Supplementary Online Content

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

eMethods

Procedures

Dosage

Dosage escalation (weeks 1-4), full dosage treatment (weeks 5-8), and wean (weeks 9-10) followed the timetable and dosing for Phase III clinical trials of ezogabine in epilepsy[1], and measurements for the neurophysiological outcomes were made while at the full dosage (average of week 6 and week 8 measurements). The 600 mg/d group dose was increased from 300 mg/d (100 mg TID) initially by 150 mg/d (50 mg TID) weekly and reached the target dose (600 mg/d; 200 mg TID) after two weeks. The 900 mg/d group dose was increased from 300 mg/d (100 mg TID) initially by 150 mg/d (50 mg TID) weekly and reached the target dose (900 mg/d; 300 mg TID) after four weeks. Blinding was ensured by identical active and placebo pills for each size: 50 mg, 200 mg, and 300 mg.

Neurophysiology Training and Protocols

Training workshops were held for site neurophysiologists for TMS at the Berenson-Allen Center for Noninvasive Brain Stimulation at Beth Israel Deaconess Medical Center, Boston and for TTNCS at the Neuromuscular Disorders clinic at Beth Israel Deaconess Medical Center, Boston. Neither TMS nor TTNCS had previously been used in a large multi-site format; five of the 12 clinical trial sites lacked TMS experience, and all sites but one lacked prior axonal TTNCS experience. Therefore, after neurophysiologists were trained at the workshop, they performed formal test-retest recordings in "unmatched" healthy controls, and the recordings were centrally reviewed for compliance with the testing protocol and to assess reproducibility prior to activating sites to recruit ALS participants. Results from healthy control participant testing to evaluate inter-site and inter-observer reproducibility and comparisons with ALS participant baselines will be published in separate manuscripts.

For TMS, 10 sites used MagVenture X100 or R30 stimulators equipped with MagOption module for paired-pulse testing, and two sites used Magstim 200[2]s with a BiStim[2] module controlled by Signal software (Cambridge, UK). Figure-of-eight coils were used for TMS (MagVenture MC-B70, Magstim D70 992500), during which motor evoked potentials (MEPs) were recorded from the abductor pollicis brevis (APB) and first dorsal interosseous (FDI) muscles (FDI as control) using an AD Instruments Powerlab 26T amplifier and LabChart software. Standard measures were used for hotspot determination. Resting motor threshold (RMT) measurement was defined as the threshold that elicited five of 10 MEP responses of amplitude greater than 50 μV[2]. TMS methods adhered to the recommendations endorsed by the International Federation of Clinical Neurophysiology[3].

The TMS protocol consisted of RMT, paired-pulse testing, input output curve, and cortical silent period measurements. For paired-pulse testing, the conditioning and test stimuli were 80% and 120% of RMT, respectively. Paired-pulse testing consisted of 98 pulses, divided into 7 blocks of 14 pulses separated by pre-specified intervals between five and seven seconds. Each block contained two single pulses with stimulation amplitude 80% of RMT, four single pulses with amplitude 120% of RMT, four paired pulses for SICI measurement (3 ms interstimulus interval), and four paired pulses for intracortical facilitation (ICF, 15 ms interstimulus interval) measurement. SICI and ICF were each defined as the ratio of the conditioned MEP amplitude to the unconditioned MEP amplitude. Note that reduced hyperexcitability due to increased inhibition or reduced facilitation from conditioning stimuli results in smaller conditioned MEP amplitudes and thus smaller SICI and ICF values. To add clarity, we reported SICI^{-1}, so that stronger inhibition is demonstrated by an increase in amplitude. Input-output curves were determined using 3 blocks of 30 pulses, with each block containing three pulses at stimulation levels 60% to 150% of RMT in 10% increments. CSP was measured from the end of the MEP until the return of EMG activity using a separate group of 10 pulses with stimulation amplitude set to 120% RMT amplitude and basal activation (approximately 30% of maximal voluntary contraction) of the APB by the participant. The order for each block was determined by an initial randomization procedure and then repeated throughout the study. Data were sampled at 40 kHz and filtered from 10 Hz to 2 kHz. TMS raw data were analyzed blindly using custom Matlab software (Mathworks, Natick, MA) by a single reviewer. TMS measurements were accepted for analysis if the overall study and individual measurements of RMT, input-output curves, and CSP were judged of good quality by the central reader, and RMT was ≤83% of maximum stimulator strength as required to perform the SICI stimulation protocol at 120% of RMT.

