Lucitanib for the treatment of HR$^+$/ HER2$^-$ metastatic breast cancer: results from the multicohort phase II FINESSE study

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Translational Relevance (147 words):

Fibroblast growth factor receptor (*FGFR)1* amplification is associated with poor prognosis and endocrine resistance in hormone receptor-positive (HR^+)/human epidermal growth factor receptor 2-negative ($HER2^-$) breast cancer patients. Lucitanib is an oral multikinase inhibitor with selective activity against FGFR1-3 and vascular endothelial growth factor receptor (VEGFR)1-3. This phase II study of lucitanib treated HR^+/ $HER2^-$ metastatic breast cancer patients with *FGFR1* amplification or 11q13 amplification or no amplification for either showed overall response rates (ORR) of 19% (95%CI: 9-35%), 0% (0-18%), 15% (6-34%), respectively. In exploratory biomarker analyses, patients with high level *FGFR1* amplification ($\geq$4 copy number variation [CNV]) had higher ORR than those without high amplification (22% versus 9%). Similarly, ORR in patients with high expression of FGFR1 (immunohistochemistry [IHC], histoscore [H-score] $\geq$50) was 25% versus 8% in FGFR1-low cancers. Further exploration of FGFR1 as a biomarker for FGFR inhibitor therapy in this patient population is warranted.

Abstract:

Purpose:

FGFR1 gene is amplified in 14% of HR^+/ $HER2^-$ breast cancer patients. Efficacy and safety of lucitanib, an inhibitor of VEGFR1-3, FGFR1-3 and PDGFRα/β, were assessed.

Methods:

HR^+/ $HER2^-$ MBC patients received oral lucitanib in 3 centrally confirmed cohorts: 1) *FGFR1* amplified, 2) *FGFR1* non-amplified, 11q13 amplified, 3) *FGFR1* and 11q13 non-amplified. Key inclusion criteria included ECOG PS ≤2, ≥1 line of anti-cancer therapy, but ≤2 lines of chemotherapy. Primary endpoint was ORR by RECIST1.1. Simon´s 2-stage design was used: if ≥2 patients responded among 21 patients, 20 additional patients could be enrolled in each cohort. *FGFR1* copy number variation (CNV) were determined by FISH and ddPCR, while FGFR1 expression by IHC.

Results:

76 patients (32/18/26 in cohorts 1/2/3) from nine countries were enrolled. The pre-specified primary endpoint was met in cohort 1 with ORR of 19% (95%CI:9-35%), but not in cohorts 2 and 3 with ORR of 0% (0-18%) and 15% (6-34%) respectively. Frequent adverse events included hypertension (87%), hypothyroidism (45%), nausea (33%) and proteinuria (32%). Exploratory biomarker analyses suggested higher ORR in patients with high *FGFR1* amplification (≥4 CNV) than those without high amplification (22% versus 9%). ORR in patients with FGFR1-high tumors (IHC, H-score ≥50) was 25% versus 8% in FGFR1-low cancers.

Conclusions:

Lucitanib had modest antitumor activity and significant hypertension-related toxicity in patients with HR^+/ $HER2^-$ MBC. Although based on small sample sizes, exploratory biomarker analyses suggested patients with high FGFR1 amplification or expression might derive greater benefit.

INTRODUCTION

Metastatic breast cancer (MBC) remains incurable, with hormone receptor-positive (HR^+)/human epidermal growth factor receptor 2-negative ($HER2^-$) being the most common subtype, accounting for 70% of all breast cancers. Endocrine therapy is the cornerstone treatment for this subtype (1), but the development of endocrine resistance is unfortunately inevitable. Although patients can be offered chemotherapy, treatment response is short-lasting and with the exception of eribulin (2), there is little value of chemotherapy after three lines of therapy. There is an urgent need for development of novel treatments.

Fibroblast growth factor receptor (FGFR) 1-4, are a family of protein tyrosine kinase transmembrane receptors with roles in development, differentiation and proliferation (3,4). Genetic aberrations in *FGFR*s have been reported in a variety of cancers including gastric, lung and breast cancer (4-6). Genetic events activating the FGFR pathway include receptor amplification, receptor mutation, and generation of aberrant receptor fusions through genetic translocation (4). The *FGFR1* gene is amplified in about 14% of breast cancers and is associated with HR^+/ $HER2^-$ disease (7,8). The 11q13 amplicon contains genes for FGF3, FGF4 and FGF19 proteins that are ligands of FGFR1. Upon binding of FGFs to FGFRs, receptor dimerization activates downward cascade signalling pathways including the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-AKT pathways, ultimately regulating cell proliferation, differentiation and survival (3,9). FGFs also induce neo-angiogenesis with a direct effect on both vessel assembly and sprouting (10). Amplification of *FGFR1* and 11q13 may lead to increased signalling in the FGF/FGFR pathway and mediation of resistance to targeted and endocrine therapies (6). Up to 25% of breast cancers have either *FGFR1* amplification or 11q13 amplification or both. The 11q13 amplicon also contains *CCND1* which is a cell cycle gene encoding cyclin D1. *CCND1* amplification, occurring in 15% of breast cancers, has been shown to be associated with estrogen receptor (ER) positivity and poor prognosis (11,12).

