Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Lee M, Lee J, Carroll MW, et al. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. N Engl J Med 2012;367:1508-18. DOI: 10.1056/NEJMoa1201964

Linezolid (Zyvox™) for the Treatment of

Chronic Extensively Drug-Resistant Tuberculosis

Supplementary Appendix

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Supplemental Material 1: Inclusion and Exclusion Criteria

(1) Inclusion criteria

1) Males and females age 20 and above
2) Documented pulmonary tuberculosis at screening
3) Radiographic evidence of tuberculous disease of the lung(s)
4) History of chronic, AFB positive sputum smears and culture positive TB
5) Mycobacterium species identification as *Mycobacterium tuberculosis*
6) Confirmed resistance to INH, RIF, kanamycin, ofloxacin, and moxifloxacin by genotypic or phenotypic testing OR subjects with documented failure to respond to treatment despite DST susceptibility
7) Failure to respond (after at least 6 months) to an anti-TB drug regimen including any known active agents
8) Willingness to be an inpatient at NMTH until 2 consecutive AFB-negative sputum smears
9) When an outpatient, willing to come back for weekly tests and scheduled follow-up visits
10) Willingness to have samples stored
11) Ability and willingness to give written or oral informed consent

(2) Exclusion criteria

1) Subjects below 20 years of age
2) Subjects who have previously been on LZD
3) Women of childbearing potential, who are pregnant, breast feeding, or unwilling to avoid pregnancy (*i.e.*, the use of appropriate contraception including oral and subcutaneous implantable hormonal contraceptives, condoms, diaphragm, intrauterine

device (IUD), or abstinence from sexual intercourse) [Note: Prospective female participants of childbearing potential must have negative pregnancy test (urine) within 48 hours prior to study entry.]

4) Men who are unwilling to use contraceptives or practice abstinence

5) People with any of the following in their current medical assessments:

   a. Absolute neutrophil count < 1000 cells/mL

   b. White blood cell count (WBC) < 3.0 X $10^3/\mu L$

   c. Hemoglobin < 7.0 g/dL

   d. Platelet count < 75,000 cells/mm^3

   e. Serum creatinine > 2.0 mg/dL

   f. Aspartate aminotransferase (AST or SGOT) >100 IU/L

   g. Alanine aminotransferase (ALT or SGPT) >100 IU/L

   h. Total bilirubin > 2.0 mg/dL

   i. Moderate or severe peripheral or optical neuropathy (or a history of)

   j. HIV-1 or HIV-2 infection

   k. Systemic lupus erythematosus, rheumatoid arthritis, or other connective tissue disease

6) Patients who, in the investigator's judgment, are too ill to participate in the study

7) History of allergy or serious adverse reaction to the LZD formulation used in this study

8) Patients with anticipated surgical intervention

9) The use of any of the following drugs within 30 days prior to study or anticipated use of these drugs within the next 60 days: (Please note, bronchodilators and cough syrup (or similar cough medicines) are allowed before and during the study if blood pressure is monitored regularly, per Contraindications, p.12, of the Zyvox Package Insert.)

   a. Selective serotonin reuptake inhibitors (SSRIs)

   b. Monoamine oxidase inhibitors (MAOIs)

c. Sympathomimetic agents (e.g. pseudoephedrine)

d. Vasopressive agents (e.g. epinephrine, norepinephrine)

e. Systemic cancer chemotherapy

f. Systemic corticosteroids

g. Systemic investigational agents

h. Antiretroviral medications

i. Growth factors

j. HIV vaccines

k. Immune globulin

l. Interleukins

m. Interferons

10) The need for ongoing therapy with antidepressants (SSRI, MAOI), hydroxyzine, dopaminergic agents (such as Sinemet, dopamine, and dobutamine), lithium, cyclosporine, tacrolimus, sirolimus, and levodopa (such as sinemet) while on study drug.

11) Any other serious systemic illness including uncontrolled hypertension requiring treatment and/or hospitalization until subject either completes therapy or is clinically stable on therapy for at least 14 days prior to study entry.

12) Patients who the physician has reason to believe may have been non-compliant in the previous 12 months of treatment.