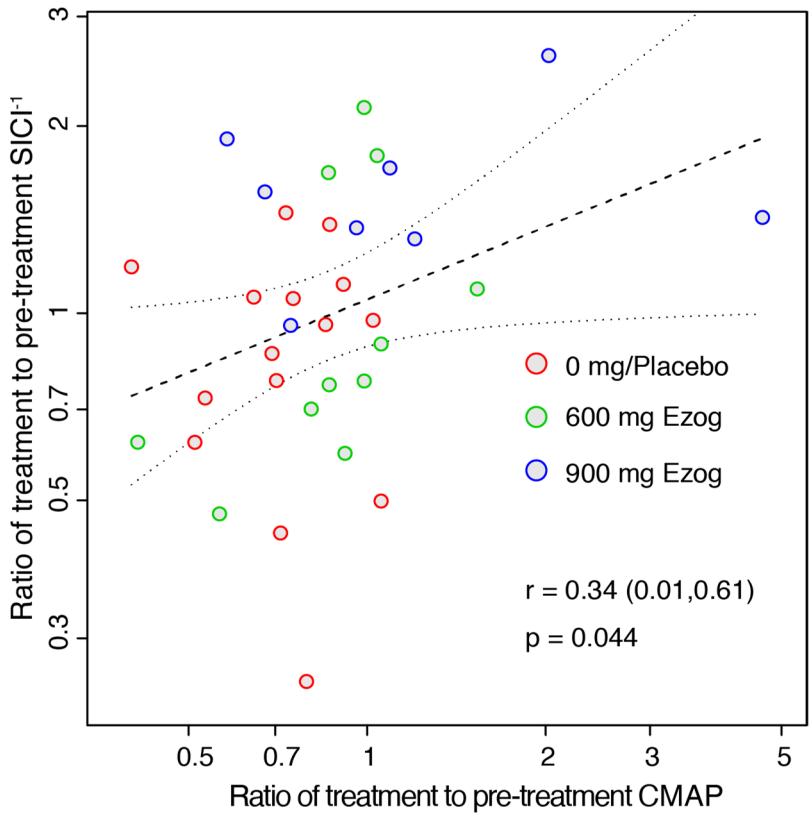
Axonal TTNCS excitability studies were performed on the median nerve according to a previously described technique[4,5]. Neuromuscular physicians used clinical EMG software and amplifiers (Synergy and Viking at 11 sites, Digitimer D440 at 1 site) for recording and a DS5 current stimulator (Digitimer, UK) controlled by Qtracs software

(Hugh Bostock, University College London). The cathode was placed 3 cm proximal to the distal wrist crease, and the anode was positioned 10 cm proximally along the median nerve and then 2 cm medially. For both TMS and TTNCS, Natus electrodes (019-415000 and 019-400500) were used. Compound muscle action potentials (CMAPs) were recorded from the abductor pollicis brevis (APB) muscle with the G1 electrode positioned over the muscle belly and the G2 electrode placed one-third of the way from the proximal to the distal protuberances of the proximal phalanx, using a belly-to-tendon montage. Data were filtered and sampled at 10 kHz using a 16-bit data acquisition card (National Instruments USB-6221). Temperature, measured at the recording site using a TempIR Body Temperature thermometer (TempIR), was required to be above 32˚C prior to testing. TTNCS measurements were accepted for analysis if judged to be of good quality by the central reader.

