At present, evaluation and screening for lung cancer are based on a combination of blood tests, computed tomography, and positron emission tomography.\textsuperscript{1-4} In early-stage tumors, imaging still yields high false-positive rates; therefore, definitive diagnosis relies on pathologic confirmation (ie, biopsy and surgery), which can result in complications.\textsuperscript{1,4-6} Moreover, up to 11\% of resected small pulmonary nodules without preoperative pathologic confirmation are ultimately benign.\textsuperscript{7,8} Therefore, to increase diagnostic accuracy and ultimately change the fate of these patients, researchers and clinicians have strived to implement innovative and minimally invasive approaches to identify diagnostic biomarkers of lung cancer that are detectable in blood, urine, or exhaled breath.\textsuperscript{3,9-13}

**Breathprinting: The Backbone of Electronic Nose Technology**

One such diagnostic biomarker is volatile organic compounds (VOCs), which are generated by various cellular metabolic pathways (ie, cytochrome P450 and oxidative stress,\textsuperscript{4,14} the Warburg effect,\textsuperscript{15,16} and genomic alterations\textsuperscript{4,17}), are altered in relation to cancer, and are excreted in the airways on the basis of the equilibrium between tissue to blood and blood to air.\textsuperscript{4} The exhaled breath typically contains \(\sim\)3000 different VOCs at concentrations of parts per million, with some present at concentrations as low as parts per billion and parts per trillion (breathprint library).\textsuperscript{4,18}

Previously, owing to the ability of the animal olfactory system to detect VOCs up to parts per trillion, canine models have been used to study exhalates to diagnose various benign and malignant diseases.\textsuperscript{19} In this setting, McCulloch and colleagues\textsuperscript{19} demonstrated that canines could accurately identify lung cancer with a sensitivity of 99\% and specificity of 99\%. However, the unreliability of the animals, the binary nature of their response, and the inability to determine the specific VOCs related to cancer limited the reproducibility and widespread use of this approach.\textsuperscript{20} Nevertheless, these efforts resulted in the conceptualization and development of engineered sensorial platforms, such as the electronic nose (e-nose), to analyze exhalates as early as the 1980s.\textsuperscript{21-23}

Historically, quantitative methods based on gas chromatography and mass spectrometry have been used to identify VOCs in exhaled breath.\textsuperscript{22,24,25} Despite the fact that they are time-consuming and require expensive laboratory equipment,\textsuperscript{4} gas chromatography and mass spectrometry allow for the creation of large breathprint libraries specific not only to lung cancer but to other cancers and benign diseases as well.\textsuperscript{8,1-3}

However, a qualitative approach for the assessment of VOCs was introduced on the basis of mimicking the combinatorial selectivity of the human nose.\textsuperscript{20} In this e-nose
model, patterns of VOC combinations to define lung cancer-associated breathprints are being studied. Current e-noses are composed of cross-reactive sensor arrays (ie, nanomaterial conductors, electroacoustic sensors, and colorimetric sensors) that can interact with a wide variety of VOCs and produce multidimensional outputs. The ability to cross-reference combinations of VOCs with the library and identify unique multidimensional patterns has yielded the concept of breathprinting. These breathprints can be detected by pattern-recognition algorithms that provide disease-specific percentage scores based on the combination of VOCs and have substantially improved the diagnostic accuracy of e-nose technology. Moreover, owing to a continuously updated library and the identification of new patterns, the diagnostic accuracy of this technology continues to improve.

In the setting of lung cancer, several investigators worldwide have confirmed the excellent diagnostic accuracy of e-nose technology in discriminating malignant from benign pulmonary nodules. Not only is the e-nose able to detect lung cancer, but further investigations have also demonstrated its ability to differentiate lung cancer histologic subtypes and genomic alterations and even to predict response to immunotherapy.

**Future Perspectives of E-Nose Technology**

Further improvements in the discriminatory ability of the e-nose, along with the availability of real-time readouts, are required to further increase the clinical implementation of this approach. Moreover, the devices should be low cost and manufactured to miniature size.

