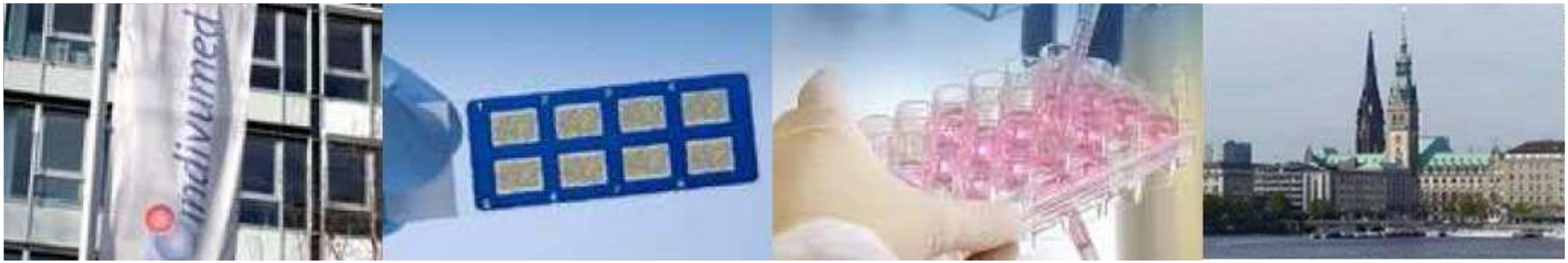

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Realizing Individualized Cancer Therapy

Effects of intra-operative ischemia on cancer and normal tissue

Prof. Dr. Hartmut Juhl
CEO of Indivumed GmbH and Inostics GmbH
Adjunct Professor, Georgetown University and Hamburg University
www.indivumed.com
juhl@indivumed.com

Hamburg:

A Federal State of Germany

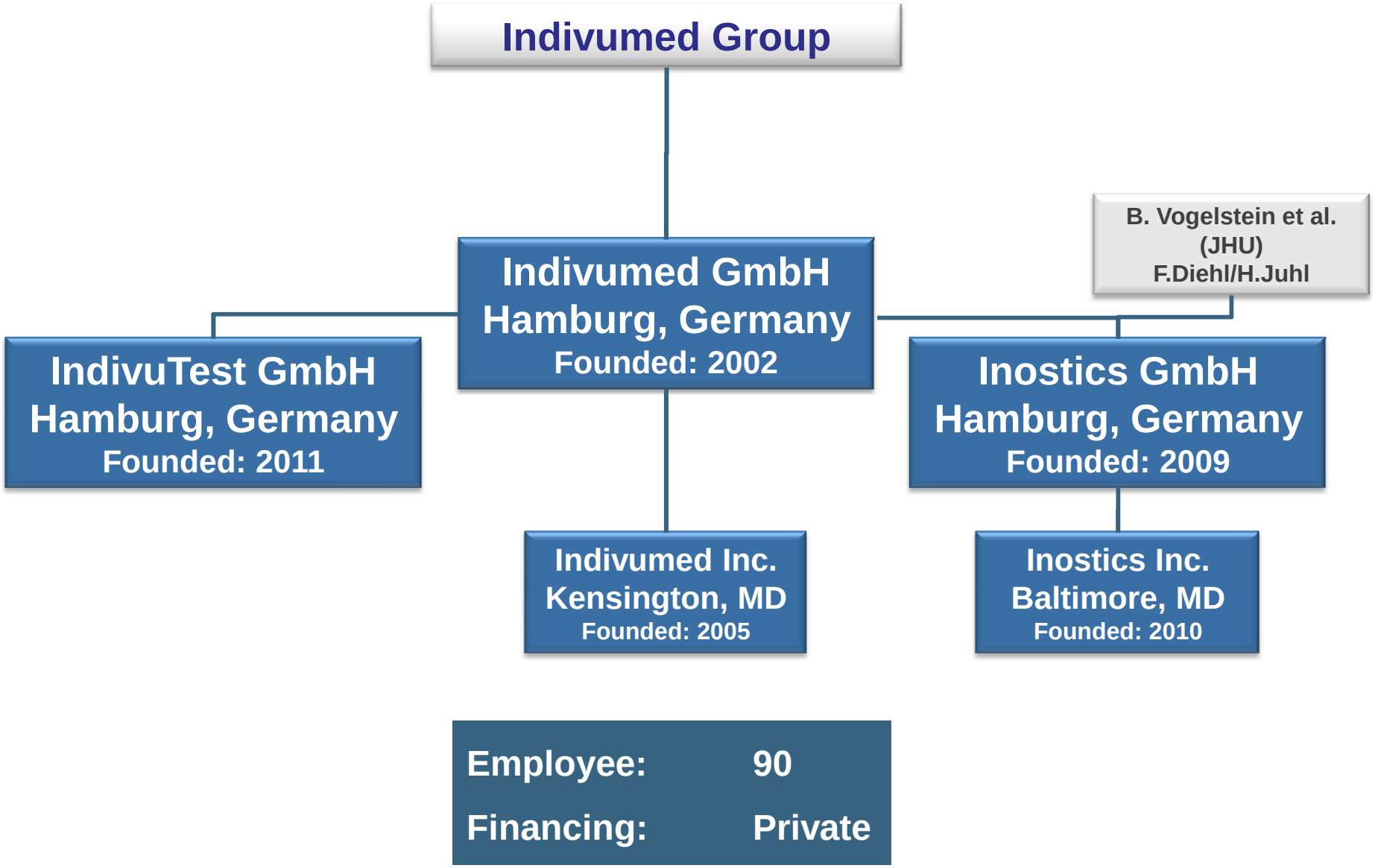
Germany‘s 2nd largest city

Metropolitan area of ~ 4 Mio people

Highest income/citizen in Europe

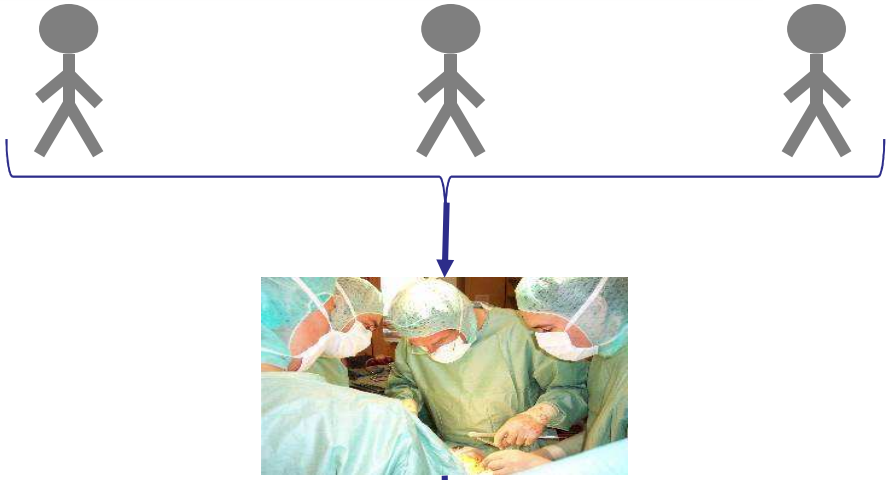
„Happiest citizen in Germany“

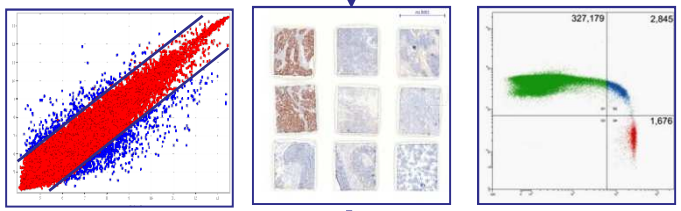
High density of outstanding cancer clinics


Indivumed Group
Indivumed GmbH
Hamburg, Germany
Founded: 2002
B. Vogelstein et al.
(JHU)
F.Diehl/H.Juhl
IndivuTest GmbH
Hamburg, Germany
Founded: 2011
Inostics GmbH
Hamburg, Germany
Founded: 2009
Indivumed Inc.
Kensington, MD
Founded: 2005
Inostics Inc.
Baltimore, MD
Founded: 2010
Employee: 90
Financing: Private

Indivumed Vision: Individualizing Cancer Medicine

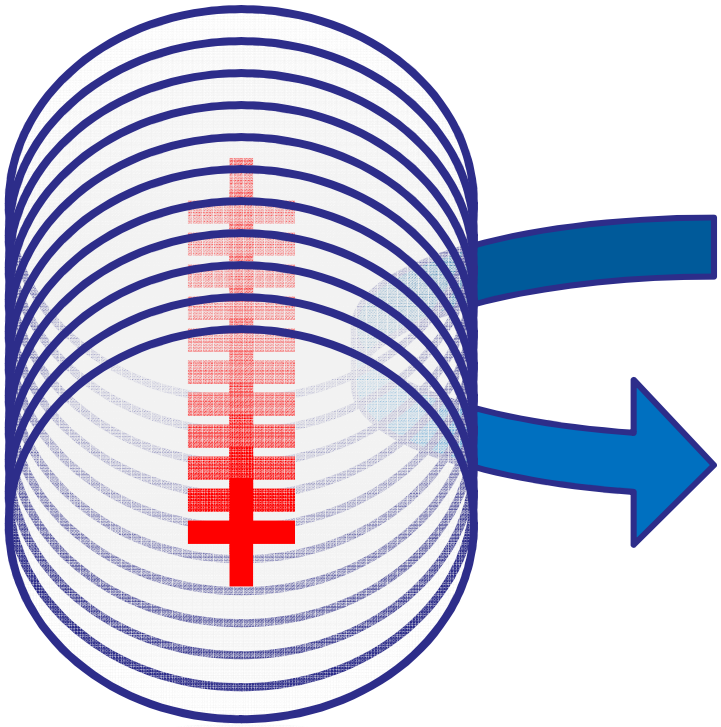
- Clinical Integration
- Biobanking
- Tissue & Blood Based Research
- Protein Based Dx (Indivumed)
- DNA-Based Dx (Inostics)
- Patient Testing (IndivuTest)





Therapy 1 Therapy 2 Therapy 3

Indivumeds Approach:
Integration of Research, Surgery and Patient Care



Partner Hospitals

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Indivumed Clinical Team

Biospecimen Direct Processing

Biospecimen
- Tumor tissue
- Normal Tissue
- Blood
- Urine

Data
- Processing Data
- Clinical Data
- Follow-up Data

Indivumed Scientific Team

Tumor-Biobank and Clinical Data Base

Indivumed Solution:
Integration of Clinical Care, Biobanking and Research

Israelitisches KH
Universitätsklinikum Hamburg-Eppendorf
MARIEN KRANKENHAUS
Krankenhaus Reinbek St. Adolf-Stift
Asklepius Klinik Harburg
Asklepius Klinik Westklinikum
Diakonie-Klinikum Hamburg
Alten Eichen Bethanien Elim
Mammazentrum Hamburg
albertinen
Georgetown University Hospital
MedStar Health
Washington Hospital Center
MedStar Health










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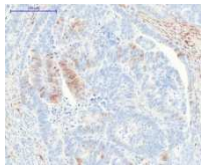
Tumor-Biobank
and
Clinical Data Base

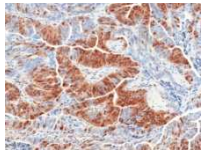
Indivumed Standard of Biobanking:

Tissue ischemia and protein phosphorylation

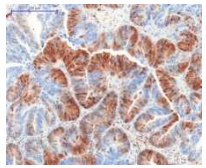
10 min



20 min

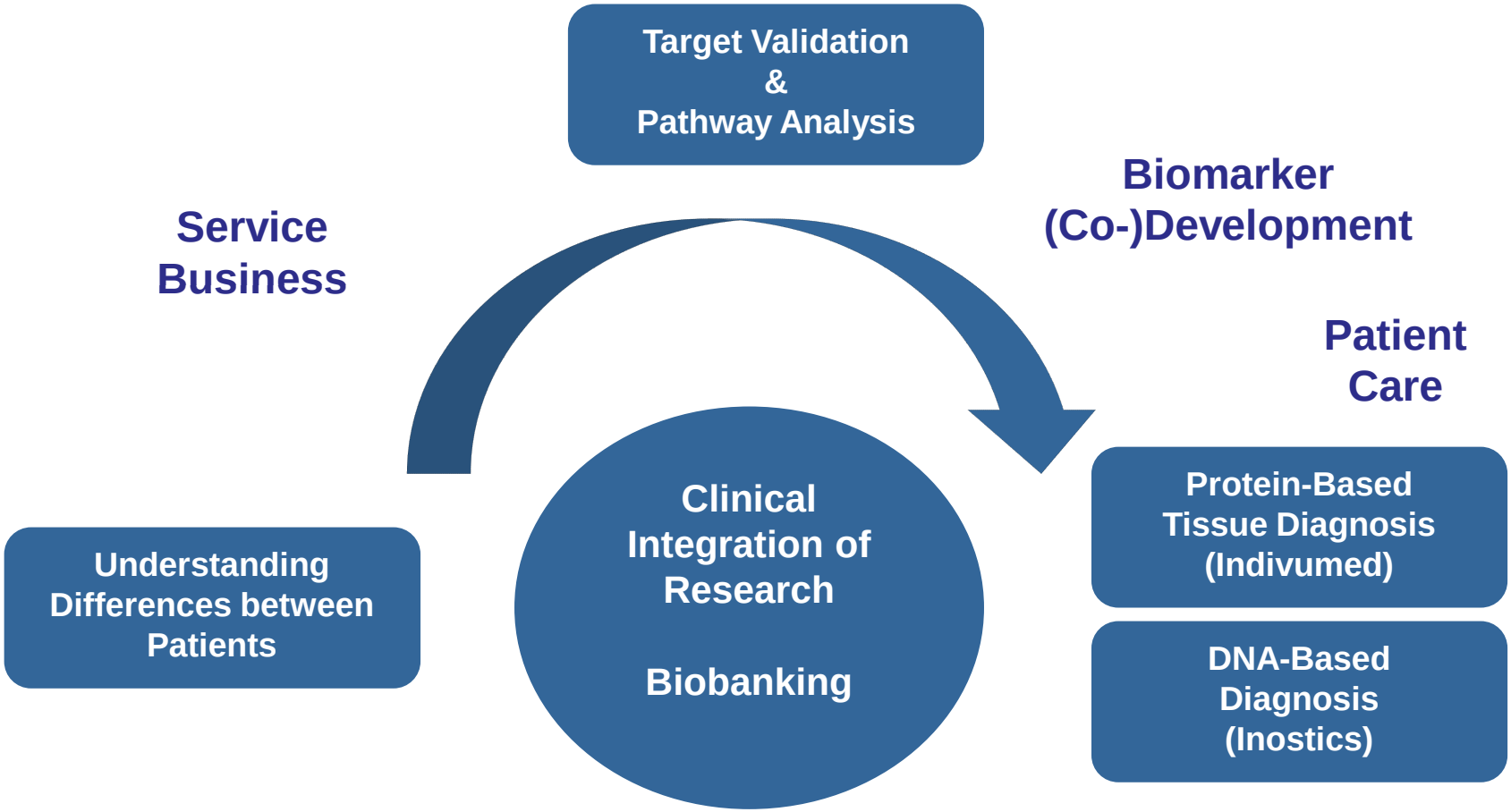


60 min



- ✓ **Exact documented and very short tissue cold ischemia times of < 12 min (mean 7 min)**
- ✓ **Exact tissue localization and standardized fixation**
- ✓ **Complete biospecimen sets**
- ✓ **Highest tissue quality monitored by visual inspection, H&E staining and microscopic assessment**
- ✓ **Native and rapid fluid preparations**
- ✓ **Complete specimen data**
- ✓ **Complete clinical data**
- ✓ **Patients' confidentiality assured following international standards**

Business Concept



Understanding the Molecular Basis of Cancer: Status 1971 (Nixon Declares „War Against Cancer“)

Understanding the Molecular Basis of Cancer: Status 2011

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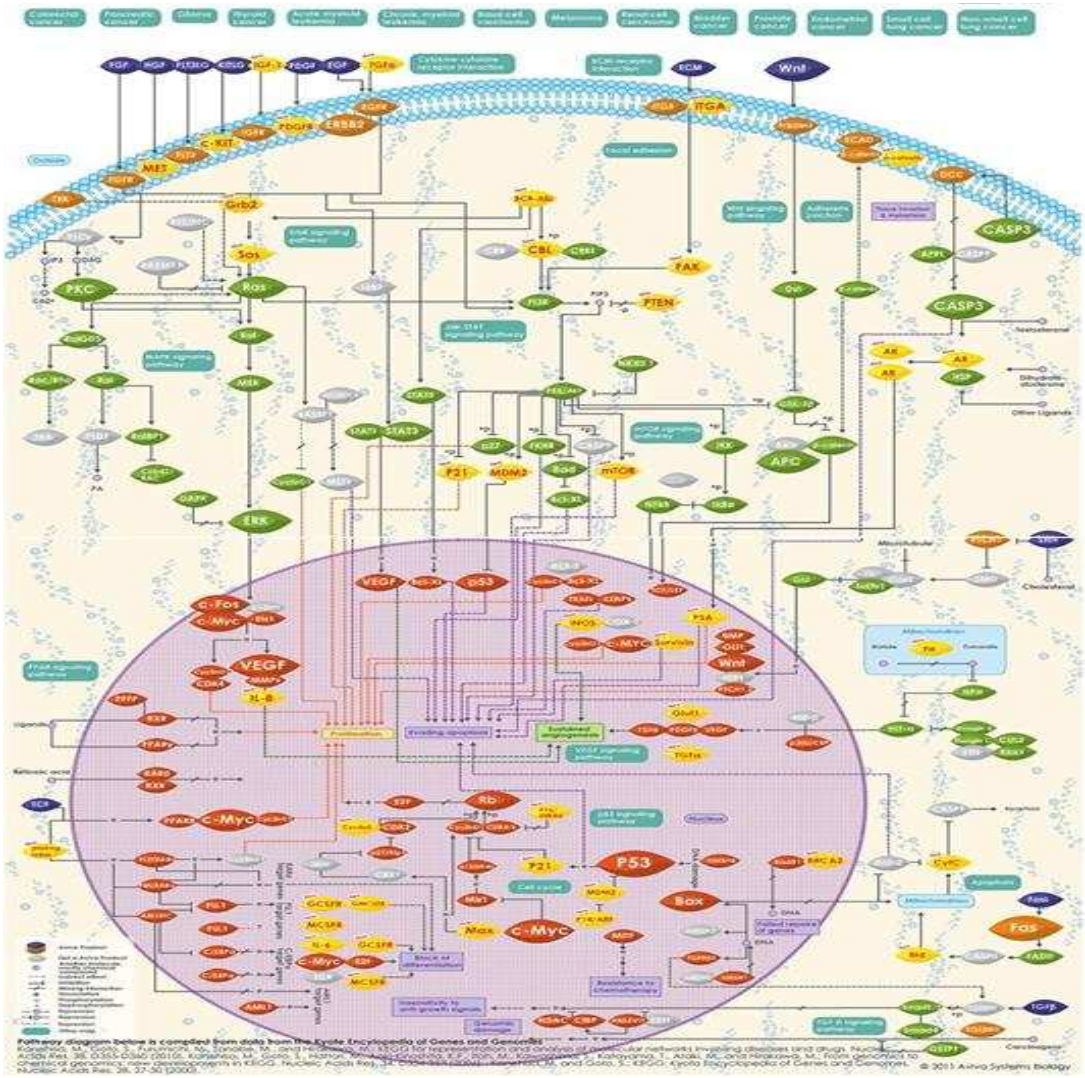
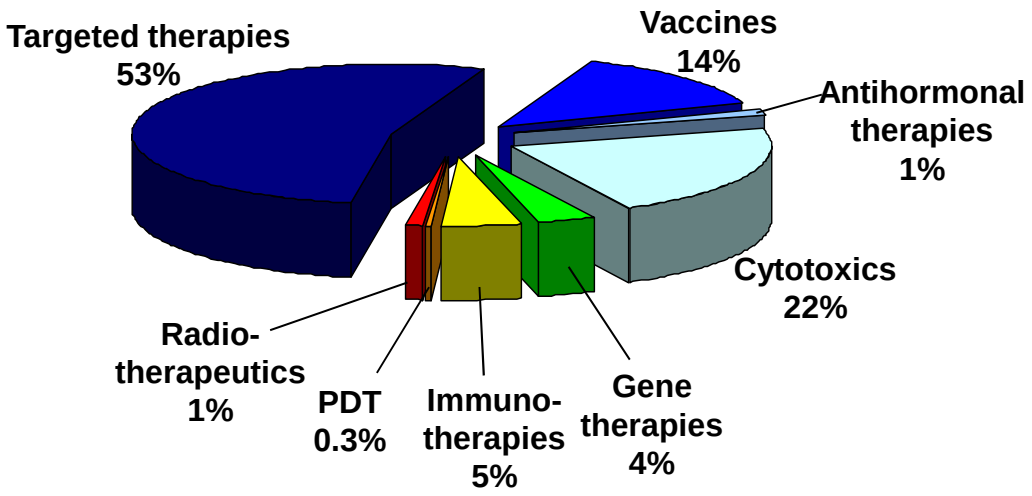
Understanding of Cancer Pathways as Basis for „Targeted Therapeutics“



