OMB Number: 4040-0001
Expiration Date: 11/30/2025

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **UEI*:** CBWYDJ3RUM85

Legal Name*: SPARK MOLECULAR DIAGNOSTICS, INC
Department:
Division:
Street1*: 6349 NANCY RIDGE DR, STE B
Street2:
City*: SAN DIEGO
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 921212247

Person to be contacted on matters involving this application
Prefix: First Name*: William Middle Name: Last Name*: Hanna Suffix:
Position/Title: CEO
Street1*: 6349 NANCY RIDGE DR, STE B
Street2:
City*: SAN DIEGO
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 921212247
Phone Number*: 6199613062 Fax Number: Email: bill@sparkmoleculardx.com

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 93-4884488

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Improving Landmark MRD Sensitivity using 4th Generation PCR

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2025 03/31/2026

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-051

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: William Middle Name: Last Name*: Hanna Suffix:
Position/Title: CEO and CSO
Organization Name*: SPARK MOLECULAR DIAGNOSTICS, INC
Department:
Division:
Street1*: 6349 NANCY RIDGE DR STE B
Street2:
City*: SAN DIEGO
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 921212247
Phone Number*: 6199613062 Fax Number: Email*: bill@sparkmoleculardx.com

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$404,246.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$404,246.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: William Middle Name: Last Name*: Hanna Suffix:
Position/Title*: CEO
Organization Name*: SPARK MOLECULAR DIAGNOSTICS, INC
Department:
Division:
Street1*: 6349 NANCY RIDGE DR, STE B
Street2:
City*: SAN DIEGO
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 921212247
Phone Number*: 6199613062 Fax Number: Email*: bill@sparkmoleculardx.com

Signature of Authorized Representative*
CHRIS Brasfield

Date Signed*
09/02/2024

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 11/30/2025

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	SPARK MOLECULAR DIAGNOSTICS, INC
UEI:	CBWYDJ3RUM85
Street1*:	6349 NANCY RIDGE DR, STE B
Street2:	
City*:	SAN DIEGO
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	921212247
Project/Performance Site Congressional District*:	CA-051

Additional Location(s) File Name:

OMB Number: 4040-0010
Expiration Date: 11/30/2025

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ● **Yes** ❍ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Summary_2024.08.31.pdf
8. Project Narrative*	Narrative_2024.8.30.pdf
9. Bibliography & References Cited	References_2024.08.30.pdf
10.Facilities & Other Resources	Facilities-2024.08.29.pdf
11.Equipment	Equipment_2024.09.01.pdf

SUMMARY

Currently, the rate of tumor recurrence following resection of a solid tumor in stage II-IV colorectal cancer (CRC) is between 20 and 50 percent, highlighting the need for more effective means of predicting and detecting relapse. By better understanding this risk, clinicians are better informed on the timeline and strategy of post-operative adjuvant therapies aimed at treating tumor relapse. As such, there is great clinical utility in the development of prognostic markers through detecting minimal residual disease (MRD). While the development of next generation sequencing (NGS) and digital PCR methods of MRD detection in liquid biopsy samples have shown promise, these methods are typically limited by their low sensitivity, making the accurate detection of circulating tumor DNA (ctDNA) and other biomarkers difficult, thereby increasing the risk of inaccurate results and false negatives. The consequences of clinical false negatives at landmark not only introduces the problem of greatly reduced lead times but can also lead to the implementation of unnecessary adjuvant therapies on patients that were effectively cured from their surgery. Therefore, there is a need for new methods of MRD detection using liquid biopsies that achieve sufficient levels of sensitivity and specificity to allow for earlier MRD detection to inform subsequent treatment strategies and improve patient outcomes. To address these limitations, Spark Molecular Diagnostics is developing “Spark MRD”, a 4th generation PCR based approach of MRD detection in blood plasma. Preliminary analyses indicated that Spark MRD has unparalleled sensitivity and can detect a single molecule of mutant DNA among 100,000 wildtype molecules (330 nanograms of plasma DNA), making it 20 to 100 times more sensitive than current NGS and digital PCR-based MRD tests for solid tumor relapse. As such, this technology has the potential to improve landmark detection and increase lead times, allowing for more effective intervention and treatment. Spark Molecular Diagnostics seeks to evaluate Spark MRD’s sensitivity in a direct comparison to the current clinical “gold standard” method of MRD detection, Natera’s Signatera NGS-based approach. To achieve this goal, one specific aim is proposed that involves the quantitative comparison of MRD detection limits of Spark MRD and Signatera in blood plasma samples obtained from stage II and III CRC patients post tumor resection.

PROJECT NARRATIVE

The detection of minimal residual disease (MRD) following tumor removal in stage II and III colorectal patients allows for clinicians to predict risk of tumor relapse, informing more appropriate and effective adjuvant therapies to improve patient outcomes. While various approaches of MRD detection such as next generation sequencing (NGS) and digital PCR have allowed for the detection of small numbers of circulating tumor DNA (ctDNA), these methods are limited by their relative lack of sensitivity that makes early MRD landmark detection difficult, leading to decreased lead times and the administration of unnecessary adjuvant therapies. This study is designed to test the efficacy of Spark MRD, a PCR-based method of MRD detection, which is expected to improve ctDNA detection sensitivity by virtually eliminating background noise from high ratios of co-isolated wildtype (WT) DNA, leading to more accurate diagnoses to inform intervention strategies and improve patient outcomes.

FACILITIES AND OTHER RESOURCES

Office and Laboratory Spark Molecular Diagnostics is located at 6349 Nancy Ridge Rd Ste B San Diego CA 92121, within the biotechnology district in San Diego. Spark Molecular Diagnostics occupies a 2,500 Sq Ft facility, with excellent room segregation with 15 lab benches. There one large office area that is suitable for up to 8 people. The laboratory space is unshared.

Computer Computer facilities include personal computers available for the technical staff. All equipment to be used in this proposal will be connected to computer systems for data collection and analysis and interfaced with an intranet server for data backup. These resources will ensure efficient data handling and communication by research team members.

Environment
The facilities and other resources available to the PI and his research team at Spark Molecular Diagnostics will ensure the successful completion of the proposed research project. The investigators on this project represent a team with multidisciplinary skills (preclinical studies, research and diagnostic development, clinical research, and other required skills) who have worked and published together over the past several years. The PI works closely with the other members of the research team and reviews experimental design, data generated, data analysis on a regular basis, along with regular group meetings at which progress and study plans are discussed. The PI has established a strong network of collaborators. Located in the San Diego biotechnology district, Spark Molecular Diagnostic's location provides access to top university resources (University of California San Diego), entrepreneurial resources, and investment firms.

Biohazards Handling and Disposal Spark Molecular Diagnostics and collaborators meet all federal environmental laws and regulations. Spark Molecular Diagnostics will use Diego Medical Waste Services, LLC for disposing of discarded blood samples and related disposables. All work involving biological agents/materials will be carried out in compliance with NIH Guidelines and the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) practices at collaborating sites (University of California San Diego and Sharp Memorial Hospital). These partners have established safe practices of biohazard handling and waste disposal as defined in their policies and procedures. Any laboratory personnel involved in the study will be required to attend annual safety training and adhere to strict safety practices. Personal protective equipment requirements will be implemented, where necessary.

Intellectual Property
1. Compositions and methods for high sensitivity detection of rare mutations. WIPO reference number WO2022108933A1
2. Composition and Methods for Affinity Directed Enrichment of Rare Species. US patent reference number US20200399683A1

Sharp Memorial Hospital: (901 Frost St, San Diego, CA 92123) Sharp Laboratory Services located at Memorial Hospital will provide a fee-for-service access to expertise and state-of-the-art instrumentation for all clinical sample collection from stage II and III colorectal cancer patients. From real time inventory interface with the blood bank to a comprehensive reference lab in hematology, histology, immunology, specimen processing, and molecular genetics, they have all the necessary equipment, facilities, and certifications to contribute to the work described in Aim 1.

Novogene: (8801 Folsom Blvd #290, Sacramento, CA 95826) Novogene will conduct whole exome sequencing on plasma samples from stage II and III colorectal cancer patients, aiming to identify patient-specific mutations for the Spark MRD assay. With their advanced sequencing technology, ISO-certified and CAP-accredited labs, and a skilled bioinformatics team, Novogene has the expertise and resources necessary to support the work outlined in Aim 1, ensuring both accuracy and reliability in their contributions.

nRichDx: (15339 Barranca Pkwy, Irvine, CA 92618) nRichDx will perform high volume sample preparation of plasma samples. They have all the necessary equipment, facilities, and certifications to contribute to the work described in Aim 1.

University of California San Diego CFAR Core Lab: (9575 Gilman Dr, La Jolla, CA 92093) The mission of CFAR is to provide researchers fee-for-service access to expertise and state-of-the-art instrumentation across a broad range of services, including molecular and cellular immunology, translational virology, biostatistics and modeling, and clinical investigation. CFAR is open to all investigators, including UCSD and their affiliates, commercial, and not-for-profit biotechnology companies, and other academic institutions. For this project, digital PCR on cell free DNA collected from each of the stage II and III CRC patient plasma samples will be performed at CFAR labs for quality check and to measure the level of wildtype DNA present in each sample. They have all the necessary equipment, facilities, and certifications to contribute to the work described in Aim 1.

Clinical
Sharp Memorial Hospital: (901 Frost St, San Diego, CA 92123) Sharp Memorial Hospital will perform all clinical sample collection from stage II and III colorectal cancer patients. They have all the necessary equipment, facilities, and certifications to contribute to the work described in Aim 1.

