Disclosures

› Research collaborations:
  - Labcyte Inc (liquid handling)
  - Pfizer, Roche
  - IMI-Predict public-private project (10 companies)

› Off-label use of drugs will be discussed
Genomic and molecular landscape in AML

23 significantly mutated genes in 200 AML patient samples

The Cancer Genome Atlas (TCGA) dataset

Panoramic view of AML, Chen and Chen; Nature Genetics, June 2013
Many leukemia patients die due to disease and/or treatment toxicity.

- AML genomes sequenced
- Clonal evolution characterized
- Increasing data on molecular pathogenesis
- Stem cell niche

Novel therapy targets and therapeutics (e.g. FLT-3, DNMTs) disease/patient-specific molecular markers

<50% survival
- Resistance common (10% survival)
- Classical chemotherapy used (from 1960s) with toxicity
- Targeted treatments not available
- Mutational profile often not helpful
Toward Precision Medicine

Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease

US National Academy of Sciences 2012
Infrastructure and collaborative environment for individualized systems medicine in AML

**Why AML?**
- Sampling over the course of disease
- Cancer always accessible
- Ex-vivo functional drug testing easier

**National Biobank**

**Clinic**

**Finnish Hematology Society**

**FiMM**

Genomic & molecular profiles
Drug sensitivity testing
Data integration & models

**Individualized and improved therapy**

Kimmo Porkka
Satu Mustjoki
Mika Kontro

Krister Wennerberg
Tero Aittokallio
Caroline Heckman
Jonathan Knowles
Olli Kallioniemi
Systems medicine to optimize drugs and drug combinations for individual AML patients

- Repeated sampling at diagnosis, therapy response, relapse and drug resistance
- Integration of genomic and molecular profiles and high-throughput ex-vivo drug testing for each patient (real-time)
- Feedback therapeutic insights back to the clinic for tailoring personalized therapy or guiding clinical trials with new agents
- Molecular and functional analysis of drug resistant samples after novel therapies
Biobanking all leukemia patients across the country:
On average 46 sample aliquots per patient
Samples across time points provide molecular insights on disease progression in patients, including drug resistance.

Diagnosis
Relapse 1
Treatment 1
Relapse 2
Relapse 3

Exome seq
p-proteomics

Drug testing
Exome seq
RNA seq
p-proteomics

Drug testing
Exome seq
RNA seq
p-proteomics

Drug testing
Exome seq
RNA seq
p-proteomics
Individualized systems medicine (ISM)

System medicine approach:
- Data integration
- Repeated sampling
- Feedback to clinic
- Learning system

Pemovska et al. Cancer Discovery, on-line Sep 20, 2013
Rapid data analysis, integration and interpretation to provide therapeutic insights to the clinic

Drug sensitivity testing

Exome sequencing

Integration & Interpretation & Feedback to clinic (4 days to 2 weeks)

Gene expression

Phosphoproteomics

Fusion genes
High throughput screening infrastructure: - “repurposed” from functional genomics/drug discovery to drug sensitivity testing for individualized medicine

BeckmanCoulter integrated robotic system – fully automated assays

Labcyte Access Workstation – nl acoustic dispensing

Wennerberg, Östling et al. HTB facility at FIMM
The most direct way to ascertain patient treatment: test drugs individually on patient cells

Drug sensitivity and resistance testing (DSRT) workflow

Leukemia cells isolated from blood or bone marrow

Pre-plated drug collection (1, 10, 100, 1000 and 10 000 nM)

Fluorescent dyes (HCS)

Measure cell growth/survival/p-proteins and calculate response

30 x 10^6 cells

37°C, 72 h

4 day turnaround
Drug sensitivity and resistance testing with dose-response curves for each drug

- Detailed dose-response curves for all oncology drugs and many emerging cancer compounds for individual patient samples

- Approved: 137
- Investigational: 103
- Probes: 66

- All conventional chemotherapeutics
- Tyrosine kinase-type inhibitors
  - Abl, Src, EGFR, FGFR, VEGFR, JAK, IGF1R, PDGFR, Met, ALK, Kit, Fli3, etc.
- S/T-type inhibitors
  - Aurora, PLK1, MEK, TTK, PDK1, Akt, Wee1, PKCs, Cdk5, Chk1
- HDACi's
- HSP90 inhibitors
- Bcl-2 inhibitors
- PI3K inhibitors
- mTOR inhibitors
- Survivin inhibitor
- Hh inhibitors
- γ-secretase inhibitors
- Farnesyltransferase inhibitor
- p53 activators
- PARP inhibitors
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Identification of cancer-specific drug responses

› Kill cancer cells more than normal cells (cancer-selective response)

› We calculate DSS scores by comparing cancer’s response to controls (Healthy bone marrow)

High DSS value => cancer cells have higher sensitivity to the drug
Summary of ex-vivo drug testing data from 27 chemorefractory AML patients

Most frequent bioactive drugs in 27 AML patients
Guidance for personalized medicine and clinical development of emerging drugs

