An In Vivo Evaluation of Docetaxel Delivered Intratumorally in Head and Neck Squamous Cell Carcinoma

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Objective: To identify activity and biological mechanisms of intratumoral (IT) docetaxel on head and neck squamous cell carcinoma (HNSCC).

Methods: Docetaxel IT therapy was tested in xenograft models of 2 HNSCC lines, HN30 and HN12. The overall and disease-free survival rates, tumor growth, and toxic effects were measured. The pharmacokinetic profiles of docetaxel in plasma and tumor were compared after IT and intravenous (IV) administration. Comparisons between common and supradoses of docetaxel with regard to expression of regulators in the cell cycle, apoptosis, and signal transduction pathways were determined using Western blot analysis.

Results: In the HN30 and HN12 xenograft models, IT docetaxel improved overall as well as disease-free survival and reversed tumor growth. The only toxic effects noted were local (alopecia and skin breakdown). Skin breakdown resolved in all cases. At equivalent dosing levels, IT docetaxel achieved a 26-fold higher peak tumor concentration and 24-fold longer tumor exposure than IV treatment. Furthermore, limited plasma exposure was noted with IT docetaxel. Supradoses of docetaxel produced distinct protein expression patterns for regulators of the cell cycle (cyclins A and B, p21, and p27), apoptosis (cleaved caspase-3 and cleaved PARP), and signal transduction (EGFR, pEGFR, pc-Jun, and pERK) in HNSCC, which supports a distinctive mechanism of action for supradora docetaxel levels. Since levels of cleaved caspase-3 and PARP, markers of apoptosis, were only elevated with lower doses, the observed cell death at supradora levels was probably due to necrosis.

Conclusions: Injections of IT docetaxel at usual and supradoses are associated with a pharmacokinetic profile and biological mechanism distinct from those observed with usual IV doses. It is calculated that IT therapy in men will increase peak concentrations of docetaxel in tumors by 1000-fold over the conventional IV dose used clinically. These preclinical results support further testing of IT docetaxel in HNSCC.


The treatment for advanced head and neck cancer includes surgery, radiotherapy, chemotherapy, or more commonly a combined treatment regimen. Platinum-based chemoradiotherapy compared with radiotherapy alone has been shown to improve survival for nasopharyngeal carcinoma and unresectable squamous cell carcinoma (SCC). When cisplatin was used together with fluorouracil, organ preservation of the larynx and hypopharynx was achieved. Chemoradiation using cisplatin after surgical resection has demonstrated a benefit in 5-year overall survival for advanced head and neck cancer (33% vs 40%, P<.02). Although systemic cisplatin chemotherapy has significantly reduced the incidence of distant metastasis, local-regional failure frequently occurs. This has led to the testing of newer chemotherapy agents. Docetaxel is a taxane that has significant antitumor effects and is currently being tested in patients with head and neck SCC (HNSCC). Taxanes promote tubulin assembly in microtubules, inhibit depolymerization, act as a mitotic spindle poison, and induce a mitotic block in proliferating cells. Furthermore, taxanes induce G2M arrest and p53-independent apoptosis. Taxanes have been shown to improve survival in breast, lung, and ovarian cancers. Although intravenous (IV) taxanes (paclitaxel and docetaxel) in combination with 1 or more agents (cisplatin, fluorouracil, and/or leucovorin) have demonstrated high clinical response rates in newly diagnosed HNSCC (75%-100%), in recurrent disease the response rates are not as high (13%-77%); most range between 20% and 40%. In recurrent and pretreated...
HNSCC, the response rates with docetaxel in combination with other agents are only 16% to 24%.\(^1\)\(^,\)\(^2\)\(^,\)\(^2\)\(^2\) Phase 3 trials are ongoing to determine the added survival benefit of taxanes. A phase 3 trial in metastatic and recurrent HNSCC comparing (1) fluorouracil plus cisplatin vs (2) taxol plus cisplatin did not demonstrate an improvement in survival with taxol plus cisplatin.\(^\)\(^2\) Phase 3 trials using taxane combinations are ongoing in newly diagnosed HNSCC as well.

Tumor cell death induced by a chemotherapeutic agent is proportional to drug concentration and time of exposure. Higher tumor concentrations and longer exposures will have a greater cytotoxic effect. Drug resistance can develop due to low drug delivery into tumor cells using traditional IV chemotherapy.\(^2\)\(^4\) Intratumoral (IT) chemotherapy focuses high drug concentrations in solid tumors and limits total body exposure to the cytotoxic agent resulting in increased dose-related cell killing and reduced systemic toxic effects. Preclinical in vivo studies have shown antitumor activities using IT chemotherapy, as recently reviewed.\(^2\)\(^5\)\(^,\)\(^2\)\(^6\) Most IT trials have used cisplatin or ethanol, whereas IT taxanes have not been studied. We have chosen to study docetaxel instead of cisplatin because IT trials with cisplatin have not demonstrated improvement in overall survival.\(^2\)\(^7\)

