The aryl hydrocarbon receptor (AhR) is a ligand-controlled transcription factor that is primarily known to be a sensor of xenobiotics and tumor-promoting activities of halogenated hydrocarbons and polycyclic aromatic hydrocarbons. Several endogenous ligands such as metabolites produced by commensal microorganisms on skin and in gut and metabolites of L-Tryptophan - produced under control of the Tryptophan dioxygenases IDO1 and TDO2 such as L-Kynurenine and Kynurenate acid are known to modulate the transcriptional activity of AhR. The activity of AhR is predominantly in different immune and epithelial cells, thereby balancing immune responses towards various signals (Fig.1).

Activation of the IDO1-Kynurenine-AhR pathway and accumulation of nuclear AhR protein is frequently seen in different tumor types and possibly linked to the observed diminished anti-tumor immune response. AhR agonizing ligands produced by cancer cells and/or lymphocytes recruited to the TME reduce the anti-tumor immune response through increasing the numbers and function of regulatory T cells while reducing that of cytotoxic CD8+ T cells (Fig. 2).

In order to reduce AhR-mediated immune-suppression in cancer patients, Phenex Pharmaceuticals initiated a program to identify small molecule AhR antagonists to block activated downstream signaling of AhR. To this end, we have identified novel AhR antagonists, which show strong antagonistic activity on mouse and human AhR. The compound series display good oral bioavailability and low clearance in mice. Our AhR antagonist molecules display effects on multiple immune cell types, reversing immune-suppression on tumors and thereby leading to tumor growth inhibition. Our molecules show single agent anti-tumor activity and increase the efficacy of Gemcitabine and anti-PD-L1 checkpoint inhibition in different syngeneic mouse tumor models. Future research is directed to determine PK-PD markers suitable for clinical development and identification of human malignancies likely of responding to AhR antagonist treatment.

**Phenex potent AhR antagonists**

**PK-A26446 reduces VAG539-induced Cyp1a1 expression in vivo**

**AhR activity in mouse and human T cells**

**Conclusions**

Phenex has generated highly potent and selective small molecule AhR antagonists with good bioavailability and PK properties.

- block activity of multiple AhR agonists in vitro
- potently inhibit the PD marker - Cyp1a1 in vivo - mouse liver
- suppress sI2 levels in human PMBCs and mouse splenocytes
- demonstrate anti-tumor activity i.e. tumor growth inhibition in various syngeneic models
- enhance chemotherapeutic drugs and anti-PD-L1 efficacy
- reverse the immune-suppressive TME to a permissive, immune-competent state, increasing intra-tumor CDB T cells and M1 macrophages

Our AhR antagonists are well tolerated and suited for combination therapies, making them very attractive for clinical development.