Animal Not applicable.

EQUIPMENT-Spark Molecular Diagnostics Inc.

The available equipment for the research to be performed in this application includes but is not limited to:

Major Equipment	Quantity	Manufacturer	Stage
Kingfisher-96	1	Thermo-Fisher	Sample Prep
Qubit Flex fluorometer	1	Thermo-Fisher	Sample Prep
QuantStudio 5 PCR	1	Thermo-Fisher	PCR
PTC-200	2	Bio-Rad	PCR
OPTO-2 Luminometers.	2	MGM Instruments	Spark Endpoint PCR
Thermomixer C	6	Eppendorf	Spark Endpoint PCR

UCSD CFAR Molecular and Cellular Immunology Core Genomics Unit
Major Equipment:
The equipment currently available for this project includes:

-Digital PCR services using Bio-Rad's QX200 digital droplet PCR instrument

-DNA shearing services using Covaris ME220 Focused-Ultrasonicator

-DNA size analysis services using Agilent 2200 TapeStation.

-Gene sequencing services using MiSeq, NovaSeq 6000, HiSeq PacBio SMRT, Nanopore, Ion S5/Ion Chef.

-qPCR analysis services using Taqman PCR platform.

Sharp Memorial Hospital
Major Equipment:
The equipment currently available for this project includes:

-Automated molecular testing platform using Roche 6800

-Automated blood screening for type and crossmatch using Ortho Vision

Novogene
Major Equipment:
The equipment currently available for this project includes:

-Whole exome sequencing using Novaseq 6000.

-Solid tumor mutation detection using NovoPM 2.0.

-mRNA sequencing using Illumina PE150.

nRichDx
Major Equipment:
The equipment currently available for this project includes:

-High volume cfDNA isolation from liquid biopsy samples using nRichDx Revolution Plus Sample Prep System

-High volume cfDNA isolation from liquid biopsy samples using nRichDx Revolution Pro Sample Prep System

-High volume liquid biopsy collection using nRicher Cartridge.

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: William Middle Name Last Name*: Hanna Suffix:

Position/Title*: CEO and CSO
Organization Name*: SPARK MOLECULAR DIAGNOSTICS, INC
Department:
Division:
Street1*: 6349 NANCY RIDGE DR STE B
Street2:
City*: SAN DIEGO
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 921212247

Phone Number*: 6199613062 Fax Number:

E-Mail*: bill@sparkmoleculardx.com

Credential, e.g., agency login: PIHANNA

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1994

Attach Biographical Sketch*: File Name: Bill-biosketch_2024.09.01.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Alexander Middle Name Last Name*: Yum Suffix:

Position/Title*: CTO
Organization Name*: SPARK MOLECULAR DIAGNOSTICS, INC
Department:
Division:
Street1*: 6349 NANCY RIDGE DR STE B
Street2:
City*: SAN DIEGO
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 921212247

Phone Number*: 0000000000 Fax Number:

E-Mail*: alex@sparkmoleculardx.com

Credential, e.g., agency login: ALEXYUM

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: BS Degree Year: 2016

Attach Biographical Sketch*: File Name: Alex-biosketch_2024.08.22.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 08/2024 Approved Through 01/31/2026)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: William Hanna, PhD

eRA COMMONS USER NAME (credential, e.g., agency login): PIHANNA

POSITION TITLE: CEO, CSO

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Northern Illinois University	BS	05/1987	Biology
University of Illinois, at Chicago	PhD	05/1994	Immunology
Northwestern University	Post-Doc	10/1998	PCR Liquid Biopsies

A. Personal Statement

I am the Chief Executive and Chief Scientific Officer of Spark Molecular Diagnostics, Inc. which focuses on next-generation technologies addressing unmet needs in the emerging field of liquid biopsies for early cancer detection. I've has been engaged in startup efforts for several years and am an inventor or co-inventor on several issued U.S. patents and patents pending worldwide. My first startup experience was with Biological Dynamics (a cancer diagnostics microchip start-up) where I held a senior scientific position and championed the cfDNA isolation utility of the early versions of the ExoVerita apparatus as a solid-state competitor to traditional cfDNA and exosome isolation techniques. Afterwards, I co-founded Radian Biotechnology where I developed the nascent versions of a novel DNA mutation detection platform with sensitivities suitable for tissue biopsies. I was also engaged in a DNA optical mapping genomics startup effort as the CSO for identifying large structural variations based with the inventor of DNA optical mapping, Professor David Schwartz at the University of Wisconsin, Madison. Prior to Spark Molecular, I was the Chief Scientific Officer at CY Molecular Diagnostics, LLC.

Before working in startups, I was at Gen-Probe, then the nation's largest stand-alone DNA Diagnostics company, from 1999 to 2008. There, I was technical lead in the Cancer and Technology Assessment Group and was instrumental in develop Gen-Probe's two cancer diagnostics for prostate and cervical cancer, the PCA3 test and the HPV test, respectively.

I received a B.S. in Biology from Northern Illinois University, a Ph.D. in Microbiology/Immunology from the University of Illinois at Chicago and performed post-doctoral work at Northwestern University.

My role in this Phase I SBIR is PI, where I will oversee all scientific activities, including the execution of the proposed experiments and coordination of clinical sample procurement and analysis.

B. Positions, Scientific Appointments, and Honors

Positions

2023 – Present	CEO & CSO, Spark Molecular Diagnostics, Inc.
2016 – 2024	CSO, CY Molecular Diagnostics, LLC
2015 – 2015	Director of Research Development, Industry3200 Inc.
2013 – 2014	Co-Founder, Radian Biotechnology, Inc.
2010 – 2013	Sr Scientist, Biological Dynamics, Inc.
2008 – 2010	Biotechnology Consultant, Self-employed
1999 – 2006	Sr Scientist, Gen-Probe, Inc.
1998 – 1999	Postdoctoral Fellow Northwestern University

C. Contributions to Science

I have been engaged in cancer diagnostics of one version or another for my entire professional life. In my view, I've worked at being a good soldier in various settings in the critical analysis of the field's existing states and have been one of the many who work to divine future trends the limits of my knowledge and accumulated wisdom. Along this road, I've been lucky to have been exposed to high level industrial science and business thinking beginning with my time at Gen-Probe and extending through my career in biotechnology startups. Cancer diagnostics, as a translational science, is brimming with the history and evolution of various and continually improved techniques, yet remains extremely complex, fast moving, and unfinished. Work remains, and the final versions of cancer diagnostics are in our collective future.

Throughout my career it has been my hope that I would be part of a team that pushes the current limits further in a genuine way. In my view this technical effort is part of that long line of phase changes that allow us to look deeper into the clinical biology of cancer for the practical improvement of patients' lives.
One of my key contributions has been in developing and refining methods to improve the analytical sensitivity of liquid biopsy tests. These improvements have correlated with increased clinical detection rates, particularly in detecting minimal residual disease (MRD) after curative-intent surgeries. Despite the advancements, MRD detection sensitivity remains at approximately 50% one-month post-surgery. This limitation may be due to the absence of ctDNA in the blood or the inability of current technologies to detect existing ctDNA.

My recent efforts have concentrated on overcoming this challenge by enhancing raw sensitivity through improvements in analytical sensitivity and increasing the amount of cell-free DNA (cfDNA) input. I have also explored strategies to expand nucleic acid-based approaches to include additional biomarkers such as methylation and RNA. These efforts aim to push the boundaries of MRD detection and significantly impact the clinical management of cancer patients.

Patent 1: Compositions and methods for high sensitivity detection of rare mutations.
WIPO (PCT) WO2022108933A1.
Patent 2: Composition and methods for affinity-directed enrichment of rare species.
WIPO (PCT) WO2019165469A1.

OMB No. 0925-0001 and 0925-0002 (Rev. 08/2024 Approved Through 01/31/2026)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Alexander Yum

eRA COMMONS USER NAME (credential, e.g., agency login): ALEXYUM

POSITION TITLE: CTO

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Nevada, Las Vegas	BS	05/2016	Biology

A. Personal Statement

I am the Chief Technical Officer of Spark Molecular Diagnostics, Inc. which focuses on next-generation technologies addressing unmet needs in the emerging field of liquid biopsies for early cancer detection and minimal residual disease. I am focused on ultra low level mutation detection that will increase landmark sensitivity for MRD and improve lead times, improving patient health. Previously, I was a scientist and Chief Technical Officer of CY Molecular Diagnostics, Inc, where I worked on the development of DNA mutation detection for Companion Diagnostics. My role on this Phase I SBIR will be to serve as co-investigator and assist with the execution of the proposed experiments involving the evaluation of Spark MRD's capabilities for MRD detection.

B. Positions, Scientific Appointments, and Honors

Position

2023 – Present CTO, Spark Molecular Diagnostics, Inc
2018 – 2023 CTO, CY Molecular Diagnostics, Inc
2016 – 2018 Scientist, CY Molecular Diagnostics, Inc

C. Contributions to Science

I have been engaged in biotechnology startup efforts since 2018 and I am the co-inventor on two patents pending. My first startup experience was with CY Molecular Diagnostics, where I distinguished myself in the lab with his analytical rigor, and as an inventive thinker on technology development leading to the creation of proprietary and patentable elements of Spark PCR's DNA mutation detection workflow using wildtype suppression and our probes. I have helped improve our assay to detect a single copy of tumor DNA which will lead to better landmark sensitivity for MRD.

