Advanced/Metastatic disease: the role of biological and molecular factors in oropharyngeal carcinoma

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Università degli Studi di Brescia

Overview

- defining the problem: progression, relapse and metastases in HNSCC
- therapeutic options in advanced disease
- the knowledge “gap” in front line treatment
- cellular heterogeneity in HNSCC: cancer stem cells and CTC
- molecular heterogeneity in HNSCC: gene expression profiles

Advanced disease in HNSCC: epidemiology

- locally advanced disease at diagnosis (AJCC stage III, IVa, IVb): 60%
- metastatic disease at diagnosis (AJCC stage IVc): 5%

Gibson MK et al. Lancet Oncology 2006;7:565-574

Advanced disease in HNSCC: outcome

- limited stage disease at diagnosis (AJCC stage I, II): 35%
- locally advanced disease at diagnosis (AJCC stage III, IVa, IVb): 60%
  - 50% locoregional control
  - 35% locoregional progression
  - 15% distant metastases
- metastatic disease at diagnosis (AJCC stage IVc): 5%
  - treatment-sensitive: median survival 6-8 months
  - treatment-refractory: median survival 3 months

Gibson MK et al. Lancet Oncology 2006;7:565-574

Advanced disease in HNSCC: outcome

Stage	% Failure (progression/relapses)
I	>10
II	30
III	50
IV	70

Prince MEP et al. JCO 2009;26:2871-2879

Advanced disease in HNSCC: to sum up

- advanced disease at diagnosis: >50%
- despite good locoregional control, relapse in >50%
- 5-year survival rate: 10-40%
- heterogeneous disease:

oral cancer	3-yrs survival	47%
oropharyngeal cancer	3-yrs survival	67%

Kumar B et al. Int J Radiat Oncol Biol Phys 2007;69:109-111

Therapeutic options for recurrent disease

- salvage surgery +/- re-irradiation
- re-irradiation alone
- chemotherapy

Gibson MK et al. Lancet Oncology 2006;7:565-574

Therapeutic options for recurrent disease (I): salvage surgery

- feasible in a limited number of patients (approx. 20%)
- high rate of locoregional failure (approx. 50%)
- high risk of distant M+ (approx. 15%)

Wong SJ et al. JCO 2008;26:5500-5501

Therapeutic options for recurrent disease (II): re-irradiation +/- CT

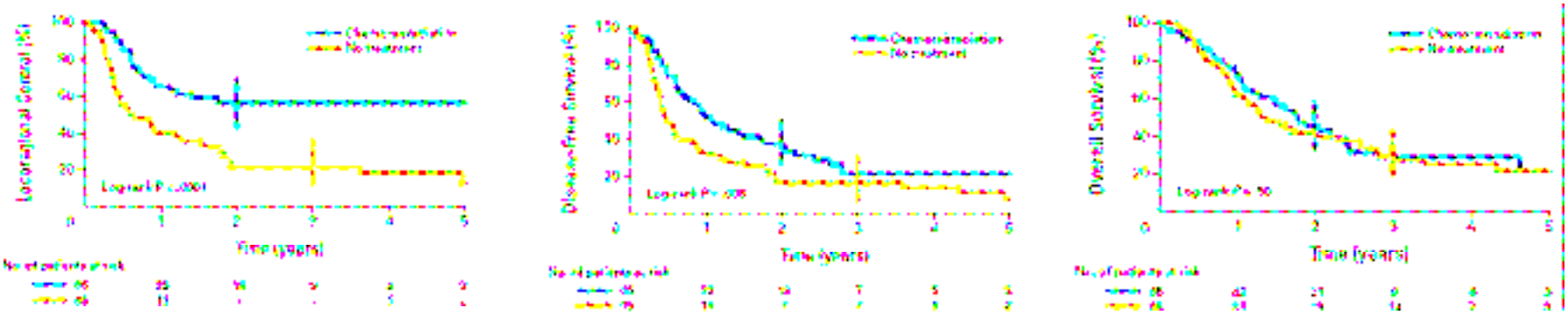
- feasible and effective in selected cases
- useful at high doses (60 Gy)
- improvement in locoregional control and DFS but not OS
- treatment-related toxicity (and toxic deaths) major concern

Wong SJ et al. JCO 2008;26:5500-5501

Therapeutic options for recurrent disease (II): re-irradiation +/- CT

Randomized Trial of Postoperative Reirradiation Combined With Chemotherapy After Salvage Surgery Compared With Salvage Surgery Alone in Head and Neck Carcinoma

François Janot, Dominique de Raucourt, Ellen Benhamou, Christophe Ferron, Gilles Dolivet, René-Jean Bensadoun, Marc Hamoir, Bernard Géry, Morbize Julieron, Marine Castaing, Etienne Bardet, Vincent Grégoire, and Jean Bourhis



Janot F et al. JCO 2008;26:5518-5523

Therapeutic options for recurrent disease (III): Chemotherapy

- in historical series, modest benefit over BSC
- Cisplatin alone: RR 15%
- Cisplatin+Fluorouracil: RR 30%
- CDDP+Taxanes: RR 33-67%
- CDDP+Cetuximab: RR 23%
- Paclitaxel+Cetuximab: RR 70%
- Cetuximab alone: RR 13%

No one of these schedules translates in OS benefit!

Licitra L et al. Ann Oncol 2008;19:200-203

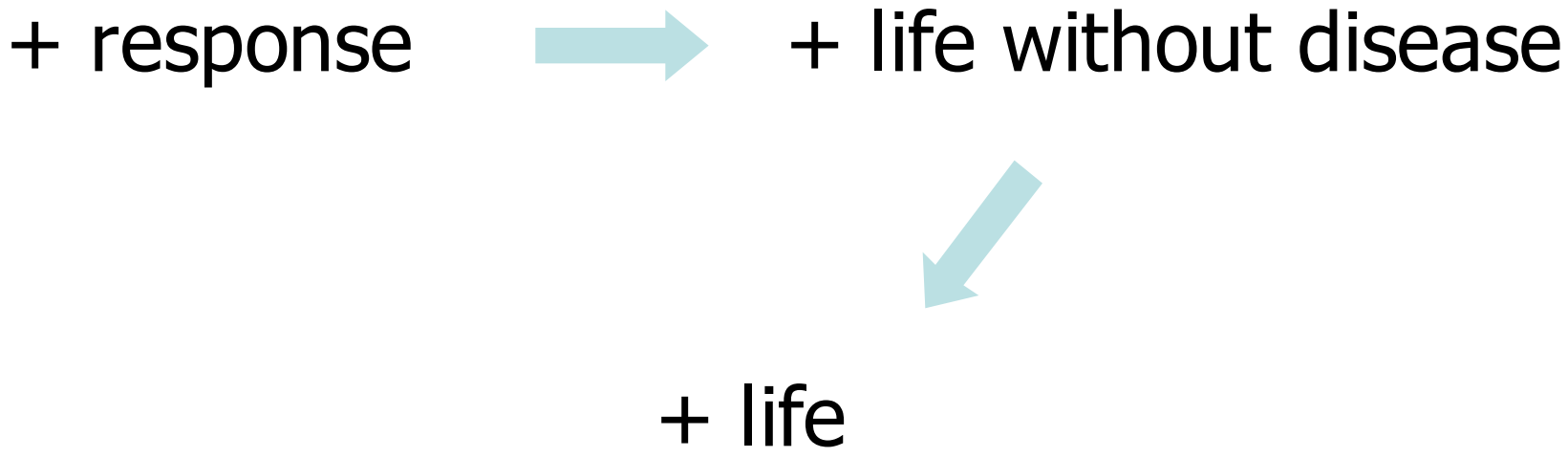
Therapeutic options for recurrent disease (IV): Chemotherapy+anti-EGFR (EXTREME trial)

- Phase III trial of CDDP/Fluorouracil +/- Cetuximab
- RR 36% vs 20%
- PFS and OS benefit
- but no benefit if PS<80 and age>70

First evidence of OS benefit offered by CT in the recurrent/metastatic setting in the last 25 years !

Licitra L et al. Ann Oncol 2008;19:200-203

The "surrogacy"rationale of treatment



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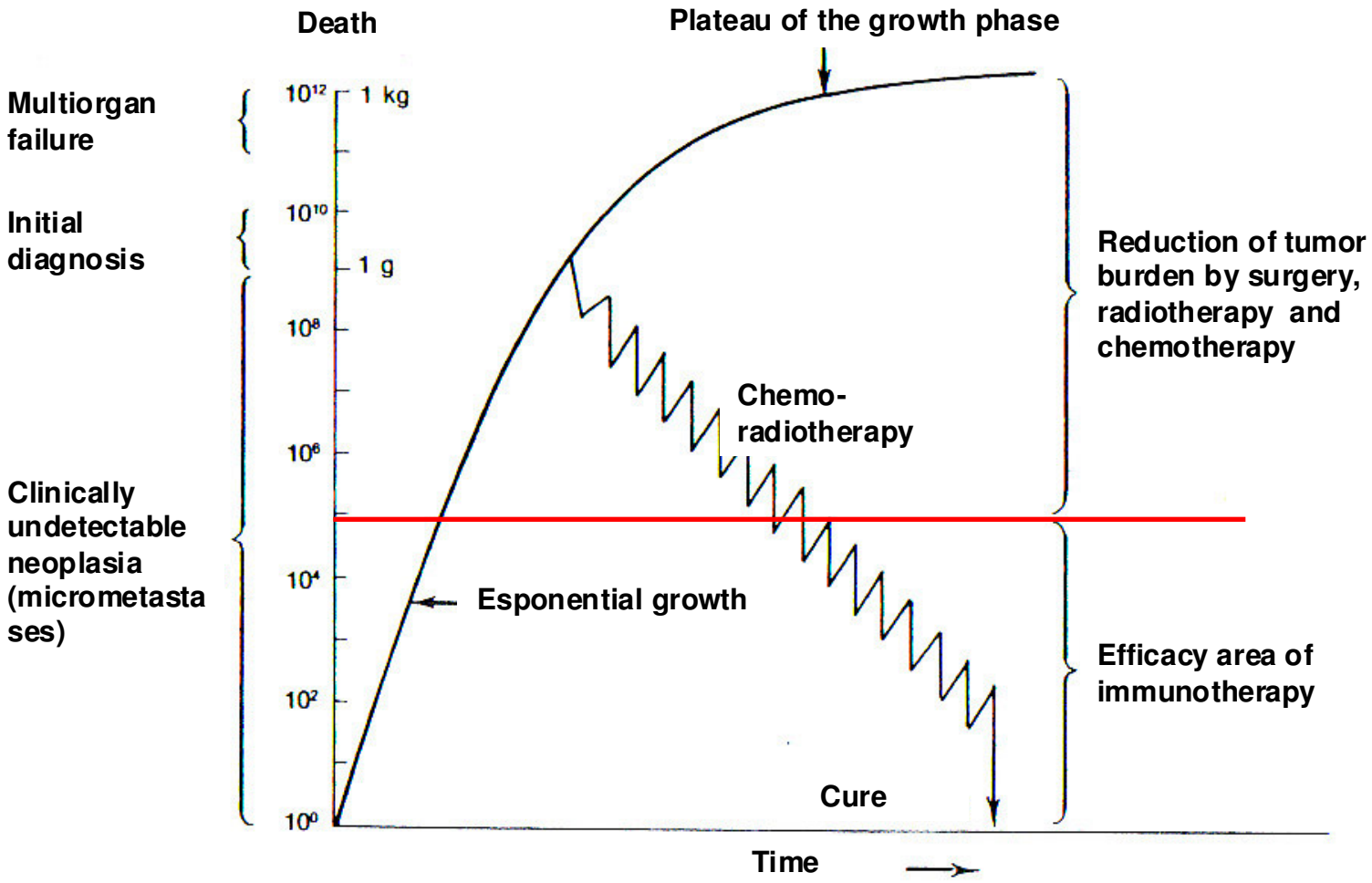
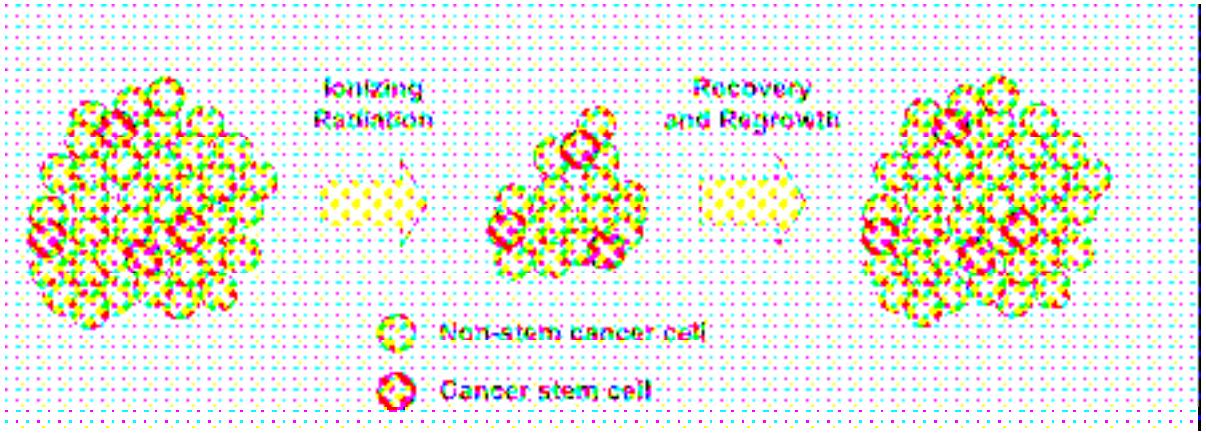


