Effects of intra-operative ischemia on cancer and normal tissue

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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

IndivuTest GmbH
Hamburg, Germany
Founded: 2011

Indivumed Inc.
Kensington, MD
Founded: 2005

Inostics GmbH
Hamburg, Germany
Founded: 2009

Inostics Inc.
Baltimore, MD
Founded: 2010

Employee: 90
Financing: Private

B. Vogelstein et al. (JHU)
F.Diehl/H.Juhl
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

Indivumed Clinical Team

Indivumed Scientific Team

Partner Hospitals

Biospecimen Direct Processing

Biospecimen
- Tumor tissue
- Normal Tissue
- Blood
- Urine
- Data
- Processing Data
- Clinical Data
- Follow-up Data

Tumor-Biobank and Clinical Data Base
Indivumed Solution: Integration of Clinical Care, Biobanking and Research

Tumor-Biobank and Clinical Data Base
Indivumed Standard of Biobanking:

- 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
Understanding the Molecular Basis of Cancer: Status 1971 (Nixon Declares „War Against Cancer“)
Understanding the Molecular Basis of Cancer: Status 2011
Understanding of Cancer Pathways as Basis for "Targeted Therapeutics"
Targeted therapies are the Focus of Current Drug Development

- Targeted therapies: 53%
- Vaccines: 14%
- Antihormonal therapies: 1%
- Cytotoxics: 22%
- Gene therapies: 4%
- Immuno-therapies: 5%
- PDT: 0.3%
- Radiotherapeutics: 1%
- Gene therapies: 4%

Cost of 900 Mio $ / new FDA approved compound
90% of compounds fail in clinical phases

Top 8 der umsatzstärksten Krebsmittel

<table>
<thead>
<tr>
<th>Rang</th>
<th>Marke</th>
<th>Hersteller</th>
<th>Umsatz 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Avastin</td>
<td>Roche</td>
<td>6 213 Mio. US$</td>
</tr>
<tr>
<td>2</td>
<td>Rituax</td>
<td>Roche</td>
<td>6 110 Mio. US$</td>
</tr>
<tr>
<td>3</td>
<td>Herceptin</td>
<td>Roche</td>
<td>5 220 Mio. US$</td>
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<tr>
<td>4</td>
<td>Gleevec</td>
<td>Novartis</td>
<td>4 265 Mio. US$</td>
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<td>5</td>
<td>Neulasta</td>
<td>Amgen</td>
<td>3 558 Mio. US$</td>
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<td>6</td>
<td>Taxotero</td>
<td>Sanofi-Aventis</td>
<td>2 814 Mio. US$</td>
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<td>7</td>
<td>Revlimid</td>
<td>Celgene</td>
<td>2 466 Mio. US$</td>
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<td>8</td>
<td>Alimta</td>
<td>Lilly</td>
<td>2 208 Mio. US$</td>
</tr>
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</table>
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)

- Mutations
  - KRAS
  - PIK3CA
  - BRAF
  - EGFR
  - Many others

- Mutations
  - Oncotype
  - AmpliChip
  - MammaPrint
  - Others

- Target-Expression
  - HER-2
  - EGFR
  - VEGFR
  - Others
  - Protein Profiling

- Functionality
  - pmTOR
  - P-AKT
  - P-MAPK
  - Many, many more

Predictive value / Impact of preanalytical factors
Diagnostic Approaches for Individualizing Cancer Therapy: Tissue and Blood

- Tumor Tissue
  - Viable Tissue
  - Frozen or FFPE Tissue

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

Time 0

- Specimen is viable and reactive
- Biomolecules may degrade

Expression of Targets (e.g., Pathways) and Biomarker depend on Individual Variables and Tissue Processing
Phosphoprotein Expression: pMAPK Immunostaining (Ventana)

Case A            Case B

10 min

Change of pMAPK expression after 10-20 min cold ischemia

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

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)

PCA mapping: grouping of warm ischemia time points

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

Gene Expression Profiling

Protein Quantification
Cellular Distribution

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-3β/total GSK-3β (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)

