Lung Cancer 142 (2020) 34–40

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

A multicenter study of thromboembolic events among patients diagnosed with *ROS1*-rearranged non-small cell lung cancer

Marliese Alexander[a,b,*], Nick Pavlakis[c,d,e,f], Thomas John[g,h,i], Rachel O'Connell[j], Steven Kao[k,l], Brett G.M. Hughes[m,n], Adrian Lee[c,d,e,f], Sarah A. Hayes[c,d], Viive M. Howell[c,d], Stephen J. Clarke[c,d,e,f], Michael Millward[o,p], Kate Burbury[b,q], Benjamin Solomon[b,r], Malinda Itchins[c,d,e,f]

[a] Department of Pharmacy, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
[b] Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia
[c] Bill Walsh Translational Research Laboratory, Kolling Institute Medical Institute of Research, Royal North Shore Hospital, St Leonards, New South Wales, Australia
[d] Northern Clinical School, University of Sydney, St Leonards, New South Wales, Australia
[e] Northern Cancer Institute, St Leonards, New South Wales, Australia
[f] Department of Medical Oncology, Royal North Shore Hospital, St Leonards, New South Wales, Australia
[g] Medical Oncology Unit, Olivia Newton John Cancer and Wellness Centre, Austin Health, Melbourne, Australia
[h] Olivia Newton-John Cancer Research Institute, Melbourne, Victoria, Australia
[i] Department of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Victoria, Australia
[j] NHMRC Clinical Trial Centre, University of Sydney, Camperdown, New South Wales, Australia
[k] Sydney Medical School, University of Sydney, Camperdown, New South Wales, Australia
[l] Department of Medical Oncology, Chris O'Brien Lifehouse, Camperdown, New South Wales, Australia
[m] Department of Medical Oncology, The Prince Charles Hospital, Chermside West, Queensland, Australia
[n] School of Medicine, University of Queensland, Brisbane, Queensland, Australia
[o] School of Medicine, University of Western Australia, Perth, Western Australia, Australia
[p] Department of Medical Oncology, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia
[q] Department of Haematology, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
[r] Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

ARTICLE INFO

Keywords:
ROS1
Non-small cell lung cancer
Thromboembolism
venous thromboembolism

ABSTRACT

Objectives: This study aimed to describe the longitudinal thromboembolism (TE) risk relative to the natural history of disease and clinical course of *ROS1* rearranged non-small cell lung cancer (NSCLC).
Materials and Methods: Cases of *ROS1*-rearranged NSCLC from six Australian hospitals were pooled and evaluated for incidence, timing, predictors and outcomes of venous or arterial TE, as well as objective response rate (ORR) to active therapy and overall survival (OS).
Results: Of 42 patients recruited, 20 (48%) experienced TE; one (2%) arterial, 13 (31%) a pulmonary emboli (PE), and 12 (29%) a deep vein thrombosis. Among those with TE, six (30%) experienced multiple events, three as concurrent and three as recurrent diagnoses. The cumulative incidence of TE over time, adjusted for death as a competing risk factor, approached 50%. TE occurred prior to, during and post the peri-diagnostic period and occurred irrespective of treatment strategy. A thrombophilia was identified in n = 3/10 (30%) cases screened: in two factor V Leiden and in one anti-thrombin III (ATIII) deficiency. Median OS was 21.3 months in those with TE vs. 28.8 months in those without; hazard ratio 1.16 (95%CI 0.43–3.15). Respective ORR to first-line therapy with TE was 50% vs. 44% without TE in the chemotherapy arm and 67% vs. 50% in the targeted therapy arm.
Conclusion: In the rare cancer subtype, *ROS1*, these real-world data demonstrate sustained TE risk beyond the diagnostic period irrespective of therapeutic strategy. High incidence of PE, concurrent TE, and recurrent TE warrant validation in larger cohorts. Consideration of primary thromboprophylaxis in *ROS1* populations is recommended.

* Corresponding author at: Peter MacCallum Cancer Centre, Locked Bag 1 A'Beckett Street, Melbourne, 8006, Victoria, Australia.
E-mail address: marliese.alexander@petermac.org (M. Alexander).

https://doi.org/10.1016/j.lungcan.2020.01.017
Received 26 September 2019; Received in revised form 16 January 2020; Accepted 20 January 2020

1. Introduction

Thromboembolism (TE) is a well-recognized complication among patients with non-small cell lung cancer (NSCLC), occurring throughout the disease course with an incidence commonly reported between 10–15% and exceeding 20% in some populations [1,2]. TE in cancer patients is associated with worse prognosis [3].

Within NSCLC, *EGFR* (16–17%) and *KRAS* (25–26%) mutations are the most commonly occurring oncogenic drivers [4], with reported TE rates (8–18%) comparable to unselected NSCLC cohorts [5,6]. *ALK* rearrangements occur in 4–8% of NSCLC [4], with mixed findings in relation to TE incidence [9]. The highest reported incidence comes from published data from small real-world cohorts (47%, n = 17 and 42%, n = 55) [7,8]; however this reduces significantly in larger real-world cohorts (17%, n = 70 and 22%, n = 193), 6,9] and even further (1–6%) in clinical trial cohorts for *ALK*-directed therapies [10–13], thus the expected true incidence is not certain.

ROS1-rearranged NSCLC biologically shares homology with *ALK* and occurs in less than 2% of all NSCLC cases [4]. A recent multicenter study has reported 35% TE incidence in the peri-diagnostic phase, a period defined as 90 days before or after an advanced cancer diagnosis [6]. There are no published data in *ROS1* NSCLC patients, with regards to longitudinal risk or lifetime TE burden. Physiologic mechanisms driving TE in this selected cohort remain unclear. However, in general cancer cohorts' presence of an underlying thrombophilia has been associated with higher incidence of TE compared to cancer patients without a thrombophilia [14], and case reports of co-occurring thrombophilia and TE in *ROS1* NSCLC are emerging [15].

