Discovery of a Potent, Orally Efficacious Small Molecule Agonist of Glucagon-Like Peptide-1 (GLP-1) Receptor

*David J Bearss[1], Chenyu Lin[1], Kyle Medley[1], Hariprasad Vankayalapati[1]
[1]Biolexis Therapeutics, Inc., Salt Lake City, UT

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ABSTRACT

Introduction: Given its physiological roles, GLP-1 agonists are considered a class of drugs used in the treatment of type 2 diabetes and obesity. These agents mimic 7-36 amide Semaglutide, increase insulin release, decrease glucagon secretion, and a slowing of gastric emptying but are injectables, week half-life, and cold chain storage limit their utility. Several small molecule agonists of the GLP-1 receptor have been investigated as potential alternatives to peptide-based GLP-1 agonists like Danuglipron, and Orforglipron. Here we present the discovery of the MLX-7005 series exhibited on target-specific GLP-1 agonist activity in its binding and cellular studies.

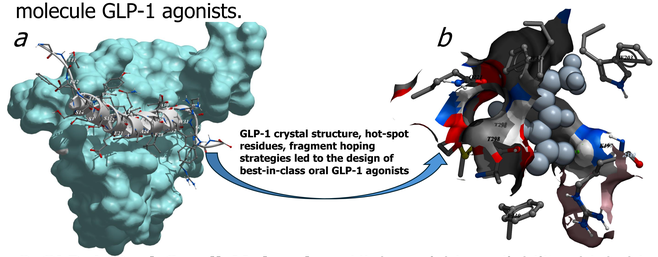
Methods: Tag-Lite GLP-1 receptor binding assay. Ca^{2+} mobilization in Min6-c4 cells. cAMP in PSC-HEK293 Cell Line Expressing GLP-1R. cAMP β-Cell Line 1.2B4. The cAMP Gs dynamic and a LANCE Ultra cAMP kit. GLP-1 agonist in db/db and DIO models of T2D and obesity.

Results: Small molecule GLP-1 agonists we discovered are orally available, stable, lower manufacturing costs than peptide-based drugs. We disclose MLX-7006 and its analogs, exhibiting potent activity in vitro MIN6 $Ca2^{+}$ assay dose-dependently with an EC_{50} of 202 nM and reference PF-06882961 (Danuglipron), GLP-1 peptide are 219 nM and 70 nM. The lead MLX-7006 additional *in vitro* and *in vivo* pharmacology profile results consistent with its on-target potency. MLX-7005, an initial lead and MLX-7006 had a high binding affinity to human GLP-1R. MLX-7006 strongly activated the GLP-1R cAMP pathway without inducing measurable β-arrestin recruitment signaling. The insulin secretion effect of MLX-7006 was evaluated in a functional human pancreatic beta cell line, in which both MLX-7006 and early lead MLX-7005 showed dose-dependent induction of insulin secretion.

Conclusion: In conclusion, MLX-7006 is a highly potent, orally available, GLP-1 agonist. Pre-clinical in vivo DIO, db/db rodent model's studies are underway along with safety, and tolerability to further evaluation as a potential therapy for T2DM and obesity will be presented.

MOLECULERN FRAGMENT-BASED DISCOVERY

1. Early Discovery: Figure 1: (a) Semaglutide peptide in complex with GLP-1 receptor and (b) Fragment-based design of oral small molecule GLP-1 agonists.

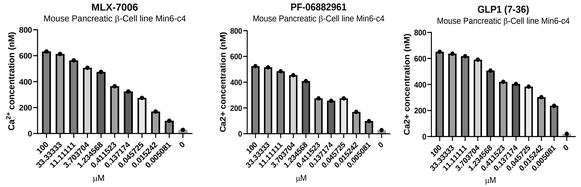


2 GLP-1 Oral Small Molecules: High oral bioavailability, high bio-efficiency. shortened half-life for a more controllable treatment, greater distribution, brain disposition characteristics, high stability, solid dosage form, room temp, developable, and reduced treatment costs.