**METHODS**

**DOCETAXEL, CELL LINES, AND HNSCC XENOGRAFT MODEL**

Docetaxel (Taxotere; Aventis Pharmaceuticals, Paris, France) was dissolved in ethanol at 10 mg/mL and stored at –20°C. Serial dilutions were prepared to make final desired concentrations. The final ethanol concentration was less than 0.05% by volume. The HNSCC cell lines (HN6, HN12, and HN30) were grown in Dulbecco Modified Eagle Medium with 10% fetal calf serum. The p53 gene is mutated in HN6 and HN12, while HN30 has a wild-type p53 gene.\(^2\)\(^8\) All mouse studies were conducted in compliance with and approval from Wayne State University Institutional Animal Care and Use Committee—Division of Laboratory Animal Research. Female CB17 SCID mice (severe combined immunodeficiency disorder), aged 6 to 11 weeks, were obtained from Harlan (Frederick, Md). Cells (15 \(\times\) 10\(^6\)) were washed twice in phosphate-buffered saline and suspended in 0.1 mL of phosphate-buffered saline and 0.1 mL of Matrigel (Becton Dickinson, Bedford, Mass) for a total volume of 0.2 mL per injection per site. One flank of each mouse was injected with the same cell line. Tumor nodule growth was monitored by weekly measurements.

**INTRATUMORAL THERAPY IN HNSCC XENOGRAFT SCID MODEL**

After tumor nodules formed (maximum diameter, 7-10 mm), the mice were randomized before tumor cell injection, and IT docetaxel injections were performed along with control injections (diluent alone). Two docetaxel dose groups (low-dose [LD], 7.5 mg/kg per injection; high-dose [HD], 15 mg/kg per injection) were IT injected twice a week for 6 weeks. The total weekly IT doses were within the range of commonly used IV doses in mice (20-40 mg/kg infused weekly).\(^2\)\(^9\) An IT therapy course consisted of 2 IT injections of docetaxel (1 each on days 1 and 3) every week for 2 consecutive 3-week cycles. Following an IT therapy course, mice were observed for 6 more weeks until day 85 if a complete or partial regression of tumor was observed or no change in tumor size was found. At day 95, all surviving mice were humanely killed. At any time point when no tumor was noted, IT therapy was discontinued and mice were observed until day 95. Weight, tumor size, survival, and toxic effects were monitored throughout the course of this study.

**ANTITUMOR ACTIVITY IN EXPERIMENTAL VS CONTROL GROUPS**

During and after IT therapy, tumor size and overall and disease-free survival rates were determined for experimental groups and compared with controls. Tumor nodules were weighed directly at the time mice were killed; otherwise, tumor weights were estimated using the following formula: length \(\times\) width in square centimeters \(\times\) 0.5 \(\times\) 1 g/cm\(^3\). Tumor length and width were measured by calipers. Tumor weight was not directly measured; rather, size (volume) was used to estimate tumor weight. The density of tissue was assumed to be 1 g/cm\(^3\). In human trials, tumor response criteria are often determined by using the sum of the largest bidimensional measurements, which is considered standard. Therefore, human trials (bi-dimensional) and mouse experiments (length and width) are similar in the use of 2 measurements. Given that the same laboratory assistant performed the IT docetaxel injections and measured the tumor size, the observer was not blinded to the treatment arm. Tumor growth inhibition was estimated also at the time the mice were killed by using a treatment-to-control (T/C) value (control group was injected with IT diluent): T/C value (percentage) = median tumor weight in the treatment group/median tumor weight in the control group \(\times\) 100. If the T/C value was greater than 42%, the treatment was considered not active; 10% to 42%, minimally active; and less than 10%, highly active.

**CLINICAL ASSESSMENTS FOLLOWING IT DOCETAXEL INJECTIONS**

Tumors were measured 3 times a week by calipers in experimental and control groups. Mice were evaluated for weight loss, scruffy appearance, listlessness, uncoordinated movements, inability to grasp a pencil, and walking splay footed. All dead mice underwent necropsy to evaluate spleen size, evidence of gastrointestinal toxic effects, and the condition of the liver and kidneys. Mice were killed by carbon dioxide narcosis and cervical dislocation if (1) tumors reached 1500 mg or 10% of the animal’s body weight, (2) any open lesion appeared, or (3) weight loss reached more than 20% of body weight.

**PILOT PK STUDY**

Because the pharmacokinetic (PK) profile of IT docetaxel was unknown, a pilot study was performed in the HN12 xenograft model to obtain data to plan the full-scale study. Sampling points were chosen in 2 groups of 6 mice at 6, 12, 24, 48, 96, and 192 hours after a single IT injection.