Blocking the FGF/FGFR pathway with multi-targeted inhibitors may enhance the anti-tumor activity by targeting pro-angiogenic and proliferative pathways. Several preclinical studies have suggested that targeting FGFR1 in *FGFR1*-amplified cell lines leads to anti-tumour effects (13,14). Furthermore, FGFR1-knock down was shown to decrease cell proliferation and reverse resistance to endocrine therapy in *FGFR1*-amplified breast cancer cell lines (15). Lucitanib is a potent inhibitor of vascular endothelial growth factor receptor (VEGFR)

1-3, FGFR1-3 and platelet derived growth factor receptor (PDGFR) α/β, with promising anti-tumor activity in xenograft models. Among the heavily pretreated FGF-aberrant breast cancer patients in a phase I first-in-human study of lucitanib at daily doses of 5 to 20mg, ORR was 50% (6/12 patients) with a median progression-free survival (PFS) of 40.4 weeks (16). This compelling clinical activity led to the initiation of this global multicentre phase II study of lucitanib in HR^{+}/ $HER2^{-}$ MBC in 3 selected populations (*FGFR1* or 11q13 amplified or non-amplified) and to explore the role of *FGFR1* or 11q13 amplifications through translational analyses.

METHODS

Study Participants and Design

FINESSE study (CL2-80881-001/ BIG2-13/ EudraCT 2013-000288-10/ NCT02053636) was an open label, multi-centre, phase II, 2-stage trial testing oral administration of single agent lucitanib in 3 cohorts of patients with histologically confirmed HR^+/ $HER2^-$ MBC: Cohort 1) *FGFR1*-amplified irrespective of 11q amplification, Cohort 2) *FGFR1*-non-amplified with 11q amplification, Cohort 3) *FGFR1*-non-amplified without 11q amplification (Supplementary Figure S1A and S1B). These patients had received at least 1 line of systemic anti-cancer therapy in the metastatic setting, but no more than 2 lines of chemotherapy. There was no limit to lines of prior endocrine therapy or targeted therapy. All patients had measurable disease at baseline and had demonstrated disease progression by radiological or clinical assessment. Men and women of at least 18 years of age, Eastern Cooperative Oncology Group (ECOG) performance status (PS) $\leq$2, a life expectancy of over 3 months and a left ventricular ejection fraction of at least 50% were eligible to enrol. Patients were ineligible if they received bevacizumab within 3 months of the first dose of lucitanib, had uncontrolled arterial hypertension requiring more than 2 anti-hypertensive agents, were at risk of developing hypertension-related complications, had a previous stroke, history of renal impairment, past history of thromboembolism in the last 6 months, uncontrolled thyroid function, uncontrolled diabetes mellitus, QTc prolongation or the use of medications with strong effect on CYP2C8 or CYP3A4 within 7 days of starting lucitanib. Central nervous system metastases without the requirement of high-dose steroid treatment were allowed if clinically stable for at least 4 weeks.

It was mandatory for all patients to submit adequate tumor tissue either obtained at the time of study or previously archived from a metastatic biopsy. For patients with non-amplification of both *FGFR1* and 11q assigned to cohort 3, if the initial submitted tissue was from archival material, a fresh biopsy from a metastatic site was required before starting study drug for subsequent confirmation of molecular status. Blood samples were collected on Cycle 1 Day 1, Cycle 1 Day 14 and end of treatment for soluble growth factor analyses.

Treatments

Lucitanib was administered orally, once daily, on a continuous schedule in fasting conditions. A mandatory checklist for optimal management of hypertension was completed by the investigator for each patient. All patients were trained to measure blood pressure

daily using the provided equipment on the first cycle and at least twice a week thereafter. Patients were advised to immediately contact the hospital if blood pressure was abnormal. After each adverse event of hypertension, daily self-monitoring of blood pressure was recommended for the subsequent 4 weeks. The starting dose was reduced from 15mg to 10mg after protocol amendment 5 due to high rates of grade $\geq$3 hypertension. For patients who enrolled prior to this protocol amendment, the dose was reduced to 10mg when starting the next 4-weekly cycle, unless the treating physician chose to continue treatment at 15mg. Following an adverse event, dose reduction to 7.5mg and 5mg daily could be considered, but dosing below 5mg was not allowed. Patients continued treatment until disease progression, intolerable toxicity, physician decision or consent withdrawal (Supplementary Table S1).

Assessments

Each of the 3 cohorts was evaluated separately. The primary endpoint was objective response rate (ORR), defined as the proportion of patients with complete response (CR) or partial response (PR) as best overall response, evaluated by the investigator every 8 weeks by computed tomography or magnetic resonance imaging according to Response Evaluation Criteria in Solid Tumours (RECIST) criteria version 1.1. Secondary endpoints included clinical benefit rate (CBR), progression-free survival (PFS), duration of response (DOR), safety and pharmacokinetics of lucitanib. CBR was defined as the proportion of patients for whom a confirmed CR or a confirmed PR or prolonged stable disease (SD, according to RECIST criteria for at least 24 weeks from inclusion) was observed during the treatment. Toxicity was graded according to the Common Terminology Criteria for Adverse Event (CTCAE) version 4.0. All patients measured their blood pressure daily at home at screening and on the first cycle, then at least twice a week thereafter during the study duration. Patients were advised to contact the hospital immediately in the event of abnormal blood pressure values. An independent data monitoring committee regularly reviewed activity and safety data during the course of the trial and made recommendations regarding changes or adjustments required to ensure patient safety and preserve study integrity. Exploratory endpoints were to characterise the biological activity of lucitanib on soluble growth factors of interest, on tumor cells and to explore biomarkers potentially predictive for lucitanib response in blood samples and in primary archived or metastatic tumors.

