XOMA 129, a Novel Insulin Receptor Negative Modulator, Is Efficacious in Treating Insulin- and Glibenclamide-induced Hypoglycemia in Animals

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Abstract

Hypoglycemia is the most common and serious complication associated with insulin-based diabetes treatments and requires prompt recognition and treatment to prevent organ damage and possible mortality. There is a need in acute hypoglycemic conditions for a safe and well-tolerated treatment with rapid onset and optimal duration. Herein, we describe a novel antibody fragment drug candidate (XOMA 129) that can promptly reverse hypoglycemia induced by either a bolus injection of insulin or oral sulfonylurea administration in animal models.

XOMA 129 is a fully human, high affinity Fab that specifically targets the human insulin receptor. It binds to an allosteric site of the receptor, a site distinct from the site for insulin binding, and dampens insulin signaling. XOMA 129 - at doses of 3 and 10 mg/kg i.v. - normalized glucose levels in rat models of hypoglycemia induced by either a bolus injection of insulin (1 IU/kg, s.c.) or oral administration of a sulfonylurea drug (glibenclamide, 10 mg/kg). Serum levels of XOMA 129 at 10 mg/kg showed a terminal half-life of ~4 hours. Intramuscular administration to normal minipigs transiently elevated blood glucose. Thus, XOMA 129 has potential as a novel treatment for treating (or preventing) acute hypoglycemic conditions.

Summary:

Insulin and insulin secretagogue-caused hypoglycemia is one of the most common complications of diabetes treatment. Hypoglycemia is typically defined by blood glucose level <70 mg/dL. Severe hypoglycemia is characterized by need for third party assistance, and the most feared consequences of severe hypoglycemia are irreversible damage to vital organs, and even death. Although the treatment for hypoglycemia is straightforward, severe hypoglycemia requires a treatment that can not only increase blood glucose rapidly, but also sustain the effect for at least a couple of hours to overcome some long-lasting hypoglycemia caused by certain medications. The existing therapy for severe hypoglycemia is either difficult to administer, may have undesirable side effects, or does not have sustained effect. Thus, there is a need for a safe and well-tolerated treatment with rapid onset and optimal duration.

XOMA 129 is a fully human, high affinity Fab that specifically targets the human insulin receptor (INSR). As a negative allosteric modulator (NAM), it binds with high affinity to a site distinct from insulin binding with high affinity and dampens insulin signaling. In vitro assays showed that XOMA 129 decreased the effect of insulin on CHOK1 cells overexpressing either human INSR or rat INSR in a dose-dependent manner. In vivo, oral administration of a sulfonylurea drug (glibenclamide, 10mg/kg) caused a gradual yet long-lasting hypoglycemia in rats. XOMA 129 - at doses of 3 and 10 mg/kg i.v. - elevated blood glucose levels rapidly, reaching the maximal effect within 2 hours; and the effect of XOMA 129 lasted for about 8 hours. When rats received bolus injection of insulin (Humulin R, 1 IU/kg, s.c.), a more challenging model due to the rapid decrease of blood glucose, XOMA 129 - at doses of 3 and 10 mg/kg i.v. – countered the blood glucose drop within 10 minutes after injection and effectively normalized blood glucose. The analysis at 6 hours post-insulin dosing showed elevation of insulin and C-peptide, which might be due to receptor-mediated insulin clearance and/or the elevated blood glucose. Serum levels of XOMA 129 at 10 mg/kg showed a terminal half-life of ~4 hours. We also tested the ability of XOMA 129 to modulate blood glucose in minipigs. A 10mg/kg intramuscular dose significantly elevated blood glucose over several hours.

Based on results presented here, we conclude that XOMA 129 is a novel NAM insulin receptor-binding antibody fragment that can rapidly reverse hypoglycemia induced by either a bolus injection of insulin or oral sulfonylurea administration. In addition to rapid onset, XOMA 129 displays optimal duration and as such has potential as a novel treatment for acute hypoglycemic conditions.

Material and Method:

Cell lines: CHOK1 cells engineered to express either human INSR or rat INSR were used in the signaling assay. These cells were maintained in EX-CELL 302 serum-free medium (Sigma-Aldrich, St. Louis, MO), supplemented with 4 mM L-glutamine, and 0.4 mg/mL GENETICIN® (Invitrogen, Carlsbad, CA).

