CROWN BIOSCIENCE

MiXeno™

PBMC humanized mouse models for a simplified approach to immunotherapeutic assessment

A JSR Life Sciences Company

FACTSHEET

V1.0

Mi*Xeno* provides a simple, fast, and costeffective approach to evaluating your test compounds' antitumor activity, making this the ideal alternative to complex, full stem cell derived humanized models.

Choose from a wide range of models already developed and benchmarked using various antitumor mAbs and immunomodulators, or let us work with you to develop your own custom model.

- PBMC humanized models generated through admixing or sequential addition of human immune cells with xenograft models
- Resulting mouse models have human T and NK cell lineages reconstituted
- B cell reconstitution occurs with production of antibodies observed upon sensitization and challenge.
- Ideal models to test T and NK cell modulating agents and assess a range of immunotherapeutics including checkpoint inhibitors, cancer vaccines, and ADCC effects.
- Variety of models available for both solid tumors and blood cancers.

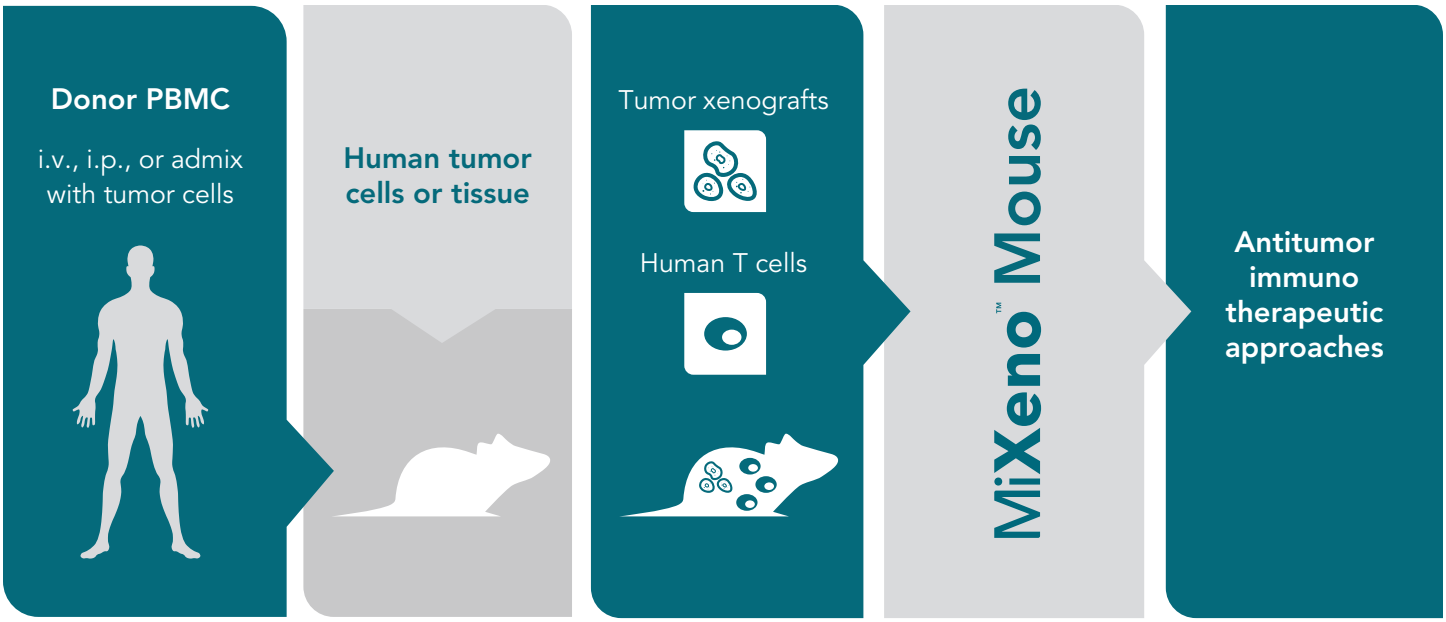
Models with human immunity are needed for preclinical evaluation of human specific immunotherapeutics, simplify your approach with PBMC humanized models:

- Generated by engrafting human peripheral blood mononuclear cells (PBMC) and xenograft tumors sequentially, or by mixing immune cells with tumor cells before subcutaneous inoculation
- Resulting mouse models show reconstitution of T, B, and NK cell populations
- Alternative approach to complex full stem cell derived humanized models
- Customized model development available
- A wide range of models across multiple tumor types have been developed and benchmarked using antitumor mAbs and immunomodulators to assess tumor response upon treatment (see Table 2 for model summary).

