Genomic progression of melanoma

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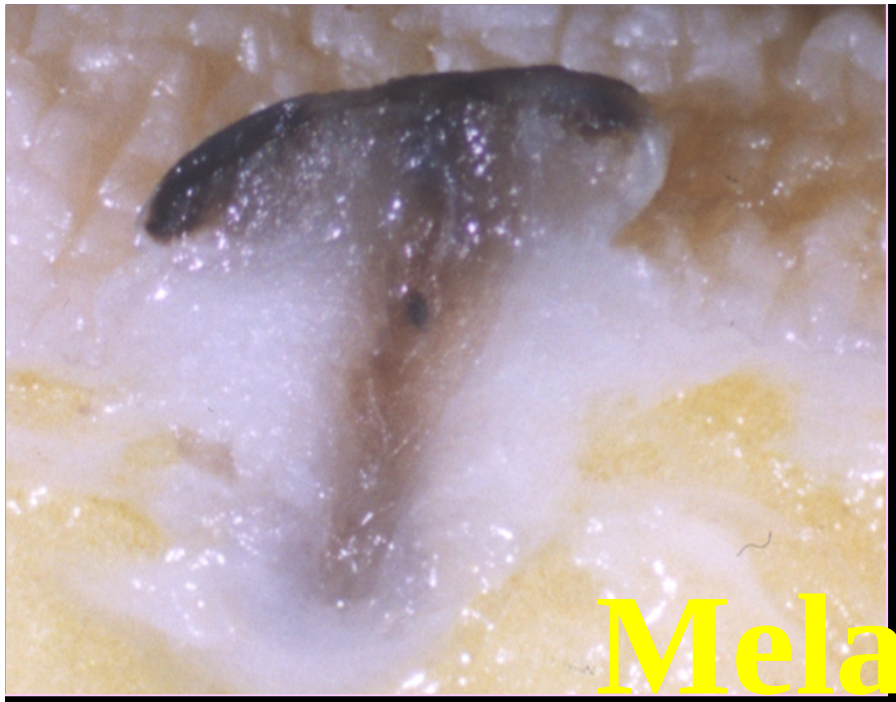
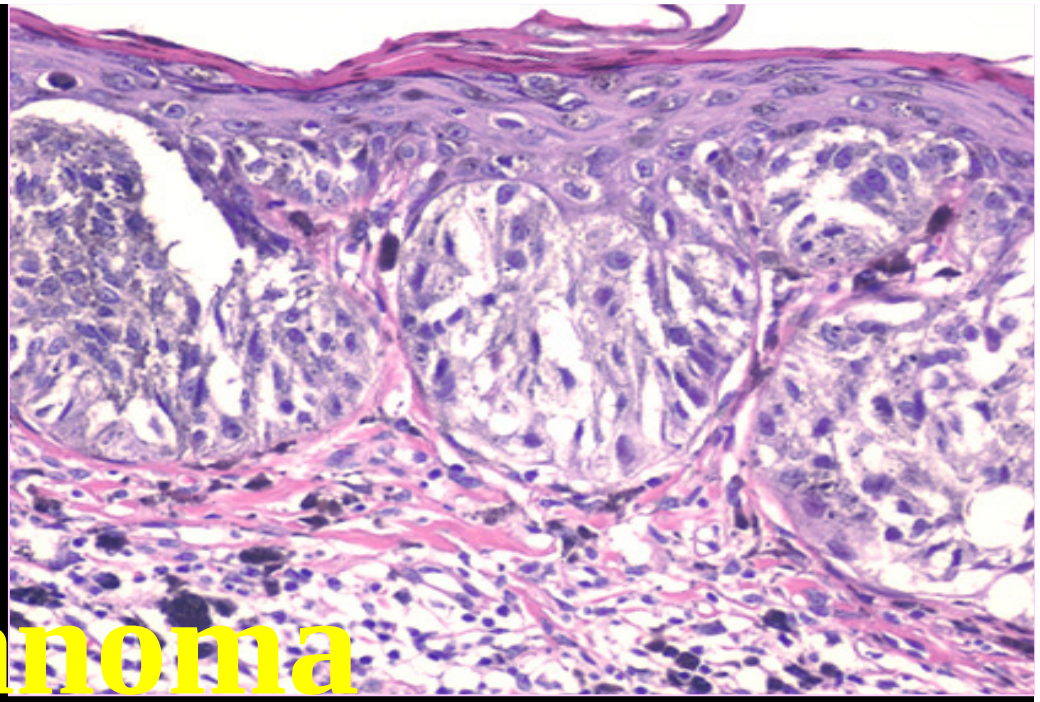
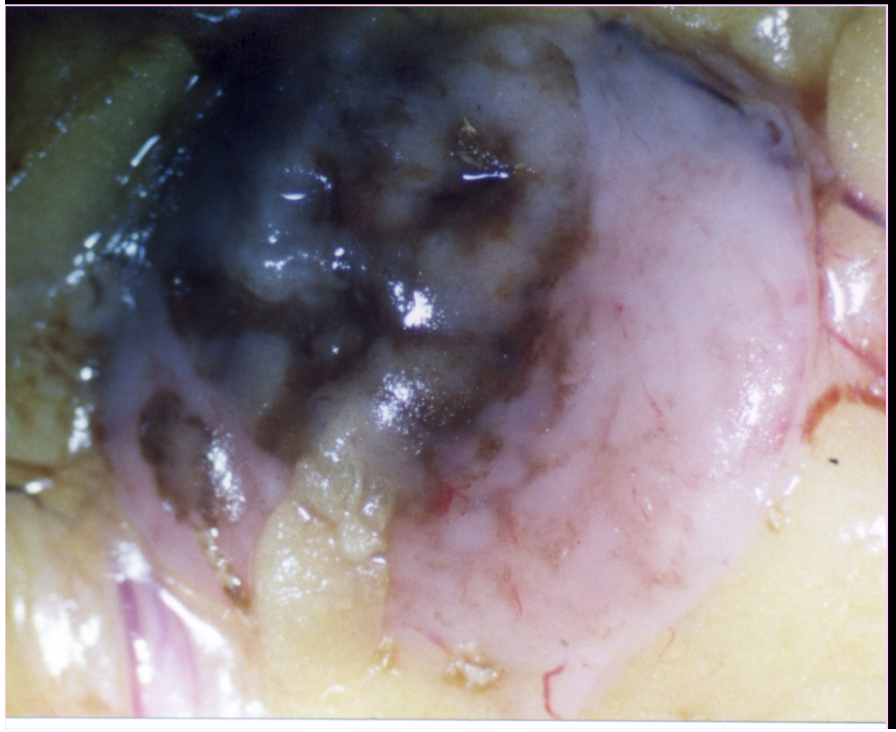
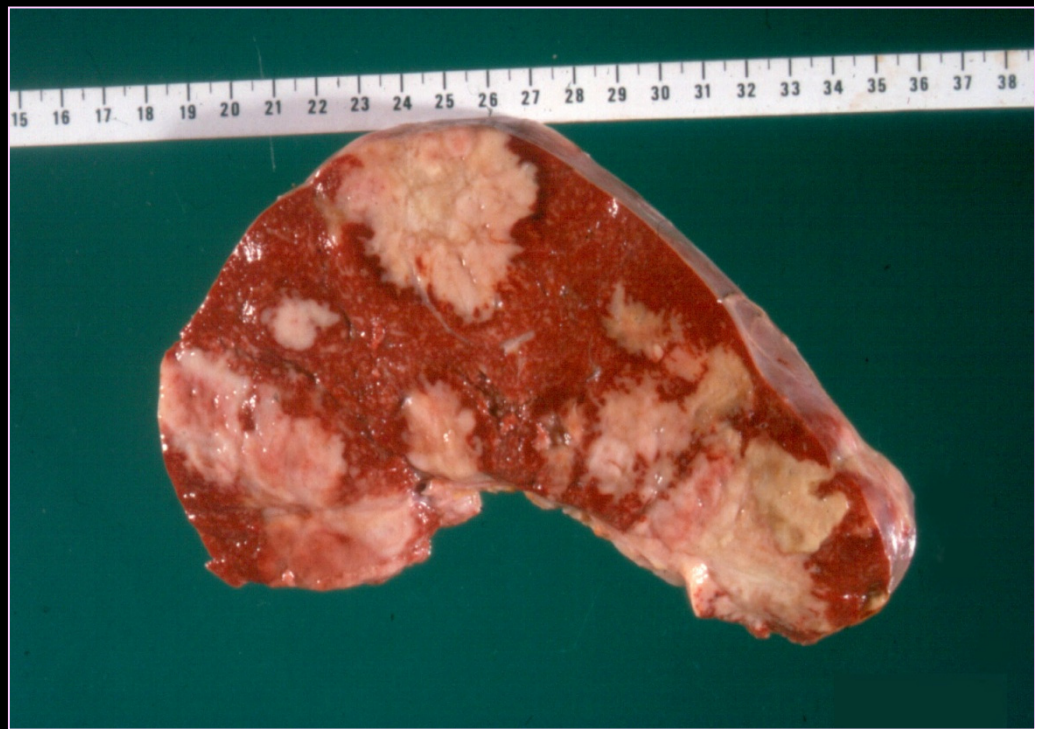
(2012-2017)





Melanoma





Tumor mutational burden (TMB)

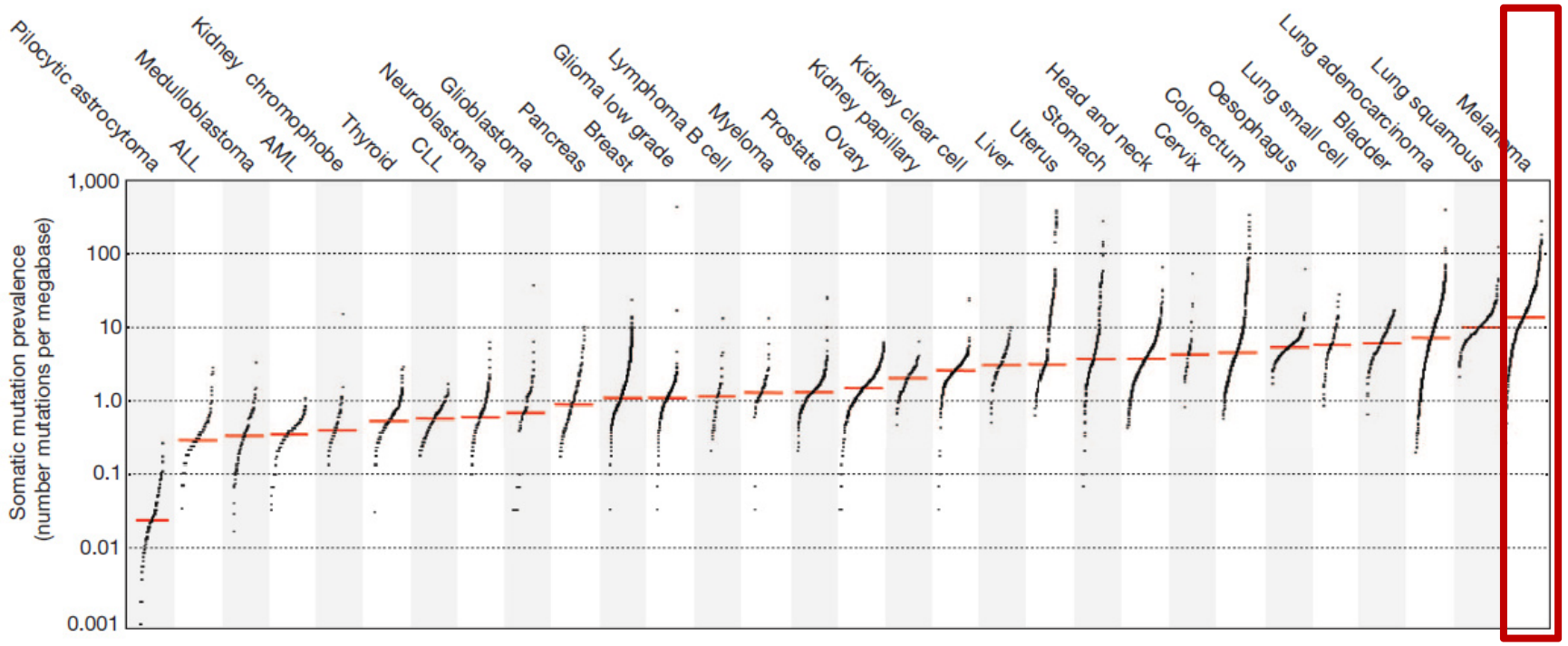
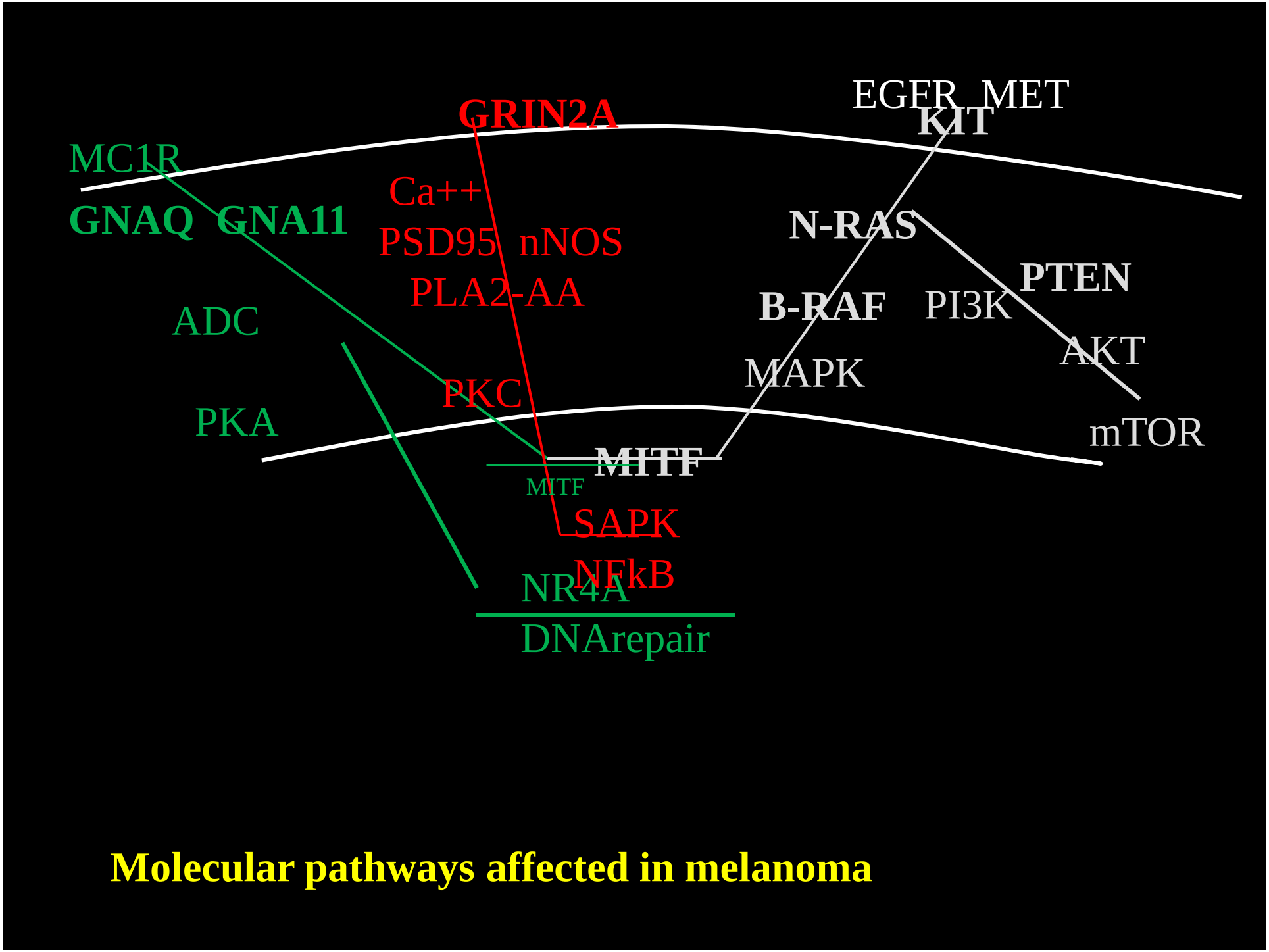


