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We have been challenging to cure intractable hematological disorders such as leukemia and lymphoma mainly with the aid of hematopoietic stem cell transplantation (HSCT). No less than 40 patients per year receive allogeneic HSCT in our facilities. Based on our achievement as a main hub of HSCT centers in Japan, we greatly contributed to establish the Japan Marrow Donor Program (JMDP) and have been continuously working for JMDP in not only transplantation but also collection of unrelated donor marrows. In recent years, unrelated cord blood has turned to be our major stem cell source in HSCT. Since 1998 we have performed up to 80 cases of unrelated CBT for adult patients, which appears a distinguished experience in the world.

1. Effect of cyclophosphamide on serum cyclosporine levels at the conditioning of hematopoietic stem cell transplantation.

Nagamura F, Asano S.

We retrospectively analyzed the factors that affect serum cyclosporine (CsA) concentrations up to day 14 after allogeneic hematopoietic stem cell transplantation (HSCT). In all, 103 transplant recipients who received MTX and CsA for acute GVHD prophylaxis were analyzed. No significant relationships between serum CsA concentrations and gender, age, serum creatinine levels, AST/ALT levels, or antibiotic/fluconazole administration were found by comparing median CsA concentrations or by using longitudinal or regression multivariate analyses. However, the mean of the median serum CsA concentration in patients (n＝54) receiving the regimen containing cyclophosphamide (CY) (149.7 ng/ml; 95% confidence interval (CI): 132.1 -167.4) was significantly (P＜0.0001) lower than that in patients (n＝49) receiving the non-CY regimen (217.3 ng/ml; 95% CI: 198.9-235.6). Longitudinal analysis and regression multivariate analysis showed that only administration of CY had a significant effect on the serum CsA concentration. Our results suggest that administration of CY during conditioning can reduce the effects on serum CsA concentrations during the 2 weeks following HSCT. The mechanism of this effect is not clear, but it may be due to the autoinduction of CY.

2. Unrelated donor transplants in adults re-

cipients using cord blood compared with bone marrow

Takahashi S, Ooi J, Tomonari J, Iseki T, Uchimaru K, Tojo A, Asano S

Cord blood transplantation from unrelated donor (CBT) has comparable efficacy to bone marrow transplantation from unrelated donor (UBMT) in children and can restore hematopoiesis with acceptable toxicities in adults. We studied the clinical outcomes of 50 adults (median age and weight, 38 years and 55 kilogram) with hematologic malignancies who received unrelated cord blood after myeloabrative chemo -radiotherapy and compared them with 37 recipients (median age and weight, 28 years and 63 kilogram) of bone marrow from unrelated donors in the single institute. We compared hematopoietic recovery, the rates of graft-versus-host disease (GVHD), the risks of transplant-related mortality (TRM) and relapse, and disease-free survival (DFS) using Cox proportional-hazard regression models. All grafts of umbilical-cord blood were mismatched for HLA antigens while 33 out of 37 (89%) bone marrow grafts were HLA matched. Median nucleated cell numbers for transplant were 2.5×10^7 per kilogram in cord blood and 34.0×10^7 per kilogram in bone marrow ($P<0.01$). Multivariate analysis demonstrated slow recoveries of neutrophil (hazard ratio, 0.91; $P<0.01$) and platelet (hazard ratio, 0.25; $P<0.01$) in CBT as compared with UBMT. On the other hand, there was almost comparable in hematopoietic engraftment with longer-term follow up. The overall myeloid engraftment rates were 90% in cord-blood and 100% in bone marrow at day 42. Platelet counts of more than 20×10^9per little at day 120 were 84% and 95%, of more than 50×10^9 per little at day 180 were 82% and 92% in CBT and UBMT, respectively. Although the incidence of acute GVHD in cord blood recipients tended to be lower than in bone marrow recipients, the rates of acute (grades II to IV) and chronic (limited plus extensive type) GVHD were similar in two groups (hazard ratio: 1.13 [$P=0.70$] and 1.26 [$P=0.45$]; respectively). However, the incidence to treat severe acute GVHD with steroid in cord blood recipients was significant lower than in bone marrow recipients (38% vs 83%, $P<0.01$) and there was no GVHD-related deaths in cord blood recipients while 6 out of 19 (32%) in bone marrow recipients. The relapse rate was not significant different (hazard ratio 0.64, $P=0.32$), but TRM and DFS were better in cord-blood recipients (hazard ratio: 0.14 [$P=0.02$] and 0.42 [$P=0.01$]; respectively) in multivariate analysis as follows: The 1-year cumulative incidences of relapse among recipients were 19% (95% CI, 7% to 31%) after CBT and 24% (95% CI, 10% to 38%) with UBMT. The 1-year cumulative incidence of TRM was 4% (95% CI, 0 to 10%) in CBT and 24% (95% CI, 10% to 38%) in UBMT. The 2-year probabilities of DFS were 73% (95% CI, 60 to 86%) after CBT and 49% (95% CI, 33 to 65%) after UBMT. In the multivariate analysis of umbilical-cord blood recipients, the presence of high number of $CD34^+$ cells in the graft was associated with faster neutrophil and platelet recoveries (hazard ratio: 2.04 [$P=0.02$] and 2.17 [$P=0.03$]; respectively). In this study, adult recipients of CBT have improved TRM and DFS compared with UBMT. These results encouraged considering CBT for adult patients with hematologic malignancies which should be needed hematopoietic stem cell transplantation, if they don't have HLA-matched sibling donor.

3. Unrelated cord blood transplantation for adult patients with advanced myelodysplastic syndrome.

Ooi J, Iseki T, Takahashi S, Tomonari A, Tojo A, Asano S.

We report the results of unrelated cord blood transplantation (CBT) for 13 adult patients with advanced MDS. The median age was 40 years, the median weight was 51 kg, and the median number of infused nucleated cells was 2.43×107 /kg. Twelve patients had myeloid reconstitution and the median time to $>5\times108/l$ absolute neutrophil count was 22.5 days. A self-sustained platelet count greater than $50\times109/l$ was achieved in 11 patients at a median time of 49 days. Acute GVHD occurred in 9 out of 12 evaluable patients and chronic GVHD in 8 out of 11 evaluable patients. Ten patients are alive and free of disease at between 171 and 1558 days after transplantation. The probability of disease-free survival at 2 years was 76.2%. These results suggest that adult advanced MDS patients without suitable related or unrelated bone marrow donors should be considered as candidates for CBT.

4. Unrelated cord blood transplantation for adult patients with de novo acute myeloid leukemia.

Ooi J, Iseki T, Takahashi S, Tomonari A, Tojo A, Asano S.

We report the results of unrelated cord blood transplantation (CBT) for 18 adult patients with de novo acute myeloid leukemia (AML). The median age was 43 years, the median weight

was 55.2 kg, and the median number of cryopreserved nucleated cells was 2.51×107/kg. Seventeen patients had myeloid reconstitution and the median time to more than 0.5×109/L absolute neutrophil count was 23 days. A self-sustained platelet count more than 50×109/L was achieved in 16 patients at a median time of 49 days. Acute graft-versus-host disease (GVHD) above grade II occurred in 11 of 17 evaluable patients and chronic GVHD in 14 of 17 evaluable patients. Fourteen patients are alive and free of disease at between 185 and 1332 days after transplantation. The probability of disease-free survival at 2 years was 76.6%. These results suggest that adult AML patients without suitable related or unrelated bone marrow donors should be considered as candidates for CBT.

5. Herpes simplex virus infection in adult patients after unrelated cord blood transplantation.

Tomonari A, Iseki T, Takahashi S, Ooi J, Tojo A, Asano S

Herpes simplex virus (HSV) infection in adult patients who underwent cord blood transplantation (CBT) from unrelated donors was studied. None of 9 HSV seronegative patients developed HSV disease after CBT. Of 28 HSV-seropositive patients, 7 (25%) developed HSV disease at a median of 92 days after CBT (range, 52 to 239 days). The cumulative incidence of HSV disease in HSV-seropositive patients was 27% at 12 months after CBT. The manifestations of HSV disease included gingivostomatitis (three patients), herpes labialis (two patients), localized herpes facialis of the nose (one patient), and disseminated eczema herpeticum (one patient). HSV disease recurred in two patients as gingivostomatitis and disseminated eczema herpeticum. All the patients responded to antiviral therapy. The presence of grade II-IV acute graft-versus-host disease (GVHD) was significantly associated with a higher rate of HSV disease after CBT (51% vs. 8%, P=0.015). These results suggest that the recovery of HSV-specific immune responses is delayed in patients who develop grade II-IV acute GVHD after CBT.

6. Varicella-zoster virus infection in adult patients after unrelated cord blood transplantation.

Tomonari A, Iseki T, Takahashi S, Ooi J, Tojo A, Asano S

Varicella-zoster virus (VZV) infection in 40 adult patients who underwent cord blood transplantation (CBT) from unrelated donors was studied. Twenty-five patients developed VZV reactivation at a median of 5 months after CBT (range, 1.7 to 26). The cumulative incidence of VZV reactivation after CBT was 80% at 30 months. Twenty-two patients developed localized herpes zoster. The remaining 3 patients developed atypical nonlocalized herpes zoster, and 1 patient accompanied with visceral dissemination. All the patients responded well to antiviral therapy. Unexpectedly, the absence of grade II-IV acute graft-versus-host disease (GVHD) was associated with a higher rate of VZV reactivation after CBT (100% vs 55%, P=0.01). These results suggest that recovery of VZV-specific immune responses after CBT is delayed even in patients without severe acute GVHD.

7. Cytomegalovirus infection following unrelated cord blood transplantation for adult patients.

Tomonari A, Iseki T, Ooi J, Takahashi S, Tojo A, Asano S.

