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A Single-Arm Pilot Phase II Study of Gefitinib and Irinotecan in Children with Newly Diagnosed High-Risk Neuroblastoma

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Abstract

Background—Gefitinib potently inhibits neuroblastoma proliferation *in vitro*, and the gefitinib/irinotecan combination shows greater than additive activity against neuroblastoma xenografts. This Phase II pilot study estimated the rate of response to two courses of intravenous irinotecan plus oral gefitinib in children with untreated high-risk neuroblastoma.

Methods—Two courses of irinotecan [15 mg/m²/day (daily×5)×2] were combined with 12 daily doses of gefitinib (112.5 mg/m²/day). Response was assessed after six weeks. A response rate >55% was sought.

Results—Of the 23 children enrolled, 19 were evaluable for response. Median age at diagnosis was 3.1 years (range, 18 days – 12.7 years). Most patients were older than 24 months (n=20; 87%), male (n=18; 78%), white (n=16; 70%), had INSS 4 disease (n=19; 83%), and had adrenal primary tumors (n=18; 78%); nine patients (39%) had amplified tumor *MYCN*. The toxicity of gefitinib/irinotecan was mild and reversible (nausea, 5/20; diarrhea, 8/20; vomiting, 7/20). Five patients had partial responses; 9 others had a 23%–60% decrease in primary tumor volume and/or improved MIBG scans or decreased bone or bone marrow tumor burden. Median (range) systemic irinotecan exposure (AUC) was 283 ng/ml*hr (range, 163–890 ng/ml*hr) and 28 ng/ml*hr (3.6–

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297 ng/ml*hr) for the active metabolite, SN-38. No relation was observed between response and tumor expression of EGFR, MRP2-4, ABCG2, and Pgp.

Conclusions—Although the gefitinib/irinotecan combination was very tolerable and induced responses, it was not sufficiently active to warrant further investigation. Initial investigational studies of this type can preclude the necessity for larger, longer, and costlier trials.

Keywords

Neuroblastoma; irinotecan; gefitinib; clinical trial; Phase II; ATP-Binding Cassette Transporters

Introduction

Our institution identified irinotecan as highly active against neuroblastoma in comprehensive preclinical xenograft models and demonstrated that irinotecan's activity is dramatically schedule- and dose-dependent.[1–4] However, it remains challenging to integrate irinotecan into the treatment of children with high-risk neuroblastoma in the era of targeted therapy.[5–7] A potential approach is to use it with “targeted agents” known to be additive to or synergistic with irinotecan, such as gefitinib, a specific epidermal growth factor receptor (EGFR) inhibitor. Gefitinib and irinotecan showed significantly greater than additive activity against several xenografted pediatric tumors, including two of three neuroblastoma xenografts.[8] The effect of gefitinib appeared to be independent of tumor ERBB1 status.[8] *In vitro*, gefitinib potently reversed resistance to SN-38 (the active metabolite of irinotecan) only in a cell line overexpressing functional ABCG2 (breast cancer resistance protein, BCRP), which actively exports cellular SN-38.[9] Four of 6 neuroblastoma xenografts were found to be immunohistochemically positive for ABCG2.[8]

Gefitinib, by inhibiting ABCG2-mediated SN-38 efflux, may modulate irinotecan cytotoxicity at the cellular level.[10,11] Another ATP binding cassette (ABC) transporter, ABCC4/MRP4, is reportedly a marker of poor prognosis in neuroblastoma and confers resistance to irinotecan *in vitro*. [12] Similarly, CI1033, a HER tyrosine kinase inhibitor, enhances the cytotoxicity of SN-38 by inhibiting ABCG2-mediated drug efflux.[13] Inhibition of EGFR signaling by gefitinib may also offer a promising strategy for treatment of neuroblastoma [14,15] and appears likely to enhance the effect of cisplatin and doxorubicin.[16–18] Clinically, the combination of irinotecan and cetuximab (Erbix, IMC-C225), an anti-EGFR monoclonal antibody, induced a 22.9% response rate in patients with irinotecan-refractory colorectal cancer.[19–21] We therefore evaluated the combination of gefitinib and irinotecan in children with high-risk neuroblastoma in an initial investigational phase before standard therapy. Although gefitinib is a “specific” EGFR inhibitor, it may also modulate irinotecan cytotoxicity by inhibiting ABC transporters. Therefore we assessed tumor expression of EGFR and of the drug efflux transporters ABCG2, ABCB1 (Pgp), ABCC2 (MRP2), ABCC3 (MRP3) and ABCC4 (MRP4) before and after the investigational treatment.

