Exploring the landscape of rheumatoid arthritis: piecing together risk factors and autoantibodies

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Autoantibody-positivity before and seroconversion during treatment with anti-PD-1 is associated with immune-related adverse events in melanoma patients

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ABSTRACT

Introduction

Treatment with the immune checkpoint inhibitor anti-programmed cell death protein-1 (PD-1) often causes immune-related adverse events (irAEs). Since irAEs resemble autoimmune diseases, autoantibodies might play a role and could potentially be used to identify patients at risk. Therefore, we investigated the association between autoantibody-positivity and toxicity as well as clinical response in patients with melanoma treated with anti-PD-1.

Materials and methods

This two-center, retrospective study included 143 patients with melanoma treated with anti-PD-1. Toxicities grade ≥2 and recurrences/responses were captured until 6 months after treatment initiation. Autoantibody measurements were performed at baseline and 3 months after treatment initiation, including IgM-rheumatoid factor (RF), antinuclear antibodies (ANA), extractable nuclear antigen, anti-cyclic citrullinated peptide antibodies (anti-CCP2) and anti-thyroid antibodies.

Results

169 irAEs were experienced by 86/143 patients (137 grades 1–2, 32 grades 3–4), the most common being thyroiditis (n=25), dermatitis (n=24), and sicca problems (n=19). Patients with autoantibodies at baseline experienced more irAEs (p=0.001), predominantly associated with anti-thyroid antibodies and thyroid dysfunction. No association was observed between any irAE and anti-CCP2, RF or ANA. In women, baseline and on-treatment anti-thyroid antibody-positivity as well as seroconversion during treatment was associated with thyroid dysfunction. In men, this association was only observed on-treatment. The presence of autoantibodies was not associated with melanoma recurrence (p=0.776) or response (p=0.597).

Conclusion

The presence of autoantibodies prior to anti- PD-1 therapy is associated with irAEs in patients with melanoma. Both baseline positivity and seroconversion of anti-thyroid antibodies were strongly associated with thyroid dysfunction. This association was stronger in women, with all women who were baseline positive developing thyroid dysfunction.

INTRODUCTION

The introduction of immune-checkpoint inhibitors (ICI) has revolutionized the treatment of patients with several types of malignancies, including melanoma.[1] ICI are able to reactivate antitumor T-cell responses by blocking the immune checkpoints cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein-1 (PD-1), or programmed death-ligand 1 (PD-L1). However, as their mechanism of action is not specific for tumor-reactive T cells, they may also activate autoreactive T cells. This activation can lead to immune-related adverse events (irAEs), which are frequently observed in patients treated with ICI, especially within the first 6 months of treatment.[2, 3] irAEs can affect any organ system, with varying severities and frequencies, and are often treated with immunosuppressive drugs.[2, 4]

Based on the resemblance between irAEs and autoimmune diseases, it has been hypothesized that autoantibodies might also play a role in the development of irAEs. Both the ICI-induced activation of autoreactive T cells, as well as the direct effects of ICI on B cells can result in the activation of autoreactive B cells, which can in turn lead to the production of autoantibodies.[5] As immune checkpoints are also expressed on B cells, treatment with ICI has been shown to increase the proliferation and activation of B cells, and in an increase in circulating plasmablasts.[6, 7] Autoantibodies can easily be measured in plasma or serum and could potentially be used to help identify patients at risk of developing irAEs. Several studies correlating autoantibodies with either toxicity or clinical response to ICI therapy have been published,[7–14] as summarized in a recent systemic literature review by Ghosh and colleagues.[15] However, the association of autoantibodies with toxicity as well as disease response has been scarcely studied in patients with melanoma, and studies with autoantibody measurement both at baseline and on treatment are limited.

De Moel and colleagues have shown that there is no significant association between the development of auto- antibodies and irAEs in patients with melanoma treated with the anti-CTLA-4 agent ipilimumab. However, they did find an association with anti-thyroid antibodies after treatment with ipilimumab and the development of thyroid dysfunction under subsequent anti-PD-1 therapy.[8] As the mechanism of action between anti-CTLA-4 and anti-PD-1 is significantly different,[7, 12] it is unknown whether this finding can be extrapolated to patients with melanoma treated with first-line anti-PD-1 therapy.

We conducted a study on autoantibody positivity, including rheumatoid factor (RF), anti-cyclic citrullinated peptides (anti-CCP2), antinuclear antibody (ANA), extractable nuclear antigen (ENA), anti-thyroglobulin (anti-Tg), and anti-thyroid peroxidase (anti-TPO), and toxicity in patients with melanoma treated with anti-PD-1.

MATERIALS AND METHODS

Patient selection

This two-center, retrospective study included patients diagnosed with stage III or IV melanoma aged ≥18 years who received at least one dose of anti-PD-1 and of whom baseline (prior to start anti-PD-1) and on-treatment/posttreatment serum or plasma samples taken approximately 3 months after treatment initiation were available. Patients were treated with the anti-PD-1 agents pembrolizumab or nivolumab, or a combination of nivolumab with the anti- CTLA-4 antibody ipilimumab as first-line treatment at either the Netherlands Cancer Institute, Amsterdam, the Netherlands, or at the Leiden University Medical Center, Leiden, the Netherlands between 2015 and 2021. Patients who received prior immunotherapy were excluded from our analyses. The study was conducted in accordance with the Declaration of Helsinki. All patients gave informed consent for the storage and use of the blood samples for research purposes.

