# Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era

**GP-TCM**

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## Report on the reviewed literature relating to CHM in animal models of disease

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## 1. INTRODUCTORY COMMENTS

In the WP5 kick-off meeting it was decided to start with a preliminary review focused on the current status of Chinese herbal Medicine (CHM) studies in animals. In a second step, it was decided that an in-depth review in cancer should be undertaken and would be considered as a significant sample of the state-of-the-art in CHM studies in animals: according to MedLine, oncology is one of the most active medical areas in CHM in the last 10 years (see Report on Deliverable D5.4, Volumes I and II). And this is because cancer is one of the common diseases but remains associated with poor prognosis. There is a long history that traditional Chinese medicine (TCM) has been used to treat human malignant diseases due to its significant efficacy in clinic. Recently more and more scientists are getting interested in the role of Chinese medicine in cancer therapy, and therefore, a large number of experimental studies assessing the anti-tumour effects of TCM have been carried out.

## MAIN TEXT

## 2. ABSTRACT

Cancer is one of the common diseases which most patients would die from. There is a long history that traditional Chinese Medicine (TCM) has been used to treat human cancer diseases due to its significant efficacy in clinic. Recently more and more scientists are getting interested in the application of TCM to the treatment of cancer diseases, and therefore, a large number of experimental studies on anti-tumour effects of TCM have been carried out. Based on the literature in the past ten years, we reviewed the status of animal models for cancer research in TCM. At present, the animal models with the widest use in experimental therapeutics of TCM are transplanted animal tumour models and induced tumour models. The diagnosis of animal models for cancer research in TCM followed mostly the criteria of Western medicine and lacked the one from TCM syndromes. In most experimental studies, quite few signs and symptoms of animal models for cancer research were determined. The signs and symptoms described in the literature were as follows: body weight, food intake, hair, activity, faeces. Moreover, these signs and symptoms were investigated generally and commonly, but not as the evaluation or the mark of the efficacy of TCM specifically. At present, in the experimental studies of animal models for cancer research, most researchers mainly determine the clinical efficacy of TCM through the following indicators: tumour size and weight, tumour growth inhibition, inhibitory rate of metastasis, and survival time. Despite numerous studies about animal models of cancer, these experimental studies are not standardised. Results are therefore inconclusive. Future studies should fulfil the following criteria: i) it is needed to focus more on standardization in terms of physical and biological parameters, ii) the diagnostic criteria of animal models for cancer research should be standardized and quantified (histological stage of the tumour), and iii) the assessment and evaluation of efficacy needs to be unified as well.

## 3. INTRODUCTION

Cancer is one of the common diseases which most patients would die from. Therefore, researches on the mechanism of its prevention and treatment are the focal points in the field of TCM and Western medicine at present. Western medicine practitioners have focused the study of cancer to the molecular biology field. Surgical operation and radio- and chemotherapy are used as primary treatment methods, but the outcome is not always good. In recent years, TCM, as a part of the comprehensive therapy, has been gradually gaining attention of oncologists. There is a long history of the use of TCM in the treatment of human cancer because of both its claimed clinical efficacy and its beneficial role as a supportive therapy to the conventional treatments involving surgical operation, radio-and chemotherapy.

Cancer is usually diagnosed according to symptoms, examination, especially image diagnosis, but pathology is the gold standard. In tumour diagnosis, the agreement between Western medicine and TCM is pathology. Biopsy is an important method of clinical diagnosis of cancer. During a biopsy, the practitioner removes a small tissue sample from the part of patient's body which is suspected to be cancerous. The tissue sample is analyzed in the laboratory and a positive diagnosis will be made when cancer cells are found. The histological staging of the cancer disease is then established using an international consensus statement.

Several herbal therapies, but not all, are related with the cancer type. There are three types of TCM for anti-tumour treatment: Chinese herbal complex formula, single Chinese herbal and bioactive compound. Chinese herbal complex formula is composed of several herbals which reflect treatment upon syndrome differentiation of TCM. Chinese herbal complex formula is commonly used in the clinic treatment of tumours and has a certain effect. Anti-tumour experiments involving Chinese herbal complex formula are designed to assess its efficacy against the tumour itself. The number of studies about the anti-tumour activity of single Chinese herbal of is growing and they are mainly based on assessing its efficacy and its pharmacological properties. Application as anti-tumour of single Chinese herbal extracts is the result of experimental studies that have usually been confirmed by pharmacological studies, which are large-scale randomized open-blinded. Currently, the formulations used in

experimental studies involving single Chinese herbal are extract and injection (with Chinese patent medicine). Bioactive compound is the active ingredient extracted from Chinese herbal medicine and is the key material to treating disease. At present, Chinese herbal compound, single Chinese herbal and bioactive compound are widely used in anti-tumour therapy. Published examples of the application of Chinese medicine in anti-tumour therapy are given in Table 1.

**Table 1**

Chinese medicine in the application of anti-tumour

| Category | | Name | Disease | References |
|---|---|---|---|---|
| Chinese herbal compound | | Sho-Saiko-to | Liver cancer | Shiota et al. (2002) |
| | | Xuefu Zhuyu Tang | Liver cancer | You et al. (2003) |
| | | Xiao-Zhi-Ling(XZL) | Liver cancer | Lu and Wu. (2003) |
| | | Kang-Lai-Te(KLT) | Liver cancer | Wu et al. (2004) |
| | | Ganfujian granule | liver cancer | Qian and Ling. (2004) |
| | | Bushen huayu jiedu recipe (BSHYJDR) | Liver cancer | Cao et al. (2005) |
| | | Mylabris Mixture | Liver cancer | Zhou et al. (2006) |
| | | ZYD88 | Lung cancer | Duan et al. (2007) |
| | | Fuzheng Jiedu Decoction | Liver cancer | Yin et al. (2008) |
| | | Weichang'an(WCA) | gastric cancer | Zhao et al. (2007) |
| | | Wei Chang An (WCA) | gastric cancer | Zhao et al. (2008) |
| | | Jinlongshe granules | gastric cancer | Yu et al. (2006) |
| | | Weichang'a | gastric cancer | Zhao et al. (2005) |
| | | Weikang-ning (WKN) | Gastric cancer | Li et al. (2005b) |
| | | Shikunshito-Kamiho (SKTK) | colorectal cancer | Yoo et al. (2001) |
| | | Active Chinese mistletoe lectin-55 (ACML-55) | colon cancer | Ma et al. (2008) |
| | | PC-SPES | colon cancer | Huerta et al. (2002) |
| | | | Prostate cancer | Bonham et al. (2002) |
| | | San-Zhong-Kui-Jian-Tang (SZKJT) | breast cancer | Hsu et al. (2006) |
| | | Juzen-taiho-to | endometrial cancer | Wang et al. (2008a) |
| | | Chengqi Shengxue prescription | Lung cancer | Zhao et al. (2008) |
| | | Shichimotsu-koka-to | melanoma | Ohno et al. (2002) |
| | | Juzen-taiho-to (JTT) | malignant glioma | Kamiyama et al. (2005) |
| | | Sijunzi | Bladder cancer | Li et al. (2005a) |
| | | Shenyang prescription | Oral cancer | Jiang et al. (2007) |
| | | Mao-Bushi-Saishin-to | Glioma | Saito et al. (2004) |
| | | Jianpiyiwei capsule (JPYW) | Gastric cancer | Shi et al. (2002) |
| | | Juzen-taiho-to(JTT) | Malignant melanoma | Dai et al. (2001) |
| Single Chinese herbal | Extract | *Salvia Miltiorrhiza (SM)* | Liver cancer | Liu et al. (2001) |
| | | *Cordyceps militaris* estract(CME) | melanoma | Yoo et al. (2004) |
| | | *Coriolus versicolor* | leukaemia | Ho CY et al. (2006) |
| | | *Panax notoginseng* | colorectal cancer | Li et al. (2007) |
| | | *Sarcandra glabra Extracts* | nasopharyngeal cancer | Kang et al. (2008) |
| | | *Wogonin* | breast cancer | Chung et al. (2008) |
| | | *Scutellaria barbata* | hepacellular carcinoma | Dai et al. (2008) |
| | | *Wedelia chinensis* | Prostate Cancer | Tsai et al. (2009) |
| | | *Haishengsu (HSS)* | Ehrlich ascites tumour | Liu et al. (2009) |
| | Injection | HMD | gastric cancer | Ji et al. (2004) |
| | | *Celastrus orbiculatus* | colon cancer | Yang et al. (2005) |
| | | astragalus injection | Lung cancer | Dong et al. (2006) |

