Fibroblast Growth Factor Receptors in breast cancer: expression, downstream
effects and possible drug targets

M. Tenhagen 1, P.J. van Diest 1, I.A. Ivanova 1, E. van der Wall 2, P. van der Groep 1,2

1Department of Pathology and 2Division of Internal Medicine and Dermatology,
University Medical Center Utrecht, The Netherlands

Address for correspondence:

Petra van der Groep, PhD
Department of Pathology
University Medical Center Utrecht
PO Box 85500
3508 GA Utrecht, The Netherlands
Email p.vandergroep@umcutrecht.nl
Tel: (31) 88 7556565
Fax: (31) 30 2544990

Supported by unrestricted educational grants from Aegon Inc and Pink Ribbon, The Netherlands.
Abstract

Cancer treatments are increasingly focusing on the molecular mechanisms underlying the oncogenic processes present in tumors of individual patients. Fibroblast growth factor receptors (FGFR) are among the many molecules, which are involved in oncogenesis and are currently under investigation for their potential as drug targets in breast cancer patients. These receptor tyrosine kinases play a role in several processes including proliferation, angiogenesis and migration. Alterations in these basal processes can contribute to the development and progression of tumors. Among breast cancer patients, several subgroups have been shown to harbor genetic aberrations in FGFRs, including amplifications of FGFR1, FGFR2 and FGFR4 and mutations in FGFR2 and FGFR4. Here we review in vitro and in vivo models that have partly elucidated the molecular implications of these different genetic aberrations, the resulting tumor characteristics, and the potential of FGFRs as therapeutic targets for breast cancer treatment.
Introduction

Breast cancer is the most commonly occurring cancer in women with 1.4 million new cases diagnosed worldwide annually (Jemal et al. 2010). Currently breast surgery and irradiation are the local therapies of choice, and chemo- hormonal- and anti-human epidermal growth factor receptor 2 (HER2) therapies are commonly applied as a systemic treatment to prevent outgrowth of distant metastases (Prosnitz et al. 2001). Progress in local and systemic treatment has clearly improved the prognosis of breast cancer patients, but still 458,000 women died from the disease in 2008 (Ferlay et al. 2010).

Therefore, the development of new therapies is focused on the specific genetic abnormalities in individual cancers, resulting in more personalized treatment (Bild et al. 2006, Alvarez et al. 2010). In order to develop these personalized therapies, it is important to fully understand the molecular basis of the oncogenic pathways that can be targeted in breast cancer.

Receptor tyrosine kinases (RTKs) regulate cell proliferation, differentiation and apoptosis: processes that are often deregulated in cancer. Several RTKs, including epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2) and platelet-derived growth factor receptor (PDGFR) have been extensively studied leading to the development of targeted inhibitors, such as trastuzumab and imatinib, that have been successfully used to treat cancer patients overexpressing HER2 and PDGFR, respectively (Arteaga 2003, Mukai 2010, Peterson 2011).
This review focuses on the fibroblast growth factor receptor (FGFR) family of RTKs. FGFR signaling cascades, the genetic aberrations in FGFRs, and their correlations with other genotypic and phenotypic features found in breast cancer will be summarized. Finally, therapies currently under development, as well as new possibilities to target the FGFR pathway will be discussed. Articles were retrieved through PubMed using the search items “breast” OR “mammary” AND “FGFR”. In addition, the references of the retrieved papers were screened for additional papers. Only papers in English were further considered.

**FGFRs: expression and signaling**

The *FGFR* family comprises four members: *FGFR1, -2, -3* and *-4* (Turner & Grose 2010), located on chromosomes 8p12, 10q26, 4p16.3 and 5q35.1-qter, respectively. All FGFRs contain an extracellular ligand-binding domain, a transmembrane domain and an intracellular tyrosine kinase (TK) domain (Eswarakumar *et al.* 2005). The extracellular domain has three immunoglobulin-like domains (IgI-III, Figure 1). The second and third immunoglobulin (Ig) domains are responsible for binding the fibroblast growth factors (FGF) ligand (Beenken & Mohammadi 2009). The FGF family of secreted glycoproteins consists of 18 members. The different FGFs and their corresponding receptors are expressed in a tissue-specific way. This tissue-specific expression pattern and the differences in binding affinity contribute to the specificity of the ligand-receptor interaction (Eswarakumar *et al.* 2005, Turner & Grose 2010). Furthermore, this specificity is also regulated by splicing. FGFR1, -2 and -3 have two splice variants of the IgIII domain, resulting in IIIb and IIIc isoforms.
The IIIb isoform is present on epithelial cells and IIIc is expressed by mesenchymal cells (Beenken & Mohammadi 2009).

FGFs are released from the extracellular matrix by heparines, proteases or specific FGF binding proteins. FGFs bind FGFRs and form a stable structure together with heparin sulphate proteoglycans (HPSGs) on the cell surface (Harmer et al. 2004, Mohammadi et al. 2005, Turner & Grose 2010). Klotho family proteins also facilitate the FGF-FGFR interaction (Wu et al. 1991, Kurosu et al. 2006) by binding to FGFRs and increasing the affinity of FGF for its receptor thus resulting in increased receptor activation (Kurosu et al. 2006). Each FGFR isoform can bind multiple FGF ligands and some ligands are common to several receptors (Eswarakumar et al. 2005). Ligand binding results in FGFR dimerization and subsequent activation of the intracellular kinase domain, leading to cross-phosphorylation of tyrosine residues present on the intracellular tail of the receptor (Turner & Grose 2010). Several docking proteins are able to bind to these phosphorylated residues, resulting in their phosphorylation and subsequent activation. Major pathways downstream of activated FGFRs include the rat sarcoma- mitogen activated protein kinase (Ras-MAPK) pathway and the phosphoinositide-3 kinase - v-akt murine thymoma viral oncogene (PI3K-Akt) pathway whose activation is mediated via fibroblast growth factor receptor substrate 2α (FRS2α) and several other adaptor molecules. Furthermore, protein kinase C (PKC) is activated via phospholipase C gamma (PLCγ), and finally activation of signal transducer and activator of transcription (STAT) signaling can take place (Acevedo et al. 2009, Turner & Grose 2010) (Figure 1).

