



Realizing Individualized Cancer Therapy



Tissue Intraoperative Factors

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Adjunct Professor, Georgetown University and Hamburg University

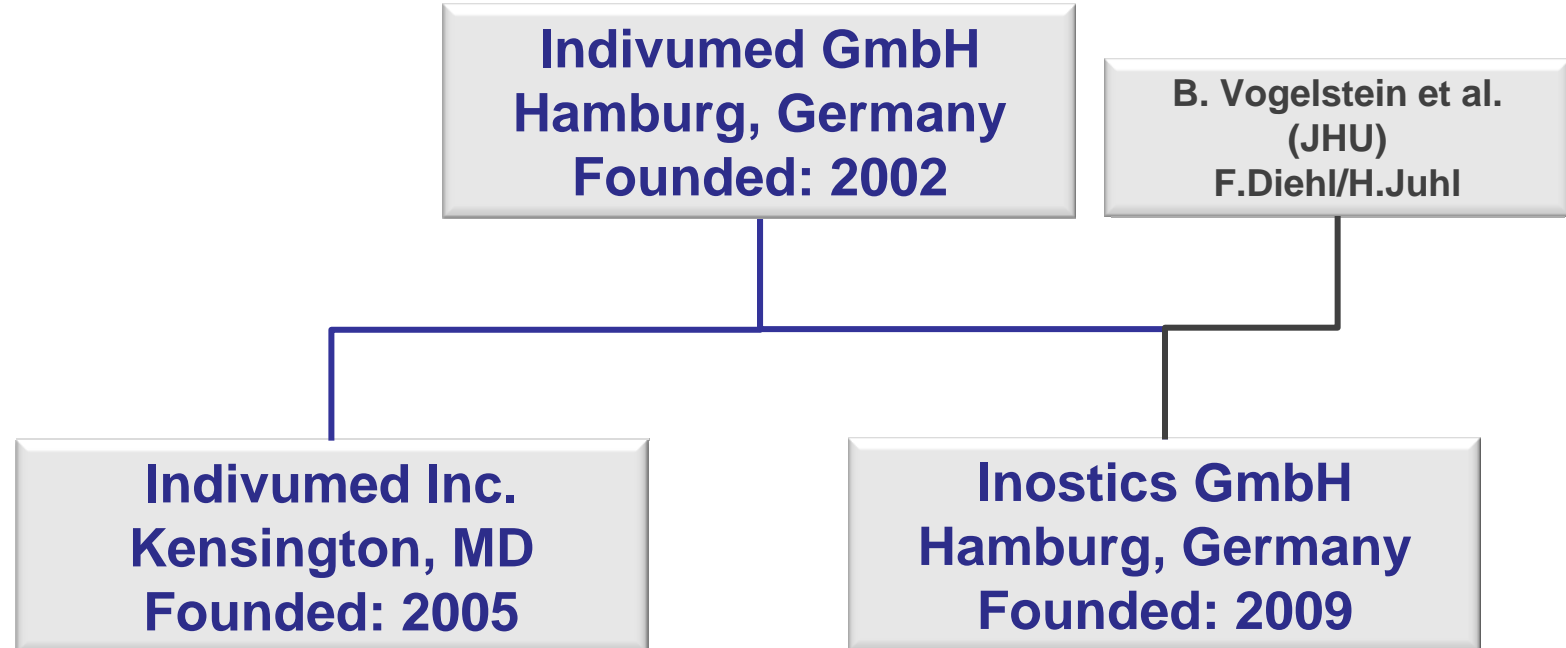
www.indivumed.com

juhl@indivumed.com

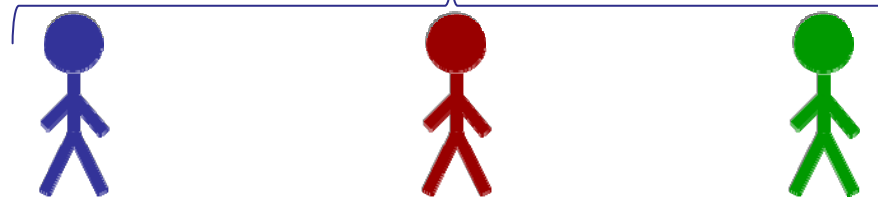
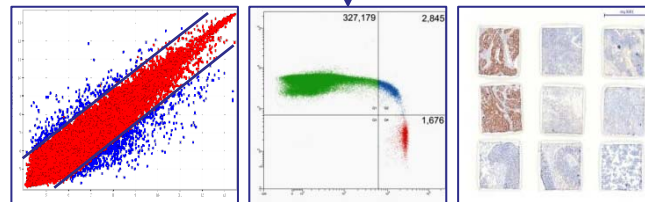
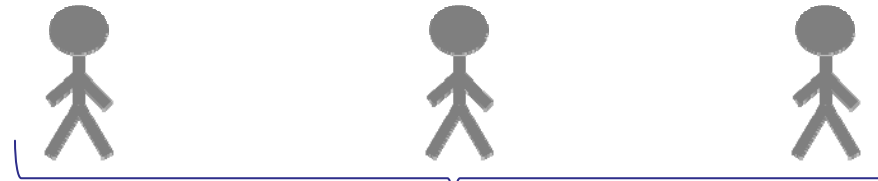
Germany
Land of Ideas



Company Structure



Indivumed Vision



Therapy 1

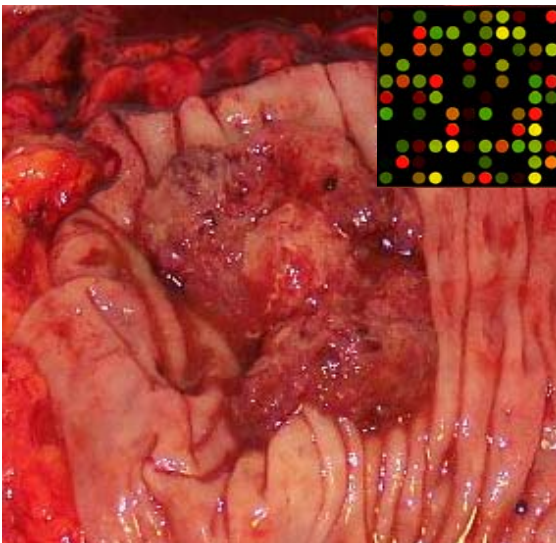
Therapy 2

Therapy 3

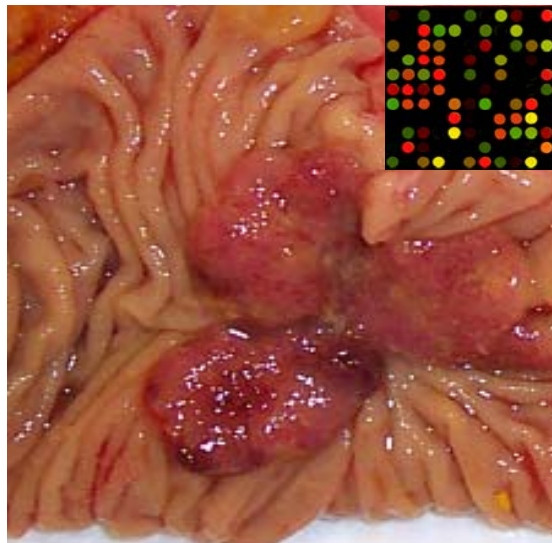
The Cancer Problem: Heterogeneity

Three colon cancer patients:
Same disease? Same therapy?

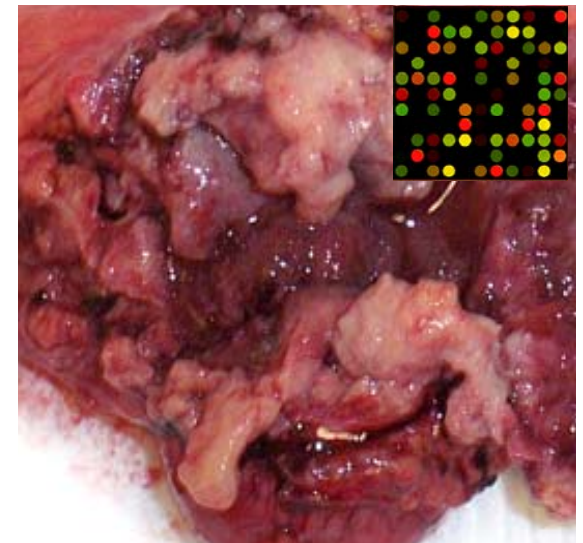
Patient 1



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

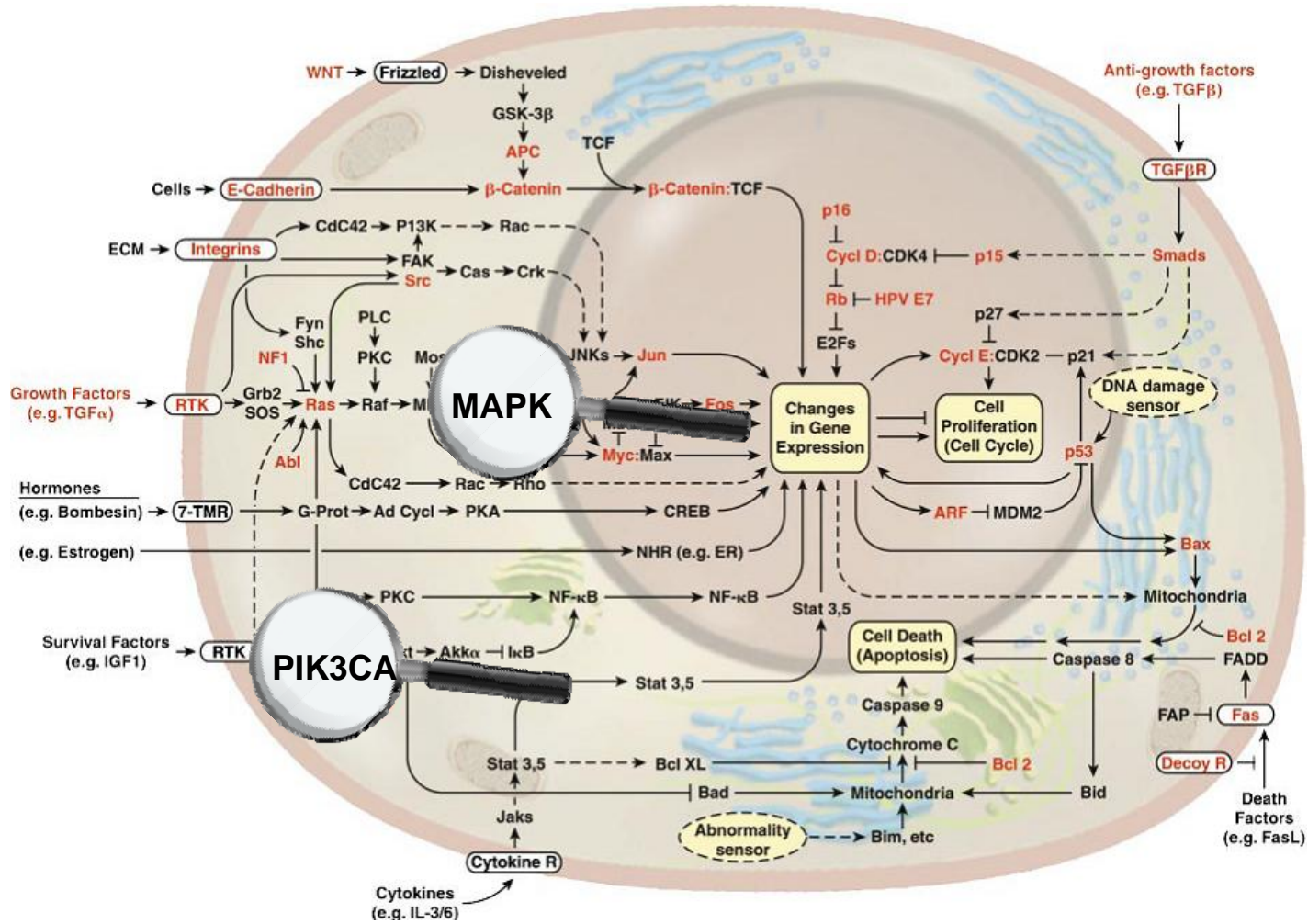
**Understanding the Molecular Basis of Cancer: Status 1971
(Nixon Declares „War Against Cancer“)**



