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A PHASE II TRIAL OF SURGERY WITH PERIOPERATIVE INGN 201 (Ad5CMV-p53) GENE THERAPY FOLLOWED BY CHEMORADIOTHERAPY FOR ADVANCED, RESECTABLE SQUAMOUS CELL CARCINOMA OF THE ORAL CAVITY, OROPHARYNX, HYPOPHARYNX AND LARYNX: REPORT OF THE SOUTHWEST ONCOLOGY GROUP

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Abstract

Objective—To assess the feasibility of treating high risk, selected Stage III and IV squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, and larynx with perioperative INGN 201 gene therapy along with surgery and chemoradiation in a multi-institutional setting.

Design—Prospective, single-arm, phase II multi-institutional trial within the Southwest Oncology Group.

Patients and Methods—From March 1, 2003 to July 1, 2006, a total of 13 patients were registered to the study with subjects enrolled from a total of 3 participating institutions. Entry criteria were: newly diagnosed, previously untreated squamous cell carcinoma of the oral cavity, oropharynx, larynx and hypopharynx; selected stage III or IV disease without distant metastases; surgically resectable disease. Patients underwent surgery, perioperative INGN 201 gene therapy and postoperative chemoradiotherapy.

Results—All 13 patients received surgery and perioperative INGN 201 injections to the primary tumor bed and to ipsilateral neck. In addition, three patients received injections into the contralateral neck. Three patients did not receive any chemoradiation. One patient had a Grade 2 fistula of the oral cavity. Among the 10 evaluable patients, two have experienced Grade 4 adverse events, one due to hypokalemia, hyponatremia, vomiting, leukopenia and neutropenia and one due to SGOT increase and SGPT increase. Seven other patients have experienced Grade 3 adverse

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events. The estimate of one-year progression-free survival is 90% (95% confidence interval: 56% – 100%).

Conclusions—Intraoperative INGN 201 gene therapy with postoperative chemoradiotherapy is technically feasible, but not logistically possibly when carried out in a multi-institutional setting. Disease control appears to be reasonable; however, no definitive conclusion can be made with this small sample size.

Introduction

In advanced squamous cell carcinoma of the head and neck (SCCHN), the five-year survival rate is less than 40%[1]. Current treatment options (surgery, radiotherapy and/or chemotherapy) are toxic as well as functionally and cosmetically debilitating. The most common cause of death in patients with advanced SCCHN is local regional recurrence[1]. After surgery removes gross disease, failure occurs due to microscopic cancer cells left in the margins of resection. Adjuvant radiotherapy and/or chemotherapy have been added to treatment regimens to kill these microscopic cancer cells. However, a 25 – 40% recurrence rate exists[1]. Since patients with advanced SCCHN have a high rate of local-regional recurrence and low survival rate with the existing treatment modalities, novel biological therapies, such as gene therapy, have to be developed and tested. Gene therapy may provide an alternative mechanism for controlling the microscopic residual disease with limited or no added toxicity.

The p53 gene is a tumor suppressor gene that is mutated in 45% of human cancers[2]. In SCCHN, genetic alterations, such as p53 mutations, have been identified in histologically normal margins and have been correlated with a higher recurrence rate[3]. Overexpression of p53 in head and neck cancer cells has demonstrated tumor growth suppression using in-vitro and in-vivo models, using both mutated and non-mutated *p53* human SCCHN cell lines[4]. After adenovirus-p53 (INGN 201) was injected into surgically resected tumor beds of mice, tumor control and survival rates were improved[4]. The mechanism of growth suppression was found to primarily be apoptosis. Additional mechanisms of actions for INGN 201 have been evoked, including Fas-mediated apoptosis and anti-angiogenesis effects.

The feasibility and efficacy of INGN 201 IW therapy as an adjuvant to surgical resection was demonstrated in a mouse model that simulates residual microscopic disease after gross tumor resection of squamous cell cancer[4]. In the single-center phase I trial, a cohort of 15 patients with recurrent/refractory (failed multi-modalities of therapy) cancer who were eligible for palliative surgical resection were enrolled[5]. This perioperative approach was found to be safe and well tolerated with no significant added wound complications.

