SUPPLEMENTAL MATERIAL

Adenosine A1R/A3R Agonist AST-004 Reduces Brain Infarction in a Nonhuman Primate Model of Stroke

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SUPPLEMENTAL METHODS

Timeline of experimental protocol and comparison to average patient timelines in HERMES
Supplementary Figure IA compares the current protocol with the average timelines of patient presentation and intervention described in the HERMES meta-analysis. Supplementary Figure IB outlines the timeline of imaging, physiological, neurological and pharmacokinetic assessments in this study. As this was an initial exploratory efficacy study utilizing a stroke model established in male macaques, only male subjects were utilized in the study. Future confirmative research may utilize both male and female subjects.

Animal quarantine and stabilization
Prior to arrival at the animal facility at Hamamatsu Pharma Research (HPR), macaques were tested and were negative for *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *M. tuberculosis* and *Cercopithecine herpesvirus 1* (B virus). Individual health certifications were obtained for each macaque from the vendor (EBS, Inc., Hashimoto, Japan). Upon arrival at HPR, macaques used in the current study underwent a period of quarantine and observation for 14 days. The veterinarian and animal care staff performed daily cursory visual checks of each macaque assessing their physical state, general behavior, appetite, and excreta. Any physical, behavioral, appetite or excreta abnormalities were to be recorded in each macaque's individual health record and may have led to exclusion. No macaques used in the current study were observed for such abnormalities during the quarantine period or during the period of housing within the general colony. An exclusion criterion was abnormal cerebral anatomy (based on T2-weighted images), but no subjects needed to be excluded on this basis.

Anesthesia
Macaques were initially sedated with ketamine HCl (10 mg/kg, i.m.) and treated with atropine sulfate (0.05 mg/kg, i.m.). Macaques were intubated, immobilized with 0.04-0.16 mg/kg (i.v.) vecuronium bromide and artificially ventilated. During the surgical procedure, animals were maintained on 0.8% isoflurane in a 7:3 mixture of N_2O and O_2. Before transient middle cerebral artery occlusion (tMCAO), the concentration of isoflurane was reduced to 0.5-0.6% and continued until 4h after ischemia. During surgery, end-tidal CO_2, body temperature, heart rate and blood pressure were monitored. During magnetic resonance imaging (MRI), macaques were anesthetized with 0.5-0.6% isoflurane.
Sugammadex (8 mg/kg, i.v.) was administered to reverse the effect of vecuronium. Post-operative antibiotic (5 mg/kg enrofloxacin, i.m., twice daily, up to four days post-surgery), analgesic (0.01 mg/kg buprenorphine, i.m., twice daily, up to four days post-surgery) and prednisolone (1 mg/kg, i.m., once following surgery, then bidaily for the following four days) were administered, and animals were observed during recovery from anesthesia. Animals showing signs of severe pain or distress at any time, as determined by the attending veterinarians and their staff, were to be euthanized, but this was not required.

Middle cerebral artery occlusion and reperfusion
Under deep anesthesia, the right eyeball was removed, and the orbital content was dissected and excised using a surgical microscope (KOM300, Konan Medical Inc., Hogo, Japan). A window of approximately 10 mm in diameter was opened just anterior to the foramen of the optic canal at the base of the skull, allowing identification of the right middle cerebral artery (MCA) main trunk beneath the dura. After opening the dura mater, tMCAO was performed using 2 microvascular clips (Sugita TII, 17-001-81, Mizuho Medical, Tokyo, Japan), one on the proximal part of the main MCA trunk and the other on the distal-to-orbitofrontal brunch (Supplementary Figure II). Four hours after tMCAO, these clips were removed. After visual confirmation of recanalization of MCA blood flow, the burr hole was closed using Clearfil New Bond (Kuraray Noritake Dental, Inc., Tokyo, Japan) and the orbital cavity was closed according to best veterinary practice.

Physiological Measurement Methodology
Key physiological parameters were monitored throughout the study period (Supplementary Figure IB).

Body weight and core body temperature
Body weight was measured before dosing using a scale (HV-200KGV-K, A&D Company, Limited, Tokyo, Japan). Body weight was used for determining dosing and measured prior to surgery, at 24h, and at 5 days after surgery (Supplementary Table I). A rectal thermometer probe (CTM-303, Terumo Corporation, Tokyo, Japan) was inserted about 5 cm into the rectum and rectal temperature was measured. Body temperature was maintained with a heating pad duringsurgery and with heated gel pads during MRI. For the current study, the acceptable core body temperature range was 36-38°C (Supplementary Table II).

Arterial blood pressure and heart rate
Either the left or right femoral artery was catheterized with a polyethylene catheter (inner/outer diameter 0.8/1.2 mm, respectively) for the measurement of mean arterial blood pressure (MABP) and heart rate, and for arterial blood sampling. The MABP and heart rate were recorded using a blood pressure amplifier (AP-641G, Nihon Kohden Corporation, Tokyo, Japan) and heart rate unit (AT-601G, Nihon Kohden Corporation). For the current study, the acceptable mean arterial blood pressure range was 80-130 mmHg and the acceptable heart rate range was 100-180 beats per minute (Supplementary Tables III and IV).

Partial pressure O_2 (pO_2), partial pressure CO_2 (pCO_2), saturated O_2 (sO_2) and pH
A 0.2 mL sample of arterial blood was collected with a heparinized syringe, and pCO_2, pO_2, and sO_2, and pH were measured with a blood gas analyzer (G3+ cartridge, i-STAT System, Abbott Japan, Tokyo, Japan). Proper pCO_2 and pO_2 was maintained by monitoring ventilation and anesthesia during surgery. The acceptable pO_2 range was 150-250 mmHg, the acceptable pCO_2 range was 30-40 mmHg, the acceptable sO_2 was ≥95%. The acceptable blood pH was 7.4-7.6 (Supplementary Tables V-VIII).

Assessment of neurological function
Twenty-four hours and five days after occlusion (after the end of dosing) neurologic deficits were scored according to the method described according to the Neurologic Deficit Score (NDS, Supplementary Table XI).

Exclusion criteria based on physiological monitoring
An exclusion criterion was prospectively set to consider exclusion of any subject with a physiological endpoint outside of the normal range for three consecutive time points. No subjects had to be excluded based on this criterion.

Magnetic resonance imaging
During imaging, macaques were anesthetized with isoflurane in N_2O and O_2 as described above. Animals were placed on a MRI bed (Signa HDxt 3.0T, GE Healthcare, Milwaukee, WI, USA). Serial coronal images (vertical plane against orbitomeatal line) were obtained as follows.

Diffusion weighted imaging (DWI)
Sequence name: PROPELLER (radial acquisition), acquisition matrix: 128 x 128, echo train length (ETL): 1, number of averages: 1.5, TE/TR: 80.79 ms/10000 ms, flip angle: 90 degrees, b-factor: 1000s/mm^2, slice thickness/gap: 3 mm/0 mm, field of view (FOV): 20 x 20 cm.

Arterial spin labeling (ASL)
Sequence name: 3D-Pseudocontinuous ASL (3D-PCASL), number of spirals: 8, points per spiral: 512, post-labeling delay (PLD): 1025 ms, ETL: 16, TE/TR: 11.8 ms/4351 ms, flip angle: 155 degrees, slice thickness: 2 mm, FOV: 20 x 20 cm.

Fluid attenuated inversion recovery (FLAIR) imaging
Sequence name: 2D inversion recovery (IR)/Spin Echo (SE), acquisition matrix: 256 x 224, TE/TR: 128.59 ms/9502 ms, inversion time (TI): 2250 ms, ETL: 1, number of averages: 1, flip angle: 90 degrees, slice thickness: 6 mm, FOV: 12 x 12 cm.

T2 weighted imaging (T2WI)
Sequence name: 2D fast spin echo (FSE), acquisition matrix: 256 x 224, TE/TR: 97.41 ms/4500 ms, ETL: 24, number of averages: 2, flip angle: 90 degrees, slice thickness: 6 mm, FOV: 12 x 12 cm.

Apparent diffusion coefficient (ADC) maps, cerebral blood flow (CBF), and perfusion deficit were generated with software (FuncTool Performance, GE Healthcare) available on the MR scanner console.

