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JX-594

Recombinant Vaccinia Virus Expressing GMCSF Oncolytic

Recombinant thymidine kinase-deleted vaccinia virus (Wyeth strain) expressing the human *GMCSF* gene

EN: 400740

ABSTRACT

JX-594 is derived by inserting human GMCSF genes under the control of early/late promoters into the thymidine kinase (TK) genes of the vaccinia virus. JX-594 was well tolerated when administered intratumorally to patients with surgically incurable melanoma twice weekly at doses up to 2 x 10^7 PFU/lesion and up to 8 x 10^7 PFU/session. Three of the seven patients had complete or partial responses in their treated and untreated lesions. In an additional dose-escalation study, intratumoral injection of JX-594 (10^8 PFU, 3 x 10^8 PFU, 10^9 PFU or 3 x 10^9 PFU) every 3 weeks (1-8 doses) into primary or metastatic liver tumors was generally well tolerated. Direct hyperbilirubinemia was the dose-limiting toxicity at 3 x 10^9 PFU. Overall safety was acceptable in the context of JX-594 replication, GM-CSF expression and systemic dissemination, and JX-594 had antitumor effects against several refractory carcinomas.

BACKGROUND

Oncolytic viruses (virotherapeutics) kill cancer cells through a novel mechanism of action (oncolysis and/or necrosis) (1-3) and can be targeted to cancer cells with activated genetic pathways and/or loss of tumor suppressor or function (4-6). Selective intratumoral replication of the virus leads to multiplication, lysis of the infected cancer cell and spread to adjacent cancer cells. In addition, these therapeutics can induce tumor-specific cytotoxic T lymphocytes (CTLs) "armed" by the expression of therapeutic transgene products (7-11). Therefore, armed virotherapeutics have the potential to effectively treat cancers that have become refractory to currently approved treatments. However, these viruses have not yet been shown to be clinically effective, having inefficient intratumoral spread and intravenous delivery.

The JX-594 oncolytic virus was derived from vaccinia virus by insertion of human *GMCSF* and β-galactosidase (*GLB1*) genes into thymidine kinase (*TK*) genes. Inactivation of the *TK* gene has been shown to decrease the virulence of vaccinia virus and increase its tumor-specific replication to destroy cancer cells with cell cycle abnormalities and epidermal growth receptor (EGFR, ErbB-1)/Ras pathway activation (11). JX-594 is capable of replicating within tumor cells, releasing relevant tumor antigens in situ as the cells are lysed, along with viral antigens and the immunostimulatory cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF). The hope was that this could direct the host immune response towards the tumor, as both an immunotherapy and a vaccine.

PRECLINICAL PHARMACOLOGY

For preclinical pharmacological studies, New Zealand white (NZW) rabbits were chosen because human GM-CSF (hGM-CSF) has activity in rabbits. In preclinical toxicity studies, i.v. administration of three weekly doses of JX-594 (10^{10} plaque-forming units [PFU]/dose; approximately 4 x 10^9 PFU/kg) to NZW rabbits (two males and two females) was well tolerated. No overt clinical signs were observed throughout the 92 days of the study, with the following exception: after the first injection of JX-594, the treated animals lost approximately 5% of their body weight by day 6. Thereafter, body weight rose intermittently until the end of measurements on day 33 (Fig. 1). Treatment with JX-594 also affected hematology (Table I). Statistically significant increases in white blood cells (WBCs) were observed beginning on day 4 of the study and continuing until day 24. This increase appeared to be due to increased numbers of monocytes on days 4 and 8 and increased numbers of lymphocytes from day 10 to day 24. The early rise in monocyte counts is probably related to the large increase in serum hGM-CSF levels observed on day 4 that likely results from expression of the *GMCSF* transgene. The hGM-CSF levels increased to 600 pg/mL on day 4, which had fallen to approximately 150 pg/mL by the time of the next assay on day 8. Levels continued to decline and reached baseline by day 18. The second injection of JX-594 on day 8 did not result in an increase in hGM-CSF levels 3 days later, probably due to the formation of antibodies to the heterologous human protein hGM-CSF by the rabbits. The rabbits also formed neutralizing antibodies to JX-594; significant titers became detectable by day 14 and appeared to plateau by the time of the last assessment on day 22. The increased lymphocyte counts from day 10 to day 24 may be a consequence of viral replication following the three injections of JX-594. Platelet numbers on day 8 were increased 5-fold over day 1 values and remained elevated until day 15. This is also likely to be a consequence of the production of hGM-CSF following JX-594 injection on day 1. In order to confirm that hGM-CSF mediated these hematological changes in the rabbit model, we determined the effects of daily dosing with recombinant human GM-CSF protein (rhGM-CSF) at doses similar to those used in humans on subpopulations of WBCs in normal rabbits. rhGM-CSF significantly increased the total WBC count on days 2, 4, 6 and 11. On WBC differential analysis, absolute neutrophil and monocyte concentrations were significantly increased. It was also notable that

