Early Detection Research Network

Part 1: Request For Specimen Reference Sets

Date of Submission:

Investigator:

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Specimen Reference Set(s) Requested

Collaborative Group Oversight	Organ Site	Specimen Type
☐ Breast & Gynecological ☒ Colorectal & Other GI ☐ Lung & Upper Aerodigestive ☐ Prostate & Other Urologic	Pancreas (e.g. lung, ovary, colon, etc)	☐ Serum ☒ Plasma ☐ Other: Specify _____

Minimum volume of each sample required:	Expected Length of Study:
100 (microliters)	12 months

Institutional Approval

Do you have IRB approval to work with the requested samples?	☐ Yes: Institution: Approval Number: ☐ No ☒ Pending: Expected Date: Sep 30, 2024

Funding

How will testing of the reference set(s) be funded?	☐ Current NIH-funded grant: Grant No. Annual Direct Costs: Funding Period: ☐ Other Sponsorship: Please provide a letter of commitment from the sponsoring agency, company, or foundation. ☒ Other: Specify: Budget of Toray industries Inc.

Part II: Scientific Proposal

I. Clinical Relationship

Early diagnosis is considered an effective strategy to improve survival of patients with pancreatic cancer, but the lack of noninvasive biomarkers to predict the risk of precursor lesions or early invasive disease hinders the detection of pancreatic cancer during this period.

We propose a biomarker study aimed at developing a novel test for pancreatic cystic lesions to detect high risk for highly invasive pancreatic cancer. The biomarker APOA2-i, which will be validated in this study, was identified by a research group at the National Cancer Center in Japan (Principal Investigator: Dr. Kazufumi Honda) and has been shown to be effective in the early detection of pancreatic cancer using various cohorts including the Pancreatic Cancer Reference Set study supported by EDRN. Notably, in 2023, an ELISA kit for APOA2-i (APOA2-iTQ®) received approval for manufacturing and marketing as an in vitro diagnostic product in Japan and is now in practical use. Additionally, an LDT (Laboratory Developed Test) has been launched at a CLIA-certified laboratory in California, USA as of February 2024.

The primary objective of this study is to assess APOA2-i as a biomarker capable of distinguishing between malignant and benign pancreatic cystic tumors. Additionally, it aims to differentiate benign pancreatic cystic tumors from healthy subjects who have been diagnosed using imaging modalities. The ultimate goal is to establish a reliable test for risk stratification in pancreatic neoplasms.

II. Background and Significance

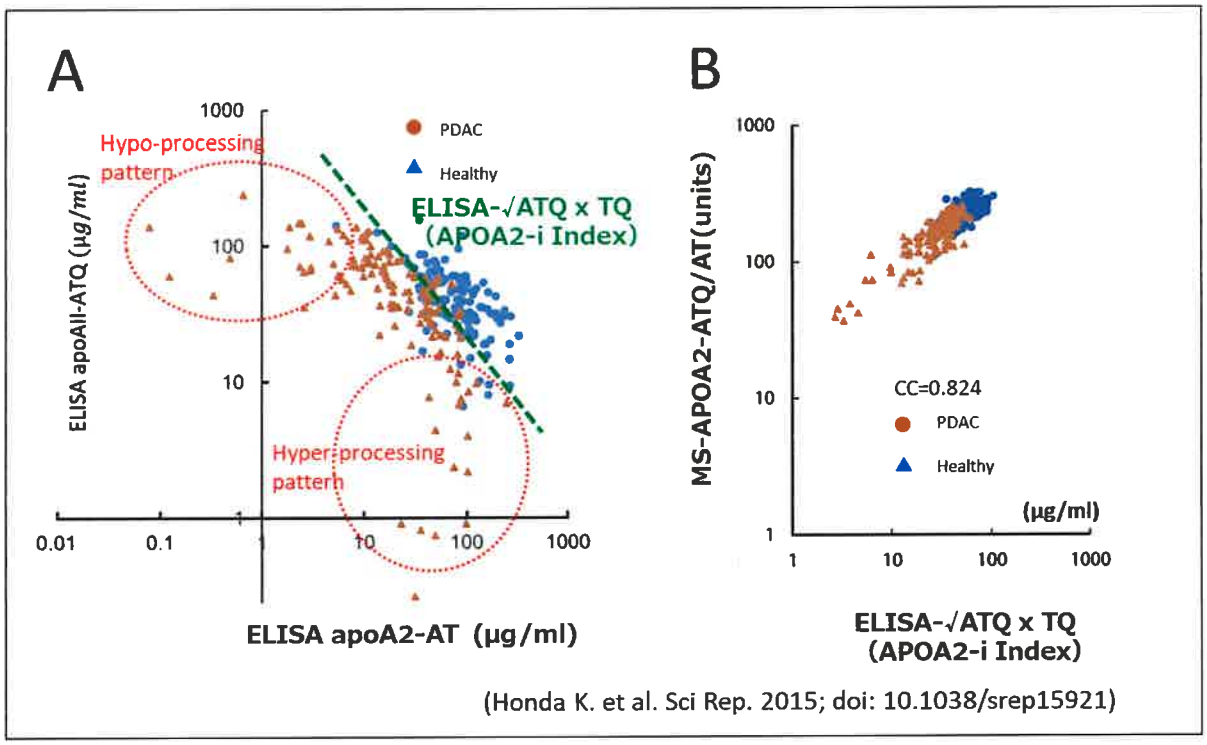
The APOA2 (Apolipoprotein A-II) exists in the blood as a dimer, held together by disulfide bonds formed by cysteine residues, and APOA2-i is its isoform protein. In 1992, Honda et al. detected five dimeric isoforms resulting from the association of three distinct monomeric units of APOA2 through top-down proteomic analysis of human plasma:

1. APOA2-ATQ: The full-length APOA2 protein.
2. APOA2-AT: A variant lacking the C-terminal glutamine.
3. APOA2-A: A variant lacking both the C-terminal glutamine and threonine.

Notably, one specific dimeric isoform, APOA2-ATQ/AT (a dimer composed of APOA2-ATQ and APOA2-AT), exhibited significant reduction in pancreatic cancer patients compared to healthy controls. The area under the curve (ROC-AUC) for APOA2-ATQ/AT values, measured via MALDI-MS, ranged from 0.877 to 0.958 across four independent cohorts, as reported in Honda et al.'s study published in PLoS One (2012;7(10):e46908)."

To facilitate clinical application, TORAY developed isoform-specific antibodies capable of distinguishing between the C-terminal structures of APOA2-ATQ and APOA2-AT. These antibodies were instrumental in creating an ELISA assay "APOA2-i ELISA RUO" designed to quantify APOA2-ATQ and APOA2-AT levels in blood. When plasma levels of APOA2-ATQ and APOA2-AT were assessed using the RUO assay in pancreatic cancer patients and healthy controls, two specific profiles were observed in pancreatic cancer cases (Fig. 1A). Additionally, the geometric mean of APOA2-ATQ and APOA2-AT (referred to as the 'APOA2-i Index') showed a high correlation (CC=0.824) with APOA2-ATQ/AT levels measured by MALDI-MS (Fig. 1B). This suggests that APOA2-i Index serves as a valuable surrogate marker for APOA2-ATQ/AT.

(Fig.1)



The APOA2-i-based diagnostic method has undergone further validation across various cohorts in Japan and the United States (Honda K. et al., Sci Rep. 2015; doi: 10.1038/srep15921). The U.S. cohort in this study was the EDRN Pancreatic Cancer Reference Set (serum), and this was the first successful collaboration with EDRN in a validation study of this marker (Reference Set Study:392 - Pancreatic Ref Set App: Honda (2014)).

