PAK4/NAMPT inhibitor KPT-9274 synergizes with Sunitinib in Pancreatic Neuroendocrine Tumor Cellular Models

Md. Hafiz Uddin[1], Mohammed Najeeb Al-Hallak[1], Husain Yar Khan[1], Sahar Bannoura[1], Amro Aboukameel[1], Erkan Baloglu[2], Rafic Beydoun[3], Ramzi M. Mohammad[1], Philip A. Philip[1], Bassel El-Rayes[4], Asfar S. Azmi[1]*

[1]Department of Oncology, Wayne State University School of Medicine, Detroit MI 48201 USA; [2]Karyopharm Therapeutics Inc., Newton, MA 02459, USA; [3]Department of Oncology, Wayne State University School of Medicine, Detroit MI 48201 USA; [4]University of Alabama, O'Neill Comprehensive Cancer Center. Birmingham, AL, 48009 USA.

Introduction

- Pancreatic neuroendocrine tumors (PanNETs) are rare islet cell tumors. Although slow growing in early stages, the 5-year relative survival rate of metastatic PanNETs is dismally low at 25%. The main treatment option for localized disease is surgical resection and for metastatic disease is either targeted therapy or chemotherapy depending on the pathological grade and other factors.
- Unfortunately, advanced PNETs show minimal response to FDA approved therapies suggesting an urgent need for the identification of novel and effective treatments.
- Previously, we showed that p21 activated kinase 4 (PAK4) and nicotinamide phosphoribosyl transferase (NAMPT) are over-expressed in PanNETs and targeted inhibition of PAK4-NAMPT by dual inhibitor KPT-9274 can suppress PanNET cell proliferation and arrest tumor growth.
- In the present study, we have tested the combination effect of PAK4/NAMPT inhibitor KPT-9274 with FDA approved RTK autophosphorylation inhibitor sunitinib (Sutent) in PanNET.

Materials and Methods

- Cellular proliferation or growth inhibition was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and cell titer Glo (Promega) assay. Inhibitory concentrations were calculated using GraphPad Prism software.
- For the determination of combination indexes (CI values) and generation of isobologram, Calcusyn 2.1 software was utilized.
- Formation of colony and spheroid in 2D or 3D culture respectively, were determined by clonogenic and spheroid formation assays respectively. Apoptosis was determined by flow cytometric analysis (annexin V-propidium iodide). Expression of key proteins were determined by western blotting technique.

Results

- The IC_{50s} for KPT-9274 and sunitinib compounds were determined as 0.056 µM and 6.6 µM respectively in BON-1 PanNET cells.
- The kinase inhibitor sulfatinib (surufatinib) that specifically that targets VEGFR and FGFR1 and is in phase III clinical trial for PanNETs. However, we did not observe any response up to 6.4 µM dose.
- Further experiments assessed the combination of PAK4/NAMPT inhibitor with RTK inhibitor. The combination of KPT-9274 and sunitinib showed synergy in PanNET cell lines (CI value was calculated as low as 0.27 suggesting strong synergy between these two inhibitors).
- Both single agents alone or in combination were reduced the number and area of the colonies significantly.
- Similar trends observed in spheroid formation assay.
- The combination showed enhanced apoptosis as well.

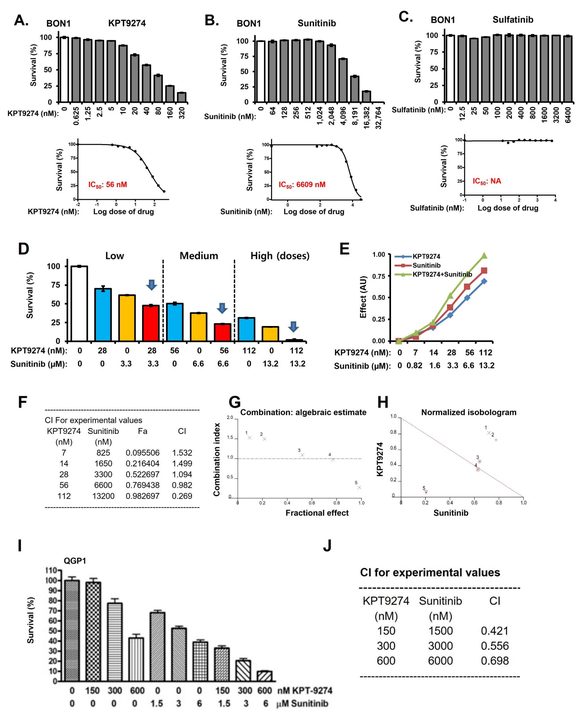

Figure 1. KPT-9274, sunitinib and sulfatinib in the inhibition of pancreatic neuroendocrine cells growth. [A–C] BON-1 cells were treated with varying indicated concentrations of KPT-9274, sunitinib and sulfatinib for 72 hrs (upper panel). MTT assay was performed to determine the growth inhibition. Lower panel is the dose-response curve generated from GraphPad Prism software, showing IC_{50} doses. [D-H] Synergistic combination of KPT-9274 and sunitinib. The combination index (CI) and isobologram were generated using Calcusyn 2.1 software. [I-J] Synergy determination of QGP-1 cells for KPT-9274 and sunitinib combination. [I] Bar diagram showing the growth inhibition in different concentrations of drug combinations. [J] Table indicates combination index values.

