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OMX-0407, a spectrum-selective kinase inhibitor drives cell-cycle arrest *in vitro* and *in vivo* – an in-depth MoA analysis by phospho-proteomics

Authors and affiliations

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Introduction

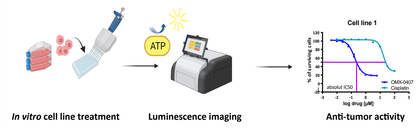
OMX-0407, an orally available, spectrum-selective kinase inhibitor targets key members of the Salt-inducible kinase (SIK) and tyrosine (-like) kinase families. An anti-tumor viability screen of >200 human cancer cell lines revealed striking effects of OMX-0407 across various cancer indications, with particularly strong effects on renal cell carcinoma (RCC) and squamous non-small cell lung cancer (sqNSCLC). Phospho-proteomics and multiplex, functional kinase activity profiling demonstrate significant anti-tumor activity of OMX-0407 via simultaneous inhibition of key cellular processes such as cell proliferation and cell cycle regulation. Orthogonal functional *in vitro* assays confirmed the association of the OMX-0407-mediated anti-tumor efficacy with the arrest of G1/ S transition and corresponding dephosphorylation of cell cycle-associated proteins downstream of the Src family kinases (SFK) and the serine/theronine kinases PAK1/2.
The inhibitory effects of OMX-0407 on cell proliferation and cell cycle regulation result in potent anti-tumor efficacy of OMX-0407 as a single agent in the RENCA-RCC mouse model, which is further enhanced in combination with axitinib, an inhibitor of the vascular endothelial growth factor receptor 2 (VEGFR2). These data further strengthen the potential of OMX-0407 for the treatment of RCC as well as other indications. In summary, OMX-0407 is a novel spectrum-selective kinase inhibitor for patients with high unmet medical need, that is currently being evaluated in a clinical Phase I trial (NCT05826600).

Results

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

- Anti-tumor monotherapy efficacy screening of OMX-0407 in 225 human cancer cell lines identifies a sensitivity profile of individual cell lines towards OMX-0407
- Squamous non-small cell lung cancer and renal cell carcinoma are emerging as key tumor indications responding to OMX-0407

A) High throughput screening of cancer cell viability with OMX-0407



B) Distinct activity profile of OMX-0407 on tumor cell lines

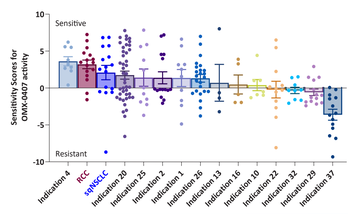


FIGURE I

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), cisplatin a 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 = $Log_2(2 \times \%max\ Inhibition)^2 - Log_2(IC50abs) + 2.5$; 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.

Phospho-proteomics analysis reveals significant dephosphorylation of key cancer signaling pathways upon OMX-0407 treatment

- Phospho-proteomics and kinase activity measurements reveal significant OMX-0407 activity in cancer cell lines
- Colorectal cancer (CRC)/gastric and adeno NSCLC cancer cell lines were treated for 4h with 200nM OMX-0407 and subjected to phospho-proteomics analysis by mass spectrometry and kinase activity measurements using multiplex, functional kinase activity profiling
- Both analyses demonstrate an OMX-0407-mediated reduction in the phosphorylation levels of many phosphosites. PamGene analysis reveals that 68% (90/133) of OMX-0407 dephosphorylated phospho-sites overlap between CRC and NSCLC cell lines
- Based on upstream kinase activity predictions, most of the 63% (73/115) OMX-0407-deactivated kinases in CRC and NSCLC cell lines are part of the the pro-tumorigenic SFK.