Clinical data

The following patient and tumor characteristics were obtained: age at the time of treatment initiation, sex, body mass index, performance score, smoking status, melanoma subtype, stage at the time of treatment initiation, history of autoimmune disease (including hypothyroidism), and the use of immunosuppressants. Regarding their treatment, the date of each cycle, the monoclonal antibody, the administered dose, and the indication (adjuvant or palliative) were registered. Furthermore, in the 6 months after treatment initiation, all toxicities were captured using the Common Terminology Criteria for Adverse Events (CTCAE) V.5.0. For our analyses, we included all immune-related toxicities grade ≥2, except for dry eyes, dry mouth and skin hypopigmentation for which grade 1 was also included because of incomplete documentation of symptoms or absence of diagnostics to accurately distinguish between grade 1 and 2. The subgroup arthralgia/arthritis comprised of all patients experiencing arthralgia or arthritis according to the CTCAE criteria; colitis of both proven colitis and diarrhea; dermatitis of rash, erythema, pruritus, and skin hypopigmentation; hepatitis of increased liver test abnormalities (either grade 3 or requiring systemic immuno- suppression); hypophysitis of hypophysitis (including relevant serum adrenocorticotropic hormone (ACTH), cortisol and thyroid stimulating hormone (TSH) abnormalities); thyroiditis of either hyperthyroidism or hypothyroidism (grade ≥2 indicating that patients require suppression/replacement therapy); uveitis of uveitis; and sicca comprised of dry eye and dry mouth. The best response to treatment, until 6 months after ICI initiation, was defined as relapse/no relapse in stage III, and the response according to Response Evaluation Criteria in Solid Tumors V.1.1. criteria in

stage IV patients. Here, clinical benefit is defined as a complete response (CR), partial response (PR) or stable disease as a best observed response.

All clinical data was collected before the autoantibody measurements were performed, to minimize the risk of bias.

Autoantibody analyses

IgM RF as well as IgG antibodies directed against CCP2, TPO and Tg were determined using the Phadia EliA system (Thermo Fisher Scientific) based on fluorescent-enzyme immuno-assay technology. Assays were performed according to the manufacturers' guidelines in an ISO15189 accredited laboratory. ANA measurements were performed on Hep2 slides (INOVA) with a serum dilution of 1:40, corresponding to a~5% positivity in the analyzed population. ENA were determined using a line blot (Euroimmun) on positive nuclear staining on ANA. Results were reported semi-quantitative (negative/weakly positive/positive) depending in the staining intensity of the line blots.

Statistical analysis

The McNemar test for paired data was used to test whether autoantibody positivity increased on anti-PD-1 treatment. Using the Fisher exact test and binary logistic regression the association between frequencies of irAEs and (1) development of autoantibodies, (2) adjuvant versus palliative treatment, (3) male versus female patients, and (4) the use of immunosuppressive drugs, was assessed. To investigate whether autoantibody development post- anti-PD-1-treatment was associated with the development of irAE's under anti-PD-1 treatment, and to see whether post-anti-PD-1 autoantibody-positivity was associated with recurrence/response, multivariate binary logistic regression was used, adjusted for age, sex, and months of ICI exposure in the first 6 months after treatment initiation. The point-biserial correlation was used to look at correlations between age and the on-treatment positivity for anti- thyroid antibodies and thyroid dysfunction.

RESULTS

Patient characteristics

The group of 143 patients with melanoma comprised 88 male and 55 female patients, with a median age of 65 years (range 21–90) (table 1). The majority of patients were diagnosed with cutaneous melanoma (85%), followed by melanoma of unknown primary (9%), acral (4%) and mucosal (1%) melanoma, respectively (table 1). 52% of patients received anti-PD-1 treatment in an adjuvant setting (resectable stages III and IV), compared with 48% in a palliative setting (unresectable stages III and IV). 128 patients received anti-PD-1 monotherapy (78 nivolumab, 50 pembrolizumab), and 15 a combination of nivolumab with ipilimumab.

irAEs

irAEs occurred in 86 (60%) of patients, who together experienced 169 events. 137 were grades 1–2, 32 were grades 3–4, and no grade 5 toxicities were observed. As listed in table 1, 5 patients experienced symptoms related to arthralgia/arthritis, 9 to colitis, 24 to dermatitis, 7 to hepatitis, 8 to hypophysitis, 25 to thyroiditis, 1 to uveitis, and 19 patients experienced sicca syndrome symptoms, respectively. There was a trend towards more irAEs in the palliative setting (66.7%) compared with the adjuvant setting (52.7%, p=0.063), which was independent of the treatments received.

Table 1. Baseline table with patient, tumor, irAE, and response characteristics

Characteristics	**N total=143**
Sex, per cent male (n)	61.5 (88)
Age, median (range)	64 (21–90)
BMI, median (range)	27 (19–42)
Smoking, per cent (n)	
Never	49.7 (71)
Former	32.9 (47)
Current	13.3 (19)
Unknown	4.2 (6)
History of auto-immune disease, per cent (n)	4.9 (7)*
Use of immune suppressants prior to start anti- PD-1, per cent (n)	7.0 (10)†
Melanoma subtype, per cent (n)	
Cutaneous	85.3 (122)
Acral	4.2 (6)
Mucosal	1.4 (2)
Unknown primary	9.1 (13)
Performance score, per cent (n)	
0	71.3 (102)
1	23.8 (34)
2	0.7 (1)
Unknown	4.2 (6)
LDH level, per cent (n)	
Normal	88.1 (126)
1–2×ULN	11.2 (16)
≥2×ULN	0.7 (1)

