A novel aryl hydrocarbon receptor antagonist (PX-A24590) with anti-tumor activity in a syngeneic mouse pancreatic cancer model

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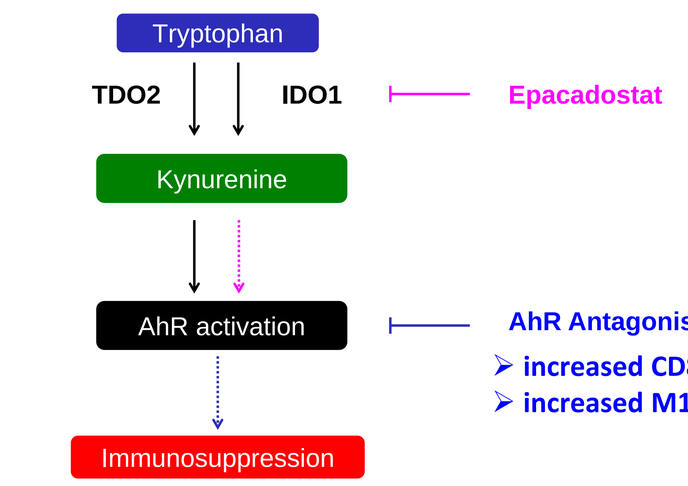
Abstract

The aryl hydrocarbon receptor (AhR) is a ligand-controlled transcription factor that is widely known for mediating toxicity and tumor-promoting activities of halogenated hydrocarbons (like Dioxin, TCDD) and polycyclic aromatic hydrocarbons (e.g. Benzo(a)pyrene and 3-Methylcholanthrene). Other ligands include metabolites produced by commensal microorganisms on the skin and in the gut and are known to modulate the transcriptional activity of AhR in different immune cells (e.g. ILC type 3 in the gut) and epithelial cells, thereby balancing the immune system´s response towards these microorganisms. In recent years, endogenous L-Tryptophan metabolites, such as L-Kynurenine and Kynurenic acid, that are produced under control of the Indole-2,3-Dioxigenases IDO1 and TDO2 pathways, were shown to activate AhR.

Constitutive activation of the IDO1/TDO2/AhR pathway and nuclear AhR protein accumulation is frequently observed in different tumor types, which is thought to be linked to the observed diminished anti-tumor immune response. It is believed that secreted AhR activating ligands aid in reducing the pressure exerted on tumors by the immune system through increasing the numbers and function of regulatory T cells and reducing the numbers and function of cytotoxic CD8⁺ T cells. Inhibition of IDO1 by Epacadostat improved anti-tumor efficacy of the anti-PD-1 checkpoint inhibitor Keytruda in a Phase II study in melanoma but unfortunately not in the recently terminated Phase III ECHO-301 study in melanoma.

In order to relieve AhR-mediated immune-suppression, Phenex Pharmaceuticals initiated a program to identify small molecule AhR antagonists to block downstream signaling of AhR due to activating endogenous ligands. To this end, we have identified a novel AhR antagonist, PX-A24590, which showed strong antagonistic activity against human AhR in a cell based CYP1A1 promoter-driven luciferase reporter assay in HepG2 cells. PX-A24590 displays good oral bioavailability and low clearance in mice. In C57BL/6 mice transplanted with syngeneic Panc02 pancreatic tumor cells, we were able to demonstrate anti-tumor efficacy with three different oral doses of PX-A24590 compared to an effective dose of the IDO1 inhibitor Epacadostat.

IDO1/TDO-Kynurenin-AhR immune suppression pathway

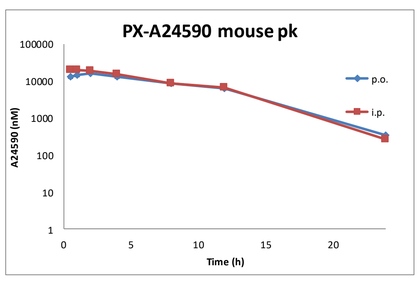


Compounds

Fig. 1

Structure guided design

Screening hits from „in-house“ library + published AhR antagonists → **PX-A24590**



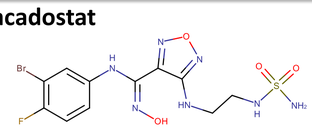
Cellular IC50
huAhR ~60nM
mAhR ~1µM

$t_{1/2}$ = 3,3 h
F~89% (po/ip)

Good PK for systemic treatment

Patents for 3 structurally related AhR modulator series have been filed

Epacadostat



Cell. hu IDO1 IC50 ~8nM
$t_{1/2}$ = 2,5 h
F~97% (po/ip)

PX-A24590 antagonizes natural and synthetic AhR agonists in HepG2-CYP1A1-Luciferase cells

