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# HER3, serious partner in crime
## Therapeutic approaches and potential biomarkers for effect of HER3-targeting

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ABSTRACT

The human epidermal growth factor receptor (HER) family members are targeted by a growing numbers of small molecules and monoclonal antibodies. Resistance against the epidermal growth factor receptor (EGFR) and HER2-targeting agents is a clinically relevant problem forcing research on optimizing targeting of the HER family. In view of its overexpression in tumors, and compensatory role in HER signaling, HER3 has gained much interest as a potential additional target within the HER family. It is the only member of the HER family lacking intrinsic tyrosine kinase activity and therefore its role in cancer has long been underestimated. Drugs that block HER3 or interfere with HER3 dimer signaling, including fully human anti-HER3 antibodies, bispecific antibodies and tyrosine kinase inhibitors (TKIs), are currently becoming available. Several compounds have already entered clinical trial. In the meantime potential biomarkers are tested such as tumor analysis of HER3 expression, functional assays for downstream effector molecules and molecular imaging techniques. This review describes the biology and relevance of HER3 in cancer, agents targeting HER3 and potential biomarkers for effect of HER3-targeting.



**Contents**



## 1. Introduction

The human epidermal growth factor receptor (HER) family comprising the epidermal growth factor receptor (EGFR) (also known as HER1), HER2, HER3 and HER4 (also known as respectively ErbB2, ErbB3, and ErbB4) is not only essential for the development and maintenance of normal tissue, but is also strongly involved in the development of many tumor types (Campbell et al., 2010). EGFR and HER2 are widely known targets for cancer therapy, with both monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs) directed against these

*Abbreviations:* 17-AAG, 17-(allylamino)-17-demethoxygeldanamycin; ADCC, ANTIBODY dependent cellular cytotoxicity; CRC, COLORECTAL cancer; Cu, COPPER; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ERK, extracellular-signal-regulated kinase; GRB7, growth factor receptor-bound protein 7; HDAC, histone deacetylase; HDACi, HDAC inhibitor; HER, human epidermal growth factor receptor; HNSCC, head and neck squamous cell carcinoma; HRG, heregulin; HSP90, heat shock protein 90; IGF-1, insulin-like growth factor 1; In, indium; mAb, monoclonal antibody; MAPK, mitogen activated protein kinase; mTOR, mammalian target of rapamycin; NRG, neuregulin; NSCLC, non-small cell lung cancer; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol trisphosphate; PYK2, proline-rich tyrosine kinase 2; RTK, receptor tyrosine kinase; SHC, SH2 containing protein; siRNA, small interfering RNA; TGF-α, transforming growth factor α; TKI, tyrosine kinase inhibitor; Zr, zirconium.

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receptors. The mAb trastuzumab which targets HER2 is now part of standard of care for patients with HER2-positive breast cancers (Yeon & Pegram, 2005). However, intrinsic or developed resistance against HER-targeting agents, is a clinical problem, and much research focuses on optimizing targeting of the HER family (Sergina et al., 2007; Garrett & Arteaga, 2011).

HER3 is the only member of the HER family lacking intrinsic tyrosine kinase activity. Therefore its role in cancer has long been underestimated. EGFR and HER2 are the preferred dimerization partners of HER3 (Graus-Porta et al., 1997; Prenzel et al., 2001). Interestingly, these two HER3 heterodimers are the most active signaling complexes in this family (Tzahar et al., 1996; Holbro et al., 2003). As such, HER3 is implicated in tumor growth and maintenance of many solid tumor types. In view of its overexpression in tumors, and compensatory role in HER signaling, HER3 is an interesting target to optimize HER family signaling inhibition. Anti-HER3-targeting agents, such as anti-HER3 mAbs and bispecific antibodies targeting both HER3 and EGFR, are currently in development. HER3 mAbs can inhibit ligand-induced phosphorylation of HER2, HER3 and downstream effector molecules, including the extracellular-signal-regulated kinase (ERK) 1, ERK2 and AKT. Blocking of HER3 dimer dependent signaling can furthermore be achieved with TKIs targeting EGFR and HER2, which indirectly inhibit HER3 phosphorylation. In the meantime potential biomarkers are tested, including tumor analysis of HER3 expression, functional assays for downstream effector molecules and molecular imaging techniques.

Therefore, the focus of this review is to describe the biology and relevance of HER3 in cancer, the currently known agents targeting HER3 and potential biomarkers for effect of HER3-targeting.

## 2. Human epidermal growth factor receptor 3, a member of the human epidermal growth factor receptor family

### 2.1. Receptor characteristics

HER3 is encoded by the *ERBB3* gene and maps to the human chromosome 12q13. Its mRNA gives rise to a 185 kDa transmembrane glycoprotein, which is composed of three regions: a NH2-terminal extracellular ligand-binding region, a transmembrane domain, and an intracellular region containing the COOH-terminal (Kraus et al., 1989; Zimonjic et al., 1995). HER3 has marginal kinase activity due to substitutions in its tyrosine kinase domain, and efficient downstream signaling reportedly happens as a result of heterodimerization (Plowman et al., 1990; Sergina et al., 2007; Shi et al., 2010). Furthermore, it is not transforming when constitutively activated by continuous ligand stimulation or when it is overexpressed (Zhang et al., 1996). Recently, *ERBB3* somatic mutations have been found in several types of human cancer, including colon and gastric cancer (Jaiswal et al., 2013).

