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The Xenobiotic-Metabolising System in Human Immune Cells

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I. Abstract

***Background*:** The ligand-activated transcription factor aryl hydrocarbon receptor (AHR) induces xenobiotic-metabolising enzymes such as cytochrome P4501 (CYP1) enzymes. CYP enzymes control degradation and metabolism of environmental and endogenous molecules. Besides this function, the AHR is now increasingly recognised as an immune-modulating factor. The activation of the AHR impacts cytokine expression and differentiation of immune cells. Several studies have indicated that AHR regulates the dichotomous development of either suppressive T regulatory T cells or pro-inflammatory T helper (Th) 17 cells. It was additionally demonstrated that AHR interacts with the signal transducer and activator of transcription 1 (STAT1). STAT1 is a negative regulator of Th17 response. Autosomal dominant gain-of-function (GOF) mutations in the *STAT1* gene result in an overactive STAT1 protein and are also reported to cause the chronic mucocutaneous candidiasis disease (CMCD). CMCD patients are characterised by isolated and recurrent infections with the fungus *Candida*, mostly *Candida albicans*, at mucosal tissues. To a large extent, these patients have an impaired Th17 pathway with a reduced production of interleukin (IL)-17 and IL-22. One common feature among various AHR ligands is the induction of IL-22. Previous studies emphasised that the stem cell factor receptor gene (*KIT*) is also an AHR target in mice. Although AHR in immunity is intensively studied, to date the role of CYP1 metabolism in immunity is unclear. However, inhibition of CYP1 activity provides a mechanism for AHR activation.

***Objectives*:** The aim of this study was to investigate the effects of a CYP1-induced AHR activation in human immune cells. Besides other target genes related to the AHR pathway, the Th17 cytokines IL-22 and IL-17, and the stem cell factor receptor (c-Kit) were selected as immunological targets. In addition, the immunological importance of this pharmacological approach of the AHR feedback loop was confirmed using splenocytes from *Cyp1a2* knockout mice. Furthermore, studies with peripheral blood mononuclear cells (PBMCs) from CMCD patients confirmed the relevance of the AHR feedback loop for IL-22 induction in these patients. A human immune cell screen for xenobiotic-metabolising enzymes was performed to identify possible susceptible immune cell populations.

***Methods*:** Activated human PBMCs from healthy donors were treated with 1-(1-propynyl)-pyrene (1-PP), a suicide inhibitor for CYP1 in the presence of a low concentration of the AHR agonist 6-formylindolo[3,2-*b*]carbazole (FICZ) alone or in combination with the AHR antagonist CH-223191. PBMCs from CMCD patients were additionally treated with a high FICZ concentration. Cytokine, *CYP* and c-Kit expression levels were analysed by quantitative real time-polymerase chain reaction (qRT-PCR), enzyme-linked immunosorbent assay (ELISA) and flow cytometry (FACS). Viability and proliferation were analysed by flow cytometry, lactate dehydrogenase (LDH) activity and ^{3}H-thymidine assay. The presence of xenobiotic-metabolising enzymes in different human immune cells was determined by qRT-PCR arrays.

***Results*:** Inhibition of CYP1 activity elevated c-Kit, IL-22 and *CYP* expression levels in activated PBMCs from healthy subjects. The addition of the AHR antagonist CH-223191 reversed these effects. Contrary to this, IL-17 was down-regulated by CYP1 inhibition and induced by the AHR antagonist. In particular, human T cells responded to CYP1 inhibition with an up-regulation of c-Kit. IL-22 and c-Kit were simultaneously induced in Th cells. Additionally, correlation analyses demonstrated that *CYP1A1* transcription was negatively, and *CYP1B1* transcription was positively correlated with *AHR* transcription in PBMCs from healthy individuals. PBMCs from CMCD patients additionally increased c-Kit and IL-22 after co-treatment with 1-PP and FICZ compared with single treatments. Both Th and cytotoxic T cells (Tc) from CMCD patients showed a stronger c-Kit induction than T cells from healthy subjects during CYP1 inhibition and in the treatments with a high FICZ concentration. Moreover, activated splenocytes from *Cyp1a2* knockout mice had a higher percentage of IL-22$^+$ cells than wild type mice upon AHR activation and in control treatments. Furthermore, gene transcription analyses of various human immune cells demonstrated that major immune cell subpopulations can be clustered according to their CYP expression. Genes encoding xenobiotic-metabolising enzymes fingerprinted human monocytes, Th cells, memory Th cells, Tc cells and B cells, human primary foreskin mast cells and basophils. However, 17 of the studied genes were transcribed in nearly all investigated immune cells.

***Conclusion*:** The classical role of the AHR is to control CYP1 and other xenobiotic-metabolising enzymes. The function of AHR in the immune system has been recently discovered. This thesis focused on AHR-regulated CYP1 enzymes in human immune cells. CYP1-mediated AHR activation is active in a range of cultured human immune cells as detected in activated PBMCs from healthy subjects and CMCD patients. These findings provide mechanisms for IL-22 and c-Kit induction by xenobiotic-metabolising enzymes. Although c-Kit and IL-22 were induced by CYP1 inhibition both are probably differently regulated by the AHR. Experiments with *Cyp1a2* knockout mice support the results of a CYP1-dependent IL-22 regulation and a function of CYP in immunity. Although human immune cell subtypes are equipped with a basic profile for xenobiotic-metabolising enzymes, each subpopulation possesses an additional specific CYP pattern. The selective CYP expression in various populations hints at cell type-specific functions of these enzymes.

II. Publications and Presentations

Publications

Cytochrome P450s in human immune cells regulate IL-22 and c-Kit via an AHR feedback loop.

Effner R, Hiller J, Eyerich S, Traidl-Hoffmann C, Brockow K, Triggiani M, Behrendt H, Schmidt-Weber CB, Buters JTM
Sci Rep.
2017 Mar 9; 7:44005. doi:10.1038/srep44005.

IL-4 and Interferon-γ orchestrate an epithelial polarization in the airways.

Zissler UM, Chaker AM, Effner R, Ulrich M, Guerth F, Piontek G, Dietz K, Regn M, Knapp B, Theis FJ, Heine H, Suttner K, Schmidt-Weber CB
Mucosal Immunol.
2016 Jul;9(4):917-26. doi 10.1038/mi.2015.110. Epub 2015 Nov 18.

IL-22 suppresses IFN-γ mediated lung inflammation in asthma.

Pennino D, Bhavasar PK, Effner R, Avitabile S, Venn P, Quaranta M, Marzaioli V, Cifuentes L, Durham SR, Cavani A, Eyerich K, Chung KF, Schmidt-Weber CB, Eyerich S
J Allergy Clin Immunol.
2013 Feb;131(2):562-70.

GSTM1, GSTT1 and GSTP1 gene polymorphism in polymorphous light eruption.

Zirbs M, Pürner C, Buters JT, Effner R, Weidinger S, Ring J, Eberlein BJ
Eur Acad Dermatol Venereol.
2013 Feb;27(2):157-62.

Human mast cells express androgen receptors but treatment with testosterone exerts no influence on IgE-independent mast cell degranulation elicited by neuromuscular blocking agents.

Chen W, Beck I, Schober W, Brockow K, Effner R, Buters JT, Behrendt H, Ring J
Exp Dermatol.
2010 Mar;19(3):302-4.

Polycyclic aromatic hydrocarbons from diesel emissions exert proallergic effects in birch pollen allergic individuals through enhanced mediator release from basophils.

Lubitz S, Schober W, Pusch G, Effner R, Klopp N, Behrendt H, Buters JT
Environ Toxicol.
2010 Apr;25(2):188-97.

Presentations

2016, 46th Annual ESDR Meeting 2016, Munich, Germany (poster presentation)

Cytochrome P450s are deactivators of the aryl hydrocarbon receptor in human immune cells.

Effner R, Hiller J, Eyerich S, Traidl-Hoffmann C, Brockow K, Triggiani M, Behrendt H, Schmidt-Weber C, Buters JTM

2013, EAACI-WAO, World Allergy and Asthma Congress, Milan, Italy (poster presentation)

Endogenous biosensors in allergy: cytochrome P450s and the aryl hydrocarbon receptor.

Effner R, Pusch G, Brockow K, Behrendt H, Schmidt-Weber C, Buters JTM

2013, 25. Mainzer Allergie-Workshop, Mainz, Germany (presentation)

Xenobiotic-metabolising enzymes and transporters in human immune cells.

Effner R, Pusch G, Eyerich S, Brockow K, Behrendt H, Schmidt-Weber C, Buters JTM

2013, Invited presentation at McMaster University, Hamilton, Canada (presentation)

Xenobiotic metabolism in human immune cells: aryl hydrocarbon receptor, cytochrome P450s and beyond.

Effner R, Pusch G, Hiller J, Eyerich S, Brockow K, Behrendt H, Schmidt-Weber C, Buters JTM

2012, 7. Deutscher Allergiekongress, München, Germany (poster presentation)

Modulation of Allergic Inflammation by Environmental Compounds: Role of the Xenobiotica Metabolising System in Target Immune Cells.

Effner R, Eyerich S, Brockow K, Behrendt H, Schmidt-Weber C, Buters JTM

2011, 7th Duesseldorf Symposium on Immunotoxicology, Biology of the Aryl Hydrocarbon Receptor**,** Düsseldorf, Germany (poster presentation)

Regulation of IL-22: Function of AHR-induced Xenobiotic Metabolism.

Effner R, Eyerich S, Schmidt-Weber C, Buters JTM

2009, 13. Treffen der Arbeitsgemeinschaft Mastzellen und Basophile der ADF, München, Germany (presentation)

Modulation of allergic inflammation in target tissue: role of xenobiotica-metabolising enzymes in mast cells and basophils.

Effner R, Brockow K, Behrendt H, Buters JTM

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VI. List of Abbreviations

1-ABT	1-Aminobenzotriazole
ABCG2	Adenosine Triphosphate (ATP)-Binding Casette Sub-Family G Member 2
AHR	Aryl Hydrocarbon Receptor
AKR	Aldo-Keto Reductase
APC	Antigen-Presenting Cells
APC	Allophycocyanin
APC-Cy7	Allophycocyanin-Cyanin7
ARE	Antioxidant Response Elements
ARNT	AHR Nuclear Translocator
BCR	B Cell Receptor
CCD	Coiled-Coil Domain
CD	Cluster of Differentiation
CD14	Antigen on Monocytes
CD3	Antigen on T Cells
CD4	Antigen on T Helper Cells
$CD4^+$ $CD45RO^+CD45RA^-$	Characterisation of Memory Th Cells
CD45RO	Antigen on Memory T Cells
CD56	Antigen on Natural Killer Cells
CD8	Antigen on Cytotoxic T Cells
CH-223191	2-Methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazo-phenyl)-amide, AHR antagonist
c-Kit	Cellular-Kit, Receptor for the Stem Cell Factor
CMC	Chronic Mucocutaneous Candidiasis
CMCD	CMC Disease
CO_2	Carbon Dioxide
CYP	Cytochrome P450
DBD	DNA-Binding Domain
DCs	Dendritic Cells
DEPs	Diesel Exhaust Particles
DMSO	Dimethyl Sulfoxide
DNase	Deoxyribonuclease
DNA	Deoxyribonucleic acid
D-PBS	Dulbecco´s Phosphate-Buffered Saline
EAE	Experimental Autoimmune Encephalomyelitis
EDTA	Ethylenediaminetetraacetate
EF1A	Elongationfactor 1 Alpha
ELISA	Enzyme-Linked Immunosorbent Assay
EPO	Erythropoietin
FACS	Fluorescence-Activated Cell Sorting
FcεR1	High affinity IgE Receptor
FICZ	6-Formylindolo[3,2-b]carbazole, AHR agonist
FITC	Fluorescein Isothiocyanate
FOXP3	Forkhead box P3

g	Gravity
GAPDH	Glycerinaldehyd-3-Phosphate Dehydrogenase
GATA3	GATA binding protein 3
GOF	Gain-of-Function
γδ T cells	Gammadelta T cells
GSTs	Glutathione-S-Transferase
ΔΔCt	Delta-Delta Cycle Treshold
°C	Degree Celsius
h	hour
HAH	Halogenated Aromatic Hydrocarbon
HIF1α/A	Hypoxia-Inducible Factor 1 Alpha
HIF1β	Hypoxia-Inducible Factor 1 Beta
HPRT1	Hypoxanthine-Guanine Phosphoribosyltransferase 1
HPC	Hematopoietic Progenitor Cell
HRE	Hypoxia Response Elements
HSC	Hematopoietic Stem Cell
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin
ILCs	Innate Lymphoid Cells
ILC2	ILC Type 2
Keap1	Kelch-like-ECH-Associating-Protein 1
K_D	Dissociation Constant
LDH	Lactate Dehydrogenase
log	Logarithm
$L^-S^+K^+$	Linage-negative Stem cell antigen-positive Kit-positive
M	Molar
MHC	Major Histocompatibility Complex
min	Minute
ml	Millilitre
mM	Millimolar
µg	Microgram
µl	Microlitre
µM	Micromolar
NF-kB	Nuclear Factor kappa B
NK	Natural Killer
NQO1	NAD(P)H: Quinone Oxidoreductase-1
NRF2	Nuclear Factor, Erythroid 2-like 2
PAH	Polycyclic Aromatic Hydrocarbon
PAS	Per-Arnt-Sim
1-PP	1-(1-Propynyl)-pyrene
PBMC	Peripheral Blood Mononuclear Cells
PCB	Polychlorinated Biphenyl
PE	Phycoerythrin
PGK	Phosphoglycerate Kinase 1
pIRES	Plasmid with Internal Ribosomal Entry Site
PM	Particulate Matter

pM	Picomolar
PMA	Phorbol-12-Myristate-13-Acetate
pO_2	Partial Pressure of Oxygen
PTGS2	Prostaglandin-Endoperoxide Synthase 2
qPCR	Quantitative Real-Time Polymerase Chain Reaction
RIN	RNA Integrity Number
RNA	Ribonucleic Acid
RORC	Retinoid Acid Receptor (RAR)-Related Orphan Receptor Gamma
ROS	Reactive Oxygen Species
RT	Reverse Transcription
RT	Room Temperature
SCF	Stem Cell Factor
STAT	Signal Transducers and Activators of Transcription
Tbet	T-box Transcription Factor
TCDD	2,3,7,8-Tetrachlorodibenzo-*para*-dioxin
TCR	T Cell Receptor
TGF-β	Transforming Growth Factor-Beta
Th	T helper
TLDA	TaqMan Low Density Arrays
TNF-α	Tumor Necrosis Factor-alpha
Tr1	Type 1 Treg cells
Treg	T Regulatory T cells
UV	Ultraviolet
VEGF	Vascular Endothelial Growth Factor
wt	Wild type
XME	Xenobiotic-Metabolising Enzymes
XRE	Xenobiotic Response Element

1 Introduction

1.1. Aryl hydrocarbon receptor (AHR)

1.1.1. AHR activation

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor mostly investigated in the context of environmentally induced xenobiotic metabolism (Nebert et al., 1993). The AHR is sequestered prior to ligand activation in a cytosolic protein complex formed by the heat shock protein 90, p23 and by the AHR-interacting protein (AIP) (Carver and Bradfield, 1997; Kazlauskas et al., 1999; Meyer et al., 1998; Perdew, 1988; Soshilov and Denison, 2011). AHR can be activated by various ligands, however, ligand-independent pathways are also described (Denison and Nagy, 2003; Denison et al., 2011; Hu et al., 2007). Ligand-binding exposes the nuclear localisation sequence and triggers the translocation of the AHR into the nucleus, where it binds to its partner AHR nuclear translocator (ARNT) in the canonical AHR pathway (Reyes et al., 1992; Soshilov and Denison, 2011; Whitelaw et al., 1993). Both AHR and ARNT belong to the basic helix-loop-helix (bHLH)/Per-ARNT-Sim (PAS) transcription factors. PAS proteins contain characteristic domains for sensing environmental stressors such as light, toxicants or oxygen (Burbach et al., 1992; Ema et al., 1994; Ema et al., 1992; Gu et al., 2000; Kewley et al., 2004; McIntosh et al., 2010).

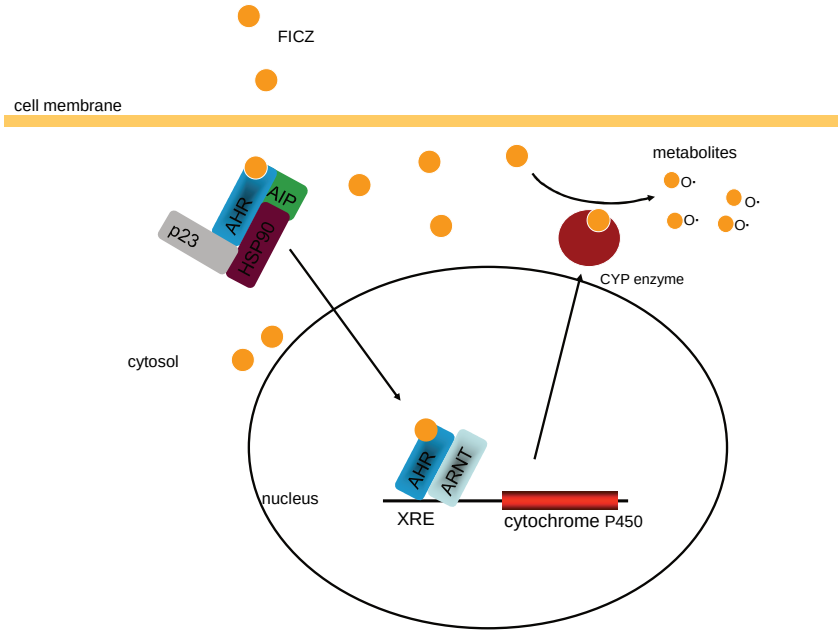
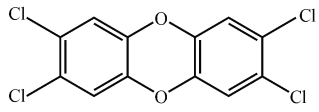


Figure 1. Induction and components of the canonical AHR pathway
AHR: Aryl hydrocarbon receptor, HSP90: Heat shock protein 90, p23: co-chaperone, AIP: AHR-interacting protein, (chaperone), ARNT: Aryl hydrocarbon receptor nuclear translocator, CYP: Cytochrome P450, XRE: Xenobiotic response element. FICZ: AHR agonist. Adapted from Stockinger et al., 2014.

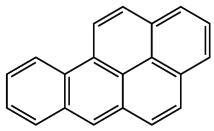
As a heterodimer AHR and ARNT directly activate xenobiotic response elements (XREs) (Bacsi et al., 1995; Whitelaw et al., 1993) with the consensus sequence 5´-TnGCGTG-3´ upstream of diverse genes (Fujisawa-Sehara et al., 1987). These genes include the commonly accepted AHR gene battery with cytochrome P450 (CYP) family 1 subfamily A member 1 (*CYP1A1*), *CYP1A2*, *CYP1B1* and NAD(P)H dehydrogenase quinone 1 (*NQO1*) (Favreau and Pickett, 1991; Jaiswal, 1991; Kobayashi et al., 1996; Ma, 2001; Nebert et al., 2004; Nebert and Jones, 1989; Whitelaw et al., 1993).

1.1.2. Exogenous and endogenous AHR ligands

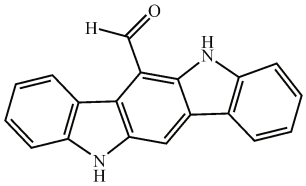
Over many decades environmental toxins have been the most studied AHR ligands, because of AHR-mediated toxic effects (Dragan and Schrenk, 2000). Persistent environmentally and ubiquitously occurring planar contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and halogenated aromatic hydrocarbons (HAHs) contain high affinity AHR agonists (Jones and Anderson, 1999; Kafafi et al., 1993a; Kafafi et al., 1993b; Kafafi et al., 1993c; Mason, 1994; Nebert and Dalton, 2006; Nebert et al., 2004; Till et al., 1999). Moreover, plant-derived dietary chemicals such as flavonoids and indoles, or endobiotics including heme metabolites, bilirubin, eicosanoids or tryptophan metabolites such as 6-formylindolo[3,2-b]carbazole (FICZ) potentially trigger AHR (Chiaro et al., 2008; Denison and Nagy, 2003; DiNatale et al., 2010; Nebert and Karp, 2008; Nguyen and Bradfield, 2008; Opitz et al., 2011; Schaldach et al., 1999; Song, 2002). Additionally, certain pharmaceuticals are also described as AHR ligands (O'Donnell et al., 2010; Prud'homme et al., 2010).



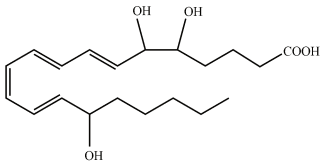
2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (TCDD), HAH, exogenous



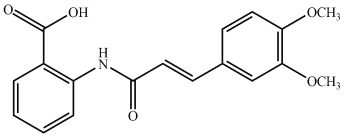
Benzo[*a*]pyrene, PAH, exogenous



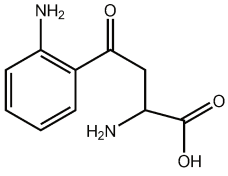
6-Formylindolo[3,2-b]carbazole, (FICZ), endogenous



Lipoxin A4, endogenous



Tranilast, drug, exogenous



Kynurenine, endogenous

Figure 2. Exogenous and endogenous AHR agonists
Denison and Nagy, 2003; Prud'homme et al., 2010

1.2. Cellular and physiological functions of the AHR

AHR was initially considered to regulate the breakdown of lipophilic, exogenous molecules (xenobiotics) and was studied to a large extent in the field of toxicology (Nebert, 1989; Nebert et al., 1993; Poland and Knutson, 1982). The receptor is highly expressed in the liver and barrier organs (Dolwick et al., 1993; Li et al., 1994). Toxicity to exogenous compounds is partially mediated by oxidative stress, genotoxic and proteinotoxic effects, and is manifested in diverse organs and multiple cancers (Nebert, 1989; Nebert and Dalton, 2006; Nebert et al., 2004). The toxicity of AHR ligands in humans is involuntary well documented and human studies exist (Baccarelli et al., 2002; Consonni et al., 2008; Landi et al., 2003; Saurat et al., 2012; Zack and Suskind, 1980). Various AHR-deficient mouse strains have been generated to study the physiological functions of the AHR. The three mostly used AHR knockout strains are resistant against 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD)-induced toxicity. Abnormal phenotypes of these strains are characterised by different as well as by common physiological changes such as increased infertility, reduced induction of xenobiotic genes and growth retardation (Esser, 2009; Gonzalez and Fernandez-Salguero, 1998; Mimura et al., 1997; Schmidt et al., 1996). That AHR has important physiological functions besides mediating adverse effects, is further supported by the conserved expression of this transcription factor among various species (Gasiewicz and Rucci, 1984; Hahn, 2002). AHR is involved in diverse cellular pathways by influencing cell cycle regulation, proliferation and chromatin structure, and by interacting with various other transcription factors (Ahmed et al., 2009; Beischlag and Perdew, 2005; Kalmes et al., 2011; Kim et al., 2000; Kolluri et al., 1999; Marlowe and Puga, 2005; Ohtake et al., 2003; Pang et al., 2008; Puga et al., 2009; Ray and Swanson, 2003; Schnekenburger et al., 2007; Tian, 2009; Tian et al., 1999; Vogel et al., 2007). Biochemical pathways are modified by integration of AHR in second messenger signalling cascades, and by controlling of the oxidative status of a cell through the expression of genes involved in the xenobiotic metabolism (Kung et al., 2009; Matsumura, 2009; Miao et al., 2005; N'Diaye et al., 2006; Nebert et al., 1993; Sciullo et al., 2009; Senft et al., 2002; Zhou et al., 2013).

1.3. AHR in immunity

The main goals of immunity are to limit expansion and to succeed in eliminating potentially harmful substances such as viruses, bacteria, fungi, parasites or transformed cells. Extremely specialised and complex mechanisms that supplement primitive defence, already found in plants, have emerged in higher vertebrates. In humans, two mechanisms referred to as innate and aquired/adaptive immunity mirror adaption to environmental complexities. The co-operation between both systems enables the optimal protection of the organism against the pathological environment (Flajnik and Du Pasquier, 2004; Fritig et al., 1998; Janeway and Medzhitov, 2002; Litman et al., 2010; Medzhitov and Janeway, 1998; Pancer and Cooper, 2006; Yuan et al., 2014)

In recent years, the focus in AHR research has been shifting from the toxicological to the endogenous functions, and an increasing number of studies examined AHR effects on the immune system (Apetoh et al., 2010; Quintana et al., 2008; Quintana et al., 2010; Veldhoen et al., 2008). Initial studies predicted that AHR over-activation by TCDD leads to immunosuppressive effects. These findings are encouraged by thymic involution, atrophy of lymphoid organs and by reduced numbers of thymocytes and lymphocytes upon TCDD exposure (Funatake et al., 2005; Holsapple et al., 1991; Marshall and Kerkvliet, 2010; Staples et al., 1998). AHR-binding sites are detected in many immunological genes such as cytokines, lineage-defining transcription factors, signal transducers and activators of transcription (STATs) and innate receptors (Frericks et al., 2007; Sun et al., 2004). Hematopoietic stem cells, leukemic cells and most cells of the innate and adaptive immunity express AHR. Naïve T cells are described with a low AHR expression but the receptor is induced at various intensities in distinct immune cell subtypes (Esser and Rannug, 2015; Martin et al., 2009; Pabst et al., 2014; Platzer et al., 2009; Singh et al., 2009; Singh et al., 2011; Veldhoen et al., 2008).

1.3.1. AHR in hematopoietic progenitor cells

Hematopoietic stem cells (HSCs) are the precursors of blood cells and the bone marrow forms microenvironmental niches for initiating quiescence, self-renewal, survival and differentiation in these cells (Ivanovic et al., 2004; Metcalf, 2007; Parmar et al., 2007; Scadden, 2006; Yahata et al., 2008). In hypoxic niches, HSCs display a high expression of the oxygen sensor hypoxia-inducible factor 1α (HIF1α) (Simsek et al., 2010). Low oxygen levels stabilise HIF1α and together with the AHR-binding partner ARNT, also referred to HIF1β, HIF1α induces genes containing hypoxia responsive elements (HREs) (Bacsi et al., 1995; Salceda and Caro, 1997; Wenger et al., 2005). HIF1α, ARNT and AHR all belong to the PAS protein family that are cellular sensors for environmental stressors including oxygen (Burbach et al., 1992; Ema et al., 1994; Ema et al., 1992; Gu et al., 2000; Kewley et al., 2004; McIntosh et al., 2010; Wang et al., 1995). Several studies investigated the interaction of AHR and HIF1α and the function of ARNT (Gassmann et al., 1997; Tomita et al., 2000; Vorrink and Domann, 2014).
Recent studies also emphasised that the stem cell factor receptor gene (*KIT*) is an AHR target in mice (Kadow et al., 2011; Kiss et al., 2011). The receptor tyrosine kinase, cellular (c)-Kit, is important for driving survival and division of bone marrow-derived hematopoietic stem cells (HSC). While c-Kit expression on progenitor cells is necessary for a sustained immune cell progression, the receptor is down-regulated in most immune cell populations during lineage commitment. c-Kit provides the response to the stem cell factor (SCF) and it is closely related to other growth factor receptors such as platelet-derived growth factor receptor or the receptor for the macrophage colony-stimulating factor 1 (Ashman et al., 1991; Blechman et al., 1993; Huang et al., 1990; Nocka et al., 1990; Ogawa et al., 1991; Qiu et al., 1988; Williams et al., 1990; Yarden et al., 1987; Zsebo et al., 1990).

Certain differentiated cells have kept or retained c-Kit expression. Thus, c-Kit is expressed on mast cells, γδ T cells, eosinophils, on subpopulations of innate lymphoid cells (ILCs) (including natural killer (NK) cells) and on non-hematopoietic cells (such as melanocytes) (Kadow et al., 2011; Kirshenbaum et al., 1999; Kiss et al., 2011; Matos et al., 1993; Spits and Di Santo, 2011; Yoshida et al., 2001). Furthermore, HSCs from AHR knockout mice display a loss of quiescence that is accompanied by premature aging and exhaustion. In accordance with these results, inhibition of AHR by AHR antagonists favours hematopoietic stem cell expansion (Boitano et al., 2010; Casado et al., 2010; Gasiewicz et al., 2014 ; Gasiewicz et al., 2010; Sakai et al., 2003; Singh et al., 2014; Singh et al., 2009; Singh et al., 2011).

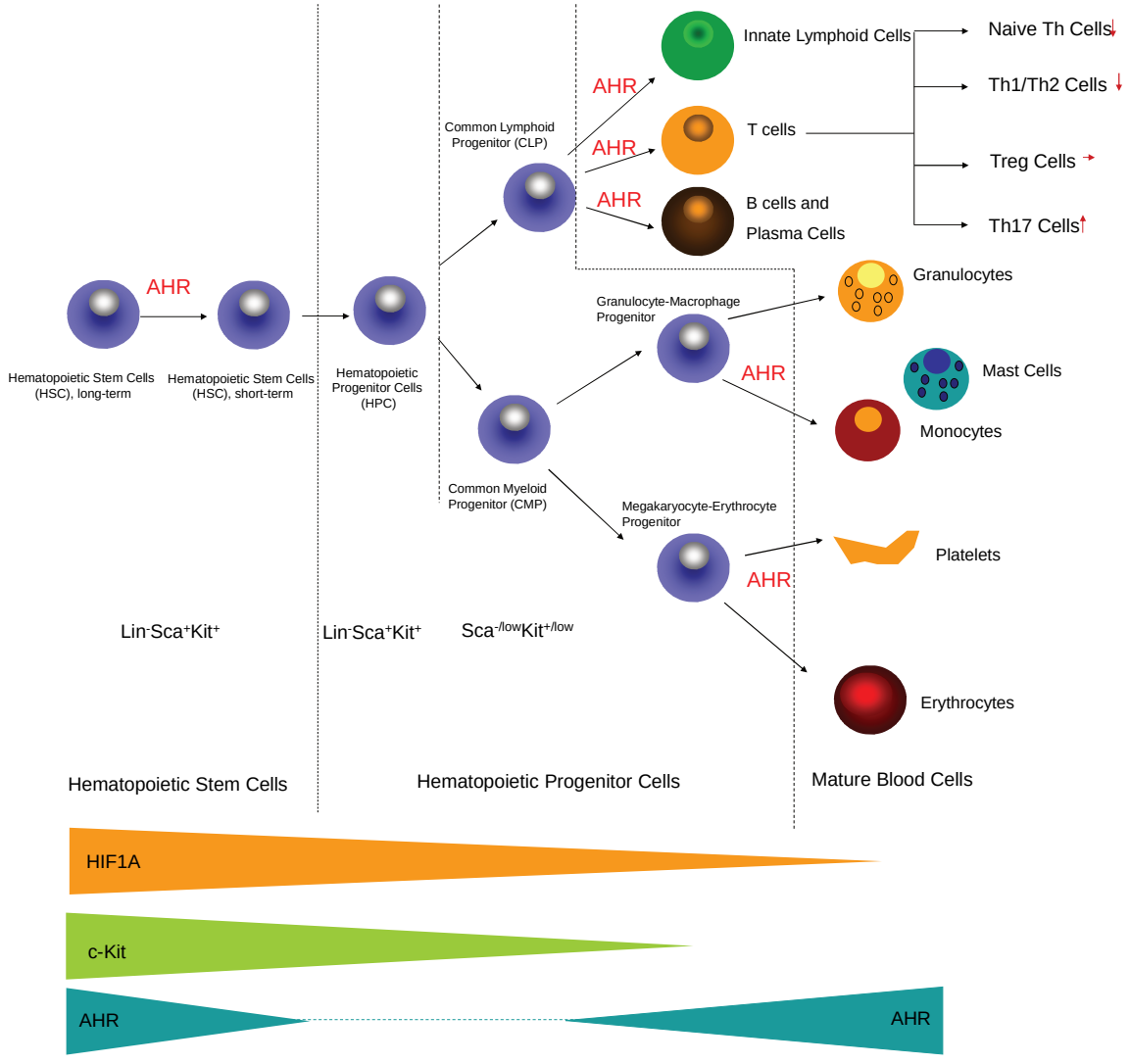


Figure 3. Overview of AHR, c-Kit and HIF1α expressions in the hematopoietic system
AHR: Aryl hydrocarbon receptor, HSC: Hematopoietic stem cells, HPC: Hematopoietic progenitor cells, Lin: Lineage, Sca: Stem cell antigen, Kit: Receptor tyrosine kinase. HIF1A: Hypoxia-inducible factor 1α. Red arrows indicate AHR expression. Adapted from Esser and Rannug, 2015; Lindsey and Papoutsakis, 2012; Singh et al., 2009.

