

**PHASE I STUDY OF THE COMBINATION OF VANDETANIB
AND DASATINIB ADMINISTERED DURING AND AFTER
RADIATION THERAPY IN CHILDREN WITH NEWLY
DIAGNOSED DIFFUSE INTRINSIC PONTINE GLIOMA**

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EXECUTIVE SUMMARY

Diffuse intrinsic pontine glioma (DIPG) is the most lethal brain tumor in children. Despite treatment with radiation therapy (RT) with or without chemotherapy, the long-term survival of affected children has remained < 10%. Radical surgery is not feasible for children with DIPG. Although RT is the mainstay of therapy, the response to treatment is generally temporary. Since chemotherapy has not been beneficial in the treatment of children with DIPG, we have been conducting clinical trials with new medications at our institution since 2001.

We have been conducting a Phase I study combining RT and vandetanib, an oral vascular endothelial growth factor receptor 2 (VEGFR-2) and epidermal growth factor receptor (EGFR) inhibitor, in the treatment of children with newly diagnosed DIPG since June 2007. Twenty-nine patients have been enrolled on this study at five dosage levels of vandetanib. Twenty-one of 29 patients were enrolled to determine the maximum tolerated dose (MTD) of vandetanib and to characterize its toxicities, particularly dose-limiting toxicities (DLTs). The remaining patients have been treated as an expansion cohort to further characterize the toxicities to therapy. The MTD of vandetanib was not reached since only one of 6 patients treated at each of the two highest dosage levels experienced DLT. Ten patients have been treated at dosage level 5; we observed further toxicities at this dosage level which was considered too toxic for further testing. We continue to treat patients at the second highest dosage level (110mg/m²). Overall, 16 patients experienced progressive disease. Four patients remain alive > 1 year from start of therapy without tumor progression (21, 21, 13, and 12 months from diagnosis).

Since we have seen some promising responses to this therapy and vandetanib seems to be well tolerated up to doses of 110mg/m² per day, we designed a new Phase I clinical trial combining local RT, vandetanib, and dasatinib. Dasatinib is an oral inhibitor of multiple tyrosine kinases (bcr-abl, src family [src, lck, yes, fyn], PDGFRA and B, c-kit, and EPHA2-5). Preliminary results of molecular analysis of tumor samples obtained from patients with DIPG demonstrated that amplification of *PDGFRA* is the most common oncogene amplification in DIPG (NBTP02 protocol). Several pre-clinical studies have also shown the importance of inhibition of PDGFRA and B, src, and c-kit in glioblastoma models. Therefore, we feel that dasatinib may inhibit key targets associated with the formation and growth of DIPG.

This is the first time that a potent VEGFR-2 inhibitor will be combined with a multi-tyrosine kinase inhibitor in the treatment of children with DIPG. The proposed clinical trial will follow a variation of the Phase I study design. Extensive correlative studies, including pharmacokinetics (PK) and pharmacodynamic analysis will be conducted in this new clinical trial. As described before, we demonstrated the ability, expertise, and commitment within our institution to perform this type of study.

DESCRIPTION OF RESEARCH PROPOSAL

1.0 OBJECTIVES

1.1 Primary Objective

- 1.1.1 To estimate the MTD of the combination of RT, vandetanib, and dasatinib in children with newly diagnosed DIPG

1.2 Secondary Objectives

- 1.2.1 To determine the chronic toxicities associated with this treatment
- 1.2.2 To characterize the PK of vandetanib and dasatinib in children
- 1.2.3 To evaluate the influence of pharmacogenomics on the PK of vandetanib and dasatinib
- 1.2.4 To evaluate the correlation between sequential levels of plasma growth factors (VEGF, bFGF, SDF-1, and PDGF) and response to therapy
- 1.2.5 To evaluate inhibition of PDGFRA and PDGFRB and downstream pathways in peripheral blood mononuclear cells
- 1.2.6 To describe the patient's and parents' perspective of the quality of life of children with newly diagnosed DIPG enrolled on this phase I trial
- 1.2.7 To describe the quality of life of parents of children with DIPG enrolled on this phase I trial

2.0 BACKGROUND AND RATIONALE

2.1 Background

Despite clinical trials spanning three decades, the outcome of children with DIPG remains dismal (long-term survival < 10%).¹ Local RT constitutes standard treatment, but unfortunately tumor progression usually occurs within 5 to 10 months from start of therapy. Fifty to 75% of patients with DIPG die within a year of diagnosis.¹ The use of conventional chemotherapy has not demonstrated any survival benefit.¹ Since tumor tissue is hardly ever available for research

purposes, little is known about the biology of DIPG.² Therefore, this lack of knowledge precludes the development of rational therapeutic approaches that target specific molecules or aberrant signaling pathways.