Antibody binding to insulin receptor: CHOK1 cells over-expressing the INSR human, rat, and minipig orthologs were plated in 96-well round bottom plates (Costar, cat# 3799). To assess parent antibody binding, cells were starved overnight in RPMI + 0.5% bovine serum albumin and then incubated with XOMA 129 in 50ul FACS buffer (PBS + 0.5% BSA + 0.1mM sodium azide) for 40 minutes at 4 ºC. Cells were stained with a secondary antibody, mouse anti-c-myc IgG (Roche, Basel, Switzerland). After washing twice with FACS buffer, cells were then stained with This secondary protocol was not used for this experiment. Replace with: Cells were washed and subsequently stained with a goat anti-human kappa FITC secondary antibody (Life Technologies) at a 1:100 dilution for 20 minutes at 4 ºC.
4oC.

pAKT assay: On the day before the assay, the cells were washed with PBS, re-suspended at 1 x 10^6 cells/mL in "Starvation Medium" containing RPMI 1640 (Invitrogen), 2 mM L-Glutamine, 0.4 mg/mL GENETICIN®, and 0.5% BSA, and incubated for 16-20 hours in a 37 ºC, 5% CO_2 incubator. The next day, cells were resuspended in PBS with 0.5% BSA and 1 x 10^5 cells were added to wells of a 96-well plate. After incubation, the treated cells were centrifuged, media decanted, and cells lysed in a buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 10 mM NaF, Phosphatase Inhibitor Cocktails 1 and 2 (Sigma-Aldrich), and Complete Mini Protease Inhibitor (Roche Diagnostics Corporation, Indianapolis, IN) for 1 hour with shaking at 4 ºC. The lysates were clarified by centrifugation at 485 x g for 3 minutes. Electrochemiluminescence ELISA using the MesoScale Discovery Multi-spot Assay System (Meso Scale Discovery, Gaithersburg, MD) was used to quantify the amount of phosphorylated AKT present within the lysates.

Animal models:

Hypoglycemia models in rat:

Sulphonylurea model: normal male Wistar rats, 7 weeks of age, were fasted for 2.5 hours. Glibencamide suspended in 0.5% methylcellulose was administered orally at a dose of 10mg /kg. XOMA 129 at either 10 or 3 mg /kg was administered intravenously. Blood glucose was measured at different time points.

Insulin Model: normal Sprague-Dawley rats, 11 weeks of age, were fasted for 2.5 hours. Humulin R (lot # C358896C, Eli Lilly and Company) was administered subcutaneously at a dose of 1IU/kg. XOMA 129 at either 10 or 3 mg /kg was administered intravenously. Blood glucose was measured at different time points The serum samples were collected at 360 min after insulin administration for analysis of insulin and C-peptide.

These experiments were performed at PreClinOmics, a Crown Bioscience Company (Indianapolis, IN).

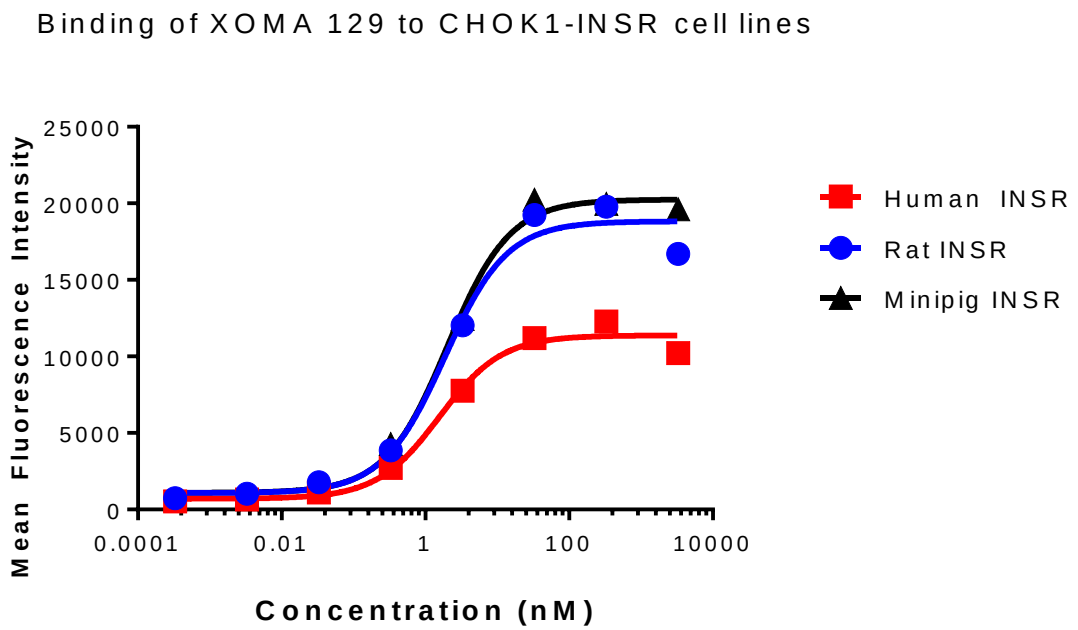
Minipig: normal male Gottingen minipigs, around 10 kg bodyweight, were fed at 6:00 am. Food was removed during the duration of the study. XOMA 129 was administered at 10 mg/kg intramuscularly. Blood glucose was detected using Abbott AlphaTRAK™.

