# Overcoming resistance to osimertinib by TUSC2 gene therapy in EGFR mutant NSCLC

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AACR 2021
Poster# 1105

## ABSTRACT

Osimertinib, the only third-generation EGFR-TKI approved for the treatment of T790M-mutant non-small cell lung cancer, shows robust clinical activity, yet patients inevitably develop secondary resistance. TUSC2, an immunogene therapy, has multifunctional activity, which a) directly blocks downstream signaling through inhibiting MAPK and mTOR, b) arrests growth and proliferation of cancer cells, c) induces direct tumor cell death, and d) activates both innate and adaptive immunity. An osimertinib resistant H1975-OsiR isogenic cell line was developed through continuous exposure to osimertinib, and an osimertinib resistant clone was selected which showed 100 fold higher resistance to osimertinib compared with its parental counterpart (H1975-parental). Xenograft tumors from both H1975-parental and H1975-OsiR cells were developed in NSG mice and were treated with osimertinib. H1975-OsiR tumors were significantly less sensitive than its parental counterpart. To maintain the resistance in H1975-OsiR, the cells were cultured in the presence of osimertinib, and the osimertinib pressure was maintained in vivo throughout the experiments. Synergistic antitumor activity of TUSC2+osimertinib was found in H1975-OsiR tumors where both TUSC2+osimertinib (5mg/kg) and TUSC2+osimertinib (10mg/kg) combinations showed a robust antitumor effect compared with single agent treatment groups. No synergistic effect was observed for H1975-parental tumors. RPPA analysis of residual tumors showed a distinct set of proteins including CD44, VEGFR-2, SHP2, Akt2, YAP-pS127 overexpressed in H1975-OsiR vs H1975-parental. Among H1975-OsiR tumors, RPPA data showed that PDK1 protein was significantly altered in osimertinib treated groups as compared with controls. PDK1 was also found to be significantly upregulated in the TUSC2+osimertinib group when compared with either control, osimertinib alone, or TUSC2 alone H1975-OsiR tumors indicating that PDK1 may be associated with osimertinib resistance. PDK1 was not altered by TUSC2 alone treatment. PDK1 was not altered in any treatment groups in H1975-parental tumors. In order to validate the role of PDK1 in H1975-OsiR, we performed XTT assays of osimertinib and TUSC2+osimertinib combinations in the presence or absence of a PDK1 inhibitor (BX-795). Only the osimertinib and TUSC2+osimertinib groups showed significantly increased sensitivity to osimertinib in the presence of BX-795 as compared with the same treatment without the inhibitor. No PDK1 inhibitor effect was found in the TUSC2 treated group validating the specific role of PDK1 in osimertinib resistance. In conclusion, TUSC2 therapy in combination with osimertinib showed synergistic antitumor efficacy in EGFR mutant osimertinib resistant NSCLC tumors and upregulation of PDK1 was associated with osimertinib resistance.

## Development and Optimization of osimertinib Resistant Xenograft Model in NSG Mice

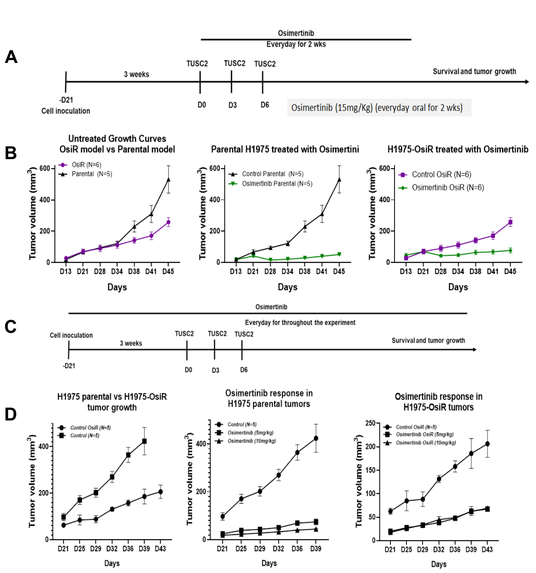



**Fig 1. Development and optimization of osimertinib resistant xenografts.** H1975-OsiR isogenic cell line was developed which had 100 fold higher resistance to osimertinib as compared with H1975-parental cell line. H1975-OsiR cells (Exon 21 L858R/T790M mutant) was maintained in 1 uM Osimertinib in culture. A) Initial treatment strategy which showed the treatment started 3 wks after tumor cell implantation. B) Tumor growth comparison between Osi-R tumors vs parental tumors (Left), effect of osimertinib on H1975-parental tumors (Middle) and H1975-OsiR tumors (Right). C) The revised treatment strategy during which osimertinib pressure was maintained throughout H1975-OsiR tumor growth. D) Tumor growth comparison between Osi-R tumors vs parental tumors (Left), effect of osimertinib on H1975-parental tumors (Middle) and H1975-OsiR tumors (Right).

