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Phase I Trial of Active Specific Immunotherapy with Autologous Dendritic Cells Pulsed With Autologous Irradiated Tumor Stem Cells in Hepatitis B-positive Patients with Hepatocellular Carcinoma

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Background and Objectives: Hepatocellular carcinoma (HCC) is often associated with chronic hepatitis due to hepatitis-B or -C viruses. Active specific immunotherapy (ASI) with autologous dendritic cells (DC) presenting antigens from autologous tumor stem cell (TC) lines is associated with promising long-term survival in metastatic cancer, but hepatitis patients were excluded. ASI might benefit high-risk primary HCC patients following surgical resection, but first it is important to show that ASI does not exacerbate hepatitis.
Methods: Previously untreated HCC patients with a solitary lesion >5 cm, or three lesions with at least one >3 cm, or more than three lesions, underwent surgical resection from which autologous TC lines were established. Irradiated TC were incubated with autologous DC to create DC-TC. After one course of trans-arterial chemoembolization therapy (TACE), three weekly subcutaneous injections of DC-TC suspended in granulocyte-macrophage colony stimulating factor were administered. Patients were monitored for eight weeks.
Results: HCC cell lines were established within five weeks for 15/15 patients. Eight patients, all with chronic hepatitis B, were treated. There was no increase in hepatic transaminases, hepatitis B antigens, or viral DNA.
Conclusion: Autologous DC-TC did not exacerbate HBV in these HCC patients. A phase II efficacy trial is being planned.
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KEY WORDS: **hepatocellular carcinoma; hepatitis B; dendritic cell vaccine; tumor stem cells; therapeutic cancer vaccines**

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common and highly lethal malignancy, with more than 500,000 cases diagnosed annually and about as many deaths world-wide [1]. Globally HCC is the 3rd most common cause of cancer death in men, and the 5th most common cause of cancer death in women. The incidence of HCC has been steadily increasing for several decades. Most HCC (70–90%) occurs in the setting of cirrhosis of the liver. In Asia cirrhosis is usually a consequence of chronic hepatitis B virus (HBV) infection [2,3], while in the United States and Western Europe, it is more often associated with hepatitis C infection (HCV), alcoholism, or non-alcoholic hepatosteatosis (NASH) [4,5]. US SEER estimates include 33,190 new HCC cases and 23,000 deaths in 2014, accounting for 2.0% of new cancers and 3.9% of deaths [6].HCC patients diagnosed during 2003–2009 and accessioned to the cancer registry of a large southern California community cancer program, had observed overall 5-year survival rate of only 12%, and a 5-year relative survival of 16%.

Because underlying liver disease with cirrhosis and portal hypertension are so common in HCC, liver function and Child-Pugh classification are important to assess suitability for surgery [7,8]. Recently the MELD (Model for End-stage Liver Disease) score has also been used, especially in patients with cirrhosis. [9] There are several other prognostic scoring systems that incorporate factors from several staging systems to help select candidates for surgery and liver transplantation. These include Okuda [10], the French Group d'Etude et de Traitment du Carcinome Hepatocellulaire (GTECH) [11], Barcelona Clinic Liver Cancer (BCLC) [12], the Cancer of the Liver Italian Program (CLIP) [13], the Chinese University Prognostic Index (CUPI) [14], and Japan Integrated System (JIS) [15].

The authors preferred the BCLC staging system because it uses key independent predictors of survival: 1) tumor staging (tumor size number of nodules, and portal vein invasion), 2) liver function (Child Pugh), and 3) overall health status. BCLC is the only system to have an independent predictive value on survival and the only system to stratify patients into treatment groups. Not only was the BCLC classification recommended in China, but it has also been approved by the European Association for the Study of Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD), and has subsequently been corroborated in

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clinical studies [16–20]. Several of these publications show the BCLC system in tabular form [17–19]. A study that validated the prognostic validity of the BCLC system led to the suggestion that BCLC-A be modified to exclude patients whose solitary lesions were >5 cm in diameter, and to include those patients in BCLC-B based on the similarity of their long-term survival [16,18].

