Discovery of A Novel EGFR-Targeting Antibody–Drug Conjugate, SHR-A1307, for the Treatment of Solid Tumors Resistant or Refractory to Anti-EGFR Therapies

Kaijie He1, Jianyan Xu1, Jindong Liang1, Jiahua Jiang2, Mi Tang2, Xin Ye1, Zhebin Zhang1, Lei Zhang1, Beibei Fu1, Yan Li1, Chang Bai1, Lianshan Zhang1, and Weikang Tao1

Abstract

Although inhibiting EGFR-mediated signaling proved to be effective in treating certain types of cancers, a quickly evolved mechanism that either restores the EGFR signaling or activates an alternative pathway for driving the proliferation and survival of malignant cells limits the efficacy and utility of the approach via suppressing the EGFR functionality. Given the fact that overexpression of EGFR is commonly seen in many cancers, an EGFR-targeting antibody–drug conjugate (ADC) can selectively kill cancer cells independently of blocking EGFR-mediated signaling. Herein, we describe SHR-A1307, a novel anti-EGFR ADC, generated from an anti-EGFR antibody with prolonged half-life, and conjugated with a proprietary toxin payload that has increased index of EGFR targeting–dependent versus EGFR targeting–independent cytotoxicity. SHR-A1307 demonstrated strong and sustained antitumor activities in EGFR-positive tumors harboring different oncogenic mutations on EGFR, KRAS, or PIK3CA. Antitumor efficacy of SHR-A1307 correlated with EGFR expression levels in vitro and in vivo, regardless of the mutation status of EGFR signaling mediators and a resultant resistance to EGFR signaling inhibitors. Cynomolgus monkey toxicology study showed that SHR-A1307 is well tolerated with a wide therapeutic index. SHR-A1307 is a promising therapeutic option for EGFR-expressing cancers, including those resistant or refractory to the EGFR pathway inhibitors.

Introduction

EGFR, a member of ErbB family of receptor tyrosine kinase (RTK), plays a central role in tumorigenesis of many types of solid tumors (1). EGFR mutations and/or upregulations were frequently identified in patients with non–small cell lung cancer (NSCLC), colorectal cancer, head and neck squamous cell carcinoma (HNSCC), and glioblastoma (2). Inhibiting EGFR activation by tyrosine kinase inhibitors (TKI) or mAbs has shown huge clinical success in suppressing tumor growth and improving patient survival (3–5). However, disease progression seems inevitable after initial responses (6, 7), possibly due to either primary or acquired resistance mechanisms, such as an EGFR secondary or tertiary mutation (8, 9), mutations on downstream mediators of the EGFR signaling (10, 11), or RTK signal bypass (12). Overcoming resistance to current EGFR-targeting agents represents a clinical challenge and a huge unmet medical need in cancer therapies.

Antibody–drug conjugates (ADC) are a new class of drugs that uses antibodies to selectively deliver cytotoxic payloads to tumors expressing high levels of tumor-associated antigens (13). The local accumulation of toxins in tumor minimized its systemic toxicity, and increased cancer cell killing in situ, thus providing a higher therapeutic index than chemotherapy or parental antibodies (14, 15). Moreover, ADCs can bypass some of the resistance mechanisms by selective delivery of antimitic or DNA-damaging toxins into cancer cells to induce apoptosis, a mechanism that is independent of inhibiting oncogenic signaling. Recently, two ADCs specifically targeting EGFR variant III (AMG-595 and ABT-414) demonstrated promising monotherapy efficacy in patients with recurrent glioblastoma (16–18), and the responses were correlated with EGFR amplification or EGFRvIII presence, providing early clinical validation for EGFR-targeting ADCs in patients with EGFR-amplified or -mutated cancer. 

Nimotuzumab (a humanized IgG1, also known as hR3) is an EGFR-targeting antibody approved for the treatment of HNSCC and glioma in many developing countries, and it is also in clinical trials for treating various solid tumors including NSCLC, colorectal cancer, gastric cancer, and pancreatic cancers (19). Because of its unique binding kinetics with EGFR receptor, hR3-related adverse events in humans were only mild to moderate in severity (20, 21). In particular, it showed much lower incidence of severe acneform rash and gastrointestinal mucosa toxicities in patients; those side effects were limiting toxicities typically observed with EGFR-targeting agents (19, 22). The excellent selectivity of hR3 for EGFR-positive cancer cells provides a strong rationale of designing a hR3-based ADC to enhance cancer cell killing while minimizing normal tissue toxicity for treating

1Shanghai Hengrui Pharmaceutical Co., Ltd., Shanghai, China. 2Jiangsu Hengrui Medicine Co., Ltd., Shanghai, China.

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: Kaijie He, Shanghai Hengrui Pharmaceutical Co., Ltd., 279 Wenjing Road, Shanghai, 200245, China. Phone: 8621-2351-1543; Fax: 8621-6430-3116; E-mail: he_kaijie@qq.com

Mol Cancer Ther 2019;18:1104–14
doi: 10.1158/1535-7163.MCT-18-0854
©2019 American Association for Cancer Research.
patients who have failed anti-EGFR therapies. Herein, we report a novel EGFR-targeting ADC, SHR-A1307, generated by using a serum half-life extended hr3 antibody, coupled to a novel auristatin analog with attenuated free drug cytotoxicity. Preclinical characterizations of SHR-A1307 in multiple drug-resistant cell lines and xenograft models demonstrated potent antitumor activities and a correlation between efficacy and EGFR expression levels regardless of oncogenic mutation status or drug resistance profiles in cancer cells. In addition, toxicology studies in cynomolgus monkeys suggested good pharmacokinetics and safety profiles, supporting the clinical development of SHR-A1307 in patients with EGFR-expressing cancer who are resistant or refractory to current EGFR-targeted therapies.

