Clinical trial

Vidofludimus calcium, a next generation DHODH inhibitor for the Treatment of relapsing-remitting multiple sclerosis

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A R T I C L E   I N F O

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A B S T R A C T

Background: Inhibition of dihydroorotate dehydrogenase (DHODH) is an established mechanism for the treatment of relapsing-remitting multiple sclerosis (RRMS). Currently approved treatments have several shortcomings. Consequently, new and effective treatments with improved safety and convenience profiles are sought after by patients.

Objective: To explore the overall profile of vidofludimus for the treatment of RRMS.

Methods: Preclinical investigations were done exploring the species-dependency of DHODH inhibition of vidofludimus. In addition, the preclinical efficacy in a rat experimental autoimmune encephalomyelitis (EAE) model and the inhibition of cytokine release from activated PBMC were investigated. Pharmacokinetic data were also obtained in a Phase 1 multiple ascending dose trial of the formulation IMU–838 (vidofludimus calcium).

Results: It was shown that vidofludimus is 2.6 times more potent in inhibiting DHO oxidation by human DHODH compared to teriflunomide. Although both compounds increased cell apoptosis, vidofludimus was more efficacious in the inhibition of T-lymphocyte proliferation compared to teriflunomide. The same was also observed for the secretion of IL–17 and IFN–γ. Interestingly, the potency of vidofludimus to inhibit rat or mouse DHODH is 7.5 and 64.4 time lower than the for the human DHODH, respectively. The rat EAE study clearly exhibited a dose-dependent inhibition of cumulative disease scores by vidofludimus. In the multiple ascending dose Phase 1 clinical trial, the serum half-life of about 30 h provides a favorable profile for once daily dosing of IMU–838, with quick dosing to steady state through levels within 5 days and the ability to wash out drug quickly, if required.

Conclusions: The investigations highlighted that the desired selective immunomodulatory properties can be separated from general antiproliferative effects seen and related adverse events in first-generation DHODH inhibitors. Based on data obtained from a series of pre-clinical as well as phase 1 and phase 2 studies, IMU–838 is a promising next-generation candidate for the oral treatment of RRMS. However, this will need to be confirmed in the currently ongoing Phase 2 study in RRMS patients.

1. Introduction

Vidofludimus calcium (IMU–838; Immunic AG, Germany) is a selective and potent second-generation dihydroorotate dehydrogenase (DHODH) oral immunomodulator being developed for the treatment of several chronic inflammatory diseases, including relapsing-remitting Multiple Sclerosis (RRMS).

Disease modifying therapies (DMT), including DHODH inhibitors such as the commercially available teriflunomide (Aubagio™) are one of the key components of comprehensive MS care in combination with symptomatic treatments.

The mechanism of action of vidofludimus calcium, a small molecule selective immune modulator, is the inhibition of the intracellular metabolism of activated immune T- and B-cells by blocking the enzyme DHODH. The inhibition of the DHODH enzyme leads to metabolic stress in metabolically activated lymphocytes resulting in reduction in pro-inflammatory cytokines and subsequently to apoptosis of activated immune cells (Fitzpatrick et al., 2010). Blocking of the DHODH enzyme activity has a selective effect to metabolically activated immune cells (Klotz et al., 2019), to malignant cells (Tan et al., 2016) and to virus-infected cells (Zhang et al., 2012). Thus, DHODH inhibition should therefore not lead to general antiproliferative effects in other cells. However, higher rates of seemingly general antiproliferative effects (neutropenia, alopecia, and diarrhea) have been observed with teriflunomide. This was initially thought to be a class effect of the mechanism of action. However, recent studies have shown that the general
antiproliferative effects of teriflunomide are most likely caused by off-target inhibition of a range of protein kinases (Buettner et al., 2019; Manna et al., 1999; Mattar et al., 2018; Siemasko et al., 1998; Xu et al., 1999). Inhibitors of such kinases, including EGFR, are characterized by the same general side effect profile as teriflunomide. IMU–838 as a second-generation DHODH inhibitor is being developed to separate the desired immunomodulatory effects from an undesirable side effect profile caused by such off-target effects.

