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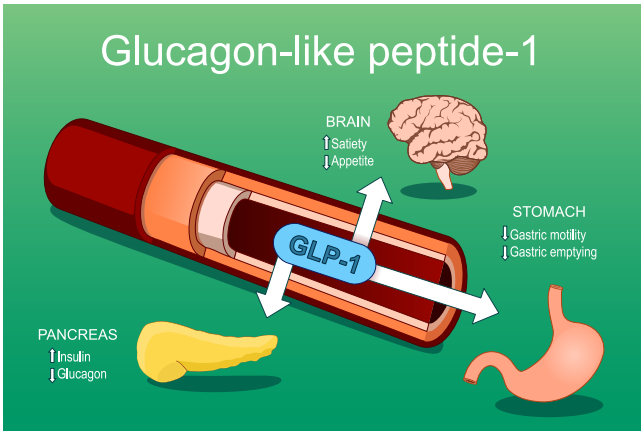
MANAGING THE COMPLEXITIES OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONIST DRUG DEVELOPMENT

Current and future development of glucagon-like peptide-1 receptor agonists (GLP-1 RAs), or simply GLP-1s, involves complexities not only in formulation but in all areas of early-phase drug development. Developing and refining preclinical models for early efficacy signals, developing and validating the bioanalytical assays necessary for quantitation, and designing the clinical studies that deliver the most robust data in this innovative therapeutic area are all key elements of a drug development program.

IN THIS ISSUE

In this issue of *The Altascientist*, we review the requirements for successful GLP-1 drug development, from the perspective of **preclinical studies**, **clinical trials**, **manufacturing**, and **bioanalysis**. Exclusive case studies are also presented.



THE GOLDEN ERA OF GLP-1 DRUGS—WHERE WE ARE AND WHAT COMES NEXT

GLP-1 RAs are entero-pancreatic hormone-based treatments, first approved in 2005 for type 2 diabetes (T2D), and now commonly prescribed for weight loss and reduction of atherosclerotic cardiovascular (CV) risk in patients with T2D.

According to a Marketsandmarkets report from July 2024, the global GLP-1 market is expected to reach $471 billion worldwide by 2032. More than one billion people worldwide are living with obesity, which increases their risk of major health complications, including T2D, high blood pressure, heart disease, stroke, metabolic syndrome, fatty liver diseases, some cancers, kidney disease, breathing problems, and sleep apnea. Most of these diseases are interrelated, with glycemic control and weight loss being a major factor in improving the overall health of sufferers.

"As we learn more about GLP-1 RAs, we realize that they target many organ systems, including the pancreas, the stomach, brain, heart, kidneys, immune system (due to reduced inflammation), and skeletal muscle. They help control the metabolism of white and brown adipose tissue and show positive effect in fatty liver disease (nonalcoholic steatohepatitis, or NASH)," says Dr. Gaetano Morelli, Chief Medical Officer at Altasciences.

WATCH NOW!

PRECLINICAL APPROACHES TO GLP-1 RA DEVELOPMENT

Preclinical studies for first generation GLP-1 RA therapeutics for treatment of diabetes have generally been standard GLP studies, conducted with normal-weight animal models. The market for GLP-1 RAs has evolved, and currently the largest growth is in the obesity market. Thus, animal models of obesity have come into play during the preclinical efficacy studies for GLP-1 RA drug development.

High-fat diets are commonly used to induce obesity in animals for studies in certain therapeutic areas, including GLP-1 RA development. Such diets can increase body adiposity and leptin, and encourage the development of hypertension and glucose intolerance, leading to conditions of human-like obesity.

Commonly Used Animal Models

Rats are frequently the species of choice in diet-induced obesity (DIO) studies. Sprague–Dawley rats are a good model for DIO, since they have a similar behavior to humans regarding excessive food consumption, which can cause weight gain and changes in lipid metabolism. Wistar rats are also a pertinent model. However, Wistar rats are more susceptible to the development of obesity through diet, since they usually consume a higher amount of high-fat diet. Differences in lipid metabolism, in the way of fatty acid uptake and lipogenesis, and the interaction between genes and diet, make Wistar rats more susceptible to DIO.

Dogs, especially certain breeds, also share metabolic characteristics with humans, making them valuable models for studying obesity, diabetes, and related metabolic disorders. Like humans, they can develop conditions like insulin resistance, glucose intolerance, and dyslipidemia, which makes them ideal candidates for obesity-related drug studies.

The most common method of achieving DIO involves feeding the dogs modified diets. Over time, this leads to weight gain and metabolic disturbances similar to human obesity. These diets are typically rich in:

- fat (often more than 30% of caloric intake);
- simple carbohydrates; and
- calorie-dense food.

Weight gain is monitored regularly, and diet composition is adjusted to achieve the target level of obesity. Limiting the physical activity of dogs is sometimes combined with a high-fat diet to accelerate weight gain. Dogs are housed in conditions where movement is restricted and exercise is limited.

