Pharmacology Discovery Services

A Eurofins Discovery Partner Lab

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White Paper

Diabetes Drug Discovery Utilizing *in vitro* panels and *in vivo* animal models to evaluate diabetes drug candidates

Diabetes mellitus is a serious chronic disease in which blood glucose levels rise due to the body's inability to produce enough insulin or to use it effectively. The global prevalence of diabetes continues to grow. Over 535 million adults (age 20-79) have diabetes, according to the International Diabetes Federation, that in 2021 caused 6.7 million deaths – 1 death every 5 seconds. The number of people suffering from diabetes is projected to grow to 643 million by 2030, and reach 783 million by 2045.[1] Among all diabetics, over 90% have Type 2 diabetes mellitus (T2DM or T2D); however, Type 1 diabetes mellitus (T1DM or T1D) is the most common form of diabetes in children (<19 years of age), and over 1.2 million children have T1D in 2021.[2]

T1D, insulin-dependent diabetes mellitus (IDDM), is primarily a chronic autoimmune disease with potential autoantigens identified. It is characterized by progressive autoreactive T cell-mediated destruction and loss of insulin-secreting pancreatic β islet cells, leading to deficient secretion of insulin and hyperglycaemia.[3,4] A combination of genetic, immunologic, and environmental factors contributes to the onset and progression of T1D. Treatment of T1D focuses on managing blood sugar levels with insulin replenishment, diet, and lifestyle to prevent further complications.[5] The research effort is underway to develop a treatment to prevent β-cell loss and/or preserve β-cell function.

T2D, noninsulin-dependent diabetes mellitus (NIDDM), is due to a progressive loss of adequate β-cell insulin secretion and the inability of insulin-sensitive tissues to respond appropriately to insulin, which is known as insulin resistance (Figure 1).[6] The pathogenesis of T2D involves polygenic and environmental factors and is strongly linked with such risk factors as obesity and being overweight. Besides lifestyle changes to reduce blood sugar levels, the treatment for T2D is more diverse (see under "Diabetes Drug Discovery") and more complicated than for T1D.[7,8]

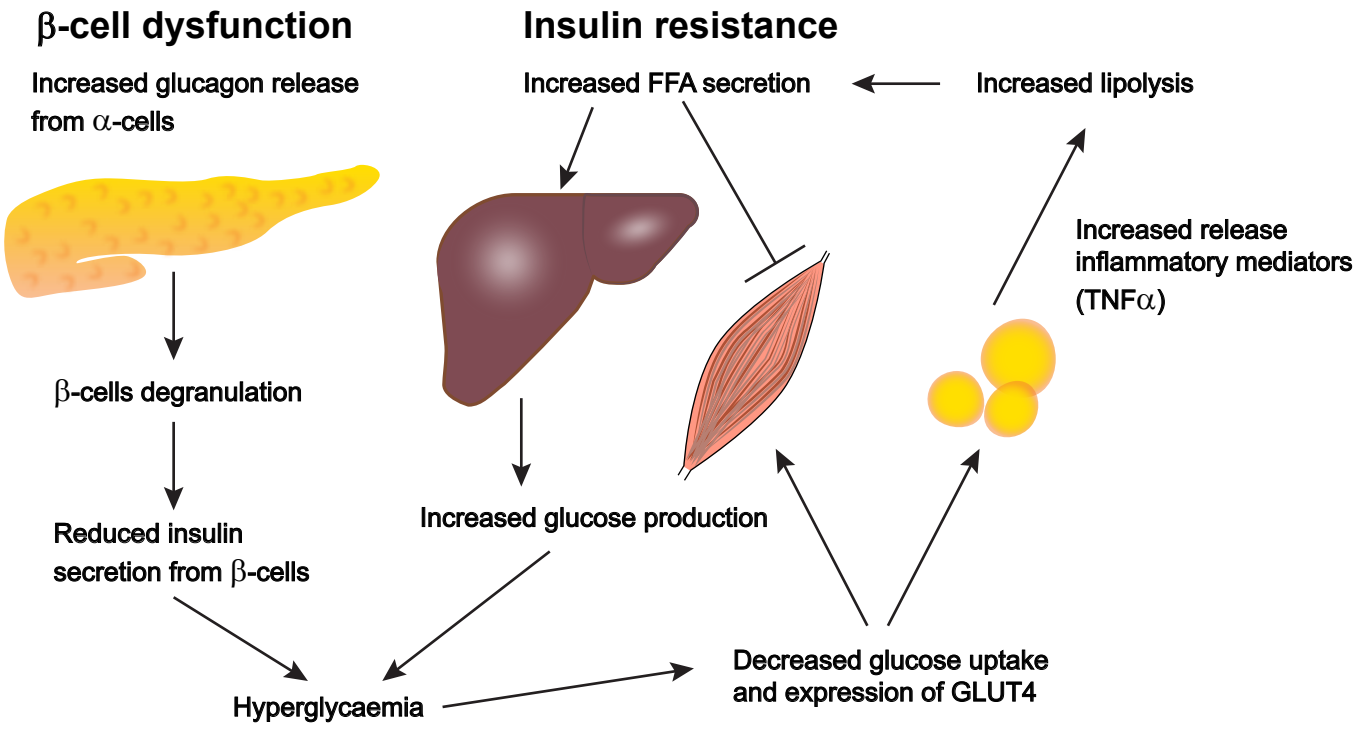

Figure 1. Pathology of T2D. Insulin is a hormone synthesized by β-cells of pancreatic islets, playing a key role in glucose homeostasis by reducing blood glucose levels. Insulin activates insulin receptors (INSR) in the liver, skeletal muscle, and adipose tissue to stimulate glucose transporter (GLUT4) translocating from the intracellular site to the cell membrane, making the cells efficiently uptake glucose. β-cell dysfunction follows damage from elevated free fatty acid (FFA) levels, obesity, insulin resistance, and inflammation. β-cells can compensate by promoting insulin release initially; however, as time passes, the compensatory mechanism declines, and β-cell mass decreases. The loss of β-cell mass is due to cell degranulation, causing increased α-cell glucagon and decreased β-cell insulin. Meanwhile, skeletal muscle and adipocytes are incapable of modulating the increased glucose levels. In addition, overloaded adipocytes increase lipolysis and cytokine release of inflammatory mediators such as TNF-α. Released FFAs also stimulate glycogenolysis in the liver. T2D is ultimately caused by a hyperglycaemia environment. Figure 1 is modified from Riddy DM, *et al*, 2018.[6]

Currently, there are nine major classes of antidiabetic agents for T1D and T2D (Table 1), including biguanide, DPP4 inhibitors (Dipeptidyl peptidase-4 inhibitor), SGLT2 inhibitors (sodium-glucose cotransporter 2 inhibitors), insulin/insulin analogues, GLP1 (glucagon-like peptide 1), amylin analogues, sulfonylureas, thiazolidinediones, and α-glucosidase inhibitors.[7,8] According to international guidelines, patients should take metformin as the first-line T2D treatment. Some classes of antidiabetic agents offer benefits in addition to glucose-lowering; in particular, they may avoid low blood glucose (hypoglycaemia), protect the heart and kidneys, and prevent weight gain. Therefore, they may be combined with metformin for T2D patients with certain risk factors.[9] Also, combination therapy with drugs of different mechanisms is often applied to better maintain blood glucose levels.[10] However, even though myriads of therapeutic agents are clinically available, various adverse side effects are still associated with current therapies (Table 1).

