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2015 Guidance on cancer immunotherapy development in early-phase clinical studies

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The development of cancer immunotherapies is progressing rapidly with a variety of technological approaches. They consist of "cancer vaccines", which are based on the idea of vaccination, "effector cell therapy", classified as passive immunotherapy, and "inhibition of immunosuppression", which intends to break immunological tolerance to autoantigens or immunosuppressive environments characterizing antitumor immune responses. Recent reports showing clinical evidence of efficacy of immune checkpoint inhibitors and adoptive immunotherapies with tumor-infiltrating lymphocytes and tumor-specific receptor gene-modified T cells indicate the beginning of a new era for cancer immunotherapy. This guidance summarizes ideas that will be helpful to those who plan to develop cancer immunotherapy. The aims of this guidance are to discuss and offer important points in early phase clinical studies of innovative cancer immunotherapy, with future progress in this field, and to contribute to the effective development of cancer immunotherapy aligned with the scope of regulatory science. This guidance covers cancer vaccines, effector cell therapy, and inhibition of immunosuppression, including immune checkpoint inhibitors.

As our understanding on the mechanism of host immune responses to cancer significantly advances, the development of cancer immunotherapies progresses at a growing pace with a variety of technological approaches. They include "cancer vaccines", which are based on the idea of vaccination, a typical immunological modality against infectious diseases by inducing active immune responses in the human body, "effector cell therapy", classified as passive immunotherapy, and "inhibition of immunosuppression", which intends to break immunological tolerance to autoantigens or immunosuppressive environments characterizing antitumor immune responses. Specifically, recent reports showing clinical evidence of efficacy of immune checkpoint inhibitors and adoptive immunotherapies with tumor-specific receptor gene-modified T cells strongly indicate the beginning of a new era for cancer immunotherapy.[1,2]

Most target antigens of previous cancer immunotherapies have been autoantigens. Various immune tolerance mechanisms inherent in the human body can suppress or prevent immune responses against autoantigens. Therefore, the immune responses induced by cancer immunotherapy are often less robust compared to those against foreign antigens such as viruses. In addition, cancer-bearing hosts create an immunosuppressive environment, especially in the tumor microenvironment, comprised of a wide variety of complex molecular mechanisms mediated by the tumor. It is reported that formation of immunosuppressive environments becomes enhanced as the cancer progresses. Recent advances in analysis of the interaction between cancer and immunity have revealed a more detailed picture of the anticancer immune response in terms of both positive and negative impacts on the molecular level.[3,4]

On the other hand, cancer immunotherapy targeting autoantigens may lead to the development of autoimmune reactions, causing tissue injuries; this has been reported in some studies.[5,6] In nature, an immune checkpoint is a biological mechanism for inducing and maintaining self-tolerance and homeostasis, suggesting that an immune checkpoint inhibitor can cause certain autoimmune reactions with varying severity. Therefore, considerations of safety in intensive immunotherapy, together with improved efficacy of cancer immunotherapy, become increasingly important.

Based on the information and the current understanding of cancer immunology, which is a rapidly evolving field, this Guidance summarizes ideas helpful to those who plan to develop useful cancer immunotherapy. The aims of this Guidance are to discuss and offer important points while carrying out clinical studies of innovative cancer immunotherapy, with future progress in this field in mind, and contribute to the effective development of cancer immunotherapy aligned with the scope of regulatory science. This Guidance explains cancer

vaccines, effector cell therapy, and inhibition of immunosuppression, including immune checkpoint inhibitors.

This Guidance has been prepared as a guide for academics, healthcare professionals, and industry engaged in clinical development in the field of cancer immunotherapy. This Guidance describes concepts of Early-Phase Clinical Studies.

Development of Cancer Immunotherapy

Cancer immunotherapy approaches: Safety and efficacy characteristics. Cancer immunotherapy intervenes the immune system composed of various immune cells and molecules in cancer-bearing hosts so that it targets cancer cells, aiming to ultimately destroy them or inhibit their growth. Although host immune responses to cancer remain largely unknown, recent progress in tumor biology, immunology, and molecular genetics and a deeper understanding of these fields have identified the involvement of various immune cells associated with both innate and acquired immunity in every stage of cancer development, and non-neoplastic and non-immune cells in a tumor microenvironment. The interaction of "cancer immunity" is being understood based on the exchange of molecular information between these cells. Based on the current understanding of "cancer immunity" mentioned above, cancer immunotherapy attempts an active intervention for cancer immune responses, which change over time and space, and has started to reveal remarkable clinical efficacy in some trials. Cancer vaccines, effector cell therapy, and inhibition of immunosuppression described here are major options for cancer immunotherapy, which are currently in development, and they have the following characteristics in terms of clinical safety and efficacy.

Cancer vaccines. Cancer vaccines are based on vaccination, a traditional immunological technology, and are intended to be administered to cancer patients as vaccine antigen(s) prepared from tumor antigen(s).[4] They can be given as various forms and by using various administration methods. Their administration will then activate the cancer antigen-specific immune responses of effector cells, mainly $CD8^+$ T cells and $CD4^+$ T cells, both of which are key players in adaptive immune responses, through antigen processing and presentation by host immune systems. It is, however, necessary to bear in mind that, unlike vaccines for infectious diseases: (i) most antigens are host autoantigens or altered autoantigens; (ii) cancer vaccines are often administered for treatment of tumors already existing in host bodies but not for prophylaxis purposes; (iii) vaccine antigen(s) is further administered to hosts while the tumor still expresses the tumor antigen(s); and (iv) the purpose of cancer vaccination is to induce an active immune response in the presence of tumor-mediated immunosuppression.[3]

Vaccine antigens administered are usually host autoantigens or altered autoantigens. For molecularly identified target antigens, vaccine antigens can be the forms of short peptides, long peptides, proteins, glycan, mRNA, or DNA.[7] For unidentified antigens, they can be components derived from cancer tissues. These antigens are administered alone or after being pulsed to, or incubated with, antigen-presenting cells (dendritic cells or other cells). Vectors such as viruses or microorganisms may be used. Recent studies have shown that it is important for enhanced immunogenicity of vaccines to administer vaccine antigen(s) in combination with a delivery system for appropriate antigen transport and/or with immuno-potentiates (i.e., adjuvants), stimulating immune responses against vaccine antigens during the course of immune responses.[8] It is generally recognized that many cancer vaccines have few adverse reactions; however, if new approaches for improving the efficacy of vaccines (e.g., delivery system, adjuvants, or immune checkpoint inhibitors) rapidly become popular, administration of cancer vaccines may offset immunological tolerance to autoantigens, resulting in adverse reactions such as normal tissue injury.

