GUIDE

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ULTIMATE PREDICTABILITY

MOUSE MODELS FOR THE ASSESSMENT OF THERAPEUTICS TARGETING THE PD-1/PD-L1 AXIS

Practical considerations for translational research



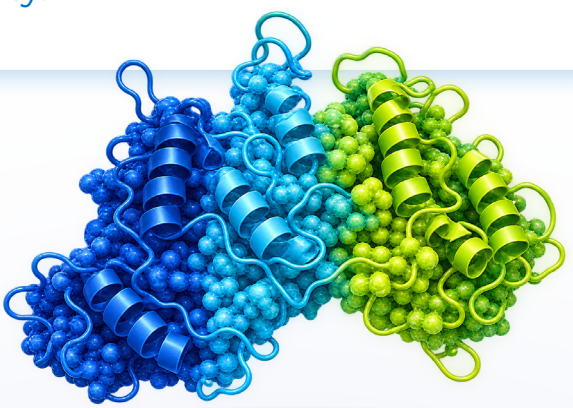
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Featuring the genO-hPD-1 (scan or click)

Featuring the genO-hPD-1/hPD-L1 (scan or click)

KEY MESSAGES

- ✓ The **PD-1/PD-L1** pathway is one of **the most clinically validated immune checkpoints in oncology**.
- ✓ Most clinical anti-PD-1 and anti-PD-L1 antibodies **bind human targets** and **do not fully cross-react with mouse PD-1 or PD-L1**.
- ✓ Wild-type mice often **require surrogate anti-mouse antibodies** and **cannot directly evaluate human-specific therapeutics**.
- ✓ Humanized PD-1 and PD-L1 knock-in mice **enable direct testing of human therapeutics in an immunocompetent setting**.
- ✓ Physiological expression of humanized targets **is critical for generating translatable efficacy and pharmacodynamic data**.
- ✓ Model selection depends on whether the objective is target engagement, efficacy testing, mechanism-of-action studies, candidate ranking, combination therapy assessment, or evaluation in a human immune system.
- ✓ Humanized immune system (HIS) models **provide access to human immune cells** but introduce additional variability, cost, and complexity.



INTRODUCTION / BACKGROUND

What is the PD-1/PD-L1 pathway?

Programmed cell death protein 1 (PD-1, CD279) is an inhibitory receptor expressed primarily on activated T cells.

Its main ligands are:
- PD-L1 (CD274)
- PD-L2 (CD273)

Binding of PD-L1 or PD-L2 to PD-1:
- Suppresses T-cell activation
- Limits cytokine production
- Reduces cytotoxic activity
- Promotes T-cell exhaustion

Tumors frequently exploit this pathway to evade immune destruction.

Why is the PD-1/PD-L1 axis important in oncology?

PD-1/PD-L1 blockade has transformed cancer treatment.

Approved PD-1 therapeutics include:
- Pembrolizumab
- Nivolumab
- Cemiplimab
- Dostarlimab
- Atezolizumab
- Durvalumab
- Avelumab

These therapies can:
- Restore anti-tumor immunity
- Increase CD8+ T-cell activity
- Promote tumor rejection
- Generate durable clinical responses

What is a humanized PD-1 mouse model?

A humanized PD-1 mouse is a genetically engineered knock-in model in which murine PD-1 is replaced by its human counterpart while preserving physiological regulation of expression.

This allows:
- Direct binding of human anti-PD-1 therapeutics
- Normal endogenous immune-cell development
- Evaluation of human-specific checkpoint inhibitors *in vivo*

What is a humanized PD-1/PD-L1 mouse model?

These models carry humanized versions of both checkpoint partners (ligand and receptor).

Advantages include:

- Simultaneous human PD-1 and PD-L1 interactions
- Direct testing of anti-PD-1 and anti-PD-L1 antibodies
- Head-to-head comparison of checkpoint inhibitors
- More comprehensive modeling of target biology

The value of double-humanized PD-1/PD-L1 mice has been demonstrated for direct comparison of FDA-approved checkpoint inhibitors (Barham et al., 2023).

