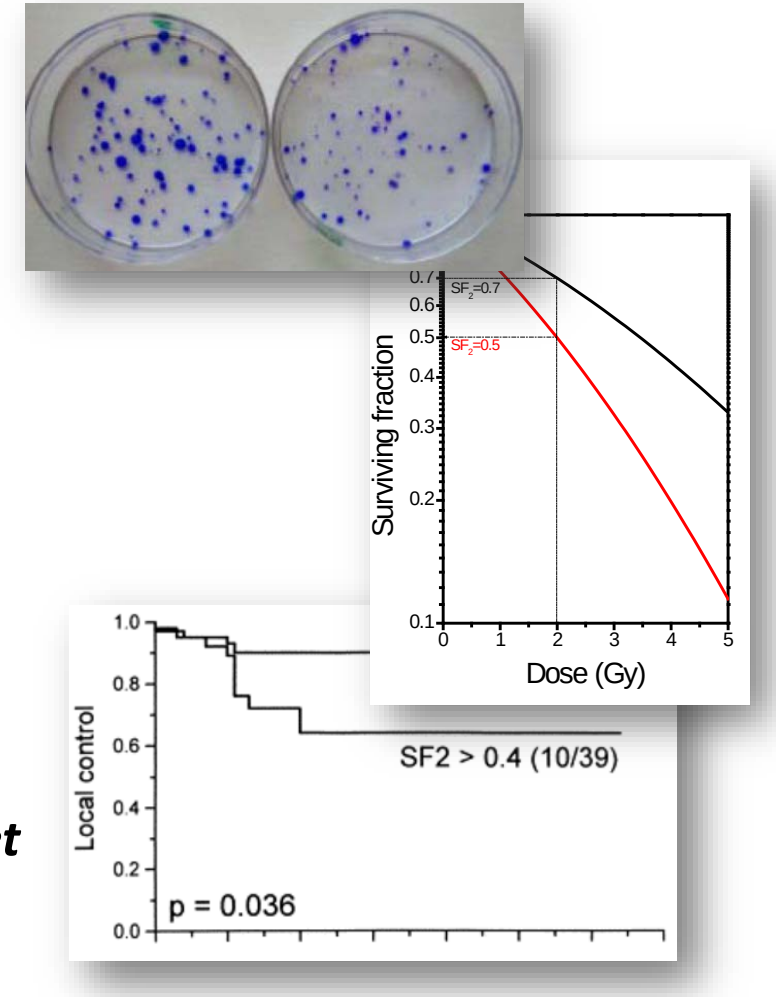
Radiobiological assays for individual tumour response



Iuliana Toma-Dasu

Medical Radiation Physics

Stockholm University and Karolinska Institutet

Predictive assays for individual tumour response

Why do we need them?

- For personalised treatments and potentially for a higher probability of cure

Predictive assays - lab analyses/tests designed to predict the response of tumours to radiotherapy based on radiobiological characteristics

=> Performance levels of the predictive assays are mechanistically based and offer the prospect of coherent selection of radiation as the therapeutic modality

$\neq$

Clinicopathologic prognostic factors - features empirically shown to correlate with the treatment outcome (i.e. tumour site, stage, type and grade)

Biological factors determining tumour response to RT

1. *Tumour cell radiosensitivity*
2. *Tumour cell proliferation kinetics*
3. *Tumour cell oxygenation*

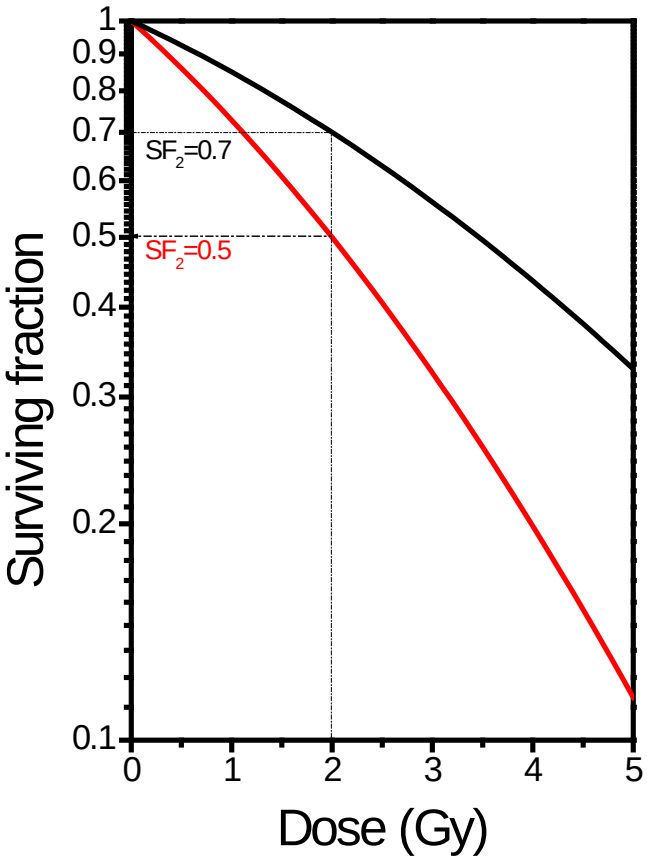
Biological factors determining tumour response to RT

1. *Tumour cell radiosensitivity*

- ***In vitro* clonogenic cell survival assay**

- Cell adhesive matrix (CAM) assay
- MTT assay
- Differential Staining Cytotoxicity (DiSC) assay
- Nucleoid light scatter on cells

- etc.



Tumour cell radiosensitivity

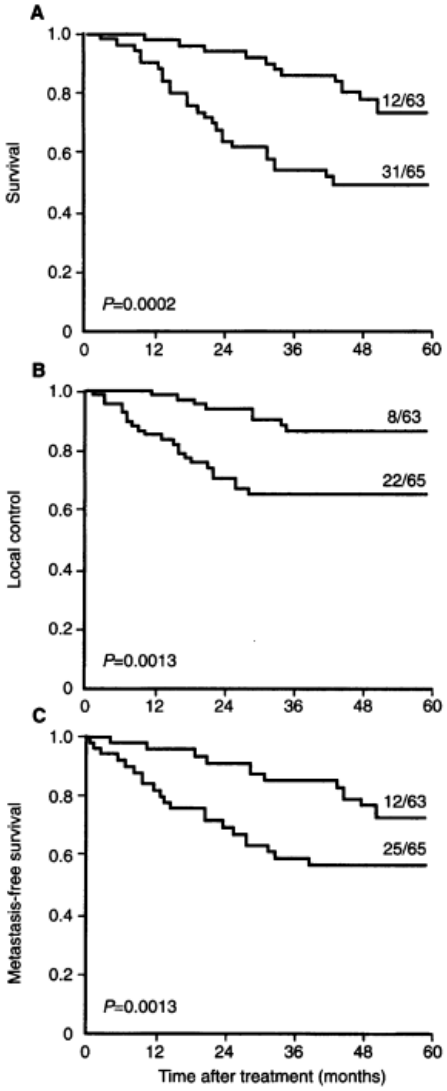
British Journal of Cancer (1997) 76(9), 1184–1190
© 1997 Cancer Research Campaign

The independence of intrinsic radiosensitivity as a prognostic factor for patient response to radiotherapy of carcinoma of the cervix

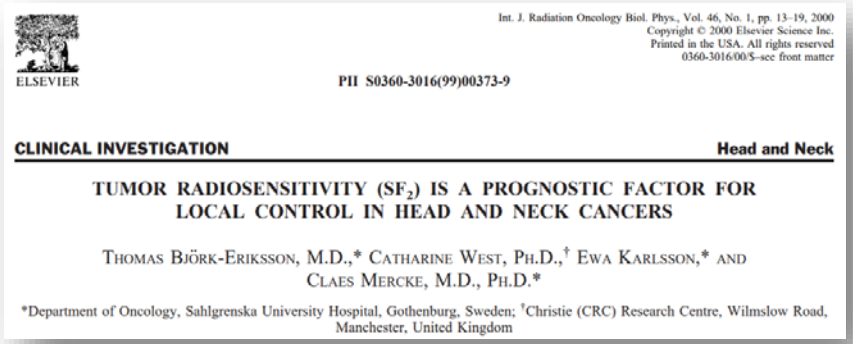
CML West[1], SE Davidson[2], SA Roberts[3] and RD Hunter[2]

[1]Cancer Research Campaign Department of Experimental Radiation Oncology, Paterson Institute for Cancer Research; [2]Department of Clinical Oncology, Christie Hospital (NHS) Trust, Wilmslow Road, Manchester M20 4BX, UK; [3]Cancer Research Campaign Department of Biomathematics and Computing, Paterson Institute for Cancer Research

