Study Report

Response to gabapentin enacarbil in RLS patients previously exposed to long-term treatment with dopaminergic agents.

Protocol Number: XP-IIT-0029

Principal Investigator:
Diego Garcia-Borreguero, MD, PhD
Sleep Research Institute
Madrid, SPAIN

Summary of findings:

1. Following a two-week treatment with GBPen administered at 600 mg/day, patients improved more with GBPen than with placebo.
2. The response to GBPen was comparatively lower for the group of patients that had been previously long-term treated with dopaminergic agents.
3. The difference between both groups of patients in the magnitude of the response to GBPen was not due to differences in age or gender, serum ferritin levels, duration of illness, severity of illness at baseline, previous existence of Augmentation, differences in the effects of GBPen on sleep or on pain, or in differences in toxicity between both groups.
4. These results indicate that a previous long-term treatment with dopaminergic agents reduces, not just the response to any other dopaminergic drugs, but also to alpha-2 delta agents.
5. In order to preserve a full response of symptoms to RLS medication, these results supports the notion that initial treatment for RLS should be started with non-dopaminergic medications.

Study hypothesis

- Dopaminergic agents have been widely used for the treatment of RLS since the 1980s (for an overview see Garcia-Borreguero et al., 2013). Although their short-term efficacy is firmly established, the main long-term complications are loss of efficacy and augmentation (Garcia-Borreguero et al, 2013).

- Loss of efficacy is a reduction of response to dopaminergic treatment in which some degree of response is still preserved (Garcia-Borreguero et al., 2010). In contrast to augmentation, the severity of symptoms is not worse than before treatment initiation. During augmentation symptoms become more severe with dopaminergic treatment than before treatment initiation. The main features of augmentation are (Garcia-Borreguero et al., 2007):
    - An earlier onset of RLS symptoms in the afternoon
    - A shorter latency of symptoms when the patient is at rest
    - A shorter duration of treatment effects
    - An increase in the intensity of symptoms
    - Presence of symptoms in previously unaffected body parts
    - Paradoxical response: An increase in symptom severity with higher doses, a decrease with dose reduction.

- There is an on-going discussion about whether loss of efficacy necessarily leads to augmentation. In such a case there would be a continuum between progressive loss of efficacy and augmentation. It is also conceivable that both clinical conditions share a common pathophysiology: long-term dopaminergic stimulation would cause a down-regulation of (pre-and/or postsynaptic dopaminergic receptors). This would ultimately dampen dopaminergic postsynaptic signaling leading to an increase in RLS symptom severity (Earley et al, 2014).

- **The question is whether such an exposure to previous long-term dopaminergic treatment would modify the fundamental pathophysiology in such a way that the future response to non-dopaminergic agents, such as alpha-2 delta ligands would also be dampened.** The existing literature does not offer any answer to such a question, as no studies have reliably compared response to alpha-2 delta ligands in untreated vs. dopamine pre-treated patients. However, ***clinical experience suggests that long-term exposure to dopaminergics reduces the likelihood of response to non-dopaminergic agents as well.***

The question we raise also has considerable practical consequences:

- On the one hand, IRLSSG guidelines have recently established that initial therapy of RLS can be started with either a dopaminergic agonists or with an alpha-2 delta ligand (Garcia-Borreguero et al, 2013).

- However, dopamine agonists have been the only treatment of choice for many years now, and despite the fact that both classes of drugs show a degree of treatment efficacy at least similar (Allen et al., 2014; Garcia-Borreguero et al, 2014, Hornyak et al., 2014), it is a fact that still today, most clinicians prefer to start treatment with a dopamine agonist and only consider the use of an alpha-2 delta ligand should treatment complications arise.

- Therefore, the key question today is to know whether the order of treatment matters, that is, ***whether previous treatment with a dopamine agonist might diminish the chances of future response to an alpha-2 delta ligand.*** Should this be the case, future treatment guidelines would have to reconsider current recommendations regarding the order of treatments. In other words, patients would have to start treatment with an alpha-2 delta ligand and only consider a

switch to a dopamine agonist if an insufficient response occurs or any treatment complications arise.

- A recent reanalysis of previous studies found no difference in response to GBP between patients previously treated with dopamine agonist and those not (Ondo et al., 2014). However, as discussed by the authors, the validity of this finding is limited by various methodological factors. Most importantly, and in contrast to the present proposal, no reliable information is provided on the duration of exposure to dopaminergic drugs, a factor that we consider critical for the hypothesis of this study.

  The purpose of this study was to compare the response to a two-week treatment with gabapentin enacarbil (600 mg/d) vs placebo in two different groups of RLS patients: a group of treatment-naïve RLS patients vs. a similar group of patients previously underwent a >5 year treatment with dopaminergics.

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2. Study objectives:

Primary objective:

- To compare the IRLS response to a two-week treatment with gabapentin enacarbil (600 mg/d) in treatment-naïve RLS patients vs. a similar group of patients previously treated with dopaminergics for at least 90% of the time during the last five years, as judged by the clinical impression of the investigator.

Secondary objectives include:

- To compare the response on the RLS-6 to a two-week treatment with gabapentin enacarbil (600 mg/d) in treatment-naïve RLS patients vs. a similar group of patients previously treated with dopaminergics for at least 90% of the time during the last five years.
- To compare the response on sleep to a two-week treatment with gabapentin enacarbil (600 mg/d) in treatment-naïve RLS patients vs. a similar group of patients previously treated with dopaminergics for at least 90% of the time during the last five years.
- To compare the response on pain to a two-week treatment with gabapentin enacarbil (600 mg/d) in treatment-naïve RLS patients vs. a similar group of patients previously treated with dopaminergics for at least 90% of the time during the last five years.
- To compare general toxicity between both groups to two-weeks treatment with gabapentin enacarbil (600 mg/d) in treatment-naïve RLS patients vs. a similar group of patients previously treated with dopaminergics for at least 90% of the time during the last five years.