All of these pathways play an important role in cell proliferation, differentiation, inhibition of apoptosis and migration. In addition, FGFR signaling is involved in angiogenesis and wound repair (Eswarakumar et al. 2005, Turner & Grose 2010).
Downstream effector activation is cell type-specific and may be dependent on crosstalk with other signaling pathways (Dailey et al. 2005). Although different FGFRs signal via similar pathways, differences in downstream effector activation have been described. Activation of downstream targets by FGFR4 is less strong than FGFR1 (Vainikka et al. 1994) and FGFR1 signaling sustains longer than FGFR2 because of faster degradation of FGFR2 after activation (Xian et al. 2007). Finally, even though FGFR isoforms have some specificity for different FGF ligands (Eswarakumar et al. 2005), no conclusive evidence has been found that specific FGFs activate specific downstream pathways (Dailey et al. 2005).

Aberrations in FGFRs found in breast cancer

Deregulation of FGFR activity could contribute to cancer development by increasing cell proliferation, angiogenesis and inhibiting apoptosis. Genetic aberrations in FGFRs have indeed been found to be associated with breast cancer and are described below.

FGFR1

No germ-line mutations in FGFR1 have been identified to date. However, a somatic mutation (K566R) was found in a sample from a patient with basal-like, triple negative breast cancer (Kan et al. 2010). Whether this mutation is present in other breast cancer patients remains to be investigated. Furthermore, a S125L mutation was described in a ductal breast carcinoma cell line (HCC1395, Stephens et al. 2005, Greenman et al. 2007). However, whether these mutations have consequences for FGFR1 function and what their role is in breast cancer development and/or progression has not been investigated.
Several studies have identified amplifications of FGFR1 in breast cancer (Chin et al. 2006, Letessier et al. 2006, Reis-Filho et al. 2006, Elbauomy Elsheikh et al. 2007, Marchio et al. 2008, Andre et al. 2009, Kadota et al. 2009, Moelans et al. 2010) (Table 1). The percentage of FGFR1 amplifications found using different techniques ranges from 7.5-17%. This variability could be caused by differences between tumor samples, which were not selected for histopathological type or clinical parameters. Furthermore, each study used a different technique to assess the copy number alterations, which can also account for the discrepancies in the results. Microarray expression analyses showed that FGFR1 amplifications were associated with increased FGFR1 RNA expression (Chin et al. 2006, Andre et al. 2009).

Several studies have described that FGFR1 amplifications were not associated with some pathological characteristics (Table 2). Associations with proliferation and HER2 status are inconsistent between studies, possibly due to differences in techniques. Proliferation was estimated by assessing the percentage of Ki67-positive cells (Letessier et al. 2006) or the mitotic activity index (Moelans et al. 2010) and HER2 expression was also evaluated in different ways (Letessier et al. 2006, Elbauomy Elsheikh et al. 2007, Kadota et al. 2009, Moelans et al. 2010). Even though estrogen receptor (ER) and progesterone receptor (PR) status were assessed in the same way in the different studies, the correlations with FGFR1 amplification were inconsistent. Confounder effects by clinical or pathological parameters could provide an explanation for the differences in association between expression of these hormone receptors and FGFR1 amplification.
In addition to pathological features, association analysis of FGFR1 amplification with clinical outcome has also been performed (Table 2). Patients with a FGFR1 amplification were more likely to develop distant metastases (Elbauomy Elsheikh et al. 2007). In addition, a trend for FGFR1 amplification and a shorter disease-free survival (DFS) was observed. Finally, amplification of FGFR1 was significantly associated with a shorter overall survival (OS), independent of other prognosticators such as grade, tumor size, lymph node invasion and ER status. Interestingly, FGFR1 amplification remained a significant independent risk factor for poor DFS and OS in ER-positive, but not in ER-negative cases (Elbauomy Elsheikh et al. 2007). This suggests that there may be an interaction between FGFR1 and ER signaling resulting in a poor prognosis. The observation that FGFR1 amplification is associated with a poor prognosis in ER-positive breast cancer was confirmed in a group of ER-positive tumor samples from patients treated with adjuvant endocrine therapy (tamoxifen). In this group, FGFR1 amplification was significantly associated with decreased metastasis-free survival as compared to samples with normal FGFR1 levels (Turner et al. 2010b). Finally, two studies showed an association between amplification of 8p11-12 where FGFR1 is located, and poor disease outcome. Although these analyses do not specifically show FGFR1 amplification in the breast cancer samples, they support the findings in previous studies (Chin et al. 2006, Letessier et al. 2006).

The function of FGFR1 as an oncogene has been disputed due to the results of functional studies in cell lines carrying 8p11-12 amplifications, which showed that FGFR inhibition did not affect the proliferation (Ray et al. 2004). Several studies have identified other potential oncogenes located on 8p11-12, including PPAPDC1B, RAB11FIP1, LSM1, BAG4, C8orf4 and WHSC1L1 (Gelsi-Boyer et al. 2005, Garcia et
These results do not exclude the possibility of multiple genes in the 8p11-12 region acting as oncogenes, which might even cooperate.

In addition to its suggested role as an oncogene, there is some evidence that FGFR1 may have a tumor suppressor function in breast cancer. Loss of FGFR1 was found in 10% of invasive breast cancer samples (Moelans et al. 2010). Another study identified a deletion of 8p11-12 in breast carcinomas, which was shown to be associated with a poor outcome (Chin et al. 2006). However, since this region contains many genes, it is not certain whether poor prognosis can be attributed to the specific loss of FGFR1.

**FGFR2**

So far, only one somatic mutation (R203C) in FGFR2 has been identified in the breast cancer cell line HCC1143 (Stephens et al. 2005, Sjöblom et al. 2006, Greenman et al. 2007). However, several single nucleotide polymorphisms (SNPs) in FGFR2 were found to be highly associated with breast cancer risk and identified FGFR2 as a breast cancer susceptibility gene (Hunter et al. 2007). Four of these SNPs are located in intron 2 of FGFR2. An independent study, using a different panel of SNPs, found an association of another SNP in intron 2 of FGFR2 with breast cancer (Easton et al. 2007). The risk allele of this SNP was significantly associated with a positive ER and PR status and a lower grade (Garcia-Closas et al. 2008). Further analysis of the SNPs located in the second intron of FGFR2 identified a haplotype block of 8 SNPs as the minor disease-predisposing allele (Easton et al. 2007, Meyer et al. 2008). This haplotype block is not in linkage disequilibrium with any coding region of FGFR2, excluding a change in protein sequence. Also, when
comparing the expression levels of the two most common splicing variations (including or excluding exon 3), no significant difference was observed between the minor and common homozygotes (Meyer et al. 2008). These results indicate that the change in the protein sequence due to the SNPs does not result in a functional difference.