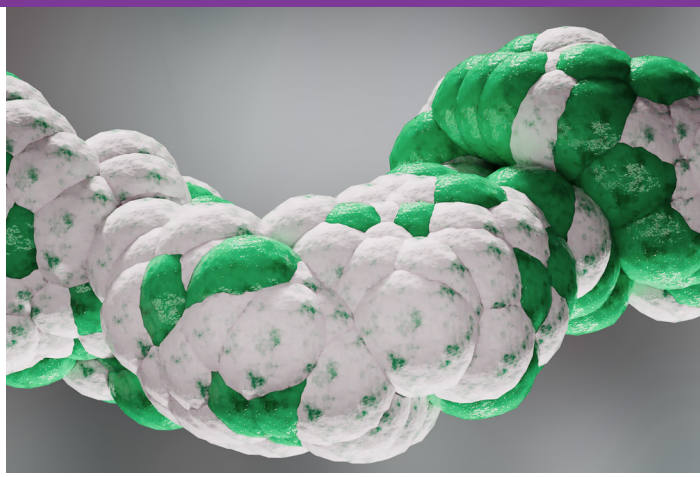
Addressing Ethical Concerns

Inducing obesity in animals raises ethical concerns, and like all studies at Altasciences, these investigations are conducted in compliance with strict ethical standards, including Good Laboratory Practice (GLP) guidelines and institutional animal care protocols to ensure humane treatment, minimize suffering, and meet regulatory requirements.

Researchers must ensure:

- minimal discomfort or stress to the animals;
- careful monitoring of body weight and overall health; and
- **humane treatment** of animals while on study.

ALTASCIENCES' PRECLINICAL CASE STUDY



GLP-1 RA: Overcoming Known Pharmacological Effects

Study Overview

Altasciences was contracted to conduct an IND-enabling study in dogs involving once-daily oral tablet administration of a GLP-1 receptor agonist. These molecules are known to decrease gastric emptying and increase satiety. In addition, they inhibit the release of glucagon from pancreatic alpha cells which results in decreased sugar liberation and production in the liver. With decreased gastric emptying and increased satiety, a common pharmacological effect observed when conducting these studies is a decrease in food consumption and body weight loss. This presents a challenge since we are evaluating the test article for potential toxicological effects, but the test article is designed to produce decreased food consumption and body weight loss. These points need to be considered to maintain the health of the animals and complete the 28-day study supporting the IND.

Study Details

- **Drug Development Phase:** IND-enabling
- **Class of Drug:** Small molecule
- **Indication:** Weight loss / type 2 diabetes (T2D)
- **Animal Model:** Dog
- **# of Animals:** 21 per sex
- **Dose Route:** Oral administration
- **Dose Regimen:** Once daily for 28 days

Study Design

The preclinical study included the following data:

- Clinical observations
- Body condition scores
- Food consumption
- Body weights
- Ophthalmology
- Electrocardiography
- Neurological assessments
- Toxicokinetics
- Clinical pathology
- Anatomic pathology

Study Purpose

To evaluate systemic toxicity and toxicokinetic characteristics of a GLP-1 RA test article and potential reversibility of any findings.

Methods

Dogs were dosed once daily for 28 days via tablets with a water flush to ensure administration.

Standard toxicological observations and measurements were performed over the course of the study, including detailed clinical observations, body weights, food consumption, ophthalmic examinations, electrocardiograms, clinical pathology, and anatomic pathology.

Animals were euthanized on Day 29. Complete necropsies were conducted, and standard organ weights recorded. A full set of tissues were collected from all animals, processed to slide, stained with hematoxylin and eosin (H&E), and evaluated by Altasciences' board-certified veterinary pathologist.

Group	Test Material	Dose Level (mg/animal)	Dose Concentration (mg/tablet)	Dose Amount (tablet/animal)	Terminal		Recovery	
					M	F	M	F
1	Placebo Tablet 1	0	0	1	3	3	2	2
2	Placebo Tablet 2	0	0	1	3	3	2	2
3	TA Tablet 1	10	10	1	3	3	0	0
4	TA Tablet 2	30	30	1	3	3	0	0
5	TA Tablet 3	100	100	1	3	3	2	2

Results

During an acclimation period (at least two weeks prior to the start of dosing) and throughout the course of the study, the dogs were provided a certified canine dry diet that contained a higher composition of fat/protein. In addition, the animals were provided daily canned and wet food. This non-standard diet was used to begin the dosing phase with animals at a higher starting body weight in anticipation of the expected weight loss.

The combination of a non-standard daily food offering (dry and wet food high in fat/protein) increased the starting weight of the dogs, which combated the weight loss due to decreased food consumption, the expected pharmacological effect of the drug. These results allowed the animals to complete the full 28 days of dosing, and the potential systemic toxic effects and toxicokinetic characteristics of the test article were evaluated without the need for a dose holiday.

The dogs' food consumption and body weight decreased over the course of the study in a dose-dependent manner with overall body weight loss at 4%, 10%, and 14% when compared to control dogs.

What Sets Altasciences Apart

Altasciences' experts used a novel approach to feeding the animals that involved a combination of dry/wet diet offering that was higher in fat/protein (compared to a standard dry diet only that has less fat/protein composition) for the duration of this study. This helped to counter the expected pharmacological effect of the GLP-1 test article (body weight loss due to decreased food consumption) from influencing/becoming so significant that intervention would be required and affect the study outcome. This approach allowed for the expected pharmacological effect to be observed while also allowing for other critical study endpoints to be met and evaluated, ensuring proper assessment of potential systemic toxic effects and toxicokinetic characteristics of the dosed GLP-1 test article.