**FULL-SCALE IV AND IT PK STUDY**

The sampling points were chosen based on the PK data from a previous pilot study conducted in 2 groups of 6 mice each at 6 time points. For each treatment (IT and IV), 45 mice bearing HN12 tumors were divided at random into 15 groups of 3. One group served as a control and received no treatment. Each animal in the remaining 14 groups was injected with either IV or IT docetaxel (15 mg/kg). For both the IV and IT study, a total of 90 mice were used. The animals were anesthetized for 2 minutes in carbon dioxide starting 2 minutes prior to each sched-
uled time before injection (no treatment group), at injection, 5, 15, and 30 minutes after injection, and 1, 2, 3, 6, 10, 24, 32, 48, 72, 96, and 192 hours after injection. Blood was collected, via intracardiac puncture, in a 1-mL tuberculin syringe pre-loaded with 0.1 mL of heparin sodium, microfuged for 5 minutes at 14,000 g at room temperature, and the plasma fraction was separated, weighed, and immediately frozen. The dilution factor due to heparin was calculated from the total volume of heparinized plasma recovered. Unconscious animals were killed by cervical dislocation, and tumor was harvested in individual preweighed tubes and quick-frozen in a dry-ice bath. All the samples were stored frozen at –80°C until assay.

**DOCETAXEL ASSAY**

Docetaxel in the biological samples was quantified using the modified, reversed-phase, high-performance liquid chromatography (HPLC) method of Lee et al with paclitaxel as internal standard. A known volume of frozen heparinized plasma (weighed at the time of storage) was spiked with 10 µL of paclitaxel solution (100 µg/mL) as internal standard and thawed under 3 mL of acetonitrile mixed thoroughly by vortex. After centrifugation at 4°C and 4500 rpm for 10 minutes, the supernatant was transferred to another glass tube and evaporated at approximately 45°C under a gentle stream of nitrogen. The residue was reconstituted in 150 µL of mobile phase, and 100 µL were injected into the HPLC system. Concentrations were corrected for the dilution with 0.1 mL of heparin used as anticoagulant; any samples diluted greater than 10-fold were excluded from analysis.

For tumor analysis, specimens were dried of surface moisture, accurately weighed, and homogenized in 3 mL of acetonitrile after adding the internal standard. A Polytron PT 2100 homogenizer (Glenmills, Clifton, NJ) fitted with a Polytron PT-DA 2112/2EC probe was used. The homogenate was centrifuged, and the acetonitrile extract was removed and evaporated as with the plasma samples. The residue was redissolved in 150 µL of mobile phase and 100 µL were injected into the HPLC system.

**HPLC CONDITIONS**

A Waters Nova-Pak C18 (Waters Corp, Milford, Mass) 4-µm (3.9×300 mm) column maintained at 25°C was used in the analysis. The mobile phase was 50% acetonitrile in 0.1% phosphoric acid in water at a flow rate of 1.0 mL/min, and the analytes were detected at 200 nm. Tissue homogenates and plasma from untreated (control) animals were analyzed to prove lack of interfering peaks. Some of these as well as some drug-containing samples were spiked with standards after initial analysis to confirm the analyte peaks by coelution with standards. The retention times for docetaxel and paclitaxel were 6.9 and 7.9 minutes, respectively. Linearity was obtained in the range of 0.1 to 100 µg/mL of docetaxel (r²=0.99) with an intraday/interday coefficient of variation lower than 9%. There were no differences in standard curve peak areas of standards from neat samples vs spiked tissue homogenates from untreated animals, which indicates complete analyte recovery from the homogenates.

**PK ANALYSIS**

The measured plasma or tumor concentration–time data were subjected to PK modeling using noncompartmental methods (WinNonlin 4.0.1 software; Pharsight Corporation, Mountain View, Calif). Values for half-life, apparent volume of distribution, drug clearance, and area under the concentration-time curve (AUC) were calculated for the single doses administered by the IT and IV routes.

**WESTERN BLOT ANALYSIS**

Cells (1.2×10⁶) were plated (100 m²) in 6 mL of Dulbecco Modified Eagle Medium with 10% fetal calf serum. After 48 hours of...
incubation with the viruses, the cells were washed with phosphate-buffered saline. Total cell lysates were prepared by sonicating and incubating the cells in RIPA buffer (150mM sodium chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.4% sodium dodecyl sulfate, 20mM EDTA, and 50mM Tris, pH 7.4) for 1 hour at 4°C. Equal amounts of protein from each sample were subjected to 7% to 14% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (BioRad, Hercules, Calif). The membrane was blocked with Blotto-Tween (5% nonfat milk, 0.05% Tween 20, 0.9% sodium chloride, and 50mM Tris, pH 7.5) and incubated with the primary antibodies. Primary antibodies were obtained from NeoMarkers, Fremont, Calif (p53 and p27 [Kip1]); Santa Cruz Biotechnology Inc, Santa Cruz, Calif (p21, cyclin A, Skp 2 [p45], and pS-Ser-c-Jun); BD PharMingen, San Diego, Calif (cyclin B1); Cell Signaling Technology Inc, Beverly, Mass (Bax, caspase-3, and cleaved caspase-3); and Zymed, South San Francisco, Calif (PARP and cleaved PARP). A secondary antibody, horseradish peroxidase–conjugated IgG was incubated with membranes and developed according to enhanced chemiluminescence protocol using SuperSignal West Pico (Pierce Biotechnology, Rockford, Ill).