Supplemental Table 1: Initial regimen, final regimen, susceptible drugs at entry*, Treatment status and follow up

Subject ID	Entry regimen	Current/Final regimen	Susceptible drugs at entry	Treatment outcome†/status, study status and follow up
1	H,R	R,Mfx,Clr	Cs, S, Mfx	Cure, completed study without relapse
2	Z,Cs,Mfx	Cs,Mfx,Clr,Amx/Clv	None	Cure, withdrew after end of therapy (EOT) plus 6 months without relapse
3	H,Pto,Cs,Lfx	Na‡	Cs	Withdrawn due to SAE (anemia) before culture conversion to negative
4	H,Lfx	Mfx,Clr,Amx/Clv	None	Cure, completed study without relapse
5	H,E,Cs,Mfx,Clr	E,Mfx	Z,PAS, S	Cure, lost to follow-up after EOT
6	Pto,Cs,PAS,Clr,Amx/Clv,Km	Cs,Mfx,Clr,Amx/Clv	Cs,Km,Cm,Am	Cure, lost to follow-up after EOT
7	H,R,Lfx	Na	Pto,Cs,S,Km,Cm,Am,Rb	Withdrawn prior to receiving study drug after being diagnosed with metastatic colon cancer
8	Pto,Mfx,PAS,Clr,Rb	Pto,Cs,Mfx,PAS,Clr,Km	Cs,Km,Cm,Am,Rb	Cure, awaiting EOT plus 6 month without relapse
9	H,R	Mfx,Clr,Amx/Clv	Pto,Mfx,PAS,S,Cm,Rb	Cure, awaiting EOT plus 12 month without relapse
10	Pto,Cs,Lfx,PAS	Pto,Mfx,Clr,Amx/Clv	None	Treatment failure, never converted sputum culture to negative
11	R,Z,Mfx,PAS	Z,Mfx,Amx/Clv,Am	Pto,PAS,Km,Cm,Am	Withdrawn due to AE (optic neuropathy) after becoming culture negative

12	H	H,Clr,Amx/Clv	E,Cs	Treatment Failure. Initially converted sputum culture to negative but reverted to positive after primary endpoint
13	Pto,Cs,Lfx,Clr	Am	Km,Cm,Am	On therapy, culture negative
14	Pto,Cs,Mfx,Clr,Amx/Clv,Rb	Cs,Mfx,Clr,Amx/Clv,S	E,S,Rb	On therapy, culture negative
15	Pto,Cs,Lfx	Na	Z,PAS,Km,Cm,Am	Treatment failure, never converted sputum culture to negative
16	H,R,Z,Pto,Cs,Mfx	Mfx,Clr,Amx/Clv,Am	Km,Am	On therapy, culture negative
17	Z,Pto,Cs,Mfx,PAS,Km	Z,Mfx,Clr,Amx/Clv,Km	PAS	On therapy, culture negative
18	Z,Pto,Cs,Mfx,Rb	Clr,Amx/Clv,S	Z,Lfx,Mfx,PAS,S	On therapy, culture negative
19	E,Cs,Lfx	Mfx,Clr,Amx/Clv,Am	Z,Mfx,PAS,Km,Cm,Am	On therapy, culture negative
20	None	Mfx,Clr,Amx/Clv,Am	Mfx,PAS,Km,Cm,Am	Withdrawn before EOT for personal reasons
21	Cs,Lfx,PAS,Clr,S	Lfx,PAS,Clr,S	Z,Ofx,Lfx,Mfx,S,Km,Cm,Am,Rb	On therapy, culture negative
22	E,Z,Cs,Mfx	Cs,Mfx,Clr,Amx/Clv,S	S	On therapy, culture negative
23	Pto,Cs,Lfx	Mfx,Clr,Amx/Clv,Am	Pto,Km,Cm,Am	On therapy, culture negative
24	Z,Cs,Mfx,Clr,Rb	Na	Mfx,PAS,S,Km,Cm,Am	On therapy, culture negative
25	Z,Pto,Cs,Lfx,Rb	Mfx,Clr,Amx/Clv,Km	Rb	On therapy, culture negative
26	H,R	R,Clr,Amx/Clv,S	S	Cure, completed study without relapse
27	Mfx,Clr	Mfx,Clr,S	Cs,Mfx,PAS,S	Cure, lost to follow-up after EOT plus 6 month without relapse

