Biomarkers in Chronic Pancreatitis?

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Lecture Outline

- **The Research Challenge**
- **Current State:**
  - **What we already know**
  - **End Stage CP**
  - ***Established CP***
  - **Definitive Evidence for CP**
- **The "Black Box"**
  - **RAP, Early CP (Indeterminate CP)**
  - **Insufficient Evidence for CP**
  - **No Evidence for CP**
- **PROCEED – Current Research Opportunities:** Cross Sectional, Discovery
- **PROCEED - Future Directions**: Prospective, Validation, Clinical Implementation

Chronic Pancreatitis in the 21st Century - Research Challenges and Opportunities

Summary of a National Institute of Diabetes and Digestive and Kidney Diseases Workshop

Aliye Uc, MD, Dana K. Andersen, MD,† Melena D. Bellin, MD,‡ Jason I. Bruce, PhD,§ Asbjørn M. Drewes, MD, PhD, DMSc,|| John F. Engelhardt, PhD,¶ Christopher E. Forsmark, MD,# Markus M. Lerch, MD,** Mark E. Lowe, MD, PhD,†† Brent A. Neuschwander-Tetri, MD,‡‡ Stephen J. O'Keefe, MD, MSc,§§ Tonya M. Palermo, PhD,|||| Pankaj Pasricha, MD,¶¶ Ashok K. Saluja, PhD,## Vikesh K. Singh, MD, MSc,¶¶ Eva M. Szigethy, MD, PhD,§§ David C. Whitcomb, MD, PhD,§§ Dhiraj Yadav, MD, MPH,§§ and Darwin L. Conwell, MD, MS****

Research Gaps and Opportunities

- Improve and accurate assessment of maldigestion and EPI.
- Establish simpler, less invasive tools to measure acinar and ductal cell function from more easily obtained biological specimens such as urine or blood to screen for pancreatic disease.
- Develop RAP and CP biomarkers that can be used to better de- fine the stage, determine prognosis, assess severity, and stratify patients for medical or surgical intervention using the mechanistic definition framework.
- Provide evidence-based recommendations for proper dietary intake and the requirements for PERT (initiation, dose, timing, follow-up).
- Develop enzyme products requiring fewer pills and with better compliance and potency.

Research Gaps and Opportunities

- Develop long-term primary acinar and ductal epithelial cell culture models.
- Explore co-culture models (eg, acinar-duct, duct-islet, acinar- islet) to identify factors that regulate exocrine cell function and restitution.
- Define mechanisms by which gene mutations/variants cause pancreatic inflammation, ductal cell malfunction, and acinar cell loss.
- Design novel therapies that target restoring pancreatic acinar cells and/or manipulate ductal cells (ie, gene and cell-based therapies, CRISPR/Cas9, *CFTR* correctors and potentiators).
- Develop experiments to determine the critical age and time for intervention to reestablish appropriate stem cell niches for cell-based therapies in diseases that damage the exocrine pancreas.

Uc A., Pancreas 2016.

Pivotal Evaluation of the Accuracy of a Biomarker Used for Classification or Prediction: Standards for Study Design (Pepe et al, 2008, JNCI)

- Prospective-specimen collection, retrospective-blinded-evaluation (**PRoBE**) design
  - ✓ Standards for pivotal studies of biomarker: both diagnosis (Phase 1-2) and prognosis (Phase 3)
  - ✓ Analogous to pivotal study of therapeutics (randomized clinical trial, or RCT)
- Biospecimens are collected prospectively from the target population envisioned for clinical application of the biomarker (eligibility of RCT)
- Specimens and clinical data are stored & collected & assayed in the absence of knowledge about patient outcome (double blinded RCT)
- Random selection of the cases and controls for assay (randomization)

PRoBE Design: Four Key Components

1. Clinical Context

- Define case-control status for all in the target population (avoid **spectrum bias**)

2. Performance Criteria

- Time between specimen collection and outcome occurrence (**prognosis**)
- Subgroups in which biomarker performs better (avoid **multiplicity**)

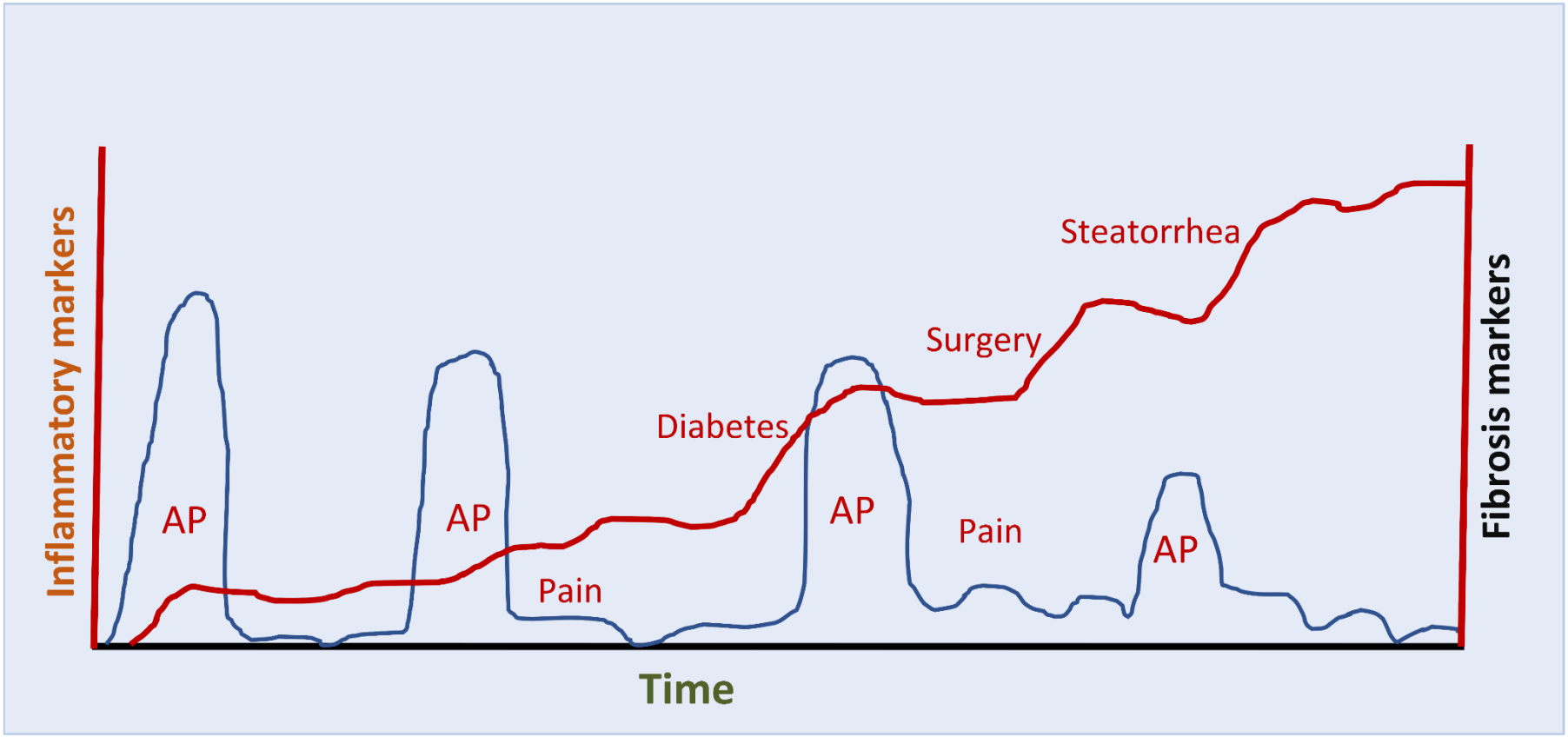
3. Biomarker test

- Specimen handling, assay & reporting blinded to outcome status (avoid **verification bias**)
- Biomarker panel or combination with other patient characteristics must be completed during discovery (avoid **overfitting bias**)

4. Study size

- *Null hypothesis*: minimally acceptable specificity given a large sensitivity; minimally acceptable sensitivity given a large specificity
- Rationale of hypothesis supported by *pilot data* (discovery studies)

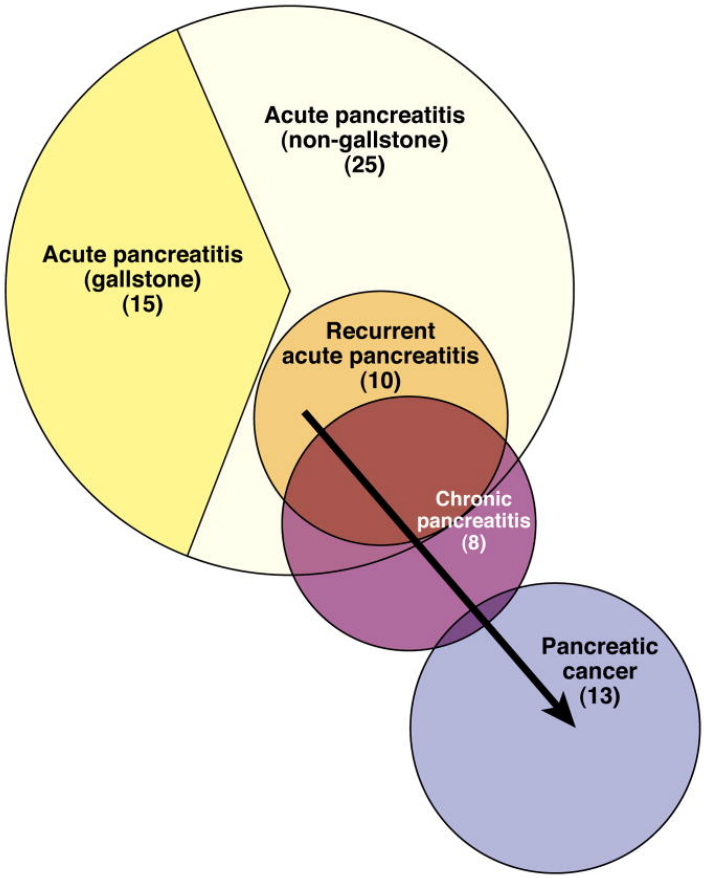
Natural history of RAP - CP results in progressive fibrosis and loss of function



