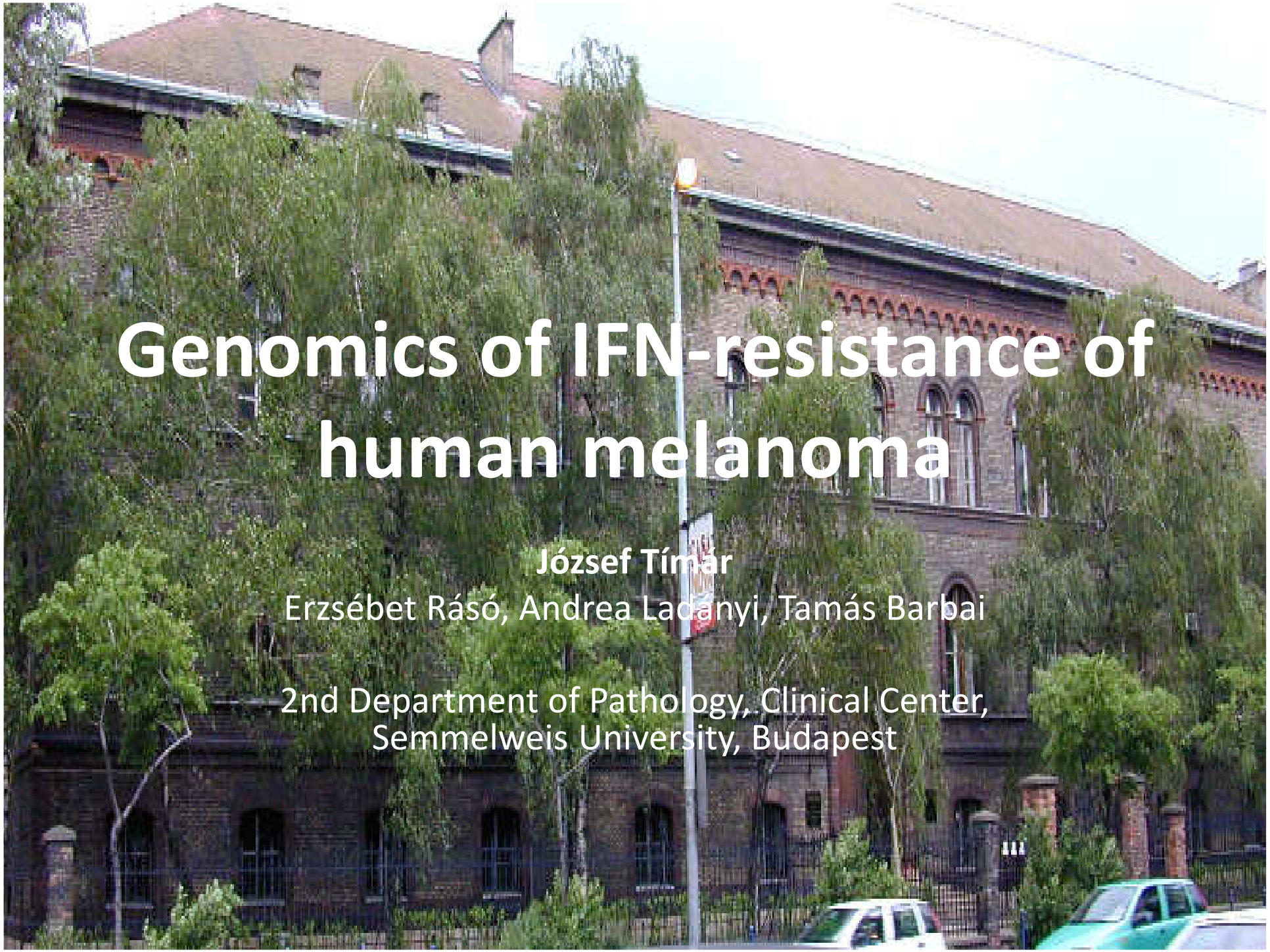


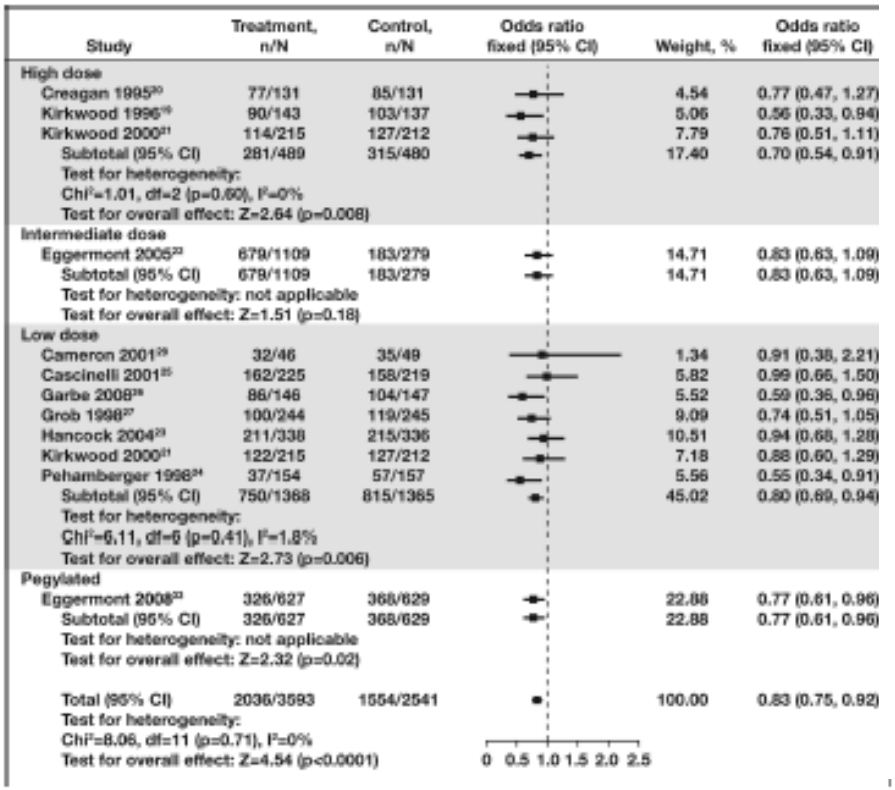
Genomics of IFN-resistance of human melanoma

