The α7 nicotinic receptor agonist ABT-107 decreases L-dopa-induced dyskinesias in parkinsonian monkeys

Danhui Zhang, Matthew McGregor, Michael W. Decker and Maryka Quik

Center for Health Sciences, SRI International, Menlo Park, California 94025, USA (D.Z., M.M., M.Q.); AbbVie, Inc, North Waukegan Road, North Chicago, IL 60064, USA (M.W.D.)
Running Title: α7 nicotinic receptor drugs and dyskinesias

Corresponding author: Address correspondence and reprint request to Maryka Quik, Center for Health Sciences, SRI International, 333 Ravenswood Ave, California 94025, USA. Tel. 1-650-859-6112; fax 1-650-859-5099. E-mail address: maryka.quik@sri.com

Number of:
pages of text, 30
tables, 2;
figures, 6;
references, 48

Number of words in:
abstract, 249
introduction, 509
discussion, 1278

ABBREVIATIONS: ABT-107, 5-((3R)-1-azabicyclo[2.2.2]oct-3-yloxy) pyridazin-3-yl)-1H-indole; ABT-894, (3-(5,6-dichloro-pyridin-3-yl)-1(S),5 (S)-3,6-diazabicyclo[3.2.0]heptane.
ANOVA, analysis of variance; LIDs, L-dopa-induced dyskinesias; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; nACHR, nicotinic acetylcholine receptor; *, the asterisk indicates the possible presence of other subunits in the nicotinic receptor complex;

Recommended section: Neuropharmacology
ABSTRACT

Previous studies in parkinsonian rats and monkeys had shown that β2 selective nicotinic acetylcholine receptor (nAChR) agonists reduce L-dopa-induced dyskinesias (LIDs), a serious complication of L-dopa therapy for Parkinson's disease. Since rodent studies also suggested an involvement of α7 nAChRs in LIDs, we tested the effect of the potent, selective α7 agonist ABT-107. MPTP-lesioned monkeys were gavaged with L-dopa/carbidopa (10 and 2.5 mg/kg, respectively) twice daily, which resulted in stable LIDs. A dose response study (0.03-1.0 mg/kg) showed that oral ABT-107 decreased LIDs by 40-60%. LIDs returned to control levels only after a 6-wk ABT-107 washout, suggesting long term molecular changes were involved. Subsequent re-administration of ABT-107 decreased LIDs by 50-60%, indicating tolerance did not develop. There was no effect of ABT-107 on parkinsonism or cognitive performance. We next tested ABT-107 together with the β2 agonist ABT-894, previously shown to reduce LIDs in parkinsonian monkeys. In one study, the monkeys were first given oral ABT-894 (0.01 mg/kg), which maximally decreased LIDs by 50-60%; they were then also treated with 0.1 mg/kg ABT-107, a dose that maximally reduced LIDs. The effect of combined treatment on LIDs was similar to that with either drug alone. Comparable results were observed in a group of monkeys first treated with ABT-107 and then also given ABT-894. Thus, α7 and β2 nAChR selective drugs may function via a final common mechanism to reduce LIDs. The present results suggest that drugs targeting either α7 or β2 nAChRs may be useful as antidyskinetic agents in Parkinson's disease.
Introduction

A critical unmet need for Parkinson's disease management is a strategy to reduce L-dopa-induced dyskinesias (LIDs). These abnormal involuntary movements are a serious debilitating side effect of L-dopa therapy, the gold-standard treatment for Parkinson's disease, and develop in most patients with continued L-dopa use (Obeso et al., 2010; Meissner et al., 2011; Schapira and Jenner, 2011; Wichmann et al., 2011; Iravani et al., 2012; Huot et al., 2013). Currently the only drug approved for LIDs is amantadine, and it is of only limited effectiveness. There is therefore an ongoing search for new therapies. Drugs targeting numerous classes of neurotransmitter receptors, including glutamatergic, serotonergic, cholinergic and others, reduce LIDs in animal models, suggesting that multiple mechanisms are involved (Fox et al., 2009; Brotchie and Jenner, 2011; Blandini and Armentero, 2012; Duty, 2012; Sgambato-Faure and Cenci, 2012; Huot et al., 2013).

More recent studies also indicate the nicotinic cholinergic system is involved in LIDs. Studies in mice, rats and monkeys showed that the general nAChR agonist nicotine consistently reduced LIDs in a dose dependent manner (Quik et al., 2007; Bordia et al., 2008; Huang et al., 2011a; Quik et al., 2013d). Tolerance did not arise to the antidyskinetic effect of nicotine, even with months of treatment. Moreover, nicotine reduced LIDs whether administered prior to L-dopa treatment or once LIDs developed.

