NEK1 IS INVOLVED IN TUMOR GROWTH THROUGH ALTERED IMMUNE SIGNALING

turbine.

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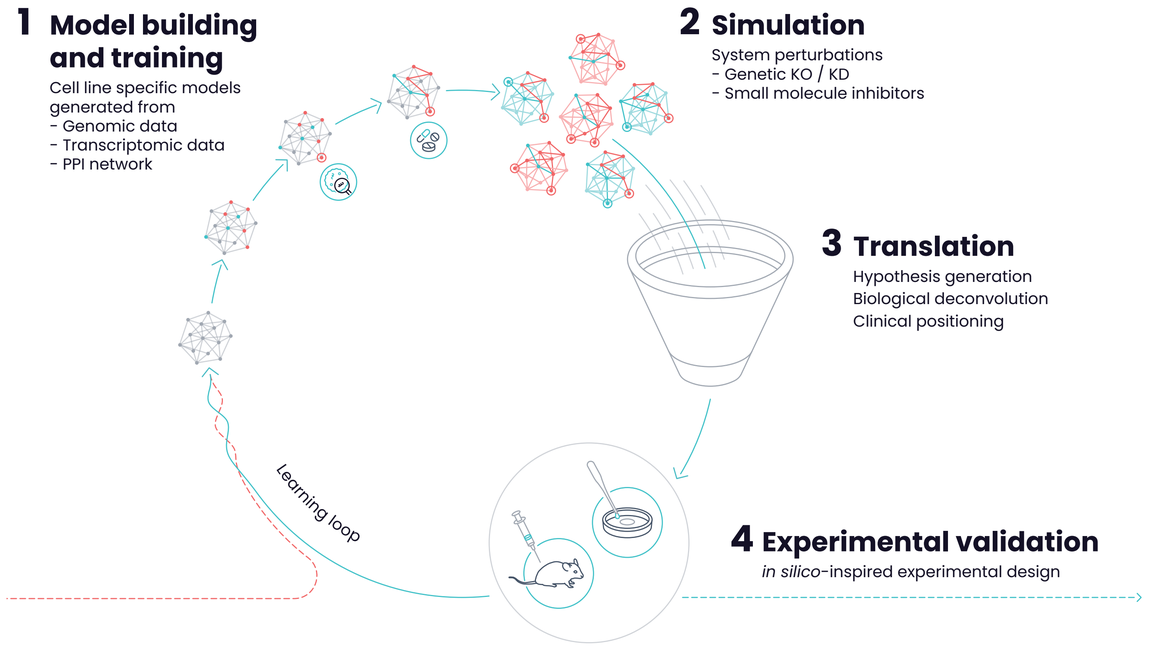
2024 ESMO TAT
Targeted Anticancer Therapies
PARIS FRANCE
26-28 FEBRUARY 2024

I. INTRODUCTION

I/a. Turbine's Simulated Cell™ platform

Turbine's Simulated Cell™ platform models cancer cell signaling to predict cell viability and to identify novel drug targets. By simulating perturbations of the DNA-damage response (DDR) pathway, we identified NEK1 as a cancer dependency in certain molecular contexts. NEK1 is part of the NIMA-related kinase family, involved in DDR, cell cycle, and mitosis, making it an interesting candidate for antitumor intervention.

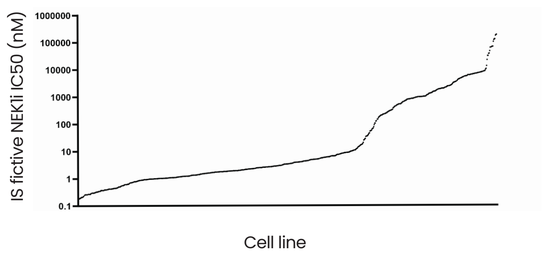


Legend: Turbine's workflow for identification of new targets and biomarkers consists of a 4-step learning loop to iteratively identify and validate novel hypotheses. The Simulated Cell™ platform allows to run high-throughput *in silico* screens for cancer target and biomarker identification in hundreds of simulated biosamples, including cancer cell lines, PDXs and patient samples. The screens consist of simulated perturbations such as gene KO/KD and use of pharmacological intervention. The simulation results are then translated into actual biology for generation of hypotheses in the context of specific cancer indications. The *in silico* results guide the planning and execution of key experiments for hypothesis testing, and the resulting proprietary data is ingested into the Simulated Cell™ as part of a continuous learning loop.

