*IMSUT Hospital*

# Center for Antibody and Vaccine Therapy
# 抗体・ワクチンセンター


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*Our center was established in April 2012, in the memory of Professor Shibasaburo Kitasato, the founder and the first director of our institute, because the year 2012 was 120th anniversary of our institute which was built in 1892. Professor Kitasato was keen to utilize 'serum therapy' for patients with infectious diseases and actually developed therapeutic sera from horses. Now, we can use monoclonal antibodies to cytokines and their receptors, growth factor receptors, cellular kinases, for treatment of autoimmune diseases and cancer. The aim of this center is to develop novel immunological therapy for patients with various diseases including cancers and autoimmune diseases. Moreover, attractive clinical trials are also ongoing in collaboration with research groups in IMSUT.*

## Tanaka Group

### 1. Clinical activities in IMSUT Hospital

**Hirotoshi Tanaka, Motohisa Yamamoto, Noritada Yoshikawa, Hiroki Yamazaki, Erika Matsubara*: *Department of Rheumatology and Allergy, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo**

Rheumatologists at our division provide state-of-the-art diagnosis and treatment for systemic autoimmune diseases (total number of patients were approximately 5,000 per year). Our physicians have active basic and clinical research projects and also are involved in training of rheumatology specialists.

Rheumatologic services offered at IMSUT Hospital include:

- Outpatient consultations
- Outpatient specialty care for patients with rheumatic diseases
- Hospital consultations
- Diagnostic and therapeutic intra-articular and soft tissue injections and aspirations
- Diagnostic ultrasonography
- Education on rheumatologic diseases and treatments
- Clinical trials

### 2. Translational Research and Clinical Trial of Division of Rheumatology

See the section of Department of Rheumatology and Allergy, IMSUT Hospital.

## Daigo Group

### 1. Novel therapeutic target discovery for solid cancers

**Yataro Daigo, Atsushi Takano, Koji Teramoto, Hi-**

**detoshi Sumimoto, Yoshinori Murakami, Phung Manh Thang, Kayo Daigo, Tomoyuki Igarashi, Masako Nakamura, Tsevegjav Bayarbat, Zhu Ming, Mbugua Regina Wachuka**

To identify molecules involved in human carcinogenesis and those which could be applied for the development of new molecular therapies and/or biomarkers, we had established a systematic screening system as follows; i) identification of overexpressed genes in the majority of solid cancers (lung, esophagus etc.) by genome-wide screening using the expression microarray in the combination of enrichment of tumor cell populations from cancer tissues by laser microdissection, ii) verification of no or little expression of each of candidate molecules in normal tissues by northern-blot analyses, iii) validation of the clinicopathological significance of its higher expression with tissue microarray containing thousands of archived solid cancers, iv) verification of a critical role of each target gene in the growth or invasiveness of cancer cells by RNAi and cell growth/invasion assays, v) evaluation of their usefulness as targets for passive immunotherapy using specific antibodies and/or as a serum biomarker for solid cancer by high throughput ELISA and proteomics analysis, if they are tumor-specific transmembrane or secretory proteins, vi) screening of the epitope peptides recognized by human histocompatibility leukocyte (HLA)-A*0201- or A*2402-restricted cytotoxic T lymphocyte (CTL) and dendritic cell (DC). This systematic approach identified dozens of molecules that appear to fall into the category of oncoantigens whose overexpression is an important feature of the malignant nature of cancer cells and that have very high immunogenicity to induce antigen-specific CTLs in cancer patients. We further validated these molecules identified as potential targets for the development of antibodies, small-molecular compounds, growth-suppressive cell-permeable peptides, and cancer vaccines that could have a more specific and strong anti-cancer effect with minimal risk of adverse events. During this screening process, we found dozens of candidate molecules to be activated in various solid cancers including lung, esophagus, oral cavity, and breast cancers, as novel prognostic biomarkers and therapeutic targets.