1.3.2. AHR in innate immunity

The innate immunity starts within minutes after pathogens penetrate body´s surface tissues and sets the first line of defence by controlling spreading of the pathogen. Besides epithelial or endothelial cells, which also facilitate the first defence, tissue resident sentinel cells such as macrophages, dendritic cells (DCs) or mast cells establish a surface tissues underlying immune cell network. Granulocytes are fast acting innate cell types supporting DCs and macrophages at infected tissues by activating scavanger functions and exudation of granules. According to the content of their granules, granulocytes could be discriminated in basophilic, eosinophilic and neutrophilic granulocytes. Neutrophilic granulocytes are an essential cell type of the acute inflammation. They curtail infections and spreading of pathogens by diverse mechanisms controlling innate and adaptive immunity (Abraham and Arock, 1998; Geering et al., 2013; Mantovani et al., 2011; Nowarski et al., 2013). A further innate mechanism, which drives the assembling of pore forming proteins in the pathogen´s membrane, is the complement system that results in the lysis and killing of the pathogen (Medzhitov and Janeway, 2000; Tomlinson, 1993; Wang et al., 2000). While most innate cell populations such as granulocytes, monocytes and macrophages emerge from myeloid progenitor cells (Akashi et al., 2000; Geissmann et al., 2010), the recently described innate lymphoid cells (ILCs) lack adaptive properties and assist epithelial and sentinel cells at body´s surfaces. ILCs emerge as a heterogeneous group of lymphocytes, lacking myeloid surface markers and specific recombined antigen receptors. On the basis of cytokine expression and presumed master transcription factors, ILCs are attempted to be classified into different groups similar to the adaptive immunity's lymphocytes (Spits and Cupedo, 2012; Spits and Di Santo, 2011). In parallel with the effective bordering and elimination of the pathogen at local sites, information about non-host invaders is transported to the proximate lymph nodes by antigen-presenting DCs. DCs bridge innate and adaptive immunity by direct cell-cell interactions with T cells. They permanently encounter extracellular molecules that are processed for optimal presentation on major histocompatibility complexes (MHC) during DCs´ migration to the lymph node. In these organs, DCs interact with lymphocytes, the effector cells of the adaptive immunity. DCs process and present molecules most efficient and are the principal antigen-presenting cells (APCs), although additional cell types such as monocytes/macrophages and B cells are also referred to as APCs (Banchereau and Steinman, 1998; Guermonprez et al., 2002; Medzhitov and Janeway, 2000).

1.3.2.1. AHR in skin epithelial cells

Epithelial boundary tissues are exposed to various environmental stressors including xenobiotics. Simultaneously, these tissues form body´s first defence lines. AHR is highly expressed in barrier organs and it is well studied in skin, gut and lung epithelial cells (Dolwick et al., 1993; Li et al., 1994; Swanson, 2004). Ultraviolet (UV) light is supposed to trigger the formation of the endogenous AHR ligand FICZ in the skin and FICZ could act as photosensitizer by mediating UV response (Fritsche et al., 2007; Jux et al., 2011; Park et al., 2015; Schallreuter et al., 2012; Syed and Mukhtar, 2015). Several observations demonstrated that AHR is an important factor for skin barrier formation by regulating the expression of structural proteins important for keratinocyte differentiation (Esser et al., 2013; Jones and Reiners, 1997; Ray and Swanson, 2003; van den Bogaard et al., 2013). The most prominent example supporting a function of the AHR pathway in the skin is the induction of chloracne by constitutive AHR activation with TCDD (Saurat et al., 2012).

1.3.2.2. AHR in antigen-presenting cells

AHR appears to have anti-inflammatory and immune-regulatory functions in antigen-presenting cells. The production of pro-inflammatory cytokines upon innate receptor activation and the initiation of the adaptive immune response make these cells to important immunological translators for environmental information. Following innate receptor activation, AHR is up-regulated and AHR knockout mice produce higher levels of pro-inflammatory cytokines than wild type mice (Kimura et al., 2009; Nguyen et al., 2010). Additionally, AHR activation by TCDD facilitates the formation of tolerogenic DCs and thereby the generation of T regulatory (Treg) T cells, whereas it suppresses the differentiation of inflammatory T helper (Th) 17. By AHR activation, DCs induce indoleamine 2,3-dioxygenase (IDO) that metabolises tryptophan to the endogenous AHR ligand kynurenine (DiNatale et al., 2010; Mezrich et al., 2010; Nguyen et al., 2010; Vogel et al., 2008). However, kynurenine and FICZ, which are both tryptophan derivatives and AHR ligands, differently affect Treg generation. For this reason, differences in the metabolism of both ligands have been hypothesised (Mezrich et al., 2010; Opitz et al., 2011; Rannug et al., 1987; Wei et al., 1998).

1.3.2.3. AHR in granulocytes and mast cells

Granulocytes and mast cells are important effector cells in innate immunity. Although murine and human mast cells highly express AHR (Sibilano et al., 2012), AHR is down-regulated during granulocyte differentiation or detected as rapidly degraded upon ligand binding. In single exposure experiments, AHR ligation aggravates allergic reactions with production of pro-inflammatory mediators in mast cells (Platzer et al., 2009; Sibilano et al., 2012; Zhou et al., 2013). However, exposure to cigarette smoke, a source of highy concentrated AHR ligands, reduces c-Kit and the high affinity immunoglobulin E (IgE) receptor (FcεR1) on mast cells and attenuates the production of pro-allergic cytokines (Givi et al., 2013). As the c-Kit receptor is an essential mast cell survival factor and controlled by the AHR, AHR knockout mice display a reduced number of mast cells and an impaired c-Kit expression. These results imply an important function of AHR for mast cell homeostasis (Kadow et al., 2011; Kiss et al., 2011; Zhou et al., 2013).

1.3.3. AHR in adaptive immunity

The adaptive immunity forms the second line of defence with a time-delayed response to pathogens. Adaptive immune system´s cell types originate from lymphoid progenitor cells, which mature in the bone marrow or the thymus. In the secondary lymphoid organs, migrated DCs from the body´s surface trigger the differentiation of naïve lymphocytes, which have not yet been in contact with an antigen, into mature lymphocytes with effector functions. Binding of antigen loaded MHC molecules to highly variable and specific T cell receptors (TCRs) of naïve T cells provides the first signal for activating T cells (Itano and Jenkins, 2003; Pancer and Cooper, 2006). The specificity of adaptive antigen receptors is also found for native, soluble and unbound antigens recognised by B lymphocytes with the membrane-bound B cell receptor (BCR) or the secreted immunoglobulins (Igs). Simultaneously, CD28, a co-stimulating receptor on T cells binds to B7 (CD80/CD86) molecules on APCs and further activate T cells (Banchereau and Steinman, 1998; Lenschow et al., 1996; Linsley and Ledbetter, 1993). The specific antigen recognition by the highly variable and randomly generated domains of BCR and TCR is one important difference between innate and adaptive immunity. Additionally, the generation of an immunological memory at both T cell and B cell level is considered to be one prominent achievement of the adaptive immune system. Memory lymphocytes are directly activated by lower antigen doses. They retain effector functions and provide an immediate adaptive immune response to previously available antigens (Gellert, 2002; Gourley et al., 2004; Lanzavecchia and Sallusto, 2009; Rogers et al., 2000; Venturi et al., 2013).

According to the route of infection and the effector functions, T cells could be roughly grouped into two distinct subpopulations; $CD4^+$ T helper (Th) cells or $CD8^+$ cytotoxic T cells (Tc). Th cells mostly recognise extracellular, phagocytosed antigens presented on MHC class II (MHC-II) whereas Tc cells are restricted to antigens derived from intracellular proteins presented on MHC class I (MHC-I) molecules. MHC-I molecules are ubiquitously expressed on nucleated cells whereas APCs express MHC-I and MHC-II for optimal antigen presentation to $CD4^+$ and $CD8^+$ T cells (Germain, 1994; Guermonprez et al., 2002; Villadangos, 2001). Tc cells constantly screen host cells for altered or infected phenotypes and they provide killing mechanisms that eliminate transformed or viral infected cells. Compared with Tc cells, Th cells support and co-ordinate innate and adaptive immune cells and are important facilitators for the immunoglobulin class switch in B cells. Diverse subpopulations of effector Th cells are raising from unprimed, naïve Th cells after antigen recognition in the secondary lymphoid organs and are classified based on their differentiation factors, signature cytokines, effector functions and the expression of associated lineage defining transcription factors (Abbas et al., 2012; Murphy et al., 2009; Zhou et al., 2009).

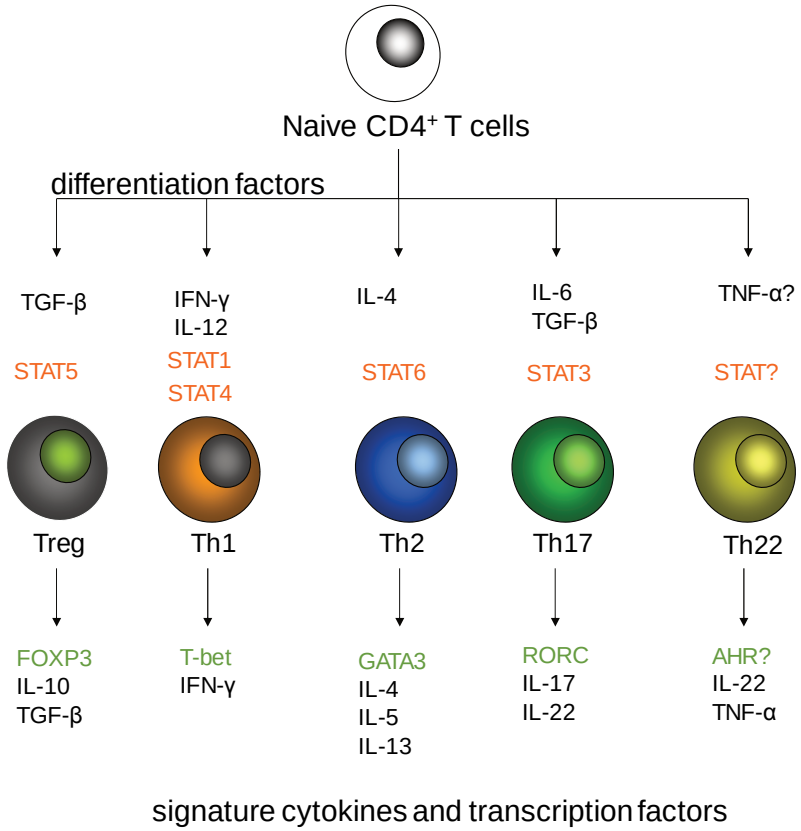


Figure 4. Classification of effector $CD4^+$ T helper (Th) cells
T helper (Th) subpopulations are mapped according to their cytokine profile and transcription factor expression. Adapted from Abbas et al., 2012; Annunziato and Romagnani, 2009; Murphy and Stockinger, 2010; Schmidt-Weber, 2010; Schmidt-Weber et al., 2007; Zhu et al., 2010.

1.3.3.1. AHR and $CD4^+$ effector T cells: AHR in Th1 and Th2 immunity

An imbalance of Th1 and Th2 cells is classically considered as the general mechanism underlying atopic diseases such as atopic dermatitis and allergy (Mazzarella et al., 2000). The dichotomous classification of Th cell populations in Th1 and Th2 cells was firstly introduced by Mosmann and Coffman in 1989 (Mosmann and Coffman, 1989). Differentiation of both subtypes is characterised by a cross-regulated inverse relationship. Thus, Th1 cytokines can suppress Th2 whereas the Th2 cytokines attenuate Th1 response (Mazzarella et al., 2000).

Traffic-related particulate matters (PM) from urban areas are a source of PAHs that are potent inducers of the AHR pathway. AHR is considered with a negligent expression in Th1 and Th2 cells, however, diesel exhaust particles (DEPs) and several PAHs could act as adjuvant factors in allergy by promoting IgE production and Th2 response to supplied allergens (Heo et al., 2001; Huang et al., 2015; Lubitz et al., 2009; Miller et al., 2010; Miller and Peden, 2014; Schober et al., 2007; Xia et al., 2015). Recent findings imply that a DEP-dependent AHR activation in antigen-presenting cells up-regulates promoters of Th cell differentiation and augments allergic airway inflammation (Xia et al., 2015). However, single AHR agonists seem to have anti-allergic functions in murine allergy models (Schulz et al., 2012; Schulz et al., 2011). The activation of the AHR by a synthetic AHR agonist suppresses Th2 immunity by skewing Th1/Th2 balance towards Th1 differentiation (Morales et al., 2008; Negishi et al., 2005). In agreement with this, AHR-deficient mice showed elevated levels of the Th2 cytokine IL-5 and of IgE (Lawrence et al., 2008; Negishi et al., 2005). When the endogenous AHR ligand FICZ is present during ovalbumine sensitisation in mice, it reduces the Th2 response at both cellular and cytokine level (Jeong et al., 2012). The function of AHR activation, during allergy particularly in humans and in concert with PM or DEPs has not yet been studied in detail.

1.3.3.2. AHR and $CD4^+$ effector T cells: AHR in T regulatory T cells, Th17 and Th22 cells

Several studies have reported that AHR regulates the dichotomous development of either pro-inflammatory Th17 cells or suppressive Treg cells (Apetoh et al., 2010; Bettelli et al., 2006; Gandhi et al., 2010; Quintana et al., 2008; Quintana et al., 2010; Veldhoen et al., 2008). Th17 cells provide benefits against extracellular pathogens mainly by IL-17-dependent recruitment of neutrophils and the induction of antimicrobial peptides (Laan et al., 1999; Liang et al., 2006). Despite of this, Th17 cells are implicated in a variety of autoimmune diseases such as collagen-induced arthritis, experimental autoimmune encephalomyelitis (EAE) and inflammatory bowel diseases (Lubberts et al., 2004; Reboldi et al., 2009; Stumhofer et al., 2006). On the contrary, Treg cells control innate and adaptive immunity by attenuating effector T cell response and inflammation (Sakaguchi, 2004). The largest subset of Treg cells are natural Treg (nTreg) cells that mature and gain their suppressive function in the thymus (Baecher-Allan et al., 2001). nTreg cells are supported by peripherally induced Treg (iTreg) that

originate from naïve T cells by the transforming growth factor-β (TGF-β) in extra-thymic organs. Common features among these cell types are the production of IL-10 and TGF-β and the expression of the transcription factor forkhead box P3 ($Foxp3^+$) (Bettelli et al., 2007; Chen et al., 2003; Sakaguchi, 2004). Currently, other diverse cell types share features with nTreg and iTreg cells. Here, $Foxp3^-$, IL-10-producing type 1 Treg cells (Tr1), $CD8^+$ Treg and IL-17-producing Treg could be noted (Cvetanovich and Hafler, 2010; Jäger and Kuchroo, 2010; Sakaguchi et al., 2010; Zhang et al., 2014).

In mice, the metabolically instable AHR ligand FICZ preferentially induces IL-17-producing pro-inflammatory Th17 cells and exacerbation of EAE, a murine model for T cell-dependent autoimmune inflammation (Veldhoen et al., 2008). Contrary, the metabolically inert TCDD tends to favour $FoxP3^+$ Treg cells and thereby represses EAE. Here, metabolic stabilities and specific affinities of AHR agonists are supposed to be responsible for diverse ligand-dependent outcomes in Th17 and Treg differentiation (Apetoh et al., 2010; Gandhi et al., 2010; Quintana et al., 2008). However, to what extent FICZ or TCDD shifts the critical development of either Treg subtypes or Th17 cells is still controversial. Recent studies considering the route of exposure and the absolute numbers of Th17 or Treg cells do not entirely agree with these research results (Chmill et al., 2010; Duarte et al., 2013). Furthermore, Gagliani et al. showed that activation of the AHR by FICZ could transdifferentiate Th17 cells into Tr1 cells (Gagliani et al., 2015).

Previously, Kimura et al. supposed an underlying mechanism for AHR-driven Th17 differentiation in mice (Kimura et al., 2009; Kimura et al., 2008). The activation of the AHR facilitates Th17 differentiation by suppressing the negative regulator signal transducer and activator of transcription 1 (STAT1). The authors hypothesised that AHR interacts with STAT1 during murine Th17 differentiation and thus AHR-dependent STAT1 inhibition favours Th17 development (Harrington et al., 2005; Kimura et al., 2008; Stumhofer et al., 2006; Wei et al., 2007). One human disease that is associated with mutations in the *STAT1* gene, and with an impaired Th17 cytokine production is a subtype of the chronic mucocutaneous candidiasis (CMC) (Liu et al., 2011; Puel et al., 2011; Puel et al., 2012). CMC determines a rare, heterogeneous group of syndromes with diverse underlying immune defects. CMC patients suffer from an impaired mucosal host defence against the in healthy individuals commensal fungus *Candida*, mostly *Candida albicans*, and they are characterised by recurrent or chronic infections of skin, nails and mucosal tissues (Eyerich et al., 2010; Kirkpatrick, 2001). CMC could occur as an associated disease to primary or secondary immunodeficiencies or accompanies infectious diseases. However, it can also occur without any other deficiencies as an isolated disease. Although the common underlying mechanisms of CMC are unclear in detail, increasing numbers of studies demonstrated that an impaired Th17 pathway is partly predisposing for CMC (Eyerich et al., 2008; Kisand et al., 2011; Maródi et al., 2012; Ng et al., 2010; Puel et al., 2011; Puel et al., 2012; Puel et al., 2010). Various inborn errors in the *STAT1* gene result in an overactive STAT1 protein and are reported as one cause for the isolated form of CMC that is referred to as CMC disease (CMCD). STAT1 is an important negative regulator of the Th17 pathway and several laboratories have analysed the coding region of *STAT1* for sequence variations in CMCD patients. Autosomal dominant heterozygous mutations, predominantly

located in the coiled-coil domain of the *STAT1* gene, could result in an overactive STAT1 protein due to an impaired nuclear dephosphorylation (Boisson-Dupuis et al., 2012; Harrington et al., 2005; Liu et al., 2011; Soltész et al., 2013; van de Veerdonk et al., 2011; Wei et al., 2007; Yamazaki et al., 2014). At present, 22 amino acid changes in the coiled-coil domain and 10 in the DNA-binding domain of *STAT1* are described for leading to gain-of-function (GOF) mutations (Yamazaki et al., 2014). To a large extent CMCD patients display a decreased Th17 cytokine production. Therefore, CMCD patients with *STAT1* GOF mutations provide a human model for an impaired Th17 cytokine regulation including IL-22 (Liu et al., 2011; Puel et al., 2011; Puel et al., 2012).

One common feature of various AHR ligands is the induction of IL-22 in T cells (Trifari et al., 2009; Veldhoen et al., 2009; Veldhoen et al., 2008). The activation of the AHR in humans, contrary to mice, facilitates the exclusive up-regulation of IL-22 without induction of IL-17A and IFN-γ (Brembilla et al., 2011; Trifari et al., 2009). These results imply that AHR could be one transcription factor important for human Th22 cells and for the regulation of IL-22. Th22 cells are a newly described Th cell subtype that specifically express IL-22 (Duhen et al., 2009; Eyerich et al., 2009; Trifari et al., 2009). According to the microenvironmental cytokines, IL-22 has pro- or anti-inflammatory properties (Besnard et al., 2011; Liang et al., 2006; Sonnenberg et al., 2010). In the skin, IL-22 and Th22 cells are essential factors for skin homeostasis and regulate epithelial barrier functions and host defence. The cytokine supports the innate defence mechanisms by induction of antimicrobial peptides in epithelial cells (Duhen et al., 2009; Eyerich et al., 2009; Liang et al., 2006; Wolk et al., 2004; Wolk et al., 2006). IL-22 is produced by many innate and adaptive lymphocytes such as Th22, Th17, γδ T cells and ILCs (Colonna, 2009; Martin et al., 2009; Spits and Di Santo, 2011; Trifari et al., 2009; Witte et al., 2010). The *IL22* gene is clustered within a conserved genomic region together with the *IL26* and the *IFNγ* genes on the human chromosome 12q14-15 (Dumoutier et al., 2000a; Goris et al., 2002). The cytokine belongs to the family of IL-10-related cytokines and its biological function is mediated through binding to the IL-10R2/IL-22R1 receptor chains mainly expressed on tissue cells (Dumoutier et al., 2000b; Kotenko et al., 2001; Wolk et al., 2004; Xie et al., 2000). Although it has been shown that IL-22 supplementation restores epithelial defence against *Candida* infections in synergy with the tumor necrosis factor-alpha (TNF-α) in human keratinocytes (Eyerich et al., 2011), the understanding of AHR-STAT1 interactions in regulating IL-22 and other Th17-associated cytokines in CMCD is rudimentary at present.

1.3.3.3. AHR in B cells

B cells are the exclusive cell type that produce immunoglobulins (Igs) in the immune system. Igs are key components of the humoral adaptive immunity. Membrane located Igs form the highly variable antigen receptors on B cells that are specific for soluble and unbound antigens. As APCs, B cells present antigens on MHC-II molecules and are activated by Th cells. Interaction with Th cells initates B cell differentiation into Ig-producing plasma cells. Cytokines that are present during B cell activation switch Ig gene expression from initially μ or δ gene expression to α, γ or ε. This changes the constant region of Igs and the accompanied effector functions (Abbas et al., 2012; Litman et al., 2010; Murphy et al., 2009; Singer and Hodes, 1983; Wall and Kuehl, 1983).
Several studies demonstrated an immunosuppressive function of AHR activation in B cells. The synthetic AHR agonist TCDD suppresses B cell differentiation, IgM and IgA syntheses and a natural occuring AHR agonist represses IgM, IgG and IgE productions (De Abrew et al., 2010; Kinoshita et al., 2006; Lu et al., 2010; Sulentic et al., 1998; Yoshida et al., 2012; Zhang et al., 2013). Noteworthy, the Th2 cytokine IL-4 induces AHR expression in B cells (Tanaka et al., 2005).

1.4. AHR and the xenobiotic-metabolising system

1.4.1. The xenobiotic-metabolising enzyme (XME) system

Xenobiotic-metabolising enzymes (XMEs) facilitate degradation and excretion of lipohilic exogenous (xenobiotics) and endogenous (endobiotics) molecules mostly by the induction of ligand-activated transcription factors (Köhle and Bock, 2009; Omiecinski et al., 2011; Tompkins and Wallace, 2007). The purpose of the xenobiotic metabolism is to change lipophilic molecules into more polar, hydrophilic compounds that can be eliminated by the kidney (Buters, 2008). The metabolism of lipophilic xenobiotics was initially considered as a biphasic reaction, however, today it is roughly separated into three phases (Ishikawa, 1992; Williams, 1971). Substrate oxygenation by CYP enzymes increases xenobiotic´s reactivity in the first phase (phase I). CYP enzymes create a reactive site in an otherwise chemically indifferent molecule, to which very hydrophilic moieties can be attached in the second phase (phase II). During CYP-dependent phase I reactions reactive or toxic intermediates can lead to cellular dysfunctions and carcinogenic effects (Buters, 2008; Guengerich, 1992, 2005, 2006; Nebert and Dalton, 2006; Williams, 1971). For detoxification of these intermediates, phase I metabolism is tightly coupled to phase II pathways (Köhle and Bock, 2007; Ramos-Gomez et al., 2001). Phase II enzymes modify the metabolite and prepare it for excretion through conjugation to hydrophilic groups such as glutathione, glucuronide or sulfate (Alexandrov et al., 2002; Gamage et al., 2006; Glatt and Oesch, 1977; Nebert and Vasiliou, 2004; Rowland et al., 2013; Wincent et al., 2009). In order to avoid intracellular trapping of the more hydrophilic, metabolised molecules, which cannot pass the lipophilic cell membane,

phase III efflux transporter proteins mediate the transmembrane transport into the extracellular space (Döring and Petzinger, 2014; Omiecinski et al., 2011; Vasiliou et al., 2009).

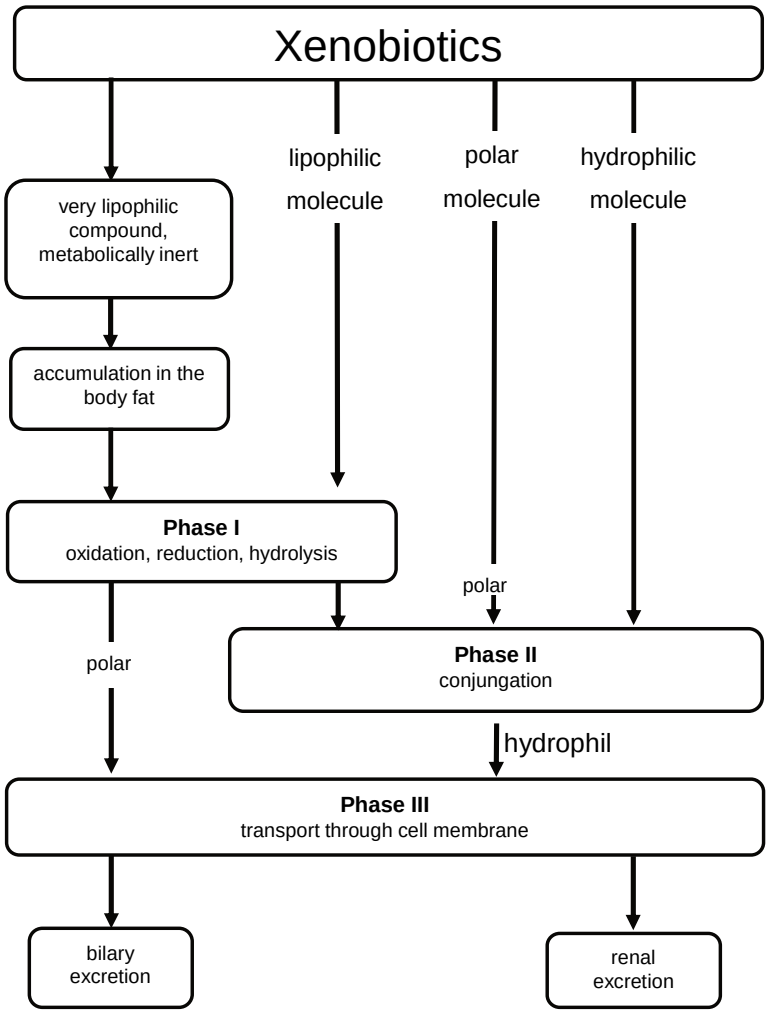


Figure 5. **Overview of the xenobiotic-metabolising system**
Different reactions of phase I (CYP) and phase II enzymes modify lipophilic or hydrophilic molecules. These molecules are prepared for cellular and for physiological excretion (phase III) (Buters, 2008).

1.4.2. Phase I metabolism and cytochrome P450 (CYP) enzymes

Majority of phase I enzymes are CYP enzymes. CYP enzymes are heme-containing mono-oxygenases. They metabolise exogenous and endogenous lipophilic chemicals including drugs by oxidative hydroxylations. The enzymes are ubiquitously expressed and use molecular oxygen and reducing equivalents for substrate oxidation. During the CYP catalytic cycle, reactive oxygen intermediates and mutagenic molecules can be generated (Bondy and Naderi, 1994; Guengerich, 1992, 2006; Nebert, 1991; Nebert and Dalton, 2006; Nebert and Karp, 2008; Nebert and Russell, 2002; Nelson et al., 1996; Puntarulo and Cederbaum, 1998).

CYP enzymes are structured into families and subfamilies according to their amino acid sequence identities. Enzymes of the same family share a sequence homology of more than 40%, and enzymes of the same subfamily of at least 55% (Nelson et al., 1996). The human genome presumably encodes for 57 functional CYP proteins that are structured according to their sequence identities in 18 families (Lewis, 2004; Nelson, 2002; Nelson et al., 2004). Most of them are polymorphic and display a complex organ-, development- and sex-specific expression. CYP enzymes are most abundantly expressed in the liver and in tissues with close proximity to the environment such as lung, skin and the gastrointestinal tract. Mapping CYP in these organs has been intensively studied in organ-specific toxicity to environmental compounds (Ding and Kaminsky, 2003; Gandhi et al., 2004; Gundert-Remy et al., 2014; Macé et al., 1998; Nebert, 2000; Swanson, 2004; Waxman and Holloway, 2009; Zanger et al., 2014; Zanger and Schwab, 2013). Preferentially members of the first three CYP families (CYP1, CYP2 and CYP3) bioactivate and detoxify foreign molecules such as drugs, carcinogens and industrial compounds such as HAHs, PCBs and PAHs, and they display overlapping substrate specificities (Guengerich, 2006; Hodgson, 2001; Nebert and Dalton, 2006; Nebert et al., 2004). CYP1 family enzymes metabolically activate PAHs. They contribute to the formation of reactive intermediates and are therefore the most investigated downstream targets of the AHR in toxicology (Gonzalez et al., 1984; Ma, 2001; Nebert et al., 2004; Nebert and Jones, 1989; Nebert et al., 1991; Nebert et al., 1993; Shimada and Fujii-Kuriyama, 2004). Additionally, CYP synthesise and catabolise endogenous compounds including steroid hormones, vitamins and fatty acids (Nebert and Dalton, 2006; Nebert and Karp, 2008; Yang et al., 2013). The expression of CYP can be greatly increased by induction. Besides AHR, various other ligand-dependent transcription factors regulate CYP expression as a response to the chemical environment (Maglich et al., 2002; Pascussi et al., 2001; Waxman, 1999; Wei et al., 2000a).

Table 1. Classification of human CYP enzymes according to their substrate specificities
Guengerich, 2006; Lewis, 2004; Nelson, 2002 and modified

Sterols	Xenobiotics	Fatty acids	Eicosanoids	Vitamins	Unknowns
1B1	1A1	2J2	4F2	5R1	2A7
7A1	1A2	4A11	4F3	24A1	2U1
7B1	1B1	4B1	4F8	26A1	2W1
8B1	2A6	4F12	5A1	26B1	3A43
11A1	2A13	2S1	8A1	26C1	4A22
11B1	2B6		2S1	27B1	4F11
11B2	2C8				4F22
17A1	2C9				4V2
19A1	2C18				4X1
21A2	2C19				4Z1
27A1	2D6				20A1
39A1	2E1				27C1
46A1	2F1				
51A1	3A4				
	3A5				
	3A7				

The AHR downstream targets CYP1A1, CYP1B1 and CYP2S1 are typically expressed in extrahepatic tissues. They are found in environmentally exposed barrier tissues such as skin, lung or the gastrointestinal tract (Baron et al., 2001; Bernauer et al., 2006; Ding and Kaminsky, 2003; Du et al., 2006; Gundert-Remy et al., 2014; Karlgren et al., 2005; Leclerc et al., 2010; Rivera et al., 2002; Rylander et al., 2001; Saarikoski et al., 2005; Swanson, 2004). Whereas the expression of AHR and CYP in these tissues is well characterised, the function of CYP metabolism in immunity has been limited investigated (Baron et al., 1998; Siest et al., 2008). The effects of inflammation on XME and transporter protein expression in the liver have been elucidated in several studies. Here, pro-inflammatory immune responses triggered by cytokines, bacterial, viral or parasitic infection models down-regulate the majority of hepatic CYP enzymes and xenobiotic transcription factors. A reduced CYP expression during inflammation depresses hepatic clearance and could result in adverse effects with an altered bioavailability of chemicals including drugs (Aitken et al., 2008; Aitken et al., 2006; Li-Masters and Morgan, 2001; Monshouwer and Witkamp, 2000; Morgan, 2001, 2009; Morgan et al., 1998; Sewer and Morgan, 1998; Theken et al., 2011; Vrzal et al., 2004). Several studies characterised CYP expression in peripheral blood mononuclear cells (PBMCs) or isolated immune cell populations after incubation with CYP-inducing agents (summarised in (Siest et al., 2008)). A comprehensive analysis of CYP expression in non-cultured, various immune cell subtypes, however, has not been investigated in detail.

1.4.3. Phase II metabolism and antioxidative response

The AHR pathway is closely linked to the antioxidative phase II response. Phase II enzymes eliminate reactive intermediates that particularly occur during CYP-mediated phase I reactions. Here, AHR co-operates with the ubiquitously expressed cytoprotective key factor nuclear factor erythroid 2-related factor 2 (NRF2) that is an important inducer of phase II enzymes (Chanas et al., 2002; Köhle and Bock, 2007; Miao et al., 2005; Ramos-Gomez et al., 2001). NRF2 is a basic leucin zipper transcription factor and sequestered in the cytoplasm by Kelch-like-ECH-associating-protein 1 (Keap1). Keap1 senses oxidative and electrophilic stress and is an adapter protein of an E3 ubiquitin ligase. When intracellular oxidative burden is low, interaction of Keap1 with NRF2 induces a permanent NRF2 degration. Rising concentrations of reactive oxygen species or electrophiles stabilise and release NRF2 from Keap1 allowing the translocation of NRF2 into the nucleus (Itoh et al., 2003; Kang et al., 2004; Kobayashi et al., 2004; McMahon et al., 2003; Nguyen et al., 2003a; Nguyen et al., 2003b). NRF2 binds onto antioxidant response elements (AREs) upstream of gene batteries coding for phase I and phase II enzymes. NQO1, aldo-keto reductases (AKRs) and glutathione-s-transferases (GSTs) are NRF2 targets and act downstream of CYP enzymes (Chanas et al., 2002; Jaiswal, 2004; McMahon et al., 2001; Nguyen et al., 2003b; Radjendirane and Jaiswal, 1999). NRF2 co-operates with AHR for inducing detoxification of potentially harmful intermediates and for regulating oxidative defence. Expression of both transcription factors is regulated mutually and activation of both factors control partly overlapping signalling pathways and XME batteries (Köhle and Bock, 2007; Ma et al., 2004; Miao et al., 2005; Wang et al., 2013).