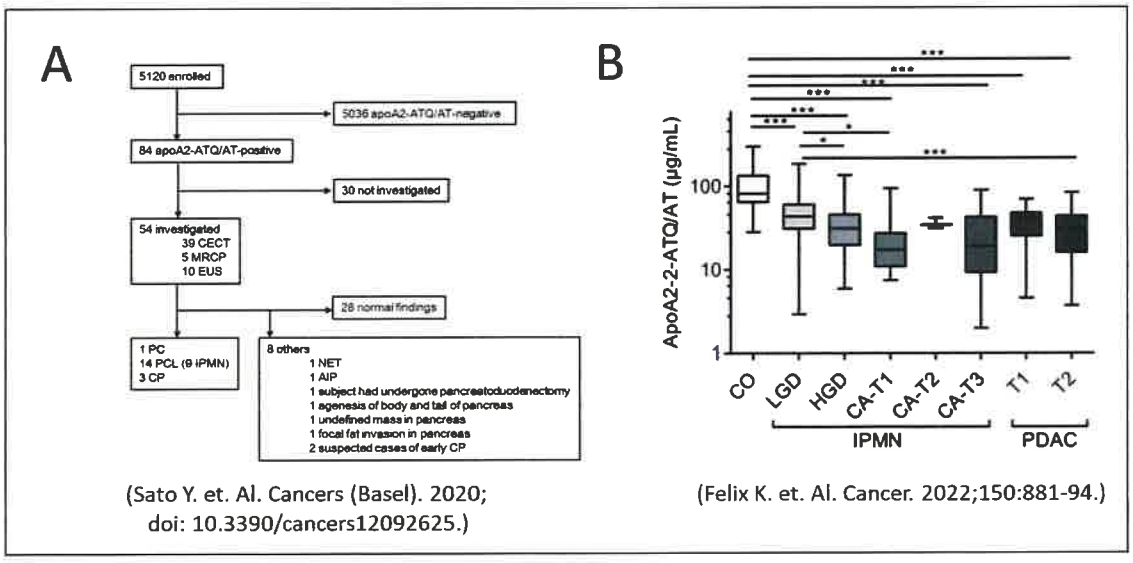
APOA2-i has demonstrated effectiveness in detecting various pancreatic cystic tumors. In the aforementioned Japanese cohort study, it exhibited high discriminatory ability (ROC-AUC):

- 0.92 for intraductal papillary mucinous neoplasms (IPMN)
- 0.816 for mucinous cystic neoplasms (MCN)
- 0.992 for chronic pancreatitis

This highlights the utility of the APOA2 isoform not only for pancreatic cancer but also for its high-risk diseases.

In a prospective study conducted by Sato et al. from 2014 to 2017, researchers aimed to screen for pancreatic cancer and related risk diseases. They measured APOA2-i in a total of 5,120 subjects from the general population in Japan. Among these subjects, 84 individuals (1.8%) were identified as positive for APOA2-i. Out of these, 54 underwent further examination using high-sensitivity imaging modalities. The results revealed the detection of 26 pancreatic diseases, including 9 cases of IPMN (intraductal papillary mucinous neoplasm), 3 cases of chronic pancreatitis, and 1 case of pancreatic cancer (Fig. 2A). This study confirmed the effectiveness of APOA2-i in detecting pancreatic cancer risk diseases. (Sato Y. et. al., Cancers (Basel). 2020; doi: 10.3390/cancers12092625)

(Fig2)



(Sato Y. et. Al. Cancers (Basel). 2020; doi: 10.3390/cancers12092625.)

(Felix K. et. Al. Cancer. 2022;150:881-94.)

Additionally, Filix et al. evaluated the risk classification and screening performance of APOA2-i using 305 IPMNs. They found that the APOA2-i Index was significantly lower in IPMNs across all stages, from low-grade dysplasia to invasive cancer, compared to healthy controls (Fig. 2B).

Specifically, (with a specificity of 96.7%) in low-grade dysplasia, the sensitivity of APOA2-i was 54.5%, whereas CA19-9 had a sensitivity of 11.4%. In high-grade dysplasia, APOA2-i showed a sensitivity of 70.6% (vs. CA19-9 at 14.5%). For IPMN-derived invasive cancer (pT1), APOA2-i demonstrated a sensitivity of 83.3% (vs. CA19-9 at 33.3%). Furthermore, the APOA2-i Index exhibited a significant decrease with disease stage progression. (Felix K. et. al., Cancer. 2022;150:881-94.)

These results indicate that the APOA2-i test holds promise for screening IPMN and assessing the malignancy grade in pancreatic diseases.

