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A New Tumor Therapeutic Vaccine: A Real-World Survey on Treatment of 68 Patients with Advanced Cancer

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Abstract

Tumor Therapeutic Vaccine (TTV) is a complex composed of a variety of bacteria or their toxin vaccines plus adjuvants. Bacterial vaccines include pertussis, typhoid/paratyphoid A/B, and *Staphylococcus aureus*, bacterial toxin vaccines include diphtheria toxin and tetanus toxin, and adjuvants include dextran and others. A total of 68 patients with advanced solid cancer, who received only TTV injections after failing conventional treatments, underwent a real-world survey.

Results: The time of receiving TTV injection treatment was 3 to 96 months, with a median of 24 months; tumor Complete Response (CR) was seen in 44.1% of cases, Partial Response (PR) 36.7%, Stable (SD) 19.1%; since TTV treatment, the overall survival period of patients is 8 to 204 months, with a median of 48 months. At the last follow-up, there are 48.5% of survivors. This survey shows that this simple and safe treatment method can effectively promote tumor regression or stabilize it, and prolong the survival of patients. In some cases, it can cause a miraculous reversal of the disease. For advanced cancer, TTV can be regarded as a promising alternative treatment.

Keywords: Immunotherapy; Bacterial vaccine; Tumor therapy vaccine; Coley toxin

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Introduction

In the past century, significant progress has been made in the treatment of cancer. However, the treatment of advanced cancer remains a huge challenge. Traditional treatments such as chemotherapy and radiotherapy can hardly get survival benefit for such patients. As early as 130 years ago, William Coley developed a heat-inactivated bacterial mixture called "Coley toxin" [1,2]. In the following 30 years, he used this "toxin" to treat more than 1,000 cancer patients and published more than 150 related papers [3]. Many patients who lost their hope of survival survived miraculously for a long time after this simple treatment [4-6]. Coley's discovery and practice have caused great controversy and have been criticized by many [3]. In the end, history sided with Coley. Many years after his death, some controlled trials re-proved that this "toxin" has anti-tumor effects [7]. He is therefore known as the "father of immunotherapy" [8].

As early as 40 years ago, Zhenyi Wang, who was known as the "father of induction-redifferentiation therapy" for inventing trans-retinoic acid therapy to successfully treat promyelocytic leukemia [9,10], proposed that cancer is heterogeneous, and cancer immune control should be "multi-target", it is recommended to use non-specific immunization methods to focus on activating innate immune function. Under the influence of the above ideas, a "Tumor Therapeutic Vaccine" (TTV) composed of a combination of bacteria and its toxin vaccines and adjuvants was manufactured in the laboratory and was voluntarily used by advanced cancer patients whose conventional therapies failed.

One of the authors of this article is a "volunteer" who received this combined vaccine TTV. In early 2006, he was confirmed to have intrahepatic cholangiocellular carcinoma (stage II). As an oncologist who has been engaged in liver cancer research for many years, he knows the poor prognosis of this cancer, and also knows that adjuvant radiotherapy and chemotherapy will not bring survival benefits [11], so in 2008 he randomly searched 38 patients with cancer in Shanghai area who had received TTV treatment due to failure of conventional treatment in the 1990s. Eleven of

them have died, but shockingly, only 5 died of cancer recurrence. So the author himself used TTV injection as an adjuvant treatment, and has so far survived disease-free for 17 years. In 2012, the author's younger sister was found to have advanced intrahepatic cholangiocarcinoma and had lost the opportunity for surgical treatment, and also only received this kind of TTV injection treatment. One year later, most of the liver tumors on CT disappeared, followed by disease-free survival for 4 years.

In 2019, we reported a case of advanced renal cancer with multiple systemic metastasis, which miraculously improved after receiving TTV treatment and had survived for 3 years [12] (in this investigation, this case was followed up again and listed as "case 9"). In order to promote further research, we conducted a "real world" survey on a total of 178 patients with advanced cancer who received TTV treatment after 2008. Among them, 68 patients had failed conventional treatment and only received TTV treatment. The results of the inquiry are now reported as follows.

Patients and Methods

Case selection

The investigated cases meet the following conditions: (1) The disease is in stage III or IV, and the diagnosis is confirmed by pathology; (2) Conventional treatment, including surgery, radiotherapy and chemotherapy, molecular targeted therapy, anti-PD-1 treatment failure or disease relapses. TTV injection started at least 4 weeks after the end of the above treatment; (3) No clinical and laboratory evidence of liver, kidney, and heart failure; (4) No special “anti-cancer” treatment was received during the TTV injection; (5) The life expectancy of the patient at the start of TTV injection was not less than 3 months; (6) There is imaging evidence that is sufficient to judge the condition; (7) Voluntary "face-to-face" field investigation.

Vaccine production: TTV is a vaccine complex of a variety of bacteria or their toxins. The bacterial vaccine is obtained from commercially available legal products or cultured in a laboratory. The main bacterial vaccines include *B. pertussis*, typhoid fever/paratyphoid A/B, and *Staphylococcus aureus*, with a bacterial content of 1.5 to 9 billion/ml; bacterial toxin vaccines include diphtheria toxin and tetanus toxin, with a content of 5 Lf/ml to 20 Lf/ml. Adjuvants include Poly I:C, glucan, fat emulsion and appropriate buffer. After being prepared according to certain procedures and techniques, it undergoes a series of sterilization and non-toxic treatments, and then tests for toxicity and tumor inhibition rate in experimental animals. At least 3 of the preparation and experiment personnel will each perform at least 2 injection tests on themselves and observe the reaction for a week.

Treatment method

TTV should be given deep subcutaneous injection into the arm or near the tumor. The best response is local redness and swelling, and slight whole body fever (Figure 1). The initial dose is 1 ml/time, depending on the degree of reaction; the dose can be appropriately increased to 2.5 ml to 4.0 ml each time. It is generally injected once a week. Depending on the patient's condition and treatment response, the interval between injections can be shortened or extended. The treatment period should be extended as much as possible, at least 6 to 12 months.