However, when correlating the expression levels of FGFR2 in invasive breast cancer samples with the haplotype of the 8 SNPS, mRNA levels of FGFR2 were significantly increased in tumor samples that were homozygous for the minor alleles when compared to samples homozygous for the common alleles (Meyer et al. 2008). This indicates that the risk genotype results in increased expression of FGFR2, which could contribute to the development of breast cancer.

In a genome-wide screen identifying copy number alterations FGFR2 was found to be amplified in only 2 out of 161 primary breast cancer samples (1.2%) (Kadota et al. 2009). Other genome-wide screens analyzing large groups of unselected breast cancer samples also showed that FGFR2 is rarely amplified (Adelaide et al. 2007), and others did not find any amplifications or losses of FGFR2 (Andre et al. 2009). However, FGFR2 was amplified in 4% of triple-negative breast cancer samples, whereas no amplification was found in other molecular subtypes. In addition, the mRNA expression levels of FGFR2 were significantly increased in amplified vs. non-amplified tumor samples (Turner et al. 2010a), suggesting a potential role for FGFR2 in triple-negative breast cancer. FGFR2 is also suggested to play a role in a subgroup of familial breast cancer patients. When comparing the RNA expression profiles of breast cancer samples of patients with a germline mutation in breast cancer 1 and 2 (BRCA1 and -2), FGFR2 expression was significantly higher in breast cancers of BRCA2 mutation carriers (Bane et al. 2009). Finally, increased FGFR2
protein levels in samples from IDC patients were found to be associated with lower (disease-free) survival rates (Sun et al. 2011). How FGFR2 is regulated in these and BRCA2-mutated patients and whether it is involved in the development or progression of breast cancer is not clear.

FGFR3

Even though activating mutations in FGFR3 have been identified in several cancer types (Cappellen et al. 1999, Sibley et al. 2001, Zhang et al. 2005, Hafner et al. 2006), no FGFR3 mutations have been found in breast cancer (Sibley et al. 2001, Koziczak et al. 2004, Greenman et al. 2007). FGFR3 mutations cause Saethre-Chotzen syndrome, consisting of premature fusion of one or more skull sutures often accompanied by facial asymmetry and fusion of digits (Sahlin et al. 2009). There is one documented case of a Saethre-Chotzen patient with a heterozygous missense mutation in exon 7 of FGFR3 (P250R) who also developed breast cancer. This mutation is located in the ligand-binding domain of FGFR3, which leads to activation of the kinase-domain (Sahlin et al. 2009). Moreover, this mutation is located near mutational hotspots found in bladder and cervical carcinomas (R248C, S249C) (Sibley et al. 2001). It is unclear if the mutation found to cause Saethre-Chotzen syndrome also increases the risk of developing breast cancer, therefore more studies are required to further elucidate this.

A possible role for FGFR3 in therapy resistance was identified in a group of 429 ER-positive breast cancer patients who received tamoxifen as primary therapy. FGFR3 protein levels were significantly increased in patients who did not respond to the treatment (Tomlinson et al. 2011).
A Gly388Arg (Arg\textsuperscript{388}) missense mutation, now known as SNP rs351855, was identified in the transmembrane domain of \textit{FGFR4} (Bange \textit{et al}. 2002). Immunohistochemical analysis of breast cancer samples did not show any correlation of the Arg\textsuperscript{388} SNP genotype with FGFR4 expression levels (Thussbas \textit{et al}. 2006). Sequence analysis of breast cancer samples revealed that the Arg\textsuperscript{388} genotype, either heterozygous or homozygous, was present in 37-43\% and 8-11\%, respectively (Table 3) (Bange \textit{et al}. 2002, Jezequel \textit{et al}. 2004, Thussbas \textit{et al}. 2006, Marme \textit{et al}. 2010). No significant difference could be identified when comparing the distribution of the Arg\textsuperscript{388} allele in healthy controls and breast cancer patients. This indicates that this SNP is not associated with the initiation of breast cancer (Bange \textit{et al}. 2002).

Arg\textsuperscript{388} did not correlate with age, HER2, ER and PR status and grade (Bange \textit{et al}. 2002, Jezequel \textit{et al}. 2004, Thussbas \textit{et al}. 2006, Marme \textit{et al}. 2010). Correlations were found for tumor stage (Thussbas \textit{et al}. 2006, Marme \textit{et al}. 2010), tumor size (Thussbas \textit{et al}. 2006) and axillary lymph node involvement (Bange \textit{et al}. 2002, Jezequel \textit{et al}. 2004, Thussbas \textit{et al}. 2006, Marme \textit{et al}. 2010), but not all of these associations were confirmed by other studies (Bange \textit{et al}. 2002, Thussbas \textit{et al}. 2006, Jezequel \textit{et al}. 2004,). Finally, when analyzing the DFS period, no correlation was found with the Arg\textsuperscript{388} allele (Bange \textit{et al}. 2002). However, in primary lymph node positive patients (N+) DFS was significantly shorter in Arg\textsuperscript{388} than Gly\textsuperscript{388} carriers (Bange \textit{et al}. 2002, Thussbas \textit{et al}. 2006). This suggests that Arg\textsuperscript{388} could be a marker for increased tumor aggressiveness in advanced breast cancer. However, another study did not find a significant association of Arg\textsuperscript{388} with DFS in unselected
N+ breast cancer cases (Jezequel et al. 2004). Arg^{388} was not associated with DFS in patients who did not receive adjuvant systemic therapy. When adjuvant systemic therapy was applied, DFS and OS were shorter in patients carrying one or two Arg^{388} alleles, making it a possible marker for therapy resistance in this patient group (Thussbas et al. 2006).

Interestingly, in patients who received neoadjuvant chemotherapy, the Arg^{388} allele was significantly and independently associated with a better clinical and pathological response (Marme et al. 2010).

In addition to the Arg^{388} germ-line mutation, a somatic mutation (V510M) in FGFR4 was identified in a tumor sample from a lobular carcinoma patient (Stephens et al. 2005, Greenman et al. 2007). This mutation has not been found in other (breast) cancer patients to date, making its significance unclear.