**RESULTS**

**SURVIVAL AND TUMOR RESPONSE USING IT DOCETAXEL IN HN30 AND HN12 HNSCC XENOGR AFT MODELS**

In the HN30 xenograft model, the overall survival at 95 days in the HD (15 mg/kg per injection), LD (7.5 mg/kg per injection), and control (diluent only) groups were 100%, 100%, and 0%, respectively (**Table 1**). The disease-free survival at 95 days for the HD, LD, and control groups were 100%, 83%, and 0%, respectively. The growth of HN30 xenografts was decreased between HD vs control and LD vs control (**Figure 1A**). The T/C ratios were 0% for both the HD and LD groups; therefore, IT therapy at HD and LD demonstrated highly active T/C values (<10%). Since HD therapy was associated with an improved disease-free survival and was well tolerated in the
HN30 xenograft model, the HD was thought to be the recommended IT dose in the HN12 model and for pharmacokinetic studies. In the HN12 HNSCC xenograft model, the overall survival at 95 days for HD and control groups were 100% and 0%, respectively (Table 1). The disease-free survival at 95 days for treated and control groups were 100% and 0%, respectively. The growth of HN12 xenografts was significantly decreased in HD vs control groups (Figure 1B). Intratumoral therapy demonstrated a highly active T/C value (H1102110%).

TOXIC EFFECTS OF DOCETAXEL
BY IT INJECTION IN SCID AND SUBCUTANEOUS INJECTION IN BALB/c MICE

Throughout the study, no SCID mice displayed a scruffy appearance, listlessness, uncoordinated movements, inability to grasp a pencil, or splay-footed walking. Weight loss, used as a broad measure of systemic toxic effects, was not significantly different between experimental and control groups (Figure 2). A noticeable difference was found in local toxic effects (skin breakdown and alopecia) between LD and HD groups compared with control groups (Table 2). Since alopecia and skin erosions can be caused by tumor progression or necrosis, subcutaneous injections were performed in BALB/c mice without tumors on the same schedule and with the same dose groups as IT injections. No significant weight loss (Figure 3A) was observed in the groups of mice injected with 7.5 or 15 mg/kg per injection (days 1 and 3 every week for 2 consecutive 3-week cycles). Although both doses caused alopecia, the alopecia resolved in more mice in the LD group than in the HD group (Figure 3B). At both doses, skin erosion occurred in 100% of mice; however, these erosions resolved more slowly in the HD group than in the LD group (Figure 3C). Therefore, the local toxic effects observed with IT and subcutaneous injections of docetaxel appear to be related to agent and not to tumor progression or necrosis.

PK PROFILE OF IT AND IV DOCETAXEL

Although plasma PKs of IV docetaxel in mice have been described,13 tumor PKs after IT injections have not. Therefore, a pilot study was performed to define appropriate PK sampling time points for a definitive PK study. In the pilot study, the PKs of docetaxel after IT delivery were compared with those after IV delivery using a single injection of docetaxel (15 mg/kg) followed by sampling at 6, 12, 24, 48, 96, and 192 hours (1 mouse per time point) in the HN12 HNSCC xenograft mouse model. The time course of plasma and tumor concentrations are shown in Figure 4A. Plasma docetaxel levels were undetectable for both IT and IV delivery even at the initial 6-hour evaluation. Since the tumor docetaxel levels were significantly higher in IT than in IV samples at early time points, a definitive PK study was designed to compare tumor docetaxel exposure following IV and IT therapy and quantify the gain in tumor drug exposure due to IT administration.

The average tumor weights for IV (257 mg; range, 87-503 mg) and IT mice (228 mg; range, 85-318 mg) were similar. Tumor and plasma were sampled at 5, 15, 30,
and 60 minutes and 2, 3, 6, 12, 24, 36, 48, 72, 96, and 120 hours. The time course of mean plasma concentrations of docetaxel and PK parameters in tumor-bearing mice following single IV and IT administration (15 mg/kg) are shown in Figure 4C. The plasma concentrations of the drug declined rapidly in a biexponential manner following IV administration, and the levels were below the limit of quantification after 2 hours (Figure 4C). In contrast, plasma levels of the drug increased with time up to 15 minutes after IT injection and declined relatively slowly, showing a half-life of 1.6 hours (vs 0.6 hours for IV injection). The level of docetaxel was quantifiable in plasma up to 3 hours after IT injection. Although IV injection resulted in very high plasma levels initially, beginning at 30 minutes after drug administration, plasma levels of docetaxel following IT injection were sustained at slightly higher concentrations, most likely due to the reservoir effect of tumor tissue. Although the overall systemic exposure (plasma AUC) and clearance for docetaxel after IT injection were equivalent to those of IV injection, the peak plasma concentration for IT delivery was just 20% of the maximum concentration of the IV injection.

