The MetAP2 Inhibitor ZGN-1061 Improves Glycaemia in High-Fat Diet-Induced Obese Mice

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ABSTRACT

Background and Aims: *Methionine aminopeptidase 2 inhibitors (MetAP2i) are a promising new therapeutic approach for the treatment of diabetes. Beloranib is a prototype MetAP2i which, when tested in obese type 2 diabetes subjects, resulted in 2.0% reduction in HbA1c and 13% body weight loss from baseline following 26 weeks of treatment. A novel MetAP2i, ZGN-1061, is in Phase 1 clinical testing. ZGN-1061 is a potent and selective inhibitor of MetAP2 enzyme activity and shows similar effects on obesity as beloranib in animal models, but has an improved safety profile in model systems of thrombotic risk. The aim of this study was to evaluate the anti-diabetic potential of ZGN-1061 in diet-induced obese (DIO) mice and relate these changes to weight loss and change of body composition.*

Materials and methods: *Male C57BL/6J mice were placed on a high fat diet (45% kcal as fat) for 34 weeks then were treated with ZGN-1061 at doses of 0.03, 0.1 and 0.3 mg/kg (SC, QD) or beloranib at 0.1 mg/kg (SC, QD) for 4 weeks (n=10/group). Body weight, food intake, DEXA body composition and oral glucose tolerance test (OGTT) were assessed.*

Results: *ZGN-1061 dose dependently reduced body weight by 4.4%, 17.0%, and 29.5% after 29 days of treatment. In comparison, beloranib achieved 31.5% weight loss. Mice in the 0.3 mg/kg 1061 and 0.1 mg/kg beloranib dose groups were still losing weight over the final week of the study suggesting dosing longer than one month could show greater weight loss. Energy intake (kJ/gram lean mass) was transiently reduced in the two higher ZGN-1061 dose groups as well as the beloranib group by 23%, 28% and 31% at day 16, respectively. However, by the end of the study, energy intake was no different from vehicle in all drug treated groups. At day 27 body composition as measured by DEXA show ZGN-1061 reduced the % fat and increased the % lean mass at both the mid and high dose groups with no change in the low dose group. On day 30 an OGTT was performed. Baseline fasted glucose was reduced relative to vehicle by 12.3%, 16.4% and 27.1% in the ZGN-1061 dose response. Beloranib had a 23.7% reduction of fasted glucose. Oral glucose tolerance was improved with treatment as shown by 12.0%, 27.2% and 33.2% reductions of the glucose AUC 0-120 and 25.4%, 44.4% and 60.1% reductions of insulin AUC 0-120 across the ZGN-1061 doses. Calculation of HOMA-IR reveal a marked drug effect to reduce the insulin resistance index at all dose levels of ZGN-1061, where the lowest dose improved HOMA-IR by 40.3% and the highest dose by 81.2%.*

Conclusions: *The MetAP2i ZGN-1061 dose-dependently improves glycemic control and insulin sensitivity in DIO mice. Weight loss at the higher doses likely contributes to the improvement of glucose control, however, glucose was reduced even at the lowest dose which was weight neutral and had no effect on fat mass after one month of dosing.*

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 a 2.0% reduction in HbA1c from baseline following 26 weeks of treatment. Beloranib development was discontinued due to an imbalance of adverse events in the treated groups compared to placebo (1-5)
- ZGN-1061 is a novel MetAP2i that was developed to achieve similar efficacy as beloranib but with an improved safety profile
- Like beloranib, ZGN-1061 is a novel potent, selective, covalent inhibitor of MetAP2 in vitro
- These studies were designed to assess the nonclinical efficacy of ZGN-1061 in a mouse model of diet-induced insulin resistance and obesity

OBJECTIVES

- **Evaluate the dose-dependent effect of ZGN-1061 on body weight, food intake, body composition, glucose tolerance, and other cardiometabolic markers compared to a maximally effective dose of beloranib**

METHODS

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. A cohort of C57BL/6J mice maintained on standard chow were used as a lean normal control.

