InvivoGen insight

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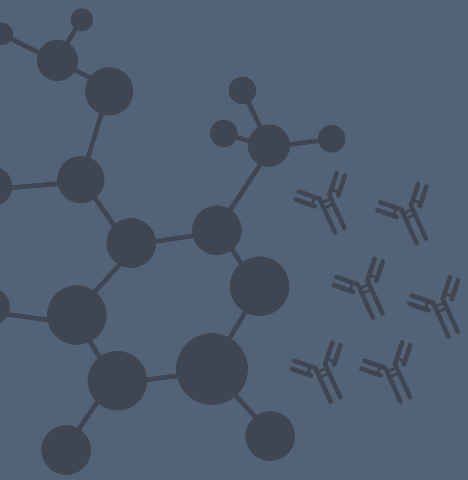


InvivoGen
www.invivogen.com
2025

Beyond PD-1/PD-L1 Blockade: Refining Antibody Approaches

Blockade of the PD-1/PD-L1 (Programmed Cell Death Protein-1/Ligand-1) immune checkpoint (IC) axis with monoclonal antibodies (mAbs) has revolutionized cancer treatment. However, due to tumor escape, only 20-30% of patients are predicted to respond favorably. This resistance is commonly associated with tumors lacking T cell infiltration characterized by a "cold" immunosuppressive tumor microenvironment (TME). Here, we highlight combinatorial antibody-based strategies to boost PD-1/PD-L1 blockade efficacy and promote "hot" immunogenic TME. These include mAb co-administration and bispecific formats.

Antibody combinations

As CTLA-4, another IC, inhibits T cell activation in a non-redundant manner to PD-1, dual checkpoint blockade strategies have been evaluated to boost antitumor immune responses[1,2]. In CheckMate studies, the combination of anti-PD-1 (Nivolumab) and anti-CTLA-4 (Ipilimumab) has demonstrated superior efficacy over monotherapies, especially in PD-L1^{low} melanoma patients[2]. However, this combotherapy induces higher-grade immune-related adverse effects than monotherapies[1-3]. Despite this concern, the FDA (USA) has approved Nivolumab and Ipilimumab co-administration in various cancers, including melanona and non small cell lung cancer (NSCLC)[1,3]. In HIMALAYA studies, the combination of anti-PD-1 (Nivolumab) and a less toxic anti-CTLA-4 (Tremelimumab)[4] has led to significantly improved overall survival in cases of advanced or unresectable hepatocellular caricoma (HCC) compared to a standard kinase inhibitor treatment. This combotherapy has been FDA approved for HCC[5], and is currently under clinical evaluation for NSCLC.

Altogether, these studies highlight the need for continuous development of new IC blocking mAbs and optimized treatment regimens to balance efficacy and safety[1,3].

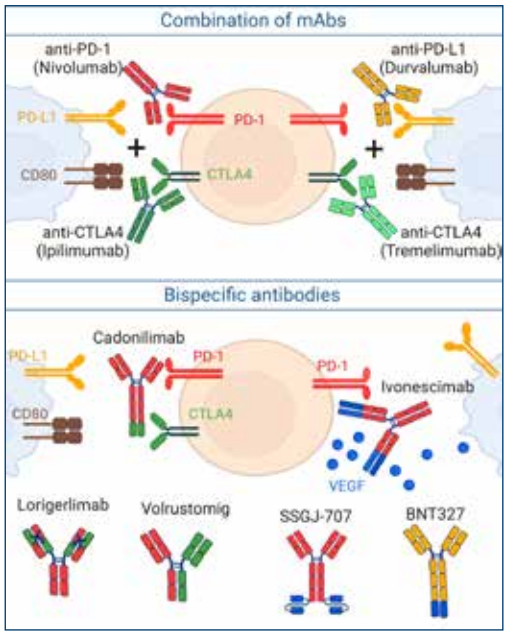
Bispecific antibodies

The success of bispecific antibodies (bsAbs) in the treatment of hematologic malignancies has spurred the evaluation of various bsAbs formats to cure solid tumors. In addition to better tissue targeting via co-localization or tumor-specific delivery, bsAbs offer multiple advantages, such as simplified dosing, optimized pharmacokinetics, and reduced toxicity. A key area of ongoing innovation lies in identifying the most effective and safest constructs among diverse engineering and targeting strategies[6,7].