Follow-up

All cases will be followed up face-to-face by at least two of the authors of this article. If the patient has died, go to the patient’s home or hospital to retrieve relevant disease data. The judgment of the condition is mainly based on ultrasound, CT and/or Magnetic Resonance (MR) scans, as well as blood tumor markers. Tumor Response is based on the Evaluation Criteria of Solid Tumor response (RECIST); the survival period is from the beginning of TTV injection to the last follow-up or death of the patient.

Result

A total of 68 patients were followed up, including 32 males and 36 females, aged 8 to 89 years, with a median of 54 years. The follow-up results are shown in Table 1. Before receiving TTV injection, 18 patients had undergone surgery, 12 had received percutaneous cryoablation, 36 had received chemotherapy, 18 had received radiotherapy, and 16 had received targeted therapy. Results: The period of receiving TTV was 3 to 96 months, with a median of 24 months. Tumor response: 30 cases (44.1%) had a Complete Response (CR), 25 cases (36.7%) had a Partial Response (PR), and 13 patients (19.1%) the Stable Disease (SD); the patient's survival period was 8 to 204 months, with a median of 48 months. At the last follow-up, 33 patients (48.5%) were still alive, and 35 patients (51.4%) had died.

Case Series

Case 1

Male, 70 years old. Diagnosis: Non-small cell lung cancer. In early 2010, the patient was diagnosed with left upper peripheral lung cancer and left iliac bone metastasis. Pathology showed undifferentiated adenocarcinoma with EGFR mutation. Received chemotherapy and targeted therapy, and radiotherapy and targeted therapy for metastasis to the left sacral area. One month later, the tumor mass in the upper left lung increased to 8.2 cm × 4.8 cm, accompanied by micro-nodules metastasis in both lungs. In August and September 2016, percutaneous cryoablation was performed on the left upper lung tumor twice. Two weeks later, a cavity was formed at the cryoablation site in the tumor (Figure 2A). One month later, he underwent thoracoscopic left upper lobectomy and mediastinal lymph node dissection due to heavy hemoptysis. The pathological examination of the surgically resected lung tissue showed a large number of cancer cells infiltration at the surgical margins (Figure 2B), and the blood tumor markers CA125, CEA, and CA19-9 were elevated. Since October 24th, 2016, he has been receiving TTV treatment. Two months later, the CT scan showed "no evidence of cancer" in the lungs (Figure 2C). The blood tumor markers CA125, CEA and CA19-9 all fell to the normal range. As of early 2019, there was no evidence of recurrence at the primary lung cancer site, tumor masses appeared in the left armpit and neck, and the biopsy was adenocarcinoma, but the gene test was negative for EGFR mutation, and it did not respond to the increased injection dose of TTV, suggesting a tumor multicentric growth and heterogeneity. The patient finally died of interstitial pneumonia complicated by anti-PD-1 therapy.

Case 2

Female, 46 years old. Diagnosis: Small cell lung cancer (confirmed by cytology) (Figure 3A). In December 2015, the patient was diagnosed with left lung cancer with mediastinal lymph node metastasis. After receiving concurrent radiotherapy and chemotherapy, the lung tumor disappeared. Eight months later, PET/CT examination revealed a new tumor in the left lung (Figure 3B), accompanied by C11 thoracic spine metastasis and multiple brain metastases. There was a large pleural effusion. The blood tumor marker CA125 was significantly increased. The patient complained of severe back pain

Table 1: TTV treatment results of 68 patients with advanced cancer.

Diagnosis	Case	TTV use mons (median)	Tumor response (cases)	Survival monms (median)	At the last follow-up* (cases)
Non-small cell lung cancer	11	5-60 (19)	CR 7, PR 2, SD 2	20-192 (38)	S 5/D 6
Small cell lung cancer	1	6	CR 1	15	D 1
Breast cancer	6	4-34 (23)	CR 2, PR 1, SD 3	28-180 (52)	S 5/D 1
Esophageal cancer	4	28-12	CR 3, PR 1	28-120	S 2/D 2
Gastric cancer	5	12-24 (14)	CR 3, PR 2		S 2/D 3
Colorectal liver metastases	6	12-34 (17)	CR 1, PR 5	24-168(45)	S 2/D 4
Hepatocellular carcinoma	6	5-42 (8)	CR 2, PR 2, SD 2	8-56 (35)	S 1/D 5
Intrahepatic cholangiocarcinoma	2	24, 6	CR 1, SD 1	60, 10	D 2
Pancreatic ductal adenocarcinoma	4	3-84	CR 1, PR 1, SD 2	17-110	S 1/D 3
Liver metastasis of pancreatic solid pseudopapilloma	1	15	PR 1	88	S 1
Pancreatic neuroendocrine neoplasm (MEN I)	1	36	PR 1	96	D 1
Renal cell carcinoma	4	33-96	CR 2, PR 2	40-176	S 4
Adrenocortical carcinoma	1	36	PR 1	84	S1
Bladder Cancer	3	14-08	PR 2, CD 1	96-144	S 3
Prostate cancer	5	12-28 (13)	CR 2, PR 2, SD 1	32-64 (36)	S 3/D 2
Uterine-cervical cancer	2	18	PR 2	20	D 2
Nasopharyngeal carcinoma	2	24	CR 1, PR 1	36-120	D 2
Tonsil cancer	1	32	CR 1	63	S 1
Melanoma	1	72	CR 1	145	D 1
Lymphoma	2	26, 28	CR 1, PR 1	120, 204	S 2
Total	68	3-96 (24)	CR 30, PR 25, SD 13	8-204 (48)	S33/D35

Note: CR: Complete Response; PR: Partial Response, SD: Stable Disease; *S: Survive; D: Died

