36. METABOLIC REPROGRAMMING IN BRAF INHIBITOR RESISTANT MELANOMA CELLS LEADS TO HYPERSENSITIVITY TO ARGININE DEPLETION AND UPREGULATION OF PD-L1 EXPRESSION

Savaraj N[1,2]; Li Y[1,2]; Wu C[1]; Suarez M[1]; Shah SS[2]; Chen S[2]; Wangpaichitr M[1]; Kuo MT[3]; Feun LG[2]
[1] Miami VA Healthcare System, Miami, FL; [2] University of Miami, Dept. of Medicine, Miami, FL; [3] Texas MD Anderson, Houston, TX

RATIONALE: The time to response to immunotherapy is long and not suitable for rapidly growing BRAF resistant (BR) or BMR (BRAF and MEK) resistant melanoma. We exploit a new approach to treat these tumors

BACKGROUND: We have previously shown that BR/BMR cells do not express argininosuccinate synthetase (ASS) and arginine deprivation induced apoptosis instead of autophagy (Oncotarget 7:14). We plan to exploited this alteration to treat BR/BMR

METHODS: Five BR cells A375, MEL-1220, A2058, UACC-62, and SK-MEL28 were established (Oncotarget) Arginine deprivation was achieved using arginine degrading enzyme (ADI-PEG20, *Polaris*) which degrade arginine to citrulline.

RESULTS: BR cells are hypersensitive to ADI-PEG20. Treatment resulted in 10-30% increase in apoptosis compared to their parental cells. The mechanisms involved are as follows: All BR cells do not express ASS, and have attenuated glucose uptake. They acquire exogenous arginine by expressing high levels of arginine transporter CAT2. The mechanism for low levels of ASS is due to diminished c-Myc, a positive regulator of ASS Additionally, AMPK-α1 (govern autophagy and glucose uptake), was attenuated in BR cells. This is proved by knockdown and overexpress AMPK-α1. Immunohistochemical staining further confirmed lower levels of AMPK-α1 in tumor tissues (average H-scores of ASS and AMPK in parental tissues vs. BR (BMR) tissues are 58.2 vs. 7.8, and 146 vs. 78.3, respectively, $p < 0.03$). Importantly, these findings also apply to tumor from BMR patients. Interestingly, treatment with ADI-PEG20 leads to robust expression of immune checkpoint PD-L1 in both parental and BR cells and PBMCs from BR patients. Importantly, macrophage polarization may involve in metabolic reprogramming.

CONCLUSION: Attenuation of AMPK-α1-in BR results in diminished autophagy and metabolic alteration. These BR cells depend less on glucose but more on arginine, and hence vulnerable to arginine deprivation. Additionally, arginine deprivation upregulates PD-L1 expression and leads to sensitivity to anti PDL-1 antibody. Combination of ADI-PEG20 with checkpoint inhibitors can lead to robust antitumor effect in BR and BMR patients. **Supported by VA Merit Review (BLR&D BX00333280-01) and R01CA152197**