An additional benefit of DHODH inhibitors such as vidofludimus (Vido) is their direct antiviral effect (Marschall et al., 2013). During long-term treatment with immunosuppressive drugs, the reactivation of latent viruses has been observed. This can lead to serious infections, such as progressive multifocal leukoencephalopathy (PML) which can have a lethal outcome. (Mills et al., 2018).

Due to the relatively high rate of undesirable effects, real world treatment cessation rates of up to 40% in the first year of treatment have been reported for teriflunomide (Johnson et al., 2017; Duquette et al., 2019).

In addition, teriflunomide has a prolonged plasma half-life of approximately 16 days in patients with MS (FDA Prescribing Information treatment cessation rates of up to 40% in the first year of treatment have been reported for teriflunomide (Johnson et al., 2017; Duquette et al., 2019).

In addition, vidofludimus calcium (IMU–838) is a new chemical entity DHODH inhibitor with no structural similarities to teriflunomide. This publication summarizes the characteristics of vidofludimus calcium (IMU–838) from preclinical and clinical studies as it pertains to its potential use in RRMS.

2. Methods

Initially, vidofludimus (Vido) was used in its free acid form. Immucin AG developed a new pharmaceutical formulation for clinical trials containing a specific polymorph of the calcium salt of Vido (INNM: vidofludimus calcium) named IMU–838 (Fig. 1).

Both formulations depend on the same active ingredient in blood (Vido) for their mechanism of action, toxicity and pharmacology (Muehler et al., 2019).

2.1. In vitro assessment of DHODH species-dependence

DHODH inhibition was measured on N-terminally truncated recombinant DHODH enzyme from mice, rats and humans as previously described (Baumgartner et al. 2006). In short, DHODH concentrations from the different species were adjusted such that an average slope of approximately 0.2 AU/min will be the positive control (e.g., without inhibitor).

The standard assay mixture contained 60 μM 2,6-Dichloroindophenol (DCIP, Sigma), 50 μM decylubiquinone (Sigma) and 100 μM dihydroorotate (Sigma). The DHODH enzymes with or without at least 6 different concentrations of Vido (3 nM–3 μM, 4SC) or teriflunomide (TFNM) were added and measurements were performed in 50 mM TrisHCl, 150 mM KCl (Merck), 0.1% Triton X–100 (Sigma), pH 8.0 at 30°C (Leban et al., 2005). The reaction was started by adding dihydroorotate and measuring the absorption at 600 nm for 2 min. For the determination of the IC50 values (concentration of inhibitor required for 50% inhibition) each data point was recorded in triplicates.

![Fig. 1. Structural formula of IMU–838.](image)

2.2. In vitro assessment of vidofludimus inhibition on activated human Peripheral Blood Mononuclear Cells (PBMC)

PBMC were isolated from healthy volunteers using density centrifugation with the Accuspin® System-Histopaque-1077 (Sigma) according to the manufacturer's manual. After isolation cells were stimulated cultured in RPMI1640 GlutaMax-I (Gibco), 10% dialyzed FCS EU (PAA Laboratories), 100 U penicillin/ 100 μg/mL streptomycin (PAA laboratories) and 2 mM L-glutamin (PAA Laboratories).

2.2.1. Cell viability

To study the effect of Vido and TFNM on cell viability, PBMC were cultured with 10 μM Vido or 100 μM TFNM or vehicle at 37 °C at 5% CO2. After 72 h, cells were harvested and stained for Annexin V (FITC; BD biosciences) and PI. After staining cells were acquired on FACS Calibur (BD Biosciences).