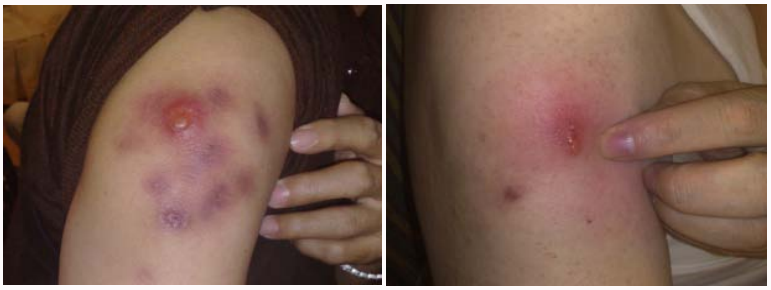

Figure 1: Local reaction of TTV injection.

and was extremely depressed. Since November 11th, 2016, the patient has received subcutaneous injections of TTV. After more than a month, the pleural effusion disappeared. Subsequently, patient's pain disappeared completely and she returned to normal life. Follow-up PET-CT showed that most of the left lung tumor disappeared (Figure C), and tumor markers such as CA125 and Neuron-Specific Enolase (NSE) completely dropped to the normal range (Figure 3, bottom panel). 15 months later, the patient died of a lung infection.

Case 3

Female, 48 years old. Diagnosis: Infiltrating ductal adenocarcinoma of the breast. In May 2009, the patient underwent breast-conserving tumor resection on the right breast. In May 2016, metastases of the ipsilateral axillary lymph nodes and T8 thoracic spine occurred. Also, the tumor recurred at the surgical site of the right mammary; the tumor mass rapidly increased to 6 cm × 8 cm in size and the surface was ulcerated. The patient underwent modified radical resection of right breast cancer. Several MRI reviews showed that T8 metastasis was active (Figure 4A). The patient received a subcutaneous injection of TTV. Three months later, MR examination showed that the T8 metastasis had lost activity (Figure 4B). After that, CT and MR follow-up were performed regularly, and no increase in metastatic activity and new metastases were found (Figure 4C). The patient has returned to normal work so far.

Case 4

Male, 73 years old. Diagnosis: Colon cancer with liver metastasis. In June 2009, the patient underwent sigmoid colon cancer resection, and multiple liver metastases were found during the operation. After that, he received 3 cycles of chemotherapy. The patient was forced to discontinue chemotherapy due to his weakness. PET-CT showed a 6 cm × 6 cm space-occupying lesion in the right liver (Figure 5A). In March 2010, the patient started receiving TTV treatment. After 2 weeks, the patient's mental state improved significantly and he was able to walk freely. After 3 months, CT examination showed a significant shrinkage in intrahepatic metastasis (Figure 5B). One

Figure 2: The course of treatment of Case 7. A: After the left upper lung tumor was subjected to percutaneous cryoablation, CT showed large pieces of residual cancer tissue and cavities in the tumor; B: The resected tissue contained large tumor masses, and histological examination of the surgical margin showed a large amount of infiltration of adenocarcinoma; C: CT scan 2 months after TTV treatment, showed "no evidence of cancer" in the lung parenchyma.

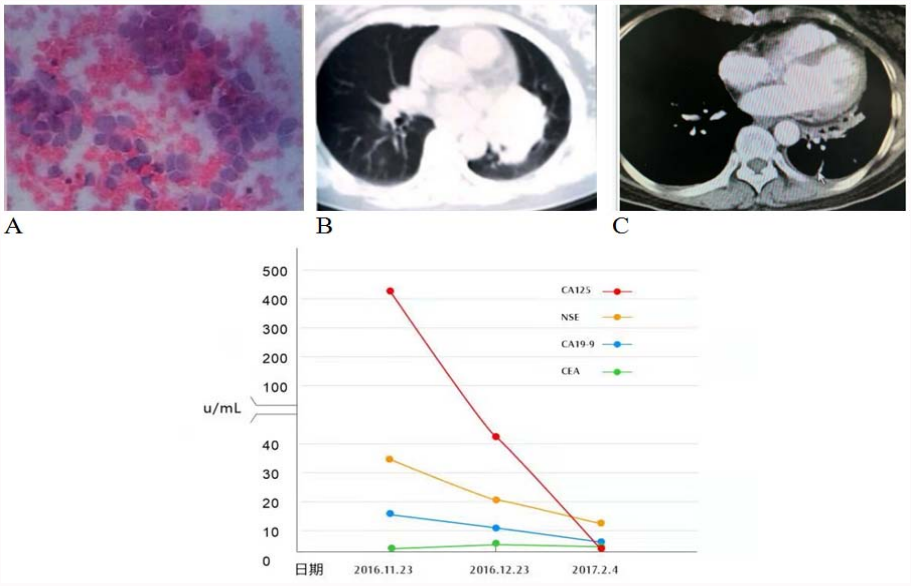

Figure 3: Imaging and tumor markers of Case 2. In the above figure, A: Intrabronchial cytology showed small cell lung cancer, B: PET-CT before TTV treatment showed a large tumor in the left lung, C: After TTV treatment, most of the tumor in the left lung disappeared. The figure below shows after TTV treatment, blood tumor markers decreased, with CA 125 and NSE decreased most significantly.

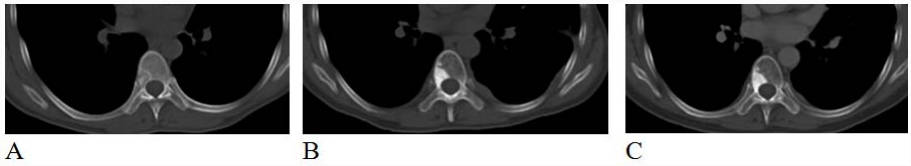

Figure 4: Bone metastasis of Case 3 with breast cancer. A: T8 thoracic spine metastasis (Arrow pointed); B: T8 metastasis had calcified after TTV treatment; C: MR follow-up 2 years later showed that T8 metastasis was still calcified.

year later, PET-CT follow-up showed that the original intrahepatic metastasis disappeared (Figure 5C). The patient survived disease-free for 6 years, finally died of a heart attack.

