Combining deep mutational scanning and Next-Generation Protein Sequencing to harness dominant protein variants to develop DNA repair inhibitors

Lina Ma[2], Anthony Oppedisano[1], Esteban Lenoir[1], Peter Stirling[1], Phil Hieter[1,2] and Nigel O'Neil [1]
[1]Arrowsmith Genetics, [2]Michael Smith Laboratories, UBC, Vancouver, Canada

Abstract

The most clinically effective DNA repair enzyme inhibitors convert the protein target itself into a therapeutic agent. Topoisomerase poisons block the completion of the topoisomerase reaction resulting in a genotoxic DNA-protein adduct. Similar mechanisms underlie clinically relevant PARP inhibitors and other emerging therapeutics targeting DNA Damage Response proteins.

Which repair proteins can be converted into gain-of-function therapeutic agents?

How can we elicit this gain-of-function phenotype with a therapeutic?

We developed an approach, **Mutational Target Mapping (MTM),** that uses **deep mutational scanning** to identify dominant missense variants that define structure-function relationships in drug targets.

Dominant missense protein variants can model the behavior of a potential drug to discover and validate mechanisms of action and define genetic dependencies.

Combining this approach with benchtop protein sequencing on the Quantum-Si Platinum® instrument, we can identify, track, and characterize the phenotypic effects of dominant missense variants in cells and biochemical assays.

We applied this approach to several therapeutic targets discovering key residues in orthosteric and allosteric sites that can be mutated to elicit a dominant phenotype.

Mutational Target Mapping

Pipeline to identify dominant protein variants as "Drug Avatars"

Assay Development	Deep Dominant Mutation Screening	Mutational Target Mapping	Mutation-Driven Drug Discovery	Compound Validation
Drug target phenotypic aim optimized screen	Permissive Selective		Biochemical HTS Computer-aided drug design	
Data integration highlights the most promising targets	Deep phenotypic scanning discovers dominant GoF variants	Dominant mutations reveal critical biophysical sites for modulation	Target Maps direct multi-faceted drug discovery campaigns	Test inhibitors in matched-pair cancer cell lines and xenografts

The engineered sensor cells at the core of Arrowsmith's platform yield functional datasets for each target

Drug Avatars can direct therapeutic development at three stages

Which targets can be turned into dominant therapeutic agents?

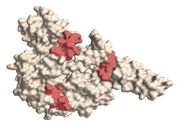
Substrate Trapping

Genotoxic stress

Synthetic lethality

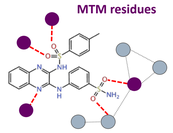
Where are the actionable sites on the target?

Catalytic residues
Allosteric sites
Protein interaction interfaces



Refining small molecules with biophysical data

Critical residues
Biophysical properties



MTM of Targets in the DDR

Across multiple DDR targets, MTM identifies dominant variants that induce DNA trapping

Example of MTM of DNA repair Target 1

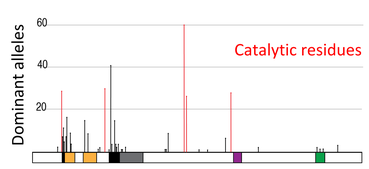


Fig1. Screening of >100K clones identified >300 dominant variants affecting 51 amino acids that define discrete catalytic and allosteric sites.

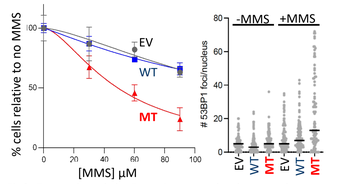
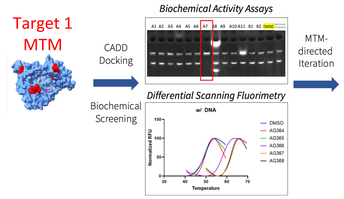


Fig2. Expression of the Drug Avatar, a dominant variant proteoform, (MT) but not the WT proteoform chemosensitizes HT29 CRC cells to the DNA alkylating agent MMS.

Translating MTM to small molecule inhibitors



Three inhibitor classes

Class 1 & 2:
- Allosteric
- Highly specific to target
- Low µM IC50

Class 3:
- Novel Pharmacophore
- On target
- nM IC50 (>10X vs benchmark)
- Traps target on DNA substrate

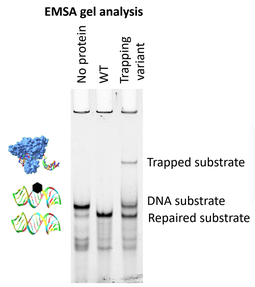
DNA Trapping Assays

Designing DNA trapping inhibitors for DDR targets other than PARP and Topoisomerases.

