**ABSTRACT**

The Aryl Hydrocarbon Receptor (AHR) has been identified as a driver of cancer progression and cancer immunity. Over-expressed in many tumor types and activated by tryptophan metabolites and other ligands, the AHR presents a novel cancer target downstream from IDO and TDO. Over the last 10 years, studies from several laboratories suggested that endogenous and environmental AHR ligand-mediated immunosuppression is effected through inhibitory T cell subsets and, potentially, other immune subsets. These results suggest that the AHR is a driver of immunosuppression and a powerful immune modulator in the tumor microenvironment. To test this hypothesis, we used pharmacologic and molecular approaches to regulate AHR activity in several murine tumor models.

We demonstrated that a novel AHR inhibitor, HP163 (Hercules Pharmaceuticals), reduces tumor growth in syngeneic models of oral (MOC1), colorectal (CT26), and skin (B16) cancers in immunocompetent hosts. CRISPR/Cas9-mediated AHR knockdown in MOC1 cells completely blocked tumor growth, decreased the percentage of CD11b⁺PD-L1⁺ tumor-infiltrating cells and increased tumor-infiltrating CD4⁺ and CD8⁺ T cells. Mice having received AHR-MOC1 cells were completely resistant to a second challenge with wildtype AHR-MOC1 cells several months after the primary inoculation. These data suggest that the presence of the AHR in the tumor is sufficient to induce immunosuppression. Furthermore, the absence of AHR in macrophages (by lysozyme promoter-driven conditional knockout) significantly slowed tumor growth and was accompanied by a decrease in CD4⁺FoxP3⁺ T cells and in the percentage of cells expressing an exhausted T cell phenotype. These data suggest that the AHR, in both malignant cells and the immune compartment, represents an attractive target for cancer immunotherapy and that HP163 may represent a novel approach to cancer therapy with single agent activity.

**RESULTS**

1. HP163 blocks murine OSCC tumor growth in orthotopic transplants.
2. HP163 reduces the percentage of draining lymph node CCR2⁺ and PD-L1⁺ MDSC-M-like and MDSC-G-like cells in MOC1-bearing mice.
3. AHR knockout in malignant OSCC cells completely blocks tumor growth, confirming that the AHR is an attractive target for cancer therapy.
4. AHR inhibitor HP163 blocks growth of multiple cancer types (OSCC, colon, melanoma), potentially with better efficacy than an IDO inhibitor Epacadostat (colon).
5. AHR inhibitor HP163 reduces the percentage of cells expressing an MDSC-M or MDSC-G phenotype in mice bearing an OSCC tumor.
6. Tumors grow more slowly in hosts in which the AHR has been conditionally deleted from macrophages.
7. Conditional AHR knockout in macrophages decreases the percentage of T cells expressing the phenotype of exhausted effector T cells and reduces the percentage of cells expressing a Treg phenotype.
8. These results are consistent with the hypothesis that the AHR represents an immune modulator as well as a regulator of malignant cell invasion, migration, metastasis and "stem-ness". As such, HP163 should be considered for targeted cancer therapy as single agent or in combination therapy.

**CONCLUSIONS**

1. AHR knockout in malignant OSCC cells completely blocks tumor growth, confirming that the AHR is an attractive target for cancer therapy.
2. AHR inhibitor HP163 blocks growth of multiple cancer types (OSCC, colon, melanoma), potentially with better efficacy than an IDO inhibitor Epacadostat (colon).
3. AHR inhibitor HP163 reduces the percentage of cells expressing an MDSC-M or MDSC-G phenotype in mice bearing an OSCC tumor.
4. Tumors grow more slowly in hosts in which the AHR has been conditionally deleted from macrophages.
5. Conditional AHR knockout in macrophages decreases the percentage of T cells expressing the phenotype of exhausted effector T cells and reduces the percentage of cells expressing a Treg phenotype.
6. These results are consistent with the hypothesis that the AHR represents an immune modulator as well as a regulator of malignant cell invasion, migration, metastasis and "stem-ness". As such, HP163 should be considered for targeted cancer therapy as single agent or in combination therapy.