USE OF ANTI-HER2 ANTIBODY-DRUG CONJUGATE IN TREATING UROTHELIAL CARCINOMA

FIELD

[0001] The present disclosure relates to use of an anti-HER2 (human epidermal growth factor receptor 2) antibody-drug conjugate in the treatment of urothelial carcinoma.

BACKGROUND

[0002] Urothelial carcinoma (UC; also known as transitional cell carcinoma, TCC) is a type of carcinoma that is usually found in the urinary system, such as kidney, bladder, and accessory organs. It is the most common type cancer among bladder carcinoma and carcinoma of ureter, urethra or umbilical duct. It is also the second most common type of renal carcinoma, accounting for 5-10% of all primary renal malignant tumors.

[0003] Urothelium (also called transitional epithelium) is the lining of the bladder, the ureter, and the inside of the urethra, and the renal pelvis (the part of the kidney where urine is collected). It is composed of urothelial cells or transitional cells. These cells can develop into cancer cells, known as urothelial carcinoma (or transitional cell carcinoma).

[0004] Depending on the invasiveness of the cancerous cells, urothelial carcinoma can be non-invasive (only in the lining of the bladder) or invasive (growing into other layers of the bladder wall). Among them, non-invasive urothelial carcinoma is only in the bladder endometrium and does not grow deeper into the bladder wall. At the time of diagnosis, 50% -60% of patients with urothelial carcinoma are non-invasive. Types of non-invasive urothelial carcinoma include: non-invasive flat urothelial carcinoma (also known as carcinoma *in situ*); non-invasive papillary urothelial carcinoma, high-grade; non-invasive papillary urothelial carcinoma, low-grade malignancy; the likelihood of non-invasive papillary urothelial tumors with low malignant potential developing into aggressive cancer is low.

[0005] In contrast, invasive urothelial carcinoma grows from the lining of the bladder into the deeper layers of the bladder wall, such as connective tissue (known as the lamina propria) and muscle layer. At the time of diagnosis, the tumors in 40% -50% of patients with urothelial carcinoma are invasive.

[0006] In theory, urothelial carcinoma can originate from any part of the urinary tract, including but not limited to the renal pelvis, ureter, bladder and urethra.

[0007] Before metastasis happens from relevant tumor cells, surgical resection is the preferred treatment regime. For patients with tumors that have metastasized, anti-cancer drugs are generally required. The current first-line therapy is: combination therapy of gemcitabine and cisplatin. Radiation therapy is not ideal for urothelial carcinoma and generally used as an adjuvant therapy. When treating cancer in the renal pelvis/ureter epithelium, BCG injection therapy (injection of *Mycobacterium Bovis* via catheter) may be an option.

[0008] Urothelial carcinoma is multi-centered and relapse-prone. For patients with tumors involving the muscle layer, total bladder resection is preferred, and strict re-examination is required after surgery. Therefore, treatment is difficult and the recurrence rate is high. (Li Xuesong, Wang Gang, Zhang Ye, eds. Essence of Urology Cases, Peking University Medical Press, 2017). The administration of mitomycin (a chemotherapeutic agent) to the bladder as a one-time dose early in the postoperative period (within 24 hours) or as a six-dose regimen a few weeks after surgery is also an option for some patients. Cisplatin-based chemotherapy is still the golden standard for treating patients with metastatic UC. The overall response rate (ORR) of cisplatin-based chemotherapy is 60-70%, overall survival (OS) is 14-15 months, and 5-year survival rate is 13-15%. Platinum-based chemotherapy was performed in relapsed patients with an ORR of approximately 15% and a median OS of approximately 7 months.

[0009] Vinflunine has been approved in Europe for the treatment of advanced urinary epithelial or metastatic TCC (Bellmunt, J. et al., J Clin. Oncol. 27 (27): 4454-4461 (2009)). Several agents have been tested for monotherapy and shown moderate activity, with median survival of 5 to 10 months (Yafi, F.A. et al. Current Oncol. 18 (1): e25-e34 (2011)). In metastatic cases, Docetaxel is administered as a relief option to patients with transitional cell carcinoma (NCCN 2014), and the medical communities in the United States and Canada have proved docetaxel treatment for advanced disease based on evidence from a phase 2 study (WO2016/064649A1).

[0010] In recent years, new drugs for the treatment of urothelial carcinoma mainly include: 1. Atezolizumab from Roche (2016) is approved to be the first anti-PD-L1 cancer immunotherapy on the market. Results of the latest phase III clinical trial (IMvigor211) of Atezolizumab for

locally advanced or metastatic urethral bladder cancer show that Atezolizumab failed to meet the primary clinical endpoint of the phase III trial IMvigor211, which is to improve overall survival (OS) in second-line treatment for patients with locally advanced or metastatic urothelial carcinoma (mUC). Among a total of 234 patients (116 in the Atezolizumab group and 118 in the chemotherapy group), the median overall survival was 11.1 months in the Atezolizumab group and 10.6 months in the chemotherapy group. The rate of confirmed objective responses in these evaluable patients was 23% and 22%, and the median duration of response was 15.9 months and 8.3 months, respectively.

[0011] The median progression-free survival in this group was 2.4 months, while 4.2 months in the control. In the exploratory analysis of the intention-to-treat population, the 12-month overall survival data is that: Atezolizumab was 39.2% and chemotherapy was 32.4%; median overall survival was 8.3 months for Atezolizumab, 7.5 months for taxane, and 9.2 months for vinflunine. (The ASCO Post, IMvigor211 Trial: Atezolizumab vs Chemotherapy in Platinum-Treated Advanced Urothelial Carcinoma, Matthew Stenger, 9/17/2018)

[0012] In addition, the U.S. Data Surveillance Commission has observed a decrease in survival of patients with PD-L1 low-expression tumors using Atezolizumab monotherapy compared with platinum-based chemotherapy. Therefore, in June 2018, the U.S. FDA issued a restriction on the use of Atezolizumab (Tecentriq) in patients with locally advanced or metastatic urothelial cancer, who is suitable for cisplatin-containing chemotherapy. It is required to detect PD-L1 expression before using Atezolizumab. This further illustrates the limitations of Atezolizumab in the treatment of urothelial cancer.

[0013] 2. Nivolumab from Bristol-Myers Squibb (2017) has been approved by the FDA for patients with locally advanced or metastatic urothelial carcinoma. Nivolumab is an anti-PD-1 monoclonal antibody. Clinical data show that Nivolumab's objective response (ORR) was 19.6% and median overall survival rate was 8.7 months. The most common serious adverse events include: urinary tract infection, sepsis, diarrhea, small bowel obstruction, and deterioration of overall health status. Nivolumab treatment was discontinued in 17% of patients due to adverse reactions, and administration of Nivolumab in 46% of patients was delayed due to adverse reactions. In clinic practice, treatment-related deaths were seen in 3 patients due to pneumonia or

cardiovascular failure.

[0014] Based on the above, the main problem of PD-L1 / PD-1 immunotherapy drugs in the current clinical stage is the poor treatment effect, which is mainly reflected in the treatment data such as objective response rate (ORR) and median overall survival, are not ideal. Another major problem is the relatively high proportion of serious adverse effects. For example, Nivolumab caused 3 deaths in related clinical trials.