2.2 **Rationale**

2.2.1 Rationale for Use of Vandetanib

EGFR overexpression is common in DIPG.² *EGFR* amplification occurs in a subset of tumors, particularly in World Health Organization (WHO) grade III and IV neoplasms.² Therefore, there is a biological rationale for targeting EGFR in the treatment of children with DIPG.

Most children with DIPG harbor high-grade gliomas at diagnosis or at autopsy.³ High-grade gliomas are one of the most vascularized cancers in humans. Angiogenesis is a hallmark of these neoplasms and its relevance to tumorigenesis is well recognized.⁴ The vascular endothelial growth factor (VEGF) is one of the most important angiogenic factors in high-grade gliomas. Extensive experimental and clinical evidence suggests that VEGF is a major contributor to the biology of these tumors.⁵ Since VEGFR-2 is the main VEGF receptor in high-grade gliomas, its inhibition is another promising target in the treatment of children with DIPG.

Vandetanib (ZD6474; Zactima; AstraZeneca) is a small-molecule that potently inhibits VEGFR-2. It also has modest inhibitory activity against EGFR, RET, and VEGFR-3. Two recent reports described the activity of vandetanib against cell lines, *in vivo* rat glioma models, and xenografts derived from patients with high-grade gliomas.^{6,7} Rich et al. tested the activity of vandetanib against the D54MG human glioma cell line and against four xenografts derived from high-grade gliomas, including two obtained from children.⁷ Vandetanib showed a concentration-dependent inhibition of proliferation and colony formation of D54MG cells.⁷ In these cells, vandetanib caused significant reduction in phosphorylated EGFR (pEGFR), but higher concentrations were required to inhibit downstream effectors (pERK and pAKT). Vandetanib also caused decreased secretion of EGF and VEGF by tumor cells, and decreased tumor cell motility and invasive capacity. Furthermore, vandetanib administered at a dose of

200mg/kg per day for 10 days displayed activity against all heterotopic (flank) and orthotopic (intracerebral) xenografts, including those resistant to EGFR inhibition and tumors harboring *PTEN* mutations.

We have been conducting a Phase I study (SJBG07) with oral vandetanib during and after RT in children with newly diagnosed DIPG. Twenty-nine patients have already been enrolled on study at the 50 ($n=3$), 65 ($n=3$), 85 ($n=3$), 110 ($n=10$), and 145mg/m² ($n=10$) dosage levels. Only two patients had DLTs which consisted of grade 3 skin rash and mucositis ($n=1$) and diarrhea ($n=1$) at dosage level 4 and 5, respectively. The first child was shown to have an active cytomegalovirus infection, which could account for the DLT. Of 4 additional children enrolled at the highest dosage level (145mg/m²) after the determination of MTD had been completed, one experienced grade 4 posterior reversible encephalopathy syndrome (PRES) with grade 4 seizures and another patient had grade 3 hypertension. One of the first 6 patients enrolled on dosage level 5 experienced grade 3 diarrhea, grade 2 QTc interval prolongation, and grade 2 proteinuria beyond the DLT-evaluation period, which required dose reduction. Other significant toxicities attributable to vandetanib consisted of grade 3/4 lymphopenia, grade 3 leucopenia ($n=1$), grade 3 hypophosphatemia ($n=1$), grade 3 neutropenia ($n=1$), grade 2 proteinuria ($n=2$), grade 2 hypertension ($n=4$) and mild QTc prolongation ($n=7$). Thirteen of 16 patients who experienced disease progression are deceased. All six patients who underwent autopsy at the time of death harbored glioblastoma (WHO grade IV). Four patients remain alive without tumor progression (21, 21, 13, and 12 months from diagnosis). We are currently expanding the cohort of patients treated at dosage level 4 (110mg/m²), which is probably the dose of vandetanib that will be recommended for Phase II studies in children.

