

DD *Up*

TRANSFORMING TOXICOLOGY PREDICTION VIA PanOmics

Leading platform to predict Drug Induced Liver Injury by combining human cell-based assays with global transcriptomics

INTRODUCTION TO
TOXICOLOGY PREDICTION AND
TRANSCRIPTOMICS

EVOTEC'S PREDICTIVE
TOXICOLOGY PLATFORM

INTERVIEW WITH
*Dr Rüdiger Fritsch and
Dr Paul Walker*

4 QUESTIONS TO
Dr Thomas Siegmund

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DEAR FRIENDS

OF EVOTEC



A message from Evotec CEO
Dr Werner Lanthaler

Welcome to this tenth issue of DDup, an Evotec publication providing you with more insights into the company and its capabilities. This edition is dedicated to our newly developed next generation predictive toxicology platform, a truly translational project across different groups at Evotec. Historically, Evotec has a long-standing expertise in *in vitro* toxicology through its wholly-owned subsidiary Cyprotex and is now successfully combining this knowledge with our new and pioneering high-throughput transcriptomics platform and proprietary data analysis platform PanHunter, to provide a smart and forward-looking solution in safety liability prediction.

Evotec is always aiming to provide not only state-of-the-art, but also new and unparalleled offerings and solutions to our partners, to optimise and smoothen the drug discovery process, leading to faster, more considered decisions and finally cost efficiency. All this development work is based on and handled by Evotec's experienced, innovative and dedicated scientists, constantly striving for cementing

our leading position as drug discovery research partner. But I would also like to mention that this unique platform will support development of our proprietary and co-owned assets, too.

The development of this platform has been a combined effort of toxicology experts, bioinformaticians and transcriptomics specialists in Germany and the UK, working together as ONE team, leading to one of our latest offerings, a unique end-to-end *in vitro* toxicology transcriptomics service that can be customised according to customer's needs.

Thank you for reading this latest edition of DDup – we hope you found it of interest. We welcome your thoughts and input, and hopefully we will get the opportunity to collaborate in this exciting area of science in the future.

Yours sincerely,
for the management of Evotec
Werner Lanthaler,
CEO of Evotec SE

INTRODUCTION

TOXICOLOGY PREDICTION AND TRANSCRIPTOMICS

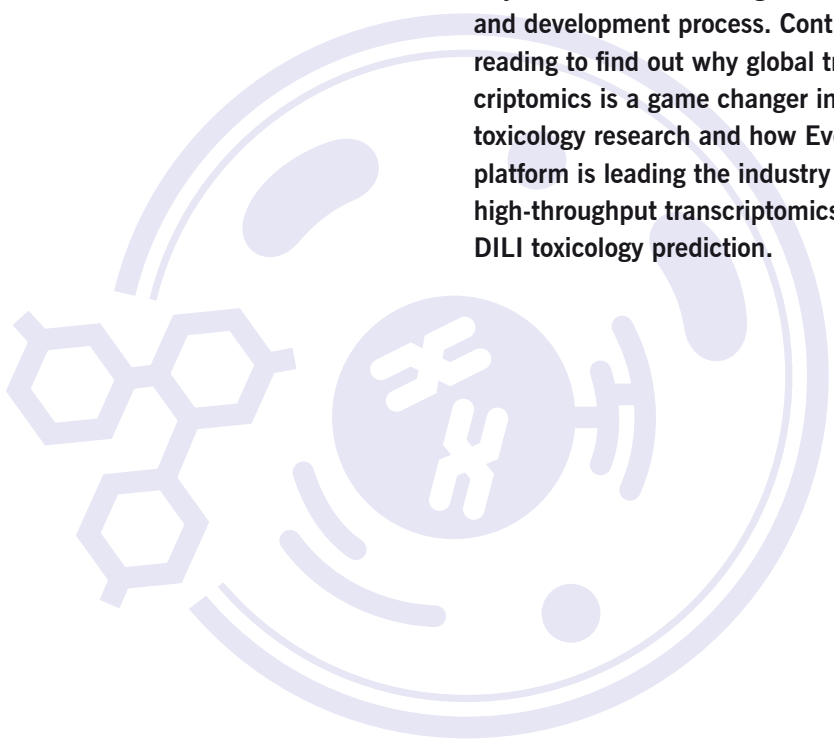
In this special DDup edition, we would like to introduce our exciting new developments in PanOmics research with a particular focus on the area of transcriptomics in predicting Drug Induced Liver Injury (DILI) in humans. With the advent of state-of-the-art technologies such as RNA-Seq, coupled with Artificial Intelligence (AI) and Machine Learning (ML), we now have the opportunity to transform the drug discovery and development process. Continue reading to find out why global transcriptomics is a game changer in toxicology research and how Evotec's platform is leading the industry with high-throughput transcriptomics and DILI toxicology prediction.

