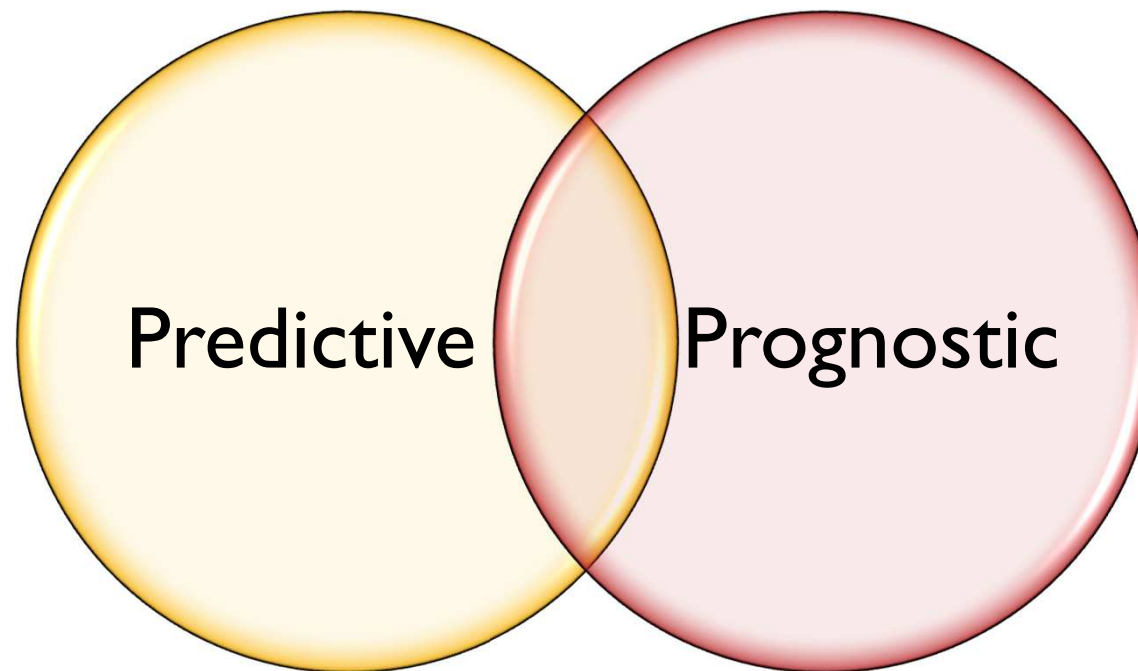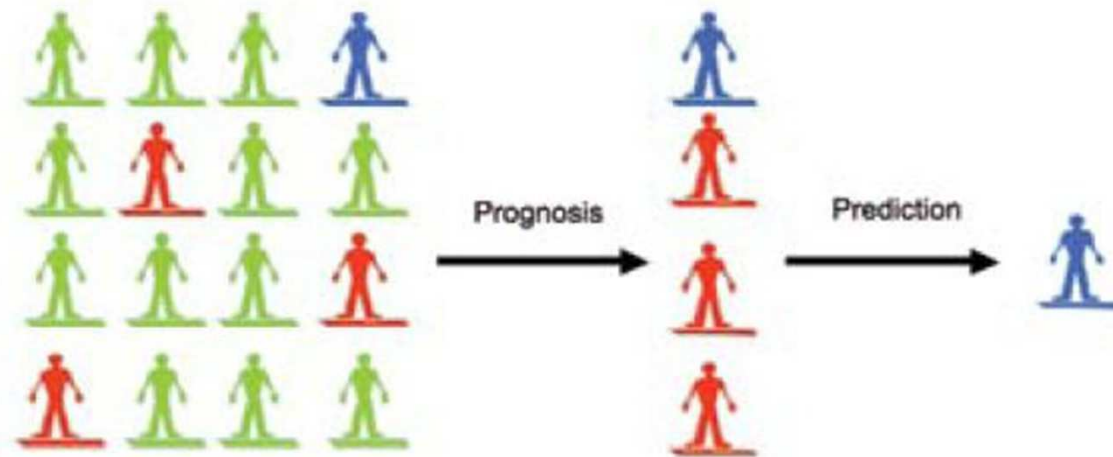# Predictive Markers

Prof Dr M S Vidyasagar,

Head of the Department of Radiation Oncolgy,

Father Muller Medical College,

Mangalore.

Predictive/Prognostic marker

Predictive | Prognostic

Prognosis

Prediction

- Prognostic -Biomarker that provides information on the likely course of the cancer disease in an **untreated individual**

- Predictive marker- marker which can be used to identify subpopulations of patients who are most likely to respond to a given therapy

Why ? Predictive assays

- Do all patients fare same for similar treatments?

- Do all tumors of certain type and site behave to treatment in the same way?

- No tumor is 100% cured till now, there are a few subsets who do not respond to the best of efforts. Shouldn't we try further?

