HYPOXIA REDUCTION IN TANDEM WITH ANTI-ANGIOGENIC THERAPY REMODELS THE PDAC MICROENVIRONMENT AND POTENTIATES CD40 AGONIST THERAPY

Arthur Liu*, Michael Curran. *The University of Texas MD Anderson Cancer Center, Houston, TX, United States*

Background The majority of patients with pancreatic ductal adenocarcinoma (PDAC) fail to derive any durable responses from single agent immune checkpoint blockade therapy. This refractory state originates from PDAC's unique tumor microenvironment that is densely populated by immunosuppressive myeloid cells while excluding most antitumor CD8 T cells.¹ In addition, PDAC is highly hypoxic and exhibits poor vascularity, both qualities which further limit antitumor immunity.^{2,3} We showed that the hypoxia-activated prodrug TH-302 (Evofofosfamide) potentiates immunotherapy responses.⁴ Mechanistically, TH-302 decreases intratumoral hypoxia and initiates normalization of the tumor vasculature. While TH-302 facilitates a cellular remodeling process that diminishes tumor hypoxia, the nature of the vascular remodeling involved remains unknown, as do the downstream consequences for the composition of the tumor microenvironment and responsiveness to immunotherapy. We hypothesized that anti-angiogenic therapy and Evofofosfamide might cooperate to normalize tumor vasculature and diminish hypoxia.

Methods TH-302 and a vascular endothelial growth factor receptor-2 (VEGFR-2) blocking antibody were used to treat several syngeneic murine models, including orthotopic pancreatic cancer and a transplantable model of prostate cancer. Immunofluorescence and flow cytometry were used to assess intratumoral hypoxia, vessel normalization, and tumor immune infiltrate.

Results We find that anti-VEGFR-2 (DC101) in combination with TH-302 demonstrates a cooperative benefit to combat both orthotopically implanted pancreatic cancer and transplantable prostate cancer. Combination therapy reduces intratumoral hypoxia, leads to pruning of the tumor vasculature, and increases the infiltration of endothelial cells into hypoxic regions. Across models, the combination of DC101 and TH-302 significantly enhance CD8 T cell function and limits their exhausted state. At the same time, tumor associated macrophages exhibit decreased expression of M2-like features. Similar to other anti-angiogenic therapies, combination DC101 and TH-302 leads to an increased frequency of PD-L1 expressing cells. Concurrent anti-PD-1 failed to provide any additional therapeutic benefit, which in part may be due poor CD8 T cell infiltration. Instead, we find that CD40 agonist therapy is improved when combined with TH-302 and DC101.

Conclusions TH-302 and DC101 utilize unique yet complementary mechanisms to improve the survival of mice challenged with pancreatic or prostate tumors. This combination relieves hypoxia and simultaneously reinvigorates T cell function and reduces macrophage mediated immunosuppression. In this setting, CD40 agonist therapy provides an additive benefit in prolonging mouse survival. Put together, these data indicate that targeted hypoxia reduction with anti-angiogenic therapy remodels the tumor microenvironment and enhances immunotherapy responses in PDAC.

REFERENCES

1. Bear AS, Vonderheide RH, O'Hara MH. Challenges and opportunities for pancreatic cancer immunotherapy. *Cancer Cell*. 2020;**38**(6):788–802. doi: 10.1016/j.ccell.2020.08.004. Epub 2020 Sep 17. PMID: 32946773; PMCID: PMC7738380.

2. Koong AC, Mehta VK, Le QT, Fisher GA, Terris DJ, Brown JM, Bastidas AJ, Vierra M. Pancreatic tumors show high levels of hypoxia. *Int J Radiat Oncol Biol Phys* 2000;**48**(4):919–22. doi: 10.1016/s0360-3016(00)00803-8. PMID: 11072146.
3. Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009;**324**(5933):1457–61. doi: 10.1126/science.1171362. Epub 2009 May 21. PMID: 19460966; PMCID: PMC2998180.
4. Jayaprakash P, Ai M, Liu A, Budhani P, Bartkowiak T, Sheng J, Ager C, Nicholas C, Jaiswal AR, Sun Y, Shah K, Balasubramanyam S, Li N, Wang G, Ning J, Zal A, Zal T, Curran MA. Targeted hypoxia reduction restores T cell infiltration and sensitizes prostate cancer to immunotherapy. *J Clin Invest* 2018;**128**(11):5137–5149. doi: 10.1172/JCI96268. Epub 2018 Oct 15. PMID: 30188869; PMCID: PMC6205399.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.730>