Understanding the Molecular Basis of Cancer: Status 2010



Targeting Key Pathways



FDA-Approved Targeted Cancer Therapeutics (2001-2006)

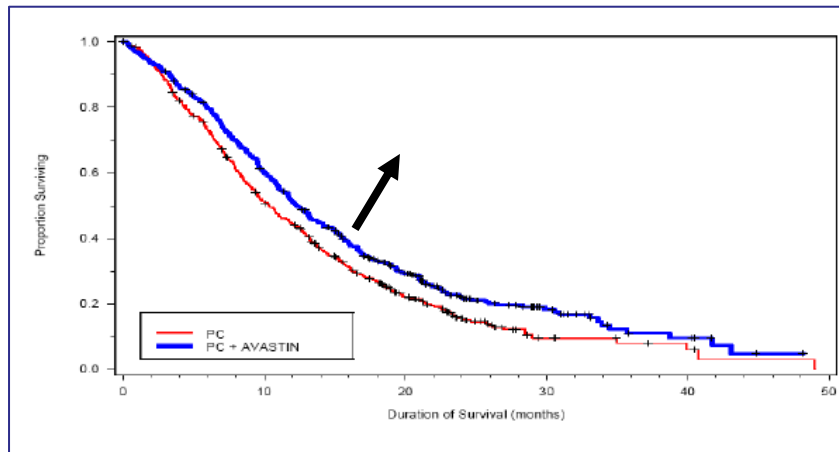
Table 1 Examples of targeted molecular cancer therapeutics receiving marketing approval by the US FDA 2001–2006

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

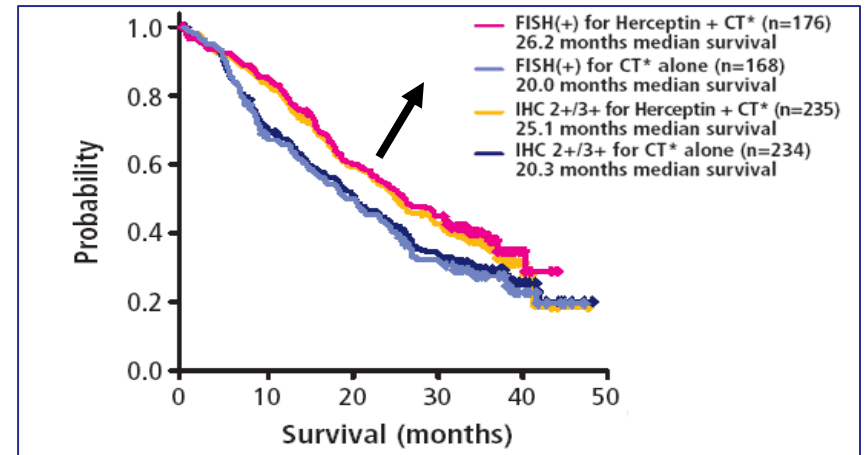
^aFirst approved 1998, use extended 2006. ^bSecond-line therapy. ^cFor people who have received at least two prior therapies. Also see CenterWatch (<http://www.centerwatch.com/patient/drugs/druglist.html>).

Cancer Therapeutics in Clinic

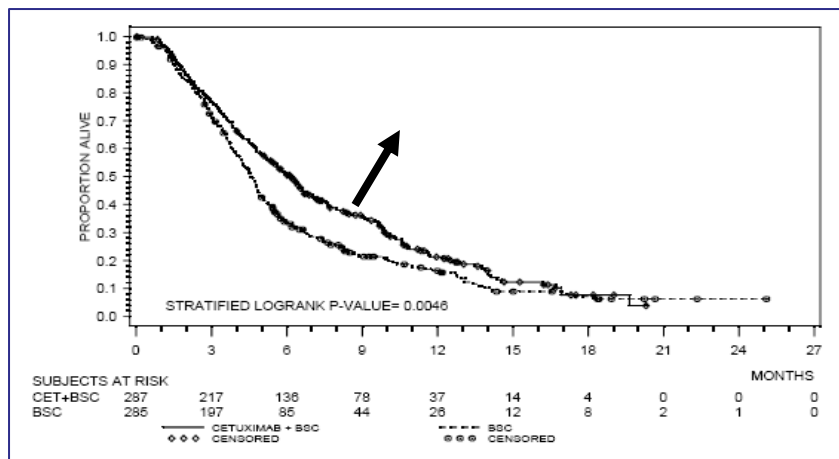
Colon cancer: Avastin



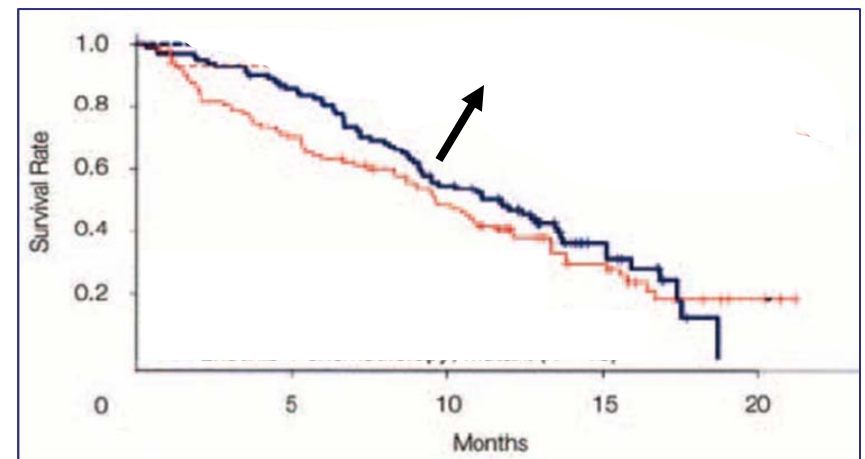
Breast cancer: Herceptin



Colon cancer: Erbitux



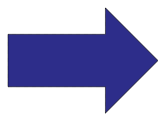
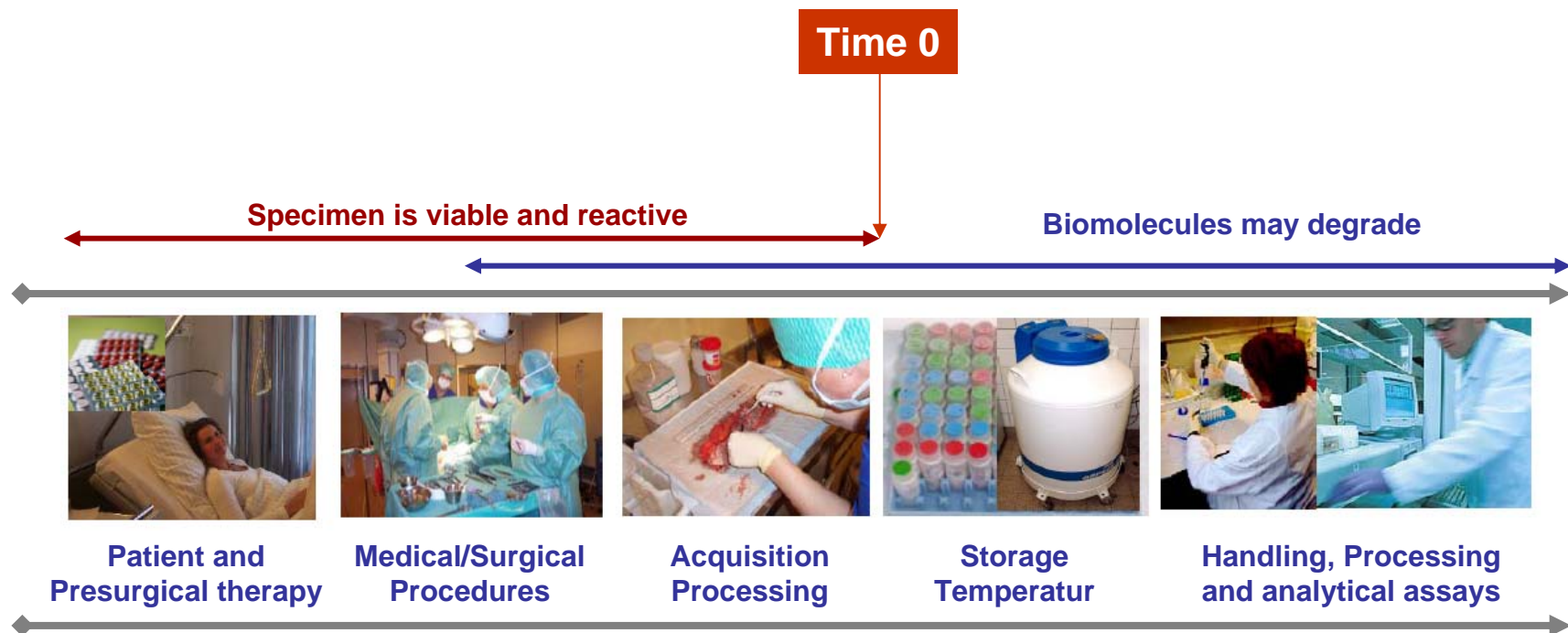
Lung cancer: Iressa