- Individual differences in inherent Radio-sensitivity

- The variables determining radiotherapy response can be grouped into three different categories:

➤ Intrinsic radiosensitivity,

➤ Tumor oxygenation status

➤ Tumor proliferative potential (Tpot)

How to Categorize

Oxygen status

Intrinsic cell radio sensitivity or resistance

Proliferative potential

Cervical cancer

- Tumor size- < 4cms or >
- Histology
- Regional spread
- Tumor hypoxia
- Interstitial Tumor Pressure

Predictive markers in breast carcinoma

- ER/PR/Her2neu
- Ki67
- p53
- bcl-2
- cyclin D1
- Molecular subtypes
- ❑ ER-positive luminal A (luminal A)
- ❑ ER-positive luminal B (luminal B)
- ❑ HER2 enriched
- ❑ basal like
- ❑ normal breast

Oxygen status

PROGNOSTIC VALUE OF HEMOGLOBIN CONCENTRATIONS AND BLOOD
TRANSFUSIONS IN ADVANCED CARCINOMA OF THE CERVIX TREATED
BY RADIATION THERAPY: RESULTS OF A RETROSPECTIVE
STUDY OF 386 PATIENTS

T. GIRINSKI, M.D.,* M. H. PEJOVIC-LENFANT, M.D.,† J. BOURHIS, M.D.,*
F. CAMPANA, M.D.,* J. M. COSSET, M.D.,* C. PETIT, PH.D.,* E. P. MALAISE, M.D. PH.D.,‡
C. HAIE, M.D.,* A. GERBAULET M.D.* AND D. CHASSAGNE, M.D.*

386 patients between 1973 and 1983.

Multivariate analysis of hemoglobin concentrations

Prognostic only during treatment for Hb <10 gm %

Significantly higher risk of local regional failure than the patients with all their values above the threshold.

70% of these high risk patients had less than half of their values below the threshold.

Possibly blood transfusions might be beneficial when given before treatment
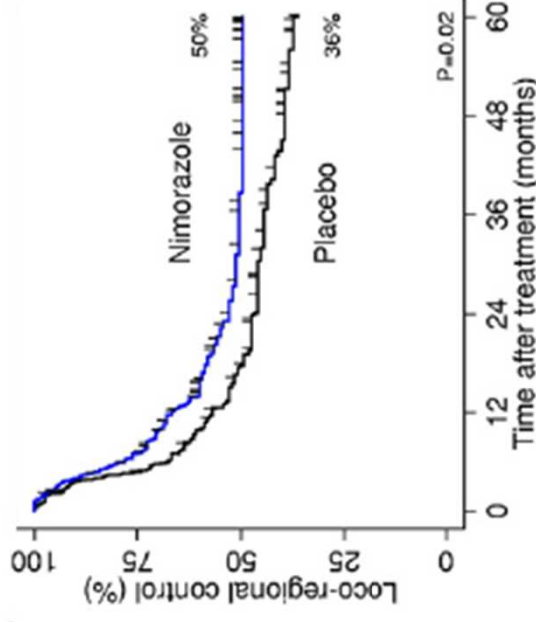
Tumor hypoxia

- pO2 readings less than 10 mm Hg
- High-risk features for parametrial spread and LVSI
- Prognostic significance with surgery as well and not only to chemo-radiation.
- High ITP
- Elevated risk of recurrence
- local and distant sites after radiation therapy
- benefit from biologically targeted agents
- modify the tumor microenvironment through platelet-derived growth factor signaling (eg, imatinib) or vascular endothelial growth factor (VEGF) signaling (eg, bevacizumab).
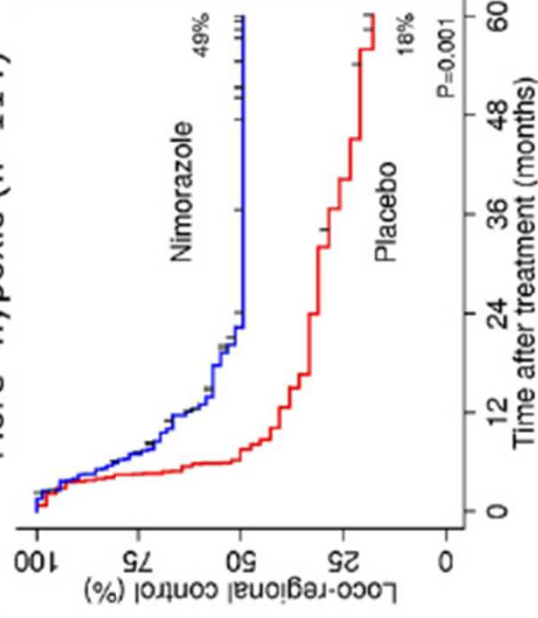
Hypoxia predictor in HN