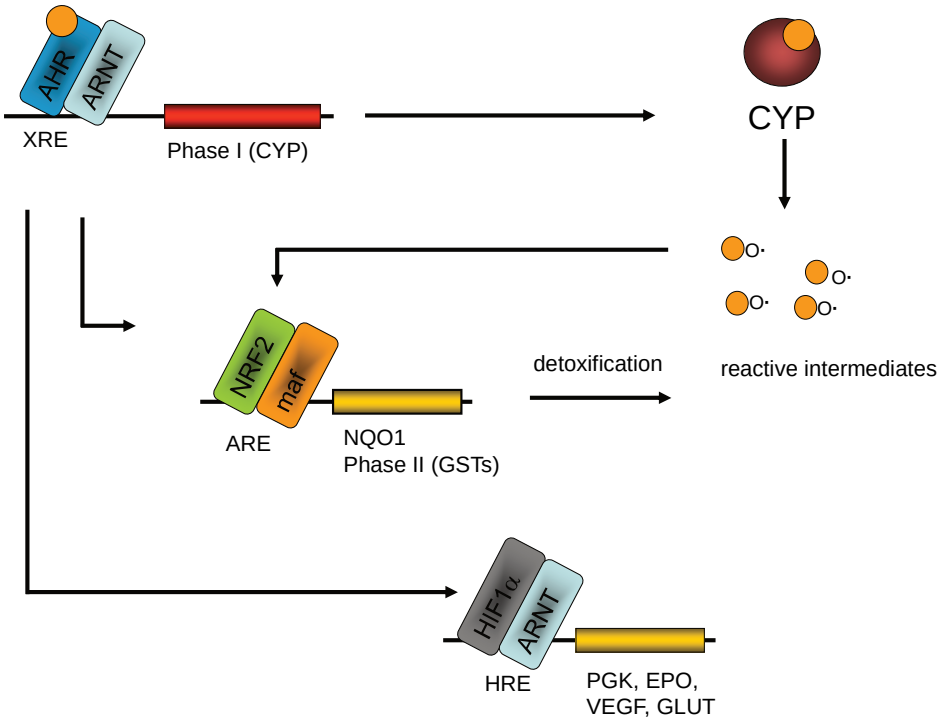


Figure 6. Interactions of CYP with transcription factors involved in the xenobiotic and oxygen response
AHR, ARNT, NRF2 and HIF1α are involved in celluar xenobiotic and oxygen response. AHR can directly regulate NRF2 and an interation between AHR and Hif1α is given by the dimerisation partner ARNT. AHR: Aryl hydrocarbon receptor, ARNT: AHR nuclear translocator, NRF2: NF erythroid 2-related factor 2, NQO1: NAD(P)H dehydrogenase quinone 1, CYP: Cytochrome P450, PGK: Phosphogylcerate kinase 1, EPO: Erythropoietin, VEGF: Vascular endothelial growth factor, XRE: Xenobiotic response element, ARE: Antioxidant response element, HRE: Hypoxia response element, GLUT: Glucose transporter.

1.4.4. CYP1-dependent AHR regulation

The AHR negatively regulates its own activation by several feedback loops such as breakdown of potential ligands through induced CYP1 expression, or by an up-regulation of the AHR repressor protein (Evans et al., 2008; Hahn et al., 2009; Mimura et al., 1999; Morel and Barouki, 1998; Morel et al., 2000; Morel et al., 1999; Wincent et al., 2012). Several observations demonstrated an auto-regulated feedback loop for AHR activation by a reduced CYP1 activity in cell lines and epithelial cells. However, evidence of this mechanism in primary human immune cells is still lacking (Chang and Puga, 1998; Chiaro et al., 2007; Hankinson et al., 1985; Wei et al., 2000b; Wincent et al., 2012).

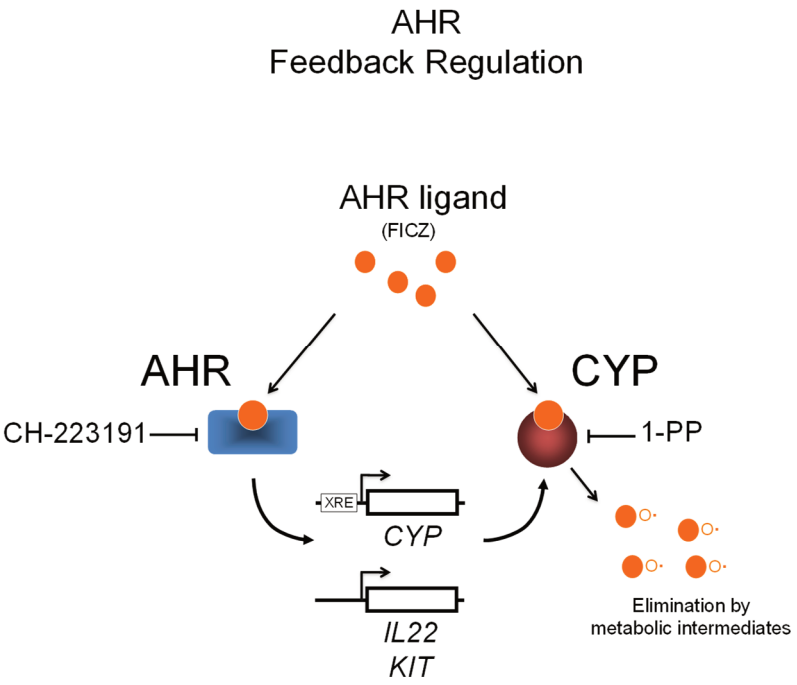
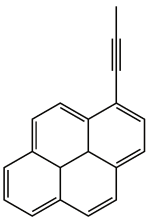
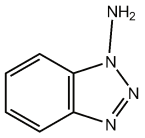


Figure 7. CYP1 activity modifies AHR by feedback regulation
Adapted from Wincent et al., 2012.

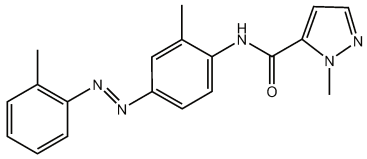
The endogenous and natural occuring AHR agonist FICZ is a planar derivative of the UV-absorbing amino acid tryptophan (Bergander et al., 2003; Helferich and Denison, 1991, Öberg et al., 2005). FICZ can be endogenously formed by UV light in the skin (Fritsche et al., 2007; Rannug et al., 1987; Wei et al., 1999; Wincent et al., 2009). In several studies, AHR ligands including FICZ induce IL-22 and down-regulate IL-17 in human immune cells (Brembilla et al., 2011; Trifari et al., 2009; Veldhoen et al., 2008). FICZ binds to the AHR with a higher affinity (K_D 0.07 nM) than the most potent environmental ligand TCDD (K_D 0.48 nM) (Bergander et al., 2003; Rannug et al., 1987). Whereas TCDD is very slowly metabolised with a half life of approximately 7 to 10 years in humans (Miniero et al., 2001), FICZ is degraded by CYP1 enzymes and induces its own phase I metabolism into hydroxylated metabolites. This is likely leading to a restricted AHR activation (Bergander et al., 2003; Bergander et al., 2004; Wei et al., 2000b; Wincent et al., 2012).



1-(1-Propynyl)-pyrene (1-PP)
(CYP1 inhibitor)



1-Aminobenzotriazole (1-ABT)
(CYP inhibitor)



CH-223191
(AHR antagonist)

Figure 8. Structures of the CYP inhibitors 1-PP and 1-ABT, and of the AHR antagonist CH-223191

The PAH 1-(1-propynyl)-pyrene (1-PP) is a highly specific substrate for the CYP1 enzyme family and acts as a mechanism-based (suicide) inhibitor. 1-PP predominantly abrogates CYP1A1 enzyme activity with probably little AHR agonistic effects (Shimada et al., 2007; Shimada et al., 1998; Zhu et al., 2011b). 1-PP and 1-aminobenzotriazole (1-ABT), another suicide inhibitor for CYP, were used in this study to investigate CYP1 inhibition. The synthetic AHR antagonist CH-223191 competitively prevents against TCDD-induced AHR translocation (Kim et al., 2006; Zhao et al., 2010-a). The molecules FICZ, 1-PP and CH-223191 were used in this study to investigate the CYP1-induced AHR activation pharmacologically in human immune cells (see Figure 7).

1.5. Aim of the Study

For many decades the ligand-activated transcription factor aryl hydrocarbon receptor (AHR) has been studied for its role in environmentally induced toxic reactions. The AHR induces cytochrome P450 (CYP) and other xenobiotic-metabolising enzymes of phase I (oxidative hydroxylation), phase II (conjungation) or phase III (excretion) reactions. However, AHR is also a mediator of immune cell differentiation.

The aim of this thesis was to investigate whether CYP1 have an impact on AHR-driven targets in human immune cells, mainly the Th17 cytokines IL-22, IL-17 and the stem cell factor receptor c-Kit. This was studied by using the AHR agonist 6-formylindolo[3,2-*b*]carbazole (FICZ) and the CYP1 suicide inhibitor 1-(1-propynyl)-pyrene (1-PP) in a pharmacological approach in human and murine immune cells. In this context, stress-related factors were investigated additionally. Based on the proposed negative interaction of the AHR with the signal transducer and activator of transcription 1 (STAT1), this study also investigated the AHR activation in PBMCs from patients suffering from an isolated form of the chronic mucocutaneous *Candida* (CMC) infection. These patients have gain-of-function mutations (GOF) in the *STAT1* gene and a reduced production of the Th17 cytokines IL-17 and IL-22. Furthermore, the study aimed to analyse the constitutive expression of genes coding for xenobiotic-metabolising enzymes in various human immune cell subtypes.

2 Materials and Methods

2.1. Material

2.1.1. Cell Culture Reagents and Buffers

Table 2. Cell Culture Reagents

Cell Culture Reagents	Cat.no	Supplier/Source/Manufacturer	
Dimethyl Sulfoxide Cell Culture Grade	A3672.0250	AppliChem GmbH	Darmstadt, Germany
Dulbecco´s Modified Eagle Medium (DMEM)	41966-029	Life Technologies Corporation	Carlsbad, USA
DMEM/F12	11320-074	Life Technologies Corporation	Carlsbad, USA
Fetal Calf Serum (FCS)	CNH0003	Perbio Science GmbH	Bonn, Germany
Human Serum	H4522-100ml	Sigma-Aldrich Chemie GmbH	Munich, Germany
L-Glutamine (200 mM)	25030-024	Life Technologies Corporation	Carlsbad, USA
MEM Non-Essential Amino Acids 100x	11140-035	Life Technologies Corporation	Carlsbad, USA
Penicillin-Streptomycin 10 000 units/ml Penicillin, 10 000 units/ml Streptomycin	11344033	Life Technologies Corporation	Carlsbad, USA
Phosphate Buffered Saline D-PBS (w/o Ca^{2+}/Mg^{2+})	14190-094	Life Technologies Corporation	Carlsbad, USA
Roswell Park Memorial Institute (RPMI)-1640 Medium + L-Glutamine	15250-061	Life Technologies Corporation	Carlsbad, USA
STEMPRO-34 Serum Free Medium (SFM) With STEMPRO-34 Nutrient Supplement	10639011	Life Technologies Corporation	Carlsbad, USA
Sodium Pyruvat (100 mM)	11360-039	Life Technologies Corporation	Carlsbad, USA
Trypan Blue Stain (0.4%)	15250.061	Life Technologies Corporation	Carlsbad, USA

Table 3. Cell Lines

Cell Lines		Source
V79	Chinese hamster lung fibroblasts	Prof Arand, Johannes Gutenberg-Universität Mainz, Germany
hCYP1A1	V79 cell line with recombinant expression of human CYP1A1	Prof Jeroen Buters, TUM, Munich, Germany
hCYP1A2	V79 cell line with recombinant expression of human CYP1A2	Prof Jeroen Buters, TUM, Munich, Germany
hCYP1B1	V79 cell line with recombinant expression of human CYP1B1	Prof Jeroen Buters, TUM, Munich, Germany

Table 4. Animals

Mice	Source
CYP1a2 knockout mice	Were a gift from Dr Daniel Nebert, Department of Environment and Center for Environmental Genetics, University of Cincinnati Medical Center, Cincinnati, OH, USA (Liang et al., 1996)
C57BL/6	Charles River Laboratories, Wilmington, Massachusetts

Table 5. Freezing Medium

Freezing Medium	
FCS	20 ml
DMSO	5 ml
DMEM/F12	25 ml

Table 6. Medium for CYP1-expressing V79 Cell Lines

Medium for CYP Cell Lines	
DMEM	200 ml
Fetal Calf Serum (FCS)	22 ml
L-Glutamine	4.6 ml
Penicillin Streptomycin	2.2 ml
Sodium Pyruvat	2.2 ml

Table 7. Proliferation Medium for Human PBMCs

Proliferation Medium for Human PBMCs and T cells	
RPMI	225 ml
Human Serum	12.5 ml
L-Glutamine	2.5 ml
Non-Essential Amino Acids	2.8 ml
Sodium Pyruvat	2.8 ml
Penicillin Streptomycin	2.5 ml
β-Mercaptoethanol 50mM	250 µl

Table 8. Medium for Murine Splenocytes

Proliferation Medium for Murine Splenocytes	
RPMI	225 ml
FCS	12.5 ml
L-Glutamine	2.5 ml
Non-Essential Amino Acids	2.8 ml
Sodium Pyruvat	2.8 ml
Penicillin Streptomycin	2.5 ml
β-Mercaptoethanol 50mM	250 µl

Table 9. Lysis Buffer for Murine Erythrocytes

10x Lysis Buffer	
NH_4Cl (Ammonium Chloride)	1.5 M
$NaHCO_3$ (Sodiumhydrogencarbonat)	100 mM
Di-sodium EDTA (Na_2EDTA)	10 mM
Adjust pH to 7,4, sterile filtered	

Table 10. Sodium Phosphate Buffer for Ethoxyresorufin Assay

50 mM Sodium Phosphate Buffer	
$Na_2HPO_4 \times 12H_2O$	50 mM
Adjust pH to 8 with phosphoric acid	

Table 11. Thermolysin Buffer

Thermolysin Protease X Buffer	
HEPES	10 mM
NaCl	142 mM
KCl	6.7 mM
NaOH	0.43 mM
$CaCl_2$	1 mM

2.1.2. FACS Reagents; Antibodies and Buffers

Table 12. Antibodies for FACS Stainings and Cell Activation

Antibodies	Clone	Cat. No	Supplier/Source/Manufacturer	
Anti-Human Antibodies				
Mouse Anti-Human CD3-FITC	OKT3	11-0037-42	eBioscience GmbH	Frankfurt, Germany
Mouse Anti-Human CD4-APC-Cy7	RPA-T4	557871	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD4-FITC	RPA-T4	555346	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD8-Pacific Blue	RPA-T8	558207	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD8-PE	HIT8a	555635	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD14-FITC	61D3	11-0149-73	eBioscience GmbH	Frankfurt, Germany
Mouse Anti-Human CD19-PE	HIB19	555413	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD40-FITC	5C3	555588	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD45RO-FITC	UCHL1	555492	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD45RO-PE	UCHL1	555493	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD45RA-PE	HI100	555489	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human CD56-PE-Vio770	AF12-7H3	130-098-132	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Mouse Anti-Human CD117-PE	A3C6E2	130-091-734	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Mouse Anti-Human CD123-PE	AC145	130-090-899	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Mouse Anti-Human CD303-FITC	AC144	130-090-510	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Mouse Anti-Human /mouse IL-22			Genentech Inc.	San Francisco, USA
Mouse Anti-Human NA/LE CD3	UCHT1	555329	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Mouse Anti-Human NA/LE CD28	CD28.2	555725	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Anti-Mouse Antibodies				
Anti-Mouse CD4-PE	RM4-5	553048	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Anti-Mouse CD8-PE VIO770	53-6.7	130-097-999	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Anti-Mouse IFNγ AF-488	XMG1.2	557724	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Hamster Anti-Mouse NA/LE CD3	145-2C11	553057	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Hamster Anti-Mouse NA/LE CD28	37.51	553294	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Purified Rat Anti-Mouse CD16/CD32 (Mouse Fc Block™)	2.4G2	553142	Becton Dickinson (BD) GmbH	Heidelberg, Germany

Table 13. FACS Buffer

FACS Buffer	
PBS w/o Ca^{2+}/Mg^{2+}	
Sodium Azide 2% (w/v)	0.02%
FCS	5%

2.1.3. qRT-PCR Primer Sequences

Table 14. Primer Sequences for qPCR

Gene	forward 5' → 3'	reverse 5' → 3'	RefSeq (NCBI)
ABCG2	TGG CTT AGA CTC AAG CAC AGC	TCG TCC CTG CTT AGA CAT CC	NM_004827
AHR	TCA GTT CTT AGG CTC AGC GTC	AGT TAT CCT GGC CTC CGT TT	NM_001621
ARNT	CTA CCC GCT CAG GCT TTT C	CAC CAA ACT GGG AAG TAC GAG	NM_001668
PTGS2	GGC GCT CAG CCA TAC AG	CCG GGT ACA ATC GCA CTT AT	NM_000963
CYP1A1	GGA ACC TTC CCT GAT CCT TG	GGA GAT TGG GAA AAG CAT GA	NM_000499
CYP1A2	ACA ACC CTG CCA ATC TCA AG	GGG AAC AGA CTG GGA CAA TG	NM_000761
CYP1B1	GCT GCA GTG GCT GCT CCT	CCC ACG ACC TGA TCC AAT TCT	NM_000104
CYP2S1	GGT CAG GCT GAG GAG TTC AG	CTC CCC GTT GGA GAA GAA A	NM_030622
EEF1A	CTG AAC CAT CCA GGC CAA AT	GCC GTG TGG CAA TCC AAT	NM_001402
HIF1A	GCG CGA ACG ACA AGA AA	GAA GTG GCA ACT GAT GAG CA	NM_001530
IFNγ	CGA GAT GAC TTC GAA AAG CTG	TCA GCC ATC ACT TGG ATG AG	NM_000619
IL17A	CCA TCC CCA GTT GAT TGG AA	CTC AGC AGC AGT AGC AGT GAC A	NM_002190
IL22	TCC AGA GGA ATG TGC AAA AG	ACA GCA AAT CCA GTT CTC CAA	NM_020525
IL26	TGC AAG GCT GCA AGA AAA TA	CTC TAG CTG ATG AAG CAC AGG A	NM_018402
KIT	ATG GCA TGC TCC AAT GTG T	GGC AGT ACA GAA GCA GAG CA	NM_000222
NQO1	ATG TAT GAC AAA GGA CCC TTC C	TCC CTT GCA GAG AGT ACA TGG	NM_000903
NRF2	CTT GGC CTC AGT GAT TCT GAA GTG	CCT GAG ATG GTG ACA AGG GTT GTA	NM_006164

2.1.4. Assays and Reference Sequences for TaqMan Low Density Arrays (TLDAs)

Table 15. TaqMan Low Density Arrays: Gene Names and Assay IDs

Assay ID	Gene Symbol	Genename	Chromosome	NCBI Gene Reference
Hs00156558_m1	CMA1	Chymase 1, mast cell	14	NM_001836.2
Hs00174029_m1	KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	4	NM_001093772.1 NM_000222.2
Hs00175232_m1	FCER1A	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide	1	NM_002001.2
Hs02576518_gH	TPSB2, TPSAB1	Tryptase beta 2 (gene/pseudogene);tryptase alpha/beta 1	16	NM_024164.5 NM_003294.3
Hs00153120_m1	CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1	15	NM_000499.3
Hs00167927_m1	CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	15	NM_000761.3
Hs00164383_m1	CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	2	NM_000104.3
Hs00258076_m1	CYP2S1	Cytochrome P450, family 2, subfamily S, polypeptide 1	19	NM_030622.6
Hs00426372_m1	CYP2A13	Cytochrome P450, family 2, subfamily A, polypeptide 13	19	NM_000766.3
Hs00868409_s1	CYP2A6	Cytochrome P450, family 2, subfamily A, polypeptide 6	19	NM_000762.5
Hs00167937_g1	CYP2B6	Cytochrome P450, family 2, subfamily B, polypeptide 6	19	NM_000767.4
Hs00426380_m1	CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide 19	10	NM_000769.1
Hs00426403_m1	CYP2C18	Cytochrome P450, family 2, subfamily C, polypeptide 18	10	NM_000772.2
Hs00426397_m1	CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9	10	NM_000771.3
Hs00164385_m1	CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6	22	NM_000106.4
Hs00559368_m1	CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1	10	NM_000773.3
Hs00167949_m1	CYP2F1	Cytochrome P450, family 2, subfamily F, polypeptide 1	19	NM_000774.3
Hs00356035_m1	CYP2J2	Cytochrome P450, family 2, subfamily J, polypeptide 2	1	NM_000775.2
Hs00430021_m1	CYP3A4	Cytochrome P450, family 3, subfamily A, polypeptide 4	7	NM_001202855.2 NM_017460.5
Hs00241417_m1	CYP3A5	Cytochrome P450, family 3, subfamily A, polypeptide 5	7	NM_001190484.1
Hs00426361_m1	CYP3A7	Cytochrome P450, family 3, subfamily A, polypeptide 7	7	NM_000765.3
Hs00426608_m1	CYP4F2	Cytochrome P450, family 4, subfamily F, polypeptide 2	19	NM_001082.3
Hs00403446_m1	CYP4F22	Cytochrome P450, family 4, subfamily F, polypeptide 22	19	NM_173483.3
Hs00168521_m1	CYP4F3	Cytochrome P450, family 4, subfamily F, polypeptide 3	19	NM_000896.2
Hs00240671_m1	CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1	15	NM_031226.2 NM_000103.3
Hs00195992_m1	AKR1A1	Aldo-keto reductase family 1, member A1 (aldehyde reductase)	1	NM_153326.2 NM_001202413.1 NM_001202414.1 NM_006066.3
Hs00164458_m1	EPHX1	Epoxide hydrolase 1, microsomal (xenobiotic)	1	NM_001136018.2 NM_000120.3
Hs00157403_m1	EPHX2	Epoxide hydrolase 2, cytoplasmic	8	NM_001979.4
Hs00265266_g1	GSTM2	Glutathione S-transferase mu 2 (muscle)	1	NM_01142368.1 NM_000848.3
Hs00356079_m1	GSTM3	Glutathione S-transferase mu 3 (brain)	1	NM_000849.4
Hs02512067_s1	GSTP1	Glutathione S-transferase pi 1	11	NM_000852.3
Hs00184475_m1	GSTT1	Glutathione S-transferase theta 1	22	NM_000853.2
Hs00155313_m1	GSTZ1	Glutathione transferase zeta 1	14	NM_145870.2 NM_145871.2
Hs00220393_m1	MGST1	Microsomal glutathione S-transferase 1	12	NM_145791.1 NM_145764.1 NM_145792.1 NM_020300.3
Hs00182064_m1	MGST2	Microsomal glutathione S-transferase 2	4	NM_001204366.1 NM_002413.4
Hs00165162_m1	MPO	Myeloperoxidase	17	NM_000250.1
Hs00168547_m1	NQO1	NAD(P)H dehydrogenase, quinone 1	16	NM_001025434.1 NM_000903.2 NM_001025433.1
Hs00287016_m1	POR	P450 (cytochrome) oxidoreductase	7	NM_000941.2
Hs00924803_m1	PTGS1	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	9	NM_080591.1 NM_000962.2
Hs00153133_m1	PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	1	NM_000963.2
Hs00184500_m1	ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	7	NM_000927.4
Hs00166123_m1	ABCC2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	10	NM_000392.3
Hs00184979_m1	ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	4	NM_004827.2
Hs99999905_m1	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	12	NM_002046.3
Hs99999909_m1	HPRT1	Hypoxanthine phosphoribosyltransferase 1	X	NM_000194.2

2.1.5. Chemicals

Table 16. Chemicals

Chemicals	Cat. No.	Supplier/Source/Manufacturer	
Ampuwa Water	00041499	Fresenius Kabi AG	Bad Homburg, Germany
Ammonium Chloride, NH_4Cl	A5666	Sigma-Aldrich Chemie GmbH	Munich, Germany
1-Aminobenzotriazole, 1-ABT	A3940	Sigma-Adrich Chemie GmbH	Munich, Germany
Calcium chloride, $CaCl_2$	C-7902	Sigma-Aldrich Chemie GmbH	Munich, Germany
Citric Acid Monohydrat	C-1909	Sigma-Aldrich Chemie GmbH	Munich, Germany
Dimethyl Sulfoxide, DMSO	A3672.0100	AppliChem GmbH	Darmstadt, Germany
Di-Sodium Hydrogen Phosphate Dodecahydrate $Na_2HPO_4 \times 12H_2O$	106579.1000	Merk KGaA	Darmstadt, Germany
Di-Sodium EDTA, Na_2EDTA	D2900000	Sigma-Aldrich Chemie GmbH	Munich, Germany
Di-Sodium Hydrogen Phosphate, Na_2HPO_4	1065860-500	Merck KGaA	Darmstadt, Germany
EDTA Ultrapure, pH 8, 0.5 M	15575-038	Life Technologies Corporation	Carlsbad, USA
Ethanol, >99.9%	1.00983.1000	Merk KGaA	Darmstadt, Germany
7-Ethoxyresorufin	E-3763	Sigma-Aldrich Chemie GmbH	Munich, Germany
6-Formylindolo[3,2-b]carbazole, FICZ	BML-GR206	Enzo Life Sciences GmbH	Lörrach, Germany
Heparin-Sodium 250 000	PZN 03874685	Ratiopharm GmbH	Ulm, Germany
2-(4-(2-Hydroxyethyl)- 1-Piperazinyl)-Ethansulfonsäure (HEPES)	11344-033	Life Technologies Corporation	Carlsbad, USA
Hydrogen Peroxide, H_2O_2	216763	Sigma-Aldrich Chemie GmbH	Munich, Germany
Ionomycin	I-0634	Sigma-Aldrich Chemie GmbH	Munich, Germany
Lymphoprep, Density 1.077 g/ml	1114547	AXIS-SHIELD PoC AS	Oslo, Norway
2-Mercaptoethanol	4227.1	Carl Roth GmbH & Co. KG	Karlsruhe, Germany
2-Methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazo-phenyl)-amide, CH-223191	C8124	Sigma-Aldrich Chemie GmbH	Munich, Germany
[Methyl-^{3}H] Thymidine, 5 mCi	MT6035/163955	Hartmann Analytic	Braunschweig, Germany
Methanol	34966	Sigma-Aldrich Chemie GmbH	Munich, Germany
Monensin Solution 1000x	004505-51	eBioscience GmbH	Frankfurt, Germany
Phorbol 12-Myristate 13-Acetate, PMA	P-8139	Sigma-Aldrich Chemie GmbH	Munich, Germany
Potassium Chloride, KCl	1049360.500	Merk KGaA	Darmstadt, Germany
Potassium Dihydrogen Phosphate, KH_2PO_4	1.048.73.1000	Merk KGaA	Darmstadt, Germany
Potassium Hydroxide, KOH	6751.3	Carl Roth GmbH & Co. KG	Karlsruhe, Germany
1-(1-Propynyl)-pyrene, 1-PP		Biochemical Institute for Environmental Carcinogens	Großhansdorf, Germany
2-Propanol, >99,5%	9866.6	Carl Roth GmbH & Co. KG	Karlsruhe, Germany
Phosphoric Acid, H_3PO_4	79606	Sigma-Aldrich Chemie GmbH	Munich, Germany
Sodium Azide, NaN_3	106688.1100	Merk KGaA	Darmstadt, Germany
Sodium Chloride, NaCl	9265.2	Carl Roth GmbH & Co. KG	Karlsruhe, Germany
Sodium Hydroxide, NaOH Pellets	1064950.250	Merck KGaA	Darmstadt, Germany
Sodium Hydroxide, NaOH, 1 M	38214	Sigma-Aldrich Chemie GmbH	Munich, Germany
Sodiumhydrogencarbonat, $NaHCO_3$	1.06329.0500	Merk KGaA	Darmstadt, Germany
Sulfuric Acid (H_2SO_4), 25%	1.00716.100	Merck KGaA	Darmstadt, Germany
Tween® 20 Detergent	655204	Merk KGaA	Darmstadt, Germany
3,3′,5,5′-Tetramethylbenzidine, TMB	87748	Sigma-Aldrich Chemie GmbH	Munich, Germany

2.1.6. Enzymes and Proteins

Table 17. Enzymes and Proteins

Enzymes And Proteins	Cat. No.	Supplier/Source/Manufacturer	
Bovine Serum Albumin (BSA)	A2137	Sigma-Aldrich Chemie GmbH	Munich, Germany
Collagenase	C7926-1G	Sigma-Aldrich Chemie GmbH	Munich, Germany
DNase	DN-25-1G	Sigma-Aldrich Chemie GmbH	Munich, Germany
Hyaluronidase	H3884-1G	Sigma-Aldrich Chemie GmbH	Munich, Germany
RNase-free DNase Set	79254	Qiagen GmbH	Hilden, Germany
Recombinant Human Stem Cell Factor (SCF)	300-07-100	PreproTech GmbH	Hamburg, Germany
Thermolysin Type X Protease	P-1512-1G	Sigma-Aldrich Chemie GmbH	Munich, Germany
Trypsin 0.05% EDTA	25300-054	Sigma-Aldrich Chemie GmbH	Munich, Germany

2.1.7. Kit Reagents

Table 18. Kit Reagents

Kit Reagents	Cat. No.	Supplier/Source/Manufacturer	
Agilent RNA Nano 6000 LabChip Kit	5067-1511	Agilent Techologies GmbH	Waldbronn, Germany
Alexa Fluor 647 Microscale Pro	A30009	Life Technologies Corporation	Carlsbad, USA
AlQshredder	79654	Qiagen GmbH	Hilden, Germany
B Cell Isolation Kit II	130-091-151	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Basophil Isolation Kit II	130-092-662	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Brefeldin A (GolgiPlug™) Solution	555029	Becton Dickinson (BD) GmbH	Heidelberg, Germany
CD117 MicroBead Kit	130-091-332	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
CD14 MicroBeads	130-050-201	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
$CD4^+$ T cell Isolation Kit	130-091-155	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
CD45RA MicroBeads	130-045-901	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
$CD8^+$ T Cell Isolation Kit	130-094-156	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
FastStart Universal SYBR Green Master	04 913 850 001	Roche Diagnostics GmbH	Mannheim, Germany
FcR Blocking Reagent	130-059-901	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Fixable Aqua Live/Dead Cell Stain Kit	L34957	Life Technologies Corporation	Carlsbad, USA
Fixation/Permeabilization Solution Kit with BD Golgi Plug	555028	Becton Dickinson (BD) GmbH	Heidelberg, Germany
High Capacity cDNA RT Kit	4368814	Life Technologies Corporation	Carlsbad, USA
LDH Cytotoxcitiy Detection Kit	11644793001	Roche Diagnostics GmbH	Mannheim, Germany
Memory $CD4^+$ T Cell Isolation Kit	130-091-893	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Rneasy Mini Kit	74104	Qiagen GmbH	Hilden, Germany
Rneasy Micro Kit	74004	Qiagen GmbH	Hilden, Germany
TaqMan Low Density Arrays (TLDAs)	Custom-made	Life Technologies Corporation	Carlsbad, USA
2xTaqMan Universal PCR Master Mix	4304437	Life Technologies Corporation	Carlsbad, USA

2.1.8. ELISA Reagents

Table 19. ELISA Kit Reagents

ELISA Kit Reagents	Cat. No.	Supplier/Source/Manufacturer	
DuoSet ELISA Human IL-22	DY782	R&D Systems GmbH	Wiesbaden-Nordenstadt, Germany
DuoSet ELISA Human IFN-γ	DY285	R&D Systems GmbH	Wiesbaden-Nordenstadt, Germany
DuoSet ELISA Human IL-17	DY317	R&D Systems GmbH	Wiesbaden-Nordenstadt, Germany
DuoSet ELISA Human TNF-α	DY210	R&D Systems GmbH	Wiesbaden-Nordenstadt, Germany
BD OptEIA™ Set Human IP-10	550926	Becton Dickinson (BD) GmbH	Heidelberg, Germany

Table 20. 20x PBS for ELISA Washing Buffer

20x PBS for ELISA	
KH_2PO_4	4 g
Na_2HPO_4 x $12H_2O$	58 g
KCl	4 g
NaCl	160 g
Adjust to 1l and pH to 7.0	
Washing buffer	
1x PBS add 0.05% tween 20	