THE COST OF DRUG FAILURE

Delivering a safe effective drug to market is the ultimate goal of the pharmaceutical industry. However, escalating costs coupled with high failure rates are a major concern.

- ▶ It is estimated that the cost of delivering a drug to market is a staggering \$ 2.6 bn
- ▶ Drug development can take up to 15 years
- ▶ 90% of drugs fail during clinical development
- ▶ Only 50% of human hepatic toxicities are identified in animal studies
- ▶ Between 1953 and 2013, approximately 18% of drug withdrawals from the market were a consequence of DILI

Drug safety has been identified as a key cause of the failure. It is now recognised that the traditional approach for pre-clinical toxicity testing using animal models has its limitations in the prediction of human toxicity. This leads to drugs with safety concerns only being picked up during clinical development or post market approval. DILI is one of the most common adverse drug reactions.



90%

of drugs fail in late clinical development

US \$ 2.6 billion
and 15 years to develop a drug

18%

drug withdrawals from the market caused by DILI

ONLY

50%

DILI picked up in animal studies

BRINGING TESTING EARLIER USING HUMAN CELL-BASED MODELS

The lack of translation from animal species to humans combined with an ethical drive to reduce animal testing has led to an industry push towards testing earlier using more human-relevant cell-based models. Major technological developments in cellular biology such as widespread access to human primary cells and human iPS cells along with liver cell lines has advanced this research. Additionally, further progress has been shown with more sophisticated and physiologically

relevant 3D liver models. Robust and highly sensitive analytical techniques have significantly advanced the predictive capabilities of these approaches.

THE CHALLENGE OF DILI PREDICTION

Pharmaceutical companies have developed various DILI screening strategies over the last decade often consisting of liver cellular models with mechanistic assays or endpoints such as:

- ▶ Oxidative stress
- ▶ Covalent binding
- ▶ Cytochrome P450 TDI

- ▶ Transporter interactions
- ▶ Mitochondrial dysfunction
- ▶ Steatosis
- ▶ Genotoxicity
- ▶ Cytotoxicity

However, there is little consistency within the industry on an optimal screening approach and multiple assays are often required to capture potential off-target effects. Limitations still exist and more sensitive and specific techniques are required to further improve our chances of successfully identifying early safety liabilities.


MECHANISTIC UNDERSTANDING IN SAFETY ASSESSMENT

Understanding the underlying mechanism of drug-induced toxicity is vital in the prevention of adverse effects. To address this, there is a need to link molecular initiating events (MIE) to possible mechanisms by assessing specific adverse outcome pathway (AOP) perturbations and how these relate to organ (or multiple organ) toxicity and eventual adverse health effects in patients. Additionally, identifying fingerprints of MIEs can be used for future hazard identification and risk assessment.

NEXT GENERATION SEQUENCING (NGS) IN TRANSCRIPTOMICS RESEARCH

Initial toxicogenomics efforts primarily involved transcriptional profiling (the study of transcriptional changes in mRNA and other RNA molecules) of *in vivo* and *in vitro* samples from pre-clinical safety studies using microarray technology. Microarrays have limited throughput and dynamic range. At the start of the millennium, the field of transcriptomics advanced significantly through the introduction of RNA-Seq, a method based on NGS. Using this technique, it is possible to sequence

the entire transcriptome of the cell efficiently and quantitatively with an unprecedented dynamic range and limited sample requirements. Molecular profiling, particularly in the field of transcriptome analysis has become omnipresent and new technologies such as high-throughput barcoding and NGS are bringing down costs. These advances allow for processing of thousands of samples in parallel and thus testing of large numbers of compounds, concentrations and individual time points.

| Microarrays | Bulk RNA-Seq | HT-RNA-Seq |
|--|--|---|
| ↓ Throughput (individual samples) | ↓ Throughput (individual samples or max 96 well format) | ↑ Throughput (multiple 384 well plates) |
| Require species- or transcript-specific probes | Whole mRNA body coverage: detects novel transcripts, gene fusions & all single nucleotide variants | 3' mRNA part-focused library prep: limited info on transcripts |
| Detected genes defined by the probes | Deep sequencing (up to 20k genes detected) | Shallow sequencing (up to 16k genes detected) |
| ↓ Semi-quantitative quantification per gene (no direct cross-gene comparison possible) | ↑ Digital quantification. No UMIs (PCR bias is not addressed) | ↑↑ Digital quantification. UMIs are used (precise mRNA quantification) |
| ↓ Limited dynamic range (10 ³) | ↑ Broad dynamic range (>10 ⁵) | ↑ Broad dynamic range (>10 ⁵) |
| ↓ Data output size | ↑ Data output size | ↑↑ Data output size |
| Commonly available | Commonly available | Proprietary  evotec platform |
| ↑ Straight forward data analysis | ↑ Straight forward data analysis | ↓ Data analysis for large sample number, requires specific tools (PanHunter platform) |
| ↑↑ \$\$\$ | ↑ \$\$ | ↓ \$ |