Statistical Analysis

For each of the 3 cohorts, sample size was estimated to assess the anti-tumor activity of lucitanib, based on a Simon's optimal 2-stage design (17) with the hypotheses H_0: p ≤ 5% versus H_1: p ≥ 20%. With a type I error at 5% (one sided) and a 90% statistical power, 21 patients were required for the first stage, with early termination if there were fewer than 2 confirmed responses in stage 1. Otherwise, 20 more patients (for a total of 41 patients in each cohort) were to be recruited. The null hypothesis would be rejected if there were at least 5 responders among all 41 patients in that cohort with responses. Therefore, the total sample size was between 63 patients (in case of early termination in each group of patients) and 123 patients (if no early termination).

The statistical analysis plan was finalised before the database lock on 19 July 2017. There was no statistical test intended to compare cohorts or dose levels. The statistical analyses were descriptive. The 95% Wilson's confidence interval for rates was computed based on inverting the normal test that uses the null proportion in the variance. The median duration and 95% confidence interval for time-dependent parameters including PFS, DOR and duration of clinical benefit were estimated using the Kaplan-Meier method.

Biomarker Analysis

Determination of FGFR1, CCND1 and FGF3/4 and 19 Copy number by FISH

The FISH analyses were performed centrally at ZytoVision (Germany) GmbH using the Zyto*Light*® SPEC *FGFR1*/CEN 8 Dual Color Probe (IVD-CE FISH probe, Z-2072-200), Zyto*Light*® SPEC *CCND1*/CEN 11 Dual Color Probe (IVD-CE FISH probe, Z-2071-200) and Zyto*Light*® SPEC FGF3,4,19/CEN 11 Dual Color Probe (IVD-CE FISH probe), all with the "Zyto*Light*® FISH-Tissue Implementation Kit".

Evaluation of FISH was performed following adapted Schildhaus criteria (18). Copy number ratio was calculated as the average number of target gene signals per cell divided by the average number of centromeric signals per cell.

For the purposes of recruitment, *FGFR1* was considered "amplified" if its gene/centromere ratio was ≥ 2 and/or if its average number of signals per tumor cell nucleus was ≥ 6. For exploratory biomarker analysis, samples were classified as high amplified (*FGFR1*/centromere ratio ≥ 4), amplified (Ratio ≥2 but <4 or average signal ≥6) or not/low amplified (Ratio <2).

Similarly, for the purposes of recruitment, *CCND1* was used as a surrogate for 11q13 amplification. All samples identified as amplified for *CCND1* were also assessed for *FGF3/4/19* copy number. Samples were considered "amplified" for *CCND1* and *FGF3/4/19* if the gene/centromere ratio was ≥ 2 and/or if the average number of signals per tumor cell

nucleus was ≥ 6. All samples identified as having *CCND1* amplification were also amplified for *FGF3/4/19* (Supplementary Figure S2).

Serum FGF23 using ELISA

The concentration of FGF23 in serum and plasma samples was determined using the FGF23 ELISA kit from KAINOS LABORATORIES, INC (cat#, CY4000), according to the manufacturer's guidelines). Performance of the FGF23 ELISA is presented in Supplementary Table S2A and range of determined concentrations in Supplementary Table S2B.

FGFR1 CNV ddPCR

Tumour content of tissue sections was determined by a pathologist from the Breast Cancer Now Histopathology Core facility, Institute of Cancer Research, London, UK. Tissue sections were stained with nuclear fast red and the tumour rich area dissected. DNA and RNA were extracted using AllPrep DNA/RNA FFPE extraction kit (QIAGEN 80234) according to the manufacturer's guidelines with an overnight digestion of the DNA containing pellet the only modification (19). DNA extracted from tumour samples was analysed to determine *FGFR1* CNV using ddPCR following the method of Pearson *et al* (19). Digital PCR was performed on a QX100 droplet PCR system (Bio-Rad). PCR reactions were prepared as previously described (20,21). Briefly, emulsified PCR reactions were run on a 96 well plate on a G-Storm GS4 thermal cycler incubating the plates at 95°C for 10 min followed by 40 cycles of 95°C for 15 sec, 60°C for 60 sec, followed by 10 min incubation at 98 °C. Plates were read on a Bio-Rad QX100 droplet reader using QuantaSoft v1.6.6.0320 software. Copy number variation was calculated as a ratio with multiplexed reference genes (Supplementary Table S2C). Copy number variation assays were performed using 1-3ng genomic DNA, to obtain a minimum of 300 reference droplets.

FGFR1 Immunohistochemistry

Immunohistochemistry for FGFR1 was performed using 3µm tissue sections, probed with anti-human FGFR1 (Abcam ab76464). Chromogenic signal was developed using ChromoMap DAB detection kit (Roche Diagnostics, 052666450010). Tissues sections were counter stained with haematoxylin and coverslips mounted using Pertex (Histolabs, 00811). Scoring for protein expression was determined according to the hybrid scoring system (H-score) criteria by a pathologist. Specimens were scored based on the different cellular compartment (e.g. cytoplasmic, membranous and total). Scoring was performed with the H-

score based on the percentage of tumor cells staining at various intensities as follows: 0x (% tumor cells with no staining) + 1x (% with faint expression) + 2x (% with moderate expression) + 3x (% with strong expression).