This experiment was performed at Charles River Laboratories, INC (Spencerville, OH).

PK analysis: The PK of XOMA 129 was studied in normal Sprague-Dawley rats, administered as single dose intravenous bolus at 10 mg/kg. A sandwich ELISA method was used to measure the concentrations of XOMA 129. Data were analyzed using WinNonlin.

This experiment was performed at Charles River Laboratories, INC.

Figure 1: XOMA 129 binds to human, rat and minipig insulin receptors



	EC50 (nM)		
Fab	**human**	**rat**	**minipig**
XOMA 129	2	2	2

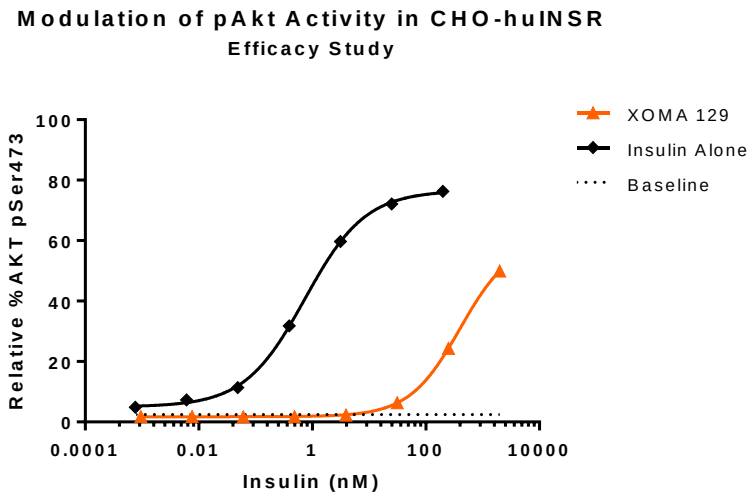
Figure 1: XOMA 129 binds to human, rat and minipig insulin receptor (INSR).

Starved CHOK1 cells over-expressing human, rat, or minipig INSR orthologs were plated at 25,000 cells per well then incubated with concentrations of XOMA129 Fab ranging from 0.001 to 100 ug/ml. Unbound antibody was washed away and cells were stained with Goat anti-human Kappa FITC secondary antibody (Life Technologies). The cells were washed and subsequently analyzed on a FACSCanto II™ flow cytometer (Becton Dickinson).

Data were analyzed using both FlowJo™ (Tristar, Paso Robles, CA) and GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Figure 2: XOMA 129 acts as a Negative Allosteric Modulator (NAM).

a



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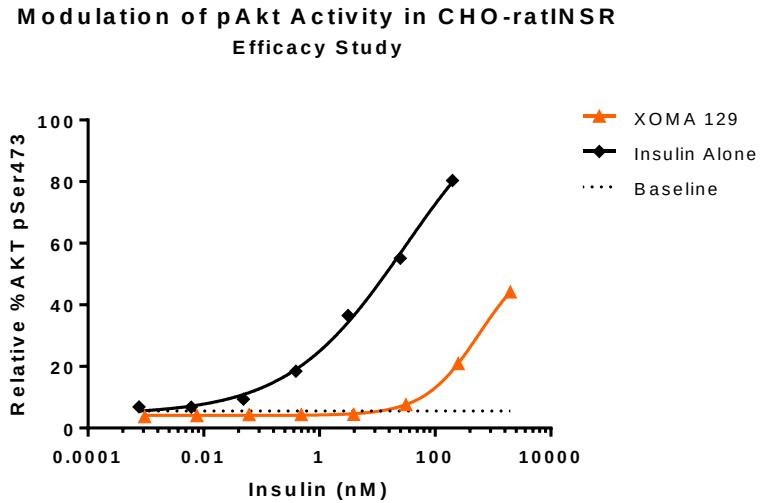