Treatment: Mice received 0.03, 0.1 or 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.

Oral Glucose Tolerance Test (OGTT): An oral glucose tolerance test was performed 60 minutes after dosing (t =0 min) at baseline and end of study. Blood samples were obtained at t =−60 min (B1), 0 min (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.

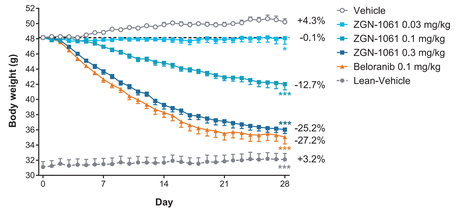
Other Assessments: Body weight and food intake were monitored daily. Body composition [analyzed using dual-energy x-ray absorptiometry (DXA)] was assessed at day 16 and day 27 of study. Lipids and cardiometabolic biomarkers were assessed at end of study. Tissue target engagement was assessed via evidence of MetAP2 inhibition (measured by the fraction of thioredoxin substrate with intact N-terminal peptide, THX 1-6).

RESULTS

1) ZGN-1061 reduced body weight and body fat in DIO mice

- Weight loss with ZGN-1061 SC in DIO mice was dose dependent and continued for the 28-day treatment period (Figure 1, Table 1)
  - The vehicle-adjusted weight loss for ZGN-1061 was −4.4%, −17.0%, and −29.5%, for the 0.01 mg, 0.3 mg, and 0.1 mg/kg treatment groups, respectively
  - Weight loss with the high dose of ZGN-1061 (0.3 mg/kg) was similar to that observed with the maximally effective dose of beloranib (0.1 mg/kg)
  - Weight loss with the mid and high doses of ZGN-1061 (0.1 and 0.3 mg/kg) was primarily due to loss of body fat. The lowest dose of ZGN-1061 did not reduce body fat
- Food intake with ZGN-1061 was reduced compared to vehicle at Week 16 but not at the end of the study (Figure 2)
  - On Day 16, food intake was reduced with ZGN-1061 compared to vehicle
  - Food intake in ZGN-1061-treated animals was similar to vehicle at the end of the study (Week 4), indicating that weight loss was partially independent of food intake

Figure 1. ZGN-1061 produced a dose-dependent reduction in body weight in DIO mice



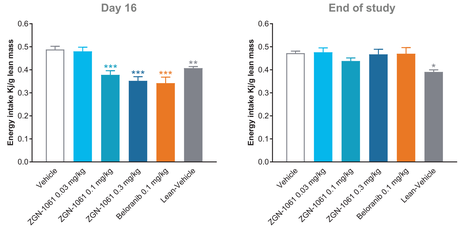
Data are mean adjusted for baseline differences between treatment groups (DIO mice) or mean (lean mice) and SEM or percent change from baseline (n=10/group). Data were analyzed by analysis of covariance with Day 0 (baseline) body weight as covariate followed by Williams' test (ZGN-1061) and multiple t-test (beloranib). *p<0.05, ***p<0.001 vs Vehicle. Abbreviations: DIO = diet-induced obesity; SEM = standard error of the mean.

Table 1. ZGN-1061 produced a dose-dependent reduction in body weight that was primarily due to loss of fat mass