| | | | |
|---|---|---|---|
| bioactive compound | honokiol | Colorectal cancer | Chen et al. (2004) |
| | gambogic acid (GA) | Gastric cancer | Liu et al. (2005) |
| | Pseudolaric acid B (PAB) | liver cancer | Wong et al. (2005) |
| | Polypeptides in bee venom (PBV) | Liver cancer | Hu et al. (2006) |
| | n-butylidenephthalide (BP) | malignant brain tumour | Tsai et al. (2006) |
| | 20(S)-25-methoxyl-dammarane-3β, 12β, 20-triol (25-OCH3-PPD), | prostate cancer | Wang et al. (2008b) |
| | active chinese mistletoe lectin-55 (ACML-55) | colon cancer | Ma et al. (2008) |
| | hydroxy safflor yellow A | gastric adenocarcinoma | Xi et al. (2009) |
| | acetylshikonin | Gastric carcinoma | Zeng et al. (2009) |

Thus, Chinese medicine has been reported to be effective in clinical cases of cancer. On the basis of this reported efficacy, a large number of animal experimental studies have been carried out, although they are still lacking of standardisation. The present article summarises results of studies in recent years about the use of TCM in animal models for cancer research.

## 4. ESTABLISHMENT OF ANIMAL MODELS FOR CANCER RESEARCH

In the study of anti-tumour medicine, a lot of types of animal models for cancer research have been established, there are as follows: transplantation tumour models, induced tumour models, spontaneous tumour models, genetically predisposed models, exercise model in animals, and so on (Hoffman-Goetz. (2003). The models with the widest use in experimental therapeutics of TCM are transplanted animal tumour models and induced tumour models. They will be discussed in some detail below.

### 4.1 Transplantation tumour models

The models with the widest use in experimental therapeutics are transplanted tumour models which include orthotopic transplantation tumour models and subcutaneous transplantation tumour models.

#### i. Orthotopic transplantation tumour models

Orthotopic implantation refers to the implantation of tumour cells into the same organ or tissue as their site of origin. This organ-specific site presumably provides the tumour cells with an

optimal environment for growth and progression. Orthotopic implantation has been used with animal and human tumour cells into mouse recipients. Because of its relevant expense and novelty, this model has as yet not been used widely. However, it is being used extensively to explore its role as an in vivo evaluation model for cytotoxic agents specific for organ sites. The greatest take (successful implant) for orthotopic tumour cell implantation is in immunodeficient animals. Because immunodeficient mice do not reject orthotopic transplants of xenogeneic (human) or allogeneic (other mouse strains) tumour cells, they are useful for studying the metastasis behaviour of tumour cells (especially those of human origin) *in vivo*.

**Liver cancer**

In the studies of animal models of liver cancer, Yin et al. (2008) established the athymic mice with indirect orthotopic transplantation tumour models, forty eight BALB/c athymic male mice were inoculated Bel-7402 hepatoma carcinoma cells with concentration of 1 million cells/mL on their waist and back, until the subcutaneously transplanted tumour grew to reach 1 cm, diameter. Then the tumour was cut down and. the necrotic tissue was removed. The tumour was cut into pieces (about 1 $mm^3$ each) in Hanks medium. The athymic mice were anesthetized by IP injection of Pentobarbital (45 mg/kg weight,). The remaining procedure was as follows: transverse incise the left upper quadrant, expose liver, take 1 piece of tumour tissue, use bodkin pinhead (20° angle of slope, deep 3 mm) imbed the tumour tissue in deep part of hepatic lobes parenchyma of athymic mouse in ex vivo 40 min, compress the incision to stop bleeding then close abdomen.

You et al. (2003) selected ICR male mice aged 6-8 weeks, and inoculated live sarcoma-180 tumour cells ($1\times10^7$) into the right lobe of the liver of normal male mice, under anaesthesia with 0.1 ml sodium pentobarbital, at a concentration of around 0.25g/15.5ml, while Lu and Wu. (2003) chose Walker-256 tumour cells to establish animal models of liver cancer. Walker-256 tumour cells were injected into the abdominal cavity of Wistar rats, tumour ascites were removed from the abdominal cavity 7 days later. After diluting them, subcutaneous injection was given in right forefoot axillary fossa of healthy Wistar rats. 8 days later they obtained the subcutaneous tumour which grew quickly, and then it was cut it into pieces of $1\times1\times1$ $mm^3$, which were placed into RPMI medium 1640. A small subcapsular incision on the left lateral lobe of the liver of healthy Wistar rats was made. Tumour fragments were gently put into the pocket and abdominal wall was then closed. Wu et al. (2004) also used tumour cell line walker-256, rats were inoculated with tumour cell line walker-256 into their abdominal cavities. On the seventh day after inoculation, tumourous ascities was sucked by a plastic needle tube (5ml) under aseptic circumstance. The tumourous ascites that had been diluted (0.5ml each one) was injected subcutaneously in the armpit of other healthy Wistar rats right forelimb. Eighty days after subcutaneous inoculation, the diameter of the tumour increased to 1cm. After the rats were anesthetized and fixed, subcutaneous tumour was cut off under aseptic condition. The

fringe fichy tissue of the tumour which grows quickly was chopped into pieces ($1mm^3$) and was put into RPMI-1640 culture liquid. These pieces were stored in ice water to keep lower temperature (within 3 hours). Forty healthy Wistar rats were anesthetized as above and were fixed falt. Then a midline incision about 1.5 cm was made and ophathalmological tweezers were used to break the membrance and enter the parenchyma of the liver. Then the preserved tumour tissue was transplanted via the access into the left liver lobe. After hemostasia by compression, the wound of abdomen was sutured.