Measurement of lesion volume, perfusion deficit, and penumbra volume

Measurement details
The infarct (lesion) areas (mm^2) of each coronal image were marked using OsiriX version 8.0.2 (Pixmeo SARL, Bernex, Switzerland). The infarct was manually delineated using DWI maps. The lesion volume (mm^3) was calculated as the sum of the product of each section's infarct area and slice thickness (3 mm). In calculating infarct area and volume, no adjustments were made for potential edema. The perfusion deficit (mm^3) was calculated from ASL maps as a reduction to <30% and <50% of the corresponding region of the contralateral side. For clarity, the perfusion

deficit includes both the penumbra volume (hypo-oxygenated tissue) and lesion volume (necrotic tissue). Penumbral volume (mm^3) was calculated by subtracting the lesion volume delineated from the DWI diffusion maps from the total calculated perfusion deficit.

Control for edema effects on volumetric datasets
In general, we did not observe major edema build-up in post-reperfusion imaging in any subjects included in the study and final analysis. Lesion volume data from those subjects included in the final analysis were nevertheless controlled for a potential impact of the edema using Gerriets' method.[1] Analysis confirmed that the impact of the edema on volumetric data was <1% and did not affect any of the performed analyses.

Subject exclusion by lesion volume
At 1.5h post-occlusion, the infarct volume for each macaque was calculated from the DWI and fell within the 90% prediction interval (PI) as determined by the linear regression model:

$$\log (\text{lesion volume, } mm^3) = 5.141 + 0.641 \times (\text{time after MCA occlusion, hours})$$

based on lesion volumes obtained over time from 3 macaques with a 3-hour occlusion and 6 macaques with a 4-hour occlusion (historical data, animals not included in this study). The 90% PI was calculated as:

$$PI = \text{mean} \pm t_{0.1,\, df=n-1} \times RSD \times \text{sqrt}(1+1/n)$$

where the residual standard deviation (RSD) was 0.614, the number of observations (n) is 57 and the *t*-value (two-tailed) for the degree of freedom of *n*-1 and the significance level of 0.1 ($t_{0.1,\, d.f.=n-1}$) was 1.68. Thus, at 1.5h post-occlusion, the mean (90% P.I.) lesion volume was 447.0 mm^3(158.6-1259.4). Macaques with calculated infarct volumes falling either below or above the 90% PI were excluded at that point from treatment and excluded from the study. Based on the exclusioncriteria, two macaques were excluded: C-134 (lesion volume at 1.5 hrs. = 2162.4 mm^3, although later review found this to be a miscalculation) and C-137 (lesion volume at 1.5 hrs. = 1390.7 mm^3). The excluded macaques were replaced with C-109 and C-114.

Euthanasia, brain collection and preparation for hematoxylin and eosin staining
Following the final NDS assessment, MRI and measurement of body weight, body temperature, heart rate and blood pressure, macaques were deeply anesthetized (pentobarbital 100 mg/kg, i.v.), exsanguinated and perfused with normal saline followed by 10% buffered formalin. Brains were harvested and cut into 9 coronal sections at 6 mm intervals with a brain matrix (MBM-2000C, ASI Instruments, Inc., Warren, MI, USA). Brain sections were stored overnight (2.3-6.5°C) in 10% buffered formalin and then transferred to phosphate-buffered saline (PBS) for storage at 2.3-6.5°C until shipment for hematoxylin/eosin (HE) staining (conducted by New Histo. Science Laboratory Co. Ltd., Tokyo, Japan). For HE staining, brain sections were dehydrated and embedded in paraffin. Standard thickness (3-4 μm) coronal sections were taken from each paraffin block and stained with HE. The HE-stained sections were then digitized with a scanner and converted to TIFF images.

Determination of lesion volume from HE-stained sections

The infarct areas (mm^2) in each section were marked using OsiriX version 8.0.2 (Pixmeo SARL, Bernex, Switzerland). The volume of the infarct (mm^3) was calculated as the sum of the infarct area of each section and thickness (6 mm) multiplied by the number of sections. Total infarct volume (the sum of infarct volumes of all regions) was also calculated. A comparison of lesion volumes 5 days after tMCAO obtained through MRI and HE was performed.

Drug administration and pharmacokinetic sampling

Blood samples (1.0 mL) were withdrawn into heparinized tubes from either the opposite cephalic or the femoral vein at 3, 6, 24, 48, 72 hours and 5 days post-occlusion. Heparinized blood was centrifuged at 4°C at 1,800xg for 10 min. In the same animals, 0.3-0.5 mL of cerebrospinal fluid (CSF) was withdrawn at 6 and 24 hours post-occlusion. Macaques were sedated with ketamine (10 mg/kg, i.m.), CSF was collected via puncture of the cisterna magna. Plasma and CSF samples were flash frozen in liquid nitrogen and then stored at -70°C until shipment for processing. Samples were shipped on dry ice. Plasma and CSF concentrations of AST-004 were determined by LC/MS/MS utilizing standard curves (performed at the Department of Bio Research, Kamakura Techno-Science, Inc. Kanagawa, Japan). Lower AST-004 limits of quantitation were 1.0 ng/mL and 0.1 ng/mL for plasma and CSF, respectively.