2.2.2. Cell proliferation

Cells were stimulated with 2 μg/mL PHA (Sigma) in the presence or absence of different concentrations (0.4–50 μM) of Vido, IMU–838 or TFNM for 48 h at 37 °C at 5% CO2 to assess the proliferation. Proliferation was assessed with the colorimetric BrdU cell proliferation ELISA as described by the manufacturer (Roche).

2.2.3. Cytokine ELISA

PBMC were stimulated as described in 2.2.2. After stimulation supernatants were collected and pro-inflammatory cytokine secretion, including interleukin (IL)–17A/F and interferon gamma (IFN–γ) was investigated using ELISA (eBioscience, Hölzel and BD Biosciences). Recombinant protein for IL–17F and IFN–γELISA standard are from eBioscience and R&D system, respectively.

2.2.4. Cytokine Luminex

PBMCs were stimulated with 2 μg/mL PHA (Sigma) with or without 5 μM Vido. After 24 h supernatant was collected and IFN–γ, IL-6 and IL–1β secretion was measured with a Luminex BioPlex 100 system, following the manufacturer's instructions (BioRad, Munich, Germany) (Fitzpatrick et al., 2010).

2.2.5. Intracellular flow cytometry

To investigate an early effect of Vido and TFNM on cytokine expression. PBMCs were stimulated with 2 μg/mL PHA (Sigma) with or without 30 μM Vido or 100 μM TFNM for 20 h at 37 °C at 5% CO2. After 16 h, 1:500 diluted Protein Transport Inhibitor Cocktail was added to the cells. Cells were harvest and stained for IL–17A APC and IL–17F PE. Cells were acquired on the FACS Calibur (BD Biosciences).

All data were acquired with FACS Calibur (BD Biosciences, Heidelberg, Germany) and analyzed using FlowJo software (Flowjo, USA).

2.3. Activity of vidofludimus in a rat demyelination model

Experimental autoimmune encephalomyelitis (EAE) is a rodent model for neuroinflammation and is acting as a preclinical human model for demyelinating diseases, including MS. To investigate the therapeutic effect of Vido, a well-established EAE model was used in which EAE is induced using spinal cord homogenate (Beeton, C et al., 2007; Burrows DJ et al., 2019; Hasseldam H et al., 2016). EAE was induced in five groups of 11-week old dark agouti (DA) female rats (n = 8 per group). The rats were immunized by subcutaneous administration (at the base of the tail) of 0.2 mL of rat spinal cord homogenate, composed of 50 mg whole rat spinal cord in Complete Freund's Adjuvant (CFA) and 10 mg/mL of Mycobacterium tuberculosis. Starting on day 7, the rats were clinically scored daily, in a blinded fashion, for progressive paralysis based on the following criteria: (Table 1).
2.4. Human pharmacokinetic evaluation of IMU–838

To assess multiple dose pharmacokinetics (PK) properties as well as safety and tolerability of IMU–838, we performed a double blind, placebo controlled, parallel group design Phase 1 study. This study was done in healthy male volunteers (n = 52) with escalating once daily doses of IMU–838 (EudraCT number: 2016–004531–21). Subjects were randomized to receive either placebo or IMU-838 for 14 days. Patients were recruited to 3 different dose groups, 30 mg IMU–838 (n = 12, placebo n = 4), 40 mg IMU–838 (n = 12, placebo n = 4) and 50 mg IMU–838 (n = 16 with or without half-dose pretreatment for six days, placebo n = 4). For all study subjects there was follow-up 10 days after receiving the last dose. Blood samples were collected and analyzed for vidofludimus concentrations using a validated LC-MS/MS method. This study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization (ICH) Guideline of GCP. Written informed consent was obtained of each healthy volunteer. The study was approved by the Ethics Committee of the Bavarian State Chamber of Physicians.

2.5. Statistical analysis

For statistical analysis, Graphpad Prism software was used. The results are shown as mean ± standard error of the mean (SEM). For experiments with two conditions, the Mann-Whitney test was performed. A repeated-measure one-way ANOVA or Friedman test followed by the Tukey's or Dunn's multiple comparisons test was performed for experiments with three or more groups. Finally, for results with missing data a mixed-effects analysis followed by the Dunn's test was used. Data were considered statistically significant when p < 0.05.