Antidiabetic Drug Class	Representative Clinical Agent	Therapeutic Target	Mechanisms of Action	Adverse Effects
Biguanide	Metformin	AMPK (Indirect)	Improve insulin sensitivity, decrease glucose production	Nausea, lactic acidosis
DPP4 Inhibitors	Sitagliptin, Saxagliptin	DPP4	Improve incretin secretion, improve insulin secretion	GI discomfort, heart failure
SGLT2 Inhibitors	Canagliflozin, Dapagliflozin	SGLT2	Inhibit renal glucose reabsorption	Ketoacidosis, genital mycosis, bone fracture
Insulin Analogues	Humulin R, Novolin R	INSR	Stimulate glucose uptake	Hypoglycaemia, weight gain, injection site reaction
GLP1 Analogues	Liraglutide, Semaglutide	GLP1R	Improve insulin secretion Increase satiety, delay gastric empty	Nausea, injection site reaction
Amylin Analogues	Pramlintide	AMY Receptor	Delay gastric empty, inhibit glucagon secretion	Nausea
Sulphonylureas	Glyburide	K_{ATP}	Improve insulin secretion	Hypoglycaemia, CV risk, weight gain
Thiazolidinediones (TZDs)	Pioglitazone	PPARγ	Improve insulin sensitivity, stimulate glucose uptake	Weight gain, edema, heart failure
α-Glucosidase Inhibitors	Acarbose	α-Glucosidase	Inhibit carbohydrate degradation	GI discomfort, diarrhea

Table 1. Current antidiabetic agents.

There are approximately 60 FDA-approved antidiabetic drugs, and many more are being evaluated in clinical trials.[7,8] The enormous heterogeneity of diabetes drives more precise and appropriate treatment. Figure 2 shows antidiabetic clinical trials from 2010 to 2021, with a total of 103 cases across 37 targets collectively.[5,8,9,11] The majority of entries identified target GLP1R, GCK, GCGR, SGLT1, and SGLT2 receptors (47% in total), as well as the combination of these targets (e.g., GLP1R/GCGP, GLP1R/GIPR, or GLP1R/GIPR/GCGR) (14%) (Figure. 2A). Small molecules and peptides are the major types of study drugs (55% and 31%, respectively) (Figure. 2B). T2D is the dominant diabetes type for antidiabetic drug development (Figure. 2C).

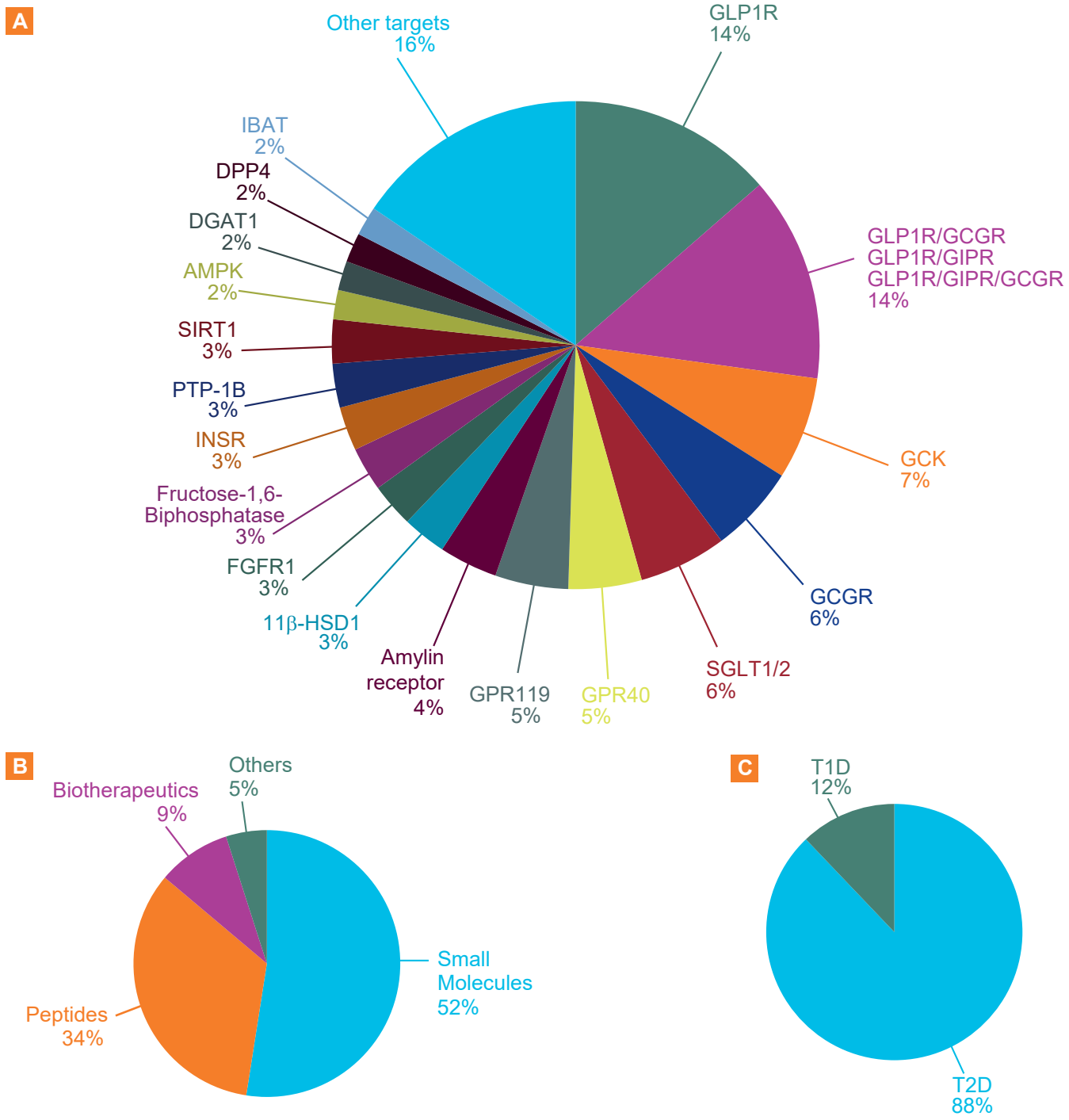

Figure 2. Diabetes drug discovery and development. Major antidiabetic drug classes are currently in various clinical phases of drug development. Percentages represent the percentage of unique compounds currently in development, per respective class based on A. Therapy targets, B. Compound types, and C. Diabetes types. Clinical trials started from 2010 to 2021, with a total of 103 cases across 37 targets collected.[5,8,9,11] The discontinued clinical trials are excluded.