In the proposed trial, we transduce *p53* in tumor - surgical microenvironment to induce apoptosis in order to improve local control. The safety and antitumor activity demonstrated in Phase I and II trials have led us to develop this perioperative Phase II trial. The feasibility and tolerability of perioperative injections into the tumor bed after surgical salvage resection of recurrent SCCHN was assessed in the multi-center Phase II setting in patients with advanced SCCHN. In addition, time to recurrence was estimated in this patient cohort.

Methods

Registration/subject population/eligibility

All patients were evaluated by Head and Neck Surgery, Radiation Oncology, Medical Oncology, and Dentistry. Eligibility criteria included the following: newly diagnosed, previously untreated squamous cell carcinoma of the oral cavity, oropharynx, larynx and

hypopharynx; selected stage III or IV disease with nodal metastasis and without distant metastases; surgically resectable disease; and a Southwest Oncology Group performance status of 0 or 1. Patients were tested negative for HIV1/2, HBV and HCV. Patients had adequate laboratory parameters, were informed of the investigational nature of this study, and gave written informed consent. The study was approved by local institutional review boards and institutional biosafety committee (IBC) and conducted under the auspices of the Southwest Oncology Group.

Surgery

Surgery is to be performed within 28 days following registration. The goal of surgery was to remove all gross tumor with margin negative resection at the time of surgery. Therefore, intraoperative frozen sections should not have invasive tumor present. All patients (with N1, N2 or N3 disease) underwent a neck dissection ipsilateral to the neck mass. The decision whether a selective, comprehensive or radical neck dissection was made by the surgeon. Surgical quality review was performed for this study.

Dose/Route/Duration of INGN 201

The injections are a relatively simple technique for which surgeons can be easily trained. A training course for participating surgeons was completed. Perioperative INGN 201 injections were performed in the intraoperative and postoperative period. One dose of INGN 201 was considered to be 2.5×10^{12} viral particles (VP) in total volume of 10 ml. Two doses of INGN 201 were administered intraoperatively. One dose of INGN 201 was injected into the surgical resection bed (mucosal and deep margin). Forty percent of the volume (4 ml) was injected into the mucosal margin within one cm around the edge of resection. Sixty percent (6 ml) was injected into the deep bed that consists of muscle and fascia. No direct injections were applied to vessels or nerves. One dose of INGN 201 was injected into the neck dissection defect. After the neck dissection is completed, half of a dose (5 ml) of INGN 201 was injected into the deep soft tissue bed of the cervical level that has clinically evident nodal metastasis. No direct injections were applied to vessels or nerves. The other half (5 ml) was placed in the neck dissection bed and allowed to sit in place for 10 minutes. If patients require bilateral neck dissections, one dose was given to each side as described above.

One dose of INGN 201 was administered postoperatively. Half dose of INGN 201 (5 ml) was given 48–72 hours postoperatively through retrograde injection into each of two drainage catheters next to the mucosal suture line and neck dissection bed. The INGN 201 was allowed to sit in place for 2 hours. If drainage catheters are inadvertently removed before 48 hours, no dose of INGN 201 was given postoperatively. If there is only 1 catheter in place, the full dose was given in one drain catheter.

Concomitant chemoradiotherapy

The chemoradiotherapy regimen was started within 56 days from surgery. Cisplatin 100 mg/m^2 was infused over 90 minutes every 21 days for three cycles, concurrent with the radiation therapy. The initial field was the total volume, which included the primary tumor, any enlarged lymph nodes, and all areas at risk for microscopic disease. Patients received radiotherapy (60 Gy in 30 fractions over a six-week period, with or without a boost of 6 Gy in 3 fractions over a period of three days to high-risk sites). During treatment patients were examined at least weekly.

Post-treatment surveillance

Once treatment ended, an evaluation was required at nine weeks, then every three months for the first year, twice annually in years 2 and 3, and annually thereafter. The patient's status, the tumor status, and adverse effects were recorded.