Cytomegalovirus (CMV) infection in 28 adult patients after cord blood transplantation (CBT) from unrelated donors was compared with that after bone marrow transplantation from HLA-matched related (R-BMT) and unrelated (U BMT) donors. Positive CMV antigenemia was seen in 19 (79%) of 24 CMV seropositive patients at a median of 42 days (range, 29 to 85) after CBT, but in 0 of 4 CMV-seronegative patients. This probability did not differ significantly from those after R-BMT and U-BMT (66%, P=0.22 and 60%, P=0.15, respectively). Based on the antigenemia results, 16 patients (67%) received pre-emptive ganciclovir therapy from a median of 47 days (range, 36 to 67) after CBT. This probability was higher than that after R-BMT (28%, P=0.0048), but did not differ from that after U-BMT (50%, P=0.21). In addition, the probability of requiring more than 2 courses of ganciclovir therapy after CBT (21%) was higher than those after R-BMT and U-BMT (0%, P=0.015 and 0.039, respectively). One patient (5%) developed CMV disease after U-BMT, while no patients developed CMV disease after CBT or R-BMT. CMV serostatus, use of steroid, and HLA disparity affected the probability of requiring ganciclovir therapy after CBT (P=0.024, 0.032, and 0.017, respectively). These results suggest that recovery of CMV specific immunity after CBT is delayed when compared with BMT.

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Department of Infectious Diseases and Applied Immunology (DIDAI) was founded in 1981. In 1986, clinic for patients with human immunodeficiency virus (HIV) infection was opened by former professor, K. Shimada. In 2003, approximately 200 patients with HIV infection visit the outpatient clinic on a monthly basis, and 5-7 beds for HIV-infected patients in the in-patient ward are usually occupied. Since the number of the staff members of DIDAI is too small to care both outpatients and in-patients, members of the Division of Infectious Diseases (DID) and the Division of Clinical Immunology of the Advanced Clinical Research Center join the clinic. Supported by clinicians of three department and divisions, basic scientists of immunology and virology in DID, and dedicated medical and paramedical stuffs, IMSUT hospital provides the most up-to-date medical treatment to HIV-infected patients in Japan. DIDAI is also a treatment center for international infectious diseases such as malaria and typhoid fever.

1. Treatment of and clinical research on HIV-infection and related diseases

Tetsuya Nakamura, Takashi Odawara[1], Takeshi Fujii[1], Tokiomi Endoh[1], Jun-ichi Takeda[1], Fuyuaki Ide[1], Takeshi Matsumura[1], Hitomi Nakamura[1], Miou Sato[1], Mieko Goto[1] and Aikichi Iwamoto[1]: [1]Department of Infectious Diseases

a. Treatment of HIV infection in IMSUT hospital

i) Statistical characteristics of HIV-infected patients in IMSUT hospital this year

Thirty-five new patients with HIV-1 infection visited our hospital this year, and as of the end of this year, 207 patients in total are under medical management in our outpatient clinic. As shown in the figure 1, the number of total patients declined in 1997 because a part of patients as well as medical stuffs were moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again with somewhat exponential curve after 1998 in accordance with Japanese statistics of HIV-infected patients. In contrast, the number of admission has decreased since 1997 because of the introduction of highly active anti-retroviral therapy (HAART) which effectively suppresses the replication of HIV. After one year of HAART, the viral loads become undetectable in more than 90% of patients, and their CD4 counts increase by approximately 200/microL in average (Figure 2). Consequently, the clinical management of HIV-infected patients changed from how to treat opportunistic infections into how to control patients with HAART.

Figure 1. Total number of HIV-infected patients who visit IMS Hospital

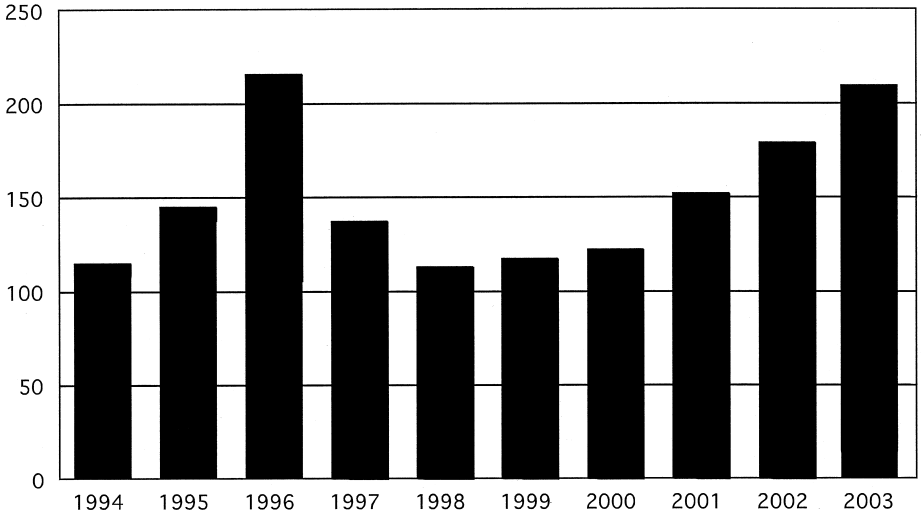
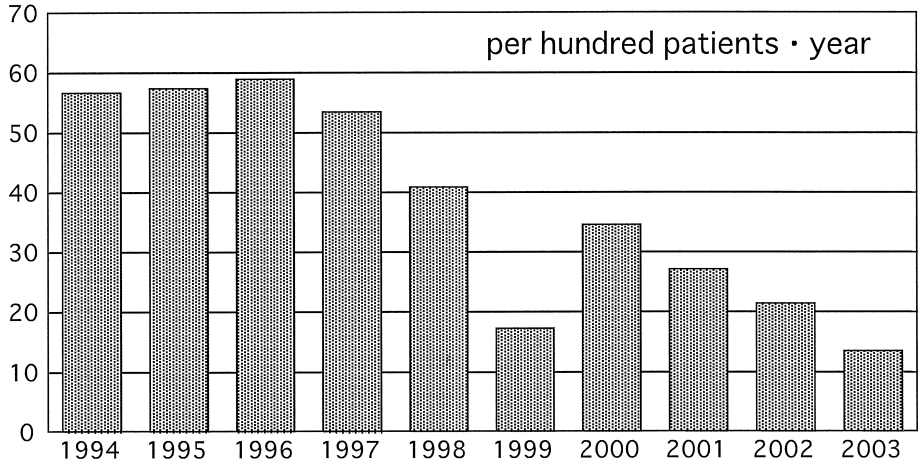


Figure 2. Total number of HIV-infected patients in addmission



ii) Specific immune therapy for human immunodeficiency virus followed by structured treatment interruption of antiretroviral therapy (Phase I study).

Great improvement has been achieved in antiretroviral therapy for HIV infection after introduction of highly active antiretroviral therapy (HAART) using three or more anti-HIV drugs. HIV-infected patients now have better survival and frequency of admission for serious opportunistic infection is decreasing (Figure 2). However, it is estimated to take more than 60 years of treatment for eradication of HIV from patients, which means that they have to continue HAART for all over their lives. Long term HAART deteriorates patients' quality of life and causes adverse effects including metabolic abnormalities.

To overcome these problems, we started a clinical trial to interrupt HAART after specific immune therapy to HIV-infected patients. We previously reported that HIV-specific immunity had quantitative and qualitative abnormality in HIV-infected patients. Consequently, if HAART is stopped, HIV quickly starts to replicate and host immunity cannot stop the proliferation. However, if HIV-specific immunity is recovered by HIV vaccine in patients who are in good viral control under HAART, HIV replication may be partially suppressed by host immunity, even after interruption of HAART, to the level that does not decrease CD4+ T cells. To test this hypothesis, we planned a phase I study: Specific immune therapy for human immunodeficiency virus followed by structured treatment interruption of antiretroviral therapy, where dendritic cells which are derived from patients and pulsed with HIV epitope peptides are used as HIV vaccine. As of end of 2003, 4 patients were enrolled, and two patients finished vaccination and are now under treatment interruption.

b. Clinical research on Infectious Diseases

i) Molecular Analysis of Human Herpesvirus

8 Using Single Nucleotide Polymorphisms in Open Reading Frame 26 (ref. 5)

Human herpesvirus 8 (HHV8) can be classified into distinct subtypes by the sequence polymorphism in several open reading frames (ORFs). We analyzed the subtypes of HHV8 in 59 HIV-infected Japanese patients using polymorphism of ORF26, and found that over two thirds HHV8 fell into major subtype A. We also found that single nucleotide polymorphisms (SNPs) at nucleotide positions 1032 (C to A substitution) and 1055 (G to T substitution) in HHV 8 ORF26 are correlated with increased susceptibility to Kaposi's sarcoma (KS) as compared to HHV8 with wild type nucleotides at these positions ($p=0.0106$). This observation suggests that molecular heterogeneity of HHV8 genome affects the biological properties of HHV8, resulting in different clinical phenotypes of HHV8 infection. Since sensitive PCR of ORF26 allows us to analyze the SNPs using peripheral blood of HHV8-infected patients, ORF26 SNPs will be a potent tool to investigate the pathogenesis of HHV8 infection.

ii) Current Concept of SARS Treatment

Severe acute respiratory syndrome (SARS) is a newly emerging, a readily transmissible, and a predominantly pneumonic disease caused by a novel coronavirus, called as SARS coronavirus (SARS-CoV). It was first appeared in Guangdong Province, China in November 2002 and has rapidly spread to total 29 countries all over the world since late February 2003. This outbreak affected 8,098 individuals resulting in 774 deaths (mortality rate: 9.6%) by 31 July 2003, and drawn enormous attention and fears worldwide. The World Health Organization (WHO) declared the termination of worldwide SARS outbreak in July.

Numerous articles on SARS have been published internationally that described its epidemiology, etiology, diagnosis, clinical features, and management. Much has been learned about SARS during this several months, however many questions remain unanswered. Among other things, the treatment of SARS has remained largely anecdotal and so far no treatment consensus has been reached, since randomized controlled treatment trials were understandably not possible during the outbreak of this novel acute disease. Until we get efficacious vaccines and specific anti-SARS-CoV agents, SARS might remain a major health threat to the world.

We have not experienced SARS outbreak in Japan and could not have any domestic information about SARS treatment. Thus, we visited to Tan Tock Seng Hospital in Singapore and Hospital and Sunnybrook and Women's College Health Centre in Toronto, where many SARS patients were treated. We asked the physicians in charge about their impression on the effectiveness of various SARS treatment and got some responses: it is difficult to make any established treatment protocol at this time, they didn't find any clinical benefit of ribavirin and have no intention of use it by now, and steroids treatment should be utilized with consideration of disease severity because some patients only have self-limiting disease.