EARLY-PHASE CLINICAL STUDY DESIGN FOR GLP-1 RAS

What we Know So Far About GLP-1 RAs

GLP-1 RA head-to-head clinical studies have demonstrated that all GLP-1 RAs are effective at reducing A1C (the average amount of glucose in a person's blood over the past three months). The initial pivotal studies that led to the first wave of GLP-1 RAs demonstrated that they work in several ways, including stimulating insulin release, slowing digestion, reducing appetite, and inhibiting glucagon release.

These actions have made GLP-1 RAs excellent candidates for obesity treatment, where much of the new development is now focused. As with most medical breakthroughs, the development and implementation of GLP-1 RA drugs is not straightforward.

Clinical Trials for GLP-1 RAs

The FDA has issued a **guidance** on the development of obesity drugs, which is not remarkably different from the clinical trials required for other innovator drug development, and the program will typically consist of the standard package of trials. With the exception of the study participants' entry criteria, the objectives and management of the trials are very similar to a standard SAD/MAD approach. Part of the early-phase trial guidance states:

> *"To increase the likelihood of identifying the most appropriate dose for the pivotal clinical trials, early-phase clinical studies should include a range of doses and be designed to identify no-effect and maximally tolerated doses. Studies should be designed to differentiate the efficacy of all the active doses versus placebo. The duration of the Phase II trials should be sufficient to capture the maximal or near-maximal weight loss effects of the active doses. Forethought should be given to whether the product will be ultimately used in a fixed-dose or dose-titration scheme, as this dosing decision will also influence the size and duration of the studies."*

The requirements for participation in weight loss trials are further defined in the guidance as follows:

> *"Patients included in the early-phase efficacy and safety studies generally should have BMIs greater than or equal to 30 kg/m2 or greater than or equal to 27 kg/m2 if accompanied by comorbidities."*

There are also a number of additional policy publications on related topics **listed here** by the B.C. Provincial Academic Detailing Service.

Adaptive Trial Designs

Clinical trial research has evolved significantly in recent years, with innovative trial designs. Referred to as adaptive trials, these designs are transforming the approach to clinical studies and are helping to expedite the drug development process.

Key features of adaptive trial designs include:

1. **Flexibility:** Changes can be made during the trial based on predefined rules. For example, the trial might stop early if the treatment is found to be highly effective or expand to include more participants if the results suggest more data is needed.
2. **Types of Adaptations:** Common adaptations include changes to dosage, patient population, endpoints, or randomization ratios. Adaptive trials can also involve the addition or dropping of treatment arms.
3. **Interim Analyses:** Regular analysis of accumulating data during the trial allows researchers to make informed adjustments. However, these analyses follow strict guidelines to prevent bias.
4. **Efficiency:** Adaptive designs often require fewer patients or shorter durations to achieve the same objectives as traditional trials, making them faster and potentially less expensive.
5. **Ethical Considerations:** Adaptive trials may expose fewer patients to less effective treatments by adjusting or terminating unproductive arms earlier than in fixed trials.

Although the primary objective of first-in-human (FIH) trials remains to determine the safe dose range using the standard SAD/MAD design, it is not unusual within an adaptive design for an innovator GLP-1 RA drug to include additional arms exploring different endpoints. A comparator arm with a marketed drug, or an arm for early proof-of-concept, are being included more frequently.

Biomarkers

The use of biomarkers in GLP-1 clinical trials is proving to meet some of the more common challenges in drug development, such as the ability to deliver clear efficacy and safety data, and to help shorten time to market. Commonly explored safety and pharmacodynamic biomarkers include HbA1c, C peptide, insulin, adiponectin, leptin and inflammatory biomarkers include CRP, IL6, TNF, and IL1B.

Side Effect Management

Reported side effects of GLP-1 RAs commonly include nausea, vomiting, and diarrhea, and clinical research teams must be trained to manage such side effects during trials. Proper side effect management helps decrease participant dropout rates, leading to more robust data collection and more efficient use of resources.

Research is now uncovering additional adverse events. The concomitant use of insulin and the sulfonylurea drug class potentiates the risk for hypoglycemia, and participants require monitoring and assessment of the concomitant medication doses and requirements. Studies must be carefully designed, and inclusion/exclusion criteria must be developed to mitigate risks of adverse events.

What Comes Next

The next generation of GLP-1 drug development will focus on dual and triple agonists, and combinations of GLP-1 with other entero-pancreatic hormones with complementary actions and/or synergistic potential, such as glucagon, amylin, and glucose-dependant insulinotropic polypeptide (GIP). Gut hormones in newer medications have different mechanisms of action and combination therapy can be used to potentially enhance the efficacy of pharmacotherapy.

Oral formulations are another future enhancement for GLP-1 RA medicines, with several pill forms in development. Various physiological barriers, such as mucus, intestinal, and enzymatic barriers interfere with the oral delivery or absorption of protein and peptide-based therapeutics. Thus, novel approaches such as nanocarriers, site-specific delivery, and stimuli-specific delivery, are being used to improve the success of oral GLP-1 RAs.