**Gastric cancer**

Yu et al. (2006) established in situ-transplanted human MKN-45 gastric carcinoma nude mouse models, before transplantation, nude mice bearing gastric adenocarcinoma MKN-45 cell lines were killed. Then the tumour was dissected and put into aseptical saline. Pieces of intact, fish meat-like tumour tissue of 1 mm × 1 mm × 1mm in diameter from margin were prepared after removal of tumour capsule. Mice anesthetized with ketamine (50 mg/kg, ip) were sterilized with caseoiodine and a transverse incision was made on the left upper quadrant. After exposure of the greater curvature of stomach, serosa and muscular layer were incised with needle. Then tumour tissues were transplanted under serosa of stomach and one drop of OB biogel was applied to the tumour tissue. After 40s, stomach was brought into abdominal cavity and abdominal wall was finally closed.

**Prostate Cancer**

In study of Tsai et al. (2009), 22Rv1 cells ($1 \times 10^6$) suspended in PBS were mixed 1:1 with Matrigel (BD Biosciences) and subcutaneously inoculated into the right flank of each mouse and allowed to reach a tumour diameter of 0.4 to 0.5 cm. For orthotopic implantation, a mouse prostate was exposed with a surgical incision and 103E cells in suspension ($3 \times 10^5$ in 20 µl PBS) were injected into the left side of prostate.

**Ehrlich ascites tumour**

Few studies about animal models of ehrlich ascites tumour were reported, Liu et al. (2009) investigated the in vivo effect of the seashell protein Haishengsu (HSS) on ehrlich ascites tumour.The ehrlich ascites tumour cells were aspirated and suspended in phosphate buffer saline. Cell viability was tested by staining with trypan blue prior to the experiment. The ehrlich ascites tumour cells were diluted with saline (1:4). Under sterile conditions, healthy mice were inoculated intraperitoneally with 0.2 ml of the diluted tumour cell solution. Abdominal tumours were palpable 3 days after the inoculation.

### ii. Subcutaneous transplantation tumour models

Subcutaneous transplantation tumour model often receive the tumour via subcutaneous routes, which is widely used for setting up a variety of tumour models, such as liver cancer, colon cancer, gastric cancer, lung cancer, breast cancer, leukaemia, prostate cancer, malignant glioma, and so on.

**Liver cancer**

In the studies of Cao et al. (2005), the established animal models of liver cancer, ascites was drawn from hepatocarcinoma mice H22 under aseptic condition, and diluted in normal saline at 1:4. Concentration of tumour cells was $1\times10^7$ cells/mL. Then 40 healthy Kunming mice weighing 18-22 g were selected, 0.2 mL tumour cells were injected into the armpit of right forelimb of each mouse. In study of Lin et al. (2003), human HCC SMMC-7721 cellular cultural suspension was centrifuged and the supernatant was removed. Then the cellular cultural liquid was added to make up $5\times10^7$ cells per millilitre. Subcutaneous injection was taken in the back of a nude mouse with 0.2 ml liquid. When the tumour grew to 1 cm in diameter, part of it was taken out under the aseptic condition and cut into small pieces in the size of 0.2×0.2×0.2 cm, which were transplanted subcutaneously at the back of other nude mice with a canula needle and underwent passages from generation to generation. In study of Chen et al. (2008), H22-bearing mice were sacrificed by cervical dislocation 7 days after intra peritoneal inoculation with H22 tumour cells. The ascitic cells were collected aseptically and stained by Trypan Blue to calculate the number of live tumour cells. H22 cells were diluted with normal saline and cell concentration was adjusted to $5\times10^5$/ml. The mice were inoculated with 0.2 ml of tumour cell solution on the right axilla to establish the solid tumour model and the same numbers of the cells were inoculated into the peritoneum to establish the ascites tumour model. While Hu et al. (2006) chosen nude Balb/c mice ,and implanted SMMC-7721 cells (200μL, $5\times10^6$) in log phase growth into the backs of the animals to establish liver cancer animal model.

**Colon cancer**

For tumour induction, colon cancer cell line CT26 cells ($5\times10^5$cells/mouse) were injected subcutaneously, and tumour growth was monitored and recorded daily for over 3 wk in study of Ma et al. (2008). Chen et al. (2004) used PKO cells to establish animal models of colon cancer. RKO cells ($5\times10^6$) were injected subcutaneously at the axilla of Balb/c nude mice. When tumours became visible about one week after implant, the animals were randomized into four groups and treated. In study of Iizuka et al. (2002), Colon 26/clone 20 cells were injected s.c. ($1\times10^6$ tumour cells/animal) with a 27-gauge needle into the right lower abdominal quadrant.

**Gastric cancer**

In studies of Ji et al. (2004), tumour cells were transplanted to 615 FC mice. Nine days after transplantation, FC mice bearing the growing tumour were placed in an ice basin inside an enclosed aseptic working table. Tumour tissue was separated and then cut into small pieces and homogenized with a glass homogenizer. The homogenate was made into a suspension containing about $5.6\times10^6$/ml of tumour cells with sterile normal saline in the conventional way, and 0.2 ml of the suspension was transplanted to the right axilla of each 615 strain mouse. Similarly, S180 tumour cells were transplanted into Kunming strain mice. After 7 days, ascites was drawn from the mice bearing well growing tumour under aseptic condition. The tumour cells were diluted with sterile normal saline in ice-bath to 4:1 to produce a suspension containing about 5.8×106/ml of tumour cells. This suspension should have a semi-transparent creamy appearance. Any ascitic fluid with blood streaks should not be used. The mice were transplanted by sc injection of 0.2 ml of the suspension to the right axilla of each Kunming mouse. Kunming mice were transplanted with H22 tumour cells. The mice bearing well growing transplanted tumour were selected after 7 d, and their ascites were drawn under aseptic condition and diluted in ice-cold sterile normal saline to a concentration of 4:1. The tumour cells were mixed well to generate a suspension containing about 6×106 tumour cells/ml, and 0.2 ml of the suspension was transplanted ip to each Kunming mouse..To determine whether acetylshikonin inhibits tumour growth in vivo, Zeng et al. (2009) injected an equal number of SGC-7901 cells into the right flanks of nude mice ($5 \times 10^6$ cells per mouse). Tumours were allowed to develop to about 7.5 mm × 7.5 mm × 7.5 mm. Liu et al. (2005) established BGC-823 nude mice xenografts, $5\times10^6$ BGC-823 cells were subcutaneously inoculated into BALB/cA nude mice. The transplantation tumours were ready to use after having been handed down three generations in nude mice. After 12-14 d, the nude mice with implanted tumour were screened for tumour volume. Tumour-bearing mice in which the tumour had reached a volume of about 100-300 $mm^3$ were selected (mice with tumours that are too large or too small were eliminated).

**Lung cancer**

In study of Duan et al. (2007), Lewis lung tumour tissues dissected from xenograft animals were washed once with RPMI-1640 medium, and then they were split up in 3 volumes of saline. C57BL/6 mice at 9-11 weeks old were injected subcutaneously in the right flank with 0.2 ml tumour cell suspension.

Ohno et al. (2002) produced Pulmonary Metastasis models, male C57BL/6 mice were injected intravenously with $2\times10^5$ or $1\times10^5$ cells/mouse of cultured B16 melanoma cells. Hong-Fen et al. (2001) selected fischer-344 male and female rats, rats were lightly anesthetized with halothane,

and $10^5$ MADB106 tumour cells were injected into their tail vein in 0.5 ml of PBS supplemented with 0.1% bovine serum albumin.

