BioDrugs (2014) 28:253–263
DOI 10.1007/s40259-014-0085-2

REVIEW ARTICLE

Management of Metastatic Breast Cancer with Second-Generation Antibody–Drug Conjugates: Focus on Glembatumumab Vedotin (CDX-011, CR011-vcMMAE)

Christos Vaklavas · Andres Forero

Published online: 5 February 2014

Abstract Exploiting the highly targeted nature of monoclonal antibodies to deliver selectively to tumor cells a cytotoxic payload is an attractive concept and the successful precedents of the recent past set the stage for broader applications in the future. Antibody–drug conjugates may currently hold an unprecedented potential; however, there are multiple unique challenges in their development, and the recent successes have come hand in hand with significant technologic advances in their chemistry and manufacturing. Over the years, multiple factors have been identified to affect the pharmacokinetic and pharmacodynamic properties of an antibody–drug conjugate, but many important details remain to be further investigated. These factors pertain to the target antigen, antibody, conjugate, linker, as well as the nature of the malignancy under treatment. Glembatumumab vedotin is an antibody–drug conjugate targeting glycoprotein nonmetastatic B (GPNMB) expressed in multiple malignancies, including breast cancer. The expression of this protein has been associated with an aggressive malignant phenotype, invasive growth, angiogenesis, and generation of skeletal metastases. Glembatumumab vedotin is currently in early stages of clinical development in melanoma and breast cancer. Although in unselected patients with metastatic breast cancer glembatumumab vedotin was not superior to other agents, by virtue of its target being frequently and highly expressed in triple-negative breast cancer, its activity was particularly promising in this subset of patients. Results from the clinical studies in breast cancer as well as companion studies in melanoma indicate that a biomarker-informed approach is the optimal pathway for the future development of this drug.

Abbreviations

ADAM10	A disintegrin and metalloproteinase 10
ADC	Antibody drug conjugate
CDX-011 or CR011-vcMMAE	Glembatumumab vedotin
CHO	Chinese Hamster Ovary
CMC-544	Inotuzumab ozogamicin
DC-HIL (GPNMB)	Dendritic cell - heparin integrin ligand
GPNMB	Glycoprotein Nonmetastatic B
GPNMB-ECD	GPNMB extracellular domain
HGFIN (GPNMB)	Hematopoietic growth factor inducible neurokinin-1 type
Ig	Immunoglobulin
MITF	Microphthalmia transcription factor
MMAE	Monomethylauristatin E
MMP-3	Matrix metalloproteinase 3
sRANKL	Soluble receptor activator of NF-κB ligand
TGF-β	Transforming growth factor beta
VEGF	Vascular endothelial growth factor

1 Introduction

Monoclonal antibodies epitomize the targeted nature of contemporary cancer therapeutics and, since their introduction in clinical practice, the outlook of multiple malignancies has improved significantly. The concept of capitalizing on their highly targeted nature to deliver

C. Vaklavas (✉) · A. Forero
Division of Hematology/Clinical Oncology, Department of Medicine, Comprehensive Cancer Center, University of Alabama at Birmingham, NP 2540M, 1802 6th Avenue South, Birmingham, AL 35294-3300, USA
e-mail: cvaklavas@uabmc.edu

selectively to the tumor cells a deleterious cargo is very appealing, and the successful precedents of the recent past [1, 2] set the stage for broader applications of this concept in the future. However, it should be recognized that developing antibody–drug conjugates (ADC) has proven to be a considerably challenging endeavor, and the recent successes have come after a series of failures and hand in hand with significant advances in the technology of ADC manufacturing and pharmacology [3].

The first step in developing an ADC is the identification and careful selection of a surface antigen, which is preferentially expressed on malignant cells [3]. Along these lines, glycoprotein non-metastatic B (GPNMB) has been recognized as a potential therapeutic target in breast cancer [4–6]; preclinical studies have shown an association between GPNMB expression and clinical outcomes [5] and placed GPNMB along the operational pathways that promote the generation of skeletal metastases [6]. In early-phase clinical studies, glembatumumab vedotin has shown promising activity particularly in triple-negative breast cancer, an aggressive malignancy where rational therapies are direly needed. Along with the fact that GPNMB is expressed in many other malignancies (melanoma [7–10], hepatocellular carcinoma [11], glioblastoma [12–14], small-cell lung cancer [15]) that are inadequately addressed with contemporary therapeutics, glembatumumab vedotin holds significant promise.

This review provides a brief outline of the underlying principles of ADC therapeutics in cancer and the potential benefits it may offer over conventional therapy. We focus on a critical analysis of efficacy outcomes of glembatumumab vedotin in metastatic breast cancer, highlighting the progress made and the future challenges.

2 The Multifaceted Challenges of Antibody–Drug Conjugates

Although the concept of ADCs is seemingly simple and not new, there are multiple and unique challenges in the development and manufacturing of ADCs [3].

2.1 Selection of Target

As with naked monoclonal antibodies, the target should be selectively and highly expressed in malignant cells with little or no expression on normal tissues. Virtually all targets of contemporary passive immunotherapy are also expressed in normal tissues, leading to adverse events that may compromise the therapeutic window of monoclonal antibodies. In the case of ADCs, whereby, besides the immune-mediated effector functions triggered by the monoclonal antibody there is also direct cytotoxicity from the highly deleterious conjugate, these adverse events may be even more pronounced, emphasizing the importance of careful target selection. On the other hand though, the surface antigen does not necessarily have to be along a pathogenetic pathway activated or upregulated in malignant cells. Recognizing that, in cancer cells, multiple driver or compensatory pathways may be operational, maintaining the malignant phenotype, the fact that ADCs bypass these interconnected molecular networks and exert a direct cytotoxic effect may constitute a long sought-after virtue in clinical practice, especially in the treatment of advanced, genetically heterogeneous malignancies.

The challenge though is that, inherent to the mechanism of action of ADCs, the target antigen on the surface of the cancer cell needs to be internalized to allow the conditional release of the conjugate. Target expression does not necessarily imply internalization and, although the same target antigen may be expressed in diverse malignancies, it may not be internalized with the same efficiency. Internalization kinetics may also be influenced by the binding epitope and affinity of the monoclonal antibody [16] or expression of other surface molecules, as has been shown to be the case with the internalization of a maytansinoid-based anti-CD19 ADC, which correlated negatively with CD21 expression levels [17]. Variations in target expression and internalization kinetics may account at least in part for the diversities in the activity of an ADC across malignancies that share the same target antigen. This can be illustrated with the example of inotuzumab ozogamicin (CMC-544), a humanized anti-CD22 antibody conjugated to calicheamicin [18]. In the phase I study that established the maximum tolerated dose of inotuzumab ozogamicin, patients with diverse CD22+ B-cell lymphomas were enrolled. At the same dose level, the response rates in follicular lymphoma and diffuse large B-cell lymphoma were 68 % and 15 %, respectively [18].

