Metabolic biomarkers enable enrichment strategies and accelerate pharmaceutical research studies
Case study: Lung cancer
Biomarkers and companion diagnostics expand drug potential

**CHALLENGES & MOTIVATION**

- **Prediction which patients are likely to respond** to a certain cancer treatment
  - Improves the **success rate** of clinical drug development
  - Saves patients from **unnecessary toxicity & enhances chance** of receiving a drug that helps
  - Reduces medical **costs**

- **Increasing demand to establish biomarkers** as companion diagnostics

- **Need of non-invasive blood-based** biomarkers:
  - Biopsies of tumors are not always accessible
  - Intratumoral heterogeneity of tumor tissues
  - Different phenotypes of primary tumor and metastasis

(Source: http://pharmaceuticalcommerce.com/lib/sitefiles/images/Biomarker_Fig4.jpg)
Executive summary (I)

**METABOLOMICS OF PLASMA SAMPLES FROM CANCER PATIENTS ENABLES**

- **Blood-based metabolic biomarker are invaluable for therapy:**
  - When biopsies of tumors are not accessible
  - To monitor the efficacy of treatment in real-time and potentially improve the choice of treatment options

- **Accelerated clinical studies** through enriched design based on metabolite profile signatures leads to **shorter time to market**

- **Higher chances to outperform standard** of care in a metabolically stratified cohort

- **Reduced attrition rate** by subgroup analysis based on metabolic phenotypes

- **Savings in time and costs** by streamlined study design and monitoring of clinical endpoints with surrogate metabolic biomarkers, which predicts clinical endpoints earlier than imaging-based technologies

- **Metanomics Health’s biomarkers MxP® LCA Progress and MxP® LCA Monitor** allow early decision making based on one plasma sample each:
  - **MxP® LCA Progress**: t0 (untreated patient) → for the prognosis of treatment response in cancer patients
  - **MxP® LCA Monitor**: t1 (after first treatment cycle) → interim monitoring of treatment response in patients
Executive summary (II)

SUMMARY – Results of case study

- Stage IV NSCLC patients receiving 1st line palliative chemotherapy
  - Comparison of 20 Non-Responders (identified at t2, t4, or after t5) vs. 49 Responders
  - Biomarkers for (i) prognosis of treatment response and (ii) interim treatment monitoring were identified

**Prognosis at t0 (untreated):**

MxP® LCA Progress - Biomarker panel:
AUC of 0.96; 92% specificity* and 95% sensitivity**

**Treatment monitoring at t1 (after 1st cycle of chemotherapy):**

MxP® LCA Monitor - Biomarker panel:
AUC of 0.95; 92% specificity* and 85% sensitivity**

*true negative = Responder  
**true positive = Non-Responder

Glossary - Biomarker definitions

- **Prognostic markers** define the outcome irrespective of treatment given
- **Treatment monitoring markers** indicate the response to given treatment (during and after therapy)
- **Predictive markers** indicate whether a particular treatment is likely to provide clinical benefit
Metabolomics provides advantages over other -omics technologies

KEYNOTES

- Enables personalized medicine
- Higher responsiveness to pathological processes
- Translation of big data into actionable biomedical knowledge
- Closest to human phenotype
- Changing through the course of treatment
- Well-accepted in scientific community
- Chance to uniquely advance discovery-driven science by metabolomics
- New highly valuable diagnostic challenges can be solved
Validated first-class clinical biomarkers by focus on high medical need and a state-of-the-art development processes

HISTORY – Metanomics Health and metanomics are pioneers of metabolomics

- **1998**: Foundation of metanomics GmbH (“MTX”) as a subsidiary of BASF, conducting metabolite profiling for gene function analysis in plants
- **2003**: Foundation of metanomics GmbH (“MTX”) as a subsidiary of BASF, conducting metabolite profiling for gene function analysis in plants
- **2008**: Launch of clinical biomarker program
- **2010**: First patent filings for the now most advanced biomarkers, HFrEF\(^1\) and PDAC\(^2\)
- **2012**: Validation of early diabetes marker
- **2013**: Validation of HFrEF\(^1\) biomarker and Quality marker
- **2014**: Validation of PDAC\(^2\) biomarker
- **2014**: Product launch of MxP\(^\text{®}\) quality control plasma
- **Today**: MxP\(^\text{®}\) LCA Progress and MxP\(^\text{®}\) LCA Monitor allows early decision making based on one plasma sample each