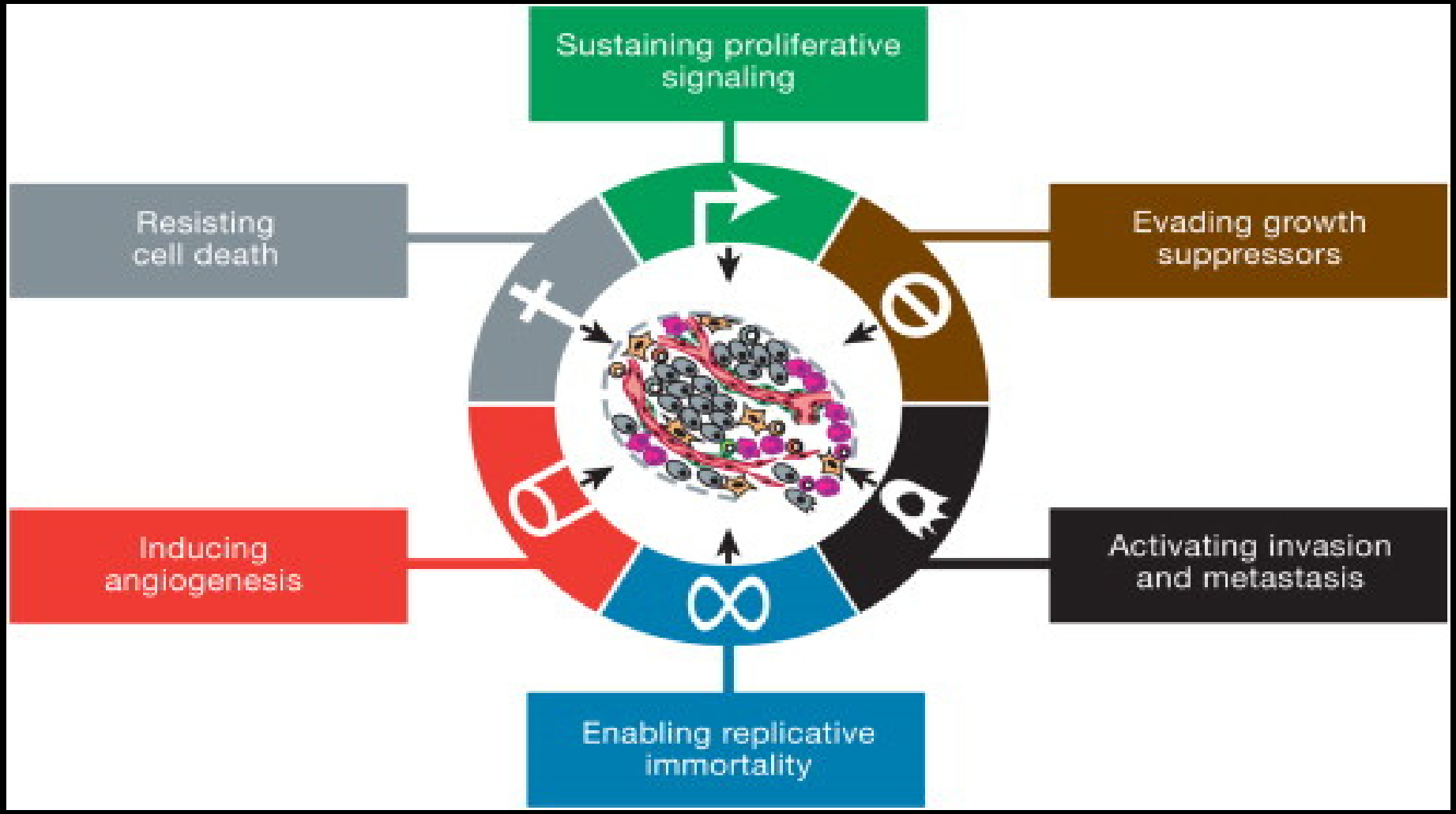
Figure 1 | The prevalence of somatic mutations across human cancer types. Every dot represents a sample whereas the red horizontal lines are the median numbers of mutations in the respective cancer types. The vertical axis (log scaled) shows the number of mutations per megabase whereas the different cancer types are ordered on the horizontal axis based on their median numbers of somatic mutations. We thank G. Getz and colleagues for the design of this figure[26]. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia.



Molecular pathways affected in melanoma

Genomic landscape of primary melanoma (skin)

mutations	amp	LOH	rearrangements
BRAF	MITF	PTEN	FHIT
NRAS (HRAS, KRAS)	BRAF		PTEN
KIT	NRAS		ETV1
NF1	KIT		MACROD1
PREX2	TERT		CSMD1
CDKN2A	CCND1		MAGI2
CDK4	CDK4		A2BP1
TP53	MET		PREX2
PTEN			TERT
selected rare events (<10%)			
ALK			ALK
ARID2			
STK19			
IDH1			
RAC1			
MAPK2K1			
RB1			
GRIN2A/NMDAR2			
EGFR4			
VEGFC			
NOTCH2NL			
ADAMTS18			
WT1			
CTNNB1			
RB1			
PPP6			

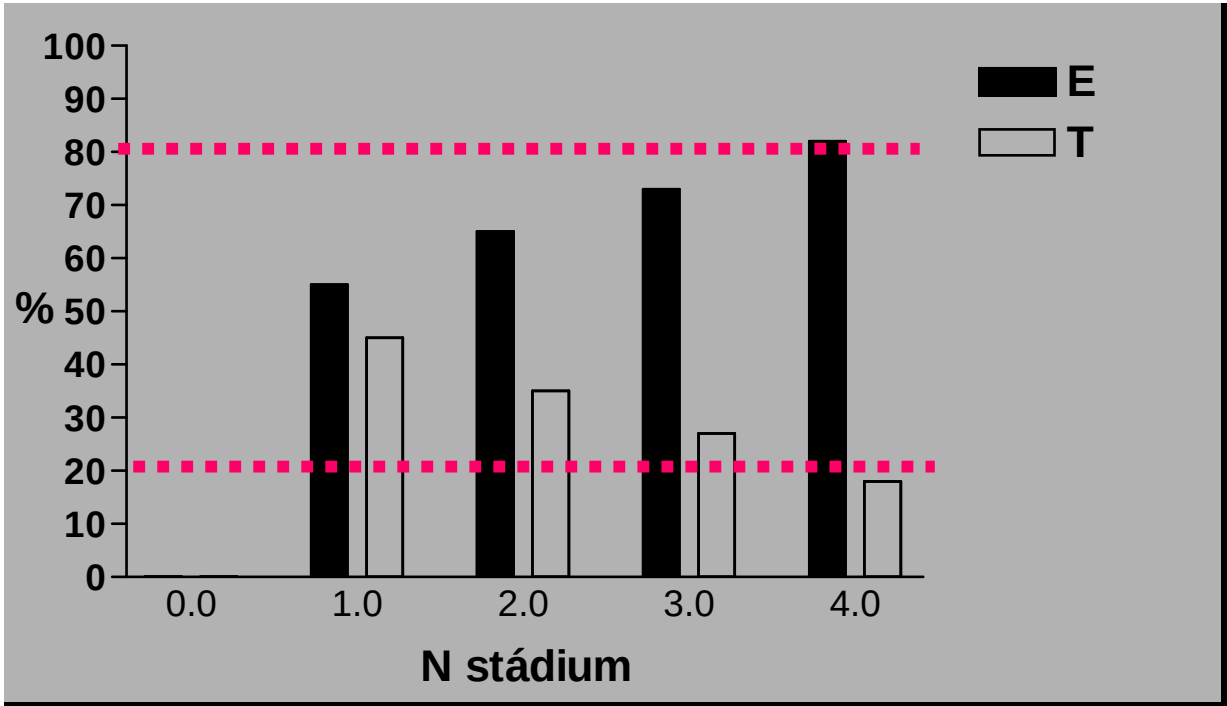


Douglas Hanahan , Robert?A. Weinberg

Figure?1 The Hallmarks of Cancer This illustration encompasses the six hallmark capabilities originally proposed in our 2000 perspective. The past decade has witnessed remarkable progress toward understanding the mechanistic underpinnings of eac...

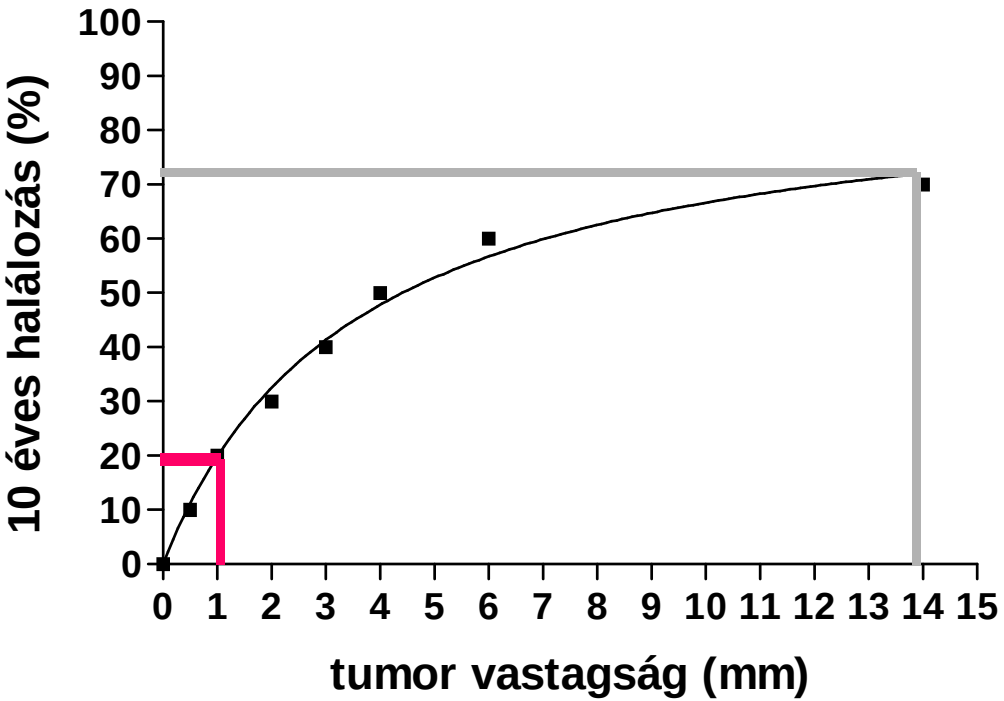
Hallmarks of Cancer: The Next Generation

