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ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ifon20

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To cite this article: Ambica Parmar, Brandon Lu, Jin Luo & Kelvin K W Chan (29 May 2024): Real-world comparative effectiveness and safety of pembrolizumab for PD-L1≥50% metastatic non-small-cell lung cancer, Future Oncology, DOI: 10.1080/14796694.2024.2342224

To link to this article: https://doi.org/10.1080/14796694.2024.2342224

FUTURE ONCOLOGY
https://doi.org/10.1080/14796694.2024.2342224

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RESEARCH ARTICLE

Real-world comparative effectiveness and safety of pembrolizumab for PD-L1 ≥50% metastatic non-small-cell lung cancer

Ambica Parmar[‡,a,c], Brandon Lu[‡,a], Jin Luo[b] and Kelvin K W Chan[*,a,b,c,d,e]

[a]Division of Medical Oncology & Hematology, Department of Medicine, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Toronto, ON M4N 3M5, Canada; [b]ICES, 2075 Bayview Avenue, Toronto, ON M4N 3M5, Canada; [c]Faculty of Medicine, University of Toronto, 27 King's College Circle, Toronto, ON M5S 1A1, Canada; [d]Canadian Centre for Applied Research in Cancer Control, 2075 Bayview Avenue, Toronto, ON M4N 3M5, Canada; [e]Ontario Health (Cancer Care Ontario), Toronto, ON, Canada

ABSTRACT

Background: Despite the demonstrated efficacy of pembrolizumab in KEYNOTE-024, effectiveness and safety in routine practice remain unclear. **Methods:** The authors identified first-line pembrolizumab or chemotherapy patients from April 2013 to March 2021. The primary outcome was overall survival; the secondary safety outcomes included rates of hospitalization, emergency department visits, specialist visits, and adverse events. Baseline differences were adjusted using propensity score matching (1:1). **Results:** The matched cohort included 2284 pairs. Median overall survival for pembrolizumab (13.0 months) was significantly longer than for chemotherapy (9.2 months), with a hazard ratio of 0.81 (95% CI: 0.71–0.92). Pembrolizumab patients reported significantly more adverse events and specialist visits, as well as a higher 1-year cumulative incidence of direct hospitalizations. **Conclusion:** The survival benefit of first-line pembrolizumab persists in the real world, although with increased toxicity and diminished benefit.

ARTICLE HISTORY
Received 3 December 2023
Accepted 9 April 2024

KEYWORDS
comparative effectiveness; comparative safety; immunotherapy; non-small-cell lung cancer; pembrolizumab; real-world evidence; safety

1. Background

The integration of immune checkpoint blockade into the therapeutic landscape for patients with metastatic non-small-cell lung cancer (mNSCLC) has led to substantial improvements in patient survival and quality of life [1–12]. With significant clinical benefit demonstrated in three phase III randomized, controlled trials (RCTs), pembrolizumab is now included as a standard-of-care, first-line treatment for all patients with mNSCLC who lack an actionable genomic alteration [1,2,7,8,10,12,13]. Across these trials, tumoral PD-L1 expression levels were used to guide the use of pembrolizumab as monotherapy or in combination with platinum-based chemotherapy [1,2,7,8,10,12]. The clinical benefit of pembrolizumab monotherapy among patients with tumoral PD-L1 expression level ≥50% was established in the pivotal KEYNOTE-024 phase III trial, in which pembrolizumab monotherapy demonstrated a significant improvement in overall survival (OS) with a median OS of 26.3 months (95% CI: 18.3–40.4) as compared with 13.4 months (95% CI: 9.4–18.3) with platinum-based chemotherapy (hazard ratio [HR]: 0.62 [95% CI: 0.48–0.81]) [10,12,14].

Although the clinical efficacy of pembrolizumab for PD-L1 high-expressing mNSCLC has been demonstrated among these phase III trials, a growing body of literature characterizing a gap in efficacy and real-world treatment effectiveness leads to uncertainty as to the expected real-world clinical effectiveness of pembrolizumab in this subpopulation of mNSCLC patients [15–18]. To date, studies examining the real-world effectiveness of pembrolizumab monotherapy in the first-line setting of PD-L1 ≥50% mNSCLC have demonstrated conflicting results regarding the efficacy–effectiveness gap between RCTs and real-world evidence [19–23]. In addition, data examining the real-world comparative effectiveness of pembrolizumab to platinum-based chemotherapy among PD-L1 high expressors have been limited [22]. Accordingly, the authors performed a real-world, population-level, cohort study to examine the comparative effectiveness and safety of pembrolizumab monotherapy for mNSCLC patients with tumoral PD-L1 expression ≥50%, as compared with first-line platinum-based chemotherapy.

2. Methods

2.1. Study cohort & design

The authors conducted a population-based, retrospective cohort study using linked administrative databases in Ontario, Canada. All adult (age ≥18 years) patients

CONTACT Kelvin KW Chan Tel.: +1 416 480 4928; kelvin.chan@sunnybrook.ca

‡Authors contributed equally

Supplemental data for this article can be accessed at https://doi/suppl/10.1080/14796694.2024.2342224

diagnosed with NSCLC, as identified through the Ontario Cancer Registry (OCR), were first identified using International Classification of Diseases for Oncology (ICD) codes, inclusive of relevant non-small-cell histologies (Supplementary Table S1). This cohort was then linked with the New Drug Funding Program database to identify patients who received first-line systemic treatment for mNSCLC between 1 April 2013 and 31 March 2021. In Ontario, first-line pembrolizumab is only funded for patients without *EGFR* mutations or anaplastic lymphoma kinase translocations. Cases were defined as mNSCLC patients who had received first-line pembrolizumab monotherapy between 17 January 2018 and 31 March 2021 (i.e., after the Ontario funding date for pembrolizumab). Ontario funding for pembrolizumab is limited to patients with tumoral PD-L1 expression $\geq$50%. Thus, cases were assumed to include only those with high tumoral PD-L1 expression levels. Controls were defined as patients who had received first-line platinum-based chemotherapy between 1 April 2013 and 17 January 2018. Platinum-based chemotherapy was identified as the receipt of one of gemcitabine, paclitaxel, pemetrexed, or vinorelbine with metastatic intent, as captured in the New Drug Funding Program database. As PD-L1 expression-level data were not collected prior to 17 January 2018, the control cohort was not restricted to patients with PD-L1 expression $\geq$50%. According to results from the KEYNOTE-042 trial, PD-L1 expression levels do not significantly affect the prognosis of mNSCLC patients receiving only chemotherapy [5]. Patients were excluded if they were nonresidents of Ontario, had another cancer diagnosis, received treatment prior to the OCR diagnosis date, and received pembrolizumab before the funding date.

Population-level administrative databases held at ICES were utilized to retrieve relevant data for study cohort identification, covariate and outcome assessment, and data analysis (Supplementary Table S2). These datasets were linked using unique encoded identifiers and analyzed at ICES, an independent, nonprofit research institute funded by an annual grant from the Ontario Ministry of Health and Long-Term Care. As a prescribed entity under Ontario's privacy legislation, ICES is authorized to collect and use healthcare data for the purposes of health system analysis, evaluation, and decision support. Secure access to these data is governed by policies and procedures that are approved by the Information and Privacy Commissioner of Ontario. To mitigate any risk for re-identification, small-cell counts (<6 patients) are suppressed herein. This study was approved by the Sunnybrook Research Ethics Board and was reported in accordance with the RECORD-PE and STaRT-RWE checklists (Supplementary Tables S3 & S4) [24,25].

2.2. Study outcomes

The primary outcome was OS defined as the time from the index date until death or censored if alive at the end of the follow-up period (31 March 2022), at the loss of Ontario Health Insurance Plan eligibility, or if alive 4 years following the index date. The index date was defined as the first treatment date with first-line pembrolizumab (for cases) or first-line platinum-based chemotherapy (for controls). Resource utilization during treatment, including hospitalization records (from the Canadian Institute for Health Information [CIHI] Discharge Abstract Database), emergency department visits (from the CIHI National Ambulatory Care Reporting System database), and specialist visits, was examined as secondary safety outcomes. Direct hospitalizations were defined as hospitalizations without an emergency department visit. All hospitalization records were assessed for immunotherapy-related complications using the admitting diagnostic ICD-10 codes, including cardiac disorders, circulatory disorders, respiratory disorders, neurologic disorders, gastrointestinal disorders, genitourinary disorders, endocrine disorders, metabolic disorders, skin disorders, and infections. Specialist visits, including visits to ophthalmologists, respirologists, rheumatologists, gastroenterologists, and endocrinologists, were identified from the Ontario Health Insurance Plan physician claims database.

2.3. Study covariates

Linked administrative datasets were used to assess baseline study cohort covariates. Patient demographic data, including age and sex, were assessed using the Registered Persons Database. Neighborhood-level income quintile, health region of residence (Local Health Integrated Network), and rurality status were assessed using Canadian 2016 census data using the patients' registered postal codes. Baseline comorbidities were assessed using the Charlson Comorbidity Index (CCI) and Adjusted Clinical Groups (ACG® system Aggregated Diagnosis Groups [ADGs], John Hopkins ACG® system, version 10.0) ADG score [26]. For both methods of comorbidity assessment, a lookback window of 2 years from the index date was used. The CIHI Discharge Abstract Database and National Ambulatory Care Reporting System were used to ascertain comorbid status for calculation of both CCI and ADG. Specific comorbidities of asthma, chronic obstructive pulmonary disease, dementia, hypertension, diabetes mellitus, congestive heart failure, and rheumatoid arthritis were also assessed. The assessed covariates that were specific to the diagnosis of mNSCLC included year of OCR diagnosis, year of treatment, and time from diagnosis to treatment. Stage of NSCLC at diagnosis, histology (squamous vs nonsquamous), and history of lung

or nonlung cancer diagnosis were assessed using data from OCR. Receipt of prior treatment inclusive of prior surgical resection of primary lung cancer, receipt of adjuvant chemotherapy, and receipt of radiation therapy were identified using CIHI Discharge Abstract Database, New Drug Funding Program, Activity Level Reporting and Ontario Health Insurance Plan databases (Supplementary Table S2).

