



INFLAMMATION AND IMMUNOLOGY

ChemPartner's immunologists are highly experienced in the evaluation of immune cell activation/proliferation/differentiation, cytokine release, signal molecules, receptor binding, and cell-cell interaction. We have helped hundreds of our global pharmaceutical and biotech clients with drug discovery for cancer immunotherapy and autoimmune diseases.

CAPABILITIES

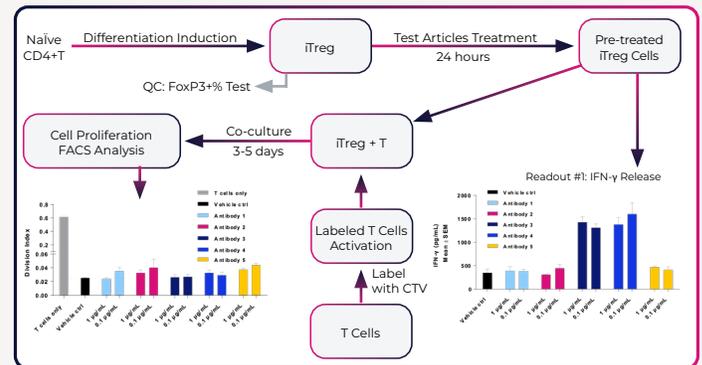
IN VITRO ANALYSIS PLATFORMS

- Primary immune cell *in vitro* assays
- Whole blood/PBMC stimulation and cytokine release assay
- Mixed lymphocyte reaction (MLR) assay
- CMV antigen recall assay
- Tumor/T cell co-culture assay
- T cells (total, CD4+, CD8+) activation and proliferation assays
- Th subsets differentiation (Th1/2/17/Treg) assays
- Treg suppressive assay (mouse, human)
- T cells killing assay (bi-specific antibody)
- OT-I mice T cell and ova-expressing tumor cell co-culture
- B cell activation assay in human blood and PBMCs/rat/mouse
- NK cell cytotoxicity assay/*in vitro* NK cell killing assay (human version K562)
- M1/M2 macrophage polarization assay
- Macrophage/T cell co-culture assay
- Phagocytosis assay
- Basophil activation assay in human blood
- JAK/STAT signaling assays in human blood
 - FACS and AlphaLISA analysis
 - PD (phosphor-STATs detection in immune cells)
- CD73-A2aR signaling assay
- NLRP3 inflammasome activation assay
- Receptor occupancy assay
- LPS-induced cytokine release in whole blood/human PBMC/THP-1 cells
- TLR7/8-induced cytokine release assay in human PBMCs
- STING antagonist assay (human/mouse version)

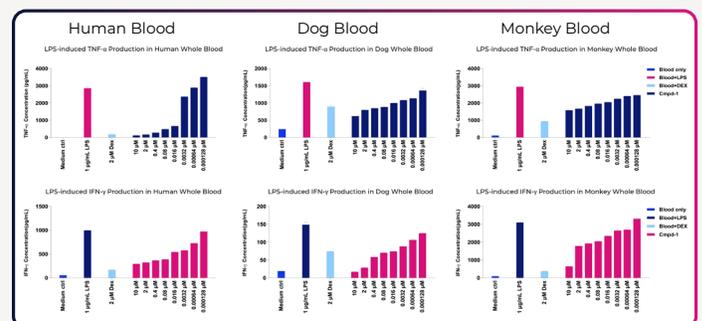
READOUTS

- Cytokine release (ELISA/Luminex/ELISPOT)
- Transcriptome (Quantigene)
- Signaling molecule (AlphaLISA)
- Multi-color flow cytometry

HUMAN iTREG SUPPRESSIVE ASSAY



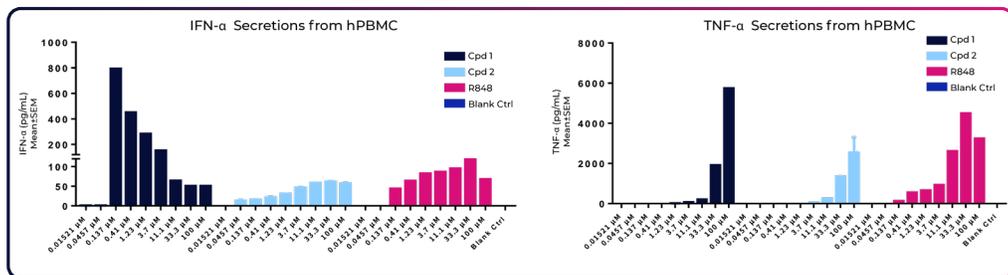
LPS-INDUCED CYTOKINE RELEASE IN WHOLE BLOOD



CASE STUDIES

TLR7/8-INDUCED CYTOKINE RELEASE ASSAY IN HUMAN PBMCs

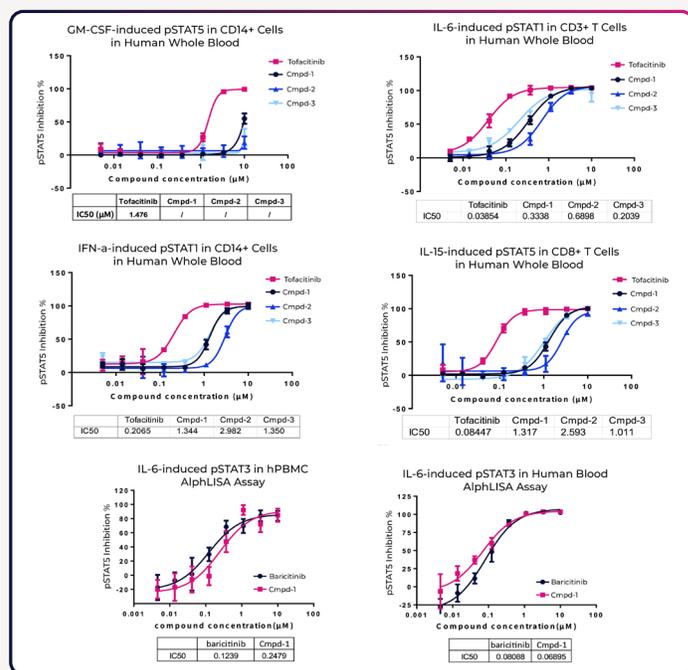
- Fresh human PBMCs treated with test articles
- R848 (Resiquimod), which induced TLR7/8 activation, served as positive control
- Supernatant harvested for TNF- α and IFN- α ELISABE, DDI, Ocular PK, etc.



