A Phase I Dose-Escalation Trial of JX-594 (Thymidine Kinase-Deleted Vaccinia Virus Plus GM-CSF) Administered by Intravenous Infusion in Patients with Refractory Solid Tumors (JX594-IV-011)

Scientific Abstract

Novel treatments are needed for cancer. Oncolytic, replication-selective viruses hold promise as novel anti-cancer therapeutics that can destroy tumors by multiple mechanisms. These viruses are engineered to replicate and spread efficiently in cancer tissues but not normal tissues. Cancer cells can be destroyed by apoptosis, virus-induced cytopathic effects, induction of cancer-specific cytotoxic T-lymphocytes and induction of anti-tumoral and anti-vascular cytokines. Vaccinia viruses have several distinct advantages as oncolytic therapeutics. These include rapid cancer cell killing and spread; large capacity for therapeutic and/or inactivating transgene insertion; extensive human experience with the virus in vaccine recipients and in melanoma patients receiving intratumoral injections.

The thymidine kinase (TK)-deleted vaccinia virus JX-594, expressing GM-CSF (granulocyte-macrophage colony stimulation factor) and humanized *Escherichia coli* β-galactosidase, holds promise as a selective oncolytic virus for the treatment of cancer. The rationale for the construction of this virus is as follows. The TK gene was deleted because TK- vaccinia viruses have been shown to replicate efficiently in cells with high nucleotide pools such as proliferating cells and cancer cells. TK- vaccinia viruses have also been shown to localize selectively in tumor cells. GM-CSF was chosen because it was the most potent stimulator of systemic anti-tumoral immunity among several tested, probably due to its unique ability to promote differentiation of hematopoietic precursors into dendritic cells. These cells act as professional antigen-presenting cells and may take up and present tumor antigens released as the tumor cells are killed by the vaccinia virus. The GM-C SF gene is under the control of a synthetic early/late promoter that contains regulatory elements from early and late vaccinia promoters resulting in constitutive expression throughout the virus replication cycle. The β-galactosidase gene was inserted to facilitate localization of replicating virus in biopsy tissues.

JX-594 Clinical Experience

Thirty-one (31) patients have been treated with JX-594 to date on three clinical research protocols, including 7 patients on a completed Phase I study in patients with unresectable melanoma [see (1) below]; 15 patients on a completed Phase I study in patients with advanced/metastatic solid tumors within the liver [see (2) below]; and 9 patients to date on an ongoing, Phase I/II study in patients with Stage 3/4 unresectable malignant melanoma [see (3) below].

(1) JX-594 was well-tolerated in a Phase I, physician-sponsored IND clinical trial of seven patients with metastatic melanoma who received multiple intratumoral (IT) injections. Injections were given twice weekly for a minimum of six weeks, up to approximately six months. Five of seven (71%) patients had tumor responses at the site of injection, including two complete...
responses (one led to complete surgical resection). GM-CSF expression and T-lymphocyte infiltration were documented in tumor biopsies. Four patients in the trial had dermal metastases at the time of entry into the trial that were not subsequently injected. Importantly, dermal metastases that were not directly injected regressed in all four of these patients. These results suggest that oncolytic virotherapy, and specifically JX-594, has the potential to treat distant metastatic disease. Two patients remained disease-free for at least one-and-one-half and six years after treatment, respectively, while the median life-expectancy of these patients prior to treatment with JX-594 was estimated to be six to nine months.

(2) A phase I, physician-sponsored, dose-escalation study of JX-594 administered by IT injection in patients with refractory solid tumor(s) located within the liver (protocol JX594T-hep001) was completed in South Korea in July 2007. JX-594 was injected into 1 - 3 tumors under radiographic guidance once every three weeks until progression at the site(s) of injection or until the patient has received a maximum of 4 treatments. Four additional cycles could be administered to patients with an objective response of the injected tumor(s) or clinical benefit (i.e. up to 8 treatments possible). Study dose levels were 10⁸ pfu (cohort 1), 3 x 10⁸ pfu (cohort 2), 10⁹ pfu (cohort 3) and 3 x 10⁹ pfu (cohort 4) per treatment. Standard Phase I dose-escalation guidelines were used, with 2-6 patients enrolled per cohort (3 if no dose-limiting toxicities are reported).

