Challenges for the Development of Individualized Cancer Therapies
**Indivumed: Corporate Facts**

<table>
<thead>
<tr>
<th><strong>Mission:</strong></th>
<th>Realizing Individualized Cancer Therapy</th>
</tr>
</thead>
</table>
| **Location:** | Hamburg, Germany (Indivumed GmbH)  
Washington DC, USA (Indivumed Inc.) |
| **Ownership:** | Private |
| **Founders:** | - Prof. H. Juhl (Lombardi Cancer Center at Georgetown University)  
- Prof. C. Zornig (Chief Surgeon, Israelitic Hospital, Hamburg)  
- Prof P. Layer (Medical Director, Israelitic Hospital)  
- F. Oertel (Economic advisor) |
| **Start of operation:** | April 2002 |
| **Inostics GmbH:** | September 2008: Indivumed and scientists from The Johns Hopkins University (Bert Vogelstein and colleagues) founded Inostics GmbH - a biotech company offering tumor-DNA analysis of tissue and bodily fluids. |
Business area:
Service and Research to accelerate
development of individualized cancer therapy

Key competence:

1. A special clinical infrastructure
2. Continuously growing unique tumor biobank of highest quality (currently > 10,000 patients)
3. A comprehensive analytical platform including special research features for drug development
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
Individualized medicine

Past

Tumor 1  Tumor 2  Tumor 3

Standard Therapy

Future

Tumor 1  Tumor 2  Tumor 3

Molecular diagnosis

Therapy 1  Therapy 2  Therapy 3

In 2001, only one of three patients benefited from cancer drug treatment (Spear et al. (2001) Trends Molec. Med. 7, 201-203)
Considering the basis for drug and companion diagnostics development:

(The Indivumed approach)

1. High quality and standardized tissues which reflect molecular reality
2. Tissues with comprehensive clinical data
3. Direct and science-guided access to patients for clinical validation
4. Cutting-edge research facility
The Challenge

Tissue is alive until fixation and reacts to the environment on the cellular and molecular level.

Specimen is viable and reactive

Biomolecules may degrade

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

Time 0
Indivumed research on critical variables for science guided biobanking

- Location of biopsy
- Drugs
- Intrasurgical ischemia
- Postsurgical ischemia
Indivumed research on critical variables for science guided biobanking

- Location of biopsy
- Drugs
- Intrasurgical ischemia
- Postsurgical ischemia
Tumor tissue varies in center and peripheral areas

Invasive growth by induction of angiogenesis

Critical: Biopsy location
Localization of tumor biopsy affects molecular pattern (Mass-spectroscopy analysis; SELDI-TOF MS)

Approx. 40% of proteins are differentially expressed between peripheral and central tumor regions
Expression of VEGF in different tissues: normal - periphery - central
(real-time RT-PCR)

N = Normal
P = periphery
C = central

Patient / tissue

A61  A157  A161  A197  A249

Critical: Biopsy location
Indivumed research on critical variables for science guided biobanking

- Location of biopsy
- Drugs
- Intrasurgical ischemia
- Postsurgical ischemia
Drugs given during surgery

Number of different commonly used active substances during surgery (Indivumed’s 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>
</tr>
<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>

**Critical: Drugs intrasurgical**
Indivumed research on critical variables for science guided biobanking

- Location of biopsy
- Drugs
- **Intrasurgical ischemia**
- Postsurgical ischemia
Patients receiving left hemicolecotony

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

Critical: Intrasurgical ischemia

Impact of time between ligation of main artery and tumor resection on gene expression in colon cancer
(NCI-Indivumed study)
Critical: Intrasurgical ischemia

Time between ligation of main artery and tumor resection affects gene expression in colon cancer
(NCI-Indivumed study)

A prospective trial collecting tissue during surgery has been initiated
Indivumed research on critical variables for science guided biobanking

- Location of biopsy
- Drugs
- Intrasurgical ischemia
- Postsurgical ischemia
Critical: Postsurgical ischemia

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
Critical: Postsurgical ischemia

Tissue ischemia and gene expression profiling
(Comparison Affymetrix data and real-time RT-PCR)

Ischemia regulated genes c-fos, HIF-alpha and HO-1

**A**

- **c-fos**
  - X-fold change (normalised intensity)
  - Graph showing time [min] vs. X-fold change for c-fos.
  - Example bars for GAPDH and CYCA.

