Preclinical Analysis of Tasidotin HCl in Ewing’s Sarcoma, Rhabdomyosarcoma, Synovial Sarcoma, and Osteosarcoma

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Abstract

Purpose: Dolastatins are a group of structurally unique peptides originally isolated from a sea hare, Dolabella auricularia, which seem to inhibit tubulin polymerization and mitosis. Tasidotin hydrochloride (tasidotin), a novel synthetic analogue of dolastatin 15, is evaluated in preclinical models of pediatric tumors.

Experimental Design: The cytotoxicity of tasidotin was evaluated in a panel of pediatric sarcoma cell lines in vitro and in vivo.

Results: The IC50 in Ewing’s sarcoma, rhabdomyosarcoma, osteosarcoma, and synovial sarcoma lines ranged from 0.002 μM to 0.32 μmol/L. In the SK-ES1 and RH30 cell lines, tasidotin induced a G2-M arrest that persisted for 48 h after the drug was washed from the cells. In vitro, more than half the cells were in the early or late phase of apoptosis 48 h after treatment with tasidotin. In vivo, a significant increase in apoptotic nuclei was apparent in xenograft tumors harvested within 24 h after a 5-day course of tasidotin. In vivo response was determined in severe combined immunodeficient xenograft models of pediatric sarcomas implanted heterotopically. Significant antitumor activity was observed in all tumor lines tested. A complete response was observed in 2 synovial sarcoma lines, 1 osteosarcoma line, 1 rhabdomyosarcoma line, and 1 Ewing’s sarcoma line. A partial response was observed in 1 rhabdomyosarcoma and 1 Ewing’s sarcoma.

Conclusions: Tasidotin induces a G2-M block in treated cells ultimately resulting in apoptosis. Antitumor activity is confirmed in vivo in preclinical xenograft models of pediatric sarcomas.

Despite great advances in the treatment of pediatric cancers, relapsed and metastatic sarcomas in children have proven refractory to most standard and experimental therapies. The need for new agents to treat these malignancies is clear. Development of new therapies, however, is hindered by the limited availability of patients for early phase clinical trials. Through the preclinical methods of Houghton et al. (1), rigorous preclinical data can assist in assigning priority to specific agents for phase I and phase II trials in pediatric patients.

Originally identified in the Indian Ocean sea hare, Dolabella auricularia (2–5), the dolastatins are groups of peptides capable of binding tubulin and inhibiting tubulin-dependent GTP hydrolysis in vitro (6). These compounds seem to inhibit the progression of cell growth through mitosis by inhibiting new microtubule assembly and inducing the polymerization of existing microtubules (7, 8). Dolastatin 10, and a synthetic dolastatin 15 analogue cemadotin, yielded limited success in phase II studies (9–11). Therapeutic efficacy of cemadotin was limited by its rapid metabolism to an active metabolite with cardiovascular toxicity (12–15).

Tasidotin HCl (Genzyme Corp), formerly known as ILX651, is a synthetic analogue of dolastatin 15. Tasidotin is a pentapeptide (N,N-dimethyl-l-valyl-l-valyl-N-methyl-l-valyl-l-proyl-l-proline-tert-butylamide hydrochloride) with metabolic stability and oral bioavailability. Tasidotin has shown marked activity in xenograft models of breast cancer, ovarian cancer, taxane-resistant ovarian cancer, prostate carcinomas, melanoma, non–small cell lung carcinoma, colon carcinoma, and P388 murine leukemia when administered both orally and i.v. (16).

