Cancer Science
Japanese Cancer Association JCA
Open Access

Report

Guidance for peptide vaccines for the treatment of cancer

Yoshiyuki Yamaguchi,[1] Hiroki Yamaue,[2] Takuji Okusaka,[3] Kiyotaka Okuno,[4] Hiroyuki Suzuki,[5] Tomoaki Fujioka,[6] Atsushi Otsu,[7] Yasuo Ohashi,[8] Rumiko Shimazawa,[9] Kazuto Nishio,[10] Junji Furuse,[11] Hironobu Minami,[12] Takuya Tsunoda,[13] Yuzo Hayashi,[14] and Yusuke Nakamura,[15] The Committee of Guidance for Peptide Vaccines for the Treatment of Cancer, The Japanese Society for Biological Therapy

[1]Department of Clinical Oncology, Kawasaki Medical School, Kurashiki; [2]Second Department of Surgery, Wakayama Medical University, Wakayama; [3]Department of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, Tokyo; [4]Department of Surgery, Kinki University Faculty of Medicine, Osaka; [5]Department of Regenerative Surgery, Fukushima Medical University, Fukushima; [6]Department of Urology, Iwate Medical University, Morioka; [7]National Cancer Center Hospital East, Kashiwa; [8]Department of Biostatistics, School of Public Health, The University of Tokyo, Tokyo; [9]Pharmaceutical Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki; [10]Department of Genome Biology, Kinki University Faculty of Medicine, Osaka; [11]Department of Medical Oncology, Kyorin University School of Medicine, Mitaka; [12]Department Medical Oncology/Hematology, Kobe University, Kobe; [13]OncoTherapy Science, Kawasaki; [14]Central Institute for Experimental Animals, Tokyo, Japan; [15]The University of Chicago, Chicago, Illinois, USA

Key words

Cancer vaccines, clinical study, guidance, non-clinical study, peptide vaccines

Correspondence

Yoshiyuki Yamaguchi, Department of Clinical Oncology, Kawasaki Medical School, Matsushima 577, Kurashiki, Okayama 701-0192, Japan.

Tel: +81-86-462-1111; Fax: +81-86-464-1134;
E-mail: shogo@med.kawasaki-m.ac.jp

The Japan Society for Biological Therapy has published Guidance for peptide vaccines for the treatment of cancer 2012 Dec; 2: (15 screens) [Cited 28 Apr 2014]. Available from URL: http://jsbt.org/guidance (in Japanese)[1]. This is the English version of that report.

Funding information

Japanese Society for Biological Therapy

Received April 8, 2014; Revised April 28, 2014; Accepted May 6, 2014

Cancer Sci **105** (2014) 924–931

doi: 10.1111/cas.12443

Recent progress in fundamental understanding of tumor immunology has opened a new avenue of cancer vaccines. Currently, the development of new cancer vaccines is a global topic and has attracted attention as one of the most important issues in Japan. There is an urgent need for the development of guidance for cancer vaccine clinical studies in order to lead to drug development. Peptide vaccines characteristically have the effect of indirectly acting against cancer through the immune system – a mechanism of action that clearly differs from anticancer drugs that exert a direct effect. Thus, the clinical development of cancer peptide vaccines should be planned and implemented based on the mechanism of action, which differs significantly from conventional anticancer drug research. The Japanese Society for Biological Therapy has created and published Guidance for peptide vaccines for the treatment of cancer as part of its mission and responsibilities towards cancer peptide vaccine development, which is now pursued globally. We welcome comments from regulators and business people as well as researchers in this area.

The molecular mechanism for the presentation and recognition of melanoma antigens was revealed through the identification of a cancer antigen gene by a Belgian group, van der Bruggen *et al.* in 1991.[2,3] Clinical research of peptide vaccines aginst melanoma using this molecular mechanism subsequently commenced in 1995.[4] Numerous studies have since been reported to show the immunological efficacy of vaccines such as inducing cytotoxic T lymphocytes (CTL);[5] however, the impact of cancer vaccines with limited tumor regression effects could not be proven in clinical study designs given that tumor regression effects are often used as an indicator of efficacy. As a result, Dr Rosenberg of the US National Cancer Institute (NCI) issued a negative report on the effect of cancer vaccines[5] in 2004. Since 2006, the inhibitory effect of cancer peptide vaccines administered as adjuvant therapy has been noted in successive reports with respect to lung cancer and breast cancer, and attention has been drawn to both the preventive effect of cancer vaccines and the subsequent improvement in survival rates.[6,7] In 2010, the cancer vaccine sipuleucel-T,[8] which demonstrated an extended effect on survival rates in cases of castration-resistant prostate cancer, was approved by the US FDA and cancer vaccines were re-unveiled as a new treatment. In 2009, prior to the approval of sipuleucel-T, the US FDA had issued guidance to companies engaged in the development of cancer vaccines, publishing important specifics on the development of cancer vaccines and seeking public comment on cancer vaccines.[9] Currently, the development of new cancer vaccines is a global topic and has

attracted attention as one of the most important issues in Japan. There is an urgent need for the development of guidance for cancer vaccine clinical studies in order to lead to drug development.

