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Phase I Clinical trial of Locoregional Administration of the Oncolytic Adenovirus ONYX-015 in Combination with Mitomycin-C, Doxorubicin, and Cisplatin Chemotherapy in Patients with Advanced Sarcomas

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Summary

Despite many advances in cancer therapy, metastatic disease continues to be incurable in the majority of cancer patients. There is an need for more efficient and less toxic treatments in this setting. Oncolytic virotherapy represents a novel promising direction in the treatment of cancer. Based on preclinical and clinical data, combination with standard chemotherapy has the potential to further increase the antitumor activity of oncolytic virotherapy in a synergistic manner. We present the design of a phase I clinical trial combining intratumoral injections of the oncolytic adenovirus ONYX-015 with systemic chemotherapy in patients with advanced sarcomas.

Keywords

Cisplatin chemotherapy; doxorubicin; mitomycin-C; oncolytic adenovirus; ONYX-015; sarcomas

1. Introduction

ONYX-015 (dl1520) is chimeric human group C adenovirus that has been genetically engineered to incorporate deletions in the E1B-55k and E3B regions (1). The E1B protein in conjunction with E4ORF6 binds to the tumor suppressor protein p53 (2). This interaction causes p53 to be degraded and prevents it from causing cell cycle arrest, thus leading to a productive viral infection. Because approximately 50% of human cancers have p53 mutations, and these are often associated with chemotherapy resistance, this could represent an attractive approach in the treatment of cancer (3, 4). Subsequent work, however, has shown that ONYX-015 can grow efficiently in cancer cells with wild-type p53 (5, 6). This can at least in part be explained by p53 inactivation due to other mechanisms, such as mdm2 amplification (7, 8).

In 1996, ONYX-015 was the first oncolytic virus to enter human clinical trials (9). The virus has demonstrated a remarkable safety profile, independent of the route of administration. The maximum tolerated dose has not been reached after intratumoral, hepatic artery, intraperitoneal, or systemic administration of the virus (8, 10–12).

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⁴Our study provided evidence that ONYX-015 can replicate in sarcomas after intratumoral administration and that it can be safely administered in combination with standard sarcoma chemotherapy. Efficacy, secondary endpoint, was observed in one patient with a malignant peripheral nerve sheath tumor who met study criteria for a partial response. Additional work to exploit this approach in patients with recurrent sarcoma is warranted.

The initial trials tested the efficacy of the ONYX-015 as a single agent. Two phase II trials evaluated the virus in patients with recurrent head and neck cancer, after intratumoral injections. An approximately 15% response rate with no objective evidence of effect in uninjected tumors was observed (9, 13). No antitumor responses were seen, however, after intratumoral administration in patients with pancreatic cancer, intraperitoneal administration in patients with ovarian cancer, or intravenous administration in patients with colorectal cancer (12, 14, 15). These results emphasize the limited activity of ONYX-015 when used as single agent.

Preclinical work both in vitro and in animal models has demonstrated synergistic activity between ONYX-015 and 5-fluorouracil (5FU) or cisplatin chemotherapy (7). In a subsequent phase II trial in recurrent head and neck cancer patients, approximately 65% of patients responded when treated with ONYX-015, cisplatin, and 5FU; this compares favorably with the historic response rate of 25–35% in this patient population (16). Injected tumors were significantly more likely to respond to the virus/chemotherapy combination as compared with noninjected tumors ($p < 0.01$). A phase III trial testing this approach in recurrent head and neck patients was aborted in the USA prior to its completion. In March of 2006, however, H101, a virus very similar to ONYX-015, was approved by the China's State Food and Drug Administration for treatment of head and neck cancer by intratumoral injection in combination with chemotherapy. The H101 virus in addition to the E1B-55k gene deletion lacks all E3 proteins. This regulatory approval was based on a phase III clinical trial, which showed a 79% response rate in patients who received intratumoral administration of H101 in combination with platinum-based chemotherapy vs 40% in controls without virus treatment ($p=0.001$). Shanghai Sunway Biotechnology, the company marketing H101, has also acquired US development rights to ONYX-015 (17).