Nicotine generally exerts its effect by acting at nAChRs of which there are multiple subtypes in both the peripheral and central nervous system. These include the αβ1*, αβ4* and α7 nAChRs in the periphery, with the αβ2*, αβ2* and α7 nAChRs being the primary ones in the brain (Millar and Gotti, 2009; Quik and Wonnacott, 2011). The asterisk denotes the possible presence of other nAChR subunits in the receptor. Studies with α4, α6, α7 and β2 nAChR

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subunits null mutant mice suggest that $\alpha 4\beta 2^*$, $\alpha 6\beta 2^*$ and $\alpha 7$ nAChRs all influence the occurrence of LIDs (Quik et al., 2013b). These findings suggest that drugs targeting $\beta 2^*$ and/or $\alpha 7$ nAChRs may yield therapies to reduce LIDs. In fact, pharmacological studies show that several $\beta 2^*$ nAChR agonists reduce LIDs in both rat and monkey models of LIDs (Huang et al., 2011b; Johnston et al., 2013; Quik et al., 2013a; Zhang et al., 2013a; Zhang et al., 2014).

In the present paper, we tested the effect of the $\alpha 7$ agonist ABT-107 since studies with $\alpha 7$ nAChR null mutant mice had indicated an involvement of $\alpha 7$ nAChRs in LIDs. We selected ABT-107 because of its potency and selectivity for $\alpha 7$ nAChRs over other available drugs (Briggs et al., 1997; Malysz et al., 2010), and because $\alpha 7$ nAChR drugs have excellent safety and tolerability profiles in clinical studies (Freedman et al., 2008; Othman et al., 2011). The results show that ABT-107 reduced LIDs ~50% with no worsening of parkinsonism and no observable side effects. We also tested ABT-107 in combination with an $\beta 2^*$ nAChR agonist ABT-894, with the drug combination yielding similar declines in LIDs as treatment with either drug along. These data suggest that drugs targeting either $\alpha 7$ or $\beta 2^*$ nAChRs have potential as antidyskinetic agents.
Materials and Methods

Animals. Squirrel monkeys (21, Saimiri sciureus) >5 years of age of either sex were obtained from World Wide Primates, Miami, FL. As required by California state guidelines, they were quarantined for 30 days upon arrival. Food consisted of monkey chow, fruits and vegetables, with water freely provided. The monkeys were singly housed in a humidity and temperature controlled room on a 12 h light/dark cycle. All studies were approved by the Institutional Animal Care and Use Committee, and were done according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Induction of LIDs. The monkeys were injected subcutaneously with 2.0 mg/kg 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Sigma-Aldrich, St. Louis, MO) dissolved in saline to render them parkinsonian (Quik et al., 2007). After a 3-4 wk recovery period from the systemic effects of MPTP injection, the monkeys were rated for parkinsonism. Parkinsonism was assessed by evaluating spatial hypokinesia, body bradykinesia, left and right manual dexterity, balance at rest and during movement, freezing during movement and action tremor, with each category rated from 0 (normal) to 4 (severely parkinsonian), as described (Quik et al., 2007; Quik et al., 2013d). The maximum parkinsonian score is 28; however, individual scores are considerably less because parkinsonism is variably expressed in the monkeys. Any one monkey may have deficits in manual dexterity, but exhibit no freezing or tremor and move well, while another monkey may exhibit freezing and tremor but have no motor deficits. If the monkeys were not parkinsonian, MPTP injection was repeated at 1.9 mg/kg. Parkinsonism was rated once weekly on Fridays throughout the study, before and 90 min after L-dopa administration.

Monkeys were then gavaged with L-dopa (10 mg/kg) and carbidopa (2.5 mg/kg) twice a day at 8:30 AM and 12:30 PM from Monday to Friday inclusive (Quik et al., 2007; Quik et al.,...
Baseline LIDs were recorded from 8:00-8:30 AM. The monkeys were then gavaged with L-dopa and recording continued. The monkeys were given only fruits and vegetables in the morning to allow for optimal L-dopa absorption, with more fruits/vegetables and monkey chow provided at ~3 PM. The LID scores provided in the Figs represent the ratings averaged over the morning L-dopa dosing period for Wednesday and Thursday of each week. LIDs were rated for a 1 min period by a blinded rater from video recordings at the times depicted in the figures. Dyskinesias were assessed on a scale of 0 (no dyskinesias) to 4 (severe dyskinesias) using the following criteria: 1 = subtle dyskinesias that were not sustained (< 3 trunk movements in a row); 2 = sustained dyskinesias (≥ 3 trunk movements in a row); 3 = moderate dyskinesias that impaired the ability to remain stationary; and 4 = severe dyskinesias that were generalized and incapacitating (Tan et al., 2002; Quik et al., 2007; Quik et al., 2013d).

