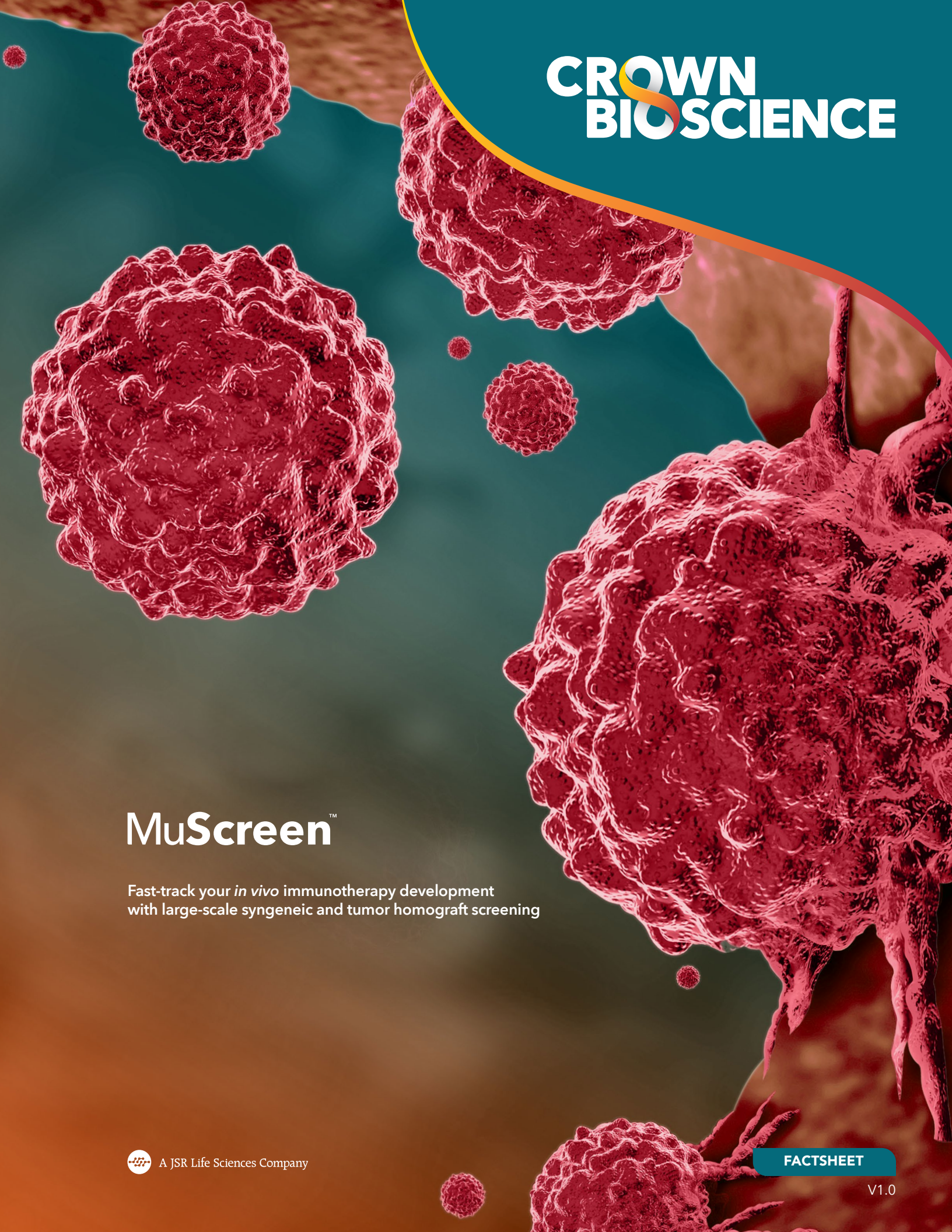


CROWN BIOSCIENCE

MuScreen™

Fast-track your *in vivo* immunotherapy development
with large-scale syngeneic and tumor homograft screening

A JSR Life Sciences Company

FACTSHEET

V1.0

Discover the benefits of running a Mu Screen™** to accelerate your single agent or combination regimen immunotherapy development programs.**

Crown Bioscience has developed **MuScreen**, a cost-effective *in vivo* screening platform to fast-track preclinical immunotherapy development.

