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A phase II multicentre study of plasminogen activator inhibitor-1 inhibitor (TM5614) plus nivolumab for treating anti-programmed cell death 1 antibody-refractory malignant melanoma: TM5614-MM trial

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Abstract

Background Anti-programmed cell death 1 antibodies (PD-1 Abs) are widely used for advanced melanoma, but information on the efficacy of anti-PD-1 Abs is limited in the Asian population. There remains an unmet need to improve the therapeutic effects of anti-PD-1 Ab-treatment, particularly in patients with melanoma who are refractory to anti-PD-1 Abs. The aim of this study was to evaluate anti-PD-1 Ab-treatment in combination with TM5614 (a plasminogen activator inhibitor-1 inhibitor) in patients with unresectable melanoma.

Methods The TM5614-MM study was a multicentre, open-label, single-arm, phase II clinical trial to evaluate the efficacy and safety of nivolumab in combination with TM5614 in patients with advanced, unresectable malignant melanoma recruited at seven Japanese institutes between 13 September 2021 and 31 March 2023. Patients with metastatic or unresectable melanoma previously treated with anti-PD-1 Abs were enrolled. Nivolumab 480 mg was administered intravenously every 4 weeks for 8 weeks, while TM5614 was administered orally at a dose of 120 mg (0–4 weeks) and 180 mg once daily (5–8 weeks). The primary endpoint was the overall response rate after 8 weeks of concomitant use of TM5614.

Results Thirty-nine patients were enrolled, and 34 patients were included in the anti-PD-1 Ab-refractory cohort. The overall response rate at 8 weeks was 25.9% (95% confidence interval 12.9–44.9%, $P=0.027$) in 27 patients who were anti-PD-1 Ab-refractory based on investigator assessment in the protocol per set cohort. Seven patients discontinued treatment owing to progressive disease or adverse events. Treatment-related grade 3 or higher adverse events occurred in 3 of 39 patients (7.7%) in the intention-to-treat cohort.

Conclusions TM5614 in combination with nivolumab is well tolerated and effective in anti-PD-1 Ab-refractory unresectable melanoma.

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Lay summary

Types of antibody called 'anti-programmed cell death 1 antibodies' (also known as 'anti-PD-1 Abs') are commonly used to treat a range of different advanced cancers, including melanoma. However, less is known about the effectiveness of anti-PD-1 Abs, especially in Asian populations. There remains an unmet need to improve anti-PD-1 Ab-treatment, particularly in people with melanoma who are 'refractory' (or do not respond) to anti-PD-1 Abs in Japan.

To evaluate anti-PD-1 Ab-treatment, we carried out a clinical trial called the 'TM5614-MM study' to investigate the safety and efficacy of using a combination of drugs (TM5614 and nivolumab). People with advanced and 'unresectable' (cannot be surgically removed) malignant melanoma were recruited from seven Japanese institutes between 13 September 2021 and 31 March 2023. Those with metastatic or unresectable melanoma who had received prior treatment with or without anti-PD-1 Abs were enrolled. The main result that we measured was the response rate after 8 weeks of treatment with TM5614.

We found that the response rate at 8 weeks was 25.9% in 27 patients who were anti-PD-1-Ab-refractory (those who had received prior treatment with anti-PD-1 Abs) and 0% in four patients in the anti-PD-1 Ab-naïve group (those who had not previously received treatment with anti-PD-1 Abs). Treatment-related severe adverse events occurred in three of 39 patients (7.7%).

Overall, our study findings suggest that TM5614 in combination with nivolumab is well tolerated and could be effective in anti-PD-1 Ab-refractory unresectable melanoma.

What is already known about this topic?

- Anti-programmed cell death 1 antibodies (PD-1 Abs) are widely used for the treatment of advanced melanoma, but information on the efficacy of anti-PD-1 Abs is limited in the Asian population.
- There remains an unmet need to improve the therapeutic effects of anti-PD-1 Abs, particularly in patients with melanoma who are refractory to anti-PD-1 Abs.

What does this study add?

- This study describes results from a phase II trial of nivolumab in combination with the plasminogen activator inhibitor-1 inhibitor TM5614 in patients with melanoma whose disease had progressed on anti-PD-1 Ab-monotherapy.
- Our study suggests that TM5614 in combination with nivolumab is well tolerated and effective in anti-PD-1 Ab-refractory unresectable melanoma.

