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ORIGINAL ARTICLE

Baseline computed tomography screening and blood microRNA predict lung cancer risk and define adequate intervals in the BioMILD trial

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Background: Large randomized trials have demonstrated that lung cancer (LC) screening with low-dose computed tomography (LDCT) reduces LC mortality in heavy smokers. We previously showed in the MILD screening trial that the combination of a prespecified circulating microRNA (miRNA) signature classifier (MSC) and LDCT improves the accuracy of LDCT alone. The primary aim of the prospective BioMILD study was to assess the additional value of the blood MSC assay at the time of baseline LDCT with the goal of personalizing LC screening intervals.

Patients and methods: The study enrolled 4119 volunteers from January 2013 to March 2016, with a median follow-up of 5.3 years. Baseline LDCT and miRNAs stratified participants into four groups: CT−/MSC− ($n = 2664$; 64.7%); CT−/MSC+ ($n = 800$; 19.4%); CT+/MSC− ($n = 446$; 10.8%); and CT+/MSC+ ($n = 209$; 5.1%). As per the protocol, those in the CT−/MSC− and CT−/MSC+ groups were allocated to LDCT repeat at 3-year and 1-year intervals; CT+ participants were allocated for 1-year or earlier intervals on the basis of LDCT features independent of MSC results.

Results: CT+ participants had a 15.8-fold higher 4-year LC incidence than CT− participants (95% confidence interval 10.34-24.05), and MSC+ participants had a 2.0-fold higher 4-year LC incidence than MSC− participants (95% confidence interval 1.40-2.90); there was no evidence that the MSC effect differed between CT+ and CT− participants. LC incidence at 4 years was 0.8% in CT−/MSC−, 1.1% in CT−/MSC+, 10.8% in CT+/MSC−, and 20.1% in CT+/MSC+ participants. LC mortality rates at 5 years in the four risk groups were 0.5 in CT−/MSC−, 1.5 in CT−/MSC+, 4.2 in CT+/MSC−, and 10.1 in CT+/MSC+.

Conclusion: The combined use of LDCT and blood miRNAs at baseline predicts individual LC incidence and mortality, with a major effect of MSC for LDCT-positive individuals. These findings may have important implications in personalizing screening intervals.

Key words: lung cancer screening, low-dose computed tomography, microRNA, risk profile

INTRODUCTION

Lung cancer (LC) is the leading cause of cancer mortality in men and women, accounting for 28% of all cancer deaths in Europe.[1] In fact, only 21% of LC patients are still alive at 5 years, as ∼70% are diagnosed with advanced disease.[2] At present, the most effective health care intervention for LC after smoking cessation is early detection by low-dose computed tomography (LDCT) screening.

In The National Lung Screening Trial (NLST), LC screening by three annual rounds of LDCT resulted in a 20% reduction in LC-related mortality.[3] Moreover, the Dutch-Belgian LC screening trial (NELSON) confirmed that LDCT screening increases LC survival, with a 26% reduction in mortality.[4] The Multicenter Italian Lung Detection (MILD) randomized trial provided additional evidence that extended intervention beyond 5 years, with annual or biennial rounds, enhances the benefit (39% mortality reduction) of screening.[5] Additionally, a recent meta-analysis of randomized LDCT screening trials found that early detection by LDCT reduces overall LC mortality by 20% (95% confidence interval 10% to 29%).[6] With regard to the application of variable screening intervals, risk prediction models based on questionnaire data of age, sex, and smoking history allow for considerable risk discrimination within screen-eligible study participants,

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which is only modestly improved by integrating CT imaging data.[7,8] Notably, we demonstrated in the MILD study that biennial screening rounds after a negative baseline LDCT result are as effective at reducing mortality as are annual rounds, which contributes to information on CT screening frequency.[9]

Despite major radiomic improvements in nodule management protocols in recent years, minimally invasive blood tests to predict LC risk and prognosis are valuable for reducing the number of LDCT repeats and unnecessary invasive work-up procedures. In our studies, we have pursued a strategy of personal LC risk refinement through blood-based biomarkers, such as circulating microRNAs (miRNAs) and inflammatory C reactive protein.[10,11] Large retrospective analysis in a subgroup of 1076 participants in the MILD trial indicated that the combination of a prespecified circulating miRNA signature classifier (MSC) and LDCT has accuracy superior to LDCT alone.[12]

The BioMILD study was launched in 2013 to evaluate whether a blood MSC assay at the time of baseline LDCT improves predictive ability in detecting LC (main aim). The primary endpoint was the proportion of LC detected within the third year screening round (i.e. 4-year LC incidence) in the entire population entering the study. Secondary endpoints were the proportion of LC detected at stage I (and of those who underwent resection) of the total number of detected LC cases, the proportion of interval cancers detected of the entire number of participants who entered the study, and the incidence rate of 5-year LC deaths.

Here, we report the results of the combined LDCT-MSC algorithm at baseline in the BioMILD trial, with a minimum follow-up of 4 years for surviving participants and a median follow-up of 5.3 years.

PATIENTS AND METHODS

Study design and participants

The BioMILD trial is a large prospective study testing the combination of plasma miRNA and LDCT to improve the efficacy of LC screening by individual risk profiling and personalized screening intervals (clinicaltrials.gov ID: NCT02247453). Volunteers were recruited from respondents to advertisements and articles published in the lay press and on television or radio broadcasts. Eligible participants were (i) aged 50-75 years and current heavy smokers of $\geq$30 pack-years or former smokers with the same smoking habits who stopped $\leq$10 years ago; (ii) aged 50-75 years and current or former smokers of $\geq$20 pack-years with family history of LC or a prior diagnosis of chronic obstructive pulmonary disease (COPD) or pneumonia. The exclusion criteria were the presence of neoplasms within the previous 5 years and suspected lung nodules under investigation.