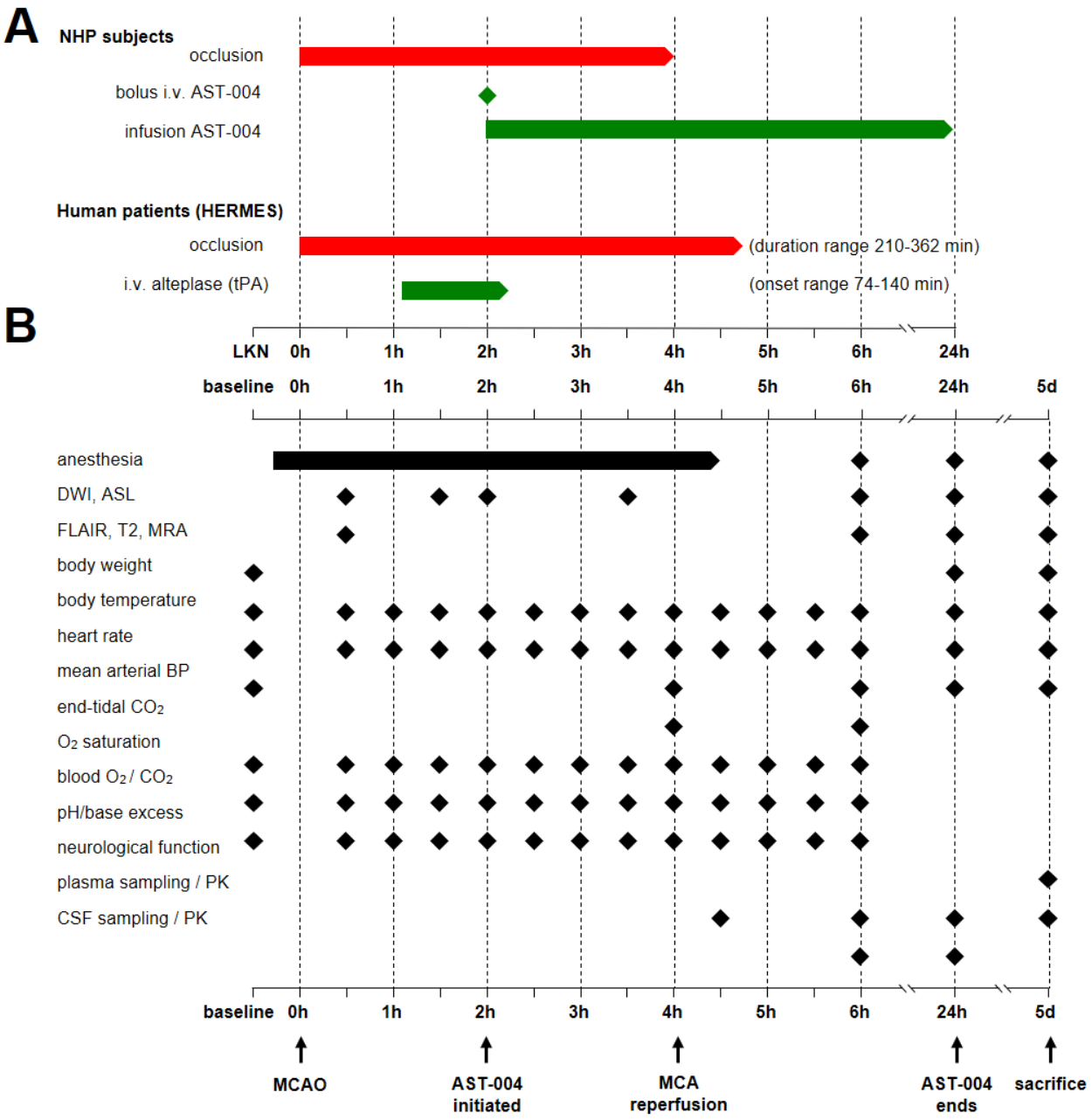
Study dropouts and replacement

Three macaques died (one subject from the vehicle, the Very Low dose and the Mid dose group each) before reaching the study endpoint on day 5 and were replaced (Supplementary Table XII). On examination of the brain of C-126 at the time of death, four days after tMCAO, intracranial hemorrhage within the basal ganglia was observed. In AST-004 Very Low dose subject C-153, an extended ipsilateral edema was observed on MRI beginning 6h after tMCAO. For AST-004 Mid dose subject C-98, no signs of illness or pathology, other than that associated with tMCAO, became obvious that would suggest a cause of death.

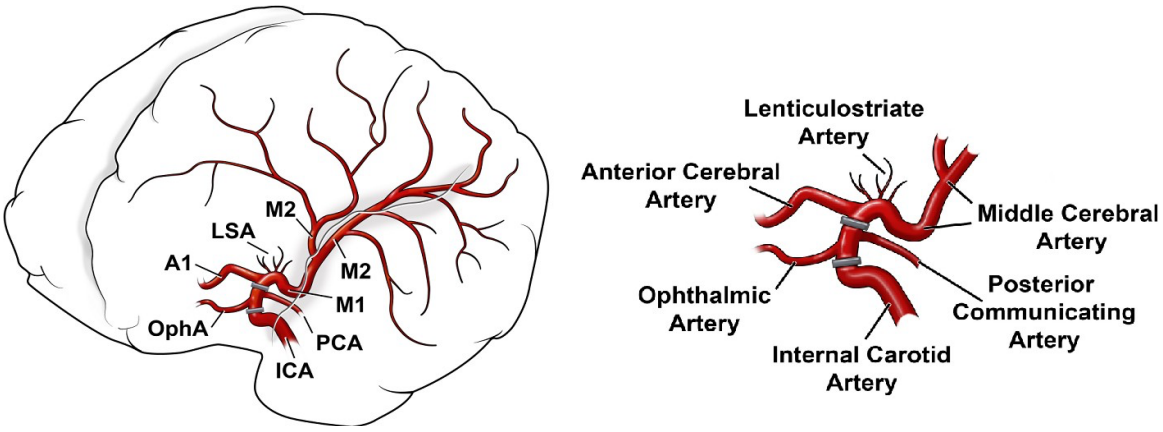
SUPPLEMENTARY REFERENCES

1. Gerriets T, Stolz E, Walberer M, Müller C, Kluge A, Bachmann A, Fisher M, Kaps M, Bachmann G. Noninvasive quantification of brain edema and the space-occupying effect in rat stroke models using magnetic resonance imaging. *Stroke.* 2004;35:566-571.

SUPPLEMENTARY TABLES AND FIGURES

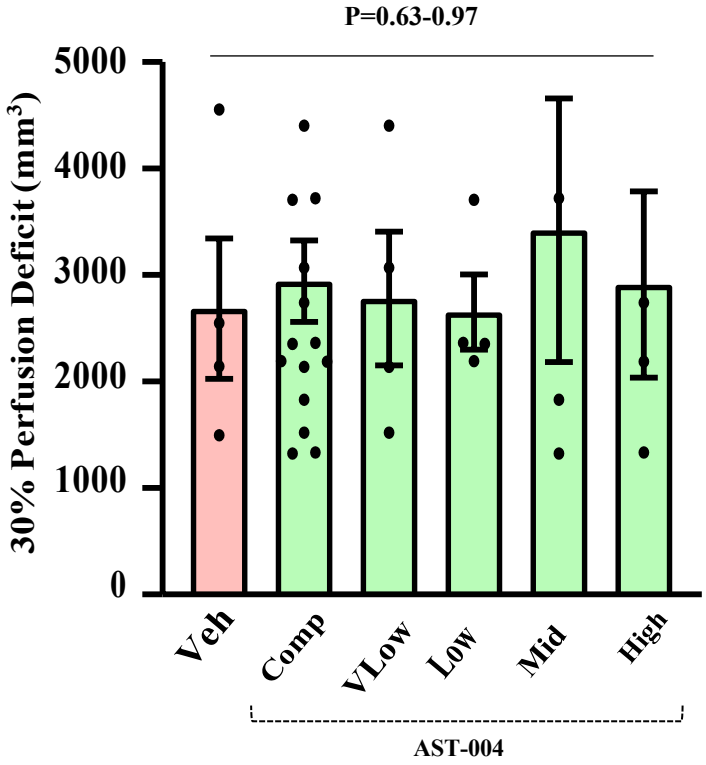


Supplementary Figure I. Overview of AST-004 study protocol in MCAO nonhuman primates and comparison to average intervention timelines for human stroke patients.
(A) Comparison of onset of occlusion, therapeutic intervention and reperfusion between AST-004 nonhuman primate efficacy study and average timelines described in the HERMES meta-analysis. **(B)** Timeline of key events and measurements in AST-004 nonhuman primate MCAO efficacy protocol. ASL, arterial spin labeling; BP, blood pressure; CSF, cerebrospinal fluid; DWI, diffusion-weighted imaging; FLAIR, fluid attenuated inversion recovery; LKN, last known normal; MRA, magnetic resonance angiography; NHP, non-human primate; PK, pharmacokinetics; T2, T2-weighted MRI imaging;



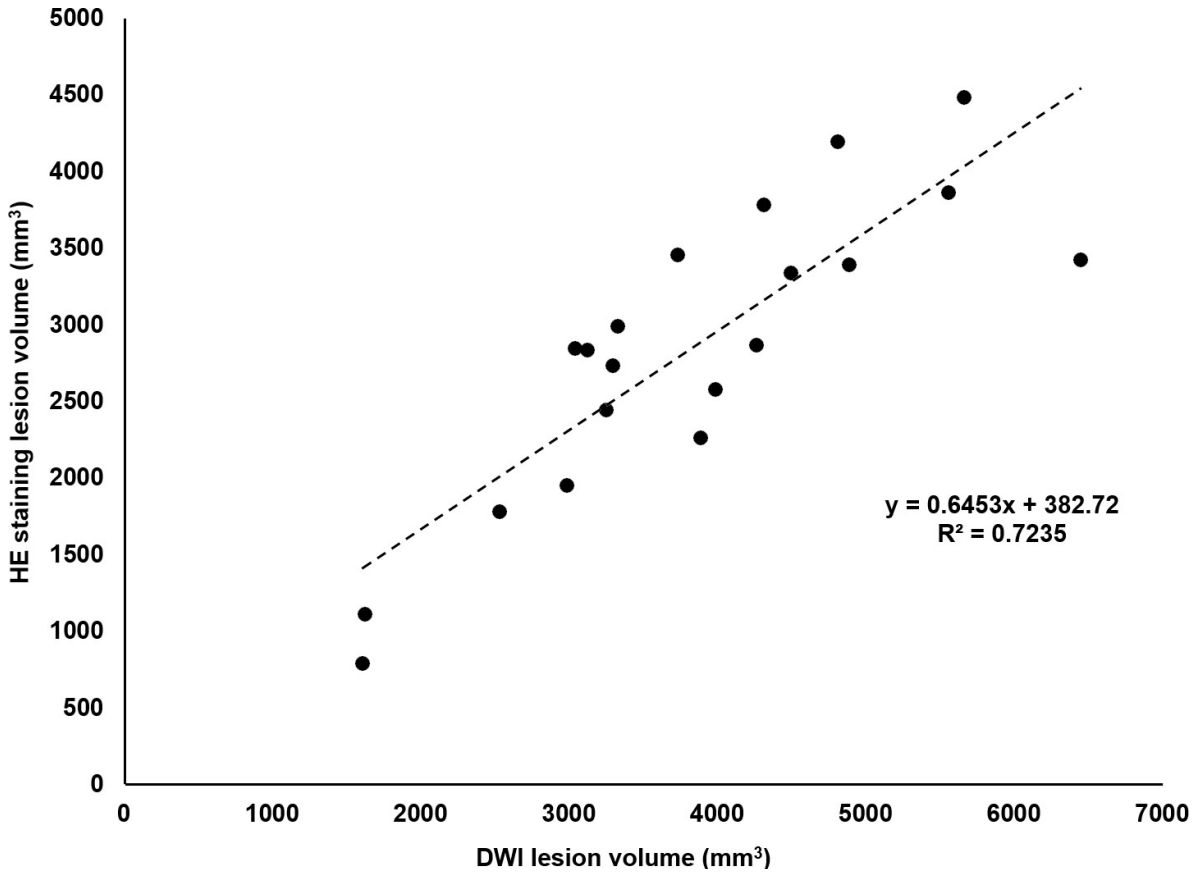
Supplementary Figure II. Methods for middle cerebral artery occlusion in cynomolgus macaques.