The Japanese Society for Biological Therapy is a group of researchers focused on the research of biological therapies to treat cancer. The Society was initially established in 1987, as a Research Group Meeting (a Kenkyukai) named The Society of Biological Response Modifiers to promote the exchange of information for the progress of new cancer treatments. The Society was renamed the Japanese BRM society in 1995, and subsequently in 1999, adopted its current name, the Japanese Society for Biological Therapy. This society has demonstrated its medical and social responsibility as the leader in this area by assembling Japanese and international researchers to discuss results pertaining to state-of-the-art biological treatment and by publishing the results of these conferences. As part of its mission and responsibilities towards cancer peptide vaccine development, which is now pursued globally, the Japanese Society for Biological Therapy has created and published these Guidance for peptide vaccines for the treatment of cancer.[1]

Characteristics of Cancer Peptide Vaccines

Cancer peptide vaccines are peptides that express pharmacological activity through utilization of the human immune system rather than being pharmacologically active themselves. Peptide vaccines administered subcutaneously reach the lymph nodes via host antigen-presenting cells and lymph flow, eventually inducing an immune response. This is accomplished through the following molecular mechanism: (i) the peptide binds to antigen-presenting cells, human leukocyte antigens (HLA) or major histocompatibility complex (MHC) molecules on the target cell surface; (ii) T-cell receptors (TCR) recognize the HLA-peptide complexes; and (iii) antigen-specific cytotoxic T-cells (specific CTL) are induced. Peptide vaccines characteristically have the effect of indirectly acting against cancer through the immune system – a mechanism of action that clearly differs from anticancer drugs and low-molecular-weight compounds that exert a direct effect. Thus, the clinical development of cancer peptide vaccines should be planned and implemented based on this mechanism of action, which differs significantly from conventional anticancer drug research. The guidances published by the US FDA Center for Biologics Evaluation and Research (CBER) in September 2009[9] were developed based on this idea. In addition, the following points should be considered in designing cancer peptide vaccine clinical research: (i) subjects allowing evaluation of the delayed effect of treatment initiated through the immune system should be selected; (ii) the study design should assume that long-term continuous administration is required and therefore focus both on survival rate and cytoreductive effects; and (iii) outcomes should be evaluated by a scientific method that allows the analysis of delayed effects.

The Concept of Non-Clinical Safety Testing for Cancer Peptide Vaccines

The purpose of conducting non-clinical safety testing. Non-clinical studies aimed at clarifying the toxicological and pharmacological properties of target compounds are necessary in the development of new drugs. Particularly, describing the toxicological properties of novel treatments is essential to ensuring the safety of humans in clinical studies. Information determining the safe initial dose in clinical studies and predicting the toxic effects that may occur with administration of the test substance can be obtained from non-clinical safety testing that has been designed and implemented properly.

Animal species selection in non-clinical safety testing. In order to obtain useful results predicting the effect of the test substance in humans from non-clinical safety testing, a suitable animal species must be identified. A suitable animal species is defined as a species in which extrapolation of the effect of the test substance to humans has been confirmed. Currently, there are no known suitable animal species for the non-clinical safety testing of peptide vaccines.

As previously mentioned, peptide vaccines are simply peptides and, not being pharmacologically active themselves, they express pharmacological activity through utilization of the human immune system, namely, antigen presentation and recognition of HLA-peptide complexes by TCR on the surface of lymphocytes and the subsequent induction of CTL. The HLA structure differs significantly between animal species; therefore, no other animal species shares an identical HLA structure with humans. Peptides used in vaccines are not able to bind to the MHC of experimental animal species, which renders antigen presentation impossible to any animal model. This indicates that there are no animal species in which peptides demonstrate pharmacological activity with a mechanism similar to that observed in humans. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline S6 (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) proposes the use of transgenic animal models in non-clinical safety testing in light of the characteristics of peptide vaccines, since it is possible to recreate transgenic MHC molecules. However, it is difficult to reproduce the necessary human-type CTL recognition and activation in order to demonstrate drug efficacy and impossible to create an animal model with completely transgenic TCR. Accordingly, it is practically impossible to use a transgenic animal model to reproduce the pharmacological activity that occurs in the human body as a result of the administration of peptide vaccines.

Pharmacokinetic properties of peptides themselves. It has been confirmed that peptides are rapidly degraded *in vivo* by dipeptidases into indigenous amino acids. Accordingly, the potential toxicity from metabolites is considered to be extremely low and non-clinical safety testing for peptide vaccines should take this characteristic of peptides into account.

The situation concerning peptide vaccine non-clinical safety testing in Europe and the United States. As described above, the requirements for non-clinical safety testing of peptide vaccines differ significantly from those required in the testing of other low-molecular-weight drugs. This is clearly shown in the guidance for non-clinical safety trials required by the regulatory authorities in Europe and the United States (the FDA and European Medicines Agency). Actually, clinical studies for peptide vaccines have been allowed to proceed in the absence of non-clinical safety testing when it has been demonstrated that information ensuring the safety of peptide vaccine administration to humans can only be obtained in humans. From the perspective of animal welfare, this avoids the unnecessary use of animals and reduces excess animal experimentation as much as possible.[10] In such cases, a logical explanation might be required as to why non-clinical safety testing is unnecessary.