The protocol we describe here investigated an ONYX-015 chemotherapy combinatorial approach in patients with advanced sarcomas. Sarcomas represent a rational target for treatment with ONYX-015. They have a high frequency of p53 mutations ranging from 40% to 75%. In addition, MDM-2 gene amplification, which can result in functional p53 inactivation, occurs in another 10–30% of sarcoma cases (18, 19). Furthermore, in preclinical experiments, ONYX-015 has been shown to replicate effectively and cause significant cytotoxicity against the sarcoma line S4052 (18). In this trial, we combined ONYX-015 with antisarcoma agents that either are synergistic with ONYX-015, such as cisplatin, or for which ONYX-015 can reverse resistance, such as doxorubicin (7).

The goals of study were:

1. To define the maximum tolerated dose (MTD) of ONYX-015 in combination with MAP chemotherapy (mitomycin C, doxorubicin, and cisplatin) in patients with metastatic sarcoma.
2. To assess ONYX-015 replication in sarcomas.
3. To assess the antitumor activity of the regimen (secondary endpoint).
4. To correlate, in a preliminary fashion, ONYX-015 replication with the presence of neutralizing antibodies.
5. To correlate any observed responses with the p53 and MDM-2 tumor status.

2. Materials

2.1 Laboratory Evaluation of Patient Samples

2.1.1. Immunohistochemistry for p53 and mdm2 Expression

1. Anti-p53 antibody M7146 (Dako, Glostrup, Denmark).
2. Anti-mdm2 antibody clone SMP14 (Dako).
3. HRP+/DAB+ chromogen substrate (Dako).

2.1.2, Plasma Neutralizing Antibody Titers

1. 12-Well plates (Fisher Scientific, Hampton, NH).
2. Agarose (Invitrogen Life Technologies, Carlsbad, CA).
3. HRK 293 cells (ATCC, Manassas, VA).

2.1.3, Detection of ONYX-015 Viral Genome in Plasma

1. QiaAmp Blood Kit (Qiagen, Alameda, CA).
2. Carrier DNA (Dualsystems, San Mateo, CA).
3. Oligonucleotide primers. The following primers and probe were employed: forward primer 5'-GCTGG CG CAGAACTAT-TCCA, reverse primer 5'-GTG CGGGTCTCATCGTACCT and the probe 5'^{FAM}-ACCTTCCAGATCCGTCGACCTGCA-^{TAMRA}.
4. DNA sequencer (Perkin-Elmer, Foster city, CA).
5. Standard polymerase chain reaction (PCR) reagents.
6. Standard reagents and apparatus for agarose gel electrophoresis.

2.1.4. Detection of Adenovirus DNA in Tumor Biopsies

1. Biotinylated adenovirus DNA probe (Enzo Diagnostics, Farmingdale, New York).
2. Proteinase K (Invitrogen, Carlsbad, CA).
3. 4% paraformaldehyde.
4. Nitroblue tetrazolium/bromochloroindolyl phosphate (NBT/BCIP) (Roche Applied Science, Indianapolis, IN).
5. Nuclear fast red (Vector Laboratories, Burlingame, CA).
6. Streptavidin-alkaline phosphatase conjugate (Vector laboratories).

3. Methods

3.1. Clinical Trial

3.1.1 Inclusion/Exclusion Criteria

Inclusion Criteria

1. Patients were required to have a histologic diagnosis of sarcoma beyond surgical cure with at least two measurable sites of metastatic disease. Injections of the viral agent were administered in one of the metastatic disease sites, while the remainder sites of metastatic disease served as control.
2. The injected lesion should have been between 1 and 10 cm in size.

3. 18 years of age or older.
4. Life expectancy ≥ 3 months.
5. Adequate hematologic, renal, and hepatic function.

Exclusion Criteria

1. Poor performance score of Eastern Cooperative Oncology Group (ECOG) 3 or 4.
2. Uncontrolled infection.
3. Metastatic disease not amenable to intratumoral administration of the virus.
4. Other concurrent chemotherapy, immunotherapy, or radiation therapy.
5. More than two previous regimens for metastatic disease.
6. To avoid cardiotoxicity patients with a previous maximum dose of doxorubicin ≥ 250 were not eligible.

The protocol was approved by the Mayo Clinic Institutional Review Board, and informed consent was obtained from all the patients.

