Biomarkers predittivi di risposta al trattamento radiochemioterapico: studio pilota monoistituzionale

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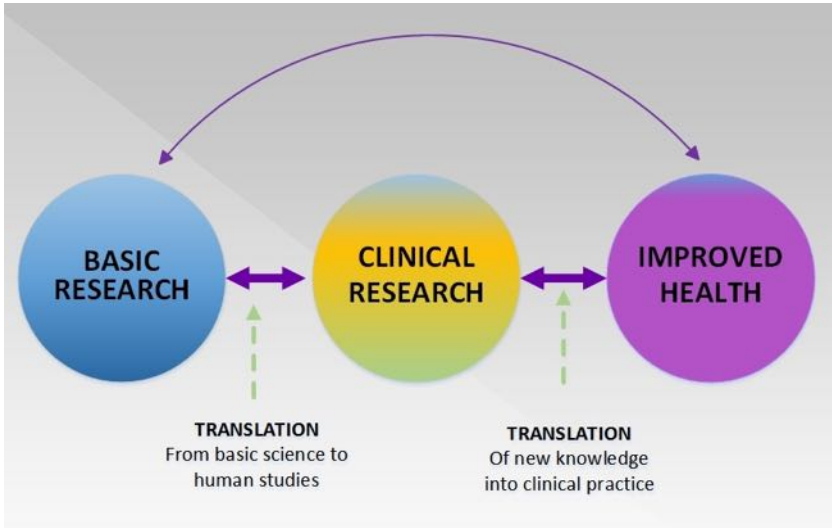
BACKGROUND: Translational Research

COMMENTARY

The Meaning of Translational Research and Why It Matters

Steven H. Woolf, MD, MPH

JAMA, January 9/16, 2008



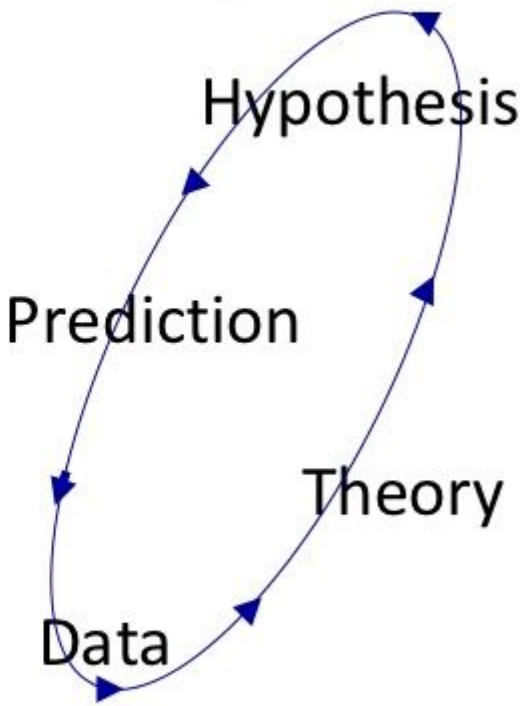
- Refers to the "from bench to bedside" enterprise of harnessing knowledge from basic sciences to produce new drugs, devices and treatment options for patients.

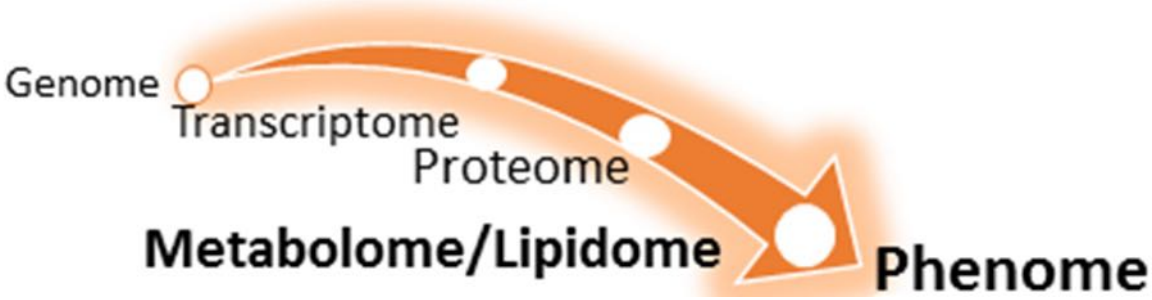
- Effective translation of the new knowledge, mechanisms and techniques generated by advances in basic science research into new approaches for prevention, diagnosis and treatment of disease is essential for improving health.

BACKGROUND: Omics Research

DATA DRIVEN APPROACH

This approach consider a large number of data which allows "**hypothesis generating**" rather than "hypothesis driven" method.





BACKGROUND: LIPIDOMICS

Phopholipids and cancer

Lipids play many essential roles in cellular functions:

- survival,
- Proliferation,
- death.

They are involved in:

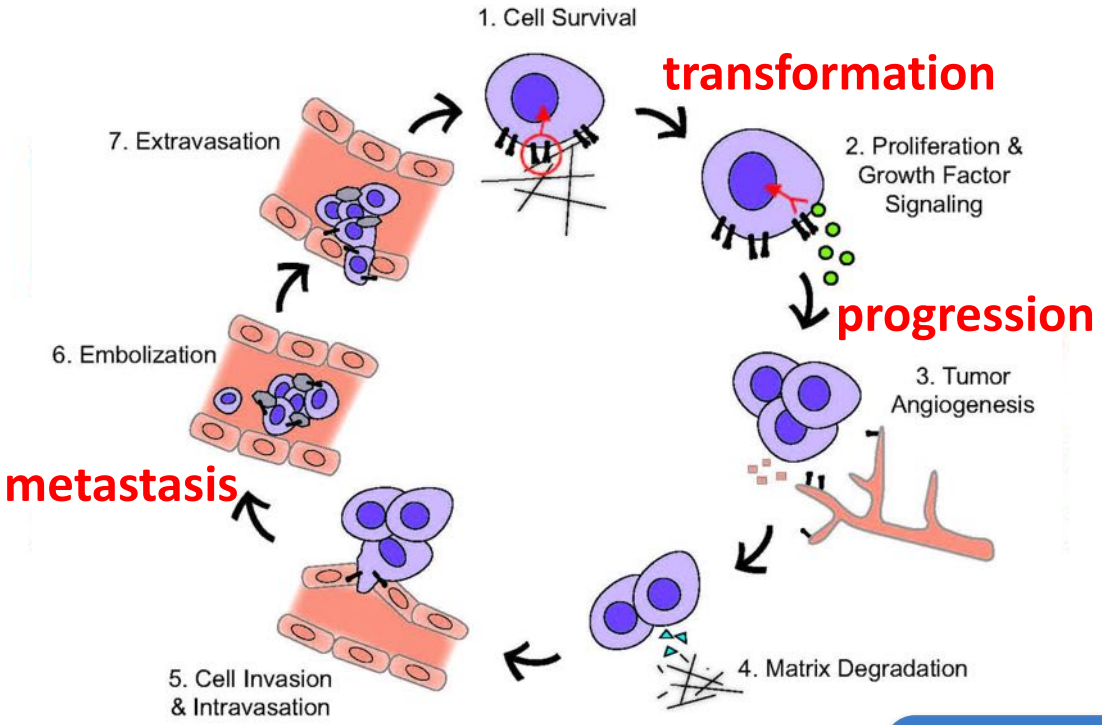
- chemical-energy storage,
- cellular signaling,
- cell membranes,
- cell–cell interactions.