Blood

Collected: 50 / 40

Colon Tissue

Normal

Cancer

Start of Surgery

Post-Surgery

10 min

20 min

45 min

Before Anesthesia

Before skin incision
Impact of intra- and postsurgical factors: Analysis of Total-control protein X by MSD (Cellular Stress Marker)
Impact of intra- and postsurgical factors: Analysis of Phospho-control protein X by MSD (Cellular Stress Marker)
Analysis of HER-family Pathway in CRC Tissue: Impact of intra- and postsurgical factors

P-protein

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

EGFR-normal

EGFR-tumor

pre resection 10 min post resection 20 min post resection 45 min post resection

relative change

Case NC7 Case NC13 Case NC17 Case NC27 Case NC35
Case NC9 Case NC11 Case NC12 Case NC18 Case NC20
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-EGFR by MSD

![Graphs showing EGFR-normal and EGFR-tumor phosphorylation over time for various cases.](image)
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-EGFR by MSD

EGFR-%Phospho-normal

Table Analyzed
Kruskal-Wallis test
P value
Exact or approximate P value?
P value summary
Do the medians vary signif. (P < 0.05)
Number of groups
Kruskal-Wallis statistic

Dunn’s Multiple Comparison Test
Difference in rank sum
Significant? P < 0.05?
Summary

EGFR-%Phospho-tumor

Table Analyzed
Kruskal-Wallis test
P value
Exact or approximate P value?
P value summary
Do the medians vary signif. (P < 0.05)
Number of groups
Kruskal-Wallis statistic

Dunn’s Multiple Comparison Test
Difference in rank sum
Significant? P < 0.05?
Summary
### Analysis of Phospho-EGFR by IHC

#### pEGFR normal

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<tr>
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<td>P value</td>
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<td>Gaussian Approximation</td>
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<td>P value summary</td>
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<td>Kruskal-Wallis statistic</td>
<td>9.194</td>
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<td>Difference in rank sum Significant? P &lt; 0.05? Summary</td>
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<td>pre resection vs 10 min post resection</td>
<td>16.45 No ns</td>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>16.45 No ns</td>
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<tr>
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<td>20.40 Yes *</td>
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<td>0.0 No ns</td>
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<td>10 min post resection vs 45 min post resection</td>
<td>3.950 No ns</td>
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<td>3.950 No ns</td>
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#### pEGFR tumor

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<td>Kruskal-Wallis statistic</td>
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<tr>
<td>Dunn's Multiple Comparison Test</td>
<td>Difference in rank sum Significant? P &lt; 0.05? Summary</td>
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<tr>
<td>pre resection vs 10 min post resection</td>
<td>22.53 Yes *</td>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>20.58 Yes *</td>
</tr>
<tr>
<td>pre resection vs 45 min post resection</td>
<td>25.10 Yes **</td>
</tr>
<tr>
<td>10 min post resection vs 20 min post resection</td>
<td>-1.95 No ns</td>
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<tr>
<td>10 min post resection vs 45 min post resection</td>
<td>3.575 No ns</td>
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<tr>
<td>20 min post resection vs 45 min post resection</td>
<td>4.525 No ns</td>
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</table>
### Analysis of Phospho-HER3 by IHC

#### Table Analyzed: pHer3 normal

<table>
<thead>
<tr>
<th>Treatment (between columns)</th>
<th>Residual (within columns)</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>pre resection</td>
<td>2550</td>
<td>31415</td>
</tr>
<tr>
<td>10 min post resection</td>
<td>3</td>
<td>850.0</td>
</tr>
<tr>
<td>20 min post resection</td>
<td>7</td>
<td>413.4</td>
</tr>
<tr>
<td>45 min post resection</td>
<td>76</td>
<td>413.4</td>
</tr>
</tbody>
</table>

#### 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
- **R square**: 0.07508

#### Bartlett's test for equal variances

- **Bartlett's statistic (corrected)**: 2.056
- **P value**: 0.5631
- **P value summary**: ns
- **Do the variances differ signif. (P < 0.05)**: No