Several phase I multi-institution clinical trials of tasidotin in adults were completed recently. Ebbinghaus et al. (17) reported neutropenia, ileus, and transaminase elevation as the dose-limiting toxicity at a dose of 36.3 mg/m2/d administered as an i.v. infusion for 5 consecutive days every 3 weeks. A patient with metastatic melanoma experienced a complete response, and 9 patients had stable disease. Pharmacokinetic analysis done with the first cycle revealed biphasic decay in the plasma with a half-life of less than 45 min. Cunningham et al. (18) reported neutropenia as the dose-limiting toxicity at a dose level of 45.7 mg/m2/d administered as an i.v. infusion on days
1, 3, and 5 every 3 weeks. Ten patients experienced stable disease. Pharmacokinetic analysis done with the first course also showed biphasic decay with a half life of less than 55 min. Mita et al. (19) reported neutropenia as a dose-limiting toxicity in a phase I trial of tasidotin administered via a 30-min i.v. infusion weekly for 3 weeks every 4 weeks. A minor response was reported in a patient with non–small cell lung cancer and stable disease for 11 months in a patient with hepatocellular carcinoma.

Tasidotin has not been explored for the treatment of pediatric malignancies. With its promising antineoplastic activity, novel mechanism of action, and metabolic stability, the agent shows potential for broad therapeutic applications. In the present report, tasidotin was investigated to determine activity in xenograft models of rhabdomyosarcoma, Ewing’s sarcoma, synovial sarcoma, and osteosarcoma.

Materials and Methods

Cell lines. The tumor cell lines used included the following: RD and RH30 rhabdomyosarcoma lines (American Type Culture Collection), SaOS-2 osteosarcoma line, SK-PN-DW and SK-ES1 Ewing’s sarcoma lines (generous gift of Mark Ladanyi, Memorial Sloan-Kettering Cancer Center, New York, NY), and HSSY-II and SYO-I synovial sarcoma lines (gift of Mark Ladanyi). The cells were grown in monolayer at 37°C, 5% CO2 in medium of MEM (SaOS-2, SYO-1, and HSSY-II), DME (SK-PN-DW, RD, and SK-ES1), or RPMI 1640 (RH30) supplemented with 10% fetal bovine serum (Invitrogen), 0.5% penicillin/streptomycin (Invitrogen), and 1% glutamine (Invitrogen). The OS1 line (a gift of Peter J. Houghton, St. Jude Children’s Research Center, Memphis, TN) is a tumor line maintained by serial passage in mice (20).

Cell proliferation assay. Growth inhibition was determined by the microculture tetrazolium method. Briefly, cells were seeded in 96-well flat-bottomed microtiter plates at a density of 500 cells per well in 100 μL of medium. After overnight incubation, 100 μL of medium containing tasidotin were added to achieve specified final concentrations and a final volume of 200 μL/well. At 120 h, the relative metabolic activities of treated and untreated cells were measured by mitochondrial conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma) to formazine. At the completion of the drug treatment, 250 μg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were added to each well and incubated at 37°C, 5% CO2 for 6 h. Formazine crystals were dissolved in DMSO and absorbance at 595 nm was measured on a VERSAmax spectrophotometer (Molecular Devices). Absorbance values were normalized to the values obtained for the vehicle-treated cells to determine the percentage of survival. The IC50 was defined as the concentration at which absorbance of the treated cells was 50% that of the controls.

Fig. 1. Inhibitory effect of tasidotin on pediatric sarcoma cell lines. RH30 and RD (A); SK-ES1 and SK-PN-DW (B); HSSY-II and SYO-1 (C); and SaOS (D) were cultured in 96-well plates at a density of 500 cells per well with 100 μL medium per well. After 24 h, medium containing tasidotin was added to achieve a final volume of 200 μL per well. Final drug concentrations in micromoles are specified in each graph. Cells were incubated with drug continuously for 120 h. Growth was assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. Data points represent the absorbance of the treated cells/control, and each point is the mean of three experiments.
Drugs. Tasidotin (Genzyme Oncology) was dissolved in PBS from lyophilized powder before each experiment.

