Considerations on the Development of Personalized Neoantigen-Targeted Therapy Products (Guidance for Industry)

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MINISTRY OF FOOD AND DRUG SAFETY
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Cell and Gene Therapy Products Division
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This guidance provides considerations on the development of personalized neoantigen-targeted therapy products in more plain language and represents the current stance of the MFDS on this topic.

This document should be viewed as only recommendations since it is not intended to be legally binding and does not impose any obligations upon industry despite the word 'should' used herein. Besides, since this guidance is written based on the established scientific and technological experiences, and valid laws as of July, 2023, its interpretation and application may vary if necessitated by revision of relevant laws and/or new scientific discoveries, etc.

※ An MFDS guidance for industry is a document published to promote understanding of applicable statues or administrative rules in more plain language or to present the current stance of the MFDS, internally and externally, on specific applications or any equivalent ones from industry filed with the agency [Article 2 of *Regulation on the Management of MFDS Guidance Documents, etc*].

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1.Introduction

Neoantigens are defined as a class of novel proteins produced by accumulated specific DNA mutations, and selectively expressed in cancer cells while absent from normal cells. Neoantigens are generated primarily by mutations including non-synonymous mutation[1], fusions, not to be limited. where changes in the sequence of a gene alter the amino acid sequence of the resulting protein; unlike tumor-associated antigens (TAAs) expressed at lower levels in non-malignant cells, neoantigens are characterized by their expression restricted to malignant cells.

Unlike conventional chemotherapies, targeted therapies, immunotherapies, or gene therapies that are designed to target shared targets or antigens across different patients or tumor types, personalized anticancer treatments are predicated on individual patient-specific neoantigens. Therefore, these products offer new therapeutic opportunities for patients with unsatisfactory responses to all existing therapeutic options, which has brought this new class of therapies into the limelight as a novel paradigm for cancer treatment, supported by recent advancements in applicable technologies including molecular biology and bioinformatics. The advent of next-generation sequencing (NGS) and advances in cutting-edge data analysis techniques has facilitated the identification of tumor-specific mutations unique to individual patients. In addition, the development of computational algorithms capable of predicting the binding affinities of peptide to major histocompatability complex (MHC) Class I/II[2] proteins has permitted the identification of putative immunogenic neoepitopes[3] out of numerous tumor-specific mutations. Leveraging these technological advancements, new personalized neoantigen-targeted clinical trials on cancer therapy are actively ongoing using various platforms, including

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1) A mutation that alters the amino acid sequence of a protein
2) Major histocompatibility complex (MHC) Class I/II: cell surface molecules that present antigens to T-cells; Class I molecules are expressed in most cell types and Class II molecules are restricted to professional antigen presenting cells
3) Neoepitopes: peptides occurring in tumor-specific mutations that bind to MHC molecules

but not limited to synthetic peptides, RNA, DNA, and dendritic cells (DCs).

Neoantigen-targeted anticancer treatments have several advantages. First, neoantigens occur exclusively in tumor cells, not expressed in normal cells. Consequently, T-cells activated by neoantigens can remove tumor cells, minimally affecting normal cells. Second, as neoantigens are novel epitopes or antigenic determinants that are derived from somatic mutations, neoantigen-reactive T-cells are not under negative selection by central immune tolerance[4] and do not elicit autoimmune responses; therefore, they possess high immunogenic potentials. Third, since T-cells stimulated by exogenous neoantigens differentiate into memory T-cells following treatment, they persist as long-lived memory T-cells *in vivo*, thereby contributing to the suppression of tumor recurrence.

Despite these advantages, further technological advancements are warranted to overcome challenges standing in the way of a broad patient access to personalized neoantigen-targeting therapies, including high manufacturing costs for personalized treatments, time-consuming manufacturing process, and uncertainties in the accurate prediction of neoantigens. In particular, complicated algorithms for identification/selection of tumor-specific neoantigens in individual patients and highly individualized subsequent custom manufacturing processes may pose hurdles to commercial use of these products. Also, unlike conventional therapies, regulatory oversight is required not only for the final products but also for other pre-manufacturing steps ranging all the way from sample collection, neoantigen identification to design and production of neoantigen-targeted therapeutics involving neoantigen prioritization. Given these challenges, this guidance has been prepared and issued to provide applicable guidance in advance for developers of neoantigen-targeted therapeutics.

