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Title: A Phase 1 Dose-Finding Study of the Novel Toll-like Receptor 8 Agonist VTX-2337 in Adult Subjects with Advanced Solid Tumors or Lymphoma

Authors:

Donald W. Northfelt*, MD¹, Ramesh K. Ramanathan*, MD²,³, Peter A. Cohen, MD¹, Daniel D. Von Hoff, MD¹,²,³, Glen J. Weiss MD, MBA²,⁴, Gregory N. Dietsch, PhD⁴, Kristi L. Manjarrez⁴, Tressa D. Randall⁴, and Robert M. Hershberg, MD, PhD⁴

Affiliations:

1. Mayo Clinic, Scottsdale, Arizona
2. Virginia G. Piper Cancer Center, Scottsdale, Arizona
3. The Translational Genomics Research Institute (TGen), Scottsdale, Arizona

* These authors contributed equally to the work.

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**Corresponding Author:**

Robert Hershberg, MD, PhD
VentiRx Pharmaceuticals, Inc.
1191 Second Avenue
Suite 1105
Seattle, WA 98101
Email: rhershberg@ventirx.com

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Donald W. Northfelt: No conflicts of interest to declare

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Kristi L. Manjarrez: Employee of VentiRx Pharmaceuticals Inc.

Tressa D. Randall: Employee of VentiRx Pharmaceuticals Inc.

Robert M. Hershberg: Employee of VentiRx Pharmaceuticals Inc.
TRANSLATIONAL RELEVANCE

Targeted modulation of immune responses is becoming an increasingly important tool in the armamentarium of novel strategies to treat cancer. Toll-like receptors recognize the conserved antigenic structures of infectious agents, and stimulate innate immune responses which can activate anti-tumor immunity. We report herein the first-in-man study of a novel small molecule (VTX-2337) that stimulates Toll-like receptor 8 (TLR8). The data from this study in patients with advanced cancer demonstrate dose-dependent pharmacology and predictable, transient adverse events associated with systemic immune activation. Pro-inflammatory cytokines and chemokines were induced by VTX-2337, provide a reliable set of biomarkers for TLR8 activation, and identified biologically active doses suitable for further evaluation. Based on these data, clinical development of VTX-2337 is being advanced in combination with various anti-cancer agents in multiple solid tumor indications. VTX-2337 highlights the possibility of modulating innate immune responses as a means to induce productive immunity in cancer patients.
ABSTRACT

Purpose: This Phase 1, open-label, uncontrolled, ascending-dose study explored the safety, maximum tolerated dose (MTD), pharmacokinetics, and pharmacology of the TLR8 agonist VTX-2337 in subjects with advanced solid tumors or lymphoma.

Experimental Design: VTX-2337 doses (0.1–3.9 mg/m²) were administered subcutaneously on Days 1, 8, and 15 of each 28-day cycle. Safety/tolerability assessments included adverse events (AEs); physical, ophthalmologic, and laboratory evaluations; and electrocardiograms. Dose-limiting toxicities (DLTs) were evaluated during the first cycle. Pharmacokinetics were evaluated after the first dose. Plasma samples were quantitatively assessed for chemokines, cytokines, and other inflammatory mediators. Anti-tumor activity was assessed.

Results: Thirty-three subjects were enrolled in 8 cohorts and received an average of 2 treatment cycles (range: 1–8 cycles). Most AEs were grade 1–2; the most common drug-related AEs were injection site reactions, chills, pyrexia, and influenza-like illness. One DLT was reported: grade 3 hypotension (3.9 mg/m²). The MTD was considered the highest dose administered. Peak drug plasma levels and total systemic exposure were generally dose-proportional. At doses ≥ 0.4 mg/m², increases above baseline levels were observed for plasma levels of G-CSF, MCP-1, MIP-1β, and TNFα. Eight subjects (24.2%) had a best response of stable disease (median duration: 54.5 days).
Conclusions: VTX-2337 is clinically well tolerated and biologically active with a predictable pharmacokinetic profile. Suitable doses for testing in combination studies were identified. Phase II placebo-controlled studies of VTX-2337 in combination with doxorubicin in ovarian cancer, and in combination with platinum chemotherapy, 5FU, and cetuximab in head and neck cancer have been initiated (NCT #01666444, NCT#01836029).
INTRODUCTION

Modulation of the host immune response is becoming an increasingly important option in the treatment of a variety of cancers. It is progressively clear that tumor antigen-specific T cell responses, while important, are only one component of anti-tumor immune responses. Ideally, an integrated, pro-inflammatory immune response—likely mediated through cells such as myeloid-derived dendritic cells (mDC) and monocytes capable of secreting inflammatory mediators such as interleukin (IL)-12, IL-1, and tumor necrosis factor-alpha (TNFα)—would link innate and adaptive pathways to regulate the priming and amplification of T cell, natural killer (NK) cell, and other cellular responses that may be relevant to the generation of anti-tumor immunity. To this end, we suggest that appropriate stimulation of the innate immune response can provide an important addition to both conventional anti-cancer therapies and some of the newer immunotherapeutic agents such as checkpoint inhibitors.