Table 21. ELISA Stop-Solution

ELISA Stop-Solution	
H_2O	266 ml
H_2SO_2 (25%)	133 ml

Table 22. TMB Solution

TMB Solution	
TMB	24 mg
ETOH	500 µl
DMSO	500 µl

Table 23. ELISA Citrate Buffer

Citrate	8.41 g
H_2O	200 ml
Adjust pH to 3.95	

Table 24. ELISA Substrate Solution

ELISA Substrate Solution	
Citrate Buffer	5500 µl
H_2O_2	2.55 µl
TMB	55 µl

2.1.9. Expendable Material

Table 25. Expendable Materials

Expendable Materials	Cat. No.	Supplier/Source/Manufacturer	
Plates			
96-well U-bottom Plates	83.1837.500	Sarstedt AG & Co	Nümbrecht, Germany
96-well F-bottom Plates	831835.500	Sarstedt AG & Co	Nümbrecht, Germany
96-well F-bottom Plates NUNC∆SURFACE	167008	Thermo Fisher Scientific GmbH	Dreieich, Germany
96-well NUNC-Immuno Plate (Maxisorb)	439454	Thermo Fisher Scientific GmbH	Dreieich, Germany
48-well Plate	353078	Becton Dickinson (BD) GmbH	Heidelberg, Germany
384-well PCR Plates	4ti-0384/C	4titude® Ltd	Wotton, Surrey, UK
Tips			
TipOne Tips 1 µl - 10 µl	S1121-3810	STARLAB GmbH	Hamburg, Germany
TipOne Tips 1 µl – 200 µl	S1120-8810	STARLAB GmbH	Hamburg, Germany
TipOne Tips 101 µl – 1000 µl	S1126-7810	STARLAB GmbH	Hamburg, Germany
Tips 50 µl -1000 µl	0030000919	Eppendorf AG	Hamburg, Germany
Tips 2 µl -200 µl	0030000870	Eppendorf AG	Hamburg, Germany
Tips 0.5 µl - 10 µl	0030000854	Eppendorf AG	Hamburg, Germany
Tips 2-200 µl	REFF161930	Gilson Internation B.V. Deutschland	Limburg, Germany
Serological pipettes			
25 ml	760 180	Greiner Bio-One GmbH	Frickenhausen, Germany
10 ml	6071 180	Greiner Bio-One GmbH	Frickenhausen, Germany
5 ml	356543	Becton Dickinson (BD) GmbH	Heidelberg, Germany
2 ml	710 180	Greiner Bio-One GmbH	Frickenhausen, Germany
Tubes			
2.0 ml Micro Tubes	72.706.400	Sarstedt AG & Co	Nümbrecht, Germany
1.5 ml Micro Tubes	72.695.406	Sarstedt AG & Co	Nümbrecht, Germany
0.2 ml PCR Tubes	951010006	Eppendorf AG	Hamburg, Germany
15 ml Centrifuge Tubes	352096	Becton Dickinson (BD) GmbH	Heidelberg, Germany
50 ml Centrifuge Tubes	62.547.254	Sarstedt AG & Co	Nümbrecht, Germany
Cell strainer			
Nylon Mesh Cell Strainer 100 (Yellow)	352360	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Nylon Mesh Cell Strainer 40 µm (Blue)	352340	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Nylon Mesh Cell Strainer 70 µm (White)	352350	Becton Dickinson (BD) GmbH	Heidelberg, Germany
AutoMacs and FACS solutions			
AutoMACs Washing Solution	130-092-987	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
AutoMACS Runnig Buffer	130-091-221	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Facs Flow	342003	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Facs Clean	340345	Becton Dickinson (BD) GmbH	Heidelberg, Germany
FACS Rinse	340346	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Additional Material			
Cryotypes, Sterile, 1.8 ml	72379	Sarstedt AG & Co	Nümbrecht, Germany
Cluster Tubes	AB-0672	Life Technologies Corporation	Carlsbad, USA
Cell Culture Flask 250 ml 75 cm (T75)	658170	Greiner Bio-One GmbH	Frickenhausen, Germany
Filtropur 250 ml, 0.2 µm	83.1822.001	Sarstedt AG & Co	Nümbrecht, Germany
Filtropur 500 ml, 0.2 µm	83.1823.001	Sarstedt AG & Co	Nümbrecht, Germany
IsoPlate 96-well Microplate	1450-514	PerkinElmer LAS GmbH	Rodgau, Germany

MeltiLex A	1450-441	PerkinElmer LAS GmbH	Rodgau, Germany
Millex-GP, Sterile Filter, 0.22 µm	SLGP033RS	Merk KGaA	Darmstadt, Germany
MicroAmp™, Optical Adhesive Film, DNA/RNA/RNAse free	4311971	Life Technologies Corporation	Carlsbad, USA
Original -Perfusor® Syringes, 50 ml	872 8810	Braun Melsungen AG	Melsungen, Germany
Printed Filtermates	1450-421	PerkinElmer LAS GmbH	Rodgau, Germany
Polystyrene Round Bottom Tube	352052	Becton Dickinson (BD) GmbH	Heidelberg, Germany
Round Bottom Polystyrene Test Tubes/ 5ml	352052	Becton Dickinson (BD) GmbH	Heidelberg, Germany
RNAse ZAP	R2020-250	Sigma-Aldrich Chemie GmbH	München, Germany
Sealing Tape ELISA/NUNC	236269	Thermo Fisher Scientific GmbH	Dreieich, Germany
Silicone Paper Envelope	1450-467	PerkinElmer LAS GmbH	Rodgau, Germany

2.1.10. Instruments and Software

Table 26. Instruments

Instruments	Supplier/Source/Manufacturer	
Agilent Bioanalyzer	Agilent Technologies GmbH	Waldbronn, Germany
AutoMACS	Miltenyi Biotec GmbH	Bergisch-Gladbach, Germany
Centrifuge 5417R	Eppendorf AG	Hamburg, Germany
Centrifuge Megafuge 1.0R	Thermo Fisher Scientific GmbH	Dreieich, Germany
Centrifuge MC 6	Sarstedt AG & Co	Nümbrecht, Germany
Elisa Washer, TECAN, Hydrospeed	Tecan Deutschland GmbH	Crailsheim, Germany
EPOCH Microplate UV-Vis Spectrophotometer	BioTek Instruments GmbH	Bad Friedrichshall, Germany
FACSCalibur Flow Cytometer	Becton Dickinson (BD) GmbH	Heidelberg, Germany
FluoStar Optima	BMG Labtech GmbH	Ortenberg, Germany
Harvester	PerkinElmer LAS GmbH	Rodgau, Germany
Incubator	Thermo Fisher Scientific GmbH	Dreieich, Germany
LSRFortessa™ Flow Cytometer	Becton Dickinson (BD) GmbH	Heidelberg, Germany
MeltiLexTMA (solid scintillant)	PerkinElmer LAS GmbH	Rodgau, Germany
Microscope, Axiovert 25	Carl Zeiss AG	Oberkochen, Germany
Multichannel pipettes, (2.5 – 25 µl, 20 -200 µl, 30-300 µl)	Brand GmbH & Co. KG	Wertheim, Germany
Nanodrop Spectrophotometer ND-1000	PeqLab Biotechnologie GmbH	Erlangen, Germany
PCR cycler, TC-412 Techne	Tecan Deutschland GmbH	Crailsheim, Germany
Reference Pipettes (0.1 µl -2.5 µl, 1 µl-10 µl, 10µl – 100 µl, 100 µl – 100 µl)	Eppendorf AG	Hamburg, Germany
Research Pipettes (1 µl-10 µl, 10µl – 100 µl, 100 µl – 100 µl)	Eppendorf AG	Hamburg, Germany
Thermomixer 5437	Eppendorf AG	Hamburg, Germany
Viaa7 PCR cycler	Life Technologies Corporation	Carlsbad, USA
HT7900 PCR Cycler	Applied Biosystems	Waltham, USA
Water bath, Julabo SW22,	Julabo Labortechnik GmbH	Seelbach, Germany

Table 27. Software and Databases

Software And Databases	Supplier/Source/Manufacturer	
BD FACSDiva™ Software, Version 7 and 8	Becton Dickinson (BD) GmbH	Heidelberg, Germany
CellQuest Pro Software	Becton Dickinson (BD) GmbH	Heidelberg, Germany
EndNote Version X2	Thompson Reuters	New York, USA
GraphPad PRISM 6	GraphPad Software Inc.	La Jolla, USA
HT7900 Software	Life Technologies Corporation	Carlsbad, USA
Microsoft Office	Microsoft Corporations	Redmond, USA
NCBI PubMed Database	National Center for Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov/pubmed
NCBI Gene Database	National Center for Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov/gene
NCBI Nucleotide Database	National Center for Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov/nuccore
qPrimerDepot	National Cancer Institute (NCI)	http://primerdepot.nci.nih.gov/
R version 3.0.0	The R foundation for statistical computing	http://www.R-project.org/
TaqMan Gene Expression Database	Life Technologies Corporation	http://www.lifetechnologies.com/de/de/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/taqman-gene-expression.html
Universal ProbeLibrary Assay Design Center	Roche Diagnostics GmbH	http://lifescience.roche.com
Viia7 Software	Life Technologies Corporation	Carlsbad, USA

2.2. Methods

2.2.1. General conditions for cell culture experiments

All cell culture experiments were carried out under sterile conditions with disposable pipettes.
Human serum was added to the cell culture medium after sterile filtration. Different adherent cell lines were cultured until they reached a confluence 70-80%. Cells in DMEM were cultured at 37 °C with 6.5% CO_2. Cells cultured in RPMI medium were cultured at 37 °C with 5% CO_2. For long-term storage, cells were resuspended in freezing medium, stored over night at -80 °C and they were frozen in liquid nitrogen (N_2).

2.2.2. Stock concentrations and storage of chemicals

6-Formylindolo[3,2-*b*]carbazole (FICZ) [10 mM], 1-(1-propynyl)-pyrene (1-PP) [100 mM], CH-223191 [60 mM] were solved in dimethyl sulfoxide (DMSO) and stored at -20 °C. 1-PP was synthesised by Prof Dr Albrecht Seidel (Biochemical Institue for Environmental Carcinogens (BIU)). 1-Aminobenzotriazole (1-ABT) [186.4 mM] was solved in aqua injectabila (Ampuwa) and stored at -20 °C. 7-Ethoxyresorufin [4 mM] was solved in methanol and stored at -20 °C.

2.2.3. Characteristics of healthy subjects and CMCD patients

Non-atopic, healthy volonteers with total IgE levels < 100 kU/l and specific IgE levels < 0.34 kU/l were included in this study. Specific IgE against at least nine common antigens and total IgE were determined in donor´s serum with ELISA-based Immuno-CAP technology at the Allergy Department of the Clinic for Dermatology and Allergology at the Technical University of Munich. Characteristics of donors used for TaqMan Low Density arrays are summarised in Table 31 to Table 36. Human primary forskin mast cells were obtained after circumcision from boys aged between 1-15 years. PBMCs from CMCD patients with *STAT1* GOF mutations were kindly provided by Prof Dr Claudia Traidl-Hoffmann (Institute of Environmental Medicine (UNIKA-T), Technical University of Munich). All experiments with PBMCs from CMCD patients were done in co-operation with Dr Julia Hiller (Institute of Environmental Medicine (UNIKA-T)). These experiments include one female patient with the amino acid exchange on position R274Q (CCD, coiled-coil domain) and one male subject with P329L (DBD, DNA-binding domain) exchange in the STAT1 protein. The genetic background of the other test persons was unknown.

2.2.4. CYP1 inhibition in V79 Chinese hamster cell lines by 1-PP or 1-ABT

Human CYP1 cell lines were generated as described previously (Schober et al., 2006; Schober et al., 2010). Briefly, V79 Chinese hamster cell lines were stable transfected with pIRES vectors containing human CYP1A1-, CYP1A2- or CYP1B1-coding cDNAs for recombinant expression. CYP1 cell lines were cultured with a density of $1x10^5$ cells/ml in 200 µl V79 growth medium in a sterile flat-bottom 96-well (NuncΔSurface) plate for 2 days. For treatment, the selective CYP1 inhibitor 1-(1-propynyl)-pyrene (1-PP) was serially diluted in a stock dilution in DMSO and further diluted in culture medium to final concentrations of 10^{-5} M to 10^{-10} M. The second CYP inhibitor 1-aminobenzotriazole (1-ABT) was diluted in medium to final concentrations of 10^{-3} M to 10^{-7} M.
On the second day, medium was removed and V79 cells were incubated with 200 µl 1-PP or 1-ABT for 30 min with the indicated concentrations. Thereafter, cells were washed twice with D-PBS w/o Ca^{2+}/Mg^{2+} and the activity of recombinant human CYP1A1, CYP1A2, CYP1B1 enzymes was analysed by addition of 100 µl 7-ethoxyresorufin [10^{-6} M] (EROD assay) in sodium phosphate buffer, pH 8, for 15 min at 37 °C as described before (Wincent et al., 2012). Methanol in sodium phospate buffer was used as control. Formation of resorufin was measured in 50 µl supernatant stopped with 50 µl ice-cold methanol in a 96-well NUNC-Immuno plate. Resorufin was quantified by the FluoStar Optima Multiplate Reader with excitiation/emission wavelengths of 544/590 nm. The percentage of inhibition was calculated relative to medium-only control and IC_{50} was determined in four independent experiments of each human CYP1 cell line.

2.2.5. Isolation of human peripheral blood mononuclear cells (PBMCs)

Human peripheral blood mononuclear cells (PBMCs) from healthy subjects and CMCD patients were isolated by density gradient centrifugation. 25 ml whole blood was diluted 1:2 in D-PBS w/o Ca^{2+}/Mg^{2+}. Whole blood was stratified on 15 ml lymphoprep with a density of 1.077 g/ml in a 50 ml tube. Cells were centrifuged at 1011 x g for 15 min on room temperature (RT) without brake. Cells were collected and transferred into a new 50 ml collection tube. PBMCs were washed three times with D-PBS w/o Ca^{2+}/Mg^{2+} supplemented with 5 mM EDTA. Isolated PBMCs were either used for immune cell purifications or stored in cryotubes with a density of 27.7 x 10^6 cells/ml in freezing media in liquid nitrogen.

2.2.6. Cell culture of PBMCs

Previously frozen PBMCs were washed with proliferation medium by centrifugation at 300 x g for 10 min at RT. Cells were cultured in 96-well flat-bottom plate for suspension cells (Sarstedt). Plates had been pre-coated with 1 µg/ml mouse anti-human CD3 for 2 h at 37 °C. Proliferation medium was supplemented with anti-CD28 to a final concentration of 1 µg/ml in all experiments. Cells were cultured with a density of 1.5 x 10^6 cells/ml in a final volume of 200 µl. Anti-CD3 and anti-CD28 treatment mimics T cell receptor activation and expands T cells.

2.2.7. Definition of appropriate FICZ and 1-PP concentrations for PBMCs

Appropriate concentrations of the CYP1 inhibitor 1-PP and the AHR agonist FICZ for IL-22 and c-Kit induction in PBMCs were determined. The chemicals were serially diluted and used in final concentrations of 10^{-5} M to 10^{-10} M. Activated PBMCs were treated for 48 h. IL-22 release was determined by enzyme-linked immunosorbent assay (ELISA) and c-Kit expression was analysed by flow cytometry (fluorescence-activated cell sorting analysis (FACS)).

2.2.8. Titration of CYP1 inhibitor 1-PP with low-dose FICZ in PBMCs

PBMCs from eight healthy subjects were treated with increasing concentrations of the CYP1 inhibitor 1-PP (10^{-5} M to 10^{-10} M) alone or in presence of a low concentration of the AHR agonist FICZ (5 x 10^{-10} M, low-dose) for 48 h. The CYP1 inhibitor 1-PP was serially diluted in a DMSO stock dilution and further diluted with the same dilution factor in proliferation medium. Control treatments include medium, DMSO, FICZ alone and FICZ with additional DMSO. Cytokine secretion, c-Kit expression, viability and proliferation were determined after 48 h with flow cytometry, ELISA, lactat dehydrogenase activity (LDH) and ^{3}H-thymidine assay.

2.2.9. Treatment of PMBCs with FICZ, 1-PP and the AHR antagonist CH-223191

For studying CYP1 inhibition and AHR-dependent effects, human anti-CD3/CD28 activated PBMCs from seven different healthy subjects were treated with the CYP1 inhibitor 1-PP in final concentrations of 10^{-7} M or 10^{-6} M for 5 days. The AHR agonist FICZ was diluted to a final concentration of 5 x 10^{-10} M and the AHR inhibitor CH-223191 was used with a final concentration of 3 x 10^{-6} M. Cells were treated with FICZ alone, 1-PP alone, 1-PP in the presence of FICZ and 1-PP, FICZ and CH-223191. Controls include medium and DMSO. DMSO concentration was adjusted to the same concentration in each treatment. In control experiments, a high FICZ concentration (10^{-7} M) was included. PBMCs were treated with FICZ alone or together with AHR inhibitor CH-223191. After 5 days, cells and supernatants were harvested. Cytokine concentrations, c-Kit expression and the

transcription of genes involved in the AHR pathway were analysed by flow cytometry, ELISA and ribonucleic acid (RNA) measurements. Viability and proliferation were determined by LDH and ^{3}H-thymidine assay.

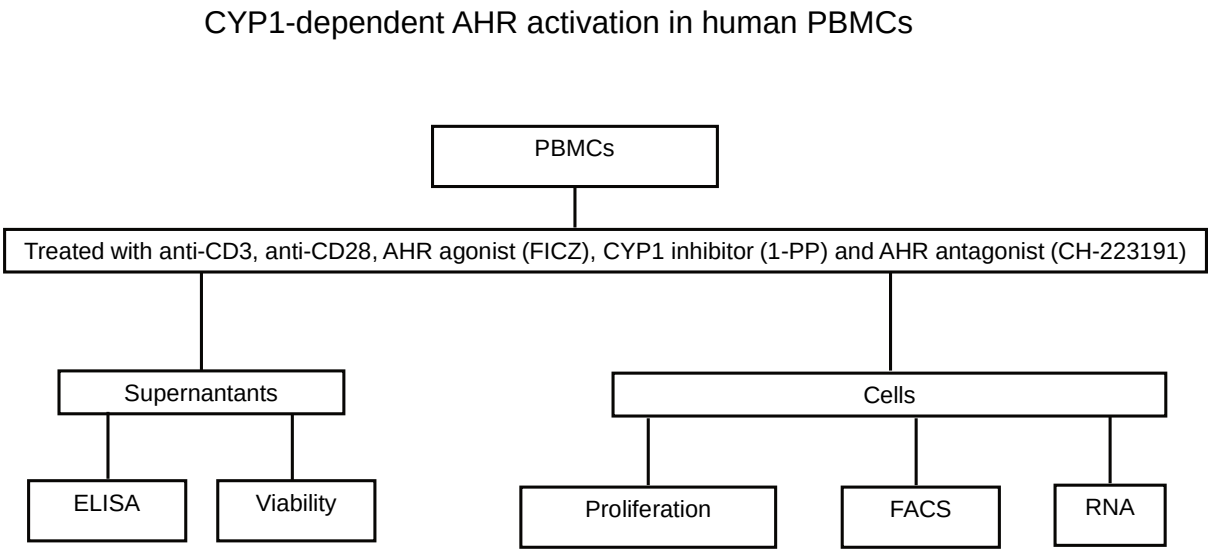


Figure 9. Experimental overview of PBMC treatment for 5 days

2.2.10. Surface receptor staining for FACS analysis at 48 h

PBMCs were treated for 48 h and stained with mouse anti-human CD3-FITC (Okt3) and mouse anti-human CD117-PE to discriminate c-Kit expression on T cells ($CD3^{+}$) and non-T cells ($CD3^{-}$). Fixable Aqua Live/Dead Cell Stain Kit was used for staining defect cells with permable cell membranes. This staining dye binds to free amine groups and could be used with fixed cells in intracellular stainings. Cells were collected and transferred to a 96-well U-bottom plate (Sarstedt). Cells were washed twice in D-PBS w/o Ca^{2+}/Mg^{2+}. Dead cells were stained with 100 µl Aqua Live/Dead solution diluted 1:1000 in D-PBS w/o Ca^{2+}/Mg^{2+}. After incubation for 30 min at 4 °C cells were washed twice with D-PBS w/o Ca^{2+}/Mg^{2+}. Surface staining reagent with mouse anti-human CD3-FITC, mouse anti-human CD117-PE and FcBlock was prepared in FACS buffer. PBMC were stained for 30 min at 4 °C in 20 µl surface staining solutions.

2.2.11. Surface repector and intracellular cytokine staining for FACS analysis at day five

After 5 days, PBMCs were collected and activated with phorbol 12-myristate-13-acetate (PMA) (50 ng/µl) and ionomycin (1 µg/ml) in the presence of 1x monensin. GolgiPlug solution was added after 2 h at concentrations recommended by the manufacturer. PMA mimics intracellular signalling pathways and ionomycin additionally increases intracellular Ca^{2+} levels. Both molecules boost activation of cells and the production of intracellular cytokines. GolgiPlug solution contains brefeldin A that inhibits

together with monensin the vesicular transport from the endoplasmatic reticulum to the cell membrane. Treatment of cells with these inhibitors stops the secretion of cytokines. After 5 h activation, Aqua Live/Dead Cell staining, and surface receptor staining were performed as described previously. For surface receptor staining, 40 µl staining solution with mouse anti-human CD3-FITC (Okt3), mouse anti-human CD4-APC-Cy7 (RPA-T4), mouse anti-human CD8-Pacific Blue (RPA-T8), mouse anti-human CD117-PE, mouse anti-human CD56-PE-Vio770 (AF12-7H3) and FcR Blocking Reagent were used. After surface staining, cells were washed twice with 100 µl FACS buffer and Cytofix/Cytoperm™ Plus was used for intracellular staining with mouse anti-human IL-22. Anti-human IL-22 had been labelled with Alexa Fluor (AF) 647 by using the Microscale Pro Kit. Anti-IL-22-AF 647 in 40 µl permeabilization buffer was used for intracellular staining. c-Kit and IL-22 in different immune cell populations were examined by flow cytometry with the LSR Fortessa™ Flow Cytometer at appropriate wavelengths. Data were analysed with FACSDiva software.

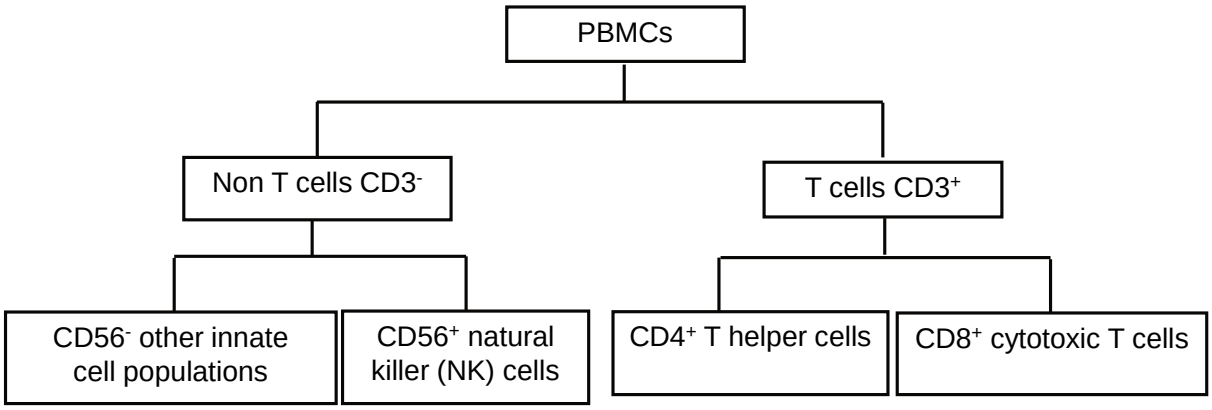


Figure 10. Selection of CD3$^+$ and CD3$^-$ cells by FACS gating

2.2.12. RNA isolation and cDNA synthesis from human PBMCs

For RNA extraction after 5 days, PBMCs were washed with D-PBS w/o Ca^{2+}/Mg^{2+} and collected by centrifugation at 153 x g for 7 min on RT. Cells were lysed in 350 µl RLT buffer supplemented with 1% 2-mercaptoethanol. Lysats were fast frozen in liquid nitrogen and stored at -80 °C until RNA isolation. RNA from treated PBMCs was isolated with Qiagen RNeasy Mini Kit with on-column DNase digestion according to the manufacturer´s protocol. RNA was stored at -80 °C previous to cDNA synthesis. RNA concentrations were determined by the Nanodrop UV/Vis spectrophotometer. Up to 1 µg RNA was reverse transcribed into cDNA with the High Capacity cDNA Reverse Transcription (RT) Kit according to the manufacturer´s protocol.

2.2.13. Quantitative real-time PCR (qRT-PCR) of AHR-regulated transcripts in human PBMCs

Relative transcription of target genes related to the AHR pathway (Cytochrome P450 (*CYP*)*1A1*, *CYP1B1*, *CYP2S1*, NAD(P)H dehydrogenase quinone 1 (*NQO1*), protaglandin-endoperoxide synthase 2 (*PTGS2*), ATP-binding cassette subfamily G member 2 (*ABCG2*)), of transcription factors (aryl hydrocarbon receptor (*AHR*), AHR nuclear translocator (*ARNT*), Nuclear Factor erythroid 2-related factor 2 (*NRF2*), hypoxia-inducible factor 1α (*HIF1A*)), of cytokines (interleukin (*IL*)*22*, *IL17A*, *IL26*, interferon-gamma (*IFNγ*)), of the stem cell factor receptor (*KIT*), and of the elongation factor 1 alpha (*EF1A*) was analysed after 5 days with FastStart Universal SYBR Green Master (ROX). qPCR primers were dissolved in H_2O at a 100 mM stock concentration and diluted to a 4 mM primer mix. cDNA was analysed in 10 µl reactions. qPCR reactions were run in 384-well plates at the Viaa7 PCR cycler with the standard cycler programme.

Table 28. Master mix for qPCR

	Primer master mix
3.4 µl	RNA [2.3 ng/µl]
5 µl	FastStart Universal SYBR Green Master
1.6 µl	Primer mix (4 mM)

Relative target gene transcription was calculated by the $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001). Ct-values were normalised to the housekeeping gene *EF1A* (ΔC_t) and relative expression ($\Delta\Delta C_t$) was calculated to the medium-only control. Data were plotted by PrismGraph software and trends in regulation were analysed by Wilcoxon signed-rank test. Fold changes were calculated as indicated.

2.2.14. Analysis of viability by lactate dehydrogenase (LDH) assay

To measure cytotoxic reactions in immune cells, supernatants of cultured PBMCs were tested for lactate dehydrogenase (LDH) activity. LDH is a cytosolic enzyme that is released into the supernatant when cells are dead or their membrane integrity is damaged (Decker and Lohmann-Matthes, 1988). LDH was quantified in the supernatants by colorimetric measurements with the EPOCH reader. To calculate viability/toxicity, medium control was set to 100%.

2.2.15. Cell proliferation with ^{3}H-thymidine assay

Proliferation of treated PBMCs was tested by adding ^{3}H-thymidine to a final concentration of 1 nCi/ml into the supernatants. Cells were pulsed for 6 h at 37 °C with 5% CO_2. Radiolabelled thymidine incorporates into the deoxyribonucleic acid (DNA) of dividing cells. Cells were harvested on printed filters. Filters were sealed with a scintillation film and radiolabelled DNA was measured with a scintillation beta-counter. Detected signals (counts per minute (cpm)) were directly proportional to the DNA synthesis.

2.2.16. Determination of cytokine concentrations by Enzyme-Linked Immunosorbent Assay (ELISA)

Commercially available sandwich ELISA Kits were used for determining IL-22, IL-17, IFN-γ, IP-10 and TNF-α concentrations in the supernantants of treated human PBMCs. Cytokine concentrations were determined with EPOCH microplate spectrophotometer according to the supplied protocol.

2.2.17. Treatment of PBMCs from CMCD patients

Isolated PBMCs from CMCD patients were treated as previously described in 2.2.9.. PBMCs were treated with a high FICZ concentration (high-dose, 10^{-7} M) or with 1-PP (10^{-6} M) in the presence of a low FICZ concentration (low-dose, 5 x 10^{-10} M). The expriments with a high FICZ concentration were included as a positive control for AHR activation. The AHR antagonist CH-223191 (3 x 10^{-6} M) was used to inhibit AHR activity. Cells and supernatants were collected for analysing CYP, cytokine and c-Kit expression levels by flow cytometry, ELISA and RNA measurements. Proliferation was measured by ^{3}H-thymidine assay as described previously.

2.2.18. Isolation of splenocytes from *Cyp1a2* knockout or C57BL/6 mice

Male *Cyp1a2* knockout or C57BL/6 wild type mice were killed by cervical fracture at the age of 12-14 weeks. Isolated spleens were disrupted through a 100 µm cell strainer with the pistil of a 3 ml syringe and washed three times with medium. Cell suspensions were centrifuged at 300 x g for 10 min at 4 °C. Erythrocytes were lysed in 10 ml 1x lysis buffer (diluted in aqua injectabila) for 6 min and 20 ml medium were added for stopping the reaction. To obtain a single cell suspension, splenocytes were washed with culture medium, centrifuged with 300 x g at 4 °C for 10 min, resuspended in culture medium and again filtered through a 40 µm cell strainer.

2.2.19. Treatment of murine splenocytes from *Cyp1a2* knockout or C57BL/6 mice

Collected single cell suspensions of splenocytes from *Cyp1a2* knockout and C57BL/6 wild type mice were cultured with a density of 1.5 x 10^6 cells/ml in a final volume of 200 µl in flat-bottom 96-well plates (NUNCΔsurface) in murine proliferation medium. Plates were pre-coated with hamster anti-mouse CD3 (1 µg/ml) for 3 h at 37 °C. Murine proliferation medium was supplemented with hamster anti-mouse CD28 (2 µg/ml). The AHR agonist FICZ was diluted to a final concentration of 5 x 10^{-10} M and the AHR inhibitor CH-223191 was used in a final concentration of 3 x 10^{-6} M. Cells were treated with FICZ alone or FICZ and CH-223191 for 72 h at 37 °C and 5% CO_2. Intracellular and surface receptor staining of IL-22 in $CD4^+$ and $CD8^+$ splenocytes was performed as described in 2.2.11 with following antibodies; anti-mouse CD4-PE, anti-mouse CD8-PE-Vio770, anti-mouse IFN-γ-AF 488 and anti-IL-22-AF 647. Dead cells were discriminated by Fixable Aqua Live/Dead Cell Stain Kit.

2.2.20. Purification of immune cell subtypes from PBMCs

Various immune cell subtypes were isolated from freshly purified PBMCs from at least seven different, healthy subjects with Miltenyi´s MicroBead technology. Human primary basophils were purified with the Basophil Isolation Kit II as described previously (Effner, 2008). $CD14^+$ cells (monocytes) were isolated using $CD14^+$ MicroBeads. Human $CD4^+CD45RO^+CD45RA^-$ T cells (memory T helper cells) were isolated using the Memory $CD4^+$ T Cell Isolation Kit and CD45RA MicroBeads. B cells were purified using the B Cell Isolation Kit II, $CD8^+$ T cells (cytotoxic T cells (Tc)) were isolated with the $CD8^+$ T Cell Isolation Kit and $CD4^+$ T cells (T helper (Th)) with $CD4^+$ T Cell Isolation Kit II. For purification the automated procedure with autoMACS seperator was used. RNA was immediately extracted from isolated cells to prevent RNA degradation.

2.2.21. Purification of human primary foreskin mast cells

Mast cells from human foreskins were isolated as described previously (Chen et al., 2010; Effner, 2008). Briefly, forskins were cut in 3 mm square pieces with a scalpel. Dermis and epidermis were enzymatically separated over night with 0.05% (w/v) thermolysin type X protease in thermolysin buffer at 4 °C. Next day, epidermis was removed and the dermis was digested and cut in RPMI containing 10% FCS, 0.1% (w/v) collagenase, 0.1% (w/v) hyaluronidase and 0.05% (w/v) DNase at 25 °C in a water bath shaker for 3-4 h. Cell suspensions were filtered through 100 nm and 40 nm cell strainer. Human primary $CD117^+$ (c-Kit^+) foreskin mast cells were enriched with anti-$CD117^+$ MicroBeads. RNA was purified immediately after cell isolation.