Table 1. *Continued*

Characteristics	N total=143
Stage at time of anti-PD-1 initiation, per cent (n)	
III	49.7 (71)
IIIa	4.9 (7)
IIIb	15.4 (22)
IIIc	22.4 (32)
IIIc (irresectable)	2.8 (4)
IV	50.3 (72)
M1a	7.7 (11)
M1b	11.2 (16)
M1c	14.7 (21)
M1d	11.2 (16)
IV (resected)	5.5 (8)
Treatment, per cent (n)	
Nivolumab	54.5 (78)
Pembrolizumab	35 (50)
Ipilimumab-nivolumab	10.5 (15)
Intention, per cent (n)	
Adjuvant	51.7 (74)
Palliative	48.3 (69)
Date OnTx blood draw in weeks after anti-PD-1 initiation, mean (range)	12.8 (7.9–20.1)
Number of patients with irAEs, per cent (n)	50.1 (86)
Total toxicities, per cent of total irAEs (n)	169
Grades 1–2	81.1 (137)
Grades 3–4	18.9 (32)
Type of toxicity, n	
Arthralgia/arthritis	5
Colitis	9
Dermatitis	24
Hepatitis	7
Hypophysitis	8
Thyroiditis	25
Uveitis	1
Sicca	19
Use of immune suppressants because of toxicity, per cent (n)	21.0 (30)

Table 1. *Continued*

Characteristics	N total=143
Response, per cent (n)	
Adj – no relapse	68.9 (51)
Adj – relapse	31.1 (23)
Pall – clinical benefit‡	69.6 (48)
Pall – progression§	30.4 (21)

*Ulcerative colitis (2), rheumatoid arthritis (1), psoriasis (1), diabetes mellitus type I (1), celiac disease (1), pre-existing hypothyroidism (1).
†Dexamethasone (brain radiotherapy, 8), mesalazine (ulcerative colitis, 1), methotrexate (rheumatoid arthritis, discontinued shortly prior to first cycle, 1).
‡Defined as complete response, partial response, stable disease.
§Defined as progressive disease.
Adj, adjuvant treatment; BMI, body mass index; irAEs, immune- related adverse events ; LDH, lactate dehydrogenase; N, number; OnTx, on treatment; Pall, palliative treatment; PD-1, programmed cell death protein-1 ; UNL, upper limit of normal.

Baseline autoantibody positivity is associated with irAEs

To investigate whether the presence of autoantibodies before the start of treatment was associated with irAEs, we determined autoantibodies in baseline samples. At baseline 37/143 (26%) patients were autoantibody positive (table 2).

As shown in figure 1, these patients experienced significantly more irAE (25/37 (68%)) than patients who were negative for autoantibodies at base- line (37/106 (35%); OR 3.89 (95% CI 1.75 to 8.61), p=0.001) (table 3).

Multivariate analysis confirmed that this difference was independent of age, sex and months of treatment received (OR 4.17 95% CI 1.65 to 10.6), p=0.003). We observed an inverse association between the severity of irAEs and baseline autoantibodies: while only 5 out of the 27 patients with grades 3–4 toxicity (19%) were autoantibody-positive, 26 of 59 patients with grades 1–2 (44%) were positive (p=0.029), but the frequency of grades 3–4 toxicity was low (table 1). Due to the low incidence of grades 3–4 toxicity, it was not possible to perform a separate analysis of the association of antibodies with irAEs of grades 3–4. Since treatment with anti-CTLA-4 is associated with the development of autoantibodies and a higher incidence of toxicities,[2, 5] an analysis excluding the patients who received combination therapy was performed. Again, autoantibody-positive patients were found to have significantly more irAEs than autoantibody-negative (p=0.002).

Table 2. Number of auto-antibody positive/negative patients at baseline and on treatment

Autoantibodies	Baseline (positive/ total)	OnTx (positive/ total)	Positive → negative	Negative → positive	Double- positive	Double- negative
RF	10/143 (6.99%)	10/143 (6.99%)	3	3	7	130
CCP2	0/143 (0%)	0/143 (0%)	0	0	0	0
Anti-Tg	7/143 (4.90%)	22/143 (15.4%)	0	15	7	121
Anti-TPO	7/143 (4.90%)	12/143 (8.39%)	1	6	6	130
ANA	19/143 (13.3%)	17/143 (11.9%)	4	2	15	122
Total	37/143 (25.9%)	45/143 (31.5%)	5	13	32	93

ANA, antinuclear antibodies; anti-Tg, anti-thyroglobulin antibodies; anti-TPO, anti-thyroid peroxidase antibodies; CCP2, anti-cyclic citrullinated peptide antibodies; OnTx, autoantibodies developed during treatment with anti-PD1; RF, rheumatoid factor.

