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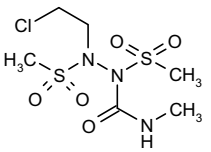
# VNP-40101M

*DNA Alkylating Agent*
*Oncolytic*

101M
Cloretazine™

1-(2-Chloroethyl)-4-methyl-1,2-bis(methylsulfonyl)semicarbazide



$C_6H_{14}ClN_3O_5S_2$
Mol wt: 307.7773

CAS: 173424-77-6

EN: 247706

**Abstract**

Carmustine (BCNU) and lomustine (CCNU) are members of the (chloroethyl)nitrosourea (CNU) class of agents that exhibit DNA alkylating and cross-linking activity and have been used clinically for years as treatments for lymphoma and many other tumor types. Upon decomposition, they generate reactive species that exert activities (*e.g.*, chloroethylation, carbamoylation, hydroxyethylation and vinylation) that can be therapeutically beneficial, unimportant or detrimental. Due to the serious toxicity associated with these agents, the search for new DNA alkylating agents with improved toxicity profiles for the treatment of cancer continues, and a novel series of bifunctional prodrugs known as 1,2-bis(sulfonyl)hydrazines (SHPs) has emerged. VNP-40101M (101M, Cloretazine™) is one such SHP prodrug that was designed to retain both the chloroethylating and carbamoylating properties but not the hydroxyethylating and vinylating effects. VNP-40101M exhibited broad-spectrum preclinical antitumor activity that was more potent than BCNU and clinical efficacy in advanced myeloid and other malignancies. It is undergoing phase III development for the treatment of acute myeloid leukemia (AML) and earlier clinical trials for a range of other cancers.

## Synthesis

VNP-40101M can be synthesized as follows:

The reaction of (2-hydroxyethyl)hydrazine (I) with MsCl in pyridine gives the trimesylated compound (II), which is treated with LiCl in refluxing acetone to yield 1-(2-chloroethyl)-1,2-bis(methanesulfonyl)hydrazine (III) (1). Finally, this compound is condensed with methyl isocyanate (IV) by means of TEA in acetonitrile to afford the target semicarbazide (2, 3). Scheme 1.

## Background

Many effective chemotherapeutic agents target tumor cell DNA, and DNA alkylating agents represent a large class currently being used in the clinic. The primary anticancer efficacy of these agents is due to the cross-linking of DNA. Two members of the (chloroethyl)nitrosourea (CNU) class of agents which exhibit DNA alkylating and cross-linking activity, carmustine (BCNU) and lomustine (CCNU), have been used clinically for many years and exhibit efficacy against lymphoma and many tumor types, including brain tumors. However, they are associated with marked toxicities due to the generation of reactive species. The generated molecules possess chloroethylating, carbamoylating, hydroxethylating and/or vinylating properties. Although actions such as chloroethylation and isocyanate generation via carbamoylation are beneficial and can potentiate the cytotoxic activity of an agent by preventing the repair of DNA alkylation and cross-links, other generated molecules which have, for example, hydroxyethylating activity, can cause carcinogenic and mutagenic events, as well as other toxicities seriously detrimental to the patient (4-13).

Researchers have therefore continued to search for new classes of DNA alkylating agents with improved toxicity profiles for the treatment of cancer. A novel series of bifunctional prodrugs known as 1,2-bis(sulfonyl)hydrazines (SHPs) emerged which, following activation, produce reactive electrophilic species exerting both DNA alkylating and cross-linking activity. These novel agents were shown to generate more of the beneficial chloroethylating species that are similar to those produced by CNUs and less of the therapeutically unimportant or detrimental species. VNP-40101M (101M, Cloretazine™) is one such SHP that was

