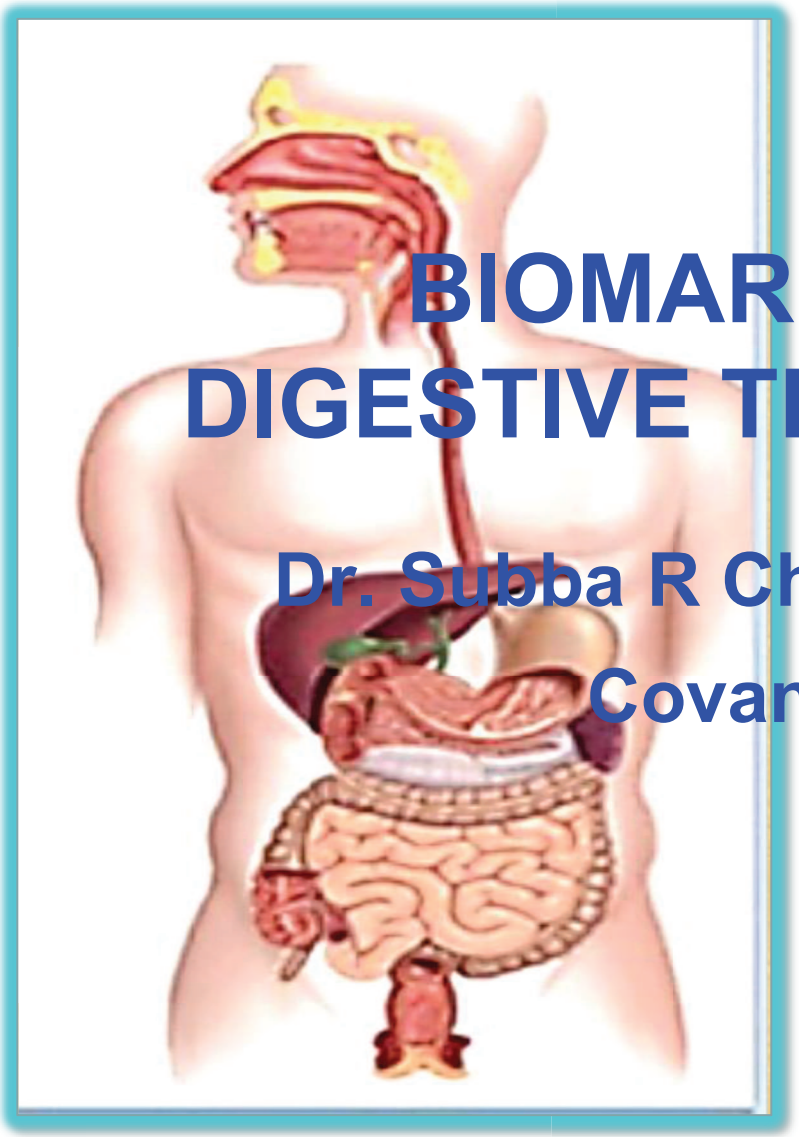
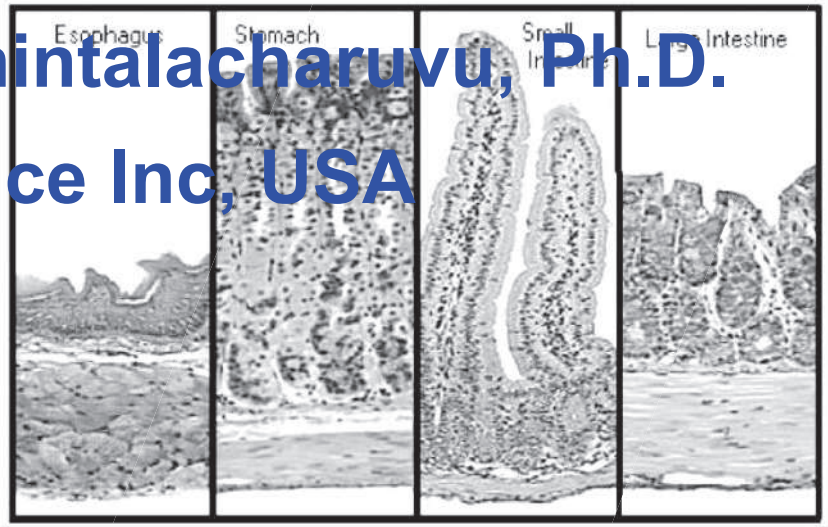


BIOMARKERS FOR DIGESTIVE TRACT TOXICITY

Dr. Subba R Chintalacharuvu, Ph.D.

Covance Inc, USA



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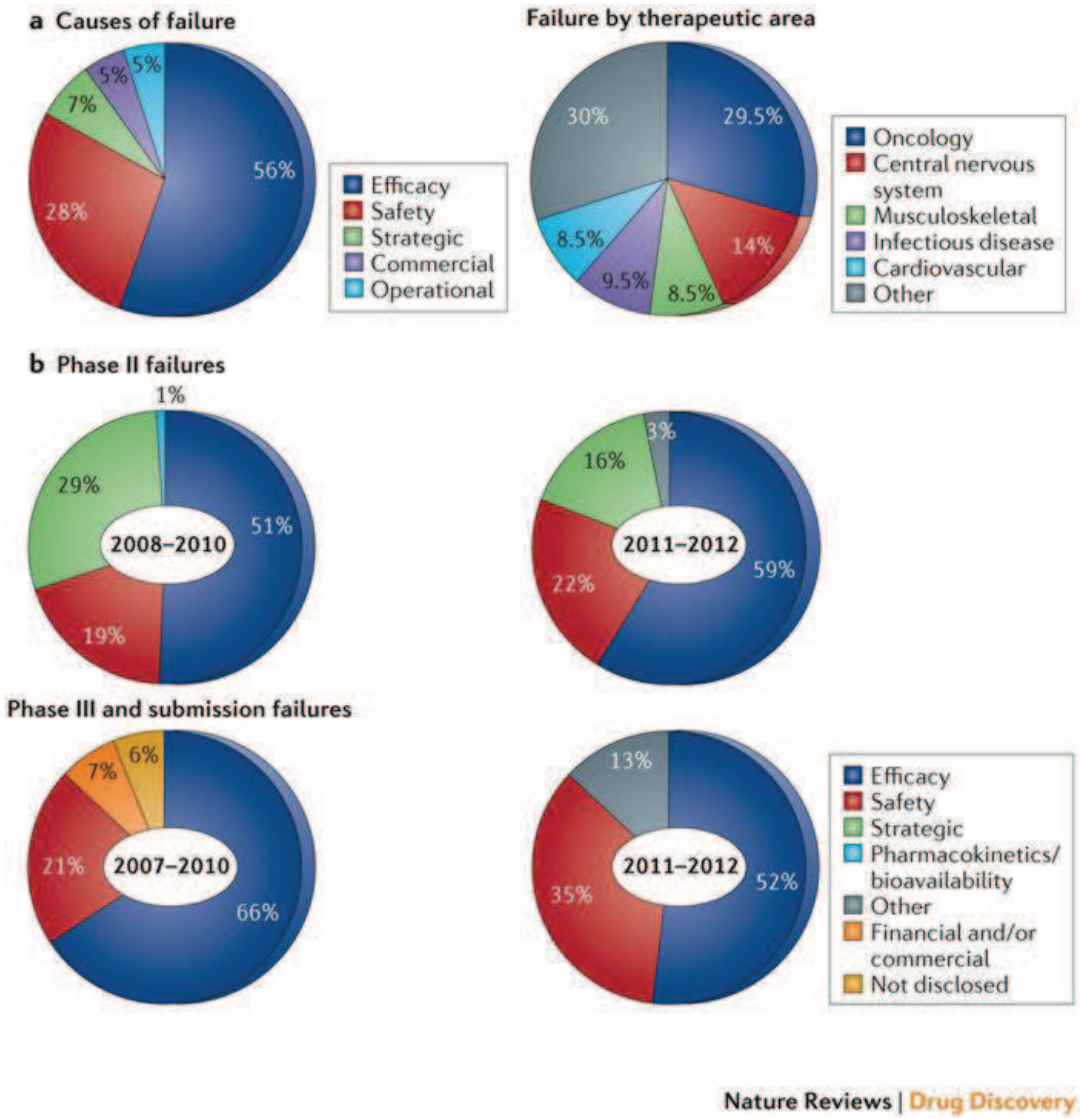
OUTLINE