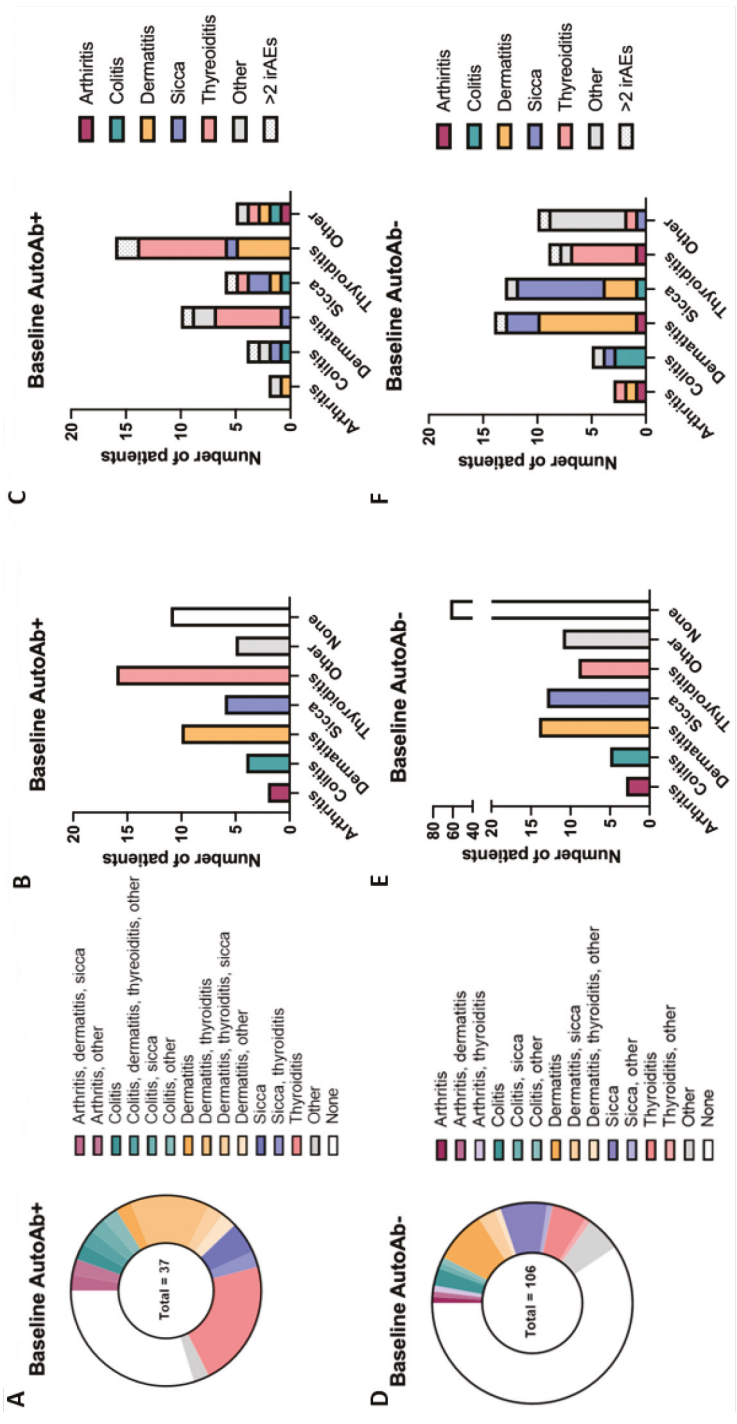

Figure 1. Frequency of irAEs based on baseline autoantibody positivity or negativity.
Overview of the types of toxicities observed in patients who are either positive (A) or negative (D) for any tested autoantibody at baseline, including the total number of patients experiencing a particular type of toxicity (B & E). To illustrate the overlap between toxicities, this is further subdivided per toxicity per column (C & F).
AutoAb: autoantibody (either ANA, ENA anti-CCP2, RF, anti-Tg or anti-TPO); Other: immune-related adverse events with low incidences and therefore not further specified; >2 irAEs: patients who developed more than two irAEs.

Table 3. Association between autoantibody positivity and irAEs.

	Baseline positive for...	Baseline negative for...	p-value	Odds-ratio (95%CI)
Any irAE/any antibody	25/37 (67.6%)	37/106 (34.9%)	<0.001	3.89 (1.75-8.61)
Thyroiditis/anti-TPO and/or anti-Tg	9/13 (69.2%)	16/130 (12.3%)	<0.001	16.03 (4.42-58.17)
Arthralgia or arthritis/anti-CCP2 and/or RF	1/10 (10.0%)	4/133 (3.0%)	0.275	3.58 (0.360-35.50)
Dermatitis/antinuclear antibodies	5/19 (26.3%)	19/124 (15.3%)	0.239	1.97 (0.636-6.12)
Sicca symptoms/antinuclear antibodies	2/19 (10.5%)	17/124 (13.7%)	0.704	0.740 (0.157-3.50)
Colitis/antinuclear antibodies	2/19 (10.5%)	7/124 (5.60%)	0.422	1.97 (0.377-10.26)

In each cell, n/N indicates the number of patients who developed the irAE (n) out of the total number of patients who were either positive or negative at baseline for the indicated antibody (N). CCP2, cyclic citrullinated peptides ; irAE, immune-related adverse event ; RF, rheumatoid factor; Tg, thyroglobulin ; TPO, thyroid peroxidase.

Baseline autoantibodies against TPO and Tg are associated with thyroiditis

Next, the relationship of autoantibodies with the corresponding irAEs was explored. Here, no association was found between anti-CCP2 and/or RF with arthralgia and arthritis (OR 3.58 (95% CI 0.360 to 35.5), p=0.28). Similarly, no association was found between ANA and dermatitis (OR 1.97 (95% CI 0.636 to 6.12), p=0.24), sicca (OR 0.740 (95% CI 0.157 to 3.50), p=0.70) or colitis (OR 1.97 (95% CI 0.377 to 10.3), p=0.42). However, anti-TPO and anti-Tg antibodies were very strongly associated with thyroid dysfunction (69% in anti-TPO and/ or anti-Tg positive patients vs 12% in anti-TPO and/ or anti-Tg negative; OR 16.0 (95% CI 4.42 to 58.2), p<0.001) (table 3). Thyroid dysfunction is more common in women than men,16 and this was also observed in our study population with 31% of women and 9% of men displaying thyroiditis. Therefore, we stratified the analyses for sex. Strikingly, all female patients who displayed either anti-TPO and/or anti-Tg at baseline developed thyroiditis (7/7, 100%), whereas only 10/48 (21%) of anti-TPO and anti-Tg negative female patients developed thyroiditis (OR could not be calculated due to 100% in women). Also in male patients, a trend towards an association between baseline anti-TPO and/or anti-Tg antibody positivity and the development of thyroiditis was observed, with 1/6 (17%) of men with baseline anti-TPO and/or anti-Tg antibody positivity developing thyroiditis, compared with 5/82 (6.1%) of the men without baseline anti-thyroid antibodies (OR 6.33 (95% CI 0.957 to 41.9), p=0.056) (figure 2A–C; figure 3). In conclusion, anti-TPO and anti-Tg autoantibody positivity at baseline was a very strong predictor of anti-PD-1-associated thyroid dysfunction in women.