Materials and Methods

Cell lines and reagents
HCC827, MCF10A, H1975, and Detroit562 cells were purchased from ATCC, A431, HC116, COLO-205, HT29, SW480, SW620, DiFi, Lovo, and HepG2 cells were from Cell Bank of the Chinese Academy of Sciences (Shanghai, China); primary human hepatocytes were purchased from Rild Biotech. Cells were maintained in incubator at 37°C, 5% CO₂ in cell culture media as indicated by provider. All cells used in this study were routinely tested and verified as Mycoplasma-negative using MycoAlert PLUS Mycoplasma Detection Kit (Lonza). EGFR-targeting agents nimotuzumab, cetuximab, panitumumab, erlotinib, and osimertinib were prepared at Shanghai Hengrui Pharmaceutical.

Generating lentivirus and stable expression cell lines
Full-length EGFR cDNA was cloned from pcDNA3.1-EGFR (Origene) into pCDH-CMV-MCS-EF1-puro plasmid, and EGFR exon 19 deletion, L858R, T790M, and C797S mutations were introduced by site-directed mutagenesis. Viral vector was generated by cotransfection of pCDH-EGFR introduced by site-directed mutagenesis. Virus was generated into pCDH-CMV-MCS-EF1-puro plasmid, and EGFR lentivirus and stable expression cell lines were established by using GenenDesign after serial in vitro passages of LUN210 tumors under long term Osimertinib pressure. Tumor-bearing mice were dosed once weekly with SHR-A1307 or control antibodies via intraperitoneal or intravenous injections; erlotinib and osimertinib were administered orally once daily; dosing schedule was described in the results. Percentage of tumor growth inhibition (TGI%) was calculated by 100 × [1 – (average Vfinal – Vinitial of treatment group)/average Vfinal – Vinitial of control group]].

In vitro cytotoxicity studies

Cells were seeded at density of 2,000–5,000/well with 90-μL assay medium in 96-well plates, added with 10 μL of medium-diluted test compounds and incubated in incubator (5% CO₂, 37°C) for about 72 hours. CellTiter-Glo agents (50 μL/well, Promega) were added into each well and incubated for additional 10 minutes. CellTiter-Glo agent is a type of luminescent dye that is activated by ATP in the living cells. Luminescent signal can be detected by microplate reader, and the signal data can be analyzed and plotted as a figure using GraphPad Prism software.

In vivo efficacy studies

In vivo experiments were conducted under an approved protocol in accordance with the institutional guidelines for the use of laboratory animals. For cell line–derived xenograft models, 1–2 × 10⁶ tumor cells (suspended with 50% Matrigel) were inoculated subcutaneously into right flank of female Balb/c nude mice (Shanghai Super B&K Laboratory Animal Corp. Ltd). Tumor sizes were measured by caliper twice weekly and tumor-bearing animals were randomized when average tumor volumes [(length × width²)/2] reached ~150 mm³. Patient-derived xenograft (PDX) models (LL2142, LL21503, LL21512, LL13075, CR0455) were established by Crownbio as described previously (24). Osimertinib-resistant PDX model, LL2120-4a, was established by GenenDesign after serial in vivo passages of LL2110 tumors under long term Osimertinib pressure. Tumor-bearing mice were dosed once weekly with SHR-A1307 or control antibodies via intraperitoneal or intravenous injections; erlotinib and osimertinib were administered orally once daily; dosing schedule was described in the results. Percentage of tumor growth inhibition (TGI%) was calculated by 100 × [1 – (average Vfinal – Vinitial of treatment group)/average Vfinal – Vinitial of control group]].
Pharmacokinetic and toxicity studies in cynomolgus monkeys

All monkey studies were conducted in Association for Assessment and Accreditation of Laboratory Animal Care-accredited research facilities, and protocols were approved by Institutional Animal Care and Use Committee. For pharmacokinetic study (Jiangsu Tripod preclinical Research laboratories), 6 male cynomolgus monkeys weighing from 3.91 to 5.56 kg were assigned (3 animals each) to receive a single dose of 5.0 mg/kg of hR3 or hR3-YTE by intravenous injection. Blood samples were collected before and after drug dosing as described in the results, and serum concentrations of antibodies were detected by ELISA.

Briefly, EGFR antigens (Sino Biological Inc.) were coated on the 96-well plates and washed with blocking buffer three times. Samples were added and incubated at 37°C for 1.5 hours, and goat Anti-Human IgG Fc (horseradish peroxidase: Abcam) and 3,3’,5,5’-tetramethylbenzidine (TMB) were added. Signals were detected by microplate reader at 450-nm wavelength. In the repeat-dose, non-CLP toxicity study (WestChina-Frontier Pharmatech), 3 cynomolgus monkeys (2 male and 1 female) were designed to receive 10 mg/kg i.v. SHR-A1307 once weekly, for up to 5 repeated doses, and a 4-week postdosing recovery phase was planned for extended observation. Vital signs, ECG, and body weight were monitored throughout the study. Blood samples were collected at various time points to measure drug concentrations, hematology, clinical chemistry, and coagulation parameters. Monkeys were euthanized by exsanguination under anesthesia for gross necropsy and histopathologic examinations at the end of study. Pharmacokinetic and toxicokinetic parameters were calculated using a noncompartmental model analysis of Phoenix WinNonlin 6.3 software.

