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Realizing Individualized Cancer Therapy

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2nd Biospecimen Research Network Symposium
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Challenges for the Development of Individualized Cancer Therapies

Indivumed: Corporate Facts

Mission:	**Realizing Individualized Cancer Therapy**
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.**

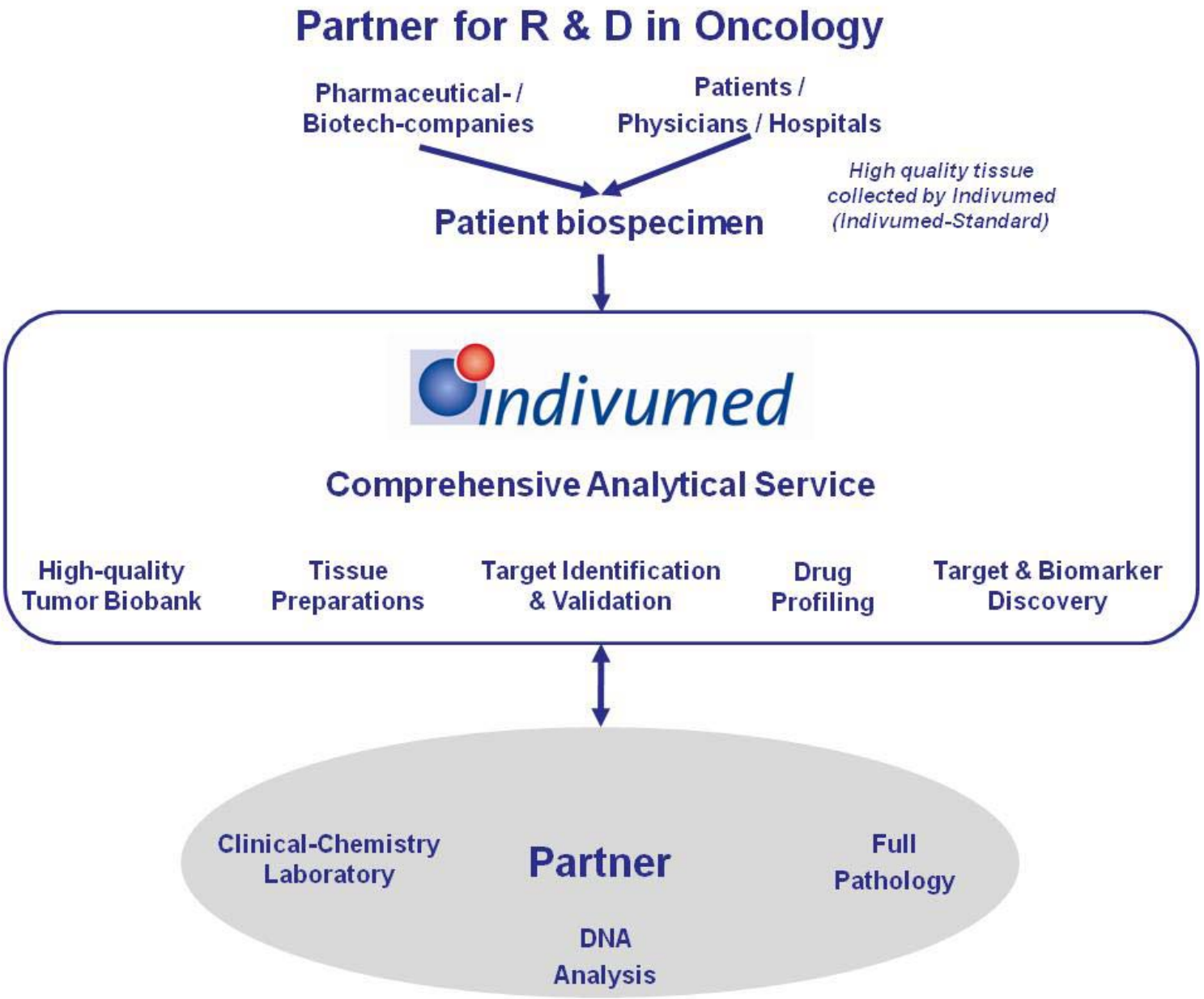
Indivumed: Corporate Facts

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

Indivumed: Corporate Facts



The cancer problem: heterogeneity

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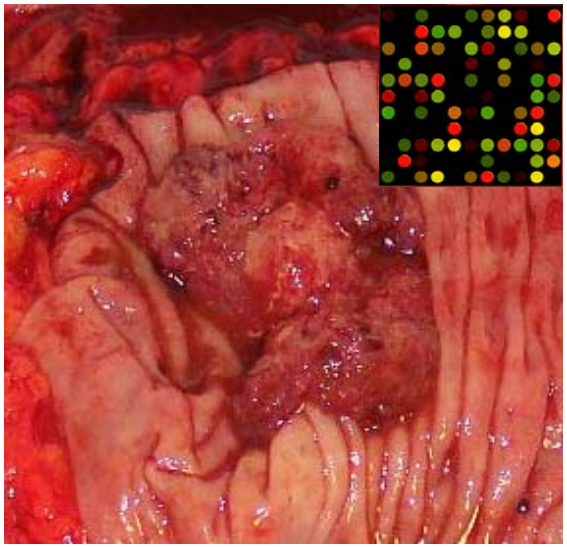
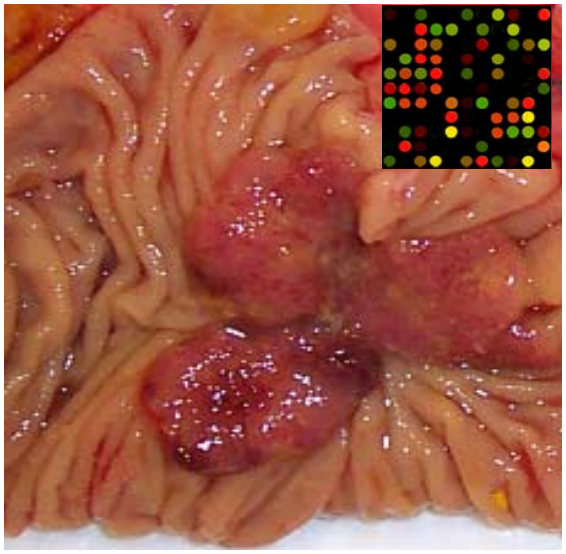
Three colon cancer patients:
Same disease? Same therapy?

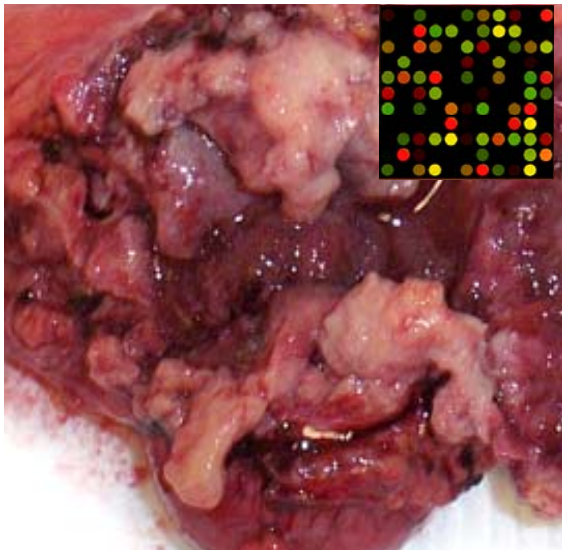
Patient 1



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

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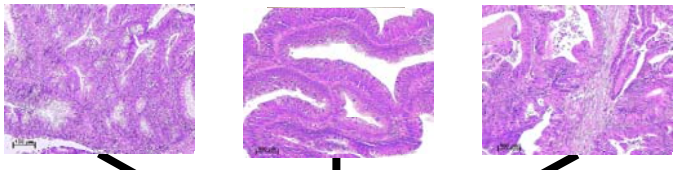
Past

Tumor 1 Tumor 2 Tumor 3



Standard Therapy

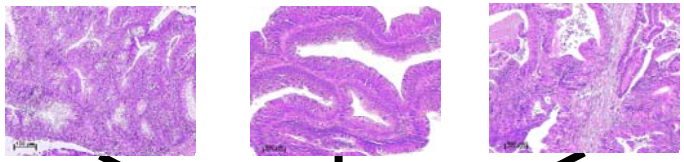
In 2001, only one of three patients benefited from cancer drug treatment

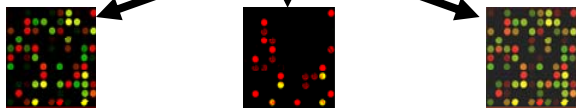
(Spear et al. (2001) Trends Molec. Med. 7, 201-203)

Effective
Less toxic
Cost reduction
FDA demand

Future

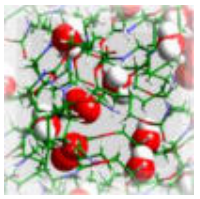
Tumor 1 Tumor 2 Tumor 3





Molecular diagnosis

Therapy 1 Therapy 2 Therapy 3



Individualized medicine

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HOW?