Immunocompetent Models Required for Immunomodulatory Agent Assessment

Immuno-oncology is presenting many new challenges to preclinical drug developers, including the need for immunocompetent models for novel agent evaluation. Many agents are assessed as mouse orthologues using syngeneic models; however, when human specific therapeutics need to be tested human targets and humanized models are required.

Humanized mouse models with a reconstituted multi-lineage immunity (hCD34+) are available, but can prove costly and complex to use. Therefore a more straightforward, rapid, and less costly humanized platform which utilizes human peripheral blood mononuclear cells (PBMC) has been developed for human specific agent assessment.



MiXeno Model Background and Concept

MiXeno models provide partially reconstituted human immunity in human tumor bearing mice through the admixing or sequential addition of human PBMC or activated T cells with xenograft models. The resulting mouse models show reconstitution of T and NK cell populations. B cell reconstitution takes place; however, they are cleared faster than T and NK cells and cannot necessarily be identified in peripheral blood samples. Antibody production can be observed upon sensitization and challenge.

The **MiXeno** platform provides a quick and simple, yet robust alternative to full stem cell reconstitution, useful when working on short timelines, or to select agents before moving to a more complex multi-lineage immunity model.

MiXeno models are appropriate for a range of immuno-oncology applications (shown in Table 1) including assessing cancer vaccines, checkpoint inhibitors, antibody-dependent cellular cytotoxicity (ADCC), or NK cell based therapeutics.

Crown Bioscience has validated the **MiXeno** platform in a number of xenograft models, summarized in Table 2 for cancer type, animal strain, and PBMC injection route. We can also establish and characterize further **MiXeno** models at client request.

COLO 205 MiXeno Case Study

Model Applications in Evaluating ADCC Effects

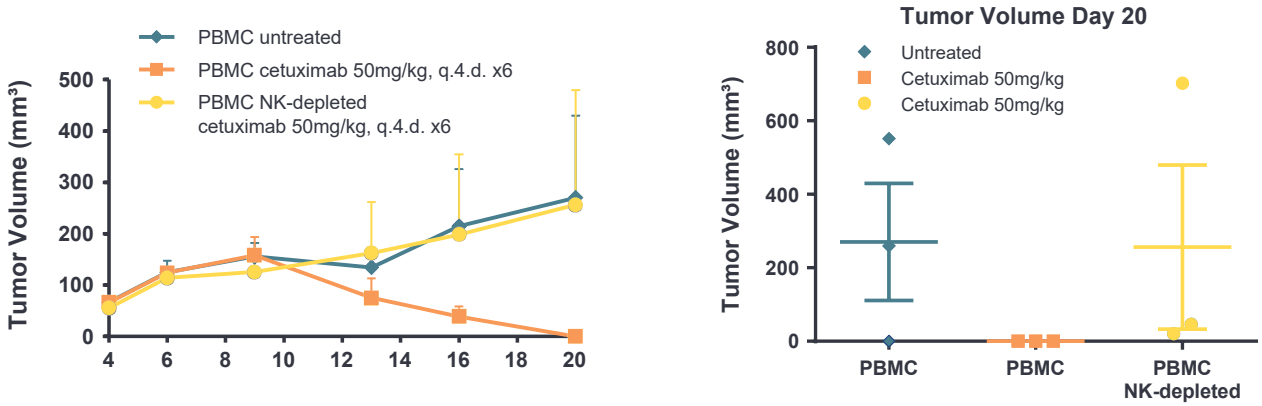
MiXeno models are the ideal platform for evaluating ADCC effects, thanks to their rapid establishment and effective reconstitution of NK cell populations. Cetuximab is a chimeric IgG1 monoclonal antibody targeting the ligand-binding domain of EGFR, which is active in metastatic colorectal cancer (mCRC). As an IgG1 antibody, cetuximab may exert its antitumor efficacy through either EGFR antagonism and/or ADCC. However, whether ADCC actually contributes to the clinical efficacy of cetuximab remains to be determined.

