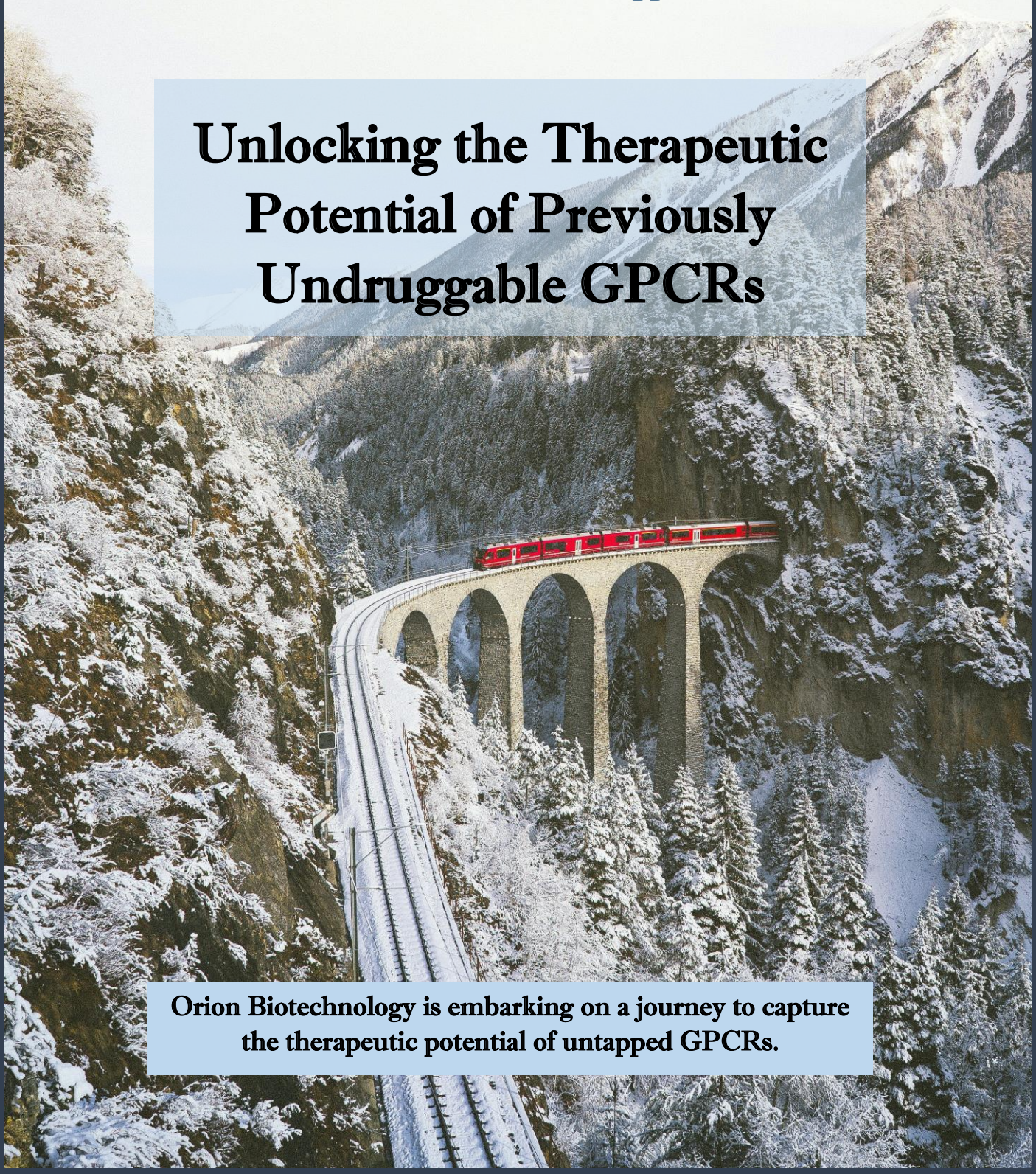
ORION biotechnology

Unlocking the Therapeutic Potential of Previously Undruggable GPCRs



Orion Biotechnology is embarking on a journey to capture the therapeutic potential of untapped GPCRs.

