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Journal of Comparative Effectiveness Research

Comparative safety and efficacy of ozanimod versus fingolimod for relapsing multiple sclerosis

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Aim: Ozanimod and fingolimod are sphingosine 1-phosphate receptor-modulating therapies for relapsing multiple sclerosis. **Patients & methods:** Comparative effectiveness was assessed by matching adjusted indirect comparisons of safety and efficacy trial outcomes at first-dose cardiac monitoring, 1 year and 2 years. **Results:** After adjustment, baseline characteristics were similar. Ozanimod was associated with a lower risk of extended first-dose monitoring, conduction abnormalities including atrioventricular block. 1-year risks of any adverse event (AE), mean lymphocyte count reductions and abnormal liver enzymes were lower with ozanimod. 2-year risks of AEs leading to discontinuation, any AEs, herpetic infections, bradycardia and abnormal liver enzymes were lower with ozanimod. Analyses of efficacy outcomes were similar. **Conclusion:** Ozanimod appears to have a favorable benefit–risk profile versus fingolimod.

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Keywords: efficacy • fingolimod • indirect comparison • ozanimod • relapsing multiple sclerosis • safety

Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative disease of the central nervous system. It is characterized by inflammation, demyelination, neuronal and oligodendrocyte loss, disruption of the blood–brain barrier, as well as debilitating symptoms such as fatigue, depressive symptoms and cognitive impairment [1,2]. MS may present as a clinically isolated syndrome, relapsing MS (RMS), secondary progressive MS or primary progressive MS [3,4]. RMS, the most prevalent type of MS, is characterized by episodes of relapses followed by a remission period. Approximately 85% of individuals with MS present with RMS [5]. Complete physical recovery from relapse can occur, but approximately 50% of relapses are associated with residual neurological deficits resulting in a sustained increase in disability [6], typically measured in Phase III MS trials using the Expanded Disability Status Scale (EDSS) [7,8]. MS often has a highly debilitating impact on quality of life for individuals and their families [9], and is associated with considerable economic burden [10].

Currently, MS has no cure, but treatment options exist. Anti-inflammatory agents, such as corticosteroids, are used as symptomatic treatment during acute relapses. Disease-modifying therapies (DMTs), such as immunomodulators, are used to alter the disease course by reducing relapses, with the goal of preventing or slowing long-term disability [11–14]. Fingolimod is a nonselective sphingosine 1-phosphate (S1P) receptor modulator that binds to the receptor subtypes $S1PR_1$, $S1PR_3$, $S1PR_4$ and $S1PR_5$, and was the first oral DMT approved (US, 2010; EU, 2011) for the treatment of RMS. However, fingolimod may be associated with cardiovascular, ophthalmologic, pulmonary and hepatic safety concerns, as listed in its drug label [15]. Ozanimod is an investigational oral DMT and a selective S1P receptor modulator designed to target only the receptor subtypes $S1PR_1$ and $S1PR_5$ [16]; it is currently under review by regulatory agencies for the treatment of RMS. The clinical efficacy, safety and tolerability of ozanimod for the treatment of patients with RMS have been demonstrated in the Phase III RADIANCE [17] and SUNBEAM [18] clinical trials.

The comparative efficacy of these two S1P receptor–modulating agents in the treatment of RMS has not yet been fully characterized. In the absence of a head-to-head randomized trial between ozanimod and fingolimod, the

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current study was designed to indirectly compare the key safety and efficacy outcomes between these two therapies for the treatment of patients with RMS using a matching-adjusted indirect comparison (MAIC) [19,20] to adjust for cross-trial differences in patient data from their pivotal clinical trials. The outcomes assessed in this analysis include first-dose cardiac monitoring outcomes as well as 1-year and 2-year safety and efficacy outcomes.

MAIC methodology was recently used to compare delayed-release dimethyl fumarate and fingolimod for the treatment of patients with RMS [21]. Results showed that the efficacy of dimethyl fumarate was similar to that of fingolimod on clinical measures of relapse and disability progression [21]. In addition, MAIC methodology was recently used to assess the comparative efficacy of cladribine versus alemtuzumab in adults with RMS [22]. Results of that study showed that the efficacy of cladribine was comparable to alemtuzumab in the treatment of patients with RMS based on 6-month confirmed disability progression (CDP) and annualized relapse rates (ARRs) [22].

Patients & methods

Data source

Individual patient data from the RADIANCE-B (NCT02047734 [RPC01-201B]) [23] and SUNBEAM (NCT02294058 [RPC01-301]) [24,25] ozanimod trials were used in this analysis. RADIANCE-B was a randomized, IFN β-1a–controlled, Phase III trial of ozanimod in patients with RMS. Eligible participants were randomly assigned in a 1:1:1 ratio to ozanimod HCI (daily oral 0.5 or 1 mg) or IFN β-1a for 2 years. The trial enrolled 1313 patients with RMS. SUNBEAM was also a randomized (1:1:1 ratio), controlled study of ozanimod HCI (0.5 and 1 mg) versus IFN β-1a in patients with RMS over a minimum 12-month period. The study enrolled 1346 patients with RMS. To attenuate potential first-dose cardiac effects with ozanimod HCI, in both trials, a dose-escalation regimen was applied for all participants, consisting of 0.25 mg on days 1–4, 0.5 mg on days 5–7 and then the assigned dose of 0.5 or 1 mg from day 8 through week 24.

In addition, the published summary-level data from the TRANSFORMS (NCT00340834 [D2302]) [26], FREEDOMS (NCT00289978 [D2301]) [27] and FREEDOMS II (NCT00355134 [D2309]) [28] fingolimod Phase III trials were used, along with pooled safety data from the TRANSFORMS, FREEDOMS and FREEDOMS II trials [29], as well as the trial data reported in clinicaltrials.gov. TRANSFORMS was a randomized, IFN β-1a–controlled, Phase III trial of fingolimod in patients with RMS. Eligible participants were randomly assigned in a 1:1:1 ratio to fingolimod (oral 0.5 or 1.25 mg) or IFN β-1a for 1 year. The trial enrolled 1292 patients with RMS. FREEDOMS I and FREEDOMS II were two randomized, placebo-controlled, Phase III trials of fingolimod in patients with RMS. Eligible participants were randomly assigned in a 1:1:1 ratio to fingolimod (oral 0.5 or 1.25 mg) or placebo for 2 years. The trials enrolled 1292 and 1083 patients with RMS, respectively. Only the approved and recommended dose of fingolimod 0.5 mg was considered for these analyses. No institutional review was required as this was a *post hoc* analysis of previously published, de-identified data.

Inclusion criteria

For the ozanimod trials, patients were required to be 18–55 years of age; have a diagnosis of RMS, as defined by the 2010 revised McDonald criteria [30]; have had at least one documented relapse in the previous year before screening (or prior 2 years with at least one gadolinium-enhancing lesion); and have a score between 0.0 and 5.0 on the EDSS [31]. In the fingolimod trials, patients were required to be 18–55 years of age; have a diagnosis of RMS, as defined by the 2005 revised McDonald criteria [30]; have had at least one confirmed relapse during the preceding 1 year (or at least two during the preceding 2 years); and have a score between 0.0 and 5.5 on the EDSS.

Study outcomes

Outcomes assessed at the first-dose cardiac monitoring included heart rate, electrocardiographic findings and change in blood pressure (BP) from baseline and whether patients received extended monitoring after 6 h, received Day 2 monitoring, or discontinued treatment on Day 1.

Safety and efficacy outcomes assessed at 1 and 2 years included adverse events (AEs), AEs leading to discontinuation, any serious AE, patient death, liver enzymes (ALT) at least three-times the upper limit of normal, macular edema, absolute lymphocyte count, lymphocyte count <0.2 K/μl, ARR and 3-month and 6-month confirmed CDP. Because data for the 6-month CDP at 1 year was not reported in the fingolimod trial, a comparison between ozanimod and fingolimod on this outcome was not feasible.

Comparisons of first-dose cardiac monitoring outcomes

Both ozanimod HCl doses (0.5 and 1 mg) from RADIANCE-B and SUNBEAM were pooled because the initial dose of ozanimod on Day 1 in both studies for both dose groups was 0.25 mg, according to identical protocol-specified dose escalation regimens [17,18]. Each fingolimod treatment group was pooled across the TRANSFORMS, FREEDOMS and FREEDOMS II studies. Before and after matching, baseline patient characteristics and selected outcomes were described and compared for the pooled ozanimod doses versus fingolimod (0.5 mg dose).

Comparisons of 1-year outcomes

Both of the ozanimod HCl 1 mg dose groups from RADIANCE-B and SUNBEAM were pooled. The ozanimod clinical trials and the TRANSFORMS trial all included a randomized comparison to IFN β-1a intramuscular (Avonex). An anchor-based comparison was conducted for 1-year safety and efficacy outcomes using the IFN β-1a arm as an anchor. Before and after matching, baseline patient characteristics and selected outcomes were described and compared for (1) the pooled IFN β-1a arms from the ozanimod trials (RADIANCE-B and SUNBEAM) versus IFN β-1a arm from the fingolimod trial (TRANSFORMS), to assess the consistency of outcomes before and after matching; and (2) the pooled ozanimod HCl 1 mg arms (RADIANCE-B and SUNBEAM) versus the fingolimod 0.5 mg arm (TRANSFORMS), with the comparison anchored on IFN β-1a.

Comparisons of 2-year outcomes

The patient group receiving ozanimod HCl 1 mg was obtained from RADIANCE-B. The fingolimod groups were pooled across the FREEDOMS and FREEDOMS II studies. Before and after matching, baseline patient characteristics and selected outcomes were described and compared for the ozanimod HCl 1 mg arm (RADIANCE-B) versus the pooled fingolimod 0.5 mg arms (FREEDOMS and FREEDOMS II). Because of a lack of data at 2 years comparing fingolimod versus IFN β-1a, the comparisons of 2-year outcomes were nonanchored.

Assessment of cross-trial similarities & differences

The trial designs included in this *post hoc* analysis were determined to be highly similar and suitable for an adjusted cross-trial comparison. Patients in all trials were 18–55 years of age and discontinued prior treatment (e.g., IFN β, glatiramer acetate and other DMTs) before randomization. For the first-dose cardiac monitoring assessment, all patients had hourly assessments of heart rate and BP available, and the duration of assessment was ≥6 h. In addition, the definitions of and assessment methodologies for relapse were similar across trials.

Several differences between the trials existed. An RMS diagnosis was based on the 2010 revised McDonald criteria in the ozanimod trials and on the 2005 revised McDonald criteria in the fingolimod trials [30]. The ozanimod trials required either one documented relapse in the prior year or one in the prior 2 years along with gadolinium-enhancing lesions, whereas the fingolimod trials required either one confirmed relapse during the prior year or at least two during the prior 2 years. The upper threshold for the EDSS score for inclusion in the ozanimod trials was 5.0, whereas the upper threshold was a score of 5.5 in the fingolimod trials. Heart rate and BP were measured hourly for the first 6 h after the first dose in both trials; however, the ozanimod trials measured patients in the supine position (as well as the standing position), whereas the fingolimod trials measured patients in the sitting position. Other differences between the trials included their time periods and geography. The ozanimod trials were multinational, conducted between 2013 and 2017, and included higher proportions of patients from Eastern Europe (~90%) than the fingolimod trials (~2%), which were also multinational but were conducted between 2006 and 2011.

Statistical methods

MAIC methodology was used to adjust for baseline patient differences for each treatment comparison and outcome period [19,20]. Individual patients in the ozanimod trials were assigned weights such that weighted mean baseline patient characteristics in the ozanimod trials exactly matched those reported for the fingolimod trials. Patients' weights were equal to their estimated odds of enrollment in an ozanimod trial versus a fingolimod trial, conditional on enrollment within either of the trial populations.

A logistic regression model using the method of moments [20] was used to estimate the weights for the propensity of enrollment in the ozanimod trials versus the fingolimod trials. Based on data availability and clinical considerations, all MAICs adjusted for cross-trial differences in the following baseline characteristics: age (mean), sex (proportion who were female), duration of MS since first symptom (mean), relapses within previous year (mean), relapses within

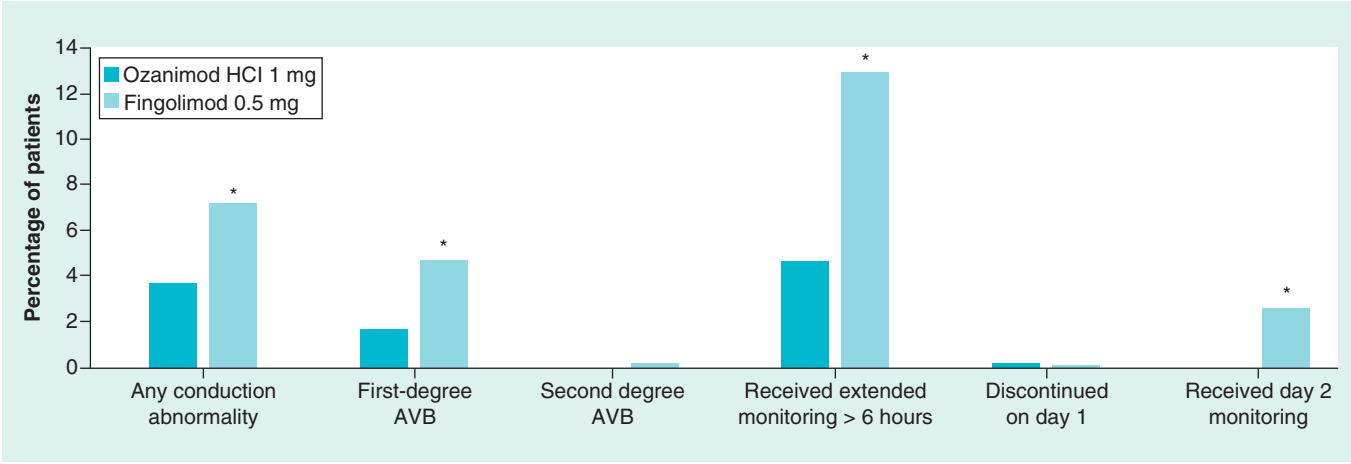

Figure 1. First-dose monitoring outcomes for ozanimod HCl 1 mg versus fingolimod 0.5 mg after baseline adjustment.
*$p < 0.05$ vs ozanimod.
AVB: Atrioventricular block.

previous 2 years (mean), EDSS score (mean), prior DMTs (percentage) and absence of gadolinium-enhancing lesions (percentage). For first-dose monitoring outcomes, resting heart rate (mean), cardiac disorders (percentage) and any conduction abnormality (percentage) were also adjusted for cross-trial differences. In addition, for 1-year outcomes, lymphocyte count at baseline was adjusted for cross-trial differences.

Baseline patient characteristics (i.e., demographic and clinical characteristics) before and after matching were compared between the ozanimod and fingolimod groups. Means and standard deviations were reported for continuous variables; frequencies and percentages were reported for categorical variables. Comparisons of binary variables before matching were conducted via χ^2 tests, and Wald tests were used for the comparisons of binary variables after matching and the comparisons of continuous variables both before and after matching. A p-value of 0.05 was used to determine statistical significance.

Results

Baseline characteristics before & after matching

Before MAIC adjustment, some of the clinically relevant differences between patients receiving ozanimod (N = 1773) compared with those receiving fingolimod (N = 1212) included shorter MS duration (6.8 vs 8.5 years, respectively) and lower likelihood of prior DMT use (29.0 vs 56.4%) (Supplementary Table 1). After adjustment, baseline averages for all included patient characteristics were balanced between the ozanimod and fingolimod trials. The anchor-based and nonanchor-based comparisons of the 1-year safety outcomes are listed in Table 1 and Supplementary Table 2, respectively.

Adjusted analyses of first-dose cardiac monitoring outcomes

Compared with ozanimod, the adjusted absolute increases in the percentages of patients whose lowest hourly recorded heart rate was <45 bpm (45–54 bpm) in the first 6 h were +1.4% (+12.1%) for fingolimod 0.5 mg ($p < 0.001$; Table 2), indicating that the adjusted risk difference (RD) was more favorable for ozanimod.

The rates of the studied safety outcomes during first-dose cardiac monitoring were generally lower with ozanimod than with fingolimod. Compared with fingolimod, ozanimod was associated with significantly lower rates of conduction abnormalities (RD: -3.5%) and first-degree atrioventricular block (RD: -3.0%), as well as a lower risk of requiring monitoring beyond 6 h (RD: -8.3%) and of requiring Day 2 monitoring (RD: -2.6%; all $p < 0.001$; Figure 1 & Table 2). Ozanimod was associated with significantly less reduction in systolic (difference in means: 2.2 mm Hg) and diastolic (difference in means: 5.0 mm Hg) BP compared with fingolimod at first dose (both $p < 0.001$; Table 2).

Table 1. Comparison of matched baseline characteristics between the ozanimod HCl 1 mg and fingolimod 0.5 mg trials for 1-year outcomes†.

Characteristic	Before matching				p-value: [A] vs [C]	p-value: [B] vs [D]	After matching				p-value: [A] vs [C]	p-value: [B] vs [D]
	RADIANCE-B (RPC01-201B) and SUNBEAM (RPC01-301)		TRANSFORMS (D2302)				RADIANCE-B (RPC01-201B) and SUNBEAM (RPC01-301)		TRANSFORMS (D2302)			
	Ozanimod HCl 1 mg (N = 882) [A]	IFN β-1a (N = 885) [B]	Fingolimod 0.5 mg (N = 429) [C]	IFN β-1a (N = 431) [D]			Ozanimod HCl 1 mg (ESS = 276) [A]	IFN β-1a (ESS = 317) [B]	Fingolimod 0.5 mg (N = 429) [C]	IFN β-1a (N = 431) [D]		
Age, mean (SD), years	35.4 (9.1)	35.6 (9.1)	36.7 (8.8)	36.0 (8.3)	<0.05‡	0.41	36.7 (9.2)	36.0 (9.4)	36.7 (8.8)	36.0 (8.3)	1.0	1.0
Female, n (%)	576 (65.3)	602 (68.0)	281 (65.4)	292 (67.8)	0.99	0.97	65.4	67.8	65.4	67.8	1.0	1.0
Duration of MS since first symptom, mean (SD), years	6.9 (6.3)	6.6 (6.0)	7.5 (6.2)	7.4 (6.3)	0.1	<0.05‡	7.5 (6.5)	7.4 (6.4)	7.5 (6.2)	7.4 (6.3)	1.0	1.0
Relapses within previous year, mean (SD)	1.3 (0.6)	1.3 (0.6)	1.5 (1.2)	1.5 (0.8)	<0.001‡	<0.001‡	1.5 (0.7)	1.5 (0.7)	1.5 (1.2)	1.5 (0.8)	1.0	1.0
Relapses within previous 2 years, mean (SD)	1.7 (0.8)	1.7 (0.9)	2.3 (2.2)	2.3 (1.2)	<0.001‡	<0.001‡	2.3 (1.2)	2.3 (1.2)	2.3 (2.2)	2.3 (1.2)	1.0	1.0
EDSS score, mean (SD)	2.6 (1.2)	2.6 (1.2)	2.2 (1.3)	2.2 (1.3)	<0.001‡	<0.001‡	2.2 (1.1)	2.2 (1.1)	2.2 (1.3)	2.2 (1.3)	1.0	1.0
Patients with prior DMTs, n (%)	252 (28.6)	276 (31.2)	237 (55.2)	243 (56.3)	<0.001‡	<0.001‡	55.2	56.3	55.2	56.3	1.0	1.0
Absence of Gd-enhancing lesions, n (%)	488 (55.3)	475 (53.7)	289 (67.4)	272 (63.1)	<0.001‡	<0.01‡	67.4	63.1	67.4	63.1	1.0	1.0
Lymphocyte count at baseline, mean (SD), K/μl	1.8 (0.6)	1.9 (0.6)	1.8 (0.5)	1.7 (0.5)	0.2	<0.001‡	1.8 (0.6)	1.7 (0.5)	1.8 (0.5)	1.7 (0.5)	1.0	1.0

†For this analysis, the fingolimod and IFN β-1a arms from the TRANSFORMS trial (Cohen *et al.* [26]) were compared to the pooled ozanimod HCl 1 mg and IFN β-1a arms from the RADIANCE-B and SUNBEAM clinical trials.
‡Denotes a statistically significant difference.
DMTs: Disease-modifying therapy; EDSS: Expanded disability status scale; ESS: Effective sample size; Gd: Gadolinium; MS: Multiple sclerosis; SD: Standard deviation.

Table 2. Comparison of first-dose cardiac monitoring outcomes for ozanimod HCl 1 mg versus fingolimod 0.5 mg: assessment of risk differences[†].

