143-LB

# The MetAP2 Inhibitor ZGN-1061 Improves Glycemia and has Weight Loss Efficacy with an Improved Safety Profile in Preclinical Models

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## ABSTRACT

*Methionine aminopeptidase 2 inhibitors (MetAP2i) are a promising new therapeutic approach for the treatment of obesity, diabetes and associated metabolic complications. Beloranib is a prototype MetAP2i which, when tested in obese type 2 diabetes subjects, resulted in 13% weight loss and 2.0% reduction in HbA1c from baseline following 26 weeks of treatment. Beloranib development was discontinued due to an imbalance of venous thromboembolism events in the treated groups. A second generation MetAP2i, ZGN-1061, has been developed and is in Phase 1 clinical testing. ZGN-1061 shows similar effects on diabetes and metabolic endpoints as beloranib in animal models, but has greatly improved safety profiling in model systems of thrombotic risk. In rodent models of obesity and diabetes ZGN-1061 (0.1 to 0.3 mg/kg, QD, subcutaneous dosing up to 5 wk) showed potent reductions of HbA1c (-2% ± 0.19% from baseline) along with improved OGTT glucose AUC (-11%), peak glucose (-57 mg/dl), weight loss (-28% at week 4) via reduced body fat (-36%) in addition to plasma LDLc (-34%) and triglyceride (-46%) improvements. In dog toxicology models which exhibit signs of coagulopathy, ZGN-1061 has a margin of >150 fold compared to only ~4 fold for similarly effective doses of beloranib. This improvement appears to be explained by a differential endothelial cell (EC) activity that shows beloranib having a rapid and lasting effect on proliferation and thereby promoting a procoagulant increase of plasminogen activator inhibitor-1 and decrease of thrombomodulin in ECs with as little as 4-hour exposure. ZGN-1061, however, requires prolonged exposure of ECs (>24h) to induce such a state. The in vivo pharmacokinetic profile of ZGN-1061 has a much shorter duration of exposure and is undetectable less than 10 hours after administration.*

## INTRODUCTION

- Methionine aminopeptidase 2 inhibitors (MetAP2i) are a promising new therapeutic approach for the treatment of diabetes, obesity, and associated metabolic complications.
- Beloranib is a MetAP2i which, when tested in patients with obesity and type 2 diabetes, resulted in approximately 13% weight loss and 2.0% reduction in HbA1c from baseline following 26 weeks of treatment. Beloranib development was discontinued due to an imbalance of venous thrombotic events in the treated groups compared to placebo (1-4).
- ZGN-1061 is a novel MetAP2i that was developed for similar efficacy to beloranib but improved toxicity and thrombotic safety.
- Like beloranib, ZGN-1061 is a novel potent, selective, covalent inhibitor of MetAP2 in vitro.
- These studies were designed to assess the nonclinical efficacy and safety of ZGN-1061.

## OBJECTIVES

- **Efficacy: Determine the efficacy of ZGN-1061 on body weight, glycemic control, and cardiometabolic markers in a mouse model of obesity and insulin resistance.**
- **Safety: Evaluate the effects of ZGN-1061 on in vitro and in vivo markers of thrombotic potential, pharmacokinetics, and toxicology.**

## METHODS

### DIO Mouse Efficacy Study

**Diet-induced obese (DIO) mice:** Male C57BL/6J mice (Charles River) were placed on a high-fat diet (45% kcal as fat) for 34 weeks. DIO mice (approximately 37 weeks of age) were maintained on high-fat diet for the duration of the study.

**Treatment:** Mice received 0.3 mg/kg ZGN-1061, 0.1 mg/kg beloranib, or vehicle (5% mannitol in 10 mM phosphate, pH 7.5) SC, QD for 28 days. (Doses of ZGN-1061 and beloranib were selected based on similar exposure in mice).

**Oral Glucose Tolerance Test (OGTT):** An oral glucose tolerance test was performed 60 minutes after dosing (t =0 min) at baseline and on Day 30. Blood samples were obtained at t =−60 min (baseline 1; B1), 0 min (baseline 2; B2), and 15, 30, 60, and 120 min. Plasma glucose was assayed using a clinical reagent (ThermoFisher hexokinase reagent TR15421). Plasma insulin was assayed using a commercial ELISA (Alpco 80-INSMU-E10) and log transformed.

## Efficacy Results: Comparable effects of maximally effective doses of ZGN-1061 and beloranib on body weight and glucose tolerance in obese mice

### 1) ZGN-1061 reduces body weight and improves glucose tolerance and cardiometabolic markers in a mouse model of obesity and insulin resistance

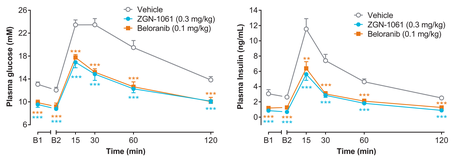
- ZGN-1061 (subcutaneous [SC], once daily [QD]) for 28 days in diet-induced obese (DIO) insulin-resistant mice resulted in:
  - Improvements in body weight, lipids, and cardiometabolic biomarkers (Table 1).
  - Improvements in glucose tolerance and insulin sensitivity (Figure 1, Table 2).