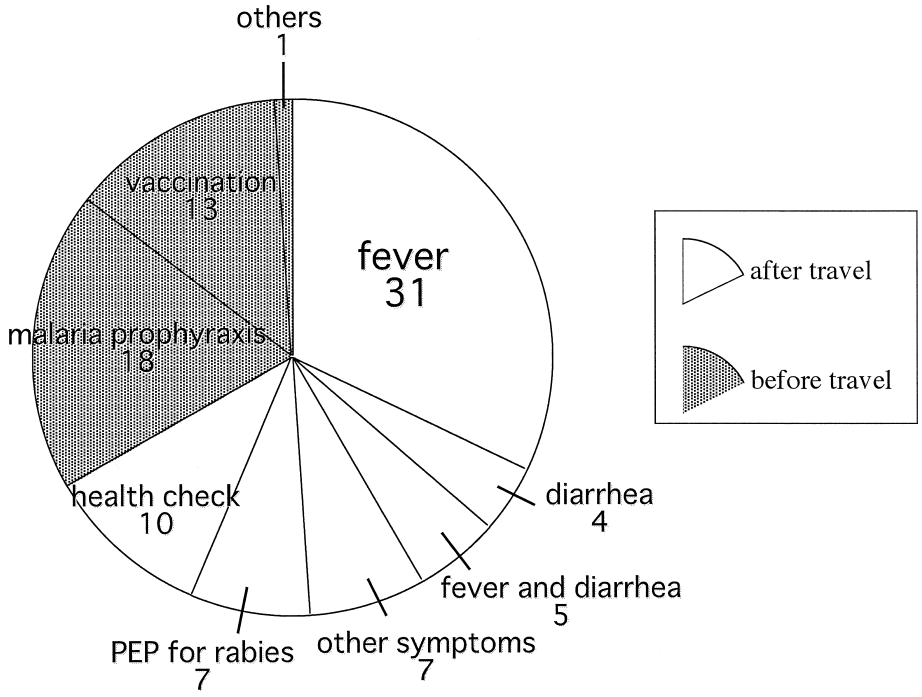
While awaiting the development of vaccines and new drugs specific for SARS, or the results of well-conducted randomized controlled studies on a sufficient number of cases, we have to rely on the existing treatment modalities, which have been overviewed in ref. 16.

2. Diagnosis and Treatment of Tropical Diseases

Tetsuya Nakamura, Takashi Odawara[1], Takeshi Fujii[1], Tokiomi Endoh[1], Jun-ichi Takeda[1], Fuyuaki Ide[1], Takeshi Matsumura[1], Hitomi Nakamura[1], Miou Sato[1], Mieko Goto[1] and Aikichi Iwamoto[1]: [1]Department of Infectious Diseases

This year, 96 patients visited our clinic for treatment or consultation of tropical diseases. Figure 3 shows reasons for their visits to our clinic. Among 96 patients, we diagnosed and treated 17 cases of tropical infections including 13 malaria (8 Pv, 2 Po, 1 Pf and 2 unclassified), 1 giardiasis, 1 typhoid fever, 1 paratyphoid fever, and 1 suspected case of Chikungunya virus infection. Since Ministry of Health, Labor and Welfare approved mefloquine for prophylaxis of malaria in September 2001, the number of people who plan to travel to areas endemic of malaria and visit IMSUT Hospital for mefloquine prescription is increasing. We not only have treated patients with tropical diseases but also have accepted consultations via telephone and E-mails from people who travel in tropical areas. We will continue this consultation activity in addition to mefloquine prescription as prophylaxis of malaria in outpatient clinic.

Figure 3. Reasons for visits to travel medicine clinic ('2003)



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Takeshi Fujii, Tetsuya Nakamura, Aikichi Iwamoto. Current Concept of SARS Treatment. J Infect Chemother. in press.

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Our major goal is to cure children suffering form a variety of life-threatening hematological disorders. Attempting to achieve it, we continue the commitment to treatment and follow-up care of such children, and to clinical and laboratory research that ultimately will help us devise better therapeutic approaches to these diseases. Currently efforts are directed toward hematopoietic stem cell transplantation including ex vivo expansion of human hematopoietic stem cells, gene therapy, immunotherapy and analysis of pathogenesis of hematopoietic disorders.

1. Hematopoietic stem cell transplantation for children with high-risk leukemia

Hirohide Kawasaki, Daisuke Hasegawa, Yoshitoshi Ohtsuka, Toshihisa Tsuruta, Atsushi Manabe, Kohichiro Tsuji

Although a standard regimen in hematopoietic stem cell transplantation (SCT) has been available for children with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), it has not been standardized for those with rare diseases including congenital bone marrow failure syndrome (CBMFS) and natural killer cell leukemia. A multi-institutional trial using regimens with a rationale should be proposed in a prospective manner. For CBMFS, we conducted in vitro and in vivo assays to assess the sensitivity of leukemic cells to G-CSF and transplanted 3 patients whose leukemic cells had a high sensitivity to G-CSF using a regime including G-CSF. Thus, we could avoid intensive chemotherapy before SCT for patients with a vulnerable normal bone marrow reserve. For patients with Fanconi anemia, we employed a regimen containing fludarabine to reduce the dose of alkylating agents and irradiation to avoid the toxicity, which was otherwise likely to occur in those patients. For patients with NK cell diseases, we used a regimen combining alkylating agents (cyclophosphamide and thiotepa) and total body irradiation based on the results that NK leukemic cells strongly expressed multidrug-resistant genes in 2 patients. Now we plan to extend our experience in nationwide collaborative studies.

2. MxA expression as a specific marker for viral infections after allogeneic stem cell transplantation

Tetsu Yoshimasu, Atsushi Manabe, Yasuhiro Ebihara, Ryuhei Tanaka, Kohichiro Tsuji

Many patients suffer febrile diseases soon after allogeneic stem cell transplantation (SCT). The symptoms of viral infections and acute graft -versus-host disease (aGVHD) after allogeneic SCT are similar and often difficult to distinguish. However, an accurate diagnosis is important since the treatments for these diseases are very different. It is known that MxA protein is

specifically induced in patients with several viral infections. We investigated the cytoplasmic expression of MxA in the peripheral blood lymphocytes of patients with fever after receiving allogeneic SCT, using a newly generated monoclonal antibody (KM1135) and flow cytometry. The level of MxA expression was significantly higher in patients diagnosed with viral infections (n=6, cytomegarovirus in 3, Epstein-Barr virus in 1, human herpesvirus-6 in 1, adenovirus in 1) than control individuals (n=9) ($p<0.05$, Student's t test). The level of MxA in patients with aGVHD (n=7) was identical to that in controls. Of note, it was demonstrated that the level of MxA well correlated with the amount of the cytomegalovirus antigen-positive cells in the presence of aGVHD in 2 patients. The measurement of MxA is simple and useful in distinguishing viral disease from other conditions including aGVHD after allogeneic SCT.

3. Cooperative clinical trial for pediatric myelodysplastic syndrome (MDS)

Atsushi Manabe, Hirohide Kawasaki, Daisuke Hasegawa, Yoshitoshi Ohtsuka, Toshihisa Tsuruta, Kohichiro Tsuji, Tatsutoshi Nakahata[1]: [1]Department of Pediatrics, Kyoto University

Pediatric MDS is a rare disease and only 50-100 children under the age of 16 suffer from the disease annually. The diagnosis and treatment has not been standardized and it should be determined in a nationwide manner. The MDS committee of the Japanese Society of Pediatric Hematology began the pathologic central review in 1999 and we reviewed all the samples of patients suspected of having MDS. At present, over 200 patients have been enrolled and standard diagnostic criteria have been proposed for juvenile myelomonocytic leukemia (JMML), a subset of MDS. We also tested in vitro cell growth for patients with JMML using diagnostic samples. The results showed that spontaneous growth and hypersensitivity to GM-CSF were observed in most children with JMML. We proposed a cooperative trial to establish the treatment for MDS (MDS99) and have enrolled over 50 patients from the whole country.

4. Molecular pathogenesis of pediatric myelodysplastic syndrome (MDS) and myeloproliferative disease (MPD)

Daisuke Hasegawa, Yoshitoshi Ohtsuka, Hirohide Kawasaki, Sumiko Watanabe[2], Kohichiro Tsuji, Takayuki Yamashita[3], Atsushi Manabe: [2]Division of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, [3]Division of Genetic Diagnosis (2), Institute of Medical Science, University of Tokyo

Pediatric MDS and MPD are very rare disorders. The diseases are commonly seen in elderly patients. It suggests that the pathogenesis of the diseases in children be of germline origins rather than of acquired process. In fact, germline mutations have been elucidated in a large proportion of pediatric MDS and MPD: GATA1 mutations in patients with MDS and Down syndrome; FANC mutations in those with MDS and Fanconi anemia; PTPN11 mutations or NF1 mutations in those with juvenile myelomonocytic leukemia (JMML). We are examining the PTPN 11 mutations in the diagnostic samples of children with MDS and MPD. We are also testing the epigenetic abnormalities. So far, over 30 samples were tested. A hypermethylation of cell cycle regulators such as p15 and p16 was observed in a substantial proportion of patients with MDS whereas it was present in virtually no cases with JMML. Finally, we will conduct a micro array analysis in patients with MDS and their parents in cooperation with the Japanese Society of Pediatric Hematology.

5. Prolactin (PRL) enhances the differentiation of erythroid progenitors from hematopoietic stem cells in Diamond-Blackfan anemia (DBA)

Hirohide Kawasaki, Yukihiro Noda[4], Takahide Nakano[4], Kohichiro Tsuji: [4]Department of Pediatrics, Kansai Medical University

DBA is a rare congenital disorder of erythropoiesis characterized by reticulocytopenia and macrocytic anemia. In colony assays of bone marrow cells of patients with DBA, colony formation of erythroid progenitors; erythroid colony-forming units (CFU-E) on day 7 and erythroid burst-forming units (BFU-E) on day 14, is usually defective or significantly impaired. Recently, Abkowitz et al. reported the results of a clinical trial, which studied the effect of PRL, via metoclopramide treatment, on the proliferation of erythroid progenitor cells in patients with DBA. We studied the effect of PRL on the proliferation and differentiation of erythroid progenitor cells from $CD34^+$ cells derived from two patients with DBA. Hemoglobinized BFU-E were formed from $CD34^+$ cells from each subject with a combination of SCF, IL-3, and EPO. Erythroid colony formation was enhanced by adding PRL to the mixture of SCF, IL-3, and EPO in a statistically significant manner. However, we failed to see any clinical effect of PRL in Patient 1 when we used metoclopramide. We

will further explore the possibility of the utility of the drug for patients with DBA, comparing the in vitro analysis.