József Tímár

Erzsébet Rásó, Andrea Ladanyi, Tamás Barbai

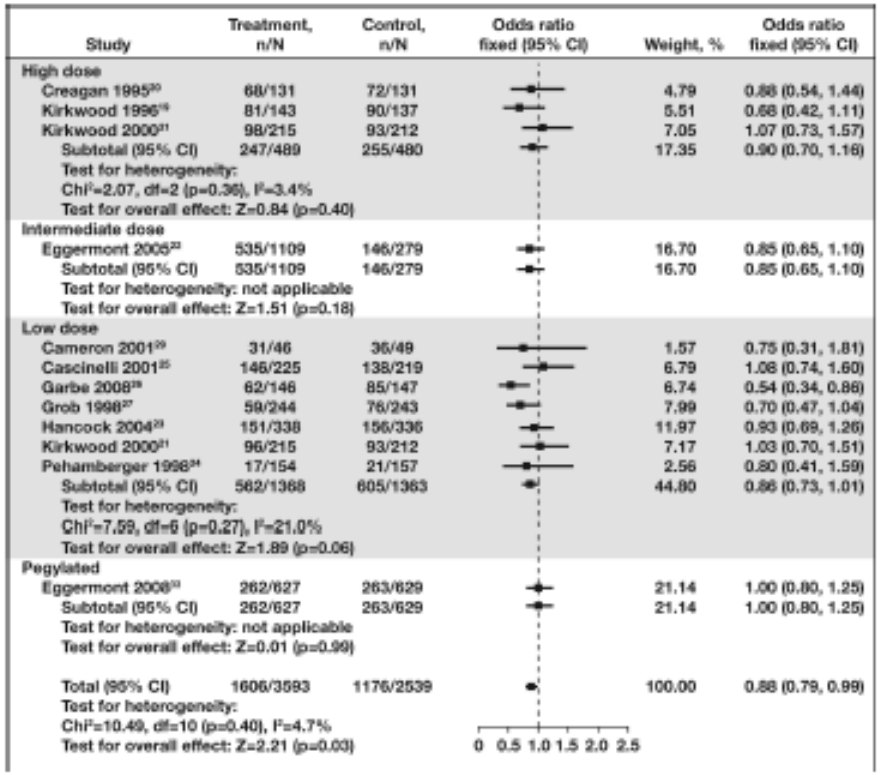
2nd Department of Pathology, Clinical Center,
Semmelweis University, Budapest

Efficacy of adjuvant IFN-α therapy on high risk melanoma patients



Study	Treatment, n/N	Control, n/N	Odds ratio fixed (95% CI)	Weight, %	Odds ratio fixed (95% CI)
High dose					
Creagan 1995[20]	77/131	85/131		4.54	0.77 (0.47, 1.27)
Kirkwood 1996[19]	90/143	103/137		5.06	0.56 (0.33, 0.94)
Kirkwood 2000[21]	114/215	127/212		7.79	0.76 (0.51, 1.11)
Subtotal (95% CI)	281/489	315/480		17.40	0.70 (0.54, 0.91)
Test for heterogeneity:					
Chi^2=1.01, df=2 (p=0.60), I^2=0%					
Test for overall effect: Z=2.64 (p=0.008)					
Intermediate dose					
Eggermont 2005[22]	679/1109	183/279		14.71	0.83 (0.63, 1.09)
Subtotal (95% CI)	679/1109	183/279		14.71	0.83 (0.63, 1.09)
Test for heterogeneity: not applicable					
Test for overall effect: Z=1.51 (p=0.18)					
Low dose					
Cameron 2001[29]	32/46	35/49		1.34	0.91 (0.38, 2.21)
Cascinelli 2001[25]	162/225	158/219		5.82	0.99 (0.66, 1.50)
Garbe 2008[28]	86/146	104/147		5.52	0.59 (0.36, 0.96)
Grob 1998[27]	100/244	119/245		9.09	0.74 (0.51, 1.05)
Hancock 2004[23]	211/338	215/336		10.51	0.94 (0.68, 1.28)
Kirkwood 2000[21]	122/215	127/212		7.18	0.88 (0.60, 1.29)
Pehamberger 1998[24]	37/154	57/157		5.56	0.55 (0.34, 0.91)
Subtotal (95% CI)	750/1368	815/1365		45.02	0.80 (0.69, 0.94)
Test for heterogeneity:					
Chi^2=6.11, df=6 (p=0.41), I^2=1.8%					
Test for overall effect: Z=2.73 (p=0.006)					
Pegylated					
Eggermont 2008[33]	326/627	368/629		22.88	0.77 (0.61, 0.96)
Subtotal (95% CI)	326/627	368/629		22.88	0.77 (0.61, 0.96)
Test for heterogeneity: not applicable					
Test for overall effect: Z=2.32 (p=0.02)					
Total (95% CI)	2036/3593	1554/2541		100.00	0.83 (0.75, 0.92)
Test for heterogeneity:					
Chi^2=8.06, df=11 (p=0.71), I^2=0%					
Test for overall effect: Z=4.54 (p<0.0001)					

