ABT-414, an Antibody–Drug Conjugate Targeting a Tumor-Selective EGFR Epitope


Abstract

Targeting tumor-overexpressed EGFR with an antibody–drug conjugate (ADC) is an attractive therapeutic strategy; however, normal tissue expression represents a significant toxicity risk. The anti-EGFR antibody ABT-806 targets a unique tumor-specific epitope and exhibits minimal reactivity to EGFR in normal tissue, suggesting its suitability for the development of an ADC. We describe the binding properties and preclinical activity of ABT-414, an ABT-806 monomethyl auristatin F conjugate. In vitro, ABT-414 selectively kills tumor cells overexpressing wild-type or mutant forms of EGFR. ABT-414 inhibits the growth of xenograft tumors with high EGFR expression and causes complete regressions and cures observed at clinically relevant doses. ABT-414 also combines with standard-of-care treatment of radiation and temozolomide, providing significant therapeutic benefit in a glioblastoma multiforme xenograft model. On the basis of these results, ABT-414 has advanced to phase I/II clinical trials, and objective responses have been observed in patients with both amplified wild-type and EGFRvIII-expressing tumors.

Introduction

EGFR plays a causal role in the development and maintenance of many human carcinomas, with mutation and overexpression observed in a number of tumor types (1). Targeting EGFR is a clinically validated therapeutic strategy, with both mAbs and small molecules, having gained widespread use in lung, head and neck, colon, and pancreatic cancers (2). These EGFR-directed therapies have improved both progression-free and overall survival in a number of indications, including lung and colorectal cancer (3, 4). Despite the success of these inhibitors, significant numbers of patients with EGFR-positive tumors fail to respond to current EGFR-targeting therapeutics as a range of mutations (e.g., EGFR, KRas, B Raf, PI3K, and PTEN) may contribute to intrinsic or acquired resistance (5).

Antibody–drug conjugates (ADC) are a rapidly growing class of cancer drugs that combine the targeting properties of mAbs with the antitumor effects of potent cytotoxic drugs (6). Currently, microtubule inhibitors are clinically validated ADC payloads. Both Kadcyla (trastuzumab emtansine; Genentech) and Ad cetris (brentuximab vedotin; Seattle Genetics) are FDA-approved ADC therapeutics, and more than 40 other ADCs have advanced to the clinic (7, 8). A microtubule inhibitor–based ADC targeting EGFR is an attractive therapeutic strategy that may improve on the activity of approved EGFR antagonists by circumventing resistance mediated by downstream signaling mutations. Nonetheless, marketed EGFR antibodies have limited potential for development as ADCs because their significant binding to normal tissue causes on-target toxicity (9). The most common toxicity of these agents is a characteristic skin rash, similar in appearance to acne, usually limited to the face, upper chest, and back. Other toxicities include diarrhea, constipation, stomatitis, fatigue, and electrolyte disturbances.

In contrast, ABT-806 is an EGFR-targeting antibody that binds a tumor-selective epitope of EGFR. ABT-806 is a humanized form of the monoclonal antibody mAb 806, which binds a cryptic epitope in the CR1 domain of EGFR that is accessible in tumors expressing amplified and overexpressed wild-type EGFR or the deletion mutant EGFR variant III (EGFRvIII; refs.10, 11). The low normal tissue binding of ABT-806 has been demonstrated in a phase I trial, where ABT-806 was well tolerated at the highest dose tested (24 mg/kg) with the absence of the characteristic EGFR inhibitor dermatologic adverse events (12). Further evidence of low normal tissue uptake of ABT-806 in patients is provided by its long half-life and dose-proportional pharmacokinetics. Other EGFR-targeting antibodies, including cetuximab and panitumumab, do not display dose-proportional pharmacokinetics and are characterized by significant target-mediated clearance (13–16). ABT-806, therefore, represents an attractive candidate for use as an ADC to deliver a potent cytotoxic payload to tumor cells expressing wild-type or mutant EGFR with limited toxicity to normal tissues.

www.aacrjournals.org

©2016 American Association for Cancer Research.
To assess the potential of ABT-806 as an antibody suitable for the development of an ADC, it was conjugated to the potent microtubule inhibitor, monomethyl auristatin F (MMAF), to generate ABT-414 (17). We describe here the preclinical characterization of ABT-414, including the assessment of activity in cell line–derived and patient-derived xenograft (PDX) models expressing either wild-type EGFR or EGFRvIII. The ability to target wild-type EGFR- and EGFRvIII-expressing tumors makes ABT-414 an attractive therapeutic candidate to treat solid tumors, including glioblastoma multiforme, where novel treatments are urgently needed (18, 19). The results presented here support further clinical development of ABT-414, which is currently in ongoing phase I/II trials, where objective responses (OR) in glioblastoma multiforme patients have been observed (20).