This study aimed to validate the association between TE and *ROS1* rearranged NSCLC and to expand on previous reports to describe the longitudinal risk profile and TE burden including impact of TE on survival and response to cancer therapies in an Australian multicenter *ROS1* NSCLC cohort. The study was founded on independent observation by clinicians of *ROS1* patients presenting with TE.

2. Methods

2.1. Participants

The *ROS1* lung cancer cohort was derived from an audit of clinical data-sets of patients attending six Australian tertiary referral hospitals. Eligible patients had a histopathological diagnosis of NSCLC with a *ROS1* rearrangement determined by fluorescence in situ hybridization (FISH) or immunohistochemistry (IHC), between 01 Jan 2010 and 28 Jan 2017. During the study period *ROS1* testing was not reflex, with IHC screen sought and FISH confirmation at the discretion of the treating physician based on clinical assessment and local practices. Antibodies for IHC varied by site, primarily D4D6 antibody concentrate at 1/50 dilution. Institutional ethics review board approval was obtained from Northern Sydney Local Health District Ethics Review Board (approval no. RESP/18/158).

2.2. Data collection

Baseline demographics (age, race, performance status, smoking, medical and TE history), pathology (neutrophil, lymphocyte, platelet, albumin, lactate dehydrogenase, calcium C-reactive protein), diagnostic data (stage, metastasis), presenting symptoms, treatment and sequencing, objective response rate (ORR) and disease control rate (DCR) (RECIST 1.1), central nervous system (CNS) ORR, TE (venous and arterial), and survival data were extracted from medical records and clinical data-sets. Screening for a thrombophilia was not mandatory but data were extracted where it had been undertaken. Routine screening for TE did not occur given no prior reported association between TE and *ROS1* NSCLC at time of patient treatment. All patients were managed at major institutions with a high proportion managed on clinical trials indicating close clinical monitoring and enabling appropriate TE investigations. TE events included in this study were diagnosed using objective methods (ultrasound, computed tomography) as performed in standard clinical care for disease assessment or clinical suspicion of TE, with acknowledged potential for underreporting of asymptomatic events [16].

2.3. Statistical analysis

Case data were de-identified and pooled for analysis. Median and range (continuous variables) and frequency and percentage (categorical variables), were used to describe clinical characteristics.

TE incidence was estimated from 1-year prior to lung cancer diagnosis until death or last study follow-up (01 Jan 2018). The association between TE and clinical variables was assessed using Fine and Gray competing-risks regression [17], with death a competing risk, and results reported as sub-distribution hazard ratio (sHR) with ninety-five percent confidence intervals (95% CI). A corresponding cumulative incidence function was obtained from the same model. Due to the small overall number of TE events (20 events) multivariate analysis was limited to a maximum of two confounding variables with strongest association by univariate analysis. Where multiple TE events occurred for an individual patient, only the first TE was assessed.

Overall survival (OS) was estimated using the Kaplan-Meier (KM) method and defined as time from lung cancer diagnosis to death by any cause (living patients censored at last follow-up). Association between TE and OS was analysed using Cox proportional hazards regression. Responses were as reported by the treating clinician according to RECIST 1.1 [18], as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Objective response rate (ORR) included patients with CR or PR while disease control rate (DCR) included patients with CR, PR or SD. Classification of ORR/DCR by TE status did not consider timing of TE.

Statistical analyses were undertaken using STATA15 software (regression and survival analyses) and SAS version 9.4 software (ORR and swimmer plot).

3. Results

3.1. Participants

At the data cut-off date 42 patients diagnosed with *ROS1* NSCLC were identified from six Australian hospitals. For 37 (88%) patients, *ROS1*-rearrangement was confirmed by FISH with the remaining five patients testing positive by IHC staining but with negative FISH (four FISH negative, one inconclusive). Of the four patients negative by FISH, one was confirmed by reverse-transcription polymerase chain reaction (RT-PCR), one confirmed via next generation sequencing (NGS), one was described as uninterpretable, and one without further explanation. Based on clinical assessment all were treated empirically and monitored closely demonstrating durable response to targeted therapies.

Median follow up for TE, treatment response and survival outcomes, was 10.9 months (range 0.1–180.4). Median age at diagnosis was 53 years (range: 31–80); 74% were female; 67% were non-Asian, 88% non-smokers and 21% had CNS disease at diagnosis. Demographic and management data are presented in Table 1.

3.2. Thromboembolism and thrombophilia

Nearly half of all patients (n = 20, 48%) experienced at least one TE event. The majority were venous (n = 19, 45%), with a high incidence of pulmonary embolism (n = 13, 31%) as well as deep vein thrombosis (n = 11, 26%). Among those with TE, six (30%) experienced multiple events, three as concurrent and three as recurrent diagnoses, Table 2. One patient presented with fatal arterial TE (*ROS1* diagnosed premortem). The cumulative incidence of TE over time, adjusted for death

Table 1
Demographic and management data.

	Number (%)
Age (years), median (range)	53 (31–80)
Female	31 (74%)
Non-Asian	28 (67%)
Non-smokers	37 (88%)
ECOG PS at diagnosis	
0-1	34 (81%)
2-3	7 (17%)
Unknown	1 (2%)
Adenocarcinoma	51 (96%)
Adenosquamous carcinoma	1 (2%)
Squamous cell carcinoma	1 (2%)
Prior early lung cancer	15 (36%)
CNS disease at diagnosis	9 (21%)
CNS relapse	3 (8%)
Enrolled in clinical trial	16 (38%)
First line ROS1 TKI	17 (40%)
ROS1 TKI any line	27 (64%)

CNS – central nervous system; ECOG PS – Eastern Cooperative Oncology Group performance status; TKI – tyrosine kinase inhibitor.

Table 2
Thromboembolic events.