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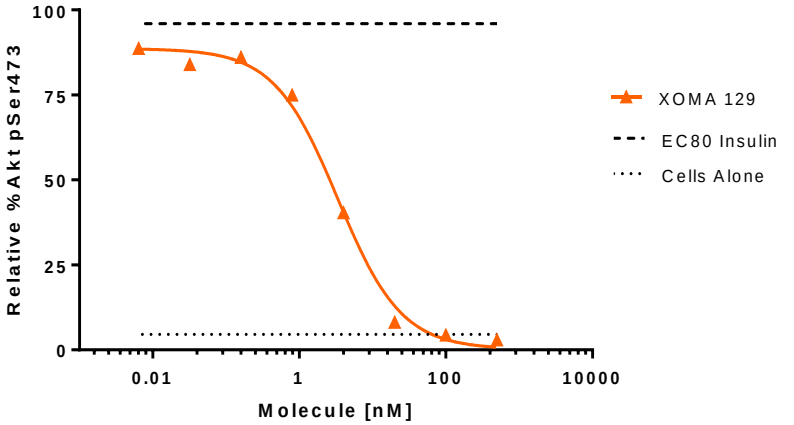

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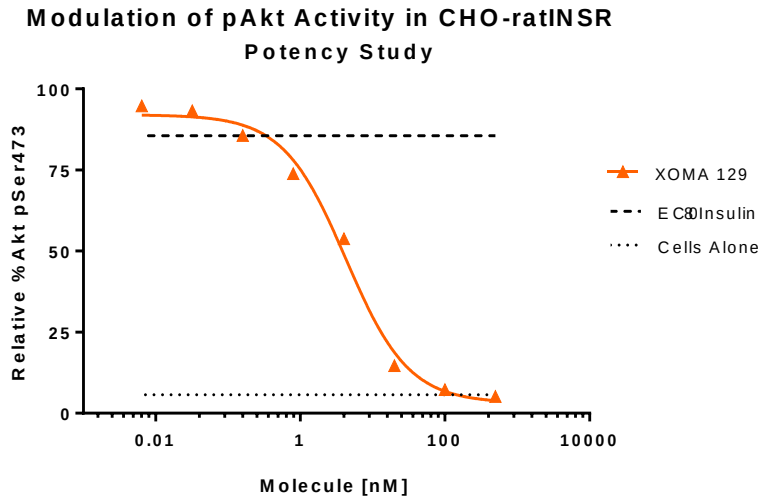

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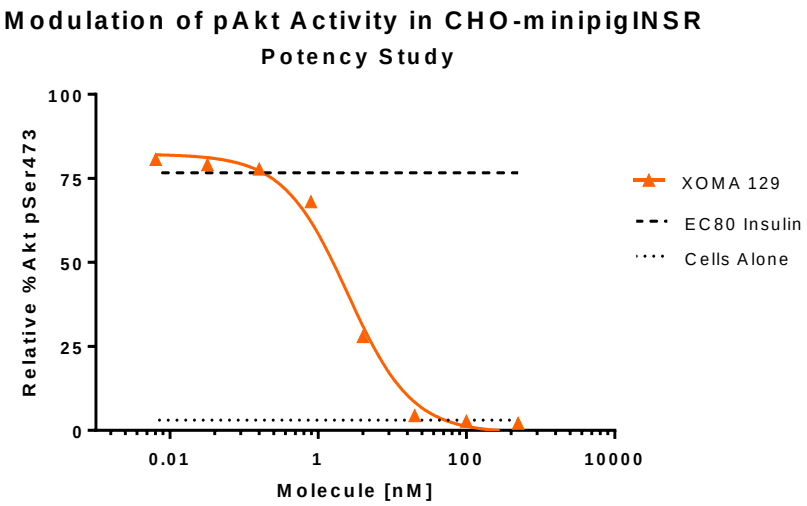


	EC50 (nM)		
Fab	**human**	**rat**	**minipig**
XOMA 129	3.4	4.3	2.5

Figure 2: XOMA 129 acts as a Negative Allosteric Modulator (NAM).

