Abstract. The present study selected two patients with lung cancer and epidermal growth factor receptor (EGFR) mutations who were treated with a programmed cell death protein 1 (PD-1) antibody and an immunomodulatory arabinomannan extracted from Mycobacterium tuberculosis. In the first case, a 67-year-old female was diagnosed with lung adenocarcinoma with an EGFR mutation (exon 19 deletion) and Stage IVB disease. Initial treatment with an EGFR mutation-targeted tyrosine kinase inhibitor (TKI), erlotinib, demonstrated a partial response. After disease progression this was followed by carboplatin and pemetrexed with bevacizumab, and re-challenged by erlotinib plus bevacizumab; however, the tumor eventually progressed. Subsequently, the patient was treated with immunomodulatory arabinomannan for 3 months. Immediately after, she was treated with nivolumab and showed a partial response. In the second case, a 57-year-old male with a history of smoking was diagnosed with stage IVB pulmonary adenocarcinoma with an EGFR mutation (exon 19 deletion). He was treated with afatinib, followed by osimertinib, when a T790M mutation was identified later. After disease progression with TKIs, cisplatin plus pemetrexed and re-challenge with erlotinib plus bevacizumab were administered subsequently. Nivolumab was administered for recurrent disease. Although he experienced tumor remission, regrowth of the tumors was observed. Under continuing nivolumab, he was treated by palliative irradiation treatments to the right pelvic bone metastasis and left adrenal metastasis with immunomodulatory arabinomannan. A chest computed tomography scan showed a reduction in the sizes of the primary site and pulmonary metastases, with a decreasing trend of carcinoma embryonic antigen. Overall, these cases may indicate that the immune adjuvant actions of immunomodulatory arabinomannan extracted from Mycobacterium tuberculosis improves the effect of PD-1 antibody treatments.

Introduction

Sequencing of DNA to identify polymorphisms has catalyzed the quest for protein kinase ‘driver’ mutations, which contribute to the transformation of a normal cell to a proliferating cancerous cell. Approximately one-third of patients in Asian populations with non-small cell lung cancer (NSCLC) harbor epidermal growth factor receptor (EGFR) mutations. For these patients, EGFR-targeted tyrosine kinase inhibitors (TKIs) have shown improved efficacy and longer progression-free survival (PFS) than standard chemotherapies (1). Currently, TKI treatment is considered the standard of care for EGFR-mutated NSCLC. However, most patients eventually develop resistance with a PFS from 10 months to 18.9 months (1,2).

For NSCLC, recent alternative treatments include immune checkpoint inhibitors (ICIs) such as programmed cell death 1 (PD-1) antibody (nivolumab or pembrolizumab) or a programmed cell death 1 ligand (PDL-1) antibody (atezolizumab or durvalumab).ICI monotherapy with nivolumab or pembrolizumab is efficacious for NSCLC, achieving response rates of ~20%, with a 5-year survival rate of ~15%. However, EGFR-mutated NSCLC is insensitive to ICIs (3,4).

Previous researchers, Coley W and Maruyama C, experienced immune responses to human malignant tumors by erysipelas and tuberculosis, respectively, indicating there might be a relationship between infection and cancer immunity (5,6). Previously, the effect of erysipelas was partially explained via the production of interleukin (IL)-12 and tumor necrosis factor (7). Regarding tuberculosis, Maruyama (6) developed Specific Substance Maruyama (SSM), a hot-water
extract from human bacillus tuberculosis containing polysaccharides including arabinomannan and mannan (8). SSM is an immunomodulatory agent and its carcinostatic potential was reported in 1966 (6). Later studies indicated that SSM acts as an immune adjuvant, resulting in a change from innate immunity to adaptive immunity (9-12).

No report has described ICI and immunomodulatory arabinomannan extracted from *Mycobacterium tuberculosis* in humans.

Material and methods

The two cases presented below gave oral consents as for presenting this case-report, and written consents were also obtained from the families. This manuscript followed a Japanese law, Act on the Protection of Personal Information.