„Targeted Therapies“ are the Focus of Current Drug Development



Top 8 der umsatzstärksten Krebsmittel

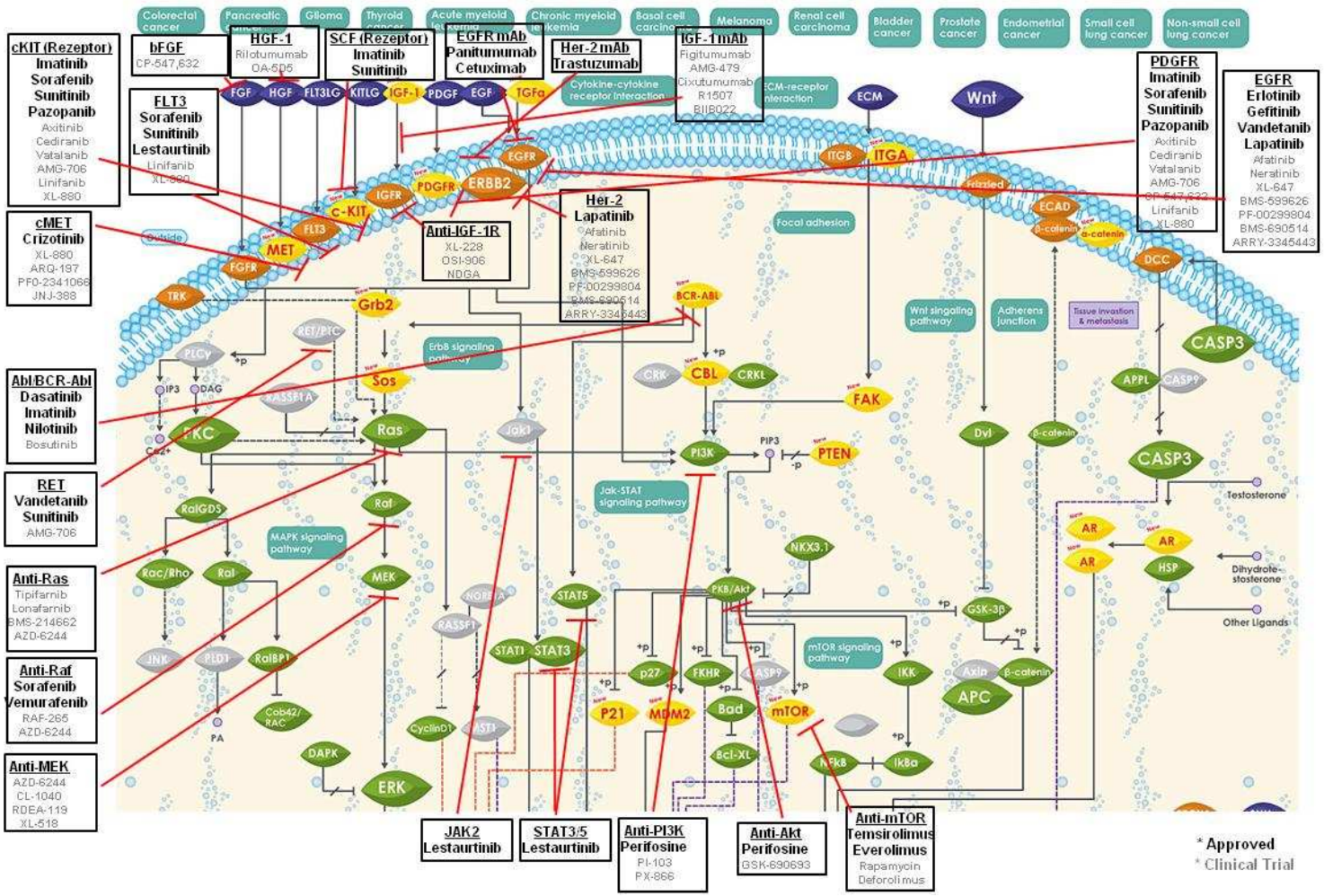
Rang	Marke	Hersteller	Umsatz 2010
1	**Avastin**	**Roche**	**6 213** Mio. US$
2	**Rituxan**	**Roche**	**6 110** Mio. US$
3	**Herceptin**	**Roche**	**5 220** Mio. US$
4	Gleevec	Novartis	**4 265** Mio. US$
5	Neulasta	Amgen	**3 558** Mio. US$
6	Taxotere	Sanofi-Aventis	**2 814** Mio. US$
7	Revlimid	Celgene	**2 466** Mio. US$
8	Alimta	Lilly	**2 208** Mio. US$

Cost of 900 Mio $ / new FDA approved compound

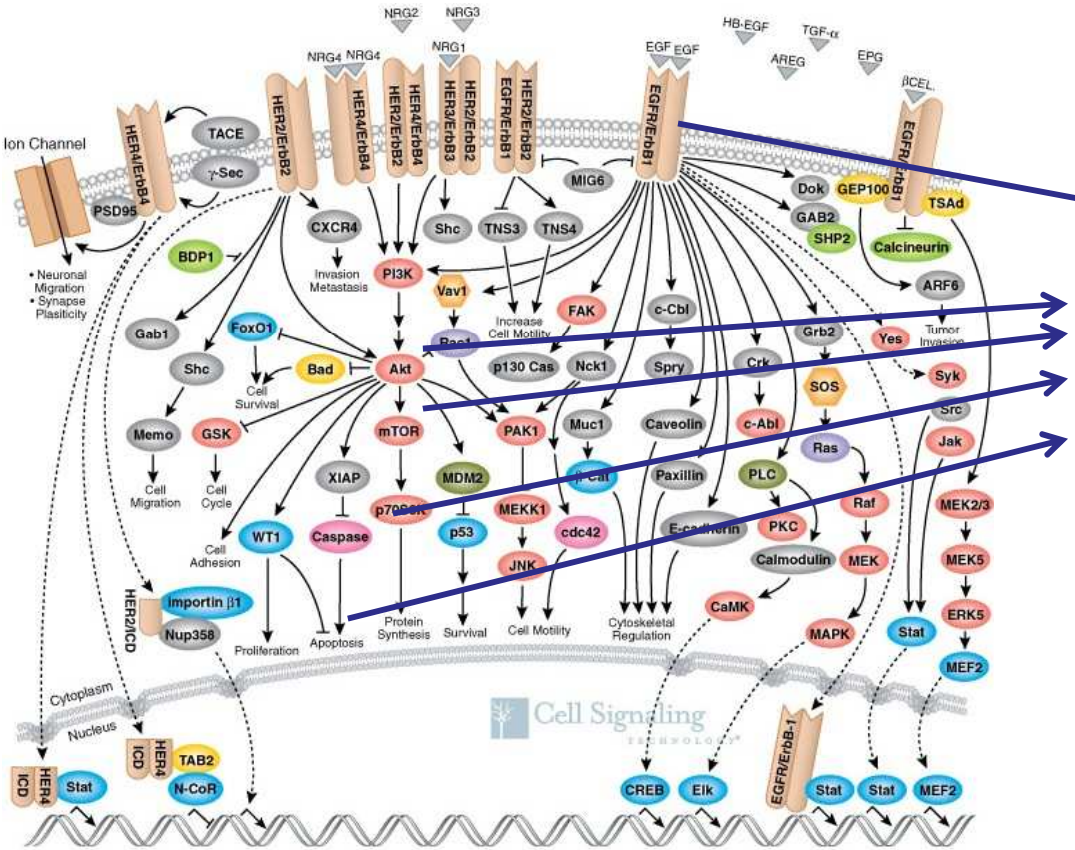
90% of compounds fail in clincial phases

Ca. 60 „Targeted Therapies“ are Approved and >800 Compounds are in Clinical Trial

(Status 2012/01)



Analysis of Pathway Activity for Individualizing Cancer Therapy



Targetexpression?

Relevant for cell biology?
(aktiv yes/no)

Which Drug?
(=Inhibitor)

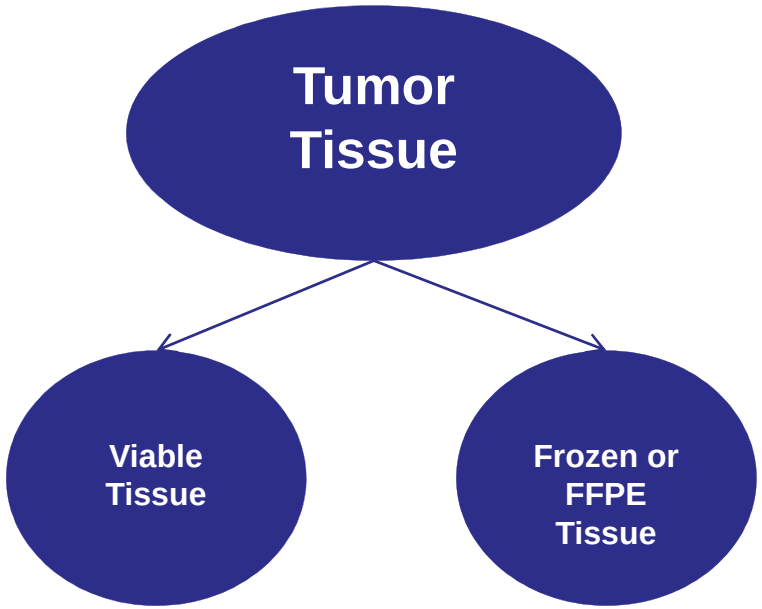
Predictive Biomarker: High Predictive Value or Robustness?

DNA → **mRNA** → **Proteins** → **Protein Modifications (e.g., phosphorylation)**

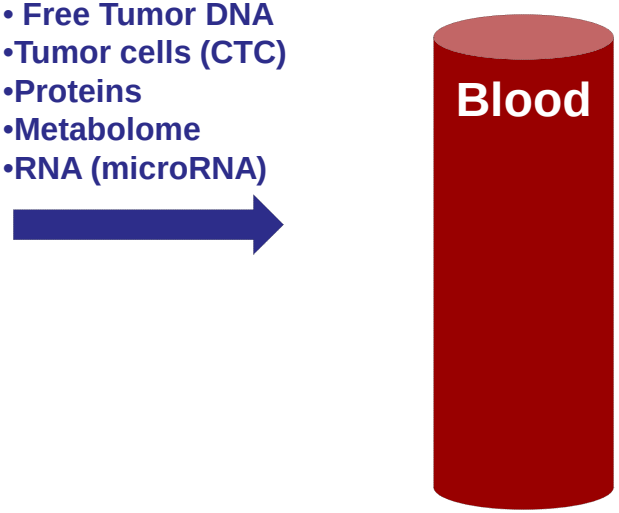
DNA	mRNA	Proteins	Protein Modifications (e.g., phosphorylation)
Mutations	**Mutations**	**Target-Expression**	**Functionality**
KRAS	Oncotype	HER-2	pmTOR
PIK3CA	AmpliChip	EGFR	P-AKT
BRAF	MammaPrint	VEGFR	P-MAPK
EGFR	Others	Others	Many, many more
Many others		Protein Profiling	

Predictive value / Impact of preanalytical factors

Diagnostic Approaches for Individualizing Cancer Therapy: Tissue and Blood



- Free Tumor DNA
- Tumor cells (CTC)
- Proteins
- Metabolome
- RNA (microRNA)



Reflects high complexity of tumor
Difficult to obtain at time of therapy
Quality matters a lot

Only low complexity of tumor
Status at time of therapy
Allows monitoring and repeated testing

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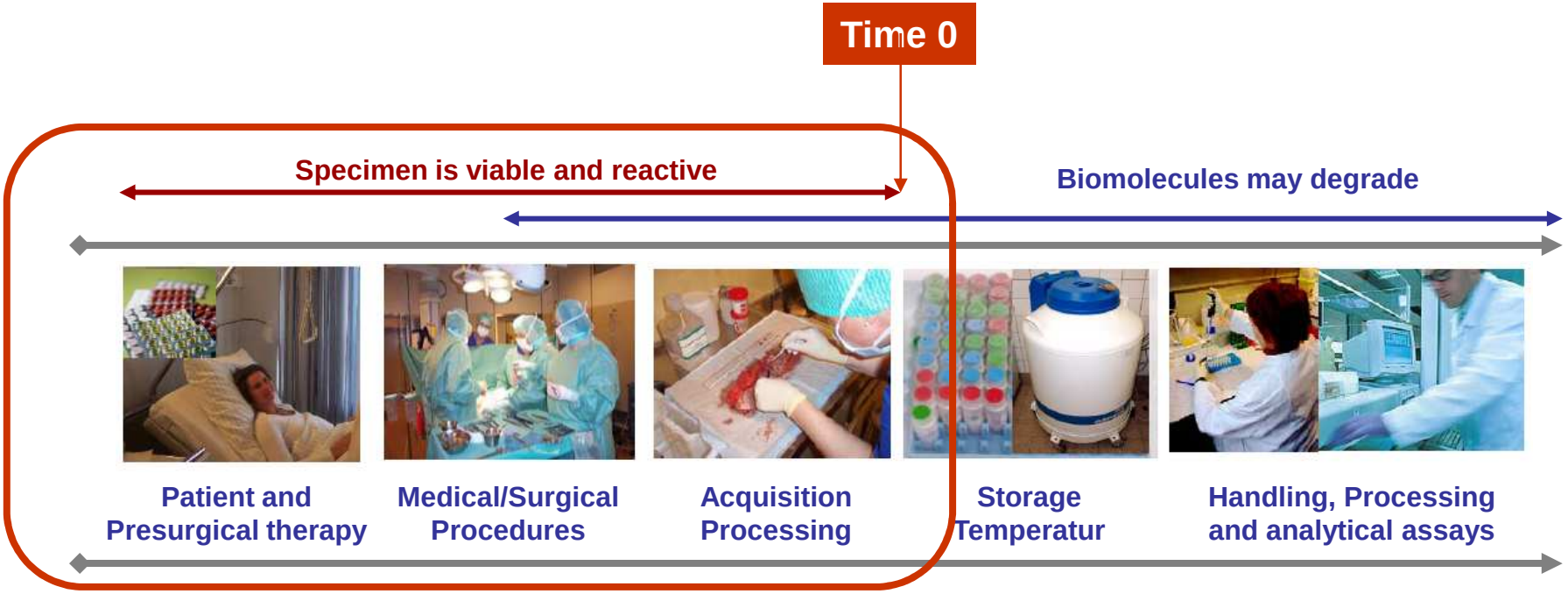
Cancer Problem #1 for Predictive Biomarker and Drug Development: : Availability of Tissue that Reflects Reality of Tumor Biology



Expression of Targets (e.g., Pathways) and Biomarker depend on Individual Variables and Tissue Processing

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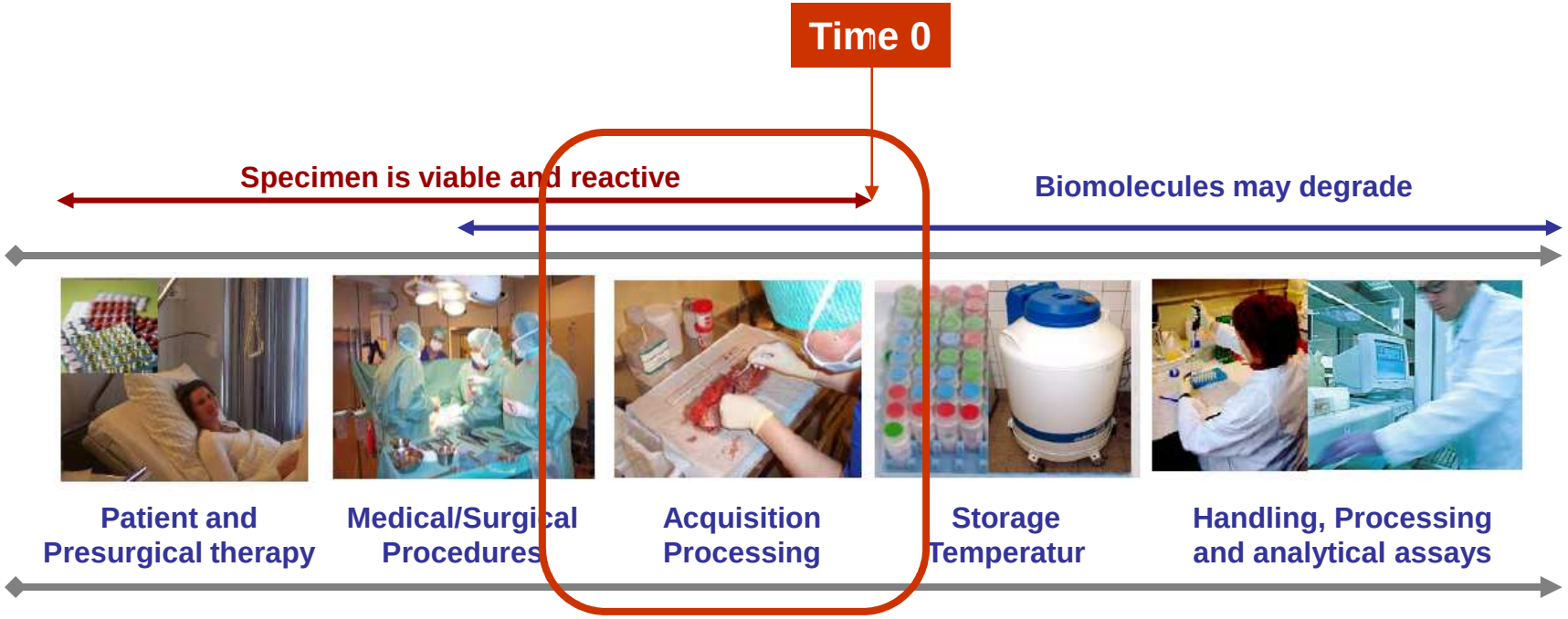
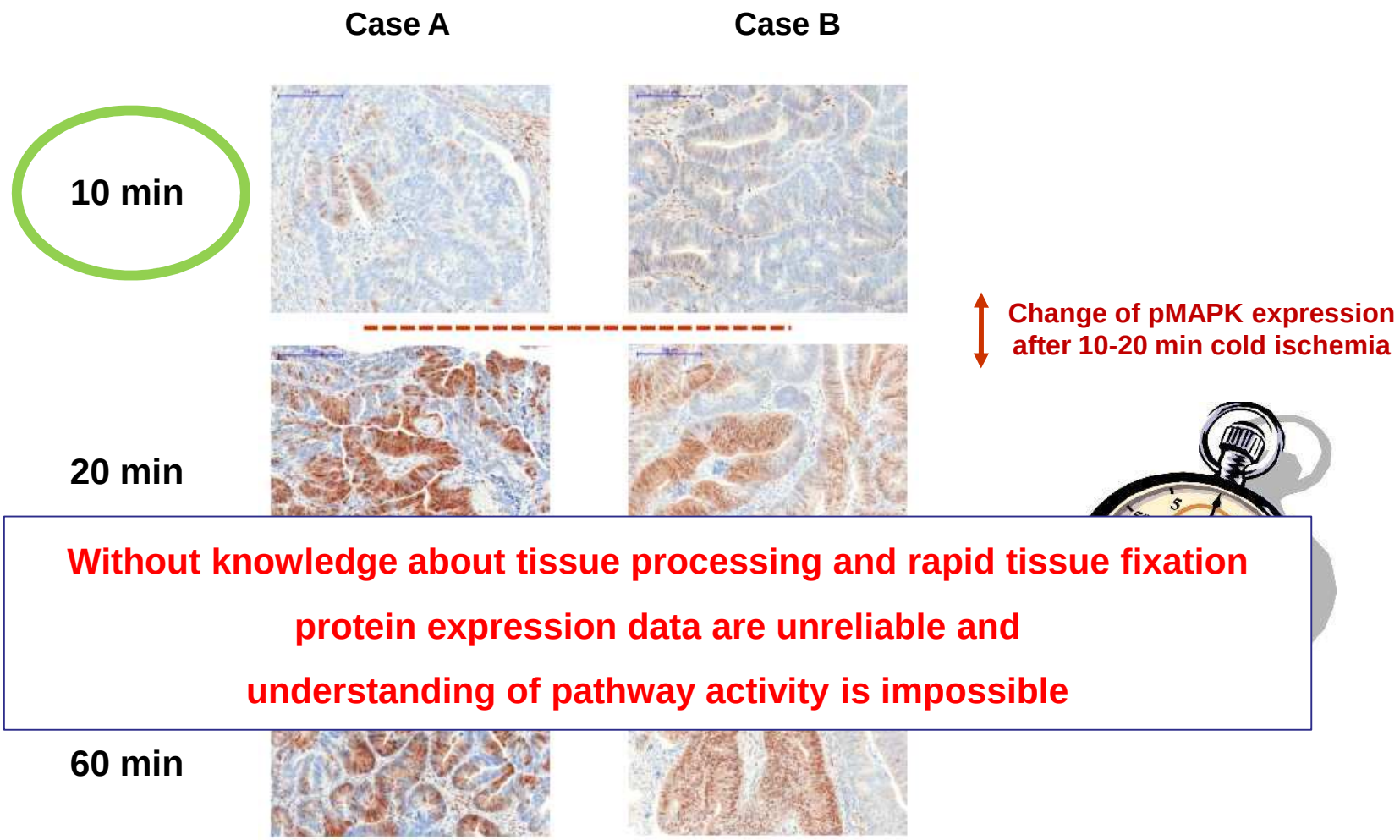
Cancer Problem #1 for Predictive Biomarker and Drug Development: : Availability of Tissue that Reflects Reality of Tumor Biology



Expression of Targets (e.g., Pathways) and Biomarker depend on Individual Variables and Tissue Processing

Phosphoprotein Expression: pMAPK Immunostaining (Ventana)



Without knowledge about tissue processing and rapid tissue fixation protein expression data are unreliable and understanding of pathway activity is impossible