S.G. Kim[1], T.H. Hwang[2].
[1]Department of Hemato-Oncology, Pusan National University Hospital (PNUYH), Busan, S. Korea; [2]Department of Clinical Pharmacology, School of Medicine of Pusan National University & PNUYH, Busan, S. Korea. Correspondence: thhwang@pusan.ac.kr.

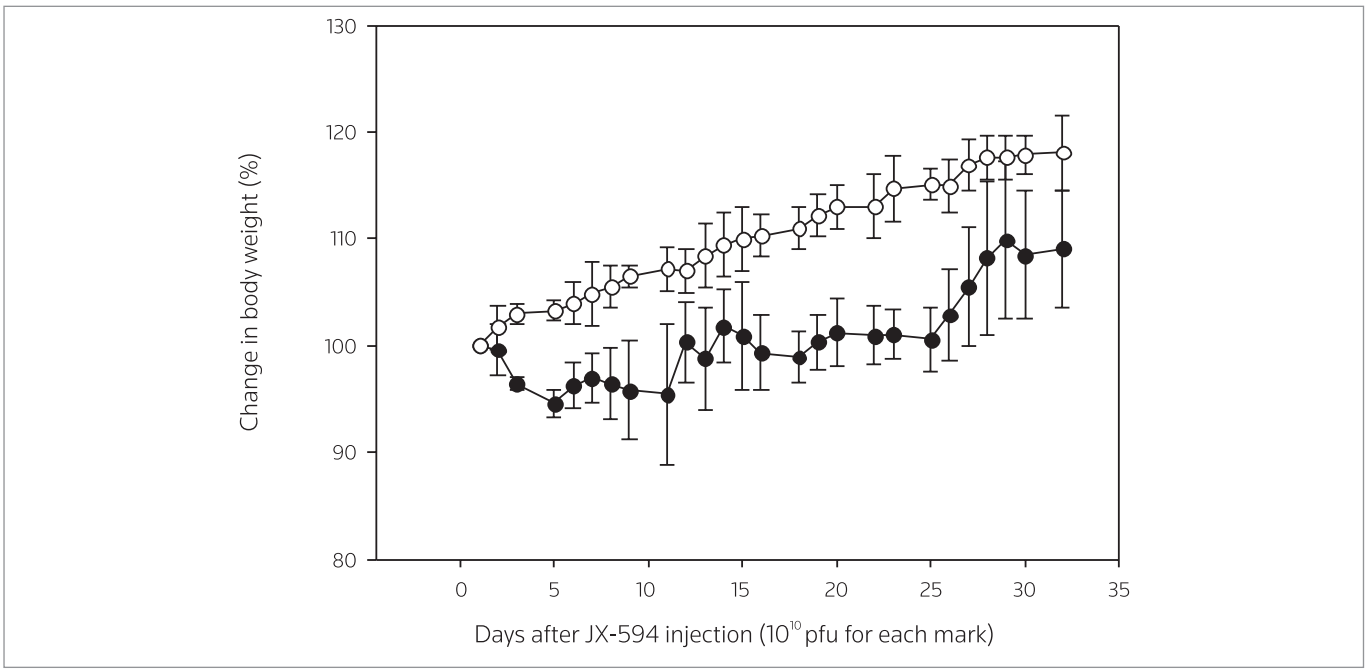

Figure 1. Effects of i.v. injection of JX-594 on body weight of rabbits. ●, JX-594; ○, phosphate-buffered saline (PBS).