Exceptions to the internalization requirement do exist and are illustrated with the example of the investigational calicheamicin-conjugated rituximab [19]. The ADC demonstrated superior antitumor activity in xenograft models of B-cell lymphoma as compared with the parent naked monoclonal antibody, rituximab, despite the fact that the target (CD20) has been shown to accumulate in lipid rafts from where it is very poorly internalized. The superiority in antitumor activity has been shown only when the toxic conjugate was appended to the antibody via an acid-labile (as opposed to an acid-stable) linker, suggesting that it is the release of the conjugate under the acidic tumor microenvironmental conditions that accounts for this superiority [19].

2.2 Selection of the Conjugate

In principle, ADCs act by binding to the respective cell surface antigen and releasing their deleterious cargo by

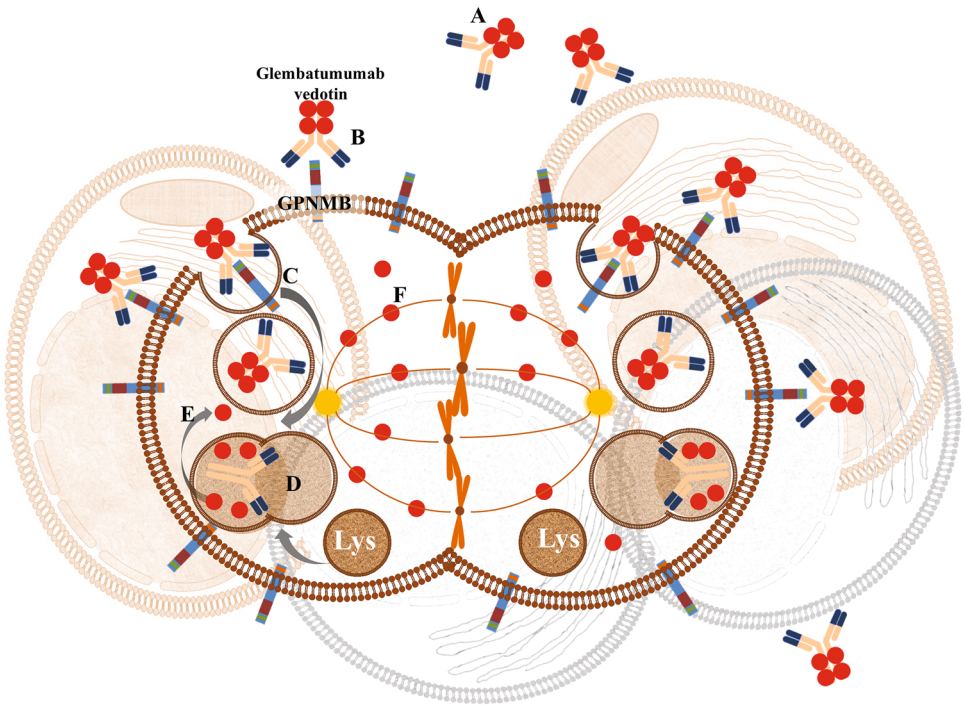

Fig. 1 There are multiple steps between administration of the ADC and final intracellular release of the active conjugate: **a** biodistribution of the drug and actual delivery to the tumor, **b** ADC-antigen binding efficiency, **c** rate of ADC-antigen complex internalization, **d** efficiency of proteolytic cleavage of the conjugate, **e** fraction of conjugate leaving the lysosome intact, **f** fraction of conjugate binding to the intracellular target. Inefficiencies along those steps lead to final intracellular release of only a small fraction of the conjugate administered. *ADC* antibody–drug conjugate, *Lys* lysosome

proteolytic cleavage upon internalization of the ADC-antigen complex and fusion with a lysosome. There are multiplicative inefficiencies in the steps between administration of the drug and final intracellular release of the conjugate. These steps are illustrated in Fig. 1 and involve biodistribution of the drug and actual delivery to the tumor, ADC-antigen binding efficiency, rate of ADC-antigen complex internalization, efficiency of proteolytic cleavage of the conjugate, fraction of conjugate leaving the lysosome intact, fraction of conjugate binding to the intracellular target, which may be variably present. In the case of glembatumumab vedotin, the intracellular target is the mitotic spindle, implying that the cancer cell must be actively dividing for the conjugate to exert its antitumor activity. These inefficiencies account at least in part for the lack of potency of the early ADCs that employed common chemotherapeutic agents such as doxorubicin [20], vinblastine [21], and methotrexate [3, 22]. To this end, the need for an exquisitely potent cytotoxic agent as a conjugate amenable to attachment to an antibody, revived the interest in auristatins [23] and maytansinoid analogs, as well as calicheamicin, for all of which clinical development stopped many years ago because of their narrow therapeutic window. Targeted delivery of these compounds to the tumor cells by appending them to an antibody has resulted in a significant improvement in their therapeutic index [3]. The improvement in the therapeutic index can be illustrated with the example of brentuximab vedotin, which employs the same conjugate as glembatumumab vedotin: while the ADC produced durable remissions in xenograft models of Hodgkin lymphoma and anaplastic large-cell lymphoma, little or no activity was seen with the administration of doses ten times the molar equivalent of free (unbound) conjugate [24].

2.3 Selection of the Appropriate Linker

The linker is a short spacer that connects the conjugate to the antibody [3]. The ideal linker should hold stably the conjugate to the antibody in the circulation and allow its conditional release in the intracellular milieu of the cancer cell. An unstable linker may result in compositional heterogeneity and a narrow therapeutic index of the respective ADC as increased fractions of unconjugated monoclonal antibody compromise the potency of the drug while increased levels of unbound cytotoxic conjugate result in non-selective cytotoxicity [23]. Advances in linker technology have been pivotal in the development of ADCs [23]. Several types of linkers are currently in use, including (1) disulfide-containing linkers (which rely on the significantly higher concentration of thiols in the intracellular as opposed to the extracellular environment to cleave their disulfide bonds); (ii) acid-cleavable hydrazine linkers; (iii) peptide-based linkers (which rely on the activity of lysosomal, endosomal, and possibly cytoplasmic proteases); and (iv) non-cleavable linkers (whereby the release of the cytotoxic moiety relies on the proteolytic degradation of the antibody moiety of the ADC). During the drug-development process, multiple linkers will be evaluated for a specific ADC [3] as they may affect the pharmacokinetic

and pharmacodynamic properties of the compound [25–27]. The effect of the linker in pharmacokinetic and pharmacodynamic properties of the ADC can be illustrated with the example of trastuzumab–emtansine: trastuzumab linked to maytansinoid through a non-reducible thioether linker displayed superior activity than the same ADC but with disulfide linkers [27]. At the pharmacokinetic level, disulfide-linked trastuzumab–emtansine was shown to be more rapidly cleared and have a shorter terminal half-life than its thioether-linked counterpart [25].