- Eppendorph oxygensensing electrode
- infusion of exogenous tracers-eg. Pimonidazole, F-MISO
- Gene expression microarray eg. 15-gene hypoxia classifier (DAHANCA)
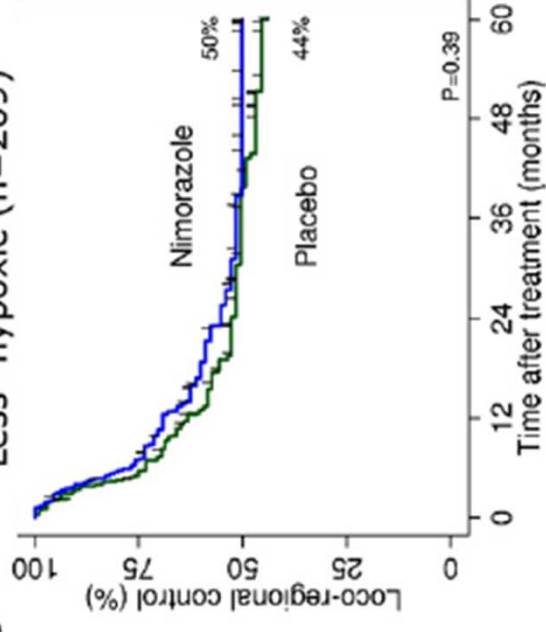
A Unclassified patients (n=323)

Loco-regional control (%)

100
75
50
25
0

Nimorazole 50%

Placebo 36%

P=0.02

0 12 24 36 48 60
Time after treatment (months)

15-gene hypoxia classification

B "More" hypoxic (n=114)

Loco-regional control (%)

100
75
50
25
0

Nimorazole 49%

Placebo 18%

P=0.001

0 12 24 36 48 60
Time after treatment (months)

C "Less" hypoxic (n=209)

Loco-regional control (%)

100
75
50
25
0

Nimorazole 50%

Placebo 44%

P=0.39

0 12 24 36 48 60
Time after treatment (months)

Tests of radiosensitivity

- SF-2 fraction of cells surviving 2Gy
- Fraction of SF-2 exceeds median the survival decreases
- Clonogenic cell survival assay has been the gold standard to measure cellular response to radiotherapy in the laboratory
- Technically difficult due to poor plating efficiency

Disease Site	No. of Patients	SF2 Cutpoint	Outcome	Positive Study	Reference
Head and Neck	99	0.4	Local control <0.4 vs >0.4 91% vs 74% $P=.036$	Yes	Björk-Eriksson et al[8]
Cervix	128	0.42	Survival <0.42 vs >0.42 81% vs 51% $P=.0002$	Yes	West et al[6]
Head and Neck	38	0.5	Local control <0.5 vs >0.5 26% vs 45% $P=NS$	No	Stausbøl-Grøn et al[9]
Glioblastoma multiforme	50	Not determined	No correlation between SF2 and survival	No	Taghian et al[10]
Head and Neck	92	Not determined	No correlation between SF2 and survival	No	Eschwege et al[11]

EGFR in Ca Cervix

- EGFR expression -inferior outcome after radiation therapy
- Worse overall survival in multivariate analysis (*P 0.011*)
- Over expression of EGFR does not correlate with outcome after surgical therapy
- Targeting EGFR with biological agents may be an effective strategy in cervical cancer in patient with EGFR amplification.
- Toxicity will have to be carefully monitored

Human Papillomavirus

- 75% to 95%, of cervical cancers are positive for human papillomavirus (HPV)
- Five-year survival rates are 45% to 50% for patients with HPV-negative oropharynx cancer, as compared with
- 75% to 80% for those with HPV-positive tumors.
- HPV status correlates with multiple molecular abnormalities
  - chromosomal changes
  - *P53* mutation
- HPV 18 has higher chances of recurrences.

HPV in HN

- Viral gene expression- E6, E7 and expression of p16
- HPV positivity have a better outcome
- HPV positivity is associated with less hypoxia
- Better response to radiation and no sign benefit from hypoxic sensitizers
- High expression of CA9 a marker for hypoxia in HPV negative tumors