Table I. *Effect of i.v. injection of JX-594 on hematology parameters in rabbits.*

Days	Control (n = 4)	1#	4	8#	11	15#	18	22	26	29
WBC (10^3/μL)	5.90 (± 0.38)	5.15 (± 0.3)	**7.4 (± 0.93)***	**7.69 (± 0.78)***	**12.85 (± 3.17)***	**7.26 (± 0.36)***	**14.95 (± 4.22)***	**7.31 (± 1.02)***	**7.87 (± 1.04)***	5.00 (± 0.6)
Neutrophils (%)	2.55 (± 0.45)	2.40 (± 0.2)	2.23 (± 0.760)	3.53 (± 0.98)	2.60 (± 1.24)	2.13 (± 0.50)	1.50 (± 0.70)	1.53 (± 0.29)	1.75 (± 0.35)	1.80 (± 0.0)
Lymphocytes (%)	40.40 (± 4.09)	**32.40 (± 2.8)***	36.00 (± 4.35)	**18.50 (± 8.52)***	**70.10 (± 6.39)***	**55.56 (± 5.10)***	**66.26 (± 9.32)***	**61.96 (± 4.15)***	**61.75 (± 2.95)***	43.80 (± 2)
Monocytes (%)	24.13 (±4.63)	21.30 (± 1.5)	**40.40 (± 5.17)***	**43.60 (± 2.79)***	**9.80 (± 0.81)***	24.20 (± 4.52)	**9.00 (± 2.61)***	26.83 (± 0.68)	29.05 (± 0.85)	**36.10 (± 3.6)***
Eosinophils (%)	0.98 (±0.05)	1.00 (± 0.0)	0.96 (± 0.06)	1.90 (± 0.15)	0.43 (± 0.08)	0.86 (± 0.18)	0.40 (± 0.10)	0.60 (± 0.25)	1.10 (± 0.00)	1.90 (± 0.0)
Basophils (%)	3.70 (±0.38)	4.10 (± 0.0)	3.26 (± 0.92)	4.10 (± 1.35)	5.30 (± 1.77)	4.23 (± 1.20)	4.76 (± 0.24)	2.56 (± 0.43)	3.20 (± 0.10)	4.60 (± 0.0)
Leukocytes (%)	7.78 (±1.38)	8.90 (± 0.9)	**18.73 (± 1.43)***	**31.93 (± 10.88)***	**14.63 (± 7.13)***	**17.10 (± 1.25)***	**22.80 (± 6.22)***	7.60 (± 4.04)	**4.80 (± 0.20)***	**14.40 (± 2.1)***
Platelets (10^3/μL)	285.50 (±19.84)	267.00 (± 12.0)	280.66 (± 10.65)	**1428.66 (± 116.20)***	**702.66 (± 43.0)***	**411.00 (± 75.2)***	**384.66 (± 62.3)***	**348.33 (± 55.04)***	**430.50 (± 17.5)***	252.00 (± 24.2)

#Days on which i.v. injection of JX-594 was given. Bold parameters are significantly different (higher) from controls. Bold underlined parameters are significantly different (lower) from controls. *$P < 0.05$.

platelet number was increased in rhGM-CSF-dosed rabbits. These results confirm similar hematological effects of hGM-CSF in NZW rabbits and humans (12).