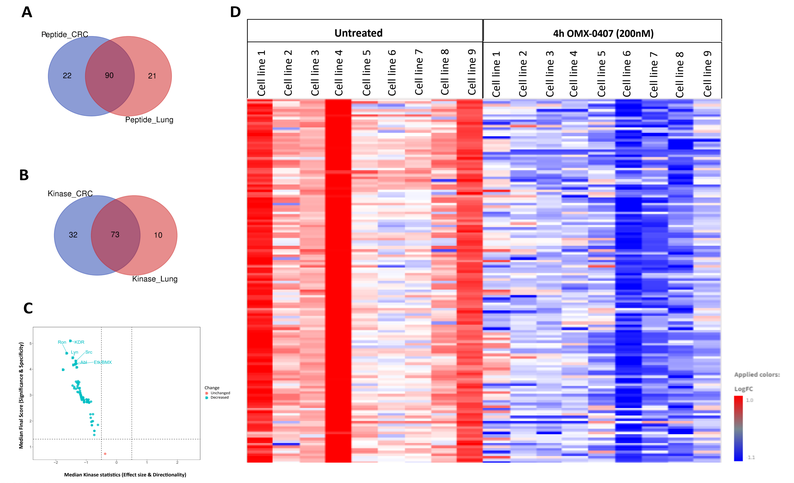


FIGURE II

A) Overlap of differentially regulated phospho-sites between CRC/Gastric and adeno NSCLC cancer cell lines. B) Overlap of predicted upstream kinases between CRC/Gastric and adeno NSCLC cancer cell lines. C) Kinase volcano Plot of OMX-0407 sensitive adeno NSCLC cancer cell lines showing the effect size and direction (x-axis, Median kinase statistic) versus significance and specificity (y-axis, Median final score) upon OMX-0407 treatment. Median Kinase Statistic is the median change in set of peptides linked to a kinase and represents change in kinase activity in a treatment (T) compared to a untreated control (U), where < 0 means inhibition, > 0 means activation in T versus U. Median final score is the sum of the Kinase Significance Score and the Kinase Specificity Score, where Kinase Significance Score is the permutation test that predicts probability of a kinase being differentially active between T and U, while Kinase Specificity Score is the permutation test that predicts probability of a set of peptides linked to a kinase. Blue spots in volcano plot are kinases that show significant difference (i.e. Median final score-log10(p)>1.3 or p<0.05) compared to untreated control. D)Heatmap comparing phosphosites in adeno-NSCLC cell lines before and after OMX-0407 treatment.

OMX-0407 inhibits tyrosine kinases involved in cytoskeletal rearrangement, cell proliferation and cell cycle

- OMX-0407 targets receptor tyrosine kinase families that are highly relevant for tumor cell proliferation
- OMX-0407 targets multiple levels within a signaling pathway ensuring a comprehensive inhibition of cancer cell growth by blocking cell proliferation and inducing a cell cycle arrest
- By targeting more than one relevant pathway of cancer cell proliferation and survival, OMX-0407 mitigates the risk of redundant pathways and the out-growth of cancer cells

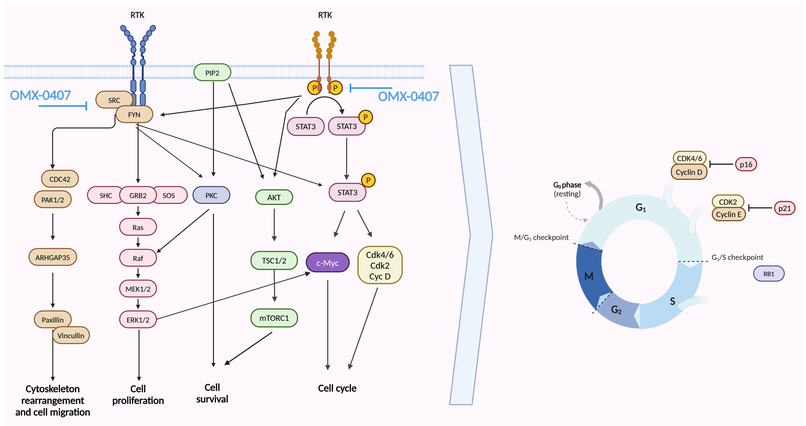


FIGURE III

Schematic representation of the different signaling pathways involved in OMX-0407 mediated cell cycle arrest. The figure shows the different key pathways, such as RAS/RAF, MAPK, PI3K/AKT/mTOR, involved in the manifestation of the OMX-0407 mediated cell cycle arrest.

