IMSUT Hospital

Department of Laboratory Medicine
検査部

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.		部長 / 病院教授	博士(医学)	長 村 登紀子
Assistant Professor	Tomohiro Ishigaki, M.D., Ph.D.		副部長 / 助教	博士(医学)	石 垣 知 寛
Project Senior Assistant Professor	Koichi Kimura, M.D., Ph.D.		特任講師	博士(医学)	木 村 公 一
Chief Technologist	Hironori Shimosaka		技師長	臨床検査技師	下 坂 浩 則

The Department of Laboratory Medicine has seven divisions: clinical hematology, biochemistry/serology, microscopy, pathology, microbiology, physiology, and a TR verification laboratory.
Clinical laboratory tests are necessary for all clinical practice steps, including disease diagnosis, stage evaluation, treatment determination, and assessment after therapy. Our department conducts most of the clinical laboratory examinations in our hospital under stringent quality control and provides investigational laboratory analysis in collaboration with many other departments.
To facilitate translational research projects in this research hospital, we established a special division named TR Verification Laboratory. This division has contributed to evaluating the safety of experimental therapeutic approaches and biopharmaceutical products for clinical trials.
As a central medical department, we also participate in many clinical trials and support many research studies conducted in our hospital.

* Only achievements related to clinical laboratory medicine are shown here, and other ones overlapping across affiliations have been omitted. Please refer to each respective affiliation.

1. Establishment of a new cytometric method to evaluate the immune function of chimeric antigen receptor T (CAR-T) cells and application for clinical testing

Tomohiro ISHIGAKI.

CAR-T cell therapy, a groundbreaking approach to hematological malignancies, can be hindered by relapses due to CAR-T cells' poor immune response or exhaustion after proliferation. Hence, evaluating the immune functions is a crucial clinical need. In this study, we have harnessed molecular imaging flow cytometry (MI-FCM) to develop a new evaluation method with joint researchers.

Our study involved the creation of eight CAR-T clones through the genetic modification of PBMCs from eight healthy donors using lentivirus to express an anti-CD19 CAR. We then assessed the cytotoxicity of these clones in vitro using a luciferase assay. Subsequently, we stimulated them with CD19 antigen for an hour and meticulously analyzed the intensity, area, and distribution (spot counts) of the CAR expression using an imaging flow cytometer. We further evaluated the cytotoxicity of some clones in vivo using a xenotransplantation model of CAR-T cells and B-cell leukemia cell-line (NALM6) cells in NOG mice.

We found superficial CAR antigens aggregate in immune responses and could detect the concentration as a decrease in CAR expression area. The percentage of CAR T-cells with a single-spot CAR concentration was significantly correlated with killing activity. We also confirmed that the clone with the

lowest concentration percentage couldn't prolong survival in xenotransplantation models. We also found that this method could be applied to remnants of CAR-T cell therapy products.

In conclusion, quantifying the superficial CAR concentration by MI-FCM could be useful for evaluating their immune function. [The 66th annual meeting of the American Society of Hematology (ASH), 2024.] [The 86th annual meeting of JSHEM, 2024.] [The 84th annual meeting of JCA, 2024.]

2. Evaluation of superior migration ability of umbilical cord-derived mesenchymal stromal cells (MSCs) toward activated lymphocytes compared to those of bone marrow and adipose-derived MSCs.

Tokiko NAGAMURA-INOUE.