<table>
<thead>
<tr>
<th>Progressive paralysis score</th>
<th>Sign</th>
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<tbody>
<tr>
<td>0</td>
<td>no sign of disease</td>
</tr>
<tr>
<td>1</td>
<td>tail paralysis</td>
</tr>
<tr>
<td>2</td>
<td>tail paralysis + paraparesis or hind leg hemiplegia</td>
</tr>
<tr>
<td>3</td>
<td>tail paralysis + paraplegia</td>
</tr>
<tr>
<td>4</td>
<td>tail paralysis + quadriplegia</td>
</tr>
<tr>
<td>5</td>
<td>moribund or dead</td>
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Once an animal reached an individual clinical score ≥ 1, it was randomly assigned to one of the five groups, treatment was started by p.o. daily and maintained for 35 days. Vido was tested in 3 cohorts at doses of 4 mg/kg, 20 mg/kg and 60 mg/kg, respectively. Leflunomide, which metabolizes to its active component teriflunomide, was tested at 4 mg/kg as a positive control; and a vehicle was used as a negative control. Throughout the 35-day treatment period, rats were weighed and scored daily.

**Table 1**

**Disease severity score.**

<table>
<thead>
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Fig. 2. IMU–838 is a more potent DHODH inhibitor (2a) DHODH inhibition by vidofludimus (Vido) was studied in vitro using N-terminally truncated DHODH. A representative graph of 4 independent experiments is shown. The delta optical density (ΔOD) per minute is plotted against the concentration (Ln[Vido]). Measurement was performed in triplicate and the IC50 was calculated (red dotted line). (2b) To study cell viability, PBMCs of healthy subjects (n = 3) were stimulated with 2 μg/mL PHA with or without 10 μM vidofludimus (Vido; inverse triangle) or 100 μM TFNM (diamond). After 72 h, cells were stained for annexin V (Anx V) and PI. The data shown are normalized to their respective controls (red dotted line). Anx V−PI− are early apoptotic cells and AnxV+PI+ cells are late apoptotic and necrotic cells. Percentages in the columns reflect the average AnxV+ cells. (2c–f) Proliferation and IL–17A, IL–17F and IFN–γ secretion were assessed by stimulating PBMCs from healthy subjects (n = 7–8) with 2 μg/mL PHA with or without different concentrations of Vido, vidofludimus calcium (IMU–838) and TFNM for 48 h. (2c) Inhibition of proliferation was assessed with a BrdU assay and EC50 for each donor was calculated and plotted. IL–17A (2d), IL–17F and IFN–γ secretion were assessed by stimulating PBMCs from healthy subjects (n = 7–8) with 2 μg/mL PHA with or without different concentrations of Vido, vidofludimus calcium (IMU–838) and TFNM for 48 h.

For statistical analysis, Graphpad Prism software was used. The results are shown as mean ± standard error of the mean (SEM). For experiments with two conditions, the Mann-Whitney test was performed. A repeated-measure one-way ANOVA or Friedman test followed by the Tukey's or Dunn's multiple comparisons test was performed for experiments with three or more groups. Finally, for results with missing data a mixed-effects analysis followed by the Dunn's test was used. Data were considered statistically significant when p < 0.05.
3. Results

3.1. IMU–838 is a more potent and selective DHODH inhibitor than Teriflunomide

At present, teriflunomide (TFNM) is approved for the treatment of multiple sclerosis. Vidofludimus calcium (IMU-838) is a potent and selective second-generation DHODH inhibitor.

First, the efficacy of vidofludimus (Vido) on human DHODH was assessed and compared to the commercially available TFNM. DHO oxidation was measured in the presence or absence of increasing concentrations of Vido or TFNM and the IC₅₀ was calculated. The IC₅₀ of Vido was ~2.6 times (160 nM; Fig. 2a) lower compared to the IC₅₀ of TFNM (420 nM) (Leban et al., 2005).

