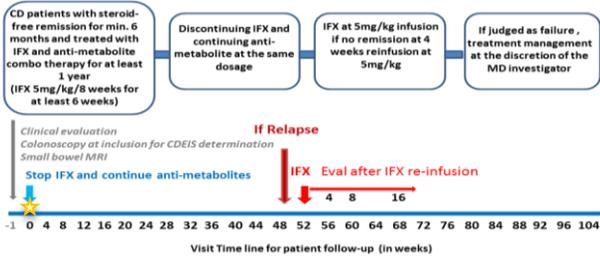


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Introduction and objectives: In Crohn's disease (CD), there is a discrepancy between clinical activity of the disease (symptoms) and intestinal healing. However absence of tissue healing (CDEIS $\geq 3^5$) is associated with the risk of relapse and tissue damage progression. Endoscopy is costly and invasive. Hence serum biomarkers correlating with intestinal healing could improve disease management. We aimed to identify potential biomarkers associated to CD mucosal healing by a shotgun proteomics label free study done on the STORI trial¹ sera analysed at base line.

STORI¹ clinical trial design



Aim: Evaluation of relapse duration when patient reach remission under infliximab and anti-metabolite treatments and following IFX treatment withdrawal.

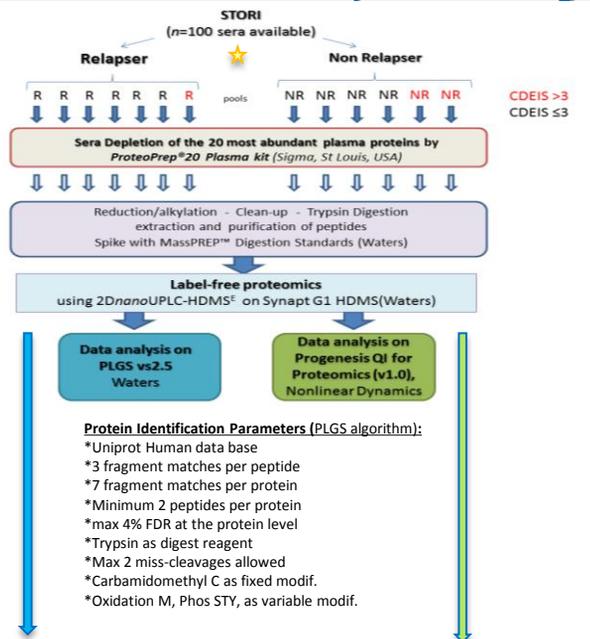
Results: For complete study, see Louis *et al*¹.

After a median 28 months follow-up, 50% of patients relapsed. Mucosal healing is monitored by endoscopy at base line and CDEIS evaluation (CDEIS ≤ 3 : mucosal healing, >3 : no mucosal healing). Serum sample are taken at base line.

Patients information

A. Patients clinical information (n=115)	
Demographic and clinical characteristics	
Male, n(%)	49 (43)
Age, yr, median (inter-quartile range)	32 (26-39)
Disease duration, yr, median (inter-quartile range)	7.8 (4.5-11.9)
Active smoker, n (%)	45 (39)
Disease site (n=114)	
Ileal, n (%)	14 (12)
Colonic, n (%)	36 (31)
Ileocolonic, n (%)	64 (56)
Previous surgical resection, n (%)	25 (22)
CDAI median (interquartile range)	37 (19-61)
Treatment history	
Antimetabolite treatment	
Methotrexate, n (%)	19 (17)
Azathioprine/6-Mercaptopurine, n (%)	96 (83)
Duration of IFX treatment, yr	2.2 (1.5-3.1)
Endoscopy	
CDEIS median (interquartile range)	0.7 (0.0-3.0)
Patients with remaining ulcers, n (%)	39 (34)
Patients with remaining deep ulcers, n (%)	4 (3)

Material and methods - Experimental design



Protein Identification Parameters (PLGS algorithm):

- *Uniprot Human data base
- *3 fragment matches per peptide
- *7 fragment matches per protein
- *Minimum 2 peptides per protein
- *max 4% FDR at the protein level
- *Trypsin as digest reagent
- *Max 2 miss-cleavages allowed
- *Carbamidomethyl C as fixed modif.
- *Oxidation M, Phos STY, as variable modif.

Differential analysis parameters:

- *Normalization: Gaussian all peptides
- *Ratio minimum: 1.5
- *Significant P value only (0 to 0.5 or 0.95 to 1)
- *Frequency of observation: 4/6 R or 4/7 NR or all pool replicates for the mucosal healing question

Differential analysis parameters:

- *Normalization: mean of all identified peptides
- *Ratio minimum: 1.5
- *Significant Anova P value only (≤ 0.05)
- *Frequency of observation: 80% of samples of all groups
- * 1 – Quantif. on peptides unique to 1 protein assignement (=« non conflictual peptides »).
- * 2 – Quantif. on all peptides

Results of differential analysis

PLGS vs2.5:

R vs NR = 18 Proteins of Interest
 CDEIS >3 vs CDEIS ≤ 3 = 17 POI



Progenesis QI for Proteomics v1.0:

R vs NR = 39 POI and 22 POI \approx distribution

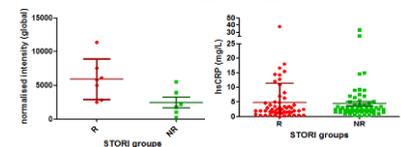


CDEIS >3 vs CDEIS ≤ 3 = 8 POI

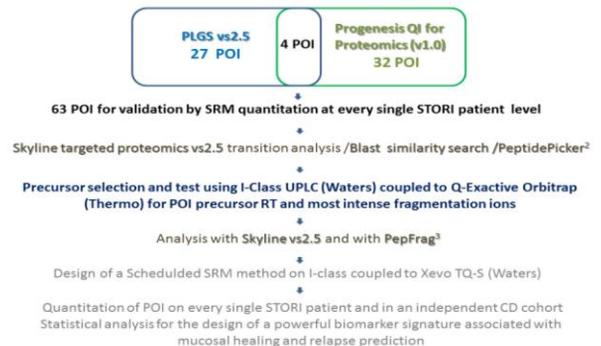


*C-Reactive Protein

(hsCRP level) is associated to relapse prediction, through a time dependent analysis together with leukocyte count, fecal calprotectin and HB levels¹



Perspectives



⁵ CDEIS = Crohn Disease Endoscopic Index of Severity

¹ Louis et al., Maintenance of Remission Among Patients with Crohn's on Anti-Metabolite Therapy after Infliximab Therapy is Stopped, in Gastroenterology, 2012 Jan;142(1):63-70.

² Y. Mohammed, et al., PeptidePicker: a Scientific Workflow with Web Interface for Selecting Appropriate Peptides for Targeted Proteomics Experiments, Journal of Proteomics, 2014, Vol106, p151-161.

³ NCRP, <http://prowl.rockefeller.edu/prowl/pepfra.htm/>