Repopulation of cancer cells as cause of treatment failure



Kim JJ and Tannock IF Nat Rev Cancer 2005;5:516-525
Rich JN Cancer Res. 2007;67:8980-8984

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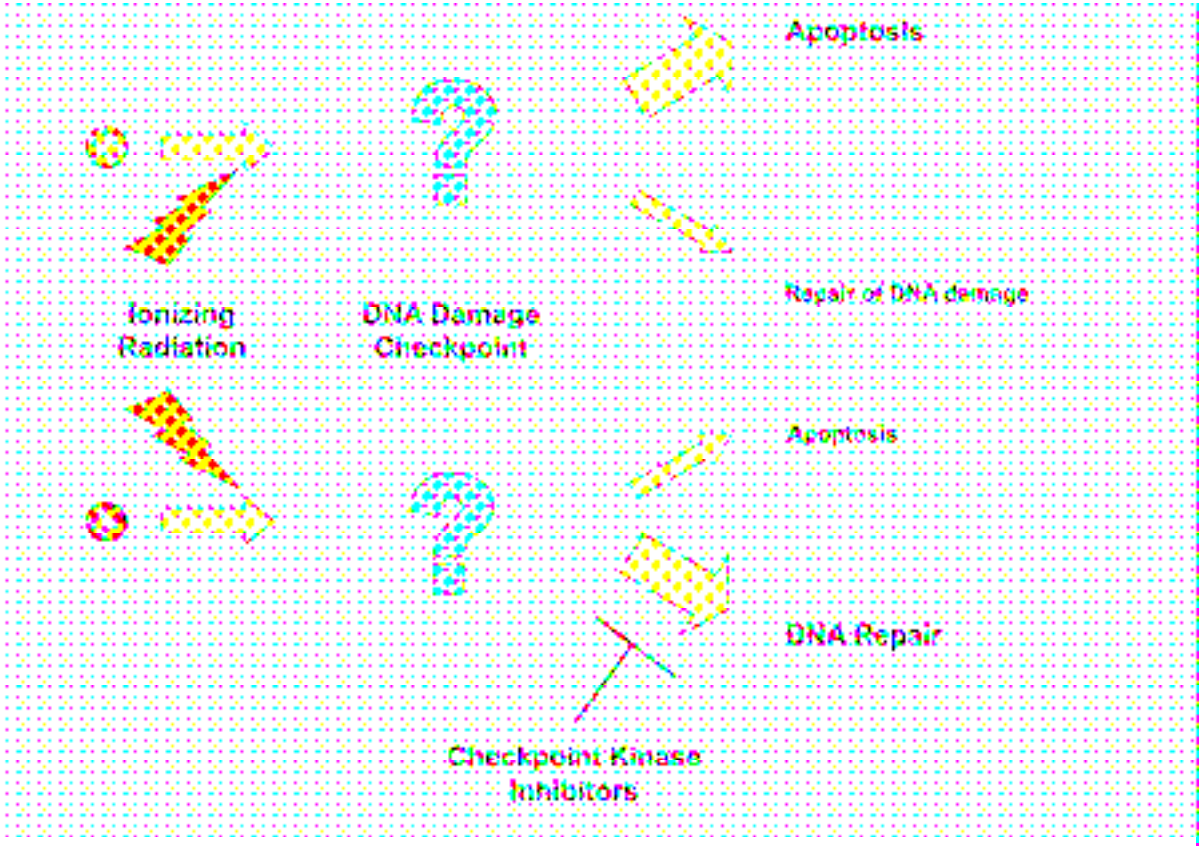

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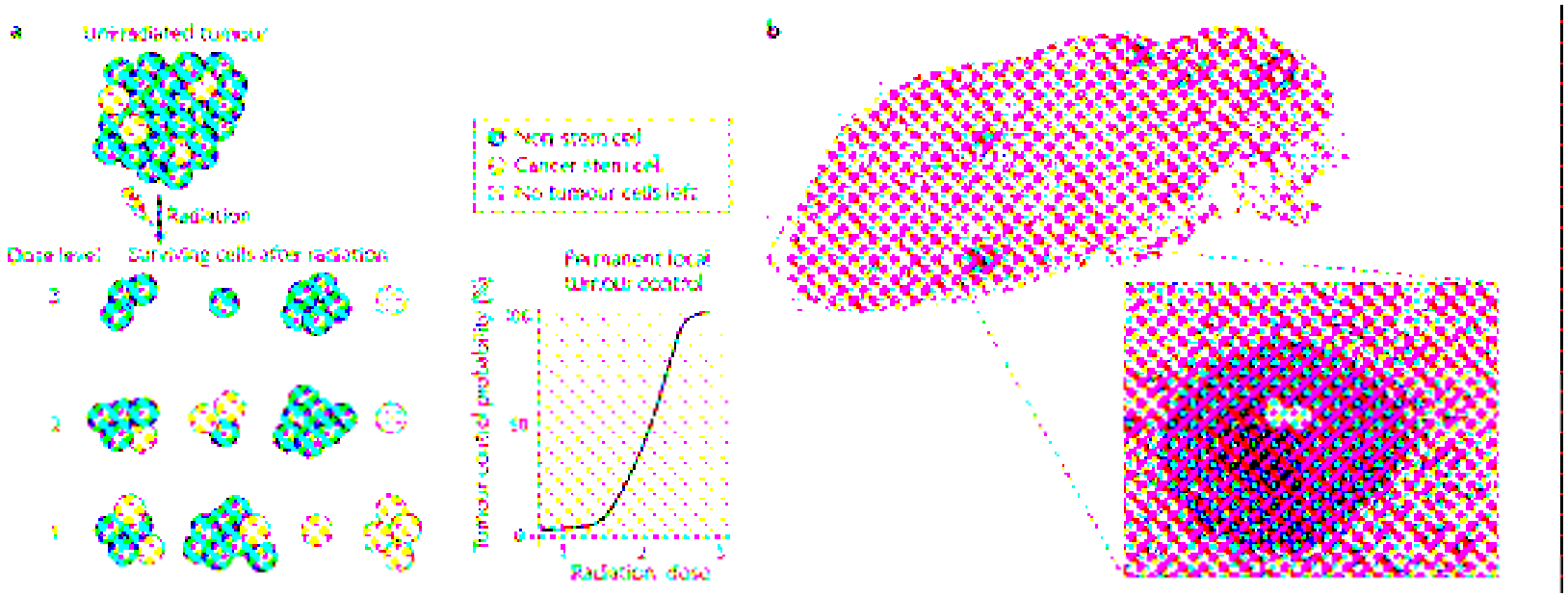
Repopulation of cancer stem cells as cause of treatment failure



Baumann M et al. Nature Rev Cancer 2008;8:545-554

Identification of cancer stem cells in HNSCC

Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma

M. F. Prince*, R. Sivanandan†, A. Kaczorowski*, G. T. Wolf*, M. J. Kaplan†, P. Dalerba‡, I. L. Weissman‡, M. F. Clarke‡, and L. E. Ailles‡§

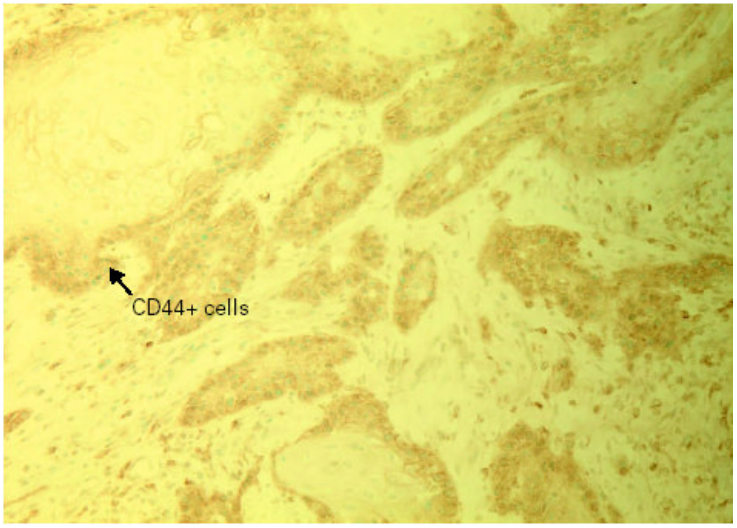
*Department of Otolaryngology–Head and Neck Surgery, University of Michigan, Ann Arbor, MI 48109; and ‡Department of Otolaryngology–Head and Neck Surgery and §Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305

