Background
Dopaminergic neurons in the basal ganglia fire in a continuous manner in normal individuals, thus striatal dopamine concentrations are maintained at a constant level. Degeneration of pre-synaptic neurons in the basal ganglia leads to dopamine deficiency and Parkinson’s disease (PD). Pulsatile stimulation of striatal dopamine receptors with short acting dopamine replacement therapies is thought to accelerate molecular and physiological changes that may have suboptimal ADME/PK such as limited water solubility, short half-life, limited oral bioavailability or are required at high doses. We have developed a POZ-polymer conjugate of rotigotine for the treatment of PD.

Technology
Polyoxazoline (POZ) polymers are non-ionic, biocompatible and are prepared by the cationic ring opening polymerization method. Molecular weights of 500 to 40,000 Da (Polydispersity Index x 1.10) have been prepared by Serina chemists in linear, branched, and pendant structures as pharmaceutical grade material. Pendent POZ is useful in delivering small molecules that may have suboptimal ADME/ PK such as limited water solubility, short half-life, limited oral bioavailability or are required at high doses. We have developed a POZ-polymer conjugate of rotigotine for the treatment of PD.

Purpose
The target product profile (TPP) for development of a POZ-rotigotine conjugate was as follows:
1. A formulation of lyophilized POZ-rotigotine conjugate that would be water soluble and one dose should fit in a standard insulin syringe (0.5 cc);
2. Prompt onset of dopaminergic tone (within one hour) following s.c. administration;
3. Near steady-state levels within a relatively narrow therapeutic window that controls symptoms;
4. Stable dopaminergic tone that would persist for > one week; and
5. Repeat dose PK that supports chronic dosing.

We evaluated whether POZ-rotigotine conjugates could provide extended duration of anti-Parkinsonian effect, compared to un-conjugated rotigotine, in the 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson’s disease (PD). Additionally, we evaluated the PK of POZ-rotigotine conjugates that would provide therapeutically relevant plasma levels of free rotigotine in both the rat and non-human primate.

Methods
POZ conjugates of rotigotine were prepared with three linker chemistries that allowed for different rates of release, i.e. "fast" (SER-212), "intermediate" (SER-213) and "slow" (SER-214). The rates of release of drug were measured in rat plasma at 37 °C and the respective half-lives of the conjugates were calculated from the release curves shown in Figure 2.

Male rats (Two groups, n=1 per group) were administered one injection (s.c.) of SER-214 (1.6 mg/kg, 0.5 mg/kg) to vehicle, rotigotine (0.5 or 3 mg/kg), SER-212 or SER-214. Plasma drug levels were measured at 6 time points, for up to 72 days post administration. In another study, male rats received unilateral 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson’s disease (PD). Additionally, we evaluated the PK of POZ-rotigotine conjugates that would provide therapeutically relevant plasma levels of free rotigotine in both the rat and non-human primate.

Normal untreated female cynomolgus macaques (n=3 per group, 3.9 ± 0.3 kg, 6.5 ± 0.2 year) were administered a single injection (s.c.) of SER-213 or SER-214 (3.5 or 4.5 mg/kg rotigotine). Plasma drug levels were assessed prior to and at 15 time points up to 14 days post drug administration.

Male rats (n=4 per group) were administered one injection (s.c.) of SER-214 (1.5 mg/kg) or vehicle (4 times/week). Plasma rotigotine levels were measured at 6 time points, for up to 72 days post administration. The weekly half-lives (t1/2) and drug exposure levels (AUC(0-7)) were calculated.

Rotigotine was measured in plasma using a qualified LC-MS/MS method. An internal standard of deuterated rotigotine was used in the assay and all response vs concentration calculations were made by weighted least squares linear regression (WLSR) method. The limit of quantitation (LOQ) was ~0 ng/mL. All pharmacokinetic calculations were determined using a non-compartmental model.

Results
The measured half-lives of SER-212, SER-213 and SER-214 in rat plasma at 37 °C were 2.4, 7.1 and 11.9 h, respectively.

In normal rats, SER-214 (1.6 mg/kg) provided plasma rotigotine levels above 0.5 ng/ml for 7 days (t1/2 ~ 40 h compared to 2.8 h for s.c. rotigotine).

In the efficacy study, the following observations were noted as shown in Figure 3:
• Day 1: SER-214-treated 6-OHDA animals showed levels of net contraversive rotations comparable to un-conjugated rotigotine (0.6 h vehicle; -56 ± 20, rotigotine (3 mg/kg) 1571 ± 312; SER-214 (1.6 mg/kg) 1408 ± 306, and (6.4 mg/kg) 1272 ± 605).
• Day 2, un-conjugated rotigotine and SER-214 showed comparable efficacy in reversing forelimb asymmetry, a measure of anti-Parkinsonian potential [asymmetry score: vehicle 68 ± 7%; rotigotine (3 mg/kg) 9 ± 13%; SER-214 (1.6 mg/kg) 8 ± 14%; and (6.4 mg/kg) 2 ± 3%].
• Day 5, only SER-214 and not un-conjugated rotigotine treated animals, continued to display greatly enhanced levels of rotations 6.4 mg/kg (5142 ± 777) vs (P<0.001 vs vehicle).
• Day 5, only SER-214 and not un-conjugated rotigotine treated animals showed robust reversal of forelimb asymmetry (1.6 mg/kg) 31 ± 13%; and (6.4 mg/kg) 6 ± 15%), (both P≤0.01 vs vehicle).

In normal macaques, SER-214 (1.5 mg/kg) was able to provide plasma levels of rotigotine above 1 ng/ml for up to 7 days following a single administration (t1/2 ~ 60 h). When the data was normalized to a dose of 0.5 mg/kg, the predicted profile in humans would achieve the attributes of the TPP.

In normal rats, repeated weekly s.c. administration of SER-214 (1.5 mg/kg) provided plasma rotigotine levels at or above 0.5 ng/ml and with comparable half-lives and drug exposure levels, (within 25% of the 1st dose). No accumulation of drug was observed at this dose of 1.5 mg/kg.

Summary
A novel POZ-polymer-conjugated rotigotine, SER-214, is able to provide significantly extended duration of anti-Parkinsonian benefit compared to that afforded by un-conjugated rotigotine or other dopamine therapies. SER-214 met our pre-specified target product profile and is being developed as a once a week injection (i.e. to treat PD patients. We will advance into Phase 1 in humans in 2013. The potential of the POZ platform technology applied to other therapeutic agents that would benefit from enhanced duration of action is considerable.