2.4. Statistical analysis

Descriptive statistics were used to summarize the baseline study cohort characteristics. Categorical variables were described with frequencies (N) and percentage (%). Continuous variables were described using means with standard deviations or medians with interquartile ranges. Comparisons of baseline characteristics between cases and controls were performed using standardized difference of the mean, median of numeric variables, and proportion of each category of categorical variables. In addition, p-values were computed from chi-squared tests for binary and categorical covariates, the Kruskal–Wallis test for median and interquartile range, one-way analysis of variance for continuous variables, and the Cochran–Armitage trend test for ordinal variables.

Propensity score matching was used to adjust for differences in baseline covariates between the case and control cohorts. A logistic regression model was used to calculate the propensity scores inclusive of the following covariates: age at index date; sex; cancer stage at diagnosis; time from diagnosis to first treatment; income quintile; rurality; Local Health Integrated Network; CCI; ADG; diagnosis of asthma, chronic obstructive pulmonary disease, dementia, hypertension, diabetes mellitus, congestive heart failure, or rheumatoid arthritis; resource utilization band; receipt of adjuvant chemotherapy; receipt of radiotherapy to the brain; receipt of radiotherapy to the lung; prior surgical resection; and prior cancer diagnosis (lung and any cancer diagnosis) within 5 years of the index date. Histology (squamous versus nonsquamous) was hard-matched. Using the logit of the propensity scores, cases and comparators were matched 1:1 with a caliper width equal to 0.1-times the standard deviation of the propensity score. Standardized differences between the adjusted covariates that were less than or equal to 0.1 were considered to represent acceptable balances [27].

The Kaplan–Meier method was used to assess OS, with median OS estimated among the living and differences in survival calculated using the log-rank test. HRs were computed using Cox proportional hazards regression models. For the safety end points, the absolute risk of safety was estimated at 1 year after the index date using the cumulative incidence function and summarized using descriptive statistics. With mortality accounted for as a competing risk, the groups were compared using Fine–Gray competing risk models [28]. A sensitivity analysis examining OS by histology subgroups (squamous versus nonsquamous) was also conducted. A p-value of <0.05 using a two-sided test was used to denote statistical significance. All statistical analyses were conducted using SAS, version 9.3 (SAS Institute, Inc., NC, USA).

3. Results

3.1. Study cohort & baseline demographics

Of the 44,049 patients diagnosed with mNSCLC between 1 April 2013 and 31 March 2021, 9723 patients were treated with first-line pembrolizumab or platinum-based chemotherapy in the metastatic setting. Following application of the inclusion/exclusion criteria and the removal of patients who had missing data on cancer stage, income quintile or rurality status, a total of 4815 patients were included in the our final cohort. In this cohort, 1190 patients received first-line pembrolizumab in the metastatic setting (i.e., cases) and 3625 patients received platinum-based chemotherapy (i.e., controls). The propensity score-matched cohort included 2284 patients (Figure 1), with all baseline patient and disease characteristics well balanced between the matched groups (Table 1). Baseline demographics of the cohort before propensity score matching are detailed in Supplementary Box S1.

3.2. Overall survival

The median follow-up time for the propensity score-matched cohort was 10.7 months (pembrolizumab: 12.8; platinum-based chemotherapy: 9.2 months). In the propensity score-matched cohort, patients treated with pembrolizumab monotherapy were found to have longer OS than patients treated with platinum-based chemotherapy (median OS: 13.0 months; 95% CI: 11.8–14.6 vs 9.2 months, 95% CI: 8.0–10.0, respectively); 1-, 2-, 3- and 4-year survival estimates are reported in Table 2. The HR for mortality for the real-world propensity score-matched cohort was 0.81 (95% CI: 0.71–0.92). Figure 2 depicts the survival curves of patients treated with pembrolizumab and platinum-based chemotherapy from the propensity score-matched cohort.

3.3. Sensitivity analysis

Overall survival by histology subgroups (squamous vs nonsquamous) was examined through sensitivity analyses in the propensity score-matched cohort. Supplementary Figure S1 depicts the Kaplan–Meier

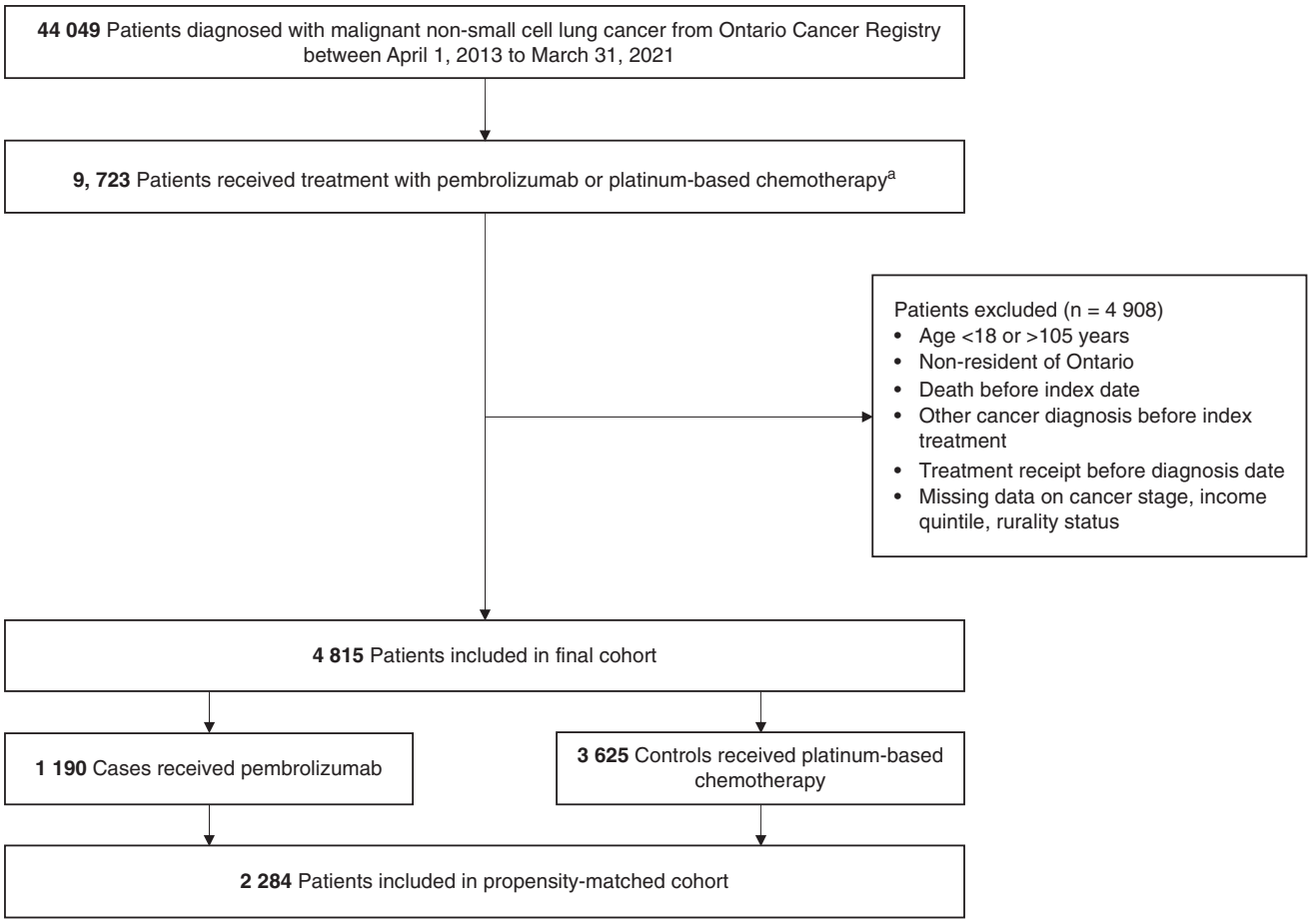

Figure 1. Cohort creation.
[a]Platinum-based chemotherapy defined as receipt of one of gemcitabine, paclitaxel, pemetrexed, or vinorelbine with metastatic intent, as identified in the New Drug Funding Program database.

survival curves. Among patients with squamous histology (n = 484), median OS for patients treated with pembrolizumab, as compared with platinum-based chemotherapy, was significantly longer at 10.7 months (95% CI: 9.1–12.8) versus 7.2 months (95% CI: 5.9–8.8); p = 0.03. Similarly, among patients with nonsquamous histology (n = 1800), OS was found to be significantly longer among patients treated with pembrolizumab (median OS: 14.2 months; 95% CI: 12.5–16.7) as compared with platinum-based chemotherapy (median OS: 9.8; 95% CI: 8.8–10.6; p < 0.0001). The HRs for mortality were 0.77 (95% CI: 0.56–1.07) and 0.81 (95% CI: 0.70–0.94) for patients with squamous and nonsquamous histology, respectively.

3.4. Safety outcomes

For all hospitalization, emergency department visit, adverse event, and specialist visit end points, resource utilization was much greater among patients who received pembrolizumab compared with patients who received chemotherapy (Table 3). The 1-year cumulative incidence of all-cause hospitalizations (42.4 vs 32.1%), direct hospitalizations (11.5 vs 6.8%), and emergency department visits leading to hospitalizations (35.7 vs 27.7%) was significantly higher in the pembrolizumab group, whereas emergency department visits not leading to hospitalization were not significantly different (37.1 vs 36.5%). The cumulative incidence curves of these end points within 180 days of the index date are depicted in Supplementary Figure S2. Compared with chemotherapy patients, pembrolizumab patients were also significantly more likely to visit a specialist and report cardiac disorder, circulatory disorder, respiratory disorder, and infection.

4. Discussion

In this population-based study, the authors compared the real-world effectiveness and safety of pembrolizumab monotherapy with tumoral PD-L1 expression ≥50% with those of first-line platinum-based chemotherapy for patients with mNSCLC in Ontario, Canada. They found that pembrolizumab monotherapy was associ-

Table 1. Baseline characteristics for matched and unmatched cohorts.