IN VITRO RESULTS

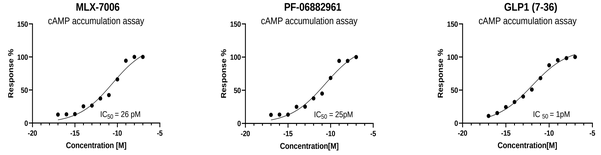
3 GLP-1R agonist mediated Ca^{2+} influx in Min6-c4 cells: Fluo-4 loaded Min6-c4 cells were treated with a range of concentration of MLX & reference agents. Fluorescence recorded as an index [Ca2+], [Ca^{2+}] was calculated using the formula: [Ca^{2+}] = Kd(F-F_{min})/(F_{max-F}), Kd if Fluo-4 taken as 345nm, F_{max} was obtained by ionomycin (2 μM) group and Fmin was obtained by EGTA (2 mM) group.

MLX Compds.	EC_{50} (nM)
MLX-7006	202
PF-06882961	219
GLP1 (7-36)	70



4 LANCE Ultra cAMP Accumulation Assay: HEK293T cells (ATCC, CRL-3216) transiently expressing GLP-1R (GLP1R-Tango plasmid bought from Addgene, cat no: 66295) for intracellular cAMP measurement performed using LANCE Ultra cAMP kit (PerkinElmer, TRF0264). LANCE Ultra cAMP assay is a homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) immunoassay designed to measure cAMP produced upon modulation of adenylyl cyclase activity.

MLX Compds.	EC_{50} (pM)
MLX-7006	26 pM
PF-06882961	25 pM
GLP-1 (7-36)	1 pM



PHARMACOKINETIC RESULTS

5 Oral Bioavailability and Brain/CSF uptake study of lead GLP-1 agonist MLX-7006 in Male Swiss Albino Mice & Male Wistar Rats:

Table 2	Male SA Mice		Wistar Rats	
Parameters	G1/IV	G2/PO	G1/IV	G2/PO
T_{max} (h)	NA	2.00	NA	0.5-1.0
C_0/C_{max}(ng/mL)	1866	3379	1445	490
AUC_{last}(ng.h/mL)	1931	3379	893	1672
AUC_{inf}(ng.h/mL)	1931	15951	912	1716
Cl(mL/min/kg)	8.60	-	19.28	NA
Vd (L/kg)	0.75	-	2.6	-
$T_{1/2}$ (h)	1.00	3.67	1.47	1.40
%F	-	100%	-	38%

DB/DB KEY PARAMETERS

- Body Weight (3x weekly)
- Fed plasma glucose (Day 1, 14, 28)
- Fasting Blood Glucose (Day 21 and 29)
- Glucose tolerance tests (Day 21 OGTT)

Time point (0, 15, 30, 60, 90 & 120)

- Urinary glucose excretion
- Test compound bioanalysis: Plasma & Liver

  Time point: 4h Post dosing (Day 29)
- Plasma Insulin, HbA1c and fructosamine
- Plasma Biochemistry (TG, Cholesterol, HDL and LDL)
- Optional: Terminal Tissue Collection for Histopathology, qPCR, Western blot, Bioanalysis or any other biomarker
- In Plasma, Pancreas, Liver, Adipose tissue, Skeletal muscle, and Heart samples collected on Day 29 for complete PK
- Urine collection for glucose (Day 27)

In plasma (4hr post-last- dosing) samples collected on Day 21 for satellite PK

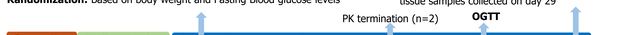
MOUSE DB/DB EFFICACY RESULTS

6: db/db Study Protocol

Study Protocol: Evaluation of Dose Response of MLX-7006 on Metabolic Parameters in db/db Male C57Bl/6 (db/db), 8-9 weeks old.