Modified from: Colombel J et al. Gastroenterology 2017;152:351-61

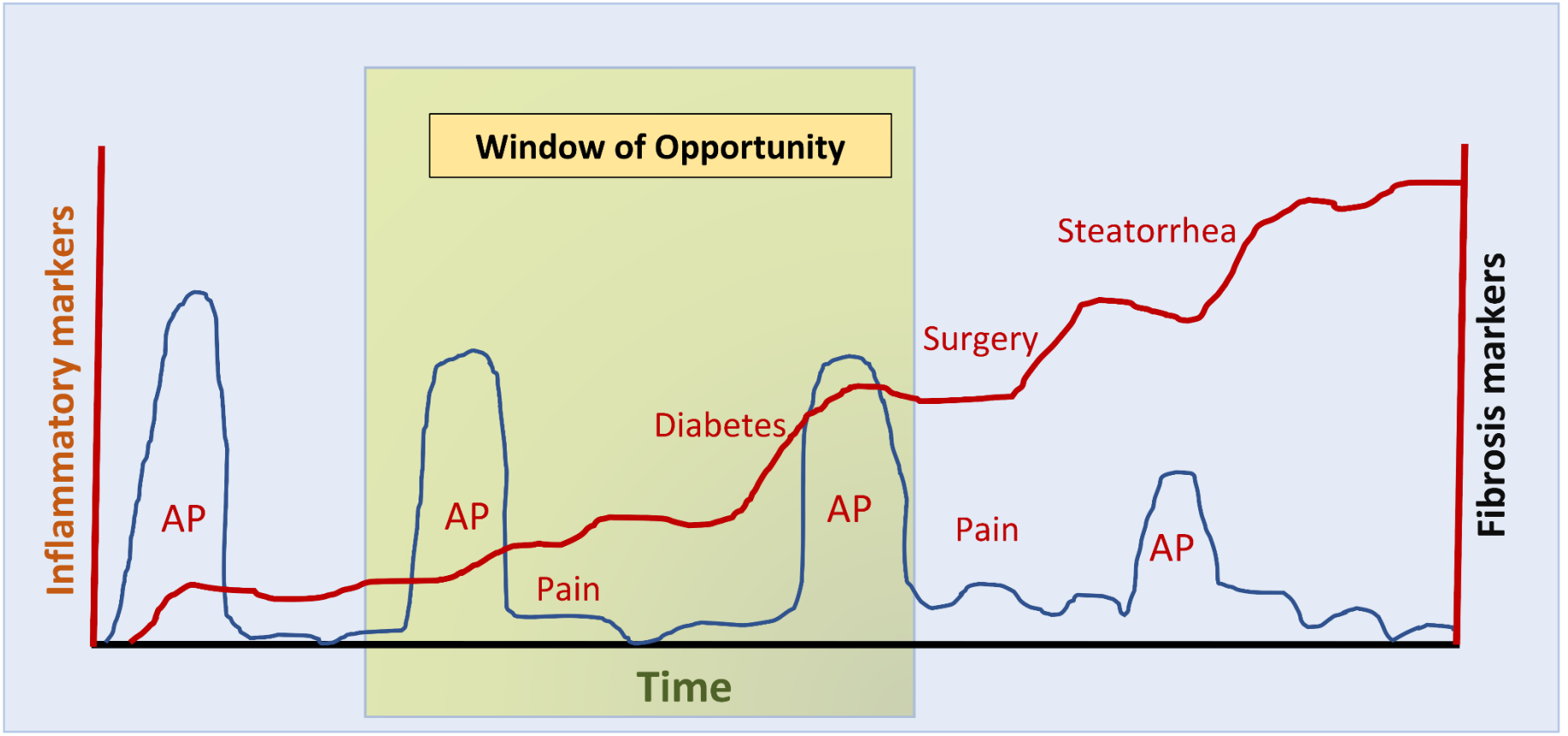
Recurrent Acute Pancreatitis

- ≥2 episodes of AP with resolution of symptomatic and imaging abnormalities between episodes
- Occurs in ~20% of AP patients
- **RAP is the strongest risk factor for progression to CP**
  - HR of 4.57 (95% CI 3.40-6.14)



Yadav. Gastroenterology 2013;144:1252.
Yadav. Am J Gastroenterol 2012; 107:1096.
Lankisch. Am J Gastroenterol 2009;104:2797.

Conceptual framework – CP



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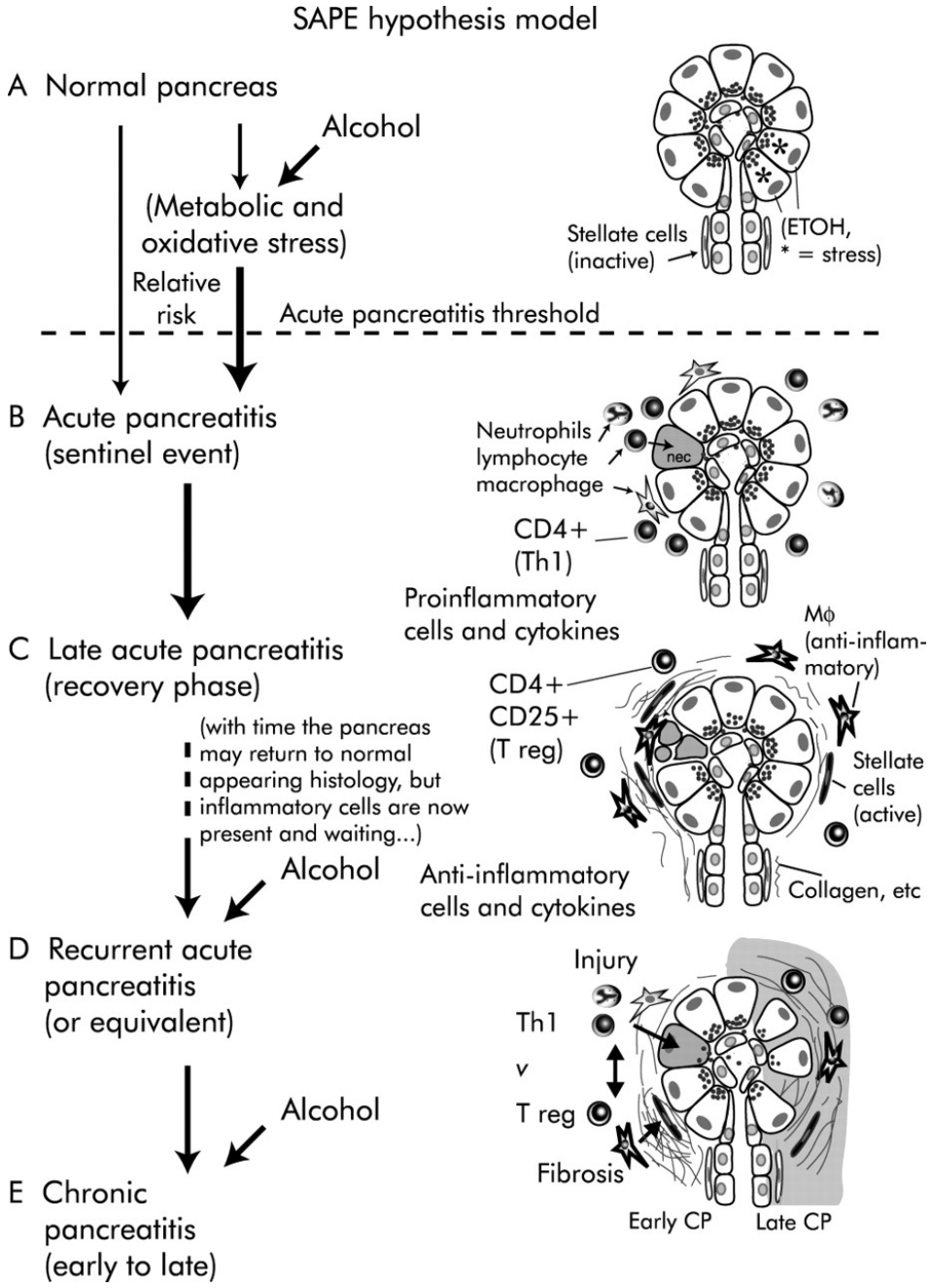
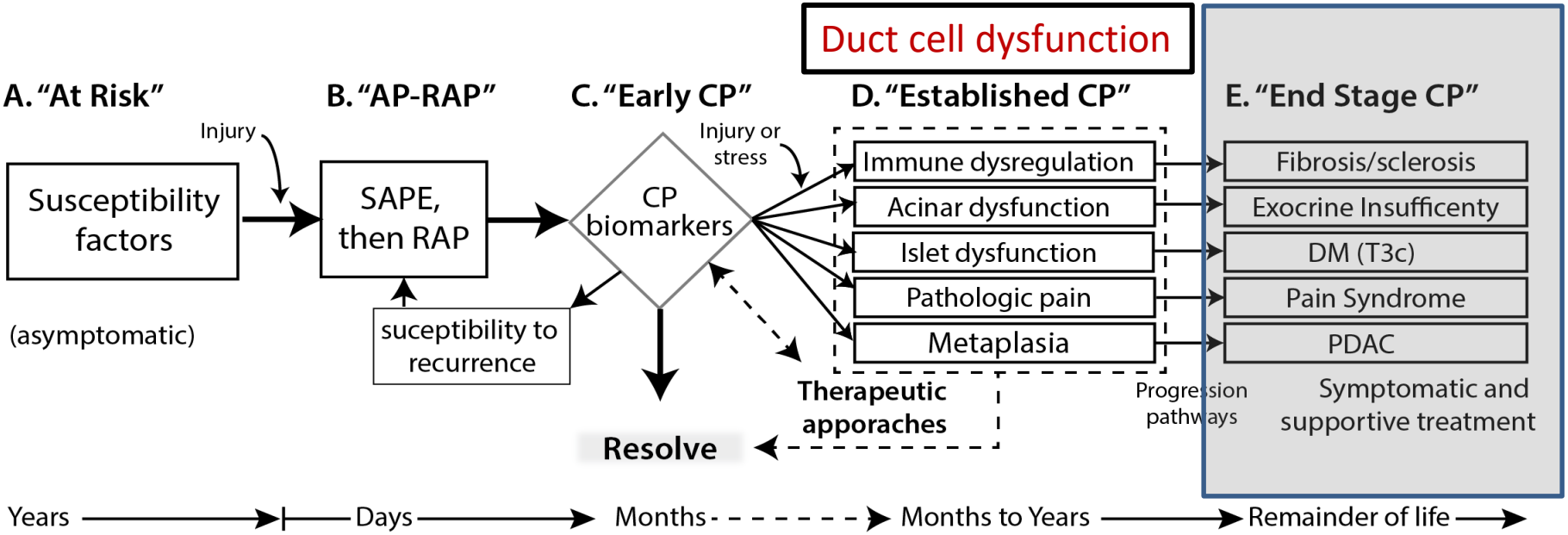


