Prognostic Value of Intrinsic Subtypesin Hormone Receptor–Positive Metastatic Breast CancerTreated With Letrozole With or Without Lapatinib

Aleix Prat MD[1,2,3,#], Maggie C.U. Cheang PhD[4], Patricia Galván[2,3], Paolo Nuciforo MD[1], Laia Paré PhD[2,3], Barbara Adamo MD[2,3], Montserrat Muñoz MD[2,3], Margarida Viladot MD[2,3], Michael F Press MD, PhD[5], Robert Gagnon PhD[6,*], Catherine Ellis PhD[7], Stephen Johnston MD[8,#]

[1]Vall d´Hebron Institute of Oncology, Passeig de la Vall d´Hebron 119-129, 08036, Barcelona, Spain; [2]Hospital Clínic of Barcelona, Villarroel 170, 08035, Barcelona, Spain; [3]Translational Genomics and Targeted Therapeutics in Solid Tumors, August Pi i Sunyer Biomedical Research Institute, Villarroel 170, 08035, Barcelona, Spain; [4]The Institute of Cancer Research, Fulham Rd, Chelsea, London, SW3 6JJ, UK; [5]USC Norris Comprehensive Cancer Center, 1441 Eastlake Ave, NOR5409, Los Angeles, CA, USA; [6]Novartis, Novartis Pharmaceuticals Corporation, 1 Health Plaza, East Hanover, NJ 07936, USA; [7]GlaxoSmithKline Oncology, 1250 S Collegeville Rd, Collegeville, PA 19426, USA; [8]Royal Marsden Hospital, Fulham Rd, Chelsea, London, SW3 6JJ, UK

[*]Robert Gagnon was an employee at GlaxoSmithKline during the time of the study conduct and initial publication development

[#]**Corresponding author**:

Aleix Prat, MD, PhD

Translational Genomics Group, Vall d'Hebron Institute of Oncology (VHIO)

Passeig de la Vall d´Hebron, 119-129, 08036, Barcelona, Spain.

Email: aprat@vhio.net

Tel: +34-646847709

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Abstract

IMPORTANCE: The value of the intrinsic subtypes of breast cancer (Luminal A, Luminal B, human epidermal growth factor receptor 2 [HER2]-enriched and Basal-like) in the metastatic setting is currently unknown.

OBJECTIVE: To evaluate the ability of the intrinsic subtypes of breast cancer to predict outcome and/or benefit in hormone receptor (HR)-positive metastatic breast cancer.

DESIGN: Unplanned retrospective analysis of 821 tumor samples (85.7% primary and 14.3% metastatic) from EGF30008 phase III clinical trial (NCT00073528), where patients with HR+ metastatic disease were randomized to letrozole with or without lapatinib, an epidermal growth factor receptor (EGFR)/HER2 tyrosine kinase inhibitor. Tumor samples were classified into each subtype using the research-based PAM50 classifier.

SETTING: HR+ metastatic disease with no prior systemic therapy for advanced or metastatic disease.

PARTICIPANTS: Postmenopausal women with HR-positive invasive breast cancer and no prior therapy for advanced or metastatic disease. Prior neoadjuvant/adjuvant antiestrogen therapy was allowed. Patients with extensive symptomatic visceral disease were excluded.

MAIN OUTCOMES AND MEASURES: Primary and secondary endpoints were progression-free survival and overall survival. Treatment effects were evaluated using interaction tests.

RESULTS: Intrinsic subtype was the strongest prognostic factor independently associated with progression-free survival and overall survival in all patients, and in patients with HER2-negative (n=644) or HER2+ (n=157) diseases. Median progression-free survival and overall survival for each subtype within clinically HER2-negative disease were: Luminal A (16.9 and 45.0 months), Luminal B (11.0 and 37.0 months), HER2-enriched (4.7 and 16.0 months) and Basal-like (4.1 and 23.0 months). Patients with HER2-negative/HER2-enriched disease benefited from lapatinib (6.5 vs 2.6 months; progression-free survival hazard ratio=0.24, 95% confidence interval: 0.07–0.86; interaction *P*=0.02).