The mean tumor concentrations of docetaxel and the tumor PK parameters are shown in Figure 4B and detailed in Table 3. After IV injection, docetaxel was rapidly taken up by tumor, and the peak levels were attained at 30 minutes. Tumor levels of the drug declined rapidly in a biexponential fashion following IT injection. The tumor levels of docetaxel always remained higher in the IT samples than in the IV samples, especially within the first 24 hours, in some cases 20 times higher (Table 4). Although the terminal elimination half-lives of docetaxel in tumor following IV and IT routes of administration were comparable, higher tumor exposure of docetaxel was achieved through IT injection. Ratios (IT-IV) of AUC and concentration of docetaxel in tumor following IV and IT injections as a function of time are listed in Table 4. Although the overall gain in tumor exposure to the drug following a single IT dose vs a single IV dose was 2.7-fold, the tumor exposure remained greater than 10-fold for up to 10 hours (Table 4). Thus, IT therapy with docetaxel results in supraexposure to the drug locally in the tumor. The time that mean tumor docetaxel levels remained greater than 0.5 or 1.0 µg/g was estimated to be substantially longer after IT than IV dosing (Table 5). These local pharmacologic goals were achieved under conditions that resulted in equivalent systemic exposure to docetaxel (Table 3).

**ACTIVITY AND MODULATION OF BIOMARKERS IN HNSCC CELLS BY DOCETAXEL SUPRADOSE LEVELS**

Since the in vivo IT docetaxel delivery data demonstrated antitumor activity and substantially higher levels, we tested the effects of supradoses of docetaxel on HNSCC cells in vitro. We performed preliminary experiments in which HNSCC cell cultures were evaluated for cell structure and adhesion at 6, 12, 24, and 48 hours after exposure to 25, 100, 500, and 1000 ng/mL of docetaxel. At 24 hours of 1000 ng/mL exposure, fewer than 10% of HN12 and HN30 cells adhered to culture plates, and 100% of cells were swollen and flat (Figure 5). At docetaxel levels of 100 ng/mL, most cells were adherent, and fewer than 50% of cells were swollen and flat. Head and neck SCC cells were healthy and 95% confluent at 0 ng/mL.

To test if different biological mechanisms mediate the effects of supradoses of docetaxel in HNSCC, we performed Western blot analysis at 6 and 24 hours using 0, 25, 100, 500, and 1000 ng/mL of docetaxel. The proteins tested were based on existing expression data.31,32 Most proteins tested did not demonstrate a differential expression pattern between typical docetaxel levels (≤100 ng/mL) and supradoses (>500 ng/mL) except for certain regulators of the cell cycle (cyclins A and B, p21, and p27) (Figure 6), apoptosis (cleaved caspase-3 and cleaved PARP) (Figure 7), and signal transduction (EGFR, pEGFR, pc-Jun, and pERK) (Figure 8).
In general, the expression pattern of cyclin A, cyclin B, p21, and p27 proteins in response to docetaxel was not consistent across the 3 HNSCC cell lines (Figure 6). In most test conditions, the expression of these proteins did not change significantly across concentrations (25-1000 ng/mL). One notable exception occurred in HN30 cells at 24 hours when the expression of cyclin A was decreased; but at 6 hours, its expression was higher, with increasing concentration in HN6 cells. For cyclin B, the expression increased with docetaxel concentration at 6 hours, especially in HN6, in a concentration-dependent manner. At 24 hours, only HN30 demonstrated altered expression of cyclin B, which peaked at 100 ng/mL. The expression of p21 decreased with increasing concentrations in HN12 (6 and 24 hours), HN30 (24 hours), and HN6 cells (24 hours; peaked at 25 ng/mL). Otherwise, p21 expression was higher with increasing concentration at 6 hours for HN6. The expression of p27 in HN12, HN30, and HN6 cells decreased between 25 and 1000 ng/mL docetaxel concentrations at 24 hours.

In the apoptosis pathway, caspase-3 and PARP expression along with their respective cleaved products demonstrated a consistent pattern of expression in all HNSCC cell lines (Figure 7) and did not change at 6 hours from 500 to 1000 ng/mL concentration of docetaxel vs the control (0 ng/mL). However, at 24 hours, the expression of cleaved caspase-3 and cleaved PARP peaked at 100 ng/mL and then subsequently decreased with increasing docetaxel concentration. Furthermore, the level of PARP and caspase-3 decreased in all HNSCC cell lines at 24 hours. Since cleaved caspase-3 and PARP are markers of apoptosis, the observed cell death was probably due to necrosis. These data suggest that a different mechanism of action for supradose levels (1000 ng/mL) is evident by expression of apoptotic regulators.

Similar to cell cycle regulators, the expression pattern of signal transduction was not consistent between HNSCC.