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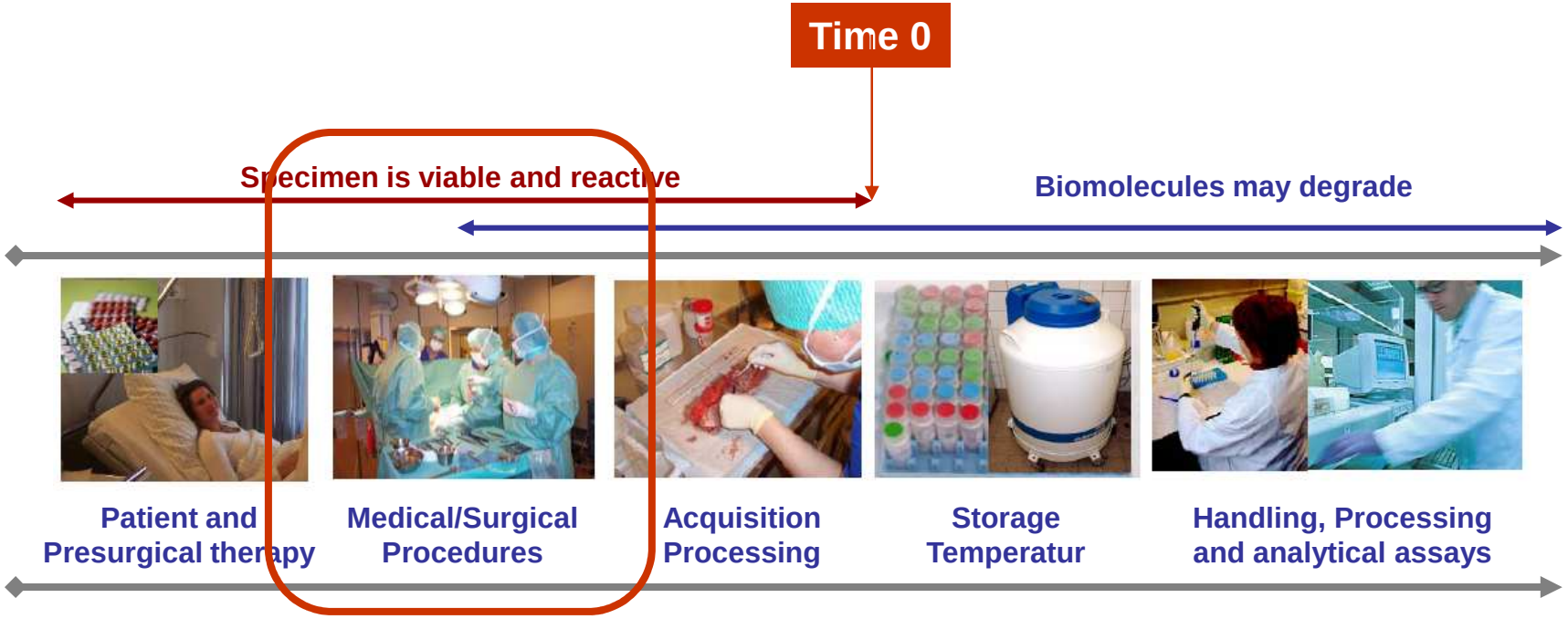
Cancer Problem #1 for Predictive Biomarker and Drug Development: : Availability of Tissue that Reflects Reality of Tumor Biology



Expression of Targets (e.g., Pathways) and Biomarker depend on Individual Variables and Tissue Processing

Retrospective Study:
Impact of Time between Ligation of Main Artery and Tumor Resection on Gene Expression in Colon Cancer

(NCI-Indivumed study)

Patients receiving left hemicolectomy

Indivumed data base / biobank:

Time (min) between artery ligation and tumor removal

20 25 30 35 40 45 50

(min)

↓

Time (min) until freezing

10 min

↓

LCM isolation of tumor cells

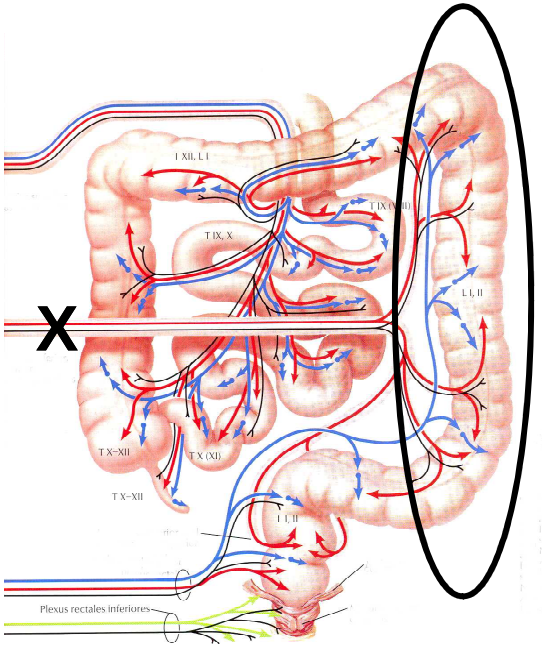
↓

Gene expression (Affymetrix)

↓

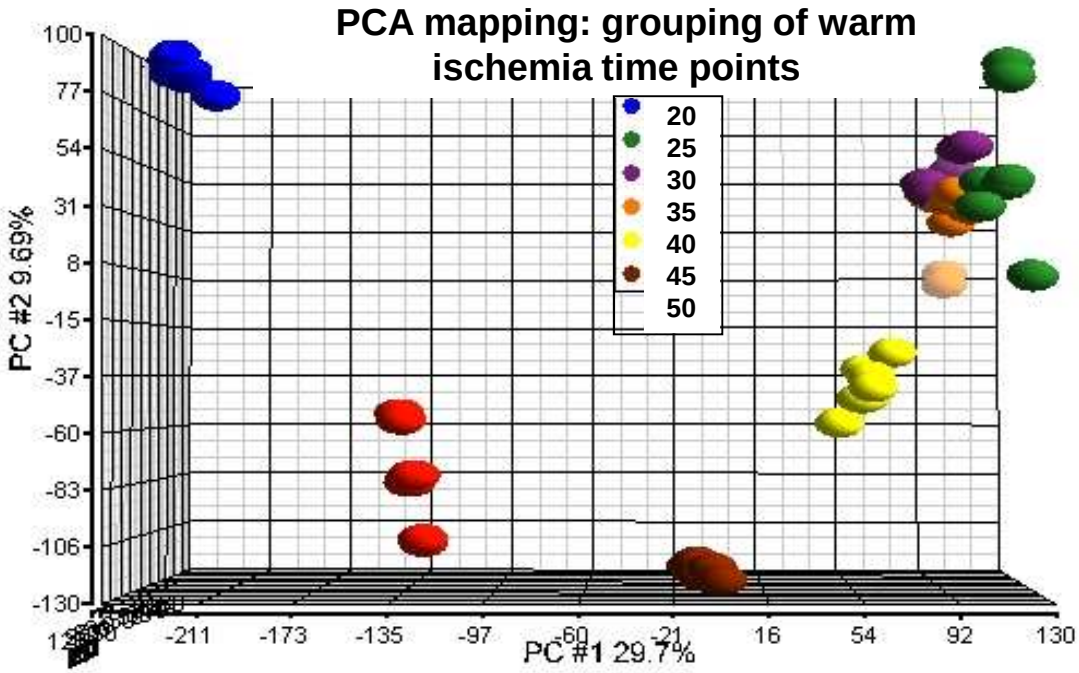
Bioinformatics

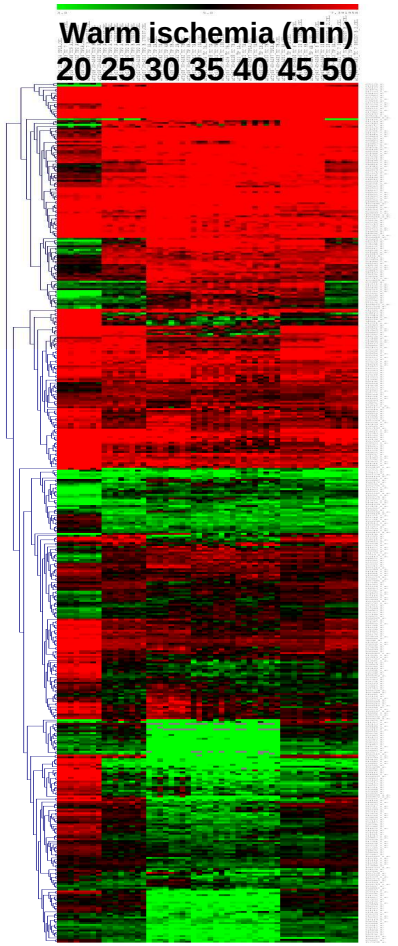
Mesenteric artery inferior



Impact of Time between Ligation of Main Artery and Tumor Resection on Gene Expression in Colon Cancer

(NCI-Indivumed study)





Prospective trial collecting tissue during surgery

Research Studies on the Effect of Intra- and Post-operative Ischemia on Gene and Protein Expression Patterns in Liver (Project 1) and Colorectal Tissue (Project 2).
An Exploratory Research Study (29XS111)

Funded by NCI Contract No. HHSN261200800001E

Partner:

OBBR/NCI

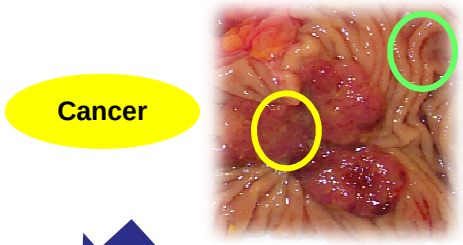
Indivumed GmbH

Department of Surgery, Israelitisches Krankenhaus (Dr. Zornig)

Department of Surgery, Diakonieklinikum Alten Eichen (Dr. Dörner)

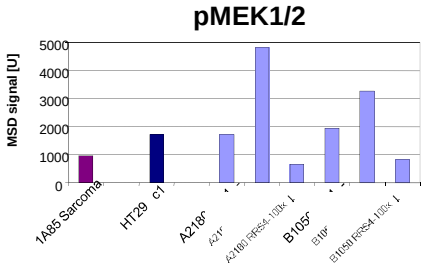
Department of Hepatobiliary Surgery, University Hospital Hamburg (PI: Dr. Nashan)

Impact of Anesthesia and Surgery on Gene and Protein Expression in Colon and Liver Tissue : Study Design

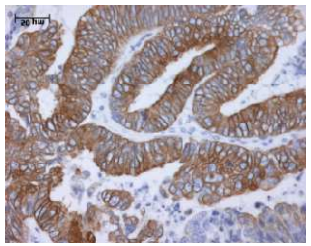


Cancer-relevant Proteins

Protein Quantification

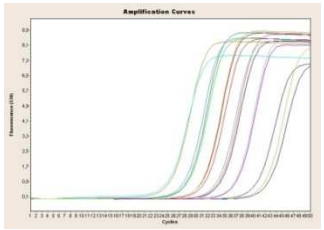


Cellular Distribution

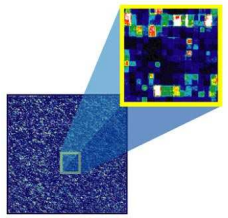


Gene Expression Profiling

Specific Genes



Comprehensive Analysis



Identification of Surgery Dependent Molecules

Analysis of Proteins:
MSD and Immunohistochemistry

Frozen Tissue:
MSD analysis

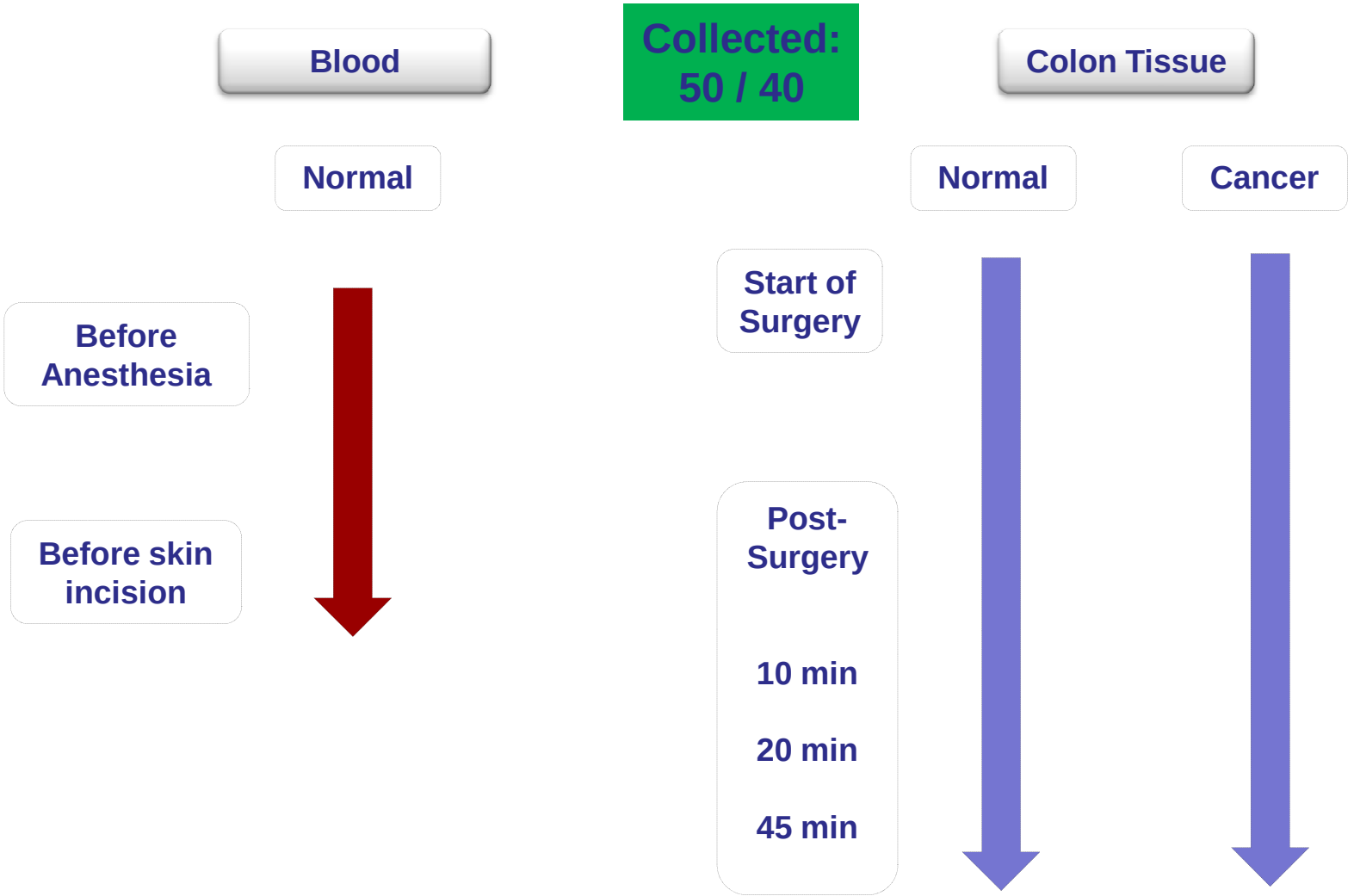
- pEGFR/total EGFR
- pMEK1/2/total MEK1/2 (Ser217/221)
- pERK1/2/total ERK1/2 (Thr202/Tyr204, Thr185/Tyr187)
- pAkt/total Akt (Ser473)
- pmTOR/total mTOR (Ser 2448)
- pP70S6K/total P70S6K (Thr421/Ser424)
- pGSK-3beta/total GSK-3beta (Ser9)

- control protein x
- Hif-1alpha

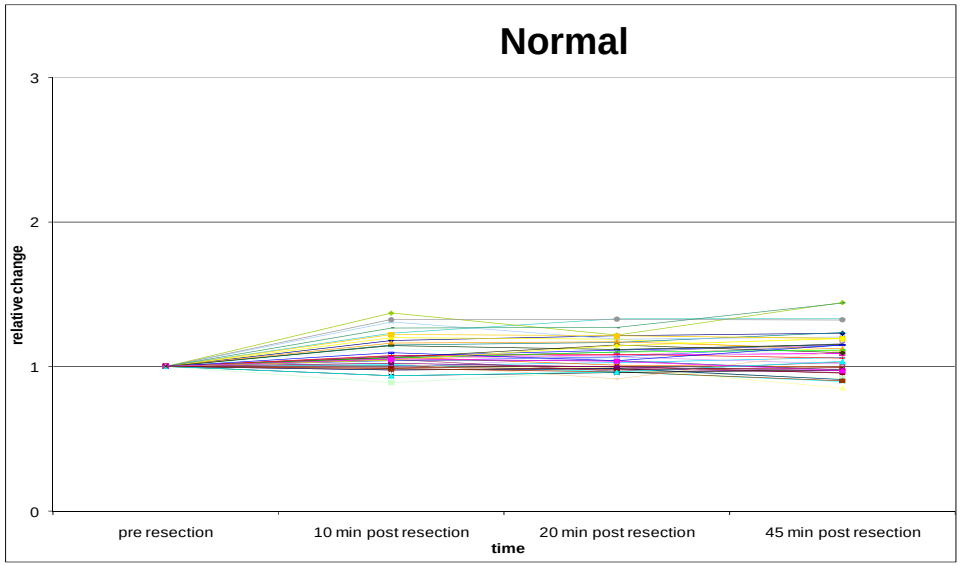
FFPE Tissue
IHC (Ventana)

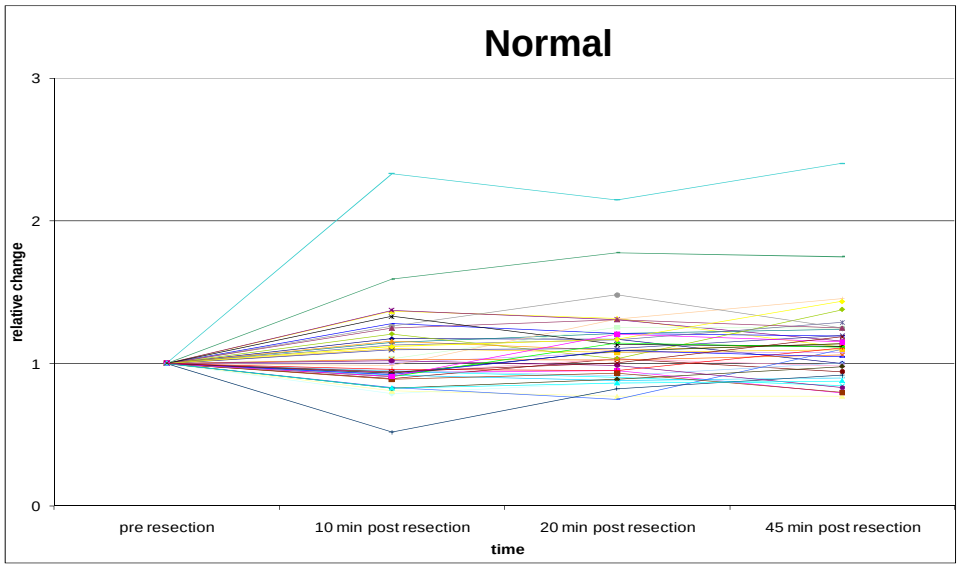
- pEGF-R
- pHER-3
- pMAPK
- pAKT
- pmTOR

Impact of Anesthesia and Surgery on Gene and Protein Expression in Colorectal Tissue (Project 2)



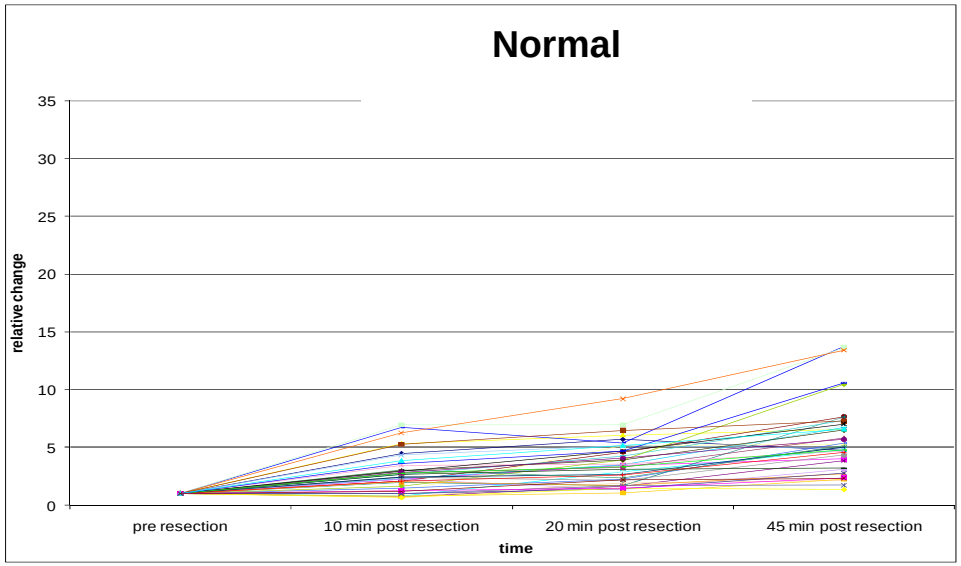
Impact of intra- and postsurgical factors: Analysis of Total-control protein X by MSD (Cellular Stress Marker)

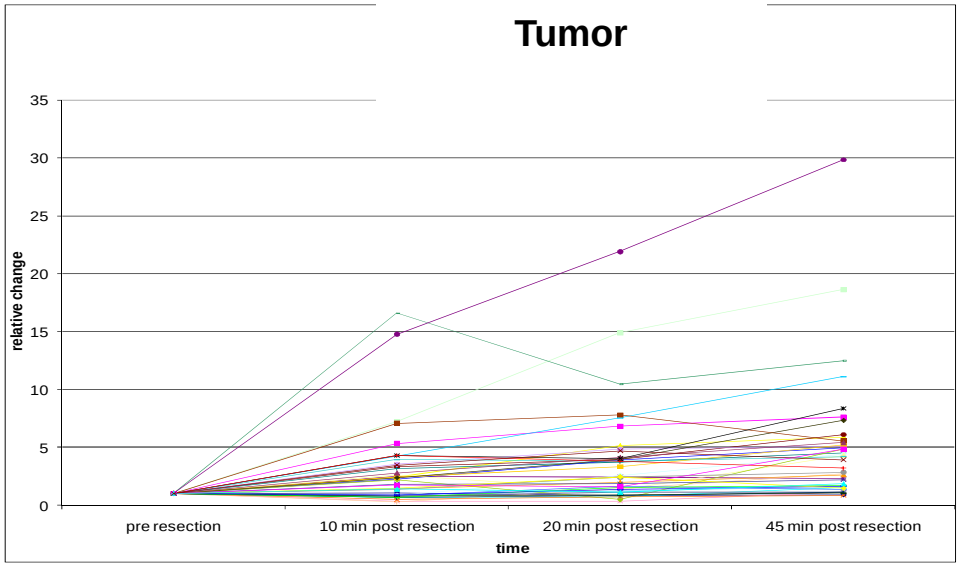




Case NC7 Case NC13 Case NC17 Case NC27 Case NC35 Case NC39 Case NC40 Case NC41 Case NC42 Case NC47
Case NC9 Case NC11 Case NC12 Case NC18 Case NC20 Case NC21 Case NC28 Case NC30 Case NC33 Case NC36
Case NC2 Case NC3 Case NC4 Case NC5 Case NC8 Case NC10 Case NC15 Case NC16 Case NC22 Case NC23
Case NC25 Case NC26 Case NC29 Case NC32 Case NC34 Case NC37 Case NC38 Case NC43 Case NC46 Case NC48