Simultaneous IC blockade with bsAbs such as anti-PD-1 x CTLA4 (Cadonilimab[7]) has been tested in clinical trials. COMPASSION studies have revealed encouraging antitumor activity and safety profile of Cadonilimab in cervical cancer patients[8].

Other promising bsAbs inhibit PD-1/PD-L1 and VEGF (vascular endothelial growth factor, a key angiogenic factor with immunosuppressive functions). HARMONi studies have shown superior efficacy of Ivonescimab, an anti-PD-1 x VEGF, compared to anti-PD-1 Pembrolizumab alone in NSCLC patients[9].

Although no comparative clinical studies have been conducted so far to assess whether bsAbs are associated with better results than the combination of two mAbs, agents such as Cadonilimab and Ivonescimab have been approved by the NMPA (China) for cervical cancer and NSCLC, respectively. While confirmatory studies outside China are needed, the approval of Cadonilimab and Ivonescimab is carrying hope for other designs of anti-PD-1 x CTLA4 (Lorigerlimab, Volrustomig), anti-PD-1 x VEGF (SSGJ-707), or anti-PD-L1 x VEGF (BNT327).



Given the heterogeneity across patients and cancer types, anti-PD-1/PD-L1-based therapies must evolve alongside a deeper understanding of the patient-specific TME to overcome insufficient T cell activation or reinvigorate exhausted T cells. This will guide rational combination strategies and molecular design of next-generation antibody formats. Among these, engineered anti-PD-1 and cytokine fusion proteins (immunocytokines) enable localized immune stimulation, enhancing efficacy while reducing systemic toxicity[10]. Together, these innovations mark a critical step toward more effective, tailored, and durable cancer immunotherapies.

1. Yi, M. *et al.* 2022. Molecular Cancer. 21(1):28. **2. Wu, K. *et al.*, 2019.** Exp Hematol. Oncol. 8(1): 26. **3. Lisi, L. *et al.*, 2022.** Pharmacol Res. 175:105997. **4. Xu, C. *et al.*, 2018.** BMJ. 363:k4226. **5. France NL., *et al.*, 2024.** Target Oncol. 19(1) :115. **6. Li, T. *et al.*, 2024.** Cell Commun Signal. 22(1):179. **7. Goebeler, M.E. *et al.*, 2024.** Nat Rev Clin Oncol, 21(7):539. **8. Gao, X. *et al.*, 2023.** Lancet Oncol. 24(10):1134. **9. Xiong, A. *et al.*, 2025.** Lancet. 405(10481):839. **11. Shi, W. *et al.*, 2024.** Acta Pharmaceutica Sinica B. 14(11):4649.

Bio-IC™ Immune Checkpoint Cellular Assays

InvivoGen's Bio-IC™ assays are bioluminescent, cell-based systems designed for the screening of antibody-, Fc-fusion protein-, or small-molecule antagonists of different inhibitory immune checkpoint (IC) axes. These assays employ engineered Jurkat and Raji cell lines to mimic the immune synapse, providing a controlled environment for evaluating the efficacy of potential therapeutic antagonists.

- **PD-1/PD-L1 Bio-IC™**
- **PD-1/PD-L2 Bio-IC™** NEW
- **CTLA4/CD80 Bio-IC™** NEW
- **(PD-1/CTLA4)/PD-L1 Bio-IC™** NEW

Key Features

- Physiologically relevant paired cell line model
- Functionally tested and validated
- Suitable for high-throughput screening

Antagonist screening assays

Assay principle

The co-culture of two cell lines, Jurkat-Lucia effector T cells and Raji B antigen-presenting cells (APCs), mimics the immune synapse where effector cells are inactivated by the IC interaction(s). The immune synapse results from the activatory interactions of the TCR/[HLA::peptide] complex and CD28/CD80 , and the inhibitory IC interactions. These three interactions prevent the Jurkat-Lucia™ cells from expressing the Lucia® reporter *(Fig. 1)*.

- Without IC inhibitors: there is no Lucia® expression.
- With IC inhibitors: there is Lucia® expression *(Fig. 2)*.

