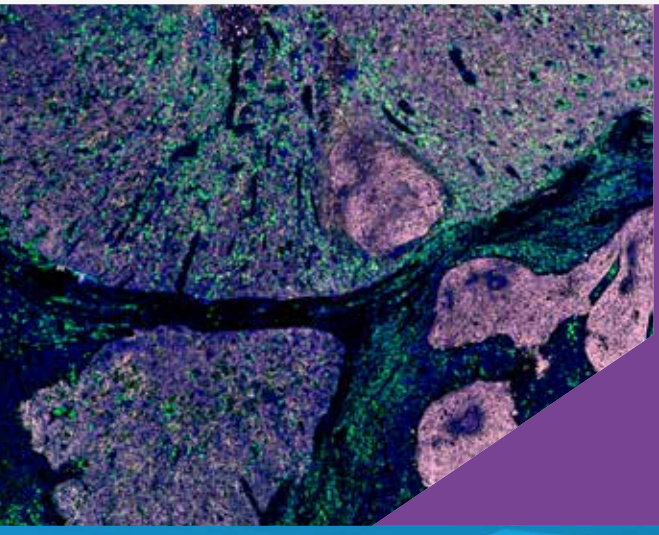
PharmaLegacy

Preclinical Specialty Pharmacology Services

INTEGRATED
IN VITRO ASSAYS
FOR IMMUNOLOGY & IMMUNO-ONCOLOGY RESEARCH:
Providing Better Data for Critical Pipeline Decisions



To advance a compound from discovery to clinical, or to halt its development, is a huge and costly decision. For the benefit of you and your company, and for the well-being of patients in need of treatment, that decision needs to be based on correct information.

PharmaLegacy works to get you the answers you need with our wide selection of carefully developed *in vivo* and *in vitro* models. In this document, you'll see a sample of the *in vitro* assays we run along with a sample of real-world data from them. See why over 300 pharmaceutical companies, including many global leaders, trust PharmaLegacy for the unbiased, accurate information they need to make critical pipeline decisions.

Building 7, 388 Jialilue Road Zhangjiang
High-Tech Park Pudong District Shanghai

+86-21-6100-2280*2188(International)
+86-131-2082-6307(International)
+1-617-803-9415(US)
+86-21-6100-2280*2658(Domestic)

+86-21-6100-2270
info@pharmalegacy.com
Michael.Zhang@pharmalegacy.com
Aimee.Duan@pharmalegacy.com

PHARMALEGACY
IN VITRO & EX VIVO ASSAYS

01

IMMUNE CELLS DETECTION WITH FACS:

- Total T cells, CD4+ T, CD8+ T, Treg, DCs, Macrophage, MDSC etc.

02

CELL ACTIVATION

- B cell activation
- NK cell activation / expansion

03

BIOMARKERS AND PD ANALYSIS

- Western Blot / IF / ELISA / IHC / Luminex / RT-PCR / RNAseq
- TIL (Tumor Infiltrating Leukocyte) profiling by FACS: T cells, DCs, Macrophage, Monocytes, MDSC, NK etc.

04

IN VITRO / EX VIVO ASSAYS IN HUMAN NHP AND RODENTS

- Mixed Lymphocyte Reaction (MLR) with allogeneic DC
- T cell activation assay
- Antibody-dependent cellular cytotoxicity (ADCC)
- NK/T cell modulating killing assay etc.