Patients and Treatment

Eligibility

Patients were eligible for the prospective, single-arm, Phase II trial (NCT00135135; AstraZeneca IRUSIERS0389) if they were less than 18 years old and had newly diagnosed, advanced-stage, high-risk neuroblastoma, defined as International Neuroblastoma Staging System (INSS) stage 2A/2B, 3, 4, or 4S disease and *MYCN* amplification (>10 copies) at any age; INSS stage 3 disease and unfavorable Shimada histology[22] at age >18 months;

INSS stage 4 disease and any unfavorable biological feature (*MYCN* amplification, unfavorable histology, or DNA index = 1) or any indeterminate/unknown biological risk feature at age 12–18 months; INSS stage 4 disease at age 18 months regardless of biological features; and progression to stage 4 after an initial diagnosis of stage 1, 2, or 4S in the absence of chemotherapy at age 365 days. Other eligibility requirements included histologic confirmation of neuroblastoma or bone marrow positive for tumor cells plus elevated urine catecholamines; adequate renal and hepatic function (serum creatinine $<3 \times$ upper limit of normal for age, AST $<3 \times$ upper limit of normal); and no prior tumor therapy with the exception of emergency local tumor treatment. The study was approved by the St. Jude Institutional Review Board, and signed informed consent was obtained from patients, parents, or guardians, as appropriate.

Drug formulation and administration

Irinotecan (Camptosar; Pfizer, Inc. New York, NY) was diluted in 5% dextrose or 0.9% NaCl to a final concentration of 0.12–2.8 mg/ml, and 15 mg/m²/day was administered intravenously over 60 min for 5 days; after 2 days, a second daily $\times 5$ course was started. Gefitinib (Iressa; AstraZeneca, Wilmington, DE) 112.5 mg/m²/day (available in 25, 100, and 250 mg tablets) was administered orally daily for 12 days, one hour before the start of irinotecan. A second, identical course of irinotecan and gefitinib was started on day 22. These dosages were established by a previously reported Phase I study of this combination in children with refractory solid tumors. [23]

Treatment after irinotecan and gefitinib

After the initial six weeks, patients received induction chemotherapy with three identical blocks of standard agents, each comprising three courses: 1) cyclophosphamide, doxorubicin, and etoposide; 2) cisplatin and etoposide; and 3) topotecan adjusted to achieve a targeted systemic exposure, daily $\times 5$ days for two weeks. During induction, patients underwent resection of the primary tumor and accessible lymph nodes and peripheral blood stem cell (PBSC) harvest. They then received intensification chemotherapy with high-dose melphalan, etoposide phosphate (Etopophos, Bristol-Myers Squibb; New York, NY), and carboplatin, with PBSC support. Upon recovery, patients underwent irradiation [24] of primary and metastatic disease sites. After consolidation therapy, patients were treated with oral retinoic acid for 8 months and then oral topotecan for eight months.[25]

Assessment of response to irinotecan and gefitinib

This report describes data from the initial investigational six-week phase of therapy. Response was assessed six weeks after the start of therapy by using the International Criteria for Neuroblastoma Response.[26] Three-dimensional CT or MR imaging plus physical exam and/or surgical measurement (when possible) were used to assess the primary tumor. Tumor volume was calculated as the product of the three maximal tumor dimensions $\times 0.523$. A complete response (CR) was defined as disappearance of all disease and normalization of the urine catecholamine metabolites homovanillic acid (HVA) and vanillylmandelic acid (VMA) (if elevated at diagnosis). Partial response (PR) was defined as $>50\%$ reduction of primary tumor volume and of measurable metastatic sites; mixed response was defined as a $>50\%$ response at one or more sites and a $<50\%$ response at one or more sites. No response (NR) was defined as $<50\%$ reduction of some or all measurable lesions with no increase $>25\%$ in any lesion and no new lesions; progressive disease (PD) indicated a $>25\%$ increase in any preexisting lesion, or any new lesion.[26]

