VISTARA NETWORK PROTEOMICS

WHITE PAPER

MISSION:

Expand the repertoire of druggable proteins and discover biomarkers/mechanistic markers with high-density protein network analyses.

DIGITIZING DRUG DISCOVERY WITH HOLISTIC VIEWS OF THE PROTEOME.

PROTEIN NETWORK ANALYSIS

- INTRODUCTION

  Cells respond to their micro-environment using a series of signals propagated thorough the web of interactions separated over time and space, thereby linking the core to the broader cellular system. Reprogramming the Interactome is a hallmark signature of cell's response to biological stimuli as well as key components of the signal transmission process. A combination of allostery, signalling complexes, ancillary small molecules, or scaffolding proteins allows key cellular proteins ('hubs') to recruit other proteins into their local sub-network and connect them to other cellular structures. While the binding of a drug to its target eventually alters biology of the cell, what happens next is poorly understood.

- OUR TECHNOLOGY

  Vistara's proteomics technologies employ *de novo* analysis using protein Mass Spectroscopy from cells, including drug-treated cells. In a single read, holistic observations of ~300 to ~2,000 proteins networking with a protein of interest is routinely achieved. Network information enables observation of the protein's behavior within its contextual framework, in a cell-specific manner, as well as high-density mapping of pathways, and cross-talk with others. Comparisons of protein networks from drug-treated and untreated cells identifies other key players with important roles in the drug's mechanistic response, which closely collaborate with the protein of interest. The networks are a rich source of information for identifying new drug candidates, targets, or biomarkers. Vistara's proteomics processes will find many uses in drug discovery research with unseen applications. All Vistara applications employ only native proteins.

OUR PLATFORMS

- **UBQuest** is a game-changing process for navigating the ubiquitin space and associating ubiquitin proteins with their cognate protein(s) targeted for degradation, such as a clinical target.

  Reversible protein ubiquitination regulates virtually all known cellular activites and their dysregulation is responsible for a large number of conditions including cancer, neurodegenerative or metabolic disease, viral infections, immune function impairments, and muscle wasting disorders.

  Unfortunately, the large repertoire of human genes encoding ubiquitins – 814 ubiquitinating enzymes and 107 deubiquitinases, and limited knowledge of cell type-, cell cycle-, or target-dependent expression of ubiquitins confounds progress in applying ubiquitin biology in drug discovery. UBQuest mitigates these problems. Patents pending.

  In a single read, UBQuest simultaneously identifies E1, E2, or E3 ligase enzymes, DUBs, any accessory proteins, and chaperones associating with the protein of interest, as well as alterations upon drug treatment. Downstream assays apply UBQuest information to identify the protein targeted for degradation, map interacting domains, and characterize any other interacting proteins. UBQuest works seamlessly with PROtac technology, being upstream of it.

  Drug discovery applications of UBQuest:
  - Direct identification of Ubiquitins expressed in cells
  - Target specificity
  - Accessory factors interacting with the UBQ system
  - UBQ alterations with drug intervention (or other physiological change)

- **STRINGseeker** is Vistara's protein discovery engine and a useful adjunct to drug discovery applications.

  STRINGseeker is a highly multiplexed, iterative, and scalable, *in vitro* physical association process for selective enrichment, purification, and sequencing (by Mass Spec) of proteins interacting with any protein of interest. In comparative mode, the process is employed to identify changes in protein network composition involving 300 – 2000 other proteins interacting with a clinical target protein or as a consequence of drug intervention. Patents pending.

  Drug discovery applications of STRINGseeker:
  - Mechanistic studies of drugs or drug candidates
  - Compound/Biologic characterization
  - Identification of new drug candidates or combinatorial targets
  - Phenotyping outcomes of CRISPR knock-outs, recombinant expression, etc.

  Protein data are analyzed using VistaLyzer, a software suite for analysis of protein information. The MS output is queried to identify pathways, domain information, protein complexes, signalling mechanisms, and kinase/phosphatase cascades, and other relevant information by comparisons with available databases.

Data from all our processes are validated using Western Blot, ELISA, recombinant protein expression, Proximity Ligation, Cell sorting, enzyme assays, or other downstream assays, some of which have been optimized for use in detecting protein interactions.

- **Phenotyper-C** provides a comprehensive proteomic fingerpriniting of a cell type

Quantitative comparisons of the proteins in cells of differing lineages provides a useful strategy for identifying molecular signatures characterizing each, as well as identifying line-specific markers specific to the sample, tumor initiation, or disease progression.