05

CELL DIFFERENTIATION / POLARIZATION

- M1/M2 macrophage
- DC/MDSC
- Th1, Th2, Th17 and Treg

06

CELL CYTOTOXICITY

- Cytotoxic T lymphocyte assay
- ADCC, ADCP
- CDC

07

MIXED LYMPHOCYTE REACTION

- DC co-culture with T cell

08

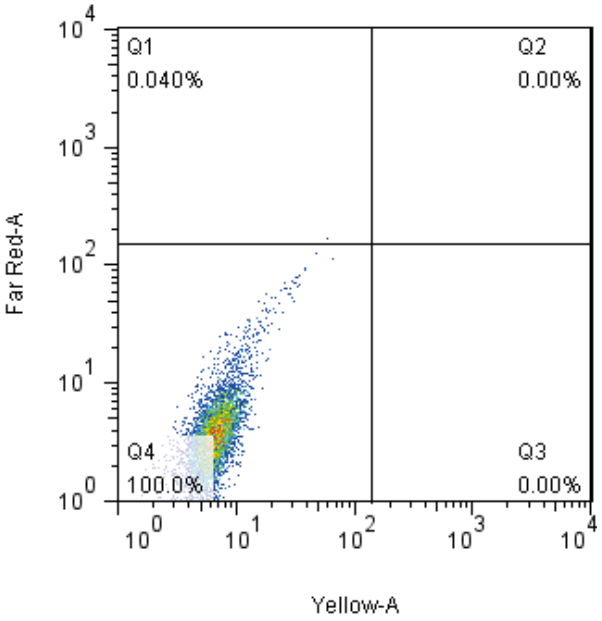
SUPPRESSIVE ASSAY

- Treg suppressive assay
- Tumor cell co-culture with immune cell

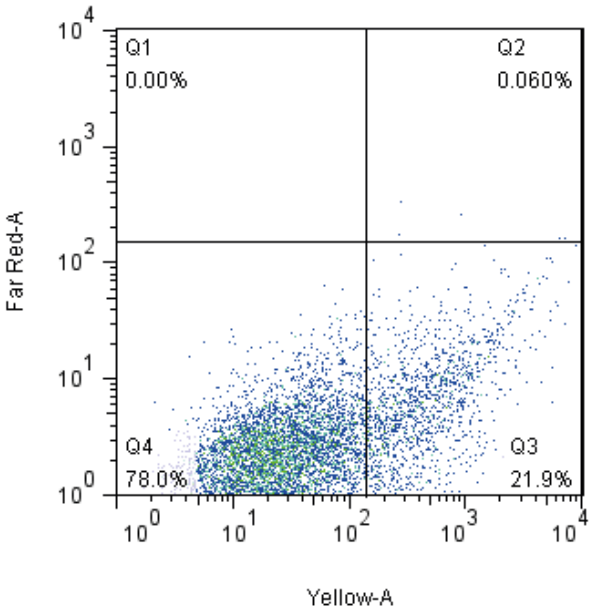
Many more + custom assay development available

Sample Assays #1: Drug-Induced Apoptosis

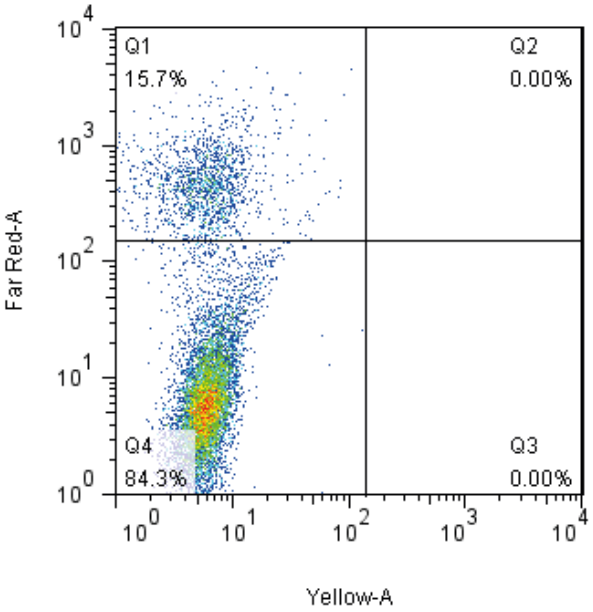
Drug-induced apoptosis is a key factor in establishing treatment sensitivity for an anti-cancer drug. For this study, we used flow cytometry and the apoptosis markers Annexin V and 7-amino-actinomycin D (7-AAD) to determine a compound's efficacy.



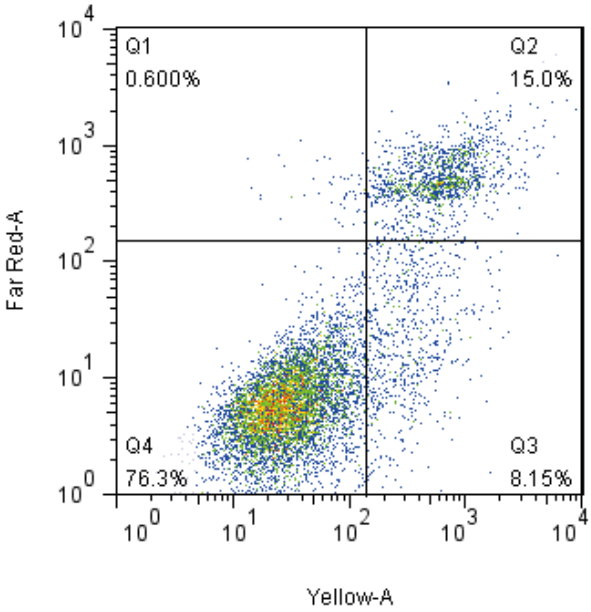
No Stain



Stained with Annexin V



Stained with 7-AAD



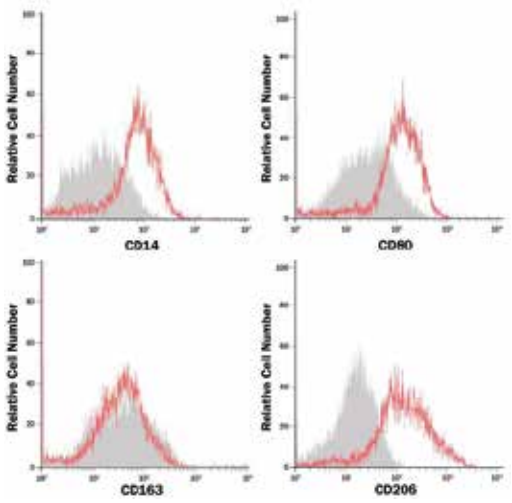
Cell Treated with Compound,
Stained with 7-AAD and Annexin V

