Appendix 1. Table A. Adverse Events and Clinical Outcomes with adjunctive Sertraline through 12 weeks

Clinical Outcomes	Sertraline 100mg/day	Sertraline 200mg/day	Sertraline 300mg/day	Sertraline 400mg/day
N enrolled	17	60	50	45
Total number Grade 4 AEs	6	14	19	7
Grade 4 AEs, cumulative incidence	5 (29%)	9 (15%)	15 (30%)	7 (16%)
Grade 5 AEs, cryptococcal related	5 (29%)	13 (22%)	14 (28%)	10 (22%)
Grade 5 AEs, non-cryptococcal related	5 (29%)	6 (10%)	7 (14%)	9 (20%)
Cryptococcal Relapse	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Paradoxical IRIS, cumulative incidence*	0 (0%)	1 (7%)	0 (0%)	1 (9%)
Nausea, Vomiting, or Diarrhea, ≥1 event	15 (88%)	44 (73%)	31 (62%)	30 (67%)
Serotonin Syndrome	0 (0%)	0 (0%)	0 (0%)	1 (2%)**
Premature sertraline dose reduction, all cause	0 (0%)	0 (0%)	1 (2%)	0 (0%)
Premature sertraline discontinuation	0 (0%)	1 (1.6%)	4 (8%)	1 (2.2%)
Lost to Follow up	0 (0%)	3 (5%)	2 (4.0%)	0 (0%)

All participants received two weeks of sertraline induction therapy in combination with amphotericin and fluconazole 800-1200mg/day. At the end of two weeks, all participants received 200mg/day until week 8 and then tapered through week 11.

[1] Cumulative incidence calculated from competing risk model for paradoxical CM-IRIS among patients with first episode of cryptococcal meningitis, who were ART-naïve at baseline and who survived to initiate ART or switched to second line ART (N=3 for 100mg/day; N=14 for 200mg/day; N=15 for 300mg/day; N=11 for 400mg/day).

**Protocol deviation by the participant taking 800mg/day for three days, resulting in a moderate serotonin syndrome.

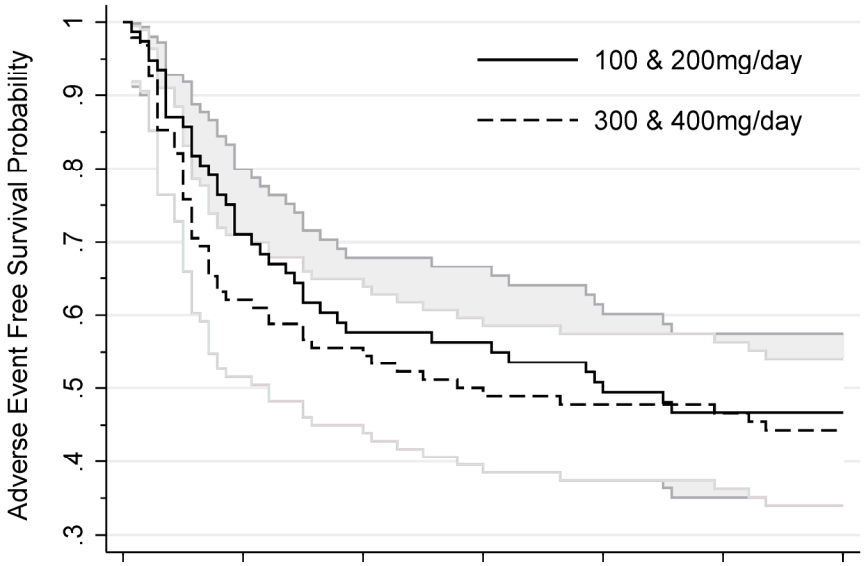
Appendix 1. Table B. Line Listing of New Grade 4-5 Adverse Events