<u>Considering the basis for drug and companion diagnostics development:</u>

(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

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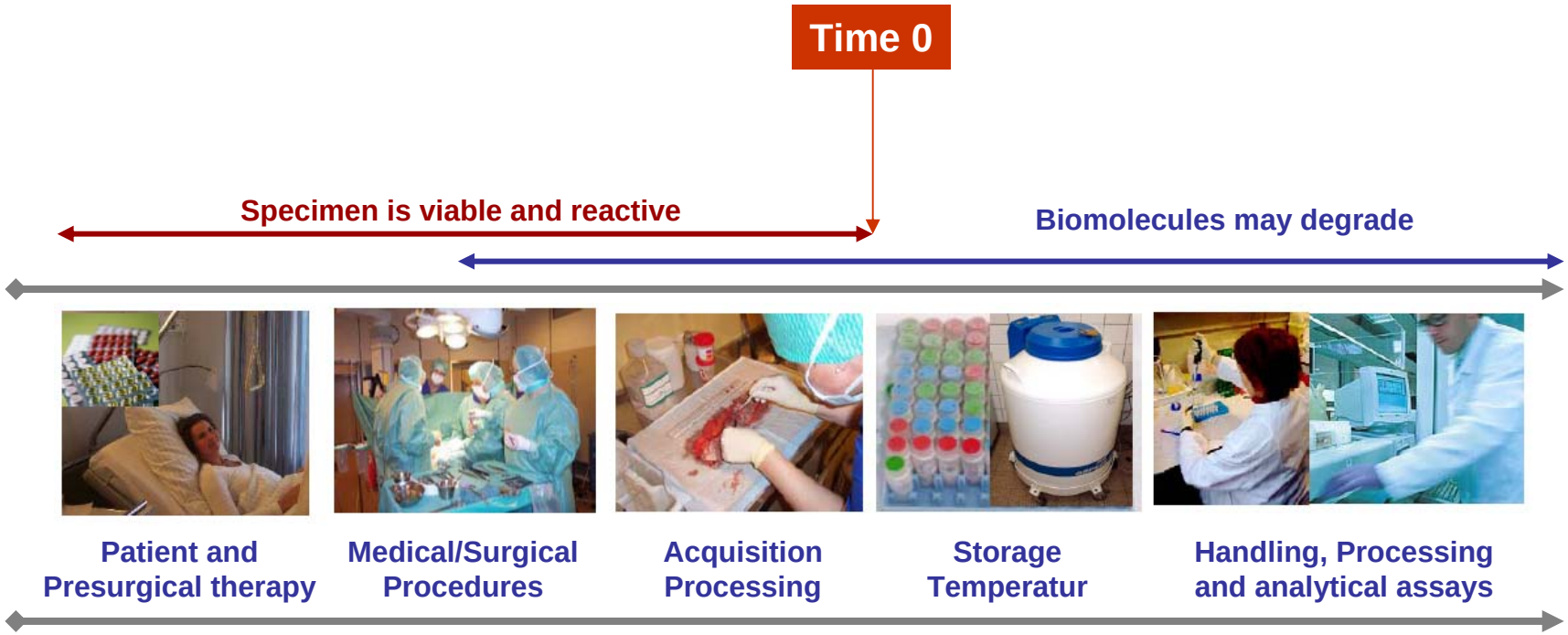
Tissue is alive until fixation and reacts to environment on the cellular and molecular level



Critical variables: Overview

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Indivumed research on critical variables for science guided biobanking

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

Critical: Biopsy location

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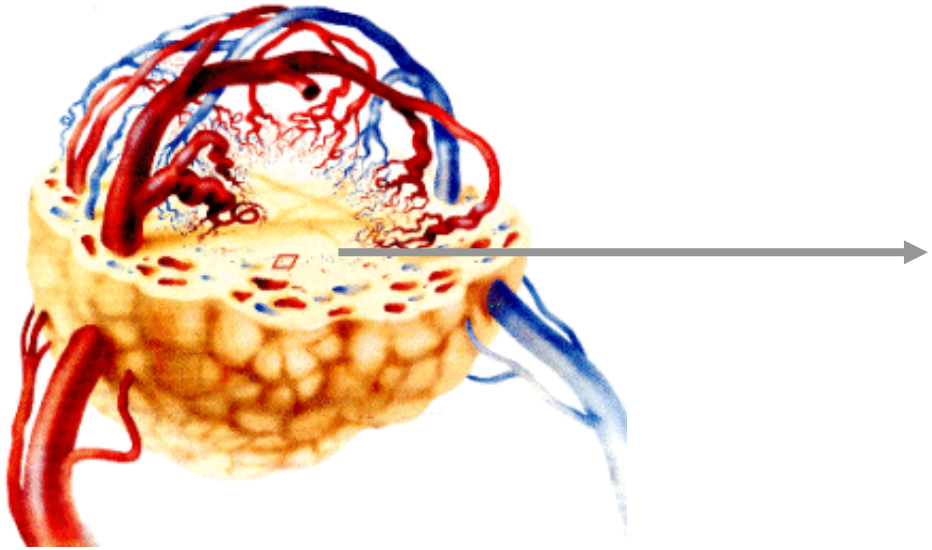
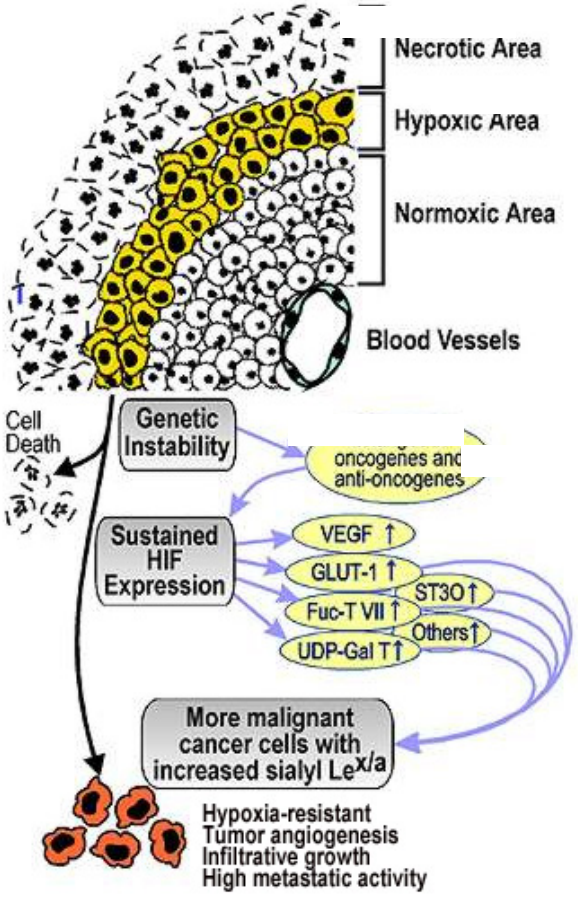
Indivumed research on critical variables for science guided biobanking

- **Location of biopsy**
- Drugs
- Intrasurgical ischemia
- Postsurgical ischemia