Amplification of FGFR4 has been reported in breast cancer samples. In a small study, 10% of breast tumors had FGFR4 amplifications, which were associated with ER and PR positivity and lymph node metastases (Jaakkola et al. 1993).

Furthermore, FGFR4 mRNA levels were elevated in 32% of breast cancer samples (Penault-Llorca et al. 1995). In a retrospective study analyzing ER positive breast cancers from patients treated with neoadjuvant tamoxifen, high levels of FGFR4 were independently associated with tumor response and survival after treatment (Meijer et al. 2008).

**Molecular implications of genetic aberrations in FGFRs on tumor characteristics**

As described above, genetic aberrations in FGFRs have been shown to contribute to breast cancer risk, tumor progression and response to therapy. These different
outcomes can be due to the fact that FGFRs signal through downstream signaling pathways whose activation depends on several factors. The *in vitro* and *in vivo* studies described below give more insight into the functional effects of abnormal FGFR signaling.

**FGFR1**

Inducible activation of FGFR1 (iFGFR1) in transgenic mice resulted in increased cell proliferation in the lateral buds and ductal branching of the mammary epithelium (Welm *et al*. 2002). Furthermore, there was recruitment of macrophages, which are required for lateral bud formation and also play a role in the formation of small blood vessels (Schwertfeger *et al*. 2006) through the production of interleukin-1β (Reed *et al*. 2009). After sustained induction of FGFR1, epithelial cells further proliferated, forming multicellular lesions which eventually became invasive. These invasive lesions were characterized by loss of cell polarity, cell detachment from the basement membrane and anoikis-resistance. Finally, the invasive lesions in iFGFR1 transgenic mice were surrounded by an increased number of highly branched small blood vessels (Welm *et al*. 2002).

The observed changes in the breast epithelium of iFGFR1 mice were recapitulated in 3D cultures of HC11 and MCF10A cells transfected with inducible FGFR1 (Welm *et al*. 2002, Xian *et al*. 2005, Xian *et al*. 2009). These models have shown that the induction of FGFR1 expression results in epithelial to mesenchymal transistion. The molecular downstream targets that are activated upon induction of iFGFR1 include ribosomal protein S6 kinase (RSK), the PI3K-Akt and the MAPK-ERK pathway (Welm *et al*. 2002, Xian *et al*. 2005, Xian *et al*. 2009). Target genes activated by FGFR1 include several clusters that are linked to tumor formation
through angiogenesis, cell cycle regulation, chemotaxis and the response to inflammation (Schwertfeger 2006).

Activation of FGFR1 does not only affect the characteristics of epithelial cells, but also influences the myoepithelium and microenvironment. Long term activation of FGFR1 in transgenic mice resulted in a disruption of the myoepithelial cell barrier and extracellular matrix (ECM) disorganization (Welm et al. 2002).

Activation of FGFR1 signaling using a small amount of FGF2 in the MDA-MB-134, CAL120, JIMT-1, MFM223, S68, SUM44 (Turner et al. 2010b), CMA, MDA-MB-361 and HCC38 (Shiang et al. 2010) cell lines, which have endogenous FGFR1 amplifications resulted in downstream activation of multiple pathways, including FRS2, ERK1/2 and RSK phosphorylation. A different study showed that these pathways were already basally activated without ligand (Turner et al. 2010b). Also, cells harboring FGFR1 amplifications seem to have an oncogenic addiction to FGFR1 signaling since they are highly sensitive to inhibition of FGFR1 by siRNA or SU5402, a FGFR1 tyrosine kinase inhibitor (Reis-Filho et al. 2006). Furthermore, FGFR1 amplified cell lines were resistant to treatment with 4-hydroxytamoxifen (4-OHT), suggesting that FGFR1 amplification is involved in resistance to endocrine therapy (Turner et al. 2010b). This notion supports the observation that amplification of FGFR1 is associated with a poor prognosis in ER positive patients that were treated with tamoxifen as an adjuvant endocrine therapy. Interestingly, inhibition of FGFR1 by siRNA increased the sensitivity of FGFR1 amplified cell lines to 4-OHT, making this a possible drug therapy (Turner et al. 2010b).

Another indication for interacting pathways comes from the mouse mammary tumor virus - wingless type 1 (MMTV-Wnt1) mouse model. Crossing these mice with iFGFR1 transgenic mice and subsequent activation of FGFR1 dramatically
accelerated tumor formation, suggesting an interaction between FGFR1 and Wnt signaling (Pond et al. 2010). Further experiments are needed to explore whether genetic aberrations in FGFRs affect tumor formation in other mouse mammary tumor models harboring mutations in oncogenes that frequently occur in breast cancer patients.

**FGFR2**

The *FGFR2* amplified SUM52PE and MFM223 cell lines depend on FGFR2 signaling for survival. This addiction to FGFR2 is due to activation of the PI3K-Akt signaling pathway that results in inhibition of apoptosis. Furthermore, downstream targets of FGFR signaling like FRS2, AKT and ERK, were phosphorylated in the absence of serum and inactivated by an FGFR-inhibitor. This indicates that the phosphorylation of these targets is independent of ligand-binding and is dependent on FGFR-kinase activity. In contrast, the HCC1143 cell line, in which a mutation in *FGFR2* has been described, showed no evidence of FGFR-dependent signaling and is not dependent on FGFR for proliferation (Turner et al. 2010a).

As described earlier, SNPs in intron 2 of *FGFR2* correlate with higher *FGFR2* mRNA expression levels (Meyer et al. 2008). Since the sequence of intron 2 includes a regulatory region, SNPs could alter one or more transcription factor binding sites, thereby regulating the expression levels of *FGFR2* (Easton et al. 2007). The SNPs rs35054928 and rs2981578 are located next to an organic cation transporter (OCT) binding site. The risk allele of rs2981578 also creates a putative binding site for runt-related transcription factor (RUNX) and the risk allele of rs10736303 one for ER (Katoh & Katoh 2009). The risk allele of rs7895676 has reduced binding capacity to (CCAAT/enhancer binding protein-beta (C/EBP-β). Whereas the rs2981578 variants
have equal affinity for OCT-1, the high risk allele has much higher affinity for RUNX2 (Meyer et al. 2008), thought to be due to differences in H3/H4 acetylation of the SNP sites (Zhu et al. 2009). Overall, the differences in binding affinity result in a higher expression of FGFR2 in cell lines carrying the high risk alleles. This is in accordance with the increased expression levels of FGFR2 in breast cancer samples that were homozygous for the high risk alleles (Meyer et al. 2008). Remarkably, ER binding was not altered for any of the SNPs, whereas the increased risk for breast cancer due to the high risk alleles of FGFR2 are highly associated with ER-positive tumors (Garcia-Closas et al. 2008). A possible explanation for this association is that ER and OCT-1/RUNX may cooperate to increase the expression of FGFR2 (Meyer et al. 2008), which is supported by the finding that OCT and ER sites often cluster together (Carroll et al. 2006). The exact role of ER in the FGFR2 allele resulting in increased breast cancer risk remains to be investigated.