Anti-programmed cell death 1 antibodies (PD-1 Abs) are important drugs for the treatment of patients with metastatic melanoma because of their effects on prolonging survival,[1] but a high incidence of immune-related adverse events (AEs) in patients with advanced melanoma is a major concern, especially when these drugs are coadministered with ipilimumab.[1–3] As anti-PD-1 Abs could be useful for the treatment of both *BRAF* wildtype and mutated advanced melanomas, they have been widely administered for the treatment of advanced melanoma, including in Asian populations.[3] However, the protocol for the treatment of *BRAF* wildtype advanced melanoma is still limited,[4] and a novel combination therapy to enhance the efficacy of the antitumour effects of anti-PD1 Abs is needed.

Plasminogen activator inhibitor (PAI)-1 is a serine protease that promotes tumour development in several types of cancer, including melanoma.[5–7] For example, PAI-1 inhibits urokinase-type plasminogen activator and tissue-type plasminogen activator, thereby reducing plasminogen activation and causing thrombosis at tumour sites. PAI-1 facilitates the migration of macrophages into tumour sites, leading to an increase in tumour-associated macrophages (TAMs) in a melanoma and colon cancer model.[5] Moreover, PAI-1 facilitates PD-L1 endocytosis of melanoma cells to abrogate the efficacy of anti-PD-L1 Abs in mouse melanoma models.[6] In addition, PAI-1 induces resistance to chemotherapy in murine B16F10 melanoma.[7] These reports suggest that PAI-1 is involved in melanoma progression in a variety of ways and that blockade of PAI-1 signalling, particularly in combination with anti-PD-1 Abs, may enhance antitumour immune responses against melanoma growth. To address these unmet medical needs, particularly in relation to Japanese patients with advanced melanoma, we designed a clinical trial to evaluate the efficacy and safety of nivolumab plus a PAI-1 inhibitor, TM5614, in Japanese patients with metastatic melanoma.

Patients and methods

Full details of the study design are provided in the protocol paper.[8]

Objectives, trial design and study setting

This was a single-arm, open-label, multi-institutional, phase II clinical trial to assess the efficacy and safety of nivolumab in combination with TM5614 (a PAI-1 inhibitor) in patients with advanced unresectable malignant melanoma. The recruitment of study participants began on 13 September

2021 (Figure 1).[8] The study was conducted initially in six centres, and one additional facility (Cancer Institute Hospital of Japanese Foundation for Cancer Research) has been added since July 2022. The trial was registered with ClinicalTrials.gov (jRCT2021210029).

Eligibility criteria

Inclusion and exclusion criteria are described in File S1 (see Supporting Information).[8]

Ethics approval and consent to participate

This study was conducted in accordance with the principles expressed in the Declaration of Helsinki, good clinical practice guidelines, and Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. The protocol version 3.0 was approved by the Institutional Review Board of Tohoku University Hospital in September 2021 and the institutional review boards of the participating centres. Written informed consent was obtained from all enrolled participants.[8]

Endpoints/outcome measures

The primary outcome of this study was the response rate based on the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1 criteria based on investigator review. Secondary outcome measures included progression-free survival (PFS), overall survival (OS), disease control rate (DCR) and AEs graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. For this study, PFS was defined as the time from initiation of treatment in the clinical trial to disease progression or death from any cause, and OS was defined as the time from initiation of treatment in the clinical trial to death from any cause.[8]

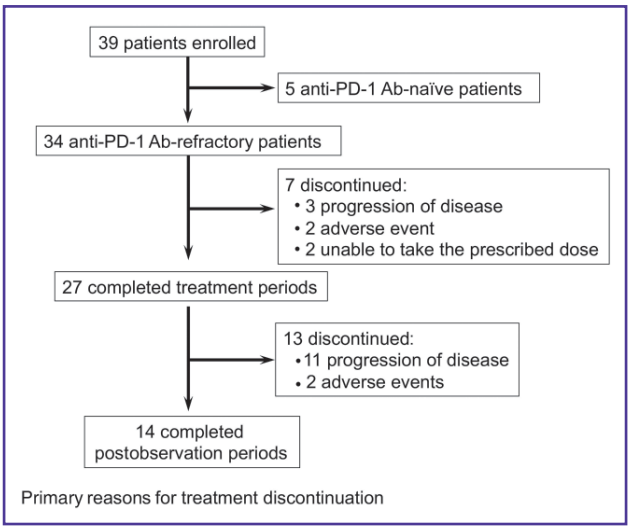

Figure 1 CONSORT diagram. The diagram includes patient enrolment and follow-up. All eligible patients were included in the intention-to-treat population. The safety population included all patients who received at least one dose of treatment. PD-1 Ab, programmed cell death 1 antibody.