N	Days from Study Entry	Event
Grade 4		
3	1*,4*,20*	*Hyperkalemia, >7.0 mEq/L
5	7,9,11,12,14	Hypokalemia, <2.0 mEq/L
28	3,3,6,6,7,7,7,7,7,7,8,9,9,10,10,10,11,11,12,12,15,16,16,20,31,50,68,84	Anemia, Hemoglobin <6.5 g/dL
1	15	Elevated Bilirubin, >5x upper limit of normal
1	8	Metabolic alkalosis, HCO_3 >20 mEq/L
1	17	Metabolic acidosis, HCO_3 <8 mEq/L
1	20	Neutropenia, neutrophils < 500/µL
1	5	Hyponatremia
3	38, 50, 84	Leukopenia / Pancytopenia
1	87	Elevated Creatinine, >5mg/dL
1	90	Respiratory distress
1	7	Sepsis
Grade 5**		
1	90	Diarrhea
1	41	Altered mental status
1	63	Hemiparesis
1	72	Seizure
3	3,4,13	Respiratory distress
1	9	Renal failure
1	34	Hyperkalemia
2	6,9	Hypokalemia
1	34	Hyponatremia

*Probable hemolysis, repeat measurements within normal limits.
**Deaths unrelated to primary diagnosis of cryptococcal meningitis.

A line listing of adverse events observed in the 177 person historical cohort (excluding 35 deaths prior to COAT trial randomization) is published as supplemental material;[5] available at: www.nejm.org/doi/suppl/10.1056/NEJMoa1312884/suppl_file/nejmoa1312884_appendix.pdf

Appendix 1. Figure A. Kaplan-Meier estimates of time to first Grade 4 or 5 Adverse Event or other death, by sertraline dose groups 100 and 200mg/day versus 300 and 400mg/day. Shading represents 95% confidence interval.



Number at risk							
100 & 200mg/day	77	53	43	42	37	34	17
300 & 400mg/day	95	58	51	45	41	40	27

Appendix 1C. Electrocardiogram QTc Intervals with Adjunctive Sertraline Therapy

Serial electrocardiogram QTc intervals were measured in 54 consecutive participants using a MAC® 1200 ECG (GE Healthcare, Wauwatosa, WI). This ECG instrument produces computer generated QTc values, calculated via the Bazett formula. Normal QTc intervals are approximately 400ms with an upper limit of normal of 440 to 470ms. In participants receiving serial ECGs, QTc interval was measured on day 1 (n=54), day 7 (n=49), and day 14 (n=36) of treatment in those receiving sertraline at doses of 100mg (n=3), 200mg (n=22), 300mg (n=13), or 400mg (n=16) daily for the first 2 weeks of participation.

Data were analyzed using Stata/IC 13.1 (StataCorp, College Station, Texas) and evaluated against a type I error rate of 0.05. Changes in QTc intervals (ms) from diagnosis to 2 weeks of follow-up between dose groups (FDA-approved 100 and 200 mg/day participants vs. 300 and 400 mg/day participants) were evaluated via linear mixed models, which employed a random effect for participant and the interaction between dose group and time. Linear mixed models were also used to evaluate the relationship between change in QTc interval in relation to sex and ART status at diagnosis, with the interaction p-values reported.

Table 1C. Mean Electrocardiogram QTc Interval with Adjunctive Sertraline Therapy

	N	Day 1 QTc (ms)	N	Day 7 QTc (ms)	N	Day 14 QTc (ms)	P-value*
Sertraline Dose							
100 – 200mg/day	25	381 (367, 395)	23	391 (383, 399)	18	367 (343, 390)	0.56
300 – 400mg/day	29	383 (372, 394)	26	392 (368, 416)	18	355 (331, 380)	
300mg/day	13	384 (367, 401)	11	393 (381, 406)	8	340 (288, 393)	0.33
400mg/day	16	382 (366, 399)	15	390 (347, 434)	10	367 (343, 391)	
Sex							
Men	39	383 (373, 394)	35	389 (371, 407)	27	369 (352, 385)	0.35
Women	15	379 (363, 395)	14	396 (386, 406)	9	337 (291, 383)	
ART at Diagnosis							
Receiving ART	24	377 (361, 393)	23	399 (373, 424)	13	365 (340, 391)	0.50
Not receiving ART	30	386 (377, 396)	26	385 (373, 396)	23	358 (336, 381)	

Data displayed as mean (95%CI).

