Thesis

Venous thromboembolism in patients with cancer treated with immune checkpoint inhibitors

submitted by

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Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 14.02.2024 Franziska Berton m.p.

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Zusammenfassung

Hintergrund: Krebspatient*innen die mit Immun-Checkpoint-Inhibitoren (ICI) behandelt werden, haben ein relevantes Risiko für venöse Thromboembolien (VTE), jedoch sind die klinischen Risikoprofile sind unklar. Das Ziel dieser retrospektiven Kohortenstudie war es, die Häufigkeit, klinische Konsequenzen, sowie Risikofaktoren für VTE während der ICI-Therapie zu identifizieren, um die Entwicklung spezifischer Thromboseprophylaxe-Strategien zu unterstützen.

Methoden: Erwachsene Patient*innen, die mit ICI an der Medizinischen Universität Graz behandelt wurden, wurden in diese Studie eingeschlossen und für das Auftreten einer VTE während der ICI-Therapie nachbeobachtet. Die statistischen Auswertungen des VTE-Risikos wurden mittels competing-risk Analyse durchgeführt, welche das Versterben als Studienendpunkt miteinbezieht. Unterschiede im VTE-Risiko wurden weiters in einem multi-state-model mit der ICI-Exposition als zeitabhängige Variable untersucht. Multivariable Modelle wurden auf potentielle Störfaktoren korrigiert.

Ergebnisse: Insgesamt wurden 417 Patient*innen eingeschlossen (häufigste Tumortypen: 41% nicht-kleinzelliges Lungenkarzinom, 16% Nierenzellkarzinom und 15% Melanom). Über eine mediane Nachbeobachtungszeit von 26.4 Monaten traten 37 VTE auf (kumulative Inzidenz: 12.2%, 95%-Konfidenzintervall [KI]: 8.7-16.4). Das VTE-Risiko war nach dem Beginn der ICI im Vergleich zum Zeitraum von der Krebsdiagnose bis zum ICI-Start erhöht (multivariable transition-hazard-ratio (THR): 3.30, 95% KI: 1.95-5.57). Ähnliche Inzidenzen und keine signifikanten Unterschiede im Risiko wurden entsprechend den Patient*innen-Charakteristika, der Komorbiditäten, dem Krebstyp und -stadium beobachtet. Der Khorana-Score hat das VTE-Risiko nicht stratifiziert (subdistribution-hazard-ratio (SHR) [Score ≥2]: 0.88, 95% KI: 0.45-1.72). Laborparameter zum Zeitpunkt des ICI-Starts, einschließlich C-reaktives Protein (CRP), sagten das VTE-Risiko nicht vorher, jedoch waren frühzeitige Anstiege des CRP (Verdopplung innerhalb von 3 Monaten nach Beginn der ICI) mit einem signifikant höheren VTE-Risiko assoziiert (adjustierte SHR: 2.31, 95% KI: 1.06-5.02), mit einer kumulativen Inzidenz von 22.9% bei Patienten mit einem frühzeitigen CRP-Anstieg.

Conclusio: Es wurde ein klinisch relevantes Risiko für VTE bei Patient*innen, die mit ICI behandelt wurden, beobachtet, gekennzeichnet durch homogen hohe Risiken unabhängig von zugrundeliegenden Patient*innen- und Krebscharakteristika. Longitudinale

inflammatorische Biomarker könnten sich für die Identifizierung von Patient*innen mit hohem VTE-Risiko eignen.

Abstract

Background: Patients with cancer treated with ICI are at substantial VTE-risk, yet clinical risk profiles are currently unclear. Our aim was to determine the incidence, clinical consequences, and risk factors for VTE during ICI-therapy to support the development of future thromboprophylaxis strategies.

Methods: Consecutive adult patients treated with ICI at the Medical University of Graz were included in this retrospective cohort study and followed for the occurrence of VTE during ICI-therapy. Statistical analyses were conducted in competing risk analysis, accounting for all-cause mortality as competing event. Risk of VTE according to time dependent exposure to ICI after cancer diagnosis was analysed in a multi-state model. Multivariable adjustment of models was conducted to account for potential confounders.

Results: Overall, 417 patients were included [non-small cell lung cancer (41%), renal cell carcinoma (16%) and melanoma (15%)]. Over a median follow-up of 26.4 months, 37 VTE occurred [cumulative incidence: 12.2% (95% confidence interval [CI]: 8.7-16.4)]. VTE-risk was increased after ICI-initiation compared to the period from cancer-diagnosis to ICI-start (transition-hazard-ratio (HR): 3.30, 95%CI: 1.95-5.57). Similar incidences and no significant differences in risk were observed according to patient demographics, comorbidity burden, cancer-type and -stage. The Khorana-score did not predict VTE-risk (subdistribution-HR [score ≥2]: 0.88, 95%CI: 0.45-1.72). Baseline levels of routine laboratory parameters including C-reactive protein (CRP) did not predict VTE-risk, yet early increases in CRP (2-fold increase within 3 months of ICI-initiation) were associated with a significantly higher VTE-risk (adjusted subdistribution-HR: 2.31, 95%CI: 1.06-5.02), with a cumulative incidence of 22.9% in patients with an early CRP-rise.

Conclusion: A substantial burden of VTE among ICI-treated patients was observed, characterised by homogenously high risks irrespective of underlying patient- and cancer-characteristics. Longitudinal trajectories of inflammatory biomarkers might identify patients at very high VTE risk.

Publications and presentations linked to this thesis

Full publication: *Early Dynamics of C-reactive Protein for the Prediction of Venous Thromboembolism in Patients with Cancer Treated with Immune Checkpoint Inhibitors*. Florian Moik, Jakob M. Riedl, Dominik Barth, Franziska Berton, Michael Fink, Cornelia Englisch, Christoph Hoeller, Thorsten Fuereder, Leyla Ay, Ingrid Pabinger, Philipp J. Jost, Armin Gerger, Angelika Terbuch, Matthias Preusser, Cihan Ay. (Under peer review)

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Poster presentation 04/2024: *Pattern of venous thromboembolism (VTE) in patients with cancer treated with immune checkpoint inhibitors (ICI).* Franziska Berton, Michael Fink, Sarah Kostmann, Dominik Barth, Lukas Koch, Nikolaus John, Robert Wurm, Christoph Wohlkönig, Erika Richtig, Philipp J. Jost, Armin Gerger, Angelika Terbuch, Jakob M. Riedl, Florian Moik. – Accepted for poster presentation at "Frühjahrstagung der österreichischen Gesellschaft für Hämatologie und Onkologie (ÖGHO) 2024", Vienna, Austria.

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Abbreviation Index

AEs *adverse events*

AKI *acute kidney injury*

APC *antigen-presenting cell*

ATE arterial thromboembolism

BMI *Body Mass Index*

CAT *cancer-associated thromboembolism*

CI *confidence interval*, *confidence interval*

CPS *combined positive score*

CRP *C-reactive protein*, *C-reaktives Protein*

CT *computer tomography*

CTLA-4 *cytotoxic T-lymphocyte antigen 4*

CTVPA *computed tomography venography and pulmonary angiography*

CV *cardiovascular*

DC *dendritic cell*

DVT *deep-vein thrombosis*

e.g *exempli gratia; for example*

ECG *electrocardiography*

ESC *European Society of Cardiology*

GI *gastrointestinal*

HF *heart failure*

i.v. *intravenous*

ICI *immune checkpoint inhibitors*, *immune checkpoint inhibitor*

IFN-γ *interferon-gamma*

IL-2 *Interleukin-2*

IQR *interquartile range*

irAE *Immune related adverse events*

iRECIST *immunotherapy-specific response evaluation criteria in solid tumors*

KI *Konfidenzintervall*

mAb *monoclonal antibodies*

MHC1 *major histocompatibility complex 1*

MI *myocardial infarction*

NK *natural killer*

NSAIDs *non-steroidal anti-inflammatory drugs*

NSCLC non-small-cell lung cancer

NT-proBNP *N-terminal pro-B-type natriuretic peptide*

OS *overall survival*

PD1 *programmed-death-1*

PD-L1 *programmed-death-ligand 1*

PD-L2 *programmed-death-ligand 2*

PE *pulmonary embolism*

QTc *corrected QT interval*

RCT *randomized controlled trials*

SHR *subdistribution-hazard-ratio*

TCR *T-cell-receptor*

THR *transition-hazard-ratio*

TNF-α *tumor necrosis factor-alpha*

Tregs *Regulatory T cells*

TTE *transthoracic echocardiography*

VEGF vascular endothelial growth factor

VTE *venous thromboembolism*

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Introduction

Immunoncology using immune checkpoint inhibitors (ICI) is an emerging method in cancer therapy. The first connection between immunology and oncology has been linked in the 19th century by surgeon William B. Coley, but it was a long way to achieve a therapeutic benefit in activating the immune system to fight cancer. (1,2) In fact, there have been observations of spontaneous tumor regression hundreds to thousands years ago, but the cause was never found until a review of some hundred case reports, which show a coincidence with tumor regression and acute infections. (3)
Anecdotally, W. Coley was one of the first who reported a potential association between acute infections and tumor regression. Coley was a young surgeon in New York and after losing his first patient because of cancer, he started to search for a new approach. He searched through the hospital records and found a patient with an egg-sized sarcoma on his cheek. The wound of the patient could not be closed after the surgical remove and skin crafts did not work. So, the patient had an open wound, the tumor progressed, and another operation was performed, but they couldn`t remove the tumor completely. Thereafter, the wound became infected with Streptococcus pyogens and the patient got high fever. The doctors couldn`t do much against the infection, but after each fever attack the tumor regressed and finally disappeared completely. Coley started to search for the patient and found him completely tumor-free. (4)
So Coley observed that cancer patients, who had a bacterial infection with fever attacks, experienced tumor regression too. He injected cultures of heat-inactivated bacteria or bacterial culture supernatants into cancer patients and some had a tumor regression and a prolonged survival after the treatment. (5) W. Coleys experiment created the basis for further research on the interplay between cancer and physiology anti-tumoral immunity, consequently fostering the development of cancer immunotherapy.