Activation of the reporter T cells can be readily measured using QUANTI-Luc™ 4 Lucia/Gaussia detection reagent

Jurkat effector cells

Jurkat human T cells are engineered to stably express a specific [HLA::peptide]-restricted TCR, the CD28 costimulatory recepor, and an NFAT-Lucia luciferase reporter. For each specific Bio-IC™, these cells also stably express PD-1 and/or CTLA4 inhibitory IC receptor(s).

Raji antigen-presenting cells

Raji human B cells are engineered to stably express the cognate TCR [HLA::peptide] complex. These cells express endogenous levels of CD80, the CD28 ligand, and PD-L1, the canonical PD-1 ligand. For each specific Bio-IC™, they stably express either PD-L1 or PD-L2 inhibitory IC ligand.

InvivoGen's Bio-IC™ assays allow the functional evaluation of IC blocking monoclonal antibodies (mAbs), including:

- **Potency comparison** of different mAb clones or isotypes targeting the same IC *(Fig. 2A)*.
- **Assessment of additive or synergic effects** of mAb combinations targeting two distinct ICs *(Fig. 2B, 2D)*.

PRODUCTS	QTY	CAT. CODE
PD-1/PD-L1 Bio-IC™	3 - 7 x 10^6 cells (x 2)	rajkt-hpdl1
PD-1/PD-L2 Bio-IC™	3 - 7 x 10^6 cells (x 2)	rajkt-pdl2
CTLA4/CD80 Bio-IC™	3 - 7 x 10^6 cells (x 2)	rajkt-ctla4
(PD-1/CTLA4)/PD-L1 Bio-IC™	3 - 7 x 10^6 cells (x 2)	rajkt-ctla4-pdl1
QUANTI-Luc™ 4 Lucia/Gaussia	500 tests (5 x P96)	rep-qlc4lg1

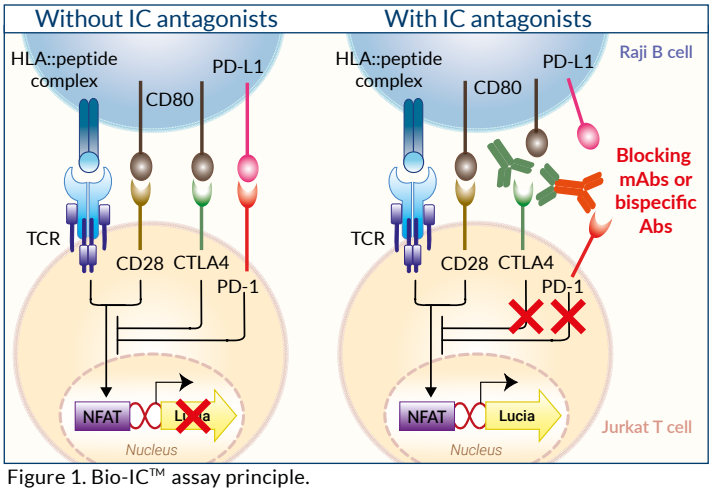


Figure 1. Bio-IC™ assay principle.