Outcome	Adjusted risk difference for ozanimod HCl 1 mg vs fingolimod 0.5 mg		
	Δ [‡]	95% CI	p-value
Heart rate (bpm)			
– <45	-1.4	(-2.0, -0.7)	<0.001[¶]
– 45–54	-12.1	(-14.7, -9.5)	<0.001[¶]
– 55–64	-3.5	(-7.9, 0.9)	0.12
– ≥65	17.2	(13.0, 21.3)	<0.001[¶]
Decrease in heart rate (bpm) from baseline, hour 5[§]	6.6	(5.8, 7.5)	<0.001[¶]
Decrease in heart rate (bpm) from baseline, hour 6[§]	7.5	(6.7, 8.3)	<0.001[¶]
ECG findings			
– Any conduction abnormality	-3.5	(-5.3, -1.8)	<0.001[¶]
– Atrioventricular block			
– First-degree atrioventricular block	-3.0	(-4.4, -1.7)	<0.001[¶]
– Second-degree atrioventricular block (Wenckebach/Mobitz type I, Mobitz type II, 2:1)	-0.2	(-0.5, 0.1)	0.12
Received extended monitoring beyond 6 hours	-8.3	(-10.6, -6.0)	<0.001[¶]
Received Day 2 monitoring	-2.6	(-3.5, -1.7)	<0.001[¶]
Discontinued on Day 1	0.1	(-0.3, 0.4)	0.72
BP, mmHg			
– Change in mean sitting systolic BP	2.2	(1.3, 3.1)	<0.001[¶]
– Change in mean sitting diastolic BP	5.0	(4.3, 5.7)	<0.001[¶]

[†]First-dose cardiac monitoring outcomes for both fingolimod arms were extracted from the pooled analysis reported in DiMarco *et al.* [29]. Patient characteristics for ozanimod were extracted from the patient-level data from the RADIANCE-B and SUNBEAM trials; data for the 0.5 mg arm and the 1 mg arm were pooled for this analysis.
[‡]Δ represents the change in risk between the two arms.
[§]Decrease in heart rate from baseline to nadir for both fingolimod arms was compared with decrease in heart rate from baseline to hours 5 and 6 for ozanimod. Hour 5 represents the nadir for ozanimod, whereas hour 6 represents the end of the monitoring period.
[¶]Denotes a statistically significant difference.
BP: Blood pressure; bpm: Beats per minute; ECG: Electrocardiogram; SD: Standard deviation.

Adjusted analyses of 1-year outcomes

After adjustment for baseline patient characteristics, ozanimod was associated with a significantly lower risk of any AEs (RD: -9.9%), higher absolute mean lymphocyte count (difference in means: 0.4×10^9/l) and lower risk of abnormal liver enzyme (ALT) elevations (ALT ≥3x upper limit of normal; RD: -6.8%) compared with fingolimod (all $p < 0.05$; Table 3). No significant differences were observed in ARRs between the two groups (ARR ratio: 1.08; $p = 0.80$), and similar proportions of patients were free of 3-month CDP (difference in proportions: 1.1%; $p = 0.72$). A comparison between ozanimod and fingolimod for 6-month CDP at 1 year was not feasible as data were not reported in the fingolimod trial.

Adjusted analyses of 2-year outcomes

After adjustment for baseline patient characteristics, ozanimod was associated with a significantly lower risk of any AE (RD: -22.7%), AEs leading to discontinuation (RD: -7.4%), herpetic infection (RD: -4.9%), basal-cell carcinoma (RD: -1.8%), bradycardia (-0.5%) and abnormal liver enzyme elevations (RD: -3.0%) compared with fingolimod (all $p < 0.05$; Table 4). No significant differences were observed in ARRs between groups (ARR ratio: 1.06; $p = 0.78$). Similar proportions of patients in both groups were free of 3-month (difference in proportions: 5.2%; $p = 0.12$) and 6-month CDP (difference in proportions: 0.9%; $p = 0.76$).

Comparisons of risk outcomes based on odds ratios were consistent with the results described for RDs.

Discussion

Evidence on the comparative effectiveness of ozanimod and fingolimod in the treatment of RMS will be important for decision makers to assess the relative clinical value of these therapies. In the absence of head-to-head randomized trials of these treatments, indirect comparisons can provide valuable comparative evidence. This analysis used

Table 3. Comparison of 1-year safety and efficacy outcomes for ozanimod HCl 1 mg versus fingolimod 0.5 mg: assessment of risk differences.

Outcome	Adjusted risk difference for ozanimod HCl 1 mg vs fingolimod 0.5 mg		
	Δ †	95% CI	p-value
AE leading to discontinuation (%)	-1.2	(-5.7, 3.3)	0.61
Death (%)	0.0	(0.0, 0.0)	–
Any AE (%)	-9.9	(-18.0, -1.8)	<0.05¶
– Herpesvirus infection	2.2	(-1.6, 6.0)	0.25
– Depression	3.1	(-0.9, 7.1)	0.13
Any SAE (%)	0.4	(-4.4, 5.3)	0.86
Infection SAE (%)			
– Appendicitis	0.6	(-0.1, 1.2)	0.1
– Herpesvirus infection (serious)	0.0	(-0.6, 0.6)	1.0
Neoplasm SAE (%)			
– Basal-cell carcinoma	-0.5	(-1.4, 0.4)	0.27
– Melanoma (including *in situ*)	-0.7	(-1.5, 0.1)	0.08
– Breast cancer (including *in situ*)	-0.5	(-1.2, 0.2)	0.14
Cardiac SAE (%)			
– Bradycardia or sinus bradycardia	-0.4	(-1.1, 0.3)	0.23
– Atrioventricular block first degree	-0.2	(-0.6, 0.2)	0.35
– Atrioventricular block second degree	-0.2	(-0.6, 0.2)	0.35
– Myocardial infarction	-0.2	(-0.5, 0.2)	0.32
Mean absolute lymphocyte count (10^9/l) at 1 year§	0.4	(0.3, 0.5)	<0.001¶
Absolute lymphocyte count <0.2 K/μl (%)	-13.8	(-17.3, -10.3)	<0.001¶
Liver enzymes: ALT ≥ 3x ULN (%)	-6.8	(-10.6, -3.1)	<0.001¶
Macular edema (%)	-0.3	(-1.0, -0.5)	0.50
Annualized relapse rate‡	1.08	(0.64, 1.82)	0.78
Proportion free of CDP, 3 months (%)	1.1	(-4.4, 6.5)	0.72

† Difference in the proportion of patients with events, unless otherwise noted.
‡ Annualized relapse rate ratios for ozanimod vs fingolimod.
§ Difference in means.
¶ Denotes a statistically significant difference.
AE: Adverse event; CDP: Confirmed disability progression; SAE: Serious adverse event; ULN: Upper limit of normal.

data from the pivotal clinical trials of ozanimod and fingolimod and adjusted for cross-trial differences in patient populations to assess the comparative efficacy of these therapies in RMS.

In this adjusted comparison, ozanimod was associated with a significantly lower risk of any AEs than fingolimod 0.5 mg. In addition, lower risks of heart rate reduction, any conduction abnormalities and atrioventricular block during first-dose monitoring were observed among patients receiving ozanimod, as well as a less frequent need for extended first-dose monitoring.

Ozanimod was also differentiated from fingolimod in the 1- and 2-year safety outcomes. Specifically, in the 1-year outcomes, patients receiving ozanimod had a lower risk of any AEs, lymphocyte count reductions and abnormal liver enzymes elevations than patients receiving fingolimod. In the 2-year outcomes, patients receiving ozanimod had a lower risk of AEs leading to discontinuation, any AEs, herpetic infections, bradycardia and abnormal liver enzymes elevation than patients receiving fingolimod. Regarding efficacy outcomes, no statistically significant differences in ARRs or rates of 3-month and 6-month CDP were found between ozanimod and fingolimod. Of note, the directions and magnitudes of differences in outcomes were generally consistent both before and after matching, indicating that the trial findings were robust to the adjustment for multiple patient characteristics.

The current analysis of pivotal trial data sheds light on the comparative safety and efficacy of ozanimod, an investigational drug and fingolimod in the treatment of RMS. While ozanimod and fingolimod were comparable in terms of effects on the ARR and CDP, ozanimod was associated with significantly lower risk of the safety outcomes currently assessed. In relation to this, it is worth noting that the mechanism by which ozanimod exerts therapeutic effects in MS is unknown but may involve the reduction of lymphocyte migration into the central nervous system.

Table 4. Comparison of 2-year safety and efficacy outcomes for ozanimod HCl 1 mg vs fingolimod 0.5 mg: assessment of risk differences.

Outcome	Adjusted risk difference for ozanimod HCl 1 mg vs fingolimod 0.5 mg		
	Δ [†]	95% CI	p-value
AE leading to discontinuation (%)	-7.4	(-12.3, -2.5)	<0.01[¶]
Death (%)	0.0	(0.0, 0.1)	0.34
Any AE (%)	-22.7	(-29.2, -16.2)	<0.001[¶]
– Herpetic infection	-4.9	(-8.9, -0.9)	<0.05[¶]
– Depression	-2.7	(-6.3, 0.9)	0.15
Any SAE (%)	-4.7	(-9.8, 0.5)	0.07
Infection SAE (%)			
– Appendicitis	-0.1	(-0.6, 0.5)	0.83
– Herpesvirus infection (serious)	-0.3	(-0.7, 0.1)	0.12
Neoplasm SAE (%)			
– Basal-cell carcinoma	-1.8	(-2.7, -0.9)	<0.001[¶]
– Melanoma (including *in situ*)	-0.1	(-0.3, 0.1)	0.38
– Breast cancer (including *in situ*)	0	(-0.3, 0.3)	0.96
Cardiac SAE (%)			
– Bradycardia or sinus bradycardia	-0.5	(-1.0, 0.0)	<0.05[¶]
– Atrioventricular block first degree	0	(0.0, 0.0)	–
– Atrioventricular block second degree	0	(0.0, 0.0)	–
– Myocardial infarction	0	(0.0, 0.0)	–
Mean absolute lymphocyte count (10^9/l) at 1 year[§]	0.2	(-,-)	–
Liver enzymes: ALT ≥ 3x ULN, %	-3.0	(-5.8, -0.1)	<0.05[¶]
Macular edema (%)	-0.4	(-0.8, 0.0)	0.08
Annualized relapse rate[‡]	1.06	(0.70, 1.62)	0.78
Proportion free of CDP, 3 months (%)	5.2	(-1.3, 11.7)	0.12
Proportion free of CDP, 6 months (%)	0.9	(-4.8, 6.7)	0.76

[†]Difference in the proportion of patients with events, unless otherwise noted.
[‡]Annualized relapse rate ratios for ozanimod vs fingolimod.
[§]Difference in means; standard errors were not reported for fingolimod and thus statistical comparison was not possible.
[¶]Denotes a statistically significant difference.
AE: Adverse event; CDP: Confirmed disability progression; SAE: Serious adverse event; ULN: Upper limit of normal.

This evidence suggests that ozanimod has a favorable benefit–risk profile compared with fingolimod in terms of first-dose monitoring and safety outcomes for S1P receptor modulation over 1–2 years.

Results of this *post hoc* analysis should be interpreted in light of several limitations. As this study included comparisons of nonrandomized treatment groups, results may be biased by differences between patient populations that could not be adjusted for, such as the differences in the location of study sites; however, adjustment for observed baseline characteristics minimizes this risk. For the 2-year comparisons, due to lack of a common comparator arm, there is less opportunity to assess confounding bias in the nonanchored based comparison of ozanimod and fingolimod. Some differences in measurement standards between trials existed. For example, the ozanimod trials measured heart rate and BP in the supine position or standing position whereas the fingolimod trials measured patients in the sitting position. Heart rate is commonly lower when measured in the supine position while BP is commonly higher when measured in the supine position. Also, there were differences in the initial dosing regimens between the ozanimod and fingolimod trials; the ozanimod trials used a dose-escalation regimen not present in the fingolimod trials, which could potentially have led to fewer first-dose cardiac AEs in the ozanimod arms. Rates of any AEs should be interpreted with caution when comparing across trials because event ascertainment and reporting may differ, especially for lower-severity events. Given the 2-year follow up data, this analysis could not evaluate the long-term safety or efficacy of these treatments.

Conclusion

In this MAIC analysis of pivotal registration clinical trial data, although ozanimod and fingolimod were comparable in terms of key efficacy outcomes, ozanimod was associated with a more favorable benefit–risk profile compared with fingolimod when considering outcomes of first-dose cardiac monitoring, potential safety outcomes for S1P receptor modulation and key efficacy outcomes over 1–2 years.

Summary points

What is already known about this subject?

- Fingolimod is a nonselective sphingosine 1-phosphate (S1P) receptor modulator for the treatment of relapsing multiple sclerosis (RMS).
- Ozanimod, a selective S1P receptor modulator designed to target only the receptor subtypes $S1PR_1$ and $S1PR_5$, is currently under investigation for the treatment of RMS.
- The superior efficacy of these two treatments compared with IFN β-1a has been demonstrated in clinical trials.
- No head-to-head trials directly compare the safety and efficacy of ozanimod versus fingolimod for the treatment of RMS.

What are the new findings?

- In this indirect comparison across separate clinical trials, ozanimod was associated with significantly lower risks of adverse outcomes during first dose monitoring outcomes and over 1–2 years of follow-up compared with fingolimod.
- Ozanimod and fingolimod were comparable in terms of reducing annualized relapse rates and the proportion of patients with confirmed disability progression.
- Overall, ozanimod appears to have a superior benefit–risk profile to fingolimod.

Impact on clinical practice

- Ozanimod is an investigational drug under review for the treatment of RMS.
- This evidence will help decision makers to assess the relative clinical value of these therapies.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/cer-2019-0169

Author contributions

All authors were involved in the design and conduction of the study, as well as in drafting the manuscript or revising it critically for intellectual content. All authors had access to the data and interpreted the data.

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Ethical conduct of research

No institutional review was required as this was a *post hoc* analysis of previously published, de-identified data. This study adhered to the principles outlined in the Declaration of Helsinki.

Data sharing statement

Celgene is committed to responsible and transparent sharing of clinical trial data with patients, healthcare practitioners and independent researchers for the purpose of improving scientific and medical knowledge as well as fostering innovative treatment approaches. For more information, please visit: https://www.celgene.com/research-development/clinical-trials-data-sharing/.

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Perspective

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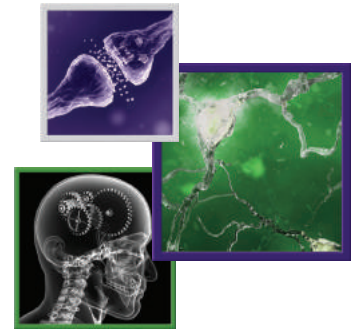

Motor-cognitive approach and aerobic training: a synergism for rehabilitative intervention in Parkinson's disease

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Practice points

- Parkinson's disease (PD) is not a mere 'movement disorder,' but rather it is a complex motor behavior disease responsible for a tremendous social and economic impact.
- The optimal management of PD should involve integrated, multidisciplinary approaches combining both pharmacotherapy and non-pharmacological interventions, such as rehabilitation.
- Bottom-up and top-down cognitive coping strategies and adaptive techniques are useful for achieving motor benefits in patients with PD.
- The aerobic exercise may promote neural rearrangements and improve cognition in patients with PD.
- Combining a 'goal-based', motor-cognitive practice with aerobic training seems to provide sustained clinical benefits rather than conventional physical therapy in patients with PD.
- Neuroplastic changes probably drive the clinical rehabilitation-induced benefits in patients with PD.
- Future studies should identify optimal parameters of intensity, frequency and duration of rehabilitation in patients with PD.

Parkinson's disease (PD) results in a complex deterioration of motor behavior. Effective pharmacological or surgical treatments addressing the whole spectrum of both motor and cognitive symptoms are lacking. The cumulative functional impairment may have devastating socio-economic consequences on both patients and caregivers. Comprehensive models of care based on multidisciplinary approaches may succeed in better addressing the overall complexity of PD. Neurorehabilitation is a highly promising non-pharmacological intervention for managing PD. The scientific rationale beyond rehabilitation and its practical applicability remain to be established. In the present perspective, we aim to discuss the current evidence supporting integrated motor-cognitive and aerobic rehabilitation approaches for patients with PD while suggesting a practical framework to optimize this intervention in the next future.

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Keywords: aerobic exercise • motor-cognitive rehabilitation • neuroplasticity • Parkinson's disease • quality of life

Parkinson's disease (PD) is the second most common neurodegenerative disorder. In the near future, the prevalence of PD is expected to exceed that of any other known neurological disorder, including Alzheimer's disease. Indeed, according to recent epidemiologic projections, the number of individuals affected by PD is poised for exponential growth [1].

The dopaminergic neuronal loss in the substantia nigra pars compacta is universally regarded as the pathological hallmark of PD [2]. The resulting altered connectivity in cortico–basal ganglia networks is believed to be involved

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in the pathophysiology of the cardinal motor symptoms of the disease, including bradykinesia, rigidity and resting tremor [3].

The clinical spectrum of PD, however, encompasses several non-motor features, which are known to be significant determinants of patients' quality of life (QoL) [4]. These include mood changes, anxiety, apathy, fatigue, sleep disturbances, chronic pain, gastrointestinal abnormalities and bladder dysfunction – among others.

Although originally described in terms of motor symptoms, it is generally acknowledged that nowadays PD should no longer be regarded as a mere 'movement disorder,' but rather as a complex motor behavior disease. Consolidated experimental evidence demonstrates how basal ganglia nuclei receive multimodal sensorimotor, cognitive and emotional information from converging cortical pathways and generate a 'compressed' and highly integrated output message to the frontal cortex, where the selection of a proper motor behavior is finally elaborated [5,6]. Notably, this highly integrated computational process encodes for any internally generated motor behavior: from the simplest movement of a single joint, to the most complex motor sequence involving the entire body in order to express emotional contents in a given cognitive setting [7].

This broad physiological complexity should indeed be appreciated by clinical scientists in order to understand the complex phenomenology of PD. Along the disease course, the neurodegenerative process spreads to other, non-dopaminergic neural systems, such as the cholinergic and the noradrenergic pathways [8,9]. At this stage, patients usually develop cognitive, motivational and more complex 'axial' disturbances affecting gait, balance and posture, whose responsiveness to dopamine replacement therapy (DRT) is almost always suboptimal, if not disappointing. Not uncommonly, these conditions may overlap in the same patient, thus exerting an overall detrimental impact on QoL as well as social and emotional well-being [10–12].

Indeed, while in early PD DRT is usually effective in improving self-perceived QoL by addressing the dominant motor features of the disease [13,14], its effectiveness in the medium-advanced stages tends to decrement. Furthermore, the potential onset of DRT-related side effects, such as motor fluctuations, dyskinesia, painful dystonia, dopamine dysregulation syndrome and impulsive–compulsive disorders conspire, over time, to reduce the overall tolerability of pharmacological therapies [15].

Given the multi-layered complexity of PD, it is generally acknowledged that its optimal management should involve multidisciplinary approaches combining both pharmacotherapy and non-pharmacological interventions [16,17]. Among the latter, growing evidence supports neurorehabilitation as an effective complementary treatment for the management of PD [18–26]. Neurorehabilitation could be defined as the set of clinical and carer interventions aimed at recovery from nervous system damage (due to acquired injuries or to degenerative diseases) by reducing or compensating for the functional disturbances by using the patients' individual neuroplastic resources.

In the past years, the complex interplay between basal ganglia and cortical–cerebellar networks in the modulation of both cognitive-motivational (non-motor) and motor aspects of action [5,27–31] has been increasingly considered in the development of novel integrated rehabilitative approaches (see Figure 1). Furthermore, emerging evidence from basic science and clinical studies suggested the potential for add-on aerobic exercise to boost brain plasticity mainly through long-term potentiation (LTP) phenomena [32].

In the current perspective, we aim to discuss the potential for integrated motor-cognitive rehabilitation practices and aerobic exercise to harness greater and more sustained clinical benefits than any uni-dimensional rehabilitative approach alone. Potential ways to combine these two methodologies within routine rehabilitation protocols will be finally discussed.

Motor-cognitive intervention in PD: scientific rationale & clinical evidence

A number of different rehabilitative protocols designed for PD were previously reported in the clinical literature [20,21]. The vast majority of them focused on improving basic motor aspects of PD [33–36]. Overall, results from these interventions were mixed and somewhat conflicting. Further, these protocols did not expressly investigate the influence of cognitive processes in modulating patients' final motor behavior [5,27–31].