- ***In vitro*** **clonogenic cell survival assay**
- OS (upper), LC (middle) and metastasis-free survival (lower)
- Data stratified according to the median SF_2 value (upper arm **SF_2<0.42**)



Tumour cell radiosensitivity



Int. J. Radiation Oncology Biol. Phys., Vol. 46, No. 1, pp. 13–19, 2000

0360-3016/00/$–see front matter

ELSEVIER

PII S0360-3016(99)00373-9

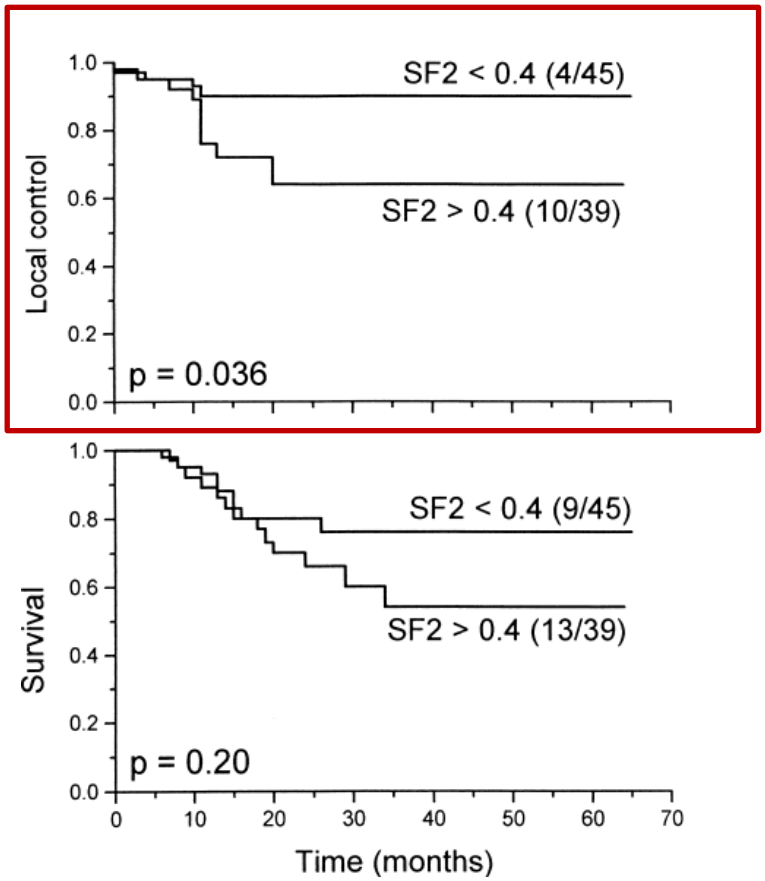
CLINICAL INVESTIGATION Head and Neck

TUMOR RADIOSENSITIVITY (SF_2) IS A PROGNOSTIC FACTOR FOR LOCAL CONTROL IN HEAD AND NECK CANCERS

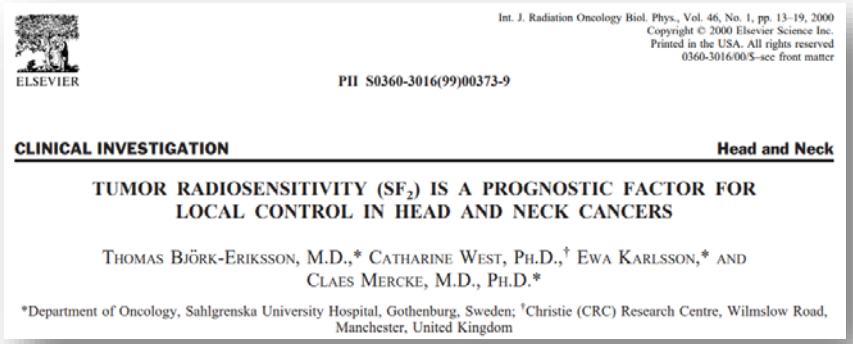
THOMAS BJÖRK-ERIKSSON, M.D.,* CATHARINE WEST, PH.D.,† EWA KARLSSON,* AND CLAES MERCKE, M.D., PH.D.*

*Department of Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden; †Christie (CRC) Research Centre, Wilmslow Road, Manchester, United Kingdom

- ***In vitro*** **clonogenic cell survival assay**
- LC (upper), OS (lower)
- Data stratified according to the median SF_2 value (upper arm **SF_2<0.40**)



Tumour cell radiosensitivity



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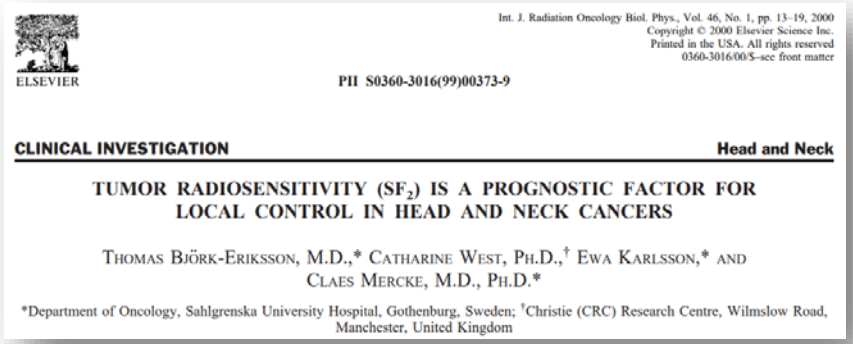
- Local control prediction for other factors

Treatment subgroup	Patients	Mean	Median	Range
1 ERT≥60Gy± surgery±CHT	20	64.3	64.6	54–68
2 ERT+IRT±CHT	51	75.5	76.6	65.8–80.8
3 IRT≥60Gy	3	63.3	60	60–70
4 ERT<60Gy+surgery+CHT	10	48.6	51	40.8–51

Variable	Value	Numbers*	*p*
SF_2	≤ 0.4	4/45	0.036
	> 0.4	10/39	
Stage	II	0/9	0.25
	III	4/16	
	IV	10/59	
Gender	Male	9/61	0.36
	Female	5/23	
Histology	PSQCC	2/24	0.24
	MSQCC	8/39	
	WSQCC	3/9	
	Undifferentiated	1/9	
	Miscellaneous**	0/3	
Site	Oral cavity	7/30	0.87
	Oropharynx	1/26	
	Nasopharynx	0/6	
	Hypopharynx	1/6	
	Larynx	1/8	
	Sinonasal	3/7	
	Skin	1/1	
Age (years)	< 62	5/41	0.21
	> 62	9/43	
Nodal status	0	12/47	0.018
	1–3	2/37	
Chemotherapy	Yes	10/62	0.44
	No	4/22	
Treatment†	1	6/20	0.001
	2	3/51	
	3	1/3	
	4	4/10	

* Numbers of local recurrences/patients.
PSQCC, MSQCC, and WSQCC = poorly-, moderately-, and well-differentiated squamous cell carcinoma, respectively.
** Two adenocarcinoma and one adenoid cystic carcinoma.

Tumour cell radiosensitivity



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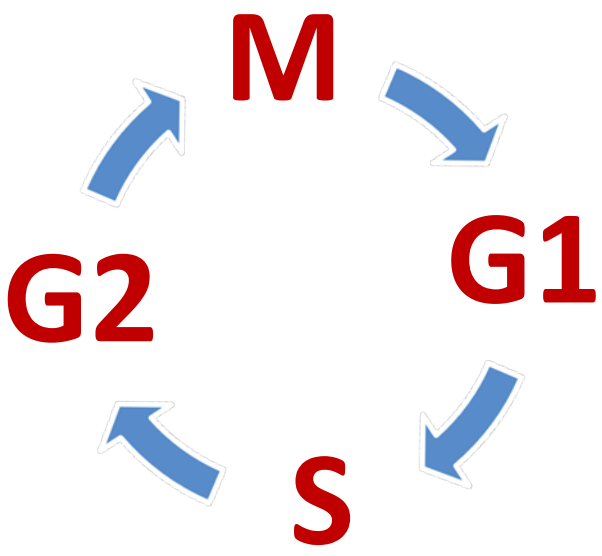
- Tumour SF_2 was an independent prognostic factor for local control

	SF_2 (median)	SF_2 (quartiles)
	0.036	0.010
Stage	0.024	0.0046
Grade	0.0044	0.21
Site (1–6)	0.12	0.085
Age	0.034	0.014
Gender	0.036	0.016
Chemotherapy	0.040	0.013
Nodal status	0.062	0.026
Treatment (1, 2, 4)	0.021	0.0031

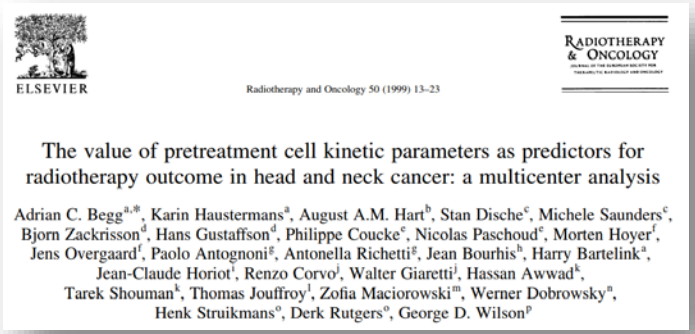
Biological factors determining tumour response to RT

2. *Tumour cell proliferation kinetics*

- **T_{pot} ≈ Ts/LI assays**
- Simultaneous measurement of DNA content in tumours, LI, and duration of S phase (Ts) using IUdR/BUdR
- etc.



