

Provisional Translation (as of March 2021)*

PSEHB/PED Notification No. 0308-1

PSEHB/MDED Notification No.0308-1

March 8, 2019

To: Commissioner of Prefectural Health Department (Bureau)

Director of Pharmaceutical Evaluation Division,
Pharmaceutical Safety and Environmental Health Bureau,
Ministry of Health, Labour and Welfare
(Official seal omitted)

Director of the Medical Device Evaluation Division,
Pharmaceutical Safety and Environmental Health Bureau,
Ministry of Health, Labour and Welfare
(Official Seal Omitted)

Guidelines on Cancer Immunotherapy Development

To facilitate development of innovative drugs, medical devices, and regenerative medical products, MHLW worked with academic and research institutions studying and developing state-of-the-art techniques to develop guidelines that aim to establish procedures for quality and safety assessment based on regulatory science, and also conducted a project to personnel exchange between those institutions and the Pharmaceuticals and Medical Devices Agency (PMDA) or the National Institute of Health Sciences (NIHS), from 2012 fiscal year.

Based on the study conducted in “Establishment of the method for evaluating safety and efficacy of cancer vaccines and immunotherapy” (representative researcher: Hiroshi Shiku, Mie University Graduate School of Medicine), we have now presented the “Guidelines on Cancer Immunotherapy Development,” as described below. Please notify the related organizations under your jurisdiction of this Notification to utilize as a reference for the marketing authorization application.

* This English translation of the Japanese Notification is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail. The PMDA will not be responsible for any consequence resulting from the use of this English version.

REMARKS

Guidelines on Cancer Immunotherapy Development

1. These guidelines show an example of matters to be considered in the development of cancer immunotherapy that can be considered at this time, and it is necessary to select the test method required for the marketing authorization application of cancer immunotherapy. Developers should utilize consultation with PMDA as needed.
2. Please refer to the PMDA website (<http://www.pmda.go.jp/rs-std-jp/facilitate-developments/0001.html>) for the details of the personnel exchange project in the innovative drugs, medical devices, and regenerative medical products

End

Note

The Japanese original version consists of four documents:

1. 2015 Guidance on cancer immunotherapy development in early-phase clinical studies
<https://onlinelibrary.wiley.com/doi/epdf/10.1111/cas.12819>
2. General principles on late-phase clinical studies
3. Basic principles in quality, non-clinical and clinical studies of cellular products used for cancer immunotherapy
4. Guidance on nonclinical study for cancer vaccine/adjuvant

This English version includes only “General principles on late-phase clinical studies” and “Basic principles in quality, non-clinical and clinical studies of cellular products used for cancer immunotherapy.”

General principles on late-phase clinical studies

I	Introduction.....	3
II	Objective and scope of this guidance	3
III	Study design in view of characteristics of cancer immunotherapy	4
	III.1. Setting of endpoints.....	4
	III.2. Control group.....	4
	III.3. Statistical analysis procedures	5
IV	Other points to be noted.....	6
	IV.1. Non-inferiority study	6
	IV.2. Biomarkers.....	6
	IV.3. Immune-related adverse events.....	6
V	Reference	7
	V.1. Comparison of key categories between RECIST1.1 and irRC	7
	V.2. Examples of search for biomarkers.....	7
	V.3. Combination cancer immunotherapy.....	8
VI	Guidelines for late-phase clinical studies.....	9
VII	Reference literature.....	10

I Introduction

Recently, information about efficacy of cancer immunotherapy has been rapidly accumulating. For immune checkpoint inhibitors, data from multiple confirmatory clinical studies were reported,¹⁻¹⁰⁾ and some have been already approved in and outside Japan. In addition, for effector cell therapies including transgenic T cell infusion therapy, the efficacy has been suggested by early-phase clinical studies.¹¹⁻¹⁴⁾ For cancer vaccines, data from clinical studies showing the efficacy of peptide vaccine alone, which is a major vaccine type under development, are limited,¹⁵⁾ and research for optimized composition of cancer vaccines in combination with other agents is being planned.¹⁶⁾

Immune checkpoint inhibitors and cancer vaccines are considered to exert an antitumor effect indirectly by inducing tumor cell specific immune response, and it may take a certain length of period to exert the effect. The effector cell therapy is a treatment in which effector cells act on the tumor directly and are reported to exert the antitumor effect as a result of proliferation of the administered cells.^{12), 13)} In addition, according to reports from early-phase clinical studies of immune checkpoint inhibitors, the antitumor response was continued for a certain period, and the tumor remission status was maintained during the follow-up period in some patients.^{17), 18)}

Before conduct of a clinical study of cancer immunotherapy, the study should be designed based on a full understanding of its characteristics different from existing chemotherapies and molecular targeted therapy, and it is important to collect reference information such as results from early-phase clinical studies and the latest knowledge about the other cancer immunotherapies.

II Objective and scope of this guidance

If the proposed cancer immunotherapy is applicable to antineoplastic drugs defined as “drugs clinically useful for patients with malignant tumor by inhibiting growth or metastasis of malignant tumor lesion, extending the survival, or controlling the symptoms” in the “Revised ‘Guideline for clinical evaluation methods of antineoplastic drugs’” (PFSB/ELD Notification No. 11010001 dated November 1, 2005), the evaluation method in accordance with the above guideline should be specified for the clinical study.

The objective of this guidance is to present general principles on late-phase clinical studies of cancer immunotherapies, especially, immune checkpoint inhibitors, effector cell therapy, and cancer vaccines as well as points to be considered for study design. Late-phase clinical studies refer to pivotal studies including confirmatory studies that mainly evaluate the efficacy and safety. Even to cancer immunotherapies not covered by this guidance, a part of this may be applicable.

For development planning, consultation with Pharmaceuticals and Medical Devices Agency (PMDA) is desirable.

III Study design in view of characteristics of cancer immunotherapy

Before conduct of a late-phase clinical study of the cancer immunotherapy to evaluate the clinical usefulness, its design should be fully examined in view of characteristics of the therapy based on information obtained from early-phase clinical studies and others.

Unlike the existing chemotherapies and molecular targeted therapies, immune checkpoint inhibitors and cancer vaccines can be characterized by delayed development and extended duration of the effect, which is exerted through immune function.^{17), 18), 19)} If the survival curves of the control and test therapy group are overlapped for the initial length of period before being separated, a proportional hazard hypothesis would not be supported, and thus procedures and schedule of interim and final analyses should be adequately examined. In addition, the follow-up period should be specified based on the cancer type, treatment line, and information obtained up to late-phase clinical studies.

III.1. Setting of endpoints

To evaluate the efficacy, clinically meaningful endpoints should be specified. The most objectively measurable and clinically meaningful endpoint is an overall survival (OS).

Cancer immunotherapy characterized by delayed development of the effect may entail transient disease progression at the beginning, and thus it should be noted that progression free survival (PFS), if specified as the endpoint, may provide underestimated results on the effect of cancer immunotherapy.¹⁹⁾ Recently, in addition to the standard response criteria for solid tumor (RECIST), application of the other response criteria, immune-related response criteria (irRC)²⁰⁾ or immune-related RECIST (irRECIST)²¹⁾ (see “V Reference-1” section), has been discussed for assessment of tumor progression in view of the periods taken to induce the immune response and for immune cells to infiltrate the tumor tissue. The new response criteria such as irRC, however, have not been fully established, requiring accumulation of evidence through clinical studies in the future. Furthermore, to a clinical study of the particular cancer immunotherapy such as effector cell therapy that is expected to exert the effect early, RECIST may be applied as the response criteria as done for conventional chemotherapy.

