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Initial Testing of VNP40101M (Cloretazine®) by the Pediatric Preclinical Testing Program

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Abstract

VNP40101M is a novel alkylating agent that yields two reactive compounds (a chloroethylating species and methylisocyanate) and has demonstrated activity against a wide spectrum of tumor xenografts. VNP40101M was tested against an *in vivo* panel of 5 pediatric brain tumor xenografts at a dose of 18 mg/kg/day administered for 5 days. O-6-methylguanine-DNA methyltransferase (MGMT) levels of xenografts were assessed by western blot analysis. Only one xenograft (GBM2), which lacked detectable MGMT expression, demonstrated an objective response to VNP40101M. VNP40101M antitumor activity was observed only in the absence of MGMT expression, with resistance to VNP40101M seen even with low MGMT expression.

Keywords

Preclinical Testing; Developmental Therapeutics; VNP40101M

INTRODUCTION

Alkylating agents are an important class of chemotherapeutic drugs that have been used to treat a wide array of solid malignancies including CNS tumors [1]. When used as a single agent or in combination therapy, alkylating agents provide an effective treatment against brain tumors. 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(methylamino)carbonyl]hydrazine (VNP40101M, Cloretazine®), is a novel bi-functional alkylating agent that spontaneously generates nucleophilic species that can efficiently alkylate DNA resulting in DNA cross-linking and cell death [2]. Unlike other chloroethylating agents, VNP40101M does not generate vinylating or hydroxyethylating species that can produce organ toxicity [2]. In addition, it has carbamoylating activity due to generation of methylisocyanate that has been shown *in vitro* to inhibit the DNA repair enzyme, O6-methylguanine-DNA methyltransferase (MGMT, also known as AGT or O6-alkylguanine alkyl transferase) [3]. Phase I and II clinical trials with VNP40101M have been conducted in adults with advanced leukemia and solid tumors using either a single-dose every six weeks or a fractionated (weekly for 4 weeks every 6 weeks) schedule [4-8]. Promising results have been observed with this drug in patients with untreated AML,

especially in combination with cytarabine [7]. Also, a recent phase I study of VNP40101M in children with recurrent malignant brain tumors demonstrated objective responses or stable disease in 10% of evaluable patients [9]. Based on the clinical studies and emerging pre-clinical data, the Pediatric Pre-clinical Testing Program (PPTP) of the National Cancer Institute (NCI) decided to explore the efficacy of this drug in a panel of pediatric brain tumor xenografts and correlate anti-tumor effects with MGMT expression.

MATERIALS AND METHODS

In vivo tumor growth inhibition studies

CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted non-glioblastoma brain and glioma tumors [10,11]. Mice bearing subcutaneous tumors, each received drug when tumors reached between 200 mm³ and 500 mm³. Mice were randomized to groups of 10 animals. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee. Tumor volumes (cm³) [solid tumor xenografts] and responses using three activity measures were determined as previously described [12]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

VNP40101M was provided to the Pediatric Preclinical Testing Program by Vion Pharmaceuticals, through the Cancer Therapy Evaluation Program (NCI). VNP40101M was dissolved in DMSO / sterile water (10%/90%) and administered i.p., once daily for 5 days at a dose of 18 mg/kg.

MGMT Western Blot

Western blot analyses were performed as previously described with minor modifications [13]. Primary antibodies to MGMT (Labvision) and β -Actin (SantaCruz Biotechnology) were used. Optical densitometry of immunoreactive bands was achieved following scanning of films using a CanoScan LiDE 35 scanner coupled to a Macintosh G5 computer using NIH Image J Software. Signal Intensity (optical density) was calculated by converting gray scale values by comparison with a calibrated photographic gray scale card (Kodak, Rochester, NY).

RESULTS

Activity of VNP40101M against the PPTP in vivo panel

Toxicity testing identified 18 mg/kg daily for 5 consecutive days as the maximum tolerated dose for VNP40101M; this dosing regimen was subsequently evaluated in 5 xenograft models with no deaths in control or treated arms. A complete summary of results is provided in Supplemental Table I, including total numbers of mice, numbers of mice with events and average times to event, tumor growth delay, numbers of responses, and T/C values.