Matters to be considered in peptide vaccine non-clinical safety testing. As previously mentioned, from the perspective of its mechanism expressing pharmacological activity, there are no

suitable experimental animal species on which non-clinical safety testing of peptide vaccines can be conducted. However, it is still necessary to consider testing in order to confirm the safety of investigational products. Impurities contained in the active ingredient or any other unintentional contamination may present safety issues when a test preparation is administered to humans. Negligible risk-based reference values have been set with respect to drug substance impurities and are listed in the guidelines; however, the possibility of unknown compounds not defined by guidelines or the unintentional contamination of compounds cannot be eliminated (ICH guideline Q3A "Impurities in New Drug Substances"[11] and ICH guideline Q3B "Impurities in New Drug Products"[12]). Therefore, chemical analysis of the peptide drug substance and animal studies to confirm any effect of exposure are useful in determining the presence or absence of adverse effects from impurities and contaminants. Finally, additional tests in experimental animal species to evaluate local irritation effects, route of administration and dosage form should also be devised when feasible.

The Concept of Quality Assurance in the Research and Development of Peptide Vaccines for the Treatment of Cancer

This guidance illustrates the concept of quality assurance in the research and development of cancer vaccines composed of chemically synthesized peptides as their active ingredient. Quality assurance also refers to the appropriateness of the drug substance or drug product for its intended use. This guidance assumes the drug substance to be peptides and the drug product to be an injectable solution composed of peptides to which adjuvants have been added (including any adjustments made at the time of administration). Furthermore, this guidance summarizes the minimum important points with respect to the quality of peptide vaccines during clinical studies; whether further examination is required will depend on the nature of each peptide vaccine, particularly in cases where the clinical study is aimed at obtaining regulatory approval.

Requirements of the laws and regulations pertaining to the quality of the test substance for clinical studies. "Investigational drugs manufactured in a plant with appropriate methods of manufacturing control and quality control as well as the structural equipment necessary to ensure the quality of said investigational drug" is the standard adopted with respect to quality assurance of test substances to be used in clinical trials (Article 17 and 26-3 of the Ministerial Ordinance on Good Clinical Practice for Drugs[13]). Compliance with investigational drug Good Manufacturing Practices (GMP[14]) is required. However, there is no mention of test substances used in clinical studies other than clinical trials in the Ethical Guidance for Clinical Studies[15] and, as such, the quality of such test substances is left up to the researchers.

The need for quality assurance during research and development. The use of a drug substance or product manufactured with a certain quality is essential in clinical studies to ensure the reliability and reproducibility of the test results and to protect the safety of the subjects. Because of the chemical and biological nature of peptide vaccines, general non-clinical safety testing does not necessarily provide information that is useful with respect to human administration and some information can be obtained only after administering the test substance to humans. For this reason, the necessity of peptide vaccine non-clinical safety testing is debatable. Even in cases where non-clinical safety testing of the peptide (the active ingredient of the peptide vaccine) is deemed unnecessary (refer to the section on non-clinical safety testing), it is still necessary to ensure the safety of impurities in accordance with the amount and type of impurities contained in the drug substance or product (refer to the section on drug substance specifications and purity testing).

Continued quality control of the peptide vaccine from the initial stages of research is a prerequisite to guarantee the quality and the results of both non-clinical and clinical studies.

The concept of quality assurance during research and development. Quality assurance of drugs is accomplished through a combination of various methods, including thorough characteristic analysis of the drug, setting appropriate standards and test methods based on these characteristics, and GMP-based quality control assessments. Quality assurance during research and development is linked with development progress and by necessity the extent of quality assurance required will change depending on the methods used, making a uniform definition difficult. Accordingly, quality assurance should be carried out in a flexible phased manner, in line with development while still taking risk into account. This guidance specifically addresses the setting of appropriate specifications and the concept of GMP-based quality control assessments.

The concept of peptide vaccine specification setting. Specifications are a list composed of the test method, a description of analysis used in the test and appropriate acceptance criteria (limits, range and other criteria) for testing to be carried out in a prescribed manner. Specifications are a manner of controlling the drug substance or product to guarantee the quality and consistency of the test substance and are an important element of quality assurance. Each item included in the specifications is intended to ensure the proper quality of the drug substance or product and any characteristics of the test substance required to ensure safety and efficacy should be set. If these characteristics change during storage, this change should be examined and appropriate specifications or storage conditions set. The Guidance for stability testing[16] serve as a reference for test conditions when conducting storage-related tests.

Drug substance specifications. The following specifications (both test methods and criteria) can be applied to the quality assurance of almost all peptide vaccine drug substances during research and development:

1 *Description*. A qualitative statement about the shape and color is necessary (for example, "white to pale yellow solid").
2 *Identification testing*. The identification tests should be specific for the drug substance. Specificity may be guaranteed through the combination of two or more methods.
3 *Assay* (*content*). It is necessary to set a specific analysis method whereby there is no interference from impurities from degraded products that may appear during storage.
4 *Purity testing*. Purity testing is a test method for identifying organic and inorganic impurities and any residual solvent. Knowing the impurity profile of a test substance also assists in determining the necessity of any safety testing.

Organic impurities are those that occur during the manufacturing process and storage and may be substances with an unknown structure. Inorganic impurities are usually substances with a known structure resulting from the manufacturing process, such as a reagent. Solvents used in the manufacturing process are organic or inorganic liquids and their toxicity is usually known.