6. Cooperative clinical trial for Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL) in children

Atsushi Manabe, Hirohide Kawasaki, Keizo Horibe[5]: [5]Department of Pediatrics, National Nagoya Hospital

Ph^+ ALL constitutes only 3-5% of ALL in children; however, its prognosis is known to be very poor despite of contemporary multiagent intensive chemotherapy. The meta-analysis of over 300 children with Ph^+ ALL demonstrated the efficacy of allogeneic hematopoietic stem cell transplantation (SCT) from matched sibling donor. Imatinib mesylate was recently produced as a specific tyrosine kinase inhibitor for bcr-abl fusion gene product in chronic myelogenous leukemia. Because the number of children with Ph^+ ALL is small, a nationwide trial for the disease is mandatory. On behalf of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG), we propose a trial, which employs intensive chemotherapy and a new drug, imatinib mesylate, to maintain a remission status, followed by allogeneic SCT at the 8th month after the diagnosis. This is a phase II study to evaluate the efficacy of imatinib mesylate. The efficacy will be assessed with molecular quantification techniques (qualitative and quantitative real-time PCR method). The toxicity of the drug will be monitored and graded by the criteria of NCI-CTC VER2.0. At present, the ethical committee of the Japanese Society of Pediatric Hematology is assessing the protocol. We expect that the study will open early in 2004.

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The goal of our team is to:
1) Improve the standards of treatment of allergic and immunological disorders
2) Promote research in the field of allergy and clinical immunology and develop novel theraputic procedures.
We participate in cutting edge studies of novel treatments for autoimmune, rheumatic and allergic diseases. In addition to conventional drug studies aimed to improve the efficacy and safety of current therapies, we are going to carry out experimental protocols of particular interest for patients not responding to conventional therapy. For these purposes, we have vigorously been collaborating with the Division of Clinical Immunology (Prof. Chikao Morimoto).

I. Therapeutically targetting transcription factors

Noritada Yoshikawa, Yuichi Makino, Hirotoshi Tanaka, et al., Rheumatology Clinic: Department of Rheumatology and Allergic Diseases

We are interested in the mechanism of eukaryotic gene expression and development of novel therapy and/or drug which target transcriptional machineries. For this purpose, our recent work is mainly focused on conditional regulation of transcription factors including the glucocorticoid receptor and hypoxia-inducible factor-1α.

a. Glucocorticoid receptor project

Glucocorticoid hormones are effective in controlling inflammation, but the mechanisms that confer this action are largely unknown. It has been shown that both positive and negative regulation of gene expression are necessary for this process. The genes whose activity is negatively modulated in the anti-inflammatory process code for several cytokines, adhesion molecules. Most of them do not carry a classical binding site for regulation by the glucocorticoid receptor (GR), but have instead regulatory sequences for transcription factors such as AP-1 or NF-κB. Considering various severe side effects of glucocorticoids, it may be pharmacologically important to dissociate these negative regulatory function of the GR from induction of metabolic enzymes, gene expression of which has been shown to be positively regulated by the GR. We propose that a certain class of compounds (surprisingly, some of them are non-steridal chemicals) may dissociate transactivation and transrepression function of the GR and offer opportunities for the design of such compounds that could function more effectively as antiinflammatory drugs. In this line, we are developing the strategy for identification of novel therapeutic strategy.

(i) Development of dissociating ligand for the glucocorticoid receptor

The GR function could be differencially regulated by ligands. We have recently shown that not only synthetic glucocorticoids but also certain bile acids could differentially modulate GR function. Moreover, the effects of those compounds are indicated to be ascrived to the ligand binding domain of the receptor. In this line, we are going to isolate the dissociating ligand that preferencially promotes transrepression function of the GR.

On the other hand, receptor specificity is another important aspect of novel GR regulator. In this line, we have shown that cortivazol is extremely specific for GR and does not bind to MR. We are studying the molecular basis for this receptor specificity of the ligand using cortivazol as a model.

(ii) Molecular biology and clinical application of a novel protein HEXIM1

We have recently cloned the cDNA encoding a novel protein HEXIM1, expression of which is induced by treatment of vascular smooth muscle cells with a differentiation inducer hexamethylane bisacetamide. We showed that HEXIM1 is a nuclear protein and represses NF-κB-dependent transcription. Since NF-κB plays a pathological role in smooth muscle cell proliferation, our study will not only unveil pathogenesis of but also contribute to therapy of atherosclerotic vascular disorders. Recently, we have also found that HEXIM1 participates in transcriptional regulation in various fashion. For example, HEXIM1 interacts with those factors related to basal transcription and splicing. Moreover, HEXIM1 interferes GR action via locking GR into a distinct compartment in the nucleus. These studies will open up a door for novel therapy for a variety of diseases.

b. Hypoxia-inducible Factor (HIF)-1α project

HIF-1 α is essential for not only angiogenesis but also development of certain organs. In this line, molecular biology of HIF-1 α will provide us possible advantage to characterize and manupilate such processes. Angiogenesis is regulated by a combination of variety factors including transcription factors. Recently, we have isolated cDNA encoding the novel protein IPAS which can squelch HIF-1 α. Its tissue-specific expression argues the physiological role of transcriptional network for orchestrated regulation of angiogenesis. We are currently studying the molecular mechanism of the interaction between HIF-1 α and IPAS. This negative regulator may also therapeutically applicable for treating a number of angiogenic disorders including cancer, diabetic retinopathy, and rheumatoid arthritis. Moreover, we have recently shown that IPAS is a splice variant of HIF-3 α, and its mRNA expression is enhanced under hypoxic conditions. This conditional regulation of splicing is our current interest.

On the other hand, we have recently identified that HIF-1 α function is regulated in a various fashion in certain physiological settings, which may be important of homeostatic control of tissue function. In this line, we are now identifying the molecular mechanism for such regulation of HIF-1 α.

II. Study on serum soluble CD26 in patients with systemic lupus erythematosus

Osamu Hosono, et al., Rheumatology Clinic: Department of Rheumatology and Allergic Diseases, Chikao Morimoto, et al., Division of Clinical Immunology

CD26 is the cell surface activation antigen with dipeptidyl peptidase IV (DPPIV) enzyme activity that is preferentially expressed on memory T cells and has a role in T cell immune responses. The soluble form of CD26 is present in serum and recombinant soluble CD26 can enhance in vitro antigen-specific T cell responses. Serum levels of sCD26 and its specific DPPIV activity were significantly decreased in SLE and were inversely correlated with SLE disease activity index score, but not with clinical variables or clinical subsets of SLE. Serum levels of sCD26 may be involved in the pathophysiology of SLE, and appear to be useful as a new disease activity measure for SLE.

III. Basic and clinical immunology of chamokine/chemokine receptors

Hiroshi Kawasaki, Osamu Hosono, Hirotoshi Tanaka, et al., Rheumatology Clinic: Department of Rheumatology and Allergic Diseases, Chikao Morimoto, et al., Division of Clinical Immunology

a. Study on chemokine receptor function in synovial fluid T cells from patients with rheumatoid arthritis

To study expression and function of the chemokine receptor CCR5 in synovial fluid (SF) T cells from patients with rheumatoid arthritis (RA). SF T cells showed an increase in the popu-

lation of CCR5, CXCR4, and CD45RO positive cells and exhibited an increase in chemotactic activity, which was not augmented with RANTES but stromal cell-derived factor-1alpha. Tyrosine phosphorylation per CasL molecule was markedly enhanced in SF T cells. In H9 cells, tyrosine phosphorylation of not only focal adhesion kinase but also CasL was induced after treatment with RANTES. Downmodulation of CCR5 by RANTES was decreased and recycling of CCR5 was accelerated in SF T cells when compared with peripheral blood (PB) T cells. When CD45RO positive PB T cells were cultured with interleukin 2, blunted responsiveness to RANTES-induced chemotaxis was reproduced as well as spontaneous chemotaxis, increased expression of CCR5, and aberrant receptor dynamics, after RANTES stimulation as observed in SF T cells. Synovial fluid T cells highly positive for CCR5 show aberrant characteristics; resistant to RANTES in terms of migration, but responsive in terms of dynamics of CCR5.

b. Structure and functional analysis of human chemokine/chemokine receptor system and identification of their roles in innate and acquired immune system

We have been pursuing the structure and functional analysis of human chemokine/chemokine receptor system in order to clearly identify their roles in innate and acquired immune system. We previously showed that CCR-1 and CCR-3 are two of the main regulators in the induction of allo-immune response. Furthermore, we have shown that the responsiveness of CCRs is intricately controlled by a panel of small GPTases. Of note was that chemokines were powerful mediators of activation of dendritic cells in the process of antigen presentation. These observations were extended to address several clinical issues such as skin inflammation and ocular autoimmune disease. Consequently we believe manipulation of chemokine system would be a tool to activate or regulate immune responses.

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Department of Advanced Medical Science was established in September 1997. We are investigating (1) A potential pro-angiogenic cell therapy for ischemic disease using hPDMCs, (2) Identification of tumor-associated antigens in melanoma patients treated by the dendritic cell therapy, (3) Differential Regulation of Cell Migration and Proliferation through Pyk2 in Endothelial Cells, (4) Analysis on mechanisms of conotruncus formation during embryonic heart development. We are planning and progressing several projects described below to develop a new therapy for several diseases, including carcinomas and ischemic disorders.

A potential pro-angiogenic cell therapy for ischemic disease using hPDMCs

Nishishita T. et al.