**nAChR drug treatments.** The monkeys had previously been treated with other nAChR agonists, followed by a 1 month washout period before initiating the current study. LID scores were at vehicle-treated levels for all monkeys groups at the start of the drug treatment regimens. The groups were as follows: a vehicle-treated group (n = 6), a nicotine-treated (n = 5) group, a group of monkeys first treated with ABT-107 and subsequently also given ABT-894 (n = 5), and a group first treated with ABT-894 and then also administered ABT-107 (n = 5). There were similar number of males and females in each group.

All monkeys received 50% diluted orange Gatorade in the drinking water as a vehicle control for nicotine treatment. The presence of the Gatorade was necessary to mask the bitter taste of nicotine ((-)-nicotine, free base; Sigma-Aldrich, St. Louis, MO) in the nicotine-treated group. The dose of nicotine was started at 50 µg/ml for 2-3 days, increased to 150 µg/ml for a further 3-4 days and then to 300 µg/ml nicotine at which it was maintained for the remainder of the study,
as described (Quik et al., 2006; Quik et al., 2013d; Zhang et al., 2013a). Gatorade with and without nicotine was also added to the dry monkey chow. This nicotine dosing regimen yielded monkey plasma nicotine and cotinine levels similar to those in moderate smokers (Quik et al., 2006; Matta et al., 2007). ABT-107 or ABT-894 was administered orally 5 days per week via a small cracker 30 min before L-dopa gavage. Each drug was applied to a small cracker in a 30-40 µl aliquot of water (depending on monkey weight) and immediately given to the monkeys. The drug had no effect on body weight; no adverse effects were noted on behavior.

**Cognitive testing.** Since nAChR drugs are known to modulate cognitive abilities, we evaluated the effect of ABT-107 on cognition. We used an object retrieval task previously used for squirrel monkeys that measures a component of prefrontal cortex-dependent cognitive control involving inhibition of an initial learned response after subsequent spatial reversals (Lyons et al., 2000; Lyons et al., 2004; Zhang et al., 2013a). The monkeys were scored for the average time and number of trials required to retrieve the marshmallow piece within a 30 sec period (Lyons et al., 2000; Lyons and Schatzberg, 2003). Cognition was evaluated once weekly on Monday.

**Data analyses.** Statistical comparisons were done using GraphPad Prism (GraphPad Software Co., San Diego, CA, USA). Total dyskinesias were determined by evaluating the area under the curve (AUC), with data analyzed two-way analysis of variance (ANOVA) followed by a Bonferroni *post hoc* test. The data are expressed as % vehicle, that is, based on the mean of the vehicle-treated group for the specific day. Differences in parkinsonian scores, which represent the total of several behavioral parameters, were determined using a t-test. The results are the mean ± SEM of the indicated number of monkeys. Differences in LID scores between groups of monkeys were determined using nonparametric tests (Mann-Whitney test) since the scores are integral values, with the median shown. *P* ≤ 0.05 was considered significant.
Results

The $\alpha_7$ agonist ABT-107 reduces established LIDs. Monkeys were treated with varying doses of ABT-107 as depicted in the time line (Fig. 1 top panel). ABT-107 (0.03 to 1.0 mg/kg) was given orally in a cracker 30 min prior to L-dopa. The results (Fig. 1 middle panel) show that ABT-107 significantly reduced LIDs by 40% at the 0.03 mg/kg dose, and maximally by 50% at the 0.10 mg/dose. The effect of nicotine on LIDs is shown for comparison (Fig. 1 bottom panel). Nicotine significantly reduced LIDs by 50-70%. Both ABT-107 and nicotine treatment reduced LIDs throughout the 4 h time course compared to vehicle, with the median scores shown (Fig. 2). The dyskinesia scores in the vehicle-treated monkeys (Fig. 2) ranged from 1.0-2.0; thus the monkeys were moderately dyskinetic. The dyskinesia scores decreased to 0-1.0 with drug treatment (Fig. 2). After the 4 week drug treatment regimen, ABT-107 and nicotine were discontinued. The results in Fig. 3 show that LIDs only returned to vehicle-treated levels by 6 wk of either ABT-107 or nicotine washout.