**Breast cancer**

To examine the in vivo effect of wogonin in breast cancer cells, Chung et al. (2008) engrafted subcutaneously T47D and MDA-MB-231 cells into athymic nude mice. In an in vivo tumour xenograft study of Hsu et al. (2006), MDA-MB-231 cells were injected subcutaneously into the flanks of nude mice ($5\times10^6$cells in 200μ l) to make breast cancer model. In Woo et al. (2007) experiments, xenografts were established by injecting 1 x $10^6$ MDA-MB-231 human breast cancer cells into the subcutaneous fat pads of female athymic nude mice to established MDA-MB-231 breast cancer models.

**Leukaemia**

Unlike solid tumours, leukaemia models are limited. Leukaemia models are transplanted ones only by the subcutaneous (SC), intravenous (IV), or intraperitoneal (IP) routes. To make animal model of human leukaemia, xenografts Suspension of $1x10^7$ of human leukaemia HL-60 cells was injected subcutaneously (s.c.) into the dorsal side of nude mice in a study of Ho et al. (2006). Wang et al. (2008a) established a murine acute promyelocytic leukaemia (APL) model: he injected FVB/NJ mice with $1\times10^5$ cells expressing PML-RARα.

**Prostate cancer**

Prostate cancer (PC3) xenograft model was established by Wang et al. (2008b), briefly, 4-6 weeks old male athymic nude mice (nu/nu) were obtained from the Frederick Cancer Research and Development Center (Frederick, MD, USA). Cultured PC3 cells were washed with and re-suspended in serum-free medium. Portions of the suspension ($5\times10^6$ cells in 0.2 ml) were injected into the left inguinal area of the mice. Bonham et al. (2002) established androgen-independent CWR22R and MSKPC9 tumour xenografts which were propagated in nude mice: he used the following ratio: one excised 2500 $mm^3$ tumour to five animals. Each animal was injected subcutaneously into the subscapular area with 500 μL of the prepared tumour suspension consisting of one part minced tumour tissue.

**Malignant glioma**

Kamiyama et al. (2005) established GL261 C57BL/6 Mouse Subcutaneous Model, three weeks after the implantation of 1×107GL261 cells in the flank of an 8-week-old C57BL/6 mouse, GL261 tumour tissue fragments were removed and then harvested tumour fragments 1mm$^3$ in size, were implanted in the flanks of other young and old mice.

### 4.2 Induced tumour models

Chemical carcinogens, radiation or viruses can induce different types of cancer in various animals. The most commonly used induced tumour model is the chemically induced tumour. The largest number of experimental animal studies on cancer has been made with chemically induced tumours. Chemical carcinogens increase cell proliferation and induce the formation of tumours in a variety of organs and tissues, depending on the route of administration, time of administration, dose, duration, frequency of administration, and sex of the animal. Chemical specificity of the carcinogen also affects the type of tumour that develops. Chemical carcinogens have been widely used in animal models to study the prevention of cancer with TCM.

**Liver cancer**

Diethylnitrosamine (DEN) is a representative chemical of a family of carcinogenic N-nitroso compounds. The International Agency for Research on Cancer concluded that DEN was carcinogenic in all animal species and that there was sufficient evidence of a carcinogenic effect to classify DEN as a probable human carcinogen. Administration of DEN to animals has been shown to cause cancer in liver (Liao et al. 2001). To test the mechanism of protective action of TJ-48, Tsuchiya et al. (2008) used a mouse model of hepatocarcinogenesis caused by diethylnitrosamine (DEN). He chosed two mouse inbred strains for these experiments, C57BL/6N and C3H/HeN, because the former is known to be resistant to spontaneous hepatocarcinogenesis, whereas the latter is prone to liver tumours.25 mice were given DEN (25 mg/l) in drinking water for 22 weeks. Before sacrificing, mice were anesthetized with diethyl ether and following exsanguination livers were removed, weighed and examined for presence of tumours. After 22 weeks of DEN administration, 22% of C57BL/6N and 63% of C3H/HeN mice developed hepatocellular carcinomas. Interestingly, when mice were coadministered TJ-48 in the food (1.6% w/w), the DEN-associated hepatocarcinogenesis was abolished completely in C57BL/6N strain, whereas tumour incidence was reduced to 41% in C3H/HeN mice. Qian and Ling. (2004)) method is different , male SD rats had free access to water containing 0.1 g/L DEN for 16 weeks to induce hepatocarcinoma. Li et al. (2007) injected DEN (200 mg/kg body weight) into the abdominal cavity of experimental rats to induce hepatocarcinogenesis.

Aflatoxin $B_1$ ($AFB_1$) is produced as a second metabolite by the mould Aspergillus flavus.

Experimental studies have shown that $AFB_1$is a potent mutagen and hepatocarcinogen in several animal species. Epidemiological evidence has also established a strong association between human exposure to $AFB_1$and a high incidence of hepatocellular cacinoma in Asia and Africa. The carcinogenic mechanism of $AFB_1$has been extensively studied. In Liu et al. (2001) study, the effect of SM on alfatoxin $B_1$ ($AFB_1$)-induced hepatocarcinogenesis was investigated in male Fischer 344 rats. $AFB_1$ (40mg/100 g body wt, by gavage) was administered once a week for 24 weeks.

**Colon cancer**

Human colorectal cancers are mainly caused by exposure to food mutagens and carcinogens.
1. 1,2-Dimethylhydrazine was studied for carcinogenicity in many experiments in rats and mice, mainly by subcutaneous, infrequently by oral and rarely by other routes of administration. 1,2-DMH is a typical colon carcinogen which conjugates with glucuronic acid in liver and is excreted in intestine via the bile. The glucuronide conjugates hydrolysed by bacterial β-glucosidase are known to increase the activities of fecal enzymes such as β-glucuronidase, tryptophanase and urease in metabolic pathways. Yoo et al. (2001) established colon carcinogenesis model induced by 1,2-dimethylhydrazine(DMH). To induce colorectal cancer 1,2-DMH was injected subcutaneously into mice.
Azoxymethane (AOM) is also a potent carcinogen used to induce colon cancer in rats and mice. It has been used in studies evaluating efficacy of preventative treatment for azoxymethane-induced carcinogenesis. Fukutake et al. (2000) used Male F344 rats, and gave them subcutaneous injections of azoxymethane (AOM) (15 mg/kg body weight) in sterile saline once a week for 2 weeks.