[0015] 3. Erdafitinib from Janssen (a Johnson & Johnson company) was granted breakthrough drug qualification by the FDA in 2018 for the treatment of patients with locally advanced or metastatic urothelial cancer who have progressed after chemotherapy and whose tumor has specific fibroblast growth factor (FGFR) gene changes. The drug is a fibroblast growth factor receptor (FGFR) tyrosine kinase inhibitor. The results of the second-phase clinical study (BLC2001, NCT02365597) showed that the overall response rate of erdafitinib treatment was 40% (complete response rate 3%, partial response rate 37%), the median progression-free survival was 5.5 months and overall survival time was 13.8 months. Out of a total of 99 patients, 7 patients discontinued due to treatment-related adverse events. Data from https://www.jnj.com/media-center/press-releases/erdafitinib-phase-2-study-results-show-promise-in-the-treatment-of-metastatic-urothelial-cancer. Because the therapeutic target of this drug is FGFR, it is only suitable for patients with urothelial cancer who have certain FGFR gene mutations, while FGFR is only overexpressed in 15% to 20% of metastatic urothelial cancer and 40% to 70% % of non-muscle invasive bladder cancer (2018 ASCO Annual Meeting, Responses Found in Advanced Urothelial Carcinoma with FGFR Inhibitor).

[0016] The current treatment results shown that advanced urothelial carcinoma has a high degree of malignancy and a poor prognosis. Especially after the failure of conventional chemotherapy, the treatment options are limited. Immunotherapy can only benefit some patients. Moreover, the number of available immunotherapeutic inhibitors is also very limited, the objective response rate is not high, the side effects of treatment are large, or there are specific genetic requirements. At present, there are not many therapeutic drugs that can be choose by patients. Therefore, it is still necessary to develop drugs with more significant therapeutic effects and wider application to meet the urgent clinical needs.

SUMMARY

[0013a] In an aspect of the present disclosure, there is provided a use of an antibody-drug conjugate (ADC) in the preparation of a medicament for treating urothelial carcinoma, wherein the antibody-drug conjugate is an anti-HER2 antibody or a functional fragment thereof coupled to a cytotoxic molecule,

wherein the antibody-drug conjugate is represented by formula Ab-(L-U)n, wherein Ab is the antibody or the functional fragment thereof, L is a linker, U is a conjugated cytotoxic molecule, and n is an integer from 1 to 8, representing the number of therapeutic agent molecules bound to the antibody,

wherein the antibody or the functional fragment thereof is capable of competing with a defined antibody against the same epitope,

wherein the defined antibody comprises a heavy chain variable region and a light chain variable region defined by Complementarity Determining Region (CDR) sequences, and wherein

(i) the heavy chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 1, 2 and 3; and

(ii) the light chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 4, 5 and 6; and

wherein L is Maleimido-Caproyl-valine citrulline p-amino-benzyloxy (mc-vc-pAB), and

U is MMAE.

[0017] The present disclosure provides a method for treating urothelial carcinoma. The method comprises injecting an effective amount of an antibody-drug conjugate (ADC) into a patient, wherein the ADC is an anti-HER2 antibody conjugated cytotoxic molecule. The cytotoxic molecule includes, but is not limited to, a tubulin inhibitor or a DNA damaging agent. The tubulin inhibitor includes, but is not limited to, dolastatin and derivatives thereof, auristatin and derivatives thereof, and maytansine and derivatives thereof; the DNA damaging agents include, but are not limited to calicheamicins, duocarmycins, anthramycin derivative PBD (pyrrolobenzodiazepine), and camptothecin derivative SN-38. The auristatin and derivatives thereof include, but are not limited

to, monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), auristatin F (AF) or derivatives thereof; the maytansine and derivatives thereof include, but are not limited to, DM1, DM3, DM4 and derivatives thereof (Research progress of bullet molecules of antibody-drug conjugates, Hu Xinyue et al., Chin Med Biotechnol, December 2017, Vol. 12, No. 6) (Research progress of maytansinoid antibody drug conjugates, Zhou Lei et al., Chinese Journal of New Drugs, Volume 25, Issue 22, 2521 -2530). The cytotoxic molecules may also be amanitins, anthracyclines, baccatins, camptothecins, cemadotins, colchicines, colcimids, combretastatins, cryptophycins, discodermolides, docetaxel, doxorubicin, echinomycins, eleutherobins, epothilones, estramustines, lexitropsins, maytansines, methotrexate, netropsins, puromycins, rhizoxins, taxanes, tubulysins, or vinca alkaloids. The cytotoxic molecules are not limited to the above-mentioned categories, and may include all drugs that can be used for ADC.

[0018] Another example of the present disclosure is to provide an antibody-drug conjugate (ADC) for use in the manufacture of a medicament for treating bladder cancer. The antibody-drug conjugate comprises an antibody or a functional fragment thereof capable of binding to HER2, wherein the antibody comprises a heavy chain variable region and a light chain variable region, and wherein (i) the heavy chain variable region comprises three CDRs, wherein the CDRs have amino acid sequences of SEQ ID NO : 1, 2 and 3, respectively; and (ii) the light chain variable region comprises three CDRs, wherein the CDRs have the amino acid sequences of SEQ ID NO: 4, 5 and 6, respectively. The antibody may also be an antibody capable of competing with a

defined antibody against the same or similar epitope, wherein the defined antibody comprises the above CDRs.

[0019] In the present disclosure, the term "antibody" may include a full-length antibody or an antibody fragment that binds to, reacts with, or composites with HER2. An antibody can be any protein, protein-like molecule, or polypeptide that binds, composites, or reacts with a portion of a population of cells seeking therapeutic modification. The antibody may be a chimeric antibody or a functionally active fragment thereof, a humanized antibody or a functionally active fragment thereof, a human antibody or a functionally active fragment thereof. It can also be an antibody or a functionally active fragment thereof of derives from another species other than the above species, for example: mouse antibody or functionally active fragment thereof, rat antibody or functionally active fragment thereof, goat antibody or functionally active fragment thereof, rabbit antibody or functionally active fragment thereof. The antibody may be a polyclonal antibody or a monoclonal antibody. In some examples, the antibody may be a bispecific antibody. Also, the antibody may be a functionally active fragment, a derivative or analog of the antibody. "Functionally/functional" means that the fragments, derivatives, or analogs can recognize the same antigen, and the antibodies that can recognize fragments, derivatives, or analogs derived from the antigen, such as but not limited to: F (ab ')$_2$, Fab, Fab ', Fv fragments and dimers of antibody heavy chains and light chains, or any minimal fragments thereof, like Fvs or single chain antibodies (SCAs). In addition, the antibody may be a fusion protein of an antibody. Antibodies may also include analogs and derivatives that are modified or unmodified (i.e., covalently linked to any molecule), as long as such covalent bonding allows the antibody to retain its antigen-binding immunospecificity. Examples include, but are not limited to, analogs and derivatives of antibodies, including further modifications such as: glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization via known protecting / blocking groups, protease cleavage, attachment to cellular antibody units or other proteins, and the like. Any bulk chemical modifications can be achieved using known techniques, including but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis in the presence of tunicamycin, and the like. In addition, analogs or derivatives may include one or more unnatural amino acids. In some examples, the antibody may have modifications (e.g., substitutions, deletions, or additions) in amino acid residues that interact with the Fc receptor. In another example,

the antibody-drug conjugate has a structure represented by formula Ab-(L-U)n, wherein Ab is the antibody or a functional fragment thereof, L is a linker, U is a coupled cytotoxic molecule, n is an integer from 1 to 8, representing the number of therapeutic agent molecules bound to the antibody.