THE PROMISE OF NGS IN DILI PREDICTION


The promise of transcriptomics and DILI prediction became evident with the pioneering studies in the early 2000's. These early studies primarily focused on rodent *in vivo* studies which were later shown to translate to surrogate gene expression markers of DILI in human blood samples. The current challenge is to detect DILI signatures early from human *in vitro* cellular models. Evotec's fully integrated platform has achieved significant improvements in the prediction of DILI using AI modelling of transcriptional data from primary

human hepatocytes. Following exposure of the hepatocytes to 128 reference compounds, an improved accuracy over established endpoint assays has been demonstrated, increasing predictability of DILI tox from 70% to 82%. The use of 3D cellular models as well as greater mechanistic understanding of the gene expression profiles from larger compound libraries will enhance our understanding of transcriptomics and its major role in toxicology prediction.

Evotec is investing heavily in generating a substantial reference database for DILI, using a large set of drugs with annotation of DILI liabilities in a number of biological

test systems including 3D liver microtissues. Combining this with transcriptomic analysis and specialised data analysis tools will further improve the prediction of DILI significantly beyond 82%.

TRANSCRIPTOME & AI DELIVER SUPERIOR DILI PREDICTION

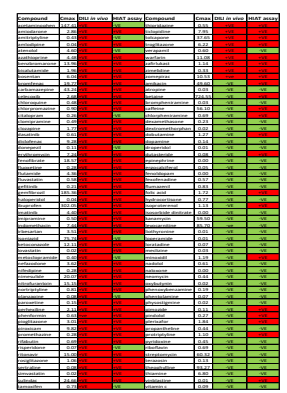
PanOmics 

- ▶ *In vitro* cellular models e.g. liver microtissues, PHH
- ▶ Broad spectrum of read-outs:
 - Phenotypic
 - Mechanistic
 - Multiparametric
- ▶ PanOmics analysis:
 - Transcriptomics
 - Proteomics
 - Metabolomics

LEADING INDUSTRIAL DILI PREDICTION PLATFORMS IN DIRECT COMPARISON

128 reference compounds tested in primary human hepatocytes (PHH).
In vitro assays vs. transcriptomics combined with AI

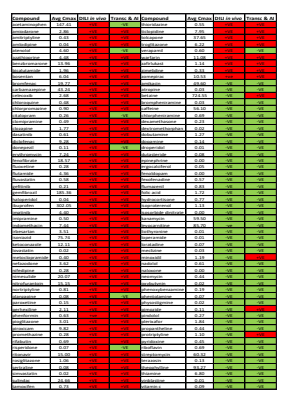
High-content imaging




Accuracy: 70%

→

Transcriptome analysis



Accuracy: 82%

PanHunter 

- ▶ Data base of toxicology profiles
- ▶ Integrated AI to enhance predictive power
- ▶ Unrivalled mechanistic insight
- ▶ Comprehensive data analysis platform
- ▶ Integrated service

Superior accuracy over current *in vitro* assay approach plus additional mechanistic insight gained from transcriptomics.

EVOTEC'S PREDICTIVE TOXICOLOGY PLATFORM

CHAPTER 02

In the second section of this DDup, you will discover how Evotec has combined forces from across its business areas to address the challenge of toxicology prediction. Driving this research is Evotec's strong expertise in cell biology and PanOmics research along with its state-of-the-art equipment, custom-built streamlined workflows, and unique ground-breaking PanHunter bioinformatics platform.

Evotec has been developing PanOmics and PanHunter as two platforms specifically for:

- ▶ **PanOmics:** High-throughput and cost effective omics data generation
- ▶ **PanHunter:** High-throughput omics data analysis

Combining PanOmics and PanHunter with our world-leading pre-clinical safety and toxicology profiling platform (Cyprotex) is opening new doors to predictive pharmacology and toxicology.