**Oral cancer**

4-nitroquinoline-1-oxide (4NQO), a water-soluble quinoline derivative, has been extensively investigated and is known to cause the formation of DNA adducts. In bacteria, 4NQO induces base pair changes (GC→AT) and deletion mutations. This mutagenic action requires the conversion of 4NQO to 4- hydroxyaminoquinoline-1-oxide, which binds covalently to nucleic acids and damages chromosomes. In eukaryotic cells, 4NQO induces diplochromosomes, indicative of disturbed chromosome replication. 4NQO is a powerful carcinogen in several organs, and it can specifically induce tongue SCC when applied in low concentrations via drinking water. The resulting sequential changes and morphological features resemble those seen during the progression of human tongue squamous cell malignancy. Therefore, 4NQO-induced rat tongue SCC is an excellent model for studying early events in oral carcinogenesis. Jiang et al. (2007) established animal model of oral cancer induced by 4NQO. 4-nitroquinoline-1-oxide (4NQO) was obtained as a powder and dissolved in water to a final

concentration of 0.02 g/l. Eighty Sprague-Dawley (SD) male rats, aged 6–7 weeks, were fed with 4NQO in their drinking water for 36 weeks. During induction, two rats were sacrificed under general anesthesia at the 6th, 12th, 18th, 24th and 30th weeks for pathological examination. Finally, 61 rats with tumours survived after this period, and tongue squamous cell carcinoma (SCC) was confirmed by pathological examination in 61 rats. Ten were sacrificed for pathological examination during induction. Nine rats had no tumours or had died of pneumonia or gastroenteritis. Morphologically, the lesions at the 6th, 12th, 18th, 24th and 30th weeks that grew in response to 4NQO demonstrated a series of squamous cell transformations from normal mucosa through epithelial dysplasia to squamous cell carcinoma. Exposure to 4NQO caused both gross and microscopic changes in the tongues. The macroscopic lesions were shown under microscope to be squamous cell carcinomas.

**Lung cancer**

N-nitrosobis (2-hydroxypropyl) amine (BHP) has been demonstrated to be a potent carcinogen with a wide spectrum of target organs in rats, and is considered to be a potential hazard for human exposure. Kashimoto et al. (2006) established an animal model of lung cancer induced by BHP. BHP was dissolved in distilled water at a concentration of 2,000 mg/l just before use. This solution was supplied to the rats ad libitum for 10 weeks from lightopaque bottles exchanged at 2- or 3-day intervals to induce pulmonary adenocarcinoma.

The above types of tumour-animal models are commonly used in researches of TCM. Nowadays there are new available animal models, which we should adopt in future research.

## 5. DIAGNOSTIC CRITERIA

At present, the diagnosis of animal models for cancer research mostly follows the criteria of Western medicine and lack the diagnosis from TCM syndromes. Following the standards of Western medicine, tumour found in autopsy was usually considered as the sign of success of setting up the model. Many published studies determine animal models for cancer research through the tumour size, and set the size of tumours.

### 5.1 Liver cancer

Lee et al. (2008) established the N -nitrosobis(2-oxopropyl)amine-induced hepatocellular carcinoma model, at the end of the 12th week, all surviving animals were sacrificed under ketamine anaesthesia, followed by macroscopical examination of the main target organs for BOP-induced tumourigenicity. The organs were fixed in 10% phosphate-buffered formalin and processed for histological examination with haematoxylin and eosin (H–E) staining. All

proliferating lesions were diagnosed histopathologically and counted using serial sections. Chen et al. (2007) established a nude mouse model with the transplantation of human hepatic carcinoma cell, and considered modelling success when the tumour reached 0.5 cm in diameter. Hu et al. (2006) recognized the animal model of hepatoma was successful when tumour diameter reached 0.5 cm. In several studies, Magnetic Resonance Imaging (MRI) examination was used to determine the incidence of cancer, Qian et al. (2005) used MRI to measure the size of tumour volume before and after treatment to determine the rate of liver transplantation.

### 5.2 Gastric cancer

To determine whether acetylshikonin inhibits tumour growth in vivo, Zeng et al. (2009) injected an equal number of SGC-7901 cells into the right flanks of nude mice ($5 \times 10^6$ cells per mouse). Tumours were allowed to develop to about 75 mm × 75 mm × 75 mm. Liu et al. (2005) established BGC-823 nude mice xenografts, 5×106 BGC-823 cells were subcutaneously inoculated into BALB/cA nude mice. The transplantation tumours were ready to use after having been handed down three generations in nude mice. After 12-14d, the nude mice with implanted tumour were screened for tumour volume. Tumour-bearing mice in which the tumour had reached a volume of about 100-300 $mm^3$ were selected (mice with tumours that are too large or too small were eliminated).

### 5.3 Breast cancer

Hsu et al. (2006) established models of breast cancer, MDA-MB-231 cells were injected subcutaneously into the flanks of nude mice ($5\times10^6$ cells in 200ml). Tumours were allowed to develop forca. 30 d until they reached 75$mm^3$, when treatment was initiated. While Chung et al. (2008) also established the animal model of breast cancer, he measured 2 diameters of tumours with calibers to permit calculation of tumour volume. Once tumour widths reached 3–6 mm, animals were randomly selected, grouped and treated.

### 5.4 Prostate cancer

Bonham et al. (2002) established androgen-independent CWR22R and MSKPC9 tumour xenografts were propagated in nude mice, he injected subcutaneously into the subscapular area of each animal with 500μL of the prepared tumour suspension consisting of one part minced tumour tissue. After 5 days, mice with established tumours of approximately 5 × 5 $mm^3$ received treatment.

### 5.5 Leukaemia

In study of Ho et al. (2006), suspension of $1x10^7$ of human leukaemia HL-60 cells was injected

Subcutaneously (s.c.) into the dorsal side of nude mice. When palpable tumours (about 100-200 $mm^3$ in volume) arose within 14-18 days after injection, the mice were treated. The above reports set the diagnostic criteria based on tumour volume, but most researches do not follow specific diagnostic criteria, and determine the model only through gross observation, experience, or autopsy. This has led to the absence of uniformity among studies. So the diagnostic criteria of animal models for cancer research needs to be quantified and standardized.

## 6. SIGNS AND SYMPTOMS

In experimental animal models for cancer research, fewer signs and symptoms were reported, and have little statistical results. These signs and symptoms were only as a general observation such as the weight changes, not as an evaluation of the indicators of efficacy of Chinese medicine.

### 6.1 Body weight

Body weight is a good indicator, which reflects the growth state of animal models of cancer.. Tumour-bearing mice gain weight very slowly ，Chen et al. (2007) confirmed qiongyugao can increase the body weight，and Dai et al. (2008) also found that scutellaria barbata extract can significantly increase the body weight, when compared with model group ($p<0.01$). This showed that Chinese medicine has a certain effect on increasing body weight of tumour-bearing mice. Kamiyama et al. (2005) measured the body weight of mice. It was found that the body weight of old mice treated with high dose Juzen-taiho-to (JTT) was significantly higher when compared to control mice, suggesting that JTT influenced good health condition especially for old mice ($p<0.05$).

However, there were a number of different results. In the experiment of You et al. (2003), the body weight of the untreated control group showed no significant change during the experiment. However, the body weight of the tumour control group increased quickly after inoculation with tumour cells compared with treated groups ($p < 0.05$). Such effects might have been due to tumour growth and ascites formation in the tumour control group. Liu et al. (2009) also found in the control group that there was a significant increase in the average body weight due to ascites and tumour growth. The body weight of the HSS 490 and 700 mg/kg groups was similar to that in the control group; however, in the HSS 1,000 mg/kg group, the average body weight was

lower than in the control group ($p<0.05$).