Biological factors determining tumour response to RT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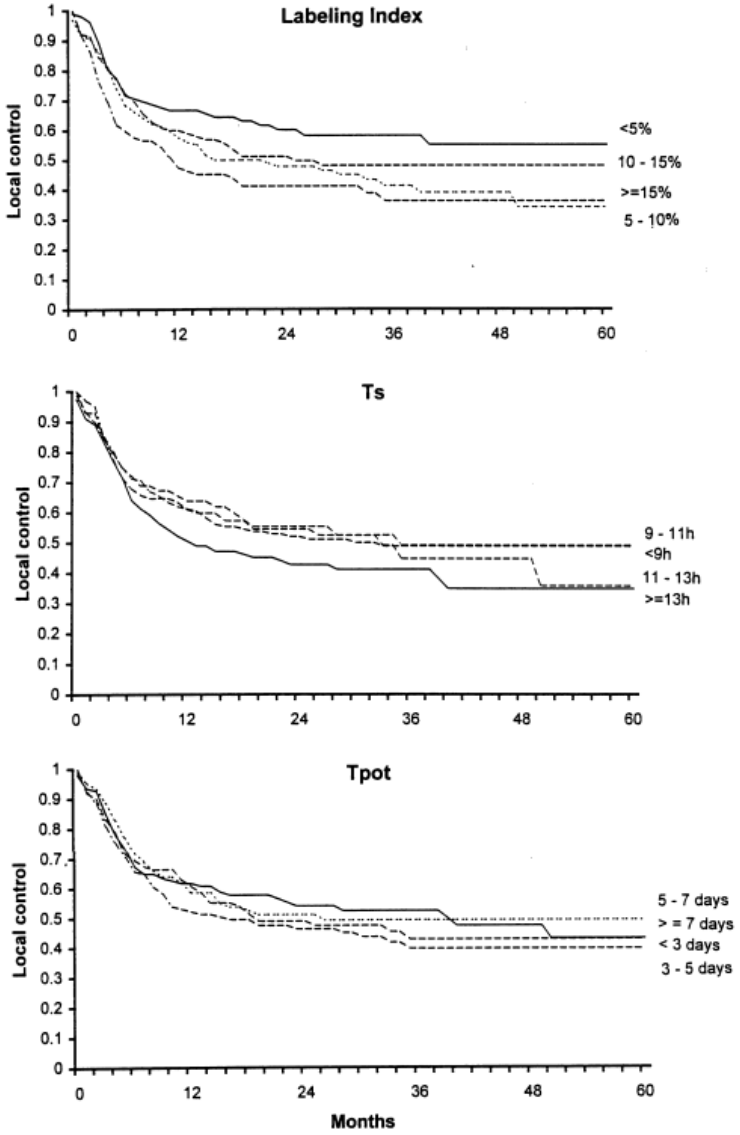
Radiotherapy and Oncology 50 (1999) 13–23

RADIOTHERAPY & ONCOLOGY

The value of pretreatment cell kinetic parameters as predictors for radiotherapy outcome in head and neck cancer: a multicenter analysis

Adrian C. Begg[a,*], Karin Haustermans[a], August A.M. Hart[b], Stan Dische[c], Michele Saunders[c], Bjorn Zackrisson[d], Hans Gustaffson[d], Philippe Coucke[e], Nicolas Paschoud[e], Morten Hoyer[f], Jens Overgaard[f], Paolo Antognoni[g], Antonella Richetti[g], Jean Bourhis[h], Harry Bartelink[a], Jean-Claude Horiot[i], Renzo Corvo[j], Walter Giaretti[j], Hassan Awwad[k], Tarek Shouman[k], Thomas Jouffroy[l], Zofia Maciorowski[m], Werner Dobrowsky[n], Henk Struikmans[o], Derk Rutgers[o], George D. Wilson[p]

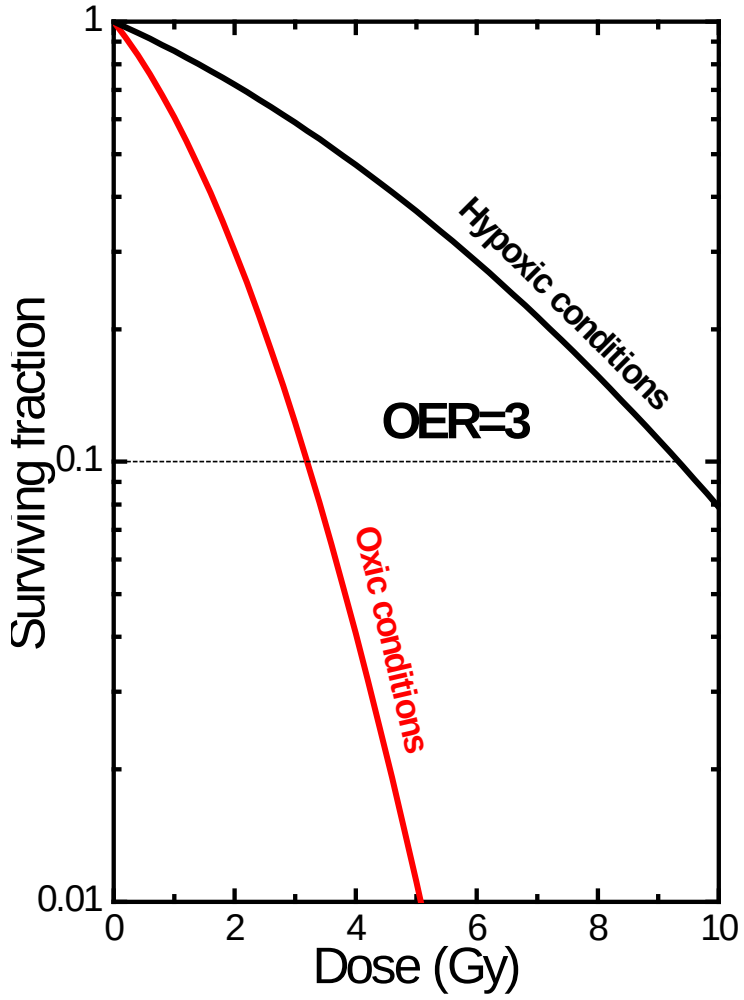
- **T_{pot} = Ts/LI assays**
- LI (upper), Ts (middle), **T_{pot}** (lower)
- Only LI showed a statistically significant association with LC in a univariate analysis, with low LI tumours associated with a more favourable outcome



Biological factors determining tumour response to RT

3. *Tumour cell oxygenation*

- **Hypoxia signature**
- **Polarographic electrodes**
- **Functional imaging**
- etc.



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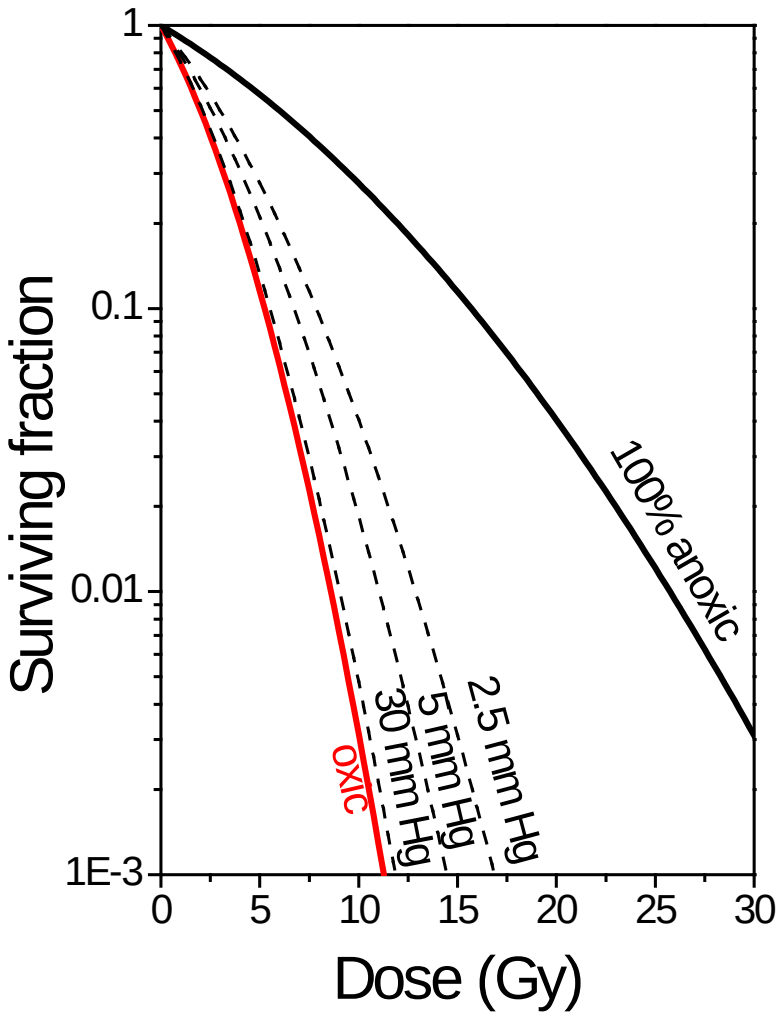