Table 3. Plasma and Tumor Pharmacokinetic Data for Docetaxel After a Single IT or IV Injection in the HN12 Xenograft Mouse Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV</td>
<td>IT</td>
</tr>
<tr>
<td>Maximum concentration, µg/mL</td>
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<td>1.5</td>
</tr>
<tr>
<td>Half-life, h</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>AUC0-1, µg × h/mL</td>
<td>3.2</td>
<td>2.7</td>
</tr>
<tr>
<td>AUC0-t, µg × h/mL</td>
<td>3.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Clearance, mL/h per kg</td>
<td>4333.0</td>
<td>4014.0</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the curve; IT, intratumoral injection; IV, intravenous injection.

Table 4. Single Intratumoral–Single Intravenous Docetaxel Injection Ratios for Tumor Area Under the Curve and Mean Tumor Concentration in the HN12 Xenograft Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.08</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>10</th>
<th>24</th>
<th>32</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>192</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under the curve</td>
<td>20</td>
<td>21</td>
<td>23</td>
<td>24</td>
<td>20</td>
<td>16</td>
<td>13</td>
<td>10</td>
<td>5.8</td>
<td>4.9</td>
<td>4.0</td>
<td>3.4</td>
<td>3.1</td>
<td>2.7</td>
</tr>
<tr>
<td>Mean tumor concentration</td>
<td>20</td>
<td>23</td>
<td>26</td>
<td>22</td>
<td>11</td>
<td>7.2</td>
<td>8.0</td>
<td>1.8</td>
<td>1.1</td>
<td>1.3</td>
<td>1.2</td>
<td>1.5</td>
<td>1.3</td>
<td>1.9</td>
</tr>
</tbody>
</table>
cell lines (Figure 8). Expression of EGFR decreased with docetaxel concentration and was concentration dependent at 24 hours for HN12 and HN30 cell lines. Otherwise, HN6 (6 and 24 hours), HN30 (6 hours), and HN12 (6 hours) cell lines showed no change in expression at any of the concentrations or time periods tested. The expression of pEGFR increased with increasing concentration at 6 hours for HN12. No EGFR expression was detected for HN30 (6 and 24 hours) or HN12 (24 hours). Expression of pEGFR decreased with increasing concentration at 24 hours in the HN6 cell line. The expression of pc-Jun in HN12 cells peaked at 25 ng/mL of docetaxel concentration and then decreased with increasing concentrations at 6 and 24 hours independent of the docetaxel concentration. At 6 hours, expression of pERK increased slightly with increasing docetaxel concentrations in HN12 and HN6 cells. In HN30 (6 hours) and HN6 cells (24 hours), pERK expression decreased with increasing docetaxel concentrations. For HN12 and HN30 cells, the expression of pERK peaked at 100 ng/mL of docetaxel at 24 hours and subsequently decreased at higher concentrations (500-1000 ng/mL).

### Figure 6
Protein expression patterns of cell cycle regulators in head and neck squamous cell carcinoma cells after exposure to different concentrations of docetaxel for 6 and 24 hours.

Chemotherapy-induced cytotoxic effects on tumor cells are proportional to drug concentration and time of exposure. Therefore, a higher deliverable IT concentration will have a greater cytotoxic effect. Using traditional IV chemotherapy, drug resistance can result from low drug delivery into tumor cells. Furthermore, wide distribution throughout the body results in a broad range of serious adverse effects. Intratumoral chemotherapy focuses high drug concentrations in solid tumors and limits total body exposure to the cytotoxic agent, resulting in increased dose-related killing of tumor cells at reduced, or at least equivalent, systemic toxic effects. In the present HNSCC xenograft mouse study, overall and
Disease-free survival rates were improved by IT injection of docetaxel. A favorable PK profile of IT delivery was observed when compared with IV tail vein injections (Tables 3, 4, and 5).

The total weekly IT doses used in this study (7.5 or 15 mg/kg per injection twice weekly) were chosen to match previously reported weekly IV doses in mice. The maximum concentrations of docetaxel delivered by IT administration were much greater than those from IV injection (132.0 vs 5.1 µg/mL; IT-IV ratio, 26). Earlier peak tumor levels were found to be 4 µg/mL after IV infusion of docetaxel (111 mg/m²) in mice with tumors. With IT delivery, tumor levels (1.9- to 26-fold) and tumor AUCs (2.7- to 24-fold) remained high throughout the study (<192 hours). Since the clearance of docetaxel was also slower with IT delivery than with IV (23.1 vs 61.6; ratio, 0.4), time of tumor exposure to docetaxel was greater with IT injections. The fact that the published IV dose of 111 mg/m² achieves a peak concentration of 4 µg/mL, whereas the single dose of 45 mg/m² in the present study achieved 5.1 µg/mL concentration, suggests that slow tumor penetration of docetaxel from the plasma may limit delivery.