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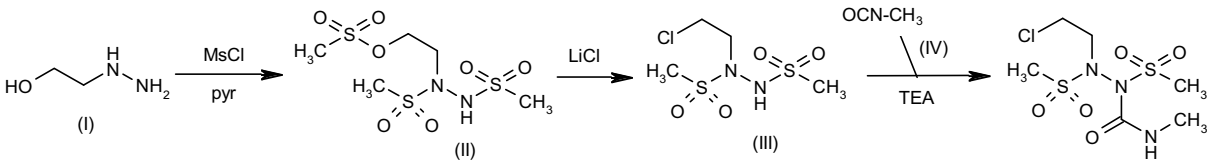
**Scheme 1: Synthesis of VNP-40101M**




designed to retain both chloroethylating and carbamoylating properties but not the hydroxyethylating and vinylating effects of existing DNA alkylating agents. The prodrug decomposes under basic conditions, forming a chloroethylating species that alkylates DNA at the $O^6$ position on guanine residues, which induces cross-linking, and a carbamoylating molecule, methyl isocyanate. It has a half-life of approximately 1 h, allowing for optimal distribution. The agent exhibited broad-spectrum preclinical antitumor activity and was generally more potent and safer than BCNU. VNP-40101M was chosen for further development as an anticancer agent (2, 14-21).

## Preclinical Pharmacology

Studies examining the effects of the electrophilic species generated by the decomposition of VNP-40101M revealed that they directly inhibited $O^6$-alkylguanine-DNA alkyltransferase (AGT). Experiments using CHO cells transfected with the human AGT gene showed that a greater degree of AGT overproduction is associated with higher resistance to VNP-40101M cytotoxicity and that the methyl isocyanate and chloroethylating species generated from VNP-40101M act synergistically to kill cells (15, 16).

Further examination of the action of VNP-40101M was performed using three murine hematopoietic $AGT^-$ leukemia cell lines sensitive to the chloroethylating species 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (L1210, P388, F-MEL; $IC_{50}$ = 6-8 μM) and $AGT^+$ bone marrow (Ba/F3) and leukemia (WEHI-3B) cells resistant ($IC_{50}$ = 50-70 μM) to this species. It has been speculated that during tumor progression, the *AGT* gene is silenced due to promoter hypermethylation. AGT expression was found to correlate with the functional status of p53, such that $AGT^-$ cells carried defective p53 and $AGT^+$ cells carried wild-type p53. VNP-40101M was selectively toxic against $AGT^-$ cells, exhibiting decreased toxicity against $AGT^+$ cells. Experiments using wild-type and *AGT*-transfected L1210 cells revealed that VNP-40101M was more selective for $O^6$-chloroethylation of guanine than BCNU. Overall, it appears that the cytotoxicity of VNP-40101M in $AGT^-$ cells is mainly due to the chloroethylating agent, while both carbamoylating and chloroethylating actions account for the compound's activity in $AGT^+$ cells (17).

The potent antineoplastic activity of VNP-40101M was determined to be due to the synergistic activity of the carbamoylating and alkylating reactive intermediates of the prodrug. Experiments were performed using CHO and human promyelocytic leukemia HL-60 cells and analogue prodrugs which exclusively generate only one of the reactive intermediates of the prodrug: 90CE, which generates the chloroethylating reactive species, or 101MDCE, which generates only the carbamoylating species. Results showed that the apoptosis observed following VNP-40101M treatment was the result of DNA cross-links produced mainly by the chloroethylating species. Furthermore, this cross-linking activity was enhanced by co-generated methyl isocyanate. Additional studies using L1210 leukemia cells and the VNP-40101M prodrug analogues 90CE and 101MDCE indicated that 90CE displayed differential cytotoxic activity against wild-type and *AGT*-transfected cells ($LC_{10}$ = 1.4 μM *vs.* 31 μM), suggesting that a major mechanism of cytotoxic action was alkylation at the $O^6$ position of guanine. Thus, the tumor-selective action of VNP-40101M may be due to the differential expression of AGT observed in tumor and host cells. 90CE alone, but not 101MDCE, induced DNA cross-links. However, more cross-links were observed with the prodrug VNP-40101M. Both 90CE and 101MDCE (5 and 80 μM, respectively) caused comparable levels of G2-M arrest and phosphorylated histone H2AX, although with differential kinetics. However, while 90CE selectively inhibited DNA synthesis after 24 h of incubation, 101MDCE rapidly and nonselectively inhibited RNA, DNA and protein syntheses (18, 19).