Recent Patents on DNA mutation detection:
Compositions and methods for high sensitivity detection of rare mutations.
WIPO (PCT)WO2022108933A1
Composition and methods for affinity directed enrichment of rare species.
WIPO (PCT) WO2019165469A1

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

UEI*: CBWYDJ3RUM85

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: SPARK MOLECULAR DIAGNOSTICS, INC

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		William		Hanna		PD/PI	150,000.00	1.8			22,500.00	5,625.00	28,125.00
2 .		Alexander		Yum		Co-Investigator	125,000.00	3.6			37,500.00	9,375.00	46,875.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **75,000.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**75,000.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

UEI*: CBWYDJ3RUM85

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: SPARK MOLECULAR DIAGNOSTICS, INC

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

UEI*: CBWYDJ3RUM85
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: SPARK MOLECULAR DIAGNOSTICS, INC

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	77,240.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Technical Assistance	4,250.00
9. Novogene	10,780.00
10. UCSD CFAR Core Lab	1,000.00
11. nRichDx	3,000.00
12. Sharp Memorial Hospital	100,000.00
13. Data Management and Sharing Costs	0.00
Total Other Direct Costs	**196,270.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**271,270.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	40.0	267,020.00	106,808.00
		Total Indirect Costs	**106,808.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**378,078.00**

J. Fee	Funds Requested ($)*
	26,168.00

K. Total Costs and Fee	Funds Requested ($)*
	404,246.00

L. Budget Justification* File Name: Budget_justification-20240901.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Upon careful review of the budget required to complete the Aims as outlined, and review of the eligible waiver topics, we respectfully request the following budget outlined that is in excess of the hard cap. On 21 May 2024, the Department of Health and Human Services published revised guidance entitled "Approved SBIR/STTR Topics for Awards over Statutory Budget Limitations" for the 2024-2025 budget period. The National Cancer Institute (NCI), as stipulated on page 15, will consider proposals focused on: B: "In Vitro and In Vivo Diagnostics (e.g., Companion Diagnostics, Prognostic Technologies, Treatment Monitoring and Diagnostic-Related Tools, and Algorithm Development)" for a budget waiver. This document justifies the budget and provides a basis for inclusion in the waiver topics.

A. PERSONNEL

(Phase I Y1: $75,000)

William Hanna, PhD (PI Y1: 1.8 months)
Dr. Hanna is the CEO and CSO of Spark Molecular Diagnostics and will serve as **PI** on this project. Dr. Hanna will coordinate all research activities and prepare final reports to the funding agency. He will oversee all scientific activities, including the execution of the proposed experiments and coordination of clinical sample procurement and analysis. Dr. Hanna has a background in molecular diagnostics and has contributed to the development of various DNA diagnostics technologies, such as co-founding Radian Biotechnologies, a company focused on developing DNA diagnostics platforms, as well as serving as Chief Scientific Officer for CY Molecular Diagnostics. His personal experience with the challenges of diagnostics assays led to the development of the Spark MRD.

Alexander Yum, BsC (Co-I, Y1: 3.6 months)
Mr Yum. is the Chief Technical Officer at Spark Molecular Diagnostics and will serve Co-I on this project. has experience and expertise PCR and diagnostics. Mr. Yum has a background in molecular diagnostics and served as a scientist and Chief Technical Officer of CY Molecular Diagnostics. He will assist Dr. Hanna in experimental design and execution of Aim 1 and its Milestones, including probe design, data analysis, and performing the Spark MRD assay on clinical samples.

The fringe benefits for personnel are 25% of the salary costs requested.

B. OTHER PERSONNEL

N/A

C. EQUIPMENT

N/A

D. TRAVEL

N/A

E. PARTICIPANT/TRAINEE SUPPORT COSTS

N/A

F. OTHER DIRECT COSTS

Materials and Supplies (Phase I Y1: $77,240) These funds are requested for all reagents needed for Spark Molecular Dx PCR assays, sample preparation reagents, and general lab supplies. Costs associated with major categories of materials and supplies are outlined below.

- DNA Probes ($20,000) – These funds are requested for probes used in Spark MRD.
- DNA Primers ($20,000) – These funds are requested for primers used in Spark MRD.
- PCR Mastermix ($1,100) – These funds are requested for mastermix used in Spark MRD.
- Peptide Nucleic Acid (PNA) ($30,000) – These funds are requested for PNA's required to perform clamp PCR used in Spark MRD.
- ddPCR Kits ($2,500) – These funds are requested to perform ddPCR as a form of quality check.

- General Lab Supplies (plastics, sample prep, reagents) ($3,640) – These funds are requested for consumables required to carry out PCR experiments.

Fee-for-Service Vendors (Phase I Y1: $114,780)

Sharp Memorial Hospital ($100,000) – Sharp Memorial Hospital will be responsible for providing de-identified stage III colorectal cancer plasma specimens to support comparison of the Spark Molecular test with Naterra. These costs include analysis of specimens with the Naterra approach. Quote will be provided at just-in-time.

Novogene ($10,780) – These funds are requested for NGS services on all plasma specimens. *Quote is attached.*

UCSD CFAR Core Lab ($1,000) – These funds are requested for PCR analysis of plasma specimens at the UCSD core lab. *Quote is attached*.

nRichDx ($3,000) – These funds are requested for high volume sample preparation of plasma samples. *Quote is attached*.

Data Management and Sharing Costs
No funds requested.

Automatic Data Processing and Computer Services
No funds requested.

Discretionary Technical Assistance (Y1: $4,250)

We are budgeting for technical assistance with marketing matters related to this SBIR proposal, to be provided by Polaris Market Research (*see Quote*), for reliable recommendations to drive Spark Molecular business strategy. Spark Molecular anticipates significant benefits will arise from this support, as it will help us make informed decisions and position our PCR assays in the current industry.

Pursuant to 84 FR 12794 published by the Small Business Administration, these funds are requested above and beyond the hard cap budget limits prescribed.

INDIRECT COSTS
An indirect cost rate of 40% has been applied to all direct costs.

FEE
A fee of 7% is requested which we believe demonstrates a reasonable profit margin for for-profit organizations performing research and development work.

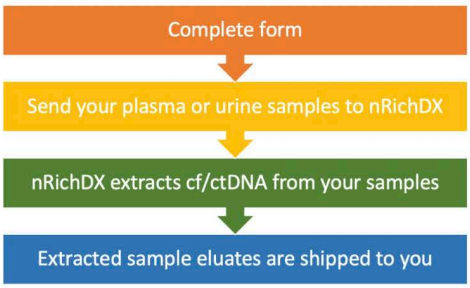
nRich[DX] APPLICATIONS PLATFORM PRODUCTS COMPANY EXTRACTION SERVICE GET A QUOTE

Let Us Extract Your cfDNA

Maximize your sample yield and get to analysis faster.

Get the expertise, convenience, and speed of nRichDX's Revolution Sample Prep as a service. Simply complete and submit the form below to get started.

Revolution Sample Prep™ Extraction as a Service Details



Send 1 mL to 20 mL of your plasma or urine samples to nRichDX. The experts at nRichDX rapidly perform the cfDNA extraction and elute in the volume you specify (25 µl - 100 µl).

Revolution Sample Prep enables extraction from up to 20 mL in a *single* extraction with no sample pooling - enabling maximum cf/ctDNA yield from your samples.

Extracted samples are typically returned to you by express overnight shipment within 7 business days from the date samples are received at nRichDX.

Qubit® and TapeStation® characterization data for your extracted sample eluates are available as an optional added service (approximately 12 µl of eluate required).

Per sample price

Extraction only:

- *<= 10mL input sample volume:* ***$99.00***
- *> 10mL - 20mL input sample volume:* ***$119.00***

Extraction with Qubit and TapeStation data:

- *<= 10mL input sample volume:* ***$129.00***
- *> 10mL - 20mL input sample volume:* ***$149.00***

To get started, please complete and submit the Service Intake Form on this page, and nRichDX will contact you shortly (usually within one business day) to verify your order, answer questions, and arrange shipment of your samples to nRichDX.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Home / Healthcare / Minimal Residual Disease Market
Market Research Report
Minimal Residual Disease Market Share, Size, Trends, Industry Analysis Report, By Detection Target (Lymphoma, Leukemia, Solid Tumors, Others); By Test Technique; By End-User; By Region; Segment Forecast, 2023-2032
Published Date:Jan-2023
Pages: 119
Format: PDF
Report ID: PM2988
Base Year: 2022
Historical Data: 2019-2021
Description
Table of Contents
Analysis Type
Research Methodology
Request Free Sample
Report Outlook
The global minimal residual disease market was valued at USD 1,973.35 million in 2022 and is expected to grow at a CAGR of 13.7% during the forecast period.
License and Pricing
Single User - $ 4250
Multi Users (Up to 20 User) - $ 5250
Enterprise License - $ 6250
Buy Now
Purchase Report Sections
Regional analysis
Segmentation analysis

Official Quotation

Company Address: 2921 Stockton Blvd. Suite 1810, Sacramento CA 95817

VAT number: GB 273975163 Phone: 916-252-0068(toll free)

Fax: 916-252-0068

Novogene Representative: Noel Chen

Representative email: noel.chen@novogene.com

Novogene Technical Support: Nick Li

TS email: nick.li@novogeneusa.com

Quote No.: NVUS2024051072

Date Issued: 2024-05-10

Validity: (30 days) 2024-06-09

Payment Terms: Net 30 days

CUSTOMER INFORMATION	
Institution	SparkMolecular Diagnostics
Shipping Address	San Diego CA United States
Customer Name	Bill Hanna
Email Address	bill@sparkmoleculardx.com