FGFR3

MCF7 cells were used to study the molecular mechanisms behind the involvement of FGFR3 in tamoxifen resistance in ER-positive breast cancer. Inducible activation of FGFR3 decreased the sensitivity of MCF7 cells to 4-OHT treatment independent of ER activity. Activation of PLCγ1 was essential for this resistance phenotype and downstream activation of PI3K signaling was an important component of this reduced sensitivity to endocrine therapy (Tomlinson et al. 2011)

FGFR4

The poor response to chemotherapy associated with overexpression of FGFR4 (Meijer et al. 2008) has been recapitulated in several FGFR4 overexpressing breast
cancer cell lines, which are resistant to doxorubicin and cyclophosphamide (Roidl et al. 2009). FGFR4 contributes to this resistance by activating anti-apoptotic signaling via activation of MAPK and subsequent increase the B-cell lymphoma–extra large (BCL-XL) levels. In accordance with this, inhibition of FGFR4 reduced ERK-phosphorylation and decreased levels of BCL-XL, ultimately leading to increased chemosensitivity to drugs (Roidl et al. 2009).

The Arg$^{388}$ SNP in FGFR4 affects its structure but tyrosine kinase activity was not increased in breast cancer cells expressing this FGFR variant (Bange et al. 2002). This suggests that FGFR4-mediated resistance to chemotherapy may not be due to increased kinase activity of the receptor. Interestingly, Gly$^{388}$-expressing cells are less invasive compared to Arg$^{388}$-expressing cells or cells that have no endogenous FGFR4 expression (Stadler et al. 2006). This indicates that the Gly$^{388}$ allele protects patients from tumor invasion, which is consistent with a better prognosis (Bange et al. 2002). The kinase domain was proven to be essential for this protective effect (Stadler et al. 2006), suggesting that downstream signaling plays an important role.

Gene expression analysis revealed upregulation of the lysophatidic acid (LPA) receptor gene (EDG-2), matrix metalloproteinase 1 (MMP-1) and downregulation of plasminogen activator inhibitor (PAI-1) in Arg$^{388}$-, as compared to Gly$^{388}$-expressing cells, possibly contributing to increased cell motility and invasion. Edg-2 induces cell migration by activation of PI3 and AKT upon binding of the LPA ligand, which is consistent with increased cell motility in cells expressing Arg$^{388}$. The observed upregulation of MMP-1 in Arg$^{388}$-expressing cells may contribute to increased invasion due to increased degradation of the ECM (Stadler et al. 2006). PAI-1 has been shown to inhibit urokinase plasminogen activator (uPA) resulting in a decrease in migration and invasion. In addition, upregulation of uPA sensitizes the Arg$^{388}$-
expressing cells to chemotherapy-induced apoptosis (Whitley et al. 2004). All of these (indirect) downstream targets of the Arg\textsuperscript{388} FGFR4 variant seem to increase tumor invasion and decrease sensitivity to chemotherapy, explaining the worse clinical prognosis of Arg\textsuperscript{388} carriers (Bange et al. 2002, Thussbas et al. 2006).

Decreased invasion and no change in proliferative capacity of Gly\textsuperscript{388} expressing breast cancer cells was also observed in the whey acidic protein – transforming growth factor alpha (WAP-TGF\textalpha) breast cancer mouse model in which either the Arg\textsuperscript{385} or Gly\textsuperscript{385} allele (the mouse equivalents of the human 388 alleles) were knocked-in. The tumors of the mice carrying the Arg\textsuperscript{385} allele were more invasive than in their Gly\textsuperscript{385} littermates due to increased migration (Seitzer et al. 2010).

In addition to increased invasion, WAP-TGF\textalpha mice carrying the Arg\textsuperscript{385} allele also developed highly proliferative lung metastases. Genes suggested to contribute to this effect are cyclin-dependent kinase inhibitor 1A (p21), which is downregulated in Arg\textsuperscript{385} tumor samples and CDK1 (cyclin dependent kinase 1), FLK-1 (fetal liver kinase-1), CD44, MMP-13 and MMP-14, which are upregulated in the Arg\textsuperscript{385} tumors and are all involved in cell invasion (Seitzer et al. 2010). Interestingly, the involvement of MMPs in Arg\textsuperscript{388}-mediated tumor progression has also been suggested in breast cancer patients (Sugiyama et al. 2010a, 2010b).

**Therapeutic targets and drugs in development**

Based on the genetic aberrations in FGFRs identified in breast cancer patients and their consequences on a molecular level, several approaches can be used to target FGFR signaling.

**Upstream intervention**
As described above, cell lines with FGFR1 amplifications and overexpression have low levels of downstream signaling in the absence of ligand. However, ligand binding strongly induces downstream activation, making inhibition of ligand-receptor interaction a useful approach to inhibit FGFR1 signaling. In FGFR2 amplified cell lines downstream signaling is ligand-independent. Whether the presence of a ligand increases signaling, making ligand interference a useful target, is yet unclear. For patients overexpressing FGFR4, the dependence on ligand binding has yet to be investigated in more detail to determine whether it is useful to target ligand binding. A possible method to inhibit ligand binding is by designing FGF ligand traps such as FP-1039 (Turner & Grose 2010): a fusion protein comprising the extracellular domain of FGFR1 and the Fc region of IgG. FP-1039 has been shown to have anti-angiogenic effects in vivo. Moreover, FP-1039 was able to block tumor formation of breast cancer cell line xenografts, depending on their expression of FGFs and FGFRs (Zhang et al. 2007). FP-1039 is currently being tested in a phase I clinical trial (Keer 2010). Furthermore, since the FGFRs have several ligands in common, not only FGFR1 activation but also activation of FGFR3 and FGFR4 is blocked by binding of FP-1039 to FGFs (Zhang et al. 2007), making FP-1039 a rather universal blocker of FGFR signaling.

