Gene signature and outcome of patients with melanoma

Alain Spatz, MD

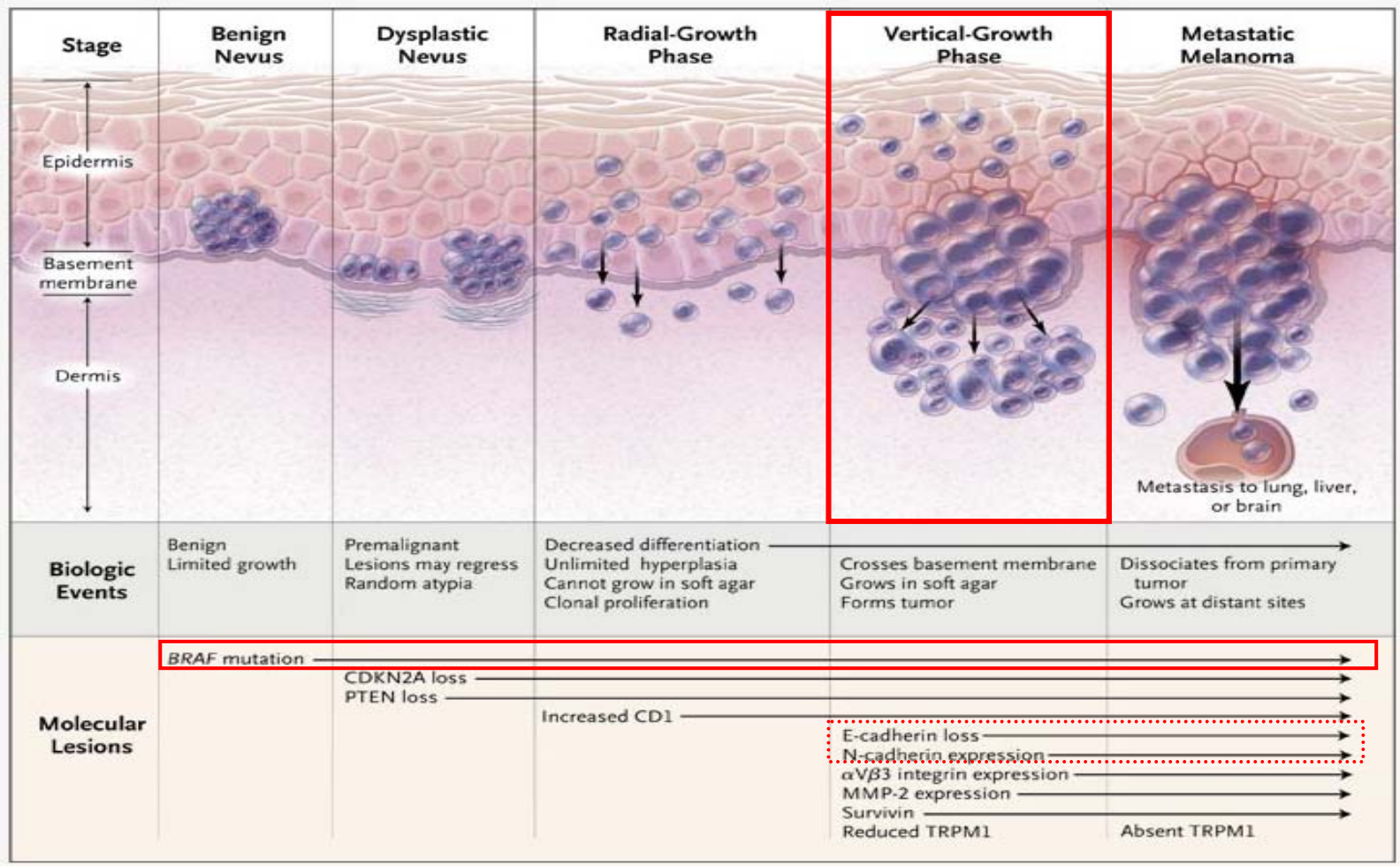
Gustave-Roussy Institute, Villejuif, France

EORTC Melanoma group Chair

Four questions

1. Does the multistep model apply to melanoma?
2. What have we learnt from genomics studies?
3. Is there a unifying concept behind molecular pathogenesis and immunomodulation of melanoma progression?
4. Where do we stand with genotypic/phenotypic correlations in melanoma?

Multistep model of melanoma progression

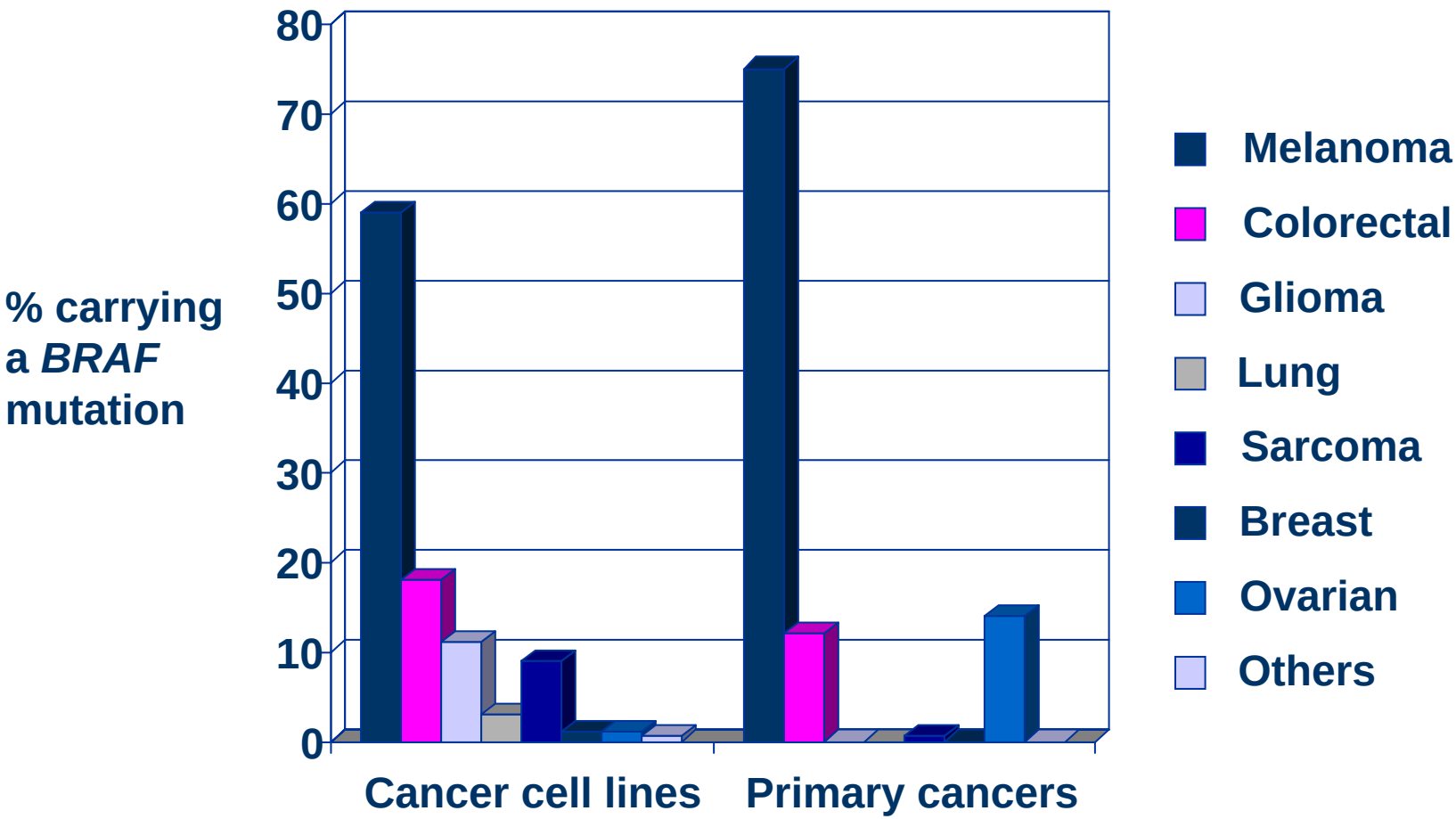


After Miller & Mihm, NEJM, 355, 2006

BRAF mutations in melanoma

- ***BRAF*** **(and *NRAS*) mutations arise early during melanoma pathogenesis.**
- **A single aa substitution (V600E) accounts for a vast majority of mutations: activ. BRAF kinase.**
- **Mutations are preserved throughout tumor progression.**
- **Oncogene if additional genetic lesions.**
- **Role for $BRAF^{E600}$ in senescence: even if *p53* is KO.**