Characteristics	Full cohort			Propensity score-matched cohort		
	Chemotherapy (N = 3625)	Pembrolizumab (N = 1190)	p-value	Chemotherapy (N = 1142)	Pembrolizumab (N = 1142)	Standardized difference
Age at treatment initiation (mean ± SD)	66.5 (9.5)	65.6 (9.5)	<0.001	68.7 (8.6)	68.8 (8.9)	0.02
Female (%)	1763 (48.6)	587 (49.3)	<0.001	566 (48.7)	566 (49.6)	0.02
Histology (%)			0.643			
Nonsquamous	2 853 (78.7)	929 (78.1)		900 (78.8)	900 (78.8)	0
Squamous	772 (21.3)	261 (21.9)		242 (21.2)	242 (21.2)	0
Cancer stage at diagnosis (%)						
I	142 (3.9)	68 (5.7)	0.004	61 (5.3)	57 (5.0)	0.02
II	146 (4.0)	68 (5.7)		57 (5.0)	58 (5.1)	0
III	694 (19.1)	217 (18.2)		201 (17.6)	207 (18.1)	0.01
IV	2643 (72.9)	837 (70.3)		823 (72.1)	820 (71.8)	0.01
Prior any cancer diagnosis[†] (%)	28 (0.8)	9 (0.8)	0.956	9 (0.8)	9 (0.8)	0
ADG[‡] (mean +/- SD)	8.4 (3.0)	8.7 (3.0)	0.002	8.8 (3.2)	8.7 (3.1)	0.02
ADG category[‡] (%)						
0–5	601 (16.6)	182 (15.3)	0.086	178 (15.6)	176 (15.4)	0
6–9	1759 (48.5)	551 (46.3)		511 (44.7)	534 (46.8)	0.04
10+	1265 (34.9)	457 (38.4)		454 (39.7)	432 (37.8)	0.04
Charlson Comorbidity Index (%)						
No hospitalization	570 (15.7)	159 (13.4)	0.008	157 (13.7)	152 (13.3)	0.01
0	2144 (59.1)	682 (57.3)		665 (58.2)	657 (57.5)	0.01
1	580 (16.0)	208 (17.5)		197 (17.3)	201 (17.6)	0.01
2+	331 (9.1)	141 (11.8)		123 (10.8)	132 (11.6)	0.03
Rural (%)	520 (14.3)	194 (16.3)	0.099	192 (16.8)	184 (16.1)	0.02
Income quintile (%)						
1 (lowest)	784 (21.6)	283 (23.8)	0.0174	268 (23.5)	265 (23.2)	0.01
2	821 (22.6)	282 (23.7)		273 (23.9)	272 (23.8)	0
3	761 (21.0)	220 (18.5)		211 (18.5)	217 (19.0)	0.01
4	670 (18.5)	228 (19.2)		228 (20.0)	217 (19.0)	0.02
5 (highest)	589 (16.2)	177 (14.9)		162 (14.2)	171 (15.0)	0.02
Prior lung surgery[§] (%)	259 (7.1)	104 (8.7)	0.071	94.8 (8.2)	95 (8.3)	0
Prior lung radiation (%)	782 (21.6)	337 (28.3)	<0.001	306 (26.8)	305 (26.7)	0
Prior brain radiation (%)	449 (12.4)	169 (14.2)	<0.001	154 (13.5)	165 (14.4)	0.03
Months from diagnosis to systemic treatment (mean ± SD)	4.9 (6.9)	7.0 (11.9)	<0.001	6.3 (8.4)	6.0 (9.4)	0.04

[†]Any cancer diagnosis in the 5 years before the index date (i.e., date of first treatment with either pembrolizumab or platinum-based chemotherapy in the metastatic setting).

[‡]ADG assessed in the 2 years before the index date. Assessment of ADG did not include ADG 31 (prevention/administrative), ADG 33 (pregnancy), and ADGE 34 (dental).

[§]Lung surgeries included complete surgical resection, as classified by the Canadian Institutes for Health Information Canadian Classification of Health Intervention codes (Supplementary Table S1 includes lung surgical resection Charlson Comorbidity Index codes).

ADG: Aggregated Diagnosis Group; SD: Standard deviation.

Table 2. Survival outcomes.

	Chemotherapy		Pembrolizumab	
	Real-world cohort[†]	KEYNOTE-024	Real-world cohort[†]	KEYNOTE-024
Median overall survival (95% CI, months)	9.2 (8.5–10.0)	13.4 (9.8–18.3)	13.0 (11.8–14.6)	26.3 (18.3–40.4)
1-year survival rate (%)	39.1	54.8	52.4	70.3
2-year survival rate (%)	21.3	34.5	34.1	51.5
3-year survival rate (%)	12.7	24.7	24.9	35.8
4-year survival rate (%)	9.8	19.8	20.6	35.8

[†]Median follow-up time and survival outcomes reported for the propensity-matched cohorts.
Survival outcomes reported from the KEYNOTE-024 trial [12,14].

ated with improved survival compared with platinum-based chemotherapy in the real-world propensity score-matched cohort (median OS: 13.0 vs 9.2 months, respectively), although the difference in median OS and the HR for mortality were notably less favorable when compared with those reported in KEYNOTE-024 (real-world difference in median OS was 3.8 months with HR: 0.81 and 95% CI: 0.71–0.92; trial-based difference in median OS was 12.9 months with HR: 0.62 and 95% CI: 0.48–0.81) [14]. The higher rates of hospitalization, emergency department visits, treatment-related adverse events, and specialist visits that the authors observed among patients

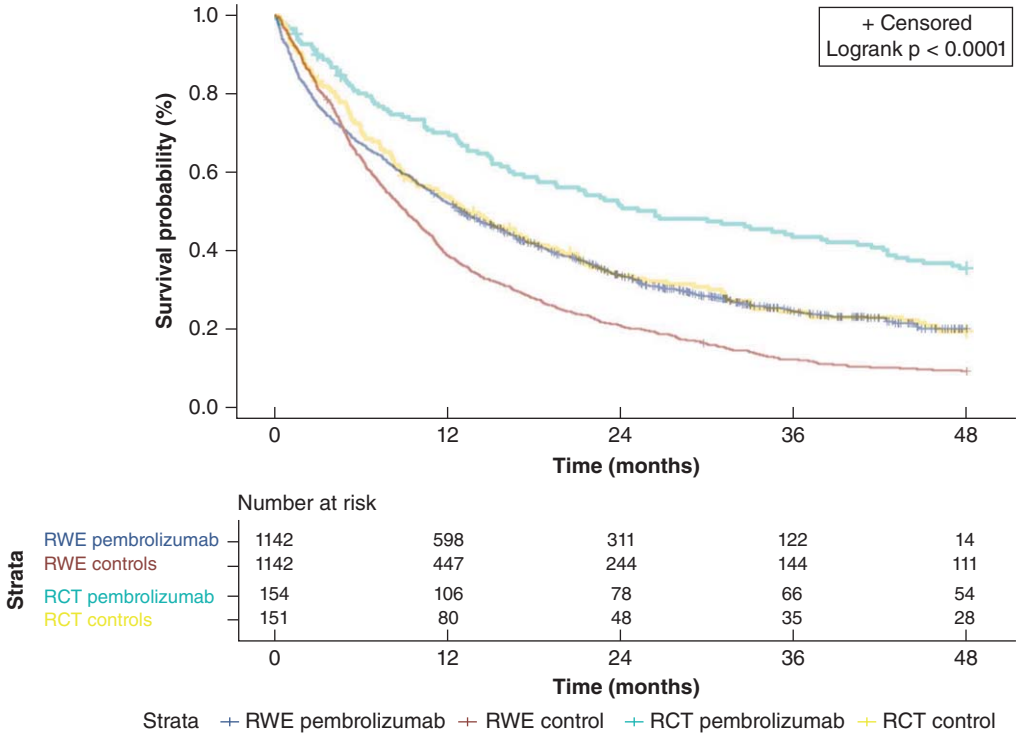

Figure 2. Overall survival curves from propensity-matched cohort. Kaplan–Meier survival curves for patients treated with pembrolizumab (cases, depicted in blue) and patients treated with platinum-based chemotherapy (controls, depicted in red). RCT: Randomized controlled trial; RWE: Real world evidence.

Table 3. Safety outcomes.

Outcome	Number of events (%)		p-value	1-year cumulative incidence, %		p-value
	Pembrolizumab (N = 1142)	Chemotherapy (N = 1142)		Pembrolizumab (N = 1142)	Chemotherapy (N = 1142)	
All-cause hospitalization	549 (48.1)	360 (32.3)	<0.001	42.4	32.1	<0.0001
Direct hospitalization	153 (13.4)	79 (6.9)	<0.001	11.5	6.8	0.0002
ED visit leading to hospitalization	465 (40.7)	319 (27.9)	<0.001	35.7	27.7	<0.0001
ED visit not leading to hospitalization	465 (40.7)	419 (36.7)	0.048	37.1	36.5	0.61
Hospital and ED visits with immunotherapy-related toxicity diagnosis						
Cardiac	166 (14.5)	93 (8.1)	<0.001	–	–	–
Circulatory	20 (1.8)	9 (0.8)	0.04	–	–	–
Respiratory	12 (11)	<6	0.002	–	–	–
Neurologic	<6	6	1	–	–	–
Gastrointestinal	125 (10.9)	110 (9.6)	0.302	–	–	–
Genitourinary	<6	<6	0.563	–	–	–
Endocrine	<6	0	0.157	–	–	–
Metabolic	123 (10.8)	104 (9.1)	0.184	–	–	–
Skin	<6	<6	0.179	–	–	–
Infection	242 (21.2)	162 (14.2)	<0.001	–	–	–
Any immunotherapy-related toxicity	468 (41.1)	352 (30.8)	<0.001	–	–	–
Specialist visits during treatment						
Ophthalmologists	124 (10.9)	64 (5.6)	<0.001	–	–	–
Respirologists	302 (26.4)	196 (17.2)	<0.001	–	–	–
Rheumatologists	52 (4.6)	19 (1.7)	<0.001	–	–	–
Gastroenterologists	114 (10.0)	43 (3.8)	<0.001	–	–	–
Endocrinologists	124 (10.9)	29 (2.5)	<0.001	–	–	–
Visited at least one of the above specialists	530 (46.4)	308 (27.0)	<0.001	–	–	–

ED: Emergency department.

who received pembrolizumab indicated a worse safety profile than expected from the trial. Nonetheless, the results from the sensitivity analysis confirmed the survival benefit of pembrolizumab monotherapy over platinum-based chemotherapy in the real world regardless of histology, albeit of smaller magnitudes of absolute and relative survival benefit.