JAK SIGNALING SCREENING WITH VALIDATED PBMCs/BLOOD PATHWAYS

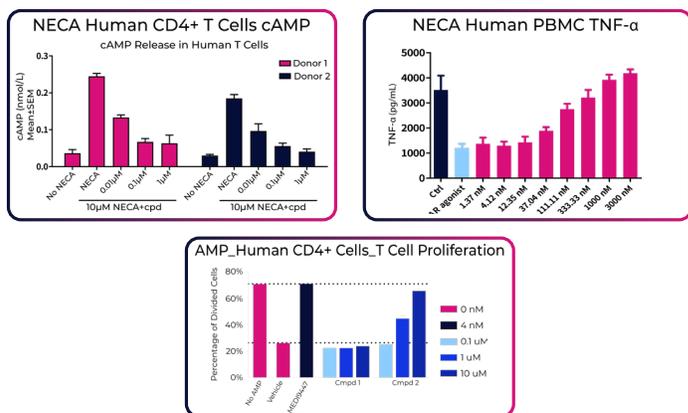
| STIMULUS | KINASE SELECTION | PHOSPHO-PR. READOUT | TARGET CELL POPULATION |
|--------------|------------------|---------------------|---------------------------|
| hIL -2 | JAK1/JAK3 | STAT5 | CD4+/CD8+ T cells |
| hIL -4 | JAK1/JAK3 | STAT6 | CD4+ T cells |
| hIL -6 | JAK1/JAK2 | STAT1 | CD3+/CD4+ T cells |
| hIL -12 | JAK2/TYK2 | STAT4 | CD3+ T cells |
| hIL -15 | JAK1/JAK3 | STAT5 | CD3+/CD4+/CD8+ T cells |
| hIL -23 | JAK1/JAK2 | STAT3 | CD3+/CD161+ cells |
| IFN α | JAK1/TYK2 | STAT1 | CD123+HLA -DR +BDCA+ pDCs |
| IFN α | JAK1/TYK2 | STAT3 | CD3+ cells |
| IFN γ | JAK1/JAK2 | STAT1 | CD4+/CD8+ T cells |
| GM -CSF | JAK2/JAK2 | STAT5 | CD14+/CD33+ |

JAK SIGNALING ASSAY IN HUMAN BLOOD_FACTS AND ALPHALISA ANALYSIS



A2AR SIGNALING RELATED ASSAYS

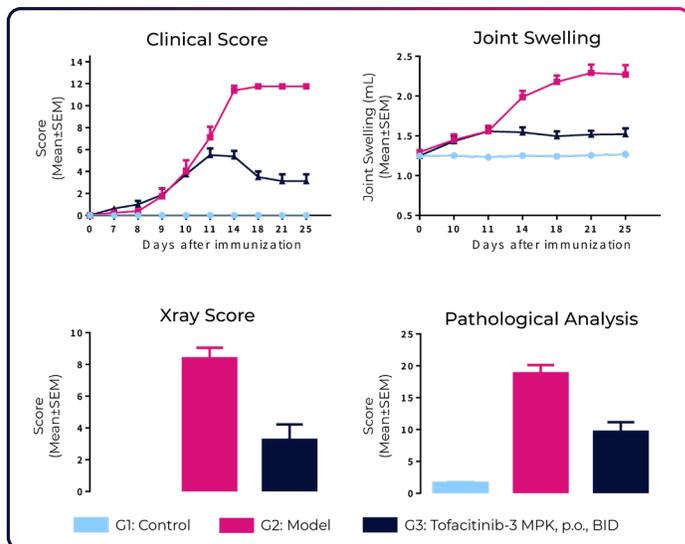
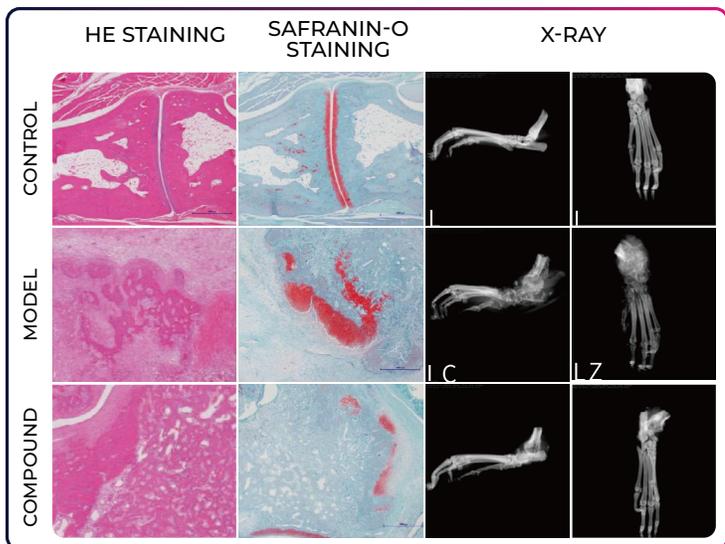
| STIMULUS | TARGET | READOUT | CELL TYPE | |
|----------|--------|---------------------------------------|--|--------------------------------------|
| NECA | A2AR | Cytokines (ELISA) | IFN- γ | mouse splenocyte |
| | | | IL-2 | hPBMC/huma nT cells/mouse splenocyte |
| | | | TNF- α | hPBMC/human T cell ls |
| | | cAMP (TR-FRET) | human T cells/mouse T cells | |
| | | pCREB (Flow cytometry) | hPBMC/mouse splenocyte/whole blood | |
| AMP | CD73 | Cytokines (ELISA) | IFN- γ /IL- α /TNF- α | hPBMC |
| | | T cell proliferation (flow cytometry) | | hPBMC/CD4+ T cell |