International Journal of *Molecular Sciences*

Review

Advances in Lipidomics for Cancer Biomarkers Discovery

Francesca Perrotti [1,2], Consuelo Rosa [1,2], Ilaria Cicalini [3,4,5], Paolo Sacchetta [4,5], Piero Del Boccio [3,4,5], Domenico Genovesi [1,2] and Damiana Pieragostino [4,5,*]



Post-translational modifications

homeostasis

adhesion

migration

Energy storage

Signal trasduction

Vesicular trafficking

apoptosis

Astigarraga et al., 2008; Shevchenko & Simons, 2010; Wenk et al. 2005

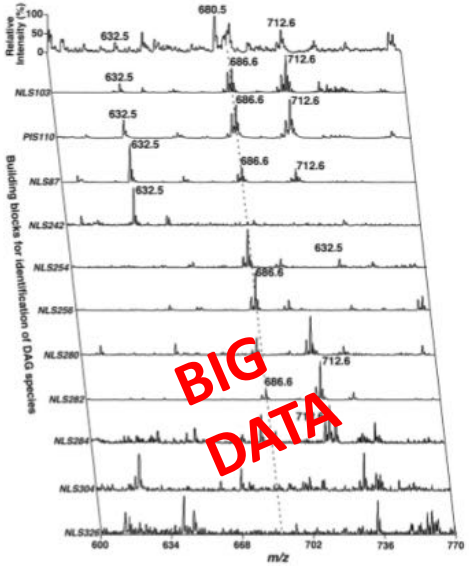
BACKGROUND: Data driven approach

1. Discovery study:

NON-TARGETED profiling by sophisticated bioinformatic tools

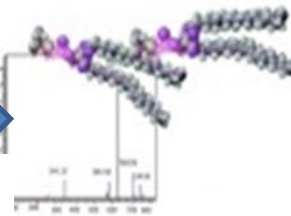
Liquid Chromatogr

Mass Spectrometry



Data processing

Statistical analysis



NEW BIOMARKERS

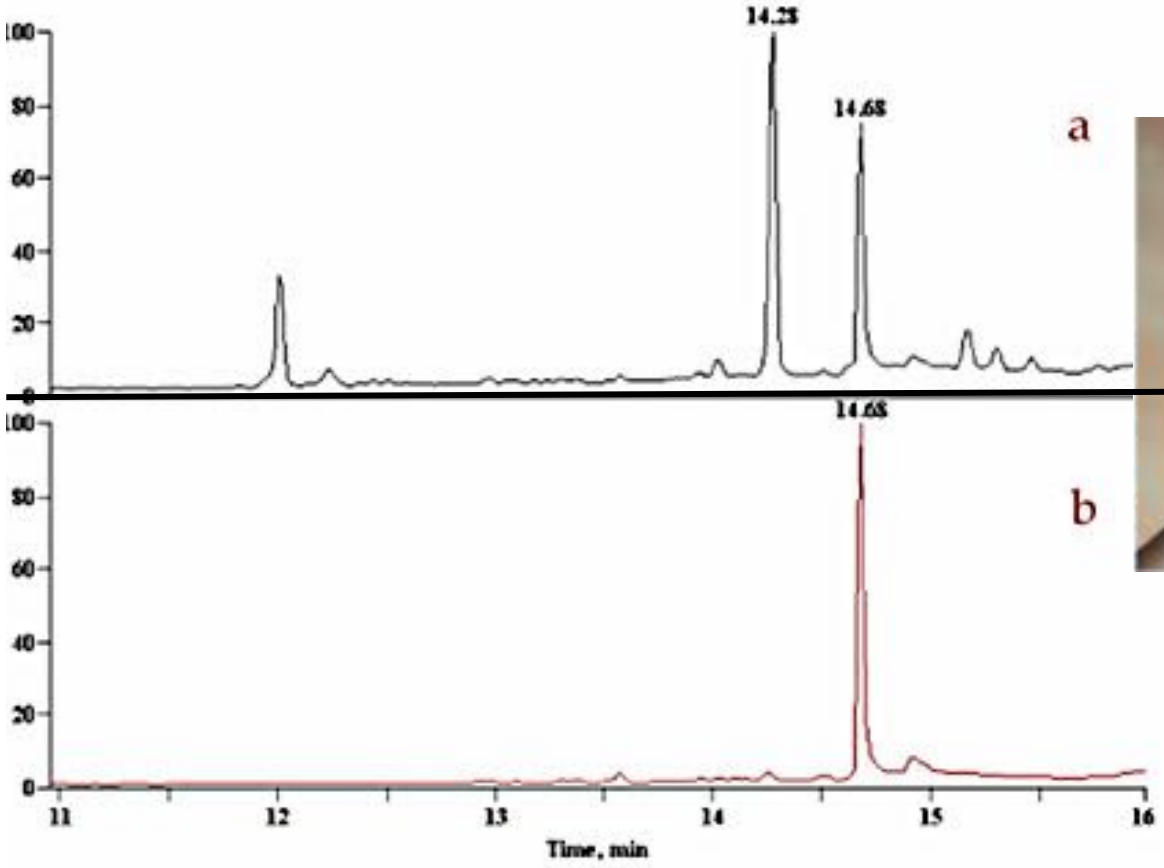
1. Discovery study:

NON-TARGETED profiling by sophisticated bioinformatic tools

APPROCCIO CLASSICO

FASE QUANTITATIVA

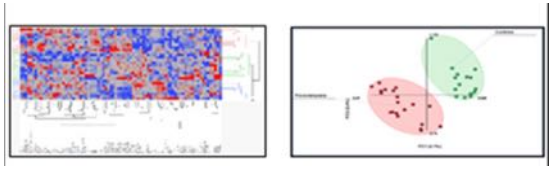
Separazione cromatografica



….SUMMARIZING….