Structure determination of individual impurities and decisions on the necessity of safety testing should be carried out

based on ICH-Q3A (R2): Impurities in new drug substances.[11] In cases where subjects will intake 2 g or less of the drug substance per day, the threshold at which impurity structure determination is required is considered to be the lower of 0.10% or 1.0 mg daily intake; the threshold at which safety confirmation is required is considered to be the lower of 0.15% or 1.0 mg daily intake. The specifications with respect to residual solvent should be set with reference to ICH-Q3C (R3): Impurities: guideline for residual solvents.[17]

Preparation specifications. Specifications for description, identification testing, assay (content) and purity testing can be applied to the quality assurance of almost all peptide vaccine products during research and development. The purity testing of drug products should control for both organic impurities produced by the decomposition of the drug substance and for impurities produced in the manufacturing process of the drug product. Impurities resulting from the manufacturing process of the drug substance are usually governed by drug substance specifications and, as such, do not need to be dealt with in drug product specifications. Decisions on the necessity of safety testing and structure determination of drug product impurities should be carried out based on ICH-Q3B (R2): Impurities in new drug products.[12]

As peptide vaccines are injectable solutions, it is also necessary to set test methods and criteria to evaluate sterility before human administration. Sterility can be evaluated through management of the sterilization process and by testing the sterility of the final product. In the event the drug product requires reconstitution at the time of administration, the method of reconstitution must be examined and confirmation must be made that the final product retains the necessary characteristics.

Any specifications necessary for either characteristics of the drug substance or product (such as moisture content) in addition to sections Drug substance specifications and Preparation specifications above can be set with reference to ICH-Q6A: Test procedures and acceptance criteria for new drug substances and new drug products.[18]

Adjuvant specifications. Peptide vaccines are usually mixed with an adjuvant at the time of administration; however, adjuvant specifications should be set independently from the specifications for the target compound. Specifications for description, identification testing, assay (content) and purity testing can be applied to the quality assurance of adjuvants as they are to drug substances.

The concept of GMP-based manufacturing control and quality control. The purpose of GMP is to create a mechanism to minimize human error, to prevent contamination and degradation of quality and to maintain quality. In order to implement this objective of GMP, manufacturing control and quality control must be carried out as a series of operations. These operations include the creation of instructions for the manufacturing method and testing method, manufacture and testing according to the instructions, and the creation and storage of records. To ensure the safety of subjects and the reliability of clinical studies, all records related to the manufacturing control and quality control of the test substance must be stored in a manner that facilitates checking at a later date. Investigational drug GMP[12] and its Q&A[19] may be referred to in the implementation of GMP-based control of the investigational drug.

Clinical Studies

The concept of early exploratory studies and late-stage confirmatory studies. The main purpose of early exploratory clinical studies on cancer peptide vaccines is to clarify the recommended dose, the recommended dosing schedule, the presence or absence of biological activity and the safety profile. In late-stage confirmatory studies, the purpose of peptide vaccine clinical trials is also to clarify the vaccine's efficacy and safety in a given population.

The following clinical points should be considered in connection with early exploratory studies and late-stage confirmatory studies:

Early or advanced-stage cancer. Many early stage clinical studies on conventional cytotoxic anticancer drugs with the purpose of determining the optimal dose, dosing schedule and maximum tolerated dose (MTD) are performed on subjects with various forms of advanced stage cancer. Because the disease progresses relatively quickly in such advanced-stage subjects, the activity of the target drugs must be observed and evaluated in a short period of time in these early stage exploratory studies. Subsequent late-stage confirmatory studies are performed as large-scale, randomized, controlled studies on subjects with a single type of cancer to determine clinical efficacy and safety. If clinical efficacy and safety are observed in studies on advanced-stage cancer patients, clinical development progresses targeting earlier stage patients and the implementation of clinical studies on adjuvant therapy is also possible.

However, if clinical studies on cancer peptide vaccines target advanced-stage subjects similar to clinical studies on conventional cytotoxic anticancer drugs, there may not be sufficient time for immune response-mediated antitumor activity to appear due to the relatively short period from the commencement of drug administration to disease progression. In addition, advanced-stage cancer subjects often undergo multiple treatments, which can damage their immune system and possibly weaken the response of the cancer peptide vaccine. Evaluating cancer peptide vaccines in earlier-stage subjects ensures enough time for the vaccine to induce an immune response and manifest effects; therefore, earlier-stage subjects are considered more suitable for the study of cancer peptide vaccines than late-stage subjects. The disadvantage of studies on earlier-stage subjects is that it generally takes a long time for a conclusion to be reached. Therefore, the pros and cons of the stage of the subjects (early stage or advanced stage) must be considered when conducting clinical studies of cancer peptide vaccines.

If a standard treatment exists, it is necessary to determine the optimal timing of cancer peptide vaccine introduction: prior to, during or after the completion of the standard treatment and, in the case of treatment during the same period, as monotherapy or combination therapy. It is also necessary to ensure the safety and biological activity of any combined treatment regimen and provide for appropriate evaluation.

Target cancer (limited to a single type of cancer or multiple types of cancer?). Phase I clinical studies of cytotoxic anticancer drugs typically targeting subjects with various types of cancer at various stages. While it is possible that the investigational drug will exhibit a different reaction in different subject populations, this is not usually a major barrier to determining the main objectives of phase I clinical studies, which are to determine the MTD and safety profile of the investigational drug. If the toxicity of the cytotoxic anticancer drug is proven to be within the allowable range in the phase I clinical study, a phase II study will be subsequently carried out on subjects with specific types of cancer.