Material and Methods

Antibodies and proteins

Recombinant forms of EGFR (sEGFR wild-type ECD; sEGFRd2-27 ECD; sEGFR(V71A,C283A) ECD) were generated by AbbVie as described previously (11). Rituximab (Roche), cetuximab (Bristol-Myers Squibb), and temozolomide (Merck & Co., Inc.) were purchased. ABT-806 was produced by transient transfection of HEK-293 cells as described previously (11). Malendocaproyl MMAF (mcMMAF) was provided by Seattle Genetics, and conjugations to generate ABT-414 and control ADCs were performed by Seattle Genetics as described previously (21).

Cell culture

The tumor cell lines A431, NR6 fibroblasts, U87MGde2-7, and U87MG were obtained from the Ludwig Institute for Cancer Research (Melbourne, Australia). NCI-H292, HCT-15, FaDu, MDA-MD-468, A549, NCI-1703, NCI-H1441, LoVo, and SW480 cell lines were obtained from the ATCC. HCC2827.ER.LMC was obtained from ATCC and serially passaged by subcutaneous injection into nude mice to enhance growth characteristics in mice. A431, NR6 fibroblast, HCT-15, FaDu, HCC2827.ER.LMC, NCI-H1703, NCI-H441, and SW480 cells were cultured in RPMI1640 supplemented with 10% FBS. U87MG and U87MGde2-7 were maintained in DMEM with high glucose, supplemented with 10% FBS and 1 mmol/L sodium pyruvate. U87MGde2-7 cells were maintained under selection with 0.4 µg/mL geneticin. MDA-MD-468 cells were maintained in DMEM supplemented with 10% FBS. A549 and LoVo cells were maintained in F-12K Nutrient Mixture supplemented with 10% FBS. SCC-15 cells were maintained in DMEM/F12K medium supplemented with 10% FBS. All cell lines were expanded in culture upon receipt and cryopreserved to provide cells at similar stage passages for all subsequent experiments. Cell lines were not authenticated in the 6 months before use; however, their EGFR expression levels were confirmed by FACS analysis.

Binding ELISA

Plates (96-well) were coated with 1 µg/mL of mouse 6x-His epitope tag mAb (A212E4; Life Technologies) at 4°C overnight and then blocked using 10% SuperBlock (Pierce) in PBS with 0.05% Tween 20 (PBST) for 2 hours at room temperature. Plates were washed three times with PBST and incubated with 100 µL of soluble EGFR (sEGFR) extracellular domain (ECD) at 2 µg/mL for 1 hour at room temperature. Plates were washed three times with PBST, incubated with ABT-806 or ABT-414 as appropriate at room temperature for 1 hour, washed three times with PBST, and incubated with 100 µL of goat anti-human IgG-horseradish peroxidase (HRP; Pierce) at room temperature for 1 hour. After washing plates three times in PBST, 100 µL of 3′,5′-tetramethylbenzidine (TMB; Pierce) was added to each well and incubated at room temperature until color developed (~20 minutes). Reactions were stopped by the addition of 100 µL 1 N phosphoric acid, and optical density (OD) was read at 450 nm.