In Vitro Panels For Diabetes Drug Discovery

Eurofins Discovery offers two *in vitro* diabetes panels – Insulin Release Panel and Insulin Sensitivity Panel, to be used to identify hits for drug discovery. Both panels cover approximately 70% of core therapeutic targets under clinical development for the treatment of diabetes (Figure 2). The Insulin Release Panel contains 10 therapeutic targets that are highly involved in insulin secretion (Table 2). The Insulin Sensitivity Panel comprises seven therapeutic targets that are highly involved in insulin resistance (Table 3).

Insulin Release Panel (Item #PP277)

This panel contains 10 target-based assays and 1 phenotypic-based assay. The biological relevance of each target is described in Table 2. Representative results from the enzymatic glucokinase (GCK) activation assay and cell-based SGLT1 inhibition assay are demonstrated in Figure 3.

Class	Target	Item#	Biological Relevance
GPCR	GLP1R	231710	GLP1R activation increases insulin secretion in a glucose-dependent manner and reduces glycaemia by inhibiting glucagon secretion.
	GIPR	By Inquiry	The combination of GLP1 and GIP analogues improves glycaemic control and weight loss. Chimeric peptides that mimic GIP and GLP1 have been developed for diabetes treatment.
	GPR40	By Inquiry	GPR40 activation stimulates the intestinal secretion of GLP1 and GIP with the pancreatic secretion of insulin in a glucose-dependent manner.
	NPY1R	257010	NPY1R activation may particularly contribute to insulin secretion after weight-loss surgery for T2D. The analogues of PYY(1-36), a selective NPY1R agonist, have been designed for diabetes treatment.
	GCGR	231680	GCGR antagonists that inhibit glucagon actions can counter high glycaemia in diabetes. Several GLP1R/GCGR dual agonists and GLP1R/GCGR/GIPR tri-agonists have been developed for diabetes treatment.
Kinase	GCK	199101-0	GCK activation promotes hepatic glucose uptake, glycogen synthesis, and enhances glucose-stimulated insulin secretion from the pancreas.
Peptidase	DPP4	199007	DPP4 plays a key role in the clearance of GLP1. DPP4 inhibition has been well-established for glycaemic control improvement in T2D patients.
Ion Channel	K_{ATP}	265600	Inhibition of pancreatic K_{ATP} channels leads to depolarization and increases intracellular calcium levels, resulting in insulin release.
Transporter	IBAT	314100-1	IBAT inhibition prevents bile acid reabsorption, which leads to GLP1 secretion and GLP1R activation.
	SGLT1	355710-1	SGLT1 inhibition delays glucose absorption in the small intestine and colon, resulting in glucose reduction, insulin secretion, and glycaemic control improvement.
Phenotypic	Insulin Release	331500-0	Pancreatic islet beta-cell line HIT-T15 is used to assess the ability to stimulate insulin release by test compounds.

Table 2. The biological relevance of each target included in the Insulin Release Panel.