However, in studies targeting patients with differing cancers of differing stages and differing prior treatment, this diversity

may significantly affect the cancer peptide vaccine-induced reaction. When targeting a variety of subject populations in an early exploratory study of cancer peptide vaccines, there is a high possibility that the safety and efficacy results will vary more widely than the respective results obtained in cytotoxic anticancer drug testing, which renders interpretation of the results difficult. Therefore, the diversity of the subject population should be considered when selecting the subject population for cancer peptide vaccine clinical studies.

Human leucocyte antigen. It is considered reasonable to measure subjects' Human leucocyte antigen (HLA) considering the molecular immunological background in which cancer peptide vaccines have been developed. As a general rule, it is common to design a study that examines subjects possessing the HLA that matches with the relevant peptide. However, the development of peptide vaccines that include the possibility of non-matching HLA as a next-generation vaccine has also commenced. Therefore, researchers are required to specify in their study design whether to measure HLA or, alternatively, whether to administer the peptide vaccine to subjects with non-matching HLA and to both specify the rationale for their decision in the study protocol and explain the possible advantages and disadvantages to the subjects.

Antigen expression. As a rule, expression of the antigen targeted by the cancer peptide vaccine in cancer tissues should be confirmed prior to the commencement of the study and its relationship with efficacy and safety data should be analyzed in detail.

Multiple antigen peptide vaccines. Cases where cancer peptide vaccine preparations contain multiple tumor-associated antigens are envisioned. In such cases, the vaccine is expected to induce multiple tumor-specific immune responses and respond to tumor heterogeneity. Generally, it is not considered necessary to evaluate the safety and activity of each component of peptide vaccine preparations containing multiple tumor-associated antigens; however, a case-by-case examination will be required.

Early exploratory studies. The main purpose of cancer peptide vaccine early exploratory studies is to clarify the safety profile of the preparation, set the recommended dose and the recommended dosing schedule, clarify potential biological activity and present scientific data to serve as the basis for future drug development.

Determination of safety—the initial dose and dosing schedule. In early exploratory studies, it is important to determine the safety of the drug and optimize the dosing schedule. To do this, the initial dose and dose escalation, followed by the recommended dose and recommended dosing schedule must all be attained. These matters are generally determined based on the data obtained via *in vitro* and *in vivo* non-clinical studies. However, as mentioned in the non-clinical safety testing section, useful data concerning the pharmacological activity and safety of the peptide vaccine preparation is unlikely to be obtained in animal studies and may only be obtained after human administration. In contrast, multiple cancer peptide vaccine clinical studies have been carried out on humans as early exploratory studies as translational researches (TR); at the present point in time, no significant toxicity has been reported. Researchers should keep this in mind and consider the need for further safety testing in humans. For clinical studies conducted with the purpose of applying for regulatory approval, even studies based on existing TR analysis, it is necessary to plan early exploratory studies to reconfirm safety in a minimum number of subjects. While implementation using a conventional "3 + 3 design" as described below is possible, a cohort of subjects can be added if necessary. If safety is confirmed, an early exploratory study for the purpose of analyzing the recommended dose, recommended dosing schedule and survival rates should subsequently be planned.

Dose escalation testing. So far, in the development of cancer treatment, a "3 + 3 design" has been used as the standard approach with respect to the dose escalation schedule. Once three subjects are registered, testing begins. If dose limiting toxicity (DLT) is not observed in any of the subjects, three additional subjects are registered and given a higher dose and the test continues. If DLT is observed in any one of three subjects, three new subjects are registered and administered with the same dose. If DLT is observed in two or more out of the six subjects administered with this dose, the maximum tolerated dose (MTD) is deemed to have been exceeded and no higher doses will be administered.

The "3 + 3 design" is used in many cancer peptide vaccine clinical studies; however, it is reportedly difficult to identify the MTD if the expression of dose-dependent toxicity is not observed. A possible recommended dose may be prescribed with consideration given to constraints in cancer peptide vaccine preparation, procedural or technical problems in administration or anatomical issues with respect to the administration site.

Accordingly, consideration of a study design other than the standard "3 + 3 design" in order to gather useful dose escalation-related information is also recommended in cancer peptide vaccine clinical studies. For example, the possibility of an approach whereby the dosage is increased in the same subject has been suggested.

In contrast, the standard "3 + 3 design" is a sure way to obtain cancer peptide vaccine safety information when administration involves combinations with other drugs, an invasive technique or a site where anatomical consideration of safety is required.

Continuous administration. In routine clinical practice for cancer, the current treatment is generally discontinued in the event of disease progression or recurrence. However, as time is required to induce an antigen-specific immune response in the administration of a cancer peptide vaccine, continuous administration of the drug with consideration of the possibility of late-onset effects is desirable. Alternatively, continuous administration of a cancer peptide vaccine even after disease progression or recurrence could also result in drawbacks: the subject losing the opportunity to undergo other treatments, an increase in adverse events or mortality during the treatment period, or deterioration in the quality of the clinical study. Accordingly, it is necessary to fully consider the criteria for continuation and discontinuation of the vaccine and formulate a study plan when conducting clinical studies of cancer peptide vaccines.

Early exploratory studies: single-arm studies and randomized controlled studies. In cancer peptide vaccine early exploratory studies, similar to clinical studies of typical anticancer drugs, the design of a study must be able to: (i) obtain data that demonstrates the cancer peptide vaccine proof of concept; (ii) validate the vaccine's relationship with the standard therapy (positioning); and (iii) clarify the recommended dose and recommended dosing schedule.