Exploratory:

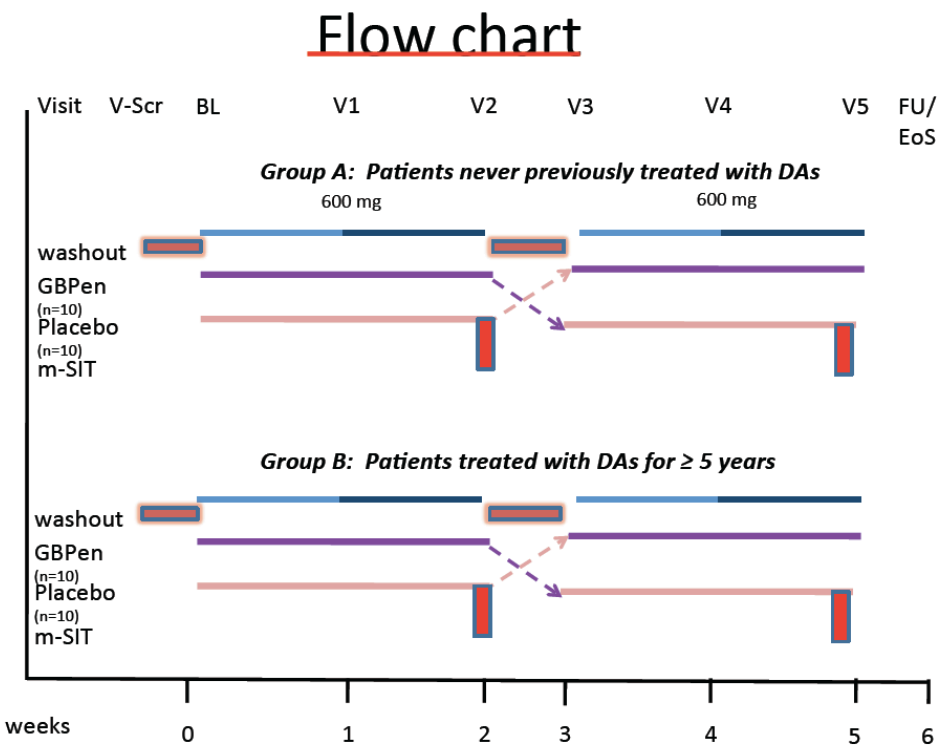
To compare the response of sensory and motor symptoms as assessed on multiple suggested immobilization tests to a two-week treatment with gabapentin enacarbil (600 mg/d) in treatment-naïve RLS patients vs. a similar group of patients previously treated with dopaminergics for at least 90% of the time during the last five years, as judged by the clinical impression of the investigator.

5. Subjects and Methods:

Design:

The study followed a double-blind, randomized, cross-over, placebo-controlled study on Gabapentin enacarbil (fixed dose: 600 mg/day) vs placebo.

Figure 1: Flow-chart



Study Population:

Forty patients with moderate to severe, idiopathic Restless Legs Syndrome were enrolled.

The patient population was stratified as:

- *Group A* (n=20): RLS patients not previously treated with dopaminergics
- *Group B* (n=20): RLS patients previously treated with dopaminergics most of the time* during at least the last five years and not meeting diagnostic criteria for augmentation.

  *: "most of the time" was defined as any continuous treatment for at least 90% of the time during at least the past 5 years, as judged by the clinical impression of the investigator.

Incl. criteria:

- Idiopathic RLS.

- A history (if currently on medication) or the presence of RLS symptoms on 3 or more days per week for at least 12 months.
- For Group A: An IRLS score ≥20 at baseline assessment

  For Group B: An IRLS score ≥15 during dopaminergic treatment and ≥20 following wash-out, during the baseline visit.
- 18 - 80 years.
- Creatinine clearance >60 ml/min

Excl. criteria:

- Any secondary forms of RLS.
- Current or previous augmentation according to MPI diagnostic criteria for augmentation
- History or current diagnosis of other clinically relevant diseases that may confound assessments or RLS symptoms.
- Serum ferritin <18 mcg/ml
- If treated with drugs likely to influence sleep architecture or motor manifestations during sleep, a wash-out period of at least 5 half-lives. If pre-treated with l-dopa or dopamine agonists, a washout of > 2 weeks.
- Employed in shift work or irregular sleep-wake schedules.
- Treatment for concurrent conditions which could interfere with efficacy assessments.

- Surgery within 180 days of baseline visit, which would negatively impact the patient's participation in the study.

- A significant medical or psychiatric disorder.

- Any clinically significant condition or laboratory assay abnormality.

- Other severe acute or chronic medical or psychiatric condition or laboratory assay abnormality that may increase the risk associated with study participation or with the interpretation of study results.

- Breastfeeding.

Outcome Parameters/Evaluation:

Primary:

Comparison between both patient groups of:

- Placebo-corrected change in IRLS total score

Secondary:

Comparison between both patient groups of:

- Placebo-corrected change in CGI-I
- Placebo-corrected change in RLS-6
- Placebo-corrected change in MOS scale
- Placebo-corrected change in Pain-VAS scale
- Incidence rate of general toxicity

Exploratory:

- Objective measurement of RLS symptoms by means of a multiple suggested immobilization test performed at 6P, 8P, 10P and 12 midnight.

Safety

Side-effects were recorded at every clinic visit.

Blood account, biochemistry and ECG will be performed on visits BL and FU-visit.

Justification of the sample size

Based on the results of a similar two week cross-over study that compared GBPen with placebo in the RLS population (Kushida et al., 2009), we assumed a clinically meaningful mean difference of 10 points (SD range: 6,1-6,5) on the IRLS-total score for GBPen compared to placebo in the treatment-naive group. Furthermore, in an exploratory manner, we assumed a placebo-corrected, clinically significant difference of at least 4 points between the treatment-naïve group and the group previously treated with dopaminergic agents. Thus, with 20 subjects per group the study had at least 80% power to detect a difference between treatment if the true difference was at least 4 points. The test assumed a Type I error of 0.025 with one-sided testing.

The expected drop-out rate had been approx. 14%.

Efficacy Analysis

All efficacy analyses will be carried out using the intent-to-treat population (ITT) which is defined as all patients who received randomized treatment.

Analysis of Primary Endpoint

The primary objective of this study is to compare the therapeutic response of two types of RLS populations (previous long-term dopaminergic treatment vs treatment naive) to a 2 week treatment period with gabapentin enacarbil by means of the International Restless Legs Scale (IRLS).