Conceptually, to achieve a successful antitumor response, the antigen presentation of tumoral epitopes has to be effective at two key points: First, dendritic cells (DCs) have to take up cancer antigens and present them for $CD8^+$ T-cell priming. Second, the tumor has to present these antigens directly, so the primed $CD8^+$ T cells can recognize the tumor and enact its cytotoxic properties. (6)

As a key prooncogenic mechanism, cancers acquire mechanisms allowing the escape of physiologic anti-tumoral immune-surveillance via a variety of mechanisms. (7) In part, immune-evasion is facilitated by different mutations and linked to effective $CD8^+$ T-cell response. (7, 8) For example, tumors can downregulate the antigen processing machinery, mostly major histocompatibility complex 1 (MHC1) pathway and thereby inhibit the presentation of tumor antigen presentation. (8) Deciphering the exact molecular mechanisms underlying these processes have been a long a process, which recently helped identify immune checkpoints abused by cancer cells to evade adaptive anti-cancer immune cells. (8)

1 Immunomodulatory pathways in cancer

Different immune checkpoints have been identified in the past that regulate the physiologic threshold of self-acceptance and prevent auto-immunity under physiologic circumstances. (9) These pathways are misused by cancer cells to evade immune cells. (8, 9) Mainly, two pathways have been identified to be involved in this process. First, a protein receptor on the surface of T-cells has been identified, which is called cytotoxic T-lymphocyte antigen 4 (CTLA-4). CTLA-4 inhibits the immune system by binding to B7 molecules on the antigen-presenting cell (APC), thereby preventing immune activation. (9)
Secondly, programmed-death-1 (PD1) is a costimulatory receptor on the T-cell. PD1 inhibits T-cells proliferation, if there is a binding to programmed-death-ligand 1 (PD-L1) and programmed-death-ligand 2 (PD-L2). (10)
These two pathways represent the main targets for current cancer immunotherapy. (2,11)

1.1.1 CTLA-4 pathway

Activation of a T-cell requires multiple stimulatory signals. The T-cell-receptor (TCR) on the T-cell binds to the MHC on the APC. This ensures specificity to T-cell activation. Furthermore, there are some other costimulatory signals needed. (10) T-cells also have the surface protein CD28, which binds to molecules B7-1 or B7-2 on the APC. (12,13) If the level of the CD28:B7 binding is sufficient, it leads to proliferation of T-cells, increased T-cell survival and differentiation, Interleukin-2 (IL-2) production, increased energy metabolism and upregulation of cell survival genes. (10,12)
Collaterally, there is CTLA-4, which is a homologous receptor to CD28, but mediates opposing functions in T-cell activation. (12) Both are ligands to the B7 molecules on the APC. CD28 has a high affinity to B7-1 and a lower affinity to B7-2. (12,13)
CTLA-4 interacting with the B7 molecules provides an inhibition to the T-cell response. CTLA-4 interacts with both ligands, B7-1 and B7-2, with higher affinity and activity than CD28. (10,13,14)
CTLA-4 is located intracellular in the naive T-cells. Stimulatory signals from TCR and CD28:B7 binding induce upregulation of CTLA-4 on the cell surface by exocytosis. (10) This process works in a graded feedback loop, in which a weak TCR signal prevents CTLA-4 expression and a strong TCR signal upregulates CTLA-4 expression. (10) By

upregulating the CTLA-4 expression the T-cell response is inhibited. In this case the IL-2 production, proliferation and survival is being reduced. (10,15)
CTLA-4 is also expressed on the Regulatory T cells (Tregs), which are important in maintaining peripheral tolerance. (10,16)

1.1.2 PD-1 pathway

The PD1 receptor and its ligands were discovered and further explored in the late 20th century. PD1 is a receptor, which can be present on T cells, B cells, natural killer (NK) T cells, activated monocytes and dendritic cells. The PD1:PDL 1&2 pathway is a costimulatory signal as the above mentioned CTLA4:B7 pathway. (11)
PD-1 belongs to the B7:CD28 family of costimulatory receptors and normally the TCR would bind to the MHC on the tumor cell and activate the T-cell, but when the ligands PD-L1 and PD-L2 bind to the PD1 receptor, the function is similar to the CTLA-4 pathway. It inhibits the T-cell proliferation, it downregulates the production of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and IL-2 production and the T-cell survival is turned down. (10,17) PD-1 is expressed on "exhausted" T-cells, so these are T-cells which experienced a high level of stimulation or a lower $CD4^+$ T cell help. An exhausted T-cell is found during chronic infections or during cancer and is characterized by T-cell dysfunction, which results in a deficient control of infections and cancer. (9,16)

2 Immune checkpoint inhibitors (ICIs)

Targeting immune-modulatory pathways has been enabled by the development of different monoclonal antibodies (mAb) directed against molecules involved in immune checkpoints (e.g,, CTLA4, PD1-PDL1-axis). These developments have led to the clinical approval of ICI for a variety of cancer types. An antibody against the CTLA-4 protein was approved first, followed by monoclonal antibodies directed at PD-1 on T cells and the lastly against its ligand PD-L1. (19) **Table 1** provides an overview of the approval history of ICI used in cancer therapy. In the following years ICIs were increasingly approved and are now broadly used. For example, against Renal cell carcinoma, Cervical carcinoma, Primary mediastinal large B-cell lymphoma, Gastric cancer, MSI-H cancers (non-colorectal & colorectal), Hepatocellular carcinoma, Head and neck squamous cell carcinoma and Hodgkin lymphoma. (28)

Table 1: ICI used in clinical practice

Target	Agent	Approvals
CTLA-4 antibody	Ipilimumab	first FDA-approval in 2011 for metastatic melanoma (20)
PD-1 antibodies	Nivolumab	first FDA-approval in 2014 for metastatic or unrespectable melanoma (21)
	Pembrolizumab	first FDA-approval in 2014 for metastatic or unrespectable melanoma (22)
	Cemiplimab	first FDA-approval in 2018 for for the treatment of metastatic cutaneous squamous cell carcinoma (23)
	Dostarlimab	first FDA-approval in 2021 for or the treatment of cancer with mismatch repair deficient recurrent or advanced endometrial cancer (24)
PD-L1 antibodies	Atezolizumab	first FDA-approval in 2016 for second line urothelial carcinoma and second line non-small-cell lung cancer (NSCLC). (25)
	Durvalumab	first FDA-approval in 2017 for locally advanced or metastatic urothelial carcinoma (26)
	Avelumab	first FDA-approval in 2017 for Merkell cell carcinoma (27)

Immune related adverse events (irAE)

2.1.1 Mechanisms and pathophysiology

Immune checkpoints are particularly responsible for humans' immune tolerance and to avoid immune associated overreactions under physiologic conditions. They are a main regulator in the peripheral immune tolerance and control the immune system, thereby preventing autoimmunity. (15,17)

Immunotherapy with mAb against CTLA-4, PD1 and its ligand PD-L1 inhibits the immune checkpoints and thereby increase the activity of the immune system to fight the cancer cells. However, by increasing the activity of the immune system against the cancer, the activity against healthy and normal working cells increases too and this can lead to irAEs. (29,30)

Beyond its antitumoral effect, ICI lead to T cells activation in different tissues, leading to a variety of immune related side effects. (29–31) The most common manifestations of irAEs involve the skin, gastrointestinal tract, muscles, joints, liver, lungs and endocrine organs, but irAEs can occur in almost every organ and organ system, mimicking known autoimmune diseases. (31)

The mechanism for every single irAEs is not clarified yet, but there are seen T cells and histiocytes in the target tissue and there is also a presumption that cross-reactive T cells (bind to the tumor and irAE affected tissue), B cells and antibodies, complement and cytokines and chemokines might play a role. (31,32)

It has shown that the irAEs occur with all tumor types and that the adverse events (AEs) depend on the ICI which is used and not which tumor is targeted. (32)

The grading for irAEs goes from 1 to 5 and is exactly and officially defined in the CTCAE 5.0. (33)