Targeting Key pathways



Challenge for using Tissue as Research and Diagnostic Tool: It is Alive and Reacts to Environmental Changes



**Highly defined quality tissues are needed
to understand cancer pathways**

Targeting Key pathways



Indivumed Research on Critical Variables for Science Guided Biobanking

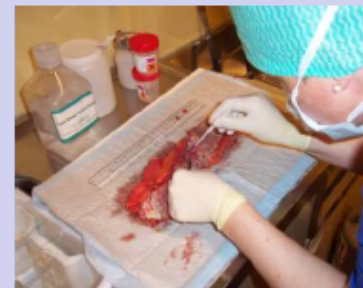


Postsurgical Processing

- Ischemia Time
- Location of Biopsy

Intrasurgical Factors

- Drugs
- Artery Ligation



Indivumed Research on Critical Variables for Science Guided Biobanking



Postsurgical Processing

- Ischemia Time
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Intrasurgical Factors

- Drugs
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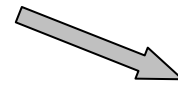
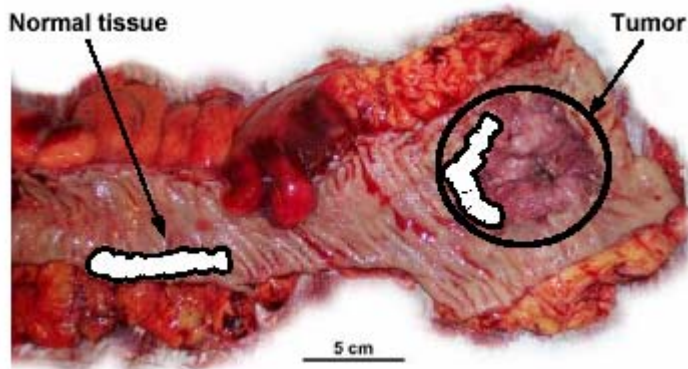


Impact of cold ischemia: controlled tissue study

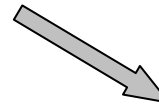
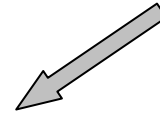


Surgical removal of rectum

Collection of normal and cancer tissue

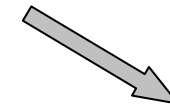


Control of warm ischemia



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



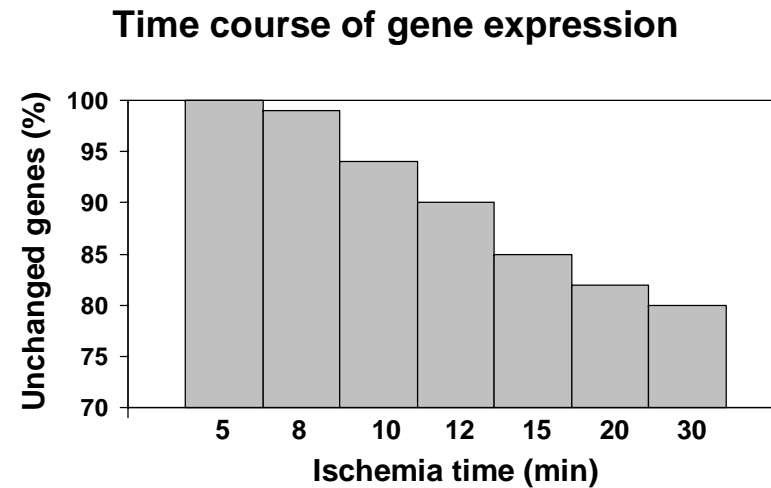
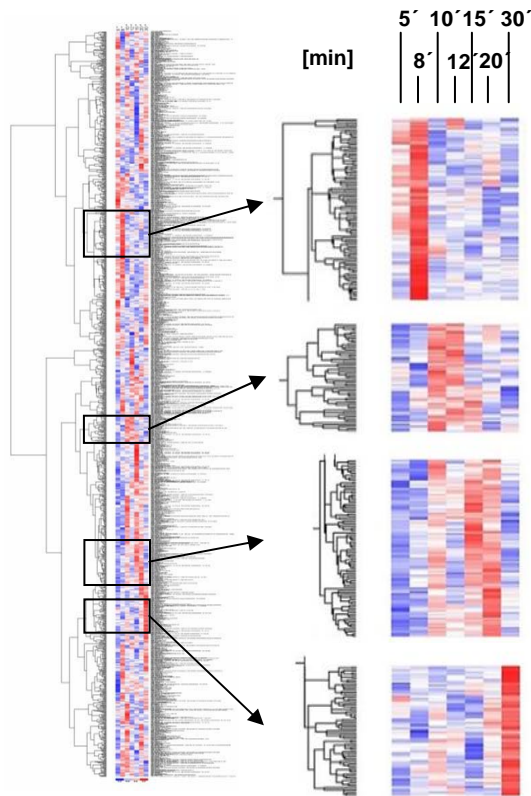
Analysis:

Affymetrix

real-time RT-PCR

SELDI-TOF-MS

Tissue Ischemia and Gene Expression Profiling (Affymetrix cDNA microarray)

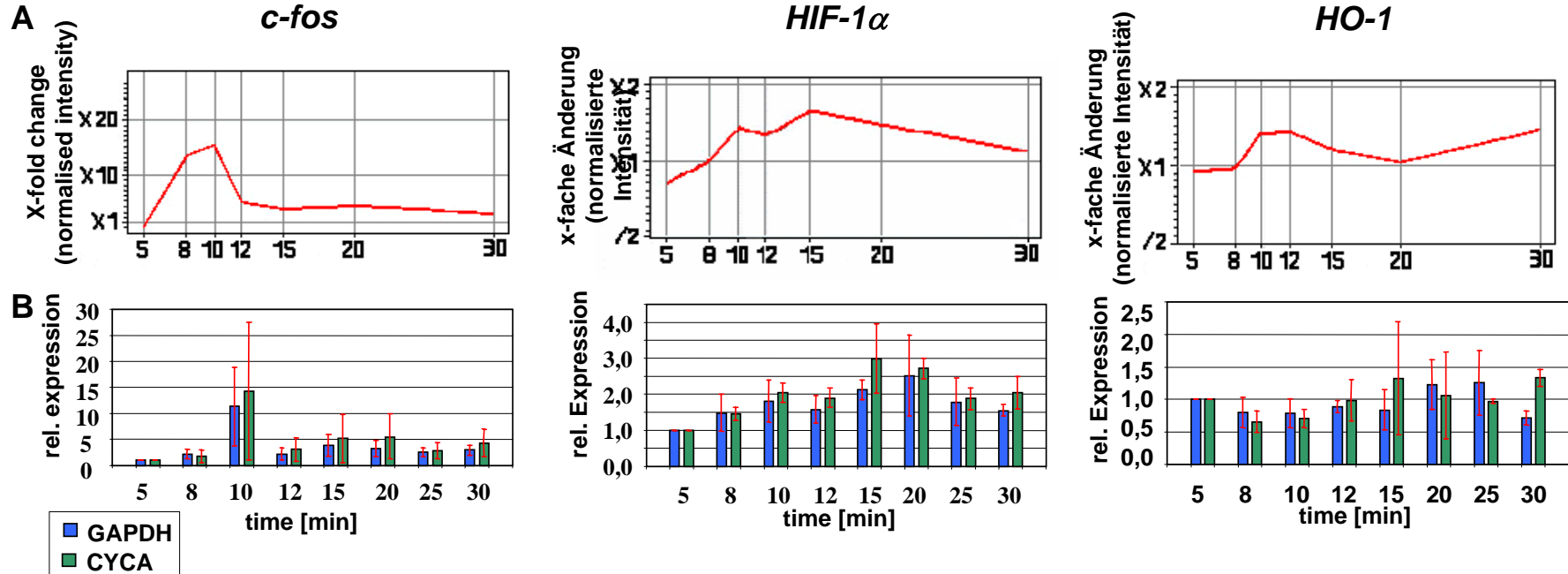


Following tumor resection ~ 20-25% of genes are differentially expressed within the first 30 minutes !

Tissue Ischemia and Gene Expression Profiling

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

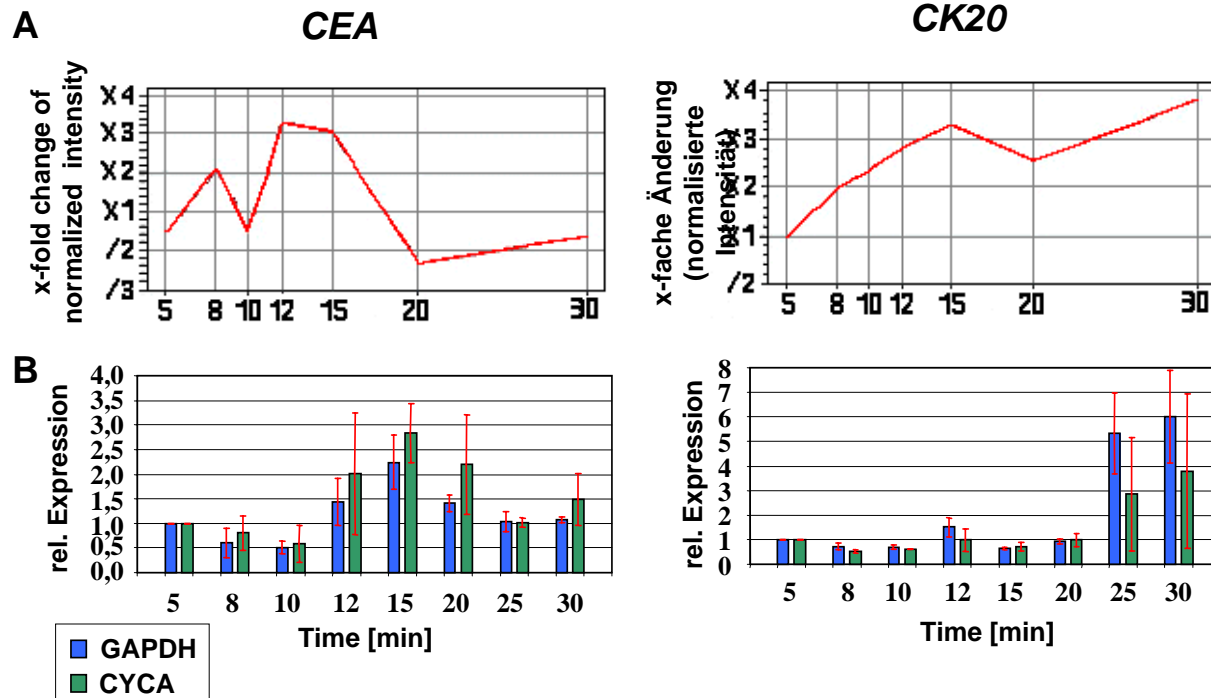
Ischemia regulated genes c-fos, HIF-alpha and HO-1