There is controversy regarding what constitutes the best management for an individual with newly diagnosed localized HCC, since both surgical resection and liver transplant can be curative in certain settings [21–23]. The best candidates for partial hepatectomy with curative intent are non-cirrhotic patients with a solitary mass <5 cm in greatest diameter without major vascular invasion (AJCC T1N0) [23], and Child-Pugh A (5–6 points) hepatic function without portal hypertension. Asymptomatic, non-cirrhotic patients with tumors less than 2 cm in diameter have a 5-year survival rate of 90% after resection alone. [1] Other patients who are candidates for resection are Child-Pugh B (7–8 points) patients with a small solitary tumor, and those with multiple tumors all $<.5$ cm (AJCC T2N0), but no portal hypertension and a suitable liver remnant with adequate hepatic reserve [19]. Patients with a solitary tumor $<.5$ cm in diameter or three or fewer lesions, all $<.3$ cm in diameter, reportedly have a 5-year survival rate of 70–75%, although 70% recur within five years [1,21,24]. It is generally felt that these patients are best managed with resection and liver transplant.

HCC patients with underlying cirrhosis are at high risk for additional HCC; so liver transplant is the most curative approach for them. The United Network for Organ Sharing (UNOS) criteria for liver transplantation include a tumor $<.5$ cm or 2–3 tumors $<.3$ cm each, no invasion of major vasculature, and no extrahepatic disease [9]. The 5-year survival rate for such transplanted patients is 75% [1,21,25,27].

Patients who are poor candidates for a curative approach (solitary tumor >5 cm in diameter, or >3 lesions or 2 or 3 lesions if one is >3 cm in diameter) often undergo debulking surgery in combination with other therapies in an effort to increase survival [28–30]. Surgically debulked HCC patients typically receive adjuvant transhepatic chemoembolization (TACE) with agents such as doxorubicin and/or cisplatin based on randomized trials demonstrating increased progression free survival in unresectable HCC patients who received TACE [31–33].The tyrosine kinase inhibitor sorafenib increased median survival from 7.9 to 10.8 months in a placebo-controlled trial that included 602 patients with advanced unresectable HCC [34,35]. However, sorafenib needs to be given indefinitely as a chronic treatment, and is associated with toxicity, and eventually tumor resistance. It appears feasible to combine sorafenib with TACE, but is unclear whether this improves survival [36].

There is clearly an unmet need for an effective, non-toxic adjuvant therapy for surgically resected HCC patients who are not considered curable with standard approaches [37]. Ideally such a therapy would induce an enduring endogenous immune response against the cells responsible for recurrence and metastasis. One promising approach is a patient-specific vaccine strategy that utilizes autologous dendritic cells that have been pulsed with tumor stem cells derived from an autologous tumor cell line [38]. This approach has yielded promising results in a 54-patient single arm phase II trial [39], and a 42-patient randomized phase II trial conducted in patients with metastatic melanoma [40]. Theoretically such a patient-specific approach could apply to any tumor type [38,41], including HCC. However, the trials in melanoma excluded patients with underlying hepatitis B or C. For this reason we conducted a phase I safety trial in HCC patients with underlying HBV to generate early evidence of safety and tolerability of this approach.

PATIENTS AND METHODS

Regulatory Assurances

This trial was conducted in accordance with the ethical standards described in the Helsinki Declaration of 1975 as revised in 1983, using Good Clinical Practices (GCP) after approval by the Institutional Review Board of Hospital 85 of the Peoples Liberation Army (PLA) of China, and in accord with assurances filed with, and approved by, the Ministry of Health (MOH) in China, and in accord with the Department of Health and Human Services and Food and Drug Administrations (FDA) of the United States (US) and China. All patients gave written informed consent prior to participation. Eligible patients signed a consent form allowing submission of a portion of their resected hepatoma to a cell biology laboratory owned by Cellular Biomedicine Group, Inc., Shanghai, China, (CBMG) and staffed by personnel who were employed and directed by California Stem Cell, Inc., (now NeoStem, Inc.). There an effort was made to establish an autologous tumor cell line using Good Manufacturing Practices (GMP) and in accord with BB-IND 5838 and BB-IND 8554 on file with the US FDA. Patients consented for a leukapheresis to obtain peripheral blood mononuclear cells and to receive the DC-TC vaccine product when it became available. The conduct of the clinical trial was monitored by the Contract Research Organization (CRO), GCP CMIC Clin Plus Co LTs (Shanghai, China) under the sponsorship and direction of CBMG.