Sample Assays #2: Phagocytosis

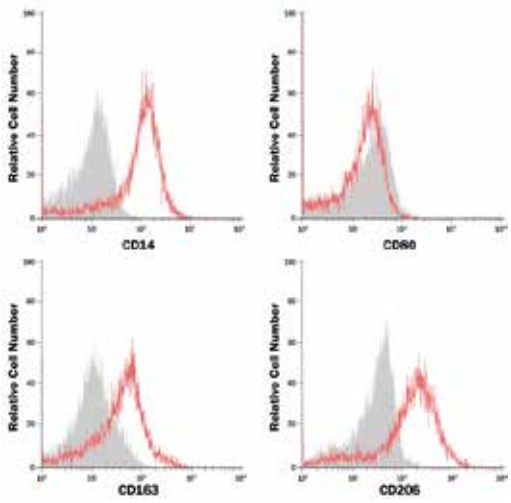
Phagocytosis plays an important yet complex role in how effective an anti-cancer of treatment may be, by determining whether it is tolerogenic or immunogenic. Tolerogenic homeostatic clearance of apoptosing cancer cells promotes tumorigenic processes, thereby suppressing antitumor activity. In contrast, cancer antigen-directed immunity is promoted through non-homeostatic clearance by antigen-presenting cells. This immunogenic, non-homeostatic clearance can lead to inflammation, however, which itself can be pro-tumorigenic or antitumorigenic.

Monocyte Differentiation into M1 or M2 Macrophages

Macrophages are recruited to all solid tumors. M1 macrophages have antitumoral effects and are associated with improved prognoses, whereas M2 macrophages are associated with tumor growth, angiogenesis, and metastasis.

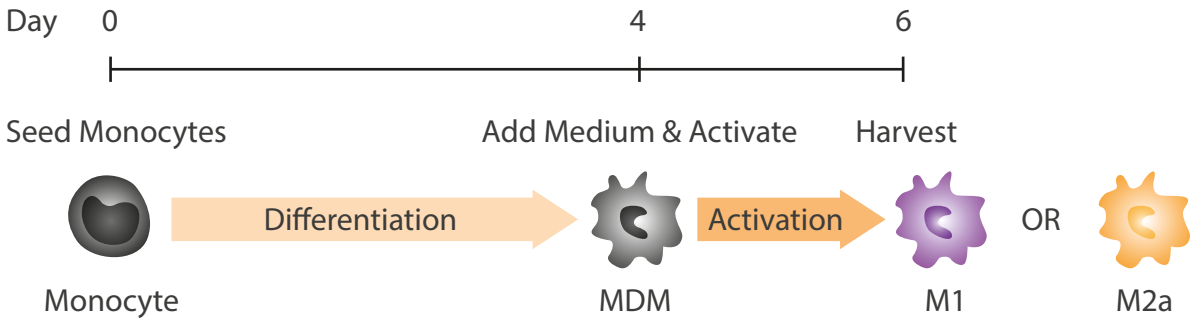


M1 Macrophages Phenotype:
CD14+ CD80+ CD163- CD206+

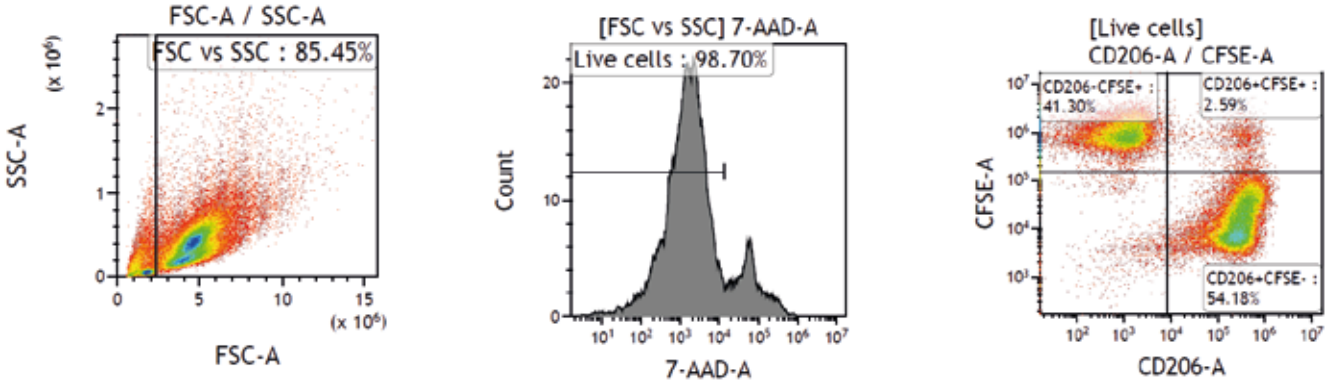


M2 macrophages phenotype:
CD14+ CD80- CD163+ CD206+

6-Day Culture Protocol



Transfer of a Label from Cancer Cells into Macrophages



FACS Gating Strategy

Phagocytosis of M1 Macrophages

Macrophage Differentiation: M1/M2 (6 days)