#### ANOVA Table

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<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
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<tr>
<td>between groups</td>
<td>2550</td>
<td>3</td>
<td>850.0</td>
</tr>
<tr>
<td>within groups</td>
<td>31415</td>
<td>76</td>
<td>413.4</td>
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<tr>
<td>Total</td>
<td>33965</td>
<td>79</td>
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</table>

#### Bonferroni's Multiple Comparison Test

<table>
<thead>
<tr>
<th>Treatment vs</th>
<th>Mean Diff</th>
<th>Significance? P &lt; 0.05?</th>
<th>Summary</th>
<th>95% CI of diff</th>
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</thead>
<tbody>
<tr>
<td>pre resection vs 10 min post resection</td>
<td>6.550</td>
<td>1.019</td>
<td>No</td>
<td>-10.87 to 23.97</td>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>10.10</td>
<td>1.871</td>
<td>No</td>
<td>-7.317 to 27.52</td>
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<tr>
<td>pre resection vs 45 min post resection</td>
<td>15.55</td>
<td>2.419</td>
<td>No</td>
<td>-1.867 to 32.97</td>
</tr>
<tr>
<td>10 min post resection vs 20 min post resection</td>
<td>3.550</td>
<td>0.5532</td>
<td>No</td>
<td>-3.841 to 20.97</td>
</tr>
<tr>
<td>10 min post resection vs 45 min post resection</td>
<td>10.000</td>
<td>1.400</td>
<td>No</td>
<td>-8.417 to 28.42</td>
</tr>
<tr>
<td>20 min post resection vs 45 min post resection</td>
<td>5.450</td>
<td>0.8477</td>
<td>No</td>
<td>-11.97 to 22.87</td>
</tr>
</tbody>
</table>

---

#### Table Analyzed: pHer3 tumor

<table>
<thead>
<tr>
<th>Treatment (between columns)</th>
<th>Residual (within columns)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre resection</td>
<td>2550</td>
<td>31415</td>
</tr>
<tr>
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<tr>
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<td>7</td>
<td>413.4</td>
</tr>
<tr>
<td>45 min post resection</td>
<td>76</td>
<td>413.4</td>
</tr>
</tbody>
</table>

#### Kruskal-Wallis test

- **Kruskal-Wallis statistic**: 9.990
- **Number of groups**: 4
- **Summary**: ns

#### Dunn’s Multiple Comparison Test

<table>
<thead>
<tr>
<th>Difference in rank sum</th>
<th>Significant? P &lt; 0.05?</th>
<th>Summary</th>
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</thead>
<tbody>
<tr>
<td>pre resection vs 10 min post resection</td>
<td>13.75</td>
<td>No</td>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>-4.025</td>
<td>No</td>
</tr>
<tr>
<td>pre resection vs 45 min post resection</td>
<td>3.275</td>
<td>No</td>
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</table>

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**Note:** The images contain graphs showing H-scores for pHer3 normal and pHer3 tumor across different time points (pre resection, 10 min post resection, 20 min post resection, 45 min post resection). The graphs illustrate the distribution and variability of H-scores across these time points.
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

Akt-normal

Akt-tumor

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

### Akt-%Phospho-normal

<table>
<thead>
<tr>
<th>Table Analyzed</th>
<th>Krukal-Wallis test</th>
<th>P value</th>
<th>Exact or approximate P value?</th>
<th>P value summary</th>
<th>Do the medians vary signif. (P &lt; 0.05)</th>
<th>Number of groups</th>
<th>Kruskal-Wallis statistic</th>
<th>Dunn’s Multiple Comparison Test</th>
<th>Difference in rank sum</th>
<th>Significant? P &lt; 0.05?</th>
<th>Summary</th>
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<tbody>
<tr>
<td>Akt-%Phospho-normal</td>
<td></td>
<td>&lt; 0.0001</td>
<td>Gaussian Approximation</td>
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<td>4</td>
<td>54.76</td>
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<td>53.74</td>
<td>Yes</td>
<td>***</td>
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<tr>
<td>pre resection vs 10 min post resection</td>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>67.75</td>
<td>Yes</td>
<td>***</td>
<td>88.21</td>
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<td>pre resection vs 45 min post resection</td>
<td>14.53</td>
<td>No</td>
<td>ns</td>
<td>6.513</td>
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### Akt-%Phospho-tumor