4) Central immune tolerance: a process occurring in the thymus to eliminate self-reactive T-cells

2. Legal Basis

Personalized neoantigen-targeted therapeutics are in development using various platforms such as peptides, mRNA, DNA and DCs. This guidance focuses on neoantigen-targeted therapeutics based on DNA, mRNA, and DCs (hereafter referred to as "personalized neoantigen-targeted therapeutics using DNA, etc."), which are classified as advanced biological products. Nevertheless, this guidance may be applicable to the development of neoantigen-targeted products involving the use of other delivery platforms, as appropriate.

Personalized neoantigen-targeted therapeutics described in this guidance, which are regulated as advanced biological products, should be licensed for marketing pursuant to Sections 23-2 and 27-1 of the *Act On The Safety Of And Support For Advanced Regenerative Medicine And Advanced Biological Products*. The dossier submitted for regulatory assessment should be prepared in the Common Technical Document (CTD) format, as required by Section 6 (Preparation of CTD) of *Regulation on Review and Authorization of Advanced Biological Products*; based on the format employed for the products, the requirements specified in Section 14 (Types of quality data for cell therapy products) or Section 15 (Types of quality data for gene therapy products), and Section 17 (Assessment criteria for nonclinical data) of the aforesaid regulation should be fulfilled.

The purpose of this guidance is to provide an overview of what should be considered for each manufacturing step and clinical and nonclinical studies for personalized neoantigen-targeted therapeutics, which comprise sequencing of tumor and normal tissue specimens, selecting desired neoantigens by screening mutations from sequencing readouts, and manufacturing anti-cancer therapeutics based on the sequences of the selected neoantigens.

3. Considerations for Quality Assessment

Depending on the manufacturing platform, the applicant may refer to one of the following guidelines; *Guideline on the Quality Control of mRNA-based Gene Therapy Products (Guidance for Industry), Guidelines on the Quality and Nonclinical Evaluation of Plasmid DNA-based Gene Therapy Products (Guidance for Industry)*, or *Guideline for Quality Evaluation of Gene Therapy Products (Guidance for Industry)* provide the basic principles for the implementation of quality control of neoantigen-targeted products. If a product is designed to use DCs primed with personal tumor-specific neoantigens, not directly delivering the genetic materials of the neoantigens, please refer to *Guideline on the Requirements for Quality Dossier of Cell and Gene Therapy products (Guidance for Industry).*

3.1 General Information

The International Nonproprietary Name for Pharmaceutical Substance (INN), drug code, and generic name for the investigational product should be presented. When the product is intended to be a gene therapy product that encodes neoantigens, the following information on the vector genome to be used should be provided: the type, size, molecular weight, schematic diagram of its construct; reference sequences of all the components (open access database and RefSeq ID numbers); function of each component, purpose of its introduction; and genomic organization showing each component. If any vector component displays a difference from its reference sequence (e.g., codon optimization, mutation, etc.), details should be described and justified. Each component of the vector genome should be integral to the activity or manufacturing of the product; if a selection marker is used, justification should be provided for its use. Although antibiotic resistance marker should be avoided in the final products as much as possible relevant justification should be provided in the inevitable case.

In addition, the general characteristics of the product under development should be described, such as physicochemical properties,

mechanism of action, and biological activity. To find out more on relevant general information, please see the guidances noted above.

3.2 Manufacturing

This guidance is written with a particular focus on the considerations for the selection of neoantigen sequences that are seen as the key element in inducing anti-tumor activity of personalized therapeutics.

Besides the selection process for neoantigen sequences, details on manufacturing process, raw material control, process validation and evaluation, and process development can be found in *Guideline for Quality Evaluation of Gene Therapy Products (Guidance for Industry)* and *Guideline on the Requirements for Quality Dossier of Cell and Gene Therapy Products (Guidance for Industry).*

For plasmid- or mRNA-based gene therapy products, *E. coli* cell banks are typically established and used to maintain consistency throughout manufacturing processes. But, , selectively reduced set of tests on an *E. coli* cell bank may be considered for personalized neoantigen-targeted therapeutics for each individual patient. Also, for personalized neoantigen-targeted therapeutics using a viral vector, virus seed stock should be prepared tailored to each patient. If consistent quality is assured with the initial batches, characterization tests may be undertaken selectively, starting from later ones onwards.

In the case of mRNA-based neoantigen therapeutics, it is possible to consider creating a DNA template to use it for mRNA production without establishing an *E. coli* cell bank, provided that sufficient quality control is performed with the DNA template, which is the starting material for *in vitro* transcription (IVT) step.

