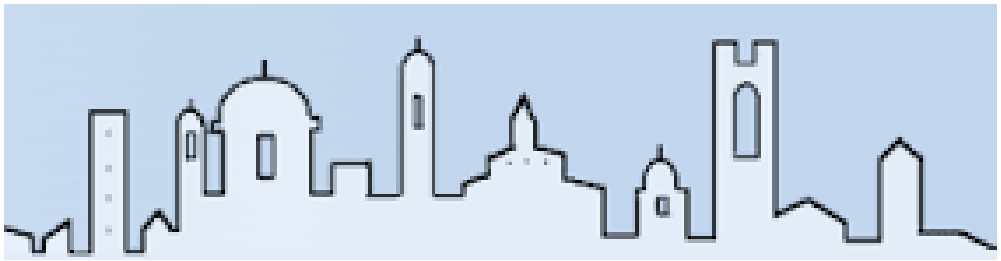
The YAP/TAZ Clinical Project

Trieste, 18 gennaio 2018



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Ospedale Papa Giovanni XXIII
Bergamo

5x1000
X AIRC = RICERCA

Extension Program 2016-2018

AIRC

Molecular basis for triple negative breast cancer metastasis: new tools for diagnosis and therapy

Giannino Del Sal - Trieste (PI)

Maurizio D'Incalci - Milano

p53-YAP/TAZ

Stefano Piccolo - Padova

Silvio Bicciato - Modena

A dense danger

Are tumors stiff because aggressive

or

aggressive because stiff ?

Nature Reviews Cancer, 2012

Outline

Rationale of YAP/TAZ in cancer (preclinical studies)

Prognosis (retrospective studies)

YAP/TAZ in TNBC

- YAP/TAZ & aggressivness
- YAP/TAZ & Mx density

Prediction (prospective studies)

YAP/TAZ as Rx target

- Pilot trial
- Ph2 RCT

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YAP/TAZ and Cancer

YAP and TAZ are the transcriptional regulators and key effectors of the Hippo pathway
YAP/TAZ are pervasively activated in human cancers, including TNBC (70%)

HIPPO

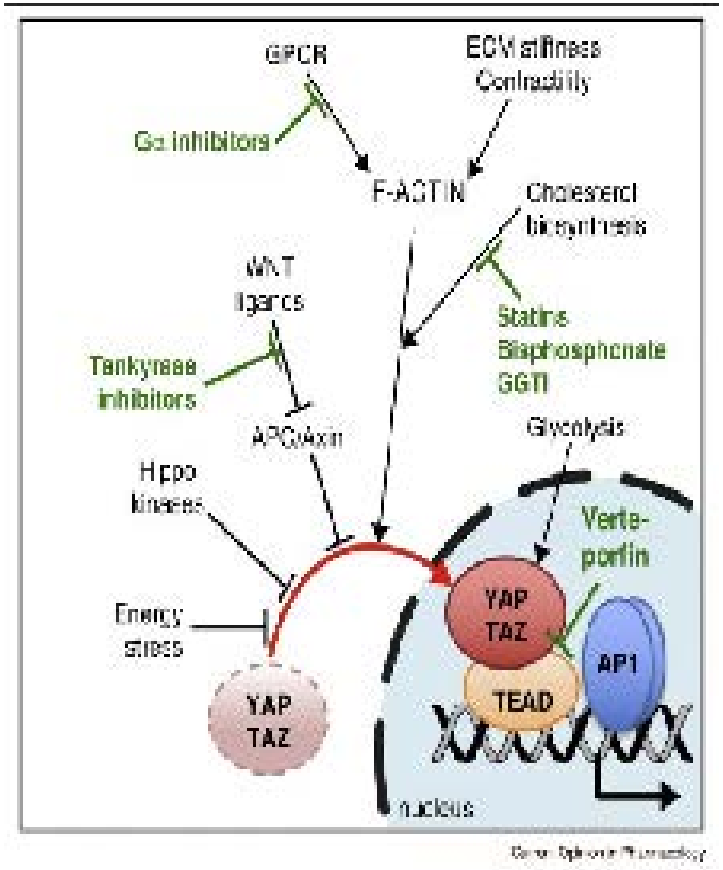
⊣

YAP/TAZ

↓

Proliferation/Organ growth

Recent work indicates that YAP/TAZ are essential for cancer initiation or growth of most solid tumors.



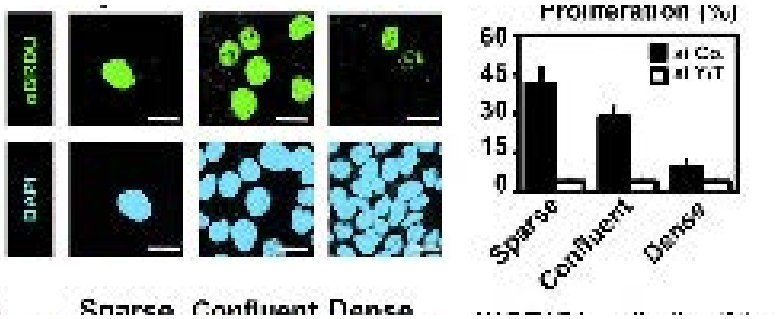
Current Opinion in Pharmacology, Volume 29, 2016, 26–33

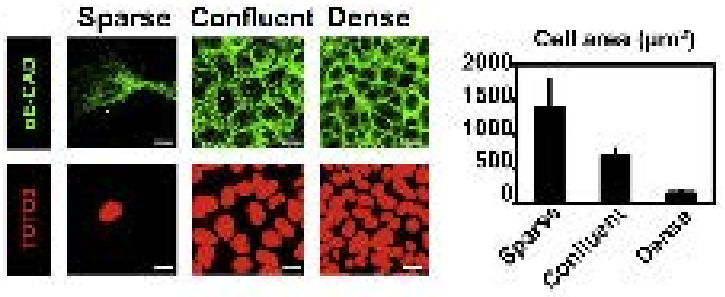
Liver YAP OE

Dong J. et al., Cell 2007

Mutations in critical geners lead to the "Hippopotamus-like" phenotype

YAP/TAZ and Cancer Bio-Mechanics





Contact inhibition of proliferation (CIP)

Loss of CIP is considered a hallmark of cancer.

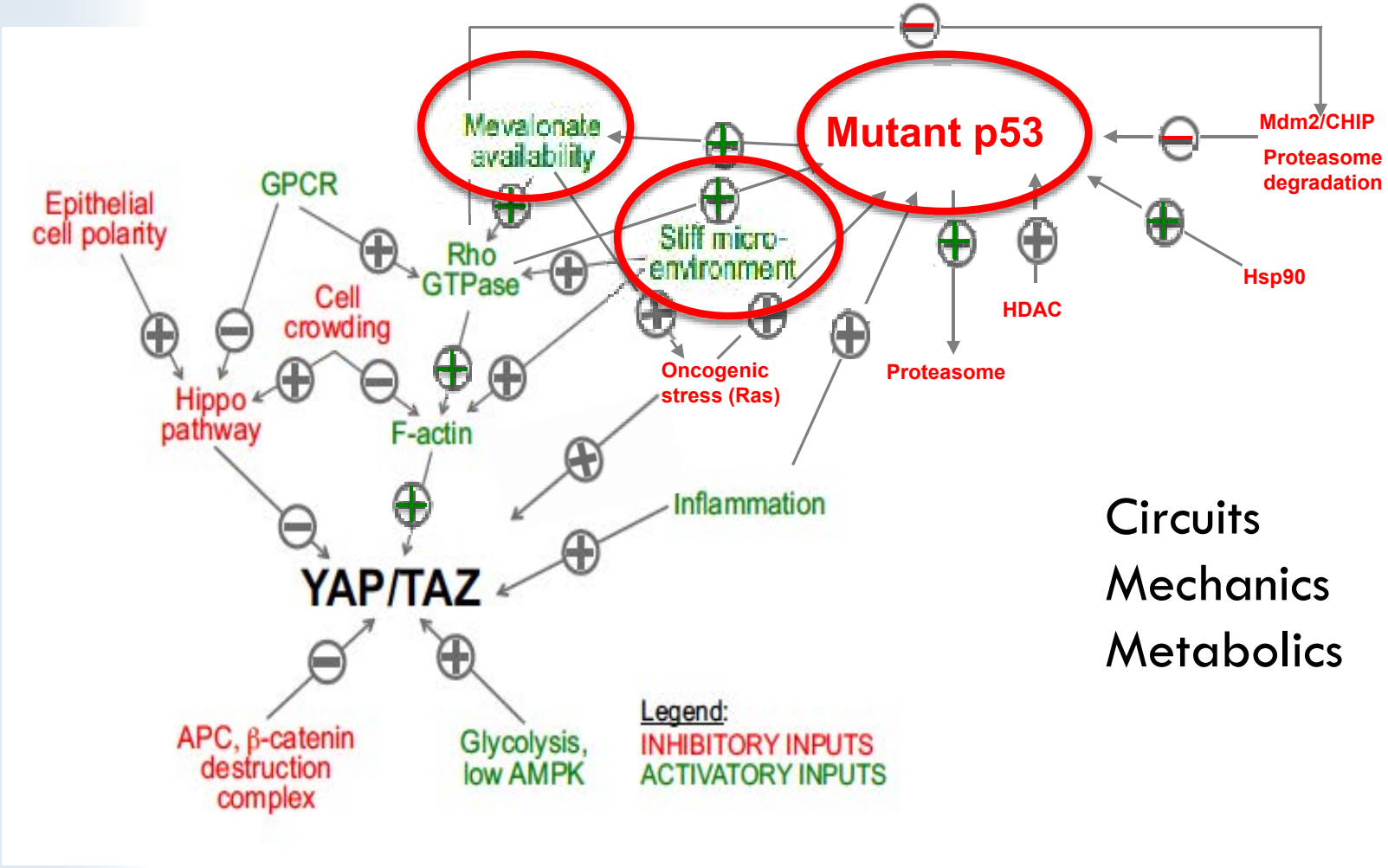
In CIP the main MoA is the regulation of YAP and TAZ, which tend to remain nuclear in cells growing at low density and relocate in the cytoplasm in confluent cultures.

CIP is associated with phosphorylation of YAP and TAZ, indicating the activation of the Hippo pathway kinases

ECM stiffness is a formidable stimulus for YAP/TAZ activity

Aragona, Piccolo Cell 2013

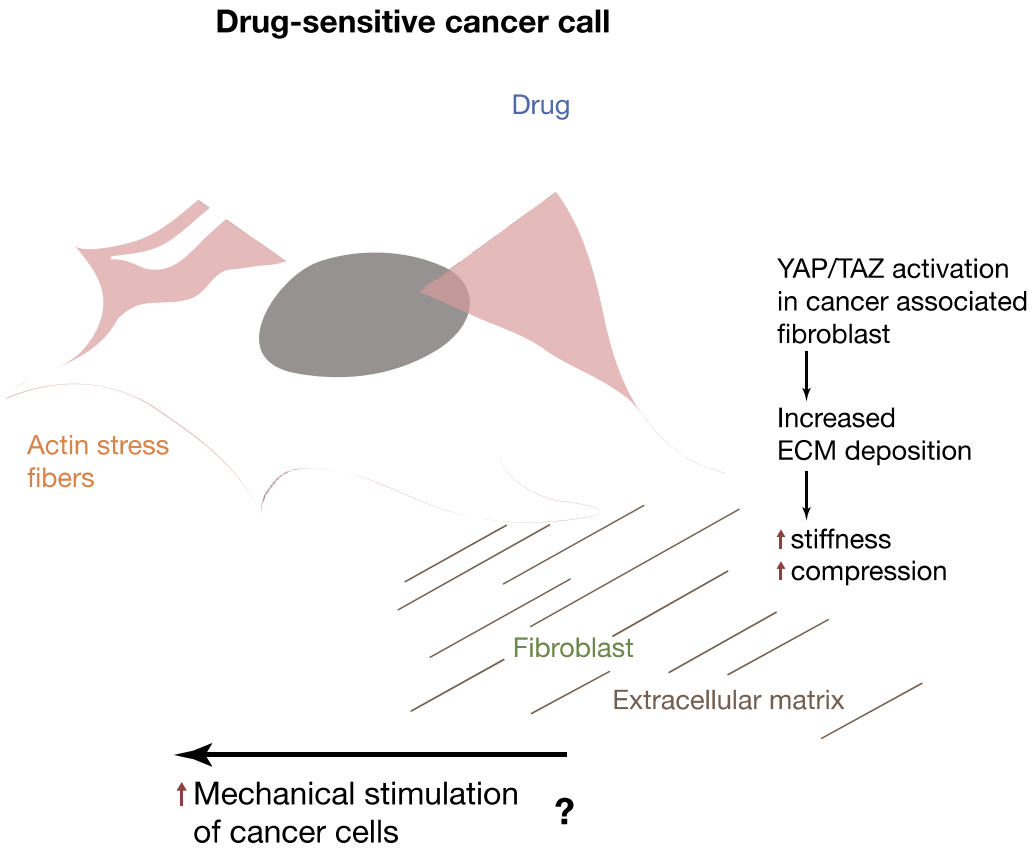
YAP/TAZ Circuits Regulation



Circuits
Mechanics
Metabolics

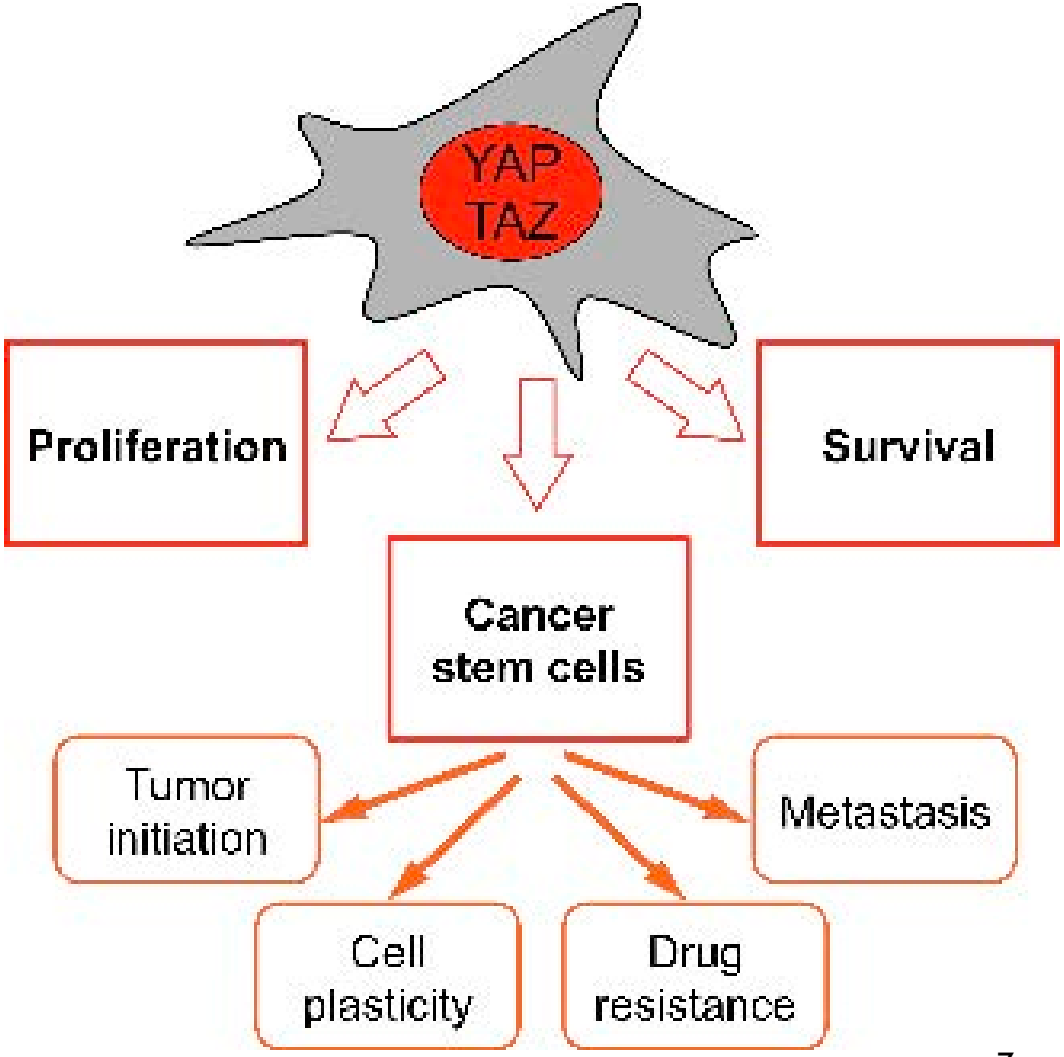
modified from Zanconato et al, *Cancer Cell,* 2016

