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ORIGINAL RESEARCH

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Preexisting autoantibodies as predictor of immune related adverse events (irAEs) for advanced solid tumors treated with immune checkpoint inhibitors (ICIs)

A Daban[a*], C Gonnin[b*], L Phan[c], A Saldmann[b], C Granier[b,d], A Lillo-Lelouet[e], C Le Beller[e], J Pouchot[f], I Weiss[g], E Tartour[b,d], E Fabre[d,h], J Medioni[a], S Oudard[a,d], YA Vano[a,i¤], MA Dragon-Durey[b,i¤], and A. Simonaggio[a¤]

[a]Department of Medical Oncology, Hôpital Européen Georges Pompidou, Institut du Cancer Paris CARPEM, AP-HP.Centre – Université Paris Cité, Paris, France; [b]Department of Immunology, Hôpital Européen Georges Pompidou, AP-HP.Centre – Université Paris Cité, Paris, France; [c]ARTIC - Association pour la Recherche de Thérapeutiques Innovantes en Cancérologie; Hôpital Européen Georges Pompidou, AP-HP. Centre – Université Paris Cité, Paris, France; [d]INSERM U970, PARCC, Université Paris-Cité, Paris, France; [e]Department of Pharmacovigilance, Hôpital Européen Georges Pompidou, AP-HP. Centre – Université Paris Cité, Paris, France; [f]Department of Internal Medicine, Hôpital Européen Georges Pompidou, AP-HP.Centre – Université Paris Cité, Paris, France; [g]Department of Clinical Immunology, Hôpital Hôtel-Dieu, AP-HP.Centre – Université Paris Cité, Paris, France; [h]Department of Medical Oncology, Hôpital Européen Georges Pompidou, Paris, France; [i]Centre de Recherche des Cordeliers, INSERM, Université Paris Cité, Sorbonne Université, Paris, France

ABSTRACT

Introduction: Immune checkpoint inhibitors (ICIs) are now standard of care in many cancers. They can generate immune-related adverse events (irAEs), but no biomarkers are available to identify patients who are more likely to develop irAEs. We assess the association between pre-existing autoantibodies and occurrence of irAEs. Patients and Methods: We prospectively collected data from consecutive patients receiving ICIs for advanced cancers, in a single center between May 2015 and July 2021. Autoantibodies testing was performed before ICIs initiation including AntiNeutrophil Cytoplasmic Antibodies, Antinuclear Antibodies, Rheumatoid Factor anti-Thyroid Peroxidase and anti-Thyroglobulin. We analyzed the associations of pre-existing autoantibodies with onset, severity, time to irAEs and with survival outcomes. Results: Of the 221 patients included, most had renal cell carcinoma (n = 99; 45%) or lung carcinoma (n = 90; 41%). Grade ≥2 irAEs were more frequent among patients with pre-existing autoantibodies: 64 (50%) vs. 20 (22%) patients (Odds-Ratio= 3.5 [95% CI=1.8-6.8]; p < 0.001) in the positive vs negative group, respectively. irAEs occurred earlier in the positive group with a median time interval between ICI initiation and irAE of 13 weeks (IQR = 8.8-21.6) vs. 28.5 weeks (IQR=10.6-55.1) in the negative group (p = 0.01). Twelve patients (9.4%) experienced multiple (≥2) irAEs in the positive group vs. 2 (2%) in the negative group (OR = 4.5 [95% CI: 0.98-36], p = 0.04). After a median follow-up of 25 months, median PFS and OS were significantly longer among patients experiencing irAE (p = 0.00034 and p = 0.016, respectively). Conclusion: The presence of pre-existing autoantibodies is significantly associated with the occurrence of grade ≥2 irAEs, with earlier and multiple irAEs in patients treated with ICIs.

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Introduction

Immune checkpoint inhibitors (ICIs) targeting anti-programmed cell-death protein 1 (PD-1) or its ligand PD-L1, used alone or in combination with ICI targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), or with chemotherapy or with VEGFR-tyrosine kinase inhibitors (VEGFR-TKI), are now standard of care in many cancers[1]. The number of patients exposed to ICIs has increased dramatically over the last few years. Almost 40% of US patients with cancer are eligible for ICI therapy[2]. These immune checkpoint molecules (ICMs) are involved in the peripheral tolerance mechanisms that prevent the immune system from reacting against the self-antigen. After binding to their ligand, ICM expressed by lymphocyte provides a negative signal leaving the cell unable to be fully activated in the presence of its antigen. The use of antagonistic antibodies targeting these ICMs or their ligands is not tumor-specific but affects all lymphocytes and may also disrupt the down-regulation of peripheral autoreactive lymphocytes. Therefore, a significant proportion of patients develop immune-related adverse events (irAEs). irAEs may potentially affect all organs including endocrine glands, lungs, skin, intestine, liver and muscles. Several mechanisms have been suggested to explain such irAEs: blocking CTLA-4 on regulatory T lymphocytes (Treg) leads to their depletion, while blocking PD-(L)1 leads to the reactivation of anergic auto-reactive T lymphocytes. Blocking PD-(L)-1 and CTLA4 may also produce pathogenic T cells, may alter B cell production and increase autoantibodies production. A systematic review found that 74% of patients treated with anti-PD(L)1 developed irAEs versus 89% of those treated with anti-CTLA-4 and 90% of those treated with

CONTACT A. Simonaggio audrey.simonaggio@aphp.fr Department of Medical Oncology, Hôpital Européen Georges Pompidou, Institut du Cancer Paris CARPEM, AP-HP. Centre – Université Paris Cité, Paris 75015, France

*These authors should be considered equally as first coauthors.
¤These authors should be considered equally as co-senior authors.