	Vehicle	ZGN-1061 0.03 mg/kg	ZGN-1061 0.1 mg/kg	ZGN-1061 0.3 mg/kg	Beloranib 0.1 mg/kg	Lean-Vehicle
Body weight						
Baseline, g	48.2 ±0.0	48.2 ±0.0	48.2 ±0.0	48.2 ±0.0	48.2 ±0.0	31.1 ±0.7
Endpoint, g	50.2 ±0.4	48.1 ±0.8	42.0 ±0.8	36.0 ±0.5	35.1 ±0.9	32.1 ±0.8
Change, g	2.1 ±0.4	−0.1 ±0.8 *	−6.1 ±0.8 ***	−12.1 ±0.5 ***	−13.1 ±0.9 ***	1.0 ±0.2
Change, %	4.3%	−0.1%	−12.7%	−25.2%	−27.2%	3.2%
Body fat mass						
Baseline, g	19.1 ±0.5	19.0 ±0.5	18.9 ±0.4	18.6 ±0.4	18.8 ±0.6	6.5 ±0.4
Endpoint, g	21.1 ±0.4	19.8 ±0.7	15.1 ±0.7 ***	9.4 ±0.5 ***	8.8 ±0.8 ***	7.1 ±0.5 ***
Baseline, % fat	40.6 ±0.7%	40.5 ±0.8%	40.1 ±0.6	39.6 ±1.0%	40.2 ±1.0%	20.9 ±0.8%
Endpoint, % fat	43.2 ±0.7%	42.5 ±1.1	37.6 ±0.9 ***	27.5 ±0.9% ***	25.9 ±1.4% ***	22.8 ±1.1% ***
Lean mass						
Baseline, g	27.9 ±0.6	27.8 ±0.7	28.3 ±0.6	28.4 ±0.7	28.0 ±0.5	24.2 ±0.4
Endpoint, g	27.7 ±0.4	26.5 ±0.3 *	24.7 ±0.5 ***	24.8 ±0.2 ***	24.6 ±0.3 ***	23.7 ±0.4 ***
Baseline, % lean	59.4 ±0.7%	59.5 ±0.8%	59.9 ±0.6%	60.4 ±1.0%	59.8 ±1.0%	79.1 ±0.8%
Endpoint, % lean	56.8 ±0.7	57.5 ±1.1%	62.4 ±0.9% ***	72.5 ±0.9% ***	74.1 ±1.4% ***	77.2 ±1.1% ***

Data are adjusted mean (DIO mice) or mean (lean mice) and SEM (n=10/group). Data were analyzed by analysis of covariance with Day 0 (baseline) body weight as covariate followed by Williams' test (ZGN-1061) and multiple t-test (beloranib). Comparisons of Vehicle (DIO) vs Lean-Vehicle was by 1-way analysis of variance and multiple t-test. *p<0.05, ***p<0.001 vs Vehicle. Abbreviations: DIO = diet-induced obesity; SEM = standard error of the mean.

Figure 2. ZGN-1061 produced a dose-dependent and transient reduction in food intake

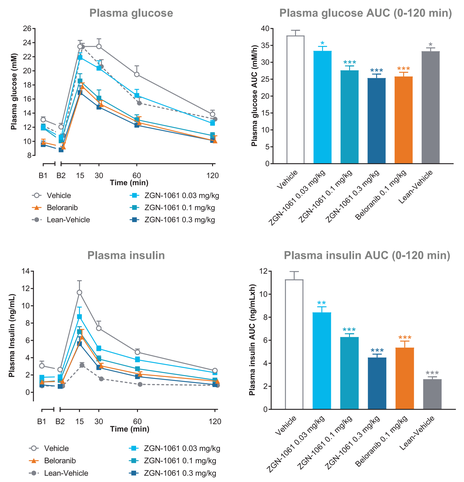


Data are mean and SEM (n=10/group), calculated from mean food intake on Day 16 (mean food intake on days 15, 16, and 17) and Day 16 DXA lean mass measurement or End of study (mean food intake on days 26, 27, and 28) and Day 27 DXA lean mass measurement. Data were analyzed by general linear model followed by Williams' tests (ZGN-1061) and multiple t-test (beloranib). Comparisons of Vehicle (DIO) vs Lean-Vehicle was by 1-way analysis of variance and multiple t-test. *p<0.05, ***p<0.001 vs Vehicle. Abbreviations: DIO = diet-induced obesity; SEM = standard error of the mean.