YAP/TAZ clinical implications



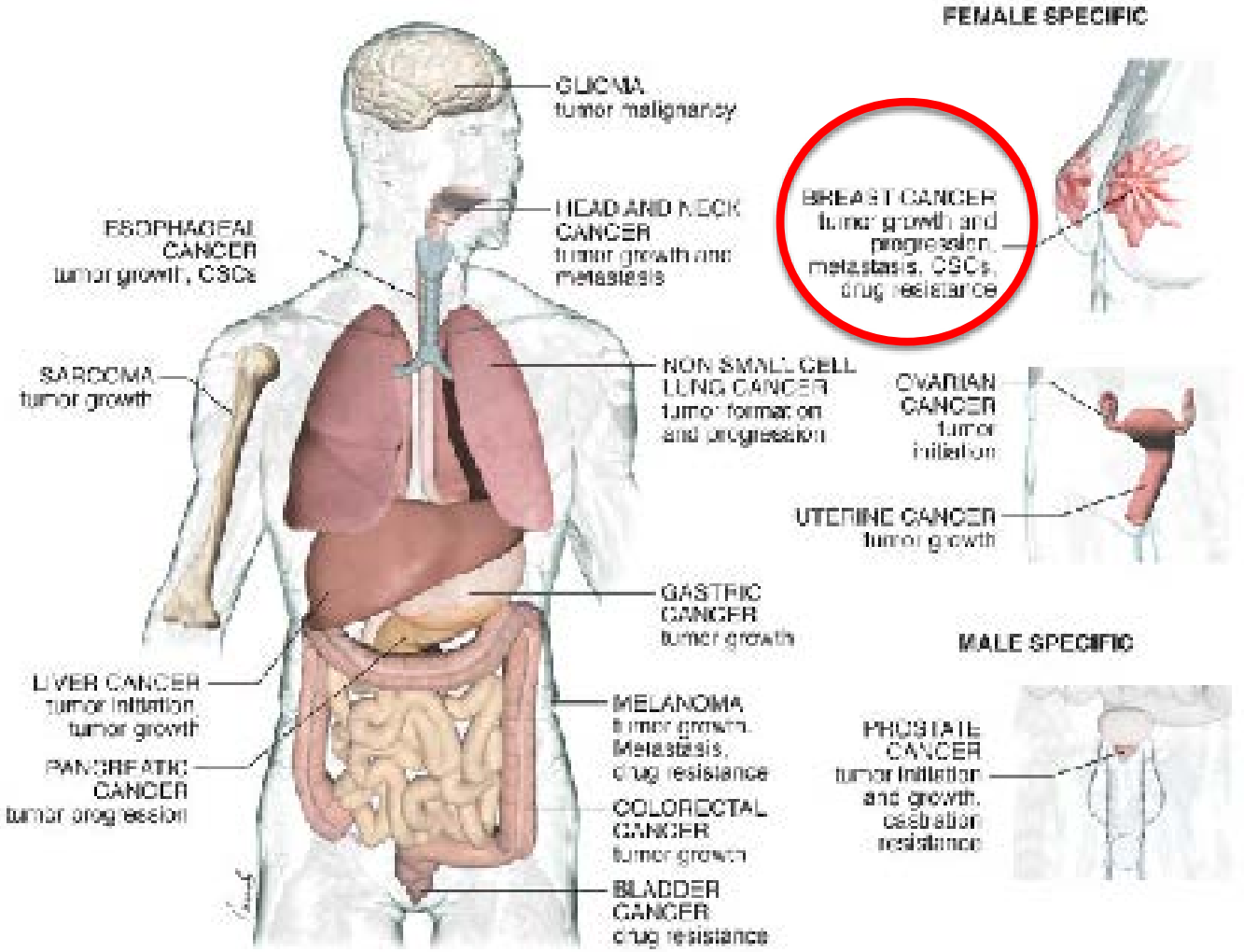
Zanconato, Cancer Cell 2016

YAP/TAZ function in cancer cell



Zanconato, Cancer cell 2016

YAP/TAZ in human tumors



Zanconato et al, *Cancer Cell,* 2016

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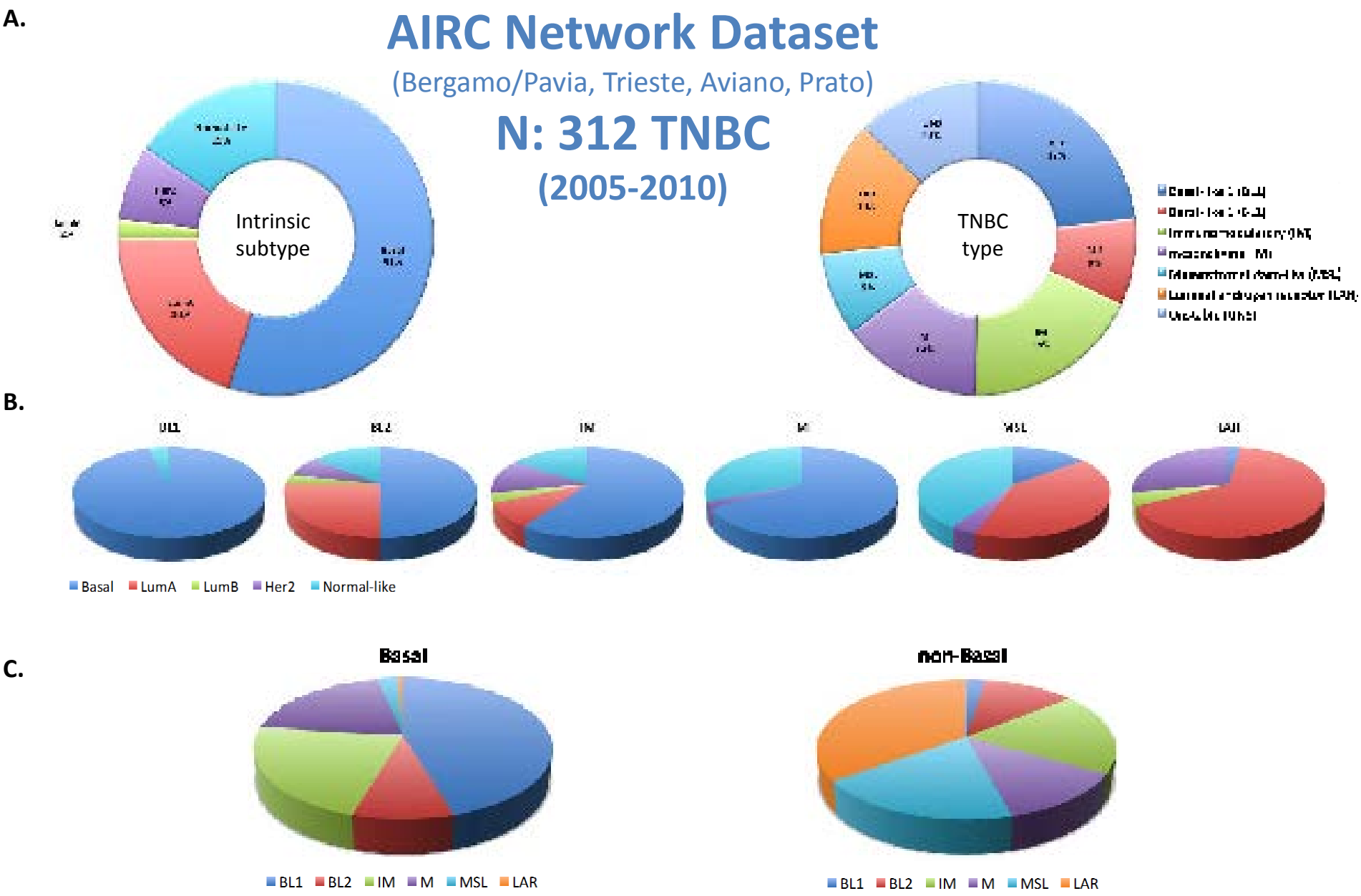


Figure 1. **TNBC molecular subtyping**.
312 TNBC gene expression profiles from the AIRC_DASL dataset were either subtyped using PAM50 (*genefu*, R package) or TNBCtype. **A.** Distribution of 312 TNBC samples using PAM50 intrinsic subtyping (left) or TNBC type (right). **B.** Intrinsic subtype composition of each TNBC subtype. **C.** TNBC type composition of either basal-like (right) or non basal-like TNBC (left).

YAP/TAZ & p53 distribution

YAP/TAZ distribution

GEP & IHC in BC molecular subtypes

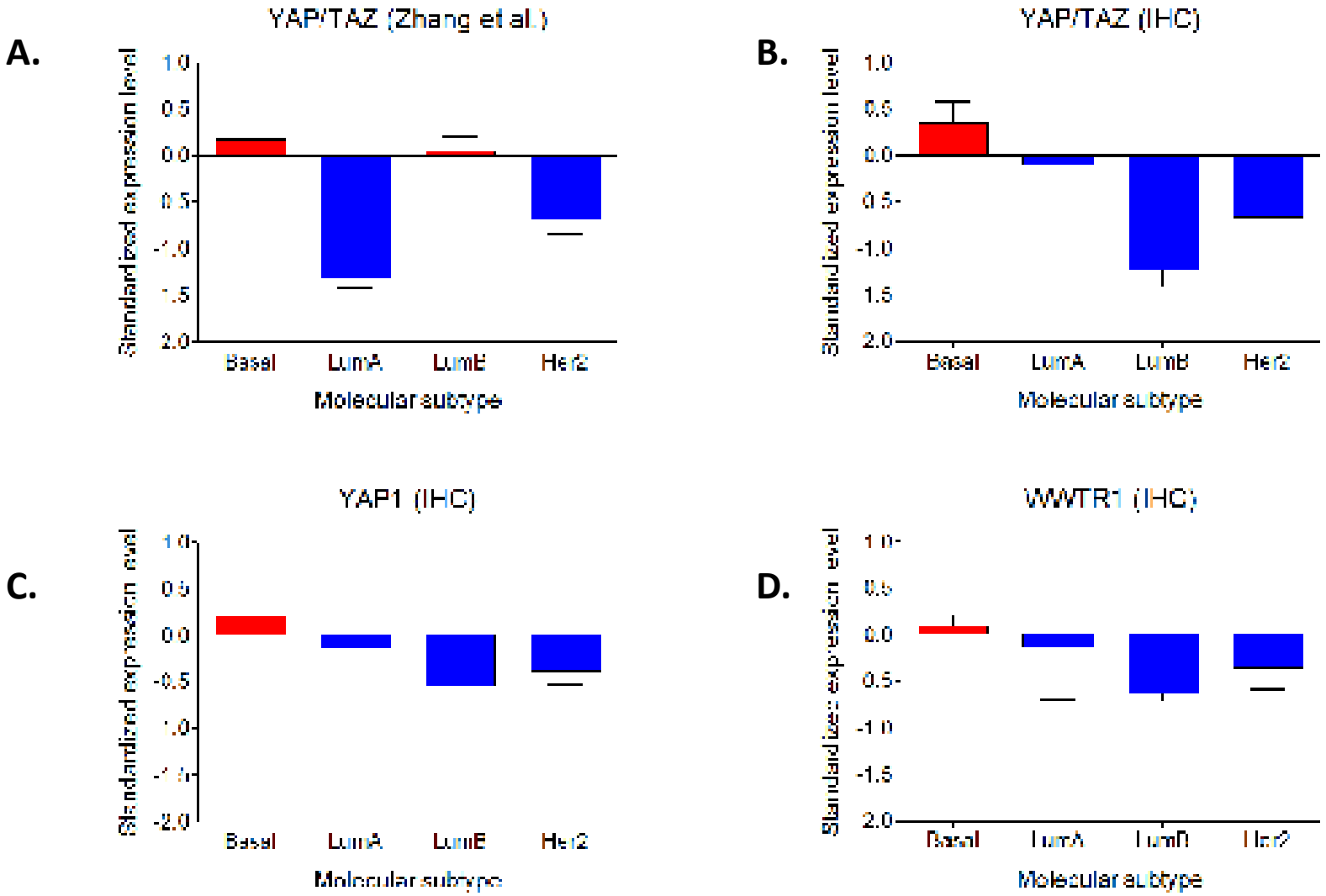
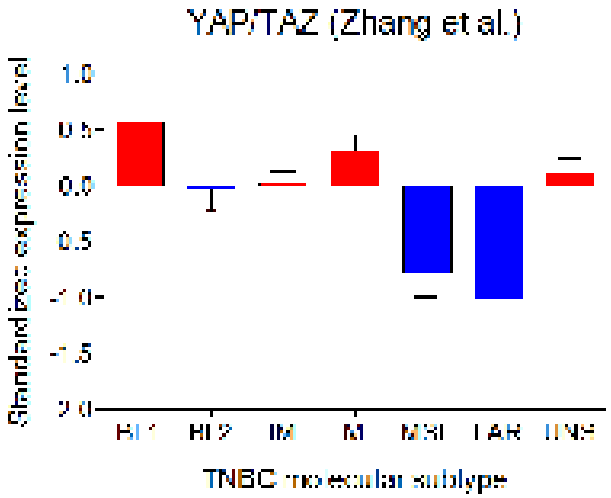


Figure 4. **YAPTAZ signature expression and YAP/TAZ, YAP1 and WWTR1 HIC levels in PAM50 molecular subtypes**.
A. YAP/TAZ gene signature as in Zhang et al. **B.** YAP/TAZ immunohistochemistry. **B.** YAP1 immunohistochemistry **C & D.** YAP1 and WWTR1 immunohistochemistry

YAP/TAZ distribution

GEP & IHC in TNBC molecular subtypes

A.