3.1.2. Pretreatment Evaluation/Follow-Up Studies

1. Pretreatment evaluation included a history and physical examination, complete blood cell count, serum chemistries, and coagulation profile.
2. Evaluation/measurement of injected and control tumors and assessment of disease status was performed by using appropriate imaging modalities, such as computed tomography (CT) scan, magnetic resonance imaging (MRI), or chest x-ray.
3. Follow-up studies included physical examination, complete blood cell counts, serum chemistries, and imaging performed every 4 weeks.
4. Neutralizing antibody, titers were obtained at baseline and before cycle 2.
5. ONYX-015 genomes in the peripheral blood were quantified at baseline, on day 5 of cycle 1, and before cycle 2. In addition, one patient had extended sampling performed for viral kinetics, consisting of additional blood samples on days 8, 10, and 12 (3, 5, and 7 days after the day 5 dose) of cycle 1.
6. Tumor biopsy for confirmation of diagnosis and determination of p53 and mdm2 status was performed at baseline, and repeat biopsy to assess viral replication by in situ hybridization was obtained on day 5 of cycle 1 (Table I summarizes the test schedule).

3.1.3. Study Drug—The ONYX-015 virus was produced in human embryonic kidney 293 cells, as previously described, formulated in Tris buffer (10 μ M Tris pH 7.4, 1 μ M MgCl₂, 150 μ M NaCl, 10% glycerol) and supplied frozen (-20°C) in 0.5-mL vials (9). The viral particle (vp) to plaque-forming units (pfu) ratio was 20. Wild-type adenovirus was undetectable in all clinical lots of ONYX-015 at a limit of detection of 1 in 10^{11} particles. Thawed ONYX-015 was maintained at $2-8^{\circ}\text{C}$ during dilution and handling and administered to patients within 2 h from viral preparation.

3.1.4. Treatment Schedule (see Note 1)—The study included following three viral dose levels per dose (Table 2):

1. Level 1: 10^9 pfu of virus per dose.

2. Level 2: 3×10^9 pfu per dose.
3. Level 3: 10^{10} pfu per dose.

The virus was administered intratumorally in multiple sites throughout the tumor mass on days 1–5 of each treatment cycle for a total dose per cycle of:

1. 5×10^9 pfu in dose Level 1.
2. 1.5×10^{10} pfu in dose Level 2.
3. 5×10^{10} pfu in dose Level 3.

The volume of viral administration was determined in conjunction with the injected tumor volume, the intent being to saturate as much of the tumor as possible with the viral solution. The injected tumor volume was calculated by multiplying the three dimensions of the lesion to be injected and dividing by 2. Subsequently the viral agent was diluted and administered in normal saline at a total volume equivalent to 30% of the calculated tumor volume (*see* Note 2).

All patients received concurrent chemotherapy: The MAP chemotherapy regimen included 8 mg/m² mitomycin-C (up to a maximum total dose of 32 mg/m²), 40 mg/m² doxorubicin, and 60 mg/m² cisplatin administered on day 1 every 28 days (*see* Note 3).

The ONYX-015/MAP treatment continued up to six treatment cycles if there was no disease progression or unacceptable toxicity for a cumulative doxorubicin dose of 450 mg/m² (including previous adjuvant doxorubicin therapy). Mitomycin-C was to be discontinued after four cycles of MAP or when a maximum dose of 32 mg/m² had been administered (Table 35.3 summarizes the treatment schedule).

3.1.5. Dose-Limiting Toxicity (DLT)—DLT was defined as follows:

1. Hematologic toxicity: neutrophil count <500 for ≥ 5 days, hemoglobin <6.5 g/dL, or platelet count <25,000.
2. Renal toxicity: serum creatinine ≥ 2 times the baseline
3. All other nonhematologic toxicity: $\geq$ grade 3 as per the National Cancer Institute (NCI) Common Terminology Criteria.

3.1.6. Response Criteria

1. Response was evaluated by the appropriate imaging modality, CT, MRI, or chest x-ray before each treatment cycle.
2. At least two measurable lesions were required according to our inclusion criteria. In addition, patient could have other evaluable sites of disease.

¹The study used a type 2 accelerated titration design as described by Simon et al. (20). This is a modification of the standard cohort of three design that has been developed to reduce the number of patients treated at potentially suboptimal dose levels. One patient is entered at each dose level until toxicity of at least grade 2 is encountered, at which point the study is converted to a standard cohort of three design with three patients treated per dose level. MTD was defined as the highest safely tolerated dose where ≥ 1 patient experiences dose-limiting toxicity (DLT) with the next higher dose level having at least two patients who experience DLT.