The maximum plasma concentrations were much lower for IT than for IV delivery (1.5 vs 6.8 µg/mL), and the plasma half-life of IT injections was longer than that of IV (1.6 vs 0.6 hours); however, the AUC and clearance were equivalent (Table 3). Since clearance of docetaxel from tumor tissue is slower than from plasma, peak plasma levels from IT injections were lower than those noted with IV delivery. The lower plasma levels seen with IT injections will decrease the concentration-dependent systemic toxic effects observed with IV delivery. Since docetaxel undergoes hepatic metabolism and biliary-fecal excretion and the 2 major metabolites are inactive against tumor cells, systemic bioactivation is not required for docetaxel activity.

After IV infusion of docetaxel in mice with tumors, the peak tumor and plasma levels were 4 and 50 µg/mL, respectively, which means that peak concentration of tumor/plasma ratio is approximately 8%. Using the rec-

![Protein expression patterns of regulators of apoptosis in head and neck squamous cell carcinoma cells after exposure to different concentrations of docetaxel for 6 and 24 hours.](image-url)
ommended IV bolus of docetaxel (100 mg/m²) in humans, the peak plasma concentration was 3.2 µg/mL at 1 hour and 1 ng/mL at 60 hours (AUC=4.81 µg·hr/mL).³³ Using this 8% ratio in humans (100 mg/m²), the estimated concentration in the tumor would peak at 261 ng/mL, and at 60 hours the concentration would be only 0.08 ng/mL. Using IT chemotherapy, the entire dose of agent can be delivered into the tumor by intralesional injections, and low tumor penetration through IV routes can be overcome. Tumor levels using an IT delivery (100 mg/m²; body surface area, 1.78 m²) would be approximately 1000-fold higher than previously reported IV delivery. The PK data, tumor-plasma ratios, collected in these preclinical models will guide the initial dose and frequency of administration in future phase 1 and 2 trials.

The toxic effects observed in the present HNSCC xenograft models were local (alopecia and skin erosion), not systemic. Higher tumor levels (up to 26-fold) and lower peak plasma concentrations (20%) with IT than with IV injection support a relationship between the type of toxic effect (local vs systemic) and the delivery approach (IT vs IV). Previous reports have demonstrated no significant systematic toxic effects using intradermal injections of docetaxel (0.9% sodium chloride, polysorbate 80) in rabbits at doses 3 times those used in humans.²⁹ The systemic dose-limiting toxic effect (neutropenia) and others (nausea, mucositis, asthenia, diarrhea, peripheral neuropathy, infection, and sodium-wasting nephropathy) may be limited with an IT approach.

Most IT trials have used cisplatin or ethanol, whereas IT taxanes have not been studied (Table 6). We have chosen to study docetaxel instead of cisplatin because IT trials with single-agent cisplatin have not demonstrated improvement in overall survival.²⁷ The results of 2 double-blind, placebo-controlled, randomized studies of IT cisplatin/epinephrine/gel in patients with recurrent HNSCC were reported.³¹ The researchers randomized 179 patients in a 2:1 fashion to receive treatment with either cisplatin/epinephrine/gel (n=119) or placebo (n=60). The overall rate of response lasting 28 days or longer was 29%

<table>
<thead>
<tr>
<th>Docetaxel Concentration, ng/mL</th>
<th>0</th>
<th>25</th>
<th>100</th>
<th>500</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR</td>
<td>HN12</td>
<td>HN30</td>
<td>HN6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pEGFR</td>
<td>HN12</td>
<td>HN30</td>
<td>HN6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pc-Jun</td>
<td>HN12</td>
<td>HN30</td>
<td>HN6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pERK</td>
<td>HN12</td>
<td>HN30</td>
<td>HN6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 8. Protein expression patterns of signal transduction molecules in head and neck squamous cell carcinoma cells after exposure to different concentrations of docetaxel for 6 and 24 hours.
in the treatment arm (19% complete response, 10% partial response) and 2% in the placebo arm (P < .001). Pain during the injection was the most common adverse event: 24% of patients in the treatment arm and 17% in the placebo arm experienced pain within 15 to 20 minutes. Local toxic effects, including inflammation, bleeding, erosion, ulceration, necrosis, and eschar, occurred in 87.4% of patients in the treatment arm and 62.7% in the placebo arm. Other adverse effects included wound infection in 5.9% vs 1.7% and fistula formation in 3.3% vs 0% for patients in the treatment arm vs placebo arm, respectively. Four cases of treatment-related cerebrovascular events (3 in the treatment arm and 1 in the placebo arm) were observed. After implementation of an amendment to exclude patients with tumors that directly involved or threatened to invade the carotid artery, no further cerebrovascular events related to treatment were reported. In clinical trials using IT docetaxel, we also expect no significant systemic toxic effects (thanks to lower plasma concentrations) and no cerebrovascular events (because no epinephrine will be involved).