A
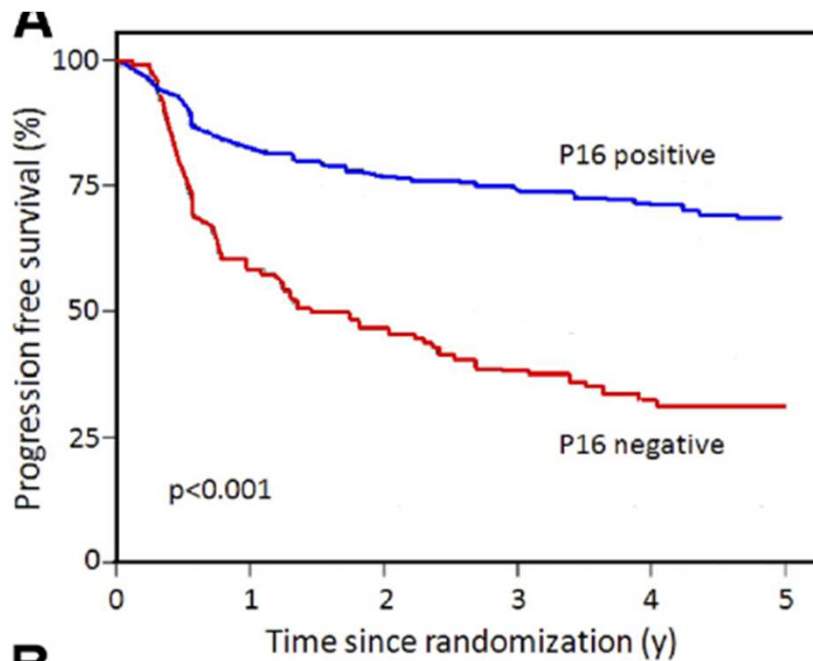
A total of 316 patients given conventional or accelerated fractioned radiotherapy, each combined with cisplatin

B

A total of 156 patients treated with conventional radiotherapy alone in the context of a randomized trial
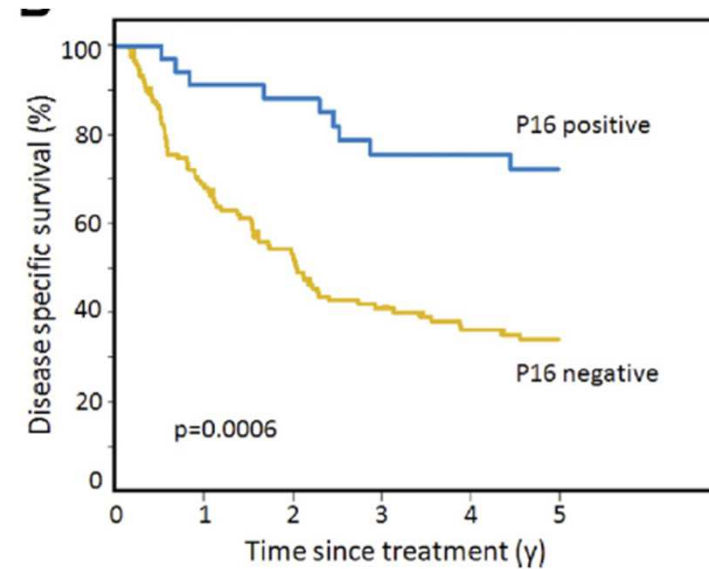
Fluoro-deoxyglucose Positron Emission Tomography

- Higher standard uptake value (SUV) for FDG in both the primary tumor and regional lymph nodes is a strong predictor of worse outcome

- OS 5yrs-

➢ 95% -SUV(max)5.2

➢ 70% -SUV(max) 5.2 - 13.3

➢ 44% -SUV(max)13.3 *(P0.0001)*

Cu-ATSM- is a new hypoxia marker showing promising results

- Carbonic anhydrase- CAIX, CAXII,
- Hypoxia-inducible factor-1
- VEGF
- EGFR
- HPV

Gene profiling

- 70-gene profile is now commercially available as the MammaPrint

- Oncotype DX-reverse-transcriptase polymerase chain reaction assay measures the expression of 21 genes (16 cancer-related genes and 5 reference genes) in RNA extracted from paraffin-embedded tumor samples from primary breast cancer