## 2. Development of therapeutic cancer vaccine

**Yataro Daigo, Atsushi Takano, Koji Teramoto, Hidetoshi Sumimoto, Koichiro Yuji, Hiroshi Yasui, Giichiro Tsurita, Kohzoh Imai, Yoshihide Fujiyama, Kazumasa Ogasawara**

Using the systematic screening system shown above, we identified concoantigens which were overexpressed in the majority of cancers derived from lung, esophagus and urinary bladder and essential for the growth and/or survival of cancer cells, as targets for therapeutic cancer vaccine treatment against various solid cancers. We screened dozens of 9- or 10-amino-acid epitope peptides recognized by human HLA-A*0201 and/or A*2402-restricted CTL by enzyme-linked immunospot (ELISPOT) assay. In IMSUT Hospital and its collaborative hospitals, International Conference on Harmonization (ICH)-Good Clinical Practice (GCP)-based clinical study using the combination of some of these peptides derived from oncoantigens in patients with lung cancer is now being conducted. In addition, new type of peptides-pulsed DC vaccination therapy is under development.

## 3. Integrated genomics-based discovery of new biomarkers for cancer immunotherapy

**Yataro Daigo, Atsushi Takano, Koji Teramoto, Hidetoshi Sumimoto, Koichiro Yuji, Hiroshi Yasui, Giichiro Tsurita, Yoshihide Fujiyama, Kazumasa Ogasawara, Yusuke Nakamura**

Immune responses play a critical role in various disease conditions including cancer. Although various immunotherapies are being developed, predictive biomarkers for the choice of effective therapy are urgently required. Using systematic cancer genomics approach on clinical materials obtained from cancer patients treated with cancer vaccine, peptides-pulsed DC vaccination therapy, or immune checkpoint inhibitors, we are clarifying how molecular profiles of cancers can be used to identify biomarkers for predicting clinical outcomes. For example, there has not been a rapid, sensitive, comprehensive, and quantitative analysis method to examine T-cell or B-cell immune responses, therefore we developed a new approach to characterize tumor mutation burdens and T cell receptor (TCR) repertoire by sequencing millions of cDNA of exomes of cancer related genes as well as TCRα and β chains in combination with a newly-developed algorithm. Using samples from lung cancer patients, we are developing detailed information of neoantigen profiles of lung cancer patients and their TCR repertoire. This newly developed next generation sequencing (NGS) platform can be applied to better understand immune responses in many disease areas including immune disorders, allergies, and organ transplantations.

## 4. Detection of neoantigen-reactive T cell clones based on the clonal expansion using next-generation sequencing of TCRβ complementarity-determining region 3

**Yataro Daigo, Hidetoshi Sumimoto, Koji Teramoto, Atsushi Takano**

Development of mechanism-driven biomarkers for immune checkpoint inhibitors in cancer immuno-

therapy requires sensitive and efficacious assays to identify tumor antigen (Ag)-specific T cells. We demonstrated the concept for a sensitive method to determine Ag-reactive T cell clones based on clonal expansion using model neoantigens rather than cytokine production. Sequential increase in T cell clonal frequencies following Ag stimulation was detected by NGS of TCRβ complementarity-determining region 3 (CDR3), with a higher sensitivity than that of ELISPOT assay by 100-fold. The TCRβ CDR3 sequences could represent useful markers to track dynamic changes during immunotherapy. The TCRβ NGS-based method could represent a novel platform both for the development of new biomarkers as well as several therapeutic options.

## 5. Molecular characterization of tumor microenvironment molecules as diagnostic and therapeutic targets

**Yataro Daigo, Koji Teramoto, Hidetoshi Sumimoto, Tomoyuki Igarashi, Atsushi Takano**

Tumor microenvironment is supposed to be involved in tumor progression and drug resistance. To identify molecules that play crucial roles in cancer cells as well as tumor stromal cells such as cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) and apply them for the development of new molecular therapies and/or biomarkers, we are characterizing various immune checkpoint molecules and cytokines in a variety of solid cancer tissues and cell lines using cell-based assays and clinical cancer materials such as tumor tissues and/or blood samples from patients with lung, breast, colon, or ovarian cancers. Studies on molecular pathological role of these molecules are in progress, however, some of them are likely to be associated with malignant potential of cancer cells.

