Center for Biomarker Research in Medicine

COMET K1 Competence Center
About CBmed

- CBmed GmbH – is a research center established in 2014 that is focused on identifying and validating new biomarkers as well as conducting translational biomarker research.
- CBmed is owned by several Austrian universities and research institutes including medical universities of Graz & Vienna.
- CBmed runs international research projects in the areas of data & technology, cancer and metabolism & inflammation, funded by several national funding agencies.
Our vision is to become the world’s most recognized center for biomarker research in personalized medicine by 2030.

This will be achieved by integrating cutting-edge technologies with international and interdisciplinary expertise in the fields of cancer, metabolism and inflammation.

Together with our scientific and industry partners, we will develop solutions and products for patient care and cure.
Mission (1)

CBmed aims to establish an internationally competitive competence centre for systematic biomarker research

**MEDICAL MISSION**

- Easy to use, targeted biomarkers
- Minimally invasive biomarkers
- Better and earlier diagnosis
- Better therapies and treatment monitoring for personalized medicine
Mission (2)

CBmed aims to establish an internationally competitive competence centre for systematic biomarker research

**SCIENTIFIC MISSION**

- International excellence in biomarker research
- Link national and international biomarker research efforts
- Combination of analytical technologies and advanced bioinformatics
- Fast identification and validation of new biomarkers
Mission (3)

CBmed aims to establish an internationally competitive competence centre for systematic biomarker research

**ECONOMIC MISSION**

- **Hub providing relevant infrastructure for biomarker research**
- **Fast and effective translation of biomarkers into marketable products**
- **Attract and link international R&D companies and research institutions**
- **Create new business areas for Austrian SMEs and industry; initiate spin-offs and start-ups**
Public/Private Partnership

- Pharma
- Diagnostic
- Biotech

- Technical expertise
- Medical/scientific expertise

Hospitals

Biobanks

Industry

For profit

Non-profit

Expert Centre

Solutions

Project Requests

Biospecimens & Data

Only research collaborations can provide a sound basis for accessing human biological samples and associated medical data
CBmed’s USPs

Biobank & Cohorts

Core Labs with cutting edge technology platforms

International network of scientific and industry partners

Fusion Technology with an innovative multi-OMICS approach

Wide scope of research & development in three main areas, Data & Technologies, Cancer and Metabolism & Inflammation

Biomarker discovery over a full clinical life cycle

Close cooperation with large hospital organizations
Biomarker discovery over a full clinical life cycle
Biomarker discovery at CBmed

- Fusion Technology
- Core Labs
- 25 Biomarker R&D Projects
Biomarker discovery at CBmed

• CBmed identifies and validates biomarker at all levels of clinical research, including:
  ▪ Early diagnostics
  ▪ Prognostic biomarkers
  ▪ Predictive biomarkers
  ▪ Translation to market
International network of scientific and industry partners
Scientific Partners

Shareholders
Main scientific partners

Other scientific consortium members
CBmed Consortium Members

Selected Industry Partners

Over 50 international partners on 4 continents
Biobank & Patient Cohorts
BBMRI-ERIC Expert Center – Bridge between Biobanks and Pharma & Diagnostic Industry

- CBmed is the first BBMRI-ERIC approved Expert Center.
- BBMRI-ERIC is the EU infrastructure providing a network for biobanks and biomolecular resources across Europe
- For retrospective cohorts, CBmed works closely with the Biobank Graz, one of Europe’s largest biobanks with ~7.5 million biospecimens.
BBMRI-ERIC — European Infrastructure for Biobanks and Biomolecular Resources

• **Members**
  - Austria
  - Belgium
  - Czech Republic
  - Estonia
  - Finland
  - France
  - Germany
  - Greece
  - Italy
  - Latvia
  - Malta
  - The Netherlands
  - Norway
  - Sweden
  - United Kingdom

• **Observers**
  - Cyprus
  - Poland
  - Switzerland
  - Turkey
  - IARC/WHO

• **515 Biobanks, over 60 Million Biospecimens**
BBMRI-ERIC Expert Centre

- Expert centres are **key intermediaries between public and private sectors** performing the analysis of biological samples under internationally standardized conditions.

- Their **main goals** are to
  - Provide access to primary data that can easily be shared in contrast to biological samples and
  - Provide high-quality information from biological samples to industry for further product development.