Second, the efficacy of Vido, IMU–838 and TFNM was evaluated on PBMC viability. Cell viability was assessed by annexin V and PI staining after 72 h of PHA stimulation. To compare clinically related meaningful results, we treated the cells with the through levels e.g. ~10 μM for Vido and ~100 μM for TFNM (Sanofi-Aventis Australia Pty, Ltd.). Our data show that Vido as well as TFNM induces approximately a 3-fold
increase in apoptosis in stimulated PBMCs as compared to the control condition (Fig. 2b). This is observed for early apoptotic cells (annexin V⁺PI⁻ cells; ~3.5x) and to a lesser extend for the late apoptotic and necrotic cells (annexin V⁺PI⁺ cells; ~ 2.4x). Therefore, Vido is ten-fold more potent on apoptosis induction in PBMCs compared to TFNM.

Third, we assessed cell proliferation and cytokine secretion, IL–17A/F and IFN–γ after 48h of stimulation. Vido and IMU–838 where shown to be more potent in inhibiting T cell proliferation compared to TFNM (EC₅₀ of 13.9 ± 2.1, 11.8 ± 1.6 and 31.3 ± 3.4 μM respectively; Fig. 2c). In addition, Vido and TFNM had a similar effect on CpG ODN 2006-PTO dependent B cell proliferation (EC₅₀ of 11.8 ± 0.8 μM and EC₅₀ of 13.5 ± 0.3 μM, respectively n = 2). Also, Vido and IMU–838 reduced the secretion of both IL–17A (Fig. 2d), IL–17F (Fig. 2e) as well as IFN–γ(Fig. 2f). Compared to TFNM, the efficacy in inhibiting these cytokines is higher for IMU–838 and Vido. Therefore, IMU–838 exhibits a higher potency than TFNM in the inhibition of activated human T cell proliferation and cytokine secretion.

Since proliferation was already inhibited at 48h, we also measured IL–17A and IL–17F expression after 20 h of 2 μg/mL PHA stimulation of PBMCs in the presence or absence of Vido or TFNM by intracellular flow cytometry. A representative gating strategy for IL–17A and IL–17F within PBMCs is given in Fig. 3A. The cell frequencies for IL-17A⁺ were lower compared to IL–17F⁺ (Fig. 3A-C). Already after 20h, the data indicated that IL–17A⁺ and IL–17F⁺ cell frequencies were reduced following Vido treatment which seems to be less pronounced following treatment with TFNM. (Fig. 3B,C). The mean fluorescent intensity (MFI) of IL–17F was decreased upon Vido treatment, but not with TFNM (Fig. 3D). Since PHA is mainly a stimulator for T lymphocytes, we investigated if Vido affected cytokines, IL–6 and IL–1β, secretion by cells not directly affected by PHA. IFN–γ (mainly a T cell cytokine), IL–6 and IL–1β were assessed in the supernatant of PHA stimulated PBMCs. Our data show that while there is no effect on IL–6 and IL–1β, the IFN–γ secretion is inhibited upon Vido treatment (Fig. 3E-G). All these measurements indicate that Vido mainly inhibits cytokine expression and secretion from directly activated cells.

3.2. Vidofludimus is more specific for human DHODH and does not have off-target effect on kinases

TFNM has general anti-proliferative side effects presumably due to off-target effect to kinases (Mattar et al., 1993). Since Vido (10 μM with 100 μM ATP) does not significantly affect other human kinases. The overall inhibition of kinase activity was < 30%.(see Fig A1 in Appendix A). We believe that IMU–838 may therefore not show general anti-proliferative effects caused by such kinases.

