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Development of a gene signature as a biomarker for prediction of response to the salt-inducible kinase (SIK) inhibitor OMX-0407

Authors and affiliations

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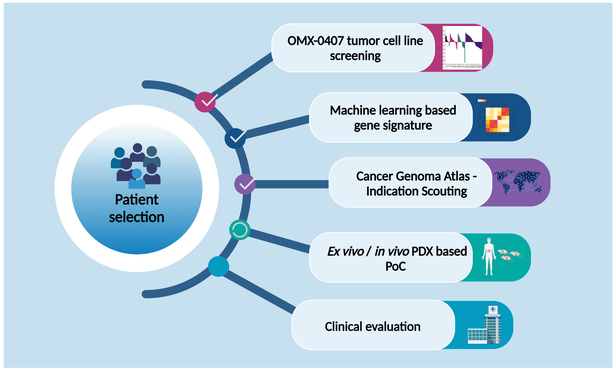
Introduction

Recently, the iOTarg genetic screening platform identified SIK3 as novel cell signaling modulator in cancer biology. SIKs are serine/threonine kinases belonging to the AMP-activated protein kinase (AMPK) family. OMX-0407, an orally available, single-digit nanomolar inhibitor was shown to inhibit tumor growth in a distinct panel of tumor models *in vitro* as well as *in vivo*.

In a comprehensive viability screen, 225 human cancer cell lines of different indications were used to identify the selective activity profile of OMX-0407 in a subset of cancers. In-depth transcriptomic analyses were performed on individual cancer cell lines and were used to identify and validate a predictive biomarker signature, that was successfully forecasting 83% of the selected cancer cell lines as responsive to OMX-0407 therapy. Following a clinical trial-like scenario, patient-derived tumor xenograft (PDX) models were selected based on their sensitivity prediction to OMX-0407 and successfully tested in an *ex vivo* tumor viability assay. Sensitivity predictions on hundreds of cell lines, PDX models, and patient datasets from The Cancer Genome Atlas (TCGA) reflect a high degree of overlap in indications sensitive to OMX-0407, independent of the analyzed raw dataset.

Representative for OMX-0407 sensitive tumors, monotherapy studies with OMX-0407 demonstrated dose-dependent anti-tumor efficacy in various syngeneic murine tumor models. With significantly prolonged overall survival and up to 90% tumor growth inhibition in individual tumor models, OMX-0407 remarkably demonstrated equivalent or superior efficacy compared to anti-PD-1 monotherapy.

We identified a response-prediction biomarker signature, which may be the basis for future development of OMX-0407 in specific indications and which will be evaluated for its potential to enrich for patients highly responsive to OMX-0407 therapy in upcoming clinical studies.



Conclusion

- OMX-0407, a potent inhibitor of salt-inducible kinase family members, demonstrated outstanding anti-tumor efficacy *in vitro* in a subset of cancer cell lines representing different indications, and in particular, remarkable monotherapy efficacy superior to anti-PD-1 monotherapy in PD-1-low or non-responding tumors.
- Using machine learning methods, the selective activity profile of OMX-0407 on individual cancer cell lines was used to identify and validate a predictive transcriptome-based biomarker signature
- The predictive biomarker signature has been successfully validated in cell lines with a prediction rate of 83% and is currently being validated in a variety of *ex vivo* and *in vivo* PDX models
- The generated predictive biomarker signature will be evaluated in the Phase I clinical trial of OMX-0407, which was started in March 2023.