CONCLUSIONS AND RELEVANCE: This is the first study to reveal that the intrinsic subtypes are strongly prognostic in first-line HR-positive metastatic disease. HR-positive/HER2- disease with a HER2-enriched profile may benefit from lapatinib in combination with endocrine therapy. The clinical value of intrinsic subtyping in hormone receptor-positive metastatic breast cancer warrants further investigation, but patients with Luminal A/HER2-negative metastatic breast cancer disease might be good candidates for letrozole monotherapy in the first-line setting regardless of visceral disease and number of metastases.

Introduction

Hormone receptor-positive (HR+) metastatic breast cancer consists of a clinically heterogeneous group of tumors with different prognoses and responses to endocrine and chemotherapy.[1,2] Except for human epidermal growth factor receptor 2 (HER2) status, little is known about its biological heterogeneity and impact on patient outcome. This is important since biomarkers that help make treatment decisions in this particular setting are urgently needed.[3] The best treatment approach (ie, endocrine therapy vs chemotherapy) in the first-line setting of HR+/HER2-negative disease is unknown and the decision today is based on patient characteristics (eg, age), tumor load (eg, number of metastases), type of metastasis (visceral vs bone-only) and prior therapy.[3]

In contrast to metastatic disease, much effort has been made to elucidate the biological heterogeneity of early breast cancer.[4,5] During the last 15 years, studies evaluating global gene expression patterns have identified four main intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched [HER2E] and Basal-like).[6-8] These entities are associated with distant recurrence and response to endocrine and chemotherapy, even within HR+ disease.[6,7,9] In fact, patients that have clinically HR+/HER2-negative disease with a HER2E gene expression profile (which represents ~5% of cases) do not seem to respond substantially, as estimated by Ki67 changes, from neoadjuvant endocrine therapy.[10,11] Although intrinsic profiles are mostly maintained during metastatic progression,[12] their prognostic and predictive value in patients with newly diagnosed HR+ metastatic disease remains largely unknown.

Despite recent advances in the treatment of HR+ metastatic breast cancer, resistance to endocrine therapies limits their success,[13] Cross-talk between pathways involving the epidermal growth factor family of receptors, epidermal growth factor receptor (EGFR) and HER2, and HRs has been implicated in resistance to endocrine therapy.[2,14] Lapatinib, a dual tyrosine kinase inhibitor of EGFR and HER2, has improved response rate and progression-free survival (PFS) in first-line HR+ breast cancer in combination with letrozole in patients with HER2+ disease (8.2 vs 3.0 months) but not in those with HER2-negative disease.[15] However, whether patients with HR+/HER2-negative disease but with a HER2E gene expression profile benefit from adding lapatinib to endocrine therapy is currently unknown.

Here we evaluated, for the first-time, the prognostic and predictive abilities of intrinsic subtypes in tumor samples from EGF30008, a Phase III randomized clinical trial of endocrine therapy with or without lapatinib in the first-line metastatic setting.[15]

Materials and Methods

Patient data

The eligibility criteria and study design for EGF30008 (NCT00073528) were reported previously.[15,16] Briefly, 1286 patients with advanced postmenopausal HR+ breast cancer (Stage III or IV) previously untreated in the metastatic setting were randomized in a blinded fashion to receive letrozole 2.5 mg daily with either lapatinib 1500 mg daily or placebo. Patients were stratified by sites of disease (soft tissue/visceral or bone-only disease) and prior adjuvant antiestrogen therapy (<6 months since discontinuation or ≥6 months since discontinuation or no prior endocrine therapy). HR+ was determined per the enrolling site and HER2 status was determined in a commercial laboratory in primary or metastatic sites

defined as either fluorescence in situ hybridization (FISH)-positive, 3+ staining by immunohistochemistry (IHC), or 2+ by IHC and confirmed HER2 FISH-positive.[17] Ethical review of the study was performed by Hospital Vall d´Hebron IRB. No additional informed consent was required beyond the original informed consent of the clinical trial.