Inhibition of cellular glutathione reductase has been reported to be involved in the pulmonary toxicity associated with BCNU and its generated 2-chloroethyl isocyanate reactive species. VNP-40101M was shown to potently inhibit purified human glutathione reductase in a manner similar to BCNU ($IC_{50}$ = 55.5 and 54.6 μM, respectively). However, while treatment of human erythrocytes with BCNU (50 μM) resulted in a decrease in cellular glutathione reductase activity of 84%, VNP-40101M inhibited activity of the enzyme by < 1%; differential inhibition of glutathione reductase activity was also observed in L1210 murine leukemia cells. Thus, in contrast to BCNU, methyl isocyanate generated during VNP-40101M decomposition acts synergistically with the co-generated chloroethylating species but does not significantly inhibit cellular glutathione reductase activity, and therefore VNP-40101M would have a reduced propensity to cause pulmonary toxicity (20).

VNP-40101M (5, 10 or 15 mg/kg i.p. once daily for 6 days starting 24 h postimplantation) exhibited marked cytotoxic activity *in vivo* against murine L1210 leukemia cells implanted in mice. Cure rates of 40% with no body weight loss and of 100% with body weight loss of < 6% were obtained with doses of 5 mg/kg and both 10 and 15 mg/kg i.p. once daily for 6 days, respectively. VNP-40101M was also active against murine B16F10 melanoma in mice (2).

The antitumor activity of VNP-40101M against L1210 leukemia was further analyzed *in vivo*, showing long-term survivor (*i.e.*, alive and tumor-free at 60 days postinoculation) rates of 60%, 100%, 100%, 100% and 80%, respectively, with single i.p. doses of 10, 20, 40, 60 and 80 mg/kg, compared to respective values for BCNU of 0%, 0%, 80% and 0%; single oral doses of VNP-40101M (60, 80, 100, 200 and 300 mg/kg) were also effective, giving long-term survivor rates of 100%, 100%, 100%, 80% and 60%, respectively. The agent was found to have a better safety profile compared to BCNU, and cure rates were obtained with little loss of body weight and no bone marrow depression. Single-dose VNP-40101M (80 mg/kg i.p.) was shown to cross the blood-brain barrier, producing a > 6.54 log kill against intracranially implanted leukemia cells in the brain. Moreover, VNP-40101M was effective against L1210 resistant to cyclophosphamide, BCNU or melphalan. Two injections of VNP-40101M (10, 20 or 40 mg/kg i.p.) resulted in antitumor efficacy against murine C26 colon carcinoma in 100% of the treated mice, and a single injection (40, 60 or 80 mg/kg i.p.) also resulted in 100% long-term survival in mice with P388 leukemia. Different regimens of the agent (10 mg/kg i.p. every 2 days x 11 or 20 mg/kg i.p. every 4 days x 6 starting 21 days after implantation of human glioma U251; 10 mg/kg i.p. every 2 days x 10 and 20 mg/kg i.p. every 4 days x 5 starting 9 days after implantation of murine lung carcinoma M109) caused complete regression of established U251 xenografts and produced significant cures in advanced murine M109 lung carcinoma (21).

The antitumor efficacy of VNP-40101M alone and in combination with fludarabine or cytarabine was evaluated in tumor-bearing mice, and it was shown to decrease tumor burden and enhance survival rates in C57BL/6 mice implanted s.c. with murine melanoma B16F10 and CD1 mice bearing human lung NCI-H460 and colon WiDr carcinoma xenografts. The agent as monotherapy (10-150 mg/kg) exerted dose-dependent antitumor activity (42.2-87%) comparable to cyclophosphamide, except that it was associated with a higher survival rate. The agent alone and in combination with fludarabine or cytarabine significantly increased survival rates in CDF1 or BDF1 mice implanted i.p. with murine leukemia L1210 cells. Survival rates were enhanced with both combination therapies as compared to monotherapy. For example, treatment with VNP-40101M (10 mg/kg) plus 5 doses of fludarabine (70 mg/kg every other day) resulted in a 90% survival rate at day 65 postimplantation compared to 40% and 0%, respectively, for VNP-40101M and fludarabine alone. Similarly, treatment with VNP-40101M (10 mg/kg) plus cytarabine (50 mg/kg) produced a survival rate of 100% at day 68 postimplantation compared to 90% and 0%, respectively, for VNP-40101M and cytarabine alone (22).