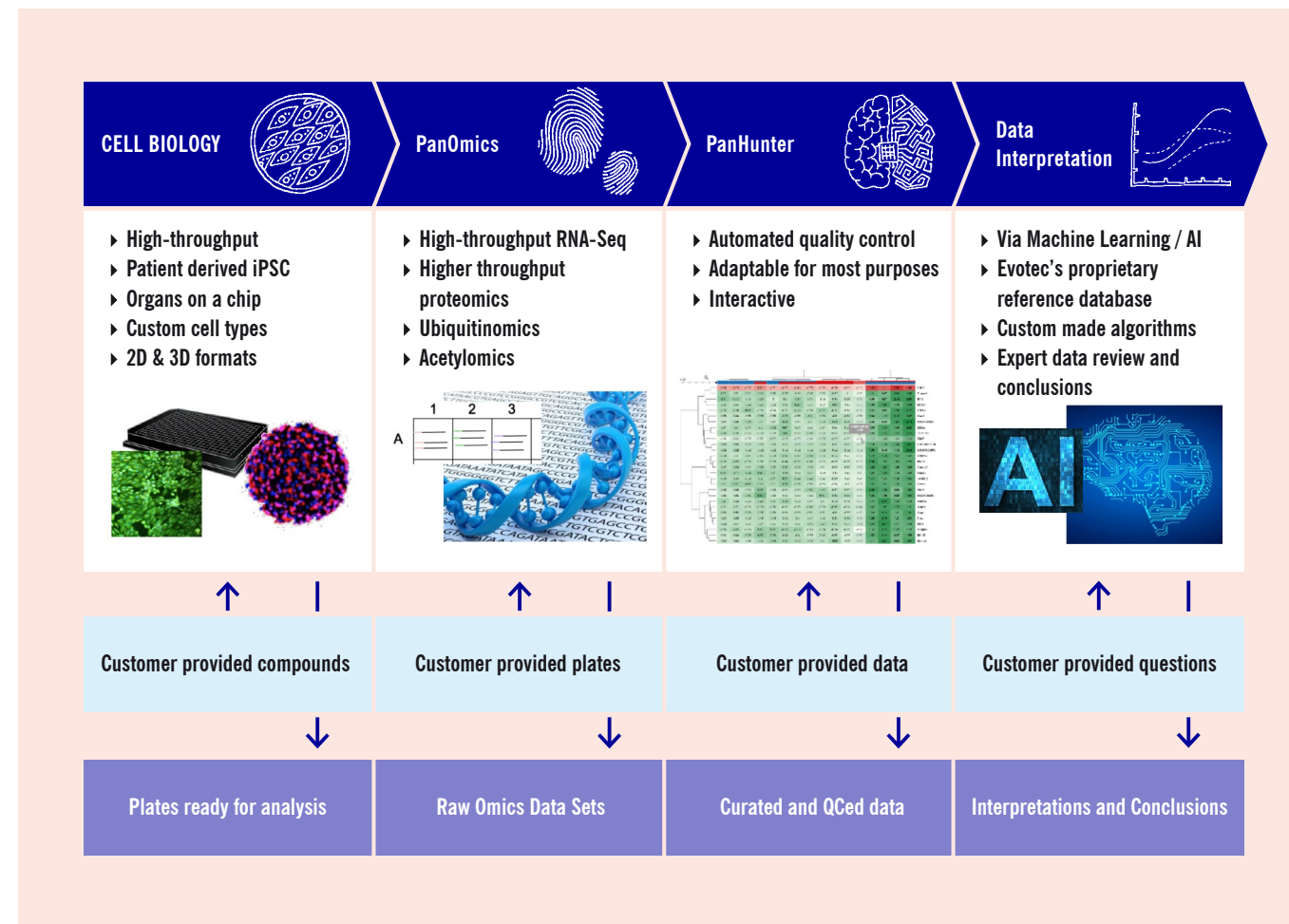
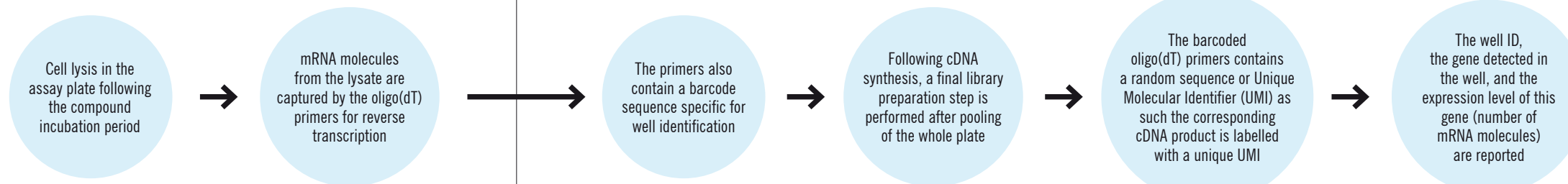
EVOTEC'S INTEGRATED CUSTOM SERVICE OFFERING

Transcriptomics is finally entering into mainstream toxicology, not only for DILI but also other areas of toxicology such as cardiotoxicity and nephrotoxicity. Through a custom transcriptomic service, Evotec can evaluate different organ-specific models to investigate mechanisms of toxicity and potential safety liabilities.

Evotec has built vast expertise in all areas relevant for the complete transcriptomic analysis workflow:

- ▶ Advanced cell culture systems for *in vitro* toxicology
- ▶ The most advanced high-throughput transcriptomics platforms
- ▶ Leading bioinformatics platforms for interactive multivariate data analysis
- ▶ Dedicated bioinformatics experts can design unique data analysis processes
- ▶ Advanced machine learning capabilities
- ▶ Data scientists to interpret large and complex data sets

OUR PROTOCOL STARTS WITH:



In practice, this service can be as flexible as needed for a given project ensuring Evotec is in a unique position to offer an end-to-end *in vitro* toxicology transcriptomics service that can be customised according to customer's needs.