The trial was designed to continue recruitment until 4000 participants were enrolled. According to the available data from the MILD screening trial in 2012,[13] 3% of participants should experience LC within 4 years of initial screening. Twenty-five percent of participants were further expected to be MSC-positive (MSC+) at baseline.[12] By accepting a 5% two-sided first-type error, the study size would be sufficient to recognize a 1.667-fold increase in the proportion of detected LC among MSC+ participants compared with MSC-negative (MSC−) participants, with 80% power. In addition, such a sample size would be able to identify an overall 20% reduction in the proportion of stage I LC and a 15% reduction in the proportion of resectable LC, assuming a 30% saving in the total number of LDCT examinations.

Our Institutional Review Board and Ethics Committee approved the study (code: INT 0021/11), and all eligible volunteers provided written informed consent. A total of 4119 participants were prospectively enrolled at the Istituto Nazionale Tumori of Milan between January 2013 and March 2016 and underwent a baseline screening round.

Risk profile and management of screening volunteers

LDCT was classified as follows. Negative test (CT−): no nodule detected, nodule with a fat or benign pattern of calcification, solid nodules (SN) $<$113 mm^3, or non-solid nodules (NSN) $<$5 mm. Positive test (CT+): indeterminate nodules (SN 113-260 mm^3, part-solid nodules (PSN) with a solid component $<$5 mm or NSN $\geq$5 mm) and positive nodules (SN $>$260 mm^3 or PSN with solid component $\geq$5 mm).[14] The MSC risk level was established as previously described,[15] whereby participants with high and intermediate risk levels were classified as MSC+ and participants with low risk levels or persisting high hemolysis levels as MSC−.

As per the protocol, CT− and MSC− individuals at baseline were allocated to repeat LDCT at a 3-year interval. CT− individuals who were MSC+ were allocated to repeat LDCT and MSC examination at 1-year intervals. Positive LDCT individuals (CT+) with SN with volume 113-260 mm^3 and/or PSN with a solid component $<$5 mm and/or NSN $\geq$5 mm were allocated to repeat LDCT and MSC examination at 1-year intervals. CT+ individuals with SN $>$260 mm^3 or PSN with a solid component $\geq$5 mm underwent further examination within 3 months [including LDCT, contrast-enhanced CT, positron emission tomography (PET), or biopsy in the case of masses], independent of the MSC results. After the 3-year screening round, all individuals were invited to continue the screening according to their risk profile and latest LDCT results. More specifically, CT−/MSC− individuals were offered a 6-year screening round if the 3-year LDCT result was negative.

Statistical analysis

The entire BioMILD population was classified into four risk profiles according to baseline LDCT and MSC results: (i) double negatives (CT−/MSC−); (ii) negative LDCT and positive MSC (CT−/MSC+); (iii) positive LDCT and negative MSC (CT+/MSC−); and (iv) double positives (CT+/MSC+). For primary analysis, the participants were *a priori* stratified by the four risk groups, and the analysis was carried out accordingly.

The percentage of LC detected among all participants screened was the main outcome, hereafter referred to as the LC incidence. The percentages of LCs detected with specific features, such as stage I, resectable and interval cancer, on the total number of participants and of LCs detected was also calculated. All these measures were derived from the entire cohort and stratified according to risk profiles.

Measures of association were evaluated by the chi-square test or Fisher's exact test for categorical data and by the Mann–Whitney *U* test for continuous variables. Mortality rates per 1000 person-years were calculated for the overall cohort and the cohorts stratified by risk profile; differences were examined using the mid-*P* test. Cumulative LC, stage I LC and late-stage LC incidence Kaplan–Meier curves were censored at 4 years and LC mortality Kaplan–Meier curves at 5 years. Selected risk profiles were compared using the log-rank test.

Cox proportional hazard regression was applied to estimate the 4-year LC incidence and 5-year LC mortality hazard ratio (HR) and 95% confidence interval after adjustment for age, sex, and pack-years (continuous) to reduce the potential effect of different baseline characteristics. Models estimated (i) the effect of LDCT measured at baseline alone (model A), (ii) the main effects of both LDCT and MSC at baseline (model B), and (iii) the main and interacting effects of LDCT and MSC (model C). The goodness of fit of each model is expressed as −2 log-likelihood (−2 logL). Because models A, B, and C were hierarchically linked, the difference in −2 logL follows chi-square statistics under the null hypothesis of model equivalence. If not otherwise specified, LC incidence was evaluated by the inclusion of all LCs detected from baseline up to 4 years of follow-up.

As supplementary analyses, different Cox regression models for LC incidence at 4 years were carried out to assess the following: (i) the predictive discrimination of LDCT exam results added by the MSC, such as nodule size and type; (ii) the predictive value of MSC for late-stage LC incidence in CT−; (iii) the predictive discrimination of the LCRAT (Lung Cancer Risk Assessment Tool)[16] and the PLCOm2012 (Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial Model 2012)[17] added by CT and the MSC risk profile; and (iv) the predictive value of the Brock risk score[18] added by the MSC in CT+.

All analyses were carried out using Statistical Analysis System Software (Release SAS: 9.04; SAS Institute, Cary, NC).

