A holistic approach to the safety assessment of exploratory drug targets

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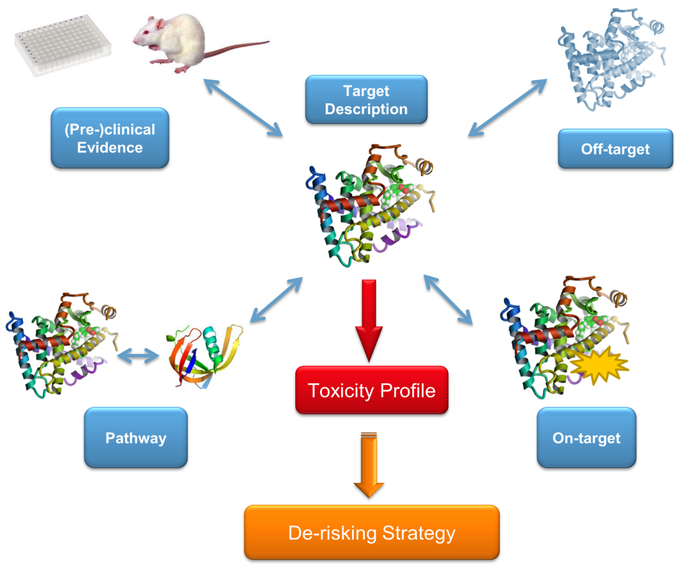
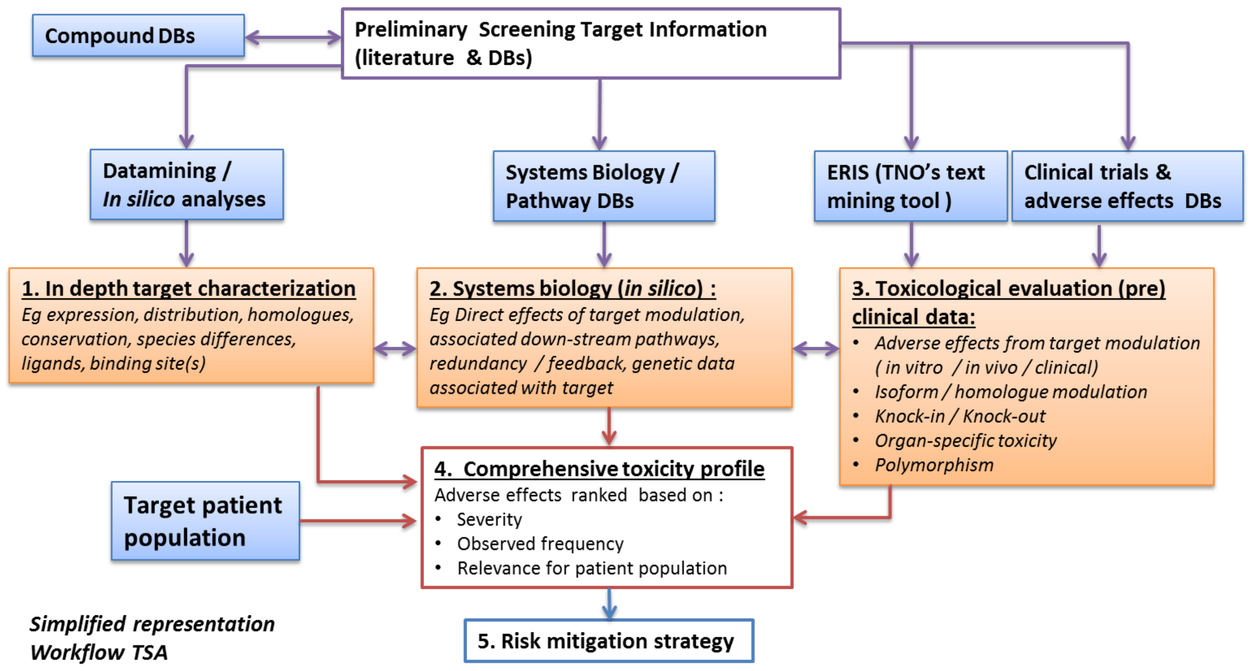
TNO innovation for life