*P-values testing for interaction between follow-up time and sertraline dose group, sex, and ART at diagnosis, respectively, from linear mixed model.

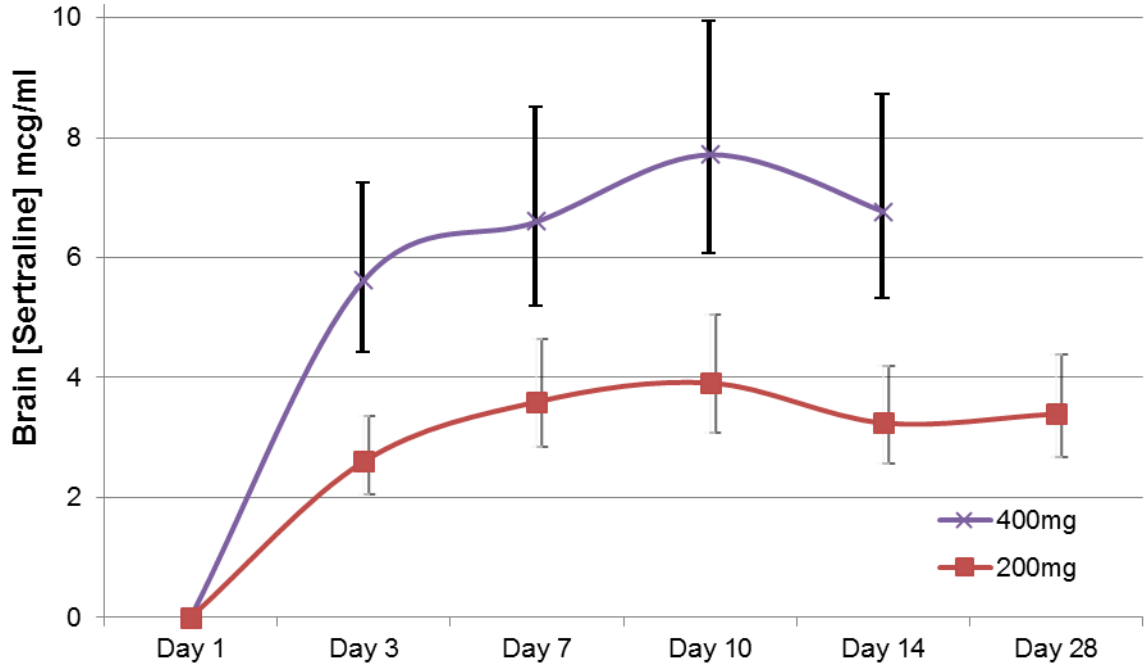
Appendix 2. Pharmacokinetics of Sertraline

A. Observed sertraline plasma concentrations (ng/mL) by administered daily dose.

Day	Sertraline Dose Cohort							
	100mg		200mg		300mg		400mg	
	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)
3	-	--	34	130 (81-245)	16	134 (99-229)	24	335 (219-445)
7	2	--	37	171 (75-314)	22	256 (131-364)	22	417 (269-539)
10	3	--	33	206 (83-325)	17	231 (98-423)	24	490 (254-621)
14	6	73 (42-206)	36	156 (77-258)	15	298 (177-420)	21	404 (173-612)
7-14	**11**	**80 (38-133)**	**49**	**201 (90-300)**	**31**	**252 (132-380)**	**30**	**399 (278-560)**
28*	-	--	46	141 (84-295)	-	--	-	--

*Experimental dose was administered for first 14 days of study, after which all participants received 200mg/day of sertraline.