2.1.2 Incidences and timing of irAEs

The reported incidence of irAEs varies depending on the type of ICI agent, combination ICI-treatment, and dose. Data out of systematic reviews and metanalyses of randomized controlled trials allow an estimation of the absolute risk of irAE in treated patients. (31) Immune related AE incidence associated with anti-CTLA-4 treatment was 72% for all grades and 24% for high-grade (3 and higher) AEs. Of those, treatment related mortality due to irAE is less than 1%. (34) Further, patients who received anti-PD1 or anti-PD-L1 have an incidence of 74% in all grades and 14% in high grade irAE. (35) Another systematic review and metanalysis reported higher incidences for combined anti-PD1/PD-L1 plus anti-CTLA4 therapy. All-grade incidence was 88% and 41% for high-grade AEs. (36)

One large-scale metanalysis also analyzed which AE occur more often with the different therapy regimes. Anti-PD1/PD-L1 have higher rates of hypo- and hyperthyroidism and pneumonitis, while colitis and hypophysitis were more common with anti-CTLA4 treatment. (35) Comparing the combined therapy with the monotherapies colitis and hypothyroidism was more often. (35)

Another recent systematic review and metanalysis pointed out that the most frequent adverse events in all grades are fatigue (18%), pruritus (11%) and diarrhea (10%). (37)

One analysis took pooled data from three trials, which treated with combined therapy and looked at the temporal kinetics of irAE. The median time for grade 3 or 4 AE was the fastest for skin toxicities at 3 weeks of onset after ICI-initiation, followed by gastrointestinal (GI) at 7 weeks, then liver at 8 weeks, lungs with 9 weeks, endocrine organs at 11 weeks and kidney at 16 weeks. (38)

Another pooled analysis, which treated melanoma patient with anti-CTLA4, showed similar results. Skin toxicities started typically after two to three weeks, GI and hepatic AEs started after six to seven weeks and AEs related to the endocrine organs started meanly after 9 weeks. (39) The high-grade AEs (grade 3 and 4) resolve in under 5 weeks, whereby endocrine events usually need prolonged hormone replacement. (38) Some of the irAEs, like inflammatory arthritis, persist even after ICI cessation. (31,40)

2.1.3 Immune related skin toxicities

Skin toxicities occur as one of the most frequent AEs by patients treated with mAb and appear mainly earlier than the other AEs. (41–43) The most frequent are skin rashes, Pruritus and Vitiligo. (30,43,44) The incidence of all grade rash with PD-1 antibody varies between 14% – 40%, depending on the used therapeutic agent. (50, 52) The incidence of all-grade pruritus with Nivolumab is ~13% and with Pembrolizumab ~20% for all tumor types. (50,52)

If a grade 3 or higher skin adverse event occurs, the ICI treatment should be interrupted immediately until it goes back to grade 1 or 2. A dermatologist should be consulted and checkups should be done. (30)

2.1.4 Immune related endocrinopathies

As hypophysitis and thyroid disorder occur more often, type 1 diabetes and adrenalitis or adrenal insufficiency are rare. Combination therapy has a higher incidence of immune related endocrinopathies than monotherapies. (46–48)

The numbers vary between the different immune related endocrinopathies, but summed up a endocrinopathy occurs in around 5-6%. (34)

A laboratory and clinically relevant hypothyroidism should be treated anyway. Beta-adrenoreceptor blockers, glucocorticoids and anti-thyroid drugs can be admired. (47) For immune related diabetes the treatment is obligatory with insulin. (47) For adrenalitis a high-dose glucocorticoid therapy should be given initially. (47)

2.1.5 Immune related hepatotoxicity

Immune-mediated liver injury clinically presents as a hepatitis or cholangitis with symptoms like fatigue, fever, nausea, and jaundice. Laboratory-chemical findings show the manifestation grade by serum liver biochemical indicators and bilirubin. (49–51)

The occurrence for an immune-mediated acute hepatitis is rare (~3,5%) and mostly not severe. (50,52) The incidences for immunomonotherapy have a range between 1-5%, for combination therapy (~18%). (51,53)

Mainly a grade ≥3 liver AE should be discontinued, a consultation by a hepatologist and a liver biopsy should be considered. Starting a corticosteroid therapy is recommended for grade ≥2. (51,53)

2.1.6 Immune related GI toxicities

Diarrhea and Colitis are one of the most reported irAE. The incidence for monotherapy is ~36%. For combined therapy the incidence is ~44%. (35,54) The incidence for colitis is ~15%, synoptical ~1/3 of the diarrhea patients suffer from colitis. (38,54)
A patient with persistent or severe diarrhea should get discontinued with ICI treatment and receive systemic corticosteroids. If a patient does not respond after three to five days, corticosteroids should be switched to a one-dose immunosuppressant (e.g. Infliximab). (30)
Immune related pancreatitis is a rare and complicated irAE. The incidence range is between ~0.5-2%. (55)
An immune related pancreatitis should be recognized (as every irAE) early. High-dose corticosteroids are indicated and tapering them should go really slow, up to some months. (56)

2.1.7 Immune related kidney toxicities

Immune related kidney toxicities mostly present as acute kidney injury (AKI) and are laboratory-chemical measured by an increase in creatinine. Electrolyte disorders can also occur and can be various (hypo-/hypernatremia, hyper-/hypocalcemia, hypo-/hyperkalemia, hypo-/hypermagnesemia, hypo-hyperphosphatemia). (57,58)
The incidence for immune mediated AKI is ~3-4%. A lot more patients experience an AKI during receiving ICI (~17%), but not verified linked to ICI. (58,59)
Patients with a grade 3 or higher AKI should be discontinued. Systemic corticosteroids are often used for the treatment (grade ≥2). (60,61)

2.1.8 Immune related pneumonitis

Immune related pneumonitis is a rare incidence, but it can be lethal, if it does not get treated immediately. Pneumonitis presents in distinct patterns and needs to get diagnosed with a computer tomography (CT). (62,63) The most frequent symptoms are cough and dyspnea. (64)

The incidences of immune related pneumonitis vary between 3.5% to 19%, depending on the tumor type and which ICI treatment is being used. (62)

If the diagnosis is confirmed ICI treatment should be delayed and corticosteroids (oral or intravenous) should be started. If there is no improvement after two days, immunosuppressive treatments (e.g. Infliximab) should be taken into consideration. (30)

2.1.9 Rare immune related toxicities

Neurological toxicity

Neurological adverse events include hypophysitis/hypopituitarism, myasthenia gravis, encephalitis/ myelitis, meningitis, Guillain-Barre syndrome, vasculitis, and neuropathy. (65) Overall ~7% ICI patients experience a neurological AE. Hypophysitis/hypopituitarism can be count into to endocrine toxicities, so taken out this the incidence of neurological AEs goes down on ~5%. (65)

For the AE treatment a neurologist should be consulted and a lumbar punction can help for the right diagnosis. ICI treatment should be stopped until the origin of the AE is found. Steroids can be administered. (30)

Rheumatological toxicity

There are some rheumatological toxicities which can occur, like arthralgias, myalgias, vasculitis, myositis, polymyositis and temporal arteritis. (66,67)

Paracetamol or/and non-steroidal anti-inflammatory drugs (NSAIDs) can be given against mild and moderate symptoms, corticosteroids can be given additionally. For severe symptoms a rheumatologist should be consulted and TNFα-blocker are indicated. (30)

Ocular toxicities

Ocular toxicities occur under ICI treatment in ~1%. They appear as uveitis, iritis, conjunctivitis, dry eyes, neovascularization and retinopathies. (66,68)
The treatment depends on the type and severity of the toxicity. Topical and systemic corticosteroids and against neovascularization intravitreal anti-vascular endothelial growth factor (VEGF) can be given. (30)

Hematological toxicities

Hematological toxicities are infrequently reported under ICI therapy. There are case reports where lethal aplastic anemia, immune thrombocytopenic purpura and autoimmune hematolytic anemia have been described. (69–71) An appropriate treatment is not known, but a hematologist should be consulted and corticosteroids and other immunosuppressive drugs can be initiated. (30,69–71)

2.1.10 Proinflammatory effect of ICI

There are studies, which show a proinflammatory effect caused by ICIs. One study (Stanford, USA) was performed in the context of myocarditis caused by ICI treatment. They found out that cytotoxic CD8+ T cells were increased in the peripheral blood in patients with ICI myocarditis. Also, CD8+ T cells exhibit increased expression of heart-tropic chemokines. (72)
Another study (Leuven, Belgium) was performed in the context of pneumonitis and ICI treatment. They found out that patients with an ICI pneumonitis have increased levels of CD8+ and CD4+ T cells in the bronchoalveolar lavage fluid. Also, levels of cytokines and cytotoxic genes were increased. (73)
There is not much data, and the causal connection is not proven, but observations were made that there is a correlation.

3 ICI-related cardiovascular (CV) toxicities

Compared to the above mentioned irAE, the characterization of cardiovascular toxicity including inflammatory, thromboembolic, atherothrombotic or rhythmologic adverse events under ICI is less clear.

