Exploratory Oncology Research & Clinical Trial Center

Preface

In 2011, our National Cancer Center (NCC) was selected as one of the five designated centers for early/exploratory clinical trials. With budget support from the Japanese Ministry of Health, Labour and Welfare (MHLW), we organized "the Exploratory Oncology Research and Clinical Trial Center" (NCC-EPOC) through the Kashiwa and Tsukiji campuses in 2012, which focused on early/exploratory clinical trials and translational research (TR). The NCC-EPOC was actually activated in April 2013, consisting of a phase I unit in each campus, central/data center function unit for clinical trials, and a translational research (TR) unit. The immunotherapy division was additionally included in the TR unit in July 2013. For innovative oncology drug developments from Japan, the NCC-EPOC has three major missions: to conduct first-in-human (FIH) trials, investigator-initiated trials (IIT) with unapproved agents, and TRs during early clinical studies. The activity in each unit in 2013 is described as below.

1) Phase I unit: The Experimental Therapeutics Department, consisting of several medical oncologists with backgrounds from each organ department, was newly organized in both hospitals in order to conduct all-comer type FIH/phase I studies. A regular weekly teleconference is held to allow the two groups in each hospital to collaborate. In 2013, 8 sponsor-initiated FIH trials have already been conducted in total at both hospitals. The number of the phase I studies in the NCC is ranked as the largest in any academic center in Asia. There were several international phase I studies including an FIH study in 2013.

2) IIT support unit: The central support/data center for IIT has been established with a total of 37 members including a project manager, monitor, data manager, biostatistician, medical writer, and auditors. Since its launch in 2012, the NCC-EPOC has initiated nine registrational IITs in accordance with ICH-GCP (so-called "ishi-syudo chiken") with an unapproved agent. Three of the nine studies have already completed accrual in collaboration with 6 major cancer centers. An additional 4 IITs, including those with new endoscopic instruments, have also been initiated. We have started a nation-wide genome screening program for the RET fusion gene, which was newly discovered in the Research Institute of our center, in patients with non-small cell lung cancer (NSCLC), for accrual to a phase II IIT with a RET inhibitor. This study is the first trial worldwide for RET positive NSCLCs with a large screening program and can be indicated for a similar very small population with driver gene alteration. The registrational IIT for GIST in accordance with a global expanded access program was conducted to improve accessibility for orphan disease. As for the academic seeds development, 10 seeds are being designated for clinical implications including three seeds already under clinical trial. An alliance contract between the NCC and the National Institute of Biomedical Innovation has been formally established for launching a nation-wide oncology seeds collection network. Intellectual property in the NCC is being integrated for efficient new seeds/drugs development.

3) TR unit: In the TR division, several projects with whole exon sequencing in lung, colorectal, and gastric cancers have been conducted to establish molecular epidemiologic data in Japanese patients. A new procedure of companion diagnosis for the RET fusion gene was established and transferred to a laboratory company, which became a basis of the nation-wide genome screening network (LC-SCRUM). Several pharmaceutical companies, who conducted similar new agent development studies for a very small population with driver gene alterations, joined this network under contract with the NCC. A similar screening system for some other driver genes has also started in colorectal and biliary tract cancers, followed by a plan for organizing a nation-wide genome screening academia-industry consortium. This consortium will facilitate new molecular targeting agent development in Japan. A genome-guided individualized therapy system in collaboration with both hospitals named the ABC and TOP-GEAR study has also been started. These studies were conducted with the aim of a better selection of on-going phase I studies for patients who are willing to enter the study. An original sequence panel is also being developed for this study. Another study named the DEF study is now under investigation to make a "cancer encyclopedia" in gastric cancer. Samples from surgically resected specimens are used for establishing human xenograft and primary culture cell lines, followed by genome sequencing analyses. This study will contribute to actual individualized therapy in the future. In the immunotherapy division, a new immune-modulating agent is being developed in collaboration with investigators in Tokyo University. Another project of new immune cell therapy with FITC-CART is also under preclinical investigations. Under the consultation with regulatory authorities, we are planning to incorporate these innovative therapies into clinical studies.

In 2013, the NCC-EPOC conducted various studies for new agent development. For the next step, we are organizing committees to launch organ-specific IIT groups and a nation-wide genome screening network. The goal of the NCC-EPOC is to establish the top innovative academic research organization in the world based on close alliances between academia, industry and government.