The GLP-1 therapeutic field is also exploding beyond the treatment of diabetes and obesity. Some recent studies have reported beneficial outcomes in cardiac and renal disease, with significant reduction in morbidity and mortality. Evidence suggests that the impact is not only related to the improvement of diabetes and weight reduction, but there may be direct effects on inflammatory markers and possibly endothelial anti-inflammatory properties.

Another area of interest and research is substance abuse disorders. Some recent studies have reported up to 40% reduction in opioid use as a result of effects of GLP-1 agonists on brain receptors involved in cravings and dependency. There is also some significant research interest in the field of fertility. In obese males, the use of GLP-1 RAs has shown enhanced sperm metabolism and motility; and in females, improved ovulation rates and regulated menstrual cycles. The mechanisms are not yet clearly understood, and more research is needed to confirm whether medical weight loss affects fertility or if there exists a possible direct action on the reproductive system.

ALTASCIENCES' CLINICAL CASE STUDY

GLP-1 RA: Rapid Phase I Study Execution

Study Overview

Altasciences was contracted to conduct a clinical study to investigate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of subcutaneous doses of a novel GLP-1 receptor agonist in overweight but otherwise healthy adults. The sponsor presented Altasciences with an aggressive timeline to determine safety and validate the half-life of the study drug with the goal of progressing to a Phase II program within six months. To meet this goal, Altasciences created an adaptive design study with an optimized and streamlined timeline, which included tightly scheduled Safety Review Committee meetings, rapid dose escalation, overlapping cohorts, and rapid turnaround of PK results.

Study Details

- **Drug Development Phase:** Phase I First-in-Human
- **Class of Drug:** Small molecule
- **Indication:** Weight loss
- **# of Participants:** 116
- **Dose Route:** Subcutaneous (SC)
- **Dose Regimen:** SAD/MAD – weekly for 5 weeks

Study Purpose

- **Primary:** To assess the safety and tolerability of single ascending or multiple ascending SC doses (administered weekly for 5 weeks) of study drug
- **Secondary:** To assess the PK profile of single or weekly SC doses of study drug
- **Exploratory:** To assess weight loss (PD measure) following single ascending or weekly ascending SC doses of study drug

Study Design

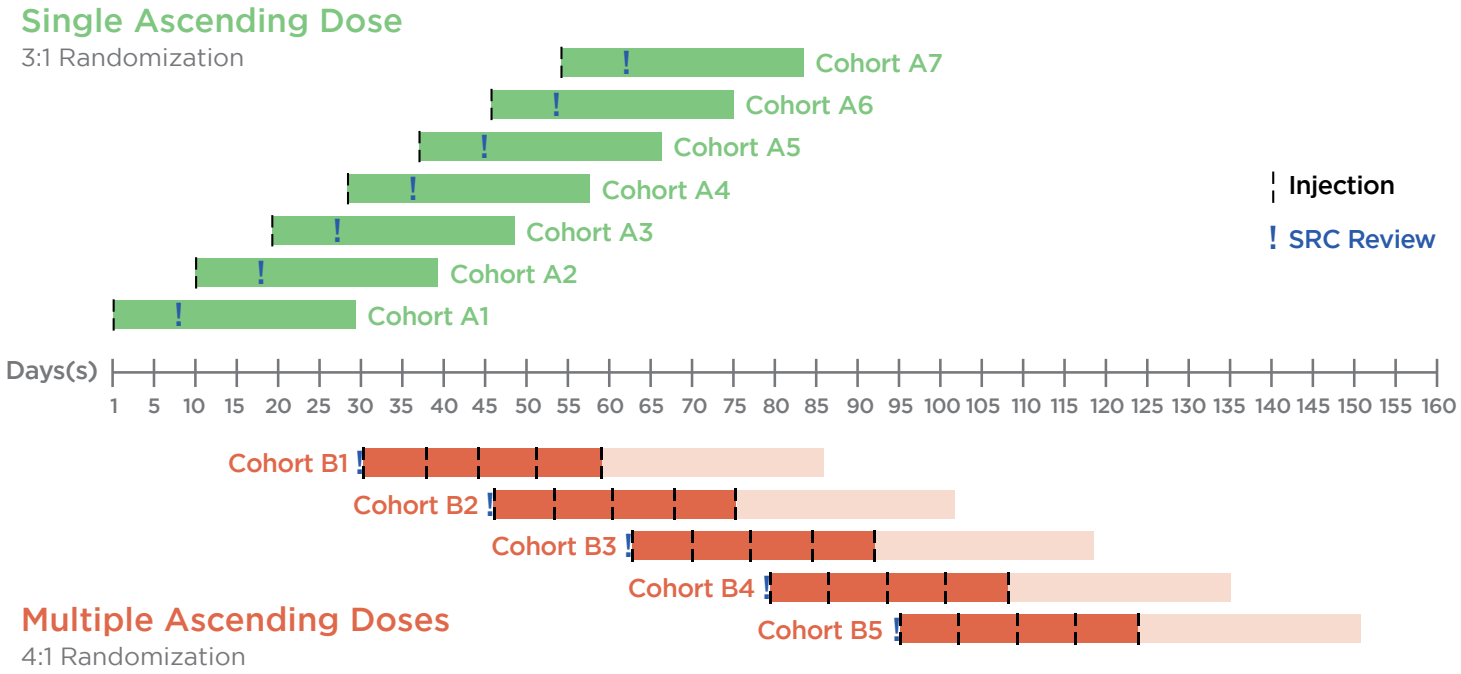
Randomized, placebo-controlled, double-blind study in healthy adult participants with a body mass index (BMI) of 27 to 38 kg/m^2.