Human Subjects

Key eligibility criteria included previously untreated hepatocellular carcinoma that was not considered curable by resection or liver transplant. Patients were eligible for tumor harvesting if they were: (1) medically fit for surgery, with an Eastern Cooperative Oncology Group (ECOG performance status of 0–1, (2) pre-operatively classified as either BCLC-A but with a solitary tumor with a diameter greater than 5 cm, or BCLC-B (two or three lesions with at least one greater than 3 cm, or more than three lesions), (3) had lesions that were felt to be completely resectable with clear surgical margins, and (4) considered to be medically fit for subsequent leukapheresis and hepatic transcatheter arterial chemoembolization (TACE) after recovery from surgery. Patients had to have well-compensated underlying liver disease with a Child-Pugh Rating of A. Patients could not have other significant co-morbidities, and had to have adequate blood counts (Hgb ≥ 9 g/dl, neutrophils $\geq 2{,}000$ ul, platelets $\geq 50{,}000$ ul) and renal and liver function including bilirubin <2.0 mg/dl, albumin ≥ 3.5 mg/dl, and up to a 3-fold elevation of liver transaminases. Having known chronic hepatitis was not an entry requirement, but it was anticipated that most, if not all, patients would have underlying hepatitis B and chronic liver disease.

Laboratory Methods

The basic methodology for production of these patient-specific DC-TC products has been previously published [39–43]. A key difference in the production of the autologous cell lines used in this trial was the use of proprietary NeoStem culture media to isolate and propagate tumor stem cells. Using sterile conditions, a sample of the resected tumor was placed into a tissue transfer kit and transferred to the cell biology laboratory where it was mechanically minced into a cell suspension and placed into stem cell media for incubation. Cells were harvested from the resulting spheroids, and expanded in tissue culture media to produce a tumor cell line. A cell line was considered successful if spheroid-derived cells had expanded to greater than 30 million cells within four weeks, the time needed for the HCC patient to recover from surgery and undergo leukapheresis, but if time permitted, the numbers were expanded to about 100 million. Post irradiation cell numbers averaged 84 million and cell proliferation assays (CPA) confirmed that tumor cells had ceased proliferating.

Monoclonal antibodies used to confirm the HCC and stem cell/progenitor cell nature of the tumor cells derived from the cell lines included: alpha-fetoprotein (AFP), cytokeratin 19 (CK19), cytokeratin 7 (CK7), epithelial cell adhesion molecule (EpCAM), neural cell

adhesion molecule (NCAM), ATP-binding cassette sub-family member 2 (ABCG2), proliferation marker Ki67, and major histocompatibility complex I (MHC class I) as a positive control. MHC I, Ki67, AFP, CK7, and CK19 were consistently expressed on all lines. EpCAM was expressed on three cell lines, NCAM on two, and ABCG2 on one line.

Autologous dendritic cells (DC) were derived from peripheral blood mononuclear cells (PBMC) obtained at the time of leukapheresis and generated over one week in GM-CSF and interleukin-4 (IL4) as previously described [39,40]. The processing included Ficoll-Hypaque separation, then plating of 2.25×10^9 PBMC for preferential adherence, differentiation into DC, and expansion in the culture flasks in the presence of the cytokines. Total PBMC could only be determined after the procedure had been finished. For patients who did not have 2.25×10^9 PBMC collected, the total number of cells available were plated.