Although the number of approved delivery systems and adjuvants used for cancer vaccines is still very few worldwide, it is expected that many products will be commercialized in the field of cancer vaccine and widely used in healthcare settings. Clinical evaluation is ongoing for various delivery systems, including physical vectors, emulsions, liposomes, polymer micelles, and nanoparticles, and biological vectors, viruses and microorganisms. In a broad sense, adjuvants can include all materials that can enhance the effect of vaccines. A wide variety of materials such as metal salts, small molecule compounds, polypeptides, nucleic acid, and proteins (e.g., cytokines and others) have been assessed and clinically evaluated as adjuvants.[9] Agonists of Toll-like receptors, which have a major effect on acquired immunity through activated innate immunity, have recently been recognized as adjuvants. While fully understanding the immunological and pharmacological properties of individual materials, the proper use of various delivery systems and adjuvants is necessary considering their benefits of enhancing vaccine immunogenicity and risks of adverse reactions induced by undesirable immune responses against autoantigens.[8] Enhanced immunogenicity and potential adverse reactions may vary depending on combinations of vaccine antigens and delivery systems and/or adjuvants, and it is desirable that appropriate combinations should be considered from early stages of development.

Regarding adverse events in cancer vaccines, previous studies on cancer vaccines with various forms of vaccine antigens have rarely reported the incidence of adverse events associated with autoimmune reactions even if immune responses against vaccine antigens were clearly detected after multiple administration of vaccines. Most reported adverse events are topical or systemic reactions seemingly towards adjuvants given with vaccines. Considering, however, the future use of cancer vaccines with highly enhanced immunogenicity by delivery systems and/or adjuvants, this may increase the incidence of autoimmune-like responses to autoantigens.

As for clinical efficacy, various forms of cancer vaccines have been evaluated in clinical studies, and only a limited number of reports have described objectively defined tumor regression.[10] Although immune responses are induced in some patients, the response in peripheral blood is not necessarily consistent with clinical effect. Indications for cancer vaccines include metastatic cancer with high tumor burden, and prevention of post-surgical recurrence with minimized tumor burden by surgical procedures and/or other treatments. Results from various ongoing late-phase studies may greatly help in determining whether cancer vaccines would be clinically efficacious against various types of cancer in terms of delayed progression and prolonged survival. Standard methods for efficacy evaluation include the Response Evaluation Criteria in Solid Tumors (RECIST), used for the evaluation of tumor regression. This is often used for conventional anticancer agents. Improvement in quality of life (QOL), such as pain relief, is also often used as a surrogate endpoint;[11] however, there is no established evaluation method with a global consensus yet.

Effector cell therapy. Effector cells directly involved in the destruction of cancer cells and inhibition of their growth include $CD8^+$ T cells, $CD4^+$ T cells, γδ T cells, natural killer (NK) cells, and natural killer T (NKT) cells. These autologous cells collected from the peripheral blood or tumor in patients are *in vitro* processed and allowed to proliferate, and are subsequently infused into the patients.[2,12–17] During *in vitro* preparation of these cells, antigen-non-specific or -specific stimulation by tumor antigens or autologous tumor cells may be provided.

Recently, efforts are ongoing to develop infusion therapy with tumor antigen-specific T cells genetically engineered by transducing antigen receptor genes for lymphocyte-specific antigens and expressing them using a viral vector or other methods. [18] T-cell receptors and chimeric antigen receptors (CARs) are mainly used as antigen receptors.

Some infusion therapies with polyclonal tumor antigen-specific T cells, prepared from tumor-infiltrating lymphocytes (TILs) or with receptor-modified T cells, are reported with serious adverse events. These adverse events include direct toxicity associated with administered cytokines, immune-suppressants used for conditioning regimen or total body irradiation in order to enhance infusion therapy effect, as well as symptoms associated with modified/enhanced activity of infused cells through the conditioning regimen. A rapid proliferation and activation of infused cells due to so-called homeostatic lymphoproliferation could lead to an increased production of cytokines and inflammatory reactions in patients who have received conditioning regimen, potentially resulting in cytokine release syndrome (CRS). As for CAR T cells, a clinical study of CD19-CAR T cells in acute lymphocytic leukemia has reported a high incidence of CRS, which may have associated with a clinical effect, suggesting the need for adequate measures to be established in advance for managing the adverse event and for carefully gathering safety information.[19–21] Rapid tumor cell damage may also lead to tumor lysis syndrome. Furthermore, it has been reported that use of artificially modified antigen receptors resulted in the incidence of adverse events including deaths due to unexpected responsiveness to target antigens or similar antigens expressed in normal tissue.

Very few serious adverse events have been reported regarding infusion therapy with non-specifically activated lymphocytes, γδ T cells, NK cells, and NKT cells, although the incidence depends on the activity and dose of infused effector cells.[22]

Objective tumor regression or disappearance have been reported with infusion of TIL-derived antigen-specific T cells and antigen receptor-modified T cells, and a number of patients are also reported to achieve long-term remission. Although these approaches are still in an early development stage, further investigations are warranted to see whether delayed tumor progression or prolonged survival can be achieved as with other standard cancer therapies.