Impact of intra- and postsurgical factors: Analysis of Phospho-control protein X by MSD (Cellular Stress Marker)





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Case NC9 Case NC11 Case NC12 Case NC18 Case NC20 Case NC21 Case NC28 Case NC30 Case NC33 Case NC36
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Case NC25 Case NC26 Case NC29 Case NC32 Case NC34 Case NC37 Case NC38 Case NC43 Case NC46 Case NC48

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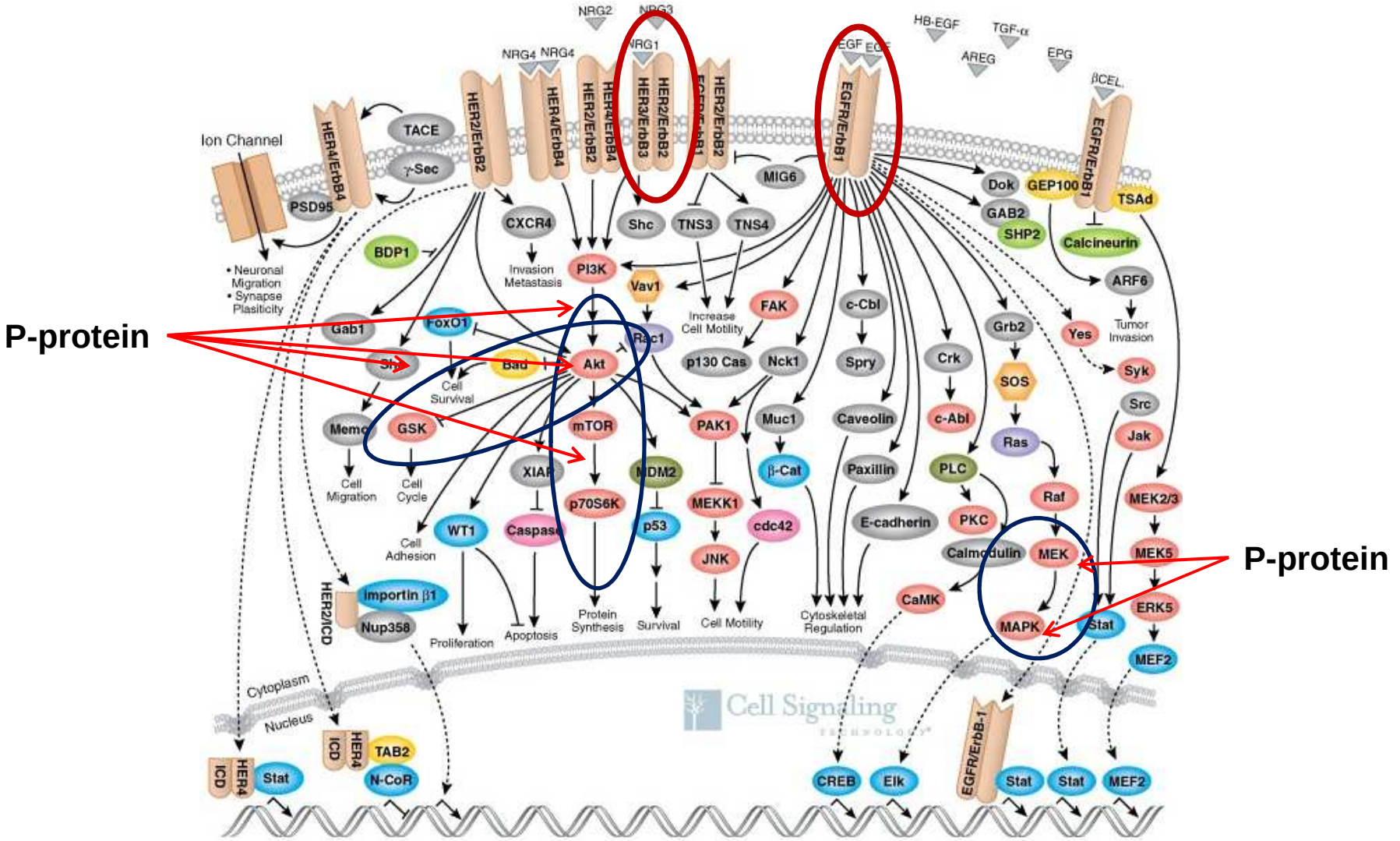
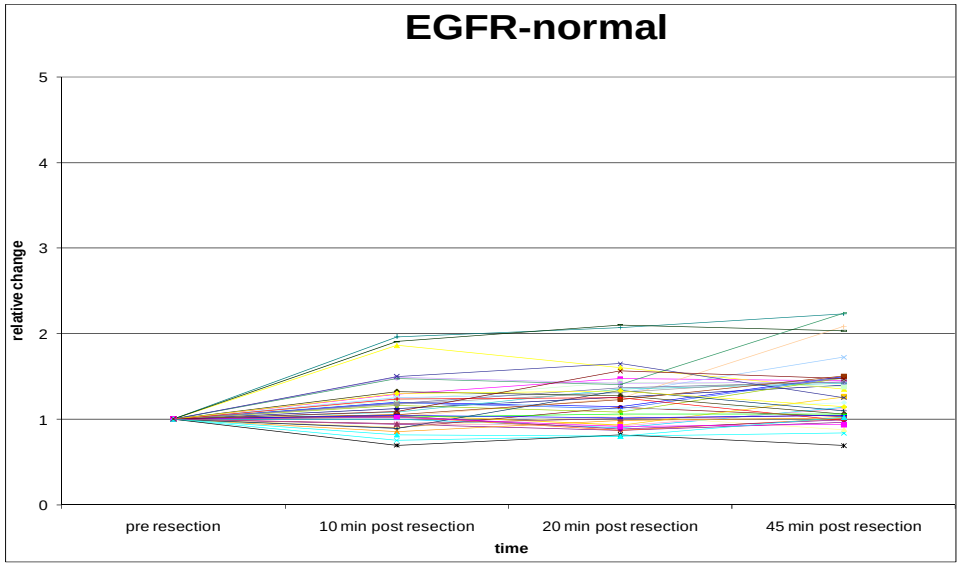
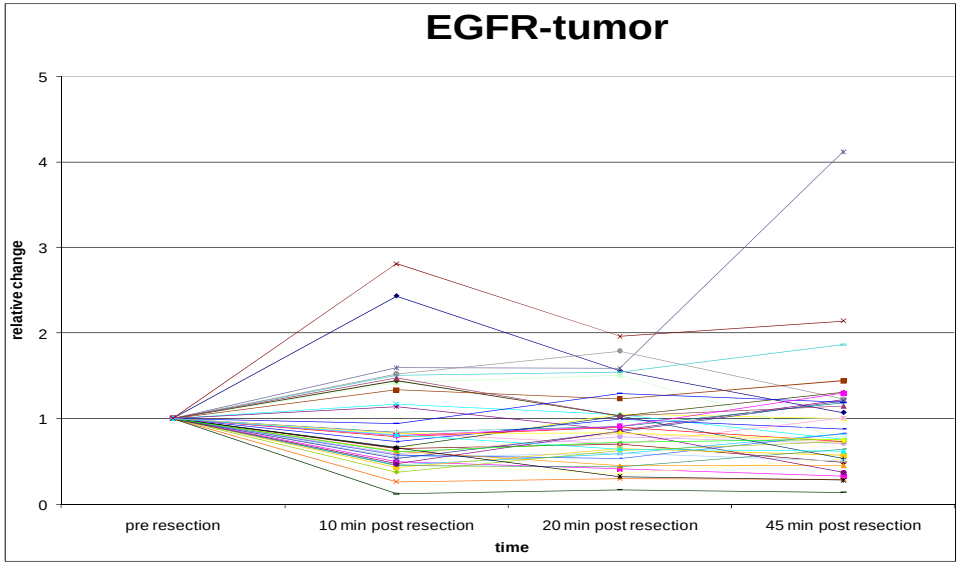
Analysis of HER-family Pathway in CRC Tissue: Impact of intra- and postsurgical factors



Impact of intra- and postsurgical factors: Analysis of Total-EGFR by MSD





Case NC7 Case NC13 Case NC17 Case NC27 Case NC35 Case NC39 Case NC40 Case NC41 Case NC42 Case NC47
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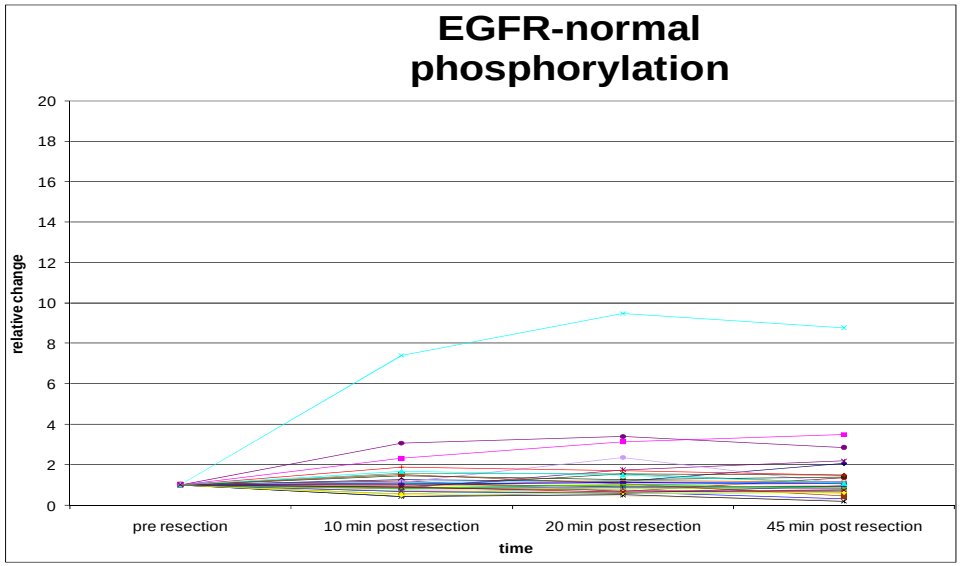
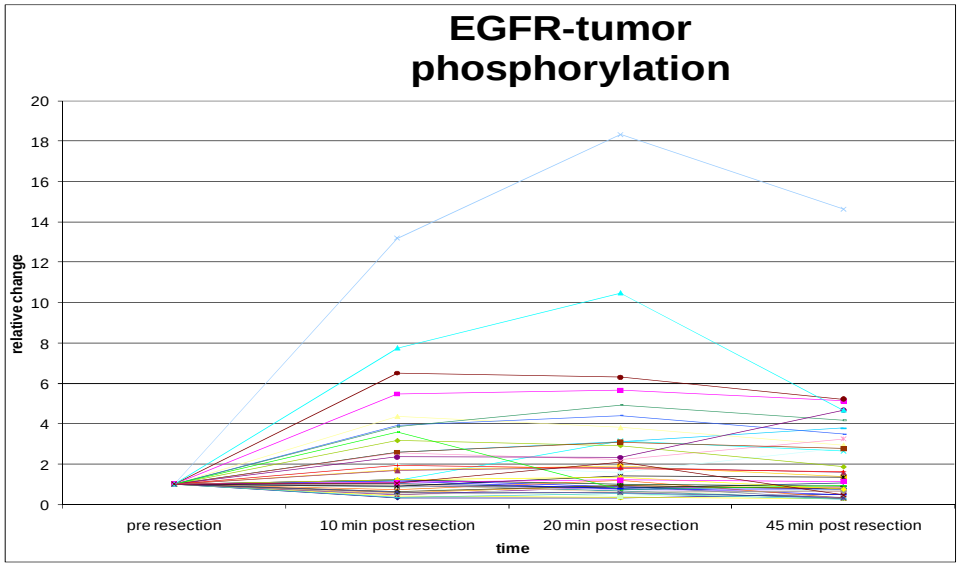
Impact of intra- and postsurgical factors: Analysis of Phospho-EGFR by MSD





Case NC7 Case NC13 Case NC17 Case NC27 Case NC35 Case NC39 Case NC40 Case NC41 Case NC42 Case NC47
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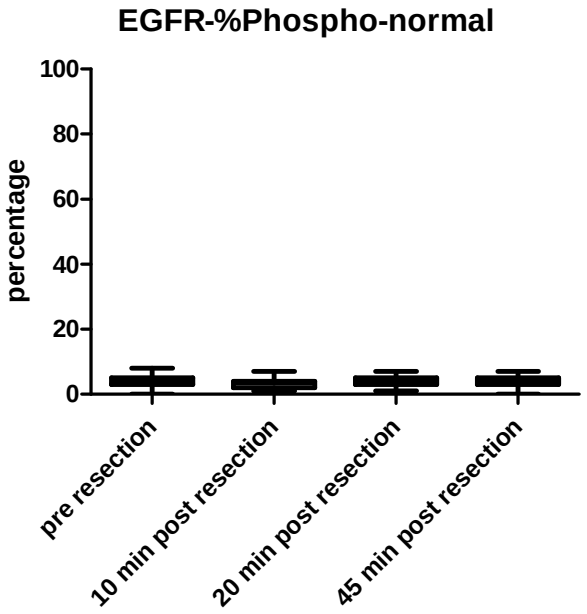
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-EGFR by MSD



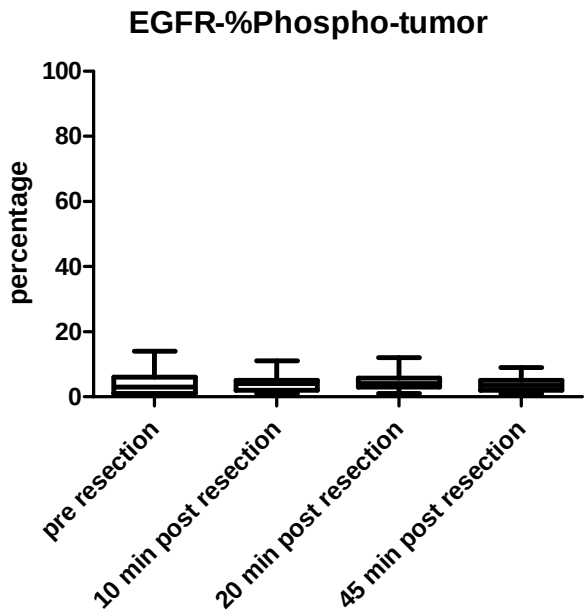
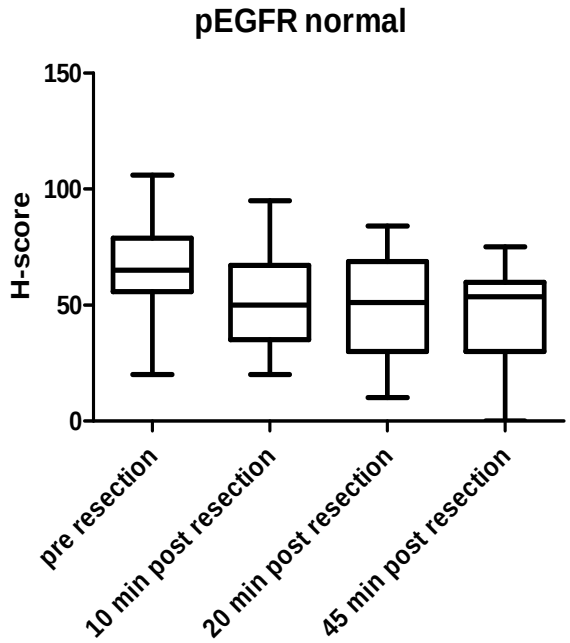
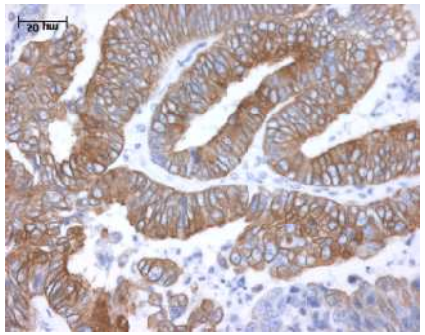


Table Analyzed	EGFR-%Phospho-normal		
Kruskal-Wallis test			
P value	0.4648		
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups	4		
Kruskal-Wallis statistic	2.559		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	16.04	No	ns
pre resection vs 20 min post resection	6.363	No	ns
pre resection vs 45 min post resection	8.900	No	ns
10 min post resection vs 20 min post resection	-9.675	No	ns
10 min post resection vs 45 min post resection	-7.138	No	ns
20 min post resection vs 45 min post resection	2.538	No	ns

Table Analyzed	EGFR-%Phospho-tumor		
Kruskal-Wallis test			
P value	0.4629		
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups	4		
Kruskal-Wallis statistic	2.569		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	-12.15	No	ns
pre resection vs 20 min post resection	-14.29	No	ns
pre resection vs 45 min post resection	-4.163	No	ns
10 min post resection vs 20 min post resection	-2.138	No	ns
10 min post resection vs 45 min post resection	7.988	No	ns
20 min post resection vs 45 min post resection	10.13	No	ns

Analysis of Phospho-EGFR by IHC





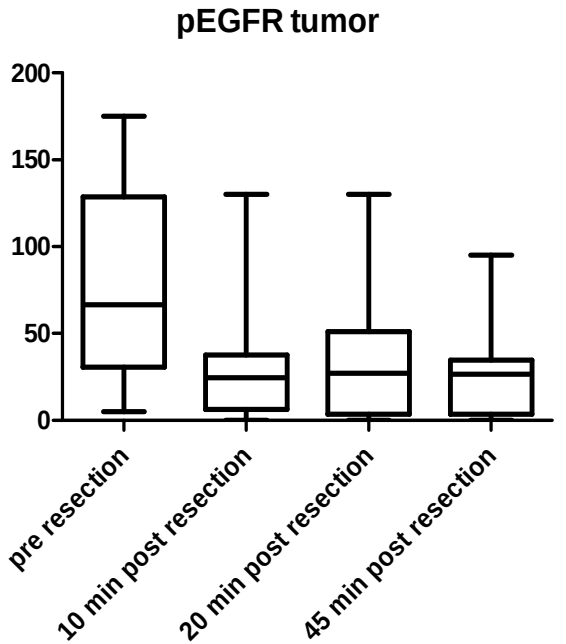
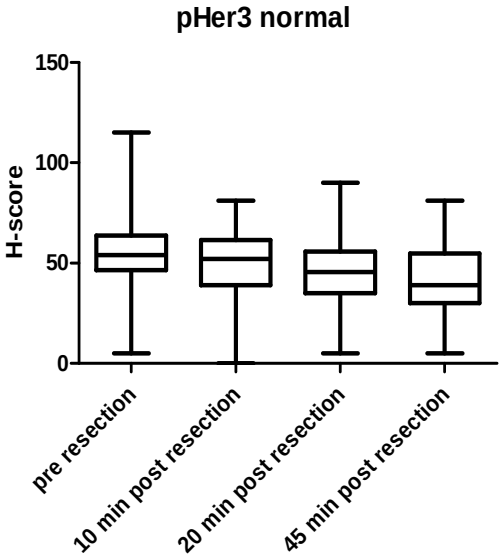
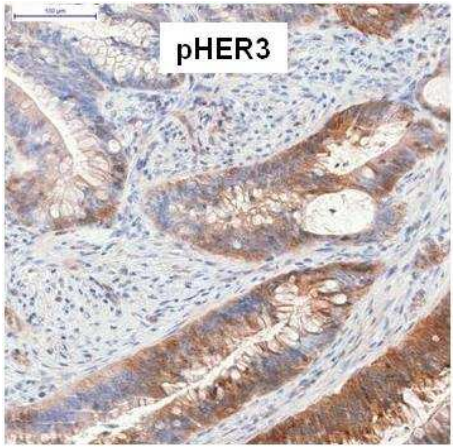


Table Analyzed	pEGFR normal		
Kruskal-Wallis test			
P value	0.0268		
Exact or approximate P value?	Gaussian Approximation		
P value summary	*		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	9.194		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	16.45	No	ns
pre resection vs 20 min post resection	16.45	No	ns
pre resection vs 45 min post resection	20.40	Yes	*
10 min post resection vs 20 min post resection	0.0	No	ns
10 min post resection vs 45 min post resection	3.950	No	ns
20 min post resection vs 45 min post resection	3.950	No	ns

Table Analyzed	pEGFR tumor		
Kruskal-Wallis test			
P value	0.0020		
Exact or approximate P value?	Gaussian Approximation		
P value summary	**		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	14.80		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	22.53	Yes	*
pre resection vs 20 min post resection	20.58	Yes	*
pre resection vs 45 min post resection	25.10	Yes	**
10 min post resection vs 20 min post resection	-1.950	No	ns
10 min post resection vs 45 min post resection	2.575	No	ns
20 min post resection vs 45 min post resection	4.525	No	ns