Tissue Ischemia and Gene Expression Profiling

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

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

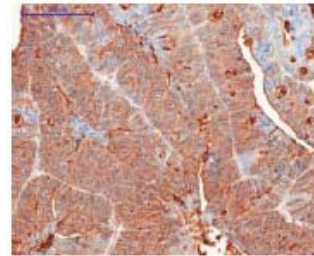
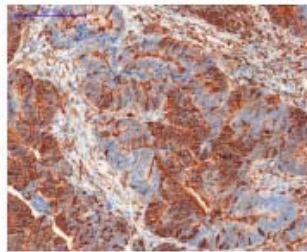


Phosphoprotein Expression: pTyr100 Immunostaining (Ventana)

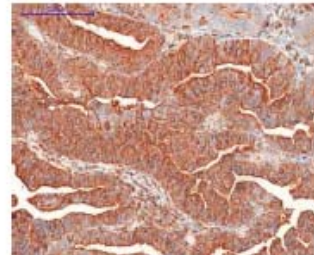
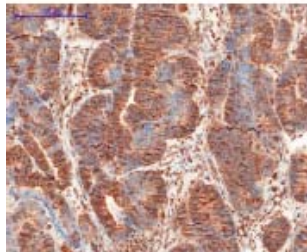
Case A

Case B

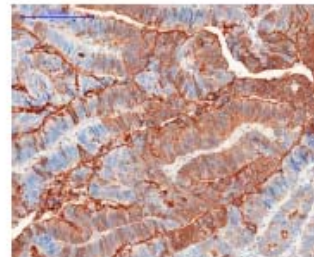
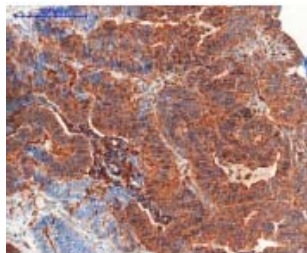
10 min



20 min



60 min



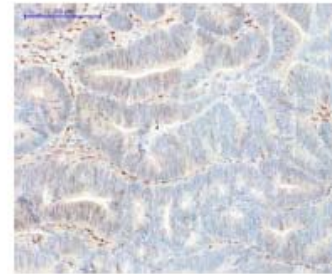
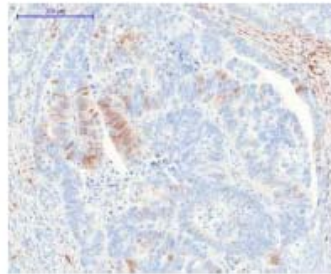
No clear trend of
pTyr100 expression
within
60 min of cold ischemia

Phosphoprotein Expression: pMAPK Immunostaining (Ventana)

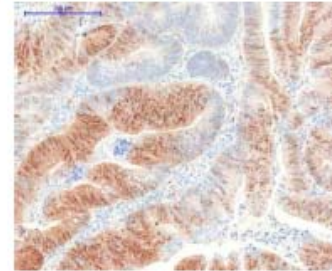
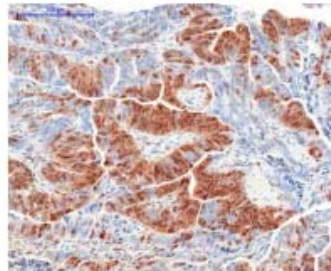
Case A

Case B

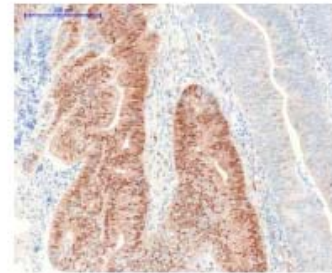
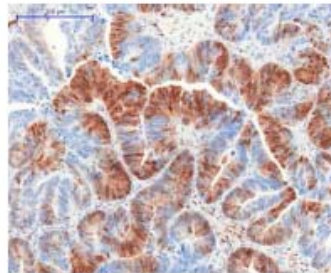
10 min



20 min



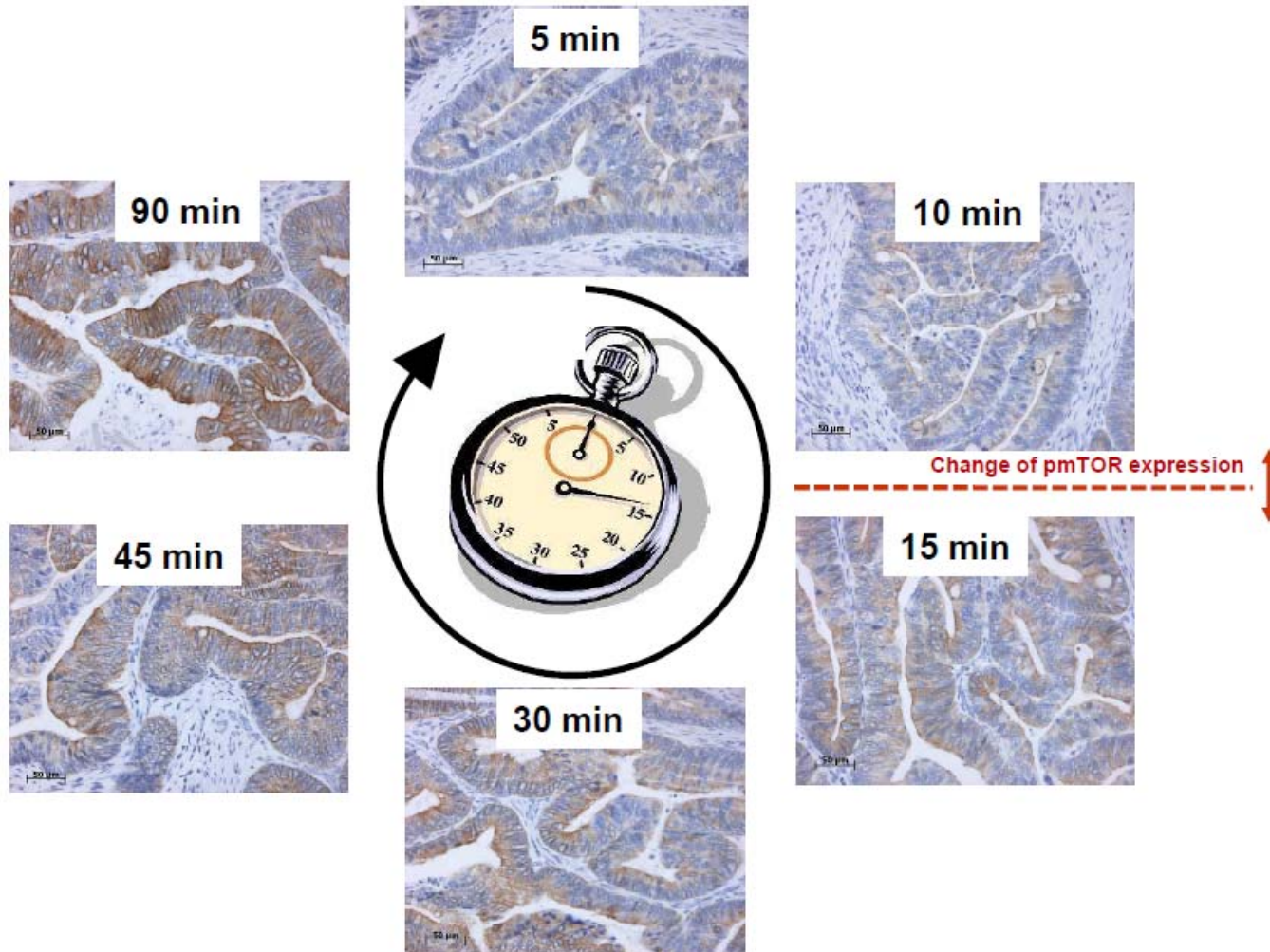
60 min



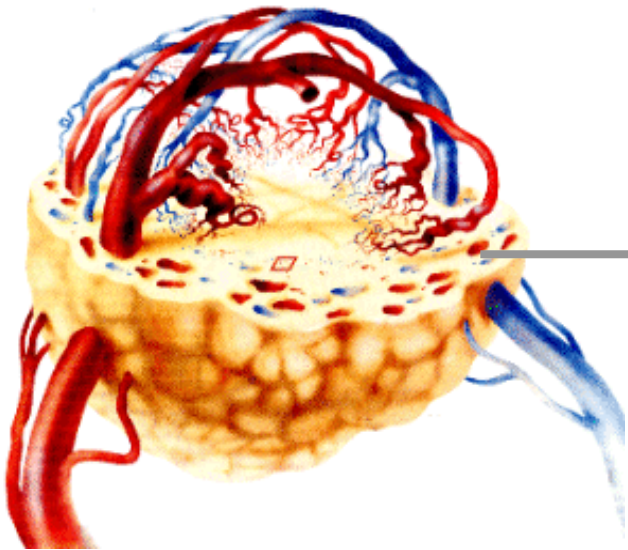
↑ Change of pMAPK expression
after 10-20 min cold ischemia



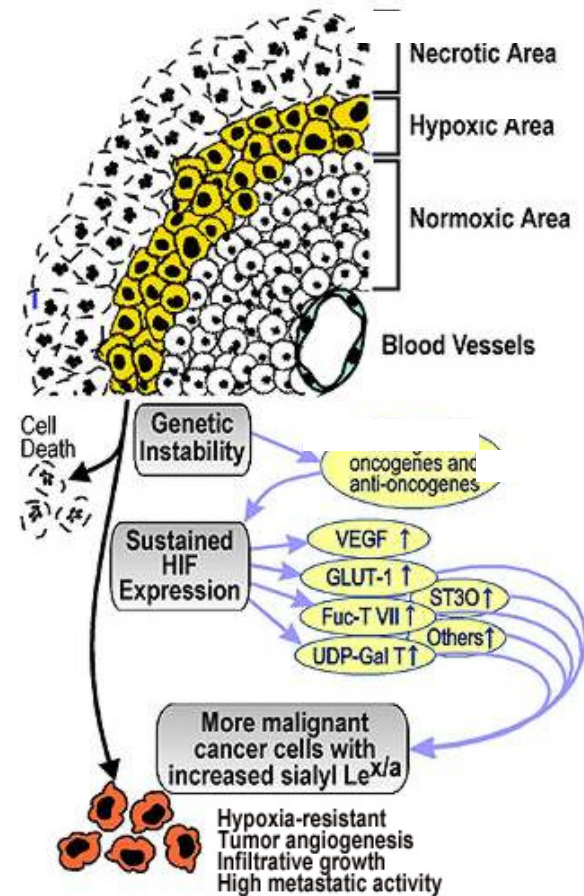
Phosphoprotein Expression: pmTOR-Immunostaining (Ventana)



