#M1097

# Toxicokinetics and Immunogenicity of CBX129801, a Pegylated C-Peptide Drug for Type 1 Diabetes Replacement Therapy, in Sprague Dawley Rats

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## Abstract

**Purpose:** To assess toxicokinetics (TK) and immunogenicity of CBX129801 in rats after subcutaneous (SC) injections for 4 weeks (5 doses) with 4-week recovery period.

**Methods:** Two groups of rats were injected with saline or CBX129801 (80 mg/kg/week) on Days 0, 7, 14, 21 and 28 followed by 28-day recovery. For TK study, blood samples were collected at predetermined times after the first and last dose. CBX129801 plasma concentrations were determined using a validated ELISA assay. TK was determined by standard model independent methods. Immunogenicity was tested for plasma on Days 29 and 56 using a validated bridging immunoassay. Cut point was determined using mean predose optical density (OD) plus 1.645xSD and then normalization factor was calculated by dividing the cut point by mean OD of naïve plasma pool.

**Results:** Following 5 doses of CBX129801 at 80 mg/kg/week, $T_{max}$ in males and females was 3 and 2 days, respectively. Corresponding $C_{max}$ values were 4260 nM and 6600 nM and $AUC_{tau}$ values were 14000 nM•day and 26000 nM•day. Apparent $T_{1/2}$ was 1.33 days in males and 1.55 days in females. $T_{max}$, $T_{1/2}$, $C_{max}$ and $AUC_{tau}$ did not change due to repeated doses. CLss/F values were 122 mL/day/kg in males and 65.6 mL/day/kg in females. Associated Vdss/F values were 234 mL/kg in males and 147 mL/kg in females. The Week 4 exposure was equal to that following the first dose. During recovery, the plasma concentrations decreased over time. Immunogenicity tests with all the samples yielded values below run specific cut point.

**Conclusions:** Following SC injection of CBX129801 at 80 mg/kg/week in rats, $T_{max}$, $T_{1/2}$ and AUC did not change due to repeated doses. Exposure of CBX129801 was higher in females than males. Exposure gradually decreased following cessation of dosing to undetectable levels at the end of recovery in both sexes. The lack of antibodies against CBX129801 in both sexes suggests that CBX129801 is not immunogenic.

## Introduction

Proinsulin C-peptide has an essential function in the synthesis of insulin as well as in regulating the nitric oxide pathway. The deficiency of C-peptide in type 1 diabetic patients causes many long-term complications even if treated with insulin. Although C-peptide does not affect blood sugar levels, it shows ameliorative effects on complications of type 1 diabetes. CBX129801 is a pegylated synthetic human C-peptide with extended half life and was developed by Cebix Incorporated as replacement therapy for type 1 diabetic patients who are deficient in C-peptide. This study assessed the TK and immunogenicity of CBX12980 in rats after SC injections for 4 weeks (5 doses) with a 4-week recovery period.

## Materials and Methods

### Study

Two groups of rats were injected with saline or CBX129801 (80 mg/kg/week) on Days 0, 7, 14, 21 and 28 followed by 28-days of recovery.

For the TK analysis, blood samples were collected at predetermined times after the first and last dose.

For the immunogenicity analysis, samples were collected on Days 29 and 56.

### Analytical Method

The plasma samples were analyzed for CBX129801 by a validated ELISA method.

Immunogenicity was tested using a validated bridging immunoassay with a rabbit anti-C-peptide serum as positive control.

The data were generated and analyzed using SoftMax Pro 5.2 (Molecular Devices, Sunnyvale, CA).

### TK and Immunogenicity Analysis

The TK parameters were determined by standard model independent methods (Gibaldi and Perrier, 1982) using WinNonlin Professional 5.2.1 (Pharsight Corp., Mountain View, CA).

For immunogenicity test, cut point was determined using mean predose optical density (OD) plus 1.645xSD and then normalization factor was calculated by dividing the cut point by mean OD of naïve plasma pool.

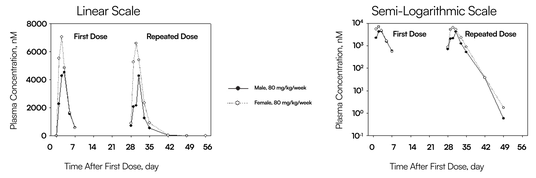
## Table 1

**Summary of CBX129801 Toxicokinetic Parameters in Rats Following 80 mg/kg/week Subcutaneous Injections of CBX129801 in a 4-week Toxicity Study**