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Organization

President:
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Director:
Atsushi Ohtsu

- Phase I Group
  - Department of Experimental Therapeutics
    Chief(Kashiwa): Toshihiko Doi
    Chief(Tsukiji): Noboru Yamamoto
    - Clinical Trial Management Office
      Chief(Kashiwa): Toshihiko Doi
      Chief(Tsukiji): Noboru Yamamoto
- IIT Support Group
  - Clinical Trial Section
    Chief: Akihiro Sato
- Translational Research Group
  - Division of Translational Research
    Chief(Kashiwa): Katsuya Tsuchihara
    Chief(Tsukiji): Takashi Kohno
    - Translational Medicine and Development Section
      Head(Kashiwa): Takeharu Yamanaka
      Head(Tsukiji): Ken Kato
  - Division of Cancer Immunotherapy
    Chief: Tetsuya Nakatsura

Activities of the Divisions

Department of Experimental Therapeutics (Phase I Group)

Toshihiko Doi, Noboru Yamamoto

Overview of the Department of Experimental Therapeutics

The National Cancer Center (NCC)-EPOC Phase I Group has been organized to promote the early drug development especially the first in human (FIH) trial, and in 2012. The phase I group consists of two sub-units (NCCE-Kashiwa & NCC-Tsukiji) which are organized by each hospital. The goal of the NCC-EPOC Phase I Group is to perform an initial clinical evaluation of promising new anti-cancer compounds emerging from the laboratory. Our Phase 1 unit is the largest program in Japan, indeed in Asia, and we contribute to the development of new cancer drugs through early phase trials.

In April 2013, the Department of Experimental Therapeutics was launched to strongly promote the EPOC missions as previously described. The members of the Department of Experimental Therapeutics comprise specialists in their particular oncology fields (Table 1).

Table 1. Staff members in the Department of Experimental Therapeutics (NCC-EPOC)

Kashiwa		Tsukiji	
Name	Area of expertise	Name	Area of expertise
Tosihiko Doi	Gastrointestinal Oncol.	Noboru Yamamoto	Thoracic Oncol.
Kouhei Shitara	Gastrointestinal Oncol.	Kenji Tamura	Breast & Medical Oncol.
Hideaki Takahashi	Hepato-biliary and pancreatic oncol	Yutaka Fujiwara	Thoracic Oncol.
Yoichi Naito	Breast & Medical Oncol.	Shigehisa Kitano	EPOC-TR group
Kiyotaka Yoh	Thoracic Oncol.	Syunsuke Kondo	Hepato-biliary and pancreatic oncol
		Satoru Iwasa	Gastrointestinal Oncol.
		Yuko Tanabe	Breast & Medical Oncol.

Routine activities

This department plays an important role in the development of new anti-cancer drugs in our center as well as all over Japan. The top priority is to conduct the FIH trials, and we also perform the phase I trials for solid tumors (*i.e.*, all-comers). Recently, we joined the global phase I trial to accelerate new drug development in Japan. Web- or tel.-conferences are held with the EU and US sites, and we are discussing patient enrollment as well as the further developmental strategy. Routine web-conference are also held between the Kashiwa and Tsukiji campus every Friday morning, and we are sharing information about adverse events, patient enrollment and are referring candidates to each other to accelerate enrollment.

Research activities

The elucidation of the proof of concept is essential in the development of new anti-cancer drugs, and especially in the early phase we conduct several translational studies in collaboration with the adjoining research institute. In each campus, comprehensive genomic analyses, known as the ABC-study and TOP-GEAR-study in Kashiwa and Tsukiji, respectively, are ongoing to facilitate the patient enrollment for the new molecular targeted drugs under investigation.

Clinical trials

In 2013, 33 phase I trials have been conducted in both campus (Table 2).