Direct inflammatory cardiac toxicities are reportedly rare (<1%) but represent a serious and potentially life-threating complication in treated patients. Cardiac irAE can manifest as myocarditis, endocarditis, pericarditis, cardiomyopathy, arrythmias and impaired ventricular function and during the treatment with pembrolizumab, nivolumab, ipilimumab and combination therapy. (30,66,74,75)

The European Society of Cardiology (ESC) firstly published in 2022 guidelines on cardio-oncology. (76) The ESC defines cancer therapy-related cardiovascular toxicities as following: cardiomyopathy and heart failure (HF), myocarditis, vascular toxicities, hypertension, cardiac arrhythmias, and corrected QT interval (QTc) prolongation. (76)

Typically, myocarditis, myopericarditis, cardiac dysfunction, arrhythmias or myocardial infarction (MI) occur under the treatment with ICI. (76–78)

The onset of symptoms for a myocarditis has a median of 30 days after ICI treatment, up to 50% of the patients died. (76,79) Late onset cardiovascular events (>90 days) include non-inflammatory HF, progressive atherosclerosis and hypertension. (76,80)

The patients baseline conditions influence the risk of an ICI-associated CV event. A double ICI therapy (e.g., Ipilimumab + Nivolumab), combination of ICI with another cardiotoxic therapy, CV events in the medical history and a non-CV irAEs rise the risk of getting an ir-cardiovascular AE. (76,81,82)

The ESC recommends a cardiac check-up before starting an ICI-treatment. This includes a cardiovascular assessment (physical examination, blood pressure measurement, lipid profile, and HbA1c), an electrocardiography (ECG), Troponin-T &
N-terminal pro-B-type natriuretic peptide (NT-proBNP) in the laboratory and for certain patients a transthoracic echocardiography (TTE). Anyhow, cardiovascular check-ups should be done regularly during the treatment with ICI. (76)

Conclusively, the management of ICI related CV events depends on the occurring symptoms and event. Firstly, the severity has to be defined (fulminant vs. not-fulminant) and ICI should be stopped. Depending on the severity immunosuppressive treatment (e.g., methylprednisolone intravenous (i.v.)) should be applied initially. For the following

treatment and management a cardiologist should be involved and a symptomatically therapy, depending on the type of the CV event, should be implemented. (30,76,81) In contrast, currently, little data is available regarding the risk of VTE associated with ICI therapy.

4 Cancer-associated venous thromboembolism (VTE)

Venous thromboembolism includes deep-vein thrombosis (DVT) and pulmonary embolism (PE). In the general population, VTE represents the third most common cardiovascular disorder following myocardial infarction and stroke. (83)
Cancer is an independent risk factor for developing VTE. Population-based studies indicate that VTE occurs in ~3% cancer patients. (84) Cancer patients have a 9-fold higher risk of a symptomatic VTE than the general population. (84–88)

4.1.1 Risk factors and biomarkers

Risk of cancer-associated VTE is highly heterogeneous based on individual risk profiles. Different patient-related factors, cancer-related factors, treatment-related factors, and biomarkers have been identified in the past which are associated with subsequent risk of VTE.

The most significant patient-related risk factor for VTE in the general population is advanced age. (86) In cancer patients age is not identified as a risk factor for VTE. In contrast. medical comorbidities, a higher Body Mass Index (BMI) and a thrombotic event in the medical history are increasing the VTE risk in cancer patients. (86,87,89,90)
The individual genetic profile has an impact on VTE, which was shown in different studies. The widespread Factor V Leiden mutation raises the risk for VTE in cancer patients. Gran et al. has summarized other genetic parameters and mutations, which have an impact on developing a VTE in cancer patients. (91,92)

Tumor-related risk factors are composed of primary site, grade, and cancer stage. (93) Various cancer types have different incidences of VTE. Pancreatic cancer, gastric cancer and brain cancer are found amongst the cancer types with the highest VTE incidences. (87,89,94) Patients with a metastatic-cancer have a higher risk for VTE than patients

diagnosed with localized cancers. (94) Further, patients with a histological high-grade tumor have higher incidences of cancer-associated thromboembolism (CAT). (93) In additional, various biomarkers were identified for the prediction of cancer-associated VTE, including plasma levels of soluble P-selectin, prothrombin fragment 1+2, D-Dimer, platelet counts, and various others. (95)

Lasty, cancer-specific therapies increases the risk of VTE. (86) For example, especially platin-based chemotherapy is commonly associated with VTE. (96,97) Besides chemotherapeutics, hormonal therapies can influence the thrombotic risk. Tamoxifen, a selective estrogen modulator, causes higher VTE incidences in patients undergoing adjuvant therapy. (98)

Recently targeted cancer therapies are increasingly used in clinical practice. For some agent groups, a prothrombotic effect has been demonstrated. Particularly, mAb targeting VEGF (Bevacizumab) has been demonstrated as prothrombotic, as shown in a meta-analysis. (99) Contradictory results have been shown in another meta-analysis, where no elevated risk for VTE with VEGF treatment was found. (100) Nevertheless, the first clinical testing of VEGF receptor inhibitor have shown a prothrombotic event. (99)

Supportive drugs, such as corticosteroids or erythropoiesis stimulating agents, also increase the risk of CAT. Radiation therapy and surgery are also reported to increase the VTE incidences. (87,89,101–104)

For ICI an increased risk of VTE has not been established, but recently published retrospective analyses of clinical cohorts have shown high VTE rates (10-30 %). Currently, it is unclear whether this risk reflects the underlying prothrombotic risk profile of treated patients, or whether ICI exhibit a distinct prothrombotic effect. (105,106)

5 ICI-associated venous thromboembolism

ICIs are increasingly used in the treatment of cancer. (28) Therefore, an enhanced understanding of the associated risk of VTE is of utmost importance. Currently, there is only limited data available about the correlation between ICI treatment and the risk of a VTE.

A retrospective study in Boston, USA showed an increase of the VTE rate after the ICI start. In numbers, the VTE risk after 6 months was 7,4% and after 1 year 13.8%. (77)

Another prospective real-world study about the clinical impact of VTE in NSCLC patients receiving ICI was lately published. They had two cohorts: One using combined computed tomography venography and pulmonary angiography (CTVPA) for screening patients (cohort A) and the other one was a retrospective multicenter control cohort without additional CTVPA screening (cohort B). The VTE rate in cohort A was much higher than in cohort B. They also showed a significant impairment on the overall survival (OS). (107)

There is a causal relationship between ICI and VTE, which needs to be more explored. Furthermore, more screenings should be done to detect VTEs earlier. (107)

Interestingly, a retrospective, multicenter study with 210 patients was performed with patients with bladder and kidney cancer and there was no correlation found between ICI treatment and developing VTE or arterial thromboembolism (ATE). (108) Recently, a retrospective, single-center study was published, which aimed to describe the clinical characteristics and outcome of patient with an ICI treatment. Unsurprisingly, they pointed out a risk between ICI and the VTE risk. For combined ICI the risk for a VTE is even higher. (109)

Moik et al. summed up cohort studies focusing on VTE linked to ICI and the numbers between the different studies were quite similar. (110) Cumulative incidences (12-month risk) between 10.9% up to 14.8% were reported. (77, 107–115)

The incidence of VTE in the above cited studies varies substantially, yet important differences exist in the design and reporting of VTE in the studies. Cohort studies report much higher VTE-rates compared to randomized controlled trials (RCT). Clinical trials with a focus on the efficacy, effect, and the general risk profile often only reported VTE as AE if it occurred as grade 3 or higher and commonly apply a frequency threshold for the reporting of adverse events. In contrast, cohort studies reporting on patients treated in

clinical practice oftentimes encompass patients with that might be at higher risk of VTE based on performance status and comorbidities. Here, a consistent risk of 10-20% of VTE was reported in ICI treated patients (107–115).

Approximately thirty years ago it was shown that cytokines influence thrombosis formation. (116) Seven years later a preclinical and clinical review confirmed the prothrombotic effect of cytokines. (117) Synoptical, previous studies found that there is an impact between ICI and cytokine release. As already mentioned above, ICI have a proinflammatory effect. (72,73) On the other hand, there is a correlation between cytokines, released by T cells, and thrombosis. (116,117)

Regarding risk factors for VTE in ICI treated patients, only limited data exist. The Khorana score is a validated tool that classifies ambulatory chemotherapy-treated cancer patients into risk groups for VTE. The Khorana score performs well for the risk stratification for patient treated with chemotherapy. (90) In contrast, different studies reported a poor performance of the score in the setting of ICI therapy. A study in Israel performed with NSCLS showed that the Khorana score could not identify VTE high-risk patients treated with ICI. (113) So a risk score adjusted to ICI patient is needed.

In summary, the risk of VTE in patients treated with ICI in clinical practice is high. This stands in contrast to a lack of reporting of VTE in the pivotal clinical trials evaluating ICI. Currently, a causal relationship between ICI and VTE has not been established, yet underlying inflammatory pathways due to ICI-associated systemic inflammation might conceptually lead to hypercoagulability. Further, the risk profiles for VTE in ICI-treated patients are currently unclear, and VTE risk stratification models developed in the pre-immunotherapy era have been demonstrated to underperform in cohorts of patients treated with ICI. Therefore, a better understanding of the incidence and risk factors for VTE in ICI remains an unmet medical need in order to develop dedicated thromboprophylaxis strategies in the future.

Therefore, our aim was to determine the cumulative incidence, clinical consequences, and risk factors for VTE in patients with cancer treated with ICI.