As the other efficacy endpoint, the durable response rate (for instance, complete response [CR] or partial response [PR] that is continued for at least 6 months within 1 year after start of the therapy) may be applicable if information obtained from early-phase clinical studies indicates that the effect is continued for an extended period.²²⁾

For the safety of cancer immunotherapy, a profile of adverse events may be different from the profiles with the existing chemotherapies and molecular targeted therapies (see “IV.3” section).

III.2. Control group

The control group included in a late-phase clinical study of cancer immunotherapy may receive the placebo, palliative care, or standard treatment depending on availability of the standard treatment for the target cancer type and disease condition. Justification of the control group should be fully examined from medical, scientific, and ethical viewpoints.

If an adjuvant is administered to the placebo group in a clinical study of the cancer vaccine with the adjuvant, it should be noted that the adjuvant may induce immune response. In addition, if autologous cells are used in effector cell therapy, the placebo-controlled study cannot be conducted from ethical and technical viewpoints because the control group in such a study would receive immune cells without antitumor immune activity added by cell preparation (processing) and thus would have to undergo an invasive blood collection procedure without any benefit, and control of a cell preparation is technically difficult.

III.3. Statistical analysis procedures

In a late-phase clinical study, information on the primary endpoint is highly important for the efficacy evaluation. In a standard analysis on the primary endpoint, Kaplan–Meier estimator on the survival, median OS value, or other parameters is obtained, and group-to-group comparison is performed by log-rank test. In addition, hazard ratio assumption and covariate adjustment are performed using Cox proportional hazards model.

Application of log-rank test to data not showing a proportional hazards profile would reduce the power of analysis. Because cancer immunotherapy characterized by delayed development of the effect is assumed to provide data not showing proportional hazards profile, an analysis method such as Harrington-Fleming test^{23), 24)} in which the late observation period is weighed may be more useful than the log-rank test. Harrington-Fleming test, however, should be appropriately planned with reasonable parameter settings in advance because the weight of Harrington-Fleming test is specified using the function with parameters, unlike the log-rank test.

Milestone survival analysis, which is a cross-sectional assessment of the survival data at the time point prespecified at the time of the clinical study planning, can be used as an analytical method.²⁵⁾ In this method, multiple time points to be covered by the analysis can be specified, but type-I error probability would increase, and actions should be taken not to unblind the study before the final analysis. In addition, a method to compare area under survival curve to specified time point (restricted mean survival times) between groups may be used.²⁶⁾ Although the milestone survival analysis method and the method to compare area under survival curve have no problem in terms of proportional hazards, it should be noted that inappropriate time points specified during planning may lead to a wrong conclusion. Setting of time points to be covered by the analysis should be appropriately planned in view of delayed development of the antitumor effect and its extended durability. Use of these analysis methods will be individually reviewed according to cancer

immunotherapy.

IV Other points to be noted

IV.1. Non-inferiority study

A non-inferiority study to show that the efficacy of cancer immunotherapy is not inferior to that of the active control drug may be conducted in some cases. For conduct of a non-inferiority study, comprehensive consideration should be given to the efficacy, safety, and the other factors (quality of life [QOL], convenience, etc.), and special attention should be paid to non-inferiority margin in view of hypothetical placebo and analytical sensitivity of the study.

IV.2. Biomarkers

At present, although diversity of tumor, interactions between tumor microenvironment, tumor, and immune function, and mechanisms of various actions provided by cancer immunotherapy remain to be elucidated, it is desirable to specify biomarkers indicative of predicted treatment effect, potential immune-related adverse events, or treatment response, and the appropriate patient population should be selected.

For this reason, biomarkers should be exploratorily investigated even at the early-phase clinical stage with reference to the latest knowledge on the other cancer immunotherapies, and the verification should be performed in the late-phase clinical study.

In addition, examples of biomarkers used for existing cancer immunotherapies are described in “V Reference-2. Examples of search for biomarkers” section.

IV.3. Immune-related adverse events

There are reports on immune-related adverse events and severity in patients receiving immune checkpoint inhibitors.^{27), 28), 29)} Important measures against immune-related adverse events include a method to detect serious immune-related adverse events, identification of markers involved in onset of adverse events, establishment of a preventive method against immunological adverse events and a treatment method, and provision of safety information to healthcare professionals and patients.

V Reference

V.1. Comparison of key categories between RECIST1.1 and irRC³⁰⁾

Category	RECIST v 1.1	irRC
Measurement of tumor lesion	Unidimensional	Bidimensional
Target lesions	Up to 5 lesions	Up to 15 lesions
New lesion	Assessed as progressive disease at first appearance	Up to 10 new visceral lesions and 5 cutaneous lesions may be added to the sum of tumor burdens
CR (complete response)	Disappearance of all target and nontarget lesions Nodes must regress to < 10 mm short axis No new lesions Reconfirmation required	
PR (partial response)	≥ 30% decrease in tumor burden from the baseline Reconfirmation required	≥ 50% decrease in tumor burden from the baseline Reconfirmation required
PD (progressive disease)	≥ 20% + 5-mm absolute increase in tumor burden compared with nadir New lesion or non-target lesion progression	≥ 25% increase in tumor burden compared with baseline, nadir, or reset baseline New lesions added to tumor burden Reconfirmation required
SD (stable disease)	Neither PR nor PD	

V.2. Examples of search for biomarkers

Although PD-L1 expression on tumor tissue is a key biomarker for anti-PD-1/PD-L1 antibody, which is classified into immune checkpoint inhibitors, but positioning of PD-L1 as a biomarker may differ depending on cancer type. When the evaluation process on PD-L1 expression including specimen collection schedule, expression level assay, and assessment method is established, a marker potentially predictive of the efficacy may be identified.

A specific cancer gene mutation pattern may play a role of a biomarker because reports have shown that the efficacy of an immune checkpoint inhibitor is correlated to both the number of somatic cell mutations in cancer cells and response of cytotoxic T cells to tumor-specific neo-epitope;³¹⁾ and an anti-PD-1 antibody achieved a high response rate in cancer types with microsatellite instability.³²⁾

For the other biomarkers, research to predict the efficacy based on DNA or RNA gene signature in tumor-infiltrating lymphocytes using tumor tissue is ongoing.³³⁾ Exploratory research to predict the efficacy of melanoma-associated antigen (MAGE)-A3 protein vaccine using specimen from early-phase clinical studies

is reported.³⁴⁾

For cancer immunotherapy, reported biomarkers potentially predictive of treatment response in addition to PD-L1 in the case of immune checkpoint inhibitors are somatic cell mutations in cancer cells, neoepitope-signal, and tumor infiltrating lymphocyte (TIL) signal.³⁵⁾

V.3. Combination cancer immunotherapy

Mechanisms of immune response to cancer such as tumor antigen presentation, activation and proliferation of effector cells, and microenvironment in tumor tissues in a patient body are being elucidated. Immunotherapies such as cancer vaccines, immune checkpoint inhibitors, and effector cell therapy are assumed to act in antigen presentation and T cell activation mechanism uniquely. With such an understanding, combination cancer immunotherapy which consists of cancer immunotherapy and the other therapy is expected to be more effective than individual cancer immunotherapy alone.

According to reports up to now, clinical studies of cancer vaccines alone rarely show the efficacy,³⁶⁾ and its use in combination with an immune checkpoint inhibitor is being investigated.^{37), 38)} On the other hand, combination with therapy of a mechanism of action different from that of cancer immunotherapy may provide synergistic effects because chemotherapy, molecular targeted therapy, or radiation therapy is shown to influence the tumor immune response through modification of antigen-presenting cells as well as the number of T cell subsets and their functions.³⁹⁾

It is desirable to conduct appropriate clinical studies of combination cancer immunotherapy based on results on usefulness from non-clinical studies.

