Report of the Fifteenth International Symposium of the Foundation for Promotion of Cancer Research: New Horizons in the Diagnosis and Treatment of Hematological Malignancies Based on Molecular Genetic Features

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The Fifteenth International Symposium of the Foundation for Promotion of Cancer Research entitled ‘New Horizons in the Diagnosis and Treatment of Hematological Malignancies Based on Molecular Genetic Features’ was held in Tokyo on January 15–17, 2002. Twenty-nine invited speakers, including 12 from abroad and 17 from Japan, presented the updated results of their research. After an overview of the classification of hematological malignancies, new findings on some disease entities based on novel immunophenotypic and molecular genetic features were presented. The results of gene expression profiling and BCL6 and C-MYC gene rearrangement in diffuse large B-cell lymphoma were presented and oncogenic mechanism of acute myeloid leukemia was discussed. In the treatment of non-Hodgkin’s lymphoma and acute leukemia, the present consensus and future directions were discussed based on the results of multicenter trials in the USA and Japan. As a molecular targeting therapy, the remarkable effect of a BCR-ABL tyrosine kinase inhibitor, STI571, in chronic myeloid leukemia and gastrointestinal stromal tumor was presented. Thereafter, promising results of active immunotherapy, chimeric anti-CD20 monoclonal antibody, anti-CD20 radioimmunoconjugate and anti-CD22 immunotoxin for B-cell lymphoma were presented. Finally, recent advances in allogeneic hematopoietic stem cell transplantation were discussed, focusing on reduced-intensity preparative regimens. The recent advances in basic and clinical research on hematological malignancies would lead to further improvement in the prognosis and quality of life of patients suffering from leukemia or lymphoma.

INTRODUCTION

The Fifteenth International Symposium of the Foundation for Promotion of Cancer Research (FPCR) entitled ‘New Horizons in the Diagnosis and Treatment of Hematological Malignancies Based on Molecular Genetic Features’ was held in Tokyo on January 15–17, 2002. Drs Tadao Kakizoe, James O. Armitage, Ryuzo Ohno and Kensei Tobinai, with Dr Takashi Sugimura as advisor, organized the symposium.

OPENING

CHAIRPERSON: DR. JAMES O. ARMITAGE

Dr Sugimura (National Cancer Center, Tokyo, Japan) began the symposium with a welcome address and a review of the history of previous cancer symposia. The Japanese government cancer control program was launched two decades ago. Annual symposia have been held since 1988 to address basic science and clinical issues in one cancer type per year. Examples of previous cancer types include lung cancer, liver cancer and esophageal cancer and have included both Japanese and international speakers. Over the course of the last 14 symposia there have been 485 speakers of whom 249 have been from Japan, 165 from the USA and the remainder from 15 additional countries (1–4).

Dr Kakizoe (National Cancer Center, Tokyo, Japan) then presented an opening address in which he discussed the annual...
mortality trends in Japan from 1930 to 1998. These data demonstrated that death due to infection was initially the leading cause of mortality in Japan. Subsequently, cerebrovascular disease became the number one cause of death. Since 1981 cancer has become the leading cause of mortality accounting for one out of three deaths on an annual basis. The incidence has also been increasing rapidly. The number of deaths due to leukemia in 2000 was approximately 6800 and the number of deaths due to lymphoid malignancy was 7900. Again, the incidence is increasing in these two categories.

Dr Kakizoe then reviewed the breakthroughs in the treatment of hematological malignancies over the past three decades. Allogeneic bone marrow transplantation, which was developed in the early 1970s, represented a major step forward in the treatment of leukemia followed by the technology to generate monoclonal antibodies in 1975. Human T-cell leukemia virus type-I (HTLV-I), a novel retrovirus, was identified as the causative agent in certain subtypes of T-cell leukemia–lymphoma in the early 1980s. Subsequently, the introduction of rituximab as the first approved monoclonal antibody for the treatment of non-Hodgkin’s lymphoma (NHL) occurred in 1997 in the USA. Most recently the approval of STI571 for the treatment of chronic myeloid leukemia (CML) occurred in 2001.

SESSION 1: NEW ASPECTS IN THE DIAGNOSIS OF HEMATOLOGICAL MALIGNANCIES

1. GENERAL VIEW

CHAIRPERSON: DR. JAMES O. ARMITAGE

Dr Elaine S. Jaffe (National Cancer Institute, Bethesda, MD, USA) presented a lecture entitled ‘The World Health Organization (WHO) Classification of Lymphomas: a Milestone and Roadmap for Future Clinical and Investigational Studies.’ The current version of the WHO classification of lymphomas represents a milestone in lymphoma hematopathology, because it represents for the first time a worldwide consensus in the complicated arena of NHL classification. This does not, however, represent a final statement, but rather the evolution of a 40-year effort to improve upon lymphoma classification. Initial schemes were more descriptive in nature, such as the Rappaport system. Subsequent schemes were more clinical and morphologically based such as the International Working Formulation (WHO) Classification of Lymphomas: a Milestone and Roadmap for Future Clinical and Investigational Studies.’ The Revised European–American Lymphoma Classification system (REAL) represented a new paradigm in the identification and classification of what were thought to be real disease entities rather than entities based upon an isolated assessment of survival characteristics, cytology, etc. These were based upon the consensus of 19 expert hematopathologists and were
based upon published data. They attempted to distinguish between what was a disease entity versus a prognostic factor. The system also introduced molecular genetics into the definition of specific entities. Dr Jaffe predicted that cytogenetics would assume a greater importance in disease definition in the future versus systems based upon histology and immunophenotype as polymerase chain reaction (PCR) techniques become more widespread. There are some genetic or molecular phenotypes, however, that can be detected by immunohistochemistry such as cyclin D1 overexpression. Also, microarray technology represents another potential tool for the classification of lymphomas.

These systems are critical for the proper definition of disease entities that are needed for the development of new therapies, especially targeted therapies. They are also necessary for the study of the pathogenesis of disease. A number of disease types have been associated with specific pathogenetic mechanisms including adult T-cell leukemia-lymphoma and HTLV-I infection; mantle cell lymphoma and cyclin D1 abnormalities; gastric mucosa-associated lymphoid tissue (MALT) lymphoma and Helicobacter pylori infection.

The recent publication of the WHO classification system was based upon a modification of the REAL system. This system takes into account morphology, immunophenotype, genetic features, the postulated normal cellular counterpart, as well as clinical features. Morphological and clinical variants are acknowledged and discussed, but their use is optional. Certain provisional entities within the REAL classification were reassessed and updated based upon new data, for example anaplastic large cell lymphoma. Similar principles were applied to the myeloid and histiocytic disorders. A clinical advisory committee was also convened such that the system would be suitable for both hematopathologists as well as clinicians in their daily practice and in the design of clinical trials.

The WHO system reassessed certain unresolved issues from the REAL classification system. These included addressing the cytological grading of follicular NHL. The decision was made to retain a three-level system. The third grade, however, was divided into 3a and 3b. The former represents a mixture where centroblasts outnumber centrocytes, while the latter consists of pure centroblasts and may be a distinct entity. Many of these tumors lack the t(14;18) translocation and are curable.

Two marginal zone B-cell lymphomas were also adopted: the splenic and nodal subtypes. High-grade B-cell NHL, Burkitt-like, was a provisional diagnosis in the REAL classification but was found not to be reproducible. These lymphomas are therefore categorized as either diffuse large B-cell lymphoma or atypical Burkitt’s lymphoma in the WHO classification system. Finally, the issues of histological variants of diffuse large B-cell lymphoma were described: thymic, intravascular, primary effusion and Epstein–Barr virus (EBV)-positive.

T-cell and natural killer (NK)-cell entities were subdivided into leukemic, nodal, extranodal and cutaneous varieties. Hodgkin’s lymphoma was divided into the classical versus the nodular lymphocyte predominant varieties. Anaplastic large cell lymphoma was first recognized based upon morphology and CD30-positive status. The advent of RT-PCR and the ALK-1 monoclonal antibody allowed greater definition. There are also continued gray zone areas: the distinction between nodal lymphocyte predominant Hodgkin’s lymphoma and T-cell rich B-cell lymphoma; also the distinction between nodular sclerosing Hodgkin’s lymphoma and Hodgkin’s lymphoma-like anaplastic large cell lymphoma and primary mediastinal diffuse large B-cell lymphoma. Thus, the WHO classification represents both a milestone and a roadmap for future investigation into the nature of lymphoid malignancies.

