Phospho-proteomic dynamics reveal DNA damage and repair signatures of drug sensitivity

Gali Arad, Avital Hay-Koren, Nitzan Simchi, Dimitri Kovalerchik, Dina Daitchman, Amit Manor, Ofer Givton, Elyad Lezmi, Iris Alchanati, Yonatan Katzenelenbogen, Shay Herman, Alon Shtrikman, Galina Otonin, Eran Seger and Kirill Pevzner
Protai Bio, Tel Aviv, Israel

American Association for Cancer Research
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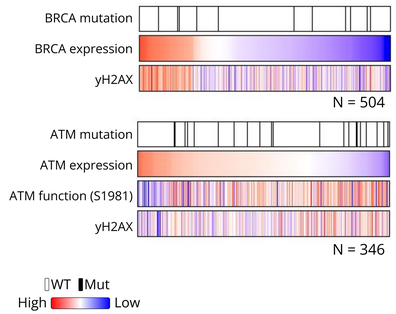
Introduction

There are numerous drug development efforts of DNA damage response (DDR) targets, such as PARP, ATR, CHK1, ATM and others.

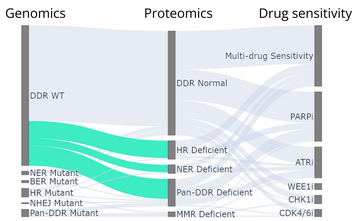
The ability to accurately identify responsive sub-populations for monotherapies and combinations is a major challenge.

DNA replication and repair are dominated by signaling events, therefore DDR deficiency might extend beyond genomic status, manifested also in the level of protein expression and modification.

Proteogenomic clinical analysis of frequent DDR mutations shows that phenotype is better described by protein expression (BRCA) or function (ATM)[1]

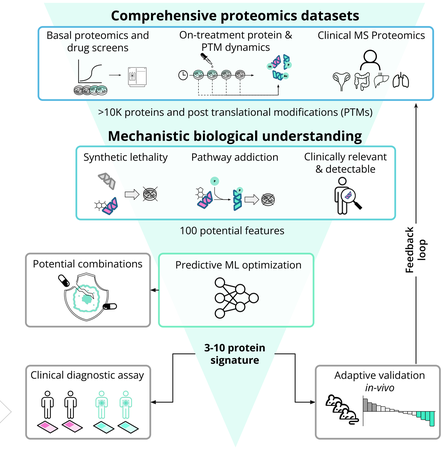


Some cell lines are sensitive to DDR drugs despite being DDR-proficient at the genomic level



Proteomic analysis of these cell lines showed that at least a third (in green) of them exhibit genomic DDR proficiency but proteomic DDR deficiency, in turn explaining DDR sensitivity beyond mutation status.

AIMS™ platform for biomarker discovery



Drug sensitivity differential analysis of basal state proteomics and PTM combined with on-treatment dynamics to identify treatment specific biomarkers

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Mechanistic understanding of treatment sensitivity through synthetic lethality or addiction

↓

Utilizing our wide pan cancer clinical atlas representing the proteomes of actual tumors

↓

Combining biological explainability with AI to translate broad signatures to 3-10 features proteomic biomarker

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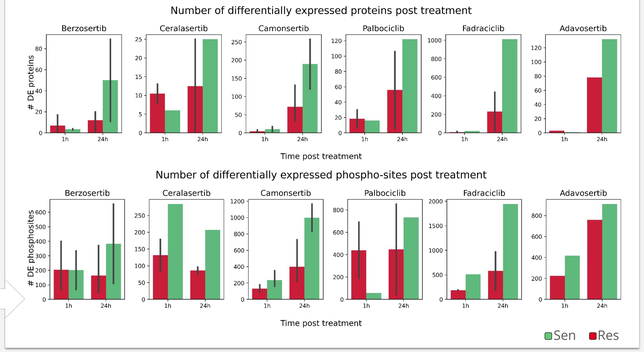
Adaptive *in-vivo* validation alongside clinical assay development

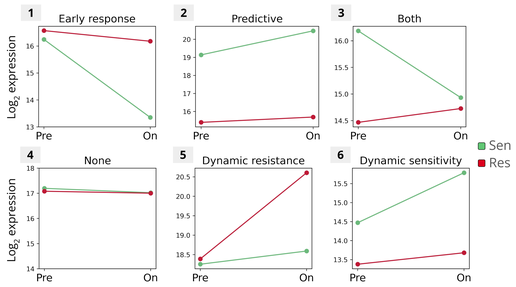
Protein dynamics profiles reflect sensitivity

Analysis of proteomic and phosphoproteomic changes upon various DDR treatments reveals that proteomic alterations are stronger in drug-sensitive cell-lines, while cell-lines that are resistant to treatment present a moderate molecular response.