Table 2. Phase I trials in the Dept. of Experimental Therapeutics in 2013

No.	Site	Target	FIH	Target	Enrollment in 2013	Status
1	Kashiwa+Tsukiji	CDK4/6		Solid tumors	10	Closed
2	Kashiwa+Tsukiji	CDDP micelles		Solid tumors	1	Ongoing
3	Kashiwa+Tsukiji	CDK4/6		Solid tumors	12	Ongoing
4	Kashiwa+Tsukiji	PD-L1		Solid tumors	5	Ongoing
5	Kashiwa+Tsukiji	FGFR	○	Solid tumors	3	Ongoing
6	Kashiwa+Tsukiji	FGFR	○	Solid tumors	12	Ongoing
7	Tsukiji	PIM	○	Solid tumors	7	Ongoing
8	Tsukiji	PI3K		Solid tumors	4	Ongoing
9	Tsukiji	Anti PRL		Breast & Prostate cancers	3	Closed
10	Tsukiji	PARP		Solid tumors	9	Closed
11	Tsukiji	PI3K		Solid tumors	10	Ongoing
12	Tsukiji	PD-L1		Solid tumors	4	Closed
13	Tsukiji	Hedgehog		Solid tumors	5	Ongoing
14	Tsukiji	PD-L1		Solid tumors	6	Ongoing
15	Tsukiji	CDK4/6		Solid tumors	3	Ongoing
16	Tsukiji	HSP90	○	Solid tumors	0	Ongoing
17	Kashiwa	c-Met		Solid tumors	7	Ongoing
18	Kashiwa	targeting hypoxia		Solid tumors	14	Ongoing
19	Kashiwa	anti-cancer-stem cell		Solid tumors	7	Ongoing
20	Kashiwa	PTK2		Solid tumors	6	Ongoing
21	Kashiwa	FGFR		Solid tumors	1	Ongoing
22	Kashiwa	epirubicin micelles		Solid tumors	2	Ongoing
23	Kashiwa	EGFR		Solid tumors	3	Ongoing
24	Kashiwa	c-Met		Solid tumors	1	Ongoing
25	Kashiwa	*****		Solid tumors	1	Ongoing
26	Kashiwa	TEM-1		Solid tumors	18	Ongoing
27	Kashiwa	PI3K		Solid tumors	5	Ongoing
28	Kashiwa	MEK		Solid tumors	3	Ongoing
29	Kashiwa	c-Met		Solid tumors	7	Ongoing
30	Kashiwa	c-Met		Solid tumors	7	Ongoing
31	Kashiwa	MEK		Solid tumors	4	Ongoing
32	Kashiwa	EGFL7		Solid tumors	1	Closed
33	Kashiwa	MET		Solid tumors	7	Ongoing

FIH: first in human trial

Publications in 2013
Journal

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Clinical Trial Section

Akihiro Sato, Yasutaka Watanabe, Kazushi Endo, Yasuko Nishikubo, Minako Honda, Harumi Nakazima, Rie Ehara, Seiko Kondo, Hiromi Hasegawa, Yoshihiro Aoyagi, Kaori Tobayama, Kyoko furuya, Ayako Sugama, Yuko Tagami, Yushi Nagai, Chika Asami, Miwa Kihara, Sakiko Fushimi, Kaoru Koike, Kashiwa office: Miki Fukutani, Kayo Toyosaki, Noriko Suzuki, Kayoko Ohsumi, Takako Tomisawa, Rieka Yamanaka, Noriko Yamashita, Tamie Sukigara, Ritsuko Nagasaka, Midori Tanaka, Shogo Nomura, Takako Kuwaki, Toshinobu Ishibashi

Introduction

Established in 2008, the Clinical Trial Section supports the Investigator Initiated Clinical Trials (IITs) Program at National Cancer Center Hospital East (NCCHE) through the Clinical Data / Coordinating Center. Our section consults on the development strategy, supports project management and protocol development. The Section consists of 5 groups (CRC for IITs, Data Management, Clinical Trial Management, Audit, and Statistics).

Routine activities

Data management group
- Data base and CRF form design
- Data management
- Central monitoring
- System administration

Clinical Trial management group
- Project management
- Study management
- Site visit monitoring
- Medical writing

Statistical group
- Study design
- Statistical analysis
- Consultation

CRC Group
- Support IITs that are conducted in NCCHE

Audit Group
- Audit
- GCP training

Research activities

CRC Office for IITs
- CRCs, in 2012 supported 32 IITs including a Sponsor Investigator IND trial. A total of 213 patients enrolled in the IITs.

Data Management, Clinical Trial Management, Audit, Statistical Groups
- In 2013 18 IITs were conducted including 9 research IND trials. A total of 187 patients enrolled.

