

Two Signatures For A New Blood-based Test For Colorectal Cancer Screening

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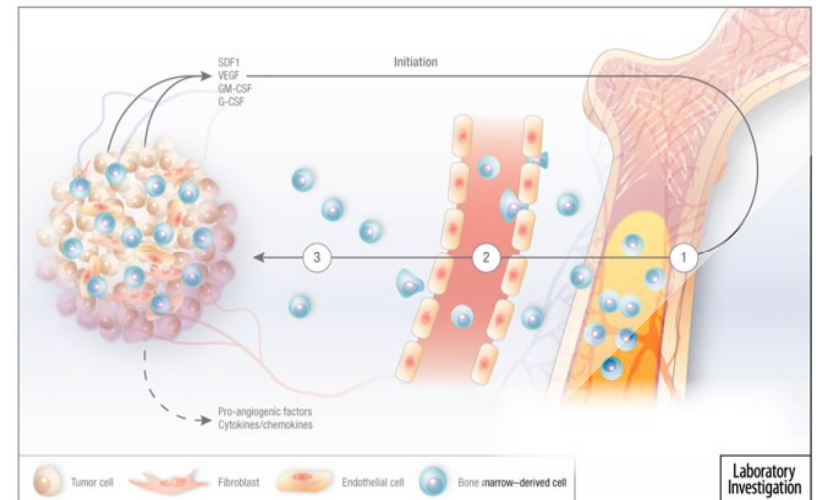
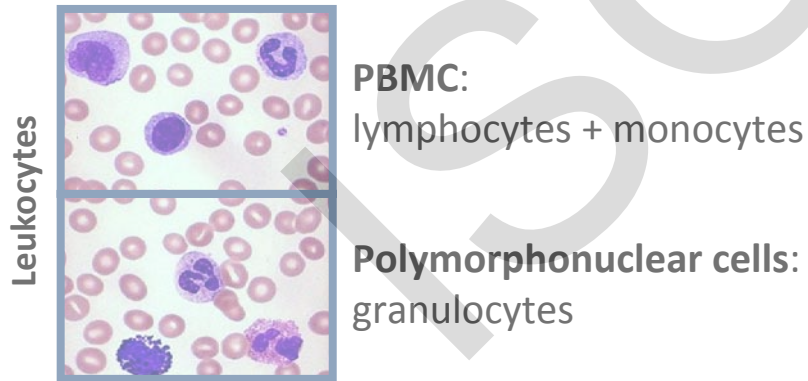
(DIAGNOPLEX SA, Lausanne, Switzerland)

Colorectal cancer screening

- **Colorectal Cancer (CRC)=650'000 death per year**
3rd most common cancer in industrialized countries
- **The paradox:**
 - Successfully cured when diagnosed in early stage: Adenoma (Polyp), Stage I-II
 - Colonoscopy and sigmoidoscopy: high sensitivity and specificity
 - Colonoscopy and sigmoidoscopy: invasive, poor compliance...
 - Non invasive tests (FOBT): low sensitivity and specificity
 - Often, CRC diagnosed in advanced stage (III-IV)
- **Non-invasive, cost effective and robust primary screening tests** must be developed to have a real impact on the mortality of CRC
- **Specific biomarkers discovery** seems to be the future of CRC diagnostic tests

COLOX: a CRC primary screening test

- **COLOX=Molecular diagnostic test** - developed by **Diagnoplex SA**:
To detect neoplastic lesions (carcinoma) and pre-neoplastic lesions (adenoma)
- **COLOX=Blood-based test based on tumor-host response concept**:
 - **Secretion of variety of cytokines/chemokines** by tumor and tumor stromal cells from early stages: mobilization, circulation, trans-endothelial migration and homing of bone marrow derived cells



- **Searching for different expression profiles in PBMC/Polymorphonuclear cells**
Comparing healthy, polyps and CRC

- **Candidate Gene Approach:**

Based on literature review and focus on the tumor-host response

Principally, we have considered:

- Genes involved in **early stage of carcinogenesis**,
- Genes involved in **specific CRC development pathway**,
- Genes involved in **immune response against tumor**,
- Genes involved in **cell-to-cell communication and interaction**,
- And few genes involved in **tumor cell circulation**

Permit to define biomarkers to **compare healthy subject with CRC patients**

- **Whole transcriptome analysis:**

Illumina® TAG profiling

Using **33 RNA samples** from **healthy subjects** and **patients with adenoma**

Permit to define biomarkers to **compare healthy with adenoma patients**

- **At the end, 103 genes were selected**

Feasibility study and biomarkers selection

- **COLOX biomarkers selection based on a pilot study: 92 subjects**
 - **CRC group, stage I to IV: CAR (n=21; 5,3,5,8)**
 - **Adenoma group, larger than 1 cm: POL (n=30)**
 - **Healthy or Control group: CON (n=41)**

Age mean=55 year old; Woman/Man=0.98

Exclusion criteria: drugs and alcohols consumers, other tumor, HNPCC and FAP

- **COLOX technology and workflow**



Blood Collection & PBMC Separation



RNA Extraction & Reverse Transcription



**“Single channel multiplex
quantitative PCR” (scmqPCR)**

Multiplex Pre-Amplification PCR

+

Specific Quantitative PCR with absolute quantification

COLOX biomarkers: biological functions

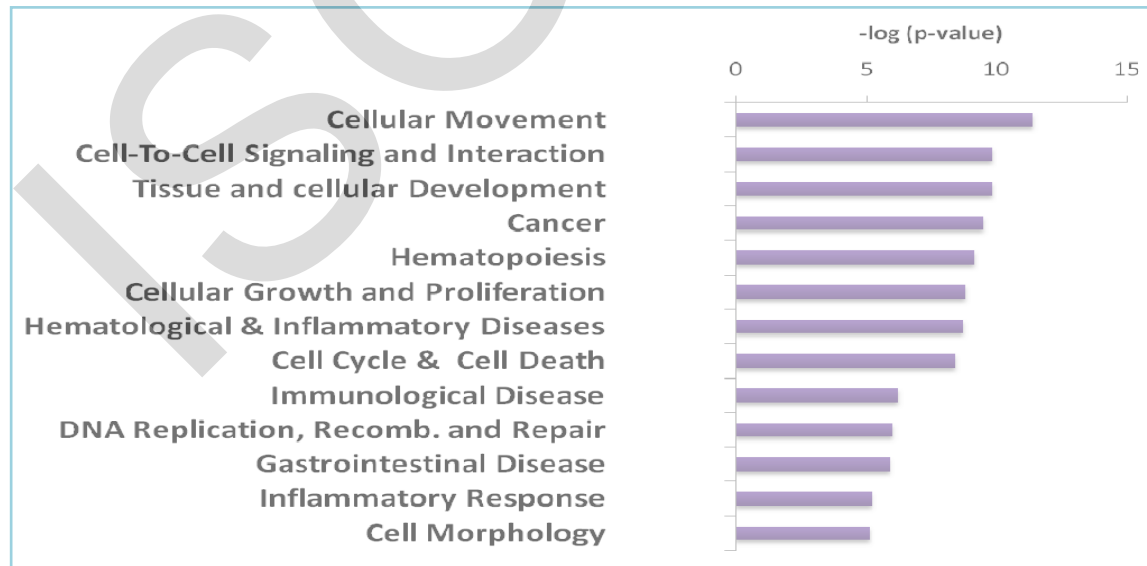
- **103 genes tested** on the cohort of 92 samples: **Training Set**

Univariate + Multivariate analysis were performed on PCR data to select the best

- **60 biomarkers validated** (with p value < 0.01 for most of the methods)

COLOX biomarkers panel can be represented by biological and functional categories:

Ingenuity Pathway Analysis (IPA) software package:

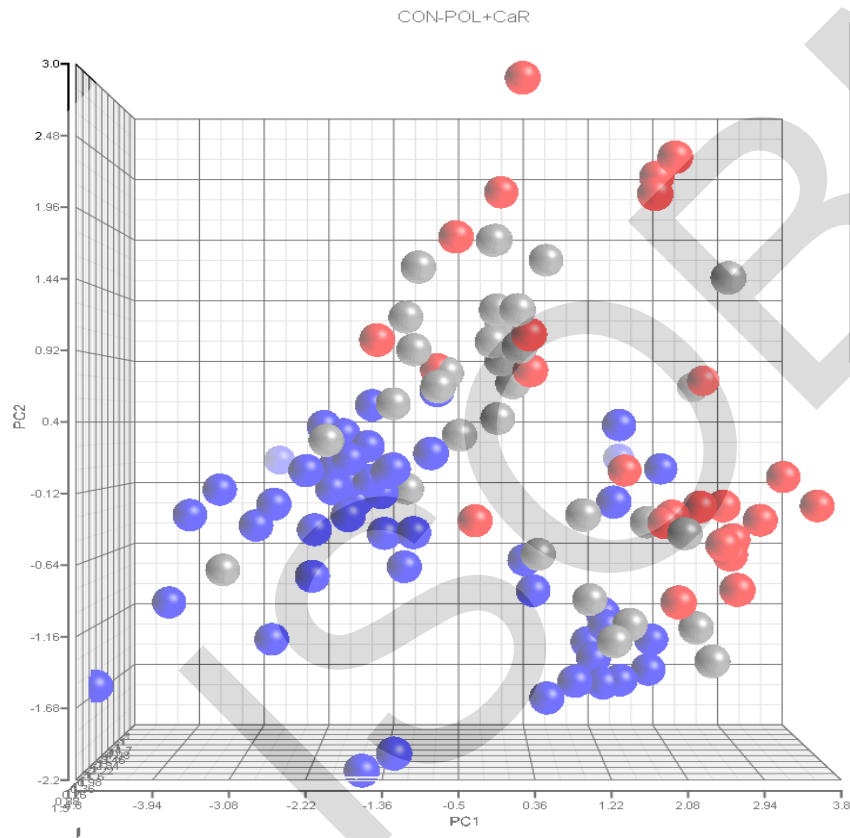


p-values measure the likelihood that the association between a set of COLOX biomarkers and a given IPA functional category is random

NI1 best what? best combination of genes?
Natsuko Imaizumi; 03.09.2010

ISOBIM

- Principal Component analysis based on the 6 best genes



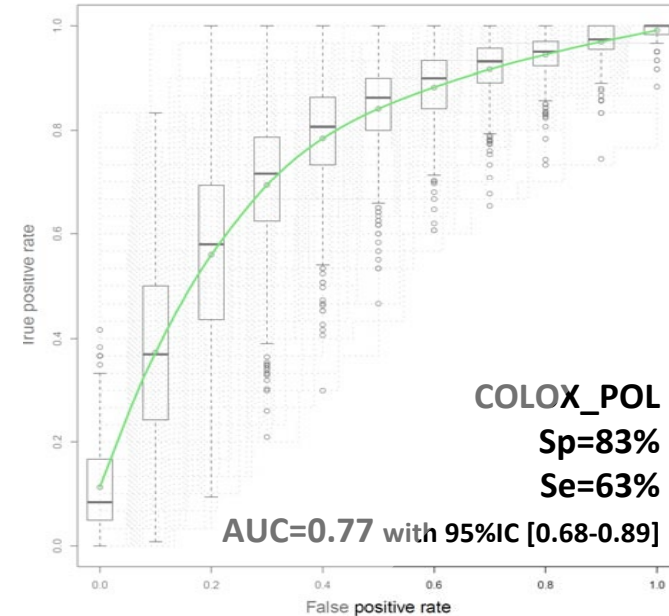
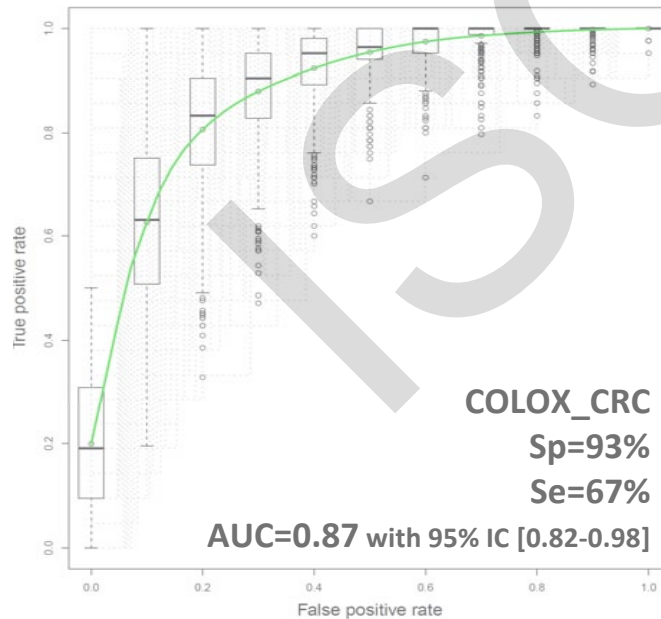
3D PCA performed with
Partek Discovery Suite Software