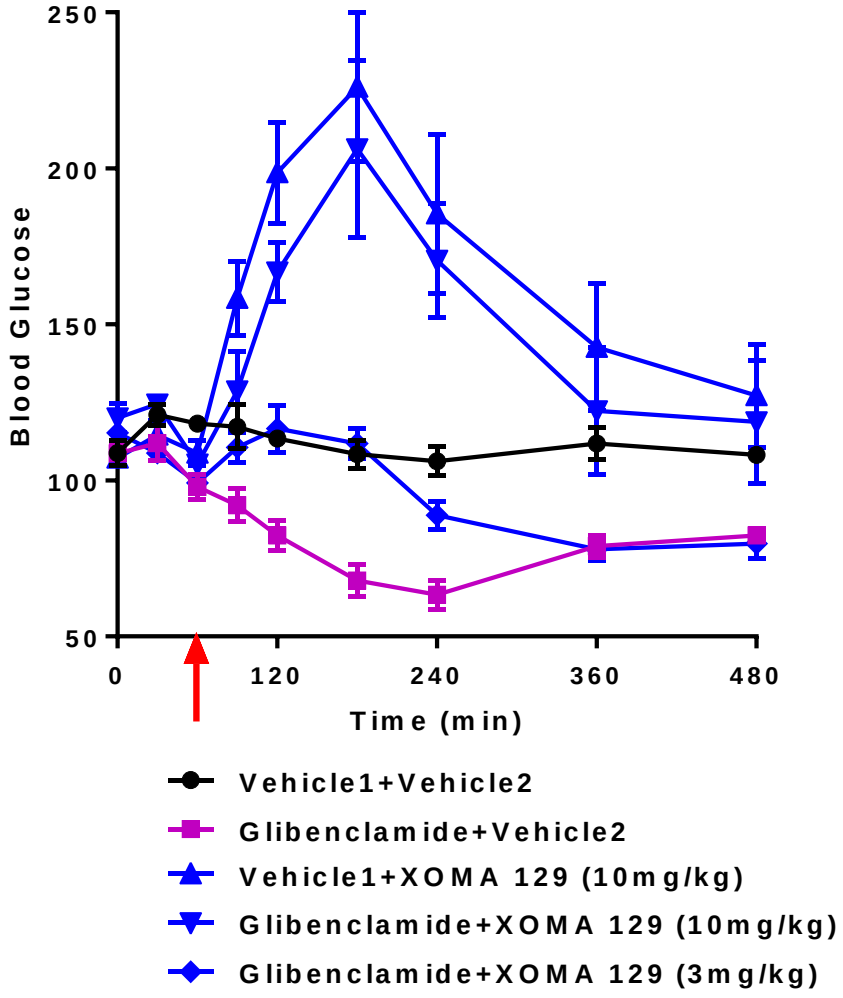
(**a-b**) Efficacy of XOMA 129. Starved CHO cells were treated with 500nM of XOMA 129 followed by incubation with various amounts of recombinant human insulin. After a 10 minute-incubation, cells were lysed and the amount of pAkt was quantified using the MesoScale Discovery Multispot Assay System.

(**c-e**) Potency of XOMA 129. Starved CHO cells were treated with an EC80 concentration of recombinant human insulin in the presence of various concentrations of XOMA 129. After a 10 minute incubation, cells were lysed and the amount of pAkt was quantified using the MesoScale Discovery Multispot Assay System.

Data were analyzed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Figure 3: XOMA 129 reverses hypoglycemia induced by glibenclamide and insulin.

a



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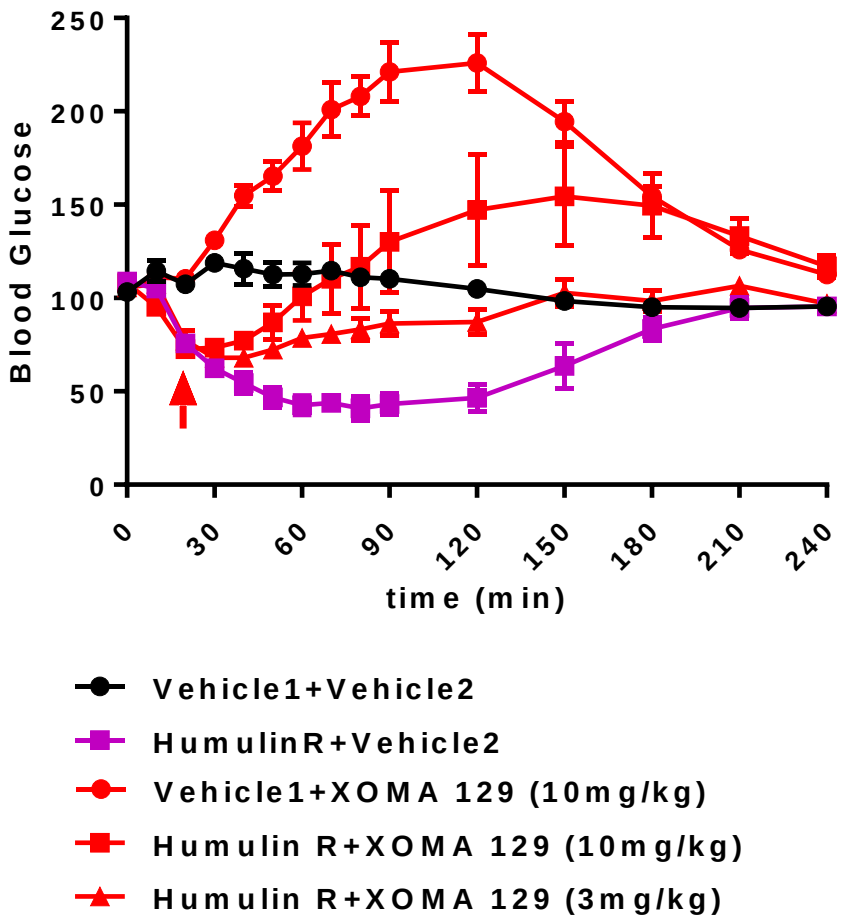