Transient middle cerebral artery occlusion (tMCAO) was performed using 2 microvascular clips, one positioned on the proximal part of the main MCA trunk, and the other on the distal-to-orbital branch. Four hours after MCAO, clips were removed.

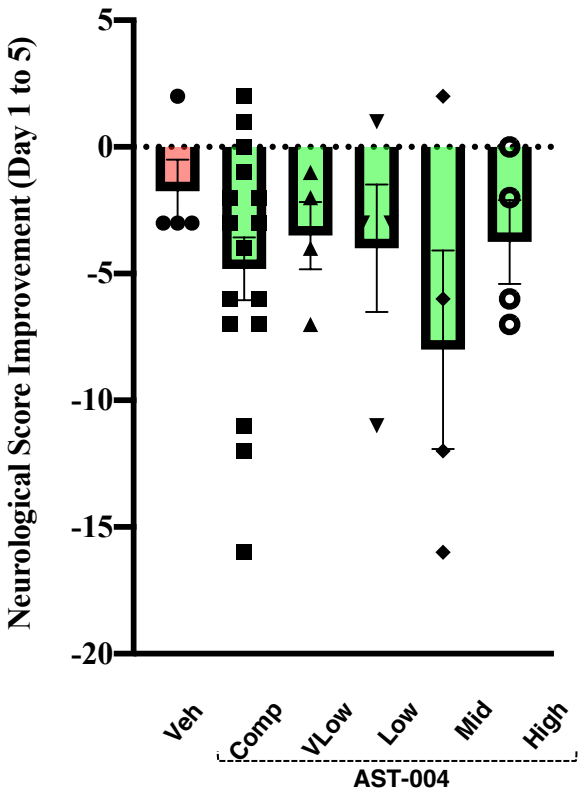


Supplementary Figure III. Baseline perfusion deficits following MCAO in nonhuman primates.

Perfusion deficits were measured at a threshold of <30% contralateral blood flow at 0.5h post-occlusion prior to initiation of AST-004 administration at 2.0h. Results are mean±SEM, n=4 per dose group and n=16 for the composite of all AST-004-treated groups combined. Statistical analyses are unpaired t-test comparing each AST-004 treatment group to vehicle group.



Supplementary Figure IV. Correlation of DWI lesion volume and HE staining lesion volume following tMCAO in nonhuman primates.
A strong correlation was observed between lesion volumes measurements by DWI MRI and lesion volumes calculated from HE stained brain slices.



Supplementary Figure V. Change in neurological deficit scores from day 1 to 5 following middle cerebral artery occlusion in nonhuman primates.

Neurologic deficits were assess using the Neurological Deficit Score (NDS). Results are presented as mean±SEM, n=4 per dosegroup and n=16 for the composite of all AST-004-treated groups combined. Statistical analysesare unpaired t-test comparing each AST-004 treatment group to vehicle group.

Supplementary Table I. Individual and group mean body weights (kg) before and after MCA occlusion.

Group	ID	Time point		
		prior to surgery	day 1	day 5
Vehicle	C-107	3.7	3.5	3.8
	C-108	4.0	3.8	3.7
	C-97	4.4	4.2	4.2
	C-103	3.6	3.5	3.5
	mean	**3.9**	**3.8**	**3.8**
	SEM	**0.2**	**0.2**	**0.1**
AST-004 Very Low	C-99	3.8	3.7	3.5
	C-113	3.6	3.4	3.6
	C-114	3.4	3.2	3.1
	C1312279	5.4	5.2	5.2
	mean	**4.1**	**3.9**	**3.9**
	SEM	**0.5**	**0.5**	**0.5**
AST-004 Low	C-158	2.9	2.9	2.6
	C-121	3.1	3.0	3.0
	C-145	2.7	2.6	2.7
	C-118	3.0	2.7	2.8
	mean	**2.9**	**2.8**	**2.8**
	SEM	**0.1**	**0.1**	**0.1**
AST-004 Mid	C-112	3.6	3.4	3.0
	C-132	2.8	2.6	2.8
	C-146	2.7	2.6	2.5
	1501051	2.5	2.4	2.3
	mean	**2.9**	**2.8**	**2.7**
	SEM	**0.2**	**0.2**	**0.2**
AST-004 High	C-122	3.6	3.5	3.9
	C-123	3.8	3.4	3.5
	C-109	3.3	3.3	3.3
	C-105	3.6	3.5	3.5
	mean	**3.6**	**3.4**	**3.6**
	SEM	**0.1**	**0.0**	**0.1**

Supplementary Table II. Individual and Group Mean Rectal Temperature (^{o}C) Before and Following MCA Occlusion.