# Validation of the 70 gene profile in 295 tumors
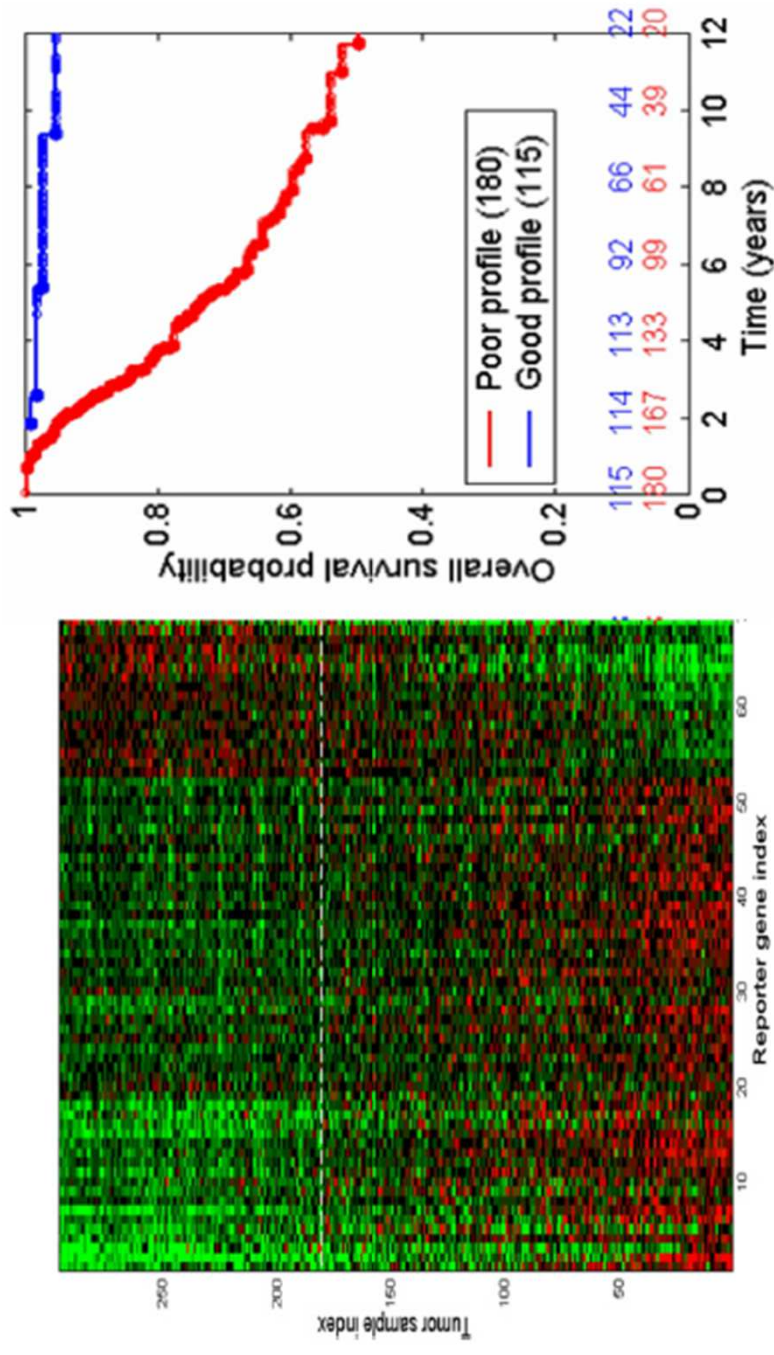
patients <53 years of age, lymph node negative or positive

Stem Cell

52 patients with early stage larynx cancer given radiotherapy alone.
High expression of the putative stem cell marker CD44 predicted poor outcome
A total of 96 patients with advanced head and neck cancer given radiotherapy combined with cisplatin.
High expression of the Chung high-risk signature genes predicted poor outcome
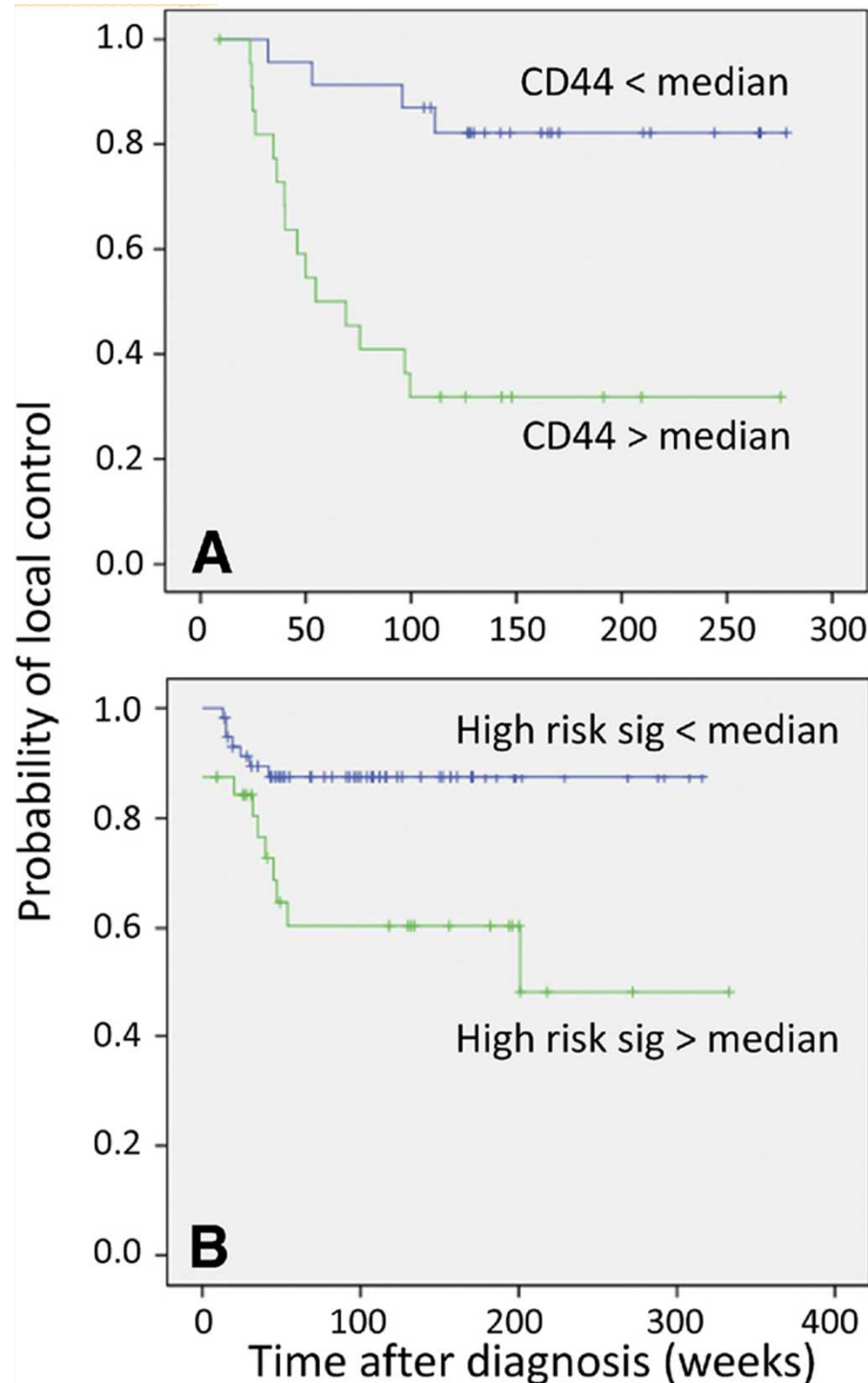
# Normal tissue effects Predictors