- **HIF-1α**
  - Graph showing time [min] vs. X-fold change for HIF-1α.
  - Example bars for GAPDH and CYCA.

- **HO-1**
  - Graph showing time [min] vs. X-fold change for HO-1.
  - Example bars for GAPDH and CYCA.

**B**

- Graphs showing relative expression vs. time [min] for GAPDH and CYCA.

Sprüssel et al, BioTechniques 2004
Critical: Postsurgical ischemia

Tissue ischemia and gene expression profiling
(Comparison Affymetrix data and real-time RT-PCR)

Tumor marker CEA (colorectal cancer biomarker) and cytokeratin CK20
Tissue ischemia time and protein expression in colon tissue
(SELDI-TOF-MS analysis)

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

Sprüssel et al, BioTechniques 2004
Critical: Postsurgical ischemia

Phosphoprotein expression:
pTyr100 immunostaining (Ventana)

No clear trend of pTyr100 expression within 60 min of cold ischemia
Critical: Postsurgical 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
Critical: Postsurgical ischemia

pmTOR-immunostaining (Ventana)

90 min

10 min

45 min

15 min

30 min

5 min

10 min

Change of pmTOR expression
A major challenge for drug and companion diagnostic development: 

*Having a valuable biobank*

Various pre-, intra- and postsurgical variables affect tissue data, e.g.:

- Drugs before and during surgery
- Tumor area
- Tissue ischemia time intrasurgical
- Tissue ischemia postsurgery
- Size of tissue block (fixation)
- Others
Solution: Overview

- Molecular analysis
- Tissue + blood + urine collection
- Biospecimen processing
- Clinical data
- Clinical validation

State-of-the-Art Technologies

- Bioinformatics analysis
- Molecular analysis

First Class Biobank

- Tissue + blood + urine collection
- Biospecimen processing

Unique clinical Infrastructure

“All in one hand“: tight integration of clinical and experimental research
Basic consideration:

Take all responsibility away from surgeons and clinical staff!
Done by Indivuméd 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

Indivuméd headcount:

Total: 75

Biobanking: 42
Research / Service: 22
Administration / Sales: 11
Clinical Infrastructure

- Cooperation agreements with 9 hospitals and 14 Surgery and Oncology Units
- Collection Centers in Germany (Hamburg) and US (Washington DC)
- Indivumed study nurses are fully integrated in day-to-day business (e.g. OR) but independent of participating hospitals
- Post-surgery, Indivumed nurses see patients annually to collect outcome information and additional blood/urine samples
Biobanking: Samples

- Tumor tissue
  - frozen
  - FFPE
- Normal tissue (matched)
  - frozen
  - FFPE
- Preparations thereof
  - DNA
  - RNA
  - protein lysates
  - membranes

- Serum
  - pre / post surgery
- Plasma
  - pre / post surgery
- MNC blood cells
  - pre / post surgery
- Urine
  - pre / post surgery
- Urine sediment

- Bladder
- Breast
- Cervix
- Colorectal
- Esophageus
- Liver
- Lung
- Ovarian
- Pancreatic
- Prostate
- Stomach

- Sets of normal
  - Tu-center
  - Tu-periphery
- Ischemia time < 12 min
- Blocks of similar size
- Simultaneous N₂-freezing
- 16h Formalin fixation

- Instant 4°C cooling
- Processing < 4 hours

Tissue:

Fluids:
Biobanking: Clinical Data

Complete, prospectively collected and verified clinical data!

- Standardized documentation
- 300 data points / patient
- Prospective collection
- Web-accessible data base
- Compatible data format (Oracle)
Patients and tumor localization (n > 10,000)

- Colon
- Rectum
- Esophagus
- Stomach
- Liver
- Pancreas
- Breast
- Ovarian
- Bladder
- Prostate
- Lung

Average cold ischemia time of Indivumed tissue: 7.48 min (± 4 min)

Biobanking: Statistics

<table>
<thead>
<tr>
<th>Patient Consent (Surgery)</th>
<th>Biospecimen (Surgery)</th>
<th>Tracking (Follow-up)</th>
<th>Biospecimen (Follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tissue</td>
<td>Blood</td>
<td>Urine</td>
</tr>
<tr>
<td>99%</td>
<td>95%</td>
<td>95%</td>
<td>75%</td>
</tr>
</tbody>
</table>

+ ~ 2,000 new patients / year !!
Consequences for research

- 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.

- To do it right needs high investments in well-trained personnel.

- It pays off to pay for high-quality.