Phospho-EGFR ELISA

Cells were plated at 2 × 10⁴ per well in collagen-coated 96-well plates in growth media. After 24 hours, cells were washed with serum-free media and serum starved for 4 hours. Where appropriate, cells were pretreated with mAb or ADC for 1 hour and then stimulated with Recombinant Human EGF (R&D Systems) for 10 minutes at 37°C. Following EGF stimulation, cells were washed twice with ice-cold PBS and lysed with 100 µL per well of cell lysis buffer (Cell Signaling Technology) supplemented with complete Protease Inhibitor Cocktail (Roche Diagnostics) and 0.1% NP40, and flash frozen at −80°C for at least 20 minutes. Phospho-EGFR levels were determined using a DuoSet IC ELISA (DYC1095; R&D Systems). Briefly, capture plates were generated by coating wells with 50 µL of an anti-EGFR antibody at 0.8 µg/mL, followed by blocking with PBS/1% BSA for 1 hour and washing three times with PBST. Cell lysates were added to capture plates and incubated at 4°C overnight. Plates were washed five times with PBST and incubated with pTyr-HRP for 1 hour. Plates were washed five times in PBST, and 100 µL of TMB peroxidase (HRP) substrate was added to each well and incubated at room temperature until color developed. Reactions were stopped by the addition of 100 µL 1 N HCl, and OD was read at 450 nm.

FACS analysis

Cells were harvested from flasks when approximately 80% confluent using Cell Dissociation Buffer (Life Technologies), washed once in PBS/1% FBS (FACS buffer), and then resuspended in 2.5 × 10⁶ cells/mL in FACS buffer. Cells (100 µL) per well were added to a round-bottom 96-well plate. Ten microliters of a 10× concentration of mAb or ADC (final concentrations are indicated in the figures) was added, and the plate was incubated at 4°C for 1 hour. For competition, FACS FITC-conjugated ABT-806 was added to a final concentration of 100 nmol/L, and then wells were washed twice in FACS buffer, suspended in 100 µL of PBS/1% formaldehyde and analyzed on a Becton Dickinson LSR II Flow Cytometer. For standard FACS, cells were washed twice with FACS buffer and resuspended in 50 µL of Alexa Fluor 488 Goat anti-Human IgG secondary antibody conjugate (11013; Life Technologies) diluted in FACS buffer. The plate was incubated at 4°C for 1 hour and washed twice with FACS buffer. Cells were resuspended in 100 µL of PBS/1% formaldehyde and analyzed on a LSR II Flow Cytometer. Data were analyzed using WinList flow cytometry analysis software.

Cytotoxicity assay

Cells were plated at 1 × 10⁴ to 3 × 10⁴ cells per well in complete growth medium containing 10% FBS in 96-well plates. The following day, medium was removed and replaced with fresh media containing titrations of antibodies or ADCs, and cells were incubated for 72 hours at 37°C in a humidified CO₂ incubator. Cell viability was then assessed using an ATPlite Luminescence
mixed with an equal amount of Matrigel (BD Biosciences) was conducted in compliance with AbbVie’s Institutional Animal Care and Use Committee and the NIH Guide for Care and Use of Laboratory Animals for a period of at least one week prior to commencement of studies. Female SCID, SCID-Beige, and nude mice were obtained from Charles River Laboratories. Ten mice were housed per cage. The body weight upon arrival was 20 to 22 g. Food and water were available ad libitum. Mice were acclimated to the animal facilities for a period of at least one week prior to commencement of experiments. Animals were tested in the light phase of a 12-hour light/dark schedule (lights on at 06:00 am). All experiments were conducted in compliance with AbbVie’s Institutional Animal Care and Use Committee and the NIH Guide for Care and Use of Laboratory Animals Guidelines in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

To generate xenografts, a suspension of viable tumors cells mixed with an equal amount of Matrigel (BD Biosciences) was injected subcutaneously into the flank of 6- to 8-week-old mice. The injection volume was 0.2 mL composed of a 1:1 mixture of S-MEM and Matrigel (BD Biosciences). Tumors were size matched at approximately 200 to 250 mm³ unless otherwise indicated. Therapy began the day of or 24 hours after size matching the tumors. Mice weighed approximately 25 g at the onset of therapy. Each experimental group included 8 to 10 animals. Tumors were measured two to three times weekly: Measurements of the length (L) and width (W) of the tumor were obtained via electronic calipers, and the volume was calculated according to the following equation: $V = \frac{L \times W^2}{2}$. Mice were euthanized when tumor volume reached a maximum of 3,000 mm³ or upon presentation of skin ulcerations or other morbidities, whichever occurred first. Host strains for each cell line and cell number in the inoculum are included in the Supplementary Table S1. For the SN0199 and SN0207 PDX models (The Jackson Laboratory), tumor fragments of 3 to 5 mm³ at passage 3 (P3) were implanted subcutaneously in the right rear flank of NSG mice (The Jackson Laboratory) with a trocar (11). For all groups, tumor volumes were plotted only until the full set of animals remained on study. If animals had to be taken off study, the remaining animals were monitored for tumor growth until they reached defined endpoints.