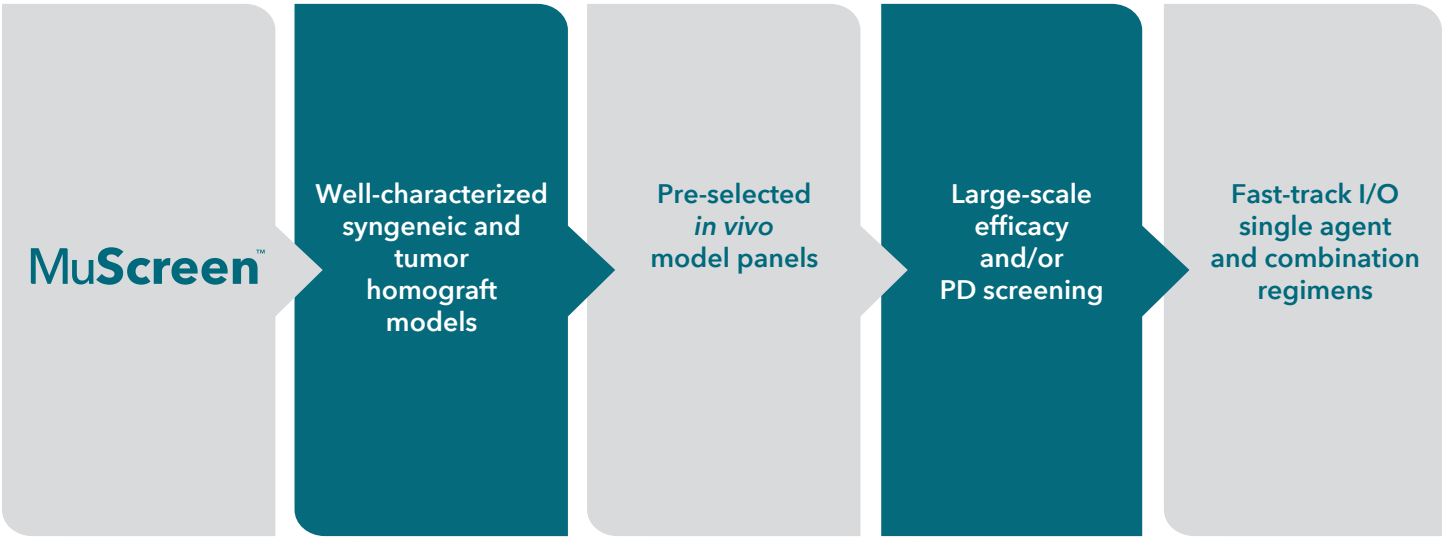
This platform overcomes common I/O screening issues, where *in vitro* platforms fail due to immunotherapies targeting the complex host immune system, and *in vivo* screening platforms prove cost prohibitive.

Choose **MuScreen** to:

- Interrogate well-characterized and validated syngeneic or tumor homograft models
- Quickly identify responder models or PD markers saving time, and improving efficiency
- Effectively evaluate combination strategies
- Accurately investigate PD effects
- Benefit from Crown Bioscience covering the cost of the vehicle group for all models and a discount on shared control groups
- Maximize the value of your MuScreen by choosing either FACS or Mouse I/O RNA-Seq Panel as an optional readout to fast-track *in vivo* preclinical immunotherapy development

MuScreen is the most experienced large-scale, *in vivo* screening platform to fast-track single agent and combination immunotherapeutics:**

- Built upon our collections of validated and well-characterized syngeneic and tumor homograft models
- Leveraging detailed model checkpoint inhibitor benchmarking data, as well as RNAseq and offering optional FACS/IHC analysis information
- Performed on a global platform across the US and China
- Comprising of predesignated syngeneic and tumor homograft panels across a range of cancer types and immune profiles
- Evaluate TGI on 6 or 12 preset syngeneic models or 6 tumor homograft models
- Evaluate PD effects (FACS analysis of TIL/TAM) with a panel of 12 syngeneic models
- Models run on a large-scale, preset schedule, with shared vehicle and common groups to improve efficiency and cost-effectiveness



Preclinical Immunotherapy Screening Challenges

The rise of immune checkpoint inhibitors (ICI) has revolutionized the cancer therapy landscape with unprecedented long term response; however, it is estimated that up to 60-70% of patients do not respond to single-agent ICI therapy. A thorough preclinical investigation of each new drug's mechanism of action and efficacy on its own and in combination with other immune sensitizing agents can help select the right candidate and treatment strategy for clinical applications. This requires relevant preclinical models and a well-established *in vivo* pharmacology platform.

Immunotherapies target the complex host immune system and *in vitro* screens fail to meet this need, while standard *in vivo* screens tend to be cost prohibitive. Instead, a large-scale, *in vivo* screening platform of syngeneic and tumor homograft models enables cost effective preclinical immunotherapy research, for both single agents and combination therapies.

Fast-track Immunotherapeutic Development with the MuScreen Platform

Panels of our syngeneic and tumor homograft models are utilized within **MuScreen**, the most experienced large-scale, *in vivo* screening platform for cancer immunotherapeutics. **MuScreen** can be used to rapidly focus immuno-oncology research efforts such as screening for efficacy, identifying PD markers, evaluating combination therapies, and qualifying drug resistance.