Two immunocompetent liver tumor models were used for evaluation of the antitumor efficacy of JX-594: a VX2 carcinoma-implanted rabbit model with time-dependent metastases to the lungs (10, 12) and a carcinogen-induced (*N*-nitrosomorpholine in drinking water for 8 weeks) rat liver cancer model (10). Weekly i.v. JX-594 (10^9 PFU/kg in both models) showed significant efficacy against intrahepatic primary tumors in both models. In addition, whereas lung metastases developed in all control rabbits, none of the animals administered JX-594 developed detectable metastases. Tumor-specific virus repli-

cation and gene expression, systemically detectable levels of hGM-CSF and tumor-infiltrating CTLs were also detected (10). Biodistribution-based quantitative PCR (Q-PCR) after systemic administration of JX-594 in the rabbit VX2 liver tumor model and mouse hepatocellular carcinoma cell (TIB-75 obtained from ATCC) model was determined. After administration of 5 x 10^8 PFU/kg, mice were sacrificed and each organ was isolated on days 1, 2, 3 and 5 after JX-594 injection. We determined the PFU/mg tissue for tumor, lung, liver, ovary, kidney, spleen, heart, skeletal muscle, bone marrow and colon. Tumor-selective deposition was demonstrated in the mouse model, but no replication was detected in any tissues, including tumor. In contrast, highly tumor-selective viral replication was demonstrated in the rabbit model, minimal or no PFUs were detected in normal tissues and no replication was demonstrated, with the exception of low levels in the ovaries.

SAFETY

Serum biochemistry parameters in NZW rabbits were unaffected by treatment with JX-594. There were remarkably few histopathological findings, none of them of major toxicological significance. Mild or very mild inflammation was observed in the central and portal veins of the liver and in the peribronchial areas of the lungs on day 4, when viral replication was expected to be at its maximum. Mild follicular lymphoid hyperplasia was also observed in the spleen at this time. By day 40, the inflammation in the liver was rated as very mild in one of the two animals (the other was normal) and had disappeared by day 92. The very mild inflammation in the peribronchial areas of the lungs on day 4 was replaced by lymphoid hyperplasia on day 40, which had resolved by day 92. In the spleen, the mild follicular lymphoid hyperplasia on day 4 had become moderate by day 40, but had resolved by day 92 (12).

CLINICAL STUDIES

In a phase I pilot trial of a JX-594 prototype, seven patients with melanoma of the skin received escalating doses injected into superficial skin metastases. JX-594 was well tolerated when administered intratumorally to these patients with surgically incurable melanoma twice weekly at doses up to 2 x 10^7 PFU/lesion and up to 8 x 10^7 PFU/session. Total doses of up to 2 x 10^9 PFU were safely administered and three of the seven patients had complete or partial responses in their treated and untreated lesions (13).