RESULTS

Study population

Of the 9735 registered volunteers, 4909 were eligible, and 4119 were actually recruited for the BioMILD study (Figure 1). The characteristics of the recruited volunteers are summarized in Table 1. Considering the entire cohort, the median age was 60 years (interquartile range: 55-64 years), and 39.3% were female. Most of the participants were current smokers (79.2%), with a median of 42 pack-years (interquartile range: 35-52 pack-years). A total of 2973 (72.2%) volunteers met the NLST eligibility requirements. At the baseline examination, 2664 (64.7%) participants were classified as CT−/MSC−, 800 (19.4%) as CT−/MSC+, 446 (10.8%) as CT+/MSC−, and 209 (5.1%) as CT+/MSC+. With a median age of 59 years, the CT−/MSC− group was younger ($P = 0.003$) and included fewer females (37.6%, $P = 0.002$). When comparing the double-negative versus all other participants, however, no differences in pack-years (<30 versus ≥30 pack-years, $P = 0.10$) or smoking status ($P = 0.85$) were observed. Furthermore, there was no statistically significant association between the MSC and smoking habits (lifetime duration, pack-years, time since quitting), as reported in Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.01.008. With 22 576 person-years and >11 600 LDCT scans carried out, the mean LDCT number for each participant in the CT−/MSC−, CT−/MSC+, CT+/MSC−, and CT+/MSC+ groups was 2.3, 3.6, 4.3, and 4.2, respectively. Adherence to the screening protocol of double-negative participants was 92% (2455/2664), with 2269 having a second LDCT at 3 years and 186 (7%) before the planned time. Among the 209 (7.8%) participants who abandoned screening after the baseline LDCT, we observed no LC-related deaths within 3 years.

LC detection and mortality

At 4 years of follow-up from the baseline round, LC was diagnosed in 119 participants (2.9%): 72 (60.5%) stage I cases and 81 (68.1%) adenocarcinomas; 96 (80.7%) were resectable (Table 2). The LC incidence was 0.8% in the CT−/MSC− participants, 1.1% in the CT−/MSC+ participants, 10.8% in the CT+/MSC− participants, and 20.1% in the CT+/MSC+ participants. Among LCs, 48 cases were diagnosed by baseline LDCT (48/4119, 1.2%); 20 in CT+/MSC− (20/446, 4.5%) and 28 in CT+/MSC+ (28/209, 13.4%) participants ($P < 0.001$). LC mortality rates (per 1000 person-years) at 5 years in the four risk groups were 0.5, 1.5, 4.2, and 10.1.

Regarding CT+ participants, the MSC− group had a lower incidence of LC ($P = 0.001$) and a lower incidence of late-stage LC ($P < 0.001$). In the CT− group, only the difference in late-stage LC incidence was very close to significant ($P = 0.05$). There was no significant evidence, however, that the incidence of interval cancer and stage I LCs differed between MSC− and MSC+ patients within the strata of the CT+ and CT− participants.

The proportion of stage I LC of LC cases was 60.5% overall: 55.0% in CT−/MSC− compared with 22.2% in CT−/MSC+ ($P = 0.13$) and 75% in CT+/MSC− compared with 54.8% in CT+/MSC+ ($P = 0.04$). No significant evidence of differences in the proportion of interval cancer (10/119, 8.4% overall) was detected.

Concerning tumor histology, adenocarcinoma was the most common type in the entire cohort. No resection for pure bronchioloalveolar carcinoma (BAC), now classified as *in situ* adenocarcinoma (AIS) or atypical adenomatous hyperplasia (AAH), was carried out.