CSC in HNSCC: <10% of the cancer cells

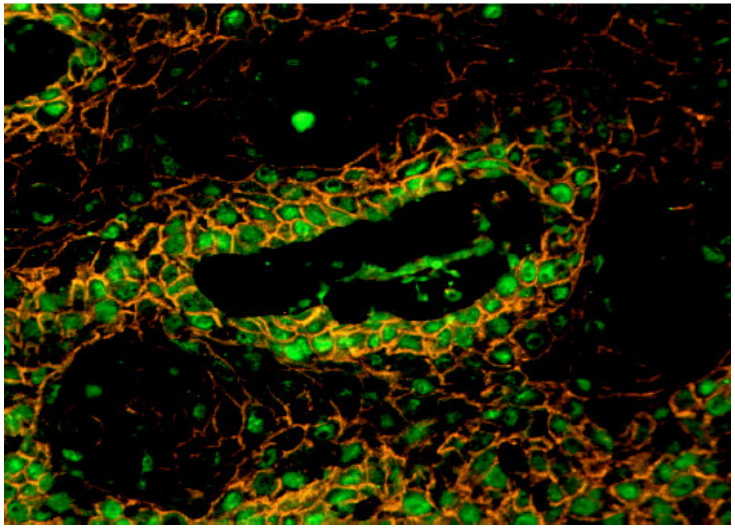
Phenotype: CD44+, cytokeratin 5 and 14 (basal cell markers), BMI1+

Prince ME et al. PNAS 2007;104:973-978

Identification of cancer stem cells in HNSCC



CD44+ (red) / BMI1 (green)



CSC in HNSCC are localized in microdomains associated with the tumor stroma

BMI-1 is a stem cell-related gene (others: Wnt, Notch, PTEN, Hedgehog)

Prince ME et al. JCO 2008;26:2871-2875

Identification of cancer stem cells in HNSCC

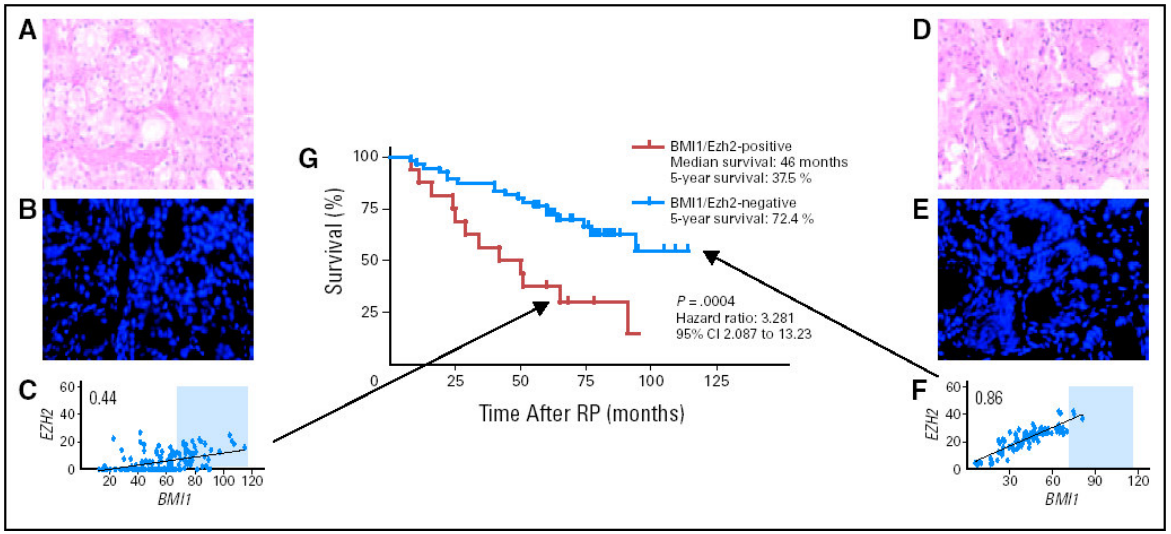
VOLUME 26 · NUMBER 17 · JUNE 10 2008

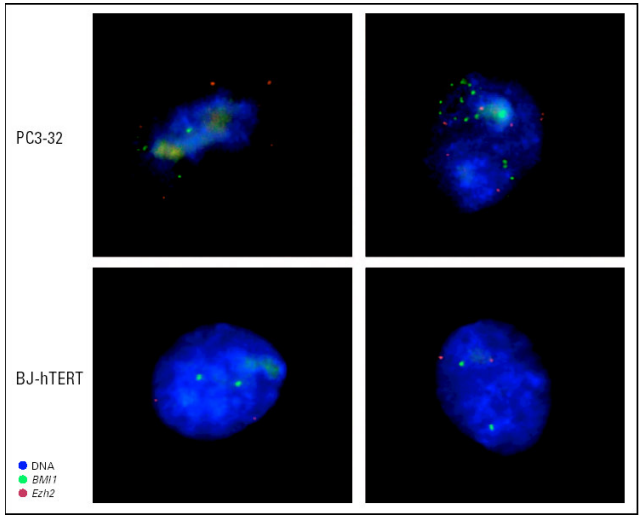
JOURNAL OF CLINICAL ONCOLOGY

REVIEW ARTICLE

"Stemness" Genomics Law Governs Clinical Behavior of Human Cancer: Implications for Decision Making in Disease Management

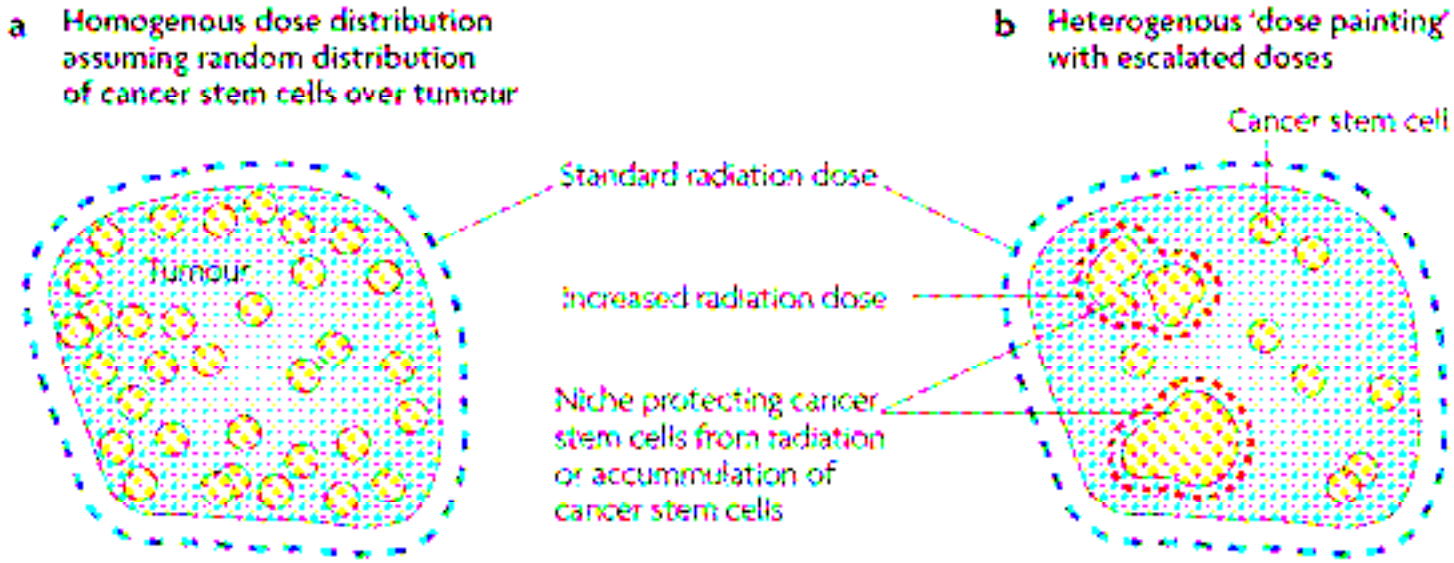
Gennadi V. Glinsky





Glinsky GV. JCO 2008;26:2846-2853

Importance of cancer stem cell niches in RT treatment planning



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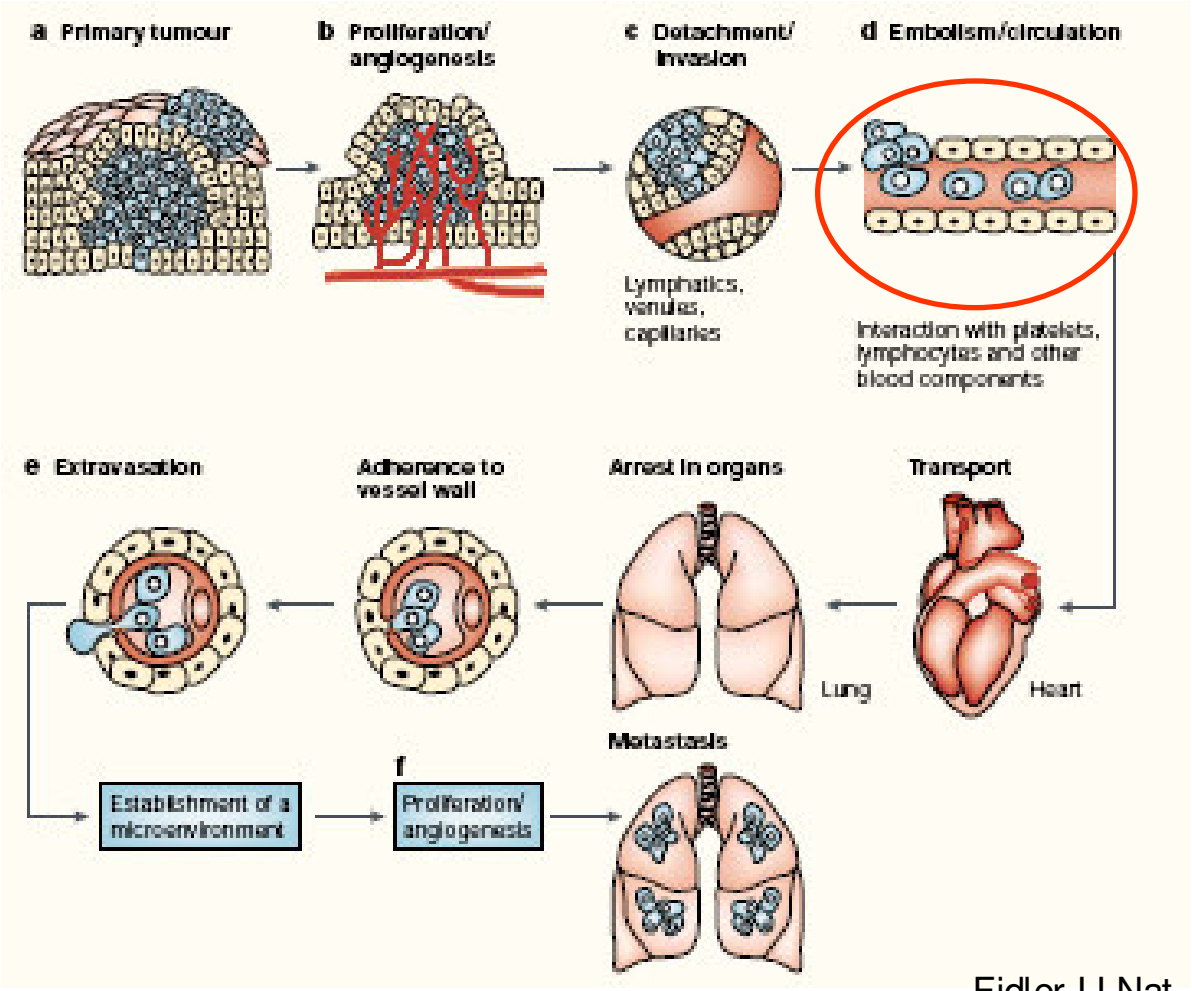
Gibson MK et al. Lancet Oncology 2006;7:565-574

