Mass Balance and ADME Properties of [^{14}C]-IMU-838 Following Oral Administration to Healthy Male Subjects

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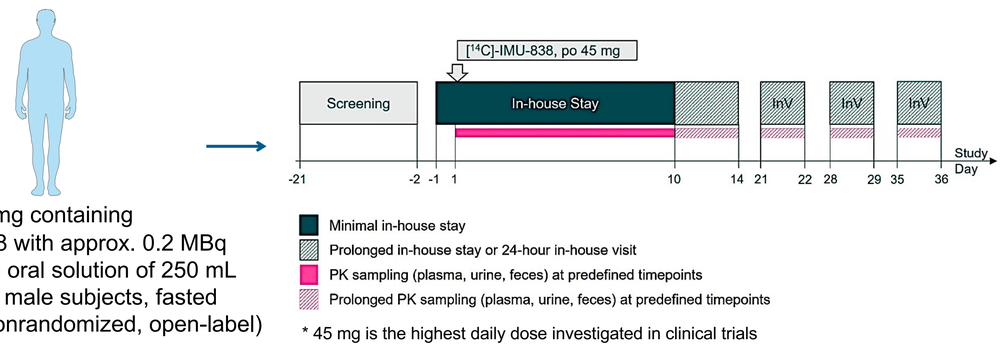
Background

Multiple sclerosis (MS) is a chronic, inflammatory, and demyelinating disease of the central nervous system and is one of the most common causes of neurological disability in young adults. There is increasing incidence and prevalence of MS in both developed and developing countries[1].

Vidofludimus calcium (IMU-838) is a novel small molecule that acts as a potent activator of nuclear receptor related 1 (Nurr1) protein, which is a neuroprotective transcription factor and an emerging target in neurodegenerative diseases[2]. In addition, it acts as a selective inhibitor of human dihydroorotate dehydrogenase (DHODH) which leads to an anti-inflammatory and antiviral effects in cells with a high need of *de novo* pyrimidine synthesis[3].

IMU-838 is in development as an oral next-generation treatment for MS patients, with ongoing phases 2 and 3 trials in progressive and relapsing MS, respectively. To fully understand the metabolic disposition and mass balance of IMU-838, a phase 1 study was conducted in male healthy volunteers who received a single oral dose of ^{14}C-radiolabeled IMU-838.

Clinical Study Design



45 mg containing
[^{14}C]-IMU-838 with approx. 0.2 MBq
(5.4 µCi) in an oral solution of 250 mL
N=7 healthy male subjects, fasted
(single-center, nonrandomized, open-label)

* 45 mg is the highest daily dose investigated in clinical trials

Biological Matrix	Time points
Plasma for TRA, parent drug PK, and MetID	Predose and 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96 (Day 5), 120 (Day 6), 144 (Day 7), 168 (Day 8), and 216 (Day 10) hours postdose*.
Whole blood for TRA	Predose, and 1, 3, 8, 12, 24, 72, and 144 hours postdose.
Urine for TRA and MetID	Predose (within 12 hours prior to dosing) and over 0-6, 6-12, 12-18, 18-24, and afterwards in 24-hour collection intervals daily through the morning of discharge.
Faeces for TRA and MetID	Predose (48 hours prior to drug administration) and in 24-hour intervals daily through the morning of discharge.

* sampling continued at 264 (Day 12), 312 (Day 14), 480 (Day 21), 648 (Day 28), and 816 (Day 35) hours if the discharge criteria were not met.
No vomit was produced and thus no such samples were analysed. Expired air samples were collected but no radioactivity was detected.
TRA: total radioactivity determination; MetID: metabolite profiling and identification

Analytical methods:

Mass Balance and TRA: liquid scintillation counting (LSC)

Parent drug PK: LC-MS/MS

Metabolites: UHPLC-HRMS-RAD with post-column split to a fraction collector for off-line AMS analysis (Accelerator Mass Spectrometry)

Pooling strategy for MetID:

Plasma: 0h-120h pooling of each subject according to Hamilton[4]. Individual pools covered >90% of total AUC. An overall AUC pool was prepared by mixing equal volumes of each individual pool.

Urine and faecal homogenate: individual subject pools prepared by combining aliquot volumes proportional to the weight of urine and faeces excreted in the respective collection intervals (up to >90% of total ^{14}C excreted). One overall urine pool was prepared by combining aliquots of each subject pool proportional to the total weight collected from t=0 h to last collection interval, and an overall faeces pool by combining equal aliquots of faeces subject pools.