Maximal tumor growth inhibition (TGI_{max}), expressed as a percentage, indicates the maximal divergence between the mean tumor volume of the test article–treated group and the control group treated with drug vehicle or isotype-matched nonbinding antibody. Tumor growth delay (TGD), expressed as a percentage, is the difference of the median time of the test article–treated group tumors to reach 1 cm³ as compared with the control group.

**Statistical analysis**

IC₅₀ and EC₅₀ values were determined by nonlinear regression analysis of concentration response curves using GraphPad Prism 6.0. Data from experiments in vivo were analyzed using the two-way ANOVA with post hoc Bonferroni correction for TGI_{max} and the Mantel–Cox log-rank test for TGD (GraphPad Prism, GraphPad Software).

**Results**

**ABT-414 retains binding and functional properties of ABT-806**

ABT-414 was generated by the conjugation of MMAF to the interchain cysteine of ABT-806 via a noncleavable maleimido-capryl linker with an average drug–antibody ratio of 3.8. To determine whether the unique tumor-selective binding properties of ABT-806 were retained following conjugation, a series of binding assays was performed to characterize ABT-414 binding. ABT-806 binding to various forms of recombinant and cell expressed EGFR has been described previously, with high-affinity binding in an ELISA format observed to a mutant form of EGFR that exposes the epitope (EGFRC271A,C283A; refs.11, 22). These binding characteristics are retained in ABT-414 with higher affinity binding to EGFR(C271A,C283A) (0.067 nmol/L for ABT-414 vs. 0.060 nmol/L for ABT-806) and lower affinity binding to wild-type EGFR (0.461 nmol/L for ABT-414 vs. 0.458 nmol/L for ABT-806). ABT-806 also has higher affinity for EGFRvIII, and this increased binding to EGFRvIII is also retained in ABT-414 (0.059 nmol/L vs. 0.060 nmol/L, respectively). FACS binding analyses of cells overexpressing wild-type EGFR, EGFRvIII, or EGFR(C271A,C283A) also confirmed the increased binding of ABT-414 for the mutant forms of the receptor, with binding characteristics essentially indistinguishable from ABT-806 (Fig. 1A and B). These results indicate that conjugation of ABT-806 to mcMMAF does not alter the binding properties of the parental antibody.
ABT-806 binds to wild-type EGFR poorly in vitro, a cell line expressing a mutant that exposes the epitope (EGFR<sup>C271A,C283A</sup>) used for these studies (11, 22). Both ABT-806 and ABT-414 bind these cells with high affinity and inhibit EGFR-mediated signaling of this receptor with a similar potency (IC<sub>50</sub> of 1.2 and 1.0 nmol/L, respectively; Fig. 1C). ABT-806 is an active therapeutic that inhibits EGFR signaling in both EGFR wild-type–overexpressed and EGFRvIII-expressing tumor cells (11). The impact of both ABT-806 and ABT-414 on EGFR signaling was compared to determine whether conjugation of the antibody to MMAF affected receptor signaling. As ABT-806 binds to wild-type EGFR poorly in vitro, a cell line expressing a mutant that exposes the epitope (EGFR<sup>C271A,C283A</sup>) was used for these studies (11, 22). Both ABT-806 and ABT-414 bind these cells with high affinity and inhibit EGFR-mediated signaling of this receptor with a similar potency (IC<sub>50</sub> of 1.2 and 1.0 nmol/L, respectively; Fig. 1C).

**ABT-414 in vitro cytotoxicity against EGFR-expressing cells**

The cytotoxic activity of ABT-414 against a panel of human tumor cell lines expressing different forms and surface densities of EGFR was evaluated in cell killing assays. A FACS-based approach used to assess the levels of EGFR across these cell lines showed EGFR densities ranging from more than 1.6 million receptors per cell for the A431 cell line to 90,000 for the HCT-15 cell line (Fig. 2A). To confirm on-target killing by ABT-414, a nonbinding control human IgG1 conjugated to MMAF was used, and minimal cytotoxic activity was observed with this negative control in these assays (Fig. 2B).

