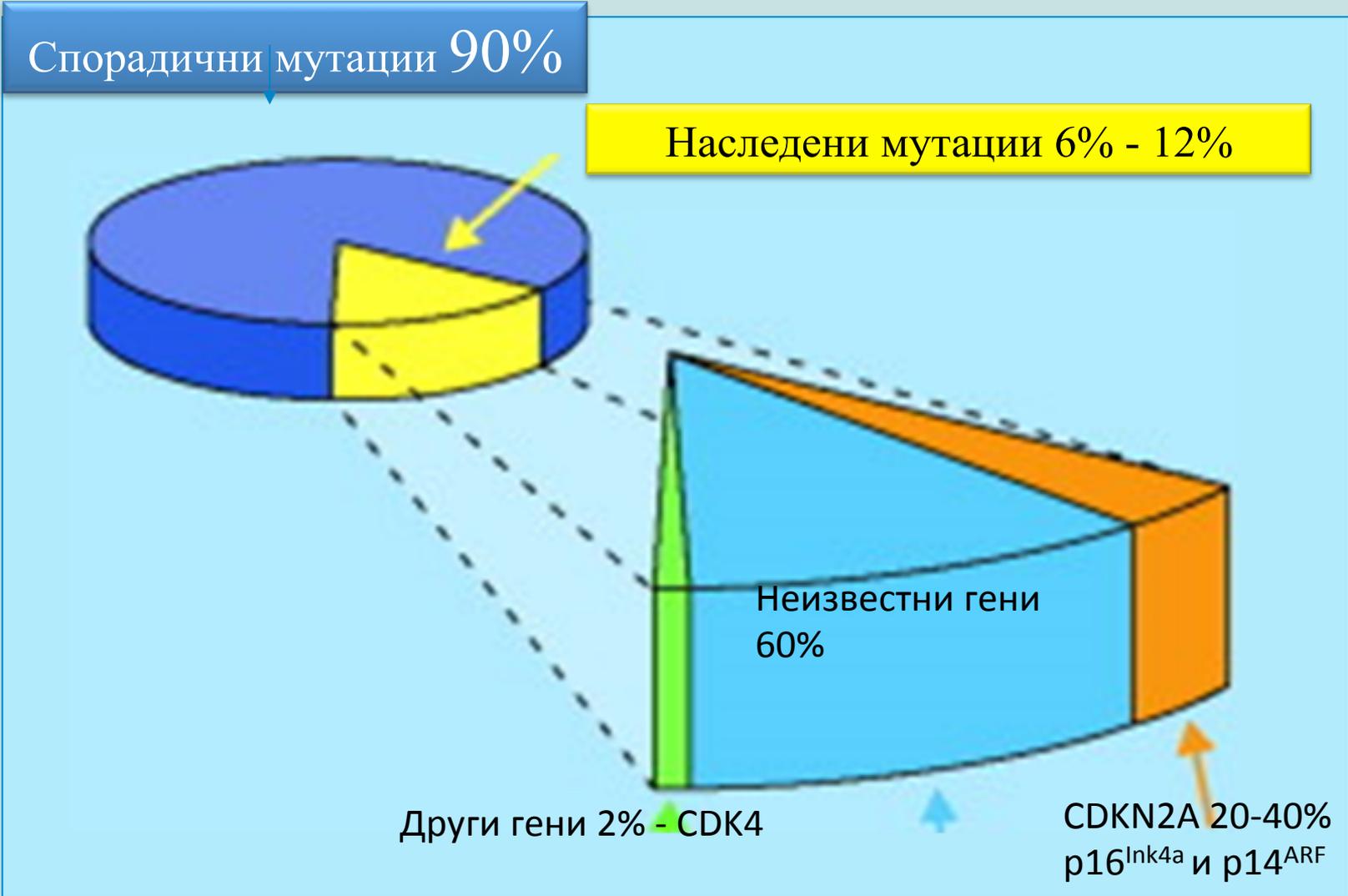


Молекулярно-патологични профили на меланом и немеланомни кожни тумори

Чл. кор. проф. д-р Д. Тончева

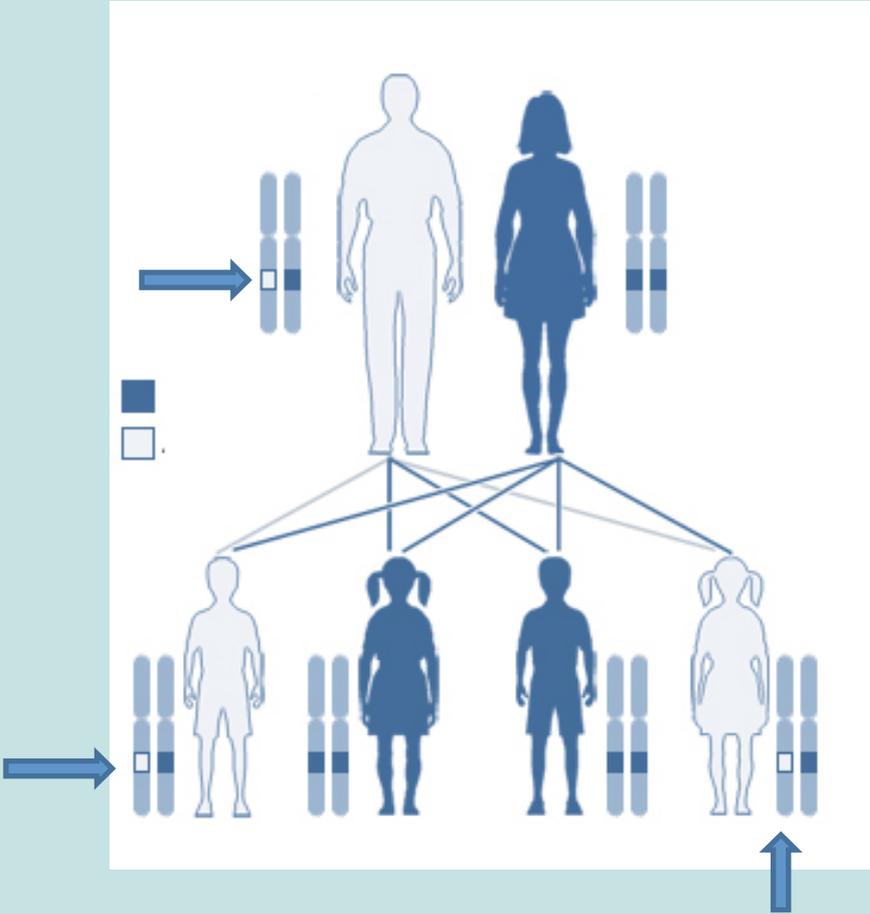


Типове мутации при меланом

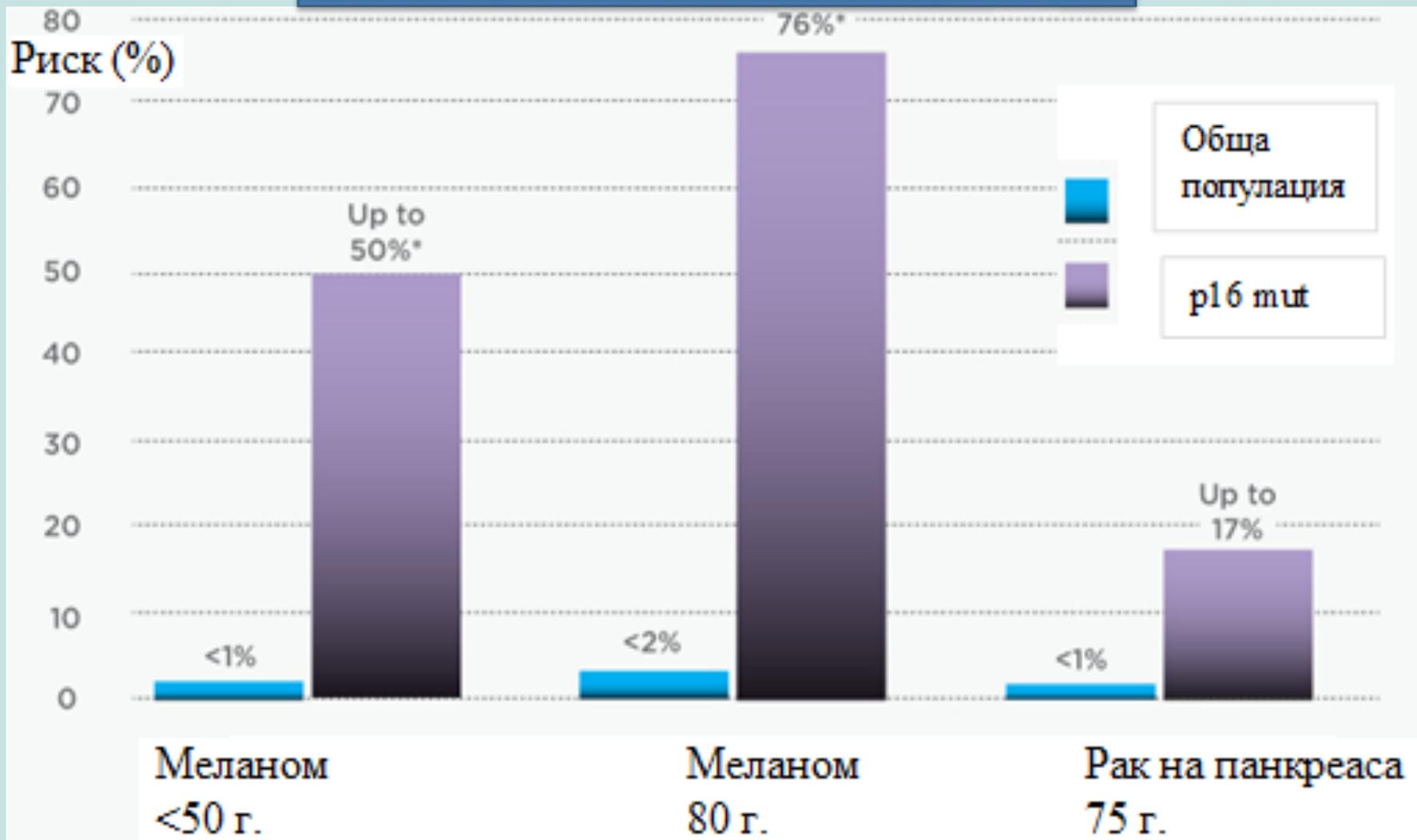


Наследствен меланом

АВТОЗОМНО-
ДОМИНАНТНО
НАСЛЕДЯВАНЕ



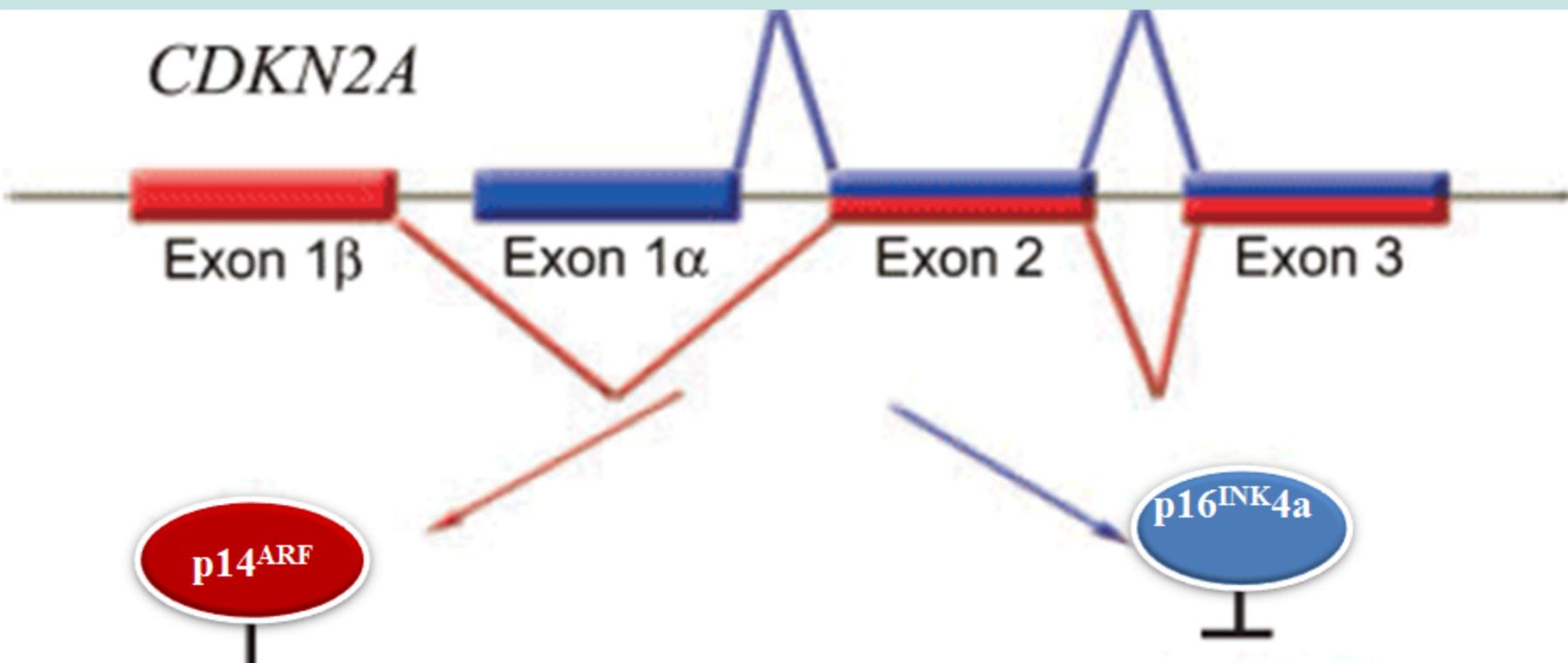
Риск при носители на мутации



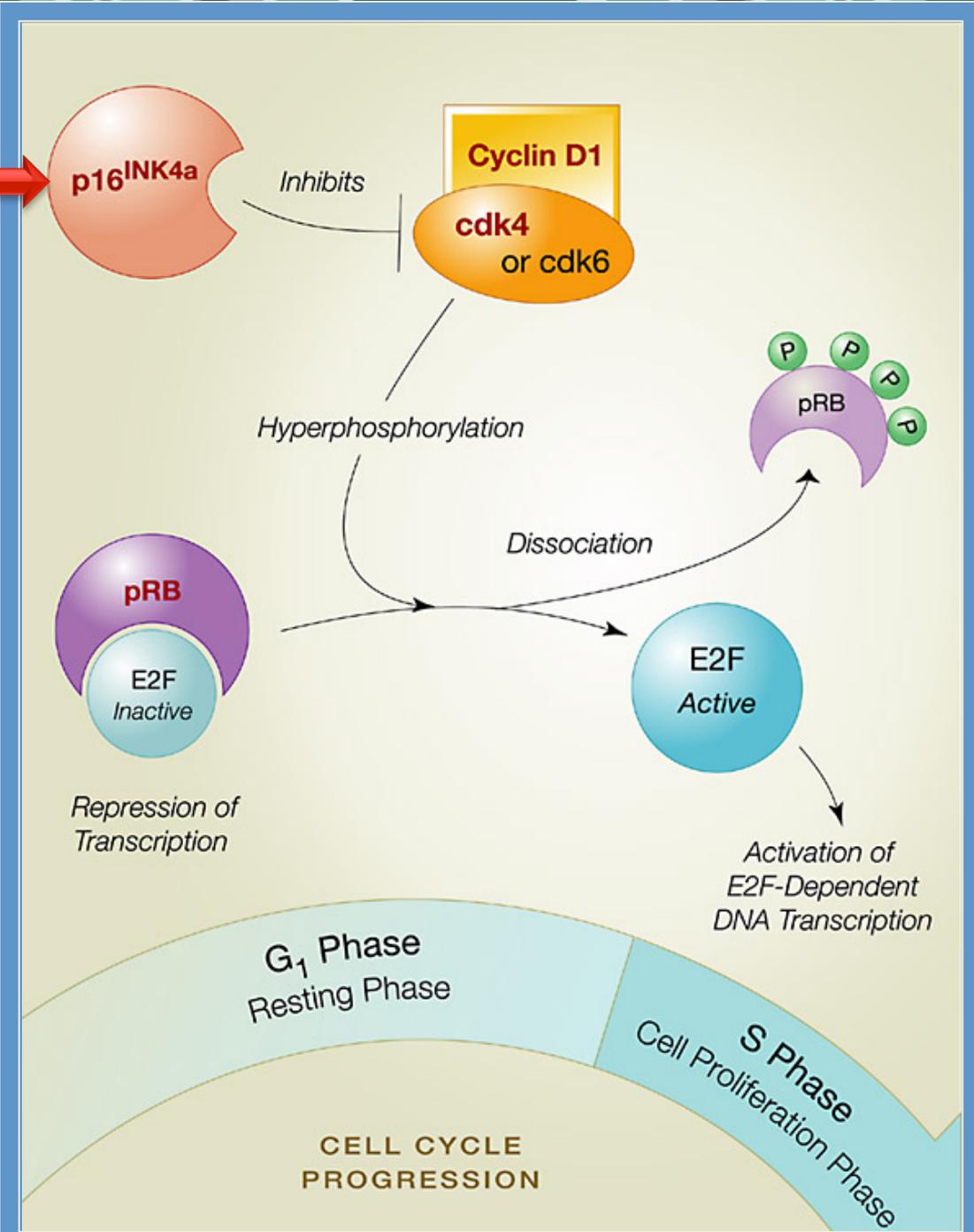


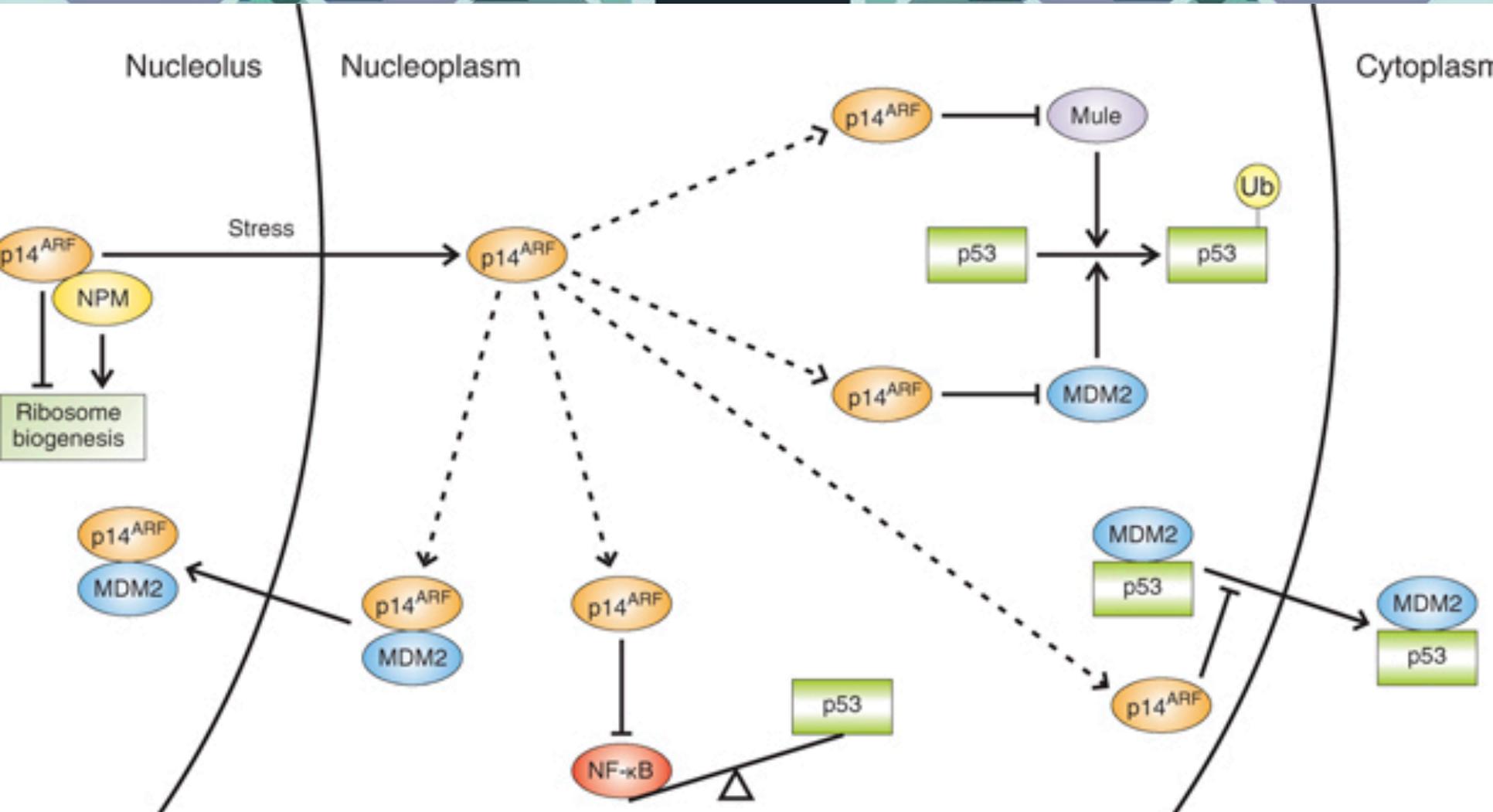
Патологични мутации при наследствени меланоми

