Evaluation of OncoK9® for Detection of Residual Disease and Cancer Recurrence in Dogs

KEY TAKEAWAYS

- Over 95% of samples with a *Cancer Signal Detected* OncoK9 result had presence of clinical disease at the same visit or at a future visit, demonstrating detection of *molecular recurrence*.
- Following excisional surgery, in the absence of clinical residual disease at the first post-operative visit, patients with a *Cancer Signal Detected* OncoK9 test result at that visit (indicative of *molecular residual disease*) were found to be twice as likely to have *clinical recurrence* within 6 months compared to patients with a *Cancer Signal Not Detected* result.
- In patients with a complete response (CR) following therapy, but with subsequent clinical recurrence during a 12-month observation period, 70% had *cancer signal detected* by the OncoK9 test prior to or concomitant with *clinical recurrence*; *molecular recurrence* was detected **prior** to *clinical recurrence* in 37% of those cases, with a median lead time of more than 2 months.
- Next-generation sequencing based liquid biopsy is a new, non-invasive tool that has the potential to improve cancer monitoring for dogs in the post-diagnosis setting. Patients with *no clinical evidence of disease* but with *molecular residual disease* or *molecular recurrence* detected by the OncoK9 test may benefit from closer clinical evaluation and monitoring.

INTRODUCTION

After a patient is diagnosed with cancer and receives therapy, the current standard of care relies heavily on physical examination and imaging to assess disease status and monitor for *cancer recurrence* (local recurrence of cancer and/or regional/distant metastasis; hereafter referred to as *cancer recurrence*). However, these monitoring tools may not be sensitive enough to detect the presence of residual disease following excisional surgery, or early evidence of *cancer recurrence* following therapeutic intervention. Additionally, access to certain imaging modalities may be limited in some care settings, and evaluation by imaging may require sedation or anesthesia, posing risks to the patient. In human oncology, next-generation sequencing-based liquid biopsy tests have been incorporated into clinical practice for cancer monitoring in the post-diagnosis setting. For example, the detection of cancer-associated genomic alterations (*cancer signal*) in a patient's blood after curative-intent surgery, indicative of the presence of molecular residual disease, has been shown to be an effective prognostic marker and recurrence predictor in multiple cancer types (solid as well as hematological).[1–3] Liquid biopsy has also been shown to detect cancer signal prior to clinical recurrence in human patients.[1,2] However, such evidence has not yet been established in veterinary medicine.

The following analyses evaluated the performance of OncoK9, a blood-based liquid biopsy test that has been clinically validated for cancer detection, as an adjunct tool for cancer monitoring in dogs. Specifically, the evaluation focused on the use of OncoK9 to detect residual disease following excisional surgery, and to detect recurrence of disease following therapy.

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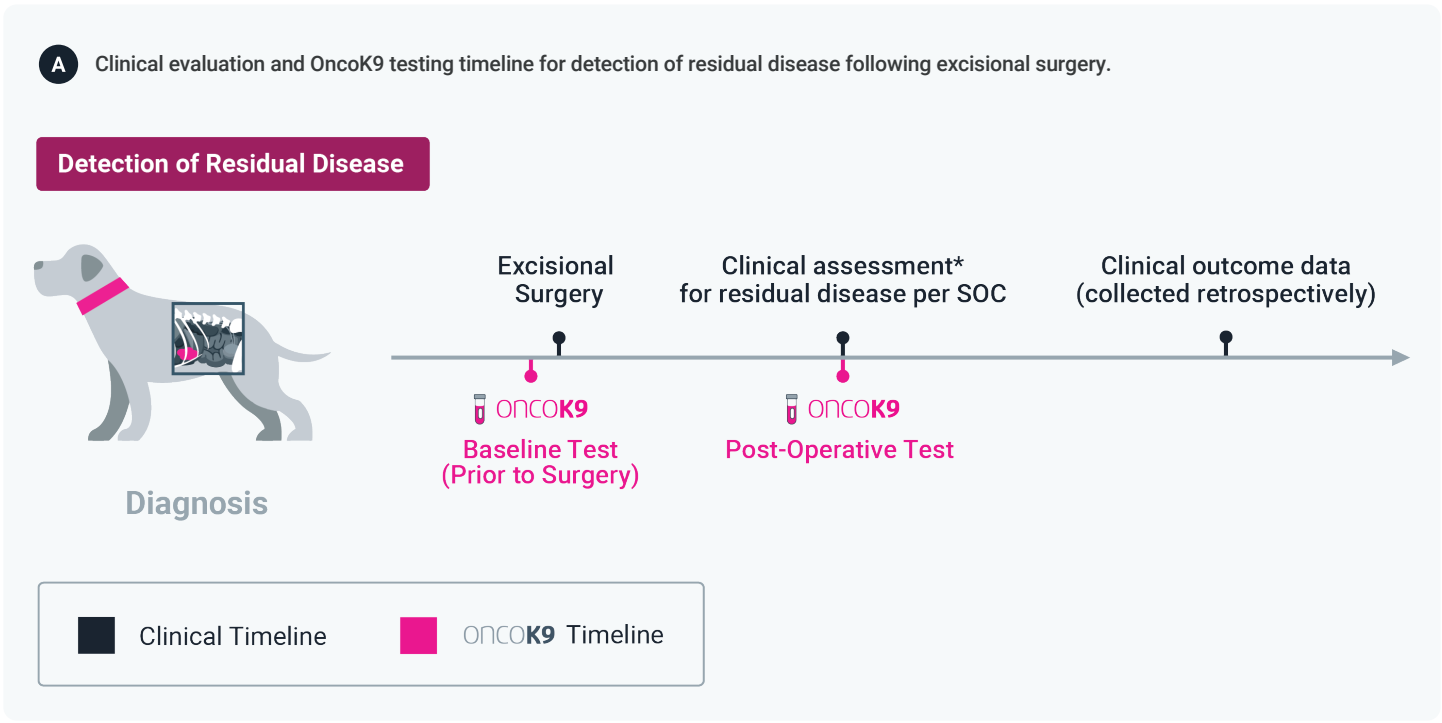
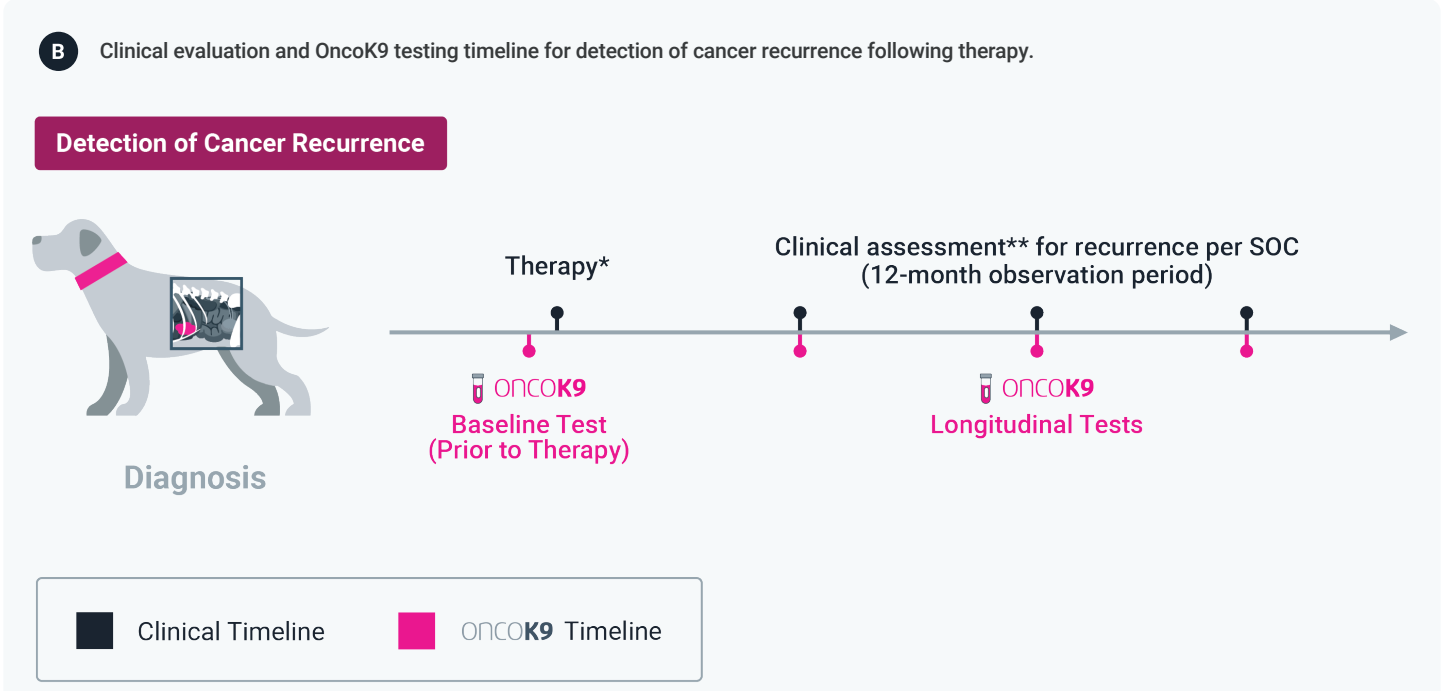
FIGURE 1. Clinical evaluation and OncoK9 testing timeline.