Upon inflammation and tissue damage, mesenchymal stromal cells (MSCs) are activated and migrate to suppress inflammation and repair tissues. Though migration is the first important step for MSCs to get functional, the migration potency of umbilical cord-derived MSCs remains poorly understood. We compared the migration potencies of umbilical cord-derived(UC-), bone marrow-derived (BM-), and adipose tissue-derived (AD-) MSCs toward allogeneic stimulated mononuclear cells (MNCs) in mixed lymphocyte reaction (MLR). UC-MSCs showed significantly faster and higher proliferation potencies and higher migration potency toward unstimulated MNCs and MLR than BM- and AD-MSCs. The amounts of CCL2, CCL7, and CXCL2 in the supernatants were significantly higher in UC-MSCs co-cultured with MLR than in MLR alone and BM- and AD-MSCs co-cultured with MLR. The amount of CCL8 was higher in BM- and AD-MSCs than in UC-MSCs, and the amount of IP-10 was higher in AD-MSCs co-cultured with MLR than in UC- and BM-MSCs. The migration of UC-MSCs toward the MLR was partially attenuated by platelet-derived growth factor, insulin-like growth factor 1, and matrix metalloproteinase inhibitors in a dose-dependent manner. UC-MSCs showed faster proliferation and higher migration potency toward activated or non-activated lymphocytes than BM- and AD-MSCs. The functional chemotactic factors may vary among MSCs derived from different tissue sources, although the roles of specific chemokines in the different sources of MSCs remain to be resolved. [Akiko HORI, Tokiko NAGAMURA-INOUE, et al. Front Cell Dev Biol. 2024.]

3. Highly sensitive detection and analysis of cells in the spinal fluid of aggressive adult T-cell leukemia/lymphoma with central nervous system infiltration.

Tomohiro ISHIGAKI.

The prognosis of adult T-cell leukemia/lymphoma (ATL) with primary central nervous system (CNS) involvement has been unclear since the advent of new therapies. We have shown that flow cytometric CD7/CADM1 analysis of CD4-positive cells is useful for detecting ATL cells that are not morphologically diagnosed as ATL cells. We investigated the role of CNS involvement in ATL using cytology and flow cytometry by analyzing cerebrospinal fluid (CSF) from aggressive ATL cases. Based on the findings in CSF, the study subjects were classified into CNS + (cytologically malignant), CNS- (cytologically non-malignant and ATL cell population negative in flow cytometry), and CNS-Micro (cytologically non-malignant and ATL cell population positive in flow cytometry) groups. As expected, the CNS + group had a shorter overall survival than the CNS- group. However, the CNS-Micro group showed no adverse impact on overall survival compared to the CNS- group, even without additional CNS-targeted treatments. Flow cytometry also demonstrated clinical utility in diagnosing CSF lesions in ATL patients with cerebral white matter lesions and detecting ATL cells on post-treatment CSF examination in patients with CNS involvement. Our study demonstrates that ATL with CNS involvement has a poor prognosis and that highly-sensitive flow cytometric analysis of CSF is useful to assist in the diagnosis of suspected CNS involvement and to detect ATL cells after treatment. [Koji JIMBO, Tomohiro ISHIGAKI, et al, Ann Hematol. 2024-2025.]

4. Retrospective analysis and search for clinical laboratory parameters associated with cardiac deterioration and shorter survival in Becker Muscular Dystrophy (BMD).

Koichi KIMURA and Tomohiro ISHIGAKI.

Becker muscular dystrophy (BMD) is an X-linked recessive disorder caused by a mutation in the dystrophin gene. It is most common in muscular dystrophies. BMD has a later onset and milder symptoms than Duchenne muscular dystrophy (DMD). Still, cardiac diseases are now one of the leading causes of morbidity and mortality in these patients. We have retrospectively reviewed biochemical examination results and echocardiographic findings. We found a clinical laboratory parameter that could be associated with cardiac deterioration and shorter survival.

5. Laboratory contribution as a central medical department and support for many clinical investigations and trials in this hospital.

Hironori SHIMOSAKA and Clinical laboratory members (clinical hematology, biochemistry/serology, physiology, and microbiology team)

We participate in clinical trials and research led by other hospital departments. Our laboratory members officially contributed to 9 clinical investigations and trials conducted in this hospital, including treatments using new drugs and new cell therapy. We also contributed to many other basic and clinical studies.