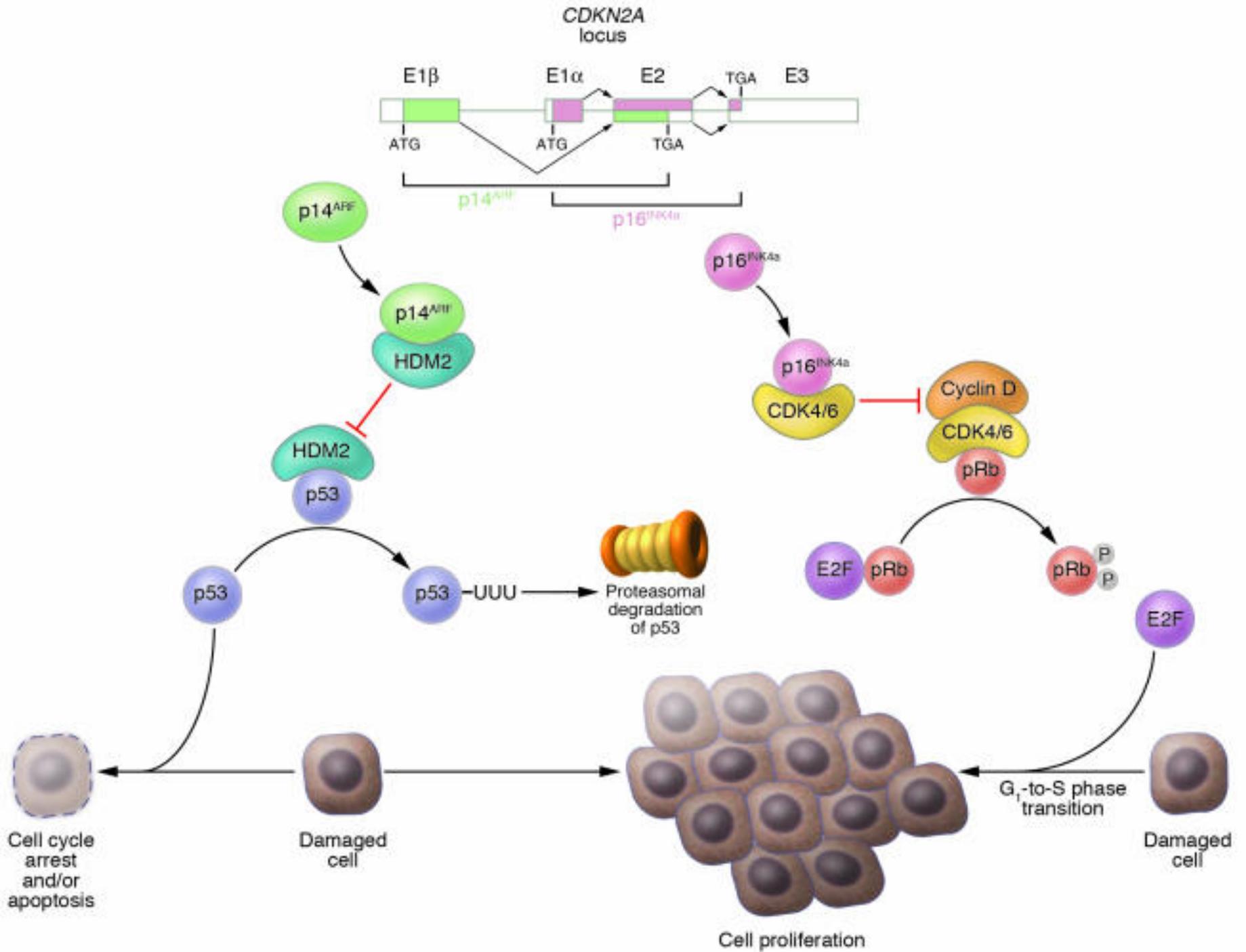
CDKN2A



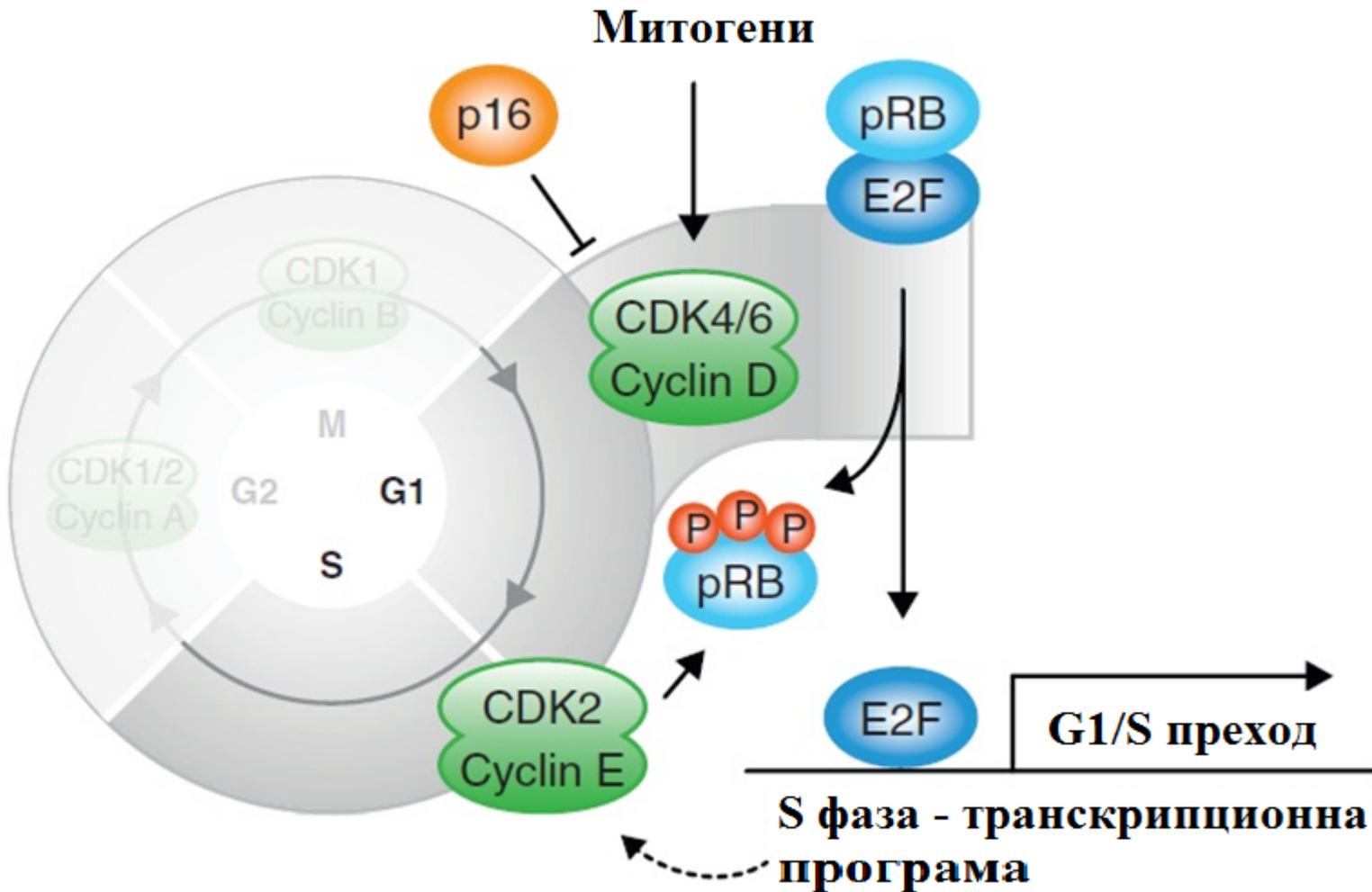
p16^{INK4a}



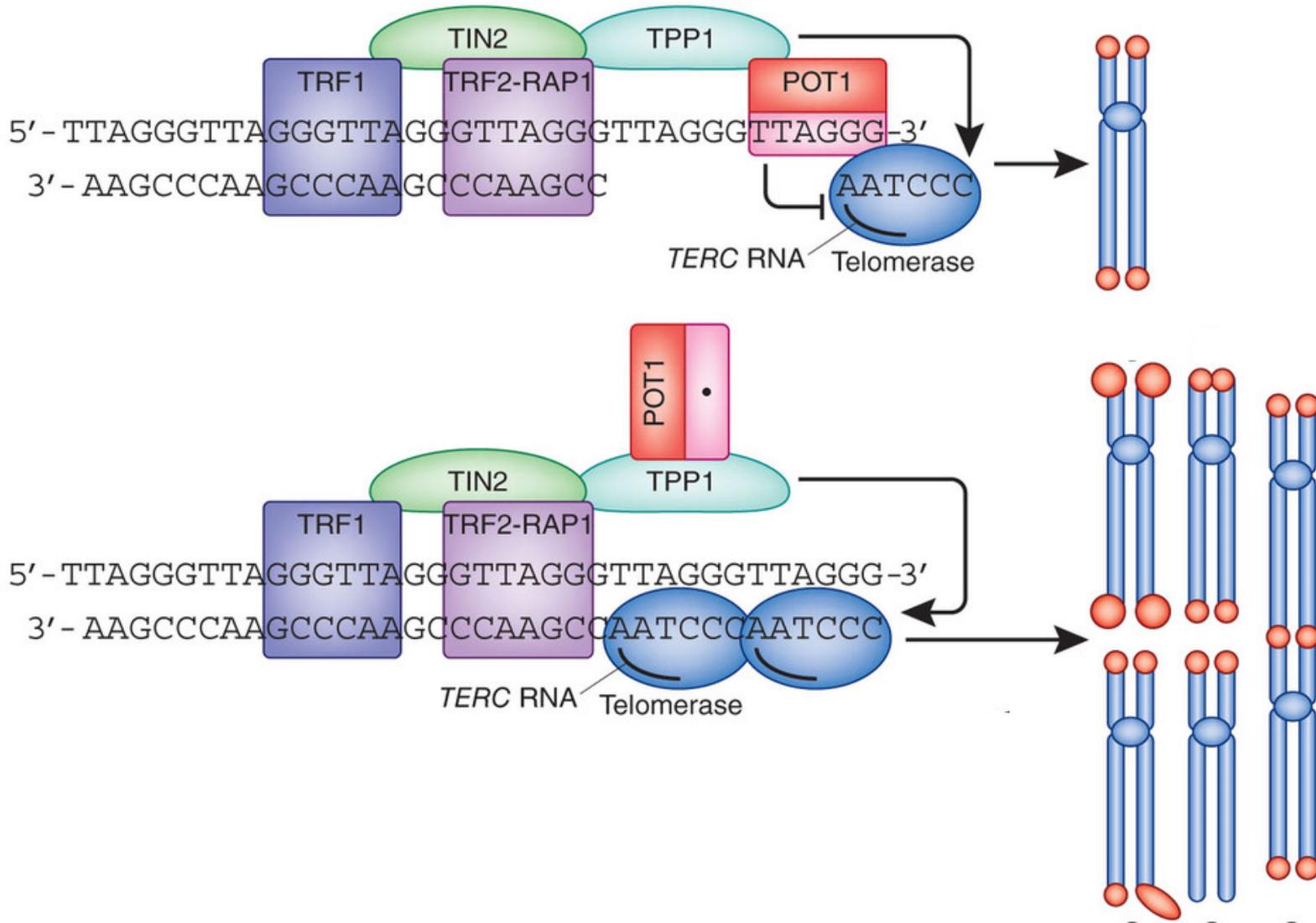




CDK4 и CDK6

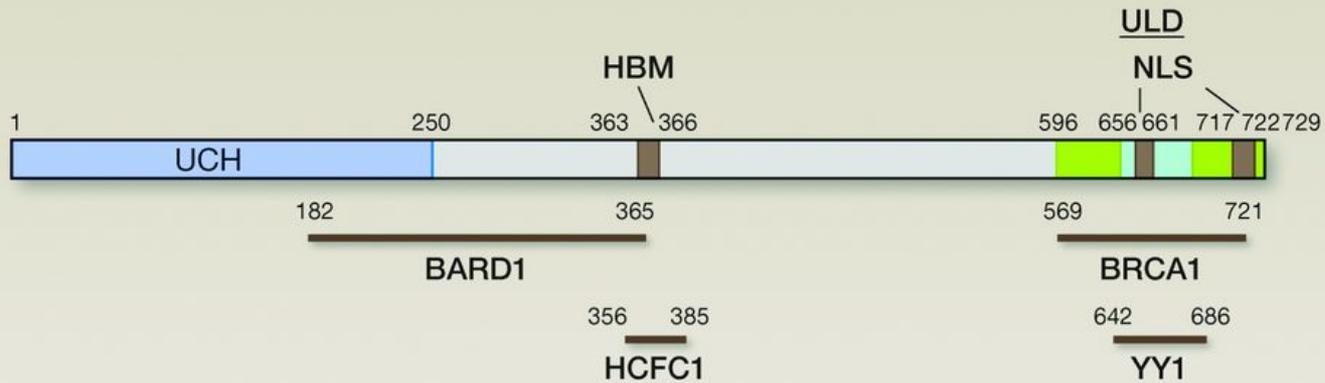


POT1



BAP1

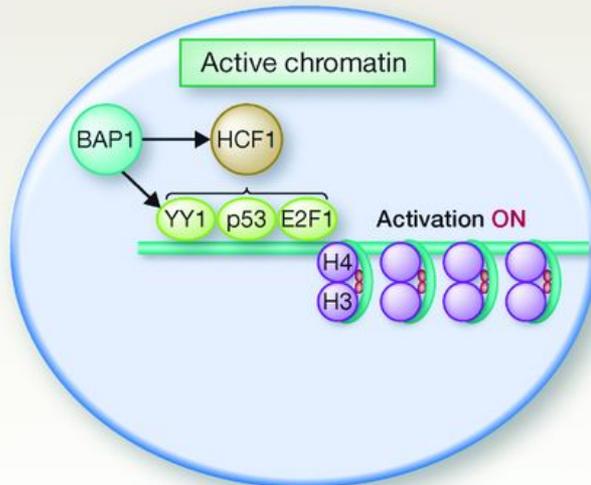
A



B

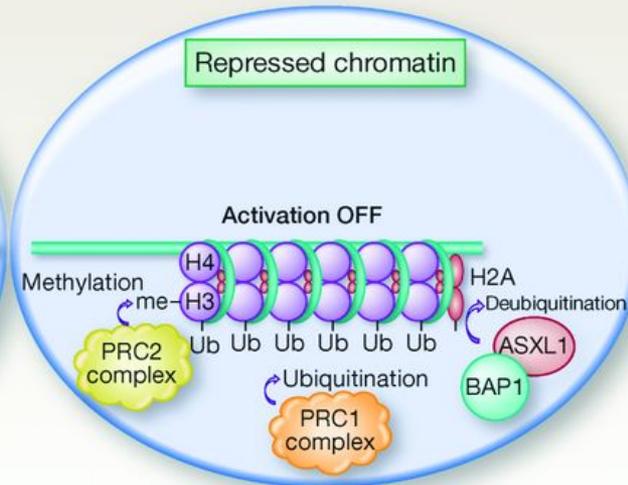
Transcription factor regulation

Interaction partners: HCF1, YY1



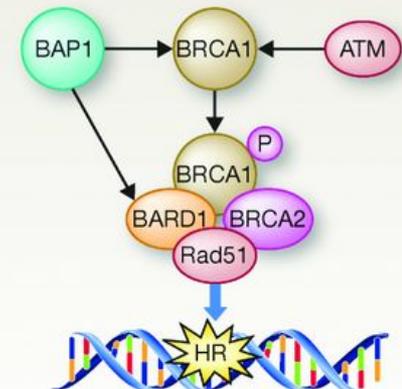
Chromatin modification

Interaction partners: ASXL1, PRC1 complex



Double-strand DNA break repair

Interaction partners: BRCA1, BARD1



Индикации за генетично изследване за наследствени мутации

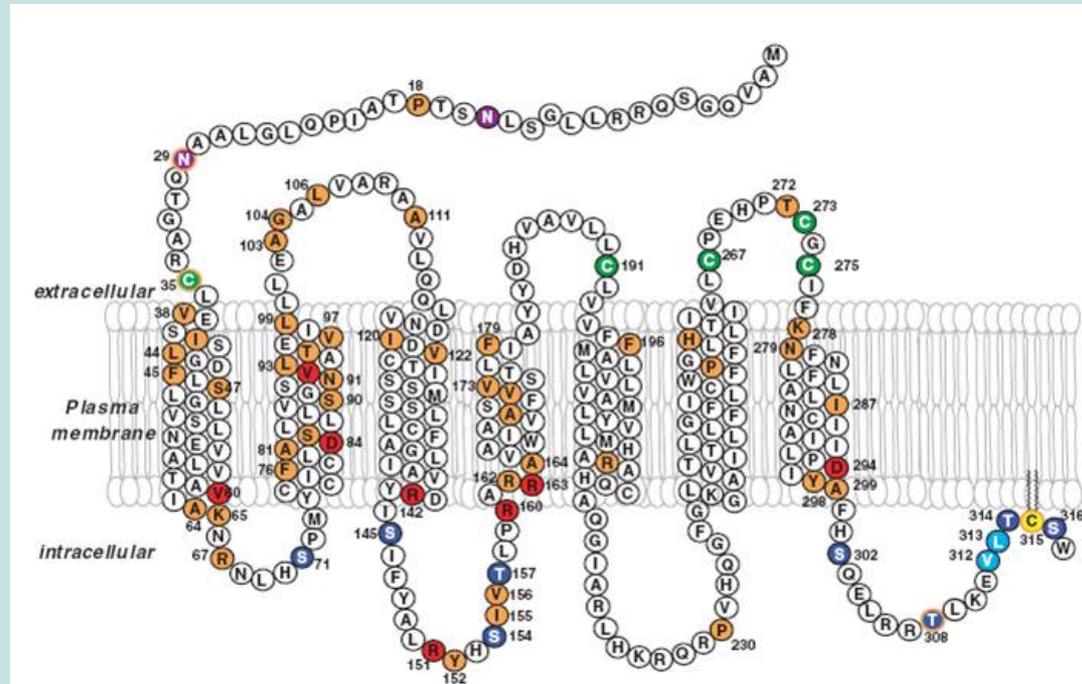
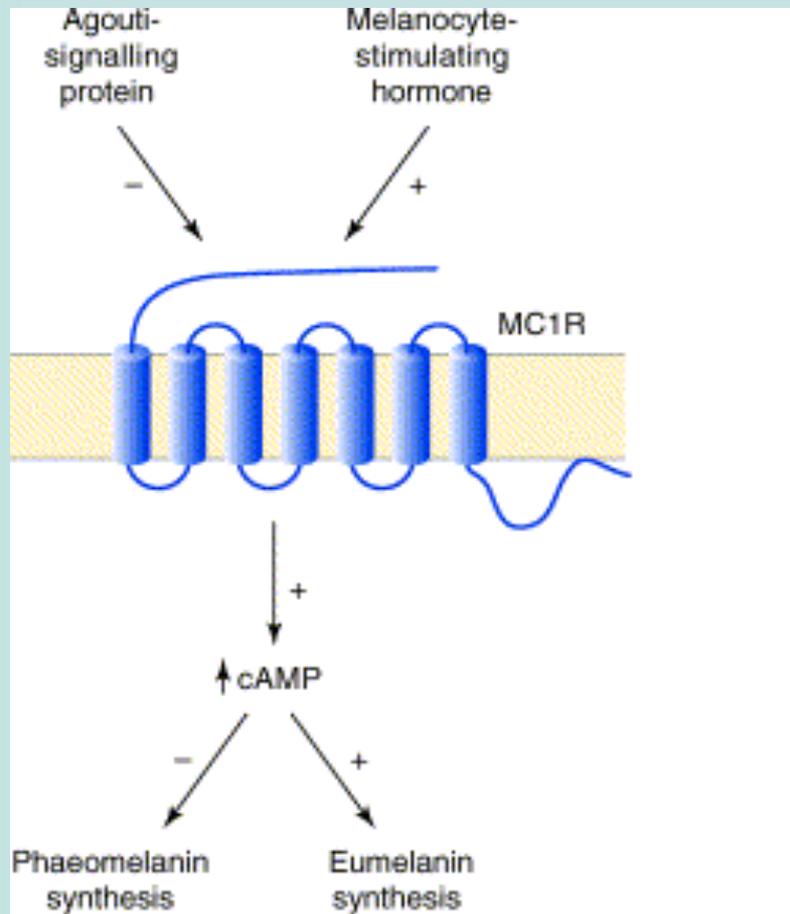