²Administration of ONYX-015 was well tolerated despite the fact that five injections per treatment cycle were performed in deep-seated lesions: patients have had up to a total of 30 injections (six treatment cycles), with practically no injection-related complications, except for one patient experiencing grade 2 injection site pain during treatment of a large recurrent chest wall liposarcoma.

³Coadministration of ONYX-015 with chemotherapy in our trial did not aggravate either toxicities caused by ONYX-015 or toxicities caused by chemotherapy.

3. Complete response was defined as complete disappearance of all evidence of tumor, maintained for at least 4 weeks.
4. A partial response was defined as a decrease of $\geq 50\%$ in the sum of the product of the perpendicular diameters of all measurable lesions maintained for at least 4 weeks with no new lesions, and no progression of evaluable disease.
5. Progression of disease was defined as an increase of $\geq 22\%$ in the sum of the products of the perpendicular diameters of all measurable lesions, appearance of new lesions, or worsening of other evaluable disease.
6. Patients with stable disease were defined as those not meeting the criteria for complete response, partial response, or progression of disease.

3.2. Laboratory Analyses

3.2.1. Immunohisto-chemistry for p53 and mdm2 Expression

1. Formalin-fixed, paraffin-embedded samples were deparaffinized with three changes of xylene, rehydrated in a series of alcohols (100%, 95%, then 7% EtOH) and rinsed well in running distilled water. Slides were then placed in a preheated 1 mM EDTA, pH 8.0, retrieval buffer for 30 min then cooled in the buffer for 5 min followed by a 5 min rinse in running distilled water.
2. Slides were then placed on the Dako Autostainer and the following procedure (at room temperature) was applied: sections were incubated with 3% H_2O_2 in ethanol for 5 min to inactivate the endogenous peroxides, then incubated with the specific antibody for 30 min, and rinsed with Tris-buffered saline with Tween (TBST) wash buffer.
3. The secondary antibody was then added for 15 min, and the slides were rinsed with TBST wash buffer, incubated in 3,3'-diaminobenzidine (DAB+) for 5 min, counterstained with modified Schmidt's hematoxylin for 5 min followed by a 3-min tap water rinse, dehydrated through graded alcohols, cleared in three changes of xylene, and mounted with a permanent mounting media.
4. Results were judged positive as 1+ when $< 10\%$ of neoplastic cells were positive 2+ when 10–50% of the neoplastic cells were positive, and 3+ when $> 50\%$ of the neoplastic cells were positive.

3.2.2. Plasma Neutralizing Antibody Titers—Neutralizing antibodies directed against adenovirus type 5/ONYX-015 were determined using a cell-based plaque assay.

1. Clinical plasma samples previously determined to produce high midrange and negative titers were designated as plasma controls.
2. Patient and control plasma samples were incubated at 55°C for 30 min to inactivate complement and serially diluted.
3. Each plasma dilution was mixed with ONYX-015 stock at a titer prequalified to produce 15–20 plaques per well in a 12-well dish format.
4. The patient's samples and controls were inoculated for 1 h at room temperature.
5. The samples were then applied to 70–80% confluent HEK(293 cells in 12-well dishes.
6. They were incubated for 2 h at 37°C .
7. The plasma–virus mix was replaced with 2 mL of 1.5% agarose.

8. Plates were read on day 7 after inoculation by counting the number of plaques per well.
9. The titer of neutralizing antibody for each sample was reported as the dilution of plasma that reduced the number of viral plaques to 60% of the number of plaques in the virus control without patient plasma.
10. The titer range of the neutralizing antibody titer assay was 1:20–1:81, 920.

3.2.3. Detection of ONYX-015 Viral Genome in Plasma (see Note 5)—Total DNA was isolated by adding 3 µg of carrier DNA to 500 µL of plasma using the QiaAmp Blood Kit.

1. PCR amplification was performed using 10 µmol/L of each primer and the probe with the following cycling conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C (denaturation), and 40 cycles of 1 min at 63°C.
2. To check for the presence of PCR inhibitors, an additional reaction was amplified using primers and probe-specific for the 18S ribosomal RNA (rRNA) gene.
3. Each PCR run contained one set of Standards, extraction controls corresponding to the test articles on the plate, and a PCR reagent control, run in duplicate reactions.