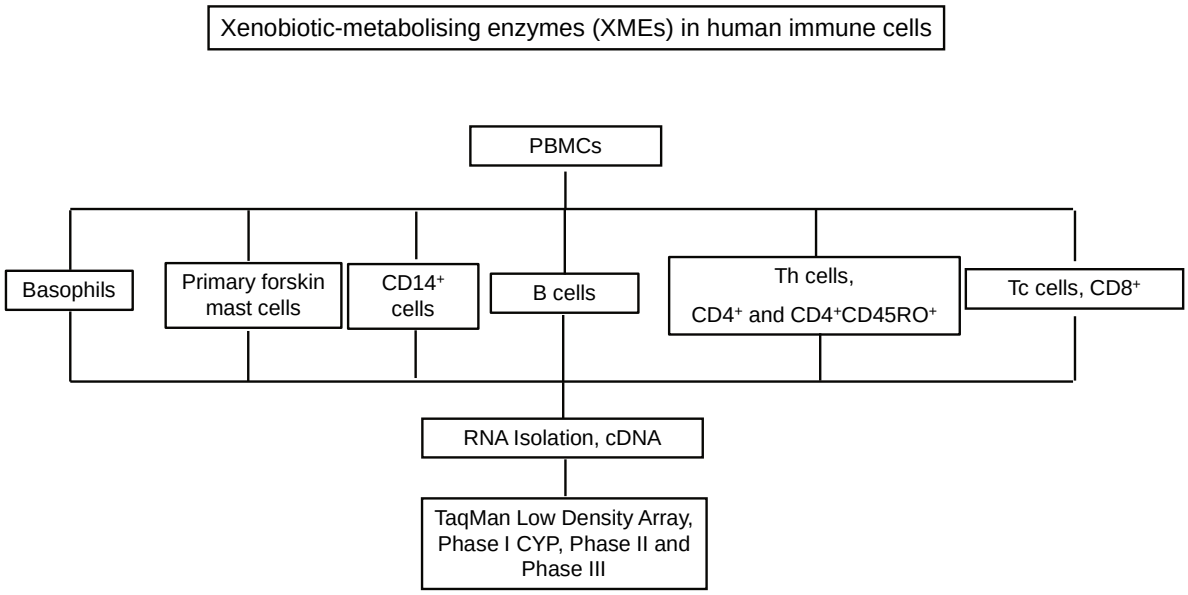


Figure 11. Experimental procedure for characterising xenobiotic-metabolising enzymes in human immune cells

2.2.22. Characterisation of immune cell purity by surface receptor FACS staining

Purity of different immune cell subpopulations was determined by flow cytometry as follows: $CD14^+$ monocytes, B cells, $CD4^+$ Th cells, $CD4^+CD45RO^+CD45RA^-$ memory Th cells and $CD8^+$ Tc cells were characterised with mouse anti-human CD14-FITC (61D3), mouse anti-human CD45RO-FITC (UCHL1), mouse anti-human CD45RA-PE (HI100), mouse anti-human CD40-FITC (5C3), mouse anti-human CD19-PE (HIB19), mouse anti-human CD8-PE (HIT8a) and mouse anti-human CD4-FITC (RPA-T4), respectively. Human primary foreskin mast cells were analysed by mouse anti-human CD117-PE (c-Kit, A3C6E2), and basophils with mouse anti-human CD123-PE (AC145) and mouse anti-human CD303-FITC (AC144). FITC and PE labelled isotypes were used as controls. Data were analysed by FACS Calibur and Cell Quest Pro software.

2.2.23. RNA isolation and quality control with Agilent Lab on a Chip Technology

RNeasy Kits with on-column DNase digestion were used to isolate RNA from immune cell subtypes. RNA was immediately isolated after cell purification. RNA concentration and quality were determined by UV/Vis spectrophotometer and by Agilent Lab-on-a-Chip technology. For gene expression analysis, a sufficient high RNA quality is necessary. RNA quality, as for TaqMan Low Density Arrays, was determined with micro-capillary gel electrophoresis by the RNA 6000 nano assay and by the Agilent

Bioanalyzer 2100 according to the manufacturer´s protocol. In only 1 µl RNA, this miniaturised laboratory measures RNA degradation and concentration. In gel-loaded microchannels, RNA is separated according its molecular weight and a fluorescent dye bound to RNA is laser excited. The detected fluorescent intensity is converted to a RNA concentration. RNA degradations and contaminations with genomic DNA were additionally analysed. RNA integrity was calculated as RNA Integrity Number (RIN). Data were plotted in an electropherogramm with the peaks of the 18S and the 28S ribosomal RNA. Low RIN values determine a high degradation whereas and a 28S/18S ratio of 2:1 and RIN values >8 depict RNA with a high integrity (Schroeder et al., 2006). Data were analysed by Agilent Technologies 2100 Expert Bioanalyzer Version B.02.03.SI307.

2.2.24. TaqMan Low Density Arrays (TLDAs)

The High Capacity cDNA RT Kit was used for reverse transcription of all RNA samples according to the manufacturer´s protocol. cDNAs from different human immune cells were screened in TaqMan Universal PCR Master Mix according to the manufacturer´s protocol on custom-made microfluidic TaqMan Low Density Arrays (TLDAs). TLDAs were run on Viaa7 or on HT7900 PCR cycler. TaqMan assays for custom-made TLDAs were selected by the assay platform of Life Technologies Corp and are summarised in Table 15. Ct-values were normalised by *GAPDH* and related to *HPRT1* as $\Delta\Delta C_t$ value for each donor and cell type. Log_2-transformed relative expression values were plotted by the heatmap2 function of the R programming environment (RCoreTeam, 2013).

2.2.25. Statistical analyses

The Wilcoxon signed-rank test for paired samples was used to compare different treatments. Spearman´s rank correlation coefficients were used to analyse relations in target gene expression. P-values < 0.05 were considered as significant (*), **: $P < 0.01$ and ***: $P < 0.001$. Statistical analysis was performed using Graph Pad Prism 6, San Diego. Heatmaps of correlation coefficients and p-values were blotted with the R programming environment. Log_2-transformed relative transcription of genes coding for xenobiotic-metabolising enzymes in different human immune cell subpopulations was plotted by the heatmap2 function of the statistical software R (RCoreTeam, 2013).

3 Results

3.1. Inhibition of CYP1 activity

Before using CYP inactivators in primary human cell cultures, two CYP inhibitors were tested in a stable cDNA-directed CYP expression system. The Chinese hamster fibroblast cell line V79 was used for recombinant expression of various human CYP1 enzymes as described previously (Schober et al., 2006; Schober et al., 2010). 1-PP is a selective inhibitor of CYP1 family enzymes and particularly inhibits CYP1A1 (Shimada et al., 1998; Zhu et al., 2011b) whereas 1-ABT is a general and non-selective CYP inactivator (Emoto et al., 2003; Linder et al., 2009).

3.1.1. Human CYP1 enzymes were selectively inhibited by 1-PP

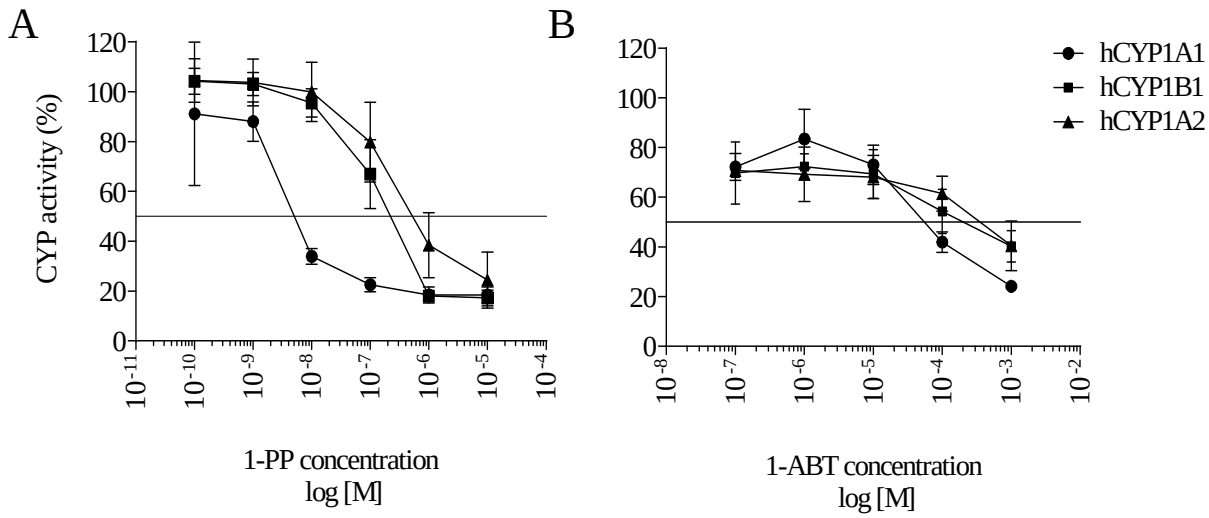


Figure 12. Inhibition of human CYP1 activity in V79 Chinese hamster cells
V79 Chinese hamster fibroblast CYP1 cell lines stable express human CYP1A1, CYP1A2 or CYP1B1 enzymes. CYP1 cell lines were pre-treated with **A**. 1-PP (10^{-10} M – 10^{-5} M) or **B**. 1-ABT (10^{-7} M – 10^{-3} M) for 30 min at 37°C. CYP1 activity was measured by incubation with the CYP substrate ethoxyresorufin (EROD) for 15 min at 37 °C. Formation of the fluorescent product resorufin was measured in triplicates in four independent experiments (n=4). Line shows 50% of CYP1 activity (IC_{50}) relative to vehicle control (100%). Means and standard deviations (s.d.) are shown.

1-PP decreased the activity of human CYP1 enzymes assayed as ethoxyresorufin deethylase (EROD) activity in a concentration-dependent manner. CYP1A1 activity was inhibited by low 1-PP concentrations (IC_{50}=5 nM), whereas CYP1A2 and CYP1B1 activities were only reduced at higher concentrations (IC_{50}=650 nM and IC_{50}=218 nM), respectively. 1-PP acts as a suicide inhibitor of CYP1A1, as a competitive inhibitor of CYP1A2 and it is considered to be metabolised by CYP1B1

(Shimada et al., 2007; Shimada et al., 1998). CYP1A1 inhibition with 1-PP was 129-fold and 43-fold more efficient compared with CYP1A2 and CYP1B1, respectively. Neither a selective nor a complete CYP1 inhibition could be detected in 1-ABT-treated cells. Therefore, 1-PP was used for abrogating CYP1 activity in human PBMCs.

3.2. CYP1-induced AHR activation in human PBMCs

3.2.1. Effects of 1-PP and FICZ on c-Kit and IL-22 expressions in PBMCs

In order to define appropiate FICZ and 1-PP concentrations for IL-22 and c-Kit inductions, increasing concentrations of FICZ and 1-PP (10^{-10} M to 10^{-5} M) were tested in human activated PBMCs for 48 h.

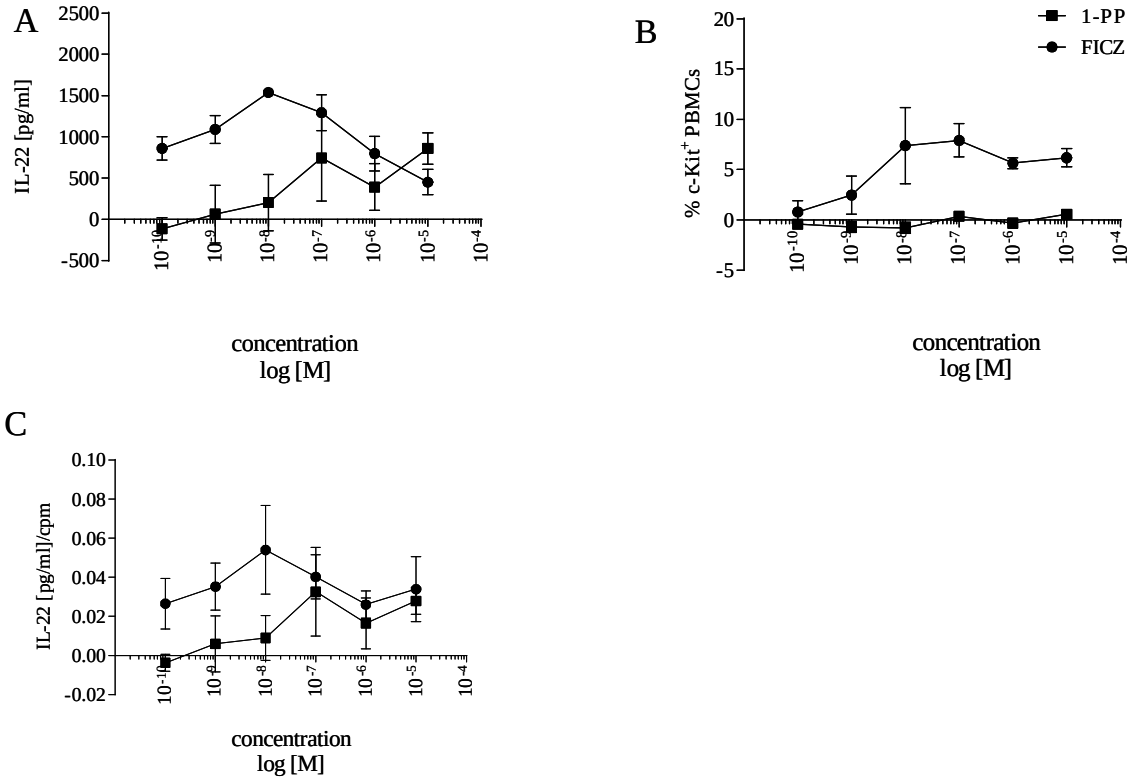


Figure 13. Induction of IL-22 and c-Kit with FICZ and 1-PP in human PBMCs

PBMCs (3 x 10^5 cells/well) were activated with anti-CD3/CD28, and treated with increasing concentrations of the AHR agonist FICZ or the CYP1 inhibitor 1-PP for 48 h. **A**. IL-22 induction by 1-PP or FICZ was determined by ELISA (n=3). DMSO controls were subtracted from treated cells. **B**. The percentage of c-Kit$^+$ PBMCs after treatment with increasing FICZ or 1-PP concentrations was determined by flow cytometry (n=2) **C**. IL-22 release normalised to proliferation. Means +/- s.e.m are shown.

Both the AHR agonist FICZ and the CYP1 inhibitor 1-PP up-regulated the cytokine IL-22 and the surface receptor c-Kit in human PBMCs (Figure 13, A and B). IL-22 was also induced by anti-CD3/CD28 activation alone in the vehicle control. To emphasise the induction by either FICZ or 1-PP, the values in the DMSO control were subtracted from values in FICZ or 1-PP treatments. IL-22 was induced in a range from 10^{-10} M to 10^{-8} M FICZ. IL-22 was down-regulated at higher FICZ concentrations ranged from 10^{-8} M to 10^{-5} M (Figure 13 A and C). IL-22 was induced by already a very low concentration of FICZ (10^{-10} M). Normalisation to proliferation was still showing a decreasing IL-22 release from 10^{-8} M to 10^{-5} M FICZ (Figure 13, C). In comparison to that, the CYP1 inhibitor 1-PP continuously increased IL-22. Similar to IL-22 expression, FICZ linearly increased c-Kit expression in the range from 10^{-10} M to 10^{-8} M. The concentration of 10^{-10} M FICZ did not induce c-Kit expression, and concentrations higher than 10^{-8} M did not result in an up-regulation of c-Kit. The CYP1 inhibitor 1-PP alone showed a weak concentration-dependent induction of c-Kit. The effects of 1-PP could be due to amplification of FICZ-like substances present in normal medium. Culture medium contains tryptophan that polymerises by light. Thus, normal culture media could contain a FICZ-like activity (for details see discussion). As IL-22 and c-Kit expression peaked at 10^{-8} M FICZ, a concentration of 5 x 10^{-10} M FICZ was choosen for further experiments in order to analyse a 1-PP-dependent up-regulation of IL-22 and c-Kit in the presence of FICZ.

3.2.2. Combination of 1-PP and FICZ induced c-Kit and IL-22 in PBMCs

Th22 cells produce the cytokines IL-22 and TNF-α, and TNF-α in combination with IL-6 additionally induce Th22 differentiation (Duhen et al., 2009; Eyerich et al., 2009; Trifari et al., 2009). IL-22 and c-Kit are known immunological targets of the AHR pathway (Kadow et al., 2011; Kiss et al., 2011; Veldhoen et al., 2008). It was tested whether anti-CD3/CD28-activated PBMCs regulated IL-22, TNF-α and c-Kit after CYP1 inhibition in the presence of a low FICZ concentration. CYP1-mediated effects were investigated with increasing concentrations of 1-PP (10^{-10} M to 10^{-5} M) in the presence of 5 x 10^{-10} M FICZ. To set the same baseline raw data were normalised by subtraction of the FICZ/DMSO control from FICZ/1-PP co-treatments, and DMSO from 1-PP measurements. Treatment of activated PBMCs with FICZ alone significantly induced IL-22 and TNF-α expression levels (Figure 14, A and B, controls). In the single treatments with a low FICZ concentration, c-Kit was significantly induced on $CD3^-$ PBMCs (Figure 14, controls C) but not on $CD3^+$ PBMCs (Figure 14, controls D). Increasing 1-PP concentrations combined with a low dose of FICZ induced IL-22 (Figure 14, A, normalised data) and c-Kit (Figure 14, C and D, normalised data) concentration-dependently, but not TNF-α (Figure 14, B). As indicated in preliminary experiments, single incubations with 1-PP alone showed a linear and significant induction of IL-22 and c-Kit (Figure 14, A,C,D, normalised data). Representative dot plots are shown in Figure 14, E.

In conclusion, an augmented CYP1 inhibition by increasing concentrations of 1-PP in the presence of a low FICZ concentration induced IL-22 and c-Kit, but not TNF-α expression in 48 h treatments. However, treatment with FICZ alone (5 x 10^{-10} M) increased IL-22 and TNF-α expression levels and weakly induced c-Kit expression on $CD3^-$ PBMCs but not on $CD3^+$ PBMCs. For analysing the AHR-dependent regulation of IL-22 and c-Kit, 10^{-6} M and 10^{-7} M 1-PP were used in the following experiments.

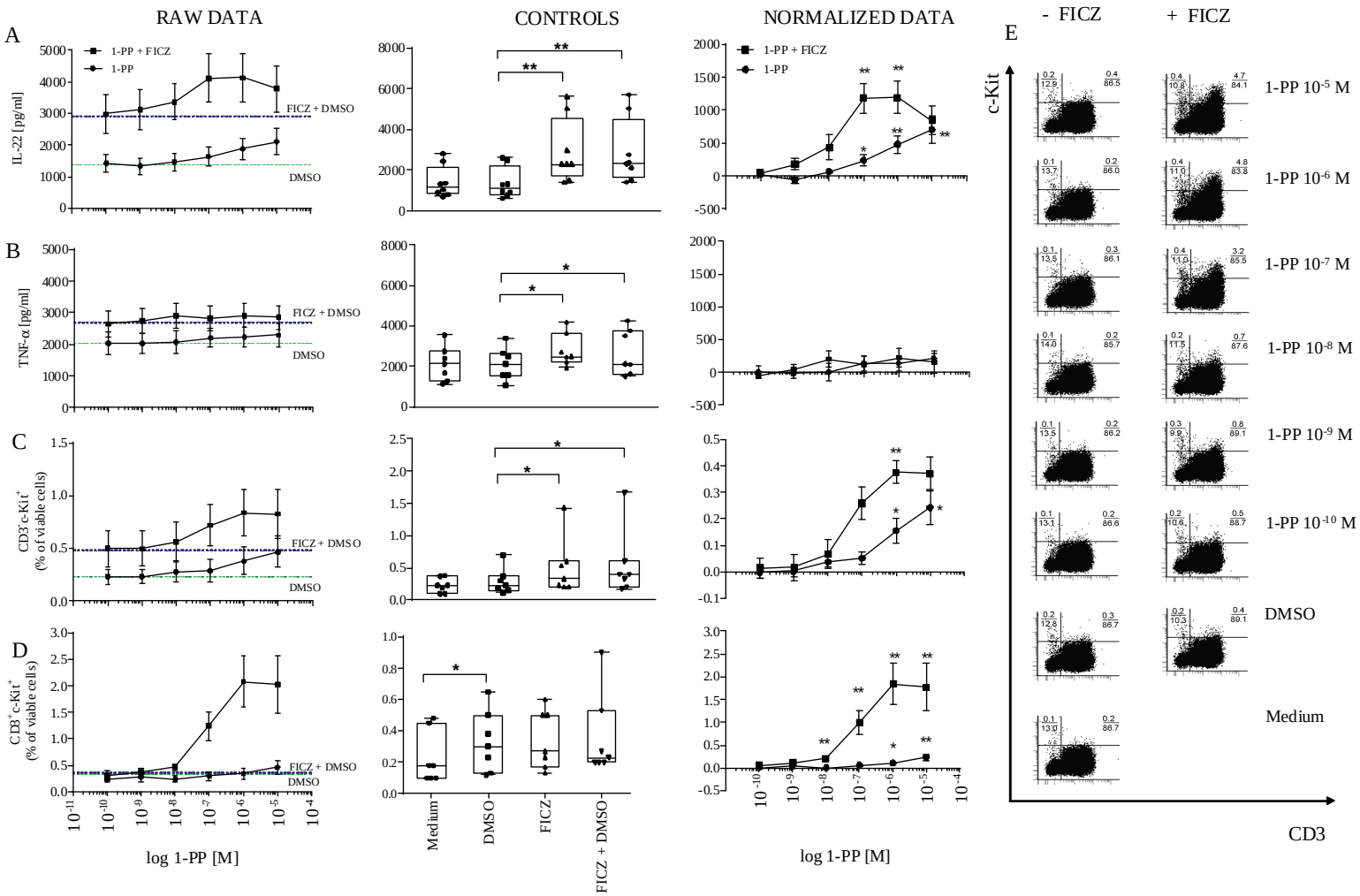


Figure 14. CYP1-dependent induction of IL-22 and c-Kit in human PBMCs

Human PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 were treated with the AHR agonist FICZ (5 x 10^{-10} M), increasing concentrations of the CYP1 inhibitor 1-PP (10^{-10} M – 10^{-5} M) alone or in combination for 48 h. DMSO was used as vehicle control. Cells were stained with anti-CD3-FITC, anti-CD117(c-Kit)-PE and viable cells were selected by flow cytometry. **A.** IL-22 was up-regulated with increasing 1-PP concentrations when a low dose of FICZ was present. **B**. Increasing concentrations of 1-PP did not increase TNF-α production (n=7). **C** and **D**. Inhibition of CYP1 activity by increasing 1-PP concentrations induced c-Kit expression on $CD3^-$ and $CD3^+$ PBMCs. Values of IL-22 and c-Kit measurements were adjusted to the same baseline by substraction of DMSO or DMSO/FICZ treatment for normalisation. **E.** Representative dot plots. **A-D** Raw and normalised data: Means +/- s.e.m. of eight different subjects are shown (n=8), controls: Boxplots show medians, interquartile ranges (box) and ranges of seven different subjects. Wilcoxon signed-rank test was used to compare differences between treatments (*:$p < 0.05$, **:$p < 0.01$).

3.2.3. Viability during 1-PP and FICZ treatments

To analyse concentration-dependent cytotoxic effects of FICZ and 1-PP in PBMCs, viability was determined by flow cytometry with Aqua Live/Dead discrimination and by the LDH assay at 48 h.

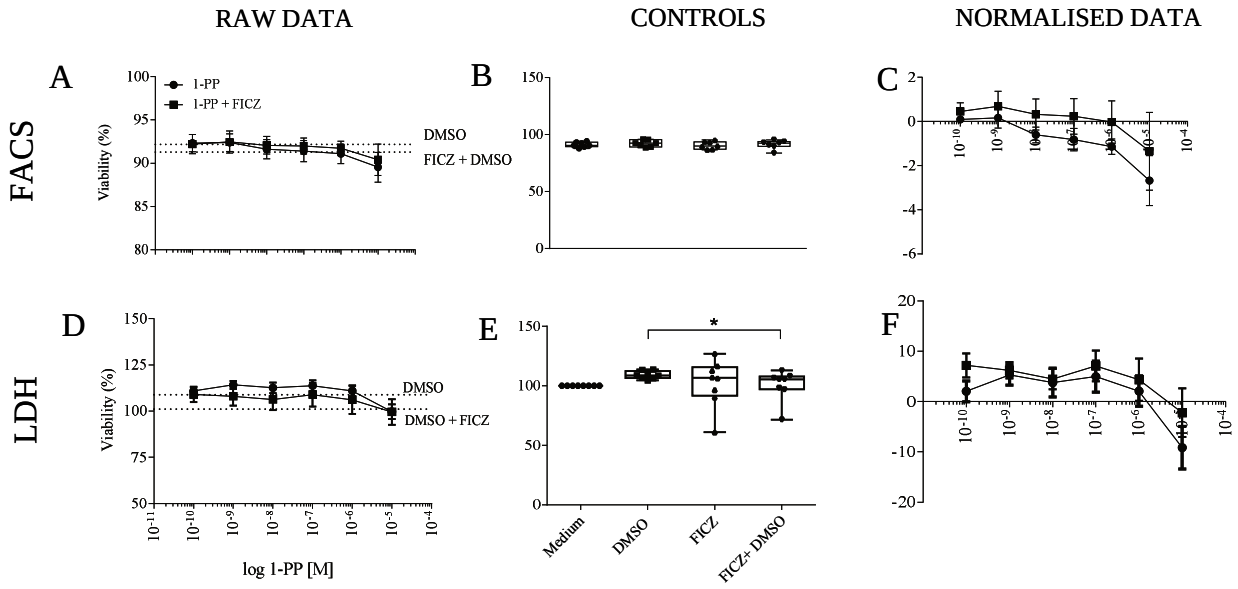


Figure 15. Viability of human PBMCs after combined treatment with 1-PP and FICZ at 48 h
Human PBMCs (3 x 10^5 cells/well) were activated with anti-CD3/CD28 antibodies in the presence of the AHR agonist FICZ (5 x 10^{-10} M) and increasing concentrations of the CYP1 inhibitor 1-PP (10^{-10} M – 10^{-5} M) for 48 h. Viability was analysed by flow cytometry and LDH assay. **A-C**. Viability in 1-PP- and in 1-PP + FICZ-treated cells was determined by flow cytometry. **A**. The percentage of viable cells in 1-PP- and in 1-PP + FICZ-treated PBMCs. **B**. The percentage of viable cells in control treatments. **C**. Normalised viability determined by flow cytometry. **D-F**. Viability in 1-PP-, 1-PP + FICZ-treated cells determined with LDH assay **D**. The percentage of viable cells in 1-PP and in 1-PP- + FICZ-treated PBMCs. Medium-only control was set to 100%. **E**. Viability in control treatments related to medium control. **F**. Normalised viability. For normalisation data were adjusted to the same baseline by substraction of DMSO or DMSO + FICZ treatment. **A, D and C, F**. Raw and normalised data: Means +/- s.e.m. of eight different subjects are shown (n=8), **B and E** controls: Boxplots show medians, interquartile ranges (box) and ranges of seven different subjects. Wilcoxon signed-rank test was used to compare differences between treatments (*:$p < 0.05$).

Neither increasing concentrations of 1-PP nor 1-PP combined with FICZ decreased viability significantly. Analyses of flow cytometry and LDH showed a small decrease in viability when PBMCs were treated with 10^{-5} M 1-PP (Figure 15, A, D and C and F). A higher variance in LDH activity was determined in the positive control samples (FICZ and FICZ with DMSO) than in the DMSO control. This effect was not detected by FACS analysis (Figure 15 B and E). To study the AHR-dependent regulation of IL-22 and c-Kit induction, 10^{-7} M and 10^{-6} M 1-PP were used in further experiments.

3.2.4. 1-PP-induced effects were dependent on AHR activation – RNA analyses

The CYP1 inhibitor 1-PP induced immunological AHR-regulated proteins such as c-Kit and IL-22, when FICZ was present with a low concentration in the culture medium. The addition of the AHR antagonist CH-223191 to 1-PP and FICZ co-treated cells was used to analyse the AHR-dependent effects. Relative mRNA levels were analysed in treated PBMCs to evaluate whether CYP1 inhibition up-regulated *CYP*, *IL22* and *KIT* after 5 days. Additionally, the expression of other target genes related to the AHR pathway (ATP-binding cassette subfamily G member 2 *(ABCG2)*, NAD(P)H dehydrogenase, quinone 1 (*NQO1)*, cytochrome P450 family 2 subfamily S member 1 (*CYP2S1*) and protaglandin-endoperoxide synthase 2 (*PTGS2)* (coding for COX-2)), of transcription factors associated with the xenobiotic pathway (*NRF2*, *ARNT*, *HIF1A*) and of cytokines (*IFNγ*, *IL17*, *IL26*) was analysed by qRT-PCR.

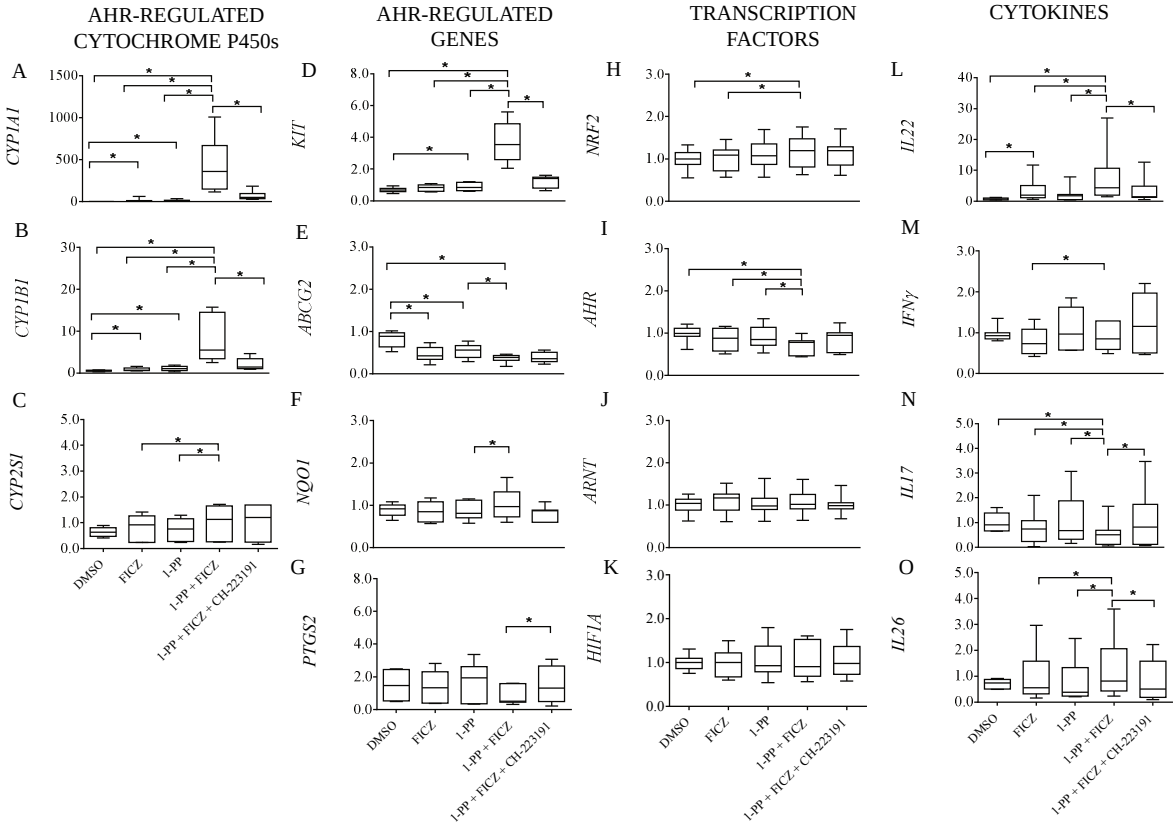


Figure 16. CYP1-induced and AHR-dependent expression of target genes related to the AHR pathway
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. **A-C**. Relative mRNA levels of AHR-regulated cytochrome P450s (*CYP1A1*, *CYP1B1*, *CYP2S1*), **D-G**. other AHR pathway-related targets (*KIT*, *ABCG2*, *NQO1*, *PTGS2*), **H-K**. transcription factors (*AHR*, *ARNT*, *NRF2*, *HIF1A*) and **(L-O)** cytokines (*IL22*, *IL17*, *IFNγ*, *IL26*) were determined with qRT-PCR. Target gene expression was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. Boxplots show medians, interquartile ranges (box) and ranges of seven different subjects. Wilcoxon rank test for paired samples was used to compare treatments (*$p < 0.05$).

Combined treatment of the CYP1 inhibitor 1-PP and the AHR agonist FICZ strongly up-regulated the genes coding for CYP1A1, CYP1B1, IL22 and c-Kit. Single treatments with FICZ or 1-PP alone induced the AHR-downstream genes *CYP1A1* and *CYP1B1* compared with DMSO control. The *KIT* gene was significantly induced with 1-PP alone, and *IL22* with FICZ alone (Figure 16, A, B, D and L). The other target genes related to the AHR pathway (*ABCG2*, *PTGS2*, *NQO1* and *CYP2S1*) (Figure 16, C, E, F, G) were slightly regulated by CYP1 inhibition in the presence of FICZ. Here, *CYP2S1* and *NQO1* were up-regulated and *ABCG2* and *PTGS2* inclinded to be down-regulated. The co-stimulation, but not the single treatments, significantly down-regulated the *AHR* gene, whereas *ARNT* and *HIF1A* (Figure 16, J, K) were not regulated. *NRF2*, a transcription factor sensitive for oxidative stress, tended to be increased in the co-treatment compared with FICZ and the vehicle control, but differences to 1-PP were not detected. 1-PP and FICZ co-treated cells significantly up-regulated *IL26*, this was similar to *IL22*. *IL17* transcription was significantly decreased (Figure 16, N, O). Congruent to the hypothesis that inhibition of CYP1 activity leads to an enhanced AHR activation, the addition of the AHR antagonist CH-223191 significantly inverted the up-regulation of the *CYP1A1*, *CYP1B1*, *KIT*, *IL22* and *IL26* genes but increased *IL17* transcription. Fold changes of compared conditions are summarised in Table 37 (supplemental material).