Dr Bennett (University of Rochester Medical Center, Rochester, NY, USA) then presented a lecture entitled ‘The WHO Classification of Acute Myeloid Leukemias and Myelo-dysplastic Syndromes.’ Dr Bennett first overviewed the general recommendations for the diagnosis of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). This includes the general requirements, which include a bone marrow aspirate and biopsy, standard Romanowski stains and cytochemistry (peroxidase; esterase), flow cytometry for immunophenotyping and cytogenetics and molecular genetics. Recent changes in classification of AML have lowered the blast count to 20% by eliminating the refractory anemia with excess blasts in transformation (RAEB-T) subtype of MDS. This was based upon clinical data that showed that some of these patients when treated with AML regimens did no worse than AML patients. There was also the recognition of special favorable categories including the M2 with t(8;21), the M3 with t(15;17) and the M4Eo with inv(16). Finally, unfavorable categories were recognized including the M4 and M5 types with 11q23, as well as AML with multi-lineage morphological dysplasia (>50% in each cell line vs >10% required for MDS) with or without a prior history of MDS. Often the latter will be associated with multiple chromosomal abnormalities, including 5q- and –7. There was also the recognition of distinct categories of primary leukemia vs secondary to MDS, as well as leukemia secondary to a history of toxic exposure or chromosomal abnormality.

The FAB classification of MDS has been shown to correlate with survival. The subtypes of refractory anemia with ringed sideroblasts and refractory anemia were subdivided into those in which there was <10% dysplasia in the granulocytic and megakaryocytic lines vs >10%. The 5q- syndrome was recognized as an independent subtype when the blast count was <5%. In addition, RAEB was subdivided into RAEB-1 (5–10% blasts) and RAEB-2 (11–20% blasts). As stated above, the RAEB-T category was eliminated, so that all patients with >20% blasts will be defined as having AML. Finally, CMMML, often considered a myeloproliferative disorder, was reclassified under the MDS/myeloproliferative diseases with two subdivisions: I and II, analogous to the blast count cutpoints of RAEB.

Thus the WHO classification of AML and MDS represents another step forward in the proper categorization of distinct hematological disease entities.
2. SOME DISEASE ENTITIES BASED ON NOVEL IMMUNOPHENOTYPIC AND MOLECULAR GENETIC FEATURES

CHAIRPERSON: WING CHUNG CHAN

Dr Shigeo Nakamura (Aichi Cancer Center, Nagoya, Japan) presented a lecture entitled ‘Molecular Diagnosis of Malignant Lymphoma: Mantle Cell Lymphoma and Anaplastic Large Cell Lymphoma.’ Based upon the evolution of malignant lymphoma classification, the emphasis on biological approaches such as immunohistochemistry and molecular biology have played an increasing role in the definition of disease entities. This is particularly evident in the testing for cyclin D1 overexpression in mantle cell lymphoma and anaplastic lymphoma kinase in anaplastic large cell lymphoma.

Mantle cell lymphoma represents a malignant proliferation of B cells in the mantle zone of lymphoid follicles. It is characterized by a monotonous proliferation of small- to medium-sized lymphocytes with slightly irregular nuclei and expresses CD5 and pan B-cell antigens on immunophenotypic analysis. Such patients typically present with advanced disease with involvement of bone marrow and extranodal sites. Despite aggressive therapy, median survival remains at 3–4 years.

A characteristic cytogenetic abnormality is the t(11;14) (q13;q32) translocation, which involves a rearrangement of the bcl-1 locus. The oncogene deregulated by this alteration has been identified as cyclin D1.

One hundred fifty cases of mantle cell lymphoma were scrutinized for cyclin D1 overexpression through immunohistochemistry techniques, and 85% of these cases were cyclin D1 positive; however, 15% were negative. These cases contained a higher prevalence of orbital disease and a lower prevalence of gastrointestinal disease. The International Prognostic Index (IPI) was lower in these patients. The tumors showed a lower mitotic index and smaller cells. These patients also appeared to have a better overall survival, unlike the cyclin D1 positive variety, suggesting that these two groups represent different entities. Dr Nakamura proposed that mantle cell lymphoma be restricted to cyclin D1 positive cases and that although cyclin D1 negative cases may resemble mantle cell lymphoma, they are not true mantle cell lymphomas.

Anaplastic large cell lymphoma (ALCL) was originally defined by the expression of CD30 as well as a peculiar anaplastic morphology with an infiltrative pattern. Morphological variabilities were subsequently proposed; however, the non-random chromosomal translocation t(2;5)(p23;q35) is highly associated with ALCL and in various studies appears to be present in 15–65% of cases. Recent cloning of this translocation has shown it to be a fusion of the NPM gene located on chromosome 5 to the newly described kinase gene, ALK, located on chromosome 2, which leads to the expression of a novel fusion protein. Although the precise function of this fusion protein is not known, it is believed to play an important role in the pathobiology of ALCLs that express it. There are now available immunohistochemistry reagents to detect this protein in lymphoma cells on paraffin sections. The detection of ALK positive versus negative cases of ALCL demonstrates that patients with ALK positive ALCL are on average younger, have a lower IPI and have overall a much longer survival than those who are ALK negative. Therefore, ALK positive cases constitute a distinct subtype of ALCL with a good prognosis. Dr Nakamura concluded that with the wide battery of monoclonal antibodies available, our understanding of lymphoma has transformed our ability to detect entities that were unresolved when addressed with hematoxylin and eosin sections alone.

Dr Ritsuro Suzuki (Aichi Cancer Center, Nagoya, Japan) presented a lecture entitled ‘CD7+ and CD56+ Myeloid/Natural Killer Cell Precursor Acute Leukemia.’ Myeloid/natural killer cell precursor acute leukemia represents a recently recognized entity characterized by frequent extramedullary involvement. The origin of this leukemia is now recognized as myeloid antigen-positive NK-cell precursors based upon their phenotypic similarity. Two distinct entities of immature CD56+ leukemia/lymphoma have been identified: myeloid/NK-cell precursor acute leukemia and blastic NK-cell leukemia/lymphoma. The myeloid/NK variety is characterized by CD7+, CD56+, myeloid antigen positive, with negative light microscopic myeloperoxidase reactivity and mostly germine configurations of T-cell receptor genes. It shares these characteristics with M0 AML, but on the other hand its localization appears to mimic that of malignant lymphoma, specifically lymphoblastic lymphoma.

To clarify the relation between myeloid/NK-cell precursor acute leukemia and M0 AML, 110 cases of M0 AML were studied and cases of CD7+, CD56+ disease were compared with the remainder. Twenty such cases were identified, while 77 were negative for both antigens and 11 could not be determined. The CD7+, CD56+ and M0 AML varieties showed a significant male predominance with a younger age of onset. Disease localization was also different with the CD7+, CD56+ variety showing more frequent extramedullary involvement, fewer circulating blasts and less anemia and thrombocytopenia. There were also different cytogenetic aberrations with no 5q abnormalities seen in the CD7+, CD56+ variety. Finally, none of the CD7+, CD56+ patients survived more than 5 years.

Hence this phenotype appears to be an independent prognostic factor for M0 AML. These findings suggest that CD7+, CD56+ M0 is a distinct subtype of M0 AML. Although it must be treated with AML-type chemotherapy, the prognosis is generally poor.

CHAIRPERSON: ELAINE S. JAFFE

Dr Peter G. Isaacson (Royal Free and University College Medical School, University of London, London, UK) presented a lecture entitled ‘Mucosa Associated Lymphoid Tissue (MALT) Lymphoma: Recent Advances in Basic and Clinical Research.’ The MALT lymphoma recapitulates the histology of normal MALT found in the body. These MALT lymphomas, however, occur in tissues normally devoid of lymphoid tissue but are preceded by chronic inflammatory process, often
autoimmune disorders, resulting in the accumulation of MALT. They can arise in a wide variety of extranodal sites, of which the stomach is the most common. The normal cell counterpart of these lymphomas is the marginal zone B cell. Normal MALT is manifested by a lymphoepithelium, which MALT lymphomas also recapitulate. Over time, these lymphomas may transform into diffuse large B-cell lymphoma.

Gastric MALT lymphoma represents a paradigm of this disease. Most gastric MALT lymphomas arise in the setting of H. pylori infection that is associated with accumulation of gastric MALT. Eradication of H. pylori results in regression of 75% of these lymphomas. In those cases where H. pylori is not demonstrable, serology is often positive. Furthermore, studies have demonstrated H. pylori gastritis that harbors the B-cell clone found in subsequent NHL, cementing the relationship between this inflammatory entity and neoplastic one.

The treatment of gastric MALT lymphoma with antibiotics can lead to regression; however, this takes months and in 50% of the cases, one can still detect the neoplastic clone via PCR. Eventually the MALT lymphoma is no longer dependent upon H. pylori. It would be useful, therefore, to be able to identify those cases unlikely to respond to eradication of H. pylori, so that conventional lymphoma therapy can be offered up front.