Schedule of the study and intervention

The trial schedule was composed of the following three periods: screening, treatment and postobservation. The screening period started from the date of consent acquisition to the day before the first administration of the study drug. In the screening period, inclusion and exclusion criteria for the study participants were evaluated. During the treatment period, nivolumab 480 mg was administered intravenously over 30 min, every 4 weeks for 8 weeks, with oral administration of TM5614 at a dose of 120 mg once daily (0–4 weeks) and 180 mg once daily (5–8 weeks). During the postobservation period, AEs were reported 28 days after the last dose of nivolumab. Subsequently, tumour lesions were monitored every 8 weeks by imaging during the follow-up period. Tumour response was assessed using imaging. To determine OS, all participants were followed up until death, withdrawal of consent, or the end of the study.[8]

Criteria for discontinuation of treatment

The administration of nivolumab plus TM5614 was discontinued in patients who experienced at least one of the following: (i) radiographic disease progression according to RECIST, version 1.1 or apparent disease progression with clinical symptoms; (ii) grade 3 or higher interstitial pneumonia; (iii) grade 3 or higher bronchospasm, diarrhoea, colitis, neurotoxicity, hypersensitivity reaction, infusion reaction or uveitis, regarded as a response to the study drug; (iv) grade 3 or higher thrombocytopenia for $\geq$5 days; (v) grade 2 or higher AEs, regarded as a response to the study drug, showing no improvement to $\leq$grade 1 after appropriate treatment; and (vi) the investigator or physician decided to discontinue administration of the drug.[8]

Sample size calculation

For the anti-PD-1 Ab-refractory cohort, assuming the null hypothesis of a response rate of 5% and an alternative hypothesis of 22.2%, a minimum of 28 patients was required to achieve a two-sided type I error of 5% and power of 70% based on the exact binomial distribution.[8]

Interim analysis

An interim analysis was conducted after the main analysis of 20 cases that had been enrolled, to select the relevant cohort from the anti-PD-1 Ab-naïve cohort or the anti-PD-1 Ab-refractory cohort. A single interim analysis was performed on a data lock on 1 June 2022.

Statistical analysis

Statistical analyses and reporting were conducted in accordance with the Consolidated Standards of Reporting Trials guidelines, with the primary analyses based on the intention-to-treat (ITT) principle without imputing any missing observations. All efficacy analyses were based primarily on the full dataset, defined as including all patients who had received at least one dose of nivolumab and were treated in accordance with the study protocol. A safety analysis was conducted using data from the safety analysis population.

For baseline variables, summary statistics were calculated as frequencies and proportions for categorical data and means and SDs for continuous variables. The primary endpoint for efficacy was the proportion of responders, based on a response threshold of 5% (H_0, null hypothesis) and an expected response rate of 22.2% (H_1, alternative hypothesis).[8] The 95% confidence interval (CI) was calculated according to the binomial distribution of the response rate. CI limits were assessed against the response threshold.

Subgroup analysis was performed to evaluate safety and efficacy by tumour subtype. All statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA). All statistical analyses were described in detail in the statistical analysis plan, which was fixed prior to database lock.

Results

A total of 39 patients were enrolled in the trial (Figure 1). Demographic data for each cohort are described in Table 1. There was one patient who had a *BRAF*^{V600} mutation in the study. Of 39 patients with evaluable data (ITT), 12 were female (31%), 27 were male (69%) and the median age was 71 years (range 44–85); 34 patients (87%) were anti-PD-1 Ab-refractory (primary resistance to PD-1 blockade) and 5 patients (13%) were anti-PD-1 Ab-naïve. Seven patients discontinued treatment during the treatment period owing to tumour progression (42%), AEs (29%) or an inability to take the prescribed dose of TM5614 (29%). A total of 27 patients had sufficient data for inclusion in the final analysis [full analysis set (FAS)]. Of the 27 patients who were anti-PD-1 Ab-refractory, 14 (52%) completed the postobservation period [per protocol set (PPS)] and 13 patients discontinued during the postobservation period owing to tumour progression (41%) or AEs (7%). Two patients were excluded from the PPS owing to low drug compliance rate for TM5614.

