ORIGINAL ARTICLE

Identification of Individual Cancer-Specific Somatic Mutations for Neoantigen-Based Immunotherapy of Lung Cancer

Takahiro Karasaki, MD,[a] Kazuhiro Nagayama, MD, PhD,[a] Mitsuaki Kawashima, MD,[a] Noriko Hiyama, MD,[a] Tomonori Murayama, MD,[a] Hideki Kuwano, MD, PhD,[a] Jun-ichi Nitadori, MD, PhD,[a] Masaki Anraku, MD, MSc,[a] Masaaki Sato, MD, PhD,[a] Manami Miyai, MSc,[b,c] Akihiro Hosoi, PhD,[b,c] Hirokazu Matsushita, MD, PhD,[b] Shingo Kikugawa, PhD,[d] Ryo Matoba, PhD,[d] Osamu Ohara, PhD,[e] Kazuhiro Kakimi, MD, PhD,[b,*] Jun Nakajima, MD, PhD[a]

[a]*Department of Thoracic Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*
[b]*Department of Immunotherapeutics, The University of Tokyo Hospital, Tokyo, Japan*
[c]*MEDINET Co. Ltd., Yokohama, Japan*
[d]*DNA Chip Research Inc., Yokohama, Japan*
[e]*Department of Human Genome Research, Kazusa DNA Research Institute, Kisarazu, Japan*

Received 9 November 2015; accepted 19 November 2015

ABSTRACT

Introduction: Two strategies for selecting neoantigens as targets for non–small cell lung cancer vaccines were compared: (1) an "off-the-shelf" approach starting with shared mutations extracted from global databases and (2) a personalized pipeline using whole-exome sequencing data on each patient's tumor.

Methods: The Catalogue of Somatic Mutations in Cancer database was used to create a list of shared missense mutations occurring in more than 1% of patients. These mutations were then assessed for predicted binding affinity to HLA alleles of 15 lung cancer patients, and potential neoantigens (pNeoAgs) for each patient were selected on this basis. In the personalized approach, pNeoAgs were selected from missense mutations detected by whole-exome sequencing of the patient's own samples.

Results: The list of shared mutations included 22 missense mutations for adenocarcinoma and 18 for squamous cell carcinoma (SCC), resulting in a median of 10 off-the-shelf pNeoAgs for each adenocarcinoma (range 5–13) and 9 (range 5–12) for each SCC. In contrast, a median of 59 missense mutations were identified by whole-exome sequencing (range 33–899) in adenocarcinoma and 164.5 (range 26–232) in SCC. This resulted in a median of 46 pNeoAgs (range 13–659) for adenocarcinoma and 95.5 (range 10–145) for SCC in the personalized set. We found that only one or two off-the-shelf pNeoAgs were included in the set of personalized pNeoAgs—and then in only three patients, with no overlap seen in the remaining 12 patients.

Conclusions: Use of an off-the-shelf pipeline is feasible but may not be satisfactory for most patients with non–small cell lung cancer. We recommend identifying personal mutations by comprehensive genome sequencing for developing neoantigen-targeted cancer immunotherapies.

Keywords: Neoantigen; Immunotherapy; Exome; Missense mutation; Genetic data bank

Introduction

Lung cancer has long been the leading cause of cancer-associated death worldwide, accounting for 1.59 million of 8.2 million deaths per year in the 2014 report.[1] Molecular profiles in non–small cell lung

*Corresponding author.

Disclosure: Dr. Kakimi received a research grant of the Project for Development of Innovative Research on Cancer Therapeutics (P-Direct), Ministry of Education, Culture, Sports, Science and Technology of Japan. Drs. Nagayama, Matsushita, Nakajima, and Kakimi received a Grant-in-Aid for Scientific Research of the Ministry of Education, Culture, Sports, Science and Technology of Japan. The remaining authors declare no conflict of interest.

Address for correspondence: Kazuhiro Kakimi, MD, PhD, Department of Immunotherapeutics, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113-8655, Japan. E-mail: kakimi@m.u-tokyo.ac.jp

ISSN: 1556-0864

http://dx.doi.org/10.1016/j.jtho.2015.11.006

cancer have been extensively analyzed, leading to the development of molecular targeting agents that inhibit the growth signals resulting from driver mutations. In particular, epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase inhibitors have become the key drugs for lung cancer treatment.[2–5] Nonetheless, these drugs are suitable for only a fraction of susceptible patients, and even then, the clinical responses may not be long-lasting.

