



WuXi AppTec Oncology and Immuno-Oncology

End-to-End Services and Solutions To Enable Cancer Drug Discovery

www.wuxibiology.com



Integrated Oncology and Immuno-oncology Platform to Enable Translational Science from Drug Discovery to Clinical Biomarker

CRISPR/Cas9 Gene Editing

- In vitro & in vivo library screening
- Genetically engineered cell-line or animal
- Genetically engineered mouse model
- Target ID/validation

In vitro Assay and HTS

- Enzymes, GPCRs, transporters, ion channels
- Biochemical and cell-based assays
- Radiometric, high content and label free
- HTS and SAR screening

Tumor Models

- 215 CDX and 1000+ PDXs
- 61 syngeneic models and IO combination experiences
- Human immune checkpoint KI mince
- Immune avatar humanized models
- (hPBMC/hHSC NOG mice + xenograft tumor)

Tumor Cell Panel Screening

- 1,000+ human/mouse cell lines
- Proliferation, apoptosis, invasion, metastasis assays
- Customized panel assembly and profiling







The World's 1st Integrated Tumor Model online Database with Mobile App Free Registration for Free Data Search



Immuno-Oncology Platform

- In vitro/Ex vivo IO assays (customizable panel: PBMC/T/B/NK/Treg/DC/ MDSC/TAM/co-culture functional assays)
- *In vivo* models (syngeneic/GEMM/humanized)
- Immune profiling (FACS/Multiplex IHC/NGS)

Clinical Biomarkers

- CAP accreditation and GCP compliance
- LIMS for project management
- Pathology, FACS and NGS

OncoWuXi Database

- Tumor model or app datebase with mobile APP
- Free registration for free data search

Discovery Chemistry

- Synthetic and medicinal chemistry
- Local project management
- Analytical and preparative scale chiral separations
- Process research and development

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CRISPR/Cas9 Gene Editing

Cell-based CRISPR library in vitro or in vivo Screening

CRISPR/Cas9 based library in vitro screening

- Customize CRISPR library and experimental design
- sgRNA synthesis, cloning and library QC
- Virus production and cell line transfection
- Perform specific cell selection (positive or negative selection)
- Target identification through next generation sequencing (NGS) and data analysis, comparing sgRNA amplitude before and after selection
- Target validation in cells as well as other tools such as lentiviral gene overexpression, RNAi knockdown





CRISPR/Cas9 based library in vivo screening

- Customize CRISPR library and expand infected cells (cancer cells or T cells)
- After *in vivo* screening, isolate tumor cells or T cells for NGS
- Ready-to-use focused libraries of human/mouse epigenetics and ubiquitin

In Vivo CRISPR for Animal Model Generation

Transgenic mice generation

- Zygotes/lentivirus infection or DNA injection
- Lentivirus infected sperm and IVF

Knock-out and knock-in

- CRISPR/CAS9 based KO/KI in mouse and rat
- ES cell based KO/KI
- Stable or conditional KO

Other services supporting animal model

- Cryopreservation
- Genotyping of cells and animals by qPCR, Southern Blotting or NGS
- Phenotyping of animals by characterization of specific disease models.
- Drug efficacy evaluation and pharmacology services
- Animal importation / exportation



In Vitro Assay and HTS

- Designated "The Center of Excellence in Hit Identification and Lead Discovery" for several global pharmaceutical companies
- Broad span of assay formats and screening scales with annual throughput of over 10 million data points
- Integrated services from compound management and assay development to screening and data management
- Full capability and capacity to support multi-clients, multiple clients, projects and platforms including operations and backup plan
- Outstanding track record of collaboration and partnership with pharmaceuticals, biotechs and startups

Assay Development & Validation

Our assay technologies include but are notlimited to: **Biochemical Assays**

- Enzymatic kinetics, receptor binding kinetics & more
- Luminescence, Fluorescent, TR-FRET, FRET, LANCE, LC/MS, Caliper, Absorbance

Radiometric Assays

• Binding, SPA, Filtration, Uptake, TLC

Cell-based Target Assays

- AlphaScreen, AlphaLisa, LANCE, Z'LITE
- cAMP, FLIPR, IP-1, beta-arrestin
- Reporter gene assay (Luciferase, β-lac, GFP, β-gal, etc.)
- Cell lines or primary cells & tissues

High Content & Multiplexing Assays

- Cell imaging and kinetic monitoring for a wide range of phenotypic assays in a quantitative and high throughput mode
- Multiplexing platform on Luminex, MSD, CBA and iQue

