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	（職名）	（学位）	（氏名）
主査	筑波大学教授（協働大学院）	博士（薬学）	伊東　洋行
副査	筑波大学教授（協働大学院）	博士（獣医学）	保富　康宏
副査	筑波大学教授（協働大学院）	博士（農学）	吉木　淳
副査	筑波大学准教授	博士（工学）	平川　秀彦

Abstract of thesis

New therapeutic strategies for the treatment of cancer harness the body's own immune system to mount an anti-tumor response. Immune checkpoint inhibitors (CPIs) such as anti-programmed death-1 (anti-PD-1) (nivolumab, pembrolizumab) and anti-cytotoxic T lymphocyte antigen 4 (anti-CTLA4) (ipilimumab) blocking antibodies improve significantly survival outcomes in some patients and are now used as therapies for cancers. Unfortunately, most patients (about 80%) still do not benefit from CPIs as a monotherapy, and the potential for serious side effects exists. Therefore, to optimize selection of suitable patients for immunotherapy and avoid unnecessary toxicity, there is a growing need to explore biomarkers. In current immunotherapy using CPIs, tumor-infiltrating lymphocytes, tumor programmed death ligand 1 (PD-L1) expression, tumor mutational burden, and several other factors are being considered as candidate biomarkers. There is no approval biomarker for anti-CTLA-4 antibody (ipilimumab). For anti-PD-1 antibody treatment, immunohistochemistry (IHC) for PD-L1 in tumor site is the only approval biomarker. However, tumor PD-L1 expression is not sufficient for predicting anti-PD-1 antibody response, because PD1/PD-L1 interaction is just one of many factors that may determine the clinical outcome of a tumor immune response. Although understanding the tumor microenvironment is important for biomarker exploration, peripheral blood biomarkers are practically becoming of major clinical utility.

Myeloid-derived suppressor cells (MDSCs), a subset of immune suppressive cells, are known to be a heterogeneous population of immature myeloid lineage cells. MDSCs have been shown to suppress responses of immune cells such as T cells, NK cells and dendric cells. Previous studies have also demonstrated that the proportion of peripheral monocytic (m) MDSCs is significantly increased in patients with various type of

cancers such as melanoma, breast cancer and colorectal cancer (CRC), and correlates positively with clinical cancer stage, tumor burden, and poor clinical outcomes. It is reported that the proportion of peripheral mMDSCs is one of important prognostic markers for current CPIs such as ipilimumab and nivolumab. For example, in metastatic melanoma patients, compared with non-responders, clinical responders to ipilimumab had a significantly lower proportion of mMDSCs in the peripheral blood. Other groups indicated that a higher number of mMDSCs before treatment was associated with a poorer outcome with nivolumab in melanoma.

Although measuring the proportion of peripheral mMDSCs is beneficial to predict clinical outcome in cancer patients, it requires a complex process of flow cytometric analysis with multiple marker staining. mMDSCs need to be studied using fresh blood because freezing blood significantly decreased the yield of mMDSCs. In addition, although mMDSCs are characterized as $CD14^{+}HLA\text{-}DR^{-/low}$ cells in humans, their HLA-DR expression typically shows wide variability, making identification of a specific subset of cells susceptible to inter-user and intra-day variability. For these reasons, measuring peripheral mMDSC levels would be difficult to incorporate as a basic clinical test, and establishment of an alternative way to predict the peripheral mMDSC proportion will be required.

In chapter 1, the author performed a correlation analysis of the proportion of freshly-drawn peripheral mMDSCs, levels of different plasma proteins, and demographic factors in CRC patients and find some predictive factors that can be used for prediction of mMDSC proportions. Freshly-drawn mMDSCs are measured using flow cytometry on peripheral blood mononuclear cells (PBMCs) from healthy donors (n = 24) and CRC patients (n = 78). The plasma concentrations of 29 different cytokines, chemokines, growth factors and enzymes are measured using a multiplex assay or enzyme-linked immunosorbent assay (ELISA). Correlation analysis to find mMDSC-associated factors is conducted using univariate and multivariate models. As a result, in multivariate analysis, considering all variables such as age, sex, and plasma proteins, inducible nitric acid synthase (iNOS) ($p = 0.013$) and platelet-derived growth factor (PDGF)-BB ($p = 0.035$) were associated with mMDSC proportion in PBMCs (mMDSC proportion (%) = 0.2929 − 0.2389 * PDGF-BB + 0.3582 * iNOS) ($p < 0.005$, $r = 0.32$). These results suggest that measuring the plasma concentrations of iNOS and PDGF-BB may be instrumental in predicting the proportion of mMDSCs in CRC patients.