After the establishment of Cord Blood Banks, more than 2,000 cord blood transplantations have been performed throughout the world. In the processing of cord blood, adjacent placenta has been so far thrown away. Recently, the Department of Cell Processing IMS, started preparation and characterization of human placenta-derived mesenchymal cells (hPDMCs), which are obtained from placental villi. One of the characteristics of placenta is that its high vascularity. So, in our laboratory, we explored the possibility that these cells might produce angiogenic cytokines and could be used for pro-angiogenic cell therapy. We measured VEGF in hPDMCs conditioned media by ELISA and found that a large amount of VEGF, comparable to the amount produced by cancer cells, is produced by hPDMCs. We confirmed this VEGF is biologically active.

In vivo studies were performed to test the efficacy of hPDMCs injection to improve ischemic status. We made an animal model for arterial occlusive disease, inducing unilateral hindlimb ischemia by binding the left femoral arteries and veins of NOD/Shi-scid mice. We transplanted hPDMCs in the ischemic muscles. Subcutaneous blood perfusion was analyzed by using a laser doppler perfusion image analyzer before and after transplantation. Transplantation of hPDMCs significantly improved the blood flow of the affected limbs. In the limbs of treated mice formation of blood vessels was more prominently observed as compared to the control. Transplanted hPDMCs produced hVEGF for at least 7 days in NOD/Shi-scid mice, which was demonstrated by real time RT-PCR. It was concluded that hPDMCs could be used to treat human ischemic diseases.

Identification of tumor-associated antigens in melanoma patients treated by the dendritic cell therapy

Yoshiura K. et al.

We explored serum antibodies in cancer patients treated by the dendritic cell (DC) therapy in search of tumor-associated antigens, which may include critical molecules in cancer biology as well as possible targets of immunotherapy. By conventional Western blot analysis using tumor lysate obtained from melanoma patients who had response to the dendritic cell therapy, two proteins (27kD and 47kD) reacted with autologous serum. We are going to analyse these proteins by two-dimension electrophoresis combined with Western blot analysis and Matrix-Assisted Laser Desorption Ionization-Time of Flight/Mass Spectrometry methods.

Differential Regulation of Cell Migration and Proliferation through Pyk2 in Endothelial Cells

Kuwabara K. et al.

Migration and proliferation of endothelial cells (ECs) constitute an essential part of angiogenesis. Chemokines and their receptors have been reported to play important roles in angiogenesis similar to inflammatory responses. Several members of the chemokine superfamily, including stromal-derived factor-1α (SDF-1α), act as potent chemo-attractants for ECs. CXC chemokine receptor 4 (CXCR4), a member of the G-protein-coupled receptor family, is a specific receptor for SDF-1α. The expression of CXCR4 on the EC membrane is stimulated by vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF), both well-known angiogenic factors. Signal transduction through CXCR4 and through another type of chemokine receptor (CC-chemokine receptor 5 (CCR5)) leads to activation of proline-rich tyrosine kinase 2 (Pyk2; also known as RAFTK, FAK2, CAKβ or CADTK), a member of the focal adhesion kinase (FAK) family. Pyk2 is also showed to be upstream of ERK 1/2; therefore it is thought to act as a key component in angiogenesis.

We studied the effect of mutant Pyk2 expression on the migration and proliferation in endothelial cells. Two types of mutant Pyk2 were examined by adenovirus vectors: AxCA-Pyk2K457 A, expressing a kinase inactive mutant, and AxCA-Pyk2Y402F, expressing a tyrosine autophosphorylation site mutant, in addition to AxCA-Pyk2, expressing wild type Pyk2. Infection with AxCA-Pyk2Y402F or AxCA-Pyk2 increased the migration of ECs, however, AxCA-Pyk2K457A did not show such an effect. Western blotting showed that both phosphorylation of Pyk2 Y881 and association of p130Cas with Pyk2 were enhanced in ECs infected either with AxCA-Pyk2Y402F or with AxCA-Pyk2, but not in ECs infected with AxCA-Pyk2K457A. In contrast, mitogenic assay showed that infection with neither AxCA-Pyk2Y402F nor AxCA-Pyk2 K457A did exert any influence on ECs proliferation. Thus, the two Pyk2 mutants revealed that Pyk2 signaling differentially regulates cell migration and proliferation pathways.

Analysis on mechanisms of conotruncus formation during embryonic heart development

Nakaoka T. et al.

The cardiac outflow tract is a frequent site for clinically relevant human heart defects. The etiology of conotruncal defects is largely unknown. The heart defect mouse (hdf), recently created by insertional mutagenesis, has proven an important opportunity for studying the segmental origin and development of the cardiac outflow tract. In the hdf mouse, most of the outflow track is absent suggesting that the hdf gene is a critical regulator in the molecular pathway leading to the to the formation of the outlet and its arterial pole. In order to address molecular mechanisms responsive for defective heart development in the hdf mouse, we performed subtractive hybridization and consequently, a novel gene, *Hag2* (hdf affected gene 2) was identified as a hypo-expressed gene in the hdf mouse.

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We have been engaged in the surgical treatment of solid tumors and the immunotherapy of various malignancies. We have also been offering services, including upper and lower endoscopic examination, ultrasonic examination, and angiography. The principal goal of our department is to develop and conduct clinical trials in the early stages (Phase I and II) on patients at Research Hospital. We have performed phase I clinical trials of melanoma vaccine using gp100 derived peptides and immunotherapy using dendritic cells in combination with local irradiation therapy. We have also initiated phase I/IIa clinical trials of epitope peptides based vaccine against gastrointestinal malignancy.

1. Summary of surgical treatment and other procedures performed in 2003

Hideaki Tahara, Takuya Tsunoda, Yoshifumi Beck, Yasutaka Takeda, Akihiko Takeda, Takuya Takayama, Yuichi Ando, Naoya Ichikawa, Hiroaki Tanaka, Juichiro Konisi, Norihiro Kokudo[1], Masatoshi Makuuchi[1]: [1]Division of Hepato-Biliary-Pancreatic Surgery and Artificial Organ and Transplantation, Department of Surgery, Graduate School of Medicine, University of Tokyo, Japan.

Surgical operations have been performed in 100 cases under general anesthesia and spinal or epidural anesthesia. As shown in Table 1, major operations were performed in 55 patients with malignant diseases and in 45 patients with benign diseases.

Procedures other than surgical operations performed in 2003 were as follows: angiography including trans-arterial embolization and trans-arterial chemotherapy (28 cases), gastroduodenal endoscopy (466 cases), and colorectal endoscopy (160 cases).

Table 1. Major operations performed in 2003

Malignant Diseases		Benign Diseases	
Cancer of the stomach	7	Cholelithiasis	14
Cancer of the colo-rectum	21	Inguinal herni	8
Cancer of the liver	2	Miscellaneous	23
Cancer of the pancreas	1	Total	45
Cancer of the breast	21		
Cancer of the thyroid	2		
Miscellaneous	1		
Total	55		

2. Phase I clinical trial of melanoma vaccine using gp100 derived peptides restricted to

HLA-A*0201 or -A*2402

Takuya Tsunoda, Toshiyuki Baba, Takuya Takayama, Yoshifumi Beck, Hiroaki Tanaka Juichiro Konishi, Hideaki Tahara

Epitope peptides derived from gp100, a melanoma associated antigen, are used for the cancer vaccine to treat the patients with advanced malignant melanoma. Patients with HLA-A*0201 were treated with a gp100 derived peptide (ITDQVPFSV) and another peptide with a mutation (IMDQVPFSV). Patients with HLA-A*2402, were treated with a gp100 derived peptide (gp 100-int4: VYFFLPDHL). All of the peptides were used with IFA in order to augment for anti-tumor immunity. Six patients with stage IV melanoma were immunized with a vaccine consisting of HLA-A*2402-restricted epitope peptide derived from gp100 melanoma differential antigen emulsified with incomplete Freund's adjuvant. And five patients were immunized with HLA-A*0201-restricted peptide. No adverse effects without grade I toxicity were observed in these patients. Patient 1 had a partial regression of multiple liver metastases and decrease of tumor maker after vaccination. In two patients (Patient 2 and 3), vitiligo was observed after vaccination. Patient 4, 5 and 6 showed disease progression at the final evaluation. Immunological monitoring was performed to determine IFN-g production and analyze A24/gp100 tetramer staining using PBMC stimulated with gp100-int4 peptide. PBMC from Patient 1, 2 and 3 was determined significant amount of IFN-g production and specific reactivity of IFN-g production to gp100-int4 peptide after vaccination. On the other hand, PBMC from Patient 4, 5 and 6 was not determined IFN-g production and specific reactivity of IFN-g production. By A24/gp100 tetramer analysis, A24/gp100 tetramer and CD8 double positive subset was detected after vaccination in PBMC from Patient 2 and 3 (both were observed vitiligo). Furthermore, melanoma-specific CTL clones were established from CD8 and A24/gp100 tetramer double positive subset in these patients. Importantly, these CTL clones were able to lyse 888mel (HLA-A24 positive and naturally expressing gp100), but not to lyse 397mel (HLA-A24 negative and naturally expressing gp100) and HT29 (HLA-A24 positive and gp100 negative). However in Patient 1, A24/gp100 tetramer and CD8 double positive subset was not detected in spite of tumor regression and melanoma-specific CTL clones were not established from the PBMC. In conclusion, our data suggested that this peptide was safe and immunogenic to the stage IV melanoma patients. In immunological monitoring, it was clarified that clinical and immunological response was inconsistent with result of HLA-tetramer analysis. It might be of significance that not only HLA-tetramer but also IFN-g production were necessary to evaluate immunological response induced by peptide-based vaccine.

3. Phase I/IIa clinical trial of melanoma vaccine using gp100 derived peptides restricted to HLA-A*2402

Takuya Tsunoda, Takuya Takayama, Yoshifumi Beck, Hiroaki Tanaka Juichiro Konishi, Hideaki Tahara

From phase I data, phase I/IIa clinical trial of melanoma vaccine using gp100 derived peptides were performed. HLA-A*2402-restricted gp100 derived peptide (gp100-int4: VYFFLPDHL) was used with IFA and interleukin (IL-2) in order to augment for anti-tumor immunity. Our goals in this clinical trial are to examine these clinical efficacy, furthermore, safety and immune responses associated with the peptide vaccination. We have enrolled 9 melanoma patients during year 2003. So far, the protocols were well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted.

