Cryptococcal Meningitis: NEW WHO Guidelines

Single-Dose Liposomal Amphotericin B Treatment for Cryptococcal Meningitis ("AMBITION"; NEJM: 2022:386:1109-20)

Robert A. Parker, ScD

rparker4@mgh.harvard.edu

Disclosures

- Nothing to disclose

Outline

- Background and current guideline treatment (WHO and US)
- Design of the AMBITION Study
    - Aside: superiority, equivalence, and non-inferiority studies
- Results and Interpretation
- Conclusions

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Background (1)

- Cryptococcal meningitis occurs in HIV+ individual with very low CD4 counts (generally under 100 cells/mm^3)
- Accounts for 10-20% of **all** HIV-related mortality in Africa
- Very poor outcomes – 24% mortality at 10 weeks in a recent clinical trial

Guideline Treatment (1)

- WHO Guidelines: Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Available for download at https://www.who.int/publications/i/item/9789241550277 (March 2018 supplement to the 2016 Guidelines)
  - **UPDATE AT END OF TALK!!!!**
- US Guidelines: https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-opportunistic-infection/cryptococcosis?view=full accessed on 24 April 2022, revised Jan 12, 2022

Guideline Treatment (2)

Three phases:

- Induction therapy: 2 weeks
- Consolidation phase (WHO: 8 weeks; US: 8 weeks or more)
- Maintenance phase

AMBITION study:

- Primary endpoint was 10 weeks after induction (2 weeks) and consolidation (8 weeks) phases
- Secondary endpoint included 16 weeks: maintenance phase

Guideline Treatment (3)
Induction Phase

Period	WHO (2018)	US (2022)
	RECOMMENDED REGIMEN (WHO) / SECOND PREFERRED REGIMEN (US)	
Days 1-7	Amphotericin B deoxycholate (1.0 mg/kg/day) + Flucytosine (100 mg/kg/day divided into 4 doses)	Amphotericin B deoxycholate 0.7–1.0 mg/kg/day + Flucytosine 25 mg/kg PO four times a day
Days 8-14	Fluconazole 1200 mg/day	SAME AS ABOVE
	ALTERNATIVE REGIMEN	
Days 1-7	Fluconazole (1200 mg/day) + Flucytosine (100 mg/kg/day divided into 4 doses)	Liposomal amphotericin B 3–4 mg/kg/day + Flucytosine 25 mg/kg PO four times a day
Days 8-14	Fluconazole (1200 mg/day) + Flucytosine (100 mg/kg/day divided into 4 doses)	Fluconazole 1200 mg/day

As I understand it, liposomal amphotericin B 3-4 mg/kg is considered equivalent to amphotericin B deoxycholate 0.7-1.0 mg/kg

Guideline Treatment (4)
Consolidation and Maintenance Phases

Both WHO and US guidelines consider

- Consolidation: fluconazole 800 mg daily after induction phase
  - WHO: 8 weeks
  - US: At least 8 weeks
- Maintenance: fluconazole 200 mg daily after completion of the consolidation phase

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Design of the AMBITION Study (1)

AMBITION Study is:

- Randomized controlled **non-inferiority** trial (RCT)
- Enrollment completed before randomization
  - 1:1 randomization between the intervention and control
  - Random blocks of different sizes (4 and 6)
  - Stratified by site
- After enrollment/randomization, participants could be excluded based on laboratory results
- Treatment was administered open-label (**not** blinded)

Design of the AMBITION Study (2)

- Primary endpoint: all-cause mortality at 10 weeks
- Secondary endpoints:
  - All cause mortality at 2 weeks, 4 weeks, 16 weeks, and time to event analysis
  - Rate of fungal clearance over 14 days
  - Clinical and laboratory adverse events (AEs)
- Endpoint assessment was collected by clinical follow-up (through week 10) or phone/visit if patient missed the visit
  - Phone/visit done by study team, so potentially endpoint assessment was **not** done by a blinded investigator
  - Primary (10 weeks) and arguably all the secondary endpoints are all "objective" so the lack of blinded assessment is unlikely to be a problem

Design of the AMBITION Study (3)

Participants:

- First episode of cryptococcal meningitis (CM)
  - Positive India ink stain or cryptococcal antigen (CrAg) flow assay [IMMY] on cerebrospinal fluid (CSF) sample
- HIV-positive adults (≥ 18 years old) (tested if not known)
- Willing to consent or, if unable to consent, has a next of kin agreeing for the patient to participate

Design of the AMBITION Study (4)

Participants (continued): exclusion criteria

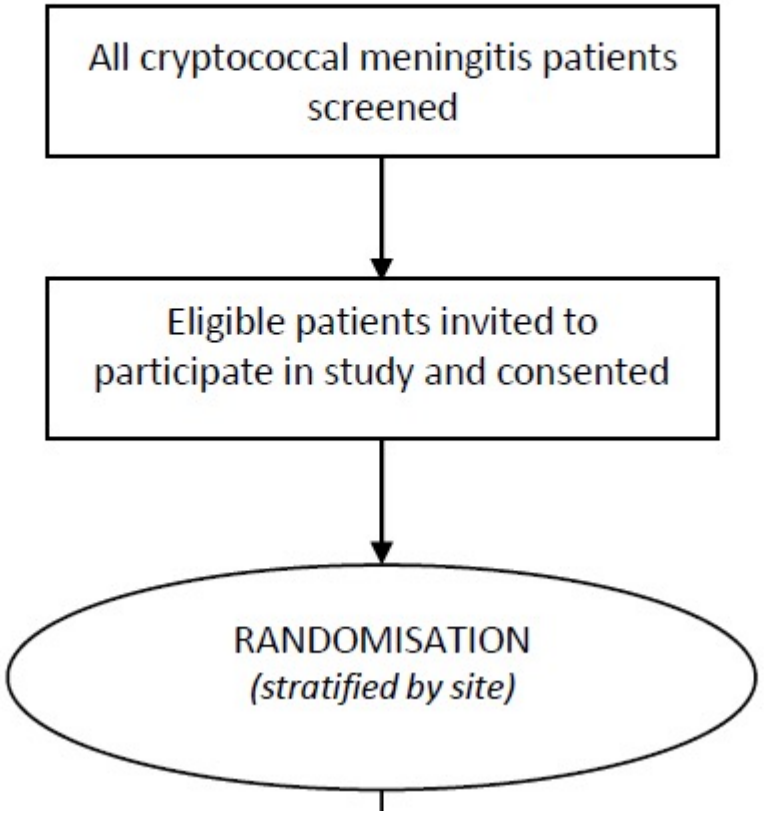
- Pregnant or breastfeeding
- Previous serious reaction to amphotericin, flucytosine, or fluconazole
- More than two doses of antifungal treatment for CM (any amphotericin B or fluconazole ≥ 800 mg/day)
- Contraindicated medication
- Late exclusion criteria:
  - HIV negative
  - Alanine aminotransferase > 5x ULN;
  - Polymorphonuclear leukocyte count < 500 / mm^3
  - Platelet count < 50,000 / / mm^3

Design of the AMBITION Study (5)