The DC-TC therapeutic product was made by incubating autologous DC with autologous TC for 18–24 h. This pulsing of DC with TC that had been previously irradiated with 100 Gy, results in antigen loading onto the DC cells. There was no effort to achieve specific DC:TC ratios, but at the start of incubation there were typically 2.00–2.25 billion PBMC from which DC were differentiated, compared to only 50–100 million TC placed into co-culture with DC. DC phenotype was confirmed by the decrease in expression of CD14 positivity, and expression of CD11c. Following co-incubation, the percentage of CD11c positive cells ranged from 86% to 98% (mean 92.9%). The final DC-TC preparation was divided into 10 aliquots for dosing and quality assurance testing, and then cryopreserved in the vapor phase of liquid nitrogen. Products were released based on DC phenotype and absence of endotoxin, mycoplasma, or bacterial contamination. At the time of treatment an aliquot of the cryopreserved DC-TC was washed and suspended in 500 mg GM-CSF just prior to each injection.

Treatment Plan

Patients underwent standard hepatic resections under general anesthesia. Approximately six weeks later, after recovery from surgery, patients underwent leukapheresis to obtain PBMC from which DC were derived. One week after leukapheresis, patients underwent TACE with epirubicin and carboplatin. During the next six weeks the DC-TC product was manufactured and underwent quality testing. Patients were then scheduled to receive subcutaneous injections weekly for three weeks. Patients were monitored up to eight weeks after initiating vaccine therapy.

Study Parameters

This was a phase I trial to determine whether injections of DC-TC were associated with toxicity, in particular, exacerbation of Hepatitis B. The plan was to treat up to eight patients if no severe or life-threatening toxicities were observed, and to stop if any severe adverse event attributed to the vaccine was documented. The US National Cancer Institute Common Toxicity Criteria v4 were used to assess toxicity. Patients had just undergone surgical resection; so there was no rationale for trying to measure tumor response rate. Because this was an open-label Phase I safety study, progression free survival and overall survival were not assessed.

RESULTS

Eighteen patients were enrolled between January and July 2013 at the Peoples Liberation Army (PLA) Hospital 85 in Shanghai, China. The study was completed in December 2013. One patient withdrew consent prior to surgery; so no tumor was obtained. Two patients were ineligible for treatment because each had a cancer other than hepatocellular (one cholangiocarcinoma; one metastasis from colon cancer). Samples from 15 HCC tumor specimens were collected from 15 patients and cell lines were successfully established within five weeks from all 15. Seven patients did not proceed with leukapheresis and the treatment phase. The reasons patients did not go on to treatment were because two had tumor recurrence prior to the treatment phase, three had deterioration in performance status following resection, and two opted for alternative treatment Table I. summarizes the characteristics of the eight patients who were treated with patient-specific DC-TC products (patient numbers 1,2, 7, 9, 11, 13, 15, and 17). The eight patients treated included 7 men and 1 woman, with ages ranging from 37 to 73 and a mean of 54 years. All patients were HBV-positive; four were receiving medication for active hepatitis, and all but one patient had cirrhosis. At the time of pre-surgical enrollment, all 8 patients were felt to have solitary lesions > 5 cm in diameter based on computerized tomography scans. Based on surgical findings, one patient had a solitary lesion that was < 5 cm in diameter, and two were reclassified as BCLC-C because of portal invasion. One of the latter already had distant metastases by the time of vaccine treatment. At the time of treatment all eight had ECOG 0.