Executive Summary

This whitepaper will provide an overview of G Protein-Coupled Receptors (GPCRs) and discuss current trends in GPCR drug discovery. GPCRs are an important class of drug targets that represent approximately 30% of global drug market sales. However, the receptors that these medicines target have been described as the 'low-hanging' fruit, and many of the remaining GPCRs have shown low tractability using established drug discovery approaches. This situation is now evolving, with recent scientific and technological breakthroughs encouraging a new wave of GPCR drug discovery.

Among the challenging GPCRs are approximately 50 that have large natural ligands. While these small protein GPCRs are valuable drug targets linked to serious diseases, many remain undrugged because they are less tractable to standard drug discovery approaches. To solve this industry-wide problem, Orion Biotechnology (Orion) is introducing a novel solution driven by its PROcisionX™ platform: precision-engineering analogs based on the natural small protein ligands of these receptors to unlock their therapeutic potential.

The GPCR Opportunity

GPCRs are the largest and most diverse group of membrane receptors. They are composed of seven membrane-spanning α-helices, comprising the transmembrane (TM) domain of the receptor, joined by alternating intracellular and extracellular loop regions that form the intracellular and extracellular faces of the receptor. In general, signaling occurs when agonists engage key microswitch structures located in the TM domain. These interactions induce local conformational changes which are amplified and transmitted to the intracellular face of the receptor, leading to the binding and activation of cytosolic effector proteins: the G proteins after which GPCRs are named, and arrestins which both shut off G protein activity and elicit G protein-independent signaling pathways.

There are approximately 800 human GPCRs in the superfamily, controlling a broad range of physiological activities. Roughly half of the receptors in the superfamily are sensory GPCRs, involved in olfaction, taste, vision and pheromone signaling. The remaining non-sensory receptors, with functions more readily linked to pathology, are currently the main focus for GPCR drug discovery.

A New Wave of GPCR Drug Discovery

GPCRs are considered highly druggable, with GPCR-targeting therapeutics contributing ~30% of global drug market sales. However only ~15% of the GPCR superfamily has been successfully drugged, due in part to the intractability of certain groups within the GPCR superfamily to standard drug discovery approaches.

Spurred on by new scientific and technological advances related to the understanding of structure and activation mechanisms of GPCRs, the biotech investment community has injected significant funding into small biotech companies developing novel technology platforms to target GPCRs in recent years. Within the last two years, three investments stand out:

- Tectonic Therapeutics: received $80 Million USD in Series A financing to develop GPCR-targeting nanobodies.
- Septerna: closed a $100 Million USD Series A round to develop small molecule drugs against difficult-to-drug GPCRs.
- Domain Therapeutics: completed a $42 Million USD Series A round to develop novel immunotherapies, including an anti-CCR8 antibody.

Small Protein GPCRs are Difficult to Drug

Among the GPCRs that are challenging to drug using standard approaches is a group of approximately 50 receptors whose endogenous ligands are small proteins. These receptors have much larger binding pockets than the majority of GPCRs that have been successfully drugged so far. The significantly increased surface area of ligand-receptor interface for these GPCRs makes modulation with small molecule drugs, when they can be identified, much more challenging.

Firstly, small molecule inhibitors only occupy a fraction of the available surface area in the large binding pocket, making them unsuitable for achieving full blockade of the natural ligand. Secondly, because the activation mechanism of small protein GPCRs involves making key contacts with the receptor at structurally distant sites in the binding pocket, it is difficult to achieve a high level of control over the quantity and quality of signaling activity using small molecule agonists.

Significant resources have been invested into the search for small molecule modulators of small protein GPCRs, but discovery is challenging and the attrition rate during clinical development has been very high.

One way to overcome the challenges presented by small protein GPCRs is to use small proteins as modalities to target them. These approaches include generating ligand analogs by precision engineering the natural small protein ligand of the receptor. The natural ligand of the target receptor provides an excellent scaffold, already with a size and shape that matches its binding pocket. The engineered analog approach involves optimizing this scaffold, adding and subtracting molecular contacts between the ligand and the receptor to obtain the required potency and signaling output.

Small protein GPCRs feature a two-component binding mechanism in which one part of the ligand engages the extracellular face of the receptor, providing an 'address' function: binding affinity and specificity. The other part of the ligand reaches in to contact the TM domain of the receptor, providing a 'message' function: engaging the microswitches that control signaling activity. As seen below in Figure 1, natural ligands do not make use of all the potential binding contacts with the TM domain of the receptor, and by modifying these key points of contact it is possible not only to strongly increase binding affinity but also to fine tune both the quantity and quality of receptor signaling.