While it has been recognized that more deeply invasive lymphoma tend not to be responsive to antibiotics, there is no certainty whether a case of mucosal disease will respond. In this context, certain genetic abnormalities have been extensively investigated. The most common of these are t(1;14) (p22;q32) and t(11;18) (q21;q21). Acquisition of these in a reactive B cell during the course of chronic H. pylori-associated gastritis may lead to its malignant transformation. The former translocation results in aberrant nuclear expression of BCL-10. Mutated BCL-10 loses its proapoptotic activity. The latter translocation is often the sole genetic abnormality and results in the fusion gene of API2-MALT1. These two translocations have been shown to be synergistic in their action. Although it is unclear how they exert their oncogenic activities, both are significantly associated with aggressive MALT lymphomas as well as with each other. They are more likely to be resistant to H. pylori eradication and would therefore merit treatment with chemotherapy up front instead of antibiotics. They are also more likely to be stage IIIE or greater. Thus gastric MALT lymphoma with t(11;18) translocation does not respond to H. pylori eradication therapy and this can be exploited clinically using an RT-PCR strategy applicable to paraffin-embedded tissue.

Dr Yukio Kobayashi (National Cancer Center Hospital, Tokyo, Japan) presented a lecture entitled ‘Systemic MALT Lymphoma Bearing t(11;18).’ A subset of MALT lymphoma has been characterized by a t(11;18) translocation, resulting in the API2-MALT chimeric fusion gene, which has been implicated in its pathogenesis. In a review of patient records of cases of B-cell malignancy with this fusion gene, 11 cases were found with the translocation detected by fluorescence in situ hybridization (FISH) and/or RT-PCR. The diagnosis was primary macroglobulinemia in two cases and MALT lymphoma in nine cases. The median age of initial manifestation was 61 years with twice as many males as females. The primary site of involvement was bone marrow in two cases, lung in five cases, stomach in two cases and the lacrimal gland and colon in one case each. Multiple sites of involvement characterized seven cases. The IgM level was elevated in six cases and morphological peripheral blood involvement in one case. In six other cases, where the peripheral blood was examined by RT-PCR, four cases were positive, suggesting a t(11;18) translocation. In these four cases morphological as well as flow cytometric analysis failed to detect tumor cells. All cases were treated with radiation or chemotherapy as well as rituximab. The remission duration was usually transient. Eight of 11 cases are alive with persistent disease at 2–14 years after diagnosis. Two patients died of unrelated causes and one patient died of disease progression 3 years after diagnosis. These data suggest that the t(11;18) lymphoma is a systemic disease and is a unique subgroup of MALT lymphoma with a tendency to disseminate to other MALT-associated organs including peripheral blood.

SESSION 2: MOLECULAR GENETIC FEATURES OF HEMATOLOGICAL MALIGNANCES

CHAIRPERSON: PETER G. ISAACSON

Dr Wing C. Chan (University of Nebraska, Omaha, NE, USA) presented a lecture entitled ‘Distinct Types of Diffuse Large B-Cell Lymphoma Identified by Gene Expression Profiling.’ Diffuse large B-cell lymphoma represents a heterogeneous entity. The IPI separates these patients based upon clinical parameters; however, these parameters represent surrogate markers of as yet unidentified biological variables of both the host and the tumor.

Gene expression profiling using the lymphochip technology utilizes competitive binding and cDNA microarray analysis to determine differential expression of a large variety of known and unknown genes. The lymphochip is based upon a collection of genes enriched with B-cell associated genes. Validation of this technology involves assessing both biological and clinical correlates.

Based upon an analysis of diffuse large B-cell lymphoma, there appears to be one gene expression pattern similar to that of germinal center B cells and another pattern similar to activated peripheral blood B cells. The overall survival of patients with the germinal center B-cell type is longer than that of those with the activated B-cell type. This difference persists even when one looks at patients stratified by IPI. The germinal center B-cell type shows evidence of ongoing systematic hypermutation, analogous to normal germinal center B cells. The BCL-2 translocation, seen in 20–30% of cases of diffuse large B-cell lymphoma, is seen only in the germinal center B-cell subtype.

Lymphochip technology is also able to identify genes that have prognostic value and form an independent set of prognostic markers within the IPI. This opens up the possibility of
generating a diagnostic chip with a smaller number of genes in order to categorize patients to a greater degree of accuracy. This diagnostic chip could provide rapid molecular categorization of every B-cell lymphoma at presentation, aiding optimal treatment decisions and prognostication. Alternatively, a panel of monoclonal antibodies may be designed based upon corresponding protein expression data to serve the same purpose. These technologies represent a new and significant method that will lead to better understanding of the pathogenesis of lymphoma and tumor progression. The insight gained may also help identify novel targets for therapeutic and preventative intervention.

Dr Hitoshi Ohno (Kyoto University, Kyoto, Japan) presented a lecture entitled ‘BCL-6 and C-MYC Gene Rearrangements and Their Clinical Implications in Diffuse Large B-cell Lymphoma.’ Diffuse large B-cell lymphoma represents the most common subcategory of B-cell NHL but is a heterogeneous disease based upon cell morphological, immunophenotypic and genetic factors. Chromosomal translocation and rearrangement of oncogenes located at identified break points have been found in certain sets of diffuse large B-cell lymphoma; however, it remains to be determined whether these abnormalities are associated with specific clinical features and treatment outcome.

Many of the translocations seen in B-cell NHL involve the immunoglobulin gene loci. Based upon a method of long distance and long distance inverse PCR, capable of amplifying up to 30 kilobases of DNA, one can detect oncogene-immunoglobulin–gene fusions. This technique allows detection of virtually all important translocations in B-cell NHL: the c-myc/IgH fusion, the BCL-2/IgH fusion and the Ig and non Ig/BCL-6 fusion.

Based upon the analysis of 203 cases of diffuse large B-cell lymphoma using these PCR techniques in addition to the Southern blot analysis, 12 cases with the c-myc/IgH fusion, 12 cases with BCL-2 rearrangement and 43 cases of BCL-6 rearrangement, as well as five cases with two of these rearrangements, were found.

Several widely accepted prognostic variables were then analyzed for correlation with each genetic subgroup as well as the remaining 141 cases that lacked these molecular lesions. The results showed no statistically significant association between pretreatment features and gene rearrangement. It was notable however that in nine out of 12 cases with c-myc/IgH rearrangement the patients were older than 60 years in contrast to the younger age associated with Burkitt’s lymphoma. The survival curve for these patients was short with poor 1- and 2-year survivals. The survival curve of the BCL-2 subgroup declined steadily, suggesting the disease to be non-curable with available treatments. The BCL-6 translocation variety was found to involve not only one of three IgH loci but also other non-Ig partners. Analysis of these cases showed an overall survival that was superior in the Ig/BCL-6 group vs the non-Ig/BCL-6, suggesting that non-Ig/BCL-6 fusion represents a poor prognostic factor. These data also suggest that the rearrangement of the c-myc, BCL-2 and BCL-6 genes are important prognostic variables related to treatment outcome of different subtypes of diffuse large B-cell lymphomas.

Dr Nozomi Nitsu (Kitasato University, Sagamihara, Japan) presented a lecture entitled ‘Serum nm23-H1 Protein, a Differentiation Inhibitory Factor, as a Prognostic Factor in Hematological Malignancies.’ Treatment approaches to NHL tend to be based upon patient risk, as represented by the IPI. However, there has been significant research on biological prognostic factors. One such factor, the nm23 gene, was first isolated as a gene coding for a nucleotide diphosphate kinase that suppresses cancer metastases based upon its lowered expression in highly metastatic cancer cells.

The most common isotype is nm23-H1. It appears to be a differentiation inhibiting factor, as well as a factor in the suppression of tumor metastases. High serum levels have been found to be a prognostic factor both in NHL and AML. Overexpression of nm23-H1 in AML correlates to a lower overall survival. These studies were based upon RT-PCR; however, a more convenient method would be to use an ELISA assay.

ELISA assays for nm23-H1 were performed on 606 cases collected over 11 years. Levels in patients with NHL were higher compared with healthy individuals and levels were higher among the aggressive NHL patients compared with those with indolent NHL. In addition, progression-free survival for patients with aggressive NHL and a high nm23-H1 serum level was significantly shorter than for those with lower serum levels. Also within IPI categories, nm23-H1 serum levels continued to be a prognostic factor.

Analysis of cases of AML, as well as CML and MDS, showed similar prognostic ability. It is therefore concluded that the serum level of nm23-H1 may provide important prognostic information that could be useful in determining an appropriate therapeutic strategy for various types of lymphoma.

**Chairperson: John M. Bennett**

Dr Misao Ohki (National Cancer Center Research Institute, Tokyo, Japan) presented a lecture entitled ‘Molecular Mechanism of Leukemogenesis by AML1–MTG8.’ The AML1 gene encoding a transcription factor is the most frequent target of leukemic chromosomal translocation and is fused to the MTG8 (ETO) gene in the t(8;21) translocation associated with M2 subtype of acute myeloid leukemia. AML1 represents a transcription factor and normally functions as a complex with histone acetyltransferases to activate transcription of target genes.