SAPE Hypothesis

- Step A: Acinar cell stimulation
  - **(Smoking / Alcohol - Pandol ; Cedars Group)**
  - Alcohol, gallstone, TG, oxidative stress, smoking
- Step B: Sentinel Event
  - **(See P1-28; Hori et al., Mayo Group)**
  - Early: pro-inflammatory response
  - Late: Stellate cells, pro-fibrotic response
- Step C: Removal of stimulus
  - Abstinence, smoking
  - cholecystectomy
  - lipid lowering agents
- **Step D: Recurrent stimulation**
  - **Stellate cell mediated periacinar fibrosis**

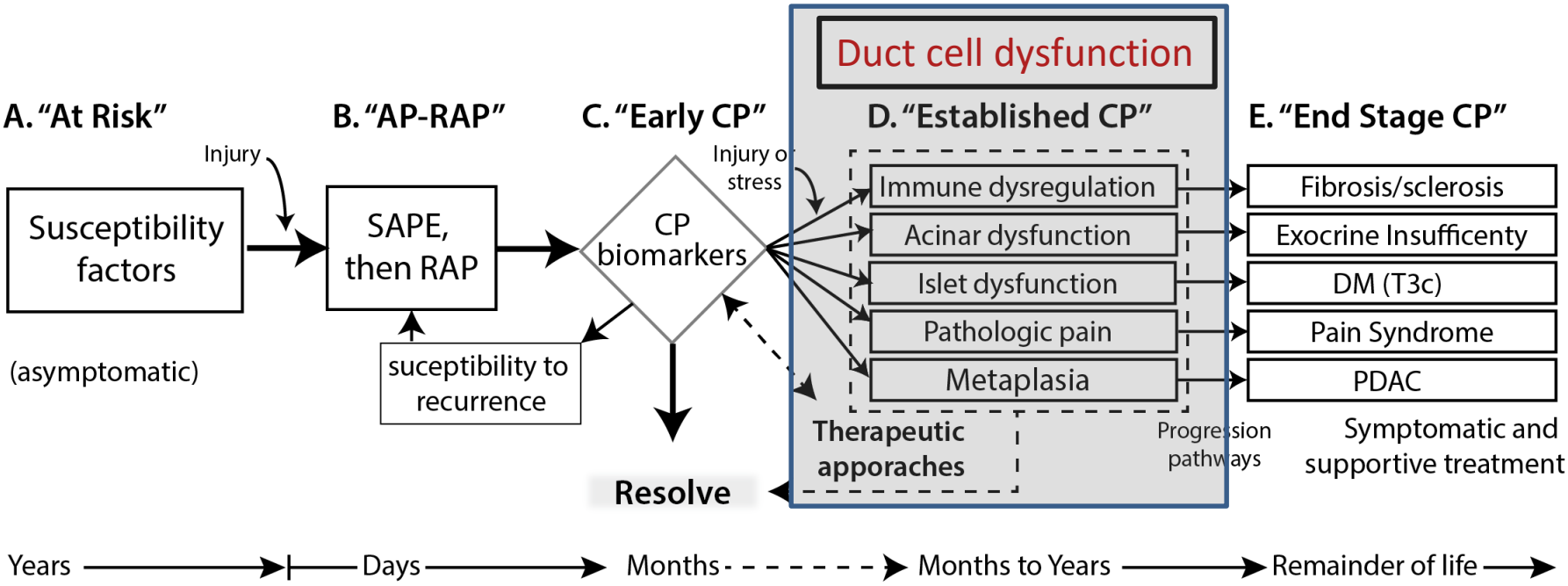
Whitcomb D, Best Prac and Res in Clin Gastro 2002; 16:347

End Stage CP: Mechanistic Definition



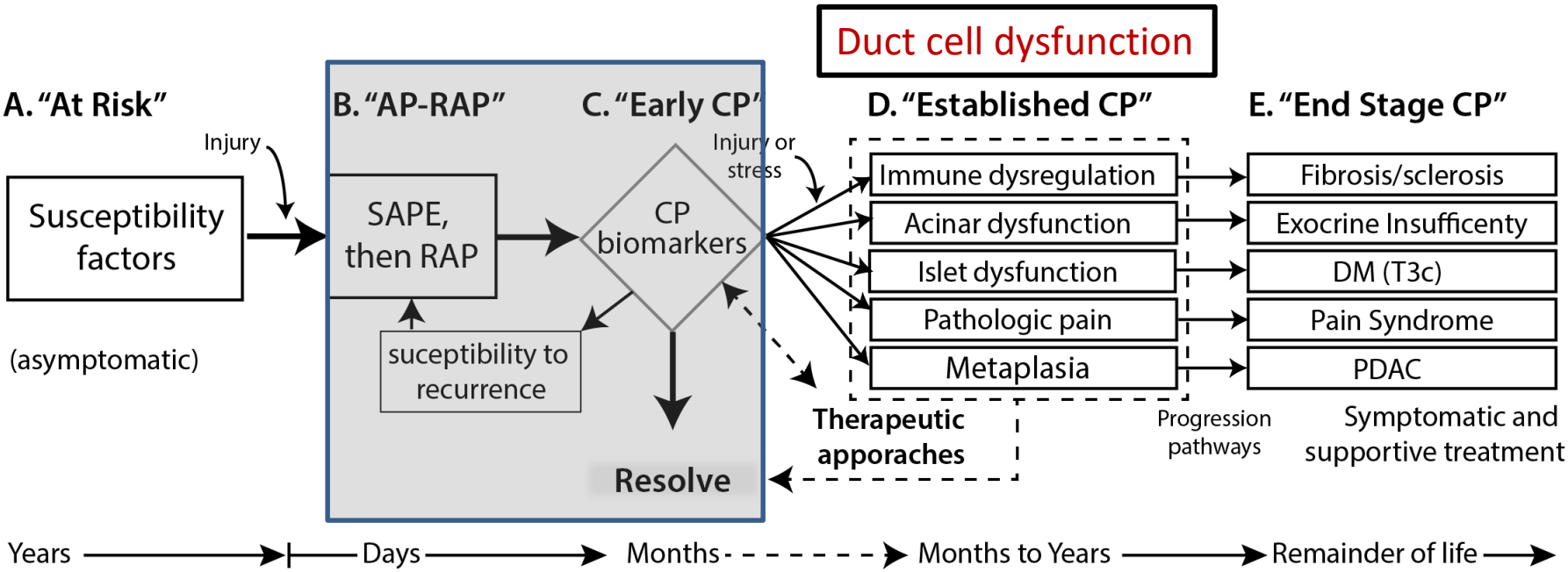
- **End Stage CP:** Highly variable and protean (various combinations of the following):
  - Fibrosis/Sclerosis/Calcifications
  - Exocrine Insufficiency, Endocrine Insufficiency
  - Type 2 DM, Type 3C DM
  - Pain Syndrome
  - PDAC
- **Research Opportunity:**
  - Concensus pathology / imaging / physiology definition based on mechanism of injury
  - **LESS LIKELY TO REVERSE DISEASE with Medical / Surgical Therapy:** **(Intervention Strategies and Therapeutics)**
    - pain modulators, diabetic medications, PERT, chemotherapy, immunomodulation, biologics, antifibrotic therapy, stone dissolution, Total Pancreatectomy +/- Islet Transplantation, Drainage, Resection