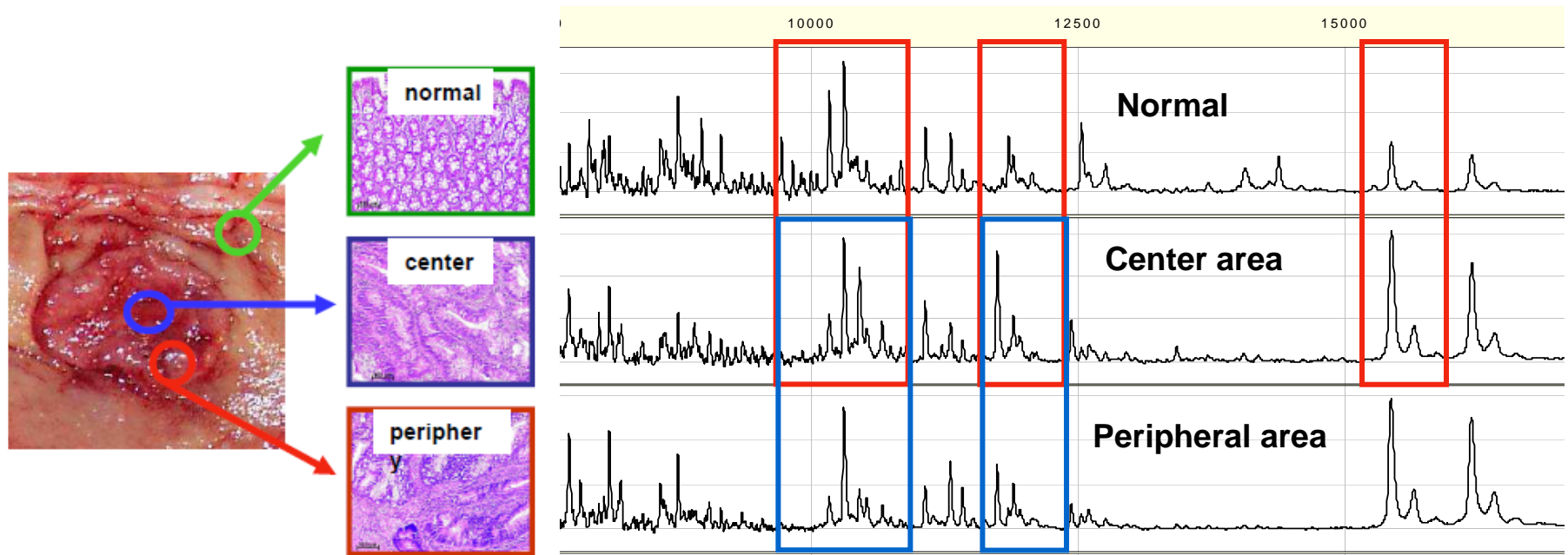
Tumor Tissue Varies in Center and Peripheral Areas



Invasive growth by induction of angiogenesis

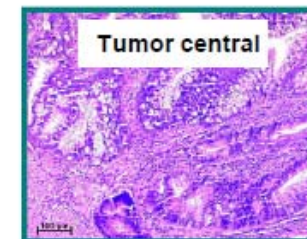
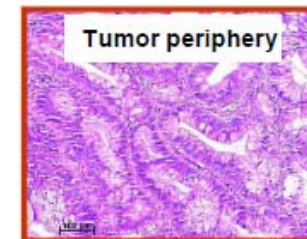
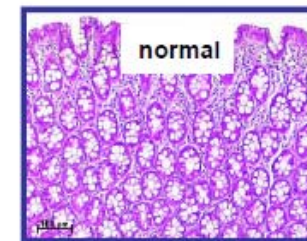
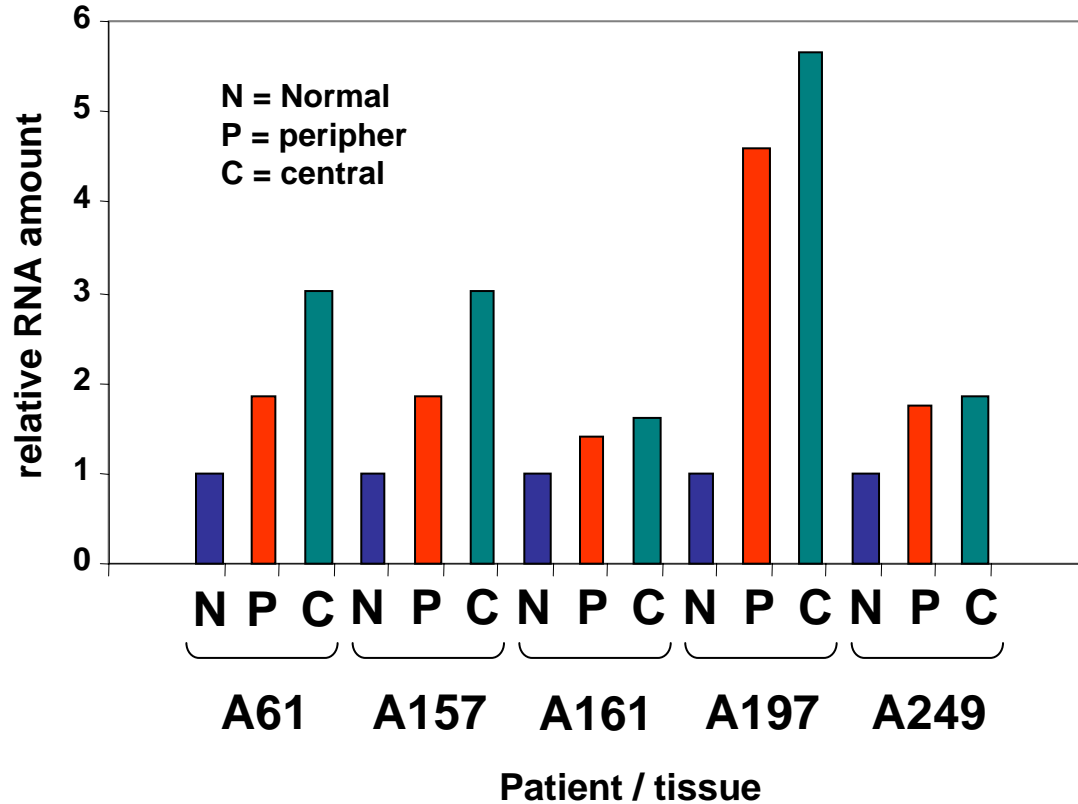


Localization of Tumor Biopsy Affects Molecular Pattern (Mass-Spectroscopy Analysis)



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)



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 (Indivumed's data base):

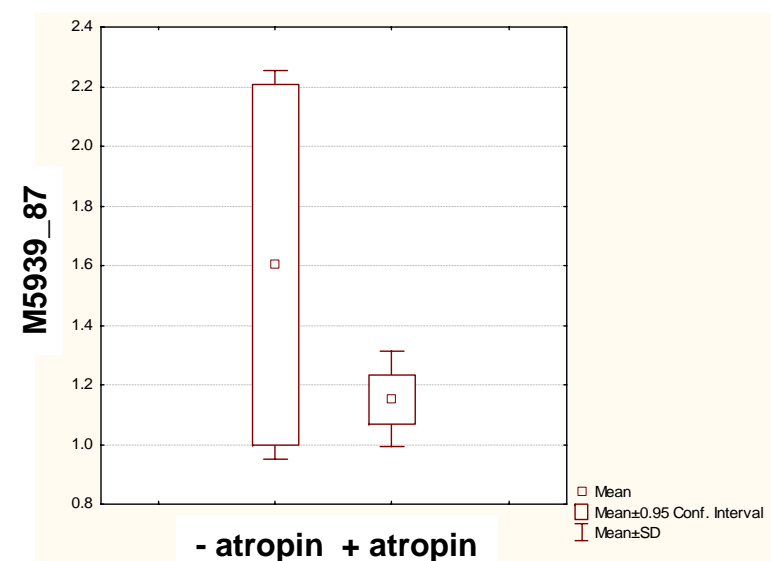
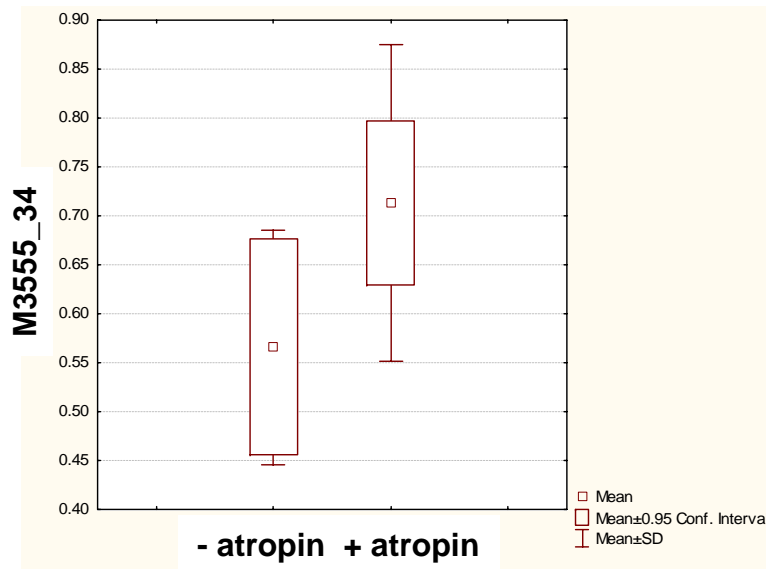
• Antibiotics:	13
• Bronchodilatator:	2
• Cardio-drugs:	17
• Diuretics & corticosteroids:	5
• GI-tract drugs & antihistaminics:	7
• Infusion & transfusion:	15
• Inhalative narcotics:	5
• Local anesthetics:	6
• Muscle relaxant:	8
• Analgetics & sedatives:	<u>34</u>
Total:	112

→ **Atropin**

Correlation of Colon Tissue Protein Expression with Intrasurgical Application of Atropin

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

T-tests; Grouping: Atropin (normal lig atropin.sta)									
Group 1: 0 + atropin									
Group 2: 1 - atropin									
Variable	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



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



(min)

