. et al. Prognostic Value of Intrinsic Subtypes in Hormone Receptor-Positive Metastatic Breast Cancer Treated With Letrozole With or Without Lapatinib. JAMA Oncol. 2, 1287–1294 (2016).
30. Prat, A., Ellis, M. J. & Perou, C. M. Practical implications of gene-expression-based assays for breast oncologists. Nat. Rev. Clin. Oncol. 9, 48–57 (2011).
31. Armitage P, Berry G, Matthews JNS (2001) Statistical Methods in Medical Research (4th Edition). Blackwell Science: Oxfor
32. R: Significance Analysis of Microarray. http://ugrad.stat.ubc.ca/R/library/siggenes/html/sam.html.
33. R Core Team. R: A language and environment for statistical computing. (R Foundation for Statistical Computing, 2017).
34. Eisen, M. B., Spellman, P. T., Brown, P. O. & Botstein, D. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. U. S. A. 95, 14863–14868 (1998).
35. Razavi, P. et al. Targeted Sequencing of tumor/normal sample pairs from 1918 Breast cancers. The cBioPortal for Cancer Genomics https://identifiers.org/cbioportal:breast_msk_2018 (2018).
36. Tan, J. et al. Whole exome sequencing of 22 phyllodes tumors. The cBioPortal for Cancer Genomics https://identifiers.org/cbioportal:bfn_duke_nus_2015 (2015).
37. Nixon, J. M. et al. Targeted Sequencing of buparlisib + letrozole and alpelisib + letrozole-treated metastatic ER+ unmatched breast tumors. The CBioPortal for Cancer Genomicshttps://identifiers.org/cbioportal:brca_mskcc_2019 (2019).
38. Eirew, P. et al. Whole genome/targeted sequencing to evaluate the clonal dynamics in 116 breast cancer patient xenografts. The cBioPortal for Cancer Genomicshttps://identifiers.org/cbioportal:brca_bccrc_xenograft_2014 (2015).
39. Shah, P. S. et al. Whole genome/exome sequencing analysis of 65 breast cancer samples. The cBioPortal for Cancer Genomics. https://identifiers.org/cbioportal:brca_bccrc (2012).

40. Banerji, S. et al. Whole-exome sequencing of 103 breast cancer tumor/normal sample pairs. Generated by the Broad Institute. The cBioPortal for Cancer Genomicshttps://identifiers.org/cbioportal:brca_broad (2012).
41. Stephens, J. P. et al. Whole exome sequencing from 100 breast cancer tumor/normal sample pairs. Generated by the Sanger Institute. The cBioPortal for Cancer Genomicshttps://identifiers.org/cbioportal:brca_sanger (2012).
42. TCGA Breast Invasive Carcinoma. Source data from GDAC Firehose. Previously known as TCGA Provisional. The cBioPortal for Cancer Genomics https://identifiers.org/cbioportal:brca_tcga(2016).
43. Lefebvre, C. et al. Whole exome sequencing of 216 tumor/normal (blood) pairs from metastatic breast cancer patients who underwent a biopsy in the context of the SAFIR01/SAFIR02 (Unicancer, France), SHIVA (Institut Curie, France) or MOSCATO (Gustave Roussy, France) prospective trials. The cBioPortal for Cancer Genomicshttps://identifiers.org/cbioportal:brca_igr_2015 (2016).
44. Pereira, B. et al. Targeted sequencing of 2509 primary breast tumors with 548 matched normal.The cBioPortal for Cancer Genomics https://identifiers.org/cbioportal:brca_metabric (2016).
45. The Metastatic Breast Cancer Project (Provisional, February 2020). The cBioPortal for Cancer Genomics https://identifiers.org/cbioportal:brca_mbcproject_wagle_2017 (2020).
46. Martelotto, G. L. et al. Whole exome sequencing of 12 breast AdCCs. The cBioPortal for Cancer Genomics https://identifiers.org/cbioportal:acbc_mskcc_2015 (2015).
47. Schettini, F. et al. Data and metadata supporting the article: Clinical, pathological and PAM50 gene expression features of HER2-low breast cancer. figshare https://doi.org/10.6084/m9.figshare.13171655 (2020).

CHAPTER 4: Endocrine treatment versus chemotherapy in postmenopausal women with hormone receptor-positive, HER2-negative, metastatic breast cancer: a systematic review and network meta-analysis

Original article reference

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Abstract

Background Although international guidelines support the administration of hormone therapies with or without targeted therapies in postmenopausal women with hormone receptor-positive, HER2-negative metastatic breast cancer, upfront use of chemotherapy remains common even in the absence of visceral crisis. Because first-line or second-line treatments, or both, based on chemotherapy and on hormone therapy have been scarcely investigated in head-to-head randomised controlled trials, we aimed to compare these two different approaches.

Methods We did a systematic review and network meta-analysis with a systematic literature search on PubMed, Embase, Cochrane Central Register of Clinical Trials, Web of Science, and online archives of the most relevant international oncology conferences. We included all phase 2 and 3 randomised controlled trials investigating chemotherapy with or without targeted therapies and hormone therapies with or without targeted therapies as first- line or second-line treatments, or both, in postmenopausal women with hormone receptor-positive, HER2-negative metastatic breast cancer, published between Jan 1, 2000, and Dec 31, 2017. Additional recently published randomised controlled trials relevant to the topic were also subsequently added. No language restrictions were adopted for our search. A Bayesian network meta-analysis was done to compare hazard ratios (HRs) for progression-free survival (the primary outcome), and to compare odds ratios (ORs) for the proportion of patients achieving an overall response (the secondary outcome). All treatments were compared to anastrozole and to palbociclib plus letrozole. This study is registered in the Open Science Framework online public database, registration DOI 10.17605/OSF.IO/496VR.

Findings We identified 2689 published results and 140 studies (comprising 50 029 patients) were included in the analysis. Palbociclib plus letrozole (HR 0.42; 95% credible interval [CrI] 0.25–0.70), ribociclib plus letrozole (0.43; 0.24–0.77), abemaciclib plus anastrozole or letrozole (0.42; 0.23–0.76), palbociclib plus fulvestrant (0.37; 0.23–0.59), ribociclib plus fulvestrant (0.48; 0.31–0.74), abemaciclib plus fulvestrant (0.44; 0.28–0.70), everolimus plus exemestane (0.42; 0.28–0.67), and, in patients with a *PIK3CA* mutation, alpelisib plus fulvestrant (0.39; 0.22–0.66), and several chemotherapy-based regimens, including anthracycline and taxane-containing regimens, were associated with better progression-free survival than was anastrozole alone. No chemotherapy or hormone therapy regimen was significantly better than palbociclib plus letrozole for progression-free survival. Paclitaxel plus bevacizumab was the only clinically relevant regimen that was significantly

better than palbociclib plus letrozole in terms of the proportion of patients achieving an overall response (OR 8.95; 95% CrI 1.03–76.92).

Interpretation In the first-line or second-line setting, CDK4/6 inhibitors plus hormone therapies are better than standard hormone therapies in terms of progression-free survival. Moreover, no chemotherapy regimen with or without targeted therapy is significantly better than CDK4/6 inhibitors plus hormone therapies in terms of progression-free survival. Our data support treatment guideline recommendations involving the new combinations of hormone therapies plus targeted therapies as first-line or second-line treatments, or in both settings, in women with hormone receptor-positive, HER2-negative metastatic breast cancer.

Introduction

The most common subtype of metastatic breast cancer is hormone receptor-positive, HER2-negative breast cancer, accounting for approximately 65% of all metastatic breast tumours [1,2]. Despite a favourable prognosis relative to other subtypes of metastatic breast cancer, outcomes of hormone receptor-positive, HER2-negative metastatic breast cancer remain poor, with a median overall survival of 36 months [2,3]. The oestrogen receptor signalling pathway is the main driver of cancer cell growth and survival in these tumours, so endocrine-based therapies are considered the most effective treatments [2]. In the past decade, randomised controlled trials have led to the introduction of several innovative therapeutic strategies into clinical practice,consisting of new targeted therapies combined with hormone treatments, both in endocrine-sensitive and endocrine-resistant metastatic breast cancer. The most relevant examples of these new targeted therapies are the mTOR inhibitor everolimus and the CDK4/6 inhibitors palbociclib, ribociclib, and abemaciclib, which are used in combination with hormone therapies. Pivotal randomised controlled trials have proven the efficacy of these combinations as first and subsequent lines of treatment for postmenopausal patients with hormone receptor-positive, HER2-negative metastatic breast cancer, with substantial improvements in patient outcomes (4-10). As a result, according to all major international oncology guidelines, a sequence of endocrine-based treatments should be the preferred strategy in hormone receptor-positive, HER2-negative metastatic breast cancer, except in instances of life-threatening visceral disease or visceral crisis [11-14]. Nevertheless, real-world data suggest that upfront use of chemotherapy is still common, even in the absence of visceral crisis [15-18]. This treatment approach might be partly due to the paucity of direct comparisons among hormone therapies and chemotherapy-based regimens for this subtype of metastatic breast cancer. To provide additional evidence to guide treatment choices in postmenopausal patients with hormone receptor-positive, HER2-negative metastatic breast cancer, we did a comprehensive systematic review and network meta-analysis to evaluate the efficacy and activity of several hormone therapy and chemotherapy regimens that have been investigated in randomised controlled trials as first-line or second-line treatments, or both [19].

Methods

Search strategy and selection criteria

For this systematic review and network meta-analysis we searched the literature on Jan 2, 2018, to identify published phase 2 and 3 randomised controlled trials evaluating the anti-tumour activity or clinical efficacy, or both, of chemotherapy with or without targeted therapies and of hormone therapies with or without targeted therapies in postmenopausal (physiological or induced by gonadotropin-releasing hormone analogues or surgery) hormone receptor-positive, HER2-negative

metastatic breast cancer, as first-line or second-line treatments, or both. The literature search was restricted to trials published from Jan 1, 2000, to Dec 31, 2017. Additional recently published randomised controlled trials relevant to the topic were added after their publication: MONALEESA 3 in August, 2018, when the main article was published; BOLERO-6 in June, 2018, when the main article was published; and SOLAR1 in October, 2018, when it was presented at the European Society of Medical Oncology (ESMO) meeting (**appendix 4, full reference list**). Randomised controlled trials exclusively enrolling premenopausal patients and those with HER2-positive or triple-negative breast cancer were excluded from the analysis. The recommendations of the Cochrane Collaboration were followed to identify all relevant randomised controlled trials [20]. The full list of search terms is provided in the **appendix 4**; we used a combination of disease characteristics, study design, treatment setting, and strategies or drugs as search terms. We searched PubMed, Embase, Cochrane Central Register of Clinical Trials, and Web of Science, as well as American Society of Clinical Oncology (ASCO) and ESMO annual meetings and San Antonio Breast Cancer Symposiums (SABCS) online archives. Some records were also retrieved via cross-references from published trials, the main international oncology guidelines, and most updated reviews or meta-analyses of therapeutic strategies in hormone receptor-positive, HER2-negative metastatic breast cancer [11-14,21-24]. Phase 2 or 3 randomised controlled trials published in the form of full papers, or as abstracts if full papers were not available, were included in the analysis. No language restrictions were adopted for our search. Two reviewers (FS and MG) independently assessed whether each selected randomised controlled trial met the predetermined criteria, and a third reviewer (DG) was consulted in case of disagreement. Additional details about the search strategy are provided in the **appendix 4**. The full reference list is reported in the **appendix 4**.

Data analysis

Details about study design, patient characteristics, interventions, and previous treatments were extracted from each paper. When duplicate publications were identified, only the most recent and complete reports of randomised controlled trials were included. Hazard ratios (HR) and associated 95%CIs were extracted for progression-free survival and time to progression, when reported. Odds ratios (ORs) for the proportion of patients achieving an overall response, and associated 95%CIs, were also retrieved. These data had to be publicly available or computable from the included studies. The primary outcomes were progression-free survival (defined as the time from randomisation to either death or disease progression, whichever occurred first) and time to progression (defined as the interval from randomisation to tumour progression). If both endpoints were

reported in a randomised controlled trial, progression-free survival was selected for inclusion in the meta-analysis [25,26]. The proportion of patients achieving an overall response, defined according to Response Evaluation Criteria in Solid Tumors (RECIST), was selected as a secondary outcome [27]. We also did an exploratory analysis reporting the proportions of patients with grade 3–5 adverse events, according to Common Terminology Criteria for Adverse Events, version 4 [28].

Because of the heterogeneity of the studies included in the systematic review, a Bayesian random-effects network meta-analysis framework was used for each outcome, and results of the network meta-analysis are reported as HRs or ORs with 95% credible intervals (95% CrIs) [19]. The parameters of the different models were estimated by use of a Markov Chain Monte Carlo method as implemented in the WinBUGS software package [29]. For further verification, all analyses were also done with a fixed-effects approach. As expected, the random-effects model provided a better fit to the data than the fixed-effects model. We assessed the risk of bias for each trial using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions [20]. All analyses were done with WinBUGS (version 1.4.3) [29].

The internal validity of eligible studies was assessed according to the Cochrane Collaboration's Risk of Bias tool in Review Manager (version 5.3). Further details on the methods used are provided in the **appendix 4**.

The project is registered in the Open Science Framework (OSF) online public database, registration DOI 10.17605/OSF.IO/496VR.

Role of the funding source

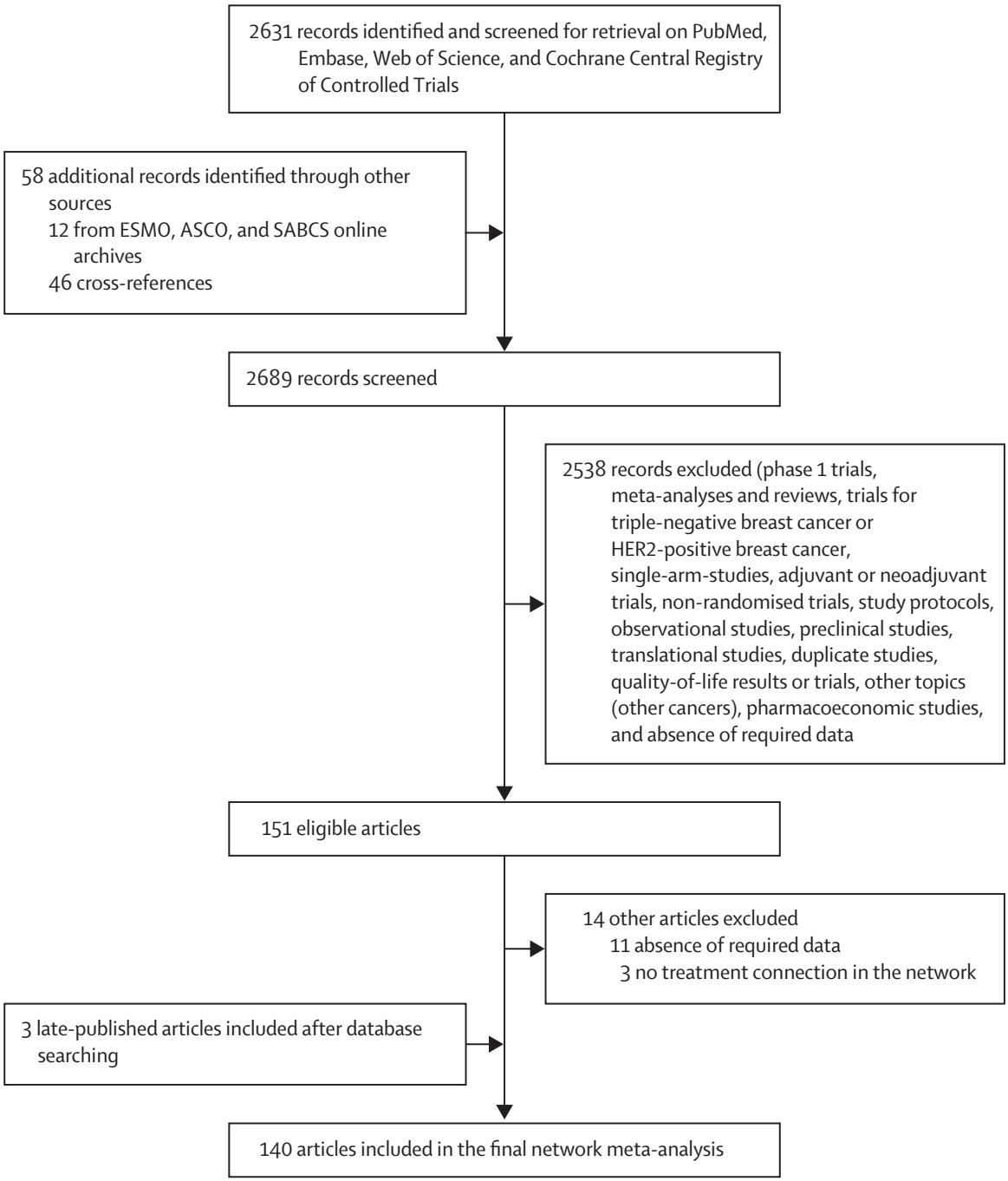
There was no funding source for this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Overall, 2,689 records were identified. 140 studies were selected as they met all the inclusion criteria and were includedinnetworkmeta-analyses (**figure 1**). A study by Dixon and colleagues was included in the meta-analysis even though the study was published in 1992, because it is the only study comparing hormone therapies with chemotherapy, aside from the BOLERO-6 trial, which was published after the initial search was done (**appendix 4**). Although randomised controlled trials specifically designed for triple-negative breast cancer were excluded from the analysis, as previously stated, several randomised controlled trials testing chemotherapy-containing regimens also included patients with triple-negative breast cancer. Moreover, older randomised controlled trials (published approximately before 2006) of hormone therapies enrolled patients with unknown hormone receptor

status. Three (2%) of 140 trials were single-centre studies, 130 (93%) were multicentre trials, and for the remaining seven (5%) trials the number of involved centres was not reported. A detailed description of all the studies included in the network meta-analysis, together with patient characteristics, is provided in the **appendix 4** (**table S1**).

Figure 1: Study selection



Legend. ASCO=American Society of Clinical Oncology. ESMO=European Society of Medical Oncology. HR=hazard ratio. OR=odds ratio. SABCS=San Antonio Breast Cancer Symposiums.

Among the 140 selected randomised controlled trials, 114 (81%) were included in a network created to analyse HRs for progression-free survival and time to progression (**figure 2**), and 135 (96%) were included in a network created to analyse ORs for the proportion of patients achieving an overall response (**figure 3**; full reference list in **appendix 4**).

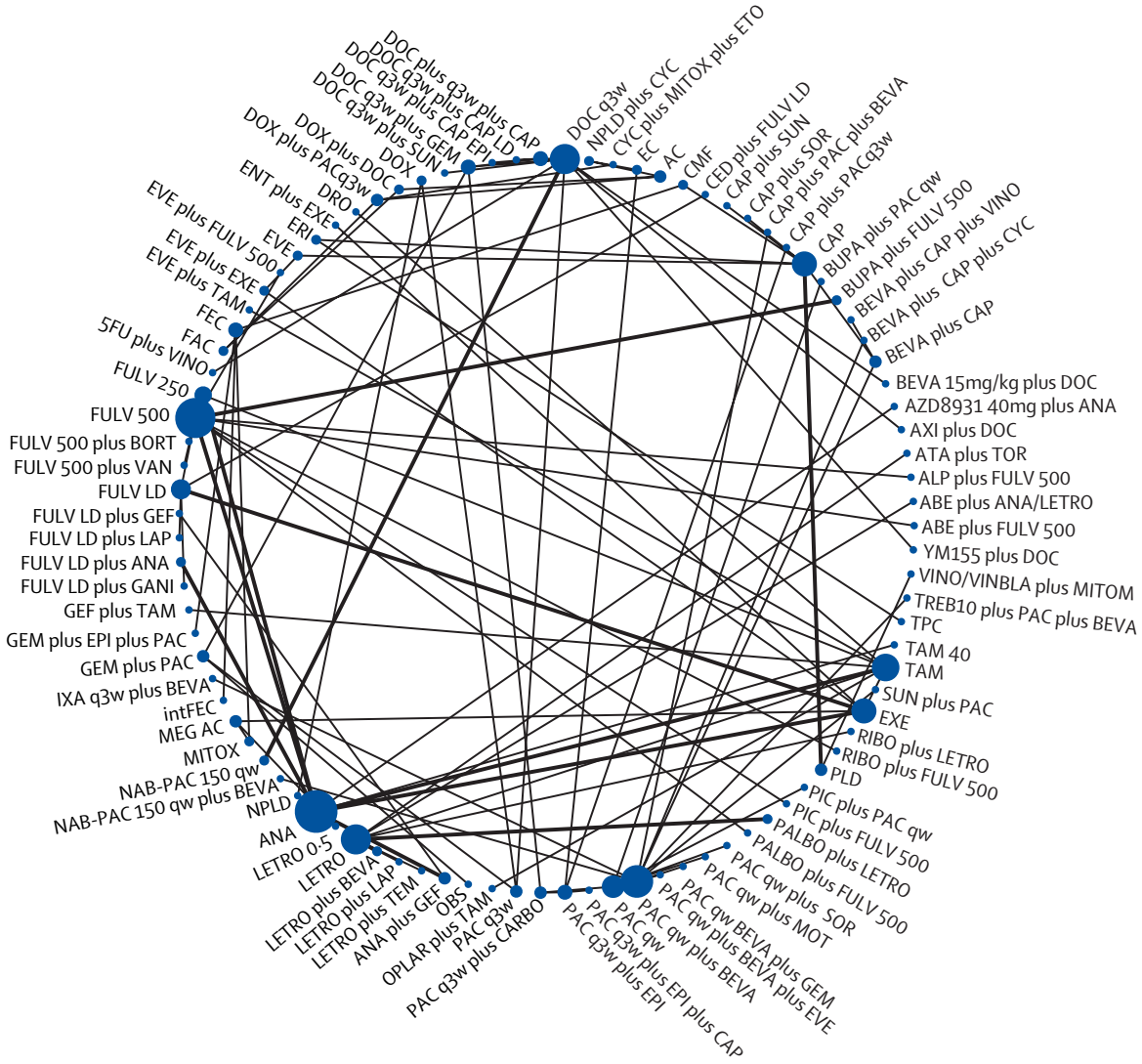
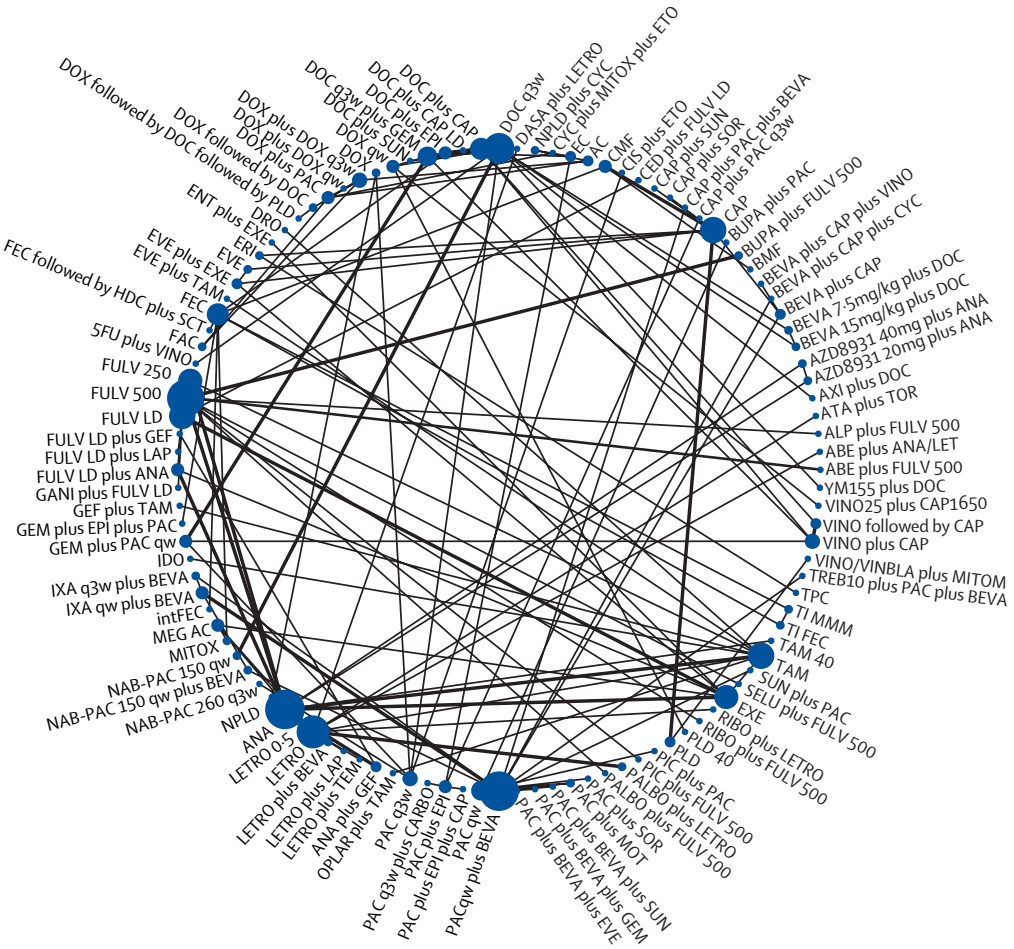


Figure 2: Network meta-analysis of progression-free survival and time to progression

Direct comparisons are represented by the black lines connecting the treatments. Line width is proportional to the number of trials including every pair of treatments, whereas circle size is proportional to the total number of patients for each treatment in the network. 5FU=fluorouracil. ABE=abemaciclib. AC=doxorubicin plus cyclophosphamide. ALP=alpelisib. ANA=anastrozole. ATA=atamestane. AXI=axitinib. BEVA=bevacizumab. BMF=bendamustine plus methotrexate plus fluorouracil. BORT=bortezomib. BUPA=buparlisib. CAP=capecitabine. CARBO=carboplatin. CED=cediranib. CIS=cisplatin. CMF=cyclophosphamide plus methotrexate plus fluorouracil. CYC=cyclophosphamide. DASA=dasatinib. DOC=docetaxel. DOX=doxorubicin. DRO=droloxifene. EC=epirubicin plus cyclophosphamide. ENT=entinostat. EPI=epirubicin. ERI=eribulin. ETO=etoposide. EVE=everolimus. EXE=exemestane. FAC=fluorouracil plus doxorubicin plus cyclophosphamide. FEC/CEF=fluorouracil plus epirubicin plus cyclophosphamide. FULV 250=fulvestrant 250 mg without loading dose. FULV 500=fulvestrant, standard dose. FULV LD=fulvestrant 250

mg with loading dose. GANI=ganitumab. GEF=gefitinib. GEM=gemcitabine. IDO=idoxifene. Int=intensive. IXA=ixabepilone. LAP=lapatinib. LD=lowdose. LETRO=letrozole, standard dose 2.5mg. LETRO 0.5=letrozole 0.5mg. MA=megestrol acetate. MITOM=mitomycin C. MITOX=mitoxantrone. MMM=mitoxantrone plus mitomycin C plus methotrexate. MOT=motesanib. NAB-PAC=nab paclitaxel. NPLD=non-pegylated liposomal doxorubicin. OBS=observation. OPLAR=octreotide pamoate long acting release. PAC=paclitaxel. PALBO=palbociclib. PIC=pictilisib. PLD=pegylated liposomal doxorubicin. q3w=every 3 weeks. qw=weekly. RIBO=ribociclib. SELU=selumetinib. SOR=sorafenib. SUN=sunitinib. TAM=tamoxifen, standard dose 20 mg. TAM 40=tamoxifen 40 mg. TEM=temsirolimus. TI=time intensive. TOR=toremifene. TPC=treatment of physician's choice. TREB=trebananib. VAN=vandetanib. VINBLA=vinblastine. VINO=vinorelbine.

Figure 3: Network meta-analysis of the proportion of patients achieving an overall response



Legend. Direct comparisons are represented by the black lines connecting the treatments. Line width is proportional to the number of trials including every pair of treatments, while circle size is proportional to the total number of patients for each treatment in the network. 5FU=fluorouracil. ABE=abemaciclib. AC=doxorubicin plus cyclophosphamide. ALP=alpelisib. ANA=anastrozole. ATA=atamestane. AXI=axitinib. BEVA=bevacizumab. BMF=bendamustine plus methotrexate plus fluorouracil. BORT=bortezomib. BUPA=buparlisib. CAP=capecitabine. CARBO=carboplatin. CED=cediranib. CIS=cisplatin. CMF=cyclophosphamide plus methotrexate plus fluorouracil. CYC=cyclophosphamide.

DASA=dasatinib. DOC=docetaxel. DOX=doxorubicin. DRO=droloxifene. EC=epirubicin plus cyclophosphamide. ENT=entinostat. EPI=epirubicin. ERI=eribulin. ETO=etoposide. EVE=everolimus. EXE=exemestane. FAC=fluorouracil plus doxorubicin plus cyclophosphamide. FEC/CEF=fluorouracil plus epirubicin plus cyclophosphamide. FULV 250=fulvestrant 250 mg without loading dose. FULV 500=fulvestrant, standard dose. FULV LD=fulvestrant 250 mg with loading dose. GANI=ganitumab. GEF=gefitinib. GEM=gemcitabine. HDC=high dose chemotherapy. IDO=idoxifene. Int=intensive. IXA=ixabepilone. LAP=lapatinib. LD=low dose. LETRO=letrozole, standard dose 2.5 mg. LETRO 0.5=letrozole 0.5 mg. MA=megestrol acetate. MITOM=mitomycin C. MITOX=mitoxantrone. MMM=mitoxantrone plus mitomycin C plus methotrexate. MOT=motesanib. NAB-PAC=nab paclitaxel. NPLD=non-pegylated liposomal doxorubicin. OBS=observation. OPLAR=octreotide pamoate long acting release. PAC=paclitaxel. PALBO=palbociclib. PIC=pictilisib. PLD=pegylated liposomal doxorubicin. q3w=every 3 weeks. qw=weekly. RIBO=ribociclib. SCT=stem-cell transplant. SELU=selumetinib. SOR=sorafenib. SUN=sunitinib. TAM=tamoxifen, standard dose 20 mg. TAM 40=tamoxifen 40 mg. TEM=temsirolimus. TI=time intensive. TOR=toremifene. TPC=treatment of physician's choice. TREB=trebananib. VAN=vandetanib. VINBLA=vinblastine. VINO=vinorelbine.

Overall, 50,029 patients were included in our network meta-analysis. Patient age ranged from 45.6 years to 72.6 years (median 58.0 years; IQR 55.0–63.0) and follow-up ranged from 4.2 months to 60.0 months (median 20.0 months; 14.9–29.1). For 47 (34%) of 140 trials, information about previous adjuvant or neoadjuvant systemic therapies was not reported. 91 (65%) randomised controlled trials were exclusively of first-line treatments, 33 (24%) included both first-line and second-line (or further-line) treatments, and 16 (11%) comprised at least second-line treatments. For patients enrolled in trials of hormone therapies, visceral involvement ranged between 9.0% and 87.0%, with a median of 53.0% (IQR 47.5–59.0). Visceral involvement for patients enrolled in trials of chemotherapies ranged between 9.0% and 91.3%, with a median of 72.6% (IQR 63.0–78.8).

All treatments were compared to anastrozole because it was the most common comparator present in the randomised controlled trials included in the network meta-analysis. All treatments were also compared to the combination of palbociclib plus letrozole, since this was the first combination of a CDK4/6 inhibitor plus hormone therapy approved for clinical practice, and remains the first-line standard of care, along with other CDK4/6 inhibitor plus hormone therapy combinations. 23 treatments were significantly better than anastrozole with regard to the primary endpoints of progression-free survival and time to progression, including the new first-line standard treatments palbociclib plus letrozole (HR 0.42; 95% CrI 0.25–0.70), ribociclib plus letrozole (0.43; 0.24–0.77), and abemaciclib plus anastrozole or letrozole (0.42; 0.23–0.76), and the second-line treatments palbociclib plus fulvestrant (0.37; 0.23–0.59), ribociclib plus fulvestrant (0.48; 0.31–0.74), abemaciclib plus fulvestrant (0.44; 0.28–0.70), everolimus plus exemestane (0.42; 0.28–0.67), and, in patients with a *PIK3CA* mutation, alpelisib plus fulvestrant (0.39; 0.22–0.66; **appendix 4, supplementary figure 1**). Among regimens comprising chemotherapy with or without targeted therapies, several regimens were better than anastrozole, including fluorouracil plus epirubicin plus

cyclophosphamide (HR 0.47; 95% CrI 0.26–0.93), paclitaxel plus bevacizumab (0.39; 0.18–0.88), capecitabine (0.41; 0.24–0.76), and eribulin (0.45; 0.23–0.89). No treatment was significantly better than palbociclib plus letrozole (**appendix 4, supplementary figure 2**). However, palbociclib plus letrozole was significantly better than fulvestrant plus anastrozole (HR 0.47; 95% CrI 0.27–0.83), fulvestrant standard dose (0.52; 0.30–0.91), anastrozole (0.42; 0.25–0.70), letrozole (0.55; 0.40–0.74), exemestane (0.43; 0.25–0.75), and tamoxifen (0.38; 0.24–0.61).

Consistent findings were observed when all treatments were compared with regimens based on CDK4/6 inhibitors (data not shown). We found no significant differences in progression-free survival among the three CDK4/6 inhibitors in combination with an aromatase inhibitor: palbociclib plus letrozole versus ribociclib plus letrozole (HR 0.98; 95% CrI 0.58–1.66), palbociclib plus letrozole versus abemaciclib plus anastrozole or letrozole (1.01; 0.59–1.70), and abemaciclib plus anastrozole or letrozole versus ribociclib plus letrozole (0.97; 0.53–1.78). Moreover, we found no significant differences among the three CDK4/6 inhibitors in combination with fulvestrant: palbociclib plus fulvestrant versus abemaciclib plus fulvestrant (HR 0.83; 95% CrI 0.47–1.46), palbociclib plus fulvestrant versus ribociclib plus fulvestrant (0.77; 0.44–1.35), and abemaciclib plus fulvestrant versus ribociclib plus fulvestrant (0.93; 0.54–1.61).

For the secondary endpoint of the proportion of patients achieving an overall response, 27 therapies were shown to be significantly better than anastrozole (**appendix 4, supplementary figure 3**). Among regimens comprising hormone therapies with or without targeted therapies, the most clinically relevant were everolimus plus exemestane (OR 4.50; 95% CrI 1.35–15.55) and abemaciclib plus fulvestrant (3.60; 1.22–10.77); palbociclib plus letrozole (1.85; 0.59–5.69), ribociclib plus letrozole (2.34; 0.65–8.48), abemaciclib plus anastrozole or letrozole (2.28; 0.62–8.29), palbociclib plus fulvestrant (2.61; 0.80–8.66), and ribociclib plus fulvestrant (1.81; 0.61–5.38) were not significantly better than anastrozole. Several chemotherapy regimens with or without targeted therapies were better than anastrozole, including paclitaxel plus bevacizumab (OR 16.48; 95% CrI 2.30–119.82), paclitaxel once weekly (15.0; 1.93–116.16), and docetaxel every 3 weeks plus epirubicin (7.64; 1.12–48.89). When compared with palbociclib plus letrozole, no treatment resulted in a significantly higher proportion of patients achieving an overall response, except for paclitaxel once weekly plus bevacizumab (OR 8.95; 95% CrI 1.03–76.92; **appendix 4, supplementary figure 4**). However, none of the other CDK4/6 inhibitor plus hormone therapy combinations was significantly different to any of the clinically approved chemotherapy-based regimens in terms of overall response (data not shown). We found no significant difference in the proportion of patients achieving an overall response with palbociclib plus letrozole versus ribociclib plus letrozole (OR 0.79; 95% CrI 0.25–2.53), with palbociclib plus letrozole versus abemaciclib plus anastrozole or

letrozole (0.81; 0.25–2.65), or with ribociclib plus letrozole versus abemaciclib plus anastrozole or letrozole (1.03; 0.27–3.91). Moreover, we observed no significant difference with palbociclib plus fulvestrant versus abemaciclib plus fulvestrant (OR 0.72; 95% CrI 0.18–2.98), with palbociclib plus fulvestrant versus ribociclib plus fulvestrant (1.44; 0.36–5.90), or with abemaciclib plus fulvestrant versus ribociclib plus fulvestrant (2.00; 0.53–7.52).
CDK4/6-inhibitors comparisons' results are fully reported in **table 1** (table not shown in the original published manuscript).

Table 1. Detailed comparisons among all CDK4/6 inhibitor-based regimens

HR (95% CrIs)	*Palbo+letro*	*Palbo+fulv*	*Ribo+letro*	*Ribo+fulv*	*Abe+NSAI*	*Abe+fulv*
Palbo+letro	-	1.14 (0.57; 2.26)	0.98 (0.58; 1.66)	0.88 (0.45; 1.70)	1.01 (0.59; 1.70)	0.94 (0.48; 1.85)
Palbo+fulv	0.88 (0.44; 1.75)	-	0.86 (0.41; 1.75)	0.77 (0.44; 1.35)	0.88 (0.42; 1.83)	0.83 (0.47; 1.46)
Ribo+letro	1.02 (0.60; 1.72)	1.16 (0.57; 2.46)	-	0.90 (0.44; 1.84)	1.03 (0.56; 1.88)	0.96 (0.47; 2.00)
Ribo+fulv	1.14 (0.59; 2.21)	1.30 (0.74; 2.25)	1.11 (0.54; 2.25)	-	1.14 (0.55; 2.31)	1.08 (0.62; 1.87)
Abe+NSAI	0.99 (0.59; 1.70)	1.13 (0.55; 2.40)	0.97 (0.53; 1.78)	0.87 (0.43; 1.81)	-	0.93 (0.45; 2.00)
Abe+fulv	1.06 (0.54; 2.08)	1.21 (0.69; 2.11)	1.05 (0.50; 2.11)	0.93 (0.54; 1.61)	1.07 (0.50; 2.20)	-
OR (95% CrIs)	*Palbo+letro*	*Palbo+fulv*	*Ribo+letro*	*Ribo+fulv*	*Abe+NSAI*	*Abe+fulv*
Palbo+letro	-	0.71 (0.14; 3.57)	0.79 (0.25; 2.53)	1.03 (0.21; 4.74)	0.81 (0.25; 2.65)	0.51 (0.11; 2.37)
Palbo+fulv	1.42 (0.28; 7.26)	-	1.12 (0.20; 6.25)	1.44 (0.36; 5.90)	1.14 (0.20; 6.59)	0.72 (0.18; 2.98)
Ribo+letro	1.27 (0.39; 4.07)	0.90 (0.16; 5.03)	-	1.29 (0.25; 6.69)	0.79 (0.15; 4.26)	0.65 (0.12; 3.36)
Ribo+fulv	0.98 (0.21; 4.69)	0.69 (0.17; 2.80)	0.77 (0.15; 4.08)	-	0.79 (0.15; 4.26)	0.50 (0.13; 1.89)
Abe+NSAI	1.24 (0.38; 4.03)	0.88 (0.15; 4.92)	0.97 (0.26; 3.68)	1.27 (0.24; 6.82)	-	0.63 (0.12; 3.30)
Abe+fulv	1.96 (0.42; 9.25)	1.38 (0.34; 5.67)	1.54 (0.30; 8.15)	2.00 (0.53; 7.52)	1.58 (0.30; 8.63)	-

Legend. Abe: abemaciclib; Palbo: palbociclib; Ribo: ribociclib; letro: letrozole; fulv: fulvestrant; NSAI: non steroidal aromatase inhibitor; HR: hazard ratio for progression-free survival; OR: odds ratio for overall response rates; CrIs: credible intervals.

The extent of heterogeneity between studies as measured by the random-effects model was assessed by inspecting the estimate of the corresponding standard deviation (SD). For the analysis of the log-HR, the average SD was 0.15 (95% CrI 0.06–0.26); for the analysis of the log-OR, the average SD was 0.43 (0.30–0.60).

Adverse events were reported differently in the included studies, so a systematic assessment of safety was not possible. However, we did an exploratory analysis of the proportions of patients with grade 3–5 adverse events [28]. We only considered grade 3–5 adverse events that were reported in 2% or more patients for each study.

The main adverse events, subdivided according to treatment categories, are reported in the **appendix 4 (tables S3-6)**. The proportions of adverse events are reported as ranges according to the values reported in different randomised controlled trials. Single-agent chemotherapy was associated with fewer adverse events than combination chemotherapy (**appendix 4, table S3**). The most frequent drug-specific adverse events were alopecia, most frequently observed with doxorubicin, docetaxel, vinorelbine, paclitaxel, and gemcitabine; stomatitis, most frequently associated with doxorubicin; febrile neutropenia, most frequently associated with docetaxel; hand-foot syndrome, mostly associated with capecitabine; and motor and sensory neurological disorders, mostly associated with taxanes. Combination chemotherapy was associated with higher frequencies of haematological and biochemical adverse events than single-agent chemotherapy (**appendix 4, table S4**). However, grade 3–5 neutropenia and leucopenia were also frequent with single-agent chemotherapy.

The most frequent grade 3–5 adverse events observed with hormone therapies were increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations, mostly with tamoxifen and exemestane; hyperglycaemia, mostly with anastrozole; pain (general), mostly with tamoxifen and exemestane; bone pain, mostly with tamoxifen and anastrozole; arthralgia, mostly with letrozole and exemestane; asthenia, mostly with exemestane and anastrozole; dyspnoea and constipation, mostly with anastrozole; anaemia, mostly with tamoxifen and exemestane; and hypoalbuminaemia, only with anastrozole (**appendix 4, table S5**). Hormone therapy plus targeted therapy combinations were associated with diarrhoea, mostly observed with abemaciclib plus anastrozole, abemaciclib plus letrozole, abemaciclib plus fulvestrant, and alpelisib plus fulvestrant; rash and fatigue, mostly observed with alpelisib plus fulvestrant; stomatitis and pneumonia, mostly observed with everolimus plus exemestane; and high frequencies of neutropenia and leucopenia were observed with the combinations of ribociclib and palbociclib plus letrozole or fulvestrant (**appendix 4, table S6**). Grade 3–5 increases in AST and ALT concentrations were observed with ribociclib plus letrozole, abemaciclib plus fulvestrant, palbociclib plus fulvestrant, and everolimus plus exemestane. Additionally, hyperglycaemia was reported with alpelisib plus fulvestrant.

A detailed risk of bias evaluation is reported in the **appendix 4** (**supplementary figure 5 and 6**).

Discussion

The findings of this large network meta-analysis confirm that the combination of CDK4/6 inhibitors plus hormone therapies is better than standard hormone therapies as first-line or second-line treatments for postmenopausal patients with hormone receptor-positive, HER2-negative metastatic breast cancer. In terms of progression-free survival or time to progression, no standard treatment schedule of chemotherapy with or without targeted therapy was significantly better than CDK4/6

inhibitors plus hormone therapies, which, in turn, showed a favourable and manageable toxicity profile. No significant differences in efficacy and overall activity were observed among the three CDK4/6 inhibitors.

In the past decade, several practice-changing randomised controlled trials have shown the efficacy of innovative therapeutic strategies as first-line or second-line treatments, or both, for patients with hormone receptor-positive, HER2-negative metastatic breast cancer, leading to substantial improvements in patient outcomes. The efficacy of hormone therapies in particular has been potentiated by combining them with new targeted therapies, such as the CDK4/6 inhibitors or mTOR and PI3K inhibitors. Median progression-free survival has almost doubled and the proportion of patients achieving an overall response significantly improved in all pivotal trials of hormone therapies combined with CDK4/6 inhibitors, mTOR inhibitors, and PI3K inhibitors, compared with standard hormone therapies alone [4-10,30]. Results of these trials have substantially changed treatment algorithms, further supporting the recommendation of oncology guidelines to adopt a sequence of all the available endocrine-based treatments and delay chemotherapy until occurrence of certain forms of endocrine resistance or clinical evidence of visceral crisis [11-14].

Nevertheless, chemotherapy-based regimens are still widely used as upfront therapy, sometimes without strict clinical justification [15-18]. To date, few data are available from randomised controlled trials directly comparing hormone therapies to chemotherapy-based treatment regimens in this disease subset. Indeed, in the past three decades, only two randomised controlled trials addressing this issue have been published (**appendix 4, full reference list**) [31,32]. Besides those two trials, only one large retrospective analysis was done of patients with hormone receptor-positive, HER2-negative metastatic breast cancer who were sensitive to aromatase inhibitors, which compared front-line hormone therapies to induction chemotherapy [33]. Moreover, the new combinations of hormone therapies plus targeted therapies have not been directly compared head to head in randomised controlled trials (i.e., palbociclib *vs* ribociclib *vs* abemaciclib) and new trials are unlikely to be designed to address this question. This research gap leaves some degree of uncertainty about the optimal treatment algorithm in patients with hormone receptor-positive, HER2-negative metastatic breast cancer. In this context, an inclusive and methodologically solid network meta-analysis could provide indirect evidence supporting physicians' treatment choice.

In terms of progression-free survival or time to progression, our results show that hormone therapies plus targeted therapies as first-line or second-line treatments, or in both settings, remain the best treatment choice, because chemotherapy was not shown to be better than endocrine therapy with targeted agents even when highly active chemotherapy regimens (i.e., taxane-based or anthracycline-based regimens, or regimens containing both drugs) were used as a comparator. Treatment strategies

involving hormone therapies plus targeted therapies, including inhibitors of tumour metabolism such as alpelisib or everolimus plus hormone therapies and the three CDK4/6 inhibitors plus hormone therapies, were all significantly better than single-agent hormone therapies with anastrozole. Several chemotherapy regimens, including some based on taxanes or anthracyclines, or both, did not show significantly better efficacy in comparison with hormone therapies alone (eg, anastrozole). This observation is valuable, especially for countries where CDK4/6 inhibitors, and targeted therapies in general, are not available yet.