1) Heart failure with reduced ejection fraction 2) Pancreatic ductal adenocarcinoma
Our well-balanced product pipeline comprises two close-to-market biomarkers as well as several promising candidates in earlier stages.

WELL-BALANCED PRODUCT PIPELINE - Biomarker development phases

<table>
<thead>
<tr>
<th>PILOT</th>
<th>IDENTIFICATION</th>
<th>VALIDATION</th>
<th>PROTOTYPE ASSAY DEVELOPMENT</th>
<th>Regulatory submission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to market</td>
<td>&gt; 6 years</td>
<td>&lt; 6 years</td>
<td>&lt; 4 years</td>
<td>&lt; 2 years</td>
</tr>
</tbody>
</table>

Killer classes
- Top mortality-causing diseases
- Top causes for hospitalization

Frequency
- Top CPT codes
- Most common diseases

Cost
- Top burden to healthcare systems
- Top rising CPT codes

Additional potential

Lung cancer marker

NAFLD marker

HFrEF marker

PDAC marker

Commercialization

1) CPT (Current procedural terminology) codes are a standardized set of codes describing medical, surgical and diagnostic services
2) Non-alcoholic fatty liver disease
3) Heart failure
4) Pancreatic ductal adenocarcinoma
5) Heart failure with reduced ejection fraction
Lung cancer is a major public health concern worldwide

**KEY FACTS**

- Lung cancer (LCA) is the **2nd most common neoplasm** worldwide in both men and women; estimated 222,500* new cases in the US for 2017
- The **leading cause of cancer death**; expected 155,870* deaths for 2017 in the US
- Most common cause is **tobacco smoke**, followed by other **carcinogens** (at work place or in environment) and **genetic factors**

Estimated cancer deaths in the US in 2016*

<table>
<thead>
<tr>
<th></th>
<th>Males 314,280</th>
<th>Females 281,400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>27%</td>
<td>26%</td>
</tr>
<tr>
<td>Prostate</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Brain &amp; other nervous system</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>All other sites</td>
<td>24%</td>
<td></td>
</tr>
</tbody>
</table>

- There are **three main types of lung cancer*** affecting treatment options and prognosis:
  - **Non-small cell lung cancers (NSCLC)**: including subtypes of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma; adenosquamous carcinoma and sarcomatoid carcinoma are much less common
  - **Small cell lung cancers (SCLC)**
  - **Lung carcinoid tumors**

NSCLC treatment & management

SURVIVAL

- **Poor outcome** is due to the difficulty of early diagnosis (~ ⅔ of cases are diagnosed at advanced stages)
- **Low 5-year survival** rates for NSCLC at late stages*

```
<table>
<thead>
<tr>
<th>Tumor stage</th>
<th>5-year survival rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>49</td>
</tr>
<tr>
<td>II</td>
<td>45</td>
</tr>
<tr>
<td>III</td>
<td>30</td>
</tr>
<tr>
<td>IV</td>
<td>31</td>
</tr>
<tr>
<td>IIIA</td>
<td>14</td>
</tr>
<tr>
<td>IIIB</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
</tr>
</tbody>
</table>
```

TREATMENT MONITORING

- Therapeutic options for late stages are restricted to chemotherapy, molecular targeted therapy, immunotherapy, radiation, (sometimes surgery)
- Tools for early estimation of therapeutic efficacy are required to save time and cost by allowing to modify the treatment strategy and avoid unnecessary side effects
- Monitoring during therapy involves imaging procedures (e.g. CT or PET scans). **Disadvantages:** low sensitivity, harmful exposure to radiation, sometimes inconclusive outcome, expensive, time-consuming evaluation

```
(Source: https://radiology.duke.edu/patient-care/specialized-services/lung-cancer-screening/)
```