INTRODUCTION

Safety liabilities of a drug and - more importantly - its associated target are often not placed in a broad enough context, which contributes significantly to the high drug attrition rate in the pharmaceutical industry. There is thus an evident need for an efficient and holistic target safety evaluation workflow that incorporates all aspects of target safety. At TNO – in collaboration with a pharmaceutical company – we have uniquely combined all necessary disciplines to develop a holistic workflow that collects, integrates and interprets all relevant information necessary to assess and rank potential toxicity issues of an exploratory target. The resulting workflow – benchmarked with HSP90 inhibition as demonstrator – serves as a blueprint for any company embarking on target nomination and validation.

FROM CONCEPT to WORKFLOW



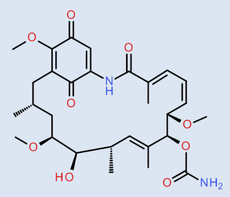


HSP90 Target Safety Assessment

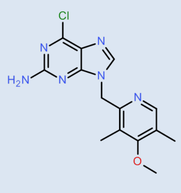
1a. HSP90 characterization

Biological function	HSP90 is an inducible molecular chaperone that functions as a homodimer. The encoded protein aids in the proper folding of specific target proteins by use of an ATPase activity that is modulated by co-chaperones. It performs key roles in the protein signaling pathway, protein folding, protein degradation, and morphologic evolution and antigen presentation.
Desired therapeutic effect	Preventing/reducing CNS diseases
Isoforms	• HSP90 alpha • HSP90 beta
Homologues ("isoforms")	• GRP94 • TRAP1
Expression profile	Ubiquitous
Ligand binding site	• ATP-binding site, N-terminal (high affinity, X-ray, eg geldamycin) • ATP-binding site, C-terminal (low affinity, no X-ray, eg novobiocin → potentially different binding sites)
Mechanism of Action	Inhibition of HSP90
Downstream effect	Up-regulation of HSP70

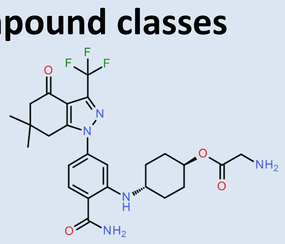
1b. Most advanced structural compound classes



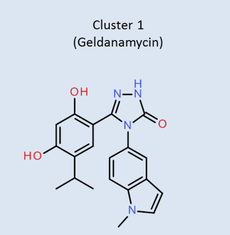
Cluster 1 (Geldanamycin)



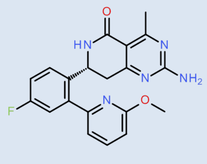
Cluster 2 (BIIB-021)



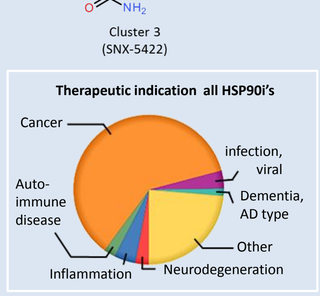
Cluster 3 (SNX-5422)



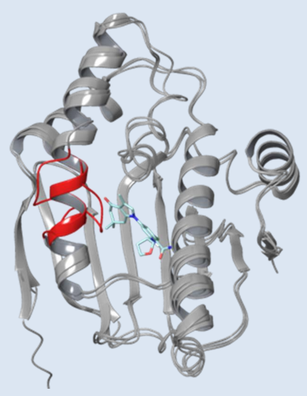
Cluster 4 (Ganetespib)



Cluster 5 (HSP-990)