Pharmacokinetics of irinotecan

Serial plasma samples were obtained on Day 8 (Day 9–12 if Day 8 fell on the weekend) of Course #1, 1 (before irinotecan infusion), 2.25, 2.5, 3, 6, and 8 hours after oral gefitinib. Plasma concentration of irinotecan, SN-38, and SN-38 glucuronide lactone were assessed by HPLC with fluorescence detection.[27] A four-compartment model was fit to the irinotecan and SN-38 concentration-time data using NONMEM (Version VI).[28] Irinotecan clearance (Cl), area under the concentration-time curve (AUC), and SN-38 AUC were determined by established approaches.[23]

Immunohistochemistry

Archived formalin-fixed, paraffin-embedded tumor sections were stained to detect ABC transporters and EGFR. The antibodies, staining methods, and instrumentation are summarized in Table 1. Appropriate internal and external positive and negative controls were consistently used. Results were interpreted as positive if any tumor cells were stained and negative controls were unstained and as negative if no tumor cells were stained but positive controls were adequately stained.

Statistical considerations

The primary endpoint was tumor response after 6 weeks of investigational therapy. The study was planned to enroll 39 patients by utilizing a four-stage group sequential design[29] to test the hypothesis that the true response rate was <35% versus >55%, with 80% power and 5% type I error ($\alpha=0.05$). Three interim analyses were planned, after 10, 20, and 30 patients had been evaluated for response. The study was closed after meeting the statistical stopping rule at the second interim analysis.

The 95% confidence interval of the response rate was calculated by the Blyth-Still-Casella method. The exact Wilcoxon signed rank test was used to compare baseline and post-treatment HVA, VMA, and LDH values. Spearman correlation coefficients were used to assess 1) the correlation of changes in HVA and VMA excretion after treatment and 2) the correlation of these changes with change in primary tumor volume. The exact Wilcoxon rank sum test was used to assess the relation of change in primary tumor volume to expression of EGFR, ABCC4, ABCG2, ABCB1, ABCC2 and ABCC3.

Results

Patients

Of 23 patients enrolled on NB2005 between August 2005 and April 2007, 20 received the investigational regimen. One patient had no measurable disease at enrollment, the clinical condition of one patient (significant loss of lower extremity function because of metastatic paraspinal tumor) contraindicated the investigational phase, and the parents of one patient (age 18 days) declined the investigational phase.

Characteristics of the 23 patients are shown in Table 2. Median age at enrollment was 3.1 years (18 days – 12.7 years). Most patients were older than 24 months (n=20; 87%), male (n=18; 78%), and white (n=16; 70%). Most patients had INSS stage 4 disease (n=19; 83%) and adrenal primary tumors (n=18; 78%). Approximately 40% of tumors (9/23) had amplified *MYCN*.

At diagnosis, the median random urine catecholamine metabolite values were HVA, 97.7 mg/g creatinine (range, 6–397 mg/g) and VMA, 139.2 mg/g creatinine (range, 4.5–334.1 mg/g). Median serum LDH activity was 847 U/L (range, 278–4070 U/L).

Outcome

Twenty of 23 patients received the gefitinib/irinotecan therapy, and 17 completed both courses. Two patients experienced progressive disease after one course and proceeded to standard induction therapy. The remaining patient started course 1 but was taken off investigational therapy because of bleeding. This patient received 3 doses of gefitinib and four doses of irinotecan before developing intratumoral bleeding and disseminated intravascular coagulation (DIC) requiring multiple transfusions. This patient had undergone biopsy of a left adrenal primary tumor eight days before starting investigational therapy. These adverse effects may have been caused by chemotherapy, but no definitive conclusion can be drawn.

Nineteen of 20 patients were evaluable for response. One patient underwent primary tumor resection before gefitinib/irinotecan therapy, and primary tumor volume could not be measured. Responses included PR in 5 patients, mixed response in 3, stable disease in 7, progressive disease in 3, and no response in 1 patient who did not complete course 1 due to toxicity (bleeding in tumor). Of the ten patients with mixed response or stable disease, 7 had a decrease in primary tumor volume (23%–60%) and two others had improved MIBG scans. The study was closed at the second interim analysis for lack of efficacy (i.e., fewer than 7 PRs among 20 patients). The observed response rate to the investigational therapy was 26.3% (5/19) (95% CI, 11.0%–50.0%).

Seventeen of 20 patients had measurement of primary tumor volume before and after investigational therapy (2 courses in 15 patients; 1 course in 2 patients who had progressive disease). Twelve patients showed reduction of primary tumor volume (23.4%–77.7%) and five showed an increase (5.6%–40.8%) (Fig. 1). Overall, the median percent change in primary tumor volume was –30.9% (range, –77.7% to +40.8%).

