

INDIVIDUALISED SYSTEMS MEDICINE

next-generation precision cancer medicine and drug positioning

A growing number of oncology drugs are currently clinically available and more than a thousand compounds are being developed for oncology indications. Thus, the challenge is to systematically develop all these drugs to optimal indications across all cancer types and subtypes as well as to tailor treatments of individual patients based on the underlying mechanisms of disease and driver signals. We describe here how coupling of genomic and molecular profiling of cancer with functional testing of drugs in patient-derived cell samples will make it possible to customise patient treatments as well as to identify biomarker patterns and genetic aberrations that explain the drug responses. This approach may also facilitate rapid clinical translation and optimised development of drug candidates and investigational drugs through identification of the patient subgroups that are most likely to respond and systematic evaluation of efficacious drug combinations.

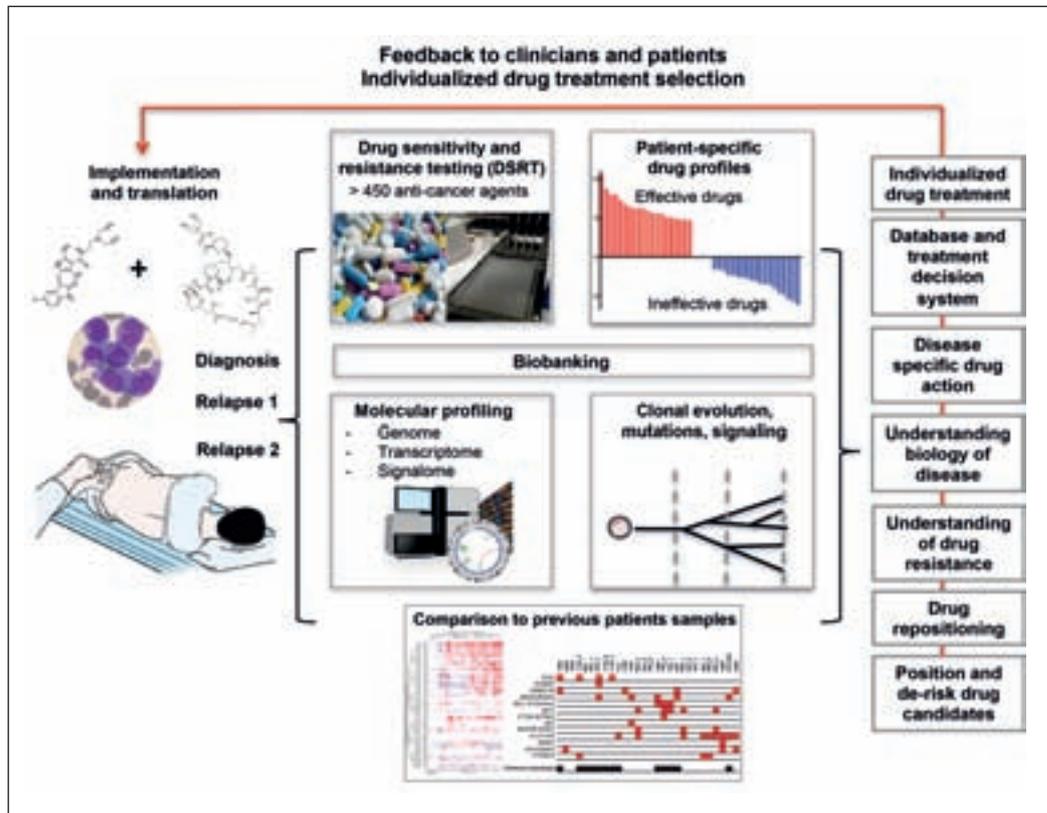
Cancer therapy increasingly involves the use of smart drugs with high specificity and low toxicity in selected patients based on a precision or personalised medicine paradigm. The targeted smart drug revolution can be considered to have started in the late 1990s and early 2000s with the approval of trastuzumab (Herceptin®) for breast cancer patients with HER2 amplifications and shortly thereafter imatinib (Gleevec®) for patients with BCR-ABL1-driven chronic myeloid

leukaemia (CML). These examples marked the beginning of rational drug design, which takes into account knowledge of disease pathogenetics and has led to the concept of personalised cancer medicine. In oncology, personalised medicine entails optimisation of the entire treatment strategy for an individual patient with the help of genetic information as well as molecular and cellular analysis. The personalised tailored treatment is expected to be highly effective and better tolerated in comparison

