T158

Evaluation of M4 Muscarinic Receptor Occupancy by CVL-231 Using [^{11}C]MK-6884 PET in Nonhuman Primates

Sridhar Duvvuri,[1] Philip Iredale,[1] Matthew Leoni,[1] John M. Kane,[2] Vasily Belov,[3] Nicolas J. Guehl,[3] Sung-Hyun Moon,[3] Maeva Dhaynaut,[3] Peter A. Rice,[3] Daniel L. Yokell,[3] Georges El Fakhri,[3] Marc D. Normandin,[3] John Renger[1]

[1]Cerevel Therapeutics, Cambridge, MA, USA; [2]Zucker Hillside Hospital, Hempstead, NY, USA; [3]Gordon Center for Medical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Presenting Author: Sridhar Duvvuri; Sridhar.Duvvuri@cerevel.com

CONCLUSIONS

- Robust quantification of CVL-231 RO in the striatum was obtained via [^{11}C]MK-6884 PET imaging and by using noninvasive pharmacokinetic modeling techniques
- These data confirm the dose-dependent target binding of CVL-231 to M4 receptors in the striatum of nonhuman primates
- Evaluation of M4 RO by CVL-231 in humans using [^{11}C]MK-6884 is being explored

ACKNOWLEDGMENTS: Editorial/Medical writing support was provided under the direction of the authors by Alexandra Kennedy, PhD and Emilia Raszkiewicz, ELS at MedThink SciCom and was funded by Cerevel Therapeutics.

REFERENCES: 1. Iredale et al. Presented at: Society for Neuroscience 2021, November 3-7, 2021; Virtual.

Presented at the American College of Neuropsychopharmacology Annual Meeting
December 5-8, 2021 • San Juan, Puerto Rico

INTRODUCTION

- CVL-231 is a novel, brain-penetrant, positive allosteric modulator selective for M4 muscarinic acetylcholine receptors (mAChRs) in development for the treatment of schizophrenia
- Preclinical characterization of CVL-231 in rodents showed favorable brain penetration, direct target engagement, and robust in vivo activity in animal models of psychosis (eg, reversal of amphetamine-stimulated locomotor activity, prepulse inhibition)[1]
- Verification of in vivo target engagement in primate brains and quantification of the exposure-occupancy relationship is useful to facilitate clinical dose selection and translation of preclinical data to humans

OBJECTIVE

- Using [^{11}C]MK-6884, an M4 positive allosteric modulator radioligand, this study evaluated M4 receptor occupancy (RO) of CVL-231 in the striatum of nonhuman primates as a function of CVL-231 dose and plasma concentration
  - The first objective was to determine the RO at M4 mAChRs in the striatum using arterial input function-based pharmacokinetic (PK) modeling methods following different intravenous doses of CVL-231
  - The second objective was to assess the accuracy of reference tissue-based PK modeling methods for quantifying the RO of CVL-231

METHODS

STUDY DESIGN

- Two male adult rhesus macaques aged 9 and 13 years were used in this study; their mean body weights on day of imaging were 12.9 kg and 15.1 kg, respectively
- Both animals had 3 imaging sessions, each consisting of a 90-min baseline scan followed by a 90-min positron emission tomography (PET)/computed tomography (CT) blocking scan with CVL-231 administration; each session was separated by >1 month to allow for sufficient recovery (**Figure 1**)
  - Before each imaging session, animals were sedated with ketamine/xylazine (10/0.5 mg/kg intramuscularly) and were intubated for maintenance anesthesia with isoflurane
  - Imaging sessions were performed on a Discovery MI (GE Healthcare, Chicago, IL) PET/CT scanner. A CT scan was acquired prior to each PET acquisition for attenuation correction. Emission PET data were acquired in three-dimensional list mode for 90 min following injection of [^{11}C]MK-6884

METHODS (CONTINUED)

  - Three-dimensional, T-1 weighted, magnetization-prepared rapid gradient-echo (MPRAGE) magnetic resonance images were also acquired for each monkey using a 3T Biograph mMR scanner (Siemens Medical Solutions USA, Inc, Malvern, PA) for anatomical reference
  - Methods for arterial blood sampling are presented in the Table

Figure 1. Diagram of a typical testing design for a single dose of CVL-231.