Phenotyper-C interrogates 12 – 20% of all proteins expressed in the cells using Mass spectroscopy, and combines structural and functional aspects of the proteins.

The process can be applied for molecular phenotyping of cells or to evaluate the similarities and differences between cell types via proteomic phenotyping.

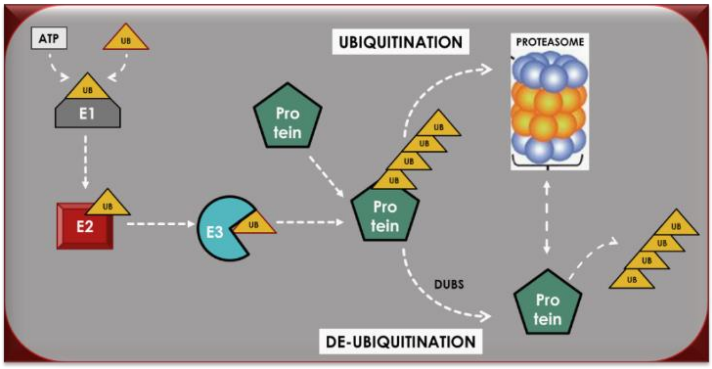
Vistara Processes & Sample Data

UBQuest: *Getting to the specifics*

Protein ubiquitination is a fundamental mechanism for recycling proteins in cells

This reversible process is inducible by various stimuli

The process affects not only the half-lives of target proteins, but also their structure, activity, localization and interaction

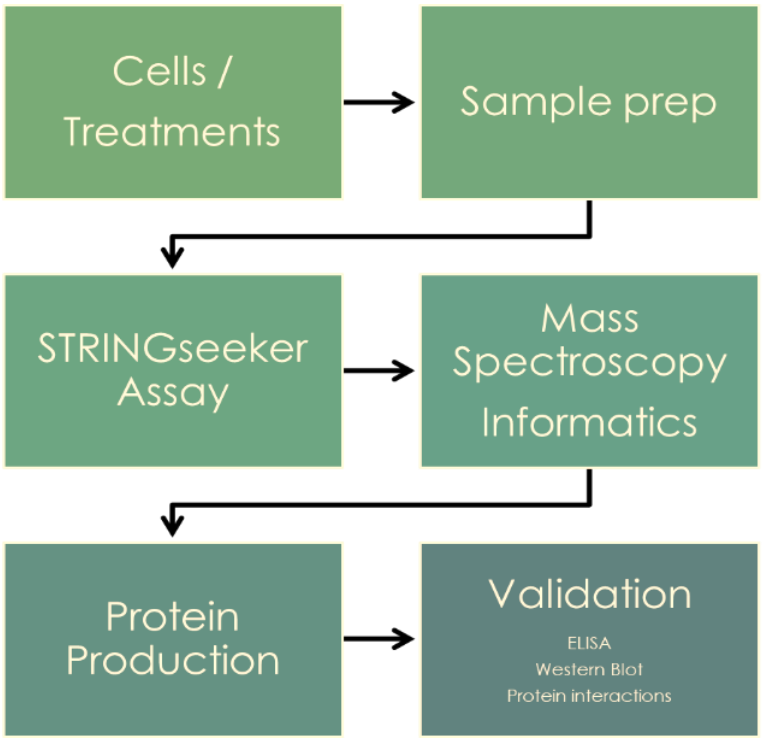


- *Navigating the Ubiquitin space is extremely challenging*
- *Large gene family of UBQs in humans; cell- and target-specificity; accessory proteins*

E3	E2	E1	DUB	ADAPTOR	SUPPRESSOR	UBQ KINASE	UBQ	SUMO	TARGET
600 - 1,000	41	2	107	?	?	?	4	4	?