Quoted from „BBMRI-ERIC-Associated Expert Centres / Trusted Partners V2.0“
Colon Cancer Cohort with Early and Late Relapse

- A project was identified for validation of immune infiltration as a biomarker for colon cancer relapse
- Inclusion and exclusion criteria were defined in an eCRF
- Feasibility request sent to the Biobank Graz for FFPE biospecimens from 100 stage II & III colon cancer patients
- In collaboration with the Oncology Department of the Medical University of Graz, the cohorts were further refined with data regarding clinical outcome
- An application for ethics approval was submitted to the Ethics Department of the medical university of Graz. The Ethics approval was successfully granted
- Tissue samples were pseudonymised and sent to CBmed for analysis
Clinical Researchers & Prospective Cohorts

Prospective cohorts have many benefits

- control sampling time points to observed patient response to therapy
- access to biospecimens not normally collected as routine
- control of sampling procedures
- collection of fresh cells and tissues required for analysis by e.g. flow cytometry, MALDI-MS imaging and development of PDX models

CBmed has built strong collaborations with clinical researchers from the Medical Universities of Graz & Vienna enabling the initiation of a wide range of prospective collections
Prospective Cohorts

• **CBmed Prospectively collects Biospecimens in the following indications**
  - Type 1 & 2 diabetes, inflammatory bowel disease, cardiovascular disease, liver disease (NAFLD, NASH, alcoholic liver disease, hepatitis C, cirrhosis), chronic kidney disease, obesity, metabolic bone disease, fertility disorders (polycystic ovarian), sepsis.
  - Healthy controls.

• **Diverse biospecimen types**
  - Blood (serum, plasma), tissue, circulating tumor cells, circulating immune cells, urine, stool, saliva, cultures.

• **Ethical Guidelines** - Collected following the ethical guidelines of the Biobank Graz, an international standard for Biobanking.

• **Clinical Data Management** - Clinical data collected in a clinical data management system in accordance with good clinical practice (GCP). CBmed has access to specimens with meticulous clinical characterization, follow-up data and biomarker results.
Close cooperation with large hospital organisations
Innovative use of Clinical Information for Biomarker Research

- Access to biospecimens alone is worthless without associated high quality, in-depth clinical data
- CBmed works together with KAGes, one of the largest hospital companies in Europe with a catchment area of 1.2 million people, with the aim of developing IT solutions for extracting the relevant clinical data from structured and unstructured sources with a view to selecting the appropriate patients for biomarker research

Our Strengths
- Expertise in
  - Data semantics
  - Biomedical Terminologies
  - Natural Language processing
  - Big data management (e.g. SAP HANA)
  - Predictive content analytics
Wide scope of R&D

Internal Biomarker Research Programmes
R&D Project Overview

**Core Labs**
- NGS
- Metabolomics
- Immunology
- Digital Pathology
- MALDI-MS
- Proteomics & In-vivo Imaging

**Technology Development** e.g. electrochemical chip-based diagnostics,

**Patient Cohorts**

**AREA 1**
- Data & Technology
- **Multi-omics**
- **Clinical Data Management**
- Software development – semantic searching of clinical data

**AREA 2**
- Cancer

**AREA 3**
- Metabolism & Inflammation

- **Early Diagnostics** including
- Tracking the Trace (CTCs in Urologic Cancer)
- Metabolomics (Idiopathic Pulmonary Arterial Hypertension)
- Sepsis & Fungal Infection

- **Prognostic & Predictive Markers** (Patient Stratification) including
- Minimal Residual Disease in AML
- Immune Cell Characterisation as a Biomarker in CRC
- Eukaryotic Initiation Factors in e.g. Glioblastoma
- Biosensors for short-lived biomarkers in diabetes
- Diabesity & Cardiovascular disease including microbiome analysis

- **Translation to Market**
- Health Technology Assessment
Internal R&D Projects

Area 1: Data & Technologies
- Clinical information system
- Semantic data management
- Knowledge discovery & data mining
- Next generation sequencing

Area 2: Cancer
- Tracking the trace
- Minimal residual disease and CAR T-cells

Area 3: Metabolism & Inflammation
- Diabesity
- Cardiovascular disease
- Biosensors
- Bone metabolism
- Fertility