However, mounting evidence suggests that the broad degenerative process underlying PD may manifest with a complex clinical phenomenology involving not only primary motor aspects of action, but also cognitive, emotional and motivational drivers [5,26,29,30,37]. This huge neural networks disruption could explain why PD motor signs and symptoms may be preceded or accompanied by a wide range of neuropsychiatric features (such as anxiety, apathy, depression, fatigue and psychosis) [38], whose potential impact on functional outcomes has been described in the rehabilitation field [39,40]. In the last years, the importance of engaging subject's cognition in order to achieve greater motor benefits began to be increasingly considered [37]. More specifically, the impairment of basal

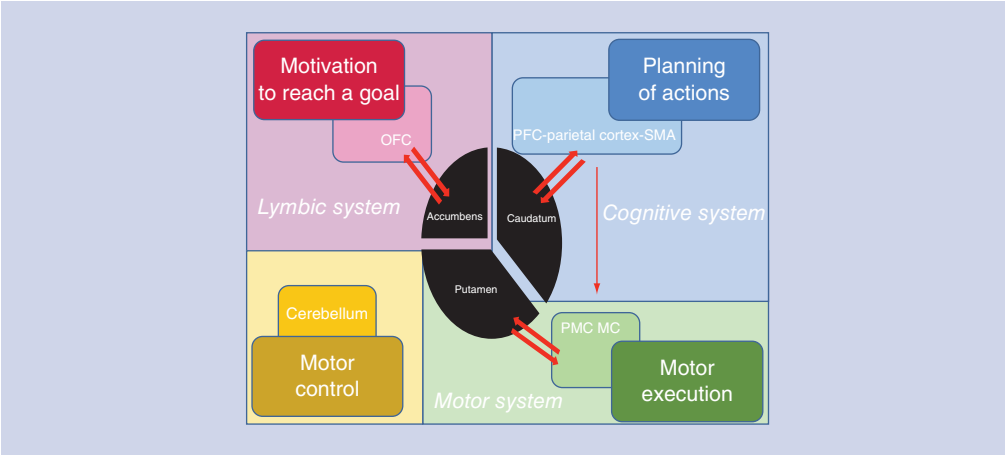

Figure 1. The role of the basal ganglia–cortico–cerebellar networks in motor behavior: implications for rehabilitation.
Schematically, the striatum can be divided into three principal nuclei with different functions: the nucleus accumbens plays a crucial role in decision-making and in reward-based learning; the nucleus caudatum is fundamental for motor learning and for action planning; finally, the putamen has a pivotal role for scheduling and executing habitual-automatic motor skills. These nuclei are strictly interconnected with limbic, associative and sensorimotor cortical areas, respectively. From a functional point of view, these corticostriatal structures are central to learn and express each single motor behavior: the motivation to move for reaching a goal is driven by the accumbens–OFC network; the network linking the caudatum with PFC, parietal cortex and SMA represents the neural system for action planning; motor execution is finally provided by the putamen–SMA–PMC–MC network. The cerebellum monitors the motor performance, contributes to error correction of the 'ongoing' action and generates a continuous signal for maintaining the congruency of the motor behavior. Considering this functional complexity, in a rehabilitative perspective, the treatment of a neurological disorder leading to the disruption of motor behavior (such as Parkinson's disease) has to be designed considering all the motor, motivational and cognitive aspects underlying the dysfunction. OFC: Orbitofrontal cortex; PFC: Prefrontal cortex; PMC: Premotor cortex; MC: Motor cortex; SMA: Supplementary motor area.

ganglia–cortical–cerebellar networks in PD has been involved in: i) an aberrant expression of habitual-automatic, goal-based actions [41–44], ii) the dysregulation of reward-based [45–47] and error-based learning processes [48,49], and iii) an impaired switching from automatic to voluntary and goal-based actions [50–53]. The aforementioned mechanisms are believed to underline the pathophysiology of bradykinesia [54], walking problems – including freezing of gait [55], postural abnormalities and balance disturbances with falls [56–58], writing and manual dexterity problems [59], speech [60] and swallowing deficits [61] in these patients.

It therefore follows that, from a cognitive and motor learning perspective, rehabilitation in PD should aim to foster the re-acquisition of lost habitual, goal-based motor behavior [37]. To achieve this result, clinicians may take advantage of multidisciplinary approaches [26] including physiotherapy, occupational therapy [62], speech and swallowing therapies [63].

Although both the implicit learning and executive control may be affected in PD [64–68], it has been shown that PD patients remain capable to apply bottom-up and top-down cognitive coping strategies and benefit from adaptive techniques. These include explicit cues, such as verbal instructions, and implicit cues, such as environmental sounds or visual signals [37,69–75].

It is believed that cues allow patients for engaging the volitional-executive control of movements, thus by-passing dysfunctional sensorimotor-habitual networks, while reinforcing the cortical mechanisms involved in motor drive [76–78]. While the explicit cues likely exploit executive functions mediated by the caudatum–prefrontal cortex network, implicit cues are thought to act through entrainment and attention focusing via the cerebellum–prefrontal cortex network [37,79,80].

An additional rehabilitative strategy involves the use of feedback-based learning. The underlying neural pathway seems to be found in the striatum–prefrontal cortex network [81]. Feedback-based motor learning involves the volitional, constant modulation of the ongoing motor behaviors based on internal and/or external signals that are strictly action-specific [82,83].

The overall effects of adaptive techniques (external cues, internal cues and feedback-based motor learning) may translate into clinical improvements on mobility, balance, gait, posture – among others [70–72,75,84].

The fact that PD patients may effectively apply to cognitive strategies in order to cope with their motor disability suggests that rehabilitative interventions engaging cognition may indeed harness motor learning schemata leading to broad clinical, motor and functional benefits [32]. As a case in point, many robotic-mechanical devices adopted in the field of neurological rehabilitation engage cognition to achieve motor benefits. Treadmill training represents the prototype among the tools used in this motor-cognitive perspective. The main goals of treadmill training are improving gait and enhancing patients' physiological reserve. Training parameters such as speed and workload can be individualized for each subject, while the intensity and challenge of the training can be dynamically adjusted overtime. While using this device, kinetic signals provided by the sliding belt allow patients for focusing their attention to the active control of gait. As such, treadmill training may exert a normalizing effect on spatiotemporal gait parameters, thus improving gait rhythmicity and reducing gait variability in patients with PD, especially when associated with cues, feedback and music cueing [85–87]. Worthy of interest in this field is the use of virtual reality (VR), a computerized simulation that allows patients to interact with a virtual environment through multiple sensorial modalities [88,89], thus stimulating both motor and cognitive processes, simultaneously [90]. VR provides augmented feedback about performance and may enable individualized repetitive practice of motor function. The use of VR provides patients with benefits in the short and long-term period [91,92].

Another effective cognitive rehabilitative technique is motor imagery (MI). This is based on a conscious access with the intention to move. A large body of evidence suggests that imagined and executed actions share the same neural structures, recruiting overlapping regions [90,93,94]. Consistently, it has been demonstrated that MI may improve both motor performance and motor learning processes [94]. In the same way, Action Observation Therapy (AOT) represents a further, interesting and effective cognitive instrument for motor rehabilitation in PD [94]. AOT is based on the observation and imitation of specific motor actions for facilitating the motor learning processes, probably through the activation of the so-called 'mirror neurons' system [95,96]. A recent study demonstrated that this technique is effective in treating freezing of gait and that benefits may be sustained over a 4-weeks follow-up period [96]. The cognitively-mediated effects of this modality of rehabilitation were recently supported by imaging studies reporting an increased recruitment of frontoparietal areas in patients undergoing AOT [97]. Over the past years, motor-cognitive training showed a great efficacy in ameliorating a lot of goal-based, automatic motor behaviors, thus corroborating the valuable role of these strategies for the management of parkinsonian patients [37].

In this scenario, dual task training is a prime example of motor-cognitive interplay and a highly appealing technique to be implemented in the management of PD [98].

In PD, gait function tends to shift from a healthy, semi-automatic pattern to a maladaptive profile where attention-based strategies are inappropriately used to maintain locomotion. These compensatory strategies are not optimized for natural walking and when a second cognitive task is applied, the resulting attention shift may decrease walking speed and smoothness, ultimately leading to gait disruption and falls [99,100].

While in PD the abrupt administration of dual tasks can exert disruptive effects on walking, compelling evidence also suggests that motor strategies based on repetitive dual task training and dual task reinforcement may improve the cognitive reserve of these patients, thus ameliorating their gait function in clinical and ecological settings [101,102].

Based on previous reports from stroke literature [103], Yang and colleagues investigated the effects from cognitive dual task training on gait function in patients with PD. A decreased double support time was found following a 12 sessions program (30 min each session, three sessions per week for 4 weeks); furthermore, motor dual task gait training led to decreased gait variability in motor dual task walking conditions [101].

Finally, the application of noninvasive brain stimulation techniques, such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), may maximize clinical benefits through mechanisms involving cortical plasticity [104,105]. The underlying molecular basis is not fully known, but it seems to involve synaptic remodeling through LTP and long-term depression (LTD) phenomena [106].

Manenti *et al.,* [107] investigated the effects of anodal transcranial direct current stimulation (tDCS) over the dorsolateral prefrontal cortex in combination with physical therapy in PD patients. Patients were either assigned to anodal tDCS plus physical therapy (2 weeks of treatment consisting of daily direct current stimulation application for 25 min during physical therapy), or to sham neuromodulation plus physical therapy. While significant improvements in motor skills and depressive symptoms were observed in both groups, performances on PD Cognitive Rating Scale and verbal fluency tests improved only following add-on neuromodulation. These benefits were sustained

over time (3 months). A number of studies emphasized the potential of tDCS and rTMS to modulate both motor and cognitive functions [108–111] and to improve neuroplasticity [104,105].

Interestingly, add-on tDCS in conjunction with dual task gait training may positively influence cognitive performances while walking under certain experimental conditions [112]. The effect of tDCS and rTMS on prefrontal cortex [108,113,114] could be related to modulation of functional connectivity on corticostriatal level, thus somehow replicating what is observed with rehabilitative interventions based on motor-cognitive training. This provides further evidence for motor benefits that are mediated, at least partially, by neuroplastic phenomena.

Using different behavioral tasks probing reaction time (RTs), Ortelli *et al.,* [115] investigated the relationship between asymmetric dopaminergic degeneration and attentional resources in PD patients undergoing motor-cognitive rehabilitation. Attention-mediated performances did not significantly differ between right-side (RPD) and left-side (LPD) predominantly affected patients. However, only LPD patients showed significant improvements on attentional tasks following motor-cognitive rehabilitation. The authors hypothesized that these different profiles of cognitive modifiability in PD patients may be related to a lateralized susceptibility of the nigrostriatal system to neuronal degeneration and brain plasticity [115]. Importantly, these findings were in agreement with a previous randomized controlled trial describing the effects of 24 weeks of structured exercise interventions in PD [116]. The study provided Class IV level evidence for Progressive Resistance Exercise or Modified Fitness Counts in improving attention and working memory in non-demented PD patients with mild-to-moderate disease severity.

Aerobic exercise in PD: scientific rationale & clinical evidence

Solid experimental evidence suggests that physical exercise may promote brain plasticity through the activation of neurotrophin signaling pathways [117], synaptogenesis [118], angiogenesis and neurogenesis [119,120]. Furthermore, beneficial effects from aerobic exercises may be mediated by reduced neuroinflammation [121] oxidative stress [122], and by improved intracellular calcium homeostasis [123].

In PD models, aerobic exercise may induce compensatory rearrangements of dopamine neurotransmissions by virtue of neuroplastic changes involving the striatal–thalamic–cortical motor circuits [32,124–127]. In neurotoxin-based models of PD, such as MPTP and 6-OHDA, physical exercise facilitates dopamine release (DA) through the modulation of DA receptors [124,128,129]. Using western immunoblotting analysis of synaptoneurosomes and *in vivo* positron emission tomography imaging with DA-D2R specific ligand [^{18}F]fallypride, Vučković *et al.,* [130] observed an increase in striatal DA-D2R expression within dorsal striatum in MPTP mice following treadmill training. Notably, the increase expression of DA-D2R in the dorsal striatum has been linked to the potential restoration of automatic motor patterns in PD models [32].

It is hypothesized that exercise-induced effects on brain plasticity may also involve other non-dopaminergic circuitries. Consolidated evidence from *in vitro* and animal models shows that following dopamine depletion, a glutamate overdrive within corticostriatal connections tends to emerge. This phenomenon has been linked to aberrant learning and memory processes [131]. In neurotoxin-based rodent models of PD, exercise seems able to reverse this aberrant hyperactive glutamatergic state by reducing the presynaptic release of glutamate. Interestingly, these changes are accompanied by changes in the firing pattern of nigral dopamine neurons, suggesting a strong correlation between these two neurochemical systems in PD [123,132].

In addition to changes in neurotransmitters, modulation of neurotrophic factors may play an important role in the exercise-induced neuroplastic changes. These proteins are pivotal in key neurobiological processes including neuronal survival, growth and synaptogenesis [133,134]. Specifically, the action of BDNF is a candidate mechanism underlying exercise-induced benefits. Literature data suggest that BDNF release can help to optimize brain plasticity outcomes via exercise interventions, which could be properly relevant in the context of multimodal training (i.e., exercise and cognitive stimulation) [135]. Interestingly, in the context of exercise, the candidate tissues for the addition of BDNF to circulation is not only the brain, but also skeletal muscle, peripheral blood mononuclear cells, vascular endothelial cells and platelets via the spleen [135]. In particular, endothelial cells rapidly secrete BDNF in proportion to the magnitude of exercise-like stimuli, including shear stress [136] and reductions in PO_2 [137]. Consistently with the upregulation of central BDNF expression in rodents, physical activity was found to increase circulating BDNF levels in both healthy humans and PD patients [138–140]. Fontanesi *et al.,* [140] tested the hypothesis that a 4-week rehabilitation program, including aerobic exercise and functional and goal-directed training, could enhance BDNF-TrkB signaling in lymphocytes in patients with PD (Hoehn & Yahr stage 2–3). Following the intervention, a significant improvement in motor and non-motor symptoms along with an up-regulation of BDNF-TrkB signaling [140] was observed. Moreover, changes in the Unified Parkinson's Disease

Rating Scale significantly correlated with the increase in TrkB signaling, thus suggesting that clinical benefits in this population may be mediated by enhanced BDNF-TrkB signaling in lymphocyte. Two randomized controlled trials tested the rehabilitation effects on functional outcomes in PD and whether the treatment increases the BDNF serum level*s* [141]. Frazzitta *et al.,* [142] enrolled 30 participants in early stages of PD who were assigned to intensive rehabilitation or to a control group (no rehabilitation). The intervention lasted 28 days and included aerobic exercise. The authors found that the intervention increased the BDNF levels and improved PD signs [142]. Sajatovic *et al.,* [143] aimed to compare changes in depression in people with PD with comorbid depression between individual versus group exercise plus chronic disease self-management. The authors selected some biomarkers of inflammation and neuronal integrity, including BDNF, as outcome measures possibly related to mechanisms involved in depression. They found a significant increase in plasma BDNF level that corresponded to the initial 12-week 'intensive' portion of the interventions [143].

Similarly to what observed in animal models, Fisher and colleagues [144] reported an increased in DA-D2R binding potential within the dorsal striatum of individuals with early stage PD following an 8 weeks training program. Interestingly, these findings specifically correlated with improved postural control, suggesting that benefits from exercise may also be task-specific. Other studies seem to indicate dose-dependent effects from physical activity. However, the specific role played by motor tasks and dose in influencing the final clinical outcomes remains a matter of investigation. Finally, mounting evidence from different neurophysiologic studies suggest that high-intensity exercise may normalize corticomotor excitability in early PD [145]. These findings are consistent with the well-known relationship between exercise intensity and BDNF levels, with higher exercise intensities inducing larger BDNF increases [146]: noteworthy, low to moderate intensity exercise is less effective than high-intensity exercise at increasing BDNF concentrations in healthy adults [147,148].

Overall, these findings in humans seem to recapitulate prior evidence from both *in vitro* and animal models, suggesting that neuroplastic re-arrangements following intensive exercise may be mediated though different, potentially interconnected molecular mechanisms [124,129].

For the scopes of the present perspective, it is noteworthy that exercise paradigms incorporating aerobic training in PD may improve cognition and motor learning in addition to their known effects on motor function [32,149].

Indeed, the dopaminergic neuronal loss in the basal ganglia may directly affect cognitive functions with specific respect to executive domains [150,151]. Different studies with functional magnetic resonance imaging suggest that aerobic exercise may increase connectivity between brain regions specifically involved in affect, reward, learning, memory, attention and executive control [152]. In this setting, Duchesne *et al.,* [153] described a functional re-organization of brain activity in cerebral regions concerned with motor learning (hippocampus, striatum and cerebellum) in 19 early stage PD patients following a 12-week progressive aerobic training. These functional changes were accompanied by improvements on behavioral outcomes observed in PD patients. Together with additional data showing improved executive functions following aerobic exercise [154], current evidence suggests that cognitive benefits following physical activity could be mediated by enhanced activation in frontal brain regions.

Silveira *et al.,* [155] compared the effects of aerobic and goal-based exercise on five cognitive domains (attention and working memory, executive functions, memory, language and visuospatial function) in cognitively normal and impaired individuals with PD who were randomly allocated into three intervention groups: aerobic, goal-based and control. The authors found that aerobic exercise was more effective than goal-based exercise in improving executive functions (i.e., inhibitory control) in both cognitively normal and impaired individuals with PD.

These findings are extremely relevant in light of the role played by frontal brain regions in modulating attentive processes putatively used to compensate for impaired motor automaticity in PD [37].

Integrating motor-cognitive rehabilitation with aerobic exercise: open questions

Mounting evidence from clinical, behavioral, brain imaging, animal models and *in vitro* studies, supports the combined use of intensive, 'goal-based', motor-cognitive practices with aerobic training to achieve greater and more sustained clinical benefits in patients with PD (Figure 2) [32,37,134]. In the attempt to optimize complementary models of care for PD, we believe that future rehabilitative approaches should integrate both motor-cognitive and aerobic interventions. While cognitive engagement seems critical to achieve motor-behavioral benefits, aerobic training may act synergistically by maximizing brain plasticity. Indeed, this perspective of care encompasses all the main strategies historically adopted in PD rehabilitation: cueing techniques [156], use of feedback [157], verbal instructions [158] external focus [75], MI [159,160], biofeedback [161], AOT [96,160], mechanical devices [85,86], VR [92,92], non-invasive brain stimulation techniques [104,105], front-to-front physical therapy [25], aquatic therapy [162,163],

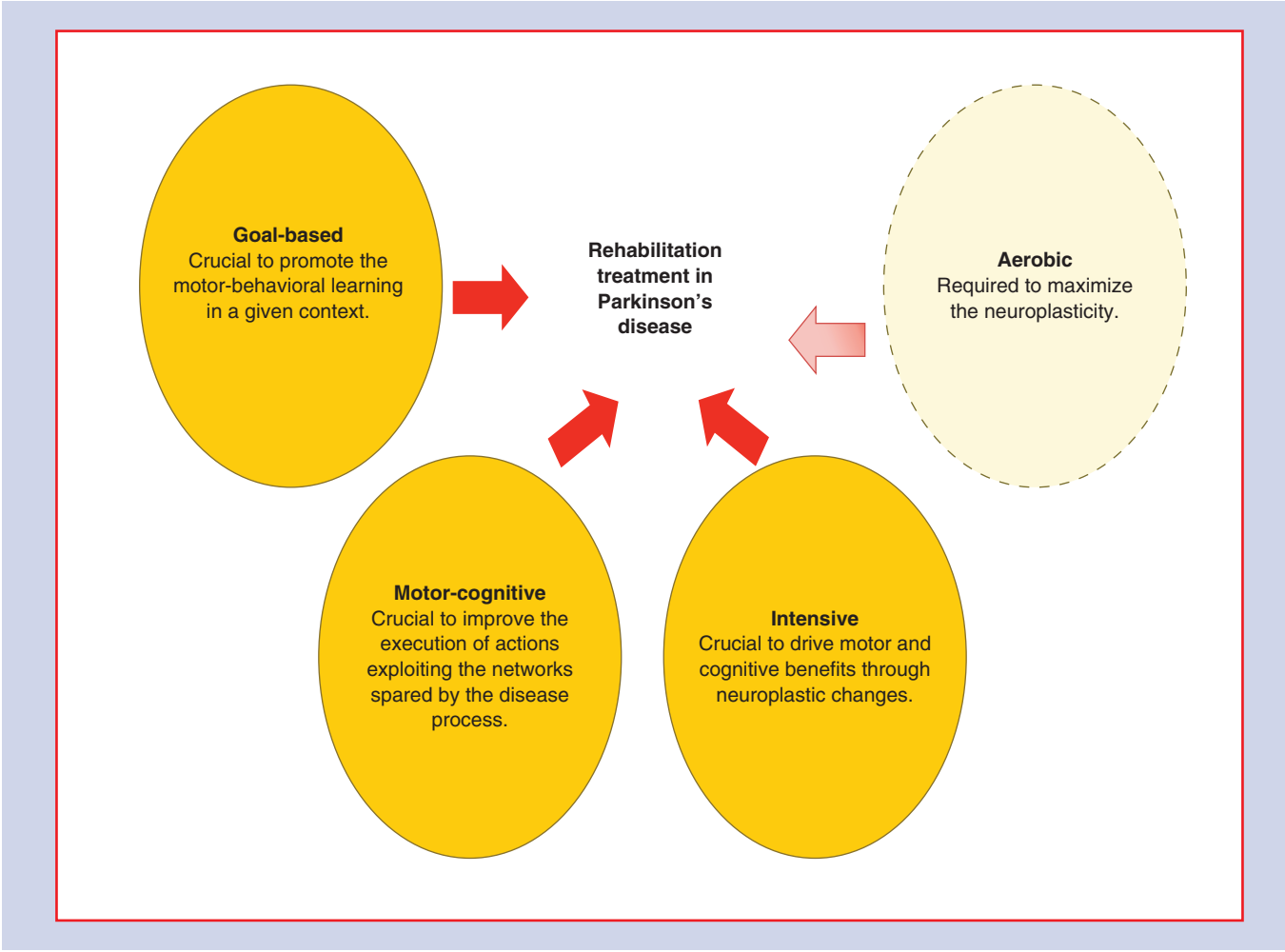

Figure 2. The theories on which rehabilitation in Parkinson disease should be based.
Combining an intensive and 'goal-based', motor-cognitive practice with aerobic training promotes neuroplasticity at the corticostriatal level, stimulates the executive resources and promotes the learning processes, thus probably representing the best way to obtain sustained improvements in Parkinson's disease (see both of the paragraphs about motor-cognitive intervention and aerobic exercise in Parkinson's disease).

speech and swallowing therapies [61] and occupational therapy [62]. Cognitive engagement may play an equally important role in other complementary activities designed for patients with PD, including dance therapy [164,165], art therapy and music therapy [166].