Table 1. Similar improvements in body weight, body fat, lipids, and cardiometabolic markers with ZGN-1061 and beloranib in DIO mice

| | Vehicle | ZGN-1061 0.3 mg/kg | Beloranib 0.1 mg/kg |
|---|---|---|---|
| **Body weight** | | | |
| Baseline, g | 48.2 ±0.0 | 48.2 ±0.0 | 48.2 ±0.0 |
| Endpoint, g | 50.2 ±0.4 | 36.0 ±0.5 | 35.1 ±0.9 |
| Change, g | 2.1 | −12.2*** | −13.1*** |
| Change, % | 4.3% | −25.2%*** | −27.2%*** |
| **Body fat** | | | |
| Baseline, g | 19.1 ±0.5 | 18.6 ±0.4 | 18.8 ±0.6 |
| Endpoint, g | 21.1 ±0.4 | 9.4 ±0.5*** | 8.8 ±0.8*** |
| Baseline, % body fat | 40.6 ±0.7% | 39.6 ±1.0% | 40.2 ±1.0% |
| Endpoint, % body fat | 43.2 ±0.7% | 27.5 ±0.9%*** | 25.9 ±1.4%*** |
| **Lipids (at endpoint)** | | | |
| Total cholesterol, mg/dL | 228.8 ±12.7 | 169.6 ±13.4** | 191.5 ±14.9 |
| LDL cholesterol, mg/dL | 48.5 ±5.2 | 25.6 ±4.0** | 29.9 ±3.4** |
| HDL cholesterol, mg/dL | 225.7 ±11.1 | 165.0 ±13.5*** | 192.6 ±14.7* |
| Triglycerides, mg/dL | 68.1 ±3.4 | 71.7 ±4.4 | 82.3 ±3.5* |
| **Cardiometabolic biomarkers (at endpoint)** | | | |
| β-hydroxybutyrate (µmol/L) | 514.0 ±57.9 | 633.5 ±50.4* | 630.7 ±41.4 |
| Leptin (ng/mL) | 31.3 ±3.6 | 4.7 ±0.9*** | 6.6 ±1.1*** |

Data are mean and SEM. Mice (n=9–10/group) received drug or vehicle SC QD for 28 days. Body fat was analyzed by DXA (dual-energy x-ray absorptiometry). Lipids and biomarkers were collected in the morning after an overnight fast at endpoint only. Comparisons vs Vehicle of treatments were by Williams' test (ZGN-1061) and multiple t-test (beloranib). *p<0.05, **p<0.01 and ***p<0.001 vs Vehicle. Abbreviations: QD = once daily; SC = subcutaneous; SEM = standard error of the mean.

Figure 1. Similar improvement in glucose tolerance with ZGN-1061 and beloranib in DIO mice




Data are mean and SEM. Mice (n=9–10/group) were fasted overnight and dosed with vehicle or study drug SC. An oral glucose tolerance test was performed 60 minutes after dosing (t =0 min). Blood samples were obtained at t =−60 min (B1), 0 min (B2), and 15, 30, 60, and 120 min. Data are calculated from the residuals of the statistical models. Comparisons vs Vehicle of treatments were by Williams' test (ZGN-1061) and multiple t-test (beloranib). *p<0.01 and **/***p<0.001 vs Vehicle. Abbreviations: SEM = standard error of the mean.

Table 2. Similar improvement in glucose tolerance with ZGN-1061 and beloranib in DIO mice

| | Vehicle | ZGN-1061 0.3 mg/kg | Beloranib 0.1 mg/kg |
|---|---|---|---|
| Fasting glucose (mM) | 12.1 ±0.5 | 8.8 ±0.3*** | 9.2 ±0.6*** |
| Glucose $AUC_{0-120\ min}$ (mMxh) | 37.9 ±1.5 | 25.4 ±1.2*** | 25.8 ±1.3*** |
| Fasting insulin (ng/mL) | 2.6 ±0.2 | 0.7 ±0.0*** | 1.3 ±0.2*** |
| Insulin $AUC_{0-120\ min}$ (ng/mLxh) | 11.3 ±0.7 | 4.5 ±0.3*** | 5.4 ±0.6*** |

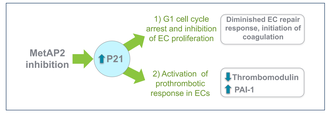
Data are shown as adjusted means and standard errors of the mean (SEM) are calculated from the residuals of the statistical models. Mice (n=9–10/group) were fasted overnight and dosed with vehicle or study drug SC, followed by an oral glucose tolerance test 60 minutes after dosing (t= 0 min). Glucose and insulin were measured at baseline (0 min). Comparisons vs Vehicle of treatments were by Williams' test (ZGN-1061) and multiple t-test (beloranib). ***p<0.001 vs Vehicle. Abbreviations: $AUC_{0-120\ min}$ = area under the curve from time 0 to 120 minutes; SEM = standard error of the mean.