Change in IRLS-Total Score from baseline (Visit BL) to week 2 (V2) will be analyzed using Analysis of Covariance (ANCOVA) with the change score as the dependent variable and the independent variables of treatment and baseline (Visit BL) IRLS-Total Score. Assumptions for the ANCOVA model will be checked using plots of predicted values versus residuals as well as plots of the baseline score versus change score for each treatment (note that the small sample size limits the usefulness of a treatment by baseline interaction term for the model).

Analysis of Secondary Endpoint

Secondary endpoints will be analyzed for treatment effect using the ANCOVA model for continuous variables, and Cochran-Mantel-Haenszel tests for categorical variables.

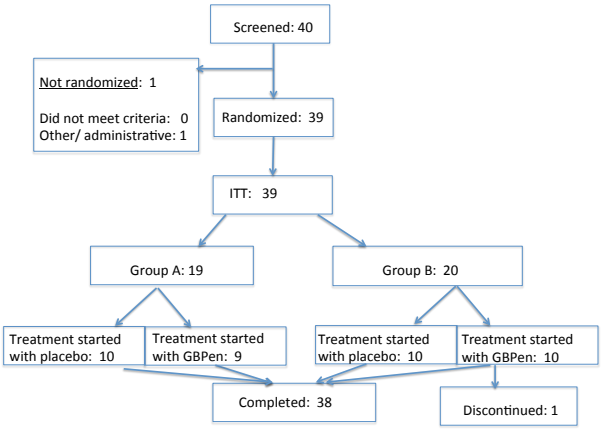
Safety Analysis

Adverse events were summarized by treatment and severity. Adverse events were coded using standardized methods. Vital signs were summarized for each visit at which they are collected. Rates of concomitant medication use were summarized using WHO-coding.

Results:

1. Patient disposition

Table 1: Patient disposition



As shown in Table 1, 40 subjects were screened and 39 were randomized. Thirty-eight subjects completed the entire study.

Two subjects were not able to complete: One of them decided to step out due to severity of symptoms during the screening period. The other discontinued the study for personal reasons at the end of the first treatment condition.

2. Demographics

Table 2: Summary of demographics (mean ± SD)

	Group A: Treatment naïve N=19	Group B: DA-treated N=19		p
Age	57,74 (7,43)	58,26 (12,42)	Z= -0,7	n.s.
Gender (% females)	63	63	N/A	n.s.
Duration of disease (yrs)	18,11 (15,47)	18,58 (15,00)	Z= -0,14	n.s.
Duration of disease -since diagnosis- (yrs)	4,84 (5,62)	7,36 (2,34)	Z= -0,3	n.s.
Family History (%)	57,90	70,00	chi_sq.= 0,62	n.s.
Serum ferritin (ng/ml)	71,59 (53,28)	85,80 (63,99)	Z= -1,09	n.s.
Duration of previous DA treatment (yrs)	N/A	7,31 (2,88)	N/A	
IRLS severity at baseline	21,68 (5,03)	21,31 (5,48)	Z= -0,8	n.s.

Concomitant diseases in both groups

Grupo A		
	Medical condition	**N**
Cardiology	Arrythmia	0
	Arterial hypertensión	4
	Thrombosis	0
Endocrinology and metabolism	Diabetes	0
	Hypercolesterinemia	3
	Hypothyroidism	1
	Hyperthyroidism	1
	Hyperuricemia	1
	Hiperglycemia	0
Ostheoarticular	Cervical arthrosis	0
	Knee surgery	1
	Dupuytren	1
	Hip surgery	0
	Ostheoporosis	0
Rheumatology	Fibromyalgia	0
	Arthrosis	0
Gastrointestinal	Hepatitis	2
	Gastroesophageal reflux	2
	Apendicitis	2
	Cholitis ulcerosa	0
	Gastric ulcer	1
	Gilbert´s Syndrome	0
Neurology	Meningitis	0
	Lumbociatic pain	1
	Cervical disk prolapse	2
	Lumbar disk prolapse	1
Gynecology	Hysterectomy	1
Psychiatry	Depression	1
	Bulimia	1
Urology	Nephrolithiasis	0
	Prostate hypertrophy	0
Nefrology	Renal disease	2
ENT	Vertigo	1
	Rhinitis	1
Respiratory	Asthma	1
Ophtalmology	Retinal detachment	0
Dermatology	Psoriasis	0

Grupo B		
Systems	**Medical condition**	**N**
Cardiology	Arrythmia	1
	Arterial hypertensión	2
	Thrombosis	1
Endocrinology and metabolism	Diabetes	1
	Hypercolesterinemia	4
	Hypothyroidism	2
	Hyperthyroidism	0
	Hyperuricemia	0
	Hiperglycemia	1
Ostheoarticular	Cervical arthrosis	1
	Knee surgery	0
	Dupuytren	0
	Hip surgery	1
	Ostheoporosis	1
Rheumatology	Fibromyalgia	2
	Arthrosis	1
Gastrointestinal	Hepatitis	1
	Gastroesophageal reflux	1
	Apendicitis	2
	Cholitis ulcerosa	1
	Gastric ulcer	0
	Gilbert´s Syndrome	1
Neurology	Meningitis	1
	Lumbociatic pain	0
	Cervical disk prolapse	0
	Lumbar disk prolapse	0
Gynecology	Hysterectomy	1
Psychiatry	Depression	1
	Bulimia	0
Urology	Nephrolithiasis	1
	Prostate hypertrophy	1
Nefrology	Renal disease	0
ENT	Vertigo	0
	Rhinitis	0
Respiratory	Asthma	0
Ophtalmology	Retinal detachment	1
Dermatology	Psoriasis	1

3. Efficacy variables

a. International Restless Legs Scale (primary endpoint)

The IRLS rating scale is intended to evaluate in a standardized way the subjective severity of major symptoms of the RLS and, in two items (9 and 10), the impact of

the disease on subjects' functioning in daytime activities by use of a five-point scale for each of a total of ten items:

1. global severity rating of restless legs symptoms in legs and arms
2. urge to move
3. relief by walking
4. sleep disturbances due to RLS
5. fatigue and somnolence due to RLS during the day
6. global severity rating of the restless legs syndrome
7. frequency of symptoms
8. severity of symptoms during an average day (24 hours), if present
9. impact of symptoms on daytime activities (family, home setting, contacts to friends, job)
10. impact of symptoms on mood (anger, dejection, sadness, anxiousness, irritation).