Activation of the innate immune system is rapid in nature, and tends to involve acute, inflammatory responses to microbial or endogenous ligands. Initiation of the innate immune response in humans and other higher mammalian species is regulated in part by Toll-like Receptors (TLRs). The effects of activating the innate response include alterations in normal leukocyte circulation through blood and tissues, release of soluble mediators, up-regulation of antigen processing and presentation to helper (CD4) and cytolytic (CD8) T-cells, and increases in NK cell function. In the context of an existing cancer, these effects should act in concert to increase the innate and adaptive immune responses to tumor cells, and TLR agonists may be potential candidates for the treatment of various malignancies in humans (1–5).
VTX-2337 is a novel, selective, small molecule agonist for TLR8 that mimics the natural ligand of viral ssRNA (6). TLR8 is interesting amongst the family of TLRs in that murine and rat cells respond weakly to synthetic or natural ligands to TLR8 (7, 8) due to a small deletion in a defined leucine-rich repeat (LRR) in the linear structure of the receptor (9). Recent X-ray crystallographic data have confirmed this LRR is associated with ligand binding (10). In all other species TLR8 is a potent activator of innate immunity, and in humans TLR8 is expressed in the endosomal compartment of monocytes and myeloid dendritic cells (6, 11). This is in clear distinction to the expression of human TLR7 and TLR9 in the endosome of plasmacytoid dendritic cells—a population of DC with a very different phenotype and biology compared to mDC (12, 13). Thus activation of TLR8 is expected to stimulate the release of distinct inflammatory mediators, including Th1-polarizing cytokines, chemokines, and other acute phase proteins (5, 11, 14–16) compared to TLR9 agonists that have previously been evaluated in cancer patients (17). Since the structure of TLR8 is highly conserved among primates (16), the cynomolgus monkey was considered a relevant species for the preliminary pharmacological assessments of VTX-2337.

Subcutaneous administration of VTX–2337 in cynomolgus monkeys at dose levels ranging from 0.1 to 30 mg/kg (1.2 to 360 mg/m²) produced transient dose-dependent changes in the plasma levels of multiple cytokines, chemokines, acute phase proteins, and shed cell surface antigens consistent with activation of the innate immune system. When assessed at 6, 12, 24, and 96 hours after dosing, large dose-related increases were observed for a number of biomarkers in response to increasing doses of VTX-2337. Peak
levels were observed at different times after dosing. Many mediators, including granulocyte colony-stimulating factor (G-CSF), IL-6, monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1β, were rapidly induced (peaking at approximately 6 hours after dosing). A few, however—including calcitonin and C-reactive protein—had delayed responses, peaking between 12 and 24 hours after dosing (VentiRx, data on file). The only unexpected observation in toxicology studies in the cynomolgus monkey was ocular inflammation, albeit at dose levels considerably higher than would be expected to be used in human clinical applications. Treatment of human peripheral blood cells in vitro with VTX-2337 resulted in increased production of tumor necrosis factor alpha (TNFα) and interleukin (IL)-12 from monocytes and mDC and increased production of interferon IFNγ from NK cells (6).

On the basis of these initial studies, it was expected that TLR8 activation in humans via VTX-2337 would bolster the body’s natural immune response by providing integrated, pro-inflammatory immunomodulatory activity. The Phase 1 study of VTX-2337 reported herein is the first-in-human study, conducted to define single agent safety and tolerability, including identification of the maximum tolerated dose (MTD) for the agent. In addition, the pharmacokinetics and pharmacodynamics of VTX-2337 were investigated, including assessment of the immune-based biomarkers that had been identified in the studies of cynomolgus monkeys and human cells. Anti-tumor activity was also explored.

SUBJECTS AND METHODS
This single-arm, open-label, dose-escalation, Phase 1 study was conducted at 2 study centers in the United States (November 2008 to October 2010). The study was conducted in accordance with good clinical practice guidelines and the ethical principles based in the Declaration of Helsinki. Approval for study procedures was obtained from the institutional review boards of each study site and all subjects provided written informed consent prior to study enrollment.