- Introduction
- Reasons for attrition in drug R&D: digestive tract liabilities
- Evolving role of biomarkers
- Digestive tract toxicity biomarkers
- Application in a pre-clinical model
- Utility of biomarkers in diagnosis and treatment of Inflammatory bowel disease
- Conclusion

REASONS FOR ATTRITION IN R&D



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Impact of adverse effects of drugs by organ function throughout the pharmaceutical life cycle

Phase	'Nonclinical'	Phase I	Phase I-III	Phase III/ Marketing	Post-Marketing	Post-Marketing
Information:	Causes of attrition	Serious ADRs	Causes of attrition	ADRs on label	Serious ADRs	Withdrawal from sale
Source:	Car (2006)	Sibille et al. (1998)	Olson et al. (2000)	BioPrint® (2006)	Budnitz et al. (2006)	Stevens & Baker (2008)
Sample size:	88 CDs stopped	1,015 subjects	82 CDs stopped	1,138 drugs	21,298 patients	47 drugs
Cardiovascular:	27%	9%	21%	36%	15%	45%
Hepatotoxicity:	8%	7%	21%	13%	0%	32%
Haematology/BM:	7%	2%	4%	16%	10%	9%
Nervous system:	14%	28%	21%	67%	39%	2%
Immunotox; photosensitivity:	7%	16%	11%	25%	34%	2%
Gastrointestinal:	3%	23%	5%	67%	14%	2%
Reprotox:	13%	0%	1%	10%	0%	2%
Musculoskeletal:	4%	0%	1%	28%	3%	2%
Respiratory:	2%	0%	0%	32%	8%	2%
Renal:	2%	0%	9%	19%	2%	0%
Genetic tox:	5%	0%	0%	0%	0%	0%
Carcinogenicity:	3%	0%	0%	1%	0%	0%
Other:	0%	0%	4%	16%	2%	2%

The various toxicity domains have been ranked first by contribution to products withdrawn from sale, then by attrition during clinical development.

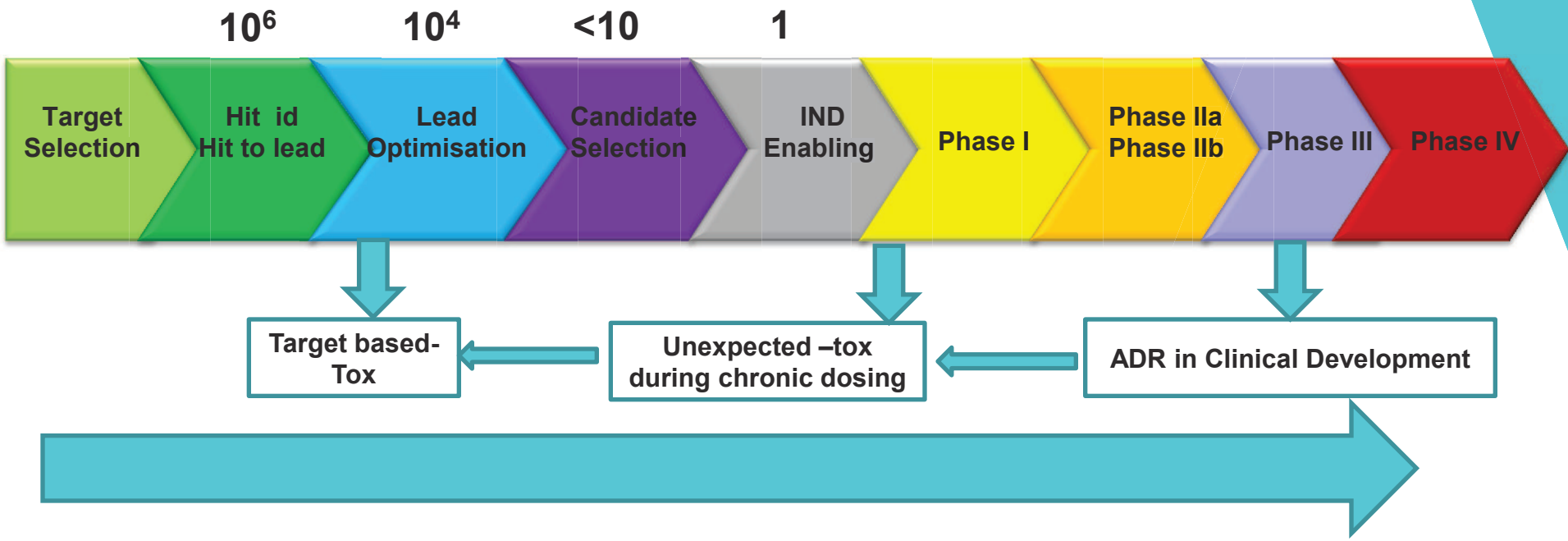
0% 1-9% 10-19% >20%

Adapted from Redfern WS et al. SOT 2010

Digestive Tract toxicity Assessment

Function	Injury
Established	**Established**
Gastric emptying	Macroscopic (ulcer index)
Gastric secretion	Histopathology
Intestinal motility	
Emerging	**Emerging**
Endoscopy	Endoscopy (CTE)
Capsule – pH, pressure	Capsule
Strain gauge for contraction	*BIOMARKERS*
In silico (PBPK modeling)	

EVOLVING USE OF BIOMARKERS



The ultimate goal is to provide drug R&D with a toolbox of qualified Biomarkers that perform well for drug candidates in animals studies and also to monitor clinical safety and efficacy.

BIOMARKER

"A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."

Biomarkers Definition Working Group, NHI, Clin Pharmacol. Ther 2001,69:89

“WHAT IS AN IDEAL BIOMARKER”

- ❑ Specific
- ❑ Sensitive
- ❑ Predictive
- ❑ Robust
- ❑ Bridge pre-clinical & clinical

- ❑ Proteomics
- ❑ Genomics
- ❑ Metabolomics
- ❑ Imaging

	Serum Biomarkers	Fecal Biomarkers
NSAIDs	CITRULLINE	Calprotectin
Cancer	DAO	Lactoferrin
IBD	CD64	Bile acids
Metabolic Disorder	Gastrin	PMN-e
	CRP	I-FABP, L-FABP
	Oral probes	miRNAs-194
	Matrix Metallo Proteinases	Fecal S100A12
	Pepsinogen I & II	Novel markers

DIGESTIVE TRACT BIOMARKERS

CITRULLINE:

- Intermediate metabolic aa produced by the enterocytes of small intestine
- Correlates with chemo-therapeutically reduced enterocytes mass
- Challenging assay (HPLC-MS), highly specific, no enzymatic assay

DIAMINE OXIDASE (DAO):

- Increased DAO levels with the increase in severity of small intestine lesion induced by anticancer drugs
- Limitations of low level of DAO and masked by heparin stimulation

CD64:

- Useful neutrophilic biomarker for digestive tract injury with inflammatory and functional disease of intestine
- CD64 expressed significantly higher in IBD patients and responds well to intervention

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DIGESTIVE TRACT BIOMARKERS

- **GASTRIN:**
  - Produced by endocrine G cells of gastric antrum and duodenum in response of digestive stimuli
  - Increased gastrin levels associated with duodenal ulcers, bacterial infections and tumors
  - Acidity of gastric lumen will decrease gastrin levels.
- **C-REACTIVE PROTEIN (CRP):**
  - Hepatic protein produced in response acute and chronic inflammation
  - Reliable biomarker for the disease severity and progression in UC patients
- **MATRIX METALLOPROTEINASES**: Tissue specific markers for chemotherapy-induced gut injury
- **PEPSINOGEN I & II:**
  - Pepsinogen I & II produced by gastric mucosa
  - Progression of gastritis to acute and pangastritis is associated with increase levels of serum Pepsinogen II