After experiment of Liu et al. (2008), no significant difference was found in mouse weight between the *Gecko* groups and the NS group ($P > 0.05$). In Liu et al. (2001) study, the initial body weight of rats in all four groups was similar(179.5±20.7g). After 28 weeks of treatment, rats in the control group gained an average of 163.7 g, whereas that of AFB1-treated rats was only 120.8 g ($p < 0.05$). On the other hand, the body weight gain of rats receiving AFB1 and SM concurrently was higher than that of rats treated with AFB1 alone, suggesting that SM could improve the general condition of rats exposed to AFB1. However, the variation between individual animals within the groups was relatively large and thus no statistical significance was found ($P>0.05$). Huerta et al. (2002) found no difference in body weight occurred between the treatment group and control group. Ho et al. (2006) found that there was no significant difference between the body weights of control and treated animals. Hu et al. (2006) also found during the experiment, physiological behaviour, appetite and weight of mice were not disturbed. Kashimoto et al. (2006) found the body weight showed no significant difference among the experimental groups after BHP treatment.

To investigate BP anti-tumour activity, Tsai et al. (2006) carried out animal experiments in vivo. There were no significant differences between the control and BP-treated groups with respect to the body weight of the rats after BP treatment.

### 6.2 Food intake

Zhang et al. (2006)、Chen et al. (2006) and Yang et al. (2006) observed the food intake of model animals and found that the model group animals had a general decline in food intake, while no significant change in the Chinese medicine treatment group. This shows that Chinese medicine can increase the food intake of tumour-bearing animals, alleviating their clinical symptoms.

Of course, there were different results, Kamiyama et al. (2005) found no difference in food intake between the control group and the “Juzen-taiho-to”group .Huerta et al. (2002) found no difference in food intake the treatment group and control group. Kashimoto et al. (2006) found the diet intake also showed no significant difference between the experimental groups after BHP treatment

### 6.3 Hair

Hair is the visual symptom which can evaluate the growth state of animal models for cancer . It

was found that the hair of tumour-bearing animals will appear dry, sparse and so on. Zhang et al. (2006) and Chen et al. (2006)observed the hair of animals and found that the hair of model group animals was dry, fluffy and dull, while Chinese medicine treatment group has somewhat alleviated. These observations suggested to authors that Chinese medicine could alleviate the loss of hair of tumour-bearing animals and increase its luster.

### 6.4 Activity

Activity reflects the physical condition of experimental animals, tumour-bearing animals always show slowly activities, and TCM can regulate tumour-bearing animals, body condition and increase activity. Zhang et al. (2006) and Chen et al. (2006) observed the model animal activity and found that the model group animals suffered from the activities of slow, unresponsive, but the traditional Chinese medicine treatment group had no significant change compared to pre-treatment. But there were also opposite results, Hu et al. (2006) found during the experiment, physiological behaviour, appetite and weight of mice were not disturbed.

### 6.5 Faeces

Very little relevant literature observed the faeces of tumour-bearing animal, Zhang et al. (2006) and Chen et al. (2006) observed the faeces of animal models and found that the model group had diarrhea, while the Chinese medical treatment group had no significant change compared to pre- treatment.

To conclude this section, the above studies observed signs and symptoms of the animal models for cancers, including body weight, food intake, hair, activity and faeces. But these signs and symptoms were investigated generally and commonly, but not as the evaluation or the mark of the efficacy of Chinese medicine. Thus, at present the description of clinical symptoms of tumour animal models is not standardized. Nor quantitative criteria nor quantitative results are available in the literature. .

## 7. EFFICACY EVALUATION

The signs and symptoms mentioned above, were used only as a clinical observation, and can not be used to evaluate drug efficacy. A range of methods can be used to evaluate drug effect

on tumours in animal models. At present, in the experimental studies of animal models for cancer research, most researchers mainly determine the clinical efficacy of TCM through the following indicators.

### 7.1 Tumour weight and size

Tumour size and tumour weight or size changes are simple and easily reproducible parameters, which can reflect the growth of the tumour to determine the therapeutic effect of TCM Ji et al. (2004) recorded the tumour weight, to evaluate efficacy, It was found that HMD demonstrated an obvious inhibitory effect on the growth of tumour in FC mice in a positive dose-effect manner, significantly inhibited the growth of S180 sarcoma in tumour-bearing mice in a positive dose-effect manner, and obviously prolonged the life span of H22 bearing mice in a positive dose-effect manner. Chen et al. (2007) also take tumour weight of tumour-bearing mice as the effect evaluation of treatment. Tumour size was measured using calipers and tumour volume was estimated according to the formula: tumour volume ($mm^3$)=$L \times W^2/2$, where L is the length and W is the width. Hsu et al. (2006) measured tumour size and tumour volume to determine whether SZKJT inhibited the tumour growth. In the experiment of Ho et al. (2006), he found the *Coriolus versicolor* (CV) extract had the significant suppression of HL-60 growth in terms of tumour volume when compared with the control group. Hu et al. (2006)also found the tumour volume after treatment with PBV was significantly reduced compared with other groups ($P<0.05$). To determine whether BP could suppress human GBM tumour growth, nude mice were inoculated with human DBTRG-05MG cells and treated with BP. Tsai et al. (2006) calculated the tumour size and the result showed that mean tumour sizes at day 89 were > 1000 $mm^3$ in the control group, 605.8 ± 98.8 $mm^3$ in the BP-70-treated group, 504.4 ± 38.9 $mm^3$ in the BP-150- treated group, 415 ± 130 $mm^3$ in the BP-300-treated group, 365.8 ± 116.7 $mm^3$ in the BP-500-treated group and 171.6 $mm^3$ in the BP-800-treated group ($p < 0.05$). After tumour inoculation, tumour masses (average volume ¼ 41.84 ± 4.45 $mm^3$) were developed on the backs of every mouse, but the tumours of mice with BP treatment shrank or completely regressed to be undetectable on day 150. Liu et al. (2008) found the tumours of the Gecko

groups shrank significantly (P < 0.01) compared with the NS group. To study the therapeutic effectiveness of intra-tumour injection of Xiao-Zhi-Ling (XZL) on transplanted hepatoma in rats. Lu and Wu. (2003) detected the hepatoma volume (HV), After 3 and 8 days, the HVs in Xiao-Zhi-Ling group were smaller than those in control group (P<0.05). Dai et al. (2001) measured the volumes of tumours that developed on the skin and/or eyes (the size of the biggest tumour in each mouse was) in juzen-taiho-to-treated and untreated control transgenic mice. The rates of tumour growth, assessed by measurements of tumour volumes, were remarkably reduced in the juzen-taiho-to-treated mice during the entire period after the initial tumour development, compared with those in nontreated littermate mice(P <0.001). Wu et al. (2004) found that after drug injection, the difference between control group and KLT-treated group was significant (P<0.05). However, there are different experimental results. Kamiyama et al. (2005) measured the size of the tumours three times in a week by caliper. The tumour volume in each group was calculated. But there was no significant difference in the tumour volume between the control group and the JTT group.

