Combination of trastuzumab and letrozole after resistance to sequential trastuzumab and aromatase inhibitor monotherapies in patients with estrogen receptor-positive, HER-2-positive advanced breast cancer: a proof-of-concept trial (SAKK 23/03)

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Abstract

A sequential treatment design was chosen in this trial to ensure complete resistance to single-agent non-steroidal aromatase inhibitor (AI) and trastuzumab both given as monotherapy before receiving the combination of a non-steroidal AI and trastuzumab. Key eligibility criteria included postmenopausal patients with advanced, measurable, human epidermal growth factor receptor-2 (HER-2)-positive disease (assessed by FISH, ratio (≥2)), hormone receptor (HR)-positive disease, and progression on prior treatment with a non-steroidal AI, e.g. letrozole or anastrozole, either in the adjuvant or in the advanced setting. Patients received standard dose trastuzumab monotherapy in step 1 and upon disease progression continued trastuzumab in combination with letrozole in step 2. The primary endpoint was clinical benefit rate (CBR) in step 2. Totally, 13 patients were enrolled. In step 1, six patients (46%) achieved CBR. Median time to progression (TTP) was 161 days (95% confidence interval (CI): 82–281). In step 2, CBR was observed in eight out of the 11 evaluable patients (73%), including one patient with partial response. Median TTP for all the 11 patients was 188 days (95% CI: 77–not reached). Results of this proof-of-concept trial suggest that complete resistance to both AI and trastuzumab can be overcome in a proportion of patients by combined treatment of AI and trastuzumab, as all patients served as their own control. Our results appear promising for a new treatment strategy that offers a chemotherapy-free option for at least a subset of patients with HR-positive, HER-2-positive breast cancer over a clinically relevant time period.

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Introduction

Estrogens are the most important stimulators of growth in breast cancer. Approximately, 70% of breast cancers are estrogen dependent and therefore amenable to endocrine therapy. The development of primary and acquired resistance to anti-hormonal agents in breast cancer is a major therapeutic problem. Several mechanisms have been proposed contributing to the
development of this resistant phenotype. Acquired resistance has been linked with different mechanisms such as down-regulation or loss of estrogen receptor (ER), ER mutations, development of ligand-independent ER-mediated transcription, and perturbation of the interactions between ER and co-activators and co-repressors of transcription (Ali & Coomes 2002, Schiff et al. 2003, Ring & Dowsett 2004). About a quarter of breast cancers over-express the epidermal growth factor receptor (EGFR; Hynes & Lane 2005) and/or the human epidermal growth factor receptor-2 (HER-2; Slamon et al. 1987), which are generally considered to confer less endocrine-sensitive disease (De Laurentiis et al. 2005, Mauriac et al. 2007, Dowsett et al. 2008). Compelling evidence suggests that several growth factor pathways cross talk and network with ER, clinically translating to impaired efficacy of endocrine therapy (Arpino et al. 2008, Prat & Baselga 2008). Trastuzumab, a monoclonal antibody directed against HER-2, is the cornerstone in the treatment of patients with HER-2 over-expressing early and advanced breast cancer. Combining chemotherapy and anti-HER-directed therapy has been established as the treatment of choice in this patient population even though approximately half of the HER-2-positive primary breast cancers co-express hormonal receptors (Piccart-Gebhart et al. 2005, Romond et al. 2005). However, endocrine therapy remains a well-established additional treatment at least for patients with potentially endocrine-responsive HER-2-positive early breast cancer (Goldhirsch et al. 2009).

Based on this strong biological rational and on the cumulating evidence for improved efficacy by targeting both ER and HER family signaling, clinical trials in metastatic disease have been conducted with trastuzumab and EGFR/HER-2 tyrosine kinase inhibitors (gefitinib, erlotinib, and lapatinib) in combination with endocrine therapy. The primary goal to improve disease control over a prolonged time and delay endocrine resistance was achieved in most of these trials with combined treatment, compared with single-agent endocrine treatment (Johnston et al. 2009, Kaufman et al. 2009, Normanno et al. 2009, Huober et al. 2009).

When this trial was designed in 2003, the clinical relevance of the combined inhibition of both hormonal and growth factor pathways was undefined, and we assumed that exploring the sequential treatment design was the optimal strategy in this proof-of-concept trial to ensure complete clinical resistance to single-agent therapy before testing the combination of a non-steroidal aromatase inhibitor (AI) and trastuzumab. We speculated that any clinical anti-tumor activity with combined treatment after progression under single-agent treatments could indicate restoration of sensitivity as a consequence of cross talk between both pathways.