CASE STUDIES

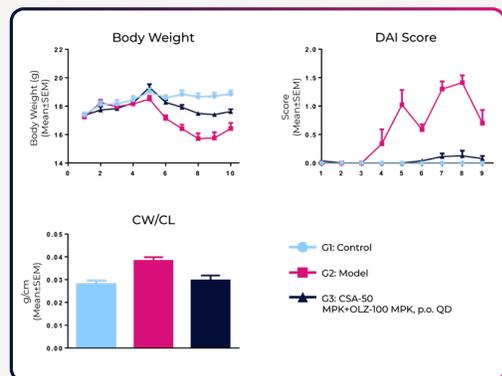
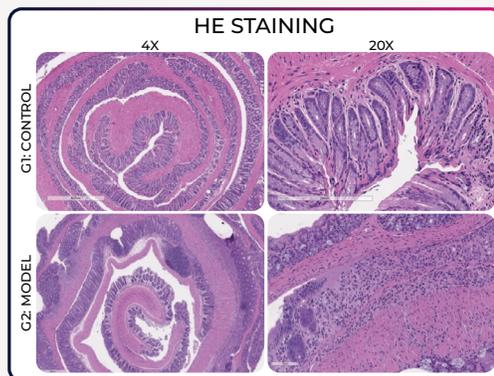
ADJUVANT-INDUCED ARTHRITIS (AIA) IN RATS

- Animal: Lewis rat, female, 180-200g
- Immunization: CFA
- Readouts: body weight, joint swelling, clinical score, pathology, x-ray



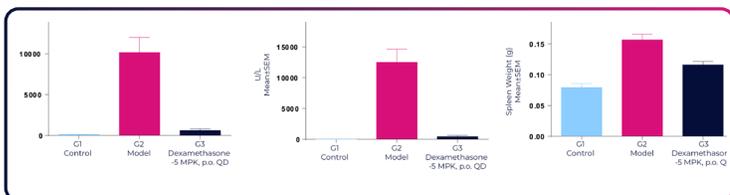
ADJUVANT-INDUCED ARTHRITIS (AIA) IN RATS

- Animal: C57BL/6 mice
- Modeling: treatment with DSS in drinking water
- Readouts: body weight, clinical score(DAI), colon weight/length, pathology

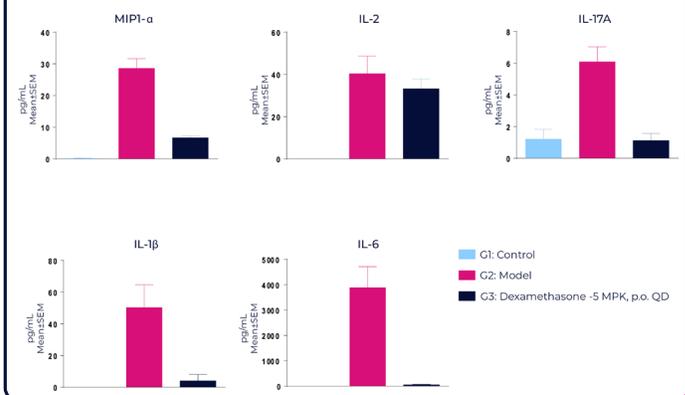


CON-A-INDUCED SYSTEMIC INFLAMMATION IN MICE

- Animal: BALB/c mice, female, 9-11 weeks
- Modeling: ConA injection through tail veins
- Reference drug: Dexamethasone
- Readouts: body weight, liver/spleen weight, ALT and AST level in serum, inflammatory cytokines release in plasma



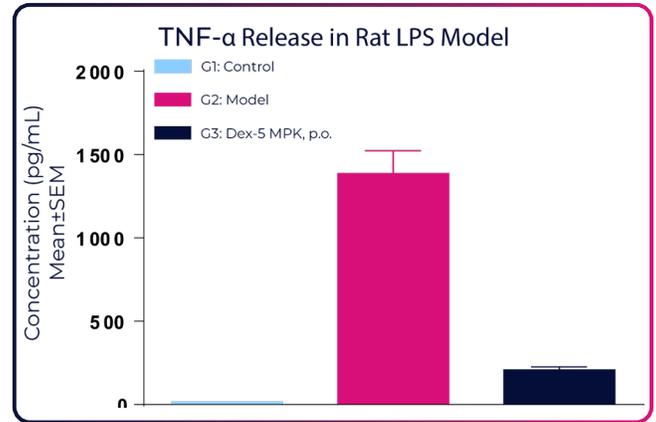
MULTIPLE CYTOKINES RELEASE LEVELS IN MOUSE PLASMA



CASE STUDIES

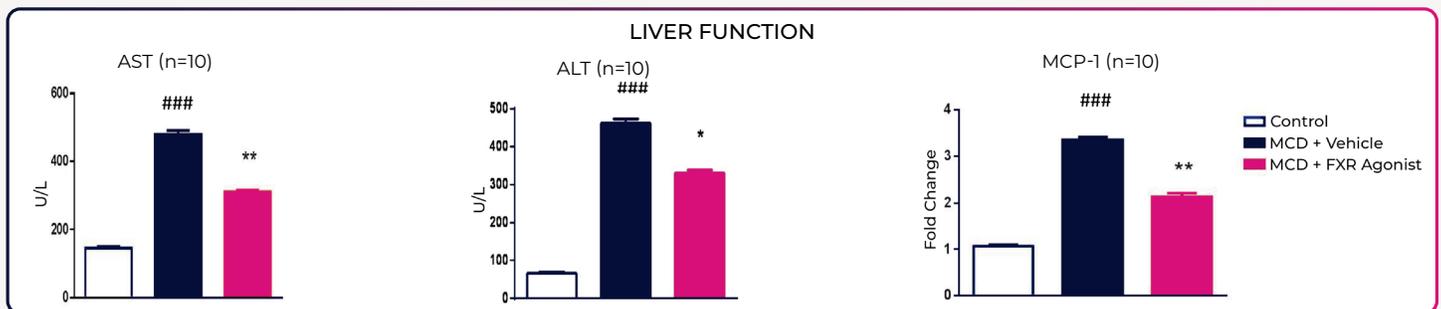
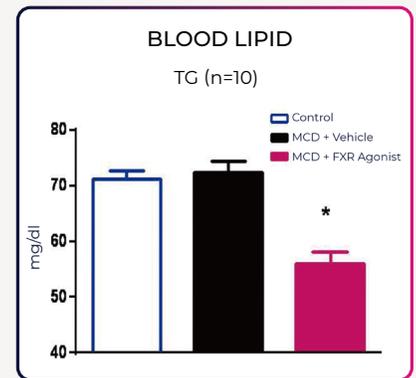
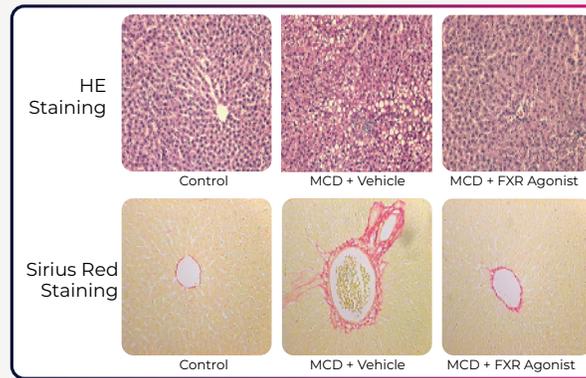
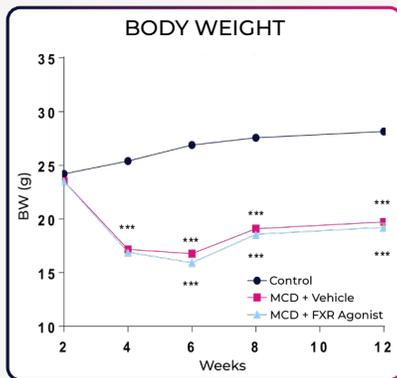
LPS-INDUCED SYSTEMIC INFLAMMATION IN RATS