VI Guidelines for late-phase clinical studies

- Revision of “Guideline for Clinical Evaluation Methods of Antineoplastic Drugs”
(PFSB/ELD Notification No. 1101001 dated November 1, 2005)
- Questions and Answers (Q&A) about Guideline for Clinical Evaluation Methods of Antineoplastic Drugs
(Administrative Notice dated March 1, 2006)
- General Considerations for Clinical Trials (ICH E8)
(PMSB/ELD Notification No. 380 dated April 21, 1998)
- “Statistical Principles for Clinical Trials (ICH E9)”
(PMSB/ELD Notification No. 1047 dated November 30, 1998)
- Concerning “Selection of Control Group for Clinical Studies and Relevant Issues”
(PMSB/ELD Notification No. 136 dated February 27, 2001)

VII Reference literature

- 1) Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol* 2015; 16:375-84.
- 2) Herbst RS, Baas P, Kim D, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; 387:1540-50.
- 3) Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet* 2016; 387:1837-46.
- 4) Seiwert TY, Burtness B, Mehra R, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol* 2016; 17:956-65.
- 5) Hamanishi J, Mandai M, Ikeda T, et al. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. *J Clin Oncol* 2015; 33:4015-22.
- 6) Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* 2015; 372:311 – 9.
- 7) Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 2016; 387:1909-20.
- 8) Muro K, Chung HC, Shankaran V, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 2016; 17:717-26.
- 9) Nanda R, Chow LQM, Dees EC, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: Phase 1b KEYNOTE-012 Study. *J Clin Oncol* 2016; 34:2460-7.
- 10) Kaufman HL, Russell J, Hamid O, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. *Lancet Oncol* 2016; 17:1374-85.
- 11) Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science*. 2006; 314:126-9.
- 12) Robbins PF, Kassim SH, Tran TL, A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. *Clin Cancer Res*. 2015; 21:1019-27.
- 13) Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015; 7 (303):303ra139.
- 14) Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N*

Eng J Med.2014;37:1507-17.

- 15) Schwartzentruber DJ, Lawson DH, Richards JM, et al.gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma.N Engl J Med.2011;364:2119-27.
- 16) Walter S, Weinschenk T, Stenzl A, et al.Multipptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival.Nat Med.2012;18 (8):1254-61.
- 17) Topalian SL, Sznol M, McDermott D.F., et al.Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab.J Clin Oncol.2014;32:1020-30.
- 18) McDermott DF, Drake CG, Sznol M, et al.Survival, durable response, and long-term safety in patients with previously treated advanced renal cell carcinoma receiving nivolumab.J Clin Oncol.2015;33:2013-20.
- 19) Small, E.J., Schellhammer, P.F., Higano, C.S., et al.Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer.J Clin Oncol.2006;24:3089-94.
- 20) Wolchok JD, Hoos A, O'Day S, et al.Guidelines for the evaluation of immune therapy activity in solid tumors:immune-related response criteria.Clin Cancer Res.2009;15(23):7412-20.
- 21) Bohnsack O, Hoos A, Ludajic K, Adaptaion of the immune related response criteria:irRECIST.Ann Oncol.2014;25(Supplement 4):361-72.
- 22) Andtbacka RH, Kaufman HL, Collichio F, et al.Talimogene laherparepvec improves durable response rate in patients with advanced melanoma.J Clin Oncol.2015; 33:2780-8.
- 23) Harrington DP, Fleming TR.A class of rank test procedures for censored survival data. Biometrika.1982; 69:553-66.
- 24) Fine GD.Consequences of delayed treatment effects on analysis of time-to-event endpoints.Drug Inf J.2007; 41:535-9.
- 25) Chen TT.Milestone Survival:A potential intermediate endpoint for immune checkpoint inhibitors.J Natl Cancer Inst.2015; 107(9).
- 26) Royston P, Parmar MK.The use of restricted mean survival time to estimate the treatment effect in randomized clinical trials when the proportion hazards assumption is in doubt.Statist Med.2011; 30:2409-2421.

- 27) Lo JA, Fisher DE, Flaherty KT. Prognostic significance of cutaneous adverse events associated with pembrolizumab therapy. *JAMA Oncol.* 2015. doi:10.1001/jamaoncol.2015.2274.
- 28) Attia P, Phan GQ, Maker AV, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J Clin Oncol.* 2005;23(25):6043-53.
- 29) Weber JS, Hodi FS, Wolchok JD, et al. Safety profile of nivolumab monotherapy: a pooled analysis of patients with advanced melanoma. *J Clin Oncol* 34 2016 Nov 14. [Epub ahead of print]
- 30) Hodi FS, Hwu W, Kefford R, et al. Evaluation of immune-related response criteria and RECIST v1.1 in patients with advanced melanoma treated with pembrolizumab. *J Clin Oncol.* 2016;34:1510-7.
- 31) Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Eng J Med* 2014;371:2189-99.
- 32) Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med.* 2015;372:2509-20.
- 33) Lopez-Chavez A, Thomas A, Rajan A, et al. Molecular profiling and targeted therapy for advanced thoracic malignancies: a biomarker-derived, multiarm, multihistology phase II basket trial. *J Clin Oncol.* 2015;33:1000-7.
- 34) Ulloa-Montoya F, Louahed J, Dizier B, et al. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. *J Clin Oncol.* 2013;31:2388-95.
- 35) Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non – small cell lung cancer. *Science.* 2015; 348:124-8.
- 36) Rosenberg SA, Yang JC, and Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med.* 2004; 10:909-915.
- 37) Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med.* 2015;373:23-34.
- 38) Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med* 2015;372:2006-17.
- 39) Galluzzi L, Buqué A, Kepp O, et al. Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell.* 2015;28:690-714.

Basic principles in quality, non-clinical and
clinical studies of cellular products used
for cancer immunotherapy

Table of contents

Introduction	15
Chapter 1 General Provisions.....	17
Article 1 Objective	17
Article 2 Definitions	17
Chapter 2 Manufacturing and quality control.....	18
Article 1 General principles on manufacturing and quality control	18
Article 2 Donors.....	18
Article 3 Matters for manufacturing method.....	18
Article 4 Matters for quality control of final product.....	19
1) Effector cell products	19
2) Non-effector cell products.....	19
Article 5 Transportation.....	19
Chapter 3 Non-clinical safety studies	21
Article 1 General principles on non-clinical safety studies	21
1) General principles on toxicity evaluation.....	21
2) Selection of animal species and special characteristics of immunodeficient animals....	21
Article 2 General toxicity studies.....	22
Article 3 Tumorigenicity studies	23
Chapter 4 Studies to support efficacy or performance	25
Article 1 General principles on studies to support efficacy or performance	25
Article 2 <i>In vitro</i> and <i>in vivo</i> tests	25
1) <i>In vitro</i> tests	25
2) <i>In vivo</i> test.....	26
Chapter 5 Biodistribution studies	27
Article 1 General principles on biodistribution studies.....	27
Article 2 Study method.....	27
Chapter 6 Clinical studies	28
Reference	29
Reference literature	32

Introduction

As evident clinical effects of immune checkpoint inhibitors and genetically modified lymphocytes have been reported recently, cancer immunotherapy is drawing high expectations and strong interest. The remarkable clinical effects, on the other hand, have been shown to entail a possibility of adverse reactions, requiring a strategy to develop cancer immunotherapies with improved efficacy and safety. In Japan, at the same time, use of human cell-based products, which are classified as regenerative medical products under the recently established Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics (hereinafter referred to as the "PMD Act"), has been spreading in various medical fields, and even in the field of cancer immunotherapy, human cell-based products (hereinafter referred to as "cellular products") are expected to be further widely used as well.