2.4 Manufacturing and Quality Control

Erratic conjugate binding may distort the affinity of the monoclonal antibody, and variations in the antibody:conjugate stoichiometry result in compositional heterogeneity and inconsistency. Overconjugated compounds may have altered target antigen-binding affinities and significantly shortened plasma half-lives, while underconjugated compounds are ineffective [3]. To this end, the development of a method to modify cysteine residues for site- and stoichiometry-specific conjugation [28, 29] has been integral in optimizing the efficacy, safety, and manufacturing process of ADCs. The implications in terms of quality control are assurance of product homogeneity and maintenance of batch-to-batch consistency [3]. The effect of antibody:conjugate stoichiometry has been investigated in vivo with an antiCD30-[30] and an antiCD70 ADC [31], both conjugated with auristatin derivatives. In the first study, an increase in potency directly proportional to conjugate loading was observed. In parallel, with the higher antibody:conjugate stoichiometry, there was an attendant decrease in the maximum tolerated dose and an accelerated clearance of the ADC [30]. Although it is anticipated that adding more conjugates to the antibody results in the generation of a bulkier ADC and may adversely affect its binding affinity to the target antigen, no such measurable impact was observed [30]. In the second study, on the other hand, the clearance of the compound was not significantly affected when the ADC was loaded with eight, as opposed to four, conjugates [31]. Further, with the addition of another four conjugates to the ADC, no significant increase in the potency of the drug was observed [31].

Collectively, with the hurdles of chemistry and manufacturing to a large extent addressed, ADCs currently hold an unprecedented potential in cancer therapeutics. The example of trastuzumab–emtansine [1, 27, 29, 32] illustrates a case of an ADC whereby the activity of the parent monoclonal antibody was significantly enhanced when its target antigen is still expressed but the cancer cells have become resistant to its blockade. Along these lines, it is anticipated in the near future to see many ADC versions of existent monoclonal antibodies entering the clinical arena, whereby the efficacy of the parent compound is restored or significantly enhanced. More intriguingly though, the precedent of brentuximab vedotin whose parent monoclonal antibody was only moderately active [2, 33] will spark the interest to pharmacologically explore multiple new targets and exploit the tumor-targeting specificity of novel or existent monoclonal antibodies to deliver cytotoxic compounds selectively to cancer cells.

3 Description of the Compound

The backbone of glembatumumab vedotin (CDX-011, CR011-vcMMAE) is a fully human immunoglobin (Ig)-G_2 monoclonal antibody (CR011) against the extracellular domain of GPNMB. The tubulin-binding cytotoxic compound monomethylauristatin E (MMAE) is appended to the parent antibody via a valine-citrulline (vc) linker, cleavable by cathepsin B as well as perhaps other lysosomal cysteine proteases; and *p*-aminobenzoic acid spacer. The average MMAE:CR011 molar ratio is approximately 4.5:1, namely, on average, 4 or 5 MMAE molecules are bound to a single antibody (Fig. 2). Its mechanism of action involves binding to the GPNMB expressed on tumor cells, internalization, fusion of the vesicle with a lysosome, proteolytic cleavage, and intracytoplasmic release of MMAE.

3.1 CR011, the Parent Monoclonal Antibody

CR011 was generated in XenoMouse strains via immunization with the recombinant extracellular domain of the human GPNMB (amino acids 23–480) [9]. The XenoMouse strains are genetically engineered mice without endogenous antibody production, in which the human heavy and kappa light chain immunoglobulin loci have been engrafted [34]. The engrafted immunoglobulin loci recapitulate all the genetic events (somatic hypermutation, rearrangement, and assembly) that occur in humans and lead to antibody generation. Accordingly, these mice with humanized humoral immune systems are able to produce high-affinity fully human antibodies to multiple antigens, including human proteins [34]. B-lymphocytes from immunized XenoMouse strains are subsequently fused with mouse myeloma cells, yielding a panel of hybridomas [35]. In the case of CR011, the generated cell lines were subsequently screened for reactivity with the extracellular domain of human GPNMB by means of enzyme-linked immunosorbent assay, and the positive hybridomas were cloned. The hybridomas selected for further characterization and expansion generated a monoclonal antibody with a dissociation constant of 52 nmol/L for the purified

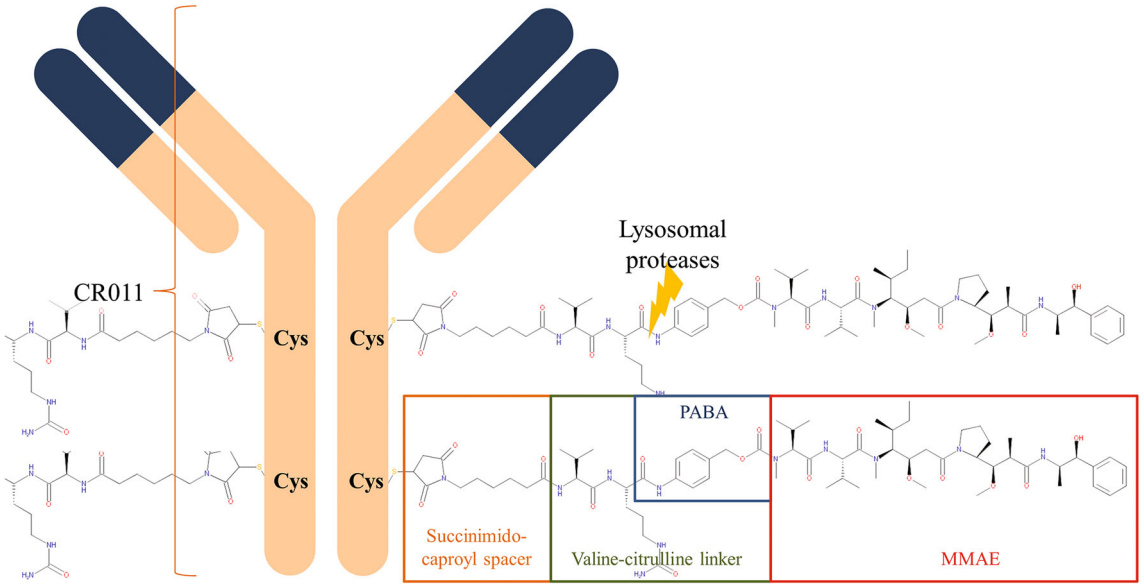

Fig. 2 Structure of glembatumumab vedotin (MMAE:CR011 stoichiometry 4:1 illustrated). *MMAE* monomethylauristatin E, *PABA* *p*-aminobenzoic acid spacer

extracellular domain of GPNMB [9]. Currently, the antibody is manufactured in Chinese Hamster Ovary (CHO) cell culture. CHO strains can be genetically engineered to produce high levels of recombinant human proteins by gene targeting [36].

3.2 Monomethylauristatin E, the Conjugate

In the initial experiments with CR011, melanoma cell lines that were positive for *GPNMB* transcript expression showed surface staining with the monoclonal IgG_2 GR011 antibody of at least 1.5 times higher than control IgG_2 antibody [9]. However, as the monoclonal antibody did not by itself inhibit melanoma cell growth, the ADC CR011-vcMMAE was generated to combine the tumor-targeting specificity of CR011 with the cytotoxic activity of MMAE [9].