Patients and methods
Eligibility criteria
Postmenopausal women with advanced breast cancer expressing ER and/or PgR (≥10 fmol/mg cytosol protein or ≥10% of the tumor cells positive by immunohistochemical evaluation) and proven HER-2 gene amplification (≥2) by FISH were eligible. Documented progression after previous treatment with a non-steroidal AI, e.g. letrozole or anastrozole, either in the adjuvant or in the advanced setting was required. Prior therapy for advanced or metastatic disease with either chemotherapy or trastuzumab within the last 12 months prior to registration was prohibited, but prior neoadjuvant/adjuvant chemotherapy was allowed. Other eligibility criteria included WHO performance status 0–1 and adequate baseline hepatic, renal, and bone marrow function. Clinical symptoms or history of CNS metastases or leptomeningeal disease, visceral involvement with risk for organ dysfunction, uncontrolled cardiac disease, and any other serious underlying medical conditions, which could impair the ability of the patient to participate in the trial, were additional exclusion criteria. In the screening phase, a maximum time window of 28 days was accepted between diagnosis of progressive disease and study inclusion.

The protocol was approved by local ethics review boards and all patients provided written informed consent. The trial was registered at the National Institute of Health (www.clinicaltrial.gov; identifier number: NCT00238290) and performed in accordance with the Declaration of Helsinki, the guidelines of Good Clinical Practice issued by ICH and Swiss regulatory authorities’ requirements.

Treatment plan
This was a multicenter, two-step, phase II trial (Fig. 1). Treatment in step 1 consisted of trastuzumab mono-therapy given at the initial loading dose of 4 mg/kg i.v. followed by a weekly dose of 2 mg/kg i.v. or at the initial loading dose of 8 mg/kg i.v. followed by 6 mg/kg i.v. doses every 3 weeks (at the discretion of the investigator) until tumor progression. After tumor progression in patients eligible for step 2 (same eligibility criteria as at trial entry), trastuzumab was continued (at the same dose and schedule as in step 1) together with letrozole 2.5 mg daily. All patients
were followed up until disease progression, at which point further treatment according to the best local practice was implemented.

**Trial assessments**

Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (Therasse et al. 2000) based on CT or MRI scans or palpation performed at baseline and every 12 weeks. Bone lesions measured with MRI were considered as measurable lesions. Objective responses (complete or partial) were confirmed after a minimum of 4 weeks. Adverse events (AEs) were graded using the Common Terminology Criteria for Adverse Event, version 3 (CTCAE v. 3.0).

**Statistical design and analysis**

Sample size calculations deduced that 20 evaluable patients were required for step 2. A CBR of at least 20% in step 2 would have been considered clinically interesting.

The primary endpoint was the clinical benefit rate (CBR), defined as the proportion of patients with a confirmed complete or partial response (PR) or stable disease (SD) for at least 6 months (24±1 week) under combined treatment with trastuzumab and letrozole (step 2). The rate of patients with CBR in step 2 and the corresponding exact 95% Clopper–Pearson confidence interval (CI) were produced.

Secondary endpoints included time to progression (TTP; for single-agent trastuzumab treatment (step 1: from registration until first progression or death); for combined treatment with trastuzumab and letrozole (step 2: from first day of combined treatment until second progression or death)) and time to treatment failure (TTF; due to unacceptable toxicity, progression, symptomatic deterioration, death, or patient refusal (step 1: from registration until treatment failure; step 2: from first day of combined treatment until treatment failure)). All time-to-event endpoints were analyzed using the Kaplan–Meier method to estimate the median values together with their 95% CIs and to produce the corresponding curves.

Other secondary endpoints included CBR in step 1, duration of CBR, and safety. AEs were summarized by event type and grade over the total number of patients (worst recorded AE grade per patient).

Prospective exploratory subgroup analyses were also planned for patients with high-level amplified HER-2 tumors (HER-2 signal:centromere ratio ≥4) compared with low-level amplified HER-2 tumors (HER-2 signal: centromere ratio =2 or 3) for CBR, TTP, TTF, and duration of CBR, and for tumor response in patients with tumors showing a HER-2 signal:centromere ratio ≥6 or clustering of HER-2 signal compared with tumors with a HER-2 signal:centromere ratio <6 in steps 1 and 2.