B. Estimated Sertraline Brain Levels by dose: Median (IQR)



Sertraline dosing at 400mg/day achieves a median 6.7 μg/mL (IQR, 4.6-9.7) in brain tissue at steady state levels averaged between days 7-14, based on Monte Carlo simulation modeling of observed plasma levels and the published fold-change concentration into brain tissue.[9]

C. Supplemental Pharmacokinetic Methods:

Sertraline and *N*-desmethyl sertraline determination in human plasma or CSF

Instrumentation

At the University of Minnesota, the chromatographic instrument used was an Agilent 1200 series HPLC pump, auto sampler and column oven. The triple quadrupole mass spectrometer used was a TSQ Quantum, manufactured by Thermo Electron Corporation. All the parameters of LC and MS were controlled by Xcalibur® Version 2·07.

Liquid chromatographic conditions

Reversed-phase liquid chromatography (LC) was performed on an Agilent 1200 series HPLC at 150 μl/min flow rate with the auto sampler temperature set at 5 °C. Chromatographic column used was from Thermo Electron Corporation type Beta Basic C-4, 150 mm × 1.0 mm (Length x inner diameter), with 5 μm particle size and was maintained at 40 °C in the column oven. The mobile phase consisted of 0·5% acetic acid in DI water: 0·5% acetic acid in methanol in ratio of 35:65 (v/v).

Mass spectrometric conditions

The mass spectrometer was run in positive mode and multiple reaction monitoring (MRM) was used to monitor the ions. The protonated precursor to product ion transitions monitored:

- Sertraline: *m*/*z* 306·2 → 159·0
- Sertreline-d3 (internal standard): *m*/*z* 309·2 → 159·0
- *N*-desmethyl sertraline: *m*/*z* 292·1 → 159·0
- *N*-desmethyl sertraline-d4 (internal standard): *m*/*z* 296·1 → 159·0

The analysis data were acquired and quantified using by Xcalibur® Version 2·07

CSF sample preservation

Human CSF samples were preserved with 0·5% Tween 20 prior to freezing at −80 °C. Without 0·5% Tween 20, sertraline would precipitate out of CSF when frozen.

Sample extraction

Human plasma/CSF samples were stored at −80 °C and shipped on dry ice (-20°C). After thawing for 30 minutes, the samples were vortexed using a vortex mixer prior to pipetting. Using a micropipette, a 0·150 mL aliquot of plasma was transferred into 2·0 ml microtubes, to which was added 50 μL of the working solution of IS (internal standard). To the same tube, 150 μl of 5% NH_4OH was added and the tubes were briefly vortexed. Next, 1·25 mL of Ethyl Acetate was added to the microtubes followed by 10 minutes of vortexing with a multi-tube vortexer. The microtubes were centrifuged at 12,000 x g, for 5 minutes at 5°C. The supernatant layer was removed and transferred to a 13 x 100 mm test-tube and dried for 10 minutes with nitrogen at 37°C. Samples were reconstituted with 100 uL of mobile phase and 5 uL was injected onto the column for analysis.

Accuracy and precision
Sertraline: Total assay accuracy 99%, variability 3.0%
N-desmethyl sertraline: Total assay accuracy 95%, variability 4·2%

D. Monte Carlo Simulation

To determine the estimated proportion of persons with steady state sertraline levels in brain at therapeutic concentrations, we performed a Monte Carlo simulation based on the distributions of:

- Steady state plasma concentrations by dose between days 7-14 of induction therapy (Appendix 2 Table A).
- Published fold-change concentration into the brain over plasma, based on gamma distribution.[9]
    - Minimum = 10-fold
    - 25th percentile = 13·0-fold
    - Median 16·5-fold
    - 75th percentile = 21·3
    - Max = 47.0-fold truncated (Published = 57·3-fold maximum)
- Sertraline Minimum Inhibitory Concentration (MIC) distribution for *Cryptococcus* clinical isolates among 150 participants with first episode of meningitis

Sertraline MIC µg/mL	N	%	Cumulative %
1	10	7·8%	7·8%
2	25	19·5%	27·3%
4	73	57·0%	84·4%
6	9	7·0%	91·4%
8	11	8·6%	100·0%
Total	128		