- Animal: Lewis rat, female, 180-200g
- Immunization: CFA
- Readouts: body weight, joint swelling, clinical score, pathology, x-ray



METHIONINE AND CHOLINE DEFICIENT (MCD) DIE T-INDUCED NON-ALCOHOLIC STEATO HEPATITIS MODEL (NASH) IN MICE

- The MCD-fed mice showed typical pathological features, including lipid degeneration and liver fibrosis
- Significant increases in ALT and AST levels in the MCD-fed mice showed liver injury
- The liver inflammation marker MCP-1 increased in the MCD-fed group
- Treatment with FXR agonists alleviated steatohepatitis score (DAI), colon weight/length, pathology



IN VIVO DISEASE MODELS

RESPIRATORY DISEASE

- OVA-induced asthma in mice
- Bleomycin-induced lung fibrosis in mice

RHEUMATOID ARTHRITIS

- Collagen induced arthritis in rats(CIA)
- Adjuvant induced arthritis in rats (AIA)

MULTIPLE SCLEROSIS (MS)

- MOG-induced EAE in mice
- PLP-induced EAE in mice

SKIN-RELATED DISEASE

- IMQ induced psoriasis in mice
- IL-23 induced hyperplasia in mice
- Passive cutaneous anaphylaxis in mice (PCA)
- DNFB/Oxa induced delayed type hypersensitivity in mice (DTH)

INFLAMMATORY BOWEL DISEASE (IBD)

- DSS-induced IBD in mice(acute)

SYSTEMIC INFLAMMATION OR OTHER

- LPS-induced cytokine release in mice/rats
- Systemic inflammation in mice (Con A)

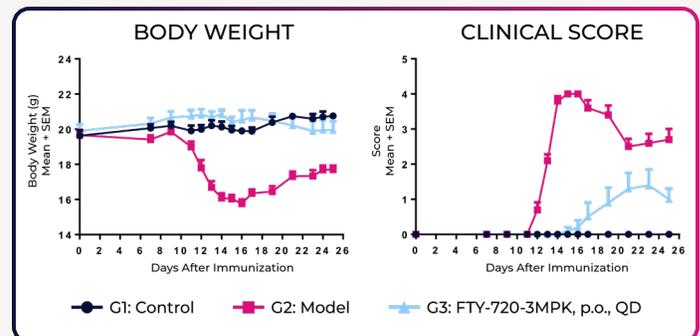
METABOLIC DISEASES

- HFD induced obesity in mice
- Diabetes in db/db mice
- Diabetes nephropathy in db/db mice

CASE STUDIES

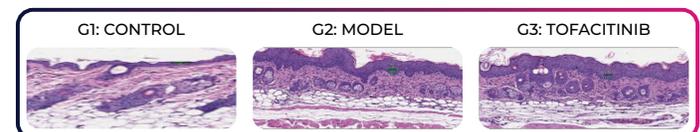
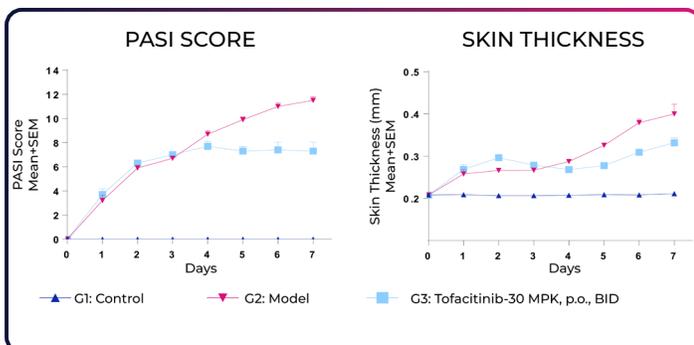
MOG35-55-INDUCED EAE IN C57BL/6 MICE

- Animal: C57BL/6 mice, female
- Immunogen: Myelin Oligodendrocyte Glycoprotein (MOG35-55) peptide
- Readouts: body weight, clinical score, pathology



