Targeting HER3 using mono- and bispecific antibodies or alternative scaffolds

Magdalena Malm, Fredrik Y. Frejd, Stefan Stahl, and John Löblom

Division of Protein Technology, School of Biotechnology, KTH-Royal Institute of Technology, SE, Stockholm; Affibody AB, SE, Stockholm, Sweden; Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

ABSTRACT
The human epidermal growth factor receptor 3 (HER3) has in recent years been recognized as a key node in the complex signaling network of many different cancers. It is implicated in de novo and acquired resistance against therapies targeting other growth factor receptors, e.g., EGFR, HER2, and it is a major activator of the PI3K/Akt signaling pathway. Consequently, HER3 has attracted substantial attention, and is today a key target for drugs in clinical development. Sophisticated protein engineering approaches have enabled the generation of a range of different affinity proteins targeting this receptor, including antibodies and alternative scaffolds that are either mono- or bispecific. Here, we describe HER3 and its role as a key tumor target, and give a comprehensive review of HER3-targeted proteins currently in development, including discussions on the opportunities and challenges of targeting this receptor.

Introduction
In the field of oncology, the ability to specifically target tumor cells using highly selective molecules is an attractive and established concept. Due to the highly diverse nature of different cancers, treatment methods and therapeutics should ideally be customized depending on the cancer subtype, and hence, the concept of personalized medicine has emerged. Technological advancements within genomics and proteomics have increased our knowledge about the mechanisms behind tumor development and growth, which has enabled identification of many new cancer-associated proteins that are overexpressed or mutated in tumors compared to normal tissues.1 As a result, improved methods for detection and diagnosis are emerging, e.g., using radiotracers specific for certain tumor-markers combined with single photon emission CT (SPECT) or positron emission tomography (PET) imaging. Unlike traditional methods, which require serum samples or biopsies, non-invasive diagnostic imaging can provide information about tumor subtypes along with detection of distant metastatic lesions, as well as discordsances between primary and secondary tumors.

Personalized treatments using targeted therapeutics, such as highly specific affinity proteins or tyrosine kinase inhibitors, are being developed as less toxic alternatives and complements to conventional chemotherapeutic drugs. In this context, members of the epidermal growth factor receptor (EGFR) family are well-established targets in many different human cancers. However, unlike its family members EGFR and HER2, the role of HER3 in tumor signaling was elucidated relatively recently. Today, HER3 is known to be an important signaling node in many human cancers, and involved in the resistance against targeted therapies toward other epidermal growth factor receptors. As a result, HER3 has emerged as a suitable target for therapeutics that might block the receptor’s function, deliver of toxic payloads and for tumor imaging agents.

Epidermal growth factor receptors
The EGFR family is a group of cell-surface receptors that have been implicated in the progression of several different cancers. The family consists of four homologous receptors: EGFR (HER1 or ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4), which normally signal for cell division, migration, survival and organ development (Fig. 1).2,3 These receptors contain four extracellular domains, a transmembrane region, an intracellular tyrosine kinase domain for signal transduction and a cytoplasmic tail with tyrosine phosphorylation residues (Fig. 1).3,4 Signaling is initiated by binding of a ligand to the extracellular domain I and III. At least 11 ligands have been...
identified, including epidermal growth factor (EGF), transforming growth factor-α (TGF-α), betacellulin (BTC) and neuregulin (NRG). Of these, some are specific for only one receptor while others are more promiscuous. Ligand binding results in structural rearrangement of the extracellular receptor domains: an inactive closed conformation rearranges to expose a dimerization arm on domain II, which can then interact with another activated receptor nearby to form homo- or heterodimers (Fig. 1). Such extracellular receptor dimerization leads to activation of intracellular tyrosine kinase activity in an asymmetric manner, where one receptor induces a structural change of its binding partner’s catalytic domain, which is then activated for subsequent phosphorylation of tyrosines at the C-terminal tail of the activating receptor. These phosphorylated residues subsequently provide docking sites for cytoplasmic signaling molecules, and are critical to the initiation of various signaling cascades, of which the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI-3K) pathways are the predominant routes, mainly stimulating proliferation and survival, respectively. The nature of the signaling mediated by these receptors mainly depends on which ligand and receptor pair are involved in the signal transduction.

Two main characteristics differentiate HER2 and HER3 from the other receptors of this family. HER2, which is the most potent and preferred dimerization partner in the HER family, has an extracellular domain that lacks activating ligands. It adopts a constantly active conformation (Fig. 1), enabling ligand-independent interactions with other activated receptors. In contrast, HER3 has an intracellular tyrosine kinase domain (Fig. 1) that is inactive or active to a very low extent compared to its family members. Normally, both HER2 and HER3 heterodimerize with another receptor to facilitate signaling, and the heterodimer formed by these two receptors has been shown to be the most potent signaling pair within this receptor family. Another notable observation is that HER3 seems unable to form homodimers upon ligand activation, whereas overexpression of HER2 can lead to homodimerization and ligand-independent signaling, a process associated with several different cancers. Aberrant signal transduction of the HER family promotes cell survival and growth, and it is naturally involved in the progression of cancer. In particular, HER2 and EGFR have been extensively evaluated in this context, and overexpression, gene amplification, autocrine signaling, activating point mutations or partial gene deletions of these receptors have been reported in many human cancers. Both monoclonal antibodies and small molecule tyrosine kinase inhibitors (TKIs) that target HER2 and EGFR have been developed. For instance, the anti-HER2 antibodies trastuzumab and pertuzumab are approved for treatment of HER2-overexpressing breast cancers, while ado-trastuzumab emtansine (trastuzumab conjugated to the chemotherapeutic agent DM-1) has been approved for treatment of HER2-positive metastatic breast cancer patients previously treated with either unconjugated trastuzumab or a taxane, or the combination of the two agents. Anti-EGFR antibodies cetuximab and panitumumab are approved for treatment of EGFR-expressing colorectal cancers; cetuximab is also approved for head-and-neck cancers.

Even though approved antibodies are efficacious in the treatment of some patients, response rates can be low. When trastuzumab is administered as a single agent to HER2-overexpressing breast cancer patients, the response rate is only 11–26%. In colon cancer, cetuximab and panitumumab only benefit a specific subgroup of patients who express the non-mutated form of the proto-oncogene KRAS. In addition to de novo non-responding tumors, resistance can develop against these targeted therapies during treatment, rendering the drugs inefficacious.

The role of HER4 in cancer is obscure, and different studies suggest that this receptor may have bifacial role, providing both pro- and anti-tumoral effects depending on the cancer subtype and the HER4 isoform being expressed. Due to this complicated nature of HER4 in the context of cancer, this receptor is not...
recognized as a validated tumor target for antibody therapy and no HER4 specific antibodies have yet been evaluated clinically.