Interim analysis

As this is the first study to use TM5614 plus nivolumab in patients with melanoma, it was not possible to decide at the start of the trial whether the untreated or the anti-PD-1 Ab-resistant group would be more suitable for the study. Therefore, at the protocol stage, an interim analysis was designed to select one of these two groups once 20 patients had been enrolled. At the interim analysis on 1 June 2022, 16 patients who were anti-PD-1 Ab-refractory and 5 patients who were anti-PD-1 Ab-naïve were enrolled. As most patients were in the anti-PD-1 Ab-refractory group at the time of the interim analysis, only patients for the nivolumab-refractory group were recruited thereafter.

Primary endpoints

Of the 27 patients who completed the treatment period (PPS cohort), the response rate of anti-PD1 Abs in combination with TM5614 was 25.9% (95% CI 12.9–44.9, $P=0.027$), including 1 case of complete response (CR) (4%), 6 cases of partial response (PR) (22%), 10 cases of stable disease (37%) and 10 cases of progressive disease (PD) (37%), and the DCR was 63% (95% CI 44.2–78.5) for patients who were anti-PD-1 Ab-refractory (Figure 2, Table 2). Of the 15 patients who completed the postobservation period (PPS group), the efficacy of anti-PD1 Abs in combination with TM5614 was 40% (95% CI 16.3–67.7) and DCR was

Table 1 Demographic data for each cohort

		Total *N*=39	Anti-PD-1 Ab-naïve *N*=5	Anti-PD-1 Ab-refractory *N*=34
Sex	Male	27 (69)	4 (80)	23 (67)
	Female	12 (31)	1 (20)	11 (32)
Stage	Unresectable stage III	13 (33)	2 (40)	0 (0)
	IV	25 (64)	3 (60)	22 (65)
	Unknown	1 (3)	0 (0)	1 (3)
BRAF mutation	Wildtype	37 (95)	5 (100)	32 (94)
	V600E	1 (3)	0 (0)	1 (3)
	V600K	0 (0)	0 (0)	0 (0)
	Unknown	1 (3)	0 (0)	1 (3)
ECOG PS	0	34 (87)	5 (100)	29 (85)
	1	5 (13)	0 (0)	5 (15)
	2	0 (0)	0 (0)	0 (0)
	3	0 (0)	0 (0)	0 (0)
	4	0 (0)	0 (0)	0 (0)
Clark's classification	Superficial spreading melanoma	7 (18)	0 (0)	7 (21)
	Lentigo maligna melanoma	0 (0)	0 (0)	0 (0)
	Acral lentiginous melanoma	13 (33)	4 (80)	9 (27)
	Nodular melanoma	8 (21)	1 (20)	7 (21)
	Other	11 (28)	0 (0)	11 (32)
Bastian's classification	CSD	6 (15)	0 (0)	6 (18)
	Non-CSD	10 (26)	0 (0)	10 (29)
	Acral	12 (31)	4 (80)	8 (24)
	Mucosal	10 (26)	1 (20)	9 (27)
	Other	1 (3)	0 (0)	1 (23)

CSD, cumulative sun damage; ECOG PS, Eastern Cooperative Oncology Group Performance Status; PD-1 Ab, programmed cell death 1 antibody. Data are presented as *n* (%).