0 0.5 1.0 1.5 2.0 2.5



Study	Treatment, n/N	Control, n/N	Odds ratio fixed (95% CI)	Weight, %	Odds ratio fixed (95% CI)
High dose					
Creagan 1995[20]	68/131	72/131		4.79	0.88 (0.54, 1.44)
Kirkwood 1996[19]	81/143	90/137		5.51	0.68 (0.42, 1.11)
Kirkwood 2000[21]	98/215	93/212		7.05	1.07 (0.73, 1.57)
Subtotal (95% CI)	247/489	255/480		17.35	0.90 (0.70, 1.16)
Test for heterogeneity:					
Chi^2=2.07, df=2 (p=0.36), I^2=3.4%					
Test for overall effect: Z=0.84 (p=0.40)					
Intermediate dose					
Eggermont 2005[22]	535/1109	146/279		16.70	0.85 (0.65, 1.10)
Subtotal (95% CI)	535/1109	146/279		16.70	0.85 (0.65, 1.10)
Test for heterogeneity: not applicable					
Test for overall effect: Z=1.51 (p=0.18)					
Low dose					
Cameron 2001[29]	31/46	36/49		1.57	0.75 (0.31, 1.81)
Cascinelli 2001[25]	146/225	138/219		6.79	1.08 (0.74, 1.60)
Garbe 2008[28]	62/146	85/147		6.74	0.54 (0.34, 0.86)
Grob 1998[27]	59/244	76/243		7.99	0.70 (0.47, 1.04)
Hancock 2004[23]	151/338	156/336		11.97	0.93 (0.69, 1.26)
Kirkwood 2000[21]	96/215	93/212		7.17	1.03 (0.70, 1.51)
Pehamberger 1998[24]	17/154	21/157		2.56	0.80 (0.41, 1.59)
Subtotal (95% CI)	562/1368	605/1363		44.80	0.86 (0.73, 1.01)
Test for heterogeneity:					
Chi^2=7.59, df=6 (p=0.27), I^2=21.0%					
Test for overall effect: Z=1.89 (p=0.06)					
Pegylated					
Eggermont 2008[33]	262/627	263/629		21.14	1.00 (0.80, 1.25)
Subtotal (95% CI)	262/627	263/629		21.14	1.00 (0.80, 1.25)
Test for heterogeneity: not applicable					
Test for overall effect: Z=0.01 (p=0.99)					
Total (95% CI)	1606/3593	1176/2539		100.00	0.88 (0.79, 0.99)
Test for heterogeneity:					
Chi^2=10.49, df=10 (p=0.40), I^2=4.7%					
Test for overall effect: Z=2.21 (p=0.03)					

0 0.5 1.0 1.5 2.0 2.5

DFS-HR: 0.83

OS-HR: 0.88

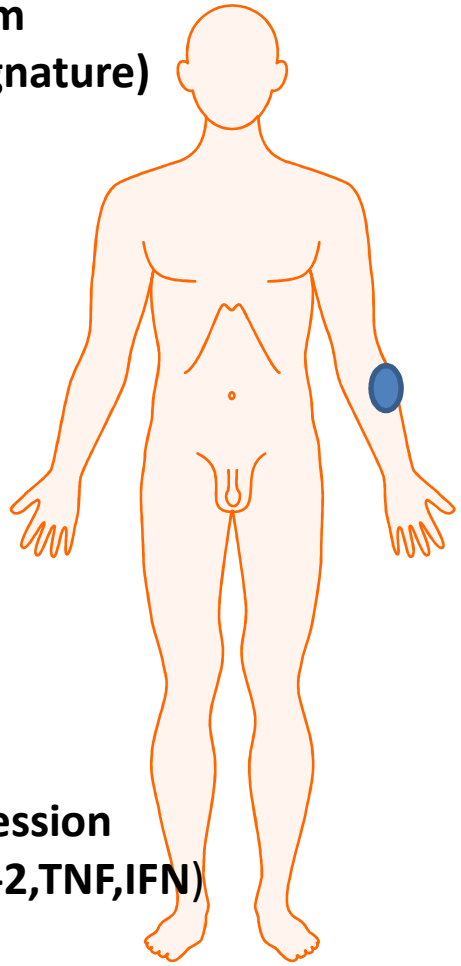
Garbe et al. Oncologist16:5,2011

Two aspects of IFN-α resistance in melanoma patients

Immune Defense Mechanism
Of the Host (Lymphocyte signature)

EIF2AK2
IFI44
IFIT3/2/1
STAT1 (reduced)
OAS1
MX1/2
IFI44L
RSAD2
HERC5

T-cell defects:
Low activation marker expression
Low cytokine expression (IL-2,TNF,IFN)



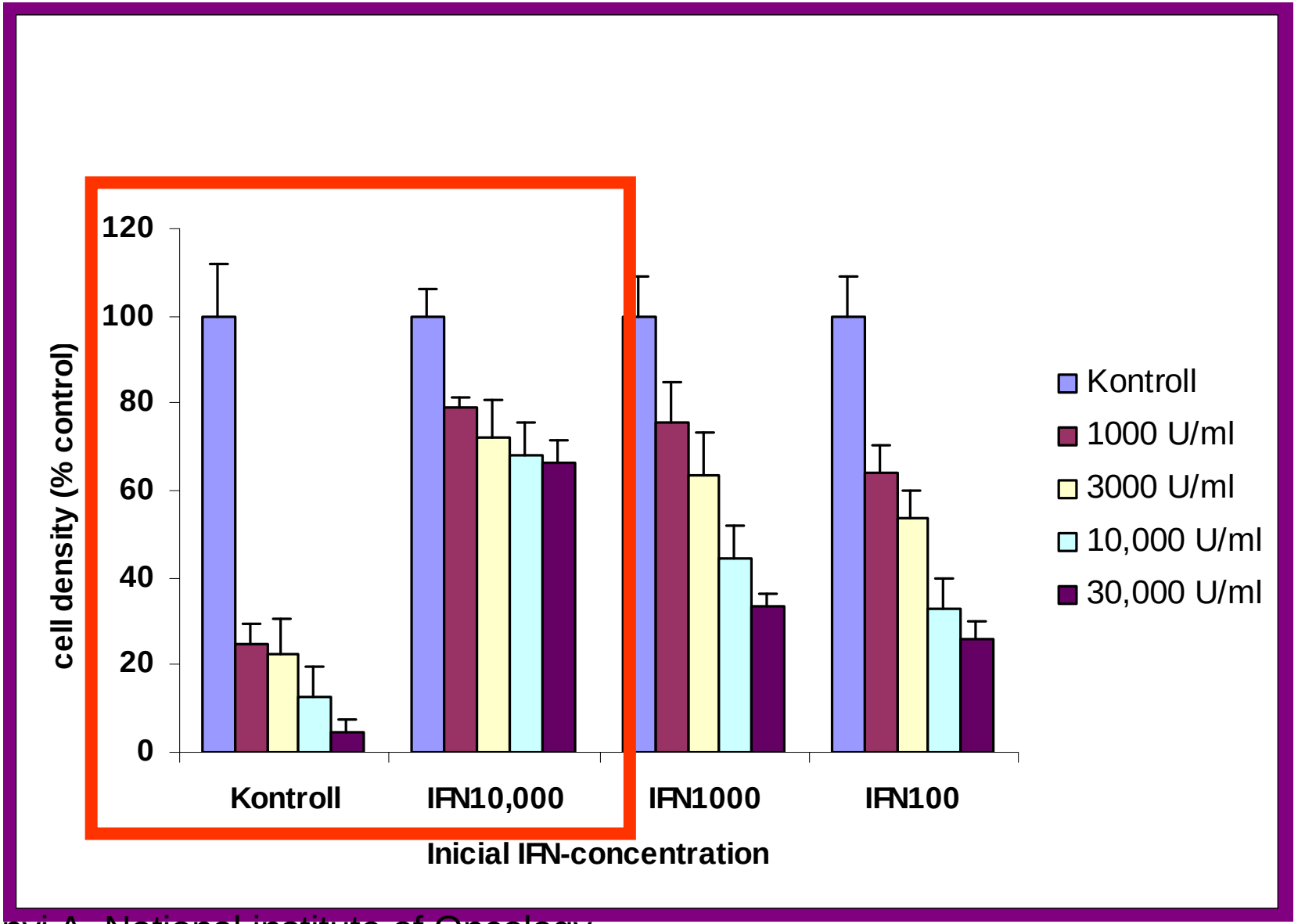
Critchley-Thorne et al. PlosMed4:e176,2007

