DISCOVERY AND CHARACTERIZATION OF BIASED AGONIST AND ANTAGONIST hCCR5 ANTIBODIES USING 3RD GENERATION ebBRET BIOSENSORS

Guilhem DUGAST[1], Claire Normand[1], Arturo MANCINI[1], Jiannan LI[2], Noel PAULI[2], William ROACH[2], Laurent SABBAGH[1]
[1] Domain Therapeutics NA Inc., Montreal, QC, Canada
[2] Adimab LLC, Lebanon, NH, USA

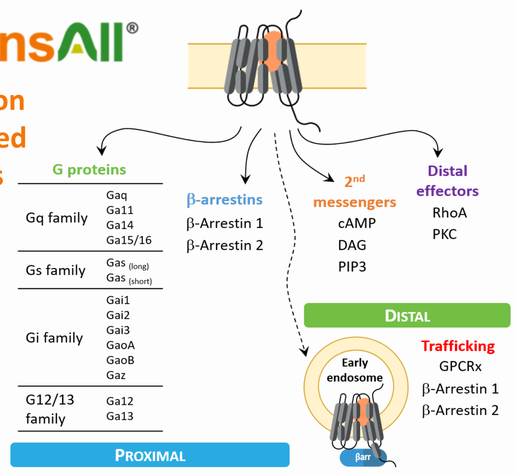
INTRODUCTION

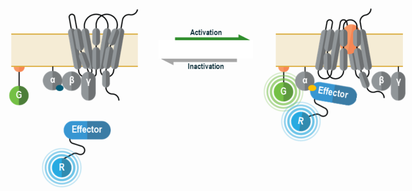
G protein-coupled receptors (GPCRs) are the most prominent drug targets with approximately 1/3 of the marketed drugs targeting these receptors. However, approximately 75% of them remain underexploited. Classically, these receptors have been targeted by small molecules. Functional antibody development for GPCRs is an innovative and promising area in drug discovery, one that may help uncover the therapeutic potential of the undrugged GPCRome. Yet, this field has thus far lagged due to the complexity of GPCR structures, their conformational plasticity and the lack of comprehensive screening approaches capable of measuring their signaling and pharmacology.

We describe herein how bioSens-All®, an enhanced bystander (eb) BRET-based biosensor platform, was used to characterize and uncover a novel class of functionally selective agonist and antagonist anti-hCCR5 antibodies developed by Adimab, whose yeast-based protein engineering platform, paired with immunized animal diversities, can solve many of the challenges of membrane protein antibody discovery.

MATERIALS AND METHODS

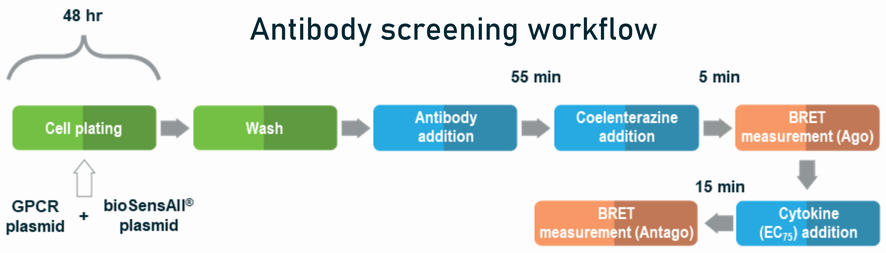
bioSens-All®
3rd generation ebBRET-based biosensors





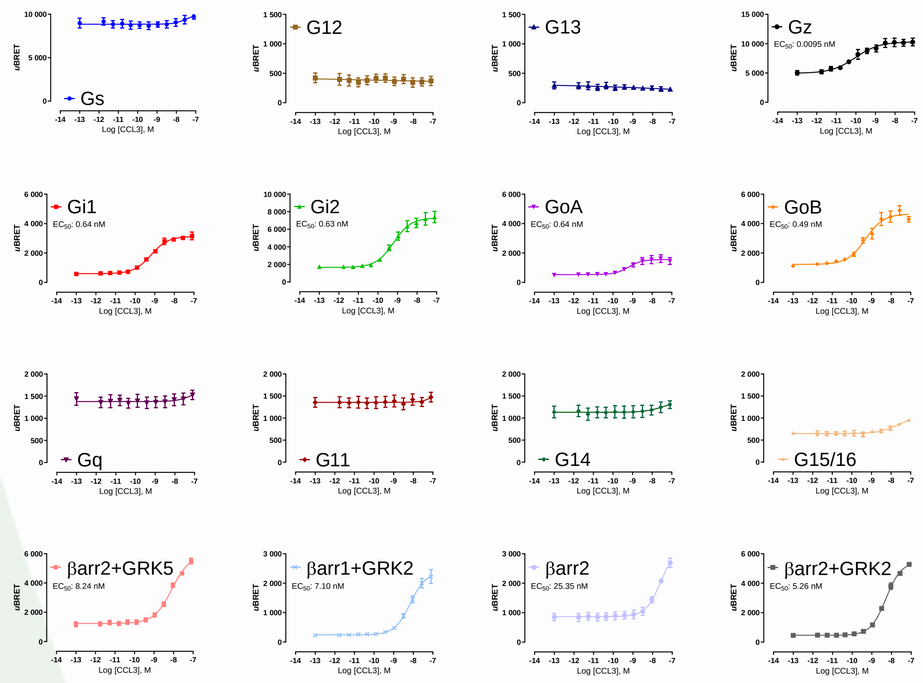
> Analysis of 61 hCCR5 antibodies
> 5 pathway-specific biosensors
> Agonist / antagonist modes