Eligibility

Eligible subjects were adult patients who had received a diagnosis of histologically or cytologically confirmed solid tumors or lymphoma, with locally advanced or metastatic disease for which there were no further treatment options known to provide clinical benefit. Subjects had a life expectancy of $\geq 16$ weeks and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Subjects also had acceptable physical examinations including ophthalmologic exams and acceptable bone marrow, renal, and hepatic function based upon screening laboratory tests. Patients were not eligible if they had received treatment with an anticancer therapy or corticosteroids (previous 2 weeks), required ongoing systemic immunosuppressive therapy; or were known to have brain metastases (unless stable for $\geq 28$ days), active autoimmune disease, or insulin-dependent diabetes. Additional exclusion criteria included clinically significant cardiac or ophthalmologic disease, QTc interval $> 450$ msec on baseline electrocardiogram, uncontrolled intercurrent illness, infection requiring antibiotic therapy
or causing fever (previous week), lack of acceptable methods of birth control, and pregnancy.

Treatment and Dose Escalation

After a 7 to 14 day screening period, subjects were assigned to the currently-open VTX-2337 dose cohort and began the first treatment cycle. If unacceptable toxicity did not occur during Cycle 1, subjects received a second cycle of VTX-2337 at the same dose level. VTX-2337 was administered SQ on Days 1, 8, and 15 of each 28-day cycle in the outpatient treatment center. Doses for each sequential cohort were escalated in a step-wise manner (0.1, 0.2, 0.4, 0.8, 1.3, 2.0, 2.8, and 3.9 mg/m\(^2\)). Lyophilized VTX-2337 was reconstituted with sterile water and diluted with normal saline prior to subcutaneous administration (volume \(\leq 0.3\) mL). Subjects received 500 mg acetaminophen within 30 minutes before each dose of VTX-2337. Tumor response was assessed radiologically by RECIST 1.0 criteria every 2 cycles (18). Following the first assessment of tumor response—performed after the initial 2 cycles of treatment)—subjects were able to continue therapy in the absence of disease progression or unacceptable toxicity (referred to as ‘extended dosing’).

At least 3 evaluable subjects were enrolled in each cohort. For this first-in-man study of this novel immunomodulatory agent, we employed a staggered enrollment approach, whereby the first subject in each dose cohort received 2 doses of VTX-2337 prior to enrollment of the remaining 2 subjects for that cohort. Cycle 1 safety data were reviewed for all 3 subjects before the next cohort was enrolled. If 1 of the first 3 subjects in a
dosing cohort experienced a dose-limiting toxicity (DLT), the cohort was expanded to 6 subjects. If 2 or more DLTs were observed, dose escalation was to be halted. The MTD was defined as the dose one level below that at which 2 or more subjects experienced a DLT. DLTs were defined as adverse events that occurred during Cycle 1, were considered by the Investigator to be related to treatment with VTX-2337, and met one of the following criteria: hematologic toxicity of Grade 4 severity, non-hematologic toxicity of Grade 3 or 4 severity (with the exception of Grade 3 hypersensitivity reactions or localized injection-site toxicities), uveitis or ocular toxicities of any grade, or death.

Study Assessments

Safety monitoring included the ongoing assessment of adverse events and DLTs. Adverse events were summarized using the Medical Dictionary for Regulatory Activities (MedDRA), version 13.0 and graded for severity using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Clinical laboratory testing and physical examinations were performed at screening, days 1, 8, and 15 of each cycle, and the end of study visit. Due to ocular toxicity previously observed in nonclinical cynomolgus monkey studies, ophthalmologic exams (including direct and indirect ophthalmoscopy) were performed by an ophthalmologist prior to each dose of VTX-2337 and at the end of study visit. An electrocardiogram was recorded at screening, on the first day of VTX-2337 dosing (before and 3.5 hours after dose administration), and the end of study visit.
Plasma levels of VTX-2337 were evaluated by validated, high-performance liquid chromatography method using tandem mass spectrometry (HPLC-MS/MS) at 0.5, 1, 1.5, 2, 4, 6, 8, and 24 hours after the first dose. The pharmacodynamic effects of VTX-2337 were explored by quantitatively assessing the plasma levels of 96 distinct analytes, including cytokines, chemokines, and other inflammatory biomarkers (HumanMAP® panel, Myriad-RBM, Austin, TX); samples were collected before and 4, 8, and 24 hours after the first dose of VTX-2337 in Cycles 1 and 2.

Adverse event monitoring and clinical laboratory and ocular toxicity assessments continued during the extended dosing period. Physical exams were performed at the beginning of the extended dosing period, and physical and ophthalmologic exams were performed thereafter as clinically indicated.

