

Development of a gene signature to predict the anti-tumor response of the salt-inducible kinase (SIK) inhibitor OMX-0407

Authors and affiliations

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Introduction

SIK3 was identified by the iOTarg genetic screening platform as a novel cell signaling modulator in cancer biology. SIKs are serine/threonine kinases belonging to the AMP-activated protein kinase family. OMX-0407, an orally available, spectrum-selective salt-inducible kinase (SIK) inhibitor, currently under evaluation in a clinical Phase I trial and was shown to inhibit tumor growth in a distinct panel of tumor models by decreasing downstream pro-survival signaling of SIK3, enhancing caspase-mediated apoptosis and repolarizing the tumor microenvironment by decreasing regulatory T cell number. OMX-0407 demonstrated dose-dependent anti-tumor efficacy in murine and patient-derived xenograft (PDX) tumor models for several indications with high unmet medical need.

In a comprehensive anti-tumor viability screening, more than 200 human cancer cell lines of different indications were used to identify a selective activity profile of OMX-0407 in a subset of cancers. In-depth transcriptomic analyses were performed and 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. By using *ex vivo* and *in vivo* patient-derived tumor models, the response-prediction gene signature was further validated and is currently under optimization on heterogeneous tumor systems for its use in human patient samples. We could show a consistent overlap of OMX-0407 efficacy prediction in hundreds of cell lines of the Cancer Cell Line Encyclopedia (CCLE) database, human PDX models as well as human cancer patient data of The Cancer Genome Atlas (TCGA). Our computational model predicted a subset of indications with a high potential for anti-tumor efficacy of OMX-0407 and consequently potential clinical benefit. For OMX-0407-sensitive indications, monotherapy studies in different murine tumor models demonstrated strong *in vivo* anti-tumor efficacy of OMX-0407 with significantly prolonged overall survival and up to 90% tumor growth inhibition.

In summary, by screening sensitive and non-sensitive tumor cell lines and PDX models, we identified a response-prediction biomarker signature, which will contribute to the future development of OMX-0407 in specific indications and which will be assessed for its potential to select patients highly responsive to OMX-0407 therapy in clinical studies. The gene signature will be evaluated as part of the ongoing first-in-human study OMX-0407-101 (NCT05826600).

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 profile of individual cell lines towards OMX-0407
- ➔ Squamous non-small cell carcinoma of the lung and renal cell carcinoma are emerging as major tumor indications 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