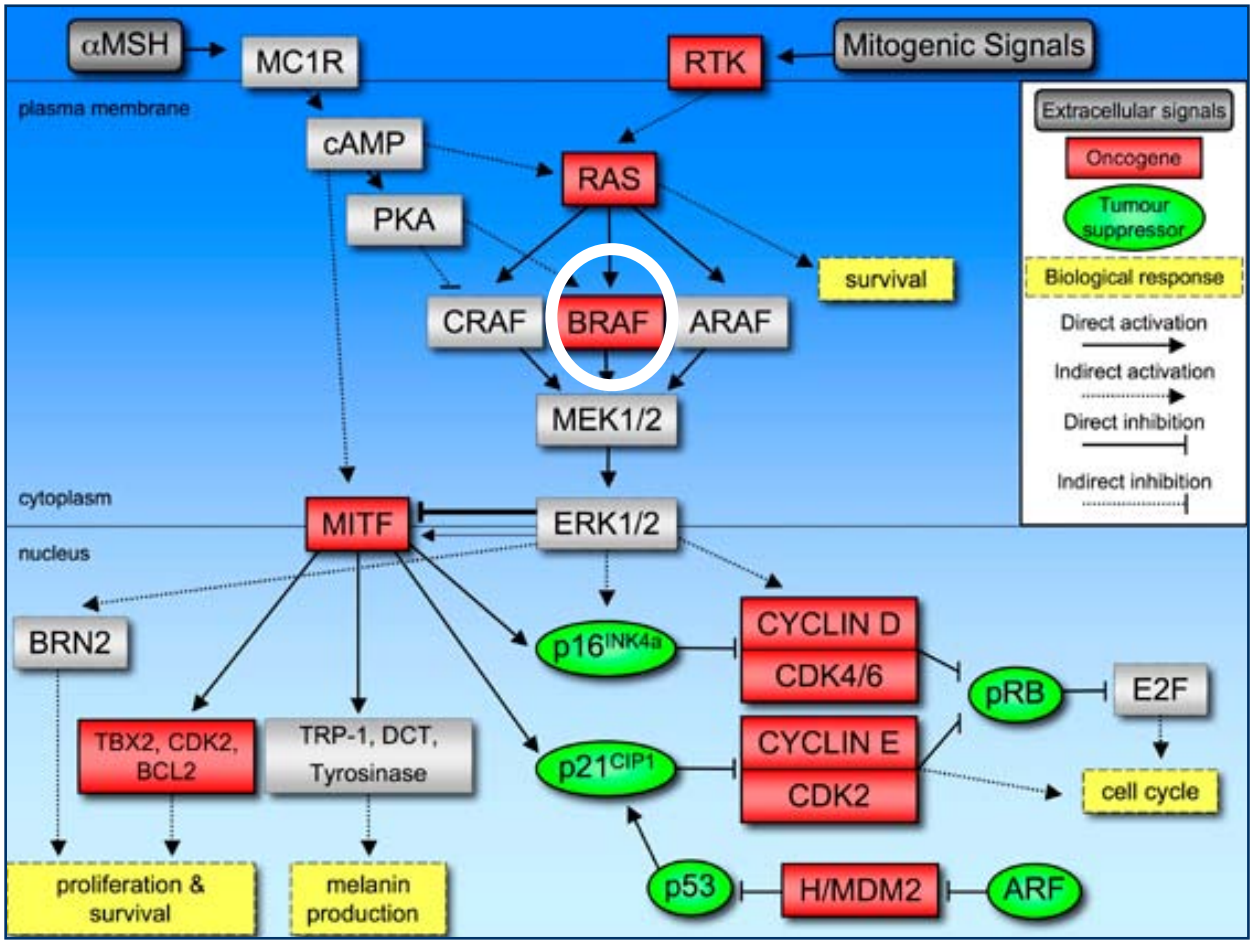
Cancers with *BRAF* mutations

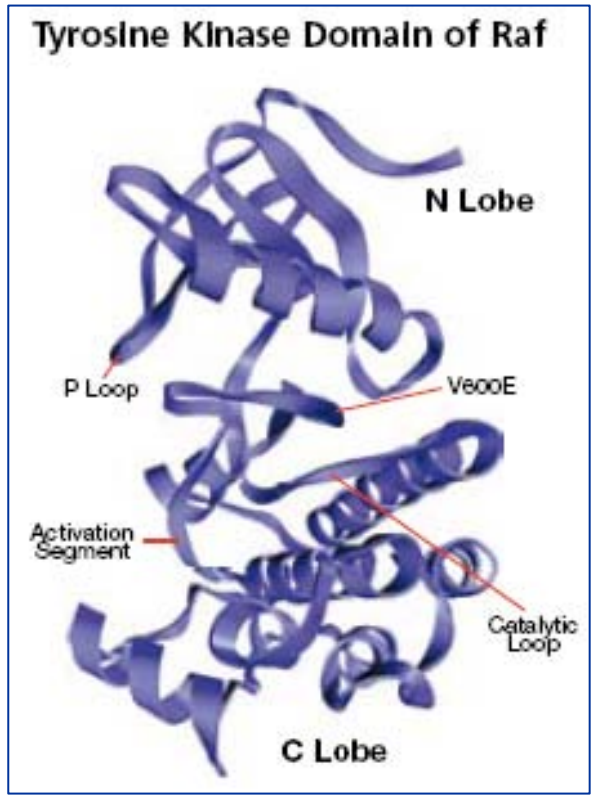


Alain Spatz

IGR

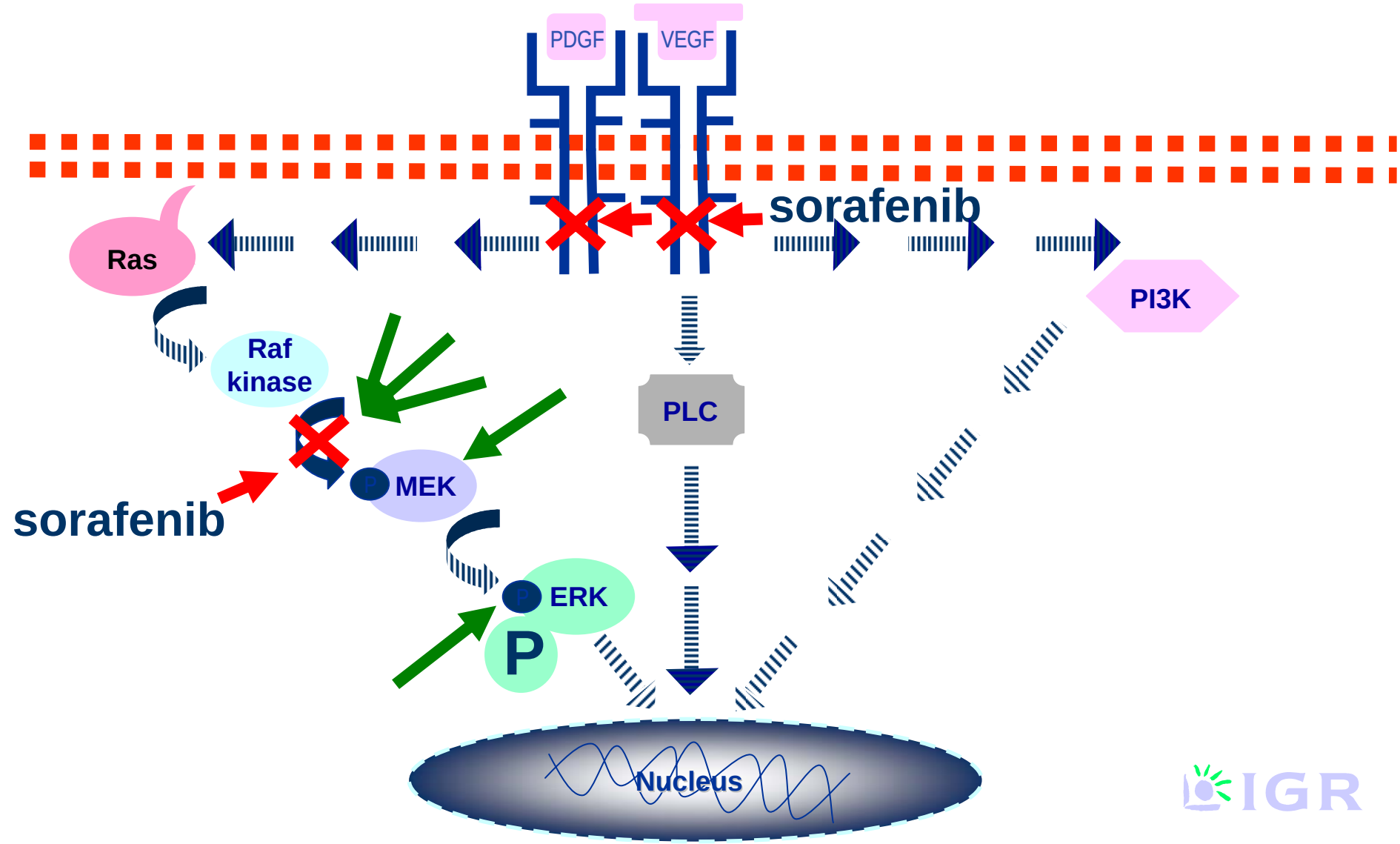
BRAF pathway





Michaloglou *et al.*, Oncogene, 2007

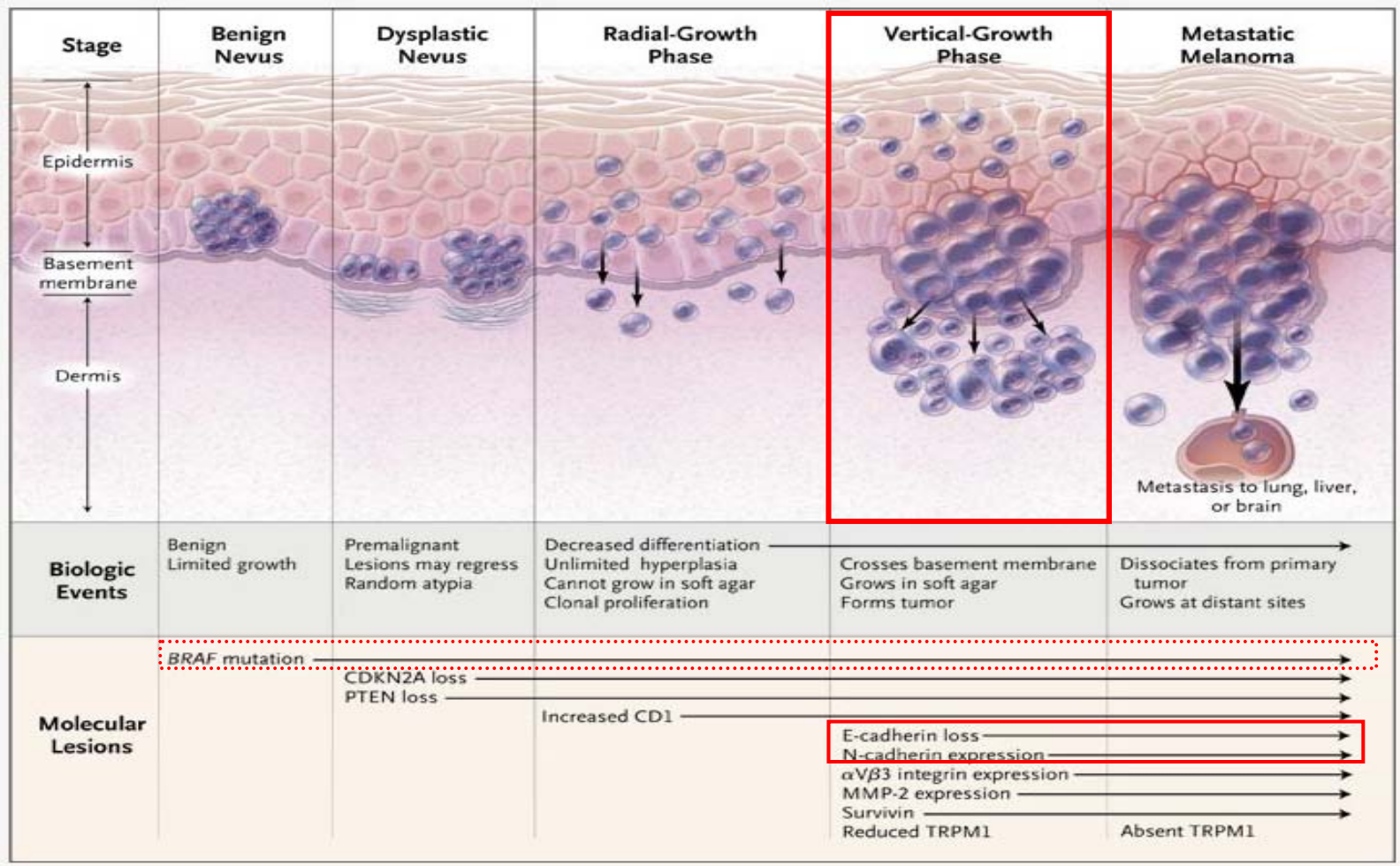
BRAF^{E600} as a therapeutical target



Is *BRAF*^{E600} a therapeutical target?

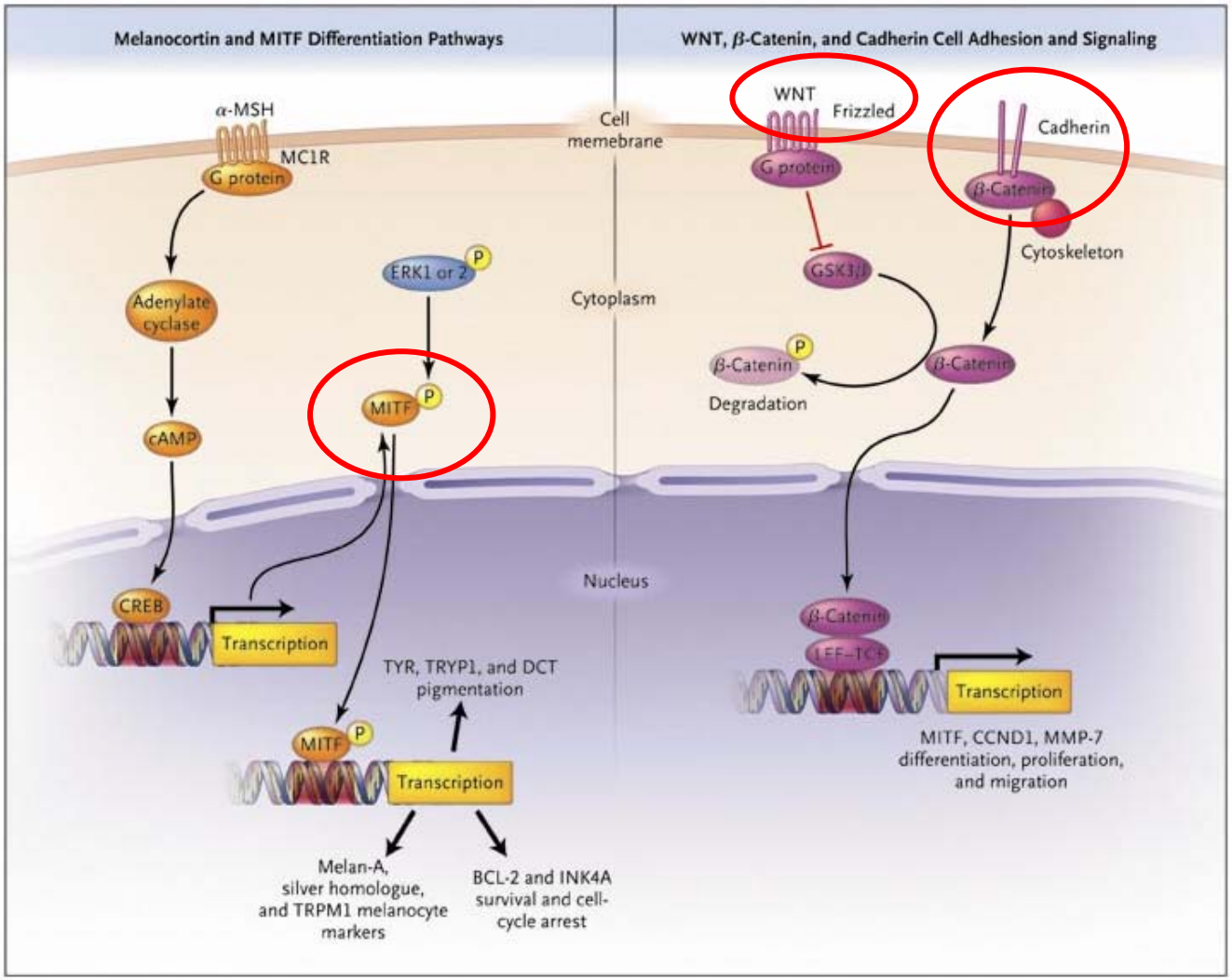
- Sorafenib is a multi-kinase inhibitor that targets ser/thr and RTKs in both the tumor cells & vasculature
- More specific anti-BRAF molecules in development.
- What is the good anti-BRAFs combination?
  - Problem of BRAF isoforms (alternative splicing): i.e., B2-RAF and B3-RAF have antagonist actions on MEK.
  - Property issues.

Multistep model of melanoma progression



After Miller & Mihm, NEJM, 355, 2006

Other pathways



After Miller & Mihm, NEJM, 355, 2006

Microphthalmia-associated transcription factor

- Master regulator of melanocyte development, function and survival.
- Activates the pigmentation machinery in the presence of P53.
- Amplified oncogene in a fraction of melanoma (5-20%).
- Role in melanoma progression *in vivo* ill-known; no clear relation with prognosis nor response.
