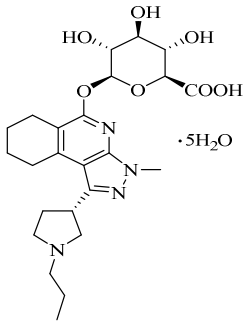
TSL-1502 COMPOUND PHARMACEUTICAL COMPOSITION

Technical Field

The invention is related to a pharmaceutical composition for treating tumor, in particular a pharmaceutical composition consisting of the compound TSL-1502 and temozolomide, cisplatin or carboplatin, irinotecan.

Background Art

TSL-1502 has a chemical name of (2S,3S,4S,5R,6S)-3,4,5-trihydroxy-6-((3-methyl-1-((S)-1-propyl-pyrrolidin-3-yl)-6,7,8,9-tetrahydro-3H-pyrazolo[3,4-c]isoquinolin-5-yl)oxy)tetrahydro-2H-pyran-2-carboxylic acid pentahydrate. TSL-1502 is first found as the compound in paragraph 0177 of the Chinese Patent ZL 201180002886.8 (Publication No. CN102510863A, Authorization No. CN102510863B) with a structural formula as follows.



Formula I

The above patent disclosed in claim 16 the specific tumor type, such as head carcinoma, thyroid carcinoma, neck cancer, eye cancer, skin cancer, oral cancer, throat cancer, esophagus cancer, breast cancer, bone cancer, leukemia, myeloma, lung cancer, colon cancer, carcinoma of sigmoid, rectal cancer, gastric cancer, prostate cancer, breast cancer, ovarian cancer, kidney cancer, liver cancer, pancreatic cancer, brain cancer, intestinal cancer, heart cancer, adrenal carcinoma, subcutaneous tissue cancer, lymph node cancer, malinant melanoma, malignant glioma and the like.

TSL-1502 is a novel oral poly (ADP-ribose) polymerase (PARP) inhibitor, and can inhibit the activity of two important members PARP1 and PARP2 in the PARP protein family at extremely low dosage. It is proposed to be used in the treatment of late-stage

solid tumor. According to the study on the mechanism of in vitro anti-tumor effect, it is indicated that the anti-tumor mechanism of TSL-1502 includes inducing tumor cell DNA damage, cell cycle arrest and cell apoptosis; and can inhibit the DNA damage repair after chemotherapy when used in combination with other cytotoxic anti-tumor drugs, such that the therapeutic effect of chemotherapeutic drugs can be enhanced. In vitro and in vivo anti-tumor spectrum study confirmed that TSL-1502 is more sensitive to various DNA repair deficient tumor (such asBRCA1/2-deficient or mutated tumor), and is a specific anti-tumor and chemotherapy enhancement drug .

The combination therapy of TSL-1502 and other drug was not reported yet. The invention studies the combination therapy of TSL-1502 and other drug, and it is surprisingly found that, the combination therapy of TSL-1502 with temozolomide, cisplatin or carboplatin, irinotecan shows synergistic effect. The invention therefore conducted the following experimental research.

The combination of TSL-1502 + temozolomide for treatingmelanoma; the combination of TSL-1502 + cisplatin, for treating breast cancer; the combination of TSL-1502 + carboplatin, for treating breast cancer; and the combination of TSL-1502 + irinotecan hydrochloride for treating colorectal cancer.

Summary of the Invention

The invention provides a pharmaceutical composition composition comprising TSL-1502 and another anti-tumor drug, wherein the another anti-tumor drug is selected from a group consisting of temozolomide, cisplatin, carboplatin, irinotecan.

The invention encompasses a pharmaceutical preparation composition comprising the two active ingredient TSL-1502 and another anti-tumor drug, or a combination wherein two pharmaceutical preparations individually containing TSL-1502 and another anti-tumor drug are packaged together.

The preparation method of the pharmaceutical preparation composition according to the invention comprises a step of mixing a mixture comprising TSL-1502 and another anti-tumor drug.

Alternatively, the TSL-1502 and another anti-tumor drug are separately prepared into pharmaceutical preparations, and the two preparations are packaged together, such

that the two drugs can be conveniently combined and administered when use. As described, regarding the combination package, TSL-1502 can be prepared into an injectable preparation, and another anti-tumor drug can be prepared into an injectable preparation, and the two unit dose injectable preparations can be packaged together in the same packaging box, and can be injected separately or together when use.

In the pharmaceutical composition of TSL-1502 and another anti-tumor drug according to the invention, the dose of each drug adopts a drug effective amount, and the "effective amount" refers to the amount effective for achieving clinical prevention or treatment of diseases when each drug is applied singly or in combination. For example, the injectable preparationis prepared in unit dose form, such as subpackaging into sterile vials of different colors, different specifications and sizes, each vial containing 0.1-2000mg of pharmaceutically active ingredient, packaging the vials, and placing into a packaging box; and can be further placed into a packaging box capable of containing 2 to 100 bottles for convenient storage and transportation.

The composition according to the invention comprises TSL-1502 and another anti-tumor drug, wherein the weight ratio of the two can be formulated according to each effective amount, such as 1:1000 to 1000:1.

Furthermore, the pharmaceutical composition according to the invention comprises TSL-1502, temozolomide formulated in an effective amount of (5-60):(10-100), preferably (10-40):(40-60); most preferably (10-30):50. The composition is used for treating melanoma.

Furthermore, the pharmaceutical composition according to the invention comprises TSL-1502, cisplatinformulated in an effective amount of (5-50):(2-10), preferably (5-40):(2-8); most preferably (6.25-25):6. The composition is used for treating breast cancer, in particular human breast cancer MX-1 type.

Furthermore, the pharmaceutical composition according to the invention comprises TSL-1502, carboplatinformulated in an effective amount of (10-90):(5-100), preferably (20-70):(50-70), most preferably 25-50:60. The combination is used for treating breast cancer, in particular human breast cancer MX-1 type.

Furthermore, the pharmaceutical composition according to the invention comprises

TSL-1502, irinotecan hydrochloride (CAMPTO) formulated in an effective amount of (5-100):(5-30) 10, preferably (5-60):(5-20), most preferably (5-50):10. The combination is used for treating colorectal cancer.

The preparation method of the pharmaceutical composition according to the invention comprises the following steps of: preparing the required amount of TSL-1502 and another anti-tumor drug together or respectively as pharmaceutical active ingredients into a pharmaceutical composition which can be taken according to the conventional technology of pharmacy, wherein the dosage form comprises an injectable preparation, preferably a dry powder injectable preparation, and particularly preferably a lyophilized injectable preparation.

The injectable preparation of the invention can be prepared without excipients or with one or more pharmaceutically acceptable excipients, such as, glucose, lactose, mannitol, sodium chloride, hydroxypropyl-B-cyclodextrin and the like, by using a proper method.

The use method of the invention comprises the following steps of: preparing the required amount of TSL-1502 and another anti-tumor drug together as two pharmaceutical active ingredients into an injectable preparation for injection. It is also possible to separately prepare the required amount of TSL-1502 and another antitumor drug into injectable preparations for separate injection.

The pharmaceutical preparation composition according to the invention can be in any ingestible pharmaceutical form, such as, tablet, sugar-coated tablet, film-coated tablet, enteric-coated tablet, capsule, hard capsule, soft capsule, oral liquid, buccal tablet, granule, electuary granule, pill, pulvis, ointment, pellet, suspension, powder, solution, injectable preparation, suppository, soft ointment, hard ointment, cream, spray, drop, patch.

The pharmaceutical preparation composition of the invention is preferably in a unit dose form of the pharmaceutical preparation. For example, in the preparation of a medicament, the unit dose of the medicament may contain TSL-1502 of the present invention, and another anti-tumor drug in the range of 0.1 to 1000 mg, the balance being pharmaceutically acceptable excipients. The pharmaceutically acceptable

excipients can be 0.01-99.99% of the total weight of the preparation by weight.

The pharmaceutical preparation composition of the present invention is used in an amount determined according to the condition of a patient, for example, 1 to 3 times a day. 1-20 tablets at a time.