Combined effect of the $\alpha_7$ agonist ABT-107 and the $\beta_2^*$ nAChR agonist ABT-894 on LIDs. Our previous studies had shown that the $\beta_2$ nAChR agonist ABT-894 reduced LIDs by 60% similar to the current results with $\alpha_7$ agonist ABT-107 (Zhang et al., 2014). This raised the question whether combined treatment with the two different classes of nAChR agonists may yield a greater decline in LIDs than either drug alone. To evaluate this possibility monkeys were treated with both drugs in combination as follows. One set of monkeys (n = 5) was first treated with 0.10 mg/kg ABT-107, a dose that maximally decreases LIDs (Fig. 4A). The monkeys were given ABT-107 orally for 5 wk to ensure the drug effect had plateaued. A 50-60% decline in LIDs was obtained throughout the entire period. At week 6, the monkeys were also orally given ABT-894 at a dose that optimally reduced LIDs (0.01 mg/kg) (Zhang et al., 2014). The decline in LIDs
with combined ABT-107 plus ABT-894 treatment was similar to that observed with ABT-107 alone (Fig. 4A).

Another set of monkeys (Fig. 4B) was first given ABT-894 orally at 0.01 mg/kg, a dose that optimally reduced LIDs (Zhang et al., 2014). The decline in LIDs plateaued by 5 wk of ABT-894 treatment. At wk 6 the monkeys were also administered ABT-107 at 0.10 mg/kg (Fig. 4B). No further decline in LIDs was observed with ABT-894 and ABT-107 in combination as compared to single drug treatment. Thus similar results were obtained regardless of the order of treatment of ABT-107 and ABT-894.

The effect of nicotine administration on LIDs in another set of monkeys, treated at the same time as in the studies above, is shown in the bottom panel for comparison (Fig. 4C). A 50-60% decline in LIDs was observed throughout.

The effect of ABT-107, ABT-894, the two drugs in combination, and nicotine on the hourly time course of LIDs is shown in Fig. 5. The average dyskinesia scores in the vehicle-treated monkeys ranged from 1.0-2.0 and in the drug-treated monkeys from 0-1.5, with the median score shown. The decline in LIDs was similar with all treatments (Fig. 5). The effect of drug washout is shown in Fig. 6. The time required for LIDs to return to vehicle-treated levels with the ABT-107 and ABT-894 drug combination was similar to that for the drugs alone (see Fig. 3).

Effect of the α7 agonist ABT-107 and the β2* nAChR agonist ABT-894 on parkinsonism. Vehicle-treated monkeys were moderately parkinsonian. ABT-107 did not significantly affect parkinsonism either on or off L-dopa at any time point compared to vehicle (Table 1). The scores shown are for the last 4 wk of treatment, with similar results throughout the study. In addition, ABT-107 had no effect on cognitive performance at any dose tested (Table 2).
Discussion

The present results are the first to show that an \( \alpha_7 \) nAChR agonist reduces LIDs in a parkinsonian monkeys, a model that exhibits many features reminiscent of the dyskinesias that occur in L-dopa-treated Parkinson's disease patients. Notably, the ABT-107-induced decrease in LIDs persisted over several months of treatment, indicating tolerance does not develop. The effect of the drug was fairly long-lasting, with 6 wk of washout required before LIDs returned to values similar to those in vehicle-treated monkeys.

The above observations suggest that \( \alpha_7 \) nAChR agonists may represent a promising class of drugs for the treatment of LIDs in Parkinson's disease patients. ABT-107 offers the advantage over other \( \alpha_7 \) nAChR agonists such as DMXB, as the former drug is a potent and selective \( \alpha_7 \) nAChR agonist. As well, \( \alpha_7 \) nAChR drugs have been tested in healthy human volunteers and in schizophrenic patients, and were shown to be safe and well-tolerated. Side effects were similar to those with placebo treatment (Freedman et al., 2008; Othman et al., 2011), despite the presence of \( \alpha_7 \) nAChRs in the peripheral nervous system. These characteristics suggest that \( \alpha_7 \) nAChR drugs may represent good candidates for further development for the treatment of LIDs in Parkinson's disease patients.