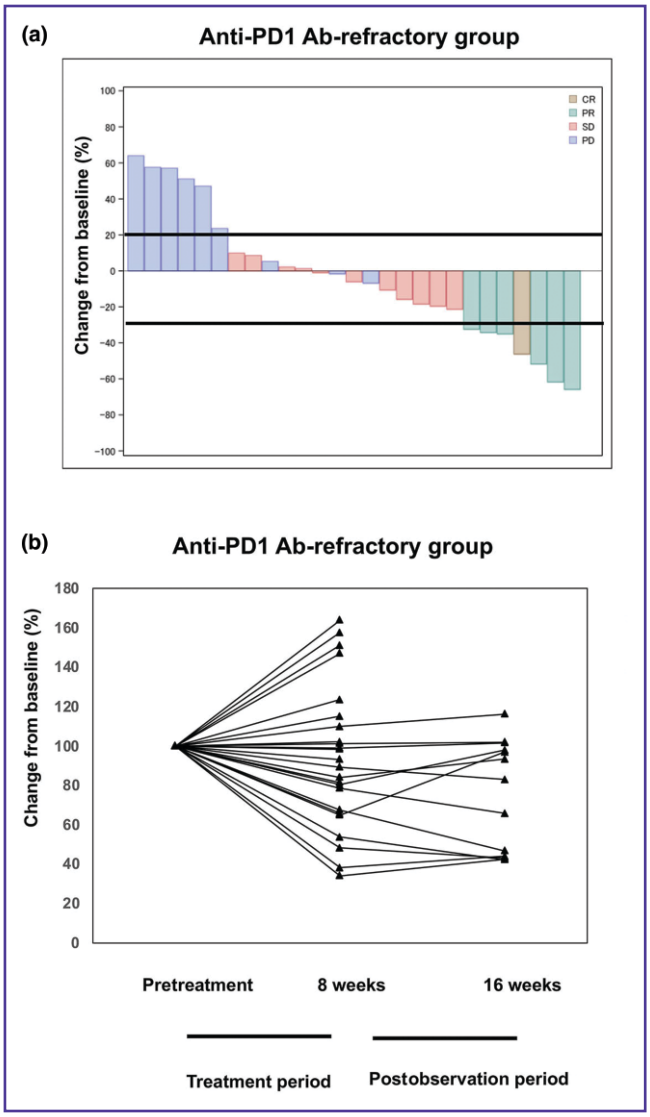

Figure 2 Depth of response of the full analysis set (FAS) cohort. (a) Waterfall plots of overall response in the anti-programmed cell death 1 antibody (PD-1 Ab)-refractory group in the FAS cohort at 8 weeks. The plots show the change in Response Evaluation Criteria in Solid Tumours target lesions. The lines indicate the threshold for objective response (≥ 30% decrease) or disease progression (> 20% increase). (b) Spider plots of overall response in the anti-PD-1 Ab-refractory group during the treatment and postobservation period. CR, complete remission; PD, progressive disease; PR, partial response; SD, stable disease.

Table 2 Summary of the efficacy of TM5614 in combination with nivolumab

	Anti-PD-1 Ab-refractory, *n* (%)
Complete response	1 (4)
Partial response	6 (22)
Stable disease	10 (37)
Progressive disease	10 (37)
Not evaluated	0 (0)

PD-1 Ab, programmed cell death 1 antibody.

100% (95% CI 76.1–103.5) for patients who were anti-PD-1 Ab-refractory. No patients were lost to follow-up.

Progression-free survival and overall survival in the full analysis set cohort

For the secondary endpoint, in the 27 patients in the FAS cohort, the DCR was 64.3% (95% CI 45.8–79.4) for patients who were anti-PD-1 Ab-refractory (Table 2). For the 14 patients in the postobservation period (FAS cohort), the DCR was 100% (95% CI 76.1–103.5) for patients who were anti-PD-1 Ab-refractory (Table 2). The median PFS of the FAS cohort, according to RECIST guidelines, version 1.1, was 174 days (95% CI 114.4–232.9) in the anti-PD-1 Ab-refractory group (Figure S1; see Supporting Information). The median OS of the FAS cohort was 273.3 days (95% CI 209.7–337.0) in the anti-PD-1 Ab-refractory cohort (Figure S1).

Toxicity

Toxicity was evaluated in 39 patients in the ITT group. Table S1 (see Supporting Information) summarizes the most commonly reported AEs of all grades (patients in either treatment cohort). In 39 patients, 26 cases of any-grade AEs were reported. Among them, the frequency of any TM5614-related AEs was 26.5% (95% CI 14.4–43.3). The most frequently reported (≥ 10%) AEs (all grades) were gastrointestinal disorder (10 cases), liver dysfunction (7 cases), cutaneous disorder (6 cases) and fibrinolytic system abnormality (4 cases).