| TK Parameter | | Male | Female |
|---|---|---|---|
| First Dose (TK) | $C_{max}$, nM | 4,350 | 7,050 |
| | $T_{max}$, day | 3.00 | 2.00 |
| | $T_{1/2}$, day | 1.35 | 1.27 |
| | $AUC_{tau}$, nM•day | 17,000 | 23,700 |
| | $AUC_{inf}$, nM•day | 18,100 | 24,700 |
| | CL/F, mL/day/kg | 94.2 | 69.0 |
| | $V_d$/F, mL/kg | 184 | 127 |
| | $C_{max}$ Male/Female Ratio | 0.643 | - |
| | $AUC_{tau}$ Male/Female Ratio | 0.733 | - |
| | CL/F Male/Female Ratio | 1.37 | - |
| | $V_d$/F, Male/Female Ratio | 1.45 | - |
| First Dose (TK) | $C_{max}$, nM | 4,260 | 6,600 |
| | $T_{max}$, day | 3.00 | 2.00 |
| | $T_{1/2}$, day | 1.33 | 1.55 |
| | $AUC_{tau}$, nM•day | 14,000 | 26,000 |
| | CLss/F, mL/day/kg | 122 | 65.6 |
| | $V_d$/SS/F, mL/kg | 234 | 147 |
| | $C_{max}$, Male/Female Ratio | 0.645 | - |
| | $AUC_{tau}$, Male/Female Ratio | 0.538 | - |
| | CLss/F Male/Female Ratio | 1.86 | - |
| | $V_{dss}$/F, Male/Female Ratio | 1.59 | - |
| | Repeated/First Dose $C_{max}$ Ratio | 0.940 | 0.936 |
| | Repeated/First Dose $AUC_{tau}$ Ratio | 0.824 | 1.10 |

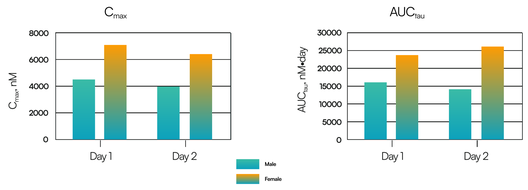
## Figure 1

**Mean Plasma Concentrations (nM) of CBX129801 in Rats Following 80 mg/kg/week Subcutaneous Injections of CBX129801 in a 4-Week Toxicity Study**



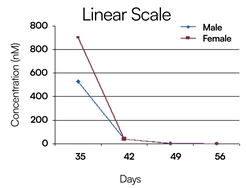

## Figure 2

**$C_{max}$ and $AUC_{tau}$ versus CBX129801 Dose in Rats Following 80 mg/kg/week Subcutaneous Injection of CBX129801 in a 4-Week Toxicity Study**



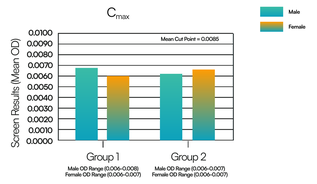

## Figure 3

**Plasma Concentrations (nM) of CBX129801 in Rats During Recovery Period Following 80 mg/kg/week Subcutaneous Injections of CBX129801 in a 4-Week Toxicity Study**



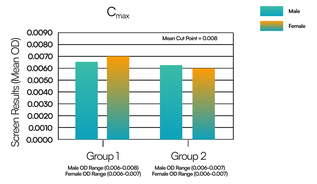

## Figure 4

**Screen ADA Results (Mean OD) from Day 29 Samples in Rat Plasma (Group 1: Saline treated; Group 2: CBX129801-treated)**




## Figure 5

**Screen ADA Results (Mean OD) from Day 56 Samples in Rat Plasma (Group 1: Saline treated; Group 2: CBX129801-treated)**




## Results

### TK Analysis

CBX129801 $T_{max}$ values ranged from 2.00 to 3.00 days in both sexes and did not change due to repeated doses (Table 1 and Figure 1).

Day 1 $C_{max}$ values were 4,530 and 7,050 nM and $AUC_{tau}$ were 17,000 and 23,700 nM•day in males and females, respectively. Apparent $T_{1/2}$ was 1.35 days in males and 1.27 days in females. The exposure in females was higher than that in males (Table 1).

CBX129801 did not accumulate following repeated dosing and its TK exposure after repeated dosing was approximately equal to the first dose (Table 1 and Figure 2).

During recovery, the plasma concentrations of CBX129801 decreased over time and there was no detectable drug at the end of the recovery period in both sexes (Day 56) (Figure 3).

### Immunogenicity Analysis

All screened Day 29 and Day 56 samples yielded values below the run specific cut point and were reported as ADA negative. Samples yielded screen ADA-negative results regardless of dose and gender designation (Figures 4 and 5).

## Conclusions

CBX129801 exhibited no accumulation following 5 once weekly subcutaneous doses.

CBX129801 $T_{max}$, $T_{1/2}$ and AUC did not change due to repeated weekly doses.

Exposure in females was higher than that in males.

Exposure gradually decreased following cessation of dosing to undetectable levels at the end of recovery in both sexes.

Immunogenicity test with the samples from Days 29 and 56 in both sexes yielded values below run-specific cut point.

The lack of antibodies against CBX129801 suggests that CBX129801 is not immunogenic.

## Acknowledgements:

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