*Clinical assessment performed according to the standard of care (SOC) of each clinic.



*Therapy may include one or more of the following: surgery, chemotherapy, radiation therapy, immunotherapy, small molecule inhibitors, and other standard-of-care cancer therapy.

** Clinical assessment performed according to the standard of care (SOC) of each clinic.

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METHODS

Blood samples were collected prospectively from an all-comers cohort of dogs with confirmed diagnoses of cancer originally enrolled in the CANcer Detection in Dogs (CANDiD) study, and were stored for liquid biopsy testing as previously described.[3] The results of the test were reported as *Cancer Signal Detected* or *Cancer Signal Not Detected*. In all analyses, the laboratory team at PetDx that issued the OncoK9 test results were blinded to the patient's clinical disease status. As the testing was performed on stored samples, months to years after collection, results were not available in real-time to the treating veterinarians to inform patient management.

All patients had a baseline sample collected after diagnosis but prior to any therapeutic intervention, which may have included surgery, chemotherapy, radiation therapy, immunotherapy, small molecule inhibitors, and other standard-of-care cancer therapy. In a majority of patients, additional samples were longitudinally collected during follow-up visits on a pre-defined schedule at 1, 3, 6, 9, and 12 months following the baseline visit. The remaining patients, all of whom received surgical excision as the only or the first interventional therapy for their tumors, had only one follow-up sample collected, at the time of the post-operative visit **(Figure 1)**. Not all patients completed all predefined visits and, in some cases, additional samples were obtained at non-predefined visits, e.g., when the treating veterinarian noted a change in a patient's clinical disease status.

Clinical data were collected over a median follow-up period of 183 days (range: 3-839 days) from the time of baseline sample collection. At each follow-up visit, the patient's clinical disease status was evaluated using available standard-of-care methods such as physical exam, bloodwork, and routine imaging (radiography and/or ultrasound); and the treating veterinarian assigned a standardized clinical disease status using cRECIST criteria for solid tumors[4], or response criteria for lymphoma[5], as follows: progressive disease (PD), stable disease (SD), partial response (PR), and complete response (CR); a *no clinical evidence of disease* status designation was considered synonymous with CR. These designations, and supporting clinical records, were subsequently reviewed by an ACVIM board-certified veterinary medical oncologist at PetDx to confirm that the treating veterinarian's clinical disease status designation was aligned with the standardized criteria at each visit. In less than 1% (5/537) of visits, the clinical disease status designation provided by the treating veterinarian did not agree with the independent evaluation of the PetDx oncologist. In these cases, a second ACVIM board-certified veterinary medical oncologist at PetDx performed an independent review, and the final disease status designation was the one in which two of the three veterinarian designations agreed. The clinical disease status assigned at each visit was further used to categorize each visit as either *presence of clinical disease* (corresponding to SD, PR or PD) or *absence of clinical disease* (corresponding to CR). The clinical team at

TABLE 1. Demographics of the 228 patients.

		Total Cohort (n = 228)	Detection of Residual Disease (n = 53)	Detection of Recurrence (n = 54)
Age	Median (years)	9.2	10.1	8
	Range (years)	1.9-15.8	1.9-14.5	3-14.2
Weight	Median (kg)	28.1	32	27.8
	Range (kg)	5-81.5	9.5-60	6.8-60
Sex	Male	125 (55%)	30 (57%)	28 (52%)
	Intact	*13*	1	3
	Neutered	*112*	29	25
	Female	103 (45%)	23 (43%)	26 (48%)
	Intact	*6*	0	1
	Spayed	*97*	23	25
Breed	Purebred*	121 (53%)	23 (43%)	23 (43%)
	Mixed-breed	107 (47%)	30 (57%)	31 (57%)

*Dogs from 46 breeds were represented; the most common were Golden Retriever (n = 25); Labrador Retriever (n = 10); and English Bulldog, Pembroke Welsh Corgi, and Doberman Pinscher (each n = 5).

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PetDx that reviewed the clinical disease status designations were blinded to the OncoK9 test results.

The performance of the OncoK9 test for detection of cancer signal has been shown to vary across cancer types.[3] Detection of cancer signal by the test at baseline (prior to any therapeutic intervention) in a cancer-diagnosed patient suggests that the test could be subsequently used in that patient as a tool for detection of residual disease following surgery, or for detection of recurrence following therapy. In order to reflect the cancer monitoring scenarios in which the test is optimally suited to add clinical value, the analyses outlined below evaluated test performance only in patients that had a *Cancer Signal Detected* result at baseline. This cohort comprised 228 dogs; demographic characteristics and cancer types represented in the cohort are summarized in **Table 1** and **Table 2**, respectively.

Statistical tests between groups were performed using one-sided Fisher's Exact Tests. Calculations and plotting were performed in R (4.0.4). A p value of < 0.05 was considered statistically significant.

TABLE 2. Cancer types represented, by analysis.

	Overall Concordance Analysis	Detection of Residual Disease Analysis	Detection of Recurrence Analysis
Anal Sac Adenocarcinoma	X	X	X
Apocrine Sweat Gland Adenocarcinoma	X		
Bone, Fibrosarcoma	X	X	
Bone, Multilobular Osteochondrosarcoma	X	X	
Bone, Osteosarcoma	X	X	X
Carcinoma, Cutaneous	X		
Ceruminous Adenocarcinoma	X		
Chondrosarcoma	X		
Heart Base Tumor	X		
Hemangiosarcoma	X	X	X
Hepatocellular Carcinoma	X	X	
Histiocytic Sarcoma	X	X	
Insulinoma*	X	X	
Leiomyosarcoma*	X		
Leukemia, Chronic Lymphoid (CLL)	X		
Lymphoma, Intermediate to Large Cell	X		X
Malignant Melanoma	X	X	
Mast Cell Tumor	X		X
Oral Osteosarcoma	X	X	
Pulmonary Carcinoma	X		
Soft Tissue Sarcoma	X	X	X
Squamous Cell Carcinoma	X		X
Thyroid Carcinoma	X		
Transitional Cell Carcinoma*	X		X
Transmissible Venereal Tumor	X		

*Present in one subject with a *Cancer Signal Detected* Result that had one other concurrent primary cancer type.