RESULTS

Patient characteristics and treatment

Between 19 Dec 2013 and 4 Aug 2016, amongst a total of 129 patients screened for the study, 76 patients were enrolled from nine countries. Thirty-two patients were recruited to cohort 1 with amplified *FGFR1* irrespective of 11q13 amplification, 18 to cohort 2 with 11q13 amplification but *FGFR1* non-amplified, and 26 to cohort 3 with both *FGFR1* and 11q13 non-amplified (Supplementary Figure S1B). Fifty-nine patients received lucitanib at starting dose of 15mg daily and 17 patients at a lower starting dose of 10mg after protocol amendment. The median age was 54 years (range 26-78), 66% of patients had ECOG PS of 0, 86% were post-menopausal and the median time from the diagnosis of MBC was 2.4 years (range 0.2-12.6). The majority (82%) of the tumors were ductal and 38% were grade 3. Of all the patients, 50% had bone metastases and 36% had liver metastases; 99% received prior endocrine therapy and 92% received at least 1 line of chemotherapy. The baseline characteristics were similar in the 3 cohorts (Table 1).

Outcome

In cohorts 1 and 3, two responses were observed during stage 1 of the study, thus additional patients were enrolled into stage 2 of the study. The ORR in the entire population was 13% (95% CI: 7–23%; Table 2). The waterfall plot on Figure 1A illustrating the best relative change in sum of the size of target lesions from baseline suggested anti-tumor activity of lucitanib in all cohorts, but partial responses were only evident in cohorts 1 and 3, with no confirmed responses observed in cohort 2 per RECIST criteria. The ORR was 19% (95%CI: 9-35%) and 15% (95%CI: 6-34%) in cohorts 1 and 3, respectively. Clinical benefit rates (CR, PR and SD$\geq$24 weeks) were 41% (95%CI: 26 - 58), 11% (95%CI: 3 - 33), 27% (95%CI: 14- 46) in cohorts 1, 2, 3 respectively and 29% (95%CI: 20-40) in the entire population (Table 2). Among the patients who achieved PR, the median time to response was 90 days and the median duration of response was 129 days (Table 2). The overall median PFS was 113 days (approximately 3.7 months; 95% CI: 69-164 days) and numerically shortest in cohort 2 (Fig 1B).

Safety

Safety was assessed in all patients who received at least 1 dose of lucitanib. The most frequent treatment-related adverse event (AE) was hypertension with 88% of any grade and 66% of grade $\geq$3. The median time to onset of grade 3-4 hypertension was 7.5 days. Other

common treatment-related AEs (all grades / grade 3-4) included hypothyroidism (45% / 0%), nausea (33% / 1%), proteinuria (32% / 0%), diarrhoea (30% / 1%) and fatigue (30% / 4%; Table 3). Due to difficulty to sustain more than 3 cycles of 15 mg daily lucitanib in the first 59 patients, mainly because of hypertension, the starting dose was reduced to 10 mg daily for the subsequent 17 patients enrolled. Despite the dose reduction to 10mg daily lucitanib, 8/17 (47%) patients still experienced grade 3-4 hypertension. However, hypertension resolved in 77% patients after drug discontinuation and proteinuria resolved in 68% at the end of the study. Most AEs were adequately managed with dose reductions, interruptions and the use of appropriate supportive treatments. A case of Posterior Reversible Encephalopathy Syndrome (PRES) at 15 mg daily lucitanib was observed in one patient, but all symptoms completely resolved after stopping the study drug. AEs led to treatment discontinuation in 16 patients (21%), of which 6 were due to hypertension and 1 was due to proteinuria. Treatment interruption and dose reduction occurred in 63% and 66% of patients, of whom 67% and 89% respectively were due to AE (Supplementary Table S1). One patient died of unknown causes.

The pre-specified primary study objective of rejecting the null hypothesis if at least 5 responders among 41 patients was achieved in cohort 1 with *FGFR1* amplified HR^+/ $HER2^-$ MBC (PR in 6/32 patients). However, on the basis of a risk/benefit analysis run on all available data of the lucitanib breast cancer clinical development program showing that lucitanib was not likely to be superior to standard of care, the sponsor decided to terminate the study. Nonetheless, patients under treatment at that moment were offered the option to continue lucitanib following discussion with their treating physician.

Biomarker analyses

Evidence of drug activity

Serum FGF23 levels after 14 days of lucitanib were significantly increased from baseline (median increase by 45%, p = 1.74e- 06), suggesting effective targeting of FGFR (5,22-24). Increases in serum FGF23 were similar in all 3 cohorts and were regardless of treatment response. Similar findings were observed in plasma samples (Fig 2A).

Relationship between FGFR amplification / expression and anti-tumor activity

Tissue was available from all 76 patients for analysis of *FGFR* amplification by FISH. Of these, 53 samples were available for *FGFR1* CNV ddPCR and 59 were available for IHC to assess FGFR1 protein expression. Exploratory biomarker analyses suggested that patients

classified as *FGFR1* highly amplified by FISH (*FGFR*1/centromere ratio ≥4, n=23) presented higher ORR than those without high level amplification (<4, n=53): 22% (5/23) versus 9% (5/53; Fig 2B). By contrast, 11q amplification might be associated with poor response (2/29= 7% responders). FISH and ddPCR showed good agreement (p=0.79) and assessment of *FGFR1* copy number using ddPCR gave similar results with ORR of 25% (4/16, ≥4) versus 8% (3/37, <4; Fig 2B). A similar level of agreement was detected between *FGFR1* FISH signals or ddPCR copy numbers and FGFR1 IHC H-score (p = 0.71, data not shown). FGFR1 over-expression was mostly detected for patients with *FGFR1* amplification (24/27; 89%). Further, in patients with high FGFR1 expression (H-score ≥50), assessed by IHC, ORR was higher (25%, 5/20) than in patients with low FGFR1 expression (8%, 3/39; Fig 2B). Interestingly, patients with *FGFR*1 high amplification (FISH ≥4) had 49 days (approximately 2 months; 158 [57-332] days vs 109 [56-165] days) nominally longer median PFS than those with no amplification (<2; Supplementary Figure S3A). Patients with higher FGFR1 expression (H-score ≥50) also had nominally longer median PFS than those with FGFR-low tumours (H-score <50) by 103 days (approximately 3 months; 212 [165-NA] days vs 109 [57-158] days; Supplementary Figure S3B). Endothelial expression of FGF2 or Ki67 was not different between cohorts (Supplementary Figure S4). Similarly, no trend of association was observed between PFS and endothelial expression of either FGF2 or Ki67 (Supplementary Figure S5A and S5B).