Gene expression analysis

A section of the formalin-fixed paraffin-embedded (FFPE) breast tissue was first examined with hematoxylin and eosin staining to confirm presence of invasive tumor cells and determine the tumor area. For RNA purification (Roche® High Pure FFPET RNA isolation kit), ≥1–3 10 μm FFPE slides were used for each tumor specimen, and macrodissection was performed, when needed, to avoid normal breast tissue contamination.[18] A minimum of ~150 ng of total RNA was used to measure the expression of 105 breast cancer-related genes and 5 housekeeping genes using the nCounter platform (Nanostring Technologies, Seattle, WA, US).[19] Data were log base 2 transformed and normalized using 5 housekeeping genes (ACTB, MRPL19, PSMC4, RPLP0, and SF3A1). Samples with ≤10 counts in ≥50% of the genes were removed. Raw gene expression data will be deposited in Gene Expression Omnibus.

Sample data and PAM50 intrinsic subtyping

Of the 1286 tumor samples, 916 were profiled and 821 meet the minimum criteria for further analysis (eFigures 1 and 2). Intrinsic subtyping (Luminal A, Luminal B, HER2E, Basal-like and Normal-like) was performed using the research-based PAM50 intrinsic subtype predictor as previously described.[7,18] Proper normalization was evaluated by a

principal component loading plot (eFigure 3). PAM50 subtyping was performed at the Translational Genomic Group at VHIO blinded from clinical data.

Statistical analysis

Estimates of PFS and overall survival (OS) were from Kaplan–Meier curves and tests of differences by log-rank test. Univariable and multivariable Cox-regression analyses were used to test the independent prognostic significance of each variable. To test the prognostic contribution of the PAM50 subtypes, changes in likelihood ratio (LR) values ($\chi 2$) were used to measure and compare the relative amount of additional prognostic information of one variable/score compared with another. To test the predictive value of the PAM50 subtypes, interaction tests between PAM50 subtypes and treatment for PFS were evaluated in uni- and multivariable models. Proportional-hazards assumption was tested on the basis of Schoenfeld residuals. A 2-sided $P < 0.05$ was used as the threshold for statistical significance.

Results

Clinical-pathological characteristics and subtype distribution

The clinical-pathological characteristics of the 821 patients with subtype data were well-balanced compared with the patients included in the original study (eTable 1). The median age was 62 years, 86% of the patients had visceral disease, 644 (80%) had HER2-negative tumors, 157 (20%) had HER2+ disease and 73% had relapsed ≥6 months since discontinuation of anti-estrogen therapy or not received prior endocrine therapy. Similar to

the original results, lapatinib showed a significant PFS benefit in HER2+ disease but not in HER2-negative disease (eFigure 4).

In both HER2-negative and HER2+ disease, all breast cancer intrinsic subtypes were identified albeit with different proportions (Table 1). Compared with HER2-negative disease, HER2+ disease had a lower proportion of Luminal A tumors (27% vs 52%) and a higher proportion of HER2E tumors (29% vs 3%). The proportion of Luminal B and Basal-like tumors remained similar in both HER2 groups (29% vs 30% and 4% vs 3%, respectively). No significant differences in the distribution of the intrinsic subtypes were identified based on number of metastases, type of metastases and treatment arm. Interestingly, a significant increase in the proportion of non-luminal subtypes (17% vs 9%, $P<0.001$), mostly HER2E (70.6%), was observed in patients that relapsed during adjuvant endocrine therapy or within 6 months of discontinuation compared with those that never received or relapsed at least 6 months after completing adjuvant endocrine therapy (data not shown).

Prognosis within HER2-negative disease

Survival data were available for 644 patients with HER2-negative disease (eFigure 5). Compared with the Luminal A subtype, the other subtypes showed a significantly worse PFS (Figure 1A) independently of other clinical-pathological variables (Table 2). When other clinical-pathological variables were held constant, patients with Luminal B, HER2E and Basal-like subtypes had a 1.457, 2.873, and 2.258 times higher risk of tumor progression, respectively. Median PFS differed across the intrinsic subtypes: Luminal A (16.85 months, 95% confidence interval [CI]: 14.09–19.9), Luminal B (10.97 months, 95%

CI: 9.56–13.6), HER2E (4.67 months, 95% CI: 2.73–10.8) and Basal-like (4.14 months, 95% CI: 2.53–13.8). Intrinsic subtype added more prognostic information regarding PFS than any of the other clinical-pathological variables evaluated in the model (LR $\chi 2 = 31.15$; $P<0.0001$) (eTable 2). The second and third most important prognostic variables were prior endocrine therapy (LR $\chi 2 = 27.842$; $P<0.0001$) and number of metastases (LR $\chi 2 = 15.377$; $P<0.0001$). Interestingly, visceral versus nonvisceral disease did not provide independent prognostic information (LR $\chi 2 = 1.512$; $P=0.22$).