- ✓ Болен с двама или повече родственици от първа/втора степен с меланом;
- ✓ Болен с три или повече първични меланоми;
- ✓ Болен с меланом или рак на панкреаса и с двама или повече родственици от първа/втора степен с меланом или рак на панкреаса;
- ✓ Здрав индивид от фамилия с известна патологична мутация в p16.



**Пигментни (минорни) гени за
предразположеност към меланом**



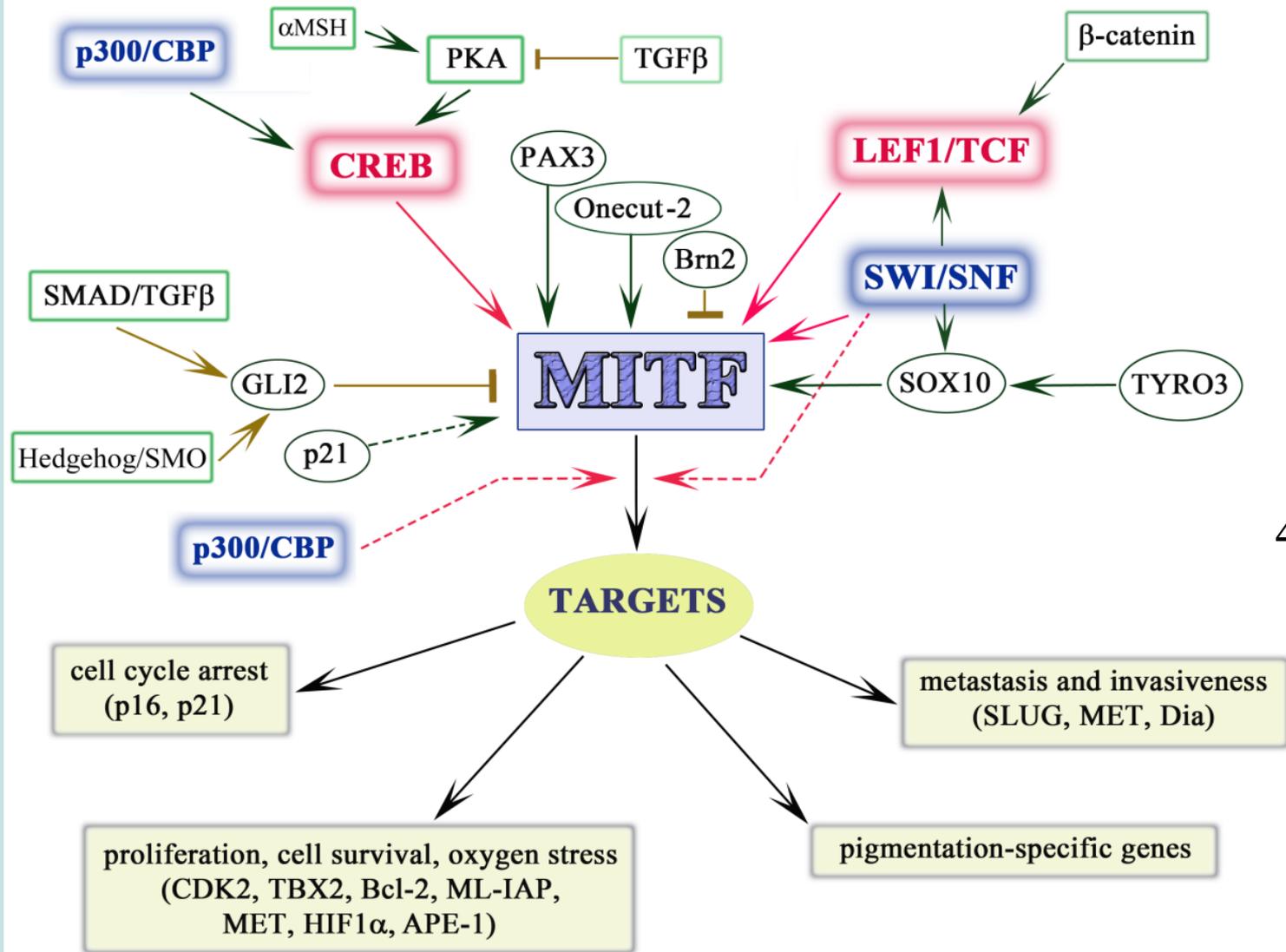
MC1R (alpha melanocyte-stimulating hormone receptor)



MC1R (R163Q) + *CDKN2A* мутации

4X риск

MITF



E318K



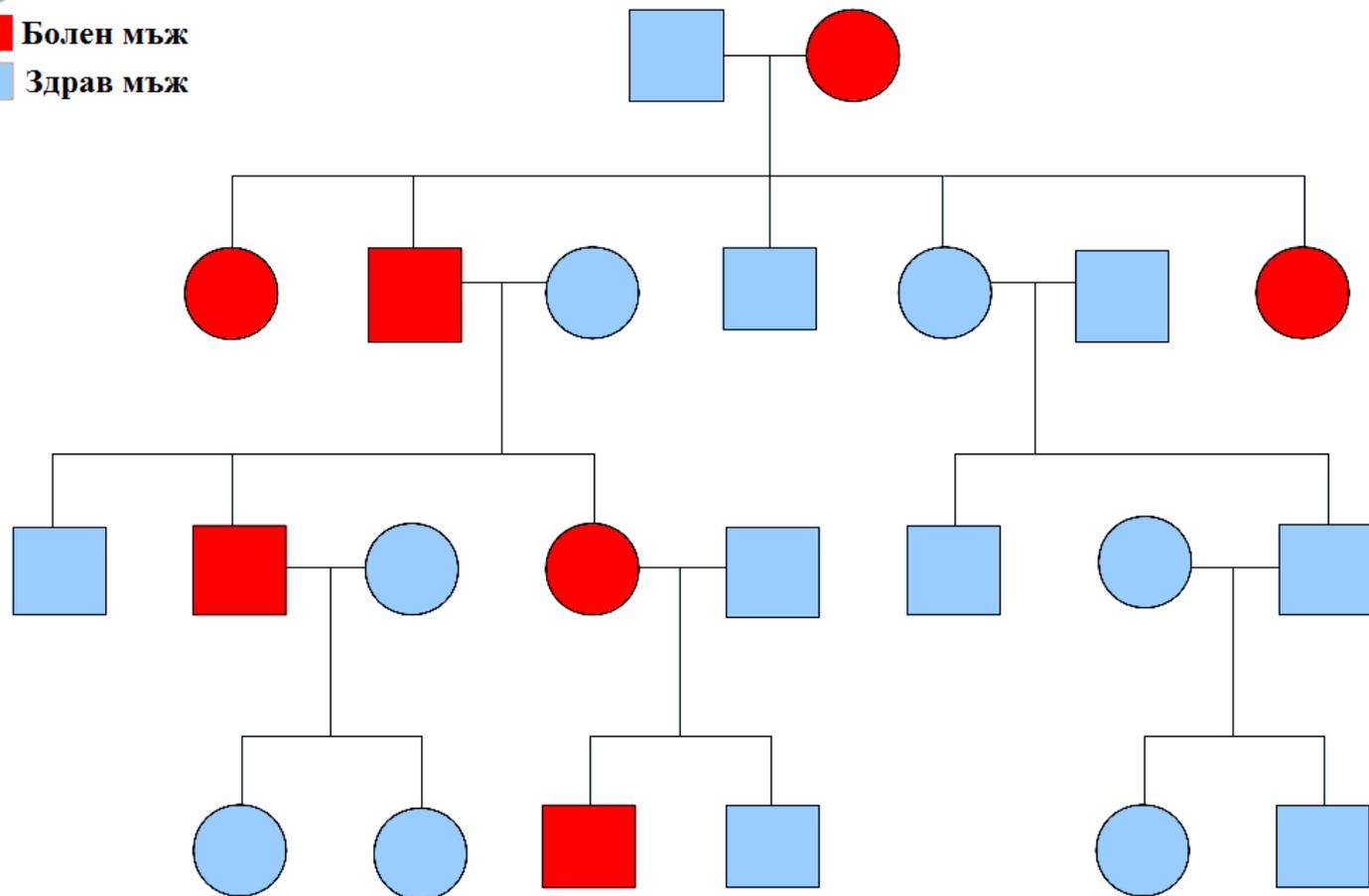
4X риск



**Наследствени синдроми, предразполагащи
към меланом**

Li-Fraumeni синдром (LFS)

- Болна жена
- Здрава жена
- Болен мъж
- Здрав мъж



Li-Fraumeni синдром (LFS)