PSORIASIS: IMQ INDUCED PSORIASIS IN MICE

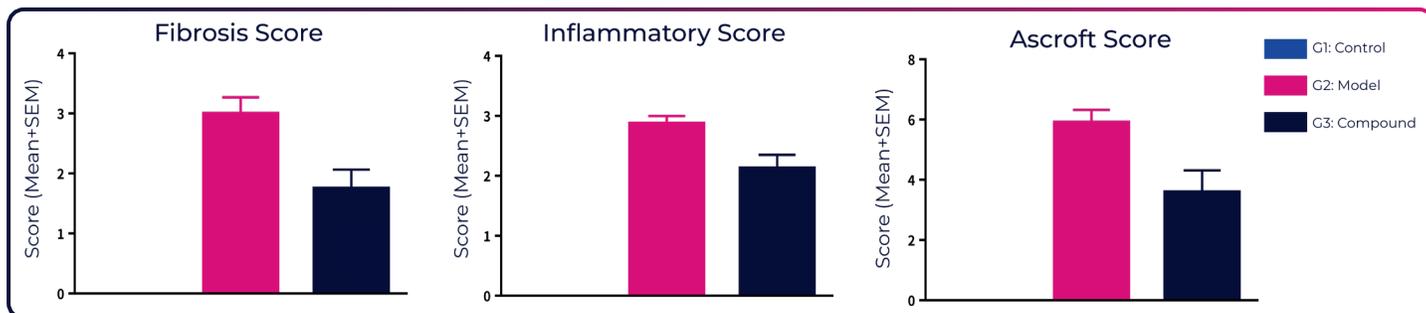
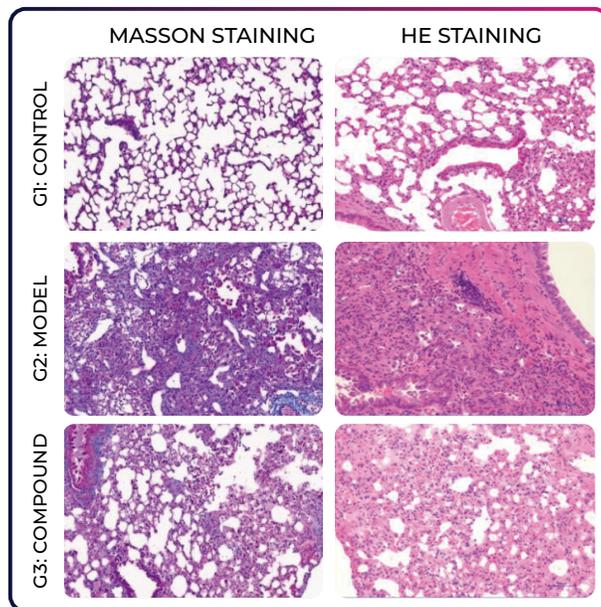
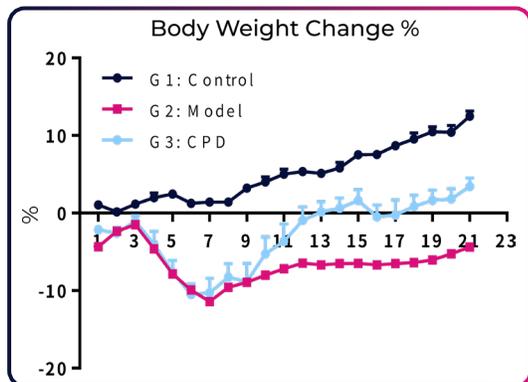
- Animal: BALB/c mice, female
- Readouts: body weight, PASI score, skin thickness, pathology
- PASI score criteria: Erythema, scale, and thickness
- 0: no; 1: mild; 2: moderate; 3: severe; 4: marked



CASE STUDIES

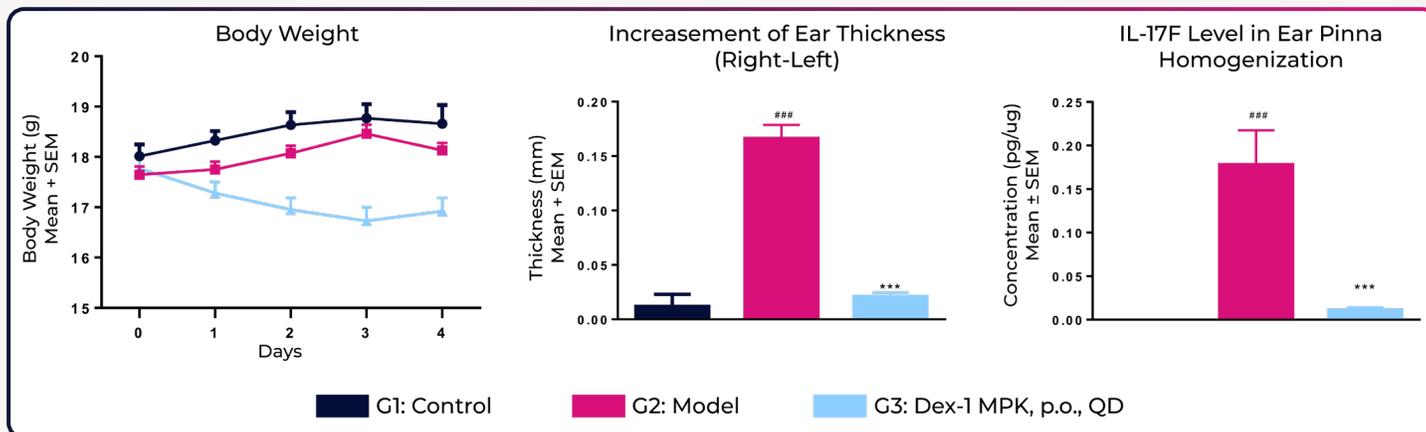
BLEOMYCIN INDUCED LUNG FIBROSIS MODEL IN MICE

- Animal: C57BL/6 mice, male, 8 weeks
- Modeling: BLM, i.t.
- Readouts: body weight, pathology (Mason and HE staining), inflammatory cells counts in BALF, total collagen (optional)



IL-23-INDUCED EAR EDEMA MODEL IN MICE

- Animal: C57BL/6 mice, female, 6-8 weeks
- Sensitization: rmIL-23, s.c.
- Reference drug: Dexamethasone, p.o., QD
- Readouts: body weight, ear thickness, IL-17F level in ear pinna homogenization



EX VIVO ANALYSIS PLATFORMS

READOUTS

- Frequency of immune cell subpopulation
- Expression of immune checkpoints and markers
- Soluble factors
- Receptor occupancy %
- PD/biomarker design, method development, method validation and clinical sample analysis