Preferably, the pharmaceutical preparation composition of the invention is an oral preparation or injectable preparation. Among others, the oral preparation is one selected from a group consisting of capsule, tablet, dripping pill, granule, concentrated pill, oral liquid. Among others, the injectable preparation is one selected from a group consisting of liquid, semi-solid, solid, powder form, preferably injectable solution, injectable powder.

In the pharmaceutical preparation composition of the invention, the orally administered preparation may comprise excipients, such as binder, filler, diluent, tableting agent, lubricant, disintegrant, colorant, flavoring agent and wetting agent, and if necessary, the tablet may be coated.

Suitable filler includes cellulose, mannitol, lactose and other similar filler. Suitable disintegrant includes starch, polyvinylpyrrolidinone and starch derivatives, for example, sodium starch glycolate. Suitable lubricant includes, for example, magnesium stearate. Suitable pharmaceutically acceptable wetting agent includes sodium lauryl sulfate.

The pharmaceutical preparations of the present invention can be prepared into solid oral compositions by conventional methods such as mixing, filling, compression, and the like. Repeated mixing allows the active substance to be distributed throughout those compositions that use a large amount of filler.

Oral liquid preparation may be in the form of, for example, aqueous or oily suspension, solution, emulsion, syrup or elixir, or may be in the form of a dried product reformulated before use with water or other suitable carrier. Such liquid preparation may comprise conventional additives, such as suspending agent, for example, sorbitol, syrup, methylcellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel or edible hydrogenated oil, emulsifying agent, for example, lecithin, sorbitan monooleate or arabic gum;

non-aqueous carrier (it can include edible oil), for example, almond oil, fractionated coconut oil, such as oily ester of the glycerol ester, propylene glycol or ethanol; preservative agent, for example, methylparaben or propylparaben or sorbic acid, and if necessary, may comprise conventional flavoring agent or colorant.

For injectable preparation, a liquid unit dosage form is prepared containing the active substance of the present invention and a sterile carrier. Depend on that carrier and the concentration, the compound may be suspended or dissolved. Solutions are typically prepared by dissolving the active substance in a carrier, sterilizing by filtration before loading into a suitable vial or ampoule, and then sealing. Excipients such as a local anesthetic, preservative and buffer may also be dissolved in such a carrier. To improve its stability, the composition may be frozen after vial loading and the water removed under vacuum.

The invention further provides the use of the composition of the present invention in the manufacture of an anti-tumor drug in which the dosage of TSL-1502 and another anti-tumor drug may be from 0.01 mg to 2000 mg per day. The tumor is selected from breast cancer, melanoma, and colorectal cancer.

The application of the invention obtains unexpected technical effects, and application examples are shown in experimental examples of the invention.

Brief Description of The Drawings

Figure 1. The therapeutical effect of TSL-1502, AZD2281 alone or in combination with carboplatin on human breast cancer MX-1 nude mice subcutaneous xenografts;

Figure 2. The effect of TSL-1502, AZD2281 alone or in combination with carboplatin on the body weights of tumor-bearing nude mice, mean±SD; solvent, n=12, treatment group, n=6;

Figure 3. The therapeutical effect of TSL-1502, AZD2281 alone or in combination with carboplatin on human breast cancer MX-1 nude mice subcutaneous xenografts (tumor photo);

Figure 4. The enhancement effect of TSL-1502, AZD2281 on CAMPTO® treated human colorectal cancer SW620 nude mice subcutaneous xenografts;

Figure 5. The effect of TSL-1502, AZD2281 alone or in combination with

CAMPTO□ on the body weights of tumor-bearing nude mice, mean±SD; solvent group, n=12, treatment group, n=6;

Figure 6. The enhancement effect of TSL-1502, AZD2281 on CAMPTO® treated human colorectal cancer SW620 nude mice subcutaneous xenografts (tumor photo);

Figure 7. The enhancement effect of the combination of HD199, veliparib and cisplatin on treated human breast cancer MX-1 nude mice subcutaneous xenografts - average tumor volume (mm^3);

Figure 8. The enhancement effect of the combination of HD199, veliparib and cisplatin on treated human breast cancer MX-1 nude mice subcutaneous xenografts - relative tumor volume RTV (%);

Figure 9. The effect of the combination of HD199, veliparib and cisplatin on the body weights of human breast cancer MX-1 nude mice (BW,g);

Figure 10. The enhancement effect of the combination of HD199, veliparib and temozolomide on treated human melanoma B16F10 nude mice subcutaneous xenografts - average tumor volume (mm^3);

Figure 11. The enhancement effect of the combination of HD199, veliparib and temozolomide on treated human melanoma B16F10 nude mice subcutaneous xenografts - relative tumor volume RTV (%);

Figure 12. The enhancement effect of the combination of HD199, veliparib and temozolomide on treated human melanoma B16F10 nude mice subcutaneous xenografts - relative tumor proliferation rate T/C (%).

Detailed Description of The Invention

The invention is further illustrated below by way of example and not by way of limitation.

Example 1

The invention provides a compositional pharmaceutical lyophilized injectable preparation consisting of TSL-1502 and another anti-tumor drug temozolomide, cisplatin, carboplatin, irinotecan.

Preparation Example:

TSL-1502 20g, and any one of the pharmaceutical drug selected from temozolomide,

cisplatin, carboplatin, irinotecan 10g, were respectively dissolved into a 1000ml aqueous solution containing 100g mannitol, and respectively filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, each one vial was taken to package into combination, such that the product was obtained.

Example 2

TSL-1502 5g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 10g were dissolved together into a 1000ml aqueous solution containing 100g mannitol, mixed, and filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, they were packaged, such that the product was obtained.

or

TSL-1502 5g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 20g were dissolved together into a 1000ml aqueous solution containing 100g mannitol, mixed, and filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, they were packaged, such that the product was obtained.

or

TSL-1502 5g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 50g were dissolved together into a 1000ml aqueous solution containing 100g mannitol, mixed, and filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, they were packaged, such that the product was obtained.

or

TSL-1502 5g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 100g were dissolved together into a 1000ml aqueous solution containing 100g mannitol, mixed, and filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, they were packaged, such that the product was obtained.

or

TSL-1502 10g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 5g were dissolved together into a 1000ml aqueous solution containing 100g mannitol, mixed, and filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, they were packaged, such that the product was obtained.

or

TSL-1502 20g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 5g were dissolved together into a 1000ml aqueous solution containing 100g mannitol, mixed, and filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, they were packaged, such that the product was obtained.

or

TSL-1502 50g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 5g were dissolved together into a 1000ml aqueous solution containing 100g mannitol, mixed, and filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, they were packaged, such that the product was obtained.

or

TSL-1502 100g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 5g were dissolved together into a 1000ml aqueous solution containing 100g mannitol, mixed, and filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, they were packaged, such that the product was obtained.

Example 3

TSL-1502 2g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 1g, were respectively dissolved into a 1000ml aqueous solution containing 100g mannitol, and respectively filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, each one vial was taken to package into combination, such that the product was obtained.

Example 4

TSL-1502 10g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 5g, were respectively dissolved into a 1000ml aqueous solution containing 100g mannitol, and respectively filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, each one vial was taken to package into combination, such that the product was obtained.

Example 5

TSL-1502 5g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 2.5g, were respectively dissolved into a 1000ml aqueous solution containing 100g mannitol, and respectively filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, each one vial was taken to package into combination, such that the product was obtained.

Example 6

TSL-1502 5g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 5g, were respectively dissolved into a 1000ml aqueous solution containing 100g mannitol, and respectively filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, each one vial was taken to package into combination, such that the product was obtained.

Example 7

TSL-1502 50g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 5g, were respectively dissolved into a 1000ml aqueous solution containing 100g mannitol, and respectively filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, each one vial was taken to package into combination, such that the product was obtained.

Example 8

TSL-15025g, and any one of the pharmaceutical drug selected from temozolomide,

cisplatin, carboplatin, irinotecan 50g, were respectively dissolved into a 1000ml aqueous solution containing 100g mannitol, and respectively filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, each one vial was taken to package into combination, such that the product was obtained.

Example 9

TSL-1502 5g, and any one of the pharmaceutical drug selected from temozolomide, cisplatin, carboplatin, irinotecan 10g, were respectively dissolved into a 1000ml aqueous solution containing 100g mannitol, and respectively filled into a 2g vial for 500 vials. Before capping, they were placed into a lyophilizer, and dried in vaccum for 24 hours. After capping, each one vial was taken to package into combination, such that the product was obtained.