In the development of typical cancer drug treatments, the primary objective of phase II clinical studies is to demonstrate the cytoreductive effect. This is because the cytoreductive effect is considered the most appropriate surrogate for the extension of a

vital prognosis. However, an extended vital prognosis can be obtained with cancer peptide vaccines even in cases where a cytoreductive effect cannot be obtained. Such fact should be considered in the design of early exploratory studies on cancer peptide vaccines. Therefore, in the development of cancer peptide vaccines it is important for the design of clinical studies, even early exploratory studies, to primarily focus on vital prognosis indicators. In cases where it is necessary to design an early exploratory study to analyze the recommended dose and recommended dosing schedule, the primary objective of inducing a cancer antigen-specific immune response – the cancer peptide vaccine proof of concept – is assumed. Ideally, the primary objective is directly specified in the protocol.

When planning early exploratory studies, the advantages and disadvantages of a single-arm study versus a randomized controlled study (Table 1)[20] should be carefully considered. The results obtained from single-arm studies must be compared against historical data, which introduces bias and other confounding variables, such as time. Since the cytoreductive effect of cancer peptide vaccines is limited, overall survival and relapse-free survival/disease-free survival become important effect indicators; however, these indicators may produce even greater variations from the differences in historical data because of evolving subject background, etc. In contrast, while randomized controlled studies are too small in size to statistically verify efficacy, they can provide feasibility information (outcome predictions, protocol adherence and sample size determination), which is useful in the design of full randomized controlled trials.

Pharmacokinetic and immune response monitoring. In general, analysis of pharmacokinetics (PK) and pharmacodynamics (PD) is required in early exploratory studies of drug development. This is because the accumulation of scientific data concerning blood concentration, tissue distribution, metabolism and excretion of a drug is considered to contribute to the understanding of the drug's efficacy. However, a cancer peptide vaccine administered subcutaneously is intended to exert an immune system-mediated effect through lymph flow and considering this mode of action it is difficult to find any meaning in measuring the concentration of the drug in the blood. In addition, because PK analysis itself is assumed to be difficult, as peptides are rapidly degraded *in vivo* by dipeptidases, etc. (refer to section Pharmacokinetic properties of peptides themselves), it is considered unlikely for useful new data to be obtained by measuring the concentration of the drug in the blood in early exploratory studies. Researchers should bear this in mind and, after examining the data obtained in non-clinical studies, scientifically and logically examine the need for pharmacokinetic analysis[21] in human studies.

It is possible to monitor the immune response expected to be induced by the cancer peptide vaccine over time. As cancer peptide vaccines are believed to cause antitumor activity by inducing a cancer antigen-specific immune response as their mechanism of action, monitoring the immune response is extremely important in PD analysis for the following reasons:

1 The dose and schedule are optimized and a determination made as to whether the cancer peptide vaccine induces its intended immune response in early exploratory studies. These results form the basis for further development of the cancer peptide vaccine and planning of future confirmatory trials.
2 The relationship between indicators of clinical efficacy and the type and strength of immune response are important in confirmatory studies and useful in analysis.

Multiple monitoring methods are required to identify an important immune response. An assay method to measure the most important and relevant immune response with respect to antitumor effect must be developed and validated. Where possible, it is recommended to use at least two immunological assay methods in order to monitor the cancer antigen-specific immune response envisioned from the research hypothesis. Methods such as cancer peptide vaccine delayed type-hypersensitivity reaction testing, peptide-specific cytotoxic testing, Interferon-γ Enzyme-Linked Immunospot peptide-specific assay and peptide-specific multimeric flow cytometry are recommended. The reproducibility of results must be validated for each measurement. The assay conditions, positive and negative controls, positive and negative cut-off values and the statistical procedure used to analyze the results should be specified in the clinical study protocol prior to the commencement of a clinical study.

Concurrent cancer peptide vaccine and target antigen test development. In the case of drugs from which a specific antigen response is expected as the mechanism of action, it is important to concurrently develop a method of measuring expression of the target antigen in the cancer tissue of individual subjects, etc. and consider the possibility of using this data in immune reaction monitoring and subject selection.

If seeking regulatory approval and a new measurement method will be developed in a clinical study, the applicant must work with the regulatory agency to propose a plan for

Table 1. Differences between single-arm exploratory studies and randomized controlled exploratory studies

	Single-arm exploratory studies	Randomized controlled exploratory studies
Advantages	More information about adverse events related to the new treatment can be obtained	Control group information can be obtained at the same time
	There is a chance to implement the new treatment to all participating subjects	The randomization increases reliability with respect to the response rate end-point
	Simple end-points can be set and results obtained quickly	The randomization also increases reliably with respect to overall survival and progression-free survival
Disadvantages	A historical control is required	Statistical analysis is difficult with the low number of cases in early exploratory studies
	The response rate does not necessarily reflect the survival time	Subjects in the terminal stages of cancer may not accept randomization
	It is difficult to obtain reliable results with respect to overall survival and progression-free survival	Not as much information about adverse events related to the new treatment can be obtained
		Implementation of confirmatory studies may be difficult if satisfactory results are obtained

the concurrent development of the assay method together with the cancer peptide vaccine. This plan must be done prior to submitting the application to the agency. At the presubmission conference, the regulatory authorities will provide scientific and institutional advice with respect to the development of *in vitro* diagnostics and medical equipment.