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1) Overall concordance between clinical disease status and OncoK9 results

Concordance between the OncoK9 result and the clinical disease status at each visit was evaluated for the 228 patients across 537 follow-up visits where both data points were available. Results are presented as the proportion of visits with OncoK9 *Cancer Signal Detected* results for each clinical disease status. Of note, although concordance was assessed on a per-visit basis, it was possible to have a *Cancer Signal Detected* OncoK9 result at a visit when there was no clinical disease identified but where the patient subsequently presented with clinical disease at a later visit; in such cases, the detection of cancer signal in blood prior to detection of clinical disease in the patient was defined as a detection of *molecular recurrence*. The proportion of patients in which the OncoK9 test detected *molecular recurrence* was additionally evaluated.

2) OncoK9 for detection of residual disease following excisional surgery

The performance of OncoK9 for detecting residual disease was evaluated in cancer-diagnosed patients managed with excisional surgery: each patient had a baseline blood sample collected immediately prior to surgery (baseline visit) and one follow-up blood sample collected after surgery (post-operative visit). At the post-operative visit, patients were assessed by the treating veterinarian for clinical residual disease **(Figure 1A)**. An independent review of available medical records (including physical examination records, surgical reports, pathology reports and/or imaging reports, if available) was subsequently performed by ACVIM board-certified veterinary medical oncologists at PetDx, as described above, to classify the post-operative clinical disease status of each patient as having *clinical residual disease* or *no clinical residual disease*, based on the presence or absence of gross clinical disease. Beyond the post-operative visit, additional clinical outcome data were retrospectively collected for each patient in this cohort through a final patient outcome report completed by the treating veterinarian. Information collected included: whether a patient had developed clinical evidence of recurrence; the documented date of recurrence; if the patient had died; and the date and cause of death (if applicable).

The analysis was based on the premise that detection of *molecular residual disease* (defined as *cancer signal detected* in blood at a post-operative visit with *no clinical residual disease*) would be associated with a higher risk for *clinical recurrence* (defined as a *presence of clinical disease* designation at any time after an *absence of clinical disease* designation) within 6 months. Of the 228 patients in the overall cohort, 66 received excisional surgery; and of those, only patients with *no clinical residual disease* at the post-operative visit (3-34 days following surgery) were included in the analysis (n = 53), in order to evaluate the ability of the OncoK9 test to detect *molecular residual disease*.

These 53 patients were stratified by their OncoK9 test results at the post-operative visit into two groups, *Cancer Signal Detected* and *Cancer Signal Not Detected*, and were compared for their (i) rate of recurrence and (ii) recurrence-free survival (RFS), in both cases within 6 months of excisional surgery. RFS results were expressed using Kaplan-Meier (KM) curves. Patients who were lost to follow-up or died due to non-cancer related causes within 6 months were censored in all calculations.

3) OncoK9 for detection of cancer recurrence following therapy

The ability of OncoK9 to detect cancer recurrence was evaluated in a subset of patients who (i) had at least 2 follow-up visits; (ii) had an *absence of clinical disease* designation at any follow-up visit, and (iii) subsequently presented with *clinical recurrence* during the observation period. Of the 228 patients in the overall cohort, this analysis included 54 unique patients who were followed for a 12-month observation period, with a corresponding total of 184 blood samples collected at 3, 6, 9, and 12 month follow-up visits **(Figure 1B)**; the majority of these patients received single or multimodal therapy during the observation period, including surgery, chemotherapy, radiation therapy, immunotherapy, small molecule inhibitors, nonsteroidal anti-inflammatory medications, and/or prednis(ol)one. The remaining 174 patients either had *presence of clinical disease* at all visits throughout the observation period, or had less than 2 follow-up visits. The aims of the analysis were to determine (i) the proportion of patients in which the OncoK9 test was able to detect *cancer recurrence* overall (i.e., cancer signal was detected concurrently or prior to *clinical recurrence*), and (ii) the proportion of patients in which OncoK9 detected *molecular recurrence*. The corresponding lead time, defined as the time difference between detection of *molecular recurrence* and *clinical recurrence*, was calculated for each case of *molecular recurrence*.

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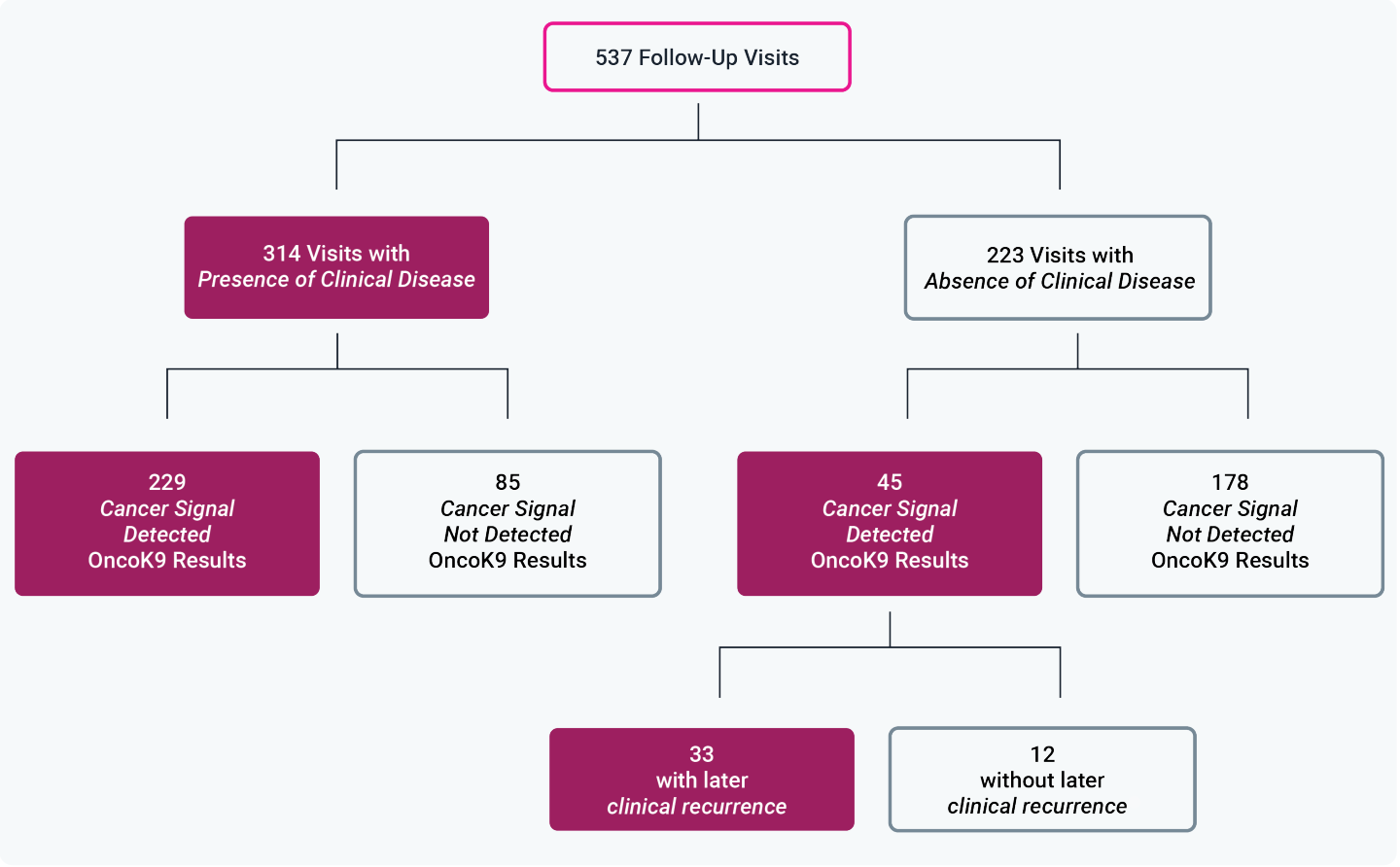
RESULTS

1) Overall concordance between clinical disease status and OncoK9 results

Of the 537 follow-up visit samples (representing 228 unique patients) that were evaluated, 314 samples (representing 161 unique patients) were collected at visits where a status of *presence of clinical disease* was assigned, and OncoK9 results were concordant (*Cancer Signal Detected*) in 72.9% (229/314) of these visits. The remaining 223 samples (representing 127 unique patients) were collected at visits where the patient was assigned a status of *absence of clinical disease*, and OncoK9 results were concordant (*Cancer Signal Not Detected*) in 79.8% (178/223) of these visits. Among the 45 samples where the OncoK9 test detected cancer signal in the *absence of clinical disease*, 33 were from patients who went on to develop *clinical recurrence* at a later time within the observation period, indicating detection of *molecular recurrence* by the test **(Figure 2)**.