3.2.4. Detection of Adenovirus DNA in Tumor Biopsies (see Note 6)

1. Biopsy samples were formalin-fixed, paraffin-embedded, and cut.
2. Slides were deparaffinized in xylene, hydrated with ethanol, digested with proteinase K, and fixed in 4% paraformaldehyde.
3. Samples were hybridized overnight at 37°C with 0.5 µg/mL biotinylated adenovirus DNA.
4. Slides were then sequentially washed in 0.5X SSC.
5. Probe detection was carried out by using a streptavidin–alkaline phosphatase conjugate.
6. Slides were stained with nitroblue tetrazolium/bromochloroindolyl phosphate (NBT/BCIP), and counterstained with nuclear fast red.
7. Three sections were stained per biopsy (20).
8. Pathology interpretation was obtained on all biopsy samples

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⁵ONYX-015 was detected by quantitative PCR 24 h after the fourth intratumoral injection in the peripheral blood of all five patients treated in the two highest dose levels. One patient in addition had repeat sampling performed on days 8, 10, and 12. A second peak in the genome was observed on day 12 after a drop on day 10. Both the in situ hybridization and viral kinetics data are suggestive of viral replication in sarcoma tumors.

⁶In situ hybridization positivity in study patients was focal. Although this could be interpreted as limited viral replication in vivo, other contributing factors could include sampling effect and small numbers of cells in biopsy specimens. This emphasizes the difficulties associated with the use of biopsies to assess viral replication in vivo.

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Fig. 1. (a,b) Focal areas of viral replication were demonstrated by in situ hybridization in the tumor of one of the study patients, (c,d) Close-up view of the positive areas. (Reproduced from (21) with permission),

Test schedule

Table 1

Test and procedures	<14 Days before registration	Cycle 1, days 5 and 8 ¹	During interval between treatment (all cycles)	Before each cycle (cycles 2-6)	Observation: every 3 mo, year 1; every 4 mo, year 2; every 6 mo, year 3
History and exam, weight, PS	X			X	X
Height	X			X	
Hematology group WBC, ANC, Hgb, PLT	X		Weekly	X	X
Chemistry group AST, total Bili., Alk phos, Creat, INR	X			X	X
Na, K, Mg	X			X	
Chest X-ray	X			X	X
Evaluation/measurement of injected and control tumors and disease status (CXR CT, MRI, etc.) ²	X			X	At 3 mo after treatment only; thereafter as clinically indicated
Serum neutralizing antibodies	X			Cycles 2, 4, and only	
Tumor biopsy for viral replication	X	X ¹			
Tumor biopsy for confirmation of diagnosis and determination of p53, mdm-2 status	X				
Viral titers (plasma)	X	X		Cycle 2 only	
Ejection fraction	X ³			X ³	
Serum pregnancy, test ⁴	X				

¹Day 8 biopsy was encouraged (if deemed safe) but was not mandatory.

²The same imaging modality should have been used for a given patient throughout the study. The study, radiologist was to review all imaging studies.

³As clinically indicated in association with MAP chemotherapy.

⁴For women of childbearing potential only. Must be done <7days before registration.

Abbreviations: PS = Performance Status, WBC = White Blood Cells, ANC = Absolute Neutrophil Count, Hgb = Hemoglobin, PLT = platelets, AST = Aspartate Transaminase, total Bili = total Bilirubin, Alk phos = Alkaline phosphatase, Creat = Creatinine, INR = International Normalization Ratio (prothrombin time), NA = Sodium, K = Potassium, Mg = Magnesium, CXR = Chest X-ray. CT = Computer Tomography, MRI = Magnetic Resonance Imaging

Table 2

ONYX-015 dose escalation

Level	Dose of ONYX-015 per treatment intratumorally
0	3×10^8 pfu
1*	10^9 pfu
2	3×10^9 pfu
3	10^{10} pfu

* Starting close level

Table 3

Treatment schedule

Agent	Dose#	Route	Day(s)	Retreatment
ONYX-015	As assigned by the Randomization Center (<i>see</i> Table 2)	Intratumoral injection *	1–5	Every 28 days
Mitomycin C _†	8 mg/m ²	IV Push	1	Upto6 Cycles
Doxorubicin _†	40 mg/m ²	IV Push	1	
Cisplatin	60 mg/m ²	IV over 2 h in 1,000 mL of 0.45 NaCl plus 25 g of mannitol		

* By a diagnostic radiologist under ultrasound or CT guidance