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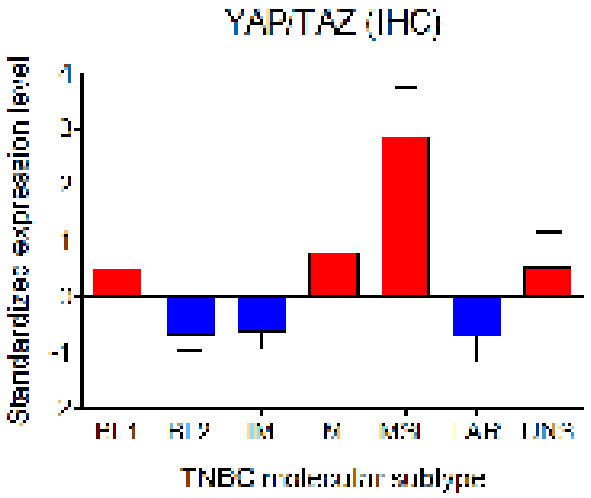

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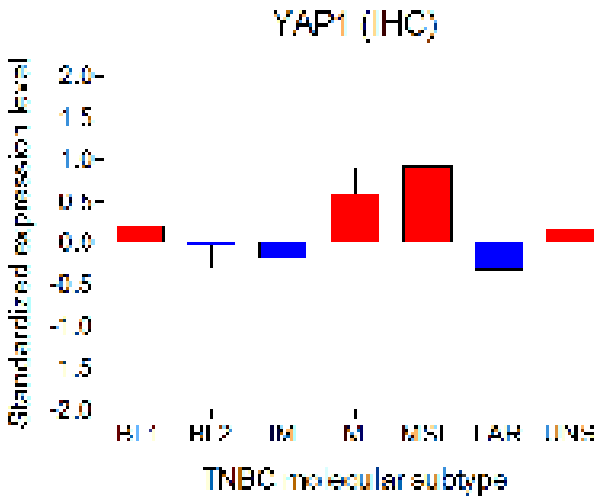

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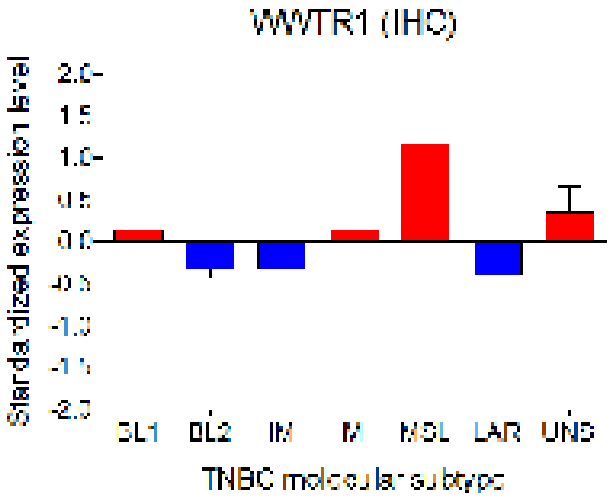


Figure 5. **YAPTAZ signature expression and YAP/TAZ, YAP1 and WWTR1 HIC levels in TNBC subtypes**.
A. YAP/TAZ gene signature as in Zhang et al. **B.** YAP/TAZ immunohistochemistry **C and D.** YAP1 immunohistochemistry; WWTR1 immunohistochemistry

p53 distribution

GEP & IHC in BC molecular subtypes

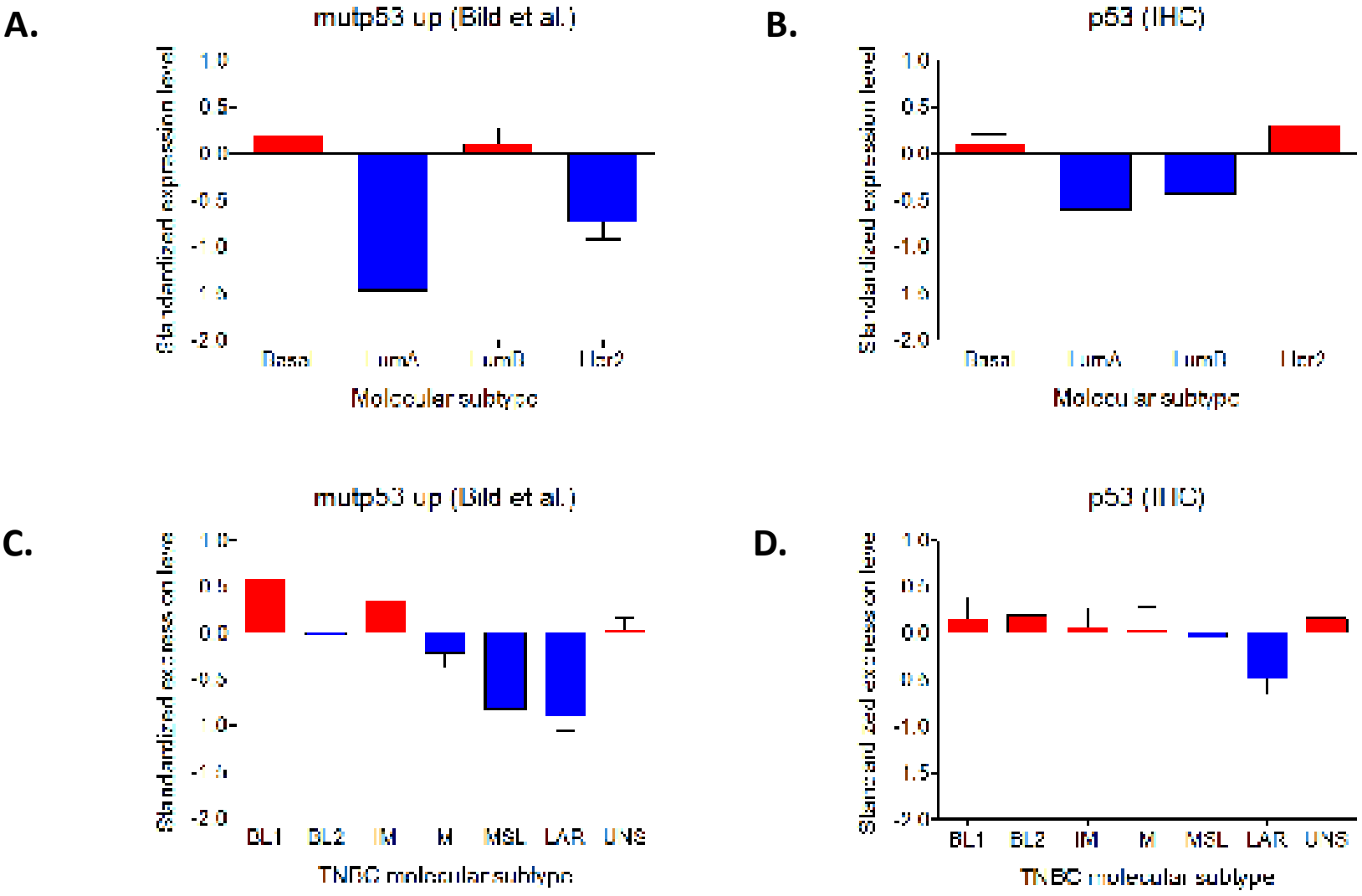
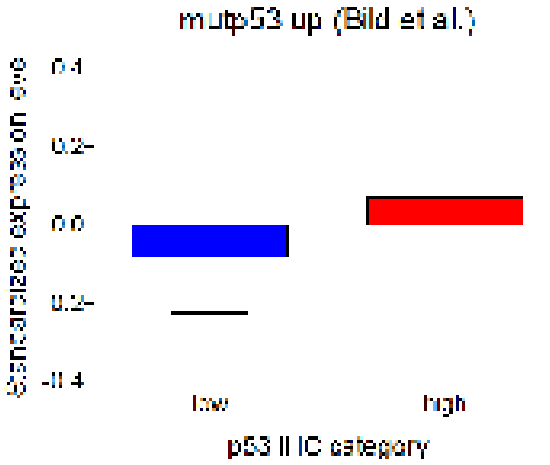


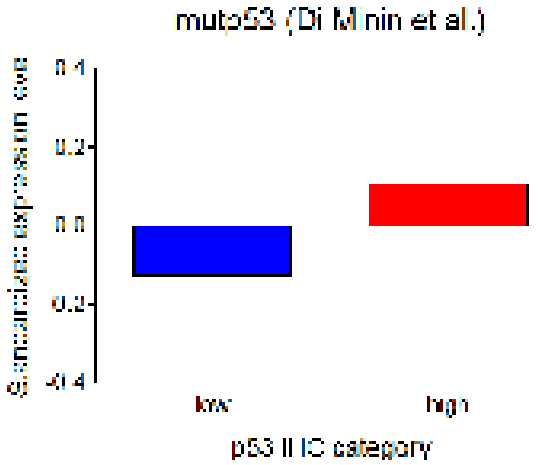
Figure 6. mutp53 signature expression and p53 HIC expression levels in PAM50 and TNBC subtypes.
A. mutp53 gene signature (Bild et al) in PAM50 subtypes (n=283). **B.** p53 immunohistochemistry in PAM50 subtypes (n=147). **C.** mutp53 gene signature (Bild et al) in TNBC subtypes (n=283). **D.** p53 immunohistochemistry in TNBC subtypes (n=147).

Correlation b/w biomarkers

YAP/TAZ & p53 correlation (I)

A.





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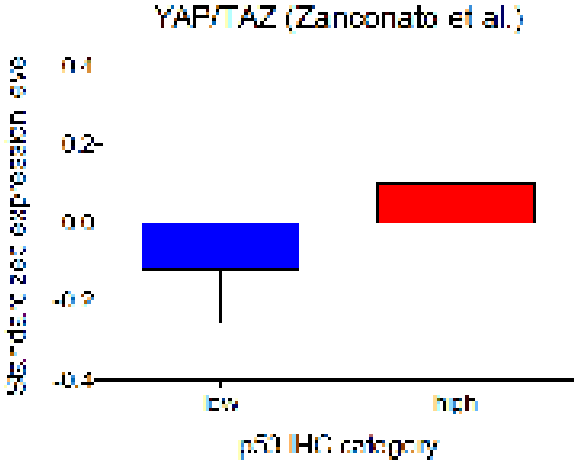


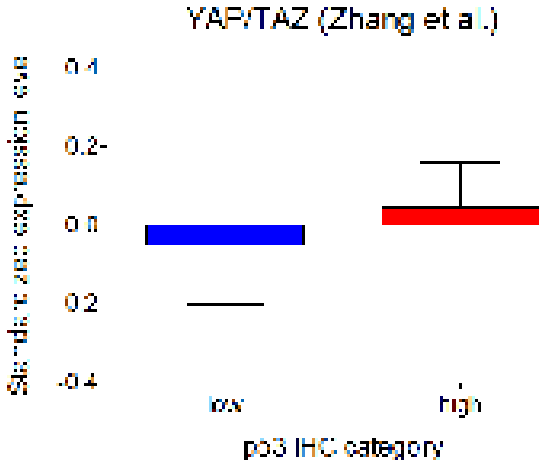


Figure 8. **mutp53 and YAP/TAZ signature expression levels in IHC p53 categories (n=118)**.
A. Standardized expression level of mutp53 signatures. **B.** Standardized expression level of YAP/TAZ signatures.

YAP/TAZ & p53 correlation (II)

wwtr1/yap1 **IHC**

p53	*>20*	*≤20*
>30	18 (21.4%)	27 (32.1%)
≤30	9 (9.5%)	31 (36.9%)

p-value=0.06

Yap/Taz (Zhang et al.) **GEP**

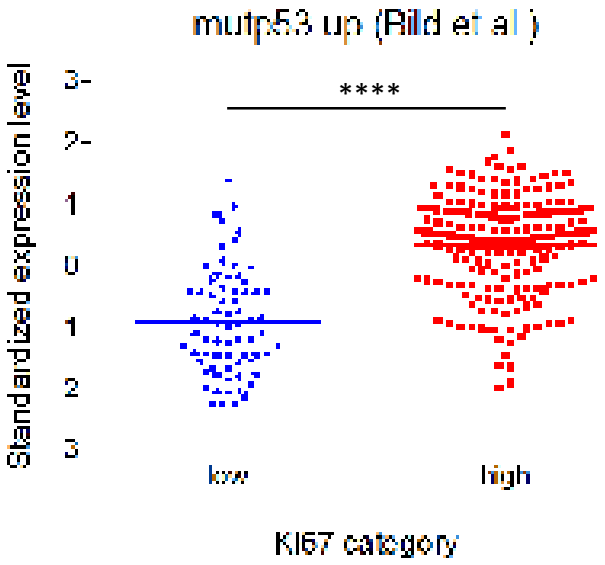
p53 (Bild et al.)	*high*	*low*
high	142 (50.2%)	12 (4.2%)
low	10 (3.5%)	119 (42.0%)

p-value<0.001

Figure 7. **Correlation between p53 status and WWTR1, YAP1, and YAP/TAZ HIC and signature levels**.
A. Fisher's Exact Test between p53 HIC and YAPTAZ HIC. **B.** Fisher's Exact Test between p53 and YAPTAZ signature levels.

YAP/TAZ, p53 and Ki67 correlation (I)

A.



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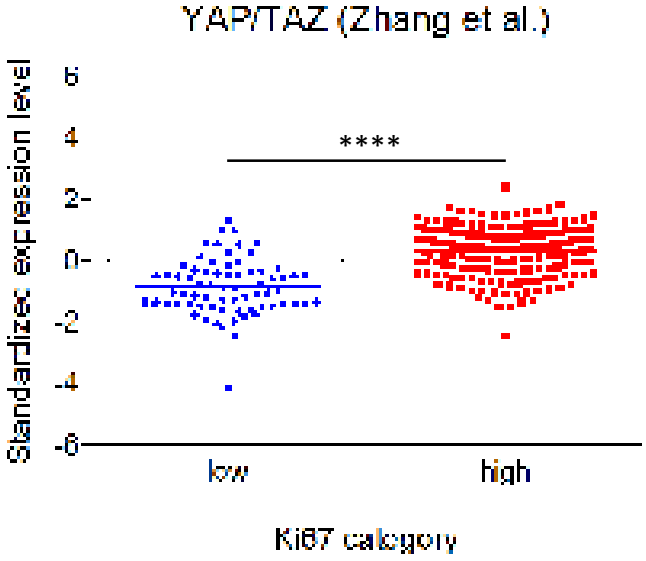


YAP/TAZ, p53 and Ki67 correlation (II)

yaptaz (Zhang et al.)

		high	*low*
ki67	*>20*	130 (49.4%)	62 (23.6%)
	≤20	10 (3.8%)	61 (23.2%)

p-value<0.001

mutp53 (Bild et al.)

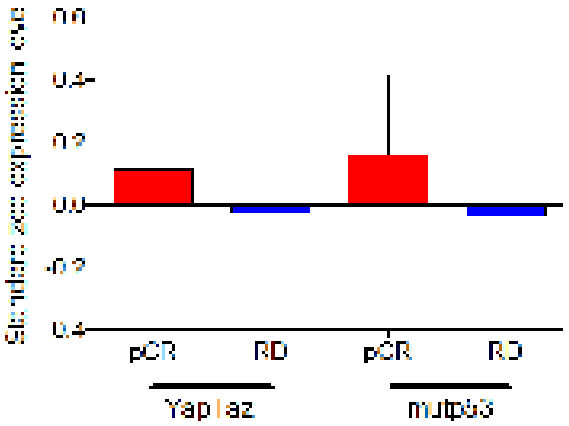
		high	*low*
ki67	*>20*	132 (50.2%)	60 (22.8%)
	≤20	9 (3.4%)	62 (23.6%)

p-value<0.001

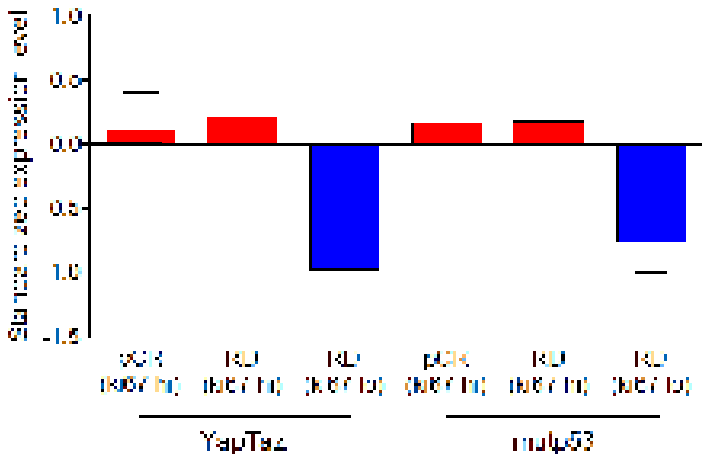
Figure 10. **Correlation between ki67 status and WWTR1, YAP1, YAP/TAZ, and p53 HIC and YAP/TAZ and mutp53 signature levels**.
Fisher's Exact Test between ki67 and YAP/TAZ and mutp53 signature levels.

Correlation with pts outcome

Neoadjuvant ORR



A.



