A Phase I/II Trial of Intratumoral Endoscopic Ultrasound Injection of ONYX-015 with Intravenous Gemcitabine in Unresectable Pancreatic Carcinoma

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Abstract

Purpose: Localized pancreatic carcinoma is rarely resectable and is resistant to conventional therapies. ONYX-015 (dl1520) is an E1B-55kD gene-deleted replication-selective adenovirus that preferentially replicates in and kills malignant cells. Endoscopic ultrasound (EUS) has the potential to conveniently and accurately deliver local therapy to the pancreas. Therefore, we undertook a trial of the feasibility, tolerability, and efficacy of EUS injection of ONYX-015 into unresectable pancreatic carcinomas.

Experimental Design: Twenty-one patients with locally advanced adenocarcinoma of the pancreas or with metastatic disease, but minimal or absent liver metastases, underwent eight sessions of ONYX-015 delivered by EUS injection into the primary pancreatic tumor over 8 weeks. The final four treatments were given in combination with gemcitabine (i.v., 1000 mg/m2). Patients received 2 × 1010 (n = 3) or 2 × 1011 (n = 18) virus particles/treatment.

Results: After combination therapy, 2 patients had partial regressions of the injected tumor, 2 had minor responses, 6 had stable disease, and 11 had progressive disease or had to go off study because of treatment toxicity. No clinical pancreatitis occurred despite mild, transient elevations in lipase in a minority of patients. Two patients had sepsis before the institution of prophylactic oral antibiotics. Two patients had duodenal perforations from the rigid endoscope tip. No perforations occurred after the protocol was changed to transgastic injections only.

Conclusions: This study indicates that ONYX-015 injection via EUS into pancreatic carcinomas by the transgastic route with prophylactic antibiotics is feasible and generally well tolerated either alone or in combination with gemcitabine. Transgastric EUS-guided injection is a new and practical method of delivering biological agents to pancreatic tumors.

Introduction

Adenocarcinoma of the pancreas is the fifth most common cause of cancer death in the United States, with over 28,000 patients expected to die from the disease this year (1). Pancreatic cancer is usually unresectable at the time of diagnosis because of metastasis or local extension, particularly to the mesenteric vasculature (2). At the time of diagnosis, patients with pancreatic carcinoma frequently have locally advanced disease either alone or with small liver metastases (3). These patients suffer from local complications including pain and biliary or intestinal obstruction. Survival for this group of patients is poor with a median life expectancy of 6–10 months (4). Historically, patients with locally advanced disease have been treated with 5-fluorouracil and radiation (5). More recently, gemcitabine has been used, although life expectancy remains short, and morbidity remains high (6). Clearly, more effective modalities of treatment for locally advanced pancreatic carcinoma are needed. New agents with novel mechanisms of action that can be safely combined with chemotherapy and/or radiotherapy would be particularly attractive.

Replication-selective viruses are novel and promising potential treatments for cancer (7). These agents have the attributes of being able to replicate selectively in and lyse cancer tissue while sparing normal tissue. Unlike replication-incompetent gene therapy approaches, these agents spread after replication and oncolysis, and they are therefore able to more efficiently target tumor cells throughout a solid mass. A variety of viruses are being studied in clinical trials, including gene deletion mutants [e.g., adenovirus (8), herpes virus (9, 10), and vaccinia], tissue-specific promoter-regulated viruses [e.g., adenovirus (11)], and inherently tumor-selective viruses [e.g., reovirus (12) and Newcastle Disease virus]. The first such engineered replication-selective virus to enter clinical trials was dl1520 (ONYX-015). This adenovirus serotype 2/5 chimera has a deletion in the E1B-55kD gene (as well as a partial E3 region deletion; Ref. 13). E1B-55kD has several functions, including the inhibition of p53 function, in complex with E4ORF6. Because p53 function is already lost in the majority of human cancers (through mutation, deletion, or functional inactivation), it was hypothesized that E1B-55kD would be expendable in tumor cells but still necessary in normal cells for viral replication. Preclinical data
have demonstrated a role for p53 in modulating the replication of this virus in most (14–16), but not all (17), cell systems. Cancer cells with normal p53 gene sequences but loss of normal p53 function have been shown to be sensitive to replication-dependent killing by this virus (18, 19). Data from nude mouse human tumor xenografts have confirmed a correlation between p53 function and the efficacy of ONYX-015. Finally, Phase I and II clinical trials of ONYX-015 in head and neck cancer patients have now been completed. These trials demonstrated clear tumor-selective viral replication, sparing of injected peritumoral normal tissues, tumor necrosis associated with viral replication, and a correlation between p53 gene mutation and tumor regression (20, 21).