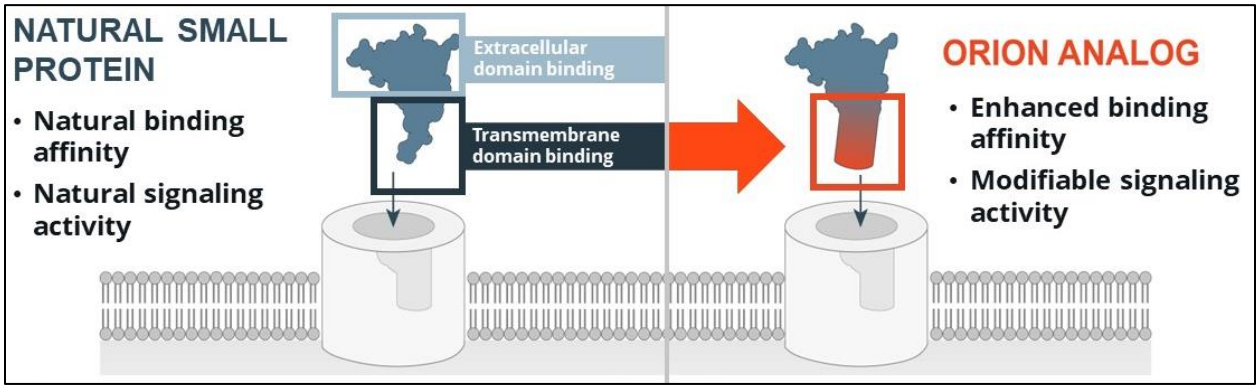


Figure 1. Left: Natural ligands of small protein GPCRs use a two-component binding mechanism, with extracellular domain binding providing a highly specific 'address' function, and the transmembrane domain binding making 'message' contacts that drive signaling activity. Right: Orion ligand analogs are engineered to optimize transmembrane domain providing significantly increased binding affinity and providing a means to control both the quantity and quality of signaling activity.

Orion's approach to engineering ligand analogs involves leaving the 'address' binding interface on the extracellular face of the receptor unchanged and searching for optimized interaction with the TM domain of the receptor. This characteristic provides a major advantage in drug discovery: massive parallel library-based screening can be carried out directly on living cells expressing the target receptor. The unmodified 'address' interaction ensures that all molecules in the library are specific for the target receptor rather than irrelevant cell surface targets, meaning that in contrast to all of the other current GPCR discovery procedures, Orion's

approach does not require the onerous and time-consuming processes of target receptor expression, purification and stabilization at the beginning of a discovery campaign.

Orion's PROcisionX™ Platform

In Orion's discovery process, shape space at the TM domain 'message' site is extensively explored by using surface display technology, with molecular diversity focused into the part of the ligand that engages the TM domain. Libraries of billions of analogs are screened in parallel for functional interaction with receptors presented in their physiological environment. Selection of the libraries typically leads to the isolation of several hundred candidate hits, enriched because they either bind with increased affinity at the cell surface, or because they have an enhanced capacity to elicit receptor internalization, thereby gaining the capacity to shelter inside the cell. Both properties enable enhanced ligands to avoid being eliminated during the stringent washing process.

All candidate hits are then rapidly produced in parallel by total chemical synthesis using Orion's proprietary multiplex synthesis technology, and then screened for pharmacological activity (receptor binding affinity, G protein and arrestin signaling) in high throughput cell-based assays. Screening directly identifies leads, and the structure-activity data obtained for the ensemble of candidate hits provides a rich resource of information to inform lead optimization, either by human and in silico-aided rational design, or by the construction of next-generation surface display libraries.

The PROcisionX™ platform (summarized in Figure 2) can be used to complete a discovery campaign within only 6-12 months, yielding optimized leads ready for preclinical validation. Hence PROcisionX™ is one of the fastest discovery platforms in industry, and one that is uniquely capable of generating drug candidates with optimal molecular pharmacological properties for targeting small protein GPCRs.