28	Z,Pto,Cs,Clr,Amx/Clv	Pto,Cs,Mfx,Clr,Amx/Clv	E,Mfx,S,Km,Am	Cure, awaiting EOT plus 12 month without relapse
29	Pto,Cs,Mfx	Pto,Cs,Mfx,Amx/Clv,S	PAS,S	Cure, awaiting EOT plus 6 month without relapse
30	Pto,Cs,Lfx,PAS,Clr,S	H,Mfx,Clr,Amx/Clv,Am	Km,Cm,Am	Cure, awaiting EOT plus 6 month without relapse
31	Pto,Cs,Mfx,PAS	Cs,Mfx,PAS	Z,Cs,PAS,Km,Cm,Am	Cure, awaiting EOT plus 6 month without relapse
32	H,E,Z,Mfx,Clr	E,Mfx,Clr,Amx/Clv,Km	E,Lfx,Mfx,PAS,S,Km,Cm,Am,Rb	On therapy, culture negative
33	E,Pto,Cs,Lfx,PAS	Amx/Clv,Am	E,Km,Cm,Am,Rb	On therapy, culture negative
34	Z,Pto,Cs,PAS,Rb	Z,Clr,Amx/Clv,Am	Km,Cm,Am	Withdrawn due to AE (optic neuropathy); initially became culture negative on LZD but reverted after discontinuation of LZD due to AE
35	Z,Mfx,PAS,Clr,Amx/Clv	No change	Cs,Lfx,PAS,Cm,Am,Rb	On therapy, culture negative
36	None	Z,Mfx,PAS,Clr,Am	H,Cs,PAS,Cm,Am	On therapy, culture negative
37	Pto,Cs	Z,Mfx,Clr,Amx/Clv,Km	E,S,Km,Cm,Am	On therapy, culture negative
38	E,Pto,Mfx,Rb	Na	PAS,Km,Cm,Am,Rb	Treatment failure, never converted sputum culture to negative
39	H,Pto,Lfx	Pto,Mfx,Clr,Amx/Clv,S	E,PAS,S,Rb	On therapy, culture negative

* Abbreviation of drugs as following: isoniazid (H), rifampicin (R), ethambutol (E), pyrazinamide (Z), prothionamide (Pto), cycloserine (Cs), ofloxacin (Ofx), levofloxacin (Lfx), moxifloxacin (Mfx), p- aminosalicylic acid (PAS), clarithromycin (Clr), amoxicillin/clavulanate (Amx/Clv), streptomycin (S), kanamycin (Km), amikacin (Am) capreomycin (Cm), rifabutin (Rb), linezolid(Lzd)

† Status as of May 1, 2012. Cure and treatment failures were defined using WHO criteria.

Supplemental Figure 1: Study timeline (Immediate arm)

Evaluation	Screening	Entry	1w	2w	3w	4w	5w	6w	7w	8w	9w	10w	11w	12w	13w	14w	15w	16w	5m	6m	7m	Every 2month	EOT	EOT +6m	EOT+ 12m	Early withdraw
Clinical examination	O	O				O								O				O	O	O	O	O	O	O	O	O
Medical history	O																									
HIV ELISA	O																									
AFB smear		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
TB culture		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
DST	O																									
Radiologic imaging	CT or PET/CT scan was performed according to the randomized arm and participation of substudy for PET/CT																									
Chemistries	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Hematology	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
PK (blood/sputum)		O		O	O	O	O									O	O	O	O							O
MTB lipid (urine/blood/sputum)		O	O		O		O		O		O		O		O		O		O				O	O	O	
T-SPOT-TB		O	O		O		O		O		O		O				O			O			O	O	O	
Whole blood killing assay		O			O				O																	O
Mitochondrial Assay		O			O		O		O		O		O		O		O		O	O	O	O				O
TB RNA		O	O	O		O																				
Neurologic examination	O					O				O				O				O	O	O	O	O	O	O	O	O
Optic test		O				O				O				O				O	O	O	O	O	O	O	O	O
Pregnancy test	O									O										O					O	O