The invention is further illustrated below by experimental data.

Experimental Example1

The therapeutical effect of TSL-1502 alone or in combination with carboplatin on human breast cancer MX-1 nude mice subcutaneous xenografts

3 Tested Drug

Drug name and Lot. No. : TSL-1502 is a white powder, purity 99.56%, water content 16.65%, Lot. No. 120301; AZD2281 is a white powder, purity 99.15%, Lot. No. 20131105; carboplatin is a white powder, purity 100.0%, Lot. No. C20141208.

Provider: TSL-1502 and AZD2281 (olaparib)

All provided by Pharmacological and Toxicological Research Center of Research Institute of Tianshili Pharmaceutical Group Co., Ltd.; wherein AZD2281 was purchased from Shanghai Demer Co., Ltd.; carboplatin was provided by in-house laboratory, from Kunming Institute of Precious Metals.

Preparation method: TSL-1502 was prepared with distilled water and diluted to the corresponding concentration. AZD2281 was formulated and diluted with 3% DMA+20% PEG 400+20% propylene glycol +57% normal saline and used as the "solvent"; Carboplatin was temporarily formulated with a 5% glucose solution.

4 Experimental animals

BALB/c nude mice, 6-7 weeks, females, purchased from Shanghai Slack Experimental Animal Co., Ltd.. Production license number: SCXK (Hu) 2012-0002; Animal Certificate No. 2015000526837. Feeding environment: SPF grade.

5 Model and Criteria for dosage selection

The subcutaneous xenografts model of human breast cancer MX-1 in nude mice and the dose of TSL-1502 (25 and 50 mg/kg, PO, BID14) were selected with reference to the in vivo pharmacodynamic study doses of FDA-approved PARP inhibitors Olaparib(LYNPARZA), Rucaparib(RUBRACA) and Niraparib(ZEJULA) [2–4], as well as the experimental results in Chapter 5.×14).

6 Experimental steps

The nude mice were subcutaneously inoculated with human breast cancer MX-1 tumor tissue, and after the tumor grew to 100-200mm^3, the animals were randomly divided into groups (D0). The administered doses and dosing regimens are presented in Table 1.

7 Results

As shown in Table 1 and Figs. 1-3, TSL-1502(25 and 50 mg/kg, PO, BIDx14) dose-dependently inhibited the growth of subcutaneous xenografts of human breast cancer MX-1 in nude mice, with growth inhibition rates of 71% and 91%(D21), respectively. AZD2281(25, 50 mg/kg, PO, BIDx14) was also effective on MX-1, and the growth inhibition rates were 42% and 72%(D21), respectively. Two out of six tumors in the 50 mg/kg group partially resolved. TSL-1502 exerted a stronger therapeutic effect on MX-1 than AZD2281, and there was a statistically significant difference between the 25 mg/kg groups ($P<0.05$), but there was no statistically significant difference between the 50 mg/kg groups ($P=0.057$).

The growth inhibition rate of carboplatin (60 mg/kg, IP, Q4D×3) on MX-1 was 149%, and two out of six tumors partially resolved and three out of six tumors completely resolved (D21). By the end of the experiment (D37), two out of six tumors still partially resolved and one out of six tumors completely resolved. When TSL-1502 25 mg/kg was combined with carboplatin 60 mg/kg, the growth inhibition rate was significantly increased, from 149% of carboplatin alone to 200%. All tumors (6/6)

completely resolved. When AZD2281 25 mg/kg was combined with carboplatin 60 mg/kg, all tumors also completely resolved (D21). By the end of the experiment (D37), no tumor recurrence was observed in the combination group. The therapeutical effect of either TSL-1502 or AZD2281 in the combination group was significantly better than that of carboplatin alone group ($P<0.05$), suggesting that both drugs had significant enhancement effects on carboplatin. The tumor-bearing mice could tolerate the above drugs well, and the maximum weight loss was 3.8% (D6) in the TSL-1502 combined with carboplatin group and 9.3%(D9) in the AZD 2281 combined with carboplatin group. The body weight could be gradually recovered after drug discontinuation. Other drugs did not cause significant weight loss in mice. In comparison, the efficacy of TSL-1502 on MX-1 was significantly stronger than that of reference drug AZD2281. Both drugs can significantly enhance the therapeutical effect of carboplatin in the treatment of MX-1.

Table 1. The therapeutical effect of TSL-1502, AZD2281 alone or in combination with carboplatin on human breast cancer MX-1 nude mice subcutaneous xenografts

			Average Tumor Volume (mm^3)		Average Tumor Volume (mm^3)		% T/C	% inhibition	P value	Partially	Completely	Experimental Start	Experimental End
Groups	Dose	Route	D0	SEM	D21	SEM	D21	D21	D21	Resolved	Resolved	Animals per Group	Animals per Group
Solvent	D0-13, BID	PO	162.0	±3.4	1080.2	±73.6	-	-	-	0	0	12	12
TSL-1502 25mg/kg	D0-13, BID	PO	156.5	±10.0	420.1	±64.9	29	71 *	0.000	0	0	6	6
TSL-1502 50mg/kg	D0-13, BID	PO	159.3	±6.6	237.9	±46.4	9	91	0.000	2	0	6	6
AZD2281 25mg/kg	D0-13, BID	PO	155.0	±5.2	687.0	±98.1	58	42	0.007	0	0	6	6
AZD2281 50mg/kg	D0-13, BID	PO	153.0	±5.7	418.4	±92.1	29	71	0.000	0	0	6	6
Carboplatin 60mg/kg	D0,4,8	IP	165.6	±8.2	84.5	±60.3	-49	149	0.000	2	3	6	6
TSL-1502	D0-13,	PO/IP	154.6	±7.5	0.0	±0.0	-100	200	0.000	0	6	6	6

25mg/kg+ Carboplatin 60mg/kg	BID/ D0,4,8												
AZD2281 25mg/kg+ Carboplatin 60mg/kg	D0-13, BID/ D0,4,8	PO/IP	162.5	±4.5	0.0	±0.0	-100	200	0.000	0	6	6	6

D0: Time for First Dose; P Value refers to the relative to solvent; *P<0.05, compared with AZD2281 25 mg/kg; Using Student's t test.

Table 1-2 King's formula calculation on the Q value of the combination of the two drugs TSL-1502 and carboplatin (Q≥1.15 indicates synergy)

Dose (mg/kg)		Q	Synergistic effect
TSL-1502	carboplatin		
25	60	1.75	Yes

Figure 1. The therapeutical effect of TSL-1502, AZD2281 alone or in combination with carboplatin on human breast cancer MX-1 nude mice subcutaneous xenografts

Figure 2. The effect of TSL-1502, AZD2281 alone or in combination with carboplatin on the body weights of tumor-bearing nude mice. mean±SD; solvent, n=12, treatment group, n=6.

Figure 3. The therapeutical effect of TSL-1502, AZD2281 alone or in combination with carboplatin on human breast cancer MX-1 nude mice subcutaneous xenografts (tumor photo) .

Experimental Example2

The enhancement effect of TSL-1502 on irinotecan hydrochloride treated human colorectal cancer SW620 nude mice subcutaneous xenografts

3 Tested Drug

Drug name and Lot. No. : TSL-1502 is a white powder, purity 99.56%, water content 16.65%, Lot. No. 120301; AZD2281 is a white powder, purity 99.15%, Lot. No. 20131105; CAMPTO® (irinotecan hydrochlorideinjectable solution), is a light yellow

clear liquid, 2ml:40mg/bottle, Lot. No. JM85B, Production Date: 2015/02, Effective Date 2018/01.

Provider: TSL-1502, AZD2281 all provided by Pharmacological and Toxicological Research Center of Research Institute of Tianshili Pharmaceutical Group Co., Ltd.; wherein AZD2281 was purchased from Shanghai Demer Co., Ltd.; CAMPTO® was provided by in-house laboratory, produced by Pfizer.

Preparation method: TSL-1502 was prepared with distilled water and diluted to the corresponding concentration. AZD2281 was formulated and diluted with 3% DMA+20% PEG 400+20% propylene glycol +57% normal saline; CAMPTO® diluted with normal saline.