**ABT-414 displays significant cytotoxicity against tumor cells overexpressing wild-type or mutant forms of EGFR, with the growth of the most sensitive cell lines inhibited by single-digit nanomolar concentrations of ABT-414 (Table 1). Generally, there was good correlation between EGFR density and sensitivity to ABT-414–mediated killing, with those cell lines with more than 5 × 10<sup>5</sup> receptors per cell having IC<sub>50</sub> values ≤0.21 nmol/L (Fig. 2C). ABT-414 also inhibits the growth of mouse fibroblasts engineered to overexpress human EGFR, whereas isogenic EGFR-null cells are resistant, further confirming the specificity of cell killing (Table 1).**

The cytotoxicity of ABT-414 in vitro results from delivery of the payload rather than from the efficacy of the antibody because unconjugated ABT-806 does not inhibit proliferation of tumor lines in vitro (Fig. 2B).

**ABT-414 in vivo efficacy in wild-type and mutant EGFR-expressing models**

In vivo efficacy of ABT-414 was characterized in multiple xenograft models derived from a variety of tumor types. ABT-414 treatment induced significant delay of tumor growth or tumor growth inhibition in 9 of 11 xenografts that were tested (Table 2). In all cases, the antitumor activity of ABT-414 was significantly enhanced when compared with vehicle control or cetuximab alone.
ABT-414: A Tumor-Selecting EGFR-Targeting ADC

greater than that of parental ABT-806. Several tumor models were very sensitive to ABT-414 treatment, with regressions observed. In particular, ABT-414 mediated regressions and cures in the A431 xenograft squamous tumor model with amplified wild-type EGFR (Fig. 3A). We have previously shown that even repeated dosing with the parental ABT-806 mAb at 40 mg/kg does not induce regressions in this model, indicating that conjugation of MMAF significantly increases the potency (11). In addition, combining ABT-806 with the cell permeable mono-methyl auristatin E (MMAE) toxin had minimal antitumor activity compared with either ABT-414 or ABT-806-MMAE (Supplementary Fig. S1B) indicating that conjugation is required for maximal efficacy.

ABT-414 was also highly effective against NCI-H1703 (squamous cell carcinoma of the lung), HCC827.ER.LMC (lung adenocarcinoma with EGFR-activating mutation), and SCC-15 (head and neck squamous cell carcinoma) xenografts (Table 2; Fig. 3B–D). In each of these models, sustained tumor regressions were observed after administration of ≤4 mg/kg ABT-414 when dosed every 4 days for a total of six doses (Q4D × 6 regimen). A distinct dosing regimen (1 mg/kg, Q4D × 3) was used to assess activity in the SCC-15 model, as higher doses of the unconjugated antibody can induce tumor regressions in this model (11). ABT-414 had a significantly greater response at 1 mg/kg than was observed with ABT-806 (Fig. 3D). A431, HCC827.ER.LMC, and SCC-15 cells harbor EGFR gene amplification, whereas NCI-H1703 overexpresses wild-type EGFR, indicating that ABT-414 can be effective against tumors with either overexpressed or amplified wild-type EGFR (23–26). In addition, HCC827 cells express the EGFR deE746-A750 deletion mutant, resulting in constitutive activation of the receptor (25). Not all EGFR-expressing xenografts were susceptible to inhibition by ABT-414 administration. HCT-15 and A549 xenografts, with lower levels of EGFR, did not respond to ABT-414 therapy (Table 2; Fig. 2).

In some models, the IgG-mcMMAF also resulted in a statistically significant TGD, although this was observed only at higher doses, and the responses were less durable than those observed with ABT-414 treatment. This growth inhibition by IgG-mcMMAF is likely a result of the enhanced permeability and retention effect resulting from antibody or ADC accumulation in tumors rather than the recognition of a tumor-associated antigen (27–29).

ABT-414 efficacy in glioblastoma multiforme models

As the prevalence of EGFRvIII and amplification of wild-type EGFR suggested glioblastoma as a potential target indication for ABT-806 and ABT-806-derived ADCs, the activity of ABT-414 was evaluated in the U87MGde2-7 glioblastoma multiforme model that expresses amplified exogenous EGFRvIII. ABT-414 elicited complete regressions and cures at 4 mg/kg dosing (Fig. 4A). In comparison, ABT-806 inhibited tumor growth but did not cause tumor regressions even when dosed at 20 mg/kg (Supplementary Fig. S1A).

