Drugs of the Future 2011, 36(9): 663-667

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.9.1678337

MONOGRAPH

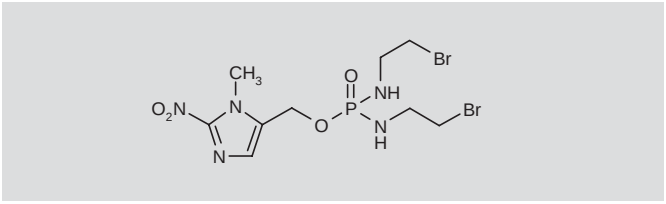
TH-302

DNA Alkylating Agent
Hypoxia-Activated Cytotoxic Prodrug
Oncolytic

HAP-302

N,*N*'-Bis(2-bromoethyl)phosphorodiamidic acid (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl ester

InChI: 1S/C9H16Br2N5O4P/c1-15-8(6-12-9(15)16(17)18)7-20-21(19,13-4-2-10)14-5-3-11/h6H,2-5,7H2,1H3,(H2,13,14,19)



$C_9H_{16}Br_2N_5O_4P$
Mol wt: 449.036
CAS: 918633-87-1
CAS: 918632-75-4 (labeled)
EN: 442503

SUMMARY

Since cancer cells do not produce blood vessels, tumors usually contain regions of hypoxia with relatively low metabolic activity and regions with relatively normal oxygenation where cancer cells grow much more rapidly. Most current cytotoxic anticancer drugs inhibit the growth of highly metabolic cancer cells, while not affecting the hypoxic cancer cells, which remain to grow more aggressively following chemotherapy. TH-302 is a phosphoramidate-based prodrug which becomes activated by electron reduction involving NADPH cytochrome P450 or other reductases present in hypoxic tumor tissue. Preclinical studies have demonstrated the unique hypoxic specificity and therapeutic efficacy of TH-302 in a variety of common human cancer cells. The results of phase I and II clinical trials indicate that this compound has clinical promise in terms of pharmacokinetics, safety and effectiveness for the treatment of various solid tumors in human cancer patients.

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*Synthesis prepared by S. ShankharaRaman, C. Estivill, R. Castañer. Thomson Reuters, Provença 398, 08025 Barcelona, Spain.

SYNTHESIS*

TH-302 is prepared by reaction of 2-bromoethylammonium bromide (I) with $POCl_3$ in the presence of Et_3N in CH_2Cl_2 to yield isophosphoramide mustard (II) (1), which then undergoes Mitsunobu reaction by means of PPh_3 and DIAD in THF (1, 2). Scheme 1.

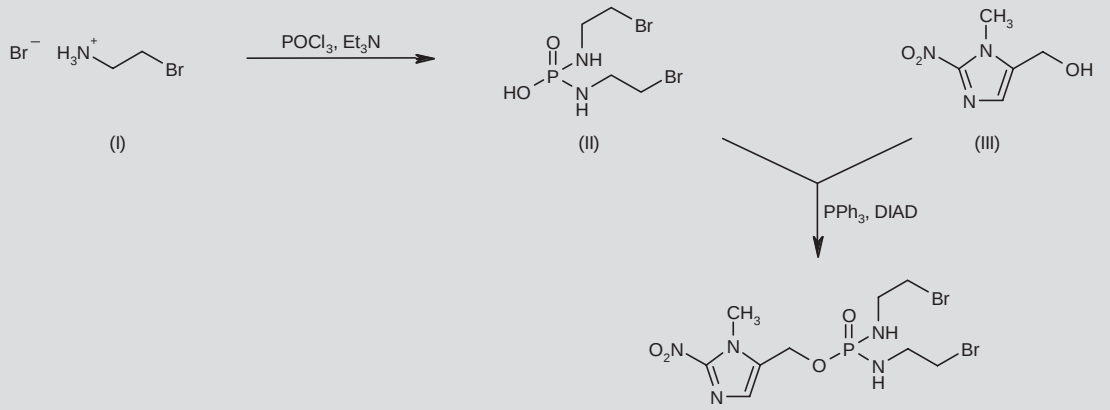
INTRODUCTION

Since the tumor or cancer cell mass cannot produce endothelial cells necessary for the formation of lymphatic and blood vessels, when the cell mass becomes large enough that oxygen and nutrients do not diffuse adequately into the tumor tissue (usually when the cell mass is 1-2 mm in diameter), the cancer cells secrete several factors that stimulate endothelial cells in the surrounding normal tissue to produce new blood vessels, which grow into the tumor mass to deliver oxygen and nutrients and to carry away waste products, thus permitting the tumor mass to rapidly grow much larger (3). Therefore, angiogenesis or neovascularization is essential for tumor growth and metastatic progression. However, the angiogenesis which occurs in tumor tissue is usually disorganized and inefficient when compared to the vascularization of normal tissue, resulting in interspersed areas of hypoxia and normoxia within the tumor microenvironment (4-6).