Our previous data had shown that \( \beta_2^* \) nAChR agonists reduced LIDs in parkinsonian monkeys to a similar extent as the \( \alpha_7 \) agonist ABT-107 in the current study. Varenicline, which acts at \( \beta_2^* \) and other nAChR subtypes, as well as \( \beta_2^* \) selective drugs including A-85380, sazetidine, TC-2696, TI-10165, TC-8831 and TC-10600 all reduced LIDs to varying extents in rats with a unilateral 6-hydroxydopamine lesion (Huang et al., 2011b; Quik et al., 2013a). Additionally, varenicline and TC-8831 decreased LIDs ~50% in nonhuman primates with no tolerance, although a limitation of these drugs was the development of emesis (Johnston et al.,
2013; Zhang et al., 2013a). We more recently tested two β2* nAChR agonists that had previously been evaluated in phase 2 clinical trials for other indications (Zhang et al., 2014). These included ABT-089, a partial agonist (Decker et al., 1997; Sullivan et al., 1997; Anderson et al., 2009; Marks et al., 2009; Apostol et al., 2012), as well as a full agonist ABT-894 (Ji et al., 2007; Rowbotham et al., 2012; Bain et al., 2013). ABT-089 maximally decreased LIDs by 40% whereas ABT-894 reduced LIDs up to 60% in nonhuman primates (Zhang et al., 2014). None of the agonists worsened parkinsonism, and there were no detectable side effects and no emesis (Zhang et al., 2014). Moreover, the effects of both the β2* nAChR drug ABT-894, the α7 nAChR drug ABT-107, as well as nicotine, persisted for months with treatment and, in addition, remained for several weeks after drug continuation.

α7 nAChRs are functionally and pharmacologically distinct from β2* nAChRs. They exhibit a greater calcium conductance and faster desensitization, and have a more direct impact on glutamatergic signaling (Albuquerque et al., 2009; Quik and Wonnacott, 2011). These differential characteristics raised the question whether combination α7 and β2* nAChR drug treatment may yield a greater decline in LIDs. However, when ABT-107 was tested together with the β2* nAChR agonist ABT-894, the improvement in LIDs was comparable to that with either drug alone. In addition, it was similar to the effect of nicotine, a general agonist that acts at both α7 and β2* nAChRs, although with lower affinity for the α7 subtype.

The finding that the β2* and α7 nAChR agonists both reduce LIDs by ~60% but show no significant additive effect with combined treatments suggests that these classes of drugs either reduce LIDs by a common mechanism or decrease LIDs by affecting different mechanism(s), which ultimately converge to produce parallel downstream changes. A key brain region involved in the generation of LIDs is the striatum, which expresses both β2 and α7 nAChRs. Although not
at high density (Quik et al., 2000), α7 nAChRs are located on glutamatergic afferents from the cortex to the striatum to regulate dopamine release (Kaiser and Wonnacott, 2000). In addition, α7 nAChRs are present in the substantia nigra to control striatal dopaminergic function (Quik et al., 2000). β2* nAChR receptors are also expressed in the striatum on striatal dopamine nerve terminals as well as other neuronal elements. Lesion studies suggest that the β2* nAChRs on the dopamine nerve terminals are important for the nAChR-mediated reduction in LIDs because nicotine and nAChR drugs most effectively reduced LIDs in parkinsonian animals with moderate nigrostriatal damage (Huang et al., 2011a; Huang et al., 2011b; Quik et al., 2013c). Thus, a common mechanisms by which α7 and β2* nAChR drugs act to reduce LIDs may involve a reduction in striatal dopamine release. Further evidence for this suggestions stems from studies showing that long term nicotine treatment reduces dopamine release from striatal synaptosomes (Bordia et al., 2013).

In addition to the nigrostriatal dopaminergic system, other neuronal pathways also appear to be involved in the nAChR-mediated decline in LIDs. Evidence for this idea stems from studies showing that nicotine and β2* nAChR drugs still diminished LIDs up to 30% in rats with severe nigrostriatal damage. Possible candidates include nAChRs on nondopaminergic striatal neurons (GABAergic or cholinergic) or nAChRs in other brain regions (Huang et al., 2011b; Quik et al., 2013e). The effects of α7 nAChR drugs may also be mediated by α7 nAChRs in cortex, thalamus, cerebellum and other brain regions linked to motor control, since there is relatively high expression of α7 nAChRs in these regions (Quik et al., 2000).

With respect to the signaling pathways affected by nAChR drugs, activation of the nicotinic acetylcholine system has been linked to alterations in cAMP-linked intracellular signaling and in extracellular signal-regulated kinases (ERK) (Ding et al., 2011; Feyder et al., 2011; Kawamata
and Shimohama, 2011). Changes in the cAMP signaling cascade including activation of cAMP, increased activity of the cAMP-dependent protein kinase (PKA) and of the dopamine- and cAMP-dependent phosphoprotein of 32 kDa (DARPP-32) have also been implicated in LIDs (Santini et al., 2007; Calabresi et al., 2008; Rangel-Barajas et al., 2010; Santini et al., 2010; Feyder et al., 2011). As well, LIDs are associated with enhanced activity of ERK (Santini et al., 2010; Feyder et al., 2011; Wang et al., 2012). These mechanisms and others may thus contribute to the nAChR-mediated reduction in LIDs.