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<th>Dunn’s Multiple Comparison Test</th>
<th>Difference in rank sum</th>
<th>Significant? P &lt; 0.05?</th>
<th>Summary</th>
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<tr>
<td>Akt-%Phospho-tumor</td>
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<td>4</td>
<td>80.39</td>
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<td>82.64</td>
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<td>pre resection vs 10 min post resection</td>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>71.80</td>
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<td>67.11</td>
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<td>10 min post resection vs 20 min post resection</td>
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<td>ns</td>
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Analysis of Phospho-AKT by IHC

**Table Analyzed**

<table>
<thead>
<tr>
<th>pAkt normal</th>
<th>pAkt tumor</th>
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</thead>
<tbody>
<tr>
<td>One-way analysis of variance</td>
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<tr>
<td>P value</td>
<td>0.6336</td>
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<td>Are means signif. different? (P &lt; 0.05)</td>
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<td>Bartlett's test for equal variances</td>
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<td>Residual (within columns)</td>
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<tr>
<td>95% CI of diff</td>
<td>-10.78 to 22.28</td>
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<tr>
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<td>pre resection vs 10 min post resection</td>
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<td>11.33</td>
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<td>ns</td>
</tr>
<tr>
<td>95% CI of diff</td>
<td>ns</td>
</tr>
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<td>95% CI of diff</td>
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<td>95% CI of diff</td>
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</table>
Impact of intra- and postsurgical factors: Analysis of Total-mTOR by MSD
Impact of intra- and postsurgical factors: Analysis of Phospho-mTOR by MSD

**mTOR-normal phosphorylation**

**mTOR-tumor phosphorylation**

[Graphs showing relative change over time for mTOR-normal and mTOR-tumor phosphorylation]
Impact of intra- and postsurgical factors:
Analysis of Phospho/Total-mTOR by MSD

**Table Analyzed**

<table>
<thead>
<tr>
<th>mTOR-%Phospho-normal</th>
<th>mTOR-%Phospho-tumor</th>
</tr>
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<tbody>
<tr>
<td>Kruskal-Wallis test</td>
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<tr>
<td>Exact or approximate P value?</td>
<td>Gaussian Approximation</td>
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<td>Do the medians vary signif. ([P &lt; 0.05)?</td>
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<td>Difference in rank sum</td>
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<td>pre resection vs 10 min post resection</td>
<td>11.78</td>
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<tr>
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<tr>
<td>Exact or approximate P value?</td>
<td>Gaussian Approximation</td>
</tr>
<tr>
<td>P value summary</td>
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<tr>
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</tr>
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<td>Kruskal-Wallis statistic</td>
<td>31.84</td>
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<td>Difference in rank sum</td>
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<tr>
<td>pre resection vs 10 min post resection</td>
<td>43.81</td>
</tr>
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<td>54.80</td>
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<td>-5.275</td>
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<tr>
<td>20 min post resection vs 45 min post resection</td>
<td>-16.26</td>
</tr>
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</table>
Analysis of Phospho-mTOR by IHC

### pmTOR tumor

- **H-score**
  - 0
  - 50
  - 100
  - 150
  - 200
  - 250
  - 300

- **pmTOR**
  - pmTOR tumor
  - pmTOR normal

- **Analysis**
  - 0.5953
  - Gaussian Approximation
  - ns
  - No
  - 4
  - Kruskal-Wallis statistic: 1.891

- **Dunn's Multiple Comparison Test**
  - No resection vs 10 min post resection: -6.100
  - No resection vs 20 min post resection: -10.80
  - No resection vs 45 min post resection: -23.40
  - 10 min post resection vs 20 min post resection: -4.700
  - 10 min post resection vs 45 min post resection: -20.30
  - 20 min post resection vs 45 min post resection: -15.00

- **Significant? P < 0.05?**
  - No resection vs 10 min post resection: No
  - No resection vs 20 min post resection: No
  - No resection vs 45 min post resection: No
  - 10 min post resection vs 20 min post resection: No
  - 10 min post resection vs 45 min post resection: No
  - 20 min post resection vs 45 min post resection: No

