Comprehensive Cell-based Assays for Characterization & Development of Agonistic Antibodies as Therapeutics for Cancer & Autoimmune Diseases

Learn more at discoverx.com/checkpoint

Venkatesh Chari, Luhan Yang, Jane E. Lamerdin, Jennifer Lin-Jones, & Gaurav Agrawal
Eurofins DiscoverX® I Fremont, CA USA

Abstract

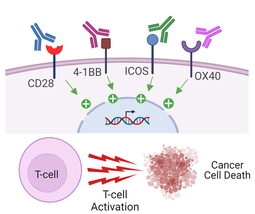
While checkpoint blockade antibodies are established cancer therapeutics, they have emerged as promising therapeutics for suppressing inflammation in autoimmune diseases. Their ability to regulate immune function targeting both co-stimulatory & co-inhibitory receptors renders them as emerging therapeutics in autoimmune diseases & for restoring tolerance in graft-versus-host disease (GVHD) complications. Existing assays frequently fail to accurately assess the agonistic activity of antibodies as they cannot replicate the necessary physiologically relevant conditions.

We present case studies demonstrating the pivotal role of cell-based assay approaches developed by Eurofins DiscoverX in driving the accurate characterization & clinical development of agonistic antibody therapeutics targeting checkpoint receptors. Additionally, we demonstrate the indispensable role of Fcγ Receptor (FcγR) mediated antibody clustering as part of the assay design in capturing the agonist potential of antibodies.

Active Areas of Clinical Development for Agonistic Antibodies

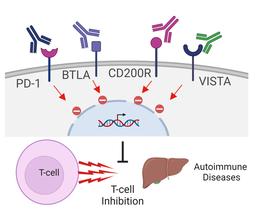
Cancer

Agonistic antibodies stimulating co-stimulatory receptors to activate T-cell to target cancer cells



Autoimmune Diseases

Agonistic antibodies stimulating co-inhibitory inhibit T-cell activation reduce inflammation in autoimmune diseases



Functional Cell-based Assays for Checkpoint Receptor Targets Based on EFC Technology

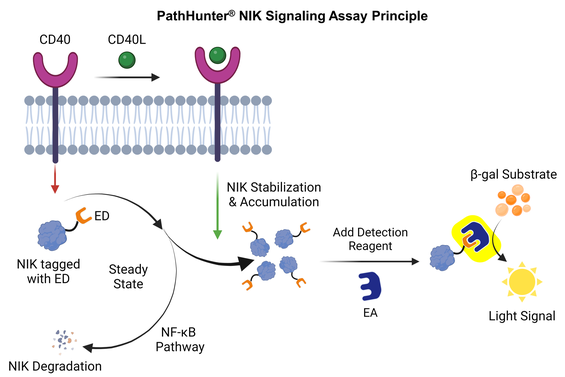


Figure 1. Eurofins DiscoverX's Enzyme Fragment Complementation (EFC) Assay Design for the CD40 Checkpoint Receptor. The EFC technology is based on a split β-galactosidase (β-gal) enzyme system. Individually, the enzyme donor (ED) peptide & enzyme acceptor (EA) fragment are inactive. When brought in proximity, the ED complements with EA forming an active β-gal enzyme that hydrolyzes substrate resulting in a chemiluminescent signal. The PathHunter NIK Signaling Cell Line was engineered to express ED-tagged NIK protein. Addition of CD40L (ligand) leads to activation of the NF-κB pathway resulting in stabilization & accumulation of the NIK protein with increasing concentrations of CD40L. Addition of substrate, EA, & buffer, lyses the cells & forces complementation of the ED & EA enzyme fragments. This results in the formation of a functional enzyme that hydrolyzes substrate to generate a chemiluminescent signal.