Sample extraction for MetID:

Plasma and faeces homogenate: 1x ACN, and 2x with a mixture of ACN/water 2:1
Urine: no sample processing; centrifugation and direct injection into the instruments.
(*Other extraction solvents were tested but none of the alternatives provided higher extraction recoveries compared to acetonitrile*)

Results and Discussion

Table 1: Demographic characteristics

	Category	Statistics
Gender – n	Male	7
Race – n	Asian	1
	White	6
Ethnicity – n	Hispanic or Latino	0
Age (yr)	Mean (SD)	27 (7)
	Median	24
	Min, Max	22, 43
Height (cm)	Mean (SD)	185 (5)
	Median	186
	Min, Max	176, 191
Weight (kg)	Mean (SD)	81.0 (13.1)
	Median	84.6
	Min, Max	58.7, 96.7
BMI (kg/m^2)	Mean (SD)	23.5 (3.4)
	Median	23.7
	Min, Max	19.0, 29.5

BMI=body mass index; max=maximum; min=minimum; n=number of subjects; SD=standard deviation.
Note: Height, weight, and BMI were determined at screening

Mean [^{14}C] recovery, 312 hours postdose (Day 14).

Total: 93%

Urine: 54%

Faeces: 39%

- The discharge criteria (collection of radioactivity) were met for 1 subject on Day 12, for 2 subjects on Day 13, and for 4 subjects on Day 14.
- The administrated dose/radioactivity was safe and well tolerated. No changes or trends in clinically significant parameters were observed, no SAEs and no discontinuations due to AEs was reported.

Pharmacokinetic

Table 2. Mean pharmacokinetic parameters

Parameter (unit)	IMU-838 in Plasma	TRA in Plasma	TRA in Whole Blood
C_{max} (ng [eq]/mL)	8336 (6840 – 11800)	11103 (8698 – 14983)	6390 (4906 – 8746)
t_{max} (h)	1.00	1.00	1.00
$AUC_{0\text{-}last}$ (h.ng [eq]/mL)	151578 (99909 – 273463)	249597 (164662 – 406468)	132879 (83409 – 234808)
$AUC_{0\text{-}inf}$ (h.ng [eq]/mL)	175320 (116604 – 306432)	258624 (172792 – 416645)	150244 (94613 – 259211)
$t_{1/2}$ (h)	29.5 (23.1 – 38.1)	40.3 (33.4 – 49.0)	32.1 (24.3 – 43.9)
CL/F (L/h)	0.257 (0.147 – 0.386)		
V_2/F (L)	10.9 (8.07 – 12.9)		
f-AUC	67.8 (63.52 – 73.55)		

(max - min) values. f-AUC=ratio of the $AUC_{0\text{-}inf}$ of IMU-838/$AUC_{0\text{-}inf}$ of TRA in plasma as a percentage.
For t_{max} median is presented (1h; first collection time-point).
CL/F is calculated as dose/$AUC_{0\text{-}inf}$; Vz/F is calculated as (CL/F)/k_{el} where mean k_{el} = 0.0235

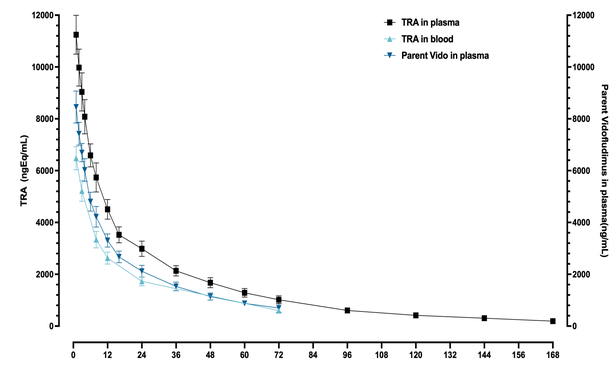


Figure 3: Geometric mean concentration-time profiles of total radioactivity in plasma and whole blood, and parent drug in plasma (linear scale)

- Exposure for total radioactivity (TRA) in plasma was higher than that of parent IMU-838 (f-AUC 67.8), indicating that approximately 1/3 of the drug-related material in the systemic circulation consists of metabolite(s).
- Metabolite(s) are apparently responsible for the longer terminal elimination half-life of plasma radioactivity (40.3 hours) compared to parent drug (29.5 hours).
- Whole blood to plasma ratio for TRA was consistent over time (0.58 - 0.59).