MMAE is a synthetic derivative of dolastatin 10, a natural cytostatic pseudopeptide originally isolated from the marine mollusk *Dorabella auricularia* [37]. MMAE exerts its potent cytostatic effect by inhibiting microtubule assembly and polymerization. MMAE by itself has potent antitumor activity in vivo [16, 24], but its clinical development ceased in 2000 [38, 39]. In the original study, the conjugation of MMAE to the monoclonal antibody CR011 significantly enhanced the inhibitory effect of the latter on GPNMB-expressing melanoma cell lines: while the naked antibody did not inhibit the growth of GPNMB-expressing melanoma cells, its ADC counterpart blocked their growth with a half-maximal inhibitory concentration (IC_{50}) at the range of 216–300 ng/mL [9].

4 Glycoprotein Nonmetastatic B (GPNMB) as a Rational Target in Cancer Therapeutics

Glembatumumab vedotin is directed against GPNMB, also known as osteoactivin, dendritic cell – heparin integrin ligand (DC-HIL), or hematopoietic growth factor inducible neurokinin-1 type (HGFIN). *GPNMB* maps on chromosome 7p15.1 and was initially identified as a gene that was differentially expressed among melanoma cell lines with high and low metastatic potential [40]. The encoded protein is a type I transmembrane protein, which shows closest homology (by 26 % amino acid sequence) to the melanocyte/melanoma-specific protein pMEL17 [40]. In sequencing and cloning the extracellular domain of GPNMB, two alternative splice variants of the protein were identified [9]. The length of the protein is 560 or 572 amino acids depending on the splice variant; the longer variant contains an in-frame 12 amino acid insertion within the extracellular domain.

4.1 Structure of GPNMB

By homology modeling, GPNMB is predicted to consist of several domains (Fig. 3) [41]. The extracellular domain of GPNMB contains a putative heparin-binding site, many *N*-glycosylation sites, a signaling (SIG) domain, an Arg-Gly-Asp (RGD) motif embedded in the N-terminal domain, a GAP1 and GAP2 domain separated by a kringle-like (KRG) domain, and a polycystic kidney disease (PKD) domain, which can fold into an immunoglobulin-like tertiary structure [41, 42]. At the N-glycosylation sites, an

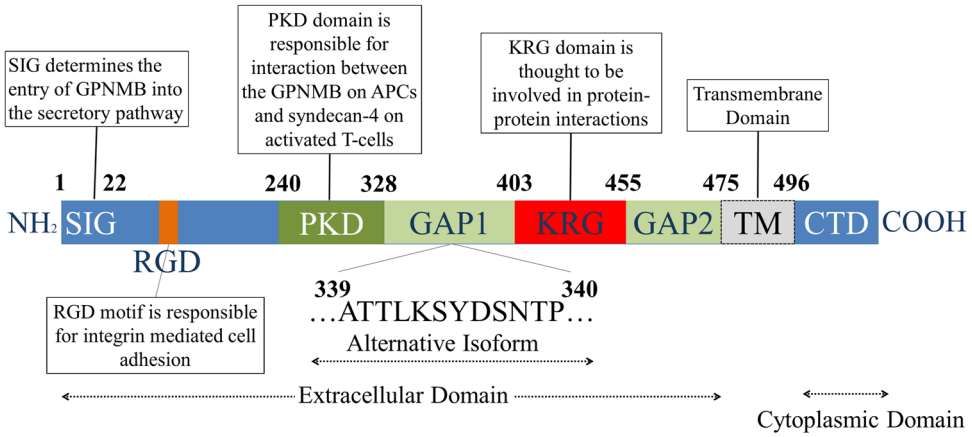

Fig. 3 Structure of glycoprotein non-metastatic B (GPNMB) [41]. The extracellular domain of the GPNMB contains a signaling (SIG) domain, an Arg-Gly-Asp (RGD) motif, a GAP1 and GAP2 domain separated by a kringle-like (KRG) domain, and a polycystic kidney disease (PKD) domain. The SIG domain is a signal peptide thought to determine the entry of GPNMB into the secretory pathway [41]. The RGD motif is responsible for integrin-mediated cell adhesion. The KRG domain is thought to be involved in protein–protein interactions [41]. The PKD domain mediates the interaction between the GPNMB expressed on antigen-presenting cells (APCs) and syndecan-4 expressed on activated T cells [45]

N-glycan is linked to an asparagine residue, a process that takes place as the protein passes through the endoplasmic reticulum and the Golgi apparatus. GPNMB is a highly N-glycosylated protein and the attached carbohydrates play an essential role in modulating protein stability, conformation, and interactions with other proteins. The SIG domain is a signal peptide thought to determine the entry of GPNMB into the secretory pathway [41]. The RGD motif is responsible for integrin-mediated cell adhesion. It mediates the adhesion of melanocytes with keratinocytes [43] as well as the adhesion of endothelial cells through recognition of heparan sulfate proteoglycans on the cell surface of the latter [44]. The KRG domain is a triple disulfide-linked autonomous structural domain thought to be involved in protein–protein interactions [41]. Lastly, the PKD domain mediates the interaction between the GPNMB expressed on antigen-presenting cells and syndecan-4 expressed on activated T cells [45]. The result of this interaction is attenuation of T-cell activation, suppression of interleukin 2 secretion, and T-cell proliferation arrest [42].

An interesting phenomenon observed in transmembrane proteins, including GPNMB, is the ectodomain shedding, i.e. the release in the extracellular milieu of the extracellular domain of the protein. Although initially believed to be a purely cell surface event, there is increasing evidence that ectodomain cleavage can also occur in the intracellular compartments [41]. In the case of GPNMB, the mechanisms that regulate its ectodomain shedding are largely unknown [41]. However, a disintegrin and metalloproteinase 10 (ADAM10) has been implicated in mediating GPNMB ectodomain cleavage [4]. Also, the GPNMB protein destined for ectodomain shedding was shown to undergo different post-translational processing, even though all GPNMB forms are eventually trafficked to the plasma membrane [41]. It has been postulated that GPNMB ectodomain shedding may compromise the efficacy of glembatumumab vedotin, as shed GPNMB extracellular domain (GPNMB-ECD) may act as decoy, attenuating the binding of the drug on malignant cells [4]. Still, as outlined below, shed GPNMB-ECD is an active molecule in cancer as it recruits endothelial cells and upregulates matrix metalloproteinase (MMP)- 3 expression, thereby promoting angiogenesis and formation of osteolytic metastases, respectively [4, 44].

4.2 GPNMB in Normal Tissues

GPNMB is normally expressed in various tissues such as bone, hematopoietic system, and skin [4].