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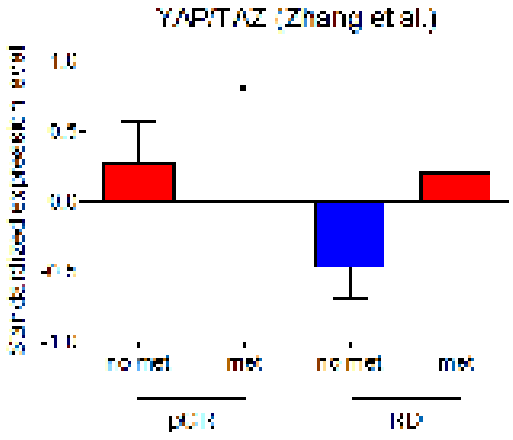

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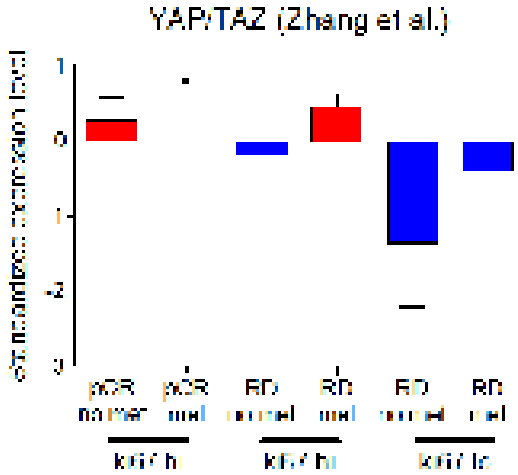


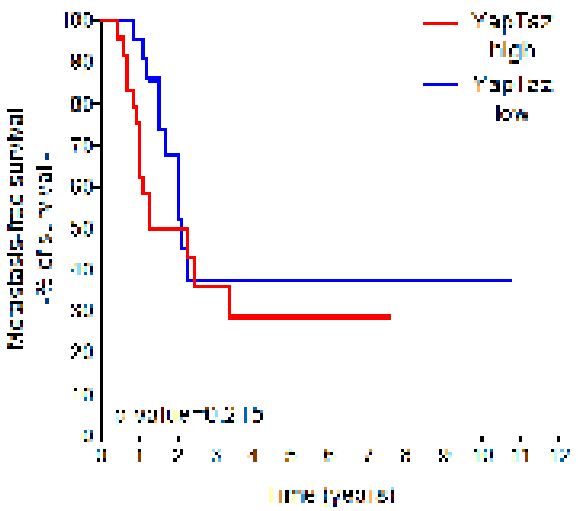
D.

Figure 12. **Signature expression levels in pCR (n=11) and RD (n=55) after neoadjuvant chemotherapy**.
A. Standardized expression level of YapTaz (Zhang et al.) and mutp53 (Bild et al.) signatures. **B.** Standardized expression levels of YapTaz (Zhang et al.) and mutp53 (Bild et al.) signatures in pCR and RD patients stratified according to Ki67. **C.** Standardized expression level of YapTaz (Zhang et al.) in non-metastatic and metastatic samples stratified by neoadjuvant response. **D.** Same as in **C.** with the additional stratification based on Ki67 level.

Neoadjuvant: DFS and OS

mFU: 46m yrs

A.



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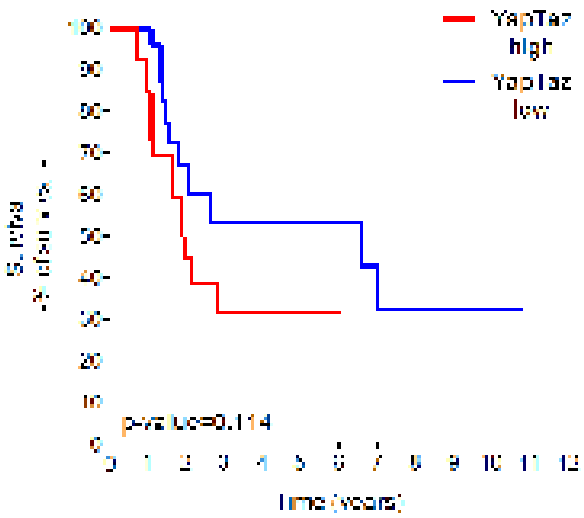

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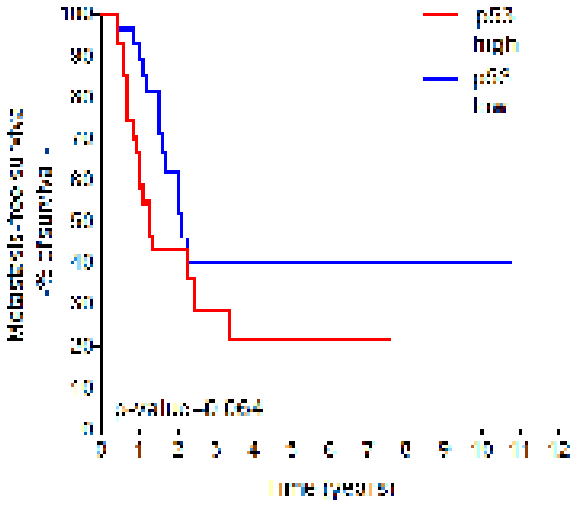

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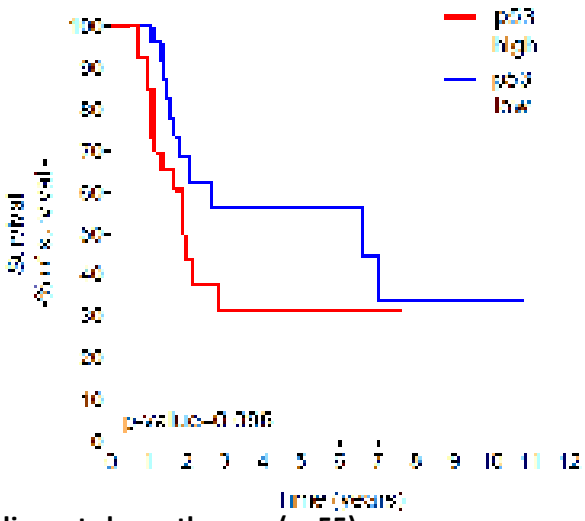


Figure 13. **Predictive power of YapTaz and p53 signatures in RD patients after neoadjuvant chemotherapy (n=55)**

A. KM curve of metastasis free survival. RD patients are stratified according to YapTaz (Zhang et al.) signature level. **B.** Same as in **A.** for overall free survival. **C.** KM curve of metastasis free survival. RD patients are stratified according to p53 (Bild et al.) signature level. **D.** Same as in **C.** for overall free survival.

Adjuvant: univariate analysis

mFU: 64m yrs

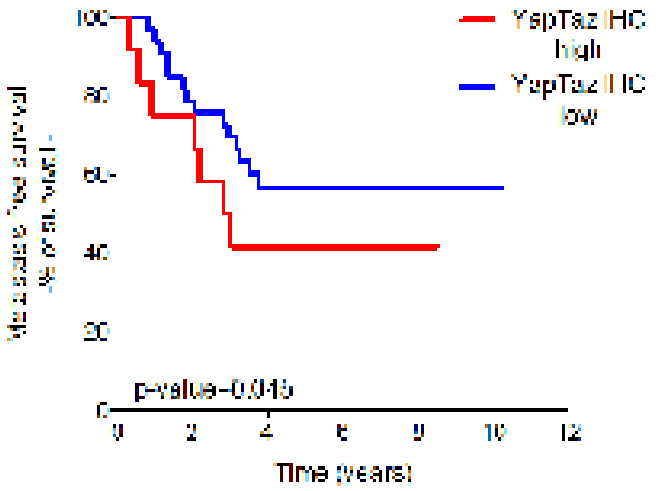
Covariate	subtype	neoadjuvant (n=101)		adjuvant (n=157)		no chemo (n=73)	
		OS	MFS	OS	MFS	OS	MFS
Grade	Grade 2	0.728	0.604	0.995	0.995	0.995	0.999
	Grade 3			0.995	0.995	0.995	0.999
pT	T2	0.115	0.275	0.006	0.002	0.135	0.112
	T3	0.002	0.002	0.997	0.990	0.995	0.999
	T4	0.110	0.075	0.002	0.003	0.995	0.999
pN	N1	0.050	0.040	0.042	0.008	0.750	0.427
	N2	0	0	0.199	0.008	0.004	0.009
	N3	0.002	0	0.036	0	0.554	0.576
PAM50	Her2	0.997	0.996	0.655	0.185	0.999	0.994
	LumA	0.891	0.913	0.548	0.921	0.288	0.554
	LumB	0.609	0.707	0.022	0.157	0.178	0.097
TNBC subtype	BL2	0.788	0.831	0.029	0.019	0.764	0.198
	IM	0.063	0.206	0.786	0.301	0.995	0.999
	M	0.158	0.121	0.239	0.057	0.995	0.999
	MSL	0.514	0.604	0.112	0.012		
	LAR	0.978	0.774	0.980	0.948	0.995	0.991
	UNS	0.983	0.929	0.792	0.995	0.452	0.522
KI67		0.800	0.637	0.178	0.351	0.148	0.115
YAP1 (IHC)		0.999	0.910	0.135	0.157	0.254	0.371
WWTR1 (IHC)		0.091	0.140	0.177	0.807	0.214	0.208
YAP/TAZ (IHC)	High/Low	0.972	0.999	0.998	0.997	0.999	0.999
	Low/High	---	---	0.107	0.079	0.050	0.060
	Low/Low	1.000	0.887	0.045	0.249	0.038	0.039
YAP/TAZ (GEP)	neoadjuvant only (n=35)	0.019	0.014				
	both (n=31)	0.105	0.066	---	---	---	---
	adjuvant only (n=152)	---	---	0.578	0.966	---	---
	none (n=73)					0.957	0.959
p53 (IHC)		0.301	0.636	0.770	0.366	0.782	0.490
p73 (GFP)	neoadjuvant only (n=35)	0.995	0.990	---	---	---	---
	both (n=32)	0.091	0.049				
	adjuvant only (n=152)			0.974	0.957		
	none (n=25)	---	---	---	---	0.835	0.970

Figure 16. Univariate analysis (Cox proportional hazards regression model) in the 3 cohorts of the DASL study.

Adjuvant: DFS and OS

mFU: 64m yrs

A



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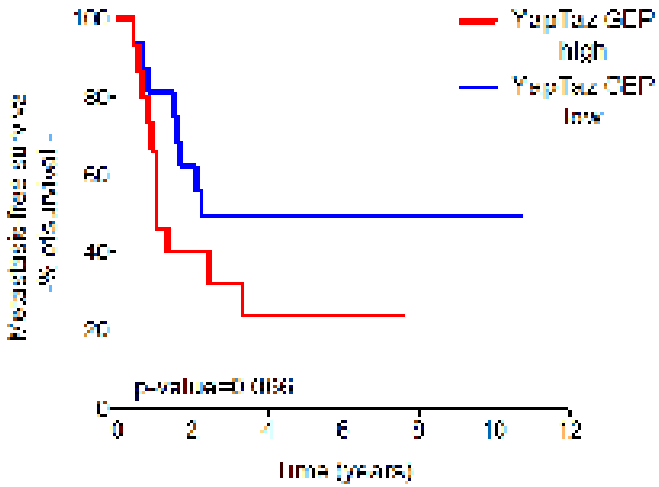


C

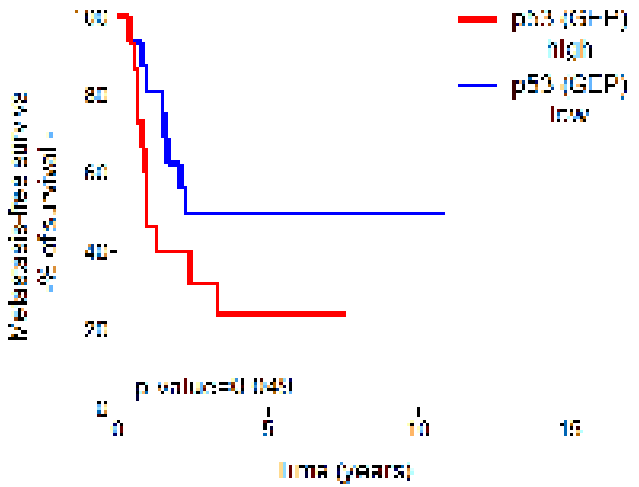
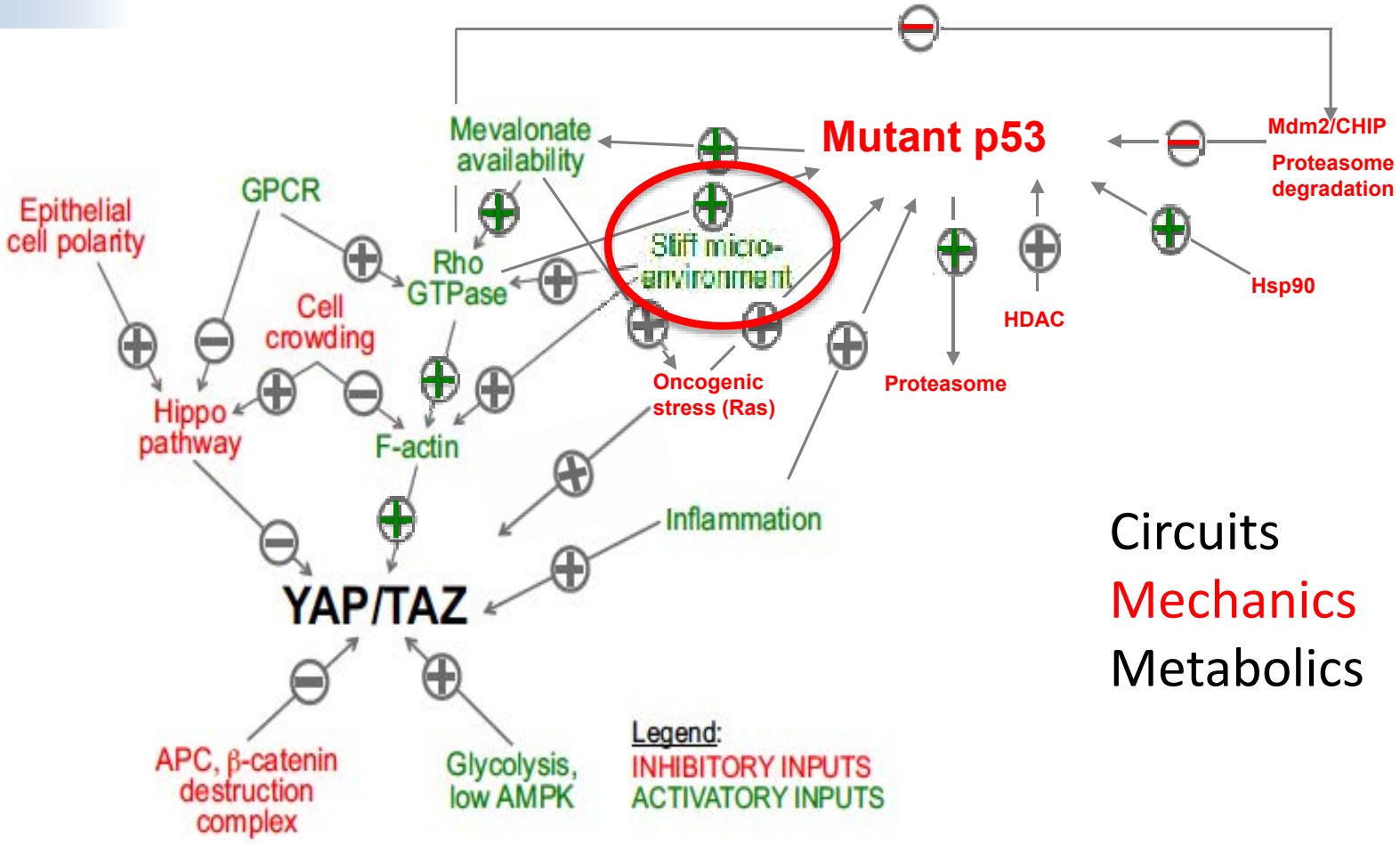


Figure 17. **Predictive power of YAP/TAZ IHC and YAP/TAZ and mutp53 signatures**

A. KM curve of metastasis free survival. Patients of the adjuvant cohort (n=45) are stratified according to YAP/TAZ IHC level. **B.** KM curve of metastasis free survival. Patients of the neoadjuvant + adjuvant cohort (n=31) are stratified according to YAP/TAZ signature level (Zhang et al.). **C.** Same as in **B.** Patients of the neoadjuvant + adjuvant cohort (n=31) are stratified according to mutp53 signature level (Bild et al.).