The real-world median OS was found to be 13.3 and 4.2 months shorter in the pembrolizumab (real-world: 13.0 months; trial: 26.3 months) and chemotherapy groups (real-world: 9.2 months; trial: 13.4 months), respectively, with the HR observed in this study (HR: 0.81) differing significantly from that of the pivotal trial (HR: 0.62) [14]. These OS estimates are congruent with previous single-arm, real-world studies that have characterized an efficacy–effectiveness gap for first-line pembrolizumab [20,22,23]. In addition, the current real-world safety results showed a greater risk for hospitalization and/or emergency department visits while receiving pembrolizumab despite the fact that both groups in this study had comparable comorbidities based on the CCI and ADG. These findings are not consistent with the KEYNOTE-024 trial, where pembrolizumab demonstrated greater magnitudes of survival benefit and a better safety profile compared with chemotherapy (trial-based incidence of grade 3–5 adverse events: 31.2 vs 53.3%, respectively) [14], illustrating an efficacy–effectiveness gap between RCT and real-world evidence [15]. However, it is worth noting that most of the adverse events observed in the chemotherapy arm of the KEYNOTE-024 trial were hematological toxicities, which may not be well captured in current administrative databases unless they result in clinically relevant events such as emergency department visits or hospitalizations. While this approach may not facilitate direct comparisons with the initial trial and may in part explain why pembrolizumab was associated with a worse safety profile, the current assessment of toxicities ensures the clinical meaningfulness and relevance of these events.

The authors find that the observed efficacy–effectiveness gap may in part be explained by the highly selective nature of RCTs, given the higher representation of older patients (real-world median age: 69.0 years; trial median age: 65.3 years) and patients with treated brain metastases (real-world: 14.0%; trial: 9.2%) in the current real-world cohort compared with the KEYNOTE-024 trial. As well, real-world patients from the current study likely had higher baseline comorbidities and less stable disease as compared with the initial trial, where patients were selected and generally free of comorbidities. Furthermore, smokers treated in real-world practice are more likely than smokers in the initial trial to have a higher burden of comorbidities, including chronic obstructive pulmonary disease, emphysema, and cerebrovascular disease. Considering that the majority of patients in the current study progressed and received subsequent therapies such as nivolumab, these differences in patient characteristics may have also impacted the tolerability of subsequent therapies and explain the observed differences in survival. For the control patients who did receive nivolumab, however, PD-L1 status was neither routinely collected nor required, as the clinical trials and funding for immunotherapy in the second-line are not dependent on PD-L1 status. Finally, though not captured in the present study, prior research has also shown how differing Eastern Cooperative Oncology Group performance status scores between real-world and RCT populations can contribute to an efficacy–effectiveness gap [29]. Therefore, the findings of this study are more relevant and generalizable to patients in routine practice and can be used to support shared decision-making among patients and physicians by providing evidence of the real-world risks and benefits of treatment with pembrolizumab.

Notably, these findings indicate the presence of an early crossover in OS between the pembrolizumab and chemotherapy groups, similar to what was observed in not only the PD-L1 $\geq$50% subgroup of the KEYNOTE-042 trial but in the PD-L1 $\geq$20, $\geq$1, and 1–49% subgroups as well [5]. While this early crossover in OS was not observed in the original KEYNOTE-024 trial, this finding suggests that there may be a subgroup of patients who do not benefit from upfront pembrolizumab monotherapy. Future studies investigating alternative biomarkers for immunotherapy response may assist with future patient treatment stratification.

While PD-L1 expression levels are a known prognostic factor for mNSCLC patients receiving pembrolizumab monotherapy [5,12], its prognostic impact on patients receiving chemotherapy has not been investigated as thoroughly. In the pivotal KEYNOTE-042 trial, the median OS of mNSCLC patients receiving chemotherapy did not significantly differ between subgroups of varying tumoral PD-L1 expression. The median OS for the chemotherapy group was 12.2 months (95% CI: 10.4–14.2), 13.0 months (95% CI: 11.6–15.3), and 12.1 (95% CI: 11.3–13.3) months in the PD-L1 $\geq$50%, $\geq$20%, and $\geq$1% groups, respectively [5]. Further, patients in the chemotherapy group with PD-L1 1–49% presented OS values comparable to those of the PD-L1 $\geq$50% population with overlapping CIs (median OS: 12.1 months and 95% CI: 11.0–14.0 vs 12.2 months and 95% CI: 10.4–14.2) [5]. Together, these findings suggest that the current study's use of a control cohort unrestricted to PD-L1 $\geq$50% mNSCLC – as PD-L1 testing was not conducted routinely historically prior to

the funding of pembrolizumab – would likely not confound the comparisons.

There are some limitations to consider in this study. First, the retrospective, nonrandomized design of this study inherently subjects the results to potential confounding. Though the use of propensity score matching ensured that measured characteristics were well balanced between the treatment groups, residual confounding owing to unmeasured variables may have remained. Second, secular changes in treatment practices or supportive care may have affected the use of a historical comparator and confounded the results, such as the increased awareness of early palliative care management [30], which might have biased the results in favor of pembrolizumab. Despite these limitations, however, this is the largest study to make real-world comparisons between the effectiveness and safety of first-line pembrolizumab monotherapy for mNSCLC patients with tumoral PD-L1 expression $\geq$50% and platinum-based chemotherapy. The OS results were found to be, in general, consistent with the estimates reported by existing analyses regarding the diminished effectiveness of pembrolizumab in the real world [31–34], though some studies have also reported estimates closer in magnitude to the KEYNOTE-024 trial findings [35,36]. Nonetheless, this study's use of population-based administrative databases enabled the authors to capture all patients treated with pembrolizumab in Ontario, Canada, providing this study with the advantages of a large sample size and including populations that are often underrepresented in RCTs. Thus, the use of real-world data confers greater external validity to these findings.

5. Conclusion

The real-world use of first-line pembrolizumab monotherapy among mNSCLC patients with tumoral PD-L1 expression $\geq$50% offers a survival benefit over platinum-based chemotherapy, though the real-world safety and magnitude of benefit were worse than anticipated compared with the pivotal phase III RCT findings. In light of this efficacy–effectiveness gap, our study demonstrates the importance of real-world evidence for the re-evaluation of a drug's comparative effectiveness that can inform clinical decision-making with data more relevant to routine practice. The absolute and relative real-world survival benefit could also be used as updated input to cost–effectiveness models to help payers and health technology assessment agencies such as the UK National Institute for Health and Care Excellence and the Institute for Clinical and Economic Review to reassess the actual real-world value for money to allow for life-cycle reassessment of drugs and technologies [37,38].

Summary points

- Previous literature examining the real-world effectiveness of first-line pembrolizumab for metastatic non-small-cell lung cancer has produced conflicting results regarding the potential for differences between clinical trial efficacy and real-world effectiveness.
- In this population-based study of 2284 propensity score-matched patients, pembrolizumab was associated with a median overall survival improvement of 3.8 months compared with chemotherapy but resulted in higher rates of hospitalization, emergency department visits, treatment-related adverse events, and specialist visits.
- This study demonstrates an efficacy–effectiveness gap for pembrolizumab. When compared with the initial KEYNOTE-024 trial, pembrolizumab resulted in lower magnitudes of absolute and relative survival benefit.
- The real-world safety profile of pembrolizumab was also worse than anticipated from the trial.
- The findings of this study can be used by clinicians to counsel patients on the magnitude of risks and benefits of treatment with pembrolizumab in routine practice.
- Additionally, the safety results support a need for more proactive and multidisciplinary approaches to the management of toxicities.
- This study demonstrates the importance of reassessing safety and effectiveness using real-world data to provide patients and decision-makers with evidence that reflects actual practice.

Acknowledgments

This study was supported by ICES, which is funded by an annual grant from the Ontario Ministry of Health (MOH) and the Ministry of Long-Term Care (MLTC). This document used data adapted from the Statistics Canada Postal CodeOM Conversion File, which is based on data licensed from Canada Post Corporation, and/or data adapted from the Ontario Ministry of Health Postal Code Conversion File, which contains data copied under license from ©Canada Post Corporation and Statistics Canada. Parts of this material are based on data and/or information compiled and provided by the Canadian Institute for Health Information, Ontario Health, and the Ontario Ministry of Health. The analyses, conclusions, opinions, and statements expressed herein are solely those of the authors and do not reflect those of the funding or data sources; no endorsement is intended or should be inferred. We thank IQVIA Solutions Canada Inc. for use of their Drug Information File.

Financial disclosure

The authors have no financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

Competing interests disclosure

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, stock ownership or options and expert testimony.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

Data availability

The data that support the findings of this study are available from ICES, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of ICES.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval (Sunnybrook Research Ethics Board approval number/ID 3004) and/or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

The study has received ethics approval from the Research Ethics Board of Sunnybrook Health Sciences Centre, Ontario, Canada.

ORCID

Ambica Parmar https://orcid.org/0000-0002-0597-3843
Brandon Lu https://orcid.org/0000-0002-7109-0862
Kelvin K W Chan https://orcid.org/0000-0002-2501-3057

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Interview

Oncology Central

Deciphering the T-cell receptor cancer-recognition code

Michael Birnbaum is an associate professor in the Department of Biological Engineering at *MIT* and a member of the Koch Institute for Integrative Cancer research. He trained as an immunologist – he completed his PhD at Stanford in Chris Garcia's lab, where he worked to create tools that allowed them to take an unprecedentedly extensive look at how TCRs recognize their peptide-MHC antigens, and how the immune system balances its need for antigen specificity and cross-reactivity. Since starting his lab at MIT in 2016, they have continued to build tools for characterizing and manipulating T cell recognition and signaling. They use these methods to understand and predict natural immunity, and to create new cancer immunotherapies.

Cancer Grand Challenges is a trailblazing research funding initiative taking on some of the toughest challenges in oncology by uniting a global network of oncologists. In this interview, we discuss the MATCHMAKER project with team lead Michael Birnbaum (Massachusetts Institute of Technology, MIT, MA, USA) to uncover how they're seeking to better understand and predict how T cells recognize tumors, paving the way for personalized immunotherapies.

What are the main aims of the MATCHMAKERS project?

The goals of MATCHMAKERs are to (first) be able to computationally predict pairings between T-cell receptor sequences from tumor-infiltrating lymphocytes with the cancer antigens they recognize, and (second) to be able to computationally design T-cell receptors specific for cancer antigen targets to create next-generation immunotherapies.

Could you explain the importance of T-cell receptors (TCRs) and peptide-major histocompatibility complexes (MHCs) in immuno-oncology?

T cells patrol our bodies for infection or cell dysfunction by using their T-cell receptors (TCR) to recognize short peptide antigens bound to MHC molecules.