4. Phase I/IIa clinical trial of epitope peptides based vaccine against gastrointestinal malignancy

Takuya Tsunoda, Takuya Takayama, Yoshifumi Beck, Hiroaki Tanaka Juichiro Konishi, Hideaki Tahara

Epitope peptides derived from MAGE3 and HER2/neu are used for the cancer vaccine to treat the patients with advanced gastrointestinal malignancy. Patients with HLA-A*0201 were treated with MAGE3 and HER2/neu derived peptide (FLWGPRALV, KIFGSLAFL). Patients with HLA-A*2402, were treated with MAGE3 and HER2/neu derived peptide (IMPKAGLLI, RWGLLLALL). All of the peptides were used with IFA and IL-2 in order to augment for anti-tumor immunity. To analyze the immune response of the vaccinated patients, HLA-Tetramer was prepared and used for staining of the peripheral blood lymphocytes taken from the patients enrolled in this protocol. Our goals in this clinical trial are to examine these clinical efficacy, furthermore, safety and immune responses associated with the peptide vaccination. We have enrolled 1 esophageal cancer patient for MAGE3-HLA-A*2402 peptide during year 2003. So far, the protocols were well tolerated, and no cardiac, hematological, hepatic, or renal

toxicity was noted.

5. A phase I clinical trial of intra-tumor injections of dendritic cells combined with local radiotherapy and systemic administration of IL-2 for advanced cancer patients.

Takuya Takayama, Marimo Sato, Hideaki Tahara

BACKGROUND: Dendritic cells (DCs) may capture the tumor antigen in situ from tumor cells in apoptosis induced with local radiotherapy. These immune responses may be further promoted with subcutaneous administration of IL-2. The goals of this phase I, dose-escalation study are to assess the feasibility, the safety, and the immune responses of this combination therapy.

METHODS: DCs were generated from peripheral blood mononuclear cells obtained by leukopheresis using the stimulation with granulocyte-macrophage-colony stimulating factor and interleukin-4. A tumor lesion was irradiated and injected with 5 or 20 million autologous DCs every one week for five times. Low dose IL-2 (0.7 million unit per body) was also injected subcutaneously after every DCs injection.

RESULTS: Five patients with melanoma, two patients with breast cancer, one patient with gastric cancer, and one patient with colorectal cancer have been enrolled. One melanoma patient couldn't accomplish the treatment schedule, due to the progress of disease. Injections were well tolerated. Transient but clear reductions of tumor sizes were observed at the injected sites in five out of six cases. However, no regressing tumors were observed at non-injected distant sites. Treatment-related adverse events were limited to grade I pain were observed at the injection site.

CONCLUSIONS: Feasibility and safety of this combination therapy were confirmed. Immune response associated with this therapy is now under examination.

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Research Hospital

Department of Radiology
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Our department consists of three major divisions: diagnostic radiology, nuclear medicine and radiation oncology. Diagnostic radiology plays a crucial role in evaluating various neoplastic and infectious diseases. Researches aim at sophisticating diagnostic procedures in clinical practice. In nuclear medicine, we develop analytic methods to estimate in vivo physiology, as well as studying the tracer kinetics and physical characteristics of detectors. Total body irradiation prior to bone marrow transplantation is a major role of our division of radiation oncology.

Primary retroperitoneal neoplasms: CT and MR imaging findings with anatomic and pathologic diagnostic clues

Manabu Minami and Nishino Mizuki[1]: [1]Department of Radiology, Kyoto City Hospital

Primary retroperitoneal neoplasms are a rare but diverse group of benign and malignant tumors that arise within the retroperitoneal space but outside the major organs in this space. Although computed tomography and magnetic resonance imaging can demonstrate important characteristics of these tumors, diagnosis is often challenging for radiologists. Diagnostic challenges include precise localization of the lesion, determination of the extent of invasion, and characterization of the specific pathologic type. The first step is to determine whether the tumor is located within the retroperitoneal space. Displacement of normal anatomic structures of the retroperitoneum is helpful in this regard. For tumors that are located within the retroperitoneum, the next step is to identify the organ of origin. Specific signs, including the "beak sign," the "embedded organ sign," and the "phantom (invisible) organ sign," are useful for this purpose. When there is no definite sign that suggests the organ of origin, the diagnosis of a primary retroperitoneal tumor becomes likely. Awareness of specific patterns of spread, specific tumor components, and tumor vascularity help in further narrowing the differential diagnosis. Attention to these diagnostic clues is essential in making an accurate radiologic diagnosis of primary retroperitoneal tumors and in obtaining clinically significant information.

Benign odontogenic tumors of the mandible and maxilla

Manabu Minami and Takashi Kaneda[2].: [2]Departement of Radiology, Nihon University at Matsudo, School of Dentistry

We have reviewed radiological strategy in diagnosing benign odontogenic tumors of the mandible and maxilla. Conventional radiography including dental films and occlusal view and panoramic tomography are useful for detection of abnormalities and analysis of the relationship of the lesion with the teeth and pres-

ence of dental displacement and erosion. Computed tomography can provide three-dimensional information of the anatomy and extension of the lesion. Influence to the surrounding bone and soft tissue can be also evaluated. Magnetic resonance imaging has high contrast resolution and multi-directional imaging capability. This modality is useful in differentiating solid lesions from cystic lesions and can provide some information of tissue characterization. Important factors influencing the diagnosis are discussed from the clinical viewpoint with the representative cases of benign odontogenic tumors in the maxillomandibular region.

Assessment of cardiac function by magnetic resonance imaging

Yusuke Inoue

Magnetic resonance imaging (MRI) has been established as an accurate tool to measure the systolic function of the left ventricle. However, it is rather time-consuming and troublesome in view of patient preparation, image acquisition, and data analysis, and is not well accepted in clinical practice. We are attempting to solve technical problems in measuring cardiac fucntion by MRI. To assess the wall motion accurately, high temporal resolution is desirable; however, high temporal resolution leads to longer breath-hold acquisition time. We assessed the effect of temporal resolution on indices of systolic function. Low temporal resolution caused mild underestimation of ejection fraction but appeared acceptable for use in most situations. Although electrocardiographic gating is preferable for cardiac imaging, it is rather troublesome and sometimes fails. We compared systolic function indices obtained with electrocardiographic gating and peripheral gating in subjects without arrhythmia, and showed excellent concordance. We have developed a program to assess detailed diastolic function using the Fourier analysis and are testing the effects of various parameters on calculated diastolic indices. The left ventricular cavity of many slices and many phases is required to be demarcated in determining diastolic function. Manual drawing of regions of interest imposes an unacceptable burden, and we are planning to develop semiautomatic methods. We are also planning to develop sophisticated techniques to display four -dimensional data obtained by cardiac MRI.

Effect of chollimator characteristics on scintigraphic assessment of cardiac sympathetic nervous system.

Yusuke Inoue, Ichiro Shirouzu[3], and Toru Machida[3]: [3]Department of Radiology, Kanto Medical Center NTT EC

Low-energy (LE) collimators are generally applied to imaging ^{123}I sources with a gamma camera. ^{123}I emits high-energy photons of more than 400 KeV, in addition to 159-KeV photons that generate signals valid for creating images. Septal penetration of high-energy photons through the septa of the collimator may impair image quality and quantitative. To determine the appropriate collimator in ^{123}I imaging, we first examined physical characteristics of three collimators: a low-energy high-resolution (LEHR) collimator, special LEHR (SLEHR) collimator, and medium-energy (ME) collimator. Both sensitivity and resolution were similar with the LE collimators, and higher sensitivity and lower resolution were observed with the ME collimator. Severe degradation due to septal penetration was shown in imaging ^{123}I with the LEHR collimator, and the degradation was reduced with the SLEHR collimator and further with the ME collimator. Next, we evaluated the effect of collimator choice on estimation of the heart-to-mediastinum (H/M) ratio from planar cardiac ^{123}I-MIBG imaging. Phantom and clinical studies demonstrated that collimator choice substantially influences estimated H/M ratios and that the use of an ME collimator is preferable in the quantitative evaluation of global cardiac sympathetic nerve function. SPECT is used to assess regional impairment of cardiac symphathetic nerve system, and we compared the three collimators in phantom experiments simulating cardiac SPECT studies. The image quality and defect contrast were the best with the SLEHR collimator, followed by the ME collimator and LEHR collimator. The use of an SLEHR collimator, which provides high spatial resolution and relatively low penetration, appears suitable for cardiac SPECT studies with ^{123}I-labelled tracers. The use of an ME collimator gives the most reliable estimate of the H/M ratio and better quality SPECT image than that of an LEHR collimator, the most common technique, and is indicated to be acceptable for both planar and SPECT imaging.

Therapeutic effect on myocardial flow reserve in metabolic diseases.

Yusuke Inoue and Ikuo Yokoyama[4]: [4]Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo

Studies have demonstrated the effectiveness of hydroxymethylglutaryl coenzyme A reductase inhibitors, statins, for the prevention of

coronary events and the regression of coronary artery stenosis. Myocardial flow reserve (MFR) have been shown to be diminished in hypercholesterolemia without evidence of ischaemia. Its impovement may be hypothesized after statin therapy; however, previous studies showed discrepant results, namely, significant improvement and lack of significant improvement. Statins used in previous studies have varied, as have patient populations, and such differences may be related to the discrepancies in results. We evaluated myocardial flow reserve before and after lipid-lowering therapy with simvastatin or pravastatin. This study aimed to clarify the effects of the two statins on impaired MFR in hypercholesterolaemia and to determine whether or not the effect differed between the two statins. Hypercholesterolemic patients with low probability of coronary artery disease were enrolled. Before and after lipid-lowering therapy with simvastatin or pravastatin, myocardial blood flow (MBF) at rest and during dipyridamole loading was measured using positron emission tomography (PET) with ^{13}N-ammonia, and MFR was assessed. Treatments with simvastatin and pravastatin similarly reduced plasma lipid fractions. Rest MBF was comparable between the two therapy groups and unchanged after therapy. MBF during dipyridamole loading and MFR were decreased in both therapy groups. They improved significantly after therapy with simvastatin, whereas no improvement was observed after pravastatin therapy. It is suggested that improvement of MFR by simvastatin therapy depends on mechanisms other than the lipid-lowering effect, so-called pleiotropic effects, and that the effect on coronary circulation differs between statins. We are also investigating reversibility of myocardial flow reserve in type II diabetes mellitus.