## Pharmacokinetics and Metabolism

An LC-ESI-MS/MS method for the quantitation of VNP-40101M and its 90CE metabolite in human plasma was reported. The method resulted in a mean recovery rate of over 90% for both VNP-40101M and 90CE and was validated over a concentration range of 1-1000 ng/ml and 0.5-100 ng/ml, respectively. High within-day and between-day accuracy rates were obtained (101.3-105.7% for VNP-40101M and 98.7-105.6% for 90CE). The development of a manufacturable, stable, parenteral formulation of VNP-40101M for use in phase I trials was also described (23, 24).

The pharmacokinetics and tissue distribution following a single 10 mg/kg i.v. dose of [$^{14}$C]-VNP-40101M were examined in rats. Pharmacokinetic parameters were similar in both males and females. The labeled agent was eliminated rapidly, with a half-life of about 20 min. Average $C_{max}$, volume of distribution at steady-state ($V_{ss}$) and total body clearance values were 11.3 μg/ml, 0.91 l/kg and 33.5 ml/min/kg, respectively. Recovery of total radioactivity after 7 days was 85% and 79% for males and females, respectively, with 70% eliminated in urine and only 6% in feces within 48 h postdosing. Relatively high levels of radioactivity were detected in kidneys, liver, lung and spleen, and also in brain, indicating penetration of the blood-brain barrier. Elimination of radioactivity from tissues was prolonged, since 9% of the dose was still detected at 7 days postdosing. VNP-40101M was extensively metabolized (25).

A phase I study in 26 patients with advanced or metastatic cancer examined the pharmacokinetics and safety of VNP-40101M as a short i.v. infusion (3 mg/m$^2$ every 4 weeks, escalated to 6, 12, 24, 40, 60, 80, 100, 125, 155, 195, 245 and 305 mg/m$^2$). A total of 50 courses were administered and pharmacokinetic parameters were determined from 23 patients who received 45 courses. Linear, dose-proportional $C_{max}$ (0.26-32.37 μM) and AUC values were obtained and the elimination half-life was short (10-25 min). The pharmacokinetics of the metabolite 90CE were determined in 14 patients. Peak plasma levels of 90CE ranged from 0.23 to 0.82 μM (26).

## Safety

The toxicity of VNP-40101M as an i.v. bolus once daily for 5 consecutive days was evaluated in rats (1, 3, 10 and 20 mg/kg) and dogs (0.3, 1 and 3 mg/kg) followed for up to 45 and 50 days, respectively. Pulmonary toxicity, including increased lung weight, pink or red fluid in the thoracic cavity, alveolar edema, vascular congestion, alveolar histiocytosis and vascular thrombi, and death were seen in rats administered doses of 10 and 20 mg/kg; these effects were also seen in about 7-30% of the rats

receiving a dose of 3 mg/kg. The low-effect level (LOEL) in rats was concluded to be 1 mg/kg, with no significant toxicity seen; the maximum tolerated dose (MTD) was concluded to be 3 mg/kg, and doses of 10 mg/kg and above were not tolerated. Comparable results were obtained in dogs, although red iliac lesions, slight body weight loss, occasional anorexia and transient reductions in white blood cell counts were also noted in animals receiving 0.3 mg/kg. The LOEL, MTD and toxic doses for dogs were determined to be 0.3, 1 and 3 mg/kg or greater, respectively (27).