Similar results were observed in OS despite only 37.6% of patients with an event (Figure 1B and eTable 3). Compared with patients with a Luminal A subtype, patients with a Luminal B, HER2E and Basal-like subtype had a 1.518, 2.528, and 2.338 times higher risk of death, respectively, when other clinical-pathological variables were held constant. Median OS differed across the intrinsic subtypes: Luminal A (45 months, 95% CI: 41–NA), Luminal B (37 months, 95% CI: 31–42), HER2E (16 months, 95% CI: 10–NA) and Basal-like (23 months, 95% CI: 12-NA). Intrinsic subtype added more prognostic information regarding OS when added to the other clinical-pathological variables (LR $\chi 2 = 20.641$; $P<0.001$) than any other variable evaluated (eTable 4), except prior endocrine therapy (LR $\chi 2 = 25.686$; $P<0.0001$). The third most important prognostic variable regarding OS was performance status (LR $\chi 2 = 14.426$; $P<0.001$).

Prognosis within HER2+ disease

Survival data were available for 157 patients with HER2+ disease (Figure 1C). Compared with the Luminal A subtype, the other subtypes showed a worse PFS independently of other clinical-pathological variables (Table 3). When other clinical-pathological variables

were held constant, patients with a Luminal B, HER2E, and Basal-like had a 1.471, 1.818, and 4.799 times higher risk of tumor progression, respectively. Median PFS differed across the intrinsic subtypes: Luminal A (11.07 months, 95% CI: 5.72–16.95), Luminal B (5.55 months, 95% CI: 3.02–8.25), HER2E (4.37 months, 95% CI: 2.83–8.64), and Basal-like (3.58 months, 95% CI: 2.27–NA). Intrinsic subtype added more prognostic information regarding PFS when added to the other clinical-pathological variables (LR $\chi 2 = 12.328$; $P=0.02$) than any other variable evaluated (eTable 5). The second and third most important prognostic variables regarding PFS were lapatinib treatment (LR $\chi 2 = 6.626$; $P=0.01$) and performance status (LR $\chi 2 = 5.339$; $P=0.02$).

In terms of OS, similar results were observed (Figure 1D and eTable 6 and 7). Compared with patients with a Luminal A subtype, patients with a Luminal B, HER2E, and Basal-like subtype had a 1.547, 1.913, and 2.919 times higher risk of death, respectively. Overall, median OS differed across the intrinsic subtypes: Luminal A (not reached), Luminal B (32 months, 95% CI: 21–NA), HER2E (28 months, 95% CI: 17–NA) and Basal-like (19 months, 95% CI: 9–NA). Intrinsic subtype added more prognostic information regarding OS when added to the other clinical-pathological variables (LR $\chi 2 = 9.955$; $P=0.04$) than any other variable evaluated (eTable 7). The second and third most important prognostic variables regarding OS were prior endocrine therapy (LR $\chi 2 = 7.996$; $P=0.005$) and number of metastases (LR $\chi 2 = 7.187$; $P=0.007$).

Benefit of lapatinib

The effect of lapatinib on PFS in HER2-negative disease was evaluated within each intrinsic subtype (Figure 2). Among the different subtypes, only the HER2E showed a

significant benefit from lapatinib in univariate (6.49 month median PFS with lapatinib vs 2.60 month median PFS with placebo; hazard ratio = 0.238, 95% CI: 0.066–0.863; *P*=0.03) and multivariate (lapatinib vs placebo hazard ratio = 0.040, 95% CI: 0.04–0.395; *P*=0.006) analyses. The interaction test between HER2E and treatment was significant in univariate (*P*=0.02) and multivariate analyses (*P*=0.006).

The effect of lapatinib on PFS in HER2+ was evaluated within each intrinsic subtype (eFigure 6). All subtypes seemed to benefit to some degree from lapatinib by looking at the estimate of the hazard ratio. The interaction tests between each subtype and treatment were not statistically significant (data not shown).