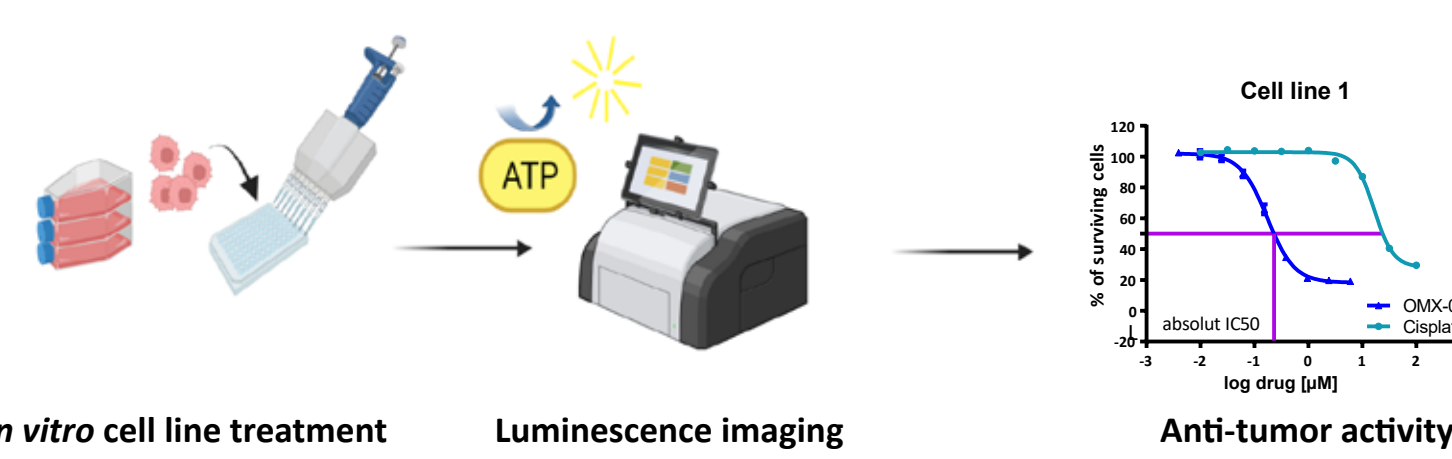


FIGURE I

A) Schematic overview of a high throughput tumor cell viability screening. Each cell line was treated for 48 h with OMX-0407 (6 μ M – 4 nM), cisplatin as standard chemotherapy drug (1 mM – 100 nM) as reference control and OMX-0407-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 = $\text{Log}_2(2 \times \% \text{max Inhibition})^2 - \text{Log}_2(\text{IC}_{50} \text{obs}) + 2.5$; sensitivity score > 0 = sensitive to OMX-0407; sensitivity score < 0 = resistant to OMX-0407. RCC = renal cell carcinoma, sqNSCLC = squamous non-small cell lung cancer.