Group	ID	Time post-occlusion (hr.)														
		Presurgery	After MCAO	1.0	1.5	1.8	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	Day 1	Day 5
Vehicle	C-107	36.0	36.8	36.0	36.1	36.0	36.1	37.0	37.1	37.6	37.5	37.5	37.6	37.7	36.5	36.4
	C-108	36.1	36.3	36.0	36.1	36.1	36.2	36.9	37.4	37.4	37.3	37.4	37.1	38.0	36.2	37.1
	C-97	36.3	37.5	36.3	36.2	36.3	36.2	36.2	36.3	36.6	36.3	36.7	37.5	38.0	37.3	36.8
	C-103	36.2	36.2	36.1	36.2	36.3	37.0	37.5	37.4	37.4	37.3	37.2	37.1	37.2	37.1	37.2
	mean	**36.2**	**36.7**	**36.1**	**36.2**	**36.2**	**36.4**	**36.9**	**37.1**	**37.3**	**37.1**	**37.2**	**37.3**	**37.7**	**36.8**	**36.9**
	SEM	**0.1**	**0.3**	**0.1**	**0.0**	**0.1**	**0.2**	**0.3**	**0.3**	**0.2**	**0.3**	**0.2**	**0.1**	**0.2**	**0.3**	**0.2**
AST-004 Very Low	C-99	36.2	36.8	36.3	36.0	37.2	37.3	37.0	37.2	37.0	37.9	37.6	37.9	37.7	37.0	37.5
	C-113	36.3	36.3	36.5	36.4	36.2	36.0	36.2	36.5	36.7	38.0	37.6	37.6	37.9	37.3	36.9
	C-114	36.1	36.0	37.1	37.0	36.8	36.5	36.5	36.5	36.5	37.6	37.5	37.5	37.1	36.4	36.3
	C1312279	36.3	36.2	36.0	36.2	36.0	36.1	36.0	36.2	36.3	36.8	37.3	37.2	37.1	37.2	37.0
	mean	**36.2**	**36.3**	**36.5**	**36.4**	**36.6**	**36.5**	**36.4**	**36.6**	**36.6**	**37.6**	**37.5**	**37.6**	**37.5**	**37.0**	**36.9**
	SEM	**0.0**	**0.2**	**0.2**	**0.2**	**0.3**	**0.3**	**0.2**	**0.2**	**0.1**	**0.3**	**0.1**	**0.1**	**0.2**	**0.2**	**0.2**
AST-004 Low	C-158	36.8	36.8	36.1	36.4	36.1	36.6	36.4	36.2	36.7	38.0	37.6	37.4	37.5	37.4	37.3
	C-121	36.0	36.0	36.3	36.5	36.3	36.7	36.5	36.6	36.4	37.8	37.2	37.2	37.3	37.7	37.3
	C-145	36.9	37.8	37.4	36.9	37.0	36.9	36.8	36.7	37.8	37.8	37.8	37.9	37.8	36.6	37.0
	C-118	37.0	37.3	37.3	37.1	36.8	36.6	36.3	36.1	36.1	36.1	36.3	36.7	36.9	36.5	36.6
	mean	**36.7**	**37.0**	**36.8**	**36.7**	**36.6**	**36.7**	**36.5**	**36.4**	**36.8**	**37.4**	**37.2**	**37.3**	**37.4**	**37.1**	**37.1**
	SEM	**0.2**	**0.4**	**0.3**	**0.2**	**0.2**	**0.1**	**0.1**	**0.1**	**0.4**	**0.4**	**0.3**	**0.2**	**0.2**	**0.3**	**0.2**
AST-004 Mid	C-112	37.0	37.7	36.8	37.0	36.8	36.4	36.1	36.2	36.3	36.8	37.0	38.0	37.3	37.1	37.0
	C-132	37.0	37.8	38.0	37.2	36.5	36.7	36.8	36.5	36.1	36.1	37.2	38.0	37.2	37.2	36.8
	C-146	36.5	36.4	37.1	37.2	37.1	37.4	37.5	37.5	37.9	37.4	37.5	37.6	37.1	37.2	37.1
	1501051	36.3	36.4	36.3	36.4	36.1	36.2	36.4	36.2	36.3	36.1	36.8	36.5	37.1	36.3	36.3
	mean	**36.7**	**37.1**	**37.1**	**37.0**	**36.6**	**36.7**	**36.7**	**36.6**	**36.7**	**36.6**	**37.1**	**37.5**	**37.2**	**37.0**	**36.8**
	SEM	**0.2**	**0.4**	**0.4**	**0.2**	**0.2**	**0.3**	**0.3**	**0.3**	**0.4**	**0.3**	**0.1**	**0.4**	**0.0**	**0.2**	**0.2**
AST-004 High	C-122	37.6	38.0	38.0	37.8	37.6	37.8	37.6	38.0	38.0	37.8	37.8	37.6	37.8	37.3	37.1
	C-123	36.5	36.7	36.3	36.7	36.8	37.0	36.5	36.7	36.5	36.4	36.4	36.6	36.7	37.0	36.8
	C-109	36.3	36.5	37.3	37.1	37.0	37.1	37.1	37.3	37.6	38.0	37.9	37.2	37.1	37.3	37.5
	C-105	36.3	36.6	36.7	36.5	36.2	36.4	36.5	37.5	37.3	37.8	38.0	38.0	37.8	37.7	37.2
	mean	**36.7**	**37.0**	**37.1**	**37.0**	**36.9**	**37.1**	**36.9**	**37.4**	**37.4**	**37.5**	**37.5**	**37.4**	**37.4**	**37.3**	**37.2**
	SEM	**0.3**	**0.4**	**0.4**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.4**	**0.4**	**0.3**	**0.3**	**0.1**	**0.1**

Normal Physiological Range 36-38 ^{o}C

Supplementary Table III. Individual and Group Mean Arterial Blood Pressure (mm Hg) Before and Following MCA Occlusion.

Group	ID	Time post-occlusion (hr.)														
		Presurgery	After MCAO	1.0	1.5	1.8	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	Day 1	Day 5
Vehicle	C-107	111	110	ND	ND	ND	ND	ND	ND	100	ND	ND	ND	111	105	81
	C-108	84	80	ND	ND	ND	ND	ND	ND	83	ND	ND	ND	88	80	85
	C-97	99	101	ND	ND	ND	ND	ND	ND	107	ND	ND	ND	115	94	86
	C-103	82	82	ND	ND	ND	ND	ND	ND	100	ND	ND	ND	109	112	106
	mean	**94.0**	**93.3**	ND	ND	ND	ND	ND	ND	**97.5**	ND	ND	ND	**105.8**	**97.8**	**89.5**
	SEM	**6.8**	**7.3**	ND	ND	ND	ND	ND	ND	**5.1**	ND	ND	ND	**6.0**	**7.0**	**5.6**
AST-004 Very Low	C-99	80	91	ND	ND	ND	ND	ND	ND	95	ND	ND	ND	89	83	105
	C-113	84	82	ND	ND	ND	ND	ND	ND	113	ND	ND	ND	100	108	91
	C-114	92	110	ND	ND	ND	ND	ND	ND	85	ND	ND	ND	84	91	98
	C1312279	85	91	ND	ND	ND	ND	ND	ND	130	ND	ND	ND	133	98	100
	mean	**85.3**	**93.5**	ND	ND	ND	ND	ND	ND	**105.8**	ND	ND	ND	**101.5**	**95.0**	**98.5**
	SEM	**2.5**	**5.9**	ND	ND	ND	ND	ND	ND	**9.9**	ND	ND	ND	**11.0**	**5.3**	**2.9**
AST-004 Low	C-158	81	81	ND	ND	ND	ND	ND	ND	92	ND	ND	ND	82	91	109
	C-121	90	94	ND	ND	ND	ND	ND	ND	84	ND	ND	ND	101	81	94
	C-145	85	121	ND	ND	ND	ND	ND	ND	96	ND	ND	ND	103	91	89
	C-118	80	96	ND	ND	ND	ND	ND	ND	100	ND	ND	ND	113	98	93
	mean	**84.0**	**98.0**	ND	ND	ND	ND	ND	ND	**93.0**	ND	ND	ND	**99.8**	**90.3**	**96.3**
	SEM	**2.3**	**8.4**	ND	ND	ND	ND	ND	ND	**3.4**	ND	ND	ND	**6.5**	**3.5**	**4.4**
AST-004 Mid	C-112	80	86	ND	ND	ND	ND	ND	ND	91	ND	ND	ND	103	85	87
	C-132	84	106	ND	ND	ND	ND	ND	ND	97	ND	ND	ND	84	93	88
	C-146	84	88	ND	ND	ND	ND	ND	ND	88	ND	ND	ND	93	100	91
	1501051	84	88	ND	ND	ND	ND	ND	ND	101	ND	ND	ND	105	107	105
	mean	**83.0**	**92.0**	ND	ND	ND	ND	ND	ND	**94.3**	ND	ND	ND	**96.3**	**96.3**	**92.8**
	SEM	**1.0**	**4.7**	ND	ND	ND	ND	ND	ND	**2.9**	ND	ND	ND	**4.9**	**4.7**	**4.2**
AST-004 High	C-122	90	80	ND	ND	ND	ND	ND	ND	90	ND	ND	ND	90	98	85
	C-123	83	90	ND	ND	ND	ND	ND	ND	95	ND	ND	ND	87	97	95
	C-109	81	94	ND	ND	ND	ND	ND	ND	108	ND	ND	ND	111	112	100
	C-105	81	86	ND	ND	ND	ND	ND	ND	83	ND	ND	ND	84	100	89
	mean	**83.8**	**87.5**	ND	ND	ND	ND	ND	ND	**94.0**	ND	ND	ND	**93.0**	**101.8**	**92.3**
	SEM	**2.1**	**3.0**	ND	ND	ND	ND	ND	ND	**5.3**	ND	ND	ND	**6.1**	**3.5**	**3.3**

Normal Physiological Range 80-130 mm Hg. Yellow highlighted cells show brief deviations outside of normal range.

Supplementary Table IV. Individual and Group Mean Heart Rate (beats/minute) Before and Following MCA Occlusion.