A second method to interfere with ligand binding is the use of antagonistic peptide mimics. These could be effective drugs for patients harboring FGFR amplifications that depend on the presence of a ligand to induce signaling. However, at present only agonistic peptide mimics have been designed for FGFR1-IIIc and FGFR2-IIIb. These were proven to be functional FGFR agonists with apparent therapeutic potential (Li et al. 2008).
**Intervention on the FGFR level**

Several methods can be used to target the FGFRs at the receptor level. First of all, numerous tyrosine kinase inhibitors (TKIs) targeting FGFRs have been developed. These small molecules compete with ATP to bind to the receptor resulting in reduced activity of the kinase domain (Katoh & Katoh 2009, Turner & Grose 2010). However, since the kinase domains of receptor tyrosine kinases are very similar, most TKIs are not specific for one FGFR and also inhibit the activity of VEGFRs and/or PDGFRs (Turner & Grose 2010). This may in fact increase their activity in cancer patients, but perhaps cause more side effects. Brivanib alaninate is an example of a TKI targeting both FGFRs and VEGFRs. Its effect in breast cancer has so far only been tested *in vitro*: cell lines with FGFR1 amplification/overexpression are more sensitive than non-amplified ones (Marme *et al.* 2010). The effect of this drug on other FGFRs and in patients remains to be investigated.

Another multi-target drug is Dovitinib, an inhibitor of FGFRs, PDGFRs and VEGFRs. This TKI was tested in a phase II clinical trial (NCT00958971) including HER2-negative breast cancer patients and only showed anti-tumor activity in patients with an FGFR1 amplification and an ER and/or PR positive status, whereas other subgroups only showed stabilization of tumor growth (Andre *et al.* 2011). Another phase II trial (NCT01262027) with Dovitinib has been started to control inflammatory breast cancer and also evaluate the safety of this drug. E-3810 is a multi-target small molecule inhibitor targeting VEGFRs and FGFR1, which is currently being tested in a phase I clinical trial. After initial testing in patients with solid tumors, breast cancer patients with *FGFR1* amplifications are included in the dose-expansion phase to obtain preliminary data on the anti-tumor activity of this drug (clinical trial:...
Finally, BIBF1120 was developed as a kinase inhibitor targeting FGFRs, PDGFRs and VEGFRs. Testing of this drug in multiple cancer mouse models and clinical trials including different cancer types has already been shown to be successful (Hilberg et al. 2008, Antoniu & Kolb 2010). A phase I/II clinical trial (NCT01484080) is currently investigating its efficacy in combination with paclitaxel (a mitotic inhibitor used to treat several types of cancer) in patients with early HER2-negative breast cancer.

PD173074 and SU5402 are the first compounds that have been designed as FGFR-specific TKIs. PD173074 was effective in ER, PR and HER2 negative cell lines with FGFR2 amplification/overexpression (Turner et al. 2010a). SU5402 decreased proliferation exclusively in cell lines with FGFR1 amplification and overexpression (Reis-Filho et al. 2006). It must be noted, however, that not every cell line with FGFR1 amplifications is sensitive to FGFR1 inhibition, which was found to depend on the oncogenic background (Reis-Filho et al. 2006). In this regard, further in vitro and in vivo studies will have to be performed in order to identify the right subgroup among patients with FGFR1 amplification and overexpression who will respond to FGFR-specific drugs.

The efficacy and safety of AZD4547, a TKI which targets FGFRs, are currently being tested in a phase IIa clinical trial (NCT01202591). The patients included are ER-positive and have FGFR1 polysomy or gene amplification and are also treated with exemestane, an aromatase inhibitor used to treat ER-positive breast cancer (Lintermans et al. 2011). Another pan-FGFR inhibitor (BGJ398) is currently also being tested in a phase 1 clinical trial to determine the maximum tolerated dose (clinical trial NCT01004224). This trial includes patients with solid tumors with FGFR1 or -2 amplifications or FGFR3 mutations.
Monoclonal antibodies may be a good solution to circumvent the side effects of unspecific TKIs. There are FGFR1-IIIc- and FGFR3-specific antibodies that have not yet been tested in any breast cancer model (Turner & Grose 2010). An anti-FGFR4 antibody (10F10) strongly increased sensitivity to doxorubicin in cell lines naturally overexpressing FGFR4 (Roidl et al. 2009).

Another potentially highly specific way to target FGFRs is by RNA aptamers. These short RNA oligonucleotides are selected for their high affinity and specificity for their target protein, thereby acting as synthetic antibodies (Ireson & Kelland 2006). Currently, RNA aptamers that target the FGFR2 kinase domain, the FRS2 interacting domain and the extracellular domain of FGFR2 are being developed, but their effectiveness remains to be tested (Katoh & Katoh 2009). Therapeutic strategies using siRNA or miRNA are still in a very early stage of development focusing on off-target effects and delivery (Katoh & Katoh 2009).

**Downstream intervention**

Many of the proteins involved in FGFR signaling also play a role in other signaling pathways and in healthy cells. This makes it very difficult to target these proteins without side effects. No extensive research has been performed on targeting the downstream effectors of FGFR signaling. The targets suggested below are therefore mostly hypothetical, whereas for some, preliminary data is present.

In FGFR1 transfected MCF10A cells, treatment with CMK, an irreversible small molecule inhibitor specific for RSK (Cohen et al. 2005), reduced proliferation and restored sensitivity to anoikis (Xian et al. 2009). Interestingly, the inhibitory effect of CMK was only observed in breast cancer cell lines overexpressing FGFR1 (Xian et
The effect of CMK in vivo remains to be investigated.

MMPs could also be possible drug targets downstream of FGFR1. Inhibition of MMPs in HC11 cells overexpressing FGFR1 with a pan MMP inhibitor (GM6001) resulted in decreased invasion and EMT, although proliferation, apoptosis and cell polarity were not changed (Xian et al. 2005). This indicates that inhibition of MMP activity may not suffice as mono treatment, but could be useful when used in combination with other drugs.

PI3-kinase is a possible drug target for patients with FGFR2 amplifications, since FGFR2-amplified cell lines are dependent on PI3K-mediated inhibition apoptosis for their survival. This idea is supported by a study in FGFR2-amplified cell lines which found that dual targeting of FGFR2 and PI3-kinase by PD173074 and BEZ235 (a PI3-kinase/mTOR inhibitor), respectively, was very effective (Adelaide et al. 2007). Since PI3K signaling is also suggested to be important in FGFR3-mediated resistance to tamoxifen, the inhibition of this pathway could also be a potential treatment for this group of patients.