To effectively recognize any possible antigen, each developing T cell recombines a unique TCR, creating a repertoire of millions of TCR specificities in each of us. While the central role of T cells in infection has been well appreciated for decades, the potential for T cells to recognize and eliminate cancer cells has become increasingly apparent over the last several years. In fact, checkpoint blockade therapy, which has provided durable responses to countless patients with melanoma and lung cancer among many others, works by reinvigorating T cells that can recognize and eliminate tumor cells.

Given the molecular diversity of T-cell receptors, what challenges do you anticipate in identifying the antigens recognized by T cells, particularly in different cancer types and patient subsets?

The same molecular diversity that makes T cells such powerful potential cancer treatments complicates their study. It is extremely labor-intensive to find an antigen target for T cells isolated from a patient, especially because there are usually many T cell reactivities per tumor, every cancer has different mutations, and we all have different MHC molecules. This is why the goal of MATCHMAKERS is to develop computational approaches that can eventually supplement or replace experiments, and be done at much larger scale. Our biggest challenge will be generating enough training data to create computational approaches that can work regardless of a patient's MHCs, cancer type or antigen target. The astounding progress in machine learning for biology in the last few years has shown how important large amounts of high-quality data are for enabling progress – to that end, we are developing and implementing multiple cutting-edge approaches to generate as much data about a wide range of TCR-pMHC interactions, including their 3D structures, as possible.

What impact do you hope this project will have on personalized immunotherapy approaches?

If successful, our team will enable three distinct advances for cancer immunotherapy. First, our computational approaches would let a clinician predict what antigens are being recognized in a tumor just by sequencing the T cells. This can guide whether immune checkpoint blockade therapies are likely to work. Second, this knowledge can be used to design personalized antigen vaccines to optimally expand a patient's tumor-specific T cells. Finally, our team spans both prediction and design – we hope to be able to design synthetic TCRs that can specifically and potently recognize a patient's tumor antigens even if their natural immune system cannot. Such an advance would pave the way for the development of personalized protein or cellular immunotherapies.

Future Oncology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ifon20

Cancer cytogenetics in the era of artificial intelligence: shaping the future of chromosome analysis

Alain Chebly

To cite this article: Alain Chebly (12 Aug 2024): Cancer cytogenetics in the era of artificial intelligence: shaping the future of chromosome analysis, Future Oncology, DOI: 10.1080/14796694.2024.2385296

To link to this article: https://doi.org/10.1080/14796694.2024.2385296

Published online: 12 Aug 2024.

FUTURE ONCOLOGY
https://doi.org/10.1080/14796694.2024.2385296

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EDITORIAL

Cancer cytogenetics in the era of artificial intelligence: shaping the future of chromosome analysis

Alain Chebly[*,a,b]

[a]Center Jacques Loiselet for Medical Genetics and Genomics (CGGM), Faculty of Medicine, Saint Joseph University of Beirut (USJ), Beirut, Lebanon; [b]Higher Institute of Public Health, Saint Joseph University of Beirut (USJ), Beirut, Lebanon

ABSTRACT

Artificial intelligence (AI) has rapidly advanced in the past years, particularly in medicine for improved diagnostics. In clinical cytogenetics, AI is becoming crucial for analyzing chromosomal abnormalities and improving precision. However, existing software lack learning capabilities from experienced users. AI integration extends to genomic data analysis, personalized medicine and research, but ethical concerns arise. In this article, we discuss the challenges of the full automation in cytogenetic test interpretation and focus on its importance and benefits.

ARTICLE HISTORY
Received 27 December 2023
Accepted 23 July 2024

KEYWORDS
artificial intelligence; cancer; chromosome analysis; cytogenetics; karyotype; machine learning techniques

Although the concept of artificial intelligence (AI) was initially introduced in the 1950s, the past 15 years have witnessed considerable advancements in the field. AI has evolved into a valuable tool, facilitating data processing and yielding better outcomes. This technology connects the power of computer systems to emulate human intelligence, showcasing remarkable attributes such as rapid learning, sophisticated analysis, predictive capabilities, self-correction and proficient decision-making [1].

Recently, AI algorithms have become integral in the realm of medicine, particularly in the analysis of medical images. This application aids healthcare providers in accurately identifying and diagnosing diseases. The ongoing AI revolution is significantly enhancing medical diagnostics by refining predictive capabilities, enhancing accuracy, expediting processes and boosting overall efficiency in the diagnostic journey [2]. The future of AI-based medical diagnostics is reportedly characterized by continuous expansion and development. More advanced AI-technologies are being introduced and tested in the research field, with the ultimate goal of implementing these cutting-edge tools into medical diagnostics.

Clinical cytogenetics is a branch of medical genetics allowing the diagnosis of constitutional and cancer chromosomal abnormalities by several techniques, mainly conventional karyotyping, FISH (fluorescent *in situ* hybridization) and microarray analysis. Cancer cytogenetic testing serves as a crucial tool for detecting and identifying chromosomal abnormalities and molecular signatures. This diagnostic approach not only provides insights into the presence of genetic irregularities but also enhances our understanding of cancer initiation, progression and ongoing monitoring of the disease [3].

Nowadays, conventional karyotyping is predominantly employed in samples from patients with hematological malignancies, alongside with FISH and microarray testing. However, when it comes to solid tumors, the primary cytogenetic methods utilized are FISH and microarrays. This preference stems from the numerous limitations associated with conventional karyotyping in solid tumor samples [4]. Irrespective of the type of sample, the analysis and interpretation of data generated by these cytogenetic techniques continue to pose significant challenges.

Undoubtedly, a primary challenge in clinical cytogenetics lies not in the techniques themselves but in the precise identification and interpretation of chromosomal anomalies. Conventional cytogenetic procedures are known for being labor-intensive and time-consuming, often necessitating highly skilled technologists and scientists for accurate analysis and interpretation of results. Notably, a significant limitation in conventional cytogenetic analysis is the capacity to correctly identify all chromosomal abnormalities, including small anomalies and complex rearrangements. For example, following the extraction of chromosomes from metaphase images, they may present as individual entities or, in most of the cases, in contact with one another; exhibiting bends or overlapping, leading to the formation of clustered chromosomes [5]. The delicate task of separating these chro-

CONTACT Alain Chebly Tel.: 00961 3 560 098; alain.chebly@usj.edu.lb

mosomes for analysis adds an additional layer of complexity to the process. Moreover, the success of this procedure hinges on the quality and quantity of the obtained metaphases, further emphasizing the crucial role and skills of the cytogeneticist's experience in navigating these intricacies. Recently, optical genome mapping technique was introduced aiming to face all these limitations and to replace all known cytogenetic tools; it has been tested in the setting of germinal and somatic anomalies [6,7]. Despite the remarkably promising results observed, several limitations were noted particularly in the cases of balanced Robertsonian translocation and the cases of mosaicism [7]. Hence, classical karyotyping is still needed in many cases.

For these reasons, the integration of AI tools, including both machines and software with learning and evolving capabilities, holds the potential to surmount the limitations inherent in traditional cytogenetic analysis. Through the automation of chromosomal data, these AI-driven solutions have the capacity to deliver more precise cytogenomic results. Presently, the majority of cytogenetic software packages, whether for G-banding or R-banding, help in classifying chromosomes by utilizing incorporated data and predefined formulas [5]. Indeed, the current situation requires users to rectify software-generated errors, complete classifications, and ultimately provide the interpretation. Notably, these existing software solutions lack the ability to learn from the experience of skilled users, a feature that could undoubtably mitigate errors and enhance overall outcomes.

While some efforts have been made in the development of robust classifiers in this domain, the existing work has encountered several limitations, particularly concerning the interpretation of metaphase images, the quality of the chromosomes, and the challenges posed by severely bent or touching chromosomes [8]. Also, the work was done using only normal metaphases from healthy individuals which represents a noteworthy limitation, especially in the context of cancer cytogenetics where many chromosomal alterations can be found within a single metaphase.

Moreover, the incorporation of AI tools into cytogenetic laboratories can significantly enhance the analysis of large-scale genomic data, providing valuable insights for treatment guidance in the era of personalized medicine. Indeed, it has been reported that AI can assist precision medicine in the field of cancer and genomics and that it can help improve a series of clinical applications against cancer [9]. Certainly, some limitations exist and need to be addressed by future research, including the lack of standardized protocols, insufficient data oversight and low trust in AI among some clinicians [9,10].

Also, AI can play a pivotal role in quality control processes, ensuring that both the analysis and reporting adhere to required criteria. Importantly, the integration of AI in cytogenetics extends benefits to researchers and instructors. It has the potential to expedite research efforts, facilitating the discovery of novel cytogenomic associations. Additionally, AI serves as a valuable resource for students and practitioners, offering opportunities to improve their skills in cytogenetics

On the other side, it is crucial to take into account the data, the storage and protection of this data [11]. It is important to acknowledge that the complete integration of AI into cytogenetics presents ethical and privacy concerns pertaining to data handling. In this context, it had been reported that with big data, generated by AI and machine-learning techniques, comes many big risks and challenges including significant questions about patient privacy, ethical and legal issues [12,13]. As we delve into this transformative approach, it becomes imperative to be mindful of possible negative aspects that could potentially outweigh all the benefits. To overcome this problem, new regulations and laws will need to be placed in order to protect individual's privacy and genetic data in the future.

Also, the application of AI in cytogenetics presents challenges for training the next generation of cytogeneticists. It can potentially limit opportunities for developing essential skills in cytogenetics, based on the current standards and practices in the field of "today's cytogenetics".

In the era of molecular biology, substantial effort is directed toward the intersection of AI and molecular tools, especially focusing on AI-based next generation sequencing technologies. Despite this emphasis, the field of cytogenetics remains relevant in contemporary medicine. Also, the "All in one technology", capable of identifying all molecular and cytogenetic anomalies during a single procedure, is still not available. Achieving full automation in the interpretation and reporting of cytogenetic tests, including karyotype, FISH and microarrays, poses a formidable challenge. Nonetheless, AI is poised to play a pivotal role in the future of cancer cytogenetics, influencing various facets of this clinical science. More efforts and research are needed for the full automation of the cytogenetic analyzing tools capable of self-learning, analyzing and interpreting cytogenetic data. Addressing challenges and innovating with AI solutions will undoubtedly pave the way for an accelerated workflow, optimal results and a transformative paradigm in cytogenetics.

Author contributions

Alain Chebly conceived this study and wrote the manuscript.

Financial disclosure

The authors have no financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Competing interests disclosure

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Writing disclosure

No writing assistance was utilized in the production of this manuscript.