HER2 IHC and FISH in HER2E/HER2-negative tumors

Fifty percent (8/16) of samples identified in the EGF30008 trial as clinically HER2E/HER2-negative showed either a lack of HER2 expression by IHC or a +1 score (eTable 8). FISH determination in 6 of these samples showed a HER2/CEP17 ratio of ≤1.6 (eTable 9). Among the other 8 HER2E/HER2-negative cases with an IHC 2+ score, 6 were tested for HER2 gene amplification; all showed a HER2/CEP17 ratio of ≤1.6 (Table S8). Thus, the HER2E/HER2-negative cases did not show evidence of HER2 gene amplification.

Discussion

To our knowledge, this is the first report to evaluate the prognostic and predictive abilities of breast cancer intrinsic molecular subtypes in postmenopausal patients with HR+ metastatic disease treated with endocrine therapy +/- lapatinib in the first-line setting.

Specifically, our results reveal that (1) all intrinsic molecular subtypes are identified within HER2-negative and HER2+ diseases, albeit with different proportions; (2) Intrinsic subtype is the most important and independent prognostic factor in this setting, even within HER2+ disease; (3) 95% of patients with Luminal A/HER2-negative disease experience long PFS periods of 14.1–19.9 months with letrozole therapy; (4) Patients with HER2E/HER2-negative disease treated with letrozole therapy may benefit from the addition of lapatinib.

The optimal systemic treatment strategy for newly diagnosed patients with HR+ advanced/metastatic breast cancer is currently unknown. National Comprehensive Cancer Network or European School of Oncology-European Society of Medical Oncology 2nd international consensus guidelines for advanced breast cancer[20,21] recommend starting with endocrine therapy but base the decision on clinical parameters such as amount of tumor burden, age, performance status, disease-free interval, or previous therapies in the adjuvant setting. However, HR+ disease is clinically and biologically heterogeneous and thus there is an urgent need to identify robust prognostic and/or predictive tumor-based biomarkers to be included with other clinical variables considered in patient management and therapy selection decisions. For example, no predictive biomarker exists to date for novel drugs such as CDK4/6 and PI3K/mTOR inhibitors that are currently being incorporated earlier in the treatment of HR+/HER2-negative metastatic disease in combination with endocrine therapy.

A limited number of studies have evaluated the prognostic value of single pathology-based biomarkers for predicting outcome in the first-line HR+ metastatic breast cancer setting following endocrine therapy.[22-25] For example, Delpech and colleagues[22] showed that high baseline Ki67 expression in 241 estrogen receptor positive (ER+) primary breast cancers

correlated with lower clinical benefit and time to progression on first-line endocrine therapy. Similar results with Ki67 were obtained by Yamashita and colleagues in a series of 73 cases.[23] Moreover, a study of the Southwest Oncology Group evaluated the prognostic ability of progesterone receptor (PR) expression in 398 patients with ER+ metastatic breast cancer treated with tamoxifen[24] and showed that elevated PR levels in the primary tumor significantly and independently correlated with increased probability of response to tamoxifen, longer time to treatment failure and OS. Finally, Rocca and colleagues[25] showed that high Ki67 or low PR expression measured in metastatic tissue of 135 patients with HR+ disease was correlated with lower time to tumor progression following first-line endocrine therapy. Overall, these data are concordant with our results since proliferation and estrogen-regulation are two key biological features that distinguish Luminal A from non-Luminal A molecular subypes.[26]

Non-luminal intrinsic subtypes (ie, HER2E and Basal-like) represent 3–10% of all HR+ breast cancers.[6-8] In patients with HR+ disease treated with 5 years of adjuvant tamoxifen only, HER2E and Basal-like subtypes had very poor disease-free survival.[9] Concordant with this finding, both subtypes had the lowest relative decrease in Ki-67 either at 2 weeks or at 4–6 months following neoadjuvant endocrine therapy.[10,11] In terms of chemosensitivity, Jimenez and colleagues recently evaluated the pathological complete response (pCR) rates of the intrinsic subtypes within 180 patients with HR+/HER2- negative disease following anthracycline/taxane-based neoadjuvant chemotherapy.[27] Interestingly, patients with HR+/Basal-like disease, which represented 7.7% of the population, achieved a pCR rate of 50%, followed by patients with Luminal B, HER2E, and Luminal A tumors who achieved a pCR rate of 20%, 14.3%, and 9.3%, respectively.