In patients with FGFR4 overexpression, targeting the anti-apoptotic pathway that activates BCL-XL via MAPK might be a possibility. Since these proteins have been shown to mediate chemotherapy resistance in this group of patients, drugs targeting them could increase the response rate. No clinical studies in this field have been performed so far, but administration of UO126 (a specific MEK inhibitor) to FGFR4-expressing breast cancer cells has been shown to decrease BCL-XL expression (Roidl et al. 2009). In addition to a broad panel of MEK inhibitors that have been developed to treat different types of cancer (Fremin & Meloche 2010), BCL-XL inhibitors are also available for clinical research (Kang & Reynolds. 2009).
Since the \textit{FGFR4-Arg}^{388} polymorphism does not result in increased tyrosine kinase activity (Bange \textit{et al.} 2002), drugs interfering with ligand binding or the tyrosine kinase activity of the receptor are unsuitable for treating \textit{FGFR4-Arg}^{388} breast cancer patients. However, as described above, numerous downstream effectors of FGFR4 play a role in the increased migration capacity of Arg^{388} expressing cells. Interference with any of these molecules could be a potential therapeutic strategy for breast cancer patients harboring the Arg^{388} allele. This is supported by the observation that siRNA targeting \textit{EDG-2} significantly reduces the migration of cells carrying the Arg^{388} FGFR4 allele (Stadler \textit{et al.} 2006).

In general, when targeting FGFR signaling, its role in proliferation, differentiation, migration and angiogenesis should be kept in mind. Interfering in these important basic cell processes could have many side effects. Further investigation of the context-dependent activation of downstream pathways is necessary to anticipate these side effects.

\textbf{Conclusion}

In the past decade our understanding of the important role of FGFRs in breast cancer has been growing. Genetic aberrations in different FGFRs have been identified in subgroups of breast cancer patients, and their (molecular) implications on tumor characteristics have been partly elucidated. Overall, it is very clear that FGFRs are promising drug targets. FGFR2 amplifications could be a very interesting drug target in triple negative breast cancer patients, since other targeted therapies are not effective. Although several mutations in FGFR3 have been found in other types of
cancer and its overexpression has been associated with endocrine-resistance, genetic aberrations affecting FGFR3 have not been identified in breast cancer patients (Sibley et al. 2001). Future experiments should be focussed on the molecular mechanism behind the increased breast cancer risk associated with the SNPs in intron 2 of FGFR2, especially addressing the role of ER. Some contradicting results regarding the involvement of the Arg388 FGFR4 allele in the prognosis and therapy resistance of breast cancer patients should be clarified by large-scale studies in which the clinical and pathological parameters are monitored closely. Furthermore, the FGFR1 gene amplification observed several different types of breast cancer makes it a very relevant drug target. Since the inducible FGFR1 models have greatly increased the understanding about its molecular mechanism, the next step should be to screen for possible drugs. Interestingly, in contrast to FGFR1, expression of FGFR2 did not induce neoplasia in the prostate (Freeman et al. 2003), suggesting that FGFR1 is more oncogenic than FGFR2. However, whether this is also the case in mammary tissue or this oncogenic potency is an issue of context, remains to be investigated.

Several FGFR targeting strategies are in different phases of development. In addition to the drugs described above several other compounds targeting FGFRs are being tested on other types of cancer, including urothelial carcinoma, non-small cell lung cancer and ovarian cancer (Katoh & Katoh 2009, Antoniu & Kolb 2010, Turner & Grose 2010, Bello et al. 2011, Lamont et al. 2011). Even though most of these drugs are not specific for FGFRs, but also target PDGFRs and VEGFRs, this might actually be an advantage since these RTKs are known for their roles in tumor angiogenesis and proliferation (Petrelli & Giordano 2008). Likely, further refinements in FGFR targeting drugs will be necessary to increase effectiveness and limit side effects. At
the same time, translational studies need to identify the subgroup of breast cancer patients that will best respond to the various FGFR targeted therapies.
References


Eswarakumar VP, Lax I & Schlessinger J 2005 Cellular signaling by fibroblast growth factor receptors. *Cytokine & growth factor reviews* **16** 139-149.


Fremin C & Meloche S 2010 From basic research to clinical development of MEK1/2 inhibitors for cancer therapy. Journal of hematology & oncology 3 8.


Lamont FR, Tomlinson DC, Cooper PA, Shnyder SD, Chester JD & Knowles MA 2011 Small molecule FGF receptor inhibitors block FGFR-dependent urothelial carcinoma growth in vitro and in vivo. *British journal of cancer* **104** 75-82.


Mohammadi M, Olsen SK & Ibrahimi OA 2005 Structural basis for fibroblast growth factor receptor activation. *Cytokine & growth factor reviews* **16** 107-137.


Ray ME, Yang ZQ, Albertson D, Kleer CG, Washburn JG, Macoska JA & Ethier SP 2004 Genomic and expression analysis of the 8p11-12 amplicon in human breast cancer cell lines. *Cancer research* **64** 40-47


Xian W, Schwertfeger KL & Rosen JM 2007 Distinct roles of fibroblast growth factor receptor 1 and 2 in regulating cell survival and epithelial-mesenchymal transition. *Molecular endocrinology* **21** 987-1000.


Figure 1: FGFR structure and downstream signalling

The extracellular domain of FGFRs consist of three ligand binding Ig domains. Binding of the ligand is stabilized by HSPG and Klotho. Intracellular, tyrosine kinase domains (TK) are present. Upon ligand binding the receptors dimerize, resulting in cross-phosphorylation of the tyrosine kinase domains. This leads to binding of several docking proteins, which can also be phosphorylated. Downstream signalling occurs through four main pathways: PLCγ, STATs, AKT and MAPK.