We focused research activities on the clinical trial methodology. We are developing a new EDC system, a sampling source document verification (SDV) method and a comprehensive information sharing infrastructure for early clinical trials.

Division of Translational Research (Kashiwa)

Katsuya Tsuchihara, Sachiyo Mimaki, Hideki Makinoshima, Shingo Matsumoto, Wataru Okamoto, Akiko Nagatsuma, Takayuki Yoshino, Atsushi Watanabe, Kazuyoshi Yanagihara, Yuka Nakamura, Atsushi Ochiai, Takeshi Kuwata, Yasuhiro Matsumura

Introduction

Basic and translational researchers at the National Cancer Center (NCC) Kashiwa Campus are involved in this Division, the aim of which is to develop novel anti-cancer therapeutics as well as to prove their concepts. The unit also closely collaborates with intramural and extramural clinical research teams to develop companion diagnostic systems and identify biomarkers contributing to individualized cancer therapy. The group is carrying out clinical sequencing programs and establishing new cancer cell line panels with multi-omics information to establish a "cancer encyclopedia".

Routine activities

Weekly conferences for the whole division and individual research groups are held with staff scientists and doctors, technical staffs, visiting scientists, and graduate students from the University of Tokyo, Tokyo Medical and Dental University, Keio University and Juntendo University. A monthly tele-conference is held with the Division of Translational Research at Tsukiji campus.

Research activities

Development of Anti-austeric Drugs

Cancer cells in solid tumors frequently encounter a hypoxic and nutrient-deficient microenvironment. Austerity, which is resistance to nutrient starvation, is a characteristic feature of various cancer cells. Since most non-cancerous tissues seldom encounter such nutrient-deficient circumstance, targeting austerity is a promising new strategy for selective cancer treatment. Arctigenin, a major component of *Arctium lappa* (the greater burdock) is one of the anti-austerity compounds identified in this division. Preclinical studies revealed that a crude extract of *Arctium lappa* exhibited anti-austeric abilities. Accompanying with a phase II clinical trial recruiting advanced pancreatic cancer patients, the mechanism of action of arctigenin has been analyzed.

Implication of biomarkers for cancer therapy

To explore more effective genomic biomarkers in anti-EGFR antibody treatment for advanced colorectal cancer, a multi-centered retrospective study combined with whole exon sequencing and copy number variation analyses (BREAC study) is being conducted. Whole exon sequencing of these characteristic anti-EGFR antibody-sensitive and –resistant cases was completed and exploration of potential new biomarkers has been conducted. To clarify the effectiveness and feasibility of multiplex trans-organ pan-cancer genomic biomarker testing, an intramural expert panel has been organized and an ABC study (Analyses of Biopsy samples for Cancer genomics) has been started. More than 180 cases were enrolled by the end of 2013 and the success rate of genetic tests was more than 90%.

Molecular epidemiology of solid tumors

Whole exon sequencing was adopted to clarify the mutation profiles of Japanese lung cancer. Somatic mutations of 55 cases of small cell lung cancer specimens were identified. Largely diverse mutation patterns of individual tumors were exhibited and potential therapeutic targets were identified. Preclinical study models that represent the wide diversity of genetic and epigenetic alterations of solid tumors have been long waited. A DEF (Discovery and Establishment of new biomarkers For gastric cancer) study, which establishes xenograft models and cell lines from primary gastric cancer specimens surgically resected in National Cancer Center Hospital East, was started. More than 90 cases were enrolled in the first 6 months.

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Division of Translational Research (Tsukiji)

Takashi Kohno, Hitoshi Ichikawa, Akinobu Hamada, Shuichi Shinma, Natsuko Hama, Tatsuhiro Shibata, Hiroki Sasaki, Ken Kato, Suga Yamagami, Atsuko Kawami, Kazumi Kanazawa, Nagako Yasuda, Kahori Takeuchi, Yukako Izoe, Sayaka Akimoto, Ayako Iwata, Izumi Kobayashi, Mari Tomoda

Introduction

This Division facilitates early phase clinical trials by conducting translational research (TR) focusing on the development of therapeutic and companion diagnostic seeds and the discovery of biomarkers. The Section of Translational Medicine and Development was established to support TR studies and the National Cancer Center Biobank (NCCBB).