The COLO 205 **MiXeno** model was established in an NPG background via subcutaneous inoculation of human COLO 205 cells admixed with either human PBMC or NK-depleted PBMC. The resulting models were treated with cetuximab to evaluate the significance of ADCC effects on the resulting *in vivo* efficacy. NK depletion is shown to prevent model response to cetuximab (Figure 1), therefore confirming that the antibody exerts its antitumor activity *in vivo* via ADCC effects.

Table 1: Immuno-Oncology Applications of MiXeno

Immune Function Involved	Test Substance	Crown Bioscience Experience
T cell function	BiTE®-like Ab	CD19, HER2, EGFR, EpCAM-CD3
	Immune checkpoint inhibitors/agonists	PD-1, PD-L1 inhibitors
NK cell function	ADCC	Cetuximab
	NK modulating agents	NA
Cell Behavior	Cancer vaccines	NA

Figure 1: COLO 205 MiXeno Model Response to Cetuximab is via ADCC Effects



NPG mice, 2 PBMC donors, tumor cells: $4x10^6$ s.c. admixed with $4x10^6$ PBMC on Day 0, treatment: from Day 4.

Treatment	Tumor Volume (mm^3)	T/C Value (%) on Day 20	p Value
Untreated	270 ± 159	--	--
Cetuximab	0 ± 0	0	0.165
Cetuximab (no NK)	256 ± 223	95	0.962

Table 2: Summary of MiXeno ModelS

Cancer Type	Model Name	Animal Strain	PBMC Injection Route	Model Utilization
Breast	BT-474 (s.c.)	NCG/NOG/NPG	s.c. (admixed with tumor cells)	CD3-bispecific antibody Herceptin®
Colon	COLO 205 (s.c.)	NCG/NOG/NPG	s.c. (admixed with tumor cells)	Cetuximab
	HCT116 (s.c.)	NOD/SCID NCG/NOG/NPG	s.c. (admixed with tumor cells), i.p.	CD3-bispecific antibody Anti-PD-1 antibody Anti-PD-L1 antibody EpCAM BiTE
	HT-29 (s.c.)	NCG/NOG/NPG	i.p.	Anti-CD137 antibody
	RKO (s.c.)	NPG NOD/SCID	i.p.	NA
	SW480 (s.c.)	NOD/SCID	s.c. (admixed with tumor cells)	CD3-bispecific antibody EpCAM BiTE
	SW620 (s.c.)	NCG/NOG/NPG	i.p.	NA
Esophageal	KYSE-150 (s.c.)	NPG NOD/SCID	i.p.	NA
	KYSE-270 (s.c.)	NPG NOD/SCID	i.p.	NA
Leukemia	NALM-6 (i.v.)	NOD/SCID	i.v.	NA
Liver	HepG2 (s.c.)	NPG	i.p.	NA
Lung	A549 (s.c.)	NCG	s.c. (admixed with tumor cells)	Anti-PD-1 antibody
	HCC827 (s.c.)	NCG/NOG/NPG	i.p., i.v.	Anti-PD-1 antibody Anti-PD-L1 antibody
	NCI-H292 (s.c.)	SCID/Beige	i.v., s.c.	NA
	NCI-H358 (s.c.)	NCG/NOG/NPG	i.p., i.v.	NA
Lymphoma	Daudi (s.c.)	NCG/NOG/NPG	i.p.	NA
	Jeko-1 (s.c.)	NCG/NOG/NPG	i.p.	CD3-bispecific antibody Ibrutinib Anti-PD-1 antibody Anti-PD-L1 antibody
	Karpas299 (s.c.)	NCG/NOG/NPG	i.v.	Anti-PD-L1 antibody CD47 BiTE
	Pfeiffer (s.c.)	NCG/NOG/NPG	i.p.	Ibrutinib
	Raji (s.c.)	NOD/SCID	i.v.	NA
Melanoma	A2058 (s.c.)	NCG/NOG/NPG	i.p., s.c. (admixed with tumor cells)	NA
	A375 (s.c.)	NCG/NOG/NPG	s.c. (activated T cells admixed with tumor cells), i.v. (PBMC)	Anti-PD-L1 antibody
Myeloma	NCI-H929 (s.c.)	NCG/NOG/NPG	i.p.	NA
	RMPI-8226 (s.c.)	NCG/NOG/NPG	i.p.	NA
Ovarian	SK-OV-3 (s.c.)	NCG/NOG/NPG	i.v.	EpCAM BiTE
Prostate	22RV1 (s.c.)	NCG/NOG/NPG	s.c. (admixed with tumor cells)	NA
	PC-3 (s.c.)	SCID/Beige	s.c. (admixed with tumor cells)	Anti-CD137 antibody
Tongue	SCC-4 (s.c.)	NOD/SCID	s.c. (admixed with tumor cells)	Anti-PD-1 antibody