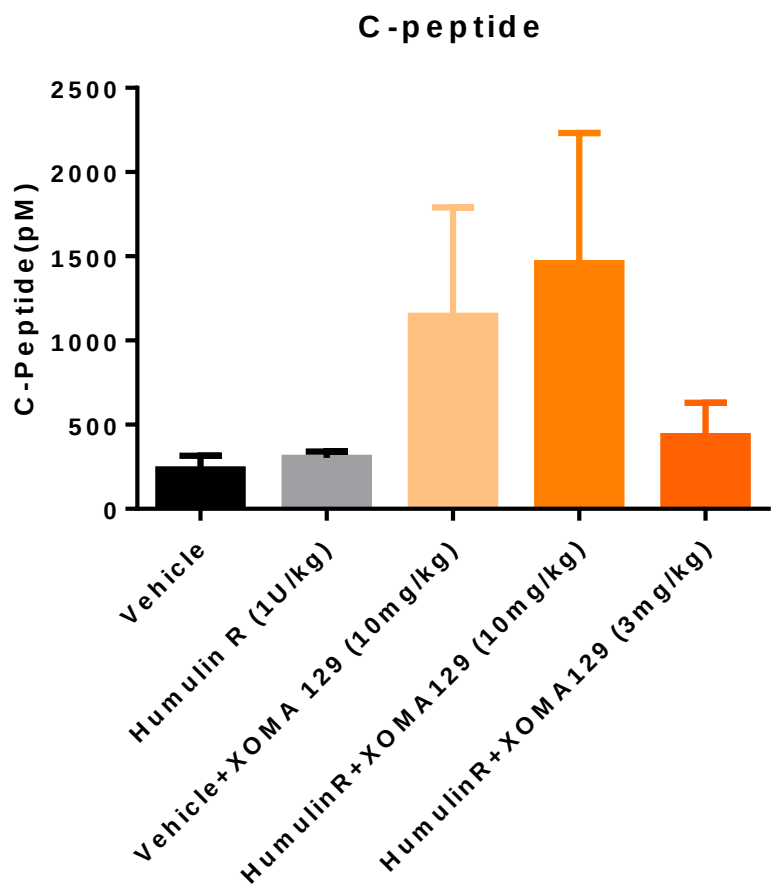
Figure 3: XOMA 129 reverses hypoglycemia induced by glibenclamide.

a) Blood glucose reading from the first 480 min in XOMA 129-treated groups. XOMA 129 was given at 10 and 3 mg/kg intravenously. Blood samples were taken from the tail before and at 30, 60, 90, 120, 180, 240, 360 and 480min after the administration of glibenclamide or vehicle for determination of the blood glucose. XOMA 129 was administered intravenously after 60 minutes (red arrow). Vehicle control group: black circle. Glibenclamide group, purple square. n=4~5 rats/group.

b) XOMA 129 was administered intravenously after 20 minutes (red arrow) at 10 and 3 mg/kg dose levels intravenously. Vehicle control group: black circle. Humulin R group, purple square. Blood samples were taken from the tail before and at 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210 and 240 minutes after Humulin R was given. n=4~5 rats/group. Data are means ±SEM.