2.2.2 Rationale for the Use of Dasatinib

We have been conducting a prospective study (NBTP02) to collect and analyze tissue samples from children with DIPG. Preliminary results showed *PDGFRA* to be amplified in a subset of tumor samples. All four platelet-derived growth factor (PDGF) ligands (PDGFA-D) and their two receptors (PDGFRA and PDGFRB) are important contributors to gliomagenesis.⁸ PDGF and PDGFR are overexpressed in cell lines and

glioma surgical samples and their increased expression is associated with higher tumor grade.^{9,10} The PDGF pathway seems to influence tumor formation by stimulating mitogenesis, de-differentiation, and increased angiogenesis via multiple cellular downstream pathways (MAPK, PI3K, src).⁸ PDGFB has also been identified as an essential factor regulating pericytes. Pericytes contribute to the stability of capillary walls and also regulate the function of endothelial cells.¹¹ This is particularly relevant to high-grade gliomas because autocrine and paracrine interactions between tumor and stromal cells are important mechanisms of tumor spread.¹²

Molecular changes in PDGF ligands and receptors in high-grade gliomas consist of gene amplification,¹³ mutation,¹⁴ and RNA/protein overexpression.¹² Less information is available about similar changes in tumor samples derived from children.^{15,16,17} *PDGFRA* amplification has been documented in a few cases of children with glioblastoma outside the brainstem.¹⁵ Liang et al. analyzed by immunohistochemistry 42 pediatric supratentorial high-grade astrocytomas (16 glioblastoma and 26 anaplastic astrocytoma).¹⁶ The authors documented *PDGFRA* and *PDGFRB* overexpression in 18 of 40 (45%) and 12 of 39 (31%) tumors, respectively. Thorarinsdottir et al. analyzed by immunohistochemistry 85 tumors (22 high- and 63 low-grade gliomas) in patients < 21 years of age.¹⁷ The authors had also previously analyzed RNA expression in 13 of these tumors. Two-thirds of these tumors originated in the supratentorial area. High expression of phosphorylated *PDGFRA* and *PDGFRB* were documented in 85.7% and 78.9% of high-grade gliomas, and 40% and 41.7% of low-grade gliomas, respectively. High expression of both *PDGFRA* and *PDGFRB* was associated with malignant histology ($P=0.005$ and 0.031 , respectively). The RNA expression analysis of 13 cases revealed that upregulation of *PDGFA* had a borderline association with high-grade gliomas ($P=0.056$). Similar to high-grade gliomas originating elsewhere in the central nervous system (CNS), the tumorigenesis and growth advantage of DIPGs are likely dependent on multiple signaling pathways.

Dasatinib (Sprycel, BMS-354825; Bristol-Myers Squibb) is an oral inhibitor of multiple tyrosine kinases (bcr-abl, src family [src, lck, yes, fyn], *PDGFRA* and B, c-kit, and *EPHA2-5*).^{18,19} Data from biochemical

assays showing the *in vitro* selectivity of dasatinib to some of these receptors is shown in Table 1.

Table1. *In vitro* Selectivity of Dasatinib

kinase	enzyme IC ₅₀ , nM	kinase	enzyme IC ₅₀ , nM
Bcr-Abl	<1.0	MEK	1700
src	0.50	VEGFR-2	>2000
lck	0.40	CDK2	>5000
yes	0.50	IKK	>10000
c-kit	5.0	AKT	>50000
PDGFR β	28	FAK	>50000
p38	100	IGF-1R	>50000
Her1	180	IR	>50000
Her2	710	MK2	>50000
FGFR-1	880	PKC $\alpha, \delta, \tau, \zeta$	>50000

Another study demonstrated that the IC₅₀ of dasatinib against PDGFRB and src in cell-based assays were 3nM and 2nM, respectively.²⁰ Imatinib is another oral small-molecule inhibitor against PDGFR. The IC₅₀ of imatinib against PDGFR was 67-fold higher than of dasatinib.²⁰

Dasatinib is approved in the United States for the treatment of chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia. Some reports suggest that dasatinib may have a better activity against leukemic involvement of the CNS compared to imatinib.²¹ Limited data about the penetration of dasatinib into the CNS is available for humans. The better activity of dasatinib against leukemic involvement of the CNS compared to imatinib seems to be due to its increased potency (and not to significantly better penetration in the CNS).

