874 OPTIMIZING A COMBINATION RADIO-IMMUNOTHERAPY REGIMEN IN A PRECLINICAL MODEL OF TREATMENT-RESISTANT, HIGH-RISK NEUROBLASTOMA

Lauren Zebertavage*, Amy Erbe, Taylor Aiken, Allison Schopf, Megan Nielsen, Sydney Katz, Zachary Morris, Alexander Rakhmilevich, Paul Sondel. *University of Wisconsin, Madison, WI, United States*

Background Neuroblastoma (NBL), a cancer derived from neural crest precursor cells, is the most common extra-cranial solid tumor in children, with a median age of diagnosis of 22 months. Patients diagnosed with NBL are segmented into prognostic categories with ~50% categorized as high-risk (HR). Of these patients, ~40% are refractory to, or relapse following, initial treatment; there are currently no effective treatment options for these patients once they have failed salvage therapy of chemotherapy combined with αGD2 therapy (dinutuximab). Our group has previously published work developing a **c**ombination **a**daptive and **i**nnate immunotherapy **r**egimen, "CAIR", to treat a murine model of treatment-resistant, HR-NBL.[1,2] CAIR utilizes αGD2 immunocytokine (hu14.18-IL2), radiotherapy (RT), αCD40, CpG, and αCTLA4 but questions remain about the relative contribution of each component. In this study, we tested if our model of HR-NBL is resistant to salvage therapy of temozolomide and irinotecan (TEM+IRI) and αGD2-based therapy and if components of our effective CAIR therapy can be removed, in order to determine their necessity.

Methods To establish 9464D-GD2 as a model for treatment-resistant, HR-NBL, tumor-bearing mice were treated with TEM+IRI and/or hu14.18-IL2. To establish the necessity of each component of CAIR, mice bearing 9464D-GD2 tumors were treated with CAIR (12Gy RT, αCD40, αCTLA4, CpG, and hu14.18-IL2) or variations of CAIR subtracting one component.

Results Salvage therapy of TEM+IRI and/or hu14.18-IL2 extended the survival of mice ($p<0.03$) but did not result in 9464D-GD2 tumor cures. Adding RT (12Gy, SARRP) improved survival ($p<0.0001$), but not more than RT alone ($p=0.29$), and still did not cure tumors.

Removing RT or hu14.18-IL2 from CAIR dramatically shortened survival ($p<0.0001$) and yielded few tumor cures (1/16, 0/16 versus 13/30). Conversely, removing αCD40, αCTLA4, or CpG did not alter survival ($p=0.81$, $p=0.85$, $p=0.70$) relative to CAIR, and resulted in similar rates of tumor cures (8/16, 9/21, 7/16 versus 13/30). Removing two of the "expendable" components (αCD40, αCTLA4, CpG) generally reduced efficacy, with only CAIR subtracting CpG and αCTLA4 (RT, hu14.18-IL2, and αCD40) curing a similar number of mice as CAIR (3/16 versus 13/30, survival $p=0.12$).

Conclusions Here we demonstrate that 9464D-GD2 tumors behave similarly to human HR-NBLs that fail to be cured by salvage therapy. In contrast, these tumors can be cured by both CAIR therapy and by several versions of a reduced CAIR therapy (CAIR subtracting αCD40, αCTLA4, or CpG). We are now exploring the role of each component of CAIR in anti-tumor immune responses in this immunologically cold model.

REFERENCES

1. Voeller J, Erbe A, Slowinski J, *et al*. Combined innate and adaptive immunotherapy overcomes resistance of immunologically cold syngeneic murine neuroblastoma to checkpoint inhibition. *J Immunother Cancer*. 2019;**7**:344.
2. Aiken T, Erbe A, Zebertavage L, *et al*. Mechanism of effective combination radio-immunotherapy against 9464D-GD2, an immunologically cold murine neuroblastoma. *J Immunother Cancer*. 2022;**10**:e004834.

Ethics Approval The study was approved by the University of Wisconsin's School of Medicine and Public Health Institutional Animal Care and Use Committee (IACUC), protocol: M005984.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0874