Phenotypic Functional Assays

- · Primary cell lines and engineering cell lines
- Various technical platforms for therapeutic focuses

Biophysical Assays

- SPR and Thermal shift Assays
- Biacore 8K and 3000 for both small and large molecule testing

Mass Spectrometry with High Throughput Option

- Enzyme inhibitor screening
- Cancer metabolite profiling
- ADME profiling

Safety Pharmacology Assays

- Standard and extended panels to cover various targets
- Both biochemical and cell based formats for receptors
- Over 400 assays can be utilized for customized panel

Hit Identification and Lead Discovery (HTS and SAR)

- Comprehensive platforms for HTS, SAR screening, off target evaluation and drug property profiling
- Primary screening
- Cherry picking and hit confirmation
- IC50/EC50 determination
- Hit follow-up
- Secondary screening
- SAR screening
- Compound ADME profiling and liability prediction

Compound Management Service

- Compound management system enables smooth operation of HTS/SAR campaigns
- Medchem supports hit follow-ups, hit expansion and lead optimization
- Compound QC with LC/MS to confirm identity and quality



Diversified Compound Library Collection

- Over 300K compounds and growing
- A combination of commercial and in-house developed libraries
- All compounds stock in DMSO in 384-well Echo plates with 1536-well plate option
- Compound and cloud-based chemo-informatics
 management system



Syngeneic Tumor Models

61 syngeneic models. Enabled with immunotherapy SOC, TIL analysis and RNAseq profiling

Cancer Type (Number of cell lines)	Name of cell line
Bladder (1):	MBT2 ^{S,GT, TIL}
Brain (2):	GL261 ^{S,GT} , GL261-luc ^s
Breast (6):	4T1 ^{S ,GC/T, TIL} , 4T1-luc ^S , JC ^{GC} , Eph4 1424 ^{GC/T} , EMT6 ^{S,GC/T, TIL} , FM3A
Colorectal (3):	Colon-26 ^{S,GT, TIL} , CT-26 ^{S,GC, TIL} , MC38 ^{S,GC/T, TIL}
Hemangioendothelioma (1):	EOMA
Liver (3):	MH-22A ^{S,GT, TIL} , H22 ^{S,GT} , Hepa 1-6 ^{S,GT, TIL}
Lung (4):	LLC1(LL/2) ^{S,GT, TIL} , KLN205 ^{S,GT, TIL} , 3LL ^{S,GT, TIL} , M109
Leukemia (4):	L1210 ^s , WEHI-3, C1498, WEHI 3BD
Lymphoma (10):	EL4, E.G7-OVA ^s , A20 ^{s,GT} , P388D1, L5178-R, WR19L, A20-Luc ^s , L5178-S(LY-S), L5178Y TK+/- clone (3.7.2C), P3/NSI/1-Ag4-1(NS-1)
Myeloma (5):	MPC-11, FO, P3X63Ag8, J558L ^{S,GT, TIL} , MOPC31C
Mastocytoma (3):	P815 ^{S,GT,} TIL, P815-luc ^S , P1.HTR
Melanoma (6):	B16-F10 ^{S,GC/T, TIL} , B16-F1B0-luc-G5, B16-F0 ^{S,GT} , CloudmanS91 ^{S,GC/T, TIL} , B16-F1 ^{S, TIL} , C57/B1
Neuroblastoma (3):	Neuro-2a, N1E-115, N18(Hamprecht)
Pancreas (1):	Pan02 ^s
Prostate (2):	RM-1 ^{S,GT} , RM-1-luc
Renal (2):	RENCA ^{S,GT, TIL} , RENCA-luc
Sarcoma (2):	WEHI164 ^{S,GT} , K7M2 wt ^{GT}
Testis (1):	MLTC-1
Ovarian cancer (1)	OV3121s
Schwannoma (1)	TR6Bc1

Note: All 61 models have growth curve data,

31 models with reference drug treatment data (either chemotherapy/target therapy or immunotherapy) were marked as S. 28 models with genomic profiling data were marked with GC (cell-line profiled), GT (Tumor tissue profiled) or GC/T (both). 17 models with immune phenotyping by Flow-Cytometry were marked with TIL

FACS Profiling Supporting IO Tumor Models

Established analyses

- Immune phenotyping of TILs or in other tissues (Spleen, Lymph node, Blood, etc.)
- Surface/intracellular biomarker analysis (in PBMC, tumor, spleen, liver, lymph node etc.)
- Receptor occupancy assay
- Cell sorting from mouse tumor, blood or spleen