FcγR Mediated Clustering Enhances Agonistic Effect of Antibodies Targeting Co-stimulatory Checkpoint Receptors

Assay Design with FcγR Clustering Cells Significantly Augments the Agonistic Effect of Anti Co-stimulatory Checkpoint Antibodies

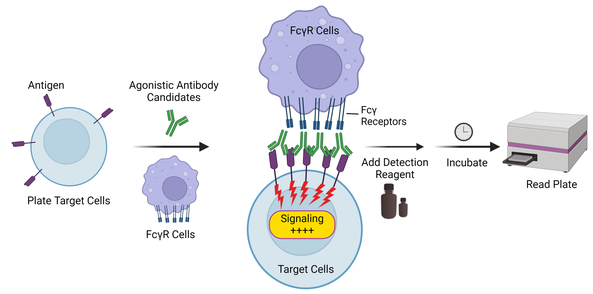
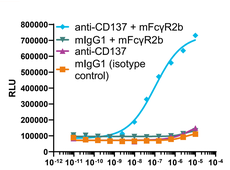


Figure 2. Modified Assay Design Including FcγR Clustering Cells. Using FcγR clustering cells (FcγR1a, 2a, 2b, or 3c) in the assay design aids in clustering the antibodies & presenting to the target cells resulting in enhanced signaling compared to standard assay design without FcγR mediated clustering.

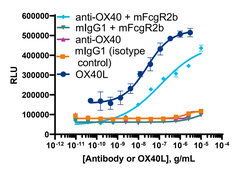
Overexpression of Target Receptor

A PathHunter CD137 Signaling Assay



	anti-CD137	mIgG1 (isotype control)	anti-CD137 + mFcγR2b	mIgG1 + mFcγR2b
S/B	6.6	1.7	8.8	1.4
EC_{50}	6.50E-06	3.10E-06	1.00E-07	3.00E-06

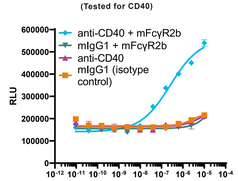
B PathHunter OX40 Signaling Assay



	anti-OX40	mIgG1 (isotype control)	OX40L	anti-OX40 + mFcγR2b	mIgG1 + mFcγR2b
S/B	1.4	1.5	3.4	9.9	2.0
EC_{50}	2.40E-06	1.90E-06	2.60E-08	1.10E-07	7.60E-06

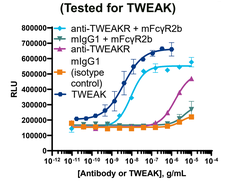
Endogenous Expression of Target Receptor

C PathHunter NIK Signaling Assay (Tested for CD40)



	anti-CD40	mIgG1 (isotype control)	anti-CD40 + mFcγR2b	mIgG1 + mFcγR2b
S/B	1.4	1.3	3.9	1.0
EC_{50}	5.90E-06	2.60E-06	2.50E-07	N/A

D PathHunter NIK Signaling Assay (Tested for TWEAK)

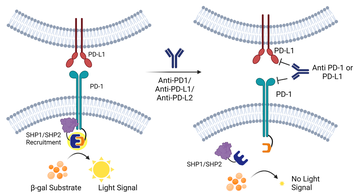


	anti-TWEAKR	mIgG1 (isotype control)	TWEAK	anti-TWEAKR + mFcγR2b	mIgG1 + mFcγR2b
S/B	3.2	1.5	3.3	3.6	1.8
EC_{50}	1.50E-06	2.90E-06	4.00E-09	9.20E-09	4.20E-06

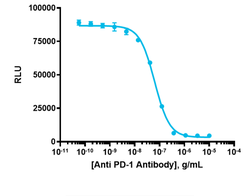
Figure 3. Augmented Agonism of Target Receptors. A. & B. Enhanced agonism observed with mouse FcγR2b (mFcγR2b) clustering cells in the PathHunter CD137 & OX40 Signaling Assays, compared to the anti-CD137 or anti-OX40 alone, respectively. C. & D. Agonistic activities of anti-CD40 & anti-TWEAKR antibodies were assessed in the PathHunter NIK Signaling Assay, where the target receptors, CD40 & TWEAKR, are endogenously expressed. Adding mFcγR2b clustering cells to the assay design significantly enhances the efficacy & potency of these antibodies compared to the antibody alone.