Case 5

Female, 45 years old. Diagnosis: postoperative recurrence of hepatocellular carcinoma with multiple metastases. In 2007, she underwent hepatectomy for hepatocellular tumor. Liver cancer recurred in 2008. Ultrasound and CT showed large diffuse liver lesions with multiple metastases in the hilar, spine, and posterior abdominal lymph nodes (Figure 6A). There was deeper jaundice, and multiple nodular metastases under the skin of the abdominal wall (Figure 6B). The patient suffered severe pain in the lower back and lower limbs, unable to walk, and had to rely on morphine to relieve pain all day long. The patient received TTV injections in the arm and abdominal wall adjacent to the metastatic nodules. After 3 days, the patient's back pain was reduced by 50%. One week later, the patient's pain was further relieved and she could get out of bed and walk on her own. Ultrasound showed that the tumor in the liver had no obvious changes and shrank, but the subcutaneous nodules in the abdominal wall disappeared (Figure 6C). After 2 months, the patient's pain disappeared completely. She survived for 14 months without progression, and finally died of hemorrhage caused by the rupture of esophageal varices.

Case 6

Female, 66 years old. Diagnosis: intrahepatic cholangiocarcinoma. In early November 2012, MR and PET-CT showed multiple low-density shadows in the left inner lobe and right posterior lobe of the liver, with unclear edges and fusion (Figure 7A). Blood CA19 and

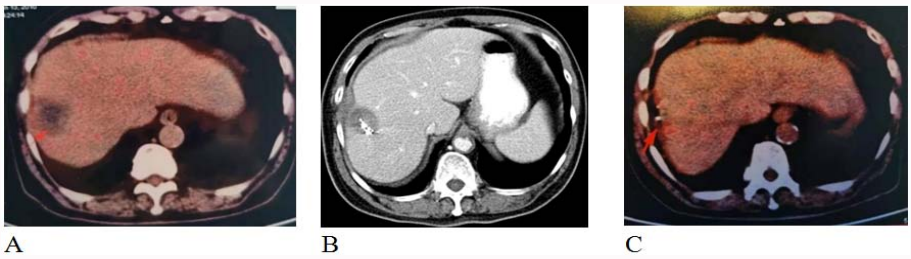

Figure 5: Imaging examination of case 4. A: Before TTV treatment, PET-CT showed a 6 cm-sized space-occupying lesion in the right liver with a dark halo; B: After TTV treatment for 3 months, MR showed that the right lobe of the liver had reduced metastasis; C: One year later, PET-CT showed that the original right lobe metastasis basically disappeared.

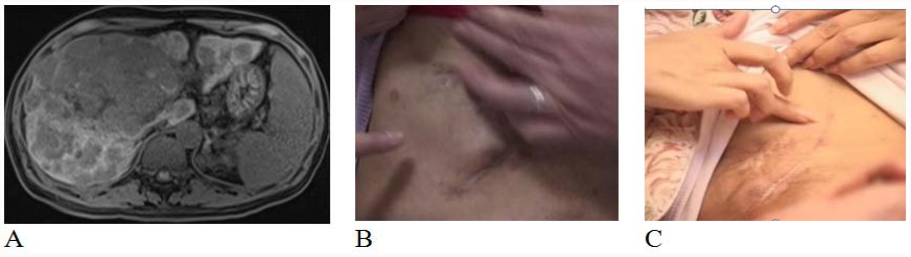

Figure 6: The imaging findings of case 5. A: CT shows large masses of multiple space-occupying lesions in the liver and hilar; B: multiple metastatic nodules under the abdominal wall before TTV treatment; C: subcutaneous nodules disappeared after TTV treatment.

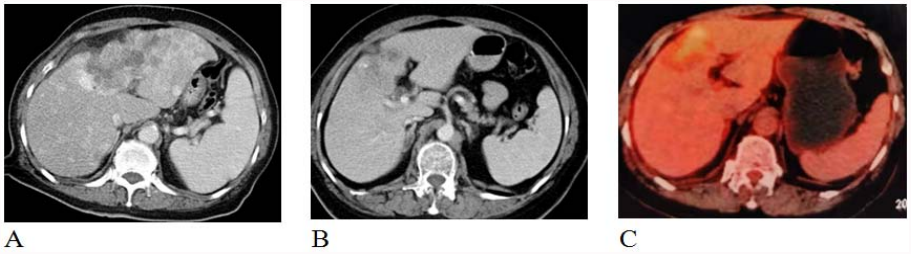

Figure 7: Liver imaging findings of case 6. A: Before TTV treatment, MR showed massive intrahepatic space-occupying lesions with multiple intrahepatic metastases; B: 90% of intrahepatic lesions disappeared after 1 year of TTV treatment; C: PET-CT after 2 years showed intrahepatic space-occupying damage remains stable.

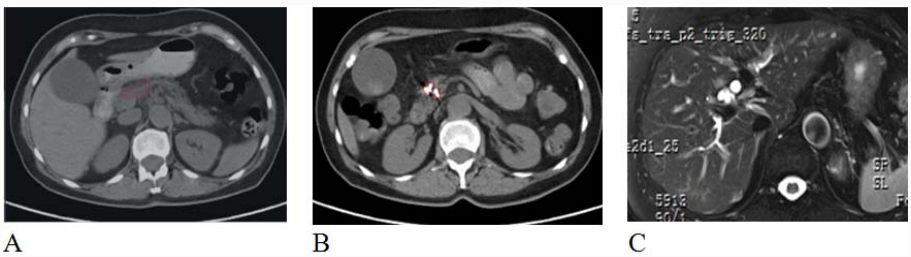

Figure 8: A: Example 7 CT showing a space-occupying lesion in the neck of the pancreas with liver metastasis (liver metastasis not shown); B: After TTV treatment, the pancreatic tumor shrank and the liver metastasis disappeared; C: MR showed recurrence of liver metastases 7 years later.