Before initiating an in vivo model, the efficacy of both Vido and TFNM on rodent (rat and mouse) DHODH was assessed. Both Vido and TFNM inhibit rat and human DHODH (Table 2). While TFNM is much more active on rat and mouse DHODH, Vido inhibits human DHODH more strongly. In order to better compare pharmacodynamic data of Vido and TFNM in animal models and extrapolate the potential efficacy in humans, an IC₅₀ ratio was calculated for the inhibition of the target DHODH. We found that Vido is about 7.5-fold and about 64.4-fold more active on human DHODH as compared to rat DHODH and mouse DHODH, respectively. In contrast, TFNM is about 46.7-fold more active on rat DHODH and about 2.7-fold more active on mouse DHODH compared to human DHODH.
4. Discussion

While the FDA-approved DHODH inhibitor teriflunomide and the investigational drug IMU–838 share the same mechanism of action, they appear to have different safety and tolerance profiles (Muehler et al., 2019). A number of characteristic general antiproliferative adverse effects (neutropenia, alopecia, diarrhea) have been observed for teriflunomide (Aly et al., 2017). These adverse effects have not been observed with the structurally unrelated vidofludimus and its calcium salt formulation, IMU–838 (Muehler et al., 2019). Many immunosuppressive drugs are known to cause myelosuppression resulting from their general antiproliferative effects. One of the key advantages of DHODH as a therapeutic target is thought to be the selectivity for metabolically activated cells with high demand for nucleotides. In an earlier published preclinical study, Vido was shown not to induce myelosuppression (Kulkarni et al., 2010). In addition, no increased rate of neutropenia has been observed in studies using either formulation of Vido (Herrlinger et al., 2013; Muehler et al., 2019). The seemingly paradoxical occurrence of neutropenia, as well as the occurrence of diarrhea and alopecia may be due to the reported off-target effects of leflunomide/teriflunomide, in particular on EGFR (Mattar et al., 1993).

Compared to teriflunomide, Vido is highly potent and selective for DHODH, with no relevant inhibitory effects when tested against a panel of more than 90 protein kinases and very low inhibitory concentrations on the target DHODH.

The safety profile of IMU–838 demonstrates that the undesirable adverse reactions are not a class effect of DHODH inhibitors, but is likely due, at least in part, to off-target effects in first-generation DHODH inhibitors. The safety profile of IMU–838 supports the hypothesis that it is possible to separate the desired immunomodulatory effects of DHODH inhibition from general antiproliferative effects.

A phase 2 randomized, double-blind, placebo-controlled trial to assess IMU–838 in RRMS patients is currently ongoing (EMPhASIS; NCT–03846219). The primary goal of this trial is to assess the efficacy of either a daily dose of 30 mg or 45 mg of IMU–838 on RRMS disease activity, as measured by magnetic resonance imaging (MRI).

5. Conclusions

The second-generation DHODH inhibitor IMU–838 (vidofludimus calcium) has the identical mechanism of action as the FDA approved teriflunomide indicated for the treatment of RRMS. However, studies have shown that these two drugs have different safety profiles and that desired selective immunomodulatory properties can be separated from general antiproliferative effects seen in first-generation DHODH inhibitors. It is well known that teriflunomide has a number of characteristic adverse effects (hypothesized to be caused by off-target effects on kinases) (Mattar et al., 1993). Preclinical as well as clinical studies have shown that IMU–838 (which is structurally unrelated to teriflunomide) does not cause these specific side effects. Based on data obtained from a series of pre-clinical studies as well as clinical trials, IMU–838 is a promising next-generation DHODH inhibitor, with a unique and favorable profile, for the oral treatment of RRMS.

Declaration of Competing Interest

All authors are employed by Immunic AG, Am Klopferspitz 19, 82152 Planegg-Martinsried, Germany.
Appendix A. The effect of vidofludimus on human kinases

Figure A1

Fig. A1. The effect of 10 mM vidofludimus on 94 human kinases was tested in the presence of 100 mM ATP. All tests were performed in duplicates and the remaining activity was depicted as percentage of the control.

References


