B-28

Therapies targeting CDK4/6 cause regression, immune cell activation, and sensitization to PD-L1 immunotherapy in pancreatic neuroendocrine tumors

Courtney Kaemmer[1], Shaikamjad Umesalma[1], Chandra Maharjan[1], Jordan Kohlmeyer[1], Joshua Lingo[1], Emily Wilkerson[2], Ryan Sheehy[1], Mariah Leidinger[1], David Meyerholz[1], Sarah Bell[1], Gideon Zamba[1], Patrick Breheny[1], Edmund Lattime[3], Chandrikha Chandrasekharan[1], Andrew Bellizzi[1], Laura Herring[2], Lee Graves[2], Benjamin Darbro[1], Ziqiang Yuan[3], Steven Libutti[3], Dawn Quelle[1].

[1]University of Iowa and Holden Comprehensive Cancer Center, [2]University of North Carolina, [3]Rutgers Cancer Institute of New Jersey.

BACKGROUND

New effective therapies are needed to improve the survival of patients with metastatic pancreatic NETs (pNETs). RABL6A is a novel oncogenic driver of pNET pathogenesis. Kinome and phosphoproteome analyses of proliferating (RABL6A-positive) pNET cells, versus arrested (RABL6A-knockdown) controls, demonstrated that cyclin-dependent kinase 4 and 6 (CDK4/6) and MEK kinases are actionable drug targets in growing pNET cells. In agreement, published studies of patient pNETs by immunohistochemistry (IHC) and RNAseq identified robust activation of CDK4/6 and MEK in tumors. In other tumor types, CDK4/6 and MEK inhibitors display synergistic antitumor activity linked with heightened CD8 T cell, plasma cell and/or NK cell activation. This drug combination has not yet been evaluated in pNETs.

METHODS

Synergistic effects of MEK inhibitor (Mirdametinib) and CDK4/6 inhibitor (Palbociclib) were measured by cell proliferation, survival, colony formation, and immunoblotting assays. Tumor suppressive effects were measured *in vivo* using 3 pNET mouse models: 1) flank xenografts in immunodeficient mice, 2) tail vein metastasis xenografts in immunodeficient mice, and 3) immune competent, *Pdx1-Cre;Men1$^{fl/fl}$;Pten$^{fl/fl}$* knockout mice that develop insulinoma by 5-6 months of age. Single cell RNAseq and multiplex IHC was performed on human pNETs.

RESULTS

In vitro, CDK4/6-MEK inhibitor therapy caused synergistic pNET cell death and pathway inactivation, as measured by retinoblastoma protein (RB1) hypo-phosphorylation. *In vivo*, the CDK4/6-MEK combination significantly slowed growth of flank pNET xenografts, yielding a 6-fold extension of average survival (~120 days versus 20 days for control). This combination likewise suppressed (but did not eliminate) pNET growth in a bioluminescence metastasis model and reduced tissue colonization relative to monotherapy controls. By comparison, dual CDK4/6-MEK inhibition in immunocompetent in *Pdx1-Cre;Men1$^{fl/fl}$;Pten$^{fl/fl}$* insulinomas caused dramatic tumor regression associated with B/plasma cell infiltration. Pilot analyses demonstrate the presence of B-lineage cells in human pNETs, which in other tumors prognose better survival and response to immunotherapy. Indeed, further tumor regression was achieved in *Pdx1-Cre;Men1$^{fl/fl}$;Pten$^{fl/fl}$* insulinomas by combining CDK4/6 inhibitors with anti-PD-L1 blockade.

CONCLUSIONS

Combination therapy targeting CDK4/6 and MEK inhibits pNET growth and metastatic colonization. Monotherapies were not effective, in agreement with lack of response to CDK4/6 monotherapy in pNET patient trials. In immune competent *Pdx1-Cre;Men1*$^{fl/fl}$*;Pten*$^{fl/fl}$ mice, CDK4/6-MEK inhibition causes significant tumor regression linked with immune activation while CDK4/6 inhibition alone sensitized insulinomas to anti-PD-L1 immunotherapy. These data reveal activation of anti-tumor immunity in pNETs following CDK4/6 and MEK or PD-L1 inhibition. Such data provide compelling pre-clinical rationale for pNET clinical trials testing these combination therapies.

ABSTRACT ID 23715