Statistical analysis

Data were analyzed by two-sided Student t test (to determine statistical significance of mean values at one point in time) or Wilcoxon matched-pairs signed-rank test (to compare statistical significance of tumor growth or plasma drug concentration over time) using Microsoft office Excel or GraphPad Prism 6.0 software. Difference with P < 0.05 was considered statistically significant.

Results

Identification of a novel EGFR-targeting ADC SHR-A1307

Nimotuzumab (hR3), compared with other approved EGFR antibodies, has demonstrated similar antitumor efficacy but significantly lower on-target, off-tumor toxicities in patients (19). Mechanistic study suggested that the moderate EGFR affinity of hR3 requires bivalent binding for stable attachment to cell surface, which leads to selectively targeting of highly expressed EGFR tumors but sparing normal tissues with low EGFR expression (20, 21, 25). In agreement with these reports, we found that hR3 had approximately 20-fold weaker binding affinity compared with cetuximab in Biacore analysis (Supplementary Fig. S1A), and approximately 5-fold weaker cell binding activity than panitumumab (Supplementary Fig. S1B and S1C). Although both antibodies showed comparable growth inhibition activities in HCC827 cells, hR3 had no impact on the proliferation of normal mammary epithelial cells (MCF10A), in contrast to the remarkable MCF10A growth inhibition caused by panitumumab (Supplementary Fig. S1D and S1E). Furthermore, when both antibodies were coupled with the potent microtubule inhibitor, MMAF (DAR = ~2.0; Supplementary Fig. S1F), hR3-derived ADC demonstrated equally potent anticancer activity but much lower MCF10A cytotoxicity (Supplementary Fig. S1G and S1H), suggesting at least 25-fold higher cancer versus normal cell selectivity than panitumumab.

Because of low tumor penetration efficiency of injected antibody (26), highly toxic payloads were utilized by ADCs to achieve maximal antitumor efficacy. However, serious toxicities may emerge if the toxins were deconjugated from ADCs and taken up by normal tissues. Accumulated clinical evidence suggested that the majority of ADC-related toxicities were derived from systemically released free drugs (27), risking their further clinical developments. To address this issue, we designed a series of auristatin analogs with intention to reduce the cytotoxicity in free drug form while maintaining their anticancer activity in ADC. Using an anti-Her2-based toxin screening platform, five lead compounds were selected on the basis of their high cancer killing potency when conjugated to pertuzumab (IC50 ranging from 0.026 to 0.33 nmol/L in Her2-positive SK-BR-3 cells; Table 1). In addition, excellent selectivity in receptor-negative HepG2 cells was also demonstrated (IC50 > 667 nmol/L in all tested ADCs), and there were no liabilities in hERG (IC50 > 30 µmol/L) or CYP450 (IC50 in 1A2, 2C9, 2D6, 3A4 and 2C19 enzymes all >10 µmol/L). Free toxin counter screening assays demonstrated that SHR151526, SHR151247, and SHR152852 had significantly reduced cytotoxicity as free drugs in multiple cell lines (IC50 value range from ~47.7 to 614.5 nmol/L), compared with 1.28–10.6 nmol/L of MMAF, indicating >30 fold higher ADC versus free toxin selectivity. These results were further confirmed using hR3-ADCs in EGFR-positive cells (Table 1). SHR152852 also demonstrated a 4-fold higher IC50 value and 20% lower minimum inhibition than MMAF in primary human hepatocytes (Supplementary Fig. S2A), suggesting a potentially lower risk of liver toxicity as free toxin. Pharmacokinetics of SHR152852 was comparable with MMAF in rat (Supplementary Fig. S2B). To analyze the plasma stability, hR3 conjugated with SHR151329, SHR152852, or MMAF at equal DAR (~2.2–2.4) was incubated in human plasma for 7 days, and all three ADCs exhibited good stability with low level of deconjugated free auristatin analogs and nondetectable MC-auristatin analog levels (Supplementary Fig. S2C). Furthermore, these ADCs were tested in HCC827 xenograft model for in vivo efficacy comparison, and SHR152852 demonstrated significantly better antitumor efficacy than SHR151329 when conjugated to hR3 (P < 0.01), and comparable efficacy with MMAF (Supplementary Fig. S2D). Given the largely improved free drug safety profile and high anticancer potency, SHR152852 was selected for later synthesis of EGFR-ADC.

hR3 antibody was reported to have relatively short half-life in human (~11/2–47.5 hours) compared with other FDA-approved EGFR antibodies (~112 hours for cetuximab and ~180 hours for panitumumab) and required weekly dosing to maintain sufficient drug exposure, which was inconvenient and cost-inefficient in clinical practice (28–30). Moreover, it is very important for ADCs to have sustained exposure and long half-life to reduce the risk of toxin accumulation caused by frequent dosing (27). To address this question, we introduced the triple mutation, M252Y/S254T/T256E (YTE), on Fc portion of hR3, which was reported to increase neonatal Fc receptor (FcRn) binding at acidic conditions (pH < 6.0) and facilitate FcRn-mediated IgG recycling (31). Consistent with previous reports, hR3-YTE exhibited at least 3-fold stronger binding to human and cyno FcRn compared...
with hR3 at pH = 6.0, but no such increase at pH = 7.4 in Biacore analysis (Supplementary Fig. S3A). To confirm the effects in vivo, pharmacokinetics was investigated in cynomolgus monkeys. The C_{max} was very similar for hR3 and hR3-YTE after a single intra-venous administration (5 mg/kg), but hR3 levels decreased more rapidly than hR3-YTE (Supplementary Fig. S3B). Plasma half-life and AUC were almost doubled in hR3-YTE group (P = 0.0001). In addition, binding and internalization capabilities were not changed in hR3-YTE (Supplementary Fig. S3C and S3D), suggesting a significantly improved pharmacokinetics profile after Fc YTE engineering without affecting EGFR-binding properties.