Abbreviations: Ig: immunoglobulin, FGF: fibroblast growth factor; HSPG: heparin sulphate proteoglycans; TK: tyrosine kinase domain; STAT: signal transducer and activator of transcription; FRS2α: fibroblast growth factor receptor substrate 2α; GRB2: growth factor receptor-bound protein 2; GAB1: GRB2 associated binding protein 2; PI3K: phosphoinositide-3 kinase; AKT: v-akt murine thymoma viral oncogene; SOS: son of sevenless; RAS: rat sarcoma; MAPK: mitogen activated protein kinase; PLCγ: phospholipase Cγ; PIP2: phosphatidylinositol 4,5-biphosphate; IP3: inositol 1,4,5-triphosphate; DAG: diacylglycerol; PKC: protein kinase C
<table>
<thead>
<tr>
<th>Breast cancer cases, N</th>
<th>Technique, whole genome /gene specific</th>
<th>Results: FGFR1 amplification/loss</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>106</td>
<td>aCGH: 70 kb interval, whole genome</td>
<td>Highly amplified in 10%</td>
<td>Andre F et al. 2009</td>
</tr>
<tr>
<td>161</td>
<td>SNP DNA microarray, whole genome</td>
<td>Amplified in 7.5%</td>
<td>Kadota M et al. 2009</td>
</tr>
<tr>
<td>1319</td>
<td>FISH, gene specific</td>
<td>Amplified in 9.4%</td>
<td>Letessier A et al. 2006</td>
</tr>
<tr>
<td>496</td>
<td>CISH, gene specific</td>
<td>Amplified in 8.7%</td>
<td>Elbauomy Elsheikh S et al. 2007</td>
</tr>
<tr>
<td>104</td>
<td>MLPA, gene specific</td>
<td>Amplified in 17% (7%: highly amplified) Lost in 10%</td>
<td>Moelans CB et al. 2010</td>
</tr>
<tr>
<td>24</td>
<td>CISH, gene specific</td>
<td>Amplified in 16.6%</td>
<td>Marchio C et al. 2008</td>
</tr>
</tbody>
</table>

aCGH: array Comparative Genome Hybridization, FISH: Fluorescent In Situ Hybridization, CISH: Chromogenic In Situ Hybridization, MLPA: Multiplex Ligation-dependent Probe Amplification.
## Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Association: yes/no</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development of distant metastasis</td>
<td>Yes: positive</td>
<td>Elbauomy Elsheikh S et al. 2007</td>
</tr>
<tr>
<td>Grade</td>
<td>No</td>
<td>Elbauomy Elsheikh S et al. 2007, Letessier A et al. 2006, Moelans CB et al. 2010</td>
</tr>
<tr>
<td>Histopathologic type</td>
<td>No</td>
<td>Kadota M et al. 2009, Letessier A et al. 2006, Moelans CB et al. 2010</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>No</td>
<td>Letessier A et al. 2006, Moelans CB et al. 2010</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>No</td>
<td>Elbauomy Elsheikh S et al. 2007, Kadota M et al. 2009</td>
</tr>
<tr>
<td>Molecular subtypes (basal-like, luminal A/B, ERBB2-positive, normal-like)</td>
<td>No</td>
<td>Letessier A et al. 2006, Moelans CB et al. 2010</td>
</tr>
<tr>
<td>P53 status</td>
<td>No</td>
<td>Letessier A et al. 2006</td>
</tr>
<tr>
<td>EGFR (epidermal growth factor receptor) status</td>
<td>No</td>
<td>Elbauomy Elsheikh S et al. 2007</td>
</tr>
<tr>
<td>Expression of low- and high-molecular weight cytokeratins</td>
<td>No</td>
<td>Elbauomy Elsheikh S et al. 2007</td>
</tr>
<tr>
<td>Androgen receptor status</td>
<td>No</td>
<td>Elbauomy Elsheikh S et al. 2007</td>
</tr>
<tr>
<td>Proliferation</td>
<td>No</td>
<td>Moelans CB et al. 2010</td>
</tr>
<tr>
<td></td>
<td>Yes: positive</td>
<td>Letessier A et al. 2006</td>
</tr>
<tr>
<td>ER (estrogen receptor) status: positive or negative</td>
<td>No</td>
<td>Elbauomy Elsheikh S et al. 2007, Kadota M et al. 2009, Letessier A et al. 2006</td>
</tr>
<tr>
<td></td>
<td>Yes: associated with a positive status</td>
<td>Moelans CB et al. 2010</td>
</tr>
<tr>
<td>PR (progesterone receptor) status: positive or negative</td>
<td>No</td>
<td>Kadota M et al. 2009, Letessier A et al. 2006, Moelans CB et al. 2010</td>
</tr>
<tr>
<td></td>
<td>Yes: trend with a negative status</td>
<td>Elbauomy Elsheikh S et al. 2007</td>
</tr>
<tr>
<td>Age (&lt;50/≥50)</td>
<td>No</td>
<td>Letessier A et al. 2006, Moelans CB et al. 2010</td>
</tr>
<tr>
<td>---------------</td>
<td>----</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Yes: if amplified, more likely to be older than 50</td>
<td>Elbauomy Elsheikh S et al. 2007</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Her2 status</th>
<th>No</th>
<th>Letessier A et al. 2006, Moelans CB et al. 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes: inverse correlation with Her2 overexpression</td>
<td>Elbauomy Elsheikh S et al. 2007</td>
<td></td>
</tr>
</tbody>
</table>

| Yes: amplification was positively associated with Her2+ status | Kadota M et al. 2009 |
Table 3

<table>
<thead>
<tr>
<th>Cases, N</th>
<th>Gly/Gly</th>
<th>Gly/Arg</th>
<th>Arg/Arg</th>
<th>Correlations with Arg-allele</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls (n=123)</td>
<td>45%</td>
<td>49%</td>
<td>6%</td>
<td>Axillary lymph node involvement</td>
<td>Bange J et al. 2002</td>
</tr>
<tr>
<td>145</td>
<td>46%</td>
<td>43%</td>
<td>11%</td>
<td>Axillary lymph node involvement</td>
<td>Bange J et al. 2002</td>
</tr>
<tr>
<td>234</td>
<td>52%</td>
<td>37%</td>
<td>11%</td>
<td>Axillary lymph node involvement</td>
<td>Jezequel P et al. 2004</td>
</tr>
<tr>
<td>372</td>
<td>49%</td>
<td>43%</td>
<td>8%</td>
<td>Tumor stage, tumor size, Axillary lymph node involvement</td>
<td>Thussbas C et al. 2006</td>
</tr>
<tr>
<td>352</td>
<td>46%</td>
<td>43%</td>
<td>11%</td>
<td>Tumor stage, Axillary lymph node involvement</td>
<td>Marme F et al. 2010</td>
</tr>
</tbody>
</table>