VNP40101M induced significant differences in event-free survival (EFS) distribution for treated animals compared to controls in 1 of 5 evaluable xenografts tested (GBM2) (Supplemental Table I). Results with GBM2 also met the criteria for high activity as defined by the PPTP tumor volume T/C activity measure, with a tumor volume T/C value of 0.10 at day 14. The remaining 4 xenografts had low activity by the tumor volume activity measure, with T/C values ranging from 0.58 to 0.89. GBM2 (Figure 1) was the only xenograft that achieved an objective response (CR), with the remaining xenografts showing progressive disease. The *in vivo* testing results for the objective response activity measure are presented in Supplemental Figure 1 in a 'heat-map' format as well as a 'COMPARE'-like format, based on the scoring criteria described in the Material and Methods and the Supplemental Response Definitions section. The latter analysis demonstrates relative tumor sensitivities around the midpoint score of 5 (stable disease).

MGMT levels

MGMT levels were determined by western blot for the xenografts evaluated. Protein levels were undetectable in GBM2 and extremely low in BT-39 (Figure 2). MGMT was detectable in the BT-44, BT-45 and BT-50 cells with the highest expression levels seen in BT-44.

DISCUSSION

Our pre-clinical testing of VNP40101M in pediatric brain tumor xenografts indicated that its activity was restricted to the one malignant glioma xenograft that had undetectable MGMT expression. In another pre-clinical study of VNP40101M in a broad panel of CNS xenografts (subcutaneous and intracranial), Badruddoja et al., reported growth delays in malignant glioma (D-245 and D-54) and medulloblastoma (D-425) xenografts that had either low or undetectable tumor MGMT activity [14]. In addition, Badruddoja et al., observed an increase in tumor growth delay following treatment with VNP40101M in MGMT over-expressing malignant glioma xenografts when the MGMT pseudo-substrate, O6-benzylguanine, was administered beforehand. Their report provides evidence that MGMT expression might be the predominant mechanism of resistance of CNS tumors to this drug. By contrast, our observation that BT-39, a malignant glioma xenograft with very low MGMT expression, did not respond to VNP40101M suggests that non-MGMT mechanisms (e.g., ERCC2 expression) may be important in conferring resistance to VNP40101M [15,16]. In addition, although methylisocyanate produced from VNP40101M breakdown inhibits free MGMT protein *in vitro*, its efficiency *in vivo* might be restricted due to low concentrations and MGMT enzyme binding to DNA in tumor cells, thereby masking the enzyme from methylisocyanate inhibition [3].

In a recent phase II trial of VNP40101M given as a single dose every six weeks in adults with recurrent malignant glioma, there were no objective responses or prolongation of survival [4]. However, in a recent pediatric phase I trial of the drug given in a fractionated schedule (i.v. daily for 5 days every 6 weeks) in children with recurrent malignant brain tumors, 4 of 40 evaluable patients demonstrated partial response (one patient each with medulloblastoma and brain stem glioma) or stable disease (one patient each with low-grade glioma, and malignant glioma,) [9]. Such variability in objective responses to the drug might be related to differences in tumor MGMT levels or to differences in the schedule of drug administration. Since MGMT over-expression is the only characterized mechanism of resistance to VNP40101M, ongoing preclinical and clinical studies will evaluate whether prior treatment with other alkylating agents that deplete MGMT (e.g., temozolomide) might improve VNP40101M antitumor activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Reference List