Overall, these data, together with our results showing a median PFS of ~4.5 months in non-luminal subtypes within HR+/HER2-negative disease, suggest that molecular subtype may better represent tumor behavior in the setting of cytotoxic or endocrine therapy than clinical hormone receptor assays.

The observation that patients with HER2E/HER2-negative tumors benefit from lapatinib is intriguing. Our prior work has revealed that HER2E/HER2-negative tumors have similar genomic and genetic alterations as HER2E/HER2+ tumors except for the HER2 amplicon, which is only overexpressed/amplified in those HER2E tumors that are HER2+.[28] Similar results were obtained in the EGF30008 study when the HER2 gene expression was evaluated across the intrinsic subtypes based on clinical HER2 status (eFigure 7). Thus, the efficacy of lapatinib in the group of patients with HER2-negative/HER2E disease might be due to EGFR inhibition rather than HER2 inhibition although this will require further investigation.

The present study has several limitations. First, this is an unplanned retrospective analysis of a prospective clinical trial. To minimize bias, we were able to profile almost 2 out of 3 tumor samples from the original population, and the population representative of this subset had a similar distribution of clinical-pathological variables and a similar outcome behavior as the original study population. In addition, the laboratory that performed and reported the gene expression results for each sample was blinded from clinical data. Secondly, the vast majority of profiled samples from this dataset come from primary tumors rather than metastatic tumor. Although we cannot predict the findings if the analysis would have been done on metastatic samples only, the reality is that metastatic tissues are not always available in clinical practice and thus a biomarker derived from primary tumors is of value,

especially if this biomarker remains stable during tumor progression such as the intrinsic subtypes.[12] However, further studies are needed to determine the concordance of intrinsic subtyping in primary versus metastatic tissues and the stability of subtype during or after therapy. Finally, in the EGF30008 clinical study, HER2 testing was performed by a high-volume, commercial laboratory to determine HER2 status using standardize testing methods in a single laboratory. Additionally, HER2 status was evaluated by an academic reference laboratory in a limited number of cases from the EGF30008 study population, revealing 93% concordance (eTable 10).[16,17]

To conclude, HR+ disease is biologically heterogeneous and intrinsic subtypes are strongly prognostic in a first-line metastatic setting. HR+/HER2- disease with a HER2E profile may benefit from lapatinib. The clinical value of intrinsic subtyping in HR+ metastatic breast cancer warrants further investigation, but patients with Luminal A/HER2-negative metastatic disease might be good candidates for letrozole monotherapy in the first-line setting regardless of visceral disease and number of metastases, whereas patients with HER2E/HER2-negative or Basal-like/HER2-negative subtypes need other treatment strategies such as chemotherapy or novel targeted drugs.

Acknowledgments

Author contributions

Dr Prat had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Dr Prat also had final responsibility for the decision to submit for publication.

Study concept and design: Drs Prat, Johnston, and Ms Ellis.

Acquisition, analysis, and interpretation of data: All authors.

Drafting of the manuscript: All authors .

Critical revision of the manuscript for important intellectual content: All authors.

Dr Prat contributed to the literature search. Dr Press conducted laboratory research related to determination of HER2 gene amplification status in tumors from the EGF30008 trial.

Conflict of interest disclosures

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work in this submission which was laboratory based analyses from tumors collected in the EGF30008 trial for which Dr Johnston was the Principal Investigator, with the main clinical trial results being completed and previously published in 2009.

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Tables

Table 1. Distribution of the intrinsic subtypes within the entire population and within HER2-negative and HER2-positive subpopulations. HER2, human epidermal growth factor receptor 2.

	All patients[a]		HER2-negative		HER2-positive	
Subtype	**N**	**%**	**N**	**%**	**N**	**%**
Luminal A	382	47	335	52	42	27
Luminal B	244	30	196	30	46	29
HER2E	61	7	16	3	45	29
Basal-like	28	3	21	3	6	4
Normal-like	106	13	76	12	18	11

[a]HER2 status was unknown in 20 patients.