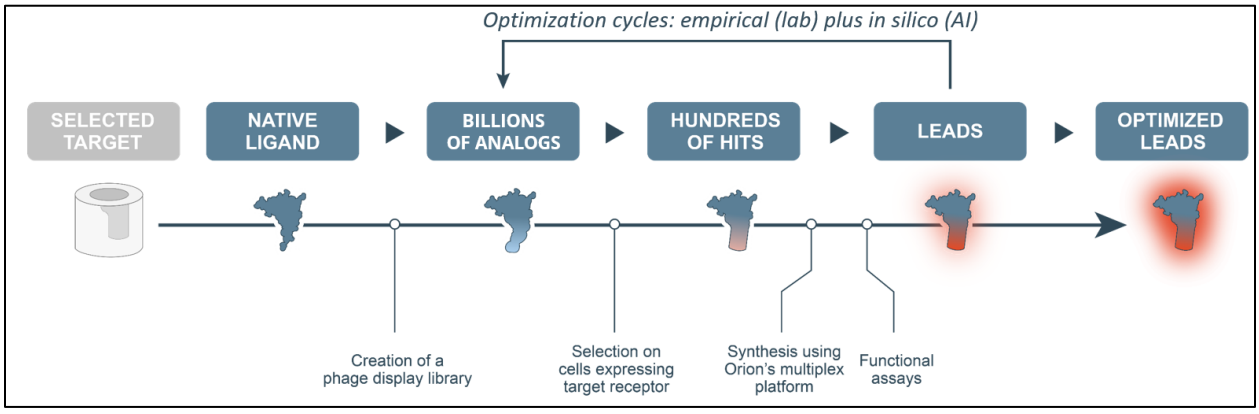


Figure 2: Workflow in Orion's PROcisionX™ platform.

A unique and important feature of Orion's discovery process is that the initial search for potent target receptor modulators is agnostic towards signaling activity because it selects for any kind of enhanced functional interaction with the receptor in the environment of a living cell. Since shape space exploration is specifically focused on the 'message' site in the TM domain, this means that lead candidates across the whole spectrum of signaling activity are identified: full antagonists, partial agonists with different levels of signaling activity, biased agonists that preferentially activate either G protein signaling or arrestin signaling, and superagonists with signaling activity higher than that of the native ligand (Figure 3).

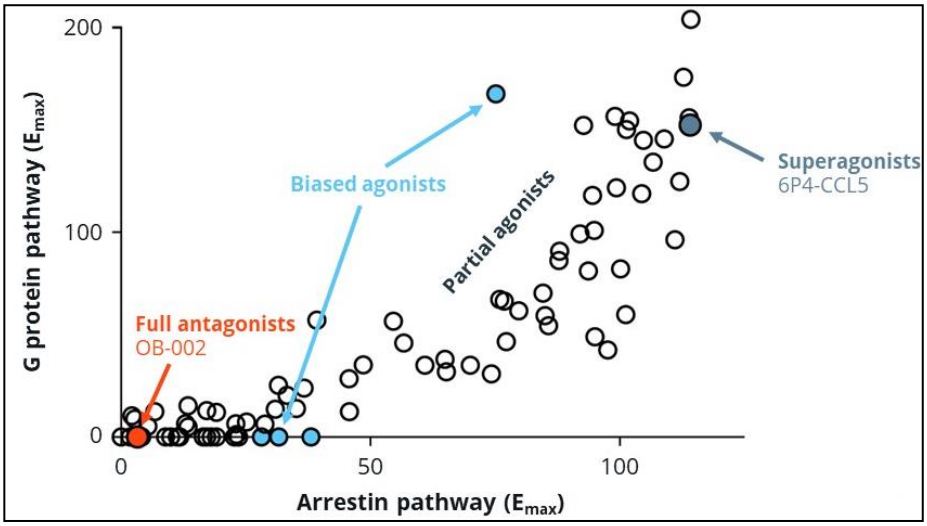


Figure 3: In a single discovery campaign (CCR5 is shown as an example), Orion's PROcisionX™ platform generates a diverse set of potent small protein ligand analogs across the whole spectrum of signaling activity.