Ectopic expression of AML1–MTG8 in L-G murine myeloid progenitor cells inhibits differentiation to mature neutrophils and induces cellular proliferation in response to granulocyte colony-stimulating factor (G-CSF). Using this model system, microarray technology was used to screen target genes whose expression was modified by AML1–MTG8 with an attempt to isolate those genes responsible for transformation. Based upon an assessment of ~12 000 genes, 68 candidate genes were identified under downstream control of AML1–MTG8. This analysis together with previous differential display analyses revealed that the alterations in expression of G-CSF receptor and
TIS11b induced proliferation of L-G cells and are partially responsible for the process of transformation. Also the genes that are responsible for azurophil granule protein were upregulated, suggesting that translocation can induce partial differentiation of myeloid progenitor cells. These features correlate well with characteristics of leukemic cells carrying the t(8;21) translocation.

Dr Hisamaru Hirai (University of Tokyo, Tokyo, Japan) presented a lecture entitled ‘Oncogenic Mechanism by EVI-1 Protein and Potential Therapeutic Application of Histone Deacetylase Inhibitors.’ Ectopic viral integration site 1 (Evi-1) was identified as a gene existing in a common locus of retroviral integration sites in murine myeloid leukemias. Evi-1 encodes a transcription factor with two sets of zinc finger domains. The temporally and spatially restricted pattern of Evi-1 expression in embryonic tissues suggests a role in murine organogenesis and morphogenesis. Mice lacking this gene die within the first few weeks of life with multiple embryonic defects.

Evi-1 expression in hematopoietic cells is restricted to a transient stage of myeloid cell differentiation. Constitutive expression of Evi-1 in hematopoietic cells, which is caused by retroviral insertions or chromosomal abnormalities, is associated with myelogenous leukemias as well as MDS. These observations suggest that inappropriate expression of Evi-1 disturbs normal cellular proliferation and differentiation in hematopoiesis, contributing to leukemic transformation.

A study was done to explore Evi-1 associated oncogenic events, based upon determining whether it perturbs signaling of TGF-β. In summary, Evi-1 represses TGF-β signaling and antagonizes its growth inhibitory effects. This appears to be through a physical interaction with Evi-1 and Smad3, an intracellular mediator of TGF-β signaling, thereby suppressing the transcriptional activity of Smad3. These results define the novel function of Evi-1 as a repressor of TGF-β signaling.

A specific histone deacetylase inhibitor, trichostatin A (TSA), blocks Evi-1 mediated repression of TGF-β signaling, suggesting that this histone deacetylase is involved in Evi-1 mediated transcriptional repression. It also highlights a potential therapeutic approach whereby histone deacetylase inhibitors may abrogate Evi-1 inhibition of TGF-β signaling and therefore may be useful in the treatment of Evi-1-induced neoplastic tumors including myeloid leukemia.

SESSON 3: STATE OF THE ART THERAPY AND NEXT QUESTIONS

Chairperson: Pratik S. Multani

Dr James O. Armitage (University of Nebraska Medical Center, Omaha, NE, USA) gave a lecture entitled ‘Treatment of Major Subtypes of NHL: State of the Art Therapy and Next Questions.’ A recent major advance in the care of patients with NHL has been the development of the WHO classification system. Based upon this new system, the frequency of NHL subtypes are as follows: diffuse large B-cell lymphoma 31%, follicular lymphoma 22%, marginal zone/MALT lymphoma 8%, peripheral T-cell lymphoma 7%, small lymphocytic lymphoma 7%, mantle cell lymphoma 6% and 2% each of mediastinal large B-cell lymphoma, anaplastic large cell lymphoma, nodal marginal zone lymphoma and T-cell lymphoblastic lymphoma.

Dr Armitage then addressed the successes, helpful hints and the unsolved problems in the treatment of NHL. The successes include the treatment of stage I and II diffuse large B-cell lymphoma. Initial studies with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) chemotherapy alone demonstrated a 98% CR rate with a 3-year progression-free survival of 80 versus 41% for disseminated disease. Fifteen-year data from the National Lymphoma Study Group showed a 5-year overall survival of 82–100%. Recently updated results from the Southwest Oncology Group (SWOG) trial of CHOP vs CHOP with external beam radiation therapy showed that although initially the radiation therapy-containing arm appeared to have a better outcome, longer follow-up reveals that this difference is no longer apparent. This finding, however, may not apply to patients with bulky disease defined as >10 cm.

Other successes have been in the identification of the etiology and potential treatment approaches to MALT lymphoma. Burkitt’s lymphoma, with the introduction of pediatric regimens in the treatment of adults with this disease, has improved event-free survival in adults to 74–85%. Similarly, improvement in the outcome of childhood lymphoma as well as in anaplastic large cell lymphoma has shown tremendous strides.

Helpful hints have also been revealed in the early incorporation of bone marrow transplantation into treatment regimens. Allogeneic bone marrow transplantation, although still with significant morbidity, has shown great potential and appears to be a very effective treatment for a number of hematological conditions. More recently, published data from the GELA study group show improved event-free survival and overall survival in the combination of CHOP chemotherapy plus anti-CD20 monoclonal antibody, rituximab, in the treatment of elderly patients with advanced stage diffuse large B-cell lymphoma. Finally, the advent of gene array and lymphochip technology has potentially expanded our insights into the pathogenesis as well as the classification of lymphoma.

There are a number of unsolved problems that remain, foremost of which are the follicular lymphomas. These malignancies continue to demonstrate a pattern of continual relapse even in the best prognostic group. In fact, patients with follicular lymphoma with an IPI of 4 or 5 have a worse outcome than those patients with diffuse large B-cell lymphoma of similar IPI score. New drugs including interferon alpha, monoclonal antibodies, vaccines, antisense molecules and allogeneic bone marrow transplantation are being tested for follicular lymphoma, but no best therapeutic approach has been determined. Mantle cell lymphoma represents another therapeutic challenge, as does testicular lymphoma.

New insights into the biology of NHL such as identification of genes involved in lymphomagenesis and lymphoma progression will provide important targets for therapy. The
management of patients with NHL can be expected to improve dramatically in the next decade.

Dr Tomomitsu Hotta (Tokai University, Isehara, Japan) presented a lecture entitled ‘Japan Clinical Oncology Group (JCOG) Trials for Lymphoid Malignancies.’ The Lymphoma Study Group (LSG) of JCOG represents a multicenter cooperative study group that was commissioned in 1978 through a grant from the Ministry of Health, Labor and Welfare, Japan. In the 1980s several other organ cancer study groups were organized and in the 1990s, these groups, including the LSG, were formally named the JCOG. Today this group has eight cancer study groups with central data management under a steering committee. All studies are conducted according to Good Clinical Practice Guidelines.

The LSG of JCOG consists of 46 institutes throughout Japan and has conducted 19 clinical studies, including five randomized controlled trials for lymphoid malignancies. These trials have been conducted in advanced stage aggressive lymphoma, adult T-cell leukemia–lymphoma, as well as multiple myeloma. Approximately 120 patients are accrued to LSG studies per year.

In 1995 a randomized phase II study of biweekly CHOP vs dose-escalated CHOP was conducted to explore a suitable dose intensity regimen for the treatment of high/intermediate risk patients with aggressive NHL. The results of this trial show that biweekly CHOP was favored because of similar CR rates and progression-free survival but with a lower toxicity profile. Based upon these results a follow on randomized phase III trial comparing biweekly CHOP with standard CHOP in newly diagnosed advanced stage, aggressive NHL is being conducted. Another new randomized phase III trial will compare rituximab plus biweekly CHOP vs rituximab plus standard CHOP for previously untreated advanced stage low-grade B-cell lymphoma. The JCOG–LSG intends to contribute to the establishment of standard therapy for these types of lymphoid malignancies.

Dr James O. Armitage (University of Nebraska Medical Center, Omaha, NE, USA) presented a lecture entitled ‘Hematopoietic Stem Cell Transplantation for Malignant Lymphoma: the Present Consensus and Future Directions.’ There are a number of different variables involved in the use of stem cell transplantation for hematological malignancies. These include such issues as choice of conditioning regimen, purging, supportive care and stem cell source. Other broader issues include the timing of transplant with respect to the overall therapeutic approach to such patients. Specifically with respect to timing, one can explore the value of transplantation as initial therapy, treatment for patients who are slow responders to initial therapy, adjuvant therapy in patients who are in complete remission, as well as in relapsed disease.