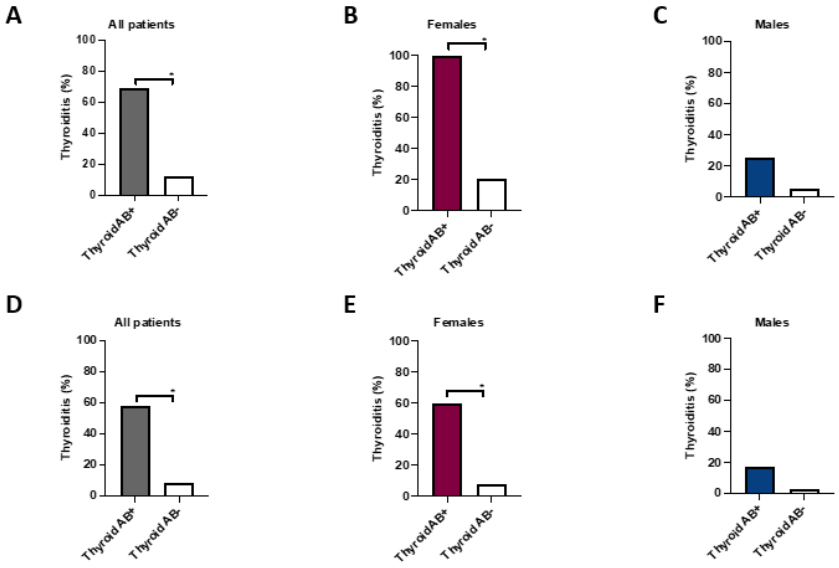

Figure 2. Frequency of thyroid dysfunction based on anti-thyroid antibody positivity at baseline or after seroconversion. Overview of the percentage of patients who experienced thyroid dysfunction after anti-programmed cell death protein-1 initiation with or without anti-thyroid antibody (anti-thyroglobulin or anti-thyroid peroxidase) positivity at baseline (A–C) or after seroconversion (D–E). *Indicates significance (p≤0.05). AB, autoantibody.

Anti-thyroid antibody seroconversion is associated with thyroid dysfunction

Due to the mechanism of action of checkpoint inhibitors, it is plausible that autoantibodies may develop during treatment. For this reason, we measured autoantibodies after on average 12 weeks of treatment with anti-PD-1. No significant increase was found in the number of patients with overall autoantibody positivity between baseline (n=37 (26%)) and on treatment (n=45 (32%); p=0.096). No association was found between autoantibody development during treatment and irAEs. IrAEs were present in 8/13 (65%) of patients that seroconverted, meaning negative for any autoantibody at baseline and autoantibody-positive for one or more autoantibodies on treatment, compared with 47/93 (51%) of the patients that remained autoantibody-negative (OR 1.57 (95% CI 0.477 to 5.14), p=0.559). The combined positivity for either anti-TPO and/or anti-Tg increased from 13/143 (9.1%) at baseline to 24/143 (17%) on treatment (p=0.003; online supplemental table 1). The frequency of patients with other autoantibodies was stable over time (table 2).

Since the frequency of patients with anti-thyroid antibodies increased during treatment, we looked into the serum levels of anti-thyroid autoantibodies. The increase in anti-thyroid antibodies was mainly seen in patients developing

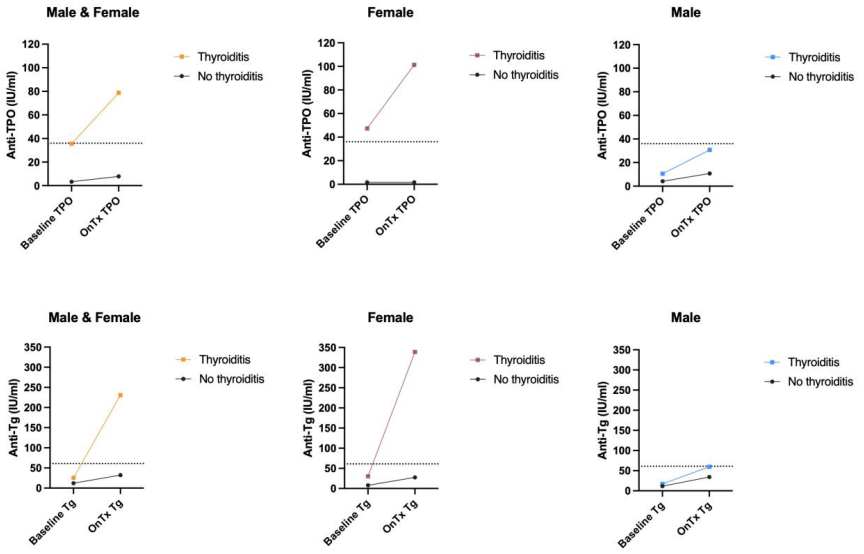

Figure 3. Anti-TPO and anti-Tg levels at baseline and on treatment for patients with and without thyroiditis. Dotted line indicates upper limit of normal. OnTx, on treatment; Tg, thyroglobulin; TPO, thyroid peroxidase.

thyroid dysfunction and was stronger in female patients compared with male patients (figure 3).