In addition, for cell-based personalized neoantigen-targeting products, including but not limited to CAR-T cell therapies, that use genetically modified T-cells through transduction with viral vectors as a main component, data on the characteristics of the vector used for genetic transduction and modification, as well as its manufacturing and quality

controls, should be provided to the satisfaction of the Regulatory Agency.

As cancer cells are characterized by an accumulation of somatic mutations, cancer-specific neoepitopes that are recognized by autologous T-cells may be generated in patients with tumor. As these neoepitopes evade central immune tolerance and are not expressed in healthy tissues, they are considered suitable targets for anti-cancer treatments, but the vast majority of somatic mutations expressed in individual patients are uniquely different among themselves. Currently, large-scale computational algorithms and machine learning tools are under development to be used for the identification of mutations in sequence data, prioritization of those likely to be recognized by T-cells, and design of personalized therapeutics comprising multiple cancer mutations. Attention should be paid to *in silico* analytical techniques that can select mutant peptides likely to bind to each MHC allele, assess the expression level of mutations, their distribution in tumors, and their similarity to self-proteins, which, in turn, provides an estimation based on which to select neoantigen candidates for patient-tailored anti-cancer therapeutics. Consequently, the relevance of these programs and algorithms should be sufficiently recognized by developers to ensure the adoption of suitable tools.

The process, which encompasses multiple steps from the manufacture of personalized neoantigen-targeted therapeutics to their administration to patients, is roughly divided into the following: acquisition of tumor tissues; the detection of patient-specific mutations in tumor; identification of potentially immunogenic neoantigens; and manufacture and administration of neoantigen-targeted therapeutics. Based on currently available emerged technologies and theories, the following sections in this document are providing guidance informative for undertaking each step described above, and dossier should be adequately prepared for clinical trial authorization and marketing approval, including detailed descriptions of each step in the “Manufactu ring” section of the quality assessment data.

3.2.1. Acquisition of Tumor Tissues

One of the characteristics exhibited by cancer tissues is that cancer cells are typified by molecular biological heterogeneity even in the same lesion, and neoantigen candidates identified in one metastatic lesion of a patient may differ from those in the primary tumor and/or other metastatic lesions. Owing to such intratumoral heterogeniety and mutation variability between the primary and metastatic cancer tissues, a biopsy of a single lesion may not accurately reflect the mutation occurring across the tumors in a patient. For the detection of tumor mutations, multi-site core needle biopsy can be performed in multiple accessible sites to ensure the representativeness of the biospecimens taken from the tumors, if necessary. Theoretically, a single biopsy might not reflect the entire mutational profile of a patient. However, since it is not feasible to conduct a multi-site biopsy in a patient, liquid biopsies that analyze circulating tumor DNA (ctDNA) in the bloodstream is proposed as a less invasive alternative to reduce the risks arising from tumor biopsy in patients. However, there is still a need for further advances in technologies that will improve detection and identification of cancer mutations.

When biopsy specimens are obtained in a clinical setting, care should be taken to ensure that the biopsied tissues contain a sufficient amount of cancer cells. Care must be taken to select normal tissue as a relevant germline control sample.

Collection of and storage conditions for malignant and non-malignant tissues may affect the resulting sequencing data; and thus careful consideration should be given to this matter. With standard operating procedures (SOPs) in place at the specimen acquisition stage, the processes for specimen acquisition and storage should be validated to ensure the reliability of the analysis outcomes. When the specimens are appropriately controlled, for example by cryopreservation, prior to sequencing, optimal analytical data are expected to be generated. However, additional efforts should be made in the storage and transport of the biospecimens. For formalin-fixed or paraffin-embedded specimens, the fixation process may affect the outcome of the DNA sequencing

data. Therefore, it is important to select appropriate biospecimens for sequencing with great care.

3.2.2 Tumor Mutation Calling

In general, Whole Exome Sequencing (WES) is employed to detect tumor-specific mutations by comparing DNA data between tumors and normal tissues in the same patient. This approach involves determining the sequences of the protein-coding regions that comprise around 1% of the entire genome. Mutations in the sequences, such as single-nucleotide variants, deletions, translocations, inversions, and insertions, should be those that lead to alterations in the sequence of amino acids in the protein. Typically, *de novo* or large-scale mutations caused by deletions, translocations, insertions, etc. are more likely to give rise to highly immunogenic peptides than changes in a single amino acid. Consequently, given that large-scale mutations may not be detected by WES, an analysis that leverages high-resolution sequencing at the whole genome level may be required.