2) ZGN-1061 improved glucose tolerance and lowered insulin levels in DIO mice

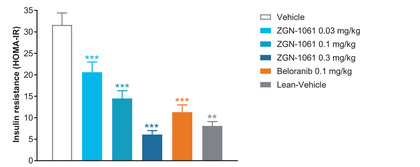
- ZGN-1061 for 28 days produced a dose-dependent reduction in glucose excursions in DIO mice, even at the lowest dose (Figure 3)
  - The middle (0.1 mg/kg) and high (0.3 mg/kg) doses of ZGN-1061 produced a similar reduction in glucose as the maximally effective dose of beloranib
  - The low (0.03 mg/kg) dose of ZGN-1061 normalized glucose to that of lean mice
- ZGN-1061 for 28 days produced a dose-dependent reduction in insulin excursions in DIO mice that was statistically significant for all doses (Figure 3)
  - The middle (0.1 mg/kg) and high (0.3 mg/kg) doses of ZGN-1061 produced a similar reduction in insulin as the maximally effective dose of beloranib
  - The low dose (0.03 mg/kg) of ZGN-1061 also produced a statistically significant reduction in insulin concentrations to levels similar to the lean animals fed standard chow (Lean-vehicle)
- All doses of ZGN-1061 for 28 days produced dose-dependent improvements in HOMA-IR, indicative of potential improvements in insulin resistance (Figure 4)

Figure 3. ZGN-1061 produced a dose-dependent reduction in plasma glucose and insulin after an oral glucose challenge



Data are mean adjusted for baseline differences between treatment groups and SEM at end of study. 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. Comparisons vs Vehicle of treatments were by robust regression and included treatment as a factor and bleeding order, baseline body weight and baseline glucose or insulin as covariates. An analysis without covariates was conducted to compare Vehicle vs Lean-Vehicle. Comparisons vs Vehicle were by Williams' test (ZGN-1061) and multiple t-test (Beloranib, Lean-Vehicle). *p<0.05, **p<0.01, and ***p<0.001 vs Vehicle. Abbreviations: AUC = area under the curve from time 0 to 120 minutes; B1 = baseline 1; B2 = baseline 2; DIO = diet-induced obese; SEM = standard error of the mean.

Figure 4. ZGN-1061 produced a dose-dependent reduction in HOMA-IR



Data are mean and SEM at end of study. 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. Comparisons vs Vehicle were by Williams' test (ZGN-1061) and multiple t-test (beloranib). Comparisons of Vehicle (DIO) vs Lean-Vehicle was by 1-way ANOVA and multiple t-test. **p<0.01 and ***p<0.001 vs Vehicle. Abbreviations: DIO = diet-induced obese; HOMA-IR = homeostatic model assessment of insulin resistance; SEM = standard error of the mean.

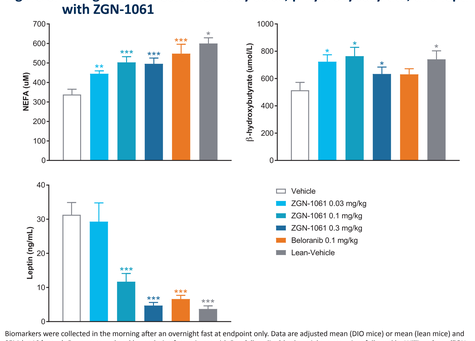
3) ZGN-1061 improved lipids and cardiometabolic biomarkers in DIO mice

- ZGN-1061 for 28 days produced improvements in total and LDL-cholesterol (Figure 5)
  - There was also a trend for reductions in HDL-cholesterol compared to Vehicle; triglycerides were generally unchanged
- ZGN-1061 for 28 days impacted cardiometabolic biomarkers (Figure 6)
  - Non-esterified fatty acids (NEFA) were increased with all doses of ZGN-1061
  - The ketone body, β-hydroxybutyrate, was elevated with all doses of ZGN-1061
  - The adipokine, leptin, was significantly reduced with the mid (0.1 mg/kg) and high dose (0.3 mg/kg) of ZGN-1061. The reduction was similar to that produced by the maximally effective dose of beloranib and consistent with the reduction in fat mass