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Figure 1

Comprehensive Individualised Systems Medicine platform integrating *ex vivo* Drug Sensitivity and Resistance Testing (DSRT) and in depth molecular profiling as an approach to tailor and optimise treatments for cancer patients



to conventional chemotherapeutics, thus resulting in an improvement of the quality of life.

The characterisation of the genome and epigenome of many tumour types has been one of the focus areas of cancer research in recent years. However, despite the progress in developing new targeted agents and increased knowledge of cancer genomics, the majority of cancers do not harbour mutations that can readily be linked to currently approved or investigational oncology drugs. For example, even though a large percentage of cancers are driven by mutations in the RAS genes (>30%), targeting RAS signalling by small molecules, directly or indirectly, remains an elusive therapeutic goal. Translation of a genotype to a phenotype (ie drug response) is not a straightforward exercise. Even when drugs that inhibit the desired target exist, meaningful responses in patients may be limited due to cell plasticity, tumour heterogeneity and compensatory signals. For instance, BRAF inhibitors (eg vemurafenib; Zelboraf® and dabrafenib; Tafinlar®) achieve rapid and effective responses in melanoma patients with V600E BRAF mutations, whereas their effects in colorectal cancer patients harbouring the same mutation are negligible (<5% response rate) due to upregulation of EGFR signalling in response to BRAF inhibitor exposure. Given that cancers are extremely hetero-

geneous between individual patients and within the tumour itself, optimised, personalised and combinatorial treatments are often required to achieve significant responses. Such treatments remain very hard to predict based solely on our current knowledge of the genomic properties of cancer cells. Therefore, there is an urgent need for increased functional understanding of cancer. This can be accomplished by directly examining cells derived from cancer patients to systematically determine both the cancer genotype and the drug response phenotype.

The Individualised Systems Medicine platform

To address these challenges, we have established and applied a translational Individualised Systems Medicine (ISM) platform to tailor treatments of cancer patients (Figure 1) at the Institute for Molecular Medicine Finland (FIMM), University of Helsinki in collaboration with the Helsinki University Hospital Comprehensive Cancer Centre¹. This project is accompanied with a national programme to biobank samples from all patients in Finland diagnosed with a hematological malignancy through the Finnish Hematology Registry and Biobank (FHRB; www.fhrb.fi). The general concept of the platform is to focus on individual

patients where personalised therapy options are identified and linked to disease- and patient-specific molecular markers. This is achieved by integrating functional profiling from *ex vivo* drug sensitivity testing of patient cancer cells with genomic and molecular profiling. Exome sequencing is used to identify somatic mutations and copy number variations, whereas RNA sequencing is used to define gene expression patterns as well as to identify gene rearrangements and fusion genes. This enables linkage of drug responses to genetic aberrations and biomarkers. Thus we carry out real-time translational research where scientists work to generate and analyse data to inform clinicians on individualised treatment possibilities. The suggested new therapies can be implemented after an informed consent and ethical approval in patients with advanced disease and no alternative treatment options. Consecutive sampling of patients over time and genomic and molecular profiling of the cancer samples will then make it possible to monitor disease progression, response to therapy, as well as to understand mechanisms of drug resistance and clonal evolution during treatment.