[0020] In another example, the linker is linked to the antibody or the functional fragment thereof via a thiol group and/or an amino group, and the cytotoxic molecule is conjugated to the antibody through site-directed or undirected conjugation. The linker of the present disclosure may be selected from the following table:

Abbreviation	Full name	Feature
mc	Maleimidocaproyl	Non-cleavable [12]
mc-vc-pAB	Maleimidocaproyl valine citrulline p-amino-benzyl	Cleavable [12]
3-MPA	3-maleimido-propionic acid	Non-cleavable [12]
Mal-di-EG-OPFP	Perfluorophenyl 3-(2-(2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)ethoxy) ethoxy)propanoate	Non-cleavable [14]
Mal-di-EG-OSu	2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy) propanoate	Non-cleavable [14]
Mal-Tri-EG-OSu	2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy) propanoate	Non-cleavable [14]
Mal-Tetra-EG-OSu	2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4 -azanonadecan-19-oate	Non-cleavable [14]
Br-di-EG-OSu	2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-bromoacetamido)ethoxy)ethoxy)propanoate	Non-cleavable [14]
Py-ds-prp-OSu	2,5-dioxopyrrolidin-1-yl 3-(pyridin-2-yldisulfanyl)propanoate	Reducible [14]
Py-ds-Prp-OPFP	Perfluorophenyl 3-(pyridin-2-yldisulfanyl)propanoate	Reducible [14]
Py-ds-dmBut-OSu	2,5-dioxopyrrolidin-1-yl 4-methyl-4-(pyridin-2-yldisulfanyl) pentanoate	Reducible [14]
Py-ds-dmBut-OPF	Perfluorophenyl 4-methyl-4-(pyridin-2-yldisulfanyl)pentanoate	Reducible [14]
SMCC	N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate	Non-cleavable [13]
MBS	3-maleimidobenzoic acid N-hydroxysuccinimide ester	Non-cleavable [13]
SATA	S-(N-succinimidyl) thioacetate	Non-cleavable [13]
SPDP	N-succinimidyl 3-(2-pyridyldithio)propionate	Reducible [13]
SMPT	(N-succinimidyl carbonyl)-1-methyl-1-(2-pyridyldithio) toluene	Reducible [13]

[0021] The linker of the present disclosure is preferably Maleimido-Caproyl-Valine-Citrulline-p-Aminobenzyloxy (mc-vc-pAB) and Maleimidocaproyl (mc).

[0022] The linker of the present disclosure may also be triglycyl peptide linker, which is a new linker for ADCs developed in recent years (Rajeeva Singh et al., A New Triglycyl Peptide Linker for Antibody–Drug Conjugates (ADCs) with Improved Targeted Killing of Cancer Cells, MCT-16-002, Published June 2016). Alternatively, a glucuronide-tubulysin linker can be used (Patrick J. Burke et al., Glucuronide-linked antibody-tubulysin conjugates display activity in MDR^+ and heterogeneous tumor models, Molecular Cancer Therapeutics, 2018).

[0023] In another example, the antibody or the functional fragment thereof is derived from an antibody secreted by the hybridoma deposited at the China General Microbiological Culture Collection Center of China Committee for Culture Collection of Microorganisms, with an accession number CGMCC No. 8102 on August 22, 2013.

[0024] In another example, the antibody is a humanized antibody, preferably, the antibody is an antibody secreted by CHO cells deposited at the China Center for Type Culture Collection under the accession number CCTCC C2013170 on November 6, 2013.

[0025] In an example, the antibody-drug conjugate used is named RC48-mc-vc-pAB-MMAE, which conforms to the structure of the general formula Ab-(L-U)n, in which RC48 (a humanized anti-HER2 monoclonal antibody) is coupled to MMAE through the linker mc-vc-pAB, and the number of coupling ranges from 1 to 8, including 1, 2, 3, 4, 5, 6, 7, 8 or a combination of antibody-drug conjugates with varying MMAE coupling numbers ranging from 1 to 8.

[0026] In the present disclosure, the urothelial carcinoma is locally advanced urothelial carcinoma that cannot be removed by surgery, locally advanced or metastatic urothelial carcinoma, HER2 (Human Epidermal Growth Factor Receptor 2, also called ErbB-2, C-erbB2, or HER2/neu) positive urothelial carcinoma, HER2 positive locally advanced or metastatic urothelial carcinoma.

[0027] The medicament described in the present disclosure may be administered intranasally, subcutaneously, intradermally, intramuscularly, or intravenously. The medicament also includes a pharmaceutically acceptable carrier; the medicament is preferably a lyophilized formulation or a liquid formulation; the carrier comprises one or more selected from the group consisting of a stabilizer, a protective agent, a buffer, a lyoprotectant, an activity protective agent, a surfactant and an adsorption carrier and an absorption promoter.

BRIEF DESCRIPTION OF DRAWINGS

[0028] Figure 1: SDS-PAGE of purified human recombinant protein HER2-ECD, stained by Coomassie brilliant blue. The loading amount was 10μg per lane.

[0029] Figure 2: SDS-PAGE analysis of cRC48 (chimeric antibody) and RC48 (humanized antibody). The loading amount was 2μg per lane.

[0030] Figure 3 shows the binding affinity of humanized antibody RC48 with HER2-ECD determined by ELISA, and the binding affinity constant Kd was calculated. Herceptin and cRC48 were used as controls in this experiment.

[0031] Figure 4A shows the binding ability of the anti-HER2 humanized antibody RC48 to $HER2^{+}$ cells SK-BR3, BT474 and $HER2^{-}$ cell MDA-MB468 by flow cytometry.

[0032] Figure 4B shows the binding ability of anti-HER2 antibodies to BT474 cell surface antigens at different antibody concentrations by flow cytometry analysis. The anti-HER2 antibodies are Herceptin, cRC48, and RC48. A total of 5×10^4 cells were analyzed.

[0033] Figure 5 shows that RC48 only shows specific binding affinity tor HER2, but not to EGFR, HER3, and HER4.

[0034] Figure 6 is the schematic diagram of the efficacy of subject No. 01001. Patient profile: female, 57 years old, with multiple lung metastases after right pelvic cancer surgery. Pathological diagnosis of urothelial carcinoma, HER2 IHC 3+.

[0035] Figure 7 is the schematic diagram of the efficacy of subject No. 01003. Patient profile: female, 45 years old, abdominal lymph node metastasis after right renal pelvis cancer surgery. Pathological diagnosis of urothelial carcinoma, HER2 IHC 3+.