No	Product Name	Description	UNIT PRICE	Currency	QTY	Total price
1	Human Whole Genome Sequencing (WBI)	Human whole genome library preparation (350bp)	419.00	USD	25	10475.00
2		NovaSeq X Plus Series (PE150) (90 G raw data per sample)				
3		Customized extraction and sample QC	70.00	USD	25	1750.00
4		Standard Analysis	50.00	USD	25	1250.00
5		FTP	0.00	USD	1	0.00
Total:						13475.00
Grand Total						13475.00

1. Overview of Service	
Product Name	Human Whole Genome Sequencing (WBI)
Species	Human - Homo Sapiens
Sample Type	Tissue(FFPE)
Sequencing Platform & Strategy	NovaSeq X Plus Series (PE150)
Q30	PE150,Q30≥85%

Data Requirement	90 G of raw data per sample
No. of Sample	25
Data analysis	Standard Analysis
Turnaround Time	15 working days starting after we receive confirmation of the sample QC report from customer

2. Sample Submission Guidelines(Human Whole Genome Sequencing (WBI))

Novogene Extraction Service Guidelines for Client

3. Bioinformatics Analysis (Human Whole Genome Sequencing (WBI)-Standard Analysis)

1. Data quality control: filtering reads containing adapter or with low quality
2. Alignment to reference genome; statistics of sequencing depth and coverage
3. Variant (SNP, InDel, CNV, and SV) calling, annotation and statistics
4. Somatic variant detection (only apply for tumor-normal paired samples)

SNP calling, annotation and statistics
InDel calling, annotation and statistics
CNV calling, annotation and statistics
SV calling, annotation and statistics
Display of Genomic Variants with Circos

4. Sample Submission

All samples must be delivered to Novogene in accordance to the sample requirements listed above. Please request for the sample submission form and sample submission instructions from the Novogene representative. Samples should be sent to the address below:

ATTN: Sample Receiving Department
Novogene Corporation Inc.
2921 Stockton Blvd. Suite 1810
Sacramento CA 95817
Tel: 916-252-0068
To order, please send us your purchase order and obtain the Sample Submission Form from us.

5. Customer Service System (CSS)

Do you know?
The Customer email address shown on this quotation will automatically become the **Project Owner** on the Novogene Customer Service System. On CSS, you can track and manage the project online 24/7. Please note that only the permittee(s) can access the project information on CSS and receive CSS email notifications. You may use the **My Team** menu on CSS to issue an authorization.

6. Terms and Conditions

By signing below, issuing a PO, and/or making a payment, I hereby confirm my agreement with the information and pricing outlined in this quotation. I acknowledge and comprehend the terms and conditions outlined in points 1-46 below. Furthermore, I acknowledge the bioinformatics specifications in the quote, and I understand that any subsequent requests for changes to the bioinformatics requirements may result in additional costs. I agree to be responsible for such incurred charges.

Client Name: _____ Date : _____

Client Signature: _____

UCSD CFAR CORE LAB QUOTE

Spark Molecular Diagnostics, Inc
6349 Nancy Ridge Drive, STE B
San Diego, CA 92121
(619) 961-3062

Purchase Order

Date	Invoice	P.O. number
7/1/2024		20240701AY

Ship date	Ship via	Terms

Vendor

Name
Veterans Medical Research Foundation
3350 La Jolla Village Drive 151A
San Diego, CA 92161

Ship to

Item #	Description	Qty	Unit price	Total price
	ddPCR for DNA samples	32	$28.94	$926.08
	mastermix provided			$0.00
				$0.00
				$0.00
			Subtotal	**$926.08**
			Shipping & handling	
			Tax rate	
			Sales tax	
				$926.08

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		75,000.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		75,000.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		196,270.00
1. Materials and Supplies	77,240.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	4,250.00	
9. Other 2	10,780.00	
10. Other 3	1,000.00	
11. Other 4	3,000.00	
12. Other 5	100,000.00	
13. Other 6	0.00	
14. Other 7	0.00	
15. Other 8	0.00	
16. Other 9	0.00	
17. Other 10	0.00	
Section G, Direct Costs (A thru F)		271,270.00
Section H, Indirect Costs		106,808.00

Section I, Total Direct and Indirect Costs (G + H)	378,078.00
Section J, Fee	26,168.00
Section K, Total Costs and Fee (I + J)	404,246.00

OMB Number: 4040-0001
Expiration date: 11/30/2025

SBIR/STTR Information

Agency to which you are applying (select only one)*

❍ DOE ● HHS ❍ USDA ❍ Other:

SBC Control ID:* 002649047

Program Type (select only one)*

● SBIR ❍ STTR

❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

Application Type (select only one)*

● Phase I ❍ Phase II ❍ Fast-Track ❍ Direct Phase II ❍ Phase IIA ❍ Phase IIB ❍ Phase IIC

❍ Commercialization Readiness Program (See agency-specific instructions to determine application type participation.)

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

Questions 1-8 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 4

1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?* ❍ Yes ● No

1d. Is your small business a Faculty or Student-Owned entity?* ❍ Yes ● No

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ❍ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and email address of the official signing for the applicant organization to state-level economic development organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ❍ No

7. Does the application include a request of SBIR or STTR funds for Technical and Business Assistance (TABA)? If yes, please follow the agency specific instructions to provide the budget request and justification. (Please answer no if you plan to use the agency TABA vendor, which does not require you to include a request for TABA funds in your application.)* ● Yes ❍ No

8. Commercialization Plan: The following applications require a Commercialization Plan: Phase I (DOE only), Phase II (all agencies), Phase I/II Fast-Track (all agencies). Include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 11/30/2025

SBIR/STTR Information

SBIR-Specific Questions:

Questions 9 and 10 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 9 and 10 blank and proceed to question 11.

9. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ❍ Yes ● No

Attach File:*

10. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ● Yes ❍ No

STTR-Specific Questions:

Questions 11 - 13 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 11 - 13 blank.

11. Please indicate whether the answer to BOTH of the following questions is TRUE:* ❍ Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

12. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ❍ Yes ❍ No

13. Provide UEI of non-profit research partner for STTR.*

OMB Number: 0925-0001

Expiration Date: 01/31/2026

PHS 398 Cover Page Supplement

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ❍ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

OMB Number: 0925-0001
Expiration Date: 01/31/2026

PHS 398 Research Plan

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Specific_Aims_2024.08.31.pdf
3. Research Strategy*	Research_Strategy_2024.08.31.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	ResourceSharingPlan_2024.08.22.pdf
11. Other Plan(s)	DMSP_2024.08.30.pdf
12. Authentication of Key Biological and/or Chemical Resources	Authentication_2024.08.29.pdf
Appendix	
13. Appendix	

Specific Aims

Current methods of minimal residual disease (MRD) detection are limited by their relatively low sensitivity when screening for biomarkers of solid tumor relapse. Spark Molecular Diagnostics is developing a 4th generation PCR based approach of MRD detection in plasma with unparalleled sensitivity capable of detecting a single molecule of mutant DNA among 100,000 wildtype molecules, making it 20 to 100 times more sensitive than current MRD tests for solid tumor relapse. As such, this technology has the potential to improve landmark detection and increase lead times, allowing for more effective intervention and treatment.

Detection of MRD is crucial for assessing the risk of relapse following surgical resection of a solid tumor, allowing for earlier and more appropriate treatment strategies leading to better patient outcomes[1]. In the last two decades, the use of liquid biopsies of blood or other bodily fluids for MRD detection has grown significantly due to the fact this approach is minimally invasive and allows for real time monitoring of MRD through measuring the presence of biomarkers such as circulating tumor DNA (ctDNA)[2]. Early landmark detection of MRD is an important predictor of tumor relapse in a variety of cancers including colorectal cancer (CRC), with MRD tests predicting relapse over 95% of the time often years before imaging[3–6]. While the use of liquid biopsies for the detection of MRD markers has shown promise, these methods are typically limited by their relatively low sensitivity, making the accurate detection of residual ctDNA from remaining tumor cells difficult, thereby increasing the risk of inaccurate results and false negatives[2,7]. This not only poses the problem of reduced landmark detection rates but can also lead to the implementation of unnecessary adjuvant therapies, further diminishing patient outcomes. Current methods of landmark (+) detection of MRD in liquid biopsies 1 month after surgery (landmark) is only 55%, irrespective of the number of mutant markers analyzed (16 to thousands), which poses the risk of reduced lead time and furthers the delay of appropriate intervention strategies[4,8–13]. Therefore, there is a need for new methods of MRD detection using liquid biopsies that achieve sufficient levels of sensitivity and specificity to allow for earlier MRD detection to inform subsequent treatment strategies and improve patient outcomes.