Fig. 2

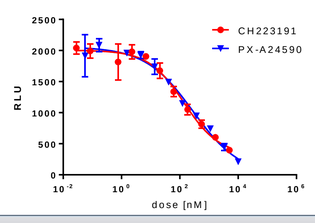
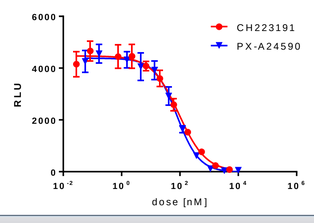
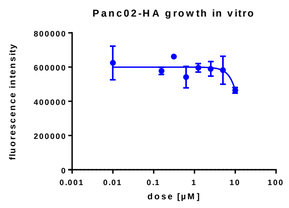


Fig. 3



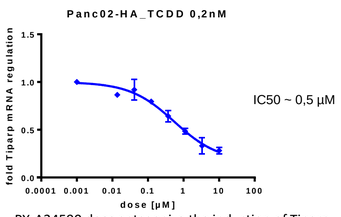
PX-A24590 activities in murine Panc02-HA cells in vitro

Fig. 4A



PX-A24590 has limited effects on Panc02-HA cell growth in vitro
Cyquant direct proliferation assay

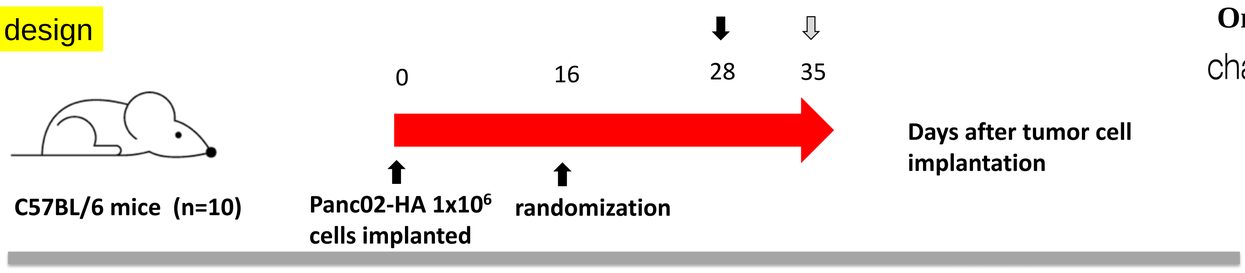
Fig. 4B



PX-A24590 does antagonize the induction of Tiparp by 0,2nM TCDD in Panc02-HA cells in vitro

Subcutaneous pancreatic cancer model with Panc02-HA cells in syngeneic C57BL/6 mice

Study design



Oncotest charles river

Fig.5

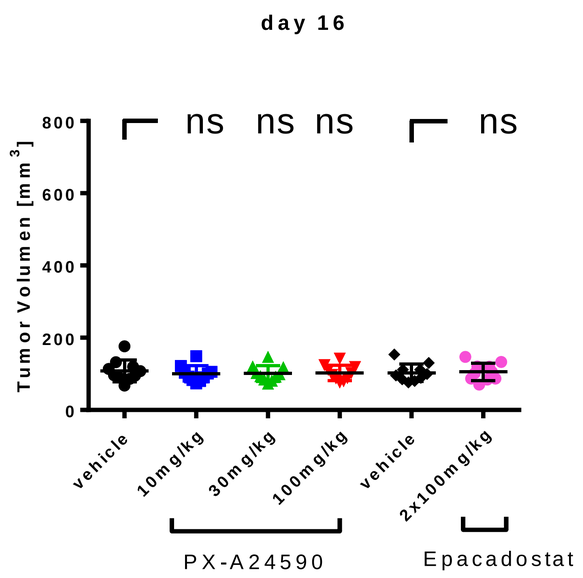
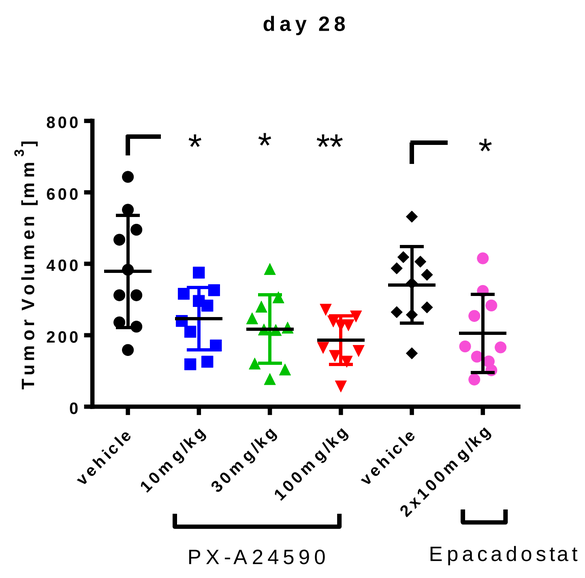


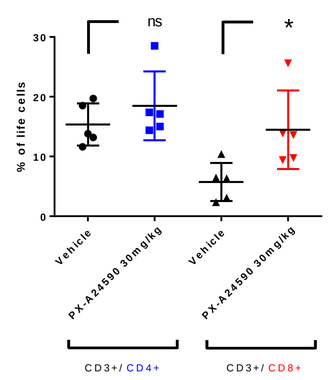
Fig.6



After randomization on day 16, tumors have an average volume of 100 mm³ (Fig.5) while significant tumor growth (caliper measurement) reduction was observed on day 28 (Fig.6) after 12 days of daily treatment with different doses (10, 30 or 100mg/kg/d) of PX-A24590 and Epacadostat (2x100mg/kg) compared to vehicle * = P<0.05; ** = P<0.01; *** = P<0.001