Table 2. Cox model progression-free survival analysis within HER2-negative[a] disease (n=644).

	Univariate analysis				Multivariable analysis			
Variables	**HR**	**Lower 95%**	**Upper 95%**	***P*-value**	**HR**	**Lower 95%**	**Upper 95%**	***P*-value**
Lapatinib vs placebo	0.925	0.766	1.118	0.42	0.905	0.745	1.100	0.32
Prior endocrine therapy								
<6 months vs ≥6 months or none	1.769	1.429	2.190	<0.001	1.903	1.513	2.394	<0.001
Performance status 1 vs 0	1.321	1.091	1.598	0.004	1.373	1.126	1.675	0.002
Visceral vs no visceral	1.120	0.837	1.451	0.49	0.891	0.656	1.209	0.46
≥3 sites vs <3 sites	1.427	1.180	1.725	<0.001	1.537	1.238	1.907	<0.001
Age	0.994	0.984	1.004	0.21	1.005	0.985	1.005	0.35
FFPE metastatic vs primary	0.753	0.564	1.006	0.06	0.677	0.500	0.916	0.012
PAM50 subtype								
Luminal A	1.000	-	-	-	1.000	-	-	-
Luminal B	1.468	1.183	1.822	<0.001	1.457	1.168	1.818	<0.001
Basal-like	2.510	1.548	4.071	<0.001	2.258	1.367	3.732	0.001
HER2E	3.193	1.814	5.620	<0.001	2.873	1.600	5.161	<0.001
Normal-like	1.784	1.327	2.397	<0.001	1.871	1.389	2.522	<0.001

[a]HER2 status was determined in a commercial laboratory in primary or metastatic sites defined as either FISH-positive, 3+ staining by IHC, or 2+ by IHC and confirmed HER2 FISH-positive.

FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IHC, immunohistochemistry.

Table 3. Cox model progression-free survival analysis within HER2-positive disease[a] (n=157).

	Univariate analysis				Multivariable analysis			
Variables	**HR**	**Lower 95%**	**Upper 95%**	***P*-value**	**HR**	**Lower 95%**	**Upper 95%**	***P*-value**
Lapatinib vs placebo	0.697	0.494	0.984	0.04	0.614	0.423	0.889	0.01
Prior endocrine therapy								
<6 months vs ≥6 months or none	1.257	0.884	1.787	0.20	1.296	0.854	1.968	0.22
PS 1 vs 0	1.486	1.049	2·103	0.03	1.543	0.584	1.729	0.02
Visceral vs no visceral	1.147	0.705	1.867	0.58	1.005	1.068	2.230	0.99
≥3 sites vs <3 sites	1.310	0.923	1.858	0.13	1.601	1.056	2.427	0.03
Age	0.985	0.965	1.005	0.13	0.983	0.962	1.004	0.12
FFPE metastatic vs primary	1.020	0.563	1.850	0.95	1.246	0.617	2.515	0.54
PAM50 subtype								
Luminal A	1.000	-	-	-	1.000	-	-	-
Luminal B	1.667	1.023	2.718	0.04	1.471	0.863	2.505	0.16
Basal-like	4.591	1.853	11.378	0.001	4.799	1.885	12.217	0.001
HER2E	1.922	1.189	3.107	0.008	1.818	1.116	2.960	0.02
Normal-like	2.356	1.302	4.265	0.005	1.762	0.938	3.310	0.08

[a]HER2 status was determined in a commercial laboratory in primary or metastatic sites defined as either FISH-positive, 3+ staining by IHC, or 2+ by IHC and confirmed HER2 FISH-positive.

FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IHC, immunohistochemistry.

Figure 1. Survival outcomes based on intrinsic subtype. (**A**) PFS in HER2-negative disease; (**B**) OS in HER2-negative disease; (**C**) PFS in HER2-positive disease; (**D**) OS in HER2-positive disease. Normal-like cases have been excluded in this plot since they are not considered a tumor subtype[4,5]. HER2, human epidermal growth factor receptor 2; OS, overall survival; PFS, progression-free survival. The results shown here are heedless of treatment arm (with or without lapatinib).