Material and Methods

Study design and patient cohort

In this retrospective cohort study, consecutive adult patients with histologically confirmed cancer were included. In detail, patients treated with ICI at the Medical University of Graz between January 2015 and November 2021 included in the AUTRICHE registry, a registry-based study collecting data from ICI-treated patients in Austria, were included. The study was approved by the institutional ethics committee (Medical University of Graz: No. 31-357 ex 18/19).

Study procedures and outcomes

Data on patient demographics, comorbidities, cancer characteristics, cancer treatment, ICI-therapy and study outcomes were obtained by electronic chart review. The primary study endpoint was the first occurrence of VTE during ICI-therapy, comprising deep vein thrombosis, pulmonary embolism, splanchnic vein thrombosis or sinus vein thrombosis. Both symptomatic and incidental VTE events were included as outcome events. The observation period for occurrence of VTE was commenced at the first day of ICI therapy and terminated in case of an event of death, start of subsequent systemic anti-cancer therapy other than ICI, or a maximum of 3 months after the last ICI cycle. Objective diagnostic imaging studies and confirmation by an independent adjudication committee were mandatory for the verification of a VTE event.

Secondary study endpoints were all-cause mortality and disease progression, comprising radiologic progression as defined by immunotherapy-specific response evaluation criteria in solid tumors (iRECIST) or death, with data obtained from the official Austrian death registry and electronic medical files. (118)

Statistical analysis

Baseline clinicopathologic characteristics and treatment specifics were summarized using absolute frequencies and percentages or median with corresponding interquartile range (IQR), as appropriate. The median follow-up time was calculated with the reverse Kaplan-Meier method. When analyzing risk of VTE, a competing risk framework accounting for all-cause death as competing outcome event was used to avoid overestimation of cumulative risks in the setting of substantial underlying mortality. (119) Cumulative incidences of VTE were obtained with the competing risk estimator and corresponding

standard errors according to Marubini and Valsecci, applying Gray's test for between-group comparisons. (120, 121) Further, modelling of the association between clinicopathologic risk factors and biomarkers with VTE risk was conducted in a proportional sub-hazard regression model according to Fine and Gray, adjusting for potential confounders in multivariable analysis (cancer type, stage, ECOG performance status, and comorbidity burden as reflected by the Charlson comorbidity index). (122) The association between ICI-exposure and VTE risk was analyzed in a time-dependent analyses, using a multi-state model, adjusting for potential confounders. Similarly, the association between VTE during ICI and risk of mortality and disease progression was analyzed in time-dependent analysis, and by using a landmark analysis.

Statistical analyses were performed using Stata version 16.1 (StataCorp LP, Collage Station, TX, USA).

Results

Patient cohort characteristics

Overall, 417 consecutive patients who receive ICI therapy between January 2015 and November 2021 at the Medical University of Graz were identified and included in the study cohort. The median age at study inclusion was 66 years (IQR: 57-72), and 255 patients were female (61.2%). The majority of patients had a good performance status (ECOG 0: n=49, 14.9%; ECOG 1: 123; 38.2%) at the start of ICI therapy. Regarding comorbidities at the initiation of ICI therapy, the median Charlson comorbidity index was 9 (IQR: 9-10), notably with 6 points allocated to patients with metastatic cancer alone. Overall, a prior history of VTE was present in 56 patients (13.4%). Of those, 41 patients had a prior diagnosis of cancer-associated VTE (9.8%) and 15 had a prior VTE diagnosis unrelated to the current cancer diagnosis (3.6%). Continuous anticoagulation therapy at ICI initiation was used in 106 patients (25.4%), whereas 68 patients received continuous platelet inhibitor therapy (16.3%).

The most frequent cancer types were non-small cell lung cancer (n=170, 40.8%), renal cell carcinoma (n=66, 15.9%) and melanoma (n=64; 15.4%), followed by urothelial cancer (n=41, 9.8%), head and neck squamous cell carcinoma (n=19, 4.6%) and different other cancer types (**Table 2**). At ICI initiation, most patients had distant metastatic cancer (i.e., stage IV disease; n=393, 94.9%). ICI were applied at median as 2nd-line systemic therapy (IQR: 1-2, range: 1-5), at a median of 13.5 months after cancer diagnosis (IQR: 4.4-38.0). The number of applied ICI treatment cycles varied substantially (median: 5, IQR: 2-12, range: 1-66). The most frequent ICI agents were pembrolizumab (n=181, 43.4%) and nivolumab (n=173, 41.5%) followed by ipilimumab-nivolumab-combination (n=29, 7.0%), atezolizumab (n= 25, 6.0%), ipilimumab (n=8, 1.9%) and durvalumab (n=1, 0.2%). Concomitant chemotherapy was used in 41 patients during ICI therapy (9.8%).

The Khorana score was calculated at ICI initiation, with higher scores indicating a higher risk of cancer-associated VTE, allocating +2 points to very high risk cancer types (gastric, pancreatic), +1 point to high risk cancer types (lung, lymphoma, gynaecologic, bladder, testicular), and +1 points each to platelet levels ≥350x10^9/L, haemoglobin levels of <10 g/dL, leukocyte counts of >11x10^9/L and a BMI ≥35 kg/m^2. The median Khorana score was 1 (IQR: 1-2), with scores of ≥2 in 174 patients (41.7%). Further details regarding characteristics of patients included in the study cohort are summarized in **Table 2.**

Table 2: Study cohort characteristics (n=417)

Variable	n (% missing)	Median [IQR] or count (%)
Demographics and clinical characteristics		
Age (years)	417 (0%)	66 [57-72]
Female	417 (0%)	255 (61.2%)
ECOG	322 (22.8%)	1 [1-2]
-ECOG 0		49 (14.9%)
-ECOG 1		123 (38.2%)
-ECOG ≥2		150 (46.6%)
Charlson comorbidity index	417 (0%)	9 [9-10]
BMI (kg/m²)	417 (0%)	25 (21-28)
History of VTE*	417 (0%)	56 (13.4%)
History of VTE during current cancer disease	417 (0%)	41 (9.8%)
Continuous anticoagulation	417 (0%)	106 (25.4%)
Continuous platelet inhibitor therapy	417 (0%)	68 (16.3%)
Khorana score	417 (0%)	1 [1-2]
-0		11 (2.6%)
-1		232 (55.8%)
-≥2		174 (41.7%)
Positive smoking history	417 (0%)	231 (55.4%)
Cancer characteristics at study inclusion		
Tumour type	417 (0%)	
-Non-small cell lung cancer		170 (40.8%)
-Renal-cell carcinoma		66 (15.8%)
-Melanoma		64 (15.4%)
-Urothelial		41 (9.8%)
-Head and neck squamous cell carcinoma		19 (4.6%)
-Colorectal cancer		10 (2.4%)
-Breast		10 (2.4%)
-Gastroesophageal		8 (1.9%)
-Cancer of unknown primary		7 (1.7%)
-Small cell lung cancer		4 (1.0%)

-Prostate cancer		3 (0.7%)
-Mesothelioma		2 (0.5%)
-Others		13 (3.1%)
Stage	414 (0.7%)	
---II		2 (0.5%)
---III		19 (4.6%)
---IV		393 (94.9%)
PD-L1 (CPS)	201 (51.8%)	
--- 0%		46 (22.9%)
--- 1-49%		75 (37.3%)
--- ≥50%		80 (39.8%)
Therapeutic management		
Immune checkpoint inhibitor agent	417 (0%)	
-Pembrolizumab		181 (43.4%)
-Nivolumab		173 (41.5%)
-Ipilimumab+Nivolumab		29 (7.0%)
-Atezolizumab		25 (6.0%)
-Ipilimumab		8 (1.9%)
-Durvalumab		1 (0.2%)
Therapy cycles	417 (0%)	5 [2-12], range: 1-66
Line of anticancer therapy	417 (0%)	2 [1-2], range 1-5
-First line immunotherapy		192 (46.2%)
Time from diagnosis to immunotherapy	417 (0%)	13.5 months [4.4-38.0]
Concomitant therapy during immune checkpoint inhibitor	417 (0%)	
- Chemotherapy		41 (9.8%)
- Radiotherapy		3 (0.7%)

Abbrevations: VTE: venous thromboembolism; PD-L1: programmed death-ligand1; CPS: combined positive score;

Risk of VTE during ICI-therapy

Patient were followed from the initiation of ICI therapy until either death, subsequent systemic therapy, or a maximum of 3 months after the last applied ICI cycle. The median follow-up period was 10.1 months (IQR: 3.7-20.1). Overall, during follow-up, 37 patients were diagnosed with VTE, and 233 patients died (55.9%). The cumulative incidence of VTE during ICI in competing risk analysis was 12.2% (95% confidence interval (CI)I: 8.7-16.4), with corresponding cumulative incidences at 3-, 6-, 12-, and 24-months of follow-up of 2.1% [95%CI: 1.1-4.0], 4.2% [95%CI: 2.6-6.6], 5.7% [95%CI: 3.7-8.3)] and 9.7% [95%CI. 6.7-13.3], respectively (**Figure 1**). The median time to VTE after ICI initiation was 7.5 months (IQR: 3.7-21.2).