Event	Incidence/Outcome
Any TE event	20/42 (48%)
Event time relative to lung cancer diagnosis, median (range)[1]	1.8 months (−7.3 months to 8.5 years) No (%) of TE (n = 20)
Type of TE/TE burden	
Any PE (alone or with DVT)	13 (65%)
Any DVT (alone or with PE)	11 (55%)
PE and DVT (concurrent or sequential)	6 (30%)
PE alone	8 (40%)[2]
DVT alone	6 (30%)
ATE	1 (5%)
TE within +/- 90 days of lung cancer diagnosis	10 (50%)
TE within +/- 90 days of metastatic lung cancer diagnosis	9 (47%)[3]
Systemic treatment at time of TE[4]	
No anticancer therapies	10 (50%)[5]
Chemotherapy +/- radiotherapy	5 (25%)
Targeted therapy	5 (25%)

1 – months from lung cancer diagnosis; 2 – includes two bilateral PEs; 3 – denominator n = 19 as one patient died with stage IIIB disease; 4 – treatment period includes time on treatment + 30 days; 5 – includes 6 TE during diagnostic phase prior to systemic treatment, 2 TE post chemotherapy (2 months and 12 months), 2 TE in context of no systemic treatment plan. ATE – arterial thromboembolism DVT – deep vein thrombosis; PE – pulmonary embolism.

as a competing risk factor, approached 45% within 24 months from lung cancer diagnosis, Fig. 1. Unadjusted modelling shows cumulative incidence increases slightly approaching 50% over the same period of time (Appendix A, Fig.A.1).

Median time to TE was 1.8 months from lung cancer diagnosis, though events occurred throughout the patient journey and expected time with lung cancer, with TE diagnoses ranging from 7.3 months before confirmed diagnosis to 8.5 years after in one patient with indolent advanced disease, Fig. 2. In six (30%) patients, the TE preceded lung cancer diagnosis with onset both prior and separate from the lung cancer diagnosis (n = 2), as well as coinciding with or leading to the diagnosis (n = 4). One of the two patients who experienced unprovoked TE prior to lung cancer diagnosis encountered recurrent TE during treatment. Routine practice dictated initiation of therapeutic anticoagulation indefinitely following TE diagnosis, with the agent selected at clinician discretion and patient preference (data not available), informed by standard practice guidelines indicating use of low molecular weight heparin (predating routine use of oral agents in malignancy).

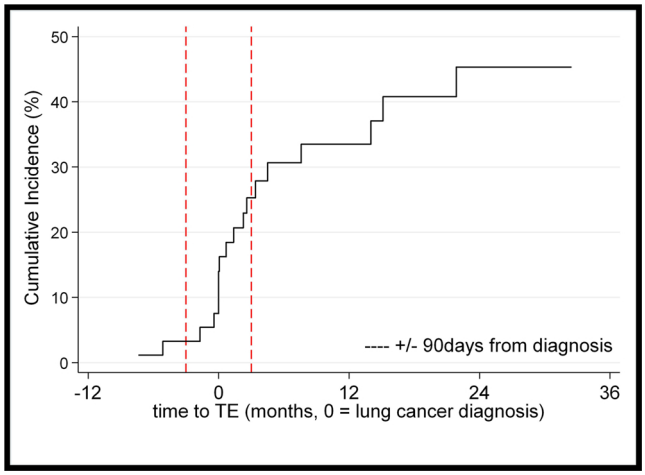

Fig. 1. Cumulative incidence of thromboembolism (TE) by Fine and Gray competing-risks regression with death a competing risk. Time zero is day of lung cancer diagnosis and dashed reference lines depict +/- 90 day interval from diagnosis.

Thrombophilia screening was conducted in n = 9/20 cases with TE and n = 1/22 without TE. Interestingly, co-occurring thrombophilia was identified in 33% (n = 3/9) of TE patients screened; two factor V Leiden; one anti-thrombin III (ATIII) deficiency. Additionally, one patient with TE had factor XII deficiency, one thalassaemia minor, and another was diagnosed with acute-promyelocytic leukaemia (without evidence of disseminated intravascular coagulation), whilst on a ROS1-inhibitor (tyrosine kinase inhibitor (TKI)), and was treated with tretinoin as standard curative intent therapy – currently in complete molecular remission.

3.3. Survival and response

Median OS for the whole patient group was estimated at 28.8 months (95% CI 16.4 – not reached (NR), range 0.1–180.4); with 38% of the cohort deceased. Survival was reduced among patients with CNS disease (median 21.3 months, 95% CI 5.7-NR, range 1.2–43.4) compared to those without (median NR, 95% CI 17.6 - NR, range 0.1 – 180.4) and increased among patients treated with TKI (median 63.5 months, 95% CI 17.6 - NR, range 2.5 – 180.4) compared to not (median 13.9 months, 95% CI 10.0 - NR, range 0.1–28.8). Prior smoking (p = 0.048) and presence of comorbidities (p = 0.032) were associated with a worse prognosis, with suggested evidence of significance in CNS disease (p = 0.079). Race, gender, age, line of therapy TKI first received, stage at diagnosis (metastatic vs. non-metastatic) and a high neutrophil to lymphocyte ratio (NLR) at diagnosis (NLR > 5) were not predictive of survival in this cohort.

Considering all treatments and all lines of therapy ORR was 41% (n = 29/70) and DCR 69% (n = 48/70). Response rates were higher for TKI compared to chemotherapy considering all lines of therapy (ORR 50%, n = 16/32 vs. 42%, n = 13/31 and DCR 81%, n = 26/32 vs. 68%, n = 21/31) and first line therapy (ORR 59%, n = 10/17 vs. 47%, n = 8/17 and DCR 88%, n = 15/17 vs. 71%, n = 12/17). Response to individual therapies is shown in Table 3.

Of the four patients negative for *ROS1* by FISH, the PCR positive patient was treated with chemotherapy, the NGS positive was treated with ceritinib on clinical trial with a partial response of unknown duration, the patient with uninterpretable result was treated with crizotinib with disease control beyond 12 months, and the patient with a negative result treated with crizotinib with 10 months' progression free survival.