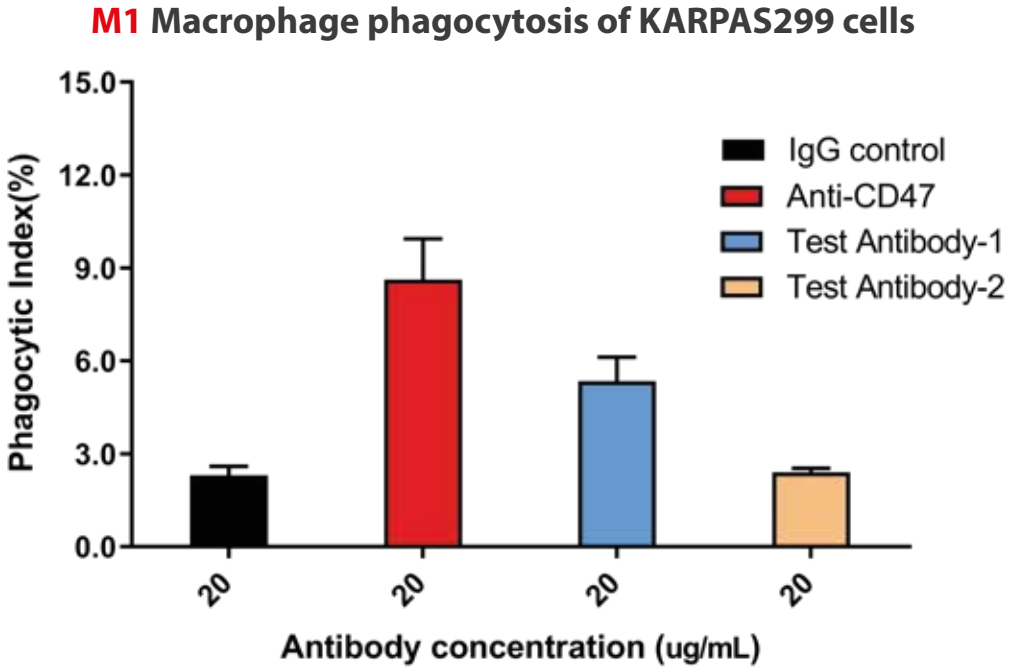
+

Target Cell Labeling (CFSE)

Phagocytosis assay CFSE+ Macs (2h, FACS)

Comparison of Two Antibody Therapeutics

Two antibody therapies were tested, using the KARPAS 299 Human Non-Hodgkin's Ki-positive Large Cell Lymphoma cell line. Anti-CD47 was used as a positive control.

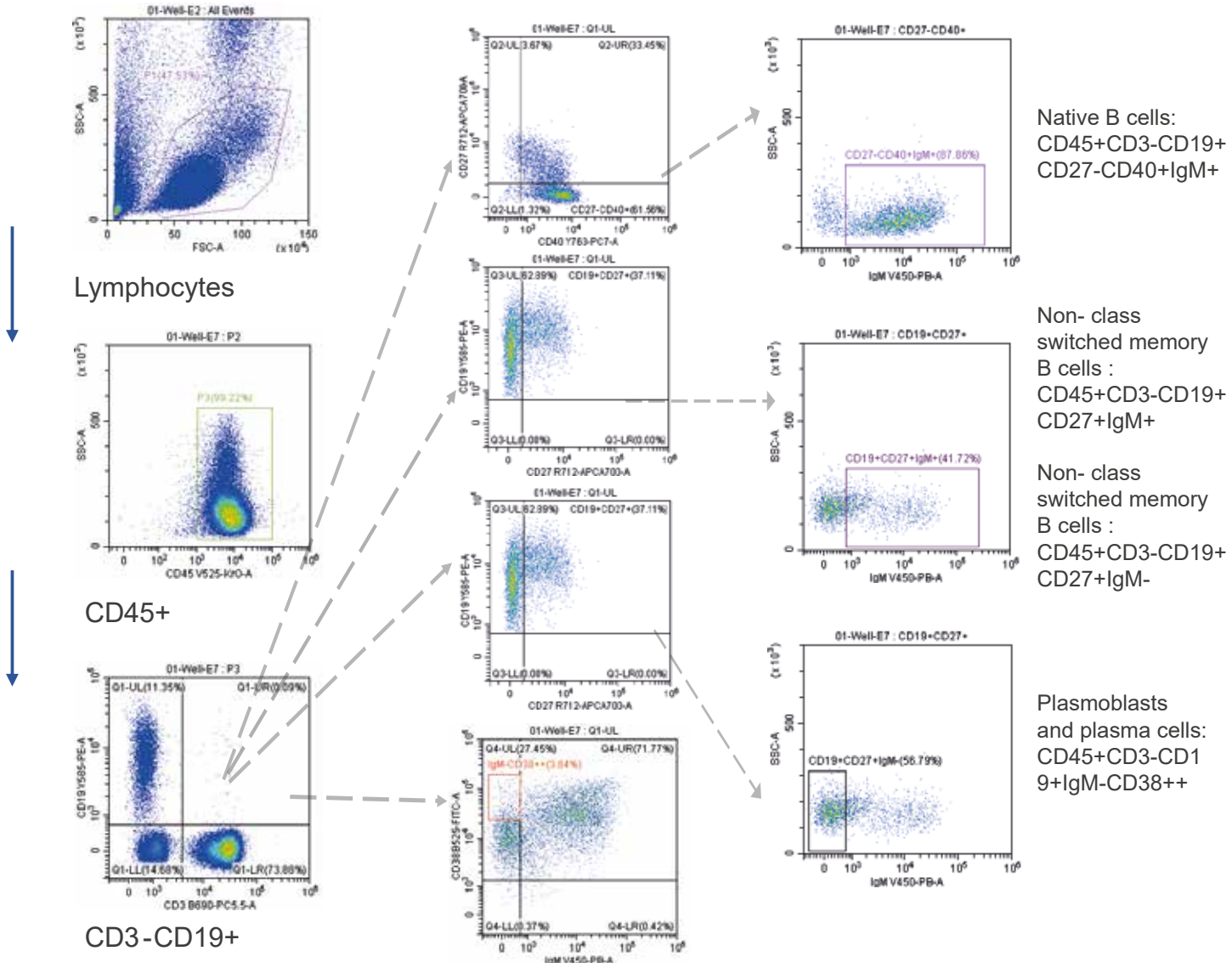


CFSE = Carboxyfluorescein diacetate succinimidyl ester]