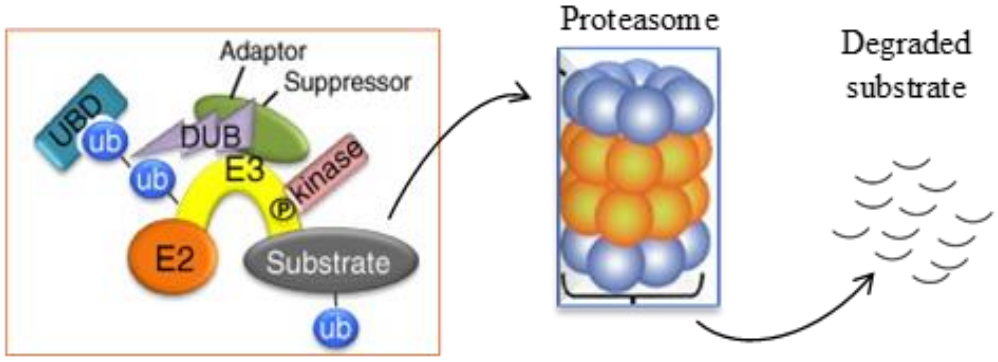
Direct identification of UBQs via Interactomics is the method of choice

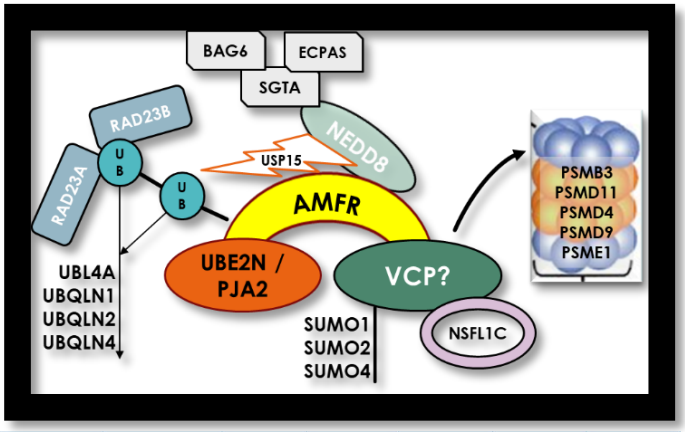
UBQuest process:



- Vistara has applied UBQuest in *in vitro* preclinical models of B cell lymphoma
- Data available upon request

UBQEST TAKES ADVANTAGE OF PROTEIN INTERACTIONS OCCURRING WITH UBQUITINS AND TARGET





TARGET	TREATMENT	E3L	E2L	PSM	DUB	Adaptor Suppressor
BRD4	Culture	1	0	21	1	6
BRD4	JQ1	7	4	6	1	24
BRD4	AZD5153	4	2	5	5	6
A2M	Culture	4	0	0	0	10
AKT1	Culture	1	0	0	0	2
DNA-PK	Culture	1	0	10	0	2
IGF1R	Culture	2	0	12	0	2
VWF	Culture	0	0	0	0	0

UBQ INTERACTIONS IN A B CELL LYMPHOMA CELL LINE

JQ1-treated cells

Interaction partners modeled with data mining and domain information

Best fit of UBQs and other proteins within dataset using Informatics

To be validated

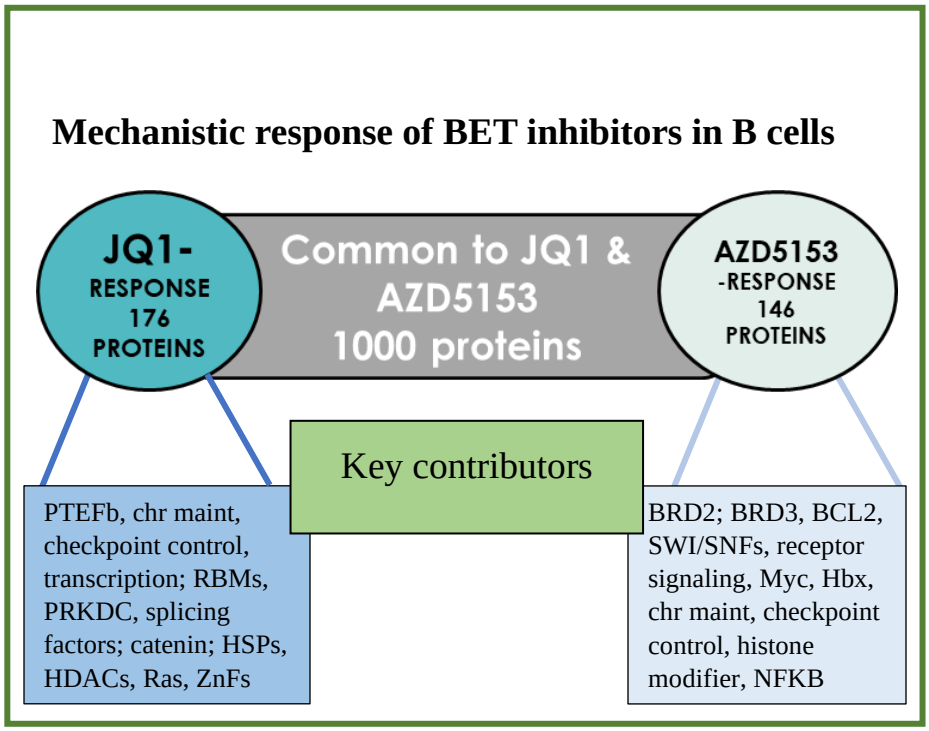
Identification of 23 E3Ls with single iteration of STRINGseeker from a B cell line

E3Ls	Putative interacting domains
Arkadia	1
BRE1B	2
CHIP	1
DTX4	1
HERC2	3
PPP1R11	1
RanBP2	1
RBBP6	1
RFWD2	1
Rififylin	1
RNF138	1

E3Ls	Putative interacting domains
RNF187	1
RNF220	1
RNF25	1
RNF8	1
SIAH1	2
Siah2	1
TRAF2	1
TRAF6	1
TRIM32	1
UBR7	1
UHRF2	1

- Similar results obtained with HepG2 and a Breast cancer cell line
- Different E3L subsets from each cell line, and with drug treatments
- Alternative splicing in some cases
- Data avaiable upon request

STRINGseeker sample data:



Interactome reprogramming in B cells treated with drug candidates

JQ1, AZD5153, or I-BET151 treated

~1200 distinct BRD4-interactants per sample

>85% identity of JQ1 & AZD5153 interactants

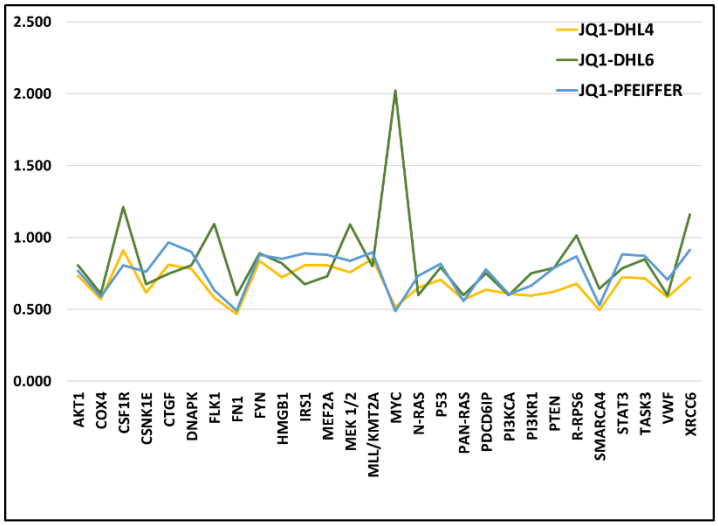
JQ1-responsive or AZD5153-responsive proteins identified

Phenotyper-C sample data

- 5 sub-lineages of human B cells lines were analyzed
- Treated with 3 BET inhibitors – AZD5153, JQ1, and I-BET151
- Proteins interacting with phospho-AKT1 in drug-treated cells were analyzed with STRINGseeker

Hu B Cell Line	Disease	Reference
su-DHL4	Follicular B Lymphoma	https://www.ncbi.nlm.nih.gov/pubmed/8639245
su-DHL6	Diffuse large B-cell lymphoma (DLBCL)	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3647059/
PFEIFFER	Diffuse large B-cell lymphoma (DLBCL)	
Z138	Mantle cell lymphoma	https://www.ncbi.nlm.nih.gov/pubmed/9669839

DIFFERENTIAL PROTEIN INTERACTIONS OF PHOSPHO-AKT1 WITH SELECTED PROTEINS IN B CELLS TREATED WITH BET INHIBITORS



HEAT MAP OF pAKT1 INTERACTIONS IN JQ1 TREATED CELLS

	su-DHL4	su-DHL6	PFEIFFER
CSF1R			
FLK1			
IRS1			
KMT2A			
MEF2A			
MEK1/2			
MYC			
PIK3R1			
PTEN			
STAT3			
TASK3			
XRCC6			

Business Model

Vistara will pursue a tripartite business model to achieve market access.

Vistara will apply its proteomics processes to identify new therapeutic candidates in Oncology, Immunology, and Infectious Disease areas, and may partner with pharma companies for these or other disease area R&D.