II. RESULTS

II/a. NEK1 KO *in silico* (IS) suggests novel dependencies in selected cell lines

Differential sensitivity of cell lines to fictive *in silico* NEK1 inhibitor



Identification of biomarkers predictive for sensitivity to NEK1 inhibition

Top biomarker hits

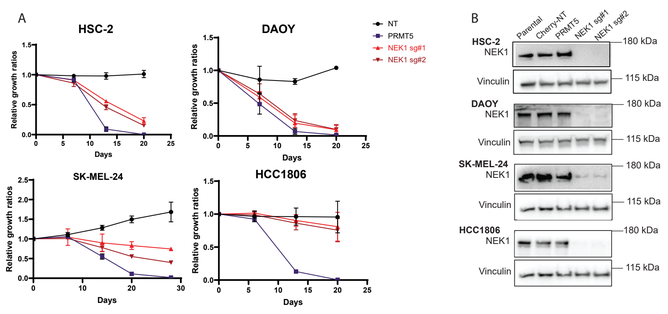
- ATM LOF
- BRCA1 LOF
- BRCA2 LOF
- RAD54 LOF
- PALB LOF

Cell line selection for *in vitro* experiments

Cell line	Disease	Chronos Dependency Score (DepMap)	Turbine's prediction	Expectation	Presence of BM
HCC1806	Breast Cancer	-0.086 No dependency	No dependency on NEK1	Negative control	None
DAOY	Medullo-blastoma	-0.57 High dependency	Dependent on NEK1	Positive control	*ATM* and *RAD54* mutant
HSC-2	Head and Neck Carcinoma	-0.21 Low dependency	Dependent on NEK1	Novel predicted dependency	*ATM* mutant *BRCA2* mutant
SK-MEL-24	Melanoma	-0.22 Low dependency	Dependent on NEK1	Novel predicted dependency	*ATM* mutant

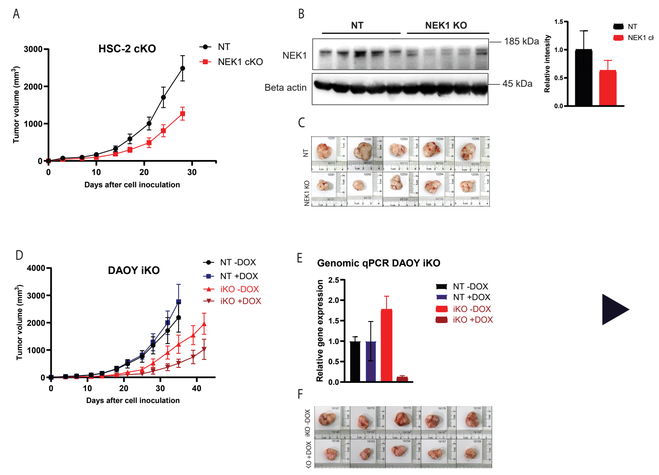
Legend: A fictional *in silico* NEK1 inhibitor was used to simulate NEK1 downregulation in a panel of 809 simulated cancer cell lines, allowing the identification of cell lines with high sensitivity to NEK1 inhibition. To investigate molecular biomarkers that indicate sensitivity to NEK1 inhibition, the fictive NEK1 inhibitor in combination with mutations on additional genes was simulated in a 1 to 1 way. Those mutations that produced a shift of NEK1 inhibitor IC50 towards sensitization in a sufficient number of cell lines were further investigated to understand their biological functions and relation to NEK1 biology until a list of top biomarker hits was obtained. Based on *in silico* NEK1 dependency and the presence of *in silico* identified biomarkers, a panel of cell lines that were predicted to be sensitive or resistant to NEK1 KO was defined for *in vitro* validation.

II/b. NEK1 KO *in vitro* validates Simulated Cell™ predictions on cell viability



Legend: The viability of constitutive NEK1 CRISPR KO cells using two different guide RNAs (sg1 and sg2) was compared to non-transfected cells (NT) or positive control (PRMT5) for reduced cell proliferation in a cell competition assay. For all experiments, n=2. **A.** The medulloblastoma cells DAOY and head and neck carcinoma HSC-2 cells were dependent on NEK1 expression for proliferation *in vitro*, while the melanoma SK-MEL-24 cell line showed intermediate sensitivity, and the negative control (predicted to be resistant *in silico*) breast cancer HCC1806 cells were not sensitive to NEK1 KO. **B.** Western blots showing NEK1 protein level reduction upon NEK1 CRISPR KO. NEK1 antibody ab229489.