### 7.2 Tumour growth inhibition percentage

Evaluation of tumour growth inhibition percentage is the classic indicator to evaluate the efficacy of anti-tumour drugs. In many studies, researchers commonly used tumour growth inhibition percentage to determine the therapeutic effect of Chinese medicine. The tumour growth inhibition percentage was calculated according to the following equation: rate of inhibition (%) = (mean tumour wt of negative control -mean wt of treated group)/mean tumour wt of negative control ×100.

Yang et al. (2005) took the tumour inhibition rate (IR)> 30% as a measure of drug efficacy standards. Ji et al. (2009) calculated the tumour growth inhibition to evaluate the anti-tumour effect of Liqi in tumour bearing mice. Hsu et al. (2006)calculated the tumour growth inhibition to evaluate the effect of San-Zhong-Kui-Jian-Tang. The study of Cao et al. (2005)showed that compared to the control group, significant inhibitory effect of Bushen huayu jiedu recipe (BSHYJDR) was found in DDP group (P<0.01) and high dosage BSHYJDR group (P<0.05). But

no significant difference of inhibitory effect was found in low dosage BSHYJDR group (P>0.05). The inhibitory effect in high and low dosage BSHYJDR groups was lower than that in DDP group (P<0.01). Chung et al. (2008) examined the in vivo effect of wogonin in breast cancer cells through the tumour growth inhibition, and the experimental result indicated the statistical significance of inhibition of tumour growth by wogonin (P<0.05), so wogonin could suppress breast cancer growth in xenograft models. Liu et al. (2008) established the transplanted tumour model of the mouse S180 sarcoma. In the Gecko groups at dosage of 13.5, 9 and 4.5 g/kg, the inhibitory rate was 49.8%, 52.8% and 43.1%, respectively. The differences of the groups of Gecko were very significant from the control group (P< 0.01). These results indicated that *Gecko* could inhibit growth of solid tumour of S180 mice. 25-OCH3-PPD was evaluated in a mouse xenograft model of androgen-independent prostate cancer. 25-OCH3-PPD was first given at 5, 10, or 20mg $kg^{-1}$ $day^{-1}$ 3 days per week for 4 weeks. The highest dose significantly inhibited PC3 xenograft tumour growth by 67% on day 27 (P＜0.001). Even the lowest dose of 5mg $kg^{-1}$ caused significant (30%, P＜0.05) tumour growth inhibition. The preclinical data indicate that 25-$OCH_3$-PPD is a potential therapeutic agent against prostate cancer(Wang et al. (2008b). Zhao et al. (2008) studied the Wei Chang An (WCA) -induced effects on tumour growth, he found the average tumour inhibition rate in the WCA group was 44.32% ±5.67% .When compared with controls, tumour growth was significantly inhibited by treatment with WCA (P < 0.01). In experiments of Woo et al. (2007), he determined whether the Coix extract preparation could affect growth of human cancer xenografts in athymic mice. For the MDA-MB-231 breast cancer cell line xenografts, he observed significant reduction in tumour growth in the animals treated with 50 mg of Coix seed extract by intraperitoneal injection. Tsai et al. (2006) investigated BP anti-tumour activity by in vivo animal experiments. There was a significantly higher inhibitory effect on RG2 tumour growth in the BP-treated group than in the control (vehicle only) group(P < 0.005).To determine whether BP can suppress human GBM tumour growth, nude mice were inoculated with human DBTRG-05MG cells and treated with BP. Significant suppression of tumour growth with respect to the untreated group was observed in the BP-70-, BP-150-, BP-300-, BP- 500- and BP-800-treated groups. Cao et al. (2005) observed the inhibition of BSHYJDR on mice hepatocarcinoma H22, and he found significant

inhibitory effect in BSHYJDR group, and BSHYJDR could significantly improve the inhibition of DDP when used in combination with DDP (P<0.001). The research showed that BSHYJDR decoction could significantly restrain transplanted hepatocarcinoma H22 in mice, enhance the effect of DDP on transplanted hepatocarcinoma H22, indicating that BSHYJDR restrains tumour and has synergistic effects when used in combination with chemotherapy. Wong et al. (2005) used the MDR liver cancer cell line QGY-TR50, which was chosen for testing the efficacy of PAB in vivo, because it could form tumours readily when implanted in nude mice, and the MDR phenotype rendered QGY-TR50 a more rigorous model for evaluating the efficacy PAB in vivo. The result showed the tumour growth inhibition was most evident in mice treated with PAB at 25 mg/kg/d, where ~50% reduction in tumour size were observed compared with mice treated with the vehicle. Liu et al. (2005) observed the inhibition of GA on BGC-823 nude mice xenografts, and the results showed that GA was observed to inhibit the growth of BGC-823 nude mice xenografts. The inhibitory effect of GA (8 mg/kg) was dramatically better than the negative control. Chen et al. (2004) observed the inhibition of solid tumour growth in nude mice bearing RKO cell. The results showed a progressive increase in tumour volume, with a growth rate of 1627.6% and 1408.2% respectively on d 28. While in treated groups, tumour growth rate was increased to 968.9% in adriamycin group and 709.9% in honokiol-treated group. There was a significant difference between honokiol-treated group and its control (treated with PEG400/dextrose) (P<0.05). These data further confirmed that honokiol had an effective anticancer activity in vivo. Bonham et al. (2002) studied PC-SPES and Paclitaxel Effects on Prostate Tumour Growth in vivo. He used the CWR22R prostate cancer xenograft model to evaluate the efficacy of PC-SPES, paclitaxel, and the combination of both agents against prostate tumour growth in vivo. Compared with tumour growth in control mice, tumour growth was statistically significantly inhibited in mice that received PC-SPES(P = .028) or paclitaxel(P<.001). Tumour growth in mice treated with the combination of PC-SPES and paclitaxel was also inhibited (P = .034). These results provide additional evidence for the potential attenuating effect of PC-SPES on paclitaxel-mediated antitumour activity in prostate cancers.

Lu and Wu. (2003) calculated the rate of tumour inhibition (RTI) by the formula: (volume of

control group –volume of test group)/volume of control group×100 %; 3 and 8 days after injecting drug, RTIs in Xiao-Zhi-Ling groups were 45.86 % and 57.73 %. The result showed that the therapeutic effects of intra-tumour injection of XZL on implanted hepatoma were obvious. XZL could evidently inhibit the growth of tumour. The result of Cao et al. (2005)study showed that compared to the control group, significant inhibitory effect of BSHYJDR was found in high dosage BSHYJDR group (P<0.05).

In addition to the above, Chen et al. (2008) took the inhibition of tumour growth as the evaluating factor，in order to work out the optimal drug proportion for QHF. He found the tumour in control group grew rapidly, while tumours in mice in the treated group grew slowly. Thus it was demonstrated that tumour growth inhibition percentage is the most commonly used indicators to determine the clinical efficacy of TCM. Almost all studies have used this indicator.

### 7.3 Tumour growth index

Tumour growth index (TGI) is calculated by the formula: volume of tumour (after treatment-before treatment) / volume of tumour (before treatment). TGI reflects the situation in tumour growth, however, few research used this indicator. Lin et al. (2003) investigated the anti-cancer effect and the immunological mechanism of ultrasound-guided intratumoural injection of Chinese medicine “Star-99” in hepatocellular carcinoma (HCC) of nude mice. The result showed that the growth index of “Star-99” group was markedly lower than that of the saline group ($P$<0.01). It was also lower than that of the ethanol group, but there was no significant difference between them ($P$>0.05). The results of this study illustrated that the compound Chinese medicine “Star-99” had the strong effect on the growth restraint of the tumour.