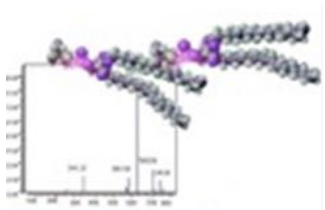
Lipidomic Discovery study

1. Un-targeted Analysis
2. Targeted Analysis



Novel hypotheses generation

3. New candidate biomarkers



Validation study

4. Indipendent validation set
5. Functional validation

Serum lipidomic study reveals potential early biomarkers for predicting response to chemoradiation therapy in advanced rectal cancer: A pilot study

Piero Del Boccio [a,b], Francesca Perrotti MD [c,d], Claudia Rossi [b,e], Ilaria Cicalini [a,b], Sara Di Santo MD [c,d], Mirco Zucchelli [b], Paolo Sacchetta [b,d], Domenico Genovesi [c,d], Damiana Pieragostino [b,e,*]

advances in radiation oncology
www.advancesradonc.org

In press

Purpose:

✓ **to highlight a typical lipidomic signature, able to predict tumor response to chemoradioterapy in patients with advanced rectal cancer**

✓ **Personalized treatment**

pCR after CRT demonstrated a significantly improved prognosis with implications for an organ preservation strategy.

For non-responding patients, other therapeutic strategies should be considered, without delaying surgery and sparing patients from useless and potentially toxic CRT

Maas M, J Clin Oncol. 2011; 29: 4633-40.

Pucciarelli S, Dis Colon Rectum. 2013; 56: 1349-56.

✓ **Translational relevance**

LIPIDS are fundamental mediators of inflammation, proliferation, and apoptosis.

The involvement of lipids in **radioresistance of rectal cancer** is also demonstrated by the *modulation of lipid bindings proteins.*

Schneider G, MCR. 2014; 12:1560-73.
Qiu J, PloS one. 2014;9:e90062.
Long J, Tumour biology. 2015.

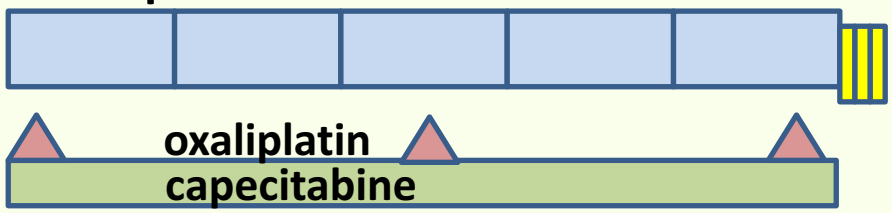
Materials and methods: Experimental Design

N= 19 pts; 18 evaluables pts

Prospective global analysis of lipids in 54 sera from 18 LARC patients

N=4 pts

RT sequential boost

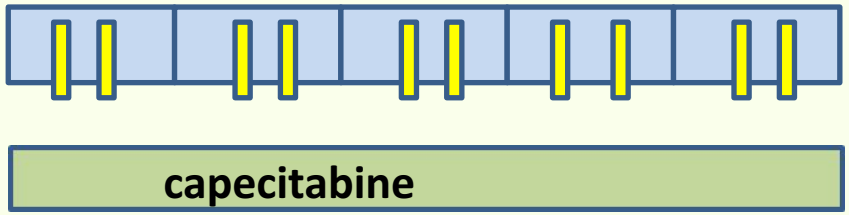


45 Gy (1,8 Gy/fr)
5.4-Gy (1,8 Gy/fr) boost

Capecitabine 1300 mg/mq/die
Oxaliplatin 130 mg/mq (d 1-19-38)

N=14 pts

RT concomitant boost



45 Gy (1,8 Gy/fr)
10 Gy (1 Gy/fr twice weekly) boost

Capecitabine 1650 mg/mq/die

T=0 **T=14** **T=28**

SERUM SAMPLES COLLECTION

Samples were collected at **3 time points**:
before (T0),
at 14° day (T14),
at 28°day (T28) of CRT

Materials and methods

Patients characteristics

- 10 pts: NRP
- 8pts: RP

TRG scale according to Mandard's classification

PTs	STADIO CLINICO	VOLUMI: PELVI + BOOST	TRG (sec. Mandard)	CATEGORIA
1	IIIB	CONCOMITANTE	2	RESP
2	IIIB	CONCOMITANTE	1	RESP
3	IIIA	CONCOMITANTE	2	RESP
4	IIIB	CONCOMITANTE	4	NON RESP
5	IIIA	SEQUENZIALE	2	RESP
6	IIIB	SEQUENZIALE	3	NON RESP
7	IIIB	CONCOMITANTE	4	NON RESP
8	IIIB	SEQUENZIALE	2	RESP
9	IIIB	SEQUENZIALE	1	RESP
10	*IIIB*	*CONCOMITANTE*	*2*	*RESP*
11	IIIB	CONCOMITANTE	4	NON RESP
12	IIIB	SEQUENZIALE	2	RESP
13	IIIB	SEQUENZIALE	2	RESP
14	IIIb	CONCOMITANTE	3	NON RESP
15	IIIB	CONCOMITANTE	3	NON RESP
16	IIIB	CONCOMITANTE	3	NON RESP
17	II	CONCOMITANTE	2	RESP
18	IIIB	CONCOMITANTE	3	NON RESP
19	IIIB	CONCOMITANTE	3	NON RESP