[0036] Figure 8 is the schematic diagram of the efficacy of subject No. 01007. Patient profile: Male, 63 years old, after bladder cancer and right pelvic cancer surgery, lung metastasis, liver metastasis, cervical lymph node metastasis, mediastinal metastasis, multiple bone metastases. Pathological diagnosis of urothelial carcinoma, HER2 IHC 3+.

DETAILED DESCRIPTION

Example 1. Preparation and sequence analysis of murine monoclonal antibody mRC48

1. Expression and purification of HER2 antigen

[0037] The cDNA fragment encoding HER2-ECD (from Thr23 to Thr652, GenBank accession No. M11730) was cloned into pcDNA3 expression vector (Invitrogen) by PCR.

[0038] The detailed method: the cDNA of HER2-ECD coding region was obtained from $HER2^{+}$ SKBR3 cell line (ATCC No. HTB-30) by RT-PCR (using ImProm-IITM Reverse Transcription System of Promega).

[0039] The primers are: P1: 5'CGGGATCCTGCCACCAGCTGTGCGCC (SEQ ID NO: 7), P2: 5 'GCTCTAGA TCAGTTGATGGGGCAAGGCT (SEQ ID NO: 8), the underlined sequences are the introduced BamHI and XbaI restriction enzyme cutting sites, respectively. The HER2-ECD cDNA obtained by reverse transcription was used as a template for PCR amplification using the above primers. The amplification conditions: denaturing at 94℃ for 30s, annealing at 60℃ for 30s, extending at 72℃ for 1 minute, for a total of 30 cycles, and then extending at 72℃ for 10 minutes. The PCR fragment was then recovered, digested with BamHI and XbaI (from NEB), and ligated with pcDNA3 vector. A polyhistidine tag was added to the C-terminus of HER2-ECD to facilitate purification. HEK293 cells (ATCC, USA) were transfected with the constructed DNA expression vector, and soluble protein HER2-ECD with his-tag was purified from the culture medium by Ni-NTA affinity chromatography (Qiagen). SDS-PAGE and Coomassie brilliant blue staining showed that the purified HER2-ECD protein had more than 95% homogeneity, as shown in Figure 1. Soluble HER2-ECD appears as a monomer with a relative molecular weight of about 75 kDa, slightly larger than the calculated molecular weight (71 kDa), indicating the protein was glycosylated in HEK293 cells. The purified HER2-ECD protein was further concentrated and transferred to a sterile pH 7.4 PBS buffer for subsequent *in vivo* and *in vitro* analysis.

2. Generating and screening of hybridoma cells

[0040] Mice were immunized with the HER2-ECD prepared above as the antigen to produce antibodies. Immunization, hybridoma cell fusion, and preliminary screening were performed according to standard procedures (Reference: WHO Technical Report Series, No. 822, 1992 Annex 3). 0.25mL of HER2-ECD protein (50-100μg) and 0.25ml of Freund's complete adjuvant (Difco Lab) were mixed in equal volume and used to immunize 4 Balb/c mice (purchased from Shanghai SLAC Laboratory Animal Co., Ltd). The second injection was performed after 2 weeks. Freund's incomplete adjuvant (Difco Lab) and antigen with the amount of 25-50μg/0.5mL/per mouse were used for the second injection. 3 weeks after, the third injection was performed at the same dose as that of the second injection. Blood was taken 10 days after the third injection. The serum of the mice was analyzed by enzyme-linked immunosorbent assay (ELISA). The cells from the spleens of the two mice with the highest anti-HER2 antibody titers were taken, and then fused with myeloma cells P3X63Ag8 (ATCC, CRL-1580). The fused cells were diluted into 96-well plate, and screened preliminarily by ELISA according to the binding ability with HER2-ECD. In a typical ELISA, Nunc Maxisorb 96-well plate was coated with HER2-ECD (0.2-1μg/mL) and then incubated with a gradient dilution of mouse serum or hybridoma supernatant (100μL). The mouse anti-HER2 antibody was detected using a horseradish peroxidase-conjugated goat $F(ab')_2$ anti-mouse IgG Fc (Invitrogen) secondary antibody.

[0041] ELISA was used to screen the supernatants of 400 hybridoma cell lines, of which 36 showed strong binding with HER2-ECD. Ten hybridoma cells with the strongest HER2 binding ability were selected and subcloned hybridoma cell lines were screened again by limiting dilution method. The subcloned hybridoma cell lines were cultured in suspension, proteins were purified, and the binding affinity with HER2 was determined by ELISA. The binding ability of the above antibodies to HER2 expressed on the surface of human breast cancer cell lines was further tested by flow cytometry (BD FACS Calibur) (see Example 4 for detailed description). Finally, a hybridoma cell line mRC48 (murine IgG1k) was identified through sequence analysis, which has strong HER2 binding capacity. The hybridoma cell mRC48 was deposited with accession No. 8102 at China General Microbiological Culture Collection Center of China Committee for Culture Collection of Microorganisms on August 22, 2013 (the date of conversion to deposit under the Budapest Treaty was October 29, 2013).

3. Sequence analysis of anti-HER2 antibody from hybridoma cell clone mRC48

[0042] The 5' ends of the heavy chain and light chain variable regions of clone mRC48 were rapidly amplified using the commercial SMARTTM RACE cDNA Amplification Kit (Clontech) for sequencing according to the instructions.

[0043] Total RNA was extracted from hybridoma cells using RNApure Tissue Kit (Beijing ComWin Biotech Co.,Ltd), and reverse transcription was performed using the SMART^{TM} RACE cDNA Amplification Kit. First-strand cDNA for RACE-Ready was obtained by reverse transcription according to the protocol provided with the kit, using the total RNA as the template, the primers provided with the kit, and reverse transcriptase SMARTScribe^{TM} Reverse Transcriptase. And then two rounds of PCR were carried out. For the first round PCR, the obtained cDNA was as template, the UPM provided with the kit was used as the 5 'end primer and mRC48-VL-1/mRC48-VH-1 as the 3' end primer. The PCR reaction conditions: pre-denaturation at 94℃ for 5 minutes; 25 cycles (denaturation at 94 ℃ for 30s, annealing at 68 ℃ for 30s and extension at 72 ℃ for 2 minutes); and extension at 72 ℃ for 10 minutes.

[0044] The second round PCR was performed using the products of the first round PCR as the template, NUP15 provided with the kit as the 5' end primer and mRC48-VL-2 / mRC48-VH-2 as the 3' end primer. PCR reaction conditions: pre-denaturation at 94 ℃ for 5min; 25 cycles (denaturation at 94 ℃ for 30s, annealing at 68 ℃ for 30s and extension at 72 ℃ for 2min); extension at 72 ° C for 10min. Both the heavy chain and light chain variable regions of the aforementioned antibody from hybridoma cell clone mRC48 were obtained.