Normal cell

A

DNA damaging effects

Damaged DNA

p53

p53 over-production

G1 inhibition

DNA restoration

G₁

S

M

G₂

G₀

Tumour cell

B

DNA damaging effects

Damaged DNA

Mutant p53

Synthesis of damaged DNA

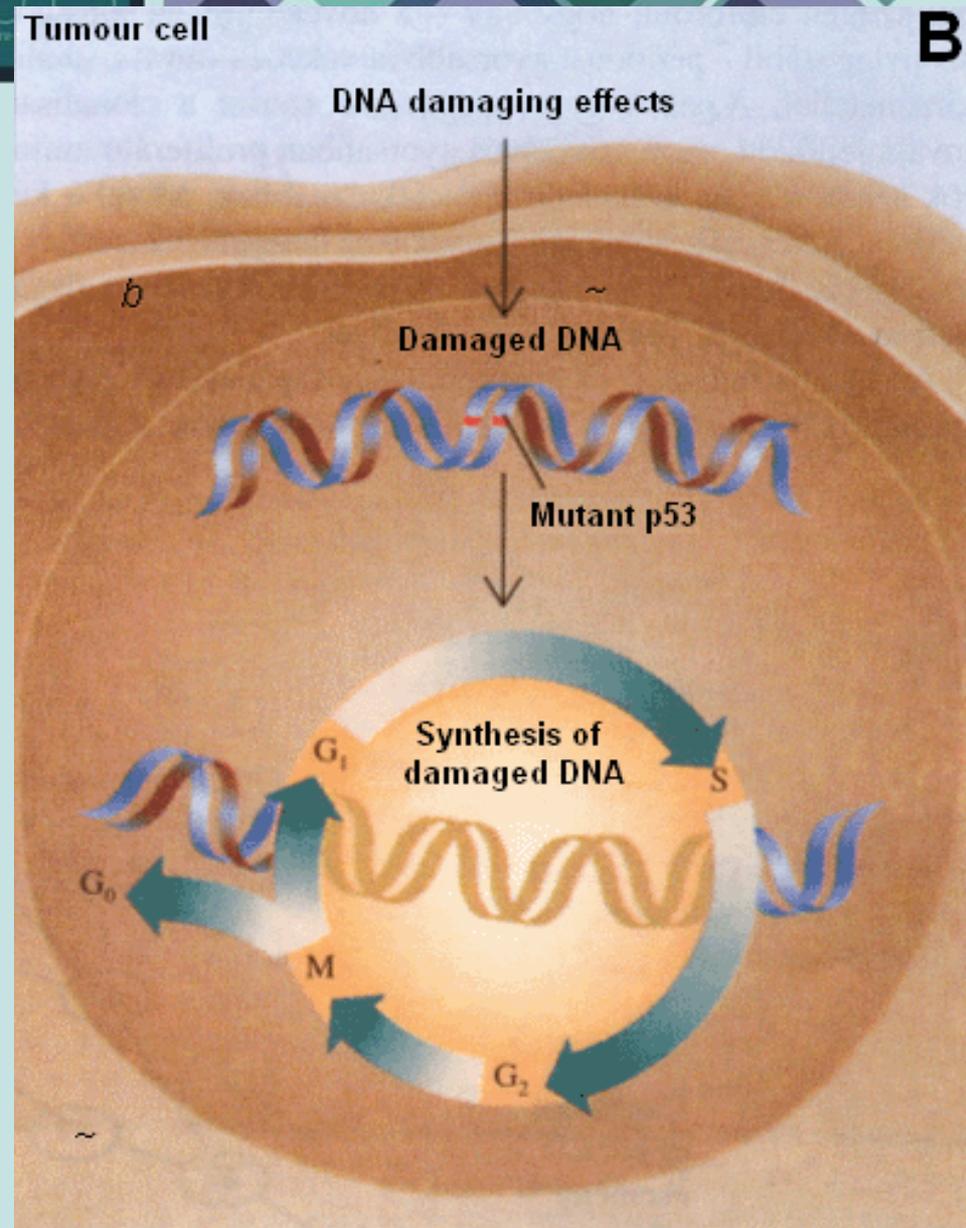
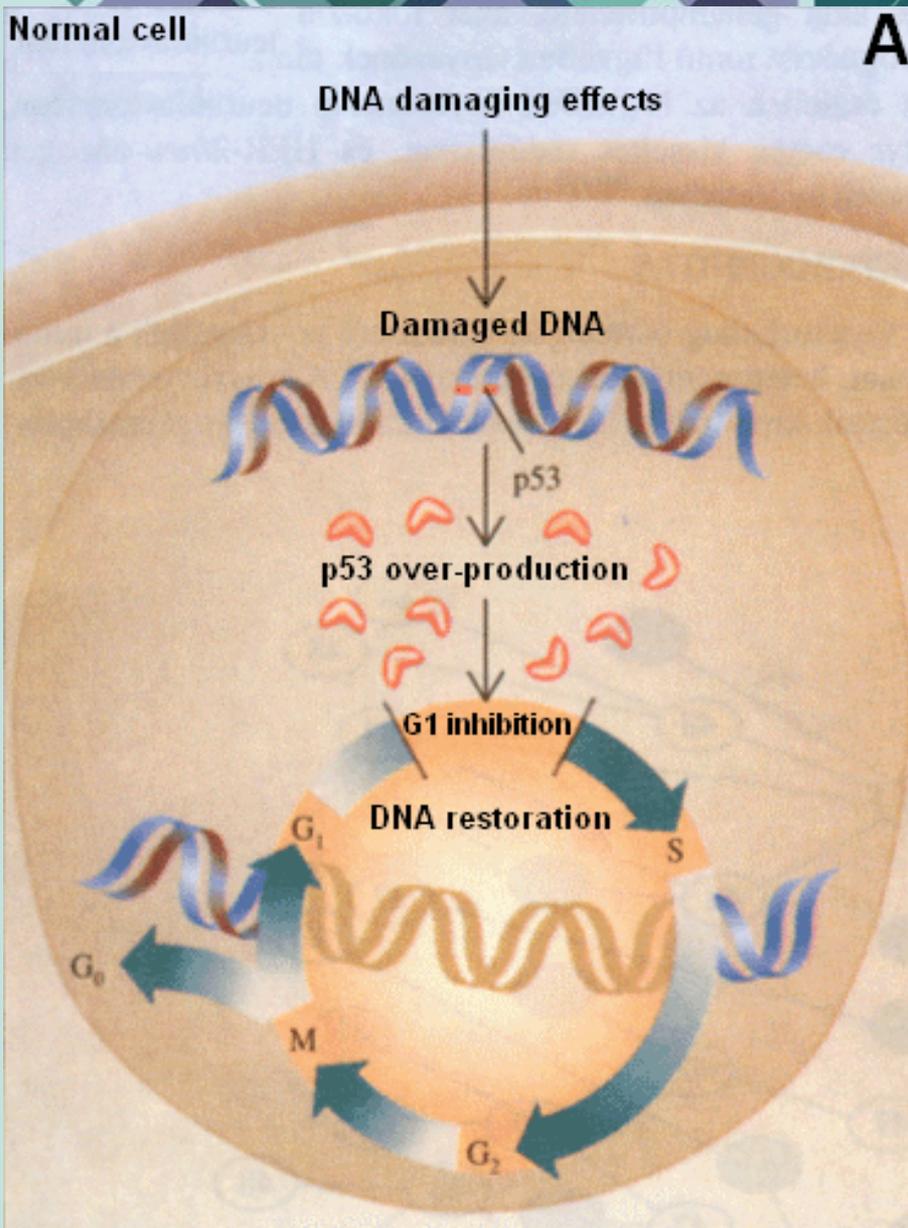
G₁

S

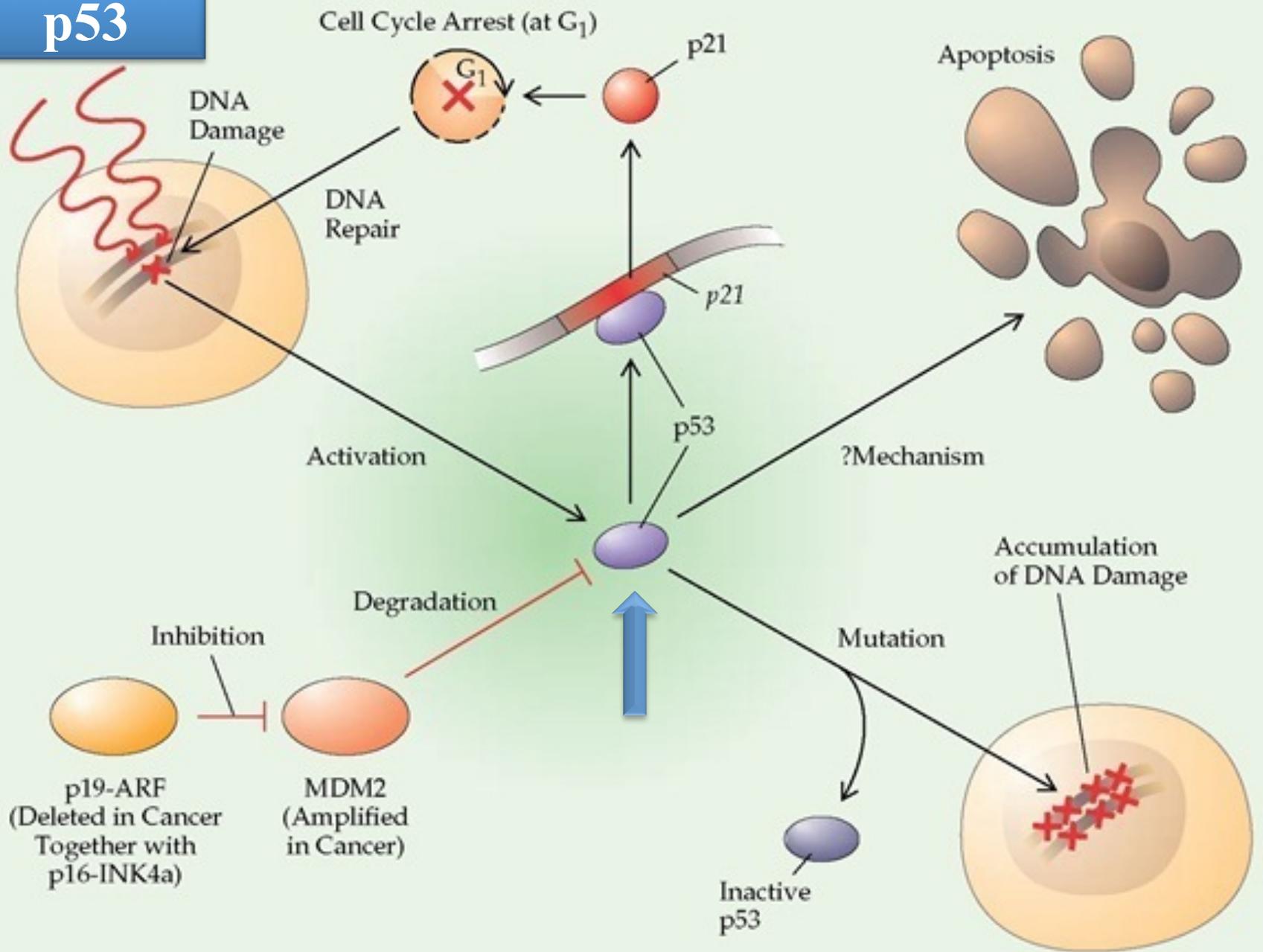
M

G₂

G₀



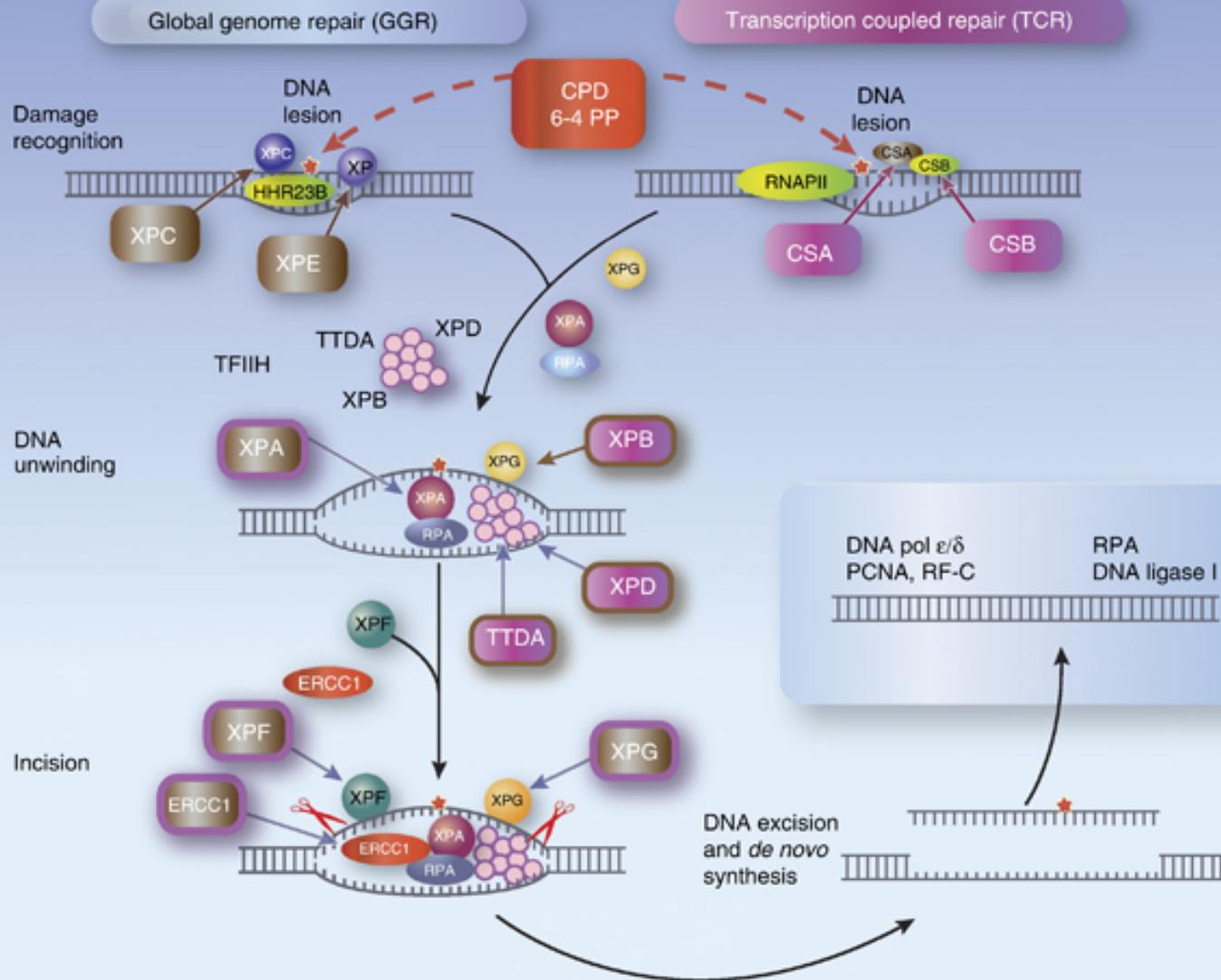
p53



Ксеродерма пигментозум (XP)



ERCC3
(*XPB*),
XPC,
ERCC2
(*XPD*),
DDB2
(*XPE*),
ERCC4
(*XPF*),
ERCC5
(*XPG*),
POLH
(*XP-V*)

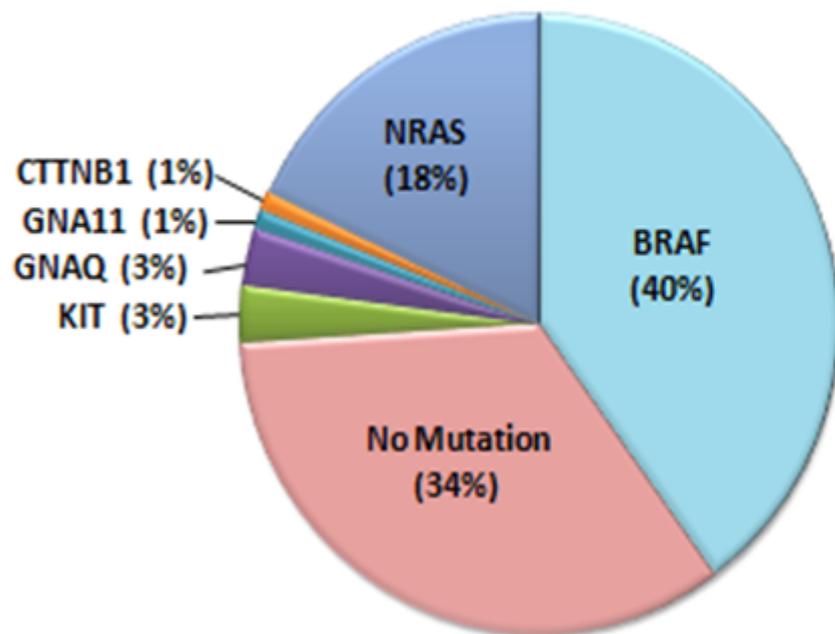




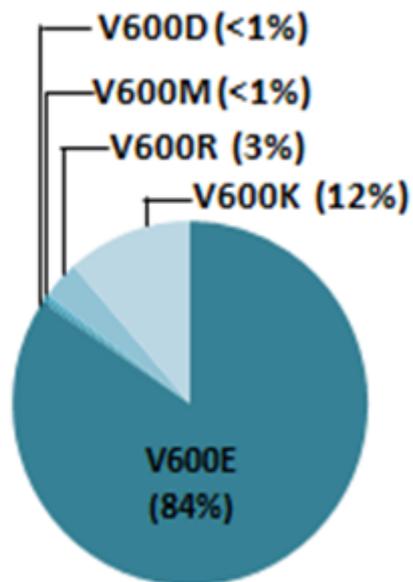
Соматични мутации при спорадични меланоми