With regard to the proportion of patients achieving an overall response, by comparison with contemporary single-agent chemotherapy and combination chemotherapy regimens with or without targeted therapies, palbociclib plus letrozole was significantly less active than bevacizumab-containing treatments only, including paclitaxel plus bevacizumab, although paclitaxel plus bevacizumab failed to show greater activity than the other combinations of CDK4/6 inhibitors plus hormone therapies. However, to correctly interpret these data, it is important to consider that studies of chemotherapy plus bevacizumab also enrolled patients with triple-negative disease, higher proportions of whom might achieve overall responses with these regimens than would be typically observed in patients with hormone receptor-positive, HER2-negative breast cancer. Among the hormone receptor-positive, HER2-negative subgroups in the TURANDOT and the CALGB 40502 trials, 35% to 46% of patients achieved an overall response with paclitaxel plus bevacizumab [34,35]. Notably, despite all the limitations of indirect comparisons, the proportion of patients achieving an overall response was not higher than that observed in trials of CDK4/6 inhibitors as first-line treatments.

None of the three CDK4/6 inhibitors, either combined with an aromatase inhibitor or fulvestrant, appeared to be better than the others in terms of both progression-free survival and the proportion of patients achieving an overall response; this observation provides new evidence for another crucial point of uncertainty regarding treatment choices in the first-line and second-line setting for hormone receptor-positive, HER2-negative metastatic breast cancer.

The exploratory analysis of safety showed that the toxicity of combinations comprising CDK4/6 inhibitors plus hormone therapies was of intermediate severity between that of standard hormone therapies and that of chemotherapy with or without targeted therapies. Moreover, although haematological adverse events were frequent with regimens containing CDK4/6 inhibitors, they were not accompanied by consistent rates of febrile neutropenia [4-10]. Some distinctive grade 3–5 adverse events differentiate the combination of abemaciclib (mostly diarrhoea) from palbociclib-containing and ribociclib-containing therapies (mostly haematological and hepatic toxicity), and from everolimus plus exemestane (mostly stomatitis and pneumonia) or alpelisib plus fulvestrant (mostly

hyperglycaemia and rash). Side-effects are reported differently in large international trials. Over-reporting or under-reporting can occur, depending on the location and setting of the study or as a result of different race-dependent safety profiles. Head-to-head comparisons are the best way to understand differences in safety profiles. The effect of different treatments on quality of life is even more complex. Fortunately, quality-of-life assessments are now systematically included as an important secondary endpoint in trials investigating different treatments in metastatic breast cancer. Despite these challenges, safety profiles, together with efficacy data and evidence of the effect of treatments on quality of life [36,37], support the use of hormone therapies plus targeted therapies and support delaying administration of chemotherapy. However, financial costs remain a major issue. Access to new drugs, as well as the direct and indirect costs of treatment, vary substantially from one country to another. High-quality pharmacoeconomic studies are therefore needed to integrate costs into treatment algorithms.

Our network meta-analysis has some limitations. First, we acknowledge the heterogeneity among the included studies, treatments, and patient populations, as a result of the long publication period considered (18 years), as also shown by the estimation of the random effects. Diagnostic advances could have produced a stage migration (i.e., improvements in diagnosis of metastatic disease over time) that might have influenced disease features and patient prognosis. Advances in histopathology, including changes to techniques for assessing hormone receptor status, might also have provided better selection of patients deriving benefit from hormone therapies.

Large phase 3 trials investigating CDK4/6 inhibitors have shown consistent benefit of these agents combined with hormone therapies when compared with hormone therapies alone, independently of clinical subgroups. However, the benefits of chemotherapy are possibly more pronounced in more aggressive and less endocrine-sensitive tumours than in slowly growing, highly endocrine-sensitive tumours. Our network meta-analysis did not allow analysis of specific subgroups to detect a differential effect according to subpopulations. It would be interesting to do this subgroup analysis in the large phase 3 PEARL trial (NCT02028507), which directly compares palbociclib plus exemestane or plus fulvestrant versus capecitabine. No information is available about the efficacy of CDK4/6 inhibitors in patients presenting with a visceral crisis, as these patients were excluded from these trials.

Additionally, we were unable to do separate analyses for first-line, second-line, and subsequent lines of therapy, since only a few studies included in the network meta-analysis (mostly recent trials) focused on one specific line of therapy (i.e., randomised controlled trials of purely first-line or second-line treatments). Additionally, although randomised controlled trials specifically designed for patients with triple-negative breast cancer had been excluded, several studies investigating chemotherapy

regimens enrolled also patients with triple-negative breast cancer, as previously mentioned. Other important endpoints cannot be analysed accurately by our network meta-analysis. In particular, whether a specific sequence affects overall survival remains a major debate. Unfortunately, to our knowledge, few trials have been designed to answer this clinically relevant question. The SONIA trial (NCT03425838) will investigate the optimal position of CDK4/6 inhibitors in the first-line or second-line setting for patients receiving a non-steroidal aromatase inhibitor in the first-line setting, and fulvestrant in the second-line setting for hormone receptor-positive, HER2-negative metastatic breast cancer.

We did not report publication bias because the approaches developed to assess this type of bias in network meta-analyses have limitations and their effectiveness is often debated. Moreover, verifying the presence of publication bias in network meta-analyses is notoriously challenging, as funnel plots within this context need a special adjustment because the studies compare different pairs of interventions [38]. However, our analysis includes most of the available literature on the topic, which might mitigate the effect of publication bias. Finally, all network meta-analyses share the same limitations of standard pairwise meta-analyses [39,40]. Moreover, these meta-analyses are based on an additional set of assumptions, the foremost being consistency between direct and indirect evidence, on which a lot of research is still ongoing [41].

Despite these limitations, we believe the results of this large network meta-analysis are timely, clinically meaningful, and methodologically reliable. The internal validity of the eligible studies was successfully assessed with the most appropriate risk of bias analysis [20]. Our data are consistent with previously published network meta-analyses, although, to our knowledge, this analysis comprises the largest number of randomised controlled trials ever reported in hormone receptor-positive, HER2-negative metastatic breast cancer and is the first comprehensive network meta-analysis to provide an indirect comparison of all CDK4/6 inhibitors plus aromatase inhibitors or fulvestrant and chemotherapy-based regimens [21-24]. Moreover, this network meta-analysis is the first to include the BOLERO-6 trial, which, despite its small sample size, represents the only contemporary study directly comparing a hormone therapy plus targeted therapy (everolimus plus exemestane) versus chemotherapy (capecitabine), a regimen that is currently used in clinical practice (**appendix 4, full reference list**) [32]. Results from the ongoing phase 3 PEARL trial are likely to provide additional evidence on this topic. According to the results of our network meta-analysis, if patients with hormone receptor-positive, HER2-negative metastatic breast cancer are treated with CDK4/6 inhibitors in the first-line setting, they might still benefit from hormone therapies such as the combination of everolimus plus exemestane, or alpelisib plus fulvestrant in patients with a *PIK3CA* mutations, and thus delay chemotherapy.

In conclusion, our results corroborate the treatment algorithms recommended by the official oncology guidelines, supporting the use of new combinations of hormone therapies plus targeted therapies in the first-line or second-line setting in patients with hormone receptor-positive, HER2-negative metastatic breast cancer without visceral crisis.

Contributors

FS, MG, and DG conceived the study. FS, MG, and DG did the literature search. FS, CR, and SV extracted the required data. CR did the analysis of bias. SV did the statistical analyses. All authors contributed to data interpretation and wrote, revised, and approved the final version of the manuscript.

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Declaration of interests

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References

1. O'Shaughnessy J. Extending survival with chemotherapy in metastatic breast cancer. *Oncologist* 2005; 10: 20–29.

2. Prat A, Pineda E, Adamo B, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. *Breast* 2015; 24: S26–35.
3. Gong Y, Liu Y-R, Ji P, et al. Impact of molecular subtypes on metastatic breast cancer patients: a SEER population-based study. *Sci Rep* 2017; 7: 45411.
4. Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med* 2012; 366: 520–29.
5. Finn RS, Martin M, Rugo HS, et al. Palbociclib and letrozole in advanced breast cancer. *N Engl J Med* 2016; 375: 1925–36.
6. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. *Lancet Oncol* 2016; 17: 425–439.
7. Hortobagyi GN, Stemmer SM, Burris HA, et al. Ribociclib as first-line therapy for hr-positive, advanced breast cancer. *N Engl J Med* 2016; 375: 1738–48.
8. Slamon DJ, Neven P, Chia S, et al. Phase III randomized study of ribociclib and fulvestrant in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: MONALEESA-3. *J Clin Oncol* 2018; 36: 2465–72.
9. Goetz MP, Toi M, Campone M, et al. MONARCH 3: abemaciclib as initial therapy for advanced breast cancer. *J Clin Oncol* 2017; 35: 3638–46.
10. Sledge GW Jr, Toi M, Neven P, et al. MONARCH 2: abemaciclib in combination with fulvestrant in women with HR+/HER2− advanced breast cancer who had progressed while receiving endocrine therapy. *J Clin Oncol* 2017; 35: 2875–84.
11. Cardoso F, Costa A, Senkus E, et al. 3rd ESO–ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 3). *Ann Oncol* 2017; 28: 16–33.
12. NCCN clinical practice guidelines in oncology-breast cancer 2018. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf (accessed June 25, 2018).
13. Rugo HS, Rumble RB, Macrae E, et al. Endocrine therapy for hormone receptor-positive metastatic breast cancer: American Society of Clinical Oncology Guideline. *J Clin Oncol* 2016; 34: 3069–103.
14. Italian Association of Medical Oncologists (AIOM) breast cancer guidelines 2017. Available from http://www.aiom.it/professionisti/ documenti-scientifici/linee-guida/1,413,1 (accessed June 25, 2018).
15. Andrè F, Neven P, Marinsek N, et al. Disease management patterns for postmenopausal women in Europe with hormone-receptor-positive, human epidermal growth factor receptor-2 negative advanced breast cancer. *Curr Med Res Opin* 2014; 30: 1007–16.
16. Bonotto M, Gerratana L, Di Maio M, et al. Chemotherapy versus endocrine therapy as first-line treatment in patients with luminal-like HER2-negative metastatic breast cancer: a propensity score analysis. *Breast* 2017; 31: 114–20.
17. Cazzaniga M, Mustacchi G, Giordano M, et al. 259P Adherence to International ESO-ESMO (ABC) guidelines in HER2-ve metastatic breast cancer (MBC) patients (pts): preliminary results of the GIM 13-AMBRA Study. *Ann Oncol* 2017; 28 (suppl 5): mdx365.022.

18. Lobbezoo DJA, van Kampen RJW, Voogd AC, et al. In real life, one-quarter of patients with hormone receptor-positive metastatic breast cancer receive chemotherapy as initial palliative therapy: a study of the Southeast Netherlands Breast Cancer Consortium. *Ann Oncol* 2016; 27: 256–62.
19. Lu G, Ades AE. Combination of direct and indirect evidence in mixed treatment comparisons. *Stat Med* 2004; 23: 3105–24.
20. Higgins JPT, Green S, eds. Cochrane handbook for systematic reviews of interventions, version 5.1.0. Updated March, 2011. London: The Cochrane Collaboration, 2011.
21. Zhang J, Huang Y, Changyi Wang, et al. Efficacy and safety of endocrine monotherapy as first-line treatment for hormone-sensitive advanced breast cancer: a network meta-analysis. *Medicine* 2017; 96: e7846.
22. Wilson FR, Varu A, Mitra D, et al. Systematic review and network meta-analysis comparing palbociclib with chemotherapy agents for the treatment of postmenopausal women with HR-positive and HER2-negative advanced/metastatic breast cancer. *Breast Cancer Res Treat* 2017; 166: 167–77.
23. Zhang T, Feng F, Zhao W, et al. Effect of first-line endocrine therapy in patients with hormone-sensitive advanced breast cancer: a network meta-analysis. *Onco Targets Ther* 2018; 11: 2647–56.
24. Generali D, Venturini S, Rognoni C, et al. A network meta-analysis of everolimus plus exemestane versus chemotherapy in the first- and second-line treatment of estrogen receptor-positive metastatic breast cancer. *Breast Cancer Res Treat* 2015; 152: 95–117.
25. Forsythe A, Chandiwana D, Barth J, et al. Progression-free survival/time to progression as a potential surrogate for overall survival in HR+, HER2– metastatic breast cancer. *Breast Cancer* 2018; 10: 69–78.
26. Li L, Pan Z. Progression-free survival and time to progression as real surrogate end points for overall survival in advanced breast cancer: a meta-analysis of 37 trials. *Clin Breast Cancer* 2017; 18: 63–70.
27. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45: 228–47.
28. National Cancer Institute. Common terminology criteria for adverse events. https://ctep.cancer.gov/protocolDevelopment/electronic_ applications/ctc.htm (accessed June 25, 2018).
29. Lunn DJ, Thomas A, Best N, et al. WinBUGS—a Bayesian modelling framework: concepts, structure, and extensibility. *Stat Comput* 2000; 10: 325–37.
30. André F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. *N Engl J Med* 2019; 380: 1929–40.
31. Dixon AR, Jackson L, Chan S, et al. A randomised trial of second-line hormone vs single agent chemotherapy in tamoxifen resistant advanced breast cancer. *Br J Cancer* 1992; 66: 402–04.
32. Jerusalem G, de Boer RH, Hurvitz S, et al. Everolimus plus exemestane vs everolimus or capecitabine monotherapy for estrogen receptor–positive, HER2-negative advanced breast cancer. The BOLERO-6 randomized clinical trial. *JAMA Oncol* 2018; 4: 1367–74.
33. Jacquet E, Lardy-Cleaud A, Pistilli B, et al. Endocrine therapy or chemotherapy as first-line therapy in hormone receptor positive HER2-negative metastatic breast cancer patients. *Eur J Cancer* 2018; 95: 93e101.
34. Brodowicz T, Lang I, Kahan Z, et al. Selecting first-line bevacizumab-containing therapy for advanced breast cancer: TURANDOT risk factor analyses. *Br J Cancer* 2014; 111: 2051–57.
35. Rugo HS, Barry WT, Moreno-Aspitia A, et al. Long-term follow-up of CALGB 40502/NCCTG N063H (Alliance): a randomized phase III trial of weekly paclitaxel (P) compared to weekly nanoparticle albumin bound nab-Paclitaxel

(NP) or ixabepilone (Ix) +/- bevacizumab as first-line therapy for locally recurrent or metastatic breast cancer (MBC). 2017 San Antonio Breast Cancer Symposium; Dec 5–9, 2017; San Antonio, TX; GS3-06 (abstr).

36. Verma S, O'Shaughnessy J, Burris HA, et al. Health-related quality of life of postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer treated with ribociclib + letrozole: results from MONALEESA-2. *Breast Cancer Res Treat* 2018; 170: 535–45.
37. Rugo HS, Finn RS, Diéras V, et al. Palbociclib plus letrozole as first-line therapy in estrogen receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer with extended follow-up. *Breast Cancer Res Treat* 2019; 174: 719–29.
38. Chaimani A, Higgins JPT, Mavridis D. Graphical tools for network meta-analysis in STATA. *PLoS One* 2013; 8: e76654.
39. Greenland S. Can meta-analysis be salvaged? *Am J Epidemiol* 1994; 140: 783–87.
40. Bailor JC III. The promise and problems of meta-analysis. *N Eng J Med* 1997; 337: 559–61.
41. Dias S, Ades AE, Welton NJ, Jeron JP, Sutton AJ. Network meta-analysis for decision-Making. Hoboken, NJ: Wiley, 2018.

CHAPTER 5 : Overall Survival of CDK4/6-Inhibitor-Based Treatments in Clinically Relevant Subgroups of Metastatic Breast Cancer: Systematic Review and Meta-Analysis

Original article reference

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Abstract

Background: Cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors + endocrine therapy (ET) prolonged progression-free survival as first- or second-line therapy for hormone receptor-positive (HR+)/HER2-negative metastatic breast cancer prognosis. Given the recent publication of overall survival (OS) data for the 3 CDK4/6-inhibitors, we performed a meta-analysis to identify a more precise and reliable benefit from such treatments in specific clinical subgroups.

Methods: We conducted a systematic literature search to select all available phase II or III randomized clinical trials of CDK4/6-inhibitors + ET reporting OS data in first- or second-line therapy of HR+/HER2-negative pre- or postmenopausal metastatic breast cancer. A random effect model was applied for the analyses. Heterogeneity was assessed with I^2 statistic. Subgroup analysis was performed to explore the effect of study-level factors. The project was registered in the Open Science Framework database (doi: 10.17605/OSF.IO/TNZQP).

Results: Six studies were included in our analyses (3,421 patients). A clear OS benefit was observed in patients without (hazard ratio [HR]: 0.68, 95% confidence interval [CI]: 0.54 to 0.85, I^2= 0.0%) and with visceral involvement (HR: 0.76, 95%CI: 0.65 to 0.89, I^2=0.0%), with at least 3 metastatic sites (HR: 0.75, 95%CI: 0.60 to 0.94, I^2= 11.6%), in an endocrine-resistant (HR: 0.79, 95%CI: 0.67 to 0.93, I^2= 0.0%) and sensitive subset (HR: 0.73, 95%CI: 0.61 to 0.88, I^2= 0.0%), for younger than 65 years (HR: 0.80, 95%CI: 0.67 to 0.95, I^2= 0.0%) and 65 years or older (HR: 0.71, 95%CI: 0.53 to 0.95, I^2= 44.4%), in postmenopausal (HR: 0.76, 95%CI: 0.67 to 0.86, I^2= 0.0%) and pre- or perimenopausal setting (HR: 0.76, 95%CI: 0.60 to 0.96, I^2= 0.0%) as well as in chemotherapy-naïve patients (HR: 0.72, 95%CI: 0.55 to 0.93, I^2= 0.0%).

Conclusions: CDK4/6-inhibitors + ET combinations compared with ET alone improve OS independent of age, menopausal status, endocrine sensitiveness, and visceral involvement and should be preferred as upfront therapy instead of endocrine monotherapy.

Introduction

Hormone receptor-positive (HR+)/HER2-negative metastatic breast cancer (MBC) represents the most frequent subgroup of advanced breast tumors [1]. The most relevant therapeutic improvement of the last few years in this subset has been represented by the introduction of cyclin-dependent kinases (CDK) 4 and 6 (CDK4/6) inhibitors (palbociclib, ribociclib, and abemaciclib) combined with endocrine therapy (ET). These drugs bind to the CDK4 and 6, preventing their correct functioning and leading to cell-cycle arrest and apoptosis. They also seem to induce a broad spectrum of immunological events, which, however, need further investigation to be fully understood [2].

Pivotal trials led to the approval of CDK4/6-inhibitors plus ET combinations after showing very similar statistically significant and clinically meaningful improvements in progression-free survival (PFS) in a first- or second-line setting of both premenopausal [3–5] and postmenopausal [3,4,6–9] patients with HR+/HER2-negative MBC. The median PFS of all the comparison arms roughly doubled, as well as overall response rates, compared with standard ET [3–9]. Notably, a recent network meta-analysis confirmed the superiority of CDK4/6-inhibitor regimens over single agent ET, showed a substantial equivalence among the 3 inhibitors and no difference with chemotherapy (CT) [10]. However, all these studies were based on PFS as their primary endpoint and, until recently, overall survival (OS) data were available only for palbociclib-containing phase II PALOMA 1 and phase III PALOMA 3 trials [11,12] and the ribociclib-containing MONALEESA 2 trial [7]. Previous studies had observed a statistically significant association between PFS and OS in MBC [13], in general and specifically in HR+/HER2-negative disease, overall suggesting that the first might be a good surrogate endpoint for the latter [14]. Nevertheless, the prediction of OS based on PFS is still matter of debate, because the number of subsequent treatment lines, cross-over from the control arm to active treatment, and nonrandomized use of second-line agents might interfere with this association [13,15]. Finally, OS results for the pivotal phase III trials MONARCH 2, MONALEESA 3, and MONALEESA 7 were recently published, providing additional data regarding abemaciclib- and ribociclib-based regimens [16-18]. Considering all available results, a 4- to 10-month improvement in median survival with a 19%-29% relative reduction in the risk of death has been observed so far [7,11,12,16–18]. However, results were not statistically significant for each trial or for each subgroup of patients, probably due to the study being substantially underpowered in demonstrating possible OS differences [19]. For these reasons and also given the current lack of effective biomarkers capable of identifying patients that might benefit most from these novel therapeutic agents, we decided to perform this meta-analysis in different clinically relevant subgroups of HR+/HER2-negative MBC.

Materials and Methods

Search Strategy and Selection Criteria

We conducted a systematic literature search on PubMed at the end of October 2019 to select all available phase II or III randomized controlled trials (RCT) of CDK4/6-inhibitors plus ET showing OS data in the first- or second-line treatment setting of HR+/HER2-negative pre- or postmenopausal MBC. European Society for Medical Oncology (ESMO) and American Society of Clinical Oncology meetings' and San Antonio Breast Cancer Symposium' online databases were also consulted. The query included the terms "palbociclib," "ribociclib," "abemaciclib," "breast," "metastatic," and "advanced." Duplicate reports were excluded. No language restriction was adopted. The research and data extraction were conducted by 2 investigators (F Schettini and F Giudici) and a third one (D Generali) was consulted in case of controversy. Details about study design, patient characteristics, interventions, and previous treatments were extracted from each article. The primary outcome was OS measured in various subgroups of interest. Hazard ratios (HR) and associated 95% confidence intervals (CI) were extracted for OS from published articles. Subgroups of interest were the following: visceral disease (yes vs no), bone-only disease (yes vs no), number of metastatic sites (<3 sites vs ≥3), endocrine sensitivity and resistance (yes vs no), previous CT for the metastatic setting (yes vs no), age (<65 vs ≥65 years), and menopausal status (pre-perimenopausal vs postmenopausal). Endocrine resistance and sensitivity were defined according to ESO-ESMO International Consensus Guidelines [20].

Data Analysis

Analyses were performed applying a priori the random-effect model from DerSimonian and Laird [21]. Pooled data were presented in forest plots. All study-specific estimates were combined using inverse variance-weighted averages of logarithmic hazard ratios in random-effects models. Statistical significance was set at *P* less than 0.05. All tests were 2-sided. The degree of heterogeneity between studies was assessed by visual inspection of the forest plots and I^2 statistic estimate [22]. Using subgroup analysis, we planned to explore the effect of the following study-level factors: visceral involvement status (no involvement vs involvement), bone-only disease condition (yes vs no), number of metastatic sites (<3 vs ≥3), endocrine sensitive status (resistant vs sensitive), previous CT for the metastatic setting (untreated vs treated), age (<65years vs ≥65years), and menopausal status (postmenopausal vs pre/perimenopausal). Subgroup analyses were performed if at least 2 studies for each of the previously mentioned subgroups of interest were available. Q-test of homogeneity (Q Statistic: Q within and Q between) was performed to compare the pooled effect in 2 or more groups. Publication bias was not assessed due to inadequate numbers of included trials to properly assess a funnel plot or more advanced regression-based assessments. Statistical analyses were performed

using R software version 3.5.0 (package *meta*) [23]. The risk of bias for each trial was assessed by using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions [24]. Internal validity of eligible studies was assessed according to the Cochrane Collaboration's "Risk of Bias" tool in Review Manager [25]. Each domain related to a risk of bias was assessed in each included trial, because there is evidence that these issues are associated with biased estimates of treatment effect. The domains were the following: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, other bias. Review authors' judgments were categorized as "low risk," "high risk," or "unclear risk" of bias.

The project was registered in the Open Science Framework online public database (http://osf.io with doi: 10.17605/OSF.IO/ TNZQP).

Results

Included Studies' Characteristics

Six out of 8 (75.0%) studies reported OS results and were therefore included in the analyses for a total of 3421 patients (7,11,12,16–18). The study selection process is summarized in **Supplementary Figure 1 (appendix 5)**. Three of the 6 (50.0%) included studies enrolled only postmenopausal patients, 1 (17.0%) study exclusively enrolled premenopausal patients, to whom an analogue of gonadotropin-releasing hormone agonist (GnRH) was administered to induce ovarian function suppression, and the 2 (33.0%) remaining studies enrolled both post- and premenopausal patients. For the latter group, a GnRH analogue was administered along with study treatments. Three out of 6 (50.0%) studies were set in first line, while the remaining (50.0%) were set in first or second line. Five (83.0%) studies were phase III RCT and 1 (17.0%) was a phase II trial. The experimental arms included in these trials were fulvestrant plus ribociclib, palbociclib or abemaciclib, letrozole plus palbociclib or ribociclib, and a nonsteroidal aromatase inhibitor or tamoxifen plus ribociclib. Trial characteristics and main outcomes are reported in **Table 1** and full results are shown in **Table 2**. An overall pooled OS benefit was observed for CDK4/6-inhibitor combinations compared with standard ET (HR: 0.76, 95%CI: 0.68 to 0.85, I^2= 0.0%; **Supplementary Figure 2, appendix 5**).

Table 1. Characteristics and results of published randomized phase II or III trials of CDK4/6-inhibiotrs combined with ET in HR+/HER2-negative MBC

Features	Published randomized Phase II or III trials								
	PALOMA 1	PALOMA 2	PALOMA 3	MONALEESA 2	MONALEESA 7	MONALEESA 3	MONARCH 3	MONARCH 2	MONARCH plus
Phase	II	III	III	III	III	III	III	III	III
No. of patients	165	666	521	668	672	726	493	669	463
Treatment	Palbociclib + letrozole vs letrozole	Palbociclib + letrozole vs letrozole -	Palbociclib + fulvestrant vs fulvestrant (+ GnRHa in pre/peri pts)	Ribociclib + letrozole vs letrozole	Ribociclib + tamoxifen or AI + GnRHa vs tamoxifen or AI + GnRHa	Ribociclib + fulvestrant vs fulvestrant	Abemaciclib + NSAI vs NSAI	Abemaciclib + fulvestrant vs fulvestrant (+ GnRHa in pre/peri pts)	Abemaciclib + NSAI or fulvestrant vs NSAI or fulvestrant
Menopausal status at moment of trial enrollment	Post	Post	Pre/post	Post	Pre	Post	Post	Pre/post	Post
Setting	1st line HR+ HER2− MBC	1st line HR+ HER2− MBC	≥1st line HR+ HER2− MBC	1st line HR+ HER2− MBC	1st line HR+ HER2− MBC	≥1st line HR+ HER2− MBC	1st line HR+ HER2− MBC	≥1st line HR+ HER2− MBC	≥1st line HR+ HER2− MBC
Median PFS, mo	20.2 vs 10.2	24.8 vs 14.5	9.5 vs 4.6	25.3 vs 16.0	23.8 vs 13.0	20.5 vs 12.8	NR vs 14.7	16.4 vs 9.3	NR and 11.5 vs 14.7 and 5.6
PFS HR (95% CI)	0.49 (0.32 to 0.75)	0.58 (0.46 to 0.72)	0.46 (0.36 to 0.59)	0.57 (0.46 to 0.70)	0.55 (0.44 to 0.69)	0.59 (0.48 to 0.73)	0.54 (0.41 to 0.72)	0.55 (0.45 to 0.68)	0.50 (0.35 to 0.72) and 0.38 (0.24 to 0.59)
ORR[a]	43% vs 33%	42% vs 35%	25% vs 11%	43% vs 29%	51% vs 36%	41% vs 9%	59% vs 44%	48% vs 21%	56% and 39% vs 30% and 8%
Median OS, mo	37.5 vs 33.3	NM	35.0 vs 28.0	NR	NR vs 40.9	NR vs 40.0	NM	46.7 vs 37.3	NM
OS HR (95% CI)	0.81 (0.49 to 1.35)	NM	0.81 (0.64 to 1.03)	0.75 (0.52 to 1.08)	0.71 (0.54 to 0.95)	0.72 (0.57 to 0.92)	NM	0.76 (0.61 to 0.95)	NM
Journal/Congress[b]	Lancet Oncol/J Clin Oncol	N Engl J Med	New Engl J Med	Ann Oncol	New Engl J Med	N Engl J Med	J Clin Oncol	JAMA Oncol	Ann Oncol
First author[b]	Finn RS	Finn RS	Turner NC	Hortobagyi G	Im S-A	Slamon DJ	Goetz MP	Sledge GW	Jiang Z
Year[b]	2014/2017	2016	2018	2018	2019	2019	2017	2019	2019

Legend. a: values are rounded; **b:** The citations refer to manuscripts with available OS results unless they have not been published yet. **AI:** aromatase inhibitor; **CI:** confidence interval; **ESMO:** European Society for Medical Oncology; **ET:** endocrine therapy; **GnRHa:** gonadotropin-releasing hormone agonist; **HER2-:** human epidermal growth factor receptor 2 negative; **HR:** hazard ratio; **HR+:** hormone receptor positive; **MBC:** metastatic breast cancer; **NM:** not mature; **NR:** not reached; **NSAI:** nonsteroidal aromatase inhibitor; **ORR:** overall response rate; **OS:** overall survival; **peri:** perimenopausal; **PFS:** progression-free survival; **post:** postmenopausal; **pre:** premenopausal.

Table 2. Full subgroup analyses results

Variables	No. of Pts	No. of studies	Pooled HR (95% CI)	I^2, %	P_{pooled}	P_H	$P_{sub.\ diff.}$
Age, y	1916	3	0.77 (0.66 to 0.88)	12.0	<.001	.34	.49
<65	1203	3	0.80 (0.67 to 0.95)	0.0	.01	.45	
≥65	713	3	0.71 (0.53 to 0.95)	44.4	.003	.17	
Menopausal status	3417	6	0.75 (0.67 to 0.84)	0.0	<.001	.95	.99
Pre- or perimenopausal	894	3	0.76 (0.60 to 0.96)	0.0	.02	.41	
Postmenopausal	2523	5	0.76 (0.67 to 0.86)	0.0	<.001	.89	
Bone-only disease	1577	3	0.74 (0.62 to 0.89)	0.0	<.001	.61	.47
Yes	492	3	0.82 (0.60 to 1.13)	0.0	.23	.45	
No	1085	2	0.71 (0.58 to 0.88)	0.0	.002	.47	
Metastatic sites	1600	3	0.77 (0.65 to 0.91)	0.0	.002	.63	.74
<3	891	2	0.79 (0.62 to 1.01)	0.0	.06	.63	
≥3	709	3	0.75 (0.60 to 0.94)	11.6	.02	.32	
Previous CT in metastatic setting	979	2	0.77 (0.62 to 0.94)	0.0	.01	.74	.42
Yes	271	2	0.85 (0.61 to 1.18)	0.0	.34	.45	
No	708	2	0.72 (0.55 to 0.93)	0.0	.01	.82	
Visceral involvement	2291	4	0.73 (0.65 to 0.83)	0.0	<.001	.89	.91
No	901	3	0.68 (0.54 to 0.85)	0.0	<.001	.96	
Yes	1390	4	0.76 (0.65 to 0.89)	0.0	<.001	.69	
Endocrine sensitivity status	2834	5	0.77 (0.68 to 0.86)	0.0	<.001	.73	.55
Resistance	1331	4	0.79 (0.67 to 0.93)	0.0	.004	.45	
Sensitive	1503	4	0.73 (0.61 to 0.88)	0.0	.001	.70	

Legend. CI: confidence interval; **CT:** chemotherapy; **HR:** hazard ratio; $\mathbf{P_H}$**:** *P* value for heterogeneity test; $\mathbf{P_{pooled}}$**:** *P* value for the pooled analysis; $\mathbf{P_{sub.diff.}}$**:** *P* value for subgroup differences; **Pts:** patients.

Visceral Involvement Status

cumulative effect was statistically significant (HR: 0.75, 95%CI: 0.60 to 0.94, *P*=0.02, I^2= 11.6%; **Figure 1F**) as well as the result obtained when joining the 2 subpopulations (HR: 0.77, 95%CI: 0.65 to 0.91, *P*=0.002, I^2= 0.0%; **Figure 1**), with no statistically significant between-group difference (*P*=0.74).

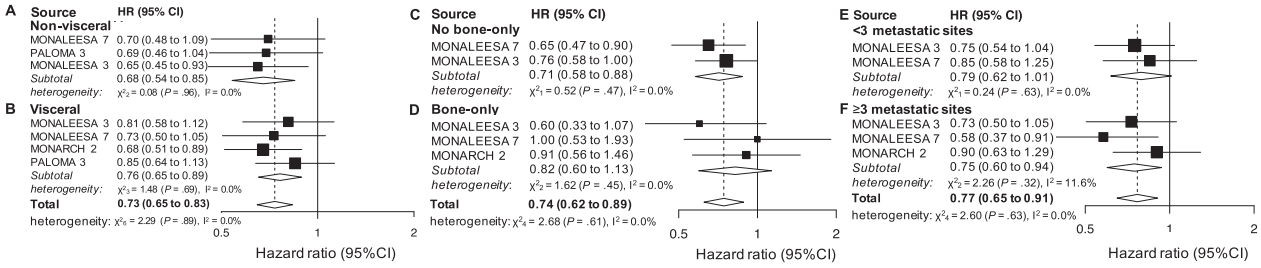
Bone-Only Status

Two studies (1,085 patients) reported OS results for patients without bone-only disease. The cumulative effect was statistically significant (HR: 0.71, 95%CI: 0.58 to 0.88, *P*=0.002, I^2= 0.0%; **Figure 1C**). Three studies reported results for bone-only disease (492 patients). A non-statistically significant cumulative benefit was observed (HR: 0.82, 95%CI: 0.60 to 1.13, *P*=0.23, I^2=0.0%; **Figure 1D**). When combining all the patients involved in the subgroup analyses, the result was overall statistically significant (HR: 0.74, 95%CI: 0.62 to 0.89, *P*<0.001, I^2= 0.0%; **Figure 1**) and there was no statistically significant difference between the 2 groups (*P*=0.47).

Number of Metastatic Sites

Two studies reported results for patients with less than 3 metastatic sites (891 patients). An almost statistically significant cumulative effect was observed (HR: 0.79, 95%CI: 0.62 to 1.01, *P*=0.06, I^2= 0.0%; **Figure 1E**). Three studies reported results for patients with at least 3 metastatic sites (709 patients). The cumulative effect was statistically significant (HR: 0.75, 95%CI: 0.60 to 0.94, *P*=0.02, I^2= 11.6%; **Figure 1F**) as well as the result obtained when joining the 2 subpopulations (HR: 0.77, 95%CI: 0.65 to 0.91, *P*=0.002, I^2= 0.0%; **Figure 1**), with no statistically significant between-group difference (*P*=0.74).

Figure 1. Pooled overall survival (OS) according to metastatic sites and tumor burden



Legend. Pooled OS in nonvisceral (A), visceral (B), no bone-only (C), or bone-only (D) disease and in case of less than 3 (E) and 3 or more metastatic sites (F). **CI:** confidence interval; **HR:** hazard ratio.

Endocrine Sensitivity Status

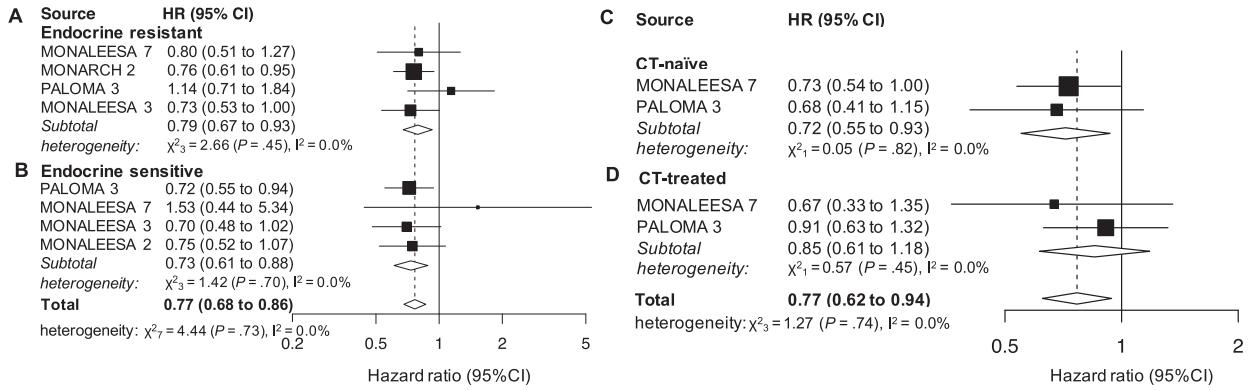
Four studies provided results for the endocrine resistant subset (1,331 patients). The effect in the subgroup was statistically significant (HR: 0.79, 95%CI: 0.67 to 0.93, *P*=0.004, I^2= 0.0%; **Figure 2A**).

Four studies (1,503 patients) reported results for the endocrine sensitive subset, which was statistically significant as well (HR: 0.73, 95%CI: 0.61 to 0.88, *P*=0.001, I^2= 0.0%; **Figure 2B**). The overall effect in the joint analysis of the 2 subgroups was also statistically significant (HR: 0.77, 95%CI: 0.68 to 0.86, *P*<0.001, I^2= 0.0%; **Figure 2**), whereas the between-group difference for the endocrine resistance and sensitive setting was not (*P*=0.55).

Previous CT for Metastatic Disease

Two studies reported results for CT-naïve (708) and CT-pretreated (271) patients in a metastatic setting. A statistically significant cumulative effect was demonstrated for the first (HR: 0.72, 95%CI: 0.55 to 0.93, *P*=0.01, I^2= 0.0%; **Figure 2C**) but not for the latter group (HR: 0.85, 95%CI: 0.61 to 1.18, *P*=0.34, I^2= 0.0%; **Figure 2D**). The joint analysis of the 2 subpopulations was statistically significant (HR: 0.77, 95%CI: 0.62 to 0.94, *P*=0.01, I^2= 0.0%; **Figure 2**), and there was no statistically significant difference between the 2 groups (*P*=0.42).

Figure 2. Pooled overall survival (OS) according to endocrine resistance status and previous chemotherapy (CT)



Legend. Pooled OS in patients younger than 65 years (A), 65 years or older (B), postmenopause (C) and pre- or perimenopause (D). **CI:** confidence interval; **HR:** hazard ratio.

Age

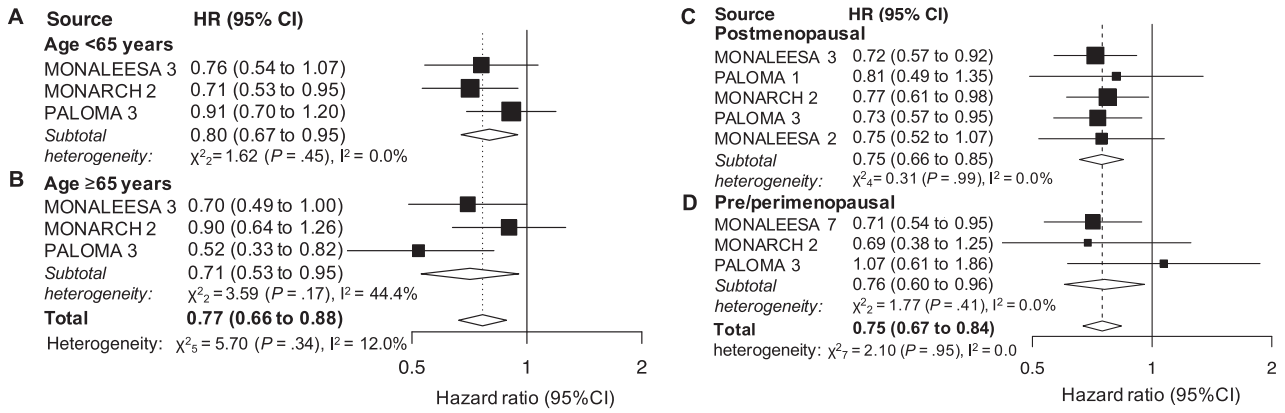
Three studies reported results for patients younger than 65 years and those 65 years and older (1,203 and 713 patients, respectively). A statistically significant effect was demonstrated in both subgroups (HR: 0.80, 95%CI: 0.67 to 0.95, *P*=0.01, I^2= 0.0% and HR: 0.71, 95%CI: 0.53 to 0.95, *P*=0.003, I^2= 44.4%; **Figure 3A and B**, respectively). The cumulative effect observed in the joint analysis was

statistically significant (HR: 0.77, 95%CI: 0.66 to 0.88, *P*<0.001, I^2= 12.0%; **Figure 3**), whereas the between group difference was not (*P*=0.49).

Menopausal Status

Five studies (2,523 patients) provided results for postmenopausal patients. The cumulative effect was statistically significant (HR: 0.76, 95%CI: 0.67 to 0.86, *P*<0.001, I^2= 0.0%; **Figure 3C**). Three studies (894 patients) provided results for the pre- or perimenopausal setting. The pooled result was statistically significant as well (HR: 0.76, 95%CI: 0.60 to 0.96, *P*=0.02, I^2= 0.0%; **Figure 3D**). The overall effect in the joint analysis was statistically significant (HR: 0.75, 95%CI: 0.67 to 0.84, *P*<0.001, I^2= 0.0%; **Figure 3**) with no difference observed between subgroups (*P*=0.99).

Figure 3. Pooled overall survival (OS)according to age and menopausal status



Legend. Pooled OS in patients younger than 65 years (A), 65 years or older (B), postmenopause (C) and pre- or perimenopause (D). **CI:** confidence interval; **HR:** hazard ratio.

Risk of Bias Analysis

The studies included in our analyses did not show any relevant risk of bias within the 7 domains considered. Risk of bias pooled results are reported in **Supplementary Figure 3 (appendix 5)**. A detailed assessment for each single study is reported in **Supplementary Figure 4 (appendix 5)**.

Discussion

We focused our meta-analysis on specific subgroups of clinical relevance. Results show for the first time, to our knowledge, that CDK4/6-inhibitors plus ET combinations, compared with ET monotherapy, improve OS in HRþ/HER2-negative MBC as first- or second-line treatment independent of age (<65 vs ≥65 years), menopausal status (pre- or peri- vs postmenopausal), endocrine sensitivity (sensitive vs resistant), and visceral involvement. More specifically, we

observed a 24% and 32% relative reduction in the risk of death for patients with or without visceral metastasis, respectively, which was also accompanied by a statistically significant 29% risk reduction in patients without bone-only disease, irrespective of other metastatic sites. The OS benefit was comparable in both pre- or peri- and postmenopausal settings, with statistically significant 24% relative decreases in the risk of death in both cases. Notably, CDK4/6-inhibitor–based therapies produced a statistically significant relative reduction in the risk of death of 20% and 29% for patients younger than 65 years and those 65 years and older, respectively. Given the acceptable and manageable toxicity profile, it is reassuring that CDK4/6-inhibitor combinations also proved to be statistically significantly effective in older patients, confirming and strengthening results from a previous meta-analysis of targeted agents combined with standard ET in elderly patients based on PFS as survival endpoint [26]. Importantly, CDK4/6-inhibitor–based treatments were also able to statistically significantly reduce the risk of death by 21% in an endocrine resistance setting and 27% in endocrine sensitive setting. Notably, in the endocrine-resistant subgroup, the only statistically significant individual result was the one obtained within the MONARCH 3 study, which specifically enrolled endocrine-resistant patients to be treated with an abemaciclib-based combination. In this trial, endocrine resistance was defined according to the previously mentioned ESO-ESMO definition [20]. Differently, in the endocrine-sensitive setting, only the palbociclib-containing PALOMA 3 trial was associated with a statistically significant result, and no abemaciclib-containing study was available for this analysis. Additionally, palbociclib and ribociclib proved, overall, to produce a statistically significant relative decrease in the risk of death for CT-naïve patients in the metastatic setting (28% death risk reduction) and also in patients with at least 3 metastatic sites (25% death risk reduction).

On the other hand, the observed OS benefits in patients with less than 3 metastatic sites (21% death risk reduction) or bone-only disease (18% death risk reduction) and in CT-pretreated patients in an advanced setting (15% death risk reduction) were not statistically significant. However, these data must be put into context. Firstly, it is important to point out that each of the clinical subsets examined had a different sample size and biological plausibility for a different effect size (as reflected by different HR). This also translates in a different power to identify a statistically significant treatment effect. In fact, it is highly likely that the analysis regarding the CT-pretreated subgroup was negatively affected by the low number of patients (94 of 572 and 177 of 521 from MONALEESA 7 and PALOMA 3 trials, respectively), probably insufficient to demonstrate a clear benefit in terms of OS. Additionally, when putting together CT-naïve and CT-pretreated patients, the cumulative effect observed was a statistically significant 23% relative reduction in the risk of death, with no statistically significant difference between the 2 groups, suggesting that CDK4/6-inhibitor–based treatments are

effective in both subsets, albeit a more pronounced effect could be obtained in CT-naïve patients. Furthermore, a posthoc subgroup analysis of the recently published Young-PEARL phase II trial comparing palbociclib + exemestane or fulvestrant vs capecitabine in premenopausal patients similarly showed a statistically significantly improved PFS for the CDK4/6-inhibitor arm in patients pretreated with CT for metastatic disease (HR: 0.62, 95%CI: 0.38 to 0.99) with a more uncertain benefit for CT-pretreated patients (HR: 0.82, 95%CI: 0.36 to 1.92) [27]. Taken together, these results clearly support the recommendation of international guidelines concerning the need to delay CT in HR+/HER2-negative MBC, except in case of visceral crisis [20,28], and support the use of CDK4/6-inhibitor–based treatments as upfront therapy.