As the maximum tolerated dose (MTD) was not reached (up to 8 × 10^7 PFU), a phase I dose-escalation study of intratumoral JX-594 was conducted in primary and metastatic liver cancer (14, 15). We aimed to define the safety and MTD at substantially higher doses, specifically after treatment in a solid organ, pharmacokinetics, including replication-dependent shedding of JX-594 into the blood over 3 weeks, and efficacy against a broad spectrum of cancer types (Table II). JX-594 was administered by imaging-guided intratumoral injection using 21-gauge multipore PEIT needles. Fourteen patients were treated with 10^8 PFU, 3 × 10^8 PFU, 10^9 PFU or 3 x 10^9 PFU JX-594. The MTD was determined as 10^9 PFU, as two patients treated with 3 x 10^9 PFU JX-594 showed dose-limiting toxicity (DLT). JX-594 was well tolerated up to the MTD and no treatment-related deaths occurred during the study. All patients had grade 1 or 2 flu-like symptoms (maximum fever at 4 h postinjection). After fever onset, dose-related hypotension (grade 2, no organ dysfunction) occurred in 2 patients within 4-12 h. The most frequent possibly treatment-related adverse events were fever, chilling, fatigue, myalgia, anorexia and headache. Only one serious adverse event (anorexia and abdominal pain) was considered to be treatment-related. Ten serious and unrelated adverse events were reported and attributed to complications associated with tumor progression. Two patients receiving 3 x 10^9 PFU JX-594 had grade III DLT of hyperbilirubinemia —due to tumor swelling and obstruction of the intrahepatic bile duct— and grade III anorexia and abdominal pain. All patients developed anti-JX-594 antibodies within 3-15 days of treatment. Anti-JX-594 antibody titers peaked after the first dose in six patients and increased further after the second dose in eight patients. No association was noted between baseline or post-treatment anti-JX-594 antibody titers and any clinical or laboratory endpoint, including JX-594 pharmacokinetics, replication, GM-CSF expression and efficacy. Treatment-related transient decreases in lymphocytes, platelets and hematocrit were noted during the first 3 days. Nine patients had an increase in absolute neutrophil count (ANC) in 2-4 days. Increases in ANC were dose-related and the largest increases were associated with GM-CSF detected in the blood. Increases in monocytes and eosinophils and dose-dependent thrombocytopenia were also observed in some patients. IL-6, IL-10 and TNF-α peaked at 3 h. Later peaks (days 3-22) were also recorded. Cytokine induction was greater in cycles 2-8 of JX-594 treatment than in cycle 1. IL-6 induction was frequently detected in patients with detectable serum GM-CSF. IL-1β and IL-4 induction was not noted. Ten patients were assessable for target tumor computed tomography (CT) responses. Three patients showed objective responses and six patients showed stable disease according to RECIST. One patient had progressive disease. Eight patients had objective responses by Choi criteria. Patients with response according to RECIST and Choi criteria had squamous cell carcinoma of the lung, hepatocellular carcinoma and melanoma. In seven patients with noninjected tumors, six patients had stable disease according to RECIST or objective response by PET-CT; only one patient had progressive disease. Time to progression of these noninjected tumors ranged from 6 to over 30 weeks. JX-594 genomes were determined in plasma and blood (Fig. 2). All patients had JX-594 genomes detected immediately after injection (49 of 50 cycles), and concentrations increased with dose. JX-594 genomes were decreased about 50% within 15 min and about 90% within 4-6 h. After initial clearance of injected JX-594 in the blood, delayed re-emergence of circulating JX-594 was frequently detected due to intratumoral replication of JX-594. Twelve of 14 patients had detectable genomes (blood or plasma) between days 3 and 22. Generally, the higher the dose the greater the secondary peak, and the pharmacokinetics of the secondary release had similar patterns as after the initial release. Lower secondary concentration peaks were detected after repeated dosing in cycles 2-7 (4 of 11 patients). Shedding of infectious JX-594 into the environment was assessed, as infectious shedding of viral units would have public health consequences, but infectious plaque was not observed in saliva and urine, suggesting no public health concerns. Three patients given the MTD had concentrations of detectable GM-CSF for over 48 h after JX-594 injection that were higher than those reported after s.c. injection of GM-CSF protein (46-16,000 pg/mL).

Table II. *Targeted tumor responses, GM-CSF levels, JX-594 replication and survival duration for each patient in a phase I study.*

Patient number	Tumor type	GM-CSF	JX-594 replication, day postinjection*	Response according to RECIST**	Response according to Choi criteria (+ or –)†	Survival (days)
103	SCC lung	No detection	D3, D8	PR	+ (51% decrease)	736
201	HCC	> LOD	D3, D5, D15, D22	Liver: PR; neck: PR	Liver : + (30% decrease); neck : + (57% decrease)	355
304	Melanoma	No detection	No detection	Liver: PR; neck: SD	Liver: + (33% decrease); neck: + (51% decrease in HU)	366
301	RCC	> LOD	D3, D8, D15	SD	+ (42% decrease in HU)	840+#
302	Colon	> LOQ	D3, D5, D8	SD	+ (15% decrease in HU)	267
202	SCC thymic	No detection	No detection	SD	+ (16% decrease in HU)	303
102	Colon	No detection	No detection	SD	+ (31% decrease in HU)	246
203	Extragonadal germ	No detection	D5, D22	SD	+ (40% decrease in HU)	135
305	Colon	No detection	D8	SD	–	54
306	Colon	No detection	No detection	PD	–	282
101	Gastric	No detection	No detection	NA***	NA***	54
303	Melanoma	> LOQ	D5, D8	NA	NA	10
401	HCC	No detection	No detection	NA	NA	90
402	HCC	> LOQ	No detection	NA	NA	18‖

RECIST, Response Evaluation Criteria in Solid Tumors; SCC, squamous cell carcinoma; PR, partial response; SD, stable disease; HU, Hounsfield units; PD, progressive disease; NA, not available; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; LOD, limit of detection; LOQ, limit of quantitation. + denotes response; – denotes no response. *Days secondary JX-594 replication was detected; **RECIST criteria: partial response is a maximum diameter decrease of ≥ 30%; PD is an increase of ≥ 20%; SD is a change in diameter between these two cut-offs for PR and PD. †Choi criteria: maximum diameter decrease of ≥ 10% or density decrease of ≥ 15%. #Still alive. ‖not cancer-related deaths. ***CT scans done at week 3 showed tumor progression.