In the skeletal system, GPNMB was shown to play a role in the differentiation and activity of both osteoclasts [46] and osteoblasts [47–49]. Experiments in murine osteoclasts have shown that *GPNMB* expression is highly induced during maturation [50], is primarily involved in the late (rather than early) osteoclast differentiation process [46], and the expression of the gene is regulated by the microphthalmia transcription factor (MITF) [50]. Comparative microarray analyses in murine osteoclasts have also shown that *GPNMB* expression was significantly upregulated by the soluble receptor activator of nuclear factor (NF)-κB ligand (sRANKL) [46]. It has been hypothesized that GPNMB mediates RANKL-dependent fusion and/or spreading of osteoclasts [46]. It is known that many processes induced by RANKL, such as osteoclast differentiation, adhesion, spreading, cytoskeletal

reorganization, and resorption activity are mediated at least in part, by integrins. The mechanistic basis for the above hypothesis is provided by the observation that GPNMB, possibly interacting with integrins via its RGD motif and thus functioning as a co-integrin receptor, coimmunoprecipitated with integrins β_1 and β_3 [46]. In osteoblasts, GPNMB was shown to mediate the effects of bone morphogenetic protein 2, a secreted growth factor that belongs to the transforming growth factor (TGF)-β superfamily, with diverse properties including osteoblast differentiation and maturation [47]. GPNMB acts downstream of bone morphogenetic protein 2 and stimulates osteoblast differentiation markers, including alkaline phosphatase activity, nodule formation, osteocalcin production, and matrix mineralization, without affecting cell proliferation or viability [47, 49].

In the hematopoietic system, *GPNMB* is expressed in dendritic cells and macrophages [4]. In macrophages, *GPNMB* expression is strongly upregulated during differentiation [51]. In addition, macrophage activation with interferon-gamma and lipopolysaccharide results in translocation of GPNMB from the Golgi apparatus to the periphery [51]. Further experiments in mice have shown that GPNMB is a negative regulator of macrophage inflammatory responses [51]. The interaction between GPNMB expressed on macrophages and dendritic cells (both potent antigen-presenting cells) through its PKD domain with syndecan-4 expressed on T cells explains the attenuated T-cell mediated inflammatory response [42, 45, 52].

Lastly, in the skin, GPNMB is expressed on epidermal Langerhans cells (which are a form of immature dendritic cells) [52] and melanocytes, where the protein can be found in melanosomes [41]. In the skin, GPNMB expression is relatively specific to melanocytes and was found to be relatively stronger in unpigmented or lightly pigmented melanocytes [41].

4.3 GPNMB in Breast Cancer

In breast cancer, *GPNMB* expression was shown to play a significant role in three major cancer-related processes: generation of skeletal metastases [5, 6], invasive growth [5], and angiogenesis [4]. Gene expression profiling studies identified *GPNMB* as a candidate gene that is highly and selectively expressed in breast cancer cells aggressively metastatic to the skeleton [6]. It has been hypothesized that expression of *GPNMB*, a gene normally expressed primarily in osteoclasts and osteoblasts, confers osteomimetic properties to breast cancer cells, thereby promoting their metastatic outgrowth in the skeletal microenvironment. GPNMB can induce the expression of MMP-3 in breast cancer cells as well as cells of the tumor microenvironment by ectodomain shedding [6]. MMP-3 in turn can cleave and solubilize RANKL [53], a key mediator of osteoclastogenesis [6], thereby giving rise to osteolytic metastases. Furthermore, the osteoclastogenic properties of RANKL are mediated at least in part by the GPNMB expressed on the surface of osteoclasts [46].

Analyses of gene expression datasets have identified a correlation between high *GPNMB* expression and features that have been associated with an aggressive phenotype, such as estrogen-receptor negative status, increasing grade, and p53 mutational status [6]. Clinically, studies on human samples have shown that tumor *GPNMB* expression as assessed by immunohistochemistry is associated with worse outcomes [5]. Experiments in breast cancer cell lines where ectopic *GPNMB* expression was shown to confer an invasive phenotype provide the biologic background for this observation. *GPNMB* expression may be identified in the stroma as well but the clinical implications of stromal *GPNMB* expression are not entirely clear [5].

Lastly, GPNMB in breast cancer can promote tumor angiogenesis by two interconnected mechanisms: upregulation of stromal vascular endothelial growth factor (VEGF) expression and chemoattraction of endothelial cells by GPNMB ectodomain shedding [4]. This chemoattraction may be mediated by the interaction between the RGD motif of the shed GPNMB-ECD and the integrins or heparan sulfate proteoglycans on the surface of endothelial cells [4, 44]. Clinically, an association between upregulated *GPNMB* expression and increased microvascular density has been observed, corroborating these experimental findings [4].

5 Clinical trials with Glembatumumab Vedotin in Breast Cancer

Two studies with glembatumumab vedotin have been completed in breast cancer, a phase I/II (NCT00704158 [54, 55]) and a phase II (NCT01156753 [56, 57]) study, both conducted in women with locally advanced or metastatic disease (Table 1).

The first study consisted of a phase I and a phase II component and, collectively, a total of 42 women were treated with the investigational agent (NCT00704158 [54, 55]). The starting dose in the phase I component was 1.34 mg/kg based on a prior phase I/II study conducted in patients with unresectable stage III or IV melanoma [58]. Due to worsening pre-existing neuropathy, the protocol had to be amended to exclude patients with baseline neuropathy grade 2 or worse (according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3 [59]). It should be noted that neuropathy has been also a common adverse event with brentuximab vedotin

Table 1 Overview of the clinical trials with glembatumumab vedotin (CDX-011, CR011-vcMMAE) in breast cancer

Author (phase)	*N*	Dose	Mean number of cycles	Response	Notes
Burris et al. (I/II) [54, 55]	42	1–1.88 mg/kg q3w	3.5	PR 13 % SD 56 %	35 % achieved a PFS >12 weeks Median PFS 9.1 weeks
Yardley et al. (RCT, II) [56, 57]	122 CDX-011: 96 (15 crossover) Control: 41	1.88 mg/kg q3w	2	PR 16 % (CDX-011) vs. 14 % (control) SD or better 57 % (CDX-011) vs. 53 % (control)	Median PFS 2.1 (CDX-011) vs. 2 (control) months Median OS 7.6 (CDX-011) vs. 7.4 (control) months Significantly better PFS and OS in high GPNMB-expressing TNBC with CDX-011

GPNMB glycoprotein non-metastatic B, *OS* overall survival, *PFS* progression-free survival, *PR* partial response, *q3w* every 3 weeks, *RCT* randomized clinical trial, *SD* stable disease, *TNBC* triple-negative breast cancer

[2], which shares MMAE as a conjugate with glembatumumab vedotin. While the median age and age distribution was similar between the two studies, the median number of prior chemotherapy regimens in the study in breast cancer was seven versus one in its melanoma counterpart. Dose escalation was resumed at 1 mg/kg and reached the predefined maximum dose of 1.88 mg/kg without further dose-limiting toxicities reported. This dose was further employed in the phase II component of the study [55].