The ability of ABT-894, ABT-107 and nicotine to reduce LIDs weeks after drug discontinuation suggests that they most likely induce their effect via long-lasting molecular adaptations. Indeed, long-term changes in gene-expression, intracellular signaling pathways, neuronal plasticity and morphology have all been linked to the expression of LIDs (Santini et al., 2009; Cenci and Konradi, 2010; Feyder et al., 2011; Huot et al., 2011; Rangel-Barajas et al., 2011; Zhang et al., 2013b). Moreover, these changes may occur via numerous neurotransmitter systems such as the dopaminergic, glutamatergic, serotonergic and others, which are all implicated in the development and maintenance of LIDs (Carta and Bezard, 2011; Huot et al., 2011; Blandini and Armentero, 2012; Duty, 2012; Rylander, 2012; Huot et al., 2013).

$\alpha_7$ nAChR agonists offer the advantage that they have also been linked to improvements in cognition in numerous animal models (Levin, 2012; Lendvai et al., 2013) and in various neurological/psychiatric disorders (Geerts, 2012; Lieberman et al., 2013). These latter observations suggest that $\alpha_7$ nAChR drugs may also be helpful for the memory deficits with Parkinson's disease, in addition to improving LIDs.

In summary, the present data show that the $\alpha_7$ nAChR agonist ABT-107 reduces LIDs in parkinsonian monkeys, without affecting parkinsonism. Our previous work showed similar
declines in LIDs with the β2* agonist ABT-894. These data suggest that development of subtype-selective nAChR drugs may not only offer greater specificity with reduced side-effect profiles, but also provide multiple therapeutic options.

Authorship Contributions

Participated in research design: Quik, Zhang, Decker
Conducted experiments: Zhang, McGregor
Contributed new agents or analytical tools: Decker
Performed data analysis: Zhang, McGregor, Quik
Wrote or contributed to the writing of the manuscript: Quik, Zhang, McGregor
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cyclase type V/VI and increased GABA release in the substantia nigra reticulata. 

*Neurobiol Dis* **41**:51-61.


Footnotes

This work was supported by the National Institutes of Health [Grants NS 59910, NS65851]. The authors thank Tanuja Bordia and Xiomara Perez for helpful comments concerning the manuscript. ABT-107 and ABT-894 were provided by AbbVie, Inc.

Request reprints to Maryka Quik, 333 Ravenswood Avenue, Menlo Park, CA 94025. E-mail address: maryka.quik@sri.com
Figure Legends

**Fig. 1.** The α7 agonist ABT-107 decreases LIDs in MPTP-lesioned monkeys. L-dopa (10 mg/kg) plus carbidopa (2.5 mg/kg) was administered at 8:30 AM and 12:30 PM 5 days per wk for 4 wk, with ABT-107 given orally 30 min prior to each L-dopa gavage. The overall treatment timeline is shown in the top panel. The effect of increasing doses of ABT-107 (0.03 to 1.0 mg/kg) on total dyskinesia scores (expressed as % vehicle) is provided in the middle panel. Any one dose of ABT-107 was given for an entire wk, with bars depicting the average score over 2 days. The bottom panel depicts the effect of nicotine (300 µg/ml in the drinking water) on LIDs in a separate group of monkeys. Values represent the mean ± SEM of 5-6 monkeys. Significance of difference from vehicle treatment, ***p < 0.001 using two-way ANOVA followed by a Bonferroni post hoc test.

**Fig. 2.** ABT-107 administration reduces the hourly time course of LIDs. The monkeys were treated with ABT-107 as outlined in Fig. 1. The data shown in the top panel were obtained using 0.1 mg/kg ABT-107, a dose that results in a maximal decline in LIDs. The effect of nicotine administration is shown in the bottom panel. The symbols depict the median of 5-6 monkeys. Significance of difference from vehicle using a Mann-Whitney test, **p < 0.01 ***p < 0.001.

**Fig. 3.** ABT-107 discontinuation leads to a return of LIDs to vehicle-treated levels. ABT-107 treatment to L-dopa-treated MPTP-lesioned monkeys (Fig. 1) was discontinued and effects on LIDs determined. After 6 wk of washout, LIDs were similar to those in vehicle-treated monkeys. The values depict the weekly average LID scores. Values are mean ± SEM of the number of monkeys indicated in parenthesis. Significance of difference from vehicle, *p < 0.05, **p < 0.01,
***p < 0.001 using two-way ANOVA followed by a Bonferroni post hoc test.

