Indivumed: Precision and quality for individualizing cancer therapy

Prof. Dr. med. Hartmut Juhl
CEO
Indivumed GmbH and IndiviTest GmbH
Adjunct Professor, Georgetown University and Hamburg University

www.indivumed.com
Control of preanalytical factors and comprehensive clinical data to individualize cancer therapy
Today, Indivumed is an integrated oncology company providing services for obtaining an in-depth understanding of the underlying mechanisms causing the development of a patient’s cancer.
OVERVIEW OF HISTORY:
THE INDIVUMED-INOSTICS STORY

Founders of „Liquid Biopsy“ Concept

Inostics Exit: Sale to Symex Corp.
First Comp.Dx agreement (Bayer Health)
CLIA-certification Inostics Inc

GCP Inostics GmbH
Foundation Inostics Inc
Opening ceremony Inostics GmbH

Start of Inostics GmbH

2002
Start of Indivumed

2003
Best company in Hamburg
Foundation Indivumed Inc.

2004
Best company in Germany

2005
New investor group

2006
„Land of Ideas“ award by Germanys President

2007
Start of US biobanking (Washington DC)

2008
Lab expansion: new facilities

2009
ISO 9001-2008 Indivumed

2010
Start of Indivumed Foundation

2011

2012
ISO 9001-2008 Indivumed

2013
GCP compliance Hamburg lab

2014
Start of Geisinger Health collaboration

Bert Vogelstein:
• Founder of molecular diagnostics in cancer
• Discovery of most cancer relevant genes
• 80% of all JHU patent applications
• Most quoted scientist

Detection and quantification of mutations in the plasma of patients with colorectal tumors

Joint publication of BEAMing

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# Indivumed Facts

<table>
<thead>
<tr>
<th>Founded:</th>
<th>April 2002</th>
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<tbody>
<tr>
<td>Funding:</td>
<td>Private investors</td>
</tr>
<tr>
<td>Employees:</td>
<td>&gt;100 (Germany: 80+, US: 20++)</td>
</tr>
<tr>
<td>Headquarter:</td>
<td>Indivumed GmbH, Hamburg, Germany</td>
</tr>
<tr>
<td>Affiliates:</td>
<td>Indivumed Inc., Baltimore, MD; IndivuTest GmbH, Hamburg Germany</td>
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</table>
| Clinical Partner: | Hamburg: 9 Cancer Clinics and >20 Oncology Practices  
Washington DC: Georgetown University Medical Center & Washington Hospital Center  
Danville, PA: Geisinger Health System |
| Academic Collaboration: | Ludwig Institute at Kimmel Cancer Center, Johns Hopkins University  
Lombardi Cancer Center, Georgetown University  
University Clinic of Hamburg University  
US-National Cancer Institute  
Stanford University  
Food and Drug Administration USA |
Leading clinicians and scientists are providing a clear assessment of the opportunities to expand and extend the research and clinical engagement opportunities.

Carolyn Compton, Chair
CMO of NBDA and Arizona State University
Former Director, Office of Biorepositories and Biospecimen Research at National Cancer Institute

David Galas
Principal Scientist
Pacific Northwest Diabetes Research Institute
Chair, Fannie & John Hertz Foundation

John Marshall
Chief Division of Hematology / Oncology,
Medstar Georgetown University Hospital

Björn Nashan
Director of Department of Hepatobiliary and Transplant Surgery, University Hospital Hamburg-Eppendorf

Wolff Schmiegel
Professor and Director in the Medical university hospital (Ruhr University of Bochum)
President of German Cancer Association

Bert Vogelstein
Director of the Ludwig Center, Clayton
Professor of Oncology and Pathology and a Howard Hughes Medical Institute investigator at The Johns Hopkins Medical Institutions

Anton Wellstein
Professor for Pharmacology and Oncology, Lombardi Cancer Center at Georgetown University
INDIVUMED CORE COMPETENCE: TO OBTAIN MEANINGFUL TISSUE

Expression of Targets (e.g., Pathways) and Biomarker depend on Individual Variables and Tissue Processing
TISSUE ISCHEMIA AND GENE EXPRESSION PROFILING
(PILOT STUDY IN RECTAL CANCER)

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
THE IMPACT OF SURGERY AND TISSUE PROCESSING ON TISSUE DATA: AN INDIVUMED - NCI STUDY ANALYZING SAMPLES FROM 90 PATIENTS

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, Diakoniekinikum Alten Eichen (Dr. Dörner)
Department of Hepatobiliary Surgery, University Hospital Hamburg (PI: Dr. Nashan)
THE IMPACT OF SURGERY AND TISSUE PROCESSING ON TISSUE DATA: AN INDIVUMED - NCI STUDY ANALYZING SAMPLES FROM 90 PATIENTS

Blood
- Normal
  - Before Anesthesia
  - Before skin incision

Colon Tissue
- Normal
- Cancer
  - Start of Surgery
  - Post-Surgery
    - 10 min
    - 20 min
    - 45 min