Routine activities

The Section of Translational Medicine and Development has routinely obtained the informed consent to participate as an NCCBB donor from patients who consult the National Cancer Center Hospital (NCCH) for the first time. Clinical research coordinators in this section coordinate the translational research in several ways, such as assistance of registration for clinical trial, logistics of pathological specimen, data collection for case report form and coordination between sections.

Research activities

Clinical sequencing for early phase clinical trials

Through a collaborative work with the Department of Clinical Genomics (Group for Translational Research Support Core), a next generation sequencer-based clinical sequencing system was developed, which enables us to identify genetic alterations, including gene fusions, using FFPE tissue DNAs (Figure 1).

Establishment of molecular diagnosis for FGFR fusion genes in pathological archives

FGFR2 fusion genes identified as a driver and druggable alteration in cholangiocarcinomas by the Division of Cancer Genomics, Research Institute, are a promising new molecular target therapy against this rare and intractable tumor. To facilitate the FGFR-targeted clinical trials including First-In-Human ones in EPOC, we have established a molecular diagnosis procedure to enrich FGFR-fusion positive patients using routine formalin-fixed tissues (Figure 2).

Novel Pharmacokinetic (PK)/Pharmacodynamic analysis for the development of new anticancer agents

Drug exposure and distribution in several tissues impact the pharmacology, toxicology, and efficacy in drug development. However, conventional PK analyses, using HPLC, LC-MS/MS and ELISA, have limitations in providing a comprehensive assessment of exact intra-tumor distribution. A newly developed MALDI (matrix-assisted laser desorption ionization) Mass Imaging system enables us to evaluate concentrations and spatial distributions of anticancer agents and metabolites within target tumor tissues (Figure 3).

Preclinical Studies Using Newly Established Gastric Cancer Cell Lines

Genome-wide genetic information in 770 cancer cell lines is available in the COSMIC database (Sanger Center, UK); however, among them, only 21 cell lines are derived from gastric cancer (GC). We possess 71 GC cell lines including 65 diffuse-type, including 43 newly established by us, and 6 intestinal-type. We have also established a peritoneal metastasis model in mice. In vitro and in vivo preclinical studies of 4 molecular-targeted drugs are being conducted through collaborative studies with two pharmaceutical companies to deriver new therapeutic agents to early phase clinical trial projects in EPOC (Figure 4).

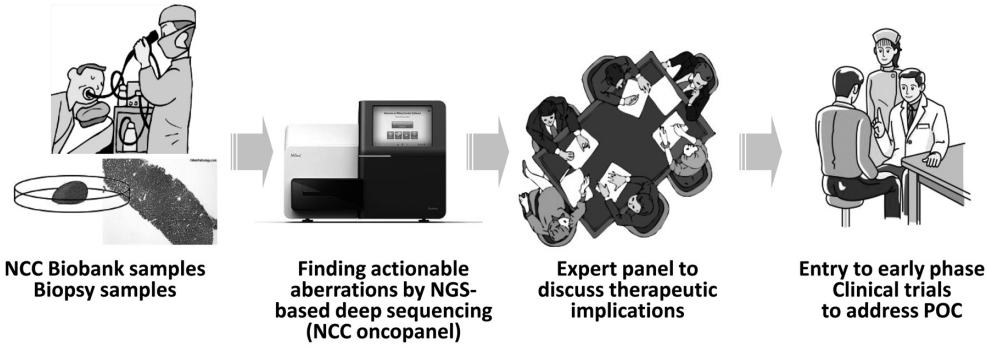
Biobank Support

We explained the purpose and aim NCCBB to 7040 patients from January to December 2013, and received consent on blood collection for research from 6095 patients (86.6% consent rate). We received consent from 6197 patients for research use of their surplus samples (88.0% of consent rate). The patient load with our assistance in filling in the preliminary-diagnosis card and so on was 8,501 (Table 1).

Clinical trials

The TOPICS-1 (Trial of Onco-Panel for Introduction into Clinical Study-Phase 1) study was launched to investigate the feasibility and utility of

this clinical sequencing system to enrich patients for early phase clinical trials based on actionable gene aberrations. We have finished analyses of > 40 patients and found gene aberrations with therapeutic implications in about 50% of the patients. The actionable gene aberrations were discussed in four Expert Panel meetings to subject patients to phase I clinical trials to address "proof-of-concept" of the relationship between gene aberrations and therapeutic effects. Data and samples for this study were managed by the Section of Translational Medicine and Development.