- Cytokines and growth factors as predictive factors-
  ✓ transforming growth factor b-1 (TGF-b1)
  ✓ interleukin (IL)-1a
  ✓ IL-6 in radiation pneumonitis.
- Radiogenomics
  ✓ SNP's

POSSIBLE ROLE OF GLUTATHIONE IN PREDICTING RADIOTHERAPY RESPONSE OF CERVIX CANCER

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PATHIRISSERY UMA DEVI, PH.D.,[‡] TATTIKONDA SEETHARAMAIAH, M.D.,*
MAMUDIPUDI S. VIDYASAGAR, M.D.,* KILARI KOTESHWER RAO, M.D.,*
CHANDRASHEKHAR S. HOSPET, M.D.* AND JOHNSON GILBERT R. SOLOMON, PH.D.*

*Department of Radiotherapy and Oncology, Shirdi Sai Baba Cancer Hospital, Manipal; [†]Department of Zoology, Sri Kerala Verma College, Thrissur; [‡]Department of Radiobiology, Kasturba Medical College, Manipal, India

- 45 patients of ca cervix on EBRT
- Blood and tumor samples taken at baseline and after 1 dose of radiation for GSH estimation
- Clinical Assessment done 1 month post RT
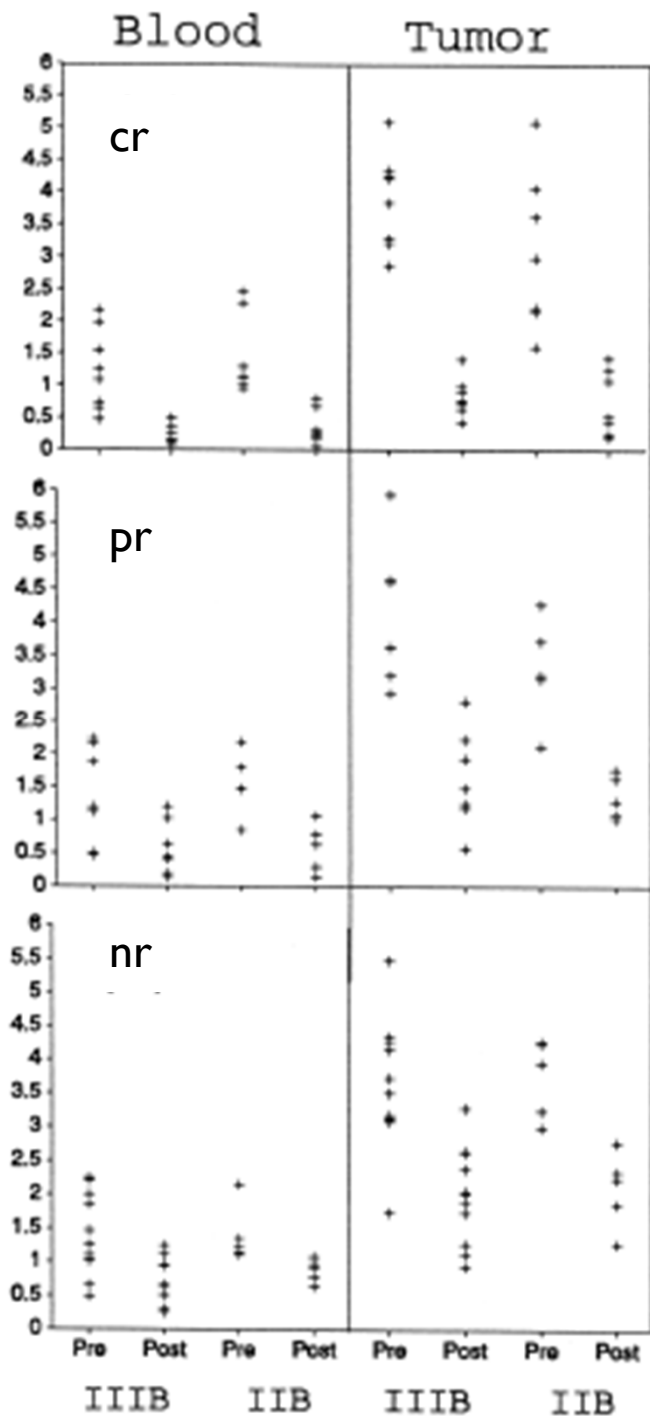
- Good correlation could be detected between the degree of GSH depletion (tumor and blood) and the tumor response.