To generate EGFR-targeting ADC SHR-A1307, hR3-YTE was coupled with SHR152852 via noncleavable L2-MC linker (Supplementary Fig. S4A). The purity of SHR-A1307 was >97% analyzed by size-exclusion chromatography (Supplementary Fig. S4B), and free SHR152852 was 0.724 ng/mL (<0.0007%) and MC-SHR152852 was 3.28 ng/mL (<0.003%) in SHR-A1307 solution (10 mg/mL). To further evaluate the in vivo stability, 3 mg/kg SHR-A1307 was injected (intravenously) in to Sprague Dawley rats for pharmacokinetic analysis. Plasma concentrations of total antibody and ADC were comparable over time, and free SHR152852 and MC-SHR152852 were both below detection limits, indicating a good in vivo stability. SHR-A1307 was produced in large scale and subjected to further characterizations.

In vitro cytotoxicity of SHR-A1307 in drug-resistant tumor cells harboring EGFR or downstream gene alterations

To explore the therapeutic potential of EGFR-ADC, we examined SHR-A1307 in a panel of cancer cell lines with different oncogenic mutations and drug resistance patterns. In NSCLC cells with EGFR-activating mutations, SHR-A1307 was very potent in cancer cell killing, with IC_{50} of 0.57 and 1.9 nmol/L in HCC827 and H1975 cells, respectively, which were about 5~15-fold more potent than erlotinib (IC_{50} = 8.95 nmol/L) or osimertinib (IC_{50} = 11.98 nmol/L; Fig. 1B). To evaluate whether SHR-A1307 was effective in cancer cells harboring Cys797Ser (C797S) mutation, a recently identified resistance mechanism to osimertinib, we constructed in-cis EGFR C797S cell models via lentiviral-delivered EGFR Del19/T790M/C797S (DT) or L858R/T790M/C797S (LTC) triple mutation genes, and examined their cell viability after treatment with different anti-EGFR agents. Consistent with previous reports, Osimertinib lost its activity in both HCC827-DTC and H1975-LTC cells (IC_{50} = 1856 and 7914 nmol/L, respectively). In contrast, SHR-A1307 demonstrated potent killing activities in these cells (IC_{50} = 0.17 and 6.5 nmol/L, respectively), which indicate potential to overcome EGFR-mediated drug resistance. In colorectal cancer and HNSCC cells harboring EGFR downstream mutations in RAS/MAPK or PI3K pathways, SHR-A1307 also exhibited effective cell killing with IC_{50} values of 8.1 and 17.2 nmol/L in Lovo (KRASG13D) and Detroit562 (PIK3CAH1047R), respectively, whereas cetuximab or panitumumab had only minimal cytotoxic effects. Moreover, control IgG1-ADC had no cytotoxic effects in any of the tested cells, suggesting a target-mediated cell killing mechanism of SHR-A1307. To correlate in vitro potency with EGFR receptor density on the cell surface, a panel of cancer cell lines with various EGFR receptor levels was selected on the basis of previously published results, and EGFR expressions were confirmed by FACS in selected cell lines (Table 2; Supplementary Fig. S5; refs. 32–35). Cell surface EGFR levels in general correlated well with in vitro potency of SHR-A1307, with IC_{50} values ranging from 0.17~17.2 nmol/L in cells with high to moderate EGFR expression, whereas in EGFR low or negative cells, SHR-A1307 had negligible effect. Together, these results suggested that...
In vitro and in vivo activities of SHR-A1307 in cancer cells harboring different EGFR mutations. A, Chemical structure of SHR-A1307. B, In vitro activities of SHR-A1307 and anti-EGFR therapies in multiple cancer cell lines with different mutations; IgG1-ADC control is chimeric human IgG1 anti-HIV antibody C25 conjugated with the same linker drug as SHR-A1307 (DAR/C24 = 2.2). C, HCC827 xenograft model with EGFR Del19–activating mutation (9 mice in each group). D, Osimertinib-resistant HCC827-DTC tumor model represents patients with acquired C797S mutation after progression on osimertinib (8 mice in each group). E, EGFR antibody–resistant Lovo tumor model represents patients resistant to cetuximab or panitumumab due to downstream RAS/RAF mutations (10 mice in each group). Tumor growth curve after treatment with small-molecule TKI (osimertinib), hR3-YTE mAb, Ab+toxin Mix, or SHR-A1307 showing the mean tumor volumes (±SEM). F, Waterfall plots of tumor size change from baseline showing individual tumor responses in mice. GW, once weekly; CR and PR of tumors are defined as tumor size change after treatment achieved 100% and >30% reduction, respectively, compared with initial tumor size before treatment. (Statistical significance: *, P < 0.05; **, P < 0.01; †††, P < 0.001; ns, nonsignificant.)
Table 2. EGFR expression and in vitro cytotoxicity of SHR-A1307 in a panel of solid tumor cell lines with different EGFR and downstream gene alterations