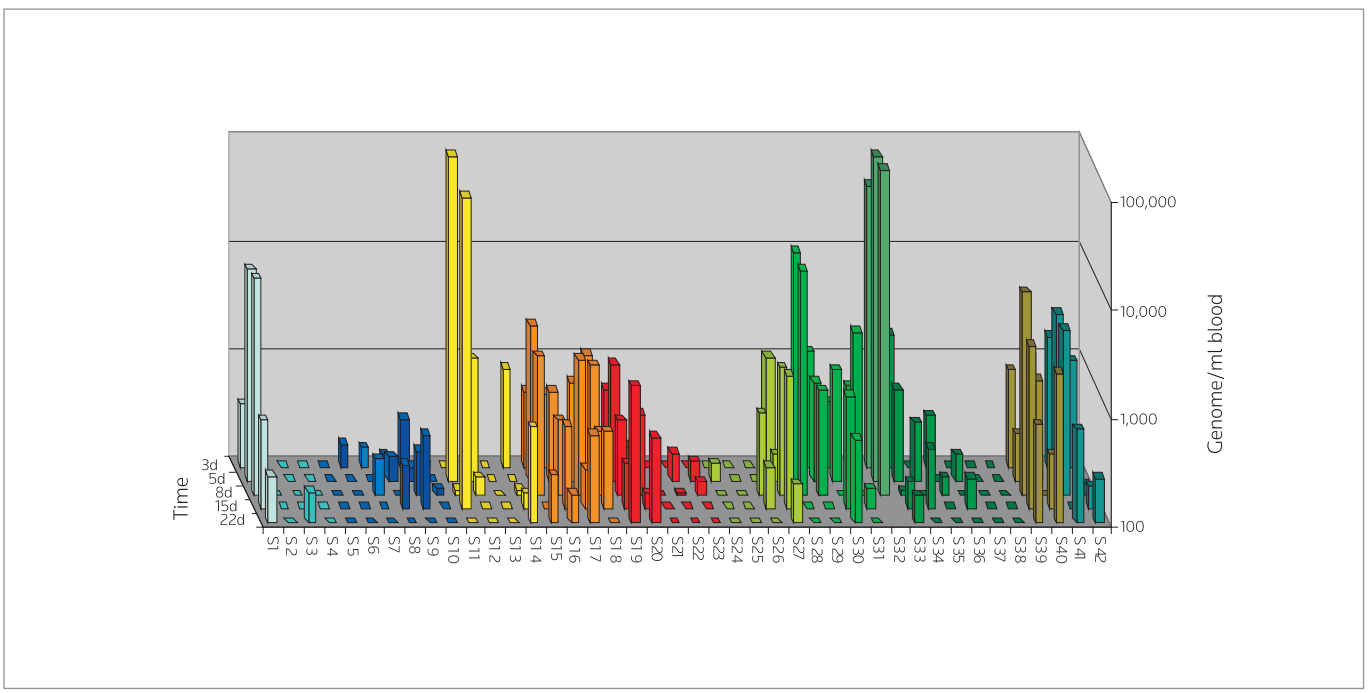

Figure 2. Real-time quantification of JX-594 genomes in whole blood from each patient.

Phase II clinical trials in liver cancer (intratumoral injection) and phase I trials in refractory solid tumors (systemic injection) are now under way in the U.S., Canada and South Korea (16, 17).

DRUG INTERACTIONS

No drug interactions have been reported for JX-594, although antiviral agents such as cidofovir can synergize or antagonize JX-594 replication.

SOURCES

Jennerex Biotherapeutics, Inc. (US); licensed to Green Cross for development and commercialization in South Korea.

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