When considering the subgroup of patients with less than 3 metastatic sites, the result was only marginally non-statistically significant (P=0.06, 95%CI: 0.62 to 1.01), reflecting a clear trend for improved survival. Additionally, when joining together patients with less than 3 and at least 3 metastatic sites, the pooled effect was statistically significant, with a meaningful 31% relative reduction in the risk of death and a statistically non-significant test for subgroup differences (P=0.74), suggesting a potentially more pronounced effect in patients with higher tumor burden compared with patients with a low tumor burden metastatic disease. Similarly, the OS benefit obtained with CDK4/6-inhibitor–based combinations in bone-only disease was not statistically significant. In fact, patients with bone-only metastatic tumors usually show a more indolent and less rapidly evolving disease, with an improved survival over patients with other metastatic sites [29]. Therefore, it is highly likely that more patients, longer follow-up, and more events might be needed to obtain more conclusive results. Moreover, within pivotal trials the interaction tests between treatment effect and metastatic sites were not statistically significant. Additionally, another meta-analysis demonstrated a statistically significantly improved PFS for CDK4/6-inhibitor–based therapies as first line in bone-only metastatic disease [29], further confirmed by a patient-level pooled analysis from the US Food and Drug Administration [30]. Furthermore, when taken together with the result obtained in the subset of patients with no bone-only tumors, the benefit was clinically meaningful (26% risk reduction) and statistically significant (P<0.001), with no statistically significant subgroup difference (P=0.55). Overall, this result should thus be interpreted carefully and be updated in the near future with still unpublished OS subgroup data from other CDK4/6-inhibitors trials in order to draw more definitive conclusions.

This meta-analysis has some limitations that need to be addressed. Firstly, some of the subgroups from published trials were not totally identical. More specifically, it was not possible to extract a clean result concerning all the patients untreated with CT in metastatic setting due to different subgroup characterization, which led to a potential underestimation of the number of patients

untreated with CT specifically in the metastatic setting (this happened for the PALOMA 3 study). Similarly, for visceral and nonvisceral disease, both MONALEESA 3 and 7 studies used the categorization “liver or lung involvement” instead of visceral and nonvisceral. Secondly, data about crossover after progression in the mono-ET arms have not been reported, except for MONALEESA 2 trial, where crossover was explicitly not admitted. Therefore, its impact on survival outcomes could not be clearly elucidated. Furthermore, subgroup analyses differed among trials; thus, for each of the subgroups considered, not all of the RCT could be included. In fact, for some of the trials included [7,17], OS data were published before final analysis, possibly because more mature results for worse subgroups (patients with visceral metastases, primary endocrine resistance) provided more OS events that drove the early stopping rules data. This might have produced a theoretical risk of “enriched” meta-analysis in positive trials because a negative trial is only published at the time of the final analysis. Therefore, intention-to-treat and subgroup OS data from the remaining PALOMA 2 and MONARCH 3 trials and final OS results from the MONALEESA 2, and MONARCH 2 trials are awaited. Nevertheless, based on PFS data and current results, we expect a substantial confirmation of the effects already observed, specifically in the subgroups with non-statistically significant results (e.g. tumors with very limited tumor burden and bone-only disease) for which current data might not be sufficiently mature. In fact, more patients and longer follow-up might be needed to observe a statistically significant effect. At the same time, due to the potentially more indolent disease course, it cannot be excluded that patients with bone-only metastases or very small tumor burden may experience prolonged PFS and OS even when receiving ET alone initially.

To our knowledge, this is the first meta-analysis exploring the impact and benefit of CDK4/6-inhibitor–based regimens on OS in specific clinically relevant subgroups. Results from our study address clinically relevant questions that might help the clinicians in better tailoring patients’ treatments. In this perspective, we also would like to point out that a study-level meta-analysis like ours, compared with patient-level meta-analysis, provides more rapid results and does not need large, time-consuming, and potentially more expensive collaborations between major competitors to obtain individual patients’ data from each trial, making it more suitable for addressing clinically relevant questions in a reasonable timeline. What most, results were not affected by significant heterogeneity and, overall, there was no truly relevant risk of bias concerning the included trials. It is also noteworthy that when the meta-analysis is based on only a few studies (2 or 3), the heterogeneity is difficult to estimate and standard random-effects meta-analysis methods are usually performed even if the obtained results may be influenced by the small number of studies (wide pooled confidence intervals). Nevertheless, it is unclear whether or to what extent small-sample-size behavior could be improved by more sophisticated modeling systems.

In conclusion, CDK4/6-inhibitors plus ET combinations are substantially effective in improving OS in HR+/HER2-negative MBC as first- or second-line treatment in young or adult (<65years) as well as in older patients independently from visceral involvement, endocrine sensitivity, and menopausal status. Ribociclib-based combinations might be preferred for the premenopausal setting, because the major contribution on the overall positive subgroup analysis result came from the ribociclib-based MONALEESA 7 trial, which specifically enrolled pre- and perimenopausal patients (a total of 672), whereas the other studies included only contributed with relatively small subgroups of the overall patients enrolled (108 and 114 for PALOMA 3 and MONARCH 3, respectively). On the other hand, abemaciclib-based combinations might be preferred for endocrine-resistant tumors, being the only CDK4/6-inhibitor clearly providing a statistically significant effect in this subset. However, it must be considered that this is only speculative, because no currently published data support the superiority of 1 of the 3 molecules, or the same CDK4/6-inhibitor with a different ET companion (AI, fulvestrant or tamoxifen), over the others [10,31]. Furthermore, the degree of benefit shown across pivotal trials for the intention-to-treat populations is quite similar [3–9]. Standard ET without CDK4/ 6-inhibitors might still be an option for bone-only and very limited disease given a more uncertain OS benefit. However, a clear PFS benefit demonstrated elsewhere [29,30] and a the current OS pooled analysis being substantially under-powered suggest that more data are needed to draw definitive conclusions. CDK4/6-based regimens should thus be considered in these subsets as upfront therapy, although they could still be used as a second-line option in case of different first-line treatment choice. Finally, it could be preferable avoiding CT as the upfront therapy in the metastatic setting. Apart from toxicity, activity, and efficacy concerns reported elsewhere [10,20,28,29,31], our analysis shows that upfront CT might also reduce the beneficial impact on OS for CDK4/6-inhibitor–based treatments.

Overall, our results strongly support the recommendations from major international treatment guidelines [20,28] and recent pooled analyses [10,30–32].

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References

1. Schettini F, Buono G, Cardalesi C, Desideri I, De Placido S, Del Mastro L. Hormone receptor/human epidermal growth factor receptor 2-positive breast cancer: where we are now and where we are going. Cancer Treat Rev. 2016;46:20–26.
2. Schettini F, De Santo I, Rea CG, et al. CDK 4/6 inhibitors as single agent in advanced solid tumors. Front Oncol. 2018;8:608.
3. Sledge GW, Toi M, Neven P, et al. MONARCH 2: abemaciclib in combination with fulvestrant in women with HR+/HER2- advanced breast cancer who had progressed while receiving endocrine therapy. J Clin Oncol. 2017;35(25): 2875–2884.
4. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol. 2016;17(4):425–439.
5. Tripathy D, Im S-A, Colleoni M, et al. Ribociclib plus endocrine therapy for premenopausal women with hormone-receptor-positive, advanced breast cancer (MONALEESA-7): a randomised phase 3 trial. Lancet Oncol. 2018;19(7): 904–915.
6. Finn RS, Martin M, Rugo HS, et al. Palbociclib and letrozole in advanced breast cancer. N Engl J Med. 2016;375(20):1925–1936.

7. Hortobagyi GN, Stemmer SM, Burris HA, et al. Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. Ann Oncol. 2018;29(7):1541–1547.
8. Slamon DJ, Neven P, Chia S, et al. PhasecIII randomized study of ribociclib and fulvestrant in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: MONALEESA-3. J Clin Oncol. 2018; 36(24):2465–2472.
9. Goetz MP, Toi M, Campone M, et al. MONARCH 3: abemaciclib as initial therapy for advanced breast cancer. J Clin Oncol. 2017;35(32):3638–3646.
10. Giuliano M, Schettini F, Rognoni C, et al. Endocrine treatment versus chemotherapy in postmenopausal women with hormone receptor-positive, HER2-negative, metastatic breast cancer: a systematic review and network meta-analysis. Lancet Oncol. 2019;20(10):1360–1369.
11. Finn RS, Crown JP, Lang I, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol. 2015; 16(1):25–35.
12. Turner NC, Slamon DJ, Ro J, et al. Overall survival with palbociclib and fulvestrant in advanced breast cancer. N Engl J Med. 2018;379(20):1926–1936.
13. Miksad RA, Zietemann V, Gothe R, et al. Progression-free survival as a surrogate endpoint in advanced breast cancer. Int J Technol Assess Health Care. 2008; 24(04):371–383.
14. Forsythe A, Chandiwana D, Barth J, Thabane M, Baeck J, Tremblay G. Progression-free survival/time to progression as a potential surrogate for overall survival in HR+, HER2+ metastatic breast cancer. Breast Cancer. 2018; 10:69–78.
15. Sargent DJ, Hayes DF. Assessing the measure of a new drug: is survival the only thing that matters? J Clin Oncol. 2008;26(12):1922–1923.
16. Slamon DJ, Neven P, Chia S, et al. Overall survival with ribociclib plus fulvestrant in advanced breast cancer. N Engl J Med. 2020;382(6):514–524.
17. Sledge GW, Toi M, Neven P, et al. The effect of abemaciclib plus fulvestrant on overall survival in hormone receptor-positive, ERBB2-negative breast cancer that progressed on endocrine therapy-MONARCH 2: a randomized clinical trial. JAMA Oncol. 2020;6(1):116.
18. Im S-A, Lu Y-S, Bardia A, et al. Overall survival with ribociclib plus endocrine therapy in breast cancer. N Engl J Med. 2019;381(4):307–316.
19. Tanguy M-L, Cabel L, Berger F, Pierga J-Y, Savignoni A, Bidard F-C. CDK4/6-inhibitors and overall survival: power of first-line trials in metastatic breast cancer. NPJ Breast Cancer. 2018;4(1):14.
20. Cardoso F, Senkus E, Costa A, et al. 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4). Ann Oncol. 2018;29(8): 1634–1657.
21. Der Simonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177–188.
22. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539–1558.
23. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2017. https://www.R-project.org/.
24. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0. The Cochrane Collaboration. http://handbook. cochrane.org. Accessed March 2011.
25. The Nordic Cochrane Centre. The Cochrane Collaboration. Review Manager (RevMan). Copenhagen, Denmark: The Nordic Cochrane Centre;2014.

26. Omarini C, Piacentini F, Sperduti I, et al. Endocrine-based targeted combination versus endocrine therapy alone as first-line treatment in elderly patients with hormone receptor-positive advanced breast cancer: meta-analysis of phase II and III randomized clinical trials. J Clin Oncol. 2019;37(15_suppl):1046–1046.
27. Park YH, Kim T-Y, Kim GM, et al. Palbociclib plus exemestane with gonadotropin-releasing hormone agonist versus capecitabine in premenopausal women with hormone receptor-positive, HER2-negative metastatic breast cancer (KCSG-BR15-10): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol. 2019;20(12):1750–1759.
28. Rugo HS, Rumble RB, Macrae E, et al. Endocrine therapy for hormone receptor–positive metastatic breast cancer: American Society of Clinical Oncology Guideline. J Clin Oncol. 2016;34(25):3069–3103.
29. Toss A, Venturelli M, Sperduti I, et al. First-line treatment for endocrine-sensitive bone-only metastatic breast cancer: systematic review and meta-analysis. Clin Breast Cancer. 2019;19(6):701–716.
30. Gao JJ, Cheng J, Bloomquist E, et al. CDK4/6 inhibitor treatment for patients with hormone receptor-positive, HER2-negative, advanced or metastatic breast cancer: a US Food and Drug Administration pooled analysis. Lancet Oncol. 2020;21(2):250–260.
31. Wilson FR, Varu A, Mitra D, Cameron C, Iyer S. Systematic review and network meta-analysis comparing palbociclib with chemotherapy agents for the treatment of postmenopausal women with HR-positive and HER2-negative advanced/metastatic breast cancer. Breast Cancer Res Treat. 2017; 166(1):167–177.
32. Generali D, Venturini S, Rognoni C, et al. A network meta-analysis of everolimus plus exemestane versus chemotherapy in the first- and second-line treatment of estrogen receptor-positive metastatic breast cancer. Breast Cancer Res Treat. 2015;152(1):95–117.

DISCUSSION

Breast cancer therapeutic scenario has been shifting towards ever-increasing personalized treatment strategies since the introduction of anti-HER2 target therapy. Tailored therapies can be represented by both drugs directed against tumor/patient-specific molecular targets or entire treatment strategies based on a number of patient's and tumor's variables that have to be collectively taken into account. Examples of the first strategy are anti-HER2 directed treatments for HER2+ tumors and the PI3K inhibitor alpelisib combined with fulvestrant for *PIK3CA*-mutant HR+/HER2-neg. disease. An example of the second approach is represented by adjuvant "post-neoadjuvant" treatments in patients not achieving pCR (e.g. capecitabine in TNBC or T-DM1 in HER2+ tumors). The correct identification of appropriate biomarkers of response to diverse treatments is crucial to further develop tumor-specific individual patient-centered treatment strategies. This was precisely one of the main focus of this thesis.

With respect to HER2+ breast cancer, in the last decade, many potential biomarkers have been explored, including PTEN, PIK3CA mutations, p95-HER2[58–60]; however, the only biomarker that has reached clinical utility to date is HR status, except for HER2 itself. Among the different biomarkers, PAM50 intrinsic subtypes have shown promising results in HER2+ disease. In particular, the HER2-E subtype, which represents approximately 50% of all HER2+ tumors, seems to identify a subgroup of highly HER2 addicted HER2+ tumors[61]. In our metanalysis, we evaluated data from 16 different studies and 3,733 patients treated in the neoadjuvant setting. Our results are clear in confirming that the HER2-E subtype is a consistent predictor of pCR in the presence or absence of CT. In addition, we showed that it provides information beyond HR status. This article has been helpful to better establish the clinical validity of the HER2-E biomarker in the neoadjuvant setting of HER2+ breast cancer. Future studies to escalate or de-escalate systemic therapy in this setting should consider this genomic biomarker.

During the last few years, practice-changing clinical trials have led to the approval of innovative endocrine-based treatments for HR+/HER2-neg. metastatic breast cancer. However, intrinsic and acquired endocrine resistance occurs in a significant proportion of patients, ultimately leading to progression and death; one of such mechanism of resistance is based on mTOR activity[62–64]. This molecule is a downstream effector of the PI3K pathway, which is implicated in cell growth and survival, angiogenesis and immune regulation and frequently contributes to breast cancer progression as a key mechanism of resistance to ET[62–64]. The mTOR inhibitor everolimus, combined with exemestane, is approved for the treatment of advanced HR+/HER2-neg. breast cancer. However, benefit from everolimus is variable and reliable biomarkers for the selection of patients who will most likely respond lack. There is emerging evidence highlighting the immunomodulatory role of

everolimus in several solid tumors, excluding breast cancer[65–68]. For this reason, we retrospectively investigated immune infiltrate and circulating immune cells in breast cancers using several cohorts of patients treated with everolimus, to find out potential easy-to-detect biomarkers of response. Albeit preliminary, our results show that lymphocytes subpopulations, CECs and NLR might serve for the scope in HR+ breast tumors. Further confirmatory studies are now warranted.

HER2 is a well-known drug target in HER2+ breast cancer. However, tumors that express some levels of it (i.e. HER2-low), but do not fulfill the definition of HER2+ disease are recently gaining a lot of attention. The reason is that new anti-HER2 ADC, such as trastuzumab deruxtecan and trastuzumab duocarmazine, are showing impressive results in HER2-neg./HER2-low disease in breast cancer and other cancer types. Thus, there was a need to better understand the clinical and molecular features of HER2-low disease and reimagine the role of HER2 as therapeutic target in a broader range of breast tumors. In our study, we have analyzed the clinical-pathological features of 3,689 HER2-neg. (HER2-low and HER2 0) breast tumors from 13 different studies, and performed gene expression analyses. We found that more than half of HER2-low breast tumors are HR+, while about a third is triple negative (65.4% vs 36.6%). Apparently, HER2-low status (collectively or by separate IHC 1+ and 2+ scores) by itself was not associated to different survival outcomes in both HR+ tumors and TNBC. We also discovered that the proportion of Luminal A and B tumors was higher in HR+/HER2-low tumors than in HR+/HER2 0. Interestingly, *ERBB2* mRNA was more expressed in HR+/HER2-low than in HR+/HER2 0 tumors, and more in HR+/HER2 2+ than in HER2 1+. Differently, within TNBC no differences in subtype distribution and *ERBB2* mRNA levels could be observed between HER2-low and HER2 0. Finally, *ERBB2* mRNA appeared to be significantly more expressed (2.5-fold difference) in HR+/HER2low than in TNBC/HER2low. Overall, our results suggest that HR status is critical to distinguish 2 main groups of HER2-low disease, with HR+/HER2-low tumors being a more distinct and defined biological entity than TNBC/HER2-low. Moreover, the reproducibility of the HER2-low identification among pathologists was suboptimal. This study thus emphasized the large biological heterogeneity of HER2-low breast cancer, and the need to implement reproducible and sensitive assays to measure low HER2 expression, as well.

Another important part of this thesis was focused on metastatic luminal tumors in postmenopausal patients, to refine metastatic treatment algorithms.

The availability of new TT combined with ET, including everolimus and CDK4/6-inhibitors, as well as the new alpha-specific PI3K inhibitors, has led to radical changes in treatment algorithms. Despite all the major international guidelines support the administration of ET ± TT in postmenopausal advanced HR+/HER2-neg. breast cancer, upfront use of CT is still a common approach, even in the

absence of visceral crisis, as previously highlighted[31,32]. Apart from some prejudice towards ET or novel TT, with respect to established CT, an important cause might be the lack of direct comparisons among ET and CT regimens. In fact, apart from few very late trials of the 70s' and 80s', before we performed our network meta-analysis, the only available evidence of comparisons between ET and CT was represented by a study published in 1992 from Dixon et al. and the BOLERO-6 trial, published in 2018[69–71]. Moreover, the newest ET + TT regimens have not been directly confronted among each other in RCTs (e.g. palbociclib vs. ribociclib vs. abemaciclib or CDK4/6inhibitors vs mTOR inhibitors etc.) nor will be in the near future. This leaves some degrees of uncertainty regarding the optimal treatment algorithm in HR+/HER2-neg. metastatic disease. In this complex scenario, we sought to compare efficacy and activity of 1st/2nd line CT- and ET-based treatments, by performing a network meta-analysis, that is the only accepted methodology capable to compare different treatments not being investigated in head-to-head randomized clinical trials. We adopted a Bayesian approach to perform all the analyses, in order to assure a solid methodology. We found that:

1. CT-based regimens are not superior to CDK4/6-inhibitors-based combinations, everolimus+exemestane and alpelisib+fulvestrant (in *PIK3CA*-mutant tumors) in efficacy;
2. Response rates with CT are mostly not superior to the ones obtained with novel ET+TT regimens;
3. Single agent ET are inferior to most of the ET+TT in efficacy and activity;
4. Some CT+/-TT are more effective than single agent ET, but most CT are not;
5. The toxicity profile of ET+TT is intermediate between single agent ET (best profile) and CT (worst profile, more with poli-CT);
6. None of the 3 CDK4/6-inhibitor is superior to the other, neither in combination with an AI, nor with fulvestrant.

After our work was published, results from randomized controlled trials on the topic were presented. The phase III PEARL evaluated palbociclib + exemestane or fulvestrant vs capecitabine in postmenopausal patients and did not show significant differences in PFS (HR: 1.09, 95%CI:0.83, 1.44), nor in response rates. Most frequent G3-4 toxicities with palbociblib + ET and capecitabine were neutropenia (~56% and 5.5%, respectively), hand-foot syndrome (0% and 23.5%, respectively) and diarrhea (~1% and 7.6%, respectively)[72]. It's interesting to note that the results observed with this trial are in line with the results of our network meta-analysis, although our study was conducted previously, therefore could not include PEARL data. Nevertheless, the phase II Young-PEARL, that evaluated in premenopausal women palbociclib + exemestane + GnRH analogue vs capecitabine in 1st-/2nd-line, showed that the palbociclib-containing regimen was significantly superior to capecitabine in PFS (p=0.024) and comparable in terms of overall response rates (37% vs 34%,

p=0.781)[73]. All in all, the current body of evidence now strongly support main international treatment guidelines in recommending ET ± TT as the main upfront treatment of option in HR+/HER2-neg. metastatic breast cancer[4,9,74].

Importantly, apart from our network meta-analysis, no comparison exist between all the available CDK4/6-inhibitors-based regimens. The only direct comparison that has been provided so far comes from the PARSIFAL phase II RCT, that compared palbociclib in combination with different ET (AI vs fulvestrant) in 1st-line. This study confirmed no differences in both survival and response rates, as also previously observed with our indirect comparisons[75]. Therefore, based on the available data, there is no evidence to support the superiority of one specific CDK4/6-inhibitor over the others in the metastatic setting, and the choice should be based on tolerability and schedule preferences (palbociclib and ribociclib require one week off, while abemaciclib is administered continuously).

After the publication of our meta-analysis, OS data regarding all 3 inhibitors became finally available, although some OS results are still awaited. Therefore, we decided to perform a meta-analysis with the aim of identifying a more precise and reliable benefit deriving from CDK4/6 inhibitors-based treatments in different clinically-relevant subgroups of HR+/HER2-neg. breast tumors. The goal was to provide more definitive conclusions and help the clinicians in better selecting the most appropriate treatment option in different clinical conditions. We investigated the following subsets: visceral/non-visceral disease, bone-only disease (yes/no), number of metastatic sites, endocrine sensitivity and resistance, previous CT for the metastatic setting, age (<65/≥65 years) and menopausal status.

Our results showed that CDK4/6 inhibitors combined to endocrine agents are superior to standard ET, independently from age, menopausal status, endocrine sensitiveness and visceral involvement, providing a clinically meaningful and statistically significant OS benefit in almost all of the subgroups examined (relative reduction in the risk of death ranging 21% - 29%). These combinations should thus be preferred as upfront therapy instead of single agent ET. The benefit was less clear in bone-only and low tumor burden (<3 metastatic sites) disease, where single agent ET might still play a role in selected cases. The OS improvement was also potentially impaired by CT-pretreatment in metastatic setting, further supporting the idea that CDK4/6-inhibitors + ET should be administered before CT in the advanced disease. Finally, in the endocrine-resistant subgroup, the only statistically significant individual result was the one obtained the with an abemaciclib-based combination that drove the overall significant pooled subgroup result. For this reason, in the specific subgroup of endocrine resistant tumors the use of abemaciclib might be favored with respect to the other inhibitors.

CONCLUSIONS

In conclusion, the articles that are part of this thesis, provided evidence to:

5. Support the use of the PAM50 HER2-E molecular subtype, if not in the clinical practice, at least in future clinical trials to assess neoadjuvant escalated or de-escalated therapeutic approaches in HER2+ tumors, irrespective of HR status;
6. Further explore CECs, NLR and lymphocytes subpopulations as biomarkers of response to select optimal candidates to everolimus in HR+ breast cancer patients;
7. Support main international treatment guidelines in recommending ET+TT as the preferred $1^{st}/2^{nd}$-line treatment of HR+/HER2-neg. postmenopausal metastatic breast tumors, especially CDK4/6-inhibitors-based regimens;
8. Support the use of CDK4/6-inhibitors-based regimens instead of single agent ET, independently from age, menopausal status, endocrine sensitiveness and visceral involvement.

Finally, we dissected for the first time the clinicopathological and molecular characteristics of HER2-low breast tumors and find out that this category do not show the features of an independent breast cancer subtype. However, the detection of HER2 low protein levels as well as the assessment of *ERBB2* mRNA levels, might play an important role from a therapeutic perspective in the near future. Moreover, HR+/HER2-low show distinct features from TNBC/HER2-low and HER2 0, as well as from HR+/HER2 0 tumors, while TNBC/HER2-low do not present substantial differences with TNBC/HER2 0. This merit further investigation for potential therapeutic implications.

REFERENCES

[1] SEER statistics for breast cancer n.d. https://seer.cancer.gov.

[2] Gong Y, Liu Y-R, Ji P, Hu X, Shao Z-M. Impact of molecular subtypes on metastatic breast cancer patients: a SEER population-based study. Sci Rep 2017;7:45411–45411. https://doi.org/10.1038/srep45411.

[3] Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol Off J Eur Soc Med Oncol 2019;30:1674. https://doi.org/10.1093/annonc/mdz189.

[4] Cardoso F, Paluch-Shimon S, Senkus E, Curigliano G, Aapro MS, André F, et al. 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5)†. Ann Oncol 2020;0. https://doi.org/10.1016/j.annonc.2020.09.010.

[5] Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, et al. Tailoring therapies--improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol Off J Eur Soc Med Oncol 2015;26:1533–46. https://doi.org/10.1093/annonc/mdv221.

[6] Howlader N, Altekruse SF, Li CI, Chen VW, Clarke CA, Ries LAG, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst 2014;106. https://doi.org/10.1093/jnci/dju055.

[7] Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J Clin Oncol 2020;38:1346–66. https://doi.org/10.1200/JCO.19.02309.

[8] Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol Off J Am Soc Clin Oncol 2018;36:2105–22. https://doi.org/10.1200/JCO.2018.77.8738.

[9] NCCN Guidelines for Breast Cancer, vers.3.2019 n.d. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf.

[10] Curigliano G, Burstein HJ, Winer EP, Gnant M, Dubsky P, Loibl S, et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol Off J Eur Soc Med Oncol 2017;28:1700–12. https://doi.org/10.1093/annonc/mdx308.

[11] Prat A, Pineda E, Adamo B, Galván P, Fernández A, Gaba L, et al. Clinical implications of the

intrinsic molecular subtypes of breast cancer. Breast Edinb Scotl 2015;24 Suppl 2:S26-35. https://doi.org/10.1016/j.breast.2015.07.008.

[12] Prat A, Carey LA, Adamo B, Vidal M, Tabernero J, Cortés J, et al. Molecular features and survival outcomes of the intrinsic subtypes within HER2-positive breast cancer. J Natl Cancer Inst 2014;106. https://doi.org/10.1093/jnci/dju152.

[13] Schettini F, Pascual T, Conte B, Chic N, Brasó-Maristany F, Galván P, et al. HER2-enriched subtype and pathological complete response in HER2-positive breast cancer: A systematic review and meta-analysis. Cancer Treat Rev 2020;84:101965. https://doi.org/10.1016/j.ctrv.2020.101965.

[14] Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011;5:5–23. https://doi.org/10.1016/j.molonc.2010.11.003.

[15] Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet Lond Engl 2014;384:164–72. https://doi.org/10.1016/S0140-6736(13)62422-8.

[16] von Minckwitz G, Huang C-S, Mano MS, Loibl S, Mamounas EP, Untch M, et al. Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer. N Engl J Med 2019;380:617–28. https://doi.org/10.1056/NEJMoa1814017.

[17] Masuda N, Lee S-J, Ohtani S, Im Y-H, Lee E-S, Yokota I, et al. Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy. N Engl J Med 2017;376:2147–59. https://doi.org/10.1056/NEJMoa1612645.

[18] Poggio F, Bruzzone M, Ceppi M, Pondé NF, La Valle G, Del Mastro L, et al. Platinum-based neoadjuvant chemotherapy in triple-negative breast cancer: a systematic review and meta-analysis. Ann Oncol Off J Eur Soc Med Oncol 2018;29:1497–508. https://doi.org/10.1093/annonc/mdy127.

[19] Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Peto R, Davies C, Godwin J, Gray R, Pan HC, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet Lond Engl 2012;379:432–44. https://doi.org/10.1016/S0140-6736(11)61625-5.

[20] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet Lond Engl 2015;386:1341–52. https://doi.org/10.1016/S0140-6736(15)61074-1.

[21] Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C, Godwin J, Gray R, Clarke M, Cutter D, et al. Relevance of breast cancer hormone receptors and other factors to

the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet Lond Engl 2011;378:771–84. https://doi.org/10.1016/S0140-6736(11)60993-8.

[22] Corona SP, Roviello G, Strina C, Milani M, Madaro S, Zanoni D, et al. Efficacy of extended aromatase inhibitors for hormone-receptor-positive breast cancer: A literature-based meta-analysis of randomized trials. Breast Edinb Scotl 2019;46:19–24. https://doi.org/10.1016/j.breast.2019.04.004.

[23] Ibrahim EM, Al-Hajeili MR, Bayer AM, Abulkhair OA, Refae AA. Extended adjuvant endocrine therapy in early breast cancer: a meta-analysis of published randomized trials. Med Oncol Northwood Lond Engl 2017;34:131. https://doi.org/10.1007/s12032-017-0986-2.

[24] Qian X, Li Z, Ruan G, Tu C, Ding W. Efficacy and toxicity of extended aromatase inhibitors after adjuvant aromatase inhibitors-containing therapy for hormone-receptor-positive breast cancer: a literature-based meta-analysis of randomized trials. Breast Cancer Res Treat 2020;179:275–85. https://doi.org/10.1007/s10549-019-05464-w.

[25] Yamashiro H, Iwata H, Masuda N, Yamamoto N, Nishimura R, Ohtani S, et al. Outcomes of trastuzumab therapy in HER2-positive early breast cancer patients: extended follow-up of JBCRG-cohort study 01. Breast Cancer Tokyo Jpn 2020;27:631–41. https://doi.org/10.1007/s12282-020-01057-4.

[26] Chumsri S, Li Z, Serie DJ, Mashadi-Hossein A, Colon-Otero G, Song N, et al. Incidence of Late Relapses in Patients With HER2-Positive Breast Cancer Receiving Adjuvant Trastuzumab: Combined Analysis of NCCTG N9831 (Alliance) and NRG Oncology/NSABP B-31. J Clin Oncol 2019;37:3425–35. https://doi.org/10.1200/JCO.19.00443.

[27] von Minckwitz G, Procter M, de Azambuja E, Zardavas D, Benyunes M, Viale G, et al. Adjuvant Pertuzumab and Trastuzumab in Early HER2-Positive Breast Cancer. N Engl J Med 2017;377:122–31. https://doi.org/10.1056/NEJMoa1703643.

[28] Chan A, Moy B, Mansi J, Ejlertsen B, Holmes FA, Chia S, et al. Final Efficacy Results of Neratinib in HER2-positive Hormone Receptor-positive Early-stage Breast Cancer From the Phase III ExteNET Trial. Clin Breast Cancer 2020. https://doi.org/10.1016/j.clbc.2020.09.014.

[29] Gianni L, Pienkowski T, Im Y-H, Roman L, Tseng L-M, Liu M-C, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:25–32. https://doi.org/10.1016/S1470-2045(11)70336-9.

[30] Giordano SH, Temin S, Chandarlapaty S, Crews JR, Esteva FJ, Kirshner JJ, et al. Systemic Therapy for Patients With Advanced Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer: ASCO Clinical Practice Guideline Update. J Clin Oncol 2018;36:2736–40.

https://doi.org/10.1200/JCO.2018.79.2697.

[31] Cazzaniga M, Mustacchi G, Giordano M, Garrone O, Donadio M, del mastro L, et al. 259PAdherence to International ESO-ESMO (ABC) guide-lines in HER2-ve metastatic breast cancer (MBC) patients (pts): Preliminary results of the GIM 13 - AMBRA Study. Ann Oncol 2017;28. https://doi.org/10.1093/annonc/mdx365.022.

[32] Lobbezoo DJA, van Kampen RJW, Voogd AC, Dercksen MW, van den Berkmortel F, Smilde TJ, et al. In real life, one-quarter of patients with hormone receptor-positive metastatic breast cancer receive chemotherapy as initial palliative therapy: a study of the Southeast Netherlands Breast Cancer Consortium. Ann Oncol 2015;27:256–62. https://doi.org/10.1093/annonc/mdv544.

[33] André F, Neven P, Marinsek N, Zhang J, Baladi J-F, Degun R, et al. Disease management patterns for postmenopausal women in Europe with hormone-receptor-positive, human epidermal growth factor receptor-2 negative advanced breast cancer. Curr Med Res Opin 2014;30:1007–16. https://doi.org/10.1185/03007995.2014.887002.

[34] Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee K-H, et al. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. N Engl J Med 2018;379:753–63. https://doi.org/10.1056/NEJMoa1802905.

[35] Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. N Engl J Med 2017;377:523–33. https://doi.org/10.1056/NEJMoa1706450.

[36] Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med 2018;379:2108–21. https://doi.org/10.1056/NEJMoa1809615.

[37] Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im S-A, Yusof MM, et al. KEYNOTE-355: Randomized, double-blind, phase III study of pembrolizumab + chemotherapy versus placebo + chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer. J Clin Oncol 2020;38:1000–1000. https://doi.org/10.1200/JCO.2020.38.15_suppl.1000.

[38] Bardia A, Mayer IA, Vahdat LT, Tolaney SM, Isakoff SJ, Diamond JR, et al. Sacituzumab Govitecan-hziy in Refractory Metastatic Triple-Negative Breast Cancer. N Engl J Med 2019;380:741–51. https://doi.org/10.1056/NEJMoa1814213.

[39] Common Terminology Criteria for Adverse Events (CTCAE) | Protocol Development | CTEP n.d. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm (accessed November 24, 2020).

[40] Giuliano M, Schettini F, Rognoni C, Milani M, Jerusalem G, Bachelot T, et al. Endocrine treatment versus chemotherapy in postmenopausal women with hormone receptor-positive, HER2-negative, metastatic breast cancer: a systematic review and network meta-analysis. Lancet Oncol 2019. https://doi.org/10.1016/S1470-2045(19)30420-6.

[41] Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of Anthracyclines. Front Cardiovasc Med 2020;7. https://doi.org/10.3389/fcvm.2020.00026.

[42] Bodai BI, Tuso P. Breast Cancer Survivorship: A Comprehensive Review of Long-Term Medical Issues and Lifestyle Recommendations. Perm J 2015;19:48–79. https://doi.org/10.7812/TPP/14-241.

[43] Kirova YM, De Rycke Y, Gambotti L, Pierga J-Y, Asselain B, Fourquet A. Second malignancies after breast cancer: the impact of different treatment modalities. Br J Cancer 2008;98:870–4. https://doi.org/10.1038/sj.bjc.6604241.

[44] Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst 1994;86:527–37. https://doi.org/10.1093/jnci/86.7.527.

[45] Sismondi P, Biglia N, Volpi E, Giai M, de Grandis T. Tamoxifen and endometrial cancer. Ann N Y Acad Sci 1994;734:310–21. https://doi.org/10.1111/j.1749-6632.1994.tb21761.x.

[46] Davies C, Pan H, Godwin J, Gray R, Arriagada R, Raina V, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet Lond Engl 2013;381:805–16. https://doi.org/10.1016/S0140-6736(12)61963-1.

[47] Pagano T, De Rosa P, Vallone R, Schettini F, Arpino G, De Placido S, et al. Fractional microablative CO2 laser for vulvovaginal atrophy in women treated with chemotherapy and/or hormonal therapy for breast cancer: a retrospective study. Menopause N Y N 2016;23:1108–13. https://doi.org/10.1097/GME.0000000000000672.

[48] Sodergren SC, Copson E, White A, Efficace F, Sprangers M, Fitzsimmons D, et al. Systematic Review of the Side Effects Associated With Anti-HER2-Targeted Therapies Used in the Treatment of Breast Cancer, on Behalf of the EORTC Quality of Life Group. Target Oncol 2016;11:277–92. https://doi.org/10.1007/s11523-015-0409-2.

[49] Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012;367:1783–91. https://doi.org/10.1056/NEJMoa1209124.

[50] Modi S, Saura C, Yamashita T, Park YH, Kim S-B, Tamura K, et al. Trastuzumab Deruxtecan

in Previously Treated HER2-Positive Breast Cancer. N Engl J Med 2020;382:610–21. https://doi.org/10.1056/NEJMoa1914510.

[51] Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med 2018;379:2108–21. https://doi.org/10.1056/NEJMoa1809615.

[52] Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im S-A, Yusof MM, et al. KEYNOTE-355: Randomized, double-blind, phase III study of pembrolizumab + chemotherapy versus placebo + chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer. J Clin Oncol 2020;38:1000–1000. https://doi.org/10.1200/JCO.2020.38.15_suppl.1000.

[53] Bayraktar S, Glück S, Darling H. Update on PARP inhibitor therapy for solid tumors. J Cancer Prev Curr Res 2019;10:98–107.

[54] Modi S, Park H, Murthy RK, Iwata H, Tamura K, Tsurutani J, et al. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low-Expressing Advanced Breast Cancer: Results From a Phase Ib Study. J Clin Oncol Off J Am Soc Clin Oncol 2020:JCO1902318–JCO1902318. https://doi.org/10.1200/JCO.19.02318.

[55] Banerji U, van Herpen CML, Saura C, Thistlethwaite F, Lord S, Moreno V, et al. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. Lancet Oncol 2019;20:1124–35. https://doi.org/10.1016/S1470-2045(19)30328-6.

[56] Schettini F, Giudici F, Giuliano M, Cristofanilli M, Arpino G, Del Mastro L, et al. Overall Survival of CDK4/6-Inhibitor-Based Treatments in Clinically Relevant Subgroups of Metastatic Breast Cancer: Systematic Review and Meta-Analysis. J Natl Cancer Inst 2020;112:1089–97. https://doi.org/10.1093/jnci/djaa071.

[57] Schettini F, Sobhani N, Ianza A, Triulzi T, Molteni A, Lazzari MC, et al. Immune system and angiogenesis-related potential surrogate biomarkers of response to everolimus-based treatment in hormone receptor-positive breast cancer: an exploratory study. Breast Cancer Res Treat 2020;184:421–31. https://doi.org/10.1007/s10549-020-05856-3.

[58] Loibl S, Majewski I, Guarneri V, Nekljudova V, Holmes E, Bria E, et al. PIK3CA mutations are associated with reduced pathological complete response rates in primary HER2-positive breast cancer: pooled analysis of 967 patients from five prospective trials investigating lapatinib and trastuzumab. Ann Oncol Off J Eur Soc Med Oncol 2016;27:1519–25. https://doi.org/10.1093/annonc/mdw197.

[59] Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, et al. Expression of p95HER2, a

truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst 2007;99:628–38. https://doi.org/10.1093/jnci/djk134.

[60] Rimawi MF, De Angelis C, Contreras A, Pareja F, Geyer FC, Burke KA, et al. Low PTEN levels and PIK3CA mutations predict resistance to neoadjuvant lapatinib and trastuzumab without chemotherapy in patients with HER2 over-expressing breast cancer. Breast Cancer Res Treat 2018;167:731–40. https://doi.org/10.1007/s10549-017-4533-9.

[61] Prat A, Pascual T, De Angelis C, Gutierrez C, Llombart-Cussac A, Wang T, et al. HER2-enriched subtype and ERBB2 expression in HER2-positive breast cancer treated with dual HER2 blockade. J Natl Cancer Inst 2019. https://doi.org/10.1093/jnci/djz042.

[62] Conciatori F, Bazzichetto C, Falcone I, Pilotto S, Bria E, Cognetti F, et al. Role of mTOR Signaling in Tumor Microenvironment: An Overview. Int J Mol Sci 2018;19. https://doi.org/10.3390/ijms19082453.

[63] Schettini F, Buono G, Trivedi MV, De Placido S, Arpino G, Giuliano M. PI3K/mTOR Inhibitors in the Treatment of Luminal Breast Cancer. Why, When and to Whom. Breast Care 2017;12:290–4. https://doi.org/10.1159/000481657.

[64] McAuliffe PF, Meric-Bernstam F, Mills GB, Gonzalez-Angulo AM. Deciphering the role of PI3K/Akt/mTOR pathway in breast cancer biology and pathogenesis. Clin Breast Cancer 2010;10 Suppl 3:S59-65. https://doi.org/10.3816/CBC.2010.s.013.

[65] Santoni M, Buti S, Conti A, Porta C, Procopio G, Sternberg CN, et al. Prognostic significance of host immune status in patients with late relapsing renal cell carcinoma treated with targeted therapy. Target Oncol 2015;10:517–22. https://doi.org/10.1007/s11523-014-0356-3.

[66] Santoni M, De Giorgi U, Iacovelli R, Conti A, Burattini L, Rossi L, et al. Pre-treatment neutrophil-to-lymphocyte ratio may be associated with the outcome in patients treated with everolimus for metastatic renal cell carcinoma. Br J Cancer 2013;109:1755–9. https://doi.org/10.1038/bjc.2013.522.

[67] Kobayashi M, Kubo T, Komatsu K, Fujisaki A, Terauchi F, Natsui S, et al. Changes in peripheral blood immune cells: their prognostic significance in metastatic renal cell carcinoma patients treated with molecular targeted therapy. Med Oncol Northwood Lond Engl 2013;30:556. https://doi.org/10.1007/s12032-013-0556-1.

[68] Personeni N, Giordano L, Abbadessa G, Porta C, Borbath I, Daniele B, et al. Prognostic value of the neutrophil-to-lymphocyte ratio in the ARQ 197-215 second-line study for advanced hepatocellular carcinoma. Oncotarget 2017;8:14408–15. https://doi.org/10.18632/oncotarget.14797.

[69] Jerusalem G, de Boer RH, Hurvitz S, Yardley DA, Kovalenko E, Ejlertsen B, et al. Everolimus

Plus Exemestane vs Everolimus or Capecitabine Monotherapy for Estrogen Receptor-Positive, HER2-Negative Advanced Breast Cancer: The BOLERO-6 Randomized Clinical Trial. JAMA Oncol 2018;4:1367–74. https://doi.org/10.1001/jamaoncol.2018.2262.

[70] Dixon AR, Jackson L, Chan S, Haybittle J, Blamey RW. A randomised trial of second-line hormone vs single agent chemotherapy in tamoxifen resistant advanced breast cancer. Br J Cancer 1992;66:402–4. https://doi.org/10.1038/bjc.1992.277.

[71] Wilcken N, Hornbuckle J, Ghersi D. Chemotherapy alone versus endocrine therapy alone for metastatic breast cancer. Cochrane Database Syst Rev 2003:CD002747. https://doi.org/10.1002/14651858.CD002747.

[72] Martin M, Zielinski, C, Ruíz-Borrego M, Carrasco E, Ciruelos E, Muñoz M. GS2-06.- Results from PEARL study (GEICAM/2013-02_CECOG/BC.1.3.006): a phase 3 trial of Palbociclib (PAL) in combination with endocrine therapy (ET) versus Capecitabine (CAPE) in hormonal receptor (HR)-positive/human epidermal growth factor receptor (HER) 2-negative metastatic breast cancer (MBC) patients (pts) whose disease progressed on aromatase inhibitors (AIs). San Antonio Breast Cancer Symp. 2019, vol. General Session 2, San Antonio, TX: 2019, p. 07.

[73] Park YH, Kim TY, Kim GM, Jung KH, Kang SY, Park IH, et al. A randomized phase II study of palbociclib plus exemestane with GNRH agonist versus capecitabine in premenopausal women with hormone receptor-positive metastatic breast cancer (KCSG-BR 15-10, NCT02592746). J Clin Oncol 2019;37:1007–1007. https://doi.org/10.1200/JCO.2019.37.15_suppl.1007.

[74] Rugo HS, Rumble RB, Macrae E, Barton DL, Connolly HK, Dickler MN, et al. Endocrine Therapy for Hormone Receptor–Positive Metastatic Breast Cancer: American Society of Clinical Oncology Guideline. J Clin Oncol 2016;34:3069–103. https://doi.org/10.1200/JCO.2016.67.1487.

[75] Llombart-Cussac A, Pérez-García JM, Bellet M, Dalenc F, Gil Gil MJ, Ruiz Borrego M, et al. PARSIFAL: A randomized, multicenter, open-label, phase II trial to evaluate palbociclib in combination with fulvestrant or letrozole in endocrine-sensitive patients with estrogen receptor (ER)[+]/HER2[-] metastatic breast cancer. J Clin Oncol 2020;38:1007–1007. https://doi.org/10.1200/JCO.2020.38.15_suppl.1007.