Cell Volume 144, Issue 5 2011 646 - 674

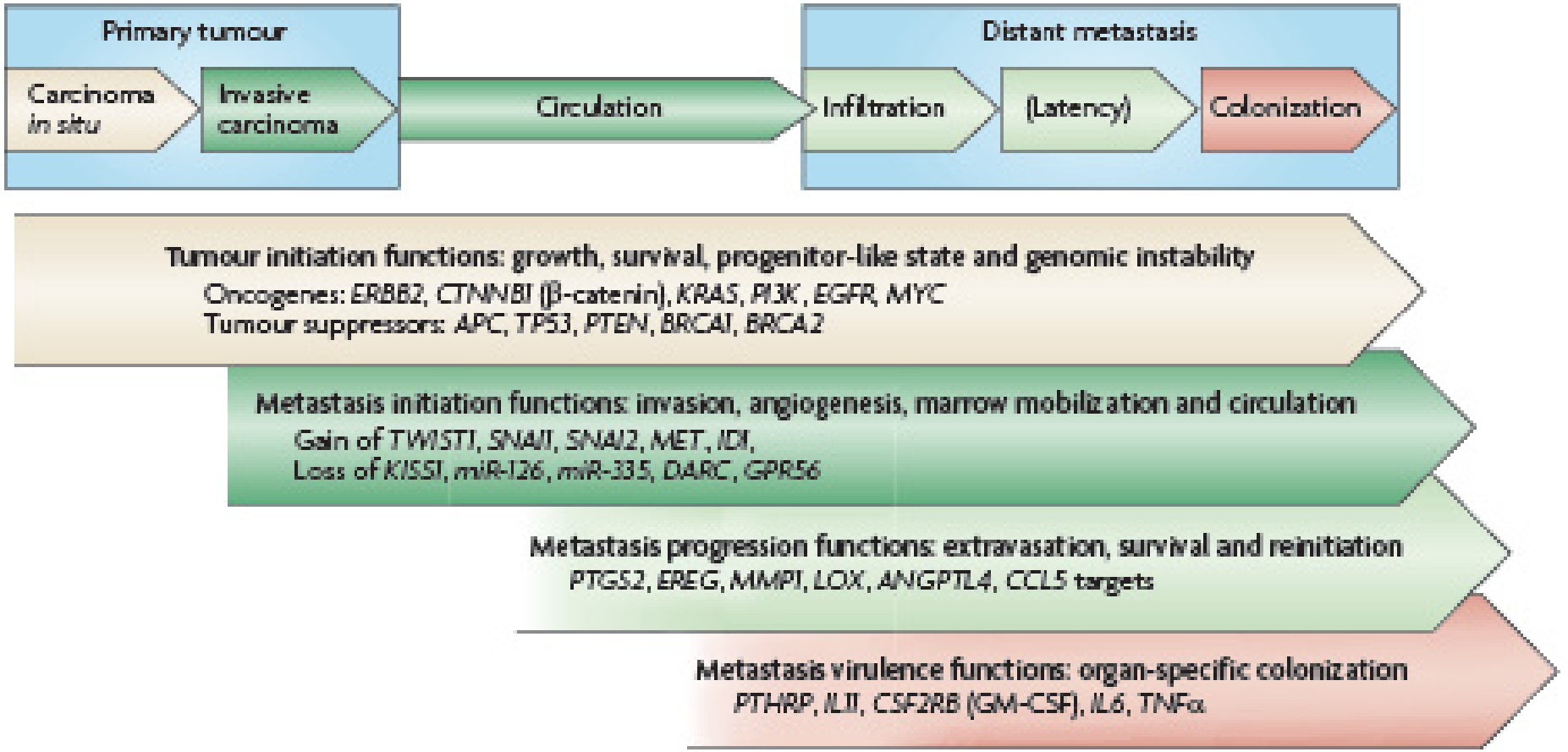


10 year survival (T)
According to N stages

10 year survival according to
Thickness of the primary



Progression of Cancer



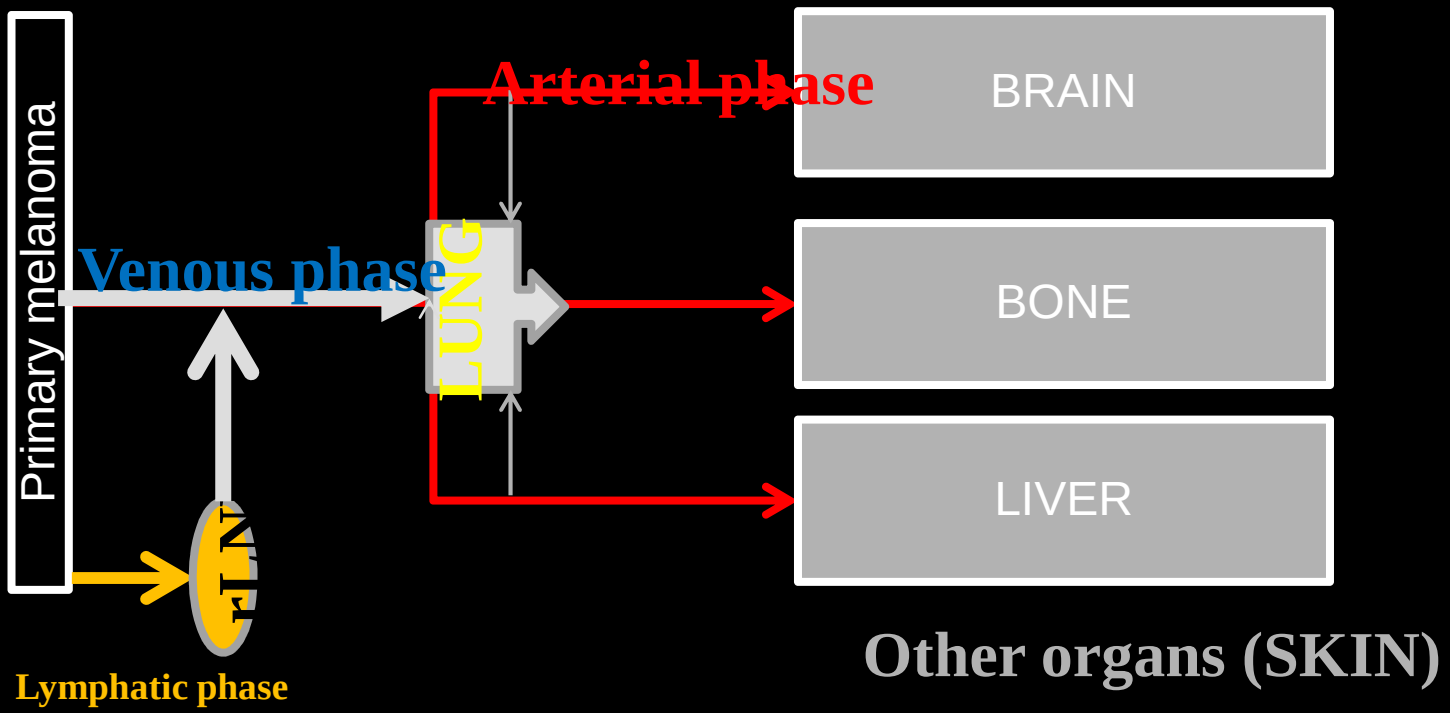
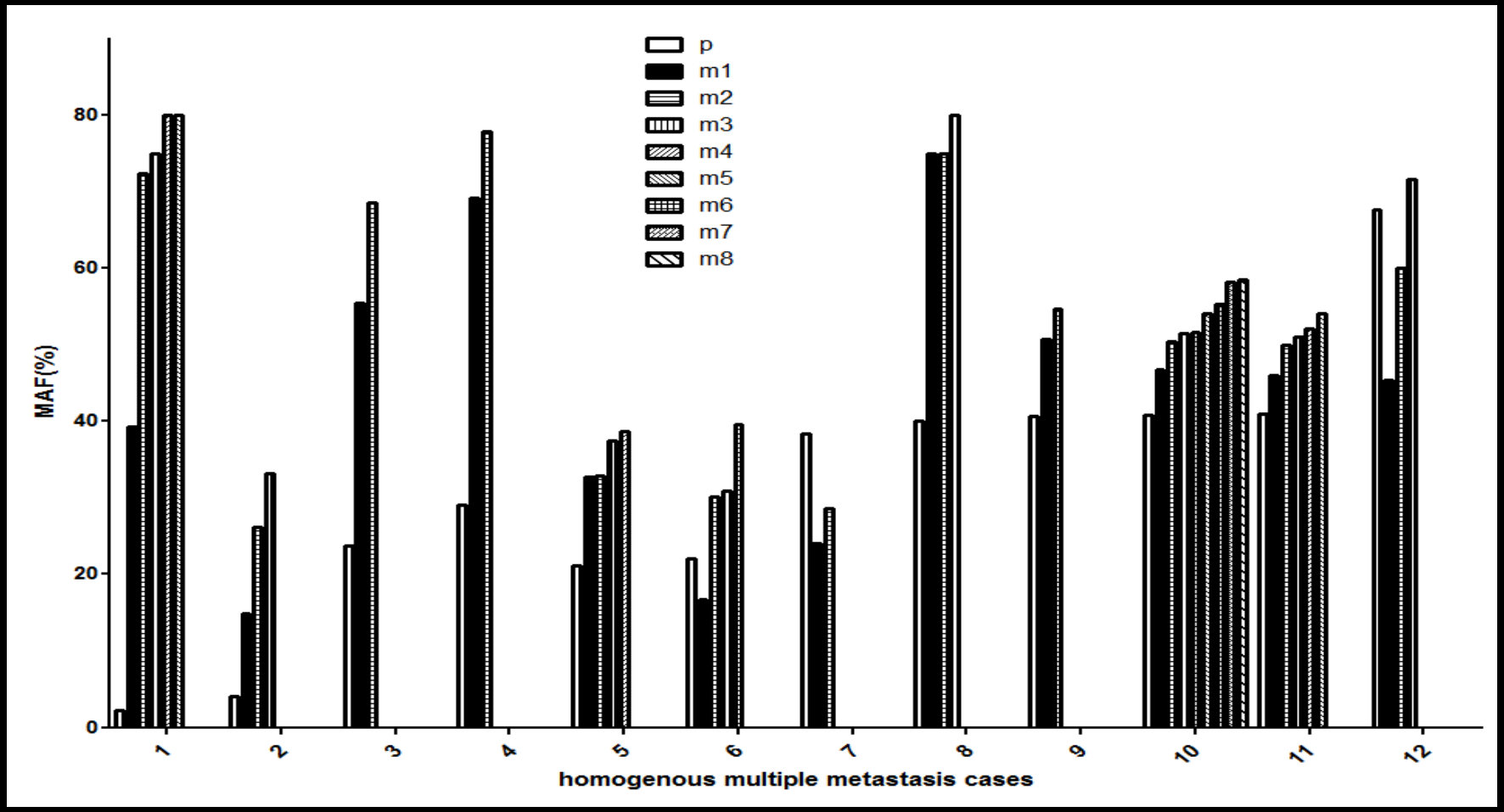

Primary melanoma
Arterial phase
Venous phase
LUNG
BRAIN
BONE
LIVER
LN
Lymphatic phase
Other organs (SKIN)

Fig.2 Timar

Mutant allelic frequency of BRAF during metastatic progression

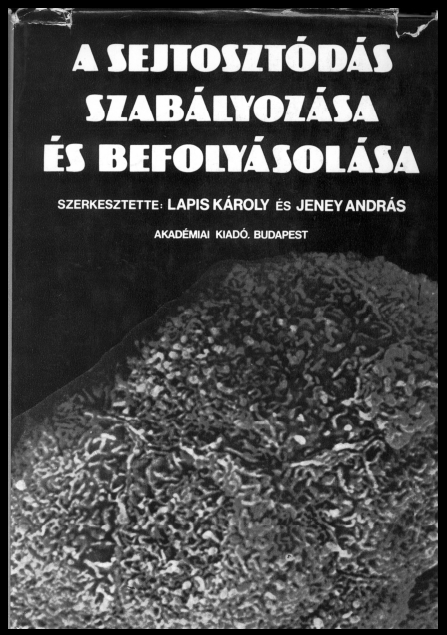


Genetic progression of Malignant Melanoma

alteration type	natural progression	target therapy (BRAFi and MEKi)	immunotherapy
mutation	**BRCA1** ERBB4 NMDAR2 **ADAM19/29** **NOTCH2**	BRAF MEK1/2 AKT1 PIK3CA PIK3R1/2	NRAS b2 microglobulin MART1 FAM3c CSMD1
amplification	MITF AP1/TEAD **BRIC5/survivin** MET VEGFA	BRAF MITF	
LOH/loss	**KISS1R** PTEN	PTEN	