In all items, the scores range from 0=not present to 4= severe. A sum score across all ten items is calculated for analysis which varies between 0=no RLS symptoms present at all to 40=maximum severity in all symptoms.

A categorial transformation of total scores of the IRLS rating scale into severity levels has been introduced by the authors of the scale. The following ranges are used for severity categories: 0 = "none", 1 to 10 = "mild", 11 to 20 = "moderate", 21 to 30 = "severe", 31 to 40 = "very severe".

Table 3 shows the mean ± SD values of the IRLS total score on both groups of patients and under both treatment conditions:

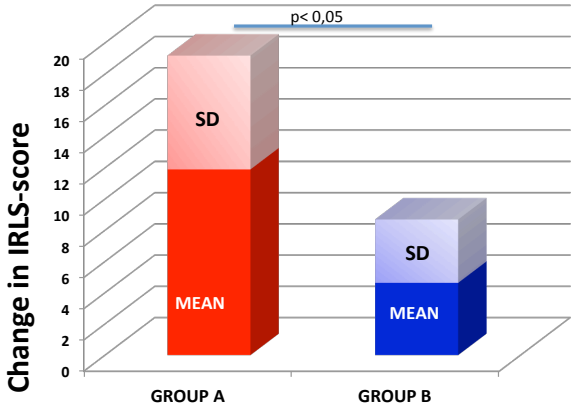
TABLE 3: IRLS mean (± SD) across treatments and groups

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*Z*	*p*	*Z*	*p*
Group A (DA-naïve)	GBPen	24,45	4,64	12,94	5,66	11,14	7,17	-3,71	0,0001	-2,005	0,045
Group A (DA-naïve)	PLB	21,85	8,23	21,22	8,71	20,51	8,92				
Group B (DA treated)	GBPen	24,40	6,26	17,52	5,93	14,27	5,88	-3,62	0,0001		
Group B (DA treated)	PLB	21,07	4,36	15,35	6,58	15,01	6,78				

Z: Mann-Whitney test

Figure 2 shows the mean (SD) change in placebo-corrected IRLS score for both groups of patients. As can be seen, treatment-naïve RLS patients improved significantly more during treatment with GBPen than the group of patients previously treated with dopaminergics.

Figure 2
IRLS: mean (±SD) PLB-corrected change



Summary:

- Both groups (A and B) showed on the IRLS scale during the two-week treatment period a greater improvement during with GBPen than with placebo.
- However, treatment-naïve patients (group A) had a larger response to GBPen (compared to PLB) than the group of patients previously treated with dopaminergics (F: 0,651; p<0.05).

b. Secondary endpoints

I. CGI

The CGI-S Scale was initially developed for a risk-benefit estimation within the treatment of mentally ill subjects. Nowadays, the four global scales (severity of illness, change in severity from baseline, therapeutic efficacy and tolerability of treatment) are used as different measures of

treatment outcome in different kinds of pharmacological studies. The CGI-S is considered also as a highly valid ("gold standard") outcome measure for evaluation of treatments in RLS subjects. Only the first ietm was used.

- CGI-Sitem 1 "severity" scores between "not at all ill" (1) to "extremely severe ill" (7).

Table 4 shows the mean ± SD values of the CGI score on both groups of patients and under both treatment conditions:

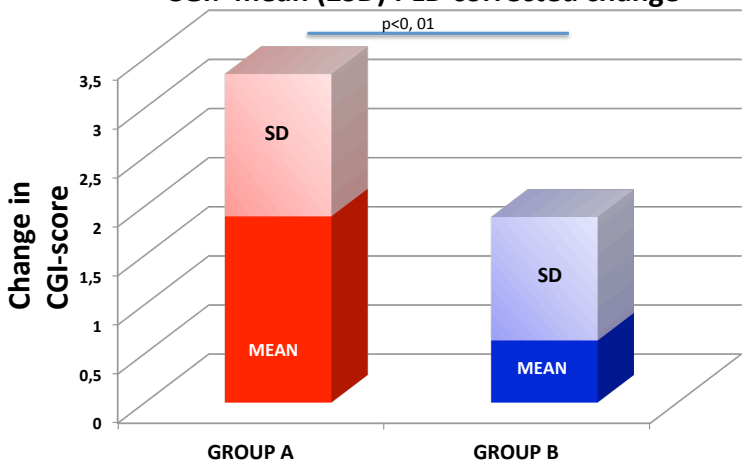
TABLE 4: CGI mean (± SD) across treatments and groups

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*Z*	*p*	*Z*	*p*
Group A (DA-naïve)	GBPen	5,00	0,93	5,13	0,83	4,75	1,28	-3,49	0,0001	-2,75	0,006
Group A (DA-naïve)	PLB	5,45	1,16	5,00	1,30	4,82	1,50				
Group B (DA treated)	GBPen	4,57	0,98	3,57	0,53	3,57	0,53	-2,04	0,041		
Group B (DA treated)	PLB	4,83	0,72	4,08	1,16	4,25	1,22				

Z: Mann-Whitney test.

Figure 3 shows the mean (SD) change in placebo-corrected CGI score for both groups of patients. As can be seen, treatment-naïve RLS patients improved significantly more during treatment with GBPen than the group of patients previously treated with dopaminergics.

Figure 3
CGI: mean (±SD) PLB-corrected change



Summary:

- Both groups (A and B) showed greater improvement with GBPen than with placebo on the CGI scale during the two-week treatment period.
- However, treatment-naïve patients (group A) had a greater response to GBPen (compared to PLB) than the group of patients previously treated with dopaminergics (F: -2,75; $p<0.01$).

II. RLS-6

The RLS-6 scale has been recently validated and is increasingly being used in clinical trials. Six 11-point scales with ranges between 0=not at all to 10=maximum are used to assess the severity of RLS in the course of treatment, they were include in diaries. These scales proved to be sensitive both for description of changes in severity during the study as well as for the

demonstration of differences between active treatment and placebo. The following four scales of the RLS-6 are designed to assess severity of RLS and be used at every visit:

- Severity of RLS at time falling asleep (item 2a)
- Severity of RLS during the night (item 2b)
- Severity of RLS during the day at rest (item 2c)
- Severity of RLS during the day when engaged in daytime activities. (item 2d)

Two further scales are added which cover sleep and daytime tiredness:

- Satisfaction with sleep (item 1)
- Severity of daytime tiredness/sleepiness (item 3)

Table 5 shows the mean-values (SD) of item 1 ("sleep satisfaction") across treatment and groups.