For patients with relapsed large cell lymphoma, a randomized trial demonstrated benefit to autologous transplantation plus continuation of conventional chemotherapy, based upon a superior overall survival (53 vs 32%) and event-free survival (46 vs 12%) at 5 years. At 8 years these differences are still significant; 47 vs 27% (OS) and 36 vs 11% (EFS), respectively. Subsequent trials have demonstrated that salvage therapy resulting in initial cytoreduction followed by transplantation is superior to immediate transplantation alone.

With respect to primary therapy, a randomized trial by the GELA group showed the need for a standard course of initial chemotherapy. In this trial, chemotherapy versus a short course of chemotherapy followed by autologous bone marrow transplantation showed an inferior event-free and overall survival for the transplant group. A trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) demonstrated that patients who were slow responders to CHOP chemotherapy and went on to autologous bone marrow transplantation fared worse with respect to disease-free (35 vs 57%) and overall survival (65 vs 83%) than those patients who continued CHOP chemotherapy.

The use of autologous bone marrow transplantation as adjuvant therapy was explored by a French study group which, based upon a retrospective analysis, showed an advantage to autologous bone marrow transplantation in terms of event-free and overall survival in patients with high risk disease, defined as advanced stage or by an elevated LDH. There was no difference between the two arms in patients with low risk disease.

Based upon these trials, it appears that full dose standard chemotherapy is necessary; that transplantation after initial cytoreduction is best performed with patients who are in as good a remission as can be obtained; and that high risk patients are the best candidates, particularly those with two risk factors in the age-adjusted IPI.

There are a number of sequelae of transplantation that currently need to be explored. These include psychosexual dysfunction such as infertility, end-organ damage, including cardiac, immune and thyroid injury and finally the risk of secondary malignancies. With respect to the last issue, an increased incidence of chromosomal abnormalities has been shown in patients who received TBI as part of their preparative regimen.

**Chairperson: Brian J. Druker**

Dr Richard A. Larson (University of Chicago, Chicago, IL, USA) presented a lecture entitled ‘Treatment of Acute Leukemias and Next Questions: Cancer and Leukemia Group B (CALGB) Studies.’ The CALGB has performed a series of studies evaluating different aspects of induction and post remission treatment in adults with AML and acute lymphoblastic leukemia (ALL). The CALGB has also recently supplemented these trials by conducting systematic studies of morphology, immunophenotype, cytogenetics and molecular genetics, leading to the identification of different risk subgroups that may warrant individualized treatments. These efforts have focused on such issues as multi-agent versus single agent post-remission therapy, modulation of multi-drug resistance with the investigational agent PSC-833, cytogenetically determined post-remission therapy, autologous stem cell transplantation in first remission and immunotherapy, specifically with interleukin-2 (IL-2).
A phase III clinical trial (CALGB 9222) examined the question of consolidation therapy in patients aged less than 60 years with de novo AML after standard induction therapy. Patients were randomized to receive either three courses of high-dose cytarabine or sequential multi-agent chemotherapy, consisting of a single course of high-dose cytarabine, etoposide and cytarabine was established. A second phase I segment established the MTD of this combination with PSC-833. These results showed that less than half the dose of daunorubicin and etoposide could be given in combination with PSC-833. The dose-limiting toxicity was grade 4 mucositis and reversible hepatotoxicity. There is now an ongoing study (CALGB 19808) comparing these two induction regimens and testing the utility of MDR modulation in young patients with AML. This study will enroll patients with favorable cytogenetics and randomize then after receiving induction therapy to high-dose cytarabine x3 followed by observation or IL-2. Patients with normal or unfavorable cytogenetics will undergo autologous stem cell transplantation, again followed by observation or IL-2.

Another trial evaluating ADE vs ADE + PSC-833 in cytogenetics with de novo AML and age greater than 60 years has also been conducted. Again, a phase I trial was conducted to determine the MTD for each arm of treatment. The phase III study was stopped early after 120 patients were accrued, however, owing to excessive toxicity on the ADEP arm. The ADE arm is still ongoing. To date the results show a 46% CR rate on the ADE arm versus 39% on the ADEP arm. Mortality was 20% on the ADE arm versus 44% on the ADEP arm. Overall survival is not statistically significantly different, although disease-free survival may be increased in the ADEP arm.

Concomitant studies were done on blood samples from 33 patients on each arm in which bone marrow or blood samples were analyzed for the presence or absence of efflux of fluorescent dyes to demonstrate whether PSC-833 was modulating MDR. Among the efflux positive patients, there appeared to be a longer disease-free survival in the ADEP versus the ADE arm (14 vs 5 months, P = 0.07). In patients receiving ADE, those who were efflux negative did significantly better than those who were efflux positive. On the ADEP arm, efflux negative and positive patients did similarly; however, the efflux negative patients fared worse on the ADEP arm than on the ADE arm. This may suggest that the efflux negative patients were under treated on the ADEP arm in which the chemotherapy drugs were dose reduced.

Studies in ALL have accrued more than 600 patients to date. These trials have demonstrated that survival varies by cytogenetic subsets. Poor subsets include those with the Philadelphia chromosome [t(9;22)], +8 and t(4;11). Favorable cytogenetics include del(12p) and t(12p). These and other studies are continuing to explore and establish new therapeutic standards for the treatment of these patients.

Dr Hisashi Sakamaki (Tokyo Metropolitan Komagome General Hospital, Tokyo, Japan) presented a lecture entitled ‘Treatment of Acute Leukemia: Experience of Japan Adult Leukemia Study Group (JALSG).’ The JALSG was organized in 1987 to establish standard chemotherapy for leukemia in Japan and contribute to the worldwide improvement of leukemia therapy. To date three trials for ALL and five for AML have been completed. The JALSG encompasses 196 institutions. ALL87 and ALL89 introduced response-oriented individualized therapy. A more intensified induction therapy was used in ALL93. With this trial the complete remission rates had risen to 78% with a 30% disease-free survival at 4–6 years.

The first AML trial, AML87, randomly assigned patients to induction therapy with or without vincristine and four or 12 courses of maintenance therapy. Cytarabine and BHAC were compared in AML89. AML92 examined the usefulness of VP-16 for induction therapy as well as a separate protocol for patients with M3 AML that used all-trans-retinoic acid (ATRA). Finally, response-oriented individualized induction therapy and standard therapy were compared in AML95. These trials demonstrated that the addition of vincristine or VP16 to AML induction therapy was not beneficial in terms of CR or disease-free survival. Also 12 courses of maintenance therapy yielded a superior disease-free survival than four courses. Finally, standard chemotherapy was comparable to response-oriented chemotherapy. Future trials will examine the utility of intensifying post-remission regimens especially in patients with AML.

SESSION 4: MOLECULAR TARGETING THERAPY OF MYELOID LEUKEMIAS

CHAIRPERSON: RICHARD A. LARSON

Dr Eric L. Sievers (Fred Hutchinson Cancer Research Center, Seattle, WA, USA) presented a lecture entitled ‘New Therapeutic Options for Patients with Recurring Acute Myeloid Leukemia.’ Although most patients with AML achieve an initial remission with combination chemotherapy, ~60–80% of patients eventually relapse. Unfortunately, only 14% of patients whose initial remission duration was less than 1 year achieve a second remission using cytarabine-containing regimens. Furthermore, these regimens require prolonged hospitalizations and roughly half the patients develop serious or fatal infections.

Based upon these observations, investigators have explored strategies to ablate leukemia cells selectively using anti-CD33 monoclonal antibodies. The CD33 antigen is normally expressed on myelo-monocytic progenitors and precursors, monocytes and myeloid dendritic cells. Leukemic blast cells in >80% of AML patients express CD33. Because this antigen is not present either on non-hematopoietic tissue or on normal
primitive hematopoietic progenitors, this antibody has been evaluated as a means of developing targeted therapy for AML. Because the unconjugated anti-CD33 monoclonal antibody has minimal efficacy, particularly in patients with a high AML blast cell burden, cytotoxic small molecules and radioisotopes have been used as conjugates as a means of increasing potency.

Gemtuzumab ozogamicin (CMA-676, Mylotarg; Wyeth-Ayerst Laboratories, Philadelphia, PA, USA) consists of a humanized anti-CD33 monoclonal antibody conjugated to calicheamicin. Studies evaluated this agent in 142 patients with CD33-positive AML in first relapse who lacked a history of an antecedent hematological disorder. The drug was given as a 2 h intravenous infusion at 9 mg/m² at 2 week intervals for two doses. Patients who received the drug had a relatively high incidence of myelosuppression as well as grade 3 or 4 hyperbilirubinemia (23%) and elevated hepatic transaminases (17%). The incidence of grade 3 or 4 mucositis and infections was relatively low (4 and 28%, respectively). Thirty percent of patients achieved a remission, defined as ≤5% blasts in the bone marrow, neutrophil count >1500 and red cell and platelet transfusion independence. Relapse-free survival tended to be brief unless consolidation with high dose therapy with stem cell support or further chemotherapy was delivered.