Statistical Analysis

Statistical analyses used SAS® (SAS Institute Inc., Cary, NC) and were descriptive in nature. All 33 treated subjects were included in the safety, pharmacokinetic, pharmacodynamic, and efficacy analysis sets. Pharmacokinetic evaluations used actual sampling times and actual doses administered. Values below the limit of quantification were set to 0; single values of “0” between 2 measureable concentrations were excluded from analyses, as were values occurring after 2 consecutive values of 0. Plasma concentration-time data were analyzed using non-compartmental methods. Pharmacodynamic assessments were conducted for each subject; fold-increases relative to baseline values were calculated for each responsive biomarker at each sampling time.
point following dosing; mean fold-increases from baseline values were then calculated for each cohort. If baseline levels were below the level of quantification for the analyte, the lowest quantifiable level for the analyte was used to calculate the fold-increase. For assessment of antitumor responses (complete response [CR], partial response [PR], stable disease [SD], or progressive disease [PD]), measurable lesions at study screening were defined as those that could be accurately measured in a least one dimension with a longest diameter ≥ 20 mm using conventional techniques or ≥ 10 mm with spiral CT scan (18).

RESULTS

Of the 35 subjects screened for this study, 33 were enrolled and treated with VTX-2337. Subjects were enrolled into 8 dosing cohorts, ranging from 0.1 to 3.9 mg/m², with 3 to 8 subjects enrolled into each cohort (Table 1). A total of 16 subjects (48.5%) completed the planned 2 cycles of treatment. Reasons for not completing 2 cycles included: disease progression (n=9, 27.3%); adverse event (n=4, 12.1%); withdrawal of consent (n=3, 9.1%); and death (n=1, 3.0%). Most subjects received 1 cycle (n=12, 36.4%) or 2 cycles (n=13, 39.4%) of VTX-2337 treatment, with 6 (24.2%) subjects entering the extended dosing period and receiving a total of 3 to 8 cycles of treatment.

Demographics

Median age was 65 years (range: 40 to 85); the majority of subjects were male (n=19/33; 57.6%) and all were Caucasian (Table 2). The most prevalent tumor types were colorectal
(n=9; 27.3%) and pancreatic cancer (n=5, 15.2%). Median time from initial pathological diagnosis to the first dose of VTX-2337 was 2.0 years (range: 0 to 15).

Maximum Tolerated Dose

Three additional subjects were enrolled into Cohort 4 (0.8 mg/m$^2$) after a subject experienced pneumonia (non-neutropenic), dehydration, and pre-renal azotemia after the third dose of VTX-2337. Although the events did not meet the DLT definition, additional subjects were enrolled as an extra measure of safety. Upon further medical review, no DLTs occurred in this cohort, and no significant unexpected VTX-2337-related events were observed.

Eight subjects, including 2 replacement subjects, were enrolled in Cohort 8 (3.9 mg/m$^2$). One of the six evaluable subjects experienced a DLT after administration of the second dose of VTX-2337: Grade 3 hypotension requiring hospitalization. The subject also experienced the non-serious but clinically significant event of Grade 2 cytokine release syndrome. The hypotension resolved the day following dosing, and the subject was discontinued from the study due to this DLT. Although the protocol specified that 2 subjects in a dose cohort experience a DLT in order to establish the MTD, the Investigators and Sponsor considered the study aims to have been met after observing a single DLT. Clear biological activity of VTX-2337 had been established across multiple dose levels (described below), and a range of doses suitable for combination studies had been identified. Thus the highest dose administered was 3.9 mg/m$^2$, and resulted in one DLT observed in a single subject.
Safety

The most common adverse events overall were injection site reactions (n=28/33; 84.4%), chills (n=19; 57.6%), pyrexia (n=15; 45.5%), fatigue (n=13; 39.4%), nausea (n=11, 33.3%), influenza-like illness (n=8; 24.2%), decreased appetite (n=8; 24.2%), and vomiting (n=7; 21.2%; Table 3). The majority of adverse events were Grade 1 or 2 in severity and were resolved with no additional treatment or with supportive care. Adverse events of Grade 3 severity that occurred in ≥ 2 subjects were anemia, abdominal pain, urinary tract infection, hyponatremia, and cancer pain (n=2; 6.1% each). One subject experienced a Grade 4 event (hepatic encephalopathy), unrelated to VTX-2337. Two (6.1%) subjects died during the study; the deaths were due to progressive metastatic malignant melanoma and hepatic failure secondary to progressive pancreatic carcinoma, respectively. Two (6.1%) additional subjects died within 30 days of receiving VTX-2337; deaths were a result of gastrointestinal hemorrhage in a subject who had received 2 cycles of VTX-2337 before going off study due to disease progression, and metastatic pancreatic carcinoma in a subject who had received 1 cycle of VTX-2337 before going off study prematurely due to rapid disease progression. All 4 deaths were considered by the Investigators to be unrelated to treatment. Four (12.1%) subjects experienced an adverse event that led to treatment discontinuation during Cycles 1 and 2: hyperbilirubinemia, Escherichia sepsis, hepatic failure, and hypotension. Only hypotension was characterized as treatment-related and it was considered a DLT, as
previously described. The most commonly occurring adverse events that were considered by the Investigators as related to treatment included injection site reactions (n=28/33; 84.4%), chills (n=19; 57.6%), pyrexia (n=14; 42.4%), and influenza-like illness (n=8; 24.2%). In general, these events appeared on the day of injection and resolved within 48 hours. The flu-like symptoms, pyrexia, and fatigue were expected and consistent with the pharmacology of VTX-2337. With the exception of a few reports of fatigue that were ongoing at the end of the study, these events resolved without additional treatment or with supportive care. While ocular toxicity was seen in cynomolgus monkeys dosed at high levels of VTX-2337 in non-clinical studies, no VTX-2337 related ophthalmologic toxicity was seen in subjects in this study at any dose level tested.