The platform is designed to fast-track the *in vivo* screening of immunotherapy compounds either as single agents or combination regimens. **MuScreen** leverages detailed profiling data on our syngeneic and tumor homograft models, including efficacy benchmarking with anti-PD-1, PD-L1, and CTLA-4 antibodies, RNAseq data, and FACS analysis on both baseline and treated tumor samples, which aids in the discovery of biomarkers to predict response.

Choose Syngeneic and/or Tumor Homograft Model Panels

Syngeneic Models for Efficacy and PD Screening

Our syngeneic 6 and 12 model panels (Table 1) represent the range of immune profiles observed clinically, providing an ideal platform for large-scale screening of immune modulating agents. The syngeneic panel can be used for proof of concept studies and to identify responder and non-responder models.

PD effects can also be studied using the syngeneic **MuScreen**, to uncover predictive biomarkers and understand the mechanism of action of a new therapeutic agent/combination regimen.

Our global syngeneic screening capabilities are supported by a wealth of historic data for these widely used I/O models.

Tumor Homograft Models for Efficacy Screening

Our tumor homograft 6 model panel (Table 2) allows large-scale I/O efficacy screening in more clinically relevant models. Tumor homografts are transplants of spontaneous or carcinogen-induced GEMM tumors in immunocompetent syngeneic hosts. The original GEMM tumors are not passaged *in vitro*, minimizing the artificial selection pressure.

Table 1: Syngeneic Models Available for MuScreen

Cancer Type	Model	Mouse Strain	Immune Cell Profiling	RNAseq	Baseline Proteomics Data
Breast	EMT6	BALB/c	Yes	Yes	Yes
Bladder	MB49	C57BL/6	Yes	Yes	No
Colorectal	CT26.WT	BALB/c	Yes	Yes	Yes
	MC38	C57BL/6	Yes	Yes	Yes
Kidney	Renca	BALB/c	Yes	Yes	Yes
Liver	H22*	BALB/c	Yes	Yes	Yes
	Hepa 1-6	C57BL/6	Yes	Yes	Yes
Lung	LL/2	C57BL/6	Yes	Yes	Yes
Lymphoma	A20	BALB/c	Yes	Yes	Yes
Melanoma	B16-F10	C57BL/6	Yes	Yes	Yes
Pancreatic	Pan02	C57BL/6	Yes	Yes	Yes
Prostate	RM-1	C57BL/6	Yes	Yes	Yes

The tumor homografts feature disease-specific oncogenic driver mutations in genes such as KRAS and p53, as well as preserving tumor architecture relevant to the original tumor microenvironment (TME). This allows the screening of immune modulating agent efficacy in the context of these oncogenic pathways and TME using the tumor homograft panel.

Model Characterization

All of our **MuScreen** syngeneic and tumor homograft models are comprehensively characterized, with available data including:

- Immune checkpoint inhibitor benchmarking
- Baseline tumor immune profile
- Tumor RNAseq

MuScreen Efficacy and PD Modes

Efficacy Screening

MuScreen Efficacy Mode is run following a preset schedule across our syngeneic and tumor homograft models along with a shared vehicle group (Figure 1). The main endpoint is TGI (with optional Mouse I/O RNA-Seq Panel, FACS and IHC). Frozen or fixed tumors are available on request.

PD Effect Screening

MuScreen PD Mode is run on a preset schedule and is currently only available with our syngeneic 12 model panel, with a shared vehicle group. Figure 1 shows an overview of a pooled screen study design. The **MuScreen** PD Mode can also be run as an individualized screen, with the study design and dosing customized to fit a given client compound's mechanism of action.

One of the main PD Mode study endpoints is FACS analysis of tumor infiltrating lymphocytes and tumor associated macrophages, using a choice of 10 or 13 marker FACS panel (Table 3):

- 10 marker panel: Live-Dead/CD45/CD3/CD4/CD8/FoxP3/CD335/CD11b/F4-80/Gr-1
- 13 marker panel: Live-Dead/CD45/CD3/CD4/CD8/FoxP3/CD335/CD11b/F4-80/Ly-6C/Ly-6G/IA-IE/CD206

The other main endpoint for the PD Mode study is the Mouse I/O RNA-Seq Panel, which comprehensively profiles 1080 genes associated with tumor immunity. (Learn more)

Additional endpoints can also include cytokine panel profiling in blood and tumor, IHC for biomarker analysis (on tumor tissues, blood cells, lymph nodes, and spleen cells), and frozen or fixed tumors are available on request.