The cornerstone of the ISM approach is the drug sensitivity and resistance testing (DSRT) platform where cancer patients cells are tested *ex vivo* against a comprehensive small-molecule collection of more than 450 approved and investigational anti-cancer compounds (Figure 2). The drugs and drug-like molecules to i) deconvolute the underlying cellular signalling pathways and molecular processes that drive cancer; ii) identify effective drugs and drug combinations; iii) position and de-risk drug candidates and investigational drugs as well as reposition existing drugs for new indications; and, ultimately, iv) use this information to

help treatment decisions. The oncology drug collection covers the majority of approved small molecule oncology substances, oncology-related approved substances, major investigational oncology compounds as well as probes with unique and cancer-relevant activities and is composed of conventional chemotherapeutics, kinase inhibitors, epigenetic and differentiating drugs, hormone therapy drugs, metabolic modifiers, apoptotic modulators and immunomodulators with kinase inhibitors being the most common drug class. About one-third of the collection are approved agents while the remaining two-thirds are investigational and experimental agents. The capabilities of the DSRT platform in terms of miniaturisation, customisation and multiplexing of assays, precision and throughput have been enabled by acoustic liquid handling technology and associated software solutions (Labcyte Inc, Sunnyvale, CA, USA). Testing across a 10,000-fold concentration range allows for the determination of dose-response information for all tested compounds. The drug sensitivity information across the oncology pharmacopeia can subsequently be used to gain understanding of the driving signals and addictions, to functionally group different samples based on oncogenic signal addictions and to identify novel clinically actionable putative therapies.

Implementation of the ISM platforms

In our case, the ISM has mostly been applied to patients with hematological malignancies, where viable cancer cells are typically extracted from bone marrow or peripheral blood samples by density gradient separation. The compounds are preprinted on 384-well plates in five different concentrations using acoustic nanodispensing (Labcyte Echo 550)