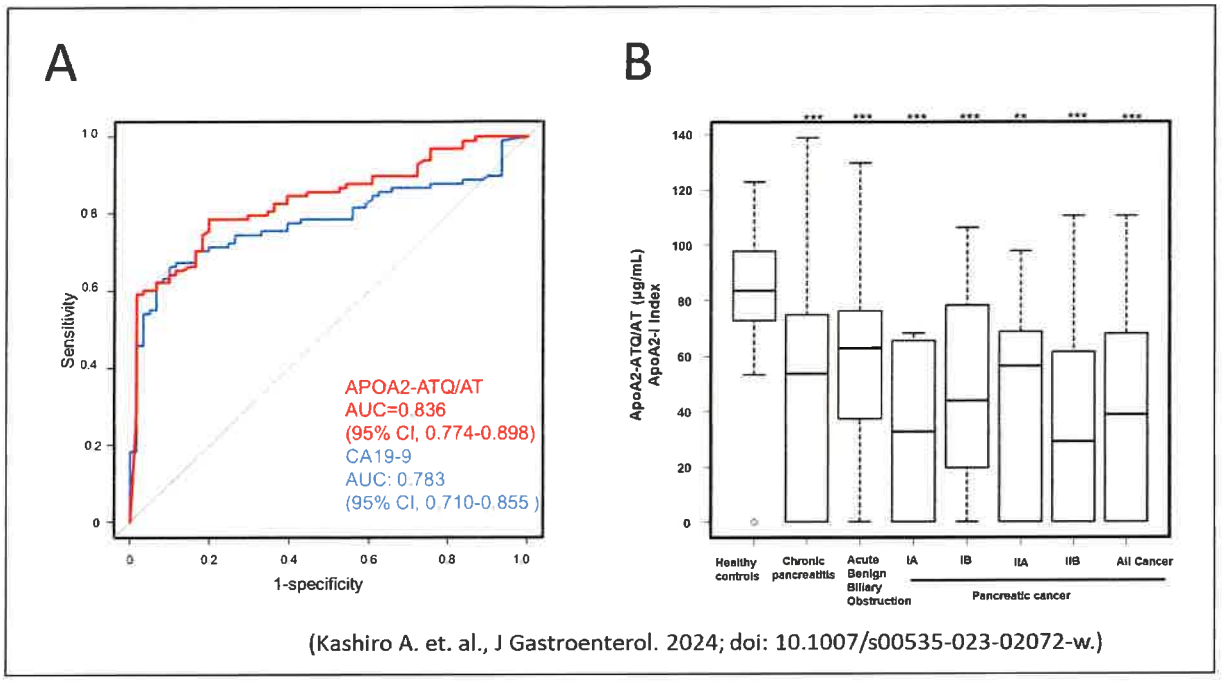
III. Preliminary Data and Methods

Recently, we have developed a new APOA2 isoform ELISA assay "APOA2-iTQ" to obtain the approval in Japan in accordance with the requirements of the Japanese medical device quality control system and conducted a clinical performance study using 2,732 plasma samples. In this study, we demonstrated that the point estimate of the area under the curve distinguishing pancreatic cancer (n=106) from healthy controls (n=106) was higher using the APOA2-i Index (0.879, 95% CI: 0.832-0.925) compared to CA19-9 (0.849, 95% CI: 0.793-0.905). These results align with previous research findings. Moreover, the sensitivity of APOA2-i Index in detecting stage I (47.4%) and stage I/II (50%) pancreatic cancer was higher than that of CA19-9 (36.8% and 46.7%, respectively). Based on these results, Toray Industries, Inc. received approval from the Japanese Ministry of Health, Labour and Welfare in June 2023 to manufacture and market in Japan as an in vitro diagnostic product to aid in the diagnosis of pancreatic cancer.