## 6. Clinical significance of PD-L1-positive cancer-associated fibroblasts in pN0M0 non-small cell lung cancer

**Yataro Daigo, Koji Teramoto, Tomoyuki Igarashi**

CAFs are a dominant cell type in tumor stroma and support the generation of pro-tumorigenic microenvironment. CAFs have frequent opportunities to interact with immune cells infiltrating the tumor stroma, but the process remains to be determined. We focused on immune checkpoint mechanism and examined the induction of programmed cell death-ligand 1 (PD-L1) on CAFs by immune cell, and the clinical significance of PD-L1-expressed CAFs in non-small cell lung cancer (NSCLC). PD-L1 mRNA and protein expression on CAFs was upregulated by exogenously supplemented interferon-gamma (IFN-γ) and downregulated through the depletion of IFN-γ. PD-L1 expression on CAFs was upregulated by co-culture with activated lymphocytes releasing IFN-γ. Immunohistochemistry revealed that PD-L1-positive CAFs were observed in 31 of 125 surgically resected pN0M0 NSCLC cases (24.8%). Postoperative relapse-free survival was significantly prolonged in patients with PD-L1-positive CAFs as compared with those with PD-L1-negative CAFs, with 5-year relapse-free probabilities of 84.5% and 66.3%, respectively ($P = 0.031$). Multivariate analysis revealed that PD-L1 expression on CAFs was an independent prognostic factor of longer relapse-free survival after surgery (hazard ratio: 3.225, P = 0.027). PD-L1 expression on CAFs is reversibly regulated by environmental stimuli including IFN-γ from activated lymphocytes. In the non-metastatic NSCLC, PD-L1 expression on CAFs suggests the induction of anti-tumor immune responses, contributing to better prognosis after surgery.

## 7. Identification of lung cancer susceptibility loci by genome-wide association studies.

**Yataro Daigo, Atsushi Takano**

To identify new susceptibility loci associated with lung cancer risk, we imputed data from genome-wide association studies (GWAS) of lung cancer. In our meta-analysis, we are identifying new loci that achieved genome-wide significance, marked by single nucleotide polymorphism (SNP). In addition, we performed Mendelian randomization and pathway analysis of genome-wide association study data from never-smoking Asian women with tuberculosis infection and lung adenocarcinoma. The results extend the catalog of regions associated with lung cancer risk and highlight the potential of genetic susceptibility alleles as a new biomarker for cancer risk prediction and prevention.

## 8. Scientific Platform of Supporting Cohort Study and Biospecimen Analysis

**Yataro Daigo, Atsushi Takano, Koji Teramoto, Kohzoh Imai, Yoshinori Murakami**

To support life science researchers in the field of basic life science, cancer diagnostics and therapeutics, we are collecting cancer and corresponding normal tissues, serum, plasma, and peripheral blood mononuclear cell (PBMC) from patients with solid cancers originated from 30 organs. To date, we collected 55,000 clinical materials. We also constructed tissue microarray system covering about 5000 archived clinical cancers. Using these clinical materials, we are validating the clinicopathological significance of various candidate disease biomarkers as requested by researchers and contributed to their clinical application

and publications in international journals.

### Nagatoishi Group

Various antibodies have been approved for therapeutic use and currently examined in clinical development. Developments and improvements of technology for the discovery and optimization of high-potency antibodies, therefore, have greatly increased to find the specific and stable antibody with desired biological properties. Biophysical analyses of a therapeutic antibody, particularly those of protein interaction and stability, are recognized as one of the critical procedures in the development of biopharmaceuticals, which would be assessed as an essential step to develop next-generation antibodies. The development of analytical methods with quantitative and high-sensitive detection of antigen interaction, protein stability and biological function of antibody, therefore, has been intriguing for the pharmaceutic companies. In this division, we study biophysical analyses of various antibodies to propose a new strategy for the development of the next-generation antibody.