Clinical implication of CSCs in HNSCC

"*It would seem that every time we treat a noncurable cancer, we increase the density of cancer stem cells and facilitate cancer to become more resistant*"

Ajani JA et al. JCO 2008;19:162-163

Metastatic multistep process



Fidler IJ Nat. Rev. Cancer 2003

Circulating Tumor Cells: background

- CTCs in the blood of patients with solid tumors have been described more than 100 years ago

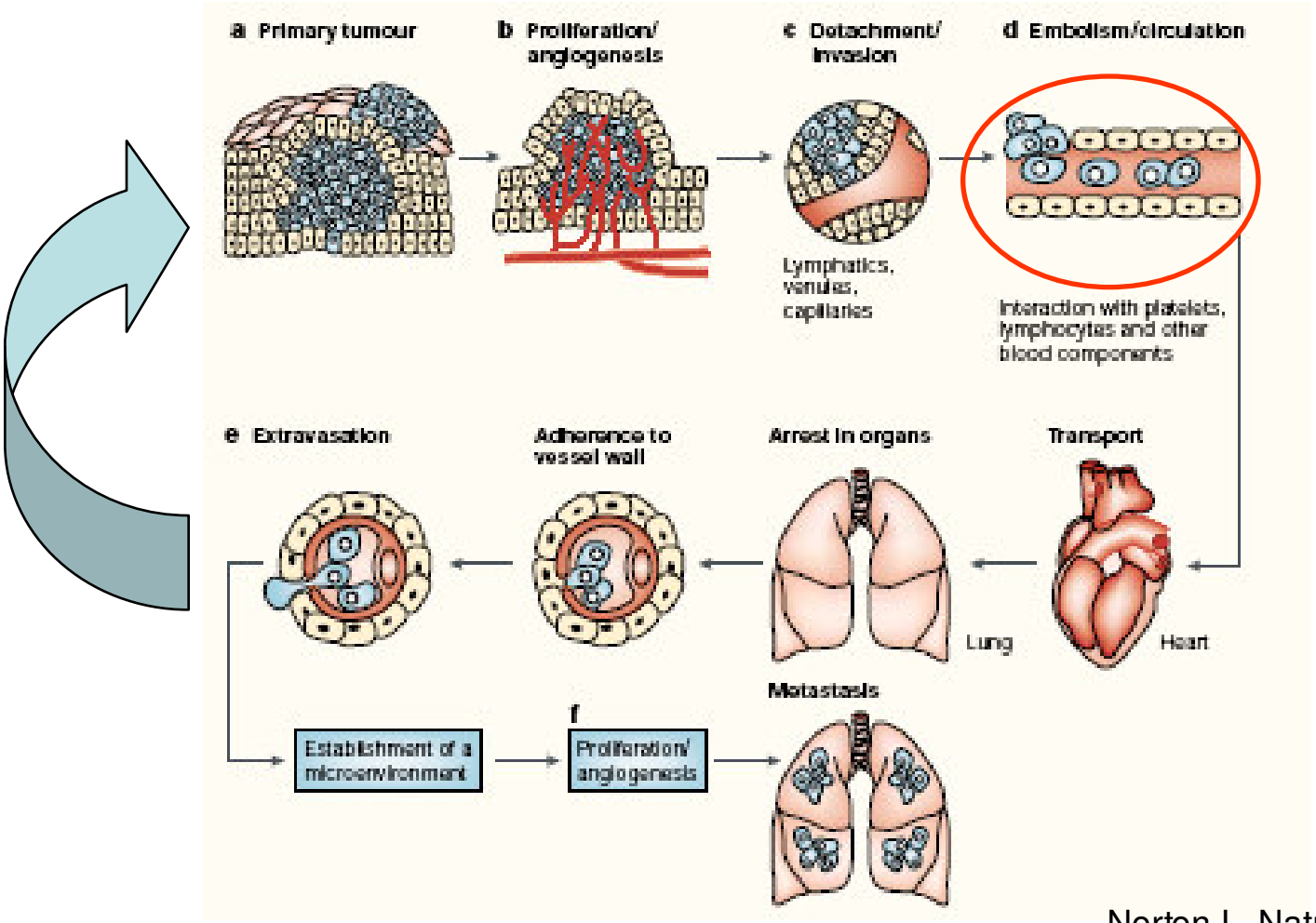
- In cancer patients CTCs can reach the peripheral blood every few hours and can remain for long periods

- They represent the first step of the (highly inefficient) metastatic process

Circulating Tumor Cells: open questions

- are CTCs responsible for metastases?
- are CTCs tumorigenic (cancer stem cells)?
- are CTCs responsible for primary tumor repopulation?
  (Self seeding hypothesis of tumors – L. Norton)

Self seeding hypothesis



Norton L. Nature Medicine 2006

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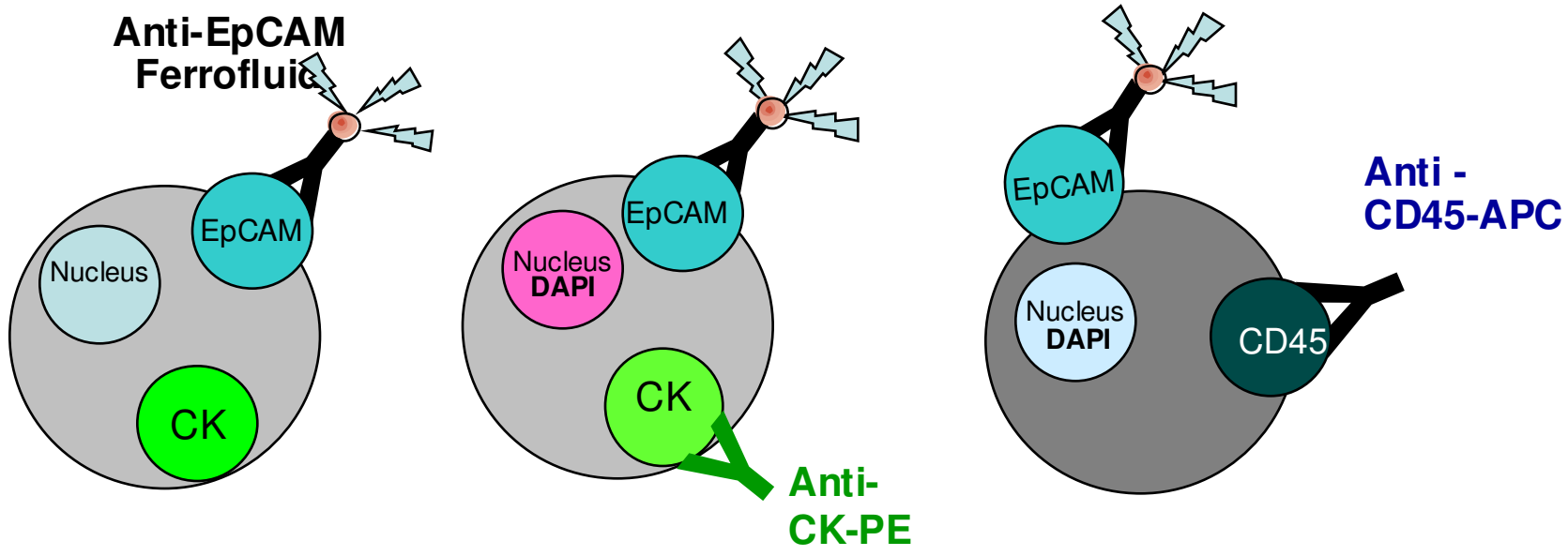
CellSearch Technology: criteria to define a CTC