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

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

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

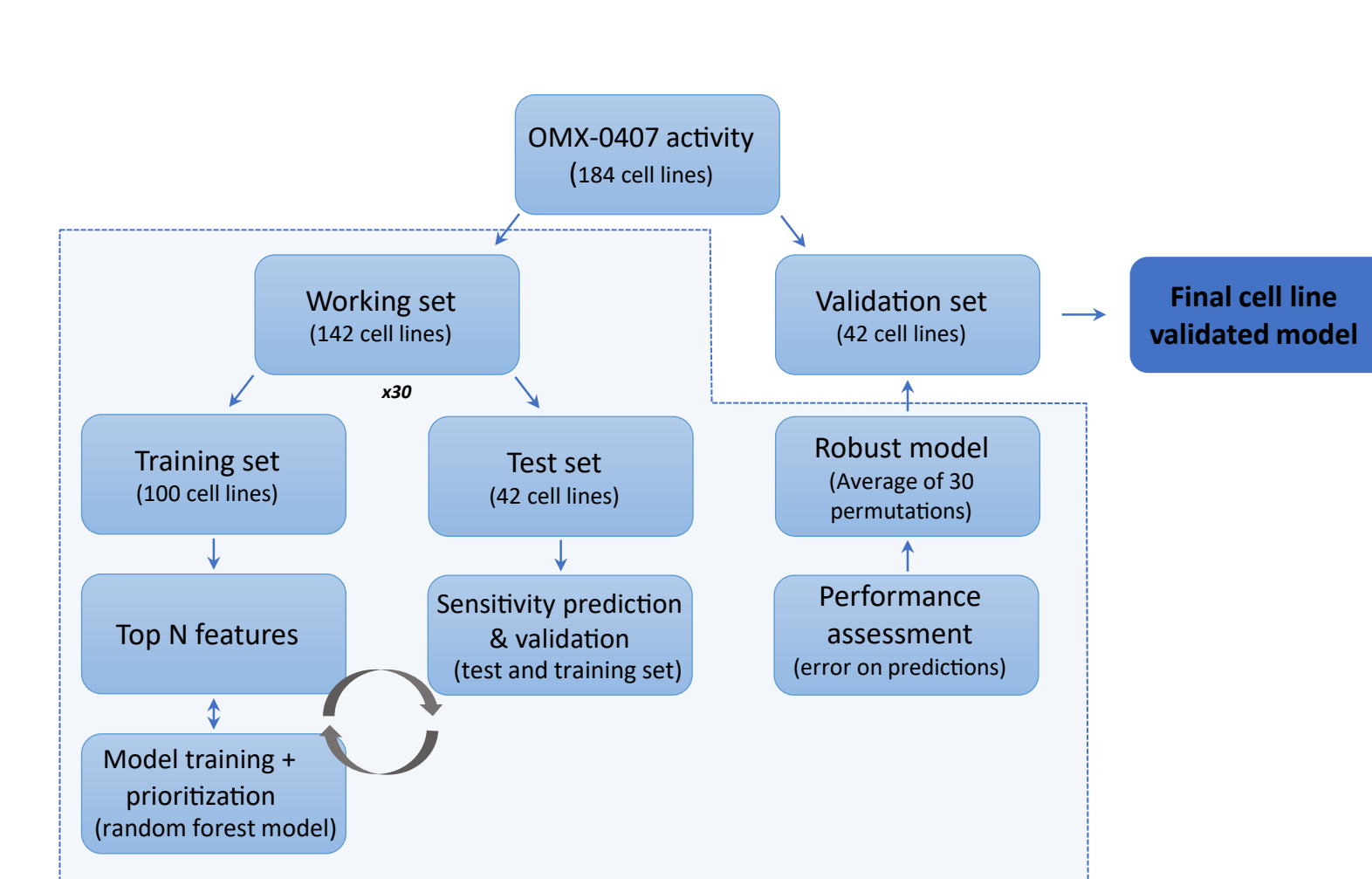


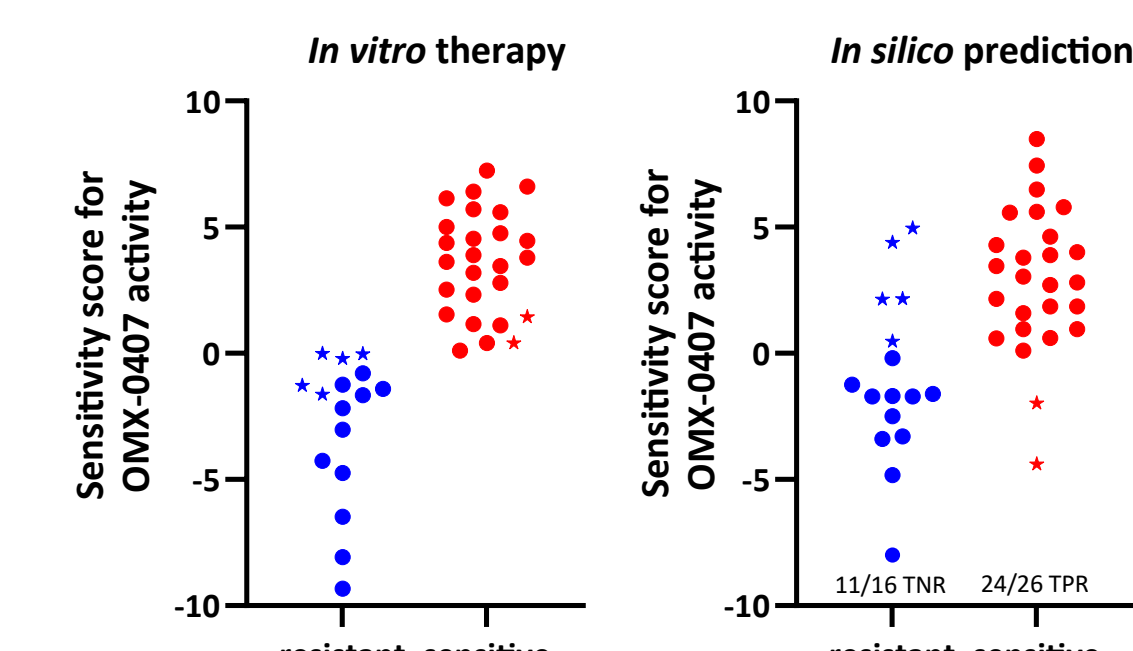
FIGURE II

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 (data shown in Figure III A). B) Heatmap of the obtained gene signature consisting of 34 features demonstrating a distinct subset of genes correlating (orange shades) or anti-correlating (blue shades) with OMX-0407 anti-tumor sensitivity.