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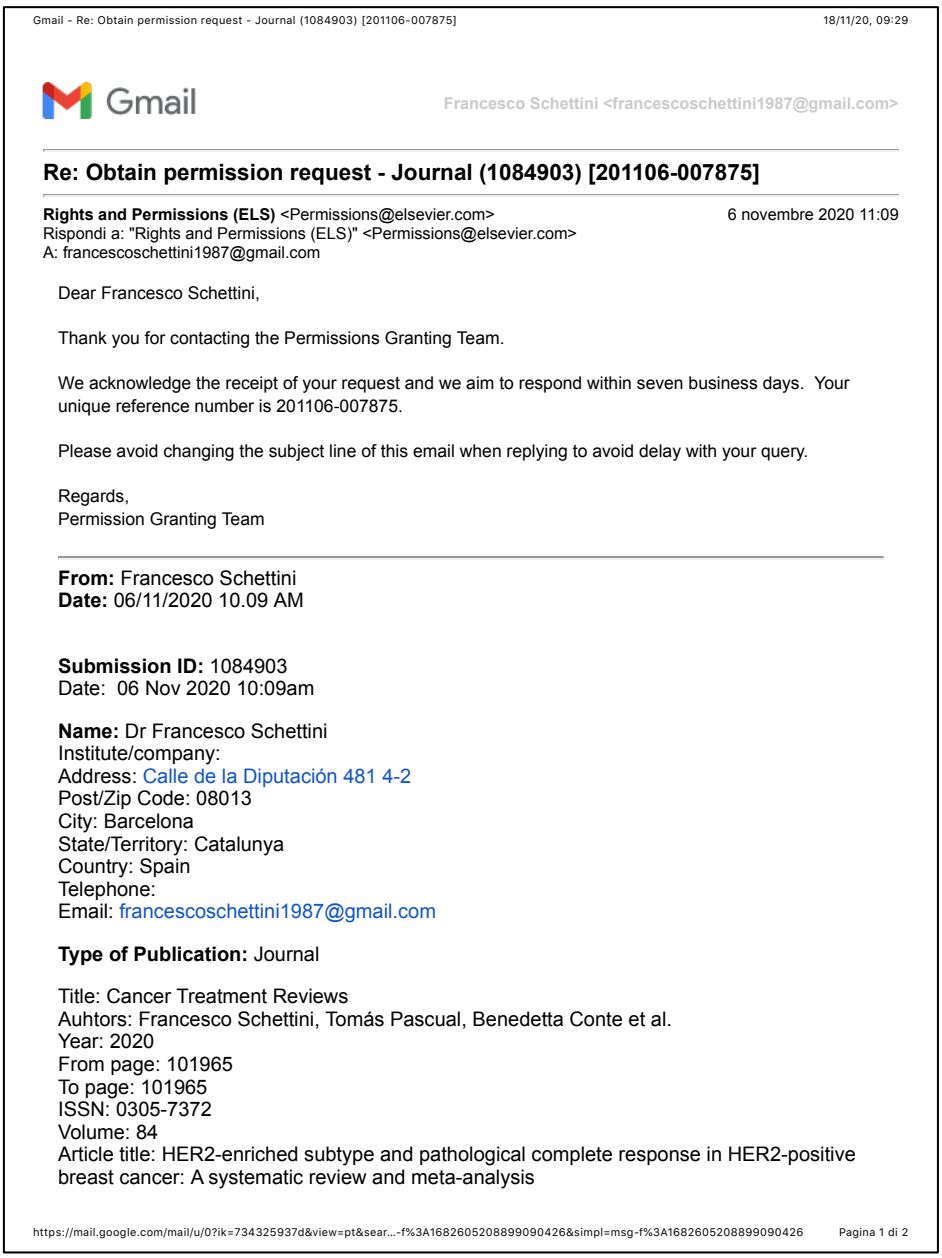

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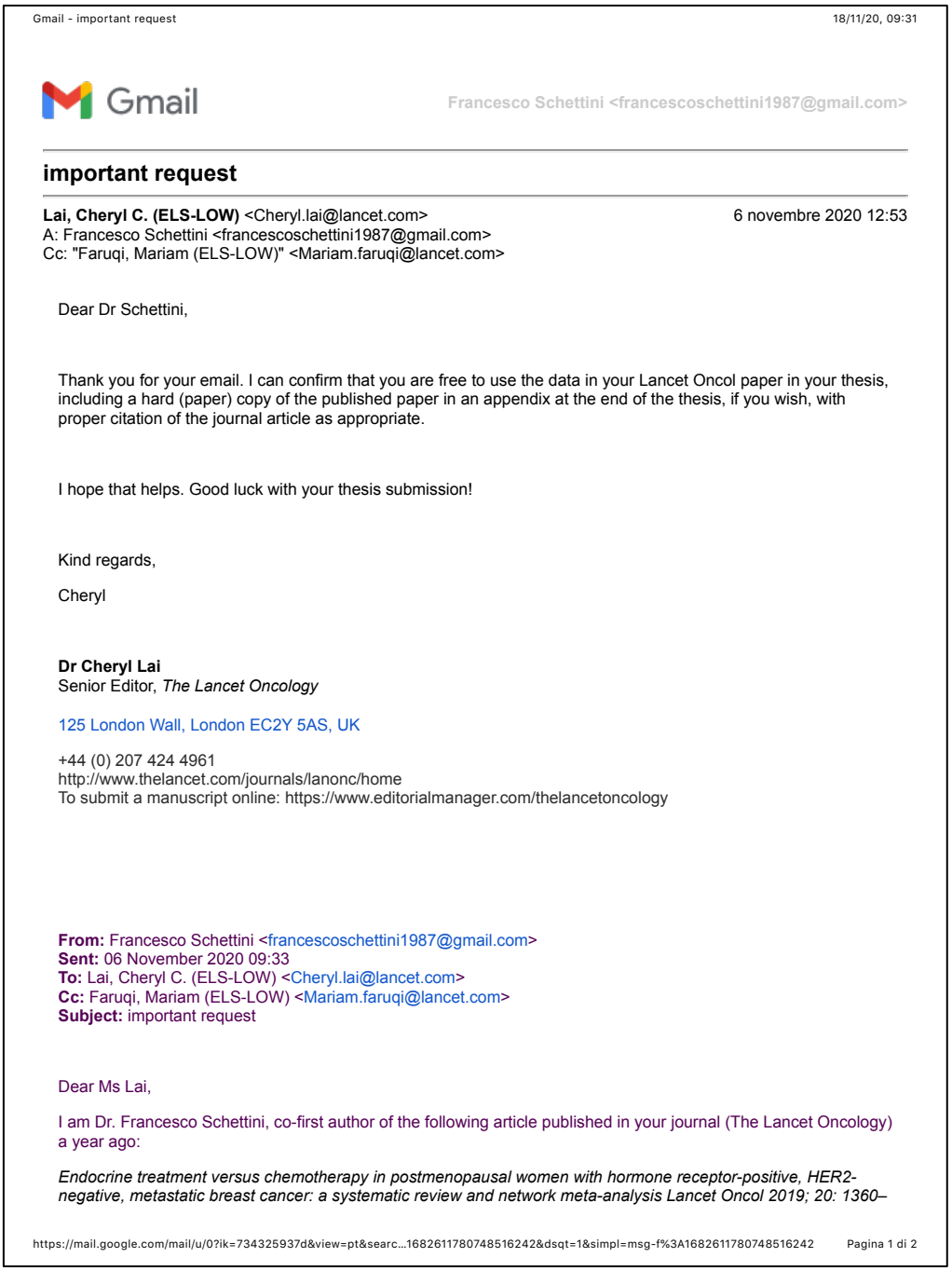

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Appendix 2 – Supplementary materials Chapter 1

Search terms for the systematic review in detail

We launched the following query in Pubmed and replicated it in Web of Science: neoadjuvant AND (breast OR mammary) AND (tumor OR cancer OR tumour) AND (HER2 OR Epidermal Growth Factor Receptor 2 OR HER-2 OR Erbb2) AND (anti-HER2 OR Trastuzumab OR Pertuzumab OR t-dm1 OR tdm1 OR Lapatinib)

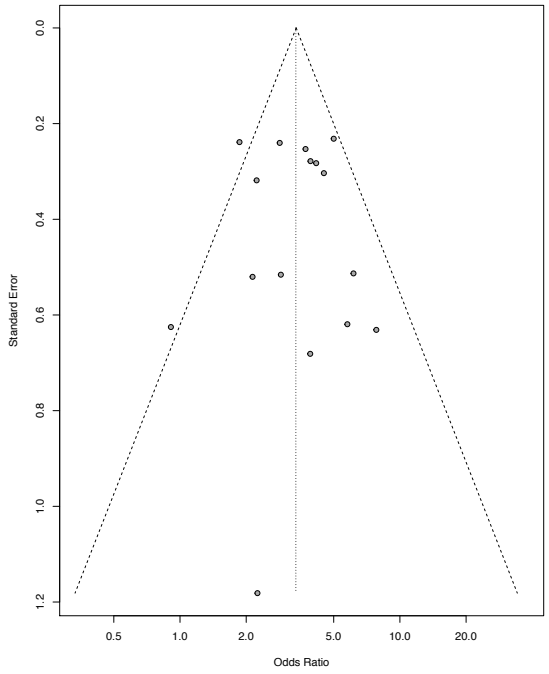
pCR and intrinsic subtypes other than HER2-E

When considering the other intrinsic subtypes separately, Basal tumors were favored over Luminal A subtype (OR=2.13, 95% CI: 1.20 – 3.79, p=0.01, I^2=36%) and Luminal A/B overall (OR=1.80, 95% CI: 1.13 – 2.86, p=0.01, I^2=25%) and unfavored when compared to all other subtypes (OR=0.70, 95% CI: 0.51 – 0.98, p=0.04, I^2=0%). No difference was observed when compared to Luminal B (OR=1.47, 95% CI: 0.96 – 2.23, p=0.07, I^2=1%).

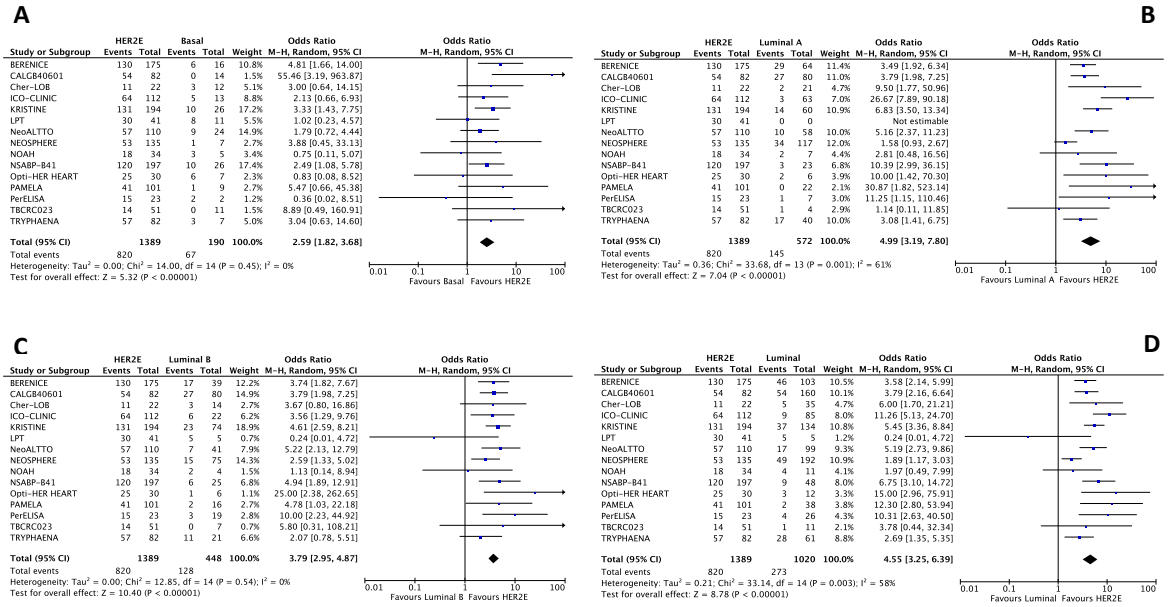
Overall Luminal tumors appeared to be less associated with pCR, compared to the other subtypes (OR=0.27, 95% CI: 0.20 – 0.36, p<0.001, I^2=48%). No significant difference was observed between Luminal A and B tumors (OR=0.88, 95% CI: 0.65 – 1.19, p=0.41, I^2=0%), Luminal A were significantly unfavored against all the others (OR=0.33, 95% CI: 0.22 – 0.48, p<0.001, I^2=55%), as well as were Luminal B (OR=0.43, 95% CI: 0.34 – 0.54, p<0.001, I^2=0%). All other comparisons have been already reported in the manuscript.

Supplementary Figures

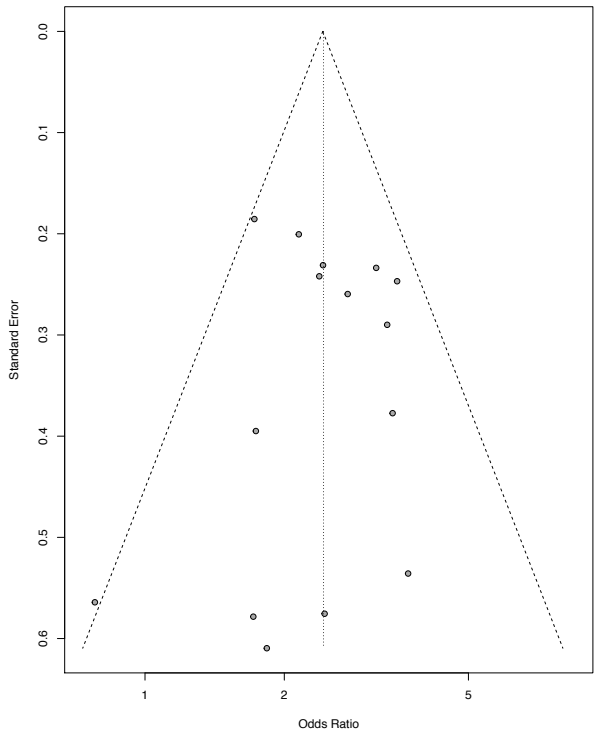
Supplementary figure 1. Funnel Plot for the primary endpoint



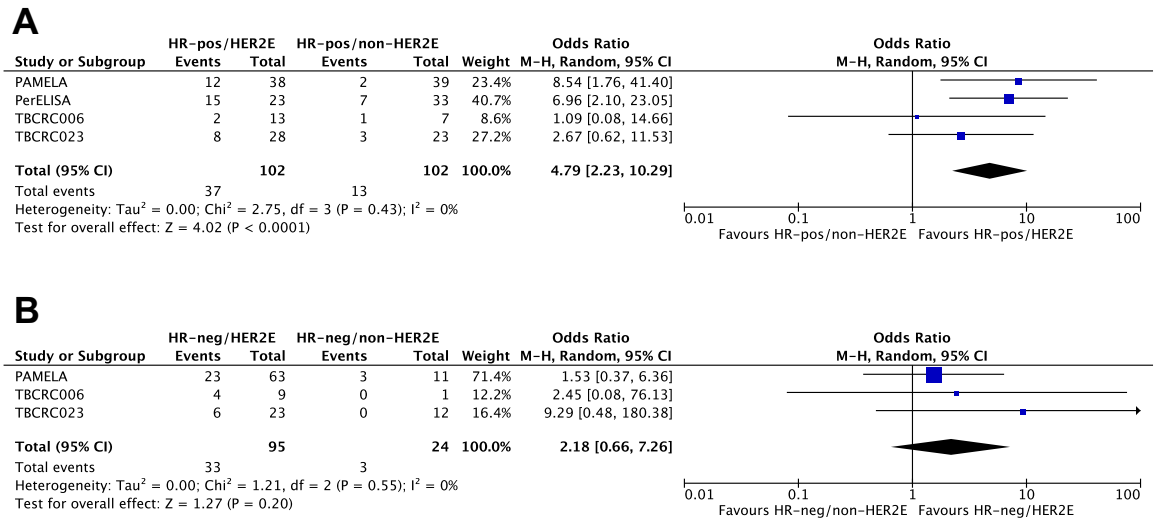
Supplementary figure 2 A-D. Forrest Plots comparing the association between HER2-E and Basal-like (A), Luminal A (B), Luminal B (C) and Luminal A/B (D) subtypes with pCR in the overall population



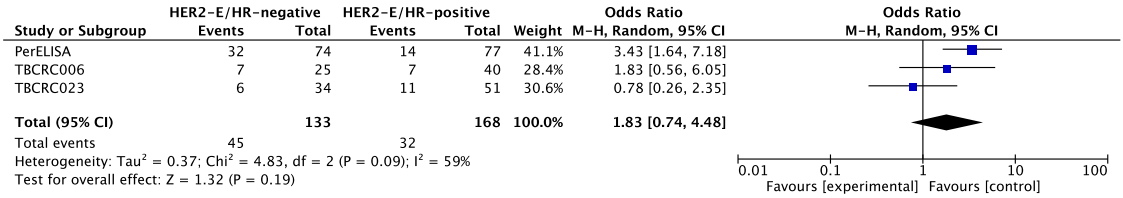
Supplementary figure 3. Funnel Plot for the analysis of the association between HR status and pCR in the overall population



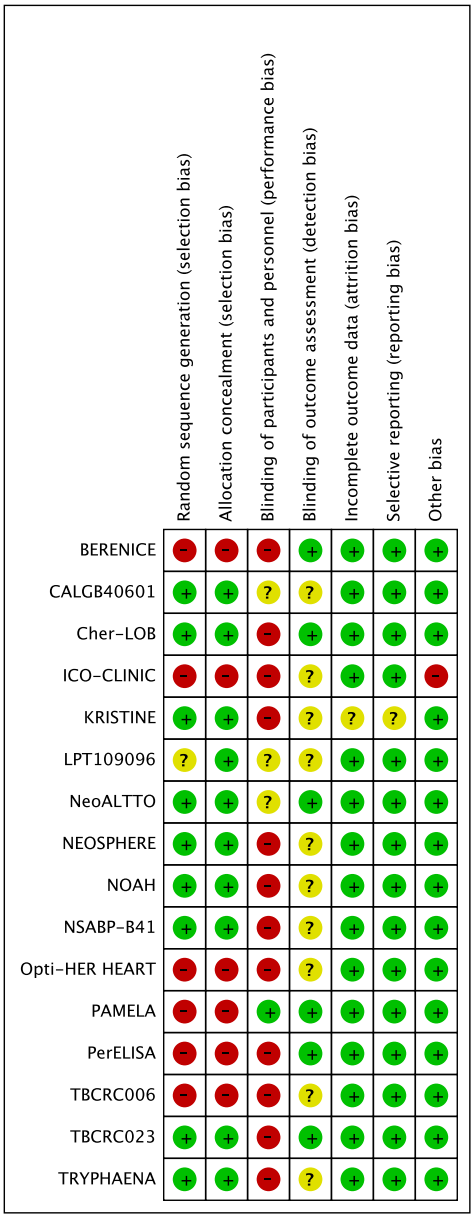
Supplementary figure 4 A-B. Forrest Plot comparing the association between pCR and HER2-E subtype vs. the others within HR-positive (A) and HR-negative (B) tumors



Supplementary figure 5. Forrest Plot comparing the association with pCR between HR positive vs. HR negative tumors in HER2-E specimens



Supplementary figure 6. Risk of bias analysis detailed per study



Appendix 3 – Supplementary materials Chapter 3

Supplementary tables

Supplementary table 1. Demographics according to HER2 IHC status

DEMOGRAPHICS	HER2-NEGATIVE								P*
	HER2 0		HER2 1+		HER2 2+ NON-AMPLIFIED		OVERALL POPULATION		
	N	%	N	%	N	%	N	%	
	1486	40.3	1489	40.4	714	19.3	3689	100	
Age at diagnosis (years)									
Median	55		58		60		58		**0.005**
IQR	46 - 65		48 - 67		49 - 68		48 - 67		
Min - max	24 - 93		26 - 96		26 - 90		24 - 96		
Pts with available data	259	27.4	427	45.2	258	27.3	944	100	
Sex									
Male	0	0	13	0.9	2	0.3	15	0.4	**0.001**
Female	1486	100	1475	99.1	712	99.7	3673	99.6	
Total	1486	40.3	1488	40.4	714	19.3	3688	100	
Menopaual status									
Pre/perimenopausal	385	37.3	454	37.4	206	36.4	1045	37.2	0.908
Postmenopausal	646	62.7	759	62.6	360	63.6	1765	62.8	
Total	1031	36.7	1213	43.2	566	20.1	2810	100	
Biospecimen									
Primary lesion	1000	73.7	959	79.1	423	74.7	2382	72.1	0.143
Other lesion	357	34.6	377	28.7	186	32.9	920	27.9	
Total	1357	41.1	1336	40.5	609	18.4	3302	100	
Histotype									
Ductal	639	70.8	799	73	415	76.9	1853	73	0.18
Lobular	194	21.5	221	20.2	93	17.2	508	20	
Other	69	7.6	75	6.8	32	5.9	176	6.9	
Total	902	35.6	1095	43.2	540	21.3	2537	100	
T									
1	509	55.8	573	50.7	234	44.5	1316	51.2	**0.005**
2	294	32.2	409	36.2	209	39.7	912	35.5	
3	71	7.8	93	8.2	49	9.3	213	8.3	
4	38	4.2	55	4.9	34	6.5	127	4.9	
Total	912	35.5	1130	44	526	20.5	2568	100	
N									
0	556	58.8	662	57.3	275	52	1493	56.8	**0.009**
1	272	28.8	300	26	164	31	736	28	
2	71	7.5	104	9	44	8.3	219	8.3	
3	46	4.9	89	7.7	46	8.7	181	6.9	

Total	945	35.9	1155	43.9	529	20.1	2629	100	
Metastatic status									
Metastatic Yes	529	65.6	601	61.3	280	64.1	1410	63.4	
Metastatic No	278	34.4	379	38.7	157	35.9	814	36.6	0.173
Total	807	36.3	980	44.1	437	19.6	2224	100	
Ab initio Yes	136	10	151	11.4	80	13.4	367	11.2	
Ab initio No	1218	90	1172	88.6	515	86.6	2905	88.8	0.087
Total	1354	41.4	1323	40.4	595	18.2	3272	100	
ER									
Positive	983	67	1261	85.9	633	89.7	2877	79	
Negative	484	33	207	14.1	73	10.3	764	21	**<0.001**
Total	1467	40.3	1468	40.4	706	19.3	3641	100	
PgR									
Positive	789	54.7	1030	70.9	512	73.7	2331	64.9	
Negative	654	45.3	423	29.1	183	26.3	1260	35.1	**<0.001**
Total	1443	40.2	1453	40.5	695	19.3	3591	100	
G									
1	67	8.8	107	11.6	32	8.1	206	9.9	
2	272	35.6	353	38.4	161	40.9	786	37.8	**0.041**
3	426	55.7	459	49.9	201	51	1086	52.3	
Total	765	36.8	919	44.2	394	19	2078	100	
Ki67									
Median	16		18		18		18		
IQR	9 - 30		10 - 26		10 - 27		10 - 27		0.811
Min - max	0.5 - 95		0.5 - 95		0.5 - 93		0.5 - 95		
Pts with available data	433	36.4	483	40.6	273	23	1189	100	
≤14%	190	43.9	193	40	101	37	484	40.7	0.176
>14%	243	56.1	290	60	172	63	705	59.3	
<20%	236	54.5	268	55.5	143	52.4	647	54.4	0.712
≥20%	197	45.5	215	44.5	130	47.6	542	45.6	
TILs									
Median	1		1		5		1		
IQR	0 - 5		1 - 5		1 - 5		1 - 5		**0.033**
Min - max	0 - 80		0 - 80		0 - 60		0 - 80		
Pts with available data	102	37.2	108	39.4	64	23.4	274	100	
PAM50 subtypes									
Luminal A	193	28.7	283	49	176	54.2	652	41.4	
Luminal B	127	18.9	162	28	98	30.2	387	24.6	
HER2-enriched	40	5.9	23	4	9	2.8	72	4.6	
Basal-like	294	43.7	88	15.2	32	9.8	414	26.3	**<0.001**
Normal-like	19	2.8	22	3.8	10	3.1	51	3.2	
Total	673	42.7	578	36.7	325	20.6	1576	100	
IHC subtypes simplified									

HR-positive	1025	69.6	1296	87.3	641	90.3	2962	80.8	
Triple Negative	448	30.4	189	12.7	69	9.7	706	19.2	**<0.001**
Total	1473	40.3	1485	40.4	710	19.3	3668	100	

Legend and footnotes. Pts: patients; HR: hormone receptors; IQR: interquartile range; IHC: immunohistochemical; TILs: tumor-infiltrating lymphocytes; *: Chi square test for differences in proportions, Kruskalis-Wallis and Wilcoxon rank sum test with continuity correction, where appropriate, for continuous variables (median comparisons)

Supplementary table 2. Demographics according to HR status

PATIENTS' AND TUMORS' CHARACTERISTICS	HER2-NEGATIVE						
	HR-POSITIVE		TRIPLE NEGATIVE		OVERALL		P*
	N	%	N	%	N	%	
	2962	80.3	706	19.1	3689	100.0	
Age at diagnosis							
Median	58.0		51.0		58		**0.001**
IQR	48.0 - 67.0		45.0 - 62.0		48 - 67		
Sex							
Male	14	0.5	1	0.1	15	0.4	
Female	2947	99.5	705	99.9	3652	99.6	0.215
Missing	2961	80.7	706	19.3	3667	100.0	
Menopaual status							
Pre/perimenopausal	895	35.9	148	47.7	1043	37.2	
Postmenopausal	1596	64.1	162	52.3	1758	62.8	**<0.001**
Missing	2491	88.9	310	11.1	2801	100.0	
TILs							
median	1		5		-		
IQR	1 - 5		1 - 40		-		0.296
Pts with available data	269		5		274		
Histotype							
Ductal	1563	70.8	279	87.7	1842	73.0	
Lobular	487	22.1	21	6.6	508	20.1	**<0.001**
Other	157	7.1	18	5.7	175	6.9	
Total	2207	87.4	318	12.6	2525	100.0	
T							
1	1194	53.0	120	39.0	1314	51.3	
2	776	34.5	133	43.2	909	35.5	
3	184	8.2	27	8.8	211	8.2	**<0.001**
4	98	4.4	28	9.1	126	4.9	
Total	2252	88.0	308	12.0	2560	100.0	
N							
0	1332	57.6	157	51.1	1489	56.8	**0.036**
1	646	27.9	87	28.3	733	28.0	

2	183	7.9	34	11.1	217	8.3	
3	152	6.6	29	9.4	181	6.9	
Total	2313	88.3	307	11.7	2620	100.0	
Metastatic ab initio							
Yes	330	12.8	36	5.3	366	11.2	
No	2248	87.2	649	94.7	2897	88.8	**<0.001**
Missing	2578	79.0	685	21.0	3263	100.0	
G							
1	200	11.0	6	2.4	206	10.0	
2	763	42.0	21	8.3	784	37.9	**<0.001**
3	852	46.9	225	89.3	1077	52.1	
Total	1815	87.8	252	12.2	2067	100.0	
Ki67							
≤14%	477	20.7	4	7.4	481	20.4	**0.01**
>14%	676	29.3	23	42.6	699	29.6	
<20%	637	27.6	6	11.1	643	27.2	
≥20%	516	22.4	21	38.9	537	22.8	**0.001**
Missing	2306	97.7	54	2.3	2360	100.0	
HER2 status							
IHC 0	1025	34.6	448	63.4	1473	40.2	
IHC 1+	1296	43.8	189	26.8	1485	40.5	**<0.001**
IHC 2+ Not Amplified	641	21.6	69	9.8	710	19.4	
Total	2962	80.8	706	19.2	3668	100.0	
PAM50 subtyes							
Luminal A	644	56.6	7	1.6	651	41.4	
Luminal B	385	33.9	1	0.2	386	24.5	
HER2-enriched	35	3.1	37	8.5	72	4.6	
Basal-like	44	3.9	370	84.7	414	26.3	**<0.001**
Normal-like	29	2.5	22	5.0	51	3.2	
Total	1137	72.2	437	27.8	1574	100.0	

Legend and footnotes. *: Chi square test for differences in proportions, Kruskalis-Wallis and Wilcoxon rank sum test with continuity correction, where appropriate, for continuous variables (median comparisons)

Supplementary Table 3. PAM50 intrinsic subtypes distribution within HR-positive and TN tumors according to HER2 status

HR-POSITIVE									
PAM50 subtypes	**HER2 0+**		**HER2 1+**		**HER2 2+ NOT AMPLIFIED**		**Overall**		**P***
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
Luminal A	187	51.8	283	57.9	174	60.6	644	56.6	**<0.001**
Luminal B	126	34.9	162	33.1	97	33.8	385	33.9	

HER2-enriched	12	3.3	16	3.3	7	2.4	35	3.1	
Basal-like	29	8.0	12	2.5	3	1.0	44	3.9	
Normal-like	7	1.9	16	3.3	6	2.1	29	2.6	
Total	361	31.8	489	43.0	287	25.2	1137	100.0	
TRIPLE NEGATIVE									
PAM50 subtypes	**HER2 0+**		**HER2 1+**		**HER2 2+ NOT AMPLIFIED**		**Overall**		**P***
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
Luminal A	5	1.6	0	0.0	2	5.4	7	1.6	
Luminal B	1	0.3	0	0.0	0	0.0	1	0.2	
HER2-enriched	28	9.0	7	7.9	2	5.4	37	8.5	0.284
Basal-like	265	85.2	76	85.4	29	78.4	370	84.7	
Normal-like	12	3.9	6	6.7	4	10.8	22	5.0	
Total	311	71.2	89	20.4	37	8.5	437	100.0	

Legend and footnotes. *: Chi square test

Supplementary Table 4. Comparisons of PAM50 intrinsic subtypes distributions within each HER2 IHC score according to HR status

PAM50 subtypes in HER2 0	**HR-POSITIVE**		**TNBC**		**OVERALL**		**P***
	HER2 0+		**HER2 0+**		**HER2 0+**		
	N	**%**	**N**	**%**	**N**	**%**	
Luminal A	187	51.8	5	1.6	192	28.7	
Luminal B	126	34.9	1	0.3	127	18.9	
HER2-enriched	12	3.3	28	9.0	40	5.9	
Basal-like	29	8.0	265	85.2	294	43.7	**<0.001**
Normal-like	7	1.9	12	3.9	19	2.8	
Total	361	53.7	311	46.3	672	100.0	
PAM50 subtypes in HER2 1+	**HER2 1+**		**HER2 1+**		**HER2 1+**		
	N	**%**	**N**	**%**	**N**	**%**	
Luminal A	283	57.9	0	0.0	283	49.0	
Luminal B	162	33.1	0	0.0	162	28.0	
HER2-enriched	16	3.3	7	7.9	23	4.0	
Basal-like	12	2.5	76	85.4	88	15.2	**<0.001**
Normal-like	16	3.3	6	6.7	22	3.8	
Total	489	84.6	89	15.4	578	100.0	
PAM50 subtypes in HER2 2+ Not Amplified	**HER2 2+ Not Amplified**		**HER2 2+ Not Amplified**		**HER2 2+ Not Amplified**		
	N	**%**	**N**	**%**	**N**	**%**	
Luminal A	174	60.6	2	5.4	176	54.2	
Luminal B	97	33.8	0	0.0	97	30.2	
HER2-enriched	7	2.4	2	5.4	9	2.8	
Basal-like	3	1.0	29	78.4	32	9.8	**<0.001**
Normal-like	6	2.1	4	10.8	10	3.0	
Total	287	88.6	37	11.4	324	100.0	

Legend and footnotes. HR: hormone receptors; **TNBC:** triple negative breast cancer; *: Chi square test

Supplementary table 5. List of genes and subtypes signatures evaluated for differential expression analysis in the overall HER2-negative population and according to HR status

GENE/SIGNATURE	OVERALL	HR-POSITIVE	TNBC
Basal-like	Evaluable	Evaluable	Evaluable
HER2-enriched	Evaluable	Evaluable	Evaluable
Luminal A	Evaluable	Evaluable	Evaluable
Luminal B	Evaluable	Evaluable	Evaluable
Normal-like	Evaluable	Evaluable	Evaluable
ACTR3B	Evaluable	Evaluable	Evaluable
ANLN	Evaluable	Evaluable	Evaluable
AR	Evaluable	Not evaluable	Evaluable
BAG1	Evaluable	Evaluable	Evaluable
BCL2	Evaluable	Evaluable	Evaluable
BIRC5	Evaluable	Evaluable	Evaluable
BLVRA	Evaluable	Evaluable	Evaluable
CCNB1	Evaluable	Evaluable	Evaluable
CCNE1	Evaluable	Evaluable	Evaluable
CD274 (PD-L1)	Evaluable	Not evaluable	Evaluable
CD4	Evaluable	Not evaluable	Evaluable
CD8A	Evaluable	Not evaluable	Evaluable
CDC20	Evaluable	Evaluable	Evaluable
CDC6	Evaluable	Evaluable	Evaluable
CDCA1	Evaluable	Evaluable	Evaluable
CDH3	Evaluable	Evaluable	Evaluable
CENPF	Evaluable	Evaluable	Evaluable
CEP55	Evaluable	Evaluable	Evaluable
CXXC5	Evaluable	Evaluable	Evaluable
EGFR	Evaluable	Evaluable	Evaluable
ERBB2	Evaluable	Evaluable	Evaluable

ESR1	Evaluable	Evaluable	Evaluable
EXO1	Evaluable	Evaluable	Evaluable
FGFR4	Evaluable	Evaluable	Evaluable
FOXA1	Evaluable	Evaluable	Evaluable
FOXC1	Evaluable	Evaluable	Evaluable
GPR160	Evaluable	Evaluable	Evaluable
GRB7	Evaluable	Evaluable	Evaluable
KIF2C	Evaluable	Evaluable	Evaluable
KNTC2	Evaluable	Evaluable	Evaluable
KRT14	Evaluable	Evaluable	Evaluable
KRT17	Evaluable	Evaluable	Evaluable
KRT5	Evaluable	Evaluable	Evaluable
MAPT	Evaluable	Evaluable	Evaluable
MDM2	Evaluable	Evaluable	Evaluable
MELK	Evaluable	Evaluable	Evaluable
MIA	Evaluable	Evaluable	Evaluable
MKI67	Evaluable	Evaluable	Evaluable
MLPH	Evaluable	Evaluable	Evaluable
MMP11	Evaluable	Evaluable	Evaluable
MYBL2	Evaluable	Evaluable	Evaluable
MYC	Evaluable	Evaluable	Evaluable
NAT1	Evaluable	Evaluable	Evaluable
ORC6L	Evaluable	Evaluable	Evaluable
PDCD1 (PD1)	Evaluable	Not evaluable	Evaluable
PGR	Evaluable	Evaluable	Evaluable
PHGDH	Evaluable	Evaluable	Evaluable
PTTG1	Evaluable	Evaluable	Evaluable
RRM2	Evaluable	Evaluable	Evaluable
SFRP1	Evaluable	Evaluable	Evaluable
SLC39A6	Evaluable	Evaluable	Evaluable
TMEM45B	Evaluable	Evaluable	Evaluable
TYMS	Evaluable	Evaluable	Evaluable
UBE2C	Evaluable	Evaluable	Evaluable
UBE2T	Evaluable	Evaluable	Evaluable

Legend and footnotes. HR: hormone receptors; **TNBC:** triple negative breast cancer

Supplementary table 6. All differentially expressed genes of HER2-low vs. HER2 0 tumors in the overall, HR-positive and Triple Negative populations

GENE ID/SIGNATURE	OVERALL		HR-POSITIVE		TNBC	
	Score(d)	FDR*	Score(d)	FDR*	Score(d)	FDR*
Basal-like	-5.67277	**0.0**	-0.00299	24.7	-0.82130	64.3
HER2-enriched	-0.39253	**0.0**	1.18400	**3.2**	-1.30300	64.3
Luminal A	4.73173	**0.0**	3.38402	**0.0**	0.14695	100.0
Luminal B	2.06840	**0.0**	2.34168	**0.0**	-1.22811	64.3
Normal-like	-0.33430	**0.0**	1.14341	**3.2**	-0.07865	68.3
ACTR3B	0.72473	**1.8**	0.74613	8.6	-0.49266	64.3
ANLN	-7.39622	**0.0**	-2.27444	**0.0**	-0.35919	64.3
AR	10.64278	**0.0**	-	-	0.39058	100.0
BAG1	-1.38371	**0.0**	0.90829	6.0	-0.69894	64.3
BCL2	8.20430	**0.0**	2.51291	**0.0**	-0.01781	68.3
BIRC5	-5.55863	**0.0**	-1.40851	**0.9**	-0.27225	68.3
BLVRA	5.14298	**0.0**	1.73118	**0.0**	0.20039	100.0
CCNB1	-3.76904	**0.0**	-1.84185	**0.0**	0.02360	100.0
CCNE1	-9.55346	**0.0**	-3.03781	**0.0**	-0.86694	64.3
CD274 (PD-L1)	2.00646	**0.0**	-	-	-0.42082	64.3
CD4	2.21548	**0.0**	-	-	0.28399	100.0
CD8A	-4.51166	**0.0**	-	-	-0.85870	64.3
CDC20	-4.47577	**0.0**	-0.87565	7.4	-1.06613	64.3
CDC6	-3.37515	**0.0**	-0.28853	21.8	-0.24001	68.3
CDCA1	-4.18599	**0.0**	-1.11544	6.0	-0.81522	64.3
CDH3	-5.87928	**0.0**	-1.06410	6.0	-0.66177	64.3
CENPF	-6.99532	**0.0**	-1.83252	**0.0**	-0.95846	64.3
CEP55	-7.83024	**0.0**	-1.28199	**3.2**	-1.14879	64.3
CXXC5	-3.61475	**0.0**	0.24982	21.8	-0.69201	64.3
EGFR	-0.82066	**0.0**	0.28270	21.8	0.60389	100.0
ERBB2	9.97921	**0.0**	5.17067	**0.0**	1.66991	100.0
ESR1	14.29386	**0.0**	4.96165	**0.0**	1.03532	100.0
EXO1	-7.12534	**0.0**	-2.43602	**0.0**	-1.17480	64.3
FGFR4	-1.39985	**0.0**	-0.07579	24.7	-0.12578	68.3
FOXA1	13.26991	**0.0**	4.92557	**0.0**	0.97629	100.0
FOXC1	-8.43702	**0.0**	-0.73815	9.7	0.20243	100.0
GPR160	7.74052	**0.0**	2.78485	**0.0**	-0.16216	68.3
GRB7	6.09456	**0.0**	2.76279	**0.0**	0.92797	100.0
KIF2C	-3.12355	**0.0**	-1.39427	**0.9**	-0.01905	68.3
KNTC2	-7.77839	**0.0**	-2.31629	**0.0**	-0.72532	64.3
KRT14	-1.86137	**0.0**	0.61877	11.4	0.42407	100.0
KRT17	-5.64524	**0.0**	0.04017	27.5	0.19936	100.0
KRT5	-2.51720	**0.0**	0.84223	7.4	0.79709	100.0
MAPT	9.94512	**0.0**	2.93645	**0.0**	-0.21882	68.3

MDM2	0.06691	5.3	1.12099	**3.2**	-0.21097	68.3
MELK	-6.64057	**0.0**	-1.49359	**0.9**	-0.59390	64.3
MIA	-5.35993	**0.0**	-0.95634	7.4	0.51230	100.0
MKI67	-8.67347	**0.0**	-2.35485	**0.0**	-0.96228	64.3
MLPH	8.78099	**0.0**	2.63999	**0.0**	-0.00518	68.3
MMP11	6.58603	**0.0**	2.60115	**0.0**	0.77140	100.0
MYBL2	-3.97998	**0.0**	-1.44618	**0.9**	-0.90301	64.3
MYC	-5.90597	**0.0**	-0.88871	7.4	-0.01975	68.3
NAT1	12.27258	**0.0**	4.10788	**0.0**	-0.28319	68.3
ORC6L	-7.61981	**0.0**	-2.32482	**0.0**	-0.75740	64.3
PDCD1 (PD1)	0.26237	5.3	-	-	-0.32005	68.3
PGR	11.22998	**0.0**	3.17887	**0.0**	0.52015	100.0
PHGDH	-8.41385	**0.0**	-1.68128	**0.0**	-1.23951	64.3
PTTG1	-4.82226	**0.0**	-1.44424	**0.9**	-0.60645	64.3
RRM2	-5.93472	**0.0**	-1.25960	**3.2**	-0.41814	64.3
SFRP1	-4.83858	**0.0**	-0.33182	16.4	1.23175	100.0
SLC39A6	11.60247	**0.0**	3.96208	**0.0**	-0.38795	64.3
TMEM45B	7.09375	**0.0**	2.02627	**0.0**	0.52291	100.0
TYMS	-3.20623	**0.0**	-0.56947	13.6	-0.49666	64.3
UBE2C	2.37535	**0.0**	-0.37100	16.4	-0.69730	64.3
UBE2T	4.54516	**0.0**	1.69281	**0.0**	-0.55916	64.3

Legend and footnotes. HR: hormone receptors; TNBC: triple negative breast cancer; FDR: false discovery rate; *: significant if FDR<5.0; Score(d): a T-statistic value that reflects a standardized change in expression and measures the strength of the relationship between gene expression and the HER2-low category (versus HER2 0)

Supplementary table 7. Differentially expressed genes among HR-positive/HER2 0, HR-positive/HER2-low, TNBC/HER2 0 and TNBC/HER2-Low

GENE ID	HR+/HER2 0 *Contrast*	HR+/HER2-low *Contrast*	TNBC/HER2 0 *Contrast*	TNBC/HER2-low *Contrast*	FDR*
ESR1	6.861742068	11.1074889	-12.90998321	-12.08492615	**0**
FOXA1	5.778217896	8.825351095	-10.4809953	-9.413459819	**0**
NAT1	4.318943613	8.027254553	-8.897426094	-9.080491687	**0**
SLC39A6	3.964146625	7.87369166	-8.600693487	-8.83063485	**0**
PGR	3.688389562	6.507937351	-7.385485991	-7.0821826	**0**
AR	3.652669581	6.241609361	-7.154953824	-6.753890554	**0**
MAPT	3.542191338	5.97143271	-6.749174571	-6.935422423	**0**
FOXC1	-4.981864542	-5.538845927	6.9775032	7.185258765	**0**
CCNE1	-3.37762021	-6.018579053	6.943340875	6.038719686	**0**
MLPH	2.581366165	4.571432528	-5.139368572	-5.143849516	**0**
PHGDH	-3.455515264	-4.906927001	6.05145693	4.868929478	**0**
MKI67	-2.985374693	-5.147177429	5.996903821	5.137457962	**0**
BCL2	3.004496715	5.226004989	-5.895454984	-5.913599185	**0**
CEP55	-3.612167568	-4.800907334	6.001214003	4.933726234	**0**
GPR160	2.295959541	4.559067178	-4.97306946	-5.141532706	**0**
ERBB2	0.853469611	5.811851031	-5.865658229	-4.065682234	**0**

KRT17	-3.053703742	-3.024321445	3.939545973	4.115344828	**0**
KNTC2	-2.521227666	-4.615649371	5.312815869	4.533560183	**0**
ANLN	-2.394303094	-4.430910963	5.010002631	4.649030645	**0**
ORC6L	-2.315621728	-4.553831424	5.153881435	4.455539376	**0**
TMEM45B	2.195984431	3.7004643	-4.316099686	-3.778937655	**0**
CENPF	-2.356851503	-3.977967627	4.718493603	3.747639361	**0**
SFRP1	-2.673552292	-2.925551032	3.491589308	4.638618441	**0**
MELK	-2.567267617	-3.980373357	4.725372162	4.171204745	**0**
MYC	-2.623452681	-3.435772806	4.15842533	4.142345807	**0**
MIA	-2.348514667	-2.976385496	3.519190449	4.071299962	**0**
EXO1	-1.689980966	-3.92999769	4.430571237	3.267594746	**0**
CDH3	-2.183753512	-3.074722651	3.770177997	3.174202065	**0**
RRM2	-2.207292008	-3.398663576	4.016121429	3.666137082	**0**
MMP11	1.018291318	3.028611945	-3.254201613	-2.548210338	**0**
BIRC5	-1.847675311	-3.098541079	3.577831485	3.323902063	**0**
GRB7	0.736362322	3.304204501	-3.433618431	-2.451544203	**0**
CXXC5	-1.912486573	-1.700216734	2.397390797	2.01123729	**0**
BLVRA	1.572120525	3.282508268	-3.616414509	-3.393016952	**0**
KRT5	-2.145753017	-1.520262446	2.119765859	2.802387954	**0**
UBE2T	1.104568388	2.625459449	-2.684460847	-3.204873418	**0**
PTTG1	-1.357405686	-2.746825946	3.129379599	2.524954233	**0**
CDC20	-1.561003057	-2.341316049	2.956453401	1.870697613	**0**
CD8A	-1.06424082	-2.222143957	2.587451424	1.75430237	**0**
CDCA1	-1.221013694	-2.220418771	2.658470223	1.797942709	**0**
CDC6	-1.826050077	-2.119404114	2.698602991	2.469801753	**0**
UBE2C	1.935072987	1.613637076	-2.113706106	-2.78929955	**0**
KRT14	-1.484755864	-1.07590958	1.498947303	1.917394719	**0**
MYBL2	-0.566292389	-1.883199658	2.063921511	1.287040354	**0**
TYMS	-1.245558217	-1.757831987	2.189600191	1.675491781	**0**
CCNB1	-0.414304024	-2.224612346	2.136840048	2.161647332	**0**
KIF2C	-0.435803157	-1.716742224	1.706395887	1.687281857	**0**
BAG1	-1.591018957	-0.745488035	1.416825439	1.030570598	**0**
CD4	1.278682017	1.372164069	-1.860963503	-1.357264788	**0**
CD274 (PD-L1)	0.750358454	1.166321248	-1.32500004	-1.450554218	**0**
EGFR	-0.885894045	-0.653610577	0.831535115	1.439230248	**0**

FGFR4	-0.62915164	-0.693176137	0.901706886	0.800116908	**0**
MDM2	-1.186448168	-0.079815829	0.607015399	0.460937749	**0.1**
ACTR3B	-0.247048402	0.516653373	-0.24331634	-0.769816605	**1.8**
PDCD1 (PD1)	0.229962949	0.162491103	-0.235653338	-0.265422996	**3.0**

Legend and footnotes. FDR: false discovery rate; *: significant if FDR<5.0; HR: hormone receptor; TNBC: triple negative breast cancer; *Contrast*: is the standardized mean difference between the gene's expression in a class vs. its overall mean expression in the overall dataset.