[0045] The primers are as follows:

mRC48-VL-1: 5 '-GTTGGTGCAGCATCAGCCCGTT-3' (SEQ ID NO: 9);

mRC48-VL-2: 5'-GTTCACTGCCATCAATCTTCCAC-3 '(SEQ ID NO: 10);

mRC48-VH-1: 5'-GCCAGTGGATAGACAGATGG- 3 '(SEQ ID NO: 11);

mRC48-VH-2: 5'-AGGTCACTGTCACTGGCTCAG-3' (SEQ ID NO: 12).

[0046] The PCR products were purified by agarose gel electrophoresis and then subcloned into the pCR2.1TOPO cloning vector (Invitrogen). Plasmid DNAs from ten independent clones were obtained by PCR, and then sequenced using M13 forward and reverse primers. DNA sequence

analysis showed that all 10 clones had cDNA encoding the same VH or VL polypeptide. The amino acid sequences of the complementarity determining regions (CDRs) are analyzed by Kabat coding table and listed in Table 1. Sequence comparison analysis showed that the CDRs of anti-HER2 mRC48 were significantly different from that of the known HER2 antibodies including Herceptin (trastuzumab).

Table 1. Amino acid sequences of the CDRs of anti-HER2 monoclonal antibody mRC48

	VH	VL
CDR1	DYYIH (SEQ ID NO: 1)	KASQDVGTAVA (SEQ ID NO: 4)
CDR2	RVNPDHGDSYYNQKFKD (SEQ ID NO: 2)	WASIRHT (SEQ ID NO: 5)
CDR3	ARNYLFDHW (SEQ ID NO: 3)	HQFATYT (SEQ ID NO: 6)

Example 2. Humanization of the anti-HER2 monoclonal antibody mRC48 (method as described in CN105008398A)

[0047] Murine anti-HER2 monoclonal antibody mRC48 was humanized by transplanting light chain or heavy chain CDRs into human IgG1κ framework regions.

[0048] The humanized RC48 antibody light chain variable region (RC48-VL) and the humanized RC48 antibody heavy chain variable region (RC48-VH) were designed and constituted the humanized anti-HER2 antibody: RC48. The similarity between the overall sequence of RC48-VH and human IgG1VH is 84%. The RC48 antibody comprises a light chain variable region RC48-VL and a heavy chain variable region RC48-VH.

[0049] Humanized anti-HER2 monoclonal antibody RC48 was obtained by CDR grafting. The nucleic acid sequences of heavy chain and light chain variable regions were directly synthesized by Nanjing GenScript Biotech Corporation. The synthetic variable region comprises Kozak consensus sequence, start codon, heavy chain or light chain signal peptide, human framework region and murine CDRs. Variable regions and human IgG1k constant region were ligated into a complete fragment by overlap-extension PCR.

[0050] The primers for overlap-extension PCR:

[0051] Heavy chain VH1: 5’ CGCGGATCC GCCGCCACCATGGGATGGAGCT 3′ (SEQ ID NO: 13)

[0052] VH2: 5’ GATGGGCCCTTGGTGCTAGCGGAGCTCACTGTCACCAGTGTT 3’ (SEQ ID NO: 14)

[0053] CH1: 5’ GCTAGCACCAAGGGCCCATC 3’ (SEQ ID NO: 15)

[0054] CH2: 5’ CCGGAATTCTTTACCGGGAGACAGGGAGA 3’ (SEQ ID NO: 16)

[0055] Light chain VL1: 5’ CGCGGATCC GCCGCCACCATGGACATGAGGGT 3’ (SEQ ID NO: 17)

[0056] VL2: 5’ GATGGTGCAGCCACAGTACGCTTTATCTCAACTTTTG TAC 3’ (SEQ ID NO: 18)

[0057] CL1: 5’ CGTACTGTGGCTGCACCAT 3’ (SEQ ID NO: 19)

[0058] CL2: 5’ CCGGAATTCACACTCTCCCCTGTTGAAGC 3’ (SEQ ID NO: 20)

[0059] For the generation of heavy chain nucleic acid, first, the heavy chain variable region was amplified using the synthetic variable region as the template and VH1 and VH2 as the primers, meanwhile, human IgG1κ heavy chain constant region was used as the template and CH1 and CH2 as the primers to amplify the heavy chain constant region. The amplification conditions are: denaturation at 94 ℃ for 30s, annealing at 60 ℃ for 30s, extension at 72 ℃ for 1 minute, 30 cycles, and extension at 72 ℃ for 10 minutes. Then the heavy chain sequence of RC48 was amplified using the above two PCR products as the template and VH1 and CH2 as primers. The amplification conditions were: denaturation at 94 ℃ for 30s, annealing at 60 ℃ for 30s, extension at 72 ℃ for 2 minutes, 30 cycles, and extension at 72 ℃ for 10 minutes.

[0060] For the generation of light chain nucleic acid, first, the light chain variable region was amplified using the synthetic variable region as the template and VL1 and VL2 as the primers, meanwhile, the human IgG1κ light chain constant region was used as the template and CL1 and CL2 as the primers to amplify the light chain constant region. The amplification conditions were: denaturation at 94 ℃ for 30s, annealing at 60 ℃ for 30s, extension at 72 ℃ for 1 minute, 30 cycles, and extension at 72 ℃ for 10 minutes. Then the light chain sequence of RC48 was amplified using the above two PCR products as the template and VL1 and CL2 as primers. The

amplification conditions were: denaturation at 94 ℃ for 30s, annealing at 60 ℃ for 30s, extension at 72 ℃ for 2 minutes, 30 cycles, and extension at 72 ℃ for 10 minutes.

[0061] Thus, the humanized anti-HER2 monoclonal antibody RC48 sequence was obtained, wherein RC48 comprises human IgG1κ heavy chain constant region and heavy chain variable region RC48-VH, and human IgG1κ light chain constant region and light chain variable region RC48- VL.

[0062] Human-mouse chimeric antibody cRC48 was also obtained by the same method. The murine variable region and human IgG1k constant region were ligated into a complete sequence by overlap-extension PCR.

[0063] Each of the amplified fragments was subcloned into the expression vector pcDNA3.0, respectively. The obtained constructs were transfected into suspension CHO cells (Invitrogen) to produce different recombinant antibodies. The antibodies were purified by Protein A and subjected to subsequent characterization. Chimeric anti-HER2 RC48 (referred to as cRC48) comprises mouse-human chimeric heavy chain and light chain. RC48 comprises the humanized heavy chain RC48-VH and the humanized light chain RC48-VL. Both cRC48 and RC48 can be expressed in cells. The antibodies were collected from the supernatants of CHO cells, purified by Protein A, and analyzed by SDS-PAGE under reducing and non-reducing conditions (see Figure 2). The CHO cells capable of secreting RC48 antibody as described above (i.e., CHO cells transfected with human IgG1κ heavy chain constant region and heavy chain variable region RC48-VH, and human IgG1κ light chain constant region and light chain variable region RC48-VL) was deposited at the China Center for Type Culture Collection on November 6, 2013 with the accession number C2013170.