Future personalized protocols aiming to integrate these modalities of rehabilitation should also identify optimal parameters of intensity, frequency, duration and task-specific exercises to improve effectiveness and tolerability. This element introduces the question regarding the actual level of standardization and reproducibility of complementary interventions in PD. Although the current methodological heterogeneity of published studies prevents from pooling the available evidence to generate conclusive recommendations, growing evidence seems to confirm and converge on the use of multidisciplinary models of care [25,26,167–171] involving different health professionals including neurologists, physiatrists, physical therapists, occupational therapists, speech therapists, psychologists, nurses, social workers – among others. This approach may succeed in shifting the pendulum from the current construct of 'one disease – one disability' to more comprehensive and integrated strategies focusing on patient's individual complexity; and functional, familiar and social needs as a whole. Hopefully, different clinical, demographical and medical aspects (including comorbidities) should be weighted to adapt the intervention to the needing and personal motor and cognitive resources of each patient. For example, 'moving' the intervention toward the goal-based cognitive side, rather than to the aerobic side, could be useful for frail, advanced-stages patients. Conversely, the aerobic exercise,

promoting plasticity, can be advantageously adopted in cognitively impaired patients for whom the cognitive, goal-based approach is hampered. Moreover, when the cognitive, goal-based approach is exploitable, it is crucial to evaluate which are the problems the patient presents in performing actions in a multi-dimension perspective (including the motor, motivational and cognitive aspects): how much the patient is motivated to perform the specific action (i.e., walking, or talking or writing)? Is it possible to increase his/her motivation? Does the patient know the correct strategies to manage the difficulties in action performing? Is it possible to modify his/her adopted strategies for ameliorating the motor performance? Which motor aspects are impaired? Finally, the time to spend in the different activities (occupational therapy, speech therapy, balance training, gait training, psychological therapy etc.) should be framed on the basis of what any single patient really needs. Therefore, algorithms to stratify the interventions and make them even more person-centered could be designed in the upcoming future. As a matter of fact, the multidisciplinary approach has been preliminarily reported to be effective in improving clinical conditions and QoL in PD [25,26,167–171], although further appropriately designed, well-powered studies are needed to corroborate these findings.

Results from these studies raise the question regarding the cost–benefit ratio and the practical applicability of these integrated interventions. Given the current demographic trend, the prevalence of PD is expected to exponentially increase in the next years, thus reaching a pandemic dimension [1]. The enormous social and economic impact for patients and their caregivers [172,173] is aggravated by the aggregate burden of pharmacological therapies in terms of direct costs, adverse reactions and side effects [174,175]. Therefore, the cost saving effect of multifaceted comprehensive non-pharmacological approaches preserving long-term functionality and improving QoL may be outstanding, especially if compared with the aggregate cost deriving from addressing every patient's comorbidity separately. This favorable aspect is also partially suggested by prior studies reporting a reduced or controlled amount of dopaminergic medications administered to PD patients receiving dedicated rehabilitation programs [25,26]. These considerations should be included in economic models evaluating the cost–effectiveness of rehabilitation.

Further questions related to the optimal outcomes chosen to measure the aggregate effect of comprehensive and integrated neurorehabilitative approaches should also be addressed. Intuitively, the simple use of clinical tools biased toward appendicular motor function may not capture clinically significant improvements occurring in multiple, interdependent functional domains. Broader, comprehensive measures should be incorporated into both clinical practice and clinical trials, including kinematic, cognitive, psychological, emotional, behavioral and social outcomes. Moreover, only few studies evaluated cost–consequences analysis of rehabilitation in PD and many limitations stand [176]. Converging literature indicates that dedicated rehabilitative interventions are associated with fewer complications and lower costs in real world settings [177].

Finally, the optimal setting in which motor-cognitive rehabilitation and aerobic interventions may be administered remains to be defined. While an inpatient setting may be optimal to ensure treatments requiring higher intensity of care, home-based activities (possibly tele-monitored by electronic devices or smartphone applications) as well as outpatient programs, should be implemented to favor the maintenance of benefits over time. The coordination of different health professionals within dedicated networks and constant interactions with patients' communities through outreach programs may successfully serve to this purpose and should therefore be encouraged [178].

Conclusion & future perspective

A large amount of data suggest that effective rehabilitative interventions for PD should incorporate motor-cognitive training with aerobic activities and be designed accordingly. The specification of the chosen rehabilitative parameters, such as intensity, specificity and complexity, should always be formally disclosed in order to promote standardization and comparability of different protocols across dedicated centers. Future studies should address these methodological considerations in order to generate high quality experimental evidence (e.g., by comparing different protocols embracing the motor-cognitive interplay in randomized controlled trials) and lead to the identification of optimal models of care in PD. The development of comprehensive, patient-centered modalities of rehabilitation may succeed in harnessing long-lived clinical benefits and improvements in QoL in patients affected by this destructive disease.

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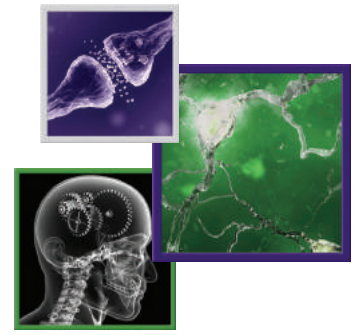

Application of the '5-2-1' screening criteria in advanced Parkinson's disease: interim analysis of DUOGLOBE

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Practice points

- Application of simple criteria to identify advanced Parkinson's disease (PD) is important because early identification of advanced PD allows doctors to adjust treatment, leading to better symptom control and improved quality of life.
- A group of experts proposed that fulfilling at least one of the '5-2-1 criteria' (taking levodopa by mouth at least five times a day, having at least 2 h of the day with 'Off' symptoms, or having at least 1 h of troublesome, uncontrolled, muscle movements (also known as dyskinesia) suggests advanced PD.
- Patients meeting at least one of the 5-2-1 criteria may also be candidates for advanced therapies, such as continuous infusion of levodopa–carbidopa intestinal gel, continuous administration of subcutaneous apomorphine, or deep brain stimulation.
- The multicountry long-term DUOGLOBE study assessed long-term effectiveness and safety of continuous administration of levodopa–carbidopa intestinal gel.
- At enrollment, almost all patients with physician-identified advanced PD in the DUOGLOBE study met at least one of the 5-2-1 criteria, and the majority (68%) met two or more of the 5-2-1 criteria.
- Patients showed improvement in motor and nonmotor symptoms following treatment with levodopa–carbidopa intestinal gel.
- As part of the physician's assessment, using the 5-2-1 criteria may be an objective way to identify patients with advanced PD using simple and reproducible measures.

Aim: A Delphi expert consensus panel proposed that fulfilling ≥1 of the '5-2-1 criteria' (≥five-times daily oral levodopa use, ≥two daily hours with 'Off' symptoms or ≥one daily hour with troublesome dyskinesia) suggests advanced Parkinson's disease (PD). **Patients & methods:** DUOdopa/Duopa in Patients with Advanced PD – a GLobal OBservational Study Evaluating Long-Term Effectiveness (DUOGLOBE) – is a single-arm, postmarketing, observational, long-term effectiveness study of levodopa–carbidopa intestinal gel (LCIG) for advanced PD. **Results:** This 6-month interim analysis (n = 139) affirms that most (98%) enrolled patients fulfill ≥1 of the 5-2-1 criteria. These patients responded favorably to LCIG treatment. Safety was consistent with other LCIG studies. **Conclusion:** In advanced PD patients, the 5-2-1 criteria generally aligns with clinician assessment.

Clinical Trial Registration: NCT02611713 (ClinicalTrials.gov)

Future Medicine

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Keywords: 5-2-1 • criteria for advanced PD • dyskinesia • LCIG • levodopa • levodopa-carbidopa intestinal gel • off time • Parkinson's disease

Levodopa, a dopamine precursor, is a key medication in the standard of care for Parkinson's disease (PD). Although oral treatment with levodopa is highly effective, there are shortcomings that become apparent over time [1]. Dose-related motor and nonmotor fluctuations often follow the dosing cycle, with patients demonstrating 'Off' related deterioration in motor function and emergence of nonmotor symptoms as the medication wears off [2,3]. As PD progresses, patients typically require more frequent levodopa dosing as the therapeutic window narrows. Dose fractioning of five or more times daily and troublesome 'Off' periods more than 1–2 h/day are critical indicators that referral to a specialist may be warranted [4].

Patients with advanced PD are required to have individual, customized therapy to manage symptoms, including nonmotor symptoms, dyskinesia and 'Off' time [5]. When modified oral regimens no longer adequately manage PD symptoms, therapies such as continuous infusion formulations of levodopa, subcutaneous apomorphine infusion and deep brain stimulation are available alternatives for consideration [4]. Levodopa–carbidopa intestinal gel (LCIG) is continuously delivered via percutaneous endoscopic gastrostomy with a jejunal extension tube and a portable pump. Results from several Phase III, observational and comparative studies have demonstrated significant improvements in motor function, nonmotor symptoms and quality of life in patients with advanced PD who are treated with LCIG [6–15]. Continuous subcutaneous infusion of apomorphine was shown in multiple open-label studies to reduce 'Off' time, extend 'On' time and improve disability and nonmotor symptoms [16–18]. Results from a double-blind, placebo-controlled study with apomorphine demonstrated significant reduction in 'Off' time and time without troublesome dyskinesia (TSD) [19]. Effective surgical procedures for advanced PD also exist, such as deep brain stimulation of the subthalamic nucleus and globus pallidus internus, which has been shown to consistently improve motor fluctuations and dyskinesia [16,20–24]. Deep brain stimulation has also been evaluated in patients with less advanced disease and has been shown to provide benefits comparable to a control group receiving the best medical treatment via drug therapy [14,16,25,26].

Efforts to establish simple criteria for the early identification of suspected advanced PD and identification of patients who would benefit from infusion or surgical therapies have been ongoing for some years. The absence of a biomarker, diagnostic test, or gold standard index makes defining the stage of advanced PD challenging, which impacts the ability to optimize therapies [4]. A Delphi expert consensus panel proposed several features across motor, nonmotor and functional-impact domains that might be useful to identify advanced PD. The chosen objective motor criteria (5-2-1 criteria) included using oral levodopa at least five times per day, having at least 2 h of the day with 'Off' symptoms, or at least 1 h of the day with TSD [4]. The 5-2-1 criteria may be useful to aid in the identification of suspected advanced PD patients who are uncontrolled with oral/transdermal therapies and may benefit from advanced treatments.

DUOGLOBE (DUOdopa/Duopa in Patients with Advanced Parkinson's Disease – a GLobal OBservational Study Evaluating Long-Term Effectiveness) is a 3-year, follow-up, observational, multicountry study. This *post hoc* analysis of an interim DUOGLOBE dataset was conducted to evaluate if patients identified by experienced clinicians as having advanced PD met the 5-2-1 criteria. DUOGLOBE also assessed the relationship of the 5-2-1 criteria to effectiveness and safety outcomes of LCIG treatment during routine care. The population was analyzed across four subgroups, divided as patients meeting the five or more times a day oral levodopa dosing criterion, two or more hours of 'Off' time criterion, one or more hours a day TSD criterion and those patients who met all three of the 5-2-1 criteria.

Patients & methods

Study design & treatment

DUOGLOBE is a global, multicountry, single-arm, postmarketing observational analysis of the long-term effectiveness of LCIG in patients with advanced PD (NCT02611713; Supplementary Figure 1).

Patients

Patients were included in the DUOGLOBE study if they were LCIG-naive at the start of the study and eligible to receive LCIG therapy in accord with the approved local LCIG product label for the region where they were enrolled

in the study (Label outside of the USA: LCIG is indicated for the treatment of advanced levodopa-responsive Parkinson's disease with severe motor fluctuations and hyper-/dyskinesia when available combinations of Parkinson medicinal products have not given satisfactory results. Label in the USA: LCIG is indicated for the treatment of motor fluctuations in patients with advanced Parkinson's disease). In addition, the physician needed to make the decision to treat the patient with LCIG before the patient was approached to participate in the study and the patient had to provide written informed consent. Patients were excluded from consideration if they had any condition included in the contraindications section of the approved local LCIG label, had current treatment with continuous apomorphine infusion, had a score <24 on the Mini-Mental State Examination, had participated in a concurrent interventional clinical trial or exhibited a lack of motivation or insufficient language skills to complete study questionnaires. Patients with history of previous surgery for PD (such as deep-brain stimulation or cell transplantation) were also excluded. However, in 2017 an amendment (only applicable to patients enrolled in the USA) allowed for inclusion of patients who had previous surgery for PD.

Assessments

Determination of patients' fulfillment of 5-2-1 criteria included frequency of daily levodopa intake, measurement of daily 'Off' time as reported by the patient and time spent with TSD as reported by the patient. TSD was defined as dyskinesia severity (Unified Parkinson's Disease Rating Scale [UPDRS] part IV item 33) with a score of 2, 3 or 4 (mild, moderate or severe) as defined in Supplementary Table 1.

Effectiveness assessments

Effectiveness in this interim analysis was evaluated from baseline to 6 months follow-up using 'Off' time as reported by the patient and by responses on the Unified Dyskinesia Rating Scale (UDysRS), the Non Motor Symptom Scale (NMSS), the Parkinson's Disease Questionnaire (PDQ-8), the UPDRS part II (activities of daily living) and the Modified Caregiver Strain Index (MCSI).

Safety assessments

Safety outcomes were assessed based on serious adverse events (AEs), pregnancies and product complaints that were monitored and reported by the physician.

Statistical analysis

This was a *post hoc* analysis of the first interim dataset of the DUOGLOBE study. Patients were stratified into subgroups who met all, one or more and each individual 5-2-1 criteria at baseline. Baseline demographics and disease characteristics were analyzed using descriptive statistics. For effectiveness outcomes, statistical comparisons within each group were assessed using a one-sample *t*-test with a p-value < 0.05 being the cut off level for significance. As this was a *post hoc* analysis of nonrandomized groups that could have underlying differences in observed or unobserved baseline characteristics, statistical comparisons between groups were not performed.

Results

Patients

Patients were enrolled in Australia, Belgium, Hungary, Israel, Italy, Romania, Slovenia, Spain, the UK and the USA. Of the 139 enrolled patients in this interim dataset, 82 had all 5-2-1 criteria status captured at baseline, had 6 month follow-up data and were included in the interim analysis. Most patients (98%) fulfilled at least one of the 5-2-1 criteria. Over 90% of patients (n = 74) reported having two or more hours of 'Off' time daily at baseline (Figure 1A). More than half (57%) of patients were taking oral levodopa five-times a day or more (n = 47) and 38% reported experiencing at least 1 h of TSD daily (n = 31). The majority (68%) of patients fulfilled two or more of the 5-2-1 criteria and 20% fulfilled all three (Figure 1B). Patients who met none of the 5-2-1 criteria (n = 2) or reported less than 2 h a day of 'Off' time (n = 8) had insufficient sample sizes for separate analysis of the effectiveness outcomes. These patients were included in the effectiveness and safety outcomes analysis for LCIG treatment.

Patients using more than five versus less than five daily doses of oral levodopa

When stratified by baseline frequency of oral levodopa dosing, patient characteristics across groups were similar, with both groups comprised mostly of males around age 70 years with approximately 11 years since being diagnosed

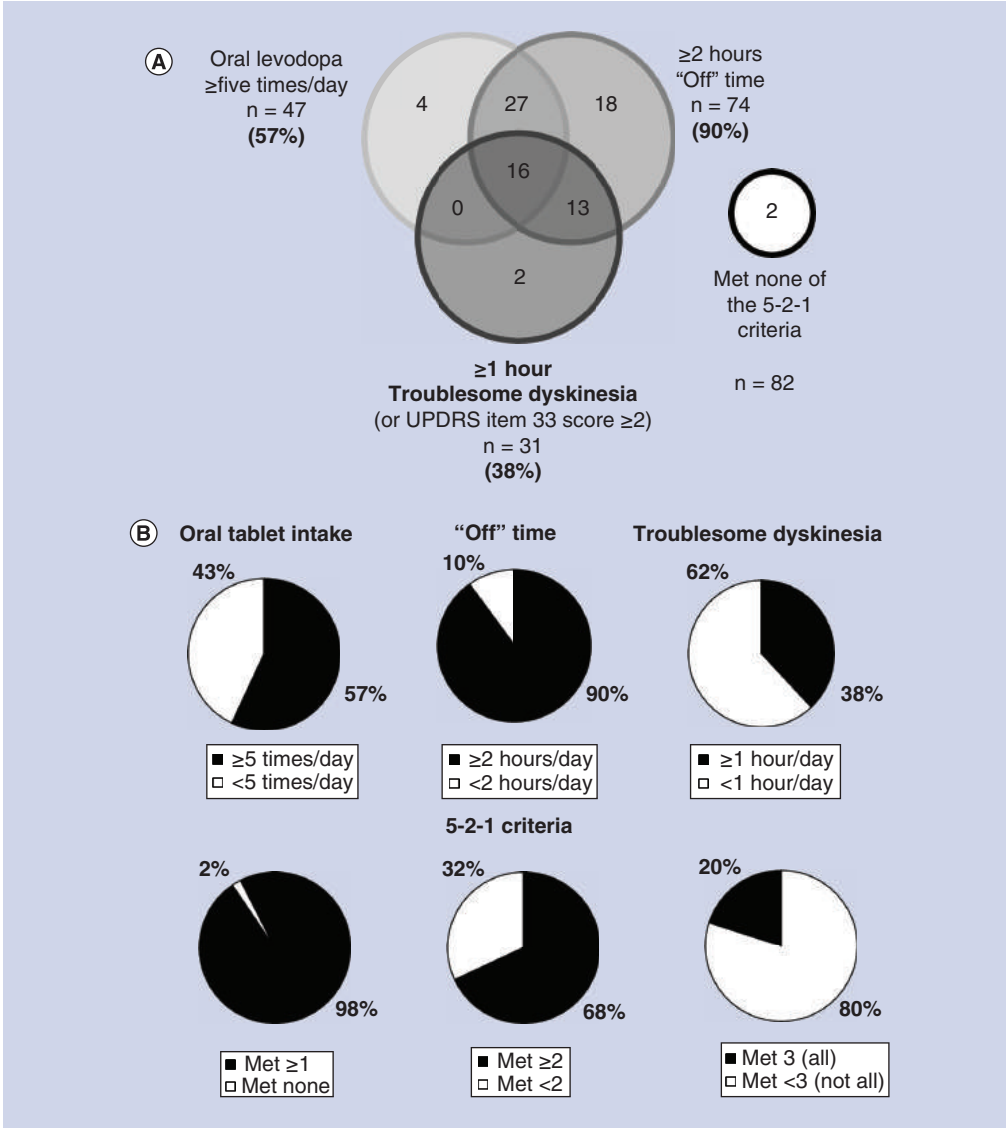

Figure 1. DUOdopa/Duopa in Patients with Advanced Parkinson's Disease – a Global Observational Study Evaluating Long-Term Effectiveness study population who met each individual, any (one or more) and all of the 5-2-1 criteria for advanced Parkinson's disease. (A) Distribution of study population at baseline. **(B)** Each graph represents the analysis population (n = 82) and if those patients met (black) or did not meet (white) the 5-2-1 criteria for advanced PD in each subgroup.
PD: Parkinson's disease; UPDRS: Unified Parkinson's Disease Rating Scale.

with PD (Supplementary Table 2). After 6 months of LCIG therapy, both groups exhibited significant reductions from baseline in 'Off' time ($p < 0.001$ for both), UDysRS ($p < 0.01$ for five or more times a day of oral levodopa and $p < 0.001$ for five or more times a day of oral levodopa) and NMSS scores ($p < 0.001$ for both). PDQ-8 summary index scores were significantly reduced in the five or more times a day oral levodopa group ($p < 0.001$; Figure 2). No significant improvements were seen in UPDRS part II scores or mean MCSI scores.