Table 2: Tumor Homograft Models Available for MuScreen

Cancer Type	Model	Mutations/ Carcinogen	Strain Background	Immune Profiling	RNAseq	Growth Curve	SoC Data
Breast	mBR6004	MMTV-PyVT TG	FVB/N	Yes	Yes	Yes	Yes
Liver	mLI9040	Alb-Cre; CAG-LSL-cMyc	C57BL/6	Ongoing	Yes	Yes	Yes
Lung	mLU6045	$Kras^{(G12D)}$; $P53^{-/-}$	C57BL/6	Yes	Yes	Yes	Yes
Pancreatic	mPA6115	$Kras^{(G12D)}$; $P53^{-/-}$; PDx-1 cre	C57BL/6	Yes	Yes	Yes	Yes
Sarcoma	mSA9003	$P53^{-/-}$	C57BL/6	Yes	Yes	Yes	Yes
Skin	mSK6005	$Apc^{Min/+}$	C57BL/6	Yes	Yes	Yes	Yes

Figure 1: Efficacy and PD Mode Study Design

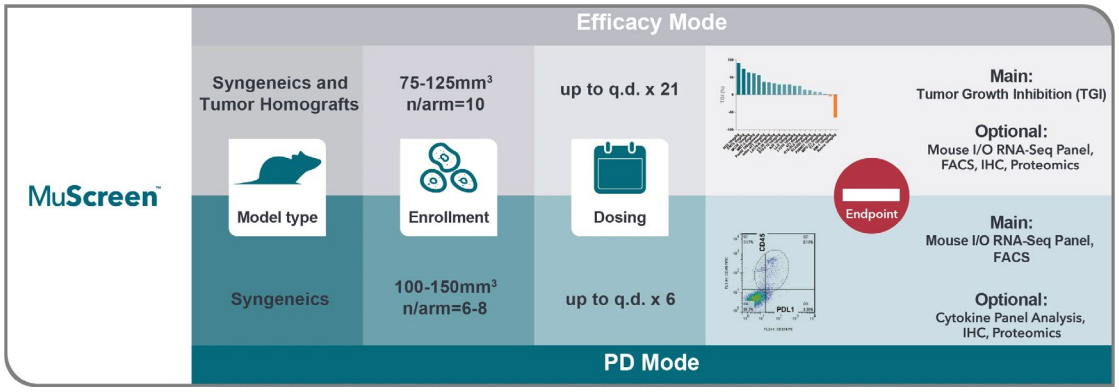


Table 3: MuScreen PD Mode FACS Panels

Markers	Immune Cell Population
$CD45^+$	Total leukocytes
$CD45^+ CD11b^- CD3^+$	Total T cells
$CD45^+ CD11b^- CD3^+ CD4^+ CD8^-$	$CD4^+$ T helper cells
$CD45^+ CD11b^- CD3^+ CD4^- CD8^+$	$CD8^+$ cytotoxic T cells
$CD45^+ CD11b^- CD3^+ CD4^+ CD8^- FoxP3^+$	Regulatory T cells
$CD45^+ CD3^- CD11b^+$ Ly-6C$^+$ Ly-6G$^-$	M-MDSC
$CD45^+ CD3^- CD11b^+$ Ly-6C$^-$ Ly-6G$^+$	G-MDSC

Markers	Immune Cell Population
$CD45^+ CD11b^+$ Ly-6C$^-$ Ly-6G$^-$ $F4/80^+$	Macrophages
$CD11b^+ F4/80^+$ Gr-1$^+$	MDSC
$CD45^+ CD11b^+$ Ly-6C$^-$ Ly-6G$^-$ $F4/80^+$ IA-IE^{high} $CD206^{low/-}$	M1 macrophages
$CD45^+ CD11b^+$ Ly-6C$^-$ Ly-6G$^-$ $F4/80^+$ IA-IE$^{low/-}$ $CD206^+$	M1 macrophages
$CD45^+ F4/80^-$ Ly-6C$^-$ Ly-6G$^-$ $CD3^-$ $CD335^+$	NK cells
$CD45^+ F4/80^-$ Ly-6C$^-$ Ly-6G$^-$ $CD3^{dim}$ $CD335^+$	NKT cells
Live/Dead (fixable)	Live/Dead

Immune cell populations in blue are available in the 13 marker panel only.

Summary

Mu**Screen** is a comprehensive platform encompassing well-characterized, immunocompetent, syngeneic and tumor homograft models. The platform enables high throughput and cost-effective screening across a diverse collection of immune profiles, tumor types, and mutations.
Syngeneic model panels are utilized to evaluate proof of concept and assess an agent's PD effect on tumor immune profiles, to reveal predictive biomarkers and understand mechanisms of action. The syngeneic model panel is ideal for interrogating heterogeneous immune phenotypes seen in the clinic, to elucidate the reasons behind the lack of immunotherapy response in certain patient subsets.

Our tumor homograft panel expands the repertoire of immunocompetent models amenable to large-scale screening, with additional beneficial features such as clinically relevant, disease specific oncogenic driver mutations and preservation of tumor architecture relevant to the original TME. The tumor homograft model panel is used to evaluate the efficacy of immunotherapies or combinations of targeted and I/O agents within the context of specific disease relevant oncogenic pathways and TME.

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