Over 200 patients have been treated with ONYX-015 by various routes of injection including intratumoral, i.p., intraarterial, and i.v. (22). Although vascular delivery of adenovirus to systemic metastases has now been demonstrated, objective responses with the first-generation adenovirus ONYX-015 (dl1520) have only been reported after intratumoral injection. In addition, whereas durable objective responses were relatively rare after ONYX-015 treatment as a single agent, encouraging durable antitumor activity was demonstrated in combination with chemotherapy in clinical trials with head and neck cancer patients. A Phase I trial of injection of ONYX-015 into locally advanced primary pancreatic tumors under EUS guidance (n = 22) demonstrated that the treatment was well tolerated without significant virus-related toxicity. Although objective responses were not demonstrated, six minor responses of injected tumors were reported (23). Unfortunately, CT-guided injection is cumbersome, and it is difficult to perform repeated intratumoral injections during a given treatment session. The efficacy of intratumoral injections has been clearly associated with the ability to spread the viral agent diffusely throughout the tumor (24). This is presumably because of the inefficient spread of adenovirus within solid tumors, in particular those with significant fibrosis and/or normal cells intercalated throughout the tumor mass. Because pancreatic tumors frequently contain significant amounts of fibrosis and normal tissue, it is unlikely that injection by single needle passes at infrequent intervals would be effective. Therefore, CT-guided intratumoral injection is not an optimal approach for repeated, diffuse intratumoral dosing of this agent. New and improved delivery methods for viral therapies for cancer are clearly needed (25).

EUS is frequently used to evaluate and biopsy the pancreas with few complications (26). Although at the time of initiation of this study EUS had not been used for the therapy of pancreatic carcinomas, we hypothesized that direct administration of a biological agent into tumors of the pancreas via EUS would be feasible. Therefore, a Phase I/II study was undertaken to evaluate the feasibility and tolerability of EUS-guided intratumoral injection of pancreatic carcinomas with ONYX-015, alone and in combination with i.v. gemcitabine.

### Materials and Methods

**Objectives.** The primary objectives of this study were the following: (a) to determine the safety and feasibility of repeated injection of ONYX-015 into primary adenocarcinoma of the pancreas under EUS guidance, alone and in combination with i.v. gemcitabine; and (b) to determine the MTD of the virus when this technique is used. Secondary objectives included the following: (a) to determine the target tumor response rate after ONYX-015 injection, alone and in combination with gemcitabine; (b) to determine the time to target tumor progression; (c) to study replication of ONYX-015 within the injected tumor and in adjacent normal pancreas after injection; and (d) to determine the survival rate of subjects at 6, 9, and 12 months after entry into the study.

**Eligibility Criteria.** Inclusion criteria included the following: (a) histologically or cytologically confirmed carcinoma of the exocrine pancreas; (b) cancer that was not considered resectable for potential cure (i.e., locally advanced or metastatic); (c) Karnofsky performance status of ≥60%; (d) life expectancy of ≥3 months; (e) ≥18 years of age; (f) use of a reliable method of contraception if sexually active; (g) total bilirubin < 2.5 mg/dl; (h) aspartate aminotransferase and alanine aminotransferase < 3.0-fold upper limit of normal; (i) international normalized ratio < 1.5 and partial thromboplastin time within normal limits; and (j) neutrophils > 1,000/ml, hemoglobin > 9 g/dl, and platelets > 100,000/ml.