TABLE 5: RLS-6 mean (±SD) across treatments and groups
Item 1: Sleep Satisfaction

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*z*	*p*	*z*	*p*
Group A (DA-naïve)	GBPen	6,13	2,10	3,25	2,38	3,88	2,90	-1,82	0,069	-0,09	n.s.
Group A (DA-naïve)	PLB	5,73	3,30	5,45	2,67	5,45	2,75				
Group B (DA treated)	GBPen	3,57	2,44	2,00	1,15	7,00	2,00	-1,56	n.s.		
Group B (DA gtreated	PLB	7,08	2,23	4,83	2,37	5,50	2,54				

z: Mann-Whitney test

- As shown, the DA-treatment naïve group improved marginally more under GBPen than under PLB. No significant differences occurred between both treatment conditions in the DA treated group.
- The response to GBPen did not differ significantly between both groups of patients.

Table 6 shows the mean-values (SD) of item 2a ("RLS severity at bedtime") across treatment and groups.

TABLE 6: RLS-6 mean (±SD values) across treatments and groups
Item 2a: RLS at bedtime

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*t-value*	*p*	*t-value*	*p*
Group A (DA-naïve)	GBPen	2,38	2,07	1,88	2,36	3,25	2,87	-2,85	0,004	-0,075	n.s.
Group A (DA-naïve)	PLB	4,36	2,77	5,00	3,37	3,55	3,11				
Group B (DA treated)	GBPen	2,14	1,46	5,88	2,75	5,63	2,92	-1,21	n.s.		
Group B (DA gtreated	PLB	3,58	2,91	3,83	2,59	4,25	2,49				

Z: Mann-Whitney test

- As shown, the DA-treatment naïve group improved more under GBPen than under PLB. No significant differences occurred between both treatment conditions in the DA treated group.
- However, the response to GBPen did not differ significantly between both groups of patients.

Table 7 shows the mean-values (SD) of item 2b ("RLS severity during the night") across treatment and groups.

TABLE 7: RLS-6 mean (±SD values) across treatments and groups
Item 2b: RLS symptoms during the night

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*t-value*	*p*	*t-value*	*p*
Group A (DA-naïve)	GBPen	3,50	3,34	2,25	1,83	1,75	1,49	-1,82	0,069	-0,29	n.s.
Group A (DA-naïve)	PLB	4,36	4,03	3,27	3,25	4,18	3,22				
Group B (DA treated)	GBPen	2,00	1,63	2,29	1,80	4,50	3,30	-0,02	n.s.		
Group B (DA gtreated	PLB	3,67	2,99	3,75	2,73	3,17	2,69				

Z: Mann-Whitney test

- As shown, the DA-treatment naïve group showed a marginal improvement under GBPen compared to PLB. No significant differences occurred between both treatment conditions in the DA treated group.
- However, the response to GBPen did not differ significantly between both groups of patients.

Table 8 shows the mean-values (SD) of item 2c ("RLS severity during the day while resting") across treatment and groups.

TABLE 8: RLS-6 mean (±SD values) across treatments and groups
Item 2c: Daytime symptoms while resting

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*t-value*	*p*	*t-value*	*p*
Group A (DA-naïve)	GBPen	1,13	1,36	0,00	0,00	0,50	0,76	-1,3	n.s.	-0,29	n.s.
Group A (DA-naïve)	PLB	1,55	2,03	1,09	1,31	1,45	1,50				
Group B (DA treated)	GBPen	1,00	1,53	0,86	1,46	2,00	1,85	-1,3	n.s.		
Group B (DA treated)	PLB	1,58	2,31	1,08	1,56	0,67	1,23				

Z: Mann-Whitney test

- No significant differences occurred between both treatment conditions in either group.
- The response to GBPen did not differ significantly between both groups of patients.

Table 9 shows the mean-values (SD) of item 2d ("RLS severity during the day while active") across treatment and groups.

TABLE 9: RLS-6 mean (±SD values) across treatments and groups
Item 2d: Daytime symptoms while active

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*t-value*	*p*	*t-value*	*p*
Group A (DA-naïve)	GBPen	5,38	1,60	2,13	1,36	2,50	1,60	-1,45	n.s.	-0,04	n.s.
Group A (DA-naïve)	PLB	3,55	2,79	2,82	1,99	3,27	2,94				
Group B (DA treated)	GBPen	3,00	2,08	1,86	1,57	5,13	2,64	-0,089	n.s.		
Group B (DA gtreated	PLB	4,58	3,18	3,42	2,50	3,17	2,76				

Z: Mann-Whitney test.

- No significant differences occurred between both treatment conditions in either group.
- The response to GBPen did not differ significantly between both groups of patients.

Table 10 shows the mean-values (SD) of item 3 ("daytime sleepiness") across treatment and groups.

TABLE 10: RLS-6 mean (±SD values) across treatments and groups

Item 3: Daytime sleepiness

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*t-value*	*p*	*t-value*	*p*
Group A (DA-naïve)	GBPen	5,38	1,60	2,13	1,36	2,50	1,60	-1,45	n.s.	-0,04	n.s.
Group A (DA-naïve)	PLB	3,55	2,79	2,82	1,99	3,27	2,94				
Group B (DA treated)	GBPen	3,00	2,08	1,86	1,57	5,13	2,64	-0,089	n.s.		
Group B (DA treated)	PLB	4,58	3,18	3,42	2,50	3,17	2,76				

- No significant differences occurred between both treatment conditions in either group.
- The response to GBPen did not differ significantly between both groups of patients.

Summary of RLS-6:

- When compared to placebo, both groups (A and B) improved regarding RLS symptoms at bedtime and during the night. However, such improvement was statistically significant only in the DA-treatment naïve group.
- No statistically significant differences existed between the groups.

III. Pain-VAS scale

The Pain-VAS scale has not been validated and measures the severity of pain in the legs on a 0-10 continuous visual analogue scale.