DISCUSSION

Breast cancer is a heterogenous disease with the largest proportion being HR^+/ $HER2^-$. Despite recent advances with the addition of targeted therapy including CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib), mTOR inhibitor (everolimus) and PI3K inhibitor (alpelisib) to endocrine therapy (25-27), ultimate treatment resistance is unavoidable. Dysregulation of FGFR/FGF pathway is often observed in human cancers including 14% of breast cancers (28) and may act as driver of tumor progression. In this study, 42% (32/76) of enrolled patients had *FGFR1* amplified breast tumours, with 30% displaying high level of amplification (≥4 CNV). This higher prevalence of *FGFR1* amplification in this study is likely due to selection bias, as some patients might have already undergone prior local molecular testing. Moreover, recruitment of patients without *FGFR1* amplification but with 11q13 amplification to cohort 2 was stopped early due to the lack of treatment response. Fifty-nine patients had adequate tissue for IHC assessment and a third of the breast cancers overexpressed FGFR1 with H-score of ≥50.

FGFR1 amplification was previously shown to be associated with resistance to endocrine therapy, shorter time to distant metastasis (15) and shorter overall survival (7) in HR^+ breast cancer. Activation of the FGFR1/FGF pathway induces neo-angiogenesis and mediates resistance to VEGFR inhibitors, highlighting the need for multi-targeted tyrosine kinase inhibitors (TKI) such as lucitanib (29,30). The modest ORR of 13% (95% CI: 7-23%) in the entire population of metastatic HR^+/ $HER2^-$ breast cancer patients in this study was higher than the ORR of the unselected metastatic breast cancer patients in another lucitanib study which included triple negative and HER2-positive subtypes (31). Furthermore, in cohort 1 patients with *FGFR1* amplification, the activity of lucitanib with an ORR of 19% and a CBR of 41% was similar to single agent CDK4/6 inhibitor abemaciclib (32) or chemotherapy including eribulin and capecitabine (33) in previously treated MBC. Although CBR might be a less reliable endpoint in a small phase II study as it could be attributed by the natural history of indolent disease, the ORR of lucitanib was higher than monotherapy palbociclib (34) or everolimus (35). Exploratory biomarker analyses showed an apparent increased ORR with higher *FGFR1* amplification (≥4 CNV) as assessed by either FISH or ddPCR as compared with low or no *FGFR1* amplification. This was consistent with the results from a phase II study of another FGFR1 multi-kinase inhibitor, dovitinib, which showed an ORR of 25% in patients with ER-positive breast cancer harbouring *FGFR1* amplification (22). *FGFR1* amplification has been reported to correlate with FGFR1 overexpression and is

associated with endocrine resistance (15). In our study, despite no definite correlation between *FGFR1* amplification and overexpression of FGFR1 protein, nominally higher ORR and longer PFS were observed in patients with high FGFR1 membrane H-score of ≥50 by IHC. FGFR1 expression has been shown to predict sensitivity to FGFR inhibitors in lung cancer as well as head and neck cancer (36,37). To our knowledge, this is the first study to suggest FGFR overexpression may be a potential biomarker of response to FGFR1 TKI in breast cancer.

Unlike selective FGFR inhibitors, but similar to another multi-target TKI dovitinib, hyperphosphataemia was not reported in patients treated with lucitanib which may suggest inadequate inhibition of the FGFR pathway (9,22) or counteractive effect of the frequently observed hypophosphatemia with VEGFR inhibitors (38). However, the increase in serum FGF23 after 14 days of treatment with lucitanib in the pharmacodynamic assay suggested lucitanib was targeting FGFRs. The toxicity profile characterised by hypertension and proteinuria was consistent with the action of lucitanib as an inhibitor of VEGFR (39). The anti-tumor activity evident in cohort 3 with non-amplification of both *FGFR1* and 11q13 was likely due to the anti-angiogenic effects of lucitanib.

Although 11q13 amplification with aberrations of the ligands (FGF3, 4 and 19) to FGFRs may lead to dysregulation of FGFR/FGF pathway, no treatment response to lucitanib was observed in cohort 2 patients with their breast cancers harboring only 11q13 amplification without *FGFR*1 amplification. This suggests that the presence of FGF ligands in the 11q amplicon may have limited significance in breast cancer. In this study, 11q13 amplification was assessed by copy number of *CCND1*. Amplification of *CCND1* is associated with increased cyclin D1 expression and poor prognosis in ER^+ $HER2^-$ breast cancer (40-42). Cyclin D1 with its catalytic subunit CDK4/6 phosphorylates retinoblastoma protein, initiating G1/S progression in the cell cycle. The key oncogenic driver of the breast cancers in the cohort 2 patients may be cyclin D/ CDK4/6 pathway instead of FGFR/FGF pathway. Hypothetically CDK4/6 inhibitors may be more effective than FGFR1 inhibitor in this group of patients; however studies thus far have shown that *CCND1* amplification is not a predictive biomarker of CDK4/6 inhibitors in breast cancer treatment (43).