EVOTEC'S HIGH-THROUGHPUT TRANSCRIPTOMICS PLATFORM

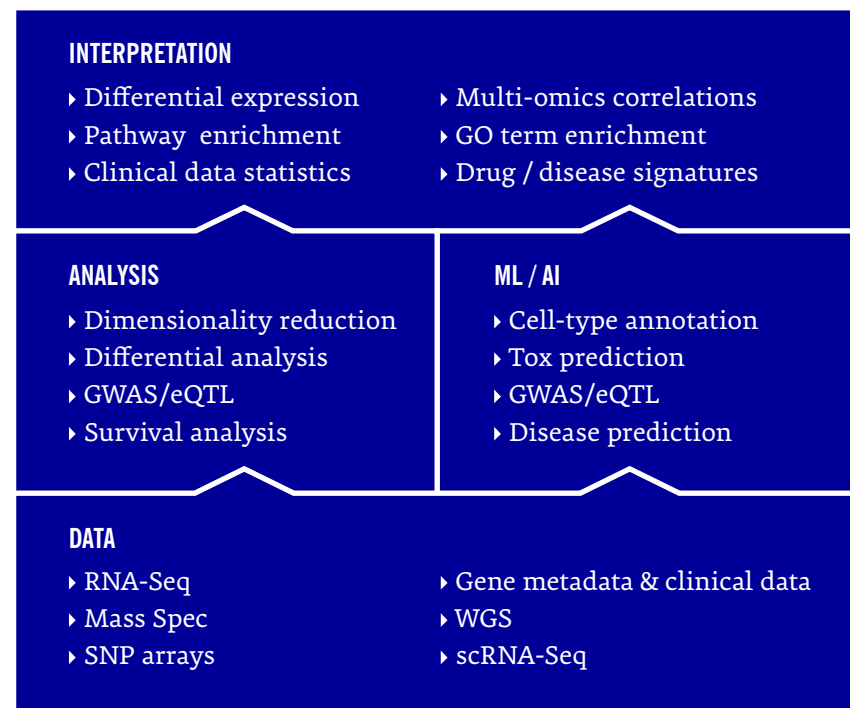
At the beginning of the millennium, the field of transcriptomics dramatically changed with the invention of RNA-Seq, a method based on next-generation sequencing

(NGS) techniques. The high-throughput transcriptomics NGS approach developed at Evotec was inspired by the elegant strategy of individual sample barcoding and pooled library preparation originally used in single cell RNA-Seq workflows.

The most important parameters of generated sequencing libraries are the diversity and the purity of the library. The diversity we define as the number of counts, or mRNA molecules, detected with a given sequencing depth. The purity shows the size of the fraction of library molecules lacking the proper well barcode sequence and reflects the specificity of enzymatic steps.

Evotec now has a robust automated cost-efficient technology able to detect up to a million mRNA molecules per well. Remarkably, the established generic protocol is performing stably over diverse set of cell systems, including more than 25 cell types and even 3D microtissues.

PanHunter architecture



EVOTEC'S PANHUNTER BIOINFORMATICS PLATFORM

As with many exciting new technologies, such as NGS the amount and complexity of data generated quickly grows to a level where specialised tools and software are necessary to manage, analyse, and interpret them in a biologically meaningful way.

Evotec has taken up this challenge by developing the PanHunter platform, which offers a comprehensive and consistent workbench for multi-omics projects at industrial scale. PanHunter combines data from a variety of different sources:

- ▶ Genomics
- ▶ Transcriptomics
- ▶ Proteomics
- ▶ Metabolomics
- ▶ Specialised CRISPR screens
- ▶ Cell painting and more ...

With NGS, for example, it covers the entire pipeline:

- ▶ Storing and managing the initial sequencing data
- ▶ Quality control and differential expression analysis
- ▶ Investigating the implications for down-stream processes, like pathway regulation or gene network analysis