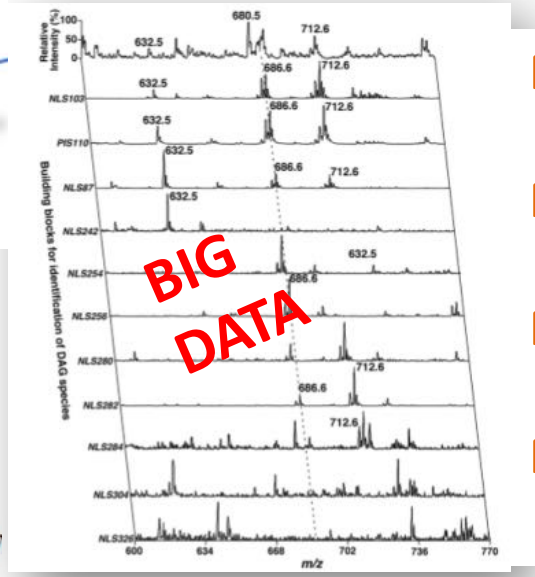
Materials and methods

2.Data acquisition

at T=0 time point

Mass Spectrometry

Liquid Chromatography



PEs:8761 signals

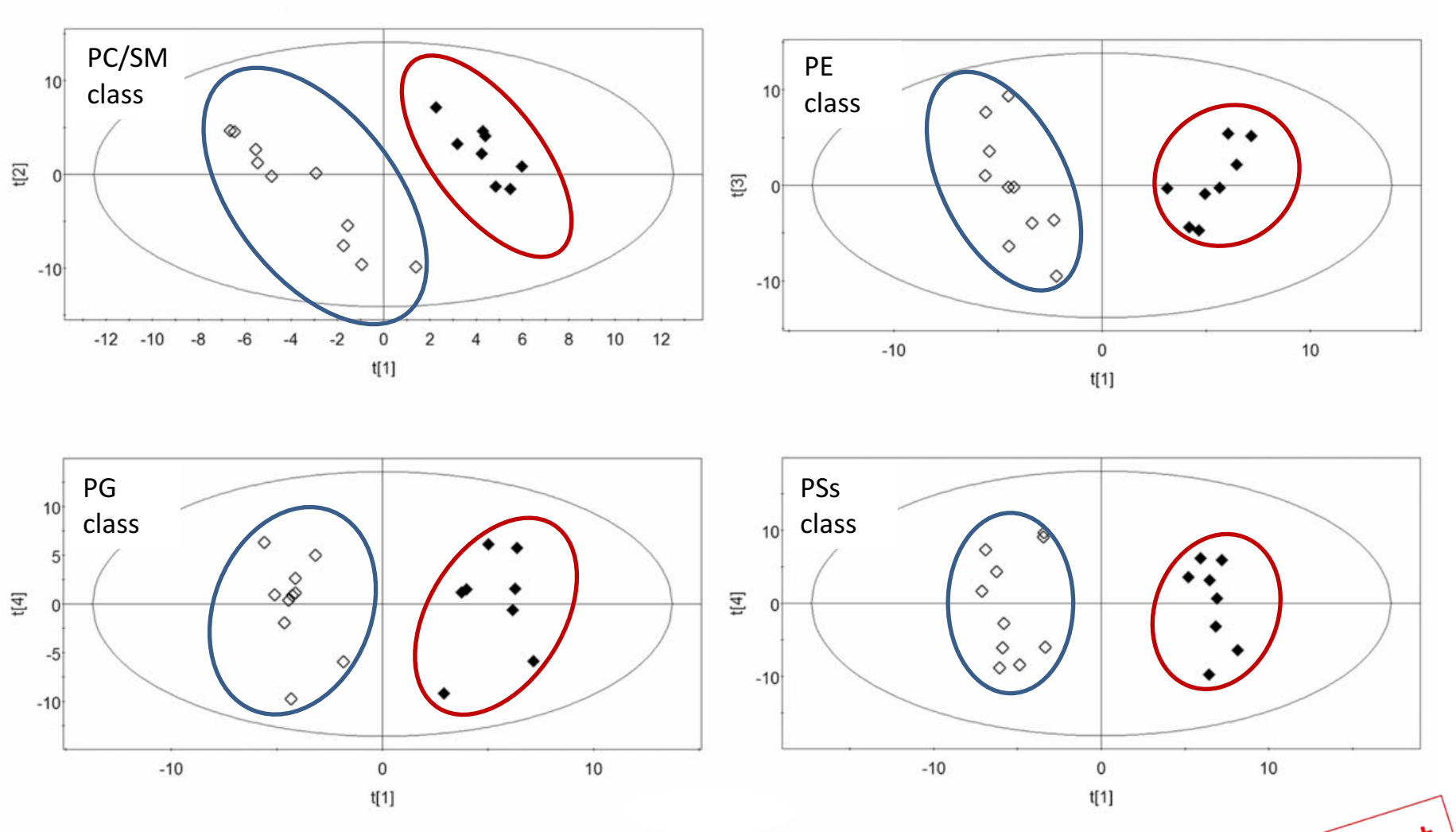
PCs, SMs:1974 signals

PSs: 14243 signals

PGs: 9653 signals

… complicated especially for PGs and PSs by a vast amount of **noise, artifacts like electronic spikes resulting in redundancy** of the data

Results: PLS-DA models from RP and NRP at T=0



RP - Responders

NRP - Non Responders

at T=0 time point

Results

3.Statistical analysis and bioinformatics

Untargeted lipidomics study from pre-treatment serum:

65 differential metabolites

12 for PC/SM class,
15 for the PE class,
13 for the PG class,
25 for PS class.

at T=0 time point

LIPID CLASS			Not Responders		Responders		P value
	RT_m/z	VIP	Mean	St.Dev	Mean	St.Dev	tTestValue
PSs	13.60_782.52	2.35	48.23	37.83	104.02	52.04	0.018
	15.05_741.50	2.44	11.11	9.65	24.79	11.20	0.013
	12.92_879.50	2.45	3.65	7.03	18.01	14.25	0.013
	12.84_815.03	2.49	2.95	5.18	18.46	16.20	0.011
	13.26_822.49	2.43	4.19	7.38	20.42	16.66	0.014
	17.99_600.69	2.13	2.40	3.97	10.04	9.65	0.036
	10.48_840.46	2.08	7.75	9.91	0.00	0.00	0.043
	13.03_844.46	2.78	12.36	10.11	0.00	0.00	0.003
	13.45_786.54	2.50	11.73	10.59	0.71	2.03	0.011
	18.56_601.86	2.11	4.04	4.40	0.39	1.12	0.038
	13.09_874.69	2.04	7.73	8.80	0.76	2.16	0.045
	14.40_838.03	2.04	10.66	13.79	0.00	0.00	0.045
	12.85_841.74	2.12	0.81	2.56	8.56	10.51	0.038
	17.02_688.96	2.23	0.56	1.79	7.07	8.23	0.026
	14.85_596.55	2.26	0.55	1.74	9.20	10.95	0.025
	13.81_716.64	2.22	0.78	2.46	6.97	7.66	0.028
	16.79_744.77	2.01	2.48	3.29	0.00	0.00	0.050
	13.76_748.40	2.47	0.00	0.00	5.36	5.99	0.012
	10.50_467.35	2.23	0.00	0.00	4.43	5.76	0.026
	14.58_798.73	2.29	0.00	0.00	3.66	4.59	0.022
	18.36_614.29	2.08	2.34	3.07	0.00	0.00	0.048
	18.81_810.59	2.03	2.71	3.58	0.00	0.00	0.049
	14.83_443.05	2.45	0.00	0.00	2.89	3.28	0.013
	19.61_732.99	2.43	0.00	0.00	3.20	3.68	0.014
	12.03_992.42	2.03	2.41	3.17	0.00	0.00	0.048