The MTD of VNP-40101M in the above-mentioned phase I study was concluded to be 305 mg/m$^2$ every 6 weeks and was recommended for phase II trials. At this dose level, 6 of 8 patients experienced grade 3 thrombocytopenia, of which 1 case was considered to be a dose-limiting toxicity (DLT). Other dose-related toxicities reported were moderate granulocytopenia, anemia and mild infusion-related syndrome (*i.e.*, acute headache and facial flushing). The granulocyte nadir was reached at a median of 34 days. Recovery of thrombocytopenia and neutropenia occurred at a median of 43 days. No objective responses were seen (26).

The safety and MTD of VNP-40101M (80, 100, 125 and 155 mg/m$^2$/week for 3 weeks as a short i.v. infusion) were determined in another phase I trial in 27 patients with metastatic cancer. The MTD was concluded to be 100-125 mg/m$^2$ weekly for 3 weeks. The majority of patients tolerated 2 or more cycles on an every 4 week schedule. No grade 2-4 nonhematological toxicities were reported. In the 18 patients evaluable for safety, 1 case each of grade 3 neutrophil and grade 3 platelet reductions was observed at the 100 and 125 mg/m$^2$ dose levels. Three grade 3 and 1 grade 4 reductions in platelets, 2 grade 4 neutrophil reductions and 2 grade 3 decreases in hemoglobin were reported at the highest dose. Platelet and neutrophil nadirs were observed at a median of 33 and 52 days, respectively. One patient receiving 5 cycles on a weekly x 3 every 4 weeks schedule developed mild, cumulative, reversible thrombocytopenia/neutropenia. Examination of patient peripheral blood mononuclear cells (PBMCs) revealed that AGT was not depleted at any of the doses tested. Evidence of antitumor activity was noted (28).

## Clinical Studies

A phase I trial in 38 patients with refractory or relapsed leukemia (including 28 with acute myeloid leukemia [AML]) and myelodysplastic syndromes (MDS; n=5) determined the safety, pharmacokinetics and preliminary activity of VNP-40101M (starting dose of 220 mg/m$^2$ by i.v. infusion over 15-70 min on day 1 every 4 weeks, escalated by about 33%). A total of 52 courses were administered. The most common adverse event was non-dose-limiting, reversible, infusion-related events reported in 24 patients during the first course. A dose of 600 mg/m$^2$ was concluded to be the recommended dose for phase II studies since dose escalation was stopped at 708 mg/m$^2$ due to prolonged myelosuppression in 1/7 patients; no extramedullary adverse events were observed at the 600 mg/m$^2$ dose level. Complete remissions (CRs) were obtained in 1 patient with MDS and another with AML at the 300 and 600 mg/m$^2$ dose levels, respectively (29).

The safety and efficacy of VNP-40101M (200, 300, 400 and 500 mg/m$^2$ i.v. on day 2 over 15-60 min, escalated in 100 mg/m$^2$ increments) in combination with fixed-dose cytarabine (1.5 g/m$^2$/day by continuous i.v. infusion starting on day 1 for 3 and 4 days in patients 65 years or older and younger than 65 years at the time of diagnosis, respectively) were examined in a phase I trial conducted in 40 patients with relapsed/refractory leukemias, including AML (n=32), acute lymphocytic leukemia (ALL; n=6), MDS (n=1) and blast-phase chronic myeloid leukemia (CML; n=1). A total of 47 courses were administered. DLTs of gastrointestinal events and myelosuppression were seen at VNP-40101M doses of 500 and 600 mg/m$^2$ combined with 4-day cytarabine; these toxicities were not seen on the 3-day schedule. Of 37 evaluable patients, complete responses were obtained in 10 at VNP-40101M dose levels of 400 mg/m$^2$ or greater. The 10 patients who responded to treatment also exhibited significantly lower AGT activity as compared to patients who did not respond. The recommended doses for phase II studies for this combination regimen were concluded to be 600 mg/m$^2$ VNP-40101M and 1.5 g/m$^2$/day cytarabine by continuous infusion for 3 days (30).