In most cases, it remains unclear if infusion therapy with non-specifically activated lymphocytes, γδ T cells, NK cells, or NKT cells would produce tumor regression. Although some studies report the efficacy in infusion therapy, it is necessary to analyze its causal relationship with the therapy. As it is inferred that a great number of patients have already received these types of therapy, it is, therefore, necessary to discuss scientific significance of the improvements in subjective symptoms and QOL. To establish scientific evidence for these treatment approaches, clinical studies should be carried out using an appropriate control group and statistical analysis.

Historically, allogeneic stem cell transplantation and associated donor lymphocyte infusion have been widely used as non-self cell-based therapy, and there have been some efforts to use non-self NK cells for infusion therapy. For effector cell therapy, patient's own lymphocytes are currently the major source. Use of non-self lymphocytes are considered to have several benefits such as ensuring homogeneity of infused cells, reducing impact on treatment outcomes due to patient's conditions, and ensuring the availability of infusion therapy. On the other hand, rejection of infused cells, graft-*versus*-host disease, and risk of pathogens are among the issues need to be overcome in order to commercialize this technology.

Inhibition of immunosuppression. Most identified cancer antigens are so-called autoantigens, and it is assumed that immunological tolerance has been developed in the host body as expression of these antigens at any point, including the fetus period. Therefore, immune responses against these antigens are weakened by various mechanisms. Moreover, recent cancer immunology studies revealed that growing cancer cells create and maintain an immunosuppressive environment around them. The induction and augmentation of immune responses against the tumor have been studied by terminating the activity of many immunosuppressive cells and molecules involved in this immunosuppression. For example, blocking tumor immunosuppression mechanism called an immune checkpoint inhibitor is one of the successful treatment methods.[1,23–28] While numerous studies have recently reported high therapeutic benefits of antibodies blocking these immune checkpoint molecules (i.e., CTLA-4, PD-1, and its ligand PD-L1), it has also become clear that development of responsiveness to normal tissues associated with the blocking of tumor immunosuppression can result in autoimmune diseases. Contrary to the approach for enhancing host immune responses against cancer using antibodies blocking immunosuppressive molecules, the method of using agonist antibodies against immune-stimulating molecules for inducing antitumor effects is currently under development. Target molecules include 4-1BB, OX-40, and GITR, which are inductively expressed mainly on activated T cells and serve as receptors transmitting stimulatory immune signals. It is also expected to develop comprehensive cancer immunotherapy by combining these treatment approaches with immune checkpoint inhibitors or cancer vaccines.

Some of these therapeutic approaches are reported to cause colitis, hepatitis, endocrine disorders, skin disorders, and other symptoms with varying incidence as well as varying extent and degree of autoimmune reactions, depending on the inhibited molecules. This clearly indicates the existence of immunological tolerance to autoantigens and suggests that use of similar approaches would inevitably lead to the incidence of various autoimmune reactions, resulting in damage to normal tissues. Adverse events associated with autoimmune reactions vary greatly among individuals, and the site of damage also differs in individuals. It is necessary to establish adequate measures to manage the adverse events in advance and carefully gather safety information as it is expected that there is a correlation between clinical efficacy and the occurrence of immune-related adverse events.[6,7]

Meanwhile, many patients have responded remarkably to these treatments, and complete tumor disappearance, tumor regression of varying degrees, and/or long-term clinical response are reported for the treatment of melanoma. Because of differences in functions between CTLA-4 and PD-1 molecules

on the surface of T cells, combination of these antibodies is reported to provide a considerable clinical effect.[29] These treatment methods are expected to slow progression and significantly prolong survival in patients with advanced cancer other than melanoma.

Development of target antigen test methods. Cancer immunotherapy often targets specific antigens; therefore, it is necessary to develop detection methods for target antigens as comprehensive as possible to select patients eligible for clinical development. Efforts should be made to develop target antigen detection systems, quantitative evaluation techniques, evaluation criteria, and their standardization from the early stages of clinical development. Considering that target antigens are often detected and quantitatively measured as proteins or mRNA encoding these proteins, it is necessary to adopt the latest techniques to the extent possible to ensure sufficient sensitivity and quantitative performance. As the information on expressions of target antigens may be used as a useful biomarker for predicting clinical response, its feasibility will be determined as the clinical development advances. Therefore, target antigen test methods should be developed in parallel with clinical studies of cancer immunotherapy. Although patients participating in clinical studies have been assessed for antigen expression in tumor tissues, the proportion and the intensity of positive results were not closely assessed in many previous studies. In enrolling eligible patients development of biomarkers, as well as implementing target antigen test methods as quantitative as possible would be beneficial in increasing the probability of success for clinical development.

Most target antigen test methods are unique to each cancer immunotherapy. If used as companion diagnostics, the test methods need to be developed with cancer immunotherapy clinical studies as their development requires considerable time and sufficiently accumulated clinical data. For antigen-specific effector cell therapy, particularly receptor gene-modified T-cell therapy, close attention should be paid in advance to expressions of antigen molecules and the potential of unexpected cross-reactivity by carrying out a detailed analysis of epitopes. It may be necessary to develop its detection method as well.

Although immune checkpoint inhibitors are not intended to target specific cancer antigens, the prior knowledge about expressions of target molecules such as PD-L1 in tumor tissues for an anti-PD-1 antibody would help in evaluating an association between the drug and tumor properties.

It is important to refer to results of target antigen test methods in the development of conventional antibody products, such as anti-HER2 antibodies, anti-EGFR antibodies, and anti-CCR4 antibodies, and to consult the guidance on the development of companion diagnostics.

Immune response. It is vitally important that immune responses are evaluated in clinical studies of cancer immunotherapies. Unlike in humans, it is often difficult to properly evaluate immune responses in non-clinical studies using animal models. While considering differences between humans and animal models, it would be important to establish a model system for verifying the mode of action of the investigational product and relevant parameters wherever possible in non-clinical studies, ultimately verifying the proof-of-concept in order to enhance the probability of success.