• Metabolomics
• Immunology
• Health technology assessment
• Digital Pathology
• Clinical MALDI Applications

• Eukaryotic initiation factors
• ADX models
• Core Lab for Target Identification and Probe Development

• Liver function
• Microbiome-gut-brain
• Sepsis
• Fungal infections
• Electrochemical biomarker detection
Disease Area Stronghold
CANCER

OUR EXPERTISE
- Experts in Clinical Oncology and Pathology
- Deep clinical phenotyping through access to standardised clinical documentation
- Pre-existing network of clinicians and researchers (basic & translational)
- Experience at clinical research including clinical trials

OUR SOLUTIONS
- Isolation & analysis of circulating tumour cells (PARSORTIX & Mobile Diagnostic System – blood and other bodily fluids)
- Detection, analysis and validation of circulating tumour DNA
- Development of discriminative multimarker panels for serum proteins and immune cell gene expression signatures
- Highly sensitive method for detection of minimal residual disease in haematological malignancies
- Drug validation and development programmes (e.g. antibodies, viral vector-based and anti-eIFs)
- Access to tumour tissue (patient & autopsy derived) for generation of xenograft models (PDX & ADX)
Disease Area Stronghold
METABOLISM & INFLAMMATION

OUR EXPERTISE
- Experts in Clinical Endocrinology & Metabolic Diseases
- \textit{In-vitro} & \textit{In-vivo} analysis
- Experience of clinical study design, planning and execution

OUR SOLUTIONS
- 16S rRNA microbiome profiling and analysis stand alone or in combination with metabolomics (mass spectrometry).
- Gut permeability measurements (zonulin, calprotectin, lactulose/mannitol)
- Assessment of neurochemical biomarkers by cerebral open flow microperfusion
- Non-invasive PET imaging methods for angiogenesis and inflammation in liver diseases
- Rodent models for various diseases (diabetes, hepatocellular).
- Human glucose triple tracer techniques with tracers in accordance with GMP, GLP and GCP guidelines for meticulous characterization of glucose metabolism
- Glycerol tracer techniques for determination of lipid fluxes
- Lipidomics techniques for biomarker research in cardiovascular disease
Blood-Based Biomarkers Are Associated with Disease Recurrence and Survival in Gastrointestinal Stroma Tumor Patients after Surgical Resection.

Point of Care Testing for the Diagnosis of Fungal Infections: Are We There Yet?
Prattes J, Heldt S, Eigl S, Hoenigl M.

Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication.

Lactobacillus casei Shirota Supplementation Does Not Restore Gut Microbiota Composition and Gut Barrier in Metabolic Syndrome: A Randomized Pilot Study.

Effect of Lactobacillus casei Shirota supplementation on trimethylamine-N-oxide levels in patients with metabolic syndrome: An open-label, randomized study.
Core Labs
CBmed Core Labs

Core Lab
Complete NGS Workflow

Core Lab
Digital Pathology

Core Lab
MALDI

Core Lab
Immunology

Core Lab
Metabolomics

Core Lab
Proteomics & In-vivo Imaging

Assoc. Prof. Armin Gerger, MD
Area Leader Cancer Clinical Oncologist

Vienna

Graz

MEDIZINISCHE UNIVERSITÄT WIEN

CBmed
BIOMARKER RESEARCH

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The earliest available signs of any disease onset are alterations of the individual's metabolome, often resulting from compensatory mechanisms which start long before clinical symptoms manifest. Therefore, the study of metabolomics is core to CBmed.

**OUR SOLUTIONS**
- GLP certified Metabolomics laboratories
- Several targeted metabolomics platforms (glycolysis, TCA, pentose phosphate pathway, AcylCoAs, Polyamines) enabling absolute quantification
- Detection of novel unknown markers by untargeted MS

**OUR EXPERTISE**
- study design
- data generation
- analysis
- interpretation & knowledge generation

**EQUIPMENT PARK CBmed & Joanneum Research**
- Agilent 6495 UHPLC TrMS/MS  • LC Triple Quad MS/MS (Thermo TSQ)  • GC Triple Quad MS/MS  • GC/MS  • LC LTQ Orbitrap XL  • LC Exactive Plus  • UHPLC Q Exactive, Robotic Liquid Handling Workstation
Core Lab Application
EARLY DETECTION OF IPAH