CA 125 are elevated. The patient received palliative percutaneous cryoablation to reduce tumor burden. TTV injection was given in December of the same year for 2 years. One month after TTV treatment, the patient's general condition was significantly improved, and subsequent CT and PET-CT follow-up showed that the intrahepatic tumor was gradually shrinking (Figure 7B, 7C). Until the end of 2017, the patient had liver tumor recurrence on CT and then died of gastrointestinal bleeding. The patient's overall survival period was 4 years.

Case 7

Female, 65 years old. Diagnosis: Pancreatic ductal cell adenocarcinoma. In 2007, she underwent an ultrasound examination showing a space-occupying pancreatic lesion. After 3 cycles of chemotherapy, the pancreatic lesions did not shrink, and multiple metastases appeared in the liver. In March 2008, the patient received a subcutaneous injection of TTV. Three months later, CT follow-up showed that pancreatic tumors shrank and liver metastases disappeared (Figure 8A, 8B). In 2015, the MR follow-up revealed scattered small metastases in the liver (Figure 8C). In 2017, the patient

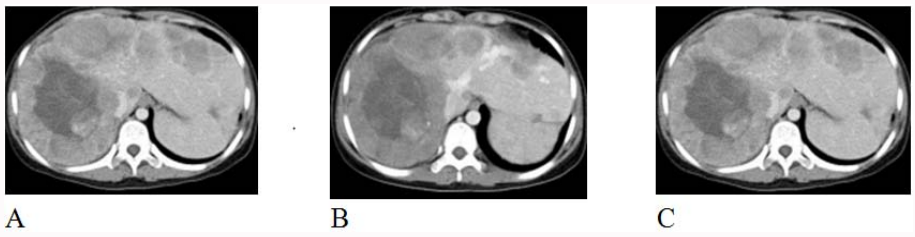

Figure 9: The liver CT of case 8 showed that within 7 years after TTV treatment, from 2015 (A), 2017 (B) to 2019 (C), there was no obvious progression of intrahepatic lesions.

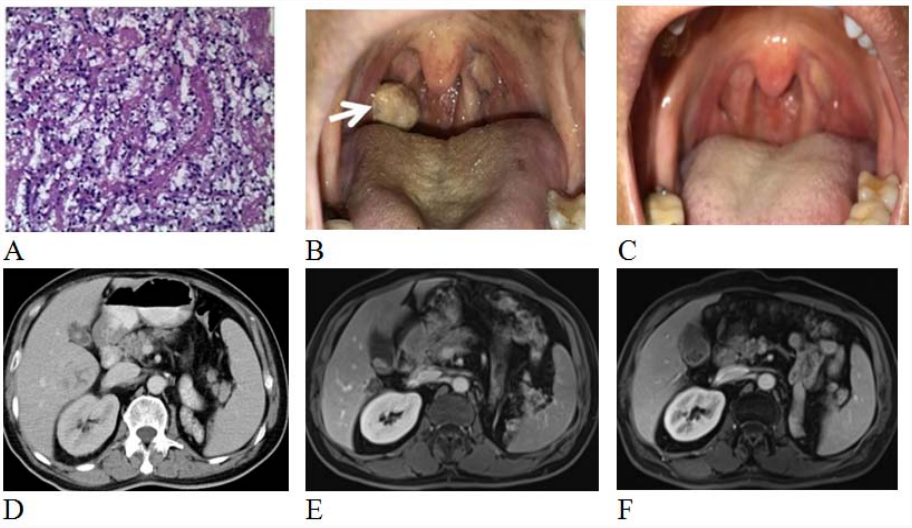

Figure 10: Image changes before and after TTV treatment of case 9. A-C shows the patient's right tonsil metastasis. A: Tonsil biopsy histology showed metastasis of renal clear cell carcinoma (HE staining), B: Gray-white tumor mass of right tonsil before TTV treatment (pointed by arrow), C: Right tonsil tumor mass disappeared 3 weeks after treatment; D-F showed liver metastasis (D, CT, indicated by the arrow) becomes smaller after TTV treatment (E, MR, after 8 weeks of TTV treatment), until it disappears (F, MR, after16 weeks of TTV).

received the last follow-up. At this time, there were still metastases in her liver, but the patient showed "living with cancer" and was generally in good condition.

Case 8

Female, 16 years old. Diagnosis: Pancreatic solid pseudopapilloma with multiple metastases in the liver and abdominal cavity. In March 2009, the patient underwent palliative tumor resection due to massive space-occupying lesions in the pancreas and abdominal cavity. The CT scan in January 2014 showed multiple nodular and massive lesions of the liver, cancerous thrombosis in the left and right portal veins, enlarged lymph nodes of the left supraclavicular, hilar, and retroperitoneal area. Liver tumor biopsy revealed pancreatic solid pseudopapillary tumor metastasis. Half a month later, the patient's condition deteriorated, with jaundice and ascites. The white blood cells and platelets were severely reduced, and the prothrombin time was prolonged by 50%. From August 25th, 2014, patients received TTV injections. One week later, the patient's general condition gradually improved, and the white blood cells and platelets rose to normal levels. After 3 weeks, the jaundice and ascites had completely subsided. The patient has been engaged in normal work for more than 7 years. Multiple CT and ultrasound follow-ups showed no significant progress of intrahepatic metastasis (Figure 9).