- Regulatory interactions RAF-MEK-ERK / Beta-cat.-MITF

Alain Spatz

Objectives of high throughput technology in melanoma

- To study if there is a transcriptional signature associated with prognosis of cutaneous primary melanomas.
- To characterize key molecular events during tumor progression (transition nevus-primary melanoma & primary melanoma-metastasis).
- To identify new therapeutical targets.

Supervized analysis: distant metastasis free survival at 4yrs

- 254 out of the 11,043 filtered genes have a differential expression ($p=10^{-4}$) between patients with DMFS< or >4yrs.
- Signature with 60 top genes highly discriminant.
- Expression clusters associated with phenotypic variables.

Winnepenninckx *et al.*, J Natl Cancer Inst 2006

IGR

Replication-controling pathways

- **Replication licensing and origins firing are key in melanoma progression**
- **hPTTG/securin overexpression is associated with VGP/RGP:**
  - **blocks sister chromatid separation: aneuploidy**
  - **induction of angiogenesis (through βFGF and VEGF)**
  - **downregulation of p53 transcription**

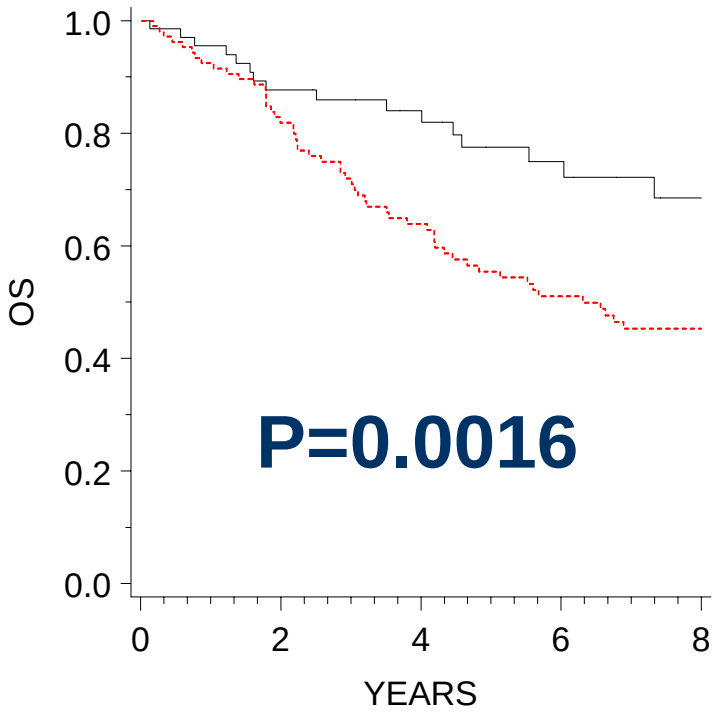
Winnepenninckx *et al.*, J Natl Cancer Inst, 2006
Winnepenninckx *et al.*, Mod Pathol, 2006