One explanation to both de novo and acquired resistance against antibodies or TKIs targeting EGFRs is the extensive cross-talk and signal plasticity observed by receptors within this and closely related families.\(^2\),\(^5\) Hence, if signaling by one receptor is blocked, another may be able to compensate. As a consequence, the idea of targeting more than one EGFR family member simultaneously has become an attractive approach. In this context, HER3 has been recognized as a key target in several different human cancers.\(^2\),\(^5\) Even though HER3 has very low tyrosine kinase activity, it is thought to be an important co-receptor and allosteric activator of other epidermal growth factor receptors.\(^1\),\(^2\),\(^6\)

**HER3 in cancer**

Because HER3 has an inactive tyrosine kinase domain, this receptor was long considered completely dependent on the activity of its family members, and therefore not crucial in the context of cancer.\(^2\),\(^5\) However, while not reported to be oncogenic on its own, HER3 is now recognized as a key player in many different cancers overexpressing HER2 or EGFR, and it is also implicated in resistance against HER-targeting therapeutics.\(^2\),\(^5\) The HER3 gene was discovered in 1989, and its expression or overexpression has since then been reported in many cancers, including breast, ovarian, lung, colon, melanoma and prostate.\(^2\),\(^7\)–\(^2\),\(^8\) However, the importance of HER3 in cancer has only recently started to be revealed, and today HER3 is associated with poor clinical prognosis in several different cancers, including breast, ovarian, lung and colon.\(^3\),\(^1\),\(^3\),\(^5\)–\(^7\) The role of HER3 is especially prominent in many HER2-driven breast cancers where the HER2/HER3 dimer is considered an oncogenic unit, as proposed by Holbro and colleagues in 2003.\(^2\),\(^6\)

One striking characteristic of HER3 is its ability to directly activate the PI-3K pathway through 6 intracellular tyrosine-containing docking sites for the p85 subunit of the PI-3K protein on its C-terminal tail.\(^3\),\(^8\)–\(^3\),\(^0\) Phosphorylation of these docking sites makes HER3 a potent activator of the PI-3K pathway, as opposed to HER4, which only contains a single p85 docking site, or HER2 and EGFR, which activate PI-3K indirectly via adaptor proteins.\(^3\),\(^2\),\(^2\) Activated PI-3K initiates downstream signaling via Akt, resulting in activation of a range of different pathways leading to increased proliferation and survival.\(^4\) Dysregulation of this pathway is observed in many human cancers. Because HER3 is the major activator of PI-3K, it often plays an important role in such tumors.\(^2\),\(^4\) For instance, HER3 expression or high levels of PI-3K signaling is often observed in HER2-amplified breast cancers, and complete inhibition of HER3-mediated PI3K/Akt-signaling is considered important for maximal therapeutic effect of HER2-targeting agents.\(^4\),\(^2\),\(^2\) A computational model of the HER-signaling network has indeed recognized HER3 as a sensitive node for the activation of Akt, where the abundance of HER3 affects ligand-induced Akt phosphorylation to a greater extent than changes in EGFR or HER2 expression.\(^4\) Moreover, compensatory signaling by HER3 is frequently coupled to acquired resistance against anti-HER treatment, potentially by providing a direct link to the PI-3K pathway. Such escape signaling has been reported to be the result of increased HER3 expression, reduced HER3 dephosphorylation, increased cell surface localization or upregulation of HER3 ligands.\(^2\),\(^4\),\(^8\)–\(^5\),\(^1\) Until recently, no HER3-gene mutations had been described to be oncogenic; however, in 2013 Jaiswal and coworkers reported the observation of oncogenic mutations of HER3 in colon and gastric cancers.\(^5\)

HER3 has two known extracellular ligands, neuregulin (NRG) 1 and 2; NRG 1 is also known as heregulin (HRG) or neu differentiation factor.\(^3\) HRG binds to HER3 with low nanomolar affinity, which is enhanced upon receptor dimerization with HER2.\(^5\) In addition to upregulation of HER3, high levels of HRG have also been reported in several cancers, indicating signaling through HER3 in an autocrine fashion. For instance, autocrine signaling has been observed in some ovarian cancer cell lines that express constitutively activated HER3.\(^5\) Downregulation of either HER3 or HRG in such cell lines, but not in cells with non-activated HER3, results in impaired in vitro tumor cell growth.\(^5\) Similar autocrine signaling has also been observed in breast cancer cells, where treatment with trastuzumab, which is able to block HER2 dimerization with other receptors including HER3, inhibits HER3/ ligand-induced tumor cell growth in vivo.\(^4\)

The same growth inhibition is not obtained by trastuzumab, which is unable to block ligand-induced receptor dimerization.\(^4\),\(^5\) Instead, the anti-tumoral activity by trastuzumab in HER2-overexpressing breast cancer tumors has been suggested to depend on its ability to disrupt PI-3K signaling activated by ligand independent HER2/HER3 dimers.\(^5\),\(^5\)–\(^5\) However, treatment of HER2-overexpressing breast cancer cells with trastuzumab in vitro can induce endogenous ligand expression and secretion, providing an autocrine signaling route via, for example, heregulin and HER3, that is potentially involved in the resistance observed in some patients against trastuzumab.\(^5\),\(^1\) Similarly, upregulation of heregulin has also been observed in colorectal cancer patients with both de novo and acquired resistance against cetuximab, suggesting that autocrine signaling via heregulin and HER3 also plays an important role in this setting.\(^5\) Moreover, HER3 has been shown to restore PI-3K signaling upon treatment of HER2-overexpressing breast cancer cells with tyrosine kinase inhibitors due to the inability of these agents to suppress long-term HER3 signaling.\(^4\) While phosphorylation of EGFR, HER2 and HER3 is initially lost upon treatment, this effect is only transient for HER3, and its activity is restored through feed-back signaling as a result of Akt-inactivation, leading to upregulation of active HER3 at the cell membrane through cell-surface re-localization and reduced HER3 dephosphorylation.\(^4\)

HER3-mediated acquired resistance has also been associated with resistance against chemotherapeutics, tamoxifen, anti-insulin-like growth factor 1 receptor (IGF-1R) therapies, as well as in castration resistance.\(^5\),\(^9\)–\(^6\) Due to the importance of HER3 in many cancers and in drug resistance, HER3 is both a promising target for therapy and an important diagnostic marker. More precise profiling of the tumor expression and dependence on epidermal growth factor receptors, in combination with other important markers, is essential to enable more personalized cancer treatments. Consequently, HER3 is an important player in the context of cancer and many affinity proteins targeting HER3 are currently in development.
Table 1. Overview of protein-based HER3-targeting agents in development.