Sample Assays #3: Flow Cytometry Panels

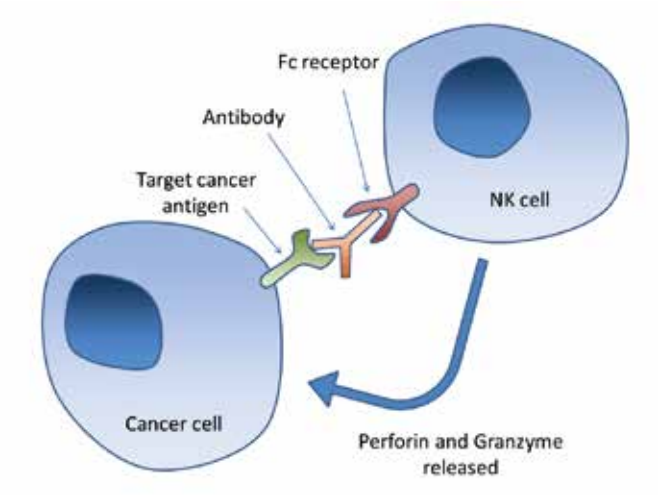
Immune cells		Human	Mouse
	B cells	CD19,CD20	B220 or CD19
T cells	Help T cell	CD3	CD3
	Help T cell	CD3,CD4	CD3,CD4
	Cytotoxic	CD3,CD8	CD3,CD8
	Treg	CD4,CD25,FoxP3,CD127	CD4,CD25,FoxP3
	Naïve/Memory/Effector T	CD3,CCR7,CD45RA	CD3,CD44,CD62L
Dendritic Cell	Classical DC	CD11b,CD11c,HLA-DR,BDCA1	CD11b,CD11c,F4/80
	Plasmacytoid DC	CD11b,CD11c,CD123,BDCA2/4	CD11b,CD11c,F4/80,Gr-1,SIGLEC-H
	NK Cell	CD56,CD16	CD49b(clone DX5),CD335,NK1.1
	NKT Cell	CD3,CD56	CD3,CD49b(clone DX5)
Macrophage	M1 Macrophage	CD11b,CD68,CD80,CD86	CD11b,F4/80,MHCII
	M2 Macropahge	CD11b,CD68,CD163,CD206	CD11b,F4/80,CD206
	Monocyte	CD14,HLA-DR	CD11b,CD115,Gr-1
	MDSC	CD11b,CD33,CD14,CD15,	CD11b,Ly6C,Ly6G

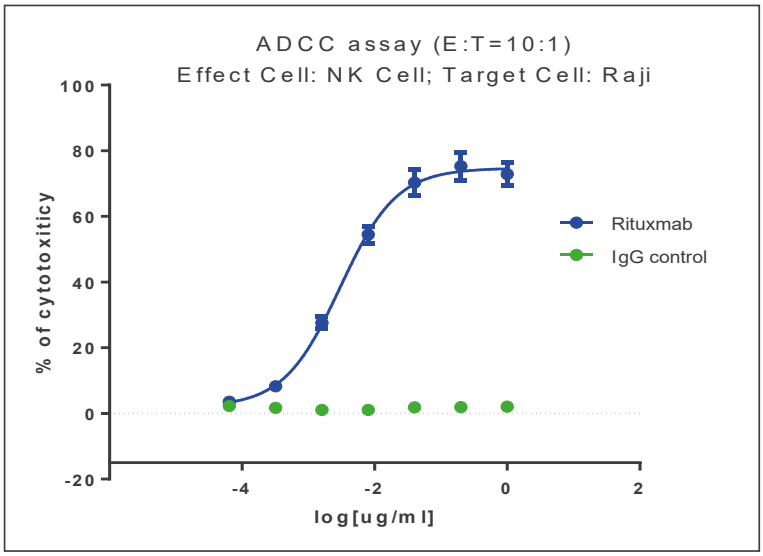
More Biomarkers coming soon…



Sample Assays #4: Antibody Dependent Cell-Mediated Cytotoxicity (ADCC)

Antibody-dependent cell-mediated cytotoxicity assays determine the efficacy of monoclonal antibody cancer therapies in triggering immune-mediated cell killing mechanisms. Here, we show the dose-dependent ability of Rituximab to recruit natural killer (NK) cells to CD20+ Raji target cells using a lactate dehydrogenase (LDH) based assay which indicates damage to the plasma membrane of the target cells.