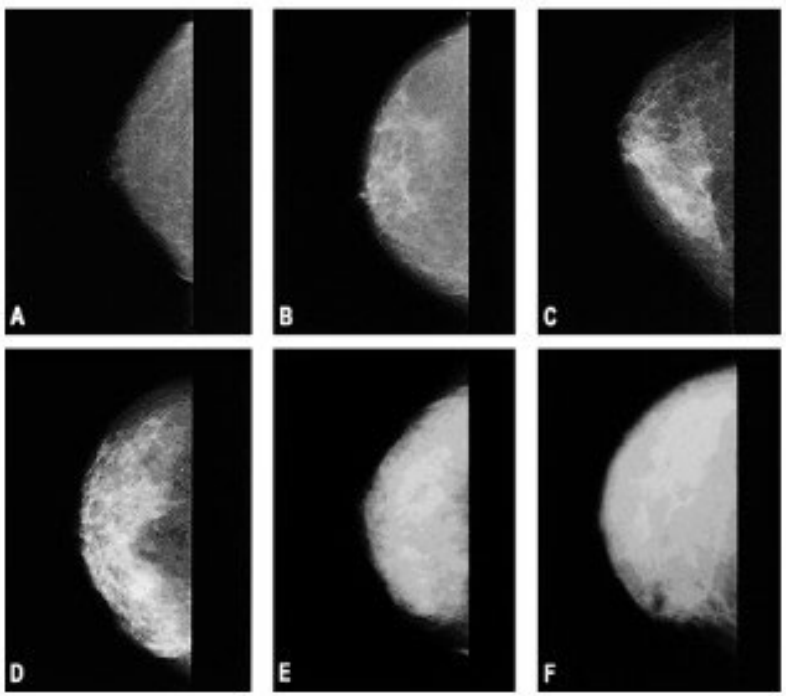
YAP/TAZ Circuits Regulation

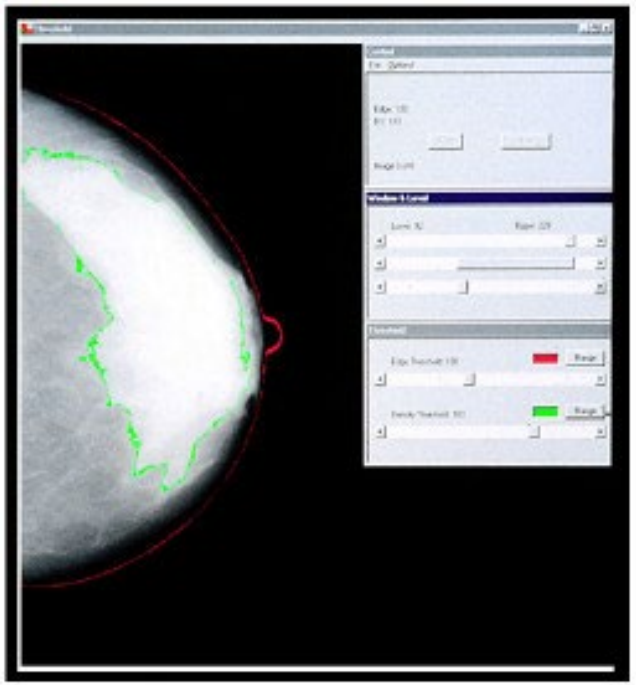


Circuits
Mechanics
Metabolics

modified from Zanconato et al, *Cancer Cell,* 2016

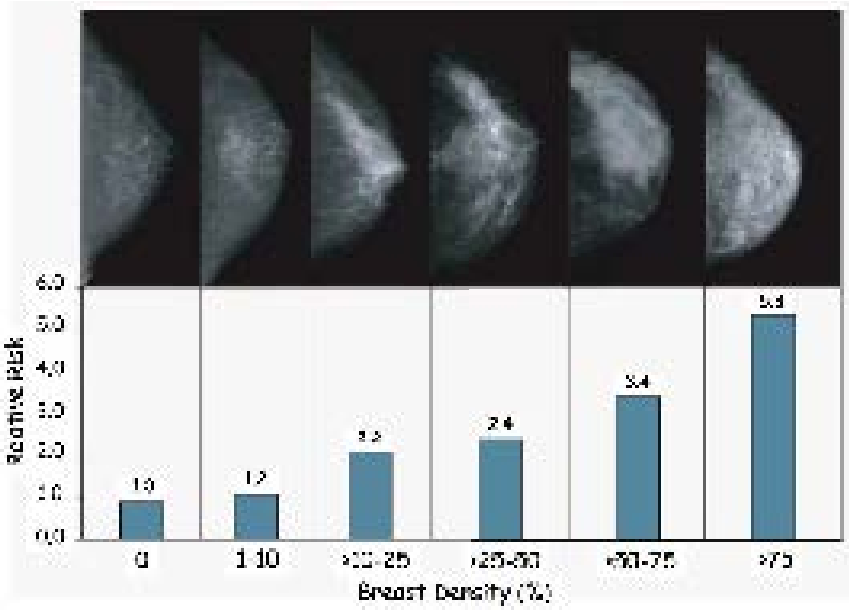
The breast stiffness & Mx density



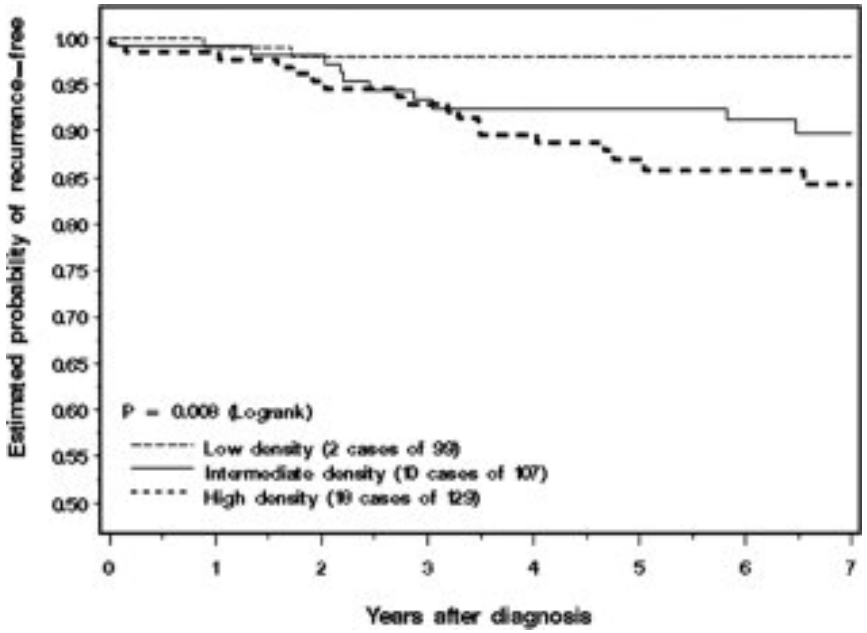


Patterns of mammographic rappresentations

Breast density a risk factor for BC and DFS



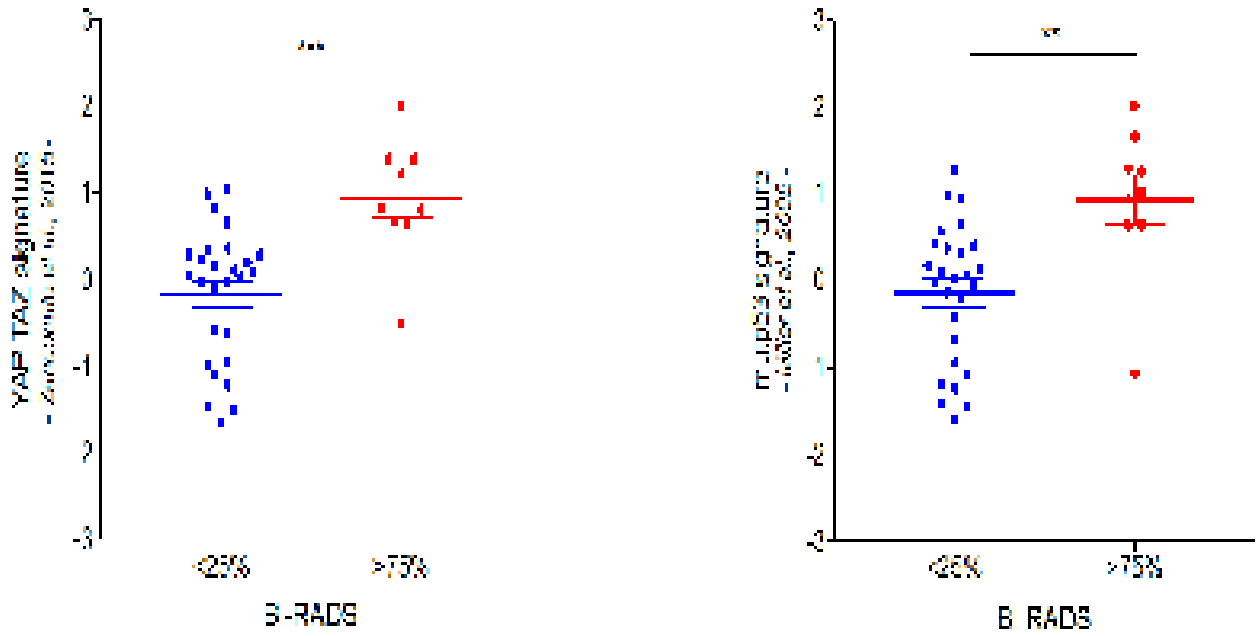
Meta-analysis of 14.000 cases and 226.000 non cases from 42 studies



High density and poor recurrence – free survival (N335)

T. Cil, Cancer, Nov 2009
McCormack Cancer Epidemiol Biom, 2006

YAP/TAZ, p53 & Mx density



Correlation of molecular signatures and mammographic density.
a. Average gene expression values of YAP/TAZ signature genes (Zanconato et al., 2015) in breast cancer samples, classified according to the increasing levels of mammographic density (BIRADS). Data are shown as individual samples (n = 36 independent breast cancer patients obtained by the clinical unit AZ; dots) and the mean ± s.e.m. (standard error of the mean; lines). P value < 0.0005 in a two-tailed unpaired t-test. **b.** Same as in **a.** for mutant p53 signature genes (Miller et al., 2005). P value < 0.005 in a two-tailed unpaired t-test.

Preliminary results

1. Higher expression TAZ/YAP in the TN population
2. Particular association with BL1-2/M TN sub-types
3. Association with p53 (IHC & GEP) and Ki67
4. No predictive role of response to chemotherapy in neoadj setting (trend in PD)
5. Potential prognostic role in DFS and OS in adj setting (double-pos)
6. Possible association higher YAP/TAZ p53 with Mx D.

Outline

Rationale of YAP/TAZ in cancer (preclinical studies)

Prognosis (retrospective studies)

YAP/TAZ in TNBC

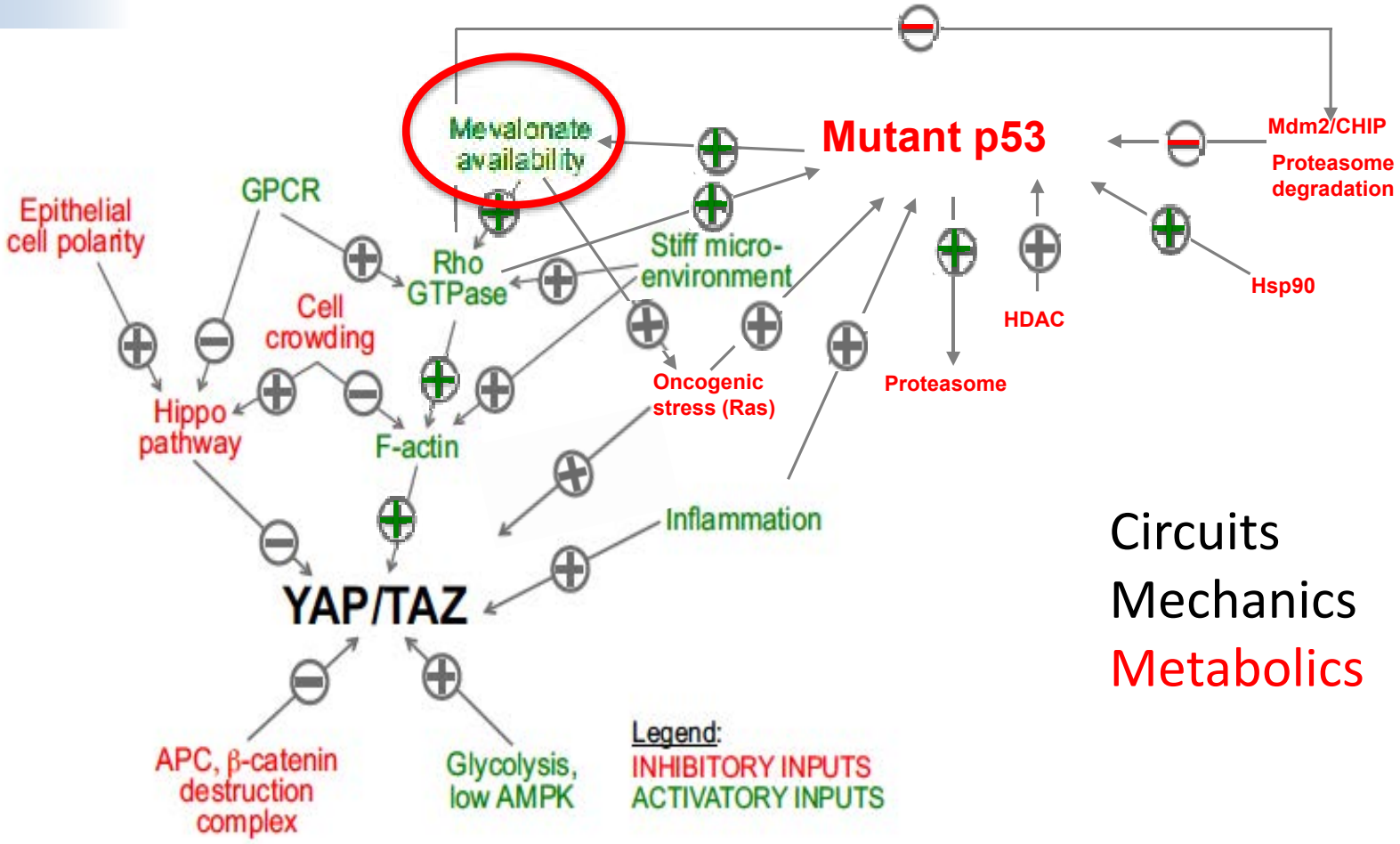
- YAP/TAZ & aggressivness
- YAP/TAZ & Mx density

Prediction (prospective studies)