| Format | Target | Most advanced clinical phase | Company | References |
|--------|--------|-------------------------------|---------|------------|
| mAbs: |        |                               |         |            |
| Patritumab (U3-1287) | Human IgG1 | HER3 | 3 | U3-Pharma, Amgen, Daiichi Sankyo | LoRusso et al.66 Li et al.59 |
| Seribantumab (MM-121) | Human IgG2 | HER3 | 2 | Merrimack Pharmaceuticals, Sanofi Aventis | Schoeberl et al.67 Schoeberl et al.70 |
| Eligentumab (LJM716) | Human IgG1 | HER3 | 1/2 | Novartis Pharmaceuticals, Ariad, Morphosys | Garner et al.74 |
| Lumretuzumab (RG7116) | Humanized IgG1 | HER3 | 1 | Roche, Glycart | Mirschberger et al.75 Meulendijk et al.77 Meezete et al.78 |
| AV-203 | Humanized IgG1 | HER3 | 1 | AVEO Oncology, Biogen | Lee et al.79 Xiao et al.80 |
| KTN3379 | Human IgG1 | HER3 | 1 | Kolltan Pharmaceuticals, Medimmune | |
| GSK2849330 | Humanized IgG | HER3 | 1 | Glaxo group | |
| TK-A3, TK-A4 | Humanized IgG | HER3 | Preclinical | Takis | Aurisicchio et al.83 Belleudi et al.84 |
| Millegen patent anti-HER3 | Nanobody | HER3 | Preclinical | MilleGen Ab Pharma, INSERM | Lazrek et al.87 |
| Sea Lane patent anti-HER3 (SL175 & SL176) | | | | Sea Lane Biotechnologies | |
| Ablynx patent anti-HER3 | | | | Ablynx, Merck Serono | |
| SG1 | Mouse IgG | HER3 | Preclinical | University of Kent | |
| Merck & Co patent anti-HER3 | | | | Merck & Co | |
| Immunogen patent anti-HER3 | Humanized IgG | HER3 | Preclinical | ImmunoGen | |
| Symphogen patent anti-HER3 | Mouse IgG | HER3 | Preclinical | Symphogen A/S | |
| KKH patent anti-HER3 | Mouse IgG | HER3 | Preclinical | Kyowa Hakko Kirin Co | |
| Trellis patent anti-HER3 | NR | HER3 | Preclinical | Trellis Bioscience | |
| AUB patent anti-HER3 | NR | HER3 | Preclinical | UAB Research Foundation | |
| Genentech patent anti-HER3 | NR | HER3 | Preclinical | Genentech | |
| Sorrento patent anti-HER3 | NR | HER3 | Preclinical | Sorrento Therapeutics | |
| University of Texas patent anti-HER3 | NR | HER3 | Preclinical | University of Texas | |
| Roche patent anti-Her3 | NR | HER3 | Preclinical | Roche | |
| Beijing Inst. of basic med. sci. anti-HER3 | NR | HER3 | Preclinical | Beijing Institute of basic medical sciences | |
| Bispecifics/multispecifics: | | | | | |
| Istritumab (MM-141) | IgG1 fused to two scFvs | HER3/GF-1R | 2 | Merrimack Pharmaceuticals | Fitzgerald et al.118 Xu et al.125 Schaefer et al.115 Huang et al.116 |
| Duligotumab (MEHD7945A) | Two-in-one symmetrical IgG1 | HER3/EGFR | 2 | Genentech, Roche | |
| MCLA-128 | Common light chain IgG1 | HER3/HER2 | 1/2 | Merus B.V. | Robinson et al.119 |
| Fox Chase patent anti-HER2/HER3 (ALM) | Bispecific scFv | HER3/HER2 | Preclinical | Fox Chase Cancer Center | |
| University of Texas patent anti-HER2/HER3 (TAB6) | IgG fused to two scFvs | HER3/HER2 | Preclinical | University of Texas | Kang et al.120 Poovassery et al.127 Gu et al.138 Hu et al.129 |
| Abbvie anti-EGFR/HER3 | DVD-ig | HER3/EGFR | Preclinical | Abbvie | |
| FL518 | Four-in-one IgG | HER3/HER2/EGFR/VEGF | Preclinical | The Second Military Medical University | |
| CRTB6 | Tetravalent DVD-ig | HER3/HER2/EGFR/VEGF | Preclinical | The Second Military Medical University | Hu et al.129 |
| Roche Glycart patent anti-HER3/chMet | NR | HER3/chMet | Preclinical | Roche Glycart AG | |
| Fox Chase patent anti-EGFR/HER3 | NR | HER3/EGFR | Preclinical | Fox Chase Cancer Center | |
| Futuximab | Chimeric human/mouse | HER3/EGFR | Preclinical | Symphogen | |
| Roche patent anti-HER3/HER4 | NR | HER3/HER4 | Preclinical | Roche | |
| Zymeworks patent anti-HER2/HER3 | NR | HER3/HER2 | Preclinical | Zymeworks | |
| Samsung patent anti-c-MET/HER3 | NR | HER3/c-MET | Preclinical | Samsung Electronics | |
| Merus patent anti-HER3/EGFR | NR | HER3/EGFR | Preclinical | Merus Biopharmaceuticals BV | |
| Merus patent anti-HER2/HER3 | NR | HER3/HER2 | Preclinical | Merus Biopharmaceuticals BV | |
| Roche patent anti-HER2/HER3 | NR | HER3/HER2 | Preclinical | Roche | |
| ZHER3-ABD-ZHER2 | Affibody/ ABD fusion | HER3/HER2 | Preclinical | Affibody AB | Malm et al.144 |
| CET-c1H | IgG/Affibody fusion | HER3/EGFR | Preclinical | Zymegnia | LaFleur et al.143 |
| Fab-PEG24-HRG | Fab/HRG crosslinkage | HER3/HER2 | Preclinical | University of Toronto | Razumienko et al.145 |
| Alternative scaffolds: | | | | | |
| ZHER3 | Affibody | HER3 | Preclinical | Affibody AB | Kronqvist et al.132 Göstring et al.138 Malm et al.139 |

* Only evaluated as an imaging agent

Abbreviations: NR = Not reported
**HER3-targeting agents**

Numerous HER3-targeting proteins, such as mono- and bispecific antibodies or alternative scaffolds, have recently been described (Table 1). These agents have various mechanisms of interfering with tumor cell growth, including inhibition of ligand binding, inhibition of receptor dimerization, downregulation of HER3 from the cell surface, recruitment of Fc-mediated functions or locking HER3 in an inactive conformation. Selected examples of HER3-targeting agents are described below.