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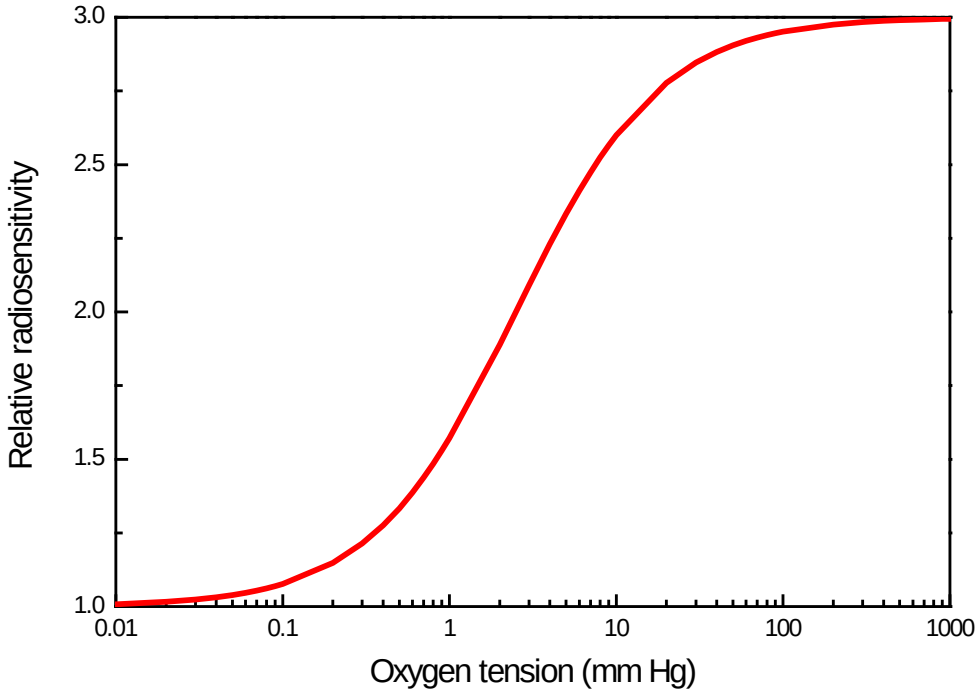


Biological factors determining tumour response to RT

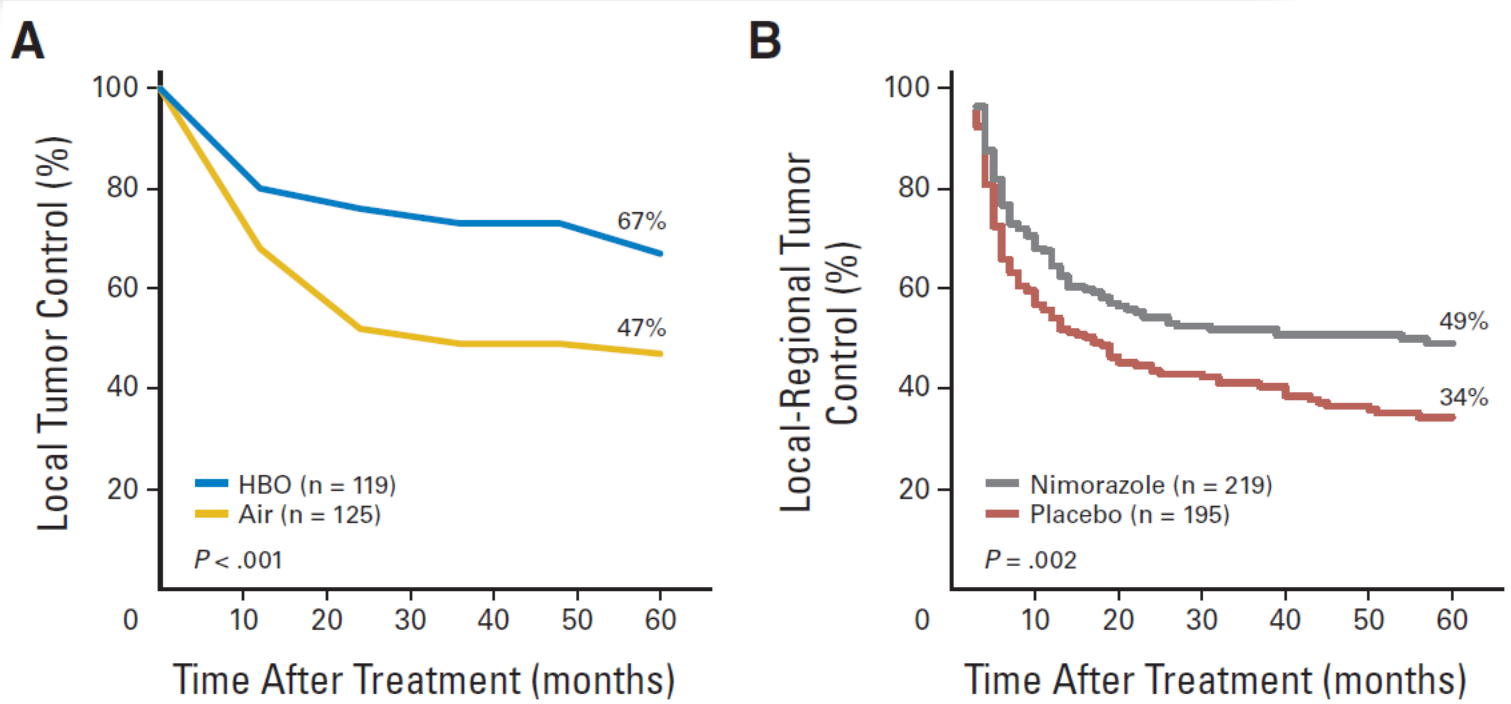
VOLUME 25 · NUMBER 26 · SEPTEMBER 10 2007

JOURNAL OF CLINICAL ONCOLOGY

REVIEW ARTICLE

Hypoxic Radiosensitization: Adored and Ignored

Jens Overgaard



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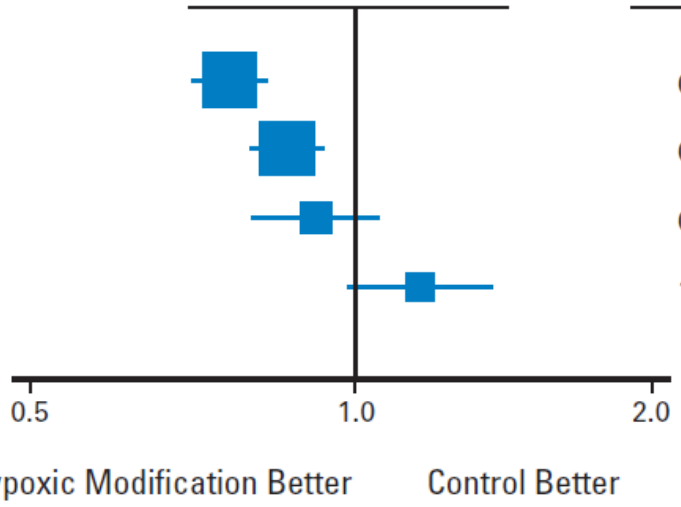
REVIEW ARTICLE