Part A—SAD

- 56 participants were randomized into 7 sequential dosing cohorts randomly assigned to the study drug or placebo (6:2).
- Participants were admitted to the clinical research unit (CRU) on Day 1 for baseline evaluations prior to dosing.
- Study drug dosing (SC) occurred on Day 1. Participants remained in the CRU under medical supervision for 7 days.
- Participants returned for 4 weekly outpatient follow-up assessments.
- A SRC convened after each dosed cohort to review all blinded clinical safety data and available PK data. The SRC assessed the safety of proceeding to the next cohort and determined the dose level for the next cohort.
- The study design allowed for the number of cohorts (maximum of 9) and dose level to be adapted based on cumulative data obtained from preceding cohorts.

Part B—MAD

- 60 participants were randomized into 6 sequential dose cohorts (10 participants per cohort) randomly assigned to the study drug or placebo (8:2).
- Participants were admitted to the CRU on Day 1 for baseline evaluations prior to dosing.
- Study drug dosing (SC) occurred weekly for 5 weeks. Participants remained in the CRU under medical supervision for 3 days after the 1st and 5th doses and remained for at least 4 hours after the 2nd, 3rd, and 4th doses.
- Participants returned for an additional 8 weekly outpatient study visits and a final visit 12 weeks post first dose.
- A SRC convened after each dosed cohort to review all blinded clinical safety data and available PK data. The SRC assessed the safety of proceeding to the next cohort and determined the dose level for the next cohort.
- The study design allowed the number of cohorts (maximum of 7) and dose level to be adapted based on cumulative data obtained from preceding cohorts.



Assessments

- Safety was assessed via evaluation of adverse events (AEs), vital signs (including temperature, heart rate, and blood pressure), 12-lead electrocardiogram (ECG), corrected QT (QTc) analysis, laboratory evaluations, physical examination, glucose monitoring, and injection site reactions.
- Tolerability was assessed via an assessment of treatment-emergent adverse events (TEAEs).
- Drug blood concentrations were analyzed via a liquid chromatography-mass spectrometry (LC-MS) bioanalytical method for PK analysis.
- Absolute and percent weight loss at pre-specified timepoints was assessed.

Results

The study drug was well tolerated by healthy participants at the doses evaluated. There were no study drug-related severe AEs or serious adverse events (SAEs) and no discontinuation or participant withdrawal due to study drug-related AEs. The most common AEs were consistent with the known safety profile of GLP-1 RAs, and consisted of nausea, decreased appetite, headache, vomiting, and dyspepsia. Most of these AEs were assessed as mild and possibly or probably related to the study treatment by the Investigator. No clinically significant changes were reported in vital signs, physical examinations, or ECGs. The half-life of the study drug was successfully calculated with concentration data from the validated LC-MS method.

What Sets Altasciences Apart

Altasciences successfully and rapidly delivered actionable data to advance the sponsor's GLP-1 RA drug candidate into a Phase II setting. The was achieved by implementing the following strategies to eliminate "white space" across all phases of the study—from start-up to reporting.

- Altasciences' scheduling team and CRU collaborated closely with the sponsor to refine and secure approval for a creative overlapping dosing schedule for both SAD and MAD cohorts.
- Our recruitment team worked tirelessly to ensure participant enrolment stayed on track.
- SRC meetings with real-time review of results allowed rapid decision-making and dose escalation.
- Constant communication between Altasciences' medical and regulatory experts and the sponsor ensured participant safety during rapid study progression.
- Effective communication between Altasciences' CRU and our bioanalytical laboratory streamlined sample analysis.
- Rapid turnaround of PK results.
- An adaptive study design allowed for determination of dose levels to take forward into the Phase II study.
- Unparalleled project management ensured coordinated activities between external stakeholders and Altasciences' internal team.

Altasciences was able to help the sponsor achieve their goals of progressing to a Phase II study within an aggressive six-month timeframe by implementing innovative and collaborative approaches to streamline study design, operations, and project management.

BIOANALYTICAL TECHNIQUES FOR GLP-1 RAS

Various bioanalytical techniques are employed to quantitate and monitor the PK and PD of GLP-1 receptor agonists, as well as their safety and immunogenicity. These techniques include ligand-binding assays (LBAs) and liquid chromatography coupled with mass spectrometry assays.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

Mass spectrometry, particularly when combined with LC-MS/MS is one of the most powerful tools for quantitating GLP-1 RA peptides. The process typically involves sample preparation techniques, like protein precipitation, solid-phase extraction (SPE), or immunoprecipitation (IP), to isolate the peptide from plasma or serum. Sample extraction is sometimes followed by enzyme digestion to generate peptide fragments more amenable for MS detection. In liquid chromatography (LC), GLP-1 analogs are separated from other peptides and proteins, typically using reverse-phase chromatography. During mass spectrometry detection, the peptide is quantified by detecting unique precursor ions (intact peptides or fragments) and their product ion after fragmentation, often using multiple reaction monitoring (MRM) mode for enhanced specificity and sensitivity. Quantitation is achieved by comparing the peptide's signal intensity with that of an internal standard, usually a labeled synthetic version of the therapeutic.