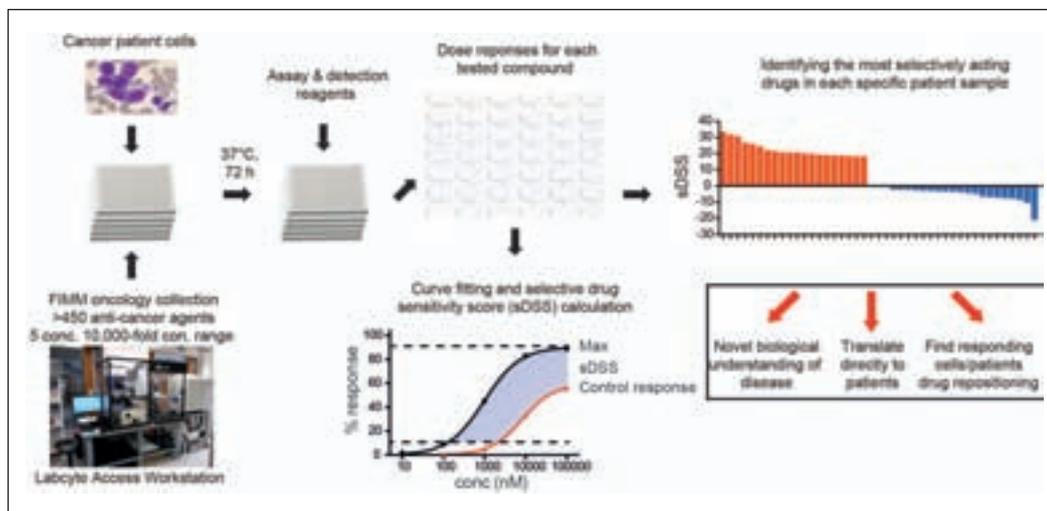
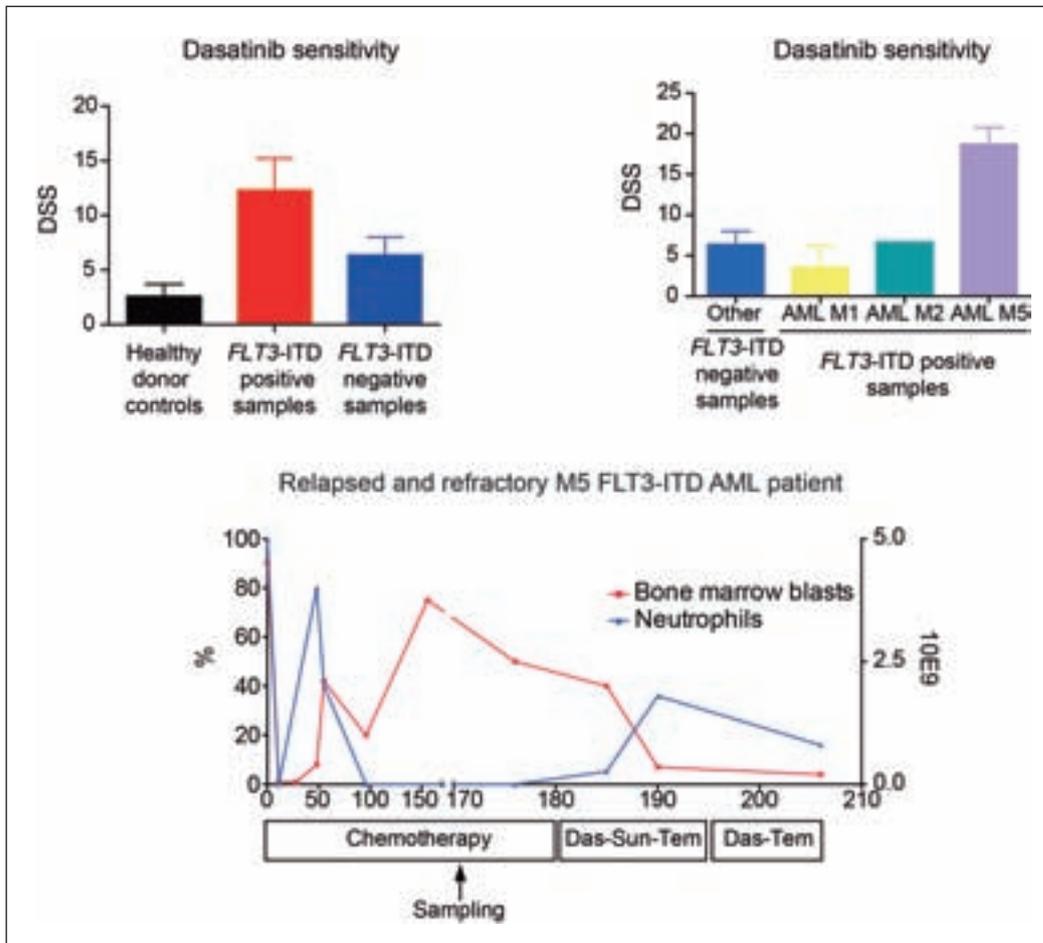


Figure 2
The Drug Sensitivity and Resistance Testing (DSRT) platform facilitates systematic and comprehensive evaluation of compound sensitivities in cancer patient cells *ex vivo* enabling novel biological understanding of disease, uncovering of patient specific molecular addictions and vulnerabilities as well as positioning and repositioning approved and investigational drugs to individual patients or subgroup of patients

Figure 3

Proof of concept example of how the ISM approach identified clinically applicable synergistic drug combination strategy of FLT3 inhibitors and dasatinib in AML M5 patients harbouring FLT3-ITD mutations



and the prepared plates are stored under nitrogen gas to maintain compound integrity until a new patient sample will be tested. The patient's cells are then added and incubated at 37°C for three days and cell response is measured with CellTiter-Glo viability and CellTox Green cytotoxicity assays (Promega). As part of the DSRT analysis pipeline, we have developed a novel drug sensitivity quantifying metric termed 'Drug Sensitivity Score' (DSS)². The DSS describes the full dose response to a compound as a modified 'area under the curve' metric so that selective responses are favoured over off-target responses². The patient-specific drug sensitivity profiles are compared with responses of cells derived from healthy bone marrow to identify drugs that exhibit cancer-selective responses. The DSRT data provide insights into novel biological understanding of disease, identify responding cells/patients to particular drugs and hence facilitate drug repurposing and can be translated directly to patients. Optimally, results are delivered to clinicians within four days.