Recently, immunological checkpoint blockade therapies targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death-1 (PD-1), and programmed death ligand-1 (PD-L1) have been shown to have quite remarkable benefits for the treatment of lung cancer, but in only a minority of patients.[6–10] Clinical responses are observed in patients whose endogenous antitumor immune responses target tumor-specific antigens generated from genes with tumor-specific somatic mutations, so-called neoantigens.[8,11–13] Combining the use of checkpoint inhibitors with strategies that actively induce neoantigen-specific immune responses may have the potential to further improve the treatment of lung cancer.[14]

Neoantigens are unique antigens derived from tumor-specific somatic mutations, regardless of whether they are driver or passenger mutations. By their de novo generation in the tumor, the induction of central immune tolerance to self-antigens in the thymus, is bypassed. Thus, neoantigens should be good targets to provoke robust immune responses.[15] Because mutant peptides with a strong binding affinity to major histocompatibility complex (MHC) are candidate neoantigens, the focus has been on computational analysis for target discovery and identification of suitable peptides to include in a vaccine.[11,14,16–20]

To establish an efficient pipeline for developing neoantigen-based cancer vaccines, here we have compared two strategies to identify suitable candidates, namely, (1) an "off-the-shelf" pipeline using a panel of missense mutations that are shared by at least 1% of patients with lung cancer and already registered in established online databases (Fig. 1*A*) and (2) a personalized pipeline using an individualized set of missense mutations obtained by whole-exome sequencing of each patient's tumor samples (Fig. 1*B*).[21–23] In the latter, tumor material must be obtained, and development of personalized vaccines would be more costly and time-consuming. All else being equal, if off-the-shelf vaccines targeting frequently shared mutations were feasible, they would be preferable in the development of new drugs for the treatment of lung cancer. Considering the high frequency of lung cancer, even mutations shared by only 1% to 2% of patients, such as the rearranged during transfection (*RET*) gene, would still represent a number of patients adequate to make drug development worthwhile.[5]

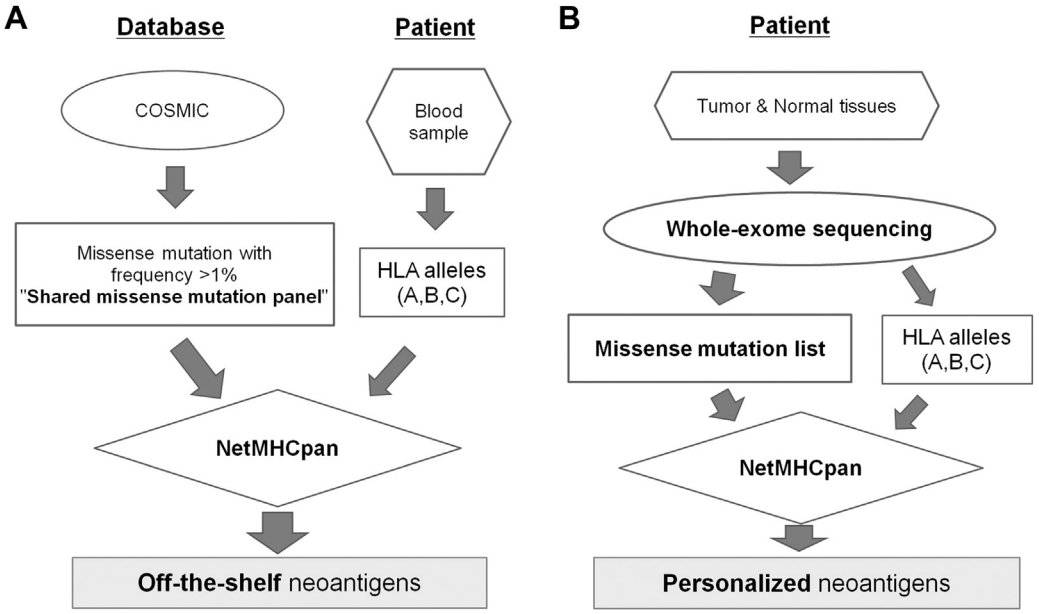

Figure 1. Pipelines to output potential neoantigens. (*A*) Off-the-shelf pipeline using a shared missense mutation panel curated from established online databases. Peripheral blood is a required sample to be collected from the patient. (*B*) Personalized pipeline using data from whole-exome sequencing. Samples of the patient's tumor and normal tissue for control (e.g., adjacent normal lung tissue or peripheral blood) are needed. In both pipelines, mutant peptides are filtered using epitope binding prediction tools to select the potential neoantigens.[21-23] COSMIC, Catalogue of Somatic Mutations in Cancer; HLA, human leucocyte antigen.