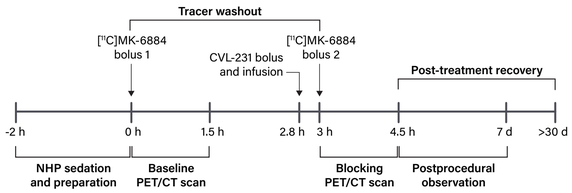


Table. Arterial Blood Sampling Methods for PET/CT Scanning Sessions

Baseline PET/CT	Blocking PET/CT
• Arterial blood sampling was performed during each dynamic PET acquisition – Samples of 1-3 mL were initially drawn every 30 s after the radiotracer injection and decreased in frequency to every 15 min toward the end of the scan • [^{11}C]MK-6884 metabolism was characterized from blood samples collected at 5, 8, 10, 15, 30, 60, and 90 min	• CVL-231 was administered intravenously as a loading dose (~48% of the total dose by bolus, 10 minutes before radiotracer) followed by a maintenance dose (~52% of the total dose continuously infused until the end of scan); doses ranged from 0.25 mg/kg to 1.7 mg/kg – Arterial blood samples for determination of CVL-231 concentration were collected at 60 min after radiotracer injection to determine plasma levels of CVL-231 for doses <1.7 mg/kg – For doses of 1.7 mg/kg, arterial blood samples were drawn at 0, 30, 60, and 90 min after radiotracer injection

CT, computed tomography; PET, positron emission tomography.

PRIMARY OUTCOMES

- The total volume of distribution (V_T) was assessed, representing the equilibrium ratio of tracer concentration in tissue relative to its plasma concentration, which is linearly related to the tracer binding to the target
  - V_T was calculated as K_1/k_2 for a one-tissue compartment model and modeled as $(K_1/k_2) \times (1 + k_3/k_4)$ for a two-tissue model
  - K_1 and k_2 are the rate constants for tracer influx and efflux, respectively, in the tissue with respect to plasma; k_3 and k_4 are the rate constants for specific binding and dissociation from the receptors in the target tissue, respectively
  - V_T was also calculated using graphical methods with arterial input functions, such as Logan plot and multilinear analysis 1 (MA1)
- Nondisplaceable binding potential (BP_{ND}) was defined as the equilibrium ratio of the concentration of specifically bound radioligand to its nondisplaceable concentration (nonspecifically bound and free in tissue)
  - BP_{ND} was a direct outcome of the simplified reference tissue model (SRTM)
  - Logan DVR and MRTM2 methods were also tested and provided distribution volume ratio (DVR) as a direct outcome: $DVR = V_T^{\text{target region}}/V_T^{\text{reference tissue}}$. BP_{ND} was then calculated as $BP_{ND} = DVR - 1$
- RO was calculated either as the slope of the Lassen plot $V_T^{\text{baseline}} - V_T^{\text{blocking}} = RO \times (V_T^{\text{baseline}} - V_{ND})$ or as $\%RO = 100\% \times (1 - BP_{ND}^{\text{blocking}}/BP_{ND}^{\text{baseline}})$, where V_T^{baseline} and V_T^{blocking} are the regional V_T at baseline and drug challenge (blocking) conditions, $BP_{ND}^{\text{baseline}}$ and $BP_{ND}^{\text{blocking}}$ are the BP_{ND} in the target region at the same conditions, and V_{ND} is the volume of distribution of nondisplaceable uptake

STATISTICAL ANALYSES

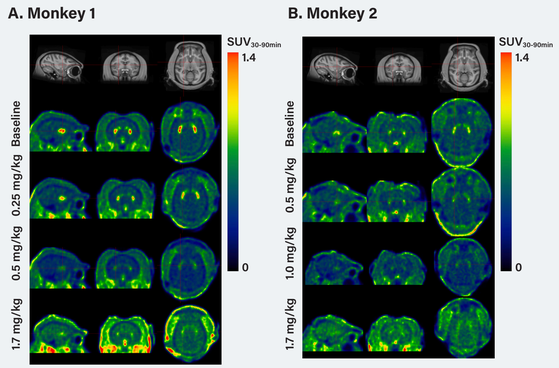
- Regional brain PET data were analyzed by various PK modeling techniques using blood-based and reference tissue (cerebellar gray matter) input functions to quantify radiotracer binding and to calculate RO at different doses of CVL-231
- Goodness of model fits, time stability of measured outcomes, and agreement between models were evaluated to assess the performance of the models
- A brain tissue suitable for use as a reference region was assessed based on the analysis of V_{ND} derived by Lassen plot method
- Estimates of RO were analyzed in a dose-response fashion against injected CVL-231 dose and plasma concentration
  - Data were fitted by the function $RO = RO^{MAX} \times D / (D + ID_{50})$, where D is the drug dose, ID_{50} is the estimated drug dose needed to achieve half-maximal occupancy, and RO^{MAX} is the estimated maximum RO that can be asymptotically attained by a high level of drug
  - An analogous fitting procedure was performed with the function $RO = RO^{MAX} \times C / (C + IC_{50})$, where C is the steady-state plasma concentration (ng/mL) at 1 hour, and IC_{50} is the estimated plasma concentration needed to achieve half-maximal occupancy