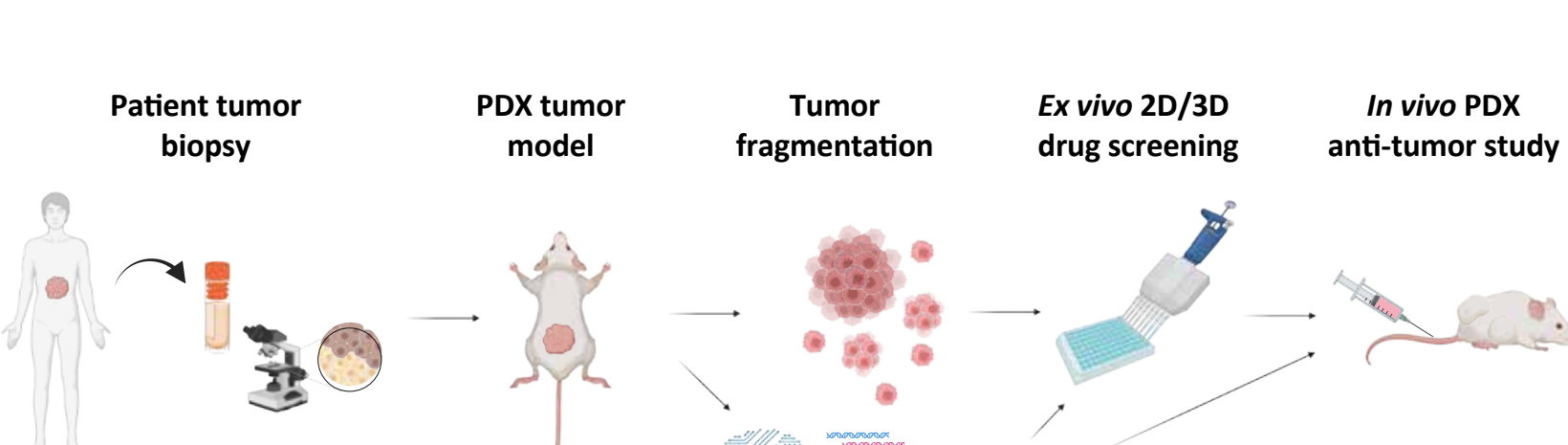
Successful validation of OMX-0407 sensitivity prediction in tumor cell lines and PDX models

- ➔ Confirmation of cell line-based predictive signature in unknown cell lines with a prediction rate of 83% (35/42)
- ➔ Testing OMX-0407 sensitivity prediction hypothesis via extensive *ex vivo* anti-tumor viability screening in 44 PDX models and *in vivo* OMX-0407 monotherapy studies in 26 PDX models compared to vehicle control
- ➔ *Ex vivo* tested PDX models of RCC, sqNSCLC and indication 4 were correctly classified with a prediction rate of 76% (10/13) by the cell line validated transcriptome signature - study ongoing

A) *In vitro* treated tumor cell lines



B) Schematic overview of *ex vivo* / *in vivo* screening for signature validation



