Discovery of Small Molecule Aryl Hydrocarbon Receptor (AhR) Antagonists for Cancer Immunotherapy

Immuno-Oncology: Emerging Targets and Therapeutics
New ways to Refocus the Immune System to Attack Cancer

Thomas Hoffmann

Boston, Sept. 17, 2019
Phenex at a glance

- Heidelberg based, private small molecule drug discovery company
- Focus on Liver / Metabolic Diseases and Cancer
- Multi-disciplinary project teams span Translational Biology and Medicinal Chemistry

Partnered projects:

- 2012 – Contract with Janssen/J&J: Licensing of Psoriasis Program on RORgt, up to 135 Mio. US$; in Phase 1

- 2014 – Contract with Gilead: Sale of NASH Program on FXR, volume up to 470 Mio. US$; in Phase 2b in NASH, in Phase 3 in PSC

- Pipeline of metabolic projects (i.e. LXR inverse agonists for NASH) and cancer projects targeting the metabolic microenvironment of tumors
Immune checkpoint inhibitors do not always overcome the suppressive metabolic alterations in solid tumors

Checkpoint inhibitors do not work in many solid tumors

- i.e. immune therapy combinations could have deleterious pro-inflammatory side effects

➢ There is a strong need for safe, controllable and affordable small molecule based TME modulating therapies to increase the efficacy of existing treatments!
Phenex aims at significantly improving response rates:

- in combination with standard of care – chemotherapy, and/or
- in combination with checkpoint inhibitor therapies, with

  - AhR Program
  - further Tumor Metabolism Programs in the pipeline
AhR - multifaceted receptor

AhR is a **ligand controlled transcription factor** (basic helix-loop-helix, bHLH family)
AhR is expressed in most cells, in particular hepatocytes and certain immune cells

Crystal structure of AhR/ARNT complex bound to its target DNA

Schulte, Daumke et al, Structure 2017, 25, 1-9

adapted from:
Zhou, Trends Immunol 2016, 37, 17-29
AhR - Sensor with Multiple Ligands & Functions

**Variety of activating ligands**

- **Environmental Ligands**: Dioxin, Biphenyls, Polyaromatic Hydrocarbons (PAHs)
- **Dietary Metabolites**: Flavonoids, Indoles
- **Microbiota derived Metabolites** of skin, gut, lung
- **Endogenous Metabolites**: Tryptophan derived via IDO1/TDO2
- **Kinases** (MEK, c-Src etc)

**Multiple biological functions**

- **Toxicity**: Dioxin
- **Xenometabolism / Chemoprevention**: CYP1A1, UGT1A1
- **Chemoresistance**: ALDH1A3, ALDH3A1, MDR1
- **Increase of Immune barrier protection**: via IL-22
- **Counterregulation of systemic immune response**:
  - **Agonist** → Immune suppression: increased \( T_{reg} \), MDSC and M2
  - **Antagonist** → Immune stimulation: increased CD8\(^+\) T cells and M1, reduced \( T_{reg} \); Reduced IL-22

Discovery on Target, Boston, Sept. 17, 2019
Activation with an Agonist ligand ----> Immune suppression

Treg cells
MDSCs

AhR

CD8+ T cells
Deactivation with an antagonist ----> Immune stimulation

AhR

CD8+ T cells

Treg cells
MDSCs

Discovery on Target, Boston, Sept. 17, 2019
AhR as a target for cancer immunotherapy in the literature

Selected publications on the role of AhR in the immune system and harnessing its potential as therapeutic target:

- Quintanta et al., *Nature* 2008, 453 (7191), 65-71: Treg and TH17 differentiation is controlled by AhR
- Opitz et al., *Nature* 2011, 478 (7368), 197-203: AhR agonism of Kynurenine, formed by IDO-1/TDO promotes tumor survival
- Bessede et al., *Nature* 2014, 511 (7508), 184-190: Aryl hydrocarbon receptor control of a disease tolerance defense pathway
- Takenaka et al., 2019 *Nat Neurosci.*, 22(5):729-740: Control of tumor-associated macrophages and T cells in glioblastoma via AHR and CD39
## AhR in tumor indications

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic cancer</td>
<td>Koliopanos et al. Oncogene 2002</td>
</tr>
<tr>
<td>Breast cancer -</td>
<td></td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>Opitz et al. Nature 2011; Gabriely et al. Trends in Endocrinology &amp; Metabolism 2017</td>
</tr>
</tbody>
</table>