Mutation calling process should accurately distinguish real mutations from artifacts introduced by sample preparation and/or sequencing steps or simple somatic variants. To achieve this, it is necessary to validate the reliability of analytical software and database, that is, to evaluate the analytical capabilities of the tools. This can be achieved by presenting analytical data from sequencing that is conducted using reference samples.

While a plethora of software tools are available for mutation calling, no single tool can be considered optimal. Consequently, a frequently employed approach is based on mutations commonly found using multiple software tools. Samples collected from tumor, in particular, may display a high degree of heterogeneity for reasons such as mixtures of healthy cells, as well as intra- and inter-tumor heterogeneity. These factors should be taken into consideration to identify an appropriate solution. To be more precise, a sophisticated approach is required to identify mutations, such as the analysis of multiple samples from a single patient or the integration of multiple data analytical methods.

In addition, it has been demonstrated that DNA methylation or post-translational modifications on histones can regulate the expression of neoantigens. Consequently, epigenetic data may be referenced to expand the scope of tumor mutation calling.

To obtain reliable data, it is necessary to collect and store tumor tissues and healthy tissues appropriately, as noted above, and DNA extraction procedures should also be conducted using validated methods. Furthermore, the quality of the specimen collected for analysis, and the quality and reliability of the reference database used for sequence alignment are important elements in mutation calling. To achieve robust sequencing outcomes of mutations, it may be necessary to analyze multi-site specimens from a single tumor tissue.

3.2.3 Selection of Neoantigens

The ultimate aim of a cancer vaccine is to promote anti-tumor immune responses until tumor cells are completely eliminated by reigniting the cancer immunity cycle through the priming of neoantigen-specific T-cells or the activation of pre-existing T-cells.

Consequently, neoantigen selection is the most important element in determining the safety and efficacy of personalized neoantigen-targeted therapeutics. Since not all somatic mutations generate neoantigens owing to MHC restriction and immune tolerance, predicting the immunogenecity of neoantigen candidates is the most critical step in the manufacture of neoantigen-targeted anti-cancer therapeutics. For this critical step, developers should qualify the expression and allele frequency of the mutations of interest and predict their MHC-binding affinity in order to select clinically effective neoantigens.

A prediction algorithm can be used to generate the abilities of neoantigen candidates to activate T-cells based on the following factors, including but not limited to 1) expression level of mutated transcripts, 2) neoepitope-MHC binding affinity and stability, and 3) T-cell recognition-associated characteristics (dissimilarity to self-antigens or similarity to pathogenic antigens and binding affinity to T-cell receptors),

in order to prioritize the list of potent candidate neoantigens. However, the weighting scheme of each neoantigen candidate is not yet established, nor are there available datasets that can accurately predict the immnogenicity of candidate neoantigens. Furthermore, existing datasets are diverse by data subject and not standardized; thus developers should provide a justification for their selection of datasets to the extent feasible.

The level of neoantigen expression in cells can be assessed using RNA extracted from a tumor biopsy using analytical methods such as RNA-seq. In general, the analysis of expression level is conducted only in tumor tissues, and the tumor specificity of the mutations can be confirmed using whole exome sequencing analyses of paired tumor and germline samples of the same patient.

The antigen presentation process by MHC molecules involves a series of key steps, including peptide-MHC binding as well as degradation of mutant proteins into smaller peptides and their transport into the endoplasmic reticulum, which can be predicted leveraging prediction algorithms.

Neoantigen-MHC binding affinity is another key feature in predicting and selecting immunogenic neoantigens. Given that the same mutation can be presented by both MHC-I and MHC-II peptides and that antigen-MHC-I complex alone is not sufficient for T-cell activation, an appropriate approach will be to combine neoantigens that are predicted to bind to both MHC-I and MHC-II molecules. Considering less stringent sequence and length requirements for binding to MHC-II compared with MHC-I, the likelihood of mutant peptides being presented on MHC-II and the diversity of neoantigen-MHC-II complexes will be higher. Neoantigens that are expressed at a robust level in tumors and provide neoepitopes with sufficiently high affinity to MHC-I or MHC-II have a higher suitability for priming naïve T-cells[5] by effective cross-presentation of

5) Naïve T-cells: T-cells that have gone through differentiation and maturation but not encountered appropriate antigens yet, which will become activated and developed into effector cells when they later on bind to peptide-MHC complexes presented by

endocytosed antigens. It is possible to exploit algorithms for predicting neoantigen-MHC binding affinities that utilize MHC binding affinity data from the wet lab process and amino acid sequence data obtained using mass spectrometry analysis of peptides eluted from MHC complexes. These methods can predict allele-specific neoantigen-MHC binding patterns. These methods are known to predict binding affinities of a neoepitope to each MHC allele.