4 Experimental animals

BALB/cA-nude mice, 5-6 weeks, females, purchased from Shanghai Lingchang Biotechnology Co., Ltd. Production license number: SCXK (Hu) 2013-0018; Animal Certificate No. 2013001817948. Feeding environment: SPF grade.

5 Model and Criteria for dosage selection

In reference to the in vivo pharmacodynamic study doses of the FDA-approved marketed PARP inhibitors Olaparib (olaparib), Rucaparib (RuCappagli) and Niraparib (NiRapali) [2–4], and the experimental results in Chapter 5, the human colorectal cancer SW620 nude mouse subcutaneous xenografts model and the dosing regimen of TSL-1502(50 mg/kg, PO, BIDx17) in combination with CAMPTO® (10 mg/kg, IP, D0,4) were selected.

6 Experimental steps

The nude mice were subcutaneously inoculated with human colorectal cancer SW620 cells, and after the tumor grew to 100-200mm^3, the animals were randomly divided into groups (D0). The administered doses and dosing regimens are shown in Table 2.

7 Results

As shown in Table 2 and Fig. 4-5, TSL-1502(50 mg/kg, PO, BID17) had no significant tumor inhibition on the growth of subcutaneous xenografts of human colorectal cancer SW620 in nude mice, with the growth inhibition rate of -5%; AZD2281(30 mg/kg, PO, QDx17) also had no significant effect on SW620, and the

growth inhibition rate was 6%. The growth inhibition rate of CAMPTO® (10 mg/kg, IP, D0,4) on the SW620 is 47%; The combination of TSL-1502(5, 15, and 50 mg/kg, PO, BIDx5) with gemcitabine significantly enhanced the efficacy of gemcitabine on SW620, with growth inhibition rates increasing from 47% to 79%, 82%, and 89%($P<0.05$ or $P<0.01$, compared with gemcitabine alone), respectively, without significant increase in toxicity. AZD2281(30 mg/kg, PO, QDx5) also significantly increased the efficacy of CAMPTO® on SW620, with the growth inhibition rate increasing to 89%, and 1/6 of the tumors partially resolved. All the above drugs were well tolerated by tumor-bearing mice, and no such symptoms as significant weight loss occurred. The results showed that, according to the current dosing regimen, TSL-1502 and AZD2281 had no significant effect on the subcutaneous xenografts of SW620 nude mice. However, they significantly enhanced the therapeutic effect of CAMPTO® on SW620 without significantly increasing its toxicity.

Table 2. The enhancement effect of TSL-1502, AZD2281 on CAMPTO® treated human colorectal cancer SW620 nude mice subcutaneous xenografts.

			Average Tumor Volume (mm^3)		Average Tumor Volume (mm^3)		% T/C	% inhibition	P value	Partially	Experimental End
Groups	Dose	Route	D0	SEM	D17	SEM	D17	D17	D17	Resolved	Animals per Group
Solvent	D0-4	IV	117.7	±2.8	1231.9	±72.3	-	-	-	0	12
AZD2281 30mg/kg+ CAMPTO® 10mg/kg	D0-4/D0,4	PO/IP	123.6	±6.0	244.8	±35.8	11	89 **	0.000	1	6
AZD2281 30mg/kg	D0-16	PO	116.7	±4.1	1164.3	±153.6	94	6	0.653	0	6
CAMPTO® 10mg/kg	D0,4	IP	120.3	±4.8	716.1	±110.5	53	47	0.001	0	6
TSL-1502 5mg/kg+ CAMPTO®	D0-4/D0,4	PO, BID/IP	115.2	±5.5	351.5	±42.1	21	79 *	0.000	0	6

10mg/kg											
TSL-1502 15mg/kg+ CAMPTO® 10mg/kg	D0-4/D0,4	PO, BID/IP	123.6	±3.4	320.6	±65.9	18	82 **	0.000	0	6
TSL-1502 50mg/kg+ CAMPTO® 10mg/kg	D0-4/D0,4	PO, BID/IP	116.2	±2.6	233.7	±22.4	11	89 **	0.000	0	6
TSL-1502 50mg/kg	D0-16	PO,BID	122.2	±4.6	1292.3	±177.9	105	-5	0.731	0	6

D0: Time for First Dose; P Value refers to the relative to solvent, #P<0.05; **P<0.01, *P<0.05 compared with CAMPTO® 10 mg/kg group; all using Student's t test. Number of mice at the experimental start: solvent group n=12, treatment group n=6.

Table 2-2 King's formula calculation on the Q value of the combination of the two drugs TSL-1502 and CAMPTO (Q≥1.15 indicates synergy)

Dose (mg/kg)		Q	Synergistic effect
TSL-1502	CAMPTO		
50	10	2.01	Yes

Figure 4. The enhancement effect of TSL-1502, AZD2281 on CAMPTO® treated human colorectal cancer SW620 nude mice subcutaneous xenografts

Figure 5. The effect of TSL-1502, AZD2281 alone or in combination with CAMPTO® on the body weights of tumor-bearing nude mice. mean±SD; solvent group, n=12, treatment group, n=6.

Figure 6. The enhancement effect of TSL-1502, AZD2281 on CAMPTO® treated human colorectal cancer SW620 nude mice subcutaneous xenografts (tumor photo).

Experimental Example3

Pharmacodynamic study of TSL-1502 combine with cisplatin in MX-1 xenografts model of human breast cancer

2.1 Cell line:

Human breast cancer cell line MX-1 was purchased from the cell bank of the Chinese Academy of Sciences.

2.2 Reagent

Fetal bovine serum (Gibco, Lot NO: 623311), RPM1640 medium (Gibco, Lot NO: 869317), L15 medium (Gibco, Lot NO: 810875), trypsin (Sigma Lot NO:27250018), penicillin G sodium salt (Shandong Lu Kang Medicine Lot NO:B100122), Streptomycin sulfate (Shandong Lu Kang Medicine Lot NO:080307), 0.25%EDTA(Trypsin (trypsin) LOT No: 252000 - 056), Hanks(NO 14170112), NaHCO3 (Chengdu Kelong LOT No.201010 - 13), HCL (Nanjing Chemical Reagent Co., Ltd.); Matrigel (matrix) (BD Lot NO:20110068), trypan blue, DMSO, PEG400, Tw-80, 0.9% sodium chloride solution, PBS powder (or NaCl, KCl, NaH2PO4, KH2PO4), etc.

2.3 Instruments:

5%CO2 cell thermostatic incubator (ThermoForma, the US), clean bench (Suzhou Purification), alcohol cotton ball, alcohol lamp, pipette, culture bottle, centrifuge tube, cryopreservation tube, glass bottle (containing culture medium), cell counting plate, cover glass, cell culture dish, cell culture dish, pressure sterilizer, inverted microscope, centrifuge, liquid nitrogen, autoclave, electronic balance, PH meter, vernier caliper, magnetic stirrer, 100mL volumetric flask, 5mL volumetric flask, 1000mL volumetric flask, 1mL syringe, gavage needle, centrifuge tube

2.4 Animals:

Sixty Balb/c female nude mice (SPF grade), 4-6 weeks old, weighing 18-22g, were purchased from Shanghai Sciple-Bikai Experimental Animal Co., Ltd. Animal production license No. was Scxk (Hu) 2008-0016, and certificate No. was 2010001601905.

2.5 Test drugs:

Test drug: TSL-1502, synthesized in the laboratory of Suzhou Henderson Pharmaceutical Technology Co., Ltd. (Batch No.: 100301), was dissolved in normal

saline. DDP (cisplatin): purchased from Shandong Platinum Source Medicine Technology Co., Ltd., and dissolved in normal saline.