Генотипиране на 652 меланоми

2015
www.more-conference.com

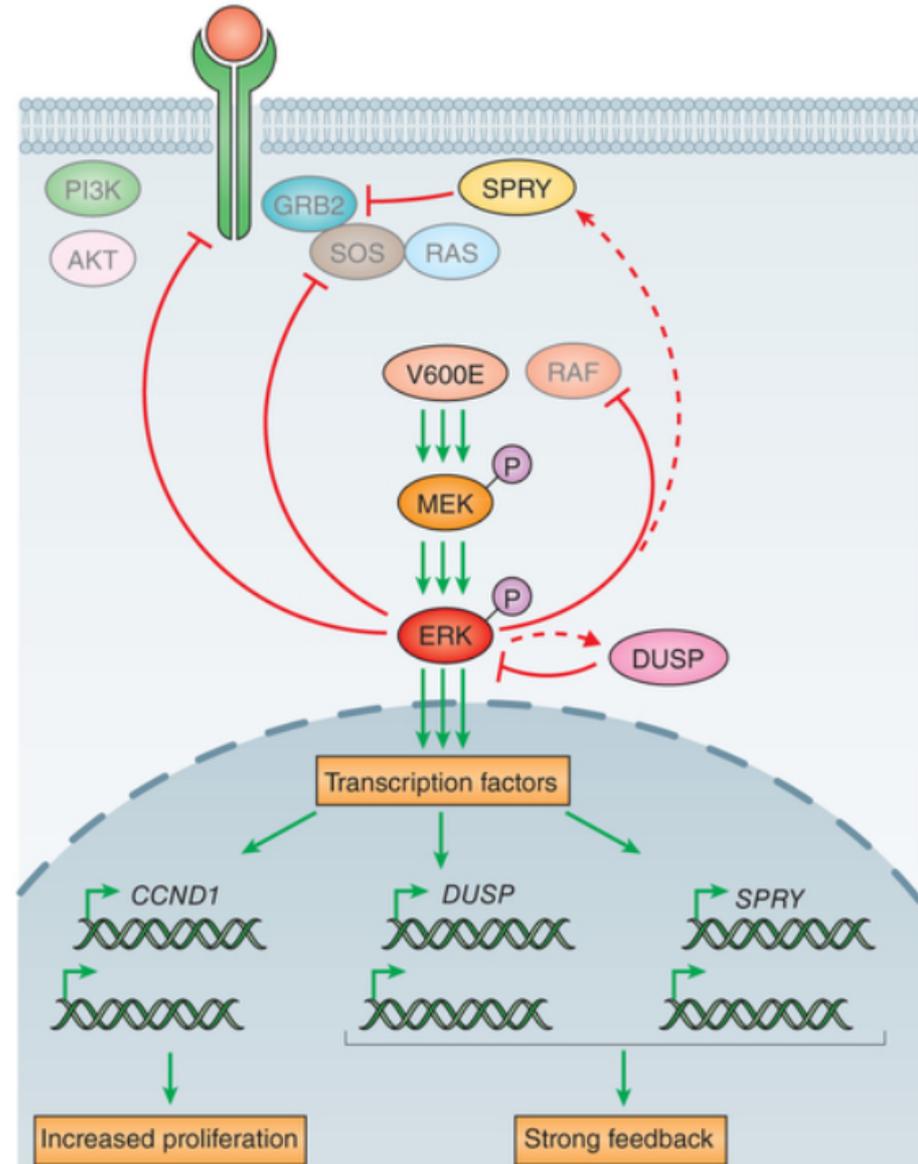
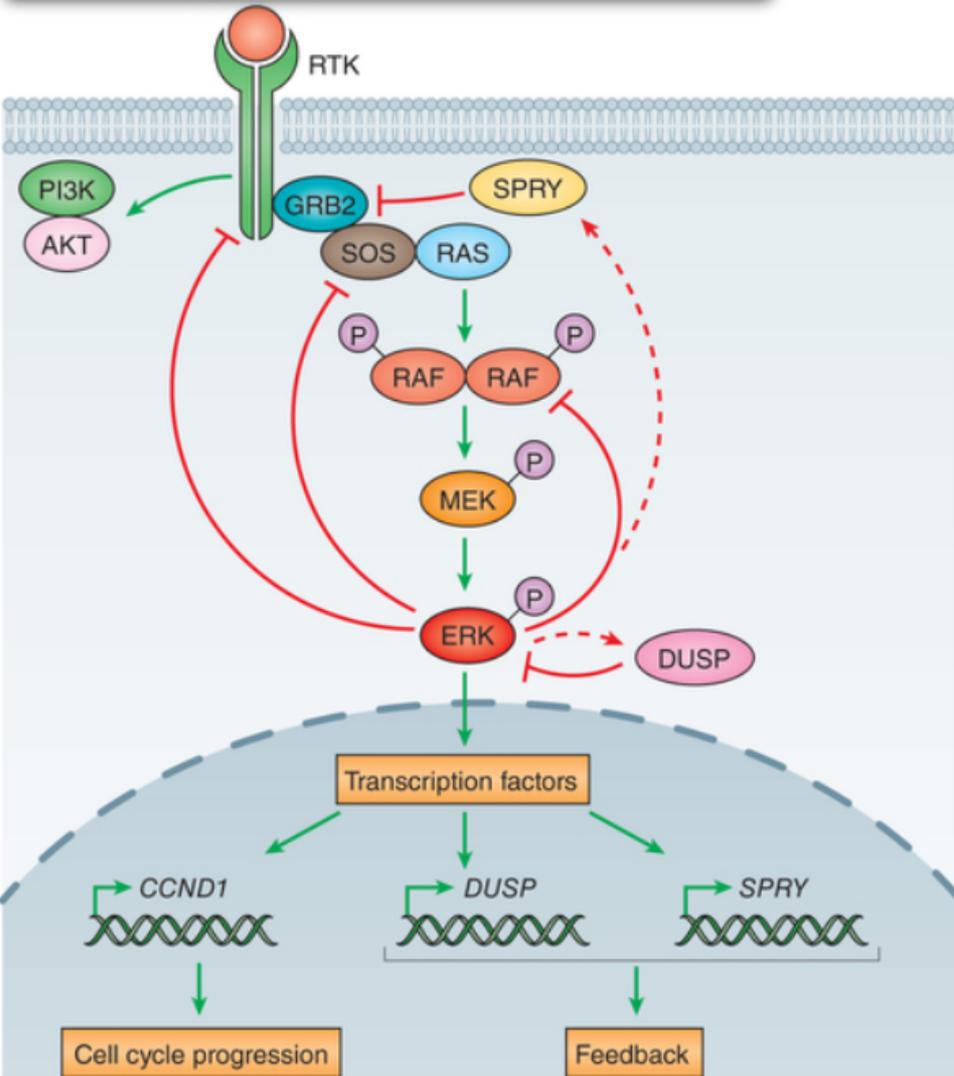


BRAF V600 мутации



BRAF

Ras / Raf / MEK / ERK сигнален път





MY CANCER GENOME

GENETICALLY INFORMED CANCER MEDICINE

Learn About Cancer Mutations

Search Clinical Trials

Find a Cancer Mutation

Melanoma

Select disease to view information about that disease and trials for that disease

BRAF

Select gene to view its significance within that disease and related clinical trials

✓ Select Variant

BRAF c.1789_1790CT>TC (L597S)

BRAF c.1790T>A (L597Q)

BRAF c.1790T>G (L597R)

BRAF c.1798G>A (V600M)

BRAF c.1798_1799GT>AA (V600K)

BRAF c.1798_1799GT>AG (V600R)

BRAF c.1799T>A (V600E)

BRAF c.1799T>G (V600G)

BRAF c.1799_1800TG>AT (V600D)

BRAF c.1799_1800TG>AA (V600E)

BRAF c.1801A>G (K601E)

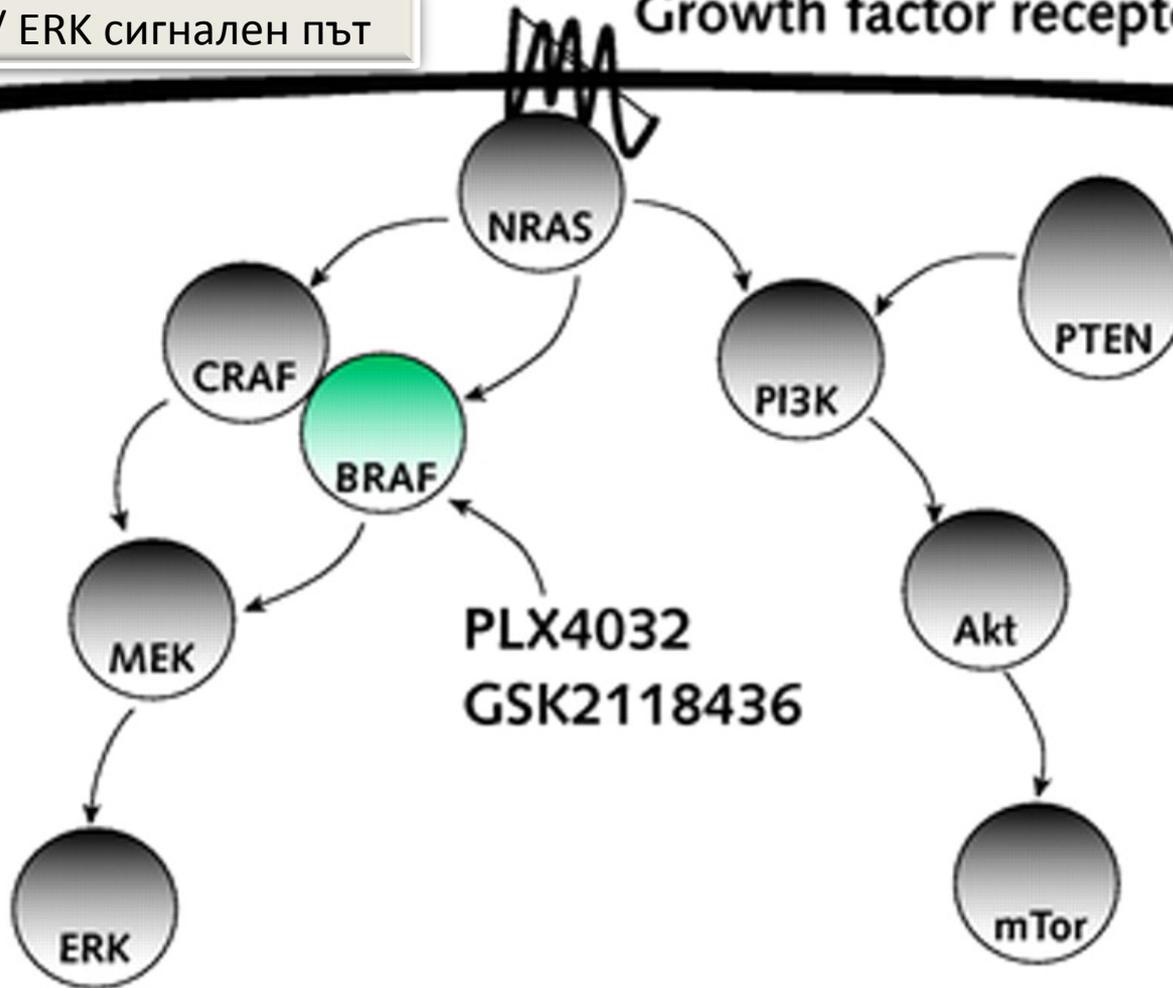
Select gene variant to see its significance within that disease and related clinical trials