Group	ID	Time post-occlusion (hr.)														
		Presurgery	After MCAO	1.0	1.5	1.8	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	Day 1	Day 5
Vehicle	C-107	167	152	135	134	128	133	136	138	149	146	147	148	149	141	131
	C-108	136	133	145	145	144	140	142	143	135	131	135	138	134	135	125
	C-97	150	149	147	151	132	127	124	123	115	121	135	143	150	151	149
	C-103	151	171	156	160	174	161	171	170	143	130	132	130	161	161	143
	mean	**151.0**	**151.3**	**145.8**	**147.5**	**144.5**	**140.3**	**143.3**	**143.5**	**135.5**	**132.0**	**137.3**	**139.8**	**148.5**	**147.0**	**137.0**
	SEM	**6.3**	**7.8**	**4.3**	**5.5**	**10.4**	**7.4**	**10.0**	**9.8**	**7.4**	**5.2**	**3.3**	**3.8**	**5.5**	**5.7**	**5.5**
AST-004 Very Low	C-99	151	162	144	144	143	134	126	122	126	140	140	143	142	123	138
	C-113	108	150	139	140	125	111	169	167	105	119	116	128	112	131	178
	C-114	184	180	165	158	165	156	158	158	152	162	166	158	163	138	158
	C1312279	150	168	155	141	135	130	131	133	187	123	131	132	180	151	135
	mean	**148.3**	**165.0**	**150.8**	**145.8**	**142.0**	**132.8**	**146.0**	**145.0**	**142.5**	**136.0**	**138.3**	**140.3**	**149.3**	**135.8**	**152.3**
	SEM	**15.6**	**6.2**	**5.8**	**4.2**	**8.5**	**9.2**	**10.4**	**10.5**	**17.7**	**9.8**	**10.5**	**6.7**	**14.6**	**5.9**	**10.0**
AST-004 Low	C-158	180	178	152	153	155	154	179	179	178	178	177	178	188	163	175
	C-121	130	179	156	161	146	151	156	145	133	141	159	164	169	139	155
	C-145	162	173	171	165	153	138	141	136	141	145	159	154	148	140	171
	C-118	171	176	168	151	140	136	154	147	137	136	149	161	162	171	151
	mean	**160.8**	**176.5**	**161.8**	**157.5**	**148.5**	**144.8**	**157.5**	**151.8**	**147.3**	**150.0**	**161.0**	**164.3**	**166.8**	**153.3**	**163.0**
	SEM	**10.9**	**1.3**	**4.6**	**3.3**	**3.4**	**4.5**	**7.9**	**9.4**	**10.4**	**9.5**	**5.8**	**5.0**	**8.3**	**8.1**	**5.9**
AST-004 Mid	C-112	169	187	174	169	172	155	180	176	171	165	172	180	179	128	131
	C-132	162	154	140	147	139	146	141	140	144	151	156	165	158	128	153
	C-146	211	214	180	179	175	174	180	168	146	158	168	167	178	178	188
	1501051	145	148	138	147	138	141	137	138	134	142	141	137	138	142	151
	mean	**171.8**	**175.8**	**158.0**	**160.5**	**156.0**	**154.0**	**159.5**	**155.5**	**148.8**	**154.0**	**159.3**	**162.3**	**163.3**	**144.0**	**155.8**
	SEM	**14.0**	**15.4**	**11.0**	**8.1**	**10.1**	**7.3**	**11.9**	**9.7**	**7.9**	**4.9**	**7.0**	**9.0**	**9.7**	**11.8**	**11.8**
AST-004 High	C-122	122	137	147	142	118	113	116	116	120	120	124	123	121	141	152
	C-123	176	159	130	129	133	115	161	155	108	131	135	131	135	141	185
	C-109	136	134	130	130	136	128	124	117	113	113	114	109	127	172	165
	C-105	175	156	148	141	143	127	123	124	120	134	174	176	180	153	142
	mean	**152.3**	**146.5**	**138.8**	**135.5**	**132.5**	**120.8**	**131.0**	**128.0**	**115.3**	**124.5**	**136.8**	**134.8**	**140.8**	**151.8**	**161.0**
	SEM	**13.7**	**6.4**	**5.1**	**3.5**	**5.3**	**3.9**	**10.2**	**9.2**	**2.9**	**4.9**	**13.1**	**14.5**	**13.4**	**7.3**	**9.3**

Normal Physiological Range 100-180 beats/minute. Yellow highlighted cells show brief deviations outside of normal range.

Supplementary Table V. Individual and Group Mean pO_2 (mm Hg) Before and Following MCA Occlusion.

Group	ID	Time post-occlusion (hr.)												
		Presurgery	After MCAO	1.0	1.5	1.8	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
Vehicle	C-107	175	167	193	193	192	184	182	173	132	163	176	166	155
	C-108	165	152	163	165	166	156	157	147	144	169	178	187	175
	C-97	153	152	183	186	179	177	180	186	138	152	160	151	127
	C-103	170	173	191	193	191	185	182	180	160	158	159	165	163
	mean	**165.8**	**161.0**	**182.5**	**184.3**	**182.0**	**175.5**	**175.3**	**171.5**	**143.5**	**160.5**	**168.3**	**167.3**	**155.0**
	SEM	**4.7**	**5.3**	**6.8**	**6.6**	**6.1**	**6.7**	**6.1**	**8.6**	**6.0**	**3.6**	**5.1**	**7.4**	**10.2**
AST-004 Very Low	C-99	165	173	184	154	172	168	170	160	159	147	148	154	130
	C-113	176	175	172	172	176	163	173	166	149	163	154	137	147
	C-114	161	182	189	203	215	171	156	209	162	157	199	156	119
	C1312279	170	174	167	210	199	203	213	198	156	158	150	195	169
	mean	**168.0**	**176.0**	**178.0**	**184.8**	**190.5**	**176.3**	**178.0**	**183.3**	**156.5**	**156.3**	**162.8**	**160.5**	**141.3**
	SEM	**3.2**	**2.0**	**5.1**	**13.2**	**10.1**	**9.1**	**12.2**	**12.0**	**2.8**	**3.4**	**12.1**	**12.3**	**10.9**
AST-004 Low	C-158	158	161	178	175	179	165	168	169	160	157	160	166	171
	C-121	174	171	179	185	185	184	186	185	164	156	160	158	165
	C-145	165	182	161	165	154	164	159	145	162	140	170	189	203
	C-118	171	166	182	187	177	168	170	187	189	165	149	163	198
	mean	**167.0**	**170.0**	**175.0**	**178.0**	**173.8**	**170.3**	**170.8**	**171.5**	**168.8**	**154.5**	**159.8**	**169.0**	**184.3**
	SEM	**3.5**	**4.5**	**4.7**	**5.1**	**6.8**	**4.7**	**5.6**	**9.7**	**6.8**	**5.2**	**4.3**	**6.9**	**9.5**
AST-004 Mid	C-112	169	158	160	167	165	168	157	160	149	150	127	142	208
	C-132	167	152	157	154	159	170	189	140	154	148	177	157	170
	C-146	153	175	179	168	151	154	136	139	170	177	174	178	181
	1501051	168	155	184	193	164	155		171	152	145	144	178	145
	mean	**164.3**	**160.0**	**170.0**	**170.5**	**159.8**	**161.8**	**160.7**	**152.5**	**156.3**	**155.0**	**155.5**	**163.8**	**176.0**
	SEM	**3.8**	**5.1**	**6.7**	**8.1**	**3.2**	**4.2**	**15.4**	**7.8**	**4.7**	**7.4**	**12.1**	**8.8**	**13.1**
AST-004 High	C-122	93	166	167	179	167	161	166	173	142	153	165	165	128
	C-123	140	149	203	188	218	187	201	187	164	144	164	164	151
	C-109	175	178	172	181	180	181	179	186	160	157	159	155	168
	C-105	176	152	157	156	163	168	183	127	157	167	163	161	172
	mean	**146.0**	**161.3**	**174.8**	**176.0**	**182.0**	**174.3**	**182.3**	**168.3**	**155.8**	**155.3**	**162.8**	**161.3**	**154.8**
	SEM	**19.5**	**6.7**	**9.9**	**6.9**	**12.5**	**5.9**	**7.2**	**14.1**	**4.8**	**4.8**	**1.3**	**2.3**	**10.0**

Normal Physiological Range 150-250 mm Hg. Yellow highlighted cells show brief deviations outside of normal range.