Effect cell: Purified NK cells

Target cell: CD20+ Raji cells

E:T ratio: 10:1

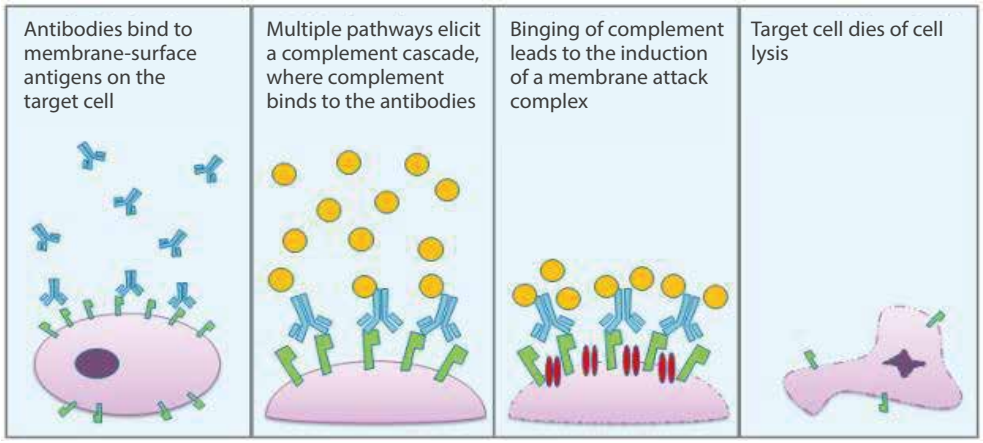
Time point: 4h

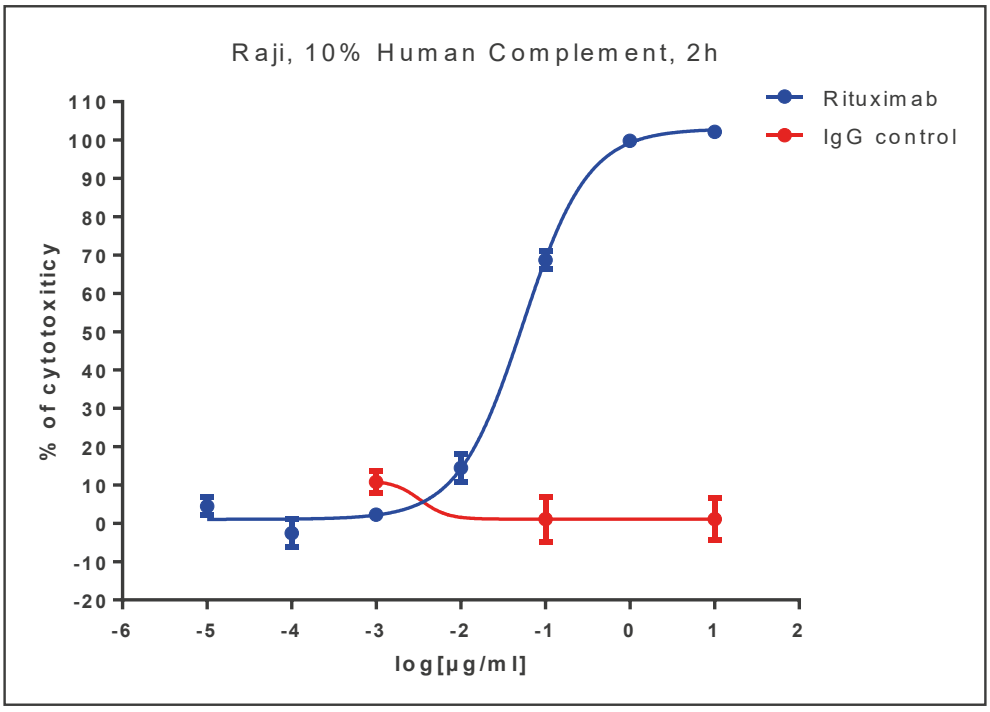
Readout: LDH based cytotoxicity assay

5% Human Compliment	Relative IC50 (μM)	Absolute IC50 (μM)	R2	HillSlope
Rituximab	0.003	0.006	0.979	1.019
IgG control	NA	NA	NA	NA

Sample Assays #5: Complement-Dependent Cytotoxicity (CDC)

One goal for monoclonal antibody therapies may be to activate complement through the classical pathway, the chain reaction beginning with complement component C1 activating upon recognition of the Fc portion of Igs, and ending in the formation of the terminal complex, or the membrane attack complex, which generates pores in the cell surface, lysing the cell. Pharmalegacy's CDC assays assess the ability of antibodies to affect cancers via this mechanism, such as in this experiment using Rituximab.





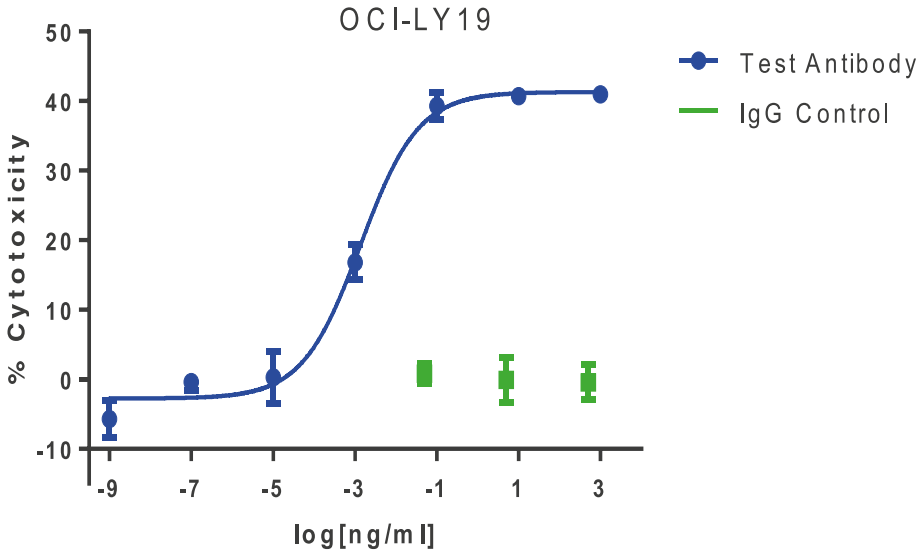
10% Human Compliment	Relative IC50 (µM)	Absolute IC50 (µM)	R2	HillSlope
Rituximab	0.054	0.051	0.997	1.128
IgG control	NA	NA	0.639	~ -2.729