Further models can be validated on request.

HCC827 Mi**Xeno Case Study**

Model Optimization and Characterization for Immunotherapy Assessment

Fresh PBMC are the ideal source of cells for humanization; however, the use of fresh PBMC requires model optimization before moving to *in vivo* efficacy studies. Model optimization includes:

- Synchronization of tumor/PBMC inoculation
- Identification of optimal inoculation routes and cell number
- Evaluation of PBMC donor dependency and specificity
- Assessment of the impact of donor HLA type on the engraftment of immune or tumor cells

One of our most highly characterized **MiXeno** models is the HCC827 lung cancer model for which we performed a series of studies to optimize PBMC and tumor inoculation, and to understand the implications of PBMC donor to donor variability on model response to therapy.

For each independent HCC827 **MiXeno** study, the best PBMC and tumor administration routes are established. This allows for favorable tumor engraftment paired with optimal immune cell reconstitution on a study-by-study basis for successful immunotherapy efficacy assessment.

To statistically power study results even in the presence of donor to donor variability, at least 2 different PBMC donors are used per inoculation, with studies repeated with multiple donors. HCC827 model response to therapy is also characterized dependent on immune cell donor.

HCC827 model response and donor variability effects have been assessed for treatment with both anti-PD-1 and PD-L1 antibodies. Figure 2 shows HCC827 model response to pembrolizumab in an efficacy study utilizing 2 PBMC donors. In this study, tumor growth inhibition (TGI) of 68% is observed.

Response to anti-PD-1 has been shown to be PBMC donor dependent, in a Keytruda® efficacy study utilizing 2 further individual PBMC donors. **MiXeno** animals humanized with Donor A PBMC showed a moderate response, while **MiXeno** animals from Donor B failed to respond (Figure 3). Similar data are observed with anti- PD-L1 antibodies.

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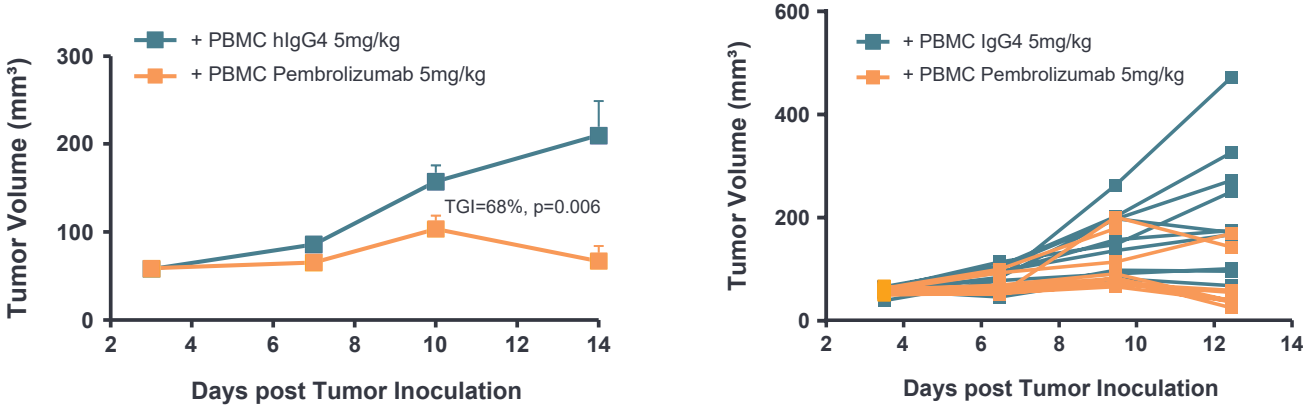
Figure 2: HCC827 Mi**Xeno Model Responds to Pembrolizumab Treatment**