Epithelial Cell

Epithelial Cell

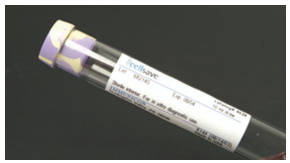
Leukocyte

Criteria for CTC definition: EpCAM+, Cytokeratins 8, 18, 19+, DAPI+, CD45-

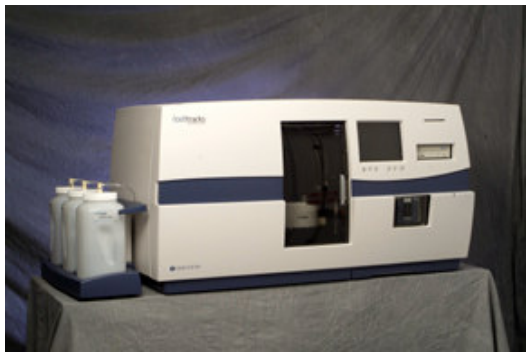
CellSearch: Technology

CellSearch™Reagents

CellSave® Tube

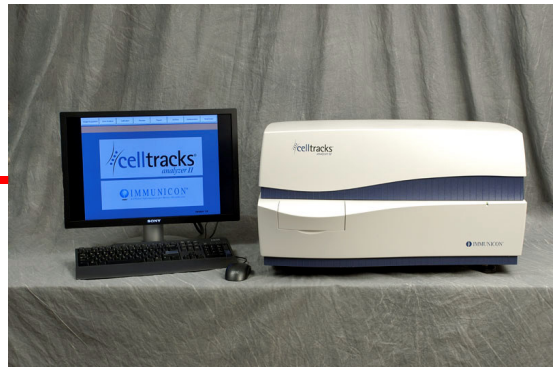


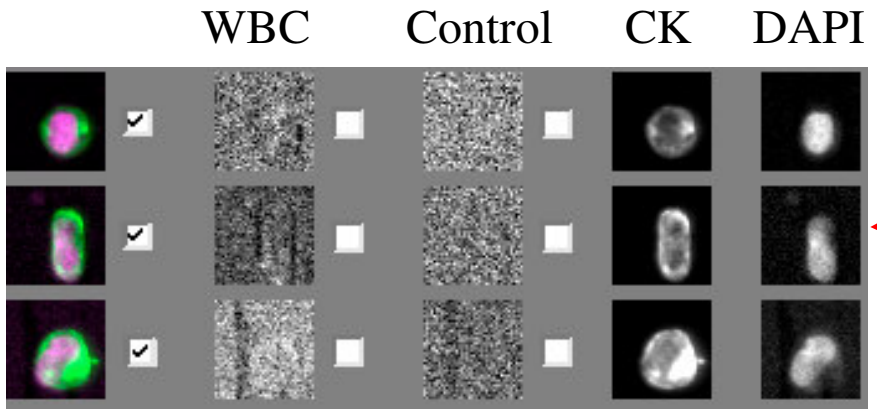
CellTracks® AutoPrep System



Magnest®

CellTracks® Analyzer II





FDA Approval 2004

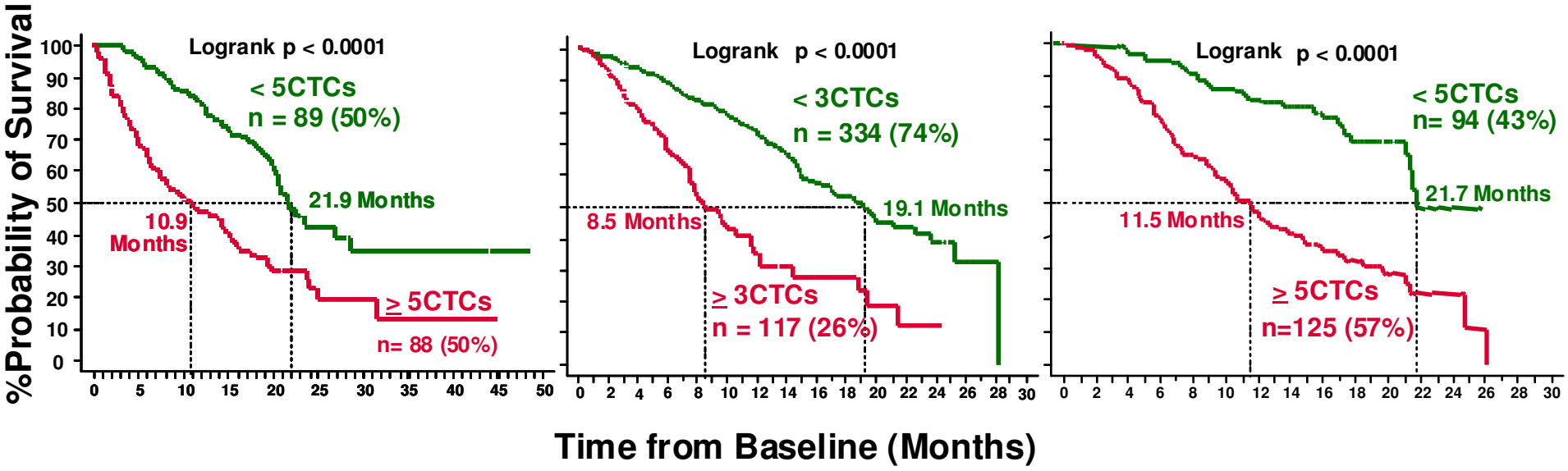
CTCs are predictive of OS

Metastatic Carcinomas

Breast
n=177

Colorectal
n=451

Prostate
n=219

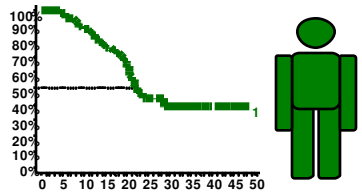


NEJM August 2004
JCO March 2005

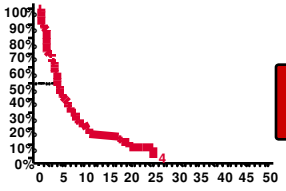
JCO July 2008

CCR October 2008

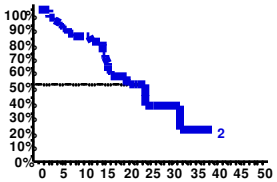
OS comparison in MBC, MCRC, MPC



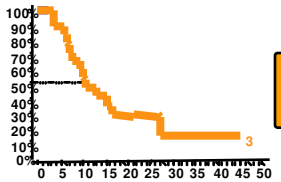
<cut-off at all time points



≥cut-off at all time points



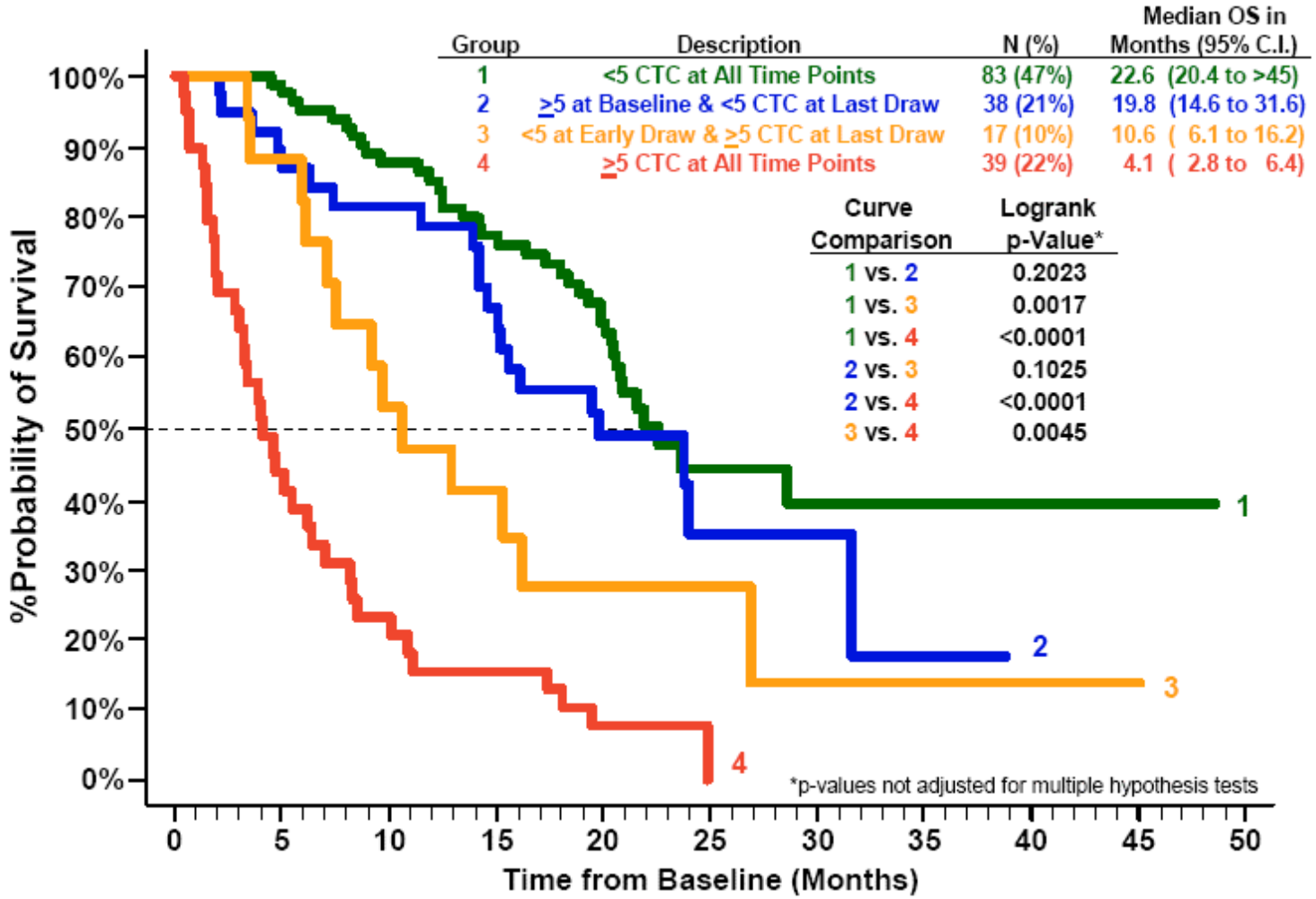
≥cut-off at baseline and <cut-off at final draw



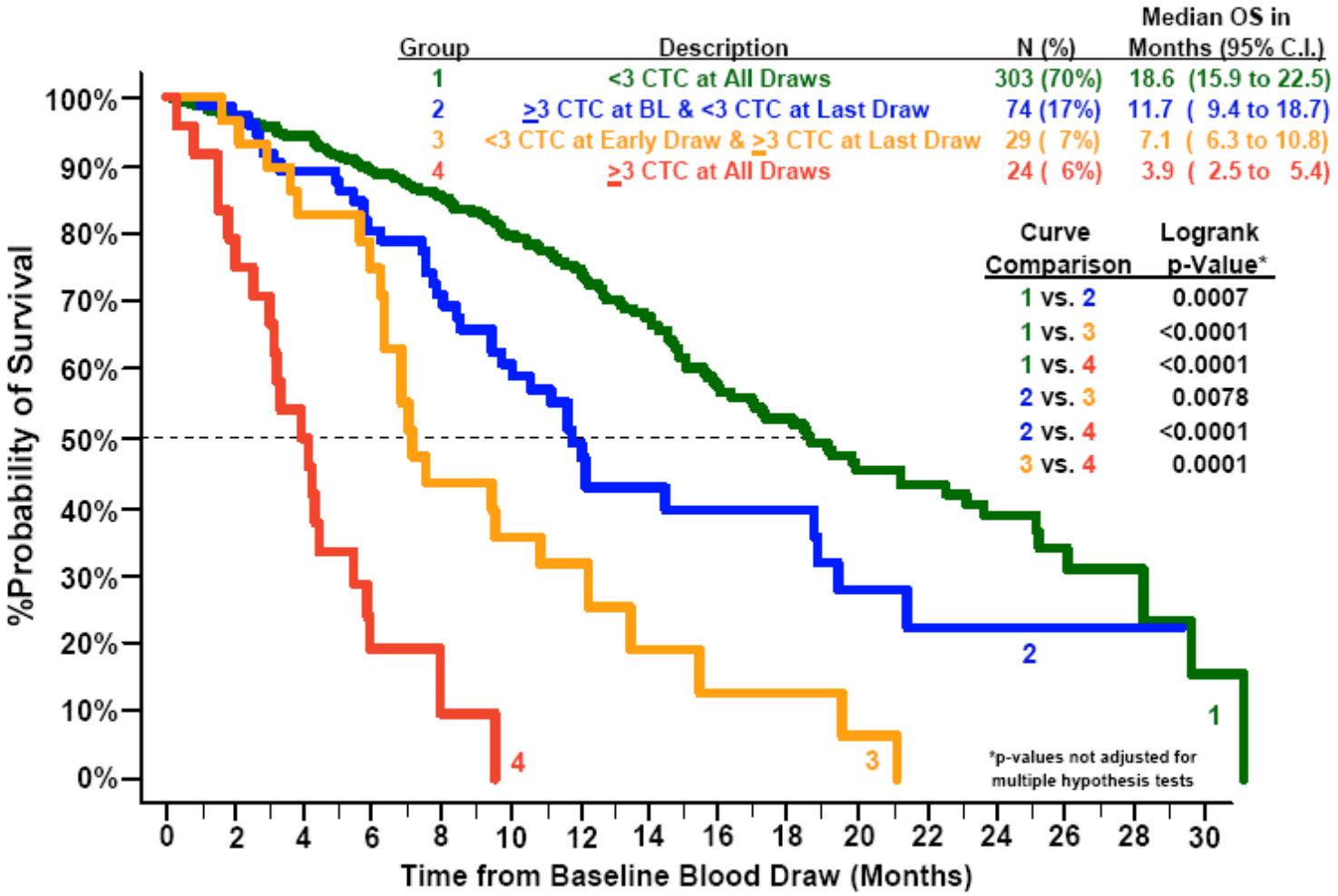
<cut-off at early draw and ≥cut-off at final draw