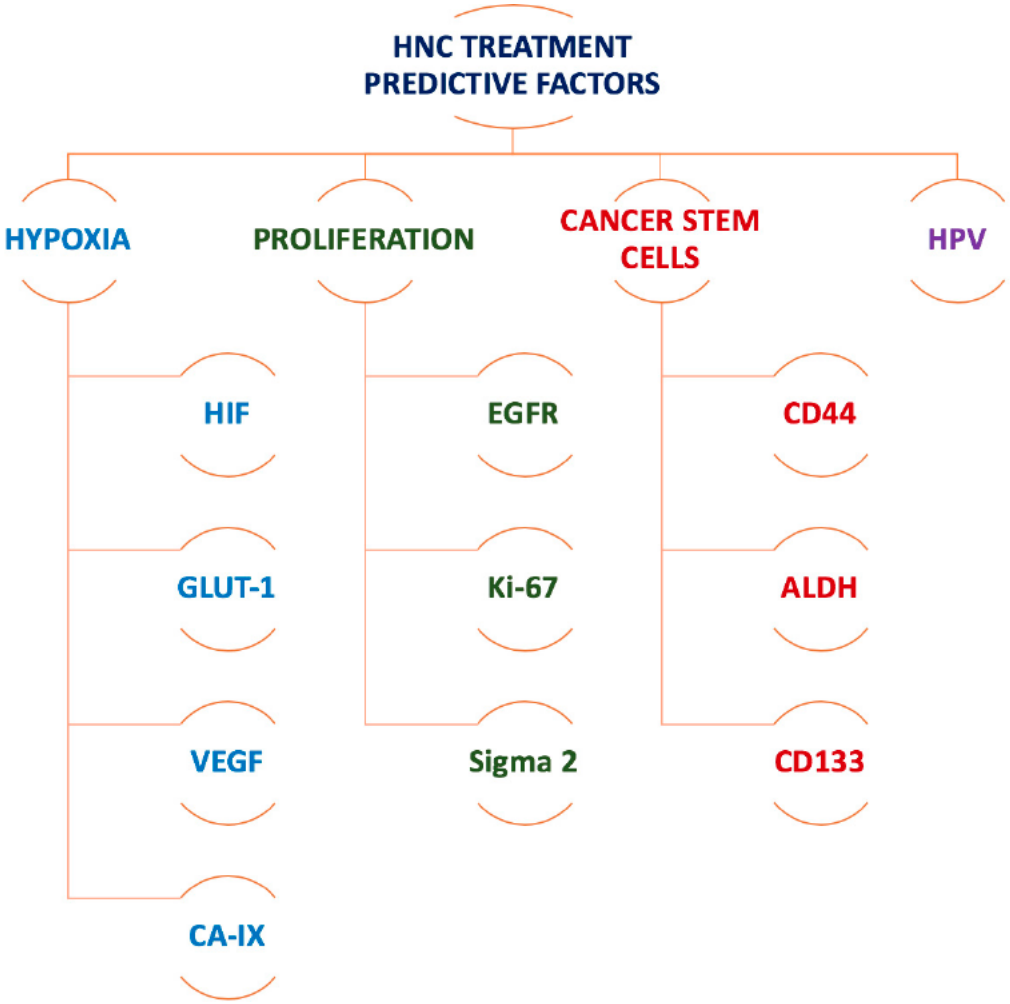
Hypoxic Radiosensitization: Adored and Ignored

Jens Overgaard

Endpoint	Hypoxic modification Events	Hypoxic modification Total	Control Events	Control Total	Odds ratio	95% CI
Loco-regional control	2,144	4,416	2,465	4,473	0.77	0.71 to 0.84
Survival	3,237	4,921	3,405	4,952	0.87	0.80 to 0.95
Distant metastasis	452	2,332	480	2,339	0.93	0.81 to 1.07
Complications	374	2,424	310	2,305	1.17	1.00 to 1.38



Biological factors determining tumour response to RT

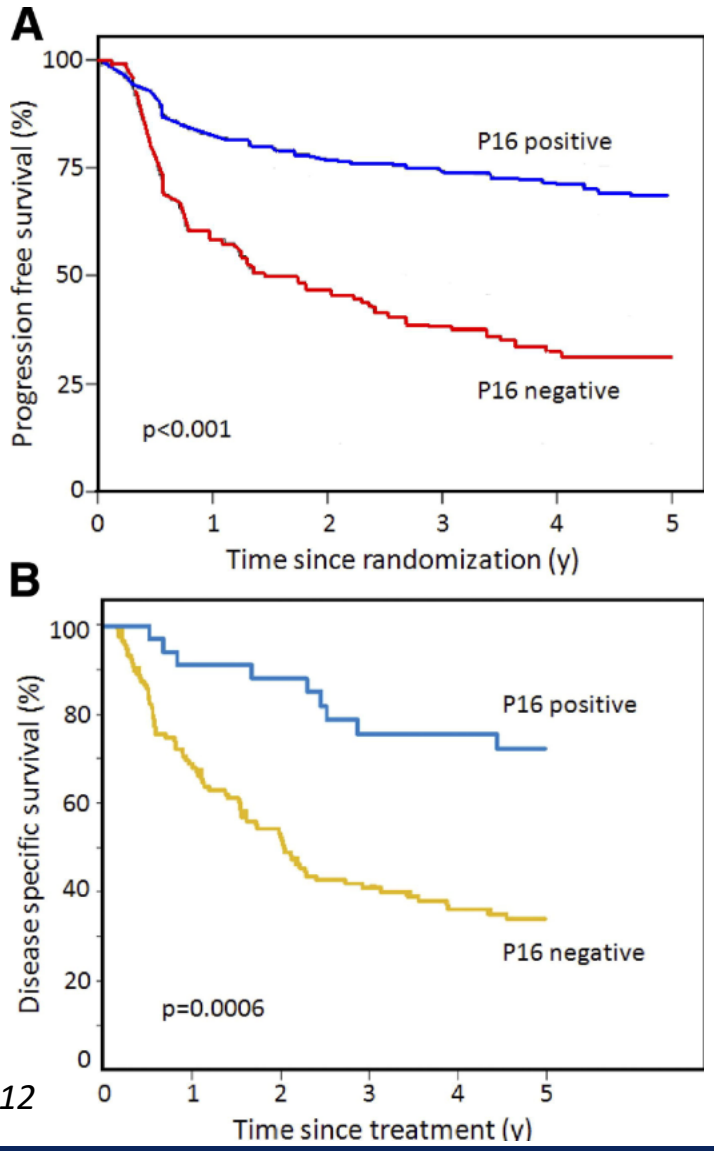


Summary of the most commonly investigated biomarkers for outcome predictions

L Marcu, P Reid and E Bezak 2018

HPV Status

- ***HPV status is a prognostic marker***
- Several methods are available for testing the HPV status:
  - Detection of viral genomic integration with polymerase chain reaction or FiSH
  - Detection of viral gene expression (E6 and E7) and the expression of p16
  - Gene expression signatures for distinguishing HPV+ and HPV-.

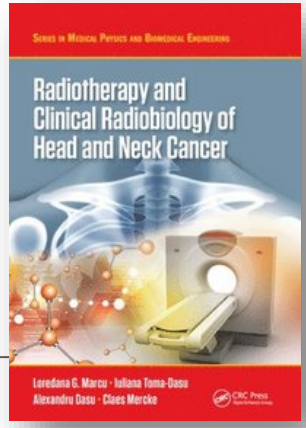


A Begg 2012

HPV Status

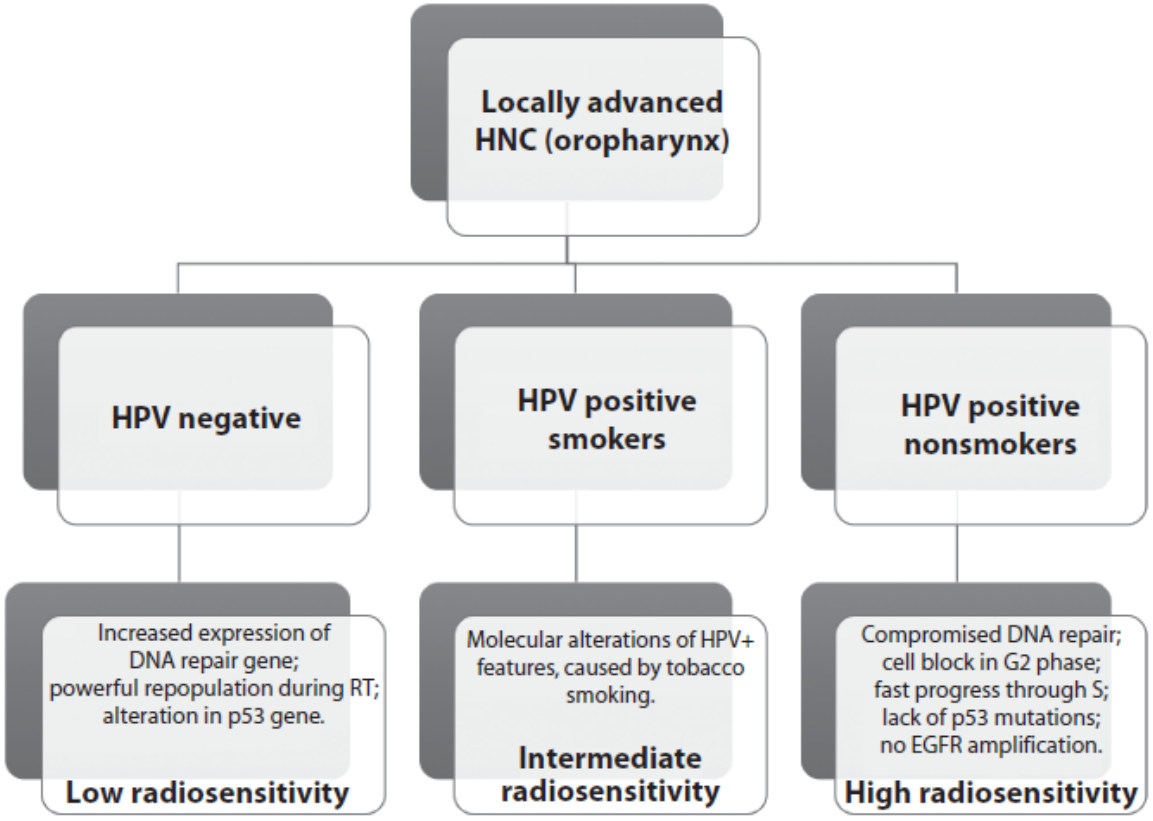
HPV testing methods with their associated advantages and drawbacks