Cellular products used for cancer immunotherapy include effector cells and non-effector cells. The former cells are $\alpha\beta$ -T cells, $\gamma\delta$ -T cells, natural killer (NK) cells, and natural killer T (NKT) cells, which are administered to induce antitumor immune response directly in patients (in an antigen-specific or antigen-nonspecific manner). The latter cells are antigen-presenting cells such as dendritic cells, which are administered to activate antitumor immune response in patients (as a kind of vaccines), and mesenchymal stem cells, which are administered to modify the immune environment in patients or the local environment in a tumor.

Sources or raw materials of these products may vary, including autologous cells, allogeneic cells, somatic stem cells, induced pluripotent stem (iPS) cells, and embryonic stem (ES) cells. These products can be manufactured through culture, in combination with non-cellular ingredients, using genetical engineering techniques, or by various cellular processing such as reprogramming, dedifferentiation, and induction of differentiation. To ensure the quality and safety of cellular products by addressing potential problems specific to the sources and manufacturing processes, relevant guidelines should be observed.

In view of information accumulating in the rapidly developing field of cancer immunotherapy and the current situation of increasing usage with cellular products as well as the future development direction of this field, points to be considered in development of cellular products for cancer immunotherapy are desired to be selected and organized. In addition, a methodology for efficient development that adequately harmonizes with the viewpoint of regulatory sciences is also desired. This guidance describes general principles on basic requirements for quality, safety, and non-clinical efficacy of cellular products in cancer immunotherapy with a focus on points to be particularly considered in development of cellular products for cancer immunotherapy and the special nature of this therapy. In addition, this guidance should be applied in a reasonable and effective manner according to the specific nature of a given cellular product.

To ensure the quality and safety of cellular products, the following announcements and notifications should be referred to.

- Standards for Biological Ingredients (MHLW Ministerial Announcement No. 375 dated September 26, 2014)
- Points to ensure the quality and safety of human (autologous) cellular and tissue-based drugs and medical devices (PFSB Notification No. 0208003 dated February 8, 2008)
- Points to ensure the quality and safety of human (allogeneic) cellular and tissue-based drugs and

medical devices (PFSB Notification No. 0912006 dated September 12, 2008)

- Points to ensure the quality and safety of human (autologous) somatic stem cell-based drugs (PFSB Notification No. 0907-2 dated September 7, 2012)
- Points to ensure the quality and safety of human (allogeneic) somatic stem cell-based drugs (PFSB Notification No. 0907-3 dated September 7, 2012)
- Points to ensure the quality and safety of human (autologous) iPS(-like) cell-based drugs (PFSB Notification No. 0907-4 dated September 7, 2012)
- Points to ensure the quality and safety of human (allogeneic) iPS(-like) cell-based drugs (PFSB Notification No. 0907-5 dated September 7, 2012)
- Points to ensure the quality and safety of human ES cell-based drugs (PFSB Notification No. 0907-6 dated September 7, 2012)

In addition, if any genetical engineering technique has been applied to the product, the following notification and administrative notice should be referred to.

- Points to ensure the quality and safety of gene therapy drugs (PFSB/ELD Notification No. 0701-4 dated July 1, 2013)
- ICH Considerations “General Principles to Address Virus and Vector Shedding” (Administrative Notice dated June 23, 2015)

The following recently issued administrative notice should be also referred to.

- Technical guidance for the quality of and conduct of non-clinical and clinical studies of regenerative medical products (human cell-based products) (Administrative Notice dated June 27, 2016)

Chapter 1 General Provisions

Article 1 Objective

This guidance describes general principles on basic requirements for quality, non-clinical safety, data supportive of efficacy or performance, disposition, and clinical studies of human cellular products for cancer immunotherapy.

Article 2 Definitions

1. Human “cellular products” are products prepared from human (autologous or allogeneic) cells as well as human (autologous or allogeneic) somatic stem cell, human (autologous or allogeneic) iPS(-like) cell, and human ES cell-based products.
2. Cellular “preparation (processing)” techniques are used for treatment of a disease or tissue repair or restoration and include drug-treatment for artificial cellular proliferation or differentiation, cell line establishment, and cellular activation, modification of biological properties, combination with non-cellular ingredients, and genetical engineering. Such preparation (processing) techniques do not include cellular separation, isolation of specified cells, treatment with antibiotics, washing, gamma-ray sterilization and others, freezing, and thawing.
3. “Manufacturing” operations include not only operations for preparation (processing) but also ones that would not modify the original nature of the cells such as cellular separation, isolation of specified cells, treatment with antibiotics, washing, gamma-ray sterilization and others, freezing, and thawing; and cover a full process from acceptance of sources of which control is required to ensure the quality of a final product to release of a human cellular product, the final product.
4. A “donor” is a human who provides cells or tissue as a raw material of a “cellular product.”
5. A “phenotype” is a morphological and physiological attribute expressed by a given gene under a certain environmental condition.
6. A “vector” is a carrier used to introduce a gene of interest into host cells. If a recombinant virus is used, however, the vector is referred to as a viral vector including the transgene. If a plasmid including a gene of interest is directly introduced into cells, the plasmid DNA is deemed as a vector.
7. A “viral vector” is a recombinant virus used as a vector and consists of viral particles in which recombinant viral genome with a gene of interest integrated is packaged instead of wild-type viral genome.
8. An *in vitro* test is a test performed outside of a human or animal body, and an *in vivo* test is a test in which a cellular product is administered into an animal body.

Chapter 2 Manufacturing and quality control

Article 1 General principles on manufacturing and quality control

For regenerative medical products including cellular products for cancer immunotherapy, the manufacturing method and quality control based on reasonable rationales reflecting the current knowledge at present point are required in view of the properties as well as the intended use, manufacturing scale, and extent affected by the risk.

In particular, many of the regenerative medical products have difficulty in ensuring the safety against infectious agents with a methodology similar to that for conventional drugs because cells used as a source may be contaminated owing to infection in the donor, and it is almost impossible to implement sterilization, viral inactivation, and removal of such agents. For this reason, the safety of a cellular product against infectious agents should be carefully investigated by not only tests on the final product but also in-process control tests according to properties of the product and the manufacturing method.

This guidance therefore describes basic requirements for and general principles on the manufacturing method and quality control of cellular products for cancer immunotherapy in light of reference notifications for ensuring the quality and safety of the cellular products presented in the “Introduction” section.

Article 2 Donors

If autologous cells are used as a source, a donor screening would not be necessarily required because of the source’s nature from a viewpoint of a risk of infectious disease transmission. Inspection items and control criteria, however, should be established according to the intended use in view of a risk specific to a given cellular product because actions can be required to rule out contamination in the manufacturing process, to address a risk of proliferation of infectious agents in the manufacturing process, or to ensure the safety primarily in operators engaged in manufacture and healthcare professionals, depending on the product. If allogeneic cells derived from an allografted donor are used, general principles on the infectious disease risk similar to those for use of autologous cells may be applicable.

Article 3 Matters for manufacturing method

It is difficult to identify critical quality attributes related to the efficacy and safety particularly at the initial development stage where experiences with and knowledge about manufacture are limited. For instance, it may not be appropriate to decide uniformly a control value on the cell number of cells used as a source, which is likely to affect the efficacy. In such a case, flexible actions may be accepted. For instance, only the initial cell number before cell expansion may be established as a minimum cell number, which is necessary to obtain the amount of cells potentially providing efficacy, based on results from non-clinical studies, in the process control of an investigational product at early clinical development stage, allowing its adjustment of the cell number within a range ensuring the safety. When the development phase is advanced, a control value that can ensure the safety as achieved in the previous phase will be required to be established.