MBC	MCRC	MPC
22.6	18.6	>26
4.1	3.9	6.8
19.8	11.7	21.3
10.6	7.1	9.3

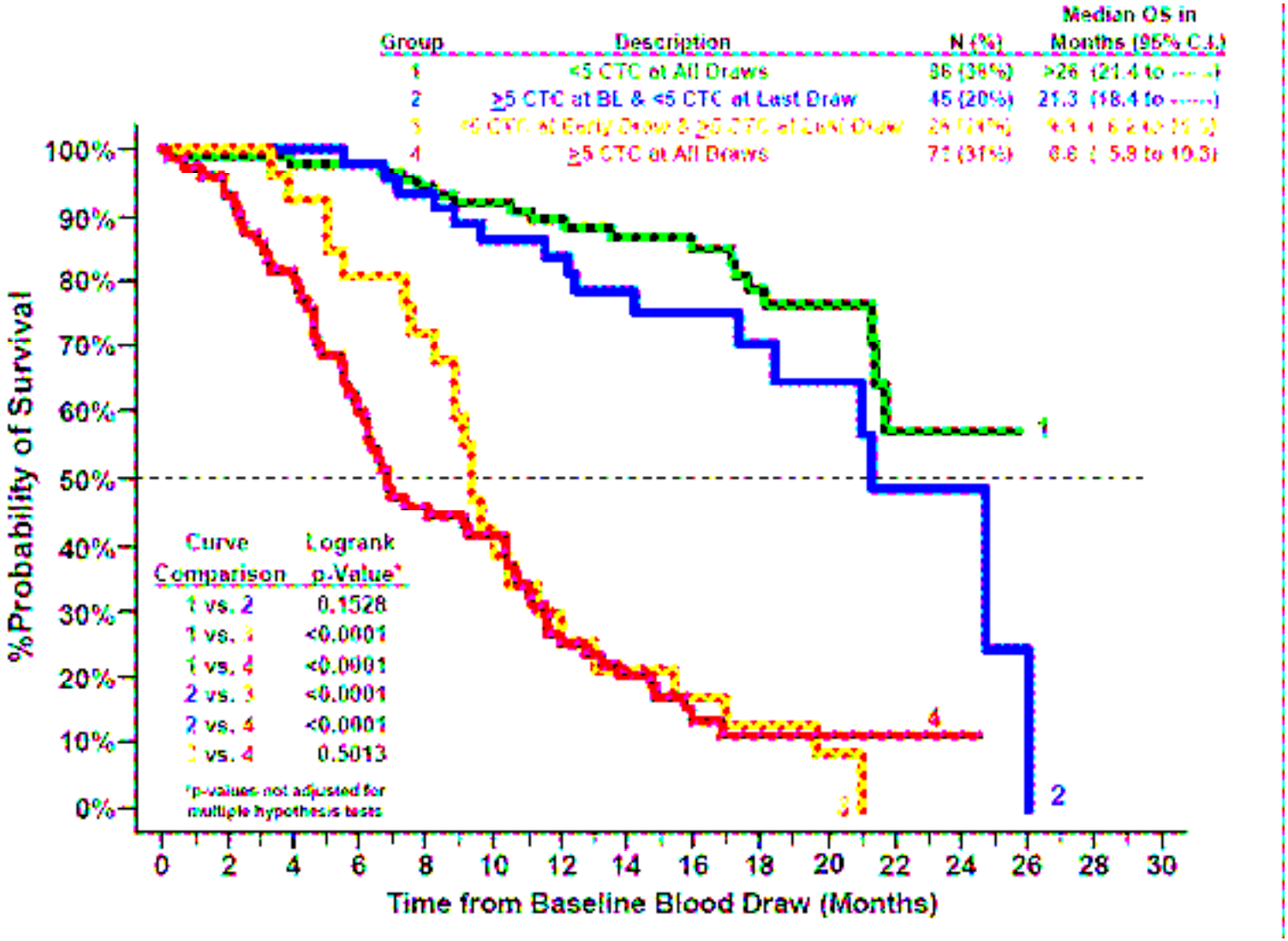
CTCs are dynamic predictors of OS in MBC



CTCs are dynamic predictors of OS in MCRC



CTCs are dynamic predictors of OS in MPC



CTCs in HNSCC: background (I)

Application of Immunomagnetic Cell Enrichment in Combination with RT-PCR for the Detection of Rare Circulating Head and Neck Tumor Cells in Human Peripheral Blood

Xiaodong Tong,[1] Liying Yang,[1] James Campbell Lang,[2] Maciej Zborowski,[3] and Jeffrey J. Chalmers[1,4*]

- 3 HNSCC cell lines
- immunomagnetic cell sorter and RT-PCR

Tong X et al. Cytometry Part B (Clinical Cytometry)2007;72B:310-323

CTCs in HNSCC: background (II)

Micrometastatic Tumor Detection in Patients With Head and Neck Cancer

A Preliminary Report

Ari Wirtschafter, MD; Michael S. Benninger, MD; Thomas J. Moss, MD; Tehila Umiel, PhD; Kathleen Blazoff, MSN, RN; Maria J. Worsham, PhD

- 18 pts, stage I-IV
- immunocytochemistry and EpCAM positive-selection (ICC assay)
- CTCs documented in 8 pts (44%)

Wirtschafter A et al. Arch Otolaryngol Head Neck Surg 2002;128:40-43

CTCs in HNSCC: background (III)

Detection of Rare Disseminated Tumor Cells Identifies Head and Neck Cancer Patients at Risk of Treatment Failure

Max Partridge,[1] Ruud Brakenhoff,
Elaine Phillips, Kulsan Ali, Rebecca Francis,
Richard Hooper, Kenneth Lavery,
Andrew Brown, and John Langdon

- 40 pts, stage I-IV, blood and bone marrow
- immunocytochemistry for E48 antigen
- CTCs documented in 10/40 pts, predictive of distant M+ and poor OS

Partridge M et al. Clin Cancer Res 2003;9:5287-5294

CTCs in HNSCC: preliminary results in Brescia

- 18 pts, stage III-IV
- immunocytochemistry and EpCAM, Cytokeratin 8, 18, 19, DAPI+, CD45-

Characteristics	N. pts
Site of T	13
Oropharynx	2
Nasopharynx	1
Laryngopharynx	5
Larynx	1
Esophagus	
Histology	
Squamous	20
Indifferentiated	2
Stage	
III-IVA-IVB	13
IVC	9

CTCs in HNSCC: preliminary results in Brescia

- Pt #1 stage IVa oropharynx
  - basal: aggregates
  - after 1 cy TPF: 3 cells/7.5 mL cPR(50%)
  - after 2 cy TPF: 0 cells/7.5 mL cCR

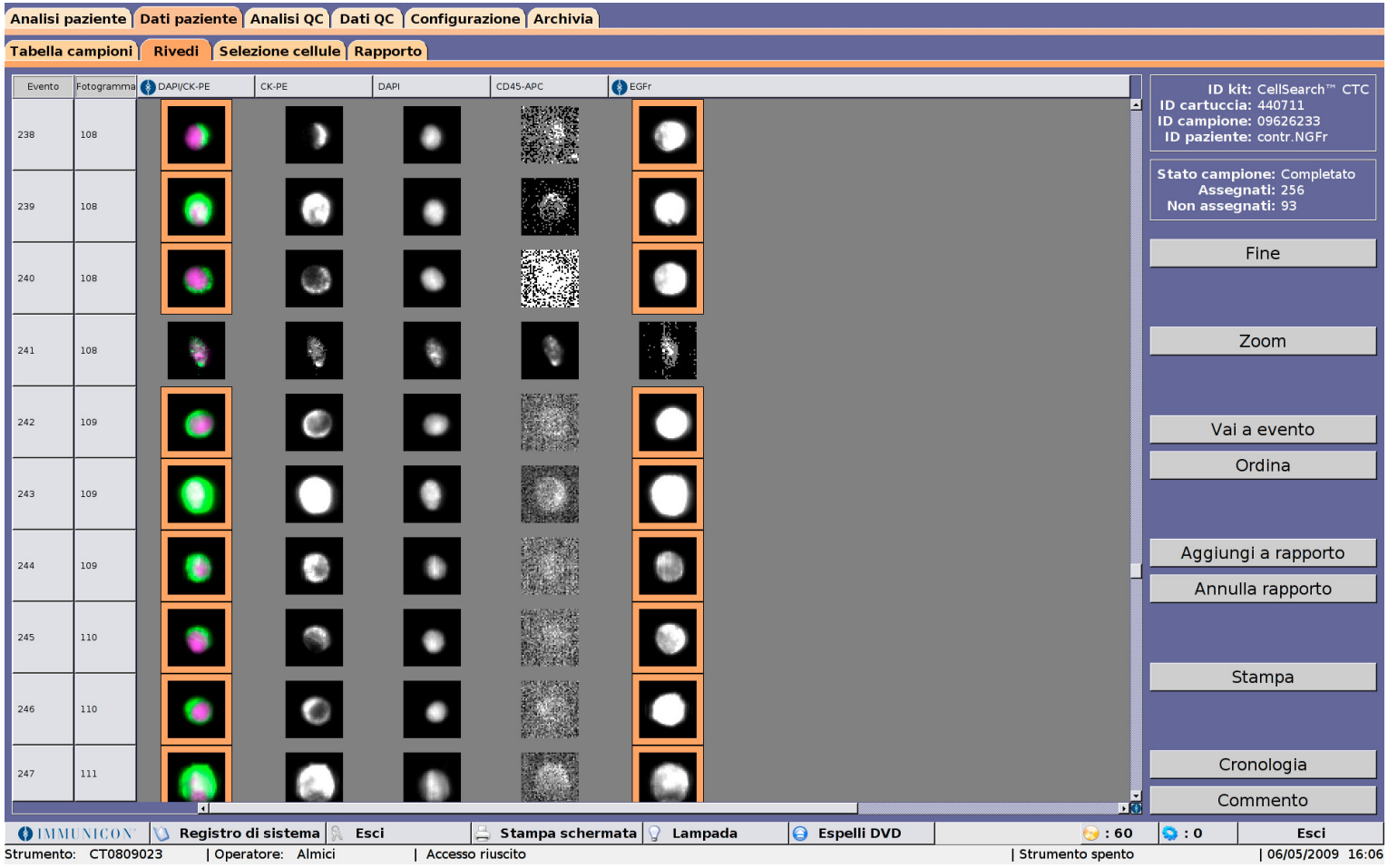
- Pt #2 stage IVc oropharynx (tonsil) with M+ (lung, liver and bone) in PD
  - basal: 25 cells/7.5 mL dead after 1 mo

CTCs in HNSCC: preliminary results in Brescia

- Pt #3 stage IVc oropharynx with M+ lymphnods (axilla + mediastinum)
  - basal: 3 cells/7.5 mL on chemo
  - after 4 cy MTX/FU: 9 cells/7.5 mL PD
  - after 1 cy CTX/FU: 5 cells/7.5 mL SD