TOPICS-1 (Trial of Onco-Panel for Introduction into Clinical Study-Phase 1)

Figure 1. Clinical sequencing for early phase clinical trials

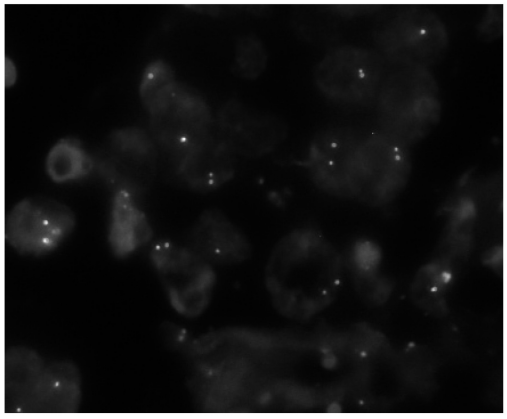
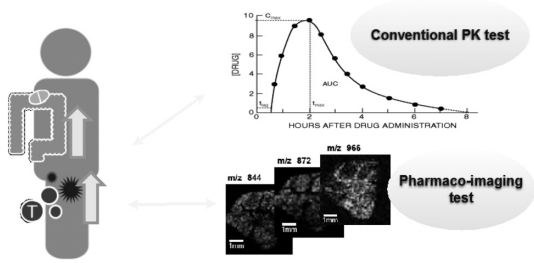


Figure 2. Diagnosis of FGFR gene fusion by FISH



- **Plasma concentration does not represent real drug exposure level.**
- **Imaging Mass spectrometry may provide complementary information to judge an optimal dose and evaluate the proof of concept in the drug development.**

Figure 3. Newly developed Mass Imaging system for PK examination

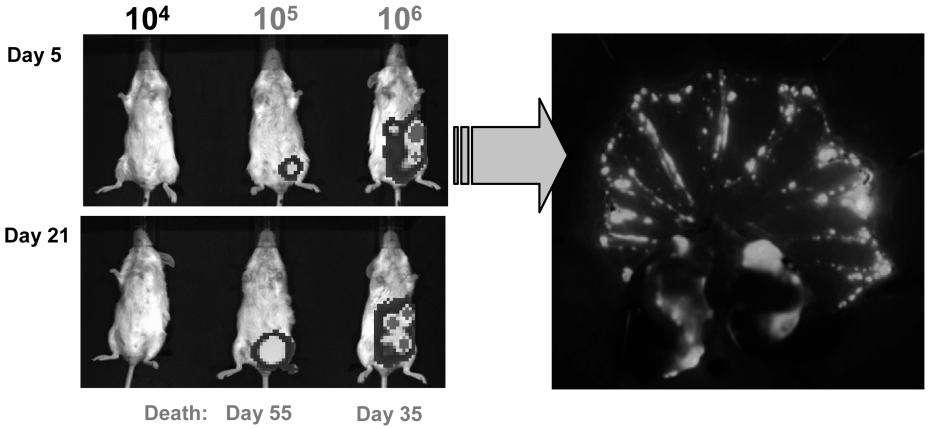


Figure 4. *In vivo* imaging of Peritoneal Metastasis

Table 1. JAN-DEC/2013

Number of patients who were informed about NCCBB	Consent for NCBB for blood collection for research (%)	Consent for NCCBB on research use of the surplus samples (%)	Patient load with our assistance
7,040	6,095 (86.6%)	6,197 (88.0%)	8,501

List of papers published in 2013
Journal

1. Kohno T, Tsuta K, Tsuchihara K, Nakaoku T, Yoh K, Goto K. *RET* fusion gene: translation to personalized lung cancer therapy. Cancer Sci, 104:1396-1400, 2013
2 Shimma S, Takashima Y, Hashimoto J, Yonemori K, Tamura K, Hamada A. Alternative two-step matrix application method for imaging mass spectrometry to avoid tissue shrinkage and improve ionization efficiency. J Mass Spectrom, 48:1285-1290, 2013
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7 Ishii H, Sasaki H, Aoyagi K, Yamazaki T. Classification of gastric cancer subtypes using ICA, MLR and Bayesian network. Stud Health Technol Inform, 192:1014, 2013