## TUSC2 enhances the osimertinib response against Osimertinib resistant tumors

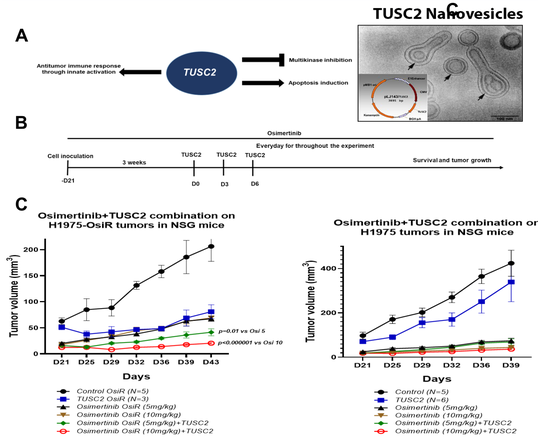
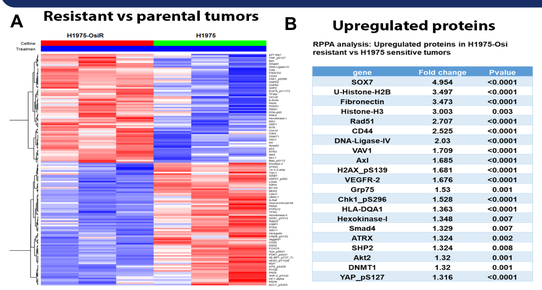



**Fig 2. Antitumor effect of Osimertinib + TUSC2 on Osimertinib resistant tumors**. TUSC2 is a tumor suppressor gene which is absent in 80% of NSCLC. The TUSC2 gene is delivered through intravenous injection of a nanovesicle formulation A) The schema shows the multifunctional activity of TUSC2 nanovesicles (Left) and electron microscopic image of TUSC2 nanovesicles (Right). B) Treatment strategies of osimertinib and TUSC2 combinations. H1975-OsiR tumors were under osimertinib pressure throughout the experiment whereas the H1975-parental tumors were grown without osimertinib pressure. C) Synergistic antitumor activity of Osimertinib + TUSC2 combination was found for only H1975-OsiR tumors (Left) whereas no synergy of osimertinib+TUSC2 combination was found against H1975-parental tumors (Right).

## PDK1 is a significantly altered protein in osimertinib resistant tumors



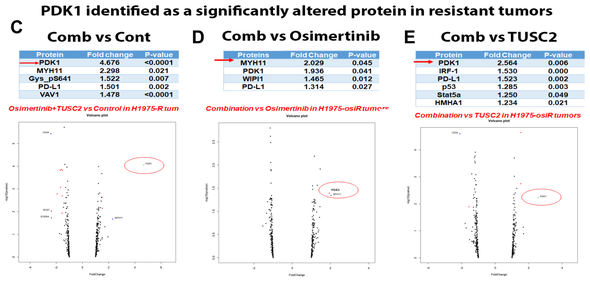




**Fig 3. RPPA analysis of protein expression in H1975-OsiR and H1975-parental tumors treated with osimertinib and TUSC2 combination.** The residual tumors were harvested by the snap-freeze method and RPPA was performed by using over 400 human antibodies. A)Heat map shows that a set of proteins are significantly altered in H1975-OsiR tumors vs H1975-parental tumors, B) table shows the list of the most upregulated proteins in osimertinib resistant tumors as compared with its parental counterpart. C-E) Volcano plots show the pair wise comparison between Combination vs Control (Left), Combination vs osimertinib (Middle) and Combination vs TUSC2 (Right) in H1975-OsiR tumors. Tables show the most upregulated proteins in each corresponding pair. PDK1 is one of the most significant altered protein.