The ISM approach has allowed us to establish

selectively responding patient subpopulations to a large number of targeted drugs and to identify compounds and compound combinations that have been utilised by our clinical collaborators on refractory acute myeloid leukaemia (AML) patients in compassionate use/off-label setting with significant success. In about 80% of the patient cases, selective responses with approved drugs are seen such that ISM tailored treatment is possible. Up to this point, translation of ISM data has led to meaningful and evaluable responses, including complete remission and morphologic leukaemia-free states in more than 40% of cases, which is a solid response rate for high-risk relapsed and refractory AML patients. Importantly, in the cases where *in vivo* resistances to the selected targeted therapies have emerged, those have also been reflected in the DSRT responses in the relapsed samples.

Drug repositioning opportunities

The ISM platform comprehensively validates candidate drugs that would only be hypothesised from genomic profiles. We have also discovered

cancer cell addictions and vulnerabilities that could not have been uncovered solely from genomic information. Systematic integrated evaluation of cancer cell functional and molecular profiles of consecutive samples from individual patients enables identification of personalised therapy options for patients with refractory and relapsed disease, aids disease monitoring and follow up of the clonal architecture of the cancer. Moreover, by studying the sensitivities to a large number of compounds in a large number of cancer patient samples, the ISM strategy facilitates de-risking, pin-pointing and repositioning drug candidates, investigational and approved drugs in terms of identifying the most strongly responding patient subpopulations, optimal drug combinations and biomarkers predicting those.

Signal transduction inhibitors display strong cancer selective effects in subgroups of patient samples with small or no effects in control samples. This is in contrast to conventional chemotherapeutics, which tend to exhibit broad effects on both patient and control cells. The sensitivity to conventional cytotoxic chemotherapy agents *ex vivo* is also highly challenging to interpret and translate to the *in vivo* setting, as their responses often correlate with *ex vivo* proliferation rates or cellular stress levels. Hence, responses to molecularly targeted drugs *ex vivo* are more likely to be predictive of the *in vivo* patient response. Still the *in vivo* predictiveness of drug responses largely depends on culture conditions and the ability to model the tumour microenvironment and its interaction with the patient's cancer cells.

In our study of adult chemorefractory AML¹, we identified to a number of approved kinase inhibitors such as dasatinib (Sprycel®), sunitinib (Sutent®), ponatinib (Iclusig®), ruxolitinib (Jakavi®), sorafenib (Nexavar®) temsirolimus (Torisel®) and trametinib (Mekinist®), none of which are currently utilised for AML treatment. Thus, these results suggest that already approved oncology drugs, for example dasatinib (CML and Ph+ ALL), sunitinib (renal cell carcinoma and gastrointestinal tumours), and temsirolimus (renal cell cancer), could be repositioned for subgroups of AML patients.