II/c. NEK1 KO *in vivo* reduces head and neck carcinoma and medulloblastoma CDX growth

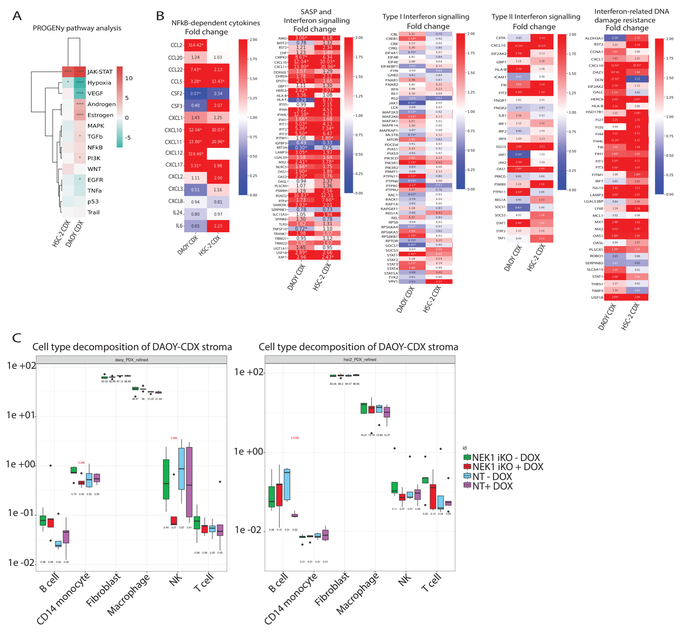


G **Summary *in vitro* and *in vivo* sensitivity to NEK1 KO**

Cell line	Disease	Chronos Dependency Score (DepMap)	Turbine's prediction	*In vitro* viability	*In vivo* CDX
HCC1806	Breast Cancer	-0.086 No dependency	No dependency on NEK1	Resistant to *NEK1* KO	NA
DAOY	Medullo-blastoma	-0.57 High dependency	Dependent on NEK1	Sensitive to *NEK1* KO	*NEK1* KO reduces tumor growth
HSC-2	Head and Neck Carcinoma	-0.21 Low dependency	Dependent on NEK1	Sensitive to *NEK1* KO	*NEK1* KO reduces tumor growth
SK-MEL-24	Melanoma	-0.22 Low dependency	Dependent on NEK1	Intermediately sensitive to *NEK1* KO	*NA*

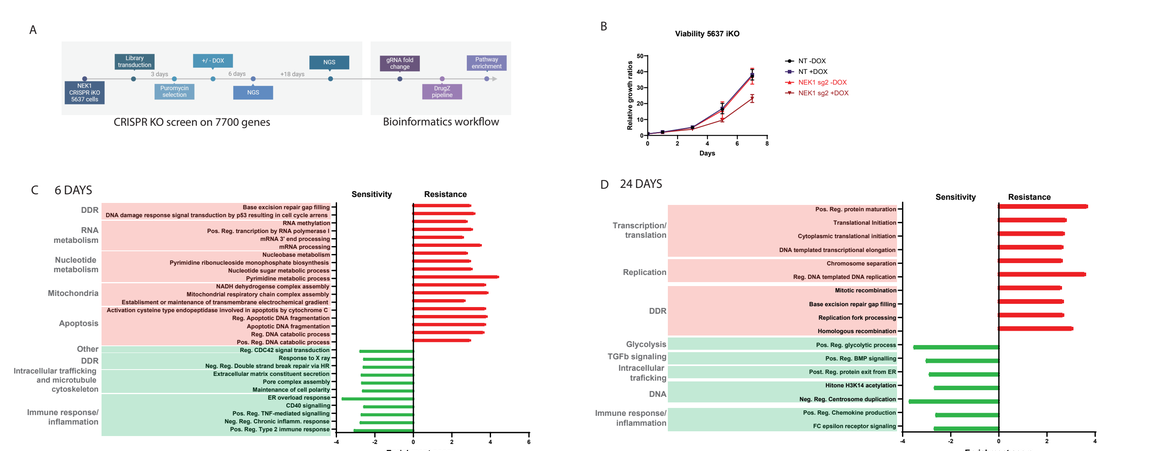
Legend: A. Head and neck carcinoma HSC-2 CDX expressing a constitutive NEK1 CRISPR KO showed reduced tumor growth in in CB17 SCID mice, n=10 per group. **B.** Western blot image and densitometry quantification show the level of reduction of NEK1 protein in the HSC-2 tumors. **C.** Representative pictures of the HSC-2 tumors at experimental endpoint. **D.** Medulloblastoma DAOY CDX expressing a doxycycline-inducible NEK1 CRISPR KO showed reduced tumor growth in CB17 SCID mice, n=10 per group. **E.** Genomic qPCR shows reduced expression of NEK1 in the doxycycline treated NEK1 iKO tumors. **F.** Representative pictures of the DAOY tumors at experimental endpoint. **G.** Summary of the *in vitro* and *in vivo* sensitivity to NEK1 KO and comparison to the original *in silico* hypotheses in the selected cell lines.