For clinical development, evaluation of immune responses in early-phase clinical studies, including first-in-human, is important for determining the immunobiological activity of the investigational therapy. Nature, magnitude, and persistency of induced immune responses, would be useful data for future clinical development. As early-phase clinical studies represent the first opportunity to observe immune responses against target antigens, their analysis should be as extensive and comprehensive as possible. Evaluation of obtained data would provide scientific rationale for relevant cancer immunotherapy; it will be vitally important to evaluate how appropriate the clinical development itself is and to determine whether to continue or discontinue the development.

In cancer vaccine and effector cell therapy, inducing and immunizing immune responses against target tumors are requirements but effective methods of immune response induction and immunization remain largely unknown for both cellular and humoral immunity. As an immune response indicative of clinical efficacy may be different in terms of its nature and magnitude, depending on the investigating therapy, it is important to clarify the thinking around its interpretation in advance.[30,31]

Antigen-specific T cells are mainly used for immune response assay during cancer vaccine and effector cell therapy. The assay should be quantitative to the extent possible as well as reflective of *in vivo* immune status. When cells are required to be incubated for a long time, it should be noted that these particular assays may not be suitable for quantitatively determining immune response. As for T-cell response assays, enzyme-linked immunospot, intracellular cytokine staining, or MHC multimers are widely used assay methods but may be variable in results depending on the sample conditions and reagents used. Furthermore, the assays would require skilled analysts, which could cause inter-laboratory variability. When multicenter clinical studies are to be carried out, it is, therefore, necessary to fully review and consider the options of centralizing analytical laboratories and standardizing procedures for consistent assay techniques for measuring T-cell responses. Other conventional assays include determining T-cell biological response using skin reactions to vaccine antigens (i.e., delayed-type hypersensitivity).

For effector cell therapy, it is important to evaluate infused cells over time, particularly analyzing the cell quantity, functions, and properties. As it is also important to assay and assess pharmacokinetic, quantitative, and functional changes of infused cells over time, it is necessary to select and develop appropriate immune response assay(s) according to the characteristics of investigational cell formulations. Although early-phase clinical studies of CAR T-cell therapy indicate remarkable antitumor effect on hematological neoplasms, there have been also reports of a high incidence of serious adverse events, including CRS; thus, taking measures to predict onset of toxicity and to consider how to manage the toxicity beforehand is critical.

Meanwhile, immune responses to various autoantigens, including tumor antigens, are sometimes enhanced through drug-induced inhibition of immunosuppression such as immune checkpoint inhibitors. Although there is a high expectation for obtaining good clinical response, it is also assumed that certain incidence of adverse events associated with injury to autologous cells is unavoidable. In addition to specific immune response assays for conventional cancer vaccine and effector cell therapy, immunological biomarkers should be, therefore, studied to select patient populations with potentially high response rates (prediction of clinical response) and those with an increased toxicity (prediction of toxicity) at an early stage. Furthermore, it is also important to analyze other properties, such as half-life of administered antibody and sustained binding to target immune checkpoint molecules (pharmacodynam-

ics). As immune checkpoint molecules are expressed not only on effector T cells but also on immunosuppressive cells, such as regulatory T cells, it is necessary to analyze immune responses by considering direct activation of effector T cells and blocking effector cell suppression after administration of therapeutic agents. Although analyses of humoral immunity responses, cytokines, chemokines, and other factors are at exploratory stage, data obtained may become important for the future development of safer and more effective therapies.

As analyses of peripheral blood samples and immune responses against "tumor lesions" are considered extremely important for all treatment approaches, it is desirable to make utmost efforts in collecting samples and closely analyzing them.

Biomarkers. It is an urgent issue to identify biomarkers for predicting efficacy and adverse events associated with cancer immunotherapy. As previous reports have revealed that target antigens and immune responses are useful but not sufficient as biomarkers, exploring other parameters is warranted.[32] Given substantial diversity in tumors, immune systems, and hosts involved in immune responses against tumor, a wide variety of parameters are candidates for biomarkers.[33] Above all, the following three factors are the main targets for evaluation: (i) tumor cells, tumor lesions, and systemic disease status; (ii) individual variability in host genomes; and (iii) analysis of immune response such as changes in immune cells and immunologically relevant molecules.

Analysis of individual cancer characteristics. Analysis of individual tumor characteristics probe into: (i) tumor cells; (ii) non-immune cells and molecules, including vessels, stromal cells, and ECM molecules in tumor tissues; and (iii) immune cells infiltrating tumor lesions. Parameters for analysis include, but are not limited to, the quality and quantity of various expressed antigens, functional properties of antigen molecules, expression of an antigen peptide–MHC molecule complex and molecules associated with the complex formation, molecules responsible for immunological synapse formation, diversity of immune-modulatory (immunosuppressive) molecules (e.g., PD-L1 and FasL) expressed on tumor cells, nature of stromal cells and composition of a tumor microenvironment, and tumor sites.

As these types of analysis mainly use tumor tissues, the collection, processing, and storage of biopsy and surgical specimens are extremely important. Pathological and immune-histochemical approaches have been widely used for analysis. Skilled and experienced analysts are required to prepare and handle the necessary antibodies and reagents for analysis. The recent widespread use of global analysis techniques for genomes and transcriptomes, such as DNA array, next-generation sequencing, and quantitative RT-PCR, is greatly improving the quality and volume of information provided by analysis of individual tumor characteristics. These analysis techniques require selecting component cells before evaluation. For all approaches, analytical procedures need to be validated and standardized. It is also necessary to carefully assess and establish systems to properly evaluate an association between the obtained data and therapeutic efficacy.