**Fig. 4.** Effect of ABT-107 in combination with ABT-894 on LIDs in MPTP-lesioned monkeys. After the nAChR drug washout shown in Fig. 3, the monkeys were re-administered ABT-107 (0.10 mg/kg) orally 30 min prior to L-dopa gavage (A). L-dopa (10 mg/kg) plus carbidopa (2.5 mg/kg) was administered at 8:30 AM and 12:30 PM 5 days per wk. After 5 wk of ABT-107 administration, at which point its antidyskinetic effect was stable, the monkeys were also given the β2* nAChR agonist ABT-894 (0.01 mg/kg). The decline in LIDs with combined ABT-107 and ABT-894 was similar to that observed with ABT-107 alone. In (B), another set of L-dopa-treated parkinsonian monkeys was first administered ABT-894 (0.01 mg/kg) until its antidyskinetic effect had stabilized (5 wk). They were subsequently also given ABT-107 (0.10 mg/kg). The decrease in LIDs resembled that seen in the monkeys treated only with ABT-894. Thus, combined drug treatment yielded a similar decline in LIDs as the use of either nAChR agonist alone. The effect of nicotine treatment in another set of monkeys is shown for comparison (C). Values are the mean ± SEM of 5-6 monkeys. Significance of difference of drug treatment from vehicle, *p < 0.05, **p < 0.01, ***p < 0.001 using two-way ANOVA followed by a Bonferroni post hoc test.

**Fig. 5.** Effect of ABT-107 in combination with ABT-894 on the hourly time course of LIDs. The monkeys were treated with ABT-107, ABT-894 or the drugs in combination as outlined in Fig. 4. The data shown for ABT-107 (A, left panel) is from wk 4 and that for the combined ABT-107 plus ABT-894 data (A, right panel) from wk 8, with similar results for the other weeks. The data shown for ABT-894 (B, left panel) is from wk 4 and that for the combined ABT-894 plus ABT-
107 data (B, right panel) from wk 8, with similar results for the other weeks. The effect of nicotine on another set of monkeys for the corresponding time period is shown in the lower panels (C). The symbols depict the median of 5-6 monkeys. Significance of difference from vehicle using a Mann-Whitney test, **$p < 0.01$.

**Fig. 6.** Discontinuation of combined ABT-107 and ABT-894 treatments led to a return of LIDs to levels in vehicle-treated monkeys. The combination ABT-107 and ABT-894 drug treatment regimen is depicted in Fig. 4. After drug discontinuation, LIDs were similar to those in vehicle-treated monkeys by wk 5 to 7. The values shown are the weekly average LID scores. Results are mean ± SEM of the number of monkeys indicated in parenthesis. Significance of difference from vehicle, **$p < 0.01$, ***$p < 0.001$ using two-way ANOVA followed by a Bonferroni post hoc test.
TABLE 1

No effect of single or multiple nAChR drug treatments on parkinsonism assessed before or after L-dopa treatment

nAChR drugs were administered orally in cracker 60 min before gavage with L-dopa (10 mg/kg)/carbidopa (2.5 mg/kg). Parkinsonism was measured 45 min before L-dopa and again 90 min after L-dopa administration once weekly. Drug doses were; 300 µg/ml nicotine in the drinking water, 0.1 mg/kg ABT-107 orally, 0.01 mg/kg ABT-894 orally, with the latter two drugs given on their own or in combination (see timelines in Fig. 4). For the nAChR drug combination studies, the drugs were administered in the order shown. Values represent the mean ± SEM of the indicated number of monkeys. Significance of difference from “before L-dopa”, *p < 0.05, **p < 0.01 and ***p < 0.001, using a t-test.

<table>
<thead>
<tr>
<th>Wk</th>
<th>Group</th>
<th># monkeys</th>
<th>Parkinsonian scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before L-dopa</td>
</tr>
<tr>
<td>----</td>
<td>-------------</td>
<td>-----------</td>
<td>---------------------</td>
</tr>
<tr>
<td>5</td>
<td>Vehicle</td>
<td>6</td>
<td>4.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>5</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>ABT-107</td>
<td>5</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>ABT-894</td>
<td>5</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>Vehicle</td>
<td>6</td>
<td>4.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>5</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>ABT-107 + ABT-894</td>
<td>5</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>ABT-894 + ABT-107</td>
<td>5</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>7</td>
<td>Vehicle</td>
<td>6</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>5</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>ABT-107 + ABT-894</td>
<td>5</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>ABT-894 + ABT-107</td>
<td>5</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>Vehicle</td>
<td>6</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
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<td>4.0 ± 0.7</td>
</tr>
<tr>
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<td>ABT-107 + ABT-894</td>
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<td>3.0 ± 0.5</td>
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<tr>
<td></td>
<td>ABT-894 + ABT-107</td>
<td>5</td>
<td>4.0 ± 0.3</td>
</tr>
</tbody>
</table>
TABLE 2