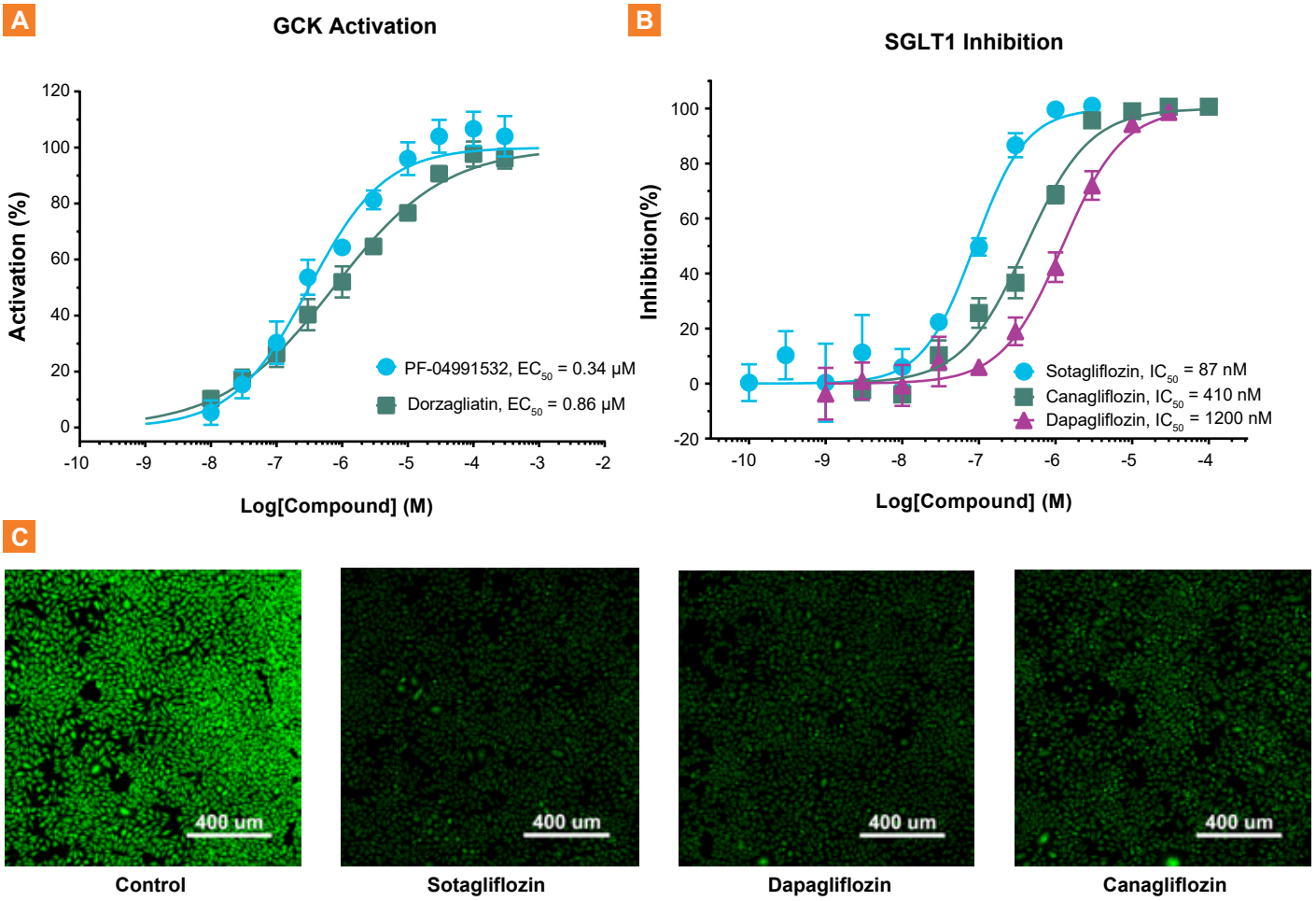

Figure 3. Demonstrated results for the Insulin Release Panel. A. Enzymatic GCK activation assay (#199101-0). GCK is a promising therapeutic target in which several clinical candidates are under clinical development for diabetes indication.[7] The EC_{50} of the two reported GCK activators were validated via enzymatic GCK activation assay. PF-04991532 and Dorzagliatin are clinical candidates for T2D treatment in Phases 2 and 3, respectively.[12,13] B. Cell-based SGLT1 inhibition assay (#355710-1). SGLT1 inhibition is an emerging antidiabetic strategy by reducing dietary glucose absorption in the intestine and increasing the release of gastrointestinal incretins like GLP-1.[14] The IC_{50} of the approved antidiabetic agent was validated through the image-based SGLT1 uptake assay. Sotagliflozin (Zynquista™), the first-in-class dual inhibitor of SGLT1/2, was approved by EMA for T1D indication in 2019.[15] Canagliflozin and Dapagliflozin, the selective SGLT2 inhibitors, are antidiabetic agents approved by FDA in 2013 and 2014, respectively.[16] C. Representative images for SGLT1 inhibition assay. Uptake of the fluorescent substrate, 1-NBDG (160 μM), was performed by stably-expressed human SGLT1 cells (Control) and was significantly inhibited in the presence of Sotagliflozin (300 nM), Dapagliflozin (3 μM), or Canagliflozin (1 μM). The substrate uptake level was analyzed via high-content imaging.

Insulin Sensitivity Panel (Item #PP278)

This panel contains seven target-based assays. The biological relevance of each target is summarized in Table 3. Representative results from the enzymatic AMPK activation assay and enzymatic 11β-HSD1 inhibition assay are demonstrated in Figure 4.

Class	Target	Item#	Biological Relevance
Kinase	AMPK	199100-0	AMPK is the center of energy metabolism that regulates glucose uptake, glucose, and lipid metabolism. The first-line antidiabetic drug metformin, an indirect AMPK activator, is widely prescribed for T2D patients.
	INSR	243000	INSR activation causes the translocation of GLUT4 to the cellular membrane. This enhances glucose uptake and metabolism. Abnormally expressed INSR is highly associated with insulin resistance.
	GSK3β	176500	GSK3β inhibition enhances glycogenesis and glucose metabolism. GSK3β signaling pathway is highly correlated with diabetes complications such as diabetic neuropathy.
NHR	PPARγ	267500	PPARγ activation improves insulin sensitivity by increasing glucose uptake through GLUT4 in muscle and reducing free fatty acid by lipogenesis. This enhances the utilization of glucose.
Phosphatase	PTP1B	192010	PTP1B Inhibition suppresses activated INSR and leptin receptors, leading to insulin sensitivity improvement.
Deacetylase	SIRT1	By Inquiry	SIRT1 activation inhibits PTP1B activity and thus increases insulin sensitivity. SIRT1 activation also enhances insulin secretion.
Dehydrogenase	11β-HSD1	125710	Abnormal high-cortisol levels are highly associated with insulin resistance and diabetes. 11β-HSD1 catalyzes the conversion of cortisone to cortisol. 11β-HSD1 inhibition reduces cortisol levels and results in insulin sensitivity improvement.

Table 3. The biological relevance of each target included in the Insulin Sensitivity Panel.

