Realizing Individualized Cancer Therapy

Tissue Intraoperative Factors

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Company Structure

Indivumed GmbH
Hamburg, Germany
Founded: 2002

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

Indivumed Inc.
Kensington, MD
Founded: 2005

Inostics GmbH
Hamburg, Germany
Founded: 2009
Indivumed Vision

Therapy 1  Therapy 2  Therapy 3
The Cancer Problem: Heterogeneity

Three colon cancer patients:
Same disease? Same therapy?

Patient 1

Patient 2

Patient 3

> 1000 different gene damages in various combinations can cause cancer

Each patient differs with respect to the molecular basis of his/her cancer
Understanding the Molecular Basis of Cancer: Status 1971
(Nixon Declares „War Against Cancer“)
Understanding the Molecular Basis of Cancer: Status 2010
Targeting Key Pathways

MAPK

PIK3CA

Changes in Gene Expression

Cell Proliferation (Cell Cycle)

Cell Death (Apoptosis)

DNA damage sensor

Anti-growth factors (e.g. TGFβ)

Integrins

β-Catenin

E-cadherin

WNT-β-Catenin

PIK3CA

GSK-3β

TCF

APC

β-Catenin:TCF

p16

Cycl D:CDK4

p15

Rb

HPV E7

p21

E2Fs

Cycl E:CDK2

p27

Smads

G-Protein

Ad Cycl

PKA

CREB

NF-κB

Stat 3,5

Bcl 2

MAPK

RTK

SOS

Grb2

P13K

PLC

NF1

Src

Fyn

Myc, Max

Cdk42

GTP

GTP

Mmr

NF-kB

Stat 3,5

Bcl XL

Bcl 2

Fas

Caspase 9

Cytochrome C

Bad

Mitochondria

TGFβR

Survival Factors (e.g. IGF1)

Hormones (e.g. Bombesin)

Growth Factors (e.g. TGFα)

PIK3CA

MAPK

RTK

Cytokines (e.g. IL-3/6)
# FDA-Approved Targeted Cancer Therapeutics (2001-2006)

Table 1: Examples of targeted molecular cancer therapeutics receiving marketing approval by the US FDA 2001–2006

<table>
<thead>
<tr>
<th>Year</th>
<th>Examples of targeted molecular therapeutics</th>
<th>Drug type</th>
<th>Disease indication</th>
<th>Primary molecular target</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>Sprycel (dasatinib)</td>
<td>Small molecule</td>
<td>Gleevec-resistant CML</td>
<td>BCR-ABL, SRC</td>
</tr>
<tr>
<td></td>
<td>Sutent (sunitinib)</td>
<td>Small molecule</td>
<td>Renal cancer and GIST</td>
<td>PDGFR, VEGFR, c-KIT</td>
</tr>
<tr>
<td></td>
<td>Herceptin (trastuzumab)</td>
<td>Antibody</td>
<td>Breast cancer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ERBB2</td>
</tr>
<tr>
<td></td>
<td>Zolinza (vorinostat)</td>
<td>Small molecule</td>
<td>Percutaneous T-cell lymphoma</td>
<td>HDAC</td>
</tr>
<tr>
<td>2005</td>
<td>Nexavar (sorafenib)</td>
<td>Small molecule</td>
<td>Renal cell carcinoma</td>
<td>VEGFR, CRAF, PDGFR</td>
</tr>
<tr>
<td>2004</td>
<td>Avastin (bevacizumab)</td>
<td>Antibody</td>
<td>Metastatic colorectal carcinoma</td>
<td>VEGF</td>
</tr>
<tr>
<td></td>
<td>Erbitux (cetuximab)</td>
<td>Antibody</td>
<td>EGFR-expressing metastatic colorectal cancer</td>
<td>EGFR</td>
</tr>
<tr>
<td></td>
<td>Tarceva (erlotinib)</td>
<td>Small molecule</td>
<td>Metastatic non-small-cell lung cancer</td>
<td>EGFR</td>
</tr>
<tr>
<td>2003</td>
<td>Iressa (gefitinib)</td>
<td>Small molecule</td>
<td>Metastatic non–small-cell lung cancer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>EGFR</td>
</tr>
<tr>
<td></td>
<td>Velcade (bortezomib)</td>
<td>Small molecule</td>
<td>Multiple myeloma&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26S proteasome</td>
</tr>
<tr>
<td>2002</td>
<td>Gleevac (imatinib)</td>
<td>Small molecule</td>
<td>GIST</td>
<td>c-KIT, PDGFR</td>
</tr>
<tr>
<td></td>
<td>Zevalin (90Y-ibritumomab tiuxetan)</td>
<td>Radiolabeled antibody</td>
<td>Non-Hodgkin lymphoma</td>
<td>CD20</td>
</tr>
<tr>
<td>2001</td>
<td>Campath (alemtuzumab)</td>
<td>Antibody</td>
<td>B-cell chronic lymphocytic leukemia</td>
<td>CD52</td>
</tr>
<tr>
<td></td>
<td>Gleevac (imatinib)</td>
<td>Small molecule</td>
<td>CML</td>
<td>BCR-ABL</td>
</tr>
</tbody>
</table>