NRAS

2015
www.more-conference.com

Ras / Raf / MEK / ERK сигнален път

Growth factor receptor

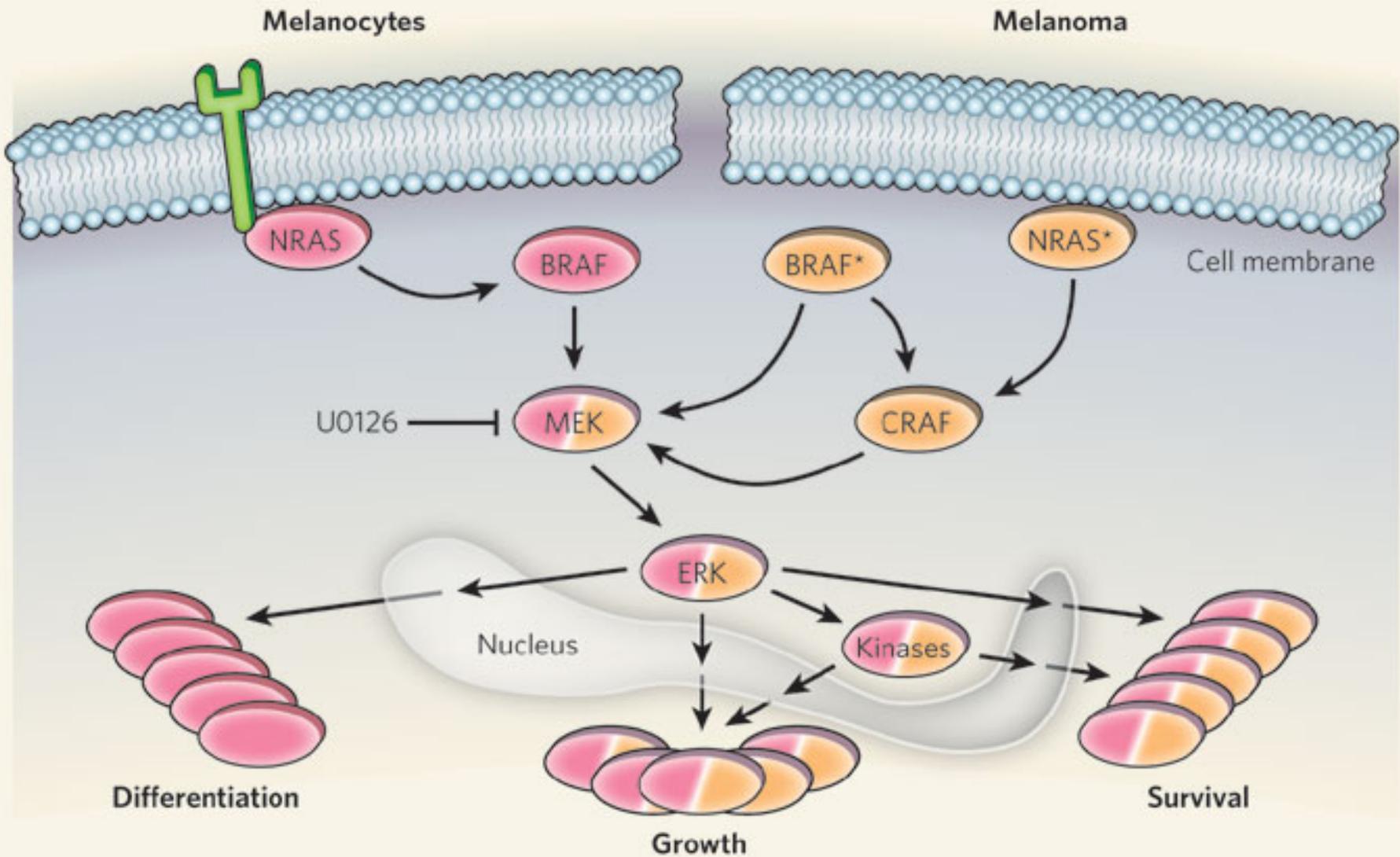


MAPK pathway

PI3K pathway

NRAS

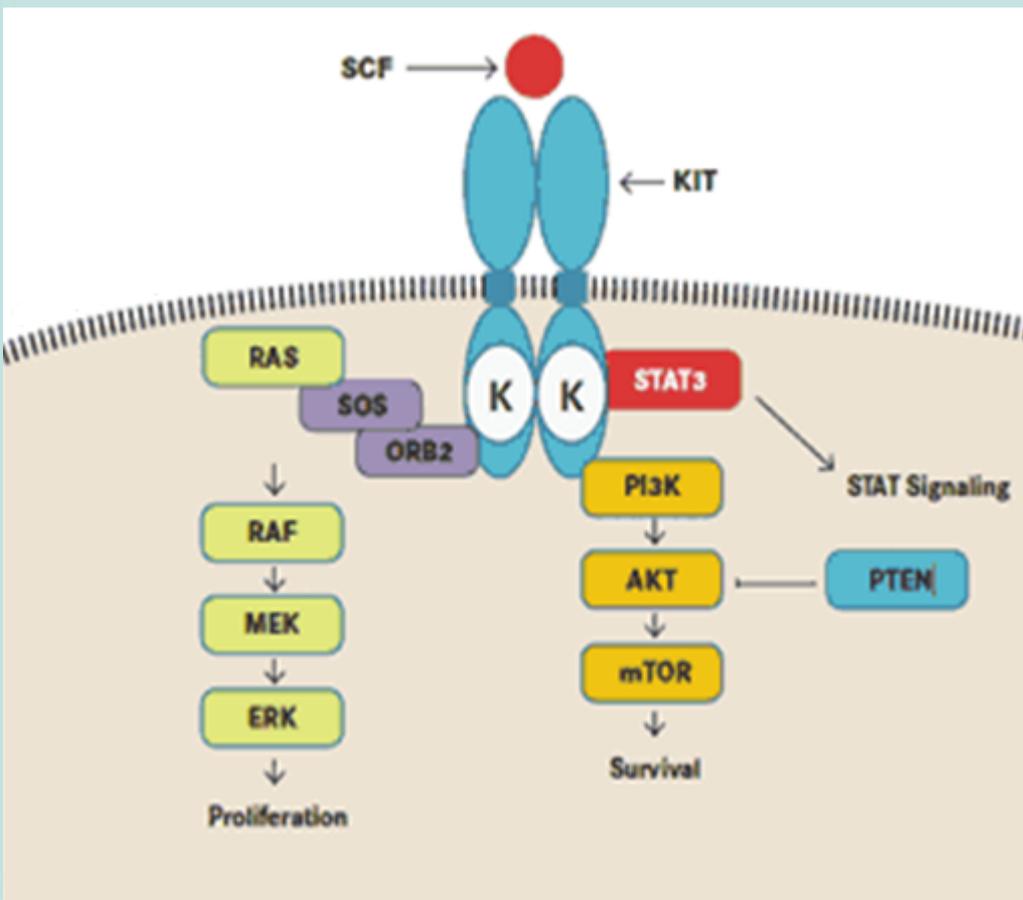
2015
www.more-conference.com



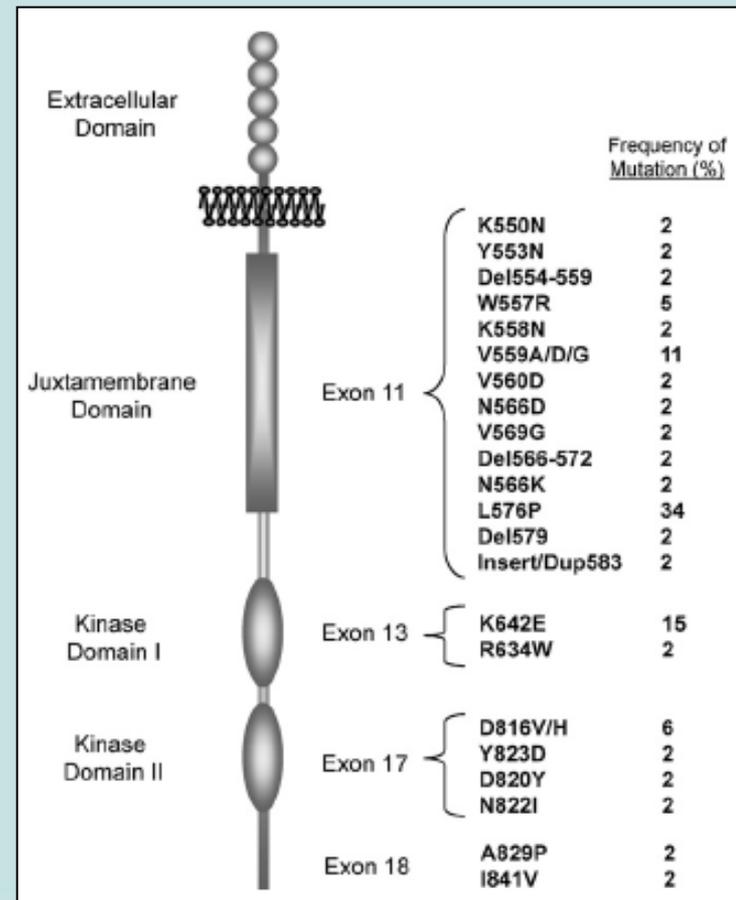
KIT

www.more-conference.com

PI3K / Akt / PTEN / mTOR сигнален път



Мутации в KIT

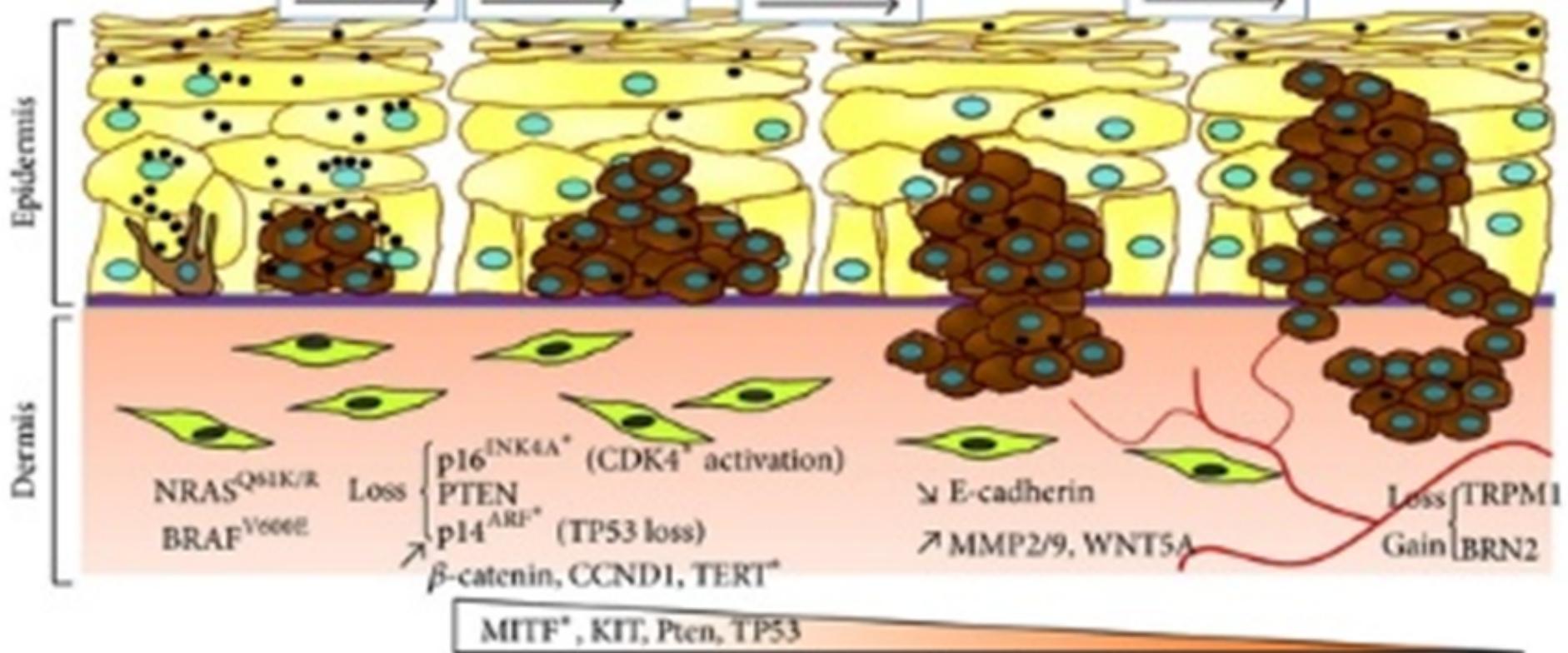


Меланоцити/диспластични
невуси/

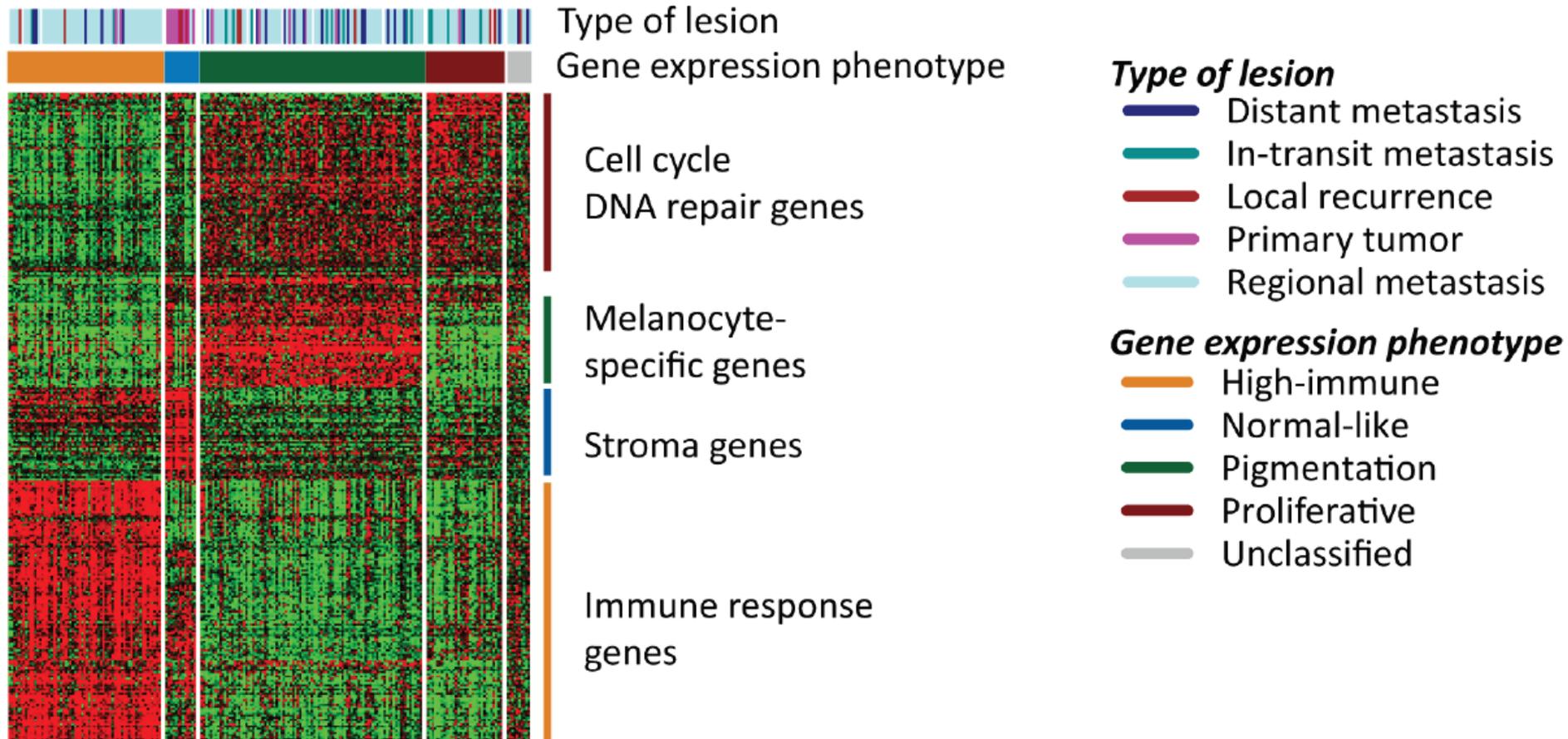
Радиален растеж

Вертикален растеж

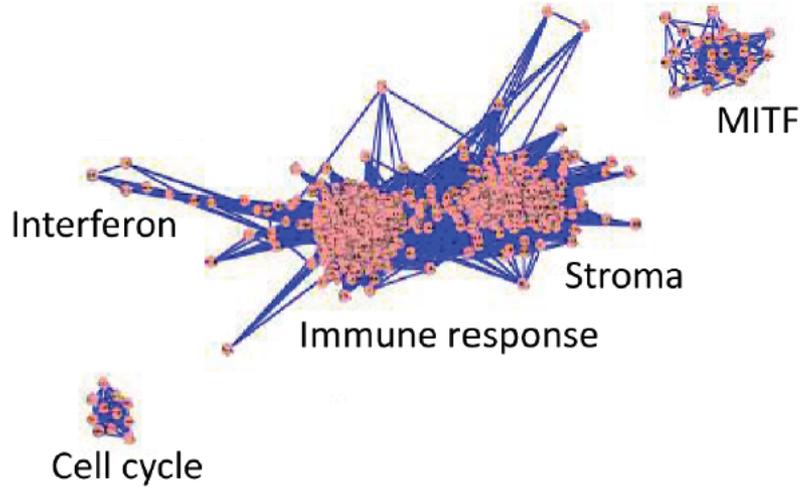
Пролиферация **Обезсмъртяване** **Инвазия** **Метастазирание**



Экспрессия на 299 гени (в редици) в 214 меланоми (в колони)



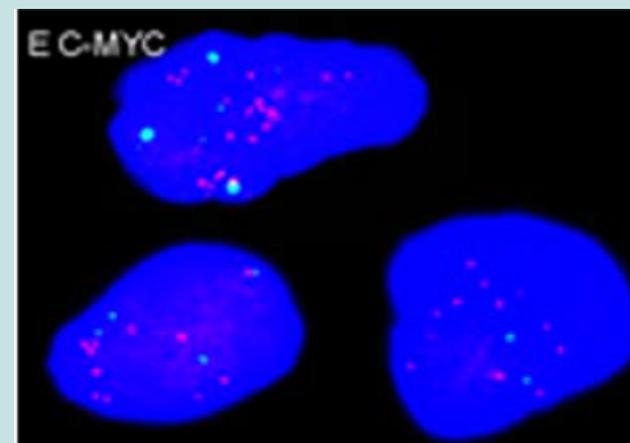
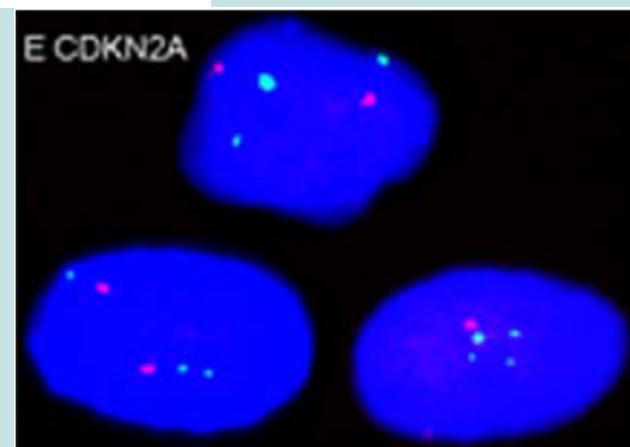
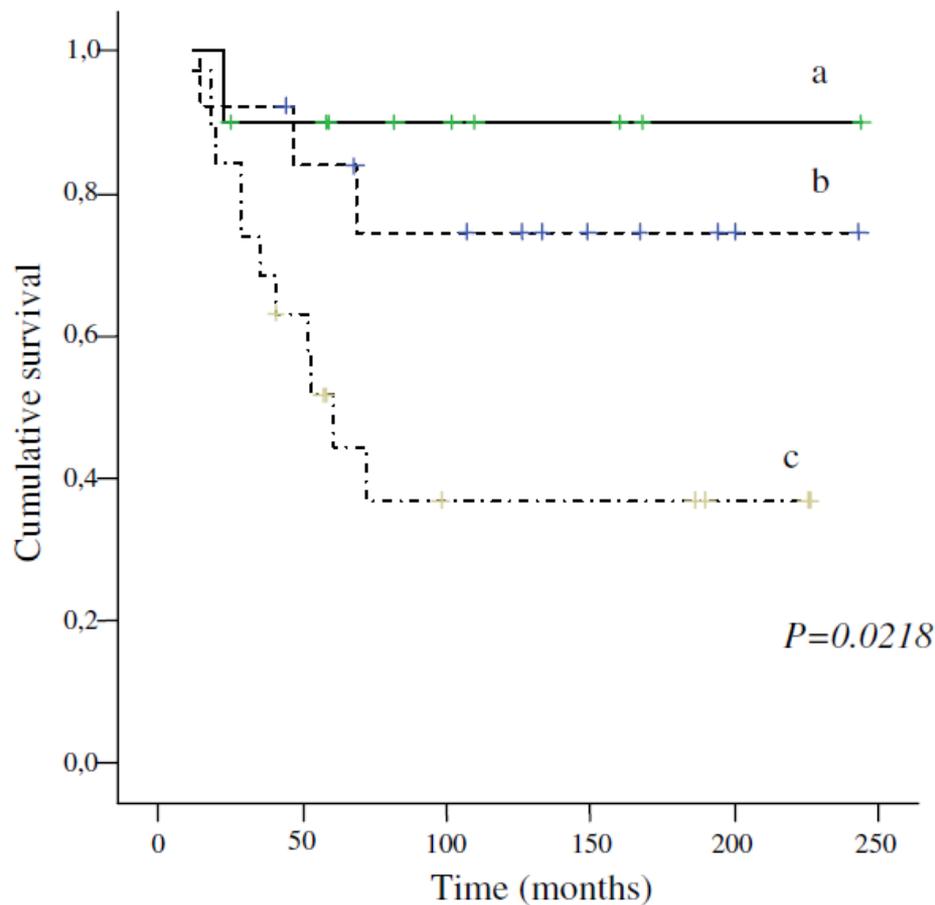
Пет клъстери от гени, свързани с биологични механизми за меланоми



MITF	Ki67	CD3	HE	
				Pigmentation tumor
				Proliferative tumor
				High-immune response tumor

Increased *C-MYC* copy numbers on the background of *CDKN2A* loss is associated with improved survival in nodular melanoma

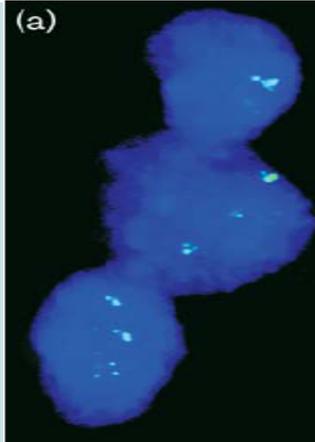
Denitsa Koynova · Ekaterina Jordanova ·
Nicole Kukutsch · Pieter van der Velden ·
Draga Toncheva · Nelleke Gruis



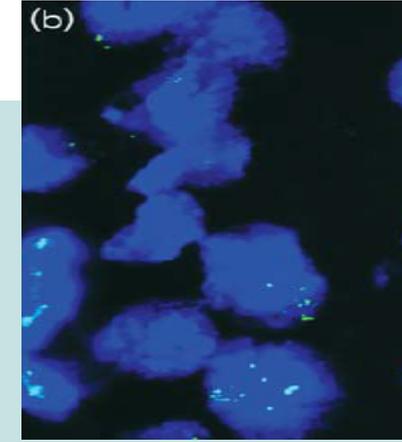
Gene-specific fluorescence in-situ hybridization analysis on tissue microarray to refine the region of chromosome 20q amplification in melanoma

Denitsa K. Koynova^a, Ekaterina S. Jordanova^d, Angel D. Milev^b, Remco Dijkman^e, Krassimir S. Kirov^c, Draga I. Toncheva^a and Nelleke A. Gruis^e