Usually, peripheral blood drawn by a conventional procedure is considered unlikely to contain microorganisms including mycoplasma. In a case where an isolated tissue is used as a raw material, however, it is useful to perform microbiology tests on the raw material according to its contamination risk as

well and thereby to determine whether to use antibiotics active against microorganisms or mycoplasma or not as a manufacturing control.

Article 4 Matters for quality control of final product

Quality control tests that should be commonly specified on cellular products for cancer immunotherapy are cell number and viability, identification, cell purity, process-related impurities, sterility, mycoplasma, bacterial endotoxins, and virus free tests. In addition, tests for efficacy and potency should be specified. Specifications and test methods should be established according to properties of a given cellular product (Reference 1).

The sterility test on a sterile drug with an antimicrobial activity is usually performed on a test sample of which the antimicrobial activity has been removed or neutralized beforehand where applicable. If an antibiotic is used in a cellular product to inhibit microbial growth during the manufacture, consideration should be given to its impact on results from a sterility test, and thereby appropriate test methods and specifications should be established. Not only a Sterility Test specified in the Japanese Pharmacopoeia but also a test method derived from one listed in Rapid Microbial Methods may be established.

In addition, to select target virus types for a virus free test, consideration should be given to a risk of viral proliferation associated with manufacture. If the risk remains unknown, a comprehensive test with a multi-assay may be established, but an informed consent should be obtained from the patient.

1) Effector cell products

Test methods for effector cell products should cover not only tests commonly specified on cellular products for cancer immunotherapy but also ones for performance and/or potency such as a cytokine production test. Furthermore, for genetically modified T cell products, a test for replication competent virus (RCV) should be established, and establishment of tests for copy number of transgene, interleukin (IL)-2 dependent growth, clonality, tetramer analysis, and vector integrity should be considered. In addition, for performance and potency of autologous effector cells, which are used for activated lymphocyte therapy, test methods are recommended to be established in a reasonable design because it is difficult to decide target cells due to the non-specific nature of the therapy, potentially leading to limited validity of the test method; and an amount of sample cells from the final product available for the tests is technically limited.

2) Non-effector cell products

Test methods for non-effector cell products should cover not only tests commonly specified on cellular products for cancer immunotherapy but also ones for performance and/or potency such as a cell growth test based on mixed lymphocyte reactions with allogeneic lymphocytes and a cytokine production test. In addition, for efficacy and potency, test methods are recommended to be established in a reasonable design because it is difficult to select target cells, potentially leading to limited validity of the test method; and an amount of a sample cells from the final product available for the tests is technically limited.

Article 5 Transportation

For transportation of blood, which is used as a starting material, conditions such as temperature, period, container, and delivery means should be controlled because these may affect the quality. If autologous serum or plasma is used in manufacture, the quality of the serum or plasma should be controlled in addition to the cells to be used as a source. For transportation of a cellular product, conditions such as storage temperature and delivery means of the cells should be controlled as well because these may affect the quality.

In addition to control of the transportation conditions, a specimen packaged separately from the cellular product for release, if prepared (as a test sample for viability determination), will enable evaluation of the cell number in the cellular product at the time of infusion, cell viability, and an impact of thawing in the case of a frozen product.

Chapter 3 Non-clinical safety studies

Article 1 General principles on non-clinical safety studies

1) General principles on toxicity evaluation

For toxicity associated with specific immune response induced by a cellular product for cancer immunotherapy or toxicity resulting from its unintended binding to receptors, evaluation using animals is mostly difficult owing to species differences. Of effector cell products, however, T cell products with a genetically modified receptor are artificially provided with cytotoxicity targeting cancer cells, and if a molecule targeted by the receptor or molecule structurally similar to such a target is expressed in vitally critical tissues or cells, severe toxicity may occur. It is therefore necessary to confirm that the products have not acquired an additional ability of responding to autologous cells (self-reactivity) (for instance, absence of an ability of producing interferon (IFN)- γ in response to autologous cells) (Reference 2). In addition, on T cell products with T cell receptor (TCR) genetically introduced (hereinafter referred to as "TCR-T cell" products), which are expected to be developed in the future, it is desirable to prevent mispairing between endogenous TCR- α/β chain and genetically introduced TCR- α/β chain, and evaluation of self-reactivity should be considered where necessary (Reference 3). In addition, on T cell products with chimeric antigen receptor (CAR) genetically introduced (hereinafter referred to as "CAR-T cell" products), evaluation for the self-reactivity *in vitro* should be considered as well, but it is theoretically unreasonable to predict a risk of adverse events, which are entailed by the desired clinical response, in *in vitro* studies, such as disappearance of normal B lymphocytes observed in anti-CD19-CAR-T cell therapy (Reference 2), and thus it may not be necessary to evaluate toxicity accompanied by cytotoxicity on target cells in *in vitro* studies.

2) Selection of animal species and special characteristics of immunodeficient animals

For conduct of nonclinical safety studies, because of species differences in active proteins such as cytokines and cell-cell interaction between human and animals, administration of human cellular product into normal immune-competent animals potentially results in rejection of the administered cells through an immunological mechanism in a short term further followed by inflammation attributable to heterologous immunoreaction, preventing acquisition of information useful in extrapolating the animal data for human safety. In addition, administration of an homologous cellular product with a comparable function to that of human cellular product (surrogate cellular product) into animals may be useful in evaluating the effect but may not provide data appropriate for toxicity evaluation because the surrogate cellular product is different from the actual product. As animals used in nonclinical safety studies, therefore, immunodeficient animals in which human cells used for cancer immunotherapy are likely engrafted should be selected (Reference 4). If a disease model mouse useful in nonclinical studies is available, it can be considered as one of the options.

Immunodeficient animals currently available for nonclinical safety studies are nude mouse (T cell deficient), severe combined immunodeficiency (SCID) mouse (T cell and B cell deficient), recombination-activating gene (RAG) deficient mouse (T cell and B cell deficient), nonobese diabetic (NOD)-SCID mouse (T cell and B cell deficient, and NK cell partially deficient), NOD/Scid/common γ -chain deficient (NOG) mouse (T cell, B cell, and NK cell deficient), and immunodeficient rat. An immunodeficient animal appropriate for the cellular type (for a transgenic human T lymphocyte cellular product, NOG mouse in which

human hematopoietic cells are likely engrafted is used) should be selected. It, however, should be noted that immunodeficient animals may be infected with microorganisms not controlled (not monitored) in the specific pathogen-free (SPF) environment because these animals are susceptible to microorganisms that are not pathogenic in normal mice. In addition, it should be also noted that conduct of a study requiring long-term observation may be difficult because the lifetime of some strains¹ of immunodeficient animals are shorter than immune-competent mice.

Article 2 General toxicity studies

General toxicity studies should be conducted in light of the number of cells enough to exert the effect and the post-dose cell growth potential with reference to the “Guideline for drug toxicity study methods,” an attachment of the “Guideline on toxicity studies necessary for marketing authorization (import) for pharmaceuticals” (PAB/ED Notification No. 1-24 dated September 11, 1989), “Revision of guideline for single- and repeated-dose toxicity studies” (PAB/NDD Notification No. 88 dated August 10, 1993), and “Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals” (PFSB/ELD Notification No. 0219-4 dated February 19, 2010). In addition, the following points should be noted for conduct of the studies.