Of the 37 observed VTE events, the index event was DVT in 12 patients, PE in 13 patients, DVT+PE in 9 patients, splanchnic vein thrombosis in 1 patient and an unspecified VTE event in 1 patient. VTE was diagnosed as symptomatic event in 27 patients (73.0%), whereas 10 patients were diagnosed with an incidental VTE (27%). Two patients died of PE, with a case-fatality rate of VTE of 5.4%. DVT was located in the lower extremity in the majority of patients (n=19, 90.5% of DVT), whereas upper extremity DVT was observed in two patients (9.5%). Of those, one patient suffered a central-catheter related DVT. Further, one patient with an index PE suffered a second VTE event during study follow up (symptomatic non-fatal PE). Details on the characteristics of VTE observed during ICI therapy are summarized in **Table 3**.

Figure 1: Cumulative incidence of VTE during ICI therapy

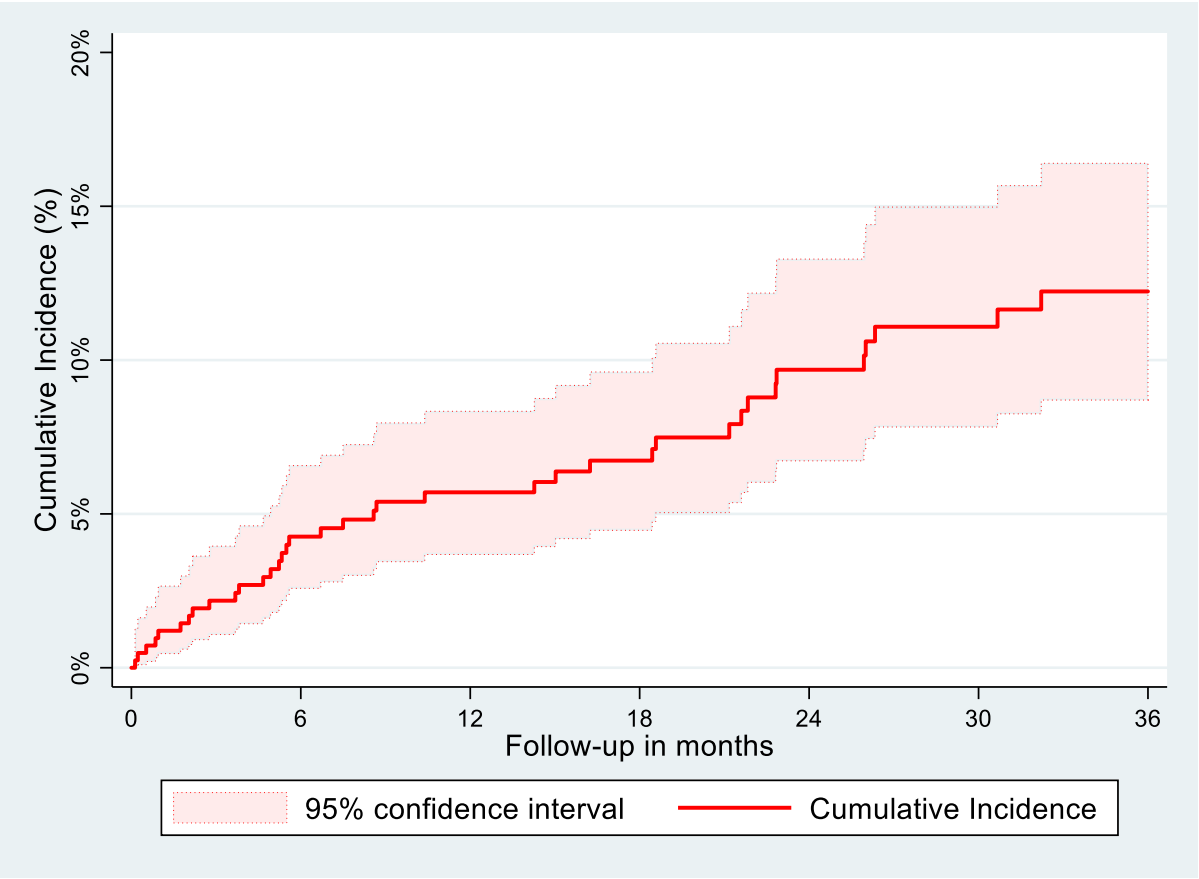


Table 3: Characteristics of VTE during ICI therapy

Patients with VTE during ICI	**N=37**
- Deep vein thrombosis (DVT)	12 (32.4%)
- Pulmonary embolism (PE)	13 (35.1%)
- DVT+PE	9 (24.3%)
- Cather-related DVT	1 (2.7%)
- Splanchnic vein thrombosis	1 (2.7%)
- Unspecified VTE	1 (2.7%)
VTE symptoms	
- Symptomatic VTE	27 (73.0%)
- Incidental VTE	10 (27.0%)
- Fatal PE	2 (5.4%)

DVT localisation	
- Lower extremity	19 (90.5%)
- Upper extremity	2 (9.5%)
PE type	
- Central	4 (18.2%)
- Segmental	10 (45.5%)
- Subsegmental	6 (27.3%)
- Unspecified	2 (9.1%)
Hospitalisation for VTE	18 (48.7%)
Second VTE during ICI	1 (2.7% - symptomatic PE, nonfatal)

Abbreviations: DVT: deep vein thrombosis; PE: pulmonary embolism

Association of ICI with VTE risk

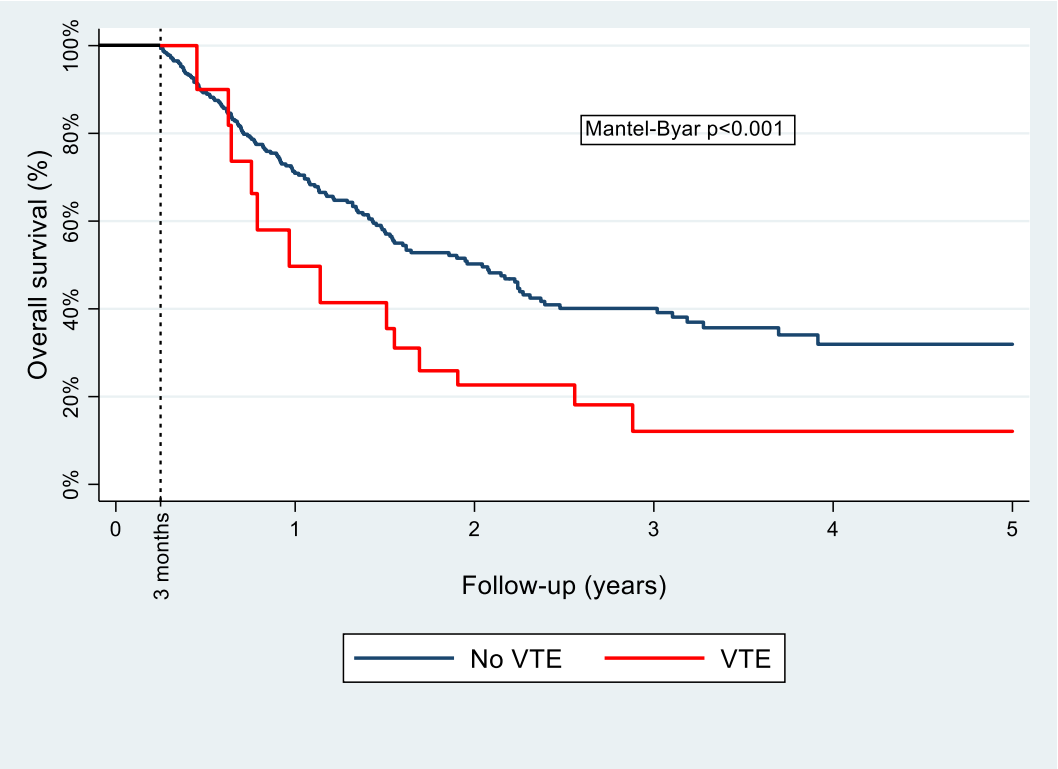
Upon analysing the association between ICI exposure with VTE risk, implementing the timeframe after ICI initiation as a time-dependent variable in a competing risk regression model of VTE risk, an increased risk of VTE was observed during ICI therapy as compared to the timeframe between cancer diagnosis and ICI start (THR for VTE: 3.30 [95% CI: 1.95-5.57], p<0.001). This association prevailed upon multivariable adjustment for cancer type and stage (adjusted THR: 3.36 [95% CI: 1.97-5.74], p<0.001).

Impact of VTE on survival and treatment response patterns

Over the duration of follow-up after ICI initiation, 233 patients died (crude mortality: 55.9%). The median overall survival of patients was 16.1 months (95% CI: 12.9-18.5). The cumulative overall survival at 6, 12, and 24 months of follow-up was 72.8% (95%CI: 68.1-76.8), 56.9% (95%CI: 51.7-61.9), and 39.3% (33.7-44.7), respectively. In a time-dependent analysis, the occurrence of VTE was associated with increased mortality (THR after VTE: 2.45 [95% CI: 1.48-4.05], p=0.0005). This association prevailed upon multivariable adjustment for cancer type, stage, age, sex, ECOG performance status, and Charlson comorbidity index score (adjusted THR: 2.03 [95% CI: 1.11-3.72], p=0.0214). In a landmark analysis, comparing overall survival estimates of patients according to the occurrence of VTE within the first 3 months of follow-up, significantly lower survival estimates were obtained for the VTE subgroup (Mantel-Byar p<0.001, **Figure 2**).