Patients who met any of the following criteria were excluded from the study: (a) known chronic liver dysfunction before the development of pancreatic cancer (e.g., cirrhosis, chronic hepatitis), which in the estimation of the principal investigator put the patient at high risk for liver complications; (b) liver metastases with a combined maximal diameter of ≥6 cm as measured by abdominal CT; (c) upper gastrointestinal bleeding in the 4 weeks before study entry; (d) obstruction of the duodenal lumen by tumor invasion; (e) ongoing active infection, including HIV; (f) any viral syndrome diagnosed within the previous 2 weeks; (g) chemotherapy within the previous 3 weeks (6 weeks for nitrosoureas or mitomycin C); (h) radiotherapy to the target tumor site within the last 4 weeks; (i) concomitant hematological malignancy; (j) chronic immunosuppressive medication; (k) pregnancy or lactation; and (l) treatment with any other investigational therapy within the last 4 weeks. The protocol was conducted according to the guidelines of the Helsinki Declaration. Written informed consent was required, and the protocol was reviewed and approved by each institution’s institutional review board.

**Test Article.** ONYX-015 (dl1520) is a chimeric human group C adenovirus (Ad2 and Ad5) that does not express the M, 55,000 protein of the E1B gene and was originally constructed in the laboratory of Arnold Berk (13). The virus contains a deletion between nucleotides 2496 and 3323 in the E1B region encoding the M, 55,000 protein. In addition, a C-T transition at position 2022 in E1B generates a stop codon at the third codon position of the protein. These alterations eliminate expression of the E1B-55kd gene in ONYX-015-infected cells. ONYX-015 was grown and titered on the human embryonic kidney cell line HEK293 as described previously (18).
Study Design. The study was an open label Phase I/II study. The Phase I component was designed to define the MTD of EUS-administered ONYX-015 and gemcitabine. Because previous Phase I trials had suggested that CT-guided injections of $2 \times 10^{12}$ particles every 4 weeks were well tolerated, the first cohort of three patients received $2 \times 10^{10}$ particles/session. If no DLT of grade 4 toxicity for flu-like symptoms, pancreatitis, or liver toxicity; grade 3 liver toxicity for $\geq 7$ days; or other toxicity related to ONYX-015 was seen, the dose was to be increased to $2 \times 10^{11}$ particles/session for the second cohort of three patients. If a DLT was seen in the first three patients, an additional three patients were to be enrolled at that dose. If fewer than two patients had a DLT at $2 \times 10^{10}$ particles/session, then the next cohort would be treated at $2 \times 10^{11}$ particles/session. The maximum practical dose was felt to be $2 \times 10^{11}$ particles/session for this preparation because of the volume needed to dilute this particular virus preparation. The highest dose shown to be safe was to be used during the treatment of 15 additional patients in the Phase II portion of the trial.

EUS Injection of Virus. The total volume of the virus solution to be injected was determined as one-tenth of the product of the three-dimensional measurements of the target tumor on CT. Previous Phase I testing with CT-guided injection into pancreatic carcinomas had shown that 20% of the tumor volume was the maximal injectable volume. EUS was performed by four experienced endosonographers at the three sites. After conscious sedation with midazolam and meperidine, the entire target tumors were visualized on EUS with a Pentax FG36UX linear array echoendoscope, using either a gastric or a duodenal approach. After two duodenal perforations occurred, the gastric approach was mandated. Each intratumoral injection consisted of 1 ml or, if the total volume to be injected was greater than 10 ml, one-tenth of the total volume. Injections were performed with a 22-gauge Wilson-Cook Echotip needle in a fan-like pattern during withdrawal of the needle under direct EUS vision. After two infections occurred that may have been related to the injection technique, the protocol was changed so that the needle was not pulled all of the way into the lumen between passes, and patients were treated with 250–500 mg of ciprofloxacin p.o. 2–3 h before the procedure and 12 and 24 h after the procedure. Patients received ONYX-015 injections on days 1, 5, 8, 15, 36, 43, 50, and 57 (Table 1).

Chemotherapy. Patients were treated with i.v. 1000 mg/m$^2$ gemcitabine over 30 min on days 36, 43, 50, and 57 (after ONXY-015 treatment on the same day). The gemcitabine dose was reduced 50% with an absolute neutrophil count of 500–999/μl or platelet count of 50,000–99,999/μl and held if below those levels. ONYX-015 injections were not held because of hematological toxicity. Chemotherapy with gemcitabine could be continued after the ONYX-015 injections were completed. Poststudy treatment was not specified.