Conclusively, these data indicated that inhibition of CYP1 activity by 1-PP in the presence of a low FICZ concentration increased *CYP*, *KIT* and *IL22* transcription levels, however, decreased the *IL17* RNA level. Moreover, the results demonstrated that these effects were dependent on AHR activation as addition of CH-223191 inverted the effects.

3.2.5. 1-PP-induced effects were dependent on AHR activation – protein analyses

Results from RNA analyses indicated an AHR-dependent regulation of the Th17 cytokines and of *KIT* during CYP1 inhibition. To confirm the AHR-feedback pathway on protein level, human activated PBMCs were identically treated with the AHR agonist FICZ, CYP1 inhibitor 1-PP, FICZ and 1-PP or FICZ, 1-PP and the AHR antagonist CH-223191 for 5 days. Cytokine concentrations were determined by ELISA. In addition, IL-22 and c-Kit in different immune cells were determined by flow cytometry. Cytokine concentrations were normalised to proliferation and fold regulations were calculated to medium-only control. In the supernatants, IL-22 was increased with FICZ alone and in 1-PP and FICZ co-treated cells. Addition of the AHR antagonist CH-223191 decreased IL-22. IP-10 and IFN-γ were significantly down-regulated by FICZ alone compared with medium-only control and IL-17 was significantly up-regulated by the addition of the AHR antagonist to co-treated cells. Additionally, 1-PP and FICZ co-treatment increased TNF-α expression compared with control treatments (Figure 17, A-E). Fold changes are summarised in Table 38 A-E.

An AHR-dependent regulation of c-Kit protein in FICZ and 1-PP co-treated PBMCs was investigated by flow cytometry, and the frequency of c-Kit$^+$IL-22$^+$ cells in human PBMCs was examined. c-Kit

expression was determined on $CD3^+CD4^+$ Th lymphocytes, on $CD3^+CD8^+$ Tc cells and on $CD3^-$ (non-T cells) cells. In $CD3^+$ cells, FICZ and 1-PP co-treatment significantly up-regulated c-Kit expression on $CD4^+$ and on $CD8^+$ T cells, but single stimulations did not. Addition of the AHR antagonist to co-treated cells significantly decreased the percentage of $c\text{-}Kit^+$ cells among $CD3^+$ T cells (Figure 17, F-H and Table 38 F-H). In order to characterise the AHR-dependent induction of $c\text{-}Kit^+IL\text{-}22^+$ cells, PBMCs were intracellularly stained with anti-IL-22. $c\text{-}Kit^+IL\text{-}22^+$ cells were detected with the highest frequency in the $CD3^+CD4^+$ Th cell compartment, whereas $CD3^+CD8^+$ Tc cells had in general a low percentage of $IL\text{-}22^+$ cells (data not shown). In agreement with the previously shown results, addition of the AHR antagonist CH-223191 reduced the frequency of $c\text{-}Kit^+IL\text{-}22^+$ cells (Figure 17, G and I).

Compared with control stimulations, the percentage of $c\text{-}Kit^+$ cells increased among $CD3^-$ cells when PBMCs were co-treated with 1-PP and FICZ, and it was reduced by the AHR antagonist. To distinguish potential natural killer (NK) cells from other non-T cell populations, $CD3^-$ PBMCs were further discriminated into $CD56^+$ (potential NK cells) and $CD56^-$ cells. Both $CD3^-CD56^-$ and $CD3^-CD56^+$ cells induced c-Kit expression during co-treatment, but not in control treatments. The addition of the AHR inhibitor reduced this up-regulation (Figure 17, J-M and Table 38 J-L).

Conclusively, protein data indicated that CYP1 inhibition by 1-PP up-regulated IL-22, TNF-α and c-Kit expression levels in an AHR-dependent manner as the addition of the AHR antagonist inverted the effects of the co-treatment. This confirmed the RNA results on protein level. Additionally, c-Kit was induced in various immune cells with lymphoid origin, and $c\text{-}Kit^+IL\text{-}22^+$ were part of $CD4^+$ Th cells showing that AHR activity affects many human immune cells.

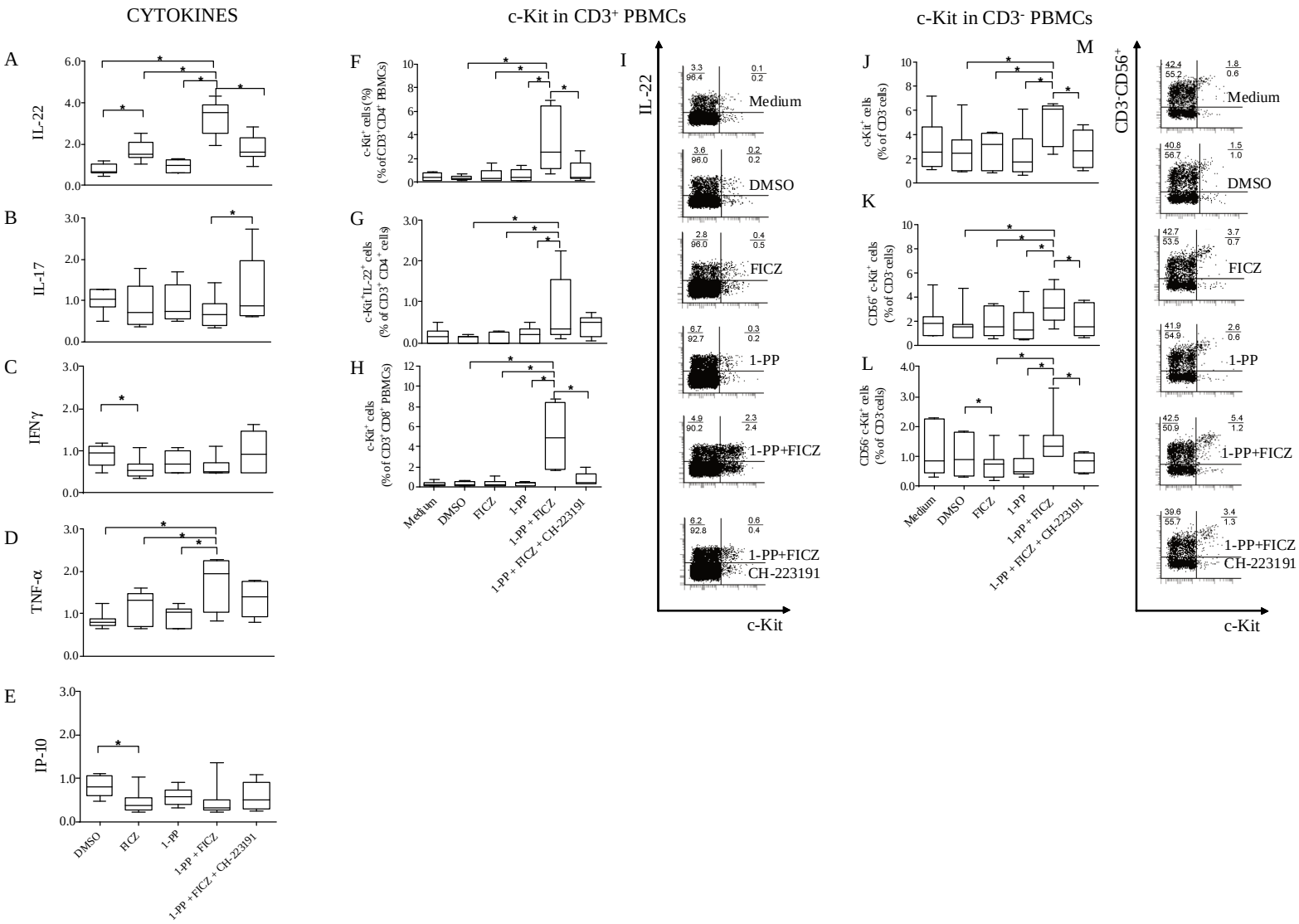


Figure 17. CYP1-dependent AHR activation regulated c-Kit protein and cytokine expression

PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M for IL-22 staining) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. **A-E**, Fold expression of cytokines (relative to medium control). **F-M**, c-Kit expression on $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes was measured by flow cytometry. **G and I**, $c\text{-}Kit^+/IL\text{-}22^+$ cells were detected among $CD4^+$ Th lymphocytes with 10^{-7} M 1-PP. **J-M**. c-Kit induction in $CD3^-$ cell populations. Boxplots show medians, interquartile ranges (box) and ranges of seven different subjects. Wilcoxon rank test for paired samples was used to compare significant differences (*$p < 0.05$).

3.2.6. Viability during 1-PP and FICZ co-treatment at day five

The activation of the AHR regulates several cellular reactions and CYP enzymes are involved in toxic responses. Therefore, viability and cell proliferation were determined by LDH, by flow cytometry and by ^{3}H-thymidine assay after treatment of PBMCs for 5 days.

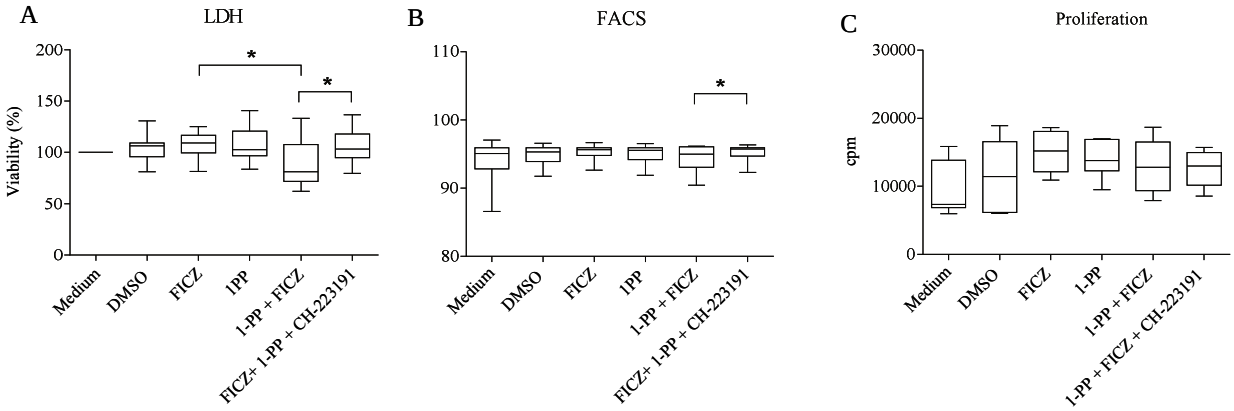


Figure 18. Viability and proliferation in 1-PP and FICZ treatments at day five
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. **A**. Viability was determined by LDH assay and by **B**. flow cytometry. **C**. Proliferation was analysed by ^{3}H-thymidine assay. Boxplots show medians, interquartile ranges (box) and ranges of seven different subjects. Wilcoxon rank test for paired samples was used to compare significant differences (*$p < 0.05$).

Combined treatment of FICZ and 1-PP significantly decreased viability (17.25% ± 15.16%) measured by LDH assay. FICZ or 1-PP alone did not. FACS analysis showed a decrease of only 1.18% ± 0.98% in co-treated cells compared with FICZ control. Addition of the AHR antagonist (CH-223191) increased viability in both LDH assay (26.19% ± 12.27%) and FACS analysis (1.13% ± 0.82%) (Figure 18, A and B). Results of proliferation did not show any trends by treatments with FICZ or 1-PP.

3.2.7. Overview of 1-PP and FICZ treatments in human PBMCs: Trends in regulation

Common regulations of investigated targets were summarised in Figure 19. The co-treatment of FICZ with 1-PP had various effects on the expression of AHR pathway associated targets.

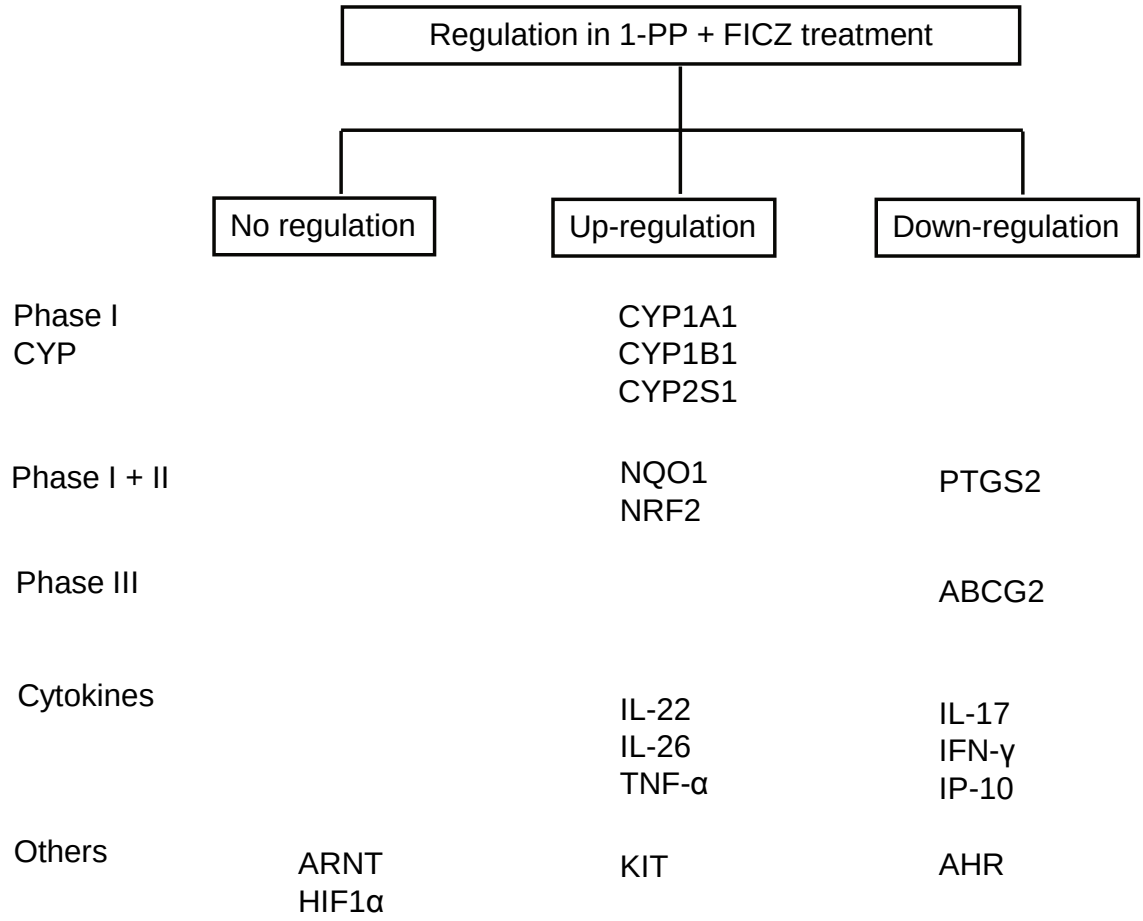


Figure 19. Trends in target gene regulation by 1-PP and FICZ co-treatment in human PBMCs

Among the compounds, which were regulated downstream of the AHR pathway, the phase I *CYP* genes, other phase I- and phase II-related genes (*NQO1* and *NRF2*), the cytokines IL-22, *IL26*, TNF-α, and c-Kit were up-regulated during CYP1 inhibition. *PTGS2*, *ABCG2*, IL-17, IFN-γ, IP-10 and *AHR* tendend to be down-regulated. No regulation was detected for the genes coding for *ARNT* and *HIF1A*.

3.3. Correlations between AHR and NRF2 pathway targets during CYP1 inhibition in human PBMCs

3.3.1. Correlations between *AHR* and the transcription of genes involved in the AHR pathway

The AHR pathway closely acts together with the NRF2 pathway for anti-oxidative and cytoprotective responses. Correlation analyses were used to study the relations between *AHR* transcription and the transcription of genes related to the AHR pathway (Figure 20) in seven healthy individuals. To analyse correlations between various targets, spearman´s rank correlation coefficients were calculated and significant p-values were marked in red.

The transcription of the *AHR* gene was strongly and positively correlated with the transcription of *NRF2, HIF1A* and *NQO1* in most treatments including vehicle control. To some extent, *AHR* transcription correlated with *ARNT*, *CYP1A1*, *CYP1B1*, *CYP2S1* and *PTGS2* transcription levels. *CYP1* genes are directly controlled by the AHR. *CYP1A1* transcription tended to correlate negatively, whereas *CYP1B1* inclined to be positively regulated with the *AHR* transcription. These effects were particularly obvious during 1-PP and FICZ co-treatment but not in the DMSO control. *CYP2S1* showed similar trends as *CYP1B1*. The *KIT* and *ABCG2* genes did not show any correlations with *AHR* transcription in PBMCs. Correlation coefficients and p-values are summarised in Table 39.

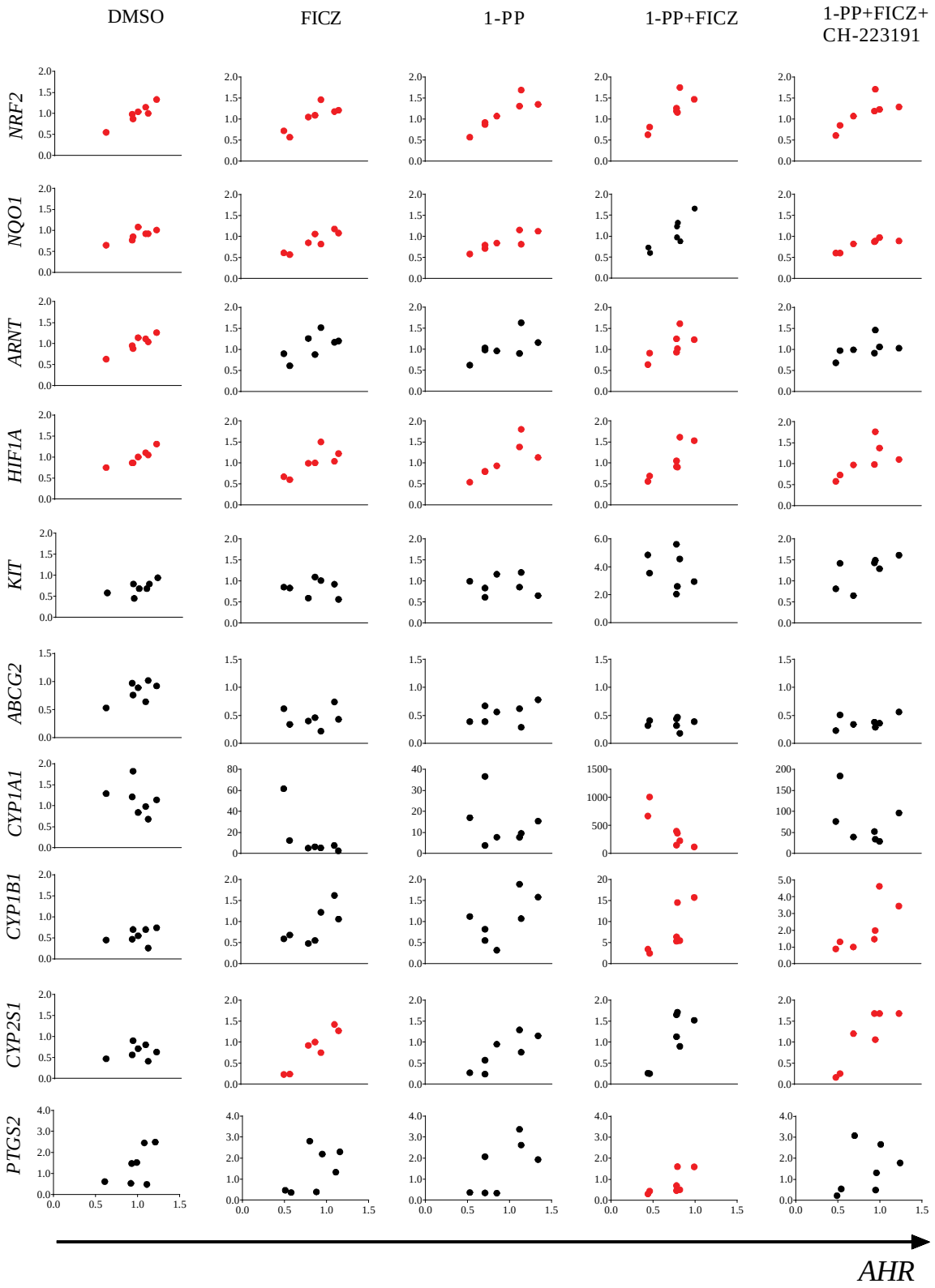


Figure 20. Correlations between *AHR* and genes coding for transcription factors, XMEs and c-Kit
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3x10^{-6} M) for 5 days. Gene transcription was analysed with qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene, relative expression was calculated to the medium-only control and correlations between various targets were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

3.3.2. Correlations between *AHR* and cytokine expression

Correlation analyses were used to study the relations between *AHR* transcription and cytokine expression. Cytokine expression was analysed by qRT-PCR (Figure 21) and ELISA (Figure 22). Gene transcription was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. Cytokine concentrations were determined in the cell culture supernatants, normalised to the proliferation and related to the medium-only control as fold regulation. Fold expressions on RNA and protein level were correlated with the *AHR* fold expression. To analyse correlations between various targets, spearman´s rank correlation coefficients were calculated and significant p-values were marked in red.

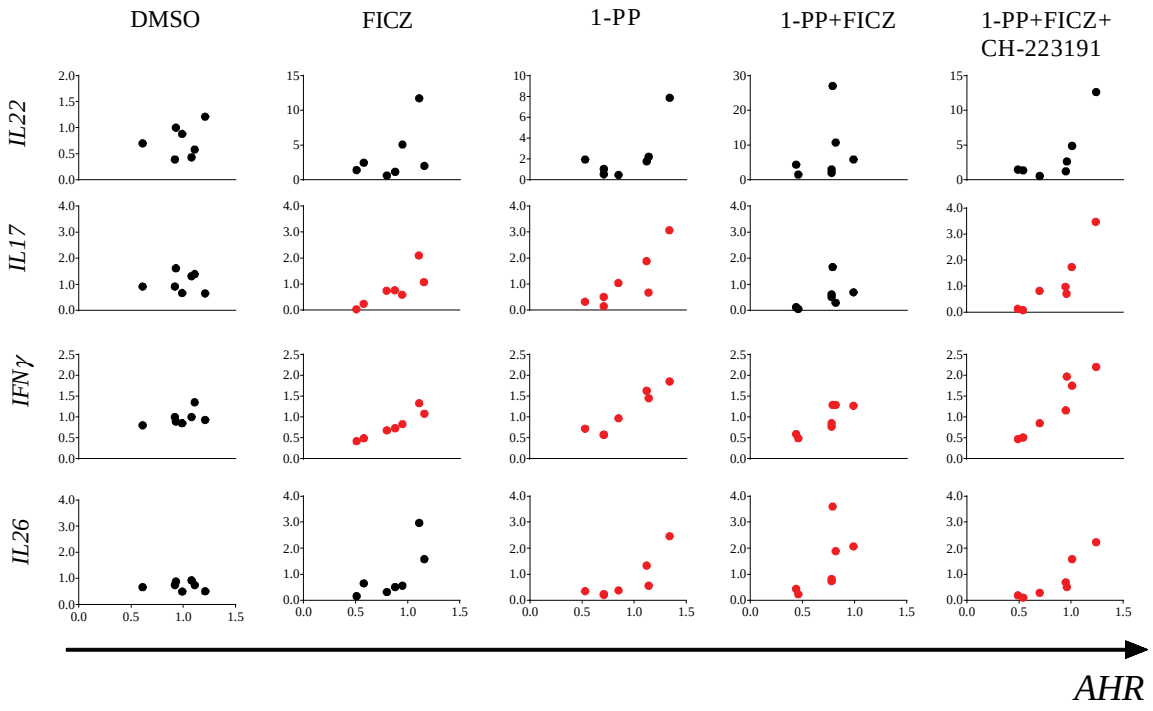


Figure 21. Correlations between *AHR* and cytokine transcription
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Gene transcription was analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene, relative expression was calculated to medium-only control and correlations between various targets were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

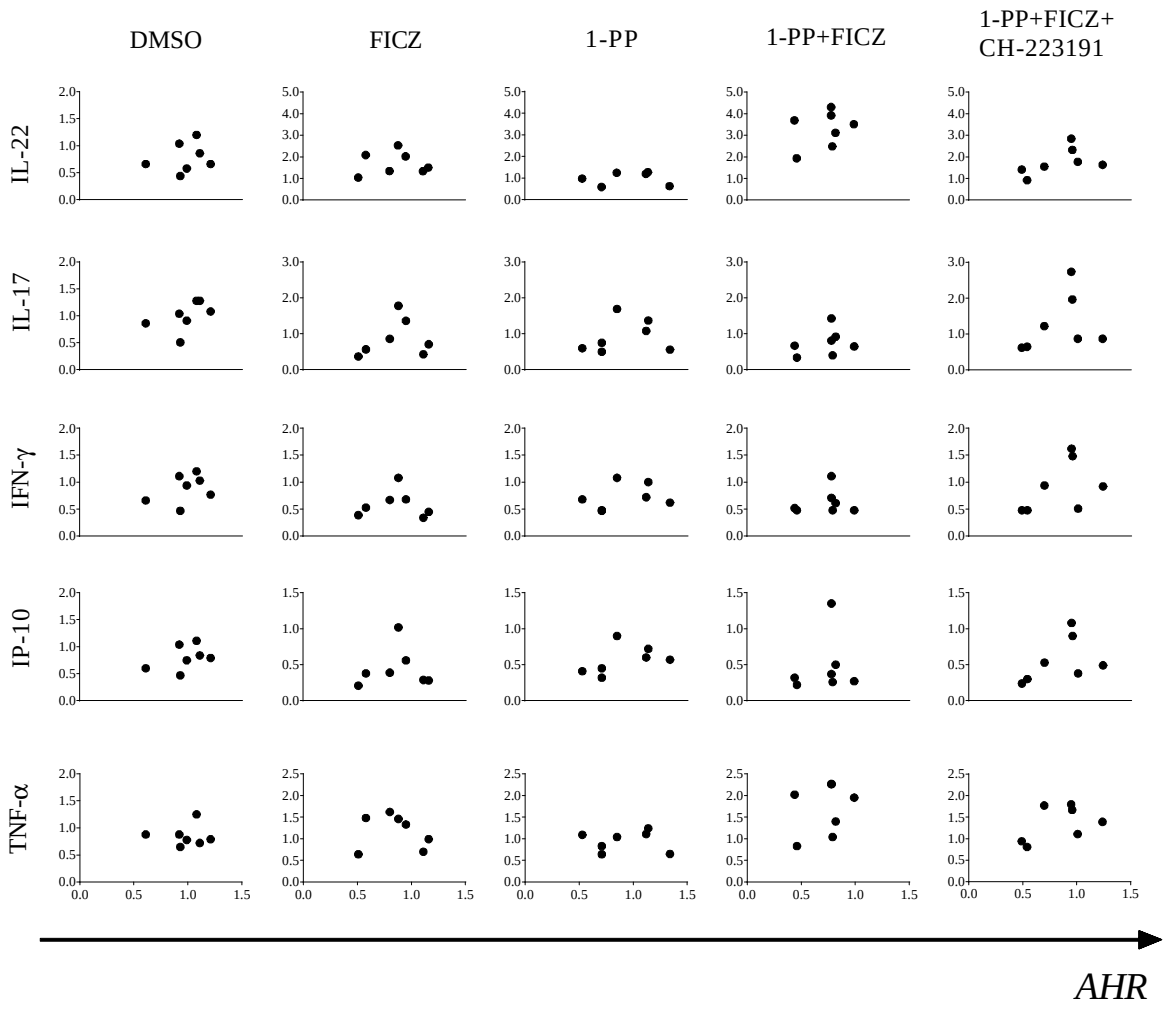


Figure 22. Correlations between *AHR* transcription and cytokine expression
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Cytokine expression was determined by ELISA and correlations between various targets were calculated with spearman´s rank correlation coefficient.

The transcription of the cytokines *IFNγ*, *IL17* and *IL26* correlated positively with the *AHR* transcription in most treatments when FICZ and/or 1-PP were present in the culture medium, but not in the vehicle control. *IL17*, *IFNγ* and *IL26* showed significant and positive correlations, but *IL22* did not correlate significantly. Whereas cytokine transcription inclined to be positively correlated with *AHR* transcription, cytokine expression on protein level did not correlate with *AHR* transcription in any treatments. Correlation coefficients and p-values are summarised in Table 39.

3.3.3. Correlations between *AHR* and c-Kit expression

Correlation analyses were used to study dependencies between the transcription of the *AHR* gene and the c-Kit protein on gated PBMCs. The c-Kit expression was analysed by flow cytometry. To analyse correlations between various targets spearman´s rank correlation coefficients were calculated and significant p-values were marked in red.

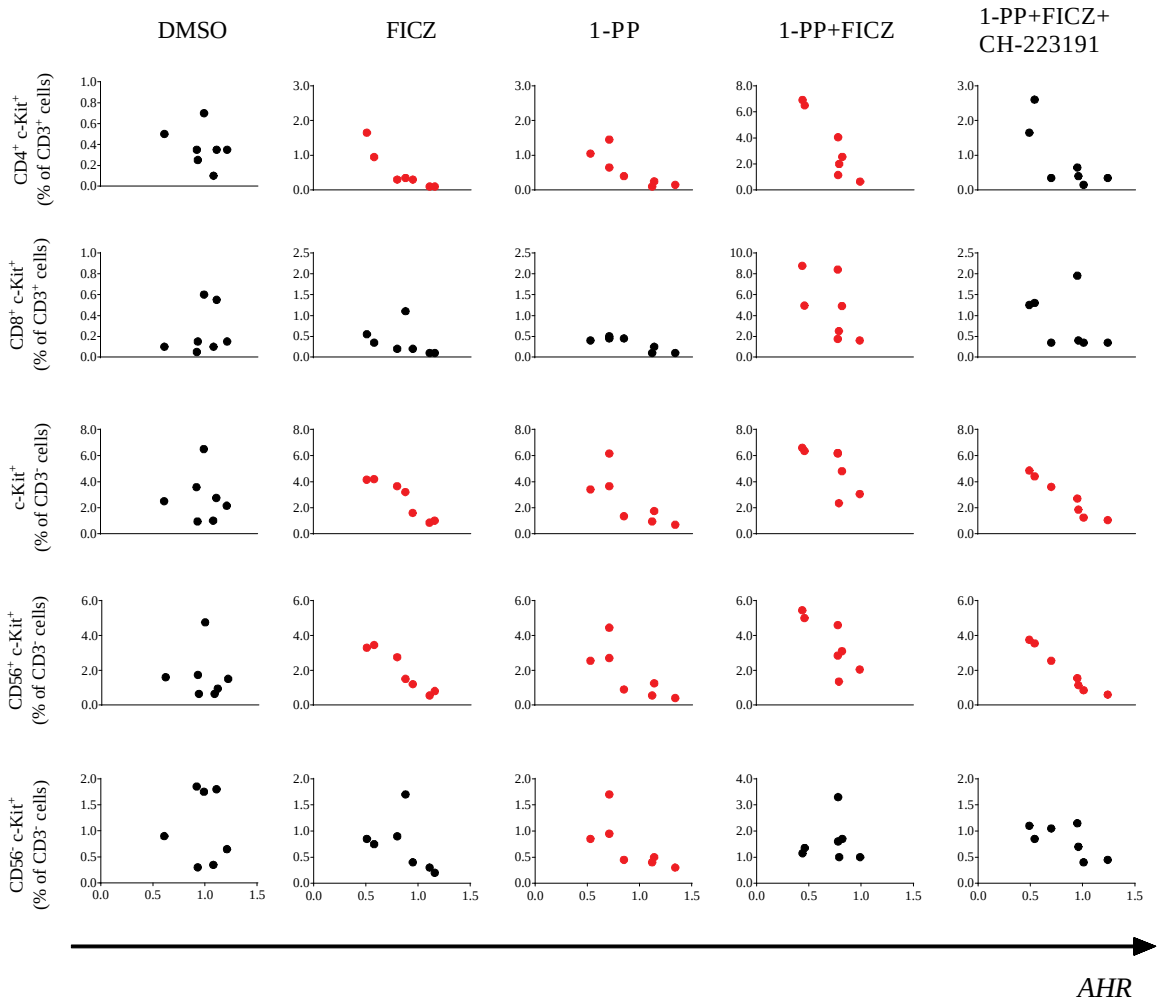


Figure 23. Correlations between *AHR* and c-Kit expression on $CD3^+$ and $CD3^-$ PBMCs
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. c-Kit expression was analysed by flow cytometry and correlations between various targets were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

The transcription of the *AHR* gene was negatively correlated with the c-Kit expression on $CD3^+$ and $CD3^-$ cell populations. Results indicated negative trends during FICZ and 1-PP single treatments and in co-treated cells, but not in treatments with DMSO. Significant changes in single treatments with FICZ were detected on $CD3^+CD4^+$, on $CD3^-$ and on $CD3^-CD56^+$ cell populations. In 1-PP and FICZ co-treated cells, significant correlations were detected in $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^-$ and $CD3^-CD56^+$ cell populations (Figure 23).