Cell cycle analysis. Cells were plated in 100 × 20-mm tissue culture dishes and allowed to grow for a period of 3 days to reach an estimated density of 1 million cells per plate. Cells were treated with tasidotin at a concentration of 0.16 μmol/L and allowed to incubate for 24 h. Following 24 h of exposure to drug, cells were washed with PBS and recultured in drug-free medium. At specified time points, cells were detached from the plates by trypsinization. Trypsinized cells were combined with the supernatant from the plate and washed twice with warm PBS. The pellet was fixed in cold 70% ethanol at -20°C overnight. After ethanol fixation, cells were washed with PBS and resuspended in 0.5 mL (1/2 10^6 cells/0.5 mL) propidium iodide/RNase staining buffer (BD Biosciences). Cells were incubated in the dark for 30 min before flow cytometric analysis (FACSCalibur, Becton Dickinson). Data were acquired with the CellQuest software (Becton Dickinson) at 10,000 events per sample and analyzed using CellQuest. Cell cycle phases and number of apoptotic cells were determined as a fraction of the total number of cells present in each individual sample. Data are representative of results confirmed in three separate experiments.

Determination of maximally tolerated dose. CB17 female scid^-/- mice were implanted with tumor subcutaneous flank tumors. For cell lines RH30, RD, SK-ES1, SK-PN-DW, HSSY-II, and SYO-1, cultured cells were harvested with trypsin/EDTA and resuspended in 50% Matrigel (Becton Dickinson) at a concentration of 1 x 10^6 cells/0.1 mL. At 5 to 6 weeks of age, the mice were injected with 0.1 mL of cells suspended in Matrigel. The OS1 tumor line was established at St. Jude Children’s Cancer Research Hospital and has been described previously (20). For transplantation with OS1 tumor, mice were anesthetized with 4% isoflurane. After a small incision was made in the flank of the mouse, a 4 x 4-mm section of tumor was implanted s.c.

When the tumors were approximately 0.20 to 0.7 cm in diameter, tumor-bearing mice were randomized into groups of five to eight mice with one treatment group and one control group. Treated mice received tasidotin at a dose of 90 mg/kg/d or vehicle control as an i.p. injection daily for 5 days starting on days 1 and 21. Assuming a spherical tumor,

Table 1. In vitro cytotoxicity of tasidotin following 120-h continuous exposure to drug

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Histology</th>
<th>Tasidotin IC_{50} (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH30</td>
<td>Rhabdomyosarcoma</td>
<td>0.02</td>
</tr>
<tr>
<td>RD</td>
<td>Rhabdomyosarcoma</td>
<td>0.02</td>
</tr>
<tr>
<td>SK-ES1</td>
<td>Ewing’s Sarcoma</td>
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</tr>
<tr>
<td>SK-PN-DW</td>
<td>Ewing’s Sarcoma</td>
<td>0.13</td>
</tr>
<tr>
<td>HSSY-II</td>
<td>Synovial Sarcoma</td>
<td>0.26</td>
</tr>
<tr>
<td>SYO-1</td>
<td>Synovial Sarcoma</td>
<td>0.32</td>
</tr>
<tr>
<td>SaOS</td>
<td>Osteosarcoma</td>
<td>0.002</td>
</tr>
</tbody>
</table>

NOTE: The IC_{50} is the concentration at which the absorbance of the treated cells is 50% that of the controls using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay.

Fig. 2. Effect of tasidotin on the cell cycle. Two tasidotin-sensitive lines [RH30 (A) and SK-ES-1 (B)] were treated with 0.16 μmol/L tasidotin for 24 h. Cells and culture supernatant were harvested at hour 0 (control), hour 24 (immediately after treatment), hour 48 (24 h after treatment), and hour 72 (48 h after treatment) fixed in ethanol, and stained with propidium iodide. DNA content was assayed by flow cytometry. Data are expressed as a percentage of the total events.