Antibody screening workflow



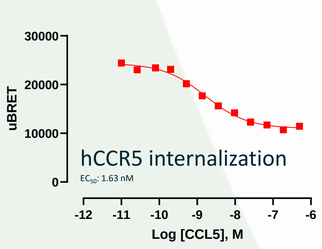
RESULTS

1 CHARACTERIZATION OF hCCR5 SIGNALING AND TRAFFICKING USING bioSens-All®

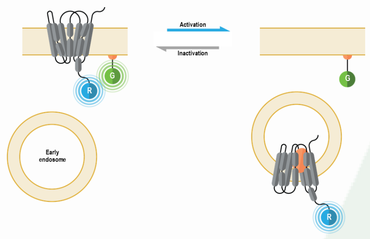


> An extensive panel of signaling (G protein and β-arrestin) and receptor trafficking ebBRET biosensors were used to define hCCR5's signaling profile.

> hCCR5 couples primarily to Gi-family G proteins (i.e., Gz, Gi1, Gi2, GoA, GoB) and β-arrestins in response to chemokine.

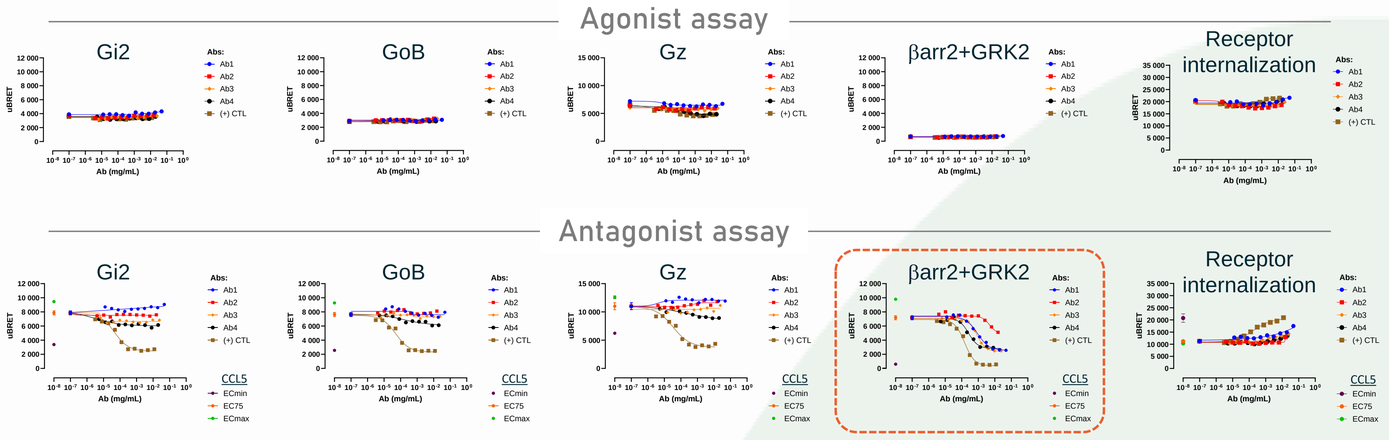


> hCCR5 undergoes internalization in a ligand dose-dependent fashion.