Figure 5. Improved total and LDL-cholesterol with ZGN-1061

Lipids were collected in the morning after an overnight fast at endpoint only. Data are adjusted mean (DIO mice) or mean (lean mice) and SEM (n=10/group). Data were analyzed by ANCOVA with Day 0 (baseline) body weight as covariate followed by Williams' test (ZGN-1061) and multiple t-test (beloranib). Comparisons of Vehicle (DIO) vs Lean-Vehicle was by 1-way ANOVA and multiple t-test. **p<0.01 and ***p<0.001 vs Vehicle. Abbreviations: DIO = diet-induced obese; LDL = low-density lipoprotein; SEM = standard error of the mean.

Figure 6. Changes in non-esterified fatty acids, β-hydroxybutyrate, and leptin with ZGN-1061

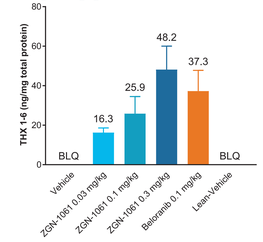


Biomarkers were collected in the morning after an overnight fast at endpoint only. Data are adjusted mean (DIO mice) or mean (lean mice) and SEM (n=10/group). Data were analyzed by analysis of covariance with Day 0 (baseline) body weight as covariate followed by Williams' test (ZGN-1061) and multiple t-test (beloranib). Comparisons of Vehicle (DIO) vs Lean-Vehicle was by 1-way analysis of variance and multiple t-test. *p<0.05 and ***p<0.001 vs Vehicle. Abbreviations: DIO = diet-induced obese; NEFA = non-esterified fatty acids; SEM = standard error of the mean.

4) Evidence of MetAP2 inhibition with ZGN-1061

- Target engagement (evidence of MetAP2 inhibition) was assessed via the fraction of a specific MetAP2 substrate, thioredoxin with intact N-terminal peptide, THX 1-6
  - MetAP2 cleaves the N-terminal peptide of thioredoxin (AA 1-6). Thus, higher fractions of THX 1-6 indicate inhibition of MetAP2
  - Consistent with evidence of MetAP2 inhibition, ZGN-1061 produced significant increases in the fraction of THX 1-6 protein in all tissues measured (liver, adipose tissue, duodenum, ileum, colon) (data not shown)
  - In the liver, even the lowest dose of ZGN-1061 (0.03 mg/kg) resulted in target engagement of approximately half of that achieved with the maximally effective dose of beloranib (Figure 7)

Figure 7. Robust target engagement with ZGN-1061 in the liver



Target engagement (MetAP2 activity) was assessed through analysis of THX 1-6 concentrations in the liver, adipose tissue, duodenum, ileum, and colon. Abbreviations: BLQ = below the limit of quantitation; THX 1-6 = thioredoxin 1-6.

CONCLUSION

- **In insulin resistant obese mice, ZGN-1061 produced similar weight loss, loss of fat mass, and improved glucose tolerance as another MetAP2 inhibitor, beloranib**
- **The improvement in glucose tolerance with ZGN-1061 can be dissociated, at least in part, from a weight loss effect or food intake effect:**
  - **Improvements in glucose tolerance were observed with the lowest dose of ZGN-1061 (0.03 mg/kg) and occurred in the absence of a change in body weight, body composition, or food intake**
  - **The improvement in glucose tolerance were similar in the middle (0.1 mg/kg) and high (0.3 mg/kg) ZGN-1061 dose groups, despite greater weight loss with the high dose**
- **Changes in the cardiometabolic biomarkers, including NEFA, β-hydroxybutyrate, and leptin, are consistent with loss of fat mass as well as increased fat mobilization and oxidation**
- **The novel MetAP2 inhibitor, ZGN-1061, represents a novel treatment for type 2 diabetes and obesity**

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