8/19 (42%) patients reported pain at baseline in Group A, and 5/19 (26.3%) patients in Group B.

Table 11 shows the mean-values (SD) of the Pain-VAS scale across treatment and groups.

TABLE 11: Pain-VAS mean (±SD) across treatments and groups

		BL-mean	SD	Week 1	SD	Week 2	SD	GBPen vs PLB		Group A vs B	
								Z	*p*	*Z*	*p*
Group A (DA-naïve)	GBPen	3,38	3,89	1,13	2,10	1,38	1,41	-0,94	n.s.	-0,73	n.s.
Group A (DA-naïve)	PLB	3,73	3,78	1,45	2,20	2,82	2,99				
Group B (DA treated)	GBPen	3,57	4,47	1,14	1,21	1,86	2,61	-0,089	n.s.		
Group B (DA treated)	PLB	1,58	2,39	1,00	1,86	0,67	1,61				

Z: Mann-Whitney test

- No significant differences occurred between both treatment conditions in either group.
- The response to GBPen did not differ significantly between the groups of patients.

IV. Medical Outcomes Sleep (MOS) Scale

The MOS scale includes questions on subjective perception of sleep initiation, sleep maintenance, perceived sleep quality, daytime somnolence and sleep breathing disorders. As RLS affects sleep, this rating scale offers the opportunity to measure any improvements in sleep and thereby, of health as a result of treatment. The MOS scale has been used before in other large therapeutic studies in RLS. The MOS scale measures specific aspects of sleep in subjects that might have different simultaneous medical disorders, and is thus adequate for patient populations that are diverse from a medical point of view.

The following subscores have been evaluated:

- Sleep disturbance: Items involved: trouble falling asleep (item 7), sleep restlessless (item 3), awaken during sleep (item 8), time to fall asleep (item1).
- Sleep adequacy: Items involved: enough sleep, feel rested (item 4), amount sleep needed (item 12),

Table 12 shows the mean-values (SD) of subscore "sleep disturbance" across treatment and groups.

TABLE 12: MOS - scale mean(±SD) across treatments and groups. Subscore "sleep disturbance"

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*t-value*	*p*	*t-value*	*p*
Group A (DA-naïve)	GBPen	2,91	1,19	2,50	1,53	3,22	1,21	-2,48	0,01	-0,91	n.s.
Group A (DA-naïve)	PLB	2,93	1,28	3,34	1,10	2,95	1,45				
Group B (DA treated)	GBPen	2,54	1,05	2,04	0,90	3,07	1,14	-0,91	n.s.		
Group B (DA gtreated	PLB	2,94	1,64	3,63	1,27	3,06	1,08				

Z: Mann-Whitney test.

- As shown, the DA-treatment naïve group improved more under GBPen than under PLB. No significant differences occurred between both treatment conditions in the DA treated group.
- The response to GBPen did not differ significantly between both groups of patients.

Table 13 shows the mean-values (SD) of subscore "sleep adequacy" across treatment and groups.

TABLE 13: MOS - scale mean (±SD values) across treatments and groups. Subscore "sleep adequacy"

								GBPen vs PLB		Group A vs B	
		BL-mean	SD	Week 1	SD	Week 2	SD	*t-value*	*p*	*t-value*	*p*
Group A (DA-naïve)	GBPen	3,13	1,46	3,75	2,19	3,25	1,56	-0,91	n.s.	-0,27	n.s.
Group A (DA-naïve)	PLB	2,59	1,77	2,50	1,75	3,18	1,45				
Group B (DA treated)	GBPen	4,50	1,38	4,33	1,51	2,86	1,68	-1,12	n.s.		
Group B (DA gtreated	PLB	2,25	1,22	2,69	1,73	2,81	1,62				

Z: Mann-Whitney test.

- No significant differences occurred between both treatment conditions in either group.
- The response to GBPen did not differ significantly between the groups of patients.

Summary of MOS-scale:

No major differences occurred between groups on sleep disturbance or sleep adequacy.

c. Exploratory endpoints

- **Multiple Suggested Immovilization Test (m-SIT):**

The m-SIT[7] is a validated test that evaluates the severity of motor and subjective RLS/WED symptoms during the daytime and evening while the patient is awake and immobile. For this study we performed immobilization tests every two hours between 6PM and 12 midnight. Leg movements were measured by surface EMG monitoring of both *m. anterior tibialis*. Every 10 minutes the patients completed

a numerical symptom severity scale (m-SIT disturbance scale [m-SIT DS]) (range: 0-10).

- MSIT discomfort scale:

Table 14 shows the mean-values (SD) of the mSIT discomfort scale across treatment and groups.

TABLE 14: M-SIT dyscomfort scale: mean (±SD) across treatments and groups

										GBPen vs PLB		Group A vs B	
		6PM	SD	8PM	SD	10PM	SD	Midnight	SD	*t-value*	*p*	*t-value*	*p*
Group A (DA-naïve)	GBPen	6,47	8,25	7,10	9,05	9,84	12,40	12,05	19,57	-2,98	0,003	-2,66	0,008
Group A (DA-naïve)	PLB	12,78	16,4	11,06	14,53	12,53	16,07	21,42	21,94				
Group B (DA treated)	GBPen	7,10	9,15	8,36	9,02	10,94	10,18	13,72	10,43	-0,98	n.s.		
Group B (DA treated)	PLB	11,16	12,97	11,66	11,61	12,55	9,92	13,22	13,51				

Z: Mann-Whitney test.

All comparative statistics were perfomed upon the baseline (6PM)-corrected area under the curve (AUC) of all tests performed following the administration of the study medication at 7PM.

- As shown, the DA-treatment naïve group improved more under GBPen than under PLB. No significant differences occurred between both treatment conditions in the DA treated group.
- Overall, treatment-naïve patients (group A) had a larger response to GBPen (compared to PLB) than the group of patients previously treated with dopaminergics (F: -2,66; $p<0.01$).

Figure 4 shows the mean-values (SEM) of the mSIT discomfort scale across treatment, groups and time of day.