- Basically, any cellular product should be administered by the same route as the proposed clinical route for evaluation. Because quantitative risk evaluation is difficult, a non-clinical study including at least 2 groups of the control and investigational cellular product may suffice for identifying hazards, but the highest dose should be the maximum dose¹ allowing evaluation of systemic toxicity. The number of doses is desirably in accordance with the regimen planned in clinical studies, but if repeated doses do not result in accumulation of the cellular product in the body and thus are unlikely to cause new toxicity findings or exacerbation of the toxicity, repeated-dose toxicity studies may not be necessarily conducted. The observation period may be specified as approximately 14 days, of which a period is considered as the minimum period allowing evaluation of systemic toxicity, but it should be appropriately specified in view of characteristics of the cellular product.
- The safety of non-cellular ingredients should be evaluated by physicochemical methods in view of the attributes and content wherever possible, and nonclinical safety studies with the focus on non-cellular ingredients should be separately conducted where necessary. Regarding impurities, it is necessary to show the residual amount of impurities (estimated residual amount) and then explain the grounds for determining that safety can be guaranteed. If the safety of the impurities at the residual amounts estimated from the existing information is hardly justified, non-clinical safety studies with the focus on the impurities should be conducted.
- Because immunodeficient animals are used, such a study may use one animal species, which does not necessarily have to be non-rodent or non-human primate.
- The negative control group should be appropriately placed. For instance, if a genetically modified human T cell product is administered into immunodeficient animals, non-modified human T cells may be used as the control to distinguish the effect of human T cells themselves.

Safety pharmacology studies are not usually required because it is poorly extrapolated to humans and

¹ The number of cells to be administered (engrafted) should be as many as possible from viewpoints of the maximum tolerated dose (MTD) and maximum feasible dose (MFD).

quantitative risk evaluation is difficult. Additional studies, however, may be considered to justify the safety in humans, if general toxicity studies (including clinical observations) have demonstrated that the product does not considerably affect vitally critical organs (cardiovascular, respiratory, and central nervous systems), but toxicity raising a safety concern in humans is observed.

Article 3 Tumorigenicity studies

With respect to cells used as a raw material, ES or iPS cells have the highest malignant transformation risk followed by somatic stem cells and somatic cells in this order, and furthermore products derived from ES or iPS cells should be evaluated for a risk of teratoma formation due to residual pluripotent stem cells.

Tumorigenicity can be evaluated by *in vitro* and *in vivo* tests, of which the former include a karyotyping test for genetic stability and a soft agar colony formation test for scaffold-independent proliferation potential, and the latter include a test for tumorigenic potential evaluated in immunodeficient animals. If a general toxicity study is conducted using immunodeficient animals, tumorigenicity *in vivo* may be evaluated as a part of the general toxicity study. The following points should be noted for conduct of *in vivo* tests.

- Evaluation of tumorigenic risk due to malignant transformed cells in a cellular product: Whether tumor cells proliferate and form tumor in the body or not, can strongly depend on the local environment conditions in the tissue, which include local histological structure, cytokine environment, blood and lymph flow status, immune surveillance status, and attributes of the extracellular matrix and interstitial cells. Predominant sites of metastatic tumor differ depending on the primary tumor type. The tumorigenic risk due to malignant transformed cells in a final product, therefore, should be evaluated using the same engraftment route as the proposed clinical route in principle.
- Evaluation of risk of teratoma formation due to residual pluripotent stem cells in a product derived from ES or iPS cells: For evaluation of a risk of residual pluripotent stem cells, it is desirable to assess a teratoma formation potential by subcutaneously engrafting the product in the back of animals.
- Because engraftment of tumorigenic cells less than the threshold of tumorigenesis may lead to a negative result for tumorigenicity, the maximum transplantable amount of cells should be engrafted by a single procedure, regardless of the clinical regimen. For determination of the tumorigenic potential, 2 groups of the control (negative) and cellular product engraftment may suffice.
- The appropriate number of animals may be 10 animals per group at the final evaluation stage in accordance with WHO Technical Report Series 978, of which scope does not include cellular products.
- The observation period to evaluate the risk of malignant transformed cells in a final product derived from ES or iPS cells should be a period extended until engrafted cells are no longer detected or period throughout which the immunodeficient animals are not affected by aging or spontaneous lesions, and the survival period in the body should be also considered. The observation period in a study to evaluate a risk of teratoma formation due to residual ES or iPS cells in a final product² should be appropriately specified as a period long enough to detect teratoma formation with reference to published literature. For products derived somatic stem cells that are considered less likely to raise tumorigenic concerns, the study period may be specified as recommended in the WHO Guideline TRS978 (4-16 weeks).

² Threshold of teratoma formation evaluated by the number of residual ES/iPS cells in a final product
Takahashi et al. Cell 2007;131,861-872: 9 weeks
Standfeld et al. Science 2008;332,945-949: 3-4 weeks
Zhou et al. Stem Cells 2009; 27,2667-2674: 4-6 weeks

Because human differentiated cells such as hematopoietic cells are considered less likely to raise concerns of malignant transformation during the manufacturing process than undifferentiated pluripotent stem cells, conduct of *in vivo* tumorigenicity studies is meaningless. For genetically modified T cells, however, attention should be paid to malignant transformation of cells associated with gene transfer, and the concern may be evaluated by an IL-2-dependent proliferation test or clonality analysis. See the “Chapter 2 Manufacturing and quality control, 1) Effector cell products” section.

Chapter 4 Studies to support efficacy or performance

Article 1 General principles on studies to support efficacy or performance

Appropriate and meaningful studies to support efficacy or performance should be effectively conducted according to cell type, autologous or allogeneic cell status, intended clinical use, and attributes based on reasonable rationales in light of rapidly advancing technological progress in this area and clinical knowledge. The following points should be noted for conduct of the concerned studies.

- Effects expected from biological activity of a product expressed by cellular preparation (processing) (Reference 5), resultant functions developed in cells, tissues, and individuals, and the duration should be evaluated using animals or cells. In addition, for the biological activity of a product expressed by cellular preparation (processing) (Reference 5), an appropriate model should be devised to elucidate the efficacy or performance based on the quality attributes.
- If the proposed cellular product is intended to introduce a gene, the effectiveness and duration of the expression as well as the biological activity of the expressed gene product should be evaluated.
- If appropriate animal cells or animal models of disease are available, studies should be conducted using these. In addition, impacts of differences in cancer type on the effect should be investigated in view of the tumor site.
- If reasonable evaluation to support the efficacy or performance of a cellular product is possible with literature or knowledge at the initial clinical stage, detailed experimental investigation is not necessarily required.

Article 2 *In vitro* and *in vivo* tests

Studies to support the efficacy or performance may be conducted *in vitro* and *in vivo* (animal model or human cell engraftment model using immunodeficient mice). For a cellular product involving multiple types of immune cells, tumor-specific cytotoxicity cells cannot be evaluated only in *in vitro* tests, and thus it is desirable to evaluate such cytotoxicity in both *in vitro* and *in vivo* tests in combination based on characteristics of the cellular product.

1) *In vitro* tests

To identify the cell type and subset, the phenotype specific to the cellular product and expression of molecules involved in the function (adhesion molecule linked to the function, expression of costimulatory, etc.) as well as the percentage of the target cells should be evaluated by a flow cytometry method. Furthermore, for a cellular product prepared by induction of differentiation, cells before and after differentiation should be compared, and the phenotype and expression of molecules involved in the function should be evaluated.

The tumor-specific cytotoxicity can be evaluated directly for effector cells and indirectly by the induced immune response for non-effector cells. An *in vitro* test of a cellular product may be performed using tumor cells or human peripheral mononuclear cells as the target cells, but for a T cell product, the target cells used in the test should be desirably tumor cell lines with human leukocyte antigen (hereinafter referred to as "HLA") gene introduced or clinical specimens from cancer patients eligible for the concerned cellular product.