Because anti-thyroid antibodies increased on treatment, we investigated if seroconversion was associated with the development of thyroiditis. Both anti-TPO and anti-Tg seroconversion were associated with the development of thyroiditis, both individually and combined (online supplemental table 1). Five of six anti-TPO positive seroconverted patients (83%) developed thyroid- itis versus 15 of 130 (12%) patients that remained negative (OR 38.3 (95% CI 4.19 to 350), p=0.001), and 9 out of 15 anti-Tg positive or seroconverted patients (60%) versus 12/121 (9.9%) that remained negative (OR 13.6 (95% CI 4.13 to 44.9), p<0.001), respectively. When looking at the combined seroconversion for either anti-TPO and/or anti-Tg, 11/19 (57.9%) of seroconverted patients developed thyroiditis, compared with 7 out of 118 (5.9%) of patients that did not seroconvert (OR 21.8 (95% CI 6.64 to 71.6), p<0.001). As there was a sex difference in the association of anti-thyroid positivity at baseline and thyroid dysfunction, this was also investigated for seroconversion. Similar to the results at baseline, an association was found between thyroiditis and seroconversion in female patients: 9 out of 12 female patients who seroconverted for anti-Tg and/or anti-TPO (75%) compared with 3/39 (8%) of patients that stayed negative developed thyroiditis (OR 36 (95% CI 6.20 to 209), p<0.001) (figure

2D–F). In male patients, no significant association was observed between thyroid dysfunction in seroconverters for anti-TPO and/or anti-Tg (2/7 (29%)) versus patients that stayed negative (4/79, (5%)) (OR 7.50 (95% CI 1.10 to 51.3), p=0.073). In conclusion, anti-thyroid antibodies develop during anti-PD-1 treatment, and are strongly associated with the development of thyroid dysfunction in female patients.

Autoantibody positivity on treatment is associated with irAEs

We next investigated if autoantibody positivity at any time during treatment (on-treatment) was related to irAEs. As expected, an association between autoantibody positivity during treatment and irAEs was observed: of the 45 patients that were autoantibody positive (baseline+ seroconverted), 35 experienced one or multiple irAEs (78%), compared with 50/98 (51%) of the patients that were negative (OR 3.36 (95% CI 1.50 to 7.53), p=0.003). This was independent of age, sex and duration of anti- PD-1 treatment (OR 2.84 (95% CI 1.24 to 6.51), p=0.014). Again, the association of antibody positivity and irAEs was based on the association between anti-thyroid antibodies and thyroiditis (OR 19.4 (95% CI 6.58 to 57.4), p<0.001) (online supplemental table 1). To see whether age played a role specifically in the observed thyroid dysfunction, we looked at the correlation between age and the on treatment positivity for anti-TPO and/or anti-Tg, between age and thyroiditis. However, with p values of 0.58 and 0.78, respectively, these correlations were not found. Furthermore, while there was no difference in autoantibody positivity at baseline between male and female patients (30.9% vs 22.7%, p=0.328), it was noted that there were more female autoantibody-positive patients on treatment (43.6% vs 23.9%; p=0.016). Interestingly, while there was no significant association between either baseline positivity or seroconversion for anti-thyroid antibodies and thyroid dysfunction in male patients, this association was observed in the on-treatment samples with 33.3% of anti-thyroid antibody-positive men developing thyroid dysfunction while this was observed in only 6.3% of men who were anti-thyroid antibody negative (OR 7.40 (95% CI 1.41 to 38.7), p=0.033). This is in line with the observation that on-treatment positivity and irAEs were independent of sex.

Association autoimmune disease or immunosuppression and irAEs

Six patients had a known history of auto-immune disease: ulcerative colitis (2), rheumatoid arthritis (1), psoriasis (1), diabetes mellitus type I (1), hypothyroidism (1), and celiac disease (1). Five out of these six patients experienced irAEs. In three patients the type of toxicity corresponded with their auto-immune disease, indicating a potential disease flare (online supplemental appendix 1). 10 patients used immunosuppressive drugs prior to anti-PD-1 initiation, either as treatment for their auto- immune disease, or in combination

with radiotherapy on brain metastases (table 1). Patients who used immunosuppressive drugs at baseline did not experience fewer irAEs than patients who did not (7/10 (70%) vs 78/133 (59%); p=0.740).

Association of autoantibody positivity and disease recurrence/ response

Of the patients receiving adjuvant treatment, 69% did not experience a relapse in the first 6 months after treatment initiation. In the palliative setting, 70% of patients had clinical benefit from the treatment, while 30% showed progressive disease as the best response. Since the development of autoantibodies during treatment with check- point inhibitors may be indicative of a stronger immune response activation, we next investigated whether this was associated with a better treatment response. Of the patients treated in an adjuvant setting, four developed autoantibodies of whom three did not relapse (p=0.776). Of the patients treated in a palliative setting, nine developed autoantibodies of whom five had clinical benefit from the treatment (p=0.597). Therefore, although the sample size is small, the development of autoantibodies was not associated with treatment response in either the adjuvant or the palliative setting.