ESCA - Esophageal carcinoma
GBM - Glioblastoma
LAML - Acute Myeloid Leukemia
PAAD - Pancreatic adenocarcinoma
STAD - Stomach adenocarcinoma

TCGA/GTEx data – GEPIA2
The AhR project @ Phenex - Assay Path

Chemical synthesis

Series 1 Series 2 Series 3 Series 4

Assay format
- Direct reporter assay
- Cellular target gene regulation assay
- Mouse splenocyte and human PBMC assay

PK/PD/ADME
- Microsomal stability
- Plasma protein binding
- Cyp inhibition
- Selectivity panel
- hERG

Syngenic models
- Series 1
  - PX-A590
  - Series 1
    - 1/30
- Series 2
  - PX-A446
    - Series 2
    - 1/69
  - PX-A548
    - Series 2
    - 1/29
- Series 3
  - PX-A590
  - Series 1,2 and 3: patents filed Feb. 2017
- Series 4: patent filed July 2018

Efficacy evaluation using Phenex immuno-oncology research platform

Series 2 Series 3
PX-A446 PX-A548

Discovery on Target, Boston, Sept. 17, 2019
Are Phenex AhR antagonists on target *in vitro*?

In HepG2 cells – AhR translocation upon ligand occupancy
Are Phenex AhR antagonists on target *in vivo*?

**PK/PD study**

In C57/BL6 female mice

- PX-A446 (antagonist) 10 mg/kg single p.o. dose
- VAG539 30 mg/kg p.o. 30 mg/kg p.o.
- Mice sacrificed 4h post-VAF539 (agonist) treatment

Bar chart showing liver Cyp1A1 expression fold over vehicle for different treatment regimens.
Effects on AhR on the immune-tumor cell axis

Aryl hydrocarbon receptor

Direct effects on the tumor

Effects on immune system / tumor microenvironment

Combination with standard of care chemotherapy

Tumor growth inhibition via IL-22 suppression

Inhibition of IDO/TDO-Kyn-AhR axis

Effects on tumor-infiltrating immune cells

Checkpoint inhibitors
Effects on AhR on the immune-tumor cell axis

Aryl hydrocarbon receptor

Direct effects on the tumor

- Combination with standard of care chemotherapy
- Tumor growth inhibition via IL-22 suppression

Effects on immune system / tumor microenvironment

- Inhibition of IDO/TDO-Kyn-AhR axis
- Effects on tumor-infiltrating immune cells

Checkpoint inhibitors
AhR antagonists enhanced the efficacy of chemostatic drugs

C57BL/6 mice

Panc02-HA tumors
(tumor vol = 50-250mm³)

randomization

Gemcitabine 50mg/kg i.p.

vehicle / PX-A275 20mg/kg/d p.o.

days after cell inoculation

0 1 8 15 22 26 29 52

RTV (%) on day 25

GS

Vehicle

PX-A275

Gemcitabine

Gemcitabine + PX-A275

GS

Discovery on Target, Boston, Sept. 17, 2019
Effects on AhR on the immune-tumor cell axis

Aryl hydrocarbon receptor

Direct effects on the tumor

Combination with standard of care chemotherapy

Tumor growth inhibition via IL-22 suppression

Inhibition of IDO/TDO-Kyn-AhR axis

Effects on immune system / tumor microenvironment

Effects on tumor-infiltrating immune cells

Checkpoint inhibitors
IL22 produced ----> ILCs, Th22 cells ----> effects on homeostasis, protection & regeneration of epithelial tissues (gut, skin, brain, pancreas, liver etc.)

- It directly participates in inflammatory diseases in the gut (IBD) and skin (Psoriasis)

- IL-22 binds to IL22RA1 to drive tumor growth and metastasis via Stat3 signaling

Bridging the immune-tumor axis via Ahr controlled IL22

Modified from Hernandez 2018, EJI, Vol. 48: 15-31
IL-22 and IL22+ lymphocytes negatively correlate with survival of colon cancer patients

Survival in Colon Cancer Patients

- IL-22 and Th22 cells are increased in the TME of various tumors (e.g. lung, gliomas, colorectal carcinoma and pancreatic ductal adenocarcinoma)

Kirchberger et al. JEM 2013
IL22 as a PD marker of AhR Antagonism

AhR agonism → Th17/Th22 cells → Increase in IL22 → Facilitates tumor progression

AhR antagonism → Th17/Th22 cells → Decrease in IL22 → Restricts tumor progression