### pmTOR normal

- **H-score**
  - 0
  - 50
  - 100
  - 150
  - 200
  - 250
  - 300

- **pmTOR**
  - pmTOR tumor
  - pmTOR normal

- **Analysis**
  - 0.0026
  - Gaussian Approximation
  - **
  - Yes
  - 4
  - Kruskal-Wallis statistic: 14.21

- **Dunn's Multiple Comparison Test**
  - No resection vs 10 min post resection: -6.100
  - No resection vs 20 min post resection: -10.80
  - No resection vs 45 min post resection: -23.40
  - 10 min post resection vs 20 min post resection: -4.700
  - 10 min post resection vs 45 min post resection: -20.30
  - 20 min post resection vs 45 min post resection: -15.00

- **Significant? P < 0.05?**
  - No resection vs 10 min post resection: No
  - No resection vs 20 min post resection: No
  - No resection vs 45 min post resection: Yes
  - 10 min post resection vs 20 min post resection: No
  - 10 min post resection vs 45 min post resection: No
  - 20 min post resection vs 45 min post resection: No
Impact of intra- and postsurgical factors: Analysis of p70s6k by MSD

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

**Table Analyzed**

<table>
<thead>
<tr>
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<th>p70s6K-%Phospho-tumor</th>
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</thead>
<tbody>
<tr>
<td>Kruskal-Wallis test</td>
<td>Kruskal-Wallis test</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Exact or approximate P value?</td>
<td>Gaussian Approximation</td>
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<td>Difference in rank sum</td>
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<td>pre resection vs 10 min post resection</td>
<td>24.31</td>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>5.88</td>
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<tr>
<td>pre resection vs 45 min post resection</td>
<td>9.75</td>
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<td>-18.83</td>
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<td>10 min post resection vs 45 min post resection</td>
<td>-31.06</td>
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<td>-12.24</td>
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**Table Analyzed**

<table>
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<th>p70s6K-%Phospho-tumor</th>
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</table>
Impact of intra- and postsurgical factors: Analysis of Total-GSK3beta by MSD

GSK3beta-normal

GSK3beta-tumor

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-GSK3beta by MSD

GSK3beta-normal phosphorylation

GSK3beta-tumor phosphorylation

Case NC7
Case NC9
Case NC2
Case NC25
Case NC13
Case NC11
Case NC3
Case NC26
Case NC17
Case NC12
Case NC4
Case NC29
Case NC27
Case NC18
Case NC5
Case NC32
Case NC35
Case NC19
Case NC8
Case NC34
Case NC39
Case NC21
Case NC10
Case NC37
Case NC40
Case NC28
Case NC15
Case NC38
Case NC41
Case NC22
Case NC16
Case NC33
Case NC42
Case NC23
Case NC29
Case NC30
Case NC36
Case NC43
Case NC46
Case NC48
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-GSK3beta by MSD

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<th>GSK3beta-%Phospho-tumor</th>
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<td>Table Analyzed</td>
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<td>exact or approximate P</td>
<td>Gaussian Approximation</td>
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<td>Significant? P &lt; 0.05? Summary</td>
<td>Difference in rank sum</td>
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<td>pre resection vs 10 min post resection</td>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>18.84</td>
<td>No ns</td>
<td>pre resection vs 20 min post resection</td>
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<tr>
<td>pre resection vs 45 min post resection</td>
<td>33.88</td>
<td>Yes **</td>
<td>pre resection vs 45 min post resection</td>
</tr>
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<td>10 min post resection vs 20 min post resection</td>
<td>4.450</td>
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<tr>
<td>10 min post resection vs 45 min post resection</td>
<td>19.49</td>
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<td>10 min post resection vs 45 min post resection</td>
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<td>20 min post resection vs 45 min post resection</td>
<td>15.04</td>
<td>No ns</td>
<td>20 min post resection vs 45 min post resection</td>
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</tbody>
</table>
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

![Graphs showing relative change in MEK-normal and MEK-tumor over time](image)
Impact of intra- and postsurgical factors: Analysis of Phospho-MEK by MSD