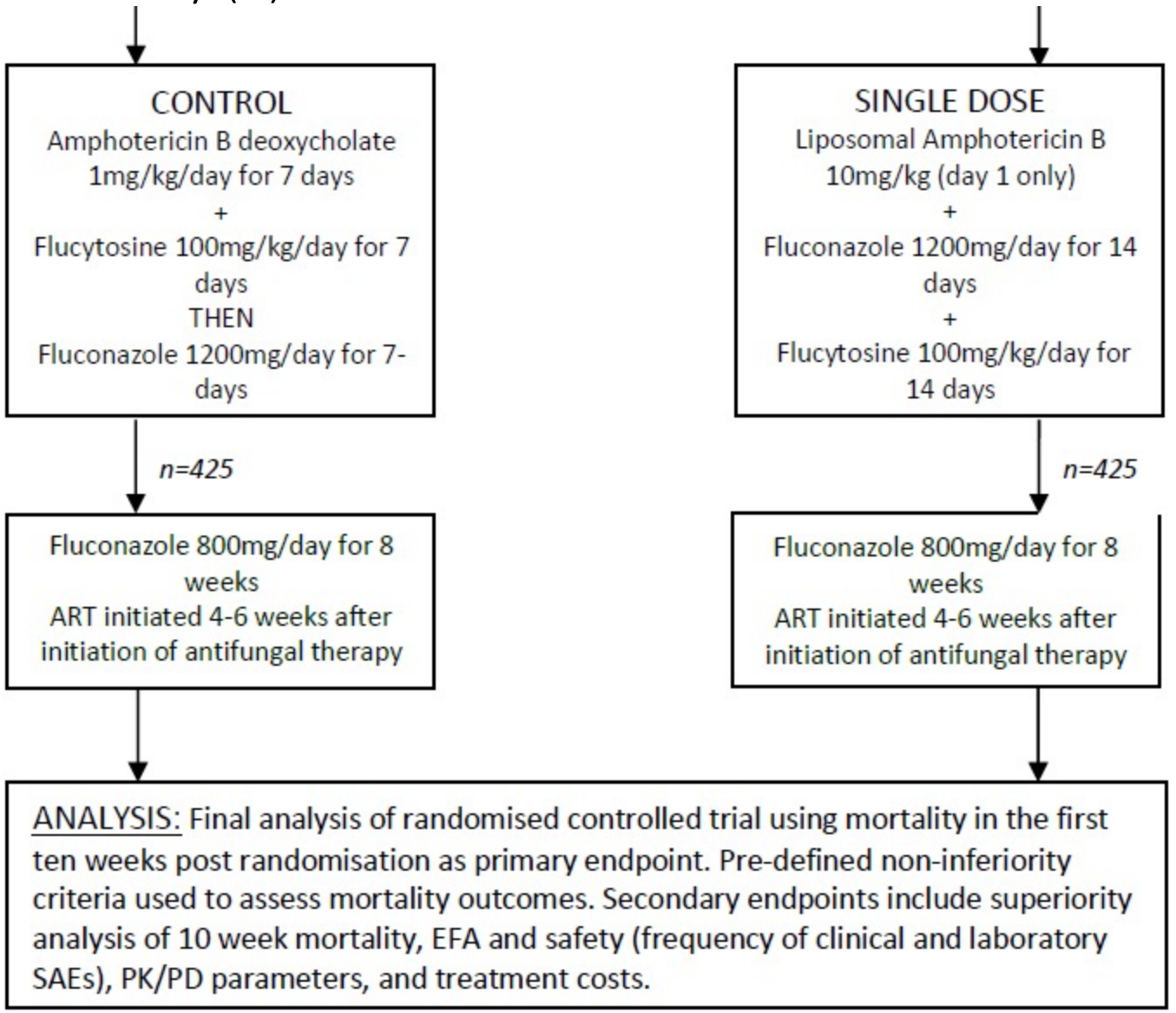
Period	WHO RECOMMENDED REGIMEN (CONTROL ARM)	INTERVENTION
Induction: Days 1	Amphotericin B deoxycholate (1.0 mg/kg/day) + Flucytosine (100 mg/kg/day *divided into 4 doses*)	Liposomal amphotericin B (10mg/kg) + Flucytosine (100 mg/kg) + Fluconazole (1200 mg)
Induction: Days 2-7	SAME	Flucytosine (100 mg/kg) + Fluconazole (1200 mg)
Induction: Days 8-14	Fluconazole 1200 mg/day	Flucytosine (100 mg/kg) + Fluconazole (1200 mg)
Consolidation: Weeks 3-10	Fluconazole 800 mg/day	Fluconazole 800 mg/day
Maintenance: After week 10	Fluconazole 200 mg/day	Fluconazole 200 mg/day
	This is the WHO preferred option	WHO alternative option

Design of the AMBITION Study (6)

Figure 1. Trial Entry, Randomisation and Treatment



Design of the AMBITION Study (7)



Design of the AMBITION Study (8)

Additional supportive care:

- Treated in-hospital for a minimum of 7 days
- Laboratory blood tests at baseline and then every 2-3 days
- Lumbar punctures at baseline, 7 and 14 days (for endpoint)
  - Daily therapeutic lumbar punctures for increased intracranial pressure (>20cmH20)
- ART therapy started weeks 4-6

Design of the AMBITION Study (9)

- Primary endpoint is that mortality at 10 weeks is **non-inferior** in the experimental arm compared to the control arm.
- Primary analysis is stated to be intention to treat (all randomized participants included in analysis independent of treatment)
- In my opinion it is a modified intent to treat (since some people excluded after randomization)

Design of the AMBITION Study (10)

Additional analyses:

- Per-protocol analysis – some participants excluded because of protocol problems
- Covariate adjustment including:
  - site (country)
  - age (<= median vs > median)
  - Sex
  - Glasgow Coma Scale (<15, 15)
  - Log10 CD4 count
  - Log10 Colony Forming Units (CFU)
  - Previous exposure to ART at enrollment

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- Background and current guideline treatment (WHO and US)
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    - **Aside: superiority, equivalence, and non-inferiority studies**
- Results and interpretation
- Conclusions

Aside: superiority, equivalence, and non-inferiority studies (1)

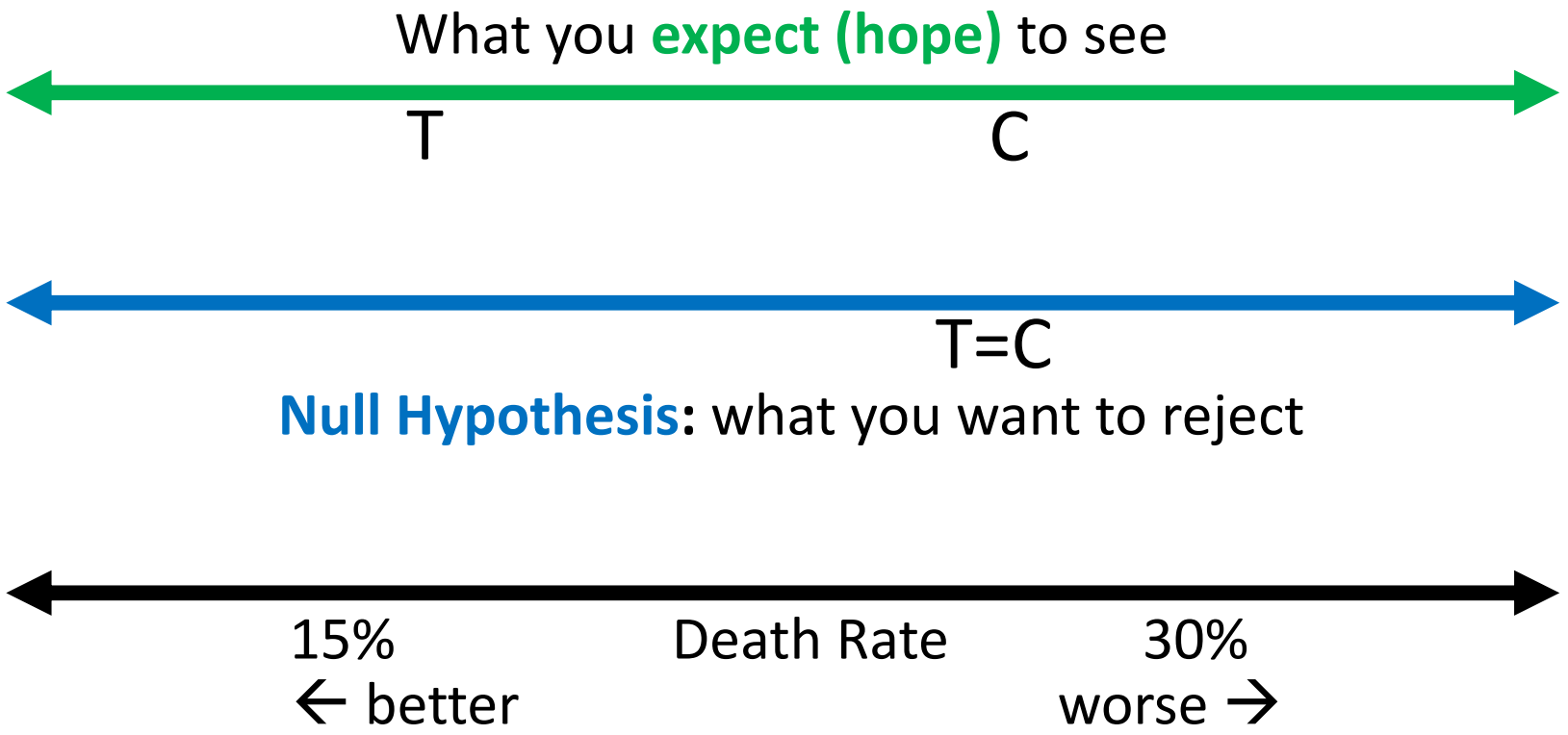
- Statistics works by setting up a “straw man” called the **null hypothesis**
- The study is done to show that the **null hypothesis** is not likely to be true, so that we can reject the **null hypothesis**
- We want to beat up the “straw man”, showing that the “straw man” is really unlikely