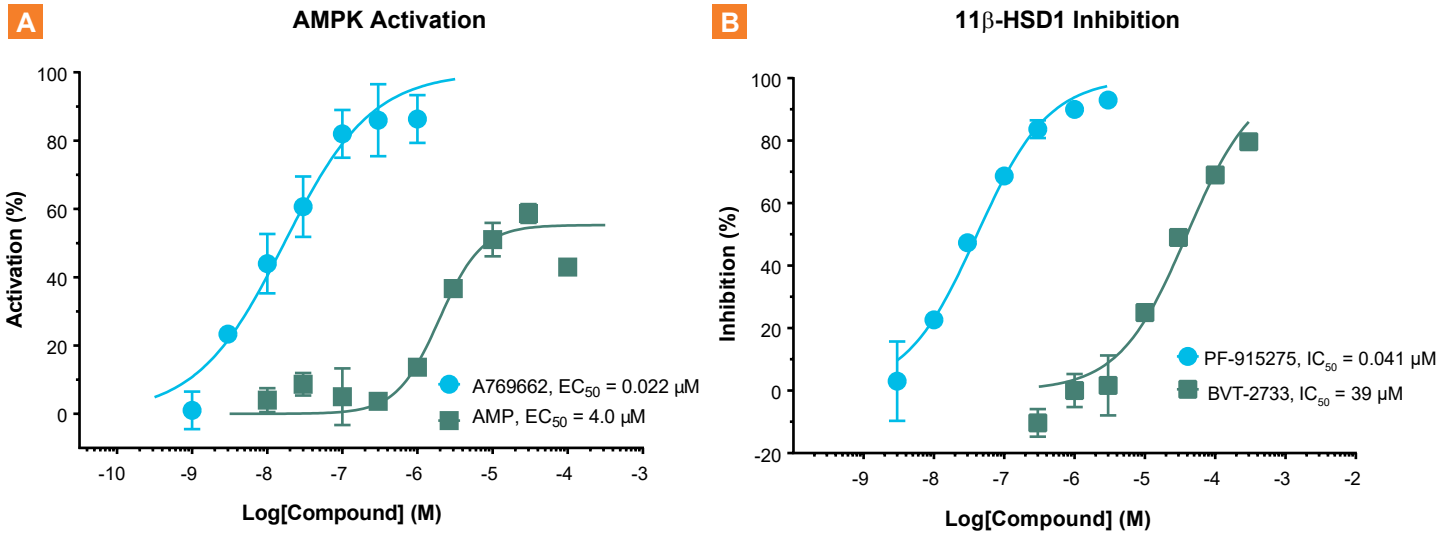

Figure 4. Demonstrated results for the Insulin Sensitivity Panel. A. Enzymatic AMPK activation assay (#199100-0). AMPK is the center of energy metabolism that regulates glucose uptake, glucose, and lipid metabolism.[17] The first-line antidiabetic drug metformin, an AMPK activator, is widely prescribed for T2D patients. The EC_{50} of the two reported AMPK activators, A769662 and AMP, was validated via the enzymatic AMPK activation assay.[18] B. Enzymatic 11β-HSD1 inhibition assay (#125710). 11β-HSD1 is a critical metabolic enzyme that catalyzes pathophysiological processes like T2D and obesity. The 11β-HSD1 inhibition represented an attractive therapeutic strategy for the treatment of T2D.[19] The IC_{50} of the selective 11β-HSD1 inhibitors, BVT-2733 and PF-915275, was validated via the enzymatic 11β-HSD1 inhibition assay.[19,20]

In Vivo Models Of Diabetes From Pharmacology Discovery Services

The ideal preclinical animal model of diabetes would reproducibly mimic the diverse disease pathogenesis that is observed in diabetic patients, and the model should be readily available and should not be a cost burden.[21] None of the currently available diabetes models fully replicates the disease.

Animal models for T1D can broadly be divided into two types: genetic models and chemical-induced models. Genetically T1D-predisposed models include nonobese diabetic (NOD) mice and diabetes-prone BioBreeding (BB) rats, which display many of the characteristics of human T1D.[22] Streptozotocin (STZ), an antineoplastic agent, when given in a single injection at a large dose induces diabetes often referred to as a model for T1D; it is used to study diabetic β-cell glucotoxicity. STZ can cause partial destruction of pancreas β-cell, leading to insulin deficiency and hyperglycaemia in mice and rats.

Rodent animal models of T2D include genetic and diet-induced. These models have been shown to recapitulate the hallmarks of the disease in humans: elevated fasting glucose and glucose intolerance. Drugs for T2D in humans are equally effective at reversing hyperglycaemia in these models. Thus, although differences in the progression of the disease exist between humans and model animals, T2D animal models are extensively used in evaluating novel anti-diabetic drugs. The most commonly used genetic T2D models include *ob/ob* mice, *db/db* mice, Zucker diabetic fatty (ZDF) rats, and KK-A^y mice.[23] These strains carry single gene spontaneous mutations in either the leptin (Lep^{ob}, *ob/ob* mice) or the leptin receptor ($Lepr^{db}$, *db/db* mice and $Lepr^{fa/fa}$, ZDF rats) genes in an inbred C57BL/6J, or a C57BLKS/J background, respectively, or *A* genes (KK-A^y mice).[24] Diabetes and obesity in humans, however, is a polygenic event and very few diabetic patients carry mutations in their *Lep, Lepr,* or *A* genes. There are other polygenic diabetic mice strains that develop diabetes due to a combination of multiple diabetes-susceptibility alleles, which may be more suitable for studies on drug candidates with different mechanisms of action.

Patients with advanced diabetes if not properly treated often develop complications due to excessive blood glucose rise, including nephropathy, neuropathy, retinopathy, liver steatosis, impaired wound healing, and increased risk of cardiovascular diseases. Researchers have developed animal models to test for these complications, including Pharmacology Discovery Services (PDS).

Pharmacology Discovery Services (PDS) currently offers one T1D model, i.e., the STZ-induced diabetes model in both mice and rats, as well as three T2D rodent models, KK-A^y mice, *db/db* mice, and ZDF rat model. In addition to both T1D and T2D diabetes models, PDS also offers rodent models of diabetic complications, e.g., diabetic nephropathy, wound healing in diabetic mice (item #595020), liver steatosis and NASH (items #546080 and #546082, learn more in our recent NASH White Paper).

In this Diabetes White Paper, we will first discuss Oral Glucose Tolerance Test (OGTT), a test commonly used in diabetic research, and then describe animal models for T1D and T2D.

Oral Glucose Tolerance Test (OGTT)

The glucose tolerance test is a medical test in which glucose is given and blood samples are taken afterward to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, impaired beta cell function, and sometimes reactive hypoglycaemia and acromegaly, or rarer disorders of carbohydrate metabolism.

OGTT Protocol In Mouse

We use groups of six male or female ICR or C57BL/6 mice weighing 22 ± 4 g. Animals are fasted overnight (~16 hours), and test article is administered to test animals 30 minutes before oral glucose (1 g/kg) loading. Blood is collected from the tail vein, and the blood glucose is measured by a Glucometer at -30 (pre-treatment), 0 (before glucose loading), 30, 60, 90, and 120 minutes after glucose loading. The area under the curve over 120 min ($AUC_{0\sim120\ min}$) is determined. In addition, the peak blood glucose is compared at all time points.

OGTT Protocol In Rat

We use groups of six male or female Wistar or Sprague Dawley (SD) rats weighing 200 ± 40 g. Animals are fasted overnight (~16 hours) and test article is administered to test animals 30 minutes before oral glucose (1 g/kg) loading. Blood is collected from the tail vein, and blood glucose is measured by a Glucometer at -30 (pre-treatment), 0 (before glucose loading), and at post-glucose loading at 30, 60, 90, and 120 minutes. The area under the curve over 120 min ($AUC_{0\sim120\ min}$) is also determined. In addition, the peak blood glucose is compared at all time points.