MEK-normal phosphorylation

MEK-tumor phosphorylation

[Graphs showing relative change over time for MEK-normal and MEK-tumor phosphorylation]
Impact of intra- and postsurgical factors: Analysis of Phospho/Total-MEK by MSD

**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)?**
- **Number of groups**
- **Kruskal-Wallis statistic**
  - 27.13
- **Dunn’s Multiple Comparison Test**
  - **pre resection vs 10 min post resection**
    - Difference in rank sum: 52.61
    - Significant? P < 0.05?: Yes
  - **pre resection vs 20 min post resection**
    - Difference in rank sum: 17.30
    - Significant? P < 0.05?: No
  - **pre resection vs 45 min post resection**
    - Difference in rank sum: 13.04
    - Significant? P < 0.05?: No
  - **10 min post resection vs 20 min post resection**
    - Difference in rank sum: -35.31
    - Significant? P < 0.05?: Yes
  - **10 min post resection vs 45 min post resection**
    - Difference in rank sum: -33.58
    - Significant? P < 0.05?: Yes
  - **20 min post resection vs 45 min post resection**
    - Difference in rank sum: 1.738
    - Significant? P < 0.05?: No

**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)?**
- **Number of groups**
- **Kruskal-Wallis statistic**
  - 27.93
- **Dunn’s Multiple Comparison Test**
  - **pre resection vs 10 min post resection**
    - Difference in rank sum: 52.68
    - Significant? P < 0.05?: Yes
  - **pre resection vs 20 min post resection**
    - Difference in rank sum: 38.94
    - Significant? P < 0.05?: Yes
  - **pre resection vs 45 min post resection**
    - Difference in rank sum: 32.39
    - Significant? P < 0.05?: Yes
  - **10 min post resection vs 20 min post resection**
    - Difference in rank sum: -13.74
    - Significant? P < 0.05?: No
  - **10 min post resection vs 45 min post resection**
    - Difference in rank sum: -20.29
    - Significant? P < 0.05?: No
  - **20 min post resection vs 45 min post resection**
    - Difference in rank sum: -8.550
    - Significant? P < 0.05?: No
Impact of intra- and postsurgical factors: Analysis of Total-ERK (MAPK) by MSD
Impact of intra- and postsurgical factors: Analysis of Phospho-ERK (pMAPK) by MSD

ERK-normal phosphorylation

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

<table>
<thead>
<tr>
<th>Table Analyzed</th>
<th>ERK-%Phospho-normal</th>
<th>ERK-%Phospho-tumor</th>
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<td>Gaussian Approximation</td>
<td>Gaussian Approximation</td>
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<td></td>
</tr>
<tr>
<td>Do the medians vary signif. (P &lt; 0.05)</td>
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</tr>
<tr>
<td>Number of groups</td>
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</table>

Dunn’s Multiple Comparison Test

<table>
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<th>Difference in rank sum</th>
<th>Significant? P &lt; 0.05?</th>
<th>Summary</th>
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<tbody>
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<tr>
<td>pre resection vs 20 min post resection</td>
<td>17.23</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>pre resection vs 45 min post resection</td>
<td>18.41</td>
<td>ns</td>
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<td>10 min post resection vs 20 min post resection</td>
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<td>10 min post resection vs 45 min post resection</td>
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<td>20 min post resection vs 45 min post resection</td>
<td>-0.8125</td>
<td>No</td>
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</tbody>
</table>
Impact of Anesthesia and Surgery on Gene and Protein Expression in Liver Tissue (Project 1)

Collected: 42 / 40 (still continued)

Blood

Liver

Normal

Normal

Met’s

Before Anesthesia

Before skin incision

Artery Clamping

0min

10 min

Post-Surgery

10 min

20 min

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

Cancer-relevant Proteins

Gene Expression Profiling

Protein Quantification

Cellular Distribution

Specific Genes

Comprehensive Analysis

Colons:

Completed

Liver:

First set of 20 completed
Final set of 20 shortly

Colon:

Completed by end of March

Liver:

Completed shortly
• 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 consistent 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