Additional Neurophysiological Outcomes

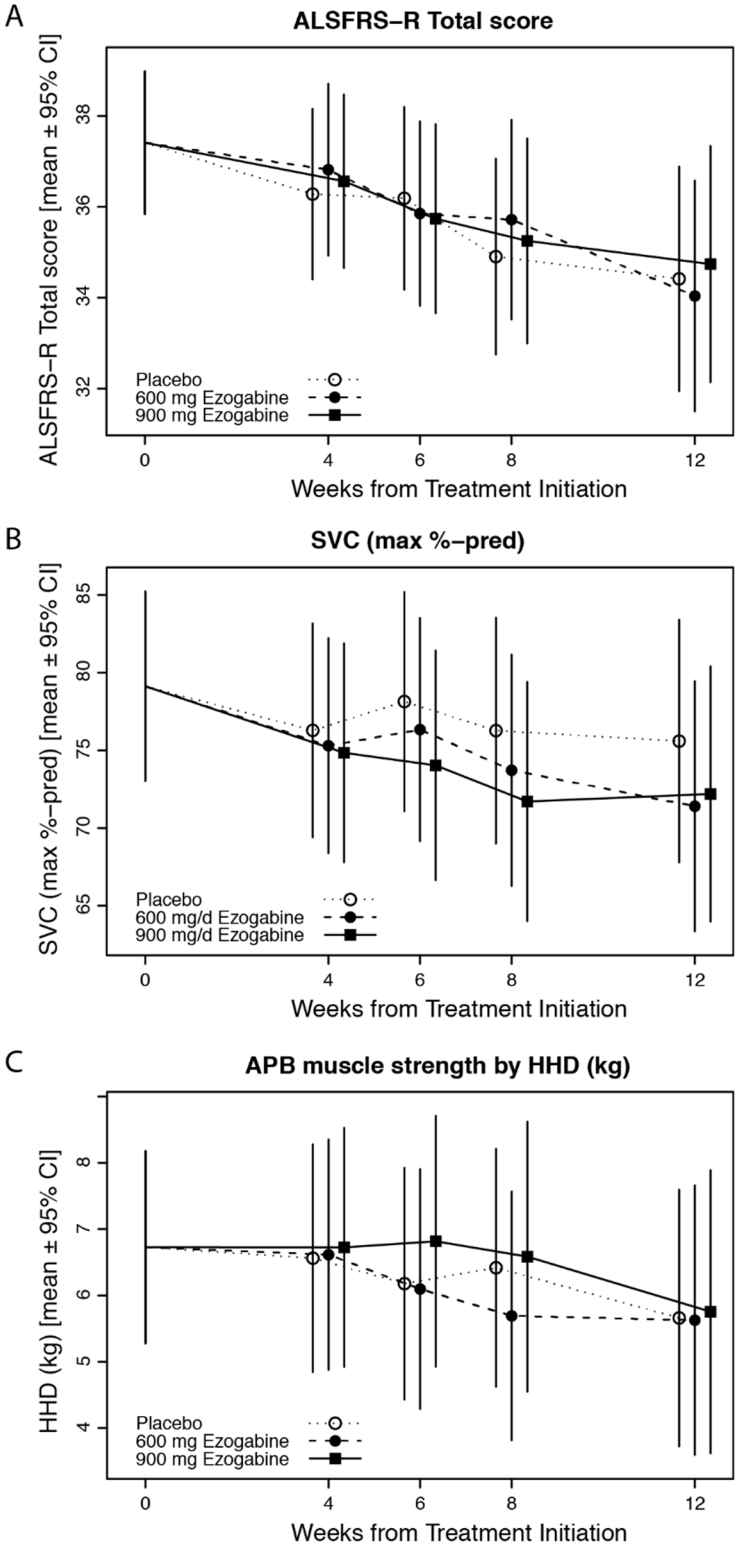
The following additional secondary outcomes were recorded with TMS: intracortical facilitation (ICF), facilitation at longer interstimulus intervals; cortical silent period, a pause in muscle activity that follows TMS stimulus and reflects both spinal cord refractoriness and cortical inhibition. The following additional secondary outcomes were measured using TTNCS: depolarizing and hyperpolarizing electrotonus (both measured at 90-100 ms), which describe how threshold for motor response changes with prolonged prepulses; recovery cycle measurements including latency, relative refractory period, superexcitabilty, and late subexcitability, which reflect different phases of threshold change in a paired stimulus protocol.

eFigure 1. Increased Cortical Inhibition Correlates With Maintained CMAP During Treatment