Across all 537 follow-up visits, the proportion of samples reported as *Cancer Signal Detected* by OncoK9 was significantly higher at visits with a *presence of clinical disease* designation compared to visits with an *absence of clinical disease* designation (72.9%, 229/314 versus 20.2%, 45/223; $p < 0.001$); this proportion was also progressively

FIGURE 2. Disposition of clinical disease status designations at follow-up visits by OncoK9 test results.



Visual representation of clinical disease status designations at follow-up visits showing the distribution of OncoK9 results. 537 follow-up visits across 228 unique patients were evaluated; all 228 patients had a prior definitive diagnosis of cancer and a *Cancer Signal Detected* OncoK9 result at their baseline visit.

314 visits had *presence of clinical disease*, and contemporaneous OncoK9 results were concordant *(Cancer Signal Detected)* in 72.9% (229/314) of these visits. 223 visits had *absence of clinical disease*, and contemporaneous OncoK9 results were concordant *(Cancer Signal Not Detected)* in 79.8% (178/223) of these visits; the remaining 45/223 visits with *absence of clinical disease* had *Cancer Signal Detected* OncoK9 results, and in 73.3% (33/45) of these cases the patient developed *clinical recurrence* at a later time within the observation period. These 33 cases demonstrate detection of *molecular recurrence* by the OncoK9 test prior to *clinical recurrence*.

This diagram represents data subsets and is not intended to reflect a chronological progression or a decision tree.

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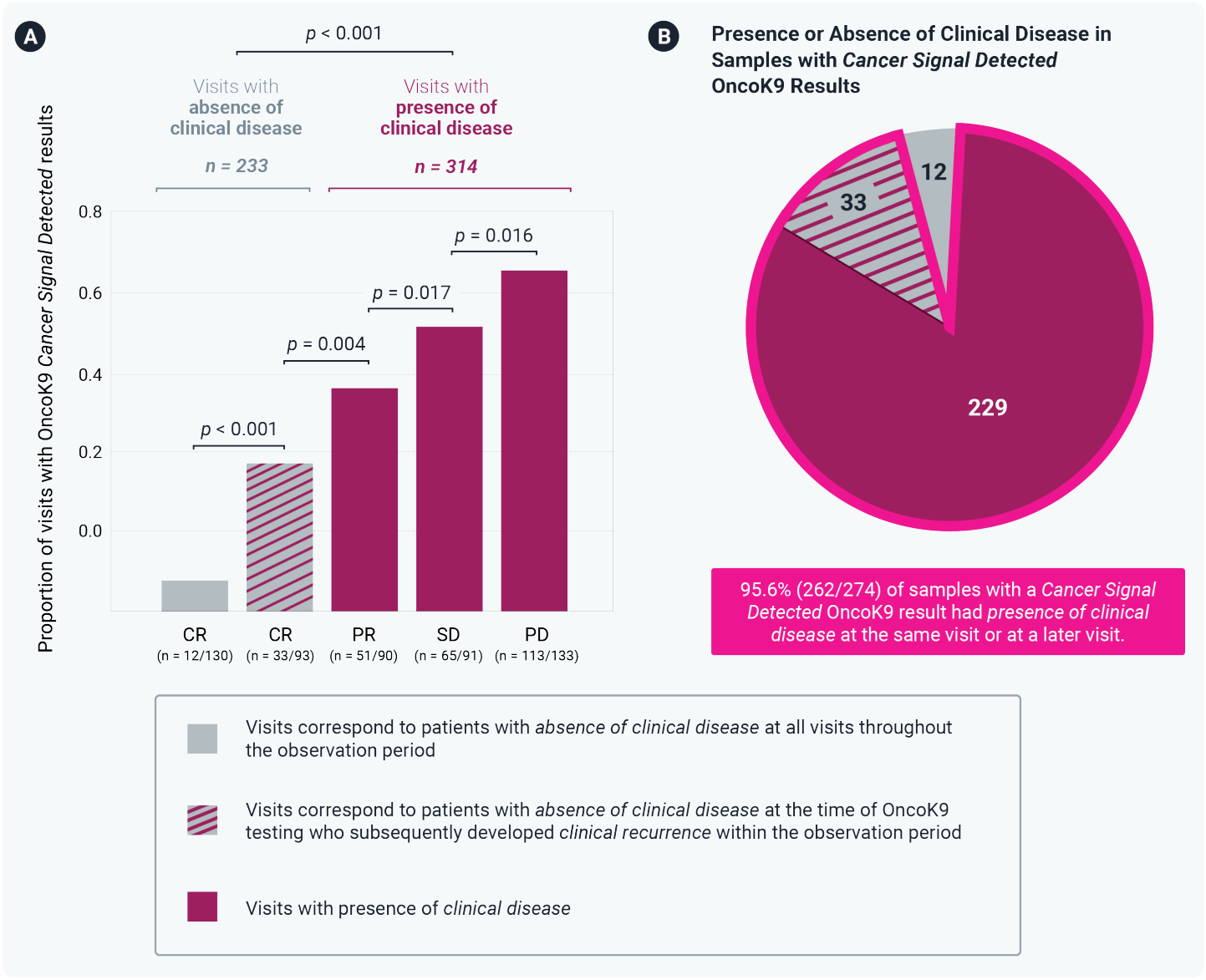
higher across each clinical disease status sub-category (PR < SD < PD) **(Figure 3A)**. Overall, 95.6% (262/274) of samples with a *Cancer Signal Detected* OncoK9 result had *presence of clinical disease* documented at the same visit (229/262) or at a later visit (33/262) **(Figure 3B)**. The lead time between detection of *molecular recurrence* and *clinical recurrence* is evaluated in the "OncoK9 for detection of cancer recurrence following therapy" analysis, below.

These results demonstrate high concordance between OncoK9 test results and clinical disease status as well as the ability of OncoK9 to detect molecular recurrence prior to clinical recurrence.

FIGURE 3. Visit-level concordance between *Cancer Signal Detected* OncoK9 test results and clinical disease status.



Proportion of visits with *Cancer Signal Detected* OncoK9 results as a function of clinical disease status designation at the same visit.

The following clinical status designations correspond to *presence of clinical disease*: partial response (PR), stable disease (SD) and progressive disease (PD). The complete response (CR) clinical status designation corresponds to *absence of clinical disease*.