## Safety Results: Differentiation of ZGN-1061 from beloranib

Background:

- MetAP2 inhibitors slow endothelial cell (EC) proliferation via activation of P21 (5, 6); this is hypothesized to contribute to the prothrombotic effects observed with beloranib (Figure 2).

Figure 2. Postulated mechanism for prothrombotic response with MetAP2 inhibition

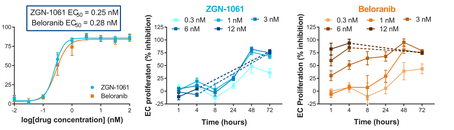



MetAP2 inhibition activates P21, which has 2 effects: 1) cell cycle arrest and inhibition of endothelial cell proliferation, and 2) activation of prothrombotic response ECs, resulting in reduced cell-associated thrombomodulin and increased PAI-1. Abbreviations: EC = endothelial cell; PAI-1 = plasminogen activator inhibitor-1.

### 2) Endothelial Cell Proliferation: Reduced effect of ZGN-1061 compared to beloranib on inhibition of cell proliferation

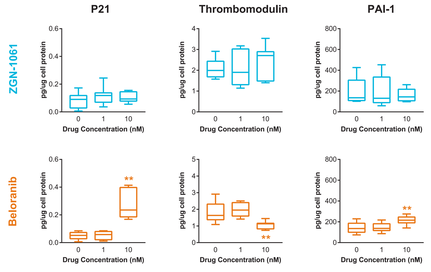
- Although both ZGN-1061 and beloranib slow proliferation in a concentration-dependent manner, ZGN-1061 requires ≥24 hours of exposure to have an effect, while an effect of beloranib can be observed after as little as 1 hour (Figure 3).
- After up to 8 hours of exposure, there was no effect of ZGN-1061 on the procoagulant markers, P21, thrombomodulin, or PAI-1, whereas changes in all markers were observed after 8 hours of exposure to beloranib (Figure 4).

Figure 3. Time- and concentration-dependent effect of ZGN-1061 and beloranib on inhibition of endothelial cell proliferation




Data are mean and SEM. Cell proliferation was studied utilizing HUVECs in the presence or absence of compound for varying amounts of time prior to washout. Proliferation was assessed at 72 hours utilizing a DNA dye (CyQUANT®) added to lysed cells to provide a fluorescent signal relative to the amount of DNA, allowing a measure of cell density. Abbreviations: EC = endothelial cell; HUVEC = human umbilical vein endothelial cell.

Figure 4. ZGN-1061 does not affect thrombosis markers that are changed by beloranib in endothelial cells after up to 8 hours of exposure

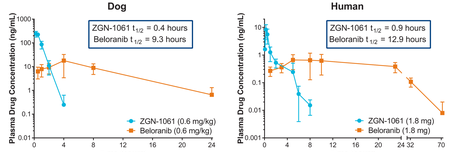



Data are mean, SEM, and min/max. Cultured HUVECs were maintained in the presence or absence of test articles 8 hours prior to compound washout. Protein levels were assessed utilizing enzyme-linked immunosorbent assays (ELISA). Abbreviations: HUVEC = human umbilical vein endothelial cells; PAI-1 = plasminogen activator inhibitor-1; SEM = standard error of the mean.

### 3) PK Profile: Lower exposure with ZGN-1061 compared to beloranib

- ZGN-1061 is rapidly metabolized and cleared following SC administration.
- ZGN-1061 has a much shorter half-life than beloranib in animals and humans (Figure 5).
- In humans, the half-life of ZGN-1061 is <1 hour and exposure durations last <12 hours. In contrast, the half-life of beloranib is 8 to 13 hours and exposure durations range from 17 to ≥24 hours.

Figure 5. Shorter half-life of ZGN-1061 in animals and humans avoids thrombotic potential while preserving cardiometabolic effects

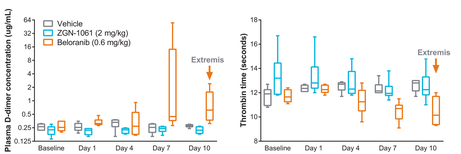



Study drug was administered SC. Drug concentrations were evaluated following repeat administration Q3D. (at steady state). Abbreviations: $t_{1/2}$ = half-life.