Clinical samples other than tumor tissue (e.g., serum, blood cells, and urine) are widely used for analysis of systemic disease status. Efforts are also underway to develop a technique to measure secretion from tumor cells or circulating tumor cells using relatively readily available clinical samples, such as blood or urine.

Analysis of individual patient characteristics. For analysis of patient's genomic background, the progress of preceding SNP analysis and PCR analysis of gene expression as well as next-generation sequencing analysis has refined the analysis of individual host characteristics. In accordance with this trend, relevant analytical procedures are also becoming more sophisticated and simplified. The classes and expression of MHC molecules involved in antigenic peptide presentation have been analyzed for determining the cause of host immune responses. Although the importance of genetic background of cells and molecules involved in a complex immune system is also suggested, its significance and usefulness should be further evaluated in most cases.

Analysis of immune cells and relevant molecules. As a result of analysis of immune cells and immune-related molecules, such as antibodies, cytokines, and chemokines, before and after cancer immunotherapy, it is suggested that pretreatment analysis data on these parameters could be biomarkers for the safety and efficacy of the investigational agents. Recent studies have focused on the behavior of antigen-specific and antigen non-specific immune responses and also emphasize the importance of measuring immunosuppressive regulatory T cells and myeloid-derived suppressor cells.[34–36] Discussions have been based on peripheral blood, but the importance of analyzing behaviors of effector cells and immunosuppressive cells in "tumor lesions" is now emphasized. To date, most studies have been carried out in an exploratory fashion, and the relevance of their findings needs to be investigated further. Current studies suggest that the number and properties of TILs may be prognostic predictors for some cancers. Results from similar studies are awaited to figure out whether the efficacy and safety of the cancer immunotherapy can be predicted.

Sampling, storage, and analysis of clinical samples. Clinical studies of cancer immunotherapy involve intervention to regulate patient's tumor immune responses, and there may be many unpredictable factors associated with post-treatment immune responses in the development of new therapeutic agents. Assessment of endpoints becomes more important in later clinical studies, and it is also important to collect, store, and analyze patient samples. Collecting samples with minimally invasive procedures over time before and after study treatments is an important issue in designing a clinical study. However, most procedures used for analysis of immune responses against tumors are not yet standardized, and only exploratory data analysis could be available. Meanwhile, tumor cytology and immune-cytology are rapidly advancing, and analysis of patient samples using new approaches is crucially important for clinical development. Advanced analytical approaches should be adopted in the developments, particularly considering that it takes ample time to conduct clinical studies from phase I to later phases. Proper collection and storage of samples are of a great significance. It is important to obtain informed consent from patients before sample collection, to register and store samples, to establish banking systems, and to build databases for data analysis.

In most cases, patient samples have been limited to surgical specimens and peripheral blood collected over time. Obtaining tumor tissues is vitally important for analyzing immune responses, and the need for collecting through biopsy pre/post treatment should be considered as much as possible.

Combination cancer immunotherapy. Immune responses against tumors comprise positive and negative feedback loops of many host immune cells and molecules around "tumor lesions." Growing knowledge of the complex mechanism strongly suggests the need for combining different approaches

to various immune responses for the development of a more effective cancer immunotherapy. Currently, developed approaches for cancer immunotherapy include an increased activity of antigen-presenting cells (e.g., immunological adjuvants), activation of effector cells (e.g., concomitant cytokines and pretreatment for lymphocyte depletion), and depletion of regulatory T cells (e.g., blocking antibodies). There is a high hope for combination cancer immunotherapy that combines therapies with different modes of action, and it is growing to be the mainstream of development approaches. Combination therapies include widely used chemotherapy, biological products, and radiotherapy; it is necessary to gather specific information on mechanisms of action, doses, and dosing regimens in terms of interaction of immunotherapies with concomitant medications as much as possible. A combination with other immunotherapies or chemotherapeutic agents may cause unexpected toxicities. Although it depends on drug properties, it is also important to investigate combination therapies with not only approved drugs but also unapproved ones from the beginning of clinical development.

Prior chemotherapy or radiotherapy could be important to ensure the efficacy of effector cell therapy. Therefore, combination use of these pretreatments should be actively investigated.

When approved drugs are combined with cancer immunotherapies, the mode of administration may differ from the approved one. Careful consideration is required in such cases. When preclinical findings suggest the basis for combination use, it is important to perform clinical studies with suggested the combination.

Personalized cancer immunotherapy. Immune responses against tumors largely depend on individual characteristics of tumor cells and hosts as well as the diversity of immune systems. It is, therefore, natural that appropriate treatment methods differ by patient. As with other cancer therapies, it is necessary to develop possible treatment algorithms, enabling the choice of an adequate treatment regimen for individual patients. A recent introduction of new technologies, such as next-generation sequencing, has greatly improved individual analysis techniques on the genomic level, providing an opportunity to consider generating a system for individualized cancer immunotherapy.[37–41]

As one of the characteristics of cancer, the type and combination of tumor-specific antigens are known to differ by patient. In this context, some cancer vaccines are being developed using a combination of different tumor antigens. Some cancer vaccines are being developed by combining antigens, such as peptides, using individual immune responses in the body as indicators to select appropriate antigens.[42] Lymphocytes that infiltrate tumor lesions in each patient are polyclonal, and their composition greatly varies among patients. The idea of effector cell therapy with TILs for cancer treatment is also based on individual characteristics of each tumor. Future development programs of individualized cancer immunotherapy include analysis of a wide range of variant antigens observed in each cancer, such as mutant proteins associated with point mutation, translocation, and splicing variant, for the production of cancer vaccines or effector cell therapy that can attack variant neo-antigens unique to each patient.

Personalized medicine, coupled with progress in supporting technologies, is expected to be widely applied to cancer immunotherapy and become a new paradigm for cancer treatments. This brings about the need to clarify the current thinking on the safety and efficacy of treatment methods based on the information gathered from each patient for the development of new therapies.