**Monospecific antibodies**

The HER3-targeting agent that has advanced furthest in clinical development is patritumab (U3-1287; U3-pharma GmbH, Amgen, Daiichi-Sankyo). Patritumab is an IgG1 that was generated by XenoMouse technology, i.e., the generation of human antibodies from transgenic mice with human immunoglobulin loci. This antibody has an affinity of ~1-3 nM and induces rapid receptor internalization and degradation as well as inhibits HER3-activation. Consequently, patritumab blocks both basal and ligand-induced HER3-signaling. In vitro studies have shown that U3-1287 can enhance sensitivity of cancer cells to radiation treatment by blocking radiation-induced activation of HER3. In a dose-finding first-in-human Phase 1 study [NCT00730470] of patritumab in patients with solid tumor-types known to express HER3, patritumab was found to be well tolerated, with mostly mild to moderate adverse events including fatigue, diarrhea, nausea, decreased appetite and dysgeusia. In addition, no human anti-human antibodies (HAMA) were detected against patritumab and preliminary findings indicated a trend toward correlation between high HER3-expression or amplification and clinical efficacy. At present, patritumab is under evaluation in combination with other agents in clinical trials of patients with cancers of the breast, head and neck, lung or non-small cell lung cancer (NSCLC) [NCT02134015, NCT02350712, NCT02633800]. In the most advanced trial (phase 3), the effect on progression-free survival of patritumab in combination with erlotinib, compared to placebo in combination with erlotinib, will be evaluated in NSCLC patients [NCT02134015]. This is an interesting clinical setting due to the previously mentioned involvement of HER3 signaling in the resistance against TKI’s observed in vitro and in vivo.

The HER3-specific seribantumab (MM-121) was developed by Merrimack Pharmaceuticals. This antibody was generated from a phage-displayed antigen-binding fragment (Fab) library. The isolated binder had a monovalent affinity of 0.8 nM, and was converted to a full-length human IgG2 format, with the aim of minimizing immunogenicity and immune effector-mediated off-target adverse effects. Seribantumab has been shown to have an anti-proliferative effect on several xenografts in vivo due to the competition with HRG for HER3 binding, as well as the ability to downregulate HER3 from the cell surface, depending on cancer cell line. Cell lines with low levels of HRG expression along with HER2 amplification were found to correlate with non-responsiveness to seribantumab therapy. Hence, cancers with ligand-dependent activation of HER3 signaling, as opposed to ligand-independent activation of HER3 in HER2-overexpressing cells, are potentially most suitable for seribantumab therapy. Seribantumab is being (or has been) evaluated in several clinical Phase 1 and 2 studies of multiple types of solid tumors, either alone or together with the oligoclonal EGFR-specific antibody MM-151, cetuximab, erlotinib, chemotherapeutics, exemestane or a PI3K inhibitor [NCT00994123, NCT01451632, NCT01447225, NCT02538627, NCT01421472, NCT01209195, NCT01151046, NCT02387216, NCT0147706, NCT00734305, NCT01436565]. In two different Phase 2 trials of seribantumab in combination with paclitaxel or exemestane in a subset of ovarian cancers [NCT01447706] or estrogen receptor/progesterone receptor positive HER2-negative breast cancers [NCT01151046], respectively, early results showed that seribantumab did not fulfill the primary endpoint of progression-free survival. However, in both studies, a subpopulation of patients that benefited from seribantumab therapy was identified based on a set of two biomarkers (high HRG levels in patients with low HER2 levels). Further clinical Phase 2 data has since confirmed that seribantumab increases progression free survival in HRG-high patients across breast, ovarian and lung cancers.

Another HER3-targeting approach is provided by the human monoclonal antibody elgemtumab (LJM716) developed by Novartis. This antibody was isolated from MorphoSys’ phage-displayed HuCal Fab library. Functional screening of selected clones for the ability to inhibit both ligand-dependent and independent HER3-signaling and cell growth ultimately resulted in the isolation of elgemtumab. This antibody binds to an epitope consisting of the interface between domain II and IV on HER3, which is responsible for the tethered inactive conformation of the receptor. Consequently, elgemtumab locks HER3 in an inactive conformation and prevents ligand-induced activation of HER3, even though it does not prevent HRG from binding to the receptor. Potent anti-tumoral effects of elgemtumab was shown in in vivo xenograft studies, and superior tumor-growth inhibition in combination with either trastuzumab or cetuximab compared to the respective monotherapies was demonstrated in relevant tumor models. Elgemtumab has been evaluated as a monotherapy in clinical Phase 1 studies of patients with advanced solid tumors, squamous cell carcinoma of head and neck (SCCHN), gastric or breast cancers [NCT01598077, NCT01911936]. Several Phase 1 or 1/2 studies of elgemtumab combined with trastuzumab or a PI3Kα-inhibitor in breast, gastric or esophageal cancers are ongoing [NCT01822613, NCT02167854, NCT01602406].

Lumretuzumab (RG7116, RO5479599) is a humanized IgG1 developed by Roche. This antibody binds to domain I of HER3 and its Fc-region has been glycoengineered to increase affinity toward the Fc-gamma receptor (FcγRIIIa) on immune effector cells. This enables a more potent activation of antibody-dependent cell-mediated cytotoxicity (ADCC) both in vivo and in vitro compared to the non-glycoengineered antibody. It also downregulates HER3 from the cell surface, and blocks HRG from binding and activating the receptor. Depending on the xenograft model, treatment with lumretuzumab, either alone or in combination with another antibody targeting either EGFR or HER2, demonstrated potent anti-tumoral effects. Lumretuzumab has been shown to block HER3-activation both in the presence and absence of HRG in vitro. Lumretuzumab thus therapeutically targets not only cells
that are dependent on active HER3 signaling, but also those that express non-activated HER3 on the cell surface. This antibody is currently being evaluated in 2 clinical Phase 1 studies. In one of these studies [NCT01482377], lumrectuzumab is being evaluated either alone or in combination with cetuximab or erlotinib in patients with HER3-expressing advanced or metastatic tumors of epithelial cell origin. In the second study [NCT01918254], lumrectuzumab is being evaluated in combination with pertuzumab and paclitaxel in HER2- and HER3-expressing metastatic breast cancer patients. Results from the first-in-human study showed that lumrectuzumab was well tolerated, and there were indications of clinical activity.77 Lumrectuzumab was also in a Phase 1 trial combined with paclitaxel and carboplatin of non-small cell lung cancer patients [NCT02204345], but this study was suspended in July 2015.