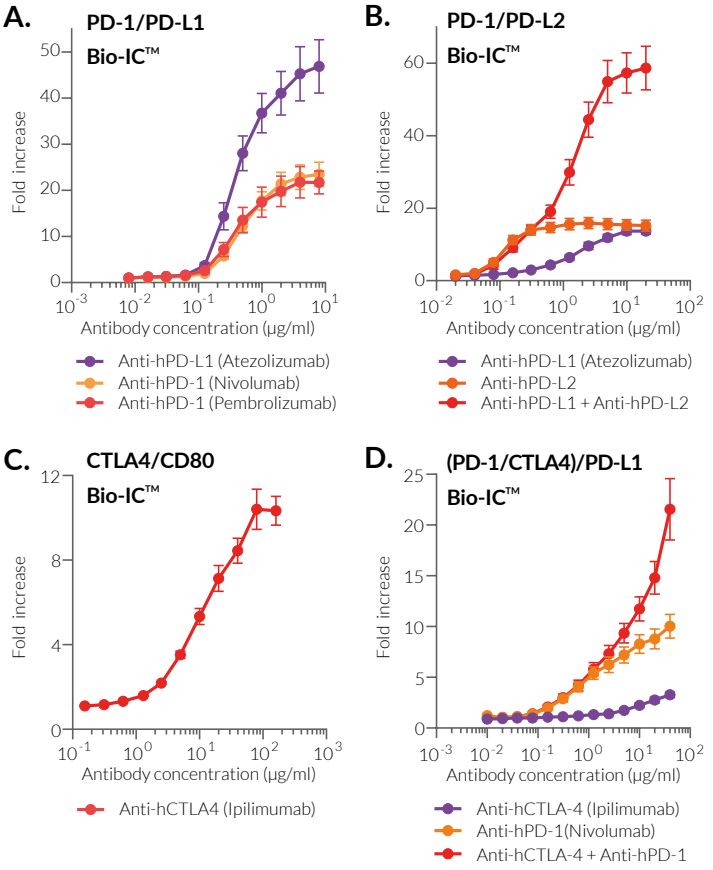


Figure 2. Activation of Jurkat-Lucia™-derived cells using IC blocking mAbs. Jurkat-Lucia™-derived effector cells and Raji-derived APCs were co-cultured in the presence of increasing concentrations of anti-hPD-1, anti-hPD-L1, anti-hPD-L2, and anti-hCTLA4 human recombinant mAbs, either alone or in combination, depending on the Bio-IC™ assay. All mAbs are derived from therapeutic biosimilar clones, except for the anti-hPD-L2. After 6 hours **(A)** or 24 hours **(B-D)**, the NFAT activation was assessed by determining Lucia® luciferase activity in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia. The fold increase over non induced cells (no mAbs) is shown (mean + SEM).

Learn more on: www.invivogen.com/immune-checkpoint-assays

Immune checkpoints blocking antibodies

InvivoGen provides high quality recombinant monoclonal antibodies (mAbs) targeting human or mouse ICs. Each lot is validated to ensure data accuracy and consistency.

• Human IC antibodies

They include well-known biosimilar antibodies *(see Table)*. Their availability in distinct IgG isotypes allows detailed analysis of Fc-mediated effector functions in the context of IC inhibition, using bioassays such as InvivoGen's BioIC™.

• Mouse IC antibodies

They include well-known clones and are specifically designed for *in vivo* studies. The InvivoFit™ grade guarantees that products are filter-sterilized (0.2 µm) and filled under strict aseptic conditions. The level of bacterial contaminants (endotoxins and lipoproteins) in each lot is verified using a LAL assay and a TLR2 and TLR4 reporter assay.

TARGETS	NAME/CLONE	ISOTYPES
Human IC antibodies		
hPD1	Pembrolizumab	hIgG1, hIgG4 (S228P)
hPD1	Nivolumab	hIgG1, hIgG1fut, hIgG4 (S228P)
h/mPD-L1	Atezolizumab	hIgG1, hIgG1 (N298A), hIgG1fut
hPD-L2 NEW	24F.10C12	mIgG2a
hCTLA4	Ipilimumab	hIgG1, hIgG1fut, hIgG4 (S228P)
Mouse IC antibodies (nvivoFit™)		
mPD-1 NEW	4C11	mIgG1, mIgG1e3
mPD-1	RMP1-14	mIgG1e3
m/hPD-L1	Atezolizumab	mIgG1e3
mPD-L1	10F.9G2	mIgG1e3
mCTLA4	9D9	mIgG2a

See cat codes on our website: www.invivogen.com/antibodies-list

IL-12 Dual Reporter Cells (SEAP & GFP)

InvivoGen introduces HEK-Blue-GFP™ IL-12, a dual-reporter cell line engineered for monitoring interleukin-12 (IL-12) activity and screening of IL-12 signaling modulators. These cells feature two STAT4-inducible reporters. The green fluorescent protein (GFP) enables real-time detection of IL-12 activity in live cells via fluorescence, making it ideal for high-throughput screening (HTS). In parallel, the secreted embryonic alkaline phosphatase (SEAP) production allows precise and quantitative analysis of the cellular response using QUANTI-Blue™ Solution.