IGR

Replication origins firing pathway

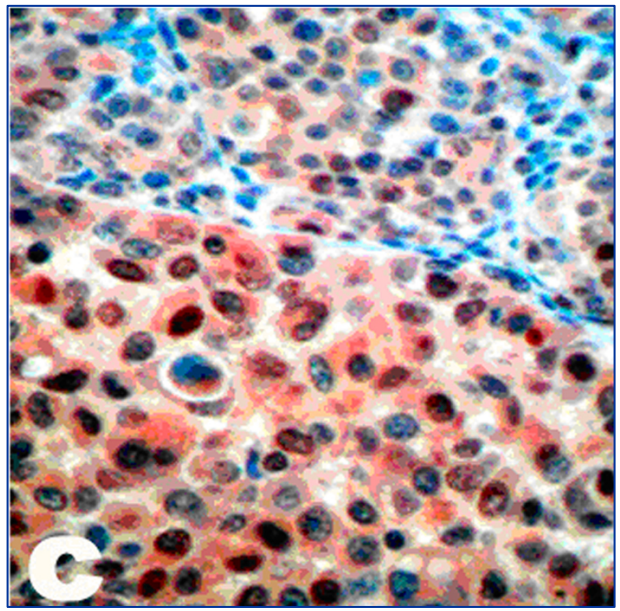
- **Global overexpression of the ROF-related genes: 4/9 *Mini-Chromosome Maintenance* genes (2, 3, 4, 6) and *geminin***
- ***MCM-4* and *-6* overexpression are prognostic for DMFS and OS independently from age, sex, ulceration, thickness (Cox model)**