Previous studies selected patients with *FGFR* amplification based on criteria used for assessment of *ERRB2* copy number (CNV ≥2) (44). In gastric cancer, tumours with high levels of homogenous amplification of *FGFR2* were found to have marked sensitivity to

inhibition of FGFR (19). Consistent with this, patients in this study with higher FGFR1 expression and/or high level copy number tended to have greater clinical benefit, suggesting that more stringent cut-offs should be applied when selecting patients based on *FGFR* status. Similarly, tumour heterogeneity, in terms of CNV and mutational burden, is recognised as a significant factor in the development of resistance by tumours in response to targeted therapies (45-47). Heterogeneity and active clonal dynamics have been characterised in the progression of early breast cancer (48). The limited anti-tumour activity reported here may in part be explained by patients being recruited to this study by *FGFR1* amplification status, without addressing the degree of heterogeneity in *FGFR1* CNV among tumour cells. Furthermore, mutations in the FGFR downstream signalling pathways, including Ras/MAPK and PI3K, confer resistance to FGFR inhibitors *in vitro* (15,45). Mutations in *PIK3CA* are among the most common in metastatic breast cancer (8) and thus pre-existing genetic events may further limit the effectiveness of drugs such as lucitanib irrespective of *FGFR1* amplification status.

Preclinical studies in cell lines and xenografts have demonstrated more effective inhibition of tumor cell growth with combined blockade of FGFR1 and ER using both lucitanib and fulvestrant (28). The efficacy of lucitanib in combination with fulvestrant in a small study showing CBR of 55.6% in metastatic HR^+/ $HER2^-$ breast cancer patients with unselected *FGFR1* status appeared to be numerically higher than monotherapy lucitanib in our study (49). Given the role of *FGFR1* aberrations in the development of endocrine resistance, it may be useful to explore the combination of lucitanib and fulvestrant as a potential treatment for HR^+/ $HER2^-$ *FGFR1* amplified breast cancers after resistance to first-line endocrine therapy.

Based on the first-in-human study results, 15 mg daily lucitanib was initially selected as the recommended phase II dose for this study (16). However, 15 mg continuous daily dosing was difficult to sustain beyond 3 cycles, with the predominant side effect of arterial hypertension related to the anti-angiogenic effect of lucitanib. Similar safety profiles across lucitanib clinical studies (16,22,31,49) have been observed. Although reduction in the starting dose of lucitanib to 10mg resulted in an improved safety profile with lower incidence of grade 3 hypertension, a substantial rate of hypertension still occurred. However, only 6 patients (8%) permanently discontinued treatment due to hypertension, as most patients could be managed with dose adjustment and supportive measures. Hypothyroidism was the second most common AE, consistent with the toxicity profile of other multi-target TKI such

as sunitinib (50), but could be easily managed with thyroid hormone supplementation. Further exploration of biomarkers in selecting patients who may benefit from a FGFR inhibitor may also avoid unnecessary side effects.

In conclusion, single agent lucitanib showed limited anti-tumor activity in HR^+/ $HER2^-$ MBC and significant hypertension-related toxicity, but with a higher ORR and CBR in a subset of patients with *FGFR1* amplification. Exploratory biomarker analyses suggested that patients whose tumors had high *FGFR1* amplification or FGFR1 expression might derive greater benefit. While the study was stopped prematurely based on a decision by the sponsor after evaluating risk and benefit of lucitanib monotherapy, the benefit of lucitanib treatment to MBC patients with *FGFR1* amplification or overexpression deserves further exploration.

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TABLES

Table 1 Baseline Characteristics of Patients Enrolled in the Three Cohorts

Characteristics	Cohort 1 *FGFR1* Amp		Cohort 2 11q13 Amp		Cohort 3 Both Non-Amp		All	
	n	%	n	%	n	%	n	%
Female	32	100%	18	100%	26	100%	76	100%
Age (median, years)	53		52		57		54	
Ethnicity								
White	24	75%	18	100%	21	81%	63	83%
Asian	2	6%	-	-	1	4%	3	4%
Other	4	13%	-	-	-	-	4	5%
Unkown	2	6%	-	-	4	15%	6	8%
ECOG								
0	21	66%	13	72%	16	62%	50	66%
1	8	25%	5	28%	9	35%	22	29%
Disease duration (median, years)	6.1 (1.4-20.1)		6.0 (1.3-20.0)		7.4 (1.8-19.4)		6.7 (1.3-20.1)	
Time since diagnosis of MBC (median, years)	2.3 (0.3-7.3)		2.0 (0.7-5.7)		3.5 (0.2-12.6)		2.4 (0.2-12.6)	
PFS of the last treatment received (median, days)	217 (20-1181)		251 (13-1884)		246 (40-1105)		241 (13-1884)	
Histology Type								
Ductal	29	91%	14	78%	19	73%	62	82%
Lobular	-	-	2	11%	3	12%	5	7%
Other	3	9%	2	11%	4	15%	9	12%
Histology Grade								
Grade 1	1	3%	2	11%	7	27%	10	13%
Grade 2	15	47%	5	28%	10	39%	30	40%
Grade 3	15	47%	6	33%	8	31%	29	38%
Unknown	1	3%	5	28%	1	4%	7	9%
HER2 status								
Positive	2	6%	-	-	-	-	2	3%

