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TITLE: Clinical validation of the role of microRNA binding site mutations in cancer risk, prevention and treatment

HIC Protocol Number: **Pro00009633**

Funding Source: **MiraKind**

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Study Center: MiraKind/MiraDx

Funding Source: MiraKind/MiraDx

Principal Investigator: Joanne Weidhaas, M.D., PhD

Study Phase: N/A

Research Hypothesis:
We have identified germ-line microRNA binding site mutations that predict an increased risk of cancer, unique tumor biology and response and toxicity to treatment. The goal of this protocol is to further determine the mechanisms of these mutations, such as the *KRAS*-variant, and their associations with human health, such as cancer. We will collect saliva, cheek swabs or blood samples from individual patients who are eligible to enroll in our studies, to test for the *KRAS*-variant and/or other mutations under study. With specific permission, we will keep excess DNA to further investigate and discover and validate additional similar mutations. We may also enroll participants in a registry, to have participants, or their doctors, answer questionnaires about lifestyle factors and treatment outcome, in an ongoing manner, to understand the impact of these mutations on cancer risk and response and toxicity to treatment.

Primary Objectives:

1) To collect individual patient samples for testing for miRNA mutations such as the *KRAS*-variant, to study the association of these mutations with human disease and response to treatment, starting with cancer. The goal is to understand modifiable factors that impact first and second cancer risk in individuals, as well as the best approach to therapy to cure these patients while minimizing toxicity.

Secondary Objectives:

1) To discover and validate other types of genetic mutations that are associated with cancer risk and biology.

Study Design:

1) Recruitment of patients.
Participants for MiraKind/MiraDx studies will be recruited through MiraKind's education efforts at conferences and events, through online marketing, through partnerships with similarly focused organizations, and through clinics that share MiraKind/MiraDx information with the appropriate patients.

Potential applicants will have an opportunity to ask questions about the trial onsite at events, through recruiting clinics, online through a contact form, or to their personal physician before enrolling. Specific study descriptions and online applications will be available at www.MiraKind.org and in clinics.

In some cases patients will be asked to join a prospective registry.

2) Screening and selection of patients.
All interested applicants will answer questions about their health history to be evaluated by a MiraKind/MiraDx physician or by their personal physician in order to determine their individual qualifications for study participation. Qualifying applicants will be either consented by their physician, or directed to a secure, encrypted and cloud-based portal where they will consent to study participation.

Patients will be given a choice as to whether or not they wish their application data to be stored for later applications. If the applicant does not consent to having MiraKind/MiraDx securely store his or her data, it will be permanently deleted.

3) Collection of germ-line DNA samples.
Once consented to study participation, individuals will either have a cheek swab DNA sample collected at their physician's office or have a sample kit delivered to them for collection. Instructions for collection are included with the collection kit. For individuals enrolling outside of a physician's office, return mailing envelopes directed to MiraKind/MiraDx's lab will be included in the test kit. After DNA is extracted it will be stored in a secure freezer at MiraKind/MiraDx.

4) Patient sample security.
For all studies, basic demographic and health history data will be collected for each participant. All data will be collected through secure, encrypted webforms online.

The MiraKind/MiraDx team will record data into a password-protected database, where identifying information will be separated from health data, with each patient being assigned a study number to ensure patient anonymity. Computers/laptops containing personal health information will be encrypted and password protected. Only patient data without any identifying PHI will be shared with the statistician.

Test Product, Dose and Mode of Administration, Duration of treatment: N/A

Statistical Method:
Dr. Telesca may evaluate the data for a significant enriched prevalence of a germ-line mutation of interest found in samples from this protocol with the expected frequency in the general population or the population being studied. Dr. Telesca may also correlate germ-line mutations of interest with disease parameters such as toxicity, local failure, disease free survival, or overall survival. Dr. Telesca will be using standard statistical analysis to perform these studies.

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1. OBJECTIVES

1.1 Primary Objectives:

1) To collect individual patient samples for testing for the *KRAS*-variant and mutations like it to study the association of these mutations with human disease, starting with cancer risk and response and toxicity to therapy.

1.2 Secondary Objectives:

1) To discover and validate other microRNA binding site mutations that are associated with cancer risk and biology.

2. BACKGROUND

Please see published articles [1-4] as well as others for background supporting the scientific and clinical basis of this investigation and the evidence of the importance of the *KRAS*-variant and other germ-line mutations in predicting cancer risk and biology.

3. PATIENT SELECTION

3.1 Eligibility Criteria

Participants must be all of the following:

1. Any individual over the age of 18 who fits specific study criteria.
2. Participant must be able to supply a cheek swab or blood sample.
3. Participant must consent to study inclusion and authorize use of their health information.

3.2 Exclusion Criteria

Patients will be excluded if they are ineligible for open studies.

2. Inclusion of Women and Minorities

Women and minorities will be included in these studies.

4. TREATMENT PLAN AND METHODS

Potential participants will fill out an eligibility form or have a physician review their eligibility with them and will be confirmed to be eligibility for a study. Once a participant has been identified as eligible for a study, they will be shown a protocol and consent, or given one by their physician, to review and sign, with contact information for MiraKind/MiraDx staff to answer any additional questions. Once they have consented, they or their physician will be asked to submit a sample.

Nucleic acid will be isolated from samples using the QIAGEN or comparable kit and following supplied directions.

Participants will not obtain individual results through this protocol.