For T-cell priming, antigen-MHC complexes should be first recognized by and bind to T-cell receptors (TCRs) expressed on the surface of T-cells. So, for prediction of antigen-MHC complex and TCR binding, analysis tools, such as structure-based neoantigen prediction and artificial intelligence (AI)-aided analysis, are applied. However, tools have not shown a sufficient level of prediction performance, yet. Furthermore, as stability may lead to an increased probability of these complexes being recognized by T-cells, it is also proposed that the stability of neoeptiope-MHC complexes could be more important for immunogenicity prediction than their binding affinities. Consequently, for neoantigen candidates, not only their binding affinities to MHC-I and MHC-II but also the stability of neoepitope-MHC complexes can be evaluated.

As T-cells are eliminated by mechanisms of central immune tolerance in the thymus based on self-reactivity, it is also advantageous for neoantigens to have a lower degree of similarity to self-antigens and a higher degree of similarity to non-self antigens for their survival and maintenance. For the identification of similarity of neoantigens to self-antigens, Basic Local Alignment Search Tool (BLAST) is commonly used to produce alignment scores as a measure of similarity to human DNA sequences. Additionally, structural similarities of neoantigens can be analyzed and compared. For neoantigens that display similarity to pathogen-derived antigens, cross-reactive immunity may be induced by pre-established memory T-cells that recognize them, as described above. For the prediction of sequence similarity between neoantigens and

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antigen presenting cells.

pathogen-derived antigens, it will be informative to analyze the likelihood of cross-reactivity of T-cells that respond to commonly encountered pathogens.

Besides the methods mentioned above, mutation clonality and loss of heterozygosity (LOH) are employed to assess the quality of neoantigens. When an essential gene undergoes LOH, resulting in the loss of one of the two alleles and the generation of neoantigens from the remaining allele, tumor cells are unable to survive in the absence of the neoantigen, thus demonstrating its indispensability for the survival of the tumor cells. It could be hypothesized that mutations in essential gene that undergo LOH might possibly be excellent targets for neoantigen-targeted therapeutics. LOH can be predicted using next generation sequencing (NGS) or microarray data analysis, and approximately 1,600-2,500 genes are known to be essential for cell survival, which can be considered for the prioritization of neoantigen candidates.

It is recommended that developers provide evidence to justify proposed strategies for the prediction of neoantigens. This can be achieved through experimental-analytical approaches, including *in silico* analyses of TCR binding affinity to each peptide-MHC complex, TCR-pMHC complex binding affinity, stability of the pMHC complex, and neoantigen similarity to self-antigens.

Moreover, to prevent the occurrence of unanticipated side effects with the administered neoantigens, it should be verified that neoantigen candidates with a high degree of expression in cells and HLA binding affinity, or proteins (peptides) with sequences similar to those of neoantigens are not expressed in other major organs or tissues.

3.2.4 Design and Delivery of Neoantigens

Current platforms in use for the delivery of personalized neoantigens include synthetic peptides, DNA, mRNA, viral vectors, and DCs. Synthetic peptides consist of 15-30 amino acids, and are typically used with adjuvants. Recently, the effectiveness of mRNA vaccines has been

demonstrated, with substantial backing from liposomal formulations. While DNA has advantage in simpler and less expensive manufacturing process, it needs to be delivered into the cell nucleus, which poses a technical challenge. Viral vectors face challenges in terms of manufacturing and handling as well as their inherent immunogenic features. However, stable gene delivery into target cells is a clear advantage of viral vectors. Considering the time and cost required starting from conduct of tumor biopsy to manufacturing of neoantigen-targeted therapeutics, developers should proceed with a development plan based on an optimized platform design for their products.