Random urine HVA and VMA measurements before and after investigational treatment were available for 15 patients (one received only 1 course of therapy). Eleven patients showed reduced HVA and VMA excretion, although we detected no significant difference from baseline values ($p=0.21$ and $p=.21$ respectively). HVA excretion decreased by a median of 27.4% (range, –76.7% to +230.3%) and VMA excretion decreased by a median of 26.2% (range, –85.1% to +110.8%) (Fig. 2). The HVA and VMA changes were highly correlated ($\rho=0.95$; $p<0.001$). Percent change in primary tumor volume was marginally associated with change in HVA ($\rho=0.58$, $p=0.029$) and VMA ($\rho=0.45$, $p=0.110$).

Pre- and post-investigational therapy LDH measurements were available for 20 patients, although 3 had received only 1 course due to early progressive disease ($n=2$) or toxicity ($n=1$). LDH activity was significantly decreased after treatment (median reduction, 51.2%; range, –76.3% to +8.0%) ($p<0.001$) (Fig. 2).

Immunohistochemistry

Expression of six drug efflux transporters was examined in available tumor samples. Baseline tumor samples were obtained from 17 patients (a median of 5 days before treatment); ten were from primary tumor. Nearly 30% (5/17) of the baseline samples expressed ABCC4 (Table 3); 27% (4/15 evaluated) expressed ABCC3 and 20% (3/15 evaluated) expressed ABCC2. Only 1 baseline sample expressed EGFR, and none expressed ABCB1. Sixteen primary tumor samples from 15 patients were obtained a median of 65 days after the start of treatment. Thirty-one percent of post-treatment samples expressed ABCC4 and 56% expressed ABCC3. There was no evidence of association between change in primary tumor volume and expression of EGFR, ABCC4, ABCG2, ABCB1, ABCC2, or ABCC3 ($p>0.227$). Both patients with baseline tumor expression of ABCG2 had large reductions in primary tumor volume (–75% and –57%), and both had PRs (Fig. 3, Table 4).

However, we were unable to draw conclusions about the relation of ABCG2 expression to response.

Toxicity

Systemic toxicities of gefitinib/irinotecan were generally mild to moderate and reversible. Grade 4 hematologic toxicity was observed in 6 patients (thrombocytopenia, 2; neutropenia, 3; anemia, 2). Grade 4 hemorrhage was observed in 1 patient. Grade 3 toxicities included vomiting (n=2), hypokalemia (n=3), hyponatremia (n=1), diarrhea (n=3), nausea (n=2), febrile neutropenia (n=1), anorexia (n=3), and DIC (n=1). Grade 2 toxicities included diarrhea (n=5), fever (n=5), hypoalbuminemia (n=4), dehydration (n=4), nausea (n=3), vomiting (n=5), abdominal pain (n=2), rash (n=2) and weight loss (n=5).

Pharmacokinetics

Serial plasma samples were collected from 18 patients. AUC values were: irinotecan, 283 ng/ml*hr (range, 163–890 ng/ml*hr); SN-38, 27.9 ng/ml*hr (range, 3.6–297 ng/ml*hr); and SN-38 glucuronide lactone, 73.9 ng/ml*hr (range, 7.2–187.3 ng/ml*hr). The median irinotecan lactone clearance rate was 42.7 L/hr/m² (range, 25.9–79.2 L/hr/m²).

Discussion

Several factors warranted evaluation of the antitumor activity and safety of irinotecan and gefitinib in newly diagnosed, high-risk pediatric neuroblastoma. First, single-agent irinotecan had shown strong activity in neuroblastoma xenograft models.[30,3,31,32] Second, the orally administered EGFR inhibitor gefitinib potently inhibited neuroblastoma proliferation *in vitro* [14,15] and enhanced topotecan activity against neuroblastoma cell lines.[33] Finally, the gefitinib/irinotecan combination exerted greater than additive activity in xenograft models.[8] Although single-agent irinotecan was only modestly active against recurrent neuroblastoma,[34,35] its preclinical synergy with gefitinib merited study of the combination in newly diagnosed patients.