Case 9

Male, 56 years old. Diagnosis: Grade II clear cell carcinoma of the kidney with multiple metastases throughout the body. In early January 2015, the patient underwent a radical resection of the left kidney due to a tumor in the left kidney. In April 2016, he had multiple metastases in the right lung and received sunitinib and anti-PD-1 therapy successively, but no significant effect was seen. CT and MRI examinations showed dozens of metastases, involving the right lung and pleura, liver, mediastinum and hilar lymph nodes, bladder wall, scapula, sixth rib, first lumbar vertebra, sacral hip, etc. The patient was severely anemic, required weekly blood transfusions, has pain in his lower limbs with difficult walking. There was a walnut-sized mass on the right tonsil, with blood oozing on the surface, and a biopsy revealed metastasis of clear cell renal cell carcinoma. On April 12th, 2017, the patient began to receive TTV subcutaneous injections. Ten days later, the patient's lower limb pain eased and the right tonsil mass shrank. After 3 weeks, the limb pain disappeared completely, the hemoglobin level gradually increased, no blood transfusion was needed, and the tonsil mass disappeared completely (Figures 10A-10C). CT and MR examinations showed that all metastases had withdrawn, and the metastases almost completely disappeared after 3 months (Figure 10D-10F). So far, he has progression-free survival for 5 years.

Case 10

Male, 54 years old. Diagnosis: nasopharyngeal carcinoma with brain and lymph node metastasis (Figure 11A). In March 2004, after receiving radiotherapy and regional chemotherapy, nasopharyngeal tumors and metastatic lymph nodes disappeared. After 3 years, the patient complained of headache and diplopia. PET-CT showed brain

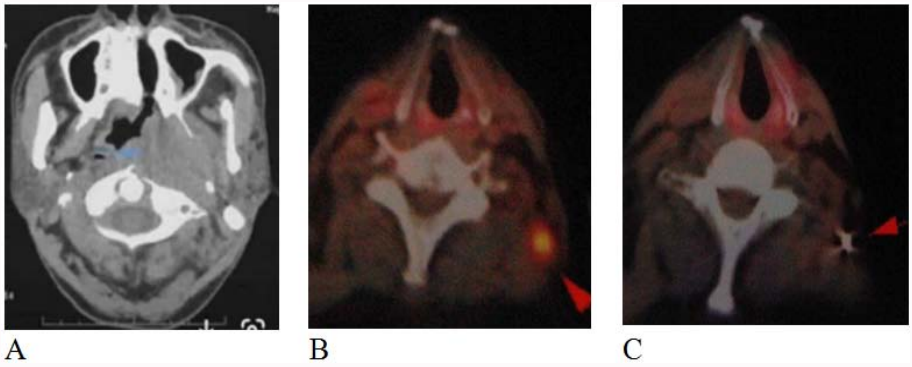

Figure 11: The imaging findings of Case 10. A: CT showed neoplasms in the nasopharynx; B: 4 years later, PET-CT showed enlarged lymph nodes in the anterior neck with enhanced activity (arrow points); C: PET-CT follow-up after one year of TTV treatment showed that metastatic lymph nodes had lost activity (arrow pointing).

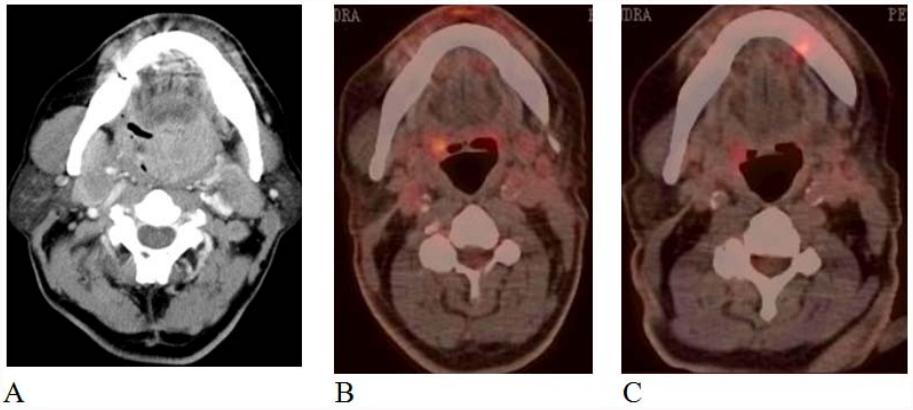

Figure 12: The imaging examination of Case 11. Before treatment, on November 5th, 2016, the tumor of the left tonsil occupied almost the entire pharyngeal cavity (A); the follow-up of PET-CT (B) on October 29th, 2019 and PET-CT (C) on November 29th, 2021 were both showed that the original pharyngeal tumor was no longer present.

metastasis and multiple lymph node metastases in the neck (Figure 11B), and lymph node biopsy confirmed metastatic squamous cell carcinoma. He has been receiving TTV treatment since April 2008. After 3 months, headache was significantly improved, and diplopia disappeared. Follow-up PET-CT in May 2009 showed that the metastatic lymph nodes disappeared (Figure 11C). After that, the patient returned to normal life and work.

Case 11

Male, 73 years old. Diagnosis: Cancer of the tonsils with extensive metastasis to cervical lymph nodes. On September 28, 2016, the PET/CT examination revealed a mass of about 4.5 cm in the left tonsil (Figure 12A). The upper boundary of the mass reached the soft palate, and the lower boundary reached the base of the left tongue, invading the left epiglottis. There were multiple enlarged lymph nodes on both sides of the neck, posterior pharynx, and right mandible. A biopsy of the tonsil mass revealed a moderately differentiated squamous cell carcinoma. The patient underwent palliative cryoablation of left tonsil tumor to reduce tumor burden. On November 20th, 2016, the patient began to receive TTV treatment. One year later, the patient underwent extensive resection of cervical lymph nodes twice. Pathological examinations showed extensive tumor necrosis and scar formation, and there was no evidence of cancer. The PET-CT follow-ups in September 2017 and November 2021 both showed “no signs of tumor recurrence” (Figure 12B, 12C). The patient has survived disease-free for 5 years so far.

