Evotec's modular target identification system: PanOmics TargetID Framework.

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The PanOmics TargetID Framework - a modular system designed to assist clients in achieving their R&D goals - is an integral part of Evotec's drug discovery program. Panomics is the overreaching term used to refer to the analysis of multiple data sets obtained from clinical studies and related biomedical sources. The TargetID Framework can be thought of as a fully flexible workflow that employs the power of panomics and enables the client to leverage additional work packages and/or -omics data, to aid the transition from target identification to IND.

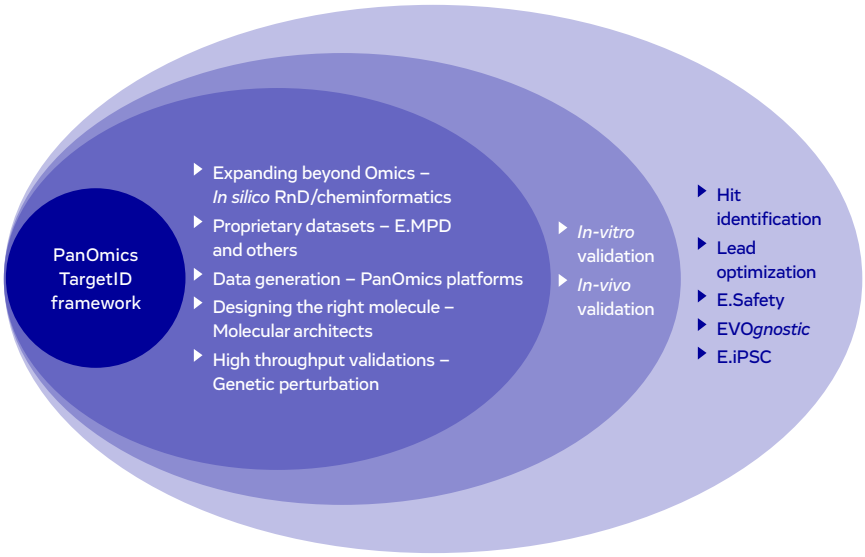

Figure 1: *The PanOmics TargetID Framework - at the heart of Evotec's drug discovery*

One of the key features of this modular process is that the final target ID strategy can be chosen by the client according to their own particular requirements; in other words, the Framework is not a rigid set of rules that is applied to every R&D project, irrespective of the indication or disease spectrum involved.

PanHunter
Interactive Omics Analysis

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The Framework

The first step of our TargetID Framework involves building a mutual understanding of the client's definition of a successful target, based on the interpretation of the indication as well as the relevance and balance of triaging criteria such as safety, novelty, and modality. This stage is supported by machine-learning and AI (LLM, NLP) to retrieve all relevant information and data sets from public and proprietary databases. This includes analysing the genomics of the patient cohort to visualize causality as well as investigating transcriptomics, proteomics and metabolomics to obtain a detailed understanding of the disease and the mechanisms of disease progression. The resulting data sets can be mined with Evotec's PanHunter dedicated workflows and assessed by an expert panel.

Inferential statistical analysis, whereby the data sets are assumed to be a sample from a very much larger population, becomes the tool of choice once a target pool has been generated. Targets in that pool can now be assessed and ranked with respect to disease specificity, safety, and novelty. By deriving targets from mined patient cohorts, not only are patient cohorts stratified, it is also possible to pinpoint the position of the target in the disease process or even the affected cell type. This spectrum of information about the target can then be used to support virtually all stages of drug discovery. Finally, the client is presented with a report that is much more than just a list of disease-relevant targets: it will contain assessments by an expert panel with data pertaining to the most significant targets and details of the processes that led to the selection of those candidates.

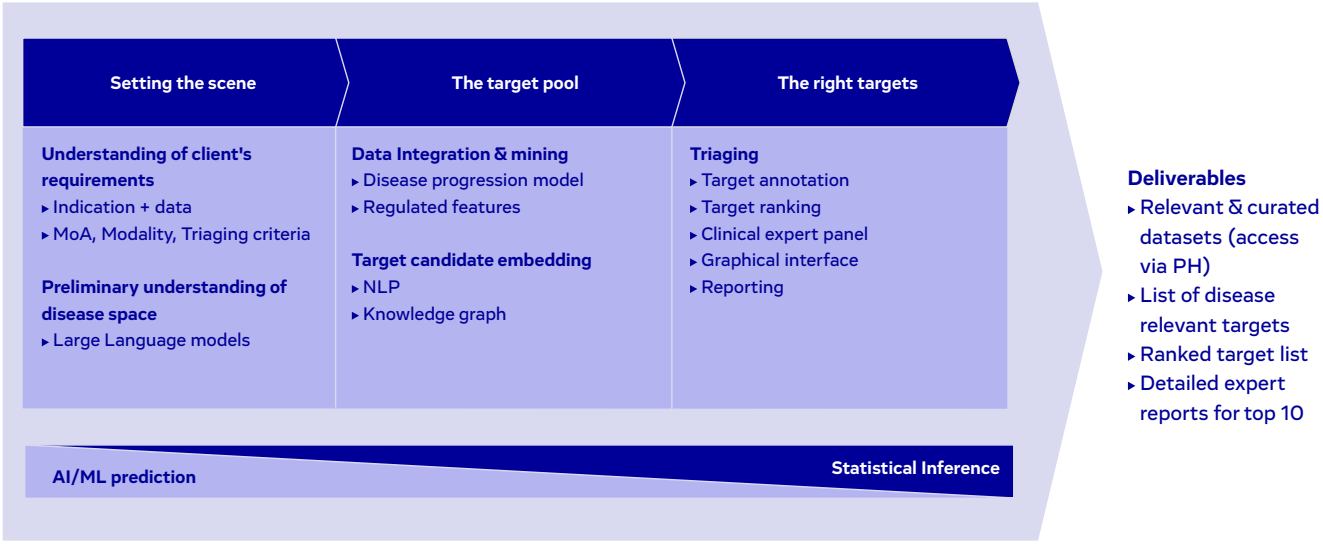

Figure 2: *Evotec PanOmics TargetID framework – A modular system adjustable to client requirements and any OMICs data*

Thus, transparency is assured and the client can concentrate on the next phase of the path towards an IND.

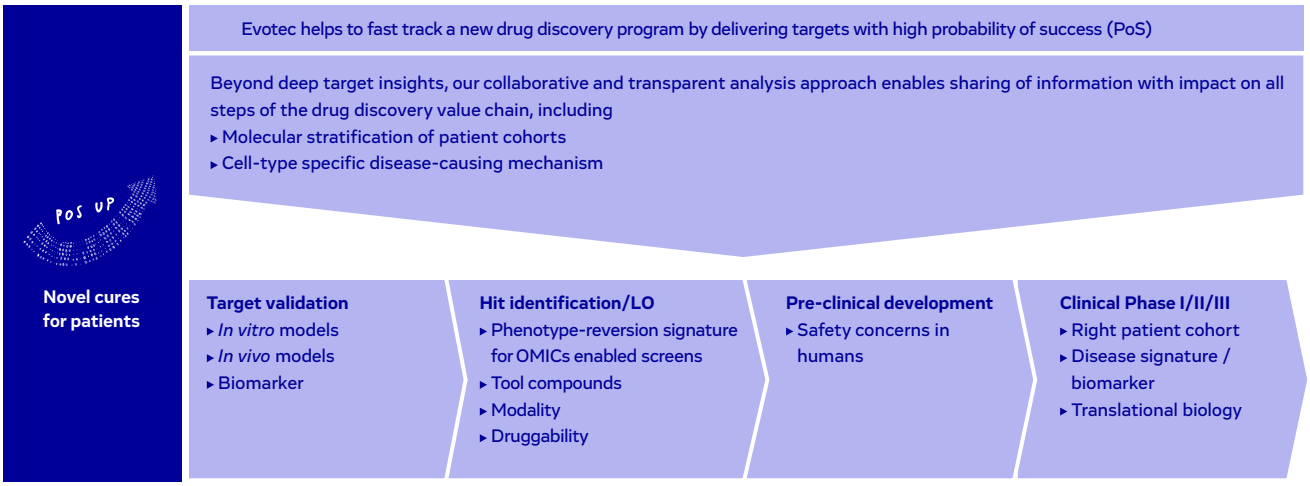

Figure 3: *Evotec PanOmics TargetID framework – A modular system adjustable to client requirements and any OMICs data*

A case study

In order to appreciate the application and relevance of the PanOmics TargetID Framework in more detail, it is useful to examine a case study that, in this example, used wide-ranging data obtained in part from the NURTuRE kidney biobank (www.nurturebiobank.org).

Data is assessed and weighted on the basis of analyses designed by an expert panel. The weights of categories such as druggability, disease association, biology, safety, commercial factors, and efficacy lead to final scores for each selected target that can then be used to rank the targets in order of probability for success and viability (figure 4).

Figure 4: *Scores of the PanOmics TargetID framework. Expert designed weights transform an ocean of information into comprehensive scores*

		Target				
		Cand. 1	Cand. 2	Cand. 3	Cand. 4	Cand. 5
Druggability	Druggable active site	0,75	0,25	0,00	0,75	0,00
	Selectivity	0,00	1,00	0,25	1,00	0,00
	Avalibility of tools	0,25	0,00	0,00	1,00	1,00
	Known ligands/receptors	1,00	0,50	1,00	1,00	0,75
Disease association	Publications linking target and disease	0,00	0,75	0,50	1,00	0,00
	Genetic disease association	0,75	0,00	0,50	0,50	1,00
	Expression in integrated data	0,25	0,25	0,00	1,00	0,25
Biology	Regulatory unit	0,25	0,75	0,00	1,00	0,50
	Cell type specificity	1,00	0,50	0,50	0,50	0,00
	Receptor ligand association	0,25	0,50	0,00	1,00	0,00
	MoA understanding	0,25	0,50	0,00	1,00	0,50
Safety	Known target-related toxicity	0,25	0,25	0,25	0,50	0,00
	Publications with adverse phenotypes	0,50	1,00	0,50	1,00	0,75
	GWAS data	1,00	0,00	0,75	0,75	1,00
	Tissue-specific expression	0,00	0,50	0,50	0,50	1,00
Competition	Clinical trials	1,00	0,75	0,00	0,00	0,25
	Limited patents about target in disease	0,25	0,25	0,00	0,00	1,00
	Targets in discovery	0,75	0,50	0,25	0,00	0,75
	Publications total	0,50	0,75	0,00	0,50	0,25
Efficacy	target modality	0,25	0,75	0,50	1,00	0,00
	In vitro data supporting activity on the target	0,00	0,00	1,00	1,00	1,00
	In vivo data supporting activity on the target	0	0,50	0,50	0,75	0,25
	In vivo data supporting activity in disease model	1,00	0,50	0,50	0,75	0,00
	Clinical trials	0,00	0,50	0,00	1,00	0,25

	Target				
	Cand. 1	Cand. 2	Cand. 3	Cand. 4	Cand. 5
Druggability	0,50	0,44	0,31	0,94	0,44
Disease association	0,33	0,33	0,33	0,83	0,42
Biology	0,44	0,56	0,13	0,88	0,25
Safety	0,44	0,44	0,50	0,69	0,69
Competition	0,63	0,56	0,06	0,13	0,56
Efficacy	0,31	0,45	0,50	0,90	0,30

Summary	
Candidate 4	0,73
Candidate 5	0,46
Candidate 2	0,45
Candidate 1	0,45
Candidate 3	0,31

Target score (weighted)

0 1

One such target, selected by analysis of the data on fibrosis and kidney disease, was Endothelin 1 (EDN1). As can be seen from the target summary (figure 5), the traffic-light system indicates a high probability of success ("green") in all categories except for commercialization (competitor activity is high) and slight safety concerns.

Figure 5: *Target Summary: Endothelin 1 (EDN1) In Fibrosis and kidney disease*

Druggability	Druggable active site		SMOL, trough a receptor (ETA/ETB) Antagonist, Peptide-based drugs have been successfully developed for various conditions.
	Selectivity		selective ETA and ETB receptor antagonists (e.g. bosentan, sitaxsentan, macitentan, and ambrisentan) are availible.
	Availability of tools		CPDs commercially available.
	Known ligands & receptors		EDN1 (ET1)1 is secreted peptide and three endogenous isoforms are known to exist (ET1, ET2 and ET3) They are ligands for G-protein coupled receptors EDNRA and EDNRB.
Disease association	Publications linking target and disease		Highly associated to fibrosis, and as well to kidney and liver fibrosis.
	Genetic disease association		Some genetic evidence for association with kidney or liver disease.
	Expression in integrated data		EDN1 widely expressed across multiple organs similarly to its receptors. Predominant expression in kidney: EDN1: Proximal tubules and Endothelial cells; EDNRB: Mesangium and Connecting tubules; EDNRA: mostly expressed in the Mesangium.
Biology	Regulatory unit		It is itself a key regulator. The downstream effects, i.e. binding to ETA/ETB are well studied and are upstream of hypertension and Vasodilation respectively. The regulation of expression of EDN1 is also well studied and significantly regulated by AP-1 with GATA-2. ProteoET1 is cleaved by furin-like proteases and ECEs both potential regulation opportunities.
	Cell type specificity		Expressed in different kidney cell types (single nuclei data).
	Receptor ligand association		ET-1 selective for ETA and ETB receptor. ETA receptors have a higher affinity for ET-1 than ETB receptors, while ETB receptors can bind to ET-1/2/3.
	MoA understanding		END1 activates ETA/ETB. ETA receptors primarily activate intracellular signaling through Gαq proteins, leading to phospholipase C (PLC) activation, release of intracellular calcium ions (Ca2+), and protein kinase C (PKC) activation. ETB receptors can activate multiple signaling cascades, including phosphoinositide 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) pathways, by coupling to Gαq, Gαi, and Gαs proteins.
Safety	Known target-related toxicity		Mild and manageable. Hypotension Fluid retention, pulmonary complications, off-tissue effects due to broad expression of ETA/ETB.
	Publications with adverse phenotypes		Minor side effects of EDN1 signaling inhibition (peripheral edema, anemia , abnormal liver function)[2].
	GWAS data		Link to cancer but antagonizing is reported to be beneficial[1].
	Tissue-specific expression		GTEx Portal: highest expression in adipose tissue, lung, breast (mammary tissue) and tibial artery; very low in liver; slightly expressed in kidney, broadly expressed according to HPA including brain, kidney.
Competition	Clinical trials (in indication space, phase)		Several within indication space of kidney disease and fibrosis (Phase 3+).
	Limited patents about target in disease		Patent present in indication space.
	Targets in discovery		Multiple commercially available compounds and drugs on the market.
	Publications total		1606 publications of EDN in Fibrosis, 343 of which are in kidney disease.
Efficacy	target modality		SMOL ETA, ETB or dual inhibitor, alternatively: Abs or sponging (ET traps).
	In vitro data supporting activity on the target		Western Blot analysis showed significant reduction in ET-1 expression levels in cell lines upon targeting endothelin receptors[3].
	In vivo data supporting activity on the target		ETA inhibitors reduced ET-1 mRNA expression levels (outer medulla) in mice post-IR injury.
	In vivo data supporting activity in relevant model		EDN1 modulation ameliorates kidney disease phenotypes in amongst others UUO and IRI.
	Clinical trials		Clinical trials have repeatedly proven efficacy of target on indication.

[1] *PMID: 12563310*
[2] *PMID: 27912207*
[3] *PMID: 22075705*

Continuing with EDN 1 as the selected target, an expert-curated deep dive was initiated into all the available data. EDN1 and its receptors, EDNRA and EDNRB, are expressed in many organs whereby, in the kidney, EDN1 is predominantly expressed in the proximal tubules and endothelial cells, EDNRA in mesangial cells and connecting tubules, and EDNRB in the mesangial cells. Thus, the expression of EDN1 and its receptors is associated with cells that are relevant to the pathology of kidney disease. Examination of further open-source

databases revealed that there was genetic evidence for the association of EDN1 expression with kidney disease, although it was also noted that there were genetic associations with cardio-vascular function as well. The latter would be flagged as a possible safety concern. A search of the medical literature also revealed evidence for association of EDN1 with fibrosis in diabetic nephropathy, cardiovascular complications in patients with renal failure, and renal survival rate in patients with chronic glomerulonephritis. Endothelin receptor antagonists were also reported to be effective in rodent models of kidney injury (figure 6).

Figure 6: *Target Efficacy and Druggability ETA antagonists are available and efficacious in rodent models of kidney injury*

Screening of drug candidates against Endothelin-1 to treat hypertension using computational based approaches: Molecular docking and dynamics simulation

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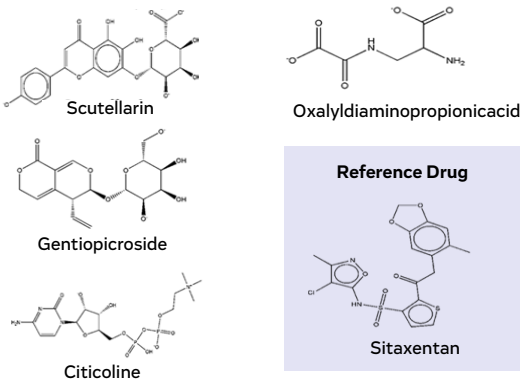
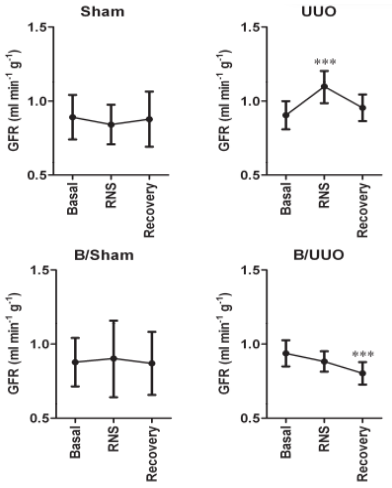


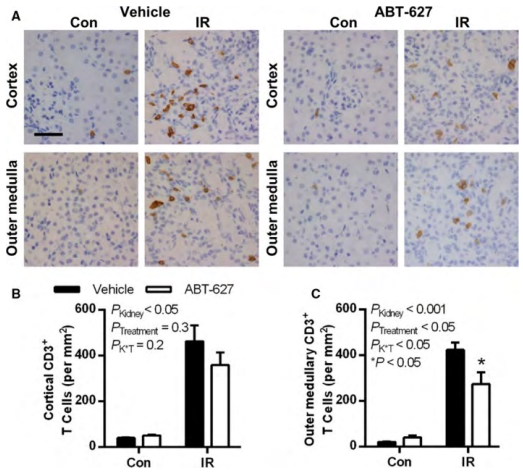
- Analysed a library of 5,000 phytochemicals against Endothelin-1
- Top 4 hits were selected and found to be potential drug targets for the inhibition of upregulated Endothelin-1[1]

Bosentan



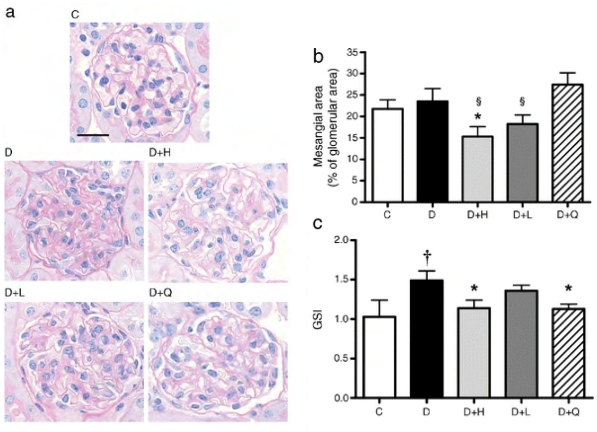
ET-1 receptor antagonist, normalized the renal response to renal nerve stimulation in the unilateral ureteric obstruction in rat model[3]

ABT-627



ET-1 receptor antagonist attenuated T-cell numbers in renal ischemia reperfusion injury mice model[2]

Avosentan



The selective, non-peptidic inhibitor of ETA, ameliorates nephropathy and atherosclerosis in diabetic mice[4]

[1] *PMID: 35981003 (paper was retracted by the authors due to unlicensed software usage)*
[2] *PMID: 30198212;*
[3] *PMID: 25157662;*
[4] *PMID: 19862499*

Thus, having identified a promising target, the detailed analysis of all available data suggests an underlying molecular mechanism for the selected disease. In this case, it appears that the inhibition of the endothelin-1 signaling pathways will directly affect three major pathological processes associated with kidney disease: vasoconstriction, inflammation, and glomerular permeability. Specific models for screening active substances can now be designed and the drug discovery program initiated.

Conclusion

This overview of Evotec's PanOmics TargetID Framework has illustrated the advantages of a flexible, client-orientated target identification process based on the application of data science to a specific indication. Analyses driven by expert assessments will provide the client with a ranked list of potential targets, and a comprehensive report detailing clinically and commercially relevant information. The transition from target identification to lead determination is both accelerated and optimized.