Of the eight treated patients, one received TACE before leukapheresis instead of after. The eight patients received all three planned injections. Table II details the number of cells utilized in the manufacturing of each patient-specific product, and the numbers of cells injected for each dose. The range in DC and TC combined for co-incubation reflects the heterogeneity among patients in terms of the numbers of PBMC derived from leukapheresis, the numbers of DC following culture in the presence of IL4 and GM-CSF, and the number of tumor cells grown and their viability following thawing after cryopreservation. Based on the presence or absence of CD11c expression, the proportion of DC in the final products ranged from 86% to 98% of the cells injected. Each of the weekly injections contained 4 –17 million cells, which reflects the division of these cells into 10 aliquots. Thus, there was about a 4-fold variation in individual

TABLE I. Clinical Characteristics of Patients at Time of DC-TC Treatment

Patient#	Age	Sex	Cirrhosis	HBV	Child Pugh	Primary HCC tumor (cm)	BCLC Stage	Portal Invasion
001	53	F	–	+	A	$5 \times 5 \times 2$	A*	–
002	37	M	+	+	A	$6 \times 5 \times 5$	C	+
007	63	M	+	+	A	$7 \times 6 \times 5$	A*	–
009	73	M	+	+	A	$7 \times 6 \times 5$	A*	–
011	41	M	+	+	A	5×4	A*	–
013	60	M	+	+	A	$6 \times 6 \times 3.5$	A*	–
015	37	M	+	+	A	$20 \times 15 \times 11$	C	+
017	69	M	+	+	A	$4.2 \times 3 \times 3$	A	–

HBV, Hepatitis B Virus; HCC, Hepatocellular Carcinoma; BCLC, Barcelona Clinic Liver Cancer; A*, Barcelona Clinic Liver Cancer classification A (solitary tumor), but greater than 5.0 cm in greatest diameter.

TABLE II. Manufacturing and Dose Summary

Patient #	PBMC from Leuka-pheresis ($x 10^9$)	PBMC seeded on to plates to genera te DC ($x 10^9$)	# Tumor Cells Grown ($x 10^6$)	# Tumor Cells Incubated with DC ($x 10^6$)	Cells Injected ($x 10^6$)			
					Wk 1	Wk 2	Wk 3	Mean ± SD
001	2.50	2.25	73	64.0	7.82	8.05	6.30	7.4 ± 0.95
002	2.46	2.25	100	37.0	12.7	12.3	15.0	13.3 ± 1.46
007	3.54	2.25	99	78.0	4.5	4.56	4.13	4.4 ± 0.23
009	2.04	2.04	32	19.5	5.10	6.00	6.40	5.8 ± 0.67
011	2.11	2.11	110	54.6	6.80	4.80	3.97	5.2 ± 1.45
013	3.60	2.25	66	54.0	4.70	4.80	4.90	4.8 ± 0.10
015	1.62	1.62	92	96.0	13.0	13.0	10.6	12.2 ± 1.39
017	4.00	2.25	100	53.0	15.0	16.0	17.0	16.0 ± 1.00

PBMC, peripheral blood mononuclear cells; DC, Dendritic cells; Wk, week; SD, standard deviation.

TABLE III. Toxicity Summary for Treated Patients, Liver Tests

Patient #	Albumin (g/L)		Total Bilirubin (μmol/L)		ALT (U/L)		AST (U/L)	
	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8
001	42	42	14.9	19.3	16	16	23	27
002	40	37	9.0	13.5	23	25	38	60
007	42	40	5.5	5.0	59	61	86	70
009	33	39	19.9	18.1	10	16	28	28
011	37	40	11.8	11.8	47	31	38	35
013	41	43	14.4	13.6	13	13	22	22
015	44	44	29.1	30.6	34	58	39	33
017	41	40	11.0	11.4	14	12	25	20

ALT, serum alanine transaminase (SGPT); AST, serum aspartate transaminase (SGOT).

TABLE IV. Hepatitis B Activity in Treated Patients

Patient #	HBV DNA (Cps/ml)		HBeAg (S/CO)		HBsAg (IU/ml)	
	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8
001	<1000	<1000	0.30	0.27	0.01	0
002	2210	1300	1.47	1.61	>250	1550
007	<1000	<1000	0.39	0.38	>250	419
009	<1000	<1000	0.38	0.42	452	>250
011	7030	<1000	1.92	1.86	1957	>250
013	<1000	<1000	0.42	0.40	0.27	0.43
015	<1000	<1000	0.44	0.46	23	23
017	<1000	<1000	0.37	0.44	16	12

HBV DNA, hepatitis B virus DNA; HBeAg, hepatitis B envelope Antigen; HBsAg, hepatitis B surface Antigen.

doses among patients, but there was little intra-patient variability in doses, as can be seen in Table II. This range of doses is similar to those administered previously in patients with renal cell cancer and melanoma in phase II trials in the United States (US) [39,40].