Pharmacokinetics and Pharmacodynamics

With subcutaneous administration, VTX-2337 was rapidly absorbed into systemic circulation; mean time to maximum concentration (T_{\text{max}}) was approximately 0.5 hours after dosing (Table 4). VTX–2337 was also cleared rapidly from circulation; the mean half-life (t_{1/2}) was approximately 5 to 7 hours for most cohorts. Peak plasma levels (C_{\text{max}}) and total systemic exposure (area under the plasma concentration time curve to the last measurable time point; AUC_{0-last}) generally increased with the administered dose. For the 8 dose levels spanning an 39-fold increase in the administered dose of VTX-2337 (0.1 to 3.9 mg/m²), mean C_{\text{max}} values increased from 1.5 to 23.0 ng/mL (15.3-fold), while mean AUC_{0-last} values increased from 3.0 to 77.8 ng•h/mL (25.9-fold).
Increases in plasma biomarker levels were observed in subjects treated with VTX-2337. Starting in subjects who received doses of 0.4 mg/m$^2$ (Cohort 3), dose-dependent increases above baseline levels were observed for a number of different biomarkers. For G-CSF, MCP-1, and MIP-1β, the largest fold-increases were generally observed 8 hours after dosing (G-CSF) or 4 to 8 hours after dosing (MCP-1 and MIP-1β), with a trend toward a maximum signal at 8 hours as VTX-2337 doses increased (Figure 1). Smaller overall fold-increases were observed for TNFα, but the data showed a trend towards increasing levels with higher doses, although the time point where maximum levels were reached was variable. There was considerable intra-subject variability, as expected for immune-based markers. At VTX-2337 doses above 0.8 mg/m$^2$ (G-CSF and MCP-1), 1.3 mg/m$^2$ (MIP-1β), or 2.0 mg/m$^2$ (TNFα), every subject showed increased biomarker levels that were consistent with activation of TLR8 by VTX-2337. The biomarkers selected for presentation herein are those that were the most consistently and reliably elevated at the time points specified in the protocol. The biomarker sampling schedule was limited by the logistical constraints of the study, which was conducted in the outpatient setting. A detailed comparison of more biomarkers from the HumanMAP panel in cynomolgus monkeys, cancer patients, and healthy human volunteers dosed with VTX-2337 will be presented in an independent manuscript (Dietsch, et al.; in preparation).

The effects of repeated administration of VTX-2337 on the pharmacodynamic response were explored by assessing the plasma levels of 96 biomarkers as described in the Methods section. Plasma levels of each biomarker were measured before and 8 hours
after the first treatment with VTX-2337 in Cycle 1 (Day 1) and Cycle 2 (Day 29). For each analyte, the magnitude of any change that was observed at Day 1 was compared to the magnitude of any change observed at Day 29 (data not shown). There was no evidence of desensitization or augmentation of the pharmacodynamic response with repeat weekly dosing for 3 weeks followed by 1 week without any treatment with VTX-2337.

Antitumor Response

Antitumor responses were assessed radiographically using RECIST 1.1, starting at 8 weeks after starting study treatment and every two cycles thereafter (approximately 8-week intervals). Stable disease was observed as the best overall response in 8 (24.2%) subjects. Subjects with SD were distributed across VTX-2337 dosing levels, with 2 subjects each receiving 0.1 mg/m$^2$, 0.2 mg/m$^2$, and 0.8 mg/m$^2$, and 1 subject each receiving 2.8 mg/m$^2$ and 3.9 mg/m$^2$. Median duration of SD was 54.5 days (range: 13 to 162). The 8 subjects who achieved a best response of SD had colorectal adenocarcinoma (n=2), adenoid cystic carcinoma of the tongue (n=1), endometrial adenocarcinoma (n=1), hepatocellular carcinoma (n=1), melanoma (n=1), ovarian adenocarcinoma (n=1), and prostatic adenocarcinoma (n=1). A total of 21 (63.6%) subjects experienced PD at their first tumor assessment (week 8), no subjects achieved a CR or PR, and disease response could not be evaluated in 4 (12.1%) subjects.