Established CP: Mechanistic Definition



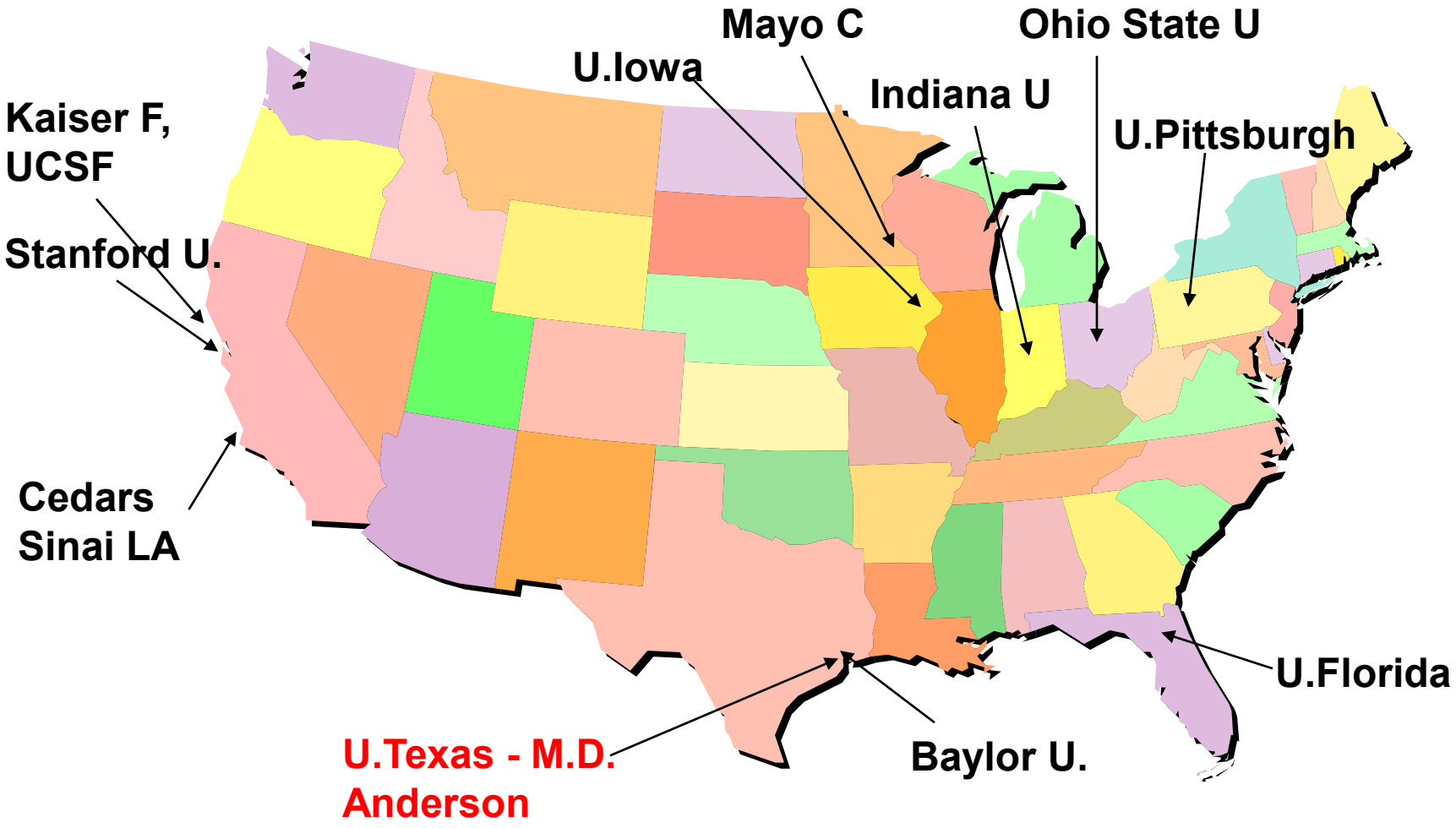
- **Established CP:**
  - Acinar Dysfunction
  - Duct Dysfunction
  - Islet Dysfunction
  - Immune Dysfunction
  - Metaplasia
- **Research Opportunity**
  - Standardization of cellular function measurements; **What defines the stage?**
  - **POTENTIAL OPPORTUNITY TO RETARD DISEASE SYMPTOMS AND PROGRESSION** with Medical / Surgical Therapy: **(Intervention Strategies and Therapeutics and Diagnostics)**
    - pain modulators, diabetic medications, PERT, chemotherapy, immunomodulation, biologics, antifibrotic therapy, stone dissolution, Total Pancreatectomy +/- Islet Transplantation, Drainage, Resection, endoscopy; Advanced Radiologic Imaging methods

The Black Box: Mechanistic Definition



- **Black Box Contents:** Highly variable and protean signs, symptoms and imaging findings, various combinations of the following:
  - *Symptom(s):* no symptoms, abdominal pain, nausea, maldigestion, glucose intolerance, IBS
  - *Pancreas Function:* normal, cellular dysregulation (duct, acinar, islets)
  - *Pancreas Imaging:* normal, EUS / MRI - minimal changes (Standard criteria, Cambridge 1-2)
  - *Histopathology:* no usually available in clinical setting; FNB, fibrosis, atrophy, inflammation, lack of concensus pathologic definitions
- **Research Opportunity: DEFINITION yet to be determined**
  - **MORE LIKELY TO RETARD DISEASE SYMPTOMS AND PROGRESSION**
  - Cross-Sectional: Biomarker Discover / Development
  - Longitudinal: Biomarker Validation / Clinical Implementation

RFA-DK- 14-027/28: Consortium for the Study of Chronic Pancreatitis Diabetes and Pancreatic Cancer



*PRO*spective Evaluation of Chronic Pancreatitis for *Epid*Emiologic and Translational Stu*D*ies

Rationale and Study Design for PROCEED From the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer

Dhiraj Yadav, MD, MPH, Walter G. Park, MD,† Evan L. Fogel, MD, MSc,‡ Liang Li, PhD,§ Suresh T. Chari, MD,|| Ziding Feng, PhD,¶ William E. Fisher, MD,# Christopher E. Forsmark, MD,** Christie Y. Jeon, ScD,†† Aida Habtezion, MD, MSc,† Phil A. Hart, MD,‡‡ Steven J. Hughes, MD,§§ Mohamed O. Othman, MD,|||| Jo Ann S. Rinaudo, PhD,¶¶ Stephen J. Pandol, MD,## Temel Tirkes, MD,*** Jose Serrano, MD, PhD,††† Sudhir Srivastava, PhD, MPH,¶¶ Stephen K. Van Den Eeden, PhD,‡‡‡ David C. Whitcomb, MD, PhD,*§§§|||||| Mark Topazian, MD,|| and Darwin L. Conwell, MD, MSc,‡‡ on behalf of the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC)*

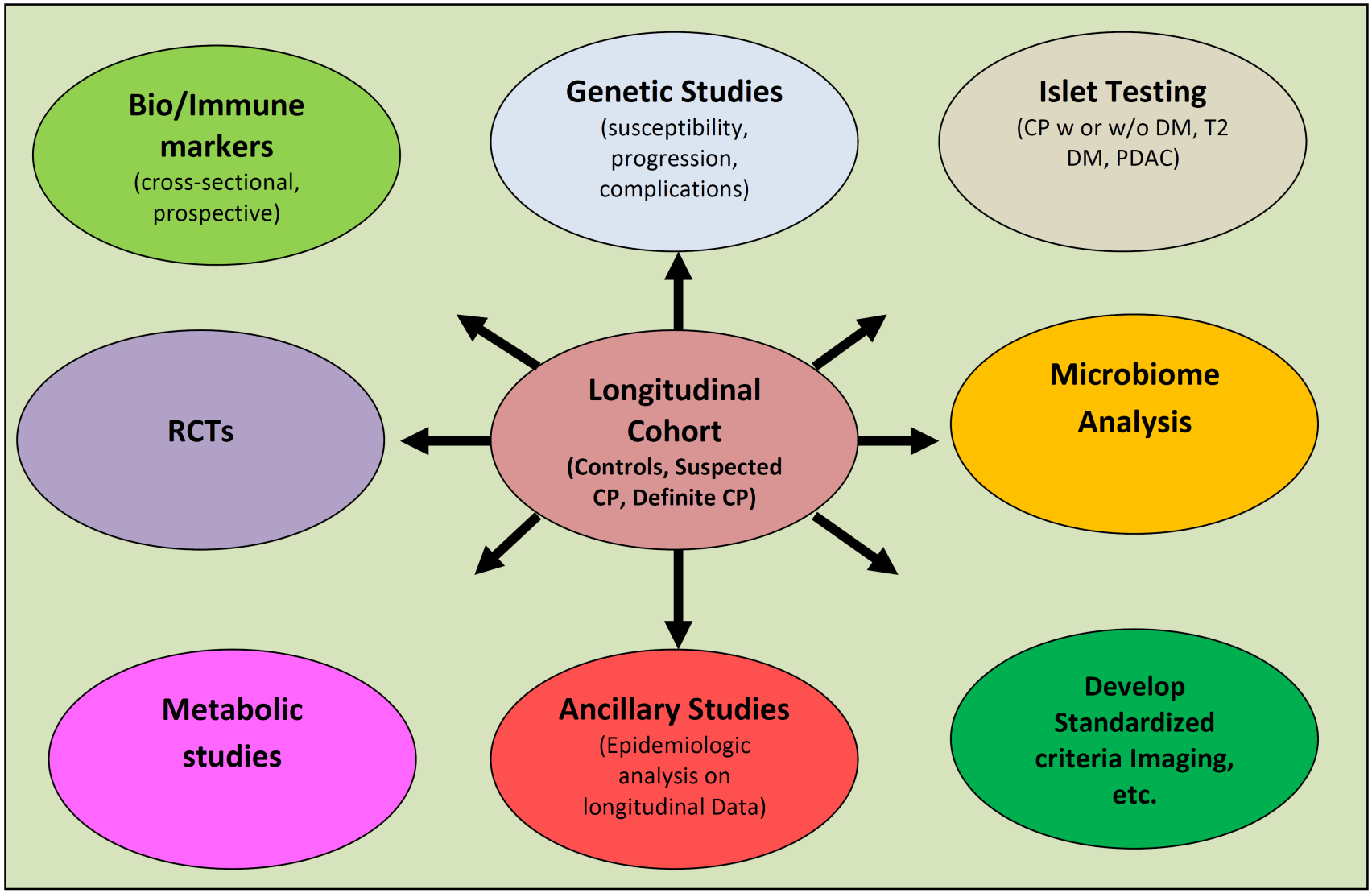
Yadav, D et al., Pancreas 2018.