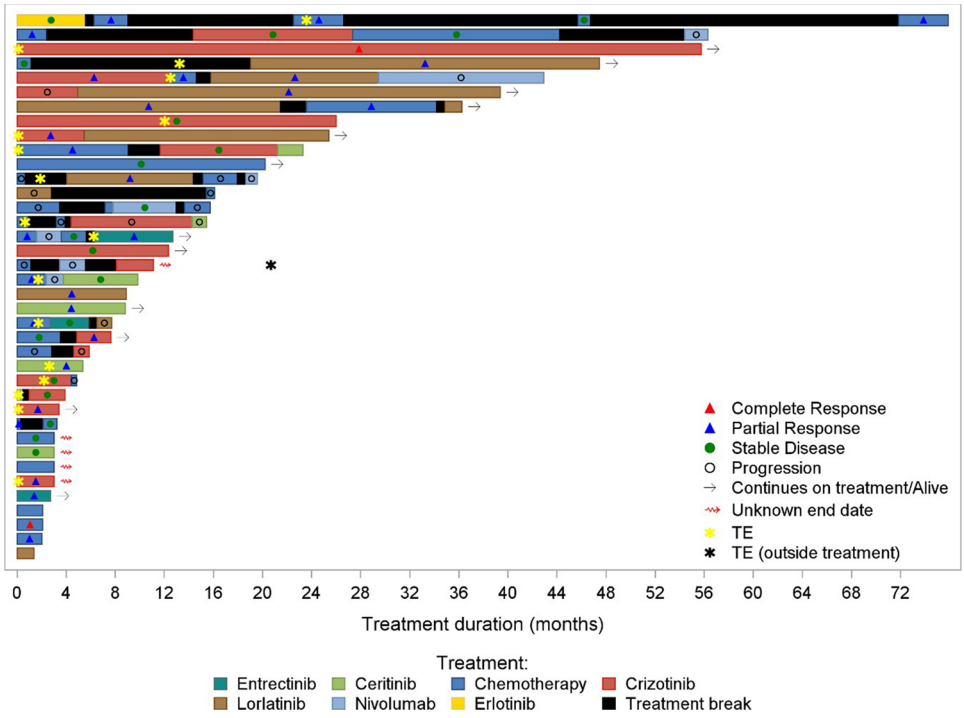

Fig. 2. Swimmer plot for treatment sequencing and thromboembolism (TE) incidence among patients who received at least one line of systemic therapy for the treatment of lung cancer.

Table 3
Best response by treatment and line of therapy overall and for patients with and without thromboembolism.

Systemic Agent	ORR[1], all		ORR, TE		ORR, no TE	
	Number	%	Number	%	Number	%
All lines of therapy						
Any agent	29/68	43%	17/40	43%	12/30	40%
Chemotherapy[2]	13/31	42%	8/17	47%	5/14	36%
TKI[3]	16/32	50%	10/20	50%	6/12	50%
Crizotinib	6/15	40%	5/10	50%	1/5	20%
Lorlatinib	6/8	60%	3/4	75%	3/4	75%
Ceritinib	2/5	40%	1/3	33%	1/2	50%
Entrectinib	2/3	67%	1/2	50%	1/1	100%
Erlotinib	0/1	0%	0/1	0%	–	–
Nivolumab	0/7	0%	0/5	0%	0/2	0%
First line therapy						
Chemotherapy	8/17	47%	4/8	50%	4/9	44%
TKI	10/17	59%	6/9	67%	4/8	50%
Any agent	18/34	53%	10/17	59%	8/17	47%

1 – ORR assessed for each line of treatment and reported as available in retrospective review of imaging with denominator showing total number of patients with PD/SD/PR/CR and numerator total number of patients with PR/CR; 2 – includes any cytotoxic chemotherapy; 3 – includes any TKI therapy. ORR – objective response rate; TKI – tyrosine kinase inhibitor.

3.4. Association between clinical variables and TE

Age; race; baseline ECOG; smoking history; treatment received; brain metastases at diagnosis; hereditary thrombophilia and NLR were not predictive of TE, Table 4. The *ROS1* fusion partner was known in two cases, both *CD74-ROS1*, one encountering TE (PE).

3.5. Association between TE and survival and response

Median OS in patients with TE was 21.3 months (95% CI 10.0-NR) versus 28.8 months (95% CI 13.9-NR) with no TE; hazard ratio (HR) 1.16 (95% CI 0.43–3.15, p = 0.77). Considering all treatments and all lines of therapy, TE did not appear to impact ORR (43% vs. 40% with and without TE) or DCR (79% vs. 78% with and without TE). However, ORR to first line chemotherapy (50% vs. 44%) and targeted therapies (67% vs. 50%) appeared marginally higher among patients with TE compared to without TE. More treatment lines were prescribed in those with TE compared to without TE (42 vs. 28 total across all patients); response according to specific treatments and lines of therapy are shown in Table 3.

4. Discussion

This multicenter series of patients with *ROS1*-rearranged NSCLC includes a representative cohort to prior published series, and intriguingly almost half the cohort developed TE. This is the highest incidence of TE reported in any subtype of NSCLC to date. Findings validate recently described high TE incidence in an international *ROS1* NSCLC cohort [6], and for the first time defines TE risk beyond the peri-diagnostic period. Additionally this paper reports on the natural history of disease and clinical course of *ROS1* patients treated at six centers across Australia.

Importantly, TE events occurred at time points across the patient's disease course, including pre and post diagnosis as well as across all therapies and throughout the disease course, a finding not previously reported. Six patients (14%) presented with TE prior to or at lung cancer diagnosis. In one further patient *ROS1* was diagnosed peri-mortem and prior to any treatment, after the presentation of a fatal arterial thrombus. This was the only patient who underwent investigation at the time of death, with no patients undergoing autopsy. More than half of the TE cohort experienced PE (n = 12/20, 60%) with overall PE incidence (n = 13/42, 31%), 8–15 times the incidence (2–4%) in general lung cancer cohorts [19,20].