Time (min) until freezing

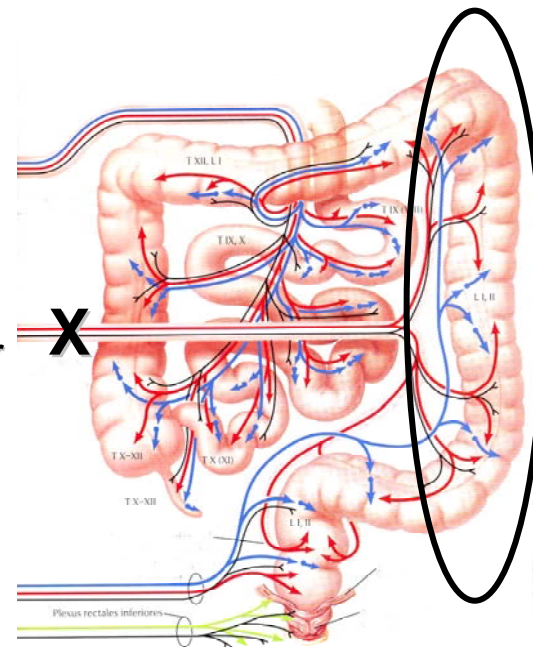
10 min

LCM isolation of tumor cells

Gene expression (Affymetrix)

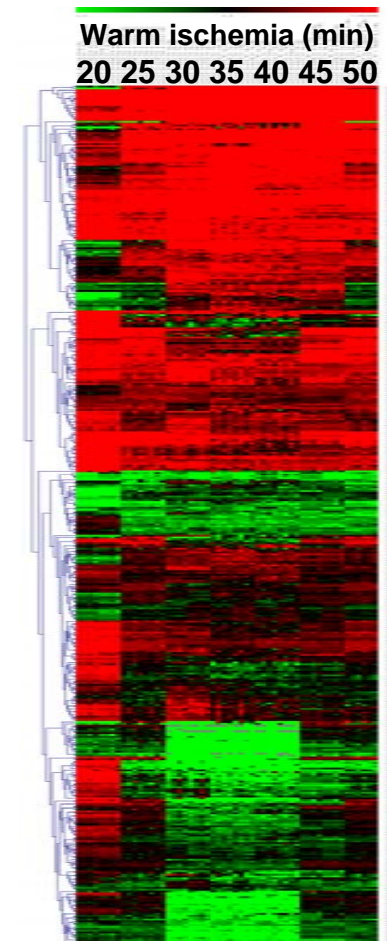
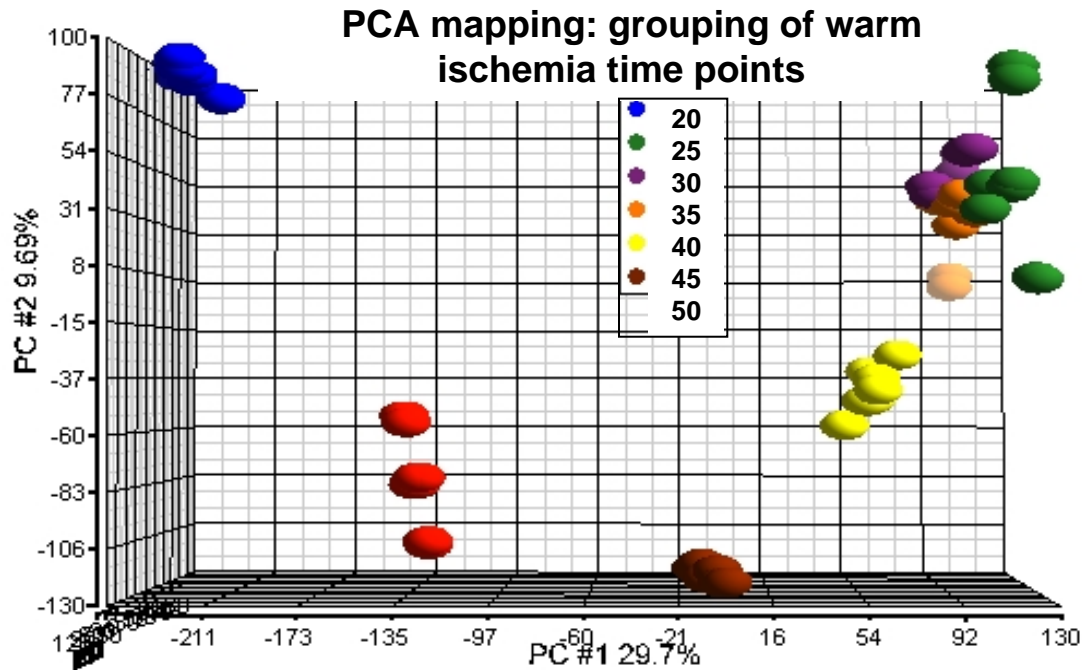
Bioinformatics

Mesenteric
artery inferior



Impact of Time between Ligation of Main Artery and Tumor Resection on Gene Expression in Colon Cancer

(NCI-Indivumed study)



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

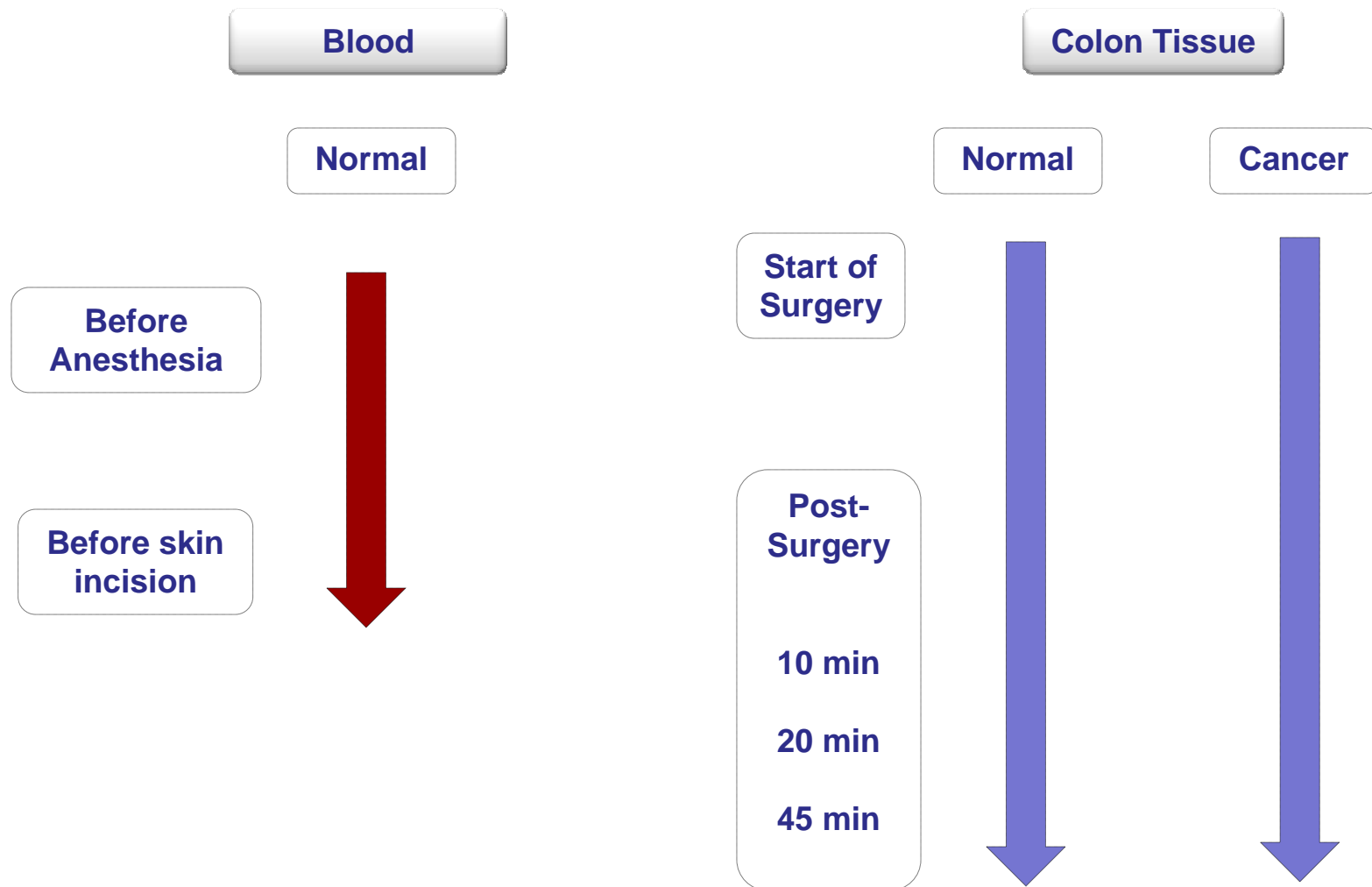


Department of Surgery, Israelitisches Krankenhaus (Dr. Zornig)