Supplementary Table 8. Gene expression of HER2-2+ compared to HER2 1+ and 0 tumors in the HR-positive subset

GENE/SIGNATURE	HR-POSITIVE/HER2-NEGATIVE			
	HER2 0 *Contrast*	HER2 1+ *Contrast*	HER2 2+ NA *Contrast*	FDR*
Basal-like	0.00283	-0.88603	1.45201	**3.6**
HER2-enriched	-1.16229	-0.44507	2.21633	**0.0**
Luminal A	-3.21810	0.46473	3.34852	**0.0**
Luminal B	-2.25388	-0.16542	3.15172	**0.0**
Normal-like	-1.05603	-0.39148	1.99252	**0.9**
ACTR3B	-0.58029	0.17146	0.45979	14.1
ANLN	1.57948	-0.47296	-1.24122	**0.0**
BAG1	-0.53348	-0.54786	1.58172	**0.0**
BCL2	-1.75095	0.37398	1.62293	**0.0**
BIRC5	0.93034	-0.64932	-0.12203	**3.6**
BLVRA	-1.36454	0.36271	1.14771	**0.9**
CCNB1	1.38774	-0.34588	-1.20499	**0.0**
CCNE1	2.22623	-0.36631	-2.24283	**0.0**
CDC20	0.61235	-0.68690	0.34603	7.2
CDC6	0.20957	-0.39872	0.38725	17.9
CDCA1	0.79716	-0.32239	-0.48896	7.6
CDH3	0.66493	-0.06819	-0.73761	7.2
CENPF	1.26956	-0.66734	-0.52587	**0.9**
CEP55	0.87016	-0.94497	0.44058	**2.3**
CXXC5	-0.13594	-0.42676	0.87480	**3.6**
EGFR	-0.18705	0.13757	0.01300	23.1
ERBB2	-3.94793	0.94579	3.49078	**0.0**
ESR1	-2.98901	0.75918	2.57206	**0.0**
EXO1	1.70203	-0.46725	-1.40718	**0.0**
FGFR4	0.04529	-0.26643	0.37983	17.9
FOXA1	-3.05763	0.93147	2.37671	**0.0**
FOXC1	0.47400	-0.61673	0.40753	7.2
GPR160	-1.88797	0.16030	2.14905	**0.0**
GRB7	-2.03291	0.49749	1.78031	**0.0**

KIF2C	0.98070	-0.63800	-0.20498	**3.6**
KNTC2	1.61274	-0.99690	-0.42297	**0.0**
KRT14	-0.35626	0.27656	0.00087	17.9
KRT17	-0.02275	0.23574	-0.35823	17.9
KRT5	-0.47377	0.25375	0.18850	14.1
MAPT	-1.77205	0.44702	1.52990	**0.0**
MDM2	-0.71511	0.04468	0.84036	**3.6**
MELK	1.02701	-0.83862	0.06545	**2.3**
MIA	0.59910	-0.58990	0.20360	7.2
MKI67	1.55384	-0.95257	-0.42053	**0.0**
MLPH	-1.51625	-0.02501	1.97851	**0.0**
MMP11	-1.55869	0.86881	0.56434	**0.0**
MYBL2	0.93321	-0.29141	-0.71369	**3.6**
MYC	0.56299	-0.63562	0.32485	7.2
NAT1	-2.40914	0.90353	1.59398	**0.0**
ORC6L	1.60517	-0.95927	-0.47510	**0.0**
PGR	-1.79970	0.93024	0.77136	**0.0**
PHGDH	1.10544	-0.67271	-0.30734	**2.3**
PTTG1	1.04078	-0.94181	0.21738	**2.3**
RRM2	0.82596	-0.84250	0.32871	**2.3**
SFRP1	0.19926	0.37772	-0.87516	7.2
SLC39A6	-2.47832	0.13383	2.94689	**0.0**
TMEM45B	-1.28927	0.80674	0.32204	**0.0**
TYMS	0.40551	-0.74150	0.70003	7.2
UBE2C	0.24692	-0.39737	0.33732	14.1
UBE2T	-1.13151	-0.22770	1.81989	**0.0**

Legend and footnotes. Neg: negative; **ISH:** in-situ hybridization; **HR:** hormone receptors; **NA:** not amplified; **FDR:** false discovery rate; *: significant if FDR<5.0; *Contrast*: is the standardized mean difference between the gene's expression in a class vs. its overall mean expression in the overall dataset.

Supplementary table 9. Included studies' description

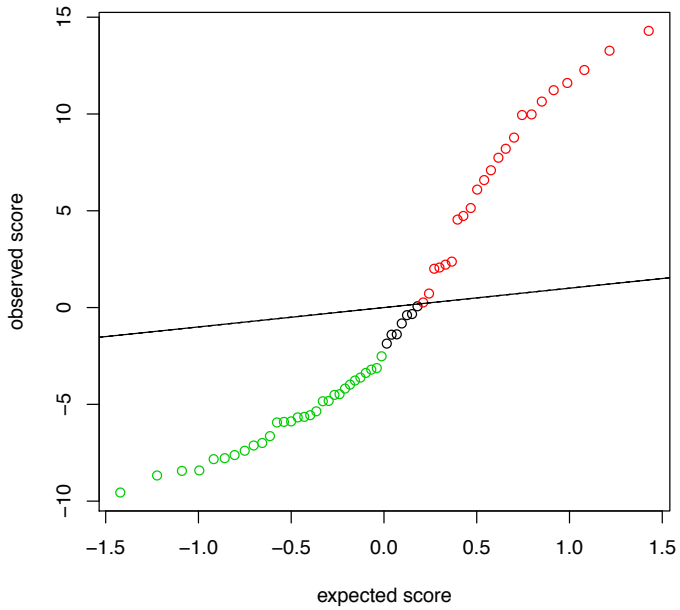
Study	Study Centers	Setting	Study type	N. of pts	Tumor types included	PAM50 type	Study reference
Cooperational spanish-italian study (including patients from the GEICAM/2012-09 study)	15 hospitals across Spain (centralized Prosigna assay and centralized pathology re-evaluation at the Gregorio Marañón Biomedical Research Institute, Madrid)	Early (Adjuvant)	Observational prospective	194	HR+/HER2-	Commercialized and standardized PAM50/Prosigna assay (NanoString Technologies, Seattle, WA) on FFPE tissues	Martin M, et al. Curr Med Res Opin. 2015; 31:1129-1137 and Fernandez-Martinez, A. et al. Oncotarget 8, 21930–21937 (2017)
	Campus Bio-Medico University (Rome)	Early (Adjuvant)	Observational prospective	159	HR+/HER2-		Fernandez-Martinez, A. et al. Oncotarget 8, 21930–21937 (2017) + unpublished
	Vall d'Hebron Institute of Oncology (Barcelona)	Early (Adjuvant)	Observational prospective	117	HR+/HER2-		Fernandez-Martinez, A. et al. Oncotarget 8, 21930–21937 (2017).
c-Bioportal	A patient-driven initiative on the Metastatic Breast Cancer Project platform	Early & Metastatic	Observational prospective	103	HR+/HER2- and TNBC	N/A	The Metastatic Breast Cancer Project. https://www.mbcproject.org/
	Memorial Sloan Kettering Cancer Center	Early	Observational retospective	1637	HR+/HER2- and TNBC	N/A	Razavi, P. et al. Cancer Cell 34, 427-438.e6 (2018).
	TCGA	Early	Observational retospective	111	HR+/HER2- and TNBC	Intrinsic subtypes were defined by applying the PAM50 predictor fom Parker, J. S. et al. J. Clin. Oncol. 27, 1160–1167 (2009)	Ciriello, G. et al. Cell 163, 506–519 (2015).

	TCGA	Early	Observational retospective	207	HR+/HER2- and TNBC	N/A	Cancer Genome Atlas Network. Nature 490, 61–70 (2012).
SOLTI 1501-VENTANA	9 hospitals in Spain (including the Hospital Clinic of Barcelona and the Vall d'Hebron Institute of Oncology)	Early (Neoadjuvant)	Randomized window-of-opportunity	46	HR+/HER2-	Breast 360TM Codeset for PAM50 assay (NanoString Technologies, Seattle, WA) on FFPE tissues. Intrinsic molecular subtypes were identified using the research-based PAM50 predictor described in Prat A, et al. JAMA Oncol 2016; 2: 1287–94 and Vidal M, et al. Mol Oncol 2015; 9: 1081–90.	Adamo, B. et al. Breast Cancer Res. 21, 108 (2019).
SOLTI 1402-CORALEEN	21 hospitals in Spain (only the Hospital Clinic of Barcelona cohort was used)	Early (Neoadjuvant)	Randomized phase II	14	HR+/HER2-	Commercialized and standardized PAM50/Prosigna assay (NanoString Technologies, Seattle, WA) on FFPE tissues	Prat, A. et al. Lancet Oncol. 21, 33–43 (2020).
CIBOMA/2004-01_GEICAM/2003-11 study	80 institutions from Spain, Brazil, Chile, Colombia, Ecuador, Mexico, Peru, and Venezuela (pathology centralized)	Early (Adjuvant)	Randomized phase III	375	TNBC		Lluch, A. et al. J. Clin. Oncol. 38, 203–213 (2020).
Hospital Clinic of Barcelona internal databases	Hospital Clinic of Barcelona	Early & Metastatic	Observational prospective and retrospective	726	HR+/HER2- and TNBC	Commercialized and standardized PAM50/Prosigna assay (NanoString Technologies, Seattle, WA) on FFPE tissues and research-based PAM50 predictor described in Prat A, et al. JAMA Oncol 2016; 2: 1287–94 and Vidal M, et al. Mol Oncol 2015; 9: 1081–90.	N/A

Legend and footnotes. N/A: not applicable; **HR+:** hormone receptor positive: **-:** negative; **TNBC:** triple negative breast cancer; **FFPE:** formalin-fixed paraffin-embedded; pts: patients.

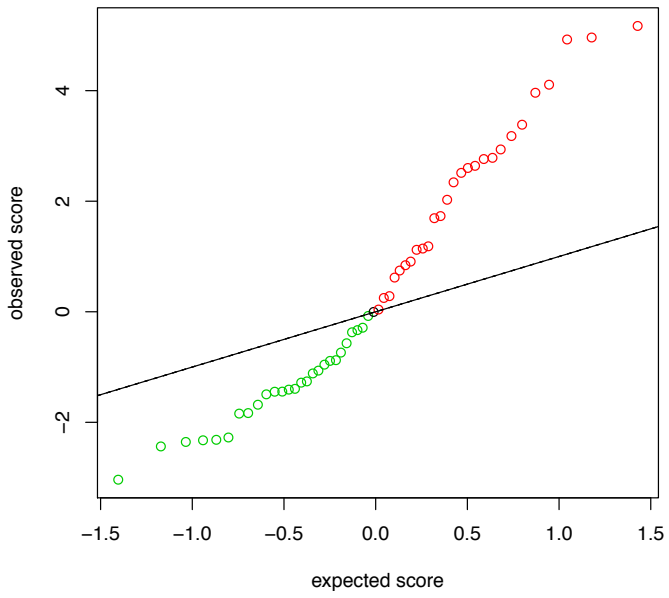
Supplementary figures

Supplementary figure 1. Plot for 2 class unpaired SAM analysis in the overall population



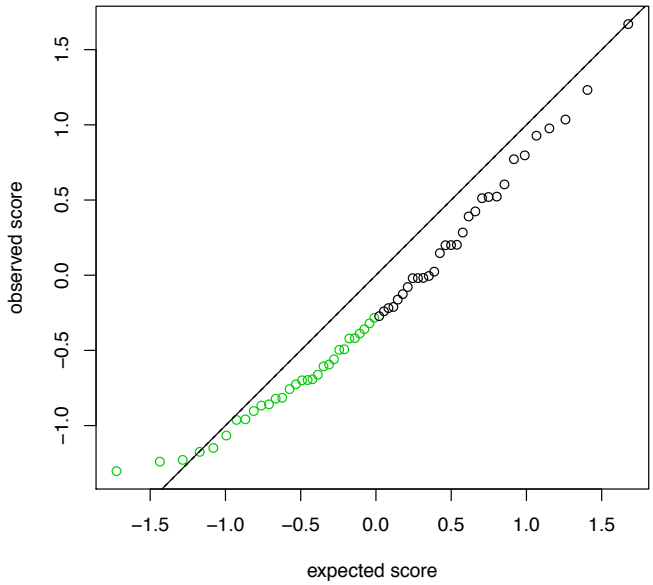
Legend: The red dots represent relative high gene expression, the green dots represent relative low gene expression, and black dots represent median gene expression for HER2-low vs. HER2 0 in the overall population.

Supplementary figure 2. Plot for 2 class unpaired SAM analysis in the HR-positive population



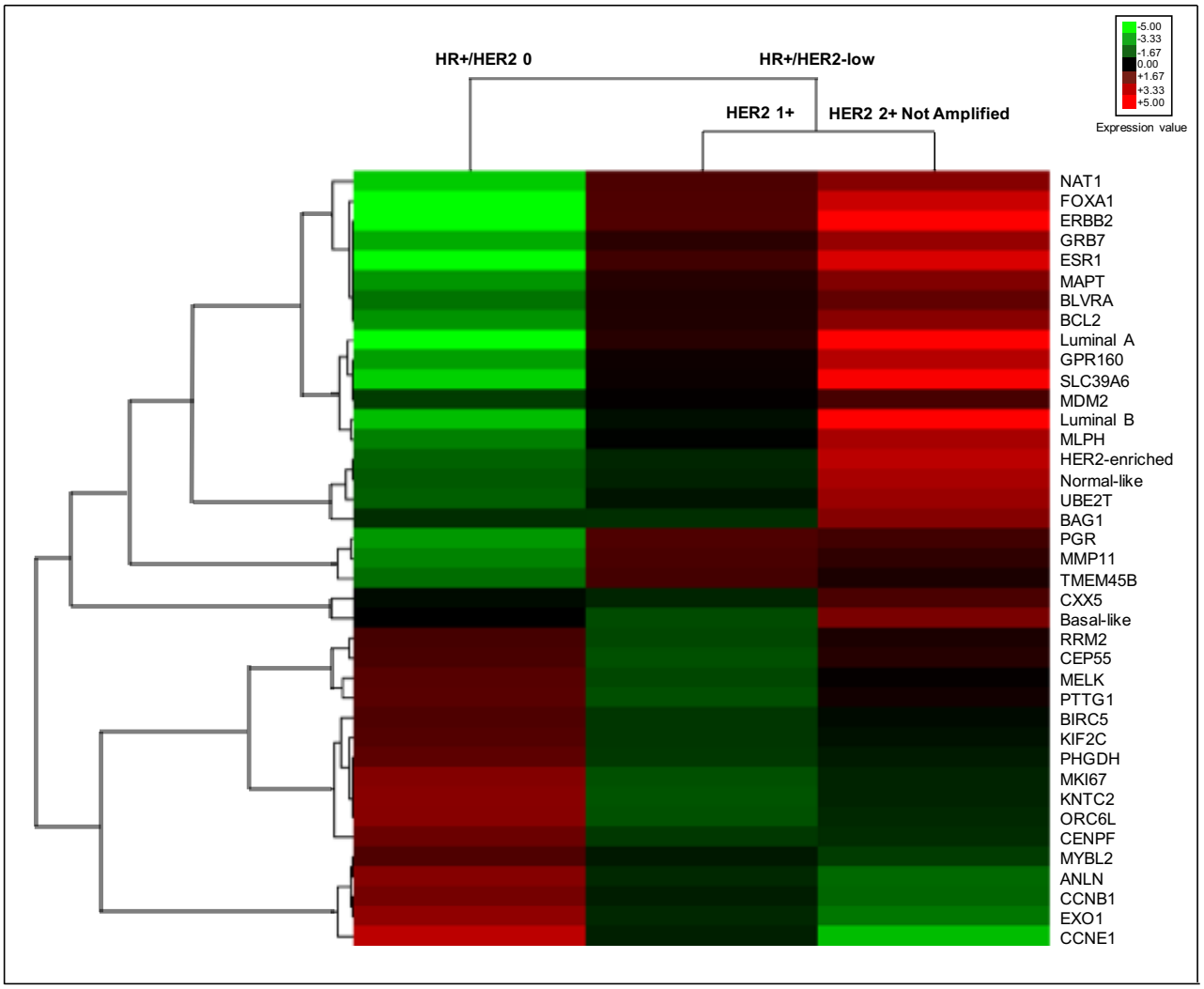
Legend: The red dots represent relative high gene expression, the green dots represent relative low gene expression, and black dots represent median gene expression for HER2-low vs. HER2 0 in the HR-positive population.

Supplementary figure 3. Plot for 2 class unpaired SAM analysis in the TN population



Legend: The green dots represent relative low gene expression, and black dots represent median gene expression for HER2-low vs. HER2 0 in the TNBC population.

Supplementary figure 4. Gene expression patterns of the HR-positive/HER2-negative breast cancers



Legend. A: supervised cluster of the HR-positive/HER2-negative breast cancers (total of 3 classes according to HER2 IHC score) with 55 variables. Sample and gene expression data from tumor samples of the same subtype have been combined into a single category. For each gene in a class, we calculated the standardized mean difference between the gene's expression in that class vs. its overall mean expression in the dataset using a 3-class Significance Analyses of Microarrays. The red color represents relative high gene expression, green represents relative low gene expression, and black represents median gene expression. **HR:** hormone receptors.

Appendix 4 – Supplementary materials Chapter 4

Additional information concerning the search strategy

We used the following search terms: breast, mammary, cancer, neoplasm, oncology, tumor, malignancy, carcinoma, adenocarcinoma, sarcoma, metastasis, metastatic, advanced, secondary, recurrent, inoperable, disseminated, incurable, trial, study, randomized, randomised, randomly, first line, second line, first-line, second-line, chemotherapy, endocrine therapy, everolimus, afinitor, sdz-rad, rad001, 159351-69-6, cyclophosphamide, methotrexate, fluorouracil, 5FU, 5-FU, doxorubicin, mitoxantrone, epirubicin, paclitaxel, docetaxel, liposomal doxorubicin, nab-paclitaxel, nab paclitaxel, pegylated, eribulin, capecitabine, vinorelbine carboplatin, cisplatin, platinum, gemcitabine, anastrozole, letrozole, aromatase inhibitor, exemestane, tamoxifen, palbociclib, PD-0332991, PD0332991, buparlisib, pictilisib, pi3k inhibitor, fulvestrant, faslodex.

No language limitations were adopted.

Data concerning the following variables were extracted from all the studies: full publication reference, publication year, line of treatment, phase of the trial, investigated treatments, single center vs multi-center studies, follow-up period (months), total number of patients, number of patients per arm, % of patients with estrogen receptor (ER)-positive tumors, median age, age range, % of post-menopausal patients, % of patients with visceral disease, lung, liver and bone metastases, main G3-5 adverse reactions rates.

The hazard ratios (HR) and associated 95% confidence intervals (CI) were extracted for progression-free survival (PFS) or time-to-progression (TTP), when reported. Odds ratios (OR) for the proportion of patients achieving an overall response and associated 95%CI: were also retrieved.

Additional details concerning the statistical analyses

A Bayesian NMA framework was used for each end-point[1-9]. All models have been implemented with both fixed and random effects to identify the best fit to the data. The parameters of the different models were estimated using a Markov Chain Monte Carlo (MCMC) method as implemented in the WinBUGS software package version 1.4.3.[10] For all the analyses, the WinBUGS sampler, using two chains, was run for 500,000 iterations that were discarded as 'burn-in', and the model was run for a further 2,500,000 iterations on which inferences were based. A thinning rate of 100 iterations was used to reduce autocorrelation of the sampled values, thus leaving 25,000 iterations per chain to use for estimation and inference.

Convergence of the chains was confirmed by the Gelman-Rubin statistic and by inspection of the trace plots.[11,12] The Deviance Information Criterion (DIC) was used to compare the goodness-of-fit of different models.[13] The DIC provides a measure of model fit that penalizes model complexity. The model with the lowest DIC was considered the model providing the best fit to the data. For the NMA of the HRs, we assumed that the log HRs were normally distributed with the log HR mean equaling the true log HR observed in each study and the variance equaling the observed variability in each study. As expected, the random effects model provided a better fit to the data compared to the fixed effect model, as confirmed by the DIC values (-1.294 for the fixed and -12.122 for the random effects model). For the NMA of the proportion of patients achieving an overall response, a binomial likelihood for the number of patients who responded was used. In addition, for this analysis the random effects model was also employed, as it provided a slightly better fit to the data than the fixed effects model (DIC for fixed effects model: 2054.18, DIC for random effects model: 1947.69).

The risk of bias for each trial was assessed by using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions.[14] Seven domains related to risk of bias were assessed in each included trial since there is evidence that these issues are associated with biased estimates of treatment effect: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, other bias. Review authors' judgments were categorized as "low risk", "high risk" or "unclear risk" of bias. Internal validity of eligible studies was assessed according to the Cochrane Collaboration's "Risk of Bias" tool in Review Manager.[15]

Full reference list

1. Hoaglin DC, Hawkins N, Jansen JP et al., Conducting indirect-treatment-comparison and network-meta-analysis studies: report of the ISPOR Task Force on Indirect Treatment Comparisons Good Research Practices: part 2. Value Health 2011; 14:429–437.
2. Jansen JP, Fleurence R, Devine B et al., Interpreting indirect treatment comparisons and network meta-analysis for health- care decision making: report of the ISPOR Task Force on Indirect Treatment Comparisons Good Research Practices: part 1. Value Health 2011; 14:417–428.
3. Cope S, Zhang J, Saletan S et al., A process for assessing the feasibility of a network meta-analysis: a case study of everolimus in combination with hormonal therapy versus chemotherapy for advanced breast cancer. BMC Med 2014; 12:93.

4. Dias S, Welton NJ, Sutton AJ et al., A generalised linear modelling framework for pairwise and network meta-analysis of randomised controlled trials. Technical support document, NICE DSU, 2011. Available from http://www.nicedsu.org.uk.
5. Gelman A, Carlin JB, Stern HS et al. Bayesian Data Analysis. 3rd edition: CRC Press, Boca Raton, Florida, USA, 2014.
6. Jansen JP, Network meta-analysis of survival data with fractional polynomials. BMC Medical Research Methodology 2011; 11: 1–14.
7. Guyot P, Ades AE, Ouwens MJNM et al., Enhanced secondary analysis of survival data: reconstructing the data from published Kaplan-Meier survival curves. BMC Medical Research Methodology 2012; 12:9.
8. Ouwens MJNM, Philips Z, Jansen JP, Network meta-analysis of parametric survival curves. Research Synthesis Methods 2010; 1:258–271.
9. Parmar MKB, Torri V, Stewart L, Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Statistics in Medicine 1998; 17:2815–2834.
10. Lunn DJ, Thomas A, Best N et al., WinBUGS — a Bayesian modelling framework: concepts, structure, and extensibility. Statistics and Computing 2000; 10:325–337.
11. Gelman A, Rubin DB, Inference from iterative simulation using multiple sequences, *Statistical Science* 1992; 7:457-511.
12. Lynch SM, Introduction to Applied Bayesian Statistics and Estimation for Social Scientists. 1st edition: Springer-Verlag, New York, NY, USA, 2007.
13. *Tomohiro A, Predictive Bayesian Model Selection.* American Journal of Mathematical and Management Sciences 2011; *31 (1-2):13–38.*
14. Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from http://handbook.cochrane.org.
15. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.
16. Baselga J, Campone M, Piccart M et al., Everolimus in Postmenopausal Hormone-Receptor–Positive Advanced Breast Cancer. N Engl J Med 2012; 366:520-529.
17. Finn RS, Martin M, Rugo HS et al., Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med 2016; 375:1925-1936.

18. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol 2016; 17: 425–439.
19. Hortobagyi GN, Stemmer SM, Burris HA et al., Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. N Engl J Med. 2016; 375(18):1738-1748.
20. Slamon DJ, Neven P, Chia S, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. J Clin Oncol. 2018; 36(24):2465-2472.
21. Goetz MP, Toi M, Campone M et al., MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. J Clin Oncol 2017; 35:3638-3646.
22. Sledge GW Jr, Toi M, Neven P et al., MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2− Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. J Clin Oncol 2017; 35:2875-2884.
23. Dixon AR, Jackson L, Chan S et al., A randomised trial of second-line hormone vs single agent chemotherapy in tamoxifen resistant advanced breast cancer. Br J Cancer 1992; 66:402–404.
24. Ackland SP, Anton A, Breitbach GP, et al., Dose-Intensive Epirubicin-Based Chemotherapy Is Superior to an Intensive Intravenous Cyclophosphamide, Methotrexate, and Fluorouracil Regimen in Metastatic Breast Cancer: A Randomized Multinational Study. J Clin Oncol 2001; 19:943-953.
25. Albain KS, Nag SM, Calderillo-Ruiz G et al., Gemcitabine Plus Paclitaxel Versus Paclitaxel Monotherapy in Patients With Metastatic Breast Cancer and Prior Anthracycline Treatment. J Clin Oncol 2008; 26:3950-3957.
26. Bachelot T, Bourgier C, Cropet C et al., Randomized Phase II Trial of Everolimus in Combination With Tamoxifen in Patients With Hormone Receptor–Positive, Human Epidermal Growth Factor Receptor 2–Negative Metastatic Breast Cancer With Prior Exposure to Aromatase Inhibitors: A GINECO Study. J Clin Oncol 2012; 30(22):2718-24
27. Bachelot T, Bajard A, Ray-Coquard I, et al., Final Results of ERASME-4: A Randomized Trial of First-Line Docetaxel plus either Capecitabine or Epirubicin for Metastatic Breast Cancer. Oncology 2011; 80:262–268.

28. Baselga J, Segalla JG, Roché H, et al., Sorafenib in Combination With Capecitabine: An Oral Regimen for Patients With HER2-Negative Locally Advanced or Metastatic Breast Cancer. J Clin Oncol 2012; 30:1484-1491.
29. Bergh J, Bondarenko IM, Lichinitser MR et al, First-line treatment of advanced breast cancer with sunitinib in combination with docetaxel versus docetaxel alone: results of a prospective, randomized phase III study. J Clin Oncol 2012; 30:921–929.
30. Bonneterre J, Roche H, Monnier A et al, Docetaxel vs 5-fluorouracil plus vinorelbine in metastatic breast cancer after anthracycline therapy failure. Br J Cancer 2002; 87:1210–1215.
31. Bonneterre J, Dieras V, Tubiana-Hulin M et al, Phase II multicentre randomised study of docetaxel plus epirubicin vs 5-fluorouracil plus epirubicin and cyclophosphamide in meta- static breast cancer. Br J Cancer 2004; 91:1466–1471.
32. Brufsky A, Hoelzer K, Beck T et al, A randomized phase II study of paclitaxel and bevacizumab with and without gemcitabine as first-line treatment for metastatic breast cancer. Clin Breast Cancer 2011; 11:211–220.
33. Chan S, Romieu G, Huober J et al, Phase III study of gemcitabine plus docetaxel compared with capecitabine plus docetaxel for anthracycline-pretreated patients with metastatic breast cancer. J Clin Oncol 2009; 27:1753–1760.
34. Crown JP, Dieras V, Staroslawska E et al, Phase III trial of sunitinib in combination with capecitabine versus capecitabine monotherapy for the treatment of patients with pretreated meta- static breast cancer. J Clin Oncol 2013; 31:2870–2878.
35. Del Mastro L, Venturini M, Lionetto R et al, Accelerated-intensified cyclophosphamide, epirubicin, and fluorouracil (CEF) compared with standard CEF in metastatic breast cancer patients: results of a multicenter, randomized phase III study of the Italian Gruppo Oncologico Nord-Ouest-Mammella Inter Gruppo Group. J Clin Oncol 2001; 19:2213–2221.
36. Fountzilas G, Kalofonos HP, Dafni U et al, Paclitaxel and epirubicin versus paclitaxel and carboplatin as first-line chemotherapy in patients with advanced breast cancer: a phase III study conducted by the Hellenic Cooperative Oncology Group. Ann Oncol 2004; 15:1517–1526.
37. Gradishar WJ, Kaklamani V, Sahoo TP et al, A double-blind, randomised, placebo-controlled, phase 2b study evaluating sorafenib in combination with paclitaxel as a first-line therapy in patients with HER2-negative advanced breast cancer. Eur J Cancer 2013; 49:312–322.

38. Gradishar WJ, Krasnojon D, Cheporov S et al, Significantly longer progression-free survival with nab-paclitaxel com- pared with docetaxel as first-line therapy for metastatic breast cancer. J Clin Oncol 2009; 27:3611–3619.
39. Hatschek T, Carlsson L, Einbeigi Z et al, Individually tailored treatment with epirubicin and paclitaxel with or without capecitabine as first-line chemotherapy in metastatic breast cancer: a randomized multicenter trial. Breast Cancer Res Treat 2012; 131:939–947.
40. Heidemann E, Stoeger H, Souchon R et al, Is first-line single-agent mitoxantrone in the treatment of high-risk metastatic breast cancer patients as effective as combination chemotherapy? No difference in survival but higher quality of life were found in a multicenter randomized trial. Ann Oncol 2002; 13:1717–1729.
41. Jones SE, Erban J, Overmoyer B et al, Randomized phase III study of docetaxel compared with paclitaxel in metastatic breast cancer. J Clin Oncol 2005; 23:5542–5551.
42. Kaufmann M, Bajetta E, Dirix LY et al, Exemestane is superior to megestrol acetate after tamoxifen failure in post- menopausal women with advanced breast cancer: results of a phase III randomized double-blind trial. The Exemestane Study Group. J Clin Oncol 2000; 18:1399–1411.
43. Langley RE, Carmichael J, Jones AL et al, Phase III trial of epirubicin plus paclitaxel compared with epirubicin plus cyclophosphamide as first-line chemotherapy for metastatic breast cancer: United Kingdom National Cancer Research Insti- tute trial AB01. J Clin Oncol 2005; 23:8322–8330.
44. Luck HJ, Du Bois A, Loibl S et al, Capecitabine plus paclitaxel versus epirubicin plus paclitaxel as first-line treatment for metastatic breast cancer: efficacy and safety results of a randomized, phase III trial by the AGO Breast Cancer Study Group. Breast Cancer Res Treat 2013; 139:779–787.
45. Martin M, Roche H, Pinter T et al, Motesanib, or open- label bevacizumab, in combination with paclitaxel, as first-line treatment for HER2-negative locally recurrent or metastatic breast cancer: a phase 2, randomised, double-blind, placebo- controlled study. Lancet Oncol 2011; 12:369–376.
46. Miles DW, Chan A, Dirix LY et al, Phase III study of bevacizumab plus docetaxel compared with placebo plus doc- etaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. J Clin Oncol 2010; 28:3239–3247.
47. Miller K, Wang M, Gralow J et al, Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007; 357:2666–2676.

48. Papadimitriou CA, Kalofonos H, Zagouri F et al, Weekly docetaxel with or without gemcitabine as second-line chemotherapy in paclitaxel-pretreated patients with metastatic breast cancer: a randomized phase II study conducted by the Hellenic Co-operative Oncology Group. Oncology 2009; 77:212–216.
49. Paridaens R, Biganzoli L, Bruning P et al, Paclitaxel versus doxorubicin as first-line single-agent chemotherapy for metastatic breast cancer: a European Organization for Research and Treatment of Cancer Randomized Study with cross-over. J Clin Oncol 2000; 18:724–733.
50. Paridaens RJ, Dirix LY, Beex LV et al, Phase III study comparing exemestane with tamoxifen as first-line hormonal treatment of metastatic breast cancer in postmenopausal women: the European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. J Clin Oncol 2008; 26:4883–4890.
51. Robert NJ, Saleh MN, Paul D et al, Sunitinib plus paclitaxel versus bevacizumab plus paclitaxel for first-line treatment of patients with advanced breast cancer: a phase III, randomized, open-label trial. Clin Breast Cancer 2011; 11:82–92.
52. Robert NJ, Dieras V, Glaspy J et al, RIBBON-1: randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor receptor 2-negative, locally recurrent or metastatic breast cancer. J Clin Oncol 2011; 29:1252–1260.
53. Rugo HS, Campone M, Amadori D et al, A randomized, phase II, three-arm study of two schedules of ixabepilone or paclitaxel plus bevacizumab as first-line therapy for metastatic breast cancer. Breast Cancer Res Treat 2013; 139:411–419.
54. Stockler MR, Harvey VJ, Francis PA et al, Capecitabine versus classical cyclophosphamide, methotrexate, and fluorouracil as first-line chemotherapy for advanced breast cancer. J Clin Oncol 2011; 29:4498–4504.
55. Zielinski C, Beslija S, Mrsic-Krmpotic Z et al, Gemcitabine, epirubicin, and paclitaxel versus fluorouracil, epirubicin, and cyclophosphamide as first-line chemotherapy in metastatic breast cancer: a Central European Cooperative Oncology Group International, multicenter, prospective, randomized phase III trial. J Clin Oncol 2005; 23:1401–1408.
56. Campone M, Dobrovolskaya N, Tjulandin S et al, A three-arm randomized phase II study of oral vinorelbine plus capecitabine versus oral vinorelbine and capecitabine in sequence versus docetaxel plus capecitabine in patients with metastatic breast cancer previously treated with anthracyclines. Breast J 2013; 19:240–249.

57. Ghosn M, Aftimos P, Farhat FS et al, A phase II randomized study comparing navelbine and capecitabine (Navcap) followed either by Navcap or by weekly docetaxel in the first-line treatment of HER-2/neu negative metastatic breast cancer. Med Oncol 2011; 28(Suppl 1):S142–S151.
58. von Minckwitz G, Chernozemsky I, Sirakova L et al Bendamustine prolongs progression-free survival in metastatic breast cancer (MBC): a phase III prospective, randomized, multicenter trial of bendamustine hydrochloride, methotrexate and 5-fluorouracil (BMF) versus cyclophosphamide, methotrexate and 5-fluorouracil (CMF) as first-line treatment of MBC. Anticancer Drugs 2005; 16:871–877.
59. O'Shaughnessy JA, Blum J, Moiseyenko V et al, Randomized, open-label, phase II trial of oral capecitabine (Xeloda) vs. a reference arm of intravenous CMF (cyclophosphamide, methotrexate and 5-fluorouracil) as first-line therapy for advanced/metastatic breast cancer. Ann Oncol 2001; 12:1247–1254.
60. Vici P, Giotta F, Di Lauro L et al, A multicenter phase II randomized trial of docetaxel/gemcitabine versus docetaxel/capecitabine as first-line treatment for advanced breast cancer: a Gruppo Oncologico Italia Meridionale study. Oncology 2011; 81:230–236.
61. Yardley DA, Burris HA III, Spigel DR et al, A phase II randomized crossover study of liposomal doxorubicin versus weekly docetaxel in the first-line treatment of women with metastatic breast cancer. Clin Breast Cancer 2009; 9:247–252.
62. Adelson K, Ramaswamy B, Sparano JA et al., Randomized phase II trial of fulvestrant alone or in combination with bortezomib in hormone receptor-positive metastatic breast cancer resistant to aromatase inhibitors: a New York Cancer Consortium trial. Npj Breast Cancer 2016; 2:16037.
63. Arpino G, Nair Krishnan M, Doval Dinesh C et al., Idoxifene versus tamoxifen: a randomized comparison in postmenopausal patients with metastatic breast cancer. Ann Oncol 2003; 14:233-241.
64. Bajetta E, Procopio G, Ferrari L et al., A Randomized, Multicenter Prospective Trial Assessing Long-Acting Release Octreotide Pamoate plus Tamoxifen as a First Line Therapy for Advanced Breast Carcinoma. Cancer 2002; 94 (2): 299-304.
65. Baselga J, Im S-A, Iwata H et al., Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2017; 18(7): 904-916.

66. Beex L, Rose C, Mouridsen H et al, Continuous versus intermittent tamoxifen versus intermittent/alternated tamoxifen and medroxyprogesterone acetate as first line endocrine treatment in advanced breast cancer: An EORTC phase III study (10863). Europ J Cancer 2006; 42: 3178-3185.
67. Bergh J, Jonsson P-E., Kerstin Lidbrink E et al., FACT: An Open-Label Randomized Phase III Study of Fulvestrant and Anastrozole in Combination Compared With Anastrozole Alone As First-Line Therapy for Patients With Receptor-Positive Postmenopausal Breast Cancer. J Clin Oncol 2012; 30(16):1919-1925.
68. Bonneterre J, Thurlimann B, Robertson JFR et al., Anastrozole Versus Tamoxifen as First-Line Therapy for Advanced Breast Cancer in 668 Postmenopausal Women: Results of the Tamoxifen or Arimidex Randomized Group Efficacy and Tolerability Study. J Clin Oncol 2000; 18:3748-3757.
69. Burstein HJ, Cirrincione CT, Barry WT et al., Endocrine Therapy With or Without Inhibition of Epidermal Growth Factor Receptor and Human Epidermal Growth Factor Receptor 2: A Randomized, Double-Blind, Placebo-Controlled Phase III Trial of Fulvestrant With or Without Lapatinib for Postmenopausal Women With Hormone Receptor–Positive Advanced Breast Cancer—CALGB 40302 (Alliance). J Clin Oncol 2014; 32:3959-3966.
70. Buzdar A, Douma J, Davidson N et al., Phase III, Multicenter, Double-Blind, Randomized Study of Letrozole, an Aromatase Inhibitor, for Advanced Breast Cancer Versus Megestrol Acetate. J Clin Oncol 2001; 19:3357-3366.
71. Buzdar A, Hayes D, El-Khoudary A et al., Phase III randomized trial of droloxifene and tamoxifen as first-line endocrine treatment of ER/PgR-positive advanced breast cancer. Breast Cancer Res Treat 2002; 73: 161–175.
72. Carlson RW, O'Neill A, Vidaurre T et al., A randomized trial of combination anastrozole plus gefitinib and of combination fulvestrant plus gefitinib in the treatment of postmenopausal women with hormone receptor positive metastatic breast cancer. Breast Cancer Res Treat 2012; 133:1049–1056.
73. Chia S, Gradishar W, Mauriac L et al., Double-Blind, Randomized Placebo Controlled Trial of Fulvestrant Compared With Exemestane After Prior Nonsteroidal Aromatase Inhibitor Therapy in Postmenopausal Women With Hormone Receptor–Positive, Advanced Breast Cancer: Results From EFECT. J Clin Oncol 2008; 26:1664-1670.
74. Clemens MR, Gladkov OA, Gartner E et al., Phase II, multicenter, open-label, randomized study of YM155 plus docetaxel as first-line treatment in patients

with HER2-negative metastatic breast cancer. Breast Cancer Res Treat 2015; 149:171–179.

75. Clemons MJ, Cochrane B, Pond GR et al., Randomised, phase II, placebo-controlled, trial of fulvestrant plus vandetanib in postmenopausal women with bone only or bone predominant, hormone-receptor-positive metastatic breast cancer (MBC): the OCOG ZAMBONEY study. Breast Cancer Res Treat 2014 146:153–162.
76. Cristofanilli M, Valero V, Mangalik A et al., Phase II, Randomized Trial to Compare Anastrozole Combined with Gefitinib or Placebo in Postmenopausal Women with Hormone Receptor–Positive Metastatic Breast Cancer. Clin Cancer Res 2010; 16(6); 1904–1914.
77. Di Leo A, Jerusalem G, Petruzelka L et al., Results of the CONFIRM Phase III Trial Comparing Fulvestrant 250 mg With Fulvestrant 500 mg in Postmenopausal Women With Estrogen Receptor–Positive Advanced Breast Cancer. J Clin Oncol 2010; 28:4594-4600.
78. Di Leo A, Seok Lee K, Ciruelos E, et al.: BELLE-3: A phase III study of buparlisib and fulvestrant in post- menopausal women with HR+, HER2-, aromatase inhibitor-treated, locally advanced or metastatic breast cancer, who progressed on or after mTOR inhibitor-based treatment. San Antonio Breast Cancer Symposium 2016, abstract S4–07.
79. Dickler MN, Barry WT, Cirrincione CT et al., Phase III Trial Evaluating Letrozole As First-Line Endocrine Therapy With or Without Bevacizumab for the Treatment of Postmenopausal Women With Hormone Receptor–Positive Advanced-Stage Breast Cancer: CALGB 40503 (Alliance). J Clin Oncol 2016; 34(22): 2602-2609.
80. Dieras V, Wildiers H, Jassem J et al., Trebananib (AMG 386) plus weekly paclitaxel with or without bevacizumab as first-line therapy for HER2-negative locally recurrent or metastatic breast cancer: A phase 2 randomized study. The Breast 2015; 24:182e190.
81. Robertson JFR, Bondarenko IM, Trishkina E et al., Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. The Lancet 2016; 388(10063):2997-3005.
82. Finn RS, Crown JP, Lang I et al., The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015; 16(1):25-35.

83. Goss P, Bondarenko IN, Manikhas GN, et al., Phase III, Double-Blind, Controlled Trial of Atamestane Plus Toremifene Compared With Letrozole in Postmenopausal Women With Advanced Receptor-Positive Breast Cancer. J Clin Oncol 2007; 25:4961-4966.
84. Howell A, Robertson JFR, Quaresma Albano J et al., Fulvestrant, Formerly ICI 182,780, Is as Effective as Anastrozole in Postmenopausal Women With Advanced Breast Cancer Progressing After Prior Endocrine Treatment. J Clin Oncol 2002; 20:3396-3403.
85. Howell A, Robertson JFR, Abram P et al., Comparison of Fulvestrant Versus Tamoxifen for the Treatment of Advanced Breast Cancer in Postmenopausal Women Previously Untreated With Endocrine Therapy: A Multinational, Double-Blind, Randomized Trial. J Clin Oncol 2004; 22:1605-1613.
86. Hyams DM, Chan A, de Oliveira C et al., Cediranib in combination with fulvestrant in hormone-sensitive metastatic breast cancer: a randomized Phase II study. Invest New Drugs 2013; 31:1345–1354.
87. Iwata H, Masuda N, Ohno S et al., A randomized, double-blind, controlled study of exemestane versus anastrozole for the first-line treatment of postmenopausal Japanese women with hormone-receptor-positive advanced breast cancer. Breast Cancer Res Treat 2013; 139:441–451
88. Johnston S, Pippen Jr J, Pivot X et al., Lapatinib Combined With Letrozole Versus Letrozole and Placebo As First-Line Therapy for Postmenopausal Hormone Receptor–Positive Metastatic Breast Cancer J Clin Oncol 2009; 27:5538-5546.
89. Johnston SRD, Kilburn LS, Ellis P et al., Fulvestrant plus anastrozole or placebo versus exemestane alone after progression on non-steroidal aromatase inhibitors in postmenopausal patients with hormone-receptor-positive locally advanced or metastatic breast cancer (SoFEA): a composite, multicentre, phase 3 randomised trial. Lancet Oncol 2013; 14: 989–98.
90. Johnston S, Basik M, Hegg R et al., Inhibition of EGFR, HER2, and HER3 signaling with AZD8931 in combination with anastrozole as an anticancer approach: Phase II randomized study in women with endocrine-therapy-naıve advanced breast cancer Breast Cancer Res Treat 2016; 160:91–99.
91. Kaufman PA, Awada A, Twelves C et al., Phase III Open-Label Randomized Study of Eribulin Mesylate Versus Capecitabine in Patients With Locally Advanced or Metastatic Breast Cancer Previously Treated With an Anthracycline and a Taxane. J Clin Oncol 2015; 33(6):594-601.

92. Kornblum N, Zhao F, Manola J et al., Randomized Phase II Trial of Fulvestrant Plus Everolimus or Placebo in Postmenopausal Women With Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer Resistant to Aromatase Inhibitor Therapy: Results of PrE0102. J Clin Oncol. 2018;36(16):1556-1563.
93. Krop IE, Mayer IA, Ganju V et al., Pictilisib for oestrogen receptor-positive, aromatase inhibitor- resistant, advanced or metastatic breast cancer (FERGI): a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol 2016; 17(6):811-821.
94. Lam SW, de Groot SM, Honkoop AH et al., Paclitaxel and bevacizumab with or without capecitabine as first-line treatment for HER2-negative locally recurrent or metastatic breast cancer: A multicentre, open-label, randomised phase 2 trial. Europ J Cancer 2014; 50:3077-3088.
95. Martín M, Loibl S, von Minckwitz G et al., Phase III Trial Evaluating the Addition of Bevacizumab to Endocrine Therapy As First-Line Treatment for Advanced Breast Cancer: The Letrozole/Fulvestrant and Avastin (LEA) Study. J Clin Oncol 2015; 33:1045-1052.
96. Mehta RS, Barlow WE, Albain KS et al., Combination Anastrozole and Fulvestrant in Metastatic Breast Cancer. N Engl J Med 2012; 367:435-44.
97. Milla-Santos A, Milla L, Portella J et al., Anastrozole Versus Tamoxifen as First-Line Therapy in Postmenopausal Patients With Hormone-Dependent Advanced Breast Cancer. A Prospective, Randomized, Phase III Study. Am J Clin Oncol (CCT) 2003; 26(3): 317–322.
98. Mouridsen HT, Letrozole in advanced breast cancer: the PO25 trial. Breast Cancer Res Treat 2007; 105:19–29.
99. Nabholtz JM for the Arimidex Study Group, Advanced breast cancer updates on anastrozole versus tamoxifen. J. Steroid Biochem. Mol. Biol. 2003; 86:321–325.
100. Ohno S, Rai Y, Iwata H et al., Three dose regimens of fulvestrant in postmenopausal Japanese women with advanced breast cancer: results from a double-blind, phase II comparative study (FINDER1). Ann Oncol 2010; 21: 2342–2347.
101. Osborne CK, Pippen J, Jones SE et al., Double-Blind, Randomized Trial Comparing the Efficacy and Tolerability of Fulvestrant Versus Anastrozole in Postmenopausal Women With Advanced Breast Cancer Progressing on Prior Endocrine Therapy: Results of a North American Trial. J Clin Oncol 2002; 20:3386-3395.