Fig. 3. Determination of the MTD of tasidotin. Non-tumor-bearing severe combined immunodeficient mice were treated with tasidotin via i.p. injection daily for 5 every 21 d for two cycles. Mouse weight is a mean calculated from treatment groups of at least five mice each.
the volume was determined by the formula: \( \text{mm}^3 = \pi / 6 \times D \times d^2 \), where \( D \) is the maximal diameter and \( d \) is the diameter perpendicular to \( D \). Volumes are expressed as relative tumor volumes (RTV), where the tumor volume at any given time point is divided by the starting tumor volume, and actual tumor volume. The RTV for treated and control mice are measured a minimum of once weekly. All experiments were conducted using protocols and conditions approved by the Albert Einstein College of Medicine Institutional Animal Care and Use Committee.

**Apoptosis analysis in vitro.** RH30 cells were grown overnight in 10-cm plates. The next day, cells were treated with tasidotin (0.16 μmol/L) for 24 h. At specified time points, cells were trypsinized, washed twice with PBS with 1% fetal bovine serum (along with the supernatant from culture plates to capture nonadherent cells), and then resuspended in binding buffer (BD Biosciences) at a concentration of 1 \times 10^6 cells/mL. Cells were incubated with 1 μg/mL of Annexin V-FITC (BD Bioscience) and 25 μg 7-amino-actinomycin D (BD Bioscience) in the dark for 10 min before flow cytometric analysis (FACSCalibur). Data are representative of the results confirmed in three separate experiments.

**Apoptosis analysis in vivo.** The apoptosis detection kit (Chemicon International) was used to assess the degree of apoptosis following treatment with tasidotin. Briefly, severe combined immunodeficient engrafted with OS1, SK-ES1, RH30, and SYO-1 subcutaneous tumors were treated with tasidotin at a dose of 90 mg/kg/d i.p. for 5 days. Control mice were treated with vehicle alone. Tumors were harvested on day 6 from the control and treated mice. Tumor tissue was fixed in formaldehyde for 24 h and then paraffin embedded. Slides containing

Fig. 4. Tasidotin inhibits growth of rhabdomyosarcoma, Ewing’s sarcoma, synovial sarcoma, and osteosarcoma xenografts. Tumor-bearing mice implanted with rhabdomyosarcoma (A), Ewing’s sarcoma (B), synovial sarcoma (C), or osteosarcoma (D) tumor lines were treated with 90 mg/kg tasidotin daily via i.p. injection for 5 d on days 1 and 21. Tumor growth is expressed as relative tumor volume (tumor volume at time \( X \)/tumor volume at time 0) and absolute tumor volume.
paraffin-embedded tissue were deparaffinized with xylene and serial washes of ethanol. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide. The specimens were incubated with the TdT enzyme for 1 h at 37°C soaking in the equilibration buffer provided in the kit. The reaction was stopped with stop buffer for 10 min at room temperature. Anti-digoxigenin conjugate was added for 30 min at room temperature. After washing with PBS, peroxidase substrate was added for 5 min at room temperature. Slides were counterstained with hematoxylin for 10 min and then mounted with a coverslip. Percentage of apoptotic cells was determined by counting the number of brown-staining nuclei per high-power field of a light microscope. Results are reported as the mean of six representative fields.

**Assessment of tumor response and statistical considerations.** Using criteria defined previously by Houghton et al. (20), progressive disease is defined as <50% regression from original tumor volume for the entire study period (RTV > 0.5) and >25% increase in tumor volume at the end of the study period (RTV > 1.25). Stable disease is defined as tumor regression that did not exceed 50% of the original tumor volume throughout the entire study period (RTV > 0.5) and ≤25% increase in tumor volume at the end of the study period (RTV < 1.25). A partial response is defined as >50% regression in tumor volume (RTV < 0.5) but with a measurable tumor mass of >0.10 cm³. Loss of measurable tumor mass (<0.10 cm³) at any point during the treatment period (6 weeks) was defined as a complete response. A sustained complete response was defined as a loss of measurable tumor mass (<0.10 cm³) at any point after initiation of therapy without regrowth during the 6-week study period. Mice that died before the week 6 and before the tumor reached four times the initial volume were censored.