RESULTS

[^{11}C]MK-6884 BRAIN UPTAKE AND TIME-ACTIVITY CURVES (TACS)

- PET images demonstrated high brain penetration 0-10 min after radiotracer injection, with baseline standard uptake value (SUV) levels in the striatum exceeding 4.2; at the dynamic equilibrium phase (30-90 min), the highest baseline SUV levels in the striatum reached 1.4, in contrast with neighboring regions (SUV ~0.5)
- A strong dose-dependent blocking effect of CVL-231 was observed on [^{11}C]M6884 binding in the striatum (**Figure 2**)

Figure 2. Individual MRI MEMPRAGE images and [^{11}C]MK-6884 PET SUV images (summed 30-90 min after tracer injection) at baseline and after blocking at different CVL-231 doses in the brains of monkey 1 (A) and monkey 2 (B).



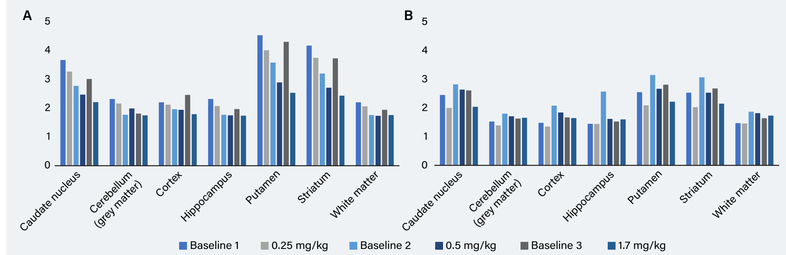
Images are presented in the MRI NIMH macaque template space. Sagittal, coronal, and transverse views are centered on the putamen. MEMPRAGE, multiecho magnetization prepared rapid acquisition gradient echo; MRI, magnetic resonance imaging; NIMH, National Institute of Mental Health; PET, positron emission tomography; SUV, standard uptake value.

- Among evaluated regions of interest (caudate, cerebellum, cortical gray matter, hippocampus, putamen, central white matter), only the caudate and putamen displayed significant blockade of [^{11}C]MK-6884 by CVL-231 (**Figure 3**)

SIMPLIFIED REFERENCE TISSUE MODELS

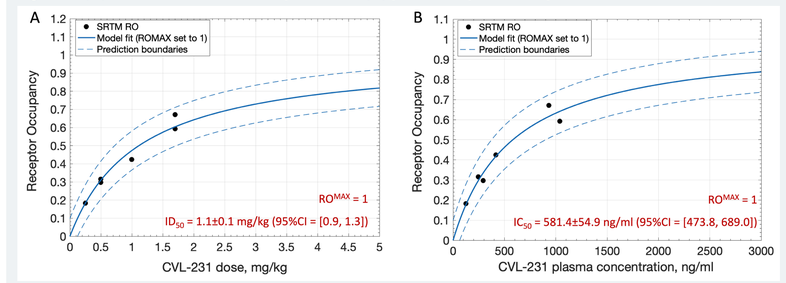
- All evaluated reference-tissue methods (simplified reference tissue model [SRTM], multilinear tissue reference model 2 [MRTM2] and Logan distribution volume ratio [DVR]) demonstrated a very strong agreement in quantifying regional BPND using cerebellar grey matter as a reference region; due to consistently high performance, the SRTM method was selected for the final quantification of RO
- Using cerebellar grey matter as a reference tissue, striatal RO was dose dependent from 18% to 67% over the range of evaluated CVL-231 doses (0.25 to 1.7 mg/kg, total of loading and maintenance components)
- The respective plasma concentrations of CVL-231 ranged from 126 ng/mL to 1040 ng/mL
  - The relationship of striatal RO with CVL-231–injected dose and plasma concentration was described by the classical Hill dose-response function, with an ID_{50} of 1.1 ± 0.1 mg/kg and an IC_{50} of 581 ± 55 ng/mL (**Figure 4**)

Figure 3. 2T4K estimates of regional V_T (mL/cc) for all studies for (A) monkey 1 and (B) monkey 2.



2T4K, 2-tissue 4-parameter; V_T, volume of distribution.

Figure 4. (A) CVL-231 mass-dose response and (B) plasma-exposure response.



RO^{MAX} is set to 1. R^2=0.96. IC_{50}, plasma concentration needed to achieve half-maximal occupancy; ID_{50}, drug dose needed to achieve half-maximal occupancy; SRTM, simplified reference tissue model; RO, receptor occupancy; RO^{MAX}, maximum receptor occupancy.