Figure 4: Levels of C-peptide and insulin in serum 6 hours post dosing.

a



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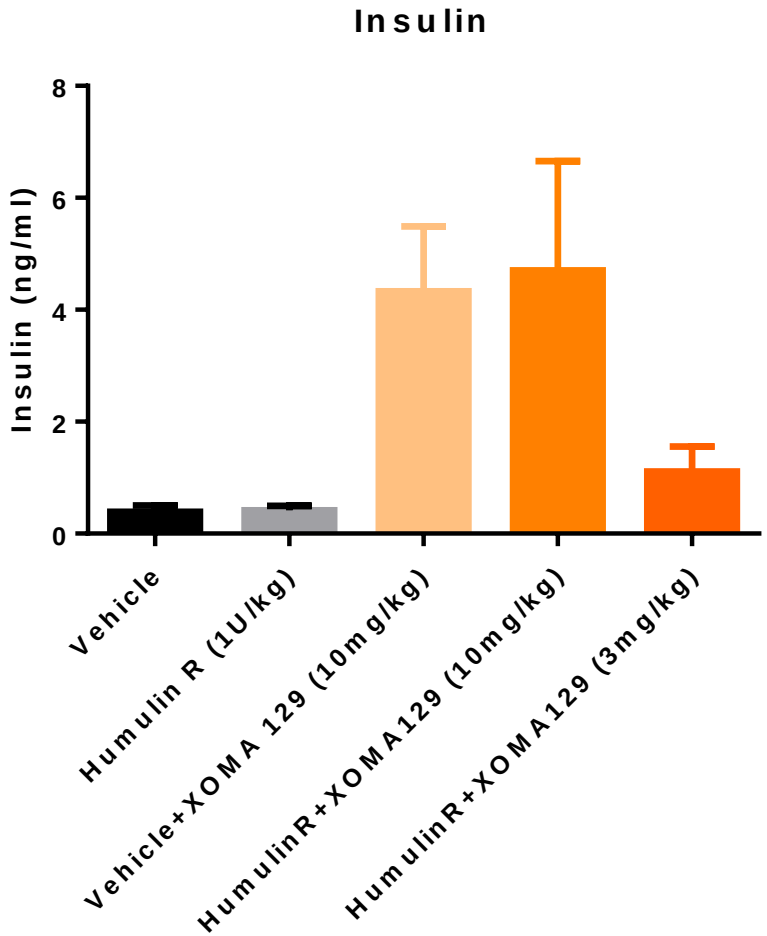


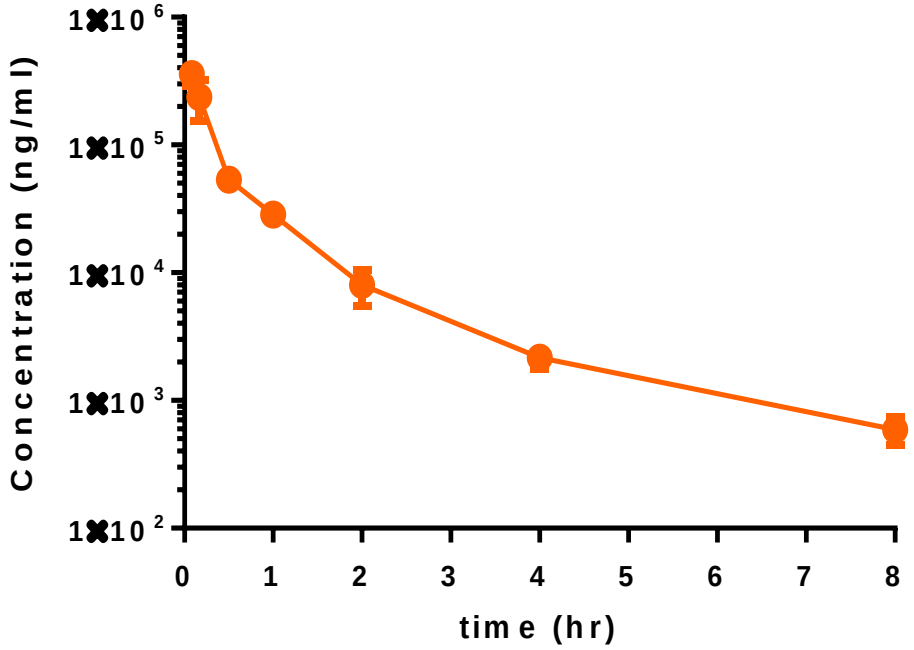
Figure 4. Levels of insulin and C-peptide in serum 6 hours post dosing.

Humulin R was administered subcutaneously at a dose of 1IU/kg. XOMA 129 was administered intravenously after 20 minutes (red arrow) at 10 and 3 mg/kg dose levels. n=4~5 rats/group.

(a - b) Blood samples were taken from cardio puncture 6 hours after Humulin R was given. Serum concentrations of C-peptide (a) and insulin (b) were determined via ELISA.

The experiments were performed at PreClinOmics, a Crown Bioscience Company (Indianapolis, IN).

Figure 5: XOMA 129 serum drug concentration vs time profile following IV administration.