For the delivery of neoantigens, manufacturing processes tailored to the chosen technology platform and a set of individual neoepitope candidates to be delivered by this platform should be selected. Some clinical studies have reported the use of a combination of between 2 and 34 mutations as MHC-presented neoeitopes for each personalized product. Depending on the product formats, since dozens of neoepitopes may be administered per patient, the products can be designed to feature different complementary categories of neoepitopes, such as MHC-I and/or MHC-II binding vs. non-binding and founding clones containing primary tumor-derived mutations vs. metastatic site-derived sub-mutations. When a combination of multiple neoantigens is selected considering these complementary features, the risk of clinical trial failure can be mitigated.

The most frequently used neoantigen formats are 15-30 amino acid-long peptides combined with poly (I:C) as an adjuvant, and mRNA formulations encoding multiple neoepitope sequences. However, viral vectors or DNA may be employed in conjunction with various adjuvants, as described above; in these cases, the need for a co-injected adjuvant and the dosing schedule (requirements and frequency of boosters following the initial priming) should be determined individually for each delivery format. In addition, for further enhancement of the immunogenicity of neoantigens, various molecular immunological agents that will act as an adjuvant can be incorporated into the design of DNA-

or mRNA-derived neoantigen-targeted therapeutics, achieving a simultaneous expression of neoantigens and these adjuvant agents. When such agents are used, a rationale should be provided to justify the selection of each agent.

The manufacturing processes for each format will impact the speed, scalability, and costs of its manufacturing. For successful commercialization of personalized neoantigen-targeted therapy, parallel, upscaled manufacturing processes that simultaneously produce multiple batches of each tailored drug product will be important. This represents a significant departure from the scale-up paradigm of manufacturing processes pursued in conventional pharmaceutical development, for which consideration should be given to building infrastructure with computerized and automated manufacturing processes.

3.3 Characterization

Characterization should include tests that can demonstrate the appropriateness of the strategies used for selecting optimal neoantigens and also the manufacturing processes for the therapeutic agent loaded with or primed against neoantigens. These tests should be conducted in a manner that allows for the assessment of relevant characteristics across multiple batches and the acquisition of an appropriate level of data that can be utilized to extrapolate the validity of the neoantigen selection strategies and manufacturing processes to the manufacturing of other neoantigen-targeted therapeutics that use each patient's own sequences. In other words, data that provide representative quality attributes of the products should be obtained by 1) selecting critical quality attributes (CQAs) and defining acceptance criteria for each CQA and 2) assessing risks for each process parameter and developing a design space for the products, through the production of multiple batches. In particular, for neoantigen selection strategies and subsequent custom manufacturing processes for each product format, analytical data on multiple batches are required to verify the efficacy of the products and consistency in their manufacturing.

For the assessment of immune responses induced by

neoantigen-targeted therapeutics, several test methods can be employed, including ELISPOT assay, intracellular cytokine staining, and p-MHC multimer staining[6]. These tests can provide useful information, such as the magnitude and phenotype of immune responses associated with polyclonal T-cells that are activated and expanded by personalized neoantigen-targeted therapeutics. For the analysis of antigen-specific T-cells at the clonal level, single cell RNA-Sequencing (scRNA-Seq), TCR-Sequencing (full-length TCR-Seq), or single cell TCR Sequencing (scTCR-Seq) are applicable.

3.4 Controls of Drug Substance and Drug Product

As neoantigens from each patient contain unique sequences, batch-specific manufacturing and controls should be implemented. More specifically, for products loaded with genetic materials for neoantigens, quality control testing should include "identity tests" to analyze the complete sequence and/or altered regions including those that encode the neoantigens. Additionally, as part of quality testing and/or characterization, tests should be conducted to verify that the genetic information encoding the neoantigens is expressed in the anticipated size at the protein level.

Detailed information on quality controls of each drug substance and drug product can be found in the above-noted guidances: *Guideline on the Quality Control of mRNA-based Gene Therapy Products (Guidance for Industry); Guidelines on the Quality and Nonclinical Evaluation of Plasmid DNA-based Gene Therapy Products (Guidance for Industry); Guideline for Quality Evaluation of Gene Therapy Products (Guidance for Industry);* or *Guideline on the Requirements for Quality Dossier of Cell and Gene Therapy products (Guidance for Industry),* and applied to the manufacturing and product platform of choice, as appropriate.