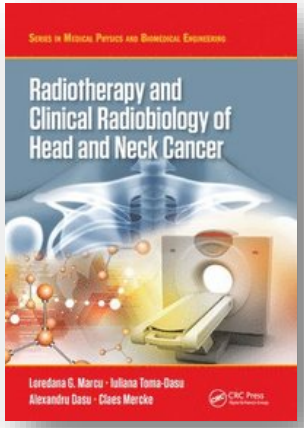
Method	Advantages	Disadvantages
p16 immunohistochemistry	Simple. Widely available. Cost-effective.	Only recommended for oropharyngeal cancers (as stand-alone test). If p16 immunostaining is lower than the cut-off value (70% tumour cells), additional tests are required.
Polymerase chain reaction	High sensitivity. Widely used and considered the gold standard.	Low specificity. Technically challenging. Time consuming. Unable to identify the anatomical origins of the HPV infection.
DNA in situ hybridization	High specificity. Allows easy integration into laboratory. Reliable detection and visualization of DNA.	Limited sensitivity for samples with low viral copy numbers.
RNA in situ hybridization	All the advantages of the DNA ISH. Identifies transcriptionally active HPV. High sensitivity.	To further increase specificity, multimodality testing is required.



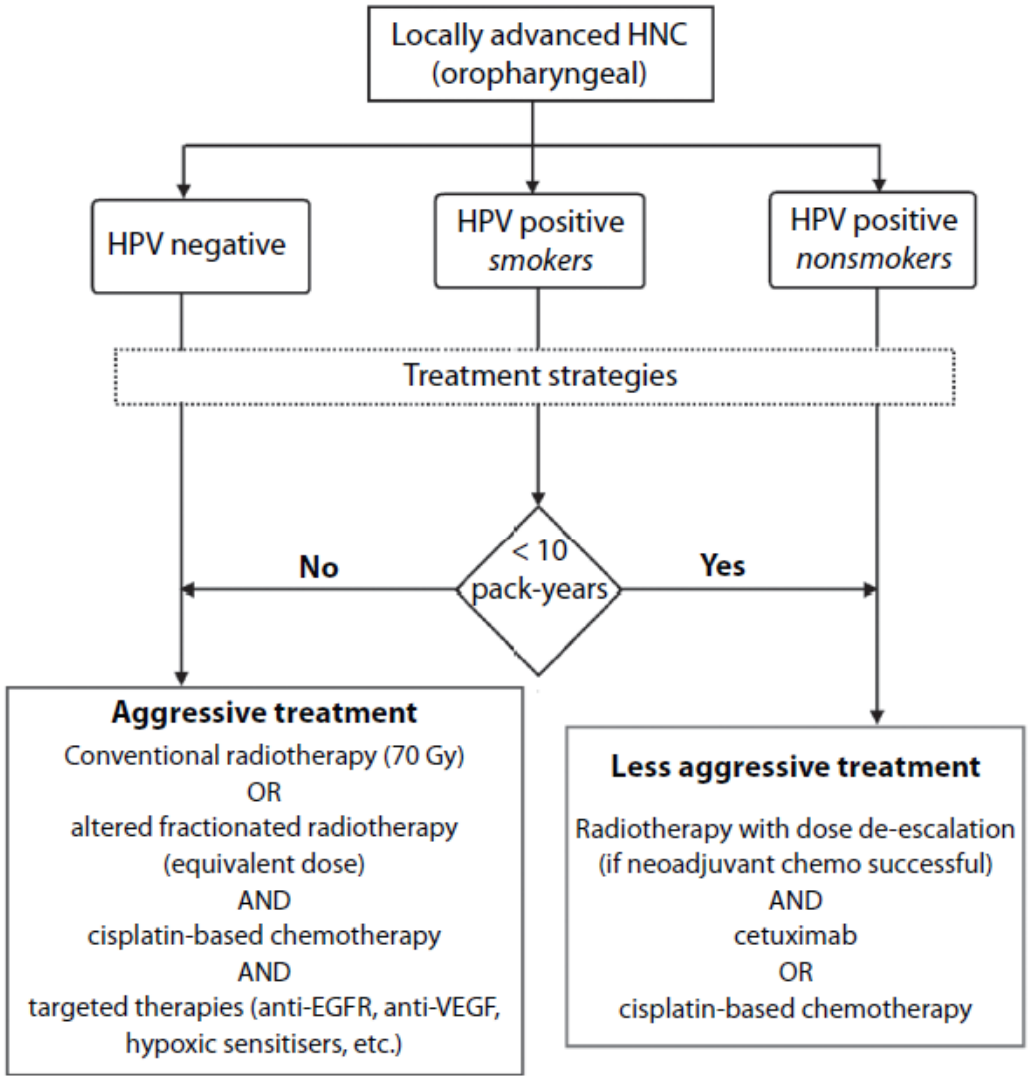
HPV Status

The multifactorial-dependent radiosensitivity of oropharyngeal squamous cell carcinoma as a function of HPV status and smoking history as a function of HPV status and smoking history

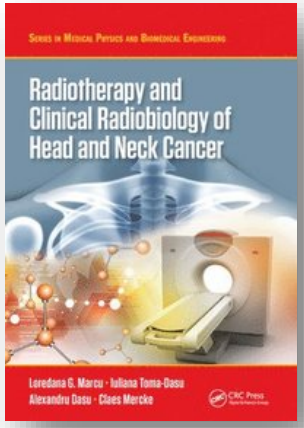




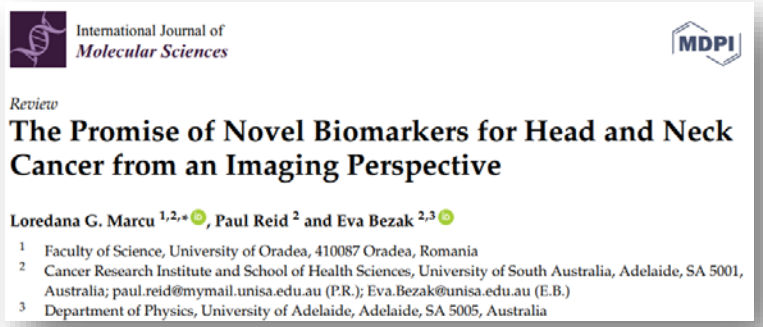
HPV Status



Current treatment approaches and projected protocols based on prognostic factors



Predictive assays for individual tumour response



International Journal of *Molecular Sciences*

MDPI

Review

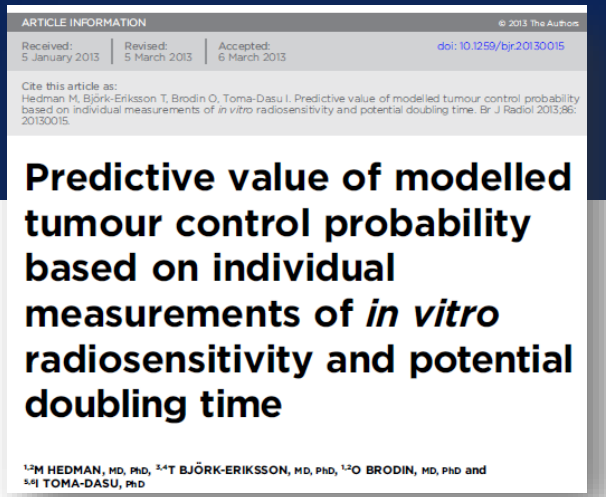
The Promise of Novel Biomarkers for Head and Neck Cancer from an Imaging Perspective