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HOW TO CHOOSE THE CORRECT MODEL FOR A STUDY

Ask yourself these questions first:

1. Is my molecule targeting human PD-1 or human PD-L1?
2. Am I evaluating efficacy, pharmacodynamics, safety, or all three?
3. Do I require an intact immune system?
4. Do I need human immune cells?
5. Am I studying combination immunotherapy?
6. Am I comparing multiple checkpoint inhibitors?
7. Do I need human tumors?



Comparison of mouse models for PD-1/PD-L1 therapy assessment

MOUSE MODEL	USE WHEN	AVOID WHEN	ADVANTAGES	KEY LIMITATIONS
Wild-type mice	· Testing surrogate anti-mouse PD-1 or PD-L1 antibodies · Early biology studies	· Human-specific antibodies · Translational studies	· Simple · Low cost · Large historical datasets	· Human therapeutics usually do not bind mouse PD-1/PD-L1 · Requires surrogate antibodies · Limited translational value
Non-genOway PD-1 humanized mice commercially available	· Testing human anti-PD-1 antibodies · Proof-of-mechanism studies	· Anti-PD-L1 studies · Comparative checkpoint studies	Enables direct engagement of human PD-1	· PD-L1 remains murine · May not fully model clinical checkpoint interactions · May display a non-physiological expression of hPD-1
Non-genOway PD-1/ PD-L1 humanized mice commercially available	· Anti-PD-1 and anti-PD-L1 assessment · Comparative efficacy studies	Studies requiring a human immune system	· Humanized receptor-ligand pair · Direct testing of approved therapeutics	· Mouse immune system remains present · Human tumors not supported · May display a non-physiological expression of hPD-1 and hPD-L1 and present clinical signs

Table continued on the next page

MOUSE MODEL	USE WHEN	AVOID WHEN	ADVANTAGES	KEY LIMITATIONS
genOway's humanized PD-1 (genO-hPD-1)	· Human anti-PD-1 antibody development · Mechanism-of-action studies · Combination therapy assessment (with chemo/ radiotherapy or with a second surrogate antibody)	Direct anti-PD-L1 assessment	· Physiological expression of hPD-1 · Immunocompetent host · Preserved endogenous immune development · Supports syngeneic tumor models	· Only PD-1 is humanized · The immune system remains of murine origin, so the response may not be fully translatable · Human immune system remains incomplete · Requires murine tumors
genOway's humanized PD-1 /PD-L1 (genO-hPD-1/hPD-L1)	· Comparative testing of anti-PD-1 and anti-PD-L1 therapeutics · Candidate ranking · Translational efficacy studies	Studies assessing a response that requires several human immune cell types	· Humanized checkpoint pair · Physiological target expression · Immunocompetent environment · Enables direct therapeutic comparisons	· The immune system remains of murine origine, so the response may not be fully translatable · Requires murine tumors
PBMC-reconstituted mice	Rapid exploratory assessment of human therapeutics	· Long-term studies · Immune toxicity interpretation	· Human T cells present · Fast implementation	· GvHD risk · Xenogeneic activation · Artificial immune responses

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MOUSE MODEL	USE WHEN	AVOID WHEN	ADVANTAGES	KEY LIMITATIONS
Non-genOway HIS mice commercially available	· Human tumor immunology · Human immune-cell studies	CRS prediction	· Human immune cell repertoire · Tumor-immune cell interactions	· Residual mouse immune environment · Variable and incomplete immune reconstitution · Overexpression of human cytokines / growth factors · Short to intermediate lifespan · Potential to develop anemia and myeloid cell hyperactivation syndrome
genO-BRGSF-HIS	·Translational assessment of efficacy and safety of mAbs or TCEs · Human checkpoint studies · Combination immunotherapy · Longitudinal immune cell monitoring · PDX studies	Requirement of tumor and stroma crosstalk	· Functional human lymphoid compartment · Functional human myeloid compartment · Long experimental window · No GvHD development	· Higher cost · Increased study complexity · Donor-to-donor variability

PRACTICAL EXAMPLES

Can I test my human anti-PD-1 antibody in a wild-type mouse?