Other HER3-specific antibodies developed by AVEO Oncology and Kolltan Pharmaceuticals in collaboration with Medimmune are in Phase 1 studies [NCT01603979, NCT02014909, NCT02456701, NCT02473731]. AVEO Oncology’s humanized IgG1, AV-203, was shown to block ligand-binding to the receptor and induce HER3 degradation.78 Kolltan Pharmaceuticals and Medimmune developed KTN3379 by phage display; this antibody locks HER3 in an inactive conformation, enabling inhibition of both ligand-dependent and independent signaling.79 The epitope of KTN3379 is found in the border between domain 2 and 3 of HER3, and the affinity of the monovalent interaction (determined using the Fab of KTN3379 in SPR measurements) is 166 pM.79 Xiao and coworkers observed that the growth-inhibitory effect of KTN3379 in vitro was reduced upon PTEN knockdown by RNAi in HER2-amplified cancers where HER3 is activated in a ligand-independent manner.80 Loss of functional PTEN is commonly observed in cancers, and is associated with resistance against trastuzumab in tumors overexpressing HER2.81 The same effect upon PTEN loss was not observed with KTN3379 in cancer cells where HER3 is activated in a ligand-dependent manner, which emphasizes the advantage of being able to identify tumor subpopulations that will benefit from a certain therapeutic agent.

Although no studies of GSK2849330 have been published, this HER3-targeting antibody is undergoing evaluation in two Phase 1 clinical studies of patients with advanced HER3-positive tumors. In the first in human trial [NCT01966445] the safety, pharmacokinetics and pharmacodynamics of GSK2849330 will be evaluated, while the second trial [NCT02345174] aims to use PET-imaging in order to evaluate the biodistribution as well as the relationship between dose and receptor occupancy.

Antibodies in preclinical development have been described in the literature. TK-A3 and TK-A4 are humanized IgG1 monoclonal antibodies developed by Takis.82 The therapeutic efficacy of these antibodies strongly correlated with their ability to promote receptor internalization and degradation.82–84 The epitope of TK-A3 has been mapped to the extracellular dimerization-domain (domain II) of HER3, while the TK-A4 epitope has not yet been successfully mapped, potentially due to a more complex nature.84 Even so, both TK-A3 and TK-A4 have been shown to inhibit ligand binding and activation of the HER3-receptor.

The humanized monoclonal IgG1 EV20 is being developed by Mediapharma.85,86 This antibody inhibits ligand-induced activation of HER3, but does not compete with HRG for receptor binding. EV20 also induces receptor degradation, and hence this antibody inhibits both ligand-dependent and independent HER3-mediated signaling. Millegen S.A. has generated HER3-specific antibodies targeting either domain 1 or 3 of the receptor by phage display or lymphocyte hybridization.87 Selected antibodies were shown to delay tumor growth of various mouse xenograft models with different HER2-expression status and HRG-dependence. The binding sites of these human or murine antibodies do not overlap with the binding sites of HRG, but the antibodies still presumably disrupt HER2/HER3-dimerization, leading to inhibition of downstream signaling via Akt.

Data on two HER3-specific Surrobodies, SL-175 and SL-176, have been reported by Sea Lane Biotechnologies.88 Surrobodies are full-length antibodies composed of a variable heavy chain and an invariable surrogate light chain, enabling straightforward addition of specificity or function by various fusions to the termini of the light chains. SL-175 and SL-176 were generated by phage display pannings of a surrobody library against HER3 and isolated binders were shown to block HRG-induced dimerization of HER2 and HER3, and decrease cell-surface expression of HER3. Moreover, these surrobodies, likely binding to a similar epitope on HER3, inhibit downstream Akt and Erk signaling, as well as proliferation of both ligand-stimulated and unstimulated cell lines.88

Although antibodies have historically been the format of choice for therapeutic affinity proteins and numerous new therapeutic antibodies are likely to reach the global market within the coming years,89 they have drawbacks. In, for instance, imaging and radiotherapy applications, the slow blood clearance results in low tumor-to-blood contrast, preventing clear tumor visualization and increasing the exposure of patients to off-target effects of the radionuclides.90,91 In addition, the large size of the antibody results in slow extravasation from the blood and poor tissue penetration, limiting the dose that can reach disease areas distant from blood vessels.92 This is an issue thought to play a role in the relatively modest clinical response that has been observed by many therapeutic antibodies targeting solid tumors in vivo.93 Moreover, the complex heterotetrameric structure of the antibody typically requires fairly costly production regimes in mammalian cells, making many antibody treatments expensive.94 Furthermore, even though ADCC and CDC are often advantageous features of therapeutic antibodies, effector functions are not always essential for achieving a therapeutic effect, and can in some applications be problematic due to unwanted immunological responses.93,95,96 These drawbacks have motivated the development and evaluation of molecules that provide a similar binding capacity as full-length antibodies but are smaller in size. Several antibody-derived formats, such as the Fab, single-chain variable fragment (scFv), and domain antibodies (Fig. 2) have been developed toward cancer-related targets, and many are currently under evaluation for clinical use.95 As will be described below, scFv antibodies targeting HER3 are being explored for bispecific targeting, but to our knowledge, no scFvs or Fabs targeting only HER3 are in clinical development. Ablynx has developed a HER3-specific nanobody (not yet described in the literature) in collaboration with Merck Serono.97 Nanobodies are single-domain antibodies derived from heavy-chain antibodies from the camelid antibody repertoire.98
Bispecific antibodies

In recent years, bispecific antibodies or alternative affinity proteins have become attractive for tumor-targeting purposes because of the potential enhanced functionality compared to monospecific targeting. For instance, simultaneous targeting of 2 distinct tumor-associated antigens, not frequently available on normal cells, could theoretically enhance the specificity toward the tumor. This requires that both proteins can be targeted simultaneously by a single agent. In cases where such dual binding is conceivable, a bispecific agent could either block two interacting entities or bring them together. A clinically validated cancer-targeting strategy of bispecific molecules is the simultaneous binding and engagement of an immune effector cell and a tumor cell for induced cytotoxic activity. Furthermore, due to the complex nature of cancers, targeting several distinct proteins is often desirable, which has led to the idea of using bispecific antibodies as alternatives to antibody cocktails for tumor therapy. The use of antibody combinations requires the generation and production of several distinct antibodies, as well as the evaluation of each antibody separately and in combination, both in preclinical and clinical studies. Hence, the use of a single protein directed against two or more targets to enable, for example, simultaneous blocking of important pathways, is an attractive two-in-one approach. Moreover, bispecific proteins could be used in pre-targeting approaches for both radiotherapeutic and imaging purposes.