Patients experiencing ≥2 h a day versus <2 h a day 'Off' time

Patients who had two or more hours a day of 'Off' time at baseline were on average 68.7 years of age with PD of 11-years' duration. Average 'Off' time was 6.3 h. No analysis was performed in the group of patients who had <2 h a day of 'Off' time because of the small group size (Supplementary Table 3). Patients with at least 2 h of 'Off' time a day represent nearly the full study population, so effectiveness data in this group provides the closet estimation

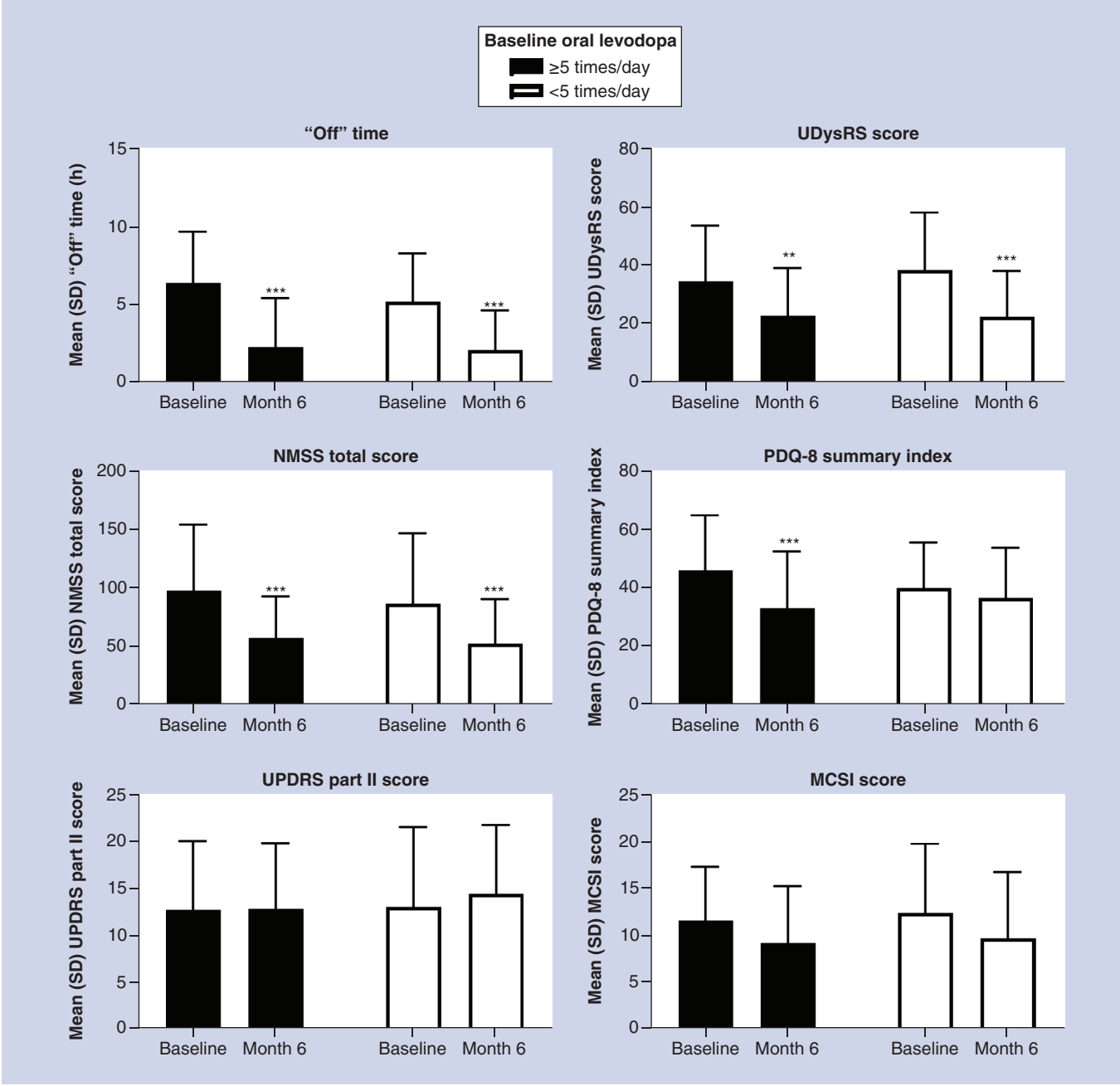

Figure 2. Effectiveness outcomes in patients stratified by baseline oral levodopa dosing frequency.
***Statistically significant at $p < 0.001$; **Statistically significant at $p < 0.01$.
MCSI: Modified Caregiver Strain Index; NMSS: Non Motor Symptoms Scale; PDQ-8: Parkinson's Disease Questionnaire-8; SD: Standard deviation; UDysRS: Unified Dyskinesia Rating Scale.

of the overall interim results. After 6 months of LCIG therapy, patients with two or more hours a day baseline 'Off' time exhibited significant reductions in 'Off' time ($p < 0.001$), UDysRS ($p < 0.001$), NMSS ($p < 0.001$), PDQ-8 summary index ($p < 0.01$) and MCSI scores ($p < 0.05$) (Figure 3). The changes from baseline in UPDRS part II score were not significant after 6 months for the group experiencing at least 2 h of 'Off' time daily.

Patients experiencing one or more hours a day versus less than 1 h a day TSD

When stratified by baseline TSD, patient characteristics across groups were similar, including UPDRS part II and PDQ-8 summary index scores, but with notable differences including more dyskinesia time and a higher UDysRS

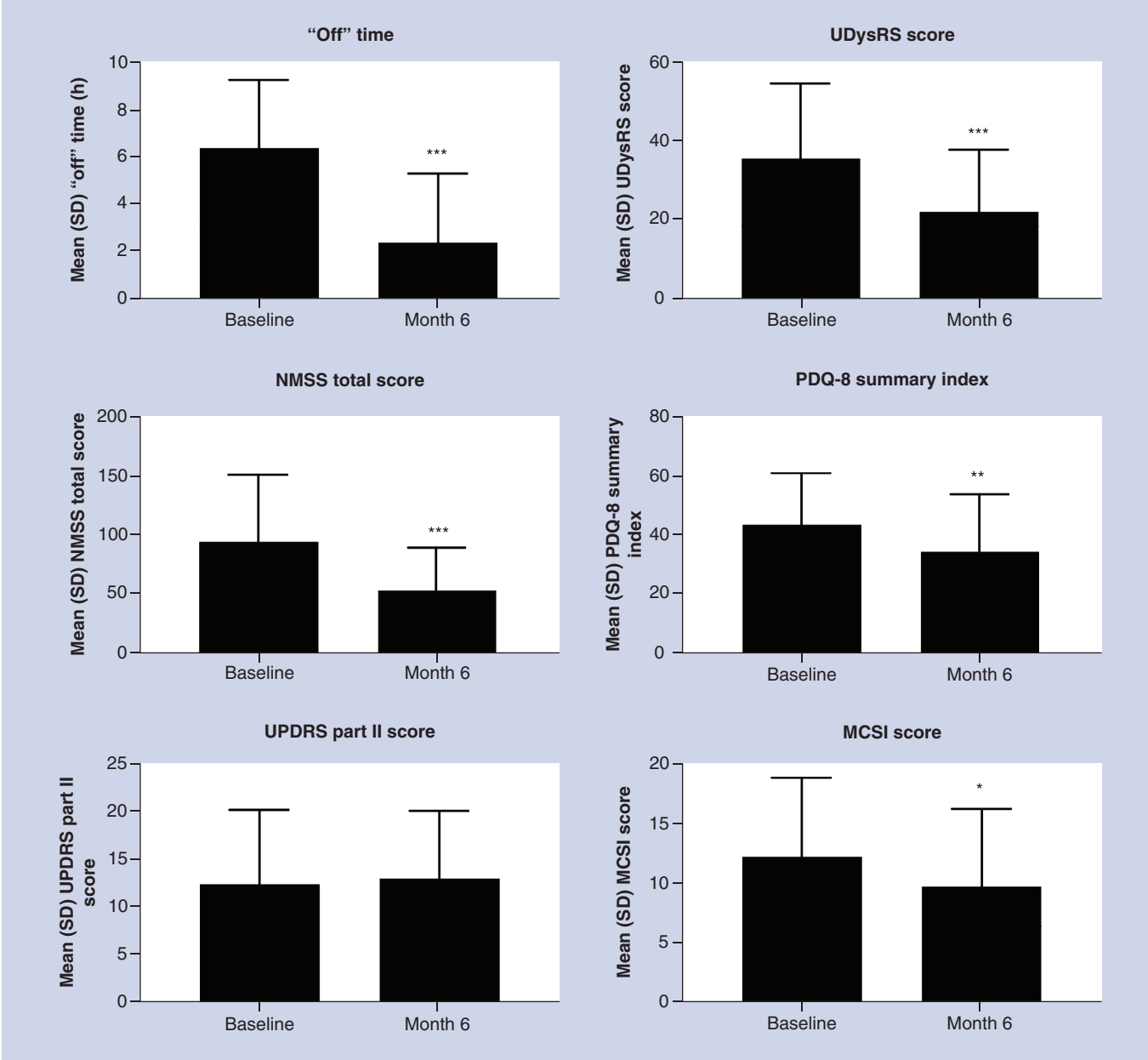

Figure 3. Effectiveness outcomes in patients with baseline 'Off' time ≥2 h/day. Patients who reported <2 h/day of 'Off' time (n = 8) had insufficient sample sizes for analysis of the effectiveness outcomes. ***Statistically significant at $p < 0.001$; **Statistically significant at $p < 0.01$; *Statistically significant at $p < 0.05$.
MCSI: Modified Caregiver Strain Index; NMSS: Non Motor Symptoms Scale; PDQ-8: Parkinson's Disease Questionnaire-8; SD: Standard deviation; UDysRS: Unified Dyskinesia Rating Scale.

score in patients with one or more hours a day TSD, although no statistical comparison was made (Supplementary Table 4). After 6 months of LCIG therapy, both groups exhibited significant reductions in 'Off' time and NMSS scores ($p < 0.001$). Patients experiencing at least 1 h of TSD a day also had significantly reduced UDysRS scores at 6 months ($p < 0.001$), as did patients experiencing less than 1 h of TSD ($p < 0.01$). PDQ 8 summary index scores were significantly reduced in the one or more hours a day TSD group ($p < 0.01$) (Figure 4). MCSI scores were significantly reduced ($p < 0.05$) in patients who experienced TSD <1 h a day. No significant differences were found in UPDRS part II scores after 6 months for either group.

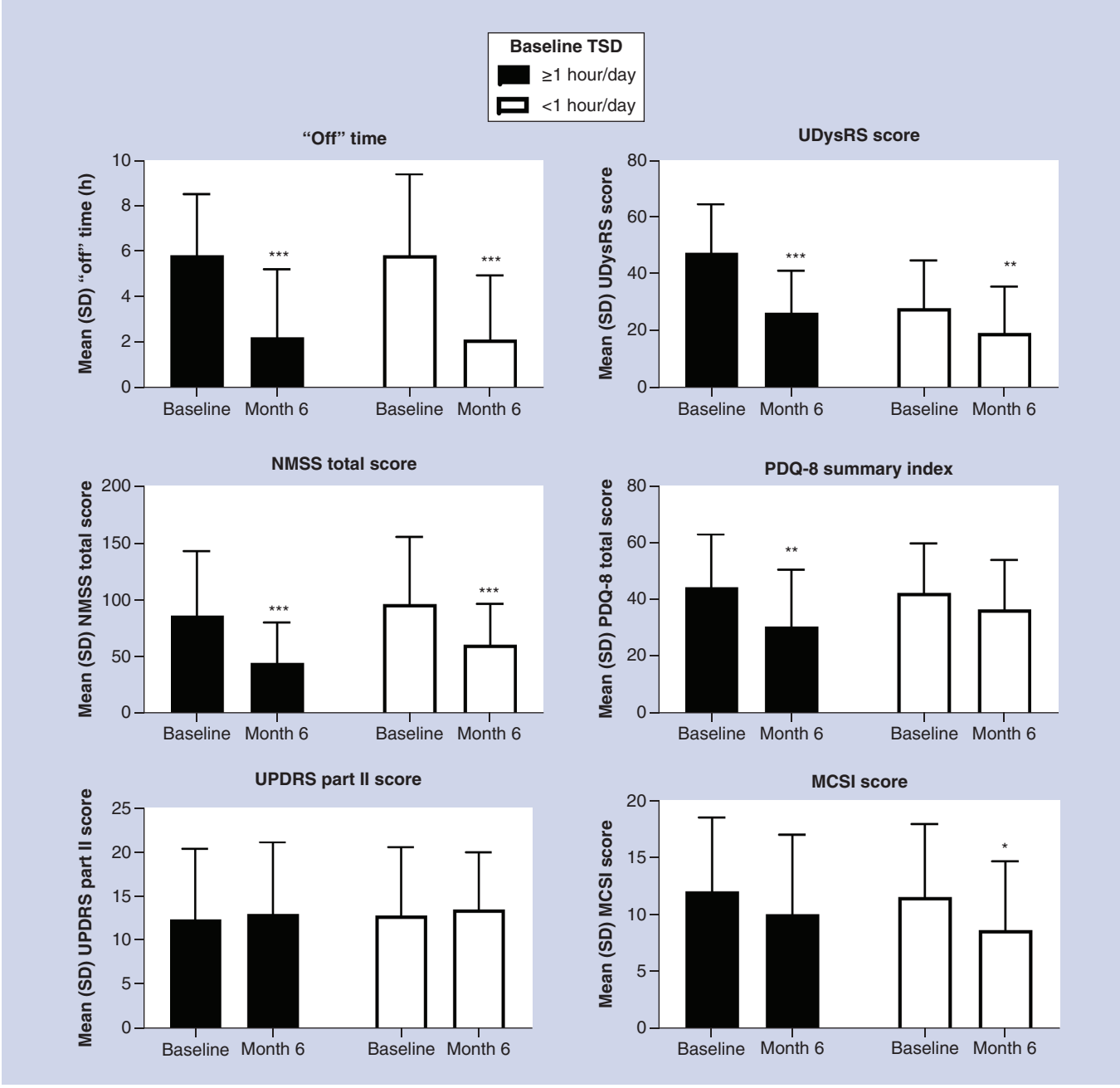

Figure 4. Effectiveness outcomes in patients stratified by baseline troublesome dyskinesia.
***Statistically significant at $p < 0.001$; **Statistically significant at $p < 0.01$; *Statistically significant at $p < 0.05$.
MCSI: Modified Caregiver Strain Index; NMSS: Non Motor Symptoms Scale; PDQ-8: Parkinson's Disease Questionnaire-8; SD: Standard deviation; TSD: Troublesome dyskinesia; UDysRS: Unified Dyskinesia Rating Scale.

Patients fulfilling all of the 5-2-1 criteria versus those not meeting all criteria

When stratified by baseline fulfillment of the 5-2-1 criteria, patient characteristics across groups were similar (Table 1). Patients who fulfilled all the 5-2-1 criteria appeared to have the highest baseline burden in terms of 'Off' time, UPDRS part II, UDysRS, NMSS, PDQ-8 summary index and MCSI scores. After 6 months of LCIG therapy, both groups exhibited significant reductions in most effectiveness measurements ($p < 0.001$ for the 'Off' time subgroup not fulfilling all criteria and both UDysRS subgroups; $p < 0.01$ for the 'Off' time and NMSS subgroups fulfilling all criteria; $p < 0.001$ for NMSS in the group not fulfilling all criteria; and $p < 0.05$ for PDQ-8 and MCSI for the subgroup not fulfilling all criteria; Figure 5). MCSI scores were significantly reduced for

Table 1. Baseline characteristics stratified by baseline fulfillment of 5-2-1 criteria.

Parameter	Mean (SD)[†]	
	Met all 5-2-1 criteria (n = 16)	Did not meet all (n = 66)
Sex, n (%)		
Female	4 (25)	21 (32)
Male	12 (75)	45 (68)
Age in years	67.6 (7.83)	69.9 (8.61)
Time since diagnosis in years	12.2 (5.50)	11.1 (4.69)
Hoehn and Yahr stage[‡]	3.0 (1.0, 5.0)	3.0 (1.0, 5.0)
'Off' time in hours	6.6 (2.10)	5.5 (3.33)
Dyskinesia time in hours	4.6 (2.34)	4.1 (3.90)
UDysRS score	50.8 (13.31)	31.8 (20.57)
NMSS total score	108.1 (51.00)	92.3 (57.76)
PDQ-8 summary index	49.8 (16.60)	42.0 (17.04)
UPDRS part II score	14.0 (7.22)	13.5 (8.02)
MCSI score	12.4 (5.62)	11.3 (6.69)

[†]Data are presented as mean (SD), unless otherwise noted.
[‡]Median (range).
LCIG: Levodopa–carbidopa intestinal gel; MCSI: Modified Caregiver Strain Index; NMSS: Non Motor Symptom Scale; PD: Parkinson's disease; PDQ-8: Parkinson's Disease Questionnaire-8; SD: Standard deviation; UDysRS: Unified Dyskinesia Rating Scale; UPDRS: Unified Parkinson's Disease Rating Scale.

Table 2. Safety.

	Daily oral levodopa frequency		'Off' time	Troublesome dyskinesia		5-2-1 criteria	
	≥Five-times/day (n = 47)	<Five-times/day (n = 35)	≥2 h/day (n = 74)	≥1 h/day (n = 31)	<1 h/day (n = 51)	All (n = 16)	Not all (n = 66)
Any serious AE	19 (40)	10 (29)	25 (34)	8 (26)	21 (41)	4 (25)	25 (38)
Any serious AE possibly related to LCIG	6 (13)	4 (11)	8 (11)	3 (10)	7 (14)	2 (13)	8 (12)
Deaths	2 (4)	2 (6)	4 (5)	3 (10)	1 (2)	1 (6)	3 (5)
Most common AEs (occurring in ≥two patients/group)							
Decubitus ulcer	2 (4)	–	2 (3)	1 (3)	1 (2)	1 (6)	1 (2)
Device occlusion	1 (2)	1 (3)	1 (1)	–	2 (4)	–	2 (3)
Femoral neck fracture	2 (4)	–	2 (3)	2 (7)	–	2 (13)	–
General physical health deterioration	2 (4)	–	2 (3)	1 (3)	1 (2)	1 (6)	1 (2)
Pneumonia	1 (2)	1 (3)	2 (3)	2 (7)	–	1 (6)	1 (2)
Pneumoperitoneum	2 (4)	–	2 (3)	–	2 (4)	–	2 (3)
Urinary tract infection	1 (2)	1 (3)	2 (3)	–	2 (4)	–	2 (3)
Discontinuations	8 (17)	3 (9)	11 (15)	3 (10)	8 (16)	1 (6)	10 (15)
Discontinuations due to AEs	4 (9)	3 (9)	7 (10)	2 (7)	5 (10)	0	7 (11)

AE: Adverse event; LCIG: Levodopa–carbidopa intestinal gel.

the group of patients who did not meet all criteria (p = 0.018). There was no significant difference for either group in UPDRS part II scores.