Discovery on Target, Boston, Sept. 17, 2019
Screening syngeneic models for AhR & IL22/IL22RA1 expression
AhR antagonist shows single agent anti-tumor activity in syngeneic pancreatic tumor model

C57BL/6 mice (n=10) Panc02-HA 1x10^6 cells implanted

Randomization 31 35 Days after tumor cell inoculation
daily p.o. treatment PX-A590

Panc02-HA

Increase in intra-tumoral CD8+ T cells and M1 macrophages (day 35)

Tumor Volume [mm^3]

% CD8+ of live cells

% CD11b+CD206+ of live cells
Effects on AhR on the (metabolic) immune-tumor cell axis

Aryl hydrocarbon receptor

Direct effects on the tumor

Combination with standard of care chemotherapy

Tumor growth inhibition via IL-22 suppression

Effects on immune system / tumor microenvironment

Inhibition of IDO/TDO-Kyn-AhR axis

Effects on tumor-infiltrating immune cells

Checkpoint inhibitors
Inhibiting the IDO-Kyn-AhR pathway

Opitz et al. Nature 2011
Murray et al. Nat Rev Cancer 2014
Platten et al. Frontiers in Immunology 2015
Lui et al. Nat Commun. 2017
Cheong et al. Trends Pharmacol Sci. 2018
Xue et al. Frontiers in Immunology 2018

Discovery on Target, Boston, Sept. 17, 2019
IDO/TDO impact --> Kynurenine are present across a spectrum of tumor

Li et al. Nature Med. 2019
AhR antagonists enhance anti-PD-L1 efficacy in MC38 tumor model

**Graph:**
- MC38 cell inoculation
- Randomization
- Vehicle / compounds 2x10mg/kg/d
- 6, 9, 12, 15 days after cell inoculation
- 25 days after cell inoculation

**Tumor Volume [mm³]**
- Vehicle
- a-PD-L1
- a-PD-L1 + PX-A25548
- a-PD-L1 + PX-A25758

**Significance:**
- *p < 0.05
- **p < 0.01
- ***p < 0.001
- ****p < 0.0001
Effects on AhR on the immune-tumor cell axis

Aryl hydrocarbon receptor

- Direct effects on the tumor
- Effects on immune system / tumor microenvironment
  - Combination with standard of care chemotherapy
  - Tumor growth inhibition via IL-22 suppression
  - Inhibition of IDO/TDO-Kyn-AhR axis
  - Effects on tumor-infiltrating immune cells
    - Checkpoint inhibitors

Discovery on Target, Boston, Sept. 17, 2019
Direct AhR antagonist activity on immune cell subsets

AhR antagonism → Immune cells → Myeloid cells → T cells → NK cells

Increase in:
- CD8 numbers
- IFNγ production
- IL2 production

IFNγ production

Increase in:

Human PBMCs

0.222 µM
0.074 µM
0.025 µM
0.008 µM
0.003 µM
DMSO

AhR compounds

Human PBMCs

0.222 µM
0.074 µM
0.025 µM
0.008 µM
0.003 µM
DMSO

AhR compounds

Mouse NK cells

5 µM
1.25 µM
0.313 µM
0.078 µM
0.020 µM
0.005 µM
DMSO

AhR compounds

Immune cells

CD8+ T cells/ul (after restimulation)

AhR antagonism

PBMC IL-2
PBMC IL-2 + TGF-β

DMSO
PX-A275

Direct AhR antagonist activity on immune cell subsets

Discovery on Target, Boston, Sept. 17, 2019
AhR is an attractive, small-molecule cancer target

AhR (as a transcription factor) contributes to a variety of cancer relevant pathways

Combining direct effects on tumor tissue with direct effects on the immune system

A series of proprietary, selective and potent AhR antagonists have been identified by Phenex

In syngeneic tumor models AhR antagonists have:
  - Single agent activity
  - Synergy with standard-of-care chemotherapy
  - Synergy with checkpoint inhibitors

Preclinical development activities for a development candidate has been started
Acknowledgements

Our AhR Team

**Biology**
Sheena Pinto
Ulrich Deuschle
Larisa Busevic-Wölfel
Michael Albers
Helen Krol
Floriane Braun
Ibrahim Sektiglu
Gisela Eisenhardt

**Chemistry/Medicinal Chemistry**
Christoph Steeneck
Aurelie Mallinger
Olaf Kinzel
Marta Czekanska

**Preclinical & Clinical Development**
Manfred Birkel
Sabine Eigenbrod-Giese