Aside: superiority, equivalence, and non-inferiority studies (2)

- The **P-value** measures how likely the observed data would have occurred if the **null hypothesis** were true.
  - A small **P-value** means that the **null hypothesis** was unlikely to occur **by chance**.
  - **It does not assess any systematic problems with a study.**
- Based on a convention from many years ago, a **P-value** < 0.05 is considered **statistically significant**, but this is an arbitrary cut-off

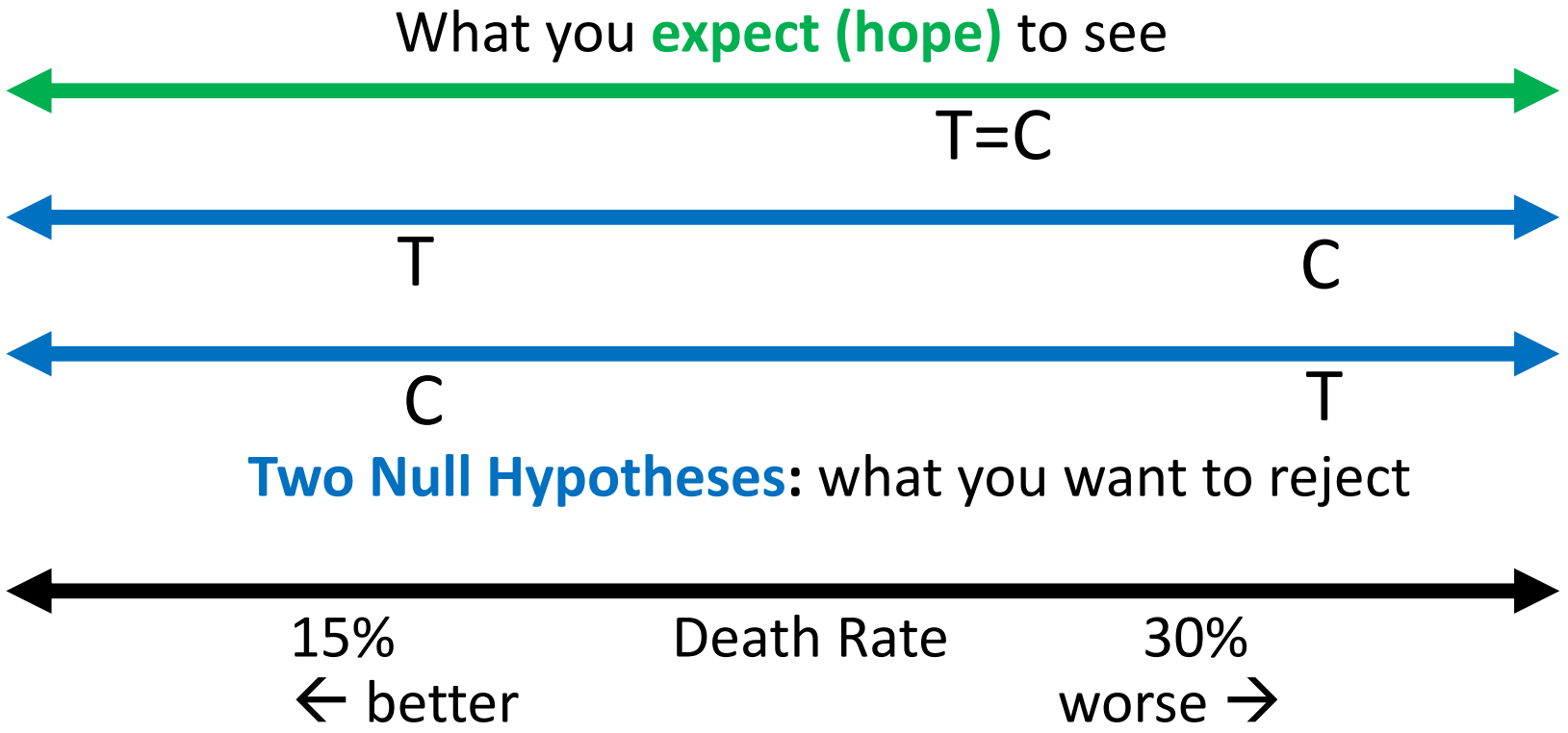
Aside: superiority, equivalence, and non-inferiority studies (3)

- Most studies are **superiority** studies: they try to show that the new treatment (T) is better than the existing standard of care (SOC; C)



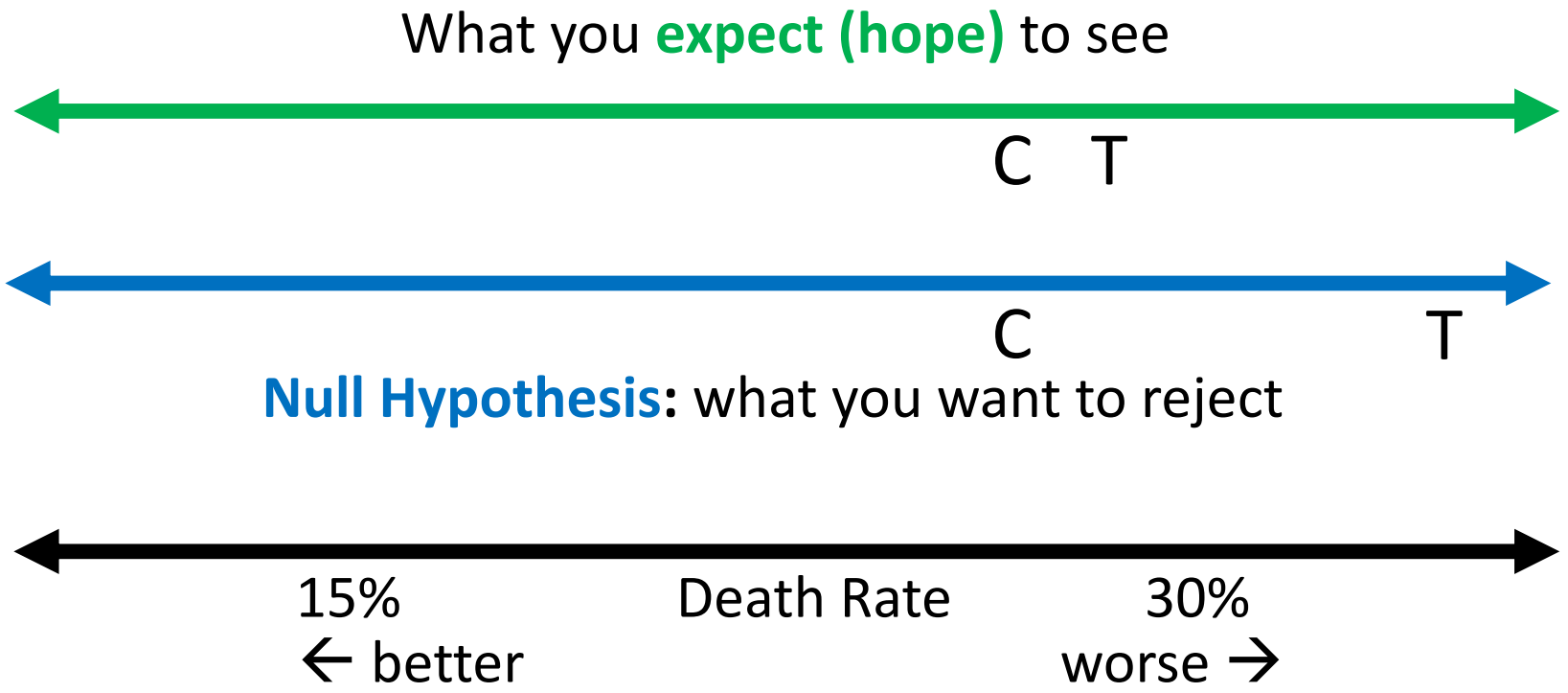
Aside: superiority, equivalence, and non-inferiority studies (4)