Interestingly, evidence of a delayed response to treatment was noted in 1 subject. This subject, an 81 year old male with recurrent visceral melanoma (abdominal, lung, and
chest wall lesions), received 2 cycles of VTX-2337 at 2.0 mg/m². The subject displayed
disease progression at the end of treatment; however, at a 2 month follow-up visit, this
subject experienced a reduction in his abdominal lesion, stabilization of the lung lesion,
and disappearance of the chest wall lesion despite the fact that no anti-cancer therapy was
given after the subject discontinued VTX-2337. Palliative radiation was directed at the
chest wall lesion. Two months following palliative radiation to the lung nodule, all 3
lesions (including the abdominal and skin lesions that were not contiguous with the chest
wall lesion and were outside of the radiation field) were undetectable and remain so more
than 3.5 years after VTX-2337 treatment (Figure 2).

DISCUSSION

Herein we report the first-in-man Phase 1 clinical study of a novel, selective TLR8
agonist (VTX-2337) in cancer patients. The goal of this study was to define the safety,
tolerability—including identification of the MTD—pharmacokinetics, and pharmacology
of this novel investigational immunotherapeutic. A key objective was to define the range
of recommended phase 2 doses for subsequent clinical evaluation of VTX-2337 in
combination with anti-cancer agents where synergy has been demonstrated in the pre-
clinical setting (e.g. anthracycline-based chemotherapy).

Treatment with VTX-2337 was well tolerated. As noted in other clinical studies of TLR
agonists, the most commonly observed AEs included injection site reactions, chills,
pyrexia, and influenza-like illness (19, 20). These events were generally transient in
nature and were consistent with activation of the immune system and with the expected
pharmacology of VTX-2337, including the release of mediators such as TNFα. Only one DLT occurred: grade 3 hypotension which transpired in a subject treated at the highest dose level test (3.9 mg/m$^2$). The subject responded well to supportive care and recovered without clinical sequelae. No ocular findings related to VTX-2337 were noted. Additionally, no instances of VTX-2337 related neurotoxicity, hematotoxicity, or cardiotoxicity were observed.

In both humans and cynomolgus monkeys, treatment with VTX-2337 induced dose-dependent increases in plasma levels of specific cytokines, chemokines, and other biomarkers of immune activation. This similarity in responses to VTX-2337 is consistent with the observation that the TLR8 receptor is highly conserved between humans and monkeys: TLR8 receptors from humans and Rhesus macaque share 96% amino acid identity and 98% amino acid similarity (16). The identification of these biomarkers of VTX-2337 activity and characterization of the dose response in cynomolgus monkeys allowed an appropriate starting dose to be selected in this initial clinical study in humans.

We have extensively characterized the in vitro response of human peripheral blood mononuclear cells (PBMC), monocytes, mDC, and NK cells to VTX-2337 in vitro (6). It should be noted that many of the prominent cytokines induced in vitro via TLR8 activation (notably, IL-12, IL-1 IFNγ) are not detected in the plasma of subjects dosed with VTX-2337. This was not unexpected due to the rapid elimination of many cytokines and chemokines from the systemic circulation, and the assumption that many important cytokines are produced and consumed locally within the immune microenvironments
throughout the body. However, in this study, significant levels of specific mediators could be readily measured in a dose-dependent manner after dosing with VTX-2337 (Figure 1), and mirror the kinetics and magnitude of the responses seen in cynomolgus monkeys (data not shown). This panel of biomarkers (e.g. G-CSF, MCP-1, MIP-1\(\beta\)) demonstrate activation of TLR8 by VTX-2337 after subcutaneous dosing, and provide an important set of markers for subsequent studies in which VTX-2337 will be combined with agents that may modulate the effects of TLR8 activation. It highlights the important distinction between biomarkers identified from in vitro studies to those identified from clinical samples, particularly for agents that modulate immune responses.

To our knowledge, this is the first clinical evaluation of a selective TLR8 agonist in humans. R848 (resiquimod, 3M Pharmaceuticals) is a TLR7/TLR8 agonist that has been studied in man. While it is an activator of TLR8 in vitro, the predominant pharmacology of R848 is consistent with the potent TLR7 agonist activity of this compound. Emerging data including our own underscore the clear differences between activators of TLR8, and TLR7 or TLR9 in human immune responses (21, 22). TLR8 is expressed on myeloid-derived DCs, monocytes, and NK cells in humans (6, 11), compared to the expression of TLR7 and TLR9 on plasmacytoid-derived DCs (6, 12, 13). We anticipate these marked differences will lead to a considerably different therapeutic profile of VTX-2337 compared to other TLR agonists previously tested.