Negative	30	94%	18	100%	26	100%	74	97%
ER status								
Positive	31*	97%	17	94%	26***	100%	75	99%
Negative	-	-	1**	6%	-	-	1	1%
PR status								
Positive	11	34%	6	33%	10	39%	27	36%
Negative	4	13%	5	28%	7	27%	16	21%
Metastatic sites								
Bone	13	41%	12	67%	13	50%	38	50%
Liver	13	41%	7	39%	7	27%	27	36%
Brain	0	0%	0	0%	1	4%	1	1%
Previous treatment								
Endocrine therapy	32	100%	17	94%	26	100%	75	99%
Letrozole or Anastrozole	27	84%	16	89%	23	89%	66	87%
Tamoxifen	24	75%	13	72%	23	89%	60	79%
Exemestane	13	41%	5	28%	15	58%	33	43%
Fulvestrant	6	19%	3	17%	5	19%	14	18%
Everolimus	7	22%	1	6%	7	27%	15	20%
Palbociclib	-	-	1	6%	-	-	1	1%
Chemotherapy	30	94%	17	94%	23	89%	70	92%
Bevacizumab	2	6%	2	11%	2	8%	6	8%

n: Number of patients with at least one medical history of breast cancer. %: (n/N)*100. *One value was missing. **One patient was first found ER^+ based on an archived biopsy (before Amendment No. 4), but was found ER^- on a new baseline biopsy (that was performed because no metastatic material was available for the inclusion in the study). A re-test confirmed the tumor status of ER^-, however the patient stayed in the study on investigator's request. ***For 1 patient there were 2 observations: one on an archived biopsy + a new baseline biopsy for ER status.

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Table 2. Summary of anti-tumour activities of lucitanib.

	Cohort 1 *FGFR1* Amp	Cohort 2 11q13 Amp	Cohort 3 Both Non-Amp	All
Objective response rate (n, %) (95% CI)	6 (19%) (9-35)	0 (0%)	4 (15%) (6-34)	10 (13%) (7-23)
Time to first response (median, days) (range)	90 (44-164)	-	82 (53-166)	90 (44-166)
Duration of response (median, days) (95% CI)	264 (106-337)	-	108 (88-392)	129 (88-337)
Clinical benefit rate* (n, %) (95% CI)	13 (41%) (26-58)	2 (11%) (3-33)	7 (27%) (14-46)	22 (29%) (20-40)
Progression free survival (median, days) (95% CI)	148 (96-212)	108 (54-140)	141 (52-214)	113 (69-164)

*** Clinical benefit rate: CR + PR + SD for ≥24 weeks**

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Table 3 Most frequently reported treatment-related adverse events (at least 5 patients overall)

	Cohort 1 *FGFR1* Amp (n = 32)		Cohort 2 11q13 Amp (n = 18)		Cohort 3 Both Non-Amp (n = 26)		All (n = 76)	
	n	%	n	%	n	%	n all grades (grade 3-4)	% all grades (grade 3-4)
Hypertension	28	88	14	78	24	92	66 (50)	87 (66)
Hypothyroidism	20	63	4	22	10	39	34 (0)	45 (0)
Nausea	14	44	2	11	9	35	25 (1)	33 (1)
Proteinuria	12	38	6	33	6	23	24 (0)	32 (0)
Fatigue	15	47	3	17	5	19	23 (3)	30 (4)
Diarrhoea	12	38	4	22	7	27	23 (1)	30 (1)
Headache	9	28	2	11	7	27	18 (0)	24 (0)
Asthenia	7	22	3	17	6	23	16 (2)	21 (3)
AST increased	11	34	2	11	2	8	15 (1)	20 (1)
ALT increased	10	31	2	11	2	8	14 (2)	18 (3)
Vomiting	6	19	2	11	5	19	13 (0)	17 (0)
Thrombocytopenia	6	19	1	6	5	19	12 (2)	16 (3)
Reduced Appetite	6	19	1	6	5	19	12 (0)	16 (0)
GGT increased	5	16	3	17	3	12	11 (6)	15 (8)
Abdominal pain	6	19	1	6	3	12	10 (0)	13 (0)
Abdominal pain upper	5	16	2	11	3	12	10 (0)	13 (0)
ALP increased	4	13	2	11	1	4	7 (1)	9 (1)
Myalgia	2	6	2	11	2	8	6 (1)	8 (1)

FIGURE LEGENDS

Figure 1. Efficacy of lucitanib in metastatic HR^+/ $HER2^-$ breast cancer by cohorts. A) Best relative change in sum of the size of target lesions from baseline; ǂ for this patient, partial response was defined after external review of the imaging. Of note, the reasons for the 10 patients who are not on the above graph showing 66 / 76 are: 1 patient only had non-target lesions; 8 patients had a BOR = NE; 1 patient had a BOR = PD but did not appear because, post-baseline, there was no evaluation on target lesions, only a new non-target lesion. B) Progression-free survival. PR: partial response; CR: complete response; PD: progression of disease.

Figure 2. A) Serum FGF23 levels at baseline and at cycle day15. p= 1.7422e-06 Wilcoxon test. B) Association of biomarkers with objective response. No statistical analyses of association were performed due to small sample size.