Dysregulation of the replication origins firing pathway: MCM-4

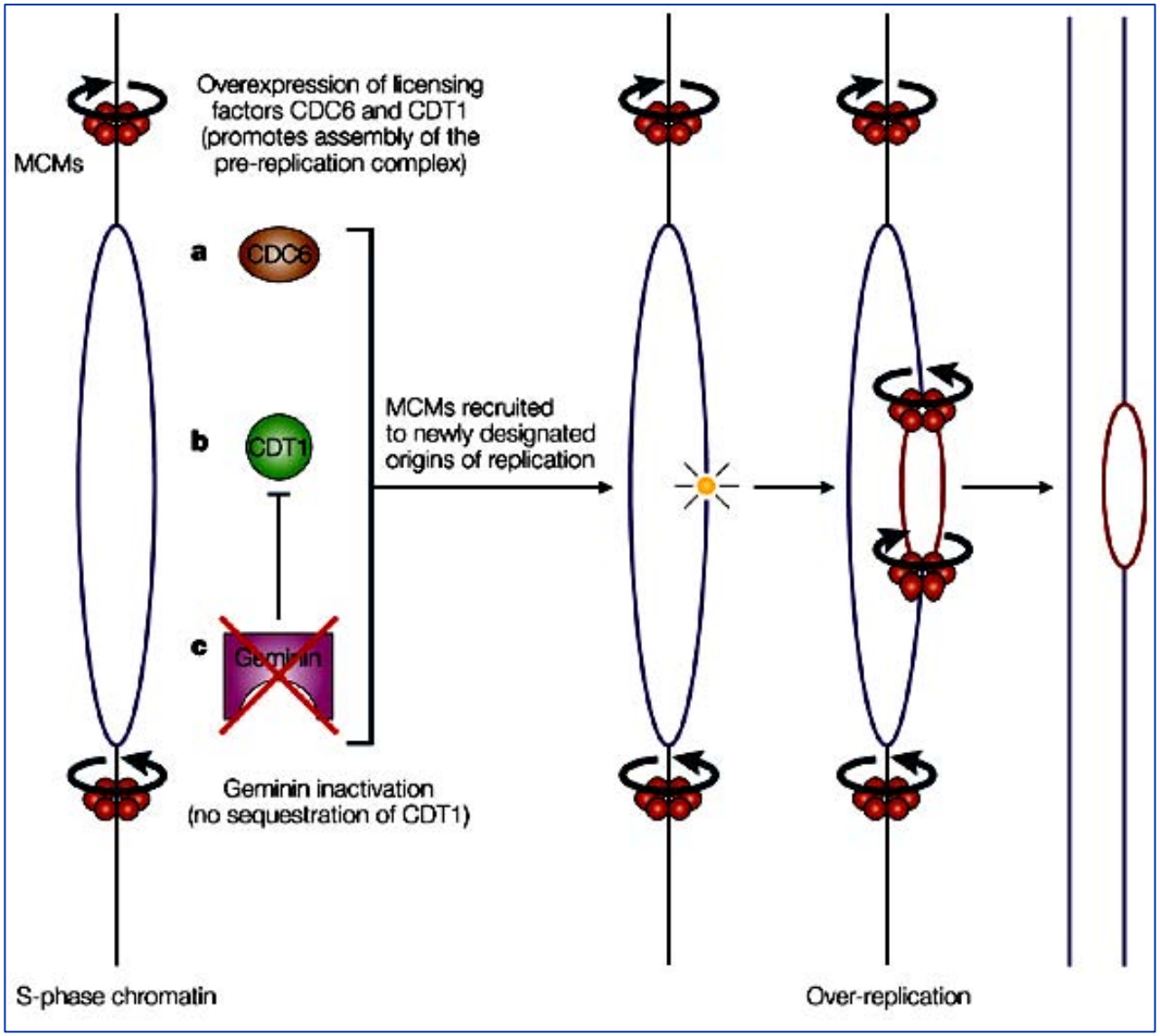


Patients at risk

<=average	68	56	41	27	18
>average	108	84	61	45	35

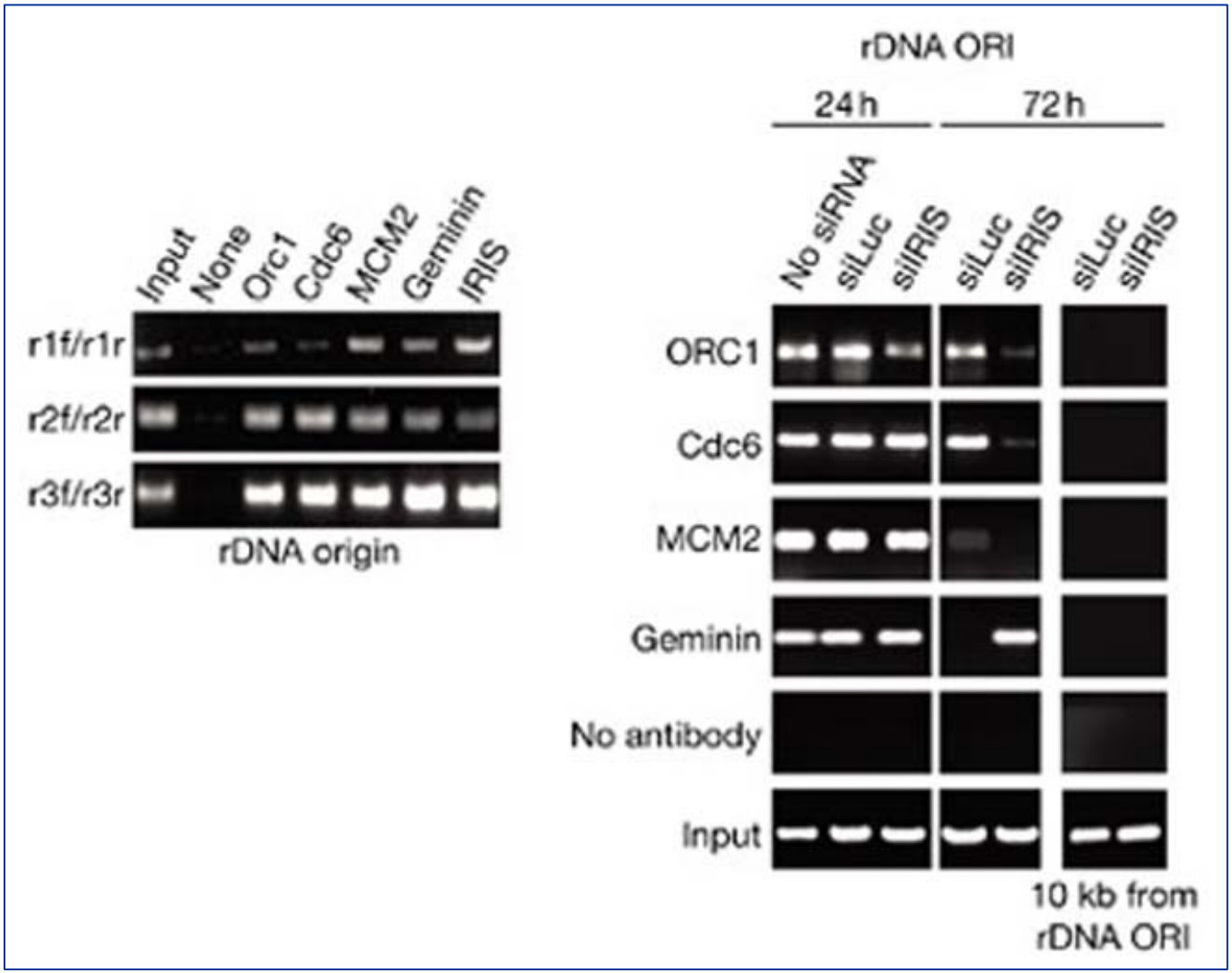


Replication origins firing pathway



After Gonzalez *et al.*, Nat Rev Cancer, 2005

BRCA1-IRIS controls GEM-CDC6 interaction and MCM2

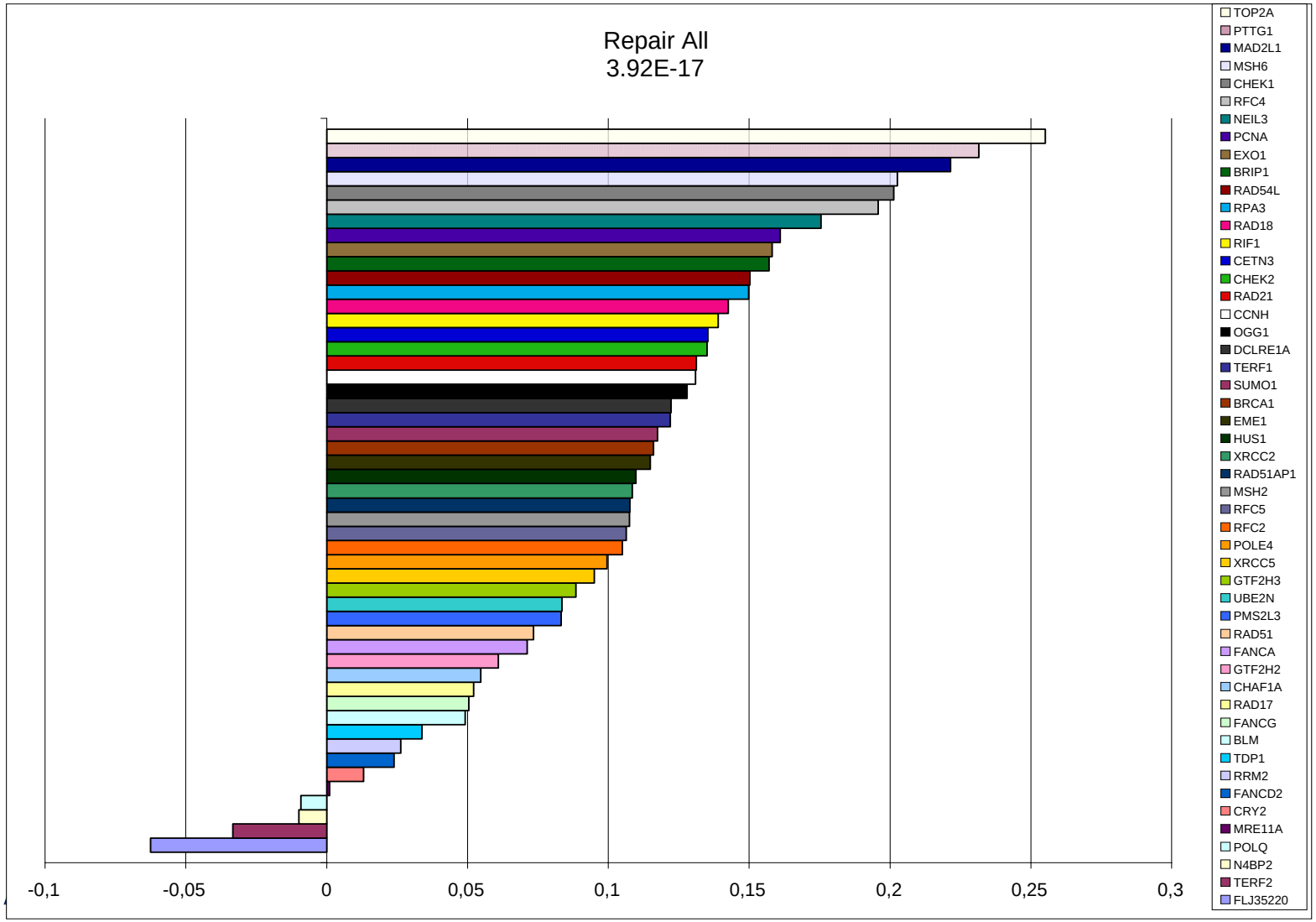


After ElShamy & Livingstone, Nat Cell Biol, 2004

Differential expressions for the DNA repair pathway

43

4



DNA-repair pathway

- **SBIME analysis of 231 genes involved in DNA repair: expression for 47 genes differs between groups**
- **Almost all genes have higher expression in the group with poor prognosis**
- ***TOP2A*** **is the most overexpressed gene in tumors with short survival: relieves DNA torsional stress, resistance to alkylating agents.**

Kauffmann *et al.*, Oncogene, In Press

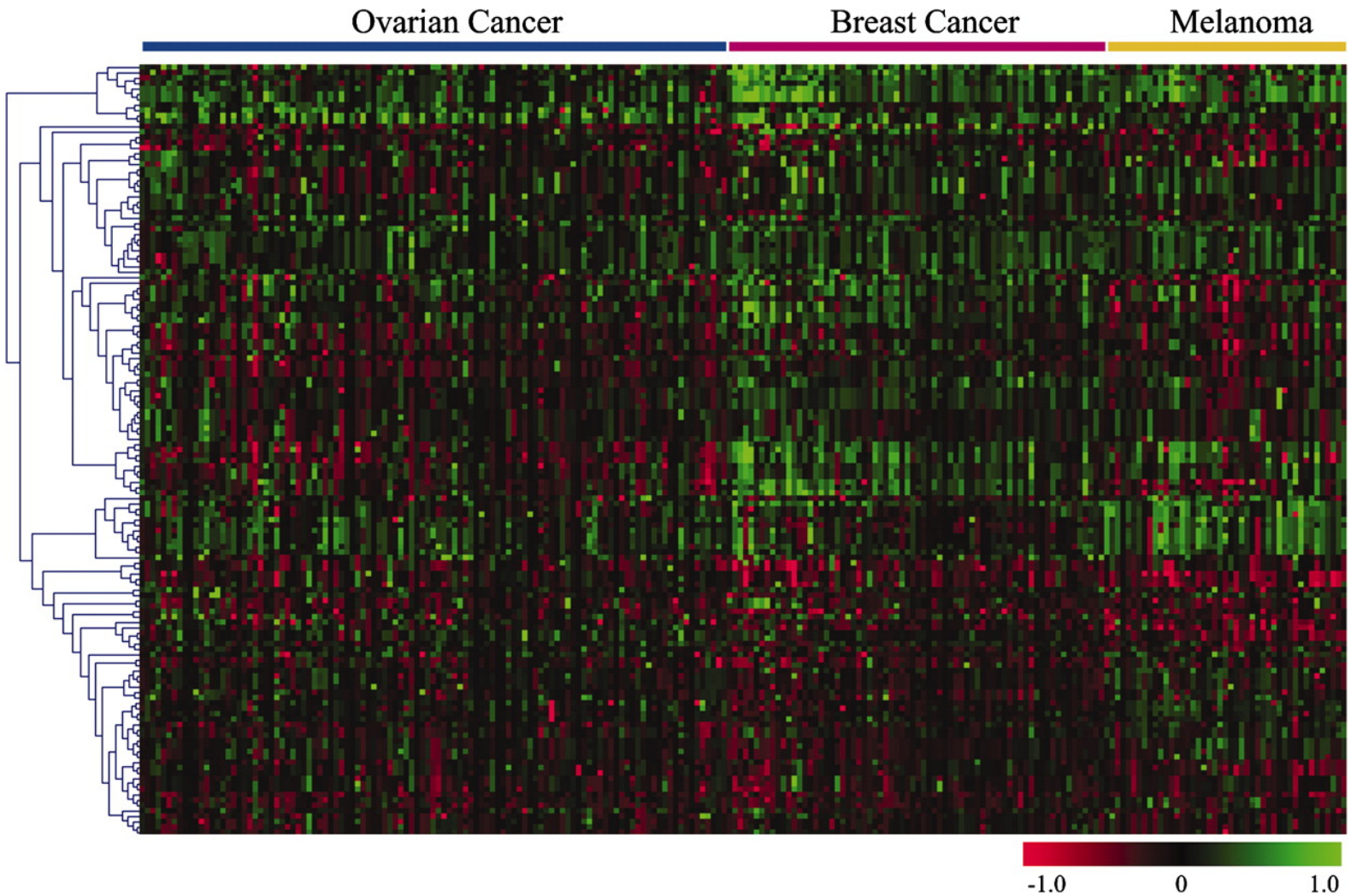
Telomeric repeat binding factor 2 gene

- **TRF2 expression strongly decreases with tumor Breslow and metastatic potential**
- **Overexpression of *TRF2* gene leads to hypersensitivity to UV or crosslinking agents and chromosome instability (Bradshaw *et al.*, Nat Genet, 2005:37)**
- **What is the mechanisme for TRF2 gene underexpression?**

Epigenetic changes in melanoma

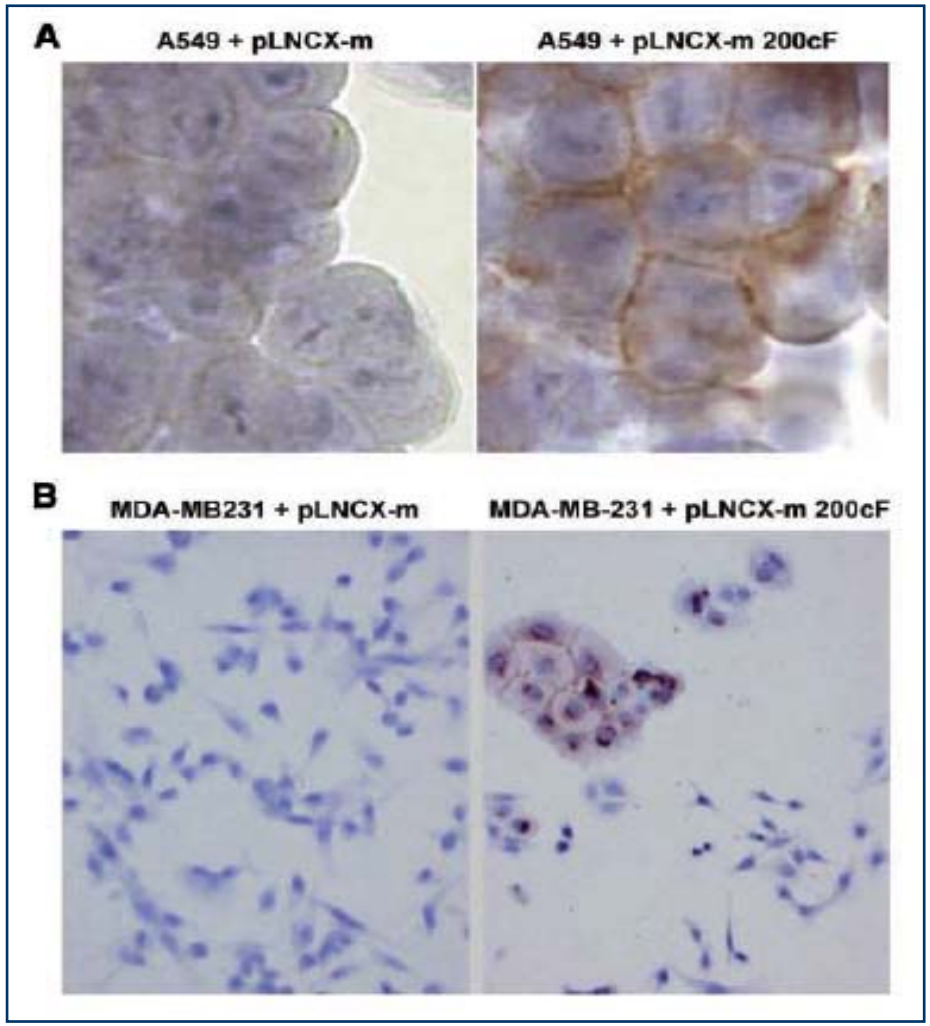
- Micro-RNAs extracted from the same specimens than RNAs and DNAs
- Three miRNAs are associated with thickness, and differentiate mets. from corresponding primary tumor (underexpressed during evolution):
  - Hsa-miR-200c (TF8, E-Cadherin): fdr *p*=0.02
  - Hsa-miR-203 (ovarian cancers, expression regulated by DNA methylation: Lorio *et al*., Cancer Res 2007): fdr *p*=0.002 for thickness, 0.005 for DMFS
  - hsa-miR-200a: fdr *p*=0.05
- Some of them are located in hot spots for aCGH modifications
- Are miRNAs related gene-silencing involved in melanoma pathogenesis:
  - genetic effects
  - but also immunomodulation (Cancer-Testis Antigens, i.e., MAGE)