No effect of single or multiple nAChR drug treatments on cognitive performance

Lesioned monkeys were gavaged with L-dopa twice daily at a 4 h interval and given nAChR drugs orally 30 min before L-dopa. Cognitive performance was evaluated 90 min after the first L-dopa gavage of the day once weekly. The drug doses were; 300 µg/ml nicotine in the drinking water, 0.1 mg/kg ABT-107 orally, 0.01 mg/kg ABT-894 orally, with the latter two drugs given on their own or in combination (see timelines in Fig. 4). For the nAChR drug combination studies, the drugs were administered in the order shown. Values are the mean ± SEM of the indicated number of monkeys.

<table>
<thead>
<tr>
<th>Group</th>
<th># Monkeys</th>
<th>Latency (s)</th>
<th>Attempts</th>
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</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>18.1 ± 2.9</td>
<td>17.0 ± 2.4</td>
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<tr>
<td>Nicotine</td>
<td>5</td>
<td>18.8 ± 2.1</td>
<td>19.0 ± 2.3</td>
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<tr>
<td>ABT-107</td>
<td>5</td>
<td>15.7 ± 3.0</td>
<td>15.5 ± 3.1</td>
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<tr>
<td>ABT-894</td>
<td>5</td>
<td>14.3 ± 3.2</td>
<td>14.6 ± 2.0</td>
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<tr>
<td>ABT-107 + ABT-894</td>
<td>5</td>
<td>16.8 ± 3.4</td>
<td>14.4 ± 1.8</td>
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<tr>
<td>ABT-894 + ABT-107</td>
<td>5</td>
<td>16.1 ± 3.2</td>
<td>16.0 ± 1.7</td>
</tr>
</tbody>
</table>
Fig. 1

**Parkinsonian ratings**

L-dopa treatment and dyskinesia ratings

<table>
<thead>
<tr>
<th>Wk</th>
<th>0.03</th>
<th>0.10</th>
<th>0.30</th>
<th>1.00</th>
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<tbody>
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</tr>
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<tr>
<td>4</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**ABT-107 dosing (mg/kg)**

- **Vehicle (n=6)**
- **ABT-107 (n=5)**

**Total Dyskinesias (% vehicle)**

<table>
<thead>
<tr>
<th>Wk</th>
<th>0.03</th>
<th>0.10</th>
<th>0.30</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
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<tr>
<td>2</td>
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<tr>
<td>4</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

**Nicotine (n=5)**

- **Vehicle (n=6)**
- **Nicotine (n=5)**

**Total Dyskinesias (% vehicle)**

<table>
<thead>
<tr>
<th>Wk</th>
<th>0.03</th>
<th>0.10</th>
<th>0.30</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>***</td>
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<tr>
<td>4</td>
<td>***</td>
<td>***</td>
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</tr>
</tbody>
</table>

300 μg/ml
Fig. 2

![Graph showing dyskinesia ratings over time for different treatments.](image-url)
Fig. 3
Fig. 4

(A) Parkinsonian ratings
L-dopa treatment and dyskinesia ratings

Repeat 0.10 mg/kg ABT-107
0.10 mg/kg ABT-107
+ 0.01 mg/kg ABT-894

(B) Parkinsonian ratings
L-dopa treatment and dyskinesia ratings

0.01 mg/kg ABT-894
0.01 mg/kg ABT-894
+ 0.1 mg/kg ABT-107

(C) Parkinsonian ratings
L-dopa treatment and dyskinesia ratings

300 µg/ml Nicotine

Vehicle (n=6)
ABT-107 (n=5)
Vehicle (n=6)
Reape ABT-107 (n=5)
Vehicle (n=6)
ABT-107+ ABT-894 (n=5)
Vehicle (n=6)
ABT-894 (n=5)
Vehicle (n=6)
ABT-894 + ABT-107 (n=5)
Vehicle (n=6)
Nicotine (n=5)
Vehicle (n=6)
Nicotine (n=5)
Fig. 5

(A) Repeat 0.10 mg/kg ABT-107

(B) 0.01 mg/kg ABT-894

(C) 300 μg/ml Nicotine

Dyskinesia Ratings

Time (min)
Fig. 6

![Graph of Total Dyskinesias (% vehicle) over Washout time (Wk) for Vehicle (n=6), ABT-107 + ABT-894 (n=5), ABT-894 + ABT-107 (n=5), and Nicotine (n=5).](image-url)