Example 3. Characterization of Anti-HER2 RC48 Antibody

[0064] HER2-binding affinity constant (Kd) of cRC48 (chimeric antibody) and RC48 antibody (humanized antibody) was measured by ELISA. The specific method can be seen in Example 1. Briefly, a 96-well plate was coated with soluble HER2-ECD, followed by incubating with diluted antibodies (Herceptin and chimeric cRC48 as controls), and HRP-conjugated goat $F(ab')_2$ anti-human IgG Fc (Invitrogen)was used as the specific secondary antibody to detect

HER2-ECD-related antibodies (all forms of human IgG1κ). The surface binding affinity constant (Kd) value for each anti-HER2 antibody was calculated by drawing a binding curve and further using a single-site specific binding nonlinear equation (Journal of Immunological Methods, 270: 155-162, 2002) (Figure 3 shows a typical HER2-binding curve obtained from three independent ELISA assays). The results of the ELISA are shown in Figure 3.

[0065] From three independent assays, it can be seen that RC48 (humanized antibody) shows an average affinity constant of 44 pM, indicating significantly improved HER2-ECD binding affinity compared to cRC48 (average affinity constant 77pM) and Herceptin (average affinity constant 97pM). The results are shown in Table 2.

Table 2. Comparison of average affinity constant between antibodies of this invention and Herceptin

Sample	Average affinity constant
Herceptin	97pM
cRC48	77pM
RC48	44pM

Example 4. Binding affinity of RC48 (humanized antibody) to HER2

1) Test for the binding affinity of RC48 antibody to HER2

[0066] Flow cytometry was used to detect the binding affinity of endogenously expressed HER2 in human breast cancer cells with the humanized anti-HER2 antibody RC48. The results are shown in Figure 3. 6μg of human IgG (as control group), Herceptin, cRC48, and RC48 were respectively incubated with two types of breast cancer cells: SK-BR-3 and BT4745 separately, as well as $HER2^-$ MDA-MB468 cells (2×10^7 cells), on ice for 30-45 minutes. After thoroughly washed with 4 mL of cold PBS twice, the antibodies binding to the cell surface were detected by R-PE-conjugated goat anti-human IgG Fc (15 μl, 25 μg/mL) secondary antibody, and then analyzed by flow cytometer (BDFACSCalibur). Human IgG1 in the control group did not show

binding to the above three cancer cells. In contrast, Herceptin, cRC48, and RC48 bind strongly to two types of HER2-positive cells, but not to HER2-negative cells, indicating that this binding is HER2-specific (see Figure 4a). By comparing the average fluorescence intensity in the same group, it was found that RC48 showed higher binding affinity than Herceptin and cRC48. By titrating the concentration of anti-HER2 antibody and the number of cells analyzed in flow cytometry, the binding curve of cell-based anti-HER2 antibody to cell surface HER2 was obtained. The results are shown in Figure 4b. The humanized anti-HER2 antibody RC48 showed obvious binding affinity to HER2, of which the binding affinity Kd to HER2 on the surface of BT474 cells was 4nM, while Herceptin and cRC48 were 10nM and 5nM, respectively. The results are shown in Table 3:

Sample	Binding affinity Kd
Herceptin	10nM
cRC48	5nM
RC48	4nM

2) Antigen binding specificity test

[0067] ELISA was used to determine the binding ability of Herceptin, cRC48, RC48 to different surface antigens: EGFR, HER2, HER3, HER4. The ELISA was carried out as described in Example 1. A 96-well plate was coated with EGFR, HER2, HER3 or HER4, 20ng for each well, incubated with different anti-HER2 antibodies, i.e., Herceptin, cRC48, and RC48, and then detected using HRP-conjugated goat F (ab')$_2$ anti-mouse IgG-Fc secondary antibody (Invitrogen). The results are shown in Figure 5. It indicates that Herceptin, cRC48, and RC48 have nearly no binding capacity for EGFR, HER3, and HER4, while have strong binding capacity for HER2, demonstrating that Herceptin and RC48 have a high binding specificity for HER2.

Example 5. Preparation of antibody-drug conjugates

1) Purification of monoclonal antibody RC48

[0068] Monoclonal antibody RC48 was collected from the supernatant of CHO cell culture using Protein A. The antibody was subjected to SDS-PAGE electrophoresis and SEC analysis and the purity was over 95%. The obtained antibody was dialyzed with a PBS buffer in a 30KD cutoff membrane bag, and concentrated. The antibody concentration was calibrated using a UV absorbance meter for subsequent conjugation.

2) Conjugation of monoclonal antibody RC48 with drug

[0069] Reducing agent and protecting agent were respectively prepared with PBS buffer as follows: 1-20mmol/L TCEP (Tris-2-carboxyethyl-phosphine), 1-20mmol/L DTPA (Diethylene triamine pentacetate acid) stock solution. The reducing agent was added within a certain concentration range according to the desired coupling ratio, mixed with a certain concentration of monoclonal antibodies (such as: 5-30mg/mL) according to a certain volume ratio (such as 1: 1), so that the final molar ratio of TCEP to antibody is 0.5-6.0 : 1, and stirred at 25℃ for 2h. The free thiol group concentration was detected by DTNB method at 412nm, and the number of free thiol groups was calculated by the molar ratio with the antibody. TCEP reduction has a good reproducibility, and the number of free thiol groups can reach 1.0-8.0 after reduction.

[0070] The antibodies can be directly subjected to conjugation after TCEP reduction. A certain concentration (10mM) of drug (vc-MMAE, vc-MMAF, or mc-MMAF) (purchased from Shanghai HaoYuan Chemical Technology Co., Ltd.) was prepared in 25% DMSO (dimethyl sulfoxide). The drug was added slowly to the antibody solution according to a molar ratio of the drugs to thiol group at 0.3-2.8 : 1, and stirred to react at 25℃ for 2h. DTNB method was used to detect the concentration of free thiol group (close to 0) at 412nm. Remaining unreacted drugs were removed by Sephadex G-25 and free small molecules such as DMSO. SDS-PAGE, R-HPLC and HIC-HPLC were used to detect the conjugation.

Example 6. Binding Affinity of the Antibody Drug Conjugates

Binding affinity test by ELISA

[0071] The ELISA plate was coated with the recombinant protein HER2-ECD (concentration:

0.5mg/ml), and incubated overnight at 2℃ to 8℃. After the plate was washed with a plate washer for 3 times, 3% BSA-PBST solution was added for blocking at 37℃ for 2h, and then the plated was washed with a plate washer for 3 times. Sample loading: the standard, starting from 1000ng/mL, was diluted with PBST buffer to obtain 11 dilution points, 100μL/well, incubated at 37℃ for 2h. The plate was washed with a plate washer for 3 times. The secondary antibody (goat anti-human IgG-Fc-HRP) was diluted 5000-fold with PBST buffer. TMB developing solution was added and incubated at room temperature in the dark for 8-10 minutes. The reaction was terminated by 2M H_2SO_4, and microplate reader was used for reading at 450/655nm. The results are shown in Table 4.