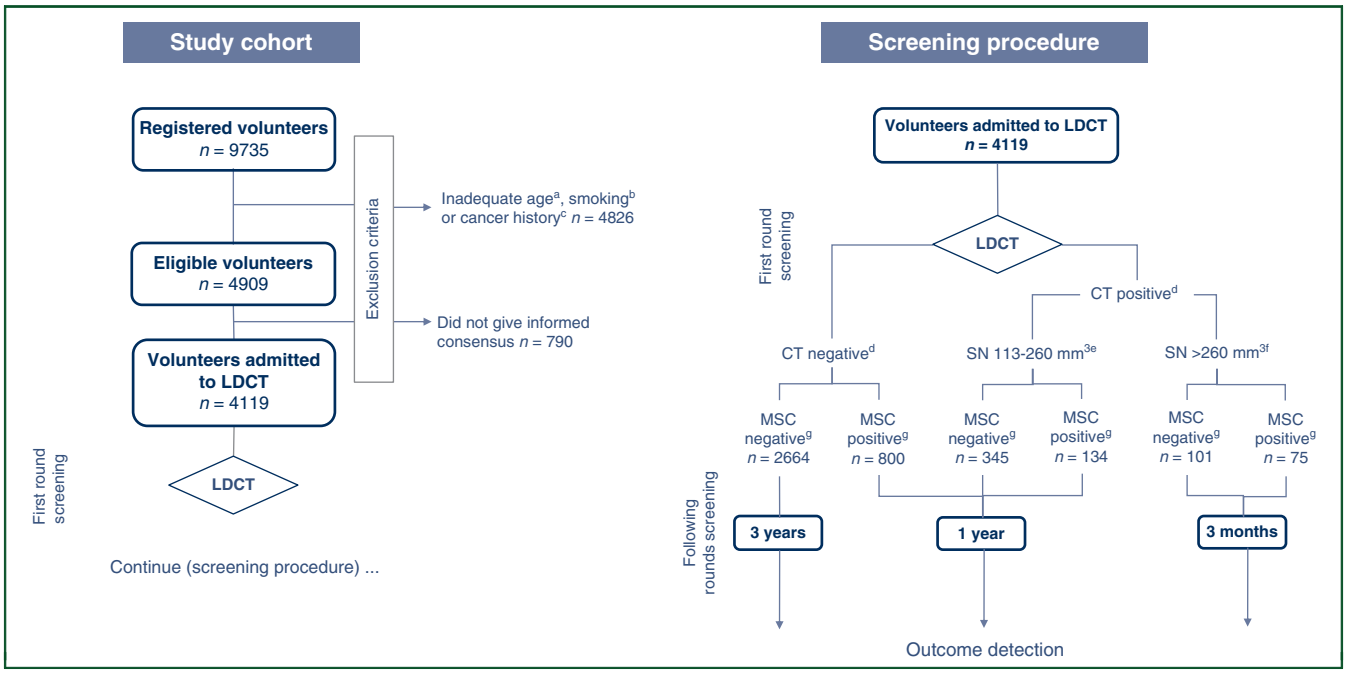

Figure 1. CONSORT diagram of the BioMILD screening trial.
LDCT, low-dose computed tomography; LC, lung cancer; MSC, micro RNA signature classifier; SN, solid nodules.
[a]Volunteers aged ≤50 or ≥75 years.
[b]Never smokers or former smokers who quit for 10 years or more or current smokers with <30 pack-years or current smokers with <20 pack-years without chronic obstructive pulmonary disease and/or family history of lung cancer.
[c]Volunteers in whom a neoplasm was diagnosed in the past 5 years.
[d]Negative LDCT: no nodule, or nodule with calcification pattern, or solid nodules <113 mm^3, or non-solid nodules <5 mm; positive LDCT: solid nodules ≥113 mm^3, or part-solid. nodules, or non-solid nodules ≥5 mm.
[e]Positive LDCT with solid nodules 113-260 mm^3, or part-solid nodules with solid component <5 mm, or non-solid nodules ≥5 mm.
[f]Positive LDCT with solid nodules >260 mm^3, or part-solid nodules with solid component ≥5 mm, or clinically significant findings.
[g]Negative MSC: low risk level or hemolyzed samples; positive MSC: intermediate or high risk level (see text).

In addition, the clinical management of indeterminate nodules was not guided by the miRNA results, even though the median time from positive LDCT to tissue diagnosis or surgical resection in CT-detected LC was 68 days in CT+/MSC+ versus 78 days in CT+/MSC− ($P = 0.04$). Overall, five participants underwent lung resection, with benign histology, representing 5% of all lung resections: none in the CT−/MSC+ group, three in the CT+/MSC− group, and one each in the other two groups.

The numbers of CT-detected and interval LCs per year/screening round are reported in Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.01.008, for each of the four MSC and LDCT groups. The total number of recalls within 4 months for suspicious baseline LDCT results was 293/4119 participants (7.1%), but the frequency of LC among 4-month recalls was lower in the CT+/MSC− group than in the CT+/MSC+ group, at 18.3% (33/180) versus 33.6% (38/113, $P = 0.003$).

Four-year LC incidence analysis

The main and interacting effects of LDCT and the MSC on the 4-year LC incidence are shown in Table 3. As expected, a strong effect of baseline LDCT was observed; a significant effect of baseline MSC was also observed, with 4-year LC incidence among MSC+ participants being 2.02-fold higher than that among MSC− participants (difference in −2 log-L models A and B = 1739.9-1726.4 = 13.5, 1 degree of freedom, $P < 0.001$). Conversely, there was no evidence that LDCT and the MSC acted synergically (difference in −2 log-L models B and C = 1726.4-1725.7 = 0.7, 1 degree of freedom, $P = 0.40$).

The results of adjusted Cox models for LC incidence stratified by LDCT nodule size and type (Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.01.008) showed a higher risk in MSC+. The restricted Cox model of CT− volunteers comparing 4-year late-stage LC incidence in strata of the MSC results (Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.01.008) revealed a nonsignificantly higher risk for MSC+ (HR 2.63, $P = 0.06$).

Moreover, 4-year LC incidence curves indicated a significant difference among the four risk groups (log-rank test $P < 0.001$), both for all LC cases (Figure 2A) and excluding prevalent LCs (Figure 2B). Comparisons were still statistically significant when comparing double negatives with all others (log-rank tests $P < 0.001$ for all cases and without prevalent cases). Models describing the effects of LDCT and MSC with the exclusion of prevalent LC cases are shown in Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2022.01.008.

Table 1. Selected characteristics of 4119 BioMILD participants by risk profile

	Total	CT-/MSC-	CT-/MSC+	CT+/MSC-	CT+/MSC+	
	N = 4119	2664 (64.7%)	800 (19.4%)	446 (10.8%)	209 (5.1%)	*P* value
Age, years, *n* (%)						
<55	980 (23.8)	664 (24.9)	191 (23.9)	88 (19.7)	37 (17.7)	<0.001[a]
55-64	2124 (51.6)	1385 (52)	428 (53.5)	206 (46.2)	105 (50.2)	0.003[b]
≥65	1015 (24.6)	615 (23.1)	181 (22.6)	152 (34.1)	67 (32.1)	
Median (IQR)	60 (55-64)	59 (55-64)	59 (55-64)	61 (56-67)	61 (56-66)	<0.001[a]
Sex, *n* (%)						<0.001[b]
Female	1618 (39.3)	1001 (37.6)	352 (44)	181 (40.6)	84 (40.2)	0.01[a]
Male	2501 (60.7)	1663 (62.4)	448 (56)	265 (59.4)	125 (59.8)	0.002[b]
Pack-years, *n* (%)						
<30	267 (6.5)	185 (6.9)	48 (6)	18 (4)	16 (7.7)	0.11[a]
≥30	3852 (93.5)	2479 (93.1)	752 (94)	428 (96)	193 (92.3)	0.10[b]
Median (IQR)	42 (35-52)	41 (35-52)	41 (34-51)	44 (37-54)	44 (35-54)	<0.001[a]
						0.08[b]
NLST eligible	2973 (72.2)	1890 (70.9)	579 (72.4)	345 (77.4)	159 (76.1)	0.02[a]
						0.02[b]
Smoking status, *n* (%)						
Current smoker	3263 (79.2)	2108 (79.1)	622 (77.8)	380 (85.2)	153 (73.2)	0.0015[a]
Former smoker	856 (20.8)	556 (20.9)	178 (22.3)	66 (14.8)	56 (26.8)	0.85[b]
Median person-years	5.3	5.3	5.5	5.2	5.6	
Total *n* of CTs	11 646	6002	2853	1918	873	
Mean CTs per participant	2.8	2.3	3.6	4.3	4.2	

CT, computed tomography; IQR, interquartile range; MSC, miRNA signature classifier; NLST, national lung screening trial.
[a]All risk profiles.
[b]CT−/MSC− versus other.