Instruments:

2x BD FACSCanto[™] (3 Lasers, 10 Channels)

61

Syngeneic model

- 2x BD LSRFortessa[™] (5 Lasers, 18 Channels)
- 1x BD FACS Aria II (4 Laser, 18 Channels, sorting)

Quick turn-around and highly motivated to develop new methods

A Case of Flow-cytometry Based PD Evaluation For Cancer Immunotherapy

Immune phenotyping in an MC38 mouse colorectal syngeneic tumor model treated with an IO drug.







Validated CDX Models

Cell Line-Derived Xenograft (CDX) Tumor Models

A large collection of cell line-derived xenograft (CDX) tumor models: 215 validated models. SOC data available. Mutation data incorporated into OncoWuXi database.

Tumor types	Cell lines
Bladder (7)	T24/83, RT112/84 , UM-UC-3, J82, HT1197, KU- 19-19, VM-CUB1
Brain (GBM; 2)	U87*, LN-229*
Breast (18)	Cal51*, BT-474*, HCC1954*, MDA-MB-231*, MDA- MB-468*, HCC1806*, MCF7*, HCC1937, MDA-MB- 436*, SUM-52PE, JIMT-1*, MDA-MB-361*, HCC-70, SUM 159PT, MFM-223, T47D*, HCC1428, ZR-75-1*
Cervical (1)	SW756
Colorectal (19)	Colo205*, HT29*, HCT-116*, HCT-15*, LS180* , LS174T*, SW620*, HT55*, DLD-1, Lovo*, COLO- 320DM, RKO, Caco-2, T84, WiDr, SW480, SW 48, LS411N, COLO 201
Duodenal (1)	НиТи80
Esophageal (2)	T.Tn, OE21*
Gastric (8)	NUGC4*, SNU-1*, SNU-16*, MGC-803, NCI-N87*, SNU-5, MKN45*, HS 746T
Head & Neck (4)	Cal 27, RPMI2650, SCC-9, Fadu*
Leukemia (13)	HEL 92.1.7*, MV4-11*, CCRF-CEM, HL-60, K562, KG-1, MOLT-4, Kasumi-1*, Nalm-6*, THP-1, SET- 2,PL-21, MKL-1z
Lymphoma (21)	Daudi*, SU-DHL-2*, SU-DHL-4*, SU-DHL-6, SU- DHL-10, Granta-519, Maver-1, Mino*, NAMALWA, Raji, Ramos, RL, WSU-DLCL2*, Z-138, U937, DoHH2, Farage*, Pfeiffer, REC-1*, U2932, Toledo

runor types	Centimes
Liver (9)	BEL-7404*, Hep3B*, Huh 7*, HepG2, SNU-182, QGY-7703, SNU-398, JHH-7*, MHCC97H*
Lung (44)	NCI-H1650, NCI-H226, NCI-H460, PC9*, A549*, Calu-1, Calu-6*, EBC-1, A427, NCI-H647, MSTO-211H, NCI-H292, NCI- H322, NCI- H358*, NCI-H441, NCI-H520, NCI-H1975*, NCI-H2228, SK-MES-1, NCI-H1299, HCC-827*, HCC4006, NCI-H1703, NCI-H727, NCI-H2122*, DV-90, NCI-H69*, NCI-H1417*, NCI-H446, NCI- H526*, NCI-H69*, NCI-H1417*, NCI-H446, NCI- H526*, NCI-H82, SHP-77, Calu-3, NCI-H2170, NCI-H820, NCI-H23, NCI-H1568, NCI-H1944, NCI-H2023, NCI-H2030, NCI-H2126, NCI- H838, NCI-H1838*, DMS114*
Lung (Small cell, 8)	NCI-H69*, NCI-H1417*, NCI-H446, NCI-H526*, NCI-H82, SHP-77, NCI-H23, DMS114*
Melanoma (6)	A375*, SK-MEL-24, SK-MEL-28, COLO 829*, SK- MEL-5 , MDA-MB-435S
Myeloma (7)	KMS-28BM, KMS-11, MOLP-8*, NCI-H929, RPMI 8226*, MM.1S*, OPM-2
Neuroblastoma (4)	CHP-134*, SK-N-AS*, BE(2)-C*, SH-SY5Y
Ovary (5)	A2780*, SK-OV-3*, ES-2*, OVCAR-3, TOV21G
Pancreas (8)	BxPC3*, Mia-pa-ca-2*, KP4*, PANC-1*, CAPAN- 1, Panc 02.13*, AsPC-1, HPAC
Prostate (5)	PC-3*, DU145*, LNCap*, VCaP , 22RV.1*
Renal (5)	786-O*, ACHN, Caki-1, SK-NEP-1, A498
Sarcoma (8)	HT1080, A673, KHOS/NP, 143B, SJSA-1* ,SAOS- 2, A204,G401
Skin (1)	A431*