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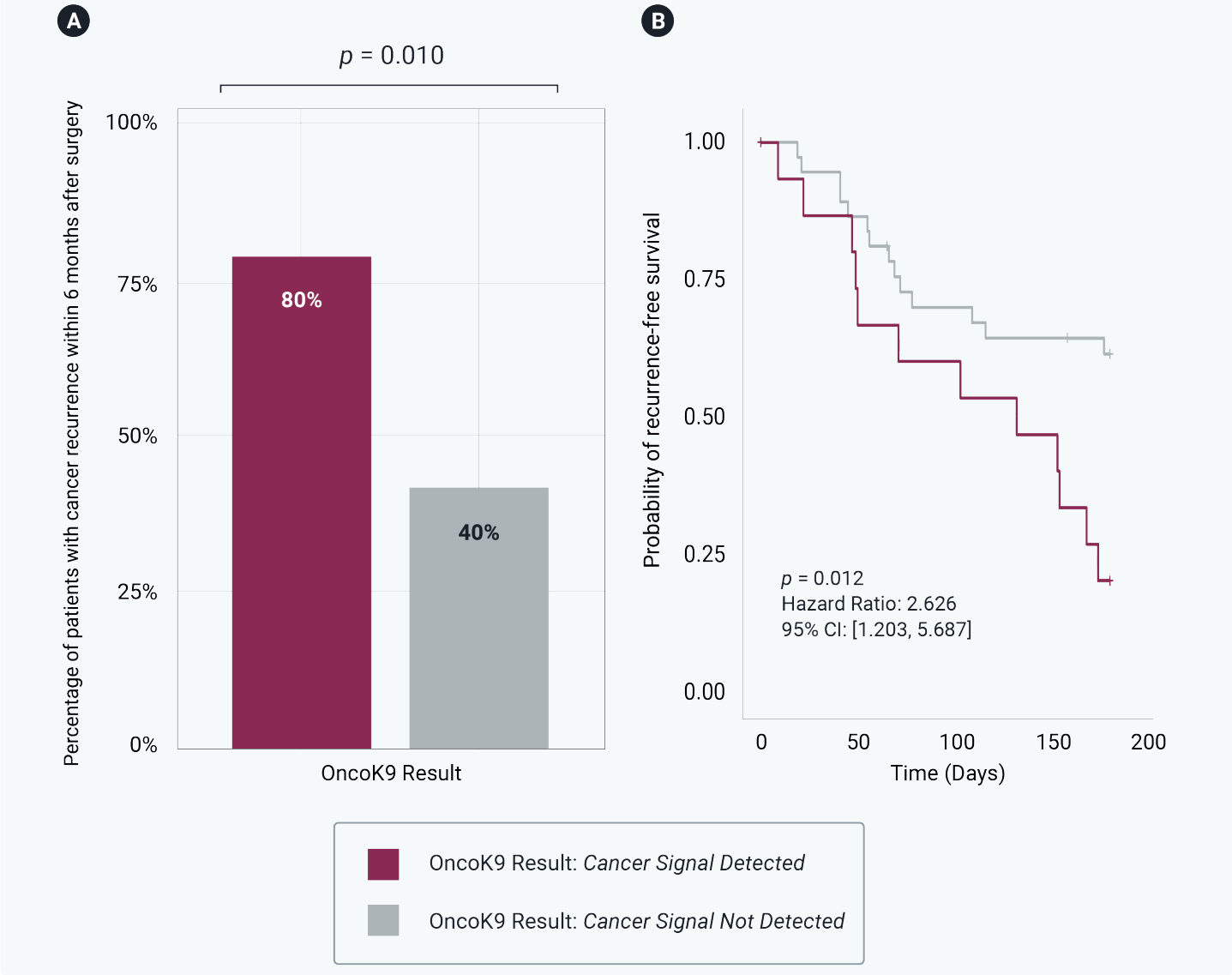
2) OncoK9 for detection of residual disease following excisional surgery

To evaluate the ability of the OncoK9 test to detect *molecular residual disease*, post-operative samples from 53 unique patients who underwent excisional surgery and had *no clinical residual disease* at the post-operative visit were analyzed.

Three patients were censored due to death unrelated to cancer or because they were lost to clinical follow-up. In the remaining 50 patients, those with a *Cancer Signal Detected* result at the post-operative visit were found to have a twofold higher likelihood of recurrence within 6 months (12/15, 80%) compared to patients with a *Cancer Signal Not Detected* result at that visit (14/35, 40%) (**Figure 4A**, p = 0.010). Patients with a *Cancer Signal Detected* result at the post-operative visit also had shorter recurrence-free survival within the 6-month observation period compared to those with a *Cancer Signal Not Detected* result (**Figure 4B**, p = 0.012; Hazard Ratio 2.626, 95% CI: 1.203, 5.687).

These results suggest that following excisional surgery, patients with no clinical residual disease but with molecular residual disease detected by OncoK9 at the post-operative visit are more likely to have cancer recurrence within 6 months. Such patients may benefit from closer clinical evaluation and monitoring.

FIGURE 4. Evaluation of the 6-month recurrence rate and recurrence-free survival based on post-operative OncoK9 results.



A. Six-month recurrence rate of patients with post-operative *Cancer Signal Detected* vs *Cancer Signal Not Detected* OncoK9 results, p = 0.010.

B. Recurrence-free survival over a 6-month observation period for patients with post-operative *Cancer Signal Detected* vs *Cancer Signal Not Detected* OncoK9 results, p = 0.012.

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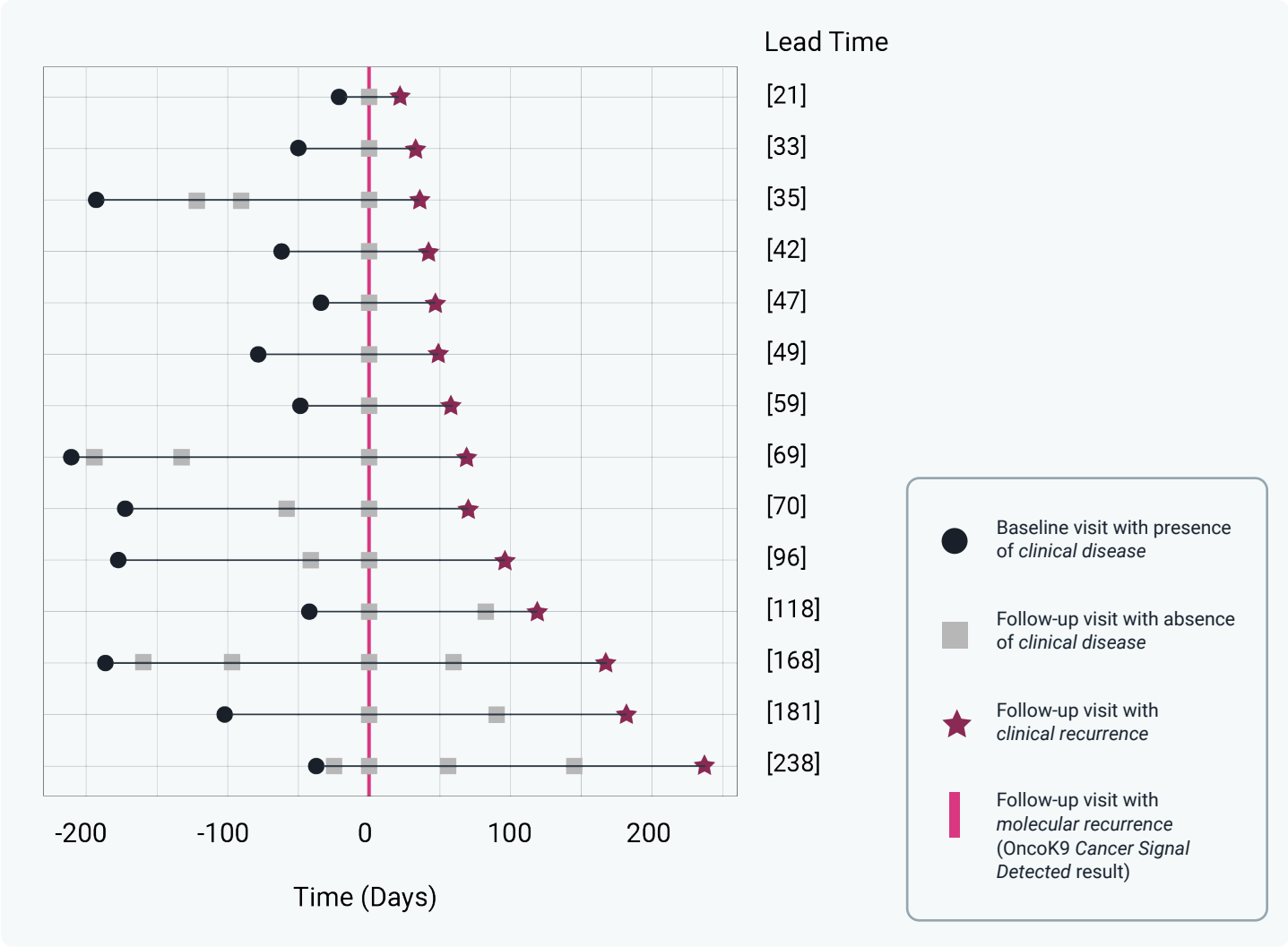
3) OncoK9 for detection of cancer recurrence following therapy