Interestingly, treatment strategy did not seem to impact TE risk. Of the 19 patients with venous TE, only one did not go on to receive anti-cancer therapy due to poor performance status (TKI not available as routine care at the time). On therapy, TE occurred during or following systemic therapy; five whilst on chemotherapy, seven whilst on *ROS1* TKI, and one > 10 months after cessation of active therapy. Recurrent

Table 4
Association between thromboembolism incidence and clinical variables by univariate and multivariate Fine and Gray competing-risks regression, with death as a competing risk.

	Univariate sHR (95% CI)	Multivariate sHR (95% CI)[1]
Asian (yes vs. no)	0.65 (0.26–1.65), p = 0.366	0.67 (0.27–1.73), p = 0.420
ROS1 confirmed at diagnosis (yes vs. no)[2]	2.06 (0.90–4.75), p = 0.089	2.72 (0.99–7.44), p = 0.052
First line TKI (yes vs. no)	1.45 (0.62–3.40), p = 0.396	1.64 (0.65–4.12), p = 0.295
Any line TKI (yes vs. no)	2.46 (0.68–8.88), p = 0.168	2.24 (0.62–8.10), p = 0.220
Metastatic at diagnosis (yes vs. no)	0.71 (0.31–1.63), p = 0.420	0.89 (0.41–1.94), p = 0.771
NLR > = 5 at diagnosis (yes vs. no)	0.94 (0.32–2.78), p = 0.911	0.94 (0.30–2.90), p = 0.909
Platelet count > 350 × 10^9/L at diagnosis (yes vs. no)	1.53 (0.55–4.26), p = 0.416	1.30 (0.42–4.00), p = 0.648
Brain metastasis at diagnosis (yes vs. no)	0.33 (0.07–1.53), p = 0.156	0.57 (0.09–3.45), p = 0.537
Prior VTE	3.36 (1.03–10.99), p = 0.045	5.30 (1.35–20.86), p = 0.017
Age > 54 (median age of cohort)	0.97 (0.39–2.39), p = 0.940	0.90 (0.36–2.28), p = 0.830
Sex (male vs. female)	1.53 (0.61–3.80), p = 0.364	1.03 (0.40–2.67), p = 0.953
Smoking (ever vs. never)	0.50 (0.13–1.92), p = 0.314	0.64 (0.16–2.52), p = 0.520
ECOG PS (> = 1 vs. 0)	0.50 (0.21–1.19), p = 0.117	0.67 (0.25–1.79), p = 0.423
ECOG PS (> = 2 vs. 0–1)	1.49 (0.44–5.02), p = 0.522	3.58 (0.91–14.13), p = 0.069

1 – Multivariate regression adjusted for *ROS1* positivity at lung cancer diagnosis and ECOG (strongest univariate predictors); 2 – *ROS1* rearrangement identified at lung cancer diagnosis compared to a later time point, usually at disease relapse or progression. ECOG PS – Eastern Cooperative Oncology Group performance status; NLR – neutrophil lymphocyte ratio; sHR – sub-distribution hazard ratio; TE – thromboembolism; TKI – tyrosine kinase inhibitor.

TE on therapeutic anticoagulation occurs in approximately 2% of the general population treated for TE and up to 8% of patients with cancer [21,22], which appear less frequent than observed in this *ROS1* lung cancer cohort with 15% (n = 3/20) of patients experiencing recurrent TE events.

The possibility of an association between TE, thrombophilia and *ROS1* is provocative and while may be coincidental given the long known high association of cancer with thrombosis, draws our attention to the importance of evaluating malignant disease status and controlling disease when TE occurs. While thrombophilia testing was only conducted on a sub-set of patients (n = 10), the identification of a 30% rate (n = 3/10) of thrombophilia in those tested, appears notably higher than the rate of hereditary thrombophilia present in 0.2–5% of the general adult population [23], and 5–10% in general cancer cohorts [14,24]. Comparative incidence in lung cancer cohorts, and specifically *ROS1*, have not been widely reported. While the recent *ROS1* TE case series does not report thrombophilia [6], population differences should be noted with thrombophilia's expressed differently among ethnic populations [25]. In our cohort 31% of patients were of Asian ethnicity (not further specified), compared to 55% in the recent report by Ng and colleagues being of Chinese ethincity [6]. The most common thrombophilia detected in the current cohort was factor V Leiden (2), which has not been reported and is less relevant in Asians and of higher prevalence in Scandinavian populations [25,26]. This is an interesting observation warranting further investigation; however, must be interpreted with the limitations associated with retrospective review and highly limited population who underwent thrombophilia screening.

ROS1 was not routinely tested for in Australia during the recruitment period, as FISH testing was not Government reimbursed as there was no health system funded TKI prior to 2019. The treatment in this group was therefore heterogeneous; however, TE occurred across treatment types, as well as disease burdens and trajectories. Thirty-eight percent (n = 15/42) were managed on a clinical trial. While incidence of TE among *ROS1* trial cohorts are yet to be reported, it must be recognized that these will likely record lower incidence relative to real-world studies given the highly selected trial cohort would likely exclude patients with recent TE either as a specific exclusion or because of resultant decline in performance status. Furthermore, clinical trial patients at progression on their investigational treatment often return to their external referring center which may compromise follow up for true longitudinal TE incidence.

Just under half of the cohort (n = 19, 45%) received TKI as first line treatment, similar numbers with TE (n = 10) and without TE (n = 9). Interestingly ORR for TKI and chemotherapy was similar across all lines of therapy (42% vs. 43%), but higher for TKI than chemotherapy in first line therapy (59% vs. 47%). While TKI is now standard of care, some patients demonstrated durable ORR and DCR for various chemotherapy regimens.

ROS1 driven NSCLC is emerging to be a diverse disease, which may in part be influenced by the various fusion partners present. The role the *ROS1* fusion partner is poorly understood; however it has recently been reported the *CD74* fusion may confer an attenuated response to crizotinib and greater CNS tropism [27]. The role of fusion partners in thrombosis risk is unknown.