Introducing FcγR Clustering in the Assay Design Helps Identify a Strong Agonistic Effect in Antibodies Previously Established as Antagonists

A PathHunter PD-1 Signaling Assay Principle (Assay Design for Antagonist Mode)

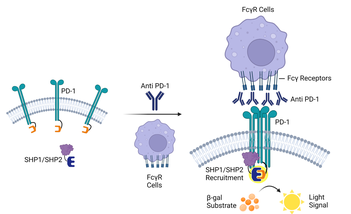


B PathHunter PD-1 Signaling Assay (Tested in Antagonist Mode)

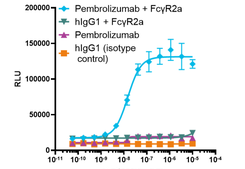


	Anti-PD1 Antibody
S/B	20.8
EC_{50}	1.02E-07

C PathHunter PD-1 Signaling Assay Principle (Assay Design for Agonist Mode)

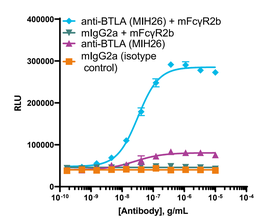


D PathHunter PD-1 Signaling Assay (Tested in Agonist Mode)



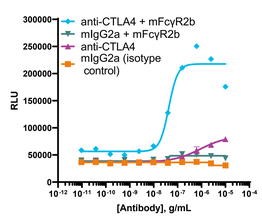
	Pembro-lizumab	hIgG1 (isotype control)	Pembro-lizumab + FcγR2a	hIgG1 + FcγR2a
S/B	1.7	1.0	7.5	1.0
EC_{50}	1.80E-08	N/A	7.00E-09	N/A

E PathHunter BTLA Signaling Assay (Tested in Agonist Mode)



	anti-BTLA	mIgG2a (isotype control)	anti-BTLA + mFcγR2b	mIgG2a + mFcγR2b
S/B	2.0	1.0	6.6	1.0
EC_{50}	1.30E-07	N/A	3.20E-08	N/A

F PathHunter CTLA4 Signaling Assay (Tested in Agonist Mode)

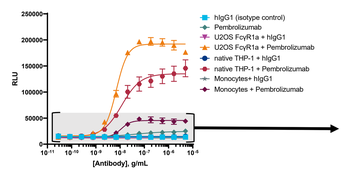


	anti-CTLA4	mIgG2a (isotype control)	anti-CTLA4 + mFcγR2b	mIgG2a + mFcγR2b
S/B	1.2	2.3	3.9	1.2
EC_{50}	2.60E-06	8.80E-07	4.30E-08	4.00E-08

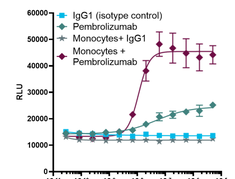
Figure 4. Checkpoint Receptors Signaling using Modified Assay Design for Capturing Enhanced Agonistic Activity of Antibodies. A. The PathHunter PD-1 Signaling Assay measures SHP1 recruitment to PD-1 ITIM motifs. When PD-L1 Ligand Cells are co-cultured with PD-1 Signaling Cells, PD-L1 activates the PD-1 receptor & SHP1 or SHP2. SH2-EA enzyme fragment fusion protein is recruited to PD-1 tagged with the complementary ED tag. Anti-PD-1 antibody disrupts the interaction between PD-1 & PD-L1, inhibiting PD-1 signaling, resulting in a loss of chemi-luminescent signal. B. Results from the PathHunter PD-1 (SHP1) Signaling Assay. Anti-PD-1 antibody (NAT105) was used to block PD-1 activation mediated by PathHunter PD-L1 Ligand Cell Line in co-culture. C. Assay design representing PD-1 Signaling driven by SHP1 or SHP2 recruitment in agonist mode when including FcγR clustering cells, in the absence of PD-L1 cells, results in enhanced signaling. D. Addition of FcγR2a cells in the assay design significantly enhances the agonistic activity of Pembrolizumab (anti-PD-1 antibody) thereby resulting in suppression of T-cell activation. E. & F. Results from the PathHunter BTLA & CTLA4 Signaling Assays, which measure SHP recruitment as an activation mechanism. mFcγR2b cells enhance the agonistic effect of anti-BTLA & anti-CTLA4 antibodies as observed by the increased efficacy & potency in the presence of clustering cells compared to the antibody alone.