The IgG molecule is a naturally bivalent protein, with two arms recognizing the same epitope. The antibody format can, however, also be rearranged in order to incorporate multispecificity by genetic engineering, which has enabled the generation of more than 45 different multispecific antibody formats. In addition to classical methods, bispecific antibodies can be built by adding a scFv to either the N-terminal or the C-terminal ends (or both) of full-length antibodies, or the binding site of an antibody can be engineered to bind two distinct epitopes instead of only one, generating a symmetric bispecific construct. Asymmetric bispecific molecules can be assembled in various ways. For example, heterodimerization of two distinct heavy chains using the knobs-into-holes technique can be combined with forced assembly of correct light chain/heavy chain pairs by CrossMab technology. Antibody derivatives such as the Fab and the scFv can be multimerized into formats such as F(ab)\(_2\), diabodies, triabodies or tetrabodies (multimeric scFvs). Non-antibody derived alternative scaffolds are usually per se amenable to multimerizations due to their small and stable characteristics, and, as a result, several bispecific alternative scaffolds have been described in the literature.

The extensive signaling cross-talk and redundancy observed within the epidermal growth factor receptor family have raised the question of whether targeting of a single receptor will be enough for therapeutic efficacy. Consequently, several of the HER3-specific monoclonal antibodies mentioned above are being evaluated in combination with other antibodies, tyrosine kinase inhibitors or chemotherapeutics. Alternatively, different bispecific HER3-targeting approaches have been generated to facilitate two-in-one receptor targeting for improved therapeutic efficiency, and to avoid the development of acquired resistance against single-targeting agents. Moreover, bispecific targeting of HER3 could also potentially provide a mechanism for improved uptake in HER3-expressing tumors since HER3 is expressed at relatively low levels on tumor cells (10^4–10^5 receptors per cell) compared to, for instance, HER2 (~10^6 receptors per cell). Improved targeting strategies may be especially critical for HER3-targeting agents because this low expression, and the presence of HER3 in normal tissues such as breast, liver, lung, small intestine, stomach and salivary gland, could complicate targeting of HER3-expressing tumors in vivo.

Developed by Genentech, duligotuzumab (MEHD7945A) represents a novel bispecific HER3-targeting approach. This symmetric antibody has two identical binding sites, each able to bind the extracellular domain III of either EGFR or HER3. The dual binding mechanism is enabled within the same binding site since most of the EGFR-targeting residues are found in the heavy chain complementarity-determining regions (CDRs), while HER3-binding residues are found within the light-chain CDRs. The antibody was isolated by phage display selections from Fab-libraries, and, after conversion to an IgG1 format, duligotuzumab was shown to efficiently inhibit ligand-binding and signaling of both EGFR and HER3 in vitro and in vivo. The antitumoral activity translated into improved growth inhibition of several xenograft models, compared to monospecific treatments, when tumor growth was dependent on both receptors. In addition, the
antibody was also shown to mediate ADCC both in vitro and in vivo. Moreover, in combination with the chemotherapeutic agent gemcitabine, duligotuzumab showed a more potent antitumoral effect than monospecific treatments, resulting in xenograft regression in vivo. Duligotuzumab also suppressed growth of cetuximab- or erlotinib-resistant tumor cells both in vitro and in vivo, and reversed cross-resistance against radiation therapy in cetuximab-resistant tumor models.\textsuperscript{116} In a first-in-human Phase 1 study, duligotuzumab showed an encouraging safety profile in cancer patients with recurrent tumors of epithelial origin.\textsuperscript{121} Moreover, a first indication of a clinical antitumoral effect was observed. Several clinical Phase 1 or two studies of duligotuzumab either alone or in combination with various chemotherapeutic agents or a MEK-inhibitor are ongoing or have been completed [NCT01986166, NCT01911598, NCT01652482, NCT01577173, NCT01207323]. In addition, due to the expression of both HER3 and EGFR in pancreatic ductal adenocarcinoma, the duligotuzumab dual-specific antibody has been evaluated for imaging of such tumors, resulting in clear visualization of the tumor, as well as good contrast between tumor and pancreas.\textsuperscript{122}

The bispecific antibody ALM was constructed by Robinson and colleagues by linking two distinct scFvs targeting HER2 and HER3.\textsuperscript{119} Results from in vitro experiments showed that such dual targeting promoted higher specificity to HER2/HER3-expressing cells compared to normal cells with lower receptor levels.\textsuperscript{119} ALM also induced growth arrest of HER2-overexpressing cells. This targeting approach was later licensed to Merrimack Pharmaceuticals, which used ALM to generate MM-111.\textsuperscript{117,125} The two scFvs of MM-111 bind to HER2 and HER3 with 0.3 nM and 16 nM affinities, respectively, and are genetically fused to one side each of a modified human serum albumin (HSA) molecule for in vivo half-life extension. This bispecific antibody was shown to be able to engage HER2 and HER3 simultaneously on the surface of cells, which resulted in the formation of an inactive receptor/MM-111 trimeric complex. The simultaneous engagement of the two receptors ultimately lead to suppression of tumor cell growth both in vitro and in vivo.\textsuperscript{117} Moreover, the HER3-binding scFv was demonstrated to block HRG from binding to HER3, and the anti-proliferative effect of MM-111 was enhanced upon stimulation of cells with HRG. However, the therapeutic effect of MM-111 was shown to be dependent on simultaneous engagement of both receptors, and the relative effect of MM-111 was demonstrated to correlate with the level of HER2-overexpression on cancer cells. Taken together, these results suggested MM-111 might be effective for targeting of HER2-overexpressing tumors that also express HER3.\textsuperscript{117} MM-111 has been investigated in clinical trials of HER2-positive breast cancers or other HER2-amplified tumors, either as a monotherapy or in various combinations with trastuzumab, lapatinib and chemotherapeutics [NCT01097460, NCT01304784, NCT00911898]. However, in 2015 Merrimack reported that a clinical Phase two trial of MM-111 in HER2-positive gastric and gastroesophageal junction cancers, in combination with trastuzumab and paclitaxel, was discontinued because patients administered MM-111 had shorter progression-free survival compared to those in the control arm [NCT01774851].\textsuperscript{124} Preliminary results suggested that the outcome of this trial could be due to too low HRG expression levels observed on tumors in a majority of patients. Merrimack subsequently reported that they would not invest further in MM-111 at this time.