FUTURE REQUIREMENTS

- **Growing need to develop non-invasive methods** to monitor the effect of treatment and predict a response to therapy

Rationale for tumor-derived markers in guidance of cancer

KEYNOTES

➢ Tumor-associated markers
  ➢ Are synthesized and/or released by cancer cells or other body cells in response to cancer
  ➢ Consider the heterogeneity and activity of the tumor mass
  ➢ Measured at t₀ (untreated patient) might be suitable for screening, diagnosis, staging, prognosis, therapy choice
  ➢ Their kinetics also reflect the effects of cytotoxic therapy
  ➢ Periodic measurement during therapy might be suitable for indication of treatment response or relapse/disease progression
  ➢ Offer rapid, sensitive, and cost-effective methods
  ➢ Detectable in tissue and non-invasive body fluids
Omics-based biomarkers in guidance of lung cancer treatment

TUMOR-DERIVED BIOMARKERS - Overview

- **Protein-based tumor markers**\(^1,^2,^3\) in the tumor tissue and body fluids are currently **not used** in clinical routine of LCA management

- **Metabolite-based tumor markers**: currently **not used** in clinical routine
  
  - Reprogramming of cellular metabolism is a hallmark of cancer; uncontrolled cell proliferation requires **energy and biomass** components in the form of metabolites. Metabolomics provides direct **pathophysiological insights** into metabolism of cancer cells plus surroundings

- **Value cases**: (i) **Carcinoids** are in routine clinical chemistry detected by **serotoninine increase** in plasma. (ii) **Pemetrexed** provides benefit in nonsquamous but has no activity in squamous NSCLC\(^4\); efficacy might be related to higher thymidylate synthase levels in squamous cancer, a rate-limiting enzyme in folate metabolism and one target of the drug\(^5\)

- Testing for **genetic mutations** and tailoring therapy accordingly is **widely accepted as standard practice** in LCA\(^7\):
  
  - **EGFR**: mutations in EGFR tyrosine kinase (TK) domain: exon 19 deletion, exon 21 L858R, exon 18 G719 \(\rightarrow\) susceptible to EGFR TK inhibitors; exon 20 T790M mutation \(\rightarrow\) resistance to TK inhibitors
  
  - **KRAS** mutations: poor prognosis

  - **ALK** mutations/rearrangements: mutually exclusive to EGFR mutations \(\rightarrow\) no benefit from EGFR TK inhibitors; ALK inhibitors: crizotinib and ceritinib

  - **ROS-1** gene alterations: susceptible to crizotinib

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\(^1\)Niklinski J and Furman N, 1995; \(^2\)Holdenrieder S et al., 2016; \(^3\)Molina R et al., 2016; \(^4\)Standfield L et al., 2011; \(^5\)Takezawa K et al., 2011; \(^6\)Mathe EA et al., 2014 and WO 2015/006657 A2; \(^7\)http://emedicine.medscape.com/article/1689988-overview
Prospective identification study to develop new biomarker candidates for prognosis and treatment monitoring of NSCLC

**NSCLC BIOMARKER DEVELOPMENT - Longitudinal study design based on clinical routine**

- **Stage IV NSCLC patients** (n=75)
- Receiving **1st line palliative treatment** (platinum-based combination chemotherapies incl. gemcitabine, vinorelbine or pemetrexed)
- Overnight **fasted plasma samples: before (t0) and during treatment (t1–t5)**
- **Treatment response (by RECIST guidelines)** was monitored slightly before or after completion of the 2nd, 4th, and 5th treatment cycle
  - **Non-Responder n=20** (n=8 at t2, n=7 at t4, n=5 after t5)
  - **Responder n=49** (incl. stable and partial remission)
  - **“unclear” patients (n=6)** with inconclusive RECIST
- The **prospective study** was performed together with collaborations partner from HELIOS clinics in Berlin (Germany)
- (Metabolite profiles of **19 SCLC patients** treated with etoposide plus either cisplatin or carboplatin were also acquired but based on RECIST only one Non-Responder was identified; of note: **metabolic subtypes** based on t0 or t1 SCLC-samples were identified → see appendix for details

**RECIST**: Response Evaluation Criteria in Solid Tumors
A prospective NSCLC patient cohort was analysed in collaboration with HELIOS clinics (Berlin, Germany).