One approach to improve the discriminatory ability of the e-nose is to combine the superior resolution of the animal olfactory system with the reliability and reproducibility of the current computational e-nose devices. In recent years, steps have been taken to integrate olfactory receptors into nanomaterials and create a bioelectronic neuron-based e-nose (bio e-nose). In this setting, Farnum and colleagues successfully connected a computational platform to an insect brain coupled to a powerful chemosensor (ie, antennae). These experiences have led to the development of a DNA-derived phage bio e-nose by Lee and colleagues, which is composed of a series of phages genetically engineered to represent the DNA of mammalian olfactory receptor cells. This bio e-nose was able to classify lung cancer through neural pattern separation with an accuracy of 87%.

Another intriguing approach to increase the discriminatory ability of the e-nose is the inclusion of spectroscopically encoded resins or barcoded resins. The interaction of VOCs with these resins results in discrete Raman and infrared spectral variations. Surface-enhanced Raman spectroscopy can detect these discrete changes in variation and consequently increase the signal-to-noise ratio, which greatly improves diagnostic accuracy.

Although these technologies are not yet ready for full implementation, they can detect concentrations up to parts per trillion (10^{12}), which compares favorably with the resolution of parts per million (10^{6}) or parts per billion (10^{9}) of the current e-nose devices. Therefore, it can be reasoned that bio e-noses or barcoded resin e-noses could detect new trace-level VOCs and patterns and consequently identify new breathprints.

A decrease in the processing time of exhalates has been made possible by further advancements in available detection techniques, such as the use of proton-transferred reaction mass spectrometry, selected ion flow tube mass spectrometry, and tunable diode laser absorption spectroscopy. As an alternative, Ricci and colleagues studied the use of thermodynamic sensor technology that allows real-time analysis by directly measuring the energetic changes related to the thermal decomposition of VOCs and the subsequent redox reduction reaction of these degradation products with metal oxide-coated sensors. By substantially accelerating processing times, these approaches will facilitate real-time readouts of the exhalates in next-generation e-noses assessed through a series of prospective trials currently ongoing at Memorial Sloan Kettering Cancer Center that are based on the technology created by the University Campus Biomedico of Rome. Table 1 highlights the advantages, disadvantages, and performance of the different e-nose technologies.

The possible clinical advancements that can be achieved through the use of e-nose technology are enormous and will continue to expand in the diagnostic paradigms of lung cancer, the identification of subtypes and genomic alterations, the prediction and evaluation of treatment response, and the development of follow-up strategies. Despite these possibilities and the current state of the technology, larger prospective standardized trials and the development of high-resolution, integrated, miniaturized, next-generation e-noses with real-time readouts are necessary for e-nose technology to establish itself as a no-touch diagnostic tool that is complementary to the current standards (ie, computed tomography, positron emission tomography, biopsy, and DNA analysis). Moreover, the prevalence of lung cancer has to be considered before e-nose technology is implemented in real-life clinical trials and practice. Indeed, in settings with a high prevalence of lung cancer, the sensitivity may increase and the specificity may decrease, owing to a decrease in the chance of encountering both true and false negatives.

**CONCLUSIONS**

The e-nose has demonstrated excellent discriminatory abilities in the diagnosis of lung cancer. The advantage of being low cost, minimally invasive, and reproducible and...
### TABLE 1. Advantages and disadvantages of the different electronic nose (e-nose) technologies