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ICI combination[3]. Most irAEs are mild to moderate (grade 1–2). Severe (grade 3 or 4) irAEs occur in almost 10% of patients receiving anti-PD-(L)1 monotherapy and 40% of those receiving anti-PD-1 plus anti-CTLA-4 combination[4]. According to the European Society for Medical Oncology's Clinical Practice Guidelines on diagnosis, treatment and follow-up, the American Society of Clinical Oncology's Clinical Practice Guidelines and the Society for Immunotherapy of Cancer clinical practice guideline, most grade 2 irAEs will require systemic steroids and the temporary discontinuation of the ICI until a grade≤2 recovery. Grade 3 irAEs require high-dose IV steroids and temporary or permanent discontinuation of the ICI. Some of them require more potent immunosuppressive treatments and may be life-threatening or cause long-term sequelae or death[5–7]. Grade 4 irAEs generally require definitive discontinuation of the ICI[8].

Some clinical or biological factors (including gender, age, smoking, past history, body mass index and biologic parameters) have been suggested for predicting irAEs, but none have been validated for routine use[9]. The search for predictive biomarkers of irAEs is a major challenge in order to avoid treatment-related deaths, improve the quality of life of patients and limit the associated financial costs[10].

There are limited data on the association between pre-existing autoantibodies and the development of irAEs in cancer patients treated with ICI without known autoimmune disease[11,12]. However, measurement of autoimmune disease-related antibodies before the ICI treatment is not recommended in clinical practice because of scarce data. We sought to investigate the association between pre-existing autoantibodies including anti-neutrophil cytoplasmic antibodies (ANCA), anti-nuclear antibodies (ANAs), rheumatoid factor (RF), anti-thyroid peroxidase (TPO) and thyroglobulin (TG) antibodies and the frequency of irAEs.

Our primary objective was to assess the association between pre-existing autoantibodies and occurrence of grade≥2 irAEs. Our secondary objectives were to assess the association between survival outcomes, pre-existing autoantibodies and grade ≥ 2 irAEs.

Patients and methods

Study design and participants

We performed a prospective analysis in a single French Hospital (Hôpital Européen Georges Pompidou, Paris) of consecutive cancer patients treated with ICIs used alone or in combination for advanced solid tumors between May 1, 2015, and July 1, 2021. Eligible patients were part of a prospective biobanking program led at our institution and named Colcheckpoint. All of them had undergone autoantibody testing before ICI initiation. Data were prospectively recorded including patients' clinical and demographic information, laboratory characteristics, tumor characteristics and outcomes at the date of radiological progression determined by the radiologist, date of death or last follow-up. Patients with known autoimmune diseases were not included in the study.

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All suspected adverse events were assessed and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Patients with suspected grade ≥ 2 irAE were referred to our multidisciplinary ICI safety board (TIMEO) to confirm causality. To be retained for this study, the causal relationship of irAE had to be qualified as certain or probable, according to the French causality assessment method[13]. Accordingly, grade 1 irAEs were excluded from this analysis because they did not justify therapeutic intervention. Patients were evaluated for treatment response every 12 weeks by CT-scan by a senior radiologist, using RECIST 1.1.

The study was approved by the local institutional review board and was conducted according to Good Clinical Practice Guidelines and the Declaration of Helsinki. All patients consented to share their clinical and biological data.

Blood sample collection

Blood samples were collected for all eligible patients before ICI initiation to identify the presence of any of the following pre-existing autoantibodies, as part of our routine practice:

- ANCA assessed by indirect immunofluorescence (IIF) on ethanol-fixed granulocytes (Euroimmun) completed, in the case of positive IIF (serum dilution 1/20), by ELISA testing (Euroimmun) for antigenic identification (anti-myeloperoxidase-MPO, and anti-proteinase 3-PR-3 antibodies). Patients were considered positive if ANCA titers were ≥1/20 associated or not with an anti-MPO or anti-PR3 positivity (>20 U/mL).
- ANAs were assessed by indirect immunofluorescence (IIF) on Hep-2 cells (Euroimmun). Patients were considered positive if ANA titers were ≥1/80 a dilution commonly used for the detection of autoantibodies for the diagnosis of systemic autoimmune rheumatic diseases, according to the recommendations from the International Consensus on ANA Patterns (ICAP) group[14]. Antigenic identification was performed when a screening test by ELISA for Extractable Nuclear Antigens (ENAscreen, Orgentec) was positive using an ALBIA technology (11 autoantigens, FIDIS connective, Theradiag) and/or when IIF was positive (serum dilution>1/500), with immunodots (myositis, sclerosis and hepatitis, Euroimmun) according to the positive IIF pattern observed. The positive thresholds recommended by the providers were applied.
- RF was assessed by nephelometry (Siemens). Patients were considered as positive if the levels were≥15 UI/mL.
- Anti-TPO and anti-TG antibodies were tested by ELISA (AESKU). Patients were considered positive if anti-TPO or TG levels were ≥60 and 120 U, respectively.