To evaluate the ability of OncoK9 to longitudinally detect *cancer recurrence*, an analysis was conducted in patients who had an *absence of clinical disease* designation at one or more of their follow-up visits but presented with *clinical recurrence* at a later time during a 12-month observation period. A total of 54 unique patients met these criteria and were evaluated, comprising a total of 184 samples collected during longitudinal follow-up. The median time from an *absence of clinical disease* visit to documentation of *clinical recurrence* was 97 days.

Overall, OncoK9 detected cancer signal prior to or concomitant with *clinical recurrence* in 38 of the 54 patients (70%). In 14 of those 38 patients (37%) the test detected *molecular recurrence* prior to *clinical recurrence*, with a median lead time of 64 days (range: 21-238 days, **Figure 5**). In this group of 14 patients, additional OncoK9 tests performed after initial detection of *molecular recurrence* consistently provided *Cancer Signal Detected* results until *clinical recurrence* was documented.

Of note, the predefined time interval (i.e., every 3 months) for the follow-up visits (where clinical assessments and blood sample collection for OncoK9 testing were

FIGURE 5. Detection of *molecular recurrence* by OncoK9 prior to *clinical recurrence*.



In 14 patients, OncoK9 detected *molecular recurrence* prior to *clinical recurrence*. All of them had *absence of clinical disease* (complete response) at one or more follow-up visits but experienced *clinical recurrence* at a later follow-up visit. The lead time between *molecular recurrence* detection by OncoK9 and *clinical recurrence* detection ranged from 21 to 238 days, with a median of 64 days. Black circles denote baseline visits with *presence of clinical disease* before therapy; grey squares denote follow-up visits when the patients had an *absence of clinical disease* designation; red stars denote follow-up visits when the patients had *clinical recurrence* documented. The magenta vertical line denotes the follow-up visit when OncoK9 detected cancer signal (indicative of *molecular recurrence*) ahead of *clinical recurrence*; all subsequent samples also had *Cancer Signal Detected* OncoK9 results. Each patient's timeline was aligned to set the time of *molecular recurrence* detection at Day 0. All patients had a *Cancer Signal Detected* OncoK9 result at the baseline visit.

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performed) may have affected lead time estimates. The date of *clinical recurrence* for each patient was established based on the clinical disease status designation at these predefined visits and the medical records provided by the study sites. Some patients may have had a change in disease status at visits outside of the predefined visit schedule, so the actual date of *clinical recurrence* may have been different from the recorded date used in this analysis. More frequent OncoK9 testing and clinical assessments may provide more accurate estimates for the lead time (from *molecular recurrence* to *clinical recurrence*) across various cancer types and therapies.

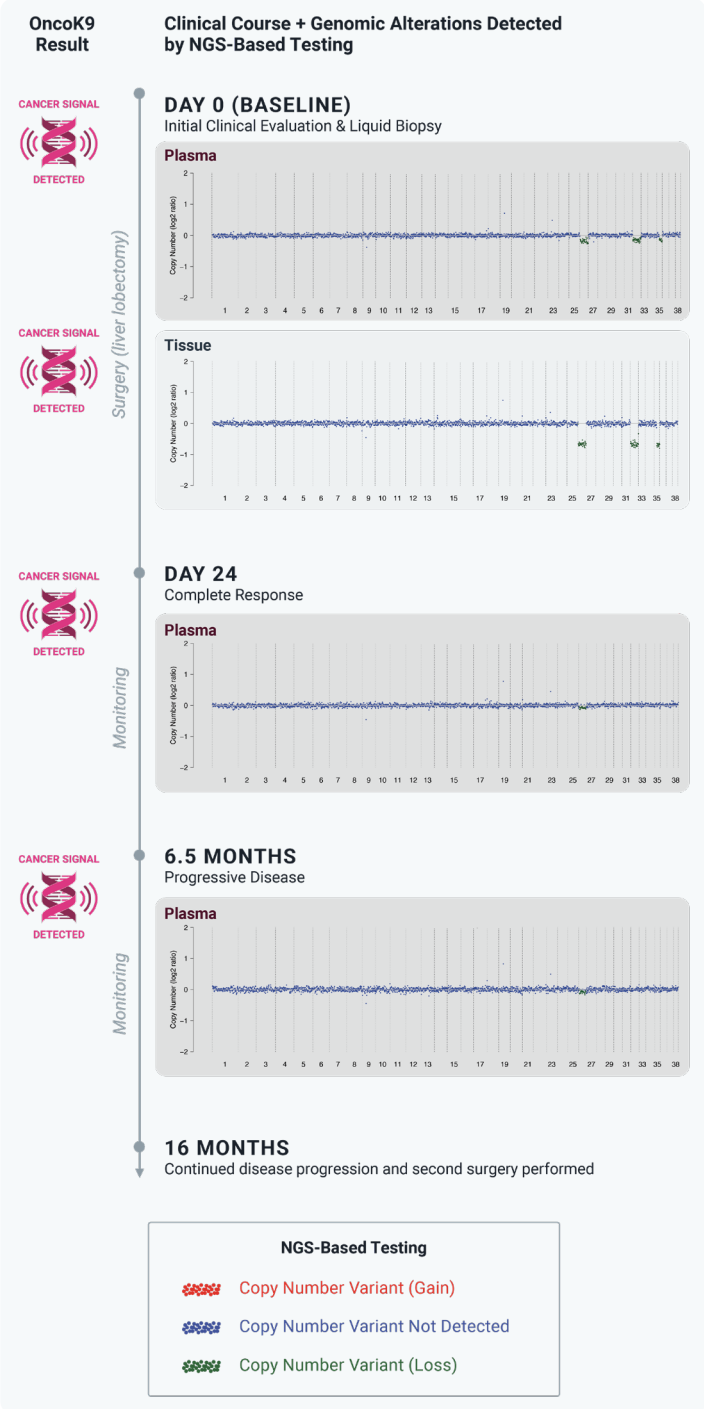
These results demonstrate that OncoK9 may be used as an adjunct tool alongside standard monitoring methods for detection of cancer recurrence following therapy. Patients with molecular recurrence detected by OncoK9 in the absence of contemporaneous clinical disease may benefit from closer clinical evaluation and monitoring.