The 4-year stage I and late-stage LC incidence (Supplementary Figure S1A and B, available at https://doi.org/10.1016/j.annonc.2022.01.008) curves illustrated a significantly lower incidence of both stage I (log-rank test $P < 0.001$) and higher-stage LC (log-rank test $P < 0.001$) in double-negative participants than in all others. These differences were confirmed when comparing stage I and late-stage LC incidence in CT− versus CT+ participants (Supplementary Figure S2A and B, available at https://doi.org/10.1016/j.annonc.2022.01.008, respectively). Notably, in CT− participants, no stage I LC and only two higher stage LCs (0.06%), namely, one in CT−/MSC− and one in CT−/MSC+, were detected at the 2-year follow-up.

Five-year LC mortality analysis

The 5-year cumulative LC mortality curves also revealed a significant difference among the four risk categories: Figure 3A with all LCs (log-rank test $P < 0.001$) and Figure 3B without prevalent LCs (log-rank test $P = 0.04$). Differences were statistically significant when comparing double negatives to all others (log-rank test $P < 0.001$ for all cases and log-rank test $P = 0.009$ without prevalent cases). Although the difference in 5-year mortality rate between CT−/MSC− and CT−/MSC+ did not reach statistical significance (0.5 versus 1.5, $P = 0.07$, Table 2), significance by the log-rank test was close ($P = 0.05$, Figure 3A), possibly

Table 2. Characteristics of LCs and study outcome by risk profile

	Total 4119	CT−/MSC− 2664	CT−/MSC+ 800	CT+/MSC− 446	CT+/MSC+ 209	*P* value
4-Year LC[a]	119 (2.9)	20 (0.8)	9 (1.1)	48 (10.8)	42 (20.1)	0.31[b]/0.001[c]
Baseline LC	48 (1.2)			20 (4.5)	28 (13.4)	<0.001[c]
Interval cancers	10 (0.2)	5 (0.2)	1 (0.1)	3 (0.7)	1 (0.5)	1.00[b]/1.00[c]
[% LCs]	[8.4%]	[25.0]	[11.1]	[6.3]	[2.4]	0.63[b]/0.62[c]
Stage I LC	72 (1.7)	11 (0.4)	2 (0.3)	36 (8.1)	23 (11.0)	0.74[b]/0.22[c]
[% on LCs]	[60.5%]	[55.0]	[22.2]	[75.0]	[54.8]	0.13[b]/0.04[c]
Higher stage LC	47 (1.1)	9 (0.3)	7 (0.9)	12 (2.7)	19 (9.1)	0.05[b]/<0.001[c]
[% on LCs]	[39.5%]	[45.0]	[77.8]	[25.0]	[45.2]	0.13[b]/0.04[c]
Adenocarcinoma	81 (2.0)	15 (0.6)	1 (0.1)	40 (9.0)	25 (12.0)	0.14[b]/0.23[c]
[% on LCs]	[68.1%]	[75.0]	[11.1]	[83.3]	[59.5]	0.003[b]/0.01[c]
LC resections [% on LCs]	96 [80.7]	15 [75.0]	4 [44.4]	42 [87.5]	35 [83.3]	0.20[b]/0.57[c]
5-Year LC deaths (MR per 1000 person-years)	32 (1.6)	7 (0.5)	6 (1.5)	9 (4.2)	10 (10.1)	0.07[b]/0.06[c]

Values are *n* (% of total participants) unless otherwise stated.
CT, computed tomography; LC, lung cancer; MR, mortality rate; MSC, miRNA signature classifier; VATS, video-assisted thoracoscopic surgery.
[a]LCs detected from baseline to 4 years of follow-up.
[b]CT−/MSC− versus CT−/MSC+.
[c]CT+/MSC− versus CT+/MSC+.

Table 3. Adjusted Cox regression models for 4-year lung cancer incidence: (i) the effect of LDCT measured at baseline alone (model A), (ii) the main effects of both LDCT and MSCs at baseline (model B), (iii) the main effects of LDCT and MSCs and their interaction (model C)

4-Year lung cancer incidence					
	4-Year LC	HR	95% CI	*P* value	−2 log-likelihood
Model A					
CT+ versus CT−	90/655 versus 29/3464	16.58	(10.88-25.28)	<0.001	1739.9
Model B					
CT+ versus CT−	90/655 versus 29/3464	15.77	(10.34-24.05)	<0.001	1726.4
MSC+ versus MSC−	51/1009 versus 68/3110	2.02	(1.40-2.90)	<0.001	
Model C[a]					
CT−/MSC+ versus CT−/MSC−	9/800 versus 20/2664	1.51	(0.69-3.32)	0.30	1725.7
CT+/MSC− versus CT−/MSC−	48/446 versus 20/2664	13.73	(8.12-23.22)	<0.001	
CT+/MSC+ versus CT−/MSC−	42/209 versus 20/2664	30.14	(17.67-51.39)	<0.001	

All 4119 volunteers were included in the models.
Models were adjusted for age, sex, and pack-years.
CI, confidence interval; CT, computed tomography; HR, hazard ratio; LC, lung cancer; LDCT, low-dose computed tomography; MSC, microRNA signature classifier.
[a]The *P* value for the interaction term is 0.41.

due to the very small number of events. The main and interacting effects of LDCT and the MSC on the 5-year LC mortality are shown in Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.01.008, with all cases included and in Supplementary Table S7, available at https://doi.org/10.1016/j.annonc.2022.01.008, without prevalent LCs.

Combination with predictive models

Supplementary analyses in Supplementary Tables S8 and S9, available at https://doi.org/10.1016/j.annonc.2022.01.008, evaluate the incremental effect of LDCT and the MSC on two 'prescreening' clinical-based predictive models, LCRAT and PLCOm2012, respectively (model 0). Models 1 (including LDCT) and 2 (including the combination of LDCT and MSC) increased the goodness of fit compared with model 0 for LCRAT and PLCOm2012. In addition, the effect of MSCs on 4-year LC was still statistically significant ($P = 0.008$) in a Cox model adjusted for the Brock risk score (Supplementary Table S10, available at https://doi.org/10.1016/j.annonc.2022.01.008).