Orion has demonstrated that optimized antagonist analogs generated using the PROcisionX™ platform have best-in-class *in vitro* functional inhibitory potency and show remarkably persistent binding. This leads to strikingly long *in vitro* receptor occupancy durations (at least seven days), meaning that lengthy pharmacodynamic activity is attained *in vivo*, despite the short circulatory half-life that is expected with small protein drugs. Orion's receptor antagonist analogs have demonstrated powerful efficacies across a range of animal models spanning different disease indications.

Orion's superagonist analogs are not only powerful candidate drugs for receptor modulation in indications where potent receptor activation is required, they are also highly effective vehicles for payload delivery. This is due to their enhanced capacity to drive receptor internalization in a process that pulls the ligand, together with its conjugated payload, inside the target cell. Orion has demonstrated that this target cell-specific delivery cannot be achieved using the native ligand of the receptor, despite its modest level of receptor internalization activity (Figure 4).

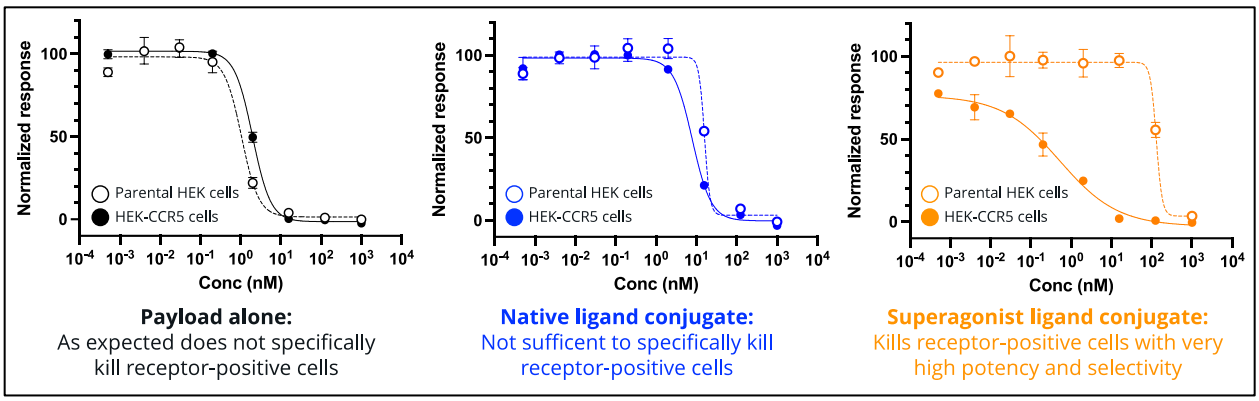


Figure 4: Payload delivery using Orion's superagonist analogs on target cells expressing CCR5. The cytotoxic drug Monomethyl auristatin E (MMAE) was used alone or conjugated to either the native CCL5 ligand or to an Orion CCL5 superagonist analog. While the native ligand conjugate exhibited poor selectivity for CCR5-expressing cells and was less effective at killing cells than unconjugated MMAE, the Orion CCL5 superagonist analog significantly increased both the potency and selectivity of the payload towards target cells.

Case study: Discovering a CCR2 Antagonist

The chemokine receptor CCR2 regulates the recruitment of monocytes to the sites of inflammation. Since many inflammatory diseases are driven by inappropriate recruitment of monocytes into tissues, CCR2 has been described as the master controller of inflammatory pathology and is considered a valuable drug target for a number of inflammatory diseases including atherosclerosis, scleroderma, multiple sclerosis and cancer.

Orion's CCR2 antagonist analog (OB-004), discovered in only 6 months, is considerably more potent than the leading small molecule competitors in *in vitro* functional inhibition assays (Figure 5). In an *ex vivo* human endothelial transmigration model, OB-004 demonstrated an unprecedented level of monocyte blockade, robustly outperforming the most potent of the competitor small molecules (Figure 6). Orion used its discovery platform technology to rapidly develop a murinized version of OB-004 that was evaluated in a thioglycolate-induced peritonitis model. In this model, OB-004 showed powerful efficacy, achieving full blockade of monocyte recruitment in response to the inflammatory challenge (Figure 7).