Generally no.

Why?

- Human PD-1 antibodies are typically engineered against human PD-1 epitopes
- Cross-reactivity with mouse PD-1 is often limited or absent
- Surrogate anti-mouse antibodies may be required

Preferred model: **humanized PD-1 knock-in mice** (**genO-hPD-1**).

Do I need humanized PD-L1 as well?

Use PD-1-only models when:

- Developing anti-PD-1 therapeutics
- Confirming target engagement
- Exploring mechanisms of action

Use PD-1/PD-L1 double-humanized models when:

- Comparing anti-PD-1 versus anti-PD-L1 agents
- Developing anti-PD-L1 antibodies
- Studying checkpoint biology more comprehensively

Can the humanized PD-1 or PD-1/PD-L1 models be used to compare different FDA-approved antibodies head-to-head or as a positive control for new therapeutics?

Yes. The single-humanized **genO-hPD-1 mouse model** has been used to develop the FDA-approved Nivolumab (Opdivo). **Double-humanized PD-1/PD-L1 models** can also be used to directly compare approved checkpoint inhibitors and reveal differences in anti-tumor activity not apparent from clinical literature alone, or as a point of comparison (positive control) for the development of new therapeutics.

Can hPD-1 or hPD-1/hPD-L1 models evaluate combination immunotherapy?

Yes. **Common combinations include:**

- Anti-PD-1 + chemotherapy
- Anti-PD-1 + radiotherapy
- Anti-PD-1 + TIGIT blockade (with a TIGIT surrogate antibody)
- Anti-PD-1 + CTLA-4 blockade (with a CTLA-4 surrogate antibody)
- Anti-PD-1 + T-cell engagers (with TCEs targeting murine CD3)
- Anti-PD-1 + cytokine therapies

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Can the humanized PD-1 mice model T-cell exhaustion?

Yes. PD-1 expression is a hallmark marker of exhausted T cells.

These models enable the study of:

- Checkpoint blockade
- T-cell reinvigoration
- Exhaustion reversal
- Memory responses
- Cytotoxic CD8+ T-cell activation

Can humanized PD-1 mice be used for inflammation and autoimmune disease research?

Yes. PD-1 is a key regulator of immune tolerance.

Beyond cancer, the PD-1/PD-L1 pathway plays a critical role in:

- Maintaining peripheral tolerance
- Limiting excessive T-cell activation
- Preventing tissue damage during chronic inflammation
- Controlling auto-reactive T cells

Disruption of this pathway is associated with multiple autoimmune and inflammatory diseases, including:

- Rheumatoid arthritis (RA)
- Systemic lupus erythematosus (SLE)
- Multiple sclerosis (MS)
- Inflammatory bowel disease (IBD)
- Type 1 diabetes (T1D)



FAQ

Which PD-1 model has the most physiological expression?

Models ensuring that the expression of humanized PD-1 is similar to the murine PD-1 expression generally provide the most physiological expression profile and preserve normal immune-cell development.

Does replacing mouse PD-1 disrupt immune development?

Both **genO-hPD-1** and **genO-hPD-1/hPD-L1** mouse models show preserved immune development and functional immune responses, supporting their use for efficacy and MoA studies.

Are these mice immunocompetent?

The knock-in humanized PD-1 (**genO-hPD-1**) and PD-1/PD-L1 mice (**genO-hPD-1/hPD-L1**) are immunocompetent.

They retain:
- Functional T cells
- Functional B cells
- Normal immune architecture
- Endogenous immune development

Unlike HIS models, they do not require human cell engraftment.