Critical: Biopsy location

Tumor tissue varies in center and peripheral areas

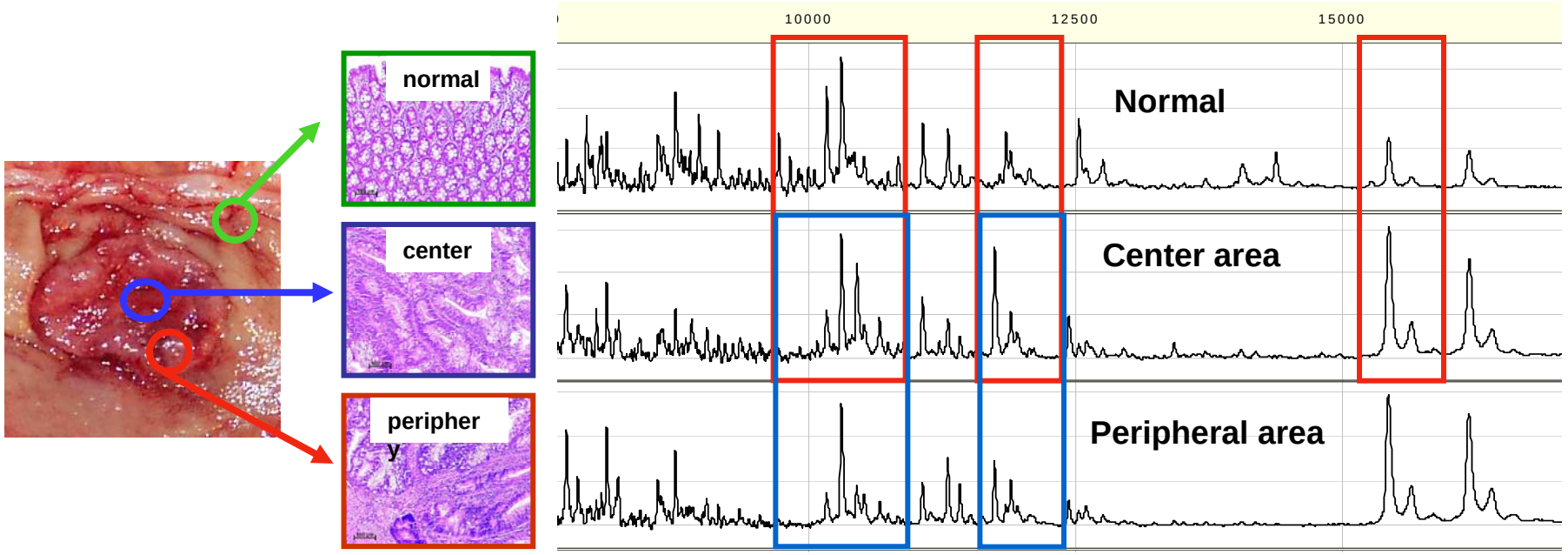




Invasive growth by induction of angiogenesis

Critical: Biopsy location

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

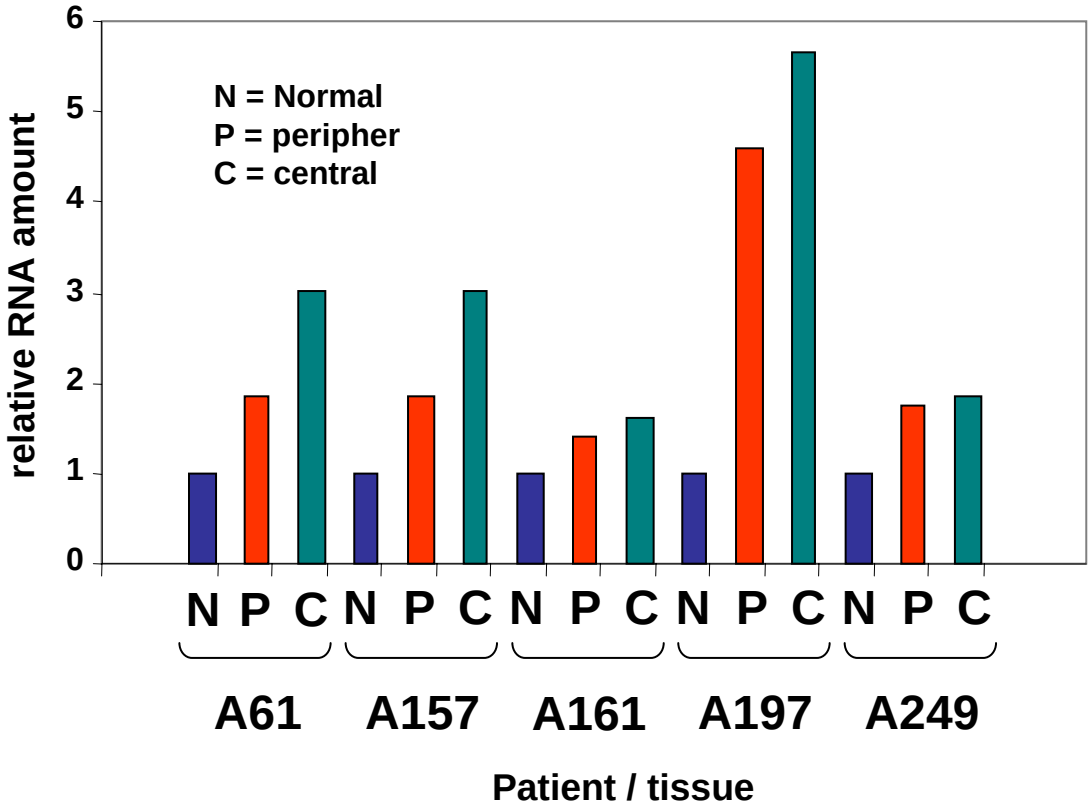


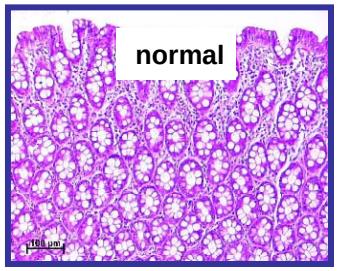
Approx. 40% of proteins are differentially expressed between peripherial and central tumor regions

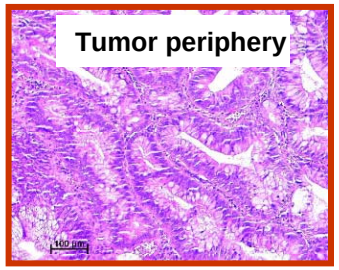
Critical: Biopsy location

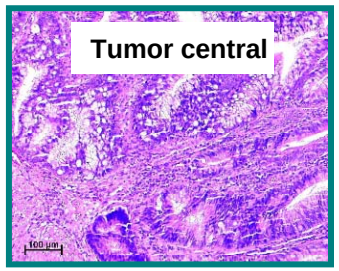
Expression of VEGF in different tissues: normal - periphery - central

(real-time RT-PCR)









Critical: Drugs intrasurgical

Indivumed research on critical variables for science guided biobanking

- Location of biopsy
- **Drugs**
- Intrasurgical ischemia
- Postsurgical ischemia