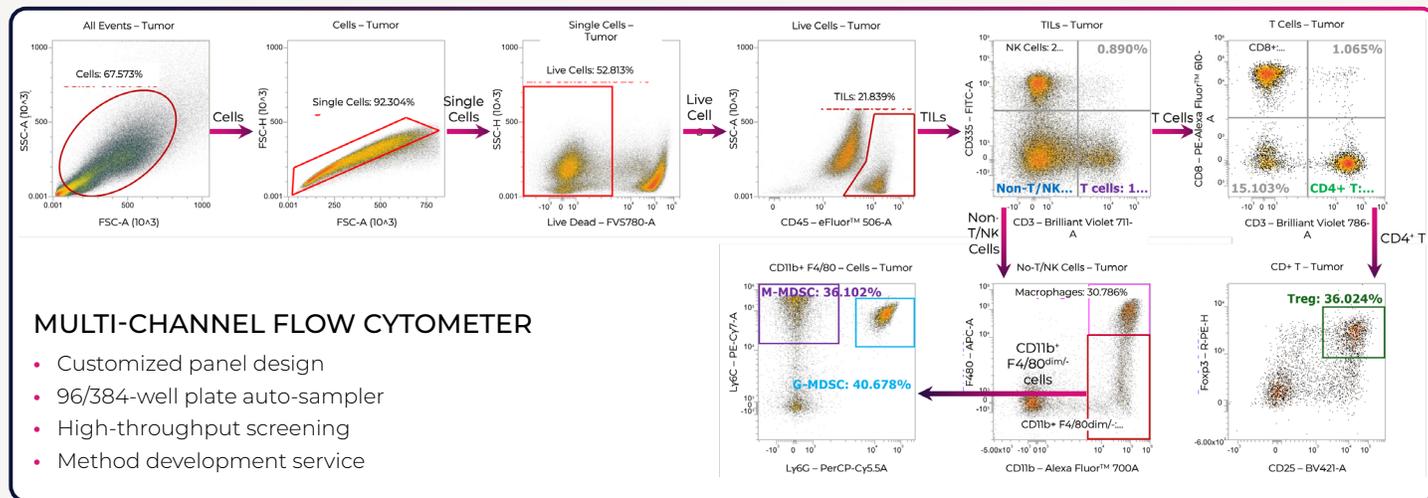
METHODS

- Flow cytometry
- Luminex/Quantigene
- ELISA, ELISpot
- Sequencing
- Western blot, qPCR

TISSUES

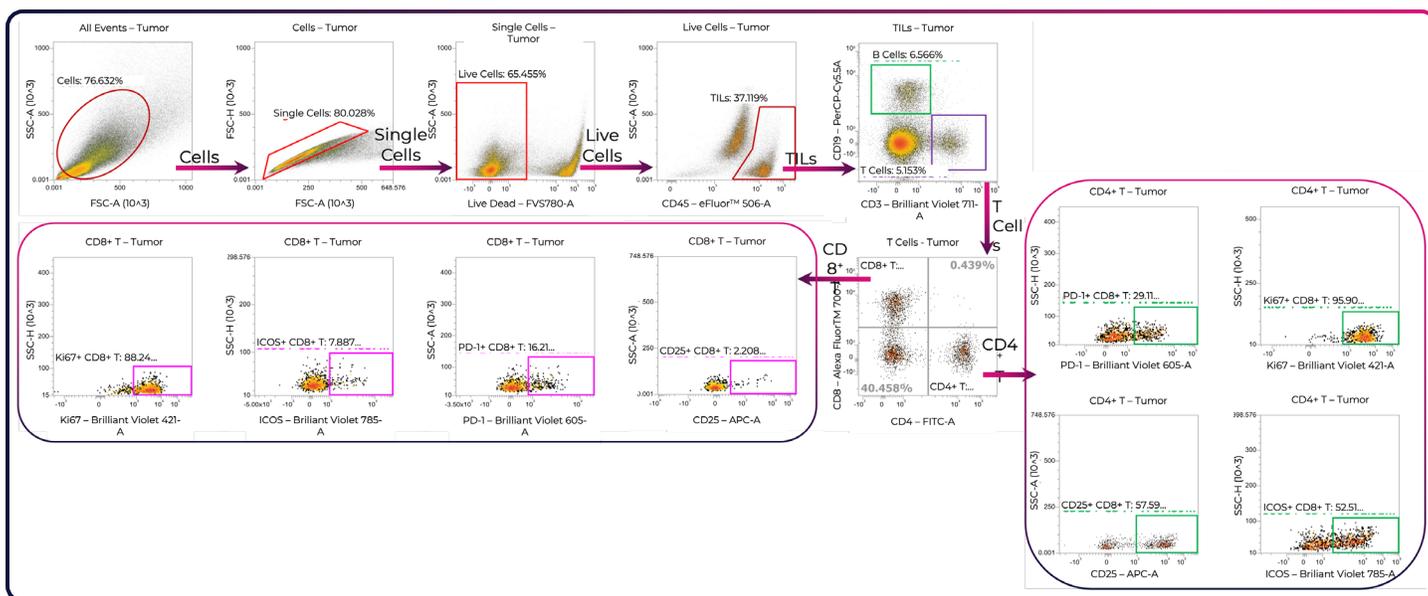
- Tumor, brain, liver, kidney
- Spleen, lymph nodes, thymus
- BALF, peritoneal cells
- Peripheral blood
- Bone marrow

MULTI-COLOR FLOW CYTOMETRY CAPABILITIES



TILS ANALYSES_ T CELL CHECKPOINT/ ACTIVATION EVALUATION PANEL

- Tumor processing with gentle MACS dissociator, then remove RBC
- Multi-color staining followed by flow cytometric analysis



MULTI-COLOR FLOW CYTOMETRIC ANALYSIS

| ITEM | | IMMUNE CELL POPULATION |
|--------------------|--------------------------|---|
| Immune Cell Typing | Mouse/Rat/Human | Lymphocytes: T cells, Treg, B cells, NK cells, memory T, effector T, naïve T... Myeloid cells: monocytes, macrophages, DCs, pDC, MDSC, granulocytes... |
| | Monkey | T cells, Treg, B cells, NK cells, monocytes, DCs, HSC, plasma cells |
| | Dog | T cells, Treg, B cells, monocytes, granulocytes |
| | | |
| Biomarker Analysis | Phosphorylation | p-CREB, p-STAT3, p-STAT6 |
| | Checkpoint | PD-1/PD-L1, Tim-3, Lag-3, CTLA-4, TIGIT |
| | Co-stimulator | 4-1BB, OX40, CD86, CD80, ICOS |
| | Intracellular cytokine | IFN- γ , IL-2, IL-4, IL-17A, IL-10, TNF- α , GranzymeB |
| | Proliferation/Activation | IFN- γ , IL-2, IL-4, IL-17A, IL-10, TNF- α , GranzymeB |