Spark Molecular Diagnostics is developing Spark MRD™, an ultrasensitive 4th generation PCR test to address the shortcomings of contemporary MRD detection methods in liquid biopsies. Spark MRD accomplishes this feat through its ability to reduce background noise from wildtype DNA using either clamp PCR or allele-specific PCR techniques, with the combination of chemiluminescent probes, allowing for greater MRD detection sensitivity and improved accuracy of results. Preliminary studies including a blinded study using over 200 samples of engineered cell line DNA, a clinical study using over 100 healthy donor samples of white blood cell and plasma DNA, and a study using an archived plasma sample compared directly with digital droplet PCR (ddPCR) demonstrate Spark MRD's sensitivity and capability to detect a single copy of ctDNA among thousands of copies of wildtype (WT) DNA. In fact, our data indicates that Spark MRD can achieve single copy sensitivity among up to 100,000 copies of WT DNA (330 nanograms of plasma DNA), translating to 20 to 100 times greater detection capabilities than various alternative ddPCR and next generation sequencing (NGS)-based MRD tests. These findings suggest that MRD landmark detection can be improved (above 50%) through a great increase in analytical detection sensitivity compatible with high plasma DNA inputs, indicating lead times in landmark and serial MRD testing can be significantly lengthened through earlier detection. The goal of this phase 1 SBIR is to demonstrate evaluate the sensitivity and accuracy of Spark MRD in CRC landmark detection at 1-month post-surgery in comparison with the current "gold standard" of liquid biopsy MRD detection, Natera's "Signatera" MRD assay. While Natera's technical approach relies on an ultra-deep NGS using a limited set of 16 truncal/clonal DNA mutation markers, new entrants into MRD using up to 1,800 DNA mutation markers are showing similar clinical landmark sensitivities[14,15]. Therefore, a clinical comparison with Natera represents a comparison to much larger NGS-based MRD tests as well.

Aim 1: Comparison of Spark MRD performance to Signatera in landmark MRD detection. We will evaluate Spark MRDs overall sensitivity and how it compares to Natera's Signatera MRD assay. This will be done in collaboration with Sharp Memorial hospital who will facilitate prospective collection of 20-30 stage II and III CRC patient plasma samples 1-month post-surgery. ctDNA from these samples will be analyzed for MRD using Natera's NGS panel of 16 mutation markers alongside Spark MRD to measure the presence of 5 truncal mutations per patient identified using whole exome sequencing (WES) NGS of the tumor biopsy. *Quantitative milestones: sensitivity and specificity for Spark MRD test ≥ Natera for landmark detection at 1-month post-treatment in statistically powered comparison study.*

Impact and Future Work: Upon completion of the proposed studies, we will pursue Phase II funding for a larger clinical study and will seek FDA clearance for the Spark MRD test. By improving the sensitivity and accuracy of MRD detection in liquid biopsy samples, Spark MRD has the potential to enable for earlier MRD landmark detection and increased lead times, allowing for more informed treatment options and better patient outcomes.

SIGNIFICANCE

Minimal residual disease detection of circulating tumor DNA is the most significant prognostic indicator of relapse in colorectal cancer. To fully realize the prognostic potential of circulating tumor DNA (ctDNA), Spark MRD is developing an improved method of PCR wildtype (WT) background reduction, which will seek to improve ctDNA detection sensitivity by improving signal to noise (S/N) ratios. This approach utilizes either clamp-PCR or allele specific PCR techniques to effectively reduce non-specific amplification and WT background noise with the combination of chemiluminescent probes, allowing for unparalleled sensitivity and detection of MRD mutation markers. Moreover, Spark MRD is optimized to analyze larger volumes of plasma samples than currently available methods, thereby increasing the chances of detecting limited quantities of ctDNA in clinical settings. As such, Spark MRD has potential to greatly impact the accuracy and lengthen leadtimes of MRD detection, improving treatment strategies and patient outcomes.

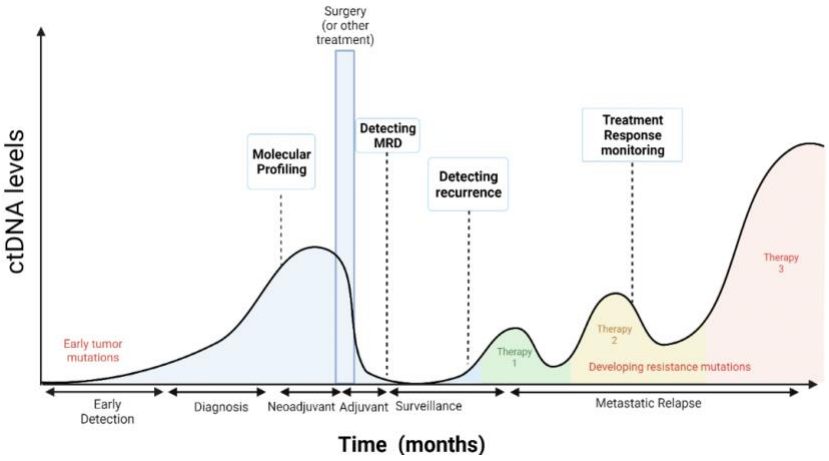

Figure 1: Timeline showing changes in ctDNA levels following surgical removal of solid tumor tissue and neoadjuvant therapy prior to recurrence and relapse. Image source: Arisi et al.

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States, with drastically increasing rate of incidence among patients younger than 55[16]. Currently, the rate of tumor recurrence following resection of a solid tumor in stage II-IV CRC is between 20 and 50 percent, highlighting the need for more effective means of predicting and detecting relapse[17,18]. By better understanding this risk, clinicians are better informed on the timeline and strategy of post-operative adjuvant therapies aimed at treating tumor relapse. As such, there is great clinical utility in the development of prognostic markers through detecting minimal residual disease (MRD). Liquid biopsies offer an effective non-invasive source of MRD detection and monitoring following solid tumor removal by allowing for the measurement of tumor biomarkers in biofluids, such as proteins, methylation markers, miRNAs, and ctDNA[19]. The measurement of ctDNA in particular has emerged as a powerful method for MRD detection in CRC and enabled for earlier and more accurate assessments of the risk of solid tumor relapse (Figure 1)[11,19,20]. This is particularly important given the detection of ctDNA at as little as 30 days post-surgical resection has been associated with a higher risk of recurrence in patients with stage I-III CRC, demonstrating the need for highly sensitive ctDNA MRD detection methods capable of detecting often minute amounts of ctDNA present at this clinically important timepoint[4,21,22]. In addition to allowing for earlier onset of treatment and adjuvant therapeutic intervention, accurate longitudinal assessment of MRD also identifies patients who are at low risk of relapse, allowing clinicians to avoid the administration of unnecessary intervention and avoid potential treatment related toxicity, further improving patient outcomes[23–25].

Company		Multiplex LOD tests using Cell Lines Or high multiplex commercial products			Reported Sensitivity		Blinded Studies?
	DNA Input	DNA Copies	Cell Line SNV # ***In Parallel***	Multiplex LOD in PPM	Multiplex LOD in %VAF	Multiplex LOD in copies	***3rd*** Party Verified
Natera	67 ng	20000	16	200 PPM	0.02% VAF	4 copies	No
			10		Not shown		
			5		Not shown		
			1	1000 PPM	0.1% VAF	20 copies	No
Invitae	60 ng	18000	50	500 PPM	0.05% VAF	9 copies	No
			18		0.05% VAF	9 copies	No
			10		Not shown		
			5		Not shown		
			1		Not shown		
Personalis	30 ng	9000	1800	3.45 PPM	0.0005% VAF	< 1 copy	No
			100		Not shown		
			50		Not shown		
			20		Not shown		
			10		Not shown		
			5		Not shown		
			1		Not shown		
IMBdx	40 ng	12000	100	450 PPM	0.04% VAF	5 copies	No
			50	500 PPM	0.05% VAF	6 copies	No
			10	1000 PPM	0.1% VAF	12 copies	No
			5		Not shown		
			1		Not shown		

Table 1: NGS Tumor-informed MRD ctDNA LODs using multi-marker simulated MRD samples.

Current methods of MRD detection are limited by a lack of sensitivity and specificity, which reduces their effectiveness at establishing early landmark detection of MRD in CRC. ctDNA based MRD detection methods in liquid biopsies utilize a variety of techniques, including next generation sequencing (NGS) and

various forms of PCR (RT-PCR, ddPCR RQ-PCR)[19]. These assays are typically “tumor informed”, allowing them to screen for patient specific tumor mutations to detect MRD[17]. Such techniques have proven to be exceptionally powerful tools as they are capable of low ctDNA LODs, allowing for early and accurate landmark detection[26–29]. Among the current industry leaders, Natera’s Signatera NGS-based MRD detection assay has been reported to detect as few as 20 copies of mutant ctDNA, positioning it as one of the more utilized MRD detection tests, and one of two commercially available MRD tests that has gained Medicare approval[30–32]. While ddPCR offers considerable sensitivity and is the main alternative to NGS-based methods of MRD detection, these assays are still limited by their low accuracy at early landmark timepoints due to relatively high levels of WT background noise and the ability to only screen for a single mutation at a time[33]. While these advances have revolutionized MRD detection in CRC patients, they have only allowed for around 55% accuracy in relapse prediction at the critical 30-day landmark detection timepoint[4]. Missing patient MRD at landmark has several consequences. First, it becomes impossible to distinguish the remaining cured patients (no future relapses) from the MRD false negative patients. Consequently, all landmark (-) patients are treated as potential relapses and receive adjuvant chemotherapy (ACT). Second, following a false negative landmark MRD test, MRD lead times in these patients are decreased by as much as 1 year since 6-month ACT programs reduce ctDNA levels below landmark levels. Therefore, it is imperative that further advancements in MRD detection are made that allow for reliable “ultrasensitive” ctDNA detection of 15 or fewer copies to enable for more informed treatment strategies. These sensitivity limitations are illustrated in Table 1. The current industry leaders, Natera’s Signatera’s ultra-deep NGS-based MRD detection assay has a reported sensitivity of 20 copies of mutant ctDNA in a single mutant sample (Table 1) and this is consistent with several recent blinded studies examining ctDNA sensitivity for prominent liquid biopsy platforms when analyzing limits of detection using single marker samples (refs). This suggests that today’s ultra-deep sequencing strategies (100k to 200k read depths) are limited to >20 ctDNA copies of a single mutant species for robust detection (ref). In response to these limitations, MRD companies have developed strategies to increase ctDNA detection sensitivity using contrived cancer plasma containing dozens to thousands of ctDNA species in the same sample (Table 1). While this approach succeeds in improving analytical sensitivities, the numbers of ctDNA mutant species in clinical MRD samples are far lower (Table 2) which greatly erodes the analytical sensitivity of the assays listed in Table 1. The utility of a higher sensitivity assay using a small set of bespoke truncal ctDNA species is further exemplified in the use of ddPCR for MRD using 2-3 ctDNA species (Table 3). Moreover, MRD sensitivity using Natera’s platform achieves ~95% of Signatera’s clinical sensitivity using only 4 truncal ctDNA species (Table 4), where tumor-informed high sensitivity tests using a few truncal mutations appear to replicate the clinical success seen in much larger assays, presumably due to ddPCR’s greater single marker sensitivity compared to NGS-based assays.