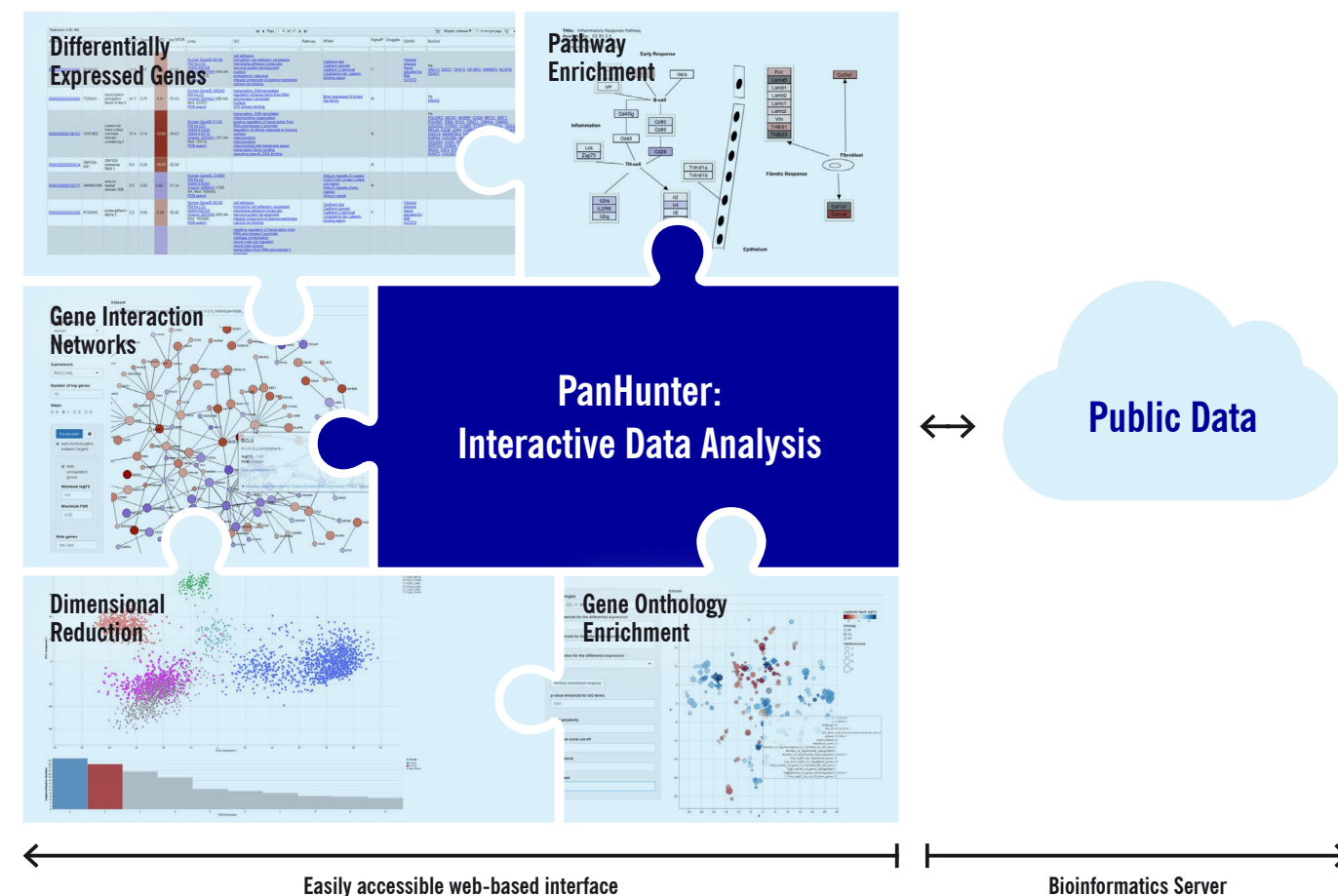
As a user-oriented and interactive web application, PanHunter provides an intuitive interface even for scientists with minimal experience in -omics data analysis. As soon as the data is pre-processed, integrated, and uploaded into PanHunter, the user can interact freely with it through a web browser, while most calculations are performed in the background. This behaviour makes it easily possible to run several operations in parallel or test different inputs or sub-sets of samples. Results are presented on-the-fly and can immediately be interpreted or used as input for subsequent steps. This direct feedback distinguishes it from other tools.

- ▶ PanHunter takes care of file management, can handle many projects in parallel, and automatically generates reports on essential aspects of data analysis. Throughout the entire PanHunter workflow, it is possible to extract the underlying data if required.
- ▶ PanHunter is accessible from virtually anywhere on the globe and features full multi-user support. Therefore, several scientists can efficiently work simultaneously on the same project, which makes PanHunter an ideal platform for interdisciplinary collaboration

within Evotec, but also with our numerous partners. When using PanHunter, scientists have a plethora of in-depth analysis tools at their disposal. Users can get an overview of large datasets with the help of well-established dimensionality reduction algorithms (e.g. PCA or t-SNE) and subsequently try to identify samples clustering due to similar characteristics. Thus, deconvolution of effects and deducing meaningful interpretations becomes significantly more comfortable with the help of PanHunter.

- ▶ New features for PanHunter are often developed in close collaboration with and tailored to the requests of power-users, such as the Evotec's transcriptomics or data scientist teams.
- ▶ PanHunter has dedicated implementations for single-cell RNA-Seq, shallow plate sequencing, deep proteomics, and genomics.

In summary, within PanHunter, we can handle multi-omics experiments starting from only a few entries up to large-scale projects with several tens of thousands of samples.



TRUE EXPERTS

IN TOXICOLOGY & TRANSCRIPTOMICS



Dr Rüdiger Fritsch

Rüdiger studied biology at the University of Göttingen and received his PhD for work done in the field of developmental biology at the Max Planck Institute for biophysical Chemistry, followed by a postdoc at EMBL in Heidelberg. In 1999, he joined the biotech industry, building a bioinformatics and data management team and infrastructure at DeveloGen, a functional genomics company. He received further postgraduate training in Pharmaceutical Medicine and technology management. Since 2017, he leads a portfolio of PanOmics projects at Evotec.