Genetic aberrations of miRNAs in human cancer



Zhang et al., PNAS, 2006

Overexpression of has-miR-200c decreases TF8 and increases E-Cadherin expression



- miR-200c seems to be strongly involved in EMT
- Loss of 200c is likely to play a significant role in invasive phenotype
- Is miR-200c downregulation linked with DNA changes?
- How to restablish miR-200c expression?

Hurteau *et al.*, Cancer Res, 67, 2007

B-Raf signature

- **Genes expression associated with BRAF status (FDR<0.01, *P*=0.95): *CD63 (FDR P=0.0001!)*, *HSP70*, *MAGE-D2, Melanoma Inhibitory Activity, SERPINE2.***
- **CD63: tetraspanins family, colocalizes with integrins**
- **HSP70B': BRAF-dependent response to *HSP70* inducers?**
- **MAGE-D2: interacts physically with $p53^{wt}$ (p53-dissector)**
- **Enhanced motility and vesiculization: modulation of adaptative UV response?**

Alain Spatz

Impact of molecular pathology on the taxonomy of melanocytic tumors.

The classification of malignant melanoma and its histologic reporting.

Vincent J. McGovern, Martin C. Mihm Jr.,

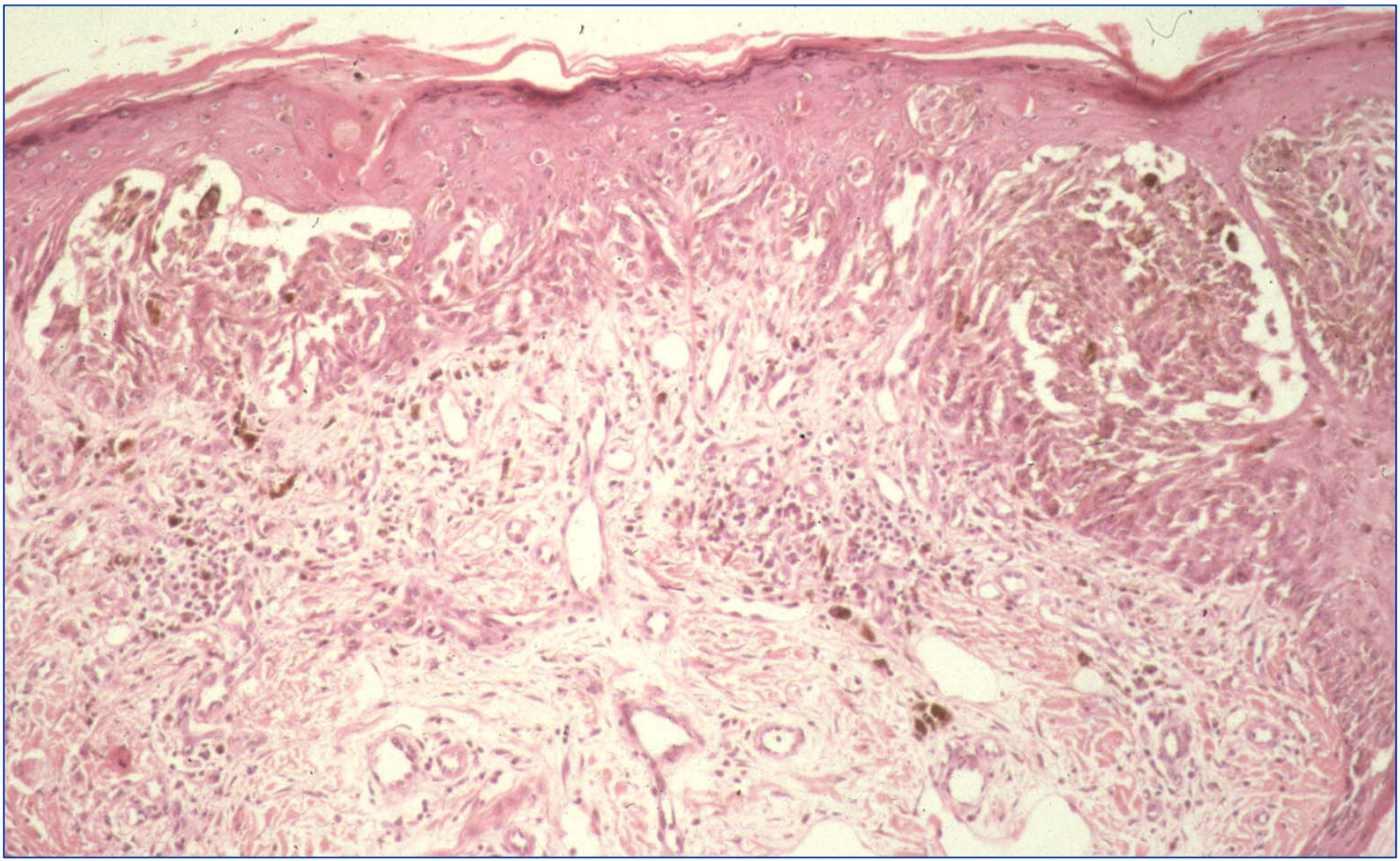
Christiane Bailly, *et al.*

Cancer 1973, *32*:1446

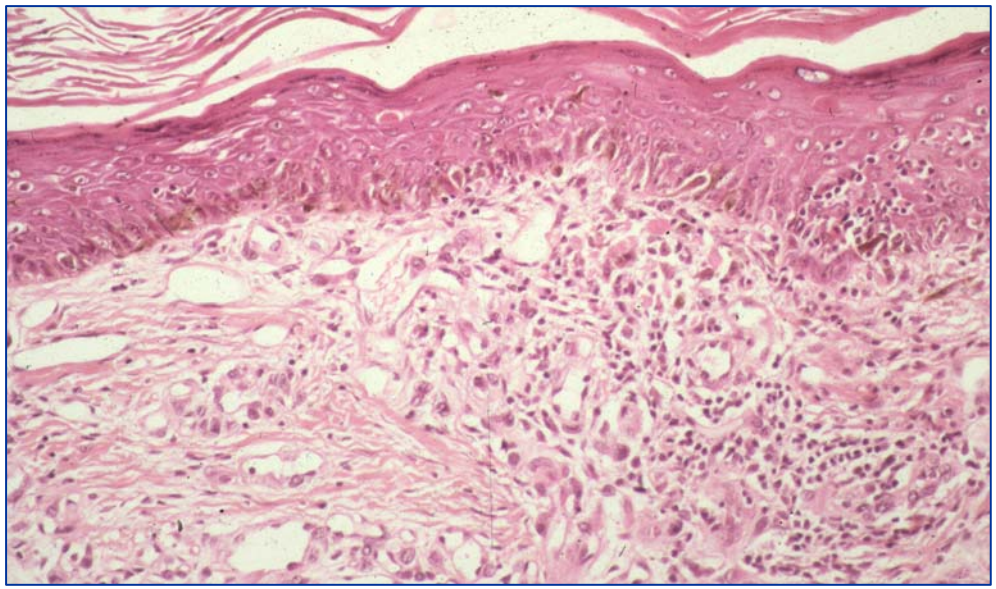
Melanomas in sun-exposed skin

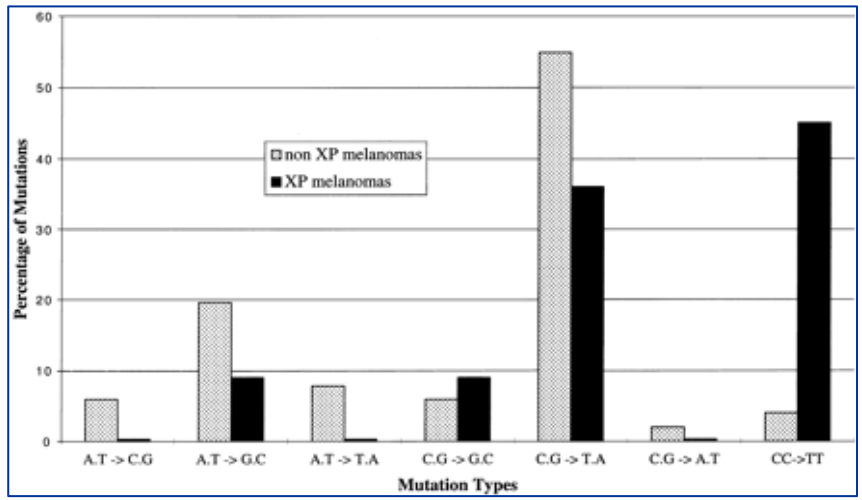
- Separation based on the lateral epidermal component is often difficult: some LMMs have prominent nesting, or epidermal spreading at later stage.
- No prognostic difference between sub-types in sun-exposed skin.