		Tumor GSH ($\mu mol/mg$)		
FIGO stage	Tumor response	Pre-RT	Post-1 RT	p values*
IIB (17)	CR (7)	2.96 ± 0.47	0.80 ± 0.22	0.02
	PR (5)	3.25 ± 0.23	1.28 ± 0.17	0.04
	NR (5)	3.51 ± 0.32	2.33 ± 0.24	0.04
IIIB (28)	CR (8)	3.55 ± 0.17	0.75 ± 0.11	0.01
	PR (7)	3.62 ± 0.22	1.59 ± 0.13	0.02
	NR (13)	3.51 ± 0.41	2.33 ± 0.26	0.001

		Blood GSH ($\mu mol/l$)		
FIGO stage	Tumor response	Pre-RT	Post-1 RT	p values*
IIB (17)	CR (7)	1.38 ± 0.21	0.31 ± 0.09	0.02
	PR (5)	1.51 ± 0.16	0.63 ± 0.13	0.04
	NR (5)	1.54 ± 0.28	0.86 ± 0.09	0.04
IIIB (28)	CR (8)	1.23 ± 0.20	0.22 ± 0.04	0.01
	PR (7)	1.50 ± 0.17	0.71 ± 0.10	0.02
	NR (13)	1.53 ± 0.14	0.86 ± 0.17	0.001

PREDICTIVE AND PROGNOSTIC SIGNIFICANCE OF GLUTATHIONE LEVELS AND DNA DAMAGE IN CERVIX CANCER PATIENTS UNDERGOING RADIOTHERAPY

Mamidipudi Srinivasa Vidyasagar, M.D.,[*] Maheedhar Kodali, M.Sc.,[†*]
Pu Prakash Saxena, M.D.,[*] Dinesh Upadhya, M.Sc.,[†] Chilakapati Murali Krishna, Ph.D.,[‡*]
Bejadi Manjunath Vadhiraja, M.D.,[*] Donald Jerard Fernandes, M.D.,[*] and
Satish Rao Bola Sadashiva, Ph.D.[†]

- ## 123 squamous cell carcinoma cervix
- ## FIGO Stage IIB-IVA
- ## 18 normal subjects undergoing hysterectomy

| Stratification of 98 cervical cancer patients (response assessment available)					
	2 Gy × 2 fractions = 4 Gy Pre- and post-treatment			2 Gy × 5 fractions = 10 Gy Pre- and post-treatment		
Study participants	29			69		
Mean age + SD (range)	47 ± 10 (27–68)			52 ± 8 (30–75)		
Stage-wise classification	Stage IIB	Stage IIIB	Stage IVA	Stage IIB	Stage IIIB	Stage IVA
	6	22	1	19	46	4

- GSH levels were measured in the normal cervix, pre-RT serum and post-RT serum of patients (2RT and 5RT arm)

- DNA damage from RT was measured with SCGE assay in the 5 RT arm.