C) *Ex vivo* treated PDX tumor fragments

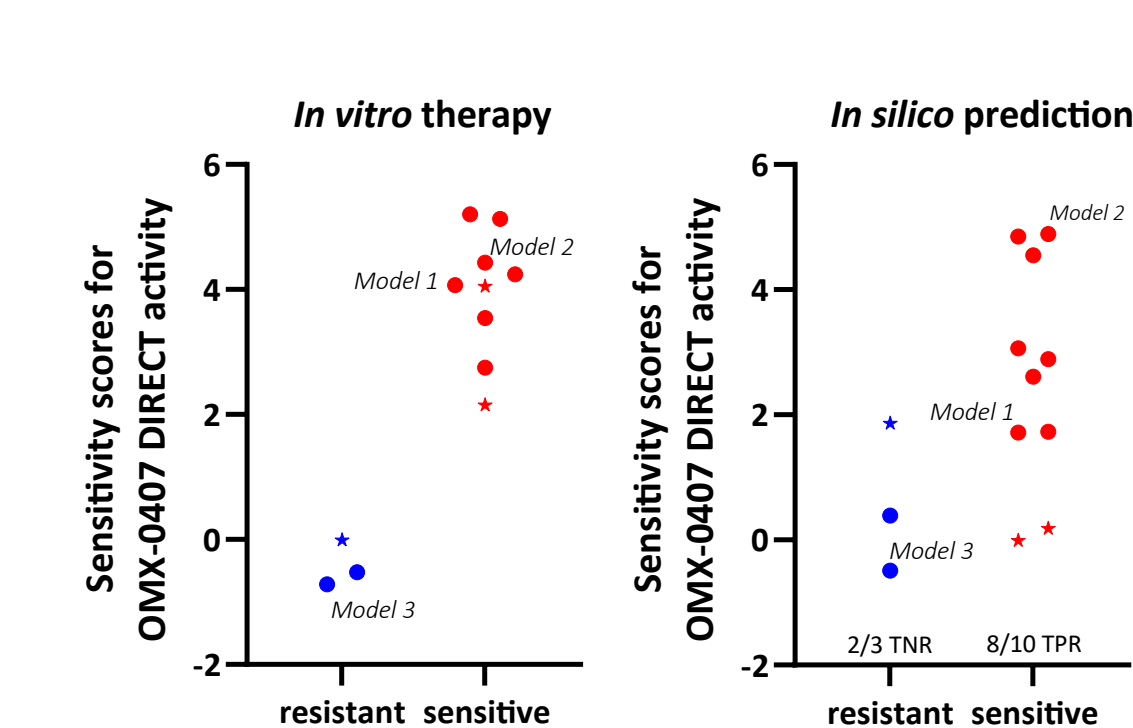


FIGURE III

A) Validation of the predictive gene signature by comparison of empirically tested anti-tumor efficacy and associated sensitivity prediction in 42 tumor cell lines of different indications. B) Schematic overview of *ex vivo* / *in vivo* PDX screening. C) Confirmation of sensitivity prediction based on baseline PDX transcriptome data, performed on $\text{log}_2(\text{TPM}+1)$ based on cell line validated gene signature depicted in figure IIB. Sensitivity score calculation for A) & C) by the following formula: Sensitivity score = $\text{Log}_2(2 \times \% \text{max Inhibition})^2 - \text{Log}_2(\text{IC}_{50} \text{obs}) + 2.5$; sensitivity score > 0 = sensitive to OMX-0407; sensitivity score < 0 = resistant to OMX-0407. TNR = true negative response; TPR = true positive response. The data points highlighted with asterisks show incorrectly predicted models. D) 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 μ M – 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.