1,000+ PDX Models

215

*: validated with SOC compound

Patient-derived Xenograft (PDX) Tumor Models

- 1,000+ PDX models covering 20 cancer types
- Well annotated with target-based SOC data
- 700+ PDX models with genetic profiling and FFPE tumor bank
- Searchable online database with mobile APP

AAALAC accredited SPF facility

Induced drug resistant model and mouse clinical trial enabling translational research

 One of the largest collection of Asia prevalent tumor types in the world » Liver - 285 » Lung - 196 » Colorectal - 105 » Gastric - 93 » Esophageal - 84 » Cholangiocarcinoma - 41 Drug resistant PDX Recurrent/metastasis PDX 	Unique >1000 PDX Panel	 Clinical information Clinical classification Pathology Treatment history PDX growth characteristics Growth curves SoC treatment >500 PDX genomic profiled Affymetrix microarray Whole-exome sequencing SNP 6.0 array RNAseq
 400 PDXs in-life for rapid study ons End-user friendly database Effective project management 	et Quality Service	



Human Immune Checkpoint Gene KI Mouse Models

KI (Knock-in) mice offer the opportunity to study clinical grade checkpoint antibodies targeting human checkpoints in the context of a fully functional immune system

- · Collection of various targets and background mouse strains
- · Validated with launched or most advanced clinical grade checkpoint antibodies
- Double checkpoint KI mice customizable for combination (PD1 + OX40, PD1 + TIM3 etc.)

Availability	Model / Strain	
Ready to use	•• hPD-1 C57BL/6 •• hPD-1 BALB/c •• hCTLA-4 C57BL/6	•• hOX40 C57BL/6 •• hTIM-3 C57BL/6
Customizable	 hCTLA-4 BALB/c hCD137 C57BL/6 hLAG3 C57BL/6 hLAG3 BALB/c 	•• hVISTA C57BL/6 •• hTIGIT C57BL/6 •• hTIM-3 BALB/c •• And Double Knock-ins

hPBMC/hCD34+ Humanized Models

hPBMC humanized models

- NOG/NSG mice based system with full immune defects
- Established models for immune-checkpoint inhibitor and bi-specific antibody candidates in a humanresembling immune system.
- Annotation for both immune cells (phenotyping, biomarker, functions) and tumor cells (expression, mutation etc.)
- Different PBMC delivery systems (systemic injection or co-inoculation with tumor)

HSC humanized models

- · Available humanized models from CIEA combined with WuXi PDX/CDX models.
- Customizable with human cytokine transgenic mice (GM-CSF/hIL-3 or hIL-2) to improve the reconstitution of myeloid lineage.

Show case of anti-PD-1 efficacy in EGFR mutant tumor models x hPBMC reconstituted mice.





Tumor Cell Panel and Screening (TCPS ™)

Diversified Collection of Tumor Cell Lines

- 1,000+ tumor cell lines covering broad tumor types with geographic diversity
 - Focused tumor type panels
 - -Lung tumor lines
 - -Hematopoietic and lymphoid tumor lines
 - -Large intestine tumor lines
 - -Breast tumor lines
 - -Skin tumor lines
 - -Liver tumor cell lines, etc.

Genetic Profiling and Analysis

- Proprietary tumor cell panel database with in-house data and genetic information from public domains
- Access to full genetic information and genome wide sequence (RNAseq, WES, and CNV)
- Collection of protein, DNA, RNA samples for further biomarker analysis



Strictly Controlled Management and QC

- Centralized tumor cell bank management with designated facility and team
- Routine mycoplasma test and STR confirmation
- Stringent cell line QC criteria

Experienced Team with Fast Turnaround Time

- Designated team with rich experience in tumor cell banking, QC, panel profiling and screening in various formats
- Track record of supporting two large pharmaceutical companies for more than 5 years
- Fast turnaround time and high quality data delivery