Timar et al. Cancer metast Rev 2016

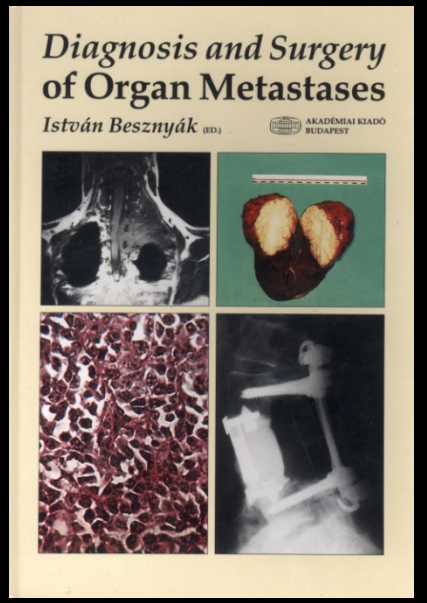
Major challenge for malignant cells

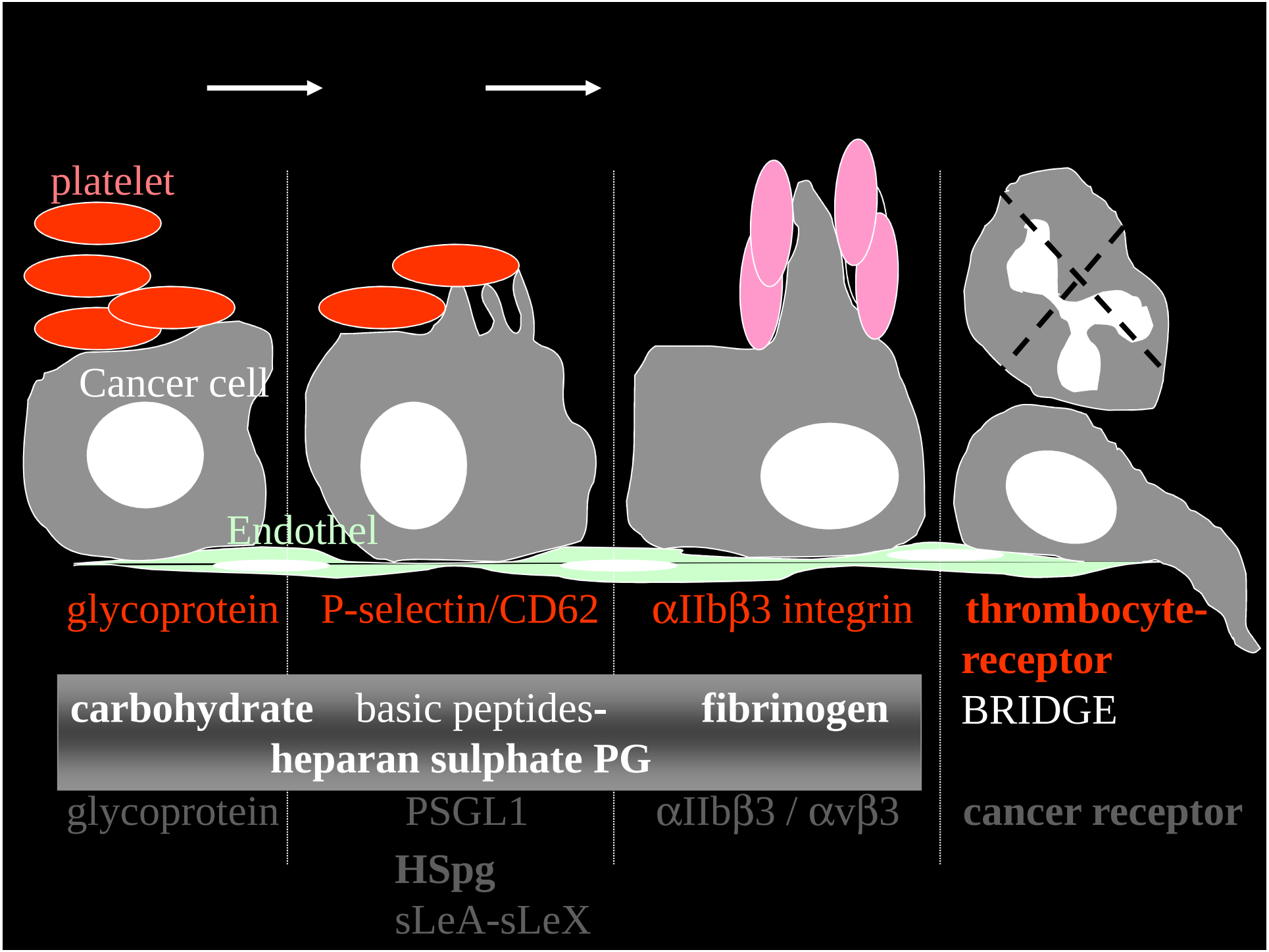


Proliferáció és/vagy

Áttétképzés

„Go or Grow”

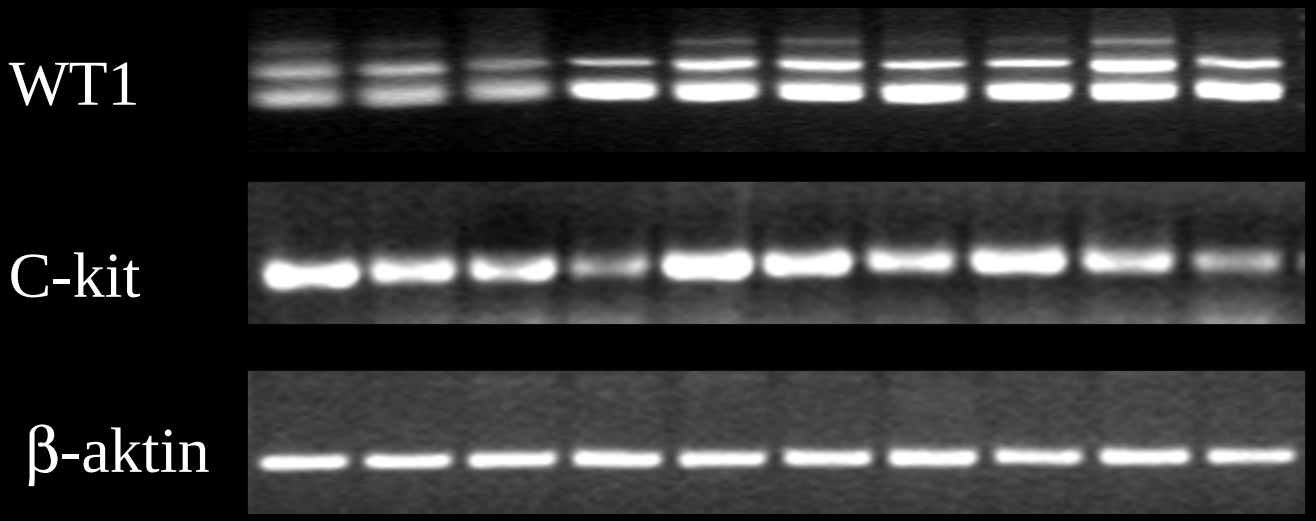



platelet
Cancer cell
Endothel
glycoprotein
P-selectin/CD62
αIIbβ3 integrin
thrombocyte-receptor
carbohydrate
basic peptides-heparan sulphate PG
fibrinogen
BRIDGE
glycoprotein
PSGL1
αIIbβ3 / αvβ3
cancer receptor
HSpg
sLeA-sLeX

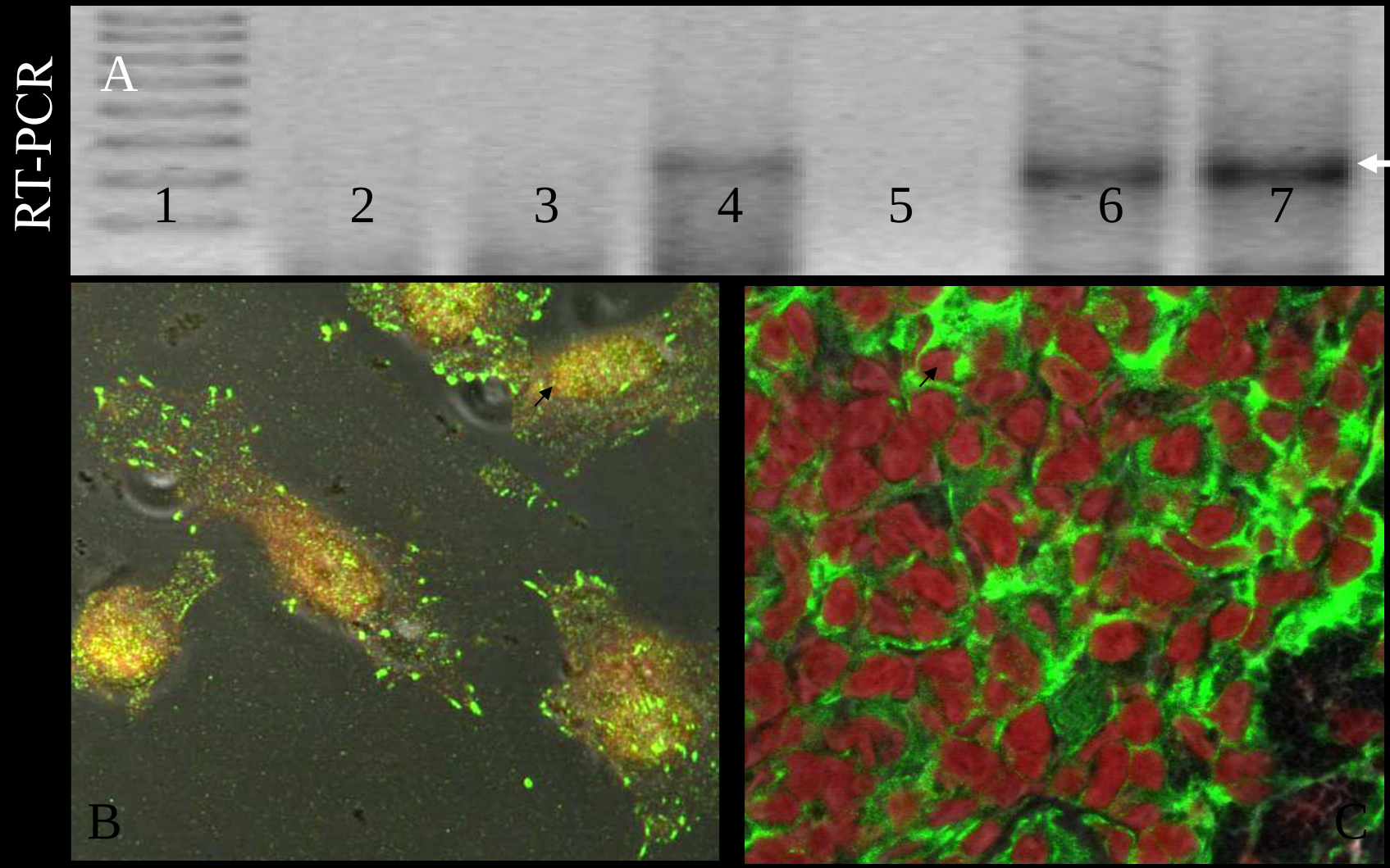
Trombocyte mimicry in melanoma

- CD41 (GPIIb, integrin αIIb)
- Thr. 12-lipoxigenase
- PECAM/CD31 (trombocyte adhesion molecule)
- Thrombin-receptor (PAR1-4)

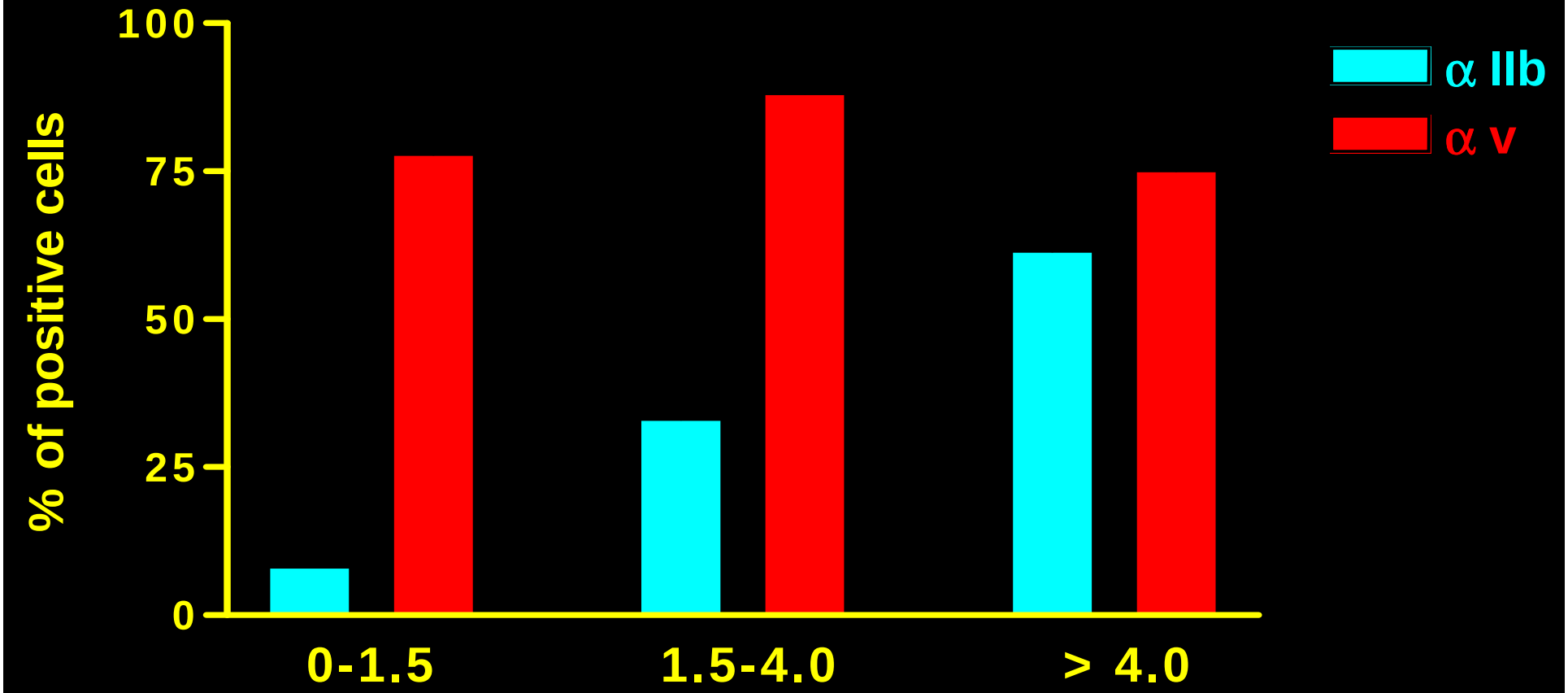
Thrombocytic mimicry of melanoma (bone marrow stem cell markers)