Analysis of Phospho-HER3 by IHC





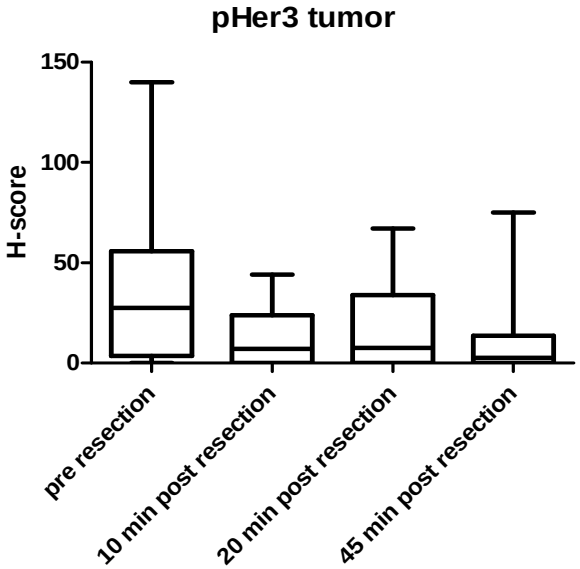


Table Analyzed	pHer3 normal				
One-way analysis of variance					
P value	0.1131				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	2.056				
R square	0.07508				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	2.045				
P value	0.5631				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	2550	3	850.0		
Residual (within columns)	31415	76	413.4		
Total	33965	79			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
pre resection vs 10 min post resection	6.550	1.019	No	ns	-10.87 to 23.97
pre resection vs 20 min post resection	10.10	1.571	No	ns	-7.317 to 27.52
pre resection vs 45 min post resection	15.55	2.419	No	ns	-1.867 to 32.97
10 min post resection vs 20 min post resection	3.550	0.5522	No	ns	-13.87 to 20.97
10 min post resection vs 45 min post resection	9.000	1.400	No	ns	-8.417 to 26.42
20 min post resection vs 45 min post resection	5.450	0.8477	No	ns	-11.97 to 22.87

Table Analyzed	pHer3 tumor		
Kruskal-Wallis test			
P value	0.0187		
Exact or approximate P value?	Gaussian Approximation		
P value summary	*		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	9.990		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	17.75	No	ns
pre resection vs 20 min post resection	13.73	No	ns
pre resection vs 45 min post resection	21.03	Yes	*
10 min post resection vs 20 min post resection	-4.025	No	ns
10 min post resection vs 45 min post resection	3.275	No	ns
20 min post resection vs 45 min post resection	7.300	No	ns

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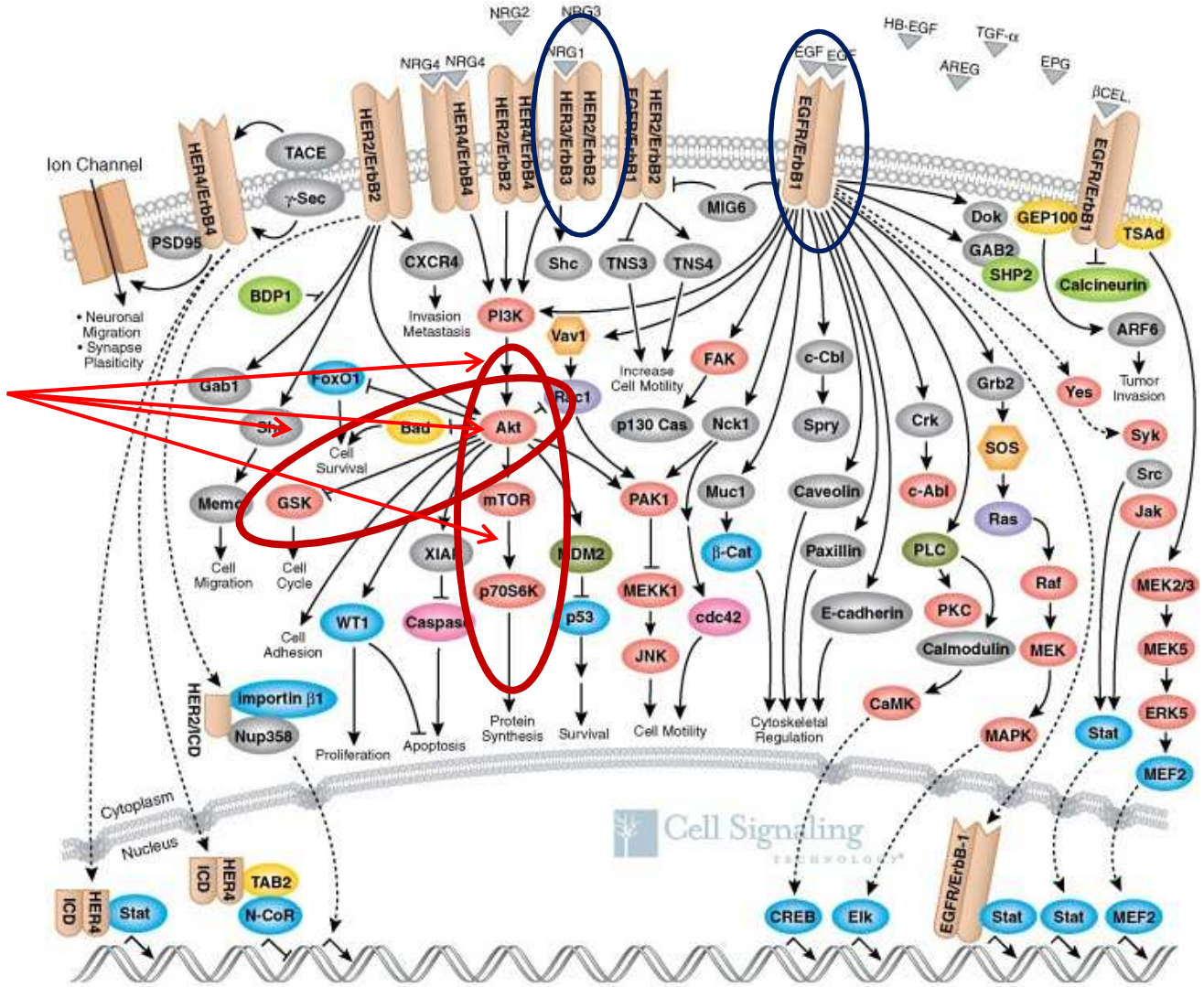
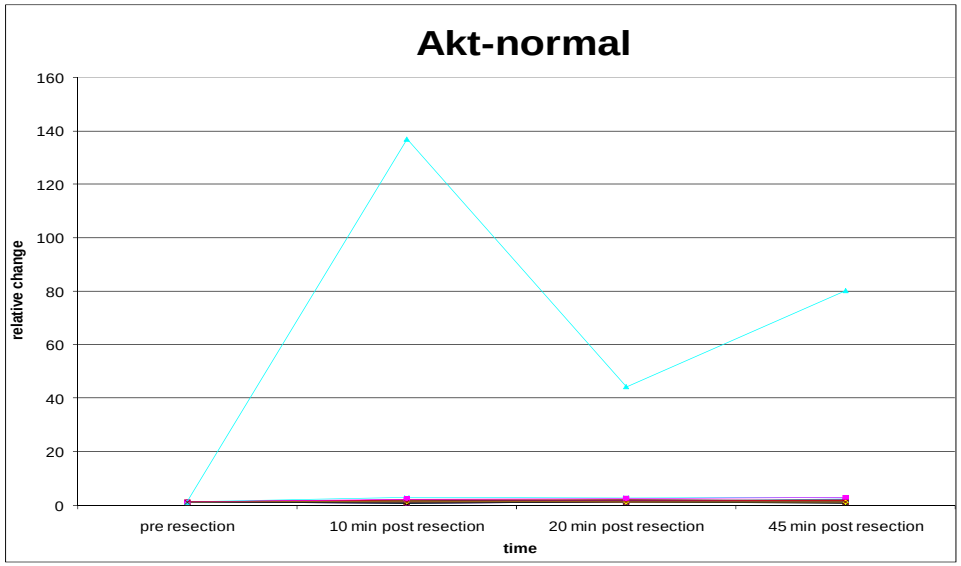
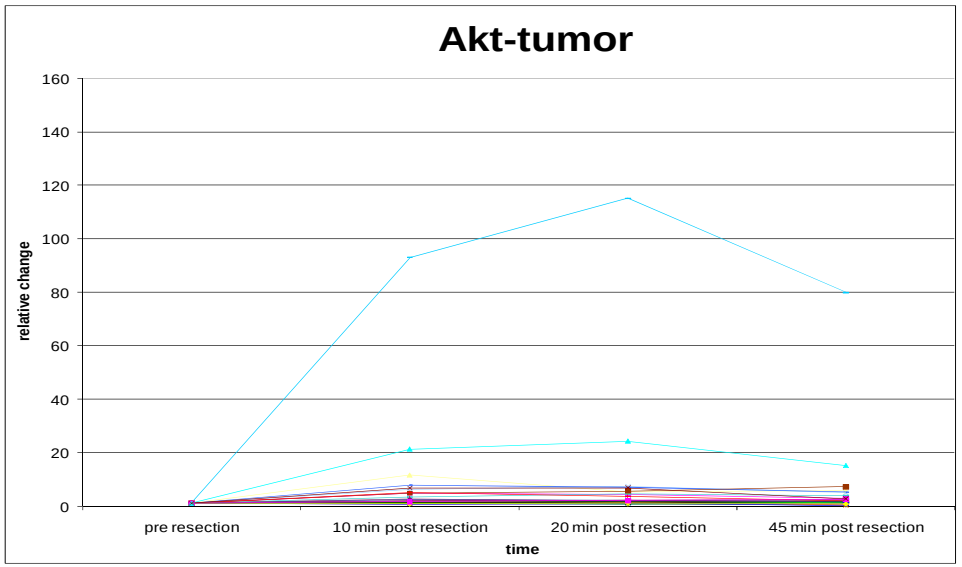
Analysis of HER-family Pathway in CRC Tissue: Impact of intra- and postsurgical factors

P-protein



Impact of intra- and postsurgical factors: Analysis of Total-AKT by MSD





Case NC7 Case NC13 Case NC17 Case NC27 Case NC35 Case NC39 Case NC40 Case NC41 Case NC42 Case NC47
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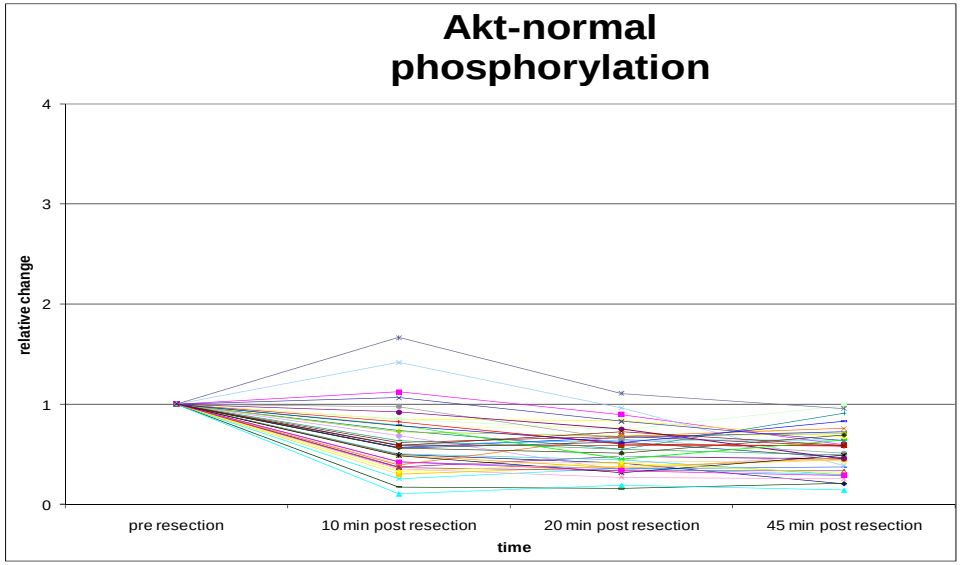
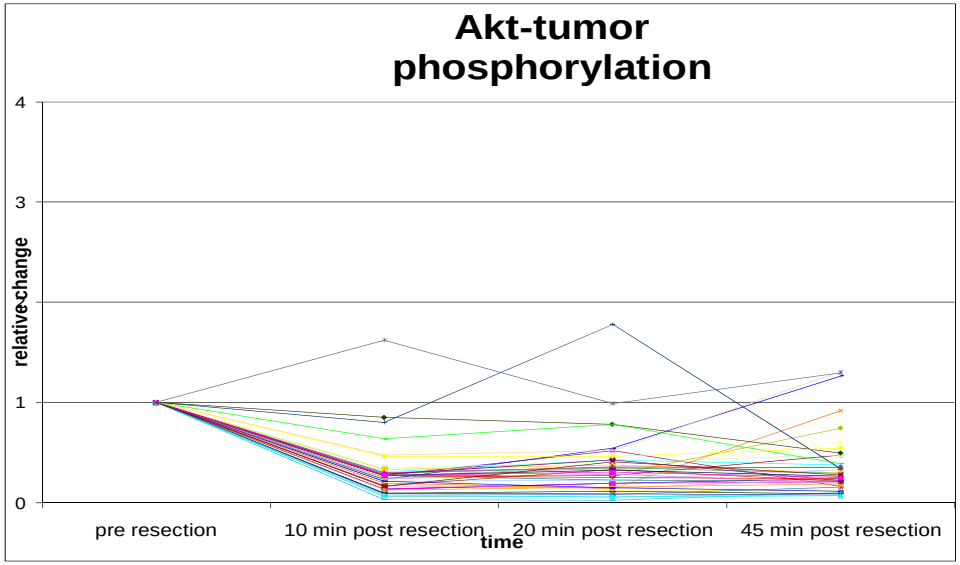
Case NC25 Case NC26 Case NC29 Case NC32 Case NC34 Case NC37 Case NC38 Case NC43 Case NC46 Case NC48

Impact of intra- and postsurgical factors: Analysis of Phospho-AKT by MSD





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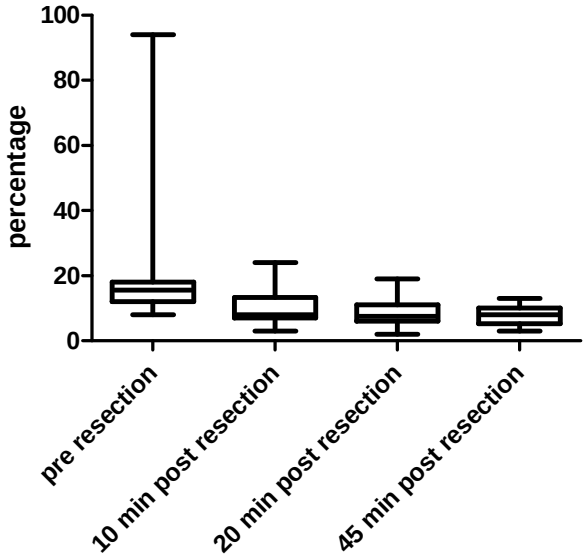
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-AKT by MSD

Akt-%Phospho-normal



Akt-%Phospho-tumor

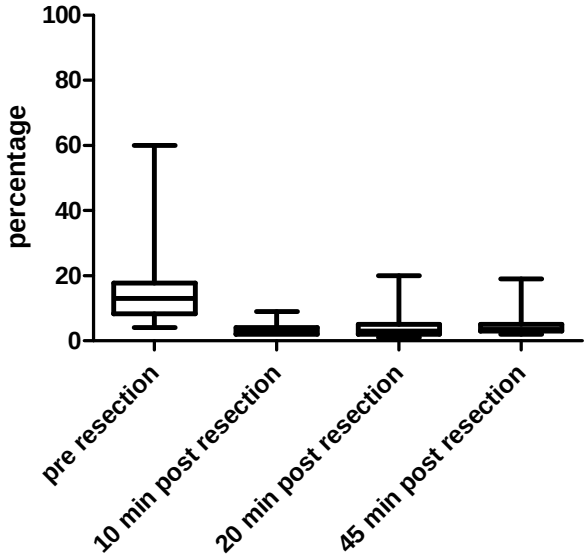
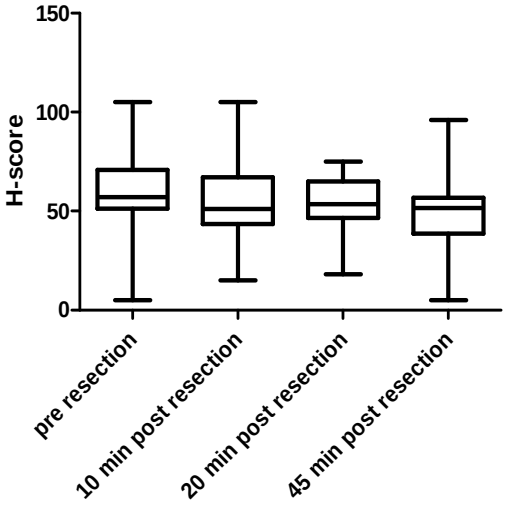
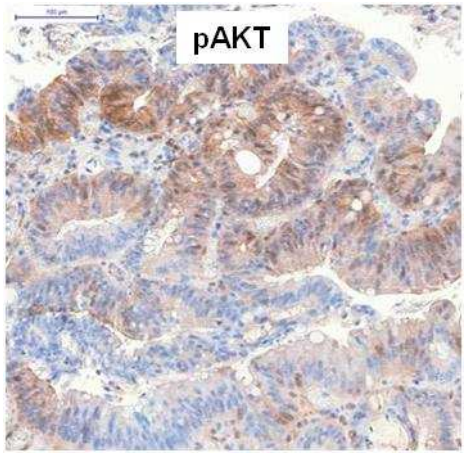


Table Analyzed	Akt-%Phospho-normal		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	****		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	54.76		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	53.74	Yes	***
pre resection vs 20 min post resection	61.75	Yes	***
pre resection vs 45 min post resection	68.26	Yes	***
10 min post resection vs 20 min post resection	8.013	No	ns
10 min post resection vs 45 min post resection	14.53	No	ns
20 min post resection vs 45 min post resection	6.513	No	ns

Table Analyzed	Akt-%Phospho-tumor		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	****		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	80.39		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	82.64	Yes	***
pre resection vs 20 min post resection	71.80	Yes	***
pre resection vs 45 min post resection	67.11	Yes	***
10 min post resection vs 20 min post resection	-10.84	No	ns
10 min post resection vs 45 min post resection	-15.53	No	ns
20 min post resection vs 45 min post resection	-4.688	No	ns

Analysis of Phospho-AKT by IHC





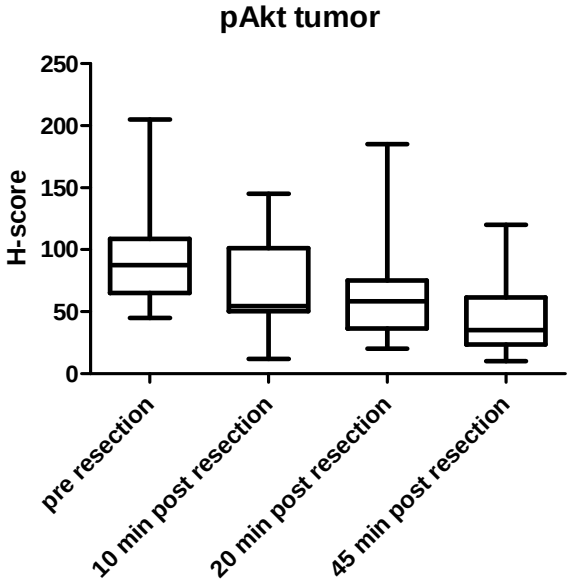
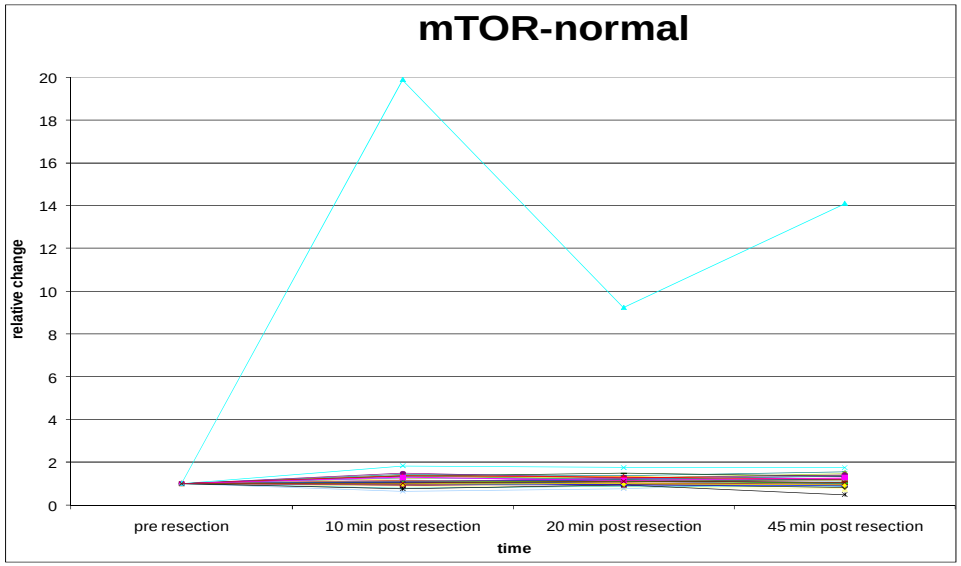
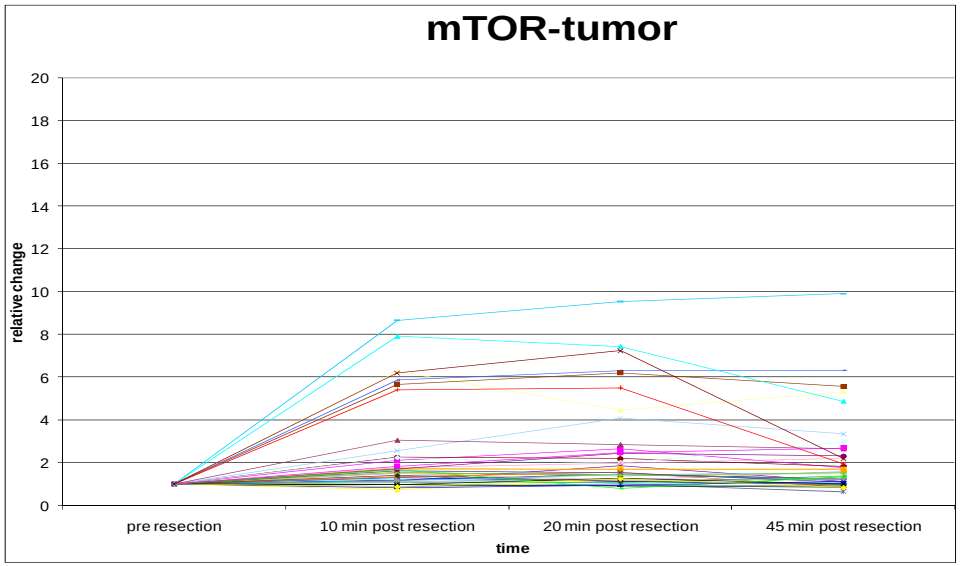