Primary & Immortalized Immune Cells with Endogenous Fcγ Receptor Expression also Augments Agonistic Activity of Pembrolizumab

A PathHunter PD-1 Signaling Assay (Tested in Agonist Mode)

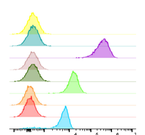


B PathHunter PD-1 Signaling Assay (Tested in Agonist Mode) Magnified from Figure A



	hIgG1 (isotype control)	Pembrolizumab	U2OS FcγR1a + hIgG1	U2OS FcγR1a + Pembrolizumab	native THP-1 + hIgG1	native THP-1 + Pembrolizumab	Monocytes + hIgG1	Monocytes + Pembrolizumab
S/B	1.0	1.8	1.0	14.6	1.0	9.5	1.0	3.5
EC_{50}	7.90E-09	1.00E-08	1.65E-08	N/A	N/A	N/A	N/A	N/A

C Clustering Mediated Agonism of Pembrolizumab Is Co-related to FcγR1a Cell Surface Expression

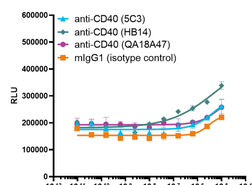


Sample Name	Antibody	Geometric Mean
FcγR1a Cell Line	Unstained	209
	Isotype Control	219
	Anti-FcγR1a	335524
Native THP-1 Cells	Unstained	191
	Isotype Control	198
	Anti-FcγR1a	14601
Primary Monocytes	Unstained	36.8
	Isotype Control	47.3
	Anti-FcγR1a	4158

Figure 5. Clustering Mediated Agonism of Pembrolizumab. A. Augmented agonistic activity of Pembrolizumab was observed with the PathHunter FcγR1a cell line, native-THP1 cells (immortalized monocytes), & human primary monocytes. B. Inset (magnified from A.) demonstrating increased agonistic activity of Pembrolizumab with monocytes compared to Pembrolizumab alone. C. Receptor density of FcγR1a in PathHunter FcγR1a cell line, native-THP1 cells, & primary monocytes using PE-labeled FcγR1a antibody.

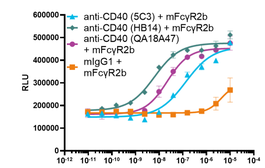
Rank Ordering Potencies of Candidate Antibodies

A PathHunter CD40 Signaling Assay (Without Fcγ Clustering Cells)



	anti-CD40 (5C3)	anti-CD40 (HB14)	anti-CD40 (QA18A47)	mIgG1 (isotype control)
S/B	1.7	2.5	1.6	1.5
EC_{50}	4.30E-06	5.90E-06	7.70E-06	2.90E-06

B PathHunter CD40 Signaling Assay (With Fcγ Clustering Cells)



	anti-CD40 (5C3)	anti-CD40 (HB14)	anti-CD40 (QA18A47)	mIgG1 (isotype control)
S/B	1.7	2.5	1.6	1.5
EC_{50}	4.30E-06	5.90E-06	7.70E-06	2.90E-06

Figure 6. RANK ordering of Antibodies. A. Low agonistic activity was observed for three different clones of anti-CD40 antibodies (5C3, HB14, QA18A47) in the absence of FcγR clustering cells. B. Enhanced agonism for all three clones of anti-CD40 antibody was observed with clustering mediated by PathHunter mFcγR2b cells, exhibiting a rank order potency with HB14 being most potent clone & the 5C3 clone being the least potent clone.

Summary

- FcγR mediated clustering significantly augments agonistic activity of anti-checkpoint antibodies offering a new avenue to design therapeutics against auto-immune diseases
- Recording direct activation of checkpoint receptors through SHP recruitment helps identify a previously unregistered agonistic activity of co-inhibitory checkpoint antibodies
- Availability of a comprehensive portfolio of cell-based assays for co-inhibitory & co-stimulatory checkpoint receptors along with all major FcγR clustering cell lines enables screening & characterization towards clinical development of agonistic antibodies

© 2024 Eurofins DiscoverX, a Eurofins Discovery Company. All Rights Reserved. 21517 022924

eurofins | DiscoverX