Fig. 4:
M-SIT dyscomfort scale: mean (±SEM) across treatments and groups

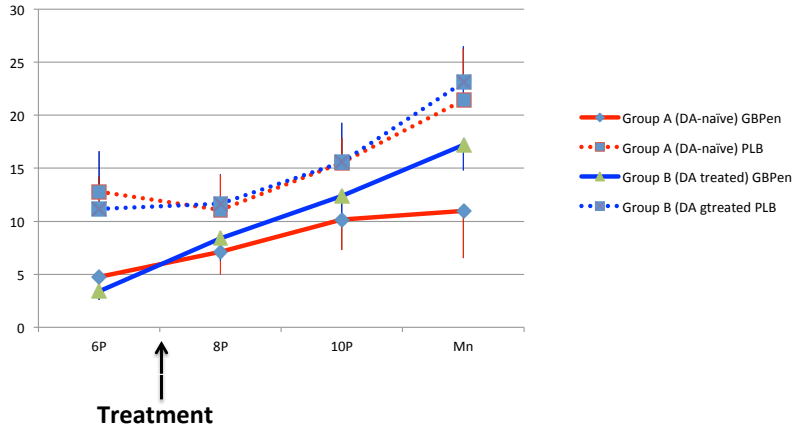
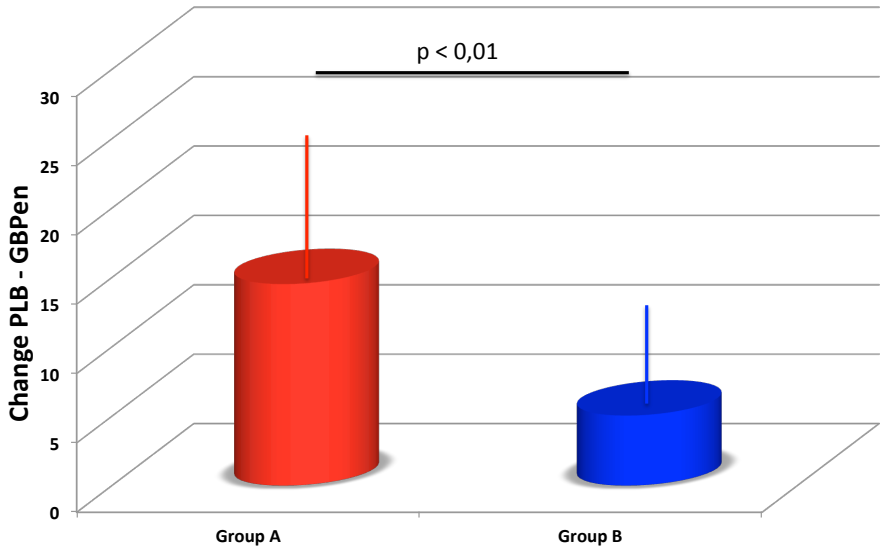


Figure 5 shows the mean (SEM) placebo-corrected change of the mSIT discomfort scale on both groups.

Figure 5
MSIT dyscomfort scale: mean (±SEM)
PLB-corrected change



- Patients in Group A responded significantly better than those on Group B on the mSIT discomfort scale.

- **PLMW-index obtained during mSIT:**

Table 15 shows the mean-values (SD) of the PLMW-index across treatments and groups.

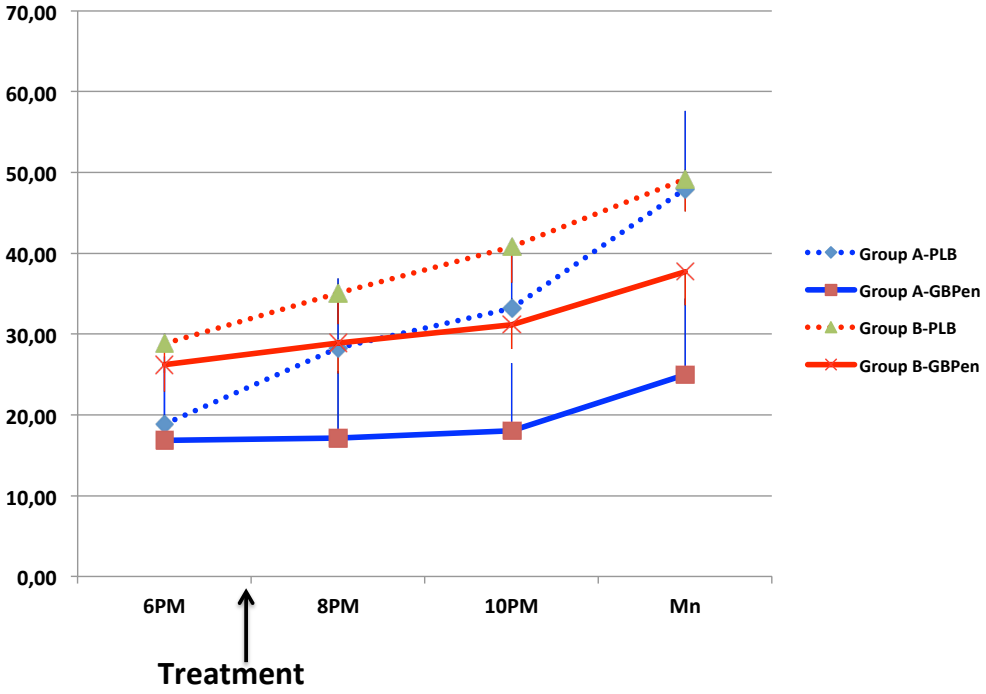
TABLE 15: PLMW mean (±SD values) across treatments and groups

		6PM	SD	8PM	SD	10PM	SD	Midnight	SD	GBPen vs PLB		Group A vs B	
										Z	*p*	*Z*	*p*
Group A (DA-naïve)	GBPen	18,84	36,56	28,26	37,51	33,18	37,55	47,95	42,19	-9,9	0,003	-1,96	0,064
Group A (DA-naïve)	PLB	16,84	16,40	17,11	14,54	18,05	16,07	24,94	21,94				
Group B (DA treated)	GBPen	28,78	3,48	35,06	9,03	40,78	10,18	49,17	10,43	-3,53	0,068		
Group B (DA treated)	PLB	26,21	12,98	28,89	11,61	31,17	9,92	37,72	13,52				

All comparative statistics were perfomed upon the baseline (6PM)-corrected area under the curve (AUC) of all tests performed following the administration of the study medication at 7PM

Fig. 6 shows the mean-values (SD) of the PLMW-index across treatments, groups and time of day:

Fig. 6:
PLMW-index: mean (±SEM) across treatments and groups



- PLMW responded in both groups better to GBPen than to PLB, although the response in group B was only marginally greater for GBPen compared to PLB.
- When comparing the placebo-corrected response in groups, the response to GBPen was marginally greater for patients in Group A compared to Group B.