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DIGESTIVE TRACT BIOMARKERS

- **CALPROTECTIN:**
  - 36.5 Kda nonglycosylated, calcium binding protein, sensitive, stable marker and plays a central role in neutrophil defense
  - Directly related to influx of neutrophils to the digestive tract damage
  - It can be measured by standard ELISA (humans)
- **LACTOFERRIN:**
  - Iron binding protein secreted by mucosa membrane and a major component of PMN neutrophils
  - Useful marker for intestinal inflammation
- **BILE ACIDS:**
  - Cathartic effect of excessive fecal bile acids may cause due to chronic diarrhea
- **POLYMORPHONUCLEAR NEUTROPHIL ELASTASE:**
  - Neutral proteinases and mediator of inflammation

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DIGESTIVE TRACT BIOMARKERS

- **I-FABP AND L-FABP:**
  - Small fatty-acid-binding protein (FABP) are released from digestive enterocytes after cellular damage
- **MICRO RNA:**
  - Small, endogenous noncoding RNAs, which act as post-transcriptional regulators of genes
  - miRNA named as miR-94 expressed in small intestine and colon
  - GI-enriched miRNA-94 is an indicative of digestive toxicity
- **FECAL S100A12**:
  - Calcium binding proteins, activates NF-kB signal transduction and enhances cytokine release
  - Fecal S100A12 has been detected in IBD patients
- **NOVEL BIOMARKERS:**
  - M2-pyruvate kinases, Adipsin- potential marker for Notch/Hes-1 signaling, increased expression of neutrophil activation markers, CD177 and CEACAM1

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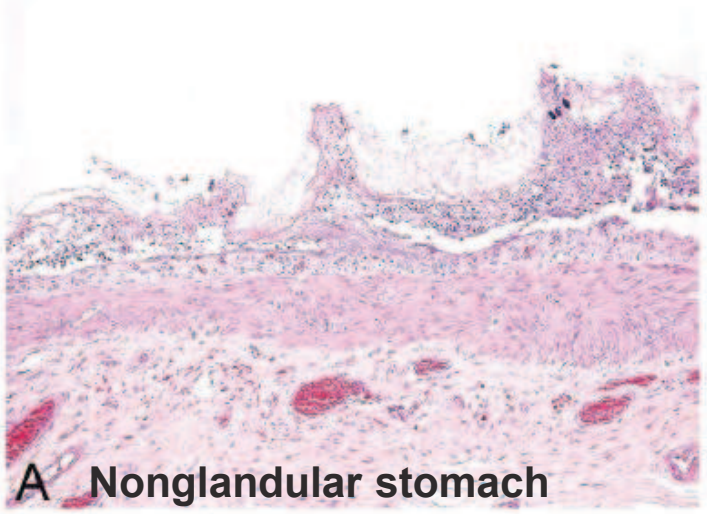
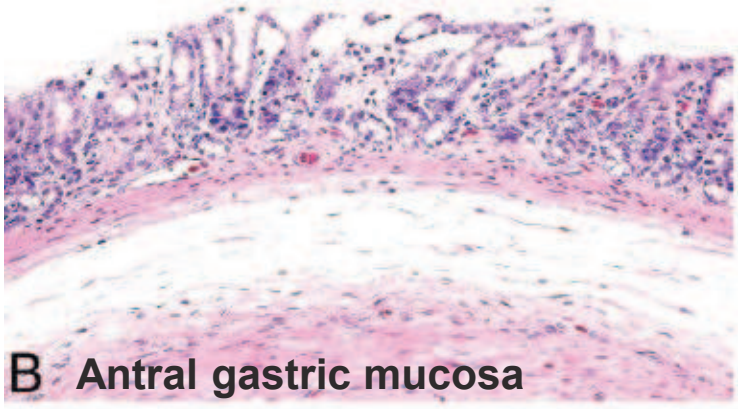
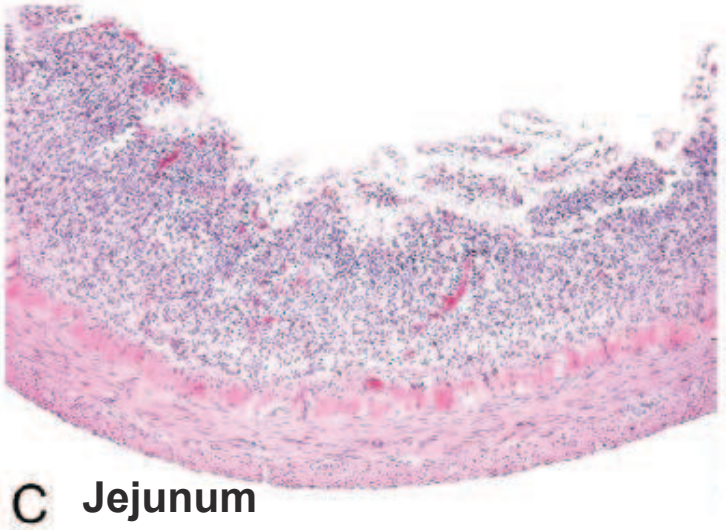
Preclinical Toxicity model: rats

- Wistar rats (n=8/group)
- PAK4 (p21-activated kinase4) inhibitor at 5 mg/kg and 15 mg/kg
- Orally, qd; 5 days
- Clinical signs, BW daily and blood and urine was collected


A Nonglandular stomach


B Antral gastric mucosa


C Jejunum

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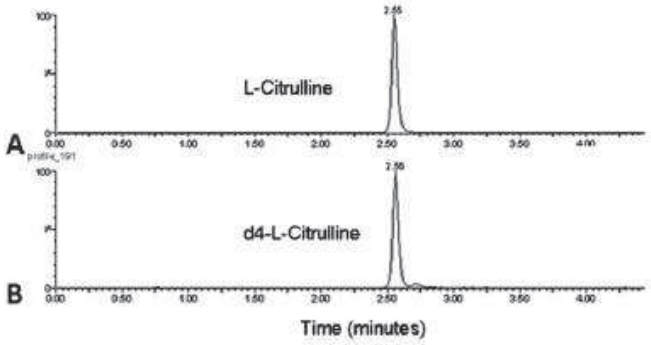
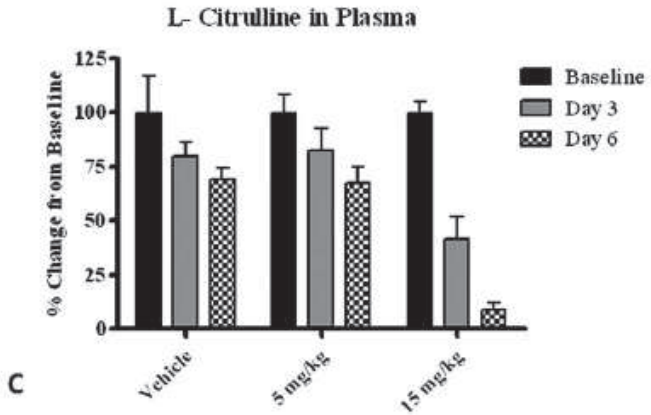
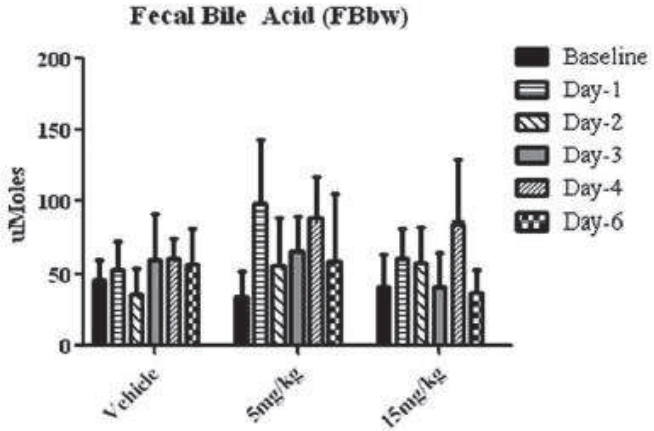
Preclinical Toxicity model: rats