FIGURE 1

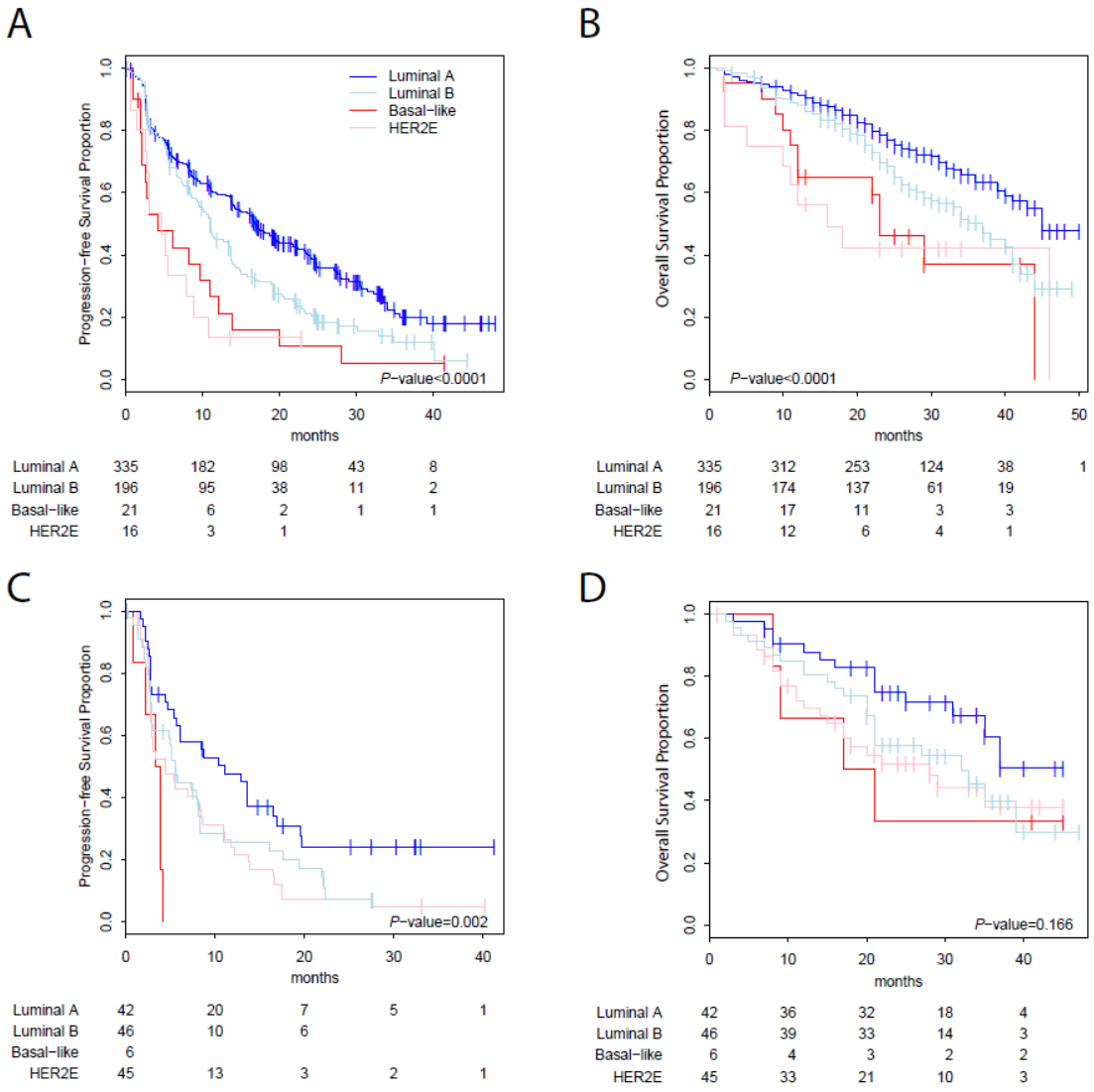

Figure 2. Effect of lapatinib within each intrinsic subtype in the HER2-negative population of the EGF30008 trial. (A) Luminal A; (B) Luminal B; (C) HER2E; (D) Basal-like.