There are a number of biochemicals produced by tumor cells and surrounding stromal cells that induce vascular growth, enhance the formation, migration and stability of endothelial cells and vascular formation referred to as vascular sprouting (7). The biochemicals known to be involved in tumor angiogenesis include vascular endothelial growth factor (VEGF), including VEGF-A to VEGF-E (8, 9), heparin-binding growth factor 1 (acidic fibroblast growth factor, aFGF) and heparin-binding growth factor 2 (basic fibroblast growth factor, bFGF) (10-12), platelet endothelial cell adhesion molecule (PECAM) (13, 14), thrombospondin-2 (15-17), various integrins (18-21), neuropilin-1 (22-25) and epidermal growth factor-like protein 7 (EGF-like protein 7, *EGFL7*) (26-28).

Bevacizumab is a humanized monoclonal antibody (MAb) that selectively binds to VEGF and inhibits tumor angiogenesis. Bevacizumab was approved in the U.S. in 2004 for the treatment of

Scheme 1. Synthesis of TH-302



advanced or metastatic colon cancer. Later it was approved for non-small cell lung cancer, glioblastoma, metastatic renal cell cancer and metastatic breast cancer (29). Continued use in breast cancer is currently undergoing NIH review.

Tumor cells in areas of vascularization grow very rapidly and are most sensitive to conventional chemotherapy. However, the hypoxic regions within the tumor are known to be resistant to standard chemotherapy and radiation (2). Thus, hypoxia represents a critical therapeutic limitation and an opportunity for the development of drugs which are selectively activated by hypoxia; thus, these compounds hold the potential to eliminate dormant cancer cells within the hypoxic tumor microenvironment (30-34). It has been proposed that the use of a hypoxia-activated drug in combination with treatments such as radiation or standard cytotoxic chemotherapy, which are most effective within normoxic tumor regions, would produce a more complete therapeutic response, reduce relapse and enhance patient survival (35).

Since the density of hypoxia in the tumor microenvironment is known to be associated with resistance to current radiation and chemotherapy (36-38), the concept of targeting hypoxic tumor cells has developed during the past 10 to 15 years (30). The first hypoxia-activated DNA alkylating mustard compounds were reported by Borch et al. in 2000 (39, 40). Other hypoxia-activated compounds that are used in the treatment of cancer or undergoing clinical testing include tirapazamine (41), mitomycin C (42) and banoxantrone (43).

TH-302 was specifically designed to enhance the cytotoxicity, selectivity and safety of hypoxia-activated alkylating agents. Duan et al. synthesized TH-302 and examined the therapeutic efficacy of this compound and related derivatives on several cancer cell lines both in vitro and in vivo (2). The results of this study demonstrated that TH-302 has a high degree of cytotoxic potency and hypoxic selectivity when compared with other hypoxia-selective isophosphoramide mustard anticancer prodrugs. This compound significantly reduced the survival of human colon adenocarcinoma HT-29 and non-small cell lung carcinoma NCI-H460 cells in a clonogenic cytotoxic assay under selective hypoxic conditions.

PRECLINICAL PHARMACOLOGY

The anticancer activity of TH-302 in vivo was examined in a mouse orthotopic xenograft model of human pancreatic carcinoma MIA PaCa-2 (2). Three days after implantation of the cancer cells, the mice were treated with i.p. injections of either gemcitabine (200 mg/kg once a week for 3 weeks) or TH-302 (30 mg/kg 5 days a week for 15 days for 11 total doses) or a combination of gemcitabine and TH-302 (given 2 hours before gemcitabine on the days that both drugs were scheduled), or vehicle alone. The doses of TH-302 and gemcitabine were approximately one-third the maximum tolerated dose (MTD) of these compounds as single agents. Gemcitabine treatment alone was observed to produce an 82% decrease in primary tumor growth and TH-302 alone produced a 41% decrease in tumor growth, while the combination produced 96% inhibition. Furthermore, the therapeutic combination enhanced survival of the animals and did not produce gross cytotoxicity.

Another study examined the therapeutic efficacy and safety of TH-302 in combination with bortezomib for the treatment of multiple myeloma in a murine xenograft model (44). Preliminary results indicated synergistic cytotoxicity related to changes in Bcl-2 family members and provided a basis for clinical trials in myeloma patients.

Other preclinical studies have demonstrated a broad range of therapeutic efficacy for TH-302 either as a single agent or in combination with standard chemotherapeutic drugs in various murine xenograft models (2, 45-47). Tumor responsiveness to TH-302 monotherapy suggests that, following hypoxia-induced activation, this compound may diffuse away from hypoxic regions and destroy cancer cells within the normoxic regions of the tumor, referred to as the "bystander effect" (2, 35, 46). Furthermore, animal toxicological studies with TH-302 established the no-observable adverse effect level (NOAEL) at 12.5 mg/kg in rats and 8 mg/kg in dogs. These results formed the basis for the allometric scaled starting dose of 7.5 mg/m^2 used in the initial phase I human trials (35).