102. Osborne CK, Neven P, Dirix LY et al., Gefitinib or Placebo in Combination with Tamoxifen in Patients with Hormone Receptor-Positive Metastatic Breast Cancer: a Randomized Phase II Study. Clin Cancer Res. 2011; 17(5): 1147–1159.
103. Paul D, Vukelja SJ, Holmes FA, et al., Letrozole plus dasatinib improves progression-free survival (PFS) in hormone receptor-positive, HER2-negative postmenopausal metastatic breast cancer (MBC) patients receiving first-line aromatase inhibitor (AI) therapy. SABCS 2013, abstract S3-07.
104. Pritchard KI, Rolski J, Papai Z et al., Results of a phase II study comparing three dosing regimens of fulvestrant in postmenopausal women with advanced breast cancer (FINDER2). Breast Cancer Res Treat 2010; 123:453–461.
105. Robertson JFR, Ferrero J-M, Bourgeois H et al., Ganitumab with either exemestane or fulvestrant for postmenopausal women with advanced, hormone- receptor-positive breast cancer: a randomised, controlled, double-blind, phase 2 trial. Lancet Oncol 2013; 14: 228–35.
106. Robertson JFR, Lindemann JPO, Llombart-Cussac A et al., Fulvestrant 500 mg versus anastrozole 1 mg for the first-line treatment of advanced breast cancer: follow-up analysis from the randomized 'FIRST' study. Breast Cancer Res Treat 2012; 136:503–511.
107. Rugo HS, Barry WT, Moreno-Aspitia A et al., Randomized Phase III Trial of Paclitaxel Once Per Week Compared With Nanoparticle Albumin-Bound Nab-Paclitaxel Once Per Week or Ixabepilone With Bevacizumab as First-Line Chemotherapy for Locally Recurrent or Metastatic Breast Cancer: CALGB 40502/NCCTG N063H (Alliance). J Clin Oncol 2015; 33. 33(21):2361-2369.
108. Smorenburg CH, de Groot SM, van Leeuwen-Stok AE et al., A randomized phase III study comparing pegylated liposomal doxorubicin with capecitabine as first-line chemotherapy in elderly patients with metastatic breast cancer: results of the OMEGA study of the Dutch Breast Cancer Research Group BOOG. Ann Oncol 2014; 25: 599–605.
109. Tryfonidis K, Basaran G, Bogaerts J et al., A European Organisation for Research and Treatment of Cancer randomized, double-blind, placebo-controlled, multicentre phase II trial of anastrozole in combination with gefitinib or placebo in hormone receptor-positive advanced breast cancer (NCT00066378). Europ J Cancer 2016; 53:144e154.
110. Welt A, Marschner N, Lerchenmueller C et al., Capecitabine and bevacizumab with or without vinorelbine in first-line treatment of HER2/neu-negative metastatic or locally

advanced breast cancer: final efficacy and safety data of the randomised, open-label superiority phase 3 CARIN trial. Breast Cancer Res Treat 2016; 156:97–107.

111. Wolff AC, Lazar AA, Bondarenko I et al., Randomized Phase III Placebo-Controlled Trial of Letrozole Plus Oral Temsirolimus As First-Line Endocrine Therapy in Postmenopausal Women With Locally Advanced or Metastatic Breast Cancer. J Clin Oncol 2012; 31:195-202.
112. Xu B, Jiang Z, Shao Z et al., Fulvestrant 250 mg versus anastrozole for Chinese patients with advanced breast cancer: results of a multicentre, double-blind, randomised phase III trial. Cancer Chemother Pharmacol 2011; 67:223–230.
113. Yardley DA, Ismail-Khan RR, Melichar B et al., Randomized Phase II, Double-Blind, Placebo-Controlled Study of Exemestane With or Without Entinostat in Postmenopausal Women With Locally Recurrent or Metastatic Estrogen Receptor-Positive Breast Cancer Progressing on Treatment With a Nonsteroidal Aromatase Inhibitor. J Clin Oncol 2013; 31:2128-2135.
114. Yardley DA, Bosserman LD, O'Shaughnessy JA et al., Paclitaxel, bevacizumab, and everolimus/placebo as first-line treatment for patients with metastatic HER2-negative breast cancer: a randomized placebo-controlled phase II trial of the Sarah Cannon Research Institute. Breast Cancer Res Treat 2015; 154:89–97.
115. Zaman K, Winterhalder R, Mamot C et al., Fulvestrant with or without selumetinib, a MEK 1/2 inhibitor, in breast cancer progressing after aromatase inhibitor therapy: A multicentre randomised placebo- controlled double-blind phase II trial, SAKK 21/08. Europ J Cancer 2015; 51:1212-1220.
116. Zielinski C, Láng I, Inbar M et al., Bevacizumab plus paclitaxel versus bevacizumab plus capecitabine as first-line treatment for HER2-negative metastatic breast cancer (TURANDOT): primary endpoint results of a randomised, open-label, non-inferiority, phase 3 trial. Lancet Oncol 2016; 17(9):1230-1239.
117. Alba E, Martín M, Ramos M et al., Multicenter Randomized Trial Comparing Sequential With Concomitant Administration of Doxorubicin and Docetaxel As First-Line Treatment of Metastatic Breast Cancer: A Spanish Breast Cancer Research Group (GEICAM-9903) Phase III Study. J Clin Oncol 2004; 22:2587-2593.
118. Alba E, Ruiz-Borrego M, Margel M et al., Maintenance treatment with Pegylated liposomal doxorubicin versus observation following induction chemotherapy for metastatic breast cancer: GEICAM 2001-01 study. Breast Cancer Res Treat 2010; 122:169–176.

119. Batist G, Ramakrishnan G, Sekhar Rao C et al., Reduced Cardiotoxicity and Preserved Antitumor Efficacy of Liposome-Encapsulated Doxorubicin and Cyclophosphamide Compared With Conventional Doxorubicin and Cyclophosphamide in a Randomized, Multicenter Trial of Metastatic Breast Cancer. J Clin Oncol 2001; 19:1444-1454.
120. Biganzoli L, Cufer T, Bruning P et al., Doxorubicin and Paclitaxel Versus Doxorubicin and Cyclophosphamide as First-Line Chemotherapy in Metastatic Breast Cancer: The European Organization for Research and Treatment of Cancer 10961 Multicenter Phase III Trial. J Clin Oncol 2002; 20:3114-3121.
121. Biron P, Durand M, Rochè H et al., Pegase03: a prospective randomized phase III trial of FEC with or without high-dose thiotepa, cyclophosphamide and autologous stem cell transplantation in first-line treatment of metastatic breast cancer. Bone Marrow Transplant. 2008; 41:555–562.
122. Blohmer J-U, Schmid P, Hilfrich J et al., Epirubicin and cyclophosphamide versus epirubicin and docetaxel as first-line therapy for women with metastatic breast cancer: final results of a randomised phase III trial. Ann Oncol 2010; 21: 1430–1435.
123. Bontenbal M, Creemers G-J, Braun HJ et al., Phase II to III Study Comparing Doxorubicin and Docetaxel With Fluorouracil, Doxorubicin, and Cyclophosphamide As First-Line Chemotherapy in Patients With Metastatic Breast Cancer: Results of a Dutch Community Setting Trial for the Clinical Trial Group of the Comprehensive Cancer Centre. J Clin Oncol 2005; 23:7081-7088.
124. Buzdar AU, Xu B, Digumarti R et al., Randomized phase II non-inferiority study (NO16853) of two different doses of capecitabine in combination with docetaxel for locally advanced/metastatic breast cancer Ann Oncol 2012; 23: 589–597.
125. Capotorto AM, Pavesi L, Pedrazzoli P et al., Randomized, Controlled, Multicenter Phase III Trial of Standard-Dose Fluorouracil-Epirubicin- Cyclophosphamide (FEC), Compared with Time-Intensive FEC (FEC-G) and Mitoxantrone-Methotrexate-Mitomycin C (MMM-G) in Metastatic Breast Carcinoma. J Chem 2003; 15(2):184-191.
126. Cassier PA, Chabaud S, Trillet-Lenoir V et al., A phase-III trial of doxorubicin and docetaxel versus doxorubicin and paclitaxel in metastatic breast cancer: results of the ERASME 3 study. Breast Cancer Res Treat 2008; 109:343–350.
127. Chan S, Davidson N, Juozaityte E et al., Phase III trial of liposomal doxorubicin and cyclophosphamide compared with epirubicin and cyclophosphamide as first-line therapy for metastatic breast cancer Ann Oncol 2004; 15: 1527–1534.

128. Cinieri S, Chan A, Altundag K et al., Final Results of the Randomized Phase II NorCap-CA223 Trial Comparing First-Line All-Oral Versus Taxane-Based Chemotherapy for HER2-Negative Metastatic Breast Cancer. Clin Breast Cancer 2017; 17(2):91-99.e1.
129. Cortes J, O'Shaughnessy J, Loesch D et al., Eribulin monotherapy versus treatment of physician's choice in patients with metastatic breast cancer (EMBRACE): a phase 3 open-label randomised study. Lancet 2011; 377: 914–23.
130. Del Mastro L, Venturini M, Lionetto R et al., Accelerated-Intensified Cyclophosphamide, Epirubicin, and Fluorouracil (CEF) Compared With Standard CEF in Metastatic Breast Cancer Patients: Results of a Multicenter, Randomized Phase III Study of the Italian Gruppo Oncologico Nord-Ouest–Mammella Inter Gruppo Group. J Clin Oncol 2001; 19:2213-2221.
131. Gradishar WJ, Tjulandin S, Davidson N et al., Phase III Trial of Nanoparticle Albumin-Bound Paclitaxel Compared With Polyethylated Castor Oil–Based Paclitaxel in Women With Breast Cancer. J Clin Oncol 2005; 23:7794-7803.
132. Harbeck N, Saupe S, Jager E et al., A randomized phase III study evaluating pegylated liposomal doxorubicin versus capecitabine as first-line therapy for metastatic breast cancer: results of the PELICAN study Breast Cancer Res Treat 2017; 161(1):63-72.
133. Harris L, Batist G, Belt R et al., Liposome-Encapsulated Doxorubicin Compared with Conventional Doxorubicin in a Randomized Multicenter Trial as First-Line Therapy of Metastatic Breast Carcinoma. Cancer 2002; 94:25–36.
134. Icli AF, Akbulut H, Uner A et al., Cisplatin plus oral etoposide (EoP) combination is more effective than paclitaxel in patients with advanced breast cancer pretreated with anthracyclines: a randomised phase III trial of Turkish Oncology Group. British Journal of Cancer 2005; 92:639 – 644.
135. Jassem J, Pienkowski T, Płuzanska A et al., Doxorubicin and Paclitaxel Versus Fluorouracil, Doxorubicin, and Cyclophosphamide as First-Line Therapy for Women With Metastatic Breast Cancer: Final Results of a Randomized Phase III Multicenter Trial. J Clin Oncol 2001; 19:1707-1715.
136. Joensuu H, Sailas L, Alanko T et al., Docetaxel versus docetaxel alternating with gemcitabine as treatments of advanced breast cancer: final analysis of a randomised trial. Ann Oncol 2010; 21: 968–973.
137. Keller AM, Mennel RG, Georgoulias VA et al., Randomized Phase III Trial of Pegylated Liposomal Doxorubicin Versus Vinorelbine or Mitomycin C Plus Vinblastine in Women With Taxane-Refractory Advanced Breast Cancer. J Clin Oncol 2004; 22:3893-3901.

138. Martín M, Ruiz A, Muñoz M et al., Gemcitabine plus vinorelbine versus vinorelbine monotherapy in patients with metastatic breast cancer previously treated with anthracyclines and taxanes: final results of the phase III Spanish Breast Cancer Research Group (GEICAM) trial. Lancet Oncol 2007; 8: 219–25.
139. Mavroudis MD, Papakotoulas P, Ardavanis A et al., Randomized phase III trial comparing docetaxel plus epirubicin versus docetaxel plus capecitabine as first- line treatment in women with advanced breast cancer. Ann Oncol 2010; 21(1):48-54.
140. Mayer EL, Dhakil S, Patel T et al., SABRE-B: an evaluation of paclitaxel and bevacizumab with or without sunitinib as first-line treatment of metastatic breast cancer. Ann Oncol 2010; 21: 2370–2376.
141. Miles D, Cameron D, Bondarenko I et al., Bevacizumab plus paclitaxel versus placebo plus paclitaxel as first-line therapy for HER2-negative metastatic breast cancer (MERiDiAN): A double-blind placebo-controlled randomised phase III trial with prospective biomarker evaluation. Europ J Cancer 2017; 70:146-155.
142. Nabholtz J-M, Falkson C, Campos D et al., Docetaxel and Doxorubicin Compared With Doxorubicin and Cyclophosphamide as First-Line Chemotherapy for Metastatic Breast Cancer: Results of a Randomized, Multicenter, Phase III Trial. J Clin Oncol 2003; 21:968-975.
143. O'Shaughnessy J, Miles D, Vukelja S et al., Superior Survival With Capecitabine Plus Docetaxel Combination Therapy in Anthracycline-Pretreated Patients With Advanced Breast Cancer: Phase III Trial Results. J Clin Oncol 2002; 20:2812-2823.
144. Park YH, Jung KH, Im S-A et al., Phase III, Multicenter, Randomized Trial of Maintenance Chemotherapy Versus Observation in Patients With Metastatic Breast Cancer After Achieving Disease Control With Six Cycles of Gemcitabine Plus Paclitaxel As First-Line Chemotherapy: KCSG-BR07-02. J Clin Oncol 2013; 31:1732-1739.
145. Rivera E, Mejia JA, Arun BK et al., Phase 3 Study Comparing the Use of Docetaxel on an Every-3-week Versus Weekly Schedule in the Treatment of Metastatic Breast Cancer. Cancer 2008; 112:1455–1461.
146. Rochlitz C, Bigler M, von Moos R et al., SAKK 24/09: safety and tolerability of bevacizumab plus paclitaxel vs. bevacizumab plus metronomic cyclophosphamide and capecitabine as first-line therapy in patients with HER2- negative advanced stage breast cancer - a multicenter, randomized phase III trial. BMC Cancer 2016; 16:780.

147. Rugo HS, Stopeck AT, Joy AA et al., Randomized, Placebo-Controlled, Double-Blind, Phase II Study of Axitinib Plus Docetaxel Versus Docetaxel Plus Placebo in Patients With Metastatic Breast Cancer. J Clin Oncol 2011; 29:2459-2465.
148. Stemmler H-J, Harbeck N, Gröll de Rivera I et al., Prospective Multicenter Randomized Phase III Study of Weekly versus Standard Docetaxel plus Doxorubicin (D4) for First-Line Treatment of Metastatic Breast Cancer. Oncology 2010; 79:204–210.
149. Vuylsteke P, Huizing, Petrakova et al., Pictilisib plus paclitaxel for the treatment of hormone receptor-positive, HER2- negative, locally recurrent, or metastatic breast cancer: interim analysis of the multicentre, placebo-controlled, phase II randomised PEGGY study Ann Oncol. 2016; 27(11):2059-2066.
150. Tamura K, Inoue K, Masuda N et al., Randomized phase II study of nab-paclitaxel as first-line chemotherapy in patients with HER2-negative metastatic breast cancer Cancer Sci 2017; 108:987–994.
151. Zhang J, Gu S-Y, Gan Y et al., Vinorelbine and capecitabine in anthracycline- and/or taxane- pretreated metastatic breast cancer: sequential or combinational? Cancer Chemother Pharmacol 2013; 71:103–113.
152. Schmid P, Schippinger W, Nitsch T et al., Up-Front Tandem High-Dose Chemotherapy Compared With Standard Chemotherapy With Doxorubicin and Paclitaxel in Metastatic Breast Cancer: Results of a Randomized Trial. J Clin Oncol 2005; 23:432-440.
153. Martín M, Chan A, Dirix L et al., A randomized adaptive phase II/III study of buparlisib, a pan-Class I PI3K inhibitor, combined with paclitaxel for the treatment of HER2– advanced breast cancer (BELLE-4). Ann Oncol 2017; 28(2): 313-320.
154. Jerusalem G, de Boer RH, Hurvitz S et al. Everolimus Plus Exemestane vs Everolimus or Capecitabine Monotherapy for Estrogen Receptor–Positive, HER2-Negative Advanced Breast Cancer The BOLERO-6 Randomized Clinical Trial. JAMA Oncology 2018; 4(10):1367-1374.
155. André F, Ciruelos EM, Rubovszky G et al., Alpelisib (ALP) + fulvestrant (FUL) for advanced breast cancer (ABC): results of the Phase 3 SOLAR-1 trial. Abstract LBA3_PR. ESMO Congress 2018. Available at: https://oncologypro.esmo.org/Meeting-Resources/ESMO-2018-Congress/Alpelisib-ALP-fulvestrant-FUL-for-advanced-breast-cancer-ABC-results-of-the-Phase-3-SOLAR-1-trial.

Supplementary tables

Table S1. Studies' and patients' characteristics

FIRST AUTHOR	YEAR	LINE	PHASE	ARM A	ARM B	ARM C	ARM D	CENTERS	FOLLOW-UP PERIOD (MONTHS)	TOT *N*	*N* PTS ARM A	*N* PTS ARM B	*N* PTS ARM C	*N* PTS ARM D	% PTS ER+ ARM A	% PTS ER+ ARM B	% PTS ER+ ARM C	% PTS ER+ ARM D
ACKLAND	2001	1st	III	FEC	CMF	-	-	multicenter	>20	460	223	237	-	-	-	-	-	-
ALBAIN	2008	1st	III	GEM+PACq3w	PACq3w	-	-	multicenter	-	529	266	263	-	-	33.1	31.9	-	-
BACHELOT	2012	1st & 2nd	II	EVE+TAM 20	TAM 20	-	-	-	-	111	54	57	-	-	98	100	-	-
BACHELOT	2011	1st	III	DOCq3w+CAP	DOCq3w+EPI	-	-	multicenter	42	68	33	35	-	-	-	-	-	-
BASELGA/PICCART	2012/2013/2014	1st & 2nd	III	EVE+EXE	EXE	-	-	multicenter	7.6	724	485	239	-	-	100	100	-	-
BASELGA	2012b	1st & 2nd	IIB	CAP+SOR	CAP	-	-	multicenter	-	229	115	114	-	-	81.7	69.3	-	-
BERGH	2012	1st	III	DOCq3w+SUN	DOCq3w	-	-	multicenter	18.0	593	296	297	-	-	74	70	-	-
BONNETERRE	2002	1st & 2nd	III	DOCq3w	FU+VINO	-	-	multicenter	30.3	176	86	90	-	-	-	-	-	-
BONNETERRE	2004	1st	II	DOCq3w+EPI	FEC	-	-	multicenter	23.8	142	70	72	-	-	54	67	-	-
BRUFSKY	2011	1st	II	PACqw+BEVA	PACqw+BEVA+GEM	-	-	multicenter	14.6 for ARM A and 17.1 for ARM B	187	94	93	-	-	63.8	72	-	-
CHAN	2009	1st & 2nd	III	DOCq3w+GEM	DOCq3w+CAP	-	-	multicenter	-	305	153	152	-	-	69	72	-	-
CROWN	2013	1st & 2nd	III	CAP+SUN	CAP	-	-	multicenter	14.3	442	221	221	-	-	66	68	-	-
DEL MASTRO	2013	1st	III	DOCq3w+GEM	GEM+PACq3w	-	-	multicenter	-	241	118	123	-	-	70.3	75.6	-	-
DIXON	1992	2nd	II	MA	MITOX	-	-	single center	-	60	30	30	-	-	27	20	-	-
FOUNTZILAS	2004	1st	III	PACq3w+EPI	PACq3w+CARBO	-	-	-	23.5	327	163	164	-	-	55	62	-	-
GRADISHAR	2013	1st	IIB	PACqw+SOR	PACqw	-	-	multicenter	-	237	119	118	-	-	41	48	-	-
GRADISHAR	2009/2012	1st	II	NAB-PAC 300 q3w	NAB-PAC 100 qw	NAB-PAC 150 qw	DOCq3w	multicenter	-	300	76	76	74	74	-	-	-	-

HATSCHEK	2012	1st	III	PACq3w+EPI	PACq3w+EPI+CAP	-	-	multicenter	-	287	143	144	-	-	72	82	-	-
HEIDEMANN	2002	1st	III	MITOX	FEC	-	-	-	13.6	260	133	127	-	-	43	68	-	-
JONES	2005	2nd	III	DOCq3w	PACq3w	-	-	multicenter	60	449	225	224	-	-	51.1	42	-	-
KAUFMANN	2000	≥2nd	III	EXE	MA	-	-	multicenter	12.2	769	366	403	-	-	67.2	68	-	-
LANGLEY	2005	1st	III	PACq3w+EPI	CYC+EPI	-	-	multicenter	-	705	353	352	-	-	-	-	-	-
LUCK	2013	1st	III	CAP+PACq3w	PACq3w+EPI	-	-	multicenter	24.9	340	170	170	-	-	70	68	-	-
MARTIN	2011	1st	II	PACqw+MOT	PACqw	PACqw+BEVA	-	multicenter	-	282	91	94	97	-	80	80	80	-
MILES	2010	1st	III	BEVA 7.5mg/kg+DOCq3w	BEVA 15mg/kg+DOCq3w	DOCq3w	-	-	25	736	248	247	241	-	78	76	78	-
MILLER	2007	1st	III	PACqw+BEVA	PACqw	-	-	-	41.6 for ARM A and 43.5 for ARM B	673	347	326	-	-	59.9	62.9	-	-
PAPADIMITRIOU	2009	2nd	II	DOCqw	DOCq3w+GEM	-	-	-	-	75	34	41	-	-	74	66	-	-
PARIDAENS	2000	1st	III	PACq3w	DOX	-	-	multicenter	-	331	166	165	-	-	27	24	-	-
PARIDAENS	2008	1st	III	EXE	TAM 20	-	-	multicenter	-	371	182	189	-	-	88	89	-	-
ROBERT	2011	1st	III	SUN+PACqw	PACqw+BEVA	-	-	multicenter	8.1	485	242	243	-	-	76	76	-	-
ROBERT	2011	1st	III	CAP	TAX/ANTHRA	BEVA +CAP	BEVA +TAX/ ANTHRA	multicenter	15.6 for Cape arms and 19.2 for Tax/Anthra arms	1237	206	207	409	415	73.7	77.4	76.9	76.1
RUGO	2013	1st	II	IXAqw+BEVA	IXAq3w+BEVA	PACqw+BEVA	-	multicenter	19	123	46	45	32	-	80.4	77.8	84.4	-
STOCKLER	2011	1st	III	CAP	CMF	-	-	multicenter	37.5	325	216	109	-	-	64	64	-	-
ZIELINSKI	2005	1st	III	GEM+EPI+PACq3w	FEC	-	-	multicenter	20.4	259	124	135	-	-	36.3	40	-	-
CAMPONE	2013	1st	II	VINO+CAP	VINO-->CAP	DOCq3w+CAP	-	multicenter	-	139	44	47	48	-	65.9	58.7	58.3	-
GHOSN	2011	1st	II	VINO25+CAP1650	DOCqw	-	-	multicenter	-	70	41	29	-	-	78	72.4	-	-

VON MINCKWITZ	2005	1st	III	BMF	CMF	-	-	multicenter	-	345	162	183	-	-	-	-	-	-
O'SHAUGHNESSY	2001	1st	II	CAP	CMF	-	-	multicenter	-	95	62	33	-	-	-	-	-	-
VICI	2011	1st	II	DOCq3w+GEM	DOCq3w+CAP	-	-	multicenter	-	72	36	36	-	-	69.4	72.2	-	-
YARDLEY	2009	1st	II	PLD40	DOCqw	-	-	multicenter	-	102	50	52	-	-	78	63	-	-
ADELSON	2016	≥1st	II	FULV 500+BORTIDO	FULV 500	-	-	multicenter	12	118	59	59	-	-	100	100	-	-
ARPINO	2003	1st	II		TAM 20	-	-	multicenter	-	219	108	111	-	-	76.8	75.7	-	-
BAJETTA	2002	1st	III	OPLAR+TAM 20	TAM 20	-	-	multicenter	-	199	99	100	-	-	100	100	-	-
BASELGA	2015	≥1st	III	BUPA+FULV 500	FULV 500	-	-	multicenter	4.2 for ARM A and 5.0 for ARM B	1147	576	571	-	-	99.1	98.6	-	-
BEEX	2006	1st	III	Intermittent TAM 40	TAM 40	Intermittent TAM 40/MED AC	-	multicenter	-	276	94	93	89	-	55.3	55.9	51.7	-
BERGH	2012	1st	III	FULV LD+ANA	ANA	-	-	multicenter	8.9	514	258	256	-	-	99.7	98.4	-	-
BONNETERRE	2000	1st	III	ANA	TAM 20	-	-	multicenter	19	668	340	328	-	-	45.3	43.9	-	-
BURSTEIN	2014	≥1st	II	FULV LD+LAP	FULV LD	-	-	multicenter	33.6	291	146	145	-	-	99	97	-	-
BUZDAR	2001	≥2nd	II	LETRO 0.5	LETRO	MA	-	multicenter	18	602	202	199	201	-	83	80	80	-
BUZDAR	2002	1st	III	DRO	TAM 20	-	-	multicenter	-	1354	681	673	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
CARLSON	2012	≥1st	II	ANA+GEF	FULV LD+GEF	-	-	multicenter	-	141	72	69	-	-	94	91	-	-
CHIA	2008	≥2nd	III	FULV LD	EXE	-	-	multicenter	13	540	270	270	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
CLEMENS	2015	1st	II	YM155+DOCq3w	DOCq3w	-	-	multicenter	-	101	50	51	-	-	68	68.6	-	-
CLEMONS	2014	≥1st	II	FULV 500+VAN	FULV 500	-	-	multicenter	-	129	61	68	-	-	92	94	-	-
CRISTOFANILLI	2010	1st	II	ANA+GEF	ANA	-	-	multicenter	14.75	93	43	50	-	-	95	85	-	-
DI LEO	2010/2014	≥1st	III	FULV 500	FULV 250	-	-	multicenter	-	736	362	374	-	-	100	100	-	-

DI LEO	2016	≥2nd	III	BUPA+FULV 500	FULV 500	-	-	multicenter	-	432	289	143	-	-	100	100	-	-
DICKLER	2016	1st	III	LETRO+BEVA	LETRO	-	-	multicenter	42	343	173	170	-	-	98	98	-	-
DIERAS	2014	1st	II	TREB10+PACqw+BEVA	TREB3+PACqw+BEVA	PACqw+BEVA	TREB10+PACqw	multicenter	16.6	228	56	57	58	57	80	82	78	-
ELLIS/ROBERTSON	2016	1st	III	FULV 500	ANA	-	-	multicenter	25	462	230	232	-	-	95.6	95.7	-	-
FINN	2015	1st	II	PALBO+LETRO	LETRO	-	-	multicenter	29.6 for ARM A and 27.9 for ARM B	165	84	81	-	-	100	100	-	-
GOSS	2007	1st	III	ATA+TOR	LETRO	-	-	multicenter	-	865	434	431	-	-	100	100	-	-
HORTOBAGYI	2016	1st	III	RIBO+LETRO	LETRO	-	-	multicenter	15.3	668	334	334	-	-	99.4	99.7	-	-
HOWELL	2004	1st	III	FULV 250	TAM 20	-	-	multicenter	14.5	587	313	274	-	-	75	74	-	-
HOWELL/JONES	2002/2005	≥1st	III	FULV 250	ANA	-	-	multicenter	14.4	451	222	229	-	-	73.4	79.9	-	-
HYAMS	2013	≥2nd	II	CED+FULV LD	FULV LD	-	-	multicenter	-	62	31	31	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
IWATA	2013	1st	III	EXE	ANA	-	-	multicenter	-	298	149	149	-	-	94.8	98.2	-	-
JOHNSTON	2013	≥1st	III	FULV LD+ANA	FULV LD	EXE	-	multicenter	37.9	723	243	231	249	-	99	98	98	-
JOHNSTON	2009	1st	III	LETRO+LAP	LETRO	-	-	multicenter	24	1286	642	644	-	-	79	78	-	-
JOHNSTON	2016	≥1st	II	AZD8931 40mg+ANA	AZD8931 20mg+ANA	ANA	-	multicenter	-	359	120	118	121	-	100 ER &/or PgR +	100 ER &/or PgR +	100 ER &/or PgR +	-
KAUFMAN	2015	1st. 2nd & 3rd	III	ERI	CAP	-	-	multicenter	-	1102	554	548	-	-	46.8	50.7	-	-
KORNBLUM	2016	≥1st	II	EVE+FULV 500	FULV 500	-	-	multicenter	-	131	66	65	-	-	100	100	-	-
KROP	2014/2016	≥1st	II	PIC+FULV 500	FULV 500	-	-	multicenter	6	168	89	79	-	-	100	100	-	-
LAM	2014	1st	II	CAP+PACqw+BEVA	PACqw+BEVA	-	-	multicenter	-	312	156	156	-	-	85	85	-	-
LLOMBART-CUSSAC	2012	1st	II	EXE	ANA	-	-	multicenter	9.1	103	51	52	-	-	100	100	-	-

MARTIN	2015	1st	III	LETRO	LETRO+BEVA	-	-	multicenter	23.7	374	184	190	-	-	100	100	-	-
MEHTA	2012	1st	III	ANA	FULV LD+ANA	-	-	multicenter	35	694	345	349	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
MILLA-SANTOS	2003	1st	III	ANA	TAM 40	-	-	-	13.3	238	121	117	-	-	100	100	-	-
MOURIDSEN	2001/ 2003/ 2007	1st	III	LETRO	TAM 20	-	-	multicenter	32	916	458	458	-	-	65	67	-	-
NABHOLTZ	2000/ 2003	1st	III	ANA	TAM 20	-	-	multicenter	17.7	353	171	182	-	-	88.2	88.4	-	-
OHNO	2010	≥1st	II	FULV 250	FULV LD	FULV 500	-	multicenter	-	143	45	51	47	-	100	100	100	-
OSBORNE	2011	≥1st	II	GEF+TAM 20	TAM 20	-	-	multicenter	-	206	105	101	-	-	98.7	98.5		-
OSBORNE/JONES	2002/ 2005	≥1st	III	FULV 250	ANA	-	-	multicenter	16.8	400	206	194	-	-	86.9	87.1		-
PAUL	2013	≥1st	II	DASA+LETRO	LETRO	-	-	multicenter	-	120	57	63	-	-	100 ER &/or PgR +	100 ER &/or PgR +		-
PRITCHARD	2010	≥1st	II	FULV 250	FULV LD	FULV 500	-	multicenter	-	144	47	51	46	-	100	100	100	-
ROBERTSON	2013	≥2nd	II	GANI+FULV LD	FULV LD	-	-	multicenter	-	156	106	50	-	-	98	94	-	-
ROBERTSON/ELLIS	2009/ 2012/ 2015	1st	II	FULV 500	ANA	-	-	multicenter	18.8 for ARM A and 12.9 for ARM B	205	102	103	-	-	96	97	-	-
RUGO	2015	1st	III	NAB-PAC 150 qw+BEVA	PACqw+BEVA	IXAqw +BEVA	-	multicenter	25	799	271	283	245	-	73	71	72	-
SMORENBURG	2014	1st	III	PLD	CAP	-	-	multicenter	39	78	40	38	-	-	62	58	-	-
TRYFONIDIS	2016		II	ANA+GEF	ANA	-	-	multicenter	18	71	36	35	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
TURNER/CRISTOFANILLI	2015/ 2016	≥1st	III	PALBO+FULV 500	FULV 500	-	-	multicenter	8.9	521	347	174	-	-	100	100	-	-
WELT	2016	1st	III	BEVA+CAP	BEVA+CAP+VINO	-	-	multicenter	22.2 for ARM A and 23.6 for ARM B	592	297	295	-	-	79.5	79	-	-

WOLFF	2012	1st	III	LETRO+TEM	LETRO	-	-	multicenter	9.5	1112	556	556	-	-	96	95	-	-
XU	2011	≥2nd	III	FULV 250	ANA	-	-	multicenter	-	234	121	113	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
YARDLEY	2013	≥2nd	II	ENT+EXE	EXE	-	-	multicenter	24 for ARM A and 26.4 for ARM B	130	64	66	-	-	98	98	-	-
YARDLEY	2015	1st	II	PACqw+BEVA+EVE	PACqw+BEVA	-	-	multicenter	-	113	56	57	-	-	79	80	-	-
ZAMAN	2015	≥2nd	II	SELU+FULV 500	FULV 500	-	-	multicenter	22	42	22	20	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
ZIELINSKI	2016	1st	III	PACqw+BEVA	BEVA+CAP	-	-	multicenter	54.3	531	266	265	-	-	75	72	-	-
ALBA	2004	1st	III	DOX-->DOCq3w	DOX+DOCq3w	-	-	multicenter	17.5	144	75	69	-	-	-	-	-	-
ALBA	2010	>1st	III	DOX-->DOCq3w-->PLD	DOX-->DOCq3w	-	-	multicenter	20	155	78	77	-	-	-	-	-	-
BATIST	2001	1st	III	CYC+NPLD	CYC+DOX	-	-	multicenter	19 for ARM A and 16 for ARM B	297	142	155	-	-	-	-	-	-
BIGANZOLI	2002	1st	III	DOX+PACq3w	CYC+DOX	-	-	multicenter	29.2	275	138	137	-	-	-	-	-	-
BIRON	2008	1st	III	FEC	FEC-->HDC+SCT	-	-	multicenter	48	179	91	88	-	-	-	-	-	-
BLOHMER	2010	1st	III	CYC+EPI	DOCq3w+EPI	-	-	multicenter	24	236	111	125	-	-	-	-	-	-
BONTENBAL	2005	1st	II/III	FU+DOX+CYC	DOX+DOCq3w	-	-	multicenter	14	216	107	109	-	-	-	-	-	-
BUZDAR	2012	1st. 2nd & 3rd	II	DOCq3w+CAP	DOCq3w+CAP LD	-	-	multicenter	16.4	470	235	235	-	-	-	-	-	-
CAPOTORTO	2003	1st	III	FEC	TI FEC	TI MMM	-	multicenter	-	135	45	44	46	-	-	-	-	-
CASSIER	2008	1st	III	DOX+DOCq3w	DOX+PACq3w	-	-	multicenter	50.2	210	107	103	-	-	-	-	-	-
CHAN	2004	1st	III	CYC+NPLD	CYC+EPI	-	-	multicenter	-	160	80	80	-	-	-	-	-	-

CINIERI	2016	1st	II	VINO+CAP	GEM+PAC q3w	DOCq 3w+G EM	-	multicenter	-	149	49	50	50	-	-	-	-	-
CORTES	2011	≥2nd	III	ERI	TPC	-	-	multicenter	-	762	508	254	-	-	-	-	-	-
DEL MASTRO	2001	1st	III	FEC	IntFEC	-	-	multicenter	-	151	74	77	-	-	-	-	-	-
GRADISHAR	2005	≥1st	III	NAB-PAC 260 q3w	PACq3w	-	-	multicenter	-	454	229	225	-	-	-	-	-	-
HARBECK	2016	1st	III	PLD	CAP	-	-	multicenter	-	210	105	105	-	-	-	-	-	-
HARRIS	2002	1st	III	NPLD	DOX	-	-	multicenter	-	224	108	116	-	-	-	-	-	-
ICLI	2005	1st. 2nd & 3rd	III	CIS+ETO	PACq3w	-	-	multicenter	-	193	96	97	-	-	-	-	-	-
JASSEM	2001	1st	III	DOX+PACq3 w	FU+DOX+ CYC	-	-	multicenter	29	267	134	133	-	-	-	-	-	-
JOENSUU	2010	1st	III	DOCq3w	DOCq3w+ GEM	-	-	multicenter	25	237	115	122	-	-	-	-	-	-
KELLER	2004	2nd & 3rd	III	PLD	VINO or VINBLA+ MITOM	-	-	multicenter	-	301	150	151	-	-	-	-	-	-
MARTIN	2007	1st. 2nd & 3rd	III	GEM+VINO	VINO	-	-	multicenter	-	251	125	126	-	-	-	-	-	-
MAVROUDIS	2009	1st	III	DOCq3w+C AP	DOCq3w+ EPI	-	-	multicenter	39.8	272	136	136	-	-	-	-	-	-
MAYER	2010	1st	II	PACqw+BEV A	PACqw+B EVA+SUN	-	-	multicenter	-	46	23	23	-	-	-	-	-	-
MILES	2016	1st	III	PACqw+BEV A	PACqw	-	-	multicenter	14.8 for ARM A and 15 for ARM B	481	242	239	-	-	-	-	-	-
NABHOLTZ	2003b	1st	III	CYC+DOX	DOX+DOC q3w	-	-	multicenter	49	429	215	214	-	-	-	-	-	-
O'SHAUGHNESS Y	2002	1st	III	DOCq3w+C AP	DOCq3w	-	-	multicenter	-	511	255	256	-	-	-	-	-	-
PARK	2013	>1st	III	GEM+PACq3 w	OBS	-	-	multicenter	-	231	116	115	-	-	-	-	-	-
RIVERA	2008	1st & 2nd	III	DOCq3w	DOCqw	-	-	single center	15.1	125	62	63	-	-	-	-	-	-
ROCHLITZ	2016	1st	III	PACqw+BEV A	BEVA+CA P+CYC	-	-	multicenter	26.1	147	73	74	-	-	-	-	-	-
RUGO	2011	1st	II	AXI+DOCq3 w	DOCq3w	-	-	multicenter	-	167	112	55	-	-	-	-	-	-
STEMMLER	2010	1st	III	DOX+DOCq 3w	DOX+DOC qw	-	-	multicenter	18.7	85	43	42	-	-	-	-	-	-
VUYLSTEKE	2016	1st	II	PIC+PACqw	PACqw	-	-	multicenter	-	183	91	92	-	-	-	-	-	-

SLEDGE	2017	≥1st	III	ABE+FULV 500	FULV 500	-	-	multicenter	-	669	446	223	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
TAMURA	2017	1st	III	NAB-PAC 150 qw	DOCq3w	-	-	multicenter	-	197	98	99	-	-	-	-	-	-
ZHANG	2013	1st	II	VINO+CAP	VINO-->CAP	-	-	single center	39.8 for ARM A and 38.2 for ARM B	60	30	30	-	-	-	-	-	-
FINN	2016	1st	III	PALBO+LETRO	LETRO	-	-	multicenter	23	666	444	222	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
MARTIN	2016	1st	II	BUPA+PACqw	PACqw	-	-	multicenter	-	416	207	209	-	-	-	-	-	-
GOETZ	2017	1st	III	ABE+ANA/LETRO	ANA/LETRO	-	-	multicenter	17.8	493	328	165	-	-	100 ER &/or PgR +	100 ER &/or PgR +	-	-
SCHMID	2005	1st	III	CYC+MITOX+ETO	DOX+PAC q3w	-	-	multicenter	22.5	93	48	45	-	-	-	-	-	-
JERUSALEM	2018	≥1st	II	EVE+EXE	EVE	CAP	-	multicenter (83 centers in 18 countries)	37.6	309	104	103	102	-	-	-	-	
SLAMON	2018	1st & 2nd	III	RIBO+FULV 500	FULV 500	-	-	multicenter	-	726	484	242	-	-	99.4	99.6	-	-
ANDRE	2018	≥1st	III	ALP+FULV 500	FULV 500	-	-	multicenter	-	341	169	172	-	-	-	-	-	-

Abbreviations. qw = weekly; q3w = every 3 weeks; ABE = abemaciclib; FULV 500 = fulvestrant, standard dose; FULV 250 = fulvestrant 250 mg without loading dose; FULV LD = fulvestrant 250 mg with loading dose; ANA = anastrozole; LETRO = letrozole, standard dose 2.5 mg; LETRO 0.5 = letrozole 0.5 mg; ATA = atamestane; TOR: toremifene; AXI = axitinib; DOC = docetaxel; BEVA = bevacizumab; CAP= capecitabine; CYC = cyclophosphamide; VINO = vinorelbine; BUPA = buparlisib; PAC = paclitaxel; SOR: sorafenib; SUN = sunitinib; CED = cediranib; LD = low dose; BMF = bendamustine + methotrexate + 5-fluorouracil; CMF = cyclophosphamide + methotrexate + 5-fluorouracil; DOX = doxorubicin; EPI = epirubicin; MITOX = mitoxantrone; CIS = cisplatin; ETO = etoposide; DASA = dasatinib; NPLD = non-peghylated liposomal doxorubicin; GEM = gemcitabine; IDO = idoxifene; ENT = entinostat; EXE = exemestane; ERI = eribulin; EVE = everolimus; TAM = tamoxifen; FEC = 5-fluorouracil + epirubicin + cyclophoshpamide; BORT = bortezomib; VAN = vandetanib; GEF = gefitinib; LAP= lapatinib; GANI = ganitumab; Int = intensive; IXA = ixabepilone; MA = megestrol acetate; NAB-PAC = nab paclitaxel; TEM =

temsirolimus; OBS = observation; OPLAR = octreotide pamoate long acting release; CARBO = carboplatin; MOT = motesanib; PALBO = palbociclib; PIC = pictilisib; PLD = peghilated liposomal doxorubicin; RIBO = ribociclib; TPC = treatment of physician's choice; TREB = trebananib; VINBLA = vinblastine; SELU = selumetinib; → = followed by; TI = time intensive; MMM = mitoxantrone + mitomycin C + methotrexate; DRO = droloxifene; HDC = high dose chemotherapy; SCT = stem cell transplant; NA = not availabl; FAC = 5-fluorouracil + doxorubicin + cyclophosphamide.