With a mean number of treatment cycles of 3.5, the most common adverse events reported with decreasing order of frequency included fatigue (48 %), rash (45 %), nausea (45 %), alopecia (33 %), neutropenia (29 %), emesis (29 %), anemia (24 %), and asthenia (7 %). Despite the protocol amendment, the incidence of peripheral sensory neuropathy was 24 %, including one case (2 %) of grade 3. In interpreting the toxicity profile of glembatumumab vedotin, one should take into account the fact that all patients had previously received treatment with a taxane, while 55 and 40 % of patients had been previously treated with vinorelbine and an epothilone, respectively. Grade 3 adverse events with decreasing order of frequency included neutropenia (19 %) and fatigue, rash, nausea, and asthenia (all 5 %). There was also one case of cutaneous bullae that led to drug discontinuation. The relationship of this event with glembatumumab vedotin is unclear; however, GPNMB is expressed in the skin and rash has been a frequent adverse event seen in the phase I/II study in melanoma [58]. In fact, the development of rash with CDX-011 in melanoma correlated significantly with better clinical outcomes [58].

The primary endpoint of the phase II component of the study was met, with 35 % of patients having achieved a progression-free interval longer than 12 weeks [55]. A total of 13 and 56 % of patients had partial response and stable disease, respectively, as their best response. The median progression-free survival was 9.1 weeks. A theme common with the study in melanoma was that patients with tumors expressing higher levels of GPNMB either in the tumor cells and/or the stroma consistently derived greater clinical benefit. Although the numbers were small and GPNMB expression was not analyzed in all patients, the progression-free survival in patients with GPNMB-expressing tumors was 18.3 weeks as opposed to 5.9 weeks in patients with GPNMB-non-expressing tumors. Intriguingly, the frequency of GPNMB expression in triple-negative breast cancer was found to be quite high (71 %) and accordingly, the progression-free survival in this subset of patients was 17.9 weeks [55, 60].

In the second study, a total of 122 women were randomized in a 2:1 ratio to receive glembatumumab vedotin or a single-agent chemotherapy selected by the investigator (NCT01156753 [56, 57]). GPNMB expression of at least 5 % in the tumor cells or stroma was required for participation. The median number of prior treatments in the investigational arm was six, with all patients having received previously a taxane; 43, 36, and 12 % had also previously received ixabepilone, vinorelbine, and eribulin, respectively [56, 57].

A total of 96 patients were treated with glembatumumab vedotin, including 15 patients from the control arm who crossed over to the investigational arm upon disease progression [57]. With a median number of treatment cycles of two, the most common adverse events reported, with decreasing order of frequency, were rash (47 %), fatigue (38 %), nausea (32 %), neutropenia (29 %), alopecia (25 %), neuropathy (23 %), pruritus (21 %), decreased appetite (19 %), emesis (18 %), constipation (14 %), and stomatitis (16 %). Grade 3 adverse events, with decreasing order of frequency, included neutropenia (16 %), fatigue (7 %), rash (4 %), peripheral neuropathy and dehydration

(3 % each), nausea and stomatitis (2 % each). Altogether, the toxicity profile of glembatumumab vedotin was highly similar to that reported in the phase I/II study [55]. There were eight cases (8 %) of drug discontinuation due to adverse events, including four cases of peripheral neuropathy and one case of rash. A diverse panel of treatment agents was used in the control arm but the most frequent agent was eribulin (37 %). When the treatment arms are compared, events of any grade that occurred more frequently in the investigational arm were rash, neuropathy, pruritus, and alopecia. Neutropenia, constipation, thrombocytopenia, and myalgia were more frequent in the control arm. Events of grade 3 or higher severity that occurred more frequently in the investigational arm were fatigue and nausea as opposed to cytopenias, which occurred more frequently in the control arm [57].

The best tumor response was partial response in 16 versus 14 % in the investigational and control arms, respectively. However, the difference was more pronounced in triple-negative breast cancer, whereby partial response was achieved in 19 % of patients in the investigational arm versus none in the control arm. In patients with triple-negative breast cancer and high GPNMB expression, the partial response rate was 33 % in the investigational arm as opposed to none in the control arm. Similarly, the difference in the progression-free and overall survival between the two arms analyzed as a whole was not significant. However, analyzing the results based on breast cancer subtype and levels of GPNMB expression, in triple-negative breast cancer with high GPNMB expression, the comparison significantly favored the investigational arm. The median progression-free and overall survival was 3 and 10 months, respectively, with glembatumumab vedotin as opposed to 1.5 and 5.5 months, respectively, with an agent of investigator's choice. Both comparisons reached statistical significance, despite the small numbers.

6 Future Directions

Collectively, based on these early-phase clinical trials, glembatumumab vedotin has shown significant promise in the treatment of GPNMB-overexpressing malignancies and in triple-negative breast cancer in particular. Further clinical investigations are necessary to confirm these encouraging results. Its eventual place in the therapeutic algorithms of breast cancer remains to be determined. A common theme arising from these early-phase clinical trials is that the development of glembatumumab vedotin should tightly incorporate correlative studies focused on GPNMB expression. This biomarker-informed or even driven development is the optimal pathway for this promising agent to consolidate its own niche in the therapeutic landscape. So far, it has been evaluated as a single agent and in patients who received multiple prior lines of therapy. As a consequence, its therapeutic potential may be underestimated. Further knowledge of the biologic functions of GPNMB, its interactions with other proteins, and the processes in which it is involved, will provide the foundation of rational combinations of glembatumumab vedotin with existing or other novel agents in the future.

Acknowledgments CV and AF wrote the manuscript. CV is supported by a Young Investigator Award of the American Society of Clinical Oncology (ASCO). The authors have no potential conflicts of interest pertaining to this manuscript.

References

1. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med. 2012;367(19):1783–91. doi:10.1056/NEJMoa1209124.
2. Younes A, Bartlett NL, Leonard JP, Kennedy DA, Lynch CM, Sievers EL, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med. 2010;363(19):1812–21. doi:10.1056/NEJMoa1002965.
3. Teicher BA, Chari RV. Antibody conjugate therapeutics: challenges and potential. Clin Cancer Res. 2011;17(20):6389–97. doi:10.1158/1078-0432.CCR-11-1417.
4. Rose AA, Annis MG, Dong Z, Pepin F, Hallett M, Park M, et al. ADAM10 releases a soluble form of the GPNMB/Osteoactivin extracellular domain with angiogenic properties. PLoS One. 2010;5(8):e12093.
5. Rose AA, Grosset AA, Dong Z, Russo C, Macdonald PA, Bertos NR, et al. Glycoprotein nonmetastatic B is an independent prognostic indicator of recurrence and a novel therapeutic target in breast cancer. Clin Cancer Res. 2010;16(7):2147–56.
6. Rose AA, Pepin F, Russo C, Abou Khalil JE, Hallett M, Siegel PM. Osteoactivin promotes breast cancer metastasis to bone. Mol Cancer Res. 2007;5(10):1001–14.
7. Okamoto I, Pirker C, Bilban M, Berger W, Losert D, Marosi C, et al. Seven novel and stable translocations associated with oncogenic gene expression in malignant melanoma. Neoplasia. 2005;7(4):303–11.
8. Tomihari M, Chung JS, Akiyoshi H, Cruz PD Jr, Ariizumi K. DC-HIL/glycoprotein Nmb promotes growth of melanoma in mice by inhibiting the activation of tumor-reactive T cells. Cancer Res. 2010;70(14):5778–87.
9. Tse KF, Jeffers M, Pollack VA, McCabe DA, Shadish ML, Khramtsov NV, et al. CR011, a fully human monoclonal antibody–auristatin E conjugate, for the treatment of melanoma. Clin Cancer Res. 2006;12(4):1373–82.
10. Williams MD, Esmaeli B, Soheili A, Simantov R, Gombos DS, Bedikian AY, et al. GPNMB expression in uveal melanoma: a potential for targeted therapy. Melanoma Res. 2010;20(3):184–90.
11. Onaga M, Ido A, Hasuike S, Uto H, Moriuchi A, Nagata K, et al. Osteoactivin expressed during cirrhosis development in rats fed a choline-deficient, L-amino acid-defined diet, accelerates motility of hepatoma cells. J Hepatol. 2003;39(5):779–85.
12. Kuan CT, Wakiya K, Dowell JM, Herndon JE 2nd, Reardon DA, Graner MW, et al. Glycoprotein nonmetastatic melanoma protein B, a potential molecular therapeutic target in patients with glioblastoma multiforme. Clin Cancer Res. 2006;12(7 Pt 1):1970–82.