Thrombocytic integrin (αIIbβ3-GpIIbIIIa) expression In human melanoma cells



αIIb integrin expression is a poor prognostic factor in skin melanoma



αIIbβ3 integrin induced gene signature: vasculogenic mimicry

d	ac.No	Name
2,002048	***D84124***	***Prostaglandin I-2 (prostacyclin) synthase***
2,014675	D21235	Human mRNA for HHR23A protein, complete cds
2,13392	D83780	Human mRNA for KIAA0196 gene, complete cds
2,156721	L76927	Galactokinase 1
2,173375	**D13168**	**Endothelin receptor type B**
2,302858	AC004262	Homo sapiens chromosome 19, cosmid R29368
2,307286	L34408	Homo Sapiens (clone B3B3E13) chromosome 4p16.3 DNA fragment
2,387747	**X62535**	**Diacylglycerol kinase, alpha (80kD)**
2,403949	**M10942**	**Human metallothionein-Ie gene (hMT-Ie)**
2,418433	Y00971	Phosphoribosyl pyrophosphate synthetase 2
2,650112	AF001862	Human SLP-76 associated protein mRNA, complete cds
2,77783	U07857	Signal recognition particle 14 kD protein
2,785849	X54232	Glypican 1
2,987556	X52943	TRANSCRIPTION FACTOR ATF-A AND ATF-A DELTA
3,07509	**S53911**	**CD34 antigen (hemopoietic progenitor cell antigen)**
4,400510	L03411	Radin blood group

14. ábra. αIIb extracelluláris domén nukleinsav és aminósav szekvencia jelölve a 19H melanómában észlelt eddig még pontosan nem jellemzett eltérést.

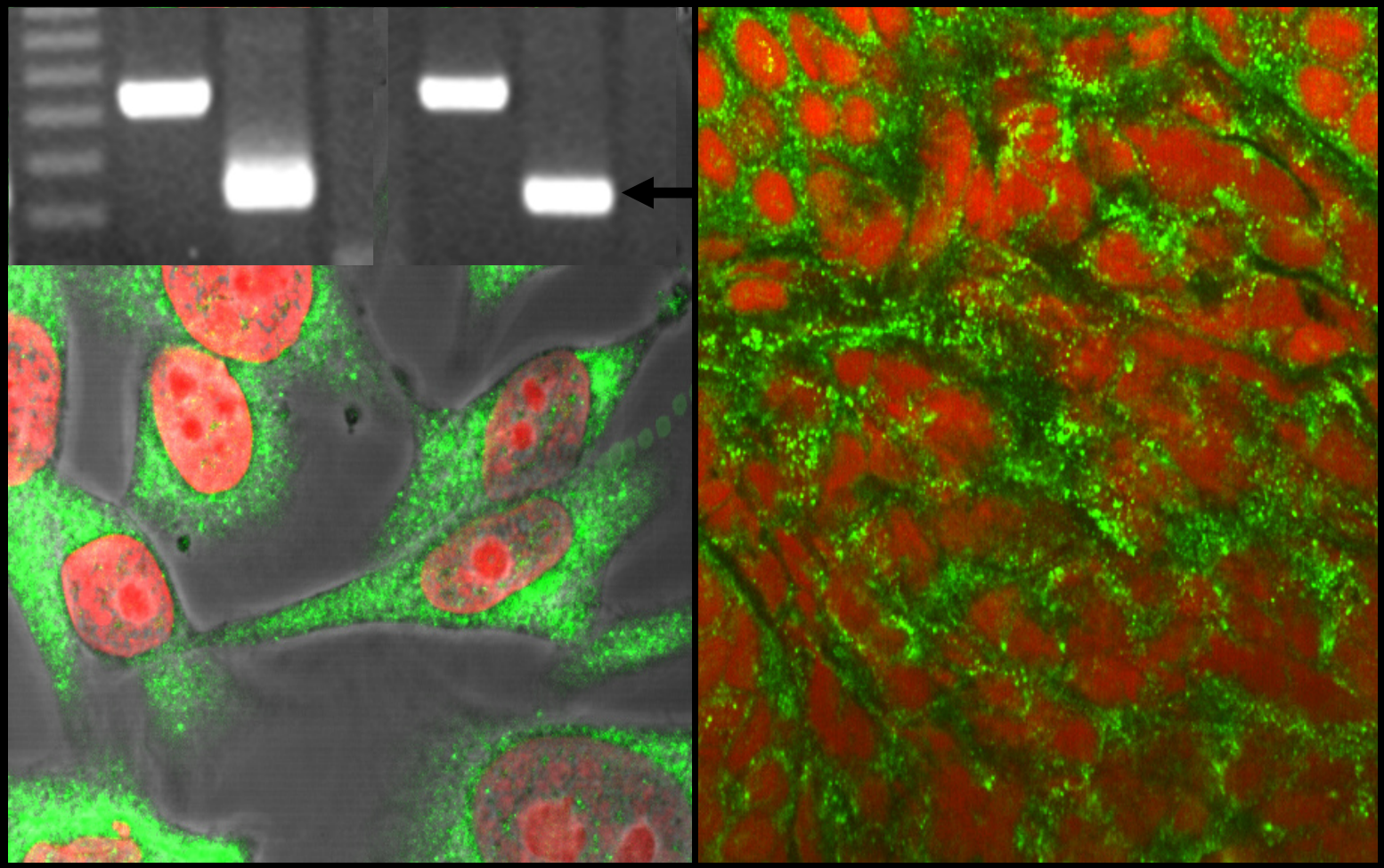
A/ nukleinsav-szekvencia

```
     1081 accgaaaact ggccgaagtg gggcgtgtgt atttgttcct gcagccgcg ggcccccacg
     1141 cgctgggtgc ccccagcctc ctgctgactg gcacacagct ctatgggcga ttcggctctg
     1201 ccatcgcacc cctgggcgac ctcgaccggg atggctacaa tgacattgca gtggctgccc
```

B/ aminósav sorrend

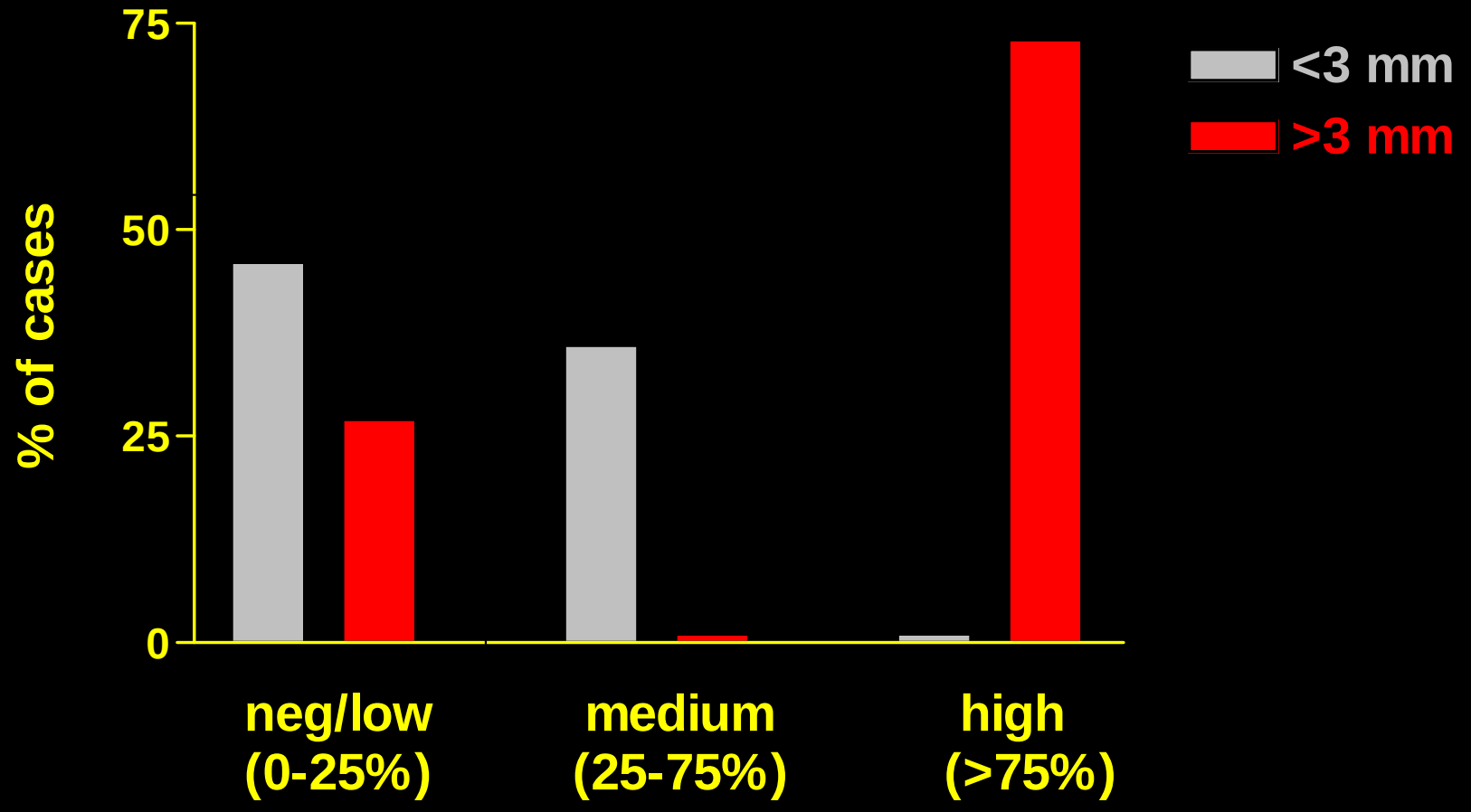
```
301 ldsyyqrlhr lraeqmasyf ghsvavtdvn gdgrhdllvg aplymesrad rklaevgrvy
361 lflqprgpha lgapsllltg tqlygrfgsa iaplgdldrd gyndiavaap yggpsgrgqv
421 lvflgqsegl rsrpsqvlds pfptgsafgf slrgavdidd ngypdlivga yganqvavyr
```

Thrombocytic 12-lipoxigenase expression in human melanoma cells

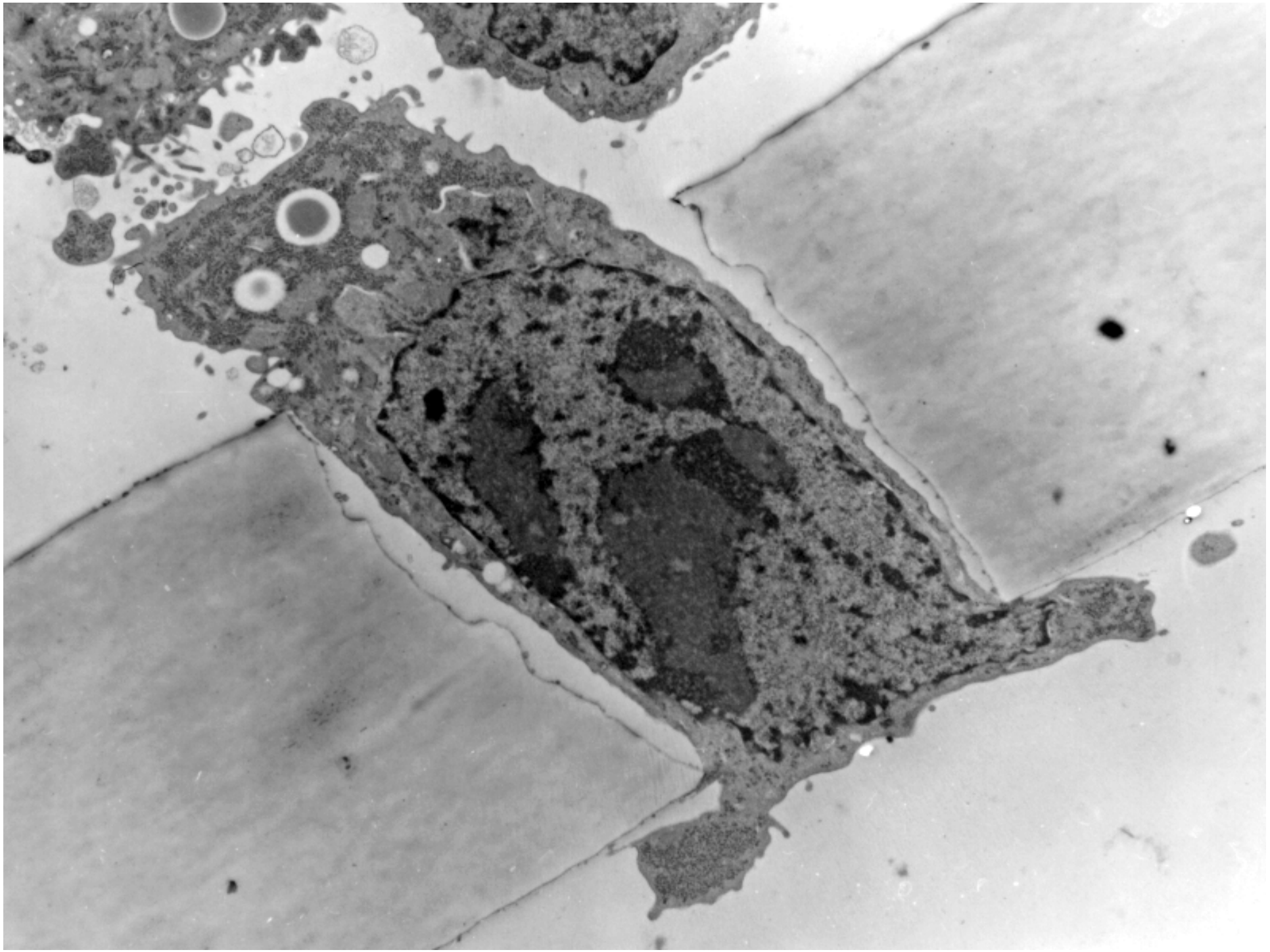


Rásó és mtsai,2004

Protein expression of p12-LOX in human skin melanoma samples (n=22, confocal microscopy)