ELISA

COMMERCIAL VENDORS

- Abcam
- R&D
- MBL
- Abclonal

TYPES OF SAMPLES

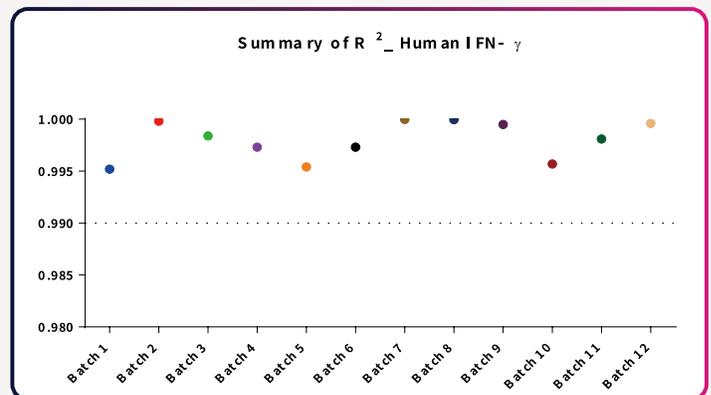
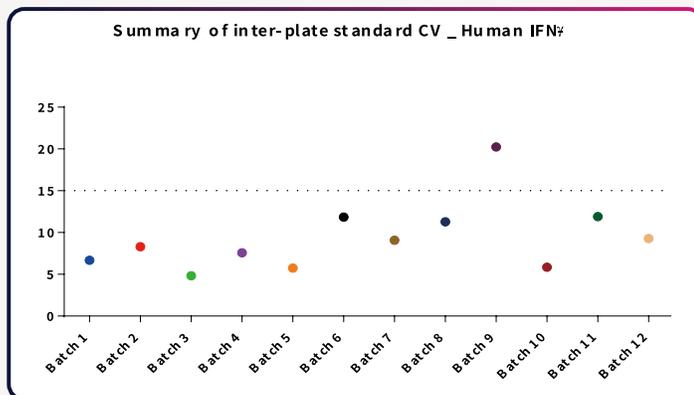
- Plasma/serum
- Cell culture supernatant
- Tissue homogenates
- Cell lysates

VALIDATED ANALYTES POOL

- Human, Mouse, rat
- IL-2, IL-6, IL-10, TNF- α , IFN- γ , IP-10, CXCL-12, MCP-1, PCSK9

STANDARD WORK FLOW

- A quick pilot to check matrix effect/ determine dilution factor
- Formal test
- QC criteria: Linear equation R² <0.9900, intra- and inter-plate standard signal CV tracking



LUMINEX

CAPABILITIES

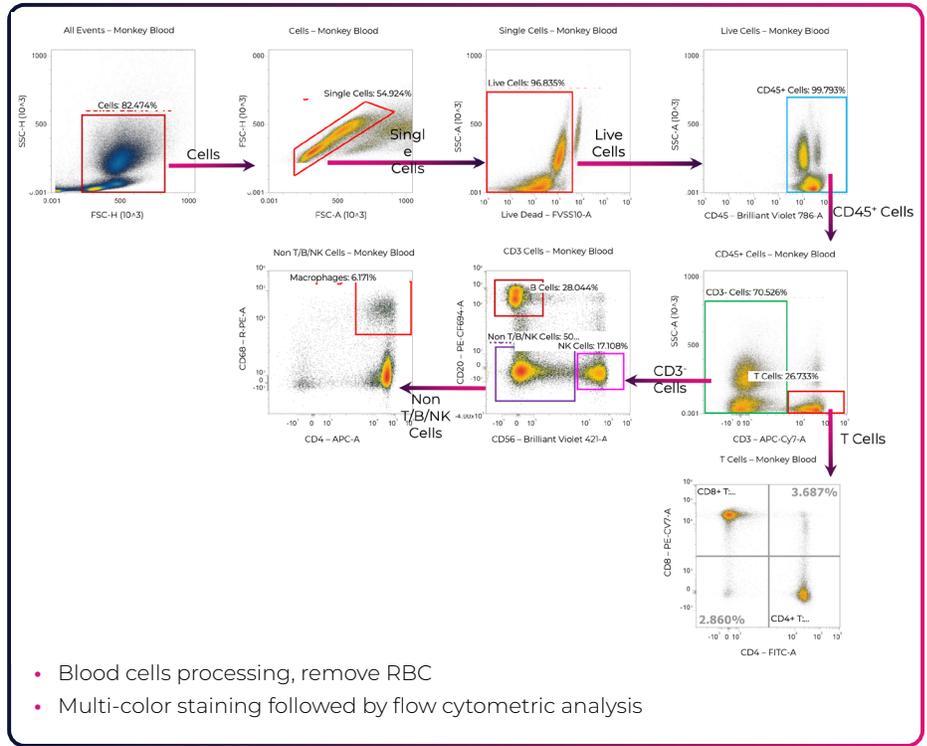
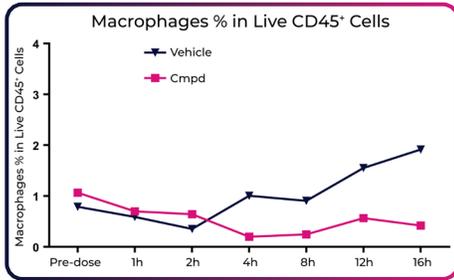
- Customized commercial kits
- Multiplexing with up to 80(2D) / 500(3D) analytes per well
- Validated analytes pool
 - 347 human targets
 - 108 mouse targets
 - 36 monkey targets
 - 17 Rat targets
- QC criterial:
 - Inner- and inter-plate standard signal CV tracking