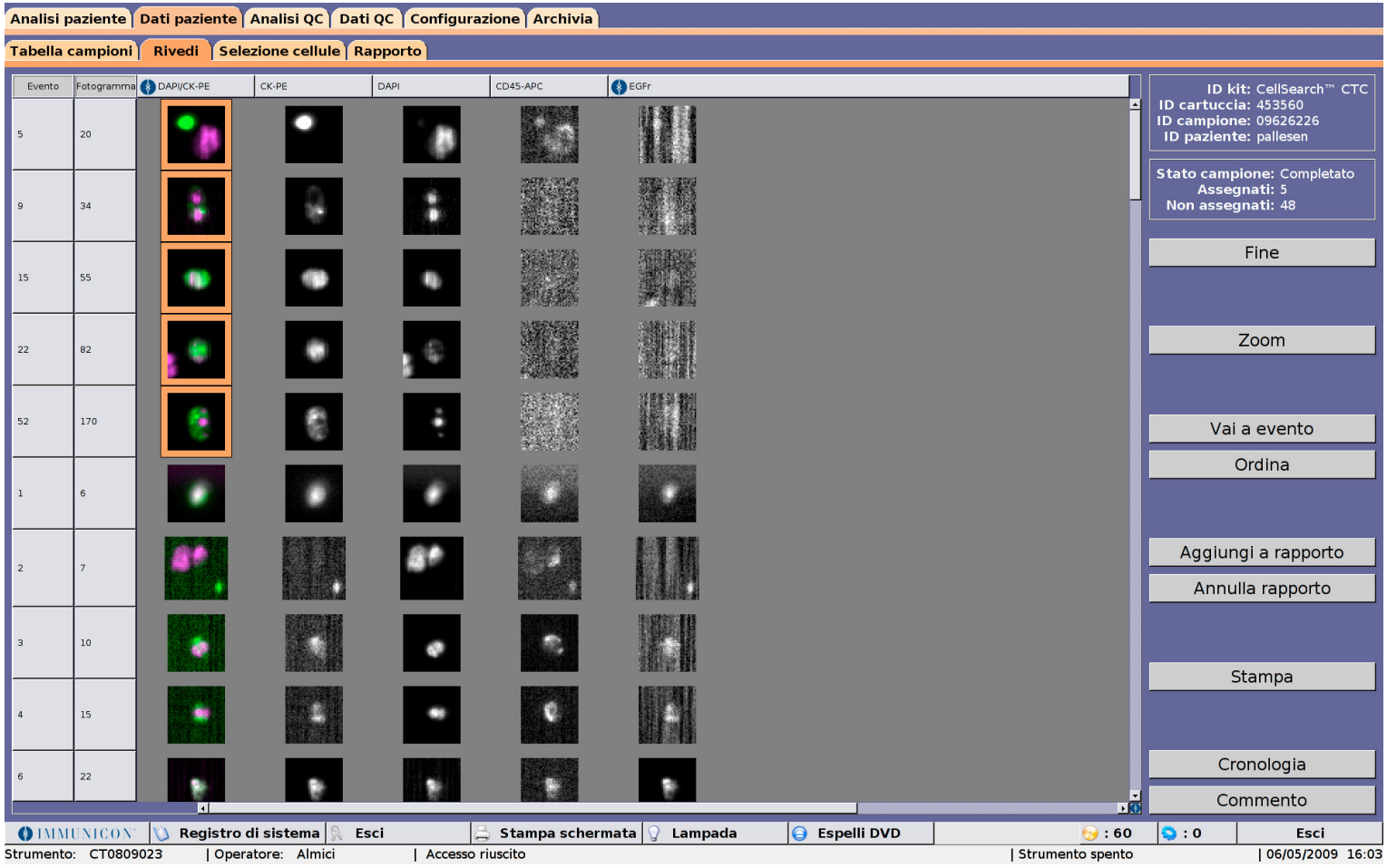
CTCs in HNSCC: are EGFR+?

Cell line SKBR EGFR+ in normal PB

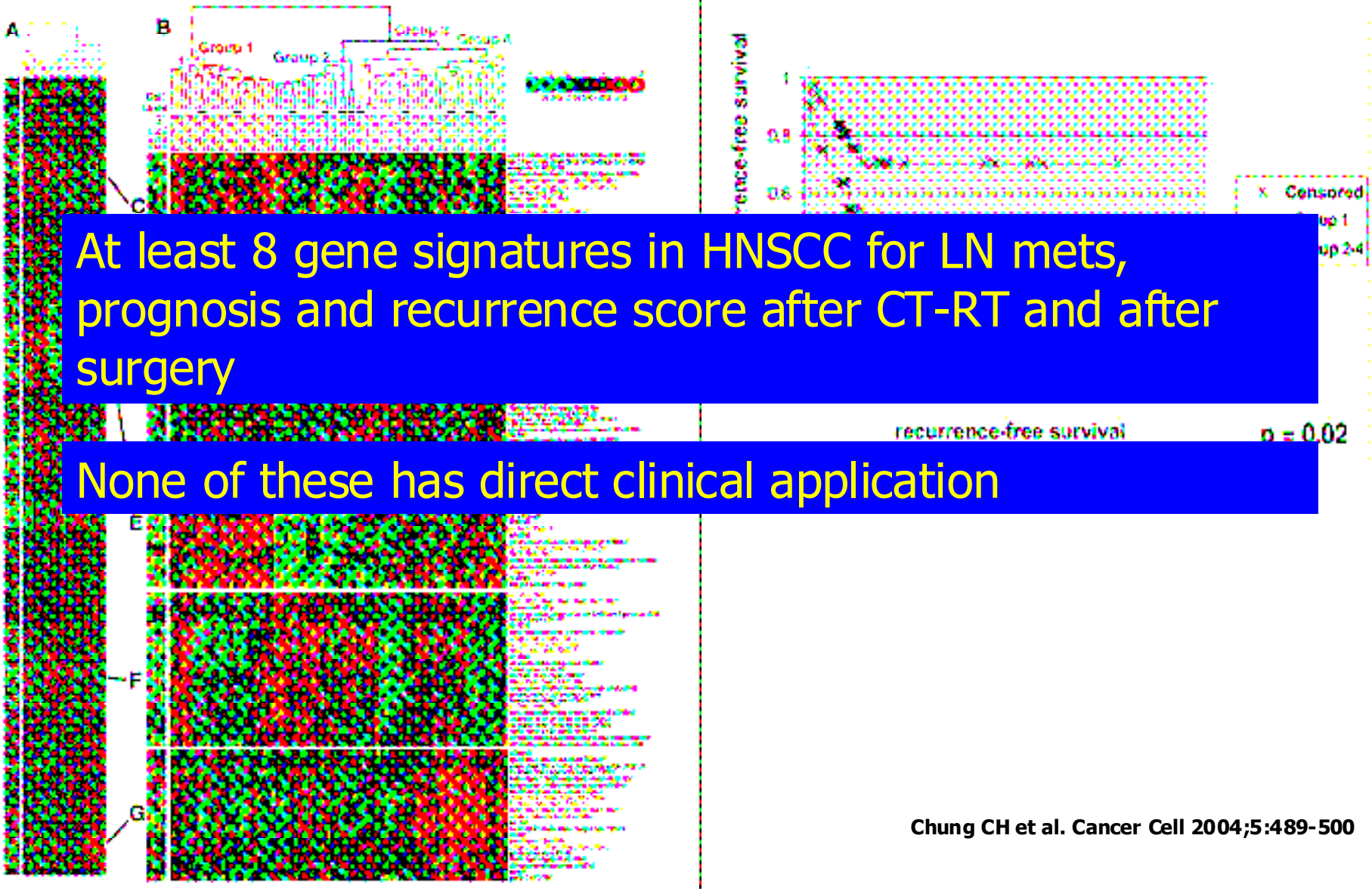


CTCs in HNSCC: are EGFR+?

Peripheral blood from patient #3



Gene profiling of HNSCC



At least 8 gene signatures in HNSCC for LN mets, prognosis and recurrence score after CT-RT and after surgery

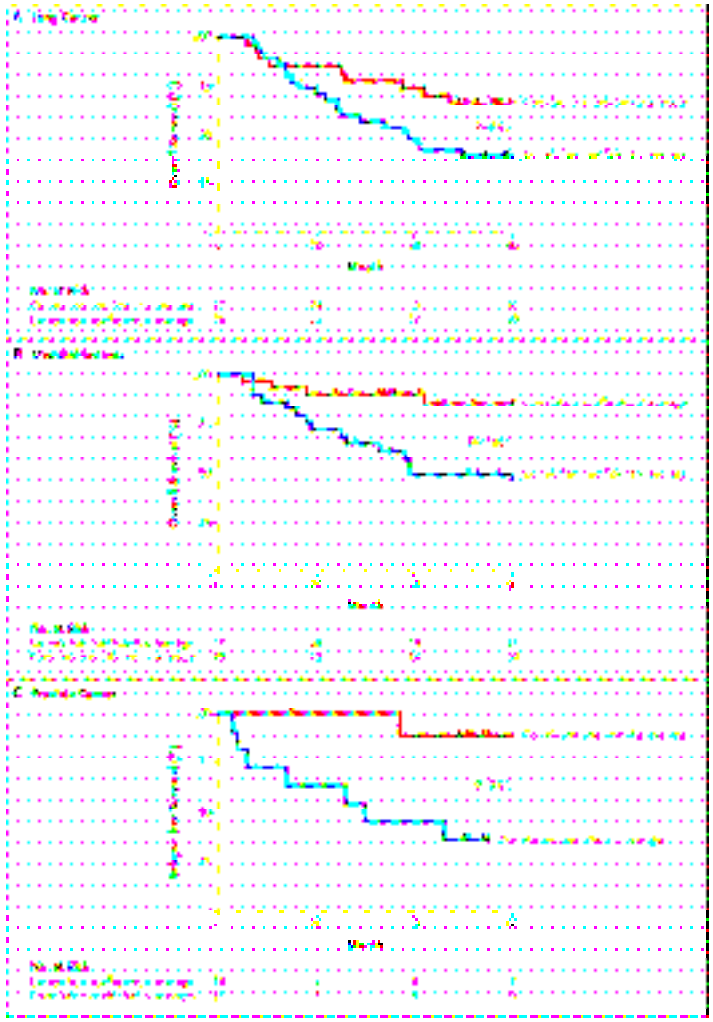
None of these has direct clinical application

Chung CH et al. Cancer Cell 2004;5:489-500

Prognostic value of invasiveness gene signature

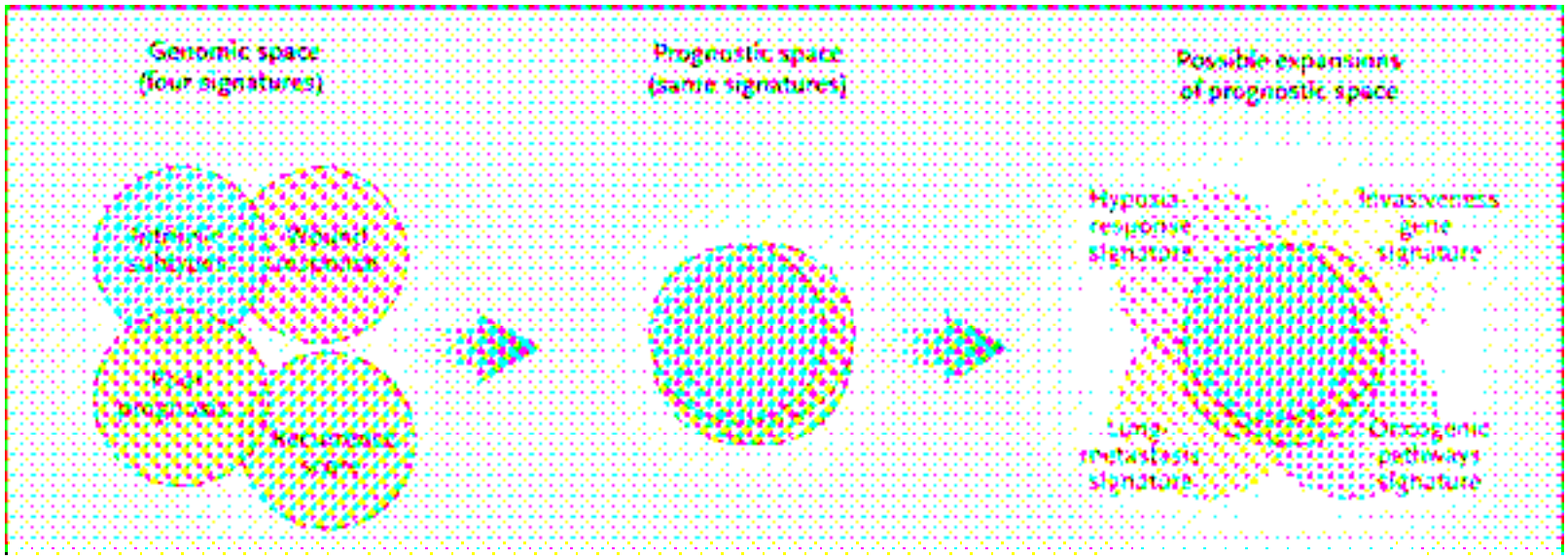
Table 1. Classification of the 186 Genes in the Invasiveness Gene Signature (IGS).