• HEK-Blue-GFP™ IL-12 cells NEW

Key Features

- Dual STAT4-reporters: GFP and SEAP
- HTS ready: GFP fluorescence, no substrate addition
- Quantitative SEAP readout
- Functionally-tested and validated

HEK-Blue-GFP™ IL-12 cells are derived from the HEK-Blue™ IL-12 cell line, featuring the human IL-12 receptor and a STAT4-SEAP reporter, by the addition of a STAT4-GFP reporter *(Fig. 1)*. Both reporters show equivalent sensitivity to IL-12.
The STAT4-activation dual readout allows:
• **Real-time monitoring** by assessing GFP fluorescence with standard plate readers or live-cell imaging systems *(Fig. 2 & 3)*.
• **Quantification** by measuring SEAP activity in the culture supernatant using the QUANTI-Blue™ detection reagent *(Fig. 3)*.

These cells respond to both human IL-12 (# rcyc-hil12) and mouse IL-12 *(Fig. 2)*. They are ideal for release assays of activatory or inhibitory molecules, such as anti-hIL-12 Ustekinumab (# hil12p40-mab1).

PRODUCT	QTY	CAT. CODE
HEK-Blue-GFP™ IL-12 Cells	3 - 7 x 10^6 cells	hkbg-il12

Do you need a specific reporter cell line?
Check our Custom Cell Line Development Service
(see next page)

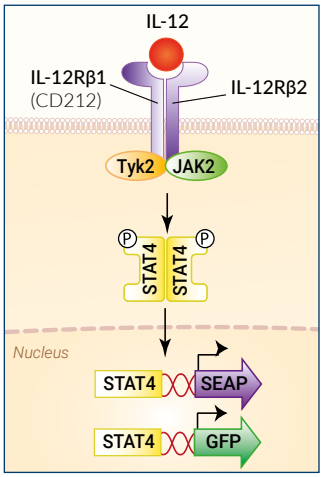


Figure 1. IL-12 signaling pathways in HEK-Blue-GFP™ IL-12 cells.

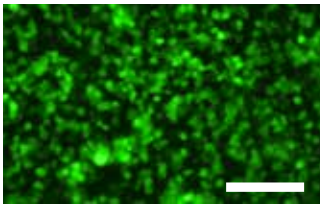


Figure 2. IL-12-induced GFP expression in HEK-Blue-GFP™ IL-12 cells. The cells were incubated with 3 ng/ml of recombinant human IL-12 for 24 hours. GFP expression was assessed using fluorescence microscopy. Scale bar: 100 µm.

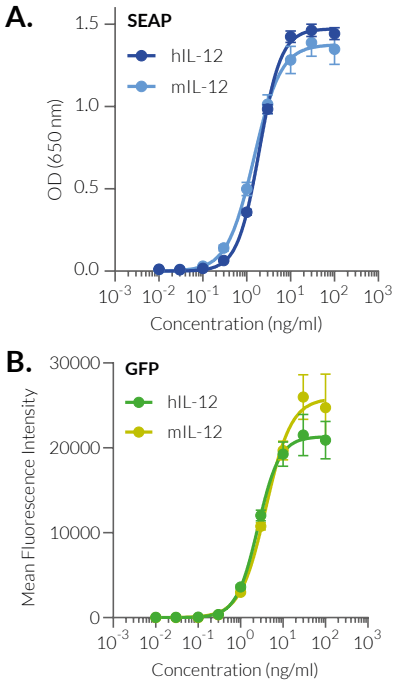


Figure 3. Cellular responses to human and mouse IL-12. HEK-Blue-GFP™ IL-12 cells were incubated overnight with increasing concentrations of human (h) and mouse (m) IL-12. **(A)** SEAP activity was measured in the culture supernatant using a spectrophotometer and reading optical density (OD) at 650 nm. **(B)** GFP mean fluoresence intensity was measured directly in the culture wells using a fluorescence plate reader. Data are shown as mean + SEM.

Custom Cell Line Development Service

Accelerate your research with InvivoGen's custom cell line development service. With more than 25 years of experience in upstream biopharmaceutical development, InvivoGen provides stably engineered cell lines tailored to your research applications. They are ideal for pathway analysis, activator/inhibitor studies, high-throughput screening systems, reporter assays, antibody-drug screenings (blocking and binding), and therapeutic development.