**CHARACTERISTICS OF INVESTIGATED NSCLC PATIENT COHORT (n=75)**

<table>
<thead>
<tr>
<th>NSCLC subtypes</th>
<th>Count</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>51</td>
<td>Male</td>
</tr>
<tr>
<td>Non-squamous</td>
<td>18</td>
<td>Male</td>
</tr>
<tr>
<td>Squamous</td>
<td>5</td>
<td>Male</td>
</tr>
<tr>
<td>N.A.</td>
<td>3</td>
<td>Male</td>
</tr>
</tbody>
</table>

- 2-Fold increase in male patients reflects the higher LCA-incidence rate for males.
- Adenocarcinoma: most common NSCLC subtype.

**Treatment at cycle 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboplatin + Gemcitabine</td>
<td>16</td>
</tr>
<tr>
<td>Carboplatin + Pemetrexed</td>
<td>2</td>
</tr>
<tr>
<td>Carboplatin + Vinorelbine</td>
<td>15</td>
</tr>
<tr>
<td>Carboplatin + Gemcitabine</td>
<td>2</td>
</tr>
<tr>
<td>Carboplatin + Pemetrexed</td>
<td>8</td>
</tr>
<tr>
<td>Carboplatin + Vinorelbine</td>
<td>11</td>
</tr>
<tr>
<td>Carboplatin + Pemetrexed</td>
<td>3</td>
</tr>
</tbody>
</table>

- Non-Responder
- Responder
- Unclear
Certain metabolite classes are relevant for prognosis of disease progression

PROGNOSIS OF CHEMOTHERAPY RESISTANCE IN NSCLC – Focus on t0 samples

- Increases in subclasses of certain complex lipids, hormones (e.g. eicosanoids), and one tumor marker protein
- Another subclass of lipids is decreased
Prognostic metabolic biomarker MxP® LCA Progress shows superior performance compared to blood-based tumor protein markers

PROGNOSIS OF CHEMOTHERAPY RESISTANCE IN NSCLC – Focus on t0 samples

Prognosis at t0 (untreated):
MxP® LCA Progress biomarker:
- AUC of 0.96
- 92% specificity* & 95% sensitivity**
- Includes 11 metabolites & one tumor protein
- Outperforms all tested tumor protein markers

AUC: area under curve; *true negative = Responder; **true positive = Non-Responder
Metabolic biomarker MxP® LCA Monitor determines disease progression after one chemotherapy cycle

**DETERMINATION OF EARLY ONSET OF DISEASE PROGRESSION IN NSCLC – Focus on t1 samples**

- Responders show decreases of a certain subclass of complex lipids, hormones, and one tumor protein while other metabolites are significantly increased.
- MxP® LCA Monitor encompasses one protein marker plus 10 metabolites and discriminates between both groups with an AUC of 0.95 (92% specificity* & 85% sensitivity**).
- MxP® LCA Monitor outperforms all tested tumor protein markers.