The weekly injections were well tolerated and associated with no acute or delayed toxicities. Similar to the experience in metastatic melanoma and metastatic renal cell cancer, mild local injection site reactions including mild pain, erythema, and pruritus were noted. There were no significant changes in any laboratory parameters before (week 0), during (weeks 1, 2 and 3) and 5 weeks after the three injections. Tables III and IV summarize the results of various tests of hepatic inflammation and function, and hepatitis B. There was no increase in hepatic transaminases, bilirubin, prothrombin time, hepatitis B antigens, or viral DNA. There was no evidence of exacerbation of hepatitis B by any parameter.

No severe or life-threatening (grade 4 or 5) toxicities were recorded. There were three adverse events reported, one each, including liver pain, fever, abdominal pain, and tumor recurrence, none of which were attributed to the study product.

DISCUSSION

This is the first trial of patient-specific DC-TC vaccines conducted in patients with HCC, and the first experience in patients with HBV. There

were three significant observations. First, the 15/15 (100%) success rate shows that short-term tumor cell lines can be reliably established from resected HCC. Second, in patients with underlying cirrhosis, the phase I trial demonstrated no significant toxicity including no worsening of hepatic inflammation, function or enzymes. Third, in HBV-positive HCC patients, there was no significant toxicity including no worsening in parameters related to hepatic inflammation, function or HBV infection. This was a feasibility and safety trial, not a therapeutic efficacy trial. The results of this trial support proceeding with a randomized phase II trial to determine whether such an immunotherapy approach provides clinical benefit.

The ability to establish a short-term cell line in every patient is important for possible further development of such patient-specific products. Historically the success rate for establishing cell lines from various tumor types using standard tissue culture techniques was only about 30%, with the highest rates of 50% in melanoma, renal cell cancer, and sarcomas [39,40,42,43]. Historically the Hoag Cell Biology Laboratory had a success rate of 0/5 from samples of hepatocellular cancer [42]. Subsequent modifications in the tissue culture media have allowed early selection of cells with the characteristics of tumor-initiating (stem) cells, and a rapid expansion to numbers sufficient for manufacturing of patient-specific DC-TC therapeutic products resulting in a success rate of 15/15 (100%) in this study.

Previous US trials with DC-TC products in melanoma and renal cell cancer excluded patients with significant underlying liver disease and specifically excluded patients with underlying HBV or HCV infection. The clinical laboratory tests of liver inflammation and function used to define eligibility for this clinical trial appear sufficient to minimize the risk of the vaccine making liver disease worse. One concern was that vaccination with an anti-cancer vaccine might distract the immune response directed against the hepatitis virus resulting in more liver inflammation or increased hepatic dysfunction. No such toxicities were observed in this trial over the dose ranges of 4 –17 million cells per dose, nor a cumulative dose of 13.2 – 48 million cells injected over three weeks.

This trial was not conducted using the classical designs for phase I dose-escalation toxicity trials. Experience has shown that classical dose escalation trials for vaccine products yield little safety information, mainly because of the minimal toxicity reported for such vaccines [44]. In addition, there are practical limitations to the numbers of cells that can be grown, which makes it difficult to escalate doses over the range of several logs. There are also practical limitations as to how many cells can be injected into one or two sites. Furthermore, for cell–based vaccines, biological variation is such that it is hard to standardize dosing because of variation in cell numbers retrieved from patients. For instance, in this trial there was variation in the numbers of tumor cells that were grown within six weeks, and biological variability among cells in their tolerance of radiation and subsequent freezing and thawing. There was also variation in the numbers of PBMC collected during leukapheresis even though the number of liters exchanged was similar for all patients. Even though an effort was made to plate 2.25×10^9 PBMC for differentiation into DC, the pheresis product from several patients contained fewer PBMC. In terms of cell counting, there is variation associated with propensity for cell clumping that can confound both sampling and counting. For these reasons, there was substantial variation in the final doses of DC-TC among patients, but doses for each unique patient were similar for each injection.