AhR antagonist increases intra-tumoral CD8⁺ T cells

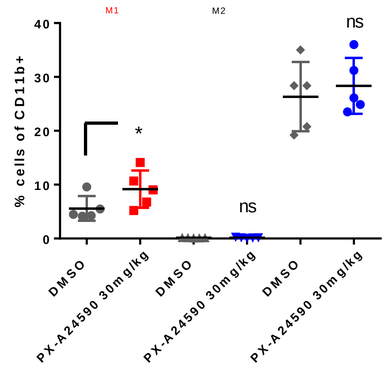
Fig. 7 CD4+ and CD8+ cells in tumors isolated on day 35



Tumors were isolated on day 35 post tumor cell implantation and CD4+ and CD8+ cell numbers determined by flow cytometry.
* = P<0.1; ** = P<0.01; *** = P<0.001

AhR antagonist increases intra-tumoral M1 macrophages

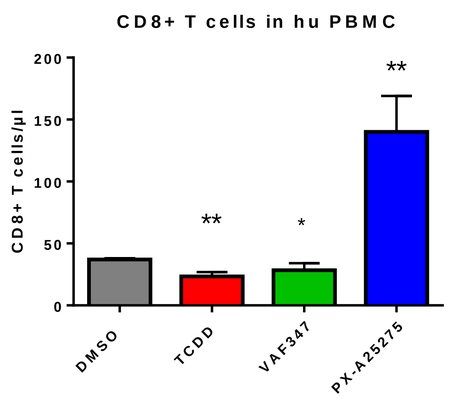
Fig. 8



Tumors were isolated on day 35 post tumor cell implantation and M1/M2 macrophages determined by flow cytometry.
* = P<0.1; ** = P<0.01; *** = P<0.001

AhR antagonist PX-A25275 increases CD8⁺ T cell numbers in human PBMC´s in vitro

Fig. 9



PX-A25275 Cellular IC50
huAhR ~8nM
mAhR ~1µM

Flow cytometric identification of CD8⁺ T cells in human PBMC, stimulated in vitro with anti-CD3/CD28 for 10 days, in presence of DMSO, 1nM TCDD, 10nM VAF347 or 500nM of PX-A25275.
* = P<0.1; ** = P<0.01; *** = P<0.001

AhR antagonist PX-A24590 enhances anti-PD-L1 efficacy in a subcutaneous CT26 tumor model

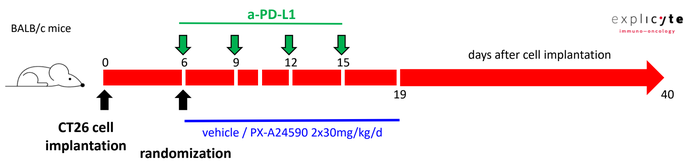
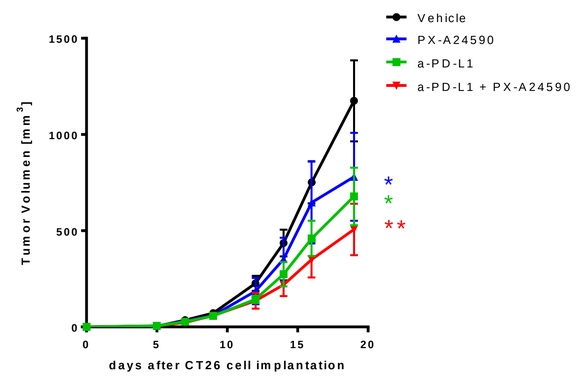


Fig. 10



Tumor volumes were determined by caliper on indicated times. Anti PD-L1 antibodies (10F.9G2, 100µg) were injected i.p. at indicated times and PX-A24590 was administered p.o. 2x 30mg/kg/d in 0,5% HPMC/saline.
* = P<0.1; ** = P<0.01; *** = P<0.001

Summary

- AhR antagonist PX-A24590 shows anti-tumor activity in syngeneic PDAC model (Panc02-HA)
- AhR antagonism increases intra-tumoral CD8⁺ T cells and M1 macrophages
- AhR antagonism increases CD8⁺ T cell numbers in αCD3/CD28 stimulated human PBMC in vitro
- AhR antagonist PX-A24590 enhances anti-PD-L1 efficacy in subcutaneous CT26 colon tumor model
- AhR antagonists may show anti-tumor efficacy in IDO1, IDO1/TDO2 and TDO2 expressing tumors

(Chen IC et al.. Expression Pattern and Clinicopathological Relevance of the Indoleamine 2,3-Dioxygenase 1/Tryptophan 2,3-Dioxygenase Protein in Colorectal Cancer. Dis Markers. 2016)