Concepts for Early-Phase Clinical Studies

The main objectives of early-phase clinical studies of cancer immunotherapy are to determine safety profile, optimal dose, dosing regimen, dosing schedule, and efficacy.

Patient population. *Target disease stage and disease state.* For early-phase clinical studies of cancer therapeutics, particularly those administered in humans for the first time, the target population is generally those patients with advanced or metastatic and recurrent cancer for whom an appropriate treatment option is not available. A clinical study of cancer immunotherapy should also be designed to target patients with similar lesions.

When an investigational product, such as cancer vaccine, is evaluated for safety and induced immune response, it should be carefully reviewed whether the patients with advanced lesions could be the target population for the study. For example, if enrolled patients have metastatic and recurrent lesions and their symptoms deteriorate shortly after the initiation of the investigational therapy, there may not be long enough time to observe any evaluable immune responses. Furthermore, the majority of patients with metastases/recurrence have received chemotherapy or radiotherapy that may have a negative impact on induced immune responses of cancer immunotherapy, especially cancer vaccines, leading to reduced immune responses. Therefore, in some cases, it may be appropriate to target a population with lower tumor burden, in whom host immune responses have been maintained. In other words, it may be necessary to evaluate the suitability of the study design; it may be necessary to consider including patients without tumor lesions after complete resection or responding to the chemotherapy/radiotherapy, or patients with only minimal lesions. If a method of minimizing the effect from prior therapy (e.g., chemotherapy), such as setting the appropriate washout period, is available, it should be considered to adopt the method when recovery of the host immune response can be expected.

When the target population has no evaluable lesions, it may be possible to fail to observe an adequate efficacy in a short term. When the progression-free survival (PFS) is evaluated, it may be difficult to decide whether to continue the development of the investigational therapy during the study. When parameters, such as disease-free survival, overall survival, and changes in some tumor markers, are used as endpoints for evaluating effects of preventing tumor recurrence after surgical removal (i.e., postoperative adjuvant setting), it is necessary to fully review suitable study designs and control groups.

In some effector cell therapies and immune checkpoint inhibitor therapies, antitumor effects such as tumor regression may be achieved relatively quickly. In such cases, it may be appropriate to target populations with evaluable metastases and/or recurrent tumor. As for drug-related adverse events, immune checkpoint inhibitors may cause autoimmune reactions; thus, the inclusion of patients with autoimmune reactions, regardless of obvious or latent, should be carefully reviewed particularly in early-phase clinical studies.

Target cancer type. In most cases, the main objectives of phase I studies of traditional anticancer agents are to determine the maximum tolerated dose (MTD) and safety profile of the investigational product; as the studies include groups of patients with a wide variety of cancers, the potentially differ-

ent clinical responses would not be a significant issue in interpreting the outcome. Therefore, multiple tumor types are commonly targeted in phase I studies for anticancer agents, such as cytotoxic drugs. After determining MTD for the investigational product, its efficacy is commonly evaluated in subsequent phase II studies targeting specific tumor type(s).

As many phase I studies have failed to determine MTD for conventional cancer vaccines, we may see increasing use of endpoints other than toxicity, particularly immune responses induced by investigational product, to determine the recommended dose moving forward. Although this is the anticipated trend, when targeting a wide range of tumor types, the previous treatment often differs among patients depending on the tumor types, which might lead to potential impact on the induction of immune responses mediated by cancer immunotherapy, hindering result interpretations of immune response analysis. This may ultimately impact the study outcomes. Therefore, if immune response is evaluated for cancer vaccines in a small group of patients, it should be noted that enrolling patients with relatively consistent tumor types and previous treatments may be desirable.

Antigen-specific cancer immunotherapies, such as cancer vaccine and effector cell therapy, are intended to induce immune responses against target antigens resulting in antitumor effects; the target cancer would be limited to those expressing the target antigens. As antigen expression is expected to be a predictive biomarker for clinical response, if the method of antigen detection is not yet established, it is important to advance the exploratory research and development of antigen detection methods in the early phase in conjunction with carrying out clinical studies (see Development of target antigen test methods).

When investigational products are evaluated in multiple tumor types with confirmed target antigens, differences in toxicity between the tumor types remain poorly understood: thus, the tumor types may not be specified for planning phase I studies. However, there may be differences in efficacy as the components of tumor cells or tissues, profiles of cytokines and chemokines produced around the tumor, and penetration of immune cells into the tumor tissue may be different depending on the tumor types. Therefore, it is necessary to review and consider all the possibilities that would result in different immune responses and antitumor effects in cancer immunotherapies in general.

For treatment with immune checkpoint inhibitors, particularly monotherapy, the target tumor type may not be limited by expressions of specific tumor antigens.

Enlargement of lesions during clinical studies. In clinical studies of conventional anticancer agents, the enlargement of tumor lesions and appearance of new lesions generally mean that the investigational product is not effective, leading to the treatment discontinuation. Meanwhile, as it is expected for cancer immunotherapy to induce biologically active immune responses to take some time, patients who receive cancer immunotherapy need to be checked for delayed responses. It should also be noted that inflammatory changes induced by immune responses at the local tumor site may trigger temporary enlargement of tumor lesions. When planning a cancer immunotherapy study in patients with evaluable lesions, the decision should be made prior to the study initiation as to whether to discontinue or continue the study in the event of lesion enlargement or appearance of new lesions. Careful review should also be carried out as to whether continuing the study participation would be disadvantageous on an individual basis. A study protocol should stipulate the criteria for continuing treatment for individual patients in the event of lesion enlargement or appearance of new lesions during protocol-driven treatment.

Before continuing the study treatment, patients must, at least, meet the following conditions:

- Comparable systemic conditions to the baseline.
- Non-life-threatening lesion(s).
- Tolerable adverse event(s), allowing continuation of the study drug.

Informed consent must be obtained from the patient after explaining an increased risk of symptom deterioration and possible switch to available alternative treatments at the time of discontinuation.