- Occasionally there are **equivalence** studies: they try to show that the new treatment (T) is the same as the existing standard of care (SOC; C)



Aside: superiority, equivalence, and non-inferiority studies (5)

- Sometimes there are **non-inferiority** studies: they try to show that the new treatment (T) is not a **lot worse** than the existing standard of care (SOC; C)



Aside: superiority, equivalence, and non-inferiority studies (6)

A conceptual approach to the statistical testing strategy is:

- **Superiority** study: conclude that the treatments are unlikely to be the same if the confidence interval excludes zero
- **Equivalence** study: conclude that there are unlikely to be big differences between the two treatments if
  - the **lower confidence limit** (one-sided) is not too low
  - the **upper confidence limit** (one-sided) is not too high
- **Non-inferiority** study: conclude that the treatment is unlikely to be substantially worse than the SOC if the upper (lower) confidence limit is not too high (low)

Design of the AMBITION Study (11)

- The investigators decided that 10% higher mortality would be too much and the new treatment would be considered inferior to the SOC
- 10% is an arbitrary decision – the sample size of the study is driven by this decision
  - a study with 5% higher mortality as too much, would have been much larger*
  - a study with 20% higher mortality as too much, would have been much smaller*
- Sample size is 425 / group
- Like most non-inferiority studies, if the inferiority hypothesis is rejected, they would then try to reject the null hypothesis that the two treatments are the same (a superiority study)

*Assuming all the other design characteristics are the same (alpha; beta; estimated mortality rate)

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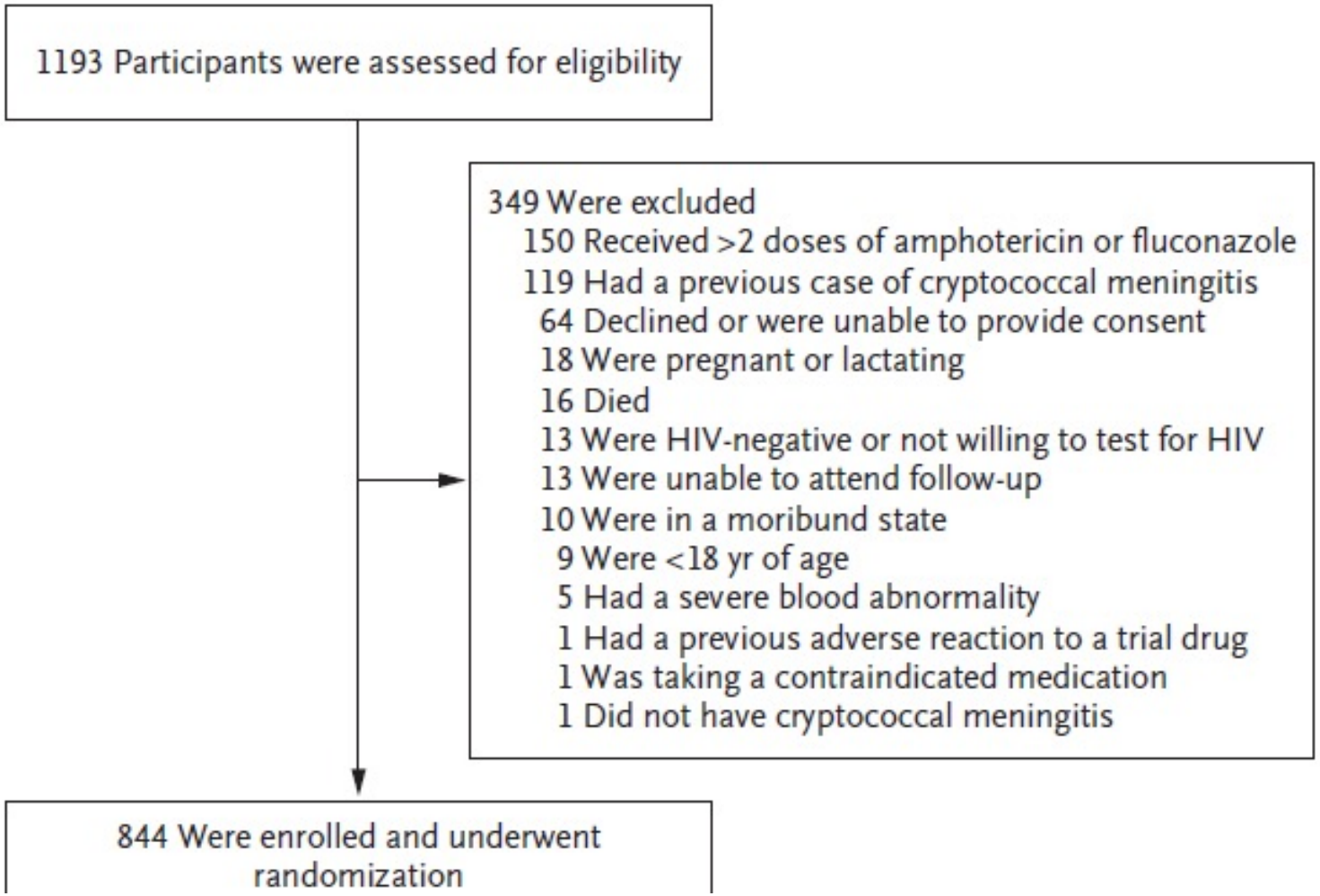
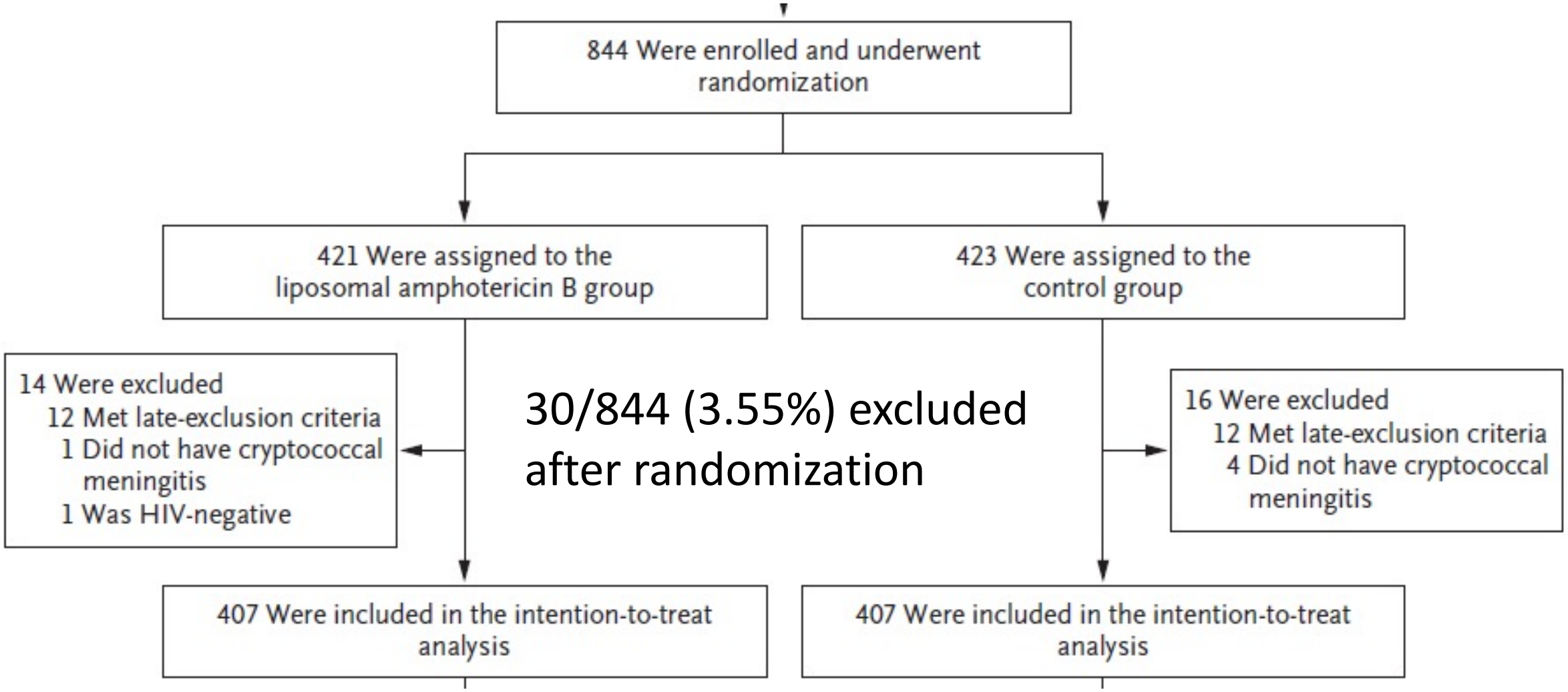
Results (1)