Dr Paul Walker

Paul is the Head of Toxicology at Cyprotex Discovery Ltd in the UK where he is responsible for the R&D strategy, operations and study management within the toxicology group. Paul obtained his Ph.D. from King's College London in Molecular Toxicology and was awarded the Tadion-Rideal prize for molecular sciences (2004). During this period he linked transcriptomic signatures to molecular initiating events (MIEs) to determine the mode of action and to distinguish signatures between different classes of toxic chemicals. Paul joined Cyprotex in 2010 with his research interests focused on the use of *in vitro* tools such as high-content imaging in safety assessment as well as the development of novel *in vitro* models. Paul works closely with Evotec parent company, on several joint collaborative research projects.

5 MINUTES WITH

DR RÜDIGER FRITSCH AND DR PAUL WALKER ON THE IMPORTANCE OF COMBINING TOXICOLOGY AND TRANSCRIPTOMICS IN DRUG DISCOVERY

What technological advances have taken place to allow high-throughput transcriptomics?

Rüdiger: Transcriptomics has undoubtedly greatly benefited from the rapid and ongoing development of next-generation sequencing techniques. Deep sequencing of RNA is becoming faster and more efficient with every new generation of instruments coming to the market. Several strategies have recently evolved which have paved the way for high-throughput transcriptomics:

1. Barcoding of samples allows for sample pooling, reducing the time and labour needed for the sample preparation steps and increasing the amount of samples processed in parallel.
2. Barcoding and multiplexing strategies allow for the optimised use of the available capacities on NGS machines.
3. Miniaturisation of steps, e.g. moving from a tube format towards a plate format, is also beneficial when it comes to moving towards high-throughput processes.

»Deep sequencing of RNA is becoming faster and more efficient with every new generation of instruments coming to the market.«

4. The data analysis and interpretation of large data sets are continuously improving.

Paul: Also on the *in vitro* assay side we have miniaturised our organ models to 96 and 384 well formats allowing the generation of a large number of samples, fulfilling the requirement to test drug dose response curves and assess multiple time points. These processes include sample management, barcoding, liquid handling, and data analysis. Therefore generate samples and/or data very rapidly and cost effectively.

Which challenges had to be overcome in order to develop a HT transcriptomics platform at Evotec?

Rüdiger: We set out to scale molecular profiling with RNA-Seq to be applicable to high-throughput workflows. With increasing instrument capacities and cost-effectiveness, we focussed on the sample preparation. We use sample barcoding and multiplexing to reduce sample numbers to be handled automating the full process and developing a robust and stable workflow. The major challenges were the compatibility with automation, and the conversion of processes from tubes or 96 well formats to a 384 well format.

Paul: A significant challenge was to develop a robust cell lysis and sequencing protocol that was compatible with a variety of cell samples, from tissues to *in vitro* organoids consisting of only a few thousand cells.

What cell types is it possible to use with the transcriptomics platform at Evotec?

Rüdiger: Since we started the development of our HT transcriptomics platform, the workflow has been used on more



»A more in-depth analysis is often required in late-stage pre-clinical projects.«

than 25 cell types as well as 3D microtissues, and every cell type tested so far has proven to be compatible with our protocol.

Paul: We are experienced with a wide range of different cell types and formats including primary cells, iPSC-derived cells and standard cell lines in either 2D or 3D format. The type of cell-based model is often driven by the organ toxicity. For example, we have different models for cardiotoxicity, neurotoxicity, nephrotoxicity and DILI. Many of our clients request specific cell lines and we work alongside our clients to source and evaluate these before proceeding with the screening protocol.

How long does the data analysis take to perform?

Rüdiger: Data analysis timelines strongly depend on scale and requirements of the experiment and are therefore project-specific. In early drug development stages, a faster turnaround is often desired, where we currently explore data analysis paths for routine use. A more in-depth analysis is often required in late-stage pre-clinical projects.

Paul: We are also exploring standardising the format of reports, which best reflect the needs of our clients and will be amenable to automation, reducing the time required. Apart from a report,

our clients can also get access to PanHunter, where they can interactively explore the generated data or collaborate with us for further data exploration.

Typically, how many transcripts are analysed and what are the number of reads?

Rüdiger: All polyadenylated mRNAs are being analysed and often several hundred genes are found to be differentially regulated in a significant way. Reads are considered less important (compared to deep RNA-Seq) as the use of UMIs allows to look for UMI counts, e.g. counting mRNA molecules.