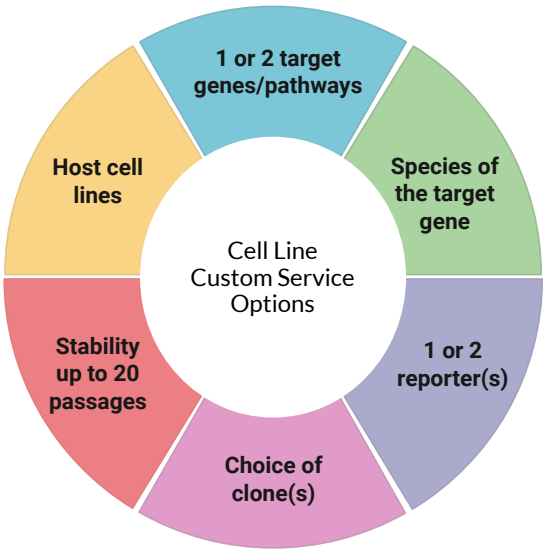
- **Customized Cell Lines** **NEW**

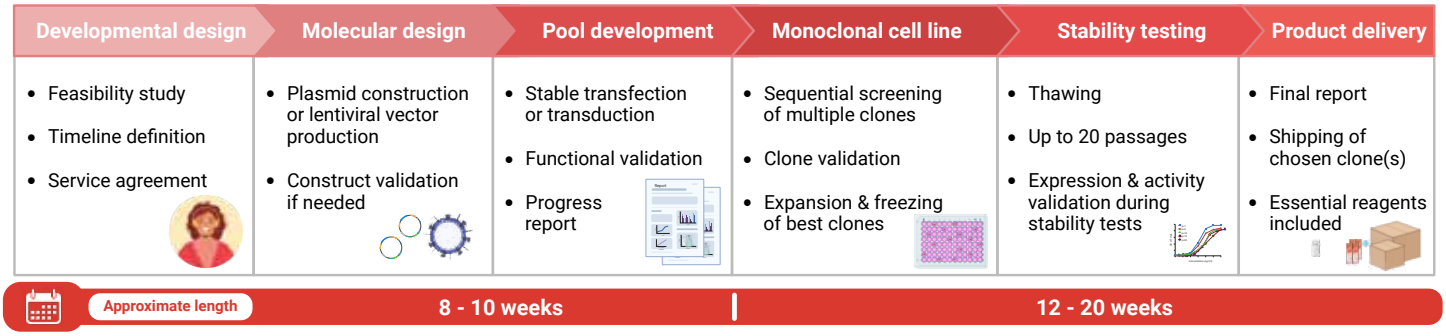
Key Features

- Dedicated support team
- Tailor-made & adaptive to your needs
- Guaranteed free of viral particles
- Fully characterized and functionally validated

Available Options

- Host cell lines: HEK293, THP-1, and others
- One or two target genes/pathways
- Species of the target gene: human, mouse, cynomolgus, ...
- One or two reporter(s): SEAP, Lucia®, GFP, ACP5
- Choice of clone(s): between two and five
- Stability: up to 20 passages





Phase I - Molecular Design & Validation (8-10 weeks)

1. Contact - Reach out to tech@invivogen.com to discuss your research needs and cell line specifications. Together, we will define the project timeline and formalize the service agreement. Once approved, we proceed with the cell line development.

2. Molecular Construction - Our experts design and construct the plasmid(s) and, if required, produce lentiviral vectors.

3. Pool Development - We perform stable transfection/transduction into your selected host cell line, validate gene expression, and assess specific activity of the cell pool. A progress report is prepared.

Phase II - Development & Delivery (12-20 weeks)

4. Monoclonal Cell Line Generation - We conduct cloning and sequential screening of multiple clones. The best-performing clones are validated, expanded, and cryopreserved.

5. Quality Control - The selected clone(s) undergo thawing and maintenance for up to 20 passages with expression and activity validation to ensure long-term stability.

6. Product Delivery - A final report is prepared, and the chosen cell clone(s) are shipped. For your convenience, we also provide complimentary samples of essential assay reagents, such as selection antibiotics and reporter detection reagents.

PRODUCT	QTY	CAT. CODE
Customized Cell Line	3 - 7 x 10^6 cells	cust-cell

www.invivogen.com/custom-service-cell-line-development

Illustrations created with BioRender.com.

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