Class	Genes
Apoptosis	*DPF2, CASP8, BCL2*
Calcium-ion binding	*SCGN, SWAP70, KIAA0276*
Cell cycle	*C10orf9, C10orf7, ALKBH, TOB2*
Cell-surface receptor	*XPR1, CD59, LRP2*
Chemotaxis	*PLP2, MAPK14, CXCL2*
Collagen catabolism	*MMP7*
Differentiation	*MGP, MLF1, FLNB*
Ion-channel activity	*SCNM1*
Membrane protein	*HSPC163, C5orf18, MGC4399, CDW92, TMC4, ZDHHC2, TICAM2, KDELR3*
Metabolism	*GNPDA1, THEM2, DBR1, FLJ90709, FLJ10774, C16orf33, GAPD, LDHA, MR-1, LARS, GTPBP1, PRSS16, WFDC2, AIM1, DHRS6, DHRS4, MGC15429, MGC45840, ECHDC2, GOLGIN-67, AFURS1, KIAA0436, CYP4V2, JTV1*
Methyltransferase	*ICMT, DNMT3A, HNMT, METTL7A, METTL2*
Morphology	*VIL2, TPD52, ARPC5*
Nucleotide binding	*NOL8, NSF, RAD23B, SRP54, HSPA2, PBP, THAP2, CIRBP, SNRPN, KIAA0052*
Phosphatase	*DUSP10*
Proliferation	*SSR1, ERBB4, EMP1, CHPT1, LRPAP1*
Protein binding	*FLJ11752, CSTF1, KLHL20, DNAJC13, APLP2, ARGBP2, DNAJB1, NEBL, SH3BGRL, NUDT5, GABARAPL1, MAPT, DCBLD1*
Protein kinase	*STK39, PAK2, CSNK2A1, PILRB, ERN1, SGKL, WEE1, MAST4, C11orf17*
Protein transport	*NUP37, CLTC, COPB2, SLC25A25*
Signal transduction	*ECOP, PDE8A, STAM, TUBB, SNX6, RAB23, PLAA, STC2, LTF*
Transcription factor	*ISGF3G, ATXN3, GTF3C3, GSK3B, KLF10, ELL2, ZBTB20, IRX3, ETS1, SERTAD1, MGC4251, MAFF, SFPQ, CITED4, CEBPD, EIF4E2*
Transferase	*HS2ST1, AGPS, PGK1, ATIC, ETNK1, ALG2*
Ubiquitination	*NCE2, MARCH8, CNOT4, RNF8, PSMA5, DPF2*
Function unknown	*AMMECR1, KIAA1287, LOC144233, LOC286505, PNAS-4, FLJ20530, THUMPD3, MGC45564, CAP350, ETAA16, HAN11, DNAPTP6, C7orf25, FLJ37953, FLJ10587, C7orf36, ELP4, NDEL1, NPD014, DKFZP564D172, FAM53C, IER5, LOC255783, KIAA0146, KIAA0792, LOC439994, LOC283481, CG018, LOC130576, NGFRAP1L1, KIAA1217, C4orf7, C21orf86, C9orf64, FLJ13456, KIAA1600, B7-H4, LOC80298, C7orf2, NUCKS, DKFZP566D1346, LOC388279, FLJ31795, C6orf107, FLJ12439, FLJ12806, FLJ39370*



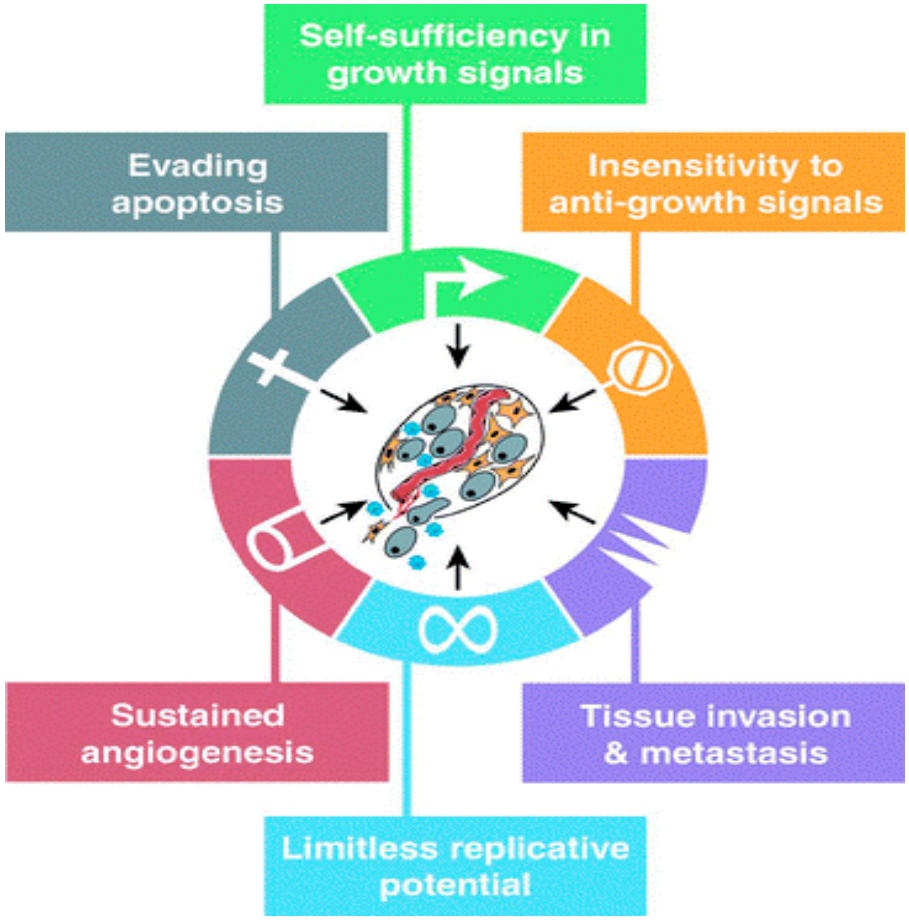
Liu R et al. NEJM 2007;356:217-226

Prognostic value of different gene signatures



Massaguè J et al. NEJM 2007;356:294-297

Prognostic value of different gene signatures



Hanahan and Weinberg. Cell 2000

Gene profiling of HNSCC:

the Agendia™ TargetPrint Discovery Gene Panel

Unigene Symbol[1]	UniGene Cluster ID	Unigene Name
AKT1	Hs.525622	V-akt murine thymoma viral oncogene homolog 1
AURKA	Hs.250822	Aurora kinase A
BCL2	Hs.150749	B-cell CLL/lymphoma 2
BRAF	Hs.550061	V-raf murine sarcoma viral oncogene homolog B1
BRCA1	Hs.194143	Breast cancer 1, early onset
BRCA2	Hs.34012	Breast cancer 2, early onset
C11orf30	Hs.352588	Chromosome 11 open reading frame 30
CCND1	Hs.523852	Cyclin D1
CCNE1	Hs.244723	Cyclin E1
CDH1	Hs.461086	Cadherin 1, type 1, E-cadherin (epithelial)
CDH3	Hs.554598	Cadherin 3, type 1, P-cadherin (placental)
CRYAB	Hs.408767	Crystallin, alpha B
CSK	Hs.77793	C-src tyrosine kinase
CXCL12	Hs.522891	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
CXCL14	Hs.483444	Chemokine (C-X-C motif) ligand 14
DHFR	Hs.83765	Dihydrofolate reductase
ECGF1	Hs.592212	Endothelial cell growth factor 1 (platelet-derived)
EGFR	Hs.488293	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
ERBB3	Hs.118681	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
ERBB4	Hs.390729	V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)
ESR2	Hs.660607	Estrogen receptor 2 (ER beta)
FANCF	Hs.651196	Fanconi anemia, complementation group F
FLT1	Hs.507621	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
FLT3	Hs.507590	Fms-related tyrosine kinase 3
FLT4	Hs.646917	Fms-related tyrosine kinase 4
FRAP1	Hs.338207	FK506 binding protein 12-rapamycin associated protein 1
GSDML	Hs.306777	Gasdermin-like
IGF1R	Hs.592020	Insulin-like growth factor 1 receptor
IGF2R	Hs.487062	Insulin-like growth factor 2 receptor
KDR	Hs.479756	Kinase insert domain receptor (a type III receptor tyrosine kinase)
KIT	Hs.479754	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
KRAS	Hs.505033	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
KRT17	Hs.2785	Keratin 17
KRT5	Hs.433845	Keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)
KRT8	Hs.533782	Keratin 8
MAP2K1	Hs.145442	Mitogen-activated protein kinase kinase 1
MAP2K2	Hs.465627	Mitogen-activated protein kinase kinase 2
NFKB1	Hs.431926	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
NFKB2	Hs.73090	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
PDGFRA	Hs.74615	Platelet-derived growth factor receptor, alpha polypeptide
PDGFRB	Hs.509067	Platelet-derived growth factor receptor, beta polypeptide
PIK3CA	Hs.85701	Phosphoinositide-3-kinase, catalytic, alpha polypeptide
PIK3R1	Hs.132225	Phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)
PITX2	Hs.643588	Paired-like homeodomain transcription factor 2
PRKCB1	Hs.460355	Protein kinase C, beta 1
PTHLH	Hs.591159	Parathyroid hormone-like hormone
RAD51C	Hs.412587	RAD51 homolog C (S. cerevisiae)
RAD51L1	Hs.172587	RAD51-like 1 (S. cerevisiae)
RAD51L3	Hs.631757	RAD51-like 3 (S. cerevisiae)
RAF1	Hs.159130	V-raf-1 murine leukemia viral oncogene homolog 1
TRIM29	Hs.504115	Tripartite motif-containing 29
TYMS	Hs.592338	Thymidylate synthetase
VEGFA	Hs.73793	Vascular endothelial growth factor A
VEGFB	Hs.78781	Vascular endothelial growth factor B
XRCC2	Hs.647093	X-ray repair complementing defective repair in Chinese hamster cells 2
XRCC3	Hs.592325	X-ray repair complementing defective repair in Chinese hamster cells 3

Conclusion I

- recurrent/metastatic disease is a heterogeneous disease
- cellular and molecular determinants of such heterogeneity are largely unknown
- the clinical oncologist (surgeon, radiotherapist, med oncol) needs to know either the rough marker for daily routine and the sophisticated pathway that modifies the naural history of a certain cancer

Aknowledgments

CTC team/SIMT

Mirella Marini
Camillo Almici
Rosanna Verardi
Andrea Bianchetti

Medical Oncology

Giovanni Marini
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Pathology Dept.

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