Mass spectrometry (MS) has several distinct advantages over other analytical platforms, making it a preferred method for peptide quantitation.

- **Higher Specificity:** MS can distinguish between closely related peptide analogs or variants.
- **Quantitative Accuracy:** MS directly measures peptide mass and ion intensity, leading to accurate and reproducible quantification. Using a labeled internal standard with identical physicochemical properties as the analyte helps to track variability at the extraction, LC and MS steps of analysis.
- **Structural Characterization:** MS not only quantifies the peptide but also identifies its structure, modifications (e.g., post-translational changes) and degradation products. This is crucial for understanding the drug's metabolism and pharmacokinetics.
- **No Need for Antibodies:** Unlike LBAs, MS does not require the development and use of specific antibodies, which can be time-consuming and expensive. This is especially important for novel peptide therapeutics where antibodies may not be available.
- **Multiplexing:** MS allows for the simultaneous quantitation of multiple peptides (e.g., therapeutic peptides and their metabolites), reducing the need for separate assays.

Developing bioanalytical methods for GLP-1 RA peptides using LC-MS presents several challenges, including the complexity of biological matrices, which can cause ion suppression and interfere with accurate quantitation. Non-specific binding of peptides to surfaces during sample preparation can lead to analyte loss and variability. At the same time, LC carryover from previous injections can reduce sensitivity and dynamic range by contaminating subsequent samples. Addressing these issues requires robust sample preparation, optimized LC separation, and careful method development.

Immunogenicity

GLP-1 RAs, being peptide-based, may elicit immune responses in some patients. Immunogenicity testing is therefore essential to assess the development of anti-drug antibodies (ADAs), which may impact the drug's safety and efficacy. LBAs are typically employed for ADA detection during the nonclinical and clinical phases of the program.

The main challenge in developing immunogenicity assays resides in the proper selection of the positive control antibody against the drug, the GLP-1 analog. The positive control antibody must demonstrate sufficient sensitivity against the drug itself and ideally cross-reacts with the endogenous counterpart in the ADA assay, as this would be required during clinical testing.

The purpose of nonclinical immunogenicity assessment of GLP-1 analogs is primarily to support the ù understanding of toxicokinetic data. For example, we may assess the impact of administering high doses of the drug following single or multiple injections, as the impact of ADAs can contribute to the planning of subsequent nonclinical toxicology studies, and ultimately impact clinical dosing decisions. In nonhuman primates, the GLP-1 analogs are perceived as foreign to the host as the biotherapeutic drugs are developed to mimic human molecules, and they may be administered at much higher doses than will be given to humans. Consequently, the immune response generated in nonclinical studies is often expected and needs to be interpreted with caution. The main challenge observed in nonclinical studies is ensuring that the assay format selected is tolerant to doses expected in nonclinical studies. Working with a peptide poses additional challenges in assay development as there are limited sites for labeling. Therefore, assay formats for GLP-1 analogs are more limited, and non-specific binding is often observed, requiring proper mitigation strategies to investigate and ensure a valid cut-point can be derived.

As the GLP-1 analog successfully moves to clinical safety testing, the sensitivity of the method being used is crucial for early immunogenicity assessments. Since the drug, in this case, has an endogenous counterpart and safety may be a concern, the neutralization capacity of the ADAs as well as the cross-reactivity to the endogenous GLP-1 is usually assessed in confirmed positive subjects. Patient samples are screened, confirmed, titered, and tested for cross-reactivity and neutralization in a three-tier approach, therefore requiring a large volume of serum to be reserved for sample analysis.

Consequently, developing a neutralizing cell-based assay (NAb assay) is required. The selected antibody positive control used for ADA assessment should ideally demonstrate neutralization with high affinity, to achieve an acceptable sensitivity and drug tolerance as per the **2019 FDA regulatory guidance**. Our strategy, in this case, is to screen several polyclonal and/or monoclonal antibodies very early in method development to select the appropriate positive control for both assays. As GLP-1 is a peptide, the binding and functional epitopes available are limited, which explains the challenge observed in raising a positive control antibody for the assays. It is recommended, when possible, to characterize well the positive control reagent for their binding affinity to GLP-1 for best outcomes throughout the program.

Biomarkers

The use of biomarkers in GLP-1 trials is helping to deliver clear efficacy and safety data which in turn helps shorten time to market. Commonly explored safety and pharmacodynamic biomarkers include HbA1c, C peptide, insulin, adiponectin, leptin, and inflammatory biomarkers CRP, IL-6, TNF, and IL-1B. Those can be assessed by LBA using either singleplex or multiplex MSD assays for fast turnaround time.

Good Laboratory Practice Compliance and Regulatory Considerations in Bioanalysis

Bioanalytical studies during GLP-1 drug development must comply with regulatory guidelines, including Good Laboratory Practice (GLP) standards. These regulations ensure that nonclinical laboratory studies are conducted with the necessary rigor, and that the resulting data is reliable and reproducible. Compliance with GLP is particularly important when generating data for regulatory submissions. The development of GLP-1 receptor agonists involves stringent regulatory oversight. Regulatory agencies, including the FDA and the European Medicines Agency (EMA), follow the ICH M10 guidelines on bioanalytical method validation. These guidelines help to ensure that the methods used to evaluate the PK, PD, safety, and immunogenicity of GLP-1 receptor agonists are accurate, precise, and reproducible.