Further, the median progression-free survival was 4.7 months (95% CI: 3.9-5.5), with a cumulative progression free survival at 6-, 12-, and 24 months of 43% (95% CI: 38.1-47.9), 22.1% (17.8-26.6), and 7.1% (4.4-10.5), respectively. In time-dependent analysis, the THR for disease progression after VTE was 1.48 (95%CI: 0.82-2.54; p=0.191).

Figure 2: Landmark analysis of overall survival according to VTE status within 3 months after ICI initiation



Risk of VTE according to cancer type and ICI agent subgroup

Risk of VTE was similarly distributed according to the underlying cancer type. Of 37 VTE events, 17 were observed in patients with NSCLC (n=170), 7 in patients with renal cell carcinoma (n=66), 6 in patients with melanoma (n= 64) and 4 in patients with urothelial cancer (n=41). The remainder of VTE events occurred in 1 patient each with small-cell lung cancer, prostate cancer, and breast cancer, respectively. The cumulative incidence of VTE was 11.9% (95%CI: 7.1-18.0) in patients with NSCLC, 18.6% (95%CI: 7.9-32.8) in patients with renal cell carcinoma, 11.5% (95%CI: 4.6-21.8) in patients with melanoma, and 13.0% (95%CI: 4.0-27.4) in those with urothelial cancer. No significant association was observed upon analysing VTE risk according to the respective cancer type sub-cohort

compared to the remainder of patients for NSCLC (SHR: 1.14 [95% CI: 0.60-2.19], p=0.684), renal cell carcinoma (SHR: 1.32 [95% CI: 0.59-2.99], p=0.493), melanoma (SHR: 0.90 [95 %CI: 0.38-2.13], p=0.829), and urothelial cancer (SHR: 1.23 [95%CI: 0.43-3.52], p=0.692).

Observed VTE events were similarly distributed among different ICI agent groups (**Table 4**). No significant difference in risk was observed upon analysing the association between VTE risk of the respective agent subgroup with the remainder of the patient cohort as reference for pembrolizumab (SHR: 0.95 [95 %CI: 0.50-1.83], p=0.888), nivolumab (SHR: 0.94 [95 CI: 0.49-1.79], p=0.854), ipilimumab/nivolumab (SHR: 0.90 [95% CI: 0.22-3.78], p=0.889), and atezolizumab (SHR: 1.04 [95% CI: 0.25-4.33], p=0.952).

Table 4: Risk of VTE according to cancer type and ICI agent

	N (patients), n (VTE)	**Cumulative VTE incidence (95% CI)**
Tumour type		
-Non-small cell lung cancer	N=170, 17 VTE	11.9% (7.1-18.0)
-Renal-cell carcinoma	N=66, 7 VTE	18.6% (7.9-32.8)
-Melanoma	N=64, 6 VTE	11.5% (4.6-21.8)
-Urothelial	N=41, 4 VTE	13.0% (4.0-27.4)
-Head and neck squamous cell carcinoma	N=19, 0 VTE	/
-Colorectal cancer	N=10, 0 VTE	/
-Breast	N=10, 1 VTE	/
-Gastroesophageal	N=8, 0 VTE	/
-Cancer of unknown primary	N=7, 0 VTE	/
-Small cell lung cancer	N=4, 1 VTE	/
-Prostate cancer	N=3, 1 VTE	/
-Mesothelioma	N=2, 0 VTE	/
ICI agent		
Pembrolizumab	N=181, 15 VTE	11.6% (6.4-18.4)
Nivolumab	N=173, 16 VTE	11.8% (7.0-18.1)
Ipilimumab/Nivolumab	N=29, 2 VTE	7.6% (1.3-21.6)

Atezolizumab	N=25, 2 VTE	12.0% (2.0-31.6)
Ipilimumab	N= 8, 2 VTE	/
Durvalumab	N=1, 0 VTE	/

Table legend: Cumulative incidences estimates for subgroups of n≥20 patients.

Abbreviations: VTE: venous thromboembolism

Exploration of risk factors for VTE during ICI therapy

Different patient-, cancer, and treatment related factors were explored regarding their predictive utility for VTE risk during ICI treatment, as described in detail in **Table 5**. Regarding cancer characteristics, no differences in VTE risk were observed according to cancer type sub-cohort, as summarized in **Table 4**. Further, the presence of distant metastatic disease (i.e., stage IV) as not associated with VTE risk (SHR: 0.95 [95%CI: 0.30-2.97], p=0.926). In patients with available PD-L1 tissue staining (n=210), no difference in risk was observed according to CPS (SHR: 0.90 [95%CI: 0.72-1.13], p=0.371). Regarding patient specific factors and comorbidities, no association with VTE risk was observed for age, sex, Charlson comorbidity burden, smoking status, prior VTE, or a higher ECOG performance status. In contrast, higher bodyweight was associated with VTE risk (SHR per point increase: 1.10 [95%CI: 1.04-1.16], p=0.001). Regarding baseline and concomitant comedication, neither the receipt of antiplatelet nor anticoagulation therapy at study inclusion was associated with subsequent VTE risk. Further, the receipt of chemotherapy during ICI therapy was not associated with an increased VTE risk.

The Khorana score was calculated for all patients at study inclusion. The median Khorana score was 1 (IQR: 1-2, range 0-4). Overall, 173 patients had a Khorana score of ≥2 (41.6%). Higher Khorana scores did not predict VTE risk after ICI initiation, both per point increase (SHR: 0.78 [95% CI: 0.53-1.16], p=0.218) and upon comparing patients with ≥2 points to those with 0-1 points (SHR: 0.88 [95% CI: 0.45-1.72], p=0.709).

Different available laboratory biomarkers were analysed for their predictive utility towards VTE risk. Levels of haemoglobin, leucocyte counts, and platelet counts ad study baseline did not predict VTE risk, neither per unit increase nor upon dichotomization according to the cut-off values used in the Khorana score (**Table 5**). Further, baseline levels of CRP, LDH and albumin did not predict future VTE risk.

Table 5: Risk factors for VTE during ICI therapy

Risk factor (at ICI initiation)	**SHR (95 %CI)**
Cancer characteristics	
Cancer type (remainder of cohort as reference)	
- NSCLC	1.14 (0.60-2.19), p=0.684
- Renal cell carcinoma	1.32 (0.59-2.99), p=0.493
- Melanoma	0.90 (0.38-2.13), p=0.829
- Urothelial cancer	1.23 (0.43-3.52), p=0.692
Stage IV	0.95 (0.30-2.97), p=0.926
PD-L1 (CPS per 10% increment)	0.90 (0.72-1.13), p=0.371
Patient and treatment characteristics	
Female	0.77 (0.40-1.46), p=0.418
Age (per year increase)	0.99 (0.97-1.01), p=0.269
Charlson comorbidity index (per point increase)	0.74 (0.53-1.03), p=0.079
BMI (per point increase)	**1.10 (1.04-1.16), p=0.001**
Positive smoking history	0.74 (0.31-1.75), p=0.495
Khorana score (per point increase)	0.78 (0.53-1.16), p=0.218
Khorana score (≥2 points vs 0-1 points)	0.88 (0.45-1.72), p=0.709
Prior VTE	1.42 (0.54-3.71), p=0.477
ECOG (≥1 vs 0)	0.56 (0.24-1.33), p=0.189
Concomitant chemotherapy	1.31 (0.46-3.76), p=0.612
Anticoagulation at ICI start	1.14 (0.55-2.36), p=0.729
Anti-platelet therapy at ICI start	0.57 (0.21-1.60), p=0.290
Biomarkers	
- Haemoglobin (per unit increase)	1.09 (0.94-1.26), p=0.241
- Haemoglobin <10 g/dL	0.42 (0.10-1.74), p=0.231
- Platelet count	1.00 (0.99-1.00), p=0.457
- Platelet count ≥350x10⁹/L	0.69 (0.29-1.67), p=0.412
- Leucocyte count	1.01 (0.93-1.09), p=0.896
- Leucocyte count >11x10⁹/L	1.10 (0.48-2.52), p=0.815