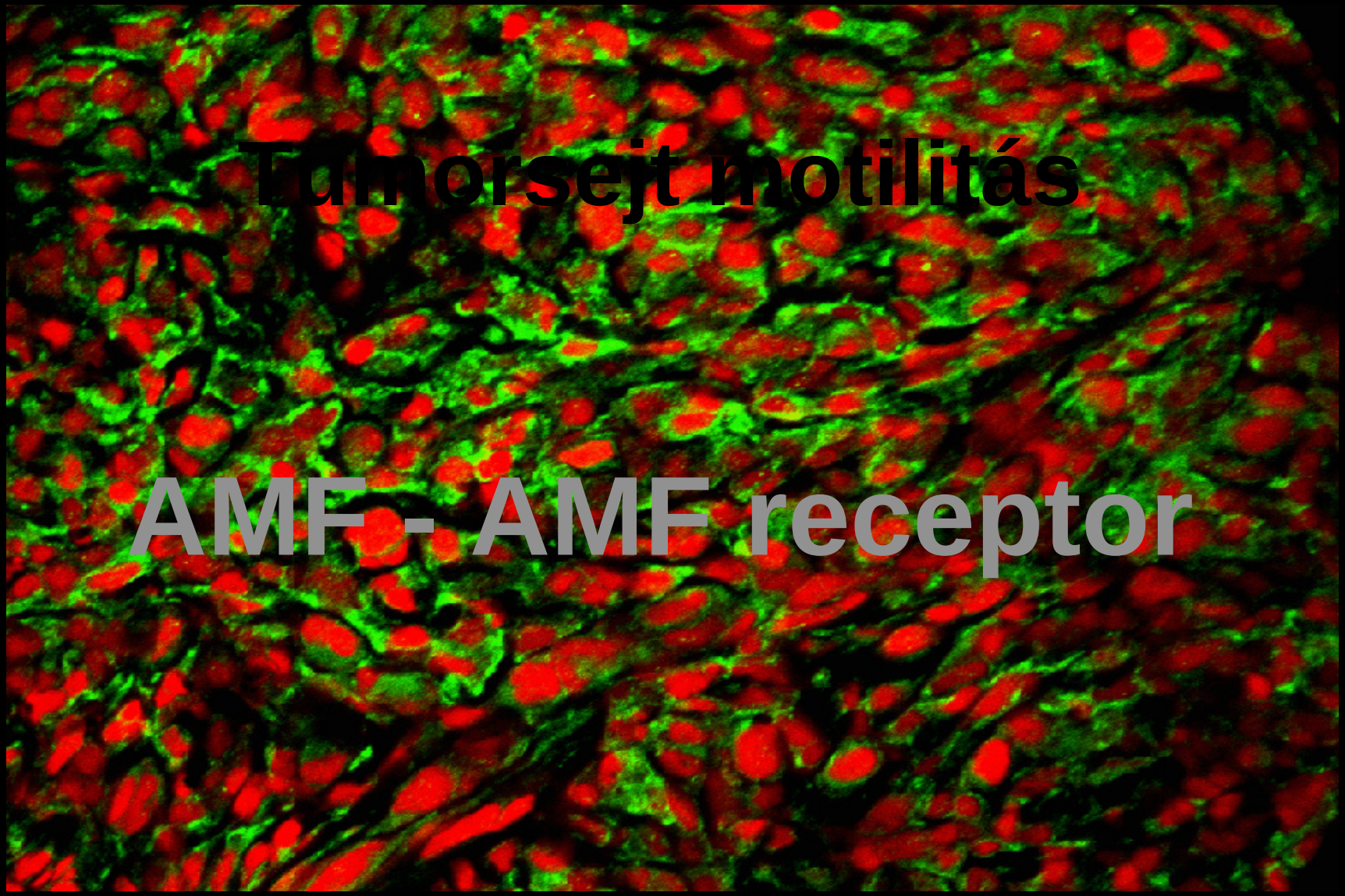


Expression ranges of p12-LOX in % of positive cells



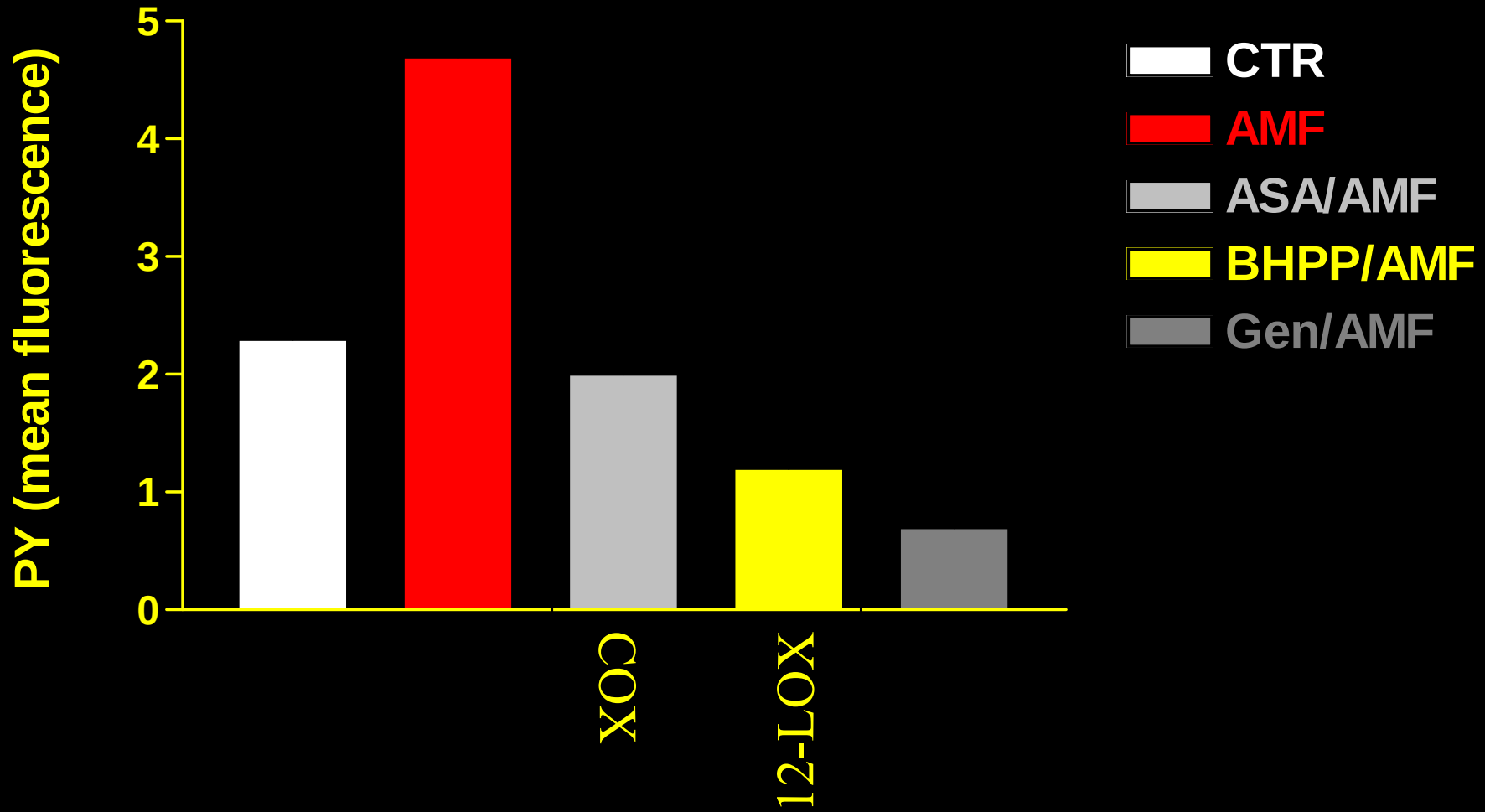
Motility cytokines and receptors

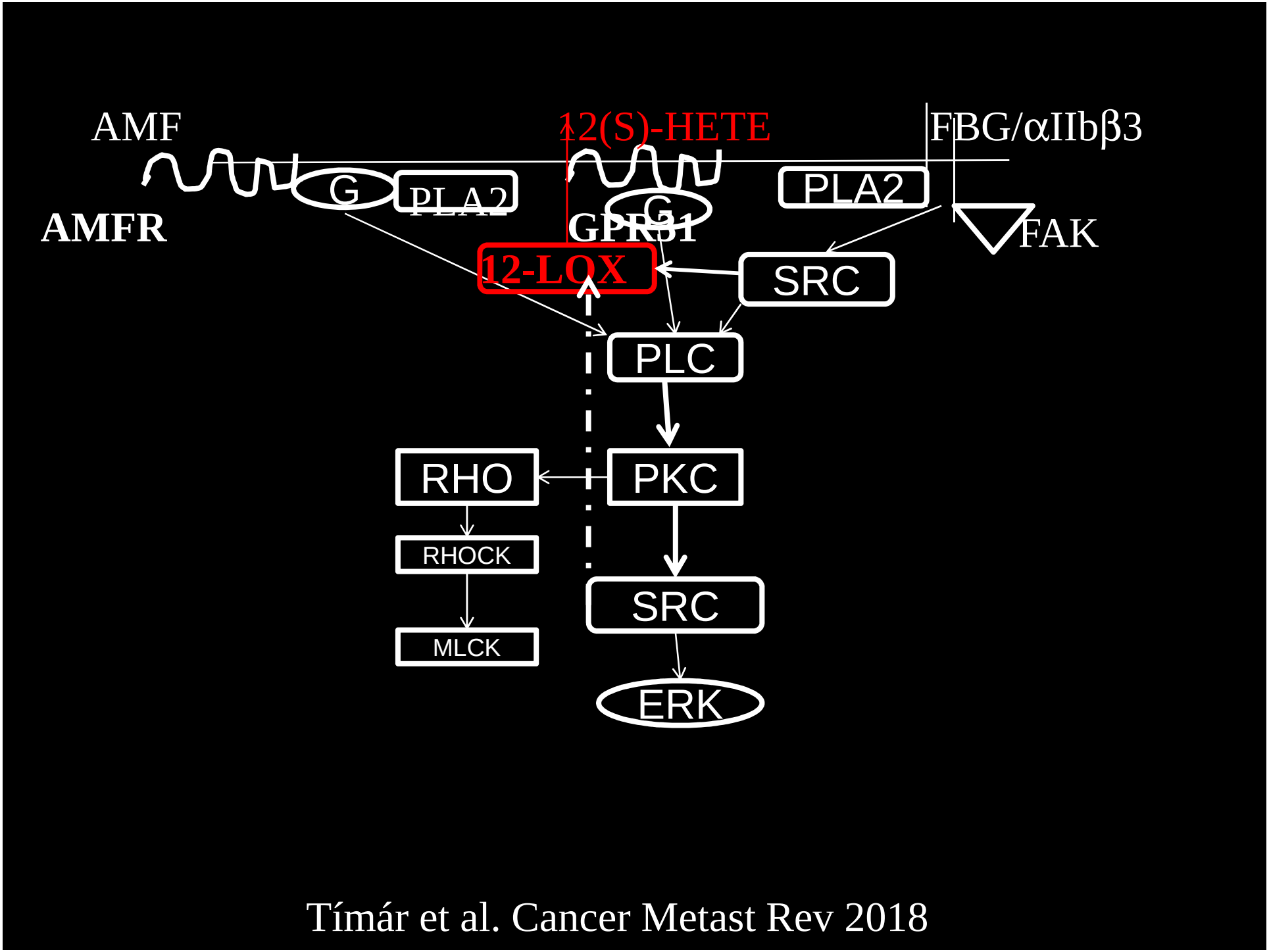
Citokine	effect	Receptor
AMF		
F-hexózizomeráz	**Motility**	**gp78 /CXCR5**
ATX: lizofoszfolipid	Motilitás	?
MSF	Motilitás	?
MIF	Motilitás	?
MCP	Motilitás	?
Inzulin	Proliferáción	IR
EGF	Proliferáción	EGF-R, c-erb
TNFα	double (motility and proliferáción)	TNF-R55, TNF-R75
TGFβ	d	TGFβ-R I., II.
HGF/SF	d	c-met
bFGF	d	TGF-R1-R4
GM-CSF	d	P80, p120
IL-6	d	IL-6R, gp130
PDGF	d	Rα p80 , Rβ p170



Tímár és mtsai. Clin Exp Metast (2001)