Considering potential alterations in physicochemical structures of each batch that is manufactured customized to each individual, key content of the strategies for selecting neoantigens, as described in "3.2

6) An assay to identify the presence of T-cells with high affinity to specific antigen-MHC complexes

Manufacturing" of this guidance, should be specified under "Definition' " and/or "Molecular Formula/Structural Formula" in the Attached Specifications of Drug Substances.

For the formulation of final products, if an excipient is used for the first time for a drug product in Korea or by a new route of administration, the safety of the excipien should be demonstrated.

3.5 Stability

Stability studies should be conducted in accordance with *Specifications for Stability Testing of Pharmaceuticals (MFDS Notification)* and *Guideline for Stability Testing of Biopharmaceuticals (Guidance for Industry)*. Detailed information is provided in *Q&As' on Specifications for Stability Testing of Pharmaceuticals (Guidance for Industry)*. Given that personalized neoantigen-targeted therapeutics display inherent structural variability per batch, it is not feasible to perform stability testing for every batch to determine the shelf-life (expiry date) of the products. Therefore, it needs to be calculated from pooled stability data on multiple batches. As such, batches used for stability testing to determine shelf-life (expiry date) should represent other batches of the investigational product, and justification should be provided to support the selection and number of representative batches included for stability assessment.

For products that require further manipulation prior to administration, such as thawing or dilution, in-use stability data, should be provided to support the validity of the conditions under which additional manipulations of the products are to be conducted.

4. Nonclinical Considerations

In general, nonclinical studies of personalized neoantigen-targeted therapeutics should be conducted in consideration of therapeutic payloads in accordance with *Guideline on Nonclinical Evaluation of Gene Therapy Products (Guidance for Industry)* or *Considerations for the*

Assessment of Dendritic Cell-based Therapy Products for Cancer (Guidance for Industry).

However, as personalized neoantigen-targeted therapeutics are custom-made for each individual patient, batches used for nonclinical studies should be those most appropriate for the assessment of the safety and efficacy of the products (hereinafter referred to as "representative batches"), and the selection of representative batches should be justified.

4.1 Efficacy Studies

In principle, submission of preclinical data on tumor reduction or *in vivo* immune response is not a mandatory requirement but recommended, but delivery efficiency *in vivo* and efficacy achieved using *in vivo* delivery should be demonstrated in representative batches. To demonstrate the efficacy attained through neoantigen selection strategies and delivery platform, it is possible to use *in vitro* immune induction studies using tumor tissues or blood taken from patients or blood from healthy donors, or *in vivo* immune response induction studies in HLA transgenic mice. Also, a murine tumor cell line similar to the type of tumor targeted by the therapy can be selected, from which neoantigens are identified using the same strategies used for the human neoantigen selection. Thereafter, a product is produced using the same manufacturing processes and injected into syngeneic tumor-bearing mice to assess the change in tumor size and occurrence of toxicological events. Furthermore, human T-cells stimulated by a product containing neoantigens derived from a patient can be administered into the patient-derived xenograft (PDX) model, which is created by the engraftment of the patient's tumor tissues in immunodeficient mice for the assessment of the anti-tumor effects and safety of the product.

4.2 Toxicity Studies

Single-dose and/or repeat-dose toxicity studies should be conducted also using representative batches. As the conduct of other toxicity studies will be determined considering the characteristics of the product under

investigation, it is very important to establish a rationale for conducting a selected set of tests based on a sufficient knowledge and understanding of the product's characteristics. If it seems infeasible to conduct toxicity studies in compliance with the criteria for such studies, they can be conducted as part of the efficacy studies with safety observations incorporated into them.

5. Clinical Considerations

The time required for the completion of the entire process, from sample collection to manufacturing and delivery to patients, is longer for neoantigen-targeted therapeutics than for other types of therapeutics. Especially for patients with metastatic or relapsed tumor, the timing of treatment administration is the most critical factor that will contribute to achieving positive clinical outcomes. Consequently, depending on target patients, careful consideration should be given to the entire duration of time required for manufacturing that encompasses multiple steps, such as the identification and screening of neoantigens and the selection and manufacturing of neoantigen delivery vehicles.

To ensure a sufficient level of immunogenicity induced by neoantigens, the route, frequency, and interval of administration and the use of treatments that boost anti-tumor immunity, such as adjuvant therapies or immunotherapies should be appropriately designed. Translational research may be considered to inform the decisions on dose selection. In addition, to shape an immune environment favorable for neoantigens, the use of pre-treatment adjuvant therapies, such as GM-CSF, and concomitant- or post-treatment with anti-cancer immunotherapies may be considered.

