Altasciences, 575 Armand-Frappier, Laval, Québec, H7V 4B3, Canada
Altasciences.com | contact@altasciences.com

Dual Purpose of a Cell-Based Assay for an Agonist of the GLP-2 Receptor

Delphine Labit, Maria Paula Laverde, Aminata Coulibaly, Maria Gabriela Aguirre Franco, Sophie Corbeil, Danielle Salha
Altasciences, Laval, QC, Canada

INTRODUCTION

During drug development, cell-based assays are used for multiple purposes. These include early proof of concept to identify a drug product's pharmacological activity, and further studies to determine whether neutralizing anti-drug antibodies (NAbs) are present. Here we present our process to develop a cell-based assay for a glucagon-like peptide-2 (GLP-2) analog drug product that could be used to support both, taking into account critical evaluations and time savings. This drug, targeting the GLP-2 receptor, has been shown to have superior efficacy on short bowel syndrome (SBS) patient response by increasing the intestine's ability to absorb nutrients. However, this peptide drug may elicit unwanted immune responses that could significantly impact safety and efficacy. These immunogenic responses can lead to the production of NAbs (Nabs). Therefore a cell-based assay was required to detect it in patient serum. In parallel, the same assay was used to support toxicological studies by evaluating the pharmacological activity of the GLP-2 analog in different matrices.

METHODS

- The GLP-2 analog promotes the expansion of the intestinal epithelium by stimulating the GLP-2 receptor (GLP2R), a G protein-coupled transmembrane receptor. A combination of various cell lines expressing the GLP-2 receptor and assay read-out were evaluated. A functional assay format was selected based on its advantages and limitations.
- Different parameters were evaluated to develop and optimize the cell-based assay for the GLP-2 analog. These include: cell density evaluation; whether stable or single-use cell preparations should be used; and assay robustness, including cell passage, precision, and incubation times.
- The neutralization assessment was performed using a positive control. In addition, serum interference, specificity and selectivity, and drug tolerance were evaluated.

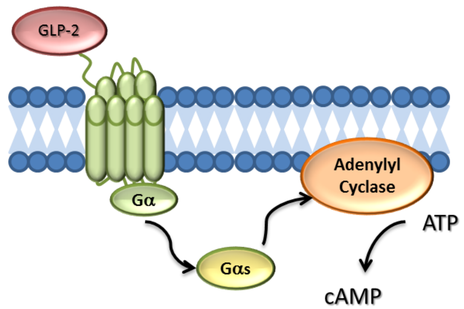


Figure 1. GLP-2R/|Gα stable cell line

RESULTS

- A cell-based assay for the GLP-2 analog was developed using a GLP-2 receptor overexpressing stable cell line.
- The activation of the GLP-2 receptor is monitored by measuring cellular cAMP levels using a homogenous competitive immunoassay based on enzyme fragment complementation technology with a cyclic adenosine monophosphate (cAMP) assay kit (DiscoverX, Fremont CA).

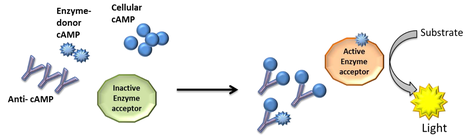
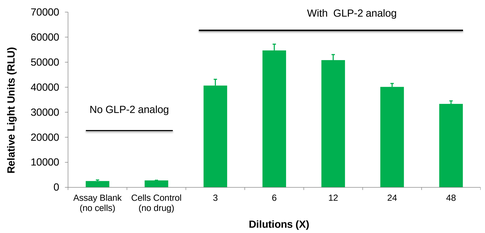


Figure 2. cAMP competitive immunoassay

- This assay requires no extra manipulation of the cells and relies on the specific early stage mechanism of action of the GLP-2 receptor.
- The bioassay measuring the amount of luminescence signal is directly proportional to the amount of cAMP produced in the cells.
- Neutralizing anti-GLP-2 analog antibodies are measured by a reduction in the amount of cAMP produced.

Serum Interference

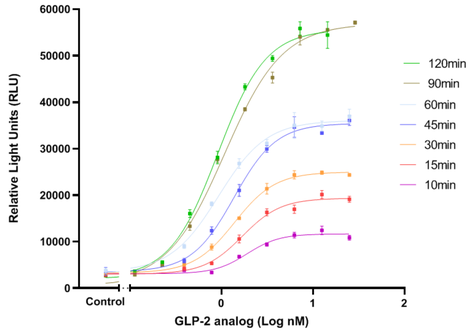
Matrix interference could lead to a nonspecific signal or inhibit the specific signal. The selected cell-based assay detects early GLP-2R activation upon GLP-2 analog binding to minimize possible matrix interference over incubation time.



Graph 1. Assessing dilutions of human serum

Controls were performed with a 3-fold dilution of serum. No serum interference was detected in human serum. A 3-fold dilution of serum was selected to perform the subsequent optimization evaluations.