The role of primary thromboprophylaxis has been investigated with interest across a number of solid tumour groups with no clear evidence for an advantage, beyond the prevention of index TE events. Most pertinent was the FRAGMATIC clinical trial in NSCLC [28], which did not demonstrate a survival advantage with dalteparin in unselected patients. However, there was a significant reduction in the incidence of radiologically confirmed venous TE but with the tradeoff of increased incidence of clinically relevant non-major hemorrhage, therefore questioning the role of universal thromboprophylaxis. Unlike in the recent series by Ng et al. which found the Khorana TE risk score to be predictive of TE in their *ROS1* lung cancer cohort [6], in the FRAGMATIC and BIOTEL lung cancer cohorts it was of limited utility in selecting patients at heightened risk [28,29], as has been the case in further lung series [30–33]. The Khorana score was not assessed in the current cohort, however further investigation of its relevance and the utility of alternate risk prediction models such as the fibrinogen/d-dimer model proposed by members of this authorship group are of interest in oncogene driven NSCLC [29]. Whether routine thrombophilia screening at *ROS1* diagnosis may add to risk-stratification is unclear but worthy of investigation given signals from highly limited data. With an approaching 50% TE rate in this cohort, *ROS1* detection may indeed be a stand-alone indication for risk-directed primary TE prevention, however the highly variable time-course would suggest some form of further risk-stratification may be beneficial. Beyond the high TE rates in this study, primary TE prevention may have even greater significance given expected extension of TE risk duration in the context of greater availability of *ROS1* TKIs in treatment enabling improved survival. The observed high arterial TE rate in Ng et al.'s report (n = 14/193, 7%) [6] differs from the present report (n = 1/42, 2%), and has implications for which prophylactic agent is most appropriate.

Limitations of this series include recruitment prior to the routine prescribing of TKI therapy, which has changed the therapeutic landscape of *ROS1* NSCLC management. In survival and response assessments relative to TKI treatment (first or subsequent lines), we acknowledge inherent bias in selecting patients with later lines of therapy and the assumption that receipt of TKI at some stage of the patient

journey would still provide survival impact. Additionally, as previously acknowledged, patient data were collected by the primary physician in each center, with potential recall and selection bias and inability to absolutely define the true denominator of patients with *ROS1* NSCLC. While the cohort size is numerically small (n = 42), this represents a significant population relative to the low incidence of *ROS1* rearranged NSCLC, and is strengthened by recruitment across six independent institutions. Also, given the retrospective nature of this study there were important data points missing, as some patients were lost to follow up, for example when returning to an outlier center after clinical trial cessation. Efforts are currently underway to ameliorate these limitations though formation of a national registry for *ROS1* lung cancer in Australia, and linked biobank, which could pool resources across Australia (and internationally) to prospectively capture clinically impactful data.

5. Conclusion

This study validates previous data indicating a high TE risk in *ROS1*-rearranged NSCLC and is the first study to report the longitudinal TE profile and burden. This is particularly relevant given the shift in standard of care to first and next generation *ROS1* TKIs enabling longer survival, however increased time at risk of TE. Real-world data have not only confirmed a high incidence of TE in the peri-diagnostic period but newly demonstrated sustained risk over time and irrespective of treatment strategy. The limited data for co-occurring thrombophilia are speculative but concordant with other *ROS1* case reports and perhaps provocative of further consideration. Further investigation and validation of these data in larger collaborative cohorts and consideration of primary preventive strategies are warranted.

Funding

This project did not receive funding.

Authors' contributions

Conception and design: MA, NP, TJ, BS, MI. Collection and assembly of data: MA, NP, TJ, RO, SK, BH, AL, SC, MM, KB, BS, MI. Data analysis and interpretation: MA, RO, MI. Manuscript writing: MA, NP, TJ, RO, SK, BH, AL, SH, VH, SC, MM, KB, BS, MI. Final approval of manuscript: MA, NP, TJ, RO, SK, BH, AL, SH, VH, SC, MM, KB, BS, MI.

Data availability

Data supporting results is archived at institutions of first and senior authors with willingness and intent for data sharing by direct contact to the corresponding author.

Declaration of Competing Interest

The authors declare no conflict of interest directly related to this work however acknowledge the following associations outside the submitted work. Dr. Alexander has nothing to disclose. Dr. Pavlakis reports personal fees from Boerhinger Ingelheim, personal fees from Takeda, personal fees from Astra Zeneca, personal fees from BMS, personal fees from MSD, personal fees and other from Roche, grants and personal fees from Pfizer, personal fees from Novartis, personal fees from Amgen, personal fees from Merck KgA, personal fees from Merck Serono, grants from Bayer, personal fees from Ipsen, outside the submitted work. Dr. John reports personal fees from Ignyta, personal fees from Roche, personal fees from AstraZeneca, personal fees from Novartis, personal fees from Pfizer, personal fees from Merck, personal fees from BMS, outside the submitted work. Dr. O'Connell has nothing to disclose; Dr. Kao reports personal fees from Pfizer, personal fees from Novartis, personal fees from Roche, during the conduct of the study; personal fees from Boehringer, personal fees from BMS, personal fees from MSD, grants and personal fees from Astra Zeneca, outside the submitted work. Dr. Hughes reports personal fees from Pfizer, personal fees from Roche, personal fees from Merck Sharpe and Dohme, personal fees from Bristol Meyers Squibb, personal fees from AstraZeneca, grants from Amgen, outside the submitted work. Dr. Lee reports personal fees from Munipharma, outside the submitted work; Dr. Hayes has nothing to disclose. Dr. Howell has nothing to disclose. Dr. Clarke has nothing to disclose. Dr. Millward reports personal fees from Pfizer, during the conduct of the study; personal fees from Astra-Zeneca, personal fees from Bristol Myers Squibb, personal fees from Roche, personal fees from Merck Sharp & Dohme, outside the submitted work. Dr. Burbury has nothing to disclose. Dr. Solomon reports personal fees from Pfizer, personal fees from Roche/Genetech, personal fees from Novartis, personal fees from Merck, personal fees from Bristol Myers Squibb, personal fees from AstraZeneca, personal fees from Loxo Oncology, outside the submitted work. Dr. Itchins reports personal fees from Pfizer, personal fees from Roche, outside the submitted work.