Malignant Melanoma

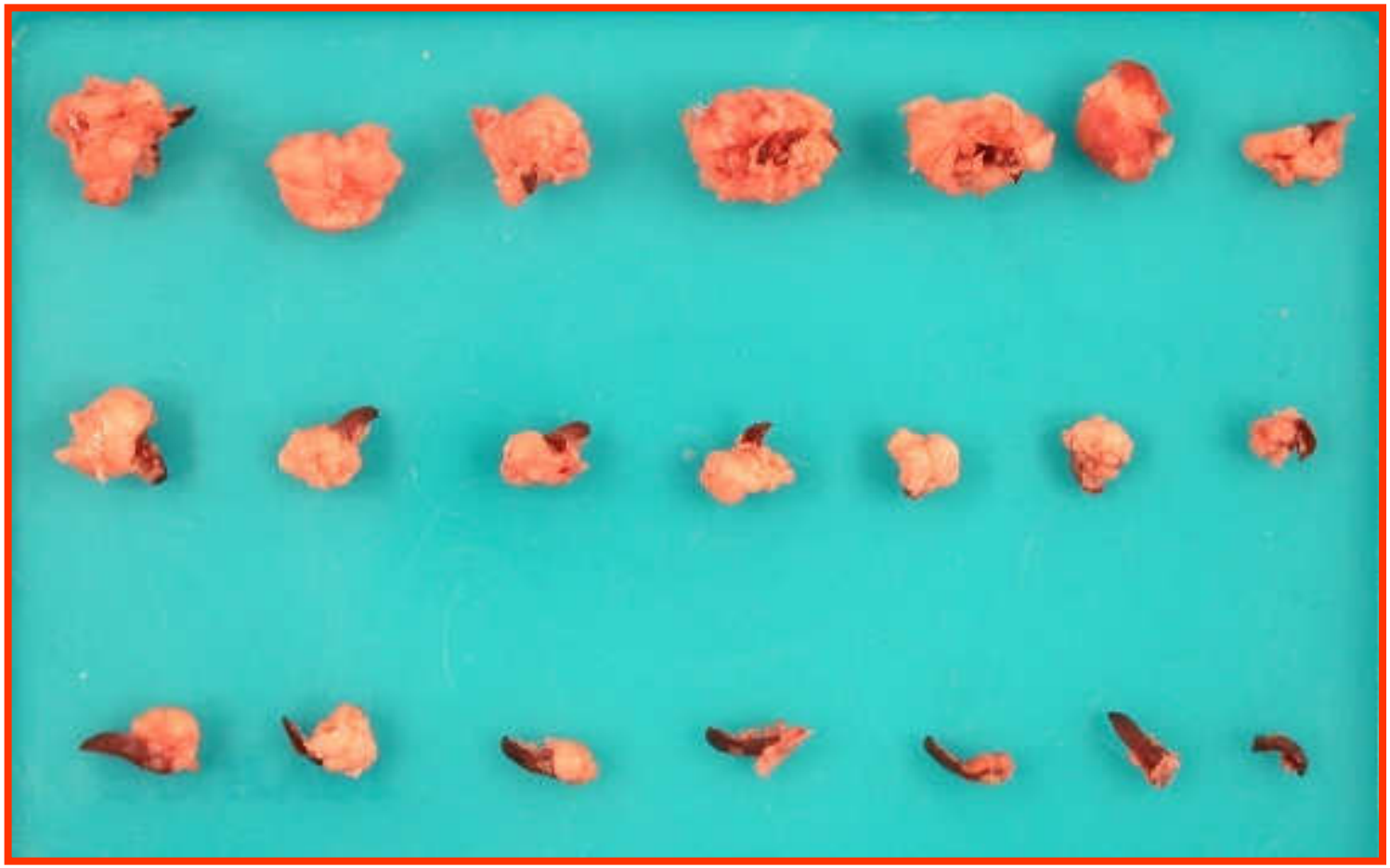
Early studies used cell lines of various sensitivity to IFN and analysed in vitro

In Vitro signatures are Completely different from In vivo……

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



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



Ladanyi A. National institute of Oncology

Material and Methods

- HT168M1-IFN-se/-rez human melanoma xenografts sc.
- Expression analysis: HGSMv2.0, Applied Biosys
- GeneSpring analysis, p>0,05 and 2x/0.5-fold
- Taqman validation
- Initial set: 115 upregulated / 97 downregulated genes
- **Final set: 93-gene IRS gene signature**