These autoantibodies were selected because they are widely available from any laboratories and also because they are well known in terms of characteristics and frequency in the population. In addition, anti-TPO and anti-TG autoantibodies are associated with autoimmune thyroiditis, a very frequent irAE.

Outcomes

Primary end point was the occurrence of grade≥2 irAEs. The study also investigated (i) the characteristics of the irAEs (severity, time to onset, occurrence of multiple toxicities, defined as two or more grade ≥ 2 irAEs occurring in the same patient, concomitantly or not) and (ii) the survival outcomes, including progression-free survival (PFS) and overall survival (OS). Progression-free survival (PFS) was defined as the time from the first administration of ICI to progression or death. Overall survival (OS) was defined from the first administration of ICI to death from any cause. Patients were monitored from the start of the treatment until last follow-up or death.

Statistical analysis

Data were extracted from the patients' medical records. Qualitative variables were reported as the frequency (percentage) and quantitative variables as the median. Comparisons were performed by using the X^2 test or Fisher's exact test for qualitative variables and the t-test or Kruskal–Wallis test for quantitative variables. Patients who were alive at the time of analysis were censored at their last follow-up. Progression-free survival (PFS) and overall survival (OS) were evaluated using the Kaplan–Meier method with 95% confidence intervals. Survival outcomes were compared by the log-rank test. For multivariate analysis for survival data, the Cox model was used. All tests were two-sided, and a p value of 0.05 or less was considered as statistically significant. All analyses were performed using R software Version 1.0.136 (Boston, MA).

Results

Patients

Between May 1, 2015, and July 1, 2021, 221 patients were included, of whom 99 (44.8%) had a renal cell carcinoma (RCC), 79 (35.7%) had a non-small-cell lung cancer (NSCLC) and 43 (19.5%) had other solid tumors. The patients' clinical characteristics are summarized in Table 1. One hundred fifty-one (68.3%) were men, the median age was 66.5 years (range 21–90 yrs) and 179 (81%) had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1. Treatment was mostly received in the first- or second-line setting ($n = 178$; 80.5%) and consisted of anti-PD-(L)1 alone in 162 patients (73.3%), anti-PD(L)-1 combined with chemotherapy in 30 patients (13.6%), anti-PD(L)-1 combined with VEGFR-TKI in 16 patients (7.2%) and anti-PD(L)-1 combined with anti-CTLA-4 for 13 patients (5.9%).

Using the defined cutoff for positivity, 129 (58%) out of the 221 patients had pre-existing antibodies distributed as follows: 120 (93%) patients with positive ANA, 11 (8.5%) with positive anti-TPO, 8 (6.2%) with positive anti-TG and 2 (1.6%) with rheumatoid factor (RF) positivity. Ten patients (7.8%) had two or more positive autoantibodies (Table 2).

Table 1. Clinical characteristics of the study population.

	Overall cohort, $N = 221$	Auto-Ab-positive group, $N = 129$	Auto-Ab-negative group, $N = 92$
Male, n (%)	151 (68.3)	80 (62)	71 (77)
Age, median (range)	68.3 (21–90)	68.6 (21–90)	64.4 (32–86)
ECOG-PS, n (%)	179 (81)	103 (79.8)	76 (82.7)
0–1	35 (15.9)	19 (14.7)	16 (17.4)
2–3	7 (3.2)	7 (5.4)	(0
Unknown			
Type of treatment, n (%)			
Anti-PD-(L)1 monotherapy	162 (73.3)	81 (62.8)	81 (88)
nivolumab,	*114 (51 .6)*	*40 (31)*	*74 (80.4)*
pembrolizumab,	*44 (20)*	*37 (28.7)*	*7 (7.6)*
atezolizumab,	*3 (1.3)*	*3 (2.3)*	*0 (0)*
others	*1 (0.4)*	*1 (0.8)*	*0 (0)*
Anti-PD-(L)1- chemotherapy	30 (13.6)	29 (22.5)	1 (1.1)
Anti-PD-(L)1- VEGFR-TKI	16 (7.2)	10 (7.8)	6 (6.5)
Anti-PD-(L)1- Anti CTLA-4	13 (5.9	9 (7)	4 (4.3)
Type of cancer, n (%)			
Renal cell carcinoma	99 (44,8)	34 (26.4)	65 (70,7)
Non-small-cell lung cancer	79 (35.7)	61 (47.2)	18 (19.6)
Mesothelioma	5 (2.3)	4 (3.1)	1 (1.1)
Other lung tumor	5 (2.3)	5 (3.9)	0 (0)
HNSCC	12 (5.4)	6 (4.6)	6 (6.5)
Urothelial cancer	13 (5.9)	12 (9.2)	1 (1.1)
Ovarian cancer	3 (1.4)	3 (1.4)	0 (0)
Other*	5 (2.3)	4 (3.1)	1 (1.1)
Line of treatment, n (%)			
1	90 (40.7)	74 (57)	16 (17.4)
2	88 (39.8)	39 (30.2)	49 (53.3)
3–4 +	42 (19)	16 (14)	26 (28.3)
unknown	1 (0.4)	0 (0)	1 (1.1)

Abbreviations: ccRCC: clear cell renal cell carcinoma; ECOG-PS: Eastern Cooperative Oncology Group- Performance Status); HNSCC: head and neck squamous cell cancer; nccRCC: non clear cell renal cell carcinoma; NSCLC: non-small cell lung cancer.
*other: Uterine cancer (1), Colorectal cancer (1), Esophagus cancer (1), prostate cancer (2).