13. Loging WT, Lal A, Siu IM, Loney TL, Wikstrand CJ, Marra MA, et al. Identifying potential tumor markers and antigens by database mining and rapid expression screening. Genome Res. 2000;10(9):1393–402.
14. Rich JN, Shi Q, Hjelmeland M, Cummings TJ, Kuan CT, Bigner DD, et al. Bone-related genes expressed in advanced malignancies induce invasion and metastasis in a genetically defined human cancer model. J Biol Chem. 2003;278(18):15951–7.
15. Li YN, Zhang L, Li XL, Cui DJ, Zheng HD, Yang SY, et al. Glycoprotein nonmetastatic B as a prognostic indicator in small cell lung cancer. APMIS. 2014;1228(2):140–6.
16. Gerber HP, Kung-Sutherland M, Stone I, Morris-Tilden C, Miyamoto J, McCormick R, et al. Potent antitumor activity of the anti-CD19 auristatin antibody drug conjugate hBU12-vcMMAE against rituximab-sensitive and -resistant lymphomas. Blood. 2009;113(18):4352–61.
17. Ingle GS, Chan P, Elliott JM, Chang WS, Koeppen H, Stephan JP, et al. High CD21 expression inhibits internalization of anti-CD19 antibodies and cytotoxicity of an anti-CD19-drug conjugate. Br J Haematol. 2008;140(1):46–58. doi:10.1111/j.1365-2141.2007.06883.x.
18. Advani A, Coiffier B, Czuczman MS, Dreyling M, Foran J, Gine E, et al. Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a phase I study. J Clin Oncol. 2010;28(12):2085–93. doi:10.1200/JCO.2009.25.1900.
19. Dijoseph JF, Dougher MM, Armellino DC, Kalyandrug L, Kunz A, Boghaert ER, et al. CD20-specific antibody-targeted chemotherapy of non-Hodgkin's B-cell lymphoma using calicheamicin-conjugated rituximab. Cancer Immunol. 2007;56(7):1107–17. doi:10.1007/s00262-006-0260-5.
20. Sjogren HO, Isaksson M, Willner D, Hellstrom I, Hellstrom KE, Trail PA. Antitumor activity of carcinoma-reactive BR96-doxorubicin conjugate against human carcinomas in athymic mice and rats and syngeneic rat carcinomas in immunocompetent rats. Cancer Res. 1997;57(20):4530–6.
21. Johnson DA, Baker AL, Laguzza BC, Fix DV, Gutowski MC. Antitumor activity of L/1C2-4-desacetylvinblastine-3-carboxhydrazide immunoconjugate in xenografts. Cancer Res. 1990;50(6):1790–4.
22. Elias DJ, Hirschowitz L, Kline LE, Kroener JF, Dillman RO, Walker LE, et al. Phase I clinical comparative study of monoclonal antibody KS1/4 and KS1/4-methotrexate immunconjugate in patients with non-small cell lung carcinoma. Cancer Res. 1990;50(13):4154–9.
23. Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cerveny CG, Chace DF, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat Biotechnol. 2003;21(7):778–84.
24. Francisco JA, Cerveny CG, Meyer DL, Mixan BJ, Klussman K, Chace DF, et al. cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. Blood. 2003;102(4):1458–65.
25. Erickson HK, Lewis Phillips GD, Leipold DD, Provenzano CA, Mai E, Johnson HA et al. The effect of different linkers on target cell catabolism and pharmacokinetics/pharmacodynamics of trastuzumab maytansinoid conjugates. Mol Cancer Ther. 2012;11(5):1133–42. doi:10.1158/1535-7163.MCT-11-0727.
26. Zhao RY, Wilhelm SD, Audette C, Jones G, Leece BA, Lazar AC, et al. Synthesis and evaluation of hydrophilic linkers for antibody–maytansinoid conjugates. J Med Chem. 2011;54(10):3606–23. doi:10.1021/jm2002958.
27. Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody–cytotoxic drug conjugate. Cancer Res. 2008;68(22):9280–90. doi:10.1158/0008-5472.CAN-08-1776.
28. Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, et al. Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index. Nat Biotechnol. 2008;26(8):925–32.
29. Junutula JR, Flagella KM, Graham RA, Parsons KL, Ha E, Raab H, et al. Engineered thio-trastuzumab-DM1 conjugate with an improved therapeutic index to target human epidermal growth factor receptor 2-positive breast cancer. Clin Cancer Res. 2010;16(19):4769–78. doi:10.1158/1078-0432.CCR-10-0987.
30. Hamblett KJ, Senter PD, Chace DF, Sun MM, Lenox J, Cerveny CG, et al. Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. Clin Cancer Res. 2004;10(20):7063–70. doi:10.1158/1078-0432.CCR-04-0789.
31. Oflazoglu E, Stone IJ, Gordon K, Wood CG, Repasky EA, Grewal IS, et al. Potent anticarcinoma activity of the humanized anti-CD70 antibody h1F6 conjugated to the tubulin inhibitor auristatin via an uncleavable linker. Clin Cancer Res. 2008;14(19):6171–80. doi:10.1158/1078-0432.CCR-08-0916.
32. Krop IE, Beeram M, Modi S, Jones SF, Holden SN, Yu W, et al. Phase I study of trastuzumab-DM1, an HER2 antibody–drug conjugate, given every 3 weeks to patients with HER2-positive metastatic breast cancer. J Clin Oncol. 2010;28(16):2698–704. doi:10.1200/JCO.2009.26.2071.
33. Vaklavas C, Forero-Torres A. Safety and efficacy of brentuximab vedotin in patients with Hodgkin lymphoma or systemic anaplastic large cell lymphoma. Ther Adv Hematol. 2012;3(4):209–25. doi:10.1177/2040620712443076.
34. Mendez MJ, Green LL, Corvalan JR, Jia XC, Maynard-Currie CE, Yang XD, et al. Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. Nat Genet. 1997;15(2):146–56.
35. Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG, Jakobovits A. Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy. Cancer Res. 1999;59(6):1236–43.
36. Kito M, Itami S, Fukano Y, Yamana K, Shibui T. Construction of engineered CHO strains for high-level production of recombinant proteins. Appl Microb Biotechnol. 2002;60(4):442–8. doi:10.1007/s00253-002-1134-1.
37. Pettit GR, Kamano Y, Herald CL, Tuinman AA, Boettner FE, Kizu H, et al. The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. J Am Chem Soc. 1987;109:6883–5.
38. Madden T, Tran HT, Beck D, Huie R, Newman RA, Pusztai L, et al. Novel marine-derived anticancer agents: a phase I clinical, pharmacological, and pharmacodynamic study of dolastatin 10 (NSC 376128) in patients with advanced solid tumors. Clin Cancer Res. 2000;6(4):1293–301.
39. Vaishampayan U, Glode M, Du W, Kraft A, Hudes G, Wright J, et al. Phase II study of dolastatin-10 in patients with hormone-refractory metastatic prostate adenocarcinoma. Clin Cancer Res. 2000;6(11):4205–8.
40. Weterman MA, Ajubi N, van Dinter IM, Degen WG, van Muijen GN, Ruitter DJ, et al. nmb, a novel gene, is expressed in low-metastatic human melanoma cell lines and xenografts. Int J Cancer. 1995;60(1):73–81.
41. Hoashi T, Sato S, Yamaguchi Y, Passeron T, Tamaki K, Hearing VJ. Glycoprotein nonmetastatic melanoma protein b, a melanocytic cell marker, is a melanosome-specific and proteolytically released protein. FASEB J. 2010;24(5):1616–29.
42. Chung JS, Sato K, Dougherty II, Cruz PD Jr, Ariizumi K. DC-HIL is a negative regulator of T lymphocyte activation. Blood. 2007;109(10):4320–7.