Verification studies. As peptide vaccines are included as drug treatments for cancer, the implementation of confirmatory studies in line with the concept of cancer drug treatment is required. Importantly, it is important to design a clinical study with an understanding of the characteristics of the cancer peptide vaccine.

Verification studies are carried out in order to establish a standard therapy and to verify the efficacy of the new treatment based on phase I and II early exploratory clinical studies. It is necessary to set an appropriate objective for the treatment in line with the subject. The purpose of many cancer drug treatments is to prolong life and mitigate symptoms.

Overall survival (OS), progression-free survival (PFS) and disease-free survival (DFS) are used as the primary end-points in validation testing. The primary end-points will differ according to the disease and pathological condition (for example, postcurative resection or unresectable, etc.). They will also differ according to whether the peptide vaccine is administered as monotherapy or in combination with antineoplastic agents. For instance, it is extremely difficult to judge progression if the peptide vaccine is administered as monotherapy and, in such cases, it is more appropriate to adopt OS or DFS as end-points. However, if the peptide vaccine is administered in combination with antineoplastic agents, it is possible to adopt PFS in addition to OS and DFS.

As objective evaluation of the symptom mitigation effect and quality of life (QOL) is difficult, and there is no established method for measuring these indicators. The end-point of quality-adjusted life year – life-years weighted by QOL – has been introduced. Evaluation of cost-effectiveness taking into account the cost of medical care must also be considered.

Safety evaluation is also an important purpose of confirmatory studies and is carried out through comparison with a control treatment. As confirmatory tests generally take the form of large-scale randomized studies and implementation of a high-quality study is required, it is necessary to prepare a sufficient study implementation system including a data center that monitors the test and manages data centrally.

Study design. The objective of the study design is to verify the non-inferiority or superiority of the developed treatment based on its efficacy and safety. Because cancer peptide vaccines, in principle, target difficult-to-cure diseases with poor prognosis, a study of superiority is considered desirable. Nevertheless, a study of non-inferiority is acceptable in the event there are safety issues with the current standard treatment. If the non-inferiority hypothesis of the test treatment is validated and not rejected (non-inferiority is demonstrated), it is possible to design a subsequent study to verify superiority or concurrent non-inferiority and superiority. In this subsequent study, superiority is concluded only if it can be demonstrated. If superiority cannot be proven, at least non-inferiority can be concluded.

Appropriate controls must be put in place to avoid bias that affects analysis of the test results and activities. As a rule, the control group in a confirmatory study is administered with the standard treatment at the time. A comparison is made with untreated subjects for diseases or pathological conditions if no standard treatment is available. In these cases, a placebo-controlled trial is desirable. Studies involving a placebo must be carefully considered and planned, because treatment with a placebo alone brings about a risk of serious adverse events such as death or irreversible morbidity through the suspension of treatment.

Necessary information, such as stratification factors, is determined and the number of subjects determined from the setting of non-inferiority or superiority, significance level, detection power and the difference to be detected.

End-points. End-points differ with respect to unresectable advanced cancer subjects (including recurrence) and post-total lesion excision subjects (adjuvant therapy).

1 Unresectable advanced cancer. As the main purpose of the treatment is to prolong life and mitigate symptoms, the main primary end-points of OS and PFS are used. It is also possible to adopt PFS under some circumstances and the setting of these primary end-points is determined by the disease and treatment (see above).
2 Postoperative adjuvant therapy. As many excisions are performed for the purpose of healing, the main purpose of adjuvant therapy is to improve the healing rate. Accordingly, the primary end-points of OS and DFS are used.

Safety evaluation. Even if the conclusion of safety was obtained in early exploratory studies, the verification study must also carefully evaluate safety through the monitoring of appropriate subjects.

In verification studies, safety is evaluated by comparison with the control group and is generally set as a secondary end-point. Arrangements must also be made in the event of unexpected adverse events and serious adverse events with respect to the reporting requirements as well as evaluations such as the relationship between the treatment and the appropriate response.

Safety is evaluated in accordance with criteria such as the Common Terminology Criteria for Adverse Events (CTCAE), which is based on adverse events, blood biochemical testing and physiological test results. Adverse events that are not listed in the CTCAE are generally evaluated by severity as mild, moderate, severe or life-threatening.

As evaluation of safety and timely feedback as to the appropriateness of study continuity is required during the study, it is necessary to establish an independent evaluation committee.

Efficacy evaluation and statistical analysis. Efficacy is evaluated mainly by the primary end-points OS or DFS. In this analysis, the survival rate is generally calculated using the Kaplan–Meier method and a comparison between treatment groups is performed using a log rank test or Wilcoxon test. The log rank test has a high detection power in cases where the hazard ratio of the test group compared with the control group is constant during the observation period. Meanwhile, the Wilcoxon test has a higher detection power than the standard log-rank test in cases where the test treatment induces a location shift for the density function of event occurrence. Of note, late-onset effects are assumed to be due to the antigen-specific immune response-mediated pharmacological efficacy of cancer peptide vaccines. Bearing this in mind, the need for analysis using new statistical methods, such as a method that weights the late period of observation as proposed in the Harrington–Fleming method,[22] is also envisioned. The statistical analysis method must be specified in the protocol along with the significance criteria.