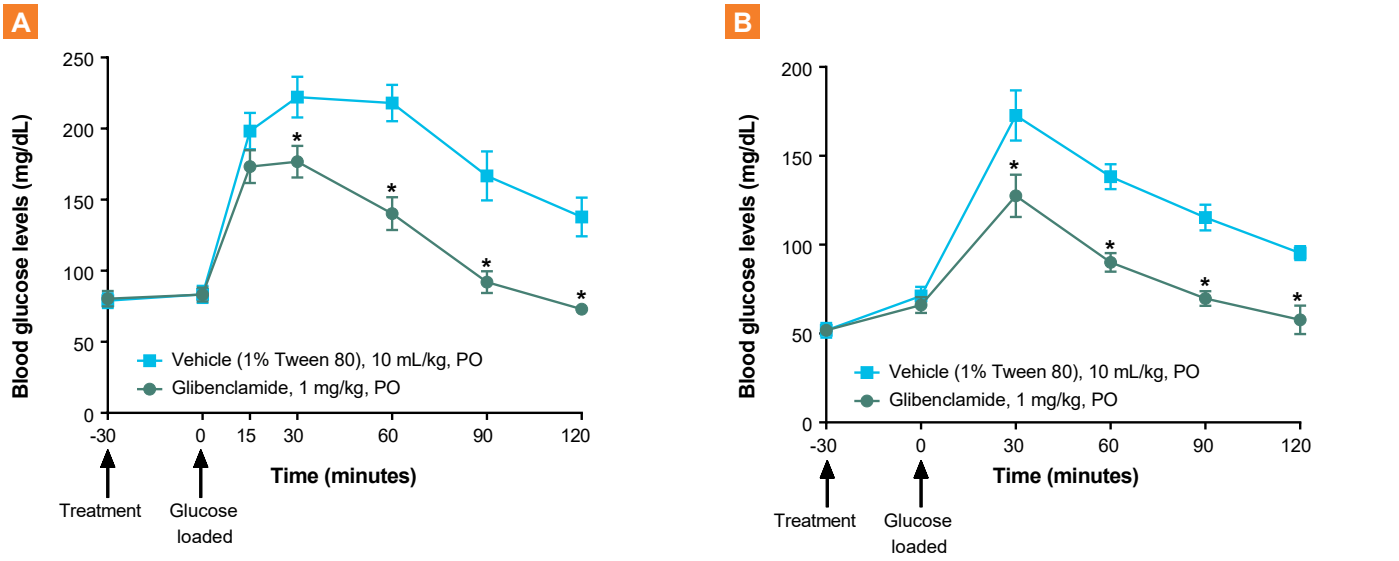

Figure 5. Demonstrated data for OGTT test. A. OGTT test in mice (#540100) or B. OGTT test in rats (#540110).

Streptozotocin-Induced T1D Models

Streptozotocin (STZ) can cause pancreatic β-cell destruction after selectively being taken up by β-cells via the GLUT2 glucose transporter, leading to insulin deficiency and hyperglycaemia in mice and rats.[25] It is currently the most used diabetogenic agent in experimental animals for inducing insulin-dependent diabetes mellitus (IDDM), also known as T1D. However, STZ-induced diabetes does not seem to be autoimmune-mediated. STZ can also produce diabetes that is similar to T2D, when injected at a lower dose in rodents fed a high-fat diet (HFD).[26]

Streptozotocin-Induced T1D Models Protocol In Mouse

We use groups of six male or female ICR mice weighing 24 ± 2 g. Test article is administered orally (PO) to a group of six male or female ICR mice weighing 24 ± 2 g, 48 hours after challenge with streptozotocin (160 mg/kg, IV). Serum glucose is determined by an automated biochemical analyzer TBA-120FR, obtained from each non-fasted animal, 5 minutes before and 90 minutes after test article administration.

Streptozotocin-Induced T1D Models Protocol In Rat

We use groups of six male or female Wistar rats weighing 250 ± 50 g. Test article is administered orally (PO) to a group of six male or female Wistar rats weighing 250 ± 50 g, 48 hours after challenge with streptozotocin (65 mg/kg i.v.). Serum glucose is determined by an automated biochemical analyzer TBA-120FR, obtained from each non-fasted animal, 5 minutes before and 90 minutes after test article administration.

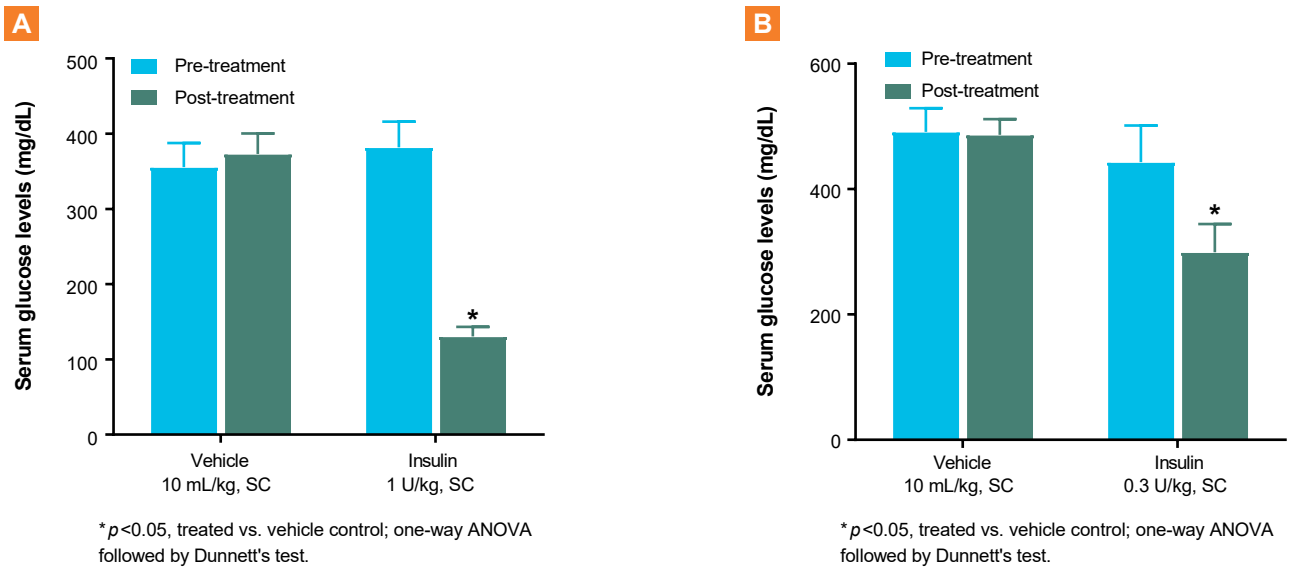

Figure 6. Demonstrated data for the Streptozotocin-induced T1D Models. Streptozotocin (STZ)-induced diabetes in A. mice (#541000) or B. rats (#541010), and the effects of insulin.

T2D Models

Some of the most commonly used genetic T2D models include *ob/ob* mice, *db/db* mice, Zucker diabetic fatty (ZDF) rats, and KK-A^y mice.[23] Mice homozygous for the Lep^{ob} (*ob/ob* mice) or $Lepr^{db}$ mutation (*db/db* mice) are hyperphagic, rapidly gain weight and become obese and hypoglycaemia. Hyperglycaemia in *ob/ob* mice is transient and mice become normoglycaemia, yet hyperinsulinemic by 14 to 16 weeks of age, and therefore can only be used for diabetic research before 14 weeks of age and not for long-term studies. The *db/db* mice have genetically determined obesity and such diabetic syndromes as hyperglycaemia, hyperinsulinemia, glucosuria, and severe insulin resistance. Diabetes in *db/db* mice is more severe than *ob/ob* mice and shows advanced stages of the disease. Mice become severely diabetic by 6 weeks of age, suffering pancreatic islet degeneration and atrophy, resulting in lethality sometimes seen as early as 16 to 20 weeks of age. Further, the *db/db* mice can also be used for studying diabetic nephropathy.[23]

The Zucker fatty (ZF) rat harboring a missense mutation (*fatty, fa*) in the leptin receptor gene (*Lepr*) develops obesity without diabetes; Zucker diabetic fatty (ZDF) rats derived from the ZF strain develop obesity with diabetes at 10 to 12 weeks of age and are most widely used for research on T2D among rat models of diabetes.[27] Only male ZDF rats develop diabetes.