ORCID

Alain Chebly https://orcid.org/0000-0001-5582-2923

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Future Oncology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ifon20

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To cite this article: Richard E Evenhuis, Ibtissam Acem, Veroniek M van Praag, Robert JP van der Wal, Michael PA Bus & Michiel AJ van de Sande (12 Jun 2024): Diagnosis of chondrosarcoma in a noninvasive way using volatile organic compounds in exhaled breath: a pilot study, Future Oncology, DOI: 10.1080/14796694.2024.2355080

To link to this article: https://doi.org/10.1080/14796694.2024.2355080

Published online: 12 Jun 2024.

FUTURE ONCOLOGY
https://doi.org/10.1080/14796694.2024.2355080

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RAPID COMMUNICATION

Diagnosis of chondrosarcoma in a noninvasive way using volatile organic compounds in exhaled breath: a pilot study

Richard E Evenhuis[*,a], Ibtissam Acem[a], Veroniek M van Praag[a], Robert JP van der Wal[a], Michael PA Bus[a] and Michiel AJ van de Sande[a]

[a]Department of Orthopaedic Surgery, Leiden University Medical Center, Leiden, The Netherlands

ABSTRACT

Aim: Aim of this explorative pilot study was to evaluate the capability of an electronic nose (aeoNose, the eNose Company) to classify healthy individuals and patients with chondrosarcoma, based on their volatile organic compound profiles in exhaled breath. **Materials & methods:** Fifty-seven patients (25 healthy controls, 24 chondrosarcoma and 8 different benign lesions) were included in the study from 2018 to 2023. An artificial neural network was used as classifier. **Results:** The developed model had a sensitivity of 75%, and a specificity of 65% with an AUC of 0.66. **Conclusion:** Results show that there is not enough evidence to include the aeoNose as diagnostic biomarker for chondrosarcoma in daily practice. However, the aeoNose might play an additional role alongside MRI, in questionable chondrosarcoma cases.

ARTICLE HISTORY
Received 27 November 2023
Accepted 10 May 2024

1. Background

Chondrosarcoma is a rare malignant neoplasm, originating from cartilaginous tissue and accounts for approximately 20% of all primary malignant bone tumors [1]. The prognosis and outcomes depend on chondrosarcoma subtype, grade, tumor location, timely and accurate identification and surgical treatment [2,3].

Most patients with high-grade chondrosarcoma present with pain at night and local swelling, while benign or atypical cartilaginous tumors are most often coincidental findings [2,4,5]. The diagnostic pathway for chondrosarcoma consists of plain radiography, followed by magnetic resonance imaging (MRI). In some cases, or based on center preferences, a biopsy may be performed, although biopsies of cartilaginous lesions are notoriously unreliable because of a substantial risk of sampling error and has the potential risk of seeding along the biopsy tract. Moreover, pathological assessment has considerable interobserver variability [2,6,7].

The diagnostic accuracy of MRI for the differentiation between a benign cartilaginous lesion and a high-grade chondrosarcoma is approximately 90% [8]. However, there are still cases in which the diagnosis remains debatable [9]. The importance of an accurate preoperative diagnosis lies in the potential consequences of the treatment. High-grade chondrosarcomas require resection with clear surgical margins and often subsequent reconstruction, carrying a substantial risk of complications and considerable morbidity. On the other hand, benign cartilaginous lesions may be followed with MRI, or treated with less aggressive surgery, in other words, intralesional curettage [3,10–13]. Therefore, there is a need for an additional diagnostic tool to differentiate between the two entities.

Promising results have been reported for using an electronic nose (eNose) in detecting various tumor types, including head and neck cancer, lung cancer, colorectal cancer and soft tissue sarcoma [14–17]. eNoses analyze volatile organic compounds (VOCs) in exhaled breath [16,18]. VOCs are degradation products of biochemical processes in the human body. Their composition can be influenced by the presence of specific pathological processes within the body and can be detected in body fluids such as urine, feces, saliva or blood [14,16,19]. To date, there is no literature on the detection of chondrosarcoma using VOC profiles.

Therefore, the objective of this explorative study is to evaluate if the aeoNose can be used to distinguish healthy individuals from patients with chondrosarcoma, based on their VOC profiles in exhaled breath.

2. Materials & methods

2.1. Design, setting & participants

Materials and methods were previously described in a similar study, employing the aeoNose (aeoNose, the eNose Company, Zutphen, The Netherlands) to detect soft-tissue sarcoma, during the same period [15]. This

CONTACT Richard Eduard Evenhuis r.e.evenhuis@lumc.nl

single-center prospective pilot study was conducted at the Leiden University Medical Center, The Netherlands (LUMC), a tertiary referral center for bone tumors. Patients were included between 2018 and 2023. At our outpatient clinic, we asked all patients who were suspected of having a high-grade chondrosarcoma to participate in this study. Patients were enrolled between 2018 and 2023. Minimum age was 18 years. Patients with any history of cancer, chronic respiratory disease and chemo- or radiotherapy were excluded. Furthermore, we excluded patients in whom metastases were found within 3 months following primary surgery, as we theorized that these were easier to identify. The goal was to identify patients with localized disease who could be considered for curative treatment. The breath test was performed in parallel with the regular diagnostic workup. As a control group, we invited people who visited the outpatient clinic for other (non-oncological) conditions, people who accompanied a patient to our outpatient clinic, and department employees. The control group underwent an eligibility check through an interview on their medical history, without additional diagnostic tests such as radiography. Suspected chondrosarcoma cases that turned out to be benign were excluded from the primary analysis (model 1) but were included in the secondary analysis as controls (model 2), to increase the number of patients. The control group was matched to our chondrosarcoma population based on age and sex in a 1:1 ratio. Both controls and chondrosarcoma cases adhered to the same in- and exclusion criteria. The patient records were reviewed by the investigator to obtain baseline characteristics, medical history and tumor characteristics.

In total, 57 patients were included (35 males, 61%). The median age for the entire cohort was 51 years (interquartile range [IQR] 48–70; Table 1). Among them, 24 patients (42%) had a histologically proven high-grade chondrosarcoma (grade 2, 3 or dedifferentiated), while eight (14%) had a benign bone tumor (atypical cartilaginous tumor [n = 3, one distal femur, two of the hand], chordoma [n = 2], fibrous dysplasia [n = 1], hemangioendothelioma [n = 1], or Langerhans cell histiocytosis [n = 1]). There were 25 (44%) healthy controls (Table 2).

2.2. Materials & procedure

The eNose used in this study is a portable, battery-powered device designed to analyze VOCs. The study participants were instructed to abstain from food, drinks (except for water), and smoking at least 3 h prior to the breath test. They were instructed to breathe through a disposable connecting mouthpiece, which included a carbon filter equipped with high-efficiency particulate air filters and one-way valves to prevent viral or bacterial contamination of the device, for 5 min. Furthermore, a nose clip was applied to prevent the entry of unfiltered air during measurements, minimizing external influences on VOCs (see appendix for setup, Figure 1). The breathing test consisted of 5 min of breathing followed by 10 min of aeoNose regeneration, thus a total of 15 min. The initial 2 min were used to clean the lungs with filtered air, to eliminate external VOCs. During the subsequent 3 min, exhaled breath interacts with three hotplate metal-oxide sensors with distinct material properties. These sensors are periodically heated between 260 and 340°C, simulating multiple identical sensors, functioning at various temperatures. This triggers a redox reaction at the surface, resulting in changes in conductivity over time. The alterations in conductivity form a distinctive VOC profile, or breath print for each patient. Detailed information on the aeNose technology was previously published elsewhere [20].

2.3. Statistical analysis

Descriptive statistics were used to report baseline characteristics. Categorical data are presented as contingency tables (frequencies and percentages). Medians including interquartile range (IQR) were reported for continuous data. To assess differences in baseline characteristics between patients and the control group, the Mann–Whitney U test, or the Chi-square test was performed for continuous and categorical variables, respectively. No formal sample size calculation was performed, since this is a pilot study with 25 chondrosarcoma patients. Data analysis was performed using SPSS version 25.0. (IBM Corp., NY, USA). The level of significance was set at a p-value < 0.05.

The breath test resulted in an aeoNose measurement, or breath print, with a unique time-series of conductivity values for every sensor. Developing an algorithm for distinguishing exhaled-breath patterns involved training an artificial neural network (ANN), with data analysis carried out using the proprietary software called 'Aethena' (The eNose Company, Zuthpen, The Netherlands). Data compression was performed using a TUCKER3-like solution, as Waltman et al. described previously [21]. Subsequently, the ANN served as a classifier for the created models, ranked by the area under the curve (AUC) through the leave-10%-out cross validation method (LOCV). The LOCV method divides data into subsets, each containing 10% of patients, allowing classifications based on 10 separate models. The validation technique assesses how results of a predictive model will be generalized to an independent dataset. Thus, participants were classified in 10 steps based on 10 separate models, resulting in an ROC based on unseen training data. Optimal AUC results, along with the corresponding sensitivity and specificity (including

Table 1. Study population.

	Model 1			Model 2		
	Chondrosarcoma (n = 24)	Controls (n = 25)	p-value	Chondrosarcoma (n = 24)	Controls (n = 33)	p-value
Age, median (IQR)	51 (40–58)	54 (41–61)	0.99	51 (40–58)	54 (45–61)	0.82
Sex, male	16 (67%)	15 (60%)	0.63	16 (67%)	19 (58%)	0.49

IQR: Interquartile range.

Table 2. Tumor details.

Diagnosis	Model 1	Model 2
Chondrosarcoma (n = 24)		
Chondrosarcoma grade 2	12 (50%)	12 (50%)
Chondrosarcoma grade 3	6 (25%)	6 (25%)
Periosteal chondrosarcoma	5 (21%)	5 (21%)
Dedifferentiated chondrosarcoma	1 (4%)	1 (4%)
Benign lesions (n = 8)		
Atypical cartilaginous tumor	–	3 (38%)
Chordoma	–	2 (25%)
Fibrous dysplasia	–	1 (13%)
Hemangioendothelioma	–	1 (13%)
Langerhans cell histiocytosis	–	1 (13%)
Tumor location		
Axial skeleton (including head)	16 (67%)	2 (25%)
Upper extremity	2 (8%)	3 (38%)
Lower extremity	6 (25%)	3 (38%)

95% CI), were reported for each analysis. The predicted response values for healthy controls and chondrosarcoma patients were presented in a scatterplot. A detailed description of the statistical method used by aeoNose, can be found in the article of Kort et al. [22].