CASE STUDIES

Case 1: Detection of molecular residual disease by liquid biopsy followed by *clinical recurrence* (Figure 6)

A 10-year-old male castrated mixed-breed dog was diagnosed with hepatocellular carcinoma of the left lateral liver lobe. A blood sample taken for liquid biopsy testing just prior to surgery revealed genomic alterations consistent with a *Cancer Signal Detected* result. The mass was completely excised with more than 6 mm histologic margins. Testing of the tumor tissue showed genomic alterations that were concordant with those observed via liquid biopsy analysis. The patient was recovering well at the post-operative evaluation visit 24 days following surgery, with *no clinical evidence of disease*. At this visit, another blood sample was collected for liquid biopsy, and a cancer signal was still detected, indicating the presence of *molecular residual disease* in the setting of *no clinical evidence of disease*. The dog continued to be monitored by the veterinary care team, and evidence of disease recurrence was documented approximately 6.5 months after the surgery, based on results of abdominal ultrasound for routine monitoring of disease status. Another blood sample for liquid biopsy was taken at the same time as the abdominal ultrasound, which again confirmed the presence of a cancer signal. Continued, slow progression of disease was noted on subsequent clinical monitoring; the dog underwent a second surgery 16 months after the first surgery, and recurrence of hepatocellular carcinoma was confirmed with histopathology. This case demonstrates the ability of liquid biopsy to detect *molecular residual disease* and predict the recurrence of disease.

FIGURE 6. Case 1: Testing for *molecular residual disease* following surgery in a dog with hepatocellular carcinoma.



Genomic testing performed in a 10-year-old neutered male mixed-breed dog diagnosed with hepatocellular carcinoma. The clinical disease status designation determined at each visit was based on standard-of-care assessment. The OncoK9 results are shown as *Cancer Signal Detected* or *Cancer Signal Not Detected*, in the baseline plasma sample, the resected tumor tissue, and the plasma samples collected at two post-operative follow-up visits.

Note: The copy number variations shown in this figure are solely provided as case-specific examples to illustrate the types of genomic information evaluated by the OncoK9 test. The current version of OncoK9 report provides the test results in a binary format of *Cancer Signal Detected* or *Cancer Signal Not Detected*.

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Case 2: Detection of *molecular recurrence* by liquid biopsy prior to *clinical recurrence* (Figure 7)

A 10-year-old spayed female mixed-breed dog was diagnosed with osteosarcoma of the right proximal humerus after evaluation by her primary care veterinarian for lameness affecting her right forelimb. On physical examination, she was found to be weight bearing, although she was painful and tense on palpation of her right proximal forelimb. Radiographs taken of the right humerus revealed a monostotic aggressive bone lesion with mixed lysis and proliferation, largely affecting the medullary cavity. Staging diagnostics, including thoracic radiographs and abdominal ultrasound, revealed no evidence of metastasis. Prior to surgery for a right forelimb amputation, a baseline blood sample was collected for liquid biopsy testing, which yielded a *Cancer Signal Detected* result. Histopathology of the lesion was consistent with a central osteoblastic osteosarcoma, and genomic testing of the tumor tissue revealed genomic alterations concordant with those

FIGURE 7. Case 2: Longitudinal monitoring following surgery and chemotherapy in a dog with osteosarcoma.

Genomic testing performed in a 10-year-old female spayed mixed-breed dog with a diagnosis of right humeral osteosarcoma. The clinical disease status designation at each visit was based on standard-of-care assessment. The OncoK9 results are shown as *Cancer Signal Detected* or *Cancer Signal Not Detected*, in the pre-operative plasma, resected tumor tissue, and plasma collected after the surgery and longitudinally every 2-3 months thereafter.

Note: The copy number variations and VAF for *TP53* shown in this figure are solely provided as case-specific examples to illustrate the types of genomic information evaluated by the OncoK9 test. The current version of OncoK9 report provides the test results in a binary format of *Cancer Signal Detected* or *Cancer Signal Not Detected*.

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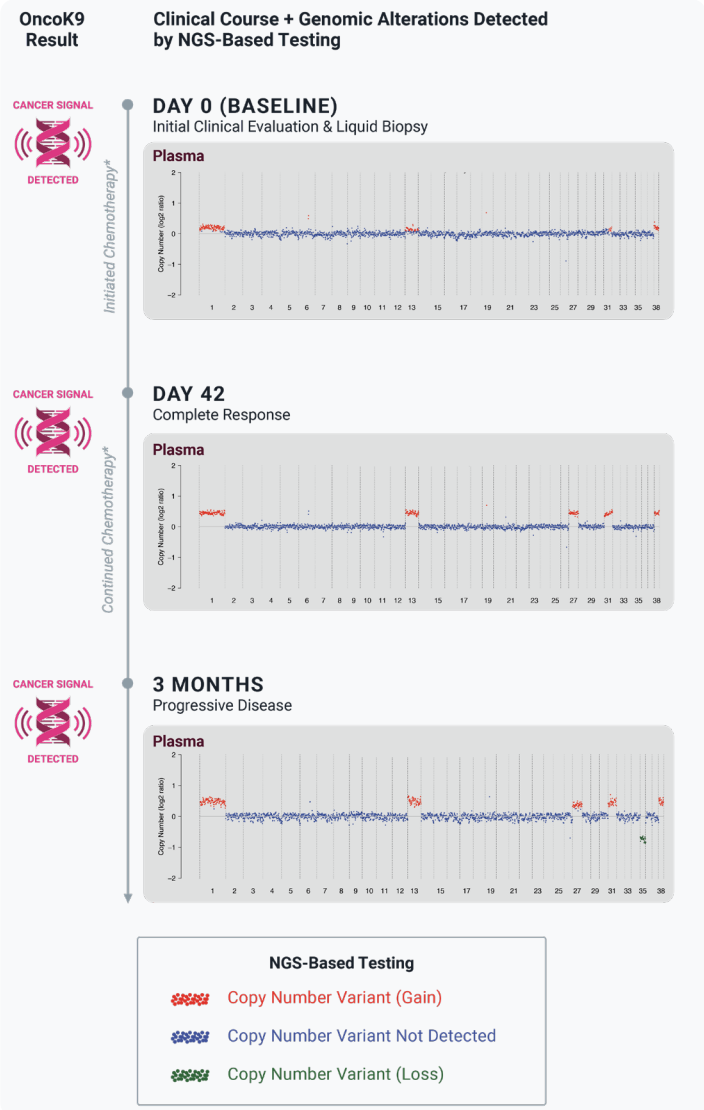
observed in the pre-operative blood sample. At the post-operative recheck visit 10 days later, she was recovering well clinically with no evidence of disease, and had another liquid biopsy test performed, which revealed a *Cancer Signal Not Detected* result. A course of adjuvant chemotherapy was initiated, with four doses of carboplatin administered every 3 weeks. At 3-months post-surgery, a complete response was noted by the treating veterinary oncologist based on physical examination and thoracic radiographs, and a liquid biopsy test performed at the same time continued to show a *Cancer Signal Not Detected* result. Another evaluation of clinical disease status at 6-months post-surgery (including thoracic radiographs) once again documented complete clinical response to therapy; however, a liquid biopsy test performed at the same time had a *Cancer Signal Detected* result, indicative of *molecular recurrence*. The *Cancer Signal Detected* result persisted at the 9-month post-surgery evaluation, and at this visit, *clinical recurrence* was noted with a new, expansile mass on the right 11th rib identified on thoracic radiographs. The patient was rechecked again at 11-months post-surgery and found to have progressive disease with pulmonary metastasis, and persistence of the *Cancer Signal Detected* liquid biopsy test result. This case illustrates the ability of liquid biopsy to detect *molecular recurrence* prior to *clinical recurrence*.

Case 3: Detection of molecular disease by liquid biopsy followed by *clinical recurrence* (Figure 8)

A 4.5-year-old neutered male English Bulldog was diagnosed with stage IVa, large, B-cell lymphoma. A baseline liquid biopsy taken at the time of diagnosis and prior to therapeutic intervention showed a *Cancer Signal Detected* liquid biopsy result. The patient was treated with a multi-agent CHOP chemotherapy protocol. At a follow-up visit 42 days after initiation of chemotherapy, the patient received a complete response status designation from the treating veterinary oncologist; however, a second liquid biopsy sample obtained at this time yielded a *Cancer Signal Detected* result. Within two weeks of this visit, the patient had a change in his chemotherapy protocol to L-asparaginase, rabacfosadine, and then lomustine. At the scheduled 3-month follow-up visit, the patient had clinically documented progressive disease; a third liquid biopsy sample obtained at this time continued to show a *Cancer Signal Detected* result.