Phase I Clinical Studies

The primary objectives of phase I clinical studies are to assess safety and tolerability.

Initial dose and dosing schedule. In development of conventional cytotoxic anticancer agents, the design of phase I clinical studies is generally based on non-clinical (*in vitro* and animal studies) data. The route of administration and dosing schedule should be examined, wherever possible, using animal models that can be extrapolated to humans before initiation of clinical studies. Unlike cytotoxic anticancer drugs, the mechanism of cancer immunotherapies is mediated by the immune response, and thus it is often difficult to establish suitable animal models. Therefore, for cancer immunotherapy, there is a limit in determining an initial dose in humans based on non-clinical data. With respect to the onset of toxicity associated with the mechanism of action of the investigated drug, historical information on similar agents given to humans may be useful.

As some effector cell therapies and immune checkpoint inhibitors are likely to produce more dose-dependent toxicity and efficacy compared to cancer vaccines, it is necessary to carefully determine an initial dose, dosing schedule, and dose-escalation scheme. In particular, effector cell therapies may cause serious adverse events even at the minimum dose level, thus dosing should be carefully carried out while monitoring predictive markers of safety such as cytokines and C-reactive protein. Therefore, it is also necessary to bear in mind that there may be an association between tumor types/tumor burden and the incidence of adverse events. As for effector cell therapy, it should be noted that infused cells would proliferate inside the body. Adverse events may persist or recur due to the long-term persistence of infused cells. Development of technique(s) to control the cells post-infusion can be one of the effective measures to address adverse events. As immune checkpoint inhibitors are likely to cause dose-dependent injury to normal tissues associated with autoimmune reactions, it is important to bear in mind that unexpected adverse events may occur.

Analysis and evaluation of pharmacokinetics of cancer therapeutics are also required.

Endpoints. In phase I clinical studies of cytotoxic anticancer agents, the incidence, type, and grade of toxicity are evaluated as primary endpoints for assessment of safety and tolerability. The National Cancer Institute's Common Terminology Criteria for Adverse Events are used to evaluate the type and grade of toxicity. For safety, MTD is found as the highest dose without unacceptable toxicity. This is because not only the toxic risk

but also therapeutic benefits would increase with higher dose of cytotoxic anticancer agents, despite their highly toxic nature; the maximum effect will be achieved at an acceptable dose in terms of toxicity. In general, MTD is based on the dose given to patients enrolled in phase I studies and resulting dose-limiting toxicity (DLT). Dose-limiting toxicity is defined as toxicity unacceptable enough to prevent an increase in dose or undesirable toxicity. In phase I clinical studies of cytotoxic anticancer agents, antitumor response and other parameters are also evaluated as secondary endpoints for assessment of efficacy.

As with cytotoxic anticancer agents, toxicity and efficacy are also expected to increase with higher dose in some effector cell therapies and immune checkpoint inhibitors. Thus, phase I studies of these treatments can be carried out using the same study population and endpoints as those for cytotoxic anticancer agents in addition to the assessment of immune responses. However, it should be noted that the onset of toxicity differs depending on the tumor types and tumor burden.

Maximum tolerated dose may not be identified for cancer vaccination because DLT rarely occurs within the dose range studied. Under such conditions, direct use of toxic reactions and other responses (such as antitumor responses, immune responses, and injection site reactions) as endpoints should also be considered in finding the dose. Advancing clinical development without triggering immune responses would lead to a fatal issue, particularly because the mode of action responsible for efficacy is mediated by the immune responses to the administered vaccine antigens. Therefore, it is necessary to consider toxicity and immune responses as endpoints (see Immune response).

Caution should be exercised when selecting the assessment period as some cancer immunotherapies may cause late-onset toxicity or produce delayed responses.

Study design. As with cytotoxic anticancer agents, a dose-finding design using toxic reactions as an indicator can be adopted for some effector cell therapies and immune checkpoint inhibitors.[43–49] So-called "3 + 3 design" is widely used, and to resolve issues pertaining to this design, many other new approaches have already been developed as a dose-finding design using toxic reactions as an indicator.[50–52] For example, they include: (i) the continual reassessment method, determining MTD based on a dose–toxicity model; (ii) accelerated titration design, where dose is escalated by using information on milder toxicity than DLT, with an option of intra-subject dose escalation, and MTD or recommended dose is determined at the completion of the study based on a dose–cumulative toxicity model; and (iii) toxicity probability interval design, where determining MTD is based on toxicity probability distribution. Any of these designs may be applied.

Maximum tolerated dose was not identified in many cancer vaccine studies because of a low incidence of DLT occurring in the studied dose range. When the dose to be evaluated may be lower than MTD, and the study is intended to determine the recommended dose for subsequent studies, more precise information on toxic reactions and other responses can be collected by increasing the size of a cohort in the above designs with toxic reactions as an indicator, e.g., use of "A + B design" that is a generalized version of 3 + 3 design or modified CRM using a large cohort size. However, increasing a cohort size has drawbacks of requiring more patients for dose escalation and treating a considerable number of patients at a low, potentially ineffective dose. It should also be noted that the targeted probability of toxicity is 20–30% for 3 + 3 design, and it is not always true for A + B design because it differs in cohort size from the former. When it is expected that the maximum efficacy can be achieved without compromising safety at the dose determined, based on a dose-finding design using toxic reactions as an indicator or the maximum dose specified because of practical restrictions such as manufacturing issues or administration site reaction (e.g., effector cell therapy and cancer vaccines using cells), expansion of cohorts with the dose level will enable a collection of highly precise information on toxic reactions and other responses.