Recent trials in the pediatric AML patient population have shown results comparable to those found in adults. Studies after transplantation have shown a high rate of veno-occlusive disease that is often fatal. Studies are also under way exploring the use of this agent in combination with chemotherapy, although these studies also show a high incidence of veno-occlusive disease. Overall gemtuzumab ozogamicin represents a major advancement in the treatment of patients with AML and represents the first introduction of targeted therapy to this patient population.

Dr Akihiro Takeshita (Hamamatsu Medical College, Hamamatsu, Japan) presented a lecture entitled ‘Cytocidal Effect of Humanized Anti-CD33 Monoclonal Antibodies (CMA-676) on Leukemia Cells.’ Gemtuzumab ozogamicin has been introduced in the therapy of patients with relapsed AML. However, its mechanism of action has not been well elucidated. The mechanism of cytotoxic effect on a number of leukemia cell lines as well as multi-drug resistant cell lines was investigated by cell cycle distribution and morphology. A dose-dependent selective cytotoxic effect was observed in cell lines that expressed CD33 and was dependent on the amount of CD33 molecules present as well as the proliferative rate of cells. Sensitive cells were temporarily arrested at the G2/M phase before increasing in hypodiploid portion and undergoing morphological changes. Gemtuzumab ozogamicin was not effective on P-gp-expressing multi-drug resistant sublines compared with parental cell lines. Multi-drug resistant modifiers, MS209 and PSC-833, restored the cytotoxic effect of gemtuzumab ozogamicin in the P-gp-expressing multi-drug resistant sublines.

Clinical samples from 24 patients with AML were also analyzed in vitro. The cytocidal effect of gemtuzumab ozogamicin was inversely related to the amount of P-gp expression as well as P-gp function. The use of MDR modifiers reversed gemtuzumab ozogamicin resistance in the P-gp-positive CD33+ leukemia cells. In CD33+ AML cells from 11 patients, gemtuzumab ozogamicin was less effective on the CD33+, CD34- subgroup than the CD33+, CD34+ subgroup. Gemtuzumab ozogamicin represents a promising agent in the treatment of patients with AML that expresses CD33. The combination of this agent with MDR modifiers may increase its selective cytotoxic effect in multi-drug resistant CD33+ AML.

Chairperson: Richard E. Champlin

Dr Brian J. Druker (Oregon Health Sciences University, Portland, OR, USA) presented a lecture ‘STI571: a Specific Inhibitor of the BCR-ABL Tyrosine Kinase in the Treatment of Myeloid Leukemia.’ The Bcr-Abl fusion protein is a 210 kDa molecule that results from a t(9;22) chromosomal translocation and is present in virtually all patients with CML. There is also a 185 kDa variant that is present in ~20% of patients with ALL. The transforming function of Bcr-Abl requires the tyrosine kinase activity of these fusion proteins, which is elevated compared with that seen with c-Abl. Hence this represents an ideal target for a therapeutic agent since an inhibitor of Bcr-Abl kinase would be predicted to give a very effective and selective therapeutic effect in CML.

STI571 (Gleevec or Glivec; imatinib mesylate) is a tyrosine kinase inhibitor optimized for its ability to competitively inhibit at the ATP-binding site of Abl, platelet-derived growth factor receptor (PDGFR) and Kit tyrosine kinases. It induces either growth arrest or apoptosis, specifically in Bcr-Abl expressing hematopoietic cells with no obvious effects on normal cells or in cells transformed by other tyrosine kinase oncogenes. It is also highly bioavailable.

A phase I clinical trial was conducted in patients with chronic phase CML who had failed interferon therapy. No dose-limiting toxicity was encountered and no MTD was achieved despite doses up to 1000 mg. Optimal responses, however, were observed at 400–600 mg. There was a 98% complete hematological response rate and 96% of these were durable. Over 50% of patients had cytogenetic responses with 31% major and 10% complete cytogenetic remissions. When studied in patients in blast crisis there was a 59% CR rate, of which 18% were durable.

These exciting data led to multi-institutional, international phase II studies. In 532 patients with chronic phase CML who had failed interferon therapy, the hematological response rate was 95%, the hematological CR rate was 95% and the cytogenetic CR rate was 41%. Of the 235 patients with accelerated phase CML, 17% achieved a cytogenetic CR, while in 260 patients in blast crisis there was a 7% cytogenetic CR rate. Most of these were achieved in the first 5–6 months and about 10% were achieved at a later period. Patients in myeloid or lymphoid blast crisis had a 20–30% incidence of severe myelosuppression representing patients whose hematopoiesis was dependent on the Philadelphia positive clone. At 18 months, progression occurred in 9% of patients in chronic phase versus 78% of patients in blast crisis.
The major adverse events were vomiting, nausea and periorbital edema, all of which were uniformly mild. There was only 1–2% incidence of grade 3 or 4 toxicity. Questions remain why some patients relapse. This may be that Bcr-Abl has been reactivated or that there are Abl kinase domain mutations. Combination studies are ongoing with STI571 and interferon as well as low-dose cytarabine in chronic phase CML. STI571 in combination with standard induction therapy is being examined in patients in blast crisis and Philadelphia+ ALL.

Dr Yuzuru Kanakura (Osaka University, Osaka, Japan) presented a lecture entitled ‘C-Kit Activating Mutations: Their Roles in the Development of Hematological and Gastrointestinal Malignancies.’ The c-kit receptor tyrosine kinase is characterized by the presence of five immunoglobulin (Ig)-like repeats in the extracellular domain and an insert that splits the cytoplasmic kinase domain into the ATP-binding and phosphotransferase regions. The interaction of KIT with its ligand, designated stem cell factor (SCF), is known to play a crucial role in proliferation, differentiation, migration and survival of hematopoietic stem cells, mast cells, melanocytes, primordial germ cells and interstitial cells of Cajal.

Point mutations in the juxtamembrane or phosphotransferase domains of the c-kit gene can lead to constitutive activation. Such constitutively activating mutations have been detected in neoplastic mast cell lines as well as in cells from patients with mastocytosis, MDS and AML. Activating mutations in the juxtamembrane domain of c-kit have been found in a number of patients with gastrointestinal stromal tumors, the most common mesenchymal tumors in the human digestive tract. These tumors originate from interstitial cells of Cajal, expressing both KIT and CD34.

When wild-type KIT and the constitutively active mutants of KIT were treated with increasing doses of tyrosine kinase inhibitors of AT1296 or STI571, the kinase activity of the juxtamembrane mutant was more effectively inhibited, whereas that of the catalytic domain mutant was not affected at all. These results suggest that some but not all KIT mutants may be effectively blocked as a therapeutic target by tyrosine kinase inhibitors.

Dr Brian J. Druker (Oregon Health Sciences University, Portland, OR, USA) presented a lecture entitled ‘Efficacy of an Oral Molecularly Targeted Therapy, STI571, in Gastrointestinal Stromal Tumors Expressing C-Kit (CD117).’ Gastrointestinal stromal tumors (GIST) have recently been recognized as a distinct clinicopathological entity. They occur in ~1 per 100 000 individuals. They likely originate from the interstitial cells of Cajal, express CD117 (c-kit) and are highly refractory to therapy, with response rates to chemotherapy of <5%. Median overall survival after tumor resection is ~66 months versus 9–12 months in locally advanced disease and 10–20 months in metastatic disease.

A multicenter phase II trial of STI571 in the treatment of patients with unresectable or metastatic GIST was initiated in July 2000. Patients in this trial were randomized to receive 400 vs 600 mg of STI571 per day. The trial enrolled 147 patients: 59% achieved a PR, 26% achieved stable disease and 13% experienced progressive disease. Gastrointestinal bleeding and neutropenia was seen in 5 and 3% of patients, respectively. Of note, there was a 76% PR rate in patients with an exon 11 mutation (activated KIT) versus 20% PR rate with wild-type KIT. Also of note was that many of the PRs with residual masses were negative on positron emission tomography (PET) scanning. These dramatic results have led to an NCI-sponsored phase III intergroup trial of STI571 for metastatic GIST comparing doses of 400 and 800 mg. The accrual of this trial was completed in September 2001 after exceeding the target goal of 600 patients. Additional clinical trials of neoadjuvant and adjuvant of STI571 are planned. As a whole these clinical trials have substantiated the concept that drugs targeted against a tumor-specific abnormality will have therapeutic utility in solid tumors as well as hematological malignancies.