NCG mice, 2 PBMC donors, tumor cells: $5x10^6$ s.c. on Day 0, PBMC: $1x10^7$ cells i.v. 3 days prior to tumor inoculation, treatment: Days 3, 7, 10.

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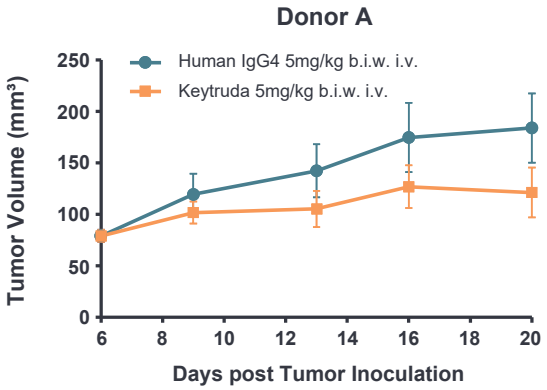
Jeko-1 MiXeno Case Study

Correlating *In Vivo* Response to Model Immunoprofile

Immunotherapy assessment using **MiXeno** models can also be combined with immunoprofiling by FACS, to truly understand the mechanisms behind response. Our Jeko-1 human mantle cell lymphoma MiXeno model has been assessed with both anti-PD-1 and PD-L1 antibodies as well as ibrutinib (a Bruton's tyrosine kinase inhibitor), with downstream immunoprofiling investigating resulting changes in immune cell populations.

The Jeko-1 model is sensitive to anti-PD-1 treatment, and partially responsive to anti-PD-L1 treatment (Figure 4). FACS analysis was used to assess subpopulations of T cells within the Jeko-1 tumors following treatment at study termination (Day 39). Consistent with the efficacy data, an increase in CD3+ and CD8+ TILs was observed following anti-PD-1 treatment, which was not observed in the anti-PD-L1 treated group, possibly explaining the more moderate level of response in these animals (Figure 5).

Figure 3: MiXeno **HCC827 Model Keytruda Response is PBMC Donor Dependent**



NOG mice, 2 PBMC donors, tumor cells: $5x10^6$ s.c. on Day 0, PBMC: $1x10^7$ cells i.v. on Day 6, treatment: b.i.w. from Day 6.

Treatment	Tumor Volume (mm^3)	T/C Value (%) on Day 20	p Value
Human IgG4 (Donor A)	184 ± 34	--	--
Keytruda (Donor A)	121 ± 24	66	0.152
Human IgG4 (Donor B)	196 ± 22	--	--
Keytruda (Donor B)	158 ± 13	81	0.163