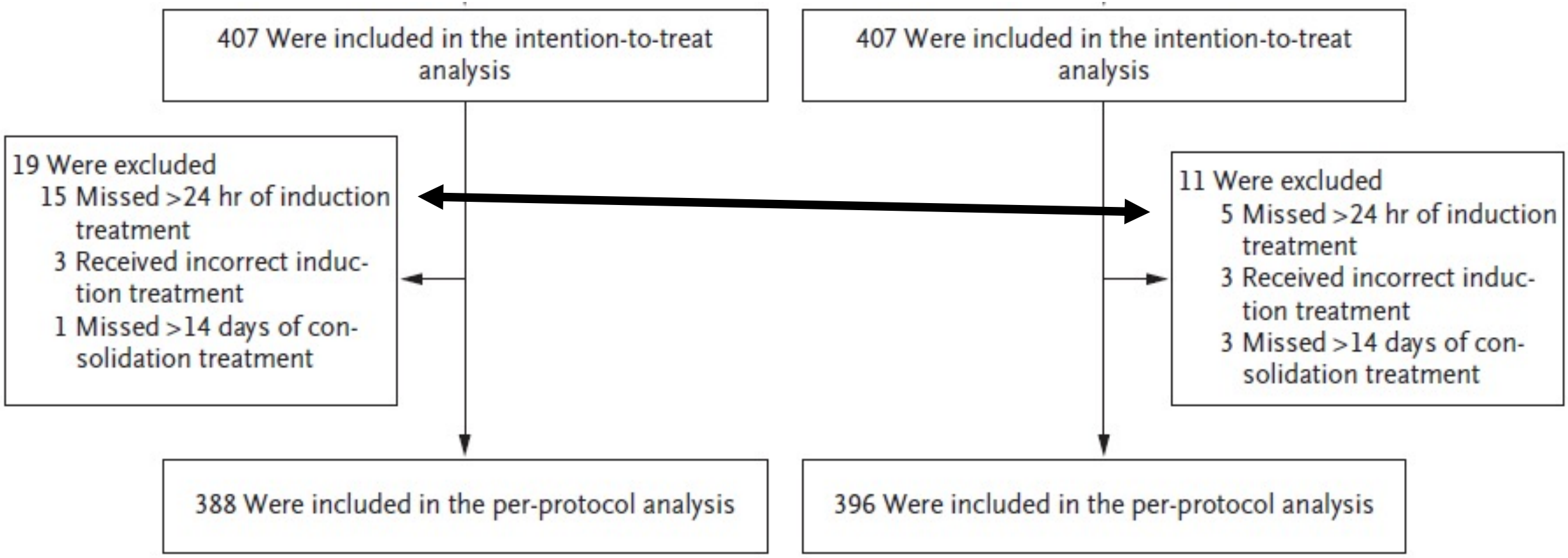
119 previous CM

Results (2)



30/844 (3.55%) excluded after randomization

Results (3)


407 Were included in the intention-to-treat analysis
407 Were included in the intention-to-treat analysis
19 Were excluded
15 Missed >24 hr of induction treatment
3 Received incorrect induction treatment
1 Missed >14 days of consolidation treatment
11 Were excluded
5 Missed >24 hr of induction treatment
3 Received incorrect induction treatment
3 Missed >14 days of consolidation treatment
388 Were included in the per-protocol analysis
396 Were included in the per-protocol analysis

Interpretation (1)

- 10% of the people were excluded for a previous case of CM – so it's not clear how to treat them, and the study does not apply to them
- Primary analysis – intention to treat population (in my opinion it is a modified ITT) – is very solid – relatively few exclusions from the total randomized
  - 24 of 30 excluded because of lab abnormalities detected after enrollment
  - 5 did not have CM, 1 did not have HIV
- Per protocol is a little more concerning – why did so many people get the wrong induction treatment in the intervention group? What were these errors?

Results (4)

Characteristic	Liposomal Amphtericin B (N=407)	Control (N=407)
Median age (IQR) — yr	37 (32–44)	37 (32–43)
Male sex — no. (%)	246 (60.4)	245 (60.2)
New diagnosis of HIV — no. (%)	127 (31.2)	118 (29.0)
Report of previous antiretroviral therapy — no. (%)†	256 (62.9)	266 (65.4)
Median weight (IQR) — kg	53 (47–60)	53 (48–60)
Headache		
Current symptom — no. (%)	390 (95.8)	394 (96.8)
Median duration (IQR) — days	14 (7–21)	14 (7–21)
Seizures within 72 hr before enrollment — no. (%)	45 (11.1)	42 (10.3)
Glasgow Coma Scale score <15 — no. (%)‡	115 (28.3)	117 (28.7)

Results (5)

	Liposomal Amphtericin B (N = 407)	Control (N = 407)
Median values from CSF sample analysis (IQR)		
Cryptococcal quantitative value — CFU/ml	48,500 (300–420,000)	42,000 (585–365,000)
CSF opening pressure) — cm of water	21 (14–32)	21 (13–31)
CSF opening pressure >25 cm of water — no./total no. (%)	165/399 (41.4)	158/400 (39.5)
White-cell count — cells/mm³	6 (4–75)	5 (3–52)
Glucose level — mg/dl	45 (29–61)	43 (27–58)
Protein level — g/l	0.90 (0.46–1.48)	0.84 (0.44–1.38)
Median blood hemoglobin level (IQR) — g/dl	11.2 (9.7–12.7)	11.2 (9.6–12.9)
Median serum creatinine level (IQR) — mg/dl	0.7 (0.6–0.9)	0.8 (0.6–1.0)
Median blood CD4+ cell count (IQR) — cells/mm³	26 (9–56)	28 (11–59)

Interpretation (2)

The two groups are pretty comparable. Possibly a little worse in the intervention group but not a lot

- Slightly more with seizures within 72 hours (11.1% vs 10.3%)
- Slightly higher proportion with high CSF opening pressure (41.4% vs 39.5%)
- Slightly higher median CSF CFU (48.5K vs 42K)
- More new HIV (31.2% vs 29.0%) and less prior ART (62.9% vs 65.4%)

But other evidence moderates any concerns

- Slightly lower proportion with headache (95.8% vs 96.8%)
- Slightly lower proportion with GCS < 15 (28.3% vs 28.7%)

Interpretation (3)

I am **really** trying to find differences between the groups and what I am finding is very small

Overall conclusion: randomization worked!