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cancer type</th>
<th>Gene alterations</th>
<th>Resistance to EGFR-targeted therapies</th>
<th>SHR-A1307 Cytotoxicity IC50 nmol/L</th>
<th>Reported EGFR expression</th>
<th>Reported EGFR number/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFi</td>
<td>Colorectal cancer</td>
<td>wtEGFR amp</td>
<td>Sensitive</td>
<td>9.9 ± 3.7</td>
<td>High</td>
<td>~1.8 × 10^5</td>
</tr>
<tr>
<td>HCC827</td>
<td>NSCLC</td>
<td>EGFR Del9</td>
<td>Sensitive</td>
<td>0.57 ± 0.09</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>HCC827-DTC</td>
<td>NSCLC</td>
<td>EGFR D730K</td>
<td>Third-generation TKI</td>
<td>0.37 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1975</td>
<td>NSCLC</td>
<td>EGFR L858R/T790M</td>
<td>First-generation TKI</td>
<td>1.9 ± 0.56</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>H1975-LTC</td>
<td>NSCLC</td>
<td>EGFR LTC</td>
<td>Third-generation TKI</td>
<td>6.5 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detroit 562</td>
<td>HNSCC</td>
<td>PIK3CA H1047R</td>
<td>EGFR mAb</td>
<td>17.2 ± 1.0</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Lovo</td>
<td>Colorectal cancer</td>
<td>KRAS G13D</td>
<td>EGFR mAb</td>
<td>8.1 ± 2.6</td>
<td>Moderate</td>
<td>1.28 × 10^5</td>
</tr>
<tr>
<td>SW480</td>
<td>Colorectal cancer</td>
<td>KRAS G12V</td>
<td>EGFR mAb</td>
<td>&gt;220</td>
<td>Moderate</td>
<td>~9 × 10^4</td>
</tr>
<tr>
<td>HCT116</td>
<td>Colorectal cancer</td>
<td>KRAS G13D</td>
<td>EGFR mAb</td>
<td>&gt;220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT-29</td>
<td>Colorectal cancer</td>
<td>B-RAF V600E</td>
<td>EGFR mAb</td>
<td>&gt;133</td>
<td>Low</td>
<td>9,000</td>
</tr>
<tr>
<td>COLO-205</td>
<td>Colorectal cancer</td>
<td>B-RAF V600E</td>
<td>EGFR mAb</td>
<td>No inhibition*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW620</td>
<td>Colorectal cancer</td>
<td>KRAS G12V</td>
<td>EGFR mAb</td>
<td>No inhibition</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Results are expressed as mean ± SD. First-generation TKI: erlotinib, gefitinib; third-generation TKI: osimertinib; EGFR mAb: cetuximab, panitumumab.

*No significant growth inhibition at the highest concentration tested (976–667 nmol/L).

SHR-A1307 can be very effective against EGFR-positive cancer cells that are resistant to current EGFR targeted therapies in vitro, regardless of the mutation status of EGFR or downstream mediators.

**Efficacy of SHR-A1307 in anti-EGFR therapy-refractory xenograft models**

To assess the antitumor activity of SHR-A1307 in vivo, we performed studies in cell line–derived xenograft models harboring various representative activating or drug-resistant mutations. In HCC827 (EGFREx19del) xenograft model, and SHR-A1307 at 1.2 and 4 mg/kg resulted in significant tumor reductions (TGI% = 78.8% and 110.9%, respectively), including 100% tumor regressions at 4 mg/kg group [8/9 complete remission (CR) and 1/9 partial remission (PR)]. To further investigate the in vivo efficacy of EGFR-ADC against drug-resistant C797S triple-mutant, SHR-A1307 was evaluated in HCC827-DTC xenograft model compared with high-dose osimertinib (50 mg/kg), and the mixture of hR3-YTE and SHR152852 at a 1:2 molar ratio (Ab+toxin Mix). As expected, osimertinib only induced transient tumor stasis at initial treatment phase, and tumors quickly progressed after 10 days even in the presence of high-drug exposure (Fig. 1D). In contrast, treatment with 4 mg/kg SHR-A1307 induced significant tumor regressions in 7 of 8 mice, including 5 CRs, and the response continued after treatment cessation for additional 41 days (Fig. 1D). As control, Ab+toxin mix only had minor impact on tumor growth, suggesting that sufficient antitumor activity of cytotoxic payloads required antibody-mediated delivery into targeted tumor cells. There were no overt toxicities or body weight loss in mice except for those treated with high-dose osimertinib (Supplementary Fig. S6C).

To investigate the therapeutic potential of SHR-A1307 in KRAS-mutated colorectal cancers, we tested it in Lovo xenograft model with KRASG12D mutation, which was reported to be intrinsically resistant to high-dose cetuximab in vivo (32). Consistent with previous studies, 40 mg/kg hR3-YTE antibody had no effect on tumor growth. However, 1.2 and 4 mg/kg SHR-A1307 caused dose-dependent TGI, including 4/10 PRs at 4 mg/kg-treated group. In addition, near maximal antitumor efficacy was observed at 12 and 40 mg/kg doses, and all mice in these two groups had tumor regressions (Supplementary Fig. S6A). No signs of adverse effects were observed in all treated mice even at the highest dose of 40 mg/kg (Supplementary Fig. S6D and S6E).