Table S2. Patients' patterns of metastasis, age and menopausal status per treatment arms

FIRST AUTHOR	YEAR	JOURNAL	*N* ARMS	ARM	TOT PTS *N*	*N* PTS/ ARM	MEDIAN AGE (Years)	AGE RANGE (Years)	POST-MENOPAUSAL (%)	VISCERAL DISEASE (%)	LUNG MET (%)	LIVER MET (%)	BONE MET (%)
ACKLAND	2001	J CLIN ONCOL	2	FEC	460	223	56	22-71	75	57	-	-	9
				CMF		237	55	26-71	69	60	-	-	8
ALBAIN	2008	J CLIN ONCOL	2	GEM+PAC	529	266	53	26-83	-	72.9	-	-	-
				PAC		263	53	27-75	-	73	-	-	-
BACHELOT	2012	J CLIN ONCOL	2	EVE+TAM	111	54	63	41-81	100	57	-	-	76
				TAM		57	66	42-86	100	49	-	-	79
BACHELOT	2011	ONCOLOGY	2	CAP+DOC	68	33	57	32-74	64	-	24	64	85
				EPI+DOC		35	59	34-71	71	-	31	49	74
BASELGA	2012/2013	N ENGL J MED	2	EVE+EXE	724	485	62	34-93	100	56	29	33	76
				EXE		239	61	28-90	100	56	33	30	77
BASELGA	2012	J CLIN ONCOL	2	CAP+SOR	229	115	55.1	-	-	75.7	-	-	-
				CAP		114	54.4	-	-	73.7	-	-	-
BERGH	2012	J CLIN ONCOL	2	DOC+SUN	593	296	54	31-84	-	74	-	-	6
				DOC		297	56	28-78	-	70	-	-	6
BONNETERRE	2002	BRITISH J CANCER	2	DOC	176	86	54.9	27.9-79.0	-	-	26.7	67.4	48.8
				FU+VIN		90	54.55	31.6-74.5	-	-	35.6	66.7	41.1

BONNETERRE	2004	BRITISH J CANCER	2	DOC+EPI	142	70	54	34-73	-	-	54	64	59
				FEC		72	54	23-71	-	-	38	57	49
BRUFSKY	2011	CLIN BREAST CANCER	2	PAC+BEVA	187	94	57.5	30.8-83.8	-	72.3	-	-	-
				PAC+BEVA+GEM		93	55.2	37.1-79.7	-	71	-	-	-
CHAN	2009	J CLIN ONCOL	2	GEM+DOC	305	153	56	26-76	-	84	41	63	44
				CAP+DOC		152	53	30-78	-	88	43	64	50
CROWN	2013	J CLIN ONCOL	2	CAP+SUN	442	221	52	27-79	-	-	-	-	-
				CAP		221	54	31-77	-	-	-	-	-
DEL MASTRO	2013	CANCER	2	GEM+DOC	241	118	58.5 A; 56 C	37-76 A; 38-77 C;	70.34	79.34	-	-	-
				GEM+PAC		123	57.5 B; 55 D	31-74 B; 43-78 D	69.11	65.85	-	-	-
DIXON	1992	BR J CANCER	2	MA	60	30	64	43-78	100	9	2	-	13
				MITOX		30	61	42-75	100	9	3	-	10
FOUNTZILAS	2004	ANN ONCOL	2	PAC+EPI	327	163	59	30-78	76	68	13.5	-	44
				PAC+CARBO		164	59	27-78	74	75	13	-	57
GRADISHAR	2013	EUROP J CANCER	2	PAC+SOR	237	119	50.6	-	-	76	-	-	-
				PAC		118	53.1	-	-	74	-	-	-
GRADISHAR	2009/2012	J CLIN ONCOL/CLIN BREAST CANCER	4	NAB-PAC 300 mg/m2	300	76	51.7	-	64	84	-	-	-
				NAB-PAC 100 mg/m2		76	55.4	-	82	80	-	-	-
				NAB-PAC 150 mg/m2		74	53.3	-	72	80	-	-	-
				DOC		74	55.4	-	81	91	-	-	-
HATSCHEK	2012	BREAST CANCER RES TREAT	2	EPI+PAC	287	143	57	-	-	-	54	45	52

				EPI+PAC+CAP		144	55.7	-	-	-	49	45	56
HEIDEMANN	2002	ANN ONCOL	2	MITOX	260	133	-	-	-	-	5.88	66.39	44.54
				FEC		127	-	-	-	-	7.56	66.39	37.82
JONES	2005	J CLIN ONCOL	2	DOC	449	225	56	22-93	88	-	-	-	-
				PAC		224	54	28-82	86.6	-	-	-	-
KAUFMANN	2000	J CLIN ONCOL	2	EXE	769	366	65	35-89	100	56.6	-	-	16.7
				MA		403	65	30-91	100	59.3	-	-	18.1
LANGLEY	2005	J CLIN ONCOL	2	EPI+PAC	705	353	55	33-78	-	65	38	40	48
				EPI+CYC		352	54	32-83	-	65	37	41	49
LUCK	2013	BREAST CANCER RES TREAT	2	CAP+PAC	340	170	57	29-75	-	56	-	-	-
				EPI+PAC		170	58	21-76	-	47	-	-	-
MARTIN	2011	LANCET ONCOL	3	PAC+MOT	282	91	55.3	-	76	-	-	-	-
				PLB		94	53	-	66	-	-	-	-
				PAC+BEVA		97	55.2	-	64	-	-	-	-
MILES	2010	J CLIN ONCOL	3	BEVA 7.5mg/kg+DOC	736	248	54	26.83	-	-	42	40	60
				BEVA 15mg/kg+DOC		247	55	27-76	-	-	48	46	55
				DOC		241	55	29-83	-	-	38	50	59
MILLER	2007	N ENGL J MED	2	PAC+BEVA	673	347	56	29-84	-	79.5	-	-	10.4
				PAC		326	55	27-85	-	87.1	-	-	7.7
PAPADIMITRIOU	2009	ONCOLOGY	2	DOC	75	34	57	38-74	-	-	41	56	56
				DOC+GEM		41	57	37-76	-	-	42	51	44
PARIDAENS	2000	J CLIN ONCOL	2	PAC	331	166	54	31-74	-	78	-	-	16
				DOX		165	55	26-75	-	75	-	-	14
PARIDAENS	2008	J CLIN ONCOL	2	EXE	371	182	63	37-86	100	47.8	-	-	11.5

				TAM		189	62	37-87	100	46.6	-	-	11.6
ROBERT	2011	CLIN BREAST CANCER	2	SUN+PAC	485	242	57	27-84	-	-	-	-	-
				BEVA+PAC		243	57	32-92	-	-	-	-	-
ROBERT	2011	J CLIN ONCOL	4	CAP	1237	206	57	23-88	-	71.4	-	-	10.2
				TAX/ANTHRA		207	55	29-85	-	73.4	-	-	3.9
				BEVA+CAP		409	56	28-91	-	67.5	-	-	8.8
				BEVA+TAX/ANTHRA		415	55	28-88	-	68.9	-	-	6.3
RUGO	2013	BREAST CANCER RES TREAT	3	IXA (16mg/m2)+BEVA	123	46	60	27-80	-	-	80.4	47.8	
				IXA (40mg/m2)+BEVA		45	59	37-83	-	-	62.2	46.7	
				PAC+BEVA		32	59	37-75	-	-	56.3	28.1	
STOCKLER	2011	J CLIN ONCOL	2	CAP (combined)	325	216	62	-	-	-	-	-	-
				CMF		109	62	-	-	-	-	-	-
ZIELINSKI	2005	J CLIN ONCOL	2	GEM+EPI+PAC	259	124	53	29-74	77.4	NC	39.5	61.3	36.3
				FEC		135	54	32-74	67.4	NC	47.4	48.9	37
CAMPONE	2013	BREAST J	3	VIN+CAP	139	44	55	31.7-73.1	-	65.9	-	-	-
				VIN->CAP		47	56.7	37.0-72.4	-	91.3	-	-	-
				DOC+CAP		48	52.2	27.4-75.0	-	64.6	-	-	-
GHOSN	2011	MED ONCOL	2	VINO+CAP	70	41	51	32-79	-	-	39	39	43.9
				DOC		29	57	35-72	-	-	51.7	20.7	34.5
VON MINCKWITZ	2005	ANTI-CANCER DRUG	2	BMF	345	162	61	-	-	30.4	-	-	-
				CMF		183	57	-	-	33.3	-	-	-
O'SHAUGNESSY	2001	ANN ONCOL	2	CAP	95	62	69	54-83	NC	NC	34	34	48
				CMF		33	70	55-80	NC	NC	22	44	44

VICI	2011	ONCOLOGY	2	DOC+GEM	72	36	61	38-70	72	50	-	-	27.8
				DOC+CAP		36	63	35-69	78	50	-	-	33.3
YARDLEY	2009	CLIN BREAST CANCER	2	LIP DOX	102	50	62	31-87	-	88	-	-	-
				DOC		52	63	34-80	-	87	-	-	-
FINN	2014	LANCET ONCOL	2	PALBO+LETRO	165	84	63	54-71	84	34	-	-	17
				LETRO		81	64	56-70	81	37	-	-	12
TURNER/CRISTOFANILLI	2015/2016	N ENGL J MED/LANCET ONCOL	2	PALBO+FULV 500	521	347	57	30-88	79.3	59.7	-	-	-
				FULV 500		174	56	29-80	79.3	59.7	-	-	-
BASELGA	2015	SABCS	2	BUPA+FULV 500	1147	576	62	29-80	100	59.2	-	-	-
				FULV 500		571	61	31-90	100	59	-	-	-
KROP	2014	LANCET ONCOL	2	PICT+FULV 500	168	89	60	36-90	89	57	-	-	21
				FULV 500		79	63	40-82	79	53	-	-	22
BONNETERRE	2000	J CLIN ONCOL	2	ANA	668	340	67	34-91	100	30.3	21.8	9.4	45.9
				TAM		328	66	41-92	100	37.8	30.5	9.5	48.2
NABHOLTZ	2000	J CLIN ONCOL	2	ANA	353	171	68		100	48.5	44.4	7.6	65.5
				TAM		182	67		100	47.8	37.4	16.5	53.8
MILLA-SANTOS	2003	AM J CLIN ONCOL	2	ANA	138	121	60.2	56-77	100	-	-	-	-
				TAM		117	60.6	55-77	100	-	-	-	-
MOURIDSEN	2000/2003	J CLIN ONCOL	2	LETRO	916	458	65	31-96	100	43	-	-	32
				TAM		458	64	31-93	100	46	-	-	29
BUZDAR	2001	J CLIN ONCOL	3	LETRO 0.5	602	202	66.5	-	100	50	-	-	28
				LETRO 2.5		199	65.5	-	100	48	-	-	34
				MEG AC 40 qid		201	65.9	-	100	48	-	-	26
OSBORNE	2002	J CLIN ONCOL	2	FULV 250	400	206	63	33-89	100	-	30.6	23.8	43.7
				ANA		194	62	36-94	100	-	30.9	22.2	43.8

HOWELL	2002	J CLIN ONCOL	2	FULV 250	451	222	63	35-86	100	-	25.2	21.6	51.8
				ANA		229	64	33-89	100	-	26.2	24.5	51.1
XU	2010	CANCER CHEMOTHER PHARMACOL	2	FULV 250	234	121	54	33-78	100	-	-	-	-
				ANA		113	54	31-77	100	-	-	-	-
DI LEO	2010/2014	J CLIN ONCOL/J NATL CANCER INST	2	FULV 500	736	362	61	-	100	66	-	-	-
				FULV 250		374	61	-	100	62	-	-	-
JOHNSTON	2013	LANCET ONCOL	3	FULV LD + ANA	723	243	63.8	57.0-72.0	100	57	-	-	15
				FULV LD		231	63.4	57.0-73.5	100	62	-	-	16
				EXE		249	66.0	59.2-75.0	100	58	-	-	13
ROBERTSON/ELLIS	2009/2012/2015	J CLIN ONCOL/BREAST CANCER RES TREAT/ J CLIN ONCOL	2	FULV 500	205	102	66	38-87	100	47.1	29.4	14.7	9.8 bone only
				ANA		103	68	36-90	100	56.3	40.8	13.6	7.8 bone only
CHIA	2008	J CLIN ONCOL	2	FULV LD	540	270	63	38-88	100	56.1	34.5	31.1	67.2
				EXE		270	63	32-91	100	57.9	36.3	32.2	66.4
BERGH	2012	J CLIN ONCOL	2	FULV LD + ANA	514	258	65	33-86	100 (physiologic or induced by GnRH a-logue)	51.9	25.6	22.1	24.4 (bone only)
				ANA		256	63	36-90	100 (physiologic or induced by GnRH a-logue)	48.4	26.6	15.6	27.7 (bone only)
HOWELL	2004	J CLIN ONCOL	2	FULV 250	587	313	67	43-93	100	-	25.2	9.6	27.5
				TAM		274	66	43-92	100	-	24.5	9.9	32.5
MEHTA	2012	N ENGL J MED	2	ANA	694	345	65	36-91	100	48.4	-	-	22% bone only

				FULV LD + ANA		349	65	27-92	100	51.9	-	-	21.5 bone only
WOLFF	2012	J CLIN ONCOL	2	LETRO+TEM	1112	556	63	36-98	100	-	-	-	-
				LETRO		556	63	28-91	100	-	-	-	-
PAUL	2013	SABCS	2	DASA+LETRO	120	57	-	-	100	-	14	18	74
				LETRO		63	-	-	100	-	16	19	70
OHNO	2010	ANN ONCOL	3	FULV 250	143	45	61	50-77	100	57.8	-	-	-
				FULV LD		51	61	43-86	100	54.9	-	-	-
				FULV		47	61	45-83	100	57.4	-	-	-
PRITCHARD	2010	BREAST CANCER RES TREAT	3	FULV 250	144	47	63	42-88	100	72.3	-	-	-
				FULV LD		51	69	38-85	100	80.4	-	-	-
				FULV 500		46	67	49-85	100	80.4	-	-	-
YARDLEY	2013	J CLIN ONCOL	2	ENTINO+EXE	130	64	63	37-85	100	53	-	-	77
				EXE		66	62	37-88	100	67	-	-	71
HYAMS	2013	INVEST NEW DRUGS	2	CEDI+FULV LD	62	31	-	-	100	-	-	-	-
				FULV LD		31	-	-	100	-	-	-	-
ZAMAN	2015	EUROPEAN JOUR-L OF CANCER	2	FULV 500+SALU	42	22	66	40-79	100	59	-	-	-
				FULV 500		20	69	46-79	100	55	-	-	-
CLEMONS	2014	BREAST CANCER RES TREAT	2	FULV 500+VANDE	129	61	61.6	-	100	-	20	23	100
				FULV 500		68	57.9	-	100	-	32	24	100
ROBERTSON	2013	LANCET ONCOL	2	GANI+FULV LD or EXE	156	106 (68% FULV . 32% EXE)	61	54-70	100	45	-	-	44
				FULV LD or EXE		50 (68% FULV	62	55-66	100	44	-	-	46

						. 32% EXE)							
CARLSON	2012	BREAST CANCER RES TREAT	2	ANA+GEF	141	72	58	34-90	100	-	45	35	65
				FULV LD+GEF		69	63	35-91	100	-	52	23	55
CRISTOFANILLI	2010	CLIN CANCER RES	2	ANA+GEF	93	43	61	41-82	100	-	33	19	70
				ANA		50	58	37-84	100	-	22	18	74
OSBORNE	2012	CLINC CANCER RES	2	TAM+GEF	206	105	61.6	40-89	>67.4	52.4	-	-	-
				TAM		101	63.1	40-86	>72.1	45.5	-	-	-
OSBORNE	2012	CLINC CANCER RES	2	TAM+GEF	83	48	-	-		52.1	-	-	-
				TAM		35	-	-		62.9	-	-	-
JOHNSTON	2009	J CLIN ONCOL	2	LAP+LETRO	1286	642	62	31-94	100	85	39	27	15 (bone only)
				LETRO		644	63	35-95	100	87	38	23	13 (bone only)
JOHNSTON	2013	J CLIN ONCOL/CLINICALTRIALS.GOV	3	ANA + AZD8931 40mg	359	120	60.4	36-85	100	-	-	-	-
				ANA + AZD8931 20mg		118	62	27-86	100	-	-	-	-
				ANA		121	60.5		100	-	-	-	-
TRYFONIDIS	2016	EUROP J CANCER	2	ANA + GEF	71	36	64	43.5-82.8	100	-	36.1	36.1	55.6
				ANA		35	64	42.8-84.4	100	-	40	22.9	60
BURSTEIN	2014	J CLIN ONCOL	2	FULV LD + LAP	291	146	-	-	100 (physiologic or induced by GnRH a-logue or oophorectomy)	-	-	-	31 (bone only)
				FULV LD		145	-	-	100 (physiologic or induced by GnRH a-logue or oophorectomy)	-	-	-	30 (bone only)

BUZDAR	2002	BREAST CANC RES TREAT	2	DRO	1350	-	-	-	not all patients were postmenopausal	-	-	-	-
				TAM		-	-	-	not all patients were postmenopausal	-	-	-	-
LAM	2014	EUROP J CANCER	2	PAC+BEVA+CAPE	312	156	56	32-76	100	-	34	57	7 (bone only)
				PAC+BEVA		156	56	34-74	100	-	26	58	9 (bone only)
KAUFMAN	2015	J CLIN ONCOL	2	ERI	1102	554	54	24-80	-	84.3	50.4	44.6	54
				CAPE		548	53	26-80	-	88.1	51.1	49.5	56.2
CLEMENS	2014	BREAST CANC RES TREAT	2	YM155+DOCE	101	50	57	34-79	-	-	-	-	-
				DOCE		51	55	25-77	-	-	-	-	-
DIERAS	2014	THE BREAST	4	TREB10+PAC+BEVA	228	56	56.5	32.72	-	-	39	43	63
				TREB3+PAC+BEVA		57	56	26-83	-	-	42	54	63
				PAC+BEVA		58	51.5	31-74	-	-	31	45	62
				TREB10+PAC		57	52	27-76	-	-	42	44	54
BEEX	2006	EUROP J CANCER	3	Continuous TAM followed by Intermittent TAM	276	94	66.9	38.5-86.9	100	30.9	-	-	44.7
				Continuous TAM		93	65.2	43.6-84.8	100	36.6	-	-	26.9
				Continuous TAM followed by Intermittent TAM/MED AC		89	66.7	41-89.7	100	30.3	-	-	41.6
SMORENBURG	2014	ANN ONCOL	2	PLD	78	40	75	65-86	100	30 (visceral only)	38	55	8 (bone only)
				CAPE		38	75	65-86	100	32 (visceral only)	45	42	11 (bone olnly)
RUGO	2015	J CLIN ONCOL	3	NAB-PAC+BEVA		271	57	-	-	77	-	-	56
				PAC+BEVA		283	57	-	-	76	-	-	60
				IXA+BEVA		245	57	-	-	81	-	-	67

WELT	2016	BREAST CANC RES TREAT	2	CAP+BEV	592	297	60.6	28.9-85.1	-	78.1	30.3	47.8	50.2
				CAP+BEV+VINO		295	62.7	34.1-88.3	-	76.3	30.2	48.5	60
LLOMBART-CUSSAC	2011	CANCER	2	EXE	103	51	67.9	45-94	100	49	-	-	-
				ANA		52	72.6	46-85	100	55.8	-	-	-
IWATA	2013	BREAST CANC RES TREAT	2	EXE	298	149	63.4	44-95	100 (physiologic or induced by GnRH a-logue or oophorectomy)	50.3	-	-	26.8 (bone only)
				ANA		149	64.0	45-94	100 (physiologic or induced by GnRH a-logue or oophorectomy)	48.3	-	-	26.8 (bone only)
MARTIN	2015	J CLIN ONCOL	2	LETRO/FULV	374	184	66	39-86	100	47.8	37.5	19.6	64.1
				LETRO/FULV+BEVA		190	64	38-85	100	47.4	32.6	20.5	65.3
YARDLEY	2015	BREAST CANCER RES TREAT	2	PAC+BEVA+EVE	113	56	61	30-77	-	-	-	-	-
				PAC+BEVA		57	57	25-79	-	-	-	-	-
SCHMID	2005	J CLIN ONCOL	2	HD PBSCT	93	48	48.9	30-60	40.9	87.1	-	-	-
				AT		45	51.6	27-60	40.9	87.1	-	-	-
DICKLER	2016	J CLIN ONCOL	2	LETRO+AVA	343	173	56	25-85	100 (physiologic or induced by GnRH a-logue)	24	-	-	24
				LETRO		170	59	29-87	100 (physiologic or induced by GnRH a-logue)	24	-	-	25
BAJETTA	2002	CANCER	2	TAM+OPLAR	199	99	62.5	33-86	89	40.4	-	-	35.4
				TAM		100	59	29-82	84	42	-	-	34
GOSS	2007	J CLIN ONCOL	2	ATA+TORE	865	434	65	-	100	30	-	-	26
				LETRO		431	63	-	100	30	-	-	25

ADELSON	2014	NPJ B CANCER	2	FULV 500+BORT	118	59	57	31-83	100	-	-	37	-
				FULV 500		59	59	31-80	100	-	-	37	-
ARPINO	2002	ANN ONCOL	2	IDO	219	108	59.1	-	100	-	28.7	18.5	44.4
				TAM		111		-	100	-	32.4	14.4	56.8
HORTOBAGYI	2016	N ENG J MED	2	RIBO+TAM	668	334	62	23-91	100	59	-	-	20.7
				TAM		334	63	29-88	100	58.7	-	-	23.4
ZIELINSKY	2016	LANCET ONCOL	2	BEVA+PAC	531	266	59	-	81	65	40	40	55
				BEVA+CAP		265	59	-	80	73	44	45	54
ELLIS	2016	ESMO 2016	2	FULV 500	462	230	64	38-87	100	58.7	-	-	-
				ANA		232	62	36-90	100	51.3	-	-	-
DI LEO	2016	SABCS 2016	2	BUPA+FULV 500	432	289	60	32-84	100	73	-	-	-
				FULV 500		143	62	37-79	100	72	-	-	-
KORNBLUM	2016	SABCS	2	EVE+FULV 500	131	66	64	39-92	100	-	42	27	65
				FULV 500		65	59	35-85	100	-	34	26	71
ALBA	2004	J CLIN ONCOL	2	A-->T	144	75	58	36-78	81	77	-	-	-
				AT		69	61	31-75	84	73	-	-	-
ALBA	2010	BREAST CANCER RES TREAT	2	A-->T-->PLD	155	78	58	30-76	-	59	-	-	-
				A-->T-->OBSERVATION		77	55	34-78	-	56	-	-	-
BATIST	2001	J CLIN ONCOL	2	NPLD+CYCLO	297	142	55	30-80	-	71	49	35	11
				DOXO+CYCLO		155	54	22-88	-	61	42	35	16
BIGANZOLI	2002	J CLIN ONCOL	2	AP	275	138	52	29-70	-	85	-	-	6
				AC		137	54	28-70	-	81	-	-	9
BIRON	2008	BONE MARROW TRANSPL	2	FEC-->OBSERVATION	179	91	46.7	-	-	-	34.1	49.5	49.4

				FEC-->HDC+SCT		88	45.6	-	-	-	29.6	56.8	50.6
BLOHMER	2010	ANN ONCOL	2	EPI + CYCLO	236	111	56	31-73	53	-	-	-	-
				EPI + DOC		125	57	35-72	60	-	-	-	-
BONTENBAL	2005	J CLIN ONCOL	2	FAC	216	107	54	31-70	-	80	36	51	64
				DOXO+DOC		109	53	30-70	-	72	25	50	65
BUZDAR	2012		2	CAPE SD + DOC	470	235	51	22-75	-	-	-	-	-
				CAPE LD + DOC		235	51	28-75	-	-	-	-	-
CAPOTORTO	2005	JOUR-L OF CHEMOTHERAPY	3	FEC	135	44	58	34-70	64	30	-	-	5
				FEC G		45	51	31-67	62	21	-	-	2
				MMM G		46	54	33-69	60	24	-	-	4
CASSIER	2007	BREAST CANC RES TREAT	2	DOXO + DOC	210	107	56	32-79	45.7	-	45.8	55.1	7.5 (bone only)
				DOXO + PAC		103	58	32-79	47.1	-	50.5	48	11.7 (bone only)
CHAN	2004	ANN ONCOL	2	NPLD + CYCLO	160	80	54	19-78	-	61	-	-	-
				EPI + CYCLO		80	54	26-82	-	60	-	-	-
CINIERI	2016	CLIN BREAST CANCER	3	VNR + CAP	149	49	58	33-76	-	80	49	47	-
				GEM + PAC		50	56	29-78	-	82	52	50	-
				GEM + DOC		50	57	33-77	-	74	48	40	-
CORTES	2011	LANCET	2	ERIBULIN	762	508	55	28-85	-	-	39	58	60
				TREATMENT OF PHYSICIAN'S CHOICE (TPC)		254	56	27-81	-	-	37	63	62
DEL MASTRO	2001	J CLIN ONCOL	2	CEF	151	74	57	33-74	78	-	-	-	-
				HD-CEF q14		77	57	38-72	92	-	-	-	-

GRADISHAR	2005	J CLIN ONCOL	2	NAB-PAC 260 mg/m2	454	229	53.1	26-79	83	-	32	40	6
				PACLITAXEL		225	53.3	30-83	83	-	35	43	6
HARBECK	2016	BREAST CANC RES TREAT	2	PLD	210	105	62	36-82	83	-	-	-	-
				CAPE		105	63	22-85	85	-	-	-	-
HARRIS	2002	CANCER	2	NPLD	224	108	58	26-85	-	71	50	42	40
				DOXO		116	58	29-82	-	72	45	41	42
ICLI	2005	BRITISH J CANCER	2	CIS+VP-16	193	96	47	26-69	-	-	47	35	45
				PACLI		97	49	24-70	-	-	47	46	39
JASSEM	2001	J CLIN ONCOL	2	DOXO+PAC	267	134	50	33-70	-	64	-	-	11
				FAC		133	50	24-74	-	68	-	-	8
JOENSUU	2010	ANN ONCOL	2	DOC	237	115	55	31-69	-	-	34	44	65
				DOC / GEM		122	54	32-70	-	-	32	46	58
KELLER	2004	J CLIN ONCOL	2	PLD	301	150	56	33-87	54	63	-	-	10
				VNR or VNB + MITOC		151	56	30-83	56	66	-	-	10
MARTIN	2007	LANCET ONCOL	2	GEM+VNR	251	125	58	28-82	-	74	38	54	-
				VNR		126	57	35-80	-	75	46	51	-
MAVROUDIS	2009	ANN ONCOL	2	DOC + EPI	272	136	60.5	30-75	88.2	-	47.8	36.7	36
				DOC + CAPE		136	63	31-75	86.8	-	49.2	37.5	44.1
MAYER	2010	ANN ONCOL	2	PAC+BEVA	46	23	52	29-80	-	-	-	-	-
				PAC+SUN+BEVA		23	58	34-81	-	-	-	-	-
MILES	2016	EUROP J CANCER	2	PAC	481	242	56	28-77	-	-	-	-	-
				PAC + BEVA		239	55	28-85	-	-	-	-	-
NABHOLTZ	2003	J CLIN ONCOL	2	DOXO + CYC	429	215	54	28-75	-	63	35	33	53
				DOXO + DOC		214	52.5	30-76	-	61	28	29	55

O'SHAUGNESSY	2002	J CLIN ONCOL	2	DOC+CAP	511	255	52	26-79	-	-	37	45	42
				DOC		256	51	25-75	-	-	39	48	46
PARK	2013	J CLIN ONCOL	2	PAC + GEM	231	116	48	30-70	48.3	-	54.3	35.3	45.7
				OBSERVATION		115	47	29-76	52.2	-	43.5	29.6	43.5
RIVERA	2008	CANCER	2	DOC q3w	125	62	56	36-82	-	-	-	-	-
				DOC q3/4w		63	54	32-86	-	-	-	-	-
ROCHLITZ	2016	BMC CANCER	2	PAC+BEVA	147	73	64	30-82	-	-	35.2	57.7	73.2
				CAPE+CYCLO+BEVA		74	62	29-81	-	-	48.5	54.4	72.1
RUGO	2011	J CLIN ONCOL	2	AXI + DOC	168	112	55	30-79	-	-	-	-	-
				DOC		56	56	34-71	-	-	-	-	-
STEMMLER	2010	ONCOLOGY	2	DOC qw + DOXO	85	43	54	29-70	74	-	47	49	49
				DOC q3w + DOXO		42	56	39-70	83	-	33	55	36
VUYLSTEKE	2016	ANN ONCOL	2	PIC+PAC	183	91	55	30-78	-	69.2	-	-	14.3 (bone only)
				PAC		92	58	33-80	-	59.8	-	-	20.7 (bone only)
SLEDGE	2017	J CLIN ONCOL	2	ABE + FULV 500	669	446	59	32-91	100 (premenopausal women were given a GnRH a-logue)	-	-	-	-
				FULV 500		223	62	32-87	100 (premenopausal women were given a GnRH a-logue)	-	-	-	-
TAMURA	2017	CANCER SCI	2	NAB-PAC q3/4w	197	98	60	25-74	-	-	50	37.8	44.9
				DOC		99	58	33-74	-	-	42.4	46.5	53.5

ZHANG	2013	CANCER CHEMOTHER PHARMACOL	2	VNR+CAPE	60	30	52	-	57	73	-	-	-
				VNR --> CAPE		30	50	-	43	67	-	-	-
FINN	2016	N ENGL J MED	2	PALBO+LETRO	666	444	62	30-89	1	48.2	-	-	23.2 (bone only)
				LETRO		222	61	28-88	1	49.5	-	-	21.6 (bone only)
MARTIN	2016	ANN ONCOL	2	BUPA+PAC	416	207	55	25-84	-	72.9	32.9	42	56.5
				PAC		209	56	24-78	-	78.0	36.8	41.6	57.4
GOETZ	2017	J CLIN ONCOL	2	LETRO or ANA + ABE	493	328	63	38-87	100 (physiologic or induced by GnRH a-logue)	-	-	-	-
				LETRO or ANA		165	63	32-88	100 (physiologic or induced by GnRH a-logue)	-	-	-	-
JERUSALEM	2018	JAMA ONCOL	3	EVE + EXE	309	104	61	32-86	100	66	42	53	85
				EVE		103	61	38-88	100	64	41	54	77
				CAPE		102	60	35-84	100	62	41	53	83
SLAMON	2018	J CLIN ONCOL	2	RIBO + FULV 500	726	484	63	31-89	100	60.5	30.2	27.7	75.8
				FULV 500		242	63	34-86	100	60.3	29.8	26.0	74.4
ANDRE'	2018	ESMO 2018	2	ALP + FULV 500	341	169	63	25-87	100	55.0	-	-	24.9 (bone only)
				FULV 500		172	64	38-92	100	58.1	-	-	20.3 (bone only)

Legend. MET = metastases; qw = weekly; q3w = every 3 weeks; ABE = abemaciclib; FULV 500 = fulvestrant, standard dose; FULV 250 = fulvestrant 250 mg without loading dose; FULV LD = fulvestrant 250 mg with loading dose; ANA = anastrozole; LETRO = letrozole, standard dose 2.5 mg; LETRO 0.5 = letrozole 0.5 mg; ATA = atamestane; TOR: toremifene; ALP= alpelisib; AXI = axitinib; DOC = docetaxel; BEVA = bevacizumab; CAP= capecitabine; CYC = cyclophosphamide; VINO = vinorelbine; BUPA = buparlisib; PAC = paclitaxel; SOR: sorafenib; SUN = sunitinib; CED = cediranib; LD = low dose; BMF = bendamustine + methotrexate + 5-fluorouracil; CMF = cyclophosphamide + methotrexate + 5-fluorouracil; DOX = doxorubicin; EPI = epirubicin; MITOX = mitoxantrone; CIS = cisplatin; ETO = etoposide; DASA = dasatinib; NPLD = non-peghylated liposomal doxorubicin;

GEM = gemcitabine; IDO = idoxifene; ENT = entinostat; EXE = exemestane; ERI = eribulin; EVE = everolimus; TAM = tamoxifen; FEC/CEF = 5-fluorouracil + epirubicin + cyclophoshpamide; BORT = bortezomib; VAN = vandetanib; GEF = gefitinib; LAP= lapatinib; GANI = ganitumab; Int = intensive; IXA = ixabepilone; MA = megestrol acetate; NAB-PAC = nab paclitaxel; TEM = temsirolimus; OBS = observation; OPLAR = octreotide pamoate long acting release; CARBO = carboplatin; MOT = motesanib; PALBO = palbociclib; PIC = pictilisib; PLD = peghilated liposomal doxorubicin; RIBO = ribociclib; TPC = treatment of physician's choice; TREB = trebananib; VINBLA = vinblastine; SELU = selumetinib; → = followed by; TI = time intensive; MMM = mitoxantrone + mitomycin C + methotrexate; DRO = droloxifene; HDC = high dose chemotherapy; SCT = stem cell transplant; NA = not available; FAC = 5-fluorouracil + doxorubicin + cyclophosphamide

Table S3. Most frequent WHO grade 3-5 adverse reactions in most used mono-chemotherapies

ADVERS REACTIONS (ADRs) WHO GRADE 3-5	MONO-CHEMOTHERAPIES										
	DOX	PAC Q3W	PAC QW	NAB-P 260 Q3W	NAB-P 150 QW	NAB-P 100 QW	GEM	CAPE	VNR	ERI	DOC
Nonhematologic events	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**
Abdominal pain	1.0	-	2.5	-	-	-	-	0.0-2.0	0.8 - 13.0	-	0.0
Allergic reactions	-	-	-	0.0	-	-	-	-	-	-	0.0 - 1.6
Alopecia	0.0 - 24.1	22.0- 100.0	-	-	0.0	0.0	55.2	0.0	0.8 - 17.0	0.0	0.0 - 44.0
Anorexia/Decreased appetite	0.4 - 3.3	-	0.3	-	3.0	-	-	0.4-1.6	0.8	0.2 - 0.6	0.0 - 3.0
Arthralgia	-	2.7 - 3.0	0.0 - 1.4	-	0.0 - 1.0	0.0	-	0.0 - 1.0	-	0.2	0.0

Asthenia/Muscle weakness	1.0 - 19.0	5.0	3.0	-	-	-	-	0.8-3.7	4.0	4.2 - 9.0	0.0 - 20.7
Back pain	-	-	-	-	-	-	-	0.4-0.5	-	1.2 - 1.5	2.0
Bone pain	-	-	-	-	-	-	-	0.9	2.0	2.0	2.0
Cardiac dysfunction/infarction/Coronary thrombosis	0.0	1.0	0.3	-	-	-	-	-	-	-	-
Cardiotoxicity	-	1.0 - 3.0	-	-	-	-	1.6	0.0	-	-	0.0
Congestive Heart Failure	-	-	0.4	-	-	-	-	-	-	-	0.3
Constipation	0.4 - 1.3	-	0.5	-	1.0	-	1.0	0.0 - 0.3	1.6 - 4.0	1.0	0.0
Deep venous thrombosis	-	-	-	-	-	-	-	-	-	-	0.0
Dehydration	-	-	-	-	-	-	-	-	-	-	2.0
Diarrhea	0.8 - 4.0	0.5-3.0	2.5 - 5.0	-	0.0	-	0.5	0.0-13.0	0.0	0.0 - 1.1	0.0 - 7.0
Disseminated intravascular disease	-	-	-	-	-	-	-	3.8	-	2.2	-
Dyspnea	-	1.0-4.0	7.0	-	-	-	-	2.0-7.0	0.0 - 6.0	4.0	21.2
Edema (not specified)	0.0	0.5-1.0	6.0	-	3.0	-	-	-	0.8	-	2.0 - 6.8
Epistaxis	-	-	0.0	-	-	-	-	0.0	-	-	0.0

Fatigue/malaise	2.0	1.2 - 8.0	3.0-6.0	-	1.0 - 3.0	0.0	-	1.0-13.0	2.0 - 17.0	2.0	0.0 - 25.0
Febrile neutropenia	9.0 - 10.0	1.2 - 7.0	0.4	<2.0	-	-	-	0.0-0.9	13.0	2.1	3.9 - 24.7
Fever / Pyrexia	1.1	-	0.0	-	0.0	-	-	0.0-0.5	2.0	0.2 - 0.4	0.0 - 2.2
Gastrointestinal	-	-	-	-	-	-	-	-	-	-	0.9
GU Infection	-	-	-	-	-	-	-	-	0.0	-	-
Hand-foot skin reaction/hand foot syndrome/PPE	0.0	-	-	-	-	-	-	4.0-26.0	0.0	0.0 - 0.2	1.0 - 3.9
Hepatic Insufficiency	-	-	-	-	-	-	-	-	-	-	-
Hepatic toxicity	-	-	-	-	-	-	-	-	-	-	-
Hepatomegaly	-	-	-	-	-	-	-	-	-	-	-
Hip fracture	-	-	-	-	-	-	-	-	-	-	-
Hypertension	-	-	4.3	-	-	-	-	1.0-2.0	-	-	0.0 - 1.8
Ileus	-	-	-	-	-	-	-	-	-	-	-
Immunology/lymphatics	-	-	2.9-6.0	-	-	-	-	-	-	-	-
Infection	2.0 - 12.0	1.8 - 7.0	-	-	-	-	3.6	4.0	6.0 - 8.0	-	2.0 - 11.5
Injection-site pain	-	-	-	-	-	-	-	0.0	0.0 - 2.0	-	-
Left ventricular systolic dysfunction	-	-	-	-	-	-	-	-	-	-	-

Lung/Lung function	-	-	-	-	-	-	-	-	-	-	-
Mouth disorders	-	-	-	-	-	-	-	-	-	1.0	-
Mucosal inflammation/Mucositis	-	0.0	-	-	-	-	7.8	1.0 - 4.0	0.0	1.0	0.0 - 2.0
Musculoskeletal pain/chest pain/Myalgia	-	2.0 - 3.0	-	-	1.0	-	-	0.3 - 4.2	0.0 - 2.0	-	-
Nail disorder	-	-	-	-	0.0	-	-	0.0	-	-	0.0 - 1.8
Nausea / Vomiting	5.0 - 24.0 / 4.0 - 24.0	0.0 - 5.0 / 0.0 - 3.0	1.0 - 3.2 / 1.5 - 3.2	-	1.0 / NA	-	7.3	1.0-7.0 / 2.0-6.0	0.0 - 7.0 / 0.0 - 3.0	0.2 - 1.0 / 0.4 - 1.2	0.0-8.6 / 0.0 - 8.6
Neurologic	-	1.0	-	-	-	-	-	-	-	-	-
Neurologic, sensory	0.0	2.0 - 12.0	3.0-24.0	10.0	14.0	8.0	-	0.0 - 0.8	0.0 - 2.0	-	1.3 - 12.2
Neurologic, motor	0.0	0.0 - 5.0	9.0	0.0	-	-	-	0.0 - 0.3	1.6	-	0.0 - 1.1
Pain	-	1.0-6.4	1.2	-	-	-	-	-	3.0	-	1.0 - 14.2
Paraesthesia/Peripheral neuropathy	-	-	2.0	-	22.0	-	-	0.0 - 1.2	0.8	7.0 - 8.2	3.9 - 10.0
Peripheral edema	-	-	0.0	-	-	-	-	-	-	-	-

Pleural effusion	-	-	-	-	-	-	-	-	-	-	0.0
Polyneuropaty	-	-	-	-	-	-	-	-	-	-	0.0
Pulmonary embolism	-	-	-	-	-	-	-	0.0	-	-	0.0
Pulmonary other	-	-	-	-	-	-	3.2	-	-	-	0.7 - 2.2
Renal failure acute	-	-	-	-	-	-	-	-	-	-	-
Sepsis	-	-	-	-	-	-	-	-	-	-	-
Skin reaction/Rash	0.0 - 1.0	-	1.0	-	1.0	-	1.0	0.0	0.0	-	0.0 - 4.5
Stomatitis	2.0 - 14.0	0.4-1.0	0.0 - 0.6	-	1.0	-	-	1.0-8.0	0.0 - 2.0	-	0.0 - 10.8
Syncope	-	-	-	-	-	-	-	-	-	-	2.0
Thrombosis or embolism	-	-	1.5	-	-	-	-	-	-	-	0.0 - 3.0
Toxic death	-	3.1	-	-	-	-	-		-	-	-
Upper respiratory tract infections	-	-	-	-	-	-	-	-	-	-	-
Venous thromboembolism	-	-	1.3	-	-	-	-	-	-	-	3.1
Hematologic events and proteinuria											
Decreased hemoglobin/anemia	1.4 - 26.0	0.0 - 7.3	3.0	-	5.0	-	5.7	1.1 - 5.0	5.0	2.0 - 2.2	0.9 - 13.5

Thrombocytopenia	0.4 - 10.0	0.0 - 2.8	0.3	-	-	-	1.5	0.0 - 4.0	2.0	-	0.0 - 5.4
Neutropenia	8.0 - 86.0	11.0 - 60.0	0.3-44.0	9.0	44.0 - 78.0	25.0	17.9	2.0 - 11.0	10.0 - 46.0	45.0 - 45.7	13.1 - 99.1
Neutrofilia	-	-	-	-	-	-	-	-	-	-	-
Leukopenia	9.0	6.0 - 32.0	-	-	58.0	-	19.3	0.9 - 7.0	-	14.0 - 15.1	4.3 - 90.0
Proteinuria	-	-	0.4	-	78.0	-	-	-	-	-	0.0
Lymphocytopenia	-	72.0	-	-	-	-	-	-	-	-	2.8
Hematologic	-	-	-	-	-	-	-	-	-	-	-
Biochemical changes											
Bilirubin/Hyperbilirubinemia	-	-	-	-	-	-	0.5	4.9	1.6	-	-
AST/SGOT	-	-	-	-	-	-	-	-	6.0	-	-
ALT/SGPT	-	0.4	0.5	-	-	-	-	0.5	2.8	3.3	3.9
ALT and or AST elevation	-	-	-	-	-	-	2.6	-	-	-	-
GGT elevation	-	-	-	-	-	-	-	-	3.0	-	0.0
ALP elevation	-	-	-	-	-	-	1.1	-	2.8	-	-
Azotemia	-	-	-	-	-	-	-	-	-	-	-
Creatinine	-	-	-	-	-	-	-	-	-	-	-
Hyperglycemia	-	8.0 - 22.0	0.5 - 5.0	-	-	-	-	-	-	-	-
Hypokaliemia	-	-	-	-	-	-	-	2.0	-	0.9	-

Legend. DOX = doxorubicin; PAC = paclitaxel; Q3W = every 3 weeks; QW = once weekly; NAB-P= nab paclitaxel; GEM = gemcitabine; CAPE = capecitabine; VNR = vinorelbine; ERI = eribulin; DOC = docetaxel Q3W

Table S4. Most frequent WHO grade 3-5 adverse reactions in most used poli-chemotherapies and chemotherapies + target therapy

ADVERSE REACTIONS (ADRs) WHO GRADE 3-5	POLI-CHEMOTHERAPIES (INCLUDING TARGET THERAPIES)																
	CMF	FEC	FAC	NPLD + CYC	AC	EC	VNR + CAPE	DOC + CAPE	DOC + GEM	PAC Q3/4W + BEVA	PAC + GEM	ET	ED	AT	AD	CARBO + GEM	CARBO + PAC
Nonhematologic events	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**
Abdominal pain	-	-	-	-	-	-	4.5 -6.0	2.1	0.0	-	2.0	-	-	-	-	-	-
Allergic reactions	-	-	-	-	0.0	-	-	1.0	-	4.0	2.0 - 2.8	2.0	0.0	-	1.4	0.0	-
Alopecia	1.1-14.0	33.3 - 95.8	-	62.0	-	-	0.0	0.0 - 6.0	-	0.0-1.0	0.0 - 2.0	71.0 - 100.0	-	-	0.0	2.1	67.0
Anorexia/Decreased appetite	8.1	-	-	-	-	-	0.0 - 4.0	0.0	0.0	0.0-0.6	0.0 - 0.8	-	-	-	0.0	-	-
Arthralgia	1.6	-	0.0	-	0.0	-	0.0	0.0	-	0.0-3.2	-	6.0	-	7.0 - 10.0	0.0	-	-

Asthenia/Muscle weakness	0.0	2.8 - 5.6	-	0.0 - 6.0	2.0 - 5.0	1.0	0.0	2.0 - 8.4	-	0.0-5.0	2.0	-	1.0	6.5	5.0 - 15.9	-	-
Back pain	-	-	-	-	-	-	4.0	-	2.0	3.0	0.0	-	-	-	-	-	-
Bone pain	-	8.3	-	-	-	-	0.0	-	2.0	1.0	4.0	-	-	-	-	-	-
Cardiac dysfunction/infarction /Coronary thrombosis	-	2.0	-	-	-	-	-	-	-	0.8-1.1	-	-	-	-	-	-	-
Cardiotoxicity	1.1	0.0 - 3.1	0.8 - 22.0	0.0	-	-	0.0	0.0	-	-	-	0.0 - 6.8	-	0.0 - 2.6	0.0 - 35.0	-	-
Congestive Heart Failure	-	-	6.0	-	1.0	0.9	-	-	-	0.4	-	-	0.8	2.0	3.0	-	-
Constipation	1.6	-	-	1.0	-	-	0.0	2.1	-	0.0	0.0	-	-	-	0.0 - 5.8	0.0	-
Deep venous thrombosis	-	-	-	-	-	-	6.0	-	4.0	-	0.0 - 2.0	-	-	-	-	0.0	-
Dehydration	-	-	-	-	-	-	4.0	-	2.0	-	2.0	-	-	-	-	-	-
Diarrhea	1.6-5.5	0.0-1.0	0.0 - 0.8	1.0 - 3.0	1.0 - 8.0	0.0 - 1.0	0.0 - 9.1	1.0 - 14.4	0.0 - 4.0	0.0-4.0	0.9 - 6.0	0.6-5.0	0.0 - 5.6	0.0 - 2.0	0.0 - 10.1	-	-
Disseminated intravascular disease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dyspnea	9.7	-	-	-	-	-	4.0	-	4.0	1.3-2.0	0.0 - 10.0	1.0	-	-	-	2.1	-
Edema (not specified)	0.5	-	0.8	-	0.0	-	-	-	-	-	-	-	-	1.0	0.0 - 2.8	-	-
Epistaxis	-	-	-	-	-	-	-	-	-	0.0 - 4.0	0.0	-	-	-	-	0.0	-

Fatigue/malaise	0.0-6.4	-	-	6.0	5.0	-	0.9 - 12.0	1.9 - 8.3	24.0	0.0-10.0	2.0 - 20.0	4.2-6.0	-	-	-	0.0	-
Febrile neutropenia	8.0-10.1	11.0	9.0	5.0 - 9.0	9.0 - 13.0	1.0 - 5.4	1.9 - 23.3	1.9 - 16.0	6.0 - 8.3	0.8-4.0	0.0 - 3.0	3.6-17.0	4.8 - 11.0	21.4 - 32.0	22.0 - 48.6	0.0	-
Fever/Pyrexia	0.5	1.4	0.0 - 4.0	-	-	-	0.0	0.0	-	-	0.0	-	-	8.0	5.8 - 21.4	-	-
Gastrointestinal	0.5	-	0.0	-	-	-	0.9	4.8	-	-	-	-	-	-	2.4	-	-
GU Infection	-	-	-	-	-	-	2.0	-	0.0	-	6.0	-	-	-	-	-	-
Hand-foot skin reaction/hand foot syndrome/PPE	0.0	-	-	-	-	-	4.5 - 8.0	4.0 - 24.0	0.0	0.0-0.3	0.0	1.0	0.0	-	-	-	-
Hepatic Insufficiency	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	2.1	-
Hepatic toxicity	-	3.0 - 3.1	2.6	-	-	-	-	0.0	5.6	-	-	-	-	0.4	-	-	-
Hepatomegaly	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	2.1	-
Hip fracture	-	-	-	-	-	-	4.0	-	0.0	-	0.0	-	-	-	-	-	-
Hypertension	1.6	-	-	-	-	-	-	-	-	1.1-19.0	-	-	-	-	-	-	-
Ileus	-	-	-	-	-	-	6.0	-	0.0	-	0.0	-	-	-	-	-	-
Immunology/lymphatics	1.1	-	-	-	-	-	-	0.9	-	-	-	-	-	-	-	-	-
Infection	3.2-8.3	0.0-12.4	0.0 - 9.0	7.0 - 11.0	2.0 - 8.0	1.0 - 6	0.9 - 4.0	-	6.0	4.6-9.3	0.0 - 0.8	0.0 - 13.0	7.2	2.0 - 7.0	2.0 - 16.7	-	2.0
Injection-site pain	-	-	-	0.0	-	1.0	-	-	-	-	-	-	-	-	-	-	-

Left ventricular systolic dysfunction	-	-	-	-	14.0	-	-	-	-	-	-	-	-	27.0	-	-	-
Lung/Lung function	-	7.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mouth disorders	-	-	-	-	-	-	-	0.0 - 5.6	-	-	-	-	-	-		-	-
Mucosal inflammation/Mucositis	15.0	0.8-12.0	5.3	-	-	-	0.0	0.0 - 5.6	8.3	0.0	-	2.0-6.0	0.0	1.3	4.7 - 7.2	-	-
Musculoskeletal pain/chest pain/Myalgia	-	-	0.0	-	0.0	-	2.0 - 2.3	2.1	2.0	0.0	4.0 - 8.0	-	-	7.0	0.0	-	-
Nail disorder	-	-	-	-	0.0	0.9	0.0	0.0	-	3.0-3.1	-	-	0.0	-	0.5	-	-
Nausea/Vomiting	0.9-14.0 / 2.7-14.0	15.3-48.0	3.5 - 19.0 / 7.0 - 19.0	2.0 - 21.0	6.0 - 18.0 / 6.0 - 18.0	5.4 - 19.0 / 6.3 - 19.0	0.0 - 8.0 / 0.0 - 10.0	0.0 - 4.0 / 0.0 - 4.0	2.0 / 2.0	0.0-6.0 / 0.0-6.0	0.0 - 4.0 / 0.0 - 2.0	3.0-10.0	0.7 - 10.4 / 0.7 - 5.6	1.9 - 8.0 / 1.0 - 8.0	4.3 - 13.0 / 2.0 - 14.0	2.1 / 0.0	-
Neurologic	1.1	-	0.0	-	-	-	0.0	0.0	-	-	-	-	0.0	12.0	0,0 - 4.3	-	-
Neurologic, sensory	-	4.0	0.0	-	0.0	-	0.0 - 4.0	4.2	2.0	2.0-23.5	2.0 - 6.0	2.4-10.0	-	3.0	0.0 - 2.0	0.0	5.0
Neurologic, motor	-	-	0.0	-	-	-	-	-	-	3.0	-	-	-	-	5.0	-	-
Pain	-	9.0	-	-	-	-	-	-	-	0.6-4.0	0.0	4.2-14.0	-	-	7.2	-	1.0

Paraesthesia/Peripheral neuropathy	-	-	-	0.0	-	0.0	0.0 - 2.0	0.0 - 2.1	0.0	9.0-25.0	2.0	0.0 - 3.9	0.0	7.8	0.9	-	-
Peripheral edema	-	-	-	-	-	-	0.0	0.0	6.0	2.0	0.0 - 0.8	-	-	-	-	0.0	-
Pleural effusion	-	-	-	-	-	-	2.0	-	2.0	-	6.0	-	-	-	-	-	-
Polyneuropaty	-	-	-	-	-	-	-	-	-	4.0	-	-	-	-	-	-	-
Pulmonary embolism	-	-	-	-	-	-	6.0	-	2.0	0.6 - 4.0	0.0	-	-	-	-	-	-
Pulmonary other	0.3	-	-	-	-	-	-	-	-	-	-	-	-	0.8	-	-	-
Renal failure acute	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	2.1	-
Sepsis	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	2.1	-
Skin reaction/Rash	2.2	3.0	-	0.0	0.0 - 1.0	0.0 - 1.0	0.0	0.0 - 2.8	0.0	0.0-2.0	0.0	-	0.8	-	0.0	-	-
Stomatitis	3.0-5.5	1.0-18.3	1.0 - 2.0	4.0 - 7.0	7.0 - 9.0	0.0	0.0 - 4.5	2.1 - 17.4	2.0	0.0-2.1	0.0 - 0.8	-	4.0	0.8 - 10.0	7.0 - 12.0	-	-
Syncope	-	-	-	-	-	-	2.0	-	0.0	0.0	6.0	-	-	-	-	-	-
Thrombosis or embolism	1.1	-	-	-	-	-	-	-	-	2.1	-	4.0	-	-	-	-	-
Toxic death	-	-	0.0	-	1.0	-		5.0	-	-	-	-	-	0.0	2.0	-	-
Upper respiratory tract infections	-	-	-	-	-	-	-	-	-	9.0	-	-	-	-	-	-	-
Venous thromboembolism	2.2	-	-	-	-	-	-	-	-	0.0 - 3.8	-	-	-	-	-	-	-

Hematologic events and proteinuria																	
Decreased hemoglobin/anemia	3.7-9.0	1.0-12.0	4.4 - 7.0	3.0 - 25.0	27.0	6.3 - 14.0	0.0 - 5.0	0.7 - 5.6	2.8 - 8.0	1.0-6.3	0.9 - 6.1	1.8 - 7.3	0.7 - 2.5	4.9 - 9.0	0.0 - 8.4	14.9	1.0
Thrombocytopenia	2.8-6.0	1.4-7.0	1.7 - 3.0	2.0 - 22.0	8.0 - 20.0	3.0 - 9.9	0.0 - 3.3	0.0 - 2.8	2.7 - 6.0	0.0-2.0	0.0 - 4.1	2.0 - 3.0	0.7 - 4.0	1.0 - 8.1	1.4 - 4.7	2.1	3.0
Neutropenia	25.7-68.0	15.0-83.9	7.0 - 84.0	61.0 - 87.0	75.0 - 88.0	67.0	10.0 - 83.3	16.0 - 83.4	13.9	0.9 - 28.0	32.7 - 61.2	19.0 - 79.1	57.0 - 70.0	89.0	13.0 - 97.0	14.9	13.0
Neutrofilia	-	-	-	-	-	-	50.0		86.0	-	46.0	-	-	-	-	-	-
Leukopenia	4.6-58.0	4.2-66.0	77.8	16.0	-	73.9	11.7 - 70.0	8.0 - 9.0	-	0.0 - 9.4	14.3 - 47.0	18.0 - 40.6	80.8	26.2 - 41.5	2.9 - 61.9	4.3	2.0
Proteinuria	-	-	-	-	-	-	-	-	-	0.0 - 4.0	-	-	-	-	-	-	-
Lymphocytopenia	-	-	-	-	-	-	-	-	-	-	8.2	20.4	-	-	-	-	-
Hematologic	-	-	-	-	-	-	-	-	-	22.0	-	-	-	-	-	-	-
Biochemical changes																	
Bilirubin/Hyperbilirubinemia	0.0	-	-	-	-	-	0.0	0.9	-	-	0.0	-	-	-	0.0	-	-
AST/SGOT	-	-	-	-	-	-	-	-	-	2.0	0..8 - 2.0	-	-	-	-	0.0	-
ALT/SGPT	-	-	-	-	-	-	-	0.9	-	-	0.0 - 8.2	-	-	-	-	2.1	-
ALT and or AST elevation	-	-	-	-	-	-	0.9	-	-	-	-	-	-	-	-	-	-
GGT elevation	-	-	-	-	-	-	-	-	-	-	4.1	-	-	-	16.7	0.0	-

ALP elevation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Azotemia	-		-		-	-	-	-	-	-	4.3	-	-	-	-	-	-
Creatinine	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	2.4	-	-
Hyperglycemia	-	-	0.8	-	-	-	-	-	-	-	0.8	-	-	-	-	-	-
Hypokaliemia	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-

Legend. CMF = cyclophosphamide + methotrexate + 5-fluorouracil; FAC = 5-fulorouracil + doxorubicin + cyclophosphamide; FEC = 5-fluorouracil + epirubicin + cyclophosphamide; NPLD = non-peghylated liposomal doxorubicin; CYC = cyclophosphamide; PAC = paclitaxel; Q3/4W = every 3 out of 4 weeks; GEM = gemcitabine; CAPE = capecitabine; VNR = vinorelbine; CARBO = carboplatin; BEVA = bevacizumab; ET = epirubicin + paclitaxel; ED = epirubicin + docetaxel; AT = doxorubicin + paclitaxel; AD = doxorubicin + docetaxel; AC = doxorubicin + cyclophosphamide; EC = epirubicin + cyclophosphamide.