AUC: area under curve; *true negative = Responder; **true positive = Non-Responder
Metabolomics – the emerging tool to investigate alterations in different aspects of lipid metabolism

FATTY ACID AND LIPID SYNTHESIS – A metabolic liability of lung cancer

- Own metabolomics data indicate altered lipid metabolism as relevant feature for LCA treatment prognosis and monitoring
- Fatty acid and lipid metabolism participates in the regulation of cellular processes (e.g. cell growth, proliferation, differentiation, apoptosis, autophagy, inflammation, membrane homeostasis, chemotherapy response, and drug resistance) and can be stimulated by the over-expression of essential enzymes (e.g. fatty acid synthase (FASN) or acetyl-CoA carboxylase (ACC) are activated in NSCLC; ATP citrate lyase (ACLY) in lung cancer)
- Fatty acid and lipid metabolism generates bioactive metabolites (e.g. eicosanoids, diacylglycerol, ceramides, sphingosines, phosphatidylinositol-3-phosphate, and cholesterol) activating different pathways such as G-protein coupled receptors, tyrosine kinases, integrin or ion channel signaling → mechanistic link between metabolite and protein networks
- The antitumor effects of the chemotherapy might occur by:
  - Direct inhibition of fatty acid and lipid synthesis in tumors
  - Inhibition of fatty acid and lipid synthesis in the tumor microenvironment and other tissues such as liver (= major contributor to levels of plasma fatty acid and lipids) → reduced overall ability of circulatory fatty acids and lipids to rescue treatment effects
- By comprehending the phenomenon of lipid/fatty acid alteration, one can obtain vital information about the pathogenesis of lung cancer and develop new biomarkers

(Figure: Currie E et al., 2013)

1Standfield L et al., 2011; 2Portilla D et al., 2006; 3Huang C et al., 2015; 4Svensson RU et al., 2016; 5Currie E et al., 2013
Metabolomics – the emerging tool for predicting response to cytotoxic drug treatments

FATTY ACID OXIDATION – The role of carnitines

- Present study demonstrates a significant increase of a specific carnitine in Non-Responders at t0 (untreated):
  - Carnitines are involved in fatty acid oxidation (→ source of NADH, FADH₂, and ATP)
  - Low levels of carnitines can induce apoptosis³, increase in levels of reactive oxygen species (→ oxidative stress), stimulate pro-inflammatory effects (e.g. via TNF-α)²,³
  - Fatty acid oxidation and carnitine palmitoyltransferase I (CPTI) are promising therapeutic targets in cancer⁴; upregulation of CPTI contributes to lung cancer chemoresistance⁵,⁶
  - Several anticancer drugs (e.g. doxorubicin, cisplatin, carboplatin, and oxaliplatin) interfere with absorption, synthesis, and excretion of carnitines and inhibit fatty acid oxidation² → results of carnitines might also indicate the likelihood and severity of side effects of chemotherapy
  - Cachectic cancer patients are especially at risk for carnitine deficiency due to decreased oral intake and/or increased renal losses²
  - Carnitine deficiency might cause fatigue due to impaired energy metabolism³

¹Takezawa K et al., 2011; ²Sayed-Ahmed MM, 2010; ³Silvério R et al., 2011; ⁴Qu Q et al., 2016; ⁵Zaugg K et al., 2011; ⁶Li J et al., 2013
Advantages of biomarker-guided clinical trials

**RAPID, COST-EFFECTIVE, AND MORE SUCCESS DRIVEN APPROVAL OF NOVEL TREATMENTS**

**Biomarker need for pharmaceutical studies**

<table>
<thead>
<tr>
<th>Long duration of studies</th>
<th>Risk of high number of false-negative results</th>
<th>Significant investment</th>
</tr>
</thead>
</table>

MTXH's biomarkers MxP® LCA Predict and MxP® LCA Monitor select explicit NSCLC cohort group

**More valuable results**

<table>
<thead>
<tr>
<th>Time-effective studies</th>
<th>Higher number of eligible patients</th>
<th>Lower costs</th>
</tr>
</thead>
</table>

19  Metanomics Health – a BASF Group Company  |  February 23, 2017  |  Public
Cancer is a heterogeneous group of diseases with respect to sensitivity to treatment. Standard trial design can generate a high number of false negative results, while studies that do yield positive results may have only small numbers of eligible patients.

MTXH’s prognosis biomarker (MxP® LCA Progress) enables streamlined research studies plus rapid, cost-effective, and more success driven approval of novel treatments.