Loredana G. Marcu [1,2,*], Paul Reid [2] and Eva Bezak [2,3]

1 Faculty of Science, University of Oradea, 410087 Oradea, Romania
2 Cancer Research Institute and School of Health Sciences, University of South Australia, Adelaide, SA 5001, Australia; paul.reid@mymail.unisa.edu.au (P.R.); Eva.Bezak@unisa.edu.au (E.B.)
3 Department of Physics, University of Adelaide, Adelaide, SA 5005, Australia

Predictive Assay	Oxygenation Status	Proliferative Potential	Intrinsic Radioresistance (Subpopulation of Cancer Stem Cells?)
Purpose	To identify the patient group that would benefit from hypoxic cell sensitisers.	To differentiate between tumours with slow and fast proliferation.	To correlate cell line radiosensitivity with tumour response to radiation.
Technique	Polarographic needle electrode Endogenous/exogenous markers; 3D models; microvessel density.	Kinetic parameter measurements: length of S phase, potential doubling time; labelling index; clonogenic survival.	Dose-response curves; Colony growth (MTT), micronucleus, chromosomal, DNA damage (Comet) assays; tumour control assay.
Limitation	Invasive; Unreliable (biopsies); Costly and time consuming; Require high level expertise.	No robust correlation between kinetic parameters and treatment outcome; Time consuming.	Highly time consuming.
Present/Future	Hypoxia-specific PET radiotracers: F-MISO; F-FAZA; Cu-ATSM; other radiotracers BOLD/TOLD (blood/tissue oxygen level-dependent) MRI	Proliferation-specific PET radiotracers: F-FLT; F-ISO-1; ^{11}C-based radiotracers.	Cancer stem cell-specific PET radiotracers; MRI; HPV-status based identification of more radioresponsive tumours.

Predictive assays for individual tumour response



Background

- There is evidence that:
  - *In vitro* measured radiosensitivity (SF_2) values correlate with the probability of local control for H&N cancer patients
  - Potential doubling time (T_{pot}) is a weak predictor of outcome of radiotherapy in H&N cancer patients
  - The tumour volume is a weak predictor of outcome of radiotherapy in H&N cancer patients

- ***What is the prediction power of SF_2 and T_{pot} measured in individual patients used in conjunction with theoretical predictions of TCP in comparison to generic parameters for the tumour radiosensitivity retrieved from the literature?***

Predictive assays for individual tumour response

Patient data

- SF_2 and T_{pot} determined for H&N patients from samples taken before treatment
  - Biopsy and surgical specimens were obtained before treatment
  - Single-cell suspensions were cultured *in vitro* using a soft-agar assay to obtain SF_2
  - T_{pot} vas determined by BrdUrd staining
- Tumour volume was assessed based on pre-treatment CT and MR images

	Range	Average	Median
EXRT dose (Gy)	40.8-68.0	61.99	64.60
BT dose (Gy)	6.0-30.0	13.71	12.00
OTT (days)	19-99	45.39	45.00

Patient no.	Tumour volume (cm^3)	SF_2	T_{pot} (days)	Local control
1	50.00	0.32	5.63	0
2	5.00	0.33	0.46	0
3	119.11	0.41	5.88	0
-	-	-	-	-
9	6.28	0.66	1.79	0
10	47.70	0.94	4.21	0
11	47.71	1.00	1.00	0
12	33.51	0.16	11.04	1
-	-		-	-
42	11.78	0.66	13.50	1
43	23.56	0.66	27.50	1
44	0.59	0.70	4.63	1
45	14.11	0.73	1.08	1
46	5.89	0.82	17.14	1
	Mean	**0.43**	**6.43**	
	Median	**0.40**	**5.06**	
	Range	**0.16 - 0.94**	**0.46 - 27.50**	

Predictive assays for individual tumour response

TCP modelling

- BED calculations

$$BED_{tot} = \left(BED_{EBRT} + BED_{BT}\right) - \frac{\ln(2)}{\alpha}\frac{T_{treat} - T_k}{T_{pot}}$$

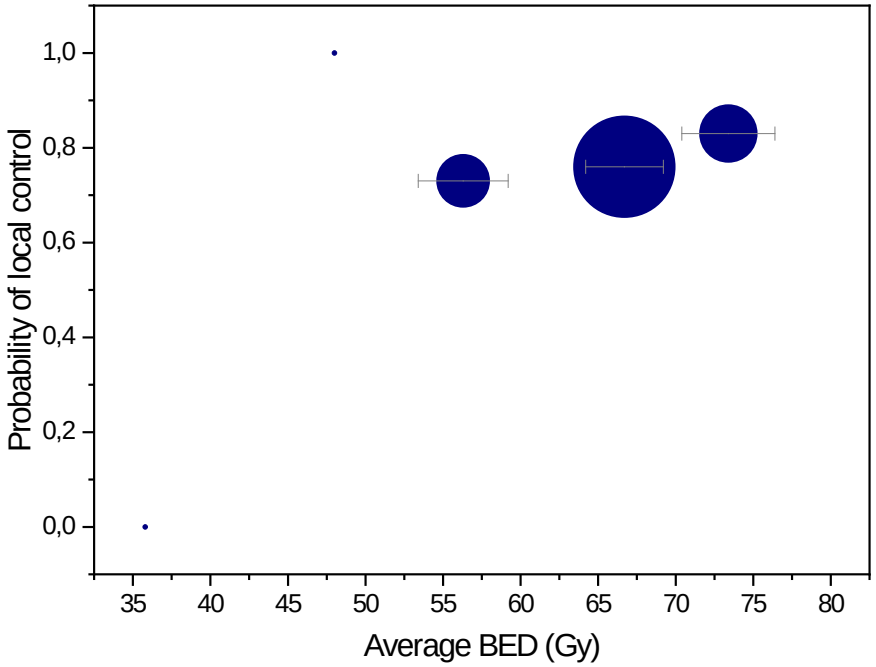
- TCP calculations

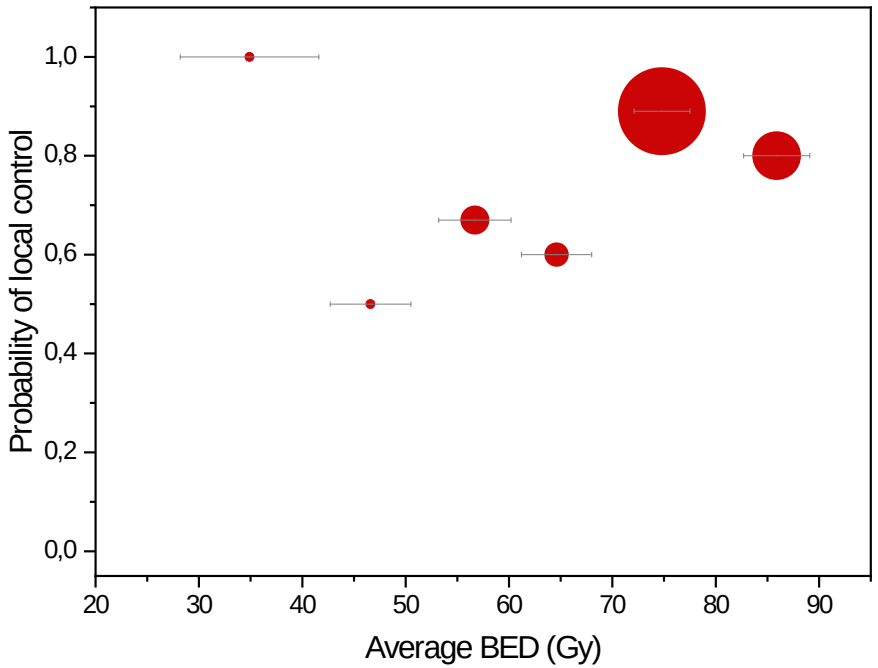
$$TCP = \exp\left\{-N_0 \cdot \exp\left[-\alpha \cdot EQD_2 \cdot \left(1 + \frac{2}{\alpha/\beta}\right)\right]\right\}$$

Calculations parameters	
α/β = 10 Gy	
T_k = 22 days	
$N_0 = 10^9 * V$	
Generic literature-based parameters	Patient specific parameters
α = 0.3 Gy^{-1}	α derived from SF_2
T_{pot} = 3 days	T_{pot}

Predictive assays for individual tumour response

Clinically observed TCP as a function of the total BED calculated using either generic (left) or patient-specific (right) α and T_{pot}