- CD20+ Raji cells were used as target cells.
- Human Serum for Complement
- Readout by CTG

Sample Assays #6: T Cell Assays

Many therapies harness the power of T cells to fight cancer. Checkpoint inhibitors have revolutionized the treatment of melanoma and many other types of cancer. Chimeric antigen receptor T cell therapy is being increasingly used for the treatment of aggressive, relapsed or refractory cancers. PharmaLegacy's T cell assays help inform the development T cell-dependent therapies.

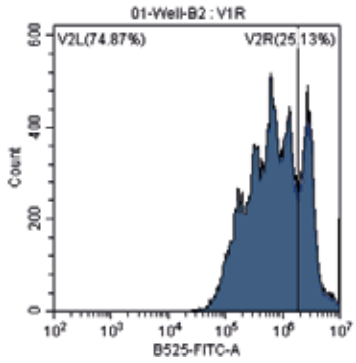
T Cell Killing Assay



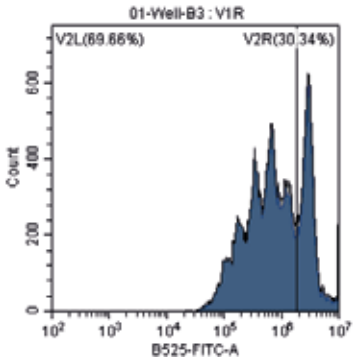
- **Test antibody: CD3/CD19 BiTE**
- Effect cell: Purified CD3+ T cells from human PBMCs
- Target cell: OCI-LY-19
- E:T ratio: 10:1
- Time point: 4h
- Readout: LDH based cytotoxicity assay

T Cell Proliferation Assay

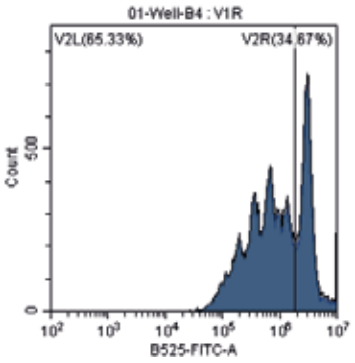
T cells were pre-stained with carboxyfluorescein succinimidyl ester (CFSE), stimulated with CD3 antibody at varying doses, and counted by flow cytometry.