93-gene IFN-resistance signature

Gene Syml	Fold change				RefSeq
WFDC1	15,21			WAP four-disulfide core domain 1	
GAGE	9,015			G antigen 2	NM_001472
TSPAN8	4,744			tetraspanin 8	NM_004616
PTRF	4,298			Polymerase I and transcript release factor	
PRG1	3,979			Proteoglycan 1, secretory granule	
SYDE1	3,51			synapse defective 1, Rho GTPase, homolog 1 (C. elegans)	NM_033025
LOC25602	3,286			Hypothetical protein LOC256021	
TYRP1	3,112			tyrosinase-related protein 1	NM_000550.1
EFHD1	3,091			EF-hand domain family, member D1	
CALM3	2,923			Calmodulin 3 (phosphorylase kinase, delta)	
SSTR5	2,84			somatostatin receptor 5	NM_001053.1
RPE65	2,818			retinal pigment epithelium-specific protein 65kDa	NM_000329.2
DOK5	2,79			docking protein 5	NM_018431.3
DEK	2,751			DEK oncogene (DNA binding)	NM_003472.2
NEU1	2,658			sialidase 1 (lysosomal sialidase)	NM_000434.2
JDP2	2,651			jun dimerization protein 2	NM_130469.2
EGR1	2,615			early growth response 1	NM_001964.2
MAPT	2,546			Microtubule-associated protein tau	
HOXD10	2,487			homeobox D10	NM_002148.3
CDKL3	2,487			cyclin-dependent kinase-like 3	NM_016508
ZNF703	2,448			zinc finger protein 703	NM_025069.1
TTY2	2,39			testis-specific transcript, Y-linked 2	AF000991.1
SLC27A4	2,359			solute carrier family 27 (fatty acid transporter), member 4	NM_005094.2
HOXC11	2,34			Homeo box C11	
IRF2	2,251			interferon regulatory factor 2	NM_002199.2
BCORL1	2,237			BCL6 co-repressor-like 1	
HES2	2,234			hairy and enhancer of split 2 (Drosophila)	BC012091.1
C21orf129	2,217			chromosome 21 open reading frame 129	NM_152506
NOX5	2,214			NADPH oxidase, EF-hand calcium binding domain 5	NM_024505.2
PVT1|COL	2,195			Collagen, type VI, alpha 1	
ATF5	2,181			activating transcription factor 5	NM_012068.2
NDRG1	2,17			N-myc downstream regulated gene 1	NM_006096.2
CDCA4	2,168			Cell division cycle associated 4	
CPXM1	2,138			carboxypeptidase X (M14 family), member 1	NM_019609.3
CTSB	2,104			cathepsin B	
DOCK11	2,076			dedicator of cytokinesis 11	NM_144658
C1QTNF3	2,064			C1q and tumor necrosis factor related protein 3	NM_030945
EFNA3	1,817			ephrin-A3; EFL2, EPLG3,Ehk1-L,HGNC:3223,LERK3	NM_004952.3
DDX10	1,68			DEAD (Asp-Glu-Ala-Asp) box polypeptide 10	NM_004398.2
WNT7A	1,602			WNT7A wingless-type MMTV integration site family, member 7A	NM_004625.3
ZNF165	1,58			zinc finger protein 165	NM_003447.2
ABCC1	1,551			ATP-binding cassette, sub-family C (CFTR/MRP), member 1	7 RefSeqs
S100A2	1,478			S100 calcium binding protein A2	NM_005978.3
HSF1	1,337			heat shock transcription factor 1	NM_005526.1
PDE6A	1,309			phosphodiesterase 6A, cGMP-specific, rod, alpha	NM_000440.1
HSPB7	1,263			heat shock 27kDa protein family, member 7 (cardiovascular)	NM_014424.3
MT2A	1,182			metallothionein 2A	NM_005953.2
HSPA1B	1,122			heat shock 70kDa protein 1B	NM_005346.3
PDE9A	1,12			phosphodiesterase 9A	20 RefSeqs
PDE1C	1,004			phosphodiesterase 1C, calmodulin-dependent 70kDa	NM_005020.1
IFIT1	0,486			Interferon-induced protein with tetratricopeptide repeats 1	NM_001001887.1
SDC2	0,468			Syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)	
SS18L1	0,468			synovial sarcoma translocation gene on chromosome 18-like 1	NM_015558
UGT2B28	0,444			UDP glucuronosyltransferase 2 family, polypeptide B28	NM_053039
SEMA3B	0,405			Sequence 920 from Patent EP1308459.	
TPD52L1	0,392			Tumor protein D52-like 1	
GPR51	0,387			G protein-coupled receptor 51	NM_005458
BST1	0,378			bone marrow stromal cell antigen 1	NM_004334
UGT2A1	0,359			UDP glucuronosyltransferase 2 family, polypeptide A1	NM_006798
MX1	0,347			Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	
PHACTR1	0,281			Sequence 133 from Patent EP1308459.	
SERPINA3	0,26			Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	
LRRK2	0,231			leucine-rich repeat kinase 2	NM_198578
IFI27	0,211			interferon, alpha-inducible protein 27	NM_005532
DKK1	0,195			dickkopf homolog 1 (Xenopus laevis)	
PAX3	-1,017			paired box gene 3 (Waardenburg syndrome 1)	7 RefSeqs
LYPLA3	-1,029			lysophospholipase 3 (lysosomal phospholipase A2)	NM_012320.3
SGK2	-1,131			serum/glucocorticoid regulated kinase 2	2 RefSeqs
SOX4	-1,179			SRY (sex determining region Y)-box 4	NM_003107.2
CLDN4	-1,243			claudin 4, CPE-R,CPER,CPETR,CPETR1,HGNC:2046,WBSCR8,hCPE-R	NM_001305.3
BEHAB/BC	-1,255			brevican, BEHAB,CSPG7,HGNC:23059,MGC13038	NM_021948.3
GPI	-1,304			glucose phosphate isomerase	NM_000175.2
ZIC1	-1,36			Zic family member 1 (odd-paired homolog, Drosophila)	NM_003412.3
EZF-2 (ZNI	-1,36			zinc finger protein 444	NM_018337.2
HDAC8	-1,439			histone deacetylase 8	NM_018486.1
WT1	-1,503			Wilms tumor 1	4 RefSeqs
FGF20	-1,53			fibroblast growth factor 20	NM_019851.1
UCP3	-1,65			uncoupling protein 3 (mitochondrial, proton carrier)	NM_003356.2
SLC17A3	-1,68			solute carrier family 17 (sodium phosphate), member 3	NM_006632.1
TNFSF10	-1,85			tumor necrosis factor (ligand) superfamily, member 10	NM_003810.2
CAMK1	-2,033			calcium/calmodulin-dependent protein kinase I	NM_003656.3
PTBP1	-2,28			polypyrimidine tract binding protein 1	NM_175847.1
AQP5				aquaporin 5	NM_001651.1
NPTXR				neuronal pentraxin receptor	NM_014293
PRSS33				Sequence 135 from Patent WO0220754.	
P2RX2				purinergic receptor P2X, ligand-gated ion channel, 2	6 RefSeqs
IFNAR1				interferon (alpha, beta and omega) receptor 1	NM_000629.2
AQP1				aquaporin 1 (Colton blood group)	NM_198098.1
AKT2				v-akt murine thymoma viral oncogene homolog 2	NM_001626.2
TNFRSF14				tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator)	NM_003820.2
ABCB1/MDR1				ATP-binding cassette, sub-family B (MDR/TAP), member 1	NM_000927.3 (M14758.1)
SORBS3,SCAM-1				sorbin and SH3 domain containing 3	NM_001018003