Table S5. Most frequent WHO grade 3-5 adverse reactions in most used hormone therapies

ADVERSE REACTIONS (ADRs) WHO GRADE 3-5	ENDOCRINE THERAPIES						
	TAM	ANA + FULV LD	ANA	LETRO	EXE	MEG AC	FULV
Nonhematologic events	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**
Anorexia/Decreased appetite	0.3-4.0	0.4	0.0	0.0-1.0	1.0	-	0.0-1.0
Arthralgia/Arthropathy/Joint pain	0.0-2.0	1.0	0.0	0.0-3.0	0.0-3.0	-	0.0
Asthenia/Muscle weakness	0.0-1.0	0.0	0.3-3.8	0.0-2.0	0.0-3.9	-	0.0-1.0
Back pain	4.0	0.4	-	0.0 - 1.0	0.1-2.0	-	2.0
Bone pain	1.5-5.0	1.0	3.9-5.7	0.0	1.0	-	1.0
Cardiotoxicity	-	0.2	0.0-2.9	-	-	-	-
Chest pain	2.0	0.4	-	-	0.0	-	0.0
Constipation	1.0	1.0	0.0-5.9	0.0-0.3	0.1-2.0	-	0.0
Diarrhea	0.0	0.4	0.0	0.0-1.4	0.0-2.0	-	0.0-1.1
Dyspnea	0.6-2.6	0.4	2.9-7.7	1.0-3.0	0.0-1.6	-	1.0
Fatigue/malaise	0.0-11.0	0.4	0.0-4.0	0.0 - 0.9	0.0-3.0	-	0.0-5.0
Flu-like symptoms/Flu syndrome	-	2.8	0.0-5.2	0.0	-	-	-
Hypertension	3.2	1.0	0.0-2.1	2.0	0.0-3.3	-	1.0-9.1
Infection	-	0.0	0.0-5.9	-	0.0-1.1	-	0.0-3.0
Lethargy	-	1.0	-	-	5.0	-	-
Pain	9.0-18.0	0.0-0.2	0.0-0.6	1.0	1.0-6.5	-	1.0-4.5
Pleural effusion	1.0-2.5	-	-	0.0	0.0	-	1.0

Hematologic events and proteinuria							
Anemia	1.1 - 4.0	-	2.7	0.0-1.8	0.7-4.0	-	1.5-2.0
Thrombocytopenia	0.5	-	-	-	0.7-2.0	-	0.0
Neutropenia	5.0	-	0.0-1.3	0.0-1.5	0.0-1.1	-	1.0
Leukopenia	0.6	-	0.7	0.0 - 1.5	0.7	-	2.0
Lymphocytopenia	4.0	-	2.0	3.9	0.7	-	1.0
Hematologic	-	2.0	1.5	0.0	0.7	-	-
Biochemical changes							
Bilirubin/Hyperbilirubinemia	1.6	-	0.0	-	3.3	-	1.0
AST/SGOT	4.8	-	0.0-1.3	1.2	1.0-4.9	-	2.0-3.0
ALT/SGPT	4.2	-	0.0	1.2	2.0-7.7	-	0.0-2.1
GGT elevation	-	-	0.0-7.3	-	-	-	-
ALP elevation	-	-	0.0-2.9	-	-	-	-
Hypercalcemia	0.6	-	0.0-2.9	-	-	-	0.0
Hyperglycemia	0.0	-	5.4	1.0	-	-	0.0-1.0
Hypoalbuminemia	-	-	6.0	-	-	-	-
Hormonal/Endocrine tox	-	2.0	0.3	-	-	-	-

Legend. TAM = tamoxifen; ANA = anastrozole; FULV = fulvestrant standard dose; FULV LD = fulvestrant 250 mg with loading dose; LETRO = letrozole; EXE = exemestane; MEG AC = megestrol acetate.

Table S6. Most frequent WHO grade 3-5 adverse reactions in most used hormone therapies + target therapies

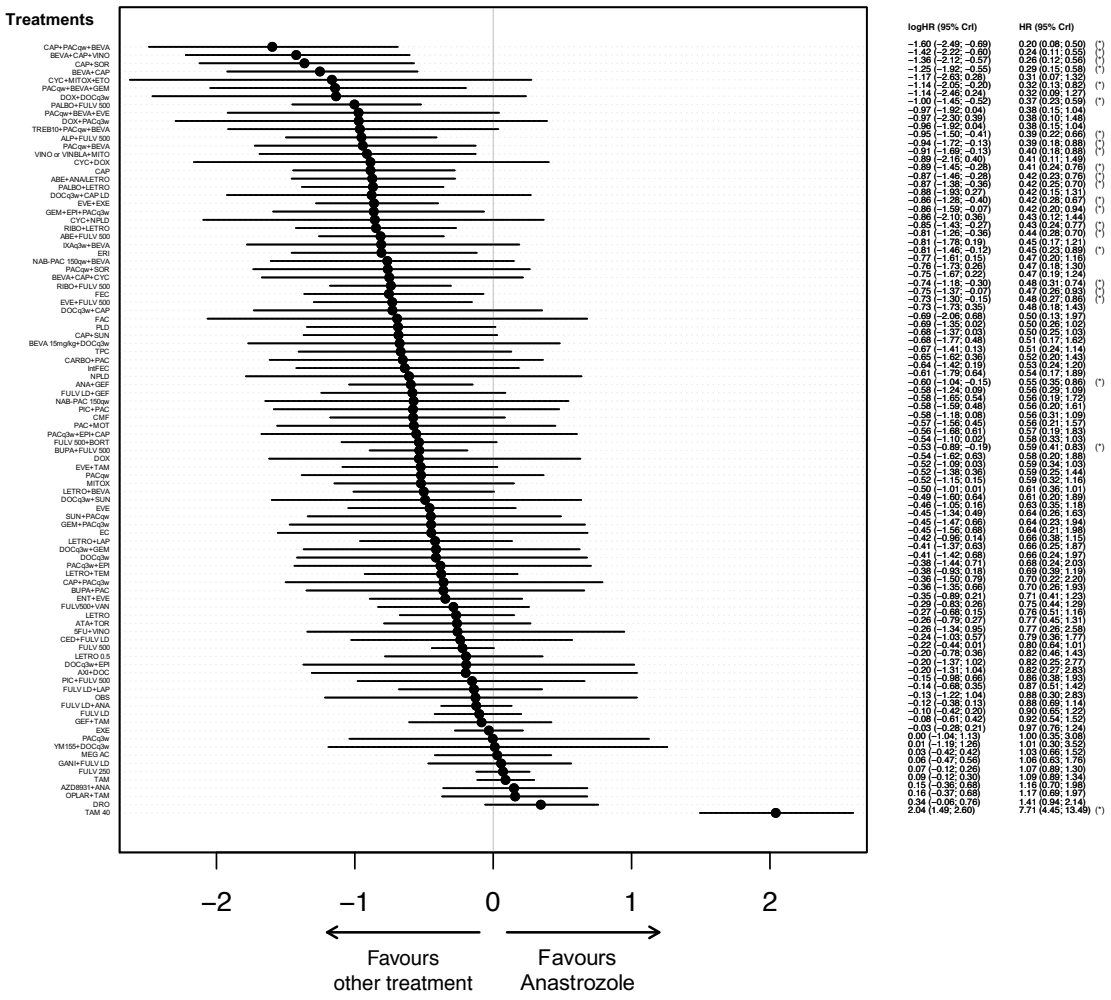
ADVERS REACTIONS (ADRs) WHO GRADE 3-5	ENDOCRINE + TARGET THERAPIES							
	PALBO + LETRO	RIBO + LETRO	ABE + ANA/LETRO	EVE + EXE	PALBO + FULV	RIBO + FULV	ABE + FULV	ALP + FULV
Nonhematologic events	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**	**% pt**
Abdominal pain	0.9	-	1.2	-	1.0	-	2.5	-
Asthenia/Muscle weakness	2.0-2.3	-	-	3.0	-	-	-	1.8
Back pain	1.0 - 1.4	2.1	-	-	1.0	1.7	0.7	-
Bone pain	2.0	-	-	-	1.0	-	-	-
Diarrhea	1.4 - 4.0	1.2	9.5	2.0	0.0	0.6	13.4	6.7
Dyspnea	1.1 - 2.0	-	-	4.0	0.2	-	2.7	-
Fatigue/malaise	1.8	2.4	1.8	3.0	2.0	1.7	-	3.5
Hypertension	-	-	-	-	2.0	-	-	-
Infection	-	4.2	-	-	2.2	-	-	-
Nausea / Vomiting	0.2 - 2.0 / 0.0 - 0.5	2.4 / 3.6	1.2 / 1.9	-	0 / 0.2	1.4 / 1.4	2.7 / 0.9	2.5 / 0.7
Rash	0.9	0.6	-	1.0	1.0	0.4	1.1	9.9
Pneumonitis / Pneumonia	-	-	-	3.0	0.2	-	-	-
Stomatitis	0.0 - 0.2	-	-	8.0	1.0	-	0.5	2.5
Hematologic events and proteinuria								
Decreased hemoglobin/anemia	5.4 - 6.0	1.2	5.8	6.0	3.0	3.1	7.2	-
Thrombocytopenia	1.6 - 2.0	-	-	2.0	3.0	-	3.4	-

Neutropenia	54.0 - 66.5	62.6	21.1	-	65.0	53.4	26.5	-
Leukopenia	19.0 - 24.8	36.8	7.6	-	28.0	14.1	8.8	-
Lymphocytopenia	-	16.2	-	-	0.5	-		-
Biochemical changes								
Hyperglycemia	-	-	-	-	-	-	-	36.6
AST/SGOT	-	5.7	-	3.0	3.0	-	2.3	-
ALT/SGPT	-	11.4	-	-	6.0	-	4.1	-

Legend. PALBO = palbociclib; LETRO = letrozole; RIBO = ribociclib; ABE = abemaciclib; ANA = anastrozole; EVE = everolimus; EXE = exemestane; FULV = fulvestrant standard dose; ALP= alpelisib

Supplementary figures

Supplementary Figure 1. NMA of the HR for PFS/TTP of all treatments versus anastrozole.

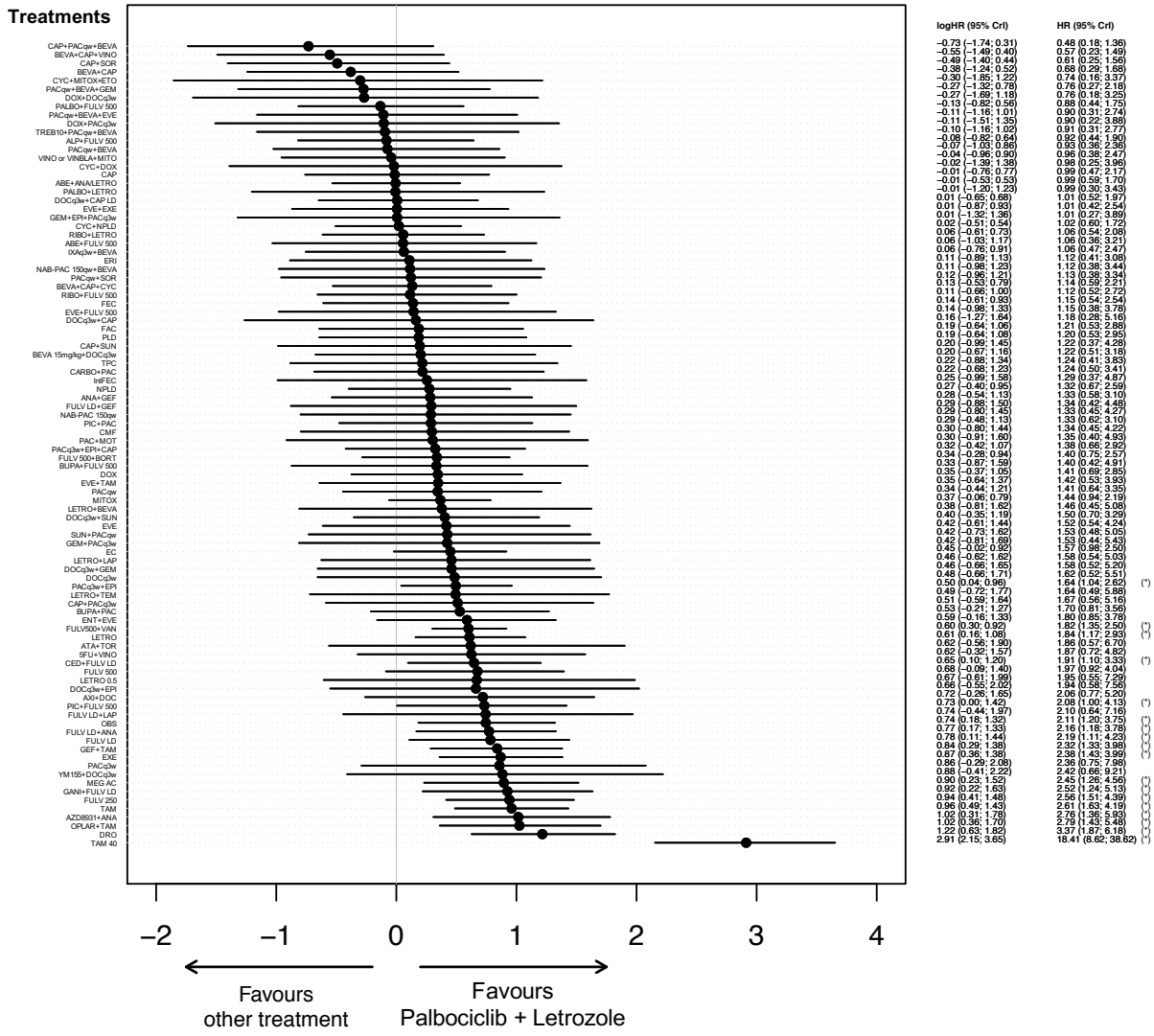


Hazard ratios (HR) of each treatment versus anastrozole. Central dots represent posterior medians; lines represent 95% credible intervals. Log scale was adopted to graphically represent the 95% credible intervals. The first column of values on the right reports the log-HR with 95% credible intervals, the second column reports HR with 95% credible intervals. Statistically significant results are highlighted by asterisks.

Abbreviations: qw = weekly; q3w = every 3 weeks; ABE = abemaciclib; FULV 500 = fulvestrant, standard dose; FULV 250 = fulvestrant 250 mg without loading dose; FULV LD = fulvestrant 250 mg with loading dose; ANA = anastrozole; LETRO = letrozole, standard dose 2.5 mg; LETRO 0.5 = letrozole 0.5 mg; ATA = atamestane; TOR: toremifene; ALP= alpelisib; AXI = axitinib; 5FU = 5-fluorouracil; DOC = docetaxel; BEVA = bevacizumab; CAP= capecitabine; CYC = cyclophosphamide; VINO = vinorelbine; BUPA = buparlisib; PAC =

paclitaxel; SOR: sorafenib; SUN = sunitinib; CED = cediranib; LD = low dose; BMF = bendamustine + methotrexate + 5-fluorouracil; CMF = cyclophosphamide + methotrexate + 5-fluorouracil; DOX = doxorubicin; EPI = epirubicin; MITOX = mitoxantrone; CIS = cisplatin; ETO = etoposide; DASA = dasatinib; NPLD = non-peghylated liposomal doxorubicin; GEM = gemcitabine; IDO = idoxifene; ENT = entinostat; EXE = exemestane; ERI = eribulin; EVE = everolimus; TAM = tamoxifen, standard dose 20 mg; TAM 40 = tamoxifen 40 mg; FEC/CEF = 5-fluorouracil + epirubicin + cyclophoshpamide; BORT = bortezomib; VAN = vandetanib; GEF = gefitinib; LAP= lapatinib; GANI = ganitumab; Int = intensive; IXA = ixabepilone; MA = megestrol acetate; NAB-PAC = nab paclitaxel; TEM = temsirolimus; OBS = observation; OPLAR = octreotide pamoate long acting release; CARBO = carboplatin; MOT = motesanib; PALBO = palbociclib; PIC = pictilisib; PLD = peghilated liposomal doxorubicin; RIBO = ribociclib; TPC = treatment of physician's choice; TREB = trebananib; VINBLA = vinblastine; SELU = selumetinib; → = followed by; TI = time intensive; MMM = mitoxantrone + mitomycin C + methotrexate; DRO = droloxifene; NA = not available; FAC = 5-fluorouracil + doxorubicin + cyclophosphamide

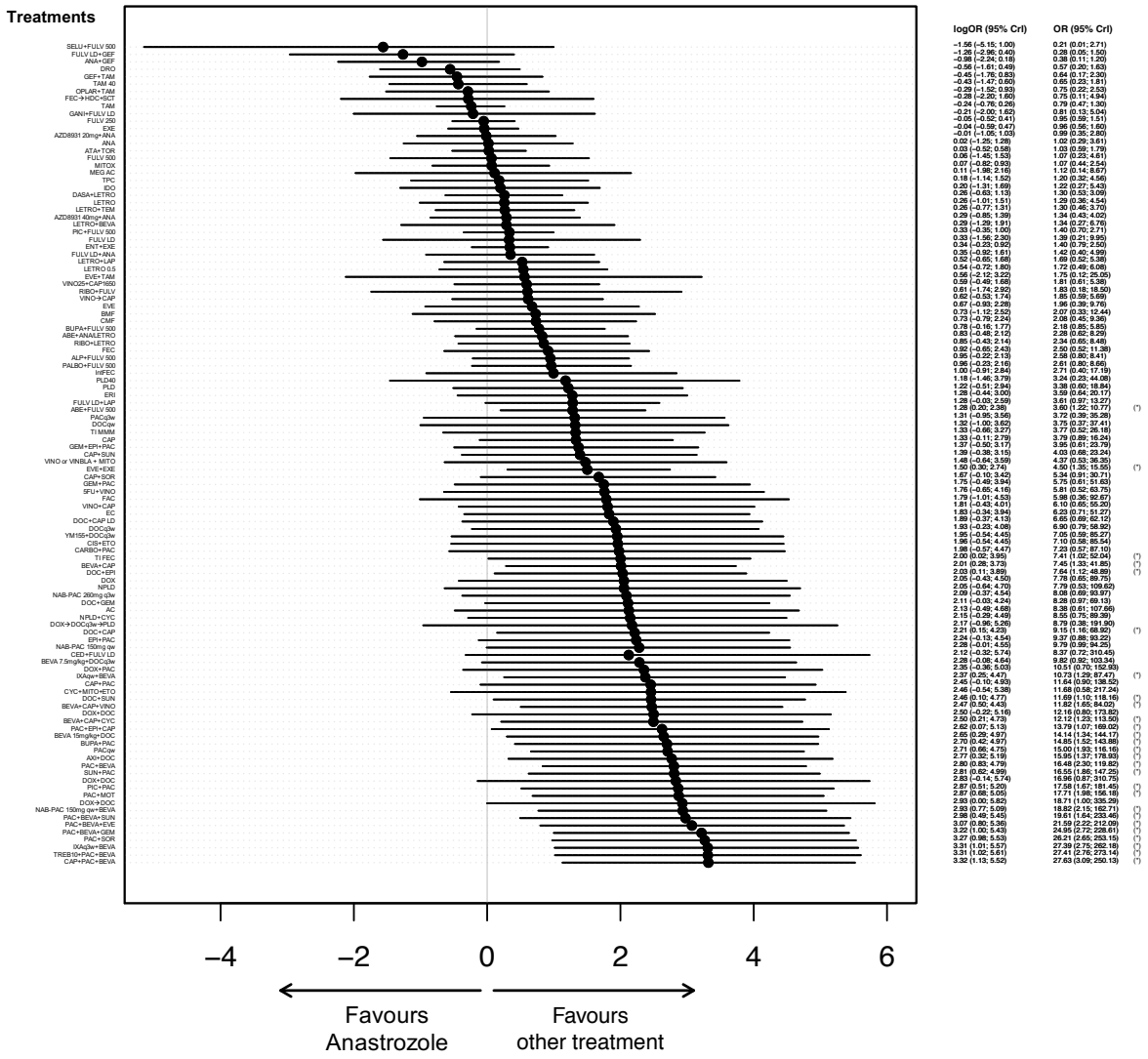
Supplementary Figure 2. NMA of the HR for PFS/TTP of all treatments versus palbociclib + letrozole



Hazard ratios (HR) of each treatment versus palbociclib + letrozole. Central dots represent posterior medians; lines represent 95% credible intervals. Log scale was adopted to graphically represent the 95% credible intervals. The first column of values on the right reports the log-HR with 95% credible intervals, the second column reports HR with 95% credible intervals. Statistically significant results are highlighted by asterisks.

Abbreviations: see supplementary figure 1 footnotes

Supplementary Figure 3. NMA of the OR for proportion of patients achieving an overall response of all treatments versus anastrozole

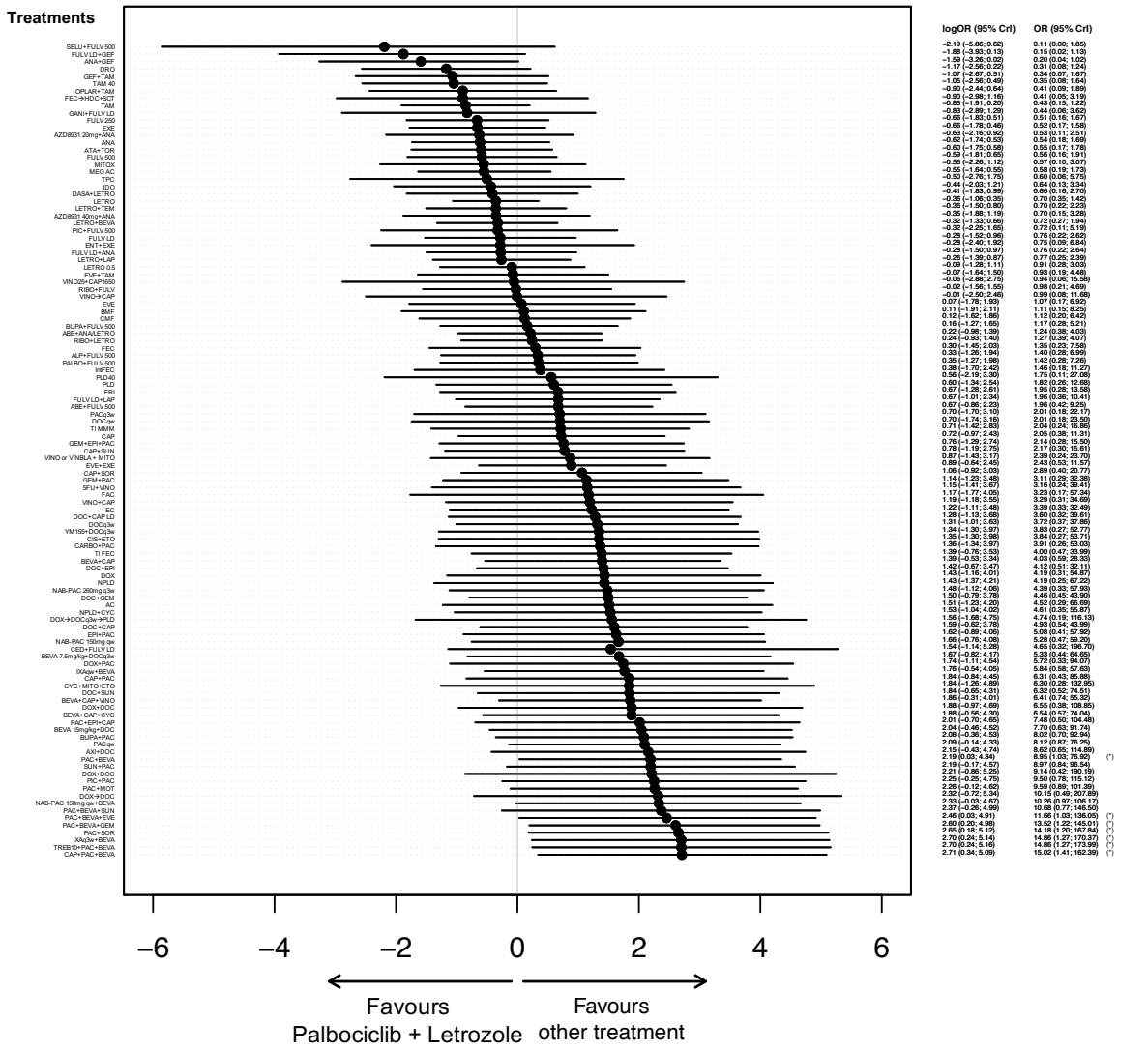


Odds ratios (OR) of each treatment versus anastrozole. Central dots represent posterior medians; lines represent 95% credible intervals. Log scale was adopted to graphically represent the 95% credible intervals. The first column of values on the right reports the log-OR with 95% credible intervals, the second column reports OR with 95% credible intervals. Statistically significant results are highlighted by asterisks.

Abbreviations: qw = weekly; q3w = every 3 weeks; ABE = abemaciclib; FULV 500 = fulvestrant, standard dose; FULV 250 = fulvestrant 250 mg without loading dose; FULV LD = fulvestrant 250 mg with loading dose; ANA = anastrozole; LETRO = letrozole, standard dose 2.5 mg; LETRO 0.5 = letrozole 0.5 mg; ATA = atamestane; TOR: toremifene; ALP= alpelisib; AXI = axitinib; 5FU = 5-fluorouracil; DOC = docetaxel; BEVA = bevacizumab; CAP= capecitabine; CYC = cyclophosphamide; VINO = vinorelbine; BUPA = buparlisib; PAC =

paclitaxel; SOR: sorafenib; SUN = sunitinib; CED = cediranib; LD = low dose; BMF = bendamustine + methotrexate + 5-fluorouracil; CMF = cyclophosphamide + methotrexate + 5-fluorouracil; DOX = doxorubicin; EPI = epirubicin; MITOX = mitoxantrone; CIS = cisplatin; ETO = etoposide; DASA = dasatinib; NPLD = non-peghylated liposomal doxorubicin; GEM = gemcitabine; IDO = idoxifene; ENT = entinostat; EXE = exemestane; ERI = eribulin; EVE = everolimus; TAM = tamoxifen, standard dose 20 mg; TAM 40 = tamoxifen 40 mg; FEC/CEF = 5-fluorouracil + epirubicin + cyclophoshpamide; BORT = bortezomib; VAN = vandetanib; GEF = gefitinib; LAP= lapatinib; GANI = ganitumab; Int = intensive; IXA = ixabepilone; MA = megestrol acetate; NAB-PAC = nab paclitaxel; TEM = temsirolimus; OBS = observation; OPLAR = octreotide pamoate long acting release; CARBO = carboplatin; MOT = motesanib; PALBO = palbociclib; PIC = pictilisib; PLD = peghilated liposomal doxorubicin; RIBO = ribociclib; TPC = treatment of physician's choice; TREB = trebananib; VINBLA = vinblastine; SELU = selumetinib; → = followed by; TI = time intensive; MMM = mitoxantrone + mitomycin C + methotrexate; DRO = droloxifene; HDC = high dose chemotherapy; SCT = stem cell transplant; NA = not available; FAC = 5-fluorouracil + doxorubicin + cyclophosphamide

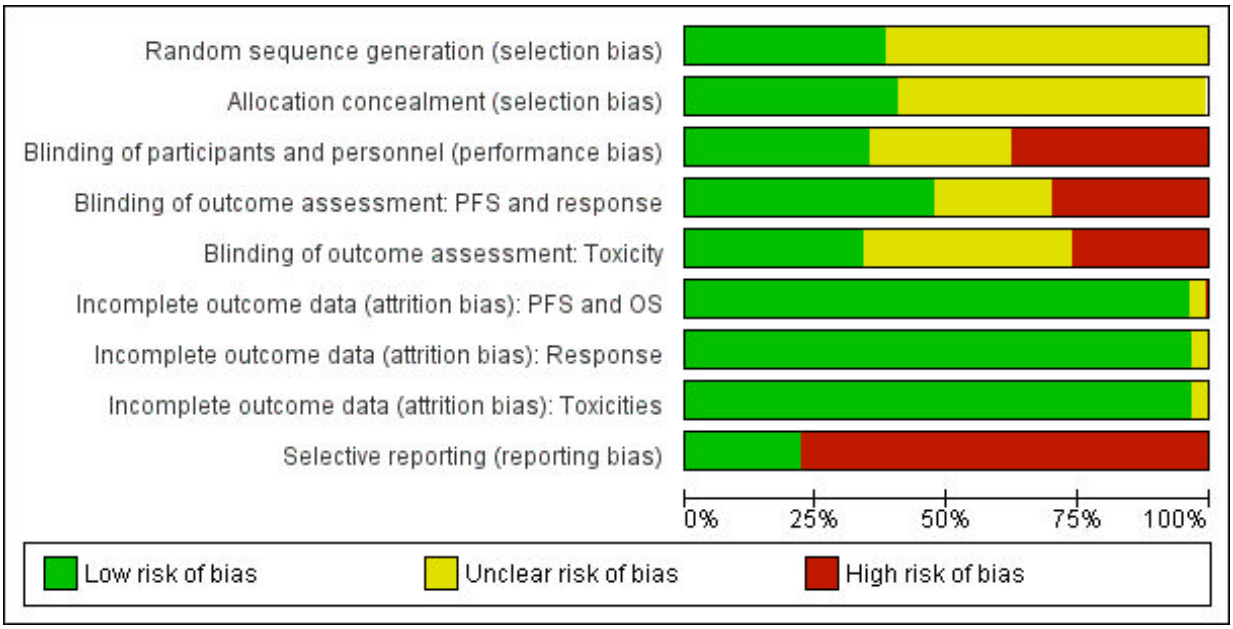
Supplementary Figure 4. NMA of the OR for proportion of patients achieving an overall response of all treatments versus palbociclib + letrozole



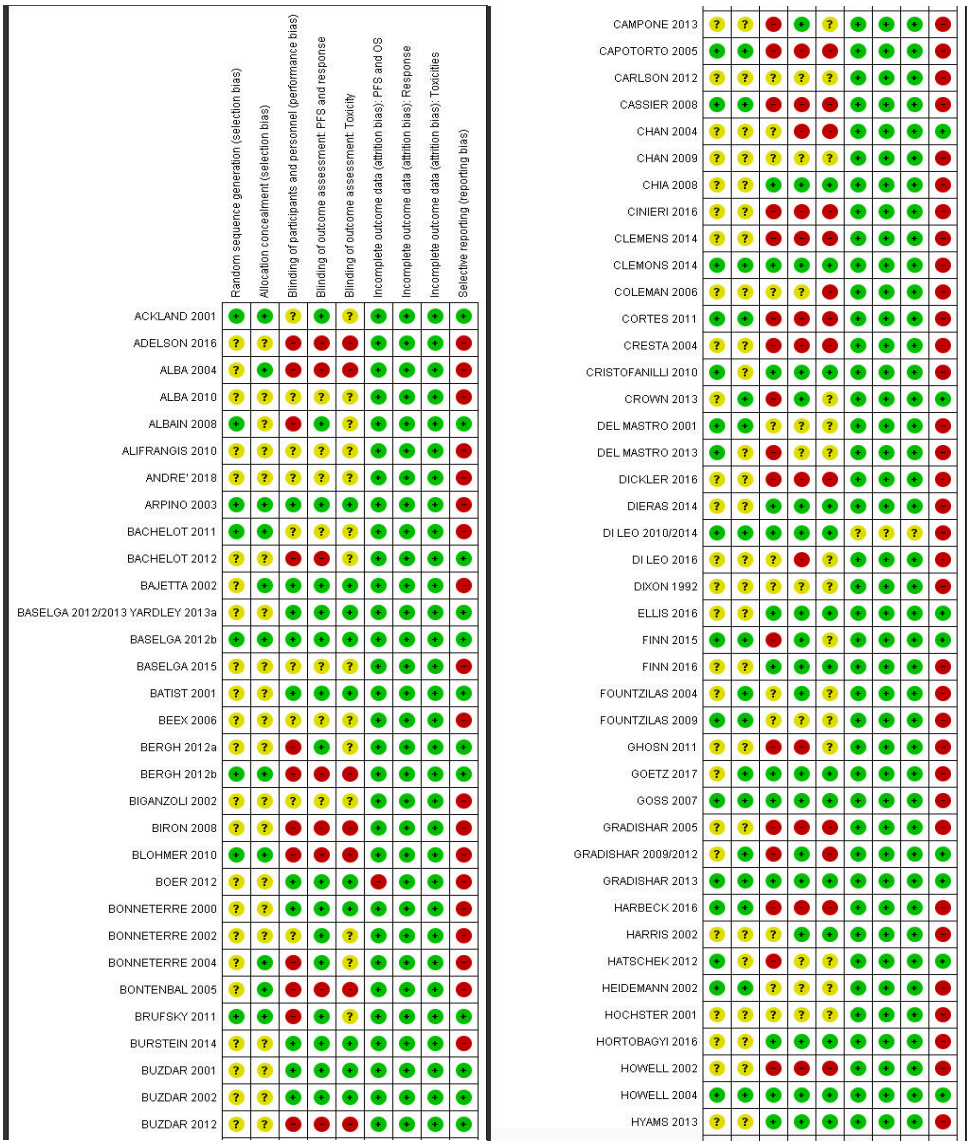
Odds ratios (OR) of each treatment versus palbociclib + letrozole. Central dots represent posterior medians; lines represent 95% credible intervals. Log scale was adopted to graphically represent the 95% credible intervals. The first column of values on the right reports the log-OR with 95% credible intervals, the second column reports OR with 95% credible intervals. Statistically significant results are highlighted by asterisks.

Abbreviations: see supplementary figure 3 footnotes.

Supplementary figure 5. Risk of bias analysis



Supplementary figure 6. Risk of bias analysis detailed for each single study included in the NMA

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KELLER 2004
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KROP 2016
LAM 2014
LANGLEY 2005
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LUCK 2013
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SCHMID 2005
SLAMON 2018
SLEDGE 2017
SMORENBURG 2014
STEMMLER 2010
STOCKLER 2011
TAMURA 2017
TRYFONIDIS 2016
TURNER/CRISTOFANILLI 2015/2016
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WRIGHT 2011
XU 2011
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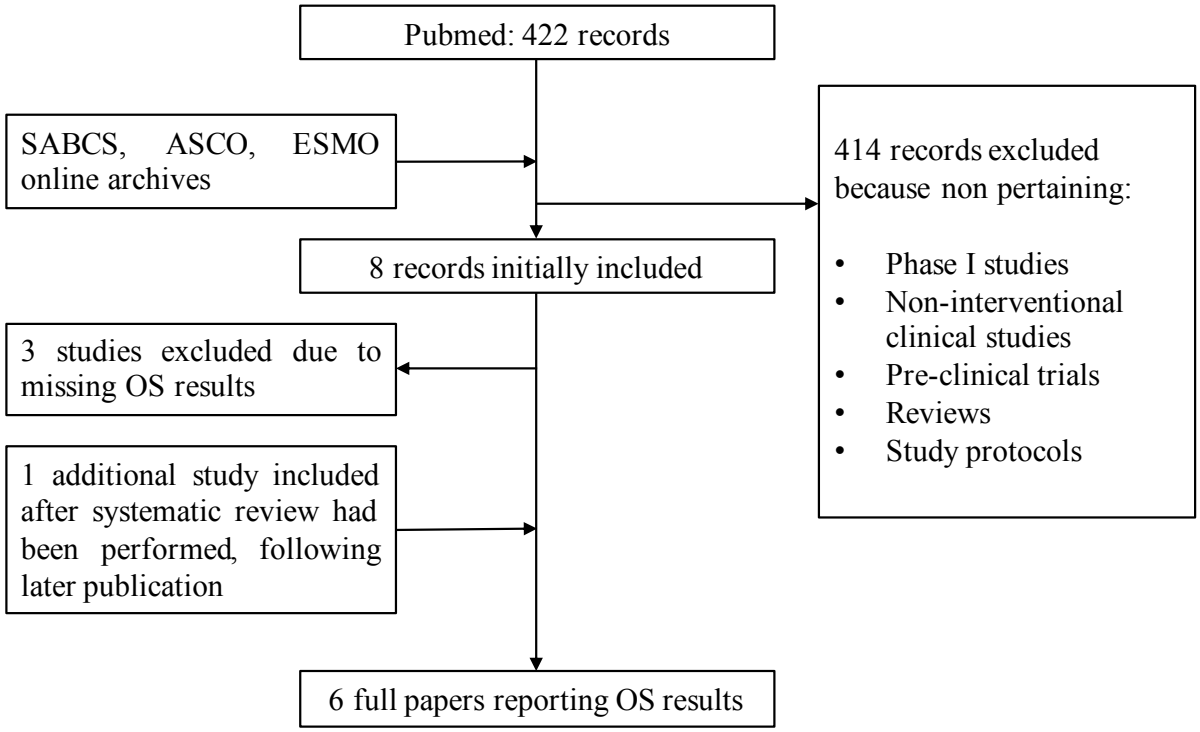
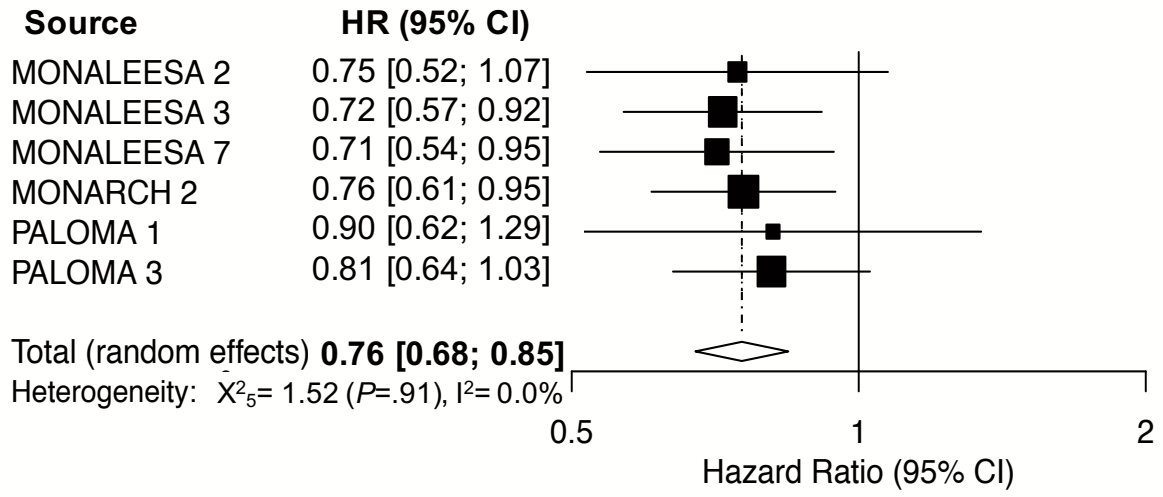
Appendix 5 – Supplementary materials Chapter 5

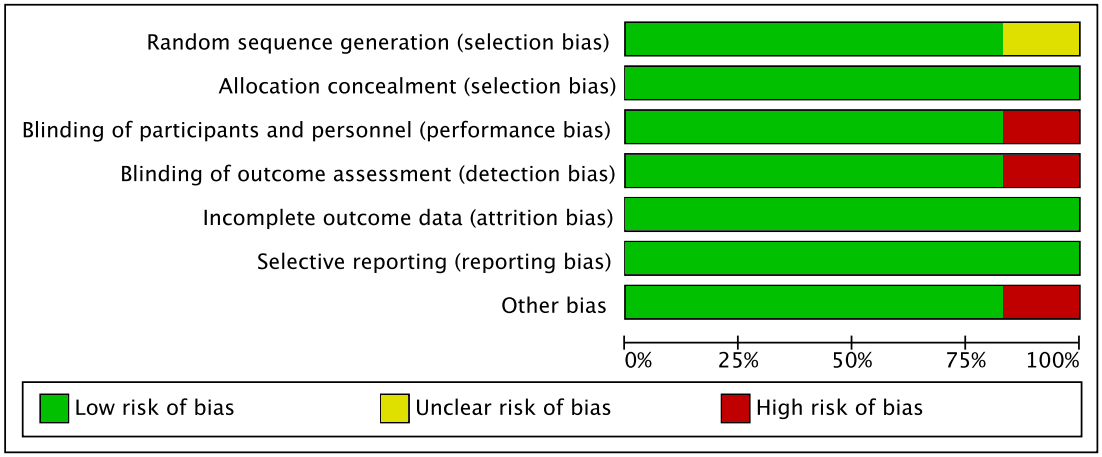
Supplementary figure 1. PRISMA diagram



Supplementary figure 2. Pooled OS effect for CDK4/6-inhibitors combinations compared to standard endocrine agents



Supplementary figure 3. Pooled risk of bias analysis



Supplementary figure 4. Risk of bias analysis for each study included