KK-A^y mice are a cross between diabetic KK mice and lethal yellow (obese) (A^y) mice, and carry a heterozygous mutation of the agouti gene from A^y mice for obesity with polygenic origin in KK mice for diabetes.[28] KK-A^y mice show altered adipokine expression, obesity, insulin resistance, hyperglycaemia, hyperinsulinemia, and dyslipidemia by 8 weeks of age, closely resembling obesity-linked T2D in humans.

T2D KK-A^y Mouse Model Protocol

We use groups of 6 non-insulin dependent diabetic mellitus (NIDDM) male or female mice (KK-A^y/Ta Jcl weighing 50 ± 5 g. Test article is administered by oral gavage (PO) once daily for three consecutive days to groups of six non-insulin dependent diabetic mellitus (NIDDM) male or female mice (KK-A^y/Ta Jcl) weighing 50 ± 5 g (12 to 14 weeks old; serum glucose = 400 ± 50 mg/dL, serum insulin = 13.0 ± 2.0 ng/mL). All animals are allowed free access to normal laboratory chow and water. Serum glucose and insulin levels are determined by automated biochemical analyzer TBA-120FR and ELISA (mouse insulin assay kit) before (pre-treatment) and 90 minutes after the last vehicle and/or test article administration (post-treatment) and percent change is determined. The mean ± SEM of pre- and post-treated values are then calculated.

T2D *db/db* Mouse Model Protocol

Groups of 6 non-insulin dependent diabetic mellitus (NIDDM) male or female mice (C57BLKS/J Iar-+$Lepr^{db}$/+$Lepr^{db}$) weighing 50 ± 10 g are used. Test article is administered by oral gavage (PO) once daily for three consecutive days to groups of 6 non-insulin dependent diabetic mellitus (NIDDM) male or female mice (C57BLKS/J Iar-+$Lepr^{db}$/+$Lepr^{db}$) weighing 50 ± 10 g (10 to 15 weeks old; serum glucose = 500 ± 50 mg/dL, serum insulin = 13.0 ± 2.0 ng/mL). All animals are allowed free access to normal laboratory chow and water. Serum glucose and insulin levels are determined by automated biochemical analyzer TBA-120FR and ELISA (mouse insulin assay kit) before (pre-treatment) and 90 minutes after the last vehicle and/or test article administration (post-treatment), and percent change is determined. Serum glucose and insulin percentage of post-treatment relative to pre-treatment group values obtained on the third day are calculated.

T2D-ZDF Rat Model Protocol

We use groups of six obese Zucker diabetic fatty rats (ZDF/Gmi-*fa/fa*) 12 ± 1 weeks of age. After pre-treatment blood collection, test animals receive treatment of vehicle and/or test article by oral gavage (PO) once daily for a total of 7 consecutive days. All animals are allowed free access to normal laboratory chow and water. Blood samples are collected again 90 minutes after the last dosing for serum glucose and insulin determination. Serum glucose and insulin levels are measured by automated biochemical analyzer TBA-120FR and ELISA (rat insulin assay kit), and percent change is determined. Serum glucose and insulin percentage of post-treatment relative to pre-treatment group values obtained on the seventh day are calculated.

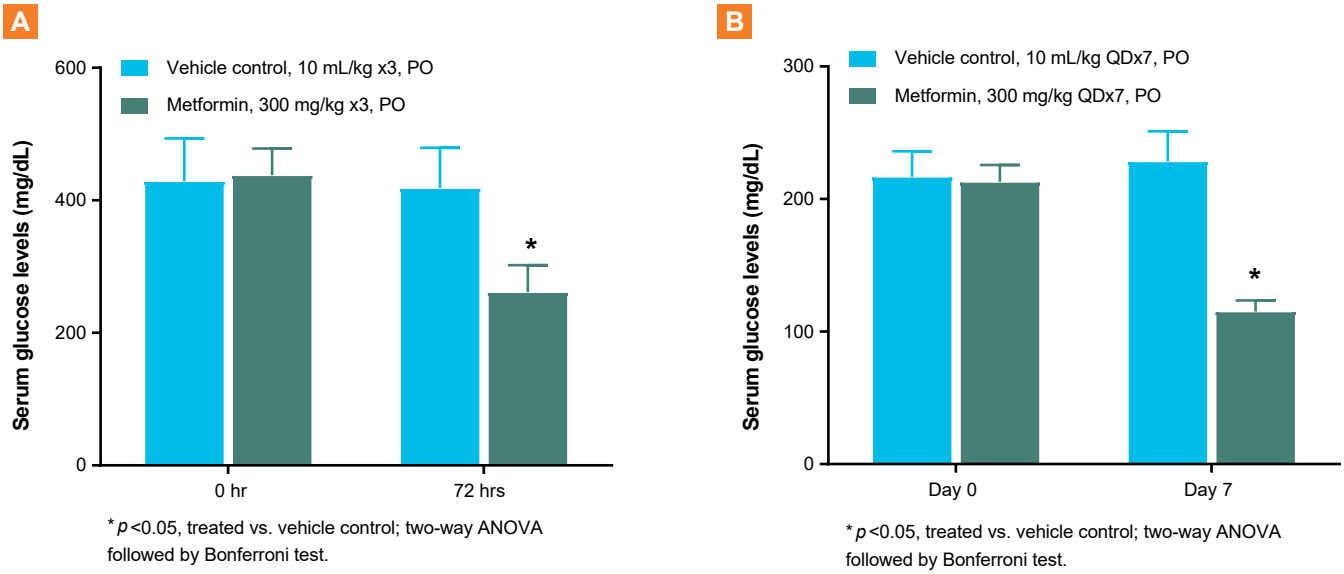

Figure 7. Demonstrated data for the T2D Models. A. *db/db* mouse model (#541630) and B. ZDF rat model (#541700) of T2D and the effects of metformin.