Table 2. Biological autoimmune characteristics of the study population.

	N (%)
Pre-existing Abs	**129 (58)**
ANA≥1/80	120 (93)
Anti-TPO≥60 Ui/mL	11 (8,5)
Anti-TG ≥ 120 Ui/mL	8 (6,2)
RF ≥ 15Ui/mL	2 (1,5)
ANCA (anti-PR3) ≥ 20Ui/mL	1 (0,4)
2 or more positive Abs	10 (7,8)
Positive	120
ANA pattern	
Speckled	67 (55,8)
Nucleolar	19 (15,8)
Homogeneous	18 (15)
Discrete nucleolar dots	7 (5,8)
Nuclear envelop	4 (3,3)
Pleiomorphic	4 (3,3)
Centrosome	1 (0,8)
ANA Titers>1:500	43
ANA-**specific antigen**	23
SSA 52 Kda	9 (39,1)
Sp100	3 (13)
Anti-DNA	2 (8,7)
Anti-PML	1 (4,3)
PM100	1 (4,3)
ARN POL III	1 (4,3)
RNP	1 (4,3)
MI2	1 (4,3)
Anti-TIF1G	1 (4,3)
SSA 60	1 (4,3)
SSB	1 (4,3)
NXP2	1 (4,3)
2 or more specific antigens	4 (23,5)

Abbreviations: ANA: Anti-Nuclear Antibodies; ANCA: Anti-Neutrophil Cytoplasmic Antibodies; TG: thyroglobulin; TPO: Thyroid Peroxidase; RF: rheumatoid factor.

Table 3. Characteristics of the immune-related adverse events according to pre-existing antibodies.

	Auto-Ab positive group N=129	Auto-Ab negative group N=92	Odds-ratio, (95%CI)	p-value
irAE, n patients-%	64 (50%)	20 (22%)	OR= 3.5 (1.8-6.8)	$p=2.4^{10-5}$
irAE type (n, %)	9	6	OR=1.07 (0.3-3.8	p=1
• skin toxicities	9	1	OR=6.7 (0.9-301)	p=0.049
• hypothyroidism	8	2	OR=3 (0.6-29)	p=0.2
• hyperthyroidism	3	0	OR=nr (0.3-nr)	p=0.2
• hypophysitis	7	2	OR=0.3 (0.5-26)	p=0.3
• colitis	11	4	OR=0.3, (0.6-9)	p=0.2
• hepatitis	5	0	OR=nr (0.7-nr)	p=0.08
• diabetes	3	0	OR=nr (0.3-nr)	p=0.3
• nephrologic events	2	2	OR=0.7 (0.06-10)	p=1
• pneumonitis	3	1	OR=2 (0.2-115)	p-0.6
• myocarditis	5	2	OR=1.8 (0.3-19)	p=0.7
• arthritis	4	2	OR=1.4 (0.2-16)	p=1
• adrenal insufficiency	3	0	OR=nr (0.3-nr)	p=0.2
• neurologic	5	2	OR=1.8(0.3-19.4)	p=0.7
• other events				
Dysthyroidism (hypothyroidism+ hyperthyroidism	17	3	*OR= 4.5 (1.2-29.5)*	p=0.01
irAE grade (n,%)	51	13		*p=0.15*
Grade 2	22	5		
Grade 3	2	2		
Grade 4	0	1		
Grade 5	5	0		
Unknown				
Multi system toxicities (n, %)	12 (9.4%)	2 (2%)	*OR=4.5(0.984-43..2)*	*p=0.047*
Time to irAE onset from ICI initiation, weeks (IQR)	13 (8.8-21.6)	28.5 (10.6-55.1)		*p=0.01*
ICI exposure, weeks (IQR)	25.6 (10-50)	19.2 (8.5-36)		*p=0.95*

Abbreviations: Ab: antibodies; irAEs: immune-related adverse events; ICI: immune checkpoint inhibitor; IQR: interquartile range.

Among patients with positive ANA at the dilution of 1:80, main fluorescence patterns are summarized in Table 2. Specific antigens identified for 23 patients are also summarized in Table 2.