In addition to a method of determining a recommended dose based on toxic reactions, direct use of immune responses and other responses (e.g., antitumor activity, administration site reactions, and restrictions) will be considered in finding the dose. For instance, it may include a dose-finding design directly using immune responses or antitumor activity as a dose-finding indicator, a design with both toxic and non-toxic reactions as an indicator, and a study design seamlessly connecting a dose-finding (phase I) part based on toxicity reactions to a randomized (phase II) part based on non-toxic reactions. For such a seamless design,[53] a toxicity-based dose-finding design is used for identifying well-tolerated dose levels and subsequently randomizing subjects to groups composed of these dose levels (see "Study design") or response-adaptive randomization, in which subjects are assigned to receive an effective dose with a high probability, is carried out to select a preferred dose in terms of non-toxic responses. However, it is necessary to carefully review clinical significance, such as reliability and validity of measurement and association with clinical outcome, particularly when using immune responses as non-toxic reactions (see Immune response).

Whether or not any of the above dose-finding designs is selected, proceeding further to subsequent phases naturally depends on whether the dose with hopefully a certain acceptable and/or the maximum efficacy together with acceptable toxicity can be identified.

In order to find a recommended dosing schedule for subsequent studies, a design intended to find a dosing schedule along with dose may be used. On delayed toxicity and responses, a design will consider the time to develop these events. The efficacy of therapies with strong antitumor activity may not be monotonically increased as the dose increases. In that case, determination of a minimum dose necessary to produce a desirable effect is required, and a study design in which dose titration starts with an extremely low level may also be used. It is also necessary to consider tumor types and tumor burden when determining eligibility criteria and a study design.

Phase II Clinical Studies

The main objectives of phase II clinical studies are to evaluate efficacy and optimize dosing regimens.

Endpoints. In phase II clinical studies of cytotoxic anticancer agents, tumor regression is often evaluated as a primary efficacy endpoint. This is because it is considered appropriate to evaluate antitumor activity for screening effective anticancer agents, although tumor regression is not used as a surrogate endpoint for prolonged survival in phase III studies, depending on the tumor type.

Using RECIST, tumor regression and delayed progression are mainly evaluated for antitumor activity in cancer immunotherapy as well; however, the onset of effect may be

delayed because of the mechanism of action specific to cancer immunotherapy. Considering an onset pattern of effect, immune-related response criteria are proposed as criteria for tumor regression, and it may be necessary to use immune-related response criteria and other new criteria in some cases.[54] Despite the lack of tumor shrinkage, some cancer immunotherapies have the potential to slow progression or improve survival; in such a case, PFS and/or overall survival will be evaluated as primary endpoints.[55] There may be a study in which patients with no evaluable tumor lesions who have received initial treatment may be mainly enrolled. The extent of a delayed progression and prolonged survival will constitute important basic data for design of confirmatory studies.

It is desirable to evaluate immune responses as data showing the biological activity of cancer immunotherapy. Dosing regimens can be optimized on the basis of the expected immune responses. As proof-of-concept for cancer immunotherapy, it is important to determine whether immune responses associated with cancer immunotherapy are induced as expected and then evaluate their association with antitumor activity and survival. Currently, however, there are no established methods to test immune responses; attention should be paid to test result interpretation as it is unclear as to what types of immune responses should be tested in some cases.

Phase II studies also need to evaluate safety as a secondary endpoint to collect more information on the incidence and grade of adverse events.

Study design. When tumor regression is used as an endpoint in cancer immunotherapy, a single-arm phase II study may be planned as in cytotoxic anticancer agents.[43–45,49] A single-arm study evaluates whether the proportion of responses significantly exceeds the response threshold and commonly adopts a two-stage design, in which an interim analysis of treatment failures and discontinuation is carried out once. This design is not applied only to the proportion of responses and can be used if binary endpoints, such as the presence or absence of immune responses, are available. However, thresholds and expected values for sample size estimation should be determined based on historical data according to immune responses to be evaluated and the tumor types.

Unlike cytotoxic anticancer agents, administration of cancer immunotherapy (e.g., cancer vaccines) may generate a relatively smooth dose–toxicity curve and not always provide a monotonous dose–response curve. Specifically, it may be unclear if the maximum effect would be achieved at MTD or the highest clinically acceptable dose. In this case, the dose used in phase III studies should be determined based on the phase II study by referring to biological activity data, such as immune responses. It may be necessary to optimize parameters other than doses, such as dosing schedules and concurrent medications. The optimization of dosing regimens in a relatively small study before initiation of phase III studies may enhance the probability of success of phase III studies.

A randomized phase II study called selection design may be performed to select the best dosing regimen amongst several treatment regimens.[56] Taking a Simon's randomized phase II study as an example, subjects are generally randomized to two to four treatment regimens and the regimen to provide the greatest tumor regression (i.e., the highest point estimates) is selected as a study treatment for phase III studies. A randomized phase II study can be planned, without using selection design, to evaluate dose–response, which is a prevalent parameter for commonly used agents other than antineoplastic agents, but the choice of a placebo group should be reviewed carefully.

A randomized controlled "phase 2.5 study design" is one of the phase II study protocol designs. One example of this design is a randomized study that compares PFS with standard of care with the one-sided significance level of 10%. Conventional randomized phase II studies are intended to "select" a study treatment used for confirmatory studies from regimen candidates, whereas the phase 2.5 studies are carried out to "make comparisons" with a control group. The phase 2.5 studies are not confirmatory studies, enabling the use of endpoints based on antitumor effect and a significance level larger than 5% commonly used for analysis. Randomized controlled studies may provide information useful for planning confirmatory studies, including the extent of responses.

A single-arm phase II study, randomized phase II study, and phase 2.5 study do not have to be carried out in order. An appropriate design should be selected according to the objective of the phase II study, depending on the situation.

Highly personalized effector cell therapies may make it difficult to incorporate placebo or blinding arm in some cases. In such cases, a comparative study with an appropriate control group is also required for efficacy evaluations. It may be necessary to make an appropriate comparison, particularly when an apparent tumor regression is not frequently observed and endpoints, such as prolonged survival and QOL, are selected.

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Disclosure Statement

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Appendix

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