Division of Cancer Immunotherapy

Tetsuya Nakatsura, Yuji Heike, Yasushi Uemura, Shigehisa Kitano, Toshiaki Yoshikawa, Keigo Saito, Manami Shimomura, Shoichi Mizuno, Yumi Tokumitsu, Kayoko Shoda, Yukiko Kozaki, Yu Sawada, Kohei Tada, Kaori Kobayashi, Megumi Ozaki, Miki Okazaki, Hikaru Kondou

Introduction

Our Division aims to investigate evidenced-based cancer immunotherapy, repeating basic research and translational research. This Division is focused on developing not only more effective immunotherapeutic strategies but also immunological methods for suppression of recurrence or for cancer prevention.

Research activities

Glypican-3 (GPC3) is overexpressed in human hepatocellular carcinoma (HCC) but not expressed in normal tissues except for the placenta and fetal liver and therefore is an ideal target for cancer immunotherapy. We identified an H2-K^b or H2-D^b restricted and murine GPC3 (mGPC3)-derived cytotoxic T-lymphocyte (CTL) epitope peptide in C57BL/6 (B6) mice, which can be used in the design of preclinical studies of various therapies with GPC3-target immunotherapy *in vivo*. First, 11 types of 9- to 10-mer peptides predicted to bind with H2-K^b or H2-D^b were selected from the mGPC3 amino acid sequence based on the binding score as calculated by the BIMAS software. We evaluated the peptide-binding affinity and confirmed that all peptides were able to bind to H2-K^b or H2-D^b with an *in vitro* cellular binding assay. Subsequently, a mixed peptide vaccine and single peptide vaccine were given to B6 mice to evaluate the immunogenic potential of the 11 selected peptides. Using the splenocytes from peptide-vaccinated mice, interferon (IFN)-γ enzyme-linked immunospot (ELISPOT) assays showed that the mGPC3-$1_{127\text{-}136}$ (AMFKNNYPSL) peptide was the most efficient for inducing CTLs among the 11 peptides. Next, we demonstrated that the mGPC3-1 peptide-specific CTL line could recognize mGPC3-expressing cancer cells, suggesting that the mGPC3-1 peptide was an endogenously presented peptide. In conclusion, we identified mGPC3-1 as an H2-K^b or H2-D^b restricted, mGPC3-derived CTL epitope peptide (1).

Previously, we performed a phase I clinical trial of GPC3 derived peptide vaccination in patients with advanced HCC, and reported that GPC3 peptide vaccination was safe and had clinical efficacy. Moreover, we proposed that a peptide specific CTL response was a predictive marker of overall survival in patients with HCC who were receiving peptide vaccination. We established GPC3 derived peptide-specific CTL clones from the PBMCs of an HLA-A*02:07-positive patient with HCC who was vaccinated with an HLA-A2-restricted GPC3 peptide vaccine and showed a clinical response in the phase I clinical trial. Established CTL clones were analyzed using the IFN-γ ELISPOT assay and a cytotoxicity assay. GPC3 peptide-specific CTL clones were established successfully from the PBMCs of the patient. One CTL clone showed cytotoxicity against cancer cell lines that expressed endogenously the GPC3 peptide. The results suggest that CTLs have high avidity, and that natural antigen-specific killing activity against tumor cells can be induced in a patient with HCC who shows a clinical response to vaccination with the $GPC3_{144\text{-}152}$ peptide (2).

We conducted a subsequent trial in advanced HCC to assess the histopathological findings before and after vaccination with the GPC3 peptide. We present herein on the clinical course and the pathological study including the autopsy of a patient with advanced HCC in the ongoing clinical trial. A 62-year old patient suffering from HCC refractory to sorafenib therapy received the GPC3 peptide vaccine. The patient had fever and remarkably impaired liver function twice after vaccination. Contrast-enhanced CT after the second vaccination showed multiple low-density areas in the liver tumor, indicating tumor necrosis. In contrast, the tumor thrombus in the right atrium increased. The patient discontinued protocol treatment due to disease progression and died 30 days after the second vaccination. An autopsy was performed to determine the main cause of death and to evaluate the antitumor effect of the vaccination. A histological examination showed central necrosis in most of the intrahepatic tumor. The main cause of death was circulatory failure due to a tumor thrombus, which occupied most of the right atrium. An immunohistochemical analysis revealed infiltration of CD8-positive T cells in the residual carcinoma, but not within the cirrhotic area. An *ex*