EQUIPMENT

- Bio-Plex® 200
- Luminex 200™
- FLEXMAP 3D

WHOLE BLOOD PHENOTYPING IN CYNO MONKEY

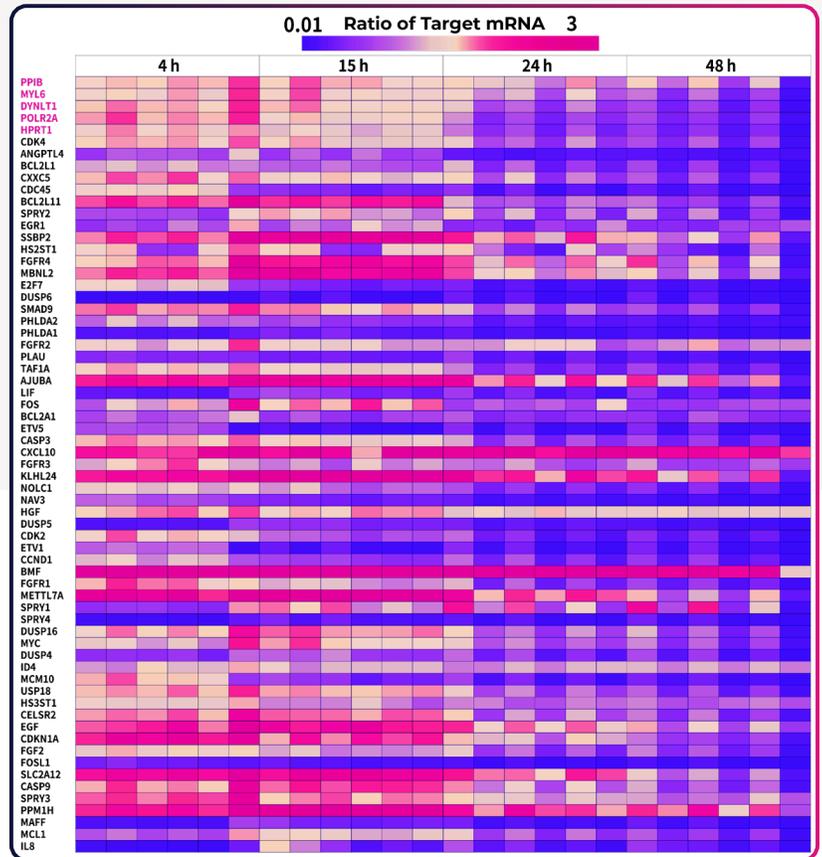
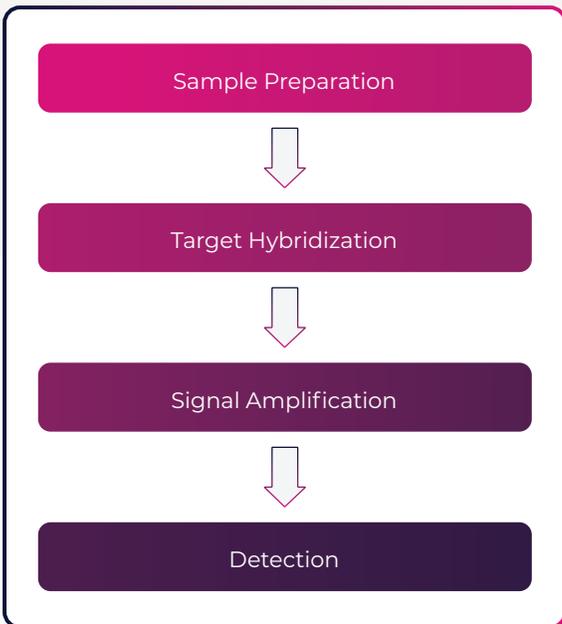
| CELL TYPES | READOUT LIST |
|-------------|--|
| T cells | Live CD45 + CD3 + cells |
| CD8 + T | Live CD45 + CD3 + CD4 - CD8 + cells |
| CD4 + T | Live CD45 + CD3 + CD4 + CD8 - cells |
| B cells | Live CD45 + CD3 - CD20 + CD56 - cells |
| NK cells | Live CD45 + CD3 - CD56 + CD20 - cells |
| Macrophages | Live CD45 + CD3 - CD56 - CD20 - CD11b + CD68 + cells |



QUANTIGENE

MULTIPLEX MRNA PROFILING

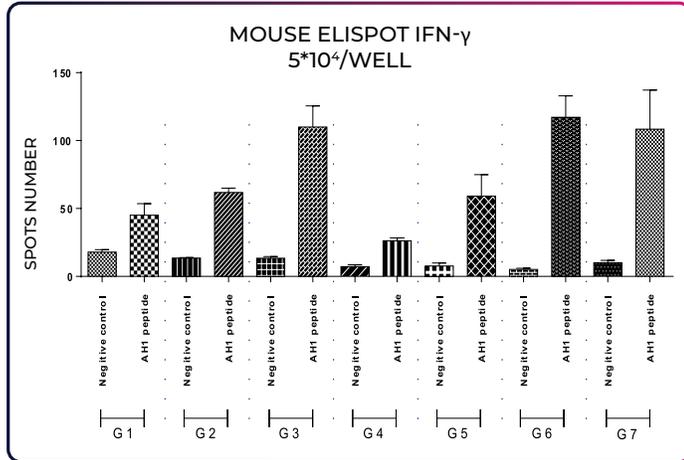
- Customized panel/probes (over 15,000 validated genes)
- Probe design service from ThermoFisher
- Multiplexing with up to 500 mRNA targets per sample



ELISPOT

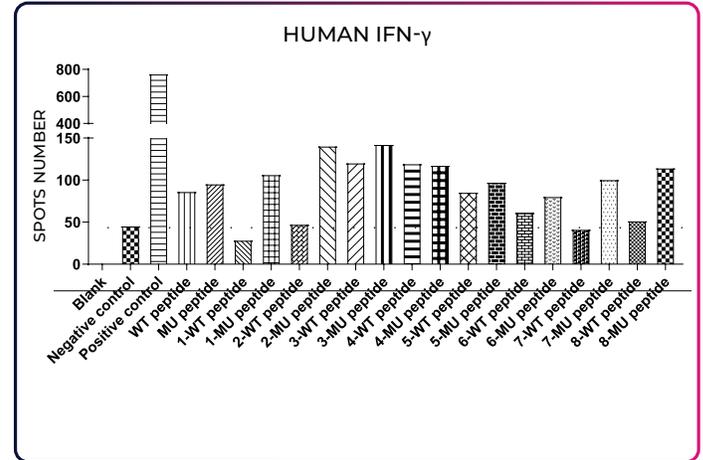
APPLICATIONS

- Mouse splenic or blood immunocytes immunogenicity evaluation
- Human fresh PBMC sourced antigen-specific T cell detection
- Neo-antigen screening by using donor contributed T cells and DCs



ANALYTES

- Human IFN-g, Gran-b, IL-1a, GM-CSF, IL-6...
- Mouse IFN-g, IgG, IgE, IgA, IL-4, IL-2...



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