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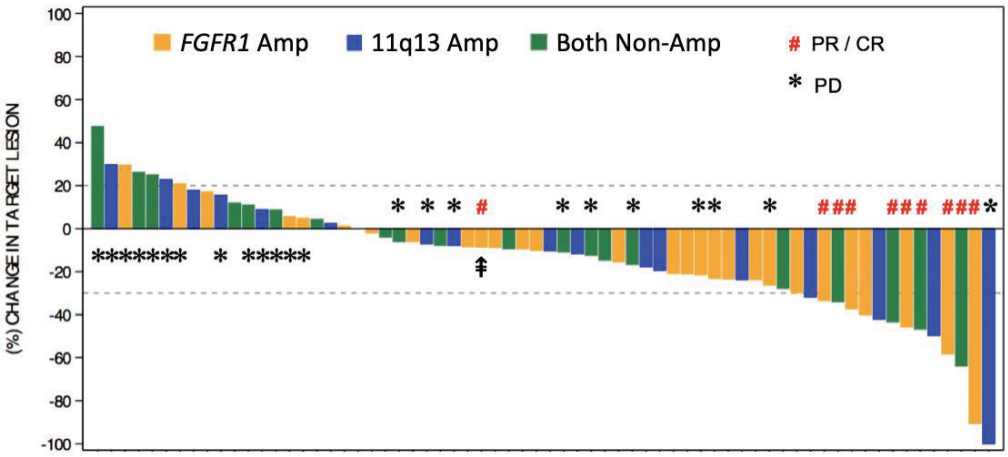

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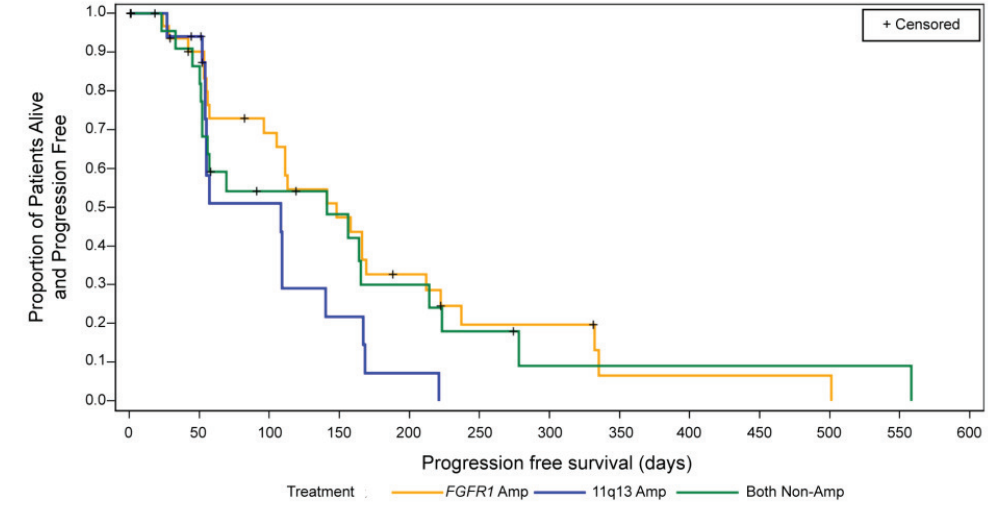

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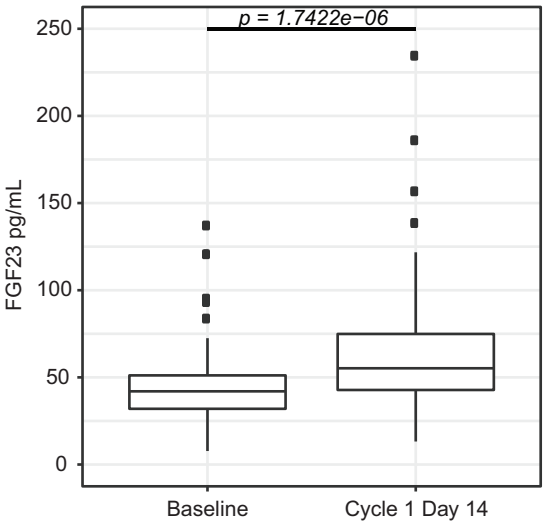

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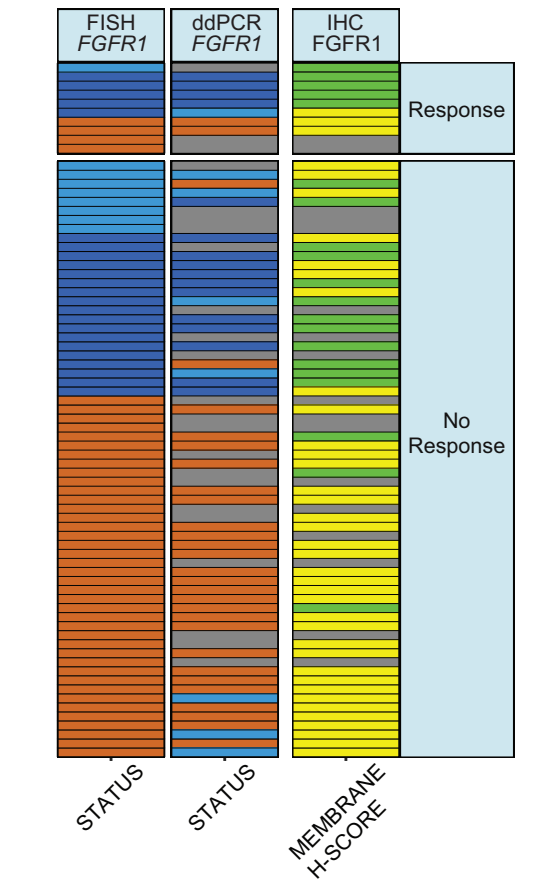

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