Idiopathic Pulmonary Arterial Hypertension
severe disease
- overall mortality: 50% in 2.8 years, life expectancy ~4 years
- frequent psychiatric comorbidities panic disorder, depression, anxiety

therapy
- PH care is very expensive - up to €300.000 / patient / year
- but currently no curative therapy!

diagnosis
- challenging with high numbers of unreported cases and late detection
- gold standard is right heart catheterization

For more info regarding our methods see,
Fröhlich et al., 2016. Brain Behav. Immun. 56:140-55

Novel minimally invasive biomarkers detected in blood.
correlation of several fatty acids, sums and ratios were found to correlate well with mPAP

Patent application submitted
Based on cutting edge Flow Cytometry phenotyping capabilities with detection of up to 18 markers simultaneously.

CBmed’s key research interest in immunology revolves around the role of Regulatory T-cells in autoimmune disease (e.g. diabetes, irritable bowel disease, rheumatoid arthritis) and cancer (e.g. colorectal cancer).

**OUR EXPERTISE**

- High Quality Workflow (to Euroflow guidelines)
- Extensive experience of analysis from clinical trials
- Prospective collection of blood specimens from a variety of disease areas.
- Other methods include ELISA, ELISpot, Chemotaxis assay, co-culture assays, proliferation and cell stimulation tests & immortalized cultures.

**EQUIPMENT PARK**

- LSR Fortessa cell analyzer, BD
- FACS ARIA II, BD (cell sorting)
FACS quantification of T- and B-cell subtypes in different diseases

• More than 50 subtypes defined by surface and intracellular markers
• 5 different panels with 12 fluorochromes per panel

• Study cohort:
  ▪ Healthy participants
  ▪ Type 1 diabetes patients
  ▪ Rheumatoid arthritis patients
  ▪ Systemic lupus erythematoses patients
  ▪ Systemic sclerosis patients
  ▪ Colorectal cancer patients
  ▪ Patients who receive renal transplantation
CBmed’s Digital Pathology workflow enables the addressing of key issues in biomarker research including heterogeneity and spatial relationship of the immune system with tumour and tumour microenvironment.

- Vectra System (PerkinElmer) is a cutting edge digital pathology system allowing indepth cell phenotyping through spectral unmixing technology and quantitative spatial analysis through image analysis and bioinformatics software (InForm & Sportfire).

OUR METHODS
- Multiplex IHC
- Insitu RNA hybridization
- Histology from Cryo & FFPE tissue

OUR EXPERTISE
- Tumour Immunology
- Image Analysis
- Close collaboration with Pathology department of the Medical Uni. Graz

EQUIPMENT PARK
- Vectra System, PerkinElmer
- Ventana Classic, XT, Ultra
- Dako Labvision, Classic
- Further autostainers, Sanovo & Menarini
- Automated TMA robot
Investigation of distance measurements between specific immune cell subtypes and tumor cells as prognostic markers in colon cancer

Comparison of early relapse vs late relapse in stage II colon cancer.

Above: CD4, CD8, CD45R0, FoxP3, Cytokeratin, DAPI
Left: Spatial distance between Cytotoxic T cells and nearest tumour cell
Core Lab Genetics & Genomics

EXPERTISE AND TECHNOLOGY

- Fast, large-scale gene mutation analysis for molecular diagnostics in cancer
- Elucidation of biological pathways by gene expression profiling
- Microbiome diversity analysis in digestive system
- Identification of bacteria and fungi in infection and sepsis

OUR EXPERTISE

- Experience of clinical & routine genetic sequencing analysis
- Biospecimen Quality - Working to relevant Molecular Diagnostics Regulations e.g. ISO15189
- Involved in working groups of the EU regarding quality regulations for Companion Diagnostics
- Close collaboration with Pathology department of the Medical Univ. Graz

EQUIPMENT PARK CBmed & Medical Univ. Graz

- Qiagen GeneReader
- Thermo Fisher Ion Torrent PGM and Proton
- Illumina MiSeq
Core Lab Application
LIQUID BIOPSY FOR FcNA BY NGS

• Comparison of gene mutations between primary tumors, distant metastases and blood with the aim of validating biomarkers from liquid biopsies

• Cohorts from lung cancer, colon cancer, melanoma & steatohepatitis

• Investigation of the mutational spectra of primary tumor, fcNA and metastases including prospective follow up studies.