Adult CP_RAP Scope of Work

Four (4) Primary Objectives

1. **Establish** a model longitudinal research cohort
2. **Estimate** the risk of disease related complications
3. **Validate** predictive and diagnostic candidate biomarkers
4. **Develop** a biorepository platform to perform genetic and mechanistic studies

Adult CP_RAP Scope of Work

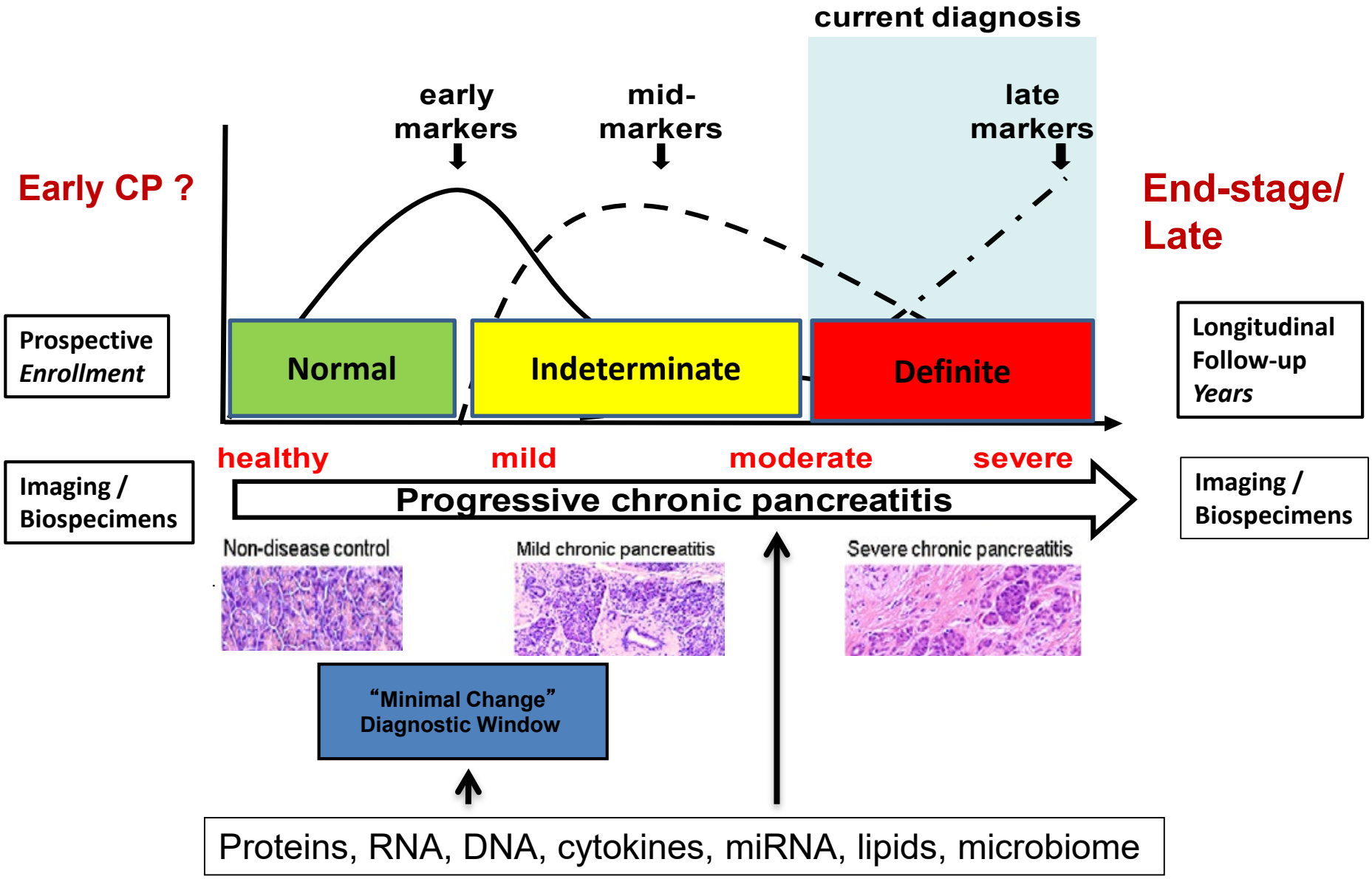


Adult Cohort Definitions

Cohort	CONTROLS		Suspected Chronic Pancreatitis		Chronic Pancreatitis
	Normal Volunteer	Suspected Pancreatic Origin	Recurrent Acute Pancreatitis	Indeterminate	Established
Minimal Inclusion Criteria	No symptoms **AND** No Risk Factors **AND** No Fam. History **AND** No DM **AND** Normal MRCP **AND** *Normal EUS (subset)	Abdominal pain **AND** No AP/CP **AND** Normal Cambridge **AND** ≤ 2 EUS Score **AND** No Sphincterotomy	At least 2 AP **AND** Normal Cambridge CT **AND** Normal MRCP **AND** ≤ 2 EUS Score **AND** Non-biliary Etiology	Clinical Presentation **AND** [Cambridge 1-2 or ≥ 3 EUS Score]	Cambridge ≥ 3 **OR** Abnormal Histology **OR** Parenchymal Calcifications
Longitudinal Follow-up	NO	YES	YES	YES	YES
Outcomes	N/A	RAP Chronic Pancreatitis	Chronic Pancreatitis	Chronic Pancreatitis	Exocrine Insufficiency Type 3cDM Cancer
Sample Size	100 (50)*	250 (100)	**660 (330)**		**660 (45)**
Clinical presentation	No symptoms	Pancreatic Type Pain	Recurrent Acute Pancreatitis (2 or more AP attacks)	AP (one or more) and/or Chronic pain	RAP ± Chronic pain or No symptoms
TIGAR-O CP Risk factors	-	±	±	±	±
Cambridge Imaging Grade (MRI/CT)	Normal	Normal	Normal	Grade I - II	Grade III - IV AND/OR Calcifications
EUS Score	0 - 2	0 - 2	0 - 2	≥3	≥5
Histology	Normal	Normal	Normal	Fibrosis (Ammann 1-6) and either inflammation and/or acinar cell loss	Fibrosis (Ammann 7-12) and Inflammation and Acinar cell loss

Translational Research Approaches

Early Disease Detection / Prediction: ***Longitudinal Follow-up***



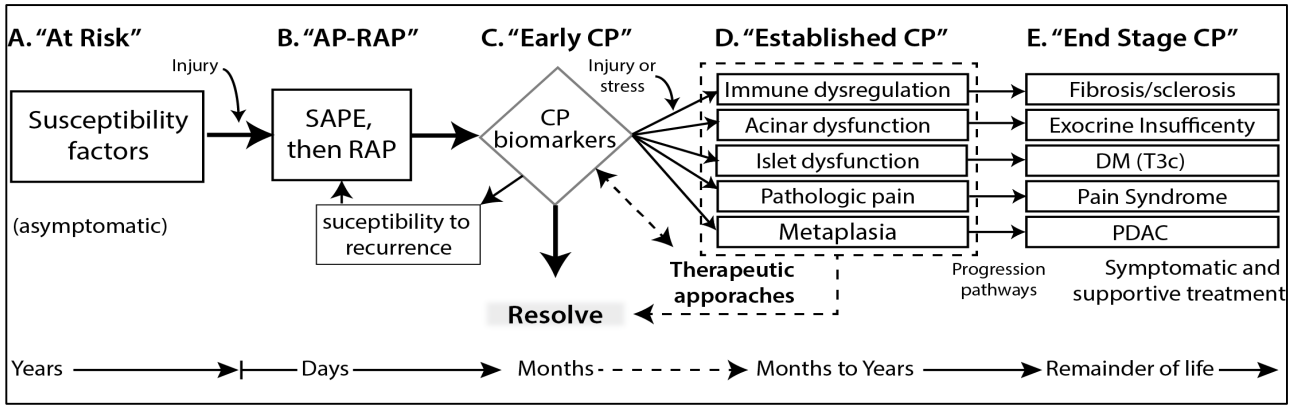
Standard Operating Procedures for Biospecimen Collection, Processing, and Storage

From the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer

William E. Fisher, MD, FACS, Zobeida Cruz-Monserrate, PhD,† Amy L. McElhany, MPH,* Gregory B. Lesinski, PhD,‡ Phil A. Hart, MD,† Ria Ghosh, MBA, MPH,§ George Van Buren, MD,* Douglas S. Fishman, MD,|| Jo Ann S. Rinaudo, PhD,¶ Jose Serrano, MD, PhD,# Sudhir Srivastava, PhD,¶ Thomas Mace, PhD,† Mark Topazian, MD,** Ziding Feng, PhD,§ Dhiraj Yadav, MD,†† Stephen J. Pandol, MD,‡‡ Steven J. Hughes, MD,§§ Robert Y. Liu, MS,|||| Emily Lu, MS,|||| Robert Orr, BS,¶¶ David C. Whitcomb, MD, PhD,** Amer S. Abouhamze, MHA,## Hanno Steen, PhD,*** Zachary M. Sellers, MD, PhD,††† David M. Troendle, MD,‡‡‡ Aliye Uc, MD,§§§ Mark E. Lowe, MD, PhD,|||||| and Darwin L. Conwell, MD,† on behalf of the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC)*

(*Pancreas* 2018;47: 1213–1221)