YAP/TAZ as Rx target

- Pilot trial
- Ph2 RCT

YAP/TAZ Circuits Regulation



Circuits
Mechanics
Metabolics

modified from Zanconato et al, *Cancer Cell,* 2016

YAP/TAZ as cancer target in TNBC

Metabolic control of YAP and TAZ by the mevalonate pathway

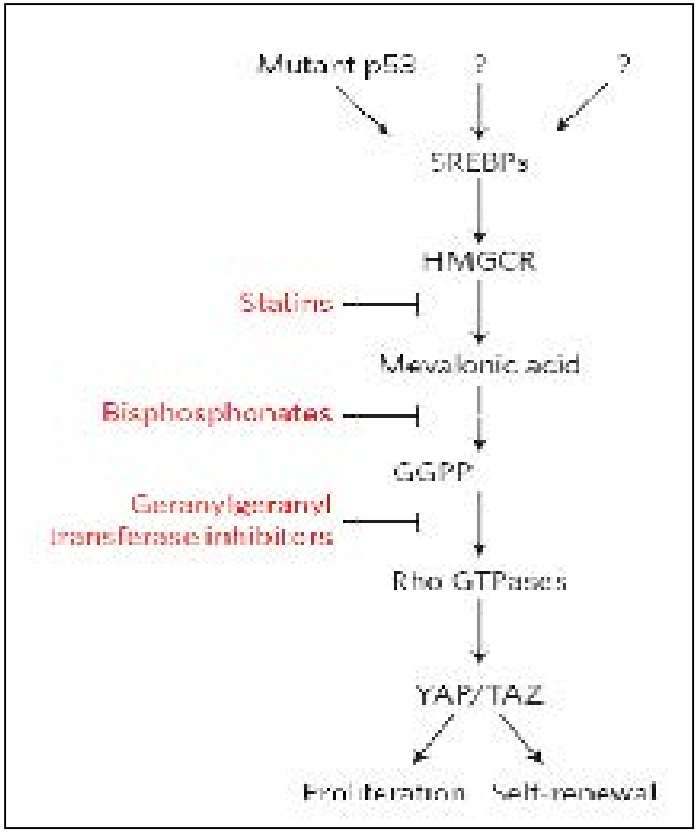
Giovanni Sorrentino[1,*], Naomi Ruggeri[1,*], Valeria Specchia[1], Michelangelo Cordenonsi[2], Miguel Mano[1], Sirio Dupont[2], Andrea Manfrin[3], Eleonora Ingallina[1,4], Roberta Sommaggio[4], Silvano Piazza[1], Antonio Rosato[4], Stefano Piccolo[3] and Giannino Del Sal[1,4]

The YAP and TAZ mediators of the Hippo pathway are controlled by the mevalonate pathway through the sterol regulatory element-binding proteins (SRBPs)

The enzymes of the mevalonate pathways are under transcriptional control of SREBPs

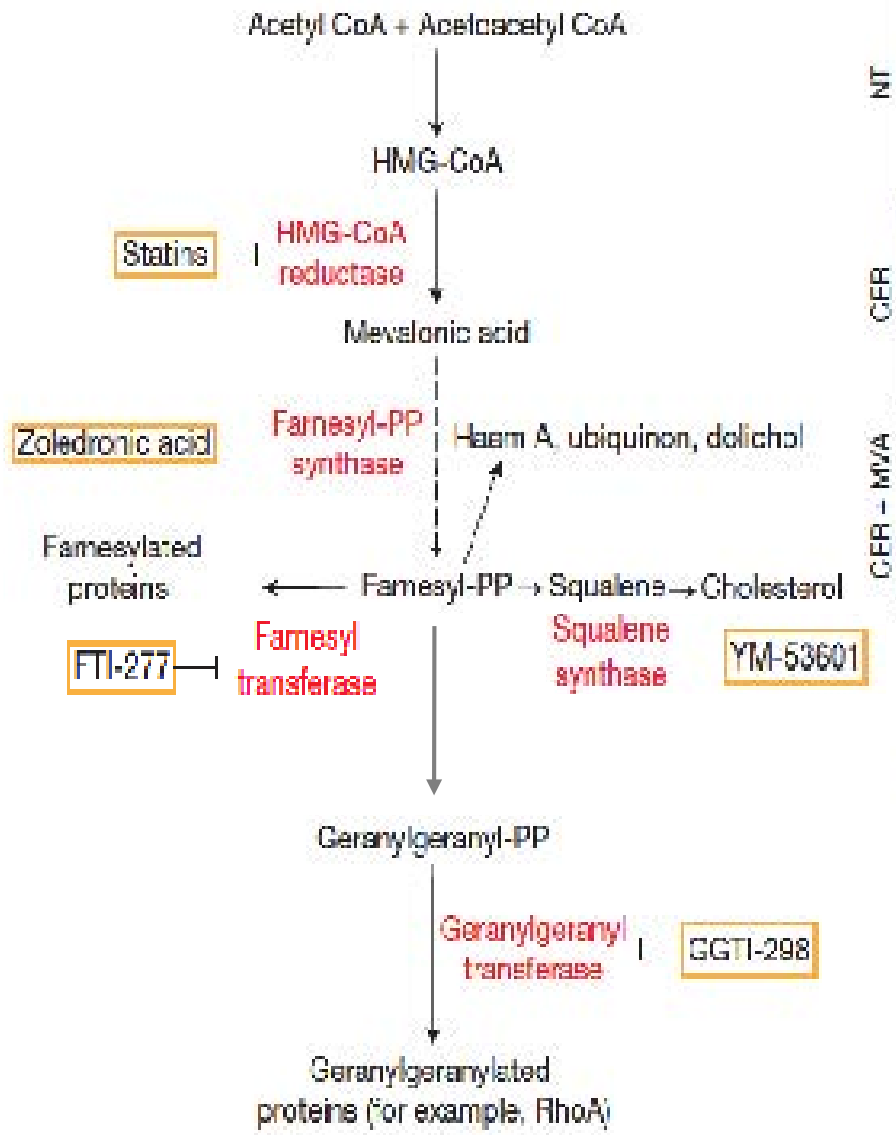
In breast cancer cells mutant p53 acts as a transcriptional cofactor for SREBPs leading to elevated expression of mevalonate enzymes and aberrant activation of YAP/TAZ

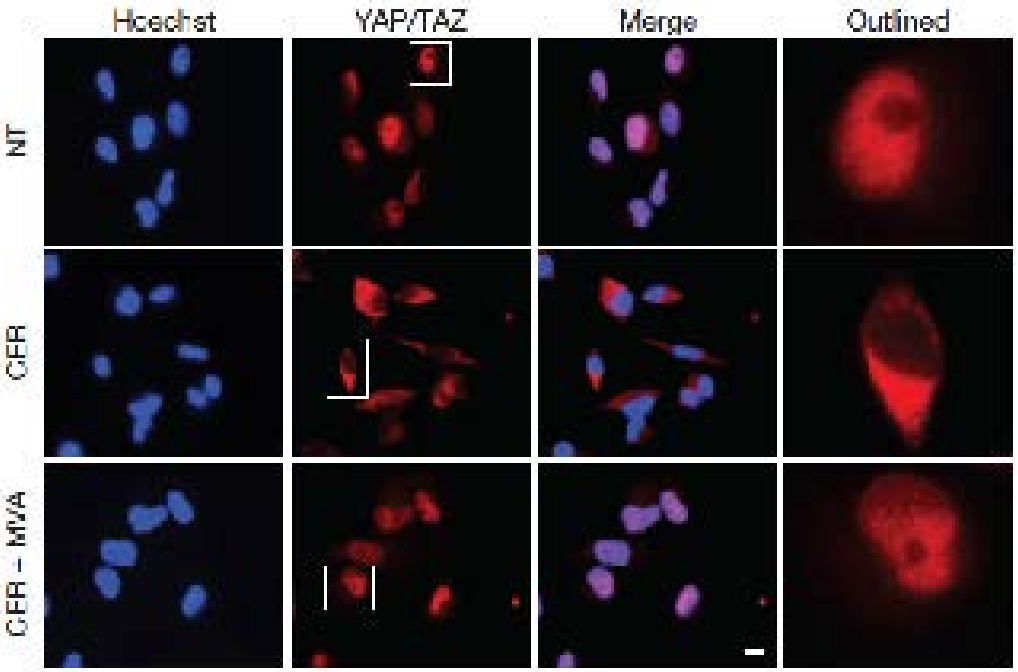
Mutant p53 and YAP/TAZ have been shown to be overexpressed in patients with TNBC, conferring an aggressive cancer behaviour and resistance to standard treatments

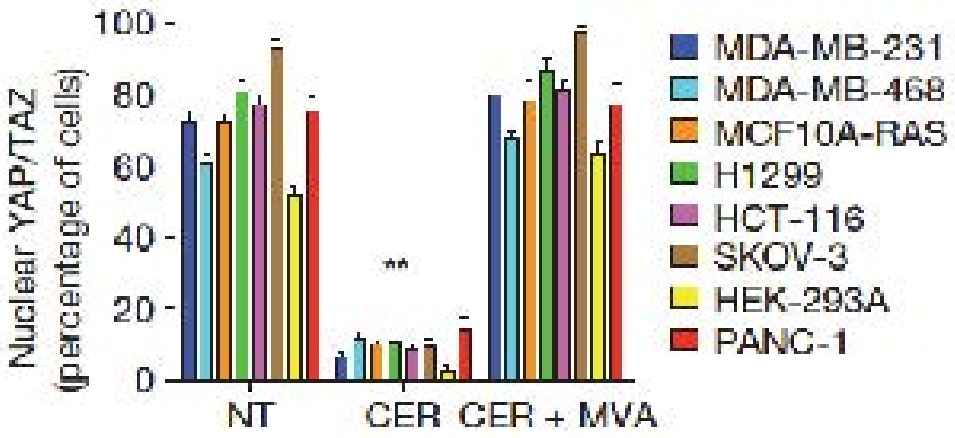


NATURE CELL BIOLOGY VOLUME 16 | NUMBER 4 | APRIL 2014

The MVA pathway sustains YAP/TAZ nuclear localization

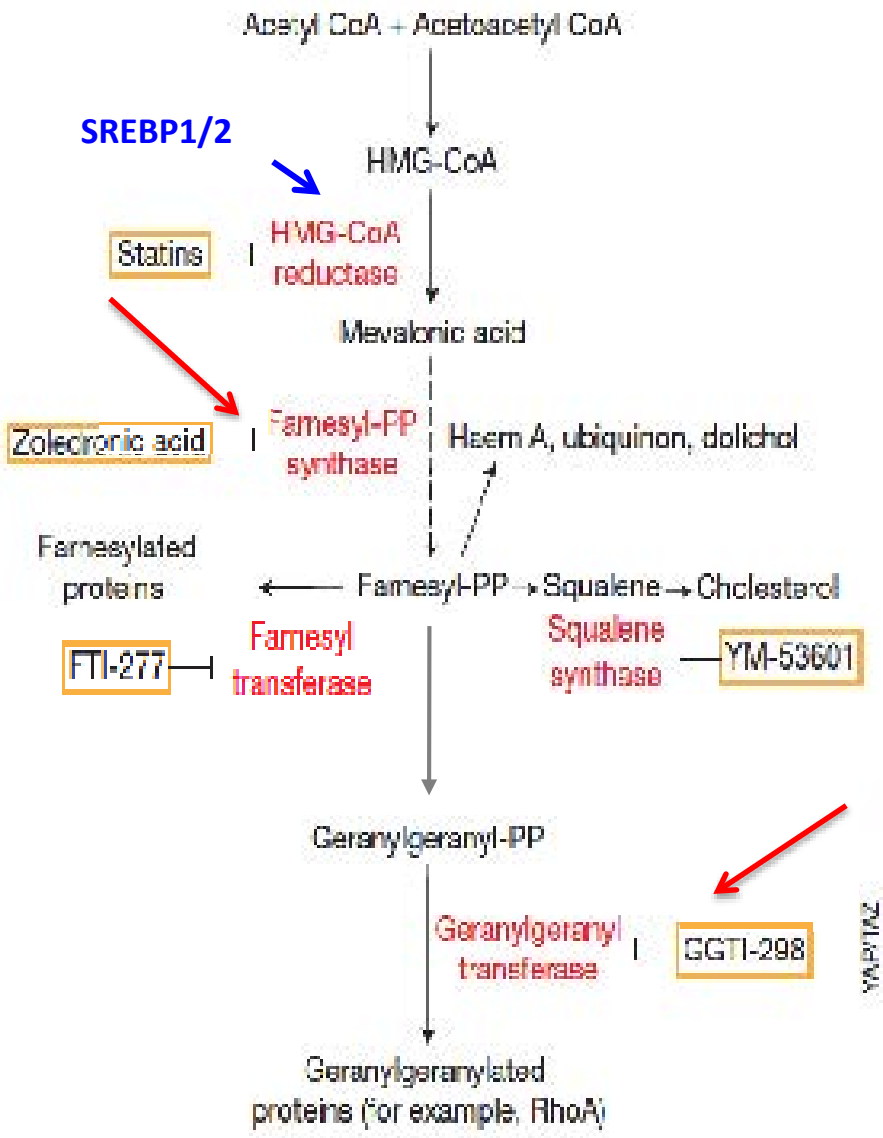


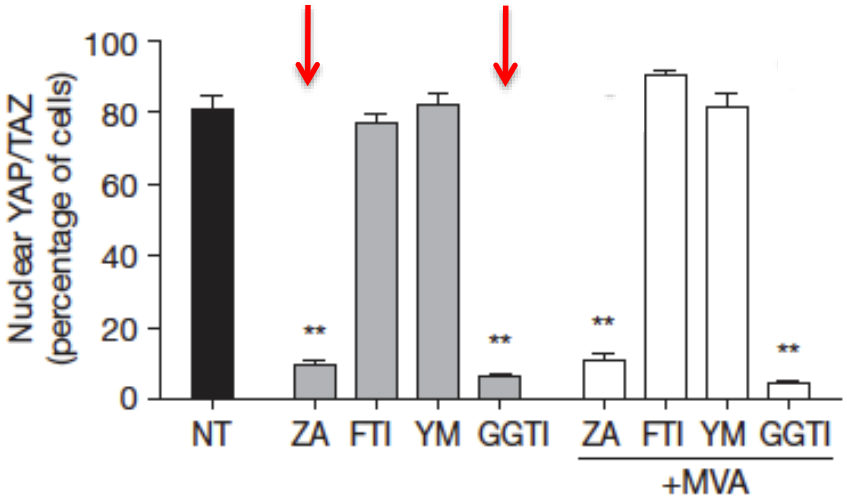


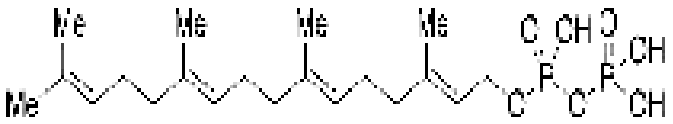


Sorrentino et al, *Nature Cell Biol*, 2014

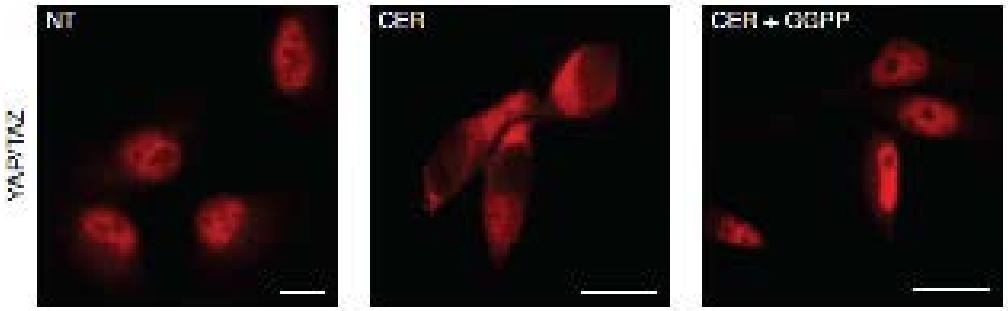
MVA pathway inhibition leading to loss of GGPP causes YAP/TAZ inhibition *(Statin/ZA)*