SESSION 5: ADULT T-CELL LEUKEMIA–LYMPHOMA AND HUMAN RETROVIRUS

Dr Shigeo Hino (Tottori University, Yonago, Japan) presented a lecture entitled ‘Updated Results of an Intervention Study against the Mother-to-Child Transmission of Human T-Cell Leukemia Virus Type I.’ It has been widely accepted that breastfeeding plays an important role in the mother-to-child transmission of human T-cell leukemia virus type I (HTLV-I), the causative agent of adult T-cell leukemia–lymphoma (ATL). However, it has not been demonstrated that refraining from breastfeeding by carrier mothers can stem the transmission of this virus. In a prefecture-wide intervention study called the ATL Prevention Program (APP), the ability to influence breastfeeding and therefore HTLV-I transmission was investigated during a 12-year period. Over 100 000 pregnant women in third trimester were screened for anti-HTLV-I antibodies in Nagasaki, Japan since 1987. The seropositive mothers were asked to refrain from breastfeeding and to bring their children in for check-ups to assess antibody status. Children with a follow-up period of at least 2 years were analyzed.

Without any intervention, the prevalence of antibody-positive women decreased significantly from 8% for those born in 1945 to below 2% for those born in 1975. Among the children born to carrier mothers, 18.4% of those who breastfed long-term were infected with HTLV-I in contrast to 3.2% of bottle-fed children. The incidence among children breastfed for 1–5 months was 11.4%. Considering the trend toward the decrease in the maternal prevalence, the prevalence among pregnant women born in 1987 is estimated to be 1%. These results confirm that breastfeeding plays the leading role in the mother-to-child transmission of HTLV-I, even for periods as short as 1–5 months. They also suggest that the APP has prevented ~800 new infections during the 12-year study period and is expected to prevent the development of 40 cases of ATL in the future.
SESSION 6: BIOLOGICAL THERAPY OF MALIGNANT LYMPHOMA

CHAIRPERSON: ROBERT KREITMAN

Dr Larry W. Kwak (National Institutes of Health, Bethesda, MD, USA) gave a lecture entitled ‘Translational Development of Active Immunotherapy for Hematological Malignancies.’ The central hypothesis of active immunotherapy of cancer is that either the tumor cell itself or antigens derived from the tumor cell that are specific or at least selective, for the tumor cell can be modified and injected back into the patient as a therapeutic vaccine. The desired result is activation of both major arms of the immune response, the host antibody response and a host T-cell response.

Recently, in support of cancer vaccine development efforts, a large number of potential tumor antigen candidates have been identified for melanomas and solid tumors. Increasingly, a number of potential tumor antigens have also been identified for hematological malignancies, including minor histocompatibility antigens, HA-1 and HA-2, proteinase-3 and WT1. However, one of the limitations of the cancer vaccine hypothesis is that this experiment has already failed in nature. That is, by virtue of the tumor’s clinical appearance, the host immune system has already failed to recognize it. Thus the primary question facing researchers at this point is whether it is even possible to immunize against an inherently weak, self tumor antigen. Cancer vaccine development must be therefore focused on answering two independent questions in the proper sequence. The first question is a scientific one: whether one can immunize against a tumor antigen. Answering this scientific question is the goal of most phase I and II cancer vaccine clinical trials.

As one example, lymphomas express a tumor-specific antigen that can be targeted by cancer vaccination. The ability of a new idiotypic vaccine formulation to elicit T-cell immunity in 20 patients in a homogeneous, chemotherapy-induced first clinical complete remission was recently studied. Nineteen of the 20 patients tested showed tumor-specific CD8+ T-cell responses using autologous tumor targets as the read-out for these assays. In addition, 11 patients had detectable t(14;18) translocations and were PCR-positive in the blood both at diagnosis and after chemotherapy, despite being in complete remission. However, eight of 11 patients converted to PCR-negative after vaccination. Taken together, these results definitively answer the scientific question of whether one can immunize against this tumor antigen.

A pivotal, multicenter, phase III randomized, controlled clinical trial with the clinical endpoint of disease-free survival, was opened in January 2000 to provide the definitive answer to the second major questions facing the cancer vaccine field: can immunization produce clinical benefit? In addition, the analysis of T-cell responses against autologous tumor targets and vaccine administration in a minimal residual disease setting provide general principles relevant to the design of future clinical trials of cancer vaccines in other tumor types.

Dr Kensei Tobinai (National Cancer Center Hospital, Tokyo, Japan) gave a lecture entitled ‘Chimeric Mouse-Human Anti-CD20 Monoclonal Antibody (Rituximab) for B-Cell Lymphoma: Clinical Trials in Japan.’ Rituximab is a chimeric monoclonal antibody with mouse variable and human constant regions that recognizes the CD20 antigen. Rituximab induces apoptosis of human B-cell NHL cell lines, in addition to cell lysis by complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC).

Twelve patients with relapsed CD20+ B-cell NHL were enrolled in a phase I trial: four at 250 mg/m² and eight at 375 mg/m², once a week for 4 weeks. Commonly observed toxicities were grade 1 or 2 infusion-related events. Of the 11 eligible patients, two showed CR and five showed PR. A pharmacokinetic analysis showed that the elimination half-life (T1/2) of rituximab was 445 ± 361 h and serum rituximab levels were still measurable at 3 months in most patients.

A subsequent phase II study enrolled 90 relapsed patients with indolent B-cell NHL and mantle cell lymphoma (MCL) and they were treated with rituximab at 375 mg/m² × 4 weekly infusions. Factors affecting response and progression-free survival (PFS) were analyzed for the 77 eligible patients with confirmed pathology of indolent B-cell NHL or MCL by the central pathology review. The overall response rates (ORR) in indolent B-cell NHL and MCL were 61 and 46%, respectively. The median PFS was 245 days for patients with indolent B-cell NHL and 111 days for those with MCL. Multivariate analysis revealed that the number of prior regimens affected ORR and the PFS was affected by the following three factors: disease type (indolent versus MCL), extranodal involvement and number of prior regimens. The PFS of patients showing higher serum rituximab levels immediately before the third infusion was significantly longer than those with lower serum rituximab levels. In conclusion, rituximab is more effective in the treatment of relapsed indolent B-cell NHL than of MCL. Several prognostic factors and serum rituximab levels are useful for predicting the efficacy of rituximab monotherapy. Subsequent rituximab studies in Japan that have completed accrual include a randomized phase II study of rituximab plus CHOP (concurrent vs sequential administration) for untreated indolent B-cell NHL and a single agent phase II study of rituximab in relapsed or refractory aggressive B-cell NHL. The latter trial demonstrated a 37% (21/57) overall response rate.

In 2001, the Ministry of Health, Labor and Welfare in Japan approved rituximab. The JCOG is ready to initiate a randomized phase II/III study of rituximab in combination with CHOP vs biweekly CHOP for untreated patients with advanced indolent B-cell NHL. Another study being planned is a phase II study of high-dose chemotherapy followed by autologous stem cell transplantation, incorporating in vivo purging of B-lymphoma cells with rituximab. The ongoing clinical trials will define the future role of rituximab in the treatment of B-cell NHL and related disorders.
Dr Pratik S. Multani (IDEC Pharmaceuticals, San Diego, CA, USA) presented a lecture entitled ‘Zevalin Radioimmunotherapy for Low Grade, Follicular or CD20+ Transformed Non-Hodgkin’s Lymphoma.’ The Zevalin (ibritumomab tiuxetan; IDEC Pharmaceuticals, San Diego, CA, USA) regimen consists of rituximab (250 mg/m²) followed within 4 h by [111In]ibritumomab tiuxetan on day 1. On day 7, 8 or 9, a second dose of rituximab (250 mg/m²) is followed within 4 h by [90Y]ibritumomab tiuxetan (0.4 mCi/kg or 0.3 mCi/kg if platelets 100K–150K; maximum 32 mCi). The regimen is administered as outpatient therapy with minimal radiation precautions required. Three hundred and forty-nine patients with relapsed or refractory low- or intermediate-grade, follicular or CD20+ transformed B-cell NHL were treated in five clinical trials: a phase I/II trial, a phase II trial in patients with mild thrombocytopenia, a phase III randomized trial of ibritumomab tiuxetan; IDEC Pharmaceuticals, San Diego, CA, USA) regimen versus rituximab (375 mg/m²) followed within 48 h by 5-fluorouracil and leucovorin, a phase I/II trial, a phase II trial in patients with mild thrombocytopenia, a phase III randomized trial of ibritumomab tiuxetan in patients with rituximab-refractory follicular NHL (defined as no response or response with time to progression of <6 months) and an expanded access trial in patients with relapsed or refractory NHL. All patients had <25% bone marrow involvement, absolute neutrophil count (ANC) >1500/mm³, platelets >100K/mm³ and no prior high-dose therapy. These patients were a refractory population: median age 60 years (range: 24–85 years); 42% with bone marrow involvement; 16% intermediate/high or high IPI risk groups; 31% with ≥4 prior therapies. Overall response rate (ORR) for the two phase III trials, using the International Workshop Response Criteria for NHL, were determined by an independent panel of oncologists and radiologists who were experts in lymphoma and were blinded to investigator assessment of response and treatment arm.