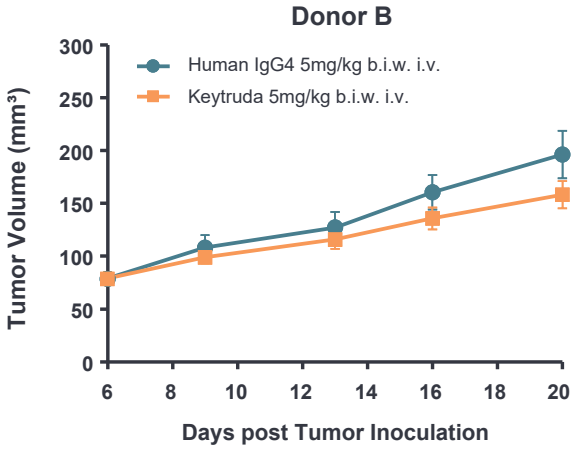
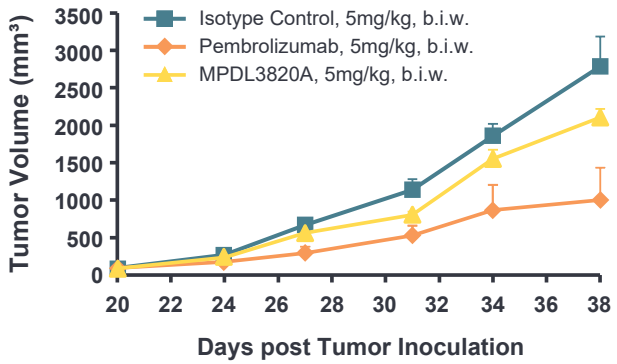


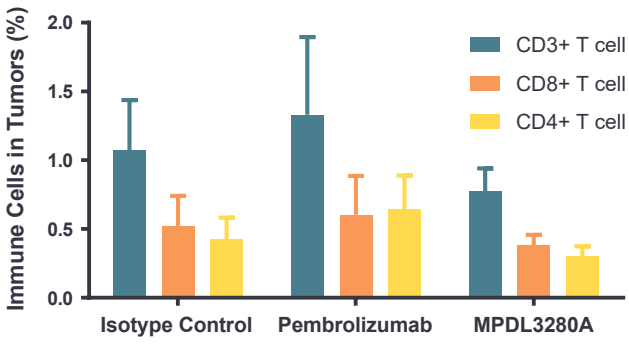
Figure 4: Jeko-15 MiXeno **Model Responds to Anti-PD-1 Treatment**



NOG mice, 1 PBMC donor, tumor cells: $5x10^6$ s.c. on Day 0, PBMC: $5x10^6$ cells i.p. 3 days post tumor cell inoculation, treatment: from Day 20.

Treatment	Tumor Volume (mm^3)	T/C Value (%) on Day 38	p Value
Isotype Control	2785 ± 398	--	--
Pembrolizumab	1002 ± 429	36	0.016
MPDL3280A	2105 ± 111	76	0.184

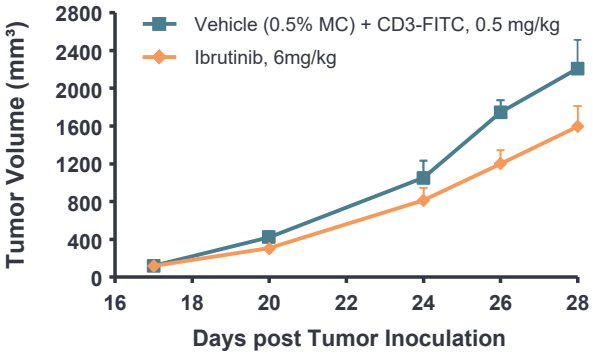
Figure 5: Tumor Immunoprofiling Data Correlate with Efficacy Response



Ibrutinib is approved as second line treatment for relapsed or refractory mantle cell lymphoma, with BTK acting as a vital component of the B cell receptor survival pathway, which is chronically activated in mantle cell lymphoma. Treatment of the Jeko-1 model with ibrutinib leads to a partial response (Figure 6).

Immunoprofiling reveals no significant effect of ibrutinib treatment on the level of B cells in peripheral blood, spleen, and tumor samples (Figure 7). A trend for increased T cell presence in the blood and spleen of treated animals on Day 25 is observed; however, the percentage of T cells in the tumor appears to be reduced upon treatment (Figure 7). These immunoprofiling data can potentially explain the lack of strong antitumor response to ibrutinib in this model.