MRD Company	MRD Assay ctDNA Species	ctDNA Species in Sample Lowest	Median	Highest
Natera	16	2	12	16
Invitae	50-200	*Not shown in publications*		
IMBdx	300	1	11	60
Personalis	1800	*Not shown in publications*		

Table 2: ctDNA species identified in landmark MRD samples.

Cancer (patients)	Stage	dPCR Marker #	Landmark MRD Se	Serial MRD Se	Hazard Ratios RFS	Median time to Recurrence
MultiMarker Digital PCR						
Colon (132) [1]	I-III	2	57%	86%	17	11.5 months
Colon (27) [2]	III	3	40%	100%	37	9.4 months
Colon (18) [3]	IV	2	92%	100%	n.d.	n.d.
Rectal (29) [4]	II-III	2	n.d.	100%	11	n.d.
Breast (43) [5]	II	2	54%	81%	18	13.6 months
Single Marker Digital PCR						
Colon (112) [6]		1	n.d.	38%	n.d.	n.d.
Breast (114) [7]		1	23%	80%	n.d.	3.9 months

Table 3: Clinical Studies: MRD by ddPCR in colon and breast cancer.

MRD Assay	ctDNA Species Used in Assay	MRD(+) Patients
Signatera	4 markers	14
Signatera	16 markers	15

Table 4: Natera’s clinical MRD sensitivity using 4 vs 16ctDNA species in assay.

Spark MRD improves upon the limitations of current MRD detection techniques. The development of more sensitive and specific ctDNA MRD detection techniques will lead to substantial improvements in the effectiveness of clinical strategies for managing tumor relapse in CRC and numerous other cancers. Spark MRD’s ability to identify a single copy of mutant ctDNA has potential to significantly improve MRD detection standards due to its unique approach to PCR that improves WT background noise reduction. This is accomplished by established peptide nucleic acid (PNA) clamp PCR techniques to selectively anneal to WT DNA sequences, preventing non-

specific amplification of mutant probes. Spark MRD can also be used with allele-specific PCR that utilizes primers designed with 3' overlap at the tumor variant's base location, allowing for more specific and selective amplification of mutant transcripts by reducing amplification of WT transcripts. Moreover, Spark MRD utilizes probes containing acridinium as a chemiluminescent reporter, which undergoes a light-generating reaction as an endpoint PCR that is more sensitive than fluorescent probes when measured in bulk PCR reactions. Collectively, the combination techniques lead to a significant increase in the sensitive of mutant DNA detection by effectively reducing WT background amplification, achieving greater S/N ratios with plasma DNA input levels 5x greater than current tests. The work proposed within this proposal will seek to further demonstrate Spark MRD exceeds current standards of MRD detection sensitivity. To the best of our knowledge, this will be the first time that a direct clinical comparison is performed between two MRD detection methods in CRC, which will further elucidate how sensitive Spark MRDs detection capabilities are compared to an established industry leading method.

Rigor of the Prior Work—The preliminary work demonstrates that Spark MRD can achieve single tumor DNA copy sensitivity in a variety of sample types, including engineered cell line DNA, healthy donor white blood cell and plasma DNA, and an archived CRC patient plasma DNA sample. Collectively, these findings highlight Spark MRD potential improve S/N ratios and enhance the sensitivity and specificity of MRD detection, while utilizing a less complex approach compared to established methods. Currently, more work needs to be done in the form of clinical studies to determine how Spark MRD compares leading methods of MRD detection in stage II and III CRC patient plasma samples, namely Signatera.

INNOVATION

Greater MRD detection sensitivity. Spark MRD will provide first-in-class diagnostic capabilities with unparalleled accuracy and sensitivity to improve MRD landmark detection timelines. Specifically, Spark MRD has the ability to detect as little as 1 to 5 copies of ctDNA in liquid biopsies samples, which has potential to significantly lengthen lead times by enabling earlier MRD landmark detection. Compared to industry leaders such as Signatera and ddPCR assays of MRD detection, Spark MRD can achieve single copy sensitivity among up to 100,000 copies of WT DNA, making it 20 to 100 times more sensitive than these techniques. This is accomplished through Spark MRD's unique approach to WT background reduction, which improves S/N ratios and allows Spark MRD to discern true signal generated from mutant ctDNA targets among large quantities of WT DNA copies. Moreover, common NGS and PCR based MRD detection methods are optimized to analyze a limited input of DNA (10-30ng and 5-35ng, respectively)[34–36]. Furthermore, industry leader Signatera can analyze inputs of up to 66ng of DNA from a volume of 9mL of plasma, while Spark MRD can analyze 200ng from up to 20mL of plasma from up to 4 tubes of blood, allowing for a larger and more comprehensive scale of analysis, improving the chances of detecting often minute numbers of copies of mutant ctDNA copies that may be present.

Enhanced MRD detection Specificity. Spark MRD's greatly increased ctDNA sensitivity is intended to improve clinical specificity by identifying clinical MRD false negatives where a sample has low levels of ctDNA but are technical false negatives due to assay insensitivity. Regarding Spark MRD assay specificity for mutant DNA, our chemiluminescent probe designs rely on hybridization-independent specific base-pair mismatching at the site of the mutation. Signals from our mutant probes on wildtype DNA are typically > 100-fold below the signal from a mutant DNA in the sample.

A less complex approach for MRD detection compared to alternative methods. Contemporary approaches to MRD detection screen for a multitude of ctDNA truncal and clonal mutation targets that are either commonly known predefined mutations, or tumor informed based on NGS of tumor tissue. Collectively, these techniques employ a wide range of ctDNA tumor markers to screen for MRD, ranging from 16-1800 tumor specific mutation markers (Signatera and Personalis, respectively) all the way up to methods employing whole genome sequencing (LabCorp and C2i Genomics). Despite the fact that there is significant variance in the range of ctDNA mutation markers used in these various MRD detection methods, there is not significant differences in their capabilities to identify MRD at landmark times of 1 month (roughly 50-60%), highlighting the fact that more markers and thus more complex methods are not always more effective for MRD detection[14,15]. Given that Spark MRD screens for only 5 truncal DNA mutation targets while achieving equivalent or greater landmark detection capabilities than these aforementioned techniques, Spark MRD's approach is far less complex without sacrificing the accuracy of the results.

Commercial Potential. Liquid biopsies have grown to be an effective noninvasive diagnostic measure of genomic alterations in solid tumor patients. Given that ctDNA is the most significant prognostic indicator of MRD in liquid biopsy samples, there has been a growing demand for ctDNA-based methods of MRD detection for clinical use. Accordingly, the market for ctDNA diagnostic methods is projected to grow to 4.3 billion dollars by 2029[37]. While there are several assays that are approved for clinical use, namely Signatera, Guardant Reveal,

and NavDX, there significant commercial potential in improving the deficiencies in sensitivity and landmark detection[38]. Spark MRD is well positioned to improve MRD detection in biofluids with its patented method of detecting rare or low frequency mutations. Key steps to commercialization include obtaining Phase II funding to perform the necessary pre-clinical evaluation studies outlined in this proposal, followed by seeking Medicare approval for laboratory diagnostic test (LDT) through the technical assessment program, or in light of the recent FDA's final decision on LDTs, build towards FDA approval.

APPROACH

Preliminary Data

Spark MRD has greater sensitivity and accuracy than ddPCR for MRD detection. To gain insight on how Spark MRD's approach to PCR compares to ddPCR (one of the most sensitive methods of PCR used for MRD detection), we performed a pilot study aimed at directly comparing the ctDNA detection sensitivities of each method. Using an archived donor plasma DNA sample of a stage IV CRC patient (n=1), we performed serial dilutions of 100, 50, 20, 10, 5, and 1 mutant ctDNA copies in WT DNA totaling up to 3000 copies of DNA, along with negative controls containing only WT DNA. Subsequently, Spark MRD's PCR was performed alongside ddPCR on each dilution. Within the comparisons of the 10 and 5 mutant copy dilutions, Spark MRD was able to achieve 100% detection in both dilutions, whereas ddPCR achieved only 80% and 46%, respectively (Figure 2). Spark MRD detected 33% of the 1 mutant copy dilutions with S/Ns of > 50, which were not run on digital PCR due to assay noise (Figure 2). Illustrative of this, most negative control wells containing only WT DNA analyzed by ddPCR had 3-4 amplification events, indicating the presence of nonspecific amplification that made it difficult to distinguish from small number of mutant copies present in the experimental groups.