Safety assessments

The safety dataset of this first interim analysis included 139 patients, but 82 patients had full baseline data on the 5-2-1 criteria. Serious AEs related to LCIG occurred at a similar rate in each group (Table 2). The most common AEs occurring in two or more patients in each group were decubitus ulcer, device occlusion, femoral neck fracture, general physical health deterioration, pneumonia, pneumoperitoneum and urinary tract infection. Polyneuropathy

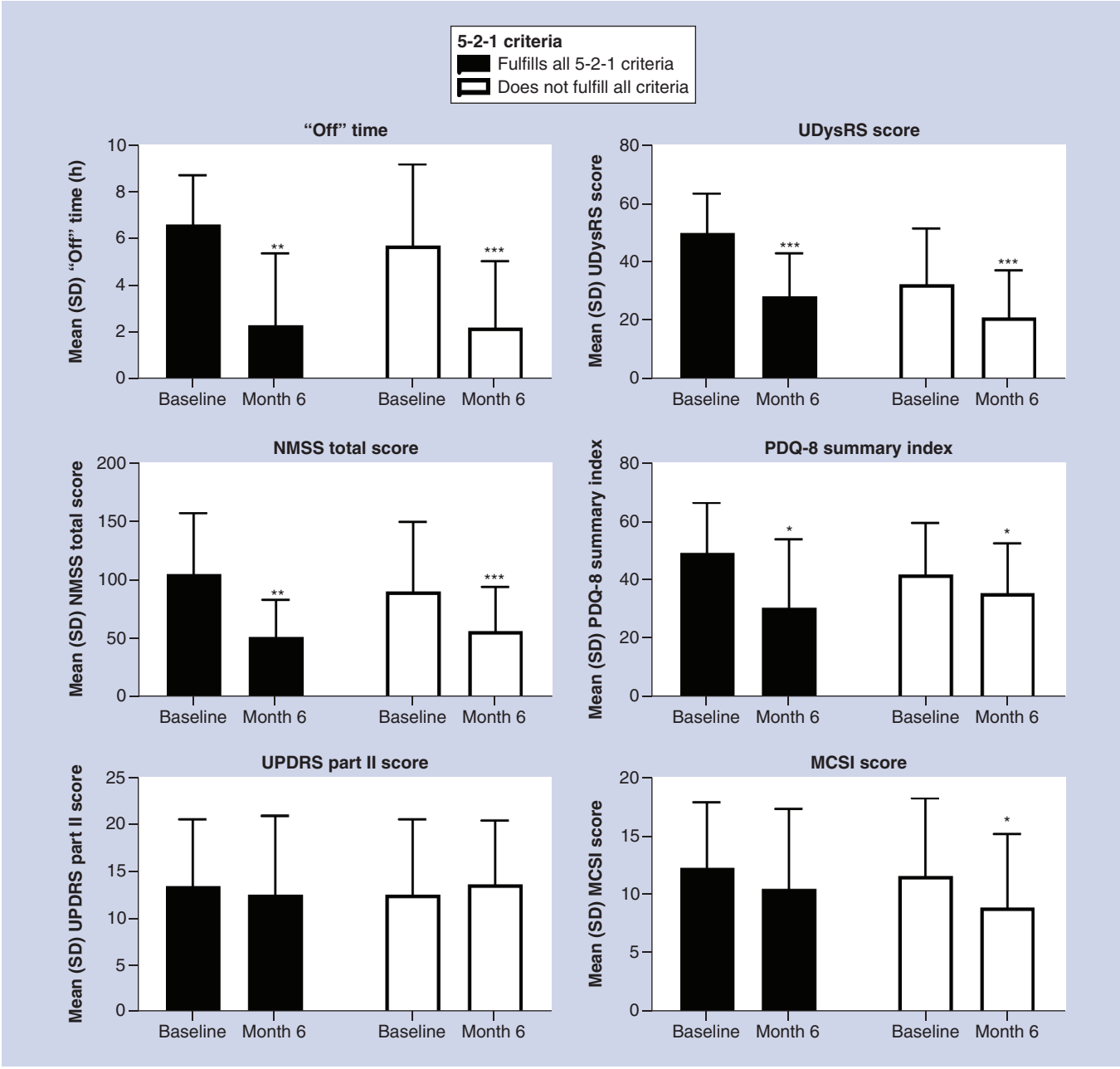

Figure 5. Effectiveness outcomes in patients stratified by fulfillment of all 5-2-1 criteria.
***Statistically significant at $p < 0.001$; **Statistically significant at $p < 0.01$; *Statistically significant at $p < 0.05$.
MCSI: Modified Caregiver Strain Index; NMSS: Non Motor Symptoms Scale; PD: Parkinson's disease; PDQ-8: Parkinson's Disease Questionnaire-8; SD: Standard deviation; UDysRS: Unified Dyskinesia Rating Scale.

occurred in one patient (between months 7 and 12). There was one report of 'sensory loss' between months 1 and 6, which may be a description of a neuropathic event. Deaths occurred in at least one patient in each subgroup (2–10%). One patient death (intestinal obstruction) in the interim dataset was deemed to be possibility related to LCIG. Across all subgroups, 6–17% of patients discontinued the study. The main reasons for discontinuation were AEs (0–9.5%), withdrawn consent (0–9%) and other reasons (5–7%).

Discussion

As PD progresses, management of motor and nonmotor symptoms becomes more difficult, particularly as administration of oral medication may provide less consistent symptom control [2,3,16,27,28]. Device-aided therapies, such as continuous subcutaneous apomorphine infusion, continuous LCIG infusion and deep brain stimulation, are used to treat advanced PD in selected patients [4]. However, there are knowledge gaps regarding what therapies are most appropriate and when they should be initiated [4,29]. Earlier identification of these patients is expected to lead to improved patient care by earlier initiation of advanced therapies [16]. Criteria to establish the identification of treatable motor symptoms of advanced PD may enable earlier and more uniform recognition of patients who might benefit from advanced therapies, but further validation is needed. Even among specialists in movement disorders, debate exists whether early treatment is based on time for duration of disease versus emergence of clinical first signs of motor fluctuations [30]. The 5-2-1 criteria may help address the clinical gap in timely identification of patients whose symptoms may be uncontrolled with oral medications [4,31]. This criteria is also found in the first section of a recently launched comprehensive screening tool (MANAGE-PD) that has been tested in the USA to screen patients whose PD is no longer controlled with oral medications (www.managepd.com) [14].

This study represents the first attempt at using the criteria with a large cohort of patients on an international scale. Interim results demonstrate that almost all patients selected for LCIG by DUOGLOBE investigators on clinical grounds fulfilled at least one of the 5-2-1 criteria. Most commonly, patients had two or more hours of 'Off' time at baseline. Patients treated with LCIG had improvements in 'Off' time, dyskinesia, nonmotor symptoms and quality of life after 6 months. Although the 5-2-1 criteria only identify patients with motor fluctuations, the improvements seen both in some dopaminergic nonmotor symptoms and in quality of life likely occur through the effects of continuously administered levodopa. Patients in this analysis experienced a LCIG safety profile consistent with the safety profile identified in the Phase III trials. Patients who fulfilled all of the 5-2-1 criteria also had the most 'Off' time and highest baseline burden according to baseline scores of UPDRS part II, UDysRS, NMSS, PDQ-8 summary index and MCSI. Results from this study confirm that the 5-2-1 criteria correlate with the established predictors of disease burden and indicate these three relatively simple screening criteria may be useful in clinical practice as part of holistic disease progression assessment and prediction of overall disease burden.

This study is limited by the observational, uncontrolled, open-label design and the fact that these criteria are being applied to patients on LCIG therapy only. The impact of the 5-2-1 criteria on nonmotor symptoms is limited by the fact that two of the three criteria are focused on motor symptoms only. The feasibility of using the 5-2-1 criteria for the selection of patients for other device-aided therapies (e.g., apomorphine subcutaneous infusion and deep brain stimulation) and their impact on effectiveness outcomes needs further evaluation. Section 1 of the emerging MANAGE-PD instrument applies the 5-2-1 screening criteria to determine whether patients are well controlled with oral medications. If patients demonstrate deficiency in any one of the questions in section 1, they are then moved to section 2, where patient eligibility for device-aided therapy is assessed [32]. The MANAGE-PD tool is available online and based on robust quantitative and qualitative data from a panel of leading PD specialists and warrants further validation studies [16,33]. Timely management of symptoms in patients with PD using a standardized and validated tool may aid in homogenizing care for patients between PD specialists and general neurologists, including the timing and need for referrals or medication change and reducing the time a patient remains inadequately controlled on oral medications [32].

There are limitations regarding sample size as a few groups in this study were too small to analyze; however, information can still be gleaned from those patients in the larger counterpart subgroup. This study is also limited by including only patients initially screened by expert clinicians as candidates appropriate for treatment with LCIG. Measurement of 'Off' time may have been limited by use of a modified UPDRS III item 39 rather than Hauser diaries and the UPDRS measurement used may cause some ambiguities as it was not the current UPDRS licensed by the Movement Disorder Society. Although application of the 5-2-1 criteria agreed with clinical judgment of advanced PD in patients in this study, results may not be generalizable to all patient populations. The criteria are focused on motor features and did not include nonmotor symptoms, although nonmotor symptoms may be very relevant in choosing therapeutic strategies in treating patients with advanced PD. Exclusion of patients with cognitive impairment limits the application of the study results to that subgroup of patients that may be considered for LCIG therapy.

Clinical scales typically used to assess patients with PD are limited in that they may not capture all patients who have PD that is considered 'advanced'. The UPDRS is a comprehensive assessment of PD; however, a physician

with a higher level of expertise (i.e., a movement disorder specialist) may be more adept at using it and use of MDS-UPDRS can be time consuming and expensive [34]. UPDRS scores may also vary over time in patients who have motor fluctuations. Other scales are strictly focused on assessing only specific aspects, such as the Parkinson Fatigue Scale, NMSS and the UDysRS [34–36]. Although, the severe disability denoted by Hoehn and Yahr stage IV and V [37] usually qualifies a patient as having advanced disease. Elucidating the full clinical picture of advancing PD requires a holistic approach.

Conclusion

Results from this interim analysis confirm that the 5-2-1 criteria apply to a population of patients identified by clinicians as having advanced PD in a large, observational, multicountry study. As part of the clinician's assessment and emerging use of the MANAGE-PD tool, applying the 5-2-1 criteria may be a practical and straightforward way to identify patients with advanced PD. While fewer than 20% of patients in this study met all of the 5-2-1 criteria, all but two (98%) met at least one criterion, suggesting that meeting any one of the criteria may identify advancing PD. Patients meeting any of the 5-2-1 criteria may also be candidates for advanced therapies, such as continuous infusion with device-aided LCIG, subcutaneous apomorphine or deep-brain stimulation, which could offer better control of motor symptom fluctuations. Patients treated with LCIG in this study had improvements in 'Off' time, dyskinesia, nonmotor symptoms and quality of life. Prompt identification of patients with advancing disease is expected to lead to improved patient care by helping recognize those patients who may benefit from referral to specialists in movement disorders and possible initiation of advanced therapies when motor fluctuations become bothersome.

Supplementary infographic

An infographic accompanies this paper at the end of the references section. To download the infographic that accompanies this paper, please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/nmt-2020-0021

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/nmt-2020-0021

Author contributions

All authors had access to the data and participated in the development, review, critique and approval of the manuscript throughout the editorial process and approved the final manuscript draft submitted for publication. All authors agree to be accountable for all aspects of the work, ensuring the accuracy and integrity of the publication. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole and have given their approval for this version to be published.

All authors critically reviewed this manuscript and provided final approval for publication. J Aldred, A Antonini, K Onuk and KR Chaudhuri contributed to study design and data interpretation. M Anca-Herschkovitsch, O Bajenaru, P Bourgeois, TL Davis, R Iansek, FE Pontieri and MS Siddiqui contributed to data interpretation. L Bergmann and W Robieson contributed to study design, data acquisition, statistical analysis and data interpretation. E Cubo and M Simu contributed to data acquisition. N Kovács contributed to data acquisition and interpretation. P Kukreja and DG Standaert contributed to study design, data acquisition and data interpretation.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, informed consent has been obtained from the all participants involved.

Data sharing statement

AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual, and trial-level data (analysis data sets), as well as other information (e.g., protocols and Clinical Study Reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications. This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.AbbVie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

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1 Article Details

Title
Application of the "5-2-1" screening criteria in advanced Parkinson's disease: interim analysis of DUOGLOBE

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2 Unmet Need

The absence of a biomarker, diagnostic test, or gold standard index makes defining the stage of advanced Parkinson's disease (PD) challenging, which impacts the ability to optimize therapies.

Early identification of advanced PD allows doctors to adjust treatment, leading to better symptom control and improved quality of life. When modified oral regimens no longer adequately manage PD symptoms, advanced treatment considerations include continuous infusion of levodopa-carbidopa intestinal gel (LCIG), continuous administration of subcutaneous apomorphine, or deep brain stimulation.

Application of objective, simple, motor criteria (5-2-1 criteria) can help identify advanced PD. Patients meeting at least one of the 5-2-1 criteria may also be candidates for advanced therapies. The 5-2-1 criteria include using oral levodopa at least 5 times per day, having at least 2 hours with "Off" symptoms, or at least 1 hour with troublesome dyskinesia.

3 Design

DUOGLOBE is an ongoing 3-year, multi-country, post-marketing observational analysis of the long-term effectiveness of LCIG in patients with advanced PD.

This post hoc analysis of an interim DUOGLOBE dataset was conducted to evaluate if patients identified by experienced clinicians as having advanced PD met the 5-2-1 criteria.

DUOGLOBE also assessed relationship of the 5-2-1 criteria to effectiveness and safety outcomes of LCIG treatment during routine care.

Patients were analyzed across 4 subgroups:

1. Patients meeting the ≥ 5 times day oral levodopa dosing criterion
2. Patients meeting the ≥ 2 hours of "Off" time criterion
3. Patients meeting the ≥ 1 hours a day troublesome dyskinesia criterion
4. Patients meeting all 5-2-1 criteria

4 Results

This study represents the first attempt at using the 5-2-1 criteria with a large cohort of patients on an international scale. Interim (6-month) results of 139 enrolled patients demonstrate that almost all patients selected for LCIG by DUOGLOBE investigators on clinical grounds fulfilled at least one of the 5-2-1 criteria.

fulfilled ≥ 1 of the 5-2-1 criteria | **fulfilled ≥ 2 of the 5-2-1 criteria** | **fulfilled all criteria**

Patients treated with LCIG who already **met at least one of the 5-2-1 criteria** had **significant improvement** after 6 months in:

- "Off" time, dyskinesia
- Non-motor symptoms
- Quality of life

Safety was consistent with other phase 3 LCIG studies.

5 Conclusions

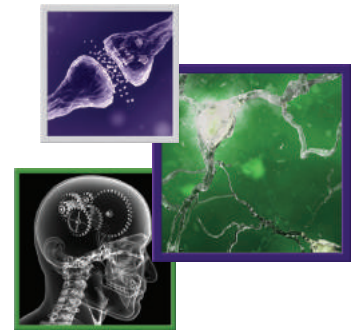
In patients with advanced PD, the 5-2-1 criteria generally aligned with clinician assessment.

In this routine clinical practice setting, treatment with LCIG led to significant improvements in "Off" time, dyskinesia, non-motor symptoms, and quality of life in patients fulfilling at least one or all of the 5-2-1 criteria.

Applying the 5-2-1 criteria may be a practical and straightforward way to identify patients with advanced PD.

Editorial

Neurodegenerative Disease Management



Comparative effectiveness studies in multiple sclerosis

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"Despite the pragmatic utility of observational studies, they are often prone to confounding by indication, and thus require methods to limit baseline imbalances in demographics and disease characteristics before direct comparisons are made."

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Multiple sclerosis (MS) is a chronic, autoimmune-mediated, demyelinating and neurodegenerative disease of the CNS that is the number one cause of nontraumatic disability in young persons. Disease-modifying therapies (DMTs) dramatically improve outcomes by reducing disease activity and progression in MS. However, the emerging DMT landscape remains complex, and as new therapies with variable efficacy and safety profiles become available, these complexities will become even more multifaceted, yielding greater challenges in DMT decision-making in clinical practice [1]. While randomized clinical trials (RCTs) provide the highest level of evidence for DMT safety and efficacy, they are costly, time-prohibitive, and have more limited applications in the clinical setting due to restrictive inclusion criteria. In contrast, observational (nonrandom) studies harnessing real-world data are cost-effective and time efficient, and allow direct comparisons of DMTs in larger, more heterogeneous patient populations to answer clinically relevant questions with broad applicability [2].

Harnessing insights from real-world observational data

Real-world comparative effectiveness studies evaluating the safety and efficacy of DMTs is a rapidly advancing area of MS clinical research. Harnessing insights from real-world data has gained growing interest in recent years and will continue to expand as the number of novel MS therapies rapidly increase. Well-designed comparative effectiveness studies in MS contribute robust real-world evidence to facilitate decision-making in clinical practice [2] and can also provide useful insights into the effectiveness of DMTs on sub-populations of interest that are not well-represented in RCTs (e.g., older patients, minority populations and those with certain comorbidities).

Observational studies, however, are susceptible to many types of biases, and therefore prone to scrutiny by regulatory agencies and the medical community. While RCTs eliminate biases through the random nature of treatment assignment to prespecified cohorts, observational studies are confounded by multiple biases that challenge their reliability and reproducibility. Such biases include: attrition, arising from between-group differences in follow-up duration, selection bias, arising from sub-populations being preferentially included in a study, immortal time bias, arising from systematic differences in the definitions of study entry, Will Rogers phenomenon, wherein diagnostic criteria are changed during the study period and indication bias, arising from subjects' exposure to nonrandom treatment [2].

In retrospective observational studies, indication bias can occur when a patient's characteristics that determine treatment selection are also associated with the treatment outcome. Providers choose treatments based on clinical judgment or 'insider information'. For example, a patient who is considered higher risk may receive a more aggressive DMT than a patient who is considered lower risk and started on a less aggressive therapy. However, the higher risk patient may have inherently poorer outcomes compared with the lower risk patient, thus appearing that the aggressive treatment does not work as well as the less aggressive treatment. In this context, individuals who receive

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one DMT might be different from those treated with another DMT, a matter of 'apples' versus 'oranges'. This is in contrast to RCTs where treatment is randomly assigned without considering individual patient factors. In this observational context, treatment groups differ systematically and are therefore noncomparable. Therefore, one must account for such systematic differences in baseline characteristics between groups of interest when estimating the effect of treatment on desired outcomes.

Propensity score analysis for mitigating limitations of observational studies

Historically, researchers have relied on the use of regression adjustment to account for differences in measured baseline characteristics between treatment groups. However, there has been increasing interest in methods based on the propensity score (PS) [3] to reduce or eliminate the effects of confounding and certain biases (e.g., indication bias) when using observational data. The PS is the probability of treatment assignment based on a prespecified selection of baseline covariates that affect treatment selection, in other words, the various 'insider information' that providers use as a basis of their clinical judgment. PS methodology addresses indication bias by balancing the distribution of covariates across treatment groups before direct comparisons are made. A robust PS model includes a complete list of covariates that are relevant to treatment selection/allocation and are typically prognostic of treatment outcomes. For example, when building a PS model for an MS study evaluating treatment A versus treatment B, important variables to incorporate into the model include demographics (e.g., age, sex and race), baseline disease characteristics (e.g., prior relapses and gadolinium-enhancing lesions, disease course, disease duration, prior number and type of DMTs) and comorbidities (e.g., vascular comorbidities, such as hypertension, hyperlipidemia, diabetes mellitus, tobacco exposure, chronic heart and lung disease). Because the PS is a function of covariates rather than outcomes, the estimated effect is therefore not biased by the desired outcome. PS methods are therefore a useful tool in MS comparative effectiveness studies to enable robust comparisons between treatment groups, approaching that of a randomized study design. In this context, comparisons of treatment groups begin to look more like 'apples' versus 'apples'.