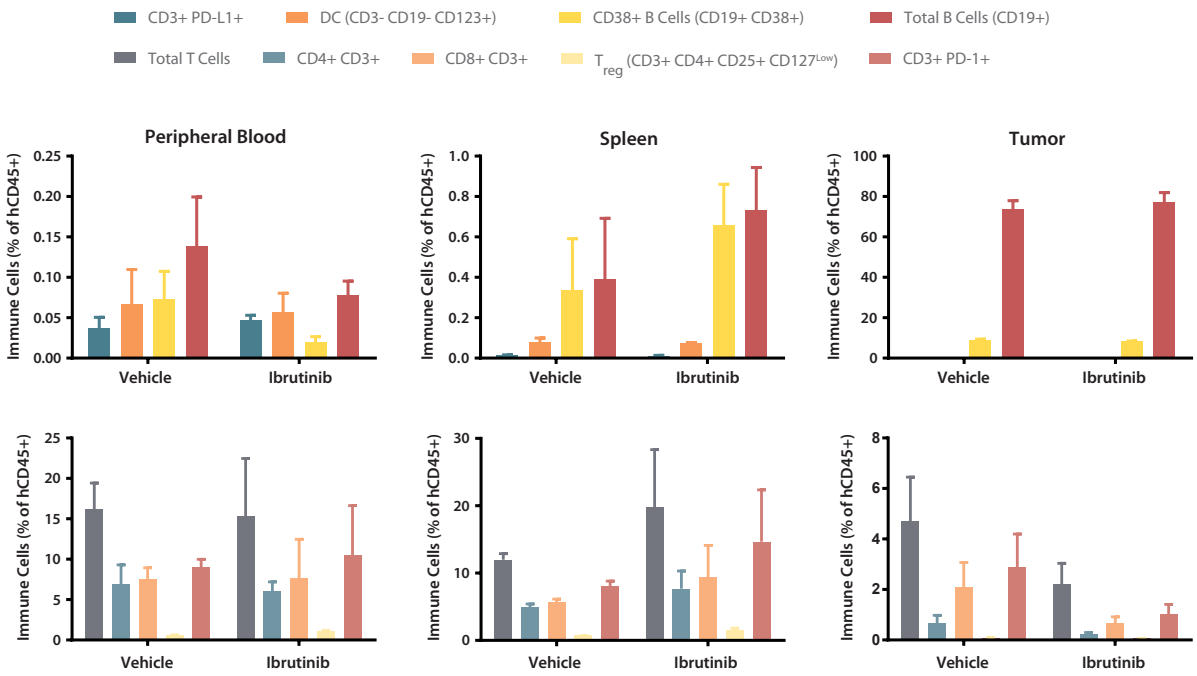
Figure 6: Jeko-15 MiXeno Model Partially Responds to Ibrutinib Treatment



NCG mice, 2 PBMC donors, tumor cells: 5 x 10^6 s.c. on Day 0, PBMC: 1 x 10^7 i.p. 3 days post tumor cell inoculation, treatment: from Day 17.

Treatment	Tumor Volume (mm^3)	T/C Value (%) on Day 28	p Value
Vehicle (0.5% MC) + CD3-FITC	2207 ± 305	--	--
Ibrutinib	1595 ± 216	72	0.925

Figure 7: Immunoprofiling Data Reveals Possible Mechanism of Lack of Response



Summary

The **MiXeno** platform provides PBMC humanized mouse models for a simplified and cost-effective approach to immunotherapy assessment. Developed by admixing or sequential addition of human immune cells with xenograft models, **MiXeno** mice feature reconstitution of human T, B, and NK cell lineages. This provides an ideal model for preclinically assessing T and NK cell modulating agents or cancer vaccines eliciting an antibody-mediated response.

Each model has been optimized to achieve the best combination of immune cell reconstitution coupled with stable tumor growth, using the most suited immunodeficient host strain for successfully investigating model response to immunotherapy. Multiple immune cell donors are employed per study to also offset donor to donor variability.

Our **MiXeno** models have been benchmarked with immunotherapeutics such as immune checkpoint inhibitors (e.g. anti-PD-1 and PD-L1) and with agents studying ADCC effects. Model response has been characterized with respect to varying immune cell donors, and efficacy studies can be combined with FACS analysis to correlate immunoprofile post-treatment with response levels and truly understand agent mechanisms of action in this platform.

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