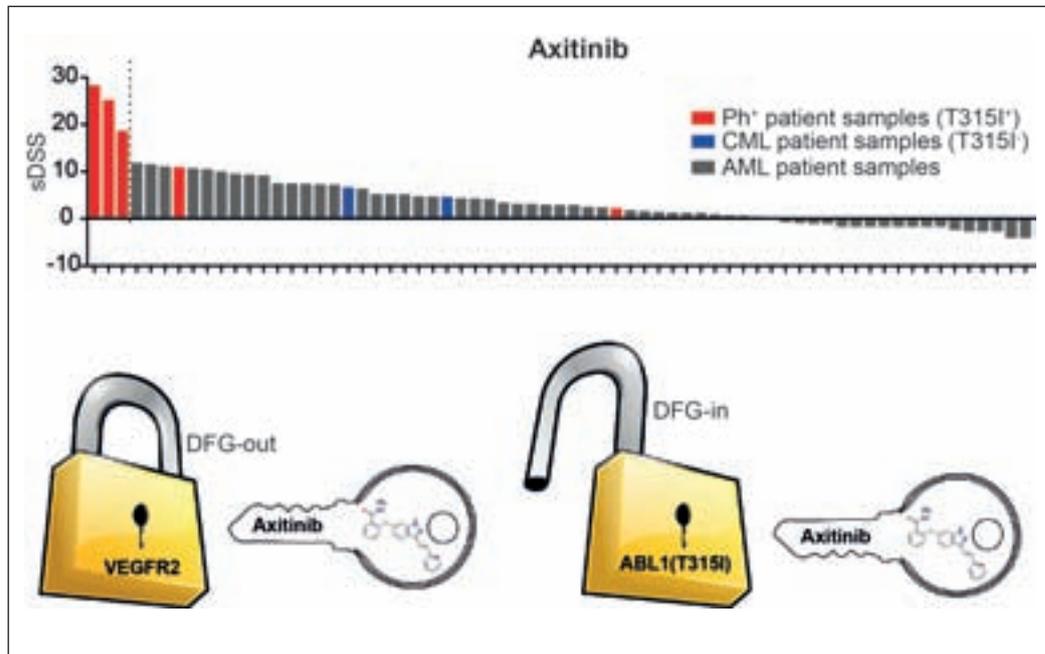
Unsupervised clustering of the drug sensitivity profiles creates functional taxonomy of both the samples and drugs tested and thus provides information on how patient samples functionally relate to each other and how the drugs relate to each other in a disease-specific context. Links between drugs whose known target profiles differ often cluster unexpectedly together, which may reflect effects

on the same or linked signalling pathways. This could provide opportunities to identify synergistic effects. For example, we have identified an association between inhibition of the FLT3 receptor tyrosine kinase and dasatinib (a tyrosine kinase inhibitor that does not target FLT3) sensitivity in AML patients with an acute monocytic leukaemia (M5) subtype with activating *FLT3*-ITD mutations. This implies that AML of the monocytic subtype driven by *FLT3*-ITD is also dependent on additional tyrosine kinase signals. Hence, combinatorial treatment of FLT3 inhibitors and dasatinib in this particular patient population could have a synergistic effect. Indeed, *in vivo* treatment of a heavily refractory AML patient harbouring a *FLT3*-ITD mutation with dasatinib and sunitinib (a tyrosine kinase inhibitor with potent activity against FLT3) led to a complete remission after failure of three consecutive induction chemotherapy regimens (Figure 3). This specific case highlights the power of the ISM platform to not only optimise treatments for patients with no treatment options in the clinic, but also to identify unexpected drug-drug interactions in a distinct patient subpopulation.

A final powerful example of ISM-driven drug repositioning is how we recently identified that the renal cancer drug axitinib (Inlyta®) exhibits anti-cancer activity in drug resistant gatekeeper mutant CML patients (Figure 4)³. The DSRT-driven discovery of the activity in drug resistant primary patient cells led to a i) new molecular understanding of kinase gatekeeper mutants and drug-target interactions; ii) proof of concept validation in patients; and, most importantly, iii) the opportunity to reposition an approved drug for a patient group with an unmet therapeutic need. More specifically, we were able to demonstrate that axitinib potently and selectively inhibits ABL1(T315I) kinase activity as well as the growth of BCR-ABL1(T315I)-driven cells. Furthermore, structural data revealed that while axitinib is an established binder of the inactive conformation of VEGFR2, it binds to the active conformation of ABL1(T315I) and the inhibitor itself takes on different conformations when binding to ABL1(T315I) versus VEGFR2. Additionally, axitinib occupies a unique binding space in ABL1 in comparison to other ABL1 inhibitors, which is expected to translate to distinct resistance profile. Overall the structural information highlights the complexity of kinase-inhibitor interactions and illustrates the importance to profile inhibitors against clinically relevant samples/assays. Most importantly, when axitinib was administered to a patient with a BCR-ABL1(T315I)-driven CML, it was able to cause a

Figure 4

Axitinib has unexpected activity in patients with BCR-ABL1(T315I) mutations and binds ABL1(T315I) and VEGFR2 kinases in a distinct and selective fashion



sharp reduction of BCR-ABL(T315I)-positive cells. Hence, these findings provide an opportunity to explore axitinib as a novel treatment strategy for patients with BCR-ABL1(T315I) mutations. Last, but not least, the discovery could be utilised to further optimise the structure of axitinib and make it even more selective towards ABL1 and/or to design gatekeeper mutant selective inhibitors to other clinically relevant kinases such as EGFR, BRAF, KIT and PDGFRs.