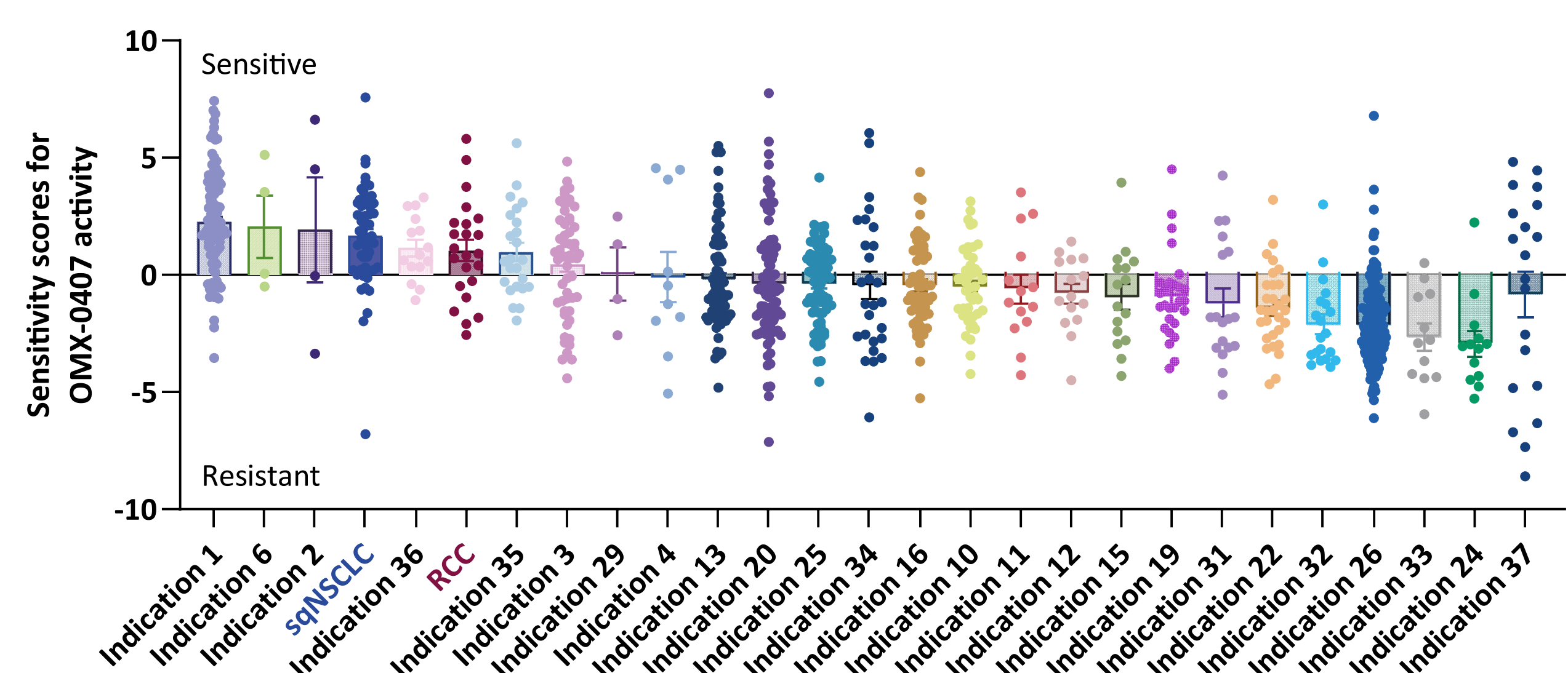
Statistical information

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 $\pm$ SEM. Significance was calculated by two-way ANOVA analysis including Tukeys multiple comparison analysis.

Consistent OMX-0407 sensitivity prediction using PDX models and human cancer patient data

- ➔ Consistent overlap of OMX-0407 sensitive-predicted indications between cell lines from CCLE (data not shown), PDX models as well as human cancer patients
- ➔ Lung squamous non-small cell carcinoma and renal cell carcinoma show strong predicted OMX-0407 anti-tumor efficacy in PDX models as well as TCGA cancer patients
- ➔ Predictions for cancer patients reveal a subset of indications with high potential for anti-tumor efficacy of OMX-0407

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



B) OMX-0407 sensitivity prediction in TCGA cancer patient data of different indications