COX and LOX inhibitors prevent AMF induced tyrosine phosphorylation cascade





Tímár et al. Cancer Metast Rev 2018

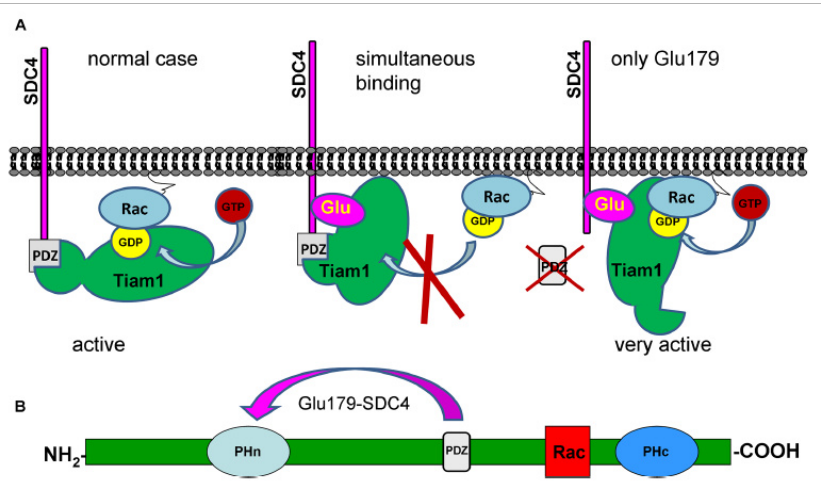


Fig 2. Schematic representation of the assumed mechanism of the inhibition of Tiam1 by phosphomimetic SDC4. (A) The only connection of SDC4 to Tiam1 via PDZ binding site or Glu^{179} does not interfere with Tiam1 activity; however the simultaneous interactions block the enzyme activity. (B) Structure of Tiam1 contains two pleckstrin homology domains (PHn and PHc). Rac1 binding site is located between the PHn and PHc domains. In pull down experiment PH domain was identified as interaction site, and PHn was shown to regulate binding of GTPases [10] thus we suppose that phosphomimetic SDC4 can interact with the PDZ and PHn domains simultaneously to exclude Rac1.

https://doi.org/10.1371/journal.pone.0187094.g002

Syndecan4 HSPG:
Expresssion in melanoma
pro-metastatic
Mutations possible

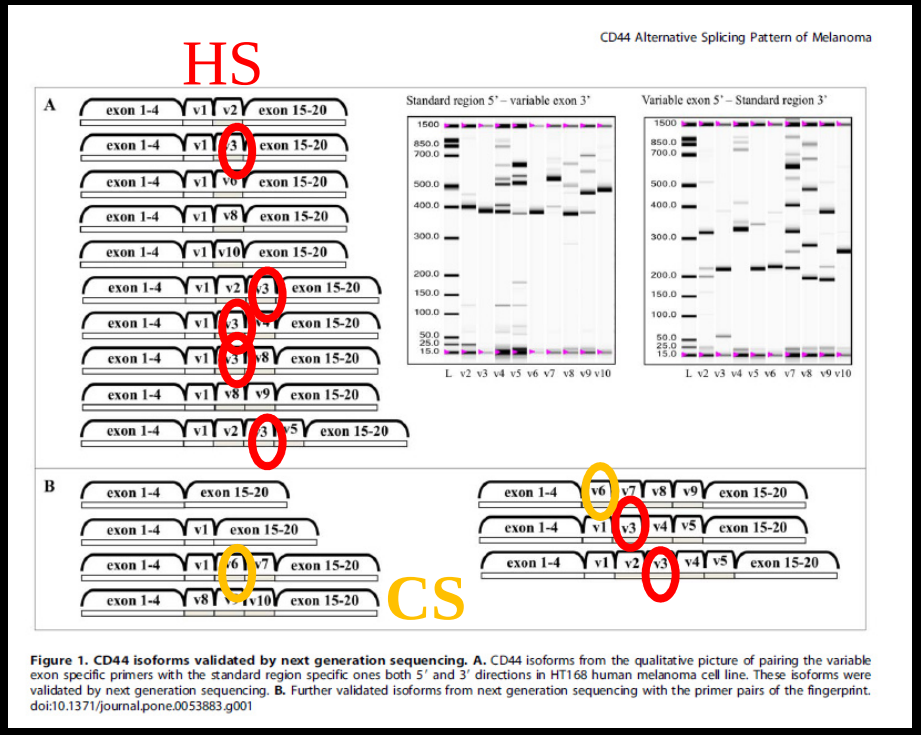


Figure 1. CD44 isoforms validated by next generation sequencing. A. CD44 isoforms from the qualitative picture of pairing the variable exon specific primers with the standard region specific ones both 5' and 3' directions in HT168 human melanoma cell line. These isoforms were validated by next generation sequencing. **B.** Further validated isoforms from next generation sequencing with the primer pairs of the fingerprint.
doi:10.1371/journal.pone.0053883.g001

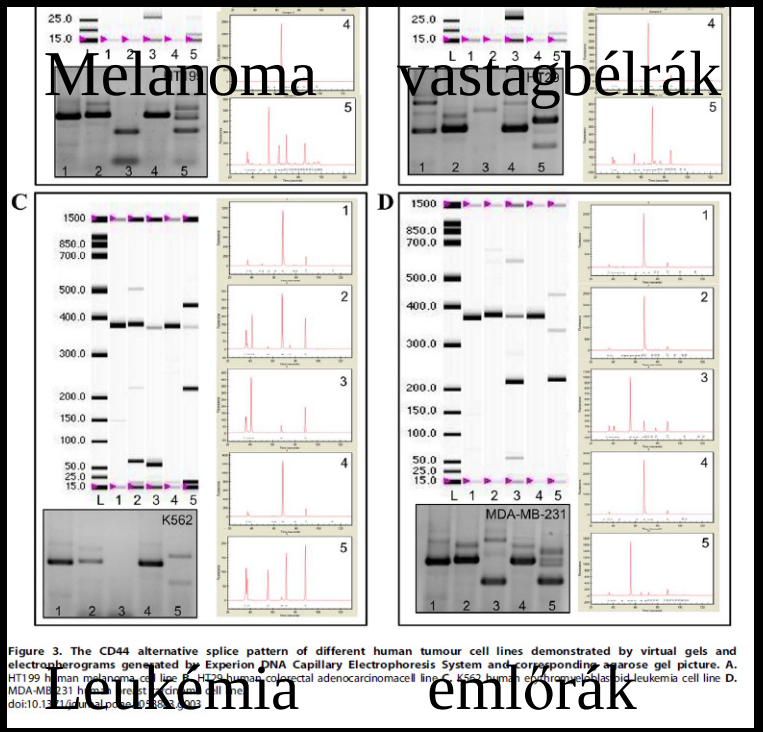


Figure 3. The CD44 alternative splice pattern of different human tumour cell lines demonstrated by virual gels and electropherograms generated by Experion DNA Capillary Electrophoresis System and corresponding agarose gel picture. A. HT199 human melanoma cell line **B.** HT29 human colorectal adenocarcinomacell line **C.** K562 human erythromyeloblastoid leukemia cell line **D.** MDA-MB231 human breast cancer cell line.
doi:10.1371/journal.pone.0053883.g003

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PLOS ONE

Demonstration of a Melanoma-Specific CD44 Alternative Splicing Pattern That Remains Qualitatively Stable, but Shows Quantitative Changes during Tumour Progression

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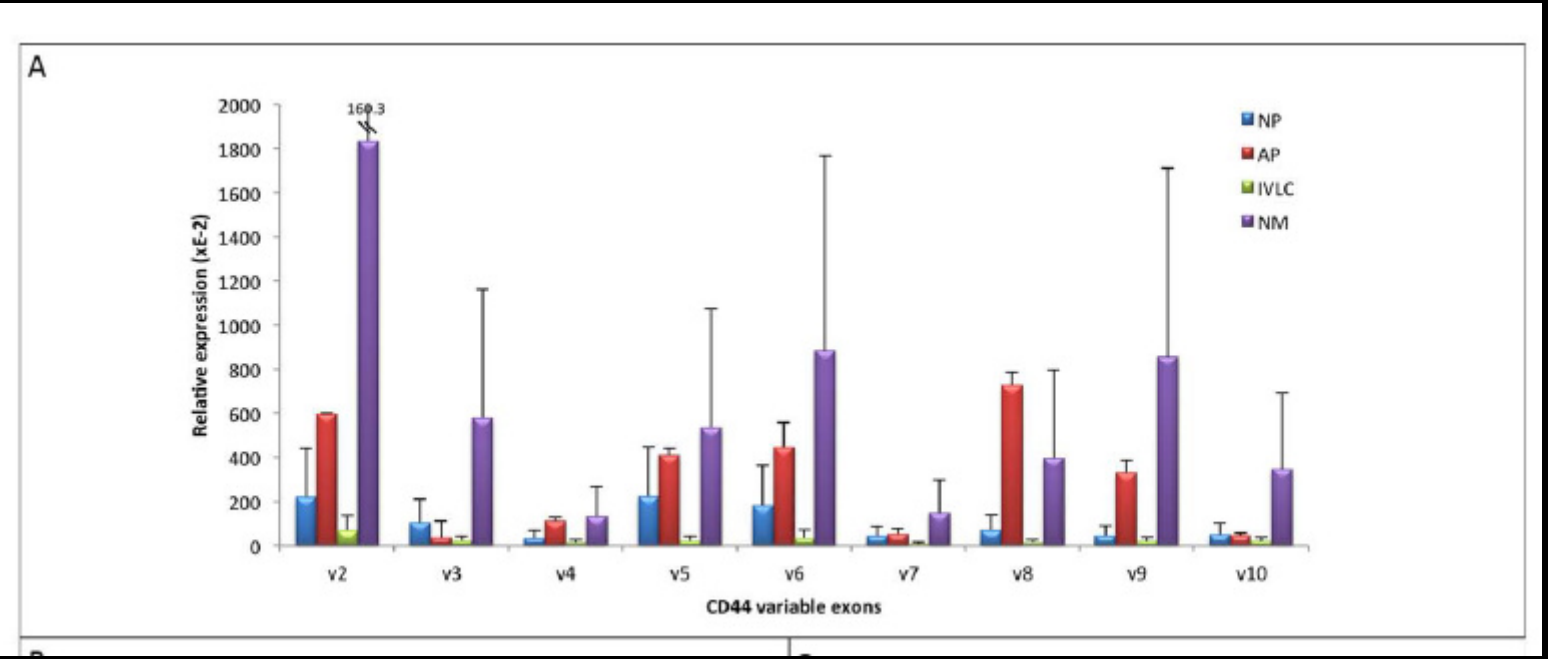
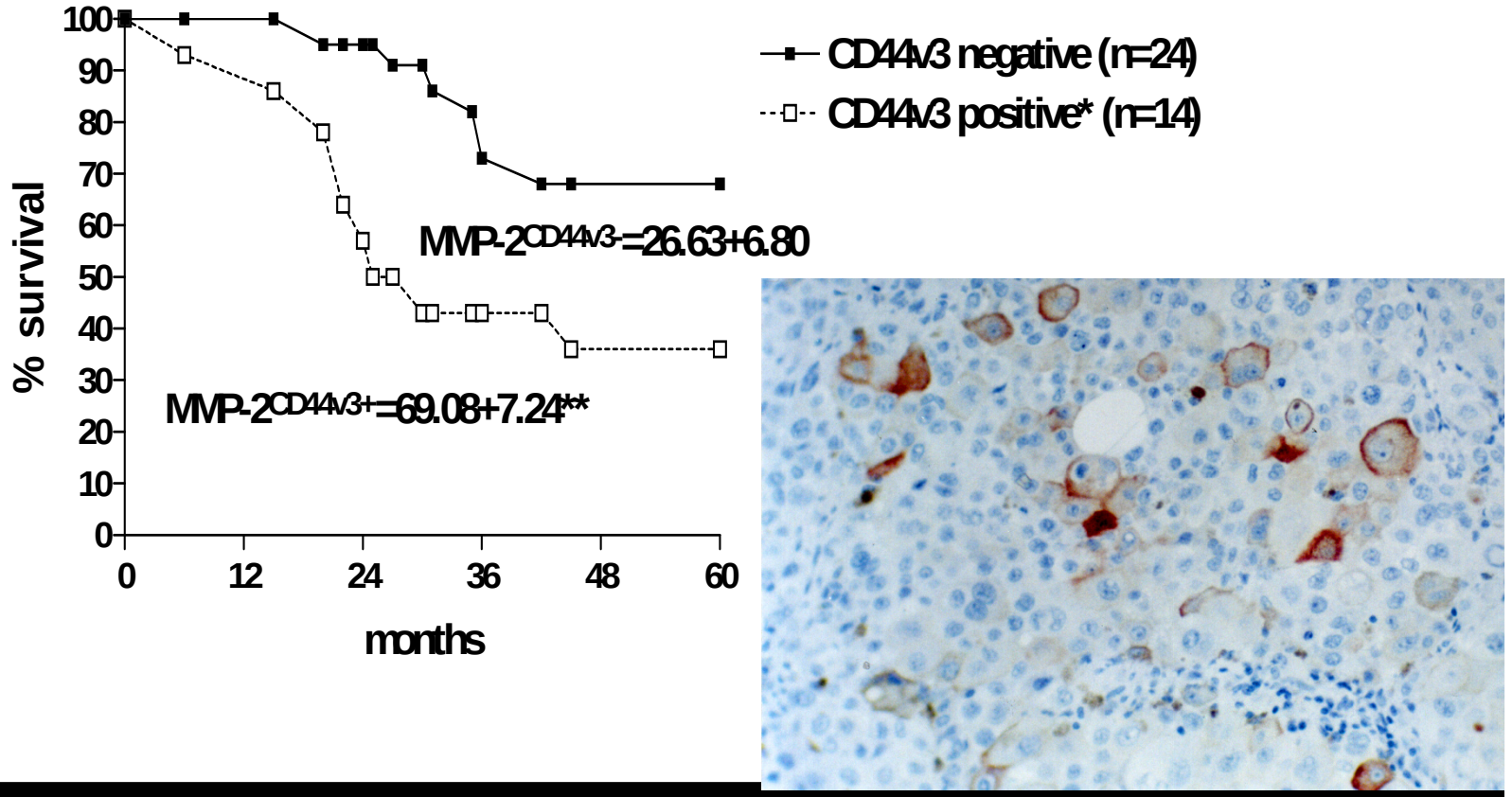
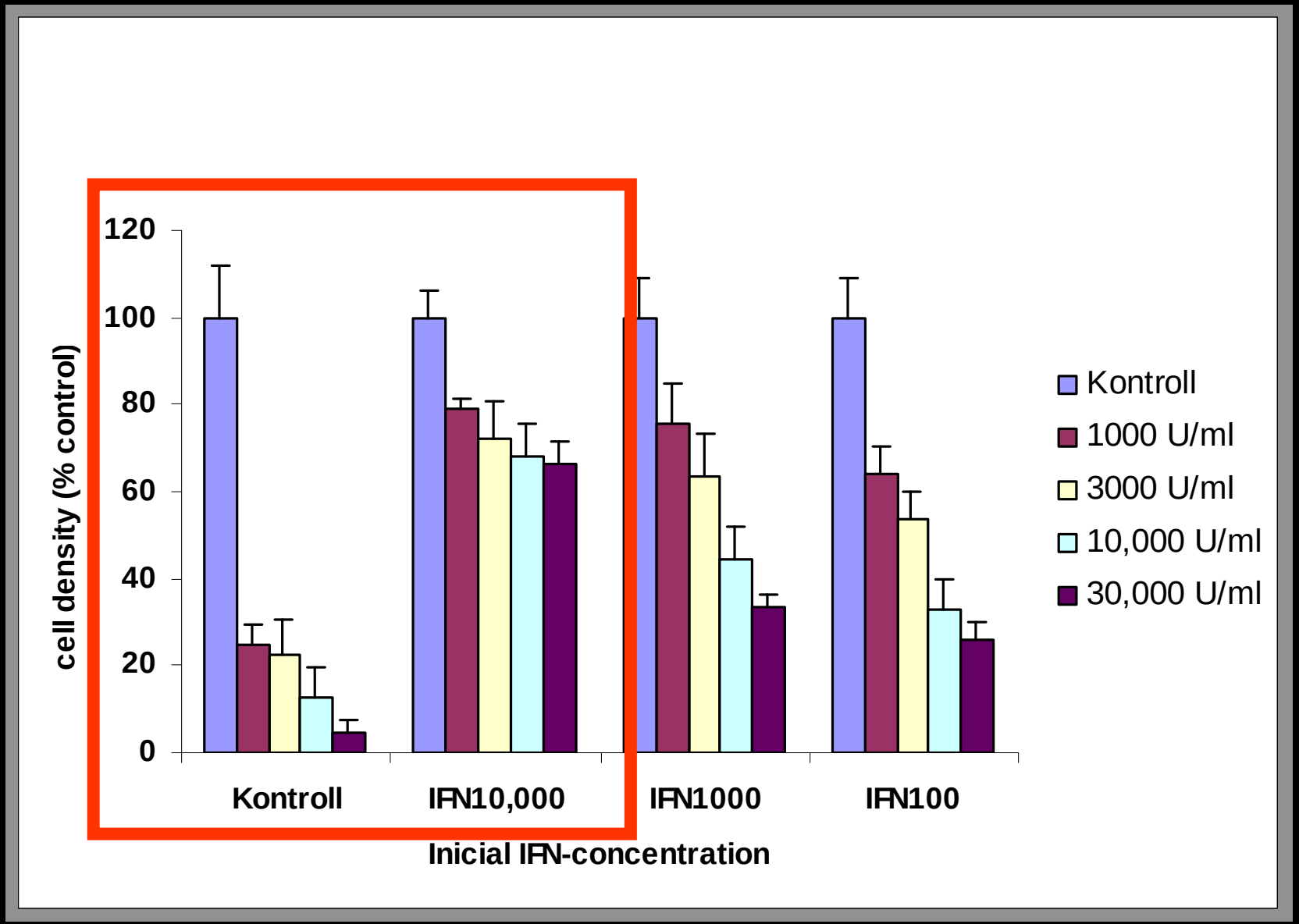
CD44 variant expression changes during metastasis of melanoma