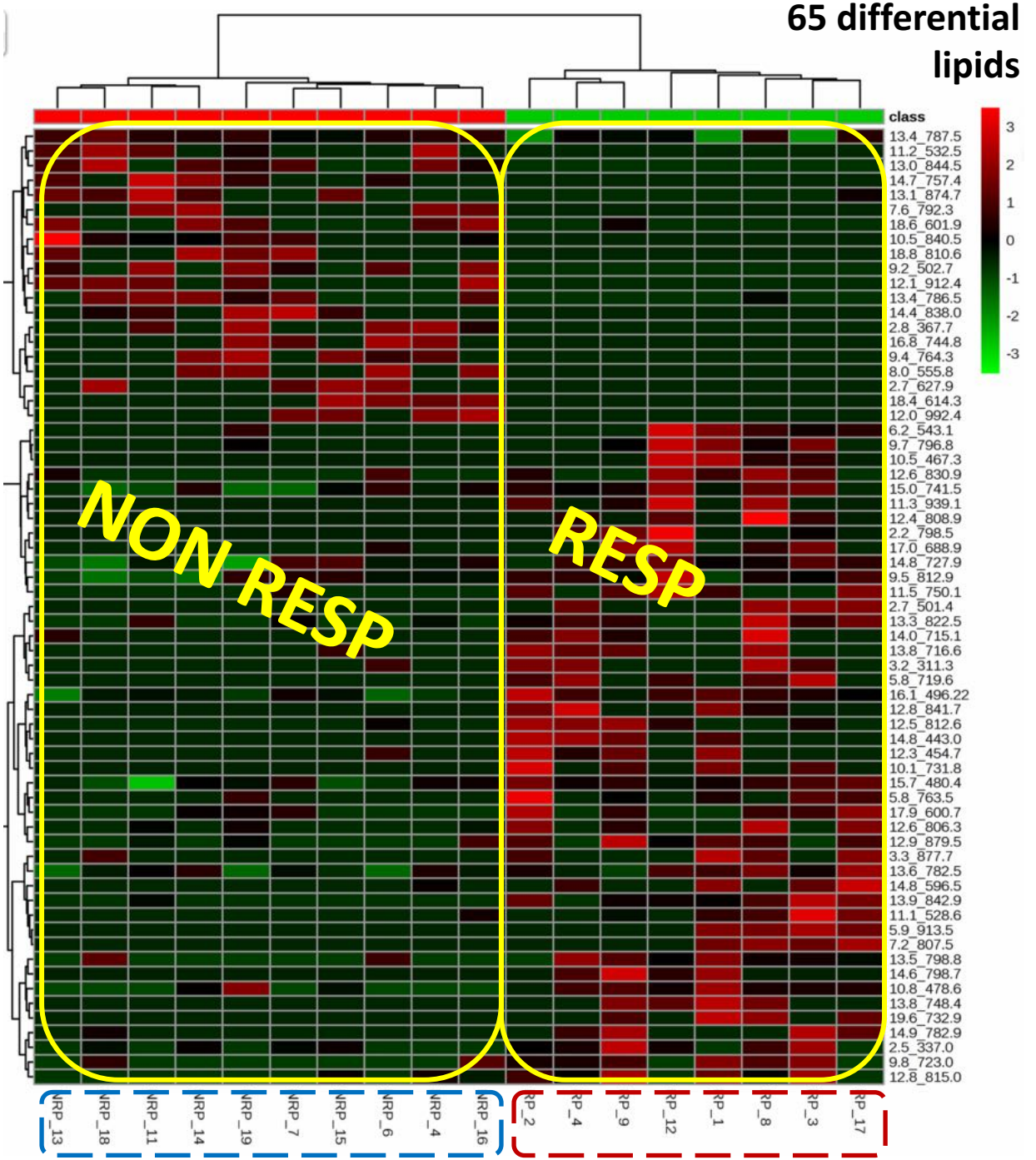
Results: HEATMAP

Overexpressed signals

Underexpressed signals

At T0,
a number of 65 lipids were identified as significant with the criteria of VIP>1,5 and p<0.05 at the univariate test

at T=0 time point



Results: Potential biomarkers validation by targeted LC-MS/MS analysis

Target identification: candidate biomarkers

Retention Time (min)	Mass (m/z) [M+H⁺]	Common name	AUC
14.79	727.86	SM(d18:2/18:1)	0.77
16.14	496.22	Lyso PC(16:0/0:0)	0.92
15.72	480.42	Lyso PC(15:1(9Z)/0:0)	0.9
13.86	842.90	PC(20:0/20:2)	0.93
		PC(20:1/20:1)	
		PC(18:0/22:2)	
		PC(18:1/22:1)	
		PC(18:2/22:0)	
		PC(16:1/24:1)	
11.08	528.61	Lyso PE(22:5/0:0)	0.78

lower levels in NRP of **5 differentially expressed lipids** ($p<0.05$)

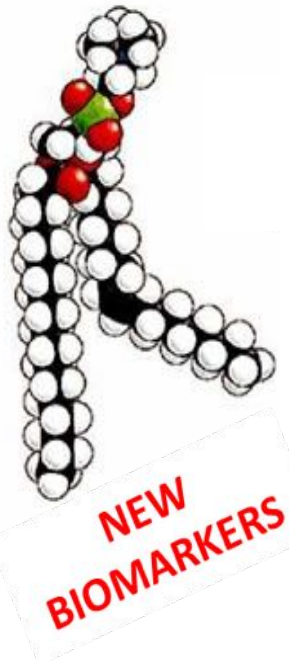
SM(d18:2/18:1)

LysoPC (16:0/0:0)

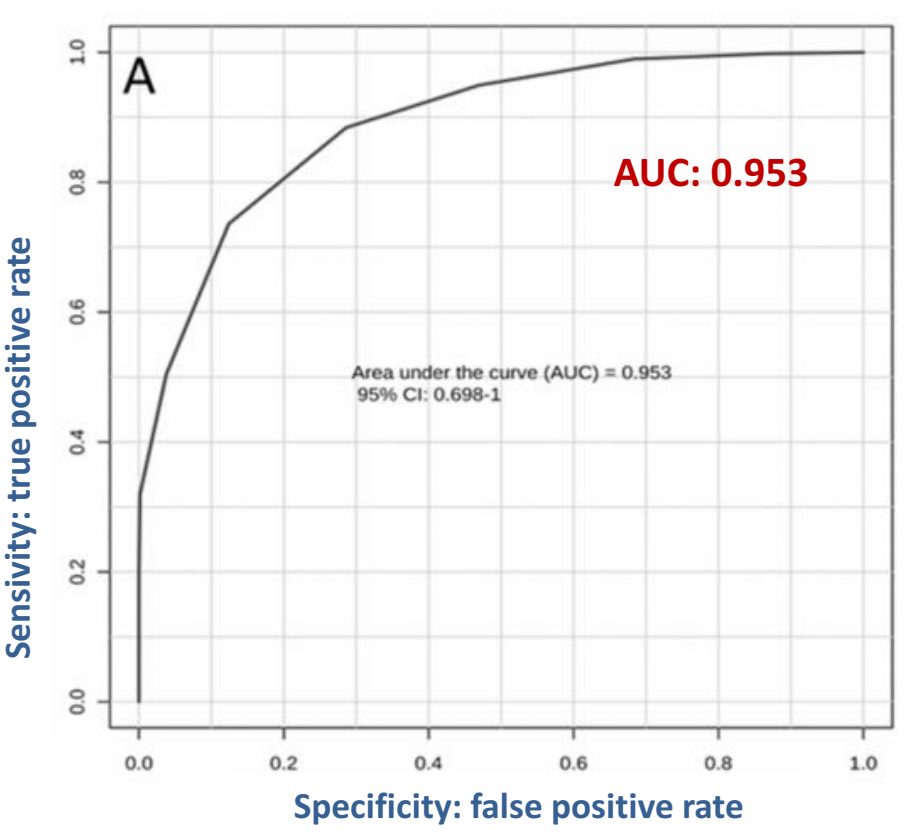
LysoPC (15:1/0:0)