Merrimack Pharmaceuticals has also developed an anti-HER3/anti-IGF-1R tetravalent antibody, istiratumab (MM-141), to target receptors within two different receptor families.\textsuperscript{118} This approach was based on the hypothesis that antibodies targeting only IGF-1R would result in low effectiveness due to compensatory signaling mediated by HER3. MM-141 consists of an anti-IGF-1R human IgG1 antibody that has been fused to two anti-HER3 scFvs at the carboxyl termini of the heavy chains.\textsuperscript{118,125} This antibody binds with monovalent subnanomolar affinities to both HER3 and IGF-1R, and blocks ligand binding and activation of both receptors. Consequently, downstream signaling via PI3K/Akt/mTOR is inhibited by MM-141. Moreover, this tetravalent antibody induces degradation of both receptors, as well as receptor complexes containing either HER3 or IGF-1R.\textsuperscript{118} This mechanism of action was shown to translate into improved growth-inhibitory effects by MM-141 compared to an antibody combination targeting HER3 and IGF-1R, both in vivo and in vitro. In addition, MM-141 was shown to enhance the antitumoral activity of chemotherapeutic agents and an mTOR-inhibitor due to control of HER3 and IGF-1R receptor levels.\textsuperscript{118} Currently, MM-141 is undergoing evaluation in a Phase two clinical study in combination with chemotherapeutics in metastatic pancreatic cancer patients [NCT02399137].

A bispecific antibody targeting HER2 and HER3 that is similar to the bispecific format described for MM-141 has recently been developed by Kang and coworkers.\textsuperscript{126} This antibody, denoted Tab6, consists of the anti-HER2 trastuzumab genetically fused at both CH3 domains with a HER3-specific scFv derived from a seribantumab biosimilar called Ab6.\textsuperscript{126} Tab6 induces receptor signaling and proliferation in HER2-overexpressing cell lines, presumably by bringing HER2 and HER3 into close proximity. However, Tab6’s superior anti-proliferative and anti-signaling effects, compared to monovalent antibodies or combinations of trastuzumab and Ab6, were observed in HRG-stimulated HER2-overexpressing cell-lines also treated with lapatinib. The antitumoral activity in combination with lapatinib in the presence of HRG could result from Tab6 locking HER3 into lapatinib-inactivated complexes, and consequently sequestering HER3 from HRG-stimulated dimerizations with other receptors.\textsuperscript{126} This is of interest due to the involvement of HER3 signaling in the resistance to TKIs observed in many HER2-overexpressing cancer cell lines. Furthermore, Tab6 has also been shown to reverse HRG-induced resistance to a PI3K-inhibitor in prostate cancer cell lines,\textsuperscript{127} again indicating a potential role for the antibody in prohibiting acquired resistance to a monotherapy.

Dual variable domain immunoglobulins (DVD-Igs) that target HER3 and EGFR have been developed by Abbvie.\textsuperscript{128} These bispecific molecules consist of an antibody with an additional variable domain genetically linked at the N terminus of both the Fab arms of an antibody. The HER3-targeting variable domains of the DVD-Igs are derived from seribantumab.\textsuperscript{128} Results from in vitro proliferation assays of cells with relatively high EGFR and HER3-expression, and in the presence of HRG, showed that the DVD-Igs had improved growth inhibition properties compared to a combination of the parental
antibodies or a conventional bispecific antibody. The potency of DVD-Igs as anti-tumoral agents may be explained by an avidity effect provided by this bispecific/multispecific format.

The DVD-Ig CRTB6 was generated by Hu and colleagues. This multispecific antibody, which targets HER3, EGFR, HER2 and VEGF was constructed by combining the variable domains of lumretuzumab, cetuximab, trastuzumab and bevacizumab into a DVD-Ig format. Moreover, Hu and coworkers also generated the four-in-one antibody FL518 by combining the two symmetric bispecific antibodies, duligotuzumab (targeting HER3 and EGFR) and bH1-44 (targeting HER2 and VEGF). These tetra-specific antibodies demonstrated superior anti-tumoral activity, both in vitro and, in vivo compared to bispecific antibodies over a range of different tumor cell lines. This may be explained by a potent inhibition of MET signaling, and thus inhibition of MET/HER crosstalk, observed only with the multispecific antibodies. In addition, another observation was that the tetra-specific DVD-Ig CRTB6 had a greater potency than FL518 in all examined models. One main difference between these two multispecific formats, which may provide a possible explanation for this result, is that one molecule of the FL518 antibody can only bind two of the four possible targets simultaneously, while CRTB6 could potentially interact with all four targets at the same time.

**Alternative scaffolds**

In addition to antibody derivatives, alternative scaffolds have been investigated as alternatives to conventional antibodies. More comprehensive descriptions of alternative scaffolds can be found in reviews by Löblom or Vazquez-Lombardi et al. General features often found among alternative binding proteins are: 1) a rigid framework, enabling diversification of surface-exposed residues or loops to facilitate alternative target binding without loss of protein structure; 2) a single polypeptide chain of a relatively small size; 3) low-cost, high-yield production; 4) high solubility and thermal stability; 5) a construct easily fused to other domains for increased valency or binding without loss of protein structure; 2) a single polymeric antibody, which targets HER3, EGFR, HER2 and VEGF was constructed by combining the variable domains of lumretuzumab, cetuximab, trastuzumab and bevacizumab into a DVD-Ig format. Moreover, Hu and coworkers also generated the four-in-one antibody FL518 by combining the two symmetric bispecific antibodies, duligotuzumab (targeting HER3 and EGFR) and bH1-44 (targeting HER2 and VEGF). These tetra-specific antibodies demonstrated superior anti-tumoral activity, both in vitro and, in vivo compared to bispecific antibodies over a range of different tumor cell lines. This may be explained by a potent inhibition of MET signaling, and thus inhibition of MET/HER crosstalk, observed only with the multispecific antibodies. In addition, another observation was that the tetra-specific DVD-Ig CRTB6 had a greater potency than FL518 in all examined models. One main difference between these two multispecific formats, which may provide a possible explanation for this result, is that one molecule of the FL518 antibody can only bind two of the four possible targets simultaneously, while CRTB6 could potentially interact with all four targets at the same time.

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Many alternative scaffolds have been described in the literature. However, to our knowledge, the only HER3-specific alternative scaffold reported is an Affibody, which is a molecule composed of 3-helix bundle proteins of 58 amino acids. In 2011, we first reported the generation of HER3-specific Affibody molecules with subnanomolar affinities toward the receptor and cross-reactivity with the murine ErbB3 receptor. These Affibody molecules were developed by combining two display methods, enabling the display and screening of a relatively large Affibody-library on the surface of phage, followed by affinity maturation by fluorescent-activated cell sorting of a staphylococcal cell surface-displayed library. These HER3-specific Affibody molecules were shown to inhibit the natural ligand HRG from binding and activating the receptor, resulting in inhibition of ligand-induced tumor cell-growth due to efficient blocking of HRG-induced phosphorylation of both HER2 and HER3, as well as downstream activation of Akt and Erk. 