Veliparib CAS:912444-00-9, purchased from Shanghai Haoyuan Chemical Technology Co., Ltd., Batch No.: HM-0698-2010112, dissolved in normal saline

3 Method Methodology

3.1 Cell culture:

The MX-1 cell lines were placed at-80 C for cryopreservation after purchase, and the cryopreservation solution was fetal bovine serum: DMSO = 9:1. After cell resuscitation, the cells were routinely cultured in RPMI-1640 complete culture medium (containing 10% fetal bovine serum, 1%(W/V) penicillin and 1%(W/V) streptomycin, of which fetal bovine serum was imported); the cells were cultured at 37 C in 5% CO2 incubator with relative saturated humidity; the growth condition was observed every day; the cells were passed on to the next generation after attachment for 2–3 days, and then subjected to passage amplification. The collected exponentially proliferating cells were digested with 0.25% trypsin +0.53mMol/L EDTA, collected in incomplete culture medium free of calf serum and gently shaken to obtain cell suspension, which was detected for cell viability (more than 95%) by cell count and trypan blue staining.

3.2 Modeling of tumor-bearing mouse:

Balb/c female nude mice (SPF grade), which are hairless and thymless congenital T cell immunodeficiency animals, lack immunologic rejection, grow well after tumor transplantation, and can maintain the original morphology of tumor cells as well as cell kinetics and biological characteristics, are the ideal and commonly used animals in tumor research. They were 4-6 weeks old, weighing 18-22g, and 60 animals. The skin at the inoculation site was disinfected with 75% alcohol. The inoculation site of the heterotopic xenografts was the groin of the left lower limb, and that of the orthotopic xenografts was the fat pad of the left second papilla of the nude mouse (as shown in the figure below). The cell inoculation cell count was 0.5×10^{7}/ ml, and the cell inoculation dose was 0.2ml(1×10^{6} cells), respectively. The cell strain was mixed with 50% matrix for inoculation (more matrix contributes to the regular shape of

tumor growth). The tumor growth was observed every day, and the tumor size (length and width) volume (length x width^2/2) was recorded. The standard deviation was calculated. After inoculation for about 14–18 days, the tumor tissue length should be about 100mm^3.

3.3 Solution Preparation

Blank solution: Add 0.9g NaCl in a 100mL volumetric flask, dissolve in distilled water, and shake to prepare 0.9% NaCl solution, and store at room temperature for subsequent use.

Cisplatin Solution: An accurate 6mg sample of cisplatin (DDP) was placed in a 100 mL volumetric flask and fixed in a 0.6 mg/ml DDP solution using 0.9% NaCl.

Veliparib solution: Accurately weigh 25mg of a veliparib sample into a 100 mL volumetric flask and dilute to 2.5 mg/ml with 0.9% NaCl.

TSL-1502 Solution: Accurately weigh 25, 12.5, and 6.25mg of Compound TSL-1502, respectively, into a 100-mL volumetric flask and dilute to 2.5, 1.25, and 0.625mg/ml TSL-1502 solutions with 0.9% NaCl.

3.4 Tumor-bearing mice grouping and dosing regimen:

Grouping of tumor-bearing mice and dosing scheme: If the tumor size of the nude mice reached about 100mm^3 (the time was about the 14th day), the nude mice with large or small tumors were removed, and the nude mice with good tumor size consistency were selected and randomly grouped into the following six groups, with at least six mice in each group.

Blank control group (0.1ml/10g, po (oral), Bid (twice a day)), after grouping, continously administered with oral gavage normal saline for nine days, wherein the third and sixth days were given intraperitoneal injection of normal saline, with continous observation for 40 days;

Cisplatin monotherapy group (6mg/kg, ip (intravenous injection), qd (once a day)), after grouping, continously administered with oral gavage normal saline for nine days, wherein the third and sixth days were given intraperitoneal injection of cisplatin solution, with continous observation for 40 days;

veliparib dosing group (25mg/kg, po (oral), Bid (twice a day)) + cisplatin (6mg/kg, ip

(intravenous injection), qd (once a day)), after grouping, continously administered with oral gavage veliparib solution for nine days, wherein the third and sixth days were given intraperitoneal injection of cisplatin solution, with continous observation for 40 days;

TSL-1502 dosing group (6.25mg/kg, po, Bid) + cisplatin (6mg/kg, ip, qd), continously administered with oral gavage TSL-1502 solution for nine days, wherein the third and sixth days were given intraperitoneal injection of cisplatin solution, with continous observation for 40 days;

TSL-1502 dosing group (12.5mg/kg, po, Bid) + cisplatin (6mg/kg, ip, qd), continously administered with oral gavage TSL-1502 solution for nine days, wherein the third and sixth days were given intraperitoneal injection of cisplatin solution, with continous observation for 40 days;

TSL-1502 dosing group (25mg/kg, po, Bid) + cisplatin (6mg/kg, ip, qd), continously administered with oral gavage TSL-1502 solution for nine days, wherein the third and sixth days were given intraperitoneal injection of cisplatin solution, with continous observation for 40 days;

3.5 Detection indicator and method:

3.5.1 Tumor volume and tumor growth profile:

The tumor volume of nude mice was detected every other day. The tumor volume was measured by measuring the length and width of the tumor tissue with a vernier caliper (assuming that the tumor tissue was elliptical). The volume was calculated using the length × width^2/2 formula, and the growth inhibition rate and relative tumor volume (RTV) were calculated. Vt: the tumor volume obtained by measuring the tumor every day, and V0: the initial tumor volume (before drug administration).

3.5.2 Relative tumor volume (relative tumor volume, RTV), with a calculation formula as:

$$RTV = V_t/V_{initial}$$

wherein $V_{initial}$ is the tumor volume when administering after grouping, and V_t is the tumor volume measured each time.

3.5.3 Relative tumor proliferation rate T/C (%), with a calculation formula as:

$$T/C\ (\%) = (T_{RTV} / C_{RTV}) \times 100\%$$

3.5.4 Tumor volume growth inhibition rate (GI)

$$GI = [1 - (TV_t - TV_{initial}) / (CV_t - CT_{initial})] \times 100\%$$

wherein TV_t is referred to the tumor volume measured each time for the treatment group;

$TV_{initial}$ is referred to the tumor volume measured when administering after grouping for the treatment group;

CV_t is referred to the tumor volume measured each time for the control group;

$CT_{initial}$ is referred to the tumor volume measured when administering after grouping for the control group;

3.5.5 Body weight change profile of tumor-bearing mice:

When measuring the volume of tumor-bearing mice and the body weight of the mice, a body weight change profile of the tumor-bearing mice was drawn, and the body weight change trend of the tumor-bearing mice was observed, and the change effect of the compound on the body weight of the tumor-bearing mice was evaluated;

3.6 Statistical analysis:

Measurement data such as tumor volume, tumor weight and tumor-bearing mice tumor were expressed as $\bar{x}\pm S$. Excel software or SPSS 12. 0 software was used for statistical analysis, and t test was used for statistical method.

3.7 Experimental results analysis

3.7.1 Analyze the tumor volume of each dosing group after administration, and draw the pharmacodynamic profile of time-volume change;

3.7.2 Analyze the relative proliferation rate of tumors in each dosing group after administration, and draw the profile of time–relative proliferation rate of tumors;

3.7.3 Analysis of tumor size inhibition after administration of each dosing group, and calculation of growth inhibition rate;

3.7.4 The weight of tumor-bearing mice in each dosing group was analyzed, and the body weight-time profile was drawn.

4 Results

4.1 The effect of TSL-1502 in combination with DDP on the growth inhibition rate of human breast cancer MX-1 xenografts in tumor-bearing mice:

Compared with the blank control group, the growth inhibition rate of cisplatin (6mg/kg) alone reached 100.33% on the d26 day of inoculation. On the 50th day of inoculation, the growth inhibition rates of TSL-1502(6.25, 12.5 and 25mg/kg) combined with cisplatin relative to cisplatin were 32%, 82% and 86%, respectively. The growth inhibition rate of veliparib (25mg/kg) combined with cisplatin at the same dose was 61%, which was significantly lower than that of TSL-1502($P<0.01$), as shown in Table 3 and Fig. 7.

Table 3. The effect of the combination of TSL-1502 or veliparib and carboplatin on the growth inhibition rate (GI)

Groups	Dose (mg/kg/d)	Tumor volume (day 26)	Growth inhibition rate (%) (day 26, compared with blank control group)	Tumor volume (day 50)	Growth inhibition rate (%) (day 50, compared with DPP group)
Blank control group	0/0	6445±1428	—	—	—
DPP(Control group)	0/6	82±21	100.33**	4626	—
veliparib + DDP	25.0/6	55±13	100.84**	1894	60.53#
TSL-1502 + DDP	6.25/6	59±15	100.75**	3191	31.79
	12.5/6	58±10	100.74**	916	82.06##
	25.0/6	54±8.7	100.81**	732	86.16##

Compared with the blank control group, * $p < 0.05$, ** $p < 0.01$; Compared with the control of DDP group: #$P<0.05$, ##$P<0.01$.