What Lies Ahead

Advances in bioanalytical technologies, such as high-resolution MS and automated immunoassays, are expected to further enhance the development of GLP-1 RAs. Innovative techniques such as microsampling can be used to reduce sample volume and possibly improve patient compliance in clinical trials. Furthermore, combining bioanalytical data with pharmacometric modeling can offer a more comprehensive understanding of the PK/PD relationships of GLP-1 drugs, allowing for optimized dosing regimens and enhanced therapeutic results.

THE MANUFACTURE OF GLP-1 RA DRUG PRODUCTS

The manufacturing of GLP-1 RA drugs is a complex undertaking, as they are biologically derived peptide-based medications that are unstable at room temperature. Currently, the vast majority of GLP-1 RA drugs are injectable due to poor oral bioavailability, a challenge that many drug developers are focused on solving.

Peptide Synthesis

GLP-1 RAs are synthetic versions of the human incretin hormone GLP-1. Peptides are chains of amino acids, and the production of GLP-1 RAs often starts with chemical peptide synthesis, commonly done through solid-phase peptide synthesis (SPPS). SPPS allows for the sequential addition of amino acids to create the desired peptide chain.

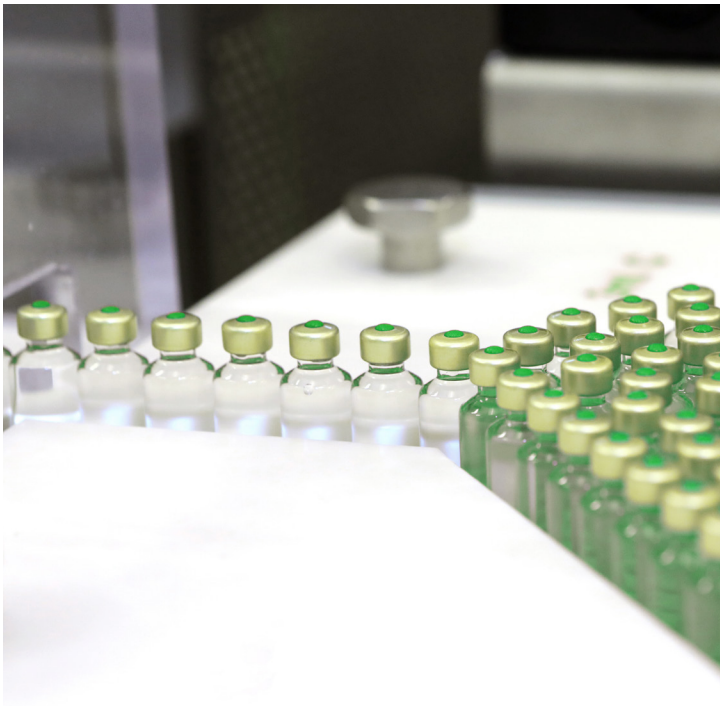
Folding and Stabilization

Peptide drugs like GLP-1 RAs are sensitive and can degrade easily. Therefore, they need to be correctly folded to maintain their biological activity after synthesis. Special techniques such as using stabilizing agents or chemically modifying the peptide ensure that the drug remains active and effective in the body. For example, some GLP-1 RAs have fatty acid chains (such as liraglutide) or are fusion proteins (like semaglutide) to increase their half-life.

GLP-1 peptides are further modified to improve their stability, prolong half-life, and reduce susceptibility to enzymatic degradation. For example, semaglutide includes modifications to increase albumin binding, reducing renal clearance and degradation by dipeptidyl peptidase-4 (DPP-4).

Biotechnological Production

More complex GLP-1 RAs are frequently produced using recombinant DNA technology in mammalian or bacterial cells. Mammalian cell systems are often preferred due to their ability to perform post-translational modifications essential for biological activity; however, bacterial systems can be simpler and more cost-effective but lack these modifications. Genes encoding the GLP-1 analog or a modified version are inserted into cells that can express the peptide. The cells are then cultured in bioreactors under controlled conditions to produce large quantities of the peptide for drug manufacture.



Purification

After production, the peptide is purified to remove any impurities, such as incomplete peptides or other byproducts. This is typically done using high-performance liquid chromatography (HPLC) or other advanced purification techniques.

Formulation

GLP-1 RAs are commonly formulated for subcutaneous injection, often with the addition of stabilizers, preservatives, and buffers, as mentioned above, to prolong the shelf-life and ensure proper drug delivery—as peptides are generally unstable at room temperature and require stabilization via the use of excipients or refrigeration.

Sterilization and Fill/Finish of Injectable Formulations

After formulation, the product is sterilized, usually through filtration, and then filled into vials, pre-filled syringes, or injection pens. The product is then subjected to quality control testing to ensure it meets all regulatory standards.

Drug manufacturers are working on enhancements to the injectable formulations, including long-lasting depot injections and microsphere formulations.