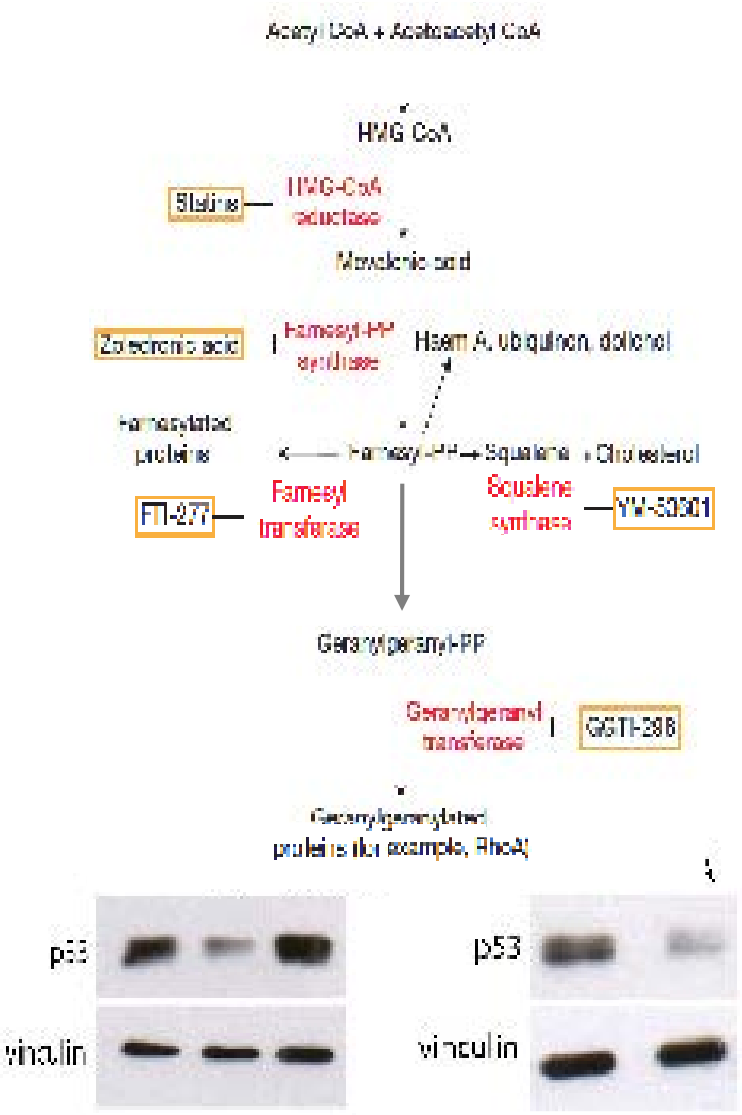


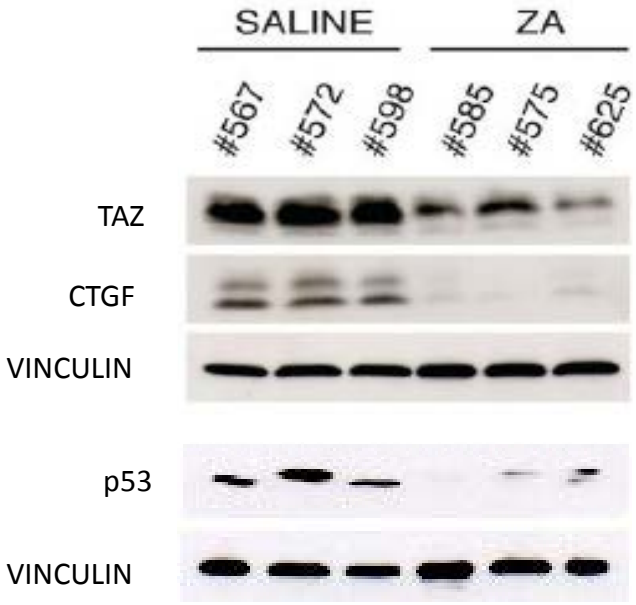
Geranylgeranyl-PhyroPhosphate (GGPP)

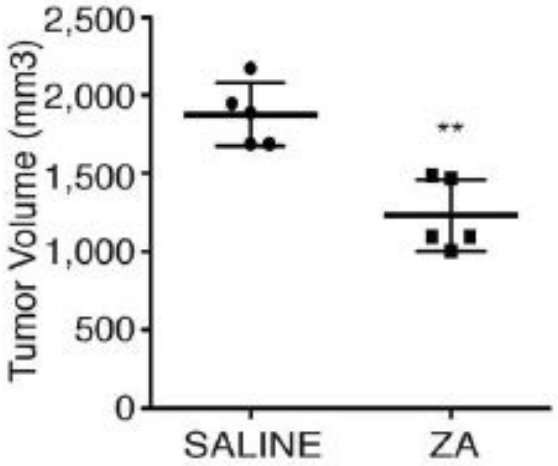


Sorrentino et al. *Nat Cell Biol*, 2014

MVA pathway inhibitors : preclinical evidence *in vivo*







Sorrentino et al. *Nat Cell Biol,* 2014
Sorrentino et al *Manuscript in prep*

Summury of Biological Evidences

- ✓ Mutant p53 and YAP/TAZ are two key pillars of cancer initiation and metastasis. Growing evidences support their role in different cancer types, including TNBC
- ✓ Mutant p53 cooperates with SREBPs leading to MVA pathway activation that leads in turn to YAP/TAZ activation.
- ✓ The MVA pathway thus promotes YAP/TAZ nuclear activities and sustains mutant p53 accumulation in cancer cells.
- ✓ Loss of Geranyl-Geranyl-Phyrophosphate induces YAP/TAZ inhibition but also mutant p53 degradation.
- ✓ Statins and other MVA pathway inhibitors (i.e. Zol) may act as potent YAP/TAZ and mutant p53 inhibitors**.**

Clinical experiences

Clinical efficacy of Statins in early Breast Cancer

N ENGL J MED 367;19 NEJM.ORG NOVEMBER 8, 2012

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Statin Use and Reduced Cancer-Related Mortality

Sune F. Nielsen, Ph.D., Børge G. Nordestgaard, M.D., D.M.Sc. and Stig E. Bojesen, M.D., Ph.D., D.M.Sc.

METHODS

We assessed mortality among patients from the entire Danish population who had received a diagnosis of cancer between 1995 and 2007, with follow-up until December 31, 2009. Among patients 40 years of age or older, 18,721 had used statins regularly before the cancer diagnosis and 277,204 had never used statins.

RESULTS

Multivariable-adjusted hazard ratios for statin users, as compared with patients who had never used statins, were 0.85 (95% confidence interval [CI], 0.83 to 0.87) for death from any cause and 0.85 (95% CI, 0.82 to 0.87) for death from cancer.

CONCLUSIONS

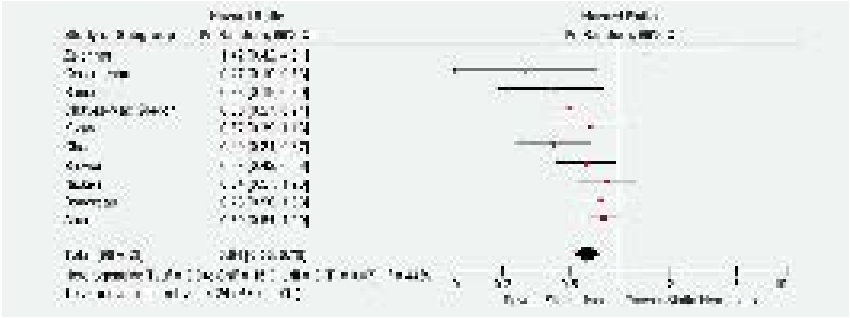
Statin use in patients with cancer is associated with reduced cancer-related mortality. This suggests a need for trials of statins in patients with cancer.

Int. J. Cancer: **139**, 1281–1288 (2016) © 2016 UICC

IJC International Journal of Cancer

Impact of statin use on cancer recurrence and mortality in breast cancer: A systematic review and meta-analysis

Sashidhar Manthravadi[1], Anuj Shrestha[2] and Sheshadri Madhusudhana[2]



BC patients who use statins (lipophilic statins) show improved RFS. Statin users also had improved OS and cancer-specific survival. (N >75.000)

Clinical efficacy of BPs in early Breast Cancer

www.thelancet.com Vol 386 October 3, 2015

Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials

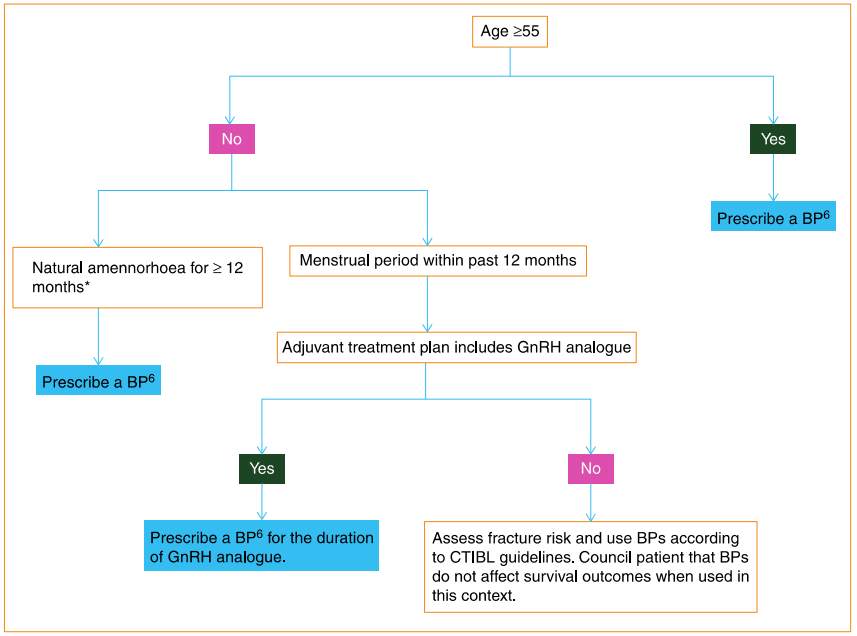
*Early Breast Cancer Trialists' Collaborative Group (EBCTCG)**

Background Bisphosphonates have profound effects on bone physiology, and could modify the process of metastasis. We undertook collaborative meta-analyses to clarify the risks and benefits of adjuvant bisphosphonate treatment in breast cancer.

Findings We received data on 18 766 women (18 206 [97%] in trials of 2–5 years of bisphosphonate)

Interpretation Adjuvant bisphosphonates reduce the rate of breast cancer recurrence in the bone and improve breast cancer survival, but there is definite benefit only in women who were postmenopausal when treatment began.

Adjuvant bisphosphonates in early breast cancer: consensus guidance for clinical practice from a European Panel



Overall
RR: 17% bone mets
Post-menopausal
RR: 14% recurrence
RR: 18% distant rec
RR: 28% bone rec
RR: 18% BC mortality

Preoperative experience of BPs and Sts in BC

British Journal of Cancer (2010) 102, 1295 – 1300
www.bjcancer.com

The effects of adding zoledronic acid to neoadjuvant chemotherapy on tumour responses: exploratory evidence for direct anti-tumour activity in breast cancer

RE Coleman[1,11], MC Winter[2,11], D Cameron[3], R Bell[4], D Dodwell[5], NN Keane[6], N Gil[6], D Ritchie[7], JL Passos-Coelho[8], D Wheatley[9], R Burkinshaw[7], SJ Marshall[10] and H Thorpe[1,3] on behalf of the AZURE (BIG01/04) Investigators

Retrospective exploratory analysis of AZURE pts (N:205) who have received NAC comparing **CT + ZOL** *vs* **CT alone**

PRIMARY ENDPOINT PATHOLOGICAL RESIDUAL INVASIVE TUMOR SIZE (mm)	
CT + ZOL	**CT**
15.5	27. 4

p=0.006

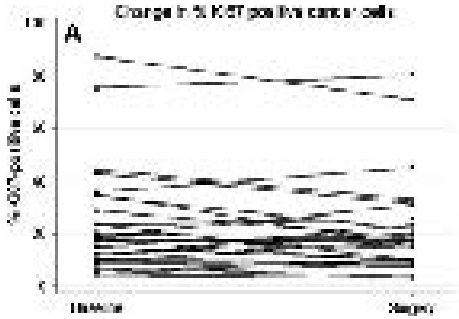
SECONDARY ENDPOINT PATHOLOGICAL COMPLETE RESPONSE RATE (%)	
CT + ZOL	**CT**
11.7	6.9

Breast Cancer Res Treat (2013) 138:499–508
DOI 10.1007/s10549-013-2473-6

CLINICAL TRIAL

Targeting HMG-CoA reductase with statins in a window-of-opportunity breast cancer trial

Olöf Bjarnadottir · Quinci Romero · Pär-Ola Bendahl · Karin Jirström · Lisa Rydén · Niklas Loman · Mathias Uhlén · Henrik Johannesson · Carsten Rose · Dorthe Grabau · Signe Borgquist



Decrease in Ki67 in eBC (N: 50) expressing HMGCR in the pre-treatment sample was **24 %** (P = 0.02)

Clinical Protocol Design

A SINGLE-ARM PILOT STUDY TO EVALUATE THE ACTIVITY OF PRE-OPERATIVE ZOLEDRONATE IN TRIPLE NEGATIVE BREAST CANCER PATIENTS ACCORDING TO P53 LEVEL

AIRC Special Program Molecular Clinical Oncology 5X1000 fase II - Molecular basis for triple negative breast cancer metastasis: new tools for diagnosis and therapy

Objectives 1

The study addresses the activity of BPs in TNBC patients according to p53 expression

Primary objective

Assessing the anti-tumor activity of pre-operative zoledronate through the Ki67 proliferative surrogate biomarker analysis in TNBC patients with high and low p53 expression.

Objectives 2

Secondary objectives

- ✓ To analyse the levels of critical genes/proteins related to p53 and to the mavelonate pathway - p53/PIN1 and YAP/TAZ - by IHC and RT-PCR performed at the time of diagnosis (core biopsy) and after ZOL treatment (definitive surgery)

- ✓ To evaluate the anti-tumor activity of zoledronate, assessed according to RECIST criteria, version 1.1

- ✓ To measure treatment compliance, defined as the proportion of patients completing treatment

- ✓ To assess the safety profile of zoledronate, evaluated by the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) scale, version 4.0 and by the occurrence of serious adverse reactions

STUDY DESIGN

DATA COLECTION AND MANAGEMENT (ELECTRONIC CRF)

Patients with newly diagnosed, untreated, operable TNBC with defined ki67 and p53 expression

Registration (20 pts)

Core biopsy tumor tissue sample: p53/PIN1, YAP/TAZ assessment by IHC and RT-PCR

Zoledronate
(4 mg, single dosing)

Seven days after ZOL adm. definitive breast surgery

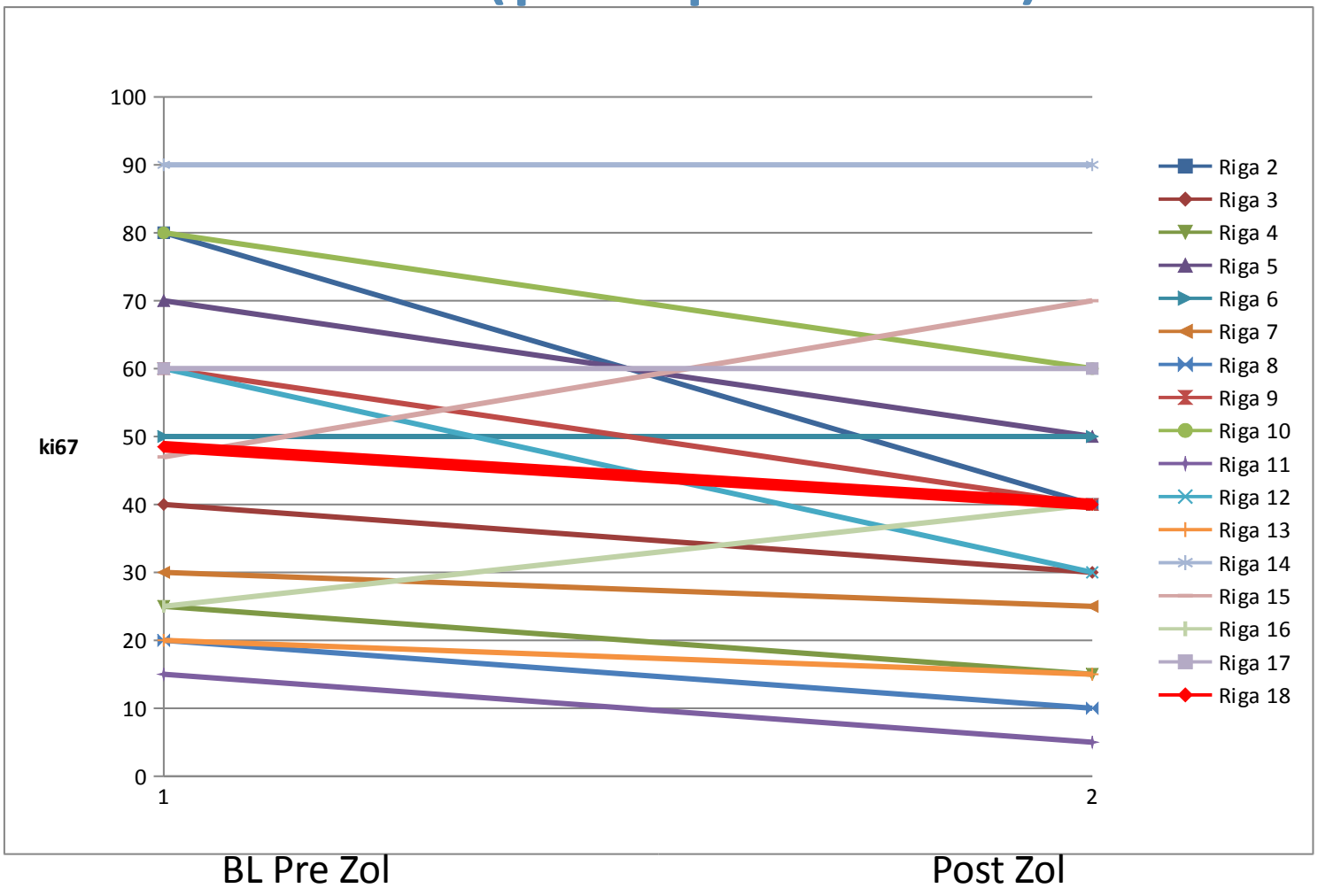
2 months of follow-up to assess adverse events