50 upregulated
43 downregulated genes

Validated 33-gene signature of IFN-resistance of human melanoma

RefSeq	Assay ID	Fold chang	Gene Symbol	
	Hs00221849_m1	5	WFDC1	WAP four-disulfide core domain 1
NM_00461	Hs00610327_m1	4,744	TSPAN8	tetraspanin 8
NM_03302	Hs00263581_m1	3,51	SYDE1	synapse defective 1, Rho GTPase, homolog 1 (C. elegans)
	Hs00368816_m1	3,091	EFHD1	EF-hand domain family, member D1
	Hs00270914_m1	2,923	CALM3	Calmodulin 3 (phosphorylase kinase, delta)
NM_00032	Hs00165642_m1	2,818	RPE65	retinal pigment epithelium-specific protein 65kDa
NM_00043	Hs00166421_m1	2,658	NEU1	sialidase 1 (lysosomal sialidase)
NM_00196	Hs00152928_m1	2,615	EGR1	early growth response 1
	Hs00213491_m1	2,546	MAPT	Microtubule-associated protein tau
NM_02506	Hs00228155_m1	2,448	ZNF703	zinc finger protein 703
	Hs00204415_m1	2,34	HOXC11	Homeo box C11
NM_02450	Hs00225846_m1	2,214	NOX5	NADPH oxidase, EF-hand calcium binding domain 5
NM_01206	Hs00247172_m1	2,181	ATF5	activating transcription factor 5
NM_00609	Hs00608389_m1	2,17	NDRG1	N-myc downstream regulated gene 1
~~NM_14465~~	~~Hs00376176_m1~~	~~2,076~~	~~DOCK11~~	~~dedicator of cytokinesis 11~~
NM_00100	Hs00356631_g1	0,486	IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
NM_05303	Hs00852540_s1	0,444	UGT2B28	UDP glucuronosyltransferase 2 family, polypeptide B28
	Hs00180099_m1	0,392	TPD52L1	Tumor protein D52-like 1
	Hs00182073_m1	0,347	MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
	Hs00153674_m1	0,26	SERPINA3	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
NM_19857	Hs00411197_m1	0,231	LRRK2	leucine-rich repeat kinase 2
NM_00553	Hs00271467_m1	0,211	IFI27	interferon, alpha-inducible protein 27
	Hs00183740_m1	0,195	DKK1	dickkopf homolog 1 (Xenopus laevis)
	Hs00367639_m1	-1,131	SGK2	serum/glucocorticoid regulated kinase 2
NM_00130	Hs00533616_s1	-1,243	CLDN4	claudin 4, CPE-R,CPER,CPETR,CPETR1,HGNC:2046,WBSCR8,hCPE-R
NM_00017	Hs00164752_m1	-1,304	GPI	glucose phosphate isomerase AMF
NM_00341	Hs00602749_m1	-1,36	ZIC1	Zic family member 1 (odd-paired homolog, Drosophila)
NM_01985	Hs00173929_m1	-1,53	FGF20	fibroblast growth factor 20
NM_00663	Hs00198361_m1	-1,68	SLC17A3	solute carrier family 17 (sodium phosphate), member 3
NM_00381	Hs00234355_m1	-1,85	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10
NM_00365	Hs00269334_m1	-2,033	CAMK1	calcium/calmodulin-dependent protein kinase I
NM_17584	Hs00738537_m1	-2,28	PTBP1	polypyrimidine tract binding protein 1

- **12/33 (36%)=IRE-genes**

IRE genes of IFN resistance signature of human melanoma

Assay ID	Fold ch	Gene Symbol	
Hs00610327_m1	4,744	TSPAN8	tetraspanin 8
Hs00165642_m1	2,818	RPE65	retinal pigment epithelium-specific protein 65kDa
Hs00228155_m1	2,448	ZNF703	zinc finger protein 703
Hs00204415_m1	2,34	HOXC11	Homeo bo C11
Hs00225846_m1	2,214	NOX5	NADPH oxidase, EF-hand calcium binding domain 5
Hs00356631_g1	0,486	IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
Hs00182073_m1	0,347	MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
Hs00411197_m1	0,231	LRRK2	leucine-rich repeat kinase 2
Hs00271467_m1	0,211	IFI27	interferon, alpha-inducible protein 27
Hs00183740_m1	0,195	DKK1	dickkopf homolog 1 (Xenopus laevis)
Hs00367639_m1	-1,131	SGK2	serum/glucocorticoid regulated kinase 2
Hs00269334_m1	-2,033	CAMK1	calcium/calmodulin-dependent protein kinase I