The following test methods may be applied to evaluation of specific cytotoxicity against tumor cells.

- Cytokine production tests: Evaluation of intracellular cytokine production (flow cytometry method) and

extracellular cytokine production (enzyme-linked immunosorbent assay [ELISA] method or enzyme-linked immunospot [ELISPOT] method)

- Cell growth assay: Evaluation of growth using ^3H -thymidine, bromodeoxyuridine (BrdU), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)
- Tumor cell-specific cytotoxicity degranulation test: Evaluation of CD107a expression
- Tumor cell-specific cytotoxicity test: Evaluation using ^{51}Cr release assay.

In addition, for a cellular product of genetically modified T cells with cancer antigen-specific TCR gene introduced (TCR-T cells), conservation of HLA restriction should be evaluated. The evaluation can be made by inhibition test for specific cytotoxicity using anti-HLA class I antibody or anti-HLA class II antibody or a specific cytotoxicity test using cells not expressing the target antigen or cells expressing the target antigen with unrelated HLA as the negative control.

2) *In vivo* test

[1] Evaluation using animal models

If an animal model of the target disease is available, and phenotype and expression of molecules involved in the functions of human cells have been demonstrated to be comparable to those of animal cells in the model, cytotoxicity can be evaluated in the animal model using the analogous animal product (model cells). It, however, should be noted that extrapolation of evaluation of results in animals to humans may not be appropriate owing to differences in immune responses between the test animal species and human, and the animal species and differences in the effectiveness between cancer types should be investigated. In addition, because the effectiveness can differ depending on presence or absence of T cell-induced inflammation at the tumor site (Reference literature 1), the tumor site in the animal model should be analyzed to evaluate the differences between the test animal species and human. Furthermore, because it has been shown that the efficacy may vary depending on cancer type, and impacts of the cancer type should be investigated (Reference 6), investigation of such impacts should be implemented in the animal model.

[2] Evaluation in human cell engraftment model

If a cellular product of which phenotype and expression of molecules involved in the functions of the human cells have not been demonstrated to be comparable to those of animal cells or an effector cell product is evaluated in a human cell engraftment model using immunodeficient mice, it should be noted that human immune responses cannot be reproduced in the immunodeficient animals (owing to differences in hematopoietic and immune systems, especially interstitial tissues which frequently serve as the origin of a tumor). Thus, an animal model appropriate for the objective must be prepared, and results from the evaluation may have limitations of the interpretation. In addition, it should be further noted that graft versus host disease (GVHD) caused by the xenogeneic engraftment may affect the cytotoxicity.

Chapter 5 Biodistribution studies

Article 1 General principles on biodistribution studies

For the cell component of a cellular product and expression product of the transgene, the survival period of the cells or tissue and duration of the effectiveness in humans should be estimated within a technically feasible and scientifically reasonable range, showing that the intended effectiveness can be adequately expected. Within a technically feasible and scientifically reasonable range, therefore, appropriateness of the administration method, and survival and locality of the administered cellular product should be clarified by studies for disposition of the product in animals and other studies.

Article 2 Study method

Disposition of the infused cellular product can be evaluated by histological analysis, polymerase chain reaction (PCR), magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and bioimaging (*in vivo* imaging using cells with luciferase gene introduced, or a dye-staining or isotope-labeling technique).

The administration method of a cellular product should be justified by evaluating disposition of the administered cells in animals. In particular, for a cellular product intended to be administered systemically, disposition of the cells systemically administered into an animal model (for instance, distribution in non-target tissues) should be evaluated and extrapolated in humans.

If the cells are directly administered into the specific site (tissue) or administered to reach the specific site to exert the effect, the locality should be clarified to discuss its impacts on the efficacy and safety of the cellular product. For an effector cell product, migration toward the tumor tissue is important, and for a non-effector cell product, distribution into tissues such as lymphoid tissues is important. Therefore, systemic distribution should be evaluated by *in vivo* imaging, etc., and then distribution of the administered cells in the target tissues, tumor tissues, or lymphoid tissues where these cells may be distributed should be analyzed in detail by immune staining or flow cytometry technique.

To evaluate survival of the administered cells, distribution of these cells as well as anti-tumor effectiveness and clinical signs in mice should be followed up over time for a certain post-dose period using immunodeficient animals. In addition, for an effector cell product, an immunodeficient mouse model in which human cancer cells (established cell line or cancer cells derived from a patient) have been engrafted may be used to evaluate appropriateness of the administration method, and survival and accumulation of the administered effector cells in the tumor(Reference 7).

Chapter 6 Clinical studies

Clinical usefulness of a cellular product will be evaluated based on information such as clinical study data on the efficacy and safety, but for a clinical study plan of a cellular product, the following points should be considered to establish an appropriate study design.

- Target disease
- Characteristics of the cellular product
- Application method
- Inclusion and exclusion criteria
- Description of treatment provided to subjects including application of the cellular product
- Justification of the clinical study in view of existing treatment
- Matters to be explained to subjects including risk and benefit expected from currently available information

Especially for patients potentially eligible for cancer immunotherapy, the risk and expected benefit should be carefully weighed. The evident risk expected for the cellular product should be eliminated by utilizing the currently available knowledge and technologies to justify the product scientifically, and then an “unknown risk” potential should be considered. Concerning the case where cancer immunotherapy is not provided to a patient with cancer resistant to conventional therapies, on the other hand, a “disadvantage that the patient may suffer from by missing the new treatment opportunity” should be considered, and both drawbacks should be weighed. Then, desirably, all the relevant information should be disclosed to the patient to whom the decision whether to receive the cancer immunotherapy or not is left.

Reference

Reference 1 Examples of preparation of homogenous cellular products

If a donor is a patient who has received highly intensified anticancer therapies or an elderly patient, complex factors involving transfection efficiency of the gene of interest and culture conditions would have impacts on the yield, and thus it may be difficult to obtain a highly purified and homogenous cell population in a large amount. To obtain a homogenous cellular product qualitatively or quantitatively, monoclonal antibody or beads can be used.

For dendritic cells, the functions should be evaluated by checking expression of a molecule group involved in T cell stimulation (HLA class I, HLA class II, CD80, CD86, and others) in the identification test and a cytokine production pattern (IL-12/IL-10 ratio and others) in the cytokine production test. In addition, for mesenchymal stem cells, evaluation should be made by checking expression of cell surface markers (CD34, CD45, CD73, CD29, CD90, CD105, CD166, and others) in the identification test, confirming cell growth (potential) and their differentiation into cartilage, bone, and fat as well as performing a karyotyping test.

Reference 2 Example of points to be considered for non-clinical safety studies of a T cell product with genetically modified receptor

1) TCR-T cells

Now that affinity of TCR applied to clinical studies has been artificially increased to a non-physiological level where fatal on-target or off-target toxicities may occur.

For example, in a clinical study where melanoma-associated antigen (hereinafter referred to as "MAGE")-A3-specific HLA-A2-restricted TCR-T cell therapy was used in treatment of melanoma, fatal central nerve destruction occurred. MAGE-A3 is one of the cancer testis antigens (CTAs) that are selectively expressed in cancer cells and normal testis tissue. In this study, a similar peptide derived from MAGE-A12, which is a MAGE-A3 family protein and slightly expressed on the central nerve tissue, was presented as the antigen with HLA-A2 and recognized and attacked by the administered TCR-T cells, leading to the fatal outcome (Reference literature 2).