HER3 is physiologically expressed in a wide variety of normal human tissue, including cells of the gastrointestinal, urinary, respiratory, and reproductive tracts as well as the skin, endocrine and nervous system (Prigent et al., 1992). In the pathological setting, overexpression of HER3 is often accompanied by overexpression of EGFR and/or HER2 (Ito et al., 2001, Giltnane et al., 2009). Furthermore, HER3 is a co-receptor for the amplified HER2 in breast cancer (Holbro et al., 2003) and is strongly implicated as a co-receptor for EGFR in a subset of EGFR-driven lung cancers and seems to be an effective predictor of sensitivity to the EGFR TKI gefitinib (Engelman et al., 2005). Immunohistochemical studies showed that HER3 overexpression is often associated with poor prognosis in various tumor types including breast, head and neck and gastric cancer (Takikita et al., 2011; Hayashi et al., 2008; Giltnane et al., 2009).

Eleven ligands bind to the HER family members, including epidermal growth factor (EGF), transforming growth factor α (TGF-α) and heregulin (HRG)/neuregulin (NRG) family members (Harris et al., 2003). HER2 has no identified ligands and exists in an open conformation that allows dimerization with other HER members. The primary ligands for HER3 are the members of the NRG family, including NRG1 (also known as HRG). Ligands can bind directly to the extracellular domain of EGFR, HER3 or HER4, which leads to a conformational rearrangement. This rearrangement exposes the dimerization domain that forms the core of the dimer interface with another HER. In HER2 amplified cancers, HER2:HER3 dimers may also be formed in a ligand-independent manner (Mukherjee et al., 2011). After the heterodimerization with other HER family members, the tyrosine kinase portion of HER3 becomes transphosphorylated. The phosphorylation creates docking sites that allow the recruitment of downstream signaling proteins. These signaling proteins include SH2 containing protein (SHC) and growth factor receptor-bound protein 7 (GRB7) that activate the RAS-mitogen activated protein kinase RAS-MAPK pathway (see Fig. 1). Apart from activating the RAS-MAPK pathway, the HER3 cytoplasmic domain contains 6 docking sites for phosphatidylinositol 3-kinase (PI3K) (Campbell et al., 2010). When PI3K is activated by a HER3 dimer, it phosphorylates membrane phosphatidylinositol bisphosphate (PIP2), which forms phosphatidylinositol trisphosphate (PIP3). This leads to the recruitment and subsequently to the activation of phosphoinositide-dependent kinase-1 (PDK1) and AKT. AKT is able to activate the mammalian target of rapamycin (mTOR)–a downstream mediator of the PI3K/AKT pathway–and thereby controls many biological processes, which are important for tumorigenesis. These biological processes include survival, translation, nutrient sensing, cell cycle control and metabolic regulation.

HER3 also plays a compensatory role in HER signaling. HER3 expression or signaling is associated with resistance to HER2 inhibitors in breast cancers. Treatment of HER2-driven breast cancer cell lines and xenograft tumors with HER-targeting TKIs led to a rapid compensatory increase in expression, signaling activity and relocalization of HER3 to the plasma membrane (Sergina et al., 2007). The compensatory increase in signaling activity is due to a compensatory shift in the HER3 phosphorylation–dephosphorylation equilibrium. This is caused by increased membrane HER3 expression (driving the phosphorylation reaction) and by reduced HER3 phosphatase activity (decreasing the dephosphorylation reaction). The knockdown of HER3 using small interfering RNA (siRNA) restored the potent pro-apoptotic activity of the TKIs. In addition, inhibition of HER3 with siRNA or a neutralizing HER3-antibody sensitized HER2-positive breast cancer cells and xenografts to lapatinib, which blocks the activation of both HER2 and EGFR, in vitro and in vivo. Therefore, persistent and complete inhibition of HER3 and its output to PI3K/AKT is likely needed for the optimal antitumor effect of therapeutic inhibitors of HER2 (Garrett et al., 2011, 2013a).

The kinase domain of HER3 has long been assumed to be inactive and has been classified as a pseudokinase (Citri, 2003; Boudeau et al., 2006). However, there is evidence that it is able to bind ATP and promote trans-autophosphorylation of the receptor's intracellular domain when it is clustered at a membrane surface (Shi et al., 2010). The tyrosine kinase activity is roughly 1000-fold weaker than that of EGFR. This might be sufficient for receptor transphosphorylation in the context of a heterodimer with another family member. In vitro, HER3 autophosphorylation was not inhibited by the TKIs lapatinib, gefitinib or erlotinib. In addition, HER3, but not HER2, is able to phosphorylate proline-rich tyrosine kinase 2 (PYK2) due to a very selective substrate specificity (van der Horst et al., 2005). PYK2 is a cytoplasmic tyrosine kinase, which is highly expressed in the central nervous system and promotes migration and invasion of glioma cells (Lipinski et al., 2005). The phosphorylation of PYK2 leads to a mitogenic response through activation of the MAPK pathway in human glioma cells (van der Horst et al., 2005). Expression of a dominant-negative PYK2 construct abrogated the HRG-induced MAPK activity, leading to the inhibition of the invasive potential of glioma cells. Activation of HER3, without detection of other activated HER family members, was reported in other cancer cell lines such as the breast cancer cell lines BT483, T47D and MCF-10A, and the ovarian cancer cell line OVCAR (Rajkumar & Gullick, 1994; Beerli et al., 1995). Other kinases such as the MET receptor may also activate HER3 under some circumstances. In vitro, MET