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amounts of ctDNA, even when extremely sensitive techniques are used to identify them (*11*, *20*). Many proteins potentially useful for early detection and diagnosis of cancer have been described in the literature (*25*–*27*). We searched this literature to find proteins that had previously been shown to detect at least one of the eight cancer types described above with sensitivities > 10% and specificities > 99%. We identified 41 potential protein biomarkers (table S3) and evaluated them in preliminary studies on plasma samples from normal individuals as well as from cancer patients. We found that 39 of these proteins could be reproducibly evaluated through a single immunoassay platform and we then used this platform to assay all plasma samples (table S3). Eight of the 39 proteins proved to be particularly useful for discriminating cancer patients from healthy controls (table S3).

We then used CancerSEEK to study 1,005 patients who had been diagnosed with Stage I to III cancers of the ovary, liver, stomach, pancreas, esophagus, colorectum, lung, or breast. No patient received neo-adjuvant chemotherapy prior to blood sample collection and none had evident distant metastasis at the time of study entry. The median age at diagnosis was 64 (range 22 to 93). The eight cancer types were chosen because they are common in western populations and because no blood-based tests for their earlier detection are in common clinical use. The histopathological and clinical characteristics of the patients are summarized in table S4. The most common stage at presentation was American Joint Commission on Cancer (AJCC) stage II, accounting for 49% of patients, with the remaining patients harboring stage I (20%) or stage III (31%) disease. The number of samples per stage for each of the eight tumor types is summarized in table S11. The healthy control cohort consisted of 812 individuals

One limitation of liquid biopsies is their inability to determine the cancer type in patients who test positive, which poses challenges for clinical follow-up. To examine whether the CancerSEEK test can help identify a cancer's tissue of origin, we used supervised machine learning to predict the underlying cancer type in patients with positive CancerSEEK tests. The input algorithm took into account the ctDNA and protein biomarker levels as well as the gender of the patient (Supplementary Materials). One of the main purposes of such predictions is to determine the most appropriate follow-up test for cancer diagnosis or monitoring after a positive CancerSEEK test. We therefore grouped together patients with esophageal and gastric cancers, as endoscopy would be the optimal follow-up in both instances. We then used this algorithm (Supplementary Materials) to study the 626 cancer patients scoring positive in the CancerSEEK Test. Without any clinical information about the patients, we were able to localize the source of the cancer to two anatomic sites in a median of 83% of these patients (Fig. 3, table S7.4 (e)-7.4 (e)-4.9 (s)-13.6 (l)-13.60.3 (re)-F 8(lefTj0.00301 (P 0.312.108(lefT8 (90401601 T 301 0

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Fig. 1. Development of a PCR-based assay to identify tumor-specific mutations in plasma samples. Colored curves indicate the proportion of cancers of the eight types evaluated in this study that can be detected with an increasing number of short (< 40 bp) amplicons. The sensitivity of detection increases with the number of amplicons but plateaus at ~ 60 amplicons. Colored dots indicate the fraction of cancers detected using the 61-amplicon panel used in 805 cancers evaluated in our study, which averaged 82% (see main text). Publicly available sequencing data were obtained from the Catalog of Somatic Mutations in Cancer (COSMIC) repository.

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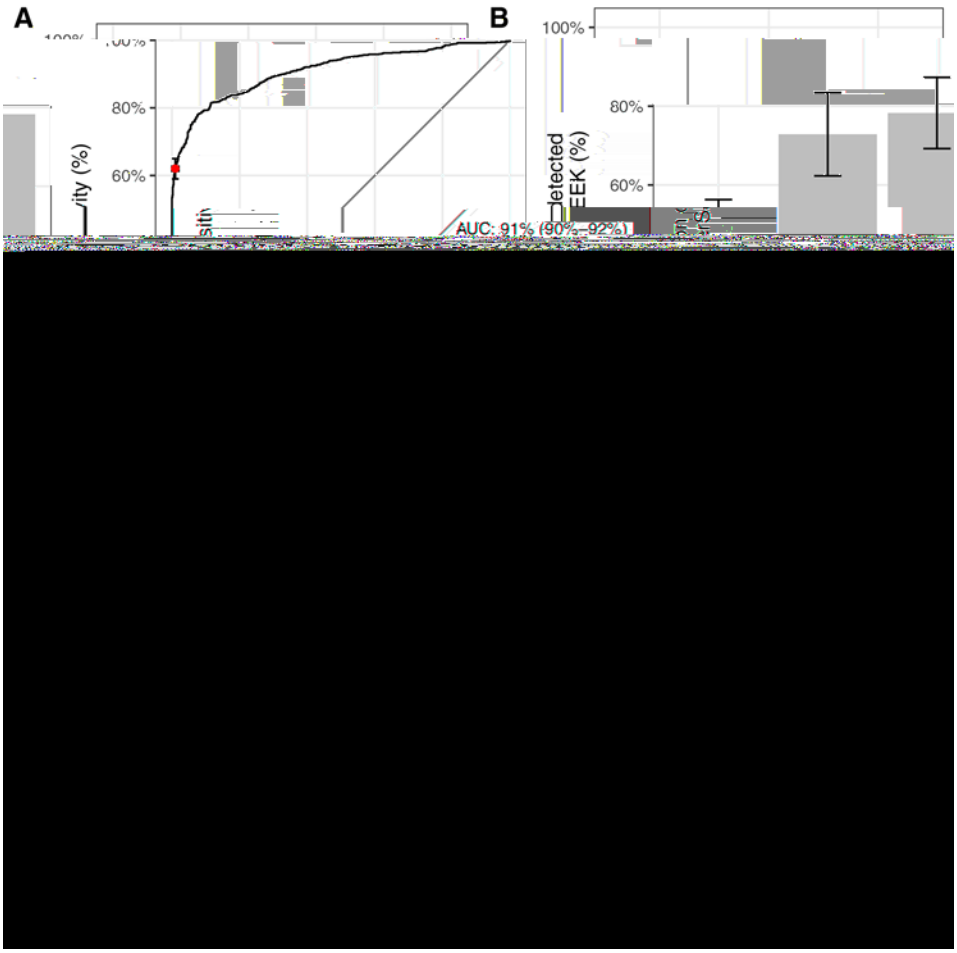

Fig. 2. Performance of CancerSEEK. (**A**) Receiver operator characteristic (ROC) curve for CancerSEEK. The red point on the curve indicate the test's average performance (62%) at > 99% specificity. Err.2 (d)-12.5 ( )]T.3

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Detection and localization of surgically resectable cancers with a multi-analyte blood test

Joshua D. Cohen, Lu Li, Yuxuan Wang, Christopher Thoburn, Bahman Afsari, Ludmila Danilova, Christopher Douville, Ammar A. Javed, Fay Wong, Austin Mattox, Ralph. H. Hruban, Christopher L. Wolfgang, Michael G. Goggins, Marco Dal Molin, Tian-Li Wang, Richard Roden, Alison P. Klein, Janine Ptak, Lisa Dobbyn, Joy Schaefer, Natalie Silliman, Maria Popoli, Joshua T. Vogelstein, James D. Browne, Robert E. Schoen, Randall E. Brand, Jeanne Tie, Peter Gibbs, Hui-Li Wong, Aaron S. Mansfield, Jin Jen, Samir M. Hanash, Massimo Falconi, Peter J. Allen, Shibin Zhou, Chetan Bettegowda, Luis Diaz, Cristian Tomasetti, Kenneth W. Kinzler, Bert Vogelstein, Anne Marie Lennon and Nickolas Papadopoulos

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