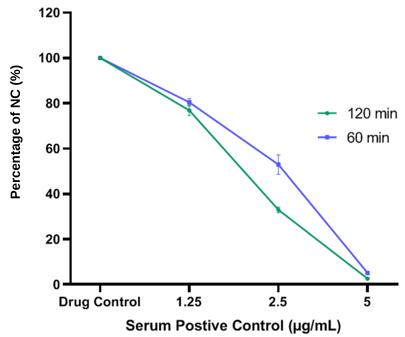
Optimization of GLP-2 Analog Incubation Time on Cells



Graph 2. Optimization of GLP-2 analog incubation time on cells

Optimal results are achieved by incubating the GLP-2 analog for 90 minutes in order to reach maximal dynamic range for the assay.

Positive Control Incubation Time with the GLP-2 Analog



Graph 3. Optimization of positive control incubation time with the GLP-2 analog

120 minutes was selected in order to ensure proper binding between the anti-drug antibodies and the GLP-2 analog.

Cell Preparation

The optimized cell-based assay was assessed in 3 inter-run evaluations with cultured and single-use cells.

Cultured cells	EC50: 2.4 nM	CV : 9.4%
Single-Use cells	EC50: 2.3 nM	CV : 4.1%

Figure 3. GLP-2 analog EC50 in cultured and single-use cells

Precision was better in single-use cells over three independent assessments. Including time and cost savings, single-use cells will also ensure a better precision and robustness, as the risk of possibly losing the GLP-2 receptor expression among cell passages is eliminated.

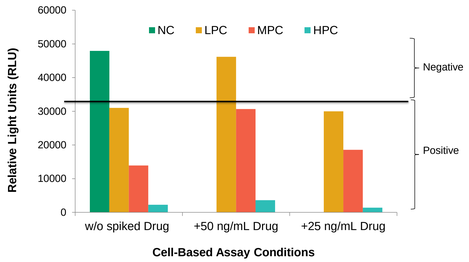
Minimum Required Dilution

Several mitigation strategies evaluated during the optimization phase to reach drug tolerance led to a decrease in sensitivity (acidification, protein A, G, L Sepharose, albumin depletion, and GLP-2 immunoprecipitation). Consequently, the approach adjusting the MRD was the most suitable option.

The final format for the cell-based assay was defined by adjusting the minimum required dilution to 18x, with a level that maintained an acceptable sensitivity and drug tolerance.

Drug Tolerance

The drug tolerance evaluation should be performed with an accurate drug concentration at the time of sampling, based on available PK data.



Graph 4. Drug tolerance assessment

The drug tolerance failed at low positive control (LPC) in the presence of 50ng/mL of drug in matrix, whereas in the presence of a lower drug concentration, established from pharmacokinetic data, the drug tolerance was reached at the LPC.

GLP-2 Analog Interspecies Matrix Plasma Binding

The compatibility of the cell-based assay with plasma was evaluated. The GLP-2 analog is characterized as having higher plasma binding capability.

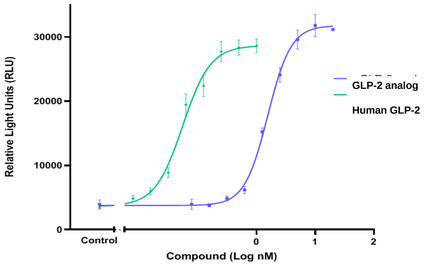


Figure 5. GLP-2 analog versus human GLP-2 in human plasma

Human GLP-2 EC50 value is significantly lower than the GLP-2 analog, and may be attributed to a different level of protein binding.

Species	Mouse	Rat	Rabbit	Minipig
Relative EC50 to human	1.0	0.8	0.9	3.1

Table 1. GLP-2 analog EC50 on various plasma species

Based on EC50 data, potency of GLP-2 analog is similar in mouse, rat, rabbit and human plasma and minimally lower, but statically significant, in minipig plasma (p=0.03). Pharmacological assessment to understand the potency of GLP-2 analog in different matrix species will also inform the design of the toxicology and clinical studies.

CONCLUSION

- The approach presented here demonstrates the versatility of a bioassay for multiple purposes, and the criticality of designing the right assay to enable the correlation of drug potency with preclinical and clinical efficacy, and therefore efficiently support the drug development process throughout.
- Designing the assay format and read-out based on the mechanism of action of the drug was very important to ensure the proper development of the cell-based assay suitable for both early pharmacological assessment and NAb assay.
- Key optimization parameters include the use of an immunoassay compatible with various matrices that allow maximizing the utility of conducting only one assay for NAb determination; and pharmacological assessments that will ensure cost and time effectiveness.
- In the future, this optimized assay could also potentially be used for lot release assays or lot comparability, and to test the cross-reactivity of the potential NAbs against the endogenous GLP-2.