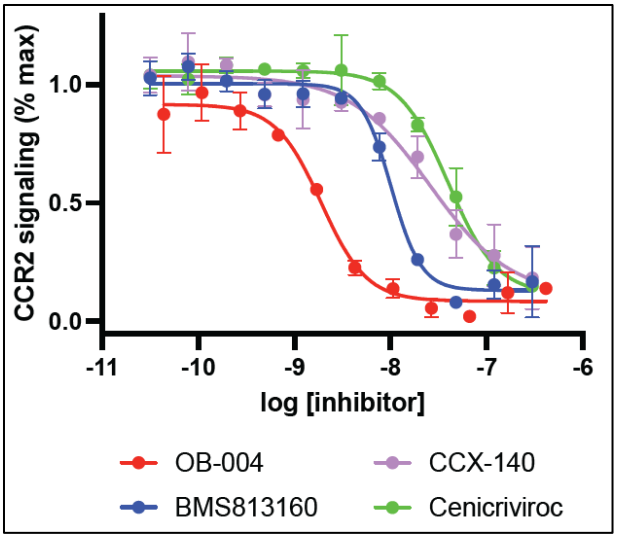


Figure 5: In an *in vitro* functional signaling assay on a human monocytic cell line, OB-004 demonstrated best-in-class potency versus a group of small molecule CCR2 inhibitors in clinical development.

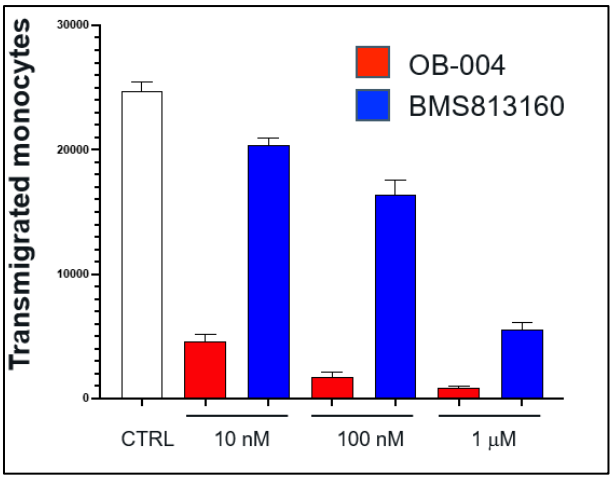


Figure 6: In a flow-based human monocyte endothelial transmigration assay, OB-004 strongly outperformed BMS813160 at all three concentrations tested, achieving full blockade of transmigration at the highest concentration in this stringent model.

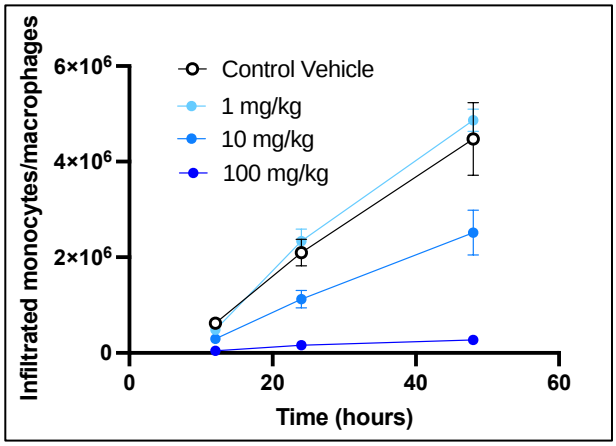


Figure 7: *In vivo* efficacy of murinized OB-004 (mOB-004) in the thioglycolate (TG) induced peritonitis murine model. Twice-daily treatment with mOB-004 dose-dependently suppressed monocyte infiltration into the peritoneum cavity, achieving complete blockade at the highest dose level.

The Future of GPCR Research

GPCRs are a very important class of drug targets, but despite historical successes, the majority of the superfamily remains undrugged, in part due to limitations in current drug discovery technology. There is a great deal of scientific and business interest in solving this problem, and the last two years have seen the emergence of a new wave of technology driven GPCR drug discovery companies.

Available structural data across the GPCR superfamily is increasing at an exponential rate, alongside understanding of the mechanisms underlying receptor activation. This, together with the rapid development of artificial intelligence (AI)-driven *in silico*-based molecular interaction prediction, will inevitably lead to a more and more central role for *in silico* methodology and AI in GPCR drug discovery. Orion has entered a strategic research collaboration with Peptilogics, a US-based AI drug discovery company to exploit the rewards that can be reaped at this exciting new frontier.