Motility associated genes in IFN resistance signature of human melanoma

RefSeq	Fold ch	Gene Symbol								
	5,01	WFDC1	WAP four-disulfide core domain 1							
NM_033025	3,51	SYDE1	synapse defective 1, Rho GTPase, homolog 1 (C. elegans)							
	3,091	EFHD1	EF-hand domain family, member D1							
	2,923	CALM3	Calmodulin 3 (phosphorylase kinase, d)							
NM_000434.2	2,658	NEU1	sialidase 1 (lysosomal sialidase)							
NM_001964.2	2,615	EGR1	early growth response 1							
	2,546	MAPT	MT-assoc. protein tau							
NM_012068.2	2,181	ATF5	activating transcription f- 5							
NM_006096.2	2,17	NDRG1	N-myc downstream regulated gene 1							
NM_144658	2,076	DOCK11	dedicator of cytokinesis 11							
NM_053039	0,444	UGT2B28	UDP glucuronosyltransferase 2 family, polypeptide B28							
	0,392	TPD52L1	Tumor protein D52-like 1							
	0,26	SERPINA3	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3							
NM_001305.3	-1,243	CLDN4	claudin 4							
NM_000175.2	-1,304	GPI	glucose phosphate isome			AMF				
NM_003412.3	-1,36	ZIC1	Zic family member 1							
NM_019851.1	-1,53	FGF20	fibroblast growth factor 20							
NM_006632.1	-1,68	SLC17A3	solute carrier family 17 (sodium phosphate), m 3							
NM_003810.2	-1,85	TNFSF10	tumor necrosis factor (ligand) superfamily, m 10							

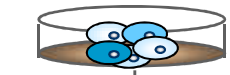
Genes involved in tumor metastasis

- Metastasis-initiating genes
- Primary role in the primary tumor (local invasion, intravasation, systemic survival)

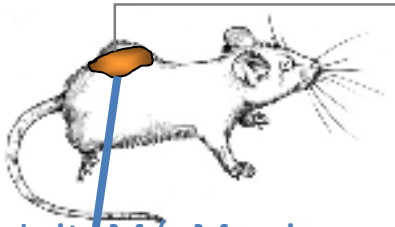
- Metastasis-maintenance genes
- Primary role in micromets (proliferation, survival, angiogenesis)

- **Metastasis-associated genes**
- Genes in volved in both initiation and maintenance

Metastatic Human Melanoma Model in SCID Mice using HT199 human melanoma



ADULT MICE
non-metastatic environment



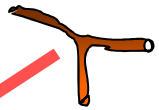
Met Init: M/nM primary

Met Maint:
Met/Primary

NEWBORN MICE
metastatic environment
(lung mets)

Stromal components

Human melanoma primary



Subcutaneous melanoma
on 7th postimplantation day

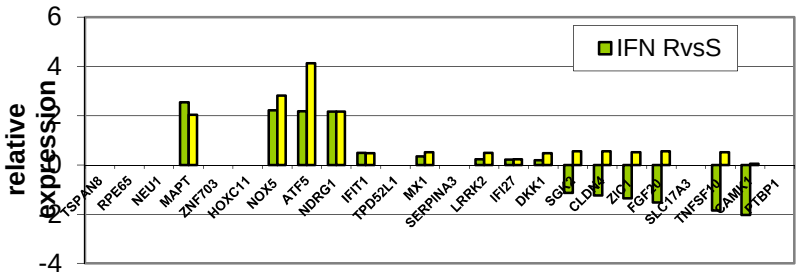
Mouse Oligo Microarray
(22,575 gén – Agilent)

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

Banky et al. Mol Cancer 2012,Raso-Barnett et al. PlosOne 2013, Barbai et al Oncotarget 2015

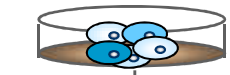
Metastasis Iniciator Genes In IFN-resistance signature (Nm-M primaries)

RefSeq	Fold chang	UP /FP	Gene Symbol	
NM_004616			TSPAN8	tetraspanin 8
NM_000329.2			RPE65	retinal pigment epithelium-specific protein 65kDa
NM_000434.2			NEU1	sialidase 1 (lysosomal sialidase)
	2,546	2,04	MAPT	Microtubule-associated protein tau
NM_025069.1			ZNF703	zinc finger protein 703
			HOXC11	Homeo box C11
NM_024505.2	2,214	2,82	NOX5	NADPH oxidase, EF-hand calcium binding domain 5
NM_012068.2	2,181	4,12	ATF5	activating transcription factor 5
NM_006096.2	2,17	2,17	NDRG1	N-myc downstream regulated gene 1
NM_001001887.1	0,486	0,48	IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
			TPD52L1	Tumor protein D52-like 1
	0,347	0,52	MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
			SERPINA3	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
NM_198578	0,231	0,49	LRRK2	leucine-rich repeat kinase 2
NM_005532	0,211	0,23	IFI27	interferon, alpha-inducible protein 27
	0,195	0,48	DKK1	dickkopf homolog 1 (Xenopus laevis)
2 RefSeqs	-1,131	0,55	SGK2	serum/glucocorticoid regulated kinase 2
NM_001305.3	-1,243	0,55	CLDN4	claudin 4, CPE-R,CPER,CPETR,CPETR1,HGNC:2046,WBSCR8,hCPE-R
NM_003412.3	-1,36	0,52	ZIC1	Zic family member 1 (odd-paired homolog, Drosophila)
NM_019851.1	-1,53	0,55	FGF20	fibroblast growth factor 20
NM_006632.1			SLC17A3	solute carrier family 17 (sodium phosphate), member 3
NM_003810.2	-1,85	0,51	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10
NM_003656.3	-2,033	0,05	CAMK1	calcium/calmodulin-dependent protein kinase I
NM_175847.1			PTBP1	polypyrimidine tract binding protein 1



MIG in IRS 15/24 (63%)