Supplemental Figure 2: Study timeline (Delayed arm)

Evaluation	Screening	Entry	Weeks																							Months			Every 2month	EOT	EOT +6m	EOT+12m	Early with
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	7	8	9					
Clinical examination	O	O				O				O				O				O				O				O	O	O	O	O	O	O	O
Medical history	O																																
HIV ELISA	O																																
AFB smear		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
TB culture		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
DST	O																																
Radiologic imaging	CT or PET/CT scan was performed according to randomized arm and participation in substudy for PET/CT																																
Chemistries	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Hematology	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
PK (blood /sputum)										O		O	O	O	O									O	O	O	O						O
MTB lipid (urine/blood / sputum)		O								O	O		O		O		O		O		O		O		O		O	O		O	O	O	
T-SPOT-TB		O								O	O		O		O		O		O		O				O			O		O	O	O	
Whole blood killing assay		O											O				O																O
Mitochondrial Assay		O			O								O		O		O		O		O		O		O		O	O	O	O			O
TB RNA		O									O	O		O																			
Neurologic examination	O					O				O				O				O				O				O	O	O	O	O	O	O	O
Optic test		O				O				O				O				O				O				O	O	O	O	O	O	O	O
Pregnancy test	O									O								O										O				O	O

Supplemental Table 2: All clinically significant adverse* events regardless of relationship to linezolid and number reported as serious adverse events (SAEs)‡

Event	Total	Reported as SAE
Peripheral neuropathy	21	6
Myelosupression	7	7
Optic Neuropathy	7	7
Hepatic dysfunction	4	3
Hyperglycemia	3	0
Pneumonia	3	3
Uric acid elevation	2	0
Rhabdomyolysis	2	2
Other †	7	5
Total	**56**	**33**

*Clinically significant adverse events were defined as follows: Peripheral neuropathy, all subjects with an SPNS score$\geq$2, or any SPNS score of one that resulted in a dose adjustment; Optic neuropathy > grade 2; other AEs $\geq$ grade 3; and other < grade 3 events reported as SAEs at the investigators discretion (dizziness n=1 and reaction to psychotropic medications n=1).

‡SAEs were defined as follows:

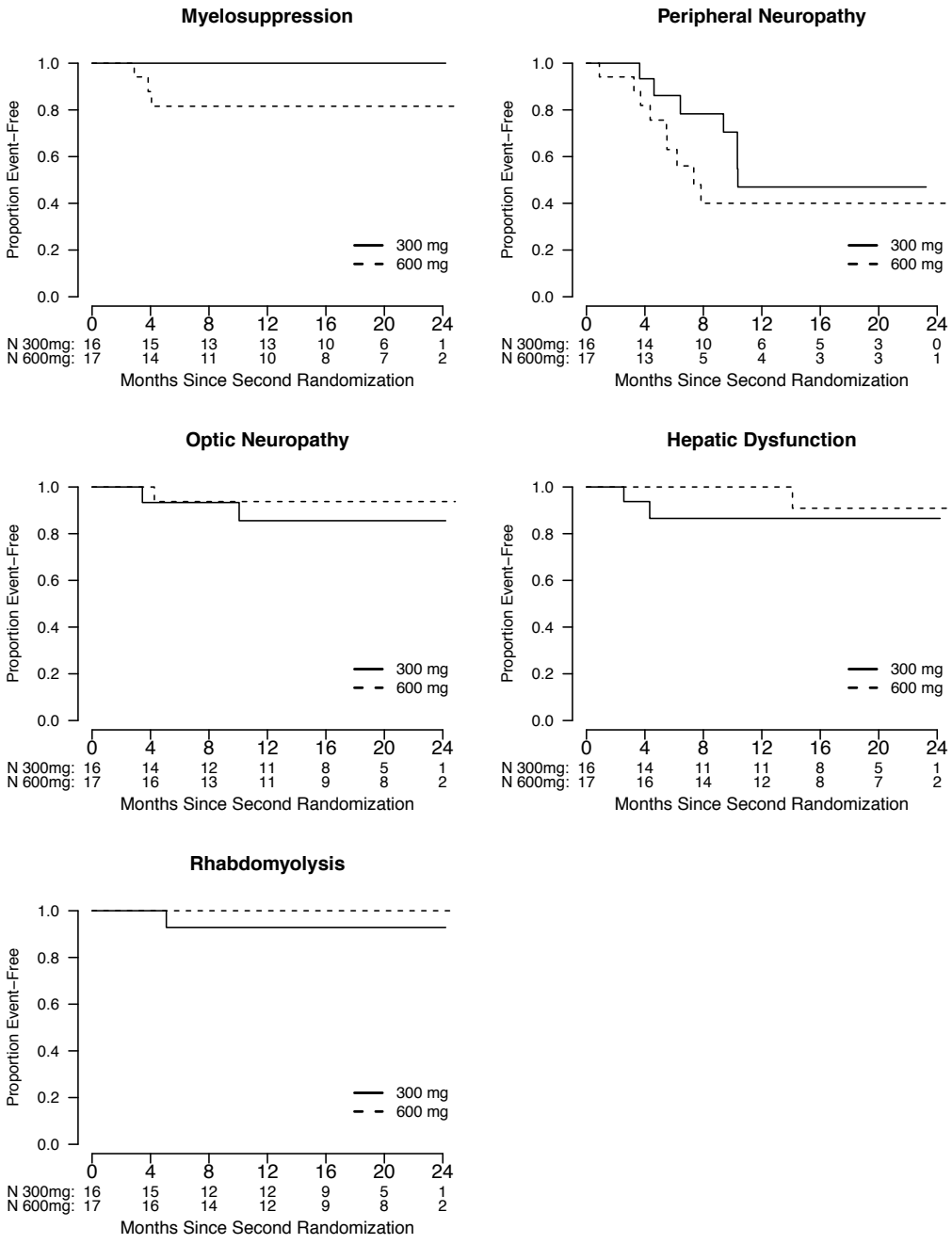
- Resulted in death (no deaths have occurred on study to date)
- Was life-threatening
- Required or prolonged hospitalization
- Resulted in persistent or significant disability
- Required intervention to prevent permanent impairment or damage

† dizziness (n=1), reaction to psychotropic medications (n=1), colon cancer with metastasis (n=1), esophageal hemorrhage due to Mallory-Weiss Tear (n=1), hemoptysis (n=1), cataract (n=1) and diarrhea (n=1)

All adverse events were graded using the DAIDS Toxicity Tables, located at http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx.

Recurrent events in a single subject were only counted once.

Supplemental Figure 3: Kaplan-Meier survival curves of type of adverse event and time since 2nd randomization, stratified by treatment arm



Supplemental Table 3: Frequency of clinically significant adverse events possibly or probably related to linezolid‖

Event	0-4 Mo						4-8 Mo						8-12 Mo					
	N*	PY†	Rate	L0‡	L300§	L600||	N	PY	Rate	L0	L300	L600	N	PY	Rate	L0	L300	L600
Myelosuppression	5	12.0	0.42	0.0	1.3	10.7	1	10.1	0.10	0.0	6.8	3.3	0	8.4	0.00	0.4	6.7	0.9
Peripheral Neuropathy	5	12.1	0.41	0.1	1.6	10.4	10	8.2	1.22	0.0	5.8	2.4	5	4.7	1.07	0.1	3.7	0.7
Optic Neuropathy	1	12.5	0.08	0.1	1.7	10.7	2	10.7	0.19	0.0	7.4	3.3	3	8.0	0.38	0.0	6.5	1.5
Hepatic Enzyme Elevation	1	12.4	0.08	0.1	1.5	10.8	1	10.9	009	0.0	7.5	3.4	1	8.6	0.12	0.4	7.0	0.9
Rhabdomyolysis	0	12.5	0.00	0.1	1.7	10.8	1	11.3	0.09	0.0	7.9	3.4	0	9.0	0.00	0.4	7.3	0.9

*N, number of events observed during the specified time interval from linezolid start; †PY, Person-Years of study interval; ‡L0, Person-Years of subjects receiving no linezolid during the study interval; §L300, person-years of subjects receiving 300 mg linezolid during the study period; ||L600, person-years of subjects receiving 600 mg linezolid during the study period