DISCUSSION

Feasibility of the 3-year interval for low-risk individuals

BioMILD is a prospective trial that offered heavy smokers a screening program combining a high threshold for negative LDCT (113 mm^3) and a predefined blood miRNA assay (MSC). At baseline, LDCT and the MSC were tested independently in 4119 volunteers with blind evaluation, with different screening intensities according to the results of the two tests. Most of the participants (64.7%) were double-negative for LDCT and the MSC and were allocated to a 3-year LDCT repeat interval. In contrast, participants with a positive MSC and/or positive LDCT result underwent annual or shorter LDCT repeats on the basis of LDCT features only.

In double-negative individuals, we found very low values for all relevant parameters: overall LC incidence at 4 years, interval cancer, stage I, and higher stages, as well as the lowest LC mortality rate at 5 years. An apparent increase in the proportion of interval cancer (5/20, 25%) was also observed for these participants, but it was not clinically relevant because three of five patients had stage I, resectable disease and were still alive.

The safety of our risk-based intervals was demonstrated by comparison with the results of other screening trials, such as the NELSON trial,[19] which reported an overall 0.5% incidence of interval cancer and 1% incidence of late-stage LC at the end of the 3-year screening round, as compared with 0.2% and 1% for the BioMILD trial, respectively.

Overall, the BioMILD results indicate that it is possible to optimize screening intensity and reduce unnecessary LDCT repeats without a significant detrimental effect on LC detection and mortality. Among CT+ participants, the discriminant power of the MSC peaked at 2 years, in keeping with our previous estimate.[12]

Identification of high-risk individuals

In the past decade, LDCT screening has resulted in a substantial reduction in LC mortality that is proportional to the screening duration.[3-5] In this encouraging scenario, the definition of individual risk to personalize LDCT intervals and other preventive measures is a central issue for improving screening benefits, avoiding unnecessary invasive procedures, and reducing cost and long-term radiation exposure. A preliminary clue in this direction can be gleaned from retrospective cohort analyses of data from the NLST and NELSON trials, which showed that participants with negative LDCT prevalence screening had a lower incidence of LC and LC-specific mortality than the overall group of participants undergoing prevalence screening.[20,21] Indeed, in the MILD trial, which tested this hypothesis with a randomized design, biennial LDCT rounds achieved a similar mortality reduction when compared with annual rounds in participants with negative baseline LDCT.[9] In the NELSON study, in which patients were screened at fixed gaps of 0, 1, 3, and 5.5 years, the final LDCT at 2.5 years from the previous timepoint detected a higher proportion of advanced