In addition, in a clinical study where MAGE-A3-specific HLA-A1-restricted TCR-T cell therapy was used in treatment of melanoma and multiple myeloma, fatal myocardial injury occurred. Although MAGE-A3 is not physiologically expressed on the myocardium at all, Titin, a myocardial structure protein, has an epitope of amino acid sequence recognized by MAGE-A3-specific TCR. The antigen epitope derived from Titin presented in the context of HLA-A1 molecule synchronized with a myocardial systole (in a context-dependent manner) was recognized and attacked by the administered TCR-T cells, leading to the fatal outcome (Reference literature 3).

At present, on-target or off-target toxicity of TCR-T cells cannot be accurately predicted. Efforts, however, have been made to predict such toxicity wherever possible. For a novel TCR-T cell therapy or TCR-based antigen epitope/HLA complex-specific CAR-T cell therapy, a method was developed to search for known amino acid sequences with the structure similar to that of the target antigen epitope from a database according to the response to a library of peptides in which amino acid residues of the target antigen epitope were replaced by alanine residue (alanine scan method) (Reference literature 4). For others, a method to check for effector cell response using a panel of vital organ cells derived from iPS cells with the intended HLA was proposed (Reference literature 3). While use of iPS cells takes time, the alanine scan method is feasible at present although the risk cannot be completely ruled out. Results obtained by the method, therefore, can serve as a part of basic data on the safety in future development of novel TCR-T cell therapies and some CAR-T cell therapies.

2) CAR-T cells

Most of the adverse events in a patient receiving CAR-T cells are attributable to on-target or off-target toxicity except for the following case: A patient who received multiple doses of CAR-T cells by infusion experienced fatal adverse events associated with anaphylactic shock mediated by human anti-mouse antibody (HAMA) developed against the extracellular domain derived from the mouse monoclonal antibody (Reference literature 5). Representative events are disappearance of normal B lymphocytes observed in anti-CD19-CAR-T cell therapy and fatal lung injury observed in anti-human epidermal growth factor receptor 2 (Her2/neu)-CAR-T cell therapy (Her2 is slightly expressed on the normal lung epithelial cells) (Reference literature 6).

Reference 3 Inhibition against endogenous TCR- α/β gene expression in TCR-T cell therapy

Autoreactivity additionally developed in TCR-T cells prepared from autologous T cells is attributable to unintended α/β chain pairing (mispairing) between endogenous TCR- α/β chain originally expressed on the host T cells and introduced therapeutic TCR- α/β chain. At present, inhibition against such endogenous TCR- α/β gene expression by various methods can reduce the risk of developing unknown autoreactivity in TCR-T cells (Reference literatures 7 and 8).

Reference 4 Example of toxicity evaluation in HLA-transgenic immunodeficient mice

For instance, Wilms Tumor 1 (hereinafter referred to as "WT1"), an cancer-related antigen of which physiological expression is found relatively high in hematopoietic progenitor cells, lung and pleura, peritoneum, and glomerular podocytes, has $\geq 95\%$ homology in amino acid sequence between humans and mouse. For on-target toxicity of WT1-specific TCR-T cells in these organs, especially the kidney, there is a report on toxicity in HLA-transgenic immunodeficient mice (Reference literature 9). Because in HLA-transgenic immunodeficient mice transplanted with human hematopoietic stem cells (so-called "humanized mouse"), hematopoietic and immune systems are partly humanized to some extent, but interstitial tissues are not, this model is not adequate for on-target toxicity evaluation. The intact mouse evaluation system rather provides more information. It is therefore very important to establish an appropriate mouse model of the disease.

Reference 5 Type of cell preparation (processing)

Cell preparation (processing) is as defined in "Points to be considered for marketing approval application of regenerative medical products" (PFSB/ELD/OMDE Notification No. 0812-5 dated August 12, 2014). Representative types are as follows:

- Combination with non-cellular component (matrix, medical materials, supportive membrane, fiber, beads, and others)
- Application of genetical modification to cells
- Introduction of protein into cells
- Reprogramming, dedifferentiation, and induction of differentiation of cells by drug treatment
- Reprogramming, dedifferentiation, and induction of differentiation of cells by physical technique

Reference 6 Example of relationship between genetic mutation and prognosis

For instance, a relationship between BRAF or NRAS genetic mutation and prognosis of melanoma has been reported (Reference literature 10).

Reference 7 Example of evaluation on survival and tumor accumulation of administered effector cells

Transfection of luciferase genes with different colors in engrafted cancer cells and infused effector cells

allows evaluation of tumor accumulation without necropsy.

Reference literature

1. Tumei PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 515 (7528):568-71, 2014.
2. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, Dudley ME, Feldman SA, Yang JC, Sherry RM, Phan GQ, Hughes MS, Kammula US, Miller AD, Hessman CJ, Stewart AA, Restifo NP, Quezado MM, Alimchandani M, Rosenberg AZ, Nath A, Wang T, Bielekova B, Wuest SC, Akula N, McMahon FJ, Wilde S, Mosetter B, Schendel DJ, Laurencot CM, Rosenberg SA. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J Immunother*. 36(2):133-51, 2013.
3. Cameron BJ, Gerry AB, Dukes J, Harper JV, Kannan V, Bianchi FC, Grand F, Brewer JE, Gupta M, Plesa G, Bossi G, Vuidepot A, Powlesland AS, Legg A, Adams KJ, Bennett AD, Pumphrey NJ, Williams DD, Binder-Scholl G, Kulikovskaya I, Levine BL, Riley JL, Varela-Rohena A, Stadtmauer EA, Rapoport AP, Linette GP, June CH, Hassan NJ, Kalos M, Jakobsen BK. Identification of a Titin-derived HLA-A1-presented peptide as a cross-reactive target for engineered MAGE A3- directed T cells. *Sci Transl Med*. 5(197):197ra103, 2013.
4. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, Wunderlich JR, Somerville RP, Hogan K, Hinrichs CS, Parkhurst MR, Yang JC, Rosenberg SA. Cancer immunotherapy based on mutation-specific CD4 + T cells in a patient with epithelial cancer. *Science*. 344(6184):641-5, 2014.
5. Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, Chew A, Zhao Y, Levine BL, Albelda SM, Kalos M, June CH. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. *Cancer Immunol Res*. 2:112-120, 2014.
6. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther*. 18:843-851, 2010.
7. Okamoto S, Mineno J, Ikeda H, Fujiwara H, Yasukawa M, Shiku H, Kato I. Improved expression and reactivity of transduced tumor-specific TCRs in human lymphocytes by specific silencing of endogenous TCR. *Cancer Res*. 69:9003-9011, 2009.

8. Torikai H, Reik A, Liu PQ, Zhou Y, Zhang L, Maiti S, Huls H, Miller JC, Kebriaei P, Rabinovitch B, Lee DA, Champlin RE, Bonini C, Naldini L, Rebar EJ, Gregory PD, Holmes MC, Cooper LJ. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. *Blood*. 119:5697-5705, 2012.
9. Asai H, Fujiwara H, Kitazawa S, Kobayashi N, Ochi T, Miyazaki Y, Ochi F, Akatsuka Y, Okamoto S, Mineno J, Kuzushima K, Ikeda H, Shiku H, Yasukawa M. Adoptive transfer of genetically engineered WT1-specific cytotoxic T lymphocytes does not induce renal injury. *J Hematol Oncol*. 7:3, 2014.
10. Bhatia P, Friedlander P, Zakaria E, Kandil E. Impact of BRAF mutation status in the prognosis of cutaneous melanoma: an area of ongoing research. *Ann Transl Med* 3:24-31, 2015.