The ORR for the randomized trial was 80% (34% CR/CRu) in the ibritumomab tiuxetan arm and 56% (20% CR/CRu) in the rituximab arm. The ORR in the rituximab-refractory trial was 74% (15% CR/CRu). Toxicity was primarily hematologic. Median nadirs: ANC = 800/mm³; platelets = 40K/mm³; and hemoglobin (Hgb) = 10.3 g/dL. The median duration below an ANC of 1000 cells/mm³ or platelets of 50K/mm³, based upon the time from the first day in grade 3 to the last day in grade 3 after nadir, was 8 and 10 days, respectively, for patients who received the 0.4 mCi/kg dose and 14 and 16 days, respectively, for those patients who received the 0.3 mCi/kg dose. Medication and transfusion data were collected in four of the trials and revealed that 22 and 20% of patients received platelet and red cell transfusions, respectively. Also, 12.8 and 8.1% of patients received G-CSF and erythropoietin, respectively. The presence of bone marrow involvement at baseline was associated with a significantly greater incidence of grade 4 neutropenia (P = 0.001) and thrombocytopenia (P = 0.013). Bleeding events were grade 3 or 4 in 1.7% of patients and 7% of patients were hospitalized with infection or febrile neutropenia. In summary, ibritumomab tiuxetan therapy was effective and well tolerated, even in this refractory population at risk for toxicity.

Dr Robert Kreitman (National Cancer Institute, Bethesda, MD, USA) presented a lecture entitled ‘Recombinant Immunotoxins for the Therapy of Chemotherapy-resistant Hematologic Malignancies.’ Recombinant immunotoxins are proteins that contain an antibody fragment genetically linked to a modified toxin. Examples of toxins employed include Pseudomonas exotoxin and Diphtheria toxin. Both these natural products effect ADP-ribosylation of elongation factor-2, resulting in cessation of protein synthesis and cell death. Anti-Tac(Fv)–PE38 (LMB-2) is a construct containing the variable light and heavy domains of the anti-CD25 monoclonal antibody, anti-Tac, fused together with a peptide linker, which is then fused to a truncated form of Pseudomonas exotoxin (PE38), which has its native binding domain removed. LMB-2 was studied in 39 patients with chemotherapy-resistant lymphoma, leukemia or Hodgkin’s lymphoma, who each received 2–63 µg/kg every other day for three doses. The MTD was 40 µg/kg QOD×3. The dose-limiting toxicity was grade 4 hepatic transaminase elevations; two patients also experienced grade 4 elevations of creatine phosphokinase with respiratory failure. The most frequent adverse events were well-tolerated hepatic transaminase elevations and fever. Antibodies to LMB-2 developed in 15% of patients after one cycle of therapy. One CR and seven PRs were observed, particularly in patients with hairy cell leukemia (HCL). LMB-2 is undergoing further testing in such patients and is also being tested in graft-versus-host disease (GVHD).

Another construct, RFB4(dsFv)–PE38 (BL22), contains the fused variable light and heavy chain domains from an anti-CD22 antibody fused to PE38. BL22 was studied in 40 patients with chemotherapy-resistant B-cell lymphoma, chronic lymphocytic leukemia (CLL) or HCL. BL22 was studied in 40 patients with chemotherapy-resistant B-cell lymphoma, chronic lymphocytic leukemia (CLL) or HCL. Patients received doses of 3–50 µg/kg QOD x3. Four patients developed hemolytic-uremic syndrome, but the most common adverse event was hypoalbuminemia. Responses were observed in 21 out of 25 patients with HCL (18 CR, 3 PR). Recombinant immunotoxins therefore represent an active therapy in patients with chemotherapy-resistant hematological malignancies. Both agents are undergoing further testing to optimize their efficacy and safety.

SESSION 7: RECENT ADVANCES IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Dr Richard Champlin (M. D. Anderson Cancer Center, Houston, TX, USA) presented a lecture entitled ‘Non-myeloablative Preparative Regimens for Allogeneic Hematopoietic Transplantation.’ Non-myeloablative regimens have approximately half the incidence and severity of graft-versus-host disease (GVHD) when compared with myeloablative allogeneic transplantation, allowing many patients to be treated who would otherwise be ineligible for myeloablative procedures. Specifically, non-myeloablative regimens have a lower rate of acute GVHD and infectious complications.
Hematological malignancies differ in their susceptibility to graft-versus-leukemia or lymphoma (GVL) effects. The most potent effects are seen in CML, low-grade NHL and CLL. Intermediate effects are seen in AML, multiple myeloma, diffuse large cell NHL and Hodgkin’s lymphoma. Little GVL activity is observed in ALL and high grade NHL. These differences may in part be due to the more rapid tumor cell proliferation seen in the less responsive diseases, leading to disease growth that outpaces the immune response. Moreover, the diseases in which GVL effects are potent constitute malignancies of antigen-presenting cells, which may augment the immune response.

A central question facing non-myeloablative transplantation revolves around the role of cytoreduction versus GVL in achieving optimal results. A regimen of fludarabine, melphalan, with or without anti-thymocyte globulin, was employed in patients with advanced, acute leukemia. This regimen was sufficiently immunosuppressive to allow engraftment of unrelated or one-HLA disparate related transplants and proved to be superior to results seen with truly non-myeloablative regimens (e.g. FLAG-Ilda) in AML/MDS patients. Follow-up demonstrated that 56% of patients with chemotherapy-sensitive disease treated with this regimen remained in continuous remission beyond 1 year. These results are similar to those achieved with ablative preparative regimens in younger, but otherwise similar patients.

A regimen of fludarabine, cytarabine and cisplatin has demonstrated durable remissions in patients with advanced CLL or transformed NHL. Another combination non-myeloablative preparative regimen consisting of fludarabine, cyclophosphamide and rituximab has also demonstrated efficacy with acceptable toxicity in 20 patients with relapsed follicular NHL, rendering 17 patients disease-free based upon PCR analysis.

Thus the use of relatively non-toxic, non-myeloablative preparative regimens allows engraftment and the generation of GVL effects, permitting potentially curative therapy for susceptible malignancies and the treatment of patients who would otherwise not be eligible for myeloablative allotransplants. The indications for a non-myeloablative vs myeloablative preparative regimen need to be defined in controlled clinical trials.

Dr Shin Mineishi (National Cancer Center Hospital, Tokyo, Japan) presented a lecture entitled ‘Allogeneic Reduced-Intensity Transplantation for Rare Solid Tumors: Update of National Cancer Center Hospital Experience.’ The recently introduced RIST program is based on the concept of intensifying immunosuppression primarily to enhance the engraftment of donor cells, rather than cytoreduction. Evidence has accumulated that stable engraftment can be achieved without myeloablation, when agents with potent immunosuppressive activities are used appropriately. Thereafter, established donor-derived lymphohematopoiesis provides a basis to exert antitumor effects.

Reported evidence of a graft-versus-tumor (GVT) effect against solid tumors has stimulated interest in the possible application of this procedure to a variety of solid tumors. Attempts have been made intentionally to induce the early occurrence of GVHD, which has been considered to be a marker of a GVL effect, through the omission or early withdrawal of prophylaxis for GVHD. However, it has become clear that the maximum induction of GVT does not necessarily rely on the clinical manifestation of GVHD.

In a phase I/II RIST study with cladribine or fludarabine + BU + ATG regimen, which was performed between April 2000 and October 2001, a total of 13 patients with a variety of metastatic solid tumors including renal cell carcinoma (RCC, n = 6), rhabdomyosarcoma (RMS, n = 2), malignant melanoma (n = 2), neuroblastoma (n = 2) and osteosarcoma (OS, n = 1) were treated. All received peripheral blood stem cell transplantation (PBSCT) from related HLA-identical or one antigen-mismatched donors. Regimen-related toxicities were mild and >90% donor chimerism was achieved before day 30 in all patients. In all three patients with non-clear cell type RCC, transient GVT effect was observed. The remaining three patients with clear cell type RCC remain in stable disease. One
patient with malignant melanoma had stable disease 1 year after transplant, although subsequently died of progressive disease on day 433. One of the two patients with RMS did not show any GVT effect even with grade III acute GVHD and died of progressive disease on day 56. In contrast, the patient with OS showed evidence of clinical response when he developed grade IV acute GVHD. This data suggest that such an immunotherapeutic approach may be available to patients with certain solid tumors.

CLOSE

Dr James O. Armitage closed the meeting by speaking on behalf of all of the participants and proclaiming the meeting a success.

Acknowledgments

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