4.2 The effect of TSL-1502 in combination with DDP on the relative tumor proliferation rate (T/C, %) of human breast cancer MX-1 xenografts in tumor-bearing mice:

Compared with the blank control group, the relative tumor proliferation rate reached to T/C(%)1.34% on the day d26 when cisplatin (6mg/kg) was used alone. On the day

50, the T/C% relative to cisplatin after the combination of TSL-1502(6.25, 12.5, and 25mg/kg) with cisplatin was 67.24%, 19.60%, and 15.50%, respectively. The T/C% relative to cisplatin after the same dose of veliparib (25mg/kg) with cisplatin was 39.10%, as shown in Table 4 and 5, Fig. 8. For human cancer xenografts model, the relative tumor proliferation rate T/C(%) is recommended as the test evaluation index. In principle, the evaluation criteria are as follows: T/C(%)>40% is effectless; T/C(%)≤40% and $P < 0.05$ was considered as effective after statistical analysis. From the results, TSL-1502 in combination with cisplatin showed a very significant inhibition of breast cancer tumors compared to cisplatin alone.

Table 4. Changes in relative tumor proliferation rate (T/C%) in tumor-bearing mice during dosing

Groups	d14	d16	d18	d20	d22	d24	d26	d28	d30	d32
Control group, DDP(6mg/kg)	99.99	109.12	29.74	9.45	3.65	1.93	1.34			
veliparib(25mg/kg) + DDP	100.01	116.26	33.45	11.05	3.97	1.80	0.86	46.15	24.35	12.35
TSL-1502(6.25mg/kg) + DDP	100.00	123.69	34.21	10.18	3.82	1.85	0.93	53.86	30.70	19.10
TSL-1502(12.5mg/kg) + DDP	100.00	117.74	33.35	10.81	4.10	1.87	0.93	47.69	23.00	12.73
TSL-1502(25mg/kg) + DDP	100.00	112.61	31.75	10.24	3.87	1.75	0.87	44.37	21.29	11.75
Groups	d34	d36	d38	d40	d42	d44	d46	d48	d50	
Control group, DDP(6mg/kg)										
veliparib(25mg/kg) + DDP	7.61	7.37	8.08	9.22	11.57	16.20	22.23	30.55	39.10	
TSL-1502(6.25mg/kg) + DDP	13.68	16.20	21.23	25.34	32.16	39.18	46.50	57.67	67.24	
TSL-1502(12.5mg/kg) + DDP	7.08	4.96	4.18	3.93	5.12	6.98	10.02	14.25	19.60	
TSL-1502(25mg/kg) + DDP	6.54	4.60	3.87	3.66	4.82	6.63	9.08	11.93	15.50	

Table 5. Summary of growth inhibition rate (GI,%) and relative tumor proliferation rate (T/C,%) in tumor-bearing mice during dosing

Groups	Dose (mg/kg/d)	RTV(%) (day 26)	Growth inhibition rate (GI,%) (day 26, compared with blank control)	T/C%	RTV(%) (day 50)	Growth inhibition rate (GI,%) (day 50, compared with DDP group)	T/C%
Blank control	--	5970.33	—	—	—	—	—

Control group, DDP	6	79.83***	100.33	1.34	4451.17	—	—
veliparib + DDP	25.0/6	51.31	100.84	0.86	1740.62##	60.53	39.10
TSL-1502 + DDP	6.25/6	55.53	100.75	0.93	2992.98#	31.79	67.24
	12.5/6	55.47	100.66	0.93	872.52###	82.06	19.60
	25.0/6	51.81	100.79	0.87	690.02###	86.16	15.50

Compared with the Control group, *p < 0.05, ** p < 0.01; Compared with the control of DDP group: #P<0.05, # # P < 0.01;

4.4 Effects of TSL-1502 combined with DDP on body weight of tumor-bearing mice:

Compared with the blank control, TSL-1502 combined with DDP had a certain effect on the body weight of tumor-bearing mice at the early stage of drug administration, but after continuous administration for more than 40 days, the body weight of animals in each dose combination group was not significantly different from that in the DDP alone group (P>0.05). See Table 6 and Figure 9. These results indicated that there was no significant increase in toxicity of TSL-1502 in combination with DDP when compared to DDP alone.

Table 6. Change in average body weight (BW) of tumor-bearing mice during dosing

Groups	d14	d16	d18	d20	d22	d24	d26	d28	d30	d32
Blank control	19.28	20.06	20.13	21.10	22.28	22.69	23.05			
Control group, DDP(6mg/kg)	19.99	20.79	20.28	19.84	18.24	19.20	19.04	19.60	20.24	20.93
veliparib(25mg/kg) + DDP(6mg/kg)	20.23	20.60	20.29	19.78	19.73	19.75	19.51	19.19	19.86	19.96
TSL-1502(6.25mg/kg) + DDP(6mg/kg)	19.74	20.26	19.90	19.19	18.98	19.04	19.29	19.55	19.75	19.98
TSL-1502(12.5mg/kg) + DDP(6mg/kg)	19.53	19.03	17.79	16.44	16.00	16.03	16.10	16.28	16.61	16.55
TSL-1502(25mg/kg) + DDP(6mg/kg)	20.81	20.44	18.75	17.11	16.58	16.38	16.20	16.08	16.33	16.70
Groups	d34	d36	d38	d40	d42	d44	d46	d48	d50	
Blank control										
Control group, DDP(6mg/kg)	21.31	21.94	22.51	22.84	23.08	23.54	24.01	25.22	26.08	
veliparib(25mg/kg) + DDP(6mg/kg)	19.94	19.89	20.19	20.49	20.61	20.19	20.16	20.69	20.97	
TSL-1502(6.25mg/kg) + DDP(6mg/kg)	20.29	20.70	21.19	21.64	22.78	23.41	24.33	24.79	25.74	
TSL-1502(12.5mg/kg) + DDP(6mg/kg)	16.46	16.46	16.99	17.26	18.16	19.51	21.29	22.83	24.28	
TSL-1502(25mg/kg) + DDP(6mg/kg)	16.48	16.50	17.03	17.19	18.09	18.89	20.89	22.53	23.80	

.Reference:

[1].Cherrie K. Donawho, Yan Luo, et al. ABT-888, an Orally Active Poly

(ADP-Ribose) Polymerase Inhibitor that Potentiates DNA-Damaging Agents in Preclinical tumor Models. Clin Cancer Res 2007;13(9) May 1, 2007.

Experimental Example 4

Pharmacodynamic study of TSL-1502 in combination with TMZ in B16F10 melanoma xenografts model

2.1 Cell line:

The melanoma cell line B16F10 was mainly purchased from the cell bank of the Chinese Academy of Sciences.

2.2 Reagent

Fetal bovine serum (Gibco, Lot NO: 623311), RPM1640 medium (Gibco, Lot NO: 869317), L15 medium (Gibco, Lot NO: 810875), trypsin (Sigma Lot NO:27250018), penicillin G sodium salt (Shandong Lu Kang Medicine Lot NO: B100122), Streptomycin sulfate (Shandong Lu Kang Medicine Lot NO:080307), 0.25%EDTA (trypsin Lot NO: 25200056), Hanks(NO: 14170112), NaHCO3 (Chengdu Kelong Lot NO:20101013), HCL (Nanjing Chemical Reagent Co., Ltd.); Matrigel (matrix) (BD Lot NO:20110068), trypan blue, DMSO, PEG400, Tween -80, 0.9% sodium chloride solution, PBS powder bag (or NaCl, KCl, NaH2PO4, KH2PO4), etc.