Summary Of *In Vivo* Models

The complex and not yet fully understood pathogenesis of diabetes makes it a challenging disease for effective therapeutic intervention. With dozens of candidates in the clinic that target different stages of pathology, efforts to further elucidate disease progression are necessary to achieve success in the clinic. It is also essential to develop novel *in vitro* assays and *in vivo* models to accurately evaluate diabetes drug candidates. Eurofins Discovery and PDS have both *in vitro* and *in vivo* services to aid clients with their diabetes drug discovery programs. The insulin release, insulin sensitivity, and glucose uptake *in vitro* panels provide the opportunity to quickly test compounds across multiple targets. To enhance research flexibility, all the assays in both panels can be ordered individually. With several targets, multiple assays (e.g., binding, enzymatic, functional, and phenotypic) may be used to fully interrogate the *in vitro* pharmacological profile of a compound.

For testing of novel anti-diabetics, we have both T1D rodent models (streptozotocin-induced diabetes in mouse, #541000/ in rat, #541010) and T2D rodent models (*db/db* mouse, #541630/ KK-A^y mouse, #541620/ ZDF rat, #541700) available. Parameters measured include serum glucose, insulin, total cholesterol (T-CHO), low-density lipoprotein (LDL), triglyceride (TG) levels, oral glucose tolerance test (OGTT), as well as fat and liver weight, etc.

Disease Model	Species	Model Number
Glucose, Blood, Oral Glucose Tolerance Test (OGTT)	Mouse	540100
	Rat	540110
Type 1 Diabetes	Streptozotocin (STZ)-Induced Mouse	541000
	Streptozotocin (STZ)-Induced Rat	541010
Type 2 Diabetes	KK-A^y Mouse	541620
	db/db Mouse	541630
	Obese Zucker Diabetic Fatty Rat	541700

Table 4. Summary of *in vivo* diabetes models.

Diabetes and NASH

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. It includes a variety of progressive liver diseases ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH).[29] Diabetes promotes the progression of NAFL to NASH and increases the risk of cirrhosis and hepatocellular carcinoma (HCC). Notably, NAFLD is associated with an increased risk of T2D, affecting approximately 45-75% of NAFLD patients with T2D.[30] The dynamic association between NAFLD and T2D is bidirectional and has led to the clinical trials of several diabetes candidates (e.g., GLP1R agonists, AMPK activators, IBAT inhibitors, PPARγ agonists) for the treatment of NAFLD/NASH.[31,32,33] Figure 8 shows the overlap of *in vitro* assay targets between Diabetes Panels and NASH Panels. Due to the extensive regulatory requirements for T2D drug development, pharmaceutical companies tend to drift their T2D clinical development to NAFLD/NASH with less strict regulatory requirements.[8] Eurofins Discovery offers both *in vitro* assay panels and *in vivo* models for Diabetes and NASH, which can be applied for new drug discovery identification or cross-verification (Tables 5 and 6).

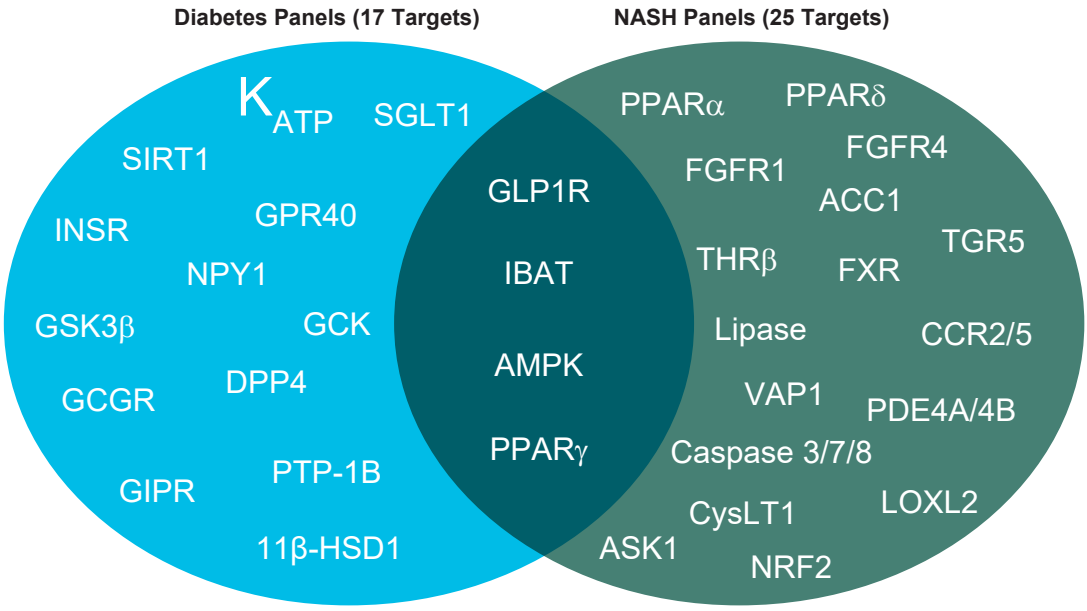

Figure 8. Therapeutic targets are included in the *in vitro* diabetes panels and *in vitro* NASH panels.

In vitro Diabetes Panels	Item #
Insulin Release Panel	PP277
Insulin Sensitivity Panel	PP278
***In vivo* Diabetes Models**	**Item#**
Oral Glucose Tolerance Test (OGTT) Mouse Model	540100
Oral Glucose Tolerance Test (OGTT) Rat Model	540110
Streptozotocin (STZ)-Induced T1D Mouse Model	541000
Streptozotocin (STZ)-Induced T1D Rat Model	541010
T2D *db/db* Mouse Model	541630
T2D KK-A^y Mouse Model	541620
T2D ZDF Rat Model	541700

Table 5. Summary of *in vitro* diabetes panels and *in vivo* diabetes models.

In vitro NASH Panels	Item #
Liver Steatosis Panel	PP270
Hepatitis Panel	PP271
Liver Fibrosis Panel	PP272
***In vivo* NASH Models**	**Item #**
MCD-induced NASH Model	546080
CDAHFD-induced NASH Model	546082

Table 6. Summary of *in vitro* NASH panels and *in vivo* NASH models.

Eurofins Discovery and our partner lab, Pharmacology Discovery Services, offer standalone and integrated Diabetes Drug Discovery Services. Eurofins Discovery focuses on the *in vitro* characterization of compounds, and drug efficacy could be further evaluated via *in vivo* diabetes models. Our Technical Directors and Study Directors are available to consult with clients for their *in vitro* and *in vivo* pharmacology studies and design studies to assess the efficacy and mechanism of action of drug candidates. We can also customize *in vitro* assays or *in vivo* studies or develop new assays/models to meet the specific needs of your diabetes programs.

For more information on *in vitro* services, please visit: eurofinsdiscoveryservices.com

For more information on *in vivo* models, please visit: pharmacologydiscoveryservices.com

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