<sup>a</sup>First approved 1998, use extended 2006. <sup>b</sup>Second-line therapy. <sup>c</sup>For people who have received at least two prior therapies. Also see CenterWatch (http://www.centerwatch.com/patient/drugs/druglist.html).
Cancer Therapeutics in Clinic

Colon cancer: Avastin

Breast cancer: Herceptin

Colon cancer: Erbitux

Lung cancer: Iressa
Targeting Key pathways
Challenge for using Tissue as Research and Diagnostic Tool: It is Alive and Reacts to Environmental Changes

Specimen is viable and reactive

Time 0

Biomolecules may degrade

Patient and Presurgical therapy  Medical/Surgical Procedures  Acquisition Processing  Storage Temperatur  Handling, Processing and analytical assays

Highly defined quality tissues are needed to understand cancer pathways
Targeting Key pathways
Indivumed Research on Critical Variables for Science Guided Biobanking

**Postsurgical Processing**
- Ischemia Time
- Location of Biopsy

**Intrasurgical Factors**
- Drugs
- Artery Ligation
Indivumed Research on Critical Variables for Science Guided Biobanking

**Postsurgical Processing**
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**Intrasurgical Factors**
- Drugs
- Artery Ligation
Impact of cold ischemia: controlled tissue study

Surgical removal of rectum

Collection of normal and cancer tissue

Control of warm ischemia

Tissue collection following resection:
Snap frozen in liquid N2
→ after 5 min
  8 min
  10 min
  12 min
  15 min
  20 min
  25 min
  30 min

Analysis:
Affymetrix
real-time RT-PCR
SELDI-TOF-MS
Tissue Ischemia and Gene Expression Profiling
(Affymetrix cDNA microarray)

Following tumor resection ~ 20-25% of genes are differentially expressed within the first 30 minutes!

Sprüssel et al, BioTechniques 2004
Ischemia regulated genes c-fos, HIF-alpha and HO-1

A. c-fos

B. HIF-1α

HO-1

Sprüssel et al, BioTechniques 2004
Tissue Ischemia and Gene Expression Profiling
(Comparison Affymetrix data and real-time RT-PCR)

Tumor marker CEA (colorectal cancer biomarker) and cytokeratin CK20

A

CEA

B

CK20

rel. Expression

Time [min]

GAPDH

CYCA
### Phosphoprotein Expression: pTyr100 Immunostaining (Ventana)

<table>
<thead>
<tr>
<th>Case A</th>
<th>Case B</th>
<th>10 min</th>
<th>20 min</th>
<th>60 min</th>
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</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>

No clear trend of pTyr100 expression within 60 min of cold ischemia.
Phosphoprotein Expression: pMAPK Immunostaining (Ventana)

Case A

10 min

20 min

60 min

Case B

Change of pMAPK expression after 10-20 min cold ischemia
Phosphoprotein Expression: 
pmTOR-Immunostaining (Ventana)
Tumor Tissue Varies in Center and Peripheral Areas

Invasive growth by induction of angiogenesis
Approx. 40% of proteins are differentially expressed between peripheral and central tumor regions.
Expression of VEGF in Different Tissues: Normal - Periphery - Center (real-time RT-PCR)

N = Normal  
P = Periphery  
C = Center

### Relative RNA Amount

<table>
<thead>
<tr>
<th>Patient / Tissue</th>
<th>Normal</th>
<th>Periphery</th>
<th>Center</th>
</tr>
</thead>
<tbody>
<tr>
<td>A61</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A157</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A161</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A197</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>A249</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

N = Normal  
P = Periphery  
C = Center
Indivumed Research on Critical Variables for Science Guided Biobanking

**Postsurgical Processing**
- Ischemia Time
- Location of Biopsy

**Intrasurgical Factors**
- Drugs
- Artery Ligation
Drugs Given During Surgery

Number of different commonly used active substances during surgery (Indivumeds data base):

- Antibiotics: 13
- Bronchodilatator: 2
- Cardio-drugs: 17
- Diuretics & corticosteroids: 5
- GI-tract drugs & antihistaminics: 7
- Infusion & transfusion: 15
- Inhalative narcotics: 5
- Local anesthetics: 6
- Muscle relaxant: 8
- Analgetics & sedatives: 34

Total: 112
Correlation of Colon Tissue Protein Expression with Intrasurgical Application of Atropin