4.1 Duration of Follow Up

The patients enrolled on registry studies will be followed by quarterly to bi-annual questionnaires, delivered via email containing a link to the secure study form, or through their physician's office, for the length of time detailed in each specific study, possibly for life. If a participant has died, then the date and cause of death will be noted if available.

5. DOSING DELAYS/DOSE MODIFICATIONS

No pharmaceutical agent is being used.

6. RISKS & BENEFITS

This study does not involve a therapeutic intervention. There is no risk of doing a cheek swab, and minimal risk of taking a blood sample.

There is a risk associated with genetic information. Such risks could include reduced access to or retention of benefits or entitlements (e.g., insurance, educational opportunities, employment, etc.) although discrimination is illegal; stigmatization; psychological distress of the patient or patient's family members in response to information; or detection of biological relationships within a family.

In addition, there is always a risk of a breach of data security. To help minimize this risk, information will be kept completely confidential and secure. Total confidentiality of patient's results will be strictly maintained, with no information being shared except to a specified personal physician of the patient, through secure means. Internally at MiraKind/MiraDx, patients will be assigned a study ID, and there will be no personal health information linked to the patient's study ID during study analysis and follow up, to further ensure confidentiality. Furthermore, MiraKind/MiraDx will take precautions to ensure data is stored in a secure, encrypted environment. Any breaches in data security will be promptly documented and reported to the patient. In the event of a data breach, swift action will be taken to mitigate risk of such breaches in the future.

Benefits of participation include advancing knowledge and understanding about genetic markers associated with cancer risk and response and toxicity to cancer treatment. Knowledge and insights emanating from this study will potentially advance research efforts surrounding cancer and ultimately may lead to better prevention, diagnosis, and treatment of this condition.

7. PHARMACEUTICAL INFORMATION

No pharmaceutical agents will be tested in this study.

8. CORRELATIVE/SPECIAL STUDIES

No other correlative studies will be performed.

9. MEASUREMENT OF EFFECT

The outcome of this study is to measure the association of the genetic biomarkers with human disease.

10. ADVERSE EVENT ANALYSIS, DEFINITION AND REPORTING

10.1 Safety Analysis

There will be no physical safety issues from this study. Patients may experience psychological distress from receiving genetic information, if they choose to receive it.

10.2 Definition of Adverse Event Terms

Adverse Event – Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). [NIH Guidelines, January 2001] There should be no adverse events from this study.

11.1 PRINCIPAL INVESTIGATOR SAE REPORTING REQUIREMENTS

11.2 Expedited Reporting of Data Breech

The PI will promptly investigate all safety information related to an adverse experience. If the results of the PI's investigation show an adverse event experience not initially determined to be reportable (based on whether the event is serious, unexpected) is so reportable, the PI will report such experience. Follow-up information to a safety report shall be submitted as soon as the relevant information is available.

12. STATISTICAL CONSIDERATIONS

The prevalence of germ-line variants of interest will be compared to the baseline prevalence found using available large human genomic DNA collections, as well as other patients. The primary statistical analysis will involve comparisons of genotypes between with (cases) and without (controls) the germline mutation. This analysis will include Pearson's $\chi 2$ square analysis or Fisher's exact test and computation of Odds Ratios (ORs) to assess the relationship of the genetic polymorphism and cancer risk, biology or association with the factors being studied. The overall genotype frequencies among the cases and expected control levels will first be compared with the frequencies expected from Hardy-Weinberg equilibrium by goodness-of-fit $\chi 2$. Odds ratios (ORs) and 95% confidence intervals (CI) will be used to estimate risk associated with the variant genotypes by using both univariate and unconditional multivariate logistic regression models.

APPENDIX A

1 LJ Chin, E Ratner, S Leng, R Zhai, S Nullur, I Babar, RU Muller, E Straka, L Su, E Burki, RE Crowell, R Patel, T Kulkarni, R Homer, D Zelterman, KK Kidd, Y Zhu, DC Christiani, SA Belinsky, F Slack, and JB Weidhaas, 'A Snp in a Let-7 Microrna Complementary Site in the Kras 3' Untranslated Region Increases Non-Small Cell Lung Cancer Risk', *Cancer Res,* 68 (2008), 8535-40.

2 T Paranjape, H Heneghan, R Lindner, FK Keane, A Hoffman, A Hollestelle, J Dorairaj, K Geyda, C Pelletier, S Nallur, JWM Martens, MJ Hooning, MJ Kerin, D Zelterman, Y Zhu, D Tuck, L. Harris, N Miller, F Slack, and J Weidhaas, 'A 3'-Untranslated Region Kras Variant and Triple-Negative Breast Cancer: A Case-Control and Genetic Analysis', *Lancet Oncology,* 12 (2011), 377-86.

3 R Pilarski, 'A Kras-Variant Is Associated with Risk of Developing Double Primary Breast and Ovarian Cancer.', *PLos ONE,* 7 (2012), e37891.

4 E Ratner, L Lu, M Boeke, R Barnett, S Nallur, L Chin, C Pelletier, R Blitzblau, R Tassi, T Paranjape, P Hui, A Godwin, H Yu, H Risch, T Rutherford, P Schwartz, A Santin, E Matloff, D Zelterman, F Slack, and J Weidhaas, 'A Kras-Variant in Ovarian Cancer Acts as a Genetic Marker of Cancer Risk', *Cancer Research,* 15 (2010), 6509-15.