Results

Tumor cell viability screening showed distinct sensitivity profile upon OMX-0407 therapy

- Anti-tumor monotherapy efficacy screening of OMX-0407 on 225 human cancer cell lines identifies a sensitivity activity profile of individual cell lines towards OMX-0407
- Squamous non-small cell carcinoma of the lung is emerging as a major tumor indication responsive to OMX-0407
- Predictive biomarkers could drive the selection of OMX-0407-sensitive patients

A) High throughput screening of cancer cell viability upon OMX-0407 treatment **B) Distinct activity profile of OMX-0407 shown on tumor cell lines**

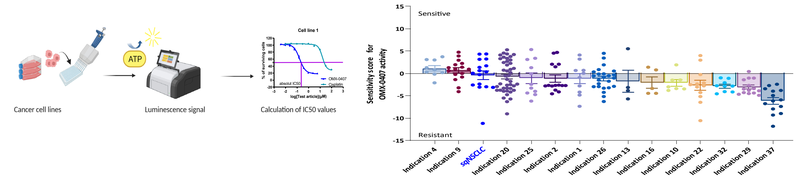


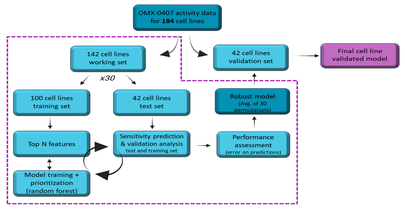
FIGURE I

A) Schematic overview of a high thoughput tumor cell viability screening. Each cell line was treated for 48 h with OMX-0407 (6 µM – 4 nM), a standard chemotherapy drug (1 mM – 100 nM) as reference control and vehicle control in a 9-point serial dilution. Tumor cell viability was analyzed using the CellTiter-Glo® assay according to the manufacturer's protocol. Dose-response curves were fitted using nonlinear, sigmoidal regression models. Absolute IC50 values were calculated according to dose-response curves generated by GraphPad Prism 5.0. B) 225 human cancer cell lines of 15 different indications were analyzed and sensitivity scores were calculated by the following formula: Sensitivity score = Log_2(2 x %max Inhibition)2 – Log_2(IC50abs) + 2.5; sensitivity score > 0 = sensitive to OMX-0407; sensitivity score < 0 = resistant to OMX-0407.

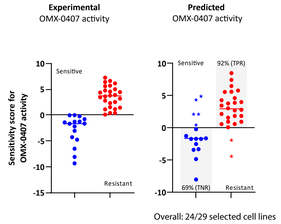
Development of a transcriptome-based signature for sensitivity prediction towards OMX-0407 therapy

- OMX-0407 efficacy-predictive, cell line-based transcriptome signature identified by using baseline RNA profile of 184 human tumor cell lines
- Machine learning (ML)-based random forest modelling revealed a subset of up- and downregulated genes, predictive for OMX-0407 sensitivity
- Further signature improvement by removal of stromal genes, filtering and prioritization of features adapted to human patient

A) General ML approach for generation of a predicitive OMX-0407 efficacy signature



B) Validation of signature in patient samples



Overall: 24/29 selected cell lines = 83% true sensitive cell lines

C) Heatmap of gene signature for predictivty of sensitive and resistant

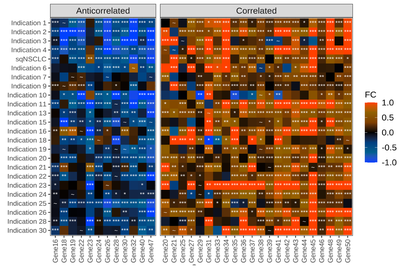


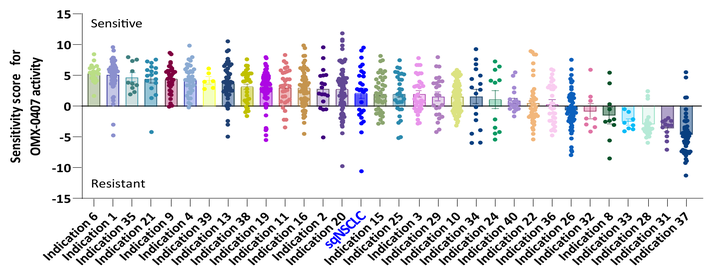
FIGURE II

A) Schematic overview of a random-forest machine learning approach for generation of a transcriptome-based OMX-0407 sensitivity signature. The workflow includes model training and internal cross validation-testing on 142 cell lines and a validation on 42 independent cell lines. B) Validation of the predictive gene signature by comparison of empirical tested anti-tumor efficacy and associated sensitivity scoring with the corresponding sensitivity prediction by the transcriptome signature. Sensitivity scores were calculated by the following formula: Sensitivity score = Log2(2 x %max Inhibition)2 – Log2(IC50abs) + 2.5; sensitivity score > 0 = sensitive to OMX-0407; sensitivity score < 0 = resistant to OMX-0407. TNR= true negative response; TPR= true positive response.