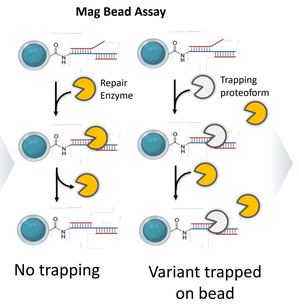
DNA trapping is emerging as an effective way to overcome DDR redundancy by generating a gain-of-function cytotoxic drug-protein intermediate. Extending this mechanism of action to other repair pathways offers an opportunity to broaden the scope of DDR-targeted therapies.

We have developed a bead-based assay that can measure DNA trapping caused by variant proteoforms or small molecules.

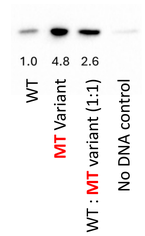
EMSA gel analysis



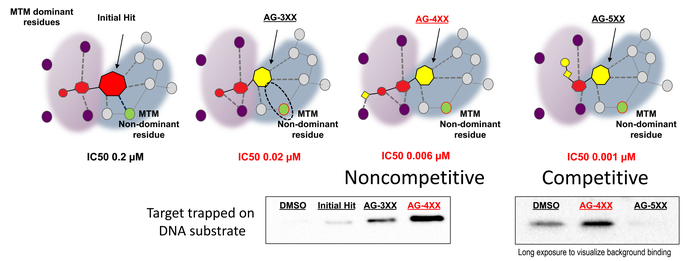
Mag Bead Assay



Western Blot of trapped pellet



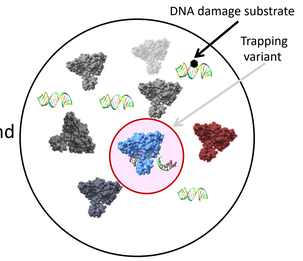
Using MTM data to improve trapping properties of small molecule inhibitor



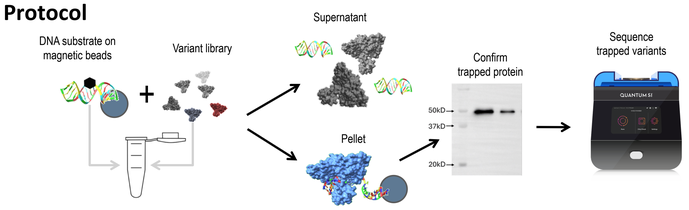
Protein sequencing to identify trapping proteoforms

Can we use emerging single-molecule protein sequencing technology to identify trapping variants from pools of proteoforms?

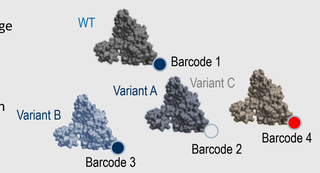
We are using Quantum-Si protein sequencing and the magnetic bead trapping assay to develop methods to directly identify trapping proteoforms.



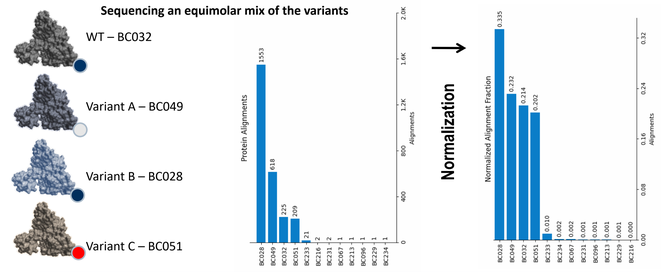
Protocol



- Some key variants fall into difficult regions to get full coverage
- To further develop this assay, we added Quantum-Si C-terminal peptide barcodes to each variant
- Barcodes can tag a wider range of variants than the common antibody recognizable tags (FLAG, 6HIS, etc.)
- Barcodes did not interfere with protein activity



Sequencing an equimolar mix of the variants



Currently improving the "*trapping assay*" with different types of DNA damage substrates

Conclusion: Single-molecule protein sequencing could be used to identify high-value variant proteoforms directly from biochemical assays and cell extracts.

Contact Information

Nigel O'Neil, Chief Scientific Officer
Arrowsmith Genetics
Room 351, 2185 East Mall
Vancouver, BC, Canada
V6T 1Z3
nigel.oneil@arrowsmithgenetics.com