Enrichment for the desired treatment response subset of a population offers possibilities to:
- Monitor recruitment process → early stop of enrollment & reduction of required overall numbers
- Increase study power by decrease of heterogeneity

Enrichment of Responders to:
- Demonstrate efficacy and safety of new chemotherapy-based treatments (in particular if no benefit is detected in the overall population)
- Avoid unnecessary treatment burden for patients who are unlikely to benefit or payers, respectively

Enrichment of Non-Responders to:
- Evaluate efficacy of alternative treatment options (e.g. immunotherapies or molecular targeted therapies)
- Study the consequence of more aggressive therapy regimen
- Investigate the mode of action of early disease progression and development of resistance to chemotherapy
Biomarker MxP® LCA Monitor to monitor chemotherapy response

INTERIM MONITORING in clinical trials using MTXH’s treatment monitoring biomarker panel

- Application of MxP® LCA Monitor enables rapid, streamlined, and cost-effective studies since efficacy can be determined early in the course of the trial → early decision making and lower overall numbers of patients
- Interim monitoring is an essential for both ethical and efficiency reasons:
  - Enables early discontinuation of a trial when the new therapy is either clearly superior to or non-superior standard therapy
  - If necessary – allows early adjustment of recruitment criteria to emphasize a better responding subgroup
  - Supports sample size planning (statistical power) and choice of study design
- Treatment response is currently monitored by evaluation of imaging procedures (e.g. CT or PET scans). Disadvantages:
  - Low sensitivity
  - Sometimes inconclusive (up to 20-30% of CTs in lung cancer RECIST evaluation provide inconclusive results)
  - Time-consuming evaluation by medical doctor
  - Expensive
  - Harmful for patients due to exposure to radiation

1 personal communication with HELIOS clinics (Berlin)
We are looking for collaboration partners to verify the quality of biomarkers for clinical applications (I)

“FIT-FOR-PURPOSE“ – Validation of prognosis and treatment monitoring biomarker panels

- **Collaborative validation** of the performance of MTXH’s biomarkers MxP® LCA Progress and MxP® LCA Monitor in
  - Human proof-of-concept studies, research studies, or randomized clinical trials (phase I, II, and III)
    - Plasma samples from untreated patients
    - If feasible: plasma samples from treated patients
- **Acquisition** of retrospective/prospective patient cohorts, human research samples
  - **Analytical validation** (accuracy, reproducibility, robustness, specificity)
  - **Clinical validation**: Do biomarkers correlate with clinical endpoint?
  - **Clinical utility**: Benefit for the patients and is it actionable?
- **Targeted assay development**
  - Mass-spectrometry-based or
  - Evaluation of commercially available enzymatic and immunological assays
- **Flexible intellectual property** strategies
- Potential for **companion diagnostic** tailored to specific drugs
We are looking for collaboration partners to verify the quality of biomarkers for clinical applications (II)

ADDITIONAL VALUE PROPOSITIONS

- Based on the application of untargeted metabolomics we can also provide information on:
  - Drug safety and drug metabolism limited to one or the other subgroup
  - In vivo mode of action analyses: primary target(s), early disease progression, and development of resistance to chemotherapy
  - Pre-analytical processing quality of the collected plasma samples (→ sample repositories/biobanks): e.g. incorrect choice of sample tubes, prolonged storage of blood or plasma and incubation at improper temperatures can have negative effects on sample quality, which in turn influences analytical reliability and reproducibility of results
- Additional future ideas:
  - Value assessment of the current biomarkers (prognosis and monitoring) for
    - SCLC patients
    - Other tumor entities
    - Novel therapies such as immunotherapy or molecular targeted therapy
    - Verify feasibility of the current biomarker to support pre-clinical in vitro and in vivo model studies (e.g. drug screening, analysis of mode of action or acquisition of drug resistance)
  - Development of new biomarker e.g. suitable for patient stratification
MTXH’s assay development process saves time and resources due to *in-silico* assay optimization and use of available RUO* kits.