The plot shows the effect of placebo (red), 600 mg/d (green), and 900 mg/d (blue) ezogabine treatment on ratio of pre-treatment to average of six and eight week treatment measurements of $SICI^{-1}$ and CMAP for individual participants. Estimated Spearman rank correlation with 95% confidence interval and p-value are reported (post-hoc analysis).

eFigure 2. 10-Week Treatment Has Little Effect on Clinical Disease Progression Metrics



Plots show effect of placebo, 600 mg/d, and 900 mg/d ezogabine treatment on ALSFRS-R (A), SVC (B), and strength (HHD) measured in the APB muscle (C).

eTable 1. Sensitivity Analyses of Treatment Effects on $SICI^{-1}$ Adjusting for Potential Confounders or Restricting to Participants on Riluzole

	Longitudinal within-group comparison: six and eight weeks on treatment vs. screening and baseline pre-treatment (ratio)			Treatment group comparison (ratio of ratio)	
	Placebo (est, CI, p)	600 mg/d (est, CI, p)	900 mg/d (est, CI, p)	600 mg/d vs placebo (est, CI, p)	900 mg/d vs placebo (est, CI, p)
Primary model for reference	0.908 (0.725, 1.137), 0.393	1.046 (0.816, 1.341), 0.715	1.388 (1.045, 1.845), 0.025	1.153 (0.874, 1.520), 0.307	1.529 (1.120, 2.089), 0.009
Adjusted models					
Sex	0.891 (0.696, 1.141), 0.354	1.021 (0.775, 1.344), 0.881	1.312 (0.965, 1.784), 0.082	1.145 (0.820, 1.600), 0.420	1.472 (1.026, 2.111), 0.036
Baseline riluzole use	0.899 (0.696, 1.160), 0.404	1.009 (0.764, 1.332), 0.951	1.317 (0.965, 1.799), 0.082	1.122 (0.795, 1.584), 0.504	1.466 (1.012, 2.123), 0.043
Time since symptom onset	0.894 (0.697, 1.147), 0.370	1.017 (0.771, 1.342), 0.903	1.314 (0.959, 1.799), 0.087	1.138 (0.813, 1.592), 0.444	1.470 (1.014, 2.129), 0.042
Age at symptom onset	0.892 (0.693, 1.147), 0.365	0.991 (0.744, 1.319), 0.947	1.304 (0.959, 1.773), 0.089	1.111 (0.792, 1.558), 0.535	1.462 (1.014, 2.108), 0.042
Baseline $SICI^{-1}$	0.882 (0.694, 1.122), 0.298	0.988 (0.755, 1.293), 0.927	1.267 (0.941, 1.706), 0.116	1.120 (0.818, 1.534), 0.472	1.437 (1.022, 2.020), 0.038
Baseline UMN score	0.870 (0.665, 1.137), 0.300	1.004 (0.743, 1.357), 0.978	1.352 (0.968, 1.889), 0.076	1.155 (0.780, 1.709), 0.464	1.555 (1.013, 2.387), 0.044
SVC	0.899 (0.696, 1.160), 0.404	1.009 (0.764, 1.332), 0.951	1.317 (0.964, 1.799), 0.082	1.122 (0.795, 1.584), 0.504	1.466 (1.012, 2.123), 0.043
Restricted sample					
Participants on riluzole only	0.861 (0.646, 1.148), 0.300	0.998 (0.719, 1.387), 0.992	1.353 (0.978, 1.872), 0.067	1.159 (0.778, 1.728), 0.459	1.571 (1.054, 2.341), 0.027

eTable 2. Treatment-Emergent Adverse Events by MedDRA System Organ Class and Preferred Terms