Results (6)

Less than 10 -- so inferiority rejected (P < 0.001)

Outcome	Unadjusted Analysis			
	Liposomal Amphtericin B	Control	Difference (95% CI)‡	Upper Boundary of One-Sided 95% CI
				percentage points
Death from any cause at 10 wk (primary end point)				
Intention-to-treat population				
Deaths — no./total no.	101/407	117/407		
Mortality (95% CI) — %	24.8 (20.7 to 29.3)	28.7 (24.4 to 33.4)	−3.93 (−10.0 to 2.2)	1.2§
Per-protocol population				
Deaths — no./total no.	95/388	113/396		
Mortality (95% CI) — %	24.5 (20.3 to 29.1)	28.5 (24.1 to 33.3)	−4.05 (−10.2 to 2.1)	1.1

Results (7)

	Unadjusted Analysis			Adjusted Analysis†	
		Difference (95% CI)‡	Upper Boundary of One-Sided 95% CI	Difference (95% CI)	Upper Boundary of One-Sided 95% CI
			percentage points		*percentage points*
Intention-to-treat population					
Deaths — no./total no.					
Mortality (95% CI) — %	.4)	−3.93 (−10.0 to 2.2)	1.2§	−5.71 (−11.4 to −0.04)	−1.0
Per-protocol population					
Deaths — no./total no.					
Mortality (95% CI) — %	.3)	−4.05 (−10.2 to 2.1)	1.1	−5.04 (−10.8 to 0.8)	−0.2

Interpretation (4)

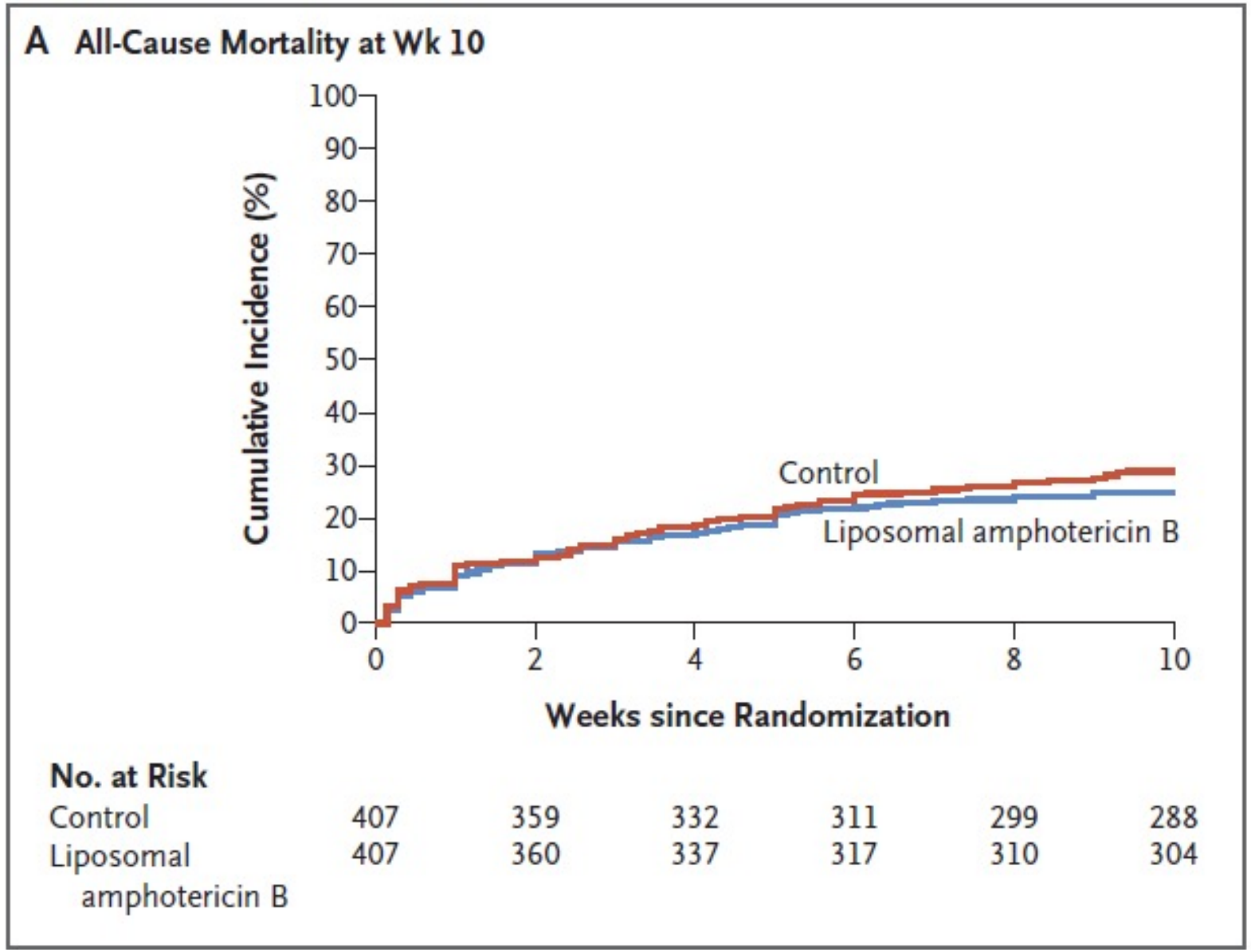
All the analyses are giving similar results:

- the null hypothesis of inferiority is rejected (all close to or below P < 0.001), then
- testing for superiority: null hypothesis is not rejected: primary analysis 95% CI for the unadjusted difference in the (modified) intention-to-treat population is -10.0 to 2.2
  - slight inconsistency with the adjusted ITT population
- **Conclusion: intervention arm is not worse than the control arm, but it is not statistically significantly better than the control arm**