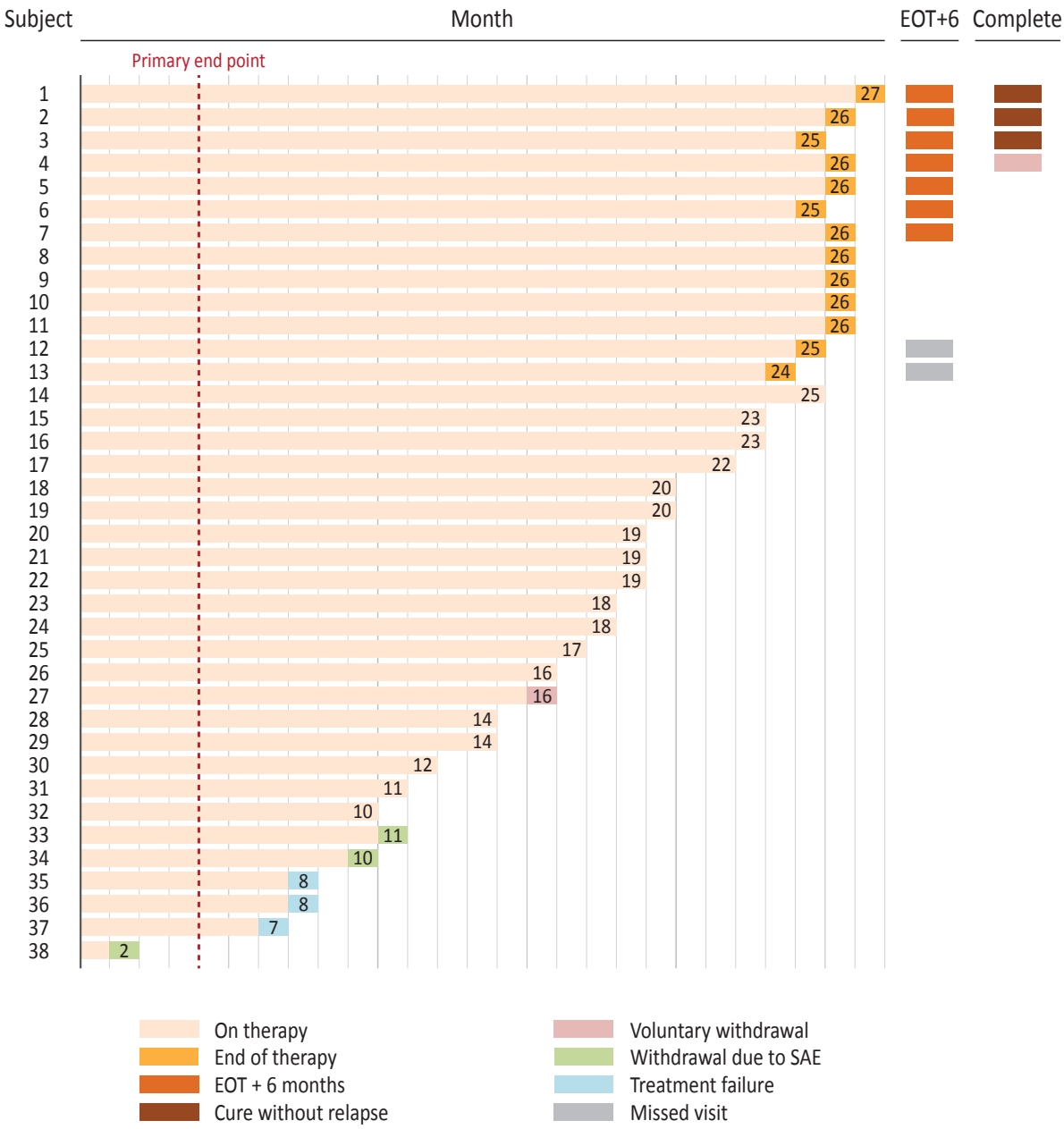
Supplemental Table 4: Linezolid stoppage and dose adjustments*