2.3 Instruments:

5%CO2 cell thermostatic incubator (ThermoForma, the US), clean bench (Suzhou Purification), alcohol cotton ball, alcohol lamp, pipette, culture bottle, centrifuge tube, cryopreservation tube, glass bottle (containing culture medium), cell counting plate, cover glass, cell culture dish, cell culture dish, pressure sterilizer, inverted microscope, centrifuge, liquid nitrogen, autoclave, electronic balance, PH meter, vernier caliper, magnetic stirrer, 100mL volumetric flask, 5mL volumetric flask, 1000mL volumetric flask, 1mL syringe, gavage needle, centrifuge tube

2.4 Animals:

Seventy C57BL/6 mice (SPF grade), half male and half female, aged 6-8 weeks, weighing 22-25g, were purchased from Shanghai Sciple-Bikai Experimental Animal Co., Ltd. The production license number of experimental animals was Scxk(Hu)2008 -0016, and the qualification certificate number was 2008001608319.

2.5 Test drugs:

TSL-1502, synthesized in the laboratory of Suzhou Henderson Medical Technology Co., Ltd., Batch No.100301, was dissolved in normal saline.

TMZ: purchased from jiangsu hengrui Pharmaceutical Technology Co., Ltd., batch No.2010805, and dissolved in normal saline.

Veliparib was purchased from Shanghai Haoyuan Chemical Technology Co., Ltd., Batch No.: HM-0698-2010112, and dissolved in normal saline.

3 Method Methodology

3.1 Cell culture:

The B16F10 cell lines were placed at-80 C for cryopreservation after purchase, and the cryopreservation solution was fetal bovine serum: DMSO = 9: 1. After cell resuscitation, the cells were routinely cultured in RPMI-1640 complete culture medium (containing 10% calf serum, 1%(W/V) penicillin and 1%(W/V) streptomycin), and then placed in 5% CO2 incubator at 37 C. The growth condition was observed under relative saturated humidity every day. The cells were transferred to next generation after attachment for 2–3 days, and then subjected to passage amplification. The cells in the exponential proliferation phase were collected and digested with 0.25% trypsin and 0.53 mmol/L EDTA. The cells were collected in the incomplete culture medium free of calf serum and gently shaken to obtain cell suspension, and the cell viability (more than 95%) was detected by cell count and trypan blue staining.

3.2 Construction of tumor-bearing mouse model:

Fifteen inbred C57BL/6 female mice of SPF (specific pathogen-free) class closed group, aged 6–8 weeks, weighing about 22 g each, were randomly divided into three groups, five for each group. The abdominal skin was disinfected with 75% alcohol, and the B16F10 melanoma cell solution was inoculated on the left first Flank of the mice, with the cell concentration of $5x10^6$/mL for each group. The doses of inoculation in the three groups were 0.2mL (inoculation of $1x10^6$ cells), 0.05mL (inoculation of $2.5x10^5$ cells) and 0.012mL (inoculation of $6x10^4$ cells), and the cells of each group were mixed with 50% matrix for inoculation (adding a little more

matrix contributes to the regular shape of tumor growth). The tumor growth was observed every day, and the volume of tumor size (length x width^2/2) was recorded. The standard deviation was calculated. The tumor volume should be about 100–200 mm^3 within 8–10 days of inoculation.

3.3 Solution Preparation

Blank Solution: Add 0.9g NaCl in a 100mL volumetric flask, dissolve in distilled water, and shake to prepare 0.9% NaCl solution, and store at room temperature for subsequent use.

TMZ Solution: Accurately weigh 50mg of temozolomide (TMZ) into a 100-mL volumetric flask and dilute to 5.0 mg/ml with 0.9% NaCl.

Veliparib Solution: Accurately weigh 25mg of a veliparib sample into a 100 mL volumetric flask and dissolve in 0.9% NaCl solution to obtain a 2.5 mg/ml veliparib solution.

TSL-1502 Solution: Accurately weigh 30, 15, and 10mg of the Henderson compound, TSL-1502, respectively, into a 100-mL volumetric flask and dilute to 3.0, 1.5, and 1.0mg/ml, respectively, with 0.9% NaCl.

3.4 Tumor-bearing mice grouping and dosing regimen:

Grouping of tumor-bearing mice and dosing regimen: SPF (specific pathogen-free) grade C57BL/6 female mice, aged 6–8 weeks, with 70 mice weighing about 22 g each, were disinfected with 75% alcohol on the abdomen skin, and the B16 F10 melanoma cell solution was inoculated on mouse Flank, respectively. The inoculation protocol selected the above optimal conditions. If the tumors were round or elliptical in shape, and the tumors of B6 mice grew to about 100mm^3 (on the 10th day after inoculation), the nude mice with large or small tumors were removed, and the nude mice with good tumor size consistency were selected and randomly divided into six groups, eight for each group.

Blank control group (0.1ml/10g, po, Bid):after grouping, continously administered with oral gavage normal saline for nine days, wherein on days 2, 3, 4, 5, and 6 respectively, normal saline was administered with oral gavage once, for continuous observation for 23 days;

temozolomide(TMZ)monotherapy group (50mg/kg, po, qd):after grouping, continously administered with oral gavage normal saline for nine days, wherein on days 2, 3, 4, 5, and 6, temozolomide solution was administered with oral gavage, for continuous observation for 23 days;

veliparib dosing group (25mg/kg, po, Bid) + temozolomide(50mg/kg, po, qd):after grouping, continously administered with oral gavage veliparib solution for nine days, wherein on day 2 to day 6 for continuous 5 days, temozolomide solution was administered with oral gavage, for continuous observation for 23 days;

TSL-1502 dosing group (10mg/kg, po, Bid) + temozolomide(50mg/kg, po, qd):after grouping, continously administered with oral gavage TSL-1502 solution for nine days, wherein on days 2, 3, 4, 5, and 6 temozolomide solution was administered with oral gavage , for continuous observation for 23 days;

TSL-1502 dosing group (15mg/kg, po, Bid) + temozolomide(50mg/kg, po, qd):after grouping, continously administered with oral gavage TSL-1502 solution for nine days, wherein on days 2, 3, 4, 5, and 6 temozolomide solution was administered with oral gavage , for continuous observation for 23 days;

TSL-1502 dosing group (30mg/kg, po, Bid) + temozolomide(50mg/kg, po, qd):after grouping, continously administered with oral gavage TSL-1502 solution for nine days, wherein on days 2, 3, 4, 5, and 6 temozolomide solution was administered with oral gavage , for continuous observation for 23 days;

3.5 Detection indicator and method:

3.5.1 Tumor volume and tumor growth profile:

The tumor volume of nude mice was detected every other day. The tumor volume was measured by measuring the length and width of the tumor tissue with a vernier caliper (assuming that the tumor tissue was elliptical). The volume was calculated using the length × width^2/2 formula, and the growth inhibition rate and relative tumor volume (RTV) were calculated. Vt: the tumor volume obtained by measuring the tumor every day, and $V_{initiall}$: the initial tumor volume (before drug administration).

3.5.2 Relative tumor volume (relative tumor volume, RTV), with a calculation formula as:

$$RTV = V_t/V_{initial}$$

wherein $V_{initial}$ is the tumor volume when administering after grouping, and V_t is the tumor volume measured each time.

3.5.3 Relative tumor proliferation rate T/C (%), with a calculation formula as:

$$T/C\ (\%) = (T_{RTV} / C_{RTV}) \times 100\%$$

3.5.4 Tumor volume growth inhibition rate (GI)

$$GI = [1 - (TV_t - TV_{initial}) / (CV_t - CT_{initial})] \times 100\%$$

wherein TV_t is referred to the tumor volume measured each time for the treatment group;

$TV_{initial}$ is referred to the tumor volume measured when administering after grouping for the treatment group;

CV_t is referred to the tumor volume measured each time for the control group;

$CT_{initial}$ is referred to the tumor volume measured when administering after grouping for the control group;

3.5.5 Body weight change profile of tumor-bearing mice:

When measuring the volume of tumor-bearing mice and the body weight of the mice, a body weight change profile of the tumor-bearing mice was drawn, and the body weight change trend of the tumor-bearing mice was observed, and the change effect of the compound on the body weight of the tumor-bearing mice was evaluated;

3.6 Statistical analysis:

Measurement data such as tumor volume, tumor weight and tumor-bearing mice tumor were expressed as $\bar{x}\pm S$. Excel software or SPSS 12. 0 software was used for statistical analysis, and t test was used for statistical method.