<table>
<thead>
<tr>
<th>Technology</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Possible improvements</th>
<th>Diagnostic accuracy</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current technology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC/MS</td>
<td>VOC identification</td>
<td>Expensive equipment</td>
<td>Miniaturized size</td>
<td>Sensitivity: 71%-85%</td>
<td>22, E11</td>
</tr>
<tr>
<td></td>
<td>Provide VOC library</td>
<td>Specialized personnel</td>
<td>Increase selectivity and resolution with ion GC/MS or 2-dimensional GC</td>
<td>Specificity: 71%-100%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Influence of contaminants</td>
<td></td>
<td>Accuracy: 71%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low portability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canine model</td>
<td>Resolution of ppt</td>
<td>No VOC identification</td>
<td>Neural-based e-nose (animal olfactory neurons + computational platform)</td>
<td>Sensitivity: 99%</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Not affected by temperature or humidity</td>
<td>Requires animal training</td>
<td></td>
<td>Specificity: 99%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Real-time readout</td>
<td>Only 1 disease at a time</td>
<td></td>
<td>Accuracy: 99%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breathprint analysis</td>
<td>Reproducibility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reliability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorimetric e-nose</td>
<td>Easy and real-time readout</td>
<td>No VOC identification</td>
<td>Humidity-independent dyes</td>
<td>Sensitivity: 70%</td>
<td>4, E6</td>
</tr>
<tr>
<td></td>
<td>Easily manufactured</td>
<td>Resolution of ppm</td>
<td>Spectroscopical analysis</td>
<td>Specificity: 86%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cost-effective</td>
<td>Sample collection</td>
<td>Sample preservation</td>
<td>Accuracy: 81%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No advanced training</td>
<td>Affected by temperature and humidity</td>
<td>Pattern-recognition AI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breathprint analysis</td>
<td>Reproducibility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electroacoustic e-nose</td>
<td>Resolution of ppb</td>
<td>No VOC identification</td>
<td>Improved chemosensitive coatings</td>
<td>Sensitivity: 86%</td>
<td>3, E31</td>
</tr>
<tr>
<td></td>
<td>Cost-effective</td>
<td>Sample collection</td>
<td>Sample preservation</td>
<td>Specificity: 95%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No advanced training</td>
<td>Affected by temperature and humidity</td>
<td>Real-time readout</td>
<td>Accuracy: 93%</td>
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<tr>
<td></td>
<td>Commercially available</td>
<td>Altersations in coatings</td>
<td>Automated coating procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Semiselective coatings</td>
<td>Reproducibility</td>
<td>Pattern-recognition AI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Miniaturized size</td>
<td>Breathprint analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanomaterial e-nose</td>
<td>Resolution of ppb</td>
<td>No VOC identification</td>
<td>Improved chemosensitive coatings</td>
<td>Sensitivity: 71%-88%</td>
<td>4, E7, E31</td>
</tr>
<tr>
<td></td>
<td>Cost effective</td>
<td>Sample collection</td>
<td>Self-calibration</td>
<td>Specificity: 86%-96%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No advanced training</td>
<td>Altersations in coatings</td>
<td>Feature selection</td>
<td>Accuracy: 85%-91%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Commercially available</td>
<td>Time-consuming calibration</td>
<td>Automated coating procedure</td>
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<tr>
<td></td>
<td>Semiselective coatings</td>
<td>Reproducibility</td>
<td>Pattern-recognition AI</td>
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</tr>
<tr>
<td></td>
<td>Miniaturized size</td>
<td>Breathprint analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Future technology</td>
<td>Resolution of ppt</td>
<td>No VOC identification</td>
<td>Additional clinical testing</td>
<td>Sensitivity: NA</td>
<td>E22</td>
</tr>
<tr>
<td>Phage e-nose</td>
<td>No advanced training</td>
<td>First-generation devices</td>
<td>Improved biochemical sensors</td>
<td>Specificity: NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Real-time readout</td>
<td>Small sample size</td>
<td>Commercial manufacturing</td>
<td>Accuracy: 87%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breathprint analysis</td>
<td>Sample collection</td>
<td>Pattern-recognition AI</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Not commercially available</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Time-consuming manufacturing</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Reproducibility</td>
<td></td>
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</tbody>
</table>

(Continued)
the promise of further improvements in diagnostic accuracy, including the availability of real-time readouts, will facilitate its implementation in current paradigms (ie, screening, diagnostics, prediction of therapy response, and follow-up) and will further support the evolution toward personalized medicine.

Conflict of Interest Statement
Dr Rocco has a financial relationship with Scanlan, Merck, and Medtronic. Dr Jones serves as a consultant for AstraZeneca and on a Clinical Trial Steering Committee for Merck. The other author reported no conflicts of interest.

The Journal policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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E-References


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