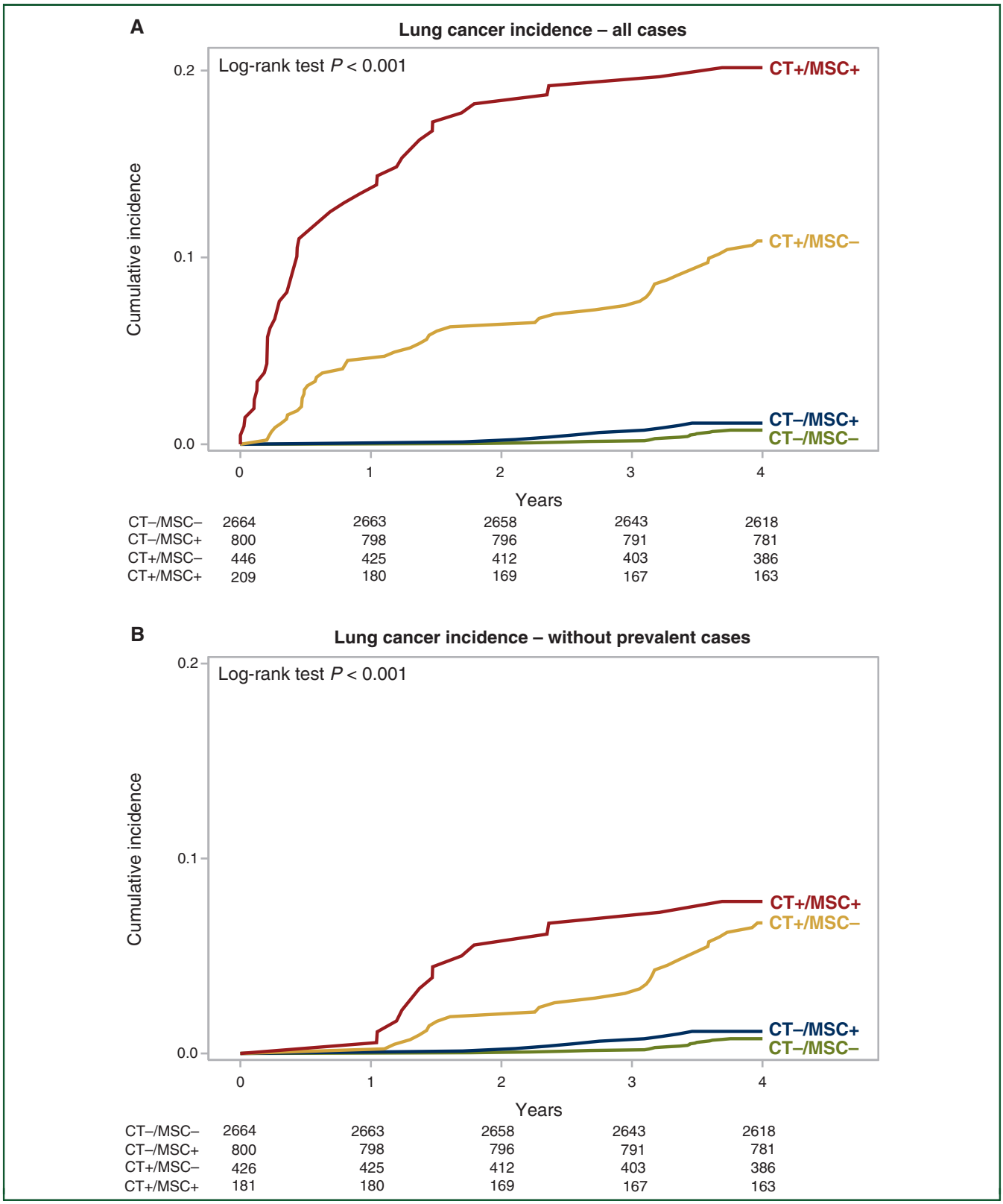

Figure 2. Lung cancer incidence curves.
Four-year cumulative lung cancer incidence with (A) inclusion and (B) exclusion of prevalent lung cancer cases diagnosed by the initial low-dose CT (baseline), as stratified by four risk groups: CT−/MSC−, CT−/MSC+, CT+/MSC−, and CT+/MSC+.
CT, computed tomography; MSC, micro RNA signature classifier.

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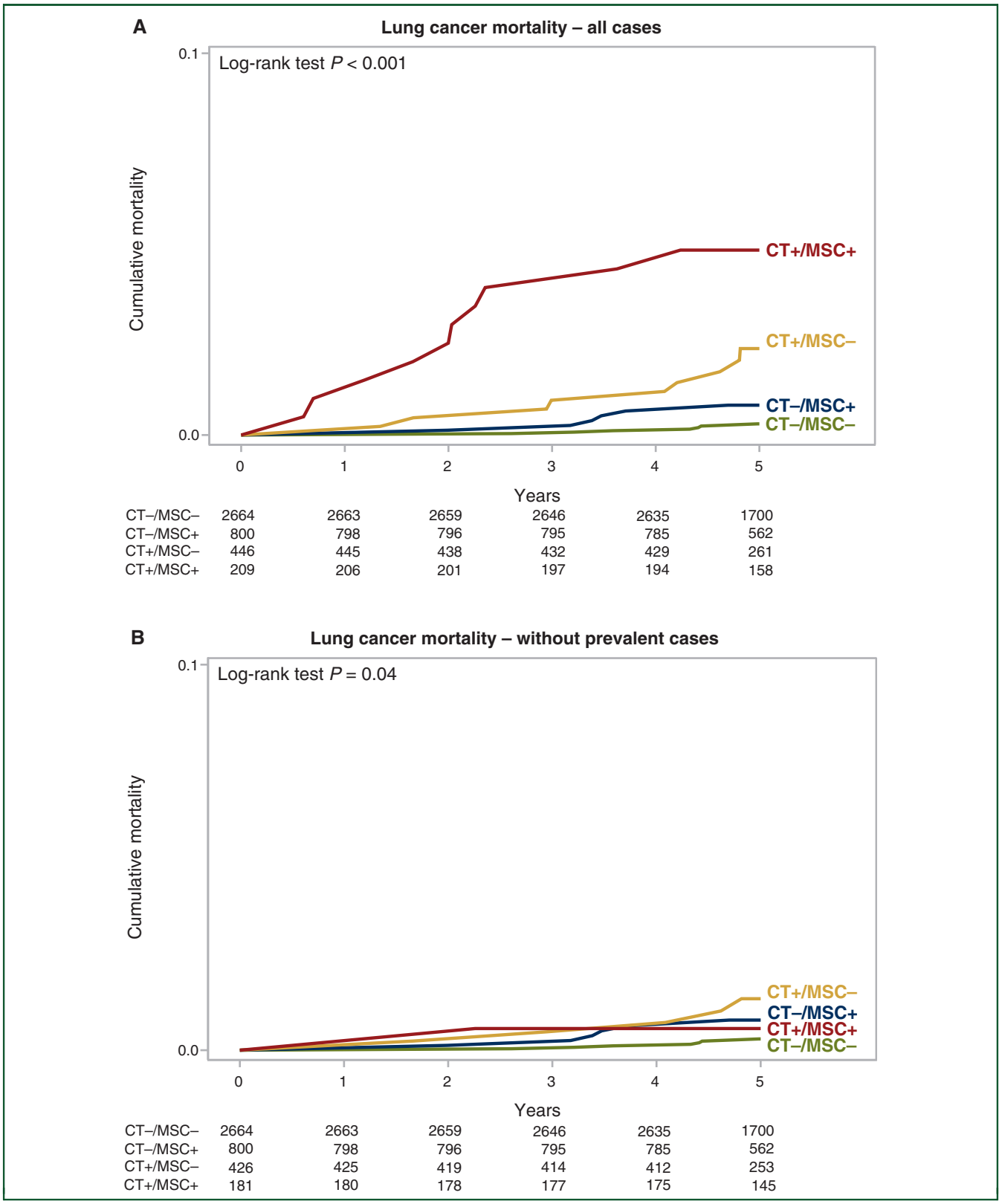

Figure 3. Lung cancer mortality curves.
Five-year cumulative lung cancer mortality with (A) inclusion and (B) exclusion of prevalent lung cancer cases diagnosed by the initial low-dose CT (baseline), stratified by four risk groups: CT−/MSC−, CT−/MSC+, CT+/MSC−, and CT+/MSC+.
CT, computed tomography; MSC, micro RNA signature classifier.

stage and interval cancers in the fourth round compared with the previous rounds.[22] Thus, the addition of effective blood markers appears to further optimize LDCT screening through baseline risk prediction and hopefully improve the management of indeterminate pulmonary nodules.