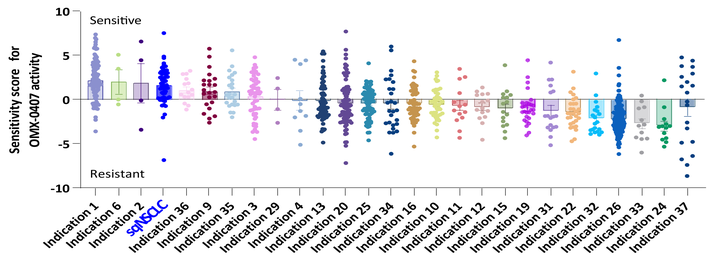
Consistent OMX-0407 sensitivity prediction using cell lines, PDX models up to human tumor patients

- The predicted sensitivity of distinct indications of 1087 cell lines of the Cancer Cell Line Encyclopedia (CCLE) database reflects the empirical tested OMX-0407 activity of direct cell screens, as shown in figure 1B
- Consistent overlap of OMX-0407 sensitive indications between cell lines (A), PDX models (B) as well as human cancer patients (C)
- Lung squamous non-small cell carinoma shows strong predicted OMX-0407 anti-tumor efficacy in PDX models as well as TCGA
- cancer patient data
- Predictions for cancer patients reveal a subset of indications with high potential for anti-tumor efficacy of OMX-0407

A) OMX-0407 sensitivity prediciton in CCLE cell lines of 32 indications



B) OMX-0407 sensitivity prediction in PDX models of 27 indications



C) OMX-0407 sensitivity prediciton in TCGA cancer patient data of 31 different indications

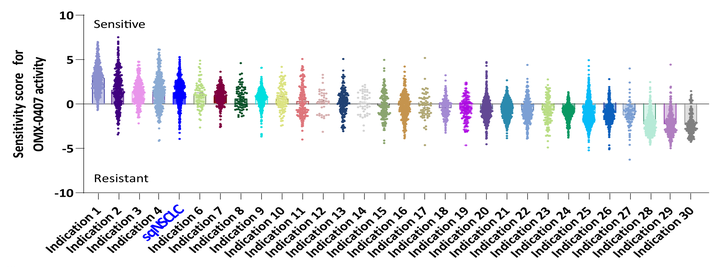


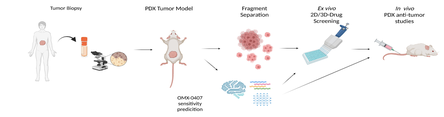
FIGURE III

Prediction of OMX-0407 sensitivity A) in 1087 cell lines including 32 indications based on baseline transcriptome data from CCLE database, B) of transcriptome data from 1045 PDX models of 27 different indications and C) of transcriptome data from 8885 human cancer patients of 31 indications from TCGA database. Sensitivity calculation for figure III A – C) performed on log_2(TPM+1) based on cell line validated gene signature depicted in figure IB. Sensitivity score = Log2(2 x %max Inhibition)2 – Log2(IC50abs) + 2.5; sensitivity score > 0 = sensitive to OMX-0407; sensitivity score < 0 = resistant to OMX-0407

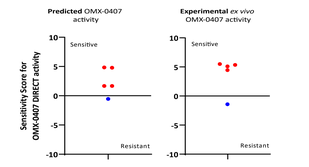
Successful clinical mimicry of OMX-0407 sensitivity prediction in PDX model