**1. Technical Capabilities and Limitations of Optical Spectroscopy and Calorimetry Using Water-Miscible Solvents: The Case of Dimethyl Sulfoxide, Acetonitrile, and 1,4-Dioxane.**

**Hirano A, Nagatoishi S, Wada M, Tsumoto K, Maluf KN, Arakawa T.**

In drug development, water-miscible solvents are commonly used to dissolve drug substances. Typical routine procedures in drug development include dilution of the stock drug solution into an aqueous solution containing target macromolecules for drug binding assays. However, water-miscible solvents impose some technical limitations on the assays on account of their light absorption and heat capacity. Here, we examined the effects of the dilution of 3 water-miscible solvents, that is, dimethyl sulfoxide, acetonitrile, and 1,4-dioxane, on the baseline stability and signal/noise ratio in circular dichroism spectroscopy, isothermal titration calorimetry, and differential scanning calorimetry. Dimethyl sulfoxide and 1,4-dioxane affect the signal/noise ratio of circular dichroism spectra at typically used concentrations due to their light absorbance. The water-miscible solvents generate interfering signals in the isothermal titration calorimetry due to their mixing heat. They show negative or positive slope in the differential scanning calorimetry. Such interfering effects of the solvents are reduced by appropriate dilution according to the analytical techniques. Because the water-miscible solvents have solubilization capacity for alkyl chain moieties and aromatic moieties of chemicals, drug substances containing these moieties can be dissolved into the solvents and then subjected to the analyses to examine their interactions with target proteins after appropriate dilution of the drug solutions.

**2. Biophysical characterization of the breast cancer-related BIG3-PHB2 interaction: Effect of non-conserved loop region of BIG3 on the structure and the interaction.**

**Chigira T, Nagatoishi S, Takeda H, Yoshimaru T, Katagiri T, Tsumoto K.**

Brefeldin A-inhibited guanine nucleotide-exchange protein 3 (BIG3) interacts with and inhibits the tumor suppressor function of prohibitin-2 (PHB2), and recent in vivo studies have demonstrated that the BIG3-PHB2 interaction is a promising target for breast cancer therapy. However, little biophysical characterization on BIG3 and its interaction with PHB2 has been reported. Here we compared the calculated 8-class secondary structure of the N-terminal domains of BIG family proteins and identified a loop region unique to BIG3. Our biophysical characterization demonstrated that this loop region significantly affects the colloidal and thermodynamic stability of BIG3 and the thermodynamic and kinetic profile of its interaction with PHB2. These results establish a model for the BIG3-PHB2 interaction and an entry for drug discovery for breast cancer.

**3. Structural features of methionine aminopeptidase2-active core peptide essential for binding with S100A4.**

**Katagiri N, Nagatoishi S, Tsumoto K, Endo H.**

Methionine aminopeptidase 2 (MetAP2) is one of the effector proteins of S100A4, a metastasis-associated calcium-binding protein. This interaction is involved in angiogenesis. The region of MetAP2 that interacts with S100A4 includes amino acids 170 to 208. A peptide corresponding to this region, named as NBD, has potent anti-angiogenic activity and suppresses tumor growth in a xenograft cancer model. However, the binding mode of NBD to S100A4 was totally unknown. Here we describe our analysis of the relationship between the inhibitory activity and the structure of NBD, which adopts a characteristic helix-turn-helix structure as shown by X-ray crystallographic analysis, and peptide fragments of NBD. We conducted physicochemical analyses of the interaction between S100A4 and the peptides, including surface plasmon resonance, microscale thermophoresis, and circular dichroism, and performed docking/molecular dynamics simulations. Active peptides had stable secondary structures, whereas inactive peptides had a little secondary structure. A computational analysis of the interaction mechanism led to the design of a peptide smaller than NBD, NBD-ΔN10,

that possessed inhibitory activity. Our study provides a strategy for design for a specific peptide inhibitor against S100A4 that can be applied to the discovery of inhibitors of other protein-protein interactions.