Statistical Methods

This study assessed the feasibility of treating patients with Stage III and IV squamous cell carcinoma of the oral cavity or oropharynx with perioperative INGN 201 gene transfer with surgery followed by chemoradiation. Primarily feasibility was assessed both by accrual rate and percentage of patients successfully receiving the required doses of INGN 201 therapy to the primary site. Sixty eligible patients were anticipated to be accrued. Success in delivering INGN 201 therapy to the primary site was defined as receiving 80% or the planned dose to the mucosal margin, deep bed and retrograde injections. The study would be considered feasible with respect to delivery if 85% of planned patients were defined as success with respect to delivery INGN 201, and would not be considered feasible if the true rate was 70% or less. With 60 patients, the power of a one sided 0.05 level test of 0.70 versus 0.85 is 0.88. If the observed success rate is greater than 0.80 the regimen was considered feasible with respect to delivery of INGN 201. Sixty patients were sufficient to estimate the 2 year progression-free survival to ± 13% (95% confidence interval) given complete follow-up. It is also sufficient local control rate to within ± 13% given complete follow-up. The local control rate for this protocol is defined as the probability of not having disease progression at the primary site given the subject is still alive. Sixty patients were sufficient to estimate any given toxicity to within ± 13%. Any adverse event occurring with at least 5% probability is likely to be seen once (95% chance).

Results

Patients/Study accrual/eligibility

From March 1, 2003 to July 1, 2006, a total of 13 patients were registered to the study with subjects enrolled from a total of 3 participating institutions. The study was closed July 1, 2006 due to diminished ongoing accrual. The anticipated total accrual of 60 patients at a rate of 30 per year was not met. As a result, perioperative gene therapy is not logistically feasible. Out of the 12 institutions initially demonstrated interested and listed on the protocol, only 5 institutions were able to open the protocol with IRB and IBC approval. In one of the seven institutions who did not open the protocol, the IBC took 5 years to review the protocol and in the end never approved the protocol. This protocol was open at 5 sites; however, only 3 institutions accrued patients. Many regulatory hurdles occurred. One institution opened the trial 36 months after submitting the application.

Completion of study interventions

All 13 patients (100%) received surgery and perioperative INGN 201 injections to the primary tumor bed and to ipsilateral neck. In addition, three patients received injections into the contralateral neck. Therefore, INGN 201 gene therapy is technically feasible to administer in the perioperative setting. Two of 13 patients had more than four milliliters injected into the mucosal margins and less than six milliliters injected into the deep surgical resection bed. Three patients did not receive any chemoradiation. Two patients decided to receive RT treatment closer to home and dropped out of the protocol. One patient was found to be pathologically node negative after surgery and institution determined that chemoradiation was not indicated.

Adverse events and protocol violations

All 13 patients have been assessed for wound-healing complications prior to chemoradiotherapy. One patient had a Grade 2 fistula of the oral cavity (Table 2, coded as "GI-other"). Three patients had major protocol violations because they did not receive any chemoradiation and were not evaluable for adverse events related to chemoradiation. Among the 10 evaluable patients, two have experienced Grade 4 adverse events, one due to hypokalemia, hyponatremia, vomiting, leukopenia and neutropenia and one due to SGOT increase and SGPT increase. Seven other patients have experienced Grade 3 adverse events. Adverse events related to study treatment are summarized in Table 3.

Disease Progression and Survival

The estimate of one-year progression-free survival (Fig 1) is 90% (95% confidence interval: 56% – 100%). This estimate should be interpreted with caution due to the small sample size. One patient has died and three progressions have been reported. The median survival among the 12 patients still alive is 21 months. The survival rate cannot be estimated because of the small sample size.

Discussion

Injection of Ad-p53 into the surgical tumor microenvironment immediately following surgical extirpation of tumor represent a novel and possible effective strategy to reduce local recurrence by targeting 33 – 45% of SCCHN[6] tumor cells that have an altered p53 gene. Genetic alterations, such as p53 mutations, in SCCHN have been identified in histologically normal margins and have been correlated with a higher recurrence rate[3]. After adenovirus-p53 (INGN 201) was injected into surgically resected tumor beds of mice, tumor control and survival rates were improved[4].