Before this study was conducted, it was not known whether a robust immune response to a TLR8 agonist could be expected in cancer patients who had been exposed to repeated
cycles of cytotoxic treatments with potential damage to their bone marrow and lymphoid tissue. The observation of a robust dose-dependent immune response, characterized by high levels of circulating cytokines and chemokines as well as clinical signs such as flu-like syndrome, demonstrate that cancer patients remain sensitive to the effects of TLR8 activation. The trial design used a once weekly dosing scheme with 3 consecutive weeks of dosing and 1 week off prior to the next treatment cycle. This dosing regimen was well tolerated. Furthermore, there was no evidence of either desensitization or augmentation of the pharmacodynamic response over 2 treatment cycles.

It was intriguing to see evidence of potentially delayed treatment response in a patient with metastatic melanoma (Figure 2). Several features of this subject are interesting. First, the initial radiographic assessment indicated progression at the 8 week time point, followed by complete resolution over the ensuing 6 months. Second, the only additional anti-cancer treatment after VTX-2337 was palliative radiation to a lesion that was not contiguous with the other measureable lesions that completely resolved (and remain clear more than 3.5 years later). Delayed responses and a potential abscopal effect of radiation have been reported with other cancer immunotherapies, most clearly with ipilimumab (23).

The clinical development focus of VTX-2337 will be in combinations with other anti-cancer therapies. We have demonstrated synergistic activity of VTX-2337 with anthracycline chemotherapy and with various monoclonal antibodies in preclinical studies (6, 22). The data from the current study provide a clinical rationale for doses of
VTX-2337 (2.5–3.5 mg/m²) that strongly activate TLR8 and have a safety and tolerability profile that is suitable for combination studies with other anti-cancer agents.

In summary, the current study has demonstrated the safety, pharmacokinetics, and pharmacologic profile of VTX-2337, a novel small molecule agonist of TLR8. The data support the conclusion that TLR8 can be activated in cancer patients with acceptable, transient toxicity consistent with expected activation of the innate immune response. Appropriate doses were identified for combination studies, and combination studies with pegylated-liposomal doxorubicin in ovarian cancer (NCT #0166644) and with cetuximab in head and neck cancer (NCT #01836029), respectively, are currently underway.

**GRANT SUPPORT**

None
REFERENCES


Table 1. Subject Disposition

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<td>0</td>
<td>0</td>
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<td>3</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

No. subjects treated: | 3 | 4 | 3 | 6 | 3 | 3 | 3 | 8 | 33
Completed 2 cycles: | 2 | 2 | 0 | 1 | 3 | 3 | 3 | 2 | 16
Did not complete 2 cycles: | 1 | 1 | 2 | 5 | 1 | 1 | 0 | 6 | 17
Disease progression: | 1 | 1 | 2 | 2 | 0 | 0 | 0 | 3 | 9
Adverse event: | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 4
Withdrawn Consent: | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 3
Death: | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1

No. cycles received, a n

<table>
<thead>
<tr>
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<th>1</th>
<th>1</th>
<th>4</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>5</th>
<th>12</th>
</tr>
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<tbody>
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<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>3–4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
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<td>6</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

a. Subjects were counted in a cycle if they received 1 or more of the 3 doses in that cycle.
Table 2. Baseline Characteristics

|                          | VTX-2337  
|--------------------------|-----------
|                          | (N=33)    |
| Age, years; median (range)| 65.0 (40 – 85) |
| Gender; n (%)             |           |
| Male                     | 19 (57.6) |
| Female                   | 14 (42.4) |
| Weight, kg; median (range)| 76.4 (46 – 126) |
| Primary disease site; n (%)|           |
| Colorectal               | 9 (27.3)  |
| Pancreas                 | 5 (15.2)  |
| Skin (melanoma)          | 5 (15.2)  |
| Gallbladder              | 2 (6.1)   |
| Kidney                   | 2 (6.1)   |
| Liver                    | 2 (6.1)   |
| Breast                   | 1 (3.0)   |
| Endometrium              | 1 (3.0)   |
| Ovary                    | 1 (3.0)   |
| Prostate                 | 1 (3.0)   |
| Skin (basal cell)        | 1 (3.0)   |
| Other a                  | 3 (9.1)   |
| Years since diagnosis    |           |
| Mean (SD)                | 3.1 (3.1) |
| Median (range)           | 2.0 (0 – 15) |
| Disease stage at study entry; n (%)|           |
| IV                       | 27 (81.8) |
| Unknown                  | 6 (18.2)  |

a adenoid cystic carcinoma of the tongue, adenocarcinoma of unknown origin, neuroendocrine carcinoma
Table 3. Adverse Events Occurring in \( \geq 10\% \) of Subjects