**ACCELERATED BIOMARKER DEVELOPMENT PROCESS**

1) **OMICS Discovery**
   - MS-based metabolomics

2) **Biomarker Validation**
   - MS-based metabolomics *in-silico* assay selection

3) **Assay Development**
   - Evaluation of commercially available enzymatic and immunological assays
   - (using RUO assays)

4) **Prototype Assay**
   - Colorimetric assay

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**Development Time**

1) **NGS** or MS-based
   - Development of PCR or targeted MS-method required for high throughput validation

2) **Targeted-Assay Development**
   - Antibody development or real time PCR development

3) **Biomarker Validation**
   - RT-PCR or ELISA

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*RUO – Research Use Only*  
§Next Generation Sequencing
Metanomics Health - Key Partnering Strengths

BENEFITS

➢ Metabolomics by BASF
➢ Most comprehensive metabolomics platform in scope and scale
➢ “Fit for purpose” tools for discovery and clinical testing providing reproducible and high quality performance characteristics (e.g. quality marker-assisted interpretation)
➢ Recognized expertise in multi-omics data interpretation (e.g. FDA)
➢ Strongest metabolomics-based biomarker pipeline
➢ Novel proprietary diagnostic biomarker assets in e.g. heart failure and pancreatic cancer
➢ Excellence in assay design and assay prototype development to guarantee simplicity, ubiquity and cost-effectiveness of final targeted assay test
➢ Metanomics Health conducts fee-for-services business with Top20 global pharmaceutical companies and is referenced as the preferred partner in metabolomics
**Literature references (I)**

**EXTERNAL READINGS (I)**

Literature references (II)

EXTERNAL READINGS (II)


SELECTED PUBLICATIONS contributed by work of MTXH


Metanomics Health translates biomarker identification to clinical utility

Metanomics Health at a glance

- Leading technology platform
- Close to market products in cardiology and oncology
- Targeting standardized clinical diagnostics
- Visionary management team
- Enormous revenue potential
- Unrivaled robustness and reproducibility
- Long-term protected IP
- Top tier scientific network in the US & Europe
Thank you!

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Appendix
Metabolism is fundamental for the origin of life

**KEY FACTS**

- **Metabolites**
  - Small endo- and exogenous molecules (<1.5 kDa) synthesized via enzyme-catalyzed reactions
  - Intermediates and/or products of an organism’s metabolism

- **Metabolome**
  - Sum of all metabolites in a cell, tissue, organ, body fluid, organism
  - Closest to the phenotype; precisely reflects the pathological status

- **Metabolomics (metabolite profiling)**
  - Systematic study of the metabolome
  - Ideal approach to understand the current status of a cell or organism
The metabolome - a unique biochemical fingerprint of the phenotype

Elucidation of disease mechanisms, prediction of toxic effects and responses to drugs or nutritional intervention

- Diverse field of scientific applications
- Physiological status is most closely reflected by metabolites
  - Responsive to genetic and environmental factors and affected by disease, drug treatment or nutritional status
- Translation of complex “big data” sets into decision-enabling results
- Many widespread diseases in humans are metabolic diseases. Benefit from metabolic biomarkers to improve your clinical studies
- Metabolic reprogramming is a hallmark of tumor cells
- A clinical biomarker is a defined characteristic measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic measures
Overview of FDA-approved NSCLC treatments by disease stage (I)

KEYNOTES*

- **Stage I or II: Early or localized disease**
  - Surgery **with and without adjuvant chemotherapy** (cisplatin, vinorelbine, etoposide, gemcitabine, pemetrexed, docetaxel, carboplatin, paclitaxel)
  - Stereotactic body radiation: patients with significant co-morbidities or not suitable for surgery

- **Stage III: Locally advanced disease**
  - Chemotherapy and radiation (cisplatin, etoposide, vinblastine, pemetrexed, carboplatin, paclitaxel)
  - (Surgery)