- Confirmation of cell line-validated predictive signature ongoing in PDX models
- Testing OMX-0407 sensitivity prediction hypothesis: 44 PDX models for extensive *ex vivo* anti-tumor viability screening and 26 *in vivo* PDX models for OMX-0407 monotherapy studies compared to vehicle control
- All currently tested *ex vivo* PDX models were correctly classified by the cell line validated transcriptome signature – study ongoing

A) Schematic overview of *ex vivo* / *in vivo* screening for signature confirmation



B) PDX *ex vivo* testing successfully confirmed OMX-0407 activity



C) Viability of PDX models upon OMX-0407 treatment

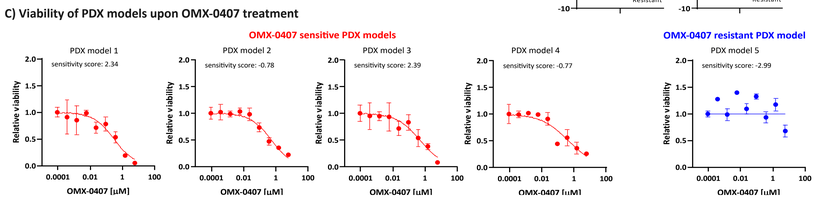


FIGURE IV

A) Schematic overview of *ex vivo / in vivo* PDX screening. B) Sensitivity calculation based on baseline PDX transcriptome data, performed on log_2(TPM+1) based on cell line validated gene signature depicted in figure IIB. Sensitivity score calculation based on following formula: Sensitivity score = Log2(2 x %max Inhibition)2 – Log2(IC50abs) + 2.5; sensitivity score > 0 = sensitive to OMX-0407; sensitivity score < 0 = resistant to OMX-0407C) PDX models were implanted in nude mice and excised at an average tumor volume of 800 – 1600 mm³. Dissociated tumor fragments were treated for 6 days with OMX-0407 (6 µM – 4 nM) or corresponding vehicle control in an 8-point serial dilution. Cell viability was analyzed by CellTiter-Glo® assay. Absolute IC50 values were calculated according to the dose-response curves generated by GraphPad Prism 5.0.

Dose-dependent anti-tumor efficacy of OMX-0407 in syngeneic tumor models of different indications

- OMX-0407 demonstrated superior anti-tumor efficacy compared to anti-PD-1 monotherapy in syngeneic PD-1-low or non-responding tumor models
- OMX-0407 monotherapy demonstrates tumor growth inhibition of more than 60% in all tested syngeneic tumor models

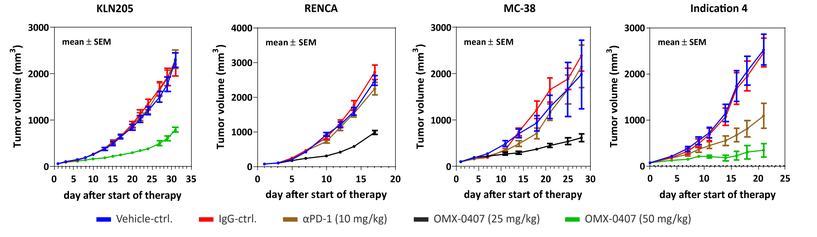


FIGURE V

Tumor cells of individual tumor models were subcutaneously implanted in their corresponding syngeneic mouse background and randomized at an average tumor volume of 50-100 mm³. Tumor bearing mice were treated twice daily with indicated OMX-0407 concentrations by oral gavage, twice weekly intraperitoneally, with 10 mg/kg anti-PD-1 mAb (clone: RMP1-14) or corresponding controls. Average tumor growth is depicted for ≤12 mice per therapy group by using the last observation carried forward method.

Statistical information

If not indicated otherwise, graphs are representative data from at least two independent experiments. Sigmoidal dose–response curves were fitted to data using 4-Parameter Logistic (4PL) non-linear curve models for in vitro dose responses. Data points show mean ±SEM. Significance was calculated with two-way ANOVA analysis including Tukeys multiple comparison analysis.

Contact