Case 12

Male, 59 years old. Diagnosis: Sinus melanoma. In November 1991, he underwent partial resection of the sinus tumor (70% of the tumor was removed) and middle turbinate resection at a hospital in Shanghai, China. During the operation, a black neoplasm of 7.5 cm × 5cm × 5 cm in the ethmoid sinus was displayed, extending to the left middle turbinate, top of the nose and the side wall of the eye socket (see surgical record, Figure 13A). Histology confirmed melanoma (Figure 13B). Postoperative radiotherapy was given with no response. Since March 1992, patients had received TTV subcutaneous injection (provided by Dr. Kong). Three years later, CT examination showed that the tumor in the sinus area was no longer visible (Figure 13C). The patient survived asymptomatically until the tumor recurred in early 2004 and finally died of massive nose bleeding. Since the TTV injection, the overall survival period is more than 13 years.

Discussion

TTV is a new type of therapeutic agent against cancer, which is a combination of common bacteria and their toxin vaccines and chemical adjuvants, but its prototype can be traced back to the "Coley toxin" developed by Coley more than 130 years ago. As mentioned earlier, the effectiveness of Coley toxin treatment had been strongly suspected and criticized, and it had not become a drug in the end. But even by today's standards, Coley's results are remarkable. Richardson et al. [13] compared 128 cases of Coley treatment with 1,675 cases

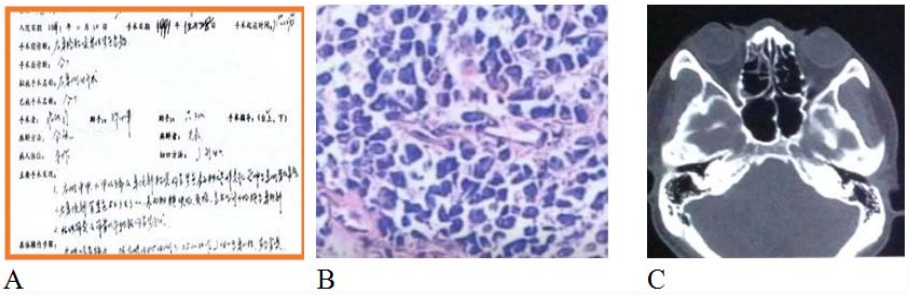

Figure 13: The treatment history of Case 12. A: Surgical records showed that the left sinus tumor was not completely removed; B: The pathological diagnosis of the removed tumor was melanoma (HE staining); C: CT follow-up after 3 years showed no obvious lesions in the sinus area.

matched with modern cancer therapy as a control group, and found that the median survival time of the "Coley toxin" treatment group was 8.9 years, while that of the control group was 7.0 years. The proportion of sarcoma patients who survive for 10 years was 50%, while only 38% of patients receiving modern treatment. The 10-year survival rate of patients with kidney cancer and ovarian cancer was also higher in the "Coley toxin" group.

In the past few decades after Coley, there have been three randomized and non-randomized controlled studies. In 1962, Johnson and Novales [14] reported the results of the 7-year study. 34 patients with advanced inoperable metastatic cancer received Coley toxin, and 37 similar patients in the control group received typhoid vaccine. The objective responses were 9/34 and 1/37 respectively. One case in the treatment group survived for 3 years, and no case in the control group survived for more than 1 year. In 1991, Zhaoyou Tang team in China evaluated 86 cases of hepatocellular carcinoma who received conventional treatment or conventional treatment combined with Mixed Bacterial Vaccine (MBV). The results showed that in patients who cannot be surgically removed, the addition of MBV can improve the 2-year survival rate. In addition, MBV improved the "second look" resection rate to 40% as compared to 17% in the control (P greater than 0.05). MBV could also prevent such immunosuppression as decrease of macrophage activity caused by radiotherapy [15]. The third randomized controlled studies were completed by the Sloan Kettering Memorial Cancer Center in the United States. Thirty cases of advanced nodular lymphoma received conventional radiotherapy and chemotherapy (conventional group), and 26 cases received mixed bacterial vaccine (combined group) before radiotherapy and chemotherapy. The results show that compared with the conventional group, the combined group has a higher complete response rate (85% *vs.* 44%), a lower recurrence rate (20% *vs.* 42%), and a longer survival period [16].

Over the years, different researchers have made more than 20 versions of vaccines, imitating Coley toxins, some of which are more effective. Among them, the "Tracy vaccine" is considered the most successful. From 2007 to 2012, 70 patients with advanced cancer, including melanoma, lymphoma, and malignant tumors of the breast, prostate, and ovary, received the vaccine. About 70% of patients had tumor shrinkage, and 20% of patients had a complete remission [17]. In Japan, OK-432, a streptococcal preparation with biological response modification activity, was approved as an anticancer agent in 1975 and has become a conventional auxiliary agent for the treatment of cancer [18].

In the past few decades, we have screened a number of effective formulations based on the inhibitory effects of vaccines against different bacteria or their toxins on experimental tumor models, and developed an anti-cancer compound vaccine called TTV. From the above retrospectively investigation the effects of TTV are as follows:

- First, the tumor response showed CR, and long-term survival of more than 5 years was obtained. For example, Case 3 breast cancer bone metastasis (5 years), Case 11 tonsil cancer (5 years), case 4 colon cancer liver metastasis (6 years), and Case 12 melanoma (13 years) belong to this category;

- Second, the tumor response is shown to be PR, and the long-term survival is more than 3 years. Case 6 intrahepatic cholangiocellular carcinoma (4 years), Case 9 kidney cancer (over 5 years) and Case 7 pancreatic ductal adenocarcinoma (over 9 years) belong to this situation;

- Third, the tumor response shows CR, but the survival period is very short, generally shorter than 3 years, such as case 1 non-small cell lung cancer (25 months), Case 2 small cell lung cancer (15 months) and Case 10 nasopharyngeal carcinoma (17 months);

- Fourth, the tumor response shows SD, but long-term survival. For example, in Case 8 with liver metastases from a solid pseudopapillary tumor of the pancreas, the intrahepatic tumor has not improved in more than 7 years, but the patient survived, and the quality of life was very good, which is a typical "coexistence with cancer";

- Fifth, the primary tumor has no obvious changes in imaging, but the patient's symptoms are significantly improved, especially effective pain relief. Case 5 liver cancer has multiple metastases, with severe pain that is difficult to stop. The pain improved rapidly after TTV treatment, and the progression-free survival was up to 14 months.