### 7.4 Inhibitory rate of metastasis

Metastasis is the major cause of mortality in cancer patients. In some experiments, researchers determined the success of transplantation by calculating the rate of tumour transplantation models, and took it as the efficacy evaluation of Chinese medicine treatment.

The liver is the most common target of the metastasis of gastrointestinal tract cancer, especially colon cancer, and the prognosis of patients with liver metastasis is extremely bad. To evaluate the efficacy of Bojung-bangam-tang against liver metastasis of cancer, Lee et al. (2003) used a

liver-metastatic variant (colon 26-L5) of the colon 26 carcinoma cells obtained by an in vivo selection method. Colon 26-L5 cells predominantly metastasize to the liver after inoculation via the portal vein of BALB/c mice. This model had provided information about the efficacy of treatments for liver metastasis, especially for occult micrometastasis. The result indicated that Bojung-bangam-tang was effective for preventing the experimental liver metastasis of the colon 26-L5 cells. In addition, a large number of experiments adopted the inhibitory rate of metastasis to evaluate the clinical efficacy of TCM. Chen et al. (2006) calculated the inhibition rate of liver metastasis to observe the effects of ethanol extracts of Panax notoginseng on liver metastasis of B16 melanoma grafted in mice. Wu et al. (2006) calculated inhibitory rate of metastasis of the 615 gastric cancer mice before lung metastasis in mice to observe the rate of effects of Jianpi Yishen Recipe on metastasis of mice transplanted with proventriculus squamous carcinoma after tumour ectomy. Zhao et al. (2005) observed the anti—metastasis effect of Weichang'an Decoction on orthotopic transplant nude mouse model of human gastric cancer through orthotopic transplantation tumour metastasis rate. Li. (2004) evaluated the efficacy of Qi—replenishing and Yin—nourishing to prevent cancer metastasis through the calculation of inhibition rate of Lewis lung cancer and melanoma metastasis rate of B16.

### 7.5 Survival time

Another parameter that can be used to assess the effect of a drug on a tumour in the animal model is the survival time. The survival time of animal models for cancer is a potentially useful indicator in order to assess the efficacy of Chinese medicine. Ji et al. (2004) calculated rate of life prolongation of through Survival days in order to determine the clinical efficacy of Haimiding, The result showed that Haimiding could significantly extend the rate of life prolongation of FC mice. Wu et al. (2006) observed the effects of Jianpi Yishen Recipe (JPYSR)，a compound Chinese herbal medicine，on recurrence，metastasis and life span of mice transplanted with proventriculus squamous carcinoma cells after tumourectomy. The result showed that the average and median life spans were obviously prolonged in JPYSR—treated group，as compared with those in untreated group. The life-prolonging rate was 100%. Liu et al. (2009) calculated the time of death and days alive were recorded, and the increased life span (ILS) using the following formula: ILS (%) = (average survival time of the treated group - average survival time of the control group)/average survival time of the control group×100%.The results showed the average survival times of the HSS groups were significantly longer than in the

control group ( P＜0.05 or 0.01). The survival time in the HSS 1,000 mg/kg group was longer than in the 490 mg/kg group (P＜0.05). The increased life span of the HSS 1,000 mg/kg group was greater than in the 490 or 700 mg/kg groups (P＜0.05). To investigate BP anti-tumour activity, Tsai et al. (2006) carried out animal experiments in vivo. Survival of rats in the BP treatment group was significantly prolonged compared with survival in the control group ($p < 0.0001$). To determine whether BP can suppress human GBM tumour growth, nude mice were inoculated with human DBTRG-05MG cells and treated with BP. Survival was significantly prolonged for nude mice in the BP treated with respect to the control group ($p < 0.001$). Chen et al. (2004) observed the prolongation of life-span in nude mice bearing RKO solid tumour, The mean survival time was 50.9 d in honokiol-treated group, with a significant prolongation compared to vehicle group (29.7 d, P<0.05). The survival rate in honokiol-treated group was 176.7%, much higher than that in vehicle group (P<0.01). The results demonstrated that honokiol could prolong the lifespan of tumour-bearing nude mice. In studies of Qian and Ling. (2004), the longest survival of rats in carcinogen-exposed control group was 20 wk, and 28 wk in Ganfujian granule treatment group. Log-rank test showed that survival of rats in Ganfujian granule treatment group was longer than rats in carcinogen-exposed control group, and risk of death in carcinogen-exposed control was lower than that in Ganfujian granule treatment group(P<0.05). Statistical results showed that Ganfujian granule prolonged survival of these animals. Sun et al. (2007), Dong et al. (2005) and Yu et al. (2004) also calculated the cancer survival in animal model evaluation effects of Chinese medicine treatment.

In addition to the above, Chen et al. (2008) took the survival as the evaluating factor，in order to work out the optimal drug proportion for QHF. He found the survival were higher in the QHF group than in the groups treated with the single drug that comprise the QHF formula.

## 8. CONCLUSIONS

Because of the significant efficacy of TCM in clinic, a large number of experimental studies on anti-tumour effects of Chinese medicine have been carried out. At present, the diagnosis of animal models for cancer research followed mostly the criteria of Western medicine and lack the one from TCM syndromes specifically. In most experimental studies, quite few signs and symptoms of animal models for cancer research were determined. The signs and symptoms

described in the literature were as follows: body weight, food intake, hair, activity, faeces. Moreover, these signs and symptoms were investigated generally and commonly, but not as the evaluation or the mark of the efficacy of Chinese medicine. In the experimental studies of animal models for cancer research, most researchers mainly determine the clinical efficacy of TCM through the following indicators: tumour size and weight, tumour growth inhibition percentage, inhibitory rate of metastasis, and survival time.

After reviewing the scientific literature on CHM in animal models of cancer, we have found that the experimental work reported in 59% of the papers published in English was below the minimal criteria of quality (see Report on Deliverable D5.4, Volume II): sample size, randomization and blinding and comparison of efficacy with Western medicine should be particularly taken into account. The analysis was focused on the animal model itself and the experimental design, without taking into account the specific problems of CHM preparation. However, the rule in most publications on CHM in animal models of disease is the generalized use of non-standardized research materials (in terms of herbs and herbal preparations), which are likely to not support reproducibility and comparability of research on the same herbs and thus significantly reduce the scientific value and impact of these studies (D5.7 and D5.10). This implies that before using an animal model it is necessary a robust control of plant mixture preparation. The current D5.10 report includes recommendations to improve the quality of research from the point of view of experimental design.

Data collected in this review illustrate that despite the large number of studies about animal models for cancer research, these experimental studies are not adequately standardized. Results are therefore inconclusive. Consequently, we should concentrate more on standardization for future studies.

The diagnostic criteria of animal models for cancer research need to be quantified and standardized, and should be added the TCM syndromes, reflecting the characteristics of TCM Syndrome Differentiation Treatment (to be defined in the Introduction). Secondly, the efficacy evaluation needs to be unified as well.

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