Critical: Drugs intrasurgical

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

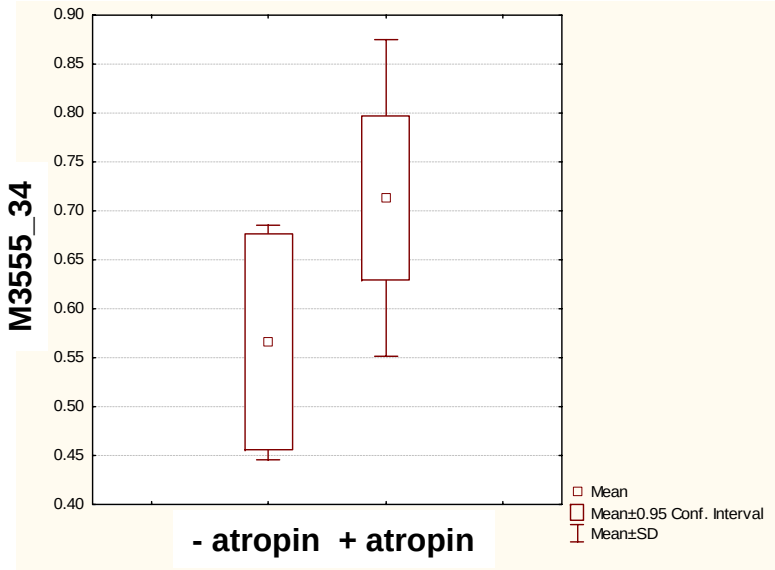
→ **Atropin**

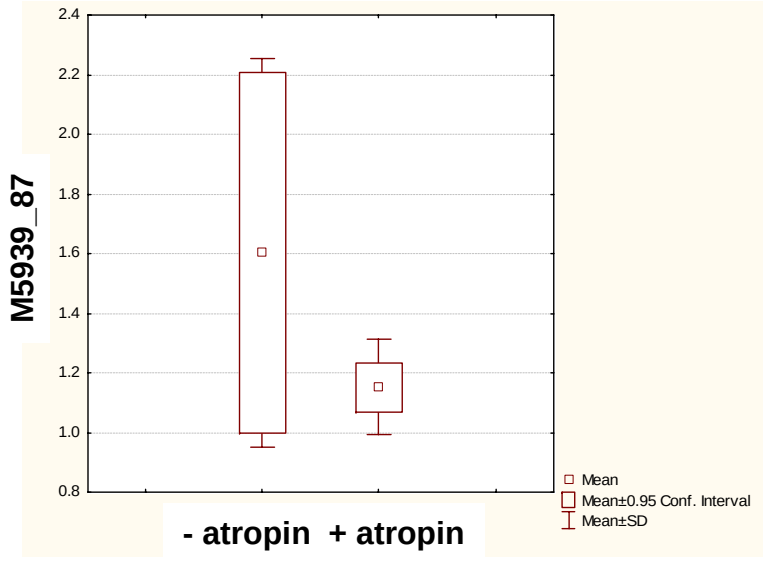
Critical: Drugs intrasurgical

Correlation of colon tissue protein expression with intrasurgical application of atropin

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

Variable	T-tests; Grouping: Atropin (normal lig atropin.sta) Group 1: 0 **+ atropin** Group 2: 1 **- atropin** Mean 0	Mean 1	t-value	df	p	Valid N 0	Valid N 1	Std.Dev. 0	Std.Dev. 1
M5939_87	1.15213	1.60276	-2.73669	22	0.012043	17	7	0.15888	0.65240
M3772_14	0.63586	1.05263	-2.34306	22	0.028574	17	7	0.25252	0.63653
M6723_51	2.17426	3.41282	-2.31784	22	0.030148	17	7	0.97299	1.63301
M3555_34	0.71346	0.56601	2.16344	22	0.041640	17	7	0.16216	0.11972





Critical: Intrasurgical ischemia

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Indivumed research on critical variables for science guided biobanking

- Location of biopsy
- Drugs
- **Intrasurgical ischemia**
- Postsurgical ischemia

Critical: Intrasurgical ischemia

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

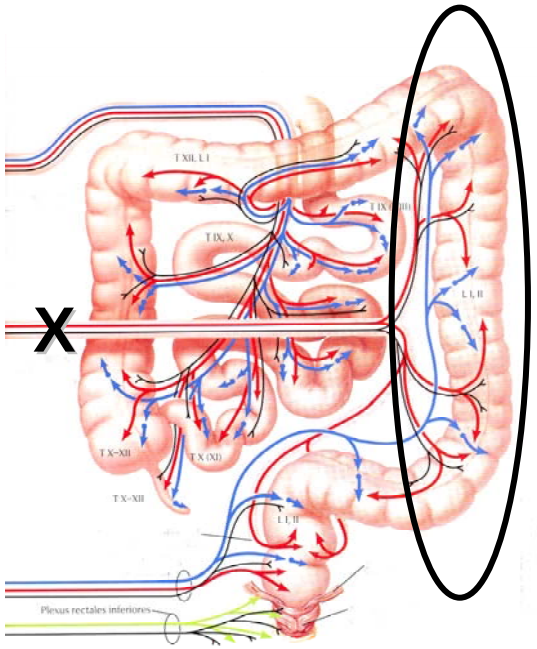
↓

Gene expression (Affymetrix)

↓

Bioinformatics

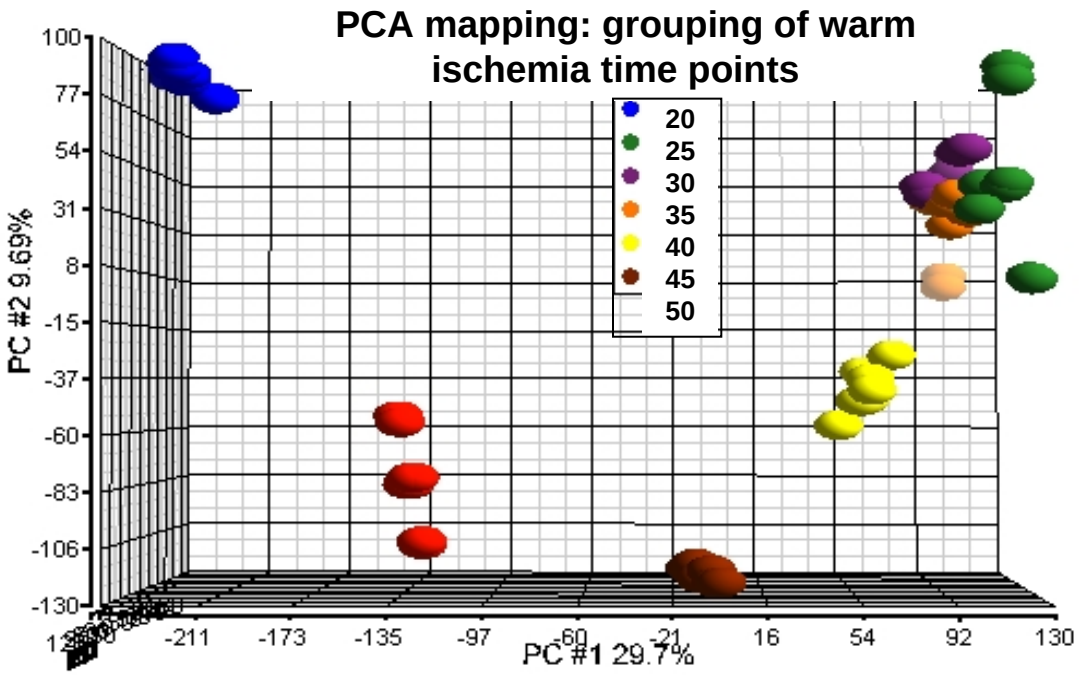
Mesenteric artery inferior

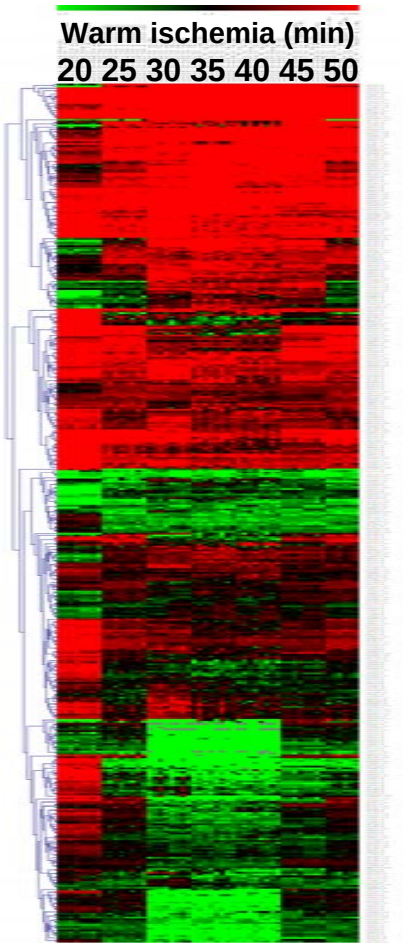


Critical: Intrasurgical ischemia

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

Critical: Postsurgical ischemia

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Indivumed research on critical variables for science guided biobanking

- Location of biopsy
- Drugs
- Intrasurgical ischemia
- **Postsurgical ischemia**

Critical: Postsurgical ischemia

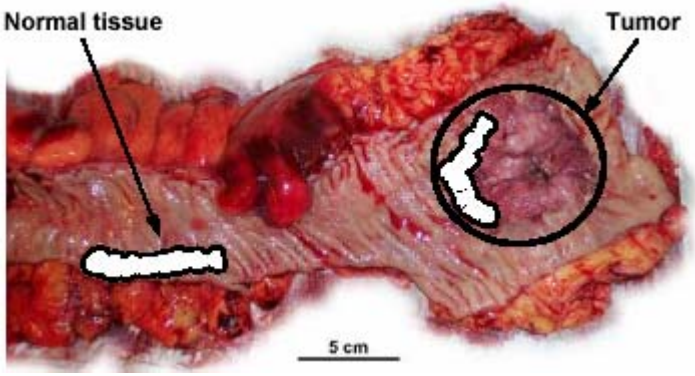
Impact of cold ischemia: controlled tissue study