Evaluation. Patients were monitored for adverse events, hematological abnormalities including lipase and amylase, physical examination changes, anti-ONYX-015 neutralizing antibody titers, viral genome shedding into the bloodstream, and tumor size by CT scan. Scans were performed on day 35, day 63, and every 6–8 weeks until progression. Response of the injected tumor was evaluated using standard WHO criteria. Responses had to be confirmed by a second scan at least 4 weeks later. Fine needle aspirate biopsies of the tumor and pancreas were attempted before viral injections during the first three EUS sessions.

In Situ Hybridization for Adenoviral DNA. In situ hybridization for adenoviral DNA was carried out on biopsy samples to determine the extent of replication of ONYX-015 in both tumor and adjacent normal tissues using a biotinylated adenovirus DNA probe (Enzo Diagnostics, Inc., Farmingdale, NY) as described previously (18).

Neutralizing Antibody Level Determination. Neutralizing antibodies for ONYX-015 were assessed at baseline, day 22, and day 50. Titters against ONYX-015 were determined on blood samples as follows. Patient and control samples were incubated at 55°C for 30 min to inactivate complement. Clinical plasma samples previously determined to produce high, midrange, and negative titers were designated as plasma controls. Each dilution was mixed with adenovirus stock at a titer prequalified to produce 15–20 plaques/well of a 12-well dish in DMEM growth medium. The patient samples and controls were inoculated for 1 h at room temperature and applied to 70–80% confluent JH393 cells in 12-well dishes. After 2 h of incubation at 37°C, 5% CO$_2$ plasma-virus mix was removed, and 2 ml of 1.5% agarose in DMEM were added to each well. Plates were read on day 7 postinoculation by counting the number of plaque-forming units/well. The titer of neutralizing antibody for each sample was reported as the dilution of plasma that reduced the number of plaques to 60% of the number of plaques in the virus control without antibody.

Results

Patient Characteristics. A total of 21 patients were enrolled from September 1998 to September 1999. The characteristics of the entire cohort of patients are listed in Table 2. The median age was 63 years (range, 34–78 years), and the median Karnofsky performance status was 90 (range, 60–100). Eight patients (39%) had received prior therapy. Nine patients (43%) only had locally advanced disease, whereas 12 patients (57%) also had metastatic disease at the start of the study. The study population enrolled was highly selected because of the entry criteria of the study. The most common reason for screened patients not to enter the study was clinical deterioration attributable to tumor progression during the screening period.

Dose Escalation. Three patients were treated with $2 \times 10^{10}$ particles/session in the Phase I portion of the study, and no virus-related DLTs were seen. Three additional patients were treated at the $2 \times 10^{11}$ particles/session dose without DLT. Fifteen additional patients were subsequently treated in the Phase II portion of the study at the MTD of $2 \times 10^{11}$ particles/session.

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Toxicity. Toxicities could be categorized as attributable to the ONYX-015 virus, to viral administration, or to gemcitabine treatment. In general, ONYX-015 itself was well tolerated, with mild and transient flu-like symptoms such as low-grade fever, chills, and myalgias being the most common. Almost all these toxicities were grade 1 or 2. Grade 3 and 4 toxicities are summarized in Table 3. Only one patient (5%) had grade 3 fever related to ONYX-015 injection. One patient had a grade 3 increase in partial thromboplastin time. Asymptomatic grade 3 and 4 increases in amylase and lipase were detected in 10% of patients, but no clinical pancreatitis was observed.

Complications from the injection process itself were more important than those from the virus. Two patients developed bacterial infections felt to be secondary to the actual EUS injection needle. Both infections were easily treated with antibiotics. One of these patients was believed to possibly have a peripancreatic abscess, but this is unlikely because of the patient’s rapid response to antibiotics. No further infections were noted after the injection technique was modified so that the needle was not drawn completely out of the tumor during repositioning and repassage, and prophylactic administration of oral ciprofloxacin was instituted.

Two patients had duodenal perforations resulting from the stiff tip of the echoendoscope rather than from the injection needle. Each perforation was quickly identified, and the small tears were oversewn without complication, resulting in complete recovery. One of these patients had additional ONYX-015 therapy without incident. The protocol then was amended to allow only transgastric injections, and no additional endoscopic complications were seen.

One patient had an asymptomatic cystic fluid collection of unclear etiology noted only by EUS after treatment began; the cystic fluid was bacterial culture negative. The patient remained asymptomatic, although he was taken off the study.