3.7 Experimental results analysis

3.7.1 Analyze the tumor volume of each dosing group after administration, and draw the pharmacodynamic profile of time-volume change;

3.7.2 Analyze the relative proliferation rate of tumors in each dosing group after administration, and draw the profile of time–relative proliferation rate of tumors;

3.7.3 Analysis of tumor size inhibition after administration of each dosing group, and

calculation of growth inhibition rate;

3.7.4 The weight of tumor-bearing mice in each dosing group was analyzed, and the body weight-time profile was drawn.

4 Results

4.1 The effect of TSL-1502 combined with TMZ on tumor volume of B16F10 model:

The results showed that TSL-1502 had a good inhibitory effect on the growth of melanoma B16F10 tumor-bearing mice. On the 33rd day after inoculation, the anti-tumor rate and significant anti-tumor effect of TMZ alone (50 mg/kg) were 37.4% and ($P<0.05$), while the anti-tumor rate of TSL-1502(10mg/kg) combined with TMZ was 76.1%, which was significantly higher than that of TMZ alone ($P<0.01$). With the increase of TSL-1502 dose, the inhibitory effect of combined TMZ was better, and the inhibitory rate was 81.2% in the (15mg/kg)+TMZ group and 87.0% in the TSL-1502 (30mg/kg)+TMZ group. See Table 7 and Figure 10.

Table 7. Average tumor volume (mm^3) and growth inhibition rate (GI, %) at day 33

Groups	Average tumor volume (mm3)	Growth inhibition rate (GI, %)
Blank control group	12660.89	0.00
TMZ(50mg/kg)	8025.33 *	37.36
Veliparib(25mg/kg) + TMZ	5991.14 #	53.61
TSL-1502(10mg/kg) + TMZ	3188.62 ##	76.13
TSL-1502(15mg/kg) + TMZ	2570.66 ##	81.17
TSL-1502(30mg/kg) + TMZ	1833.32 ###	87.02

Compared with control, *$p<0.05$, **$p<0.01$; compared with TMZ control: #$P<0.05$, ##$P<0.01$;

4.2 The effect of TSL-1502 combined with TMZ on the relative tumor proliferation rate (T/C%) of B16F10 melanoma model:

Table 8, 9, and Figure 11, Figure 12 results showed that, on day d33 after inoculation, the relative tumor proliferation rate T/C% of TSL-1502(10, 15, and 30mg/kg)

combined with TMZ increased from 61.5% with TMZ alone to 26.5%, 20.7%, and 15.4%(T/C%≤40%, P < 0.05 was effective), respectively. Compared with TMZ alone, TSL-1502 at 10 mg/kg and 15mg/kg significantly increased the tumor inhibition effect of TMZ (P < 0.01), and TSL-1502 at 30 mg/kg exerted a better synergistic effect (P<0.001).

Table 8. Change in relative tumor proliferation rate (T/C%) of tumor-bearing mice during dosing

Group	d11	d13	d15	d17	d19	d21	d23	d25	d27	d29	d31	d33
TMZ(50mg/kg)	100.00	78.62	65.03	66.79	69.17	66.43	66.23	66.42	70.26	68.64	67.52	61.55
Veliparib(25mg/kg) + TMZ	100.00	77.22	70.74	74.75	71.78	65.24	59.45	56.41	57.32	49.93	52.46	49.07
TSL-1502(10mg/kg) + TMZ	100.00	75.81	60.54	60.20	60.91	57.83	52.01	46.28	46.90	38.12	32.09	26.48
TSL-1502(15mg/kg) + TMZ	100.00	72.84	54.41	51.26	49.53	46.97	43.27	36.77	37.09	29.00	26.17	20.68
TSL-1502(30mg/kg) + TMZ	100.00	75.35	55.44	55.24	50.89	43.77	36.88	29.60	27.00	21.31	17.80	15.37

Table 9. Summary of relative tumor proliferation rate (T/C,%) and growth inhibition rate (GI,%) at day 33

Groups	RTV	T/C%	%GI
Blank control	5400.71	0.00	0.00
TMZ(50mg/kg)	3323.94 *	61.55	37.36
veliparib(25mg/kg) + TMZ	2649.89 #	49.07	53.61
TSL-1502(10mg/kg) + TMZ	1430.26 ##	26.48	76.13
TSL-1502(15mg/kg) + TMZ	1116.71 ##	20.68	81.17
TSL-1502(30mg/kg) + TMZ	830.31 ###	15.37	87.02

Compared with control, *p<0.05, **p<0.01; compared with TMZ control group: #P<0.05, ##P<0.01, ###P<0.001.

Reference:

[1].Cherrie K. Donawho, Yan Luo, et al. ABT-888, an Orally Active Poly (ADP-Ribose) Polymerase Inhibitor that Potentiates DNA-Damaging Agents in Preclinical tumor Models. Clin Cancer Res 2007;13(9) May 1, 2007.

[2]. JOANN P. PALMA, LUIS E. RODRIGUEZ, et al. The PARP Inhibitor, ABT-888

Potentiates Temozolomide:Correlation with Drug Levels nd Reduction in PARP Activity In Vivo. ANTICANCER RESEARCH 28:2625-2636 (2008).

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What is claimed

1. A pharmaceutical composition comprising TSL-1502 and another anti-tumor drug, wherein the compositional pharmaceutical combination includes a pharmaceutical preparation composition comprising the two active ingredient TSL-1502 and another anti-tumor drug, or a combination wherein two pharmaceutical preparations individually containing TSL-1502 and another anti-tumor drug are packaged together.

2. The pharmaceutical composition according to claim 1, charaterized in that, the dose of each drug is in a pharmaceutically effective amount.

3. The pharmaceutical composition according to claim 1, wherein the compositional pharmaceutical combination is in any ingestible pharmaceutical form, preferably pharmaceutical form being injectable preparation, in unit dose form, with each unit dose comprising 0.1-2000mg of pharmaceutical active ingredients.

4. The pharmaceutical composition according to claim 1, wherein the combination comprises TSL-1502 and another anti-tumor drug, with a weight ratio of the two of 1:1000 to 1000:1.

5. The pharmaceutical composition according to claim 1, wherein the another anti-tumor drug is selected from a group consisting of temozolomide, cisplatin, carboplatin, irinotecan.

6. The pharmaceutical composition according to claim 5, wherein the compositional pharmaceutical combination comprises TSL-1502, temozolomide formulated in an effective amount of (5-60):(10-100); preferably (10-40):(40-60); most preferably (10-30):50;

or that the pharmaceutical composition comprises TSL-1502, cisplatinformulated in an effective amount of (5-50):(2-10); preferably (5-40):(2-8); most preferably (6.25-25):6;

or that the pharmaceutical composition comprises TSL-1502, carboplatinformulated in an effective amount of (10-90):(5-100); preferably (20-70):(50-70), most preferably 25-50:60;

or that the pharmaceutical composition comprises TSL-1502, irinotecan

hydrochloride (CAMPTO) formulated in an effective amount of (5-100):(5-30) 10, preferably (5-60):(5-20), most preferably (5-50):10.

7. The pharmaceutical composition according to any one of claims 1 to 6, wherein the use method comprises the following steps of: preparing the required amount of TSL-1502 and another anti-tumor drug together as two pharmaceutical active ingredients into an injectable preparation for injection, or separately preparing the required amount of TSL-1502 and another antitumor drug into injectable preparations for separate injection.

8. The preparation method of the pharmaceutical composition according to claim 1, comprising the following steps of: preparing the required amount of TSL-1502 and another anti-tumor drug together or respectively as pharmaceutical active ingredients into a pharmaceutical composition which can be taken according to the conventional technology of pharmacy.

9. The preparation method according to claim 8, wherein the dosage form of the compositional pharmaceutical combination is injectable preparation and is prepared without excipients or with one or more pharmaceutically acceptable excipients, selected from a group consisting of glucose, lactose, mannitol, sodium chloride, and hydroxypropyl-B-cyclodextrin.

10. Use of the pharmaceutical composition according to any one of claims 1 to 6 in the manufacture of a medicament for treating anti-tumor, wherein preferably the tumor is selected from a group consisting ofbreast cancer, melanoma, colorectal cancer.