To produce a hot-water extract from human bacillus tuberculosis, *Mycobacterium tuberculosis*, Aoyama B strain, grown on the surface of Sauton’s media for 5 weeks at 37 degrees Celsius is diluted in distilled water. To extract polysaccharides, it is kept at 100˚C for 120 min, and, after filtering it to delete *Mycobacterium tuberculosis*, the filtrate is adjusted at the following concentrations (8). There are three types of hot water extracts from human *Mycobacterium tuberculosis* strain Aoyama B: SSM A at a dose of 2 mg as D-arabinose, SSM B at a dose of 0.2 mg as D-arabinose, and Ancer®. 1 ml ampoule containing Z-100 for subcutaneous injection, at a dose of 20 mg as D-arabinose. Ancer® has been approved in Japan as a granulocyte-macrophage (GM)-colony stimulating factor (CSF) under radiation therapy. SSM and Ancer® are supplied by Zeria Pharmaceutical Co. Ltd.

The cases were treated in clinical practice, and computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), and measurement of carcinoembryonic antigen (CEA) by a kit of CEA-ABOTT JAPAN were performed when needed in clinical practice. CT was performed by Light Speed VCT (GE, USA), and Biograph mCT 16 (Siemens Healthcare, Erlangen, Germany) was used for PET-CT using 193.8MBq of F-18 FDG.

Case reports

Case one. In 2016, a 67-year-old woman was diagnosed as NSCLC (adenocarcinoma) harboring a sensitive EGFR mutation, exon 19 deletion, of the right lower lung. TNM and staging were cT1bN3M1c (lymph nodes metastasis, bone metastases, and adrenal metastasis) and Stage IVb disease. Thus, an EGFR-TKI, erlotinib, was started in February 2016, and achieved a partial response (PR). However, her disease progressed again in both the primary site and distant metastases in October 2020. He also felt pain and disturbance of motility in the bilateral legs, and it was later diagnosed as leptomeningeal metastases by MRI. Under continuing nivolumab, he was treated with palliative irradiation to the right pelvic bone metastasis at a total dose of 20 Gy in 4 fractions and left adrenal metastasis at a total dose of 30 Gy in 10 fractions with a subcutaneous injection of Ancer® for two months. In December 2020, a chest computed tomography scan showed a reduction in the sizes of the primary site and pulmonary metastases, which were not irradiated, with a decreasing trend in carcinoma embryonic antigen (CEA) blood levels (Fig. 2). However, active treatments stopped and he moved another hospital for palliative care because of progressing leptomeningeval metastases, which made him bed rest in January 2021 and died in late January 2021, indicating that the patient with leptomeningeval metastases could survive for 3 months.

Discussion

We experienced two lung cancer patients harboring EGFR mutations in whom the immunomodulatory arabinomannan extracted from *Mycobacterium tuberculosis* might act as an immune adjuvant under ICI treatment. Although these cases might be considered random or serendipitous, we think these
cases provide interesting information. Possible mechanisms to the events described below are shown in Fig. 3.

Polymannan of tuberculosis is an immunogenic ligand for Toll-like receptors (TLRs) (14). Although signaling pathways in DCs by arabinomannan extracted from Mycobacterium tuberculosis is unknown at present, TLR ligands act as adjuvants in adaptive immune responses (15,16). It was reported that SSM induced cluster of...
differentiation (CD)80, CD86, and major histocompatibility complex (MHC) class II expression on bone marrow-derived dendritic cells (DCs), and that SSM treatment of mice increased the number of activated DCs in target lesions (9-12). Z-100 promoted a change in helper T-cell responses from a type 2 dominant state to a type 1 dominant state via the upregulation of interferon-\(\gamma\) and IL-12 production (9-11). These reports support that arabinomannan extracted from \textit{Mycobacterium tuberculosis} acts as an immune adjuvant for TLRs on DCs in cancer immunity.