### 4) Safety Margins: Greatly improved for ZGN-1061 compared to beloranib

- In contrast to beloranib, which produced increases in the coagulation markers D-dimer and thrombin time at 0.6 mg/kg, ZGN-1061 had no effect at 2 mg/kg (Figure 6).
- ZGN-1061 has a substantially greater safety margin for morbidity and coagulopathy in dogs, testicular toxicity in rats, and embryofetal development in rabbits and rats (Table 4).

Figure 6. ZGN-1061 does not alter coagulation markers in dogs




Arrow indicates day that beloranib-treated animals were in extremis (animals euthanized due to morbidity).

Table 4. Favorable improvements in safety with ZGN-1061 compared to beloranib

| | ZGN-1061 NOAEL (AUC ngxh/mL) | Beloranib NOAEL (AUC ngxh/mL) | Fold improvement for ZGN-1061 vs Beloranib | Clinical Safety Margins for ZGN-1061 |
|---|---|---|---|---|
| **Dog safety** | 3 mg/kg (1030) | 0.2 mg/kg (43) | ~25x | 300 |
| **Dog coagulopathy** | >2 mg/kg (619) | 0.2 mg/kg (43) | >14x | 200 |
| **Rat testis** | >25 mg/kg (2100) | 0.3 mg/kg (38.1) | >55x | >600 |
| **Rabbit embryofetal** | 0.9 mg/kg (230) | <0.1 mg/kg (<22.2) | >10x | 70 |
| **Rat embryofetal** | 0.5 mg/kg (32.9) | 0.3 mg/kg (18.5) | 1.8x | 10 |

Result are shown for in vivo toxicology studies conducted in rats and rabbits with dosing Q3D. Safety margins were calculated by dividing repeat dose $AUC_{0-t}$ from the toxicology study by the AUC from repeat dose $AUC_{0-t}$ from clinical results and are based on exposure of an estimated 0.9 mg clinical dose. Abbreviations: $AUC_{0-t}$ = area under the curve from time 0 to last measurable timepoint; $C_{max}$ = maximum observed concentration; NOAEL=no observed adverse effect level.

## SUMMARY: Comparison of ZGN-1061 with Beloranib

| | ZGN-1061 | Beloranib |
|---|---|---|
| **Efficacy** | • Weight loss<br>• Improved glucose tolerance<br>• Improvements in cardiometabolic markers | • Weight loss<br>• Improved glucose tolerance<br>• Improvements in cardiometabolic markers |
| **Safety: EC Proliferation** | • ≥24 h exposure required for inhibition | • As short as 1 h exposure sufficient for inhibition |
| **Safety: PK Profile** | • Half-life rat: ~0.5 h<br>• Half-life dog: ~0.5–1 h<br>• Half-life human: <1 h<br>• Exposure in humans above HUVEC $EC_{50}$: ≤6 h | • Half-life rat: ~3–4 h<br>• Half-life dog: ~2–9 h<br>• Half-life human: 8–13 h<br>• Exposure in humans above HUVEC $EC_{50}$: ≥24 h |
| **Safety: Safety Margins** | • Much higher safety margins for known beloranib toxicities<br>• No evidence of coagulopathy or testicular toxicity | • Lower safety margins<br>• Causes coagulopathy in dogs (preceded by increases in D-dimer and thrombin time) |

## Overview of ZGN-1061 Efficacy and Safety

- The efficacy of ZGN-1061 is similar to beloranib in DIO mice.
  - Similar potency for inhibition of MetAP2 as beloranib.
  - Similar improvements in body weight, glucose tolerance, and other metabolic endpoints in vivo.
- ZGN-1061 has an improved safety profile for markers of thrombosis compared to beloranib in vitro and in vivo
  - ZGN-1061 requires longer exposure duration (≥24 hours) than beloranib (<4 hours) to inhibit endothelial cell proliferation.
  - After up to 8 hours of exposure, beloranib affected procoagulant factors, but ZGN-1061 had no effect.
- ZGN-1061 exposure is brief compared to beloranib.
  - ZGN-1061 has a much shorter half-life than beloranib in animals and humans.
- ZGN-1061 has improved safety margins (NOAELs) compared to beloranib in vivo for:
  - Morbidity
  - Coagulopathy
  - Testicular toxicity
  - Embryofetal toxicity

## CONCLUSION

- **ZGN-1061 shows similar effects on glucose tolerance, body weight, and cardiometabolic markers as beloranib in animal models, but has a greatly improved safety profile.**
- **Notably, safety margins for morbidity and coagulopathy for similarly effective clinical doses are over 150-fold for ZGN-1061 compared to approximately 4-fold for beloranib.**
- **ZGN-1061 represents a potential new treatment for diabetes and obesity. Clinical studies designed to investigate the safety and efficacy of ZGN-1061 in treatment of diabetes and metabolic disease are underway.**

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