To better predict clinical response of SHR-A1307 in patients with cancer that are resistant or refractory to current anti-EGFR therapies, PDX models representing different resistance mechanisms were utilized for efficacy evaluation. Tumor EGFR expression was analyzed by IHC-based scoring system, ranging from 0 to 3 points in EGFR positivity; gene amplification and mutations were detected using RNA sequencing (Supplementary Table S1). In an erlotinib-resistant model, LU1429, SHR-A1307 caused remarkable tumor regression at 3 mg/kg and tumor stasis at 1 mg/kg with TGI% of 119% (P = 0.005) and 70% (P = 0.038), respectively. Erlotinib (50 mg/kg) showed only marginal antitumor efficacy; and unconjugated antibody and low dose of SHR-A1307 (0.3 mg/kg) were undistinguishable from IgG1 control (Fig. 2A). In another EGFR-positive NSCLC PDX model, L1210/4a, with acquired resistance to osimertinib (mutation not defined), treatment with 10 mg/kg osimertinib had no impact on tumor growth, but twice weekly administration of 3 mg/kg SHR-A1307 led to significant TGI (111%, P = 0.001) and partial tumor regressions in 4/5 of the animals (Fig. 2B). In NSCLC PDX model, L12053, high EGFR levels along with MET amplification were detected in tumors that were insensitive to high-dose erlotinib or cetuximab treatment, suggesting a RTK bypass mechanism of EGFR inhibitor resistance (36). Notably, treatment with 3 mg/kg SHR-A1307 resulted in strong inhibition of tumor growth with 92% TGI (P = 0.0005; Fig. 2C), suggesting a promising strategy of using EGFR-ADC to overcome RTK bypass instead of EGFR/MET dual blocking (37). Furthermore, the antitumor efficacy of SHR-A1307 was analyzed in additional PDX models (L13075 and L12512), representing several difficult to treatment mutations (Supplementary Table S1). Although tumors didn't regress, SHR-A1307 caused a trend of TGI in both models (Fig. 2D and E). The weaker potency of EGFR-ADC in these two models was possibly due to the relatively low expression of EGFR receptors or some intrinsic resistance to antimitotic agents in these tumors. In an EGFR-negative colorectal cancer PDX model, CR0455, no TGI was noted even at highest level of SHR-A1307 dose (40 mg/kg once weekly; Fig. 2F), suggesting that high tumor EGFR levels are required for antitumor efficacy of SHR-A1307. Together, SHR-A1307 exhibited much better antitumor efficacy than EGFR antibodies or TKIs in multiple tumor models, and may provide a therapeutic opportunity to treat EGFR-positive tumors that are resistant or refractory to current anti-EGFR therapies due to inherent or acquired mechanisms.
Preclinical toxicology study of SHR-A1307 in cynomolagus monkeys

Results from mice xenograft studies have demonstrated antitumor efficacy of SHA-1307 in the absence of overt toxicities after repeated drug administration (up to 40 mg/kg), indicating an excellent small-animal safety profile. But because hR3 does not cross-react with murine EGFR (20), mouse is not a suitable species for on-target toxicity evaluation. Cynomolagus monkey is a more human relevant species as monkey EGFR has high amino acid homology with human, and hR3 antibody cross-binds monkey EGFR (20), mouse is not a suitable species for on-target toxicity evaluation. Cynomolagus monkey was selected to assess preclinical safety in a non-GLP toxicology study. SHR-A1307 (10 mg/kg) was administered via weekly intravenous infusion, and high exposure was achieved in toxicokinetics analysis after first dosing (Fig. 3A), which were about 3-fold higher than hR3 exposure in human under 200-mg clinical dose (C_{max} = 68.55 + 10.345 \mu g/mL, AUC_{0\text{-}C_{24}} = 4521.9 + 1181.6 \mu g*h/mL; ref. 28). SHR-A1307 was generally well tolerated in monkeys. Mild to moderate hair loss, desquamation, and scratches on the forearms were observed, in accordance with previously reported on-target toxicity of EGFR inhibition in epithelial cells. One monkey had nose bleeding after fourth dosing, and was recovered after medical treatments. Liver enzymes were normal after first administration of SHR-A1307, but significant elevation of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were detected after fourth dosing, including 3 and 1 monkeys exhibited >3 × upper limit of normal AST and ALP levels, respectively (Fig. 3B). Hematologic parameters (neutrophil, lymphocyte, platelet counts) fluctuated near normal range of variations during drug treatment. One case of thrombocytopenia was observed and considered related to the nose bleeding in this monkey. Most of the toxicities emerged after fourth and fifth dosing of 10 mg/kg SHR-A1307, consistent with the observation of increased exposure levels of SHR-A1307 (near doubling C_{max} and 4-fold AUC levels after fifth dosing vs. day 0) due to slow clearance and drug accumulation (Fig. 3A). Necropsy revealed mild changes in appearances of internal organs, as decreased thymus, gastrointestinal tract inflammation, and dark
red spots in lung and heart. Overall, SHR-A1307 exhibited well-tolerable safety profile under high drug exposure and therapeutic index was adequate for further development.