2.4. Study ethics

This study was approved by our institutional review board (P19.046). All study participants signed an informed consent form, prior to participation.

3. Results

3.1. Model 1

The primary analysis consisted of 24 patients with a chondrosarcoma, and 25 healthy controls. This model predicted the correct diagnosis in 34/49 patients, yielding an accuracy of 70%. A threshold of -0.22 was used, to maximize sensitivity and specificity; 18 out of 24 chondrosarcoma patients were correctly identified by the aeoNose, and six false negative results were observed. Sixteen out of 25 healthy controls were correctly identified, and nine false positive results were observed. This resulted in a sensitivity of 75% (95% CI: 53–89%), and a specificity of 64% (95% CI: 43–81%). The corresponding ROC curve for the best fit of model 1 comprised of an area under the curve (AUC) of 0.66 (Figure 2A). The scatterplot shows the predicted value of each measurement (Figure 2B).

3.2. Model 2

The second analysis was performed using an expanded study population. The same 24 chondrosarcoma and 25 control patients were included. However, eight patients who were initially suspected of having a chondrosarcoma (but turned out to have a benign bone tumor), were added to the control-group. This model predicted the diagnosis correctly in 39/57 patients, yielding an accuracy of 68%. Eighteen out of 24 chondrosarcoma patients were correctly identified by the aeoNose, and six false negative results were observed. Twenty-one out of 33 controls were correctly identified, and 12 false positive results were observed. This resulted in a sensitivity of 75% (-95% CI: 53–89%), and a specificity of 64% (95% CI: 45–79). The corresponding ROC curve for the best fit of model 2 comprised of an AUC of 0.69, with a threshold of -0.22 (Figure 3A). The scatterplot shows the predicted value of each measurement (Figure 3B).

4. Discussion

Electronic noses potentially offer a noninvasive, easy-to-use method for the diagnosis of various cancer types by analyzing volatile organic compounds in exhaled air. This is the first pilot study exploring the potential of electronic noses in the diagnostic process of chondrosarcoma of bone. Our model shows an AUC of 0.66 with a 75% sensitivity and a 64% specificity.

Oxidative stress, inflammation and cell death can change VOC profiles. An electronic nose, as well as gas

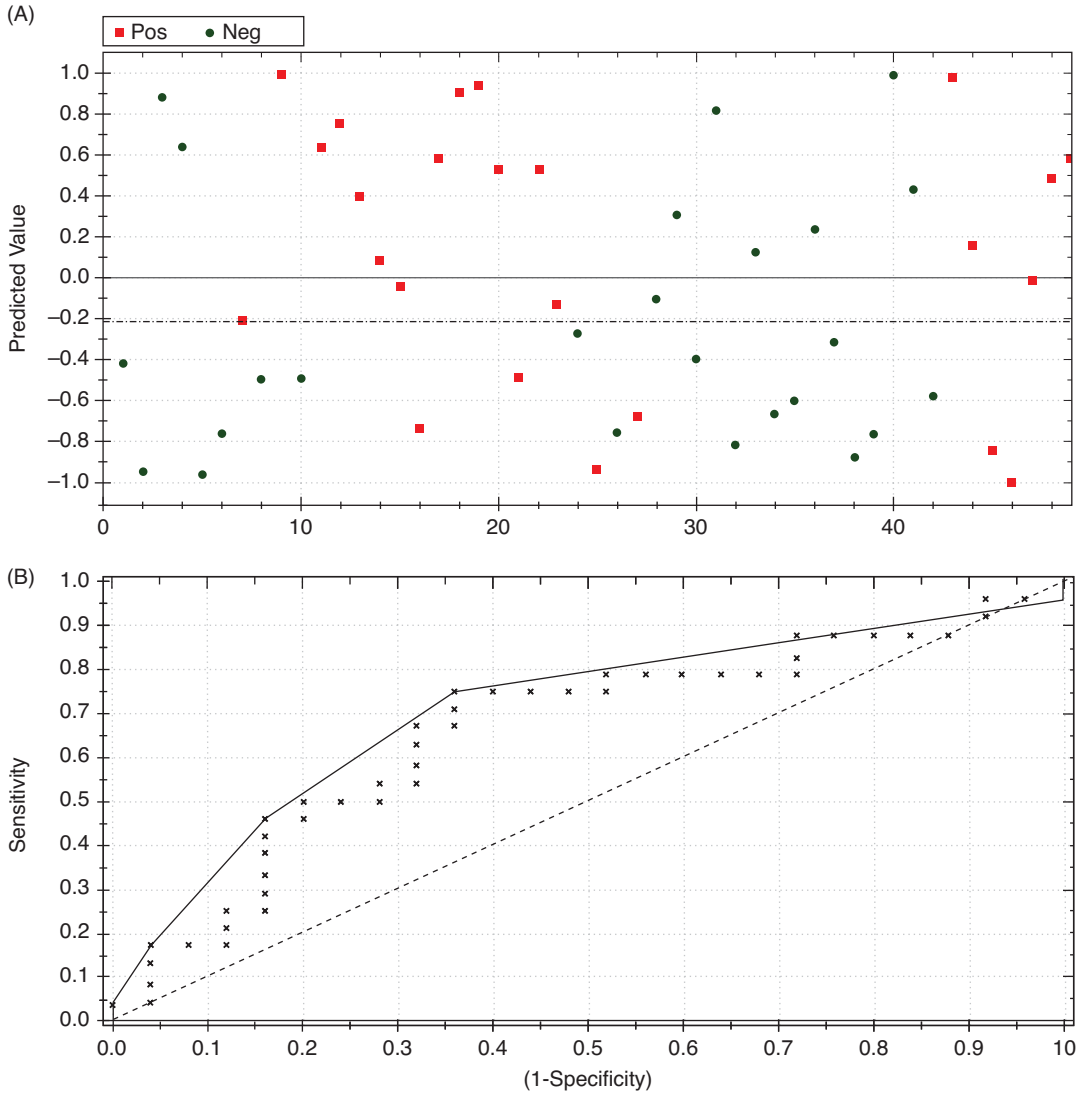

Figure 1. Model 1: **(A)** ROC curve (AUC: 0.66). **(B)** Scatter plot of individual predicted values based on the cross-validated model 1. The red squares represent patients with chondrosarcoma, the green circles represent healthy controls.
AUC: Area under the curve; ROC: Receiver operating characteristic.

chromatography-mass spectrometry (GC-MS), can detect changes in these VOC profiles. Metabolic VOC profiling could therefore be a potential tool for diagnostic purposes, since it reflects the metabolic state of cells or even organisms [23]. GC-MS is a standardized method which is suitable for the detection of individual VOC profiles based on molecular weight. However, this method is expensive and requires trained personnel [24]. The aeoNose uses VOC profile pattern recognition and needs to be trained by a training dataset. Accuracy of the diagnostic tool depends among other things on the size of the training dataset, and the representativeness of the sample population [25]. Moreover, this instrument is user-friendly, inexpensive, and, once trained and validated, capable of real-time analysis for chondrosarcoma detection. This technique holds potential as a screening method to achieve a higher pretest probability, upon validation and availability of other models.

Although Alhumaid et al. demonstrated an adequate efficacy of MRI in differentiating between ACTs and high-grade chondrosarcoma, the SLICED study group found a relatively low reliability in grading cartilaginous neoplasms (benign, low-grade malignant or high-grade malignant) in the long bones. The interobserver reliability for pathologists was 0.443, and for radiologists, it was 0.345, judging the reliability as fair to moderate reliability in differentiating between benign and malignant cartilaginous lesions [8,26]. Again, this emphasizes the need for an additional diagnostic test to enhance the diagnostic workup and prevent unnecessary overtreatment or undertreatment. While the diagnostic accuracy of the aeoNose, as observed in this explorative study, is currently