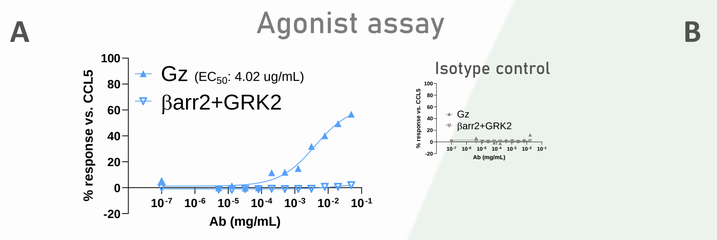
RESULTS

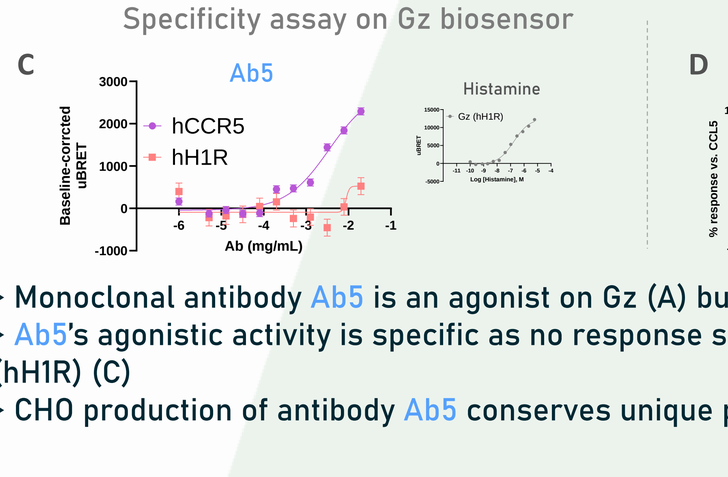
2 DISCOVERY AND CHARACTERIZATION OF β-ARRESTIN 2 -BIASED INHIBITORY ANTIBODIES



> G protein (Gi2, GoB, Gz), β-arrestin 2 (+GRK2) and internalization assays selected for screen
> (+) Control hCCR5 antibody blocks CCL5 activity across all pathways tested
> Adimab monoclonal antibodies Ab1, Ab2 and Ab3 only inhibit β-arrestin 2 activity

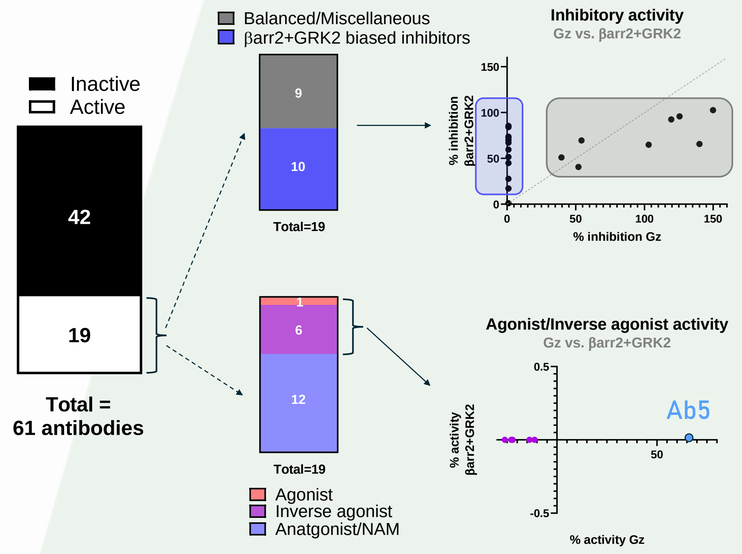
3 DISCOVERY AND CHARACTERIZATION OF A UNIQUE ANTIBODY THAT ACTIVATES Gz WHILE BLOCKING β-ARRESTIN 2 RECRUITMENT





> Monoclonal antibody Ab5 is an agonist on Gz (A) but inhibits β-arrestin 2 activation (B)
> Ab5's agonistic activity is specific as no response seen on human histamine H1 receptor (hH1R) (C)
> CHO production of antibody Ab5 conserves unique pharmacological activity (D)

4 SUMMARY OF FUNCTIONAL DIVERSITY EXHIBITED BY hCCR5 ANTIBODIES



> 19 of 61 hCCR5 monoclonal antibodies modulated receptor signaling

> An enrichment in β-arrestin 2-biased inhibitory antibodies was detected (10/19 functional antibodies)

> 6 antibodies displayed inverse agonist activity (i.e., blocked basal hCCR5 activity)

> Antibody Ab5 selectively activated Gz signaling while blocking CCL5-induced hCCR5 coupling to β-arrestin 2

CONCLUSIONS

> Multiparametric profiling of Adimab's anti-hCCR5 antibodies with bioSens-All® highlighted substantial diversity in antibody activity

> Multiple differentiated β-arrestin 2-biased inhibitory antibodies were identified

> The identification of Ab5 highlights the ability to develop unique antibodies displaying pathway-selective agonist and inhibitory properties

> The development of <<biased>> anti-GPCR antibodies, such as those highlighted in this study, may provide therapeutic advantages and reduced liabilities vs. unbiased antibodies

> bioSens-All® technology paired with Adimab's antibody generation platform, allows for the discovery of functionally differentiated and diverse anti-GPCR antibodies