Surgical removal

of rectum

Control of warm ischemia

Collection of normal and cancer tissue



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

<u>Analysis:</u>

Affymetrix

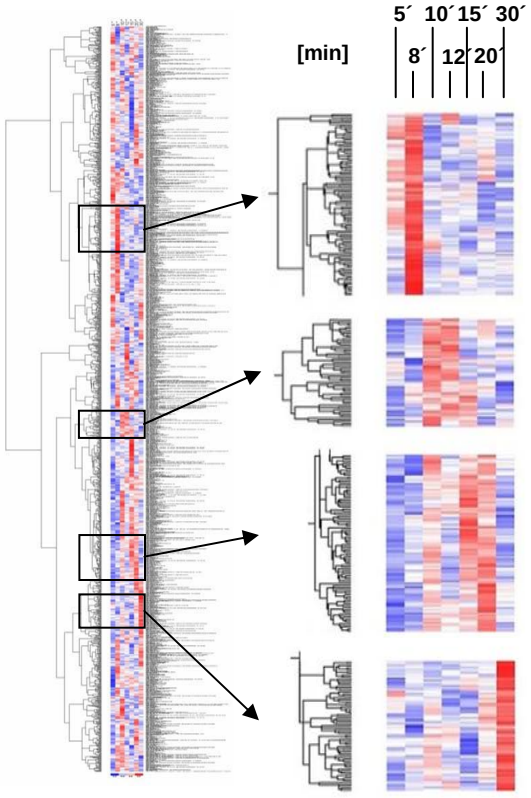
real-time RT-PCR

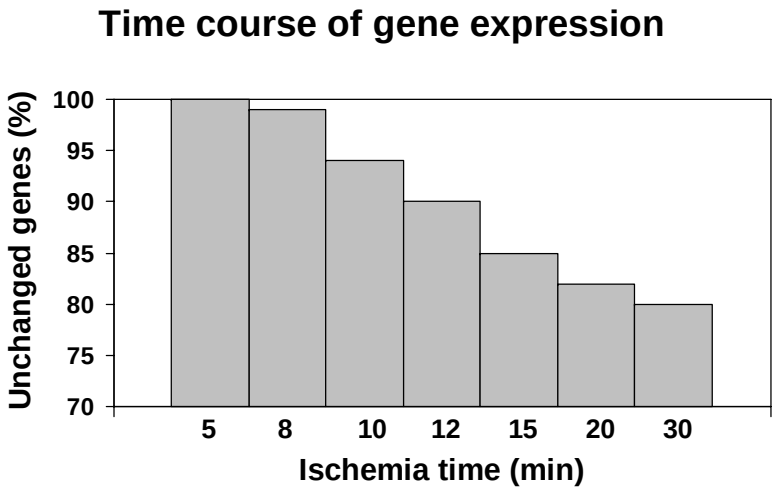
SELDI-TOF-MS

Critical: Postsurgical ischemia

Tissue ischemia and gene expression profiling

(Affymetrix cDNA microarray)