This study establishes the feasibility of producing DC-TC therapeutic vaccines in patients with hepatocellular cancer, and provides preliminary evidence for the safety of such patient-specific products in patients with cirrhosis and hepatitis B infection. This suggests that a phase II efficacy trial of such a product can be performed in patients with HCC, including those with cirrhosis, and those with HBV or HCV infection, whose performance and hepatic inflammation and function tests are similar to those used for patient selection in this trial.

CONCLUSIONS

Vaccination with autologous DC-TC in GM-CSF was not associated with a worsening of hepatitis B infection, or exacerbation of hepatic inflammation or liver dysfunction. This approach appears to be sufficiently safe to justify further testing in a phase II trial for efficacy, and to expand observations regarding safety.

AUTHOR CONTRIBUTIONS

Xiaojin Wang, MD (Hospital 85 People's Liberation Army of China) acted as the lead sub-investigator at the clinical site, managed the research subjects and supervised their medical treatment and leukapheresis procedures, and collated clinical data from the trial. Michael E. Bayer MD (NeoStem, Inc.) wrote the original protocol and consent form with Dr. Dillman, taught local Chinese staff how to perform leukapheresis and provided quality oversight for the leukapheresis procedures performed in China, provided sponsor oversight for conduct of the clinical trial in China, collected data from the trial, and provided substantial input into the content of the manuscript, and confirmed the contributions of various co-authors. Xiaosong Chen MD (Renji Hospital) identified and consented subjects for the trial, and performed the surgical resections. Craig Fredrickson BS (NeoStem, Inc.) was the onsite director of laboratory operations and manufacturing in Shanghai. This included production of tumor cell lines, generation of dendritic cells, and production of the dendritic cell/tumor cell products, and provided significant input into the content of the manuscript. Andrew N. Cornforth PhD (NeoStem, Inc.) set up the laboratory in Shanghai and provided all of the operating procedures for the production of tumor cell lines, generation of dendritic cells, and production of the dendritic cell/tumor cell products, collected and collated laboratory cell manufacturing data, and provided significant input into the content of the manuscript. Greg Liang, MD (NeoStem, Inc.) provided local regulatory oversight, quality assurance, and clinical compliance, and translated the English versions of protocol and the manuscript into Chinese for the Chinese investigators and co-authors. Jessica Cannon BS (NeoStem, Inc.) was the onsite laboratory research associate in Shanghi for the production of tumor cell lines, generation of dendritic cells, and production of the dendritic cell/tumor cell products, and in charge of quality control. Jia He MD, PhD (Second Military Medical University of China) provided the statistical design for the protocol. Qingchun Fu MD (Hospital 85 People's Liberation Army of China) acted as a sub-investigator at the local site. Jia Liu MD (Cellular Biomedicine Group, Shanghai, China) provided clinical research associate duties for this trial. Gabriel I. Nistor MD (NeoStem, Inc.) developed the modifications used in the production of the tumor cell lines used in this trial, and provided significant input into the content of the manuscript. Wei Cao PhD (Cellular Biomedicine Group, Shanghai, China) provided the space for the laboratory operations to produce products for this trial. Chengwei Chen MD (Hospital 85 People's Liberation Army of China) served as the principle investigator at the local site including submission of the protocol for IRB approval. Robert O. Dillman MD (NeoStem, Inc.) developed the cell-based technology used in this trial, wrote the original protocol with Dr. Bayer, was the Principle Investigator of this protocol, and wrote the initial draft of the manuscript, and all subsequent revisions based on input from co-authors and reviewers.

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