Table 4. Comparison of binding affinity of antibody-drug conjugates (VC is short for mc-vc-pAB) and T-DM1

Sample	Binding affinity ng/mL	Mole equivalent
RC48-VC-MMAE	2.237	15.22
RC48-VC-MMAF	3.349	13.51
RC48-MC-MMAF	2.902	16.42
T-DM1	2.376	15.16

[0072] As shown in the results, of RC48-VC-MMAE (i.e. RC48-mc-vc-pAB–MMAE), RC48-VC-MMAF, and RC48-MC-MMAF have binding affinity to HER2-ECD equivalent to T-DM1.

Example 7. Efficacy and safety test of monotherapy for HER2-positive locally advanced or metastatic urothelial carcinoma

[0073] The purpose of this experiment was to evaluate the efficacy and safety of monotherapy for HER2-positive locally advanced or metastatic urothelial carcinoma. The antibody-drug conjugate for test was RC48-mc-vc-pAB-MMAE. RC48 was coupled with MMAE via linker mc-vc-pAB, and the number of coupling drugs varied from 1 to 8.

[0074] The selection criteria for the subjects are:

[0075] Age: 18 years (minimum age) to 80 years (maximum age) old;

[0076] wherein the selection criteria are:

[0077] 1. The subjects agree to participate in the research and sign an informed consent;

[0078] 2. Male or female, 18 to 60 years old;

[0079] 3. Expected survival time is 12 weeks or more;

[0080] 4. Locally advanced or metastatic bladder urothelial carcinoma which cannot be completely removed by surgery based on pathological diagnosis;

[0081] 5. Subjects who have been diagnosed as locally advanced or metastatic carcinoma that cannot be removed by surgery, and still have disease progression or resistance after receiving at least a first-line systemic chemotherapy;

[0082] 6. At least have measurable lesions specified by RECIST 1.1 standard;

[0083] 7. Positive HER2 expression confirmed by the test laboratory;

[0084] 8. ECOG physical status is 0 or 1 score;

[0085] 9. Adequate heart, bone marrow, liver, and kidney functions;

[0086] 10. Female subjects should be surgically sterilized or postmenopausal patients, or agree to use at least one medically approved contraceptive measures (such as an intrauterine device, contraceptive pills or condoms) during the treatment period and within 6 months after the treatment; male subjects should agree to use at least one medically approved contraceptive measures (such as condoms, abstinence, etc.) during the treatment period and within 6 months after the end of the treatment;

[0087] 11. Be willing and able to follow trial and follow-up procedures.

[0088] Meanwhile, the exclusion criteria are:

[0089] 1. Known to be allergic to recombinant humanized anti-HER2 monoclonal antibody-MMAE conjugate and components thereof;

[0090] 2. Received other anti-tumor treatments within 4 weeks before the treatment;

[0091] 3. Previously received a recombinant humanized anti-HER2 monoclonal

antibody-MMAE conjugate;

[0092] 4. Subjected to major surgery within 4 weeks before the start of administration and not fully recovered;

[0093] 5. Received with live vaccine within 4 weeks prior to administration or plan to receive any vaccine during treatment period;

[0094] 6. Having other severe and uncontrollable concomitant diseases that may affect protocol compliance or interfere with interpretation of results;

[0095] 7. Having other malignant tumors within 5 years before the start of administration;

[0096] 8. Suffering from CNS metastatic and/or cancerous meningitis;

[0097] 9. Having active autoimmune disease that requires systemic treatment in the past 2 years;

[0098] 10. Previously received allogeneic hematopoietic stem cell transplant or solid organ transplant;

[0099] 11. Having a large amount of pleural fluid or ascites with clinical symptoms or requiring symptomatic treatment;

[0100] 12. Pregnant or lactating women;

[0101] 13. Positive HIV test results;

[0102] 14. Patients with active hepatitis B or C;

[0103] 15. History of active tuberculosis;

[0104] 16. Suffering from any other diseases, abnormal metabolism, abnormal results of physical examination or abnormal laboratory test, according to the researcher's judgment, it is reasonable to suspect that the subject has a certain disease or condition unsuitable for using the research drug, or affect the interpretation of the research results, or put the patient at high risk;

[0105] 17. Who is estimated to have enough patient compliance to this clinical study.

[0106] Experimental methods:

[0107] This study include subjects with locally advanced or metastatic urothelial carcinoma

who had previously failed or resistant to at least first-line systemic chemotherapy , and had measurable lesions, acceptable physical conditions and organ functions. The tumor tissue pathological sections of the subjects were submitted to the laboratory for HER2 expression confirmation, and the positive result was defined by score 2+ or 3+ in immunohistochemistry (IHC) assay (regardless the results of fluorescence in situ hybridization [FISH] detection).

[0108] The results determination of HER2 in immunohistochemistry (IHC) was according to the criteria for HER2 interpretation in "Guideline for HER2 detection in breast cancer (2014 Edition, China)". The details are shown in Table 5.

Table 5 Criteria for HER2 interpretation in "Guideline for HER2 detection in breast cancer (2014 Edition, China)".

Scoring criteria	IHC results	Up-regulated HER2 expression level
No cell membrane staining or ⩽ 10% of invasive cancer cells showed incomplete, weak cell membrane staining.	0	negative
> 10% of invasive cancer cells showed weak and incomplete cell membrane staining.	1+	negative
>10% of invasive cancer cells showed incomplete and/or weak to moderate cell membrane staining or ⩽10% of invasive cancer cells showed strong and intact cell membrane staining.	2+	positive
>10% of invasive cancer cells showed strong, complete, and uniform cell membrane staining.	3+	positive

[0109] Subjects meeting all the criteria received RC48-ADC treatment (2.0 mg/kg, intravenous infusion, once every 2 weeks), and the efficacy was evaluated every 6 weeks until disease

progressed, intolerable toxicity was produced or the subject dropped out. The primary endpoint of the study was the objectively assessed objective response rate (ORR). The secondary endpoints were progression-free survival, overall survival, and safety of treatment.

[0110] Research results:

[0111] The study was launched in December 2017. As of July 31, 2018, a total of 18 subjects received treatment, including 15 males and 3 females, with a median age of 63 years. The primary lesions included the bladder (50.0%), renal pelvis (27.8%), and ureter (22.2%). The main metastatic sites were lung, liver, and lymph node. 16 patients (88.9%) had previously received first-line platinum therapy. The immunohistochemical (IHC) results for HER2 expression (performed by the laboratory) showed that there were 11 (61.1%) IHC2+ subjects and 7 (38.9%) IHC3+ sucjects.

[0112] Efficacy evaluation was performed on 13 of the 18 subjects, and 10 subjects had partial remission (PR) (wherein 4 subjects had confirmed PR (two consecutive PRs are called confirmed PRs)). According to the RECIST standard: "when every subject meets the criteria for partial or complete remission and the efficacy is confirmed again at a later time point (usually four weeks later), then the complete or partial remission can be established". That is, two consecutive evaluations of PR for a subject is called confirmed PR. The other 6 cases have not yet reached the time point for confirming the efficacy, and only one confirmation has been completed. The objective response rate (ORR) is 76.9% (10/13), and the disease control rate (DCR) (10 cases out of 13 subjected to efficacy evaluation were PRs and 2 cases were stable disease (SD)) were 92.3% (12/13). The current maximum treatment period received by the subjects was more than 7 months. Among the subjects achieved remission, 7 (53.8%) had received taxane treatment and 4 (30.8%) had received PD-1/PD-L1 treatment.