	Placebo			600 mg Ezog			900 mg Ezog		
	#E	#S	% S	#E	#S	% S	#E	#S	% S
Blood And Lymphatic System Disorders	0	0	0%	1	1	4%	0	0	0%
Anaemia	0	0	0%	1	1	4%	0	0	0%
Cardiac Disorders	0	0	0%	2	1	4%	0	0	0%
Supraventricular Tachycardia	0	0	0%	2	1	4%	0	0	0%
Ear And Labyrinth Disorders	1	1	4%	1	1	4%	1	1	5%
Tinnitus	0	0	0%	1	1	4%	0	0	0%
Vertigo	1	1	4%	0	0	0%	1	1	5%
Eye Disorders	4	3	13%	3	3	13%	2	2	11%
Diplopia	0	0	0%	1	1	4%	0	0	0%
Eye Irritation	0	0	0%	1	1	4%	0	0	0%
Photophobia	1	1	4%	0	0	0%	0	0	0%
Vision Blurred	2	2	9%	1	1	4%	2	2	11%
Visual Impairment	1	1	4%	0	0	0%	0	0	0%
Gastrointestinal Disorders	9	6	26%	24	9	39%	12	10	53%
Abdominal Pain	0	0	0%	1	1	4%	0	0	0%
Abdominal Pain Lower	0	0	0%	0	0	0%	1	1	5%
Abdominal Pain Upper	0	0	0%	1	1	4%	0	0	0%
Constipation	2	2	9%	3	3	13%	4	4	21%
Diarrhoea	1	1	4%	2	2	9%	2	2	11%
Dry Mouth	0	0	0%	1	1	4%	1	1	5%
Glossodynia	0	0	0%	1	1	4%	0	0	0%
Haematochezia	0	0	0%	1	1	4%	0	0	0%
Hypoaesthesia Oral	0	0	0%	1	1	4%	2	2	11%
Nausea	3	3	13%	6	3	13%	0	0	0%
Paraesthesia Oral	0	0	0%	5	3	13%	1	1	5%
Retching	1	1	4%	0	0	0%	0	0	0%
Salivary Hypersecretion	2	2	4%	2	2	4%	0	0	0%
Tongue Disorder	0	0	0%	0	0	0%	1	1	5%
General Disorders And Administration Site Conditions	10	7	30%	20	13	57%	14	11	58%
Asthenia	0	0	0%	3	3	13%	2	2	11%
Catheter Site Pain	0	0	0%	1	1	4%	0	0	0%
Chest Discomfort	1	1	4%	0	0	0%	0	0	0%
Chest Pain	1	1	4%	0	0	0%	0	0	0%
Fatigue	6	6	26%	12	11	48%	7	7	37%
Feeling Jittery	1	1	4%	0	0	0%	0	0	0%

Gait Disturbance	1	1	2%	1	1	2%	2	2	5%
Infusion Site Bruising	0	0	0%	1	1	4%	0	0	0%
Oedema Peripheral	0	0	0%	0	0	0%	1	1	5%
Pain	0	0	0%	0	0	0%	1	1	5%
Peripheral Coldness	1	1	2%	1	1	2%	0	0	0%
Puncture Site Pain	1	1	4%	0	0	0%	0	0	0%
Pyrexia	0	0	0%	1	1	4%	1	1	5%
Immune System Disorders	0	0	0%	1	1	4%	0	0	0%
Seasonal Allergy	0	0	0%	1	1	4%	0	0	0%
Infections And Infestations	3	3	13%	6	3	13%	4	4	21%
Bacterial Infection	0	0	0%	0	0	0%	1	1	5%
Gastroenteritis Viral	0	0	0%	1	1	4%	0	0	0%
Influenza	0	0	0%	1	1	4%	0	0	0%
Nasopharyngitis	2	2	9%	0	0	0%	1	1	5%
Pneumonia	0	0	0%	1	1	4%	0	0	0%
Sinusitis	1	1	4%	2	1	4%	0	0	0%
Upper Respiratory Tract Infection	0	0	0%	1	1	4%	2	2	11%
Injury, Poisoning And Procedural Complications	11	5	22%	13	9	39%	9	5	26%
Back Injury	0	0	0%	1	1	4%	0	0	0%
Contusion	1	1	4%	0	0	0%	1	1	5%
Fall	7	4	17%	7	7	30%	5	4	21%
Head Injury	0	0	0%	1	1	4%	1	1	5%
Incision Site Erythema	1	1	4%	0	0	0%	0	0	0%
Incision Site Pain	1	1	4%	0	0	0%	0	0	0%
Joint Injury	1	1	4%	0	0	0%	0	0	0%
Poisoning	0	0	0%	0	0	0%	1	1	5%
Post Lumbar Puncture Syndrome	0	0	0%	2	2	9%	0	0	0%
Skin Abrasion	0	0	0%	1	1	4%	1	1	5%
Skin Wound	0	0	0%	1	1	4%	0	0	0%
Investigations	2	2	9%	12	6	26%	5	3	16%
Alanine Aminotransferase Increased	0	0	0%	0	0	0%	1	1	5%
Aspartate Aminotransferase Increased	0	0	0%	0	0	0%	1	1	5%
Bacterial Test	0	0	0%	0	0	0%	1	1	5%
Bilirubin Conjugated Increased	0	0	0%	1	1	4%	0	0	0%
Blood Urine	0	0	0%	1	1	4%	0	0	0%
Crystal Urine Present	0	0	0%	0	0	0%	1	1	5%
Electrocardiogram Qt Prolonged	0	0	0%	0	0	0%	1	1	5%
Haemoglobin Decreased	0	0	0%	1	1	4%	0	0	0%