Summary of mSIT:

Overall, dopaminergic treatment-naïve patients responded better to GBPen than those previously long-term treated with dopoaminergics. Such improvement in response

involved not only a greater response of dysesthesias to GBPen in the former group, but also of motor symptoms (PLMW).

4.Toxicity

Table 16 shows the incidence of side-effects for both treatment groups.

Table 16
Side-effects in both groups

	Group A					Group B			
Side effects	GBPen		PLB			GBPen		PLB	
	N	%	N	%		N	%	N	%
Drowsiness	3	15,79	2	10,53		6	31,58	2	10,53
Ataxia	3	15,79	3	15,79		3	15,79	2	10,53
Headache	3	15,79	2	10,53		1	5,26	1	5,26
Dry mouth	2	10,53	0	0		1	5,26	0	0
Dizziness	3	15,79	0	0		0	0	0	0
Rinitis	1	5,26	0	0		1	5,26	1	5,26
Hypertension	0	0	0	0		1	5,26	0	0
Fluid retention	1	5,26	0	0		1	5,26	0	0
Bronchitis	0	0	0	0		1	5,26	1	5,26
Acute gastroenteritis	0	0	0	0		1	5,26	1	5,26
Nausea	1	5,26	0	0		1	5,26	0	0
Gastroesophageal reflux	1	5,26	1	5,26		0	0	0	0
Stomach ache	0	0	0	0		1	5,26	1	5,26
Tachycardia	0	0	0	0		1	5,26	0	0
Conjuntivitis	1	5,26	1	5,26		0	0	0	0
Photopsies	0	0	1	5,26		0	0	0	0
Nightmares	0	0	1	5,26		0	0	0	0
Fainting	0	0	0	0		1	5,26	0	0
Cervical pain	0	0	0	0		0	0	1	5,26
Infection	0	0	0	0		1	5,26	1	5,26
Tinnitus	0	0	0	0		0	0	1	5,26
Feeling of satiety	1	5,26	0	0		0	0	0	0
Retroesternal pain	1	5,26	1	5,26		0	0	0	0
Hot flushes	1	5,26	1	5,26		0	0	0	0
Myocardial infarction	0	0	0	0		0	0	1	5,26
Leg cramps	0	0	0	0		0	0	1	5,26
TOTAL	22		13			21		14	

Both groups of patients suffered more side-effects during treatment with GBPen than during placebo, with drowsiness, ataxia (postural instability), headache, dry mouth, dizziness, occurring in more than 10% of the sample. Side effects were similarly distributed across both groups.

Table 17 shows the severity of side-effects under the active treatment in both groups.

Table 17
Severity of side-effects

	Group A			Group B	
	N	%		N	%
severe	2	10,53		1	5,26
moderate	7	36,84		8	42,10
mild	13	68,42		12	63,16
Total	22			21	

In 3 cases (7.89%), side effects became severe at least during one of the visits. One of these three patients suffered an upper airway infection, which was considered unrelated to the study medication. Another patient suffered form a severe headache before any study medication had been administered. Finally, a third patient suffered a myocardial infarct one day after the placebo condition had been completed (19 days after the administration of the last dose of GBPen), and was considered as unrelated to the study medication. *As can be seen on Table 17, the distribution of severity of side-effects was fairly similar across groups.*

Table 18 shows the number (%) of cases that suffered side-effects caused by the study medication.

Table 18
Percentage of side-effects attributed to study medication

	Group A			Group B	
	N	%		N	%
none	4	21,05		5	26,32
unlikely	3	15,79		3	15,79
possibly	5	26,32		5	26,32
most lilkely	10	52,63		8	42,11
definitely	0	0		0	0
Total	22			21	

As can be seen, no side-effects were definitely attributed to the study medication. However, in 18 cases, side-effects were most likely attributed by the investigator to the study medication. *There were no major relevant differences in the distribution of side-effects across both groups*.

CONCLUSIONS:

1. Both gorups of patients improved more following a two-week treatment with GBPen administered at 600 mg/day, than under placebo. This could be clearly observed on the IRLS scale (main endpoint), CGI, and on the sleep laboratory testing with mSIT. These results were replicated, although only partially, on some of the items of the RLS-6 scale (Tables 3, 4, 6, 7, 14, 15).
2. The response to GBPen was comparatively lower for the group of patients who had been previously long-term treated with dopaminergic agents (Tables 3, 4, 14, 15) (main hypothesis)
3. The difference between both groups of patients in the magnitude of the response to GBPen was not due to differences in:

- Age or gender (Table 2)
- Serum ferritin levels (Table 2)
- Duration of illness (Table 2)
- Severity of illness at baseline (Table 2)
- Previous existence of augmentation (exclusion criterion #2)
- Concomittant conditions at baseline (Table 2)
- Differences in the effects of GBPen on sleep (Tables 12, 13, and 14) or pain (Table 11) between both groups.
- Differences in toxicity between both groups (Tables 16-18)

4. These results support the notion that previous long-term treatment with dopaminergic agents reduces, not just the response to any other dopaminergic drugs, but also to alpha-2 delta agents.
5. Possible explanations for this loss of response are:
    - Included patients never met criteria for augmentation, but might well have been already suffering from initial changes in dopaminergic function as a result of the long-term exposure to dopaminergic medication. Such latent dopaminergic dysfunction would prepare the ground for future episodes of augmentation, assuming dopaminergic treatment is maintained.
    - Given the short period of treatment, it is possible that, for the group previously treated with dopaminergics, the response to GBPen might recover after a given period of time, and transform into a full response, similar to the treatment-naïve group.

6. These results also have far-reaching clinical implications: In order to preserve a full response of symptoms to medication, this study supports the notion that initial treatment for RLS should be started with non-dopaminergic medications, something in line with the most current treatment recommendations of the IRLSSG expert group (Garcia-Borreguero et al., 2016).

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