Randomization: Based on body weight and Fasting Blood glucose levels

End of Study: In fasted conditions plasma & tissue samples collected on day 29

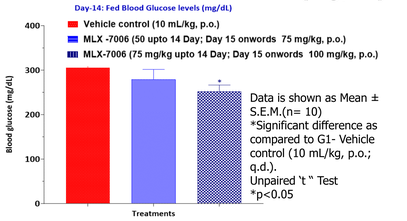


Group	Treatment Groups, n =10
G1	Disease Control + Vehicle 10 mL/kg, p.o. X 1-14 days, Q.D
G2	MLX-7006 50 mg/kg, p.o., X 1-14 days,Q.D
G3	MLX-7006 75 mg/kg, p.o., X 15-29 days,Q.D

Group	Treatment Groups, n =10
G1	Disease Control + Vehicle 10 mL/kg, p.o. X 15-29 days, Q.D
G2	MLX-7006 75 mg/kg, p.o., X 15-29 days,Q.D
G3	MLX-7006 100 mg/kg, p.o., X 15-29 days,Q.D

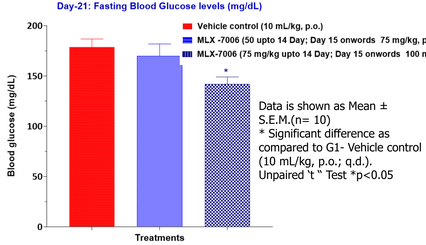
Group	Treatment Groups, n =2
G1	MLX-7006 50 mg/kg, p.o., X 1-14 days,Q.D
G2	MLX-7006 75 mg/kg, p.o., X 1-14 days,Q.D

7: Day 14-Fed Blood Glucose (mg/dL)



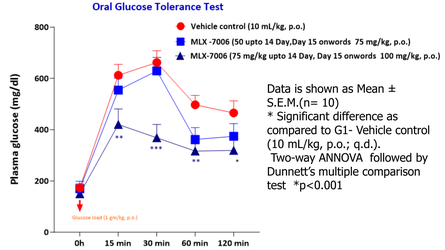
Data is shown as Mean ± S.E.M.(n= 10)
*Significant difference as compared to G1- Vehicle control (10 mL/kg, p.o.; q.d.).
Unpaired 't " Test
*p<0.05

8: Day 21 Fasting Blood Glucose (mg/dL)



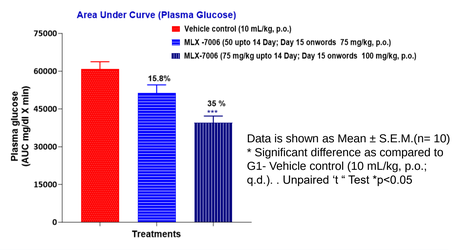
Data is shown as Mean ± S.E.M.(n= 10)
* Significant difference as compared to G1- Vehicle control (10 mL/kg, p.o.; q.d.).
Unpaired 't " Test *p<0.05

9: Day 21 Oral Glucose Tolerance Test (OGTT) (mg/dL)



Data is shown as Mean ± S.E.M.(n= 10)
* Significant difference as compared to G1- Vehicle control (10 mL/kg, p.o.; q.d.).
Two-way ANNOVA followed by Dunnett's multiple comparison test *p<0.001

10: Day 21 OGTT Area Under Curve (min X mg/dL)



Data is shown as Mean ± S.E.M.(n= 10)
* Significant difference as compared to G1- Vehicle control (10 mL/kg, p.o.; q.d.). . Unpaired 't " Test *p<0.05

DISCUSSION AND CONCLUSIONS

- MLX-7006 showed on target efficacy in cAMP and Min6-c4 cellular activities.
- Induced glucose dependent insulin secretion on Min6-c4 and cAMP assays.
- C57B/6 db/db mouse model study demonstrated significant difference in tis fed/fast blood glucose levels when compared with vehicle groups.
- OGTT and AUCs tests show significant dose dependent effect with 35% at dose of 100 mg.
- Mice, Rat & Dog species PK results are in line with compound characteristics as a clinical candidate.