Supplementary Table VI. Individual and Group Mean pCO_2 (mm Hg) Before and Following MCA Occlusion.

Group	ID	Time post-occlusion (hr.)												
		Presurgery	After MCAO	1.0	1.5	1.8	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
Vehicle	C-107	34	37	35	35	38	36	36	37	37	34	35	36	34
	C-108	32	37	38	33	35	32	30	36	31	36	37	35	34
	C-97	36	41	40	38	30	36	31	34	31	31	38	35	35
	C-103	31	31	39	35	27	37	37	33	31	25	33	28	31
	mean	**34**	**37**	**38**	**35**	**32**	**35**	**34**	**35**	**33**	**32**	**36**	**33**	**34**
	SEM	**1**	**2**	**1**	**1**	**3**	**1**	**2**	**1**	**2**	**2**	**1**	**2**	**1**
AST-004 Very Low	C-99	32	31	37	46	39	30	39	36	31	32	28	28	33
	C-113	34	33	37	43	38	43	38	36	34	32	32	36	32
	C-114	35	36	37	34	44	30	36	43	34	34	26	26	38
	C1312279	32	34	34	33	34	35	33	30	39	30	34	36	32
	mean	**33**	**34**	**36**	**39**	**39**	**34**	**36**	**36**	**35**	**32**	**30**	**31**	**34**
	SEM	**1**	**1**	**1**	**3**	**2**	**3**	**1**	**3**	**2**	**1**	**2**	**3**	**1**
AST-004 Low	C-158	35	33	31	38	37	41	37	40	31	30	35	32	35
	C-121	38	36	44	40	35	38	33	41	37	35	34	33	38
	C-145	35	60	36	40	41	37	29	46	33	32	32	34	33
	C-118	29	30	39	41	28	34	50	36	29	36	39	32	40
	mean	**34**	**40**	**38**	**40**	**35**	**38**	**37**	**41**	**32**	**33**	**35**	**33**	**37**
	SEM	**2**	**7**	**3**	**1**	**3**	**1**	**4**	**2**	**2**	**1**	**1**	**1**	**2**
AST-004 Mid	C-112	30	29	34	34	33	34	32	33	35	29	35	37	28
	C-132	30	39	41	40	33	38	38	41	41	37	34	34	30
	C-146	37	33	41	40	44	28	37	43	32	30	26	35	33
	1501051	31	32	48	44	34	40	-	30	37	37	40	20	28
	mean	**32**	**33**	**41**	**39**	**36**	**35**	**36**	**37**	**36**	**33**	**34**	**32**	**30**
	SEM	**2**	**2**	**3**	**2**	**3**	**3**	**2**	**3**	**2**	**2**	**3**	**4**	**1**
AST-004 High	C-122	35	27	31	30	31	33	37	33	33	34	25	34	31
	C-123	38	33	33	29	33	31	38	38	46	47	37	33	35
	C-109	37	33	43	40	36	35	38	34	34	32	32	36	34
	C-105	33	41	50	36	43	38	29	42	37	32	35	36	39
	mean	**36**	**34**	**39**	**34**	**36**	**34**	**36**	**37**	**37**	**36**	**32**	**35**	**35**
	SEM	**1**	**3**	**4**	**2**	**3**	**1**	**2**	**2**	**3**	**4**	**3**	**1**	**1**

Normal Physiological Range 30-40 mm Hg. Yellow highlighted cells show brief deviations outside of normal range.

Supplementary Table VII. Individual and Group Mean sO_2 (%) Before and Following MCA Occlusion.

Group	ID	Time post-occlusion (hr.)												
		Presurgery	After MCAO	1.0	1.5	1.8	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
Vehicle	C-107	100	100	100	100	100	100	100	100	99	100	100	100	100
	C-108	100	100	100	100	100	100	100	100	100	100	100	100	100
	C-97	100	99	100	100	100	100	100	100	99	100	100	99	99
	C-103	100	100	100	100	100	100	100	100	100	100	100	100	100
	mean	**100.0**	**99.8**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**99.5**	**100.0**	**100.0**	**99.8**	**99.8**
	SEM	**0.0**	**0.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.3**	**0.0**	**0.0**	**0.3**	**0.3**
AST-004 Very Low	C-99	100	100	100	99	100	100	100	100	100	100	100	100	99
	C-113	100	100	100	100	100	99	100	100	99	100	100	99	99
	C-114	100	100	100	100	100	100	99	100	100	100	100	100	99
	C1312279	100	100	100	100	100	100	100	100	99	100	100	100	100
	mean	**100.0**	**100.0**	**100.0**	**99.8**	**100.0**	**99.8**	**99.8**	**100.0**	**99.5**	**100.0**	**100.0**	**99.8**	**99.3**
	SEM	**0.0**	**0.0**	**0.0**	**0.3**	**0.0**	**0.3**	**0.3**	**0.0**	**0.3**	**0.0**	**0.0**	**0.3**	**0.3**
AST-004 Low	C-158	100	100	100	100	100	100	100	100	100	100	100	100	100
	C-121	100	100	100	100	100	100	100	100	100	100	100	100	100
	C-145	100	97	100	100	99	100	100	99	100	99	100	100	100
	C-118	100	100	100	100	100	100	99	100	100	100	99	100	100
	mean	**100.0**	**99.3**	**100.0**	**100.0**	**99.8**	**100.0**	**99.8**	**99.8**	**100.0**	**99.8**	**99.8**	**100.0**	**100.0**
	SEM	**0.0**	**0.8**	**0.0**	**0.0**	**0.3**	**0.0**	**0.3**	**0.3**	**0.0**	**0.3**	**0.3**	**0.0**	**0.0**
AST-004 Mid	C-112	100	100	100	100	100	100	100	100	99	100	99	99	100
	C-132	100	99	99	99	99	100	100	99	99	99	100	99	100
	C-146	100	100	100	100	99	100	99	99	100	100	100	100	100
	1501051	100	100	100	100	100	99		100	99	99	99	100	100
	mean	**100.0**	**99.8**	**99.8**	**99.8**	**99.5**	**99.8**	**99.7**	**99.5**	**99.3**	**99.5**	**99.5**	**99.5**	**100.0**
	SEM	**0.0**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.0**
AST-004 High	C-122	98	100	100	100	100	100	100	100	99	99	100	100	99
	C-123	99	100	100	100	100	100	100	100	99	99	100	100	99
	C-109	100	100	100	100	100	100	100	100	100	100	100	100	100
	C-105	100	99	99	99	99	100	100	99	100	100	100	100	100
	mean	**99.3**	**99.8**	**99.8**	**99.8**	**99.8**	**100.0**	**100.0**	**99.8**	**99.5**	**99.5**	**100.0**	**100.0**	**99.5**
	SEM	**0.5**	**0.3**	**0.3**	**0.3**	**0.3**	**0.0**	**0.0**	**0.3**	**0.3**	**0.3**	**0.0**	**0.0**	**0.3**

Normal Physiological Range >95%.

Supplementary Table VIII. Individual and Group Mean pH Before and Following MCA Occlusion.