**4. Highly sensitive biomolecular interaction detection method using optical bound/free separation with grating-coupled surface plasmon field-enhanced fluorescence spectroscopy (GC-SPFS).**

**Kaya T, Nagatoishi S, Nagae K, Nakamura Y, Tsumoto K.**

Grating-coupled surface plasmon field-enhanced fluorescence spectroscopy (GC-SPFS) with optical bound/free (B/F) separation technique was developed by employing a highly directional fluorescence with polarization of surface plasmon-coupled emission (SPCE) to realize highly sensitive immunoassay regardless of the ligand affinity. A highly sensitive immunoassay system with GC-SPFS was constructed using a plastic sensor chip reproducibly fabricated in-house by nanoimprinting and applied to the quantitative detection of an anti-lysozyme single-domain antibody (sdAb), to compare conventional washing B/F separation with optical B/F separation. Differences in the affinity of the anti-lysozyme sdAb, induced by artificial mutation of only one amino acid residue in the variable domain were attributed to higher sensitivity than that of the conventional Biacore surface plasmon resonance (SPR) system. The detection limit (LOD; means of six replicates of the zero standard plus three standard deviations) of the GC-SPFS immunoassay with optical B/F separation, was estimated to be 1.2 ng/ml with the low-affinity ligand (mutant sdAb Y52A: KD level was of the order of 10-7 ~ 10-6 M) and was clearly improved as compared to that (LOD: 9.4 ng/ml) obtained with the conventional washing B/F separation. These results indicate that GC-SPFS with the optical B/F separation technique offers opportunities to re-evaluate low-affinity biomaterials that are neither fully utilized nor widespread, and could facilitate the creation of novel and innovative methods in drug and diagnostic development.

**5. An epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout.**

**Eguchi A, Nakakido M, Nagatoishi S, Kuroda D, Tsumoto K, Nagamune T, Kawahara M.**

Upon developing therapeutically potent antibodies, there are significant requirements, such as increasing their affinity, regulating their epitope, and using native target antigens. Many antibody selection systems, such as a phage display method, have been developed, but it is still difficult to fulfill these requirements at the same time. Here, we propose a novel epitope-directed antibody affinity maturation system utilizing mammalian cell survival as readout. This system is based on the competition of antibody binding, and can target membrane proteins expressed in a native form on a mammalian cell surface. Using this system, we successfully selected an affinity-matured anti-ErbB2 single-chain variable fragment variant, which had the same epitope as the original one. In addition, the affinity was increased mainly due to the decrease in the dissociation rate. This novel cell-based antibody affinity maturation system could contribute to directly obtaining therapeutically potent antibodies that are functional on the cell surface.

**6. Exploring designability of electrostatic complementarity at an antigen-antibody interface directed by mutagenesis, biophysical analysis, and molecular dynamics simulations.**

**Yoshida K, Kuroda D, Kiyoshi M, Nakakido M, Nagatoishi S, Soga S, Shirai H, Tsumoto K.**

Antibodies protect organisms from a huge variety of foreign antigens. Antibody diversity originates from both genetic and structural levels. Antigen recognition relies on complementarity between antigen-antibody interfaces. Recent methodological advances in structural biology and the accompanying rapid increase of the number of crystal structures of proteins have enabled atomic-level manipulation of protein structures to effect alterations in function. In this study, we explored the designability of electrostatic complementarity at an antigen-antibody interface on the basis of a crystal structure of the complex. We designed several variants with altered charged residues at the interface and characterized the designed variants by surface plasmon resonance, circular dichroism, differential scanning calorimetry, and molecular dynamics simulations. Both successes and failures of the structure-based design are discussed. The variants that compensate electrostatic interactions can restore the interface complementarity, enabling the cognate antigen-antibody binding. Retrospectively, we also show that these mutational effects could be predicted by the simulations. Our study demonstrates the importance of charged residues on the physical properties of this antigen-antibody interaction and suggests that computational approaches can facilitate design of antibodies that recognize a weakly immunogenic antigen.