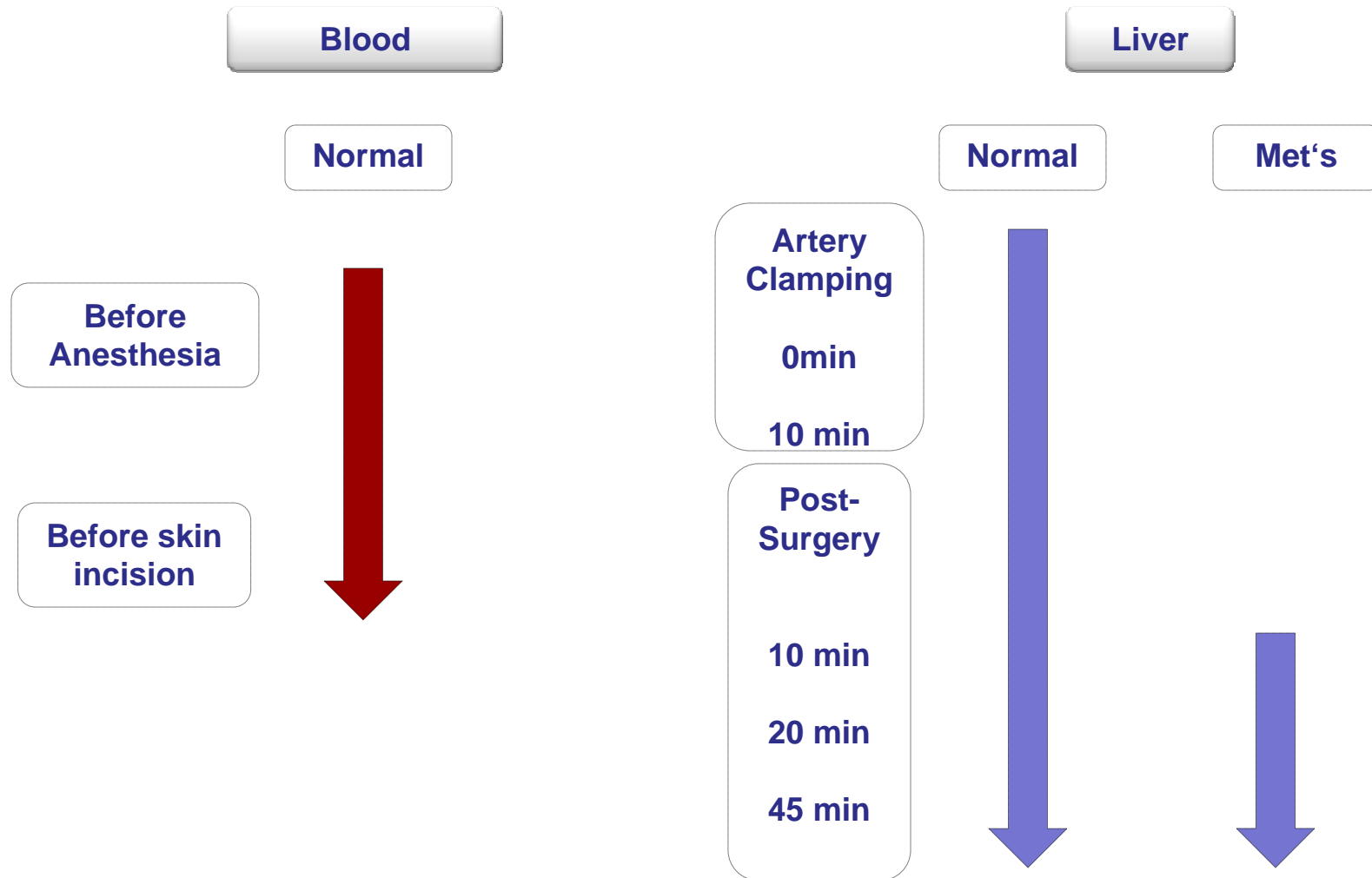
Department of Surgery, Diakonieklinikum Alten Eichen (Dr. Dörner)

**Department of Hepatobiliary Surgery, University Hospital Hamburg (PI: Dr.
Nashan)**

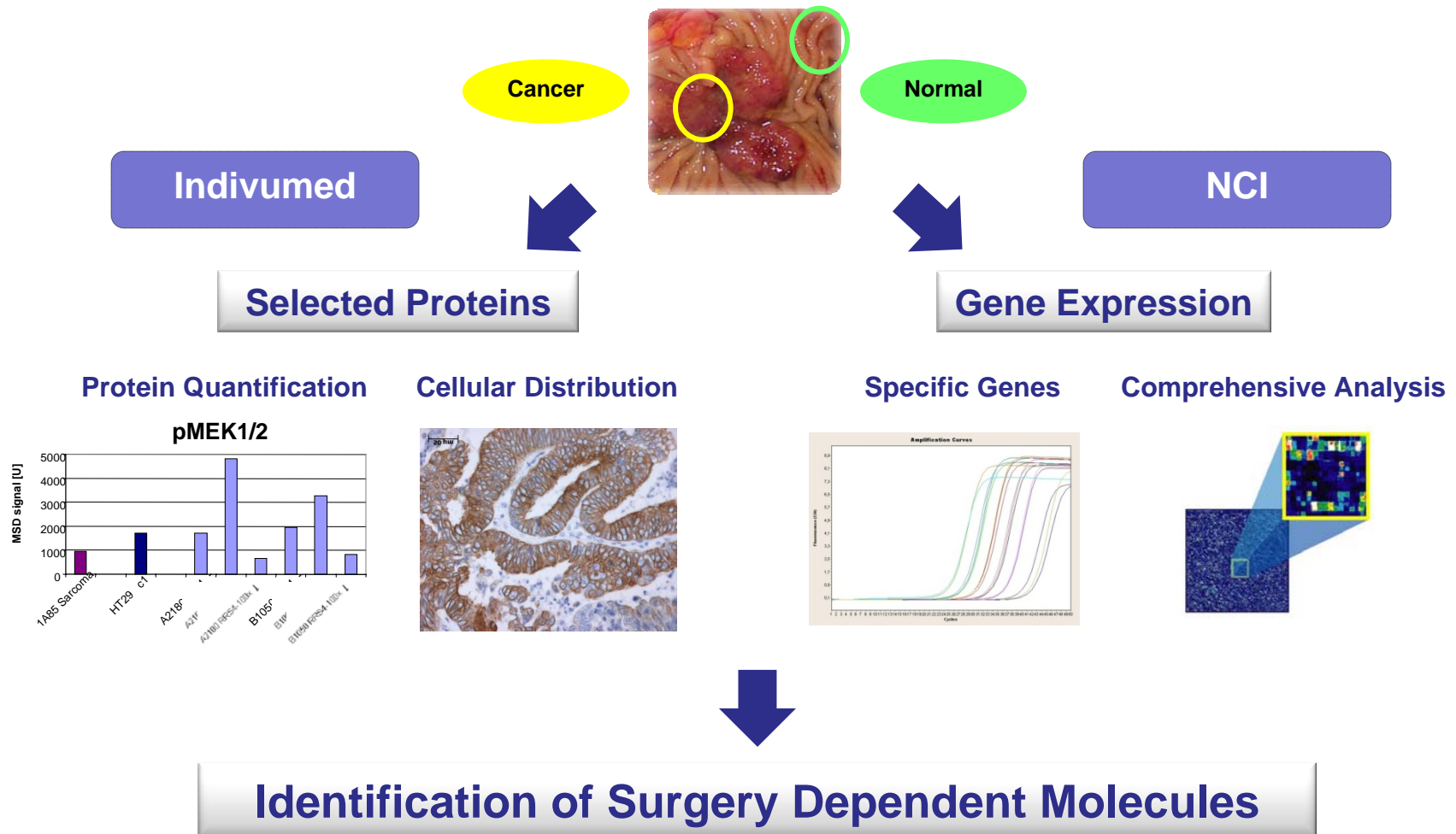
Impact of Anesthesia and Surgery on Gene and Protein Expression in Colon and Liver Tissue: Study Design



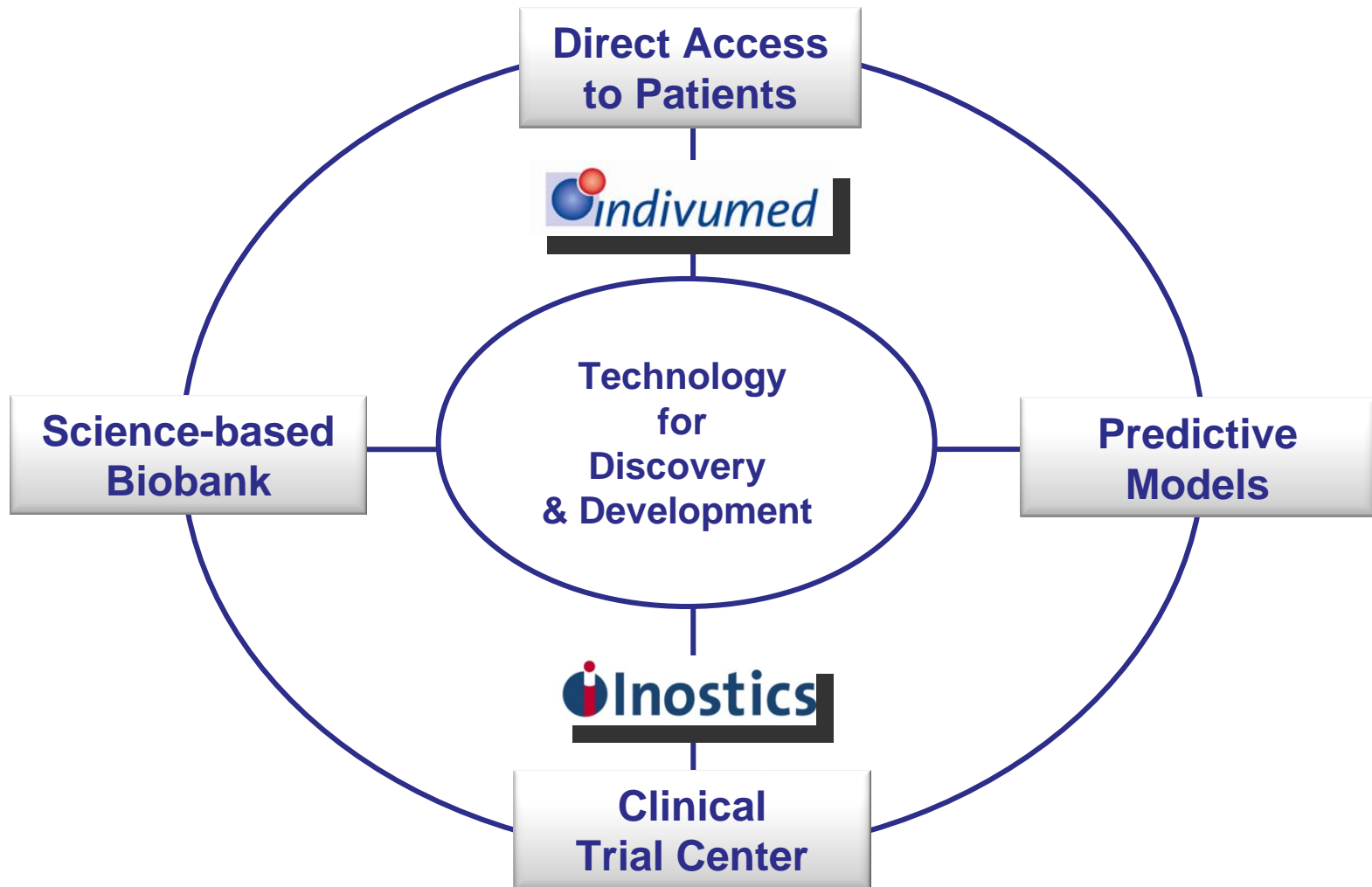
Impact of Anesthesia and Surgery on Gene and Protein Expression in Colon and Liver Tissue: Study Design



Impact of Anesthesia and Surgery on Gene and Protein Expression in Colon and Liver Tissue : Study Design



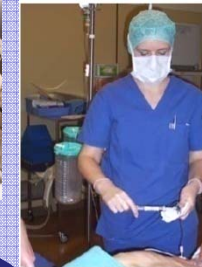
Basic Considerations for the Development of Individualized Therapies and Predictive Biomarkers