	1 copy	5 copies	10 copies
Spark PCR	33% Positive	100% Positive	100% Positive
ddPCR	n.d.	46% Positive	80% Positive

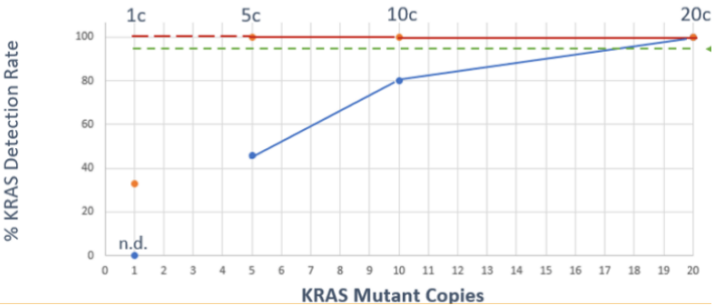

Figure 2: Results of the comparison of KRAS detection rate of Spark MRD to ddPCR at various dilutions.

Spark MRD can detect a single copy of ctDNA. To further demonstrate Spark MRD's detection capabilities, we performed a series of experiments to evaluate Spark MRD's sensitivity to detect the presence of KRAS G12A mutant DNA (a mutation that is associated with CRC) among various amounts of gDNA. This was demonstrated in two separate experiments that used Spark MRD to detect 3 and .3 copies of KRAS G12A in 100ng of gDNA. In both cases, we observed S/N ratios of over 100. In the first experiment, we were able to detect 3 copies in 9 out of 10 wells, indicating sensitivity of detecting a single mutant transcript among 10,000 WT transcripts (Figure 3). In the second experiment, we demonstrated the ability to detect a single copy of ctDNA by detecting 3 out of 10 replicates in wells containing an average of 0.3 copies each (1 mutant among 100,000 WT), further demonstrating Spark MRD's capability to detect a single copy of mutant DNA among large amounts of WT DNA (Figure 4).

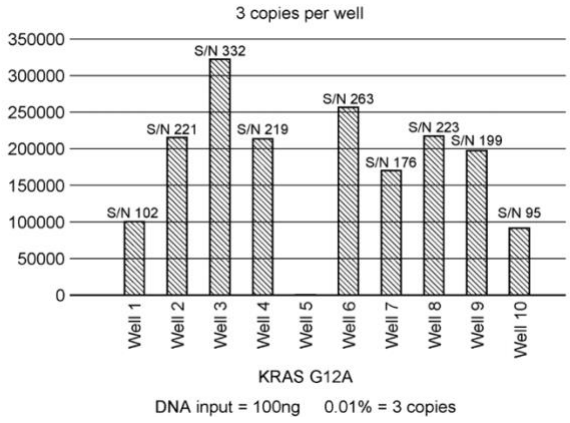

Figure 3: Results of Spark MRD performed using samples containing 100 ng per test sample, where the samples contain 0.01% KRAS G12A mutation (mean of 3 copies per well) relative to corresponding wild type DNA (99.99%).

Summary of Preliminary Data—Our preliminary studies highlight Spark MRD's superiority over ddPCR methods of MRD detection. Furthermore, they demonstrate Spark MRD's ability to detect a single copy of mutant DNA among amounts of WT DNA, making it more sensitive and accurate than established MRD detection methods. In addition to these studies, we have also performed preclinical validations of Spark MRD's sensitivity and specificity using DNA from an engineered cell line and healthy donor plasma DNA samples. Regarding the former, we tested Spark MRD's sensitivity in DNA collected from an engineered cell line containing C6223 and KRAS G12A mutations that were diluted with WT cell DNA to represent ~3mL plasma and 10ng DNA with various

mutant copy levels (0, 0.3, 3, 30, and 150 copies) among 3,000 copies of WT DNA. Afterwards, a blinded analysis using Spark MRD was conducted to detect the presence of the mutant DNA at the various concentrations. Upon analyzing the WT group with 0 mutant copies, Spark MRD correctly identified the presence of 0 copies (n=30), indicating no false positives occurred. Within the least concentrated mutant group (0.3 copies), Spark MRD was able to identify the presence of EGFR C6223 (6 experimental out of 9 expected) and KRAS G12A (7 experimental out of 9 expected) copies of mutant DNA within these samples (n=30). It is important to note however that at such low levels of copy numbers, there is a statistical likelihood based on Poisson distribution that some samples will have 0 copies, hence the reason that the number of observed positive readings was lower than expected in some instances.

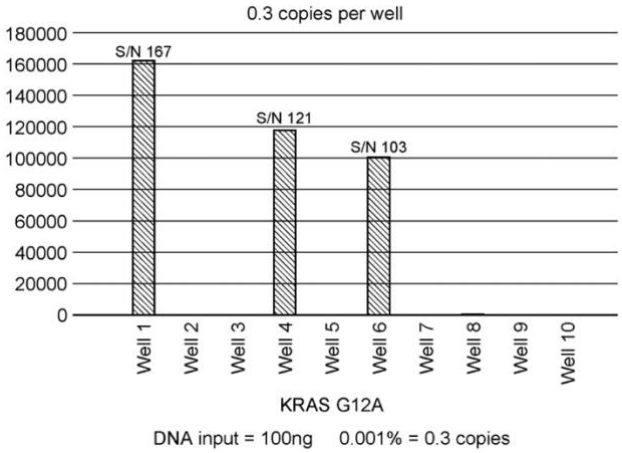

Figure 4: Results of Spark MRD performed using samples containing 100 ng per test sample, where the samples contain KRAS G12A mutation at 0.001% (mean of 0.3 copies per well) relative to corresponding wild type DNA (99.999%).

In another study, we further demonstrated Spark MRD's ultra-sensitive MRD detection capabilities using 114 male and female heathy donor plasma samples obtained from the San Diego Blood Bank of various ages with and without a spiked-in KRAS G12A mutation. When measuring the samples without a spike-in, we observed only 3 positives among the 3 oldest donors, indicating the possibility that these positives were due to the presence of a KRAS mutation, or benign clonal hematopoiesis of indeterminate potential (CHIP) since KRAS mutations commonly overlap with CHIP markers. In the analysis of the same samples containing a spiked-in KRAS G12A mutation (10 copies), we observed a 100% mutation detection rate in all 114 samples. Collectively, these results indicate that the use of Spark MRD results in a low number of false positives, as well as a robust (100%) mutation detection rate in truly positive samples.

Phase I

Milestone	Method	Quantitative Metric
1.1 Identify ct-DNA based tumor markers in stage II and III CRC patient plasma samples to inform panels used for analysis.	Whole exome sequencing, QC (Qubit and TapeStation).	Identification of patient-specific tumor mutations (NGS). Quality scores and quantity of WT transcripts (QC).
1.2 Generate mutation-specific DNA probes to be used for Spark MRD.	Probe design, PCR Deisgn.	Completion of 5 patient-specific probes targeting detected mutations.
1.3 Perform quantitative comparison of MRD detection sensitives between Spark MRD and Signatera.	NGS (Signatera), Spark MRD.	Sensitivity (rate of positive mutation detection).

Table 5: Milestones.

Aim 1: Comparison of Spark MRD performance to Signatera in landmark MRD detection.

Study Design: We will use Spark MRD alongside Signatera to measure the presence of ctDNA for purposes of comparison of these two techniques. Briefly, we will conduct a tumor informed analysis on prospectively collected stage II and III CRC patient tumor samples of patient specific ctDNA mutations that will be separately identified using whole exome sequencing (WES) using both MRD detection methods to conduct a statistically powered comparison study to determine their sensitivity at a landmark time of 1-month. Patients' disease status will be determined through longitudinal clinical monitoring.

Samples: Through a collaboration with Sharp Memorial hospital, we will perform prospective collection of plasma from up to 40mL of blood collected from 20-30 stage II and III CRC patients 1-month post-surgical resection of tumor tissue using the nRichDx system. Given that the relapse rate of stage II and III CRC is around 35%, we expect that with 30 patients we should see an average of 10 relapses. Tumor biopsies will be collected at the time of resection to be used for WES NGS to allow for downstream tumor informed analysis. Given the differences due to age and sexual dimorphism in CRC incidence, mutation type and burden, and tumor location, it is critical that both male and female patients of different ages are included in this study[39]. Therefore, the proposed research will seek to represent both sexes as equally as possible.

WES: WES of patient tumor tissue biopsy samples will be performed separately for both groups of samples (Spark MRD and Signatera). The reason for this is because Signatera's MRD assay is optimized to work in

conjunction with their proprietary WES protocols, which can't be adapted to work with Spark MRD. Therefore, WES of the tumor biopsy samples in the Signatera group will be performed by Natera in preparation for further analysis. WES will be used to identify and inform Signatera's panel of 16 mutations, which will be specific to each patient. For the Spark MRD samples, we will contract Novogene to perform WES, which will inform us on which set of 3 to 5 patient specific truncal mutations that we will screen for using Spark MRD. Prior to WES, these samples will undergo quality control using Qubit and TapeStation DNA analysis.

Signatera NGS MRD Assay: The Signatera NGS based MRD detection assay will be performed by Natera on 9mL of patient plasma DNA samples using 16 bespoke ctDNA markers identified from WES NGS performed on each respective sample.