There are various approaches to PS analyses, including matching, weighting and stratification [3,4]. The most common implementation of PS matching is one-to-one or pair matching, in which pairs of treated and untreated subjects are formed, such that matched subjects have similar values of the PS. Thus, in a set of subjects with the same PS, the distribution of observed baseline covariates between treated and untreated groups is the same. This method allows for comparisons between individuals with a similar probability (PS) of receiving the same treatment but in fact received different treatments, thereby mimicking randomization. However, one notable limitation of PS matching is that if the groups of interest have different sample sizes, patients may be unmatched and therefore excluded from the analysis, potentially introducing selection bias. A second approach of PS analysis, inverse probability of treatment weighting, uses weights based on the PS to create a synthetic sample in which the distribution of measured baseline covariates is independent of treatment assignment. A third approach, stratification on the PS involves stratifying subjects into mutually exclusive subsets based on their estimated PS. Within each PS stratum, treated and untreated subjects will have approximately similar values of the PS. Therefore, when the PS has been correctly specified, the distribution of measured baseline covariates will be roughly similar between treated and untreated subjects within the same stratum [3,4].

However, PS methodologies can only adjust for known sources of bias. In the context of 'hidden bias', certain methods can be used to estimate the minimum effect size of an unmeasured covariate to determine if the apparent treatment effect differences were actually attributable to other confounders. For example, the Rosenbaum bounds/Hodges-Lehmann Γ quantifies the robustness of the outcome measurement based on hypothetical unmeasured covariates. This sensitivity analysis identifies the proportion of hidden bias that would nullify a statistically significant finding, though it does not identify the specific unmeasured variables [5].

Comparative effectiveness studies for evaluating DMTs in MS

Several recent, noteworthy comparative effectiveness studies, many of which applied PS methodologies, contributed to the MS literature by reporting on real-world treatment effect differences between DMTs. Comparative effectiveness studies to date have primarily focused on commonly used oral DMTs, specifically dimethyl fumarate (DMF), fingolimod (FTY) and teriflunomide. However, there are some publications reporting on head-to-head comparisons of oral versus infusion DMTs (e.g., FTY versus natalizumab [NTZ]) and multiple pairwise comparisons of injectable, oral and infusion therapies. DMT sequencing studies after discontinuation of DMF, FTY and NTZ have also been reported. As large datasets become increasingly available through multicenter studies and those

using large, heterogeneous population-based databases that will comprise the newer infusion therapies, the MS literature will inevitably expand to include more comparative effectiveness studies investigating infusion versus infusion DMTs (e.g., NTZ vs ocrelizumab/rituximab).

DMF, FTY & teriflunomide

A two-center, 36-month, PS weighting-adjusted analysis by Vollmer *et al.*, showed similar effectiveness of DMF (n = 737) and FTY (n = 535), measured via proportion with clinical relapses (odds ratio [OR] = 1.27; 95% CI: 0.90–1.79), gadolinium-enhancing lesions (OR = 1.25; 95% CI: 0.85–1.84) and new T2 lesions (OR = 0.99; 95% CI: 0.74–1.32) with higher DMF discontinuations (OR = 1.81; 95% CI: 1.41–2.31), largely driven by intolerance (OR = 1.63; 95% CI: 1.18–1.73) [6]. To comment on DMT sequencing, patients who switched from DMF or FTY to highly effective therapy (HET; alemtuzumab, ocrelizumab, rituximab) showed decreased disease activity compared with those who switched to injectable/oral therapies (glatiramer acetate, IFNβ, DMF, FTY and teriflunomide). Vollmer *et al.* also investigated MS sub-populations of interest through stratification (e.g., relapsing-remitting MS [RRMS], patients younger and older than 40 years of age, male and female patients, first-line and non-first-line users, those with and without baseline gadolinium-enhancing lesions, and direct switchers from NTZ) in a 24-month DMF versus FTY study [7]. Overall, results showed comparable probability of absence of MS disease activity (measured via absence of clinical relapses and new MRI lesions) across all sub-groups, except for males (OR = 0.58; p = 0.035) and first-line users (OR = 0.67; p = 0.023) who experienced less risk with FTY treatment.

A 24-month comparative effectiveness study of DMF versus FTY by Fox *et al.* showed similar results [8]. The authors compared patient data across multiple studies with a matching-adjusted indirect method. Cross-trial differences were minimized by adjusting data from trials where individual patient data were known to match aggregate data from trials in which patient data were unknown. The matching-adjusted indirect comparison approach showed comparable efficacy of DMF and FTY measured via annualized relapse rate (ARR) (RR = 1.11; 95% CI: 0.88–1.40), though DMF was associated with better patient-reported outcomes. There were no significant differences in the percentages of patients with no evidence of disease activity (NEDA; RR = 0.92; 95% CI: 0.51–1.64). Of note, confounding from unknown differences between trials and variations in trial length might have obscured treatment effect differences between the two groups.

A comparative effectiveness study by Boster *et al.* utilized a health claims database to assess the real-world effectiveness of patients (n = 6372) newly-initiating DMF, IFNβ, GA, teriflunomide and FTY [9]. Using a Poisson and negative binomial regression model, the adjusted incidence rate ratio (IRR) of relapses was comparable between DMF and FTY (IRR = 1.03; 95% CI: 0.88–1.21), though significantly improved in DMF compared with IFNβ (IRR = 1.27; 95% CI: 1.10–1.46), glatiramer acetate (IRR = 1.34; 95% CI: 1.17–1.53) and teriflunomide (IRR = 1.23; 95% CI: 1.05–1.45) [10].

Ontaneda *et al.* used PS matching to compare the ARR of patients with MS (n = 20,311) who switched from injectable therapy to either DMF, FTY or teriflunomide using a commercial health claims database [11]. Similar to previously reported DMF versus FTY studies, results of the current investigation showed a significant decrease in ARR for patients treated with DMF as compared with teriflunomide (rate ratio [RR] = 0.667; 95% CI: 0.486–0.914) and comparable postindex ARR compared with FTY (RR = 1.07; 95% CI: 0.861–1.328).

Laplaud *et al.* compared the effectiveness of teriflunomide (n = 713) and DMF (n = 1057) using inverse probability weighting [12]. At 24-month follow-up, teriflunomide and DMF demonstrated comparable clinical effectiveness measured via relapses and disability progression, but better MRI-based outcomes measured via new T2 lesions (OR = 0.60; $p < 0.001$) [13]. Overall, this study provided Class III evidence that for patients with RRMS, teriflunomide and DMF demonstrated similar clinical effectiveness over 24 months of therapy. Another PS-adjusted analysis by Buron *et al.* compared DMF (n = 767) versus teriflunomide (n = 1469) in a real-world clinical setting [14]. The relapse RR for DMF versus teriflunomide favored DMF (RR = 0.58; 95% CI: 0.46–0.73; $p < 0.001$). Further, patients treated with DMF had a higher relapse-free survival proportion compared with those treated with teriflunomide at 48 months ($p < 0.05$). As opposed to Lauplad *et al.*, this study provided Class II evidence that for patients with RRMS, DMF was more effective in preventing relapses compared with teriflunomide.

FTY versus natalizumab

A comparative effectiveness study by Lorscheider *et al.* applied PS matching to evaluate FTY (n = 179) versus NTZ (n = 179) as second-line therapy for RRMS patients who were nonresponders to first-line injectable DMTs [10].

Results showed that patients treated with NTZ had a lower risk of relapses compared with those treated with FTY (IRR = 0.5; 95% CI: 0.3–0.8). Further, NTZ patients had higher probability of Expanded Disability Status Scale (EDSS) improvement versus FTY patients (hazard ratio [HR] = 1.8; 95% CI: 1.1–2.7). The authors concluded that NTZ was more effective in reducing relapse rates and disability progression as measured by the EDSS versus FTY.

A study by Baroncini *et al.* evaluated relapse data, EDSS scores and MRI data among RRMS patients treated with NTZ (n = 102) or FTY (n = 102) as second-line treatment using PS matching [15]. More patients discontinued NTZ compared with FTY, mostly due to safety concerns (33 vs 11%; $p < 0.001$). Patients treated with NTZ had a higher percentage of relapse-free status versus FTY (66 vs 80%; p = 0.015), higher percentage of EDSS improvement (6 vs 15%; p = 0.033), lower percentage of MRI activity (38 vs 14%; p = 0.001) and higher percentage of NEDA (44 vs 70%; $p < 0.001$). Similar to the Lorscheider *et al.* study, the investigators concluded that NTZ was superior to FTY across clinical and radiographic measures in RRMS patients nonresponding to first-line injectable therapies.

Multiple pairwise comparisons investigating injectable, oral, & infusion DMTs using a large heterogeneous MS population

Using a large multicenter, multinational database, Kalincik *et al.* used PS matching to compare a number of DMTs. In 2017, the authors investigated alemtuzumab (n = 189) versus IFNβ (n = 2155), FTY (n = 828) and NTZ (n = 1160) [16]. Patients treated with alemtuzumab showed lower ARR compared with IFNβ (0.19; 95% CI: 0.14–0.23 versus 0.53; 95% CI: 0.46–0.61) and FTY (0.15; 95% CI: 0.10–0.20 versus 0.34; 95% CI: 0.26–0.41) and comparable ARR compared with NTZ (0.20; 95% CI: 0.14–0.26 versus 0.19; 95% CI: 0.15–0.23). Alemtuzumab patients demonstrated similar probabilities of disability accumulation versus IFNβ (HR = 0.66; 95% CI: 0.36–1.22), FTY (HR =1.27; 95% CI: 0.60–2.70) and NTZ (HR = 0.81; 95% CI: 0.47–1.39). However, in terms of disability improvement, while alemtuzumab patients demonstrated similar probabilities compared with IFNβ and FTY, they had a lower probability versus NTZ (HR = 0.35; 95% CI: 0.20–0.59). Overall, patients treated with alemtuzumab demonstrated superior relapse rate reductions compared with IFNβ and FTY, while similar to that of NTZ. Results also demonstrated that NTZ appeared superior to alemtuzumab in allowing disability recovery.

In 2018, Kalincik *et al.* used PS matching to compare the effectiveness of cladribine (n = 37) versus IFNβ (n = 1940), FTY (n = 1892) and NTZ (n = 1410) [17]. Overall, patients treated with cladribine demonstrated a lower probability of relapses compared with IFNβ (p = 0.05), similar probability compared with FTY (p = 0.31) and higher probability compared with NTZ (p = 0.042). Further, patients treated with cladribine showed similar probability of disability accumulation compared with IFNβ (p = 0.37) and FTY (p = 0.089), but demonstrated higher risk of disability compared with NTZ patients (p = 0.021). However, cladribine patients had a higher probability of disability improvement compared with IFNβ ($p < 0.001$), FTY (p = 0.0025) and NTZ ($p < 0.001$).

In 2019, Kalincik *et al.* compared teriflunomide (n = 614), DMF (n = 782) and FTY (n = 2332) over 2.5 years using PS matching [13]. Patients treated with FTY demonstrated lower ARR compared with both teriflunomide (0.18 versus 0.24; p = 0.05) and DMF (0.20 versus 0.26; p = 0.01); while ARR were similar between DMF and teriflunomide (0.19 versus 0.22; p = 0.55). There were no differences in disability accumulation nor improvement across all three DMTs.

DMT sequencing following natalizumab discontinuation

Recently, Hersh *et al.* published a two-center study using PS weighting that compared the effectiveness of switching from NTZ to a moderate-efficacy DMT (DMF n = 130; FTY n = 140) versus high-efficacy DMT (ocrelizumab n = 106; rituximab n = 17, alemtuzumab n = 7) [18]. By 24 months post-NTZ, there were no significant differences in ARR across the two switched treatment paradigms (OR = 1.44; 95% CI: 0.69–1.59). However, patients who switched to moderate-efficacy DMT had more gadolinium-enhancing lesions (OR = 3.62; 95% CI: 1.56–5.21), lower proportion with absence of disease activity (OR = 0.41; 95% CI: 0.21–0.71) and higher risk of disability progression (p = 0.043). Similar results were observed across reasons for NTZ discontinuation (e.g., safety risks, breakthrough disease on NTZ), though they seemed to be driven more by those who switched from NTZ due to disease activity. The authors concluded that patients switching from NTZ to moderate-efficacy versus high-efficacy DMT were at higher risk of clinical and radiographic disease activity and progression by 24 months of follow-up.

Overall, these comparative effectiveness studies contributed to the MS literature by demonstrating DMT performance in real-world settings across a number of different databases (e.g., single- and two-center studies, health claims databases, large population-based databases). While they all differed in sample sizes, patient populations

and outcome measures; they reported on similar conclusions that DMF and FTY have similar effectiveness and out-perform injectable therapies; and alemtuzumab and NTZ are highly efficacious therapies that demonstrate superiority over injectable and oral therapies in real-world practice. While FTY appears to be superior over teriflunomide, conflicting results were reported on the comparative effectiveness of DMF versus teriflunomide, for which more large, heterogeneous studies are warranted to further investigate this relationship. Such differences in reporting underscore the importance of designing robust observational studies that maximize data quality; incorporate a diverse patient population; are all-inclusive of relevant baseline demographic, clinical and paraclinical factors for statistical model building; and incorporate sensitivity analyses.

Conclusion

Clinical trial data, while valuable from a regulatory standpoint for the approval of new medications, have limited applications in clinical settings. Observational studies, on the other hand, can leverage large, heterogeneous data to derive real-world evidence for MS patients and providers. These studies use data from clinical practice that do not have the rigid constraints of clinical trials. As such, the results are more generalizable for the neurologist in the clinic and provide patients more realistic insights into how DMTs perform in a real-world setting. In the rapidly advancing landscape of novel neurotherapeutics for MS, it has become increasingly challenging, yet crucial, to compare the effectiveness and safety of DMTs. RCTs are cost- and time-prohibitive, making such trials impractical for providing these head-to-head comparisons. Comparative effectiveness studies using retrospective observational data are thus valuable for reporting on these treatment effect differences to inform decision-making in routine practice. Despite the pragmatic utility of observational studies, they are often prone to confounding by indication, and thus require methods to limit baseline imbalances in demographics and disease characteristics before direct comparisons are made. PS analysis is a unique statistical method that reduces the impact of indication bias, thereby approximating a randomized study design. With the utility of PS methodologies, real-world comparative effectiveness studies in MS can answer a wide array of clinically relevant questions with broad applicability, contributing to real-world evidence that not only support but complement RCTs.

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Research Article

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Future NEUROLOGY

Identifying the mechanisms of α-synuclein-mediated cytotoxicity in Parkinson's disease: new insights from a bioinformatics-based approach

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Aim: A large body of evidence has implicated the cytotoxicity of α-synuclein in Parkinson's disease (PD). We planned to use a bioinformatics-based approach to gain further insight into this process. **Materials & methods:** Using STRING version 10, we identified interacting proteins of α-synuclein. Using α-synuclein and one of these interactors involved in apoptosis as query proteins, we identified other linked proteins. We further analyzed the interactions between some of these proteins by Protein–Protein Docking using ClusPro. **Results:** We identified BAX as an interacting protein of α-synuclein. Interactions of α-synuclein and BAX as well as BAX and BCL2L1 were determined. **Conclusion:** The interaction of α-synuclein and BAX could play a crucial role in the cell death process of PD where apoptosis and mitochondrial permeability transition-driven necrosis may coexist.

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Keywords: cell death • docking • Parkinson's disease • protein–protein interaction • α-synuclein

Neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease, amyotrophic lateral sclerosis etc., are characterized by the abnormal accumulation of different proteins, which are held responsible for an array of toxic actions leading to neuronal death [1,2]. In PD, abnormal accumulation of α-synuclein (coded by the *SNCA* gene) has been considered as a driving force of the disease pathogenesis, based on extensive findings from both animal and human studies [3,4]. α-synuclein is a small acidic protein with a natively unfolded structure having N-terminal lipid-binding segment with a tendency to form α-helix on membrane binding, a central hydrophobic segment called non-amyloid β component domain and a C-terminal acidic tail [4]. In the brain, it is located mainly at presynaptic terminals and is possibly involved in vesicular transport, neurotransmitter release and synaptic plasticity [4]. There has been some experimental evidence of localization of the protein in the neuronal nucleus and the mitochondria-associated endoplasmic reticulum membrane [5]. The role of accumulated monomeric or oligomeric forms of α-synuclein (wild or mutant type) in causing mitochondrial dysfunction, endoplasmic reticulum stress, altered ER-Golgi transport, impaired vesicular transport, altered cytoskeletal dynamics, calcium dysregulation and oxidative stress have been suggested in multiple studies, but the molecular mechanisms of α-synuclein action remains uncertain [3,6–11]. One suggested mechanism is the interaction of α-synuclein with lipid biomembranes and disruption of membrane structure and integrity by oligomeric α-synuclein [12]. On the other hand, α-synuclein could interact with important proteins regulating cell structure, metabolism, differentiation and death [7,10,13–15]. The unequivocal identification of such interacting protein partners of α-synuclein through isolation and characterization of protein complexes in different experimental models or in the actual disease

Future Medicine

condition has remained a formidable challenge and thus, a unique pathway of cell death mediated by α-synuclein in PD is yet to be established. Added to this complexity is the uncertainty of the nature of neuronal death in PD. Although apoptosis has been advocated as the mechanism of cell death in PD, there is substantial evidence of involvement of other pathways of regulated cell death, including necrosis [16,17]. There has been interest in identifying the mechanism and nature of α-synuclein-mediated cell death in model systems that could be relevant to PD pathogenesis [3,7,18,19]. One particularly interesting aspect in this context is the probable role played by α-synuclein and mitochondria in cell death pathways like apoptosis, ferroptosis or regulated necrosis. In addition to the conventional experimental approach, we thought it interesting to use the vast bioinformatic resources available to predict a sequence of interactions involving α-synuclein that may result in cell death, and then more effectively plan experimental designs to optimize resources and time.

The availability of high-throughput data collection techniques has resulted in the development of many databases and tools with varying degrees of complexity in which protein interactions are annotated at various levels of details. These can be utilized to understand protein–protein interaction (PPI) within the cells in normal and abnormal conditions [20,21]. Many complex cellular mechanisms, including programmed cell death pathways, take place through multiple and sequential interactions among different proteins. Such interactions generally lead to conformational changes in the interacting proteins causing activation of enzymatic activity (e.g., protein kinase) or translocation of the proteins to new compartments (e.g., from cytosol to mitochondria or nucleus) [22,23]. Such PPI networks may be generated with α-synuclein as the query protein in STRING database to identify various interacting partners of α-synuclein with links to cell death pathways. The STRING database, which is available online, has been designed in a user friendly and comprehensive manner covering nearly 5090 organisms and 24.6 million proteins. It provides us interactions, exact and predicted, based on experimental data as well as bioinformatics-based studies like gene fusion, gene co-occurrence, co-expression and text mining [24]. This database also provides us with information about predicted secondary and tertiary structures of large numbers of interacting proteins and the nature of their interactions through colored nodes and edges. For our present purpose, the most relevant PPI networks would be those that focus on experimentally proven interactions of α-synuclein with proteins of programmed cell death pathways. However, such PPI networks would at best be predictive. We, therefore, thought it prudent to perform protein–protein docking, which could demonstrate the actual probabilities of physical interactions of proteins of interest. Protein docking is the prediction of the 3D structure of a protein–protein complex from the coordinates of its component structures. Of the various available approaches, rigid body docking of two proteins is commonly performed in the first stage among large number of possible conformations and in subsequent stages a small subset of structures are refined and re-ranked using other energy functions [25,26]. Various algorithms have been optimized for this purpose [27]. Using both these approaches in the current study, we have attempted to gain new insight on interactions of α-synuclein with other proteins that may culminate in neuronal death.

Materials & methods

Creation of PPI network using STRING version 10

The STRING version 10 database was used online to produce PPI network using α-synuclein as the query protein and selecting *Homo sapiens* as the species. The PPI network obtained in STRING, shown as nodes connected by edges, was successively modified and refined by changing the parameter setting such as the number of interactor proteins, active interaction sources and minimum required interaction score. The PPI network from STRING was based on various actual and predicted interactions among α-synuclein and its interacting partners. These included actual experimental physical interaction as well as predicted interactions based on gene fusion, gene co-expression, gene co-occurrence, text-mining, etc. After getting the initial PPI networks of α-synuclein, we finally selected a network based on actual physical interactions with five of the most significant interacting proteins in the first shell. The database provided us the important details of the interacting proteins and we selected one protein linked with a programmed cell death pathway. We used the STRING database again utilizing two query proteins (i.e., α-synuclein and the newly identified interacting partner of α-synuclein) to obtain a final PPI network consisting of these two query proteins and their five significant interacting partners in the first shell.