DISCUSSION

While there is evidence that certain patient characteristics, including M-stage and baseline lactate dehydrogenase (LDH) level,[17 18] and tumor signatures, such as tumor mutational burden, interferon-gamma signature and PD-L1-expression, could serve as biomarkers to predict which patients benefit most of the treatment,[19–21] there is currently no predictive biomarker available to identify patients at risk of developing irAEs. We hypothesized that in patients with melanoma with a subclinical autoimmune profile characterized by baseline autoantibody positivity, treatment with ICI might lead to more frequent irAEs. We therefore investigated whether autoantibodies are associated with irAEs on anti-PD-1 treatment in patients with malignant melanoma. Our study reveals that autoantibody positivity prior to anti-PD-1 treatment is associated with the development of irAEs. This finding could be explained by the strong association of anti-Tg and anti-TPO antibodies with the development of thyroid dysfunction. The association between anti-thyroid antibodies at baseline with thyroiditis was stronger in female patients than in male, with all baseline anti-TPO and/or anti-Tg positive female patients developing thyroid dysfunction on treatment. Furthermore, in female patients, seroconversion of anti-Tg and anti-TPO antibodies during anti-PD-1 treatment was also associated with thyroiditis. These data indicate that measurement of anti-thyroid antibodies both prior to and 3 months after anti-PD-1 initiation is an extremely useful marker to identify patients at risk of developing thyroid dysfunction. Especially the finding that anti-thyroid antibody positivity at baseline is associated with the risk of developing lifelong thyroid dysfunction requiring supplementation

can be used in the discussion with the patient about the risk-benefit-ratio of anti-PD-1 treatment, particularly in the (neo)adjuvant setting.

Several recent studies across various countries have looked at the association between anti-thyroid antibodies and ICI-induced thyroid dysfunction. Given ethnic differences in anti-thyroid antibody prevalence, the association in Asian populations might differ from that in Caucasian populations.[22]

While findings on specific antibodies (anti-Tg vs anti-TPO) and their role in predicting dysfunction during ICI treatment vary, baseline anti-thyroid antibodies consistently correlate with a higher risk of dysfunction across countries and tumor types.[10, 12, 23–25] Furthermore, one study found that patients who seroconverted had a higher rate of thyroid dysfunction compared with the group that did not.[25] Additionally, in a Dutch patient cohort it has been shown that patients who developed anti-thyroid antibodies after treatment with ipilimumab had a significantly higher risk of developing thyroid dysfunction under subsequent anti-PD-1 treatment.[8] These findings are supported by an immune-related-thyroiditis mouse model, demonstrating that mice with pre-existing Tg antibodies had a high risk of anti-PD-1-induced thyroid dysfunction, and that this could be prevented by depletion of CD4+T cells.[26] Interestingly, depletion of CD8+T cells only leads to partial prevention, and depletion of B cells was unable to prevent thyroiditis. Moreover, the destructive anti-PD-1-induced thyroiditis was only observed in mice with previous immunization of Tg. This indicates that pre-existing autoimmunity against the thyroid gland is essential for the development of immune-related thyroid dysfunction, and that the cytotoxic memory CD4+T cells play a key role in the pathogenesis.[26] The onset of overt thyroiditis could be triggered by treatment with anti-PD-1 therapy in patients with pre-existing thyroid anti- bodies because autoreactive T cells directed against the thyroid gland that without treatment of anti-PD-1 therapy would have remained quiescent, can be (re-) activated by this treatment and can subsequently lead to inflammation and destruction of the thyroid gland. While the association between anti-thyroid anti-

bodies and ICI-induced thyroiditis has been shown before in patients treated with ICI, this is to our knowledge the first study that shows that this association is strongest in female patients, and more importantly, that all female patients who are either anti-Tg or anti-TPO positive at baseline develop immune- related thyroid dysfunction. However, the finding that all female patients with anti-thyroid antibodies at baseline did develop immune-related thyroid dysfunction needs further replication since the number of patients in this group is small. Important to note is that thyroid disease in the general population is also more common in women,[27] indicating that treatment with ICI can be the final nudge tipping the balance toward thyroid dysfunction in women who were already more prone to develop thyroid dysfunction.

In our study, we only found a significant association between anti-thyroid antibodies and thyroid dysfunction, and no association between organ-specific irAEs and RF, ANA, ENA and anti-CCP. The latter is in line with the study by Ghosh and colleagues,[28] who did not find significant associations between organ-specific irAEs and autoantibodies (ANA, RF, CCP) in patients with melanoma, and with a study by Yoneshima et al,[29] who did not find associations between ANA and irAEs. In contrast, other studies found an association between ANA and colitis,[30] and between RF with skin reactions.10 Furthermore, Giannicola and colleagues found a higher frequency of irAEs in patients that became autoantibody positive (ANA, ENA, anti-smooth muscle antibodies (ASMA), antineutrophil cytoplasmic antibodies (ANCA), anti-Tg or anti-TPO) on treatment, but this was not further associated with any of the individual autoantibodies.[31] There are several explanations for the discrepancies with our study. First, the frequencies of certain irAEs, such as arthritis and colitis, are low and potentially require a larger cohort to find this association. Second, ANA and RF are both also found in healthy individuals, ANA in approximately 5% (depending on the dilution) and RF in 1–4% (increasing with age), and are therefore not necessarily associated with autoimmune diseases.[32, 33] Third, patients with grade 1 sicca-related irAEs were also included as it was difficult to distinguish grade 1 from grade 2 for these symptoms. By including these cases, the association with ANA could have been weakened. Lastly, it is debatable whether one would necessarily expect an association between autoantibodies such as ANA, anti-CCP and RF and the development of specific complaints given that for these autoantibodies it is unknown whether they have a pathogenic role in the development of autoimmune diseases or whether they are an epiphenomenon.[34]