We established the MTD of the combination on the basis of previous studies. Daw et al described the single-agent maximum tolerated dose (MTD) of gefitinib, in children with solid tumors on a daily $\times$ 28d schedule as 400 mg/m²/day, with rash as the dose-limiting toxicity. Diarrhea was observed but was mild.[36] We defined the MTD of protracted (daily $\times$ 5 $\times$ 2) intravenous irinotecan as 20 mg/m²/day, with diarrhea as the dose-limiting toxicity. When the two agents were combined in a subsequent Phase I study in children with solid tumors, the initial dose-level of 15 mg/m²/day of irinotecan and daily gefitinib at 150 mg/m²/day for 21-d was not tolerated.[23] The MTD of the combination was established as 15 mg/m²/day of irinotecan (daily $\times$ 5 $\times$ 2) with 112.5 mg/m²/day of gefitinib (daily $\times$ 12) and was the dosage used in this Phase II study. [23]

The pharmacokinetic results of this study were consistent with those we reported previously. [23] In the present study of intravenous irinotecan with gefitinib, the median irinotecan lactone systemic clearance was 42.7 L/hr/m², which was consistent with the value (40.6 L/hr/m²) obtained in our prior study of irinotecan and gefitinib. [23] Also as noted in our previous study, SN-38 and SN-38G lactone exposure increased when irinotecan was combined with gefitinib. The AUC values for irinotecan, SN-38, and SN-38G lactone in the present study were very comparable with the median (range) values from our prior study (irinotecan 230 (121–370 ng/ml*hr, SN-38 27.7 (9.1–132 ng/ml*hr, and SN-38G 54.6 (24–309 ng/ml*hr) (unpublished data). Finally, although other investigators[37] have reported the use of irinotecan with gefitinib, our results cannot be compared with theirs because of

the large difference in dosage (200 mg/m² vs. 15 mg/m²) and in the parameters reported (lactone parameters vs. combined lactone and carboxylate parameters).

In the preclinical models, the synergy of the gefitinib/irinotecan combination was independent of EGFR expression[8] and was postulated to reflect gefitinib-mediated inhibition of ABC transporters, such as ABCG2.[10,8] Neuroblasts express a number of these transporters.[38,12,39–43] Therefore, we immunohistochemically evaluated the expression of EGFR, ABCC4, ABCG2, ABCB1, ABCC2, and ABCC3 in available tumor samples to better understand the biology of the clinical results. Expression of EGFR was low in the newly diagnosed tumors, as was expression of most members of the multidrug resistance protein (MRP) family of transporters. ABCG2, which reportedly confers resistance to the active metabolite of irinotecan,[9] was detected in 4 of 6 xenograft models. [8] However, in 13 of our primary pediatric tumor samples, ABCG2 expression was not well correlated with change in tumor volume. ABCC4 was detected in 5 of 17 baseline samples (29%). ABCC4 expression has been found to be associated with poor outcome and irinotecan resistance.[12] We observed a median tumor volume decrease of 23% in the 5 tumors that expressed ABCC4 at diagnosis versus a 55% decrease in the 8 found to be negative. Therefore, ABCC4 may confer partial resistance to irinotecan *in vivo*. Although gefitinib inhibits ABCC4, other ABC transporters, such as ABCG2, are inhibited to a greater extent (C. Stewart, unpublished observation). Thus, the low rate of response to gefitinib/irinotecan in our study may be associated with the high rate of expression of ABCC4. Interestingly, the 2 neuroblastoma xenografts[44] that responded to this combination[8] were derived from previously treated tumors, while the one that did not respond was derived from an untreated tumor.[44] Although this combination was very promising in a preclinical model, it may be less effective against newly diagnosed tumors, which appear to express gefitinib targets less frequently. Gefitinib does modulate the bioavailability of oral irinotecan in children,[23] probably by inhibiting ABCG2 in intestinal cells. An alternative explanation for our results is that gefitinib reaches adequate concentrations in the intestine, but not in the tumors, to inhibit ABCG2.