Table Analyzed	pAkt normal				
One-way analysis of variance					
P value	0.6336				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	0.5745				
R square	0.02217				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	4.075				
P value	0.2534				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	641.2	3	213.7		
Residual (within columns)	28279	76	372.1		
Total	28920	79			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
pre resection vs 10 min post resection	5.750	0.9426	No	ns	-10.78 to 22.28
pre resection vs 20 min post resection	4.600	0.7541	No	ns	-11.93 to 21.13
pre resection vs 45 min post resection	7.700	1.262	No	ns	-8.825 to 24.23
10 min post resection vs 20 min post resection	-1.150	0.1885	No	ns	-17.68 to 15.38
10 min post resection vs 45 min post resection	1.950	0.3197	No	ns	-14.58 to 18.48
20 min post resection vs 45 min post resection	3.100	0.5082	No	ns	-13.43 to 19.63

Table Analyzed	pAkt tumor		
Kruskal-Wallis test			
P value	0.0010		
Exact or approximate P value?	Gaussian Approximation		
P value summary	**		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	16.21		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	16.63	No	ns
pre resection vs 20 min post resection	17.98	No	ns
pre resection vs 45 min post resection	29.30	Yes	***
10 min post resection vs 20 min post resection	1.350	No	ns
10 min post resection vs 45 min post resection	12.68	No	ns
20 min post resection vs 45 min post resection	11.33	No	ns

Impact of intra- and postsurgical factors: Analysis of Total-mTOR by MSD





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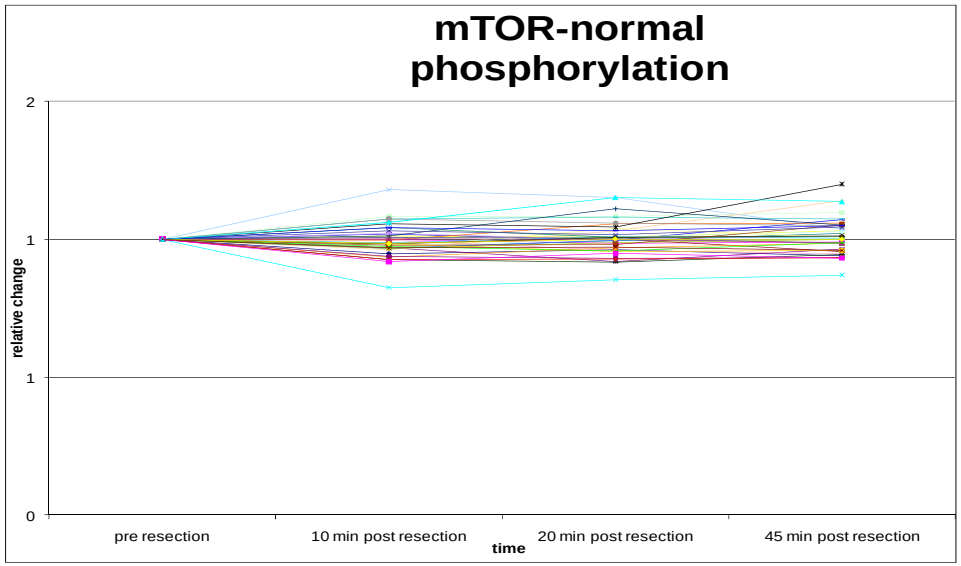
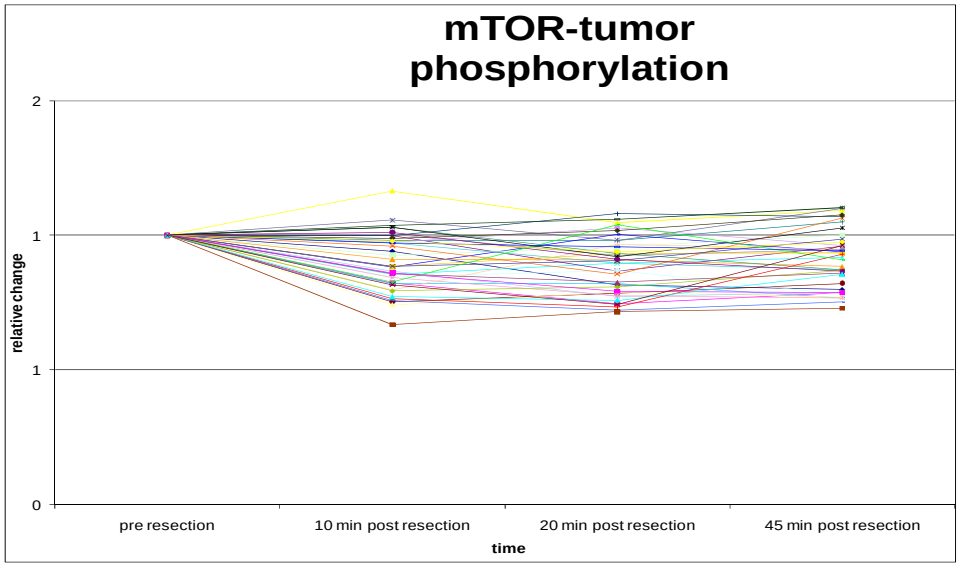
Impact of intra- and postsurgical factors: Analysis of Phospho-mTOR by MSD





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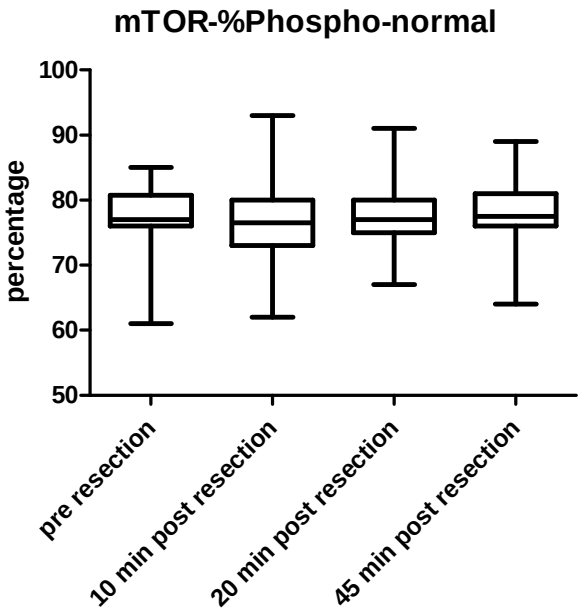
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-mTOR by MSD



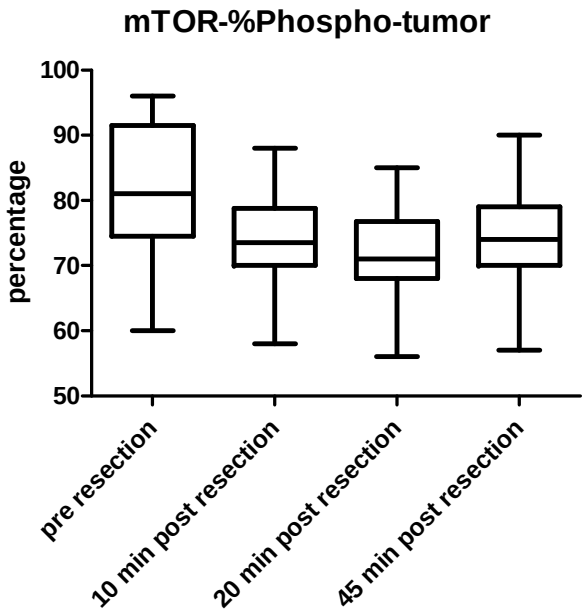
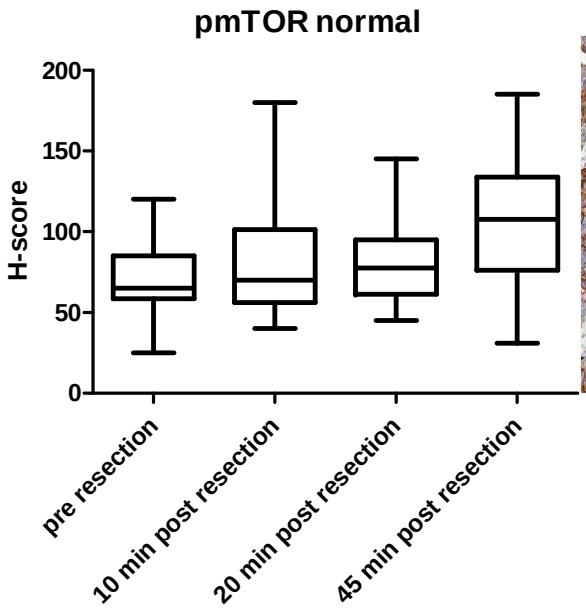
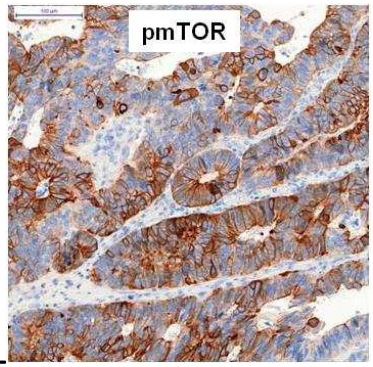


Table Analyzed	mTOR-%Phospho-normal		
Kruskal-Wallis test			
P value	0.5191		
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups	4		
Kruskal-Wallis statistic	2.266		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	11.78	No	ns
pre resection vs 20 min post resection	7.588	No	ns
pre resection vs 45 min post resection	-1.663	No	ns
10 min post resection vs 20 min post resection	-4.188	No	ns
10 min post resection vs 45 min post resection	-13.44	No	ns
20 min post resection vs 45 min post resection	-9.250	No	ns

Table Analyzed	mTOR-%Phospho-tumor		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	****		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	31.84		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	43.81	Yes	***
pre resection vs 20 min post resection	54.80	Yes	***
pre resection vs 45 min post resection	38.54	Yes	**
10 min post resection vs 20 min post resection	10.99	No	ns
10 min post resection vs 45 min post resection	-5.275	No	ns
20 min post resection vs 45 min post resection	-16.26	No	ns

Analysis of Phospho-mTOR by IHC





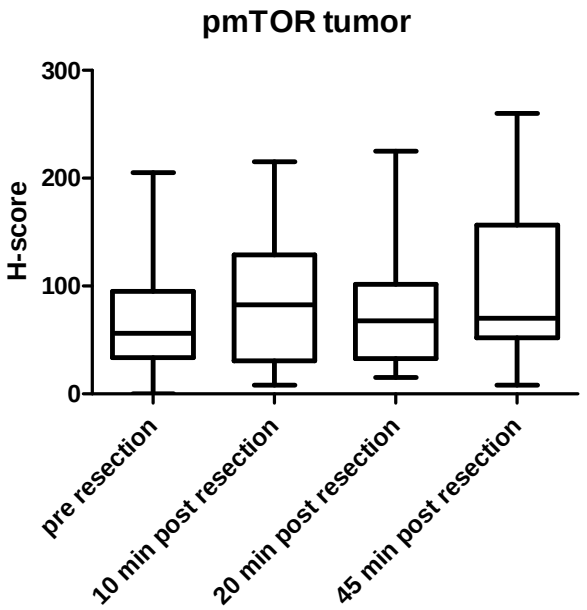
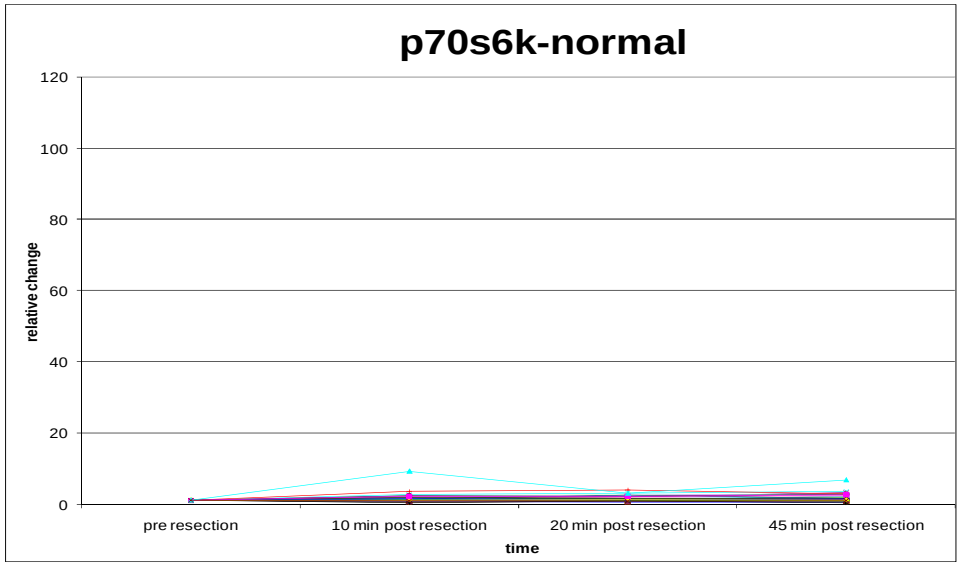
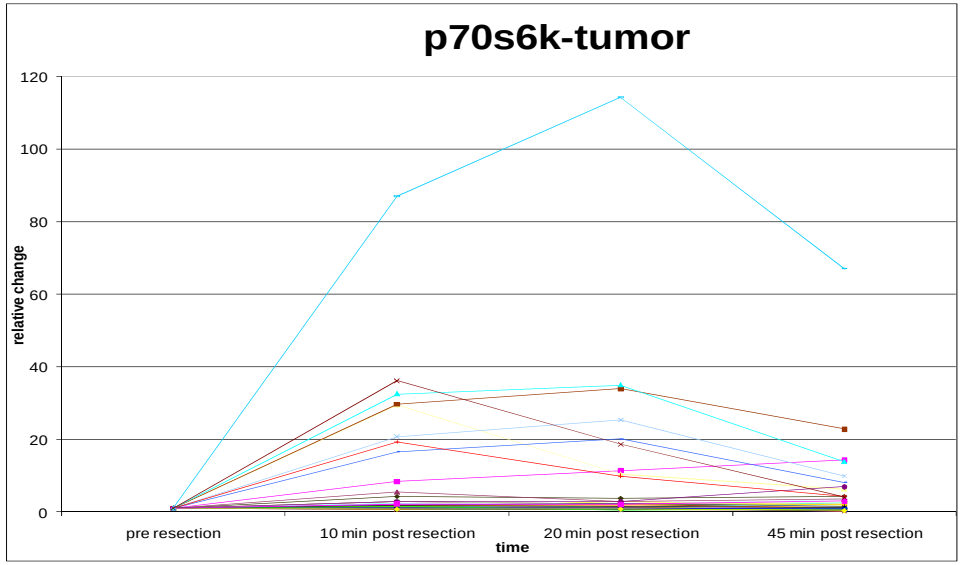


Table Analyzed	pmTOR normal		
Kruskal-Wallis test			
P value	0.0026		
Exact or approximate P value?	Gaussian Approximation		
P value summary	**		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	14.21		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	-6.100	No	ns
pre resection vs 20 min post resection	-10.80	No	ns
pre resection vs 45 min post resection	-26.40	Yes	**
10 min post resection vs 20 min post resection	-4.700	No	ns
10 min post resection vs 45 min post resection	-20.30	Yes	*
20 min post resection vs 45 min post resection	-15.60	No	ns

Table Analyzed	pmTOR tumor		
Kruskal-Wallis test			
P value	0.5953		
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups	4		
Kruskal-Wallis statistic	1.891		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	-7.250	No	ns
pre resection vs 20 min post resection	-4.975	No	ns
pre resection vs 45 min post resection	-9.675	No	ns
10 min post resection vs 20 min post resection	2.275	No	ns
10 min post resection vs 45 min post resection	-2.425	No	ns
20 min post resection vs 45 min post resection	-4.700	No	ns

Impact of intra- and postsurgical factors: Analysis of p70s6k by MSD





Case NC7 Case NC13 Case NC17 Case NC27 Case NC35 Case NC39 Case NC40 Case NC41 Case NC42 Case NC47
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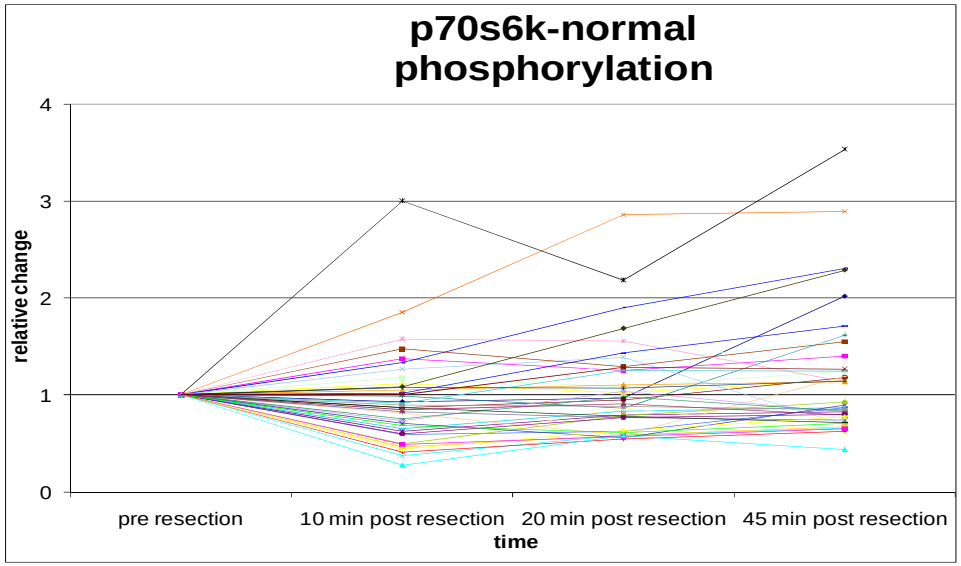
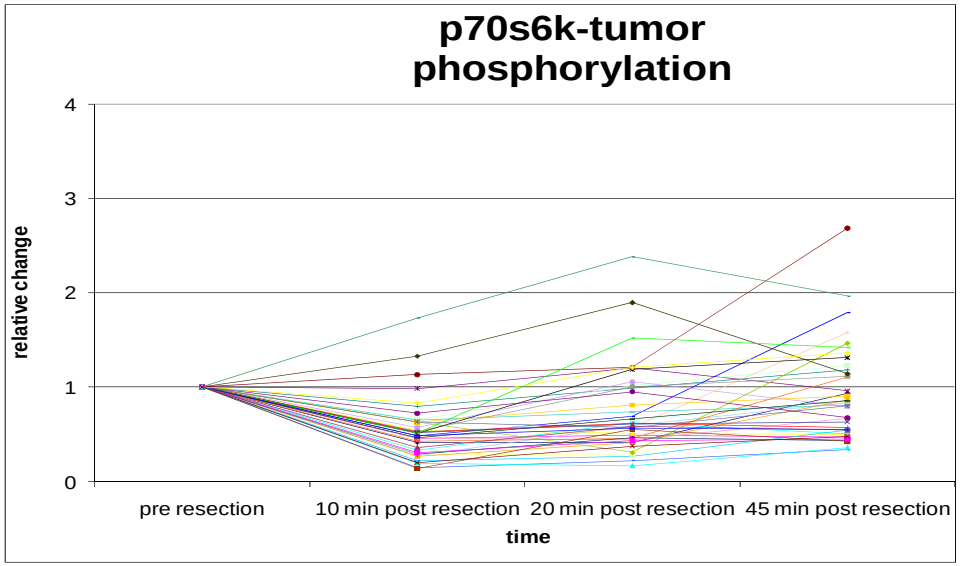
Impact of intra- and postsurgical factors: Analysis of Phospho-p70s6k by MSD





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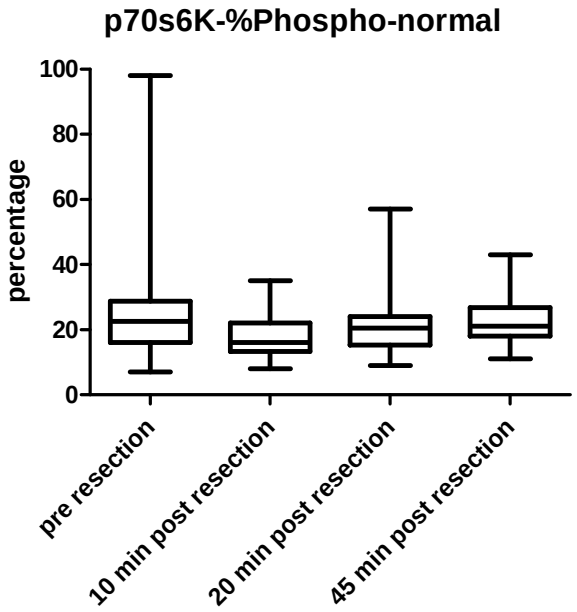
Case NC25 Case NC26 Case NC29 Case NC32 Case NC34 Case NC37 Case NC38 Case NC43 Case NC46 Case NC48

Impact of intra- and postsurgical factors: Analysis of Phospho/Total-p70s6k by MSD