**7. Control of Protein Adsorption to Cyclo Olefin Polymer by the Hofmeister Effect.**

**Fujita R, Nagatoishi S, Adachi S, Nishioka H, Ninomiya H, Kaya T, Takai M, Arakawa T, Tsumoto K.**

Cyclo olefin polymer (COP) is an attractive plastic because it has low protein adsorption despite its hydrophobic chemical structure. Here, the adsorption of model proteins to the COP was evaluated in comparison with a representative plastic, polystyrene (PSt), using reflectometry interference spectroscopy (RIfS) technology. The effects of different salts on adsorption were then examined. The adsorption of bovine serum albumin onto COP increased in the presence of kosmotropic salts, whereas adsorption of IgG increased in the presence of chaotropic salts. By contrast, the adsorption of these 2 proteins to PSt was unaffected by these Hofmeister salts. Langmuir-Freundlich model of COP adsorption suggested that the COP surface is more homogeneous for protein binding than the PSt surface. Furthermore, RIfS and sum frequency generation analyses indicated that water molecules bind more weakly to COP than to PSt. Our data propose a novel viewpoint of the way protein binds to COP surface that is different from the way it binds to PSt.

### 8. Affinity Improvement of a Cancer-Targeted Antibody through Alanine-Induced Adjustment of Antigen-Antibody Interface.

**Yamashita T, Mizohata E, Nagatoishi S, Watanabe T, Nakakido M, Iwanari H, Mochizuki Y, Nakayama T, Kado Y, Yokota Y, Matsumura H, Kawamura T, Kodama T, Hamakubo T, Inoue T, Fujitani H, Tsumoto K.**

To investigate favorable single amino acid substitutions that improve antigen-antibody interactions, alanine (Ala) mutagenesis scanning of the interfacial residues of a cancer-targeted antibody, B5209B, was performed based on X-ray crystallography analysis. Two substitutions were shown to significantly enhance the binding affinity for the antigen, by up to 30-fold. One substitution improved the affinity by a gain of binding enthalpy, whereas the other substitution improved the affinity by a gain of binding entropy. Molecular dynamics simulations showed that the enthalpic improvement could be attributed to the stabilization of distant salt bridges located at the periphery of the antigen-antibody interface. The entropic improvement was due to the release of water molecules that were stably trapped in the antigen-antibody interface of the wild-type antibody. Importantly, these effects of the Ala substitutions were caused by subtle adjustments of the binding interface. These results will be helpful to design high-affinity antibodies with avoiding entropy-enthalpy compensation.

### 9. Phospholipid Membrane Fluidity Alters Ligand Binding Activity of a G Protein-Coupled Receptor by Shifting the Conformational Equilibrium.

**Yoshida K, Nagatoishi S, Kuroda D, Suzuki N, Murata T, Tsumoto K.**

The affinity of a ligand for a receptor on the cell surface will be influenced by the membrane composition. Herein, we evaluated the effects of differences in membrane fluidity, controlled by phospholipid composition, on the ligand binding activity of the G protein-coupled receptor human serotonin 2B. Using Nanodisc technology to control membrane properties, we performed biophysical analysis and employed molecular dynamics simulations to demonstrate that increased membrane fluidity shifted the equilibrium toward an active form of the receptor. Our quantitative study will enable development of more realistic in vitro drug discovery assays involving membrane-bound proteins such as G protein-coupled receptors.

## Publications

### Tanaka Group


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**Daigo Group**

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