Immune-related adverse events

One hundred and one ≥ grade 2 irAE were experienced by 84 (38%) of the 221 patients, as follows: skin disorders (n = 15, 15%), hypothyroidism (n = 10, 10%), hyperthyroidism (n = 10, 10%), hypophysitis (n = 3, 3%), colitis (n = 9, 9%), hepatitis (n = 15, 15%), autoimmune diabetes (n = 5, 5%), pneumonitis (n = 4, 4%), nephrologic events (n = 3, 3%), myocarditis (n = 4, 4%), arthritis (n = 7, 7%), adrenal insufficiency (n = 6, 6%), neurologic events (n = 3, 3%), other events (n = 7, 7%). Fourteen patients experienced multiple organ involvement. There were 64 grade 2 events (64%), 27 grade 3 events (27%), 4 grade 4 events (4%) and 5 (5%) events of unknown grade. Patients experiencing irAE were mainly male (70%) reflecting the baseline characteristics of the study population. Median age was similar between the two irAEs-positive and irAEs-negative groups. During follow-up, one ccRCC patient receiving nivolumab for a month died from a grade 5 hepatitis.

irAEs occurrence according to pre-existing autoantibodies

Immune checkpoint inhibitor exposure was not different between pre-existing and non-pre-existing antibodies group (p = 0.95) (Table 3). irAEs were statistically more frequent in patients with pre-existing antibodies: 64 patients (50%) in the positive group vs. 20 patients (22%) in the negative group, odds-ratio (OR) = 3.5 (95% CI = 1.8–6.8), $p < 0.001$. irAEs occurred earlier in the positive group with a median time to onset from ICI initiation of 13 weeks (IQR = 8.8–21.6) in the positive group vs. 28.5 weeks (IQR = 10.6–55.1) in the negative group (p = 0.01). Twelve patients (9.4%) experienced multiple toxicities (range: 2–3) in the positive group vs. 2 (2%) in the negative group, OR = 4.5 (95% CI = 0.98–43), p = 0.047. No particular multisystem irAE pattern was identified in these patients. Toxicity grades were not different between the pre-existing and non-pre-existing antibodies group (p = 0.4).

The distribution and the characteristics of irAEs among the two groups of patients are summarized in Figure 1 and Table 3. Dysthyroidism, including both hypo- and hyperthyroidism was the only statistically more common irAE in patients with pre-existing autoantibodies (17/129 vs. 3/92): OR = 4.5 (95% CI = 1.2–29.5), p = 0.01. Among the 17 patients with pre-existing autoantibodies who experienced dysthyroidism ≥ grade 2, 13 (76%) had≥1:80 ANA positivity, 5 (29%) had anti-TPO or anti-TG antibodies positivity and 1 (6%) had both ANA and anti-TPO/TG antibodies positivity. Focusing on the 12 patients with TPO and/or TG antibodies positivity, five (45%) of them developed dysthyroidism ≥ grade 2 (42%) compared to 3 of the remaining 211 patients (1.4%). Hence, pre-existing anti-thyroid antibodies were predictive of future autoimmune dysthyroidism: OR = 27 (95% CI = 4.8–200), $p = 6.10^{-5}$. Excluding dysthyroidism, the occurrence of irAEs remains statistically more frequent among patients with pre-existing antibodies: 50 pts (39%) in the positive group vs 19 pts (20%) in the negative group, OR = 2.4 (1.2–4.8), $p = 0.005$.

Focusing on severe irAEs, 8 (8.7%) out of 92 patients experienced grade ≥ 3 irAE in the negative group vs. 19 (14.7%) out of 129 in the positive group, OR = 0.5 (95% CI = 0.2–1.4), p = 0.2. Grade ≥ 3 irAEs were more frequent for patient receiving anti-PD(L)-1-anti CTLA4 combination or anti-PD1-VEFGR TKI than for those receiving anti-PD(L)1 monotherapy: 17/161 (10.6%) for patients treated with PD(L) 1 monotherapy vs 5/13 (38.5%) [OR = 0.2 (95% CI = 0.05–0.8),