Clinical-based risk models, such as PLCOm2012, provide personalized risk scores to identify individuals at high risk of developing LC.[17] In keeping with previous studies, integrating LDCT outcome with prescreening characteristics increases diagnostic yield.[7,8] Notably, our BioMILD results also showed that the combination of CT and the MSC outperforms the predictive risk discrimination of LCRAT and PLCOm2012 and nodule malignancy Brock models.[16-18]

Management of SN and NSN

In the BioMILD trial, we set higher LDCT cut-offs for SN sizes, as optimized on the basis of the MILD trial experience,[5,9] which now mirror the new categories proposed by the American College of Radiology Lung CT Screening Reporting and Data System (Lung-RADS1.1). The population prevalence was 84% for nodules <113 mm^3 (or absent), 11.7% for nodules 113-260 mm^3 or NSN >5 mm, and 4.3% for nodules >260 mm^3. Such a high volumetric cut-off for indeterminate pulmonary nodules (113 mm^3) resulted in an 84% frequency of negative LDCT and a very high negative predictive value.

Moreover, these cut-offs led to an overall 7.1% recall rate at 4 months for suspicious baseline LDCT results, with a significantly lower frequency of LC in the CT+/MSC− group versus the CT+/MSC+ group (18.3% versus 33.6%, $P = 0.003$). For the CT+ participants, we did not use miRNA test results to define the likelihood of malignancy, the time of LDCT repeat or immediate diagnostic work-up, for both indeterminate and positive LDCT nodules. Compared with the 27.3% recall rate at 4 months in the NLST[3] and 20.8% in the NELSON[23] trials, our 7.1% recall rate represents a further benefit of the BioMILD design. These results expand the prospects for conservative management of indeterminate pulmonary nodules in future screening programs.

By implementing active surveillance of NSNs, the BioMILD protocol resulted in a low risk of lung resections for benign nodules, well below the recommended threshold of 10%.[24] Together with the absence of resection for indolent disease (AAH, AIS, and pure BAC), the BioMILD outcomes set a new standard for containing overtreatment in LDCT screening.

Blood-based biomarkers in LC screening

A few blood-based biomarkers have reached the prospective validation stage, among these the Early CDT-Lung test in the Early Detection of Cancer of the Lung Scotland (ECLS) study.[25] The latter assessed the utility of seven autoantibodies (Early CDT-Lung test) for the detection of early LC compared with standard clinical practice in >12 000 participants. The results at 2 years showed a 36% reduction in stage III/IV LC incidence in participants randomized to the interventional arm. Given the short follow-up period, however, no significant reduction in mortality between the two arms was observed. Nevertheless, such a study design caused LDCT screening to be unavailable to the vast majority (90%) of participants, because of a negative Early CDT-Lung test.

A 13-miRNA serum signature and a plasma circulating C4d complement fragment for LC screening have been tested in retrospective studies, with potential for early detection of LC.[26,27] The DETECT-A study evaluated the feasibility and safety of blood testing coupled with PET-CT imaging to detect all types of cancer in a nonregistered, prospective, interventional study of 10 006 women. By combining cell-free DNA and protein biomarkers, the authors suggested that blood testing can be safely incorporated into routine clinical care.[28] According to the AIR study, however, which evaluated 614 individuals with COPD at high risk of developing LC, circulating tumor cell detection is not suitable for LC screening.[29]

Epigenetic markers appear to be more informative for cancer risk than circulating tumor markers. Two studies on participants from the large Circulating Cell-free Genome Atlas (CCGA) trial assessed the performance of targeted methylation analysis of cell-free DNA to detect and localize multiple cancer types across all stages at high specificity (99.5%). Methylation patterns detected more than 50 cancer types, and although the sensitivity for stage I LC was only 23%, further evaluation of this test in prospective screening trials is warranted.[30,31]

The algorithm composing the MSC was derived from high-throughput profiling of miRNA circulating in the plasma of high-risk smokers.[10] The MSC is composed of 24 miRNAs that originate mostly from lung stromal and hematopoietic cells[32] and identify a subgroup of patients who do not benefit from immunotherapy treatments.[33]

An miRNA-based test is affordable, efficient, and feasible in standard clinical laboratories. The main limitation of such a test is sensitivity to hemolysis.[34,35] Indeed, nonspecific release of miRNAs due to white and red blood cell lysis leads to a negative MSC result. Implementing the miRNA test by excluding the miRNAs most affected by hemolysis or adjusting for the degree of hemolysis, however, is crucial to implement the test into clinical practice.

Implications of BioMILD findings

Regarding the prospect of long-term screening programs, exceeding 10 years, a 3-year interval for low-risk individuals would save more than half of the total numbers of LDCT scans or double the number of participants at the same cost. Such a personalized strategy would also reduce unnecessary radiation exposure to the majority of low-risk participants.

Indeed, the use of the miRNA signature to complement baseline screening in all subjects is not supported by the data. In fact, the unexpectedly high negative predictive value of LDCT, as achieved by the use of a higher cut-off size for SNs, limits the utility of MSC in individuals who undergo CT. The observed LC incidence at 3 years was so low (<1%) among CT− participants, regardless of MSC, that they

would not even be eligible for LDCT screening according to the current recommendations,[36-38] which justifies less intense (triennial) monitoring.

Instead, in individuals with baseline indeterminate or positive LDCT results, the multiplicative effect in the risk conferred by MSC may improve the overall performance of screening by guiding the decision to take a biopsy or target 4-month recalls and subsequent intervals; in our view, this population represents the best setting for blood biomarker analysis.

CONCLUSIONS

BioMILD is a screening trial that prospectively tested LDCT and a blood biomarker panel in combination, showing their clinical utility in targeting screening intervals on the basis of initial risk prediction. This combination identified individuals with major differences in LC risk despite similar age and tobacco exposure.

This study therefore provides specific guidance to future studies and priorities for implementing CT screening biomarkers. The findings also establish a basis for the adoption of personalized screening and prevention programs.

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DISCLOSURE

UP, MB, and GS are coinventors of three patent applications regarding the miRNA signature classifier. These patents were licensed to a private company, Gensignia Life Science, under the regulations of Fondazione IRCCS Istituto Nazionale dei Tumori of Milan. All other authors have declared no conflicts of interest.

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