In conclusion, *AHR* transcription was strongly and positively correlated with the transcription of genes involved in the anti-oxidative response downstream of CYP (*NRF2, NQO1*) and with *HIF1A* in most treatments including DMSO control. Cytokine transcription was positively correlated with *AHR* transcription, whereas a correlation on protein level was not found. The transcription of *CYP* genes and of cytokines clearly changed in human immune cells when FICZ was present in the culture medium. Additionally, *AHR* transcription was negatively correlated with *CYP1A1* transcription. In contrast to this, cells with a high *AHR* transcription showed a high *CYP1B1* transcription. Significant negative correlations were detected between *AHR* and c-Kit expression levels. Correlation coefficients and p-values are summarised in Table 39. The correlations of *CYP1A1*, *IL17*, *IFNγ* and c-Kit with the transcription of *AHR* were unexpected and inconsistent with the experiments of AHR activation by FICZ.

3.3.4. Correlations between *NRF2* and the transcription of genes involved in the AHR pathway

The AHR pathway closely acts together with the NRF2 pathway for anti-oxidative and cytoprotective responses. Correlation analyses were used to study the relations between the transcription of *NRF2* and the transcription of genes related to the AHR pathway. Relative gene expression was calculated to the medium-only control and correlated with *NRF2* transcription. To analyse correlations between various targets, spearman´s rank correlation coefficients were used and significant p-values were marked in red.

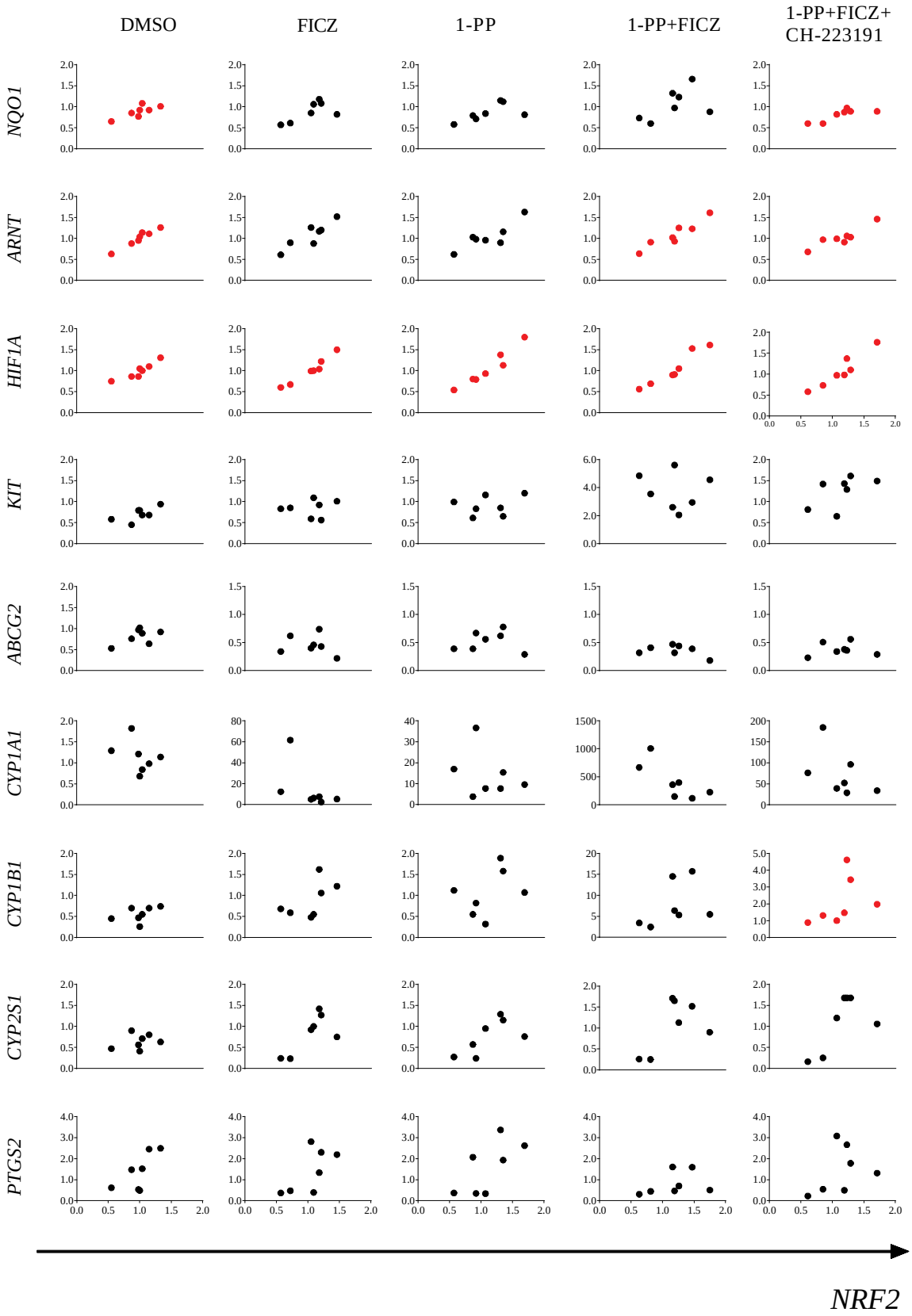


Figure 24. Correlations between *NRF2* and genes coding for transcription factors, XMEs and c-Kit
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Gene transcription was analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene, relative expression was calculated to medium-only control and correlations between various targets were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

Similar to the correlations with the *AHR* transcription, an increased *NRF2* transcription showed significant and positive correlations with *HIF1A* transcription in most treatments including vehicle control, and positive correlations with *NQO1* and *ARNT*. The transcription of the *KIT* and the *ABCG2* genes did not correlate with *NRF2* transcription. The transcription of the *CYP1* genes showed only a weak correlation with *NRF2* gene expression, however, the transcription of *CYP1A1* and *CYP1B1* genes showed inverse trends. Although transcription of *CYP2S1* and *PTGS2* were not significantly co-regulated with *NRF2* transcription, both showed trends for positive correlations. Correlation coefficients and p-values are summarised in Table 39.

3.3.5. Correlations between *NRF2* and cytokine expression

Correlation analyses were used to study relations between the transcription of the *NRF2* gene and cytokine expression. Cytokine expression was determined by qRT-PCR (Figure 25) and ELISA (Figure 26). Gene transcription was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. Cytokine concentrations were determined in the cell culture supernatants, normalised to the proliferation and related to the medium-only control as fold regulation. Fold expressions on RNA and protein level were correlated with the *NRF2* fold expression. To analyse correlations between various targets spearman´s rank correlation coefficients were calculated and significant p-values were marked in red.

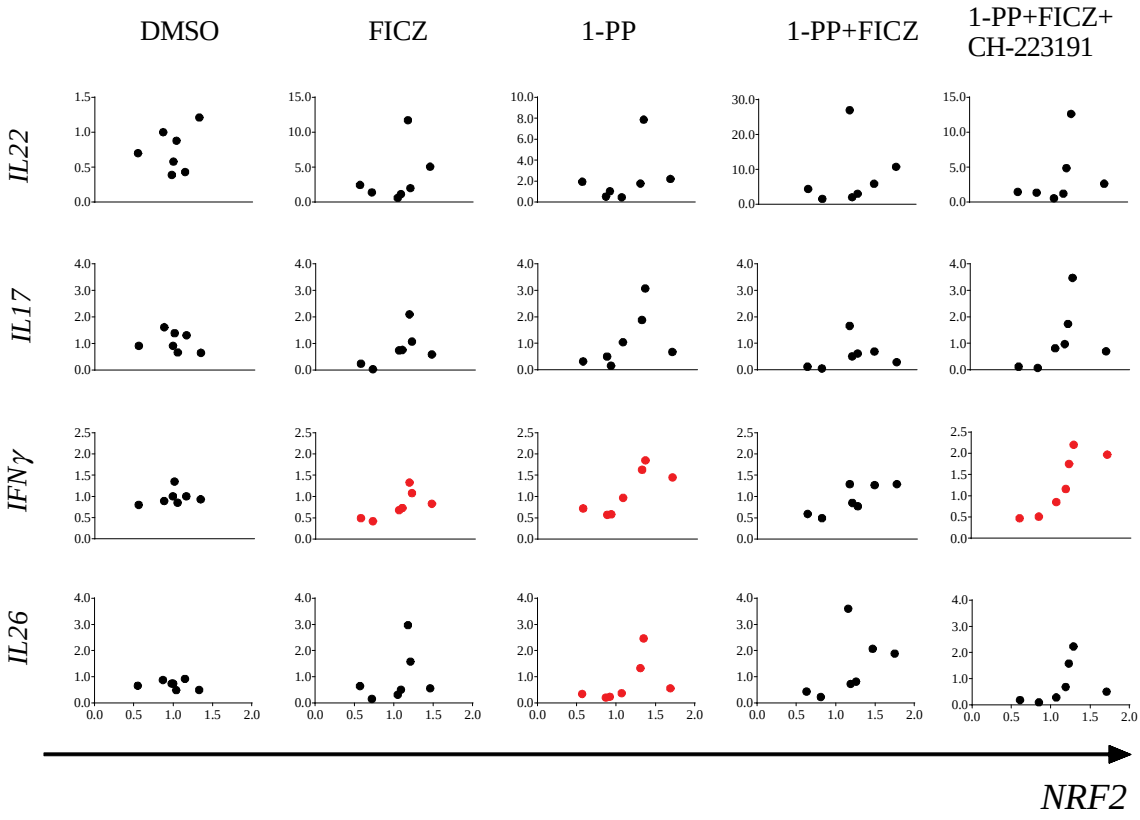


Figure 25. Correlations between *NRF2* and cytokine transcription
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Cytokine transcription was analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene, relative expression was calculated to medium-only control and correlations between various targets were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

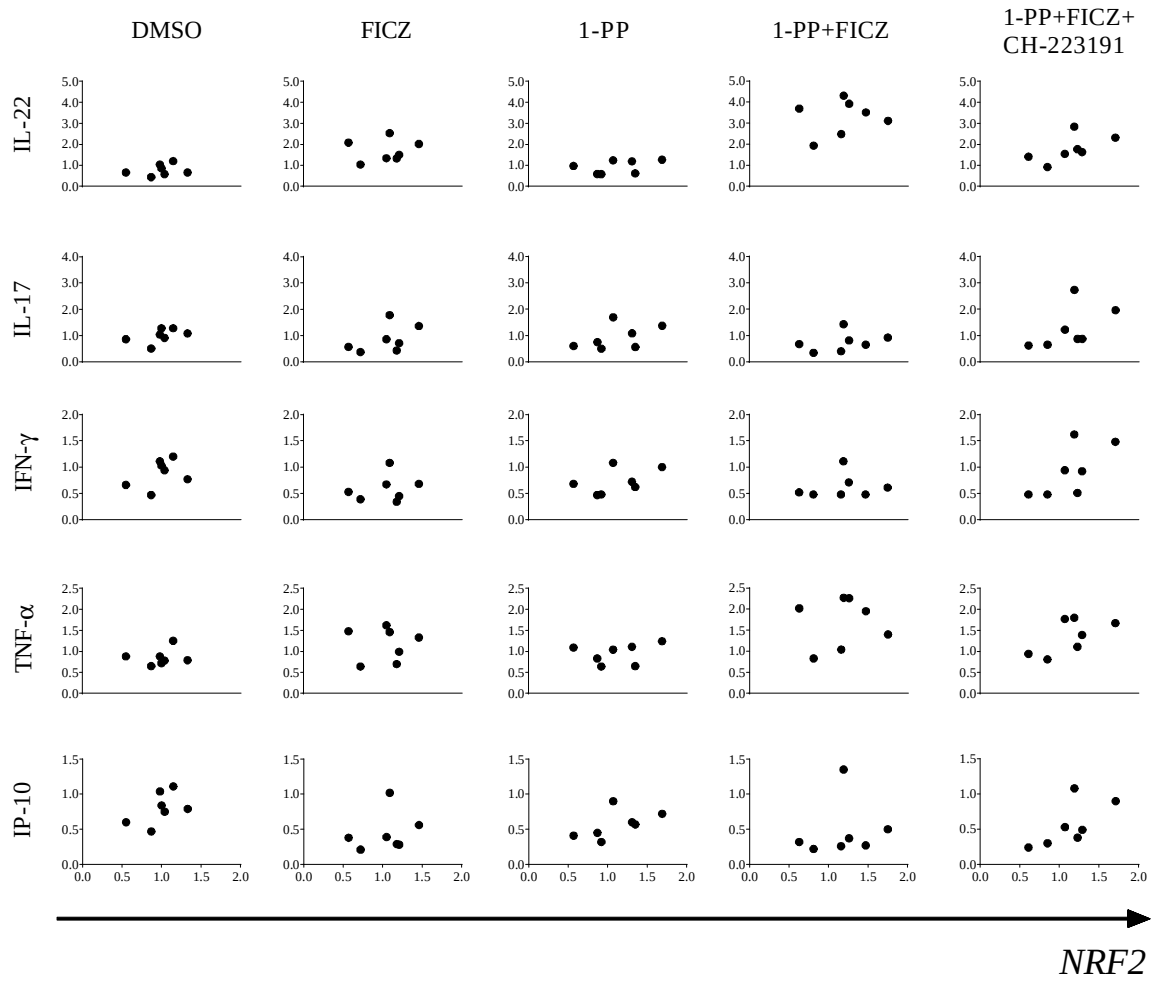


Figure 26. Correlations between *NRF2* and cytokine expression
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Cytokine release was analysed by ELISA, normalised to proliferation and fold regulation was calculated to medium-only control. Correlations between various targets were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

The transcription of the *IFNγ* and the *IL26* genes tended to be positively correlated with the *NRF2* transcription when FICZ or 1-PP were present in the culture medium, but not in the vehicle control. Although *IFNγ* and *IL26* showed significant and positive correlations, transcription of *IL17* and *IL22* was not significantly co-regulated with *NRF2* expression (Figure 25). Whereas cytokine transcription inclined to be positively correlated with *NRF2* transcription, cytokine expression on protein level did not show any correlation with *NRF2* transcription (Figure 26). Correlation coefficients and p-values are summarised in Table 39.

3.3.6. Correlations between *NRF2* and c-Kit expression

Correlation analyses were used to study relationships between the transcription of the *NRF2* gene and of c-Kit expression on gated PBMCs. The c-Kit expression on various target cells was analysed by flow cytometry. To analyse correlations between various targets spearman´s rank correlation coefficients were calculated and significant p-values were marked in red.

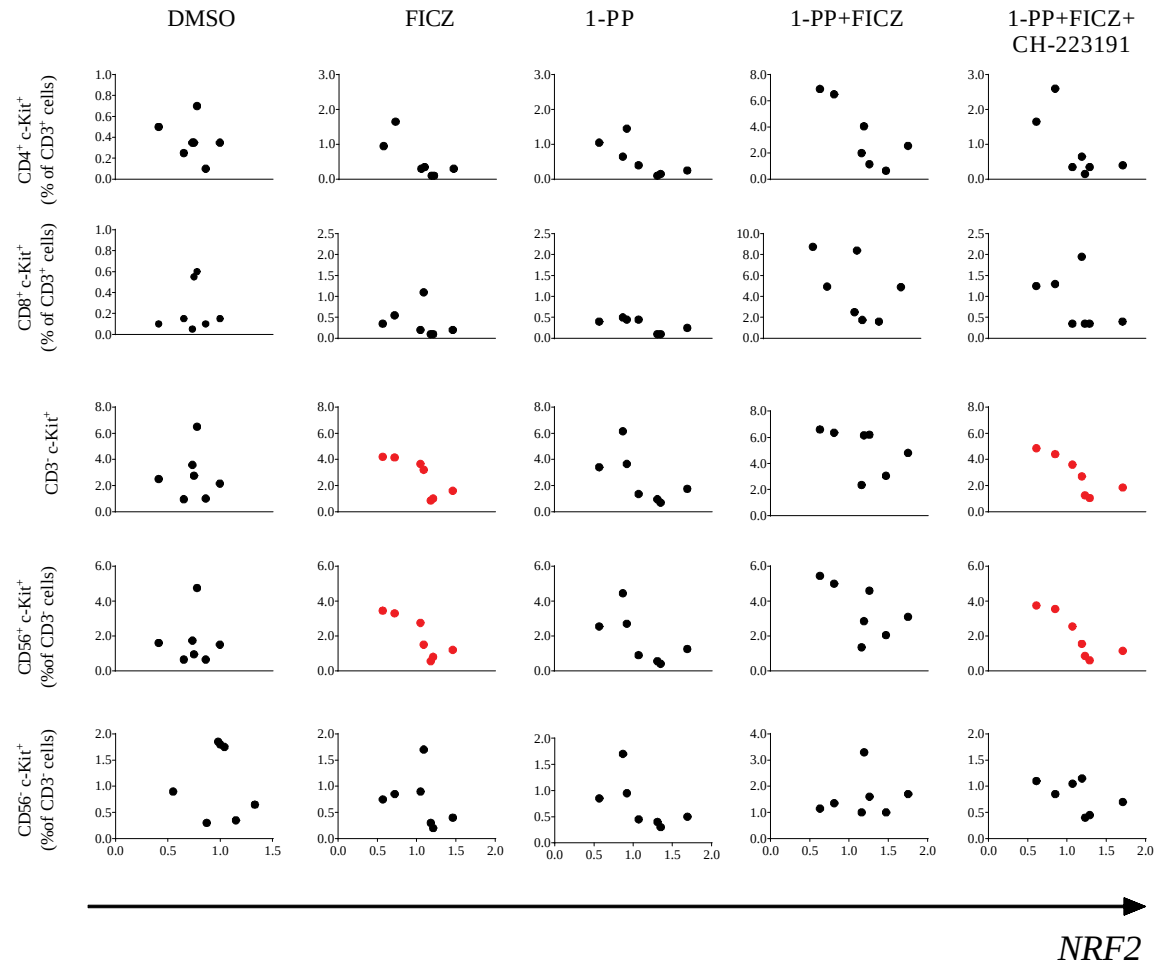


Figure 27. Correlations between *NRF2* and c-Kit expression on $CD3^+$ and $CD3^-$ PBMCs
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. c-Kit expression was analysed by flow cytometry and correlations between various targets were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

The transcription of the *NRF2* gene tended to be negatively correlated with c-Kit expression on $CD3^+$ and $CD3^-$ PBMCs. Significant negative correlations of c-Kit expression with *NRF2* transcription were only calculated in the $CD3^-$ cell compartment, however, trends were detectable in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells (Figure 27). Correlation coefficients and p-values are summarised in Table 39.

In conclusion, *NRF2* transcription was positively correlated with the transcription of *NQO1*, *ARNT* and *HIF1A*. Correlations of *NRF2* transcription with *CYP* and cytokine transcription levels changed in individuals when FICZ was present in the culture medium. Cytokine transcription inclined to be positively correlated with *NRF2* transcription whereas a correlation was not found on protein level. The *KIT* and *ABCG2* transcription levels did not correlate with *NRF2*. Additionally, *CYP1B1* transcription was positively correlated, whereas *CYP1A1* transcription was negatively correlated with *NRF2* transcription. Significant negative correlations were found between *NRF2* and c-Kit expression in the $CD3^-$ compartment.

3.3.7. Correlations between viability and the transcription of genes involved in the AHR pathway

The AHR pathway closely acts together with the NRF2 pathway for anti-oxidative and cytoprotective responses. To examine whether viability and target gene expressions were co-regulated, correlations between viability and gene or protein expression levels were analysed. Viability was measured with LDH assay and medium control was set to 100%. To analyse correlations between various targets spearman´s rank correlation coefficients were calculated and significant p-values were marked in red.

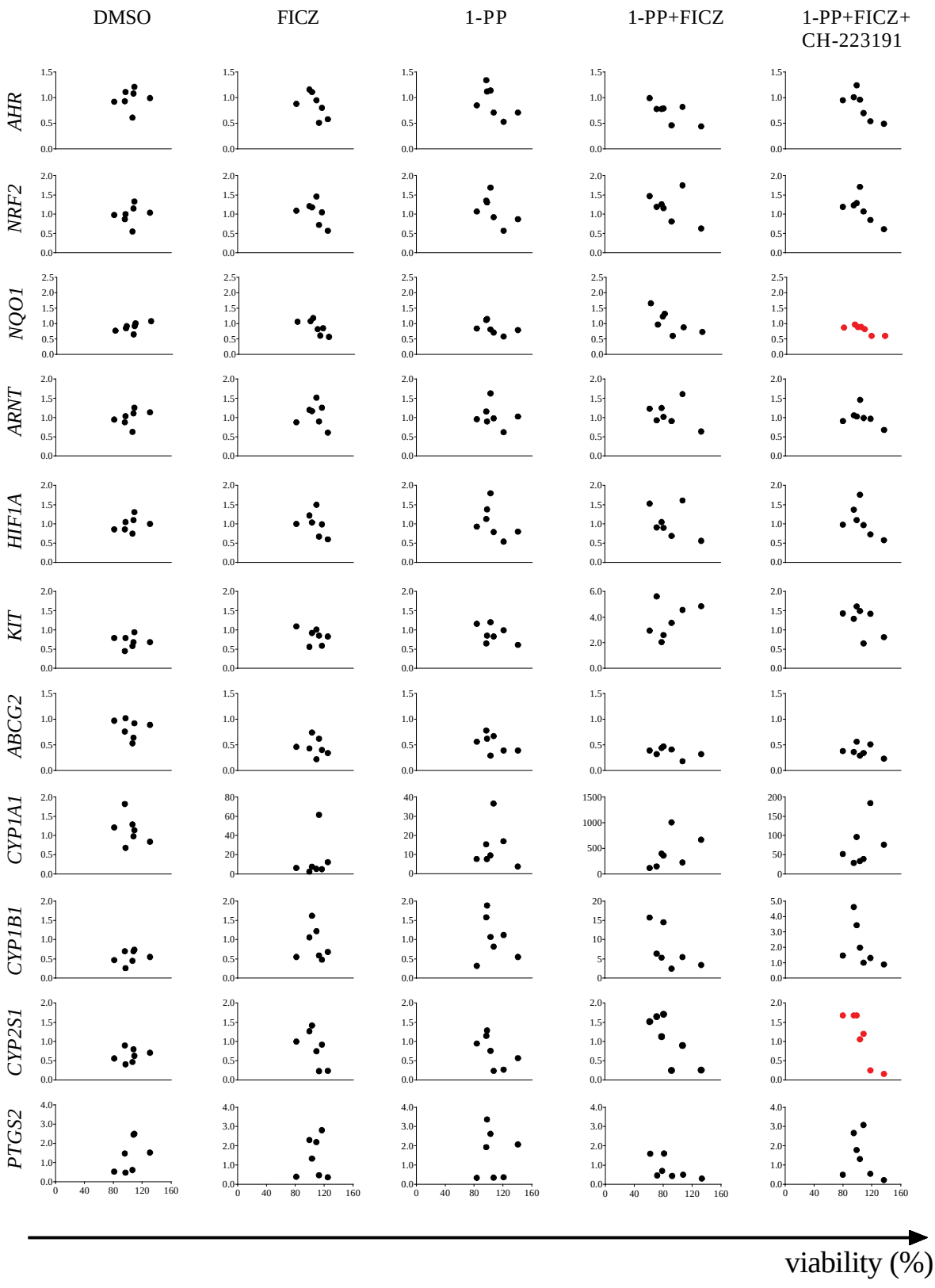


Figure 28. Correlations between viability and genes coding for transcription factors, XMEs and c-Kit
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Gene transcription was analysed with qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. Viability was measured by LDH assay. Correlations between viability and target gene transcription levels were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

Whereas significant correlations between viability and target gene transcription were only detected in the correlations with the transcription of *NQO1* and *CYP2S1*, negative trends could be also detected in the correlations between viability and *AHR*, *NRF2*, *NQO1*, *ARNT* and *HIF1A* gene expression. The transcription levels of *CYP1B1* and *CYP2S1* were slightly and negatively correlated with the viability, whereas the transcription of *CYP1A1* indicated positive trends. No trends were detected for *KIT* and *ABCG2* transcription levels with viability (Figure 28). Correlation coefficients are summarised in Table 39.

3.3.8. Correlations between viability and cytokine expression

Cytokine expression was determined by qRT-PCR and ELISA. Gene transcription was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. Cytokine concentrations in the supernatants were normalised to proliferation, related to medium-only control and fold regulations were correlated with viability measured by LDH. To calculate correlations between various targets spearman´s rank correlation coefficients were used and significant p-values were marked in red.

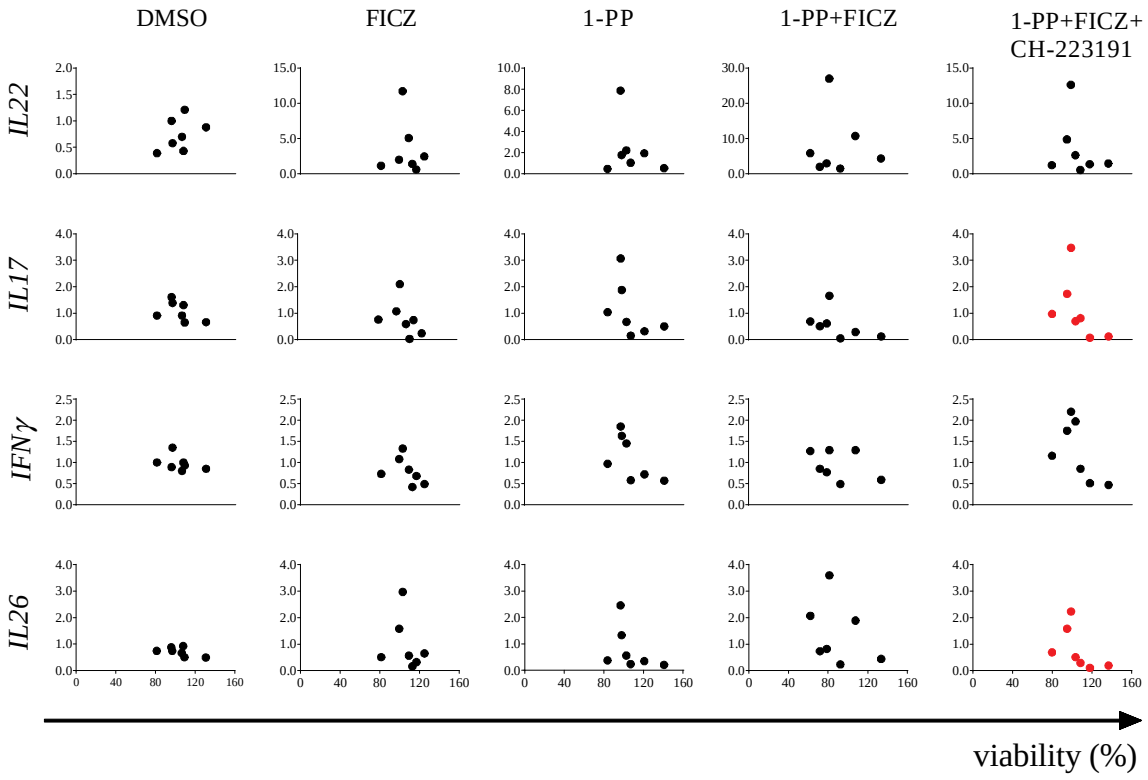


Figure 29. Correlations between viability and cytokine transcription
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Cytokine transcription was determined by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. Viability was determined by LDH assay. Correlations between viability and various cytokines were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

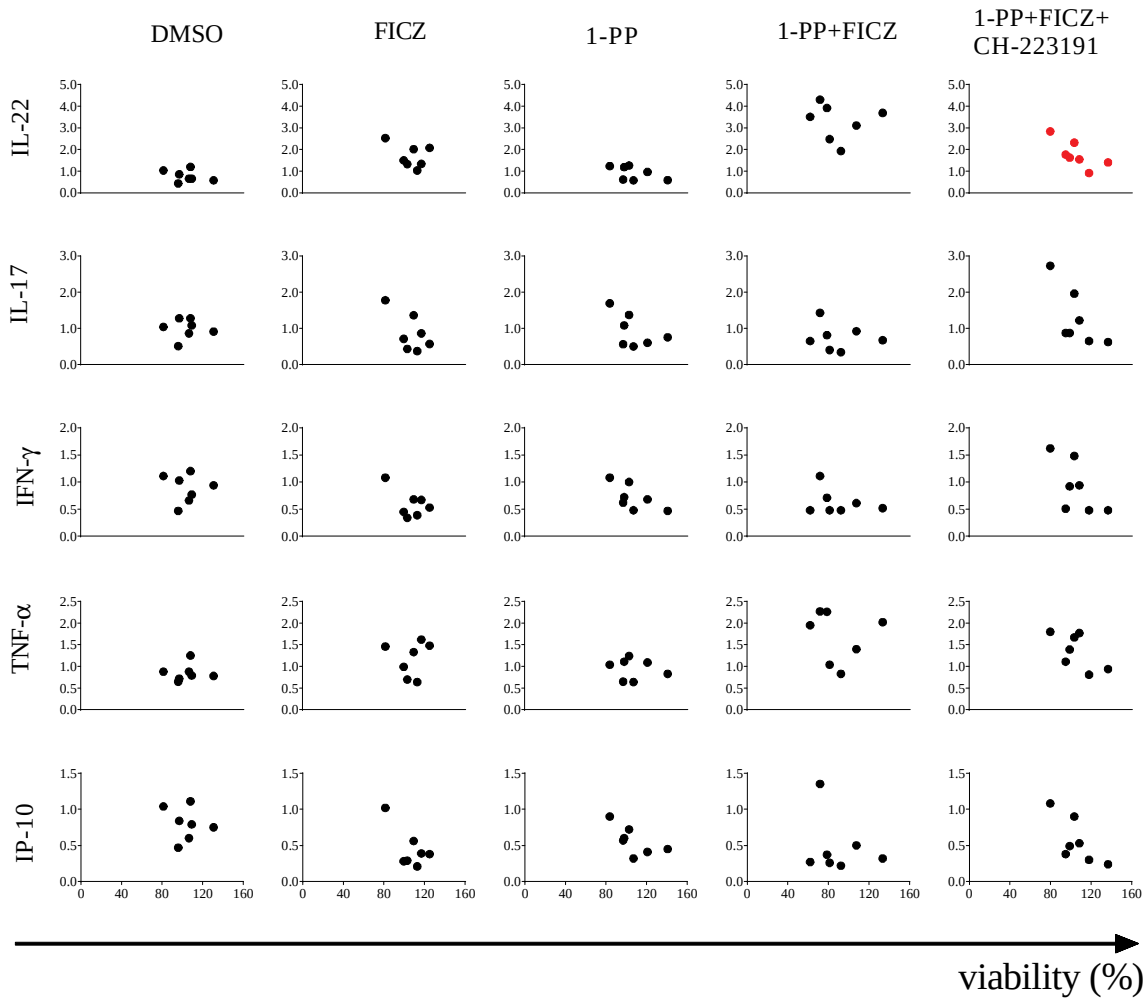


Figure 30. Correlations between viability and cytokine expression
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Cytokine expression was determined by ELISA and viability by LDH assay. Correlations between viability and cytokines were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

On RNA level the cytokines *IL22*, *IL17*, *IFNγ* and *IL26* inclined to be negatively correlated with viability (Figure 29). By trends, IL-22, IL-17, IFN-γ and IP-10 proteins were negatively correlated with viability (Figure 30) in co-treated cells. Correlation coefficients and p-values are summarised in Table 39.

3.3.9. Correlations between viability and c-Kit expression

Correlation analyses were used to study dependencies between viability and c-Kit expression on gated PBMCs. The c-Kit expression was analysed by flow cytometry. Viability was measured by LDH assay and medium control was set to 100%. Spearman´s rank correlation coefficients were calculated and significant p-values were marked in red.