Fourteen patients were enrolled (as well as 1 additional patient on a compassionate use basis), including hepatocellular (HCC), colorectal, melanoma, gastric, renal, lung, germ cell and thymic cancers. The mean number of treatments was 3.5 (range 1-8). One possibly treatment-related serious adverse event was reported (anorexia and RUQ abdominal pain). Asymptomatic grade III hyperbilirubinemia, grade III anorexia, and grade III RUQ abdominal pain were the dose-limiting toxicities at the highest dose level. It is presumed that biliary obstruction due to tumor necrosis and subsequent swelling was the cause of the resulting DLTs; direct toxicity to liver parenchyma was not demonstrated. All patients experienced mild to moderate flu-like symptoms and dose-related thrombocytopenia (days 1-5). Viral genomes were detectable in the blood of all patients within the first 30 minutes after injection. A secondary wave of viremia consistent with JX-594 replication was demonstrated in 11 of 14 patients (day 3 – 22; cycles 1-7). Replicating JX-594 and/or gene expression (GM-CSF, lac-Z) was demonstrated at distant, non-injected tumor sites (including malignant effusions, ascites). Dose-related increases were noted in GM-CSF blood concentrations and in white blood cell counts; neutrophil counts increased to >20,000 in multiple patients with detectable GM-CSF in blood.

Ten patients were evaluable for efficacy. Acute necrosis and vascular shutdown were demonstrated in multiple patients (day 5-8). Using RECIST or Choi criteria, anti-tumoral effects were demonstrated in multiple patients (n=9) with diverse cancer types: HCC, RCC, melanoma, squamous cell (2), extragonadal germ cell, and colorectal (3). Three patients had objective injected tumor responses. Patients with serum tumor markers had reductions of 40-95% (n=4). Efficacy against non-injected tumors was demonstrated by PET-CT (10-100% decrease SUV). Clear efficacy was demonstrated in all three HCC patients (blood tumor marker decreases of 60-95%; RECIST, Choi and/or Choi responses). Squamous cell carcinomas and melanomas were also permissive for JX-594 replication and/or efficacy (n=4 total).

JX-594 treatment was associated with high-level replication and gene expression, systemic dissemination and distant tumor targeting, and reproducible efficacy against a spectrum of refractory carcinomas.
A Phase I/II study of JX-594 is currently ongoing in the United States (protocol JX594-IT-MEL005) in patients with Stage 3/4 unresectable malignant melanoma. Patients are treated at a dose of $10^8$ pfu per treatment weekly for 6 total treatments. Nine patients have been treated to date. Preliminary data indicate that JX-594 has been safe well-tolerated in study patients. No treatment-related serious adverse events have been reported.

**JX-594 Preclinical Toxicology**

**Exploratory Study**

In an exploratory toxicity study, New Zealand White rabbits (2.5 kg) received one (n = 1M, 1F) or three (n = 3M, 3F) weekly IV doses of JX-594 ($10^{10}$ pfu/dose; approximately $4 \times 10^9$ pfu/kg) starting on Day 1. The animals that received the single dose were euthanized on Day 4 and those that received multiple doses were euthanized (n = 1M, 1F/time point) at Days 29, 45 and 92. The treatments were well tolerated: no overt clinical signs were observed throughout the 92 days of the study, with the following exception. For the animals receiving multiple doses, following the first injection of JX594 on Day 1, 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. Control animals gained weight steadily throughout the study.

The IV treatment with JX-594 affected hematology as follows. Statistically significant increases in WBC were observed beginning at Day 4 of the study and continuing until Day 24. This increase appears to be due to increased numbers of monocytes on Days 4 and 8 and to 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 result from expression of the GM-CSF transgene (see below). 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 at 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 GM-CSF following the JX-594 injection on Day 1. Serum biochemistry parameters were unaffected by the treatment with JX-594.

The first injection of JX-594 resulted in a large increase in hGM-CSF levels to 600 pg/mL (at Day 4), which had fallen to approximately 150 pg/mL by the time of the next assay, 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 level three 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, Day 22.