Another rationale for this study was based on reports that EGFR expression is an adverse prognostic indicator in neuroblastoma[45]; that increased EGFR expression is associated with multi-drug resistance[46]; that EGFR mediates proliferation of neuroblastoma[15]; and that gefitinib, a “selective” EGFR inhibitor, should be clinically evaluated.[15,14] Ho et al[15] documented EGFR expression in 10/13 neuroblastoma cell lines and in 18/18 primary tumors assessed, including 13 biologically unfavorable (all *MYCN* amplified, 4 stage II, 3 stage III, 4 stage IV and 2 stage IVs) tumors. They also showed gefitinib was a potent inhibitor of neuroblastoma cell proliferation. In contrast, another group tested gefitinib against six neuroblastoma cell lines and concluded that “gefitinib as monotherapy” was not warranted because its inhibitory effects were not seen at clinically achievable concentrations. Interestingly, they also noted that the inhibitory effects of gefitinib were increased by combining it with another camptothecin (topotecan), although the increase was modest.[47] Despite this exception, most of the available reports predicted EGFR expression in most newly diagnosed neuroblastomas, suggesting that EGFR inhibition might produce clinical activity. We identified EGFR expression in only 1 of 17 tumors.

Expression of EGFR in neuroblastoma is commonly assessed by RT-PCR detection of mRNA, using RNA from neuroblastoma cell lines as a template. [47,15] However, one study using this method detected surface EGFR protein in only one neuroblastoma cell line, although four expressed EGFR mRNA. [47] Thus, it is unclear whether mRNA expression *in vitro* reflects the expression of a functional protein. There have been multiple studies of tumor expression of EGFR mRNA [15] or protein, [48,45] but these reports did not distinguish newly diagnosed tumors from treated tumors. In cell culture, development of

chemotherapy resistance appears to be related to increased expression of EGFR.[49] However, we detected no change in EGFR expression after the initial six weeks of therapy.

We reasoned that the length and cost of a prospective randomized trial[50] would be justified only if the activity of the irinotecan/gefitinib combination observed in the preclinical models[8] was significantly greater than in most previous Phase II “window” trials (CR+PR rate, 17% – 54%) in neuroblastoma.[51,52] Such a finding would require a response rate of at least 55%. There is no evidence that this approach was detrimental, as the 2-year EFS estimate in our study patients was 65.2% (SE, 9.6%), similar to that in a recently published COG study.[53] Although this approach did not allow us to evaluate the relative roles of the two agents, it did preclude the necessity for a larger, longer, and costlier trial. Most patients experienced oncolytic effects in this study, but only 26% of evaluable patients achieved a PR. Therefore, a prospective, randomized clinical trial of intravenous irinotecan and gefitinib is not warranted.

Despite our results with intravenous irinotecan and oral gefitinib, the combination of oral irinotecan and gefitinib may be worth pursuing for several reasons. First, irinotecan is more efficiently converted to its active metabolite, SN-38, when given orally than when given intravenously,[54–57] probably through presystemic conversion by carboxylesterases in the GI tract. Second, gefitinib, inhibits ABCG2 as well as EGFR [58] and when given with oral irinotecan it increased irinotecan bioavailability by 4-fold compared to historical controls (median, 0.09 vs 0.42, $P < .000001$).[23] Third, although the objective response rate in this trial was disappointing, a majority of these children showed some reduction in tumor volume (Fig 1). Clinical benefit was also seen when Donfrancesco et al used a similar rationale in combining gefitinib with oral topotecan and cyclophosphamide in ten children with relapsed neuroblastoma.[59,60] It is possible that patients with less extensive neuroblastoma may derive greater therapeutic benefit from oral irinotecan/gefitinib, as observed in patients with hepatoblastoma.[61] Oral irinotecan combined with temozolomide [62] has also demonstrated activity against refractory neuroblastoma. It is possible that the addition of gefitinib to this combination would provide higher SN-38 exposure and potentially improve response.

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Fig. 1. Individual change in primary tumor volume after gefitinib/irinotecan therapy in 17 patients

Two patients who had early progressive disease (gray bars) had received only 1 course of window therapy.

Fig. 2. Individual change in urinary homovanillic acid (a, n = 15), urinary vanillylmandelic acid (b, n = 15), and serum LDH (c, n=20) after gefitinib/irinotecan therapy

Urine was randomly obtained. In panels a and b, one patient with early disease progression (gray bar) had received only one 1 course of gefitinib/irinotecan. In panel c, three patients with early disease progression (n=2) or severe toxicity (n=1) (gray bars) had received only one course of gefitinib/irinotecan.

Fig. 3. Individual change in primary tumor volume after investigational therapy, according to baseline ABC transporter expression

(N = 13; N = 12 for ABCC2). Red bars indicate immunohistochemical detection of a) ABCC4, b) ABCG2, and c) ABCC2 in primary tumors. Antibodies, staining methods, and instrumentation are summarized in Table 1.