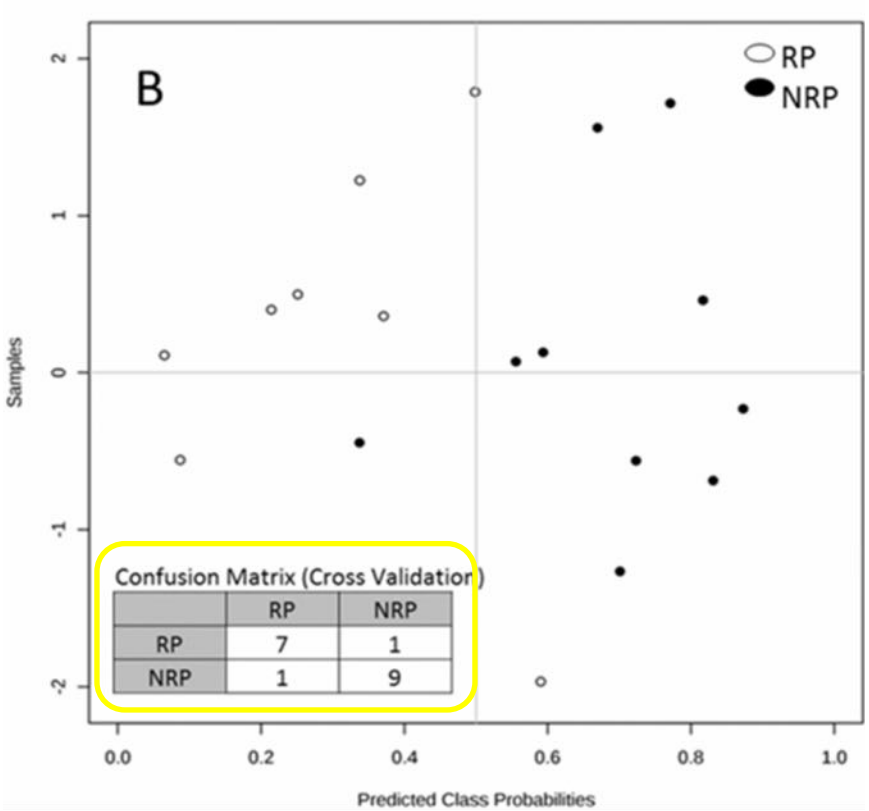
PC (40:0)

Lyso PE (22:5/0:0)



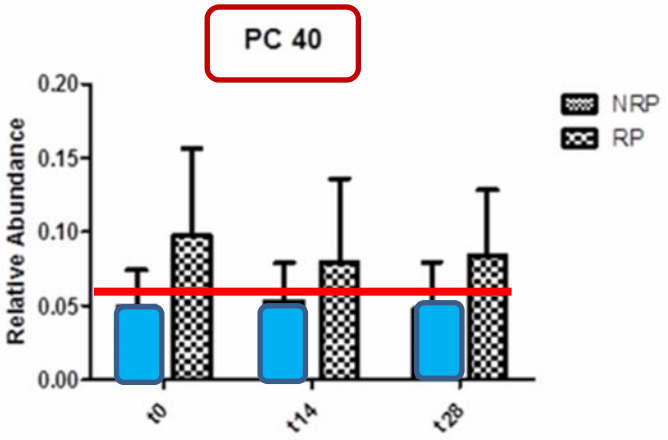
Results: predictive power of the confirmed lipid pattern (T=0)

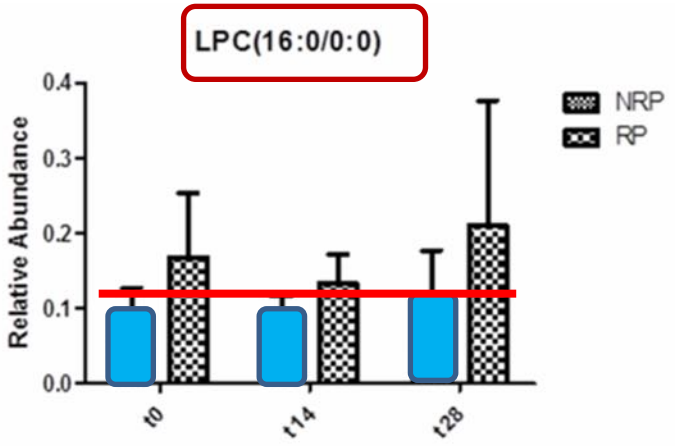


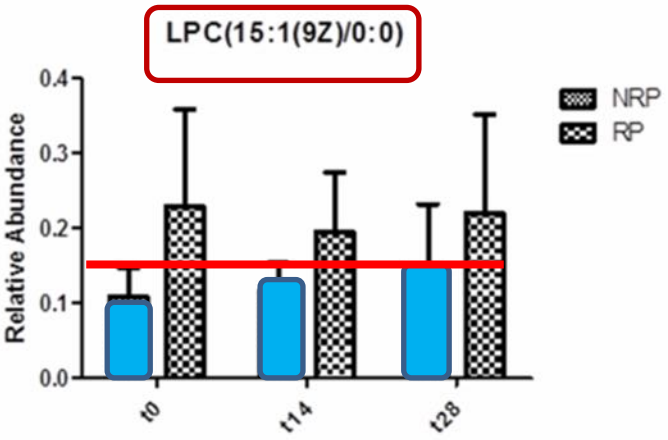


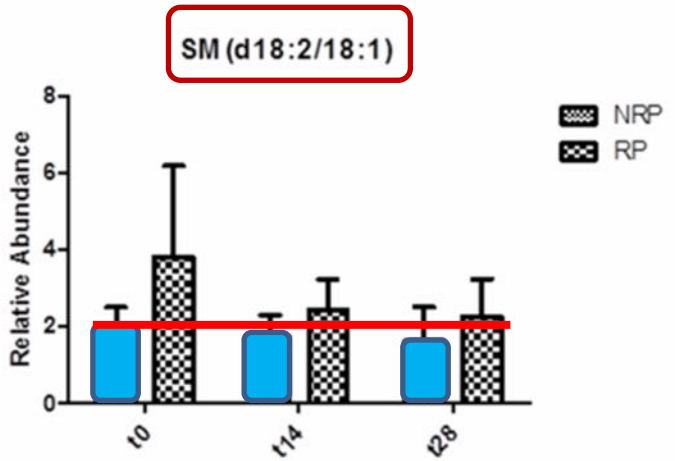
Results:

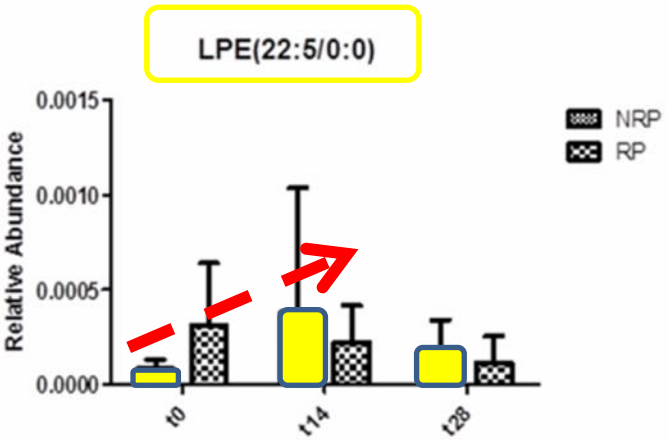
The **5 validated lipids** were analyzed at **T14** and **T28** time points to evaluate their **prognostic value** and their possible **modulation** during treatment.











Results: data driven approach

- Lyso PE (22:5/0:0)
- PC (40:0)
- LysoPC (15:1/0:0)
- LysoPC (16:0/0:0)
- SM(d18:2/18:1)

Novel hypotheses generation

Results: data driven approach

Lyso PE (22:5/0:0)

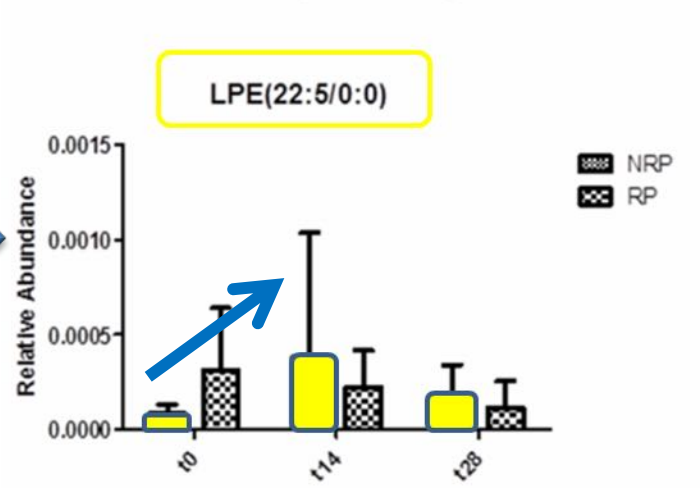
PC (40:0)

LysoPC (15:1/0:0)

LysoPC (16:0/0:0)

SM(d18:2/18:1)

Novel hypotheses generation



Lyso-PE is the unique signal that seems to modulate its expression in response to CRT.

We observed that the lyso-PE level significantly increases in NRP in respect to RP during the CRT, suggesting its role in **response inhibition**.

- human phosphatidylethanolamine-binding protein 4 **(hPEBP4) →inhibition of apoptosis**

Li P, International journal of molecular medicine. 2006;18:505-10.

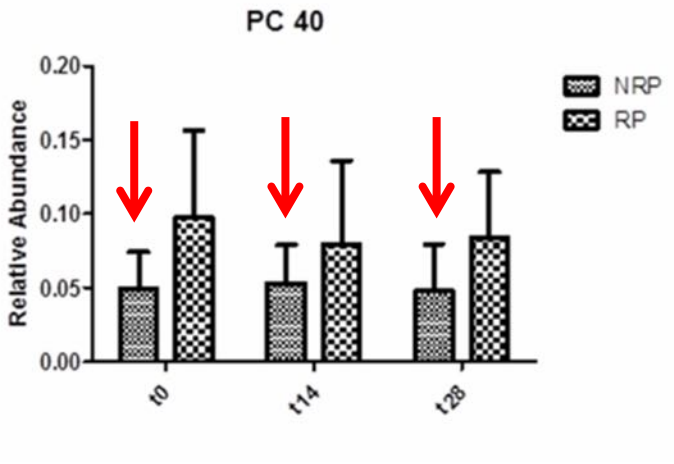
- **hPEBP4** is a predictive marker for the pathological response of rectal cancer to radiotherapy, because it **promotes the radioresistance of human rectal cancer by activating Akt** in an ROS-dependent way

Qiu J, International journal of colorectal disease. 2013;28:241-6.

Results: data driven approach

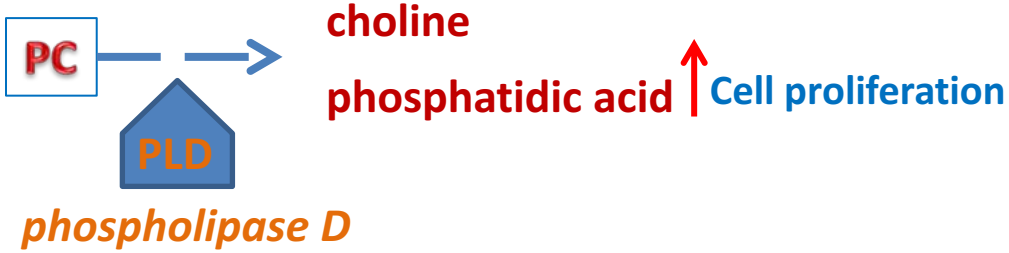
- Lyso PE (22:5/0:0)
- PC (40:0)
- LysoPC (15:1/0:0)
- LysoPC (16:0/0:0)
- SM(d18:2/18:1)

Novel hypotheses generation



PC40 is significantly **lower in NRP** sera in respect to RP ones before and during the whole treatment.

The different levels of PCs may be explained by the **modulation of enzymes** that control anabolic and catabolic pathways .



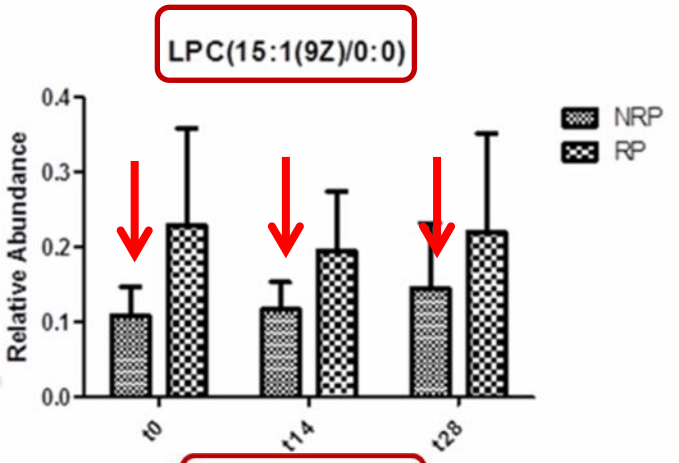
- ✓ **PLD** high expression carcinoma had significantly **poorer survival** than those with PLD low expression carcinoma
- ✓ elevation of PLD expression and activity in **human CRC**.