Discussion

EGFR alterations are among the best studied oncogenes in a variety of solid tumors, and EGFR-targeted therapeutics have resulted in marked antitumor responses and significant survival benefit in patients with cancer with advanced diseases (2). However, not all patients responded effectively to EGFR inhibitors, as in patients with NSCLC with EGFR-insensitizing mutations and patients with colorectal cancer with RAS family mutations, no clinical benefit was found due to primary resistance (6, 39). Moreover, despite the initial clinical efficacy in some patients, acquired resistance invariably develops and disease relapses typically occur within 1~2 years (12). To overcome resistance to current anti-EGFR therapies, many novel compounds targeting resistance mutations (MET, HER2, PIK3CA) or downstream mediators (MEK, AKT, mTOR) were in clinical development, as single agent or in combinations. Notably, although erlotinib plus anti-MET antibody, onartuzumab, showed significant improvements in both progression-free survival and overall survival in MET-positive patients in a retrospective phase II analysis, phase III trial of the combination therapy was disappointing, failing to show improved outcomes in the same patient population (40). In addition, MEK inhibitors were tested for the treatment of patients with refractory RAS-driven NSCLC or colorectal cancer with

<table>
<thead>
<tr>
<th>TK (mean ± SD)</th>
<th>10 mg/kg (First dosing)</th>
<th>10 mg/kg (Fifth dosing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (µg/mL)</td>
<td>266.9 ± 15.4</td>
<td>452.8 ± 8.0</td>
</tr>
<tr>
<td>AUC (0→t) (µg·h/mL)</td>
<td>5838 ± 3571.3</td>
<td>22979.2 ± 1243.5</td>
</tr>
<tr>
<td>AUC (0→inf) (µg·h/mL)</td>
<td>10030.3 ± 7496.3</td>
<td>29736.2 ± 7488.1</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>120.8 ± 25.9</td>
<td>79.9 ± 21.8</td>
</tr>
<tr>
<td>CI (mL/h/kg)</td>
<td>0.3 ± 0.2</td>
<td>0.35 ± 0.08</td>
</tr>
</tbody>
</table>

Figure 3.
Toxicology study of SHR-A1307 in cynomolgus monkey. A, Monkey plasma drug concentrations and toxicokinetic parameters after first and last (fifth) drug dosing. Data are shown as mean ± SEM. B, Clinical chemistry and hematology parameters with significant changes after dosing. Gray areas denote the normal range of laboratory values. ALT, alanine aminotransferase.
limited success due to insufficient signal blockade as single agent or increased toxicities as combination (41, 42). An alternative approach is to reinvigorate anti-EGFR antibodies by coupling them with highly potent cytotoxic payloads. Herein, we described SHR-A1307 as an example of novel EGFR targeting ADC that is potent, tumor selective and well tolerable in mice and monkeys. SHR-A1307 has multiple features enabling it to effectively kill tumor cells while minimizing toxicities, including (i) intermediate EGFR affinity of hR3 to selectively bind EGFR-expressing cancer cells while sparing normal cells; (ii) noncleavable linker to increase ADC stability and decrease toxin deconjugation in circulation; (iii) free drug cytotoxicity attenuated payload SHR152852 to further decrease the risk of systemic toxicity; and (iv) pharmacokinetics improvement by Fc domain engineering to reduce dosing frequency and potentially decrease toxin accumulation. Moreover, the ability of SHR-A1307 to efficiently kill cancer cells that were nonresponsive to current EGFR inhibitors provides an attractive therapeutic opportunity to overcome drug resistance in patients with EGFR-overexpressing tumors. Particularly, SHR-A1307 had demonstrated low nanomolar in vitro cytotoxic activities in a broad spectrum of cancer cells harboring different drug-resistant mutations. This finding implies that potent cancer killing effect of the antimitotic payload, SHR152852, is independent of EGFR or downstream mutations. Remarkably, in engineered C797S-mutated (DTC and LTC) NSCLC cells that are resistant to available EGFR TKIs, SHR-A1307 demonstrated >1,000-fold higher potency than osimertinib. This antitumor activity was further confirmed in HCC827-DTC mice xenograft models, and sustained tumor regressions were observed after only three weekly injections of SHR-A1307. Lovo and Detroit562 cells represent patients refractory/relapsed from EGFR–antiEGFR antibody or increased toxicities as combination (41, 42). Proliferation of both cells was significantly inhibited by SHR-A1307 in vitro, and dose-dependent tumor growth response was observed in SHR-A1307–treated Lovo xenograft tumors. More importantly, we found that the in vivo and in vitro antitumor activities of SHR-A1307 were closely associated with surface EGFR levels, suggesting tumor cell–associated EGFR expression could be a useful biomarker to predict clinical response in patients. These findings were further substantiated using PDX tumor models. SHR-A1307 demonstrated potent antitumor activity in multiple PDX tumors regardless of their gene mutation or drug resistance status, and a trend of correlation between EGFR IHC intensity scores and in vivo tumor responses was also established.