Furthermore, in collaboration with the NCI/EDRN, the performance of the APOA2-iTQ was independently verified through blind testing using the EDRN's Pancreatic Cancer Reference Set (Reference Set Study:513 - Pancreatic Ref Set App: Jung-Toray (2023)). The AUC of APOA2-i Index, which distinguishes stage I and stage II pancreatic cancer (98 cases) from healthy controls (61 cases), was 0.836 (95% CI 0.774-0.898), which is higher than that of CA19-9 (0.783, 95% CI 0.710-0.855) (Fig.3A). When the cut-off value was set at the point at which specificity is 95%, the positive rates for APOA2-i Index and CA19-9 are 57.1% and 28.6% for stage-IA, 55.0% and 55.0% for stage-IB, 37.5% and 50.0% for stage-IIA, 69.0% and 57.1% for stage-IIb, and 60.2% and 54.1% for all stages (Fig.3B). These results were published in the January 2024 edition of the Journal of Gastraenterology. (Kashiro A. et. al., J Gastroenterol. 2024; doi: 10.1007/s00535-023-02072-w)

Based on these successful results, Toray International America Inc., a subsidiary of Toray Industries Inc. in the United States, initiated a Laboratory Developed Test (LDT) at a CLIA-certified laboratory (Toray Molecular Oncology Lab./TMOL) in California using APOA2-iTQ in February 2024.

(Fig.3)



(Kashiro A. et. al., J Gastroenterol. 2024; doi: 10.1007/s00535-023-02072-w.)

IV. Data Analysis Plan

This study will evaluate the performance of APOA2-i as a biomarker for selecting patients with pancreatic cysts that require surgical removal and careful surveillance. EDRN sends pancreatic cyst reference set (plasma) to TMOL. TMOL measures APOA2-AT and APOA2-TQ concentrations using APOA2-iTQ® and calculates APOA2-i Index for each specimen. TMOL sends back the measurement data from the reference set to EDRN. EDRN analyzes the following items (1) to (5)

(1) Validate the ability of APOA2-i as a biomarker to distinguish cysts with high malignant potential from those with low malignant potential, diagnosed according to pathologic criteria.

(2) Validate the ability of APOA2-i as a biomarker to distinguish cysts with low malignant potential from those with no-malignant potential, diagnosed according to pathologic criteria.

(3) Validate the ability of APOA2-i as a biomarker to distinguish mucinous cysts from non-mucinous cysts, diagnosed according to pathologic criteria.

(4) Validate the ability of APOA2-i as a biomarker to determine whether cysts contain invasive carcinomas or cystic neuroendocrine carcinomas, diagnosed according to pathologic criteria.

(5) Validate the ability of APOA2-i as a biomarker to distinguish cysts with low malignant potential from those with no-malignant potential, diagnosed by EUS.

V. Future Plans

If the biomarker is found to have promising performance characteristics, the EDRN might be interested in working with you to proceed to phase II clinical validations. Address each specific scenario below according to your intentions.

Q1: Do you plan to approach EDRN for funding and collaboration in proceeding to a phase II validation study? If not, do you have other resources where validation can be accomplished? Describe clearly other resources at your disposal and how they are sufficient to complete a larger phase II validation study if you will not seek help from EDRN.

A1:Yes.

Q2: Are you amenable working within the collaborative framework of EDRN in proceeding to phase II studies?

A2:Yes.

Q3: If deemed beneficial, will you be amenable to including your biomarker into a larger panel of biomarkers for Phase II validation.

A3:Yes.

Q4: If refinements will improve the performance of the biomarker test, will you concur with further development of the test? Will it be advantageous to include resources of EDRN for this purpose?

A4:Yes.

Part III: Conditions for the Release of Reference Sets

	Yes	No
• I agree not to resell or release the reference set or sub-aliquots from this set to an investigator not directly connected with this application.	☒	☐
• I agree to complete the assays on the reference set specimens and return results to the EDRN DMCC within 4 months of their receipt.	☒	☐
• I agree to release assay results for posting on eCAS, a secure domain on the EDRN website, 3 months after I have received the unblinded results back from the DMCC for my review.	☒	☐

- **The EDRN DMCC agrees not to release your data to anyone outside the immediate analytic team until after the 3 month interval has passed.**
- **The EDRN reserves the right to post the data to its public website at 12 months after the unblinded results have been provided to the investigator.**

Signature

April. 24. 2024

Date

Investigators applying for use of an EDRN reference set will be notified within 3 months about approval for use of a set. Investigators who successfully apply must also complete a separate MTA with the National Cancer Institute. Neither NCI nor the EDRN DMCC will claim any rights to your data including the statistical analysis conducted by the EDRN DMCC

Please save a copy of this application for your records.