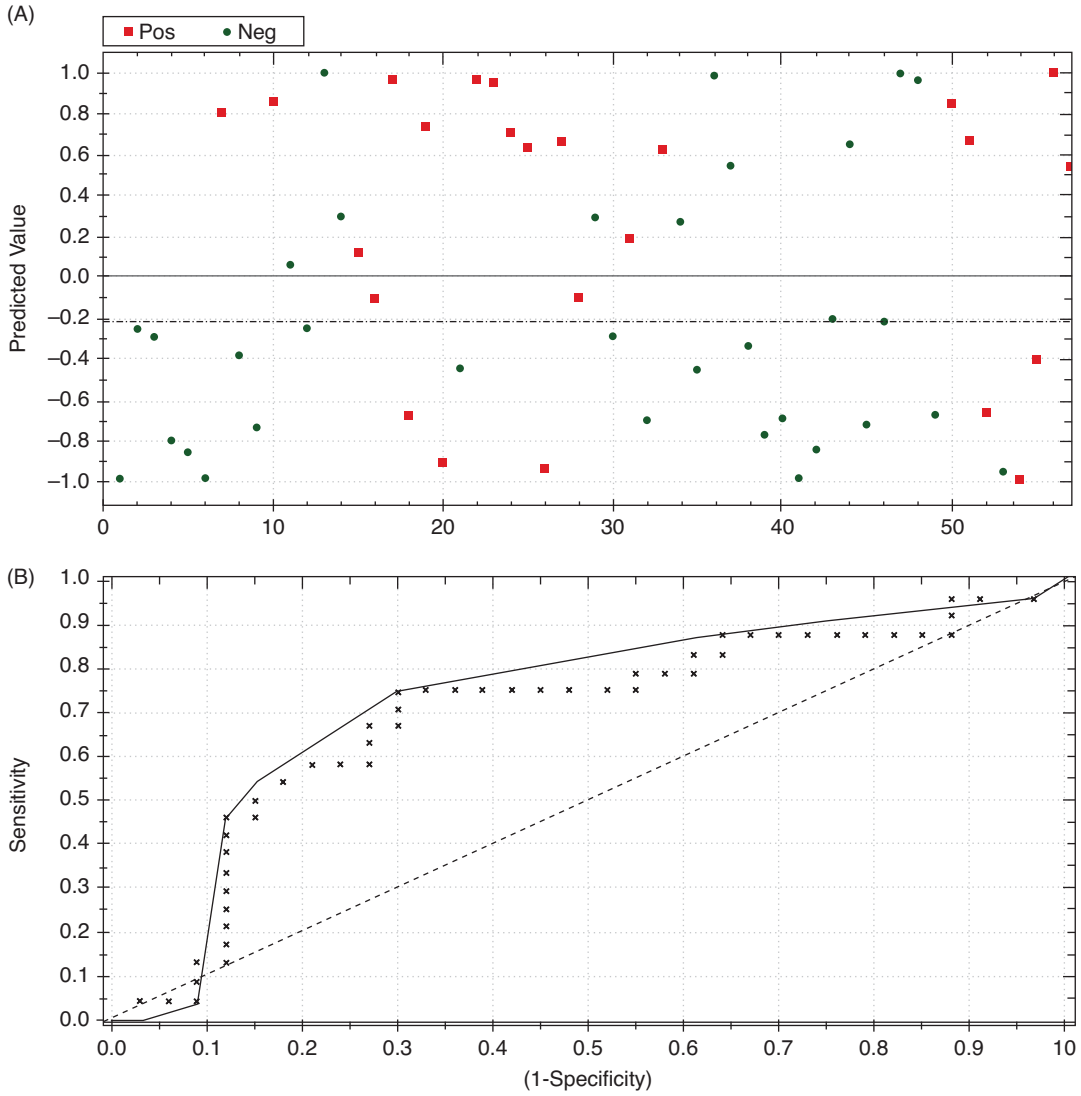

Figure 2. Model 2: **(A)** ROC curve (AUC: 0.69) **(B)** Scatter plot of individual predicted values based on the cross-validated model 2. The red squares represent patients with chondrosarcoma, the green circles represent healthy controls.
AUC: Area under the curve; ROC: Receiver operating characteristic.

insufficient for a reliable diagnosis or exclusion of chondrosarcoma of bone, it could potentially serve as an additional diagnostic tool in addition to MRI, particularly in cases involving uncertain high-grade chondrosarcoma. However, the question remains whether the accuracy of the aeoNose can be improved in the future with a larger study population.

To date, there are no studies on the use of VOC profiles in exhaled breath for the diagnosis of high-grade chondrosarcoma. Previous studies have shown that eNoses are capable to differentiate between healthy controls and conditions such as; head and neck cancer, lung cancer, breast cancer, gastric cancer, colorectal cancer, prostate cancer and soft tissue sarcoma with an AUC ranging from 79 to 90% [15,17,21,27–31]. The lower AUC observed in our study, might be attributed to the heterogeneity of chondrosarcoma, which includes various subtypes such as periosteal chondrosarcoma, chondrosarcoma grade 2 and 3 and dedifferentiated chondrosarcoma, each distinguished by unique characteristics [32]. The development of a model that encompasses VOCs from all these diverse chondrosarcoma subtypes might potentially lead to reduced diagnostic accuracy.

4.1. Limitations

This study has a number of limitations. First, our model may have been affected by artifacts unrelated to chondrosarcoma, resulting from exogenous VOCs, primarily due to the limited sample size. We attempted to overcome this limitation by employing a mouth-piece with carbon-filters to eliminate exogeneous VOCs, and by per-

Figure 3. Setup aeoNose.

forming all measurements in the same room. Future larger study populations are needed to explore the true differences in VOC profiles. Second, the population of chondrosarcoma patients is heterogenous with regard to gender, age, BMI, diet, smoking behavior and medical history, while these factors may influence VOC profiles [21,33,34]. We tried to overcome this limitation by matching our control group to the chondrosarcoma group by age and gender. However, the limited sample sizes hampered us from matching for the other potential influencing factors. Furthermore, we instructed participants to abstain from food, drinks and smoking 3 h prior to testing and only included patients without a history of chemo- and radiotherapy, which causes oxidative stress. Third, our model was internally cross-validated, limiting its reliability and generalizability to a broader study population in diverse clinical settings. Acknowledging the necessity for future larger, externally validated studies to enhance the reliability and accuracy of the model, this explorative pilot study might offer direction for future more comprehensive research on this topic. Furthermore, it would be of particular interest to study whether the diagnostic accuracy can be improved by combining information from the aeoNose and MRI in debatable cases.

5. Conclusion

This pilot study contributes to the growing body of research investigating noninvasive diagnostic methods for chondrosarcoma. While the relatively low observed accuracy is currently insufficient to introduce VOC analysis as a diagnostic biomarker in daily practice, it might play a future role as an additional diagnostic tool in combination with MRI features to guide in questionable high-grade chondrosarcoma cases. Future research based on larger cohorts, with external validation is warranted before the aeoNose can be considered a reliable diagnostic tool for chondrosarcoma of bone.

Article highlights

- It is important to differentiate between high-grade chondrosarcoma and benign cartilaginous lesions, due to the potential consequences of the treatment.
- Diagnostic accuracy on high-grade chondrosarcoma can be challenging.
- This explorative study aimed to assess the feasibility of the aeoNose to differentiate between high-grade chondrosarcoma, and healthy controls.
- 24 high-grade chondrosarcoma patients, 25 controls and 8 benign cartilaginous lesions were included for breath analysis, and were matched on age and gender.
- The aeoNose uses volatile organic compound profile pattern recognition, and an artificial neural network appeared to be the most optimal classifier.
- This pilot study contributes to the growing body of research investigating noninvasive diagnostic methods for high-grade chondrosarcoma.
- The current accuracy is not sufficient to include the aeoNose as diagnostic biomarker for high-grade chondrosarcoma in daily practice, although the aeoNose might play an additional role in questionable high-grade chondrosarcoma cases.
- Future larger externally validated studies are warranted to improve the reliability, accuracy and to determine the role of the aeoNose in high-grade chondrosarcoma patients.

Acknowledgments

The eNose Company, Zutphen, The Netherlands, provided the eNose and corresponding software.

Author contributions

Conceptualization: VM van Praag, MAJ van de Sande, methodology: RE Evenhuis, I Acem, VM van Praag, MPA Bus, investigation: RE Evenhuis, I Acem, MPA Bus, data curation: RE Evenhuis, I Acem, writing (review and editing) RE Evenhuis, I Acem, MPA Bus, MAJ van de Sande, visualization: RE Evenhuis, I Acem, MPA Bus, RJP van der Wal, MAJ van de Sande, supervision: MPA Bus, RJP van der Wal, MAJ van de Sande.

Financial disclosure

The authors have no financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Competing interests disclosure

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment,

consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval (Medical Ethical Committee of the Leiden University Medical Center (P19.046)) or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Data sharing statement

The study protocol can be requested at the corresponding author (RE Evenhuis). Anonymized data is available on request.

ORCID

Richard E Evenhuis https://orcid.org/0000-0001-6022-7096
Ibtissam Acem https://orcid.org/0000-0002-8042-9856
Veroniek M van Praag
https://orcid.org/0000-0003-1159-6711
Robert JP van der Wal
https://orcid.org/0000-0002-2570-6988
Michael PA Bus https://orcid.org/0000-0003-0023-6016
Michiel AJ van de Sande
https://orcid.org/0000-0002-9156-7656

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Interview

Oncology Central

The more the better? Quadruplets in newly diagnosed multiple myeloma

Dr Carl Ola Landgren is a Professor of Medicine, Chief of the Myeloma Division, and Co-Leader of the Translational and Clinical Oncology (TCO) Program at NCI-designated Sylvester Comprehensive Cancer Center (Sylvester). As a Cancer Center Support Grant member, he participates in research activities as an independent investigator, as a leader of research governance committees, and serves on multi-disciplinary research teams. He is a frequent speaker and faculty member for national and international meetings on myeloma and hematologic malignancies.

Dr Ola Carl Landgren from Sylvester Comprehensive Cancer Center (FL, USA) recently presented at the 2024 ASCO Annual Meeting (May 31–June 4, IL, USA) about the potential of quadruplet therapies in newly diagnosed multiple myeloma. In this interview, he provides a breakdown of key takeaways from his talk and discusses challenges and opportunities in this area.

What were the key takeaways from the discussion on quadruplet therapy approaches in multiple myeloma presented at this years ASCO?

By adding a fourth drug to an existing backbone with three drugs, it provides a higher degree of efficacy (more MRD negativity and longer progression free survival (PFS)) than the three drugs alone. This is true for both younger, fit (so-called transplant eligible) and older, frail (so-called transplant ineligible) patients. We are seeing higher and higher rates of deep treatment responses (i.e., MRD negativity) and longer PFS with novel 4-drug combinations “quadruplets”, independent of transplant status. It raises the question of whether it is time to retire the more than 40-year old terminology “transplant eligible/ineligible” and instead just use the terminology “newly diagnosed multiple myeloma”.

Interview

What are the challenges of using quadruplet therapies?

Adding more drugs always comes with adverse events. Adding immunotherapy may increase the risk of infection, which is all manageable. Adding the proteasome inhibitor bortezomib to a 3-drug combination with an IMiD, a CD38 targeted antibody, and dexamethasone, bortezomib increases the risk of peripheral neuropathy. Bortezomib has a quite high rate of peripheral neuropathy that is clinically relevant (grade 2 or higher), somewhere around 50% of all patients treated with bortezomib have peripheral neuropathy. Alternative strategies, to avoid bortezomib, include the use of newer proteasome inhibitors, such as carfilzomib; or potentially using bispecific monoclonal antibodies, which is currently being investigated in ongoing clinical trials. The field is moving fast forward..

What elements of multiple myeloma make it a good candidate for this and what other cancer types could this approach be used for?

As stated above, by adding a fourth drug to an existing backbone with three drugs – a quadruplet, provides a higher degree of efficacy (more MRD negativity and longer PFS) than the 3 drugs alone. Also, it raises the question if transplants are truly needed in the modern era. Large randomized studies done in the modern era show that transplants do not prolong overall survival. Perhaps, using more effective four-drug combinations (quadruplets) is the new way going forward, and – overall – transplants will have more of a role in the relapse setting? Importantly, CAR T cell therapy was recently US FDA approved in the setting of a first replace in multiple myeloma. As previously mentioned, the field is moving fast forward.

What are you most looking forward to at ASCO this year?

I look forward to hearing all the presentations on new therapies on multiple myeloma. I also look forward to giving my talk as a discussant, summarizing and discussing the talks on novel quadruplets in the setting of newly diagnosed multiple myeloma. I also look forward to networking with colleagues in the field.

Contact us

Editorial Department

Senior Editor
Jade Parker
j.parker@oncology-central.com

Business Development and Support

fsg.advertising@tandf.co.uk