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>( \leq \text{Grade 2} )</th>
<th>( \geq \text{Grade 3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection site reaction(^a)</td>
<td>28 (84.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chills</td>
<td>19 (57.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>15 (45.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>13 (39.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nausea</td>
<td>11 (33.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>8 (24.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Influenza like illness</td>
<td>8 (24.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (21.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>4 (12.1)</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>4 (12.1)</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>3 (9.1)</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>2 (6.1)</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>Pain</td>
<td>4 (12.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dehydration</td>
<td>4 (12.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>3 (9.1)</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td>Hypomagnesaemia</td>
<td>4 (12.1)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

\(^a\) 'Injection site reaction' includes MedDRA terms injection site nodule, injection site pruritus, injection site reaction, injection site erythema, injection site irritation, and injection site pain.
Table 4. Summary of Mean Pharmacokinetic Parameters by Dose Cohort

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N</th>
<th>VTX-2337 dose</th>
<th>T(_{\text{max}})</th>
<th>t(_{1/2})</th>
<th>C(_{\text{max}})</th>
<th>AUC(_{0-\text{last}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>hours</td>
<td>n</td>
<td>hours</td>
<td>n</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.1 mg/m(^2)</td>
<td>0.53</td>
<td>3</td>
<td>1.73</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.2 mg/m(^2)</td>
<td>0.75</td>
<td>4</td>
<td>3.07</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.4 mg/m(^2)</td>
<td>0.51</td>
<td>3</td>
<td>6.97</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.8 mg/m(^2)</td>
<td>0.52</td>
<td>6</td>
<td>5.54</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>1.3 mg/m(^2)</td>
<td>0.49</td>
<td>3</td>
<td>7.14</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>2.0 mg/m(^2)</td>
<td>0.51</td>
<td>3</td>
<td>7.04</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>2.8 mg/m(^2)</td>
<td>0.51</td>
<td>3</td>
<td>5.71</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>3.9 mg/m(^2)</td>
<td>0.50</td>
<td>8</td>
<td>5.55</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td></td>
<td>0.54</td>
<td>33</td>
<td>5.45</td>
<td>28</td>
</tr>
</tbody>
</table>

\(T_{\text{max}}\) = maximum concentration; \(t_{1/2}\) = half-life; \(C_{\text{max}}\) = peak plasma levels; \(AUC_{0-\text{last}}\) = area under the plasma concentration time curve to the last measurable time point.
Figure 1. Increase in Plasma Levels for Select Biomarkers after VTX-2337 Dosing on Cycle 1 Day 1.
Figure 2. Case Report
FIGURE LEGENDS

Figure 1. The administration of VTX-2337 to cancer patients produced a dose-dependent increase in plasma levels of G-CSF, MCP-1, MIP-1β and TNFα. The 0.4 mg/m² dose was considered the lowest pharmacologically active dose. At dose levels of 2.0 mg/m² and higher, these 4 biomarkers were increased following dosing with VTX-2337 in all subjects. These biomarkers of TLR8 activation were measured prior to dosing with VTX-2337, and then 4, 8, and 24 hours post-dose. Biomarkers typically peaked at the 8 hour time point, then returned to near baseline levels by 24 hours.

Figure 2. Serial CT images show the initial progression of a visceral abdominal lesion followed by a delayed, sustained complete response in a subject with metastatic melanoma. The first image shows the abdominal lesion pre-treatment (3.3 cm). Additional pre-treatment lesions (not shown) include right lower lung (RLL; 1.25 cm) and left chest wall (1.1 cm). After 2 cycles of VTX-2337, the abdominal lesion measures 4.5 cm, the RLL nodule is 2.0 cm, and the left chest wall lesion measures 1.2 cm. The subject discontinued VTX-2337 treatment per protocol but opted to not initiate new anti-cancer therapy. CT obtained 3 months after VTX-2337 treatment demonstrates spontaneous shrinkage of the abdominal mass (3.8 cm); the RLL nodule is stabilized (2.0 cm), and the chest wall mass was no longer detectable. The subject received stereotactic body radiation therapy (SBRT) only to the RLL nodule at this time. Five months after VTX-2337 (2 months after SBRT), all 3 lesions are undetectable. The final CT image, taken approximately 15.5 months after VTX-2337, shows no evidence of the abdominal lesion; the RLL and chest wall lesion are also undetectable. The subject remains in CR as of January 2014.
Figure 2

11/20/2009
Initiation of VTX-2337

1/26/2010
End of cycle 2

4/5/2010
2.5 mo post treatment

6/14/2010
5 mo post treatment

5/9/2011
15.5 mo post treatment
Clinical Cancer Research

A Phase 1 Dose-Finding Study of the Novel Toll-like Receptor 8 Agonist VTX 2337 in Adult Subjects with Advanced Solid Tumors or Lymphoma

Donald W. Northfelt, Ramesh K Ramanathan, Peter A Cohen, et al.

Clin Cancer Res  Published OnlineFirst May 7, 2014.

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