Acknowledgements

The authors acknowledge the contribution of clinical staff involved in the management of these patients and in particular Ms. Ann Officer who provided major contribution to data collection at Peter MacCallum Cancer Centre.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2020.01.017.

References

[1] V. Tagalakis, D. Levi, J.S. Agulnik, V. Cohen, G. Kasymjanova, D. Small, High risk of deep vein thrombosis in patients with non-small cell lung Cancer: a cohort study of 493 patients, J. Thorac. Oncol. 2 (8) (2007) 729–734, https://doi.org/10.1097/JTO.0b013e31811ea275.

[2] J.W. Blom, C.J. Doggen, S. Osanto, F.R. Rosendaal, Malignancies, prothrombotic mutations, and the risk of venous thrombosis, JAMA 293 (6) (2005) 715–722, https://doi.org/10.1001/jama.293.6.715 e-pub ahead of print 2005/02/11.

[3] H.T. Sørensen, L. Mellemkjær, J.H. Olsen, J.A. Baron, Prognosis of cancers associated with venous thromboembolism, N. Engl. J. Med. 343 (25) (2000) 1846–1850, https://doi.org/10.1056/nejm200012213432504.

[4] F. Barlesi, J. Mazieres, J.P. Merlio, D. Debieuvre, J. Mosser, H. Lena, et al., Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT), Lancet 387 (10026) (2016) 1415–1426, https://doi.org/10.1016/s0140-6736(16)00004-0.

[5] F. Dou, H. Li, M. Zhu, L. Liang, Y. Zhang, J. Yi, et al., Association between oncogenic status and risk of venous thromboembolism in patients with non-small cell lung cancer, Respir. Res. 19 (1) (2018), https://doi.org/10.1186/s12931-018-0791-2 88-88.

[6] T.L. Ng, D.E. Smith, R. Mushtaq, T. Patil, A. Dimou, S. Yang, et al., ROS1 gene rearrangements are associated with an elevated risk of peridiagnosis thromboembolic events, J. Thorac. Oncol. 14 (4) (2019) 596–605, https://doi.org/10.1016/j.jtho.2018.12.001 e-pub ahead of print 2018/12/14.

[7] A. Zer, M. Moskovitz, D.M. Hwang, A. Hershko-Klement, L. Fridel, G.J. Korpanty, et al., ALK-rearranged non–Small-Cell lung Cancer Is associated with a high rate of venous thromboembolism, Clin. Lung Cancer 18 (2) (2017) 156–161, https://doi.org/10.1016/j.cllc.2016.10.007.

[8] M. Verso, R. Chiari, S. Mosca, L. Franco, M. Fischer, L. Paglialunga, et al., Incidence of Ct scan-detected pulmonary embolism in patients with oncogene-addicted, advanced lung adenocarcinoma, Thromb Res. 136 (5) (2015) 924–927, https://doi.org/10.1016/j.thromres.2015.09.006.

[9] M. Alexander, B. Solomon, K. Burbury, Thromboembolism in anaplastic lymphoma kinase–Rearranged non–Small cell lung Cancer, Clin. Lung Cancer 19 (1) (2018) e71–e72, https://doi.org/10.1016/j.cllc.2017.07.001.

[10] B.J. Solomon, T. Mok, D.-W. Kim, Y.-L. Wu, K. Nakagawa, T. Mekhail, et al., First-line crizotinib versus chemotherapy in ALK-Positive lung Cancer, N. Engl. J. Med. 371 (23) (2014) 2167–2177, https://doi.org/10.1056/NEJMoa1408440.

[11] A.T. Shaw, D.-W. Kim, R. Mehra, D.S.W. Tan, E. Felip, L.Q.M. Chow, et al., Ceritinib in ALK-Rearranged non–Small-Cell lung Cancer, N. Engl. J. Med. 370 (13) (2014) 1189–1197, https://doi.org/10.1056/NEJMoa1311107.

[12] A.T. Shaw, L. Gandhi, S. Gadgeel, G.J. Riely, J. Cetnar, H. West, et al., Alectinib in ALK-positive, crizotinib-resistant, non-small-cell lung cancer: a single-group,