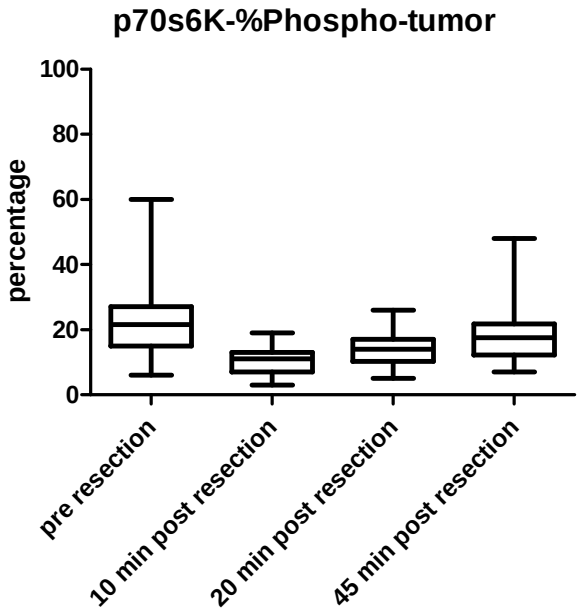
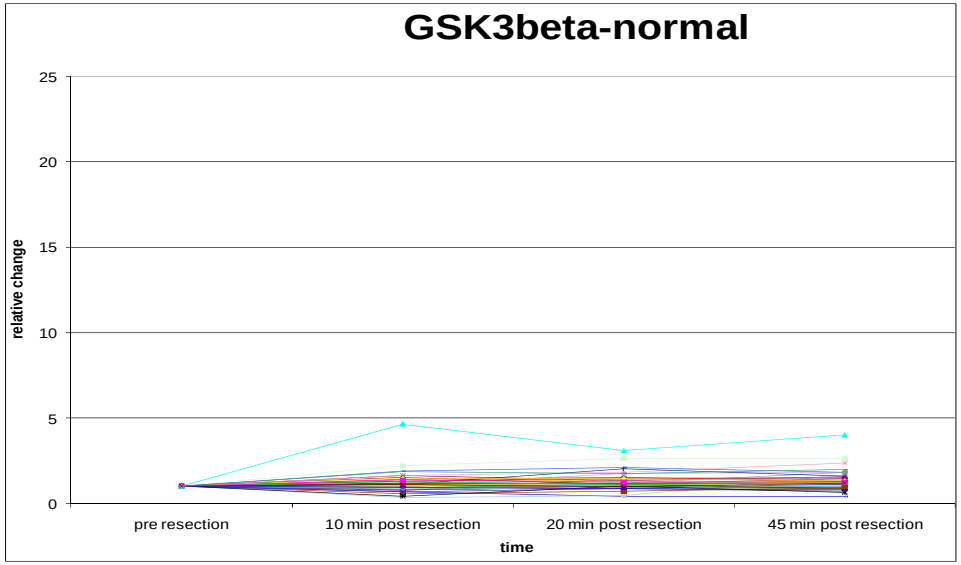
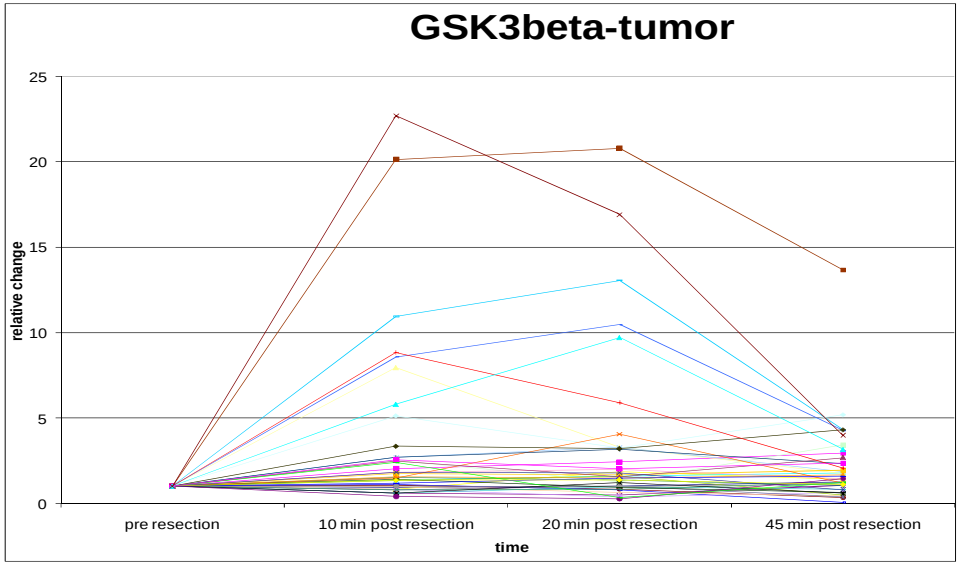


Table Analyzed	p70s6K-%Phospho-normal		
Kruskal-Wallis test			
P value	0.0188		
Exact or approximate P value?	Gaussian Approximation		
P value summary	*		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	9.969		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	24.31	No	ns
pre resection vs 20 min post resection	5.488	No	ns
pre resection vs 45 min post resection	-6.750	No	ns
10 min post resection vs 20 min post resection	-18.83	No	ns
10 min post resection vs 45 min post resection	-31.06	Yes	*
20 min post resection vs 45 min post resection	-12.24	No	ns

Table Analyzed	p70s6K-%Phospho-tumor		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	****		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	50.80		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	69.29	Yes	***
pre resection vs 20 min post resection	40.74	Yes	***
pre resection vs 45 min post resection	16.88	No	ns
10 min post resection vs 20 min post resection	-28.55	Yes	*
10 min post resection vs 45 min post resection	-52.41	Yes	***
20 min post resection vs 45 min post resection	-23.86	No	ns

Impact of intra- and postsurgical factors: Analysis of Total-GSK3beta by MSD





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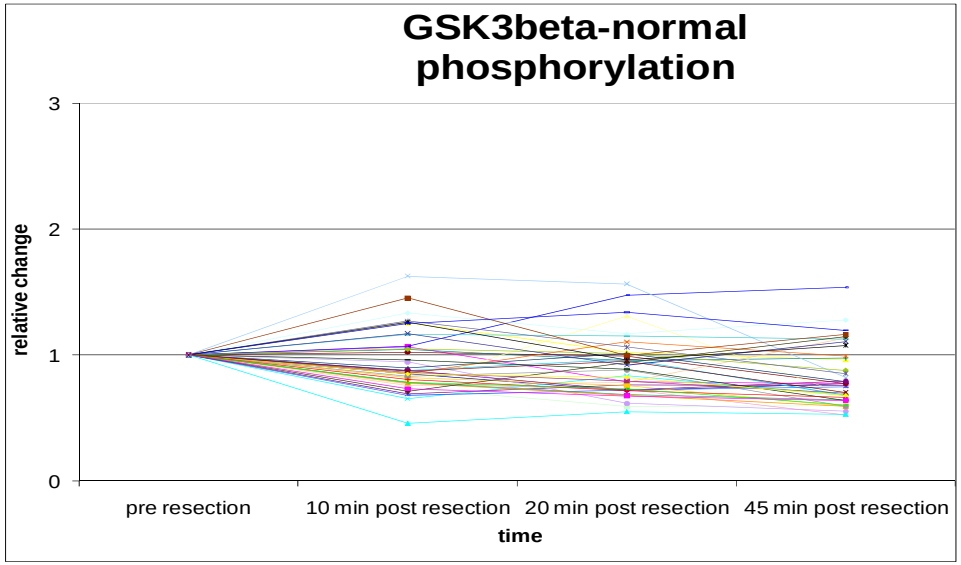
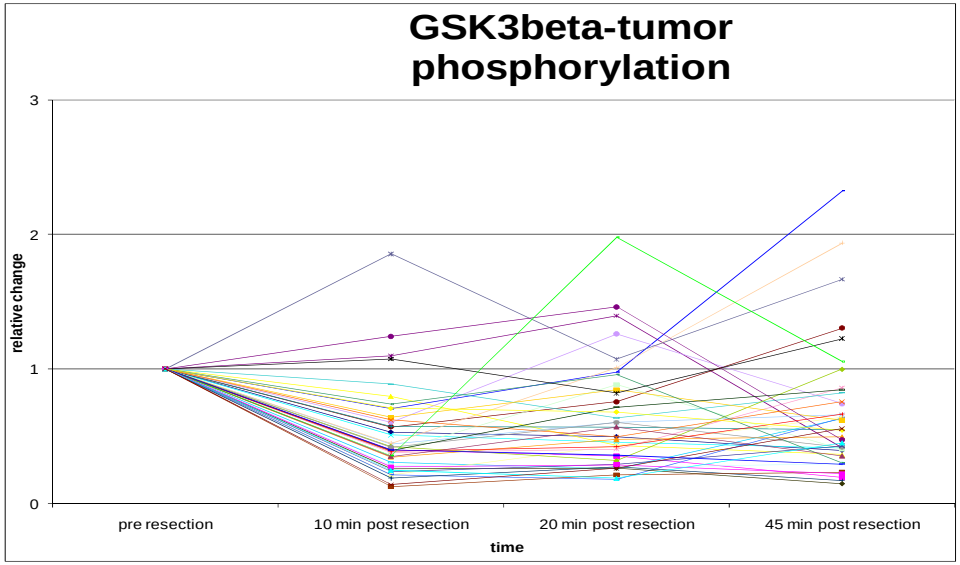
Case NC25 Case NC26 Case NC29 Case NC32 Case NC34 Case NC37 Case NC38 Case NC43 Case NC46 Case NC48

Impact of intra- and postsurgical factors: Analysis of Phospho-GSK3beta by MSD





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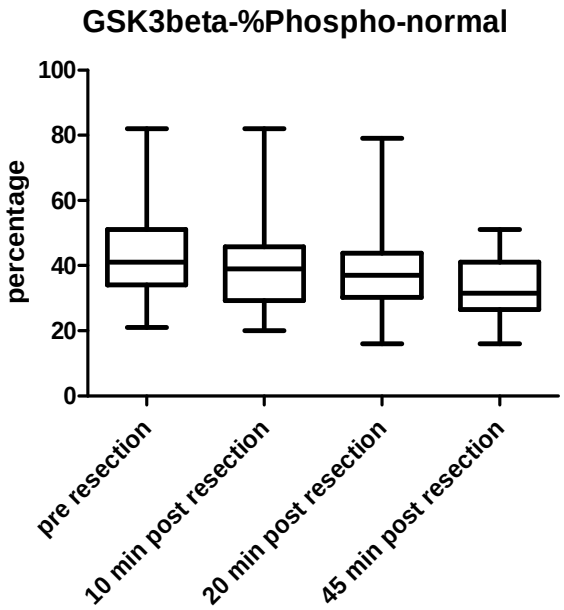
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-GSK3beta by MSD



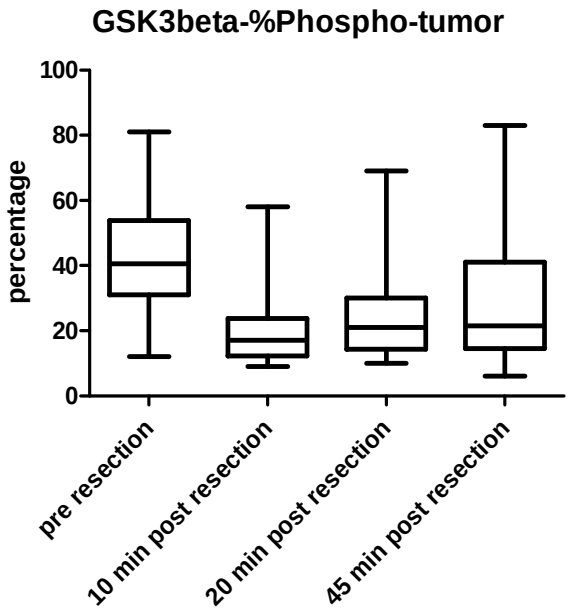


Table Analyzed	GSK3beta-%Phospho-normal		
Kruskal-Wallis test			
P value	0.0123		
Exact or approximate P value?	Gaussian Approximation		
P value summary	*		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	10.89		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	14.39	No	ns
pre resection vs 20 min post resection	18.84	No	ns
pre resection vs 45 min post resection	33.88	Yes	**
10 min post resection vs 20 min post resection	4.450	No	ns
10 min post resection vs 45 min post resection	19.49	No	ns
20 min post resection vs 45 min post resection	15.04	No	ns

Table Analyzed	GSK3beta-%Phospho-tumor		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	****		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	44.38		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	64.91	Yes	***
pre resection vs 20 min post resection	51.25	Yes	***
pre resection vs 45 min post resection	45.79	Yes	***
10 min post resection vs 20 min post resection	-13.66	No	ns
10 min post resection vs 45 min post resection	-19.13	No	ns
20 min post resection vs 45 min post resection	-5.463	No	ns

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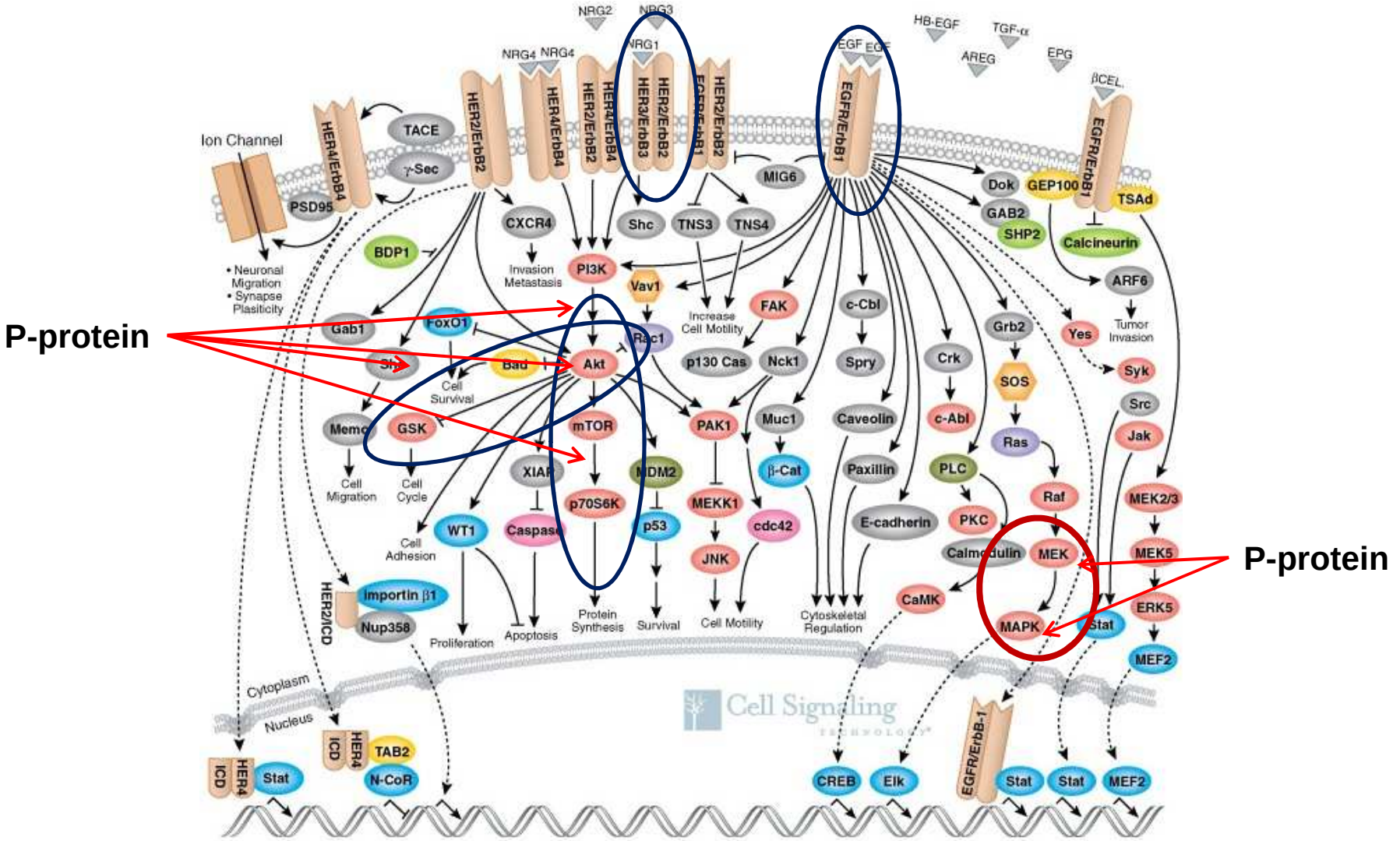
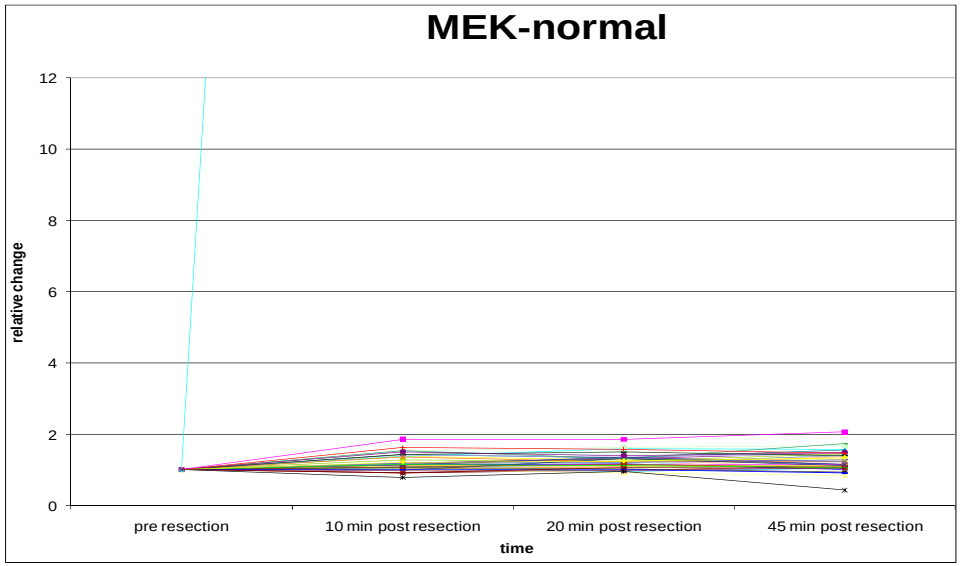
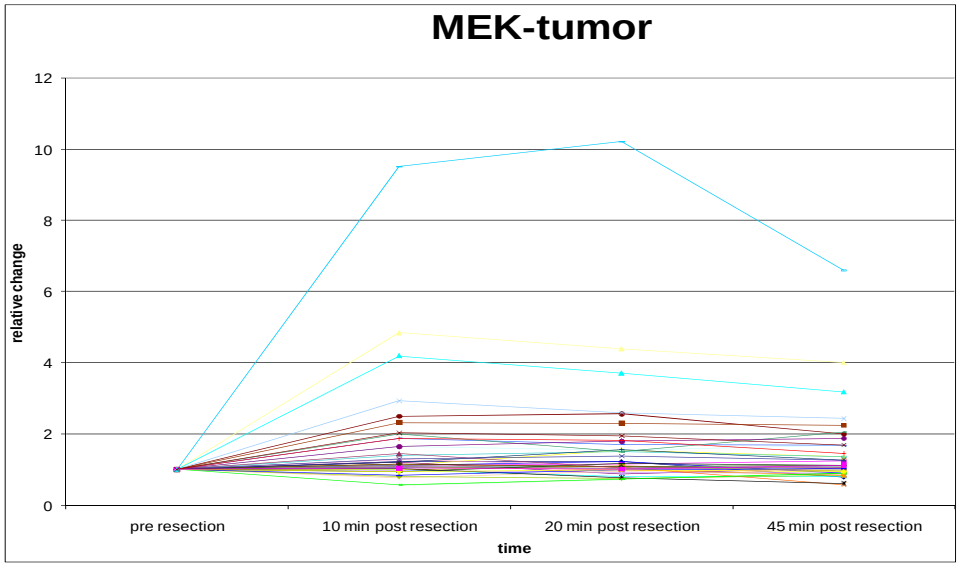
Analysis of HER-family Pathway in CRC Tissue: Impact of intra- and postsurgical factors