Mean Cell Volume Increased	1	1	4%	0	0	0%	0	0	0%
Protein Urine	0	0	0%	1	1	4%	0	0	0%
Transaminases Increased	1	1	4%	1	1	4%	0	0	0%
Urine Leukocyte Esterase	0	0	0%	1	1	4%	0	0	0%
Urine Output Decreased	0	0	0%	1	1	4%	0	0	0%
Urodynamics Measurement Abnormal	0	0	0%	1	1	4%	0	0	0%
Weight Decreased	0	0	0%	2	2	9%	0	0	0%
White Blood Cell Count Decreased	0	0	0%	1	1	4%	0	0	0%
White Blood Cells Urine Positive	0	0	0%	1	1	4%	0	0	0%
Metabolism And Nutrition Disorders	0	0	0%	6	4	17%	0	0	0%
Decreased Appetite	0	0	0%	2	2	9%	0	0	0%
Dehydration	0	0	0%	1	1	4%	0	0	0%
Hyperphagia	0	0	0%	1	1	4%	0	0	0%
Hypokalaemia	0	0	0%	1	1	4%	0	0	0%
Increased Appetite	0	0	0%	1	1	4%	0	0	0%
Musculoskeletal And Connective Tissue Disorders	18	8	35%	13	8	35%	9	6	32%
Arthralgia	1	1	4%	2	2	9%	1	1	5%
Back Pain	1	1	4%	1	1	4%	1	1	5%
Joint Swelling	0	0	0%	1	1	4%	0	0	0%
Muscle Spasms	6	5	22%	2	2	9%	2	2	11%
Muscle Twitching	1	1	4%	0	0	0%	0	0	0%
Muscular Weakness	4	3	13%	2	2	9%	3	2	11%
Musculoskeletal Chest Pain	0	0	0%	0	0	0%	1	1	5%
Musculoskeletal Discomfort	0	0	0%	0	0	0%	1	1	5%
Musculoskeletal Pain	1	1	4%	0	0	0%	0	0	0%
Musculoskeletal Stiffness	1	1	4%	1	1	4%	0	0	0%
Myalgia	1	1	4%	2	2	9%	0	0	0%
Pain In Extremity	1	1	4%	1	1	4%	0	0	0%
Synovial Cyst	1	1	2%	1	1	2%	0	0	0%
Nervous System Disorders	18	11	48%	31	10	43%	34	16	84%
Aura	0	0	0%	0	0	0%	1	1	5%
Balance Disorder	0	0	0%	1	1	4%	2	2	11%
Cerebrovascular Accident	0	0	0%	1	1	4%	0	0	0%
Cognitive Disorder	1	1	4%	2	1	4%	2	1	5%
Coordination Abnormal	0	0	0%	1	1	4%	0	0	0%
Depressed Level Of Consciousness	1	1	4%	1	1	4%	1	1	5%
Dizziness	3	3	13%	6	5	22%	9	8	42%
Dropped Head Syndrome	0	0	0%	1	1	4%	0	0	0%