Expression of 4 protein peaks (1.7%) correlates with atropin treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean 0</th>
<th>Mean 1</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
<th>Valid N 0</th>
<th>Valid N 1</th>
<th>Std.Dev. 0</th>
<th>Std.Dev. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>M5939_87</td>
<td>1.15213</td>
<td>1.60276</td>
<td>-2.73669</td>
<td>22</td>
<td>0.012043</td>
<td>17</td>
<td>7</td>
<td>0.15888</td>
<td>0.65240</td>
</tr>
<tr>
<td>M3772_14</td>
<td>0.63586</td>
<td>1.05263</td>
<td>-2.34306</td>
<td>22</td>
<td>0.028574</td>
<td>17</td>
<td>7</td>
<td>0.25252</td>
<td>0.63653</td>
</tr>
<tr>
<td>M6723_51</td>
<td>2.17426</td>
<td>3.41282</td>
<td>-2.31784</td>
<td>22</td>
<td>0.030148</td>
<td>17</td>
<td>7</td>
<td>0.97299</td>
<td>1.63301</td>
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<tr>
<td>M3555_34</td>
<td>0.71346</td>
<td>0.56601</td>
<td>2.16344</td>
<td>22</td>
<td>0.041640</td>
<td>17</td>
<td>7</td>
<td>0.16216</td>
<td>0.11972</td>
</tr>
</tbody>
</table>

- atropin + atropin

- atropin + atropin
Impact of Time between Ligation of Main Artery and Tumor Resection on Gene Expression in Colon Cancer (NCI-Indivumed study)

Patients receiving left hemicolecotomy

Indivumed database/biobank:
Time (min) between artery ligation and tumor removal

\[
\begin{align*}
20 & \quad 25 & \quad 30 & \quad 35 & \quad 40 & \quad 45 & \quad 50 \\
\end{align*}
\]

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)

A prospective trial collecting tissue during surgery has been initiated.
Prospective Trial: Impact of Anesthesia and Surgery on Gene and Protein Expression in Colon and Liver Tissue

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

Blood
- Normal
- Before Anesthesia
- Before skin incision

Colon Tissue
- Normal
- Cancer
- Start of Surgery
- Post-Surgery
  - 10 min
  - 20 min
  - 45 min
Impact of Anesthesia and Surgery on Gene and Protein Expression in Colon and Liver Tissue: Study Design

Blood

- Normal
- Before Anesthesia
- Before skin incision

Liver

- Normal
- Met’s
- 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: Study Design

Selected Proteins

Cancer

Normal

Indivumed

Gene Expression

Protein Quantification

Cellular Distribution

Specific Genes

Comprehensive Analysis

Identification of Surgery Dependent Molecules
Basic Considerations for the Development of Individualized Therapies and Predictive Biomarkers

Direct Access to Patients

Science-based Biobank

Technology for Discovery & Development

Predictive Models

Clinical Trial Center

Inostics
All done by Indivumed staff:

- IRB approval
- Patient consent
- Collection of blood/urine
- Documentation of surgery
- Documentation of anesthesia
- High-speed collection and processing of biospecimen
- Clinical data accrual
  - medical history
  - around surgery
  - annual follow-up
    - treatment
    - outcome
- Blood/urine during follow-up
- Quality control / SOPs
- Molecular analysis
- R&D / Service / M&S

Quality Management System
ISO 9001:2008 Certified

Full Integration of Indivumed Staff for Biobanking and Research:
Collaboration with 8 Hospitals in Hamburg and 1 in Washington DC
Indivumed Standard of Biobanking:
> 11,000 cases in the biobank / +2,000/year

✓ 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
Drug Profiling Platform
Drug Response and Predictive Biomarker Development

Piece of tumour tissue after resection (NSCLC, colon, breast)

Preparation of tissue slices (400 µm)

Cultivation in 24 well plates and drug treatment

Drug Response tests:
- ATP assay
- Caspase 3/7 assay
- IHC staining
- Antibody based assays (MSD)

Proteomic profiling:
- Selected proteins (e.g., phosphoproteins)
- Unselected proteins
Identification of Predictive Biomarker within Clinical Trials
Collaboration with the Otto J. Ruesch Center for the Cure of GI-tract Cancer

Otto J. Ruesch Center
Lombardi Cancer Center, GUMC

Screening/Diagnosis

Surgical Therapy

Drug Therapy (Standard)

Drug Therapy (Targeted)

Biobank/Data and diagnostics (research only)

Innovative Diagnostics

Therapy recommendation
Partner for Drug Discovery and Development: A Comprehensive Platform for Discovery and Development

**Biospecimen Preparations:**
- Tumor Biobank (> 11,000 Patients)
- Primary Cells
- Fresh Tissue

**DNA Analysis:**
- Tissue Analysis
- Blood-Based Assays (BEAMing)
- Mutation/Methylation/Amplification

**Protein Analysis:**
- Immunohistochemistry
- Pathway Analysis
- Drug Profiling

**Pharma/Biotech:**
Research Service
&
Co-Development (Companion Diagnostics)

**Direct Patient Access in 9 Clinical Center**
Standardized Biobanking for R&D
More research is needed to distinguish instable and robust molecules.

High-quality biobanks need to have highly standardized processes and complete documentation of all critical factors.

Short ischemia is crucial for analyzing sensitive molecules such as phosphoproteins.

Control of preanalytical factors are a prerequisite to utilize tissue as diagnostic tool for targeted therapies.