A multicenter phase II trial in 53 patients with relapsed AML who had an initial CR of < 12 months concluded that VNP-40101M (600 mg/m$^2$ as a single i.v. infusion) had very little activity in this population. Of the 44 patients with evaluable baseline karyotype analysis, 41 were determined to have intermediate- or high-risk cytogenetics. The median duration of the first CR in this group was 6 months (0.75-11 months), with 32 of the patients having an initial CR duration of 6 months or less. A total of 5 patients died within 30 days of initiation of VNP-40101M therapy. Of the 53 patients who were evaluable for response, only 1 patient achieved a second CR (duration of 20+ months) and another patient had clearance of leukemic blasts from the marrow with adequate myeloid and erythroid recovery but with incomplete platelet recovery (CRp) after the first course of therapy. Thus, from this patient population, 50% of whom had an initial CR of less than 6 months, only 4% achieved a second CR. Mean overall survival was 2.3 months, which was similar to rates obtained for 233 matched patients treated with other monotherapies (31).

The efficacy and safety of VNP-40101M (600 mg/m$^2$ as a single 30-60-min i.v. infusion, with a second induction for patients with a partial response or hematological improvement and a 400 mg/m$^2$ consolidation course for patients achieving CR or CRp) were examined in a multicenter phase II study in 105 elderly patients (median age = 72 years) with newly diagnosed AML or high-risk MDS. Treatment was well tolerated, with a low incidence of severe drug-related nonhematological toxicities. A total

of 19 patients died within 30 days of initiation of treatment. However, 28 and 5 patients achieved CR and CRp, respectively, for an overall response rate of 31%. The response rates in the patients with newly diagnosed AML (n=45), secondary AML (n=45) and high-risk MDS (n=15) were 49%, 11% and 40%, respectively. Response rates in 57 patients with intermediate-risk disease and 41 patients with an unfavorable prognosis according to baseline cytogenetics were 42% and 22%, respectively. The 1-year overall survival rates for the total population, patients achieving CR and patients with *de novo* AML and achieving CR (median survival = 5 months) were 12%, 28% and 32%, respectively (32-35).

The efficacy and tolerability of VNP-40101M (300 mg/m$^2$ i.v. every 6 weeks) were examined in a phase II trial in 38 patients with recurrent malignant glioma, recurrent anaplastic astrocytoma or anaplastic oligodendroglioma. Treatment was generally well tolerated. Grade 4 thrombocytopenia developed in 4 patients and grade 3 neutropenia was reported in 3 patients. Of the 36 evaluable patients, no objective radiographic responses were seen. Fifteen patients had a best response of stable disease. It was concluded that this dosing schedule had very little activity in this patient population (36).

VNP-40101M is in clinical development for the treatment of various cancers. Phase I or I/II studies are under way examining the efficacy and safety of VNP-40101M as monotherapy in young patients with recurrent, progressive or refractory primary brain tumors, in patients with Richter's syndrome, relapsed CLL or other lymphoproliferative disorders, and in combination with temozolomide in patients with relapsed or refractory leukemias (37-39). Preliminary results from the latter study demonstrated 3 CR and 1 CRp and manageable toxicity (40). Phase II trials are being conducted evaluating VNP-404101M as monotherapy for the treatment of AML or high-risk myelodysplasia, in elderly patients with poor-risk AML, in patients with relapsed or refractory, locally advanced or metastatic small cell lung cancer, and in combination with cytarabine in older patients with AML (41-44). A phase III study is recruiting patients with relapsed AML to compare the efficacy of VNP-40101M plus cytarabine with cytarabine alone. According to data from 110 patients who have completed the treatment course with cytarabine 1.5 g/m$^2$ on days 1-3 plus either VNP-40101M 600 mg/m$^2$ or placebo on day 2, early deaths have occurred in only 12% of patients, and the most common serious adverse event has been infection (50%) (45, 46).

## Source

Vion Pharmaceuticals, Inc. (US).

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