Although cancer cells of EGFR-mutated NCSLC often express PDL-1 on their cell-surface, ICIs have a poor therapeutic effect (3,4). This PDL-1 expression is induced directly by activated EGFR signaling, not by tumor immunity (17). Therefore, PDL-1 on cancer cells harboring EGFR mutations is not exposed to any cytokines (18). Type 1 cytokine (IL-2, IFN-\(\gamma\)) production was increased in tumor-bearing mice treated with arabinomannan extracted from \textit{Mycobacterium tuberculosis} (9,10). In case one, in addition to SSM acting as an immunologic adjuvant, pretreatment with SSM caused tumor cells to be exposed to cytokines.

It was reported that novel proteins were generated in response to \(\gamma\)-irradiation, resulting in new peptides presented by MHC molecules expressed by DCs (19). Formenti \textit{et al} hypothesized that ‘abscopal response’ might be related to the radiation-induced exposure of immunogenic mutations to the immune system (20). To produce the abscopal response, both antigen presentation by MHC and co-stimulation by an immunologic adjuvant are critical. A previous study reported the administration of Z-100 in combination with radiation showed the inhibitory action of pulmonary metastasis in tumor-bearing mice model, and prolonged survival time (11). In humans, a phase III placebo-controlled double-blind randomized trial of radiotherapy for stages IIB-IVA cervical cancer with or without Z-100 was reported a trend in the improvement of overall survival (OS) in locally advanced cervical cancer (21). The 5-year OS rate was 75.7% with Z-100 and 65.8% without Z-100 (hazard ratio: 0.65, P=0.07). In our second case receiving nivolumab treatment, palliative irradiation to distant metastases was administered with Ancer®, and effects on the primary site and pulmonary metastases, which were not irradiated, were observed.

In conclusion, these two cases might indicate immunomodulatory arabinomannan extracted from \textit{Mycobacterium tuberculosis} has immune adjuvant effects under PD-1 antibody treatment. This treatment strategy should be validated by prospective clinical studies, and the NEJ 046A trial is currently underway.

**Acknowledgements**

The authors are especially grateful to Dr Hiroyuki Tajima and Dr Kenji Fukushima (Saitama Medical University, Saitama, Japan) for performing the CT/PET imaging.

**Funding**

No funding was received.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors’ contributions**

KuK provided the clinical data included in the text and wrote the manuscript draft. KuK, KyK, HI, SS, KH, YM, AS, FN and HK treated the two patients and interpreted the PET-CT, CT imaging and the laboratory test results. KyK critically revised the manuscript and modified the text. KuK and KyK confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.
Osimertinib in Untreated - Radiation modulates the peptide

References

Received research grants from AstraZeneca Co., and received Japan Co., Nihon Medi-Physics Co., Taiho Pharmaceutical Co., Eli Lilly Pharmaceutical Co., Boehringer Ingelheim Co., Chugai Pharmaceutical Co., and AstraZeneca Co. HK received research grants from AstraZeneca Co., and received personal fees from Ono Pharmaceutical Co., Taiho Pharmaceutical Co., Eli Lilly Japan Co., Nihon Medi-Physics Co., and AstraZeneca Co. HK received research grants from AstraZeneca Co., and received personal fees from AstraZeneca Co. All other authors declare that they have no competing interests.

Written consent for publication was provided by the patients’ families.

Written consent for publication was provided by the patients’ families.

Competing interests

KuK received research grants from AstraZeneca Co., and received personal fees from AstraZeneca Co. KyK received personal fees from Ono Pharmaceutical Co., Boehringer Ingelheim Co., Chugai Pharmaceutical Co., Taiho Pharmaceutical Co., Eli Lilly Japan Co., Nihon Medi-Physics Co., and AstraZeneca Co. HK received research grants from AstraZeneca Co., and received personal fees from AstraZeneca Co. All other authors declare that they have no competing interests.

1. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, et al: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362: 2380-2388, 2010.
2. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, Dechaphulkun A, Imamura F, Nogami N, Kurata T, et al: Osimertinib in Untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 378: 113-125, 2018.
3. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Goldago E, et al: Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 373: 1627-1639, 2015.
4. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csósz T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, et al: Pembrolizumab versus chemotherapy for PD-L1-positive Non-Small-Cell lung cancer. N Engl J Med 375: 1823-1833, 2016.
5. Busch W: Einfluß von Erysipel. Berliner Klin Wschr 3: 245-246, 1866 (In German).
6. Maruyama C: On the treatment of malignant tumor with an extract from tubercle bacilli. Jpn J Dermatol 76: 399-404, 1966 (In Japanese).
7. Tsung K and Norton JA: Lessons from Coley’s Toxin. Surg Oncol 15: 25-28, 2006.
8. Kobatake H, Suekane T, Murakami Y, Niwa S, Okahira A and Kushida H: Studies on hot water extract of Mycobacterium tuberculosis. I. Structural analyses of polysaccharides (author's transl). Yakugaku Zasshi 101: 713-722, 1981 (In Japanese).
9. Oka H, Shiraishi Y, Sasaki H, Yoshinaga K, Emori Y and Takei M: Antimetastatic effect of an immunomodulatory arabinomanann extracted from Mycobacterium tuberculosis strain Aoyama B, Z-100, through the production of interleukin-12. Biol Pharm Bull 26: 1336-1341, 2003.
10. Oka H, Emori Y, Sasaki H, Shiraishi Y, Yoshinaga K and Kurimoto T: Anti-tumor mechanism of Z-100, an immunomodulatory Arabinomanann extracted from Mycobacterium tuberculosis strain Aoyama B, on pulmonary metastases of B16F10 melanoma: Restoration of helper T cell responses via suppression of glucocorticoid-genesis. Microbiol Immunol 46: 343-351, 2002.
11. Oka H, Sasaki H, Shiraishi Y, Emori Y, Yoshinaga K and Takei M: Z-100, an immunomodulatory arabinomanann extracted from Mycobacterium tuberculosis strain Aoyama B, augments anti-tumor activities of X-ray irradiation against B16 melanoma in association with the improvement of type 1 T cell responses. Biol Pharm Bull 27: 82-88, 2004.
12. Mitsuishi T, Kabashima K, Tanizaki H, Ohsawa I, Oda F, Yamada Y, Halifu Y, Kawanu S, Kato T and Iida K: Specific substance of Maruyama (SSM) suppresses immune responses in atopic dermatitis-like skin lesions in DS-NH mice by modulating dendritic cell functions. J Dermatol Sci 63: 184-190, 2011.
13. Kobayashi K, Kaira K and Kagami H: Recovery of the sensitivity to Anti-PD-1 antibody by celecoxib in lung cancer. Anticancer Res 40: 5309-5311, 2020.
14. Murphy K and Weaver C: Chapters: 3-5. In: Janeway’s Immunobiology. 9th edition. W.W. Norton & Company, Inc., New York, 2017.
15. Shah RR, Hassett KJ and Brito LA: Overview of vaccine adjuvants: Introduction, history, and current status. Methods Mol Biol 1494: 1-13, 2017.
16. Reed SG, Orr MT and Fox CB: Key roles of adjuvants in modern vaccines. Nat Med 19: 1597-608, 2013.
17. Hsu PC, Jablons DM, Yang CT and You L: Epidermal Growth Factor Receptor (EGFR) Pathway, Yes-Associated Protein (YAP) and the Regulation of Programmed Death-Ligand 1 (PD-L1) in Non-Small Cell Lung Cancer (NSCLC). Int J Mol Sci 20: E3821, 2019.
18. Teng MW, Ngio SF, Ribas A and Smyth MJ: Classifying cancers based on T-cell infiltration and PD-L1. Cancer Res 75: 2139-2145, 2015.
19. Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, Camphausen K, Luiten RM, de Ru AH, Neijssen J, et al: Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 203: 1259-1271, 2006.
20. Formenti SC, Rudqvist NP, Golden E, Cooper B, Wennerberg E, Lhuiller C, Vanpouille-Box C, Friedman K, Ferrari de Andrade L, Wucherpfennig KW, et al: Radiotherapy induces responses of lung cancer to CTLA-4 blockade. Nat Med 19: 1597-608, 2013.