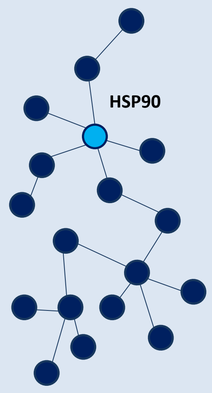


1c. Isoform selectivity



Overlay of HSP90 (1YET, blue), GRP94 (1YT2, orange), TRAP1 (4IVG , green), displaying high conservation of the N-terminal domain and inhibitor binding site *(left).* However, the ligand-induced fit of HSP90 residues 104-111 (red) allows for isoform-selective inhibition *(right).*

2. Systems biology



Network Analysis

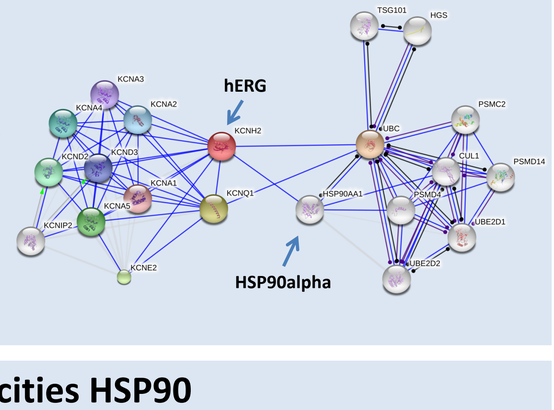
Pathways → Pathologies

Identified safety liabilities
- Cardiotoxicity
- Hepatotoxicity
- Nephrotoxicity

Mechanistic toxicity:

HSP90 related cardiotoxicity is partly due to the role of the alpha isoform in the proper folding and trafficking of hERG.

Right: StringDB plot showing protein-protein binding



3a. Major toxicities HSP90

cluster				
	1		?	• QTc interval prolongation • Chest pain • Hypertension • Cardiac arrest
	2	?	?	
	3	?	?	
	4	?		
	5	?	?	
	1			• Changes of hepatic enzyme levels • Necrosis • Lesions
	2	?	?	
	3	?	?	
	4		?	
	5	?		
	1		?	• Renal failure • Glomerular Injury • Metabolic disturbance
	2	?	?	
	3	?	?	
	4	?	?	
	5	?	?	
	1		?	• Visual disorders • Loss of pupillary light reflex • Abnormal waveforms • Changes in retinal cell layers
	2	?	?	
	3	?	?	
	4		?	
	5		?	
	1		?	• Nausea • Vomiting • Diarrhea • Anorexia
	2	?	?	
	3		?	
	4		?	
	5	?	?	

Green: no toxicity found; Red: toxicity reported; Green/Red: mixed profile in compound cluster

3b. Role of related proteins

GRP94 function	Cell defense mechanisms Angiogenesis Intestinal homeostasis Platelet activation & aggregation Muscle physiology & myogenic cell differentiation Embryogenesis
GRP94 potential tox	Gastrointestinal system Heart
TRAP1 function	Mitochondrial integrity & calcium homeostasis Cytoprotective pathways Anti-apoptotic activity
TRAP1 potential tox	Heart
HSP70 function	Protein folding Degradation Regulation Transport Prevention of Aggregation
HSP70 potential tox	None (beneficial effects for heart, GI, skeletal muscle)

4. Toxicity profile

Risk ranking of HSP90-related toxicity based on severity, frequency, evidence and Alzheimer's patient co-morbidities:

Cardiotoxicity & hepatotoxicity

Retinal, gastro-intestinal & renal toxicity

Immune toxicity, CNS effects

Osteoclast formation

Pulmonary & skeletal muscle effects

Reproductive effects

5. Risk mitigation

Risk mitigation encompasses:

- Specific screening assays for the identified (mechanistic) toxicities
- PK analysis (retinal toxicity)
- Assessment of reactive metabolite formation (liver toxicity)
- Optimization of compounds towards isoform-selective inhibitors

ACKNOWLEDGEMENTS

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