ABT-414 activity was also evaluated in the glioblastoma multiforme PDX models SN0199 that coexpresses amplified EGFR wild-type and EGFRvIII and SN0207 that expresses wild-type EGFR. ABT-806 was not efficacious in the SN0207 model (Fig. 4B) and minimally affected SN0199 growth delay when dosed at 10 mg/kg, although it was more potent at higher doses (Fig. 4C; ref. 11). In both SN0199 and SN0207 models, ABT-414 treatment caused significant tumor growth inhibition (SN0207, 87% TGDmax; SN0199, 96% TGDmax) and regression (Fig. 4B and C).

The ability of ABT-414 to combine with glioblastoma multiforme standard-of-care chemotherapy and radiotherapy was also evaluated in the U87MGde2-7 xenograft model. Suboptimal doses of ABT-414, temozolomide, and radiation were used in the U87MGde2-7 xenograft model. Suboptimal doses of ABT-414, temozolomide, and radiation were used in these studies to permit assessment of the triple combination. Addition of ABT-414 (1 mg/kg) to the clinical combination of temozolomide (1.5 mg/kg) and fractionated radiation (2 Gy)

### Table 1. ABT-414 cytotoxicity in human tumor cell lines

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>Tumor type</th>
<th>EGFR Genotype</th>
<th>Cytotoxicity (nmol/L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431</td>
<td>Vulvar epidermoid carcinoma</td>
<td>Wild-type amplified</td>
<td>8</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>TN breast cancer</td>
<td>Wild-type amplified</td>
<td>12</td>
</tr>
<tr>
<td>U87MGde2-7</td>
<td>Glioblastoma multiforme</td>
<td>EGFRde2-7 (ectopic amplified)</td>
<td>0.3</td>
</tr>
<tr>
<td>SCC-15</td>
<td>HNSCC</td>
<td>Wild-type amplified</td>
<td>21</td>
</tr>
<tr>
<td>HCC827.ER.LMC</td>
<td>Lung adenocarcinoma</td>
<td>Wild-type, E746_A750 amplified</td>
<td>10</td>
</tr>
<tr>
<td>FaDu</td>
<td>Squamous cell carcinoma of the hypopharynx</td>
<td>Wild-type</td>
<td>150</td>
</tr>
<tr>
<td>A549</td>
<td>Lung carcinoma</td>
<td>Wild-type</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>NCI-H1703</td>
<td>Lung squamous cell carcinoma</td>
<td>Wild-type</td>
<td>23</td>
</tr>
<tr>
<td>SW48</td>
<td>Colorectal adenocarcinoma</td>
<td>Wild-type</td>
<td>30</td>
</tr>
<tr>
<td>U87MG</td>
<td>Glioblastoma multiforme</td>
<td>Wild-type</td>
<td>222</td>
</tr>
<tr>
<td>NCI-H441</td>
<td>Lung adenocarcinoma</td>
<td>Wild-type</td>
<td>121</td>
</tr>
<tr>
<td>LoVo</td>
<td>Colorectal adenocarcinoma</td>
<td>Wild-type</td>
<td>234</td>
</tr>
<tr>
<td>HCT15</td>
<td>Colorectal adenocarcinoma</td>
<td>Wild-type</td>
<td>494</td>
</tr>
<tr>
<td>NR6</td>
<td>Mouse fibroblasts</td>
<td>EGFR-null</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>NR6 EGFR wild-type</td>
<td>Mouse fibroblasts</td>
<td>EGFR wild-type</td>
<td>4</td>
</tr>
</tbody>
</table>

Abbreviations: TN, triple-negative; HNSCC, squamous cell carcinoma of the head and neck.

*aCell viability was determined following incubation with ABT-414 for 72 hours. The values represent IC50s. Parental ABT-806 does not inhibit growth of any of these cell lines.