CLINICAL STUDIES

Weiss et al. completed a phase I dose-escalation study to determine the dose-limiting toxicity (DLT), MTD, safety, pharmacokinetics and

preliminary therapeutic oncolytic activity of TH-302 (35). The study was conducted in 57 patients with advanced solid tumors in whom standard anticancer therapy had failed. In this two-arm study, TH-302 was administered i.v. over 30-60 minutes. In arm A patients received doses that were escalated from 7.5 to 670 mg/m^2 (3 times weekly followed by 1 week off) and in arm B they received doses of 670-940 mg/m^2 (every 3 weeks). Adverse side effects most often observed were nausea, skin rash, fatigue, vomiting and mild hematological toxic events. In arm A the DLT was found to be 670 mg/m^2 and the MTD was reported to be 575 mg/m^2. In arm B the DLT was found to be 940 mg/m^2 and the MTD was reported to be 670 mg/m^2. Of the 57 patients enrolled in this study, partial responses were observed in 2 patients, with stable disease in 27 patients. The results of this study demonstrated a wide range of therapeutic effectiveness and served as the basis for other clinical trials.

In a separate phase I clinical trial, Ganjoo et al. conducted a study to determine the DLT, MTD, safety, pharmacokinetics and therapeutic efficacy of TH-302 when used in combination with doxorubicin in 16 patients with high-grade advanced soft tissue sarcoma (STS) (48). This study employed Response Evaluation Criteria in Solid Tumors (RECIST) criteria methodology for response assessment. In this study, TH-302 was administered i.v. on days 1 and 8 at a starting dose of 240 mg/m^2 and using a classic 3 + 3 dose escalation. Doxorubicin was administered at 2 hours following TH-302 at a dose of 75 mg/m^2 on the first day of every 3-week cycle. In several patients, prophylactic growth factor support was added due to grade 4 neutropenia. The MTD for TH-302 was determined to be 300 mg/m^2. The DLTs were neutropenia-associated infection and thrombocytopenia at a dose of 340 mg/m^2. Common adverse side effects included fatigue, nausea and skin rash. A partial response was observed in 5 patients. TH-302 appeared to enhance the hematological toxicity of doxorubicin, which could be alleviated with prophylactic growth factor support.

In a follow-up phase II trial, Cranmer et al. (49) examined the efficacy and safety of the combination of TH-302 and doxorubicin in patients with advanced or metastatic STS. The results of this study demonstrated that treatment with TH-302 at a dose of 300 mg/m^2 in combination with full-dose doxorubicin (75 mg/m^2) was associated with an acceptable safety profile, which included hematological, skin and mucosal adverse effects. In addition, the therapeutic response and progression-free survival were observed to be better than with single-agent doxorubicin. Based on these results, a trial was initiated to evaluate the efficacy of the combination as compared to doxorubicin alone, the current standard treatment for patients with STS.

In a phase IB study, Borad et al. examined the combination of TH-302 with gemcitabine, docetaxel or pemetrexed to determine the efficacy, MTD and DLT of these therapeutic combinations (50). The MTDs were determined to be 340 mg/m^2 for TH-302 plus gemcitabine, 340 mg/m^2 for TH-302 plus docetaxel and 480 mg/m^2 for TH-302 plus pemetrexed. Hemorrhagic toxicities were dose-limiting, while skin and mucosal side effects were common but manageable. The results indicate that TH-302 may be used to enhance or to complement standard cancer chemotherapy.

Borad et al. are conducting a multicenter, crossover phase II trial with TH-302 in combination with gemcitabine in 165 first-line pancreatic cancer patients (51). Three groups of patients are being treated with either TH-302 240 mg/m^2 plus gemcitabine, TH-302 340 mg/m^2 plus gemcitabine or gemcitabine alone. Patients who successfully complete six cycles without treatment-induced toxicity or disease progression may continue therapy. The results of this study will be analyzed for response rate, survival, event-free survival and safety, with the completion of a minimum of 144 events. Preliminary results indicate that TH-302 can be used safely with a full therapeutic dose of gemcitabine and that greater response rates are observed with the combination than with gemcitabine alone.

CONCLUSION

TH-302 is a cytotoxic prodrug that is selectively activated in hypoxic regions of the tumor microenvironment. When used in combination with radiation and/or standard cytotoxic chemotherapy, the therapeutic combination appears to effectively inhibit the growth, invasiveness and progression of a number of clinically important cancers. Initial clinical trials indicate that this agent is well tolerated and may be effective in combination chemotherapy for a broad range of human cancers.

SOURCE

Threshold Pharmaceuticals, Inc. (US).

DISCLOSURES

The author states no conflicts of interest.

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