multicentre, phase 2 trial, Lancet Oncol. 17 (2) (2016) 234–242, https://doi.org/10.1016/S1470-2045(15)00488-X.
[13] S.N. Gettinger, L.A. Bazhenova, C.J. Langer, R. Salgia, K.A. Gold, R. Rosell, et al., Activity and safety of brigatinib in ALK-rearranged non-small-cell lung cancer and other malignancies: a single-arm, open-label, phase 1/2 trial, Lancet Oncol. 17 (12) (2016) 1683–1696, https://doi.org/10.1016/S1470-2045(16)30392-8.
[14] A. Heraudeau, A. Delluc, M. Le Henaff, K. Lacut, C. Leroyer, B. Desrues, et al., Risk of venous thromboembolism in association with factor V leiden in cancer patients - the EDITH case-control study, PLoS One 13 (5) (2018), https://doi.org/10.1371/journal.pone.0194973 e0194973-e0194973.
[15] A. Lee, V.M. Howell, M. Itchins, H.R. Wheeler, Pavlakis N. ROS1 rearranged non–Small-Cell lung Cancer, factor V leiden, and recurrent venous thromboses, Clin. Lung Cancer 19 (5) (2018) 457–459, https://doi.org/10.1016/j.cllc.2018.05.015.
[16] D. Thaker, E. Douglas, J. Blazak, W. Xu, B. Hughes, M. Burge, et al., An analysis of incidental and symptomatic pulmonary embolism (PE) in medical oncology patients, Asia. J. Clin. Oncol. 13 (3) (2017) 243–248, https://doi.org/10.1111/ajco.12650.
[17] J.P. Fine, R.J. Gray, A proportional hazards model for the subdistribution of a competing risk, J. Am. Stat. Assoc. 94 (446) (1999) 496–509, https://doi.org/10.1080/01621459.1999.10474144.
[18] L.H. Schwartz, S. Litière, E. de Vries, R. Ford, S. Gwyther, S. Mandrekar, et al., RECIST 1.1-Update and clarification: from the RECIST committee, Eur. J. Cancer (62) (2016) 132–137, https://doi.org/10.1016/j.ejca.2016.03.081 e-pub ahead of print 2016/05/14.
[19] R.D. Malgor, T.V. Bilfinger, N. Labropoulos, A systematic review of pulmonary embolism in patients with lung Cancer, Ann. Thorac. Surg. 94 (1) (2012) 311–316, https://doi.org/10.1016/j.athoracsur.2012.03.025.
[20] J.M. Sun, T.S. Kim, J. Lee, Y.H. Park, J.S. Ahn, H. Kim, et al., Unsuspected pulmonary emboli in lung cancer patients: the impact on survival and the significance of anticoagulation therapy, Lung Cancer 69 (3) (2010) 330–336, https://doi.org/10.1016/j.lungcan.2009.11.015 e-pub ahead of print 2009/12/17.
[21] T. van der Hulle, J. Kooiman, P.L. den Exter, O.M. Dekkers, F.A. Klok, M.V. Huisman, Effectiveness and safety of novel oral anticoagulants as compared with vitamin K antagonists in the treatment of acute symptomatic venous thromboembolism: a systematic review and meta-analysis, J. Thromb. Haemost. 12 (3) (2014) 320–328, https://doi.org/10.1111/jth.12485 e-pub ahead of print 2013/12/18.
[22] S. Piran, S. Schulman, Management of recurrent venous thromboembolism in patients with cancer: a review, Thromb. Res. 164 (Suppl 1) (2018) S172–S177, https://doi.org/10.1016/j.thromres.2017.12.019 e-pub ahead of print 2018/04/29.
[23] P. MacCallum, L. Bowles, D. Keeling, Diagnosis and management of heritable thrombophilias, BMJ 349 (2014) g4387, https://doi.org/10.1136/bmj.g4387 e-pub ahead of print 2014/07/19.
[24] I. Pabinger, C. Ay, D. Dunkler, J. Thaler, E.-M. Reitter, C. Marosi, et al., Factor V Leiden mutation increases the risk for venous thromboembolism in cancer patients – results from the Vienna Cancer And Thrombosis Study (CATS), J. Thromb. Haemost. 13 (1) (2015) 17–22, https://doi.org/10.1111/jth.12778.
[25] D.C. Rees, M. Cox, J.B. Clegg, World distribution of factor V Leiden, Lancet 346 (8983) (1995) 1133–1134 e-pub ahead of print 1995/10/28.
[26] B. Zoller, O. Melander, P.J. Svensson, G. Engstrom, Factor V Leiden paradox in a middle-aged Swedish population: a prospective study, Vasc. Med. 23 (1) (2018) 52–59, https://doi.org/10.1177/1358863x17745591 e-pub ahead of print 2018/01/13.
[27] Z. Li, L. Shen, D. Ding, J. Huang, J. Zhang, Z. Chen, et al., Efficacy of crizotinib among different types of ROS1 fusion partners in patients with ROS1-Rearranged non-small cell lung Cancer, J. Thorac. Oncol. 13 (7) (2018) 987–995, https://doi.org/10.1016/j.jtho.2018.04.016 e-pub ahead of print 2018/04/29.
[28] F. Macbeth, S. Noble, J. Evans, S. Ahmed, D. Cohen, K. Hood, et al., Randomized phase III trial of standard therapy plus low molecular weight heparin in patients with lung Cancer: fragmatic trial, J. Clin. Oncol. 34 (5) (2016) 488–494, https://doi.org/10.1200/jco.2015.64.0268 e-pub ahead of print 2015/12/25.
[29] M. Alexander, D. Ball, B. Solomon, M. MacManus, R. Manser, B. Riedel, et al., Dynamic thromboembolic risk modelling to target appropriate preventative strategies for patients with non-small cell lung Cancer, Cancers (Basel) 11 (1) (2019), https://doi.org/10.3390/cancers11010050.
[30] A.S. Mansfield, A.J. Tafur, C.E. Wang, T.V. Kourelis, E.M. Wysokinska, P. Yang, Predictors of active cancer thromboembolic outcomes: validation of the Khorana score among patients with lung cancer, J. Thromb. Haemost. 14 (9) (2016) 1773–1778, https://doi.org/10.1111/jth.13378 e-pub ahead of print 2016/06/09.
[31] I. Vathiotis, E.P. Dimakakos, P. Boura, A. Ntineri, A. Charpidou, G. Gerotziafas, et al., Khorana score: nuew predictor of early mortality in patients with lung adenocarcinoma, Clin. Appl. Thromb. Hemost. 24 (8) (2018) 1347–1351, https://doi.org/10.1177/1076029618777153 e-pub ahead of print 2018/05/29.
[32] J. Rupa-Matysek, M. Lembicz, E.K. Rogowska, L. Gil, M. Komarnicki, H. Batura-Gabryel, Evaluation of risk factors and assessment models for predicting venous thromboembolism in lung cancer patients, Med. Oncol. 35 (5) (2018) 63, https://doi.org/10.1007/s12032-018-1120-9 e-pub ahead of print 2018/04/05.
[33] H. Abdel-Razeq, A. Mansour, H. Abdulelah, A. Al-Shwayat, M. Makoseh, M. Ibrahim, et al., Thromboembolic events in cancer patients on active treatment with cisplatin-based chemotherapy: another look!, Thromb. J. 16 (2018) 2, https://doi.org/10.1186/s12959-018-0161-9 e-pub ahead of print 2018/03/07.