Statistical analysis was based on event-free survival (EFS). An event is defined as a relative tumor volume of four times (i.e. quadruple the starting tumor size) or death. EFS is defined as the time from the initiation of the study to an event. For those tumors not reaching an event by 6 weeks, the end of the study period, the EFS time was censored at that time. The exact log-rank test was used to compare EFS distributions between treatment and control groups. Additionally, the day 22 RTVs for the control and treated mice were compared using the Wilcoxon-Mann-Whitney test. This allows for comparison of tumor volume after one cycle of tasidotin and at or near the time of event for the untreated mice.

**Results**

*Tasidotin inhibited the proliferation and viability of pediatric solid tumor cell lines.* In the each of the rhabdomyosarcoma, Ewing's sarcoma, osteosarcoma, and synovial sarcoma lines, significant growth inhibition was shown at 120 h when comparing treated cells to untreated controls (Fig. 1). In each
of these seven cell lines, cell viability approaches 0 after 120 h of continuous drug exposure at concentrations >0.16 μmol/L (100 ng/mL). The IC₅₀ of tasidotin for each line tested is listed in Table 1.

**Tasidotin leads to accumulation of cells in G₂-M phase of cell cycle.** Cell cycle distribution of the RH30 and SK-ES1 following exposure to tasidotin is shown in Fig. 2. Following treatment for 24 h with 0.16 μmol/L tasidotin, the RH30 line and SK-ES1 line each showed an accumulation of cells in the G₂-M phase. At hour 24, nearly all the RH30 cells were in the G₂-M phase. Over the next 48 h, no significant increase in the number of cells reentering the cell cycle was observed, despite the fact that the drug was washed off the cells. A similar pattern was seen in the SK-ES1 cell line, with the exception of the fact that maximal accumulation in G₂-M occurs at hour 48. For both cell lines, a significant fraction (27% for RH30 and 60% for SK-ES-1) of the cells was sub-G₁ at 72 h, with only a small fraction of the cells reentering the cell cycle. This irreversible block in G₂-M, ultimately leading to cell death, is consistent with the proposed mechanism of action through interference with tubulin function.

**Murine MTD.** Mice treated with 100 mg/kg had a mean weight loss of >20% with no return to their baseline starting weight, and one mouse died before the second treatment course (Fig. 3). The mice treated with 90 mg/kg/d tasidotin had a mean weight loss of <16% following each 5-day treatment of tasidotin. A full recovery to their starting weight was noted within 3 weeks of treatment. Therefore, the MTD in severe combined immunodeficient mice (i.e. the dose at which no toxic event occurred for all mice) was 90 mg/kg/dose daily for 5 consecutive days.

**Tasidotin inhibits growth of heterotopic human tumor implants in severe combined immunodeficient mice.** In the rhabdomyosarcoma, synovial, osteosarcoma, and Ewing’s sarcoma cell lines, tasidotin showed significant antitumor activity in xenograft models compared with the untreated controls (Fig. 4). Tasidotin treatment of mice with RD, SYO-1, and OS1 tumor implants yielded a complete response that was maintained for the entire study period. A complete response with tumor regrowth is shown in the SK-PN-DW and HSSY-II xenografts. A partial response is seen in mice bearing the RH30 and SK-ES1 tumor implants. Statistically significant differences in the RTV at day 22 (the 1st day of the second course of treatment) are seen in all tumor lines. A statistically significant difference in median time to event is seen in all tumor lines with the exception of the RD rhabdomyosarcoma line. The RD tumor-bearing mice achieved and maintained a complete response following treatment with tasidotin, but the growth of the RD tumor is slow and the difference in EFS does not reach statistical significance. The response data are summarized in Table 2.

**Apoptotic cell death is observed following tasidotin exposure.** Apoptosis in vitro following tasidotin exposure in the RH30 cell line is assessed by the Annexin V binding assay (Fig. 5). Cells harvested at hour 24 (immediately after 24-h exposure to tasidotin HCl in Sarcoma.