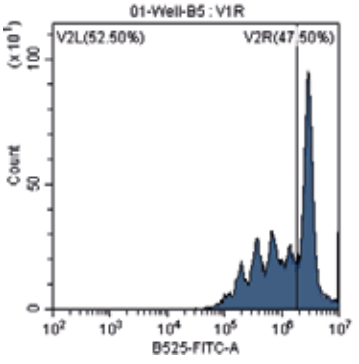
5ug/mL CD3 antibody



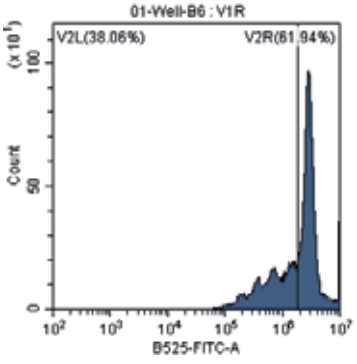
2.5ug/mL CD3 antibody



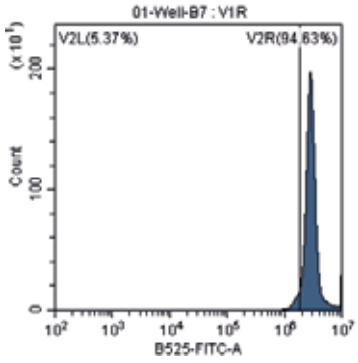
1.25ug/mL CD3 antibody



0.625ug/mL CD3 antibody



0.3125ug/mL CD3 antibody

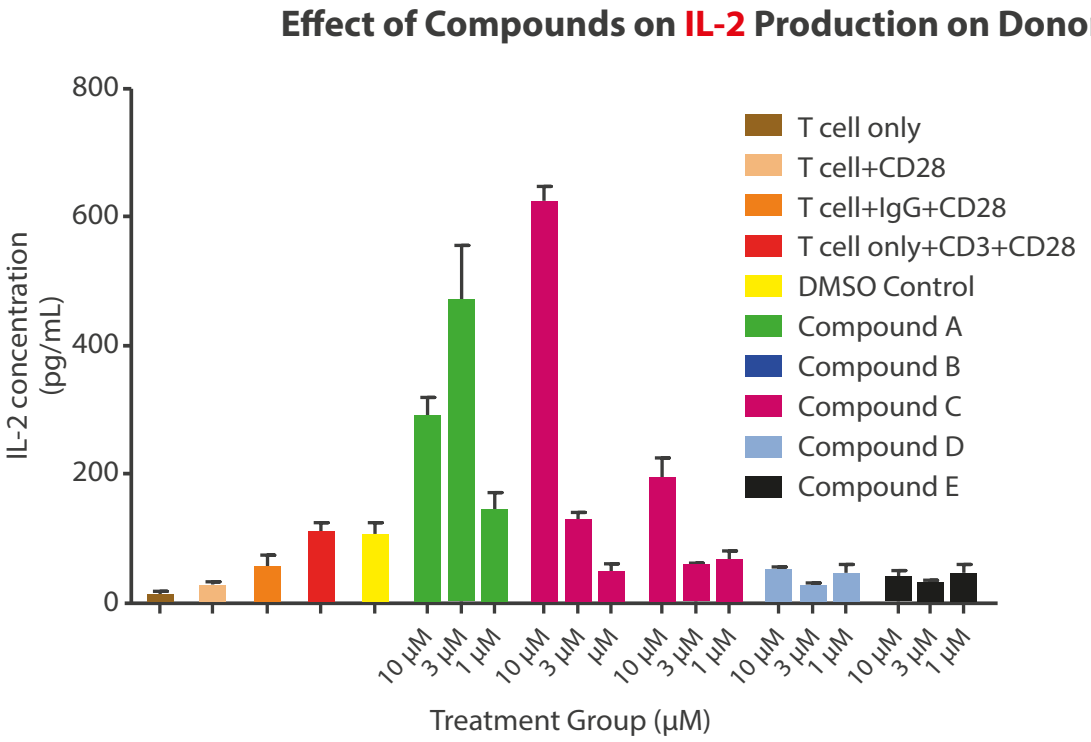


Without CD3 antibody

- T cells were pre - stained with CFSE.
- T cells were stimulated with CD3 antibody at different dose.
- Readout by FACS

T Cell Stimulation Assay

Purified CD3+ T cells from human PBMCs were pre-stimulated with CD3/CD28 antibody, then treated with one of five experimental compounds or a DMSO control.

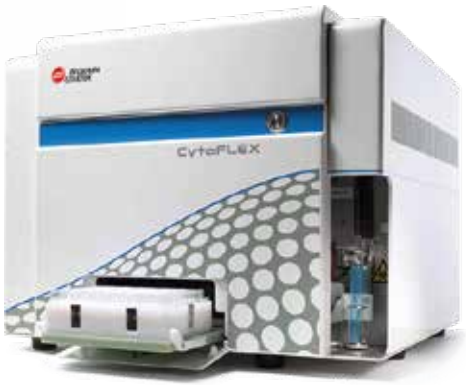


- Purified CD3+ T cells from human PBMCs.
- Pre-stimulated the T cell with CD3/CD28 antibody.
- Treated the T cell with compounds and the compounds could stimulate the T cells.

Cytometry Core

BD LSRFortessa™
(5 lasers, 18 channels)

BD FACSCanto™
(3 lasers, 8 channels)



CytoFLEX LX
(3 lasers, 13 channels)

Laser	CHANNELS		
	FACSCanto II	**LSRFFortessa**	**FACS Aria II**
UV	BV421	BUV395	BUV395
	BV510	BUV496	BUV496
	BV605	BUV737	BUV661
Violet		BV421	BV421
		BV510	BV510
		BV605	BV605
		BV650	BV650
		BV711	BV711
		BV786	BV786
Blue	FITC	BB515, FITC	FITC
	PE	PerCP-Cy5.5	PE
	PerCP-Cy5.5	PE	PE-CF594
	PE-Cy7	PE-CF594	PerCP-Cy5.5
Yellow-Green		PE-Cy5	PE-Cy7
		PE-Cy7	
Red	APC	APC	APC
	AF700	AF700	AF700
	APC-Cy7	APC-Cy7	APC-Cy7

Being Correct is Everything

To advance a compound from discovery to clinical, or to halt its development, is a huge and costly decision. For the benefit of you and your company, and for the well-being of patients in need of treatment, that decision needs to be based on correct information.

PharmaLegacy has more capability to provide rich, correct answers to pharmacological questions due to our huge repository of in vivo models, the rich experience of our company and scientists, our intense focus on pharmacology, and our proprietary technological platforms.

There's a lot on the line. Let PharmaLegacy get the correct answers.

Quick Facts:

- Over 300 validated animal models of disease spanning over 40 di¬fferent diseases
- Scientific staff average over 15 years of pharmacology experience
- FDA Part 11 compliant
- 45,000 ft2 facility with 22,000 ft2 of SPF and conventional vivarium to house 10,000 rodents and large animals
- Capacity to run 200 animal studies concurrently while strictly following AAALAC and ILAC guidelines
- Research data is electronically managed by BioBook (IDBS, UK)
- Web-based live video streaming allows remote monitoring of operations from any location worldwide
- Operations structured for maximum protection of clients' work and intellectual property
- 24/7 access to PharmaLegacy representatives
- More than 200 FDA / CFDA IND filings

Building 7, 388 Jialilue Road Zhangjiang
High-Tech Park Pudong District Shanghai

+86-21-6100-2280*2188(International)
+86-131-2082-6307(International)
+1-617-803-9415(US)
+86-21-6100-2280*2658(Domestic)

+86-21-6100-2270
info@pharmalegacy.com
Michael.Zhang@pharmalegacy.com
Aimee.Duan@pharmalegacy.com