Liver tissue
- Normal
- CRC Met's
  - Artery Clamping
    - 0min
    - 10 min
THE IMPACT OF SURGERY AND TISSUE PROCESSING ON TISSUE DATA: AN INDIVUMED - NCI STUDY

Gene Expression (Affymetrix-Analysis) in CRC Patient

Heatmap of top 100 differential expressed genes

Principal Component Analysis (PCA)

Pre
45 min
10 min
20 min
GENE AND PROTEIN EXPRESSION CHANGE DRAMATICALLY DURING AND AFTER SURGERY

Change of gene expression >2x (colon/liver)

Expression of up to 20% of genes change during surgery and postsurgical processing time >2x

List of

Stable Genes

Instable Genes

Tissue Quality Marker Panel

David et al., OncoTarget, Nov. 2014
IDENTIFICATION OF POTENTIAL TISSUE QUALITY MARKER

David et al., OncoTarget, Nov. 2014
EGFR PATHWAY IS SIGNIFICANTLY AFFECTED BY TISSUE PROCESSING

Comparison:
Tumor biopsy
---
10 min postsurgery
- 45 min postsurgery
SCIENTIFIC EVIDENCE: KNOWLEDGE OF TISSUE PROCESSING IS EXTREMELY IMPORTANT

Without knowledge about tissue processing and rapid tissue fixation, protein expression data are unreliable and understanding of pathway activity is impossible.
INDIVUMED: STANDARDIZATION OF BIOBANKING

The Black Box

Patient

HOSPITAL

Biospecimen
Quality control data
Clinical data

Clinical testing

R & D process:
Experimental Research
Data mining

Experimental control
IDENTICALLY PROCESSED BIOSPECIMEN AND CLINICAL DATA: A UNIQUE APPROACH FOR SERVICE, DX AND RX DEVELOPMENT

Indivumed Clinical Team

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 Scientific Team

Indivumed R & D / IP

Customers:
Industry and Academia

Partner Hospitals and Private Oncologist
INDIVUNET:
A DATA BASE WITH IDENTICAL CLINICAL DATA SETS COLLECTED FROM DIFFERENT CLINICAL SYSTEMS
IDENTICALLY PROCESSED BIOSPECIMEN AND CLINICAL DATA: A UNIQUE APPROACH FOR SERVICE, DX AND RX DEVELOPMENT

Indivumed Standard at all sites, e.g.:
- integration of Indivumed staff in clinics
- primary access to all patients
- tissue frozen in ~7 min (mean value)
- comprehensive clinical data
- identical clinical data format
THE WORLD’S LEADING CANCER DATA BASE AND BIOBANK

All major tumor entities identically processed

- Tumor tissue
  - Frozen
  - FFPE

- Normal tissue
  - Frozen
  - FFPE

- Blood Preparations
  - Serum
  - Plasma
  - PBMC

- Urine Samples
  - Supernatant
  - Sediment

Comprehensive clinical data identically processed

- Family History
- Disease History
- Living style
- Diagnostic tests
- Other diseases
- Neoadjuvant Therapy
- Lab test results
- Surgery
- Sample processing (ischemia time intra- and postsurgery)
- Histopathology
- Postsurgical follow-up (complications?)
- Outcome (annually)

> 24,000 Patients

IndivuNET

Understanding Cancer & New Therapies
INDIVUMED – LONGITUDINAL BLOOD-BIOBANK AND CLINICAL DATA BASE

Correlation of molecular events (e.g., DNA mutation profile) with clinical data (e.g., drug treatment and response)
Circulating tumor DNA (ctDNA) is cell-free DNA released from a solid tumor

cDNA ≠ CTCs

Origin: Necrotic or apoptotic tumor cells

Concentration: 0.01% to 60% of total DNA

Nature: small DNA fragments (<120 bp)

Clearance: Kidney → Urine

Markers: Mutations, methylation, translocations, copy number variations
Advantages of Plasma DNA Testing

- High compliance
- Fresh DNA
- Accessible
- No selection bias
- Monitoring possible
Advantage of BEAMing Technology

Detection Capability
(mutant DNA/ total DNA)

- 100% Sanger Sequencing
- 10% Pyrosequencing
- 1% Real-Time PCR
- 0.1% BEAMing
### Required Analytical Sensitivity for Tissue and Blood-Based Molecular Testing

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Technique</th>
<th>Tissue</th>
<th>Plasma</th>
<th>Sensitivity in Tissue</th>
<th>Sensitivity in Plasma</th>
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<tbody>
<tr>
<td>25%</td>
<td>Sanger Sequencing</td>
<td>4</td>
<td>-</td>
<td>27%</td>
<td>0%</td>
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<tr>
<td>2%</td>
<td>Deep Sequencing</td>
<td>15</td>
<td>10</td>
<td>100%</td>
<td>67%</td>
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<tr>
<td>1%</td>
<td>Real-time PCR</td>
<td>15</td>
<td>10</td>
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<td>0.01%</td>
<td>BEAMing</td>
<td>15</td>
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<td>100%</td>
<td>100%</td>
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</tbody>
</table>
Application 1
Prediction