QIAGEN NGS complete workflow
MALDI MS is a sensitive and rapid technique for biomarker discovery in both tissues and liquids.

CBmed aims to develop a MALDI-based technology platform for high throughput analysis of clinical samples.

OUR EXPERTISE

- Biomarker discovery in tissue – tumor-infiltrating immune cells by MALDI MS imaging coupled with immunohistochemistry and micromanipulation.
- Biomarker discovery in liquids – protein and lipid biomarkers in blood from cardiovascular disease patients.
- Close cooperation with Medical University of Vienna.

EQUIPMENT PARK

- MALDI MS imaging
- LC-ESI
- MALDI-MS, Kratos
- Micromanipulator, TissueGnostics
Tumor cells release excessive amounts of exosomes, which are suggested to influence tumor growth, progression, metastasis and drug resistance.

Due to their easy accessability and representation of the parental (tumor) cells, exosomes are promising biomarkers for diagnosis and prognosis.

MALDI-MS for characterization of tumor exosomes

MALDI-TOF-MS screening was developed on exosomes isolated from colon cancer cell lines with different degrees of drug resistance.

Screening detects molecule types in the mass range of m/z 300-18000.

Exosome screening will be applied to colon cancer patients with the view of developing novel prognostic biomarkers.
A complete and integrated workflow from target identification to probe development and correlation of *in-vivo* location with histopathological analysis. Tumor biomarkers are investigated by whole proteome platforms for development of Imaging probes that visualize the individual tumor situation.

**Our expertise:**

- **Biomarker discovery** through IHC-guided, mass spectrometry-based proteomics on huge archives of FFPE tissue
- **Studies** - strong research background in prostate and colorectal cancer
- **Application** - open for any input of novel target testing or stratification of known targets by above listed methods
- **Incorporation** of tissue samples / biomarker discovery from other CBmed core labs
- Close cooperation with Medical University of Vienna

**TECHNOLOGIES CBmed & Medical University of Vienna**

- TissueFAXSSORT guided micro-digestion
- Mass Spectrometry based Proteomics
- *In-vivo* Probe Development
- *In-vivo* Imaging by PET or SPECT
Biomarker discovery via mass spectrometry-based proteomics in FFPE tissue

Use of novel biomarkers for in-vivo probe design

Imaging of new biomarkers in-vivo and comparison with ex-vivo histology.
Fusion Technology
Fusion Technology

Proteomics

NGS

Digital Pathology

Flow Cytometry

Metabolomics

MALDI-MS

Integrated Analysis

disease without relapse
disease with relapse

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Proper use of biomarkers in clinical research increases the likelihood of approval by three times [1].

Fuse CBmed Core Lab Technologies to increase the likelihood of identifying relevant prognostic/predictive biomarkers in specific clinical settings
Clinical need: Colon cancer (CC) is the 3rd most common cancer worldwide. In stage II CC surgical resection is the treatment of choice. The clinical benefit of postoperative chemotherapy is unproven for stage II patients. The existence of prognostic and predictive biomarkers would provide a strategy for stratifying patients for risk of tumor relapse, which in turn would allow treatment options to be tailored to the individual.

Aim: Identifying a biomarker panel using a multi-OMICS approach to predict tumor recurrence in stage II colon cancer

Results are expected in mid 2017
Fusion Technology - Advantages

- Integrated approach to biomarker discovery by
  - Using modern CBmed Core Lab technologies
  - Combining the complimentary advantages of both targeted and untargeted methods for biomarker discovery
  - Integration of heterogeneous data through data management system and biostatistics
  - Standardising the workflow from feasibility check to cohort assembly, Core Lab analysis and integrated data interpretation
- Fast identification of biomarkers through parallel analysis
- Novel insights into disease mechanisms through detection of biomarker patterns on a multibiomics level
- Multiple site – single contact point – complete solution
- Applicable and easily transferable for different research questions (various diseases, identification of prognostic, predictive and drugable biomarkers)
- Network of Medical Universities and European biobanks
Organization CBmed GmbH
Valid as of October 04th, 2016

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AREA 2 Cancer
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