Spark MRD Probe Design: Following WES, we will generate mutation-specific DNA probes that contain a chemiluminescent reporter called acridinium (Ae), and this type of chemiluminescence is ~50-100x more sensitive than equivalent fluorescence probes. The mutation-specific probes are constructed using standard DNA phosphoramidite chemistry with the insertion of an amine-containing internucleotide L1 linker before or after the base which corresponds to the target's mutant single nucleotide variant in the DNA hybrid[41-42]. The Ae reporter is added to the DNA probe in a post-synthesis reaction where the Ae's N-Hydroxysuccinimide (NHS) ester group is covalently attached to the probe's amine internucleotide L1 linker. In addition to hybridization-based DNA probe specificity, the resulting Ae-labeled probe has added layer of mutant/wildtype target discrimination in endpoint reactions where background reduction steps destroy excess Ae signals on unbound probes (not bound to amplicon) and mismatched probes (mutant DNA probe bound to wildtype target) due to the increased pH lability in each as comparted to the pH stability of a perfectly matched mutant probe on a mutant target[41-42]. The unique combination of the >50x signal output from the acridinium probe versus fluorescent probes, the low cross-reactivity to wildtype targets based on chemical destruction of Ae in mismatched probes, and the additional layer of specificity from "Wildtype Suppression PCR" (WTS-PCR) reactions enables our single copy sensitivity. While WES NGS is expected to identify up to 30 or more mutations per sample, we will be developing a maximum of 5 probes based off of mutations with the highest allele frequencies (i.e. truncal mutations). These 5 mutations will be used to design probes of 20-25 bases in length and will be selected to avoid poor performing base mismatches in Ae-DNA probes.

Spark MRD PCR: We will use whole tissue sample PCR to enrich for the mutant allele through PCR cycles. The WTS variant we most often use is PNA-Clamping using a PNA with a WT sequence that attaches (clamps) to WT sequences at the site of the tumor mutation, preventing amplification. Or, we will use allele-specific PCR in some cases, wherein one primer is designed with a 3' end overlap at the tumor's variant base position where a mismatch to WT base prevents the polymerase from amplifying the WT target. Through the use of these two techniques, we will be able to effectively reduce WT noise background signal, allowing for enhanced sensitivity in our Spark MRD PCR analysis of the 5 patient-specific truncal mutations that we will screen for. Endpoint mutation detection will be conducted using a PTC-200 and dual injection luminometer to quantify the data output of relative light units following the addition of two light solutions (nitric acid and sodium hydroxide).

ddPCR: Cell-free DNA collected from each of the stage II and III CRC patient plasma samples will undergo ddPCR to measure the level of WT DNA present in each sample. ddPCR services will be performed at the University of California San Diego CFAR core lab.

Expected outcomes, limitations, and alternative approaches: At completion of this Aim, equivalence or supremacy of Spark MRD will be demonstrated compared to Signatera in terms of sensitivity, specificity, and accuracy of ctDNA detection of MRD. The heterogenous patient population by stage and treatment may limit the generalization of our data. Sharp hospital has access to diversity of patient populations and we believe this will be sufficient for demonstrating feasibility. We will ensure a more rigorous study will be completed in phase II.

Summary and Future Directions: At completion of this Phase I proposal, we will have demonstrated Spark MRD's ability to meet and/or Signatera's NGS-based approach of MRD detection. Success of these goals would provide a direct comparison of these two methods, which will inform how Spark MRD performs in MRD detection compared to other methods that are equivalent or less sensitive than Signatera. In Phase II, we perform a larger clinical study and will seek FDA clearance for the Spark MRD test. Successful commercialization of Spark MRD has significant potential to improve cancer patient outcomes following tumor removal by decreasing false negative rates, increasing lead times, and informing more appropriate treatment strategies.

Activity	Q1	Q2	Q3	Q4
Patient plasma sample collection and cell free DNA isolation.				
WES to inform Signatera panel and Spark MRD Probe Design				
Compare MRD detection sensitivites of Signatera and Spark MRD				

Table 6: Timeline.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 01/31/2026

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *	● Yes ❍ No
Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.	Not_Human_Subjects_2024.08.19.pdf
Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Other Requested Information	

HUMAN SUBJECTS

This work **does not** qualify for human subjects research due to the following criteria:

- The specimens were not collected specifically for this study
- No one on our study has access to the subject identifiers linked to the specimens or data

De-identified blood plasma specimens from stage II and III colorectal cancer patients will be provided by Sharp Memorial Hospital to be used for the validation of Spark MRD's sensitivity and specificity.

The samples are identified by reference number and we are unaware of who has access to the subjects' identities. As such, we are also not privy to how the privacy and/or confidentiality has been protected other than to say that it has been protected from us.

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

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RESOURCE SHARING PLAN

The proposed scope of work will not result in the production of (1) unique model organism research resources and/or (2) unique research tools as defined by the NIH in their guidance documents entitled “Model Organisms Sharing Policy” and “Research Tools Policy” available on the NIH website.

Consistent with Bayh-Dole Regulations Spark Molecular Diagnostics is committed to the timely development of a commercial product with support from the NIH SBIR/STTR funding mechanism. Commercialization of the Spark MRD method of detecting circulating tumor DNA (ctDNA) will lead to its broad dissemination and use by the research community and the general public, thereby enhancing the value of NIH-sponsored research.

Spark Molecular Diagnostics will make the results and accomplishments of this research available to the research community and to the public at large by the timely release and sharing of data. As a means of sharing knowledge, the investigators supported by this grant will seek to publish the original research in primary scientific journals. For each publication that results from the grant-supported research, we will include an acknowledgment of NIH grant support and follow guidelines regarding free access to published materials. Information on each publication resulting from work performed under the NIH grant-supported project will be included in the annual and/or final progress report submitted to the NIH awarding office. Dr. William Hannah will work with other investigators to respond to requests for data for reanalysis or assistance replicating the research, and all reasonable requests will be accommodated given a sound scientific rationale and purpose, appropriate data and privacy protections, feasibility of complying with the request, and compliance with the policies of all participating institutions and organizations. Spark Molecular Diagnostics is also open to collaboration with outside groups who express interest in this approach.

INTELLECTUAL PROPERTY RIGHTS

The investigators will assert copyright in scientific and technical articles based on data produced under the grant where necessary, but we will also make every effort to keep technologies developed as a result of this research project widely available and accessible to the research community. If additional patents are filed and the technology licensed, we will only seek exclusivity in cases where this approach is determined to be the best route for successful development of the technology for public use and benefit.

NIH Generated message:

The Other Plan(s) attachment included with the application is not evaluated during the peer review process but will be evaluated prior to a funding decision. Although part of the official submission, the attachment is maintained as a separate document in eRA Commons viewable by authorized users and is not part of this assembled application.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Overview:

• We apply the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation, and reporting of results. Dr. William Hannah will advise on all statistical analyses described in the proposal.

• Data are analyzed identically across experiments.

1. Specialty Chemicals

- Reporter: Acridinium NHS Ester.
    - Cayman Chemicals, Ann Arbor, MI, USA. p/n 200200.
    - Purities > 95%. Batch specific CoA available.
    - cGMP capable
    - Analytical techniques: Elemental analysis, IR and melting point, LC-MS
    - Functional Tests: Acridinium-labelled mutation-specific DNA Probes are subjected to a simple sensitivity and specificity tests using short (40 base) single-stranded DNA targets that replicate the endpoint Spark PCR reaction conditions. Wildtype single stranded DNA oligonucleotide targets are included as negative controls. Positive controls are mutation-specific oligonucleotide targets. Negative control readings should be consistently low (> 100x lower than positive controls).
- DNA linker.
    - DNA Linker name: “L1” , in patent reference below.
    - Tenova Pharmaceuticals, San Diego, CA, USA
    - Purities > 95%. Batch specific CoA available.
    - Analytical techniques: Elemental analysis, IR and melting point, LC-MS
    - DNA Linker structure from 1996 patent Linking reagents for nucleotide probes” US Patent 5,585,481.
- DNA oligos. PCR Primers, single strand DNA targets, DNA probes
    - IDT, Coralville, IA, USA
    - GMP capable.
    - Analytical techniques: ESI mass spectrometry, and capillary electrophoresis
- Peptide nucleic acids.
    - Genscript Biotech, Piscataway, NJ, CA, USA
    - cGMP capable
    - Analytical techniques: RP-HPLC, and MALDI-TOF mass spectrometry
- Buffers: Custom endpoint PCR buffers
    - Teknova, Hollister, CA, USA
    - GMP capable.
    - Analytical techniques: pH, conductivity, osmolality, density, DNAase, RNase, protease, sterility

2. Nucleic acids (e.g. siRNA, shRNA, gRNA, etc.)

- Engineered cell line gDNA
    - EGFR L858R Reference Standard. Horizon Discovery, Waterbeach, UK. p/n HD254
    - EGFR E746-A750 Reference Standard. Horizon Discovery, Waterbeach, UK. p/n HD251
    - KRAS G12A Reference Standard. Horizon Discovery, Waterbeach, UK. p/n HD265
    - KRAS G12R Reference Standard. Horizon Discovery, Waterbeach, UK. p/n HD287
    - KRAS G12V Reference Standard. Horizon Discovery, Waterbeach, UK. p/n HD289
    - Seraseq ctDNA MRD Panel Mix. Seracare, Gaithersburg, MD, USA, p/n 0710-2146
    - QC: Digital PCR or NGS for mutant VAFs
- Negative controls
    - Pooled Human Genomic DNA. Promega, Madison WI, USA. p/n G1521
    - Pooled Human Plasma (blood derived). Innovative Research, Novi, MI, USA.
- Validation tests
    - Spark Molecular’s QC:
        i. Digital PCR using Bio-Rad QX200 Digital Droplet PCR instrument at UCSD Core Lab