In a single-center Phase I trial, a cohort of 15 patients with recurrent/refractory cancer (failed multi-modalities of therapy) who were eligible for palliative surgical resection were enrolled[7]. These patients were resectable, but thought to be incurable. Preoperatively, a patient's tumor was injected six times in a two week period. Patients underwent a surgical resection and were given an intraoperative injection of INGN 201 in the resected tumor bed and in the neck dissection site. Three days later, their drainage catheters were injected (retrograde) with INGN 201. All patients had extensive surgery and required flaps for closure. The surgical complications (one vascular anastigmatic anastomotic thrombosis and one delayed wound healing) were expected and unlikely caused by INGN 201 therapy. Fever (6), injection site pain (5) and flu-like symptoms (4) were the only complications observed in these patients. Otherwise, it was felt to be safe and well tolerated. After long term follow-up, four (27%) were still alive and free of disease after 18 months[5].

A phase II INGN 201 intratumoral injection multicenter trial enrolled heavily pretreated, recurrent and unresectable patients with HNSCC[8]. A 22% (25/112) anti tumor activity (PR +CR+SD) with median survival of 10.2 months was observed [CR=complete response, PR=partial response & SD=stable disease]. A 6% (7 out of 112) response rate (PR+CR) with median survival of 40.8 months was detected. Two Phase II monotherapy studies using two dosing schedules [Days 1, 2, 3 q 4 weeks or Days 1, 3, 5, 8, 10, 12 q 4 weeks] enrolled patients with recurrent SCCHN heavily pretreated, unresectable resistant to all therapies[9, 10]. The related adverse events were fever/chills (74%), injection site pain (45%), asthenia (13%), nausea (1%) and injection site bleeding (10%). Twelve related severe adverse effects were reported (fever [4], tumor hemorrhage [3], chills [1], injection site pain [1], dehydration [1], Guillain-Barre syndrome [1] & infection [1]). No treatment related deaths were reported.

This trial was closed on July 1, 2006 due to poor accrual. Routine emails and phone calls were made to the investigators to improve accrual. Furthermore, accrual was discussed and encouraged at the SWOG-Head and Neck Surgical Subcommittee semiannual meetings. In the United States only 2–4% of patients are enrolled onto clinical trials[11]. Physicians have been shown not to consider patients for a clinical trial because of an unavailable protocol and poor performance status[11]. Patients declined to participate because of a desire for other treatment, distance from the cancer center, and insurance denial[11]. This trial has the complexity of gene therapy-related regulatory hurdles, which includes Institutional Biosafety Committee (IBC) approval. One institution took 3 years to obtain IBC approval. Furthermore, the initial excitement with gene therapy was lessened with low tumor response rates[12]. In this limited institutional trial, only 3 out of the initial 12 institutions accrued patients into this protocol. Five institutions had the protocol approved. There was also perceived unrelated gene therapy toxicity with the death of a subject treated with gene therapy[12] and development of leukemia[13].

Conclusions

Intraoperative INGN 201 Gene Therapy with postoperative chemoradiotherapy is technically feasible, but not logistically possibly when carried out in a multi-institutional setting. Disease control appears to be reasonable; however, no definitive conclusion can be made with these small numbers.

Acknowledgments

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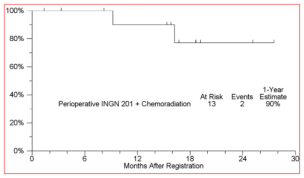

Figure 1.
Progression-free Survival

Table 1

Patient Characteristics

	Periop INGN 201 + chemo RT (n=13)	
AGE		
Median	52.2	
Minimum	40.1	
Maximum	71.0	
SEX		
Males	8	62%
Females	5	38%
HISPANIC		
Yes	1	8%
No	6	46%
Unknown	6	46%
RACE		
White	12	92%
Native American	1	8%
N STAGE		
0	0	0%
1	6	46%
2a	1	8%
2b	4	31%
2c	2	15%
PERFORMANCE STATUS		
0	9	69%
1	4	31%
PRIMARY SITE		
Oral cavity	11	85%
Oropharynx	2	15%
Hypopharynx	0	0%
Larynx	0	0%
T STAGE		
1	3	23%
2	1	8%
3	3	23%
4a	6	46%