OMX-0407 inhibits tumor cell growth by blocking the G1 to S transition, resulting in cell cycle arrest

- OMX-0407 induces a significant dose-dependent inhibition of tumor cell growth *in vitro* across many indications
- OMX-0407 significantly arrests cancer cells in G1 / G1-S phase as demonstrated by live cell imaging of cell cycle dynamics over time
- OMX-0407 dependent cell cycle arrest is associated with the inactivation or downregulation of key regulators of the cell cycle, such as SFKs, pPAKs, pERK1/2 or CDC42

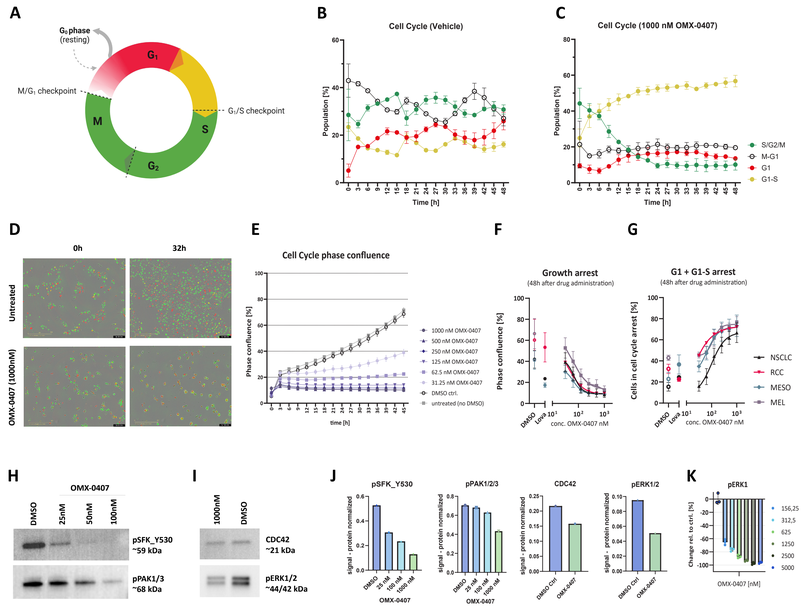


FIGURE IV

A) By using the Incucyte® Cell Cycle Lentivirus, a single-cassette, genetically encoded ubiquitination-based indicator that takes advantage of cell cycle dependent changes in the expression patterns of Geminin and Cdt1 stable cell lines were produced. Fluorescent proteins TagGFP2 and mKate2 (green/red), or TagGFP2 and TagRFP (green/orange) were linked to fragments of Geminin and Cdt1. G1 (red), the transition from G1 to S (yellow) and S/G2/M (green) phases were monitored in realtime for an untreated (B) or an 1000 nM OMX-0407 treated RCC cell line (C). D) Representative images of untreated and 4h OMX-0407 (1000 nM) treated renal cancer cells. E) OMX-0407 dose-dependent growth kinetics analyzed by phase confluence in a live cell imaging system. OMX-0407 dose-dependent growth (F) and G1 / G1-S arrest (G) of representative cell lines from different indications. G) OMX-0407 dose-dependent inhibition of cells from different indications in G1 / G1-S arrest upon OMX-0407 treatment. Phospho-protein Western Blot analyses of Caki-1 renal cancer cell lysates. Representative images of blots for SFK-Y530 (upper band), pPAK1/2/3 (lower band) (H), CDC42 (upper band) and pERK1/2 (lower band) (I) pre-and post treatment with OMX-0407. J) Signal to noise ratio after total protein normaization of pSFK-Y530, pPAK1/2/3, CDC42 and pERK1/2. K) Lumit® Immunoassay detection kit for pERK1 (Thr202) in Caki-1 renal cancer cells with different concentrations of OMX-0407 or DMSO as control (ctrl.), data shown as fold change normalized to control.

Remarkable anti-tumor efficacy of OMX-0407 in the syngeneic RCC tumor model

- OMX-0407 treatment significantly inhibits tumor growth in the RENCA RCC mouse model. This effect is further enhanced in combination with the anti-angiogenic therapy Axitinib targeting vascular endothelial growth factor receptor 2 (VEGFR2)
- Tumor growth inhibition by OMX-0407 was associated with dose-dependent reduction in the phosphorylation of SFKs & pPAKs both in the tumor and peripheral blood mononuclear cells, which correlated well with OMX-0407 plasma levels.
- OMX-0407 treatment resulted in a significant reduction of Ki67+ proliferating cells within the tumor area, likely driven by OMX-0407-induced cancer cell cycle arrest.