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**Table 2. Response analysis**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Response</th>
<th>Median day 22 RTV</th>
<th>P</th>
<th>Median time to event (d)</th>
<th>P</th>
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<tr>
<td>RH30</td>
<td>PR</td>
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<td>Control 11</td>
<td>0.0008</td>
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<tr>
<td></td>
<td></td>
<td>Treated 0.23</td>
<td></td>
<td>Treated &gt;SP</td>
<td></td>
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<tr>
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<td>mCR</td>
<td>Control 2.4</td>
<td>0.0013</td>
<td>Control &gt;SP</td>
<td>0.1515</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated 0</td>
<td></td>
<td>Treated &gt;SP</td>
<td></td>
</tr>
<tr>
<td>SK-ES1</td>
<td>PR</td>
<td>Control 13.6</td>
<td>0.0079</td>
<td>Control 18</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Treated 0.87</td>
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<td>Treated &gt;SP</td>
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<td>SK-PN-DW</td>
<td>CR</td>
<td>Control 12.8</td>
<td>0.0022</td>
<td>Control 11</td>
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<td></td>
<td></td>
<td>Treated 0</td>
<td></td>
<td>Treated &gt;SP</td>
<td></td>
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<tr>
<td>SYO-1</td>
<td>mCR</td>
<td>Control 20.2</td>
<td>0.0022</td>
<td>Control 9</td>
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<td></td>
<td>Treated 0</td>
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<td>HSSY-II</td>
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<td>Treated 0.43</td>
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</table>

**Abbreviations:** mCR, maintained complete response; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; SP, study period.

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Fig. 5. Apoptotic cell death in the RH30 cell line following exposure to tasidotin. Cells and supernatant were harvested following 24-h exposure to 0.16 μmol/L tasidotin, with anti Annexin V and 7-amino-actinomycin D, and assayed by flow cytometry. After exposure to tasidotin for 24 h, a majority of treated cells stain for Annexin V and/or 7-amino-actinomycin D when compared with untreated controls, indicating an activation of apoptotic pathways. Data are expressed as a percentage of the total events.
0.16 μmol/L tasidotin) were similar to untreated cells. However, >50% of the cells harvested at hour 48 were either in the early (Annexin V positive) or late (7-amino-actinomycin D positive) phases of apoptosis. Apoptosis in vivo is assessed in one tumor line of each histology by the terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay (Fig. 6). An increase in the number of apoptotic nuclei is observed in all lines tested within 24 h of the completion of a 5-day course of tasidotin. Images of representative high-power fields are shown.

Fig. 6. Tumors were harvested day 6 after mice received either 90 mg/kg/d tasidotin or vehicle control for 6 d. Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay was done on the RH30 (A and B), SK-ES1 (C and D), SYO-1 (E and F), and OS1 (G and H). Apoptosis was quantified by counting apoptotic nuclei per high-powered field. Images of the stained tumors at magnification ×400 (A, C, E, and G). Graphs showing the mean number of apoptotic nuclei counted in six high-powered fields (B, D, F, and H).
Discussion

Tubulin-targeted chemotherapeutic agents are common in the treatment of adult and pediatric cancers. In general, these agents lead to a disruption of the normal functions of tubulin assembly or disassembly, rendering cells unable to divide and resulting in apoptosis. The Vinca alkaloid and taxanes are the most widely used tubulin-targeted agents. The broad clinical applications of these agents and the successful targeting of tubulin have prompted the identification and evaluation of newer agents that disrupt tubulin and the microtubule system. The mechanisms by which different classes of tubulin-targeting agents can inhibit the microtubule systems are varied and so too are the potential mechanisms of resistance.