Group	N	Cmax (ug/ml)	AUClast (ug*hr/ml)	Tmax (hr)	T1/2 (hr)	CL (ml/kg/hr)	Vss (ml/kg)
10 mg/kg IV	3	360	175	~0.1	4.9	59	82

Figure 5: XOMA 129 serum drug concentration vs time profile following IV administration.

Normal male Sprague-Dawley rats, administered as single dose intravenous bolus at 10 mg/kg. Blood samples were taken from the tail before and at 5, 10, 30 min, 1, 2, 4 and 8 hours after the administration of XOMA 129. Data are means ±SEM.

Figure 6: XOMA 129 modulates glucose in intramuscularly-treated minipigs.

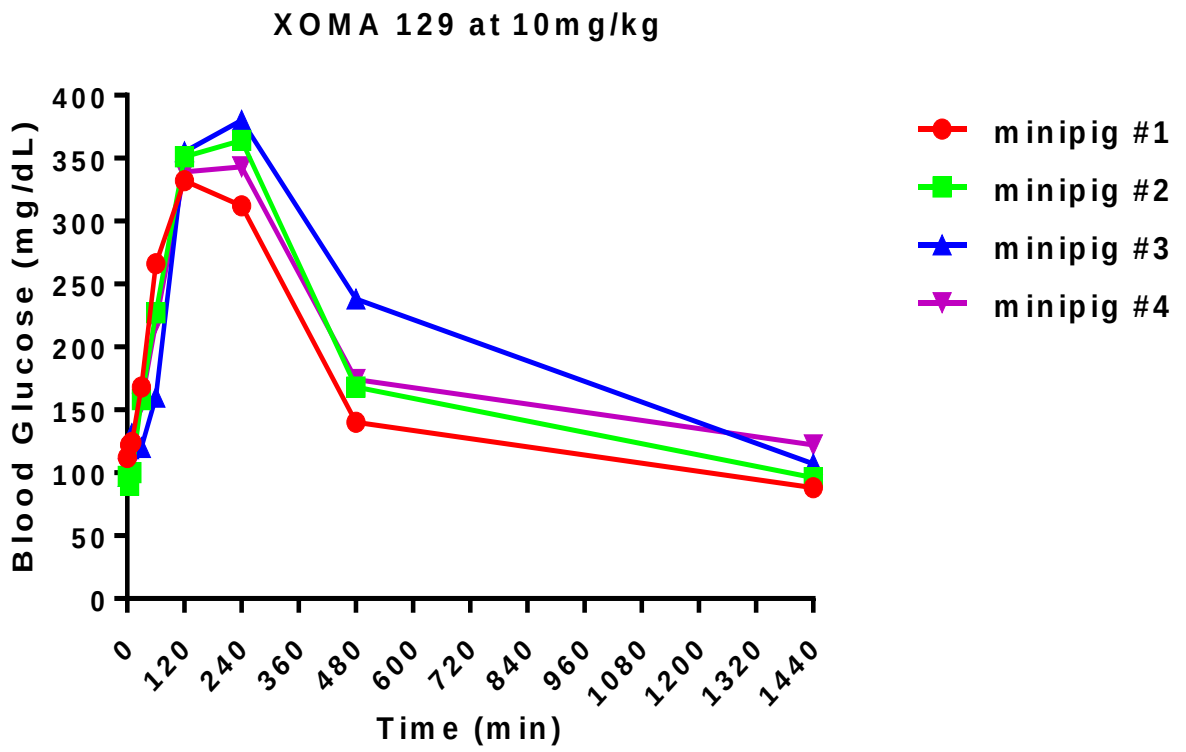


Figure 6: XOMA 129 modulates glucose in intramuscularly-treated minipigs.

Blood glucoses of 4 normal male Gottingen minipigs were plotted individually. XOMA 129 was administered at 10 mg/kg intramuscularly. Blood glucose was detected using Abbott AlphaTRAK™ before and at 5min, 10 min, 0.5, 1, 2, 4, and 8 hours after the administration.

Conclusions:

- XOMA 129 is a fully human, high affinity Fab, which acts as a NAM at the INSR.
- XOMA 129, at 10 and 3 mg/kg, reverses glibenclamide-induced hypoglycemia in a rat model.
- XOMA 129, at 10 and 3 mg/kg, reverses insulin-induced hypoglycemia in a rat model.
- Intramuscular administration of XOMA 129 can modulate blood glucose levels in minipigs for several hours.
- Results of the preliminary pharmacokinetics and pharmacodynamics of XOMA 129 in animals to support continued development as a novel targeted pharmacotherapy for the treatment of exogenous- or endogenous-induced hypoglycemic conditions.