II/d. NEK1 KO *in vivo* reveals immune signaling changes based on RNAseq analysis of CDX tumors



Legend: A. Pathway enrichment analysis in RNAseq samples collected from HSC-2 and DAOY CDXs show enriched activity of signaling pathways in NEK1 KO tumors respective to NEK1 WT. The JAK-STAT signaling pathway is significantly upregulated in NEK1 KO tumors in both CDX models. **B.** Heatmaps representing fold change gene expression in NEK1 KO tumors respective to NEK1 WT tumors. From left to right, a gene set of NFkB-dependent cytokines, genes involved in Senescence-Associated Secretory Phenotype (SASP) and interferon signaling, Type I Interferon signaling, genes involved in Type II interferon signaling, and in interferon-dependent DNA damage resistance. The expression of NFkB-dependent cytokines, SASP and Interferon response, Interferon Type II signaling, and Interferon-dependent DNA damage elements is increased in NEK1 KO CDXs, while the same pattern is not observed in the case of Type I Interferon signaling. Low type I interferon signaling, increased NFkB signaling, and DDR malfunctioning are all phenotypes related to chromosomal instability (CIN), which has been reported to occur in NEK1 KO mouse-derived cell lines. **C.** RNAseq analysis of the tumor stroma allowed the decomposition of cell components in the tumor microenvironment. NK cells are significantly diminished in the case of DAOY NEK1 KO CDX stroma, but not in HSC-2 NEK1 KO CDX stroma.

II/e. CRISPR KO screen reveals silencing of immune / inflammatory signaling elements as sensitizers to NEK1 KO



Legend: An *in vitro* CRISPR KO screen was performed on 7700 genes in combination with NEK1 KO in inducible NEK1 CRISPR KO 5637 bladder cancer cells. This cell line was selected due to its mild sensitivity to NEK1 KO, to allow for detection of further sensitization upon secondary gene KO. **A.** Overview of the CRISPR KO screen and subsequent bioinformatic analysis. After performing double CRISPR KO for 6 or 24 days, cells were collected for NGS. Detection of enrichment or loss of guide RNAs allowed to identify genes that, when knocked out, can increase or diminish the sensitivity to NEK1 KO. Pathway enrichment analysis was performed to analyze the processes in which these genes were involved. **B.** Effect of doxycycline inducible NEK1 CRISPR KO on the viability of 5637 bladder cancer cells. **C.** and **D.** Pathways involved in either sensitization (green) or increased resistance (red) to NEK1 KO-induced reduction on cell viability at 6 days or 24 days of double KO, respectively. The silencing of genes involved in immune response and inflammation related pathways sensitized the cells to NEK1 KO. All processes shown were enriched with statistical significance (p value < 0.05).

III. CONCLUSIONS

Turbine's Simulated Cell™ platform predicted NEK1 dependency in cell lines in which the DepMap dataset suggested otherwise. We confirmed our prediction of reduced cellular proliferation *in vitro* and reduced tumor growth *in vivo*. Additionally, we revealed that NEK1 effects on tumor growth are accompanied by changes in immune regulation that phenotypically overlap with CIN-mediated inflammatory responses. CIN has been observed in ex vivo NEK1 KO mammal models due to deregulated DDR and mitosis. An extensive CRISPR-KO screen revealed that co-targeting pathways involved in immune regulation may increase the efficacy of NEK1 inhibition. These findings are incorporated into the Simulated Cell™ to complete our learning loop and improve the predictive capacity of our Platform. These data validate Turbine's platform as a target identification solution and suggest that NEK1 is a promising anticancer target.