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 at 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 29, the livers were normal in both animals and on Day 40, very mild inflammation was observed in one of the two animals (the other was normal); the livers of both animals were normal on Day 92. The very mild inflammation in the peribronchial
areas of the lungs at Day 4 was replaced by lymphoid hyperplasia at Days 29 and 40 which had resolved by Day 92. In the spleen, the mild follicular lymphoid hyperplasia at Day 4 had become moderate by Days 29 and 40 but had resolved by Day 92.

While the numbers of animals in this exploratory study were limited, the findings in this study are consistent with mild vaccinia virus infection (inflammation in the liver and lung at Day 4) and with expression of the hGM-CSF transgene (lymphoid hyperplasia in the spleen at Day 4 and the lung and spleen at Day 40). All of these findings appeared to be resolved by Day 92 and none are considered to be of toxicological significance.

GLP Study

A study entitled “Toxicity and Biodistribution of Recombinant Vaccinia Virus JX-594 in the Rabbits” has recently been conducted in compliance with GLP guidelines. Nine groups of 5 rabbits/sex/group received intravenous administrations of the current clinical lot (Lot C173-3) at doses of $3 \times 10^6$ and $3 \times 10^7$ pfu/kg (30 and 300-fold, respectively, the proposed starting clinical dose ($10^5$ pfu/kg). Two groups each of low and high dose animals received 3 weekly doses (Days 0, 7, 14) and were sacrificed 3 and 30 days after the last dose. Four groups received a single high dose ($3 \times 10^7$ pfu/kg) on Day 0 and one of the groups was sacrificed on each of Days 3, 7, 14 and 44. A control group was treated with vehicle on Days 0, 7 and 14; sacrifice was on Day 17. Gross necropsies with organ weights were conducted for all animals and a full range of tissues was collected for micro-pathological examination. Blood samples for clinical pathology, hGM-CSF levels, viral titers, and antibody titers were taken from all animals alive on Days 0, 3, 7, 17, 21 and 44. Blood and tissue samples were also collected from the single dose animals for determination of the distribution of JX-594 DNA by quantitative polymerase chain reaction (Q-PCR).

In summary, JX-594 treatment-related inappetence, and dose-related, reversible, early loss of body weight was observed at both dose levels. There were some mild, dose-related, reversible changes in hematology and clinical chemistry parameters. Reversible lymphoid depletion in the thymus and lymphoid hyperplasia with red pulp expansion in the spleen were also considered physiological adaptations. Mild anemia was considered secondary to spleen enlargement. At the high dose only, abscesses were observed in the testes. All of the observed changes appear to be reversible.

JX-594 Preclinical Efficacy

Efficacy studies were conducted in the VX-2 carcinoma model in the rabbit. The VX-2 tumor was implanted under the liver capsule as a model for liver tumors and metastases. This model was used to test the efficacy of JX-594 when given either directly into the liver parenchyma (IT) or IV against the primary liver tumor or the lung metastases that invariably develop in untreated animals.

VX-2 tumors were grown beneath the liver capsule and allowed to grow for 14 days until primary tumors were established. Groups of animals were treated intra-tumorally (IT), using ultrasound guidance, with a dose of $10^5$ pfu JX-594 or intravenously (IV) with $10^3$ pfu JX-594; control animals (n =7) were treated with PBS. Tumor sizes were monitored by CT scanning and by
ultrasound. During the following 7 weeks, tumors progressed in the control animals and numerous tumor metastases became detectable within the lungs and livers of those animals. By Week 7, the mean primary tumor volume was $91.0 \pm 16.4 \text{ cm}^3$ and all control animals had detectable metastases (mean $n = 17 \pm 2.3$). In contrast, the mean primary tumor volumes in rabbits treated IT or IV with JX-594 were $6.5 \pm 0.8$ or $11.2 \pm 2.5$, respectively. Importantly, IT or IV treatment with JX-594 completely prevented the appearance of metastases, as determined by CT scans. However, when animals were sacrificed at Week 8, 2 small pulmonary nodules were observed in one rabbit. Survival times were significantly increased with IV or IT treatment; by 70 days post-VX-2 implantation, the median survival had not been reached for the IT or IV treatment groups; in contrast, the median survival was only 50 days for controls.