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

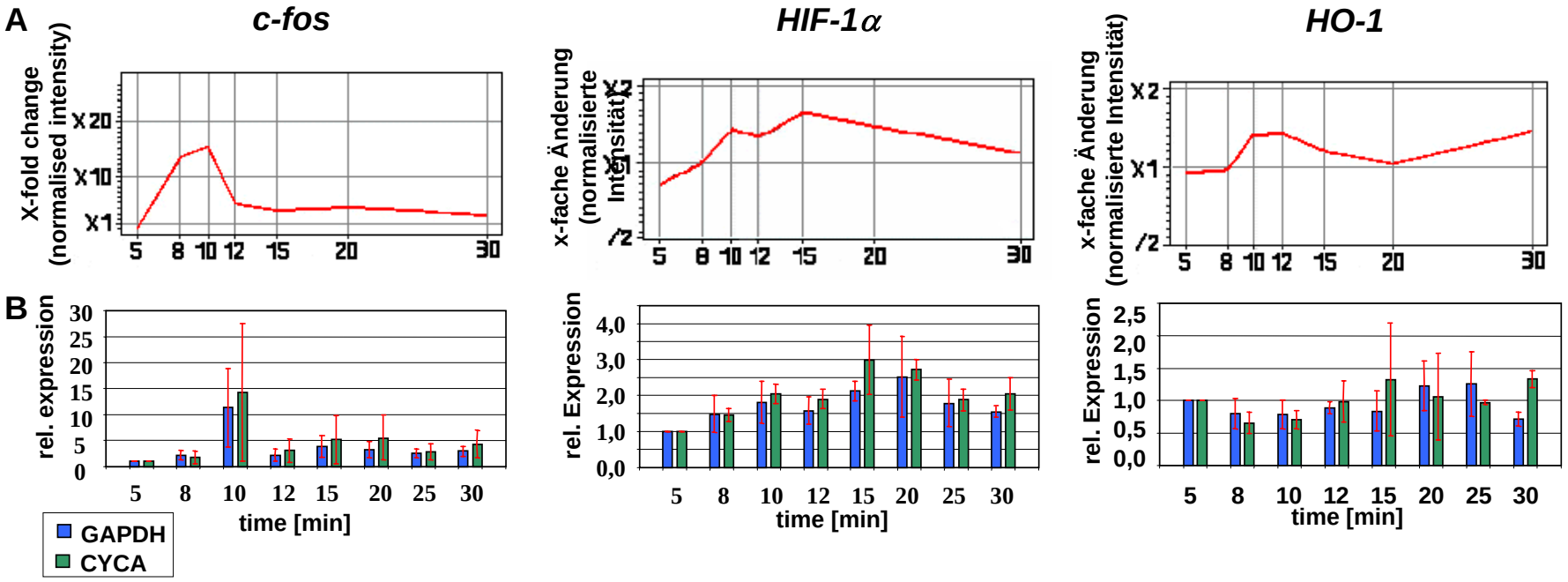
Sprüssel et al, BioTechniques 2004

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



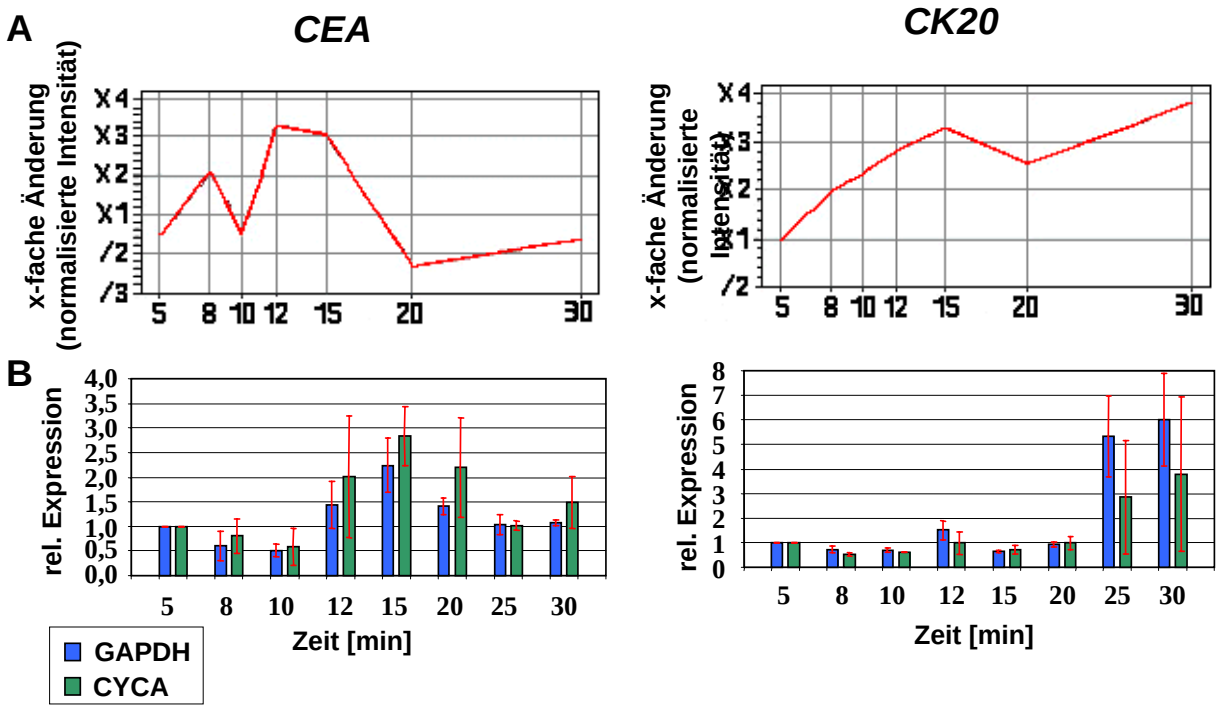
Sprüssel et al, BioTechniques 2004

Critical: Postsurgical ischemia

Tissue ischemia and gene expression profiling

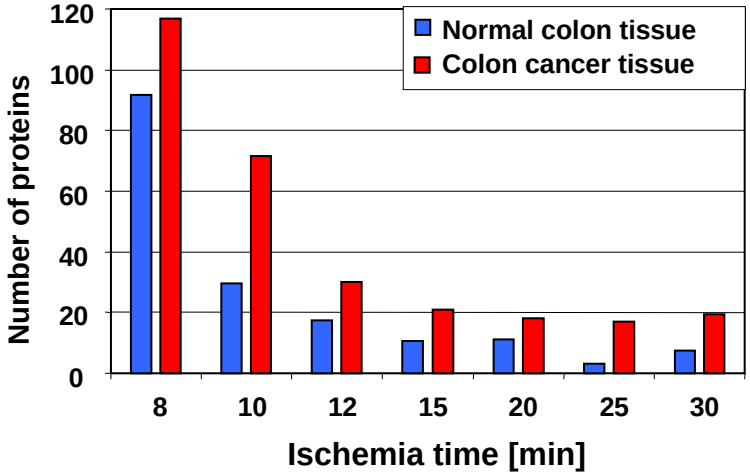
(Comparison Affymetrix data and real-time RT-PCR)

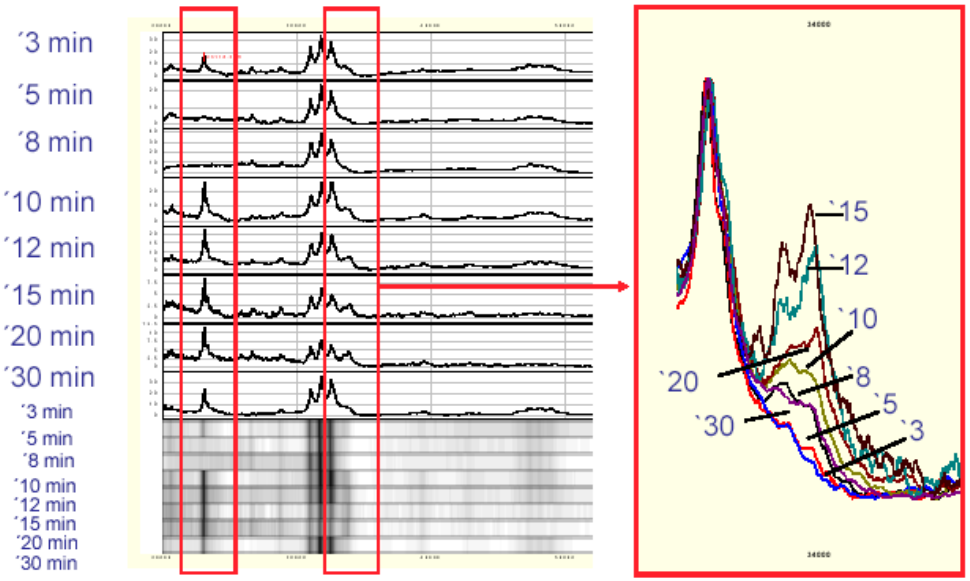
Tumor marker *CEA* (colorectal cancer biomarker) and cytokeratin *CK20*



Critical: Postsurgical ischemia

Tissue ischemia time and protein expression in colon tissue
(SELDI-TOF-MS analysis)



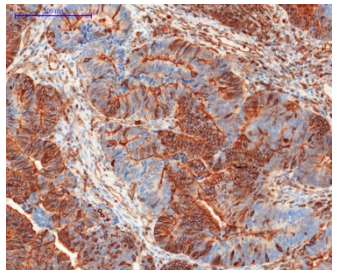
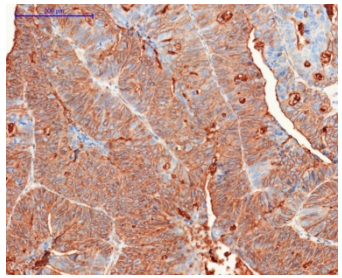
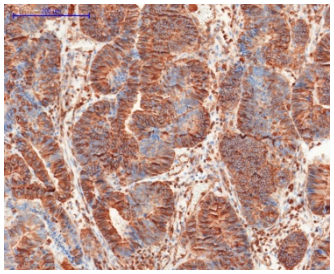
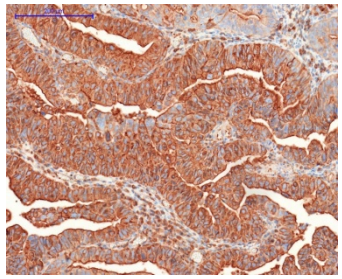
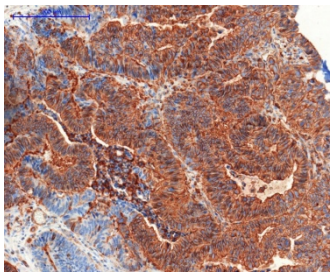
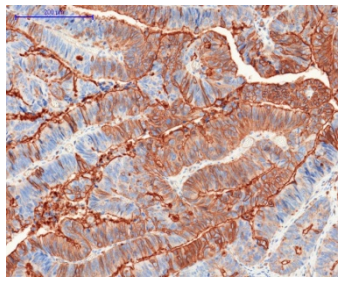


Following tumor resection ~ 25-30% of proteins are differentially expressed within the first 30 minutes !

Sprüssel et al, BioTechniques 2004

Critical: Postsurgical ischemia

Phosphoprotein expression: pTyr100 immunostaining (Ventana)