Does human PD-1 preserve downstream signaling?

The objective of knock-in humanization is to maintain physiological signaling while enabling binding of human therapeutics.

Key validation includes:
- Immune-cell frequencies
- PD-1 expression kinetics
- Functional T-cell responses
- Tumor growth inhibition after checkpoint blockade

Are there available cell lines that can be used in syngeneic tumor studies with these models?

Yes. Humanized PD-1 and PD-1/PD-L1 mice can be combined with many established murine syngeneic tumor models. Because these mice remain immunocompetent, they can be implanted with tumor cell lines originating from the same genetic background, allowing evaluation of anti-tumor immune responses in the presence of a functional immune system. genOway has developed a range of cell lines that can be used for tumor growth inhibition studies or MoA studies, such as: **genO-MC38-hPD-L1-hCD47-LZ**, **genO-MC38-hPD-L1-hHER2-LZ**, **genO-MC38-hPD-L1-hFAP-LZ**, **genO-MC38-hFAP-LZ**, **genO-MC38-hPD-L1** and **genO-MC38-hPD-L1-LZ** for a C57BL/6 background, and **genO-CT26-hPD-L1** and **genO-CT26-Pd-l1KO** for a BALB/c background.

What is the advantage of humanizing both PD-1 and PD-L1?

Humanizing both PD-1 and PD-L1 creates a model in which the entire therapeutic target axis is humanized, allowing direct evaluation of both anti-PD-1 and anti-PD-L1 antibodies under physiologically relevant conditions. This increases translational relevance compared with a PD-1-only model and avoids artifacts caused by human-mouse receptor mismatch.

How can the Fc effector function of anti-PD-1 antibodies be assessed?

The **genO-hPD-1** model can be intercrossed with the extensively validated **genO-hFcγR** model (Van Damme et al., 2026). The resulting model (**genO-hPD-1/hFcγR**) expresses the humanized target of the Fab region in the target cells, as well as all hFcγ receptors in effector cells. This provides a more comprehensive evaluation of therapeutic antibodies.

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Humanized PD-1 mouse versus HIS mouse: what is the difference?

Humanized PD-1 knock-in mouse (**genO-hPD-1**)

- Mouse immune system
- Human therapeutic target (PD-1)
- Lower variability
- Better reproducibility
- Lower cost
- Suitable for candidate ranking

HIS mouse (**genO-BRGSF-HIS**)

- Human immune cells
- Human tumor compatibility
- More clinically relevant immune interactions
- Greater variability
- Higher cost

How predictive are humanized PD-1 models of clinical efficacy?

They improve translational relevance by enabling:

- Human target engagement
- Physiological immune responses
- Direct evaluation of clinical antibodies

However, they do not completely recapitulate:

- Human tumor biology
- Human immune-cell diversity
- Human immune-related adverse events
- Therefore, they are most powerful when integrated with complementary HIS or PDX studies.

Are there also variants of the genO-hPD-1 model that target other immune checkpoints?

Yes. genOway has developed a range of validated double humanized mouse models combining hPD-1 with other immune checkpoints. For dual checkpoint blockade, models such as **genO-hPD-1/hCTLA-4** have been exploited by AstraZeneca (Dovedi et al., 2021), while models such as **genO-hPD-1/hLAG-3** (Weber et al., 2026), **genO-hPD-1/hTIM-3** (Rohrberg et al., 2026), **genO-hPD-1/hPD-L1**, and **genO-hPD-1/hVISTA** also enable the evaluation of therapies targeting complementary inhibitory pathways involved in T-cell exhaustion and resistance to PD-1 blockade. For immune activation approaches, the **genO-hPD-1/hICOS** and **genO-hPD-1/hGITR/hGITRL** models support the assessment of agonistic therapies designed to enhance effector T-cell responses and modulate regulatory T-cell activity.

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