GPCRs with small protein ligands are among the receptor groups that have proven to be particularly challenging, and Orion has developed a unique and powerful solution to the problem, based on using the natural ligands of target receptors to generate precision-engineering analogs. Orion's PROcisionX™ platform has been validated by rapid success, obtaining not only best-in-class antagonists with strong in vivo efficacy, but also potent signaling molecules for indications where subtle tuning of receptor signaling is required, or as vehicles for payload delivery. In this way Orion has established itself as one of the leaders in the exciting new wave of GPCR drug discovery.

About Orion Biotechnology

Orion's mission is to unlock the therapeutic potential of previously undruggable GPCRs. Orion has world-renowned expertise in GPCR pharmacology and protein engineering, and its proprietary drug discovery platform (PROcisionX™) has been used to rapidly and efficiently advance a diversified portfolio of GPCR-targeted drug candidates for the treatment of cancer and other serious diseases.

Orion's objectives include expanding internal and external pipelines and continuing to innovate technologies to unlock GPCRs. For more information, follow Orion Biotechnology on LinkedIn or visit www.orionbiotechnology.com.

Relevant Publications

Development of Orion's platform technology

Hartley, O. *et al.* (2004) *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 16460-16465. https://doi.org/10.1073/pnas.0404802101

- Initial work on precision engineering of a small protein GPCR ligand using new peptide chemistry technology and rational design

Gaertner, H. *et al.* (2008). *Proceedings of the National Academy of Sciences*, *105*(46), 17706–17711. https://doi.org/10.1073/pnas.0805098105

- Development and use of new technology to gain full control of signaling activity through a small protein GPCR via library-based screening

Dorgham, K. *et al.* (2016) *Methods in Enzymology*, **570**, 47-72. https://doi.org/10.1016/bs.mie.2015.09.014

- Library-based screening methodology for engineering small protein GPCR ligands

Paolini-Bertrand, M. *et al.* (2018) *The Journal of Biological Chemistry*, *293*(49), 19092–19100. https://doi.org/10.1074/jbc.RA118.004370

- New technology to rapidly synthesize candidate hits from library-based screening so that they can be tested in cell-based assays

Akondi, K. B. *et al.* (2021) *Chimia*, *75*(6), 489–494. https://doi.org/10.2533/chimia.2021.489

- Description of the fully integrated PROcisionX™ discovery platform

Structure-based validation of Orion's approach

Zheng, Y. *et al.* (2017) *Immunity*, **46**, 1005-1017.e1005. https://doi.org/10.1016/j.immuni.2017.05.002

- Structural explanation of the receptor binding mechanism of a highly potent antagonist analog

Isaikina, P. *et al.* (2021). *Sci Adv*, **7**. https://doi.org/10.1126/sciadv.abg8685

- Structural explanation of the binding and activation mechanism of a highly potent superagonist analog

***In vivo* efficacy validation of Orion's optimized leads:**

Lederman, M.M. *et al.* (2004) *Science, 306*, 485–487. https://doi.org/10.1126/science.1099288

- Use of a first precision-engineered analog to validate topical inhibition of CCR5 as a strategy for HIV prevention

Veazey, R.S. *et al. Journal of Infectious Diseases*, **199**, 1525-1527. https://doi.org/10.1086/598685

- Demonstration that topically administered precision engineered small proteins show full *in vivo* efficacy in a highly stringent model of HIV transmission

Steinbach, K. *et al.* (2019) *Science Translational Medicine*, **11**. https://doi.org/10.1126/scitranslmed.aav5519

- Demonstration of the *in vivo* efficacy of a systemically administered antagonist analog in a model of neuroinflammation

Feasibility validation of Orion analogs in clinical development

Cerini, F. *et al.* (2016) *Protein Expression and Purification*, **119**, 1-10. https://doi.org/10.1016/j.pep.2015.10.011

- Demonstration of the feasibility of manufacturing clinical grade small protein analogs

McGowan, I.M. *et al.* (2021). *AIDS Res Hum Retroviruses*, **37**, 453-460. https://doi.org/10.1089/aid.2021.0010

- Successful first-in-human clinical study of a topically administered small protein analog