Subject ID	Stoppage period (days)	Reason	Relationship to LZD	Dose adjustment
1	2	Hepatic enzyme elevation	Possibly related	Re-challenge with 600mg
2	–	Anemia	Probably related	Permanently stopped
3	1	Joint pain	Unrelated	Re-challenge with 600mg
	18	Neutropenia	Probably related	De-escalation to 300mg
4	18	Peripheral neuropathy	Probably related	Re-challenge with 300mg
5	11	Peripheral neuropathy	Probably related	De-escalation to 300mg
6	–	Optic neuropathy	Possibly related	Permanently stopped
7	21	Peripheral neuropathy	Probably related	De-escalation to 300mg
8	5	Hepatic enzyme elevation	Possibly related	Re-challenge with 300mg
	20	Rhabdomyolysis	Possibly related	Re-challenge with 300mg
9	23	Hepatic enzyme elevation	Possibly related	Re-challenge with 300mg
10	17	Optic neuropathy	Probably related	De-escalation to 300mg
11	34	Peripheral neuropathy	Probably related	De-escalation to 300mg
12	22	Anemia	Probably related	De-escalation to 300mg
	12	Peripheral neuropathy	Probably related	Re-challenge with 300mg
13	14	Peripheral neuropathy	Probably related	De-escalation to 300mg
	33	Optic neuropathy	Probably related	Re-challenge with 300mg
14	20	Anemia	Probably related	De-escalation to 300mg
15	21	Peripheral neuropathy	Probably related	De-escalation to 300mg
16	8	Gastrointestinal bleeding	Unrelated	Re-challenge with 600mg
	14	Peripheral neuropathy	Probably related	De-escalation to 300mg
17	25	Neurologic reaction to psychological medicines	Unlikely related	Re-challenge with 600mg
18	10	Peripheral neuropathy	Probably related	De-escalation to 300mg
	13	Pneumonia	Unlikely related	Re-challenge with 300mg
19	28	Optic neuropathy	Possibly related	Re-challenge with 300mg
20	16	Peripheral neuropathy	Probably related	De-escalation to 300mg
21	25	Optic neuropathy	Possibly related	De-escalation to 300mg
22	26	Optic neuropathy	Probably related	Re-challenge with 300mg

	-	Optic neuropathy	Probably related	Permanently stopped
23	25	Anemia	Probably related	De-escalation to 300mg
24	26	Peripheral neuropathy	Possibly related	De-escalation to 300mg
	14	Peripheral neuropathy	Possibly related	Re-challenge with 300mg
25	11	Anemia	Probably related	De-escalation to 300mg
	3	Total bilirubin elevation	Possibly related	Re-challenge with 300mg

*For this table 3 cases of brief drug stoppages for personal reasons (unrelated to the study) were excluded

Supplemental Figure 4: Current status of study subjects



Supplemental Material 2: Subjective Peripheral Neuropathy Score (SPNS)

1. Instruction for recording; ask the patient to rate the severity of each symptom (a-f) on a s cale of 1 (mild)-10 (most severe). Enter the score for each symptom in the column marke d Presence/Severity. If symptom has been present in the past, but not since the last visit, enter"00"/Currently Absent. If the symptom has never been present, enter "11"/Always Been Normal.

Always been normal	Currently absent	Mild …… Severe									
11	00	01	02	03	04	05	06	07	08	09	10

2. Symptom(s) Presence/Severity
   a. Pain, aching or burning in hands, arms …… _____
   b. Pain, aching or burning in feet, legs …….. _____
   c. "Pins and needles" in hands, arms …… _____
   d. "Pins and needles" in feet, legs …….. _____
   e. Numbness (lack of feeling) in hands, arms …….. _____
   f. Numbness (lack of feeling) in feet, legs …… _____

3. Instructions for grading subjective patient-elicited symptoms:
   Use highest severity score recorded in (a-f) above to obtain a subjective peripheral neuropathy grade :

   Presence/Severity Score of
   01-03 = Grade of 1
   04-06 = Grade of 2
   07-10 = Grade of 3
   11 or 00 = Grade of 0

4. Subjective peripheral neuropathy grade?…… _____