### Table 2. ABT-414 growth inhibition of xenograft tumors

<table>
<thead>
<tr>
<th>Xenograft</th>
<th>Dose (mg/kg)</th>
<th>TGImax (%)</th>
<th>TGD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431</td>
<td>10</td>
<td>98</td>
<td>&gt;792</td>
</tr>
<tr>
<td>FaDu</td>
<td>10</td>
<td>99</td>
<td>&gt;792</td>
</tr>
<tr>
<td>HCC827.ER.LMC</td>
<td>10</td>
<td>99</td>
<td>&gt;694</td>
</tr>
<tr>
<td>SCC-15</td>
<td>10</td>
<td>99</td>
<td>&gt;133</td>
</tr>
<tr>
<td>LoVo</td>
<td>10</td>
<td>95</td>
<td>196</td>
</tr>
<tr>
<td>NCI-H1703</td>
<td>10</td>
<td>99</td>
<td>&gt;546</td>
</tr>
<tr>
<td>NCI-H441</td>
<td>10</td>
<td>91</td>
<td>213</td>
</tr>
<tr>
<td>SW48</td>
<td>10</td>
<td>97</td>
<td>494</td>
</tr>
<tr>
<td>U87MGde2-7</td>
<td>10</td>
<td>99</td>
<td>&gt;792</td>
</tr>
<tr>
<td>A549</td>
<td>10</td>
<td>99</td>
<td>0</td>
</tr>
<tr>
<td>HCT-15</td>
<td>10</td>
<td>24</td>
<td>23</td>
</tr>
</tbody>
</table>

*A comprehensive summary including results from different dosing regimens is available in the Supplementary Table S2.
resulted in significant increase in tumor growth inhibition and tumor growth delay (Fig. 4C and D). The triple combination displayed significant benefit over the current standard of care, supporting the potential of enhanced efficacy of this combination regimen.

**Discussion**

The tumor specificity of ABT-806 makes it an attractive antibody for the development of an ADC. An auristatin payload was selected to conjugate to ABT-806 as this is the most widely used class of cytotoxins in clinical development (6). As glioblastoma multiforme was the most likely initial indication for successful clinical development, the cytotoxin MMAF was selected for clinical development because its low cell permeability relative to MMAE may minimize accumulation of free toxin in surrounding normal brain tissue and reduce the potential for neurotoxicity. In addition, although both MMAE and MMAF conjugates of ABT-806 demonstrated similar antitumor activity in multiple human tumor xenograft models, ABT-414 with the MMAF cytotoxin had slightly improved potency in a glioblastoma model (Supplementary Fig. S1A).

On the basis of the properties of the parental ABT-806, we anticipated that ABT-414 would be most effective in tumor cells with high levels of EGFR or those expressing EGFRvIII. This premise was supported by the results presented here, where all tumor models expressing more than 500,000 EGFR that were assessed in vivo showed potent responses to ABT-414. Models with fewer than 500,000 receptors showed variable responses. The differential response to ABT-414 may also reflect the sensitivity of different tumor types to the auristatin payload. For example, NCI-H1703 was highly responsive, whereas A549 with a similar receptor number was unresponsive. Of note, the most responsive xenografts to ABT-414 treatment also harbor amplified EGFR. In addition, the triple-negative breast cancer cell line MDA-MD-468 with amplified EGFR was highly sensitive to ABT-414 in vitro (30). These results suggest that amplification may be a useful selection tool to identify patients most likely to respond to ABT-414. A FISH-based assay to identify cancers harboring EGFR amplification has been implemented retrospectively as part of the ongoing ABT-414 phase I trials and is now being used prospectively in a phase II trial (20).

The efficacy of ABT-414 in models with amplified EGFR or EGFRvIII supports development of this model in glioblastoma multiforme, where approximately 50% of tumors have amplified EGFR and approximately 25% express EGFRvIII. Preclinical results show that ABT-414 is highly effective as monotherapy in the EGFRvIII-amplified U87MGde2-7 and the SNO199 PDX tumor models. ABT-414 is also very potent in the SN0207 glioblastoma multiforme PDX tumor model that expresses wild-type EGFR. Interestingly, the SNO207 model was unresponsive to both ABT-806 and cetuximab (11). ABT-414 also combines with
standard-of-care temozolomide/radiation in significantly delaying tumor growth of a glioblastoma xenograft model, providing additional support for development in glioblastoma multiforme.