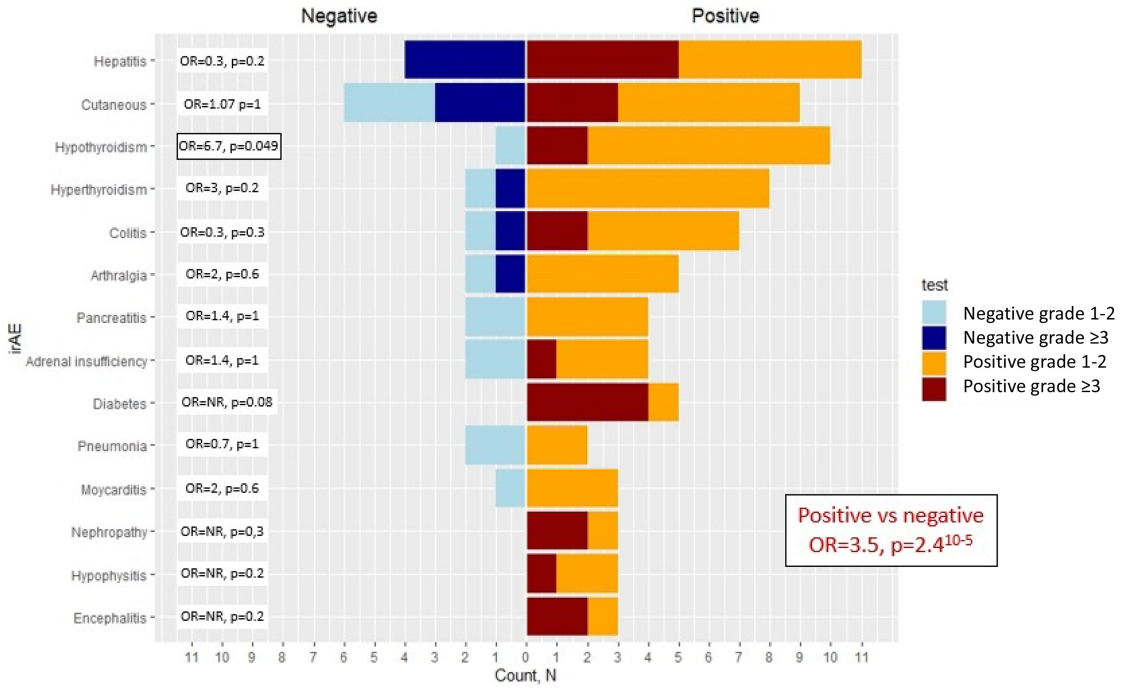

Figure 1. Distribution of the immune-related adverse events.

$p = 0.013$] and vs. 6/16 (37.5%) [OR = 0.2 (95% CI = 0.07–0.8), $p = 0.015$] for patients receiving anti-PDL1 anti-CTLA4 combination or anti-PD1-VEFGR TKI, respectively. Only one patient receiving anti-PD(L)-1 and chemotherapy experienced grade ≥ 3 irAE.

We also observed that the delay before irAE was not statistically different between the different treatments: 23 weeks (IQR = 10–49) for ICI monotherapy, 30 weeks (IQR = 9–101) for anti-PD(L)-1-anti-CTLA4, 25 weeks (IQR = 18–35) for ICI-VEGFR TKI and 26 weeks (IQR = 16–45) for ICI-chemotherapy, t-test >0.05 for all comparisons.

Survival outcomes *according to irAes and pre-existing antibodies*

After a median follow-up of 25 months (95% CI 18.8–31.4) for the overall cohort, median PFS and OS were significantly longer for patients who experienced grade ≥ 2 irAE than for those without irAE: 12.6 months (95% CI = 11–22.7) vs 5 months (95% CI = 4.2–7.0), $p = 0.00034$ (Figure 2A) and 30 months (95% CI = 22.7-NR) vs 21 months (95% CI = 15–34.6), $p = 0.016$ (Figure 2B), respectively. Regarding patients with pre-existing antibodies or not, no association was found with survival outcomes, $p = 0.09$ and $p = 0.66$ for PFS and OS, respectively (Figure 2C-D). In multivariate analyses including age, gender, type of cancer, type of treatment and line of treatment, the occurrence of irAEs remains statistically associated with better survival outcomes (OR = 0.6 [95% CI: 0.4–0.8], $p = 0.012$ for PFS and OR = 0.57 [95% CI: 0,4–0.9], $p = 0.0145$ for OS, respectively).

Sub-group analyses in ccRCC and NSCLC patients receiving anti-PD(L)-1 monotherapy or anti-PD1- anti-CTLA4 combination.

With NSCLC and ccRCC being the two most frequent cancer types in our cohort, subgroup analysis appears pertinent. Thus, we assessed irAEs occurrence according to pre-existing autoantibodies in these two subgroups of patients receiving ICI monotherapy or ICI–ICI combination. ICI-chemotherapy and ICI-VEGFR TKI regimens were excluded from this analysis to homogenize the results. This enables us to focus on a more homogeneous population and to limit bias. One hundred and twenty-six patients were analyzed. irAEs were significantly more frequent in patients with pre-existing autoantibodies (51% vs. 25%, $p = 0.003$). Concordant results were observed in both ccRCC and NSCLC cohorts, when analyzed separately: 50% vs. 29% ($p = 0.06$) in ccRCC and 50% vs. 11% ($p = 0.007$) in NSCLC. irAEs occurred earlier in the positive group with a median time interval between ICI initiation and irAE of 13 weeks vs. 30 weeks in the negative group, $p = 0.05$.

In ccRCC patients, median PFS and OS did not differ according to the presence or absence of pre-existing autoantibodies ($p = 0.21$ and $p = 0.8$, respectively). There was a trend for longer median PFS and OS in the case of irAE, respectively, 11.2 months (95% CI = 5.6-NR) vs. 7 months (95% CI = 4.2–10.4), $p = 0.16$ and 28.2 months (95% CI = 20.9-NR) vs. 19.6 (95% CI = 16.7-NR), $p = 0.16$ (Online only Figure 2). Hazard ratios for PFS and OS according irAEs were 0.9 (95% CI = 0.4–1.8) and 0.9 (95% CI = 0.5–1.8). irAEs occurred numerically earlier in the presence of pre-existing autoantibodies: 13.3 weeks vs. 29.6 ($p = 0.3$).