Dysarthria	0	0	0%	2	2	9%	2	2	11%
Dysgeusia	0	0	0%	2	1	4%	0	0	0%
Dyskinesia	1	1	4%	1	1	4%	0	0	0%
Dysstasia	0	0	0%	1	1	4%	1	1	5%
Headache	6	5	22%	3	1	4%	2	2	11%
Language Disorder	1	1	4%	0	0	0%	1	1	5%
Memory Impairment	0	0	0%	0	0	0%	1	1	5%
Muscle Contractions Involuntary	1	1	4%	2	2	9%	3	3	16%
Muscle Spasticity	0	0	0%	1	1	4%	0	0	0%
Paraesthesia	0	0	0%	1	1	4%	0	0	0%
Restless Legs Syndrome	0	0	0%	1	1	4%	0	0	0%
Somnolence	1	1	4%	2	2	9%	7	7	37%
Speech Disorder	1	1	4%	0	0	0%	1	1	5%
Syncope	1	1	4%	0	0	0%	1	1	5%
Tremor	0	0	0%	2	1	4%	0	0	0%
Psychiatric Disorders	5	2	9%	5	3	13%	15	9	47%
Affect Lability	1	1	4%	1	1	4%	0	0	0%
Anxiety	2	1	4%	1	1	4%	0	0	0%
Confusional State	0	0	0%	1	1	4%	3	3	16%
Depression	1	1	4%	0	0	0%	1	1	5%
Disorientation	0	0	0%	2	1	4%	3	1	5%
Euphoric Mood	0	0	0%	0	0	0%	1	1	5%
Hallucination, Visual	0	0	0%	0	0	0%	5	4	21%
Mental Status Changes	0	0	0%	0	0	0%	1	1	5%
Panic Attack	1	1	4%	0	0	0%	0	0	0%
Perseveration	0	0	0%	0	0	0%	1	1	5%
Renal And Urinary Disorders	2	2	9%	13	8	35%	3	3	16%
Chromaturia	0	0	0%	2	2	9%	2	2	11%
Dysuria	0	0	0%	2	1	4%	0	0	0%
Micturition Urgency	0	0	0%	1	1	4%	0	0	0%
Nocturia	0	0	0%	1	1	4%	0	0	0%
Pollakiuria	1	1	4%	0	0	0%	0	0	0%
Polyuria	0	0	0%	1	1	4%	0	0	0%
Urinary Hesitation	1	1	4%	0	0	0%	1	1	5%
Urinary Retention	0	0	0%	5	4	17%	0	0	0%
Urine Flow Decreased	0	0	0%	1	1	4%	0	0	0%
Respiratory, Thoracic And Mediastinal Disorders	4	2	9%	10	7	30%	5	4	21%
Cough	1	1	4%	0	0	0%	0	0	0%
Dry Throat	0	0	0%	1	1	4%	0	0	0%
Dyspnoea	0	0	0%	5	4	17%	1	1	5%

Increased Viscosity Of Bronchial Secretion	0	0	0%	0	0	0%	1	1	5%
Nasal Congestion	2	1	4%	1	1	4%	0	0	0%
Oropharyngeal Pain	0	0	0%	1	1	4%	1	1	5%
Sinus Congestion	0	0	0%	0	0	0%	1	1	5%
Snoring	0	0	0%	1	1	4%	0	0	0%
Throat Tightness	1	1	4%	0	0	0%	0	0	0%
Upper-Airway Cough Syndrome	0	0	0%	1	1	4%	1	1	5%
Skin And Subcutaneous Tissue Disorders	3	1	4%	9	6	26%	3	2	11%
Dermatitis Allergic	0	0	0%	0	0	0%	1	1	5%
Erythema	3	1	4%	5	2	9%	2	1	5%
Hyperhidrosis	0	0	0%	1	1	4%	0	0	0%
Rash	0	0	0%	2	2	9%	0	0	0%
Skin Disorder	0	0	0%	1	1	4%	0	0	0%
Surgical And Medical Procedures	1	1	4%	0	0	0%	0	0	0%
Cyst Removal	1	1	4%	0	0	0%	0	0	0%
Vascular Disorders	2	2	9%	0	0	0%	0	0	0%
Hypotension	1	1	4%	0	0	0%	0	0	0%
Overall	93	22	96%	170	22	96%	116	19	100%

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