Eleven cases of grade 3 or higher AEs were reported (Table 3) in 10 patients (26%), including liver dysfunction (4 cases, 10%), pleural effusion (2 cases, 5%), ascites (1 case, 3%), cellulitis (1 case, 3%), sclerosing cholangitis (1 case, 3%), ileus (1 case, 3%) and jaundice (1 case, 3%). However, except for three cases of liver dysfunction, these AEs were not previously reported as a side-effect of TM5614.[9] The cases of pleural effusion, ascites, ileus, and jaundice with liver dysfunction were due to tumour progression. As sclerosing cholangitis was previously reported as an anti-PD-1 Ab-related AE, it was decided that sclerosing cholangitis was a TM5614-unrelated AE. The distinction between TM5614-related and TM5614-unrelated AEs was made by the physician-in-charge. On aggregate, the frequency of TM5614-related grade 3 or higher AEs was 7.7% (95% CI 1.9–21.0). In the present study, there were no discontinuations owing to toxicity that appeared to be causally related to anti-PD-1 Abs during the follow-up period. Two discontinuation AEs during the follow-up period were interrupted owing to AEs associated with worsening of the underlying disease.

Discussion

Immune checkpoint inhibitors, such as anti-PD-1 Abs and anti-cytotoxic T-lymphocyte-associated protein 4 antibody, are widely used for the treatment of advanced melanoma,[3] and nivolumab plus ipilimumab combination therapy is one of the most effective immunotherapies for unresectable advanced melanoma with or without *BRAF* mutations.[1] As Asian regions, including Japan, tend to have a higher prevalence of acral melanoma, which have higher BRAF-wild

Table 3 Summary of the profiles of grade 3 or higher adverse events (AEs)

Case	Treatment group	Details of AEs	Grade	Causal relationship with TM5614
7	Refractory	Ileus	3	No (postoperative adhesions)
14	Refractory	Pleural effusion	3	No (tumour progression)
17	Naïve	Liver dysfunction	3	Yes
18	Naïve	Sclerosing cholangitis	3	No (due to nivolumab)
23	Refractory	Ascites retention	4	No (tumour progression)
30	Refractory	Liver dysfunction	3	Yes
31	Refractory	Cellulitis	3	No (infection without leucopenia)
33	Refractory	Jaundice	3	No (tumour progression)
33	Refractory	Liver dysfunction	3	No (tumour progression)
35	Refractory	Liver dysfunction	3	Yes
38	Refractory	Pleural effusion	5	No (tumour progression)

status than other cutaneous types (acral 3.6–33.3% vs. other cutaneous subtypes 40–60%),[10,11] and the number of structural variant mutations is significantly lower,[11] the efficacy of anti-PD1 Abs for advanced disease is limited in the Asian population,[12] even in combination with ipilimumab.[13,14] Moreover, the efficacy of nivolumab plus ipilimumab combined therapy in second-line treatment or beyond (anti-PD-1 Ab-refractory cohort) is much lower in Japan than in Western European countries.[15–17] In addition, the frequency of severe AEs is much higher with this combination therapy than with anti-PD1 Ab-monotherapy.[1] Therefore, the use of nivolumab plus ipilimumab combination therapy is limited, especially in the anti-PD-1 Ab-refractory cohort. Collectively, there remains an unmet need to improve the therapeutic effects of anti-PD-1 Abs, particularly for patients with melanoma who were refractory to anti-PD-1 Abs.

PAI-1 is highly expressed in various types of tumours including melanoma,[18] and various protumorigenic functions of PAI-1 in cancer progression and metastasis have been widely reported.[19] Of these, modulation of tumour-associated inflammation is one of the key antitumour functions of PAI-1.[19] Indeed, PAI-1 stimulates the recruitment of fibrosis-inducing cells and macrophages. For example, tumour-derived PAI-1 promotes the migration of monocytes and polarized tumour-associated macrophages (TAMs) towards an M2 phenotype that possesses protumorigenic function by increasing levels of interleukin (IL)-6 production.[20] IL-6 activates signal transducer and activator of transcription 3 in monocytes via an autocrine loop leading to an increase in the expression of arginase, IL-10 and CD163, which are well-known markers of M2 macrophages.[21] Importantly, PAI-1 modulates the profiles of tumour-infiltrating leucocytes (TILs) such as $CD8^+$ cytotoxic T cells and regulatory T cells by the decrease of chemokine production from M2-polarized TAMs.[22] As the efficacy of anti-PD1 Abs correlates with the number of TILs at the tumour site in various cancers,[23] the decreased chemokine production from TAMs might induce resistance against anti-PD-1 Ab-therapy for melanoma. In addition, PAI-1 facilitates endocytosis and decreases the expression of PD-L1 in melanoma cells,[6] suggesting that TM5614 might increase the expression of PD-L1 on melanoma cells to enhance the antimelanoma effects of nivolumab. Collectively, the inhibition of PAI-1 signals could recover the effects of nivolumab in patients with melanoma who were anti-PD-1 Ab-refractory.