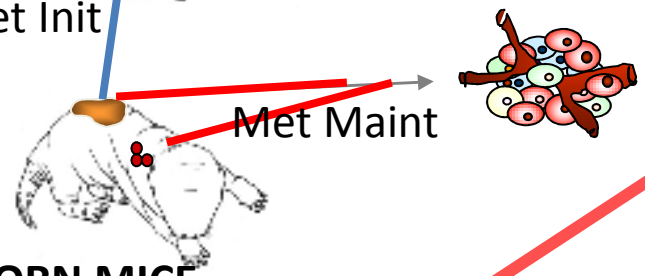
Metastatic Human Melanoma Model in SCID Mice using HT199 human melanoma



ADULT MICE
non-metastatic environment

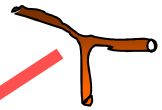
Met Init

Met Maint



NEWBORN MICE
metastatic environment
(lung mets)

Stromal components



Human melanoma primary

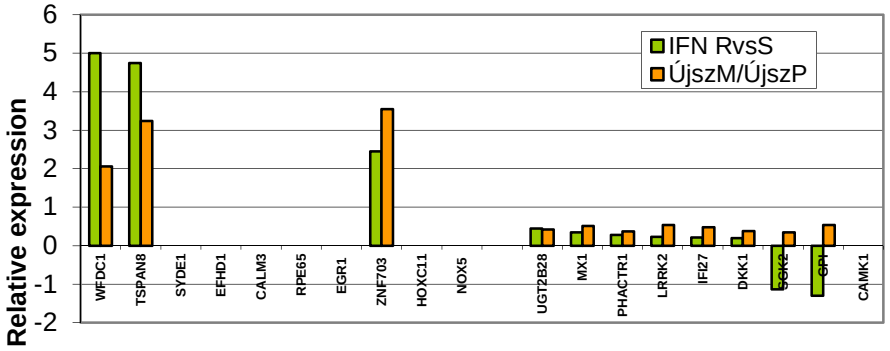
Subcutaneous melanoma
on 7th postimplantation day

Mouse Oligo Microarray
(22,575 gén – Agilent)

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

Metastasis Maintenance Genes in IFN-resistance signature (M-mP)

RefSeq	Assay ID	Fold chang	UM/UP		Gene Symbol	
	Hs00221849_m1	2,06	2,06		WFDC1	WAP four-disulfide core domain 1
NM_00461	Hs00610327_m1	3,24	3,24		TSPAN8	tetraspanin 8
NM_03302	Hs00263581_m1				SYDE1	synapse defective 1, Rho GTPase, homolog 1 (C. elega
	Hs00368816_m1				EFHD1	EF-hand domain family, member D1
	Hs00270914_m1				CALM3	Calmodulin 3 (phosphorylase kinase, delta)
NM_00032	Hs00165642_m1				RPE65	retinal pigment epithelium-specific protein 65kDa
NM_00196	Hs00152928_m1				EGR1	early growth response 1
NM_02506	Hs00228155_m1	3,55	3,55		ZNF703	zinc finger protein 703
	Hs00204415_m1				HOXC11	Homeo box C11
NM_02450	Hs00225846_m1				NOX5	NADPH oxidase, EF-hand calcium binding domain 5
NM_14465	Hs00376176_m1					dedicator of cytokinesis 11
NM_05303	Hs00852540_s1	0,42	0,42		UGT2B28	UDP glucuronosyltransferase 2 family, polypeptide B28
	Hs00182073_m1	0,51	0,51		MX1	Myxovirus (influenza virus) resistance 1, interferon-induc
	Hs00286959_m1	0,37	0,37		PHACTR1	Sequence 133 from Patent EP1308459.
NM_19857	Hs00411197_m1	0,54	0,54		LRRK2	leucine-rich repeat kinase 2
NM_00553	Hs00271467_m1	0,48	0,48		IFI27	interferon, alpha-inducible protein 27
	Hs00183740_m1	0,38	0,38		DKK1	dickkopf homolog 1 (Xenopus laevis)
2 RefSeqs	Hs00367639_m1	0,35	0,35		SGK2	serum/glucocorticoid regulated kinase 2
NM_00017	Hs00164752_m1	0,54	0,54		GPI	glucose phosphate isomerase



MMG in IRS 11/21 (55%)

Metastasis associated genes in IFN-resistance signature

upregulated	downregulated
Met-ini	Met-ini
MAPT **ATF5** **NDRG1** **NOX5**	**CLDN4** **ZIC1** **FGF20** **TNFSF10** **CAMK1** **SGK2**
Met-maint	Met-maint
WFDC1 **TSPN8** **ZNF703**	**UGT2B28** **PHACTR1** **MX1** **IFI27** **DKK1** **SGK2**

Representation of IRE+genes in various functional gene sets of human melanoma

	total	IRE-specific
IRE/IRS	12/33 (36%)	12/33 (36%)
IRE/MIG	7/24 (29%)	7/15 (47%)
IRE/MMG	7/21 (33%)	7/11 (64%)
p	n.s.	n.s.

IRE= IFN-responsive element
MIG= metastasis iniciator genes
MMG= metastasis maintenace genes

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

upregulated			downregulated		
		function			function
NOX5	NADPHoxydase	Ca-dependent SOD	**MX1**	dynamin-family	**MOTILITY**
TSPAN8	tetraspanin8	integrin-assoc **MOTILITY**	**LRRK2**	S/Tkinase MAPKKKcasc	**MOTILITY**
ZNF703	transcription factor	*ER-regulated*	**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

Genetic factors involved in melanoma sensitivity to anti-CTLA4/anti-PD1 immune therapies

	sensitivity	resistance
mutation	NRAS MART1 FAM3c CSMD1	B2 microglobulin
Protein expression	PDL1	

Herbst et al. Nature, 2014, Snyder et al. N Engl J Med 2015

Conclusion

- Human melanoma is characterized by an IFN-resistance gene signature, the majority of those genes are not IFN-regulated
- Metastasis-associated gene signature of human melanoma contains a significant number of IFN-regulated genes
- **Development of IFN-resistance and melanoma progression may contain overlapping molecular pathways (*IFN-, motility- and sex hormone signaling*)**

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