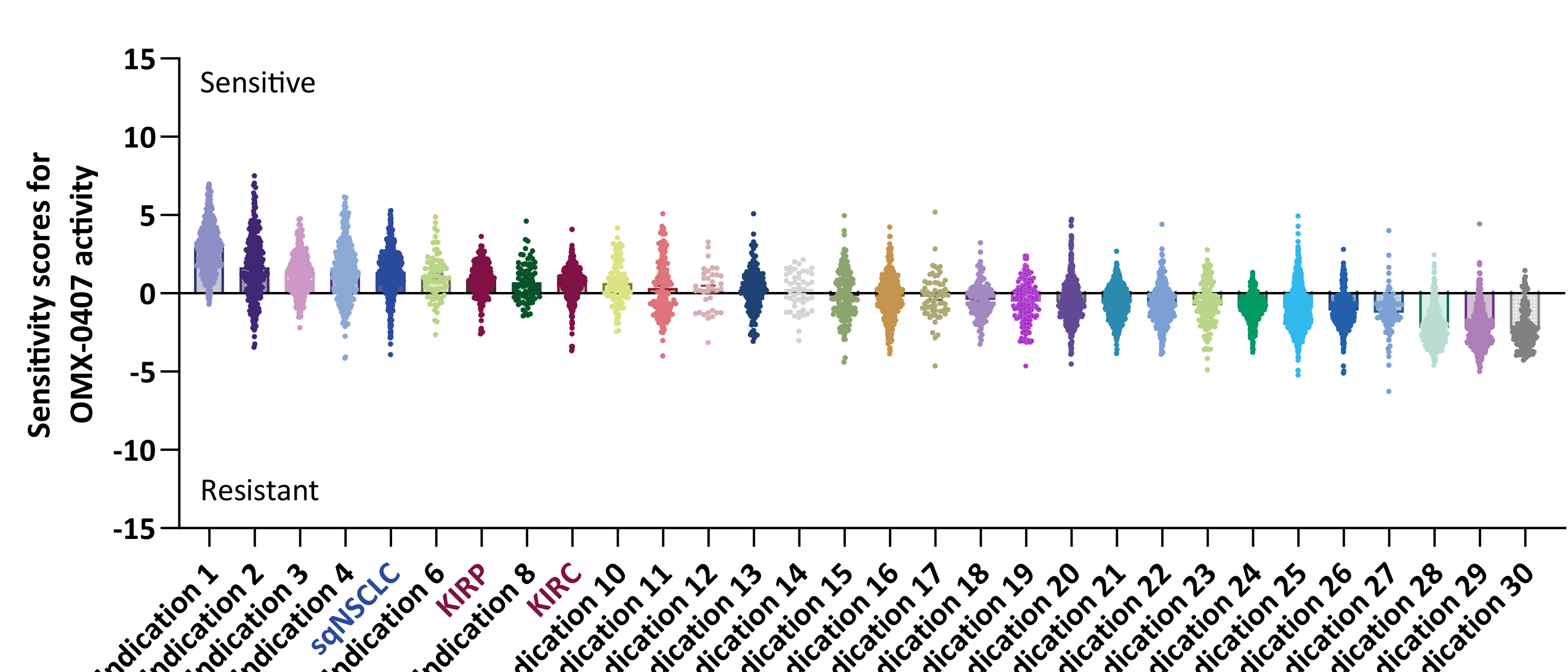


FIGURE IV

Prediction of OMX-0407 sensitivity A) in 1045 PDX models of 27 different indications and B) of transcriptome data from 8885 human cancer patients within 30 indications based on TCGA database. Sensitivity calculation for (A) & (B) performed on $\text{log}_2(\text{TPM}+1)$ based on cell line-validated gene signature depicted in figure IIB. Sensitivity score = $\text{Log}_2(2 \times \% \text{max Inhibition})^2 - \text{Log}_2(\text{IC}_{50} \text{obs}) + 2.5$; sensitivity score > 0 = sensitive to OMX-0407; sensitivity score < 0 = resistant to OMX-0407. RCC = Renal cell carcinoma; KIRP = Kidney renal papillary cell carcinoma; KIRC = Kidney renal clear cell carcinoma; sqNSCLC = Squamous non-small cell lung cancer.