TM5614 is a PAI-1 inhibitor that has been safely used in clinical trials for several malignancies including chronic myeloid leukaemia.[9] As the clinical trials described above involved TM5614-based combination therapy, accurate evaluation of TM5614-related AEs is difficult. Indeed, the previous safety profile of TM5614-related AEs is limited.[9] Only 8 cases of nonsevere AEs related to TM5614 in 6 patients, including lymphopenia (2 cases), eyelid oedema (1 case), conjunctival bleeding (1 case), stomatitis (1 case), oral hypersensitivity (1 case), liver dysfunction (1 case) and headache (1 case), have been described in 59 enrolled patients,[9] suggesting that TM5614 is well tolerated and very safe.

In light of the findings from these previous studies, we evaluated the efficacy and safety profiles of anti-PD-1 Abs in combination with TM5614, a PAI-1 inhibitor, in patients with unresectable melanoma. As 80% of enrolled cases were in the anti-PD-1 Ab-refractory cohort at the interim analysis, this study focused on the anti-PD-1 Ab-refractory cohort. Although the duration of this combination therapy was only 8 weeks, the efficacy of anti-PD-1 Abs in combination with TM5614 for anti-PD-1 Ab-refractory unresectable melanoma was 25.9% (95% CI 12.9–44.9, $P=0.027$) in the PPS group, which met the proof-of-concept requirements for this study. Indeed, one patient with stable disease during the treatment period achieved PR 8 weeks after the observation period, and furthermore, six of seven patients who responded to treatment maintained PR with subsequent nivolumab administration.

These results suggest that long-term administration may further improve the therapeutic effects of this combination therapy. Moreover, the median PFS was 174 days (95% CI 114.4–232.9), DCR was 64.3% (95% CI 45.8–79.4) and the median OS was 273.3 days (95% CI 209.7–337.0) in the anti-PD-1 Ab-refractory cohort. Furthermore, a previous meta-analysis to evaluate OS of patients with anti-PD-1 Ab-refractory unresectable melanoma treated with nivolumab plus ipilimumab showed an OS of 7.9 months.[24] In addition, a phase II trial showed that 6-month PFS of nivolumab plus ipilimumab for patients with anti-PD-1/PD-L1 Abs-refractory melanoma was 34% (90% CI 25–43).[17] As the duration of combination therapy was only 56 days, and because the median PFS in the FAS cohort was greater than 5 months, extending the treatment period might prolong PFS. The safety profile of nivolumab plus TM5614 combination therapy was evaluated in 39 patients in the ITT group. In 26 patients, 39 cases of any-grade AEs were reported (66.7%), and the frequency of TM5614-related any-grade AEs was 26.5% (95% CI 14.4–43.3) and that of grade 3 or higher AEs

was 7.7% (95% CI 1.9–21.0), suggesting that TM5614 is well tolerated, even in combination with nivolumab.

Our study had some limitations. The study was an exploratory phase II study, the combination treatment period was short and the number of patients was limited. Efficacy was assessed according to RECIST v.1.1 only by an investigator review, not a central review.

Overall, the present study suggests that TM5614 in combination with nivolumab is a well tolerated and effective protocol for anti-PD-1 Ab-refractory unresectable melanoma. Further evaluation of OS and PFS for anti-PD-1 Ab-refractory unresectable melanoma cohorts should be performed in an important phase III trial in the future.

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Conflicts of interest

T.M. declares research funding from Astellas Pharma Inc., Daiichi-Sankyo Biotech Co. Ltd., and Kowa Company, and holds stocks from Renascience Inc.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Ethics statement

The protocol was approved by the institutional review board of Tohoku University Hospital and the institutional review board of each participating centre (No. 203011).

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

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