Data analysis was performed using SAS 9.2 (SAS Institute, Inc., Cary, NC, USA).

**Results**

**Patient disposition**

Although it was planned to include a maximum of 20 patients evaluable for step 2, the trial was stopped early due to slow accrual after the inclusion of 13 patients from five centers in Switzerland, between September 2005 and June 2009.

Of the 13 patients, two withdrew from the trial at the end of trastuzumab treatment (step 1) after disease progression; one (85 years of age) was deemed ineligible for further treatment after 76 days of treatment; a further patient withdrew consent during step 1 but continued on trastuzumab; this patient was on treatment for 627 days when seen at last follow-up. These two last patients were, therefore, not considered evaluable for the primary endpoint, as they did not proceed to step 2.
Patient characteristics

All patients entering step 2 were eligible and fully evaluable for safety and efficacy analyses. Table 1 lists the patients’ demographic and pretreatment characteristics. Most patients had bone and soft tissue metastases, and approximately half of the patients had visceral metastases. Out of 13 patients, seven were pretreated with either anastrozole or letrozole for metastatic disease; all the others received one of these AIs in the adjuvant setting. Median age was 63 years (range: 47–85) and most patients (85%) had a WHO performance status of 1.

Efficacy

There were six (46%) out of 13 patients who showed clinical benefit (CB) in step 1 (Fig. 2). Out of 11 patients, eight (73%) showed CB during step 2 (Table 2). Patients who showed CB in step 1 were not necessarily identical to those in step 2; only four patients had CB in both steps. CBR was based on SD in all but one patient, who achieved PR in step 2. Furthermore, one patient achieving SD in step 2 lasting 169 days, progressing only in supraclavicular lymph node metastases. As all other sites of disease were not progressing, the investigator decided to continue trastuzumab and letrozole outside of the trial protocol and to radiate the progressing lymph node metastases. A PR of the non-irradiated tumor lesions was achieved afterward. The median TTP in step 1 and step 2 was 161 and 188 days respectively (Table 2 and Fig. 3). Mean time on trial treatment (TTP in step 1 plus TTP in step 2) for patients entering step 2 was 420 days (range: 174–990).

Out of six patients, five with CB during trastuzumab treatment had high-level amplified HER-2 tumors. Tumors showing an HER-2 signal:centromere ratio $\geq 6$ or clustering of HER-2 signal were diagnosed in eight (62%) out of 13 patients; however, no clear association to efficacy outcomes was found (data not shown).

The median follow-up time was 23.0 months (range: 3.0–35.0) using the inverse Kaplan–Meier method.

Safety

Overall, trial treatment was well tolerated. Most AEs were grade 1 and 2, and there were no unexpected toxicities attributable to trial medication. In each treatment step, four grade 3 AEs occurred. Grade 3 AEs were anorexia/weight loss, dyspnea, arthralgia, and infections (dental, herpes zoster, and pulmonary). In total, two patients had four
serious AEs, one of which occurred in step 1 and the rest in step 2. None were related to trial treatment. One patient permanently discontinued treatment as a result of a pulmonary embolism after cycle 13 in step 2.

Discussion

This trial was designed to test the clinical activity of combined treatment of letrozole and trastuzumab after progression of sequential single-agent treatment with a non-steroidal AI and trastuzumab in patients with advanced or metastatic breast cancer co-expressing both HER-2 and ER. The results of this trial, although they must be interpreted with caution because of the small number of patients, indicate that dual targeting with trastuzumab and letrozole is feasible, well tolerated, and active in this population with resistant disease to single agents. Notably, a majority of patients (73%) achieved a CB, largely achieving durable SD, with combined treatment. Our trial was closed early for accrual. However, the goal of the trial – proof-of-concept for clinical relevant activity of combination AI plus trastuzumab in patients with resistant disease to single-agent exposure – was achieved with less patients accrued than initially planned due to the surprisingly high CBR in the first 11 patients included.

This observation is in concordance with the CBR of 65%, observed in 26 patients with ER+/HER+ breast cancers receiving first-line treatment with letrozole and trastuzumab within a randomized trial (Huober et al. 2009). Furthermore, this finding is in line with results of the anastrozole plus trastuzumab arm (CBR 43%) of the randomized TAnDEM trial (Kaufman et al. 2009) and of another phase II trial using letrozole and trastuzumab (CBR 52%) as first- or second-line therapy (Marcom et al. 2007).