Melanoma Research 2007, Vol 17 No 1



ZNF217



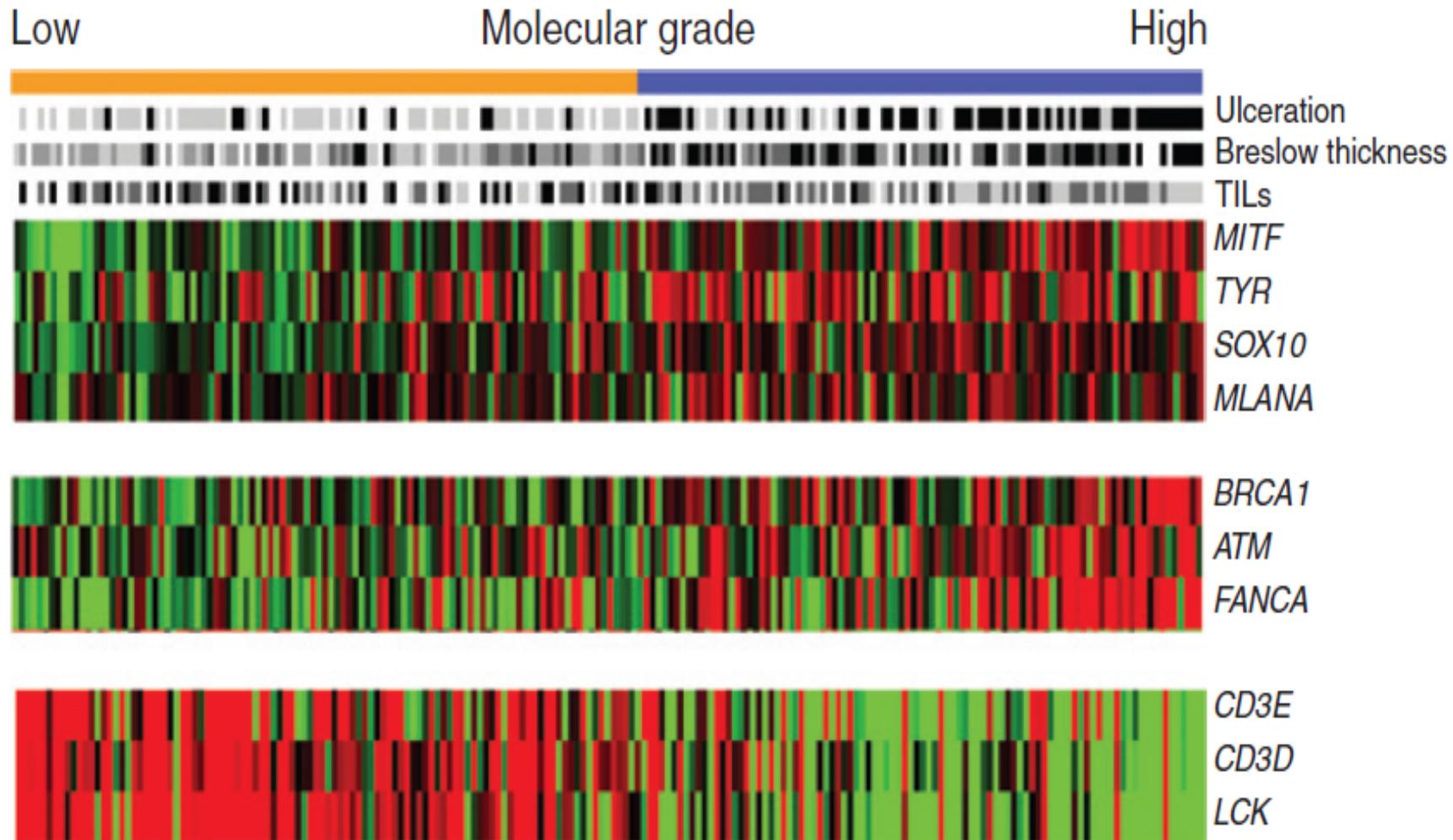
MYBL2

Параметри	Анеупл (%)	STK6		CYP24		ZNF217		MYBL2	
		Гейн (%)	Ампл. (%)						
Първични меланомни	(23.44)	(5.94)	(0.99)	(3.64)	(0.91)	(5.68)	(1.14)	(2.88)	(2.88)
Меланомни метастази	(37.93)	(7.55)	(1.89)	(10.34)	(1.72)	(11.90)	(2.38)	(11.9)	0
Общ брой	(28.34)	(6.45)	(1.29)	(5.85)	(1.17)	(7.63)	(1.53)	(5.41)	(2.03)

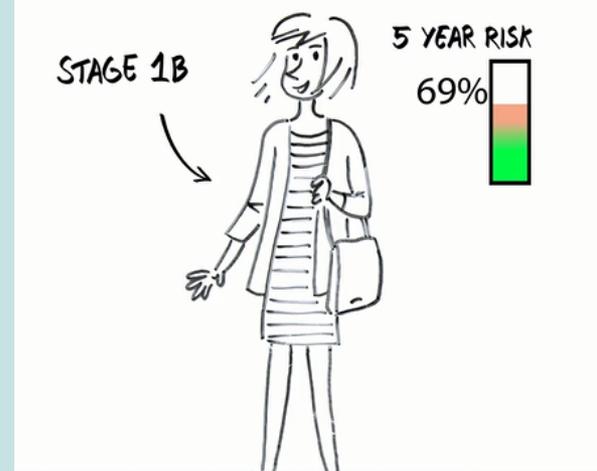
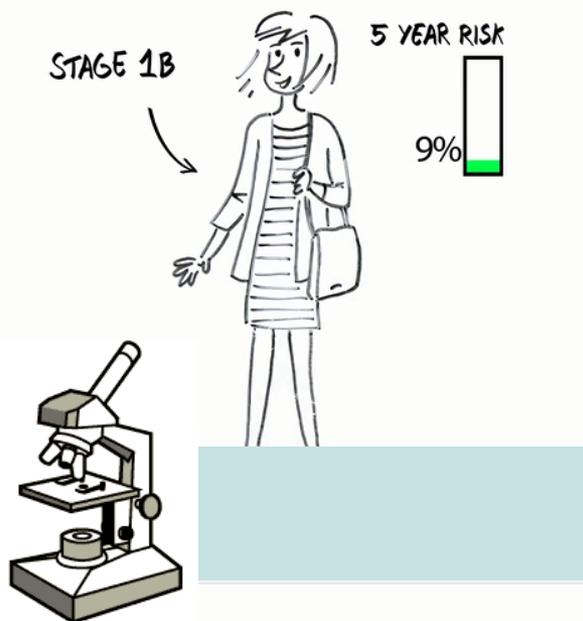
Прогностични маркери при меланоми

Молекулни профили на първични меланоми с ниска и висока степен на туморно развитие

www.more-conference.com



Риск за прогресия на меланома до 5 г. DecisionDx-Melanoma™ генно експресионен профил (GER)



Предиктивни маркери при меланоми

43 соматични точкови мутации в 6 гена, свързани с таргетна терапия на меланоми

NRAS

Position	AA mutant	Nucleotide mutant
G12	p.G12C	c.34G>T
	p.G12S	c.34G>A
	p.G12R	c.34G>C
	p.G12V	c.35G>T
	p.G12A	c.35G>C
	p.G12D	c.35G>A
G13	p.G13A	c.38G>C
	p.G13V	c.38G>T
	p.G13R	c.37G>T
	p.G13D	c.38G>A
Q61	p.Q61E	c.181C>G
	p.Q61H	c.183A>T
	p.Q61H	c.183A>C
	p.Q61L	c.182A>T
	p.Q61L	c.182_183AA>TG
	p.Q61K	c.181C>A
	p.Q61P	c.182A>C
	p.Q61R	c.182A>G
p.Q61R	c.182_183AA>GG	
Q209	p.Q209P	c.626A>C
	p.Q209L	c.626A>T
	p.Q209R	c.626A>G

GNAQ

BRAF

Position	AA mutant	Nucleotide mutant
V600	p.V600R	c.1798_1799GT>AG
	p.V600K	c.1798_1799GT>AA
	p.V600E	c.1799T>A
	p.V600E	c.1799_1800TG>AA
	p.V600M	c.1798G>A
	p.V600G	c.1799T>G
	p.V600D	c.1799_1800TG>AT

CTNNB1

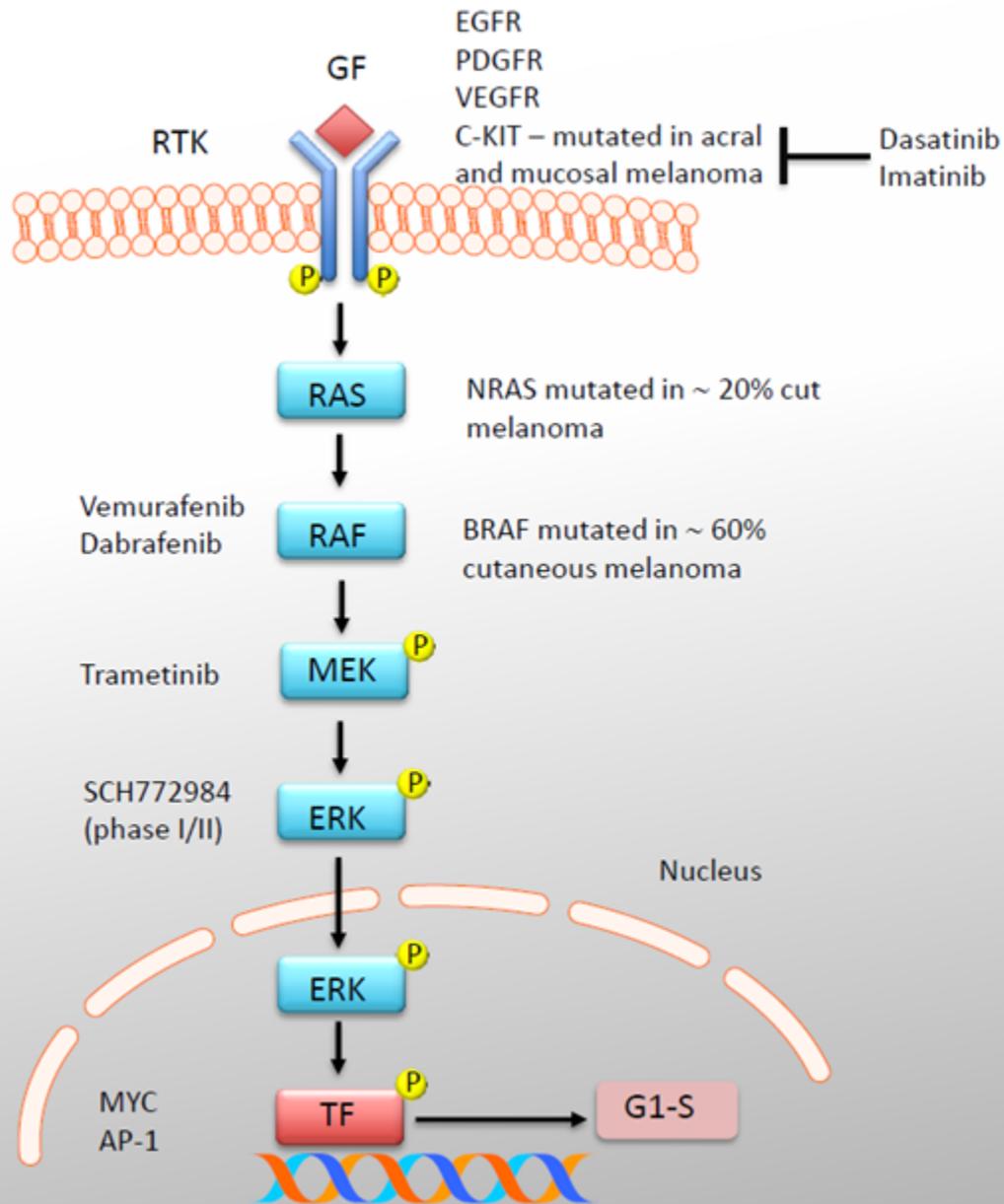
S37	p.S37F	c.110C>T
	p.S37Y	c.110C>A
S45	p.S45P	c.133T>C
	p.S45F	c.134C>T
	p.S45Y	c.134C>A

KIT

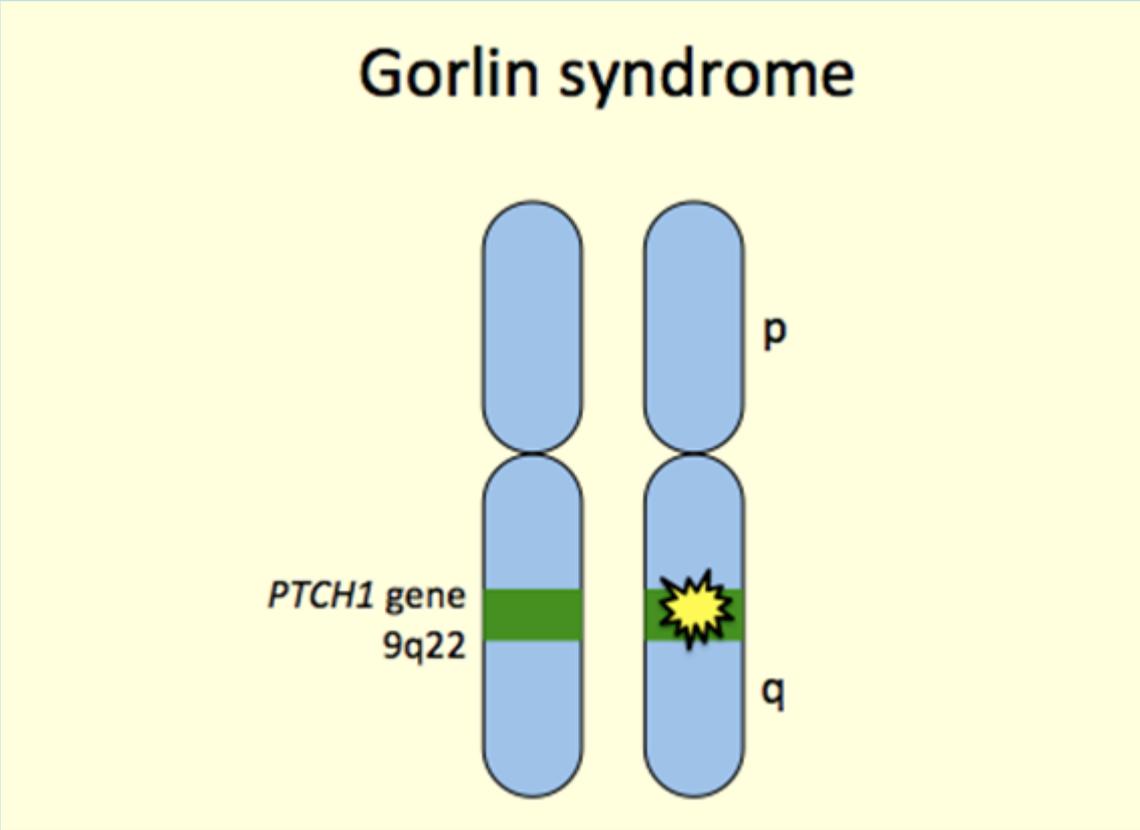
W557	p.W557R	c.1669T>C
	p.W557R	c.1669T>A
V559	p.V559A	c.1676T>C
	p.V559D	c.1676T>A
L576	p.L576P	c.1727T>C
K642	p.K642E	c.1924A>G
D816	p.D816H	c.2446G>C