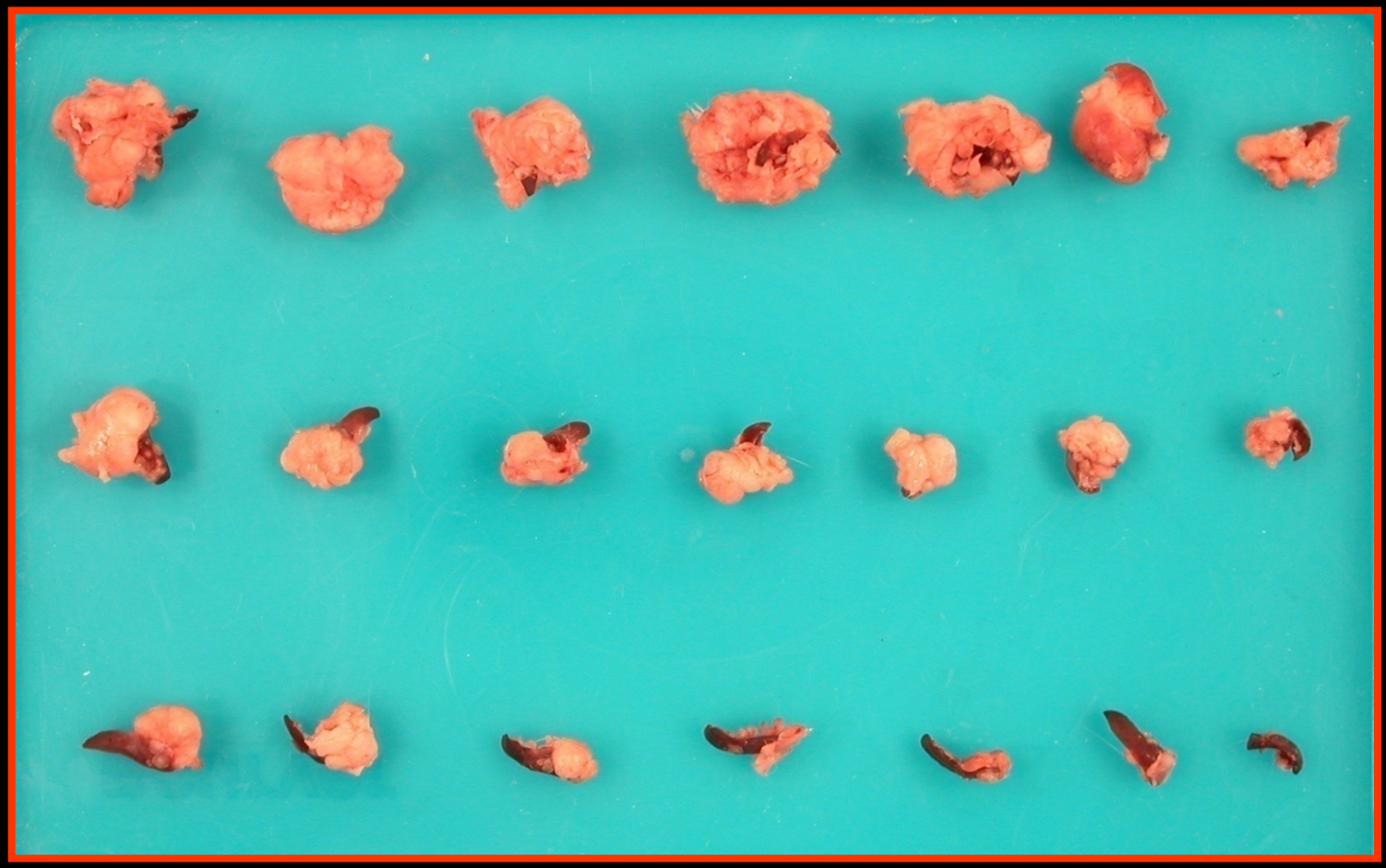
CD44v3 expression of malignant melanoma and the 5 year survival of patients



Selection of IFN-resistant clones in vitro from HT168-M1



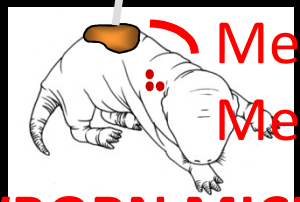
Effect of IFN-treatment in vivo on growth of sensitive melanoma line M^{sens}



Metastatic Human Melanoma Model in SCID Mice using HT199 human melanoma

ADULT MICE
non-metastatic environment

Met Init: M/hM primary

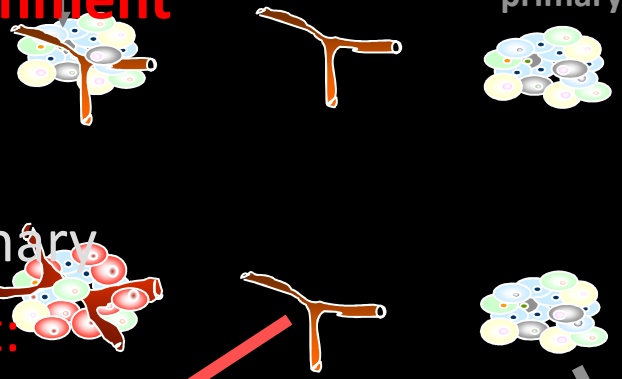


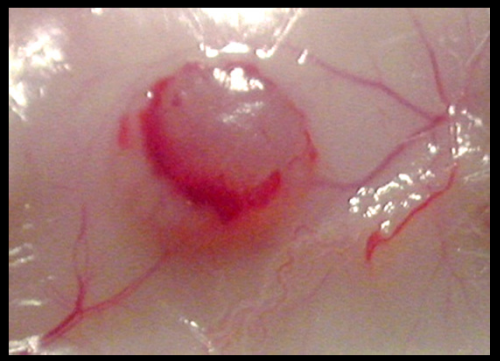
Met Maint:
Met/Primary

NEWBORN MICE
metastatic environment
(lung mets)

Stromal components

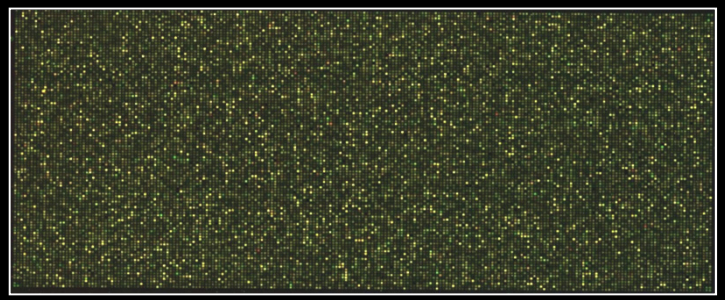
Human melanoma primary



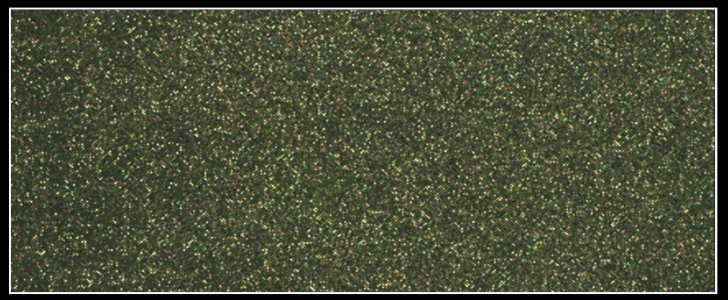


Subcutaneous melanoma
on 7th postimplantation day

Mouse Oligo Microarray
(22,575 gén – Agilent)



Whole Human Genome Oligo Microarray
(41,000 gén – Agilent)



Consensus Metastasis-associated IRE-genes of human melanoma (9)

upregulated		
		function
NOX5	NADPHoxydase	Ca-dependent SOD
TSPAN8	tetraspanin8	integrin-assoc **MOTILITY**
ZNF703	transcription factor	*ER-regulated*

downregulated		
		function
MX1	dynamin-family	**MOTILITY**
LRRK2	S/Tkinase MAPKKKcasc	**MOTILITY**
IFI-27	*estrogen-BRCA1 regulated!*	apoptosis-inducer
DKK1	WNT inhibitor	**INVASION**
SGK2	Se/glucocorticoid regulated kinase *ANDR-induced* PI3K-activated	apoptosis?
CAMK1	Ca-calmodulin-dependent kinase	Ca++ and RAS pathway

Take home messeges

- Skin melanoma is the most metastatic malignancy
- Metastatic potential is driven by high mutation rate, DDR defects?
- Metastatic potential is closely linked to ectopic expression of megakariocytic linage genes
- Melanoma is the most immunogenic tumor but development of IFN-resistance leads not only to immunresistance+ increase of the metastatic potential