Table 1

Antibodies and Detection Methods Used for Immunohistochemical Staining

Antibody specificity	Vendor	Clone	Positive titer	Detection kit	Antigen retrieval	Automated stainer
ABCC2 (MRP2)	Abcam	M2III-6	1:100	Envision	Citrate buffer	DAKO
ABCC3 (MRP3)	Novocastra	DTX	1:25	IVIEW	CC1-Standard	Benchmark XT (Ventana)
ABCC4 (MRP4)	Axxora	M4 1-10	1:250	LSAB-2	EDTA-TRIS pH 9.0	DAKO
ABCG2 (BCRP)	Signet	BXP-21	1:50	IVIEW	CC1-Standard	Benchmark XT (Ventana)
ABCB1 (PgP)	RDI	JSB-1	1:20	LSAB-2	EDTA-TRIS Ph 9.0	DAKO
EGFR	Zymed	31G7	1:10	IVIEW	Protease 1 (4minutes, 37°C)	Benchmark XT (Ventana)

Vendor legend: Abcam (Cambridge, MA); Novocastra (Newcastle upon Tyne, UK); Axxora (San Diego, CA); Signet (Dedham, MA); RDI Research Diagnostics (Fitzgerald Industries, Acton, MA); Zymed (San Francisco, CA); DAKO (Carpinteria, CA); Ventana (Phoenix, AZ).

Table 2

Patient Characteristics at Study Enrollment (n=23)

	n	%
Age (months)		
0 to <12	1	4.3
>18 to 24	2	8.7
>24	20	87.0
Sex		
Male	18	78.3
Female	5	21.7
Race		
White	16	69.6
Black	4	17.4
Other	3	13
INSS Stage		
Stage 3	3	13.0
Stage 4	19	82.6
Stage 4S	1	4.3
Primary Tumor Site		
Adrenal	18	78.3
Celiac axis	3	13.0
Paraspinal thorax	2	8.7
MYCN Status		
Amplified	9	39.1
Not amplified	14	60.9
Shimada Classification		
Unfavorable	17	73.9
Not available ^a	6	26.1
DNA Index		
1	7	30.4
>1	8	34.8
Not available	8	34.8

^a In 6 patients, the diagnosis was made by bone marrow examination only.

Table 3

Expression of MRP2-4, ABCG2, EGFR, and ABCB1 in baseline and post-treatment neuroblastoma samples

	Baseline Expression N=17	Post-treatment Expression N=16
MRP4		
Positive	5 (29.4%)	5 (31.2%)
Negative	12 (70.6%)	11 (68.8%)
ABCG2		
Positive	2 (11.8%)	0 (0%)
Negative	15 (88.2%)	16 (100%)
EGFR		
Positive	1 (5.9%)	1 (6.2%)
Negative	16 (94.1%)	15 (93.8%)
ABCB1		
Positive	0 (0%)	1 (6.2%)
Negative	14 (82.4%)	15 (93.8%)
Not done	3 (17.6%)	0 (0%)
MRP2		
Positive	3 (17.6%)	0 (0%)
Negative	12 (70.6%)	16 (100%)
Not done	2 (11.8%)	0 (0%)
MRP3		
Positive	4 (23.5%)	9 (56.2%)
Negative	11 (64.7%)	7 (43.8%)
Not done	2 (11.8%)	0 (0%)

Table 4
Percent tumor volume change after the initial six-weeks of therapy according to expression of EGFR and ABC transporters

	ABCC4	ABCG2	EGFR	ABCB1	ABCC2	ABCC3
Pre-treatment expression						
	+	-	+	-	+	-
N	5	8	0	12	10	11
Mean	-20%	-44%	-	-36%	-40%	-36%
Median	-23%	-55%	-	-42%	-42%	-53%
Minimum	-63%	-78%	-	-78%	-78%	-78%
Maximum	11%	24%	-	24%	11%	24%
Post-treatment expression						
	+	-	+	-	+	-
N	4	9	1	12	13	4
Mean	-38%	-23%	-31%	-28%	-28%	-19%
Median	-43%	-31%	-31%	-35%	-31%	-12%
Minimum	-78%	-75%	-31%	-78%	-78%	-63%
Maximum	11%	41%	-31%	41%	41%	11%