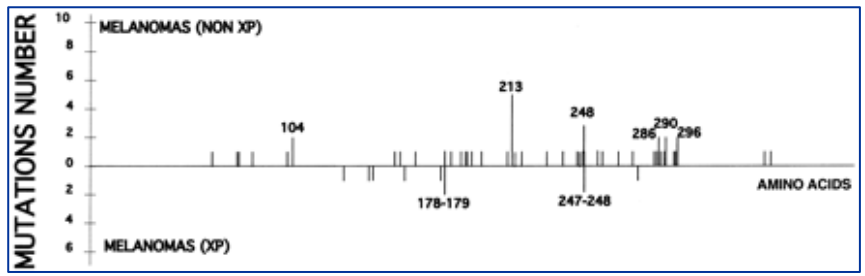
Melanomas in sun-exposed skin: morphological overlapping



Melanomas in sun-exposed skin : *p53* pathway (*xeroderma pigmentosum* model)

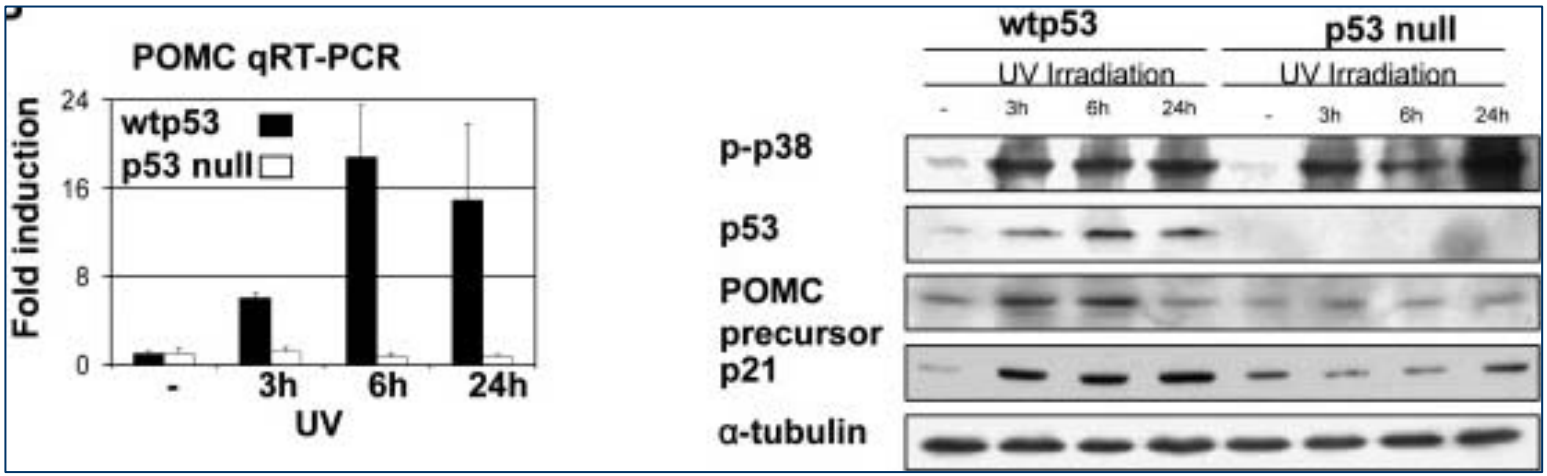


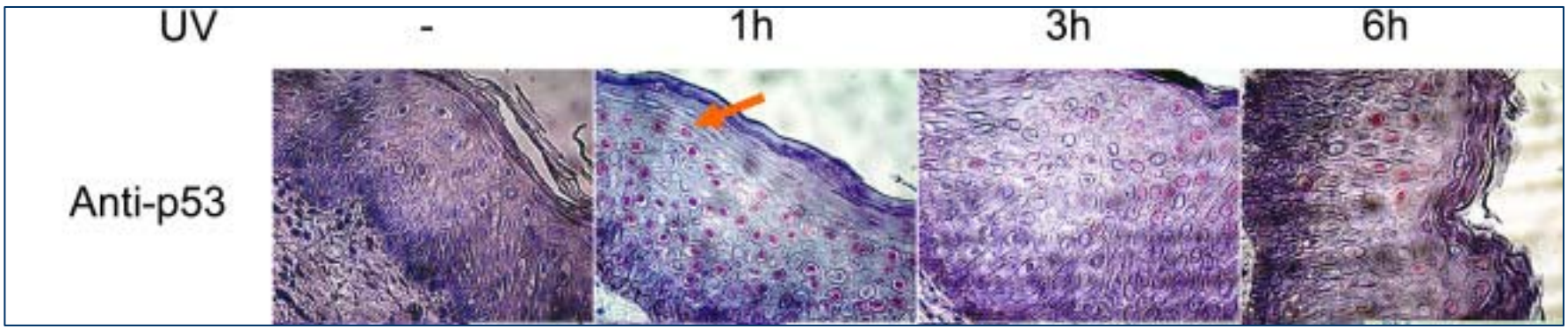




Alain Spatz

Role of *p53* in the suntan response





Alain Spatz

Cui *et al.*, Cell, 2007

Melanomas in sun-exposed skin

- **Solar elastolysis is strongly and independently associated with better prognosis**
- **Melanomas in sun-exposed skin have *p53* gene mutations (UV-induced).**
- **Mutations in *KIT* (26%) and MAP kinase-related genes**

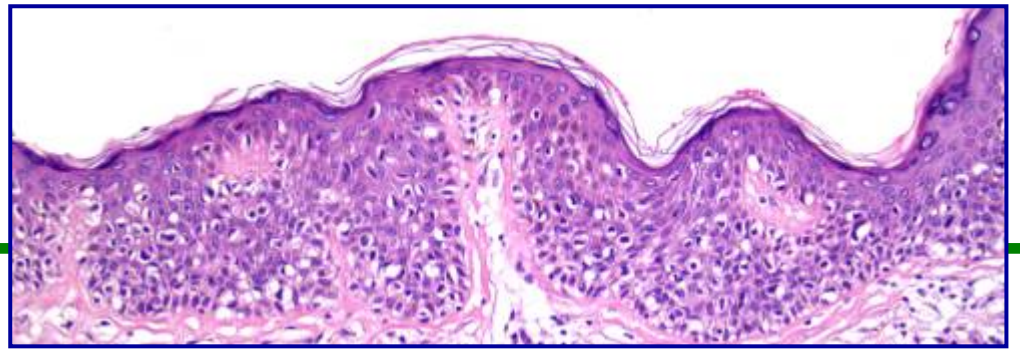
No justification to maintain LMMs in a separate category!

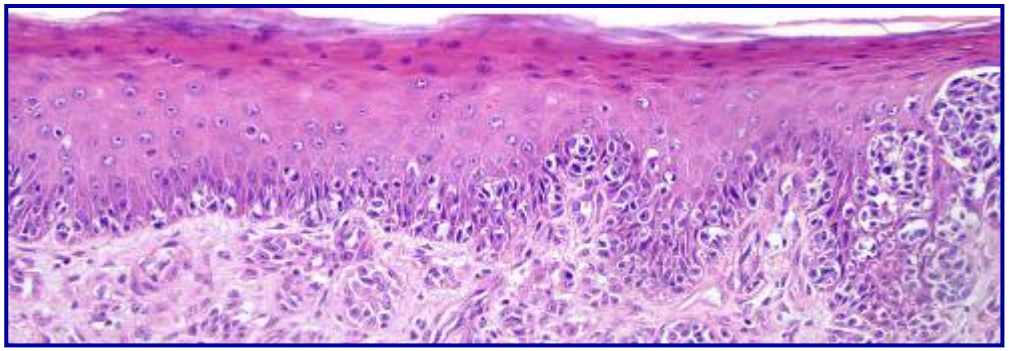
Spatz *et al.* Cancer Res, 2003
Berwick *et al*., JNCI, 2005
Cutin *et al.,* NEJM 2005 & J Clin Oncol 2006

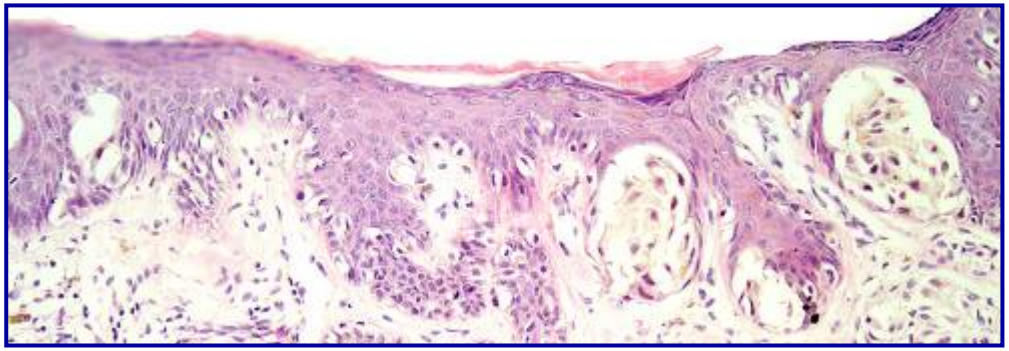
At least three diseases

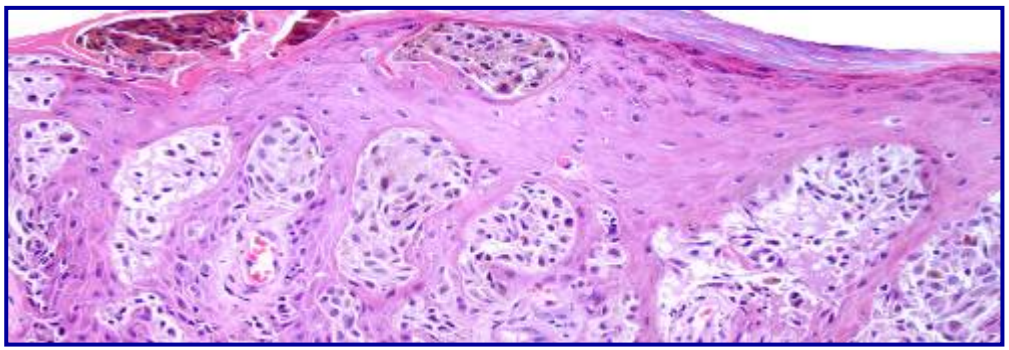
- **Solar melanomas:** better prognosis, UV-induced *p53* mutations, *KIT* mutations, XP melanomas as a model (involvement of XP genes?)
- **Acral melanomas (glabrous skin):** gains in chromosomes 5p and 11q (oncogenes?), less frequency of *BRAF* mutations
- **Other melanomas:** Growth phase pattern overcomes histogenetic classification. Dysregulation of replication origins firing.