Table 2

Wound Healing Complications Adverse Events Unlikely or Not Related to Treatment Excluded Data as of May 18, 2007

	Perioperative INGN 201 (n=13)					
	Grade					
ADVERSE EVENT	**0**	**1**	**2**	**3**	**4**	**5**
GI-other	12	0	1	0	0	0
Surgery-wound healing	12	1	0	0	0	0
Surgery-wound infection	12	0	1	0	0	0
MAXIMUM GRADE ANY ADVERSE EVENT						
Number	11	0	2	0	0	0

Arch Otolaryngol Head Neck Surg. Author manuscript; available in PMC 2011 May 19.

Table 3

Number of Patients with a Given Type and Grade of Adverse Event

	Perioperative INGN 201 + Chemoradiation (n=13)					
	Grade					
ADVERSE EVENT	**0**	**1**	**2**	**3**	**4**	**5**
Abdominal pain/cramping	12	1	0	0	0	0
Alopecia	12	1	0	0	0	0
Anemia	6	4	2	1	0	0
Anorexia	9	2	1	1	0	0
Constipation/bowel obstruction	8	5	0	0	0	0
Cough	12	0	0	1	0	0
Creatinine increase	10	0	3	0	0	0
Dehydration	10	0	2	1	0	0
Diarrhea without colostomy	11	1	1	0	0	0
Dizziness/light headedness	11	1	1	0	0	0
Dyspepsia/heartburn	11	2	0	0	0	0
Dyspnea	12	0	0	1	0	0
Ear-other	12	1	0	0	0	0
Edema	12	1	0	0	0	0
Fatigue/malaise/lethargy	5	1	6	1	0	0
Febrile neutropenia	12	0	0	1	0	0
Fever without neutropenia	12	1	0	0	0	0
GI-other	12	0	1	0	0	0
GU-other	12	0	1	0	0	0
Hypocalcemia	12	1	0	0	0	0
Hypokalemia	12	0	0	0	1	0
Hyponatremia	12	0	0	0	1	0
Infection w/o 3–4 neutropenia	12	0	1	0	0	0
Inner ear-hearing loss	12	0	1	0	0	0
Insomnia	11	2	0	0	0	0
Leukopenia	5	2	3	2	1	0

Arch Otolaryngol Head Neck Surg. Author manuscript; available in PMC 2011 May 19.

	Perioperative INGN 201 + Chemoradiation (n=13)					
	Grade					
ADVERSE EVENT	**0**	**1**	**2**	**3**	**4**	**5**
Lymphopenia	9	0	1	3	0	0
Nausea	6	3	2	2	0	0
Neutropenia/granulocytopenia	7	2	2	1	1	0
Osteonecrosis	12	0	0	1	0	0
Pain-other	12	0	1	0	0	0
RT-GI mucositis, NOS	7	2	1	3	0	0
RT-focal dermatitis, not recall	7	3	2	1	0	0
RT-pain	9	1	3	0	0	0
RT-pharyngeal dysphagia	8	0	3	2	0	0
Rash/desquamation	12	1	0	0	0	0
Renal failure	12	0	0	1	0	0
SGOT (AST) increase	12	0	0	0	1	0
SGPT (ALT) increase	12	0	0	0	1	0
Salivary gland changes	12	0	1	0	0	0
Stomatitis/pharyngitis	7	1	4	1	0	0
Surgery-wound healing	12	1	0	0	0	0
Surgery-wound infection	12	0	1	0	0	0
Thrombocytopenia	12	1	0	0	0	0
Vomiting	7	2	1	2	1	0
Weight loss	11	0	1	1	0	0
MAXIMUM GRADE ANY ADVERSE EVEN						
Number	1	1	2	7	2	0