Multiple Formats and Flexible Panels of Profiling

- Gene expression evaluation and signaling pathway analysis (WB, ELISA, IHC, qPCR)
- 2D cell growth (CTG, Cyquant WST1/MTT, SRB)
- 3D cell growth (soft agar, spheroid formation)
- IncuCyte for kinetics and long term treatment
- Apoptosis
- Cell cycle analysis (BrdU assay)
- Invasion and migration
- HCA and imaging
- Histopathology, IHC, ELISA, FACS, etc.
- Single agent or combination studies



Immunology and Immuno-oncology Platform

Outstanding Platform to Enable Cancer Immunotherapy With One Stop Service:

- Immunological functional assays
- In vivo efficacy evaluation on animal models
- Tumor microenvironment analysis

- NGS and histopathology for immunological profiling
- Patient TILs analysis

IO in vitro functional assays

- · Comprehensive and flexible assay panel involving multiple immune cell types
- Analysis by ELISA/CBA based cytokine detection, flow-cytometry based proliferation and biomarker analysis and molecular biology (Wester Blot, qPCR etc.)

Established in vitro immunology assays

Assay		WuXi capability (Human/Mouse)
Cell activation	T cell	Yes (H/M)
	B cell	Yes (H)
	NK cell	Yes (H)
Cell differentiation/ polarization	M1/M2 macrophage	Yes (H/M)
	MDSC	Yes (H)
	DC	Yes (H/M)
	Treg, Th1, Th2, Th17 polarization	Yes (H)
Mixed Lymphocyte Reaction	DC co-culture with T	Yes (H/M)
Suppressive assay	MDSC suppressive assay	Yes (H)
	Treg suppressive assay	Yes (H)
	Tumor cell+immune cell co-culture	Yes (H)
Cell cytotoxicity	Cytotoxic T lymphocyte assay (OT-1 system)	Yes (M)
	NK cytotoxicity	Yes (H)
	ADCC, ADCP	Yes (H)
	CDC	Yes (H)
Cell exhaustion	T cell exhaustion assay	Yes (M)

A well validated 96-well based MLR assay system (hPBMC derived DC co-cultured with allogenic CD4+ T cells).

1000+ compounds have been screened with this assay, which is also solid for combination screening:



T cell proliferation (%) by FACS (CellTrace violet)





Clinical Biomarker

CAP accreditation and GCP compliance for clinical trials.

CAP pathology lab

Team.

- 6 board certified pathologists and consultants •
- 12 senior technicians with rich experience •
- Partnership with Targos

Instrument platforms.

- Leica Microtome & Cryotome •
- Roche Ventana Benchmark Ultra •
- Dako Link48 Auto stainner
- Zeiss multi-head microscope(20 heads)
- Zeiss and Olympus fluorescence microscope • with image capture 4X
- Leica Bond Rx Auto stainner
- Zeiss Axio Digital Scanner and PE Vectra analysis
- Aperio Versa 8 and HALO-LINK system ٠

Technology platforms.

- Anatomic pathology: H&E, IHC, IF, ICC, special stains, enzymohistochemistry
- Molecular pathology: FISH & DISH, RNAscope •

GCP compliance/ CAP accredited.

Rapid TAT - About 3-5 business days from receipt of sample.



Automated Station Leica Autostainer XL



Ventana BenchMark ULTRA



Aperio VERSA 8



PE Vectra



Leica BOND RX



Fluorescence

Microscope

Olympus BX 43/63



Fluorescence Microscope ZEISS Axio



BD FACSCanto II 3 laser, 8 color, 96/384 well plate



BD LSRFortessa 5 laser, 18 color, 96/384 well plate



BD AriaSorp 4 laser, 18 color, 4 way, high speed, aseptic sorting

CAP FACS lab

Instruments.

- **BD FACSCanto II 2X**
- BD LSRFortessa 1X •
- BD AriaSorp sorter 1X •

Comprehensive immunoprofiling of all immune cells from blood, lymphoid organs, & tumor tissues.

- Cell activation & differentiation markers ٠
- Cytokine & chemokine receptor analysis •
- Signaling pathways

Cell sorting of immune cell subsets.

GCP/CAP compliance for clinical trials.

Rapid TAT - About 3-5 business days from receipt of sample.

Developed and validated:

- PD/PK assay for a CAR-T clinical study
- Receptor occupancy assay for PD-L1/PD-1 binding

ZEISS-Axio Scanner Z1-100





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