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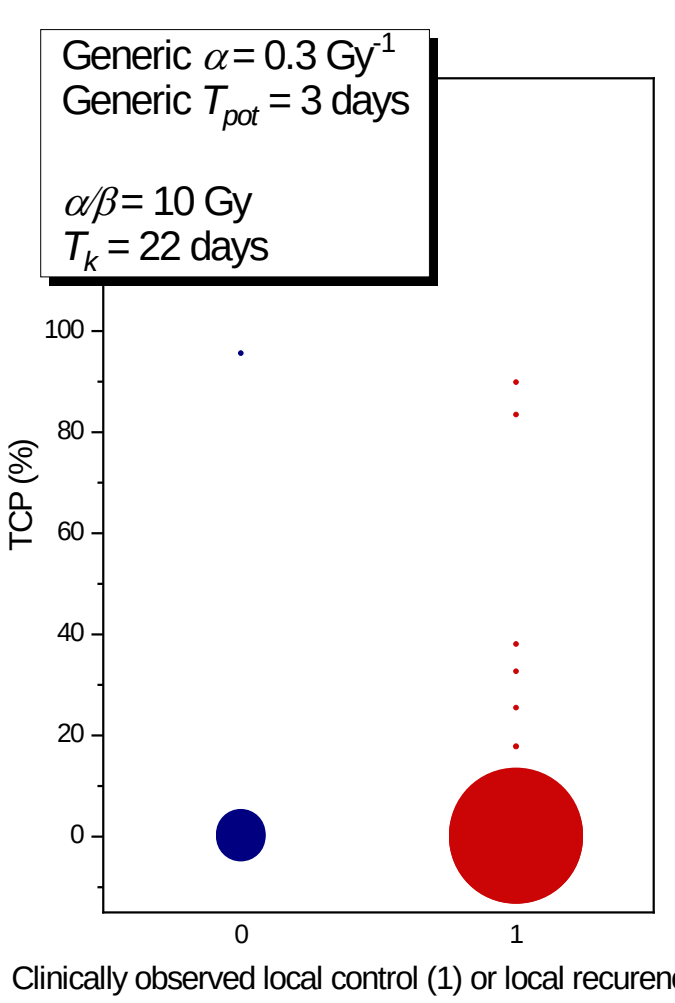
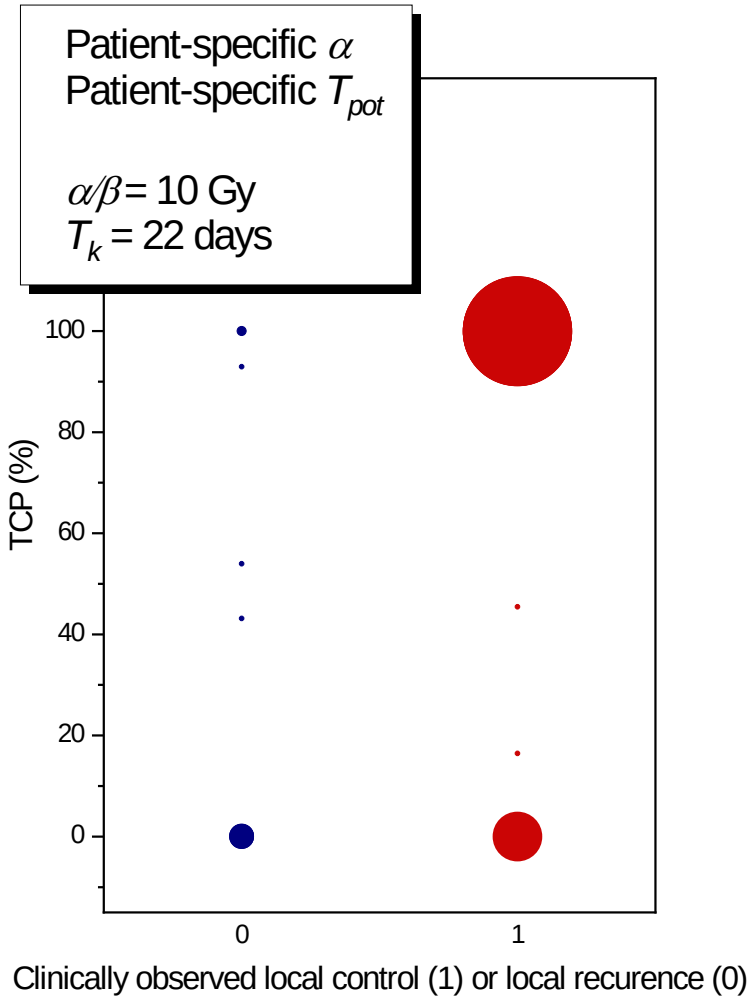
Predictive assays for individual tumour response





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Predictive assays for individual tumour response

Sensitivity, specificity, positive predictive value (PPV) and the negative predictive value (NPV) for the different ways of calculating the TCP for a threshold of 95%

	Sensitivity %	Specificity %	PPV %	NPV %
TCP calculated based on ***generic*** values for α and T_{pot}	**0**	**91**	**0**	**22**
TCP calculated based on ***mean*** values for α and T_{pot}	**94**	**27**	**80**	**60**
TCP calculated based on ***patient specific*** values for α and T_{pot}	**63**	**80**	**92**	**38**

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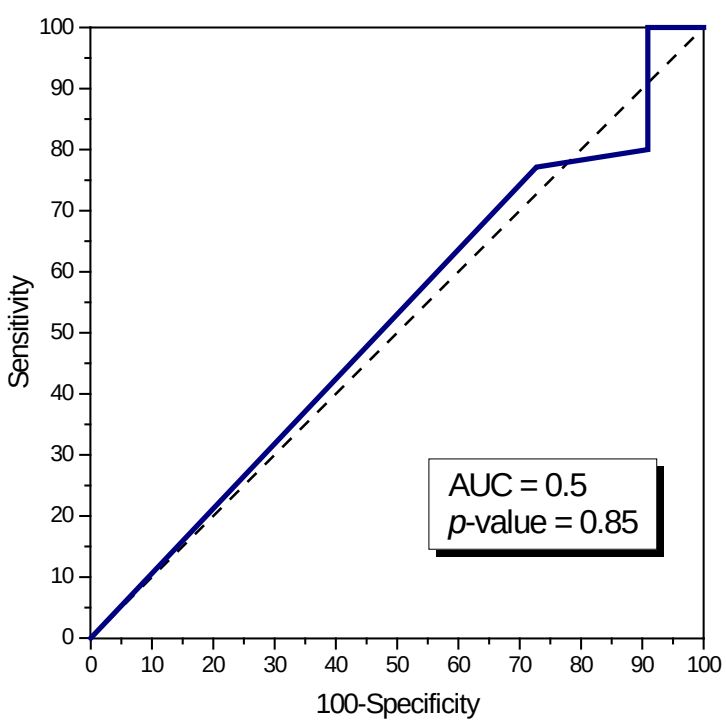
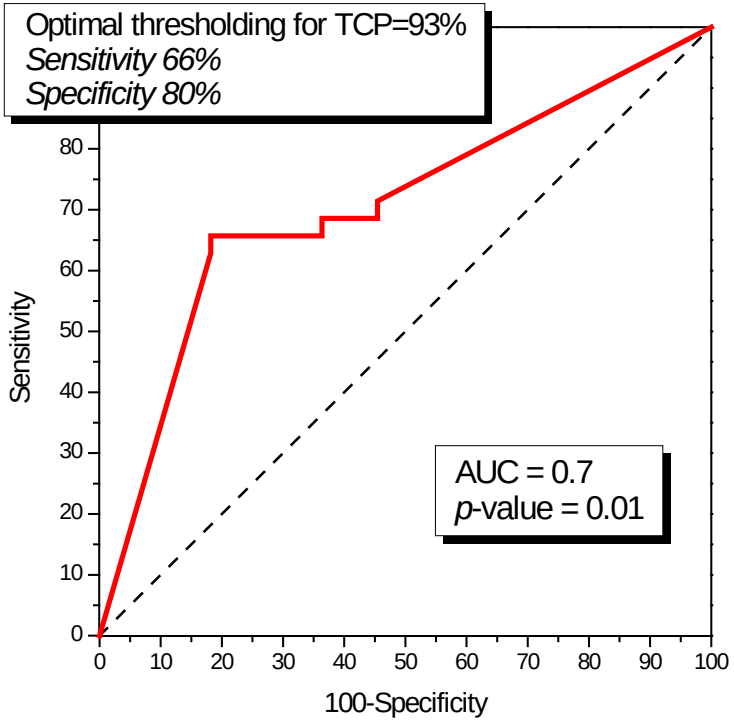
Predictive assays for individual tumour response

ROC curves for TCP calculated using either generic (blue) or patient-specific (red) α and T_{pot}





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Conclusion

- Individually derived radiobiological parameters used for the modelling of TCP are better predictors of the radiation treatment outcome in individuals than the literature-based generic parameters

- This information can be used clinically to tailor individually prescribed treatment schedules, but these results should be verified in prospective clinical studies in the future