- **Stage IV: First-line chemotherapy, metastatic or recurrent disease**
  - Platinum-based doublets (cisplatin, carboplatin, paclitaxel, gemcitabine, docetaxel, vinorelbine)
  - Squamous: *necitumumab*, gemcitabine, cisplatin
  - Nonsquamous: carboplatin, cisplatin, *bevacizumab*, paclitaxel, gemcitabine, docetaxel, pemetrexed, dexamethasone
  - Tumors with **epidermal growth factor receptor (EGFR) immunohistochemistry**: cisplatin, vinorelbine, cetuximab
  - Tumors with **EGFR exon 19 deletions or exon 21 (L858R) substitution**: erlotinib, afatinib, gefitinib
  - Treatment recommendations for **anaplastic lymphoma kinase (ALK)–positive** locally advanced or metastatic tumors: crizotinib, ceritinib, alectinib
  - Tumors with **high PD-L1 expression** (Tumor Proportion Score (TPS) ≥50%), with no EGFR or ALK genomic tumor aberrations: *pembrolizumab*

Overview of FDA-approved NSCLC treatments by disease stage (II)

KEYNOTES*

- **Stage IV: Second-line chemotherapy, metastatic or recurrent disease:**
  - Nivolumab, pembrolizumab, docetaxel, pemetrexed, dexamethasone, erlotinib, afatinib

- **Stage IV: Third-line chemotherapy, metastatic or recurrent disease:**
  - Nivolumab, ramucirumab, erlotinib

- **Stage IV: Single-agent therapy, metastatic or recurrent disease:**
  - Paclitaxel, docetaxel, gemcitabine, vinorelbine, pemetrexed
  - EGFR T790M mutation positive and after EGFR TKI therapy: osimertinib
  - ROS-1 mutation positive tumors: crizotinib
  - Patients with EGFR or ALK genomic tumor aberrations should have disease progression prior to receiving atezolizumab

- **Stage IV: Maintenance chemotherapy, metastatic or recurrent disease** (given after completing first-line chemotherapy until disease progression or unacceptable toxicities occur):
  - Docetaxel, erlotinib, pemetrexed (nonsquamous)

- **Stage IV: Continuation maintenance chemotherapy, metastatic or recurrent disease** (chemotherapy that was part of the first-line therapy; given until disease progression or unacceptable toxicities occur):
  - Cisplatin, vinorelbine, cetuximab, carboplatin, paclitaxel, bevacizumab, pemetrexed, dexamethasone, pemetrexed

*summarized in http://emedicine.medscape.com/article/2007153-overview#showall
Metabolomics reveals metabolic subtypes of untreated SCLC patients

**UNTREATED SCLC PATIENTS – Hierarchical clustering: t0 plasma samples**

- **Significant metabolic subtypes** are identified within the subgroup of untreated (t0) SCLC patients
- Key metabolic features of these metabolic SCLC subtypes might be used to
  - Gain a deeper understanding of the **pathophysiological disease mechanism** and the heterogeneity of SCLC
  - Identify **suitable therapeutic options or new treatment targets** for one or the other subtype
  - **Predict sensitivity** to therapies
  - Develop biomarker for **early diagnosis or screening**

**AU**: approximately unibased probability value; **BP**: bootstrap probability value
Metabolomics reveals two major metabolic subtypes of SCLC patients

SCLC PATIENTS AFTER THE FIRST CYCLE OF PLATINUM-BASED THERAPY WITH ETOPOSIDE (t1)

Hierarchical clustering with AU/BP values [%]:
method: Ward; distance: Pearson
Significant (p-value < 0.05), stable cluster AU>95%

- Significant metabolic subtypes are identified within the subtype of SCLC patients after one chemotherapy cycle
- These metabolic subtypes do not correspond to the metabolic SCLC subtypes of untreated patients and thus might suggest differences in overall response to therapy → patient’s individual follow-up information during the therapy-free interval (= after completion of all chemotherapy cycles) might be helpful to investigate metabolic features of early/late disease progression