1. Dropcho EJ. Novel chemotherapeutic approaches to brain tumors. *Hematol Oncol Clin North Am.* 2001; 15(6):1027–1052. [PubMed: 11770297]
2. Finch RA, Shyam K, Penketh PG, et al. 1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-(methylamino)carbonylhydrazine (101M): a novel sulfonylhydrazine prodrug with broad-spectrum antineoplastic activity. *Cancer Res.* 2001; 61(7):3033–3038. [PubMed: 11306484]
3. Baumann RP, Shyam K, Penketh PG, et al. 1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(methylamino)carbonyl]hydrazine (VNP40101M): II. Role of O6-alkylguanine-DNA alkyltransferase in cytotoxicity. *Cancer Chemother Pharmacol.* 2004; 53(4):288–295. [PubMed: 14685775]
4. Badrudoja MA, Penne K, Desjardins A, et al. Phase II study of Cloretazine for the treatment of adults with recurrent glioblastoma multiforme. *Neuro Oncol.* 2007; 9(1):70–74. [PubMed: 17108065]
5. Giles F, Thomas D, Garcia-Manero G, et al. A Phase I and pharmacokinetic study of VNP40101M, a novel sulfonylhydrazine alkylating agent, in patients with refractory leukemia. *Clin Cancer Res.* 2004; 10(9):2908–2917. [PubMed: 15131024]
6. Giles F, Verstovsek S, Faderl S, et al. A phase II study of cloretazine (VNP40101M), a novel sulfonylhydrazine alkylating agent, in patients with very high risk relapsed acute myeloid leukemia. *Leuk Res.* 2006; 30(12):1591–1595. [PubMed: 16574225]
7. Giles F, Verstovsek S, Thomas D, et al. Phase I study of cloretazine (VNP40101M), a novel sulfonylhydrazine alkylating agent, combined with cytarabine in patients with refractory leukemia. *Clin Cancer Res.* 2005; 11(21):7817–7824. [PubMed: 16278404]
8. Murren J, Modiano M, Kummur S, et al. A phase I and pharmacokinetic study of VNP40101M, a new alkylating agent, in patients with advanced or metastatic cancer. *Invest New Drugs.* 2005; 23(2):123–135. [PubMed: 15744588]
9. Gururangan S, Turner C, Stewart CF, et al. Phase I trial of VNP40101M in children with recurrent brain tumors--A Pediatric Brain Tumor Consortium (PBTCT) study. *Proc Am Soc Clin Oncol.* 2007; 25(18s):89. (asbtr# 2059).
10. Graham C, Tucker C, Creech J, et al. Evaluation of the antitumor efficacy, pharmacokinetics, and pharmacodynamics of the histone deacetylase inhibitor depsipeptide in childhood cancer models in vivo. *Clin Cancer Res.* 2006; 12(1):223–234. [PubMed: 16397046]
11. Peterson JK, Tucker C, Favours E, et al. In vivo evaluation of ixabepilone (BMS247550), a novel epothilone B derivative, against pediatric cancer models. *Clin Cancer Res.* 2005; 11(19 Pt 1): 6950–6958. [PubMed: 16203787]
12. Houghton PJ, Morton CL, Tucker C, et al. The pediatric preclinical testing program: description of models and early testing results. *Pediatr Blood Cancer.* 2007; 49(7):928–940. [PubMed: 17066459]
13. Kurmasheva RT, Harwood FC, Houghton PJ. Differential regulation of vascular endothelial growth factor by Akt and mammalian target of rapamycin inhibitors in cell lines derived from childhood solid tumors. *Mol Cancer Ther.* 2007; 6(5):1620–1628. [PubMed: 17483438]

14. Badruddoja MA, Keir ST, King I, et al. Activity of VNP40101M (Cloretazine) in the treatment of CNS tumor xenografts in athymic mice. *Neuro Oncol.* 2007; 9(3):240–244. [PubMed: 17522334]
15. Chen ZP, Malapetsa A, McQuillan A, et al. Evidence for nucleotide excision repair as a modifying factor of O6-methylguanine-DNA methyltransferase-mediated innate chloroethylnitrosourea resistance in human tumor cell lines. *Mol Pharmacol.* 1997; 52(5):815–820. [PubMed: 9351972]
16. Chen H, Shao C, Shi H, et al. Single nucleotide polymorphisms and expression of ERCC1 and ERCC2 vis-a-vis chemotherapy drug cytotoxicity in human glioma. *J Neurooncol.* 2007; 82(3): 257–262. [PubMed: 17151930]

Figure 1. VNP40101M activity against GBM2 solid tumor xenografts. Kaplan-Meier curves for EFS, median relative tumor volume graphs, and individual tumor volume graphs are shown.

Figure 2.
MGMT levels for xenografts as determined by western blot, upper panel, and scan densitometry quantitation of MGMT levels, lower panel.