The safety profiles of SHR-A1307 were assessed in small and large animals. In xenograft studies, we found no overt toxicities in mice repeatedly dosed with up to 40 mg/kg SHR-A1307, which was more than 10–20-fold of in vivo efficacious doses. Although SHR-A1307 lacks cross reactivity with murine EGFR and on-target toxicities were not evaluable, the excellent safety profiles in mice indicated low risk of nonspecific ADC uptake and negligible free toxin–related toxicities, which were usually considered as important contributing factors to “antigen-independent” adverse effects (43). To evaluate the tolerability of SHR-A1307 in a more human-related species, toxicology study was performed in cynomolgus monkeys. SHR-A1307 was generally well tolerable in monkeys, with low incidence of serious adverse effects. EGFR target-related toxicities were observed, but mostly well manageable and low grade in severity. Because of slow clearance and drug accumulation, the exposure level of SHR-A1307 in monkeys significantly increased after repeated weekly dosing, and late onset of thrombocytopenia and liver function abnormalities were identified after fourth or fifth dosing. One monkey had severe platelet counts drop, manifested as nose bleeding and mucosal hemorrhage. Because severe thrombocytopenia was uncommon in human patients treated with hR3 or other EGFR inhibitors (3–5, 19, 21), this adverse event is not likely an EGFR on-target effect. Rather, thrombocytopenia induced by ADCs is thought to be related to megakaryocyte progenitor inhibition by tubulin-acting agents (27), and mechanistic study of T-DM1 suggested that FcγRIIa expressed on megakaryocyte progenitors may contribute to target-independent ADC uptake and impairment of megakaryocyte differentiation (44). As SHR-A1307 is an IgG1-based ADC like T-DM1, it was possible that at high plasma concentration, SHR-A1307 may trigger transient FcγRIIa-mediated internalization to impair platelet production. Other common ADC-related hematologic adverse effects, like lymphopenia or neutropenia, were not observed in 10 mg/kg SHR-A1307–treated monkeys, suggesting a low risk of bone marrow suppression, thanks in part to the attenuated free drug cytotoxicity of payload SHR152852 (27). Elevated liver enzymes were also observed as common adverse events after SHR-A1307 administration. It has been proposed that mannose receptor expressed on hepatic sinusoidal cells can mediate target-independent uptake of ADC into the liver (45), which could be a major contributing factor of SHR-A1307–caused hepatic toxicity.

The assessment of pharmacokinetic results suggested that dosage optimization of SHR-A1307 could be applied to further improve therapeutic index. We noticed high peak and trough plasma concentrations in SHR-A1307–dosed monkeys, which were much higher than the efficacious concentration required for cancer killing. Dose detitration was practicable to reduce peak concentration and minimize Cmax-related toxicity, while maintaining sufficient plasma levels required for efficacy. Furthermore, weekly administration of SHR-A1307 resulted in accumulation of the drug, due to slow clearance and prolonged t1/2 of 4–5 days after Fc YTE engineering, which caused late onset of toxicities (after four or five cycles). Adjusting the dosing frequency from weekly to biweekly administration may reduce the drug accumulation and keep plasma drug exposure under toxicity threshold.

In conclusion, SHR-A1307 has demonstrated robust and sustained antitumor efficacy in multiple cell lines and xenograft tumor models that were resistant to current anti-EGFR therapies. Toxicology studies in monkeys suggested a well-acceptable safety profile and sufficient therapeutic index to support future clinical testing in human.

Disclosure of Potential Conflicts of Interest

W. Tao has ownership interest (including stock, patents, etc.) in Hengrui Pharmaceutical Co. Ltd. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: K. He, J. Liang, J. Jiang, M. Tang, C. Bai, L. Zhang, W. Tao


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. He, J. Liang, J. Jiang, M. Tang, Z. Zhang, L. Zhang, B. Fu, Y. Li, C. Bai

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. He, J. Liang, J. Jiang, M. Tang, Z. Zhang, L. Zhang, C. Bai, W. Tao
Writing, review, and/or revision of the manuscript: K. He, J. Liang, J. Jiang, M. Tang, C. Bai, W. Tao

Study supervision: K. He, J. Liang, J. Jiang, M. Tang, W. Tao

Other (design and development of novel toxins and toxin-linkers for ADCs): J. Xu, J. Jiang

Acknowledgments

The design, conduct, and financial support for the study were provided by Shanghai Hengrui Pharmaceutical Co., Ltd (a subsidiary of Jiangsu Hengrui Medicine Co., Ltd). The authors thank contributions by the SHR-A1307 project team, including, but not limited to, Xiaodong Qu, Xiaoyan Zhu, Limin Zhang, Juan Luo, Jingwen Zeng, Zairan Chen, Yanbin Guan, Changyong Yang, Zhendong Xue, Yuchang Mao, and Penghui Sun.

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 July 31, 2018; revised December 26, 2018; accepted April 3, 2019; published first April 8, 2019.

References


Dall’Acqua WF, Kiener PA, Wu H. Properties of human IgGs engineered for enhanced binding to the neuronal Fc receptor (FcRn). J Biol Chem 2006;281:23514–24.


www.aacajournals.org  Mol Cancer Ther; 18(6) June 2019 1113


Molecular Cancer Therapeutics

Discovery of A Novel EGFR-Targeting Antibody–Drug Conjugate, SHR-A1307, for the Treatment of Solid Tumors Resistant or Refractory to Anti-EGFR Therapies

Kaijie He, Jianyan Xu, Jindong Liang, et al.

Mol Cancer Ther 2019;18:1104-1114. Published OnlineFirst April 8, 2019.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-18-0854

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2019/04/06/1535-7163.MCT-18-0854.DC1

Cited articles
This article cites 45 articles, 14 of which you can access for free at:
http://mct.aacrjournals.org/content/18/6/1104.full#ref-list-1

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, use this link http://mct.aacrjournals.org/content/18/6/1104.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.