Protein–protein docking

In order to verify further the physical interactions among these proteins, we performed protein–protein docking using the software ClusPro. ClusPro is a widely used software for direct docking of two proteins [28]. ClusPro first performs rigid body docking by sampling billions of conformations and then clusters 1000 lowest-energy structures

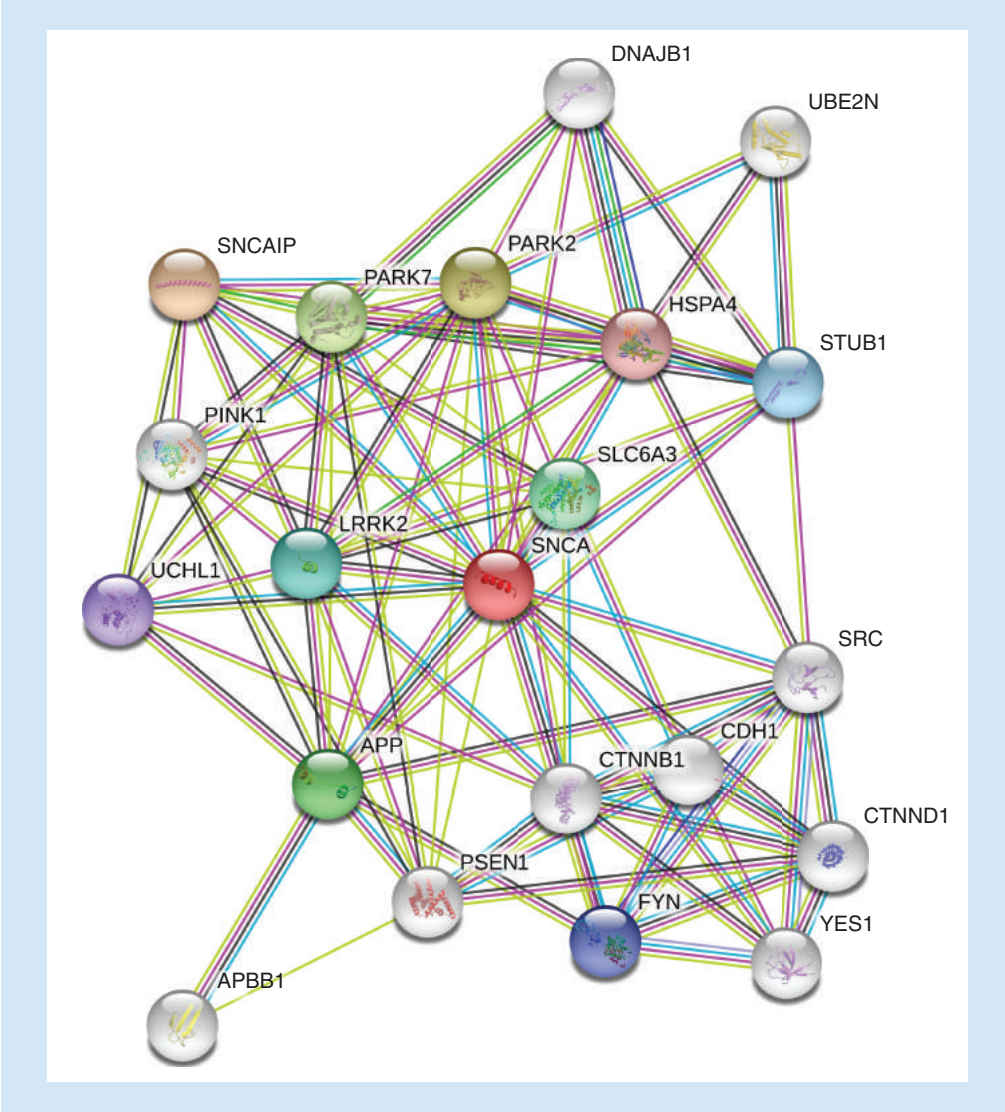

Figure 1. Expanded protein–protein interaction network of α-synuclein obtained from STRING version 10. Nodes represent proteins and edges represent PPI. The meanings of node color and node content and color and thickness of edges are detailed in the database (https://string-db.org). Parameter setting: 20 interacting proteins (ten in the first shell, ten in the second shell); interaction score 0.40 (medium confidence); all active interaction sources as mentioned in the database.
PPI: Protein–protein interaction.

based on root-mean-square deviation. It also refines the selected structures by minimizing the CHARMM energy. The top ten docking structures were selected based on lowest energy, and the protein interactions calculator was used to identify different types of interactions including hydrophobic (interaction within 5 A), hydrogen bonds, cation-pi, ionic and aromatic–aromatic interactions in all the ten models [29]. For the current study, the PDB structures PDB: 1XQ8, 1F16 and 1R2D for α-synuclein, BAX and BCL2L1, respectively, were downloaded and used for docking.

Results

When the STRING version 10 database was searched using the query protein α-synuclein selecting *Homo sapiens* as the species, a complex PPI network with 20 interacting proteins (ten in first shell and ten in second shell) could be seen (Figure 1). The nodes indicated the different interacting partners and the edges represented the nature of interaction. After changing the parameter setting of PPIs to include only co-expression and experimental physical

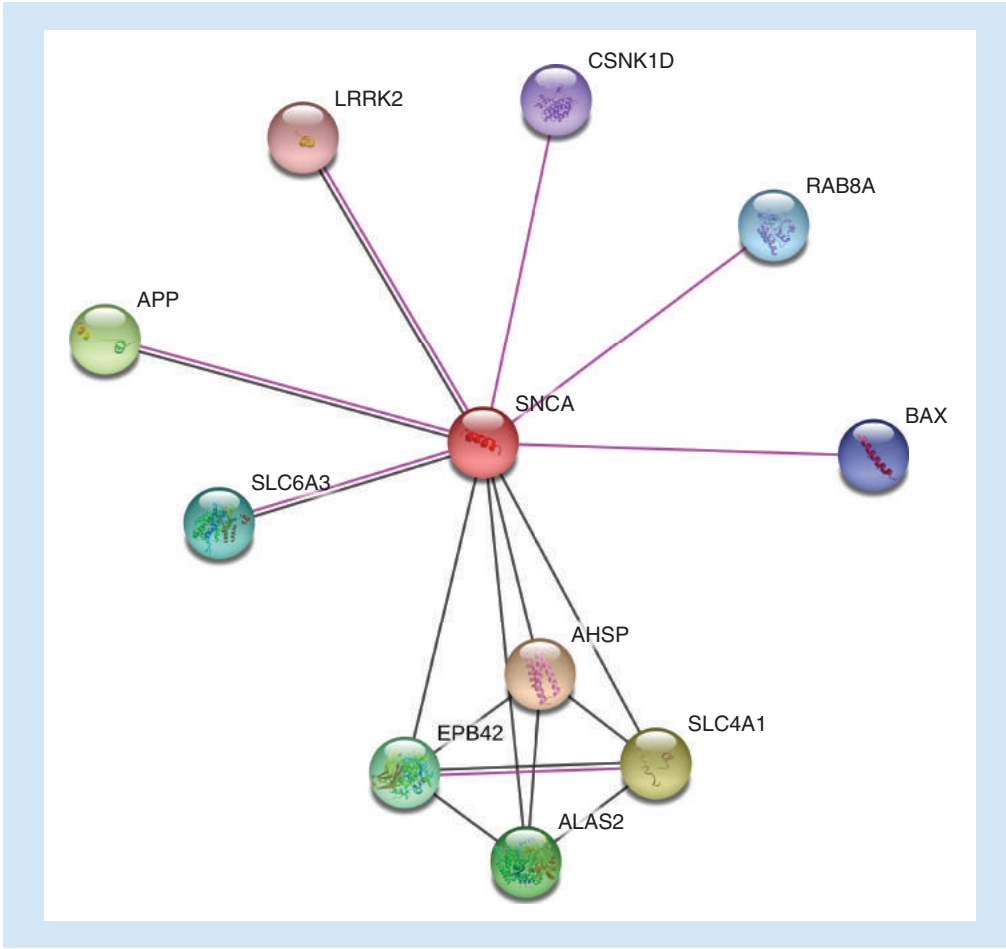

Figure 2. Refined protein–protein interaction network of α-synuclein. Ten interacting proteins are seen; interaction score (0.40) and active interaction source includes experimental physical interaction and gene co-expression.

interaction, the parameters most expected to be involved in actual pathophysiologic process, we obtained a PPI network shown in Figure 2. In this PPI network, there is experimental physical interaction between α-synuclein and BAX which is a proapoptotic protein. In Figure 2, an important PD-related protein, LRRK2, was seen to interact with α-synuclein through actual physical interaction and co-expression. We further refined the search by selecting most significant five interactor proteins having only experimental physical interactions (Figure 3). We checked the functions of the five interacting proteins present in Figure 3 from the STRING database, and the most important protein among these which was related to cell death was BAX. When the STRING database was searched with the two proteins BAX and α-synuclein simultaneously with number of interactors set at five, we obtained the PPI network shown in Figure 4. In Figure 4, we observed that two important proteins of programmed cell death pathways, BCL2L11 and BCL2L1, physically interact with BAX with high interaction scores of 0.828 and 0.97, respectively. On the other hand, BAX has direct physical interactions with α-synuclein with moderate interaction score of 0.52.

We further analyzed the protein–protein docking results between α-synuclein and BAX, as well as between BAX and BCL2L1. The models obtained by docking showed that the 5th and 6th helices of BAX (PDB: 1F16) within BCL2 homology domain (Pfam: 63–158) were involved in interaction with α-synuclein (PDB: 1XQ8). The interacting residues are 142Asp, 143Phe, 147Arg, 151Trp and 154Asp of BAX (Figure 5). Phe at position 4 in the first helix (3–11) of α-synuclein also seemed to be important for the interaction with BAX. Amino acids 71–97 of five β-strands in the C-terminal of α-synuclein protein appeared to be another stretch that seemed to be interacting with BAX. The protein–protein docking between BAX (PDB: 1F16) and BCL2L1 (PDB: 1R2D) again showed

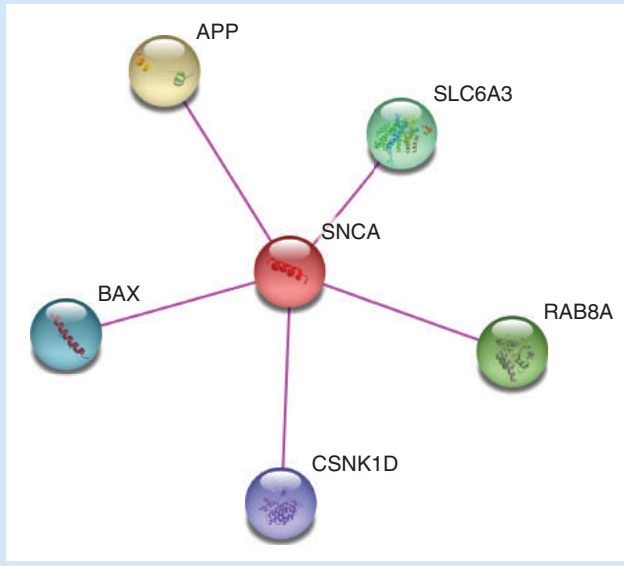

Figure 3. α-Synuclein and limited interacting proteins obtained from STRING version 10. Five interacting proteins in protein–protein interaction network; interaction score set at 0.40 and active interaction source includes only experimental physical interaction.

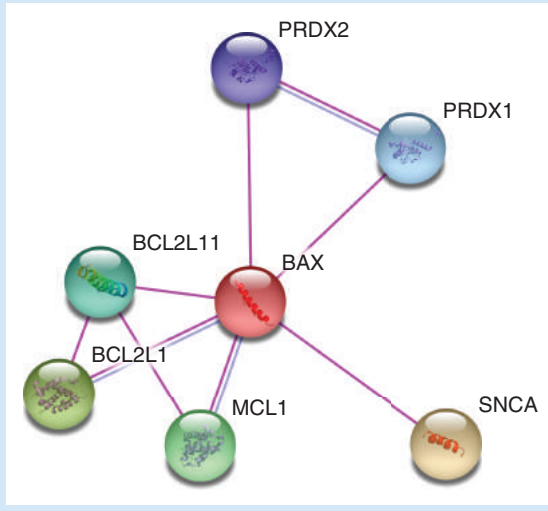

Figure 4. STRING database search results with two query proteins: α-synuclein and BAX. Five interacting proteins of α-synuclein and BAX can be seen; interaction score set at 0.40 (medium confidence) and active interaction source is only experimental physical interaction. Apart from BAX, two more proteins of BCL2 family BCL2L1 and BCL2L11 are also seen.

BCL-2 domain of BAX involved in interaction with BCL2L1. Furthermore, in BCL2L1 the residues Asp132 and Asp133 were found to be involved in interaction in more than 50% of the top ten docking models.

Discussion

The understanding of pathogenesis, identification of risk factors and development of biomarkers and neuroprotective drugs for PD remains a challenging job [3,30]. In particular, there are many areas of uncertainties related to the cause, mechanisms and nature of neuronal death in PD. However, the involvement of α-synuclein and

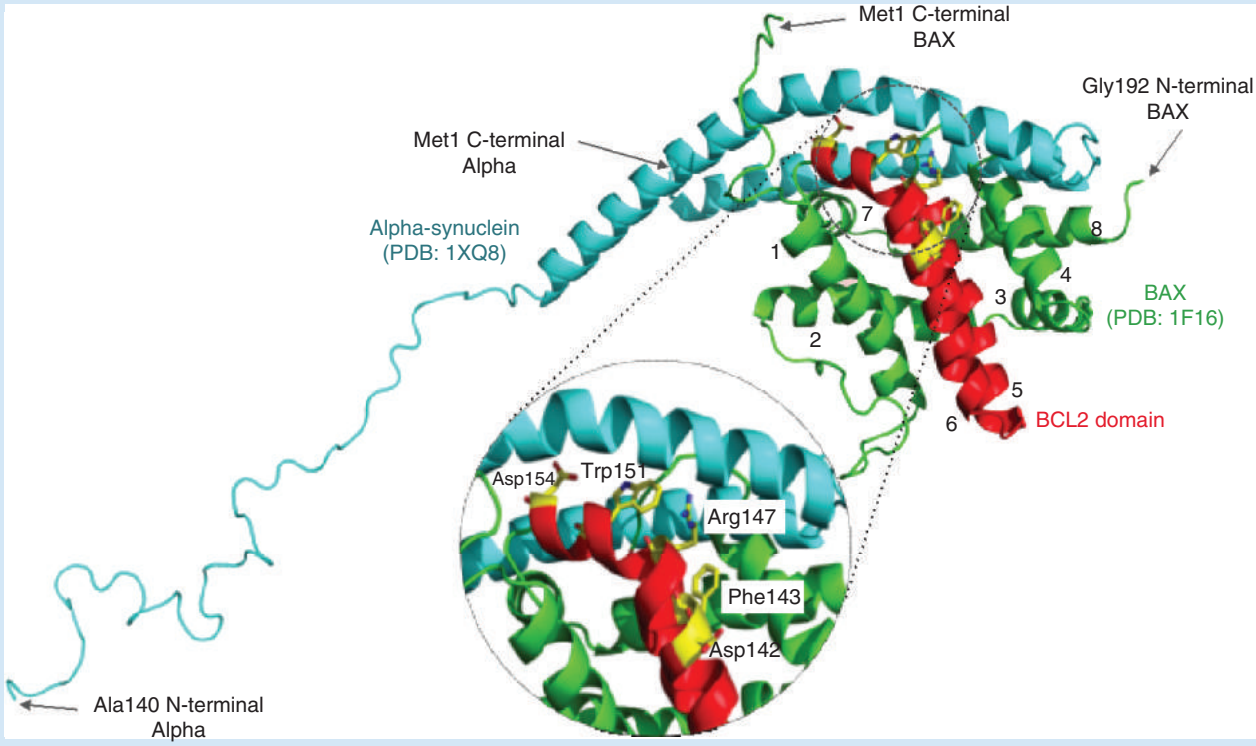

Figure 5. Docking study of α-synuclein (PDB: 1XQ8) and Bax (PDB: 1F16) using ClusPro. ClusPro best model obtained after docking is shown as ribbon diagram using PyMol (an open source molecular visualization system). α-synuclein is shown in cyan color and BAX in green color, whereas BCL2 homology domain is highlighted in red. The numbers represent the helices (1–8) in BAX starting from the N-terminus. The residues involved in interaction are shown with line and stick model Asp142, Phe143, Arg147, Trp151, Asp154 of BAX.

mitochondrial dysfunction in this process has been widely documented. We have been exploring this aspect in a model of neural cell death in which the intracellular level of α-synuclein is raised by different nongenetic manipulations [7,18,19]. In the current study, we have attempted to find out how a bioinformatics-based search could assist us in identifying the mechanisms and nature of cell death in PD neurodegeneration. Our results first showed that α-synuclein has an interacting partner, BAX, which is a proapoptotic protein belonging to the BCL2 family [31]. This interaction of α-synuclein and BAX was further validated by protein–protein docking studies, which showed that BH3 domain of BAX is involved in the interaction process. BAX is a cytosolic protein which upon activation translocates to the mitochondria, undergoes oligomerization and induces apoptosis by permeabilization of mitochondrial outer membrane and releasing proteins, such as cytochrome c, Smac/DIABLO, AIF, etc., leading to apoptosis [31]. The trigger for the activation of BAX and subsequent translocation to mitochondria is unknown but a conformational change of BAX is possibly required [31,32]. Our results tend to suggest that the binding of α-synuclein to BAX with a presumable conformational change in BAX may be the initiating event. Furthermore, α-synuclein, though a cytosolic protein, is capable of entering mitochondria and thus it is plausible that a complex of α-synuclein–BAX would reach the mitochondria as the initiating event of cell death. It is interesting that the interaction of α-synuclein with BAX takes place at the BCL2 homology domain, which is generally considered to be the region where BH3-only protein can bind and activate BAX [32,33]. The interaction of BAX with BCL2L1 that is an antiapoptotic protein is also validated by protein–protein docking in our current study. This interaction in normal conditions might lead to inactivation of pro-apoptotic function of BAX. However, it is plausible that, under various stressful conditions α-synuclein binds with BAX and prevents BAX–BCL2L1 interactions, leading to uninhibited pro-apoptotic action of BAX.

Another interesting area of controversy for PD neurodegeneration is related to the nature of cell death. Apoptosis has been widely recognized as the pathway of neuronal death in PD, but the evidence for other modes of cell death in PD is also substantial [16,17]. It has been shown that, under a variety of conditions, the death of SH-SY5Y

cells is mediated by α-synuclein-induced mitochondrial permeability transition (MPT) pore activation, which can be prevented by cyclosporine [7,18,19]. This mode of cell death is recognized as MPT-necrosis which could be, in our opinion, a potential mechanism of PD neurodegeneration [34]. It appears that different cell death mechanisms may function in concert in the degenerating dopaminergic neurons in the PD brain. Extending our hypothesis of translocation of α-synuclein–BAX complex to the mitochondria, it is thus tempting to speculate that α-synuclein initiates an apoptotic type of cell death indirectly through BAX, which causes mitochondrial outer membrane permeabilization, and directly through a MPT-necrosis process.

Conclusion

Our bioinformatics-based study has provided new clues to understand the neurodegeneration of PD in model systems. However, these possibilities of neuronal damage envisaged from bioinformatics-based analysis involving α-synuclein, BCL family of proteins and mitochondria are only predictive in nature, and experimental validation and biochemical characterizations of PPIs are necessary in suitable models to establish their role in actual PD pathogenesis.

Future perspective

Utilizing bioinformatics-based approaches to understand the pathogenesis of diseases, especially neurodegenerative diseases, is an emerging area with great potential. The current study has indicated how information gained from database searching and protein–protein docking studies can be utilized to formulate a pathway of neuronal death in PD. This will help us to design suitable experiments, optimizing resources and time, to obtain definitive information on the molecular pathogenesis of PD. Without this information, experimental studies usually progress slowly by trial and error approach. Thus, bioinformatics-based methods are an attractive alternative that may be utilized for other neurological diseases as well. We anticipate more such similar studies in the future, but many refinements and critical analyses will be required to develop this system fully.

Summary points

- This bioinformatics-based study aimed to identify a pathway of neuronal damage initiated by α-synuclein, which could be relevant to Parkinson's disease (PD) pathogenesis.
- Protein–protein interaction (PPI) networks were constructed on STRING version 10 using α-synuclein as the query protein.
- The PPI networks were refined successively by changing parameter settings and focusing on experimental evidence and co-expression data. A final network of α-synuclein and five interacting proteins, including BAX, BCL2L1 and BCL2L11, involved in the apoptotic pathway was obtained.
- Protein–protein docking studies were carried out between α-synuclein and BAX as well as between BAX and BCL2L1 to suggest possible molecular interactions among these proteins.
- Analyzing the data, we suggest that α-synuclein and BAX interactions followed by translocation of the complex to mitochondria may trigger apoptotic neuronal death. This may have implications in the neurodegeneration of PD.
- These predictive data need experimental validation in suitable models.

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