## References & Disclosures

*Meraz IM et al, Cancer Immunol Res. 2018 Feb;6(2):163-177.*
*Meraz IM et al, Cancer Immunol Res. 2019 Aug;7(8):1267-1279*

Jack A. Roth is a consultant, stock owner (including pending patent) in Genprex, Inc. All other authors have declared that no competing interests exist.

## PDK1 inhibition increases the efficacy of the osimertinib+TUSC2 combination in resistant cells

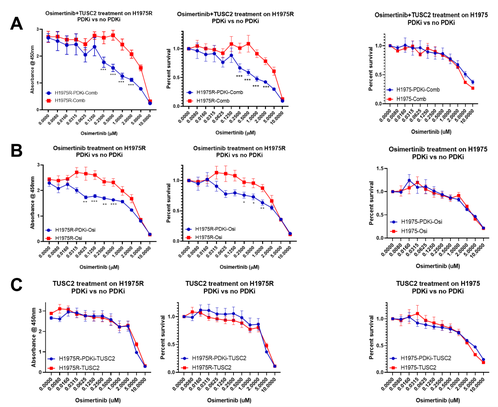



**Fig 4. Role of PDK1 on osimertinib resistant H1975-OsiR cells.** *In-vitro* XTT assays were performed to determine the cytotoxicity of osimertinib and osimertinib + TUSC2 combination in presence of BX-795, a PDK1 inhibitor, on H1975-parental and H1975-OsiR resistant cells. Cells were transfected with TUSC2 and treated with osimertinib for 24hr followed by osimertinib XTT assays. A) Cytotoxicity of Osimertinib+TUSC2 combination in presence or absence of PDKi (BX-795); H1975-OsiR (left panel) and H1975-parental (Right panel) and, B) Cytotoxicity of Osimertinib alone in presence or absence of PDKi (BX-795); H1975-OsiR (left panel) and H1975-parental (right panel), C) Cytotoxicity of TUSC2 alone in presence or absence of PDKi (BX-795); H1975-OsiR (left panel) and H1975-parental (Right panel). * p<0.05, ** p<0.005, *** p<0.0005

## PDK1 inhibition enhances the antitumor effect of osimertinib+TUSC2 treatment on H1975-OsiR tumors

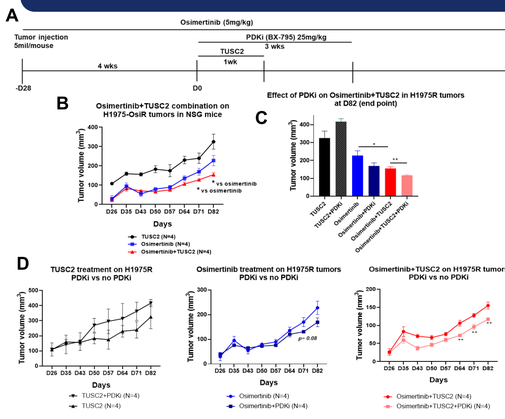



**Fig 5. Role of PDK1 in *in-vivo* antitumor activity of osimertinib + TUSC2 on H1975-OsiR tumors**. H1975-OsiR cells were cultured in presence of osimertinib and injected into NSG mice subcutaneously and mice were treated with osimertinib to maintain pressure in-vivo throughout the experiment. Mice were treated with TUSC2 and BX-795 (PDKi) 4 wks after tumor inoculation when tumors were 50-100mm3 size A) Treatment strategy for the experiment. B) validation of Osimertinib+TUSC2 antitumor effect, C) Tumor volumes in bar diagram compared the tumor volume differences with and without PDKi among different treatment groups at day 82. D) growth curves showed the differences in growth by the treatment of TUSC2, osimertinib and combination in presence or absence of PDKi. * p<0.05, ** p<0.001

## CONCLUSIONS

H1975-OsiR cells are 100 fold resistant to osimertinib in-vitro and also developed tumors in in-vivo which are significantly resistant to osimertinib as compared with that of parental tumors.

TUSC2+osimertinib treatment showed synergistic antitumor activity against H1975-OsiR resistant tumors

RPPA analysis showed a distinct set of proteins were significantly upregulated and downregulated in H1975-OsiR tumors as compared with H1975-parental counterpart.

PDK1 has been identified as one of the most significantly upregulated proteins in osimertinib+TUSC2 treated residual tumors.

PDK inhibition *in-vitro* significantly increases the cytotoxicity of combination treatment in H1975-OsiR cells

PDK inhibition in *in-vivo* significantly enhances the antitumor activity of combination treatment against resistant tumors