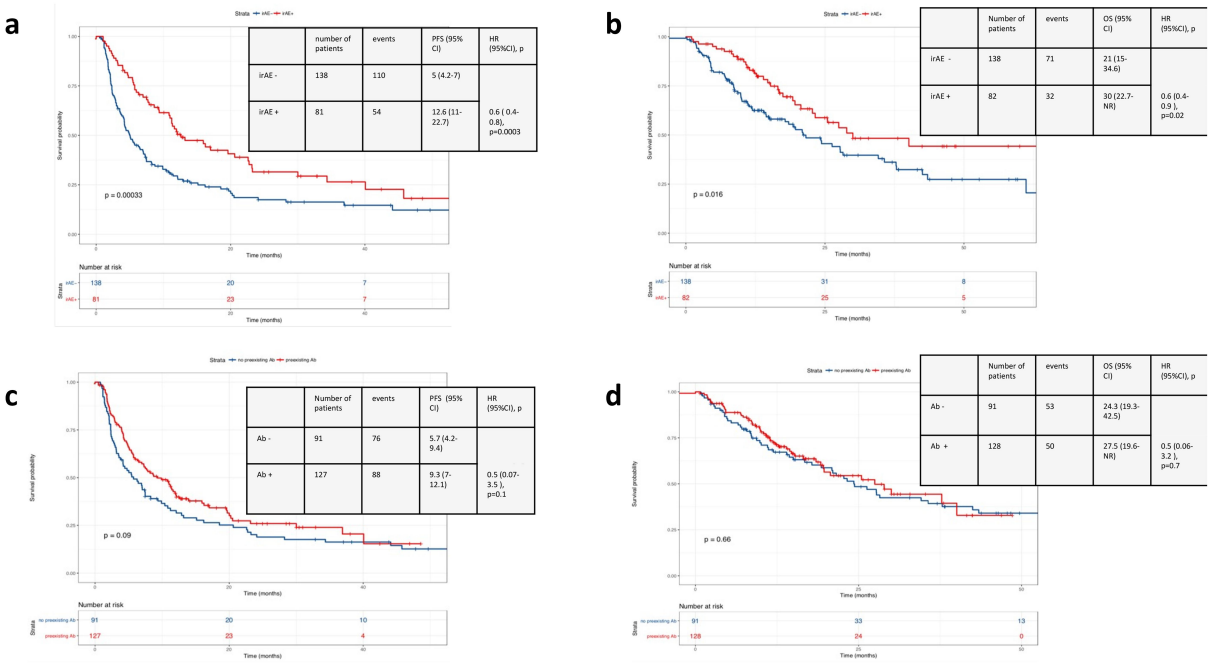

Figure 2. PFS and OS according to irAEs and pre-existing antibodies on the whole cohort.

In NSCLC patients, median PFS and OS did not differ according to the presence or absence of pre-existing autoantibodies ($p = 0.78$ and $p = 0.93$, respectively). Median PFS was statistically longer in the case of irAE: 15.9 months (95% CI = 11-NR) vs. 4 months (95% CI = 2.8-NR), $p = 0.042$, and a trend for longer OS was observed in the case of irAEs: 25.4 months (95% CI = 19.2-NR) vs. 12.8 (95% CI = 9.3–22), $p = 0.12$ (Online only Figure 2). Hazard ratios for PFS and OS according irAEs were 0.5 (95% CI = 0.3–0.98) and 0.5 (95% CI = 0.3–1.1). irAE occurred numerically earlier in the presence of pre-existing autoantibodies: 14.7 weeks vs. 47.3 ($p = 0.2$).

Discussion

To our knowledge, this is the largest prospective cohort study to assess the association between pre-existing antibodies, occurrence of grade ≥ 2 irAEs and survival outcomes for patients having no history of autoimmune disease presenting with advanced solid tumors treated with ICIs, used alone or in combination. We found that patients with pre-existing routinely searched autoantibodies (including ANA, ANCA, RF, anti-TPO or anti-TG antibodies) experienced significantly more irAEs, with a shorter time to onset and more multiorgan toxicities than patients without pre-existing autoantibody. Consistent with our results, Toi et al. reported more irAEs (OR:3.25, $p = 0.001$ in multivariate analysis) and a better prognosis (HR for disease progression or death: 0.53, $p = 0.002$) in NSCLC patients with pre-existing autoantibodies (ANA, RF, anti-TPO, anti-TG) and treated with ICI[11]. Similarly, Giannicola et al. found in 92 NSCLC patients receiving nivolumab that early detection of ANA, ENA, and anti-smooth cell antigens was associated with the risk of irAEs ($p = 0.002$) and survival outcomes (HR: 0.23, $p = 0.004$ for PFS and HR: 0.28, $p = 0.03$ for OS)[15]. Both studies were retrospective, which remains an important limitation with possible selection bias. Labadzhyan et al.[16] prospectively evaluated the impact of endocrine autoimmunity on endocrine-related adverse events (EREA) development and overall survival. Their presence was significantly associated with ERAE ($p < 0.001$) and the presence of ERAE was associated with a longer OS ($p = 0.001$).