vivo IFN-γ enzyme-linked immunospot analysis revealed vaccine-induced immune-reactivity against the GPC3 peptide. A histopathological examination at the estimated time of a strong immunological response demonstrated a GPC3 peptide vaccination-induced cytotoxic T-lymphocyte response with an anti-tumor effect (3).

Antigen-specific cancer immunotherapy is a promising strategy for improving cancer treatment. Recently, many tumor-associated antigens and their epitopes recognized by CTLs have been identified. However, the density of endogenously presented antigen-derived peptides on tumor cells is generally sparse, resulting in the inability of antigen-specific CTLs to work effectively. We hypothesized that increasing the density of an antigen-derived peptide would enhance antigen-specific cancer immunotherapy. We successfully demonstrated that intratumoral peptide injection leads to additional peptide loading onto major histocompatibility complex class I molecules of tumor cells, enhancing tumor cell recognition by antigen-specific CTLs. In *in vitro* studies, human leukocyte antigen (HLA)-A*02:01-restricted glypican-$3_{144-152}$ (FVGEFFTDV) and cytomegalovirus$_{495-503}$ (NLVPMVATV) peptide-specific CTLs showed strong activity against all peptide-pulsed cell lines, regardless of whether the tumor cells expressed the antigen. In *in vivo* studies using immunodeficient mice, glypican-$3_{144-152}$ and cytomegalovirus$_{495-503}$ peptides injected into a solid mass were loaded onto HLA class I molecules of tumor cells. In a peptide vaccine model and an adoptive cell transfer model using C57BL/6 mice, intratumoral injection of ovalbumin$_{257-264}$ peptide (SIINFEKL) was effective for tumor growth inhibition and survival against ovalbumin-negative tumors without adverse reactions. Moreover, we demonstrated an antigen-spreading effect that occurred after intratumoral peptide injection. Intratumoral peptide injection enhances tumor cell antigenicity and may be a useful option for improvement in antigen-specific cancer immunotherapy against solid tumors (4).

Clinical trials

We are performing a Phase II study of GPC3 peptide vaccine as adjuvant treatment for HCC after surgical resection or RFA, a phase II study with a GPC3 peptide vaccine in ovarian CCC patients, and a phase I study with a GPC3 peptide vaccine in pediatric cancer patients. We are also performing a Phase I study of a peptide cocktail vaccine for patients with refractory pediatric sarcoma.

List of papers published in 2013
Journal

1. Iwama T, Horie K, Yoshikawa T, Nobuoka D, Shimomura M, Sawada Y, Nakatsura T. Identification of an H2-K^b or H2-D^b restricted and glypican-3-derived cytotoxic T-lymphocyte epitope peptide. Int J Oncol, 42:831-838, 2013
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11. Kondo S, Demachi-Okamura A, Hirosawa T, Maki H, Fujita M, Uemura Y, Akatsuka Y, Yamamoto E, Shibata K, Ino K, Kikkawa F, Kuzushima K. An HLA-modified ovarian cancer cell line induced CTL responses specific to an epitope derived from claudin-1 presented by HLA-A*24:02 molecules. Hum Immunol, 74:1103-1110, 2013

12. Nishimura T, Kaneko S, Kawana-Tachikawa A, Tajima Y, Goto H, Zhu D, Nakayama-Hosoya K, Iriguchi S, Uemura Y, Shimizu T, Takayama N, Yamada D, Nishimura K, Ohtaka M, Watanabe N, Takahashi S, Iwamoto A, Koseki H, Nakanishi M, Eto K, Nakauchi H. Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation. Cell Stem Cell, 12:114-126, 2013

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14. Nakatsura T, Nakamura Y. Chapter21. Immunotherapies for Liver Tumors. In: Yuman F, Jia-Hong D (eds), Hepatobiliary Cancer, People's Medical Publishing House-USA, Ashland Ohio, pp 607-638, 2013

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