Furthermore, additional DNA analyses and booster vaccinations should be considered in cases of disease recurrence. For parameters to be used in clinical assessment, not only traditional endpoints, such as changes in

tumor size, progression-free survival (PFS), but also others including post-treatment immune responses should be considered.

To design clinical studies for personalized neoantigen-targeted therapeutics, information including considerations for early and late phase clinical studies and endpoint selection can be obtained from *Guidelines on Clinical Considerations for Therapeutic Cancer Vaccines(Guidance for Industry)*.

6. Glossary

1. Non-synonymous mutations
A mutation that alters the amino acid sequence of a protein

2. Major Histocompatibility Complex (MHC) Class I/II
Cell surface molecules that present antigens to T-cells; Class I molecules are expressed in most cell types and Class II molecules are restricted to professional antigen presenting cells

3. Neoepitopes
Peptides occurring in tumor-specific mutations that bind to MHC molecules

4. Central immune tolerance
A process occurring in the thymus to eliminate self-reactive T-cells

5. Naive T-cells
T-cells that have gone through differentiation and maturation but have not yet encountered appropriate antigens, which will become activated and develop into effector cells when they later on bind to peptide-MHC complexes presented by antigen presenting cells.

6. Peptide-Major Histocompatibility Complex (p-MHC) multimers staining
An assay to identify the presence of T-cells with high affinity to specific antigen-MHC complexes

7. References

1. Blass, Eryn, and Patrick A. Ott. "Advances in the development of personalized neoantigen-based therapeutic cancer vaccines." Nature Reviews Clinical Oncology 18.4 (2021): 215-229.
2. Castle, John C., et al. "Exploiting the Mutano me for Tumor VaccinationB16 Melanoma T-cell–Druggable Mutanome." Cancer research 72.5 (2012): 1081-1091.
3. Fritah, Hajer Guiren, et al. "The current clinical landscape of personalized cancer vaccines." Cancer Treatment Reviews (2022): 102383.
4. Hu, Zhuting, et al. "Personal neoantigen vaccines induce persistent memory T cell responses and epitope spreading in patients with melanoma." Nature medicine 27.3 (2021): 515-525.
5. Kreiter, Sebastian, et al. "Mutant MHC class II epitopes drive therapeutic immune responses to cancer." Nature 520.7549 (2015): 692-696.
6. Lang, Franziska, et al. "Identification of neoantigens for individualized therapeutic cancer vaccines." Nature Reviews Drug Discovery 21.4 (2022): 261-282.
7. Li, L., S. P. Goedegebuure, and William E. Gillanders. "Preclinical and clinical development of neoantigen vaccines." Annals of Oncology 28 (2017): xii11-xii17.
8. Ott, Patrick A., et al. "An immunogenic personal neoantigen vaccine for patients with melanoma." Nature 547.7662 (2017): 217-221.
9. Sahin, Ugur, et al. "Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer." Nature 547.7662 (2017): 222-226.
10. Shetty, Keerthi, and Patrick A. Ott. "Personal neoantigen vaccines for the treatment of cancer." Annual Review of Cancer Biology 5 (2021): 259-276.
11. Wells, Daniel K., et al. "Key parameters of tumor epitope immunogenicity revealed through a consortium approach improve neoantigen prediction." Cell 183.3 (2020): 818-834.
12. Zhang, Xiuli, et al. "Breast Cancer Neoantigens Can Induce CD8+ T-Cell Responses and Antitumor ImmunityBreast Cancer Neoantigens Can Induce Antitumor Immunity." Cancer immunology research 5.7 (2017): 516-523.
13. Clinical Considerations for Therapeutic Cancer Vaccines (FDA , 2010)
14. Guidelines on the Quality and Nonclinical Evaluation of Plasmid DNA-based Gene Therapy Products (MFDS, 2022.6)
15. Guideline on Quality Evaluation of mRNA-based Gene Therapy Products (MFDS, 2022.8)

16. Guidelines on the Requirements for Quality Dossier of Cell and Gene Therapy Products (MFDS, 2022.4)

17. Guideline for Non-clinical Assessment of Gene Therapy Products (MFDS, 2021.10)

18. Considerations for the Assessment of Dendritic Cell-based Therapy Products for Cancer (MFDS, 2017.6)

19. Guidelines on Clinical Considerations for Therapeutic Cancer Vaccines(MFDS, 2020.)

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