Correlating drug sensitivity with genomic data:
- Predictive biomarkers
- Novel therapeutics
- Drug combinations
- Novel drug targets
- Insights on drug resistance

Navitoclax
Ruxolitinib
Dexamethasone
MEKi
PI3K/mTORi
Quizartinib
Sunitinib
Dasatinib
Topoisomerase IIi

Activating FLT3 mutations link to sensitivity to FLT3 inhibitors and dasatinib

Link between MEK and JAK inhibition sensitivity in DSRT subtype II

MLL fusions link to MEK and PI3K inhibitor sensitivity
Individualized systems medicine for AML
to define optimal drugs for patients

Using drugs as biological probes => direct opportunity for translation
Drug sensitivity testing report: summary of drugs showing selective cancer efficacy in an individual AML patients based on ex vivo testing.
Clinical data: A treatment-refractory AML patient receiving a novel combination of clinical drugs based on ex-vivo profiling: Complete clinical response and subsequent progression

Ex-vivo drug response data after relapse
Clinical implementation of ex-vivo drug-testing data in chemorefractory AML patients

Leukemia-selective responses observed in ex-vivo drug testing
Drugs available for human use (on- or off-label)

14/17 (81%): ex-vivo guided treatment possible

10 patients received personalized combinatorial treatments designed according to predictions

2 x CRi, 2 x leukemia free (hypoplasia)
40% response rate

Drug combinations for patients:
- Clo-Dasatinib-Vin
- Sorafenib-Clo
- Dasatinib-Sunitinib-Temsirolimus
- Azacytidine-Sunitinib
Individualized systems medicine for AML to define optimal drugs for patients

- Molecular profiling to understand drug resistance and tx failure
- Drug-testing to understand drug (cross)-resistance and new vulnerabilities
Genomics as a measure of drug response in patients

Understand resistance mechanisms and evolution of multiple subclones

The clones causing clinical resistance often pre-exist before therapy
Evidence of vulnerability to previously ineffective drugs after resistance has emerged in patients => An opportunity to design curative drug combinations

New drug vulnerabilities = Therapeutic options for patients

Drugs whose efficacy is not altered by the clinical therapy resistance

Cross-resistance to other drugs

Sensitivity of cancer cells before resistance

Sensitivity of cancer cells after resistance

Compare drug vulnerabilities after relapse
Understanding altered cell signaling after treatment

Kinase signalling networks predicted from drug response
Systems medicine to optimize drugs and drug combinations for individual AML patients

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- Molecular and functional analysis of drug resistant samples after novel therapies
Future development

Clinical:
› Drug sensitivity testing in primary AML
› Stratified, biomarker driven trials for new drugs in AML
› Comparing treatment paradigms: Individualized vs. conventional clinical therapy

Biological:
› Progenitor / single cell assays
› Mimicking the bone-marrow niche
› Larger libraries, combinations to facilitate drug repositioning
Slide content not available for publication
Slide content not available for publication
Slide content not available for publication
Slide content not available for publication
Tissue slice cultures to assay short-term drug effects (e.g. DNA damage, phosphorylation events)

Towards analysis of solid tumors?

Reprogrammed primary cell culture (Rock-inhibitor & feeder layers)

Primary prostate cancer organoid cultures in 3D (Clevers protocol)
Precision Cancer Medicine Network

**FIMM**

Personalized Cancer Medicine
Caroline Heckman
Jonathan Knowles
Samuli Eldfors
Riikka Karjalainen
Jarno Kivioja
Ashwini Kumar
Heikki Kuusanmäki
Muntasir Mamun Majumder
Alun Parsons
Minna Suvela

Chemical Systems Biology
Krister Wennerberg
Tea Pemovska
Arjan van Adrichem

Computational Systems Biology
Tero Aittokallio
Petteri Hintsanen
Agnieszka Szwajda
Bhagwan Yadav

Individualized Systems Medicine
Olli Kallioniemi
Taija af Hällström
Henrik Edgren
Poojitha Kota Venkata
Disha Malani
John Patrick Mpindi
Astrid Murumägi
Päivi Östling
Maija Wolf

Technology Center
Janna Saarela
Evgeny Kulesskiy
Laura Turunen
Anna Lehto
Ida Lindenschmidt
Pekka Ellonen
Maija Lepistö
Sonja Lagström
Sari Hannula
Pirkko Mattila
Aino Palva

**HUCH/HRU**

Kimmo Porkka
Satu Mustjoki
Pekka Anttila
Mika Kontro
Erkki Elonen
Hanna Koskela
Mette Ilander
Emma Anderson
Paavo Pietarinen
Jaakko Vartia
Minna Lehto
Mervi Saari

Kuopio University Central Hospital
Raija Silvennoinen

Turku University Central Hospital
Tuija Lundán

Tampere University Central Hospital
Hannele Rintala
Tero Pirttinen
Marja Sankelo

University of Bergen
Bjørn Tore Gjertsen