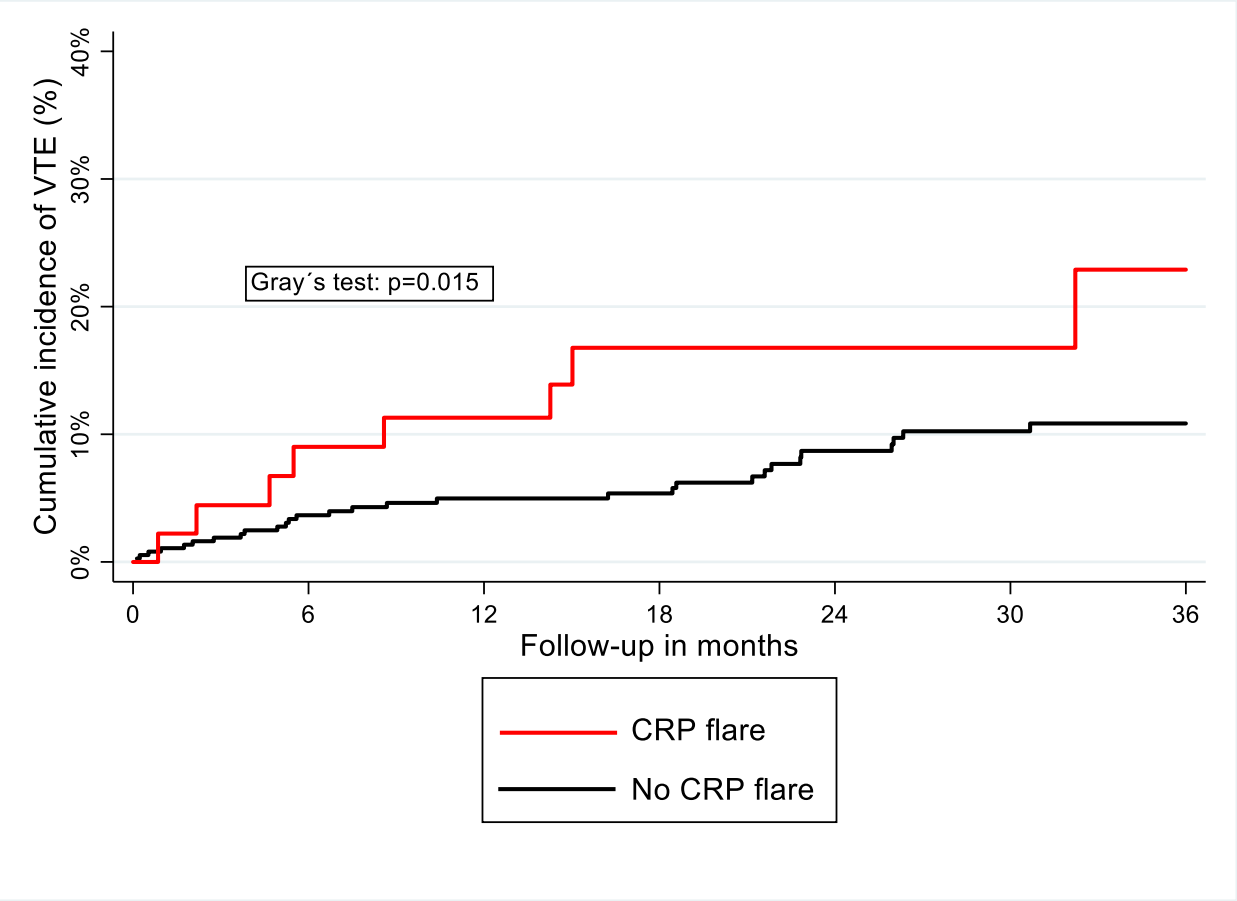
- CRP (per unit increase)	1.00 (0.99-1.00), p=0.307
- CRP (above ULN*)	0.77 (0.40-1.47), p=0.428
- LDH (per unit increase)	1.00 (1.00-1.00), p=0.467
- Albumin (per unit increase)	1.28 (0.66-2.49), p=0.460

Abbreviations: VTE: venous thromboembolism; ICI: Immune-checkpoint-inhibitors; CI: confidence interval; NSCLC: non-small-cell lung cancer; PD-L1: programmed death-ligand1; CPS: combined positive score; BMI: Body mass Index; ULN: upper limit of normal

CRP dynamics and VTE risk after ICI initiation

Lastly, early trajectories of CRP levels were evaluated for their predictive utility towards VTE risk. In patients with a doubling of CRP levels within 3 months after ICI initiation compared to baseline values (i.e., "CRP-flare"; n=45, 11% of study population), a higher risk of VTE was observed (SHR: 2.31 [95%CI: 1.06-5.02], p=0.035), which prevailed upon multivariable adjustment for age, sex, cancer type, stage and comorbidity burden (SHR: 2.25 [95%CI: 1.03-4.94], p=0.043). The cumulative risk of VTE in patients with an early CRP flare was 22.9% (95% CI: 9.7-39.3), compared to 10.8% (95% CI: 7.4-15.1) in those without a CRP-flare (Gray´s test: p=0.015; **Figure 3**).

Figure 3: Cumulative incidence of VTE according to CRP flare after ICI initiation



Discussion

In this retrospective cohort study, we provide comprehensive data on the clinical risk profiles of VTE in patients with cancer treated with ICI, including the incidence, clinical outcomes, and risk factors. We observed a clinically relevant risk of VTE after ICI initiation, indicating that approximately 1 in 8 patients treated with ICI develops VTE during therapy. The occurrence of VTE was associated with a dramatic increase in risk of all-cause mortality, indicting the identification of a sub- cohort of patients with an unfavorable clinical course of disease with a VTE diagnosis. In addition, risk of VTE after ICI initiation was increased compared to the time frame from cancer diagnosis to ICI-start. During ICI therapy, risk of VTE was homogenous according to patient- and cancer specific factors and the established risk prediction model for cancer-associated VTE, the Khorana score, did not stratify VTE risk. Lastly, in routine laboratory biomarkers measured at ICI start did not predict VTE risk, yet an early rise in CRP levels was associated with higher risk of VTE.

The high observed risk of VTE might be explained by underlying patient-, and cancer specific characteristics and reflect the underlying thrombotic risk of treated patients. However, speculatively, ICI might increase VTE risk via inflammation-induced hypercoagulability. Compared to other retrospective studies and cohort studies, which focused on ICI-associated AE and especially on cardiovascular AEs, the increase in risk of VTE corelates well with our results. (77, 109, 110) To date, a potential causal prothrombotic effect of ICI is unclear, yet underlying pathophysiologic pathways remain elusive. Recently, first murine studies were performed, which demonstrated hypercoagulability and higher levels of prothrombotic and atherosclerotic factors in ICI treated mice. (123-125) A preclinical study indicated a link between ICI and an enhanced atherosclerosis through different proinflammatory pathways, more precisely through plaque inflammation and progression. (126) In our study the CRP-flare group had a higher risk for developing a VTE, which could indicate a potential contributing role of ICI-induced inflammation for the high observed VTE risk. Opposingly, patients treated with ICI are at substantial VTE risk a priori based on cancer types, stage, and mostly advanced treatment setting. Synoptically, both underlying patient- and cancer specific factors together with ICI-induced inflammation might explain the high observed burden of VTE.

Importantly, our data suggest limited clinical utility of established clinical risk factors and risk prediction models for cancer-associated VTE in the setting of ICI therapy. Specifically, we observed a homogeneously high VTE risk irrespective of cancer type, stage, comorbidities, or baseline biomarkers. Further, the Khorana score, which is recommended to select ambulatory patient with cancer at high risk of VTE for primary thromboprophylaxis, failed to stratify VTE risk in our cohort of ICI treated patients, which is in line with previous reports.(88, 90, 127) The Khorana score, which was developed in the pre-immunotherapy era, therefore seems of limited use in the growing population of ICI-treated patients, raising the necessity of novel dedicated risk prediction models to help select patients for potential primary prophylactic interventions. In our study, we observed a promising signal regarding the predictive utility of longitudinal trajectories of inflammatory biomarkers for the prediction of ICI-associated VTE. In detail, early doubling of CRP levels on the first three months of therapy were associated with a markedly increased VTE risk. Upon external validation, this strategy might be used to predict and ultimately prevent VTE in patients treated with ICI.

Finally, several limitation warrant considerations upon interpreting our findings. First, data were collected in a retrospective manner from existing medical files, raising the possibility of missing data. However, in this consecutive cohort from a tertiary care academic center with routine clinical follow-up and documentation of adverse events, the quality of the collected data is thought to be representative. Further, data on outcome events might be limited by the unavailability of health care data from outside medical providers, which might affect the number of reported VTE. Further, the observed number of outcome events limits the potential for subgroup analyses based on small sample sizes. Concerning our observation of CRP rise as risk factor for VTE, several additional limitations apply. The longitudinal nature of CRP-trajectories is subject to a complex pattern of potential confounding factors that might affect the association between CRP-levels and VTE risk. These factors might include increased CRP levels resulting from infection, clinical deterioration, or cancer progression and would be better controlled for in a prospective study. However, the previously reported association between CRP-flare response patterns with improved clinical outcomes in patients with cancer argues against clinical deterioration and disease progression as driving factor of an increase in VTE risk in those with a CRP flare. (128) Lastly, despite the role of CRP as an established surrogate of systemic inflammation, in the present retrospective design, a more thorough

characterization of the involved inflammatory pathways and more specific immune-mediated biomarkers could not be explored. Therefore, future studies should focus on deciphering the complex granularity of the ICI-associated inflammatory response and its correlation with biomarkers of hypercoagulability and association with VTE risk.

Conclusion

Our findings support a high risk of VTE in patients with cancer treated with ICI. Given the consistently high VTE risk irrespective of underlying cancer type and stage, and the failure of the Khorana score to predict VTE, novel risk-prediction models specifically in patients treated with ICI are needed. Our data support the concept of longitudinal assessment of inflammatory biomarkers including CRP as potential risk assessment tool for immunotherapy associated VTE.

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