Other pharmacogenomics approaches

While drug-testing methodologies have been tried with limited success by other groups in the past, those have typically focused on conventional chemotherapeutics whose response is strongly cell proliferation-related and dependent on assay conditions. However, the smart drug revolution and the rapidly increasing number of clinical and novel emerging signal transduction inhibitors makes it possible to substantially improve the clinically predictive value of drug sensitivity testing. ISM efforts at FIMM have initially been focused on hematological malignancies. Studies on solid tumours, on the other hand, are typically limited by the fact that functional profiling often can not be performed directly on the patient’s cancer cells, as sufficient numbers of primary cells rarely can be extracted upfront. Instead, patient-derived models typically have to be used that may not fully recapitulate the original tumour and whose establishment takes a significant amount of time, making

them not ideal for diagnostic or treatment decision purposes. Addressing some of these problems, conditional reprogramming of cells⁴ and organoid 3D culture⁵ are two emerging technologies for expanding primary cancer cells and two recent publications report drug screening and genomic profiling of patient-derived solid tumour models using these strategies^{6,7}. Crystal and co-workers at the Massachusetts General Hospital Cancer Center introduced a pharmacogenomic platform that enabled identification of effective drug combinations that overcome resistance in conditionally reprogrammed cell culture models obtained from biopsy samples of lung cancer patients resistant to EGFR or ALK inhibitors⁶. On the other hand, van de Wetering and co-workers at the Hubrecht and Wellcome Trust Sanger Institutes established a living biobank of colorectal carcinoma patients by generating tumour organoid 3D cultures amenable to molecular profiling and high throughput drug screens facilitating identification of gene-drug links⁷.

Conclusion

The ISM strategy and DSRT technology represents a powerful method to: 1) Discover novel ways to treat individual and stratified groups of cancer patients; 2) Facilitate discovery of biomarkers associated with selective responses to targeted drugs and drug combinations; 3) Identify new targets and uses of already existing drugs; 4) De-risk the development investigational drugs and drug combina-

tions where the appropriate use can be pinpointed to the patient groups most likely to respond; and 5) Optimise lead compounds and their target profiles in cancer-relevant tissue models. Hence, we believe the ISM approach is a powerful method for molecular dissection of cancer progression in individual patients and identifying putative treatments in real time where new drugs could be more systematically developed to the best indications and tumour subgroups. **DDW**

Tea Pemovska is a cancer researcher at the Institute for Molecular Medicine Finland, FIMM, at the University of Helsinki. She is interested in investigating signalling networks driving tumour development and progression with small molecules to uncover biology of disease, drug repositioning opportunities and tailored treatment strategies for individual or subgroups of patients.

Päivi Östling is a senior researcher at FIMM. Her goal is to bring the drug sensitivity and resistance testing and in-depth molecular profiling from bench to bedside. Her research aims to develop the ex vivo drug testing not only for leukaemia patients but to enable the platform also for solid tumours.

Caroline Heckman is principal investigator and leader of the Translational Research and Personalized Cancer Medicine group at FIMM focused on hematologic malignancies. The group applies state-of-the-art research technologies and a systems-wide approach to understand disease progression and drug resistance mechanisms, directly translating results to patient care.

Olli Kallioniemi is professor and director of FIMM, a part of the Nordic EMBL Molecular Medicine Partnership. His research group is focusing on translational cancer research and molecular precision medicine approaches to leukaemia and solid tumours.

Krister Wennerberg has been a FIMM-EMBL Group Leader since 2010. His Cancer Chemical Systems Medicine research group at FIMM focuses on delineating individualised molecular vulnerabilities in cancers, how these vulnerabilities relate to the cancer cell genetics and how this can be turned into new effective therapeutic strategies.

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