In the present study, docetaxel's mechanisms of action at supradose levels with respect to cell cycle regulation, apoptosis, and signal transduction were examined. In a future study, we are planning a detailed histologic and protein expression analysis using immunohistochemical analysis, since only in vitro studies were performed in this pilot study. The docetaxel-induced altered protein expression pattern of cell cycle regulators (cyclin A, cyclin B, p21, and p27) and signal transduction molecules (EGFR, pEGFR, pc-Jun, and pERK) was not consistent between HNSCC cell lines. However, the expression of cleaved caspase-3 and cleaved PARP peaked at 100 ng/mL and then subsequently decreased with increasing concentration at 24 hours in all HNSCC cell lines. This peak of apoptosis at 100 ng/mL did not correlate with extensive cell death (cellular swelling and lack of adherence) observed at 500 and 1000 ng/mL. Therefore, cellular death at supradose levels appeared to be predominantly due to necrosis and not apoptosis.

Cell death can occur through apoptosis and/or necrosis. Apoptosis and necrosis are separate entities with overlapping mechanisms and probably represent a continuum of cell death mechanisms (Table 7). Apoptosis and necrosis can occur in the same cellular population. Apoptosis cells first will shrink and the nuclei become condensed. Necrosis has distinct morphologic features (cellular swelling) that are accompanied by rapid permeabilization of the plasma membrane and lyses. Necrosis usually occurs secondary to physiologic and pathophysiologic stimuli such as infection, ischemia, and injury. The mechanism of chemotherapy has been focused on enhancing apoptosis. Optimizing necrosis (eg, through treatment with IT docetaxel) is another pathway to achieve tumor cell death.

In conclusion, an antitumor effect independent of apoptosis pathways and a distinct pharmacokinetic profile (supradosing) are attainable with IT delivery of docetaxel. The identification of altered gene expression induced by docetaxel at supradose levels demonstrates different mechanisms, possibly necrosis, in HNSCC cells. The initiation of a phase 1/2 trial using IT docetaxel in patients with HNSCC is justified by these data.

Table 6. Intratumoral Chemotherapy Trials for Solid Tumors, Excluding Trials for Skin Cancer, Immunotherapy, and Gene Therapy

<table>
<thead>
<tr>
<th>Agent, Reference</th>
<th>Tumor Type</th>
<th>No. of Patients</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin25</td>
<td>Head and neck cancer</td>
<td>82</td>
<td>Doses, 1 to 6 mg/cm² of tumor; response rate, 50%; complete response, 49%</td>
</tr>
<tr>
<td>Cisplatin27</td>
<td>Head and neck cancer</td>
<td>178</td>
<td>Response rate, 29 vs 2% (P = .001); overall survival, no significant difference</td>
</tr>
<tr>
<td>Cisplatin34</td>
<td>Liver cancer</td>
<td>6</td>
<td>Pharmacokinetic study; time to peak and half-life longer with IT than IV chemotherapy</td>
</tr>
<tr>
<td>Cisplatin36</td>
<td>Solid</td>
<td>4</td>
<td>Tumor regression or stabilization</td>
</tr>
<tr>
<td>Cisplatin38</td>
<td>Esophageal cancer</td>
<td>9</td>
<td>Tumor volume reduced in 3 patients</td>
</tr>
<tr>
<td>P-3237</td>
<td>Solid</td>
<td>17</td>
<td>Response rate, 71%; complete response, 41%</td>
</tr>
<tr>
<td>Ethanol38</td>
<td>Liver cancer</td>
<td>207</td>
<td>1-, 2-, and 3-y survival, 90%, 69%, and 63%, respectively</td>
</tr>
<tr>
<td>Ethanol39</td>
<td>Liver cancer metatases</td>
<td>30</td>
<td>Reduction in tumor, n = 20; decreased AFP and CEA levels</td>
</tr>
<tr>
<td>Ethanol40</td>
<td>Liver cancer</td>
<td>210</td>
<td>1-, 3-, and 5-y survival, 93%, 65%, and 41%, respectively</td>
</tr>
<tr>
<td>Ethanol41</td>
<td>Liver cancer</td>
<td>115</td>
<td>1-, 2-, and 3-y survival, 96%, 63%, and 43%, respectively</td>
</tr>
</tbody>
</table>

Abbreviations: AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; IT, intratumor; IV, intravenous.

Table 7. Summary of Apoptosis and Necrosis Characteristics*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Apoptosis</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell death</td>
<td>Programmed</td>
<td>Irreversible</td>
</tr>
<tr>
<td>Morphologic properties</td>
<td>Cell shrinkage, nuclear chromatin condensation</td>
<td>Cell swelling, membrane collapse</td>
</tr>
<tr>
<td>Inflammation</td>
<td>None</td>
<td>Present</td>
</tr>
<tr>
<td>Injury</td>
<td>Response to mild injury</td>
<td>Response to severe injury</td>
</tr>
<tr>
<td>Energy dependence</td>
<td>Energy dependent</td>
<td>Energy independent</td>
</tr>
<tr>
<td>Pathways</td>
<td>Predictable and predetermined</td>
<td>Independent biochemical events</td>
</tr>
<tr>
<td>Prevention</td>
<td>Possible</td>
<td>Difficult to prevent</td>
</tr>
</tbody>
</table>

*Data reported in Kanduc et al42 and Proskuryakov et al.43

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REFERENCES