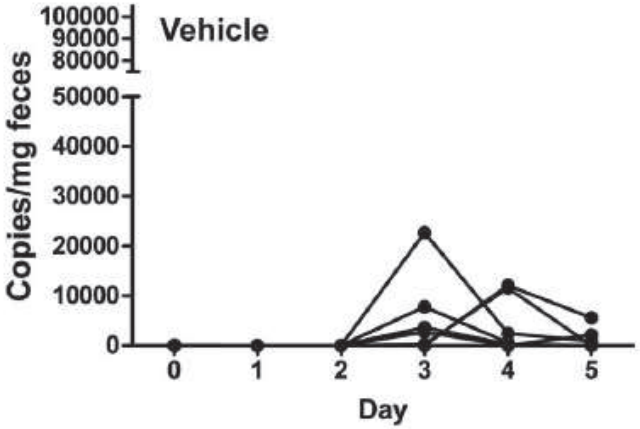




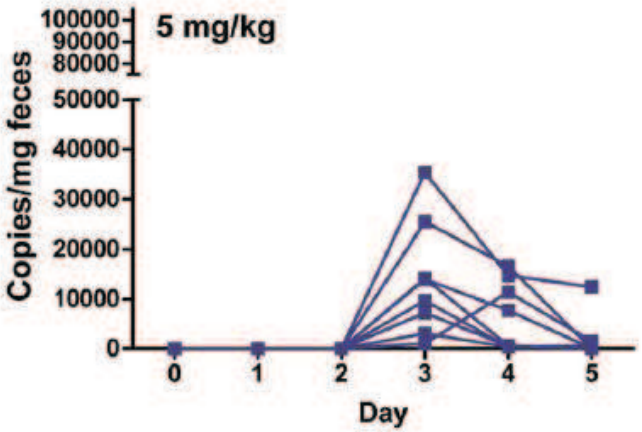


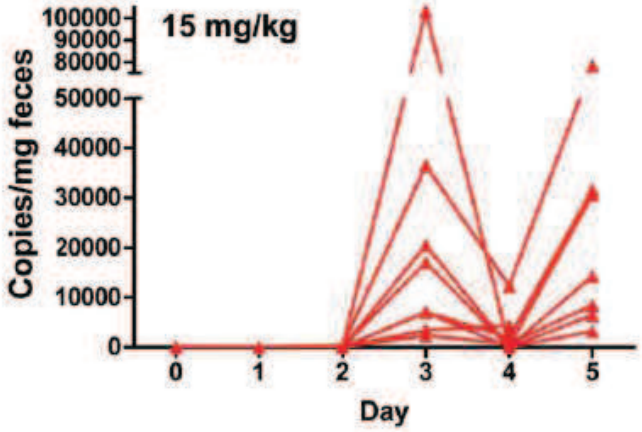
John-Bapiste et al. Tox Path 2012,40:482

Preclinical Toxicity model: rats



- ✓ Identification of reliable digestive tract biomarkers such as blood Citrulline and fecal miR-194
- ✓ Blood DAO, fecal bile acids and fecal Calprotectin were undetectable.
- ✓ Detection of biomarkers for digestive tract toxicity: limitations and gaps.





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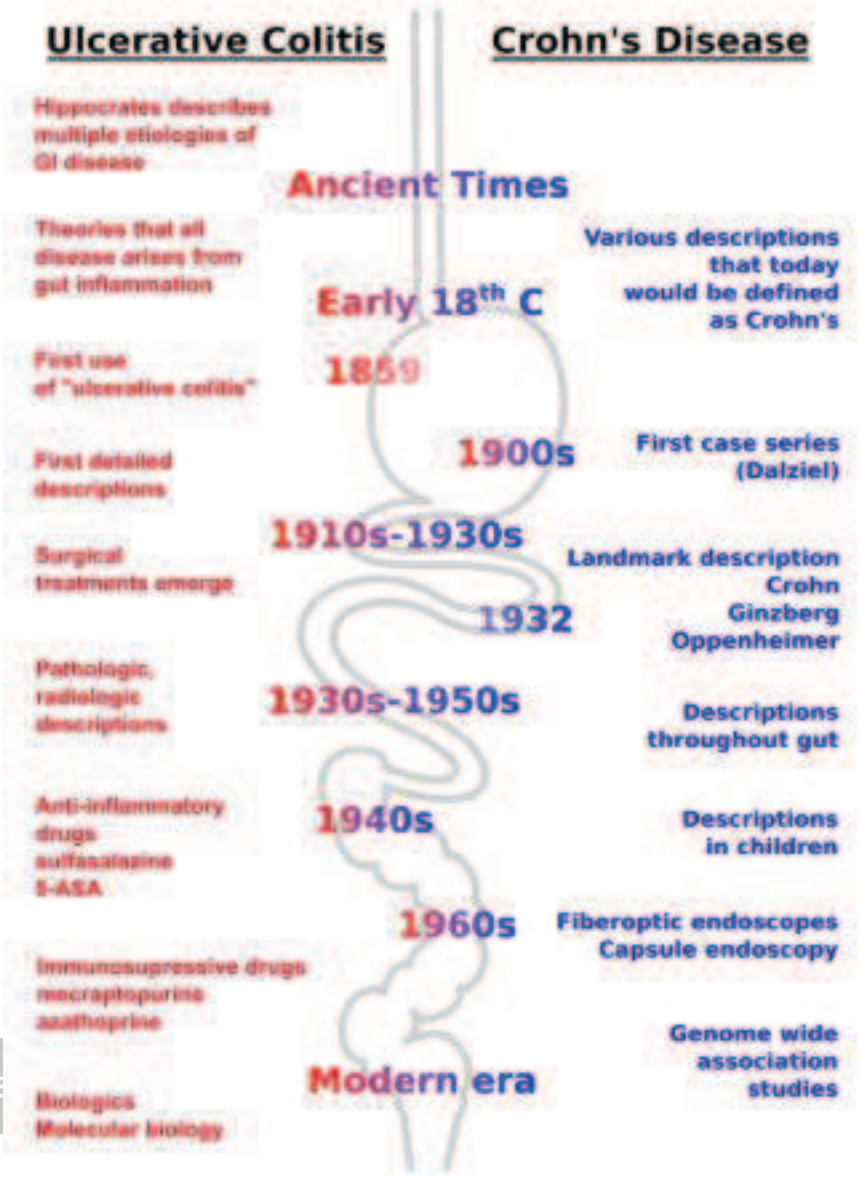
- NSAIDs
- Cancer
- IBD
- Metabolic Disorder

Serum Biomarkers	Fecal Biomarkers
CITRULLINE	Calprotectin
DAO	Lactoferrin
CD64	Bile acids
Gastrin	PMN-e
CRP	I-FABP, L-FABP
Oral probes	miRNAs-194
Matrix Metallo Proteinases	Fecal S100A12
Pepsinogen I & II	Novel markers

A tale of two diseases: Inflammatory Bowel disease



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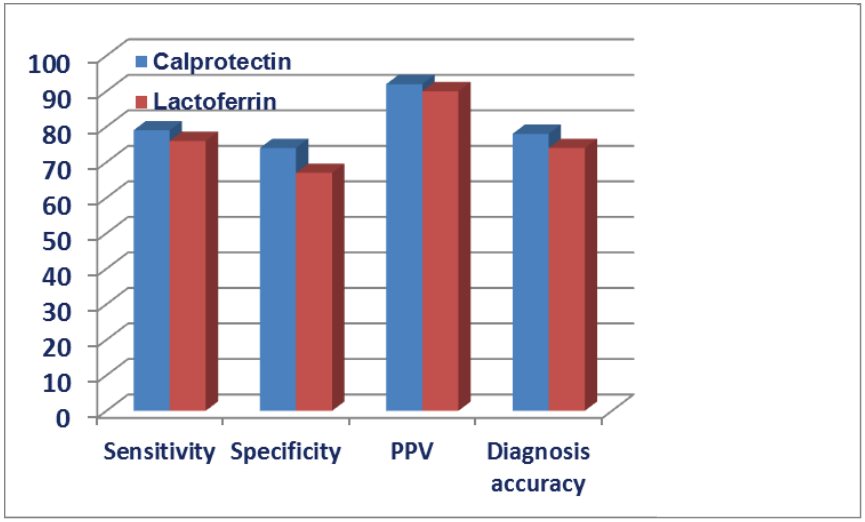
Biomarkers in managing IBD