While on gemcitabine treatment, five patients developed grade 3 leukaemia or neutropenia, one developed grade 4 neutropenia, one developed grade 3 lymphopenia, and one developed grade 4 anemia. Hematological toxicity only occurred during gemcitabine treatment. One patient was diagnosed with gemcitabine pulmonary toxicity, treated with corticosteroids, and subsequently recovered.

Response. Results of the effect of treatment on the target tumor are summarized below. No objective responses were demonstrated on day 35, following four injections of ONYX-015 as a single agent. After combination treatment with virus plus gemcitabine, objective partial regressions of >50% were seen in 2 of the 21 (10%) patients treated. The best target tumor responses were seen on the next scans that were obtained during continued treatment with gemcitabine, approximately 10 weeks after ONYX-015 treatment. During this interval, one patient had an increase in the size of a nontarget lesion, whereas the other had disappearance of two nontarget lesions. Unfortunately, neither of these two patients underwent confirmatory CT scans, but each survived for several months after their best scan.

Eight patients (38%) had stable disease, and 11 (52%) had progressive disease or had to go off study because of treatment toxicity. The median time to injected tumor progression was approximately 6 weeks, and 14% of patients were free from local progression at 6 months. Sixty-seven percent of patients were alive at 6 months, 29% were alive at 1 year, and the median survival time was 7.5 months. One patient is still alive 3 years after starting treatment.

Neutralizing Antibody Development. Seventy-six percent of patients had positive low-titer neutralizing antibody titres to Ad5 (and therefore to the coat of ONYX-015) at baseline as expected. After treatment, all patients with posttreatment values available developed positive titres and/or had a significant increase in titer.

Viral Replication and Shedding. Posttreatment aspirates generally consisted of necrotic debris or were hypocellular and did not show adenoviral DNA by in situ hybridization.

Discussion

Local complications of pancreatic carcinoma result in significant morbidity and mortality. Although systemic therapy is ultimately needed for cure, an effective locoregional therapy for the treatment of the pancreatic primary and/or regional metas-
tases to the liver would be beneficial to patients without extensive extrahepatic disease at the time of presentation. Current therapies, however, are of limited benefit to most patients. External beam radiation has been used for many years, but this has significant gastrointestinal toxicity that limits its application (5). The direct injection of anticancer therapy into the pancreas is a theoretically appealing approach but has been limited by the relative inaccessibility of the organ and the potential for causing pancreatitis. Pancreatic inflammation can lead to ongoing pancreatitis that can result in pain, metabolic derangements, scarring, pancreatic insufficiency, and even death. The safety and feasibility demonstrated in a Phase I trial with CT-guided injection of ONXY-015 (23) led to the search for more practical ways to deliver the virus into pancreatic tumors. EUS was used because it has the potential to simultaneously image the pancreatic bed and perform multiple injections into pancreatic neoplasms in real time. After this study was finished, Chang et al. (27) reported that a single injection of allogeneic mixed lymphocyte culture into pancreatic cancers under EUS guidance was well tolerated. Our study is the first to show the feasibility and tolerability of repeated administration of a replication-selective virus by EUS directly into carcinomas of the pancreas, with up to 80 injections in some patients. This may be a model for the delivery of other potential anticancer treatments.

The ONXY-015 virus itself was well tolerated, as was its combination with gemcitabine. This is consistent with a favorable safety profile of ONXY-015 alone and/or in combination with cytotoxic chemotherapy after intratumoral, intra-arterial, and i.v. administration in over 200 patients to date (22). Nevertheless, there were several significant complications from the injection procedure, which is not surprising in the development of a new technique for cancer treatment. Of these, infection and perforation were the most serious. Infection with bowel flora is always a possibility when the integrity of the bowel wall is violated, even with a small 22-gauge needle, the standard needle for aspiration to diagnose pancreatic neoplasms. No additional cases of bacteremia were seen after the injection protocol was modified to minimize disruption of the bowel wall and patients were given prophylactic oral antibiotics. The two perforations seen were serious, although manageable, complications. Wiersema et al. (26) reported a single duodenal perforation in a diagnostic study of EUS with a similar number of sessions. Both perforations were in the duodenal bulb and were almost certainly caused by the rigid tip of the echoendoscope. The tears were located on the antimesenteric side of the duodenum, which may have been because of the presence of angulation and narrowing of the lumen by the adjacent tumor in the head of the pancreas. The stomach, by comparison, is capacious, and most pancreatic tumors are amenable to injection by this approach. The protocol was changed to mandate transgastric injections only, without the occurrence of any further complications.