The dolastatins are cyclic and linear peptides derived from the sea hare, Dolabella auricularia, found in the Indian Ocean. Dolastatin 10 is a noncompetitive inhibitor of Vinca alkaloid binding to tubulin and leads to accumulation of cells in metaphase by inhibiting microtubule assembly. Dolastatin 15 competitively inhibits Vinca alkaloid binding to tubulin and disrupts tubulin polymerization. Cardiac toxicity and limited response data have hindered the development of dolastatin analogues to date. Tasidotin is an orally active synthetic analogue of dolastatin 15. In three recent clinical trials, tasidotin has shown a favorable toxicity profile to previous studies with dolastatin 15 analogues (17–19). The agent has not been evaluated for antineoplastic activity in pediatric sarcomas. In the current study, we show promising preclinical antitumor activity in rhabdomyosarcoma, Ewing’s sarcoma, synovial sarcoma, and osteosarcoma tumor lines.

For all cell lines tested, tasidotin showed significant cytotoxic activity with a IC_{50} in the range of 0.002 to 0.32 μmol/L. Although the exact cytotoxic mechanism of action for tasidotin is unknown, as a derivative of the dolastatin family, it seems to act as an inhibitor of tubulin polymerization and therefore mitosis. In the cell lines tested in the current study, tasidotin leads to an accumulation of cells in the G_2-M phase of the cell cycle. In both the RH30 and SK-ES1 cell lines, nearly all the cells were ultimately blocked in G_2-M. Even after the drug was washed from the cell, there was no increase in the G_0-G_1 and S phases. Rather, there was an increase in the sub-G_1 fraction, suggesting an irreversible mitotic block leading to cell death. In the RH30 line, cell death by apoptosis was confirmed with Annexin V and 7-aminocoumarin D staining of treated cells. A majority of cells were in either early or late apoptosis within 48 h of treatment with tasidotin. In vivo, an increase in apoptotic nuclei was seen in each of the histologies after one course of tasidotin.

Using the dosing schedule used in the clinical studies (17), we determined the MTD in severe combined immunodeficient mice to be 90 mg/kg daily via i.p. injection for 5 days repeated once every 3 weeks. A complete response is reported in 5 of the 7 lines tested (2 synovial sarcoma lines, 1 rhabdomyosarcoma, 1 Ewing’s sarcoma, and 1 osteosarcoma) and a partial response in the remaining 2 lines (1 rhabdomyosarcoma and 1 Ewing’s sarcoma). Although this is a limited panel of childhood sarcomas, the activity is significant in each of the lines.

The tubulin-targeted Vinca alkaloid, vincristine, is a well-established staple in the treatment of many childhood sarcomas. A recent phase II trial of docetaxel in recurrent solid tumors suggest that, even in patients heavily pretreated with vincristine, tubulin may still be an effective target in the relapse setting as well (21). The activity of these agents may be enhanced when used in combination with other agents. For example, the combination of gemcitabine and docetaxel is effective therapy in a wide array of malignancies. Future in vivo studies of tasidotin in combination with other cytotoxic agents may identify advantageous combinations.

Phase I studies in adult patients with relapsed or refractory solid tumors to date have yielded several objective clinical responses with acceptable toxicities. Bone marrow suppression seems to be the dose-limiting toxicity (17–19). Clinical trials in children are not yet under way. The activity of this agent in early trials in adult patients, combined with the preclinical data in the current report, suggests a potential for clinical development in children.

In summary, tasidotin is a novel synthetic dolastatin 15 analogue with promising activity in recent clinical trials in adult patients. In a panel of pediatric solid tumors, we showed in vitro activity in rhabdomyosarcoma, Ewing’s sarcoma, osteosarcoma, and synovial sarcoma cell lines with an IC_{50} ranging from 0.002 to 0.32 μmol/L. Tasidotin seemed to lead to a G_2-M block resulting ultimately in apoptosis. Treatment of murine xenograft models of rhabdomyosarcoma, Ewing’s sarcoma, osteosarcoma, and synovial sarcoma resulted in significant responses in nearly all tumor lines tested at the murine MTD for tasidotin. These results suggest that tasidotin may yield positive results in clinical trials in pediatric sarcomas.

References

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