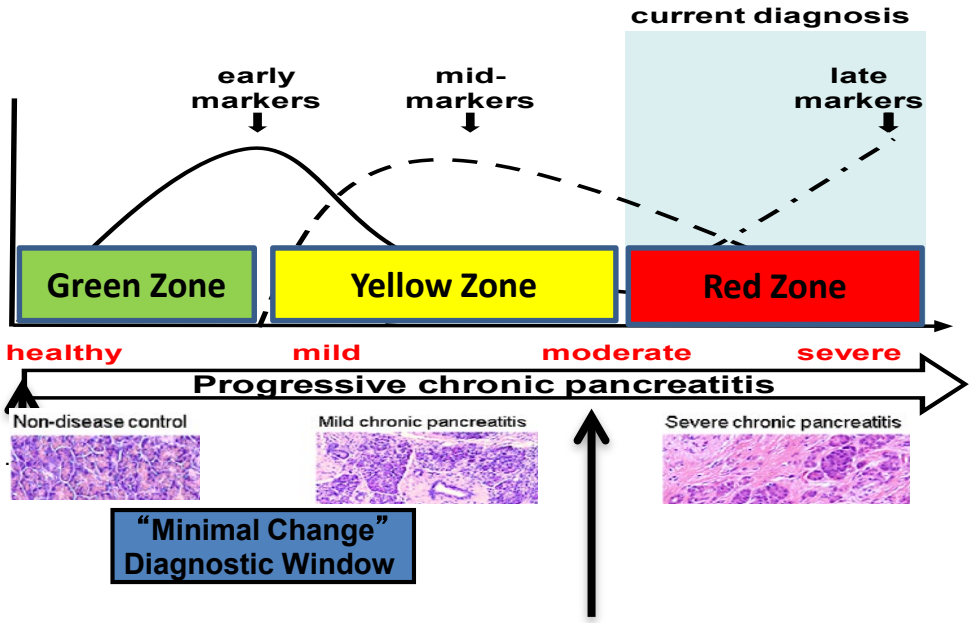
PROCEED STUDY



Early

Late

Imaging / Biospecimens



Imaging / Biospecimens

Proteins, RNA, DNA, cytokines, miRNA, lipids, microbiome

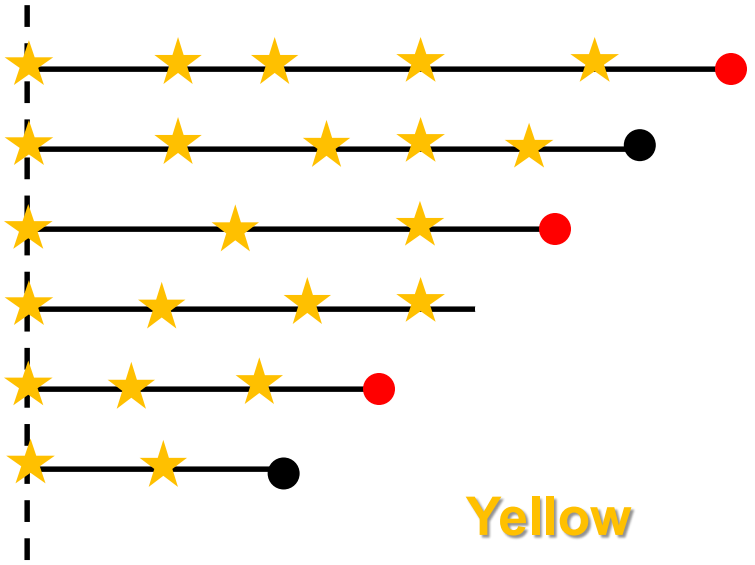
PROCEED Cohort Study

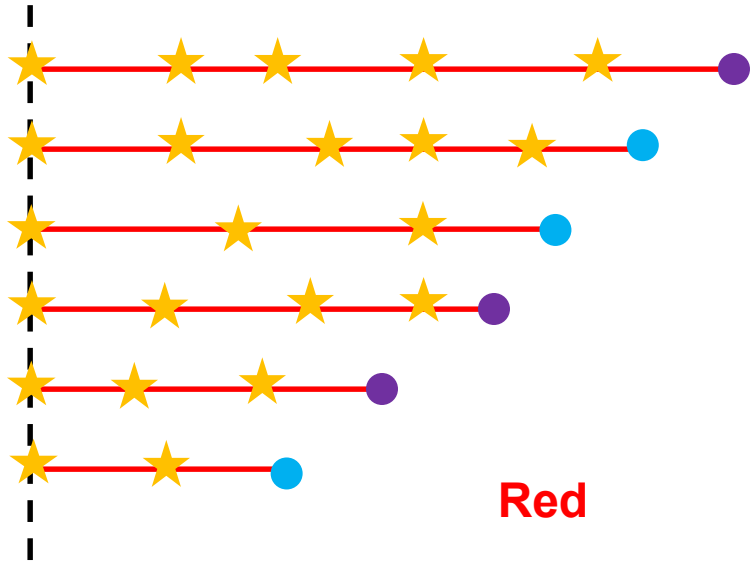
Prospective collection

- **Controls (250)**
- **Acute Pancreatitis (660)**
- **Chronic Pancreatitis (660)**

- Case Report forms
  - Patient, physician
- Labs
- Imaging: CT and MRI/MRCP and DEXA
- Endoscopic Ultrasound
- Biospecimens
  - Urine
  - Blood
  - Saliva
  - stool

PROCEED Cohort Study

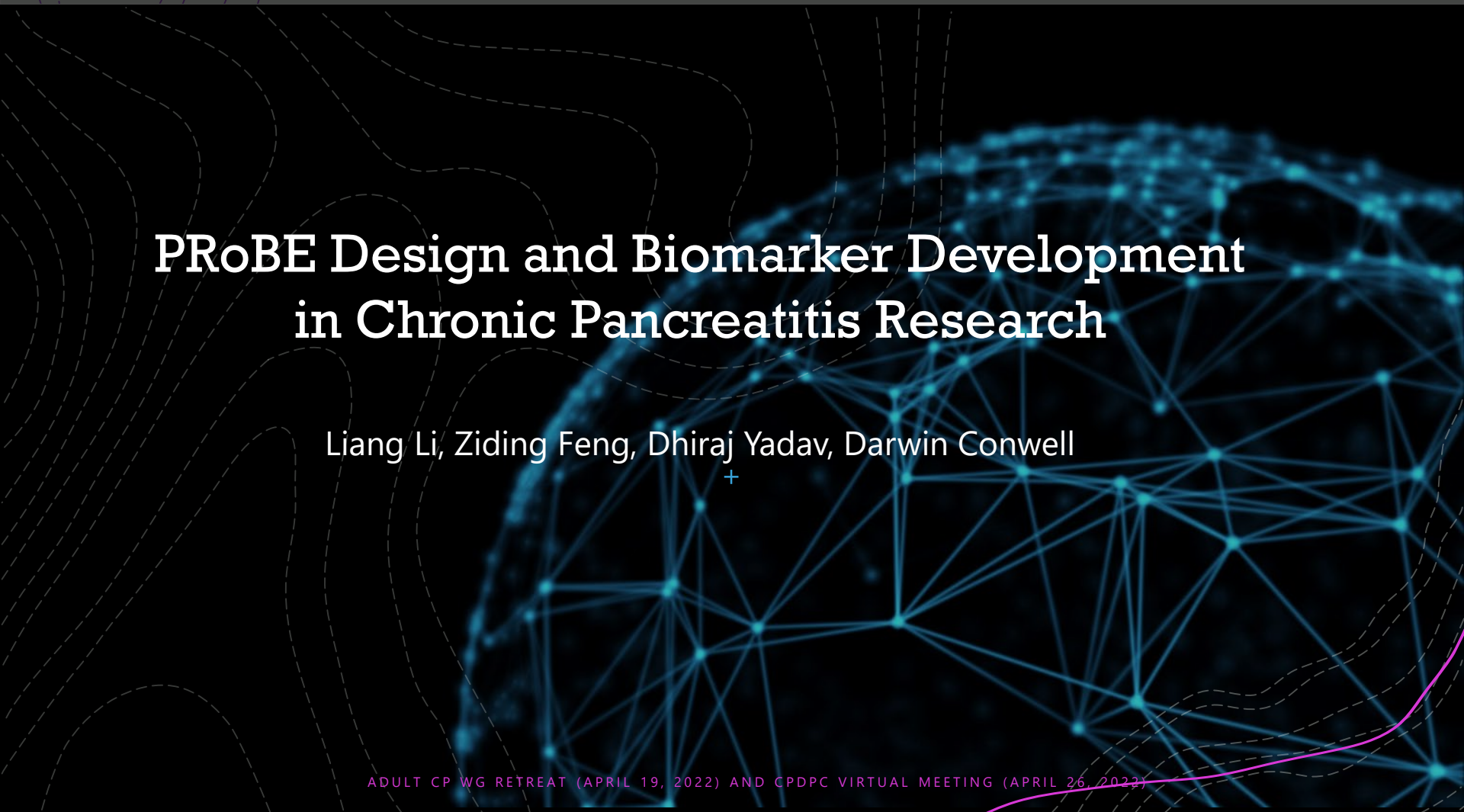




Sub-cohort	Yellow (suspected CP)	Red (definite CP)
Endpoint	Progression to definite CP	PDAC, new-onset DM
Longitudinal data	AP/RAP, biomarkers, morphology, pain/symptoms, endocrine/exocrine pancreatic insufficiency	

PRoBE Design and Biomarker Development in Chronic Pancreatitis Research

Liang Li, Ziding Feng, Dhiraj Yadav, Darwin Conwell



ADULT CP WG RETREAT (APRIL 19, 2022) AND CPDPC VIRTUAL MEETING (APRIL 26, 2022)

Adaptation of PRoBE Strategy in CP Research

- CP is characterized by persistent **inflammation** of the pancreas and **fibrosis** resulting in irreversible changes, loss of pancreatic function, and increased risk of pancreas cancer
- Early disease clinical manifestations are **non-specific** and may include pain, dyspepsia and nausea and are often overlapping with common gastrointestinal conditions such as GERD, gastritis and peptic ulcer disease
- There are no reliable early-stage diagnostic or prognostic biomarkers of CP
- Pathogenesis of CP is likely **multifaceted** and not fully understood
  - ✓ A number of ancillary biomarker studies target various aspects of the diseases
  - ✓ Biomarker may not go in one direction with the disease progression (e.g., PGE2)
  - ✓ Justify a **composite biomarker** that includes image, liquid, behavioral markers and other patient characteristics
- **Three phases**: Phase 1 (discovery/diagnosis), Phase 2 (validation/diagnosis), Phase 3 (prognosis)

PROCEED Study Used PRoBE Design

- A longitudinal cohort study that resembles Phase 3
- Stored longitudinal biospecimens processed by standardized protocol
- Baseline biospecimens for Phase 1-2 studies
- Divide the Clinical Centers into discovery and validation sites
- CDMC assisted blinding in biospecimen requests
- Conducted interobserver study of image markers (more needed)
- Recommended quality control/replication in biospecimen handling
- **Most ancillary biomarker studies are Phase 1 (discovery); one advanced to Phase 2 (NGAL, validation); none in Phase 3 yet**

Specific Issues in Applying PRoBE Design for CP Research

- **Phase 2 (validation/diagnosis)**:
  - ✓ Important to target the right clinical population (NGAL in development)
- **Phase 3 (prognosis)**:
  - ✓ The outcome is transition to CP. **Composite progression outcomes** may be used to improve sample size: transition to CP + PDAC + CP related death + etc.
  - ✓ May need sophisticated statistical model for composite biomarker that includes patient characteristics and image, liquid, behavioral markers and probably longitudinal history (and avoid overfitting bias)
  - ✓ Adjust biomarker threshold with patient characteristics and between Phase 2 & 3

Assessment of Neutrophil Gelatinase-Associated Lipocalin as a Diagnostic Biomarker for Chronic Pancreatitis and Characterization of its Role in Disease Progression