Impact of intra- and postsurgical factors: Analysis of Total-MEK by MSD





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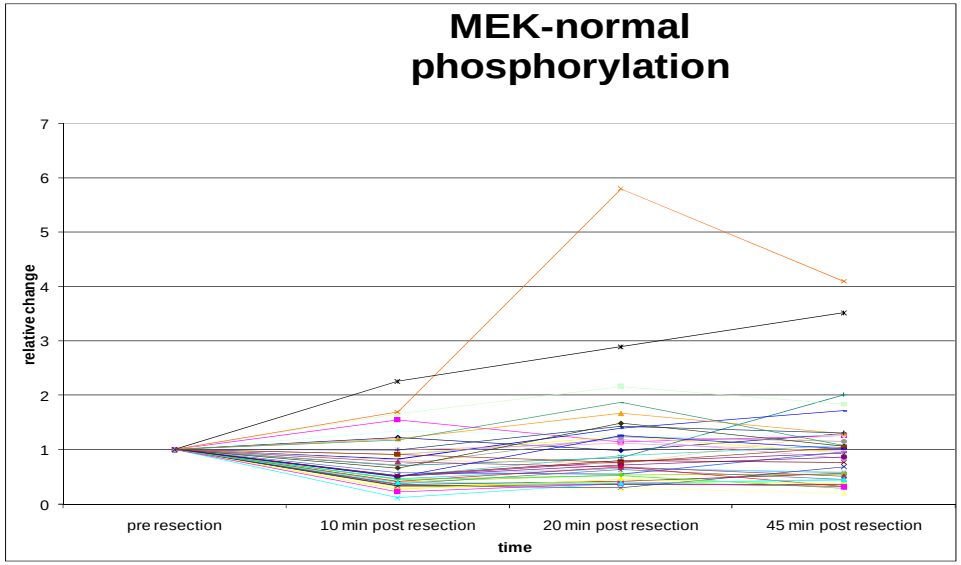
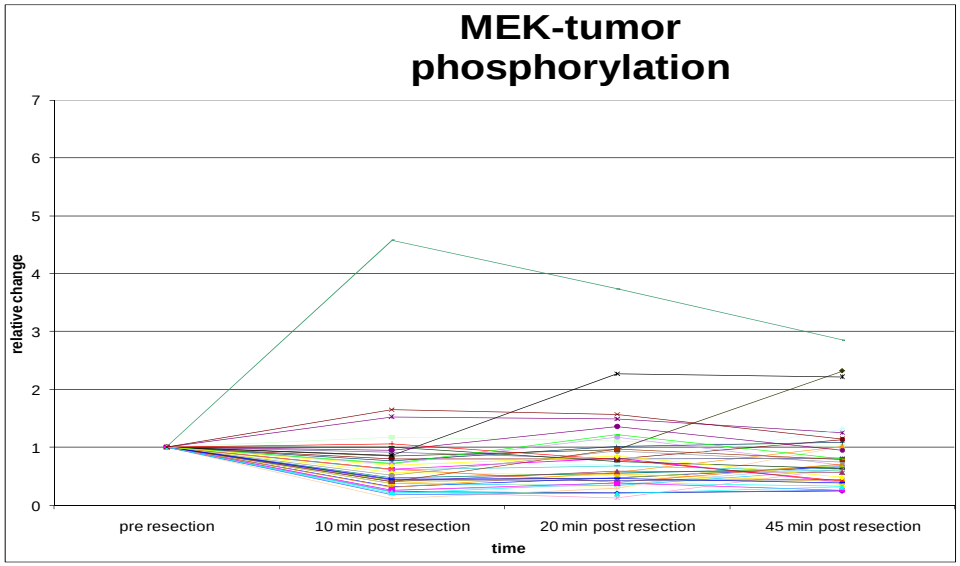
Impact of intra- and postsurgical factors: Analysis of Phospho-MEK by MSD





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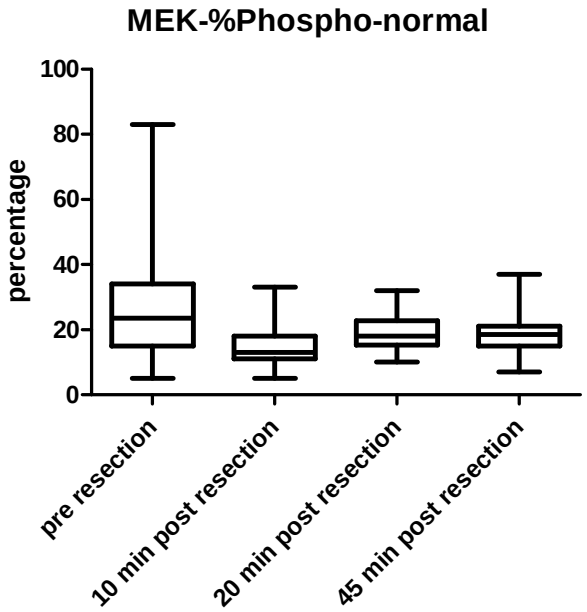
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-MEK by MSD



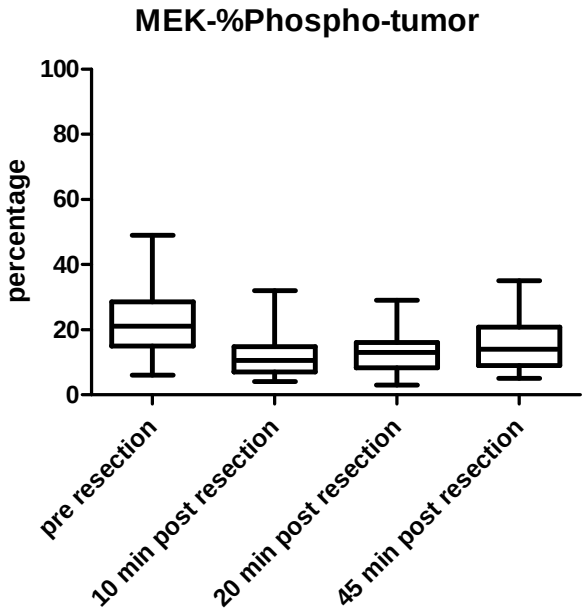
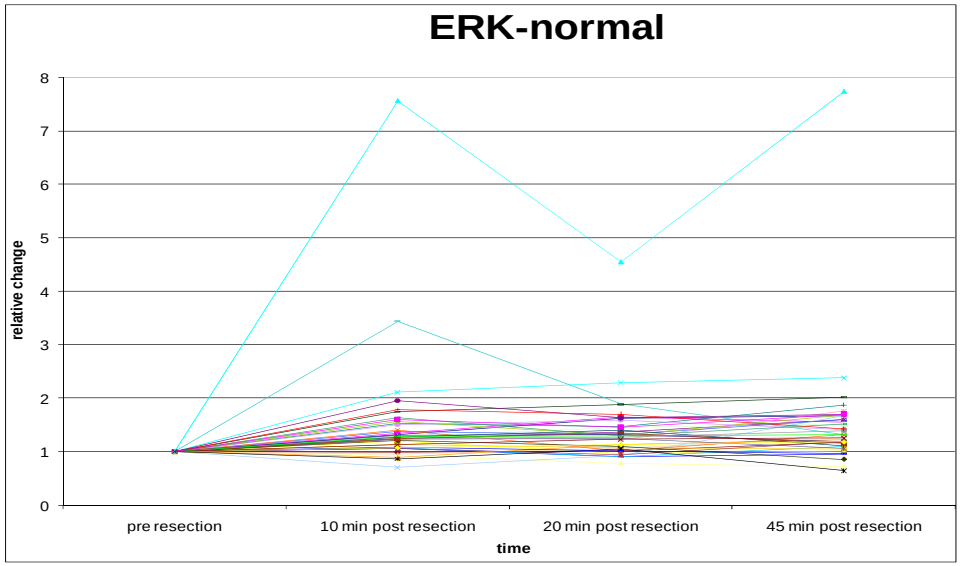
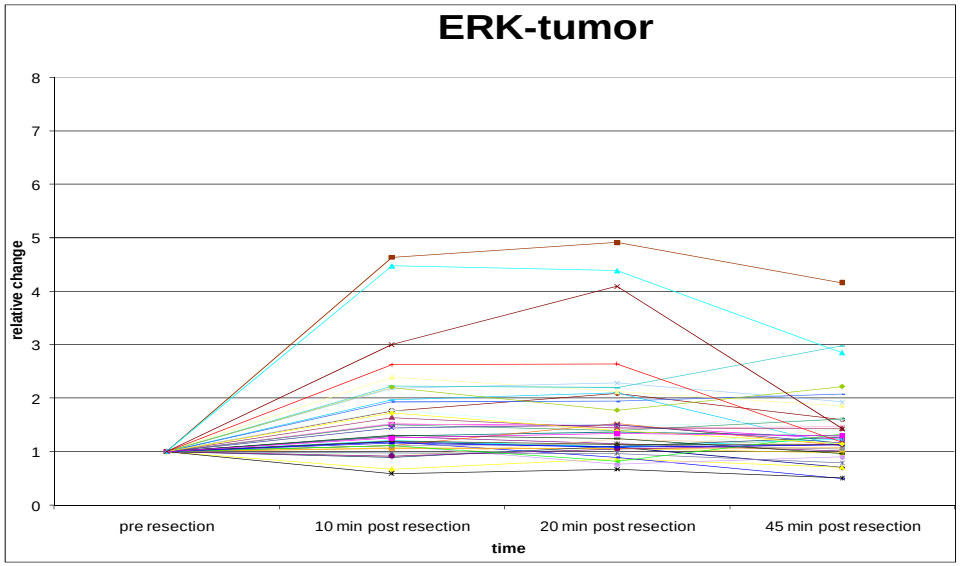


Table Analyzed	MEK-%Phospho-normal		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	****		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	27.13		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	52.61	Yes	***
pre resection vs 20 min post resection	17.30	No	ns
pre resection vs 45 min post resection	19.04	No	ns
10 min post resection vs 20 min post resection	-35.31	Yes	**
10 min post resection vs 45 min post resection	-33.58	Yes	**
20 min post resection vs 45 min post resection	1.738	No	ns

Table Analyzed	MEK-%Phospho-tumor		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	****		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	27.93		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	52.68	Yes	***
pre resection vs 20 min post resection	38.94	Yes	**
pre resection vs 45 min post resection	32.39	Yes	*
10 min post resection vs 20 min post resection	-13.74	No	ns
10 min post resection vs 45 min post resection	-20.29	No	ns
20 min post resection vs 45 min post resection	-6.550	No	ns

Impact of intra- and postsurgical factors: Analysis of Total-ERK (MAPK) by MSD





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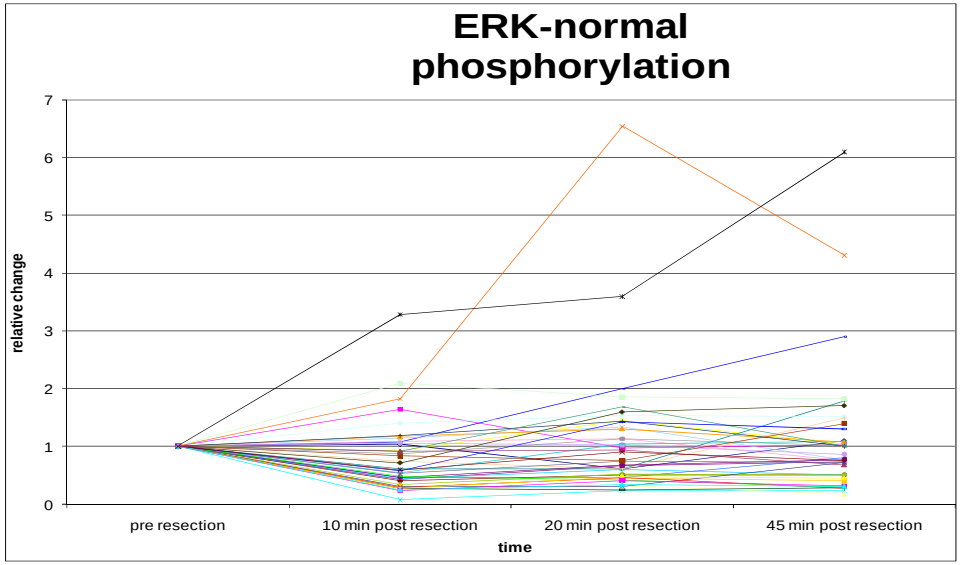
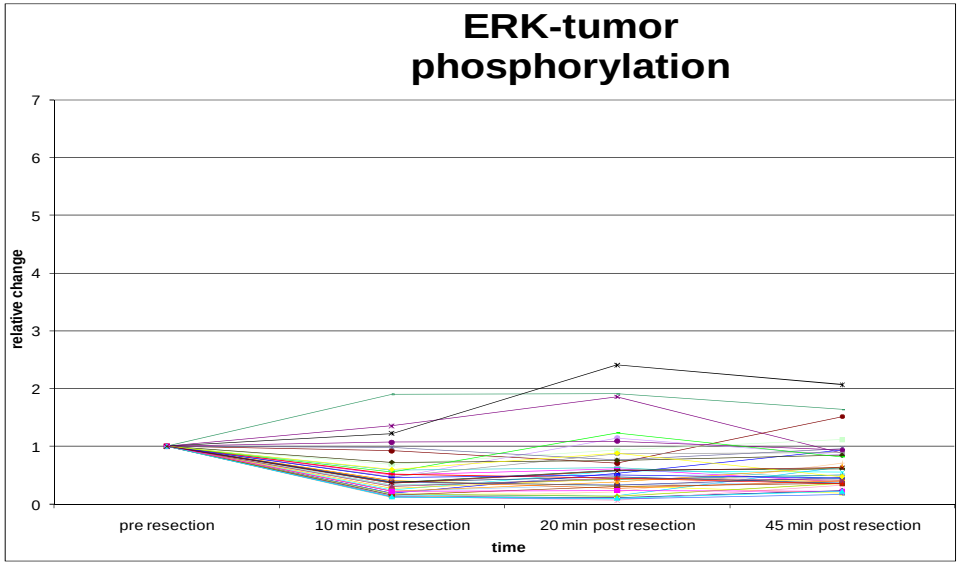
Impact of intra- and postsurgical factors: Analysis of Phospho-ERK (pMAPK) by MSD





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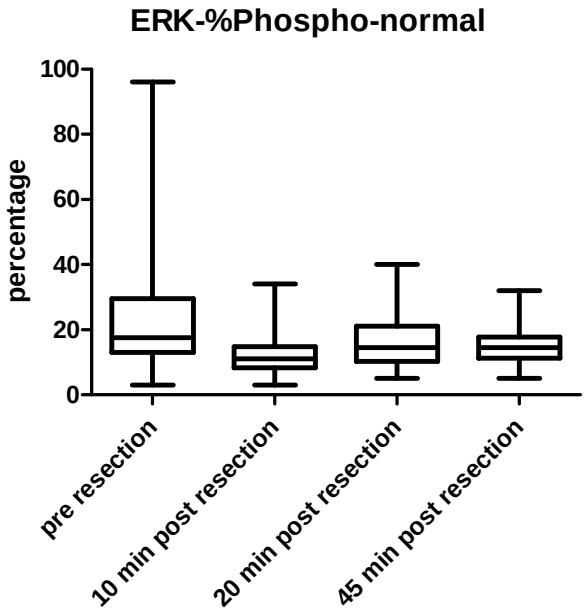
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-ERK (pMAPK) by MSD



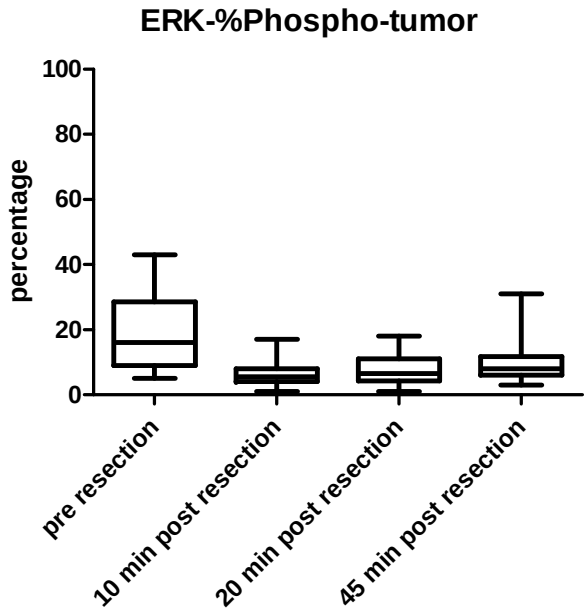
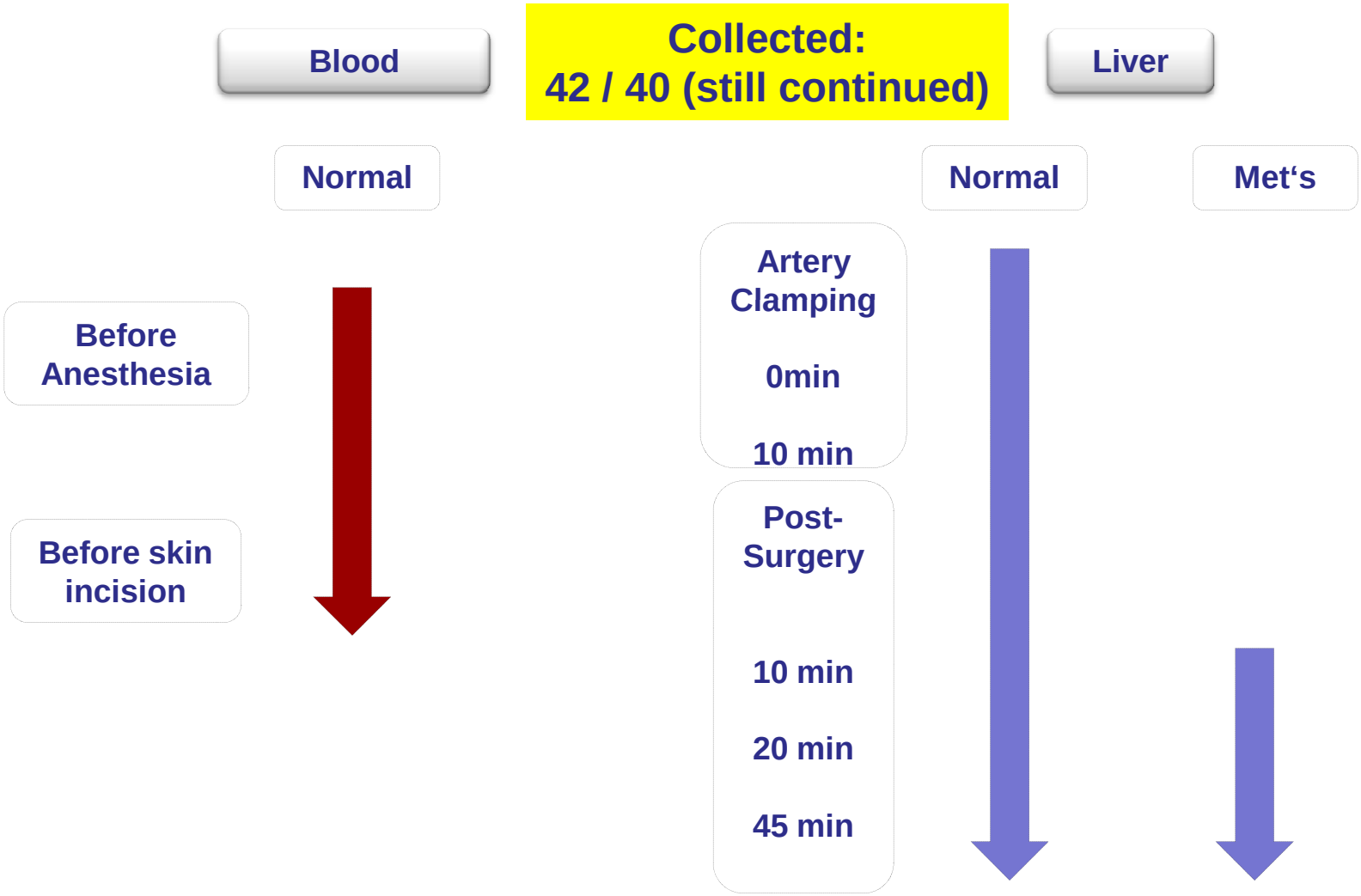


Table Analyzed	ERK-%Phospho-normal		
Kruskal-Wallis test			
P value	0.0002		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	19.30		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	44.76	Yes	***
pre resection vs 20 min post resection	17.23	No	ns
pre resection vs 45 min post resection	16.41	No	ns
10 min post resection vs 20 min post resection	-27.54	Yes	*
10 min post resection vs 45 min post resection	-28.35	Yes	*
20 min post resection vs 45 min post resection	-0.8125	No	ns

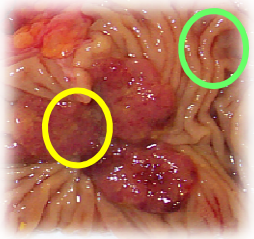
Table Analyzed	ERK-%Phospho-tumor		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	****		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	42.23		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
pre resection vs 10 min post resection	63.91	Yes	***
pre resection vs 20 min post resection	49.81	Yes	***
pre resection vs 45 min post resection	37.58	Yes	**
10 min post resection vs 20 min post resection	-14.10	No	ns
10 min post resection vs 45 min post resection	-26.34	No	ns
20 min post resection vs 45 min post resection	-12.24	No	ns

Impact of Anesthesia and Surgery on Gene and Protein Expression in Liver Tissue (Project 1)



Impact of Anesthesia and Surgery on Gene and Protein Expression in Colon and Liver Tissue : Next Steps

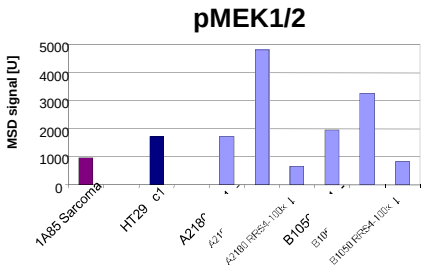
Cancer



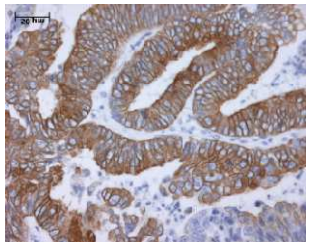
Normal

Cancer-relevant Proteins

Protein Quantification

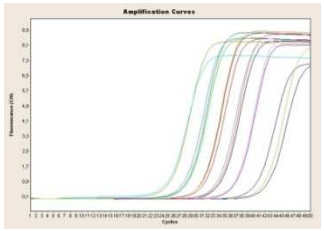


Cellular Distribution

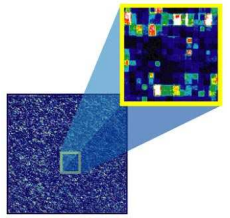


Gene Expression Profiling

Specific Genes



Comprehensive Analysis



Colon:
Completed

Liver:
First set of 20 completed
Final set of 20 shortly

Colon:
Completed by end of March

Liver:
Completed shortly

indivumed

Summary

- Positive control markers demonstrate that cold ischemia as well as surgery can have a significant impact on expression and activity of functional proteins
- Cancer and corresponding normal tissue react different (normal<cancer)
- High variability of functional protein expression and effects of intra- and postsurgery between patients
- Overall, MSD data and IHC analyses are constistent although in some markers (e.g., p-mTOR) differences can be seen
- A comprehensive data analysis – including gene expression data – will follow when results of the liver study are completed. This will include correlative analysis of clinical and anesthesiological data


Thank you very much for your attention!
Greetings from Hamburg