43. Tomihari M, Hwang SH, Chung JS, Cruz PD Jr, Ariizumi K. Gpnmb is a melanosome-associated glycoprotein that contributes to melanocyte/keratinocyte adhesion in a RGD-dependent fashion. Exp Dermatol. 2009;18(7):586–95.
44. Shikano S, Bonkobara M, Zukas PK, Ariizumi K. Molecular cloning of a dendritic cell-associated transmembrane protein, DC-HIL, that promotes RGD-dependent adhesion of endothelial cells through recognition of heparan sulfate proteoglycans. J Biol Chem. 2001;276(11):8125–34.
45. Chung JS, Dougherty I, Cruz PD Jr, Ariizumi K. Syndecan-4 mediates the coinhibitory function of DC-HIL on T cell activation. J Immunol. 2007;179(9):5778–84.
46. Sheng MH, Wergedal JE, Mohan S, Lau KH. Osteoactivin is a novel osteoclastic protein and plays a key role in osteoclast differentiation and activity. FEBS Lett. 2008;582(10):1451–8.
47. Abdelmagid SM, Barbe MF, Arango-Hisijara I, Owen TA, Popoff SN, Safadi FF. Osteoactivin acts as downstream mediator of BMP-2 effects on osteoblast function. J Cell Physiol. 2007;210(1):26–37.
48. Abdelmagid SM, Barbe MF, Rico MC, Salihoglu S, Arango-Hisijara I, Selim AH, et al. Osteoactivin, an anabolic factor that regulates osteoblast differentiation and function. Exp Cell Res. 2008;314(13):2334–51.
49. Selim AA, Abdelmagid SM, Kanaan RA, Smock SL, Owen TA, Popoff SN, et al. Anti-osteoactivin antibody inhibits osteoblast differentiation and function in vitro. Crit Rev Eukaryot Gene Expr. 2003;13(2–4):265–75.
50. Ripoll VM, Meadows NA, Raggatt LJ, Chang MK, Pettit AR, Cassady AI, et al. Microphthalmia transcription factor regulates the expression of the novel osteoclast factor GPNMB. Gene. 2008;413(1–2):32–41.
51. Ripoll VM, Irvine KM, Ravasi T, Sweet MJ, Hume DA. Gpnmb is induced in macrophages by IFN-gamma and lipopolysaccharide and acts as a feedback regulator of proinflammatory responses. J Immunol. 2007;178(10):6557–66.
52. Chung JS, Bonkobara M, Tomihari M, Cruz PD Jr, Ariizumi K. The DC-HIL/syndecan-4 pathway inhibits human allogeneic T-cell responses. Eur J Immunol. 2009;39(4):965–74.
53. Lynch CC, Hikosaka A, Acuff HB, Martin MD, Kawai N, Singh RK, et al. MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. Cancer Cell. 2005;7(5):485–96.
54. clinicaltrials.gov. Study of CR011-vcMMAE to treat locally advanced or metastatic breast cancer; 2013. http://clinicaltrials.gov/ct2/show/NCT00704158.
55. Burris H, Saleh MN, Bendell J, Hart L, Rose AAN, Dong Z, et al. A phase I/II study of CR011-vcMMAE (CDX-011), an antibody–drug conjugate, in patients with locally advanced or metastatic breast cancer. Cancer Res. 2009;69(24 Supplement 3); Thirty-second annual CTRC-AACR San Antonio breast cancer symposium, San Antonio; 2009.
56. clinicaltrials.gov. A study of CDX-011 (CR011-vcMMAE) in patients with advanced GPNMB-expressing breast cancer (EMERGE); 2013. http://clinicaltrials.gov/ct2/show/NCT01156753.
57. Yardley DA, Weaver R, Melisko ME, Saleh MN, Arena FP, Forero A, et al. A randomized phase 2 study of the antibody–drug conjugate CDX-011 in advanced GPNMB-overexpressing breast cancer: the EMERGE study. Cancer Res. 2012;72(24, Supplement 3); Thirty-fifth annual CTRC-AACR San Antonio breast cancer symposium, San Antonio; 2012.
58. Hamid O, Sznol M, Pavlick AC, Kluger HM, Kim KB, Boasberg PD, et al. Frequent dosing and GPNMB expression with CDX-011 (CR011-vcMMAE), an antibody–drug conjugate (ADC), in patients with advanced melanoma. 2010 ASCO annual meeting Chicago. J Clin Oncol. 2010;28:15s (suppl; abstr 8525).
59. http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). National Cancer Institute; 2006. http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf. Accessed 31 Dec 2013.
60. Saleh MN, Bendell JC, Rose A, Siegel P, Hart LL, Sirpal S, et al. Correlation of GPNMB expression with outcome in breast cancer (BC) patients treated with the antibody–drug conjugate (ADC), CDX-011 (CR011-vcMMAE). J Clin Oncol. 2010;28:15s (suppl; abstr 1095).