Group	ID	Time post-occlusion (hr.)												
		Presurgery	After MCAO	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
Vehicle	C-107	7.51	7.49	7.51	7.49	7.46	7.49	7.45	7.46	7.44	7.44	7.46	7.44	7.47
	C-108	7.55	7.51	7.54	7.58	7.57	7.59	7.59	7.54	7.57	7.55	7.53	7.56	7.51
	C-97	7.55	7.49	7.52	7.56	7.55	7.50	7.59	7.52	7.55	7.51	7.50	7.47	7.55
	C-103	7.47	7.48	7.35	7.54	7.55	7.49	7.48	7.50	7.50	7.48	7.47	7.47	7.50
	mean	**7.52**	**7.49**	**7.48**	**7.54**	**7.53**	**7.52**	**7.53**	**7.50**	**7.51**	**7.49**	**7.49**	**7.48**	**7.51**
	SEM	**0.02**	**0.01**	**0.04**	**0.02**	**0.02**	**0.03**	**0.04**	**0.02**	**0.03**	**0.03**	**0.02**	**0.03**	**0.02**
AST-004 Very Low	C-99	7.54	7.51	7.46	7.39	7.44	7.46	7.43	7.45	7.52	7.52	7.53	7.56	7.47
	C-113	7.47	7.46	7.40	7.38	7.38	7.36	7.42	7.43	7.49	7.49	7.49	7.46	7.50
	C-114	7.52	7.47	7.46	7.46	7.38	7.45	7.45	7.43	7.47	7.46	7.57	7.46	7.43
	C1312279	7.56	7.54	7.54	7.55	7.53	7.56	7.56	7.57	7.47	7.50	7.52	7.47	7.51
	mean	**7.52**	**7.49**	**7.46**	**7.44**	**7.43**	**7.46**	**7.47**	**7.47**	**7.49**	**7.49**	**7.53**	**7.49**	**7.48**
	SEM	**0.02**	**0.02**	**0.03**	**0.04**	**0.03**	**0.04**	**0.03**	**0.04**	**0.01**	**0.01**	**0.02**	**0.02**	**0.02**
AST-004 Low	C-158	7.58	7.62	7.66	7.59	7.59	7.53	7.51	7.52	7.61	7.60	7.54	7.52	7.52
	C-121	7.50	7.52	7.43	7.45	7.48	7.50	7.48	7.47	7.48	7.51	7.50	7.52	7.48
	C-145	7.50	6.67	7.47	7.45	7.45	7.47	7.43	7.40	7.49	7.52	7.48	7.50	7.50
	C-118	7.44	7.49	7.45	7.45	7.54	7.47	7.28	7.41	7.52	7.46	7.46	7.51	7.45
	mean	**7.50**	**7.33**	**7.50**	**7.49**	**7.52**	**7.49**	**7.43**	**7.45**	**7.53**	**7.52**	**7.50**	**7.51**	**7.49**
	SEM	**0.03**	**0.22**	**0.05**	**0.03**	**0.03**	**0.02**	**0.05**	**0.03**	**0.03**	**0.03**	**0.02**	**0.01**	**0.02**
AST-004 Mid	C-112	7.54	7.56	7.56	7.53	7.49	7.49	7.51	7.50	7.46	7.52	7.47	7.45	7.49
	C-132	7.45	7.42	7.39	7.38	7.37	7.41	7.37	7.32	7.35	7.38	7.42	7.44	7.47
	C-146	7.50	7.50	7.42	7.45	7.42	7.60	7.46	7.39	7.52	7.53	7.55	7.48	7.49
	1501051	7.50	7.50	7.41	7.44	7.52	7.45		7.53	7.45	7.44	7.42	7.43	7.55
	mean	**7.50**	**7.50**	**7.45**	**7.45**	**7.45**	**7.49**	**7.45**	**7.44**	**7.45**	**7.47**	**7.46**	**7.45**	**7.50**
	SEM	**0.02**	**0.03**	**0.04**	**0.03**	**0.03**	**0.04**	**0.04**	**0.05**	**0.04**	**0.04**	**0.03**	**0.01**	**0.02**
AST-004 High	C-122	7.51	7.54	7.53	7.52	7.51	7.52	7.49	7.51	7.49	7.47	7.42	7.47	7.46
	C-123	7.46	7.54	7.57	7.57	7.56	7.55	7.51	7.48	7.43	7.42	7.50	7.53	7.49
	C-109	7.54	7.50	7.45	7.49	7.53	7.53	7.49	7.49	7.52	7.52	7.49	7.48	7.51
	C-105	7.45	7.43	7.38	7.34	7.37	7.44	7.48	7.41	7.47	7.52	7.46	7.48	7.45
	mean	**7.49**	**7.50**	**7.48**	**7.48**	**7.49**	**7.51**	**7.49**	**7.47**	**7.48**	**7.48**	**7.47**	**7.49**	**7.48**
	SEM	**0.02**	**0.03**	**0.04**	**0.05**	**0.04**	**0.03**	**0.01**	**0.02**	**0.02**	**0.02**	**0.02**	**0.01**	**0.01**

Normal Physiological Range 7.4-7.6. Yellow highlighted cells show deviations outside of normal range.

Supplementary Table IX. Pharmacokinetic parameters of AST-004 in normal and tMCAO nonhuman primates following intravenous bolus administration.

Dose Group	Intravenous Bolus (mg/kg)	C_0 (ng/ml)	Vdss (L/kg)	Plasma Clearance (mL/min/kg)	Half-Life (hr)	Plasma;Brain Unbound Fractions (%)
Normal (n=3)	0.5	329 ± 60	3.1 ±0.1	29 ±3	1.3 ±0.1	26±5; 56±5
tMCAO (n=1)	0.5	544	3.0	28	1.5	N.D.

Results are Means ± SEM
N.D. Not Determined

Supplementary Table X. AST-004 Plasma and cerebrospinal pharmacokinetics and predicted/actual steady state plasma concentrations in tMCAO cynomolgus monkeys following intravenous bolus/constant rate infusion regimens in a pre-efficacy pharmacokinetic study

Group	Bolus Dose (mg/kg)/ Infusion (mg/kg/hr)	Predicted Steady-State [Plasma] (ng/mL)	Actual Time-Averaged Steady-State [Plasma] (ng/mL)	[Plasma]; [CSF] At 22-hour End of Dosing (ng/mL)	CSF/Plasma At 22-hour End of Dosing (%)
tMCAO (n=1)	**0.11/0.0582**	35	25	18; 1.7	9.4
tMCAO (n=1)	**1.7/0.9**	540	363	179; 15	8.4
tMCAO (n=1)	**5.2/2.76**	1660	1812	987; 64	6.6

Supplementary Table XI. Neurological Deficit Score (NDS)

Category		Score
Consciousness		
	normal, consistently alert	0
	consciousness and aggressive	4
	consciousness and evasive	6
	consciousness and clouded and tolerant	8
	drowsiness, aroused with stimulation	10
	lethargy, eyes opened by intense stimulation	16
	stupor, aroused with persistent stimulation	20
	light coma, reflex movement only	24
	deep coma, no movement	28
Sensory System		
Facial sensation		*(ipsi-, contralateral)*
	reacts consistently to touch in any facial area	0/0
	absent, does not react to touch in any area of face	3/3
Pinna reflex		
	twitch ear in response to outer/inner hair stimulation	0/0
	absent, does not move ear in response to touch	3/3
Pain reflex		
	strong, quick, complete withdrawal from toe pinch	0/0
	weak, slow, incomplete or inconsistent withdrawal from toe pinch	3/3
	absent, no withdrawal from toe pinch	5/5
Motor System		
Upper limb		
	Normal	0/0
	reduced strength and skilled movement	2/2
	movement possible, but not against gravity	4/4
	paralyzed	6/6
Lower limb		
	Normal	0/0
	raise with flexion of knee against gravity	2/2
	movement possible, but not against gravity	4/4
	paralyzed and useless	6/6
Upper limb tone		
	Normal	0/0
	overtly spastic or flaccid	3/3
Lower limb tone		
	Normal	0/0
	overtly spastic or flaccid	3/3
Musculoskeletal Coordination		
	walks normally	0
	minimal incoordination, walks with some gait impairment	4
	uncoordinated, but able to climb a wire net	6
	stands independently, falls within a few steps	10
	sits, just able to circle	12
	posed lateral or dorsal recumbence	16
	no movement	18

The maximum score is 28 for consciousness, 22 for sensory system, 32 for motor system and 18 for musculoskeletal coordination, the maximum total score is 100.

Supplementary Table XII. Study excluded subjects and their replacements				
Group	Subject ID	Time of Exclusion	Reason	Replacement Subject
Vehicle	C-126	Day 4	Death: Basal ganglial hemorrhage	C-107
Very Low	C-153	8 hours post-Occlusion	Death: Ipsilateral edema	C-146
Very Low	C-137	1.5 hours post-Occlusion	1.5-hour lesion volume >90% PI exclusion criterion	C-114
Mid	C-98	Day 1 post-MRI	Death: Unknown	C-114
High	C-134	1.5 hours post-Occlusion	1.5-hour lesion volume >90% PI exclusion criterion	C-109