C-kit and the src family of proto-oncogenes are also important in the formation of high-grade gliomas.^{22, 23} C-kit and its ligand (stem cell factor) are important in the pathologic angiogenesis of high-grade gliomas.²³ Src has been implicated in the invasiveness of high-grade glioma.²² Du et al. recently confirmed that src activation occurs in about 2/3 of glioblastomas in adults.²⁴ These authors tested the effects of dasatinib against glioma cell lines and orthotopic U87 xenograft models. Dasatinib produced decreased proliferation, apoptosis, and inhibited motility in glioma cell lines. It also

produced statistically significant inhibition of growth of xenografts compared to vehicle. Furthermore, the authors showed that the activity of dasatinib was due to src inhibition. A Phase I study of dasatinib in children with recurrent or refractory solid tumors showed that the MTD of dasatinib was 85mg/m² twice a day.

2.2.3 Rationale for a Pediatric Study Combining RT, Vandetanib, and Dasatinib in Children with DIPG

EGFR and VEGF play an important role in the radioresistance of high-grade gliomas.^{26, 27} Damiano et al. evaluated the combination of RT and vandetanib in a high-grade glioma model.²⁸ The authors tested the activity of RT, vandetanib, and the combination of the two against two glioblastoma-derived cell lines and subcutaneous xenografts (D54 and U251). Whereas vandetanib or RT caused a dose-dependent inhibition of growth of the cell lines, the treatment combination demonstrated a synergistic effect. Vandetanib (50, 75, or 100mg/kg per day, 5 days a week, for 3 weeks) and/or RT (2Gy per dose daily, twice a week for 2 weeks, 2 hours following vandetanib) were used to treat animals harboring xenografts. A cooperative and long-lasting effect was observed with combination treatment compared to each treatment alone.

Pre-clinical studies have described synergistic responses to VEGFR-2 and PDGFR inhibition in high-grade gliomas.²⁹ Timke et al. analyzed the effects of RT, PDGFR, and VEGFR-2 inhibition isolated or in different combinations against endothelial and U87 cell lines, and subcutaneous xenografts.³⁰ A consistent synergistic effect was seen when RT was combined with PDGFR and VEGFR-2 inhibitors based on endothelial cell viability, clonogenic survival, function, and apoptosis compared to either RT alone or the combination of the two agents.⁶⁵ RT induced the production of PDGF, particularly PDGF-B and PDGF-C, in endothelial cells; the authors hypothesized that inhibition of this induction may account for some of the benefits of combination therapy. Combination of RT, PDGFR and VEGFR-2 inhibitors also caused significantly increased inhibition of U87 cell lines and xenografts compared to RT or drug combination only. Combination of RT, PDGFR, and VEGFR-2 inhibitors caused significant reduction in vessel count and mitotic activity compared to other treatments in U87 xenografts.

Since all medications used to date in the treatment of children with DIPG did not show benefit, we have provided compelling data for adding dasatinib to the backbone of therapy used in our previous clinical trial (SJBG07).

2.2.4 Rationale for Correlative Studies

2.2.4.1 Pharmacokinetic Studies

We plan to conduct extensive PK of vandetanib and dasatinib to provide an understanding of the inter- and intra-patient variability of the disposition of both drugs in children. This PK may also be useful in evaluating toxicity to treatment, biologic activity of study agents, disease response, and for refining dose parameters in future pediatric studies.

We plan to start dasatinib therapy on day 1 of therapy along with RT. Vandetanib therapy will start 1 week later. Since we anticipate a pharmacokinetic interaction between vandetanib and dasatinib, the pharmacokinetic studies will be mandatory. We plan to perform PK of dasatinib without (after first dose) and with vandetanib (after dose on day 8) so that we can better understand the interaction between the two study drugs. By comparing trough steady state vandetanib plasma concentrations (after 6 weeks of continuous therapy) in the current study to historical controls (SJBG07), in which a similar population received vandetanib only, we will also be able to evaluate how dasatinib co-administration may impact the PK of vandetanib.