As the presence of autoantibodies might be indicative of a better immune response, we also looked at the association between autoantibody positivity and treatment outcome. However, we were unable to identify an association between autoantibody positivity, either at baseline or on treatment, and melanoma recurrence or treatment response in our trial. This is in contrast with a recently published study by Johannet and colleagues who showed that, by using a HuProt Human Proteome Microarray containing 21,000 unique proteins and protein isoforms, a predictive baseline autoantibody signature for recurrence and severe toxicity could be identified and validated in patients with melanoma receiving adjuvant ICIs in the CheckMate 238 and 915 trials.[14] A possible explanation for this difference could be that the autoantibodies associated with response were distinct from the ones associated with toxicity, as they observed minimal overlap between these two groups of autoantibodies in their microarray signature used to predict either irAEs or disease recurrence.[14] If their hypothesis is true, this could explain the lack of association found

between autoantibody positivity and response in our study, considering that we measured levels of autoantibodies with known associations with irAEs. Furthermore, in melanoma, the association between irAEs and response or survival is not as well-defined as in other tumor types, including non-small cell lung cancer, renal cell carcinoma, urothelial cell carcinoma and gastrointestinal tumors, as summarized in a review by Das and Johnson.[35] This could also support the lack of association between autoantibody positivity and response found in our trial.

In our patient cohort, six patients had pre-existing auto-immune diseases prior to ICI initiation. Interest- ingly, although the numbers are small, five out of six patients (83%) developed irAEs. This is higher than the previously reported incidence of 5–20% of any-grade toxicities in patients treated with anti-PD-1.[2] However, because of the immune dysregulation associated with auto-immune diseases, this patient population is often excluded from clinical trials to minimize the risk of severe irAEs or disease flare,[36] limiting our knowledge about this subgroup. Despite the fact that a high incidence of toxicities was observed in these patients, 2/3 of adjuvantly treated patients remained disease-free, and both patients with stage IV disease had clinical benefit (CR and PR). While our patient numbers are too small to draw conclusions, these findings are in line with a previous hypothesis that patients with a tendency toward immune activation, such as in patients with auto-immune diseases, may derive more benefit from ICI.[37]

This study should be interpreted in light of its limitations. First, to increase the size of our patient cohort, 15 patients who received combination treatment with ipilimumab and nivolumab were included (10% of our total study population). However, the analyses investigating the association of autoantibodies with irAEs remained significant when these patients were excluded in a subanalysis, indicating that they did not have a major influence on the results. Second, despite including an additional 15 patients, the cohort size is still relatively small with a low prevalence of several autoantibodies and more rare irAEs, resulting in a lack of power to perform certain (subgroup) analyses. Specifically, the number of male patients who are positive for anti-thyroid antibodies was much lower than the corresponding female group, which could explain the lack of significance despite the high OR for the association between these autoantibodies and thyroid dysfunction. The results of our study should there- fore be confirmed in other, larger studies. Furthermore, the incomplete documentation or diagnostics performed to accurately distinguish between dry eye, dry mouth and skin hypopigmentation grade 1 or 2, may have resulted in a potential overrepresentation of patients with skin- related or sicca-related immune-related toxicities. The observation period of this study was chosen because it has previously been observed that most irAEs occur in the first 6

months after treatment initiation,[2] however, patients could have developed irAEs after the 6-month follow-up period meaning there is a potential underestimation of the irAEs associated to the autoantibodies. And finally, it is possible that associations between irAEs and autoantibodies have been missed due to the autoantibody panel chosen in our study. Despite these limitations, our data significantly expand the knowledge of the potential predictive value of baseline autoantibodies for the development of irAEs in patients with melanoma treated with anti-PD-1.

CONCLUSIONS

In our study, autoantibody positivity prior to anti-PD-1 therapy was associated with the development of irAEs in patients with advanced-stage melanoma. While we did not observe an association between anti-CCP2, RF and/ or ANA (ENA) with arthritis, dermatitis, sicca or colitis, a very strong association between anti-thyroid antibodies and thyroid dysfunction was seen both at baseline as well as after 3 months of treatment, especially in female patients. In our patient cohort, autoantibody positivity was not associated with disease recurrence or response.

Our findings therefore indicate that measuring anti-thyroid antibodies at baseline and after 3 months of treatment is a very potent biomarker for predicting the development of thyroid dysfunction.

Supplementary material
Supplementary material available at:
https://jitc.bmj.com/content/12/6/e009215

Declarations
Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki, and was approved by the institutional review boards of both participating centers. All patients gave informed consent for the storage and use of the blood samples for research purposes.

Availability of data and material
The datasets generated and/or analyzed for this manuscript from the NKI and LUMC are available via the corresponding author on reasonable request.

Competing interests
EK has consultancy/advisory relationships with Bristol Myers Squibb, Novartis, Pierre Fabre, Immunocore and Lilly, and received research grants not related to this paper from Bristol Myers Squibb, Delcath, Novartis and Pierre-Fabre. Not related to current work and paid to institute. JH received compensation for advisory roles for Achilles Therapeutics, AZ, BioNTech, BMS, CureVac, Eisai, Gadeta, Imcyse, Immunocore, Instil Bio, Iovance Biotherapeutics, Ipsen, MSD, Merck Serono, Molecular Partners, Neogene Therapeutics, Novartis, Pfizer, Roche/Genentech, Sanofi, Scenic, Third Rock Ventures, and T-knife, and has received grants (all paid to the institute) from Amgen, Asher Bio, BioNTech, BMS, MSD, Novartis, Neogene Therapeutics and Sastra Cell Therapy.
All other authors declare to have no competing interest with respect to this manuscript.

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Authors' contributions and consent for publication
All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

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