	Case A	Case B
10 min		
20 min		
60 min		

No clear trend of pTyr100 expression within 60 min of cold ischemia

Critical: Postsurgical ischemia

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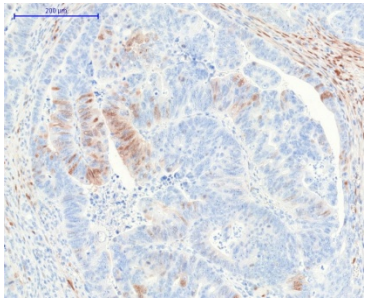
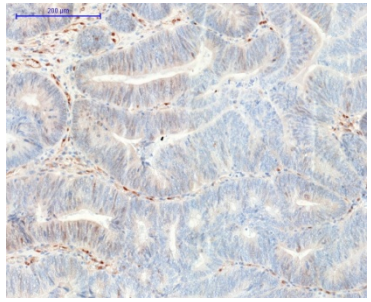
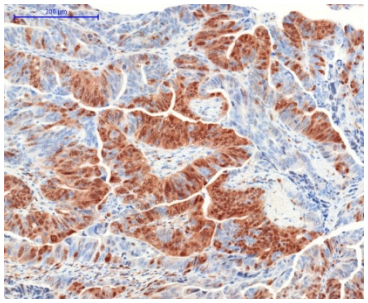
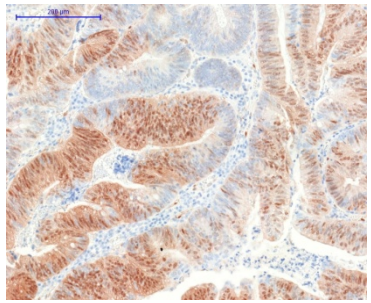
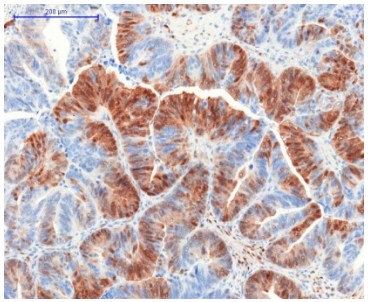
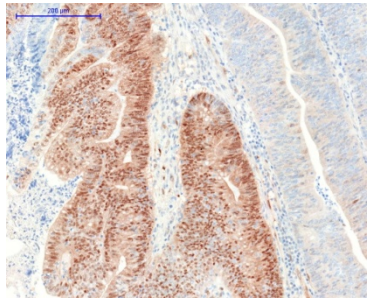
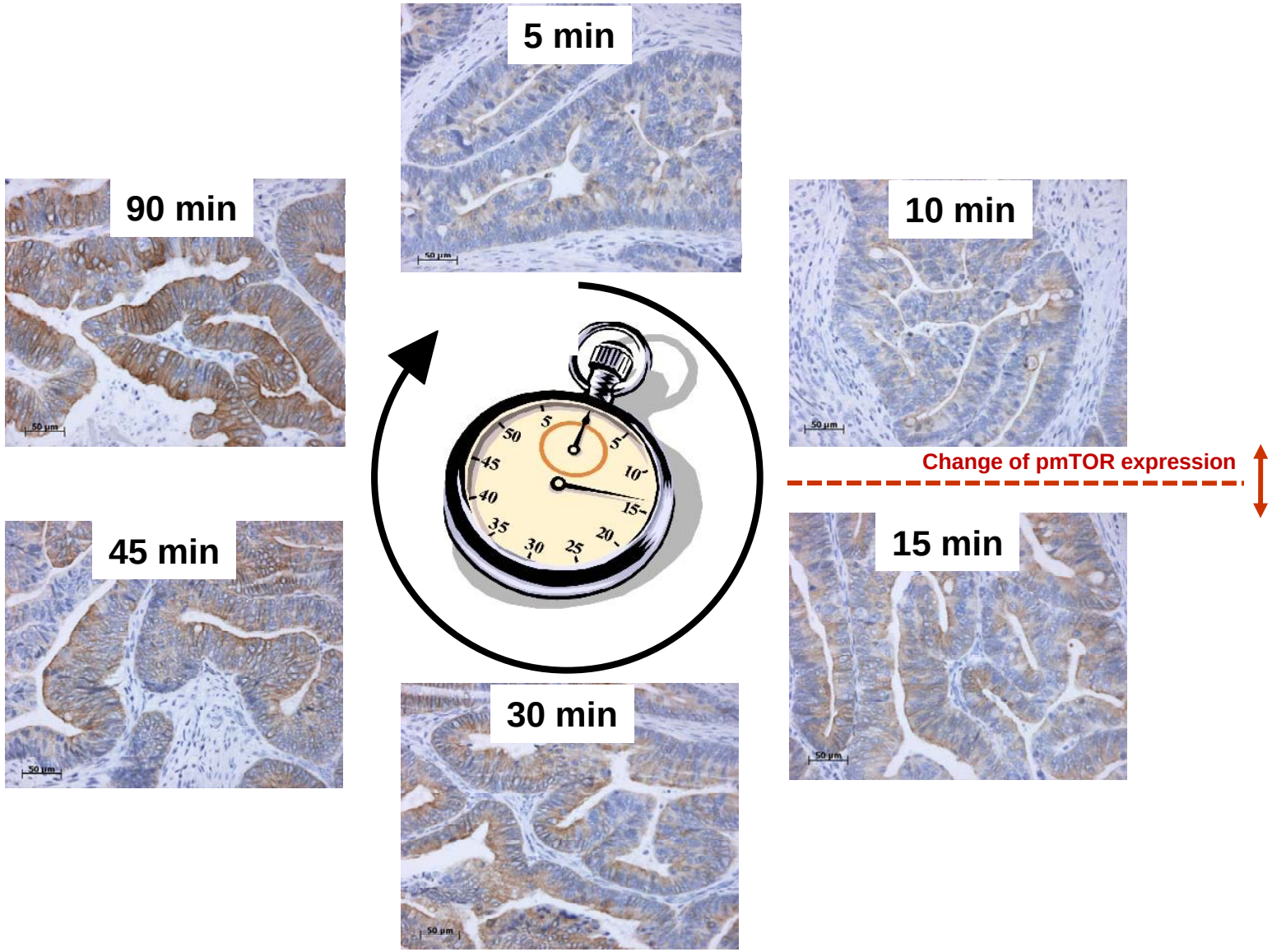
Phosphoprotein expression: pMAPK immunostaining (Ventana)

	Case A	Case B
10 min		
20 min		
60 min		

Change of pMAPK expression after 10-20 min cold ischemia

Critical: Postsurgical ischemia

pmTOR-immunostaining (Ventana)



Summary

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

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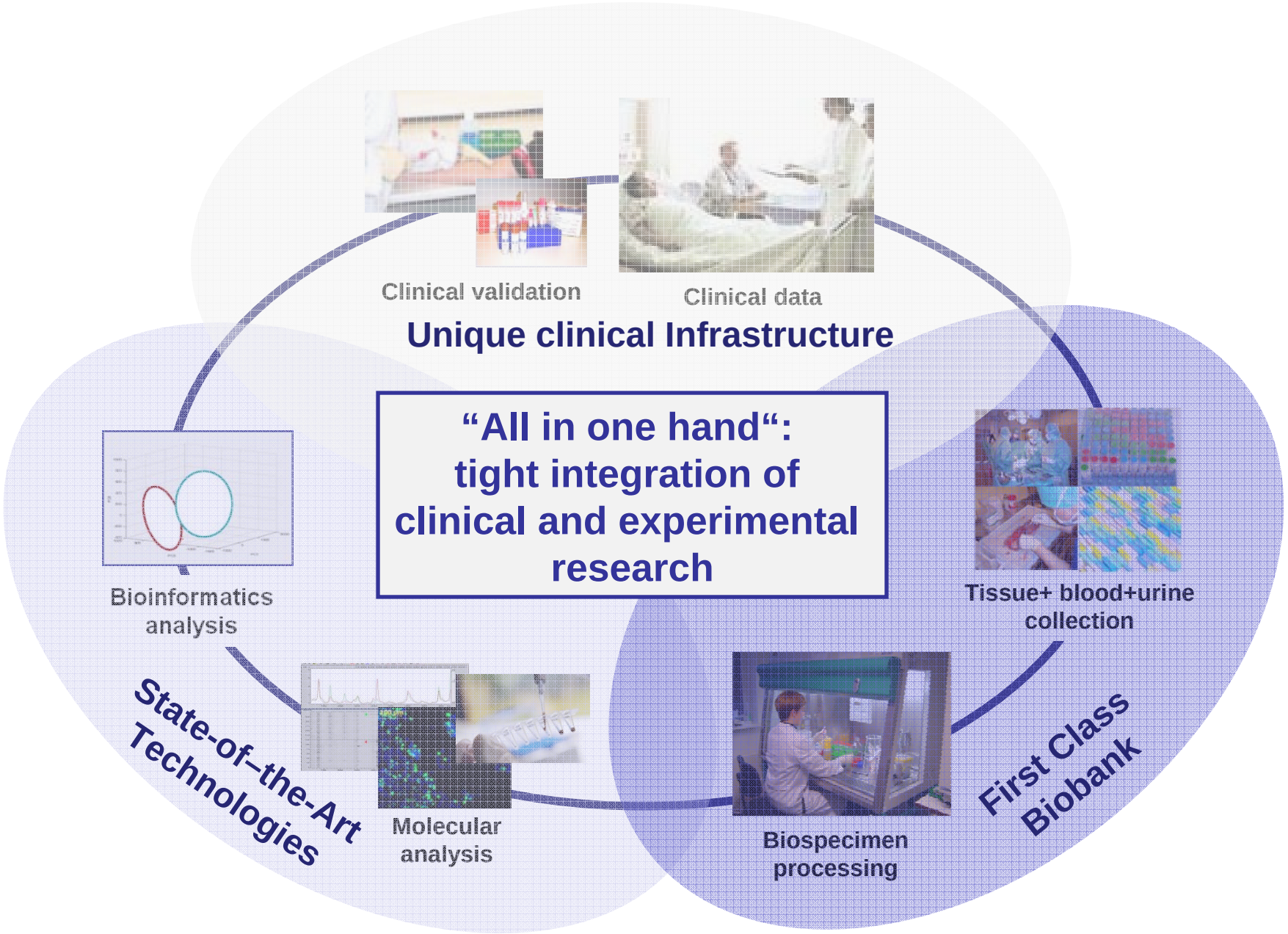

Biobanking

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Basic consideration:

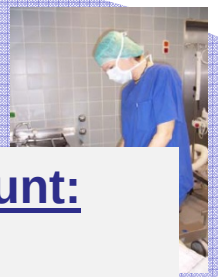
Take all responsibility away from surgeons and clinical staff !