ADC therapy of glioblastoma multiforme may potentially be limited by the blood brain barrier (BBB), which restricts transport of large molecules in the circulation to the brain. Previously, however, it was demonstrated that indium-labeled ABT-806 ([111In]ABT-806), the radiolabeled parental antibody of ABT-414, showed specific tumor uptake in the brain in a glioma model grown orthotopically (11). In addition, phase I studies with both [111In]chimeric 806 and [111In]ABT-806 showed specific uptake in patients with brain tumors, demonstrating that this mAb crosses the BBB in these patients or that the BBB is sufficiently comprised or disrupted by the disease to enable uptake (31, 32).

Collectively, these results provided a sound rationale for the investigation of ABT-414 in glioblastoma multiforme patient populations, and phase I and II trials are currently ongoing in this indication. ABT-414 has shown early clinical promise in recurrent glioblastoma multiforme with ORs, including complete responses observed, both as monotherapy and in combination with temozolomide in EGFR-amplified patients (20).

ABT-414 may also be efficacious in a subset of EGFR-expressing tumors other than glioblastoma multiforme. As ABT-414 efficacy in tumor models correlates with high EGFR expression levels, patients most likely to benefit from ABT-414 treatment are expected to be those with amplified or very highly overexpressed wild-type EGFR-tumors. EGFR amplification occurs in many tumor types, including lung and head and neck cancers, although with a typically lower copy number and at a frequency much less common than observed in glioblastoma multiforme (33, 34). Consistent with these observations, in the ABT-414 phase I studies outside of the glioblastoma multiforme setting, a single partial response was observed in a patient with triple-negative breast cancer, and the tumor was retrospectively determined to have wild-type amplified EGFR (35). EGFRvIII expression has also been reported in several tumor types in addition to glioblastoma multiforme, suggesting utility of ABT-414 in these settings, although our data are consistent with low prevalence outside of glioblastoma multiforme (36–40). In addition, lung cancer tumors harboring EGFR-activating mutations that render them sensitive to tyrosine kinase inhibitors may also be sensitive to ABT-414, as the site of the mutation in the kinase domain of EGFR is distinct from the ECD recognized by the antibody (11, 22). Preclinical results demonstrate that ABT-414 is active against the HCC827 lung tumor model harboring the EGFR-activating E746_A750 EGFR deletion mutation and a PDX model (LG0703)
expressing the L858R mutation (data not shown). The efficacy of ABT-414 against tumors with different forms of EGFR distinguishes it from EGFRvIII-specific ADCs, such as the recently described AMG 595 [41].

In summary, ABT-414 is a promising therapeutic with unique targeting capabilities. The preclinical data support the continued clinical evaluation of ABT-414 in EGFR-expressing malignancies. In this context, it will be interesting to monitor the ongoing frequency and durability of responses in ABT-414 clinical trials.

Disclosure of Potential Conflicts of Interest

N.C. Goodwin is the Vice President of Corporate Research and Development at Champions Oncology, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.C. Phillips, K.S. Vaidya, M.J. Mitten, S. Norvell, H.D. Falls, P.J. DeVries, D. Cheng, J.A. Meulbroek, L.M. McKay, N.C. Goodwin, E.B. Reilly


Writing, review, and/or revision of the manuscript: A.C. Phillips, E.R. Boghaert, K.S. Vaidya, D. Cheng, J.A. Meulbroek, F.G. Buchanan, N.C. Goodwin, E.B. Reilly

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.D. Falls, D. Cheng, E.B. Reilly


Acknowledgments

The authors thank Lenette Paige for her excellent technical assistance.

Grant Support

The design, study conduct, and financial support for the study were provided by AbbVie.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Acknowledgments

The authors thank Lenette Paige for her excellent technical assistance.

Grant Support

The design, study conduct, and financial support for the study were provided by AbbVie.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 11, 2015; revised January 22, 2016; accepted January 26, 2016; published OnlineFirst February 4, 2016.

References


Molecular Cancer Therapeutics

ABT-414, an Antibody–Drug Conjugate Targeting a Tumor-Selective EGFR Epitope


Updated version
Access the most recent version of this article at:
doi: 10.1158/1535-7163.MCT-15-0901

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2016/02/04/1535-7163.MCT-15-0901.DC1

Cited articles
This article cites 40 articles, 11 of which you can access for free at:
http://mct.aacrjournals.org/content/15/4/661.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/15/4/661.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.