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

- ➔ OMX-0407 demonstrates strong anti-tumor efficacy in monotherapy in syngeneic tumor models of indication 4, RCC, sqNSCLC and RCC
- ➔ OMX-0407 significantly promotes tumor growth inhibition in RCC in combination with the anti-angiogenic therapy Axitinib targeting vascular endothelial growth factor receptor 2 (VEGFR2)

A) OMX-0407 monotherapy in syngeneic tumor models of different indications

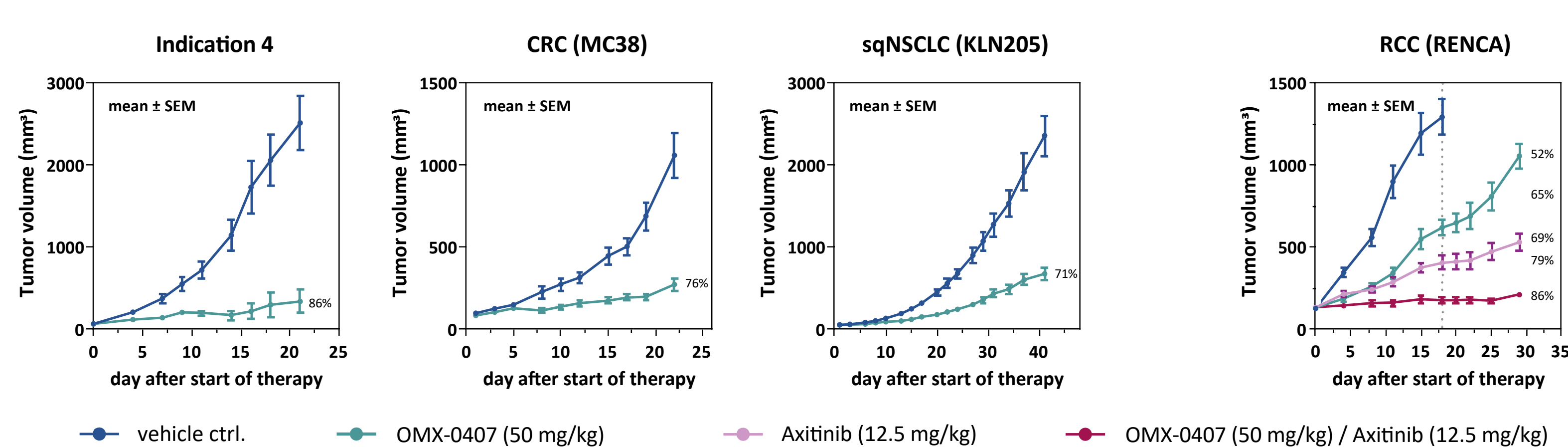


FIGURE V

Tumor cells of individual tumor models were subcutaneously implanted in their corresponding syngeneic mouse background and randomized at an average tumor volume of 50-150 mm³. A) Tumor bearing mice for monotherapy studies in indication 4, MC38 and KLN205 were treated twice daily with 50 mg/kg OMX-0407 or corresponding vehicle control by oral gavage. Graphs are demonstrating maximal tumor growth inhibition of OMX-0407 with 50 mg/kg representative of independent studies. B) RENCA tumor bearing animals were treated twice daily with both 50 mg/kg OMX-0407 or the appropriate vehicle control and 12.5 mg/kg axitinib or the appropriate vehicle control in combination via oral gavage. Average tumor growth is depicted as mean $\pm$ SEM for ≤ 12 mice per therapy group by using the last observation carried forward method.

Conclusion

- ➔ OMX-0407, a potent spectrum-selective salt-inducible kinase (SIK) inhibitor demonstrates outstanding anti-tumor efficacy *in vitro* as well as *ex vivo* in a subset of cancer cell lines and patient-rived tumor xenograft fragments, representing different indications.

- ➔ OMX-0407 shows remarkable efficacy in monotherapy as well as in combination with VEGFR2 inhibition in tumor models of indications previously identified as highly sensitive towards OMX-0407.

- ➔ 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 further validated in a variety of *ex vivo* and *in vivo* PDX models.

- ➔ The predictive gene signature will be evaluated in the ongoing first-in-human study OMX-0407-101 (NCT05826600), which was initiated in March 2023.

Contact