The important difference between our trial and all other trials investigating combined trastuzumab with an AI in HER-2+/ER+ disease was that all patients in this proof-of-concept trial received and progressed under the same sequential treatment, thus representing

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<th>Table 2 Summary of efficacy data</th>
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<td>Patients with CB (n (%))</td>
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<td>Step 1 (N=13) Trastuzumab</td>
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<td>Step 2 (N=11) Trastuzumab + letrozole</td>
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NC, not calculable; CI, confidence interval.
a true population presenting with ‘double-resistant’
tumors. Our results suggest that complete clinical
resistance to both AI and trastuzumab can be overcome
in a proportion of patients by combined treatment, as
all patients served as their own control. It also indicates
at least partial restoration of sensitivity following this
treatment sequence, possibly as a consequence of a
bidirectional cross talk between the endocrine and the HER-2 signaling pathways.

Our strategy to target different growth pathways in
order to circumvent acquired resistance represents a
new therapeutic approach, alternative to upfront
combination therapy. Moreover, recent preclinical
studies have investigated the molecular basis for endocrine resistance. Several reports suggest that a
bidirectional cross talk between ER, HER-2, and other
members of the EGFR family play a key role in the
resistant model systems (Osborne et al. 2005, Sabnis
et al. 2005, Arpino et al. 2008) and that inhibition of
only one pathway leads to the activation of the other.
Loss of response to letrozole was accompanied by
up-regulation of the HER-2/mitogen-activated protein
kinase pathway and down-regulation of ER and aromatase activity (Sabnis et al. 2010). In addition,
preclinical models with trastuzumab (Sabnis et al.
2009), gefitinib (Massarweh et al. 2006), or lapatinib
(Xia et al. 2006) have consistently shown that inhibition of the growth factor activity in endocrine-resistant cells could be associated with an adoptive increase in ER signaling and subsequent restoration of sensitivity to endocrine treatment. Up-regulation of intratumoral aromatase was also seen with trastuzumab, which made the cells more responsive to AIs (Sabnis et al. 2009). Furthermore, anecdotal evidence from a clinical case series also suggest that a proportion of originally ER-negative, HER-2+ tumors revert to the ER-positive phenotype after treatment with trastuzumab allowing a subsequent endocrine-based treatment (Munzone et al. 2006). There is also evidence for the superiority of the combination of letrozole plus trastuzumab over letrozole or trastuzumab alone in xenograft studies of letrozole-refractory tumors (Sabnis et al. 2009).

Based on the therapeutic potential to subvert endocrine resistance in ER+/HER-2+ breast cancer clinical trials with combined anti-HER-2 and hormonal therapy have been conducted. These trials when interpreted together suggest that single-agent endocrine therapy in HER-2+ disease has low efficacy and is clearly inferior to combination therapy (Huober et al. 2009, Johnston et al. 2009, Kaufman et al. 2009).

Upfront combination on targeted therapy, especially in elderly patients, patients declining chemotherapy, or without extensive, life-threatening visceral involvement, might become a reasonable treatment option in the near future in light of these trials. The comparison of sequential treatment as opposed to upfront combination therapy still needs to be investigated.

The definitive impact of upfront chemoimmunotherapy is still a matter of debate. A randomized first-line trial studying trastuzumab plus docetaxel compared with the sequential treatment of trastuzumab monotherapy followed by trastuzumab plus docetaxel at disease progression was prematurely stopped after a significant overall survival benefit was observed in the combination arm (Inoue et al. 2010). However, it should be noted that the population in this trial consisted mainly of patients with visceral metastases and those with predominantly bone or soft tissue disease were not included. The question of combination versus sequential chemoimmunotherapy is also addressed in the SAKK 22/99 trial (NCT00004935).

In summary, this proof-of-concept trial provides additional clinical evidence for a dynamic interdependence of the ER and HER-2 pathways. It further indicates that sequential blockade of these pathways is feasible without compromising the effectiveness of a combined (horizontal) treatment of targeted therapeutics, thus offering a chemotherapy-free option for at least a subset of patients with ER+/HER-2+ breast cancer. Further trials comparing sequential versus combination therapy will need to corroborate this finding.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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