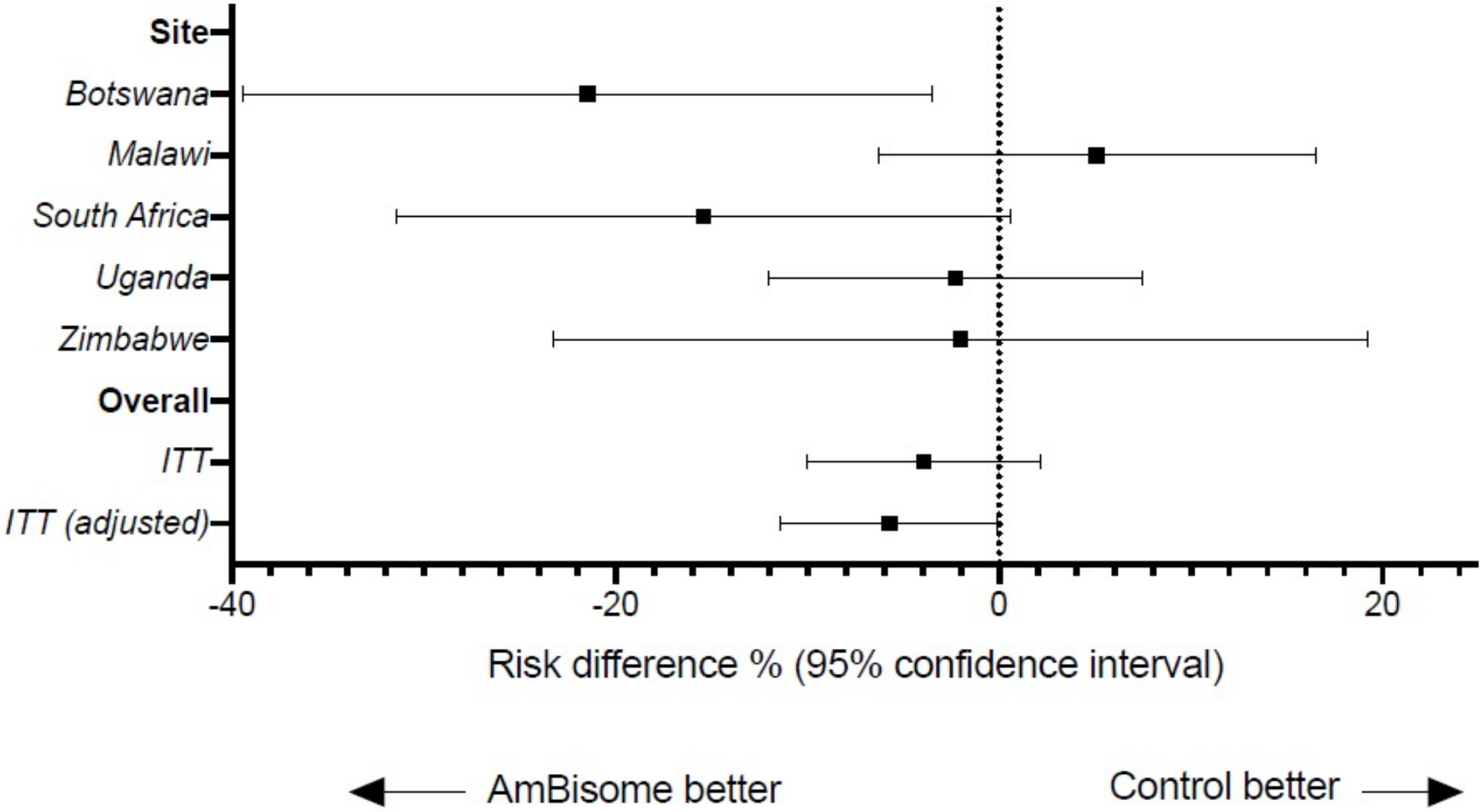
Results (8)

	Liposomal Amphtericin B	Control	Difference (95% CI)‡
Early fungicidal activity (key secondary end point)			
Participants with available data in the intention-to-treat population — no.¶	363	381	
Rate of fungal clearance over the course of 14 days — $\log_{10}$ CFU/ml/day			
Mixed-effects model	−0.40±0.13	−0.42±0.13	0.017 (−0.001 to 0.036)
Linear-regression model‖	−0.41±0.19	−0.44±0.21	0.0270 (−0.004 to 0.058)

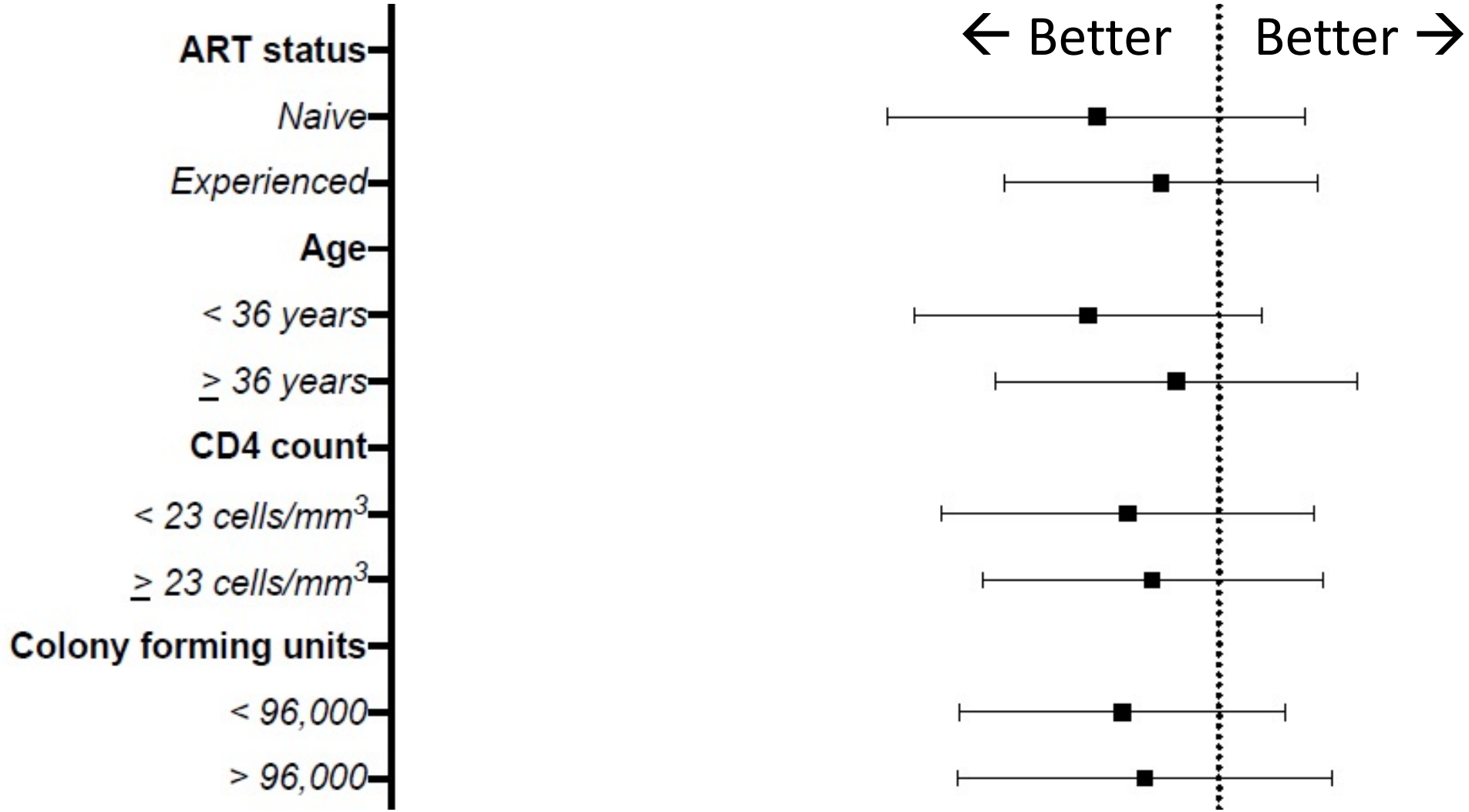
Results (9)


A All-Cause Mortality at Wk 10
Cumulative Incidence (%)
100
90
80
70
60
50
40
30
20
10
0
Control
Liposomal amphotericin B
0
2
4
6
8
10
Weeks since Randomization
No. at Risk
Control 407 359 332 311 299 288
Liposomal amphotericin B 407 360 337 317 310 304