As well as biomarker exploration to select right patients for current CPI therapy, it is vital to create a novel drug that can be effective against current CPI resistant patients and to guide optimal treatment strategy including combination therapy with current CPIs. Although the resistant mechanism of the current CPI therapies remains to be fully elucidated, it is reported that increased numbers of immune suppressive cells such as MDSCs, regulatory T cells (Tregs) have been involved in primary resistance. Several groups showed that targeting MDSCs and Tregs increased response to CPIs in preclinical studies. Therefore, it is considered that MDSCs and Tregs are potential targets for current CPI resistant patients. Besides CTLA-4 and PD-1, novel inhibitory checkpoint molecules have been discovered. It is reported that one of the next generation check point molecules, T cell immunoglobulin and ITIM domain (TIGIT) has been implicated in tumor immunosurveillance. TIGIT is mainly expressed on T cells, NK cells and Tregs. TIGIT binds to its ligands, PVR and PVRL2 which are expressed on cancer cells, MDSCs and dendric cells, resulting in induction of an immune suppressive signal to both the receptor- and ligand-expressing cells. Several groups demonstrated that TIGIT signal blockade using anti-TIGIT blocking antibody abolished the immunosuppressive activity of MDSC and Tregs against $CD8^{+}$T cells. Another group reported that diminished tumor cell killing activity of NK cells cocultured with MDSCs can be reversed by TIGIT signal blocking. It is known that both TIGIT and PD-1 are co-expressed on T cells in

a variety of different cancers. Given these considerations, there is a possibility that TIGIT signal blocking shows efficacy as single agent against anti-PD-(L)1 antibody resistant patients, and additive or synergistic effect with anti-PD-(L)1 antibodies.

In chapter 2, the author generated anti-TIGIT blocking antibodies (ASP8374: therapeutic antibody and mSEC1: mouse surrogate antibody) and conducted non-clinical pharmacological studies to see monotherapy effect and combination therapy effect with anti-PD-(L)1 antibodies. As a result, ASP8374 induced T cell activation as monotherapy by blocking TIGIT/ligand interaction in several in vitro functional assays. The combination of ASP8374 and anti-PD-1 antibody (pembrolizumab) led to an increase in secreted cytokine levels when compared with either ASP8374 or pembrolizumab treatment alone. mSEC1 displayed anti-tumor efficacy in an MC38 colon cancer model (anti-PD-(L)1 sensitive model [MDSC low model]). In this model, mSEC1 in combination with an anti-PD-L1 antibody produced comparable anti-tumor efficacy to anti-PD-L1 antibody alone. In other colon cancer model, CT26 (anti-PD-(L)1 refractory model [MDSC expansion model]), the mSEC1 alone did not demonstrate anti-tumor efficacy, but mSEC1 combined with an anti-PD-1 antibody enhanced anti-tumor efficacy over anti-PD-1 antibody alone, with an increase in the number of tumor-free mice. Taken together, these results support the development of ASP8374 in combination with PD-(L)1 blockade for effective treatment of solid tumors.

In summary, the author concluded that measuring the plasma concentrations of iNOS and PDGF-BB may be useful in predicting the proportion of mMDSCs in CRC patients' peripheral blood. This predictive model might contribute to patient stratification in current CPI therapy and should guide further research on other populations with different types of malignancy. The author also suggested that ASP8374 may improve clinical response with existing immunotherapies such as anti-PD-(L)1 antibodies in the clinic. Clinical trials of ASP8374 are currently ongoing, investigating TIGIT blockade for the treatment of patients with several advanced solid tumors.

Abstract of assessment result

【批評 Review】

Important findings in this dissertation are; 1) Identification of iNOS and PDGF-BB as predictive factors of blood mMDSC proportion in CRC patients, 2) Demonstration of T cell activation by ASP8374 as either monotherapy or in combination with anti-PD-1 antibody in human primary immune cell functional assay and 3) Demonstration of enhancement of anti-tumor activity by combination of mSEC1 and anti-PD-1 antibody in anti-PD-1 insensitive mouse tumor model (MDSC expansion model). Taken together, these findings showed that measuring plasma iNOS and PDGF-BB levels in CRC patients may be beneficial for the prediction of the clinical outcome of immunotherapy and that ASP8374 has therapeutic potential to amplify the activity of anti-PD-(L)1 antibodies in the clinic where PD-1 blockade is not fully efficacious. This research will contribute to patient stratification in the current CPI therapy and improvement of clinical response with existing immunotherapy in the clinic.

The applicant showed enough ability to explain as an expert scientific and clinical backgrounds of the study, and limitations of his findings and provide the future plan for improvement and challenge. He gave a convincing explanation and responded properly to questions regarding updated global competitive situation of the relevant drug development.

【最終試験の結果 Result】

The final examination committee conducted a meeting as final examination on 14 June 2021. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Disease Mechanism.