Picomolar affinity HER3-specific Affibody molecules were later generated by semi-rational affinity maturation of the first-generation binders. The affinity-matured binders demonstrated improved ability to inhibit HRG-induced cell growth in vitro. Furthermore, evaluation of the biodistribution of these binders in mice suggested specific targeting of HER3 in vivo and rapid clearance from circulation via the kidneys.

A good contrast between tumor and other organs was observed in xenografted mice, enabling clear visualization of a HER3-expressing tumor by microSPECT, which demonstrates the potential of these Affibody molecules for in vivo imaging applications. Moreover, the HER3-specific Affibody molecules were also shown to be suitable for microPET imaging of HER3-expressing tumors in vivo, yielding tumor-to-blood ratios of >20 at 3 hours post injection in xenografts with high HER3-expression. The fact that Affibody molecules gave a higher uptake in tumors of high HER3 expression compared to low-expressing xenografts could potentially enable in vivo evaluation of the HER3-expression status on tumor cells. Due to the current emergence of several therapeutic agents targeting HER3, a diagnostic agent able to establish the global HER3 status with anatomical location by non-invasive imaging could be a valuable clinical tool. Moreover, the first evidence of in vivo therapeutic efficacy of the HER3-specific Affibody molecules, when evaluated as antibody-fusions, or so-called Affinibs or Zybodies, was observed by La Fleur and coworkers. These high-affinity HER3-specific Affibody molecules provide a promising alternative to conventional monoclonal antibodies both for imaging and therapeutic applications, especially considering the extremely small size that could enable an improved tumor-penetration capacity, as well as the possibility of straightforward reformattting to generate multispecific binding proteins.

As a minimized alternative to bispecific antibodies, bispecific Affibody molecules targeting both HER3 and HER2 have been generated recombinantly by fusing two Affibody molecules on either side of a gene encoding a femtomolar affinity albumin-binding domain (ABD035). This bispecific construct is 22.5 kDa, and hence smaller than a scFv, but targets two different receptors, as well as HSA. The HER2-specific Affibody molecule has no therapeutic effect alone, but was incorporated into the bispecific format to facilitate improved uptake of the therapeutically active HER3-specific Affibody molecule in HER3-expressing tumors that also overexpress HER2. Moreover, ABD035 acts as a handle for protein purification by affinity chromatography, but is also exploited in this bispecific format for in vivo half-life extension through its affinity for HSA. This bispecific Affibody molecule has been shown able to interact with HER2, HER3 and HSA simultaneously and could specifically target cells overexpressing both receptors. Moreover, initial evaluations have demonstrated that the bispecific...
Affibody molecule Z_{HER3:05417} possess improved inhibitory effect of ligand-induced receptor-activation in vitro compared to monospecific HER3-targeting with a HER3-specific Affibody molecule.

Due to the small size and high stability of the Affibody molecule, it can easily be fused to other affinity proteins, such as antibodies, to generate novel multispecific constructs. Recently, the first generation subnanomolar affinity HER3-specific Affibody molecule Z_{HER3:05417} (described above), was fused to the C-terminal heavy chain ends of the EGFR-specific monoclonal antibody cetuximab.\(^{137,143}\) This Affibody-antibody fusion (AffiMab or Zybody) was shown to more efficiently inhibit growth of BxPC-3 tumor cells both in vitro and in vivo compared to treatments using either cetuximab alone or Z_{05417} fused to an irrelevant antibody. These first studies of the anti-tumoral effects of the HER3-specific Affibody molecules in vivo provide promising data of the potential use of these molecules either alone or in combination with antibodies. Furthermore, the bispecific Zybody molecule had superior anti-proliferative effect on cell growth in vitro compared to a combination of the respective monospecific agents.\(^{143}\)

**Prospects for the future**

Evidence that emphasizes the importance of HER3 signaling in several different human cancers is mounting, and today over 40 different HER3-targeting agents are already in development (Table 1). Furthermore, the role of HER3 in acquired resistance against targeted therapies toward other epidermal growth factor receptors family, along with the implications of signaling routes, it may be essential for many cancer therapies to target HER3.\(^{5,6,66,77,121}\) Due to the complexity of the signaling network often found in cancer cells that can escape therapeutic agents, by for instance upregulation of alternative signaling routes, it may be essential for many cancer therapies to act on several targets. The extensive cross-talk within the epidermal growth factor receptor family, along with the implication that targeting of HER3 may be required for efficient inhibition of the PI3K/Akt pathway, suggests that HER3-targeting agents may be highly suitable for combination therapies targeting other receptors. It is thus not surprising that many of the HER3-targeting mAbs are being evaluated in clinical trials in combination with, for instance, trastuzumab, pertuzumab, cetuximab and erlotinib. Alternatively, simultaneous targeting of more than one receptor can be provided by the use of bispecific molecules, which has become an attractive target in recent years. Since both the number of available formats and different target combinations for multispecific proteins are vast, the concept of multispecificity opens possibilities for a tremendous number of potential clinical applications. The ability of tumor cells to escape signaling through upregulation of other receptors warrants development of bispecific HER3-targeting agents.

Moreover, the complex signaling network often found in cancer cells may require the identification of tumor subpopulations that can benefit from a certain targeting agent. Hence, there is also a need for tumor-associated biomarkers along with suitable diagnostic agents to allow for so-called personalized patient treatments. At least six HER3-targeting agents (U3-1287, RG7116, GSK2849330, HER3-specific Affibody molecules, duligotuzumab, and Fab-PEG24-HRG) have been or are being evaluated in vivo imaging settings.\(^{65,122,139-141,145,146,147}\)

Today, 10 HER3-targeting agents are in clinical Phase 1, two or 3 studies (Table 1), and early safety data of some of these agents indicate that they are well tolerated, without reported severe adverse effects.\(^{66,77,121}\) However, the relatively low expression level of HER3 on tumor cells, along with the expression of this receptor in normal tissues such as liver, lung, small intestine, stomach and salivary gland, makes it a more challenging tumor antigen compared to, for instance, HER2. These aspects could potentially complicate in vivo tumor targeting, and needs to be considered to facilitate optimal tumor uptake while avoiding off-target toxicity.

Different targeting agents can provide different pros and cons in different settings. The use of combinatorial protein engineering is a powerful method in this context, enabling the generation of new affinity proteins of essentially any scaffold of choice. The exploration of a wide range of different tumor markers in combination with different targeting strategies may facilitate the development of substantially improved cancer drugs.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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