While PFS and response rate are sometimes set as secondary efficacy end-points, it is important that the secondary efficacy end-points are set according to the characteristics of the peptide vaccine. Reduction in the lesion size and progression are

other important points for objective evaluation and, as a rule, are evaluated by an independent evaluation committee based on Response Evaluation Criteria in Solid Tumors, etc.

Conclusion

The active promotion of clinical studies is essential in the development of cancer peptide vaccines and the creation of appropriate clinical study guidance is necessary for the active promotion of these clinical studies. This Guidance for peptide vaccines for the treatment of cancer has been published by the Japanese Society for Biological Therapy. Needless to say, periodic review of this guidance may be necessitated with the advancement of cancer vaccine research in the future. The Japanese Society for Biological Therapy welcomes comments from regulators and business people as well as researchers in this area.

Acknowledgments

This work was supported by the Japanese Society for Biological Therapy.

Disclosure Statement

The authors have no conflict of interest.

References

1 Yamaue H, Yamaguchi Y, Okusaka T *et al.* Guidance for peptide vaccines for the treatment of cancer (in Japanese). *Jpn Soc Biol Ther* 2012; **2**: (15 screens). [Cited 28 Apr 2014.] Available from URL: http://jsbt.org/guidance
2 van der Bruggen P, Traversari C, Chomez P *et al.* A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991; **254**: 1643–7.
3 Brodsky FM, Guagliardi LE. The cell biology of antigen processing and presentation. *Annu Rev Immunol* 1991; **9**: 707–44.
4 Marchand M, Weynants P, Rankin E *et al.* Tumor regression responses in melanoma patients treated with a peptide encoded by the gene MAGE-3. *Int J Cancer* 1995; **63**: 883–5.
5 Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med* 2004; **10**: 909–15.
6 Vansteenkiste J, Zielinski M, Dahabre J *et al.* Multi-center, double-blind, randomized, placebo-controlled phase II study to assess the efficacy of recombinant MAGE-A3 vaccine as adjuvant therapy in stage IB/II MAGE-A3-positive, completely resected non-small cell lung cancer (NSCLC). *J Clin Oncol* 2006; **24**(18S): 7019.
7 Holmes JP, Gates JD, Benavides LC *et al.* Optimal dose and schedule of an HER-2/neu (E75) peptide vaccine to prevent breast cancer recurrence: from US Military Cancer Institute Clinical Trials Group Study I-01 and I-02. *Cancer* 2008; **113**: 1666–75.
8 Kantoff PW, Higano CS, Shore ND *et al.* Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 2010; **363**: 411–22.
9 U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research. Guidance for Industry. Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications 2010. Available from URL: http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM202439.pdf
10 Singh H. Development of peptide-based cancer vaccines. from discovery to phase II clinical studies. Results of a randomized phase II study investigating multi-peptide vaccination with IMA901 in advanced renal cell carcinoma. The 48th Annual Meeting of the Japan Society of Clinical Oncology. Kyoto, Academic Seminar #5, 2010.
11 ICH-Q3A (R2): Impurities in New Drug Substances. Pharmaceutical Affairs Bureau, Notification #1216001, December 16, 2002. Partially revised: Pharmaceutical and Food Safety Bureau, Notification #1204001, December 4, 2006.
12 ICH-Q3B (R2): Impurities in New Drug Products. Pharmaceutical Affairs Bureau, Notification #0624001, June 24, 2003. Partially revised: Pharmaceutical and Food Safety Bureau, Notification #0703004, July 3, 2006.
13 Ministerial Ordinance on Good Clinical Practice for Drugs. Ordinance of Ministry of Health, Labour and Welfare, No. 36, March 23, 2005.
14 Standards for Manufacturing Control and Quality Control of Investigational Products. Pharmaceutical and Food Safety Bureau, Notification #0709002, July 9, 2008.
15 Ethical Guidance for Clinical Studies (Fully revised, July 31, 2008). Ministry of Health, Labour and Welfare, Notification No. 415, July 31, 2008.
16 ICH-Q1A (R2): Guidelines for Stability Testing of New Drug Substances and Products. Pharmaceutical Affairs Bureau, Notification #0603001, June 3, 2003.
17 ICH-Q3C (R3): Impurities: Guideline for Residual Solvents. Pharmaceutical Affairs Bureau, Notification #307, March 30, 1998. Partially revised: Pharmaceutical Affairs Bureau, Notification #1225006, December 25, 2002. Partially revised: Pharmaceutical and Food Safety Bureau, Notification #0221/No.1, February 21, 2011.
18 ICH-Q6A: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products. Chemical Substances, Pharmaceutical Affairs Bureau, Notification #568, May 1, 2001.
19 Q&A Concerning Standards for Manufacturing Control and Quality Control of Investigational Products (investigational drug GMP). Ministry of Health, Labour and Welfare, Pharmaceutical and Food Safety Bureau, Compliance and Narcotics Division, Liaison Office, July 2, 2009.
20 Chang AE, Ganz PA, Hayes DF *et al.* , eds. *Oncology: An Evidence-Based Approach*. New York: Springer-Verlag, 2006.
21 Arato T. Issues in peptide drug discovery: non-clinical animal studies. In: Kangawa K, Minamino N, eds. *Peptides and Drug Discovery*. Osaka: Medical Do, 2007; 213–23.
22 Zucker DM, Yang S. Inference for a family of survival models encompassing the proportional hazards and proportional odds models. *Stat Med* 2006; **25**: 995–1014.