GNA11

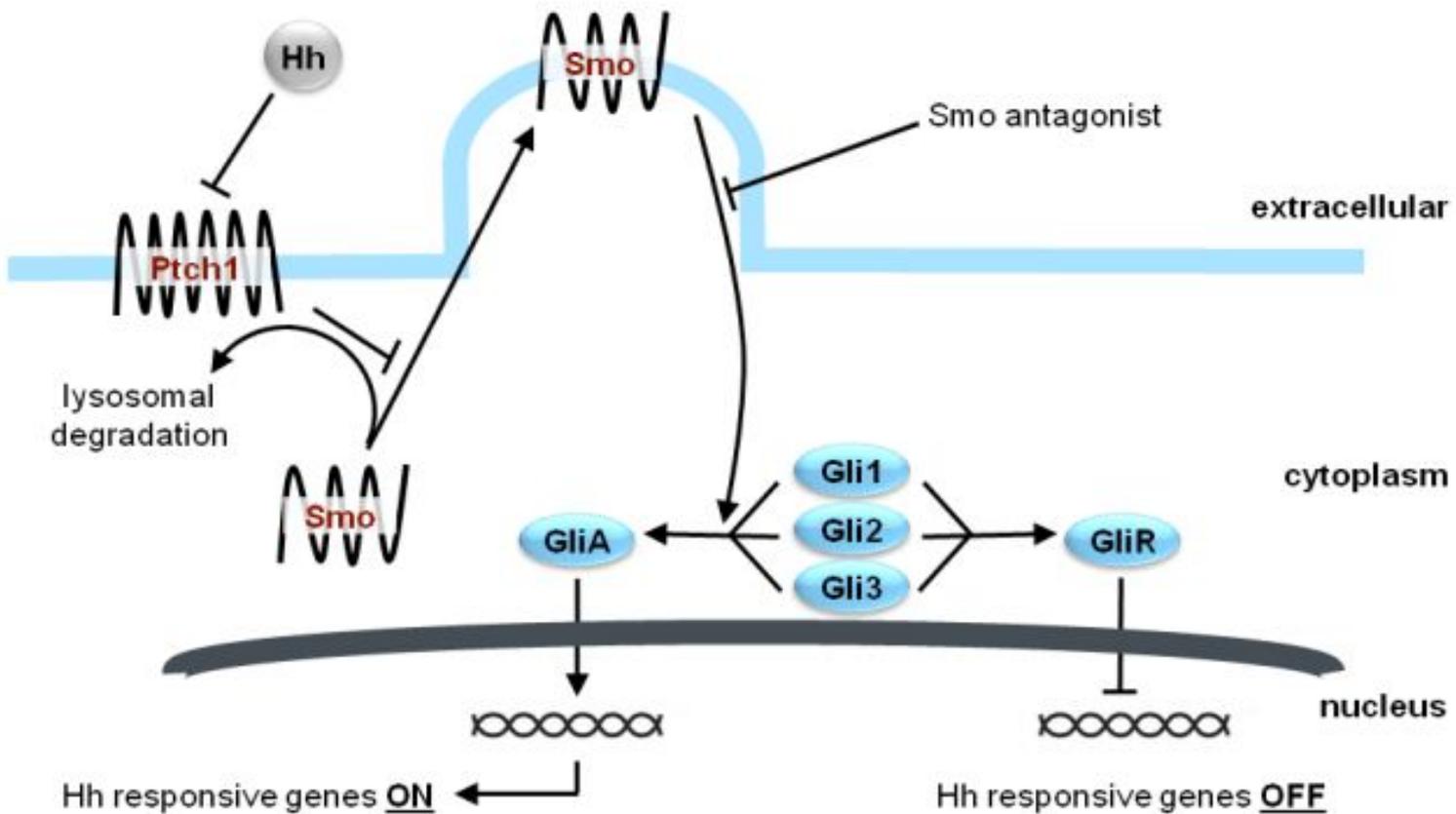
Q209	p.Q209P	c.626A>C
	p.Q209L	c.626A>T



Синдром на Gorlin (NBCCCS - Nevoid Basal Cell Carcinoma Syndrom)

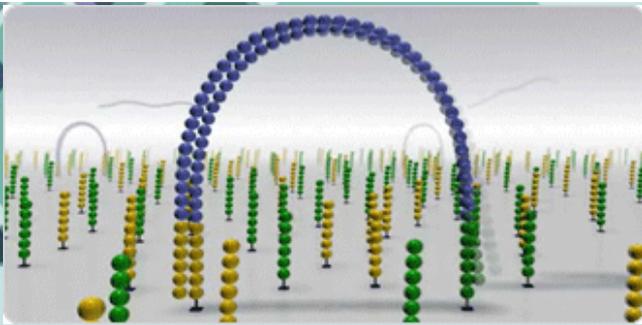


Синдром на Gorlin





NGS ТЕХНОЛОГИЯ



Ново поколение секвенатор – апарат MiSeq на фирмата Illumina



Генен панел

- Design: Illumina Design Studio
- Targets: All exons of 66 genes

Panel 1 = 34 genes

Targets = 594 (exons)

Target bp = 195838 bp

Amplicons = 1494 (max 1536)

Coverage = 95%

Low-Scoring Targets = 13

AKT1	IDH1	NF1
ALK	IDH2	NF2
BRAF	KIT	NRAS
CDK4	KRAS	PDGFRA
DDR2	MAP2K1	PIK3CA
EGFR	MAP2K2	PTEN
ERBB2	MET	RICTOR
FGFR1	MLH1	RPTOR
FGFR2	MLH3	SMO
FGFR3	MSH2	TSC1
GNA11	MTOR	TSC2
GNAQ		

Panel 2 = 32 genes

Targets = 457 (exons)

Target bp = 210570 bp

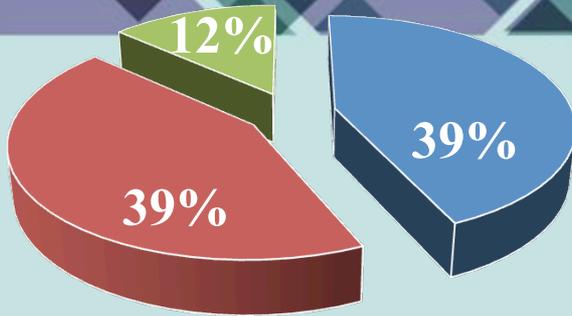
Amplicons = 1448 (max 1536)

Coverage = 93%

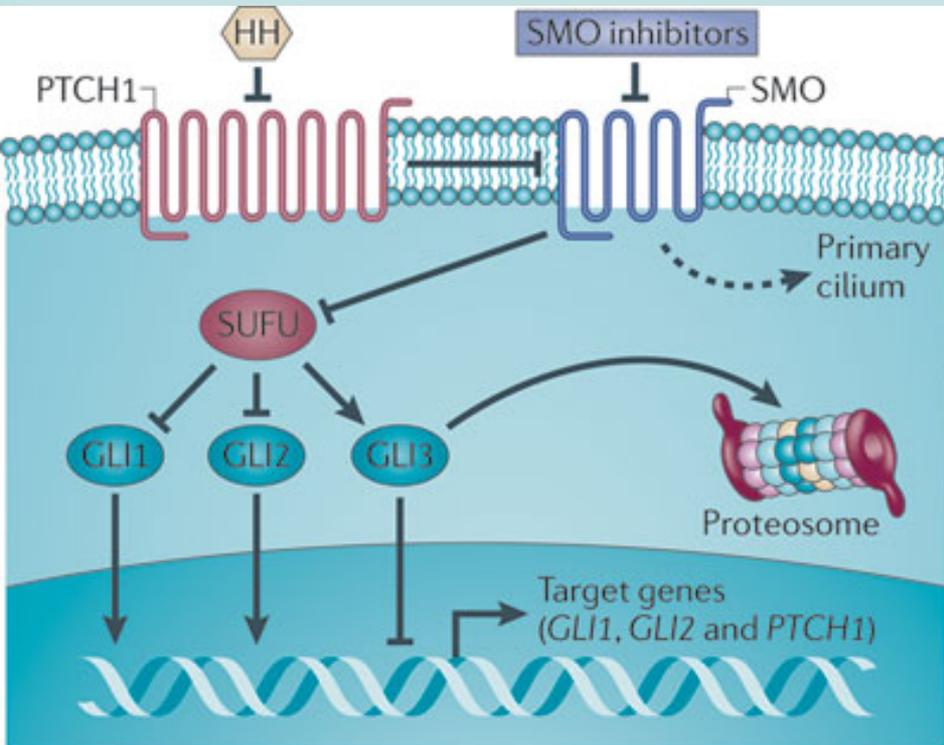
Low-Scoring Targets = 13

AKT2	JAK3	NTRK3
AKT3	KDR	PTCH1
ARAF	MCL1	PTCH2
BCL2	MYC	RAF1
BCL2L1	MYCL1	RB1
ERBB3	MYCN	RET
ERBB4	NOTCH1	SMAD4
FGFR4	NOTCH2	STK11
HRAS	NOTCH3	TP53
JAK1	NTRK1	IGF1R
JAK2	NTRK2	

Молекулен профил на базално-клетъчен карцином (ВСС)



■ TP53 ■ PTCH1 ■ SMO



SMO c.203_204delCCinsTT (A68V)

SMO c.595C>T (R199W)

SMO c.1046_1047delCCinsTT (T349I)

SMO c.1417G>C (D473H)

SMO c.1452_1453delCCinsTT (R485W)

SMO c.1542_1543delCCinsTT (L515F)

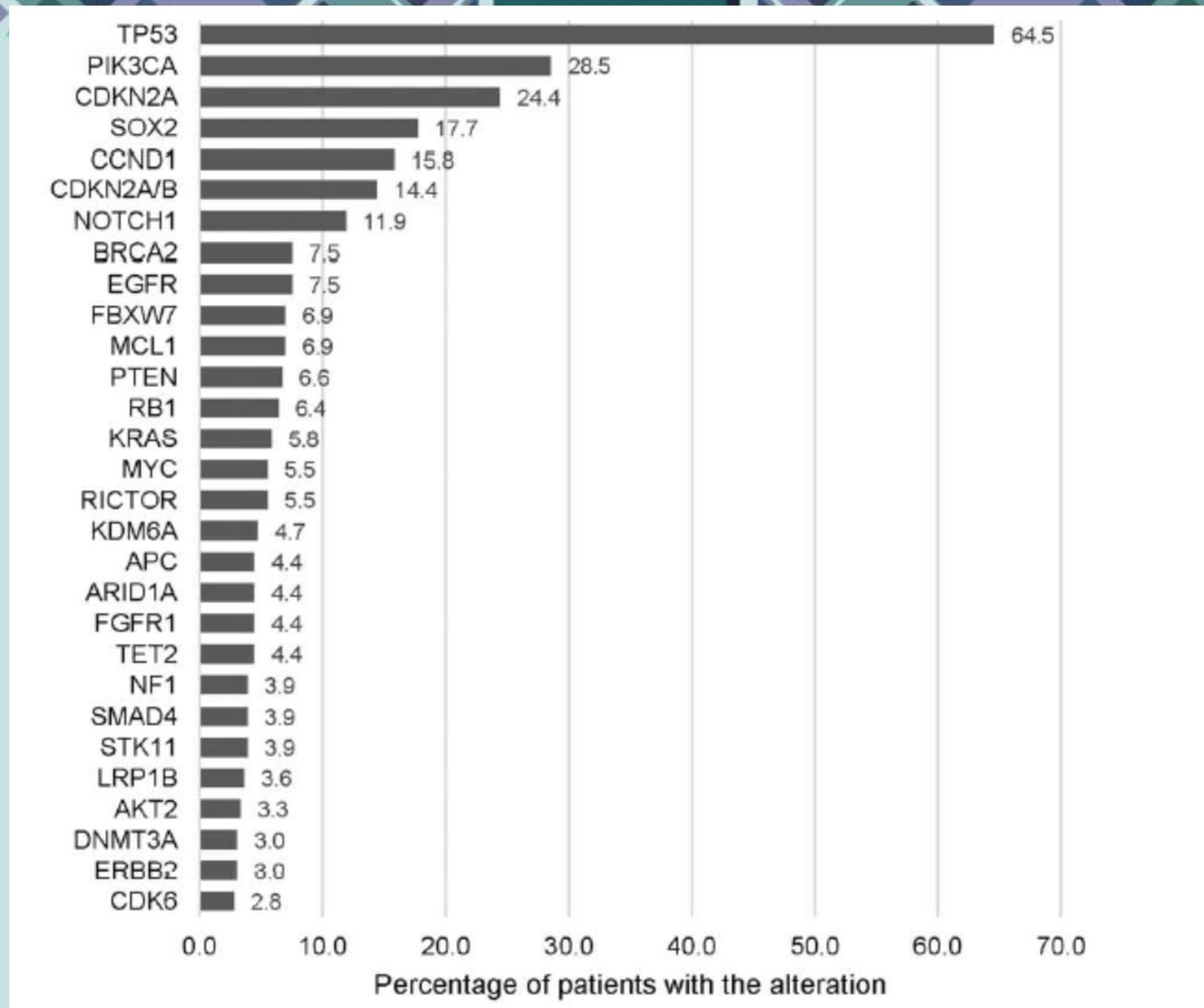
SMO c.1604G>T (W535L)

SMO c.1685G>A (R562Q)

SMO c.1955C>T (A652V)

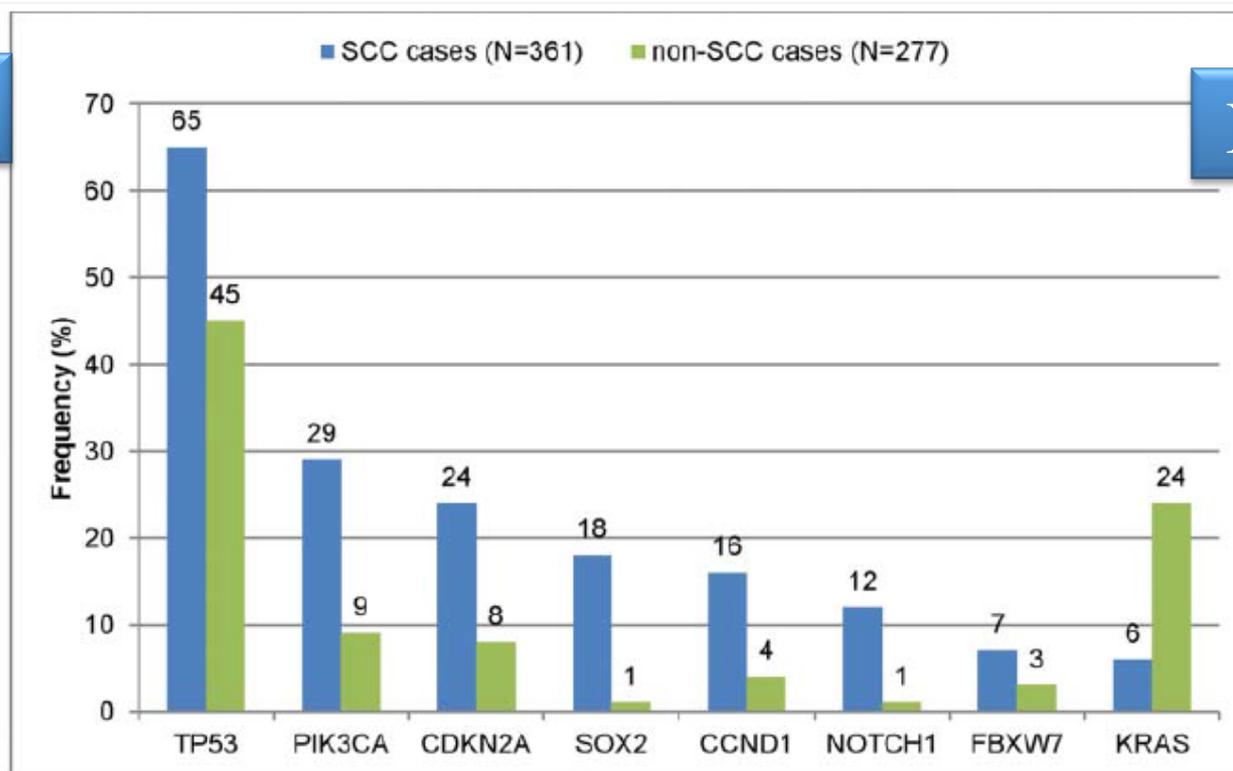
SMO c.2263_2264delCCinsTT (P755F)

Молекулен профил на 361 пациенти със сквамозно-клетъчен карцином (SCC)

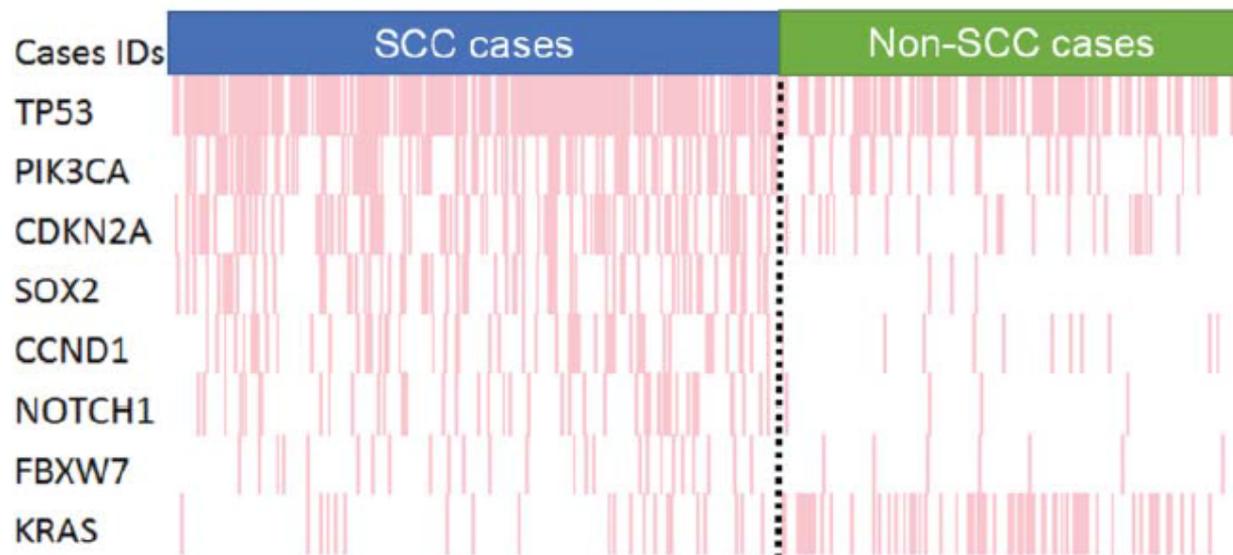


SCC

Non-SCC



B.



- Рутинно мултигенно профилиране на меланомни и не-меланомни тумори
 - Определя пациенти с клинично значими водещи мутации
 - Въвежда генетичната информация в клиничната практика на онколозите
 - Важен фактор в клиничните проучвания
 - Позволява бързо разкриване на нови, потенциални генни мишения при ‘pan-negative’ случаи
 - BRAF L597 mutations and MEK inhibitors



БЛАГОДАРЯ