[0113] 16 of the 18 subjects were subject to safety evaluation. The most common treatment-related adverse reactions (TRAEs) in safety evaluation were elevated ALT (50.0%, grade 1-2), hypoesthesia (50.0%, grade 1-2) and reduced white blood cell count (50.0%, grade 1-2); TRAE which is greater than grade 3 is a decrease in neutrophil count (12.5%, grade 3). No drug-related serious adverse events (SAE) occurred.

[0114] Descriptions of the obvious effects of the treatment in some cases are shown as follows:

[0115] 1. Patient 01001: Female, 57 years old, with multiple lung metastases after right pelvic cancer surgery. Pathological diagnosis of urothelial carcinoma, HER2 IHC 3+;

[0116] According to Figure 6, after 12 weeks of treatment, the tumor lesions in the lower lobe of the right lung were reduced from 54 × 31mm (6 weeks of treatment) to 37 × 23mm (12 weeks of treatment), decreased by 49%.

[0117] 2. Patient 01003：Female, 45 years old, with abdominal lymph node metastasis after right pelvic cancer surgery. Pathological diagnosis of urothelial carcinoma, HER2 IHC 3+;

[0118] According to Figure 7, after 6 weeks of treatment, the tumor lesion located beside the right psoas major muscle has been reduced from 56 × 48mm to 33 × 22mm, decreased by 72.9%.

[0119] 3. Patient 01007: Male, 63 years old, with lung metastasis, liver metastasis, cervical lymph node metastasis, mediastinal metastasis and multiple bone metastasis after bladder cancer, and right pelvic cancer surgery. Pathological diagnosis of urothelial carcinoma, HER2 IHC 3+;

[0120] According to Figure 8, it can be seen that after 6 weeks of treatment, the tumor lesions located in the left upper lobe, liver metastasis and mediastinal lymph nodes were significantly reduced. The tumor located in the left upper lobe was reduced from 45 × 36mm to 28×22mm, decreased by 61.9%; the tumor in the liver metastasis site was reduced from 38×32mm to 25×21mm, decreased by 56.8%; and the tumor in the mediastinal lymph nodes was reduced from 29×15mm to 18×7mm, decreased by 71.03%.

[0121] The above clinical data and visual pathological changes have shown that the anti-HER2 monoclonal antibody-MMAE conjugate of the present disclosure has a very significant therapeutic effect. It can also be seen through comparison with the currently marketed drugs that it is significantly better than the current similar drugs approved by the European Union and the United States. For example, Atezolizumab (Roche) has an ORR of only 23% in Phase III clinical data and Nivolumab (Bristol-Myers Squibb) merely 19.6%. Erdafitinib (Jansen) only targets patients with urothelial cancer with certain FGFR gene mutations. In contrast, the ORR of the recombinant humanized anti-HER2 monoclonal antibody-MMAE conjugate of the present disclosure is 76.9%, and the disease control rate (DCR) is 92.3%, which is significantly better than similar drugs currently on the market, and the side effects are also significantly smaller than

similar drug, without any serious adverse effects (SAE). These make it more available to patients, providing another option for patients with urothelial cancer in need of treatment. It can be seen that the anti-HER2 antibody conjugate of the present disclosure has excellent application prospects in the treatment of urothelial carcinoma, can effectively improve, or even reverse the disease development process in patients, and has achieved unexpected technical effects.

CLAIMS

1. Use of an antibody-drug conjugate (ADC) in the preparation of a medicament for treating urothelial carcinoma, wherein the antibody-drug conjugate is an anti-HER2 antibody or a functional fragment thereof coupled to a cytotoxic molecule,

wherein the antibody-drug conjugate is represented by formula Ab-(L-U)n, wherein Ab is the antibody or the functional fragment thereof, L is a linker, U is a conjugated cytotoxic molecule, and n is an integer from 1 to 8, representing the number of therapeutic agent molecules bound to the antibody,

wherein the antibody or the functional fragment thereof is capable of competing with a defined antibody against the same epitope,

wherein the defined antibody comprises a heavy chain variable region and a light chain variable region defined by Complementarity Determining Region (CDR) sequences, and wherein

(i) the heavy chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 1, 2 and 3; and

(ii) the light chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 4, 5 and 6; and

wherein L is Maleimido-Caproyl-valine citrulline p-amino-benzyloxy (mc-vc-pAB), and

U is MMAE.

2. The use according to claim 1, wherein the antibody or the functional fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein

(i) the heavy chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 1, 2 and 3; and

(ii) the light chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 4, 5 and 6.

3. The use according to claim 1 or claim 2, wherein the antibody or the functional fragment thereof is murine, chimeric or humanized.

4. The use according to any one of the preceding claims, wherein the antibody or the functional fragment thereof is derived from the antibody secreted by the hybridoma deposited at the China General Microbiological Culture Collection Center of China Committee for Culture Collection of Microorganisms on August 22, 2013, with an accession number of CGMCC No. 8102.

5. The use according to any one of the preceding claims, wherein the antibody or the functional fragment thereof is derived from the antibody secreted by the CHO cells deposited at the China Center for Type Culture Collection on November 6, 2013, with an accession number of CCTCC C2013170.

6. The use according to any one of the preceding claims, the urothelial carcinoma is selected from the group consisting of locally developed urothelial carcinoma which cannot be removed by surgery, locally advanced or metastatic urothelial carcinoma, HER2-positive urothelial carcinoma and HER2-positive locally advanced or metastatic urothelial carcinoma.

7. The use according to any one of the preceding claims, wherein the medicament further comprises a pharmaceutically acceptable carrier; the medicament is preferably a lyophilized formulation or a liquid formulation; the carrier is one or more selected from the group consisting of a stabilizer, a protective agent, a buffer, a lyoprotectant, an activity protective agent, a surfactant, an adsorption carrier and an absorption promoter.

8. The use according to any one of the preceding claims, the medicament can be administered intranasally, subcutaneously, intradermally, intramuscularly, or intravenously.

9. The use according to claim 6, wherein the urothelial carcinoma is a locally developed urothelial carcinoma which cannot be removed by surgery.

10. The use according to claim 6, wherein the urothelial carcinoma is a locally advanced or metastatic urothelial carcinoma.

11. The use according to claim 6, wherein the urothelial carcinoma is a HER2-positive urothelial carcinoma.

12. The use according to claim 6, wherein the urothelial carcinoma is a HER2-positive locally advanced or metastatic urothelial carcinoma.

USE OF ANTI-HER2 ANTIBODY-DRUG CONJUGATE IN TREATING UROTHELIAL CARCINOMA

ABSTRACT

The present invention relates to the use of an anti-HER2 antibody-drug conjugate (ADC) in preparing a drug for treating urothelial carcinoma. The drug is safe and effective for patients with urothelial carcinoma, especially locally advanced or metastatic urothelial carcinoma, and can effectively prolong the survival time of the patients.

[No suitable figure]