Alain Spatz

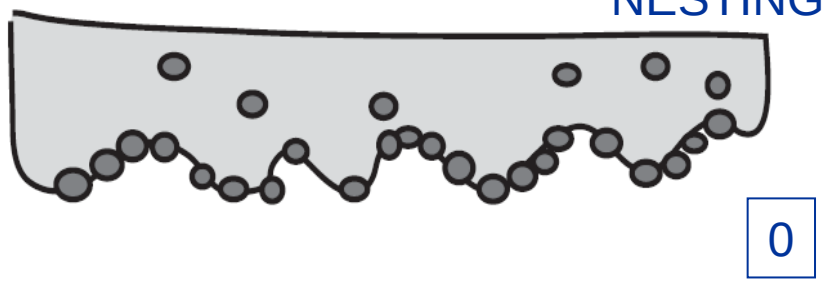


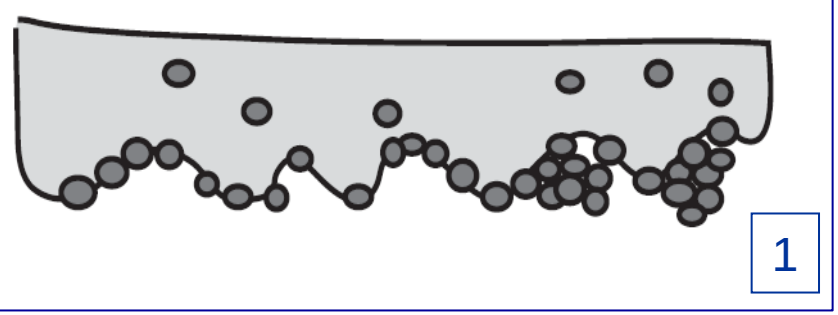


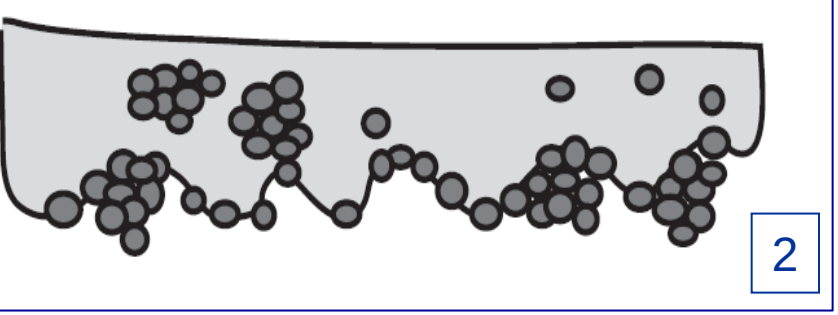


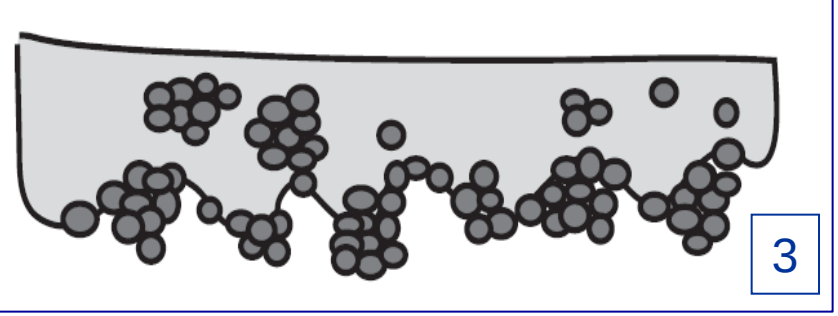


NESTING

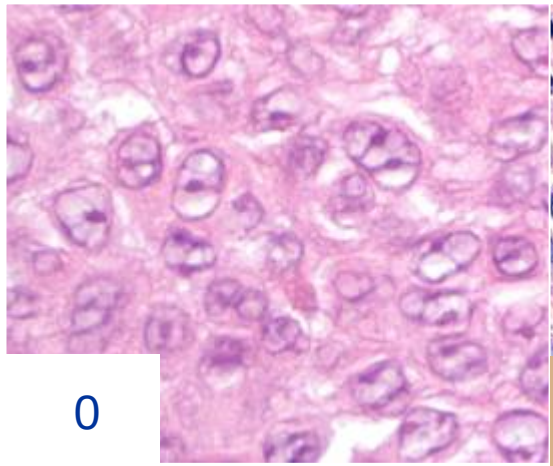




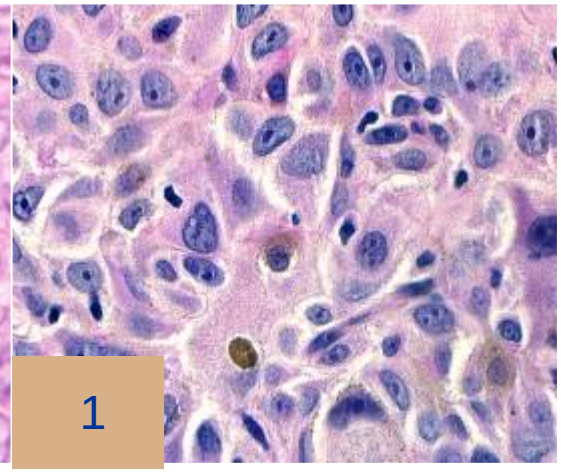




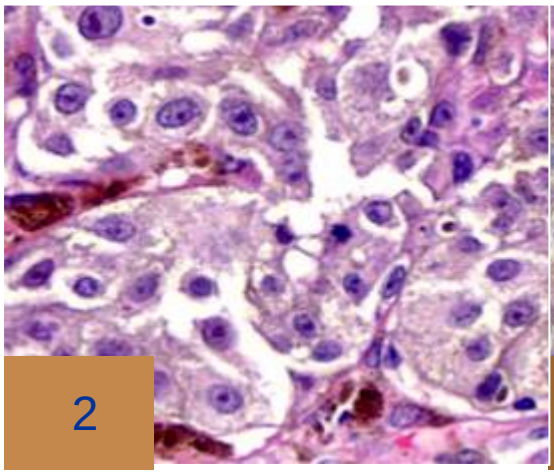
PIGMENTATION



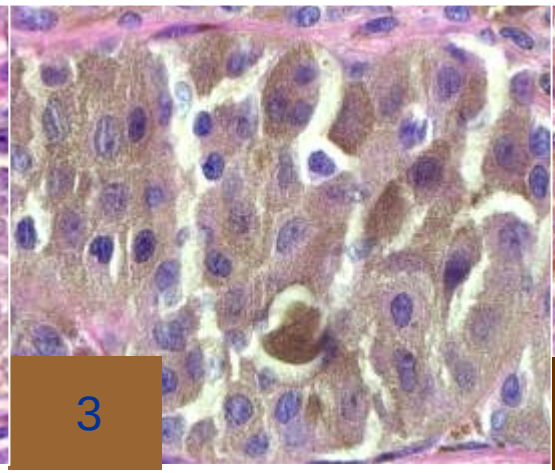
0



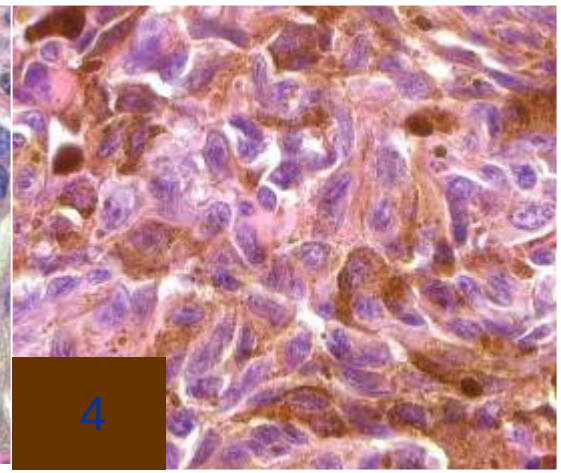
1



2



3



4

Conclusion

- A primary melanoma that is going to metastasize is a machinery to replicate DNA: maximum efficiency and minimal mistakes are necessary to overcome dysfunctional replication or telomeres maintenance.
- The increased expression of repair proteins may partly explain melanoma resistance toward radiotherapy and chemotherapy.

Conclusion (cont'd)

- Mitotic activity is the phenotypic reflect of dysregulation in the replication regulation: ROF, chromatid separation
- Stabilization of Geminin/CDT1/CDC6 is key to regulation replication
- Impact of *BRAF* mutations on ser kin –related genes (CD63?)
- Role of hsa-miR-200c downregulation during melanoma progression
- Melanoma is a heterogeneous group of diseases: phenotypic variables to discriminate between categories

Alain Spatz

COLLABORATORS

Joost van den Oord

Dirk Schadendorf

Ghanem Ghanem

Alexander Eggermont

Veronique Winnepenninckx

Vladimir Lazar

Alain Sarasin

Anne Dumay

Veronique Laville

Catherine Richon

EORTC Melanoma group

Marguerite Stas (Leuven)

Soledad Alonso (CNIO)

Paolo Ortiz (Madrid)

Caroline Robert (IGR)

Maria Debiec-Rychter

Stefan Michiels

Philippe Dessen

Sebastien Job