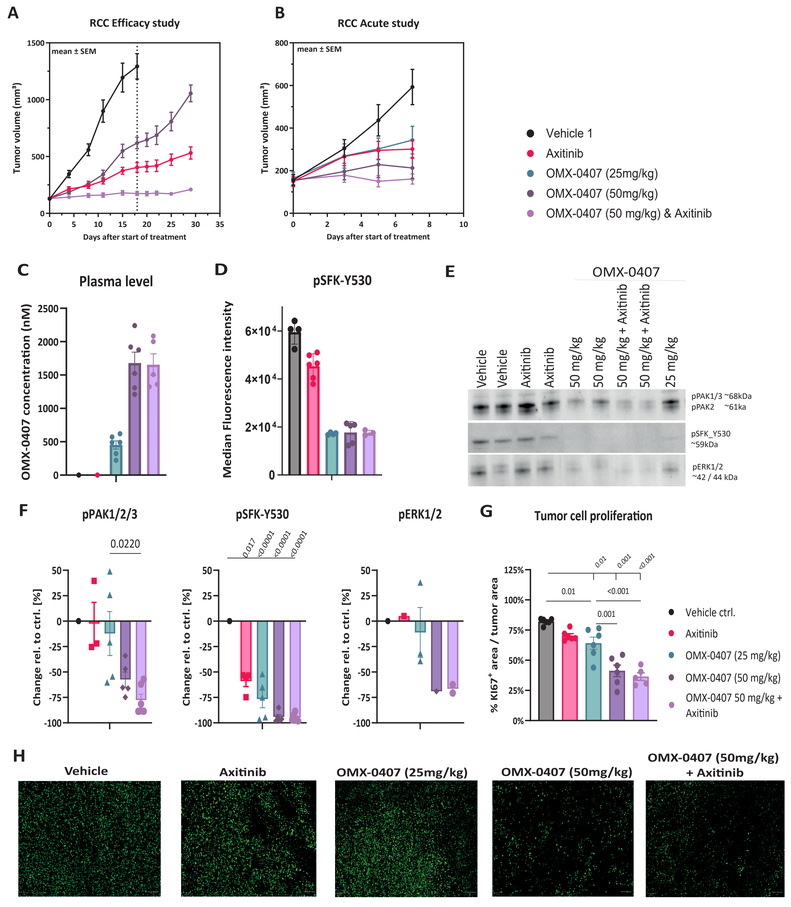


FIGURE V

RENCA tumor cells were subcutaneously implanted and randomized at an average tumor volume of 130 mm³. Tumor bearing animals were treated twice daily with both 50 or 25 mg/kg OMX-0407 and the appropriate vehicle control for Axitinib and 12.5 mg/kg Axitinib or the appropriate vehicle control for OMX-0407 in combination by oral gavage. A) Tumor growth kinetics of the efficacy animals. Average tumor growth is depicted as mean ± SEM for n≤12 mice per therapy group by using the last observation carried forward method. B) Tumor growth kinetics of separate randomized cohorts terminated for tissue sampling after 7 days of therapy. Sampling of EDTA-plasma, peripheral blood mononuclear cells (PBMCs) and tumor tissue for pharmacodynamics analyses shown in Figure IV C-H. C) OMX-0407 plasma concentration. D) Flow cytometry analysis of median fluorescence intensity of phospho-SFK-Y530 in monocytes of isolated PBMCs. E) Representative Western blot protein detection for phospho SFK-Y530 (upper row) and phospho PAK1/2/3-S144/S141/S139 (lower row). F) Signal to noise ratio after total protein normalization of anti-human p-PAK1/2/3; SFK-Y530 and pERK1/2 G) Quantification of percentage Ki-67⁺ area in viable tumor cell area. Individual data points represent the average of 5 independent field of views of a KI-67 immunofluorescence staining. I) Representative images of immunofluorescence staining of proliferation marker Ki-67 in tumor sections. Data of C, E, F, G, H are shown as mean ± SEM for n= 6 animals per treatment group.

Conclusion

- OMX-0407, a potent spectrum-selective kinase inhibitor demonstrates outstanding anti-tumor efficacy *in vitro* as well as *ex vivo* in a variety of cancer cell lines across different indications.
- OMX-0407 shows remarkable efficacy both as a monotherapy and in combination with VEGFR2 inhibition in the RENCA tumor model, supporting the potential of OMX-0407 in the treatment of RCC patients.
- OMX-0407 treatment arrests tumor cells in vitro and in vivo by inhibiting both cell cycle and proliferation.
- The ongoing comprehensive studies on the mechanism of action of OMX-0407 in inhibiting tumor cell proliferation will guide the further clinical evaluation of OMX-0407, which is currently being investigated in the ongoing first-in-human trial OMX-0407-101 (NCT05826600).

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 by two-way ANOVA analysis including Tukeys multiple comparison analysis.

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