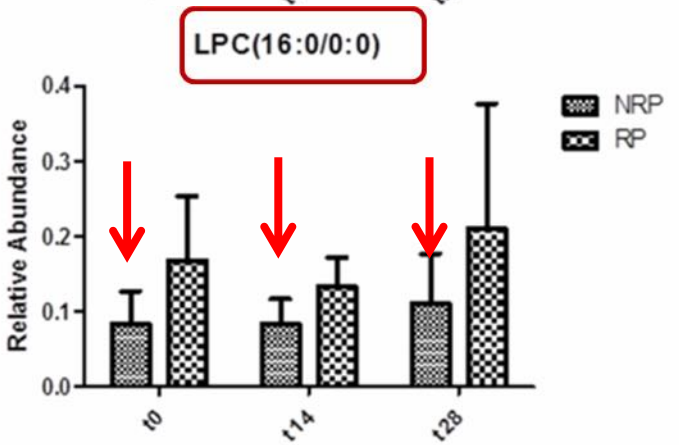
Saito M, Oncology reports. 2007;18:1329-34.
Oshimoto H, Oncology research. 2003;14:31-7.

Results: data driven approach

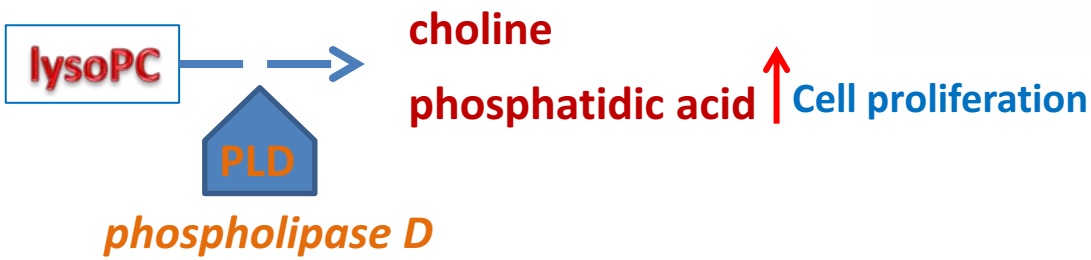
- Lyso PE (22:5/0:0)
- PC (40:0)
- LysoPC (15:1/0:0)
- LysoPC (16:0/0:0)
- SM(d18:2/18:1)

Novel hypotheses generation





Lower levels of **LysoPC (16:0/0:0) and LysoPC (15:1/0:0)** in NRP in respect to RP.



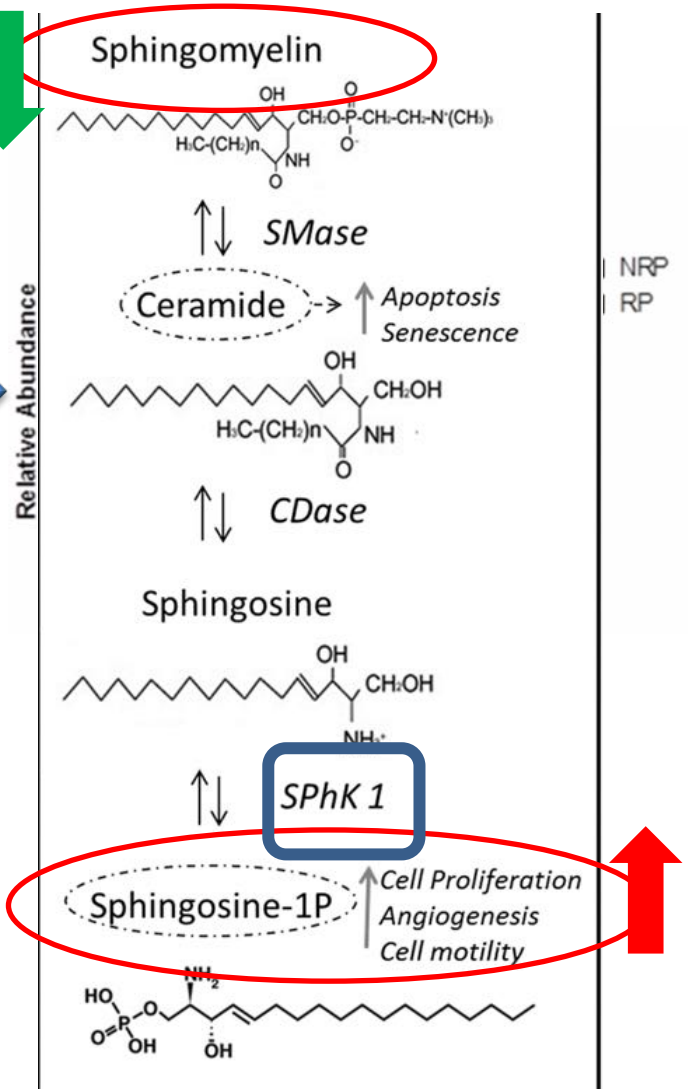
✓ Plasmatic levels of different lysoPC forms (including the 16:0) **are significantly reduced in CRC in respect to healthy controls**.

Zhao Z, JCO. 2007;25:2696-701.

Results: data driven approach

- Lyso PE (22:5/0:0)
- PC (40:0)
- LysoPC (15:1/0:0)
- LysoPC (16:0/0:0)
- SM(d18:2/18:1)

Novel hypotheses generation



Lower levels of **SM (d18:2/18:1)** in NRP in respect to RP.

- ✓ **S1P** induces cell **proliferation, angiogenesis and trigger cell motility**.
- ✓ lower levels of SM in NRP could underlie high levels of S1P, sustained by an **overexpression of SPhK1 in colorectal cancer**.

Hannun YA, The Journal of biological chemistry. 2011;286:27855-62.

Long J,et al. Tumour biology. 2015.

Lipidomic Discovery study: lipidic analysis (untargeted/ targeted)

Validation Study

Novel hypotheses generation

Indipendent validation set

- ✓ Ongoing
- ✓ N=31 pts

Functional validation

- ✓ Ongoing
- ✓ Biopsies T=0
- ✓ Serum:
  - **lipidomic analysis**
  - **enzymatic confirmation**

Conclusions:

1. Personalized treatment

2. To confirm results in *indipendent set of validation*

3. Cost-benefit analysis :
   - Invasive procedure?
   - Time consumig?
   - Costs?

Sample size…*overparametrization risk* → multicentric research?

Grazie per l'attenzione!