*Approved on 01-22-2019
OSU CPDPC Clinical Center

CPDPC Adult CP WG

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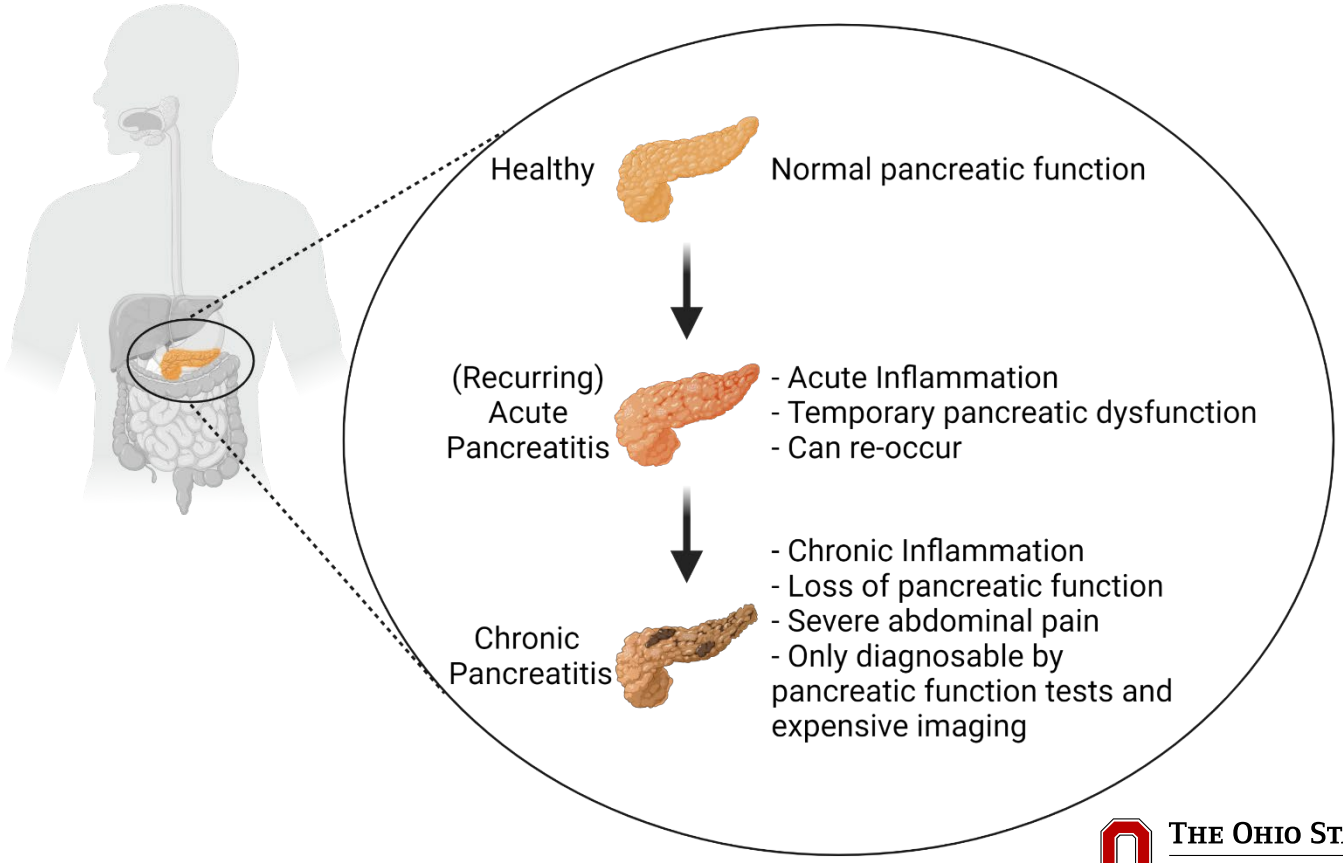
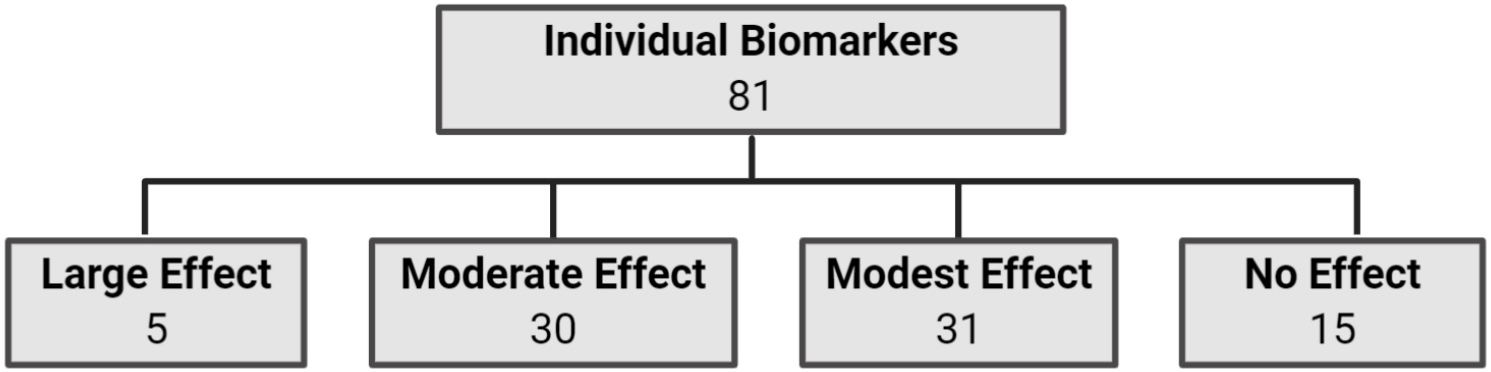
Department of Internal Medicine
Division of Gastroenterology, Hepatology and Nutrition (GHN)
Director, GHN Molecular and Cellular Biology Program and Laboratory
Assistant Director, CAMELOT (Cancer Research Training and Education), OSU Comprehensive Cancer Center
The Ohio State University Wexner Medical Center
Member, Molecular Carcinogenesis and Chemoprevention Program at the OSUCCC – James

Long-Term Goal:

Develop a method for the accurate detection of CP by using molecules expressed in biosamples from a stratified group of CP subjects based on Cambridge score (0, 1 / 2, and 3 / 4).



81 potential biomarkers for CP have been identified with varying levels of likely effectiveness



Biomarker	Biospecimen Type	N (Cont.)	N (CP)	AUROC	p-value	Effect Size	Comments	Ref(s)
Adenosine	Urine	5	5	0.971	0.048	Large		28
Des-Leu albumin	Plasma, Serum	34	9	0.996	-	Large	Measured in patients hospitalized with acute flares. No p-value presented in article	34
Oxidized Fatty Acids: 5-HETE:AA, 11-HETE:AA, 15-HETE:AA, 9-HODE:LA, 9-oxoODE:LA, 13-oxoODE:LA	Serum	5	5	0.877 – 1	0.03 - <0.001	Moderate – Large	Values shown for "severe CP" vs. control	38
Adiponectin	Plasma, Serum	13-30	27-44	0.514 – 0.994	NS - <0.0003	None - Large	Heterogeneity among study results: 2 studies of serum with null results, one study with plasma showing large effect size	30, 31, 33
IL-6	Plasma, Serum	8-72	8-56	0.507 – 0.997	NS - <0.001	None – Large	Heterogeneous results, only one study shows more than a modest effect size.	32, 36, 37, 48, 61-63

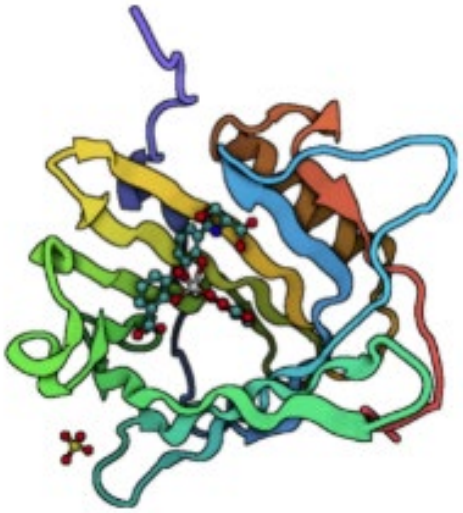
Contents lists available at ScienceDirect
Pancreatology
journal homepage: www.elsevier.com/locate/pan

Biomarkers of Chronic Pancreatitis: A systematic literature review

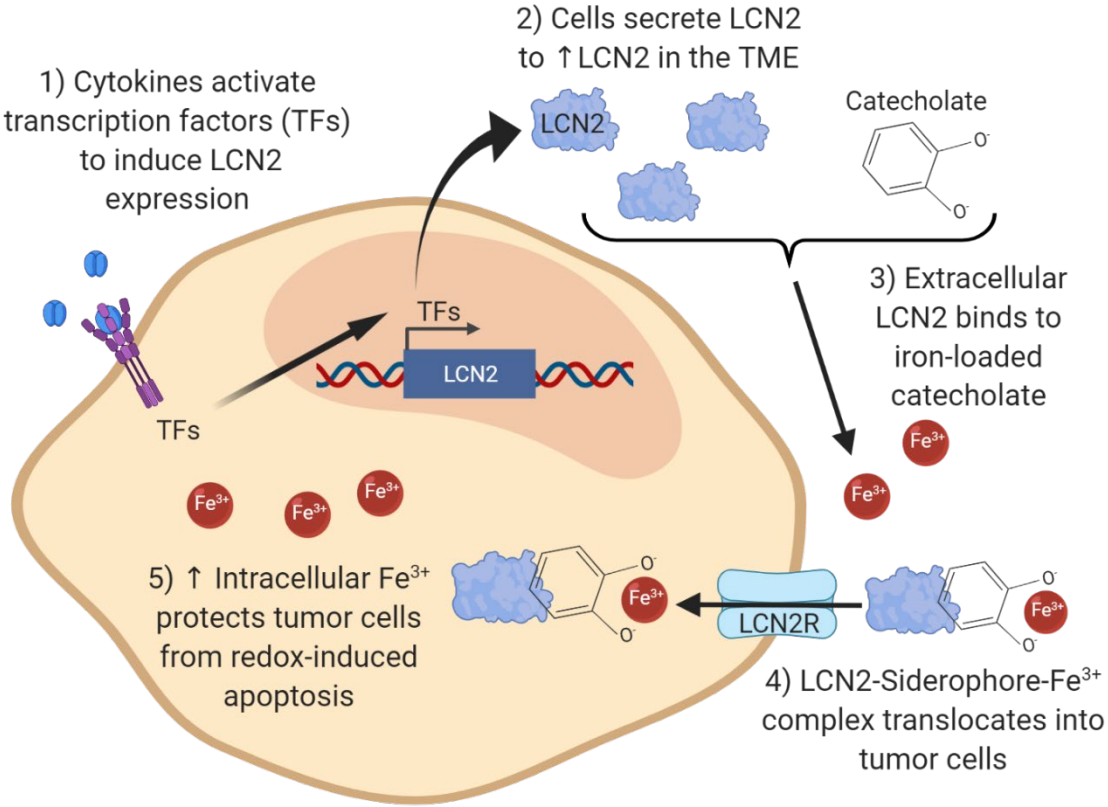
Zobeida Cruz-Monserrate [a, b, *, 1], Kristyn Gumpper [a, b], Valentina Pita [a, b], Phil A. Hart [a], Christopher Forsmark [c], David C. Whitcomb [d], Dhiraj Yadav [d], Richard T. Waldron [e], Stephen Pandol [e], Hanno Steen [f, g], Vincent Anani [h], Natasha Kanwar [h], Santhi Swaroop Vege [h], Savi Appana [i], Liang Li [i], Jose Serrano [j], Jo Ann S. Rinaudo [k], Mark Topazian [h, 1], Darwin L. Conwell [a, 1], on behalf of the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer

Cruz-Monserrate et.al, Pancreatology; 21 (323-333) 2021

Lipocalin 2 is a Biomarker for PDAC, and Potential Therapeutic Target



(LCN2, NGAL, 24p3)



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Lipocalin-2 expression and function in pancreatic diseases

Kristyn Gumpper [a, b], Andrew William Dangel [a, b], Valentina Pita-Grisanti [a, b], Somashekar G. Krishna [a, b], Luis F. Lara [a], Thomas Mace [a, b], Georgios I. Papachristou [a], Darwin L. Conwell [a], Phil A. Hart [a], Zobeida Cruz-Monserrate [a, b, *]

International Journal of *Molecular Sciences*

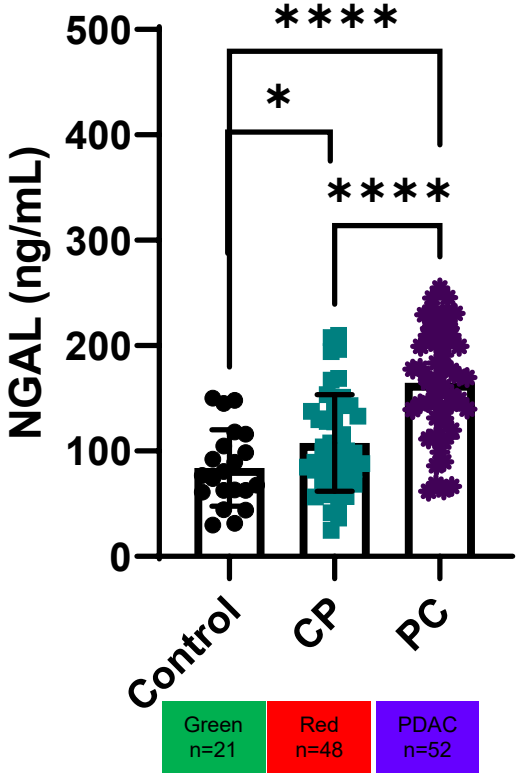
MDPI

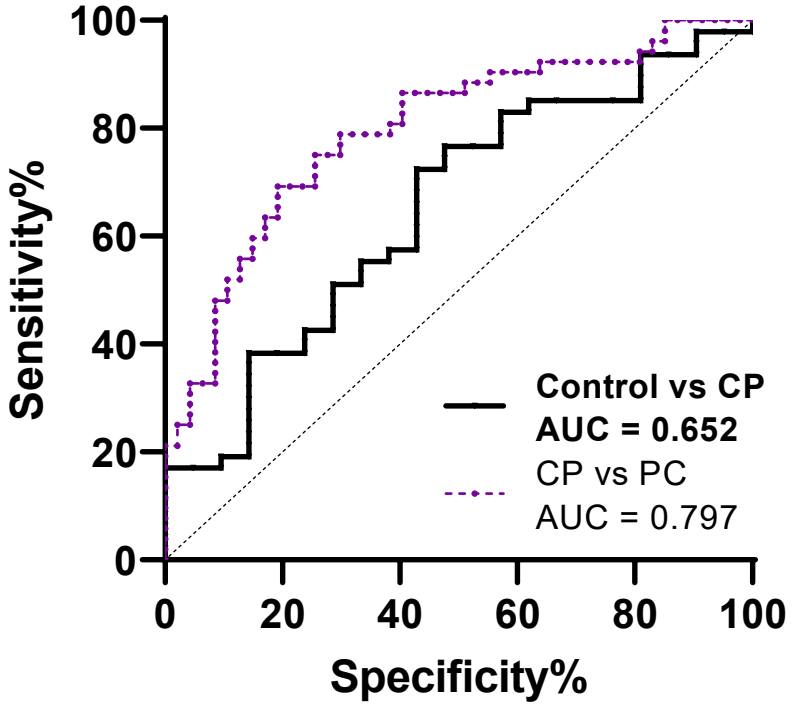
Review

Biological Functions and Therapeutic Potential of Lipocalin 2 in Cancer

Ginette S. Santiago-Sánchez [1], Valentina Pita-Grisanti [2,3], Blanca Quiñones-Díaz [1], Kristyn Gumpper [2,3], Zobeida Cruz-Monserrate [2,3,*] and Pablo E. Vivas-Mejía [1,4,*]

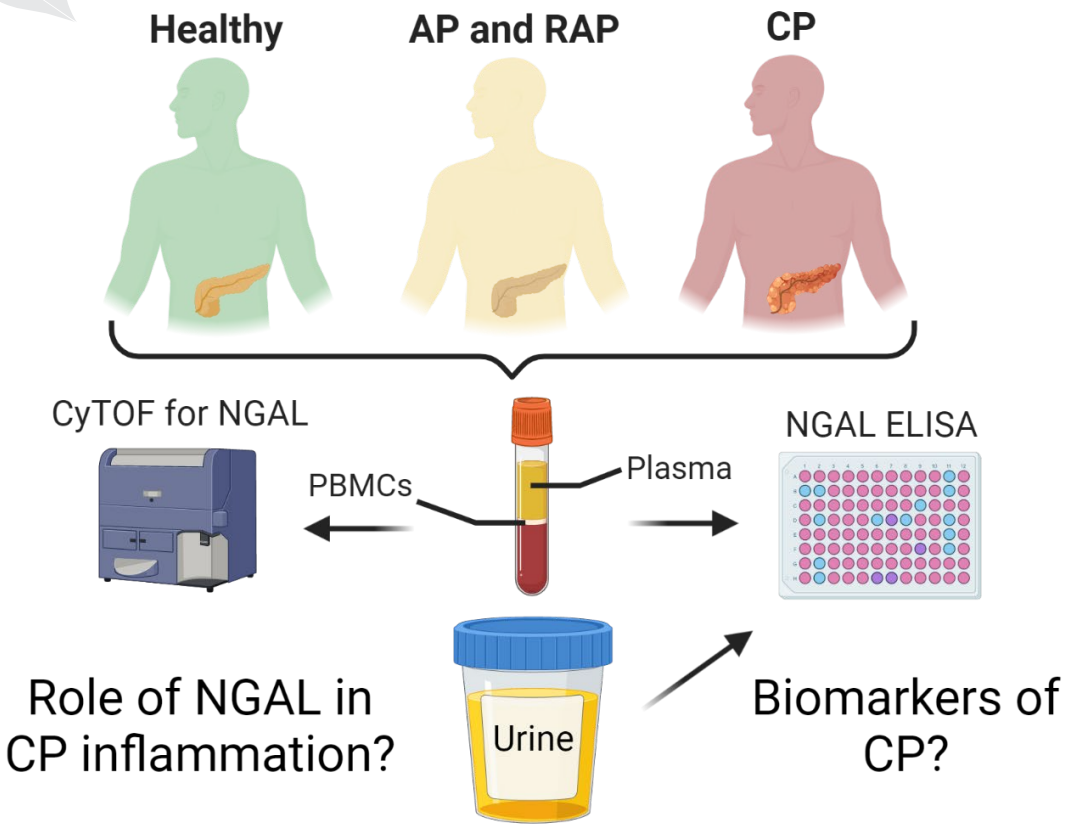
Preliminary Data: OSU pre-discovery samples show elevation of blood NGAL in CP and PDAC compared to healthy controls **POWER CALCULATION**



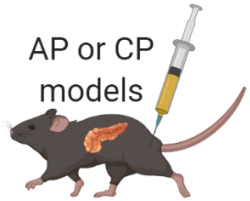


	Minimum Sample Size per Group
Control vs. Chronic Pancreatitis	50

Project Overall Goals



- To validate the expression of a neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker of CP.
- Correlated with patient- and disease-related variables, including sex, age, smoking status, body mass index, disease etiology, disease radiologic (Cambridge) stage, and presence of complications (such as diabetes, exocrine pancreatic insufficiency, and metabolic bone disease)
- To understand its role in disease progression (normal, mild-moderate and severe CP).



Finale

- **The Research Challenge – Biomarker development**
- **Current State – Definite CP**
- **The "Black Box"**
  - **RAP, Early CP (Indeterminate CP)**
  - **Outcome Prediction / progression – EPI, DM, Pain, QOL**
- **PROCEED – Current Research Opportunities**
  - **Biorepository, Imaging repository, Clinical trials**
  - Cross Sectional, Discovery, biomarker
  - Scientific Collaborations – content expertise
- **PROCEED - Future Directions**
  - **Clinical Outcomes, Surrogate endpoints, Clinical trials**
  - Prospective, Validation, Clinical Implementation
  - Scientific Collaborations – content expertise