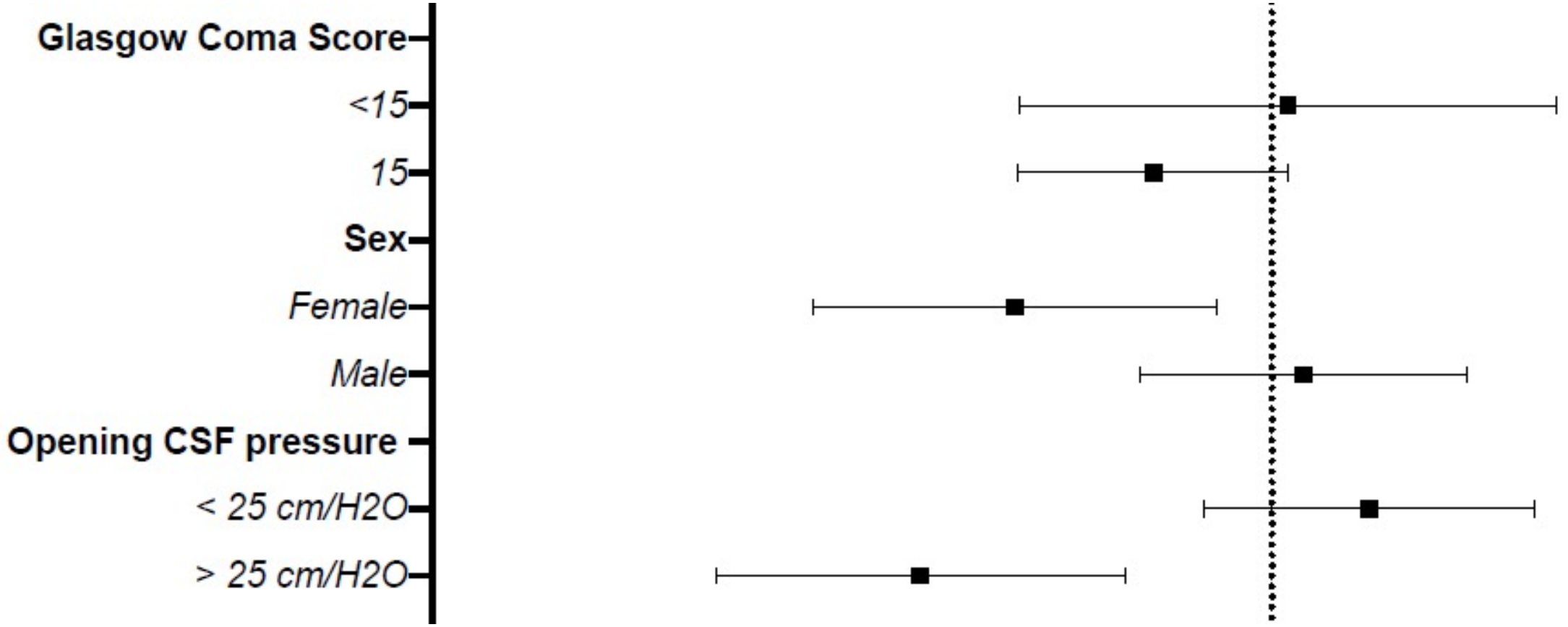
Results (10)


Site
Botswana
Malawi
South Africa
Uganda
Zimbabwe
Overall
ITT
ITT (adjusted)
-40
-20
0
20
Risk difference % (95% confidence interval)
AmBisome better
Control better

Results (11)


Intervention
Control
← Better
Better →
ART status
Naive
Experienced
Age
< 36 years
≥ 36 years
CD4 count
< 23 cells/mm3
≥ 23 cells/mm3
Colony forming units
< 96,000
> 96,000

Results (12)


Intervention
← Better
Control
Better →
Glasgow Coma Score
<15
15
Sex
Female
Male
Opening CSF pressure
< 25 cm/H2O
> 25 cm/H2O

Interpretation (5)

Again, consistent results

- Fungicidal clearance similar (CI includes zero)
- More mortality in the control group
- Across all the different subgroups, nothing stands out where the control group is clearly better than the new treatment

Results (13)

Event	Liposomal Amphtericin B (N=420)	Control (N=422)	P Value†
Grade 3 or 4 adverse events — no. of events	382	579	
Any grade 3 or 4 adverse event — no. of participants (%)			
Grade 3 or 4	210 (50.0)	263 (62.3)	<0.001
Grade 3	173 (41.2)	225 (53.3)	<0.001
Grade 4	91 (21.7)	127 (30.1)	0.005
Anemia — no. of participants (%)‡			
Grade 3	44 (10.5)	108 (25.6)	<0.001
Grade 4	12 (2.9)	62 (14.7)	<0.001
Mean change in hemoglobin level from baseline to day 7 — g/dl§	−0.3±1.39	−1.9±1.8	<0.001
Receipt of blood transfusion — no. of participants (%)	32 (7.6)	76 (18.0)	<0.001

Results (13)

	Liposomal Amphtericin B (N=420)	Control (N=422)	P Value†
Neutropenia — no. of participants (%)¶			
Grade 3	27 (6.4)	21 (5.0)	0.36
Grade 4	20 (4.8)	16 (3.8)	0.49
Thrombocytopenia — no. of participants (%)‖			
Grade 3	9 (2.1)	17 (4.0)	0.11
Grade 4	4 (1.0)	6 (1.4)	0.75
Creatinine increase — no. of participants (%)**			
Grade 3	17 (4.0)	22 (5.2)	0.42
Grade 4	5 (1.2)	3 (0.7)	0.51

Results (14)

	Liposomal Amphtericin B (N=420)	Control (N=422)	P Value†
Mean relative increase in creatinine level from baseline to day 7 — %††	20.2±48.1	49.7±70.8	<0.001
Hypokalemia — no. of participants (%)‡‡			
Grade 3	6 (1.4)	27 (6.4)	<0.001
Grade 4	0	3 (0.7)	0.25
Elevated ALT — no. of participants (%)§§			
Grade 3	6 (1.4)	4 (0.9)	0.52
Grade 4	1 (0.2)	1 (0.2)	1.0
Thrombophlebitis requiring antibiotic therapy — no. of participants (%)	8 (1.9)	28 (6.6)	<0.001
Other grade 3 or 4 adverse event — no. of participants (%)¶¶	167 (39.8)	173 (41.0)	0.72

Interpretation (6)

- Significantly fewer Grade 3 or Grade 4 AEs with the intervention than the control
  - 382 vs 579 events
  - 210 (50.0%) vs 263 (62.3%) of people
- Significantly less anemia, fewer blood transfusions (and smaller drop in hemoglobin from baseline to day 7)
- Significantly less hypokalemia
- Significantly less thrombophlebitis requiring antibiotic therapy
- Significantly smaller relative creatinine increase from baseline to day 7

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- **Conclusions**

Conclusions (1)

- The intervention is as effective as the control
- **This conclusion is not rigorous because they did not test this statistically. If they had used a sequential strategy of testing for inferiority, testing for equivalence, and then testing for superiority, they could have concluded it. But they did not test for this, so this is not a rigorous statistical conclusion.**
- The intervention has less toxicity than the control group

Conclusions (2)

The study was done very well – in my opinion an exceptionally well done study.

- no major issues in the post-randomization exclusions (most based on laboratory test results available after randomization)
- no loss to follow-up (which is incredible)
- major weakness was that it was open-label, but
  - primary endpoints were objective so less of a concern
  - this was almost essential given the treatments

Conclusions (3)

It appears that others share my view. From a **20 April 2022** WHO press release:

> New guidelines developed by WHO strongly recommend a single high dose of liposomal amphotericin B as part of the **preferred*** induction regimen for the treatment of cryptococcal meningitis in people living with HIV.

*My emphasis

World Health Organization

Credits

New guidelines from WHO recommend a simpler, safer treatment for cryptococcal disease in people living with HIV

Rapid Advice

20 April 2022 | Departmental news | Reading time: 1 min (338 words)

Cryptococcal disease is one of the most important opportunistic infections among people living with advanced HIV disease and is a major contributor to illness, disability and mortality, particularly in sub-Saharan Africa.

New guidelines developed by WHO strongly recommend a single high dose of liposomal amphotericin B as part of the preferred induction regimen for the treatment of cryptococcal meningitis in people living with HIV.

Thank you

Robert A. Parker, ScD

rparker4@mgh.Harvard.edu