Assessment of regional insulin resistance by ^{18}F-FDG PET

Yusuke Inoue and Ikuo Yokoyama[4]: [4]Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo

Skeletal muscle glucose utilization (SMGU) during hyperinsulinemic euglycemic clamping can be measured by dynamic PET imaging with ^{18}F-fluorodeoxy-glucose (FDG) to characterize insulin resistance. PET method has a potential to evaluate regional differences in insulin resistance. Dynamic PET for 30-60 min combined with frequent arterial blood sampling is commonly performed to measure SMGU. We attempted to modify the method for the assessment of the regional differences and developed techniques to calculate SMGU from static PET of 10-min duration with or without a single venous blood sampling. The technique provided SMGU values with acceptable accuracy, and it is suggested that a single injection of FDG followed by static imaging at multiple positions can offer SMGU of muscles of different compositions. Myocardial insulin resistance is not always parallel to skeletal muscle insulin resistance, and PET measurement of myocardial glucose utilization (MGU) and SMGU may aid in characterizing insulin resistance in relation to various heart diseases. We developed a method to accurately measure femoral muscle SMGU and MGU without arterial blood sampling by sequential PET imaging of the thoracic and femoral regions. In this method, input function is estimated from thoracic images during the early phase and venous blood samples during the late phase, and accumulation in the femoral muscle is measured only during the late phase. We demonstrated that omission of early dynamic data does not degrade the estimation of femoral muscle SMGU and that input function late after injection can be assessed by venous blood sampling with no substantial distortion. SMGU values obtained using thoracic images and venous blood samples as input function were almost identical to those obtained with frequent arterial sampling. These results indicate that our method permit the simultaneous estimation of femoral muscle SMGU and MGU in a single subject. We are planning to apply our method of evaluating regional insulin resistance to the evaluation of therapeutic effects.

Verification of diffusion tensor tractography using phantom data

Makoto Watanabe, Yoshitaka Masutani[5] and Shigeki Aoki[5]: [5]Department of Radiology, University of Tokyo

Fiber tractography based on diffusion tensor (DT) is a useful method to recognize white matter tracts in human central nerve system. Our aim of this study is to apply this technique to data phantoms and evaluate its validity. Materials were botanical phantoms (Asparagus officinalis, Ananas comosus, etc) and computer-synthesized data phantoms. On botanical phantoms, DT imaging was acquired on a 1.5T MRI (Signa Horizon Lx, ver 9.0) using EPI and/or PROPELLER sequences with 6 or more different motion probing gradient directions. Synthesized phantoms were designed to model various trajectories: straight cylindrical structure, circular arc, helix and crossed columns. In the cylindrical synthetic data, homogeneous tensor was

supposed. DT tractography with seeding and line-tracking method was carried out by PC based software created by one of the members. Partial volume effect of intersectional nerve fibers was simulated with crossing phantoms: seed and target areas were placed in the cylinder across the intersection, 2-ROI method was applied and the ratio of reached fibers was estimated. As a result, DT tractography of botanical phantoms could trace the anisotropic structure of their tissue. Longitudinal straight fibers of the Asparagus officinalis, and radial fibers of Ananas comosus were demonstrated. About synthesized data phantoms, intended straight, circular or spiral trajectories were displayed appropriately. On crossed cylinder phantoms, the ratio of reached fibers declined at the intersection, which shared the problem common to the tractography of pyramidal decussation. Data phantoms are useful for evaluation of the validity of DT tractography.

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Research Hospital

Department of Laboratory Medicine
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Associate Professor Naoki Oyaizu, M.D., D.M.Sc. 助教授 医学博士 小柳津 直 樹

Our department consists of five subdivisions of clinical physiology, hematology, biochemistry, bacteriology and pathology, and engages in laboratory analysis and diagnosis of clinical materials submitted from the hospital. Since our department is constructed to examine clinical samples on routine bases, our scientific output is currently quite limited. However, along with the ongoing practice of translational research projects in the research hospital, we are now under reconstruction process to evolve into the unit that functions as an integrated diagnosis & monitoring laboratory.

General Scheme

Our basic research strategy is to characterize molecular mechanisms underlying pathology, develop a way to measure this in the clinical materials or in human disease *in situ*. In particular, developing molecular-based laboratory assay, which enables to assess the effectiveness of the experimental clinical trials, is our urgent target. We believe that such an approach is indispensable to direct experimental medicine in a correct way as well as to promote translational research. Developing molecular-based assays in clinical materials requires expertise in pathology and molecular biology; we are thus focusing our specialty to achieve this goal.

1. Pathological evaluation of cancer immunotherapy

We have initiated to analyze the surgical specimen obtained from the patients under cancer immuno-therapy conducted in the research hospital. By applying sophisticated immunohistochemical techniques, we are now intensively analyzing materials from cases including GM-CSF-based gene therapy for renal cell carcinoma and dendritic cell-based or peptide-pulsed anti-melanoma immuno-therapy. Our goal is to evaluate the effectiveness and to elucidate the mechanisms of anti-tumor immune response elicited by the therapy *in situ*.

2. Elucidation of immunopathological mechanisms of autoimmune-based hematological disorders

We found the presence of characteristic pathological findings in bone marrow specimen from some patients with MDS-RA, aplastic anemia, or pure red cell aplasia, which implicate that common immunopathological mechanism may be operative in these hematological abnormalities; that is destruction of erythroid precursors by immune-based mechanisms in the bone marrow. In collaboration with the Department of Hematology, we are going to elucidate molecular mechanisms on the ground of pathology thereby establish new disease entity and develop new therapeutic interventions.

3. Analysis of the chimeric gene expression of hematological disorder

We have initiated to analyze bcr-abl gene expression in specimen from patients with CML and Ph1+ve ALL by real-time PCR and nested RT-PCR techniques. In addition, we sequenced the amplified products to provide information for the molecular resistance to STI571 treatment.

4. Developing quick & inclusive diagnosis system for infections disease

Since the introduction of new therapeutic maneuver, host-pathogen interactions altered drastically and came into aspects. This results in altered recognition and molecular interaction of infected cells with immune cells, which leads to atypical pathological as well as clinical manifestations. To distinguish infectious disease and immunological disorder is a critical issue, however as a result of modified manifestations, it is difficult to achieve this in some occasions. To circumvent this, we are pursuing to establish a quick and inclusive diagnosis system of infectious disease.

5. Developing new immunological laboratory methods to predict and monitor Graft versus. Host Disease (GVHD)

GVHD is a life-threatening immunological disorder associated with bone marrow transplantation. Diagnosis of GVHD is now solely depending on pathological examination of biopsy specimen, and there is no reliable laboratory indicator predicting the onset of GVHD. We are planning to establish new immunological methods by examining gene expression profile *in situ* thereby pinpointing the molecular markers specifically expressed in GVHD.

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Research Hospital

Applied Genomics
ゲノム診療部

Associate Professor Noriharu Sato, M.D., D.M.Sc
Clinical Associate Naoyuki Takahashi, M.D., D.M.Sc.

助教授 医学博士 佐 藤 典 治
助 手 医学博士 高 橋 直 之

Our department was established in April, 2001 to support the translational researches of our hospital. We are participating in the clinical study to examine the SNPs in genes responsible for metabolism of medicines used for hematological malignancies. We are also conducting researches to find responsible SNPs in genes related to drug sensitivity, disease progression, and prognosis in various hematological disorders. In December, 2002 we opened a clinic of genetic counseling in collaboration with the divisions of pediatrics, genetic diagnosis, nursing and so on.

1. Genetic study on CML

Naoyuki Takahashi and Noriharu Sato

Before the advent of STI571, interferon has been the first choice drug for patients with CML who had no HLA-identical sibling donors. Since the long-term effect of STI is still unclear, interferon may have some position in the treatment of CML. We are going to determine SNPs in genes related to the sensitivity of interferon in patients with CML.

2. Genetic study on MDS

Naoyuki Takahashi and Noriharu Sato

Myelodysplastic syndrome (MDS) is heterogeneous diseases with different prognosis and different drug sensitivities. Some MDS respond to steroid therapy and some do so to cytokine therapies. We are studying on the SNPs in genes related to cytokine signaling or apoptosis whether there is some association between these SNPs and drug sensitivities or disease prognosis. Immature stem cells from MDS may have different expression profiles from that of normal individuals. We are now preparing oligonucleotide arrays to examine the hypothesis.

3. Genetic study on GVHD

Naoyuki Takahashi and Noriharu Sato

In hematopoietic stem cell transplantation, we sometimes observe severe GVHD (graft-versus-host disease) in HLA-matched transplant. There are reports suggesting an important role of cytokines and non-classical HLA molecules in GVHDs. In fact, promoter polymorphism of TNF gene is reported to be involved in severe GVHD. In order to find other genes affecting the severity of GVHD in addition to TNF, we are studying SNPs in genes related to immunologic responses.

4. Genetic study concerning NK cell receptors and ligands.

Naoyuki Takahashi and Noriharu Sato

Natural killer (NK) cells have gained an increasing concern from hematologists since a report that allogeneic transplantation between killer immunoglobulin-like receptor (KIR)-ligand mismatch pairs has remarkably favorable outcome in acute myeloid leukemia. We have started to search for new alleles for MICB gene and found a new allele.

Research Hospital

Division of Clinical Trial Safety Management
医療安全管理部

Professor	Naohide Yamashita, M.D., Ph.D.	教　授　医学博士	山　下　直　秀
Clinical Associate	Fumitaka Nagamura, M.D., Ph.D.	助　手　医学博士	長　村　文　孝

Division of Clinical Trial Safety Management (DCTSM) was established on April 2001 in the Research Hospital to watch the safety and ethics of clinical trials. DCTSM also deals with the risk management of diagnosis and treatment for general medicine. The staffs of DCTSM (doctors and nurses) are doing their work in collaboration with Translational Research Coordinators (TRC), which are organized by co-medical staffs, including pharmacist, dietician, psychologist and clinical laboratory technologist. The aim of DCTSM is to carry out the safe and ethically-protected clinical trials in the Research Hospital in addition to the data management. In order to accomplish it we are doing the following activities.