»Apart from a report, our clients can also get access to PanHunter, where they can interactively explore the generated data or collaborate with us for further data exploration.«

Paul: The breadth of the cellular pathways we have found to be perturbed and importantly linked to modes of action of the drug is very exciting to see, and holds great promise to advance our capabilities of improved risk assessment from the mechanistic information.

What other technologies are on the horizon?

Rüdiger: The technological breakthrough of sequencing techniques in the early 2000s is closely followed by the development of sophisticated instrumentation for mass spectrometry. Therefore, with increasing cost-efficiency other -omics fields are advancing. Therefore, both HT-proteomics and HT-metabolomics technology as well as single cell approaches will be the next logical steps.

Paul: Often cellular responses to toxic compounds can be affected by disease or genetics. Access to patient-derived iPSC will help us greatly in understanding toxicology in different populations and help us explain why some patients experience an adverse effect and others don't. This is especially important in DILI where effects can be idiosyncratic in nature and only affect a very small percentage of the population and are difficult to identify.

Thank you for your time!



4 QUESTIONS

DR THOMAS SIEGMUND

CHAPTER
04



SHORT SUMMARY OF SCIENTIFIC CAREER

Thomas received his PhD in biology from the Free University of Berlin, working on developmental biology and neuroscience in *Drosophila*. As a postdoc Thomas moved into bioinformatics – at a time when there was no formal bioinformatics training yet, but a strong need to analyse the first animal genome data sets. He soon provided some of the first bioinformatics seminars at the FU Berlin. In 2002, he joined the former DeveloGen, now Evotec International, in Göttingen. Since this time, he has supported target ID and screening projects with bioinformatics expertise and tools. Since January 2019, Thomas is responsible for bioinformatics and biostatistics globally within Evotec.

1 How are you involved in this project?

About six years ago, I developed the first prototype version of PanHunter. Looking back, that was just a small collection of straightforward tools. Nevertheless, this early PanHunter version turned out to be very useful for a first complex RNA-Seq project.

We were then fortunate enough to hire excellent bioinformaticians and programmers who advanced the software development – turning PanHunter into the unique multi-omics data analysis platform it is today. My role is to align the development of PanHunter with the needs of our scientific projects.

2 So what was then your initial idea when setting up PanHunter?

At Evotec, we are invested in generating very rich, high-quality data in transcriptomics, proteomics, and genomics from tens of thousands of samples. The goal being to achieve a much better understanding of human diseases, our biological models, and compound activities. Data processing, analysis and interpretation on this scale can be challenging.

Therefore, the key idea behind PanHunter is very simple: we want to enable the project scientists to dive into complex data sets without getting lost. These colleagues, often biologists by training, have deep insights into the biological systems. They understand what is known about the molecular biology of a

disease and where the gaps in our knowledge are. PanHunter enables them to interact directly with the data to get faster and better answers.

To allow for this, we need to:

1. Remove much of the complexity of common bioinformatics tools and hide it behind a user-friendly interface.
2. Results need to be presented in a very convenient way.

For this reason, PanHunter uses lots of interactive graphics and tables to present the results.

3 What is so special about Evotec's approach?

If you look for bioinformatics tools supporting data analysis of transcriptomics or genomics data, you will very often find the concept of a "pipeline": At one end, you feed your raw data into this pipeline. Then there are algorithms moving the data through this tube, and at the other end, you get a ranked list of, for example, differentially expressed genes. This approach is similar to how bioinformaticians, like me, have worked for many years.

About five years ago at Evotec, we did the first study with hundreds of RNA-Seq samples in a multivariate design – different tissues, different treatment conditions, and time points. We quickly realised that a simple pipeline tool would not do the trick. In such a complex data set, there is a considerable number of different ways to look at the data. PanHunter is different from these pipeline tools. It allows the users to explore the data along a very individual path of research, and it encourages them to do so.

Very often in biology, it is all about the details. A small number of dysregulated proteins can play a crucial role in a disease context. In such cases, you may simply want to look up the abundance of one or more proteins in a set of samples. PanHunter has table views for this, but also very versatile interactive visualisations, like corresponding pathway maps that can be accessed through the interface or by clicking a hyperlink. PanHunter is also very open: at any point, you can download the data in various formats.

There is another, deeper reason why PanHunter is different from other tools: right from the start, the users and the specific needs of

our internal research programmes have driven the development of the various apps in PanHunter. If we receive positive feedback from those users, we can quickly integrate new features into the main platform. This is another way to enable the data analysts to be more productive – they help to design their own tools.

4 Tell us about your vision – what do you expect from PanHunter in the future?

That is a tough question. Right now, PanHunter is expanding in many different directions. We scale it to handle larger and larger data sets, which brings computational challenges but also forces us to re-think parts of the user interface.

Currently, we have decent support to integrate multi-omics data. However, I am pretty sure we will discover new ways to do this in a deeper and more meaningful way. With the rapid growth of our data sets, there are more and more opportunities to apply machine-learning techniques.

*For any further questions on Evotec's new
tox prediction platform, please contact:*



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