- The SCGE was measured in Olive tail Moments(OTM)
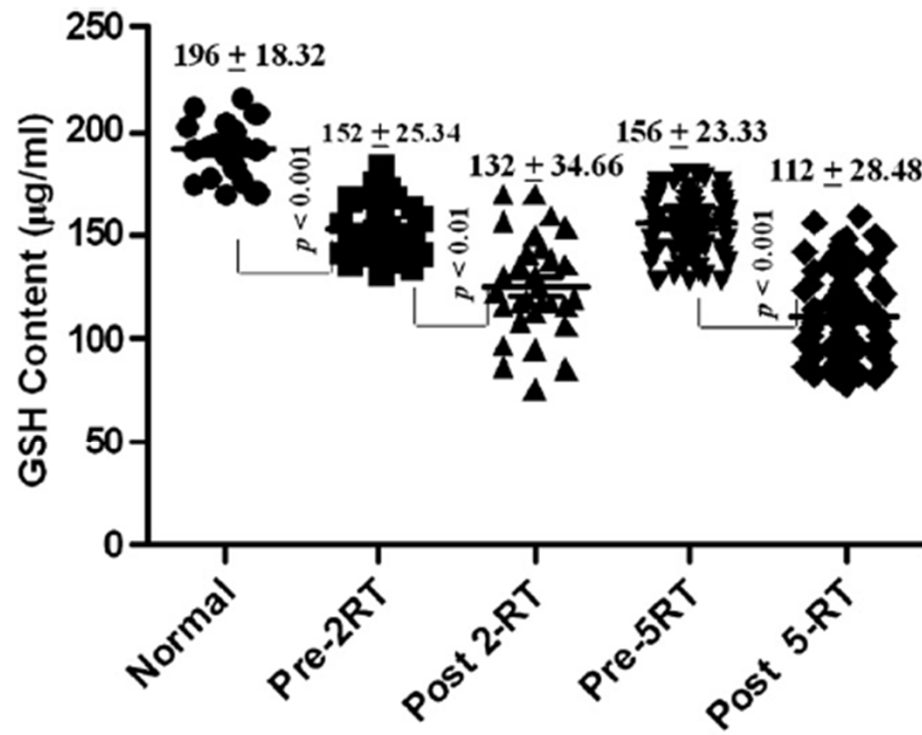
GSH estimation in serum sample

OTM estimation in tumor sample
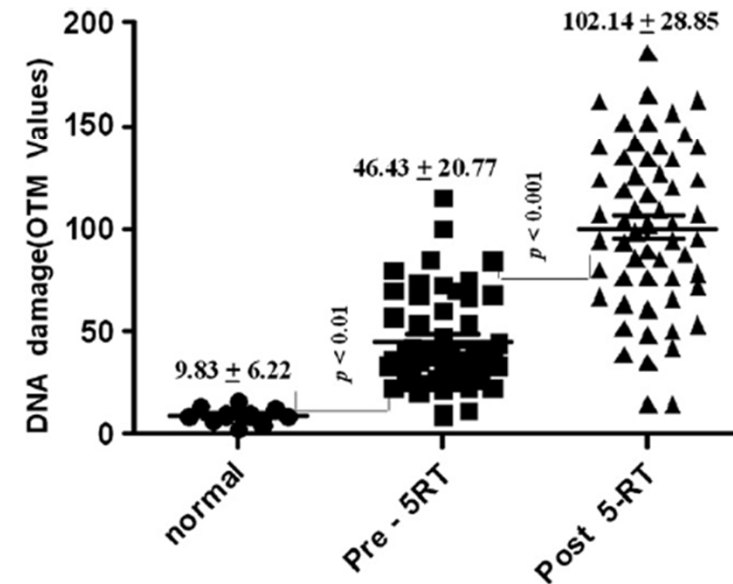
Significant fall in the p value of baseline GSH in the complete responders as well as the partial responders.
The fall in GSH was significantly high in the responders.

Tumor response $n = 29$	Serum glutathione (μg/mL) 2-RT samples	
	Pretreatment levels	Posttreatment levels
Complete responders (21)	148.63 ± 15.64*	$110.67 \pm 6.1^{\S}$
Partial responders (4)	$167.79 \pm 2.81^{\dagger}$	$144.56 \pm 15.45^{\P}$
Nonresponders (4)	172.69 ± 7.61	$151.93 \pm 1.01^{\Vert}$

Tumor response $n = 69$	Serum glutathione (μg/mL) 5-RT samples	
	Pretreatment levels	Posttreatment levels (5-RT)
Complete responders (51)	149.12 ± 7.2*	$99.52 \pm 11.76^{\S}$
Partial responders (16)	$159.94 \pm 6.24^{\dagger}$	$138.04 \pm 13.82^{\P}$
Nonresponders (2)	167.66 ± 3.45	158.15 ± 2.3

OTM was significantly high in the responders

Tumor response $n = 58$	Olive Tail Moment values (mean $\pm$ SEM)	
	Pretreatment	Posttreatment (5-RT)
Complete responders (40)	65.9 ± 5.9*	$115.8 \pm 12.0^{\S}$
Partial responders (16)	$34.9 \pm 4.6^{\dagger}$	$60.9 \pm 6.56^{\P}$
Nonresponders (2)	11.3 ± 4.6	18.0 ± 2.19

conclusion

- Predictive assays may help in modifying the treatment for select group of individuals.

- Delivery of personalized treatment.

- Field is in nascent stage and need further studies.

- Need for youngsters to involve in more radiobiology studies.

- Thank you