Risk management of Research Hospital

Naohide Yamashita, Fumitaka Nagamura, Yuko Ogami

The occurrence of medical accidents is the major problem at the hospitals. The requirement to avoid and to reduce the accidents has been increasing year by year. The aim of Research Hospital is to promote the translational researches, and reliance of the Hospital is indispensable to promote them. Staffs of DCTSM engage in the risk management at the Research Hospital. Medical accidents and incidents are reported to DCTSM by written forms. When the urgent action is required, the meeting is immediately held to discuss the first lines of action to protect the involved patient. This meeting also determines the preventive measures. This kind of meeting was held for 38 cases in this year. Medical accidents and the responses of DCTM are reported in the Council of Risk Management in the Research Hospital, which is held regularly.

Educational seminars on risk management are required to avoid the medical accident. DCTSM and Nursing Quarters took place two seminars this year. One was the lecture by a lawyer on risk management. The other was the role playing act based on the medical accidents actually happened. After reviewed the act, staffs at the Research Hospital discussed on the causes and preventions. Through these educations, consciousness for risk management will be tightened. Although medical accidents were reported, no serious adverse events and no prolonged influences were seen this year, and no suit had seen.

Food and Drug Administration (FDA) acceptance of data from outside the United States (U.S.) for oncology drug approvals.

F. Nagamura et al.

We examined the sources of data for registration studies to support oncology drug approvals from 1986 to September 2002. We queried an

FDA database of drug approvals listing studies and study characteristics and examined source documents. One hundred seventy-seven studies supporting approvals for 110 marketing claims (involving 57 new drugs) were reviewed. Of these registration studies 77 enrolled patients exclusively in the U.S, 23 in the U.S. and Canada, 35 in the U.S. and Europe and 42 without any participation from U.S. sites with the majority coming from Europe. A trend toward increasing use of partial U.S. and non-U.S. patient enrollment in registration trials has been accelerating. The 1998-1999 cohort of approvals had approximately 50% of the studies using non-U.S. sites in the submission data and the 2000-2002 cohort had about 75% of the studies using non-U.S. sites. These findings reflect the increasing globalization of oncology therapeutic drug development and the willingness of the FDA to accept quality data using objective endpoints for submissions independent of geographic origin

Review of Dose Escalation Scheme in Oncologic Phase I studies of Gene Therapy: Does it work?

F. Nagamura

The major purposes of Phase I study are to determine the dose for Phase II study and to clarify the toxicological and pharmacological profiles of agents. Dose increments, patient cohorts and stopping rule (dose limiting toxicity: DLT and maximum tolerated dose: MTD) are the critical elements to determine the recommended dose for Phase II study. However, little is known on the efficiency of dose escalation scheme on oncologic Phase I gene therapy. To clarify the efficiency of the concept of dose escalation scheme in oncologic Phase I study. To apply the results of this study for the design of the future studies. We performed literature review on reports published until the end of 2002. Eligible studies are: not gene marking; not administered with cytotoxic agents; not purging of hematopoietic stem cells; not pediatric study. Fifty-three study arms in 47 reports were eligible. Agents were administered locally in 39 studies out of 53 (73.6%): 20 studies (50.9%) used intratumoral administration. Major target diseases were malignant melanoma (11 studies: 20.0%), solid tumors (10 studies: 18.2%), and ovarian cancer (10.9%). Agents were classified into 21 kinds based on their concepts. The median and the mean of the number of enrolled patients per study were 13 and 14.4, respectively (range: 4-37). Three patient cohorts was applied in 41 studies (77.3%). The maximum number of patient per each cohort was five. The median and the mean of the number of dose levels were 4 and 4.1, respectively (range: 2-8). The number of studies used half-log based, full-log based, and full-log to half log based dose increments were 11 (20.8%), 11 (20.8%) and 10 (18.9%), respectively. DLTs were observed in only 7 studies (13.2%). Only six studies (11.3%) in three reports (6.4%) reached MTD. All the studies which reached MTD were not immunotherapy. The number of reports mentioned the definition of DLT and MTD were 15 (31.9%) and 12 (12.8%), respectively. Overall response rate was 1.3% (2 CR and 8 PR/762 pts). One CR and 5 PR were observed (2 doses below the highest dose. Low incidence of studies reached MTD indicates that the design to seek MTD in oncologic Phase I gene therapy has not been worked well. The result that more than half of responders were observed at the relatively lower dose levels suggest the question for pursuing the MTD and the necessity for surrogate endpoints other than tumor shrinkage. In addition to improve the basic technology for gene therapy, we have to reconsider the design and the concept on Phase I study of oncologic gene therapy.

Review of clinical study protocols before the review of Institutional Review Board (IRB: Chiken-Sinsa-linkai)

Fumitaka Nagamura and Naohide Yamashita.

One of the roles of our division is to keep the quality of protocols. To perform this task, we discuss and advise on a protocol with principal investigators, and made it a rule to submit a protocol and written consent form before submitting to the Institutional Review Board.

From January 2003 to December 2003, we received nine protocols and numerous questions within the research hospital. All the protocols were Phase I, Phase I/IIa studies, or Phase IIa. Pre-review of these protocols were finished within two to three weeks from the receipt. The format of pre-review is based on the style of applied in the U.S. Food and Drug Administration. Our opinion is summarized into three sections: safety issue (most concern), major problem, and minor problems/suggestions. These opinions are not obligations to have enforcement, but those to improve clinical studies. Final decision should be made at the Institutional Review Boards.

To assist the planning of clinical studies and writing protocols, we disclosed "Guideline". New Good Clinical Practice and Guidelines of International Conference on Harmonization has been settled, and many guidelines for gene therapy and gene analysis have been announced.

Our division prepared these guidelines, and tries that intra-institutional clinical studies respect such guidelines.

Psychological changes in subjects and analysis of ethical problems associated with translational research.

Momoyo Ohki

In translational research, results of basic medical studies are applied to the development of novel treatments for patients with conditions for which no therapies currently exist. At the Institute of Medical Science Research Hospital at the University of Tokyo, Translational Research Coordinators (TRCs) comprise pharmacists, nurses, certified clinical psychologists, registered dietitians, and clinical technologists. TRCs monitor and oversee ethical and scientific compliance. The present study investigated psychological changes accompanying changes in patient conditions from the perspective of TRC-certified clinical psychologists, and clarified ethical problems that should be addressed in the future were clarified.

Eighteen of the patients who participated in any of the five types of translational research at the hospital served as subjects. Each subject was interviewed by a certified clinical psychologist once a week during participation in the research. Ten of the 18 subjects consented to complete a survey on autonomy and anti-stress measures and undergo psychological tests such as Rorschach test and Profile of Mood Status.

Initially, all patients participating in the research reported high expectations. Levels of anxiety were slightly high for subjects, but were controllable. However, when research proved ineffective, levels of anxiety, depression and confusion increased. Some of them displayed anger towards themselves and others, and pessimistic outlooks towards life.

Excessive expectations towards translational research and emotional intervention at the time of research termination were identified as ethical problems that should be addressed in future.

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大木桃代，福田直子，小瀧　一，長村文孝：トランスレーショナルリサーチにおけるトランスレーショナルリサーチ・コーディネーターの役割と医療倫理遵守の取り組―インフォームド・コンセント関連業務を中心とした活動―応用薬理（印刷中）

大木桃代，小瀧　一，尾上裕子，福田直子，村山明美，畠山高年，宮崎菜穂子，中村アキエ，遊佐希，三浦洋子，佐田礼子，松本和史：トランスレーショナルリサーチ・コーディネーター業務の経過と各職種における専門性および問題点の明確化　文教大学紀要（印刷中）

長村文孝　化学療法に伴う苦痛とその対策「末梢神経障害」がん患者と対症療法　第14巻1号 p. 37–41，2003

Research Hospital

Surgical Center
手術部

Associate Professor	Masakazu Hayashida	助教授	林 田 真 和
Clinical Associate	Shin-ichi Watanabe	助 手	渡 邉 慎 一

Our clinical practice and clinical as well as experimental studies have been focused on (1) anesthetic management in patients undergoing major cardiovascular surgery, (2) management of intraoperative and postoperative pain, and (3) management of chronic intractable pain. We have published several works on these subjects last year.

1. Anesthetic management in patients undergoing major cardiovascular surgery especially focusing on monitoring of cerebral oxygenation and cerebral function

The Bispectral Index (BIS) is a recently developed derivative of processed electroencephalogram that has been proven to closely correlate with level of consciousness during natural sleep and general anesthesia. It has been widely used in the area of anesthesia to evaluate sedative/hypnotic state in patients undergoing surgery under general anesthesia.

We have also found that BIS is also useful to detect cerebral ischemia during pediatric and adult cardiac surgery especially when used in combination with the near-infrared spectroscopy (NIRS) to measure oxygen saturation of the brain. Simultaneous monitoring with BIS and NIRS revealed that in children, especially in infants, cerebral ischemia seemed to occur frequently during cardiac surgery presumably due to immaturity of the cerebral vascular autoregulation. We also reported successful anesthetic management of critically ill patients.

2. Management of intraoperative and postoperative pain

We have published several works on management of intraoperative and postoperative pain. We have developed a rabbit model of surgical anesthesia/analgesia, which allows for repeated and quantitative evaluation of depth of surgical anesthesia/analgesia provided by a variety of anesthetics/analgesics. We also published several review articles on how to manage postoperative pain, and original articles comparing various modalities of postoperative pain management.

3. Management of chronic intractable pain

We published several works on new treatment modalities for chronic intractable pain syndrome with various drugs including ketamine and ATP, after application of drug tests to differentiate the mechanisms underlying the pain. We also reviewed usefulness of epiduroscopy in pain management in patients with chronic intractable low back and leg pain.

We will continue to research on these subjects and publish several additional reports this year.

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