Proposed Clinical Study

Objectives

- Determine the maximally-tolerated dose (MTD) and/or maximum-feasible dose (MFD) of JX-594 administered by intravenous (IV) infusion
- Determine the safety of JX-594 administered by IV infusion
- Determine the JX-594 pharmacokinetics and pharmacodynamics over time following IV infusion
- Determine the immune response to JX-594 following IV infusion
- Determine the delivery of JX-594 to, and concentration within, solid tumors following IV infusion

Patient Population

This is a Phase I, open-label, dose-escalation trial in patients with advanced/metastatic solid tumors refractory to standard therapy; tumors may include malignant melanoma, non-small cell lung cancer, renal cell carcinoma, and squamous cell carcinoma of the head and neck. The estimated sample size will be 18-24 patients; the actual size will be determined based upon toxicity results.

Study Design

Phase I, open label, dose escalation trial

Treatment Plan, Dose and Schedule

Eligible patients will receive 1 infusion of JX-594 on Day 1 and will be observed for 24 hours. Patients will be enrolled in a sequential, dose-escalation design into 5 cohorts. The starting dose (cohort 1) is $10^5 \text{ pfu/kg}$. The Cohort 5 dose is $3 \times 10^7 \text{ pfu/kg}$. An independent DSMB will review safety data and make recommendations regarding dose escalations.

The dose-finding plan is designed to ensure that no more than 1 out of 6 patients, experience a DLT at the MTD. All eligible patients that have initiated treatment will be considered evaluable for toxicity. All adverse events will be coded and tabulated using the MedDRA classification scheme based a standard set of terms (NCI Common Terminology Criteria for Adverse Events, Version 3.0). The incidence of treatment-emergent AEs will be tabulated; in
addition, the data will be stratified by severity and relationship to JX-594.

A DLT is defined as any of the following treatment-related adverse events (AEs) and is evaluated and reported through the 4-week evaluation period:

- Any Grade 4 toxicity (except isolated Grade 4 lymphopenia lasting ≤ 7 days)
- Grade 3 or 4 hypotension, disseminated intravascular coagulation (DIC), or allergic reaction/hypersensitivity
- Grade 3 non-hematologic toxicity persisting for > 7 days except for the following:
  - Transaminitis, which may last > 7 days if total bilirubin is normal or Grade 1
  - Flu-like symptoms that respond to standard treatments
- Grade 3 hematologic toxicity persisting for > 7 days (except isolated lymphopenia – see above)

Patients will visit the clinic on an outpatient basis on Day 4, Day 8, and weekly thereafter through Day 29. Safety assessments, including blood testing, adverse event collection, and physical examinations (including performance status assessment) will be carried out at specified study visits. A response assessment will be conducted at Day 29. Patients who complete the study, have stable disease or a response to treatment and have not gone on to other cancer therapy, will return to the clinic for follow-up visits every six weeks for the first six months. Thereafter, patients will be followed a minimum of every three months or according to current standard of care guidelines until patients have documented tumor progression, go on to other cancer therapy, or die. If a measurable radiological response is observed at any time, the same response assessment will be repeated after 4 weeks to confirm the response. After disease progression or initiation of new cancer therapy, patients will continue to be monitored for survival.

Endpoints
Primary endpoints include: Maximally-tolerated dose (MTD) and/or maximum-feasible dose (MFD) and safety/toxicity. Secondary and other endpoints include pharmacokinetics and pharmacodynamics, immune response to JX-594, histology, and anti-tumoral activity.

Product
JX-594 is a replication-competent, transgene-armed therapeutic vaccinia virus derived from the commonly used Wyeth vaccine strain (Dryvax®, Wyeth Laboratories). It was designed to selectively replicate in and destroy cancer cells, while at the same time stimulating a systemic anti-tumoral immune response through the expression of its transgene, hGM-CSF, in the context of tumor lysis. Three genetic modifications are included in JX-594: 1) thymidine kinase gene deletion, 2) GM-CSF gene insertion under the control of the synthetic early-late promoter, and 3) lac-Z gene insertion.

JX-594 is considered a Biosafety Level 2 (BSL-2) infectious substance. JX-594 is shipped internationally and interstate as a “Biological Substance, Category B” in compliance with International Air Transport Association (IATA) and Department of Transportation (DOT) regulations.