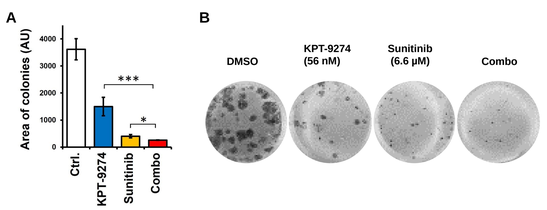

Figure 2. Colony formation with single or combinations of KPT-9274 and sunitinib. [A] Bar diagram showing comparative colony areas. [B] Representative wells showing colonies. KPT-9274 was used at 56 nM and sunitinib was used at 6.6 uM doses. Larger sized higher number colonies were appeared in vehicle control dimethyl sulfoxide (DMSO) treated group. Colonies became tiny and less in number in the combination treatment group. AU, arbitrary unit.

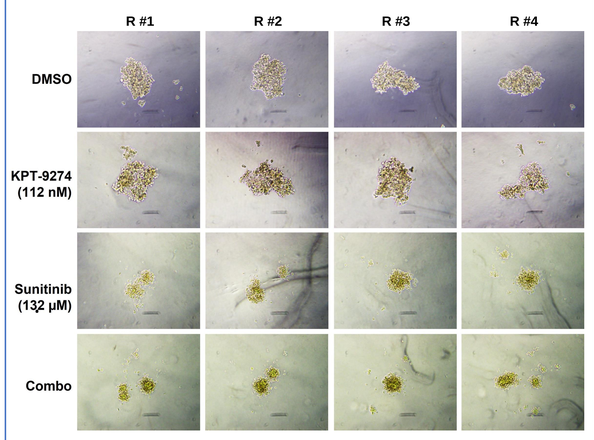

Figure 3. Effect of KPT-9274 and sunitinib on the formation of 3D spheroids of BON1 cells. In brief, 200 cells/well were seeded in 96 well ultra low attachment plate in 100 µl media supplemented with growth factors. After 7 days, images were obtained using digital microscope with 10x objective.

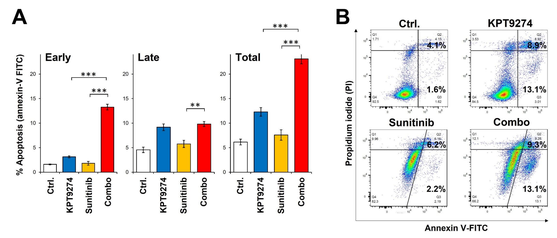

Figure 4. Apoptotic cell death with KPT-9274 and sunitinib in BON-1 PanNET cells. Apoptosis was determined by the flow cytometric detection of annexin-V and propidium iodide (PI). [A] Early, late and total apoptosis of KPT-9274 and sunitinib treated cells. [B] Representative flow cytometric image of different treated groups as shown in Fig. A.

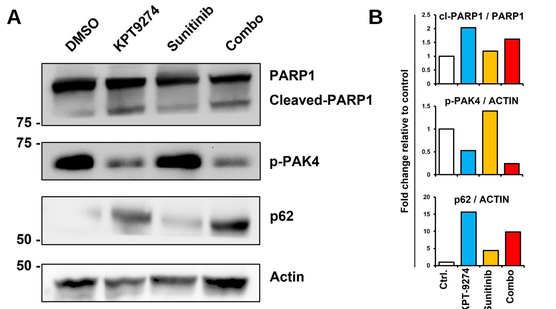

Figure 5. Western blot analysis of apoptosis and autophagy associated proteins [A]. Cells were treated with KPT-9274 and sunitinib either alone or in combination for 72 hrs. DMSO used as vehicle. Phospho-PAK4 protein analyzed to confirm the activity of KPT-9274. Actin was detected as loading control. Cell lysate was prepared in RIPA buffer with protease and phosphatase inhibitors. A total of 40 ug of total protein was separated in SDS-PAGE. [B] Band densities relative to control.

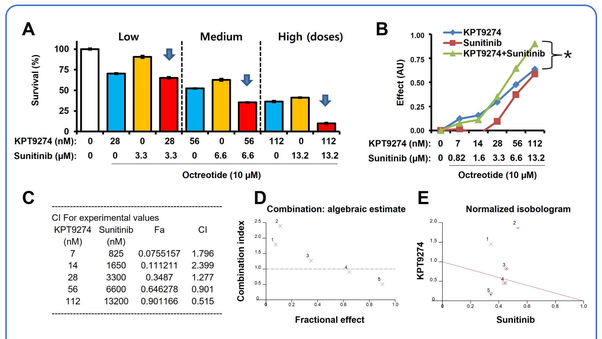

Figure 6. KPT-9274 and sunitinib in the inhibition of pancreatic neuroendocrine cells growth in the presence of octreotide. BON1 cells were treated with varying concentration of KPT-9274 and sunitinib and fixed concentration of octreotide for 72 hrs. MTT assay was performed to determine the growth inhibition. [A] Bar diagram showing growth inhibition in low, medium and high doses combination. Arrows indicate combination treatments. [B] Effect of all doses used in the experiment. *, indicates well separation of combination treatment compared to single agents. [C] Combination index (CI) values are shown, where below 1 indicates synergy. [D] CI values are plotted against affected fractions. [E] Normalized isobologram was generated using Calcusyn 2.1 software.

Conclusion

This is the first study to reveal the therapeutic potential of novel PAK4/NAMPT inhibitor KPT-9274 and RTK inhibitor sunitinib combination for the treatment of PanNETs. Currently FDA approved drug sunitinib has demonstrated its synergistic effect with KPT-9274 in growth inhibition. The combination also effective in the reduction of colony size and size of 3D spheroids. The combination induces apoptosis and could induce autophagy and might be effective in the presence of somatostatin analog octreotide. The in vivo evaluations of this novel combination in cell derived xenograft (CDx), patient derived xenograft (PDx) models are underway.

References

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