Antitumoral activity of ONXY-015 plus gemcitabine was demonstrated, although no responses to single-agent ONXY-015 were noted after four weekly sessions of injections. Response determination is complicated by the difficulty in measuring primary pancreatic tumors radiologically. Some treatment studies have abandoned measurement of the primary lesion, opting instead to use more easily visualized hepatic metastases as indicator lesions. This approach, however, ignores the many patients who only have locally advanced disease. Furthermore, primary pancreatic carcinomas have a great deal of nonmalignant fibrous tissue, so the neoplasm may be only a minority of the volume of the mass. Direct injection may also potentially cause edema in the pancreatic lesion from either the injection or virus-related inflammation, obscuring possible antitumor response. Therefore, tumor response may not represent the extent of the antitumor results of treatment. The 67% 6-month survival rate and the number of patients with stable disease are greater than expected with this population and may be a better gauge of the activity of this combination in locally advanced pancreatic cancer. This patient population, however, was a highly selected group based on the ability to undergo weekly EUS, and no efficacy conclusions can be drawn from the survival data.

What additional studies should be undertaken with ONXY-015 and other replication-selective adenoviruses in pancreatic cancer? Encouraging results have been reported recently after intratumoral injection of squamous cell cancers of the head and neck, both with (25) and without (28) concomitant chemotherapy. A logical next step is the use of higher doses of ONXY-015, because few virus-related toxicities were noted. Newer, more concentrated, virus formulations now available would make this possible. Measures of viral replication and cytopathic effect should be vigorously examined. New EUS-guided core biopsy needles may obtain significantly more tissue for study than the scanty material provided by older aspiration needles used in this study. Other possibilities include lengthening the dosing interval and including a maintenance schedule. Alternative means of delivery such as i.v. and intra-arterial routes also could be used (29). To truly examine efficacy, a randomized trial examining time to progression or survival would have to be performed.

Viral replication detection is extremely difficult in this patient population. In patients with recurrent head and neck cancers, for example, in which tumor biopsies are easily obtained after viral treatment, intratumoral ONXY-015 replication was documented by in situ hybridization in approximately 70% of patients on days 5–8 after initiation of five daily injections. Viral genome analysis by Q-PCR on peripheral blood samples (obtained on days 5–8) gave similar results. Interestingly, although Q-PCR results on blood samples were indicative of viral replication in approximately two-thirds of patients with colorectal carcinoma metastatic to the liver, tumor fine-needle aspiration results after treatment were both necrotic and negative for viral replication. Therefore, tumor fine-needle aspiration appears to be a relatively insensitive method for detection of ONXY-015 replication (30). The fact that the Q-PCR testing of blood samples for ONXY-015 on this trial were negative might reflect several possibilities. First, viral replication may have occurred either not at all or at levels below the level of detection (10,000 genomes/ml) in these tumors. Second, because the liver is a very effective “sink” for the clearance of adenovirus from the bloodstream (t½ = 12 min), it is possible that low-level viral shedding from a tumor into the portal venous system would not be detected in the peripheral blood. Perhaps the optimal method for answering this question for any viral agent would be to perform neoadjuvant injections into pancreatic tumors.
before surgical resection. Neutralizing antibody development has been uniformly demonstrated in studies of ONYX-015. To date, no correlation has been demonstrated between neutralizing antibody levels and efficacy, replication, or toxicity after intratumoral injections (22). Viral replication and antitumoral activity may be relatively "shielded" from antibodies in this location, whereas intravascular virus will almost certainly be neutralized by circulating antibodies (31).

To summarize, this Phase I/II study used a previously untested delivery system for anticancer agents and demonstrated that direct injection of ONYX-015 adenovirus into pancreatic tumors under EUS guidance is both practical and tolerable by the transgastric route. Alterations in the injection methodology, as noted above, may significantly reduce endoscopic complications. Further testing of this approach to the delivery of novel biological agents, including replication-selective viruses, is warranted.

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References


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