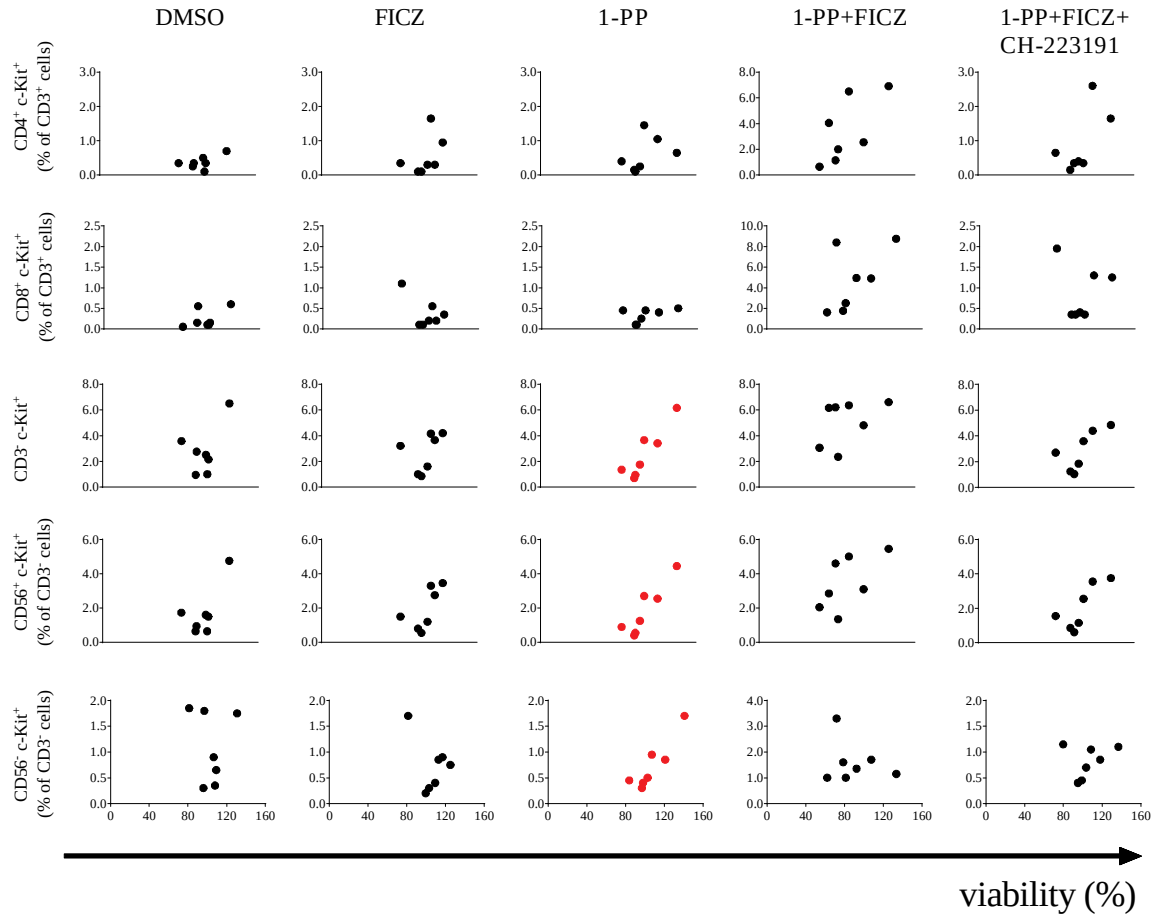


Figure 31. Correlations between viability and c-Kit expression on $CD3^+$ and $CD3^-$ PBMCs
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. c-Kit expression was analysed by flow cytometry and viability by LDH assay. Correlations between viability and c-Kit expression were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

Although positive trends were determined, neither $CD4^+c\text{-}Kit^+$ nor $CD8^+c\text{-}Kit^+$ cells showed significant correlations with viability (Figure 31). But viability was significantly and positively correlated with c-Kit on $CD3^-$ cell populations during 1-PP treatment.

In conclusion, less significant correlations were found for viability with XME, cytokine and c-Kit expression levels. By trends, viability correlated negative with *AHR*, *NRF2*, *NQO1*, *HIF1A*, *ARNT*, *IL22*, *IL17*, *IFNγ*, *IL26* and *CYP1B1* transcription levels, particularly during AHR inhibition. Similar trends were detected when viability was correlated with the cytokine protein expression. A significant negative correlation with IL-22 was not detected on RNA level but on protein level, and trends were

shown for IL-17, IFN-γ and less for TNF-α and IP-10. The transcription of the *KIT* gene did not show a correlation on RNA level, however, positive trends were detected with c-Kit expression on protein level.

3.3.10. Correlations between *CYP1A1* and *CYP1B1* during 1-PP and FICZ treatments

The AHR agonist FICZ is a potent substrate for the CYP1 enzymes. Both CYP1A1 and CYP1B1 enzymes are preferentially expressed in extrahepatic tissues. Previous data examined that *CYP1A1* and *CYP1B1* gene transcriptions correlated differently with *AHR* and *NRF2* expression levels and with viability. Following these results, correlations between *CYP1A1* and *CYP1B1* gene expression levels were analysed in seven healthy subjects during FICZ and 1-PP treatments. It was also invesigated in detail whether the ratio of *CYP1A1* and *CYP1B1* correlated with the expression of AHR pathway compounds during CYP1 inhibition.

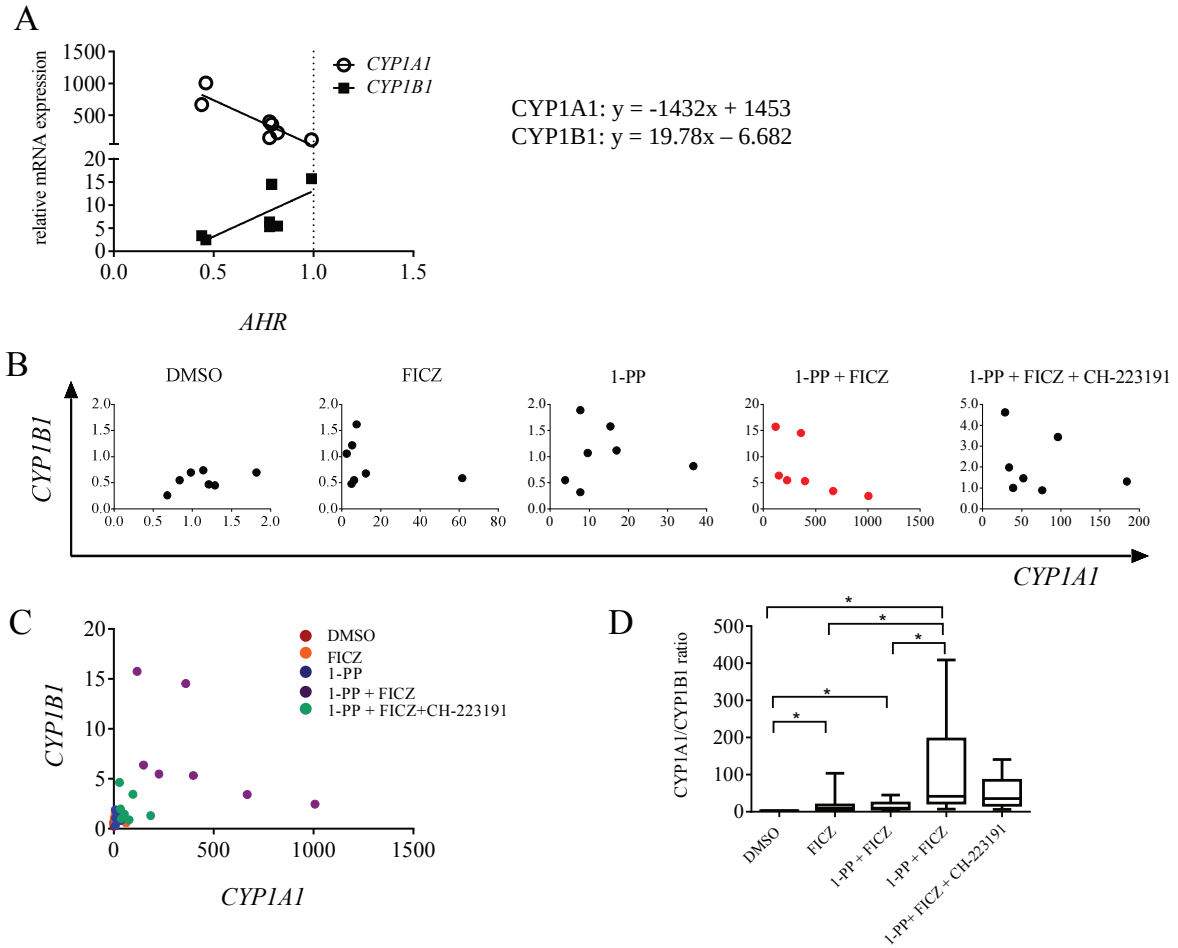


Figure 32. Correlations between *CYP1A1* and *CYP1B1* transcription
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Gene transcription of *CYP1A1* and *CYP1B1* was analysed by qRT-PCR. Target gene expression was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. **A.** Correlations of *CYP1A1* and *CYP1B1* with *AHR* transcription in co-treated (1-PP + FICZ) PBMCs. **B.** Correlations between *CYP1A1* and *CYP1B1* in all treatments. Dot plots with significant p-values are indicated in red. **C.** Summarised figure of B. **D.** Ratios between *CYP1A1* and *CYP1B1* during treatment. Boxplots show medians, interquartile ranges (box) and ranges of seven different subjects. Wilcoxon rank test for paired samples was used to compare treatments (*$p < 0.05$).

Table 29. *CYP1* ratios

Test subject	DMSO	FICZ	1-PP	1-PP + FICZ	1-PP + FICZ + CH-223191
1	2.60	103.89	44.77	408.53	140.84
2	1.52	10.34	6.99	74.71	39.05
3	2.61	4.70	9.75	24.81	27.99
4	2.65	11.46	24.04	23.34	35.47
5	1.54	4.33	8.97	41.34	17.12
6	1.41	2.35	4.07	7.42	6.21
7	2.85	18.16	15.22	195.63	85.34

The transcription of the *AHR* gene was down-regulated during FICZ and 1-PP co-treatment. In all individuals the *CYP1A1* and *CYP1B1* genes were induced during FICZ and 1-PP co-treatment. Subjects with an unregulated *AHR* gene (Figure 32, A, x=1) showed a stronger induction of the *CYP1B1* gene than individuals with a down-regulated *AHR* (Figure 32, A, x<1). The *CYP1A1* gene indicated the opposite. When cells were co-treated with 1-PP and FICZ, a significant negative correlation was detected between *CYP1A1* and *CYP1B1* transcripts (Figure 32, B). Non-significant trends were analysed in the FICZ and 1-PP single treatments, and in the 1-PP, FICZ and CH-223191 co-treatment (Figure 32, B). Addition of the CYP1 inhibitor 1-PP to a low FICZ concentration significantly increased the *CYP1A1*/*CYP1B1* ratio (Figure 32, D). This means a stronger induction of the *CYP1A1* gene than of the *CYP1B1* gene. Figure 32 and Table 29 summarised *CYP1A1/CYP1B1* ratios of all treatments.

3.3.11. Correlations between *CYP1* ratio and viability

Inverse correlations between the transcription of the *CYP1A1* and *CYP1B1* genes were examined in previous experiments. The transcription of the *CYP1A1* gene was negatively correlated with the *CYP1B1* transcription and differences in the regulation of the *CYP1* ratio were analysed. As CYP produce reactive intermediates, which could have cytotoxic effects, viability was correlated with *CYP1* ratio.

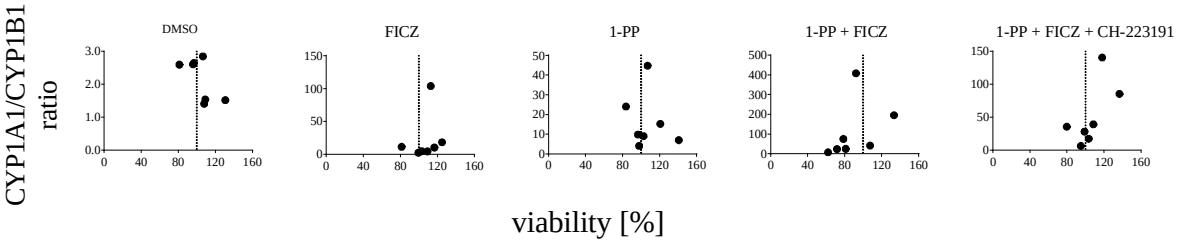


Figure 33. Correlations between *CYP1* ratio and viability
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. *CYP1A1* and *CYP1B1* gene transcriptions were analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene. Relative expression was calculated to medium-only control and ratios between both were calculated. Viability was detected with LDH assay. Correlations between *CYP1A1/CYP1B1* ratio and viability were calculated with spearman´s rank correlation coefficient. Line indicate 100%.

The correlations between the *CYP1A1/CYP1B1* RNA ratio and viability showed positive but not significant trends in 1-PP and FICZ single and co-treated cells. An increased ratio with a high transcription of the *CYP1A1* gene correlated with an increased viability particularly in co-treated cells, and when the AHR antagonist CH-223191 was present in the culture medium Figure 33.

3.3.12. Correlations between *CYP1* ratio and the transcription of genes involved in the AHR pathway

Inverse correlations between the transcription of the *CYP1A1* and *CYP1B1* genes were examined in previous analyses. *CYP1A1* transcription was negatively correlated with *CYP1B1* transcription. To study the relationship between CYP1 and *AHR*, *NRF2*, *ARNT*, *HIF1A*, *XME* and cytokine transcription levels, *CYP1* ratio was correlated with the transcription of these target genes.

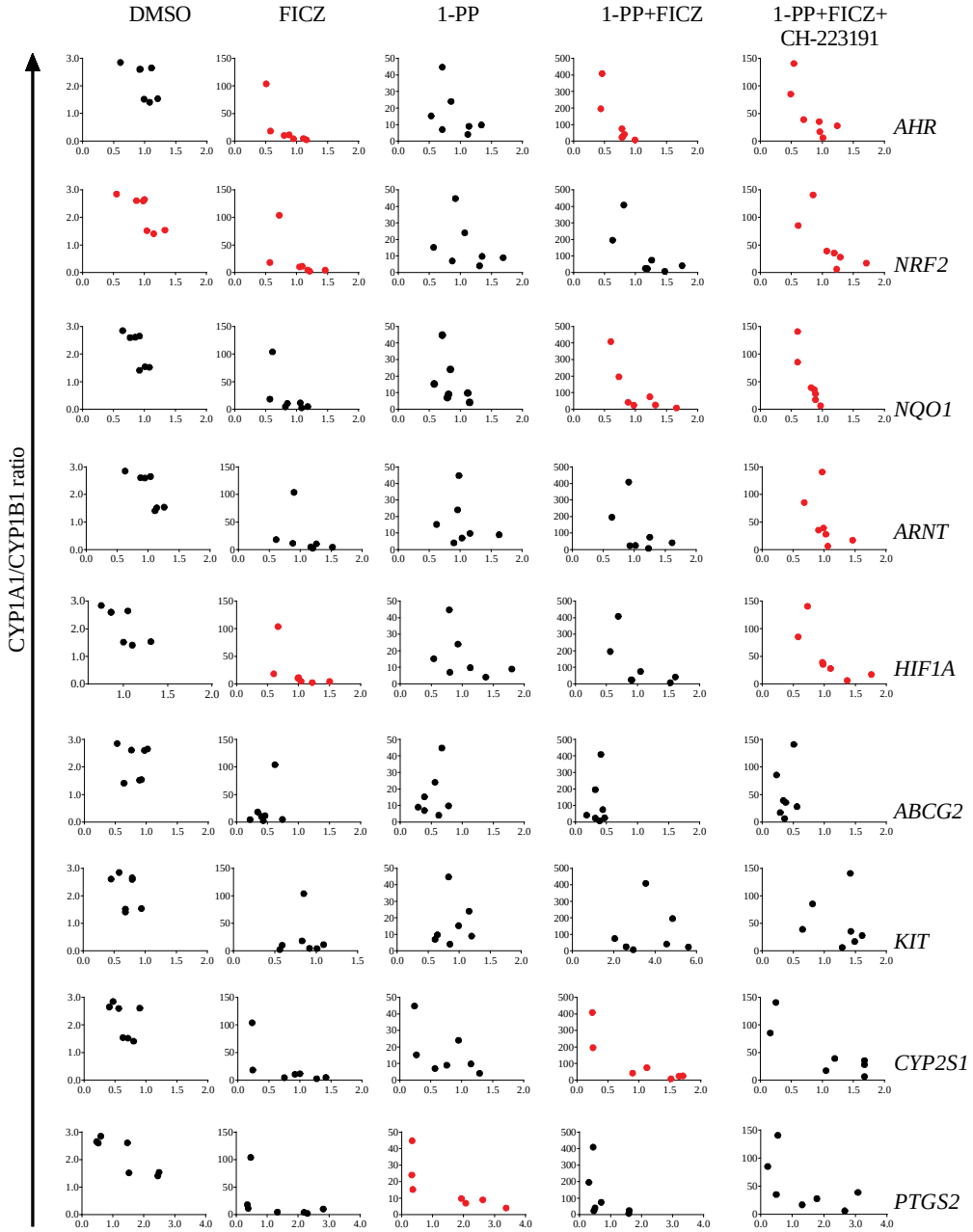


Figure 34. Correlations between *CYP1* ratio and genes coding for transcription factors, XMEs and c-Kit
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. *CYP1A1* and *CYP1B1* transcriptions were analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to the medium-only control. Ratios between both were calculated. Correlations between *CYP1A1/CYP1B1* ratio and target gene transcription levels were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

3.3.13. Correlations between *CYP1* ratio and cytokine expression

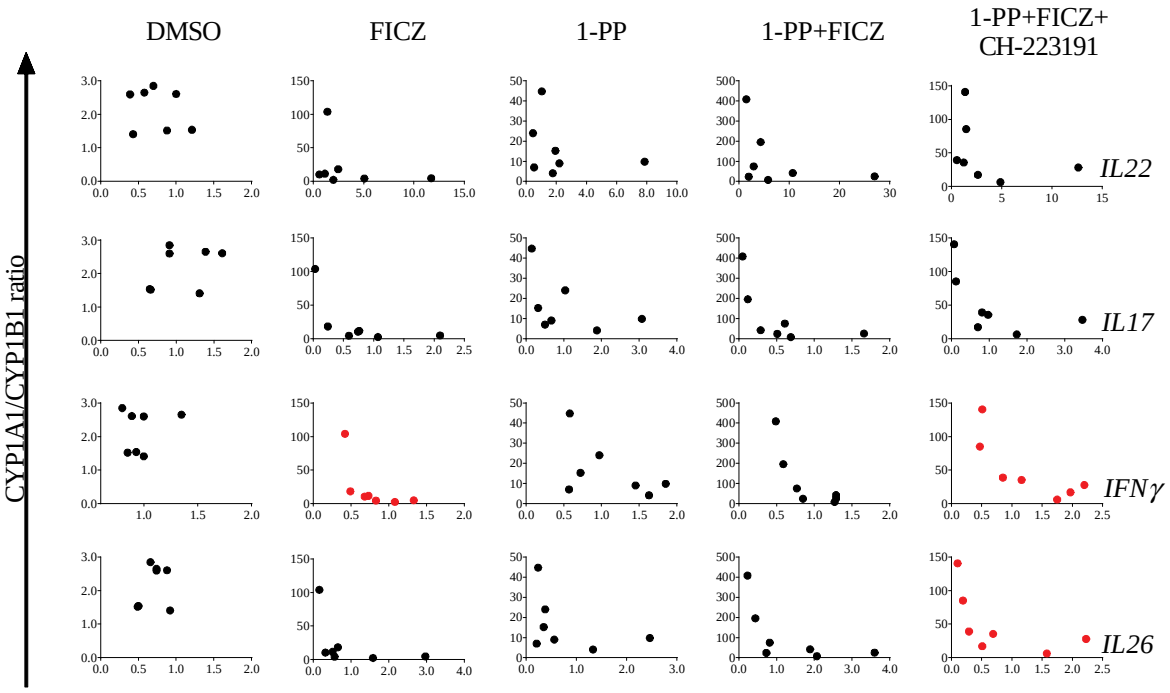


Figure 35. Correlations between *CYP1* ratio and cytokine transcription
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Transcription of *CYP1A1*, *CYP1B1* and cytokines was analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene, relative expression was calculated to medium-only control and ratios between both were calculated. Correlations between *CYP1A1/CYP1B1* ratio and cytokine transcription were calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

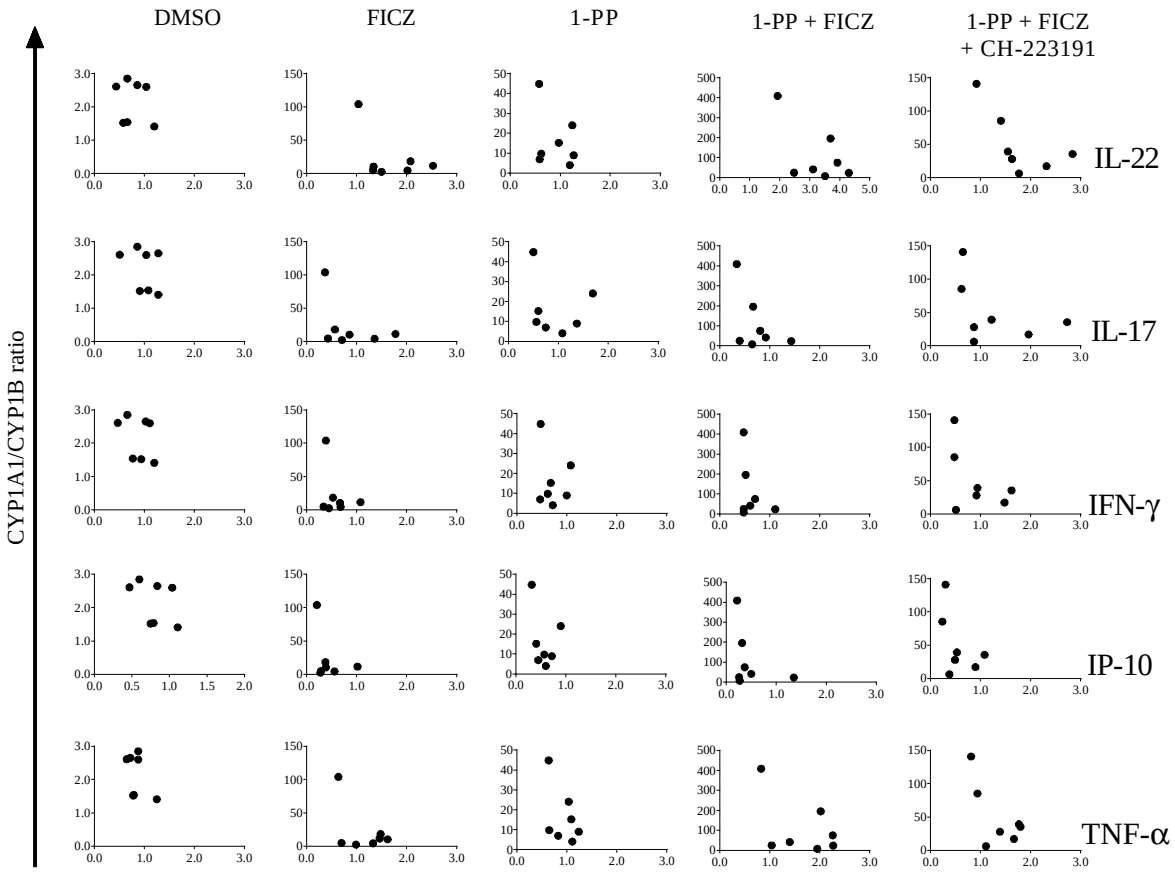


Figure 36. Correlations between *CYP1* ratio and cytokine expression
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. *CYP1A1* and *CYP1B1* gene transcriptions were analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene, relative expression was calculated to the medium-only control and ratios between both were calculated. Expression of cytokines was determined by ELISA. Correlations between *CYP1A1/CYP1B1* ratio and cytokine protein expression was calculated with spearman´s rank correlation coefficient. Dot plots with significant p-values are indicated in red.

The correlations between the *CYP1* ratio and the transcription of genes related to the AHR pathway indicated negative decays with almost all targets (Figure 34). The correlations with cytokine transcription indicated negative decays when FICZ was present in the culture medium, but not in treatments with the vehicle control (Figure 35 and Figure 36). These effects were less present on protein level. On RNA level but not on protein level, addition of the CYP inhibitor 1-PP alone reorganised correlations compared with DMSO control (Figure 35 and Figure 36). The *CYP1* ratio was significantly correlated with the transcription of the *IFNγ* and *IL26* genes (Figure 35). The protein expression of cytokines did not show any significant correlations.

3.3.14. Correlations between *CYP1* ratio and c-Kit expression

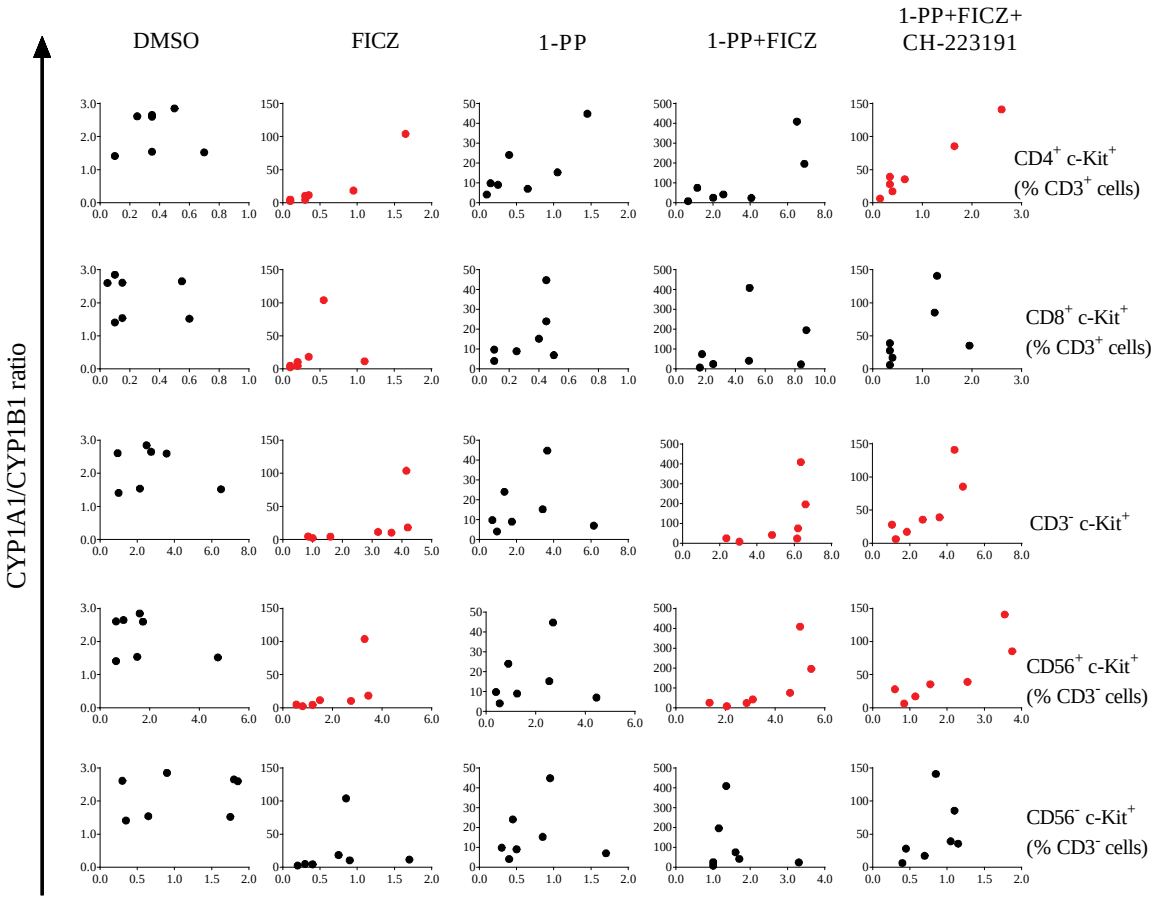


Figure 37. Correlations between *CYP1* ratio and c-Kit protein expression
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. *CYP1A1* and *CYP1B1* gene transcriptions were analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene, relative expression was calculated to medium-only control and ratios between both were calculated. Correlations between *CYP1A1/CYP1B1* ratio and c-Kit protein expression were calculated with spearman´s rank correlation coefficients. Dot plots with significant p-values are indicated in red.

Whereas *KIT* transcription did not correlate with *CYP1* ratio on RNA level, positive trends of *CYP1* ratio with c-Kit protein were detected during treatments. Significant correlations between *CYP1* ratio and c-Kit on $CD3^+$ and $CD3^-$ cell populations were detected when FICZ was present in the culture medium (Figure 37).

3.3.15. *CYP1* ratio correlated with the expression of AHR target genes and cytokines

Correlation coefficients and p-values were summarised as heatmaps.

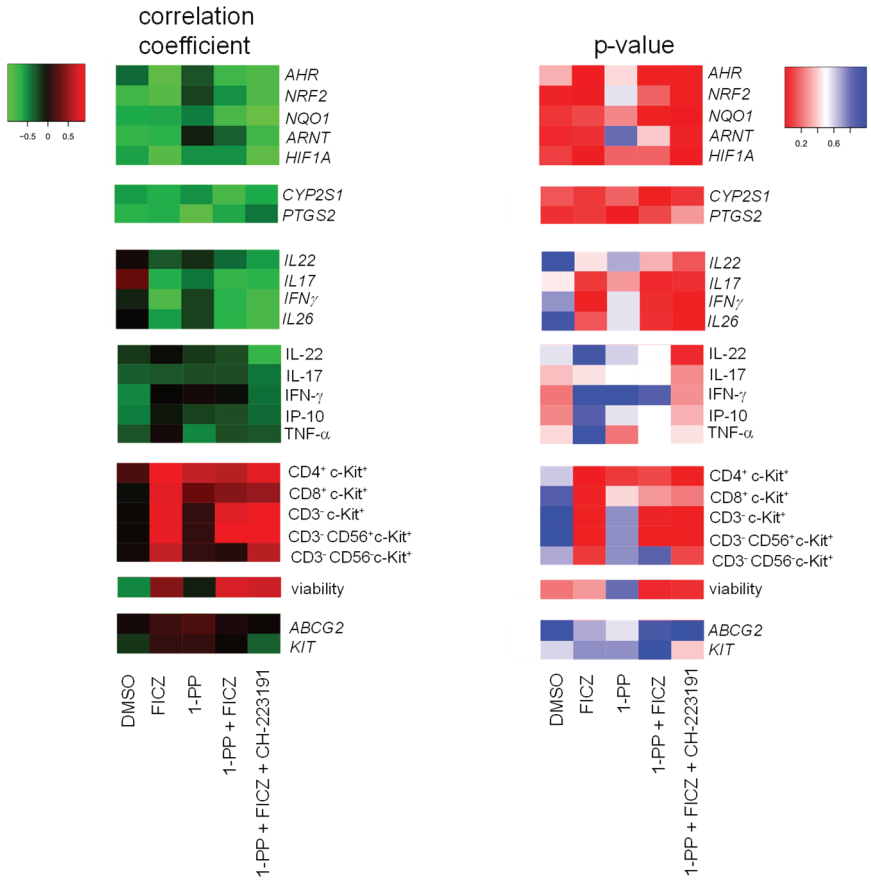


Figure 38. Correlation coefficients and p-values between *CYP1A1/CYP1B1* ratio and target gene expression
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Correlations between *CYP1A1/CYP1B1* ratio and target gene expression on RNA and protein level were calculated with spearman´s rank correlation coefficient. Coefficients and p-value were plotted as heatmaps. Shown are correlations between *CYP1* ratio and *AHR*, *NRF2*, *NQO1*, *ARNT*, *HIF1A*, *CYP2S1*, *PTGS2*, *IL22*, *IL17*, *IFNγ*, *IL26*, *ABCG2* and *KIT* genes, between *CYP1* ratio and IL-22, IL-17, IFN-γ, IP-10 and TNF-α cytokines, viability, and c-Kit receptor expression on $CD3^+$ and $CD3^-$ PBMCs.

The correlations of *CYP1* ratio with target gene transcription were blotted as heatmaps and summarised previous findings. Subgroups of investigated target genes correlated either stronger with a high *CYP1B1* and a high *AHR* transcription (green), or with a high *CYP1A1* and a low *AHR* transcription (red). Negative trends were detected with *AHR*, *NRF2*, *NQO1*, *ARNT*, *HIF1A*, *CYP2S1* and *PTGS2* in all treatments and vehicle control. Correlations of the cytokines *IL22*, *IL17*, *IFNγ* and *IL26* with the *CYP1* ratio changed to a negative correlation when FICZ was present in the culture medium. This was less in single treatments with 1-PP. Significant p-values between *CYP1* ratio and *AHR*, *NRF2*, *NQO1*, *HIF1A*, *ARNT*, *CYP2S1*, *PTGS2*, *IFNγ* and *IL26* reflected these correlations. c-Kit expression clearly changed to a positive correlation when FICZ was added to the culture medium. These effects were also detected

in co-treated cells and when the AHR antagonist CH-223191 was added. c-Kit on both $CD3^+$ and $CD3^-$ cells significantly correlated with the *CYP1* ratio. Correlation coefficients and p-values are summarised in Table 39. Conclusively, correlations of target gene expression with the *CYP1* ratio indicated that the selected compounds were differently correlated with the *CYP1* ratio and as shown previously with a high or low *AHR* transcription.

3.4. Analyses of 1-PP-dependent effects on the regulation of AHR pathway targets in human PBMCs