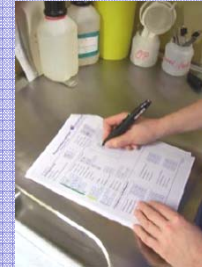
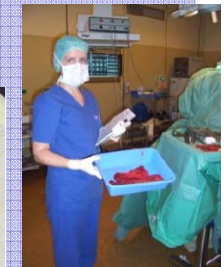
Full Integration of Indivumed Staff for Biobanking and Research: Collaboration with 8 Hospitals in Hamburg and 1 in Washington DC

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**

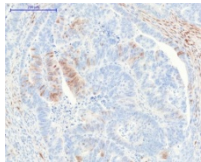


Indivumed Standard of Biobanking: > 11,000 cases in the biobank / +2,000/year

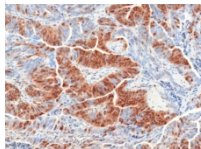
Tissue ischemia
and protein
phosphorylation



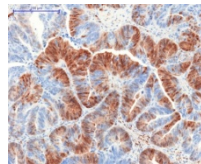
10 min



20 min

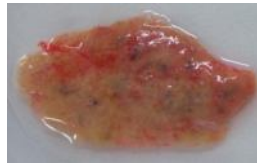


60 min

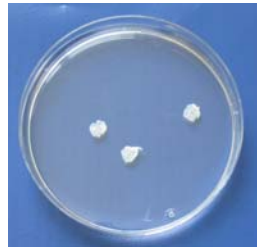


- ✓ Exact documented and very short tissue cold ischemia times of < 12 min (mean 7 min)
- ✓ Exact tissue localization and standardized fixation
- ✓ Complete biospecimen sets
- ✓ Highest tissue quality monitored by visual inspection, H&E staining and microscopic assessment
- ✓ Native and rapid fluid preparations
- ✓ Complete specimen data
- ✓ Complete clinical data
- ✓ Patients' confidentiality assured following international standards

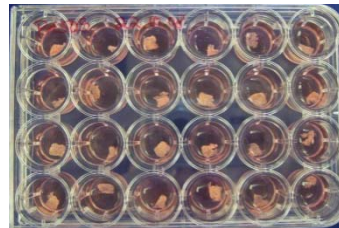
Drug Profiling Platform Drug Response and Predictive Biomarker Development



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



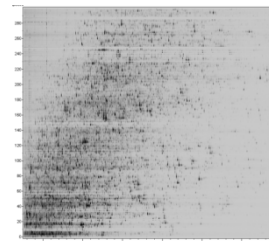
Preparation of tissue
slices
(400 μ m)



Cultivation in 24 well plates
and drug treatment

Drug Response tests:

- ATP assay
- Caspase 3/7 assay
- IHC staining
- Antibody based assays (MSD)



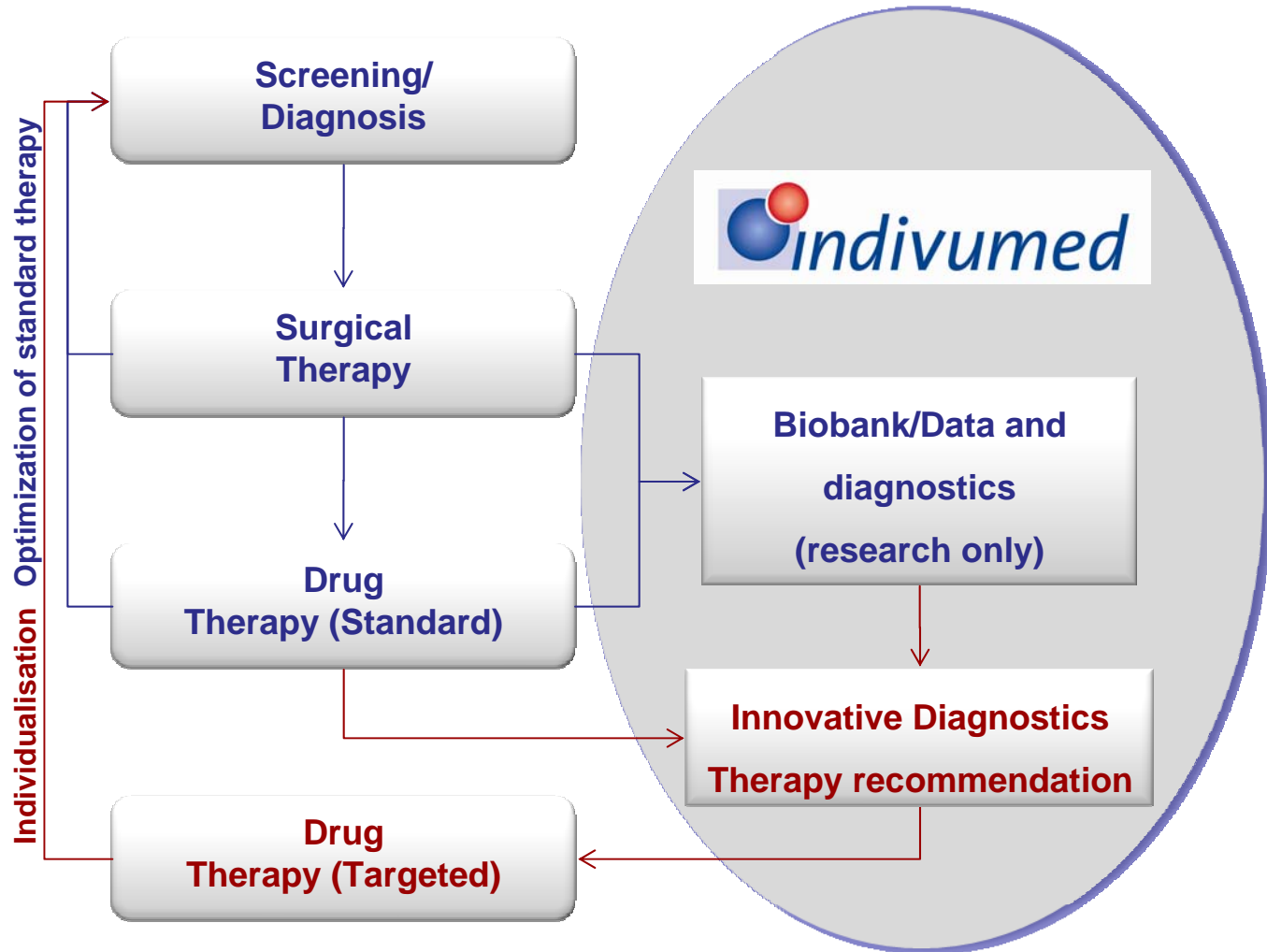
Proteomic profiling:

- Selected proteins
(e.g., phosphoproteins)
- Unselected proteins

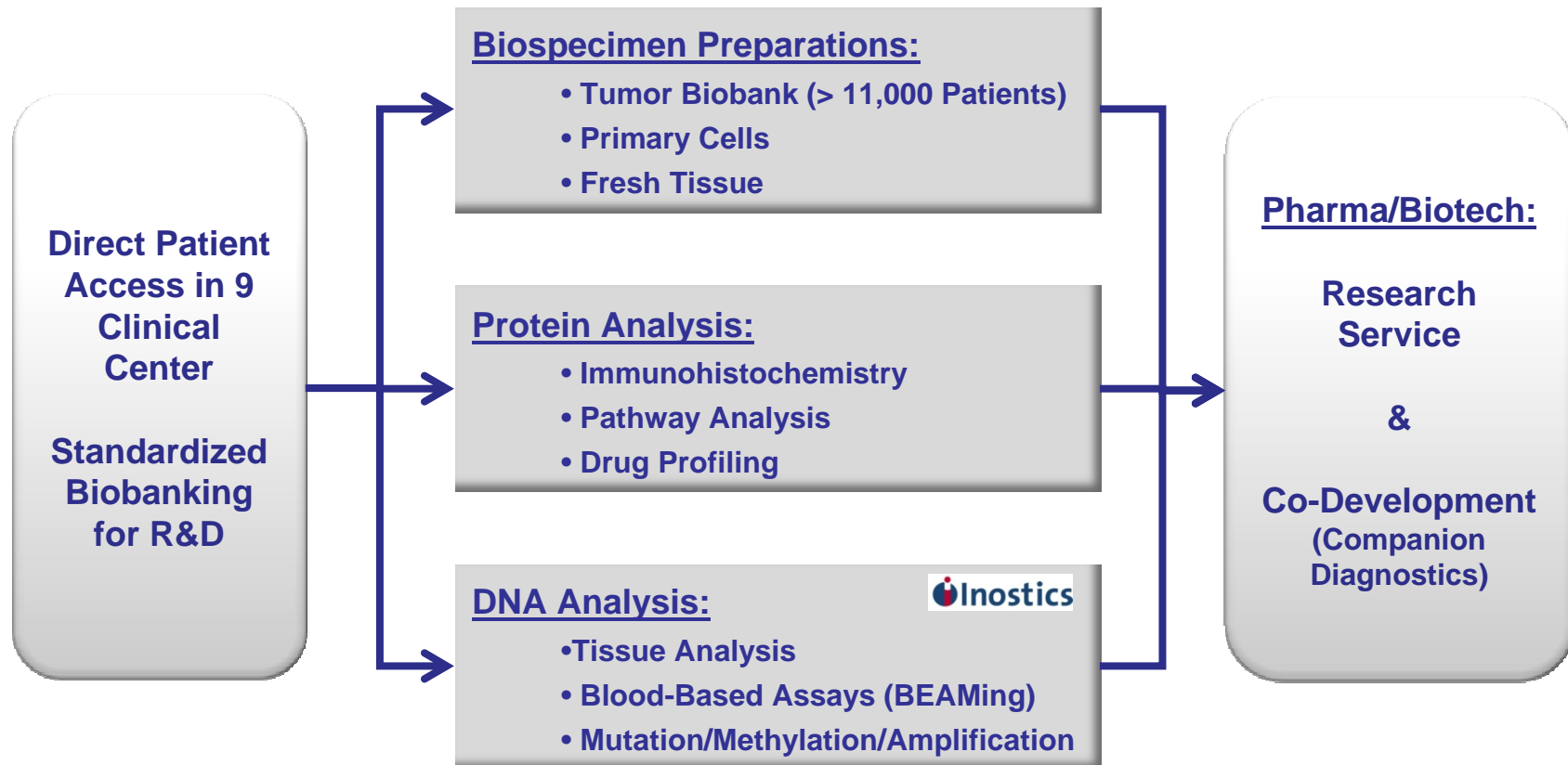
Identification of Predictive Biomarker within Clinical Trials Collaboration with the Otto J. Ruesch Center for the Cure of GI-tract Cancer



**Otto J. Ruesch Center
Lombardi Cancer Center, GUMC**



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



Consequences for Research and Patient Care

- **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**