Concordance Between Tissue and Plasma DNA Analysis in mCRC

<table>
<thead>
<tr>
<th>Gene</th>
<th>Positive Tissue / Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>15/15</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>3/3</td>
</tr>
<tr>
<td>KRAS</td>
<td>10/10</td>
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<tr>
<td>TP53</td>
<td>4/4</td>
</tr>
<tr>
<td>Total</td>
<td>32/32</td>
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</table>

Diehl et al. PNAS 2005
Diehl et al. Gastroenterology 2008
Prediction of Drug Response – Theranostics

Example: Blood-based KRAS testing for colorectal cancer

No tissue required

Standard therapy

Anti-EGFR therapy
cetuximab or panitumumab
Application 2: Monitoring of Tumor Response

Circulating mutant tumor DNA (copies / 2 ml plasma)

Surgery 1  Chemo-therapy 1  Surgery 2  Chemo-therapy 2

Tumor burden (cm)

CEA (ng/ml)

Application 3: Monitoring of Resistance Monitoring during the Treatment with Targeted Therapies

Initial Tumor → Targeted therapy → Response → Recurrence due to resistant mutations

- Normal cell
- Tumor cell
- Tumor cell with resistance mutation
Application 3: KRAS monitoring of colorectal cancer patients Undergoing anti-EGFR therapy

KRAS mutant alleles were detectable as early as 10 month before radiographic documentation of disease progression

Misale et al. Nature 2012
Ultrasensitive NGS Technology:
Plasma-Sequencing System (Plasma-Seq)

- Based on NGS technology
- Specifically designed for high sensitivity mutation discovery
- Allows the analysis of tumor suppressor genes from plasma DNA

Plasma DNA specific locus

Mutation

Universal & Unique Identifier Assignment

Library Amplification & Redundant Sequencing

Random errors

Wild-type

Mutant

UID family (= all sequence reads carrying the same UID)
Application of the Plasma-Seq System

RESEARCH ARTICLE

OVARIAN CANCER

Evaluation of DNA from the Papanicolaou Test to Detect Ovarian and Endometrial Cancers

Isaac Kindé,1,* Chetan Bettegowda,1,2,8 Yuxuan Wang,1,8 Jian Wu,1,8 Nishant Agrawal,1,4 Le-Ming Shih,3 Robert Kurman,3 Fanny Dao,6 Douglas A. Levine,6 Robert Giuntoli,7 Richard Roden,6 James R. Eshleman,6 Jesus Paula Carvalho,5 Suley Kazue Nagahashi Marie,8,9 Nicholas Papadopoulos,1 Kenneth W. Kinzler,1 Bert Vogelstein,1 Luis A. Diaz Jr.17

Papanicolaou (Pap) smears have revolutionized the management of patients with cervical cancers by permitting the detection of early, surgically curable tumors and their precursors. In recent years, the traditional Pap smear has been replaced by a liquid-based method, which allows not only cytologic evaluation but also collection of DNA for detection of human papillomavirus, the causative agent of cervical cancer. We reasoned that this routinely collected DNA could be exploited to detect somatic mutations present in rare tumor cells that accumulate in the cervix once shed from endometrial or ovarian cancers. A panel of genes that are commonly mutated in endometrial and ovarian cancers was assembled with new whole-exome sequencing data from 22 endometrial cancers and previously published data on other tumor types. We used this panel to search for mutations in 24 endometrial and 22 ovarian cancers and identified mutations in all 46 samples. With a sensitive massively parallel sequencing method, we were able to identify the same mutations in the DNA from liquid Pap smear specimens in 100% of endometrial cancers (24 of 24) and in 41% of ovarian cancers (9 of 22). Prompted by these findings, we developed a sequence-based method to query mutations in 12 genes in a single liquid Pap smear specimen without previous knowledge of the tumor's genotype. When applied to 14 samples selected from the positive cases described above, the expected tumor-specific mutations were identified. These results demonstrate that DNA from most endometrial and a fraction of ovarian cancers can be detected in a standard liquid-based Pap smear specimen obtained during routine pelvic examination. Although improvements need to be made before applying this test in a routine clinical manner, it represents a promising step toward a broadly applicable screening methodology for the early detection of gynecologic malignancies.

Kinde et al. Sci Transl Med 2013
A LONG WAY TO CURE CANCER:
STATUS 1971: NIXON DECLARED „WAR AGAINST CANCER“
A LONG WAY TO CURE CANCER:
STATUS 2015: TRANSLATION OF KNOWLEDGE TO INDIVIDUALIZED CANCER THERAPIES