At the surgery tumor tissue sample collection for
- **Ki67 IHC,**
- **p53/PIN1, YAP/TAZ IHC and RT-PCR**

Primary endpoint:
Zol anti-tumor activity (Ki67 expression)

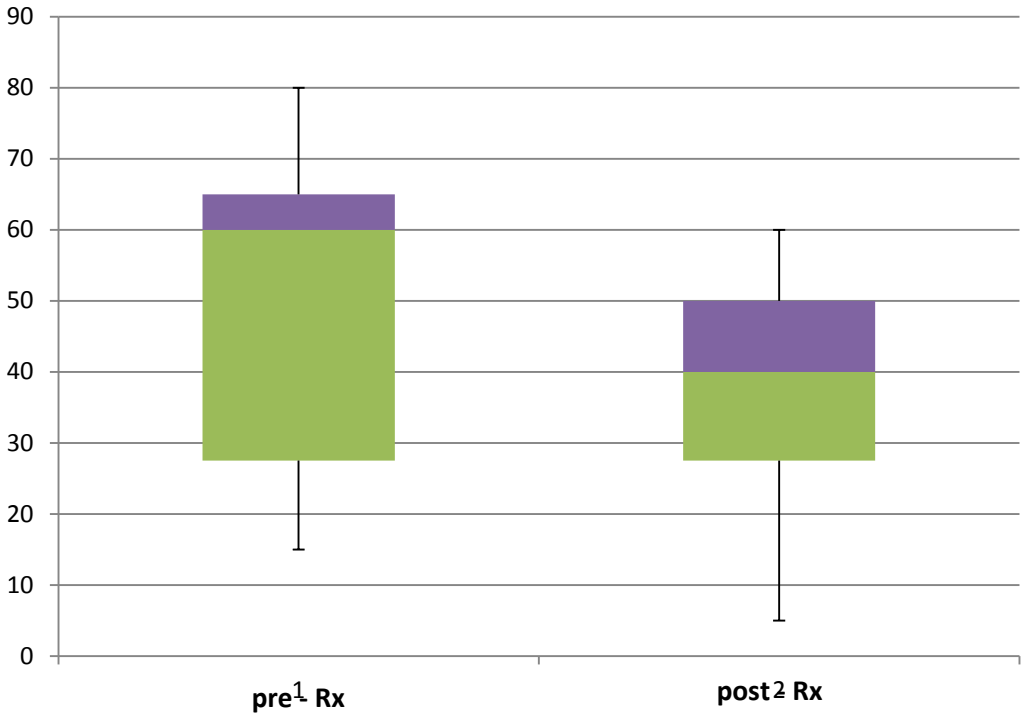
Secondary endpoint:
- p53/PIN1 and YAP/TAZ expression (IHC and RT-PCR)
- Zoledronate safety and tolerability

Ki67 (pre-post Zol)



Median: 48.5 vs 40

Box Plots Ki67

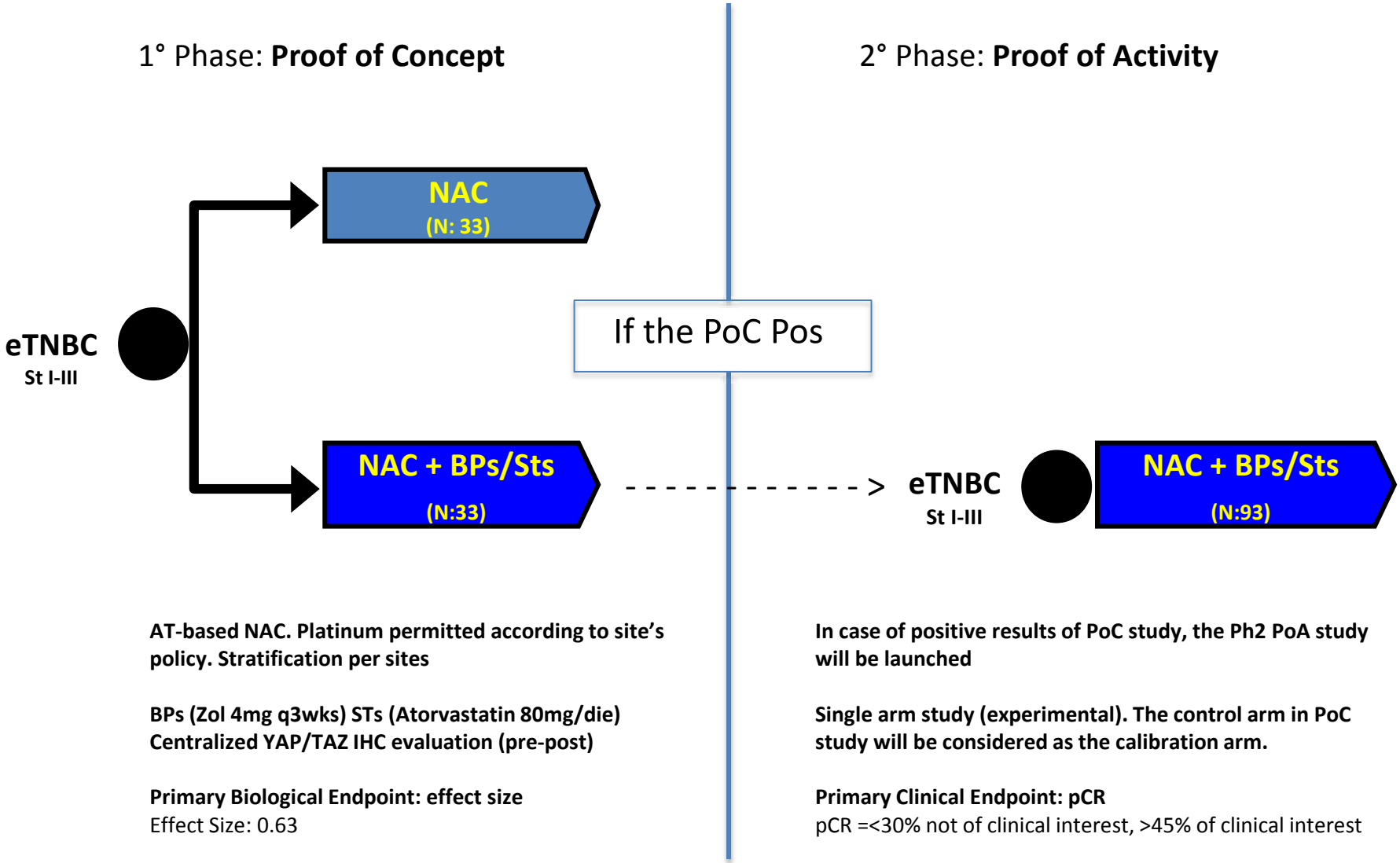


Correlation with p53 status, YAP/TAZ are pending

A MULTICENTER, RANDOMIZED, PHASE II STUDY TO EVALUATE THE ACTIVITY OF NEOADJUVANT ZOLEDRONATE + ATORVASTATION IN TRIPLE NEGATIVE BREAST CANCER PATIENTS

AIRC Special Program Molecular Clinical Oncology 5X1000 fase II - Molecular basis for triple negative breast cancer metastasis: new tools for diagnosis and therapy

YAPPETIZER Study



AT-based NAC. Platinum permitted according to site's policy. Stratification per sites

BPs (Zol 4mg q3wks) STs (Atorvastatin 80mg/die)
Centralized YAP/TAZ IHC evaluation (pre-post)

Primary Biological Endpoint: effect size
Effect Size: 0.63

In case of positive results of PoC study, the Ph2 PoA study will be launched

Single arm study (experimental). The control arm in PoC study will be considered as the calibration arm.

Primary Clinical Endpoint: pCR
pCR =<30% not of clinical interest, >45% of clinical interest

Breast Cancer Res Treat (2014)
Ann of Oncol (2012)

Two main objectives

Biological objective: to assess the anti-tumor activity of the combination of pre-operative standard chemotherapy associated or not with zoledronate and statins in terms of relative reduction in YAP/TAZ IHC-expression at surgery with respect to core-biopsy analysis.

Clinical objective : to assess the anti-tumor activity of the combination of pre-operative standard chemotherapy associated with zoledronate and statins in terms of proportion of patients obtaining a pCR at surgery.

1° Phase: Proof of Concept

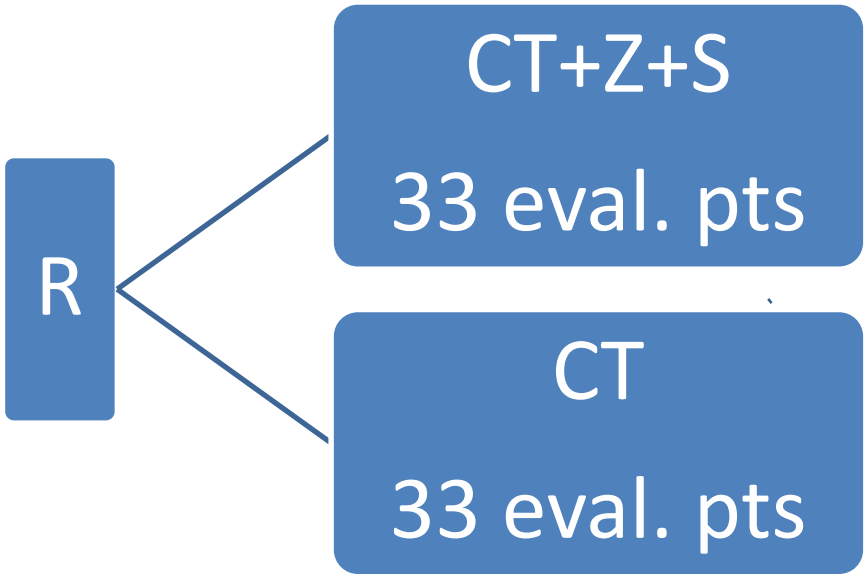
- According to the biological primary endpoint, the experimental arm will be considered deserving of further development if a significant difference between arms in terms of relative reduction of YAP/TAZ IHC-expression at surgery with respect to core-biopsy analysis will be identified. The relative red is calculated as:

  $$[\text{YAP/TAZ IHC}_{\text{at surgery}} - \text{YAP/TAZ IHC}_{\text{at baseline}}] / \text{YAP/TAZ IHC}_{\text{at baseline}}$$

- Setting a type I error at 5% one-sided for the unpaired Student's T test, with 33 pts per arm the study will have a power of 80% to detected an effect size of 0.63.

- In pts responders without a residual disease (expected to be about 30%) the value of relative reduction is set = 0.
- However, in order to have the possibility to evaluate YAP/TAZ IHC-expression in a sufficient number of pts, as second analysis, 47 patients will be randomized in each arm.

1° Phase. Proof of concept



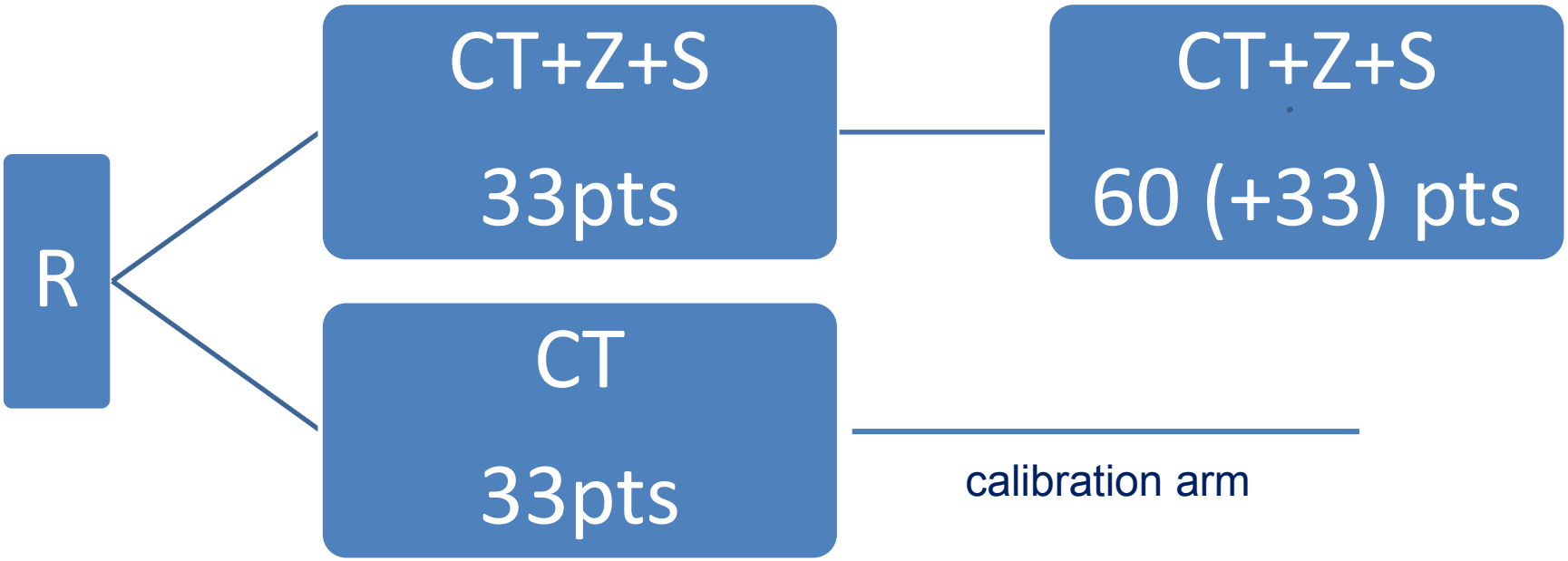
Effect Size=0.63
T-Test, α=5% $_{1\ side}$; power= 80%

2° Phase: Proof of activity

- A'hern single stage design will be used.
- The null hypothesis is that the true response rate, considered not interesting, is 30%.
- The design is planned to yield a type I error rate of 5% and power of 90% when the true response rate is 45%.
- 93 patients need to be accrued, including the patients evaluated in the I° phase. The null hypothesis will be rejected if 36 or more responses are observed in 93 patients.

- Expecting a 10% of patients not evaluable for the primary analysis the total number of patients to be entered in this part of the study is about 103.

2° Phase. Proof of activity



P_0=.30; p_1=.45
α=5% 1 side; power= 90%
pCR=≥36/93

Invitation to Partecipate !