Given that the subjects of the retrospective investigation in this article are all patients with a short expected survival period, the results of the above-mentioned TTV treatment, no matter which one is, are encouraging. The biggest flaw of the study is that there is no control group, but with the increase of follow-up cases, especially when the cases that enter the statistics are limited to "routine treatment failure" and "only receiving TTV", we firmly believe that the effect of TTV is sure. Of more importantly, its high safety, except for local redness and swelling and mild fever for 1 to 3 days after injection (it seems that these reactions are necessary to produce curative effects), almost no patients complained of serious adverse reactions ease of use is also its greatest advantage.

The treatment principle of TTV is unclear. It is known that certain

malignant tumors such as breast cancer, renal adenocarcinoma, neuroblastoma, melanoma, bladder sarcoma or carcinoma can spontaneously shrink after spontaneous fever or infection by bacteria, fungi, viruses or protozoa [19]. Some experiments have also shown that many facultative and absolutely anaerobic bacteria have anti-tumor effects, but these microorganisms that colonize the tumor will not directly dissolve the tumor [20]. It is currently believed that immunity, especially innate immunity, plays a role [21,22].

As early as 1909, Paul Ehrlich put forward the view that the immune system has the ability to recognize and eliminate cancer cells [23]. More than 50 years later, Burnet and Thomas further proposed the "immune surveillance" hypothesis [24,25]. Subsequently, the interaction between the immune system and cancer was further refined into an "immune editing" process involving the intersection of elimination, balance and escape [26]. In the elimination phase, the immune system recognizes and destroys many (but not all) abnormal cells. During the balance period, the immune system and the tumor exert opposing forces to suppress the tumor. TTV is composed of a variety of bacterial components and is likely to be used as a non-specific immunostimulant for elimination and balance immunity.

It is currently believed that cancer is not a disease, but a complex, heterogeneous disease in which different cancer cells affect each other as the environment changes [27]. Moreover, even the same type of cancer cells mutate from time to time. In fact, "cancer is a moving target" [28]. Effective immunotherapy requires a better systemic immune status [29]. "Precision treatment" is relative [30]. In a sense, "fuzzy" treatment for multiple targets may be more important [31]. The advantage of TTV lies in its "fuzziness". In addition to different bacterial components, the glucan contained in it is itself an effective and safe immune response stimulant [32].

According to our investigation, in most cases, TTV cannot cure cancer, but "controls" the tumor within a certain period of time. The tumor response to TTV is heterogeneous. First, different cancers respond differently to TTV. Non-small cell lung cancer, kidney cancer, and melanoma have a significant response to TTV, while small cell lung cancer has a rapid response, but the duration is very short, suggesting that the characteristics of cancer itself play an important role in the response to TTV. Furthermore, even for the same type of cancer, the response of cancer cells can vary from place to place. The primary lung cancer of Case 1 had a particularly rapid and significant response to TTV. It showed CR after only 2 months of TTV treatment, but the therapy failed to prevent metastasis in the armpit and neck after one year, which had no response to TTV treatment.

For TTV, there are many questions that need to be studied, such as (1) the best dose and route of treatment? According to Coley's report, Coley toxin was administered intravenously, and high fever was required for effectiveness after injection, and it was injected daily. Fever was an important sign of effectiveness. Patients with sarcoma treated by Coley who survived for more than 5 years had a fever of 38°C to 40°C. TTV treatment is relatively simple, using subcutaneous injection, the fever after injection is controlled not to exceed 38.5°C, and it is generally injected once a week. What needs to be explored is to increase the number of injections or doses to induce higher heat, which will have a better therapeutic effect? (2) The most suitable course of treatment? In our follow-up cases, some have been continuously using TTV for up to 8 years, while others have only been used for a few months. It seems that there is no clear correlation between the course of treatment and the efficacy; (3) The best combination of TTV components? Coley toxin and others the version of the mixed bacterial vaccine basically only contains *Streptococcus* and *Serratia*, while TTV is composed of a vaccine of 6 kinds of bacteria or their toxins plus chemical adjuvants. It has been proved that some microorganisms such as diphtheria, *Staphylococcus aureus*, *Bacillus pertussis*, *Bacillus diphtheria*, and even yeast have shown the effect of anti-cancer immunity. Some viruses, including *Measles* virus and herpes virus, also have the ability to stimulate immunity [33,34]. Therefore, it is a very meaningful work to screen the most effective combination; (4) There have been some studies showing that there is a correlation between the intestinal microbiome and the clinical response of anti-PD-1 immunotherapy [35]. Whether the anti-tumor immunity produced by microbial vaccines, like TTV, has a synergistic effect with cancer immunotherapy against immune checkpoints is worthy of further study.

In conclusion this "real-world" survey of 68 patients with advanced cancer shows that TTV, which is a combination of vaccines from a variety of bacteria or their toxins and adjuvants, can promote tumor regression or stabilization, and bring survival benefits to patients, in some cases, can cause a miraculous reversal of the disease. Although this is an uncontrolled retrospective study, considering that all cancer patients under investigation have received conventional treatment and failed, the effect of this simple treatment on patients should be encouraging and sufficient to prompt more researchers continue to conduct more studies, especially multicenter randomized controlled trials.

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