Oral Formulations

The main challenge in formulating oral GLP-1 drugs is poor bioavailability. The large molecule GLP-1 peptides are prone to degradation by stomach acid and digestive enzymes. Therefore, they require special formulations to survive the gastrointestinal (GI) environment. Stabilizers are added to prevent degradation during manufacturing, storage, and transit. Some peptide drugs are manufactured using lyophilization (freeze-drying) to preserve the peptide's structure and function until it is administered.

Semaglutide, the only oral GLP-1 RA currently on the market, is co-formulated with salcaprozate sodium (SNAC), which facilitates the drug's absorption in the stomach by transiently neutralizing gastric acids and promoting transcellular absorption of the peptide across the gastric lining. Manufactured by Novo Nordisk, semaglutide represents a breakthrough in GLP-1 drug delivery.

Specialized tests are conducted to simulate the drug's dissolution and absorption in the GI tract, ensuring that the absorption enhancer (e.g., SNAC) effectively increases bioavailability, often conducted by LC-MS/MS.

Granulation and Compression

The powder blend can be granulated to improve its flow properties and ensure consistent tablet formation. The choice of granulation can impact the drug's release profile and overall bioavailability, which is crucial for oral formulations with the sensitivity of these peptides. Wet granulation, dry granulation, or direct compression can be used depending on the formulation requirements, and the final mixture is compressed into tablets or filled into capsules. High-precision machinery is used to ensure uniformity in dosage and tablet weight.

Oral formulations may also involve enteric coatings that protect the drug from being broken down in the stomach's acidic environment, releasing it only in the more neutral pH of the small intestine.

The Future of GLP-1 RA Manufacturing

The field of GLP-1 RA manufacture is evolving to meet market needs, and is poised for significant advancements, driven by ongoing innovations in drug delivery, formulation, and production technology.

Key developments include:

1. **Improved Oral Formulations:** Ongoing efforts will focus on enhancing bioavailability using more efficient absorption enhancers and delivery platforms. This includes designing novel carriers such as nanotechnology-based systems to protect the peptide from degradation and improve GI tract absorption. Alternatively, using microencapsulation techniques could further protect the peptides and control their release in the GI tract.
2. **Sustained-Release Technologies:** Advances in sustained-release formulations, including biodegradable polymers and depot injections, aim to reduce dosing frequency. This could lead to monthly or less frequent dosing schedules, improving patient compliance.
3. **Peptide Stability:** Enhanced peptide stabilization techniques, such as novel excipients and peptide modifications (e.g., PEGylation), will extend shelf life, reduce degradation, and minimize the need for refrigeration.
4. **Biotechnology-Driven Production:** Biotechnological innovations, such as recombinant DNA technology and cell-free protein synthesis, are expected to streamline peptide manufacturing, reduce costs, and scale up production while maintaining high quality and purity.
5. **Personalized Medicine and Combination Therapies:** As precision medicine evolves, manufacturing may shift towards personalized GLP-1 RA formulations tailored to individual patient profiles, potentially combined with other therapeutic agents (e.g., SGLT-2 inhibitors) in a single dosage form. However, there will likely be challenges in regulatory approval, manufacturing complexities, and costs associated with personalized formulations.

These advancements will drive more patient-friendly, cost-effective, and accessible GLP-1 RA therapies, broadening their use for metabolic and cardiovascular diseases.

HOW ALTASCIENCES CAN HELP

Altasciences' integrated, end-to-end CRO/CDMO solutions play a critical role in the development of GLP-1 RA drugs by offering comprehensive services and support throughout the early phases of drug development. We bring you expertise in preclinical studies, including DIO and other modified models, clinical trial design, conduct, and reporting, regulatory support, and bioanalytical testing.

With proactive communication and seamless handoffs, we ensure that your GLP-1 RA drug development program or study is efficiently managed. This includes optimizing study designs, managing compliance with regulatory standards like GLPs, and ensuring timely data collection and analysis. Such seamless coordination accelerates timelines, reduces costs, maximizes animal and participant safety, and ensures that the data meets the stringent requirements for regulatory approval.

ALTASCIENCES' RESOURCES

Webinar

The Golden Era of GLP-1 Drugs

Scientific Journal

The Altascientist—The Global Challenge of Metabolic Disorders

The Altascientist—Navigating the IND Submission Process

The Altascientist—Planning Your First-in-Human Trial

eBooks

Altasciences' Proactive Drug Development Solution: Large Molecules

One Integrated Solution for Meeting Your Preclinical to Clinical Drug Development Needs

First-in-Human Solution for Small and Large Molecules

Webpage

Altasciences' Early-Phase Drug Development Solutions

ABOUT ALTASCIENCES

Altasciences is an integrated drug development solution company offering pharmaceutical and biotechnology companies a proven, flexible approach to preclinical and clinical pharmacology studies, including formulation, manufacturing, and analytical services. For over 25 years, Altasciences has been partnering with sponsors to help support educated, faster, and more complete early drug development decisions. Altasciences' integrated, full-service solutions include preclinical safety testing, clinical pharmacology and proof of concept, bioanalysis, program management, medical writing, biostatistics, clinical monitoring, and data management, all customizable to specific sponsor requirements. Altasciences helps sponsors get better drugs to the people who need them, faster.