Workflow: Biobanking

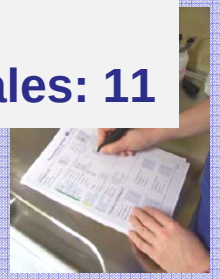
Done by Indivumed staff:

- 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







Indivumed headcount:

Total: 75

Biobanking: 42

Research / Service: 22

Administration / Sales: 11

Clinical Infastructure

- 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
- Postsurgery, Indivumed nurses see patients annually to collect outcome information and additional blood/urine samples

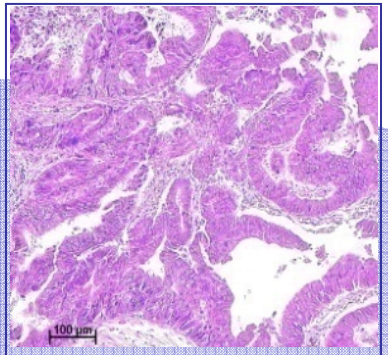
Asklepius Klinik Harburg

Biobanking: Samples

indivumed

- ✓ 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

CE

Tissue:

- ✓ Sets of normal +Tu-center +Tu-periphery
- ✓ Ischemia time < 12 min
- ✓ Blocks of similar size
- ✓ Simultaneous N_2-freezing
- ✓ 16h Formalin fixation

Fluids:

- ✓ Instant 4°C cooling
- ✓ Processing < 4 hours

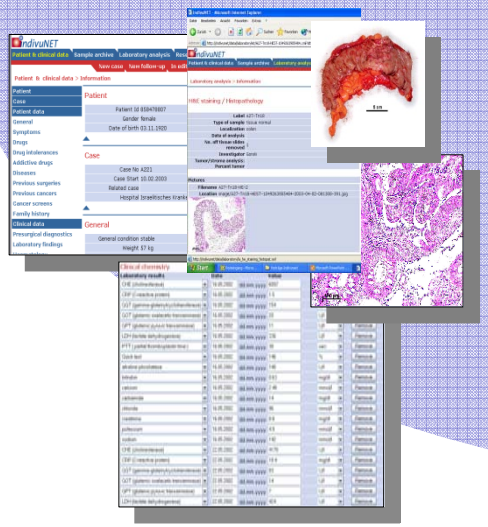
Biobanking: Clinical Data

Complete, prospectively collected and verified clinical data !

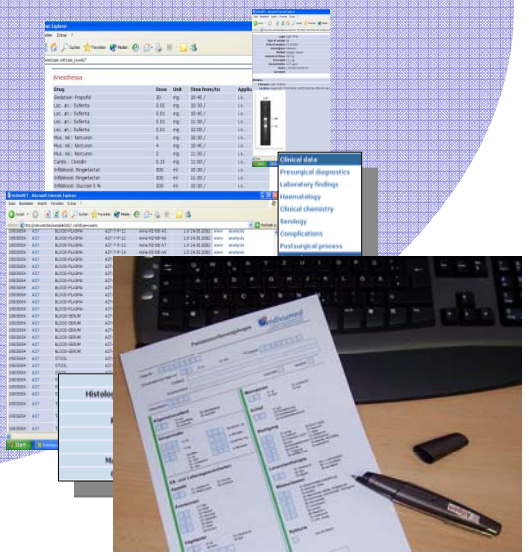
Surgery:
Procedure/drugs

Patient:
History/hospital

Patient:
Therapy/outcome

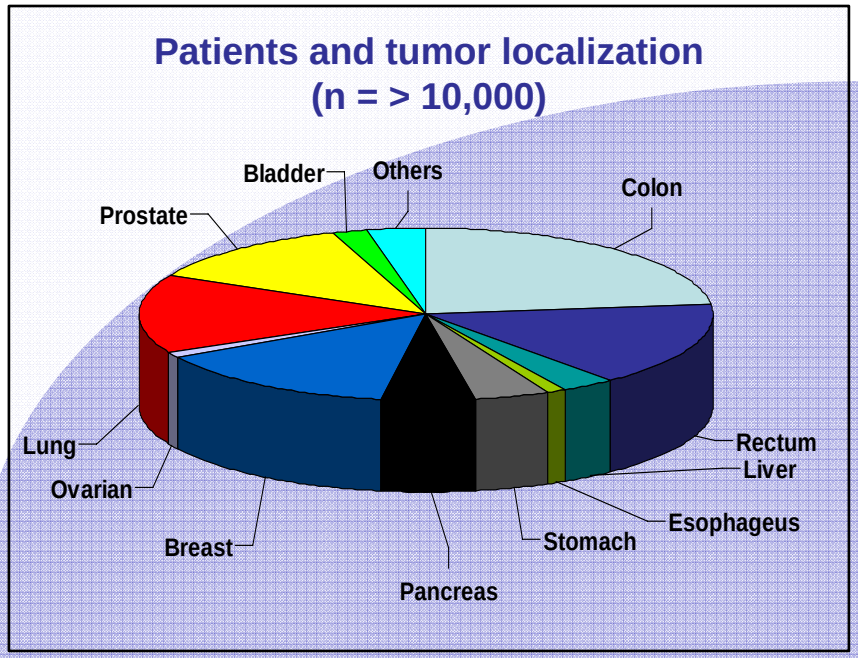


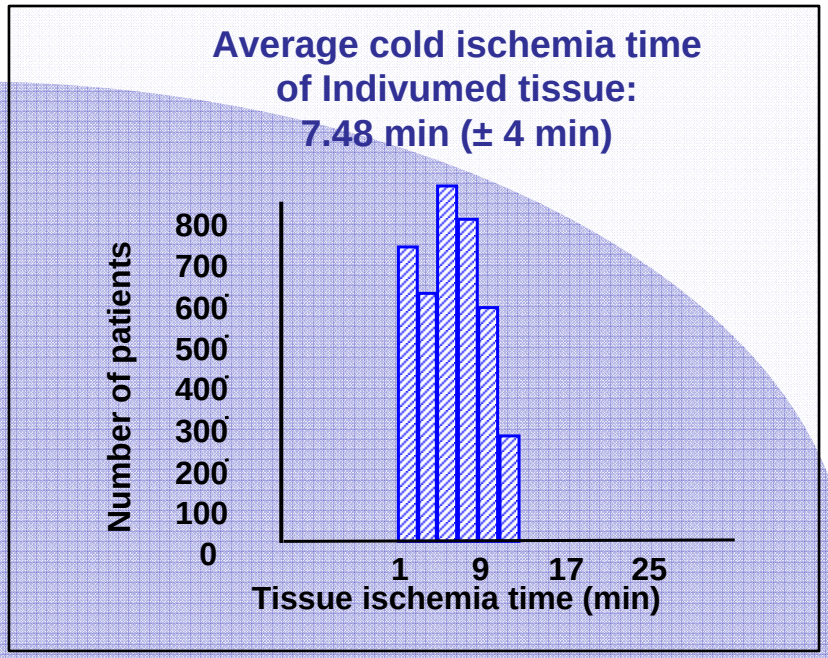
- ✓ Standardized documentation
- ✓ 300 data points / patient
- ✓ Prospective collection
- ✓ Web-accessible data base
- ✓ Compatible data format (Oracle)



Biobanking: Statistics

indivumed





Patient Consent (Surgery)	Biospecimen (Surgery)			Tracking (Follow-up)	Biospecimen (Follow-up)	
	Tissue	Blood	Urine		Blood	Urine
99%	95%	95%	75%	70%	60%	50%

+ ~ 2,000 new patients / year !!

Summary

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 personell
- It pays off to pay for high-quality