This case demonstrates the concordance between liquid biopsy results and clinical disease status designation at two of the three timepoints. However, liquid biopsy testing one month into therapy continued to detect cancer signal despite the patient having a complete response status based on clinical assessment. The patient was noted to have progressive disease shortly after receiving this positive test result. This case illustrates the ability of liquid biopsy to detect molecular disease prior to the development of *clinical recurrence*.

FIGURE 8. Case 3: Longitudinal monitoring in a dog with lymphoma undergoing chemotherapy.



Genomic testing performed in a 4.5-year-old neutered male English Bulldog with a diagnosis of stage IVa, large B-cell lymphoma. The clinical disease status designation determined at each visit was based on standard-of-care assessment. The OncoK9 results are shown as *Cancer Signal Detected* or *Cancer Signal Not Detected* in plasma samples at baseline and at the 1 and 3 month time points.

*Chemotherapy protocols administered included CHOP, L-asparaginase, rabacfosadine, and lomustine.

Note: The copy number variations shown in this figure are solely provided as case-specific examples to illustrate the types of genomic information evaluated by the OncoK9 test. The current version of OncoK9 report provides the test results in a binary format of *Cancer Signal Detected* or *Cancer Signal Not Detected*.

Test failures and false positive or false negative results may occur. To review OncoK9 test limitations and risks, please visit: oncok9.info/disclosures

TEST USE CONSIDERATIONS

While positive OncoK9 results and clinical disease status designations are highly concordant, false negative OncoK9 results may occur in the *presence of clinical disease*. OncoK9 is intended to be used as an adjunct tool for cancer monitoring in dogs, and should not replace standard-of-care clinical assessment methods. Furthermore, a *Cancer Signal Detected* OncoK9 test result should not be considered as definitive confirmation of residual or recurrent disease, as false positive OncoK9 results may occur in the *absence of clinical disease*; also, the test cannot currently distinguish between recurrence of the original cancer and development of a new cancer in the patient. ***Therefore, a positive test result should always be followed by a full clinical evaluation before any changes are made to the patient's management plan.***

The OncoK9 test reports results in a binary manner (i.e., *Cancer Signal Detected* or *Cancer Signal Not Detected*). The test report does not indicate the extent of disease (such as whether the patient has PR, SD, or PD) or whether a particular therapy is effective in reducing tumor burden. ***The test is not currently indicated for quantitative assessment of therapeutic response.***

In the post-diagnosis setting, the OncoK9 test is most useful for the detection of residual disease and the detection of disease recurrence in patients who had a *Cancer Signal Detected* result at baseline (prior to any therapeutic intervention). ***The test is not currently indicated for patients with a baseline result of Cancer Signal Not Detected.***

Significant trauma, including tissue damage secondary to surgical procedures, can result in the temporary release of high amounts of cfDNA from necrosed normal cells and/or tumor cells. In dogs undergoing surgery for tumor removal, high release of normal (non-cancer) cfDNA from damaged healthy tissues immediately after the procedure could dilute the residual tumor cfDNA fraction and impair the test's ability to detect a cancer signal. Previous studies in humans and in dogs have shown that cfDNA fragments are usually cleared from circulation within a few days.[6,7] Therefore, it is recommended to wait a minimum of 7 days after surgery to perform the OncoK9 test for the purpose of residual disease detection.

CONCLUSIONS

The results presented herein demonstrate for the first time in veterinary medicine in a large longitudinal cohort of cancer-diagnosed patients that next-generation sequencing-based liquid biopsy can be used as a non-invasive adjunct tool for cancer monitoring in dogs, with high concordance (>95%) between positive test results and clinical findings at contemporaneous or later follow-up visits.

Surgically managed patients with *no clinical residual disease* but with a *Cancer Signal Detected* OncoK9 result (indicating presence of *molecular residual disease*) at the post-operative visit had a significantly higher probability of *clinical recurrence* within 6 months. In patients undergoing longitudinal monitoring following therapy, OncoK9 detected *molecular recurrence* prior to *clinical recurrence* in a significant fraction of patients, with a median lead time of over 2 months.

Longitudinal testing using OncoK9 has the potential to improve the clinical monitoring of dogs with cancer by detecting residual and recurrent disease with a simple blood draw. Patients with no clinical evidence of disease but with *molecular residual disease* or *molecular recurrence* detected by the OncoK9 test may benefit from closer clinical evaluation and monitoring. Future studies are needed to evaluate the impact of liquid biopsy testing on clinical decision-making and on clinical outcomes across various cancer types and therapeutic interventions.

INTENDED USE

OncoK9 is a multi-cancer early detection (MCED) test for the detection and characterization of cancer-associated genomic alterations in DNA isolated from canine whole blood samples, using next-generation sequencing (NGS) technology. OncoK9 is intended for use in dogs who are at higher risk of cancer. It is recommended as an annual screening test for all dogs starting at 7 years of age, and starting at younger ages for dogs belonging to breeds in which cancer tends to develop earlier in life. It is also recommended as an aid-in-diagnosis test for dogs in which cancer is suspected based on clinical signs or other clinical findings. It is also recommended as a test for detection of residual disease following excisional surgery, and detection of recurrence following therapy, in dogs previously diagnosed with cancer. As with any laboratory test, OncoK9 results should be interpreted by a veterinarian in the context of each patient's medical history and clinical presentation.

Test failures and false positive or false negative results may occur. To review OncoK9 test limitations and risks, please visit: oncok9.info/disclosures

REFERENCES

1. Henriksen TV, Tarazona N, Frydendahl A, Reinert T, Gimeno-Valiente F, Carbonell Asins JA, et al. Circulating Tumor DNA in Stage III Colorectal Cancer, beyond Minimal Residual Disease Detection, toward Assessment of Adjuvant Therapy Efficacy and Clinical Behavior of Recurrences. Clin Cancer Res. 2022;28(3):507–17.

2. Centers for Medicare & Medicaid Services. Local Coverage Determinate (LCD) - MolDx: Minimal Residual Disease Testing for Cancer (L38779) [Internet]. [cited 2022 Nov 17]. Available from: https://www.cms.gov/medicare-coverage-database/view/lcd.aspx?lcdid=38779

3. Flory A, Kruglyak KM, Tynan JA, McLennan LM, Rafalko JM, Fiaux PC, et al. Clinical validation of a next-generation sequencing based multi-cancer early detection "liquid biopsy" blood test in over 1,000 dogs using an independent testing set: The CANcer Detection in Dogs (CANDiD) study. Plos One. 2022;17(4):e0266623.

4. Nguyen SM, Thamm DH, Vail DM, London CA. Response evaluation criteria for solid tumours in dogs (v1.0): a Veterinary Cooperative Oncology Group (VCOG) consensus document. Vet Comp Oncol. 2015;13(3):176–83.

5. Vail DM, Michels GM, Khanna C, Selting KA, London CA, Group VCO. Response evaluation criteria for peripheral nodal lymphoma in dogs (v1.0)–a veterinary cooperative oncology group (VCOG) consensus document. Vet Comp Oncol. 2010;8(1):28–37.

6. Kustanovich A, Schwartz R, Peretz T, Grinshpun A. Life and death of circulating cell-free DNA. Cancer Biol Ther. 2019;1–11.

7. Wilson IJ, Burchell RK, Worth AJ, Burton SE, Gedye KR, Clark KJ, et al. Kinetics of Plasma Cell-Free DNA and Creatine Kinase in a Canine Model of Tissue Injury. J Vet Intern Med. 2018;32(1):157–64.

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