We also identified an association between thyroid toxicities and positive specific antibodies before treatment. In particular, pre-existing anti-thyroid antibodies were predictive of future autoimmune dysthyroidism. Similar results were reported by Kobayashi et al.[17] with a significant association between baseline TG or TPO antibodies and destructive thyroiditis (3/4 vs. 3/62, $p = 0.062$), in a cohort of 66 patients treated with nivolumab for solid cancer or Hodgkin lymphoma. Nevertheless, in our study, the difference in frequency of irAEs between the autoantibody-positive and autoantibody-negative groups is not based on autoantibodies and thyroid dysfunction since in a sensitivity analysis excluding those patients with thyroid dysfunction, the difference in irAE frequencies between the two groups remains statistically significant. In contrast, in a prospective analysis, Ghosh et al.[18] tested 60 metastatic melanoma treated with nivolumab (1 mg/kg) + ipilimumab (3 mg/kg) for 120 autoantibodies associated with autoimmune disease. Considering ANA, RF and cyclic citrullinated peptide autoantibodies, there was no significant difference between the seropositive and seronegative patients in irAE development, severity, time of onset, or survival. Of note, patients received a high regimen of ipilimumab (3 mg/kg) which may explain that all of them experienced irAEs, independent of their serologic profile[9]. Similarly, Izawa et al.[12] analyzed 275 Japanese patients treated with ICIs for solid tumors and did not find any significant association between the whole pre-existing autoantibodies (ANA, anti-TG, anti-TPO, anti-GAD, anti-acetylcholinesterase antibodies) and the occurrence of irAEs. However, a significant association was reported between the detection of pre-existing TPO antibodies/TG antibodies and the incidence of thyroid dysfunction ($p < 0.01$). In contrast to our study, the threshold of ANA positivity was 1:40, and all toxicities grade levels (including grade 1) were recorded, which may explain these discrepant results.

Eastern Cooperative Oncology Group PS 2 and long duration of exposure to ICI were previously identified as independent factors associated with the occurrence of multisystem toxicities[19]. In our study, multiple toxicities were significantly higher in the positive group compared to the negative group, but ECOG PS and ICI exposure were not different between these two groups, supporting the impact of pre-existing autoantibodies on the occurrence of multisystem toxicities.

Regarding irAE and survival outcomes, we found a significant improvement in OS and PFS in patients experiencing irAEs. When analyzed separately, a significant improvement in PFS was also observed in NSCLC patients experiencing irAEs. The improvement in OS observed for RCC and NSCLC patients was not significant. This could probably be due to the small sample size of these two populations. A large amount of data is consistent with our finding, confirming that irAEs predict survival and response in solid malignancies treated with ICI[20–22,23,24,25]. In contrast, pre-existing autoantibodies were not associated with survival outcomes, which differs from Toi et al.'s results. Toi et al. reported a significantly better PFS, objective response rate and disease control rate among patients with antibodies, while OS was not significantly improved. These divergent results may be due, first, to the study population: Japanese and mainly female in Toi et al. study versus Caucasian and mainly male in our study. Second, the ANA positivity cutoff was 1:40 in the Japanese study versus 1:80 in our study. Anyway, this question remains controversial since many studies report divergent results. The difference between analyzed autoantibodies and varying cutoff for positive ANA titers makes all comparisons difficult. In addition, our pre-treatment autoimmune exploration was limited to the very routinely used assays. These results support the extension of screening for other antibodies such as those implicated in autoimmune diabetes, myasthenia or other organ-specific autoimmune diseases. Larger prospective study is necessary to answer this question. We recommend to measure autoantibodies prior to ICI initiation to identify patients at higher risk of developing irAE. Isolated pre-existing autoantibodies do not contraindicate ICI initiation but require a closer clinical and biological monitoring.

Our study has several strengths. First, to our knowledge, it is the largest prospective study, which reports the impact of pre-existing antibodies on irAEs during ICI treatment. Second, the causality of suspected irAEs was evaluated by our

multidisciplinary ICI safety board, which limits the bias of evaluation. Third, using routinely researched autoimmune markers, we enable clinicians to easily reproduce our assessment. Our study also has some limitations. First, the monocentric design requires external validation to generalize the results. Second, our cohort was heterogeneous in terms of cancer type and treatment type. Nevertheless, focusing on a more homogeneous population including ccRCC and NSCLC cancer patients receiving ICI monotherapy or ICI-ICI combination, we found concordant results. Third, we do not know the specificity of the detected autoantibodies or the exact mechanism linking their presence to the occurrence of irAEs. A dedicated more in-depth biological study is clearly needed.

Conclusion

Our study confirms the value of testing for autoantibodies prior to the initiation of ICI therapy to identify patients at higher risk of developing irAE. Closer clinical-biological monitoring should be offered to those patients, especially in the first weeks of treatment.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Authors Contribution

A.S., A.D., Y.A.V., M.A.D.D., and C.G. contributed to conception and design; A.S., M.A.D.D., and Y.A.V. contributed to supervision; A.D., A.S., C.G., M.A.D.D., and Y.A.V. contributed to identification of patients; A.S., C.G., and A.D. contributed to acquisition of data; L.P. and A. S. contributed to statistical analysis; A.S., A.D., L.P., S.O., M.A.D.D., Y. A.V. contributed to analysis and interpretation of the data; A.S., A.D., M. A.D.D., and Y.A.V. contributed to drafting of the manuscript; All authors contributed to critical revision of the manuscript: C.G. and M.A.D. D. contributed to technical or material support. The investigators A.D., C.G., M.A.D., and A.S. had access to the raw data. All authors approved the final manuscript. The corresponding author had full access to all the data and takes final responsibility for the manuscript submitted for publication.

Ethics approval and consent to participate

The study was approved by the local institutional review board (CERAPHHP Centre, *Comité d'éthique de la recherche AP-HP Centre*) and was conducted with Good Clinical Practice Guidelines and the Declaration of Helsinki.

All patients gave their oral consent

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Consent for publication

Informed consent for publication has been obtained.

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