2.2.4.2 Pharmacogenetic Studies

Pharmacogenetic studies will be performed in all consenting patients enrolled on this clinical trial to evaluate polymorphisms in enzymes critical in the metabolism and disposal of vandetanib and dasatinib [e.g., CYP3A5, CYP3A4, P-glycoprotein (P-gp) and BCRP/ABCG2].

2.2.4.3 Collection of Quality of Life Data

Investigation of quality of life (QOL) among children with the diagnosis of brain tumors has emerged as an important health-related outcome.³¹ However, to date very few studies have been published that describe the QOL of children with brain tumors.^{31, 32} A recent report indicated that the QOL of children with CNS tumors was significantly worse than in healthy children.³³ Another study which evaluated the QOL of parents of children with cancer has demonstrated that these parents have poorer QOL compared with population norms as well.³⁴

2.2.4.4 Other Correlative Studies

We will also perform sequential pharmacodynamic studies to evaluate the effects of study treatment on plasma growth factors, and the effects of dasatinib on PDGFRA and PDGFRB, and downstream pathways in peripheral mononuclear cells.

3.0 TREATMENT PLAN AND ELIGIBILITY CRITERIA

This Phase I clinical trial is designed to estimate the MTD and to determine the DLT(s) of the combination of vandetanib and dasatinib administered concurrently with local RT. Dasatinib (administered twice a day) and RT should start on the same day. The start of vandetanib (administered once a day) will be delayed by one week from the start of RT and dasatinib to assess the PK of dasatinib without and with concurrent administration of vandetanib. Conformal local RT will be administered as 1.8-Gy fractions over 30 days for a total dose of 54Gy. Treatment with oral vandetanib and dasatinib will extend for the entire duration of RT, and then will be continued after completion of RT for a maximum duration of 2 years (counting from the start of RT and dasatinib). Compliance will be monitored by follow-up of drug diaries which will be completed by the patient's families every 28 days.

This study will follow a variation of the traditional Phase I study design. We plan to treat at least 6 evaluable patients in all proposed dosage levels, but only 3 patients will be enrolled on study at each time. At the starting dosage level (level 1), patients will receive vandetanib and dasatinib at a dosage of 65mg/m² per dose. We plan to escalate vandetanib to a maximum dose of 110mg/m² per day and

dasatinib to a maximum dose of 85mg/m² twice a day. Dosage levels 0 and -1 are provided in case we encounter significant toxicity. Ten additional patients will be treated at the MTD (or highest dosage level if the MTD is not reached). We expect that 18 to 28 evaluable patients will be needed to complete this study. A maximum of 34 patients will be enrolled on this trial. We expect that the duration of the current clinical trial will be between one and a half and two and a half years.

Inclusion and exclusion criteria for this study will be standard for this type of clinical trial

4.0 IMPORTANCE OF THE PROPOSED STUDY

We believe that we have provided compelling rationale for the combination of RT, vandetanib, and dasatinib in the treatment of children with DIPG.

The design of this protocol also includes correlative studies which will enhance our understanding of the effects of therapy and its impact in the quality of life of patients and their parents..

Our institutional track record demonstrated the feasibility of this study and our commitment to developing promising and innovative therapies for children with this devastating cancer.

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BUDGET

This clinical trial is sponsored by St. Jude Children's Research Hospital and will receive partial support by AstraZeneca. AstraZeneca will also provide vandetanib that will be used by all research participants. Dasatinib will be obtained from commercially available sources for all research participants.

We plan to use any funds obtained from the current grant to buy dasatinib for the research participants.

The median age of children with DIPG is between 6 and 8 years of age. We expect that the majority of research participants in the proposed clinical trial will receive doses of dasatinib between 50 and 70mg.

The monthly supply of 50- and 70-mg tablets of dasatinib (60 tablets) costs \$5898.29.

Our request of \$50,000 will help support the acquisition of dasatinib for the participants in this study.

COLLABORATIONS AND CONFLICT OF INTEREST

The proposed study will be conducted only at St. Jude Children's Research Hospital.

Disclosure of Conflict of Interest:

1. I am part of the Medical Advisory Council of The Cure Starts Now Foundation.
2. This study will be partially supported by AstraZeneca, which will also provide one of the study drugs (vandetanib).