- Elevated serum CRP was associated with increase in inflammation and presence of active disease of UC patients measured by ilecolonoscopy. CRP levels >12 mg/L is indicative of severe and extensive disease[1,2]
- Fecal Lactoferrin and Calprotectin were useful tools in detecting bowel inflammation in symptomatic UC patients [3,4]



1. Solem et al. Inflamm. Bowel Dis. 2005, 11:707
2. Pepys et al. J Clin Invest. 2003, 111:1805
3. Kane et al. Am J Gastroenterol. 2003, 98:6
4. Schoefer et al. Inflamm.Bowel Dis. 2009, 15:1851

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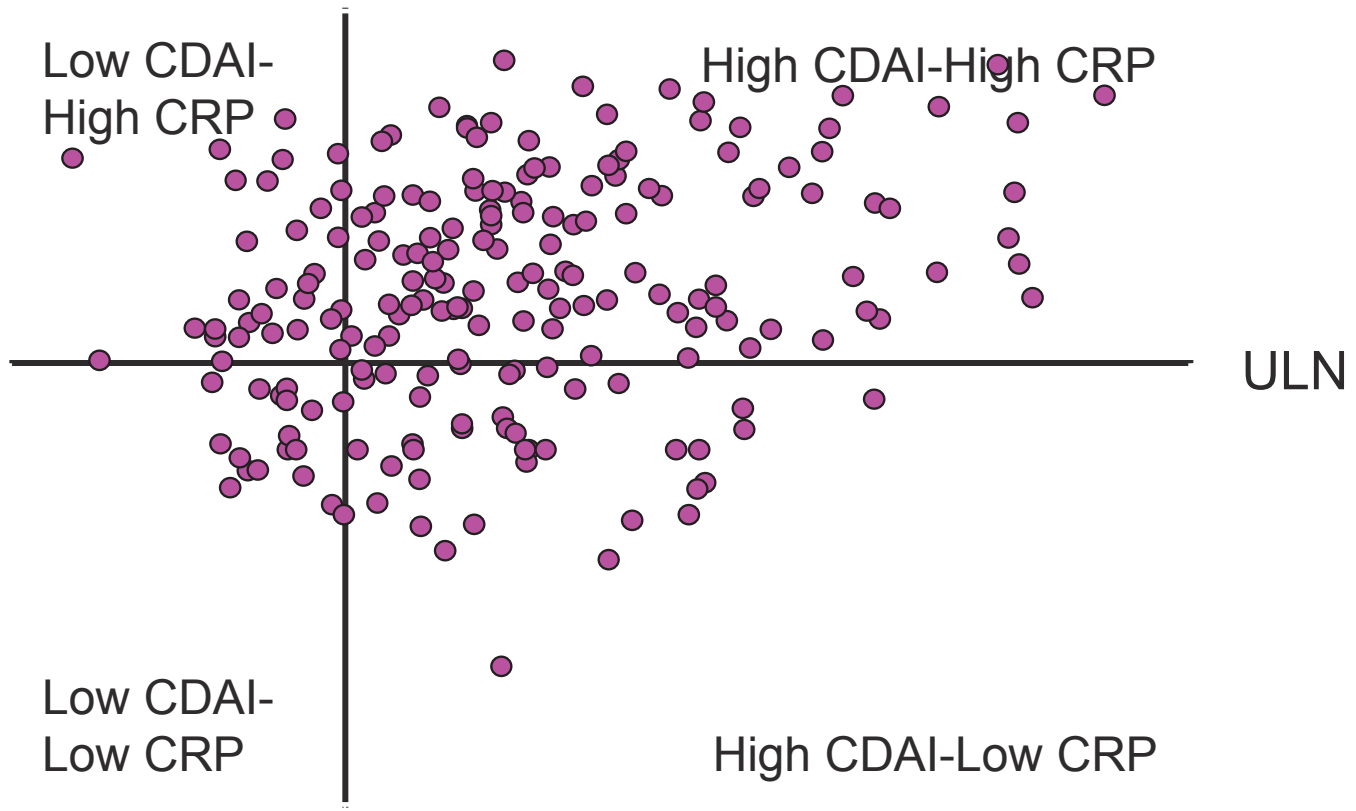
CDAI/CRP relationship: managing IBD patients



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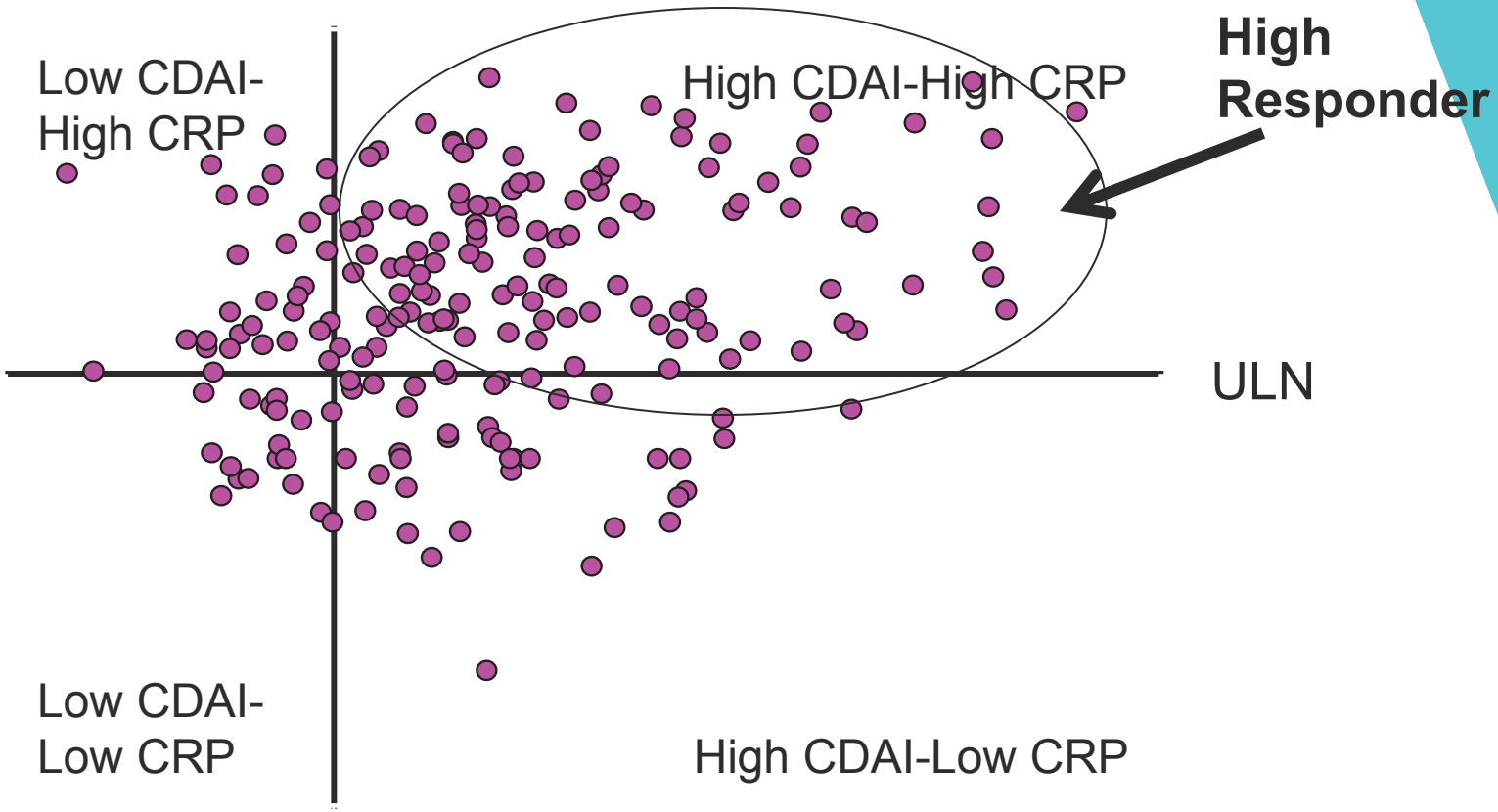
CDAI/CRP relationship: managing IBD patients



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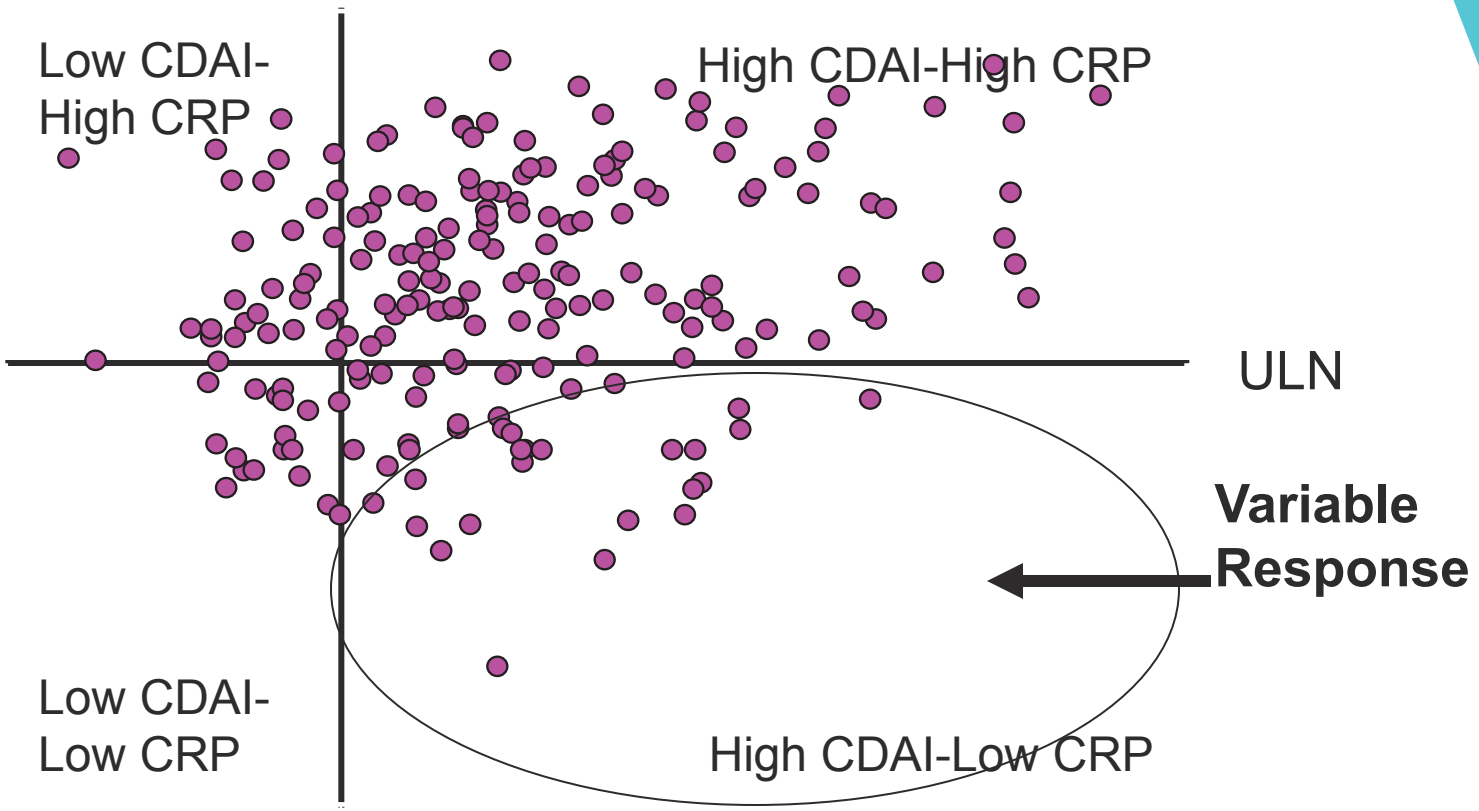
CDAI/CRP relationship: managing IBD patients



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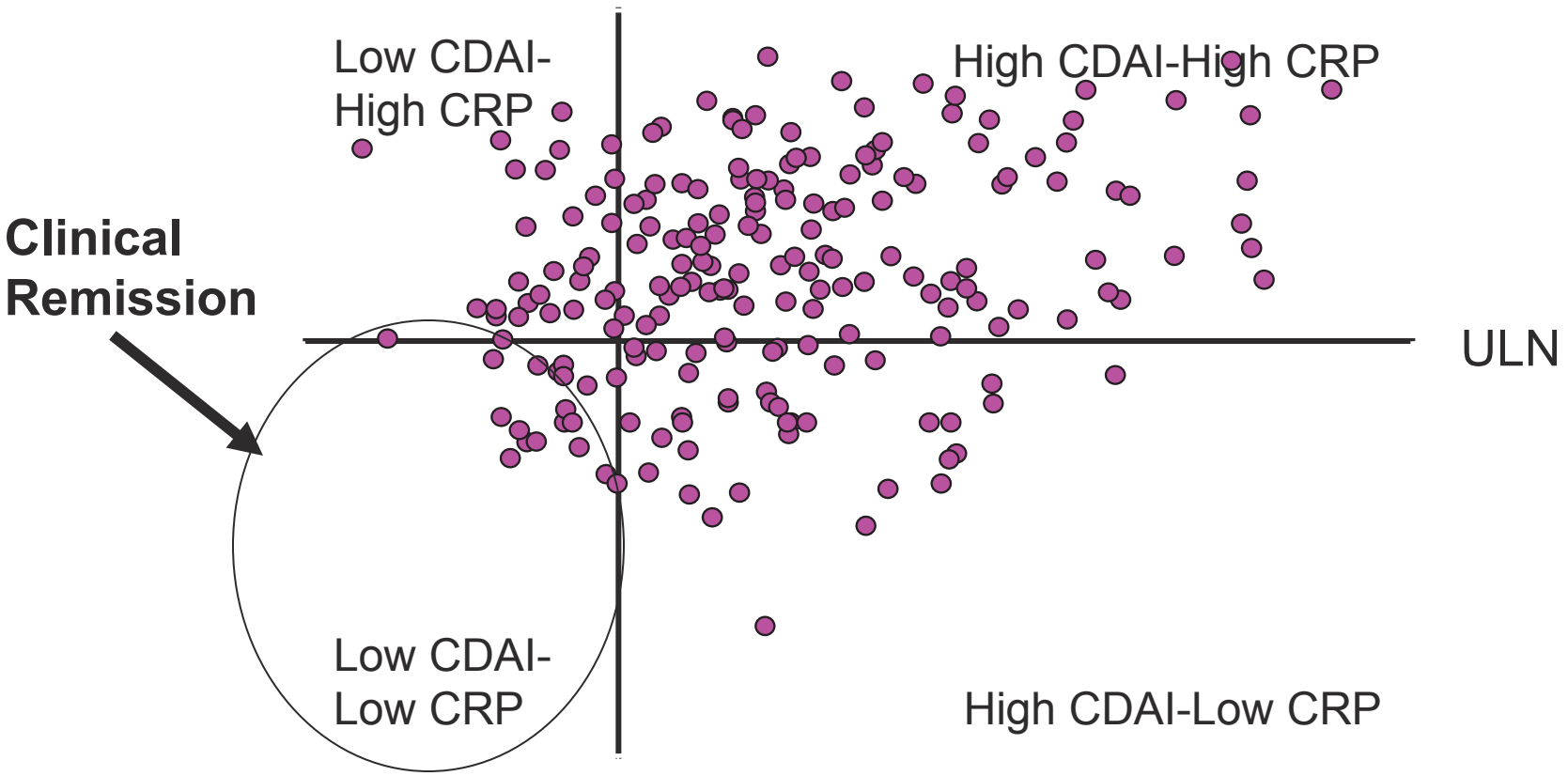

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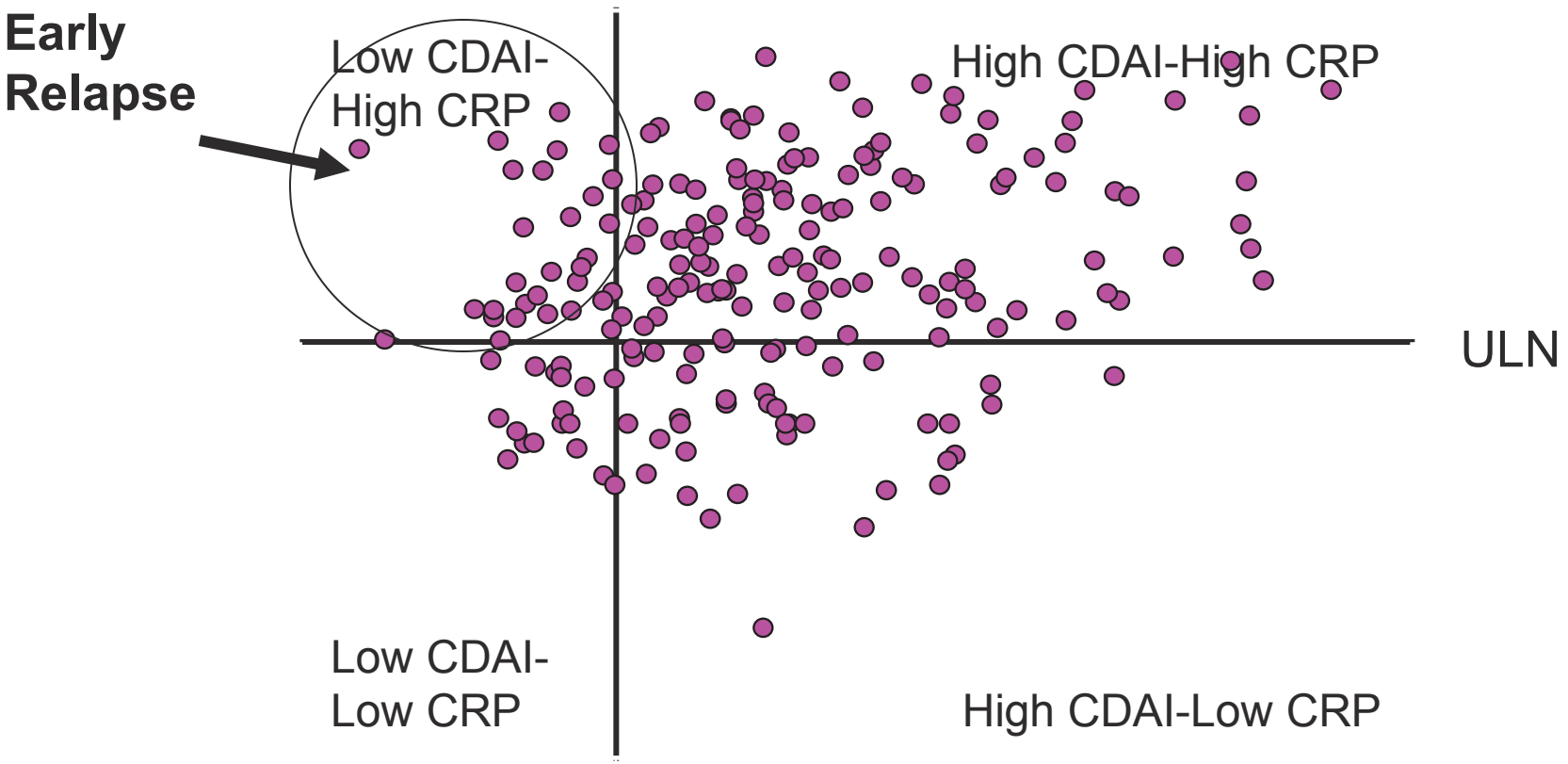
CDAI/CRP relationship: managing IBD patients



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Conclusions CRP

- ✓ CRP is a predictor of placebo response
- ✓ Efficacy signals in recent clinical trial may have been obscured by placebo responses in CRP_{low} patients.

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EMerging Biomarkers in IBD (EMBARK study)

- UC (n=107) and CD (n=157) underwent ileocolonoscopy (ICO) and subset of CD (n=66) underwent computed tomography enterography (CTE).
- Serum and fecal biomarkers were evaluated and correlated with inflammation scores.

Faubion et al. Am. J Gastroenterology 2013, 108:1891

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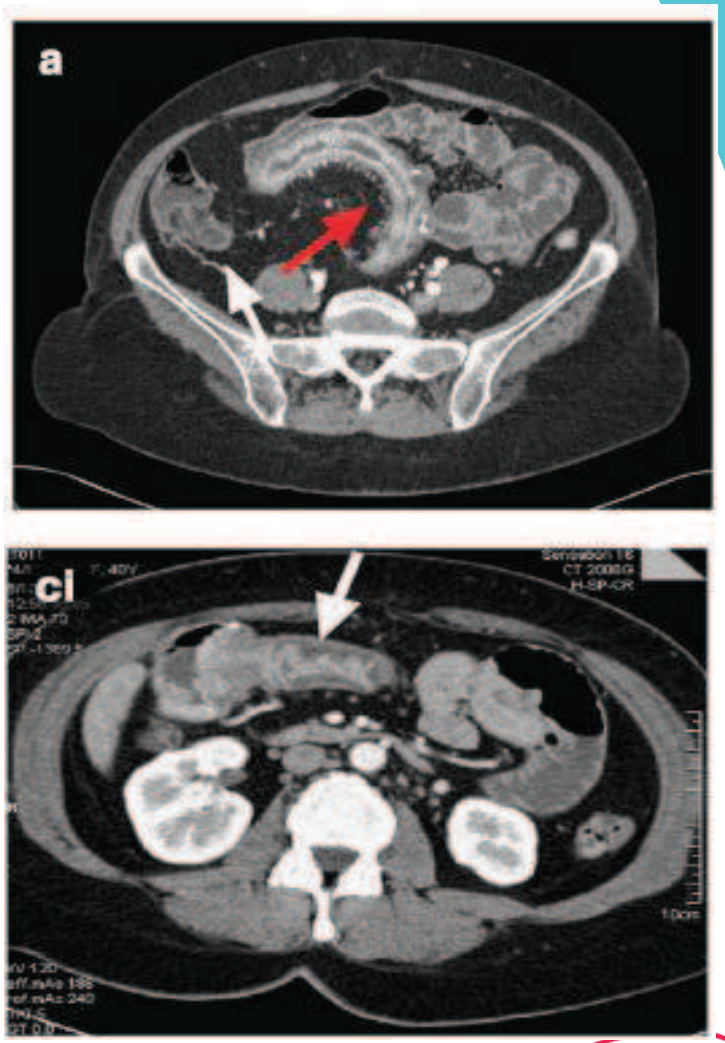
UC patients: Evaluation of different biomarkers in correlation with endoscopic scores

Biomarker	*P* value	Biomarker	*P* value
Fecal lipocalin	1.48E−07	MMP1	0.005
Fecal lactoferrin*	7.67E−07	CXCL1*	0.022
Fecal calprotectin*	3.15E−06	YKL40*	0.034
CRP*	2.84E−05	MSP	0.124
MMP9	0.0001	sICAM-1	0.163
MMP8	0.0003	Anti-ASCA IgG*	0.167
MMP3*	0.001	IFN-γ*	0.181
RegIII*	0.003	MMP7*	0.234
SAA1*	0.003		

CRP, C-reactive protein; IFN, interferon; IgG, immunoglobulin G; MMP, matrix metalloproteinase; sICAM-1, soluble intracellular adhesion molecule-1; UC, ulcerative colitis.

All biomarkers were measured in blood unless specified as fecal biomarkers. Biomarkers with a skewed distribution were log transformed before analysis and are marked with an asterisk (*).



Faubion et al. Am. J Gastroenterology 2013, 108:1891

CD patients: Evaluation of different biomarkers in correlation with endoscopic scores

Biomarker	r	P value	Biomarker	r	P value
Fecal calprotectin*	0.613	5.65E−08	MADCAM	−0.199	0.112
Fecal lactoferrin*	0.567	1.06E−06	IL-6	0.18	0.152
Fecal lipocalin	0.504	1.86E−05	Bv8	0.172	0.17
MMP3*	0.408	0.001	MSP	−0.168	0.18
CXCL13*	0.305	0.014	Anti-ASCA IgG	0.163	0.193
MMP9	0.283	0.023	sCD14	0.162	0.2
PDGF-BB	0.278	0.025	β2 Microglobulin	0.159	0.205
IL-22*	0.26	0.036	Anti-Cbir Ig	0.149	0.237
sIL2R*	0.224	0.072	MMP1	0.134	0.29
CRP	0.21	0.091			

CRP, C-reactive protein; CTE, computed tomography enterography; ICO, ileocolonoscopy; IFN, interferon; IgG, immunoglobulin G; IL-6, interleukin-6; MMP, matrix metalloproteinase; PDGF-BB, platelet derived growth factor-BB.

All biomarkers were measured in blood unless specified as fecal biomarkers. Biomarkers with a skewed distribution were log transformed before analysis and are marked with an asterisk (*).

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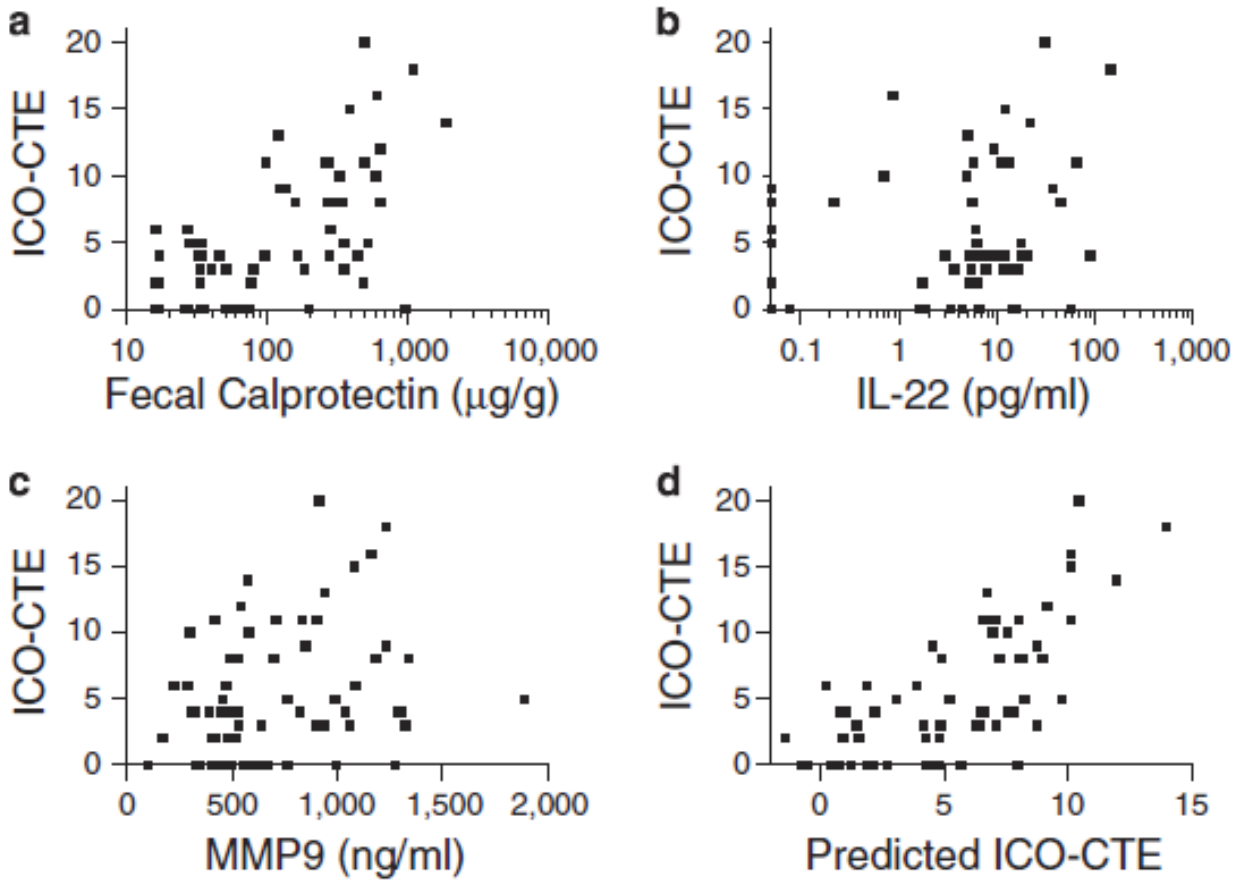
Relationship of top biomarkers with ICO-CTE



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What is new here?

- ✓ Combining ICO with CTE provides a more accurate assessment of the overall inflammatory burden
- ✓ Fecal Calprotectin and serum MMP9 correlated with the disease activity in UC patients
- ✓ Fecal Calprotectin, serum MMP3 and serum IL-22 are directly related with increased severity of the CD patients

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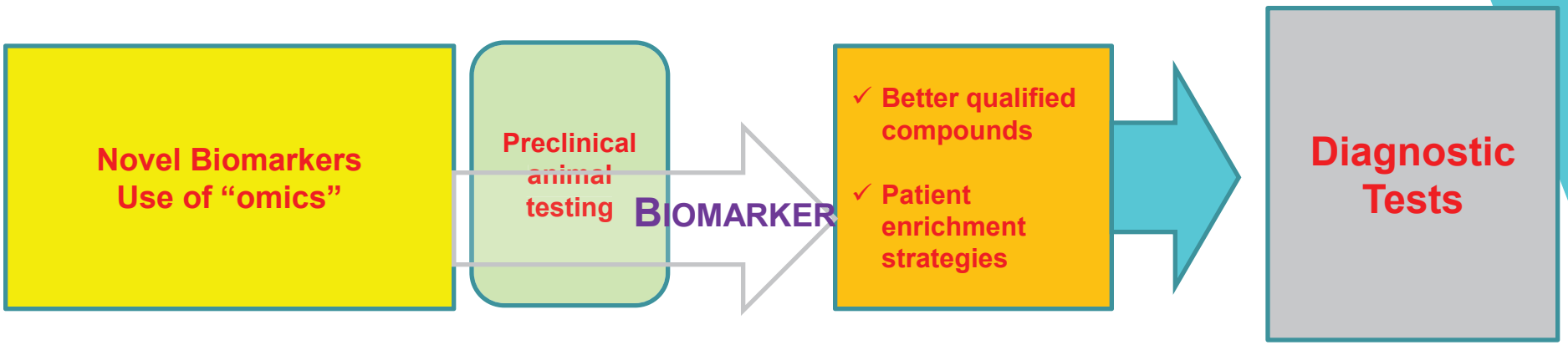
Biomarkers connect Bench to Bedside



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Questions?

"I'm afraid that your irritable bowel syndrome has progressed. You now have furious and vindictive bowel syndrome."

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