Metabolism

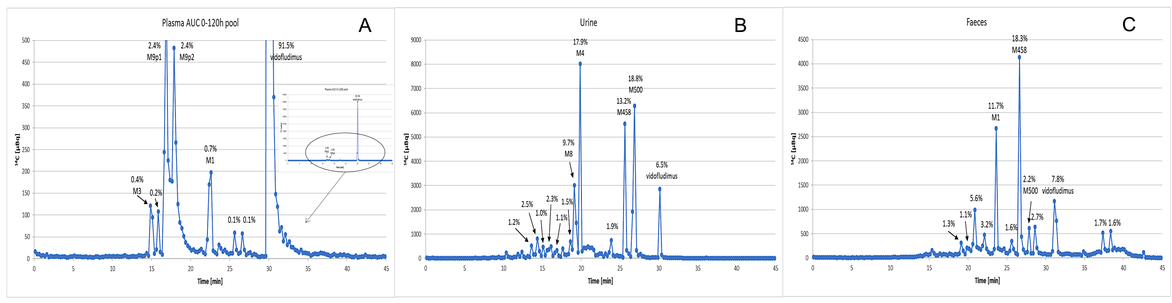


Figure 7: Representative radio-chromatograms of human plasma AUC 0-120h pool (A), urine (B) and faeces pools (C) as µBq per fraction (corrected for post-column split). Total integrated peak area relative to total 14C in the profile was 97.8% (plasma), 81.7% (urine), and 66.9% (faeces). All profiles contained an elevated baseline being more pronounced for urine and faeces. 14C sample extraction recovery was of 91.8% for plasma and 43.3% for faeces. Chromatographic column recoveries >90% indicated that the applied analytical conditions resulted in elution of all 14C injected into the system.

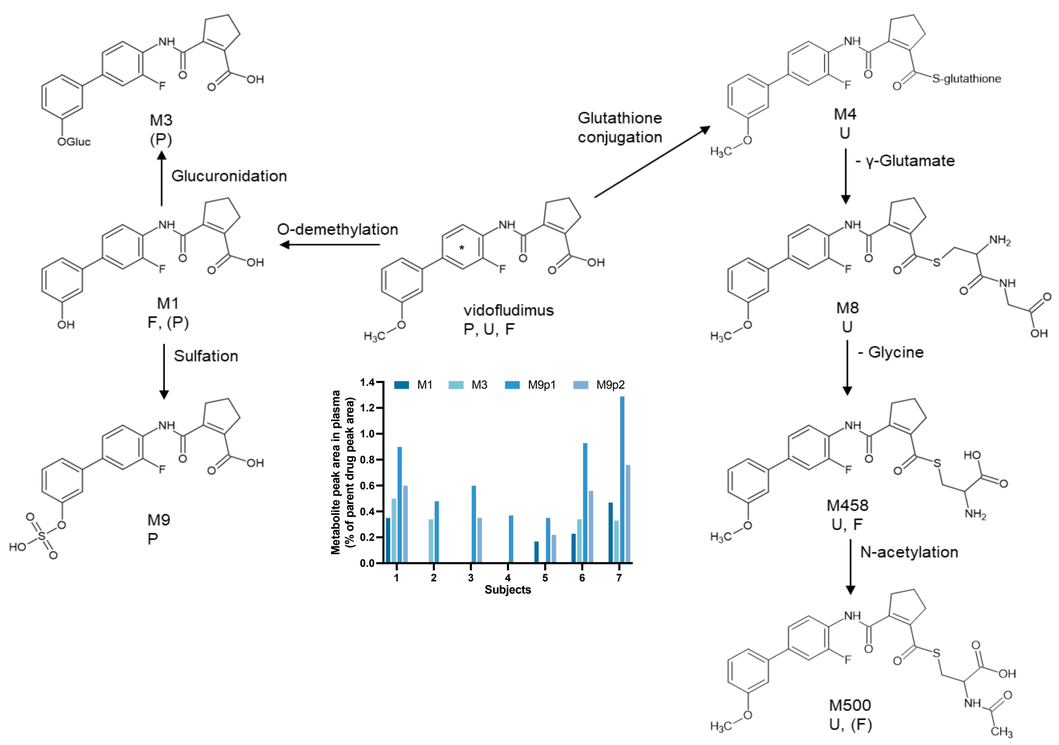


Figure 5: Proposed human metabolic pathway of IMU-838. The presence of parent drug and metabolites in plasma (P), urine (U), and faeces (F) is included in the scheme. Brackets indicate a relative abundance less than 5% (except for M9 in plasma at 4.8%). Graphic representation of the relative metabolite abundance for the seven individual plasma pools.

- Parent IMU-838 was the major circulating compound in the plasma (91.4%); followed by circulating metabolites M9 (4.8 %), M1 (0.7 %) and M3 (0.4 %).
- Previous CYP phenotyping data demonstrated that CYP2C8 was the major enzyme involved in IMU-838 metabolism (>45%), where M1 was mostly formed via CYP2C8, with minor contributions of other CYP enzymes.
- In total, 48.6 % of administered radioactivity was eliminated through the main metabolites detected in the urine and faeces profiles, where 5.1% was eliminated as parent drug (expressed as % of dose).

Conclusions

- Radioactivity was mainly excreted via urine and to a lesser extent via faeces.
- None of the metabolites circulating in plasma exceeded 10% of the total drug-related AUC.
- Identified metabolites in plasma were known and previously detected in preclinical tox species.
- No clinically meaningful inter-subject differences in metabolism were observed.
- This study confirms the favourable PK and safety profile of IMU-838.

References:

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3. Muehler A, et al. Vidofludimus calcium, a next generation DHODH inhibitor for the treatment of relapsing-remitting multiple sclerosis. *Mult Scler Relat Disord* 2020, 43: 102129.
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