- Determine 2 problems: **CON vs CAR // CON vs POL**
- Build **statistical models** based on adapted **logistic regression equations**

- **2 different signatures:**
 - **COLOX_CRC** for **CAR** detection
 - **COLOX_POL** for **POL** detection
- **Model validation by Random resampling** (*Example for Glmpath*)

Method based on the bootstrap technique in the non-overlapped way

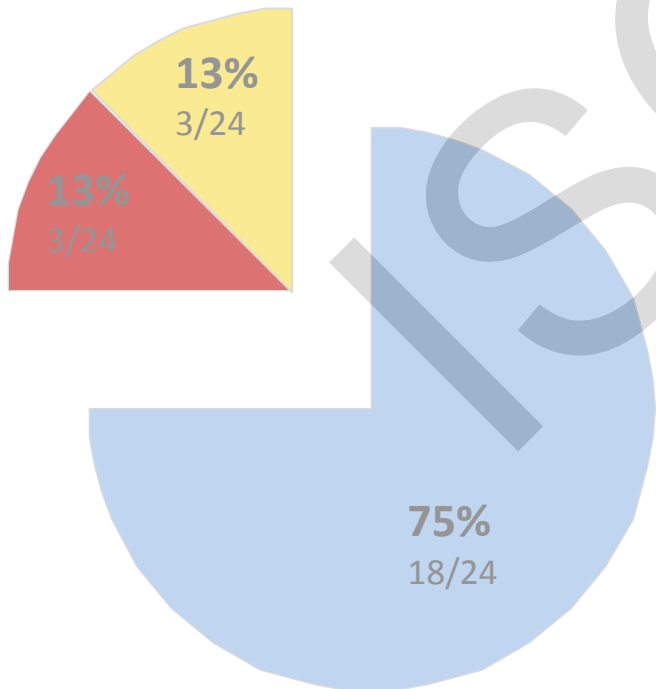
ROC Curve analysis for



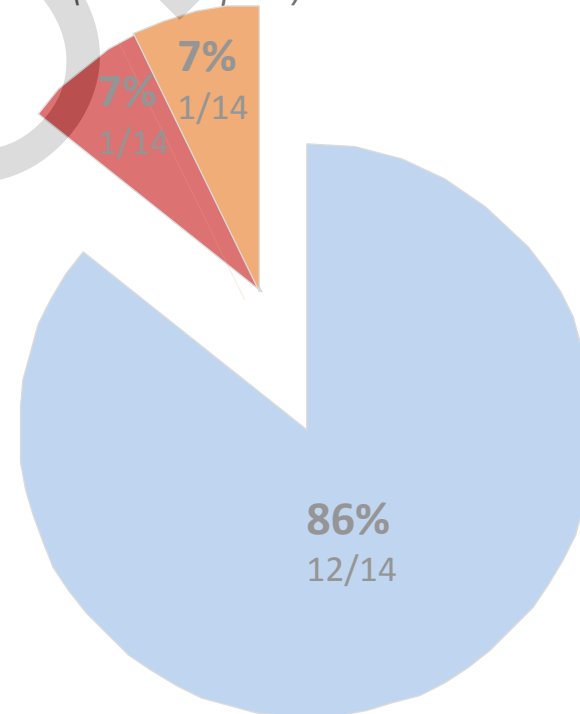
COLOX cancer and organ specificity

- **Both signatures (COLOX_CRC and COLOX_POL) were tested on independent samples (test set):**
 - **Inflammatory Bowel Disease Group: IBD (n=24)**
 - **Others Tumors Group: OT (n=14)**

IBD results:
(with Glmpath)



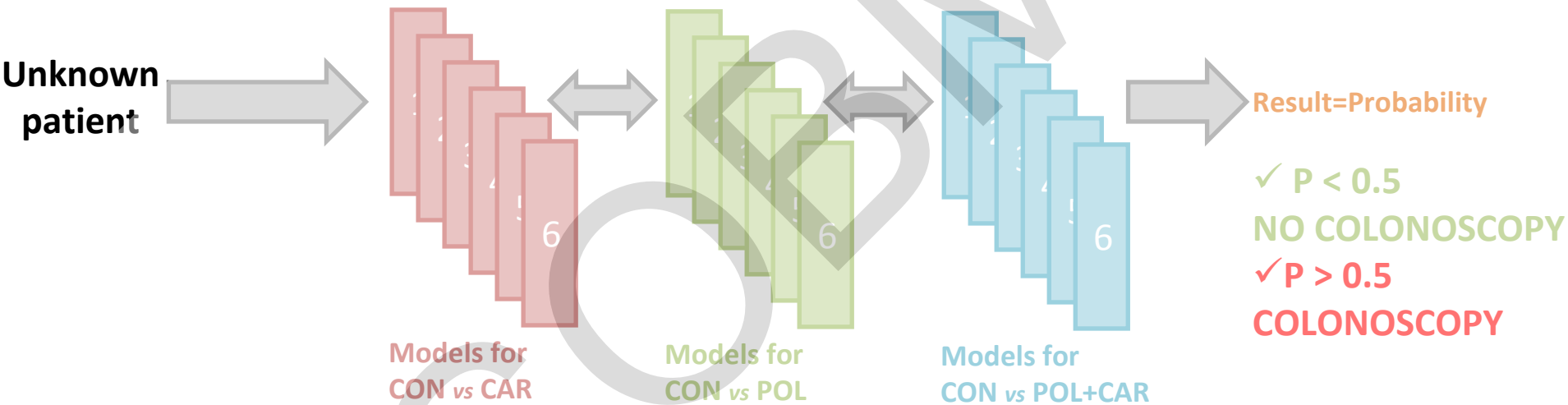
OT results:
(with Glmpath)



- ▲ Patients NEG with both COLOX
- ▲ Patients POS with both COLOX
- ▲ Patients POS with COLOX_CRC
- ▲ Patients POS with COLOX_POL

- **COLOX is a MGMC concept: Multi-Genes & Multi-Classifer**

Several statistical models can be applied in parallel for decision



- Validate COLOX MGMC by random resampling to predict performances

- Test COLOX MGMC on independent samples of test set:

IBD group, OT group and “blinded samples” group (including CON, POL, CAR, IBD) (n=50)

- **Clinical validation of COLOX**

COLOX: the future for CRC detection?

- **COLOX specificity and sensitivity meet clinical expectations**
- **COLOX high organ and disease specificity**
- **COLOX is simple, fast, non invasive, affordable and scalable**
- **A multi-centric clinical study is ongoing to validate COLOX results**

Diagnoplex Team:

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All the personal and nurses of the endoscopy department...

And all the patients...