Integration of basal and dynamic phosphoproteomics reveals distinct patterns of molecular treatment response

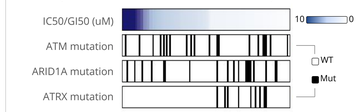
We integrated basal and dynamic phosphoproteomic data of sensitive and resistant cell lines treated with ATRi (at baseline and 1h time points). We show here selected features demonstrating distinct patterns of molecular treatment response. For example, Pattern 1 (top left) is significantly downregulated only in ATRi-sensitive cell line, representing a potential early response marker. Pattern 3 (top right) behaves similarly in response to ATRi, but is also differential in basal state, thus representing both a dynamic and predictive marker.



Predictive biomarker for ATRi outperforms known genomic markers

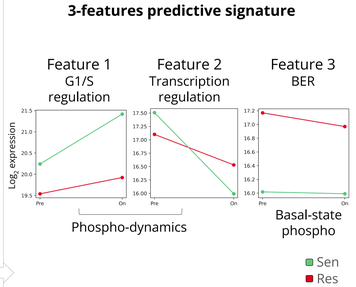
Basal state and dynamic cell line data used for discovery

4 proteomic resources, **110** cell lines, **19** tissues
➔ (CCLE[1], NCI60[3], Champions, internal data)
3 drug sensitivity resources
➔ (Wilson et al.[4], 2 internal datasets)

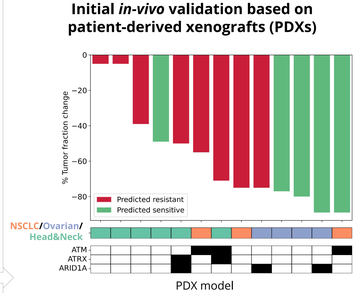


Known genomic biomarkers are enriched to some extent in sensitive cell lines, however none covers the entire spectrum of sensitivity.

3-features predictive signature



Initial *in-vivo* validation based on patient-derived xenografts (PDXs)



Summary and future steps

➔ We demonstrate here the benefit of utilizing (phospho)-proteomic data, both basal and dynamic, to tackle clinical challenges in DDR drug development, such as predictive biomarkers and combination strategies.

➔ Protai's biomarker prediction pipeline showed that sensitivity to targets such as ATR require comprehensive integration of data from multiple sources.

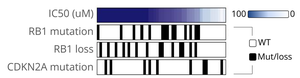
➔ Protai's combination prediction pipeline enabled distinguishing between cell lines with different resistance mechanisms.

➔ In the future, Protai will further optimize predictions by expanding in-vivo validation panels, followed by clinical validation.

Predictive biomarker for CDK4/6i

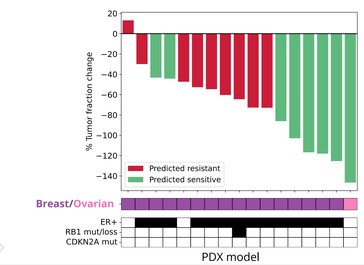
Basal state and dynamic cell line data used for discovery

2 proteomic resources, **50** cell lines, **14** tissues
➔ (CCLE[1], internal data)
2 drug sensitivity resources
➔ (GDSC2[2], internal data)



Our data highlights known mechanisms of CDK4/6 resistance such as RB1 mutation or loss and CDKN2A (p16) mutation.

Initial *in-vivo* validation
Protai biomarker outperforms RB1-related genomic events

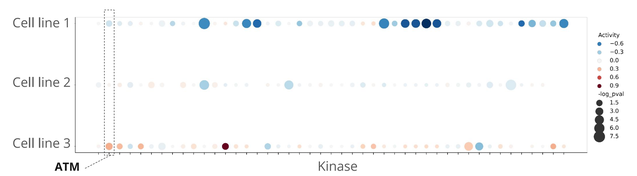


Discovery of novel ATR combinations enabled by protein dynamics

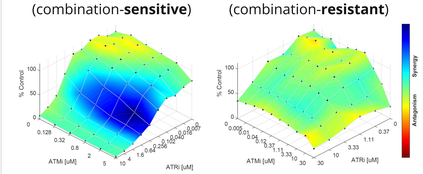
ATRi monotherapy has shown limited efficacy in clinical trials, therefore increasing the need for finding suitable and effective drug combinations.

Using our phospho-dynamic data and kinase activity estimation, we compared kinase activity upon ATRi treatment in both sensitive and resistant cell lines.

Our analysis identified ATM as well as several other druggable kinases to be upregulated upon treatment in a selective manner.



In-vitro validation showed synergy of ATMi and ATRi in a predicted sensitive cell line but not in a predicted-resistant one



References

1. Simchi et al. A large scale proteogenomic atlas for precision oncology [abstract]. In: Proceedings of the 115th Annual Meeting of the American Association for Cancer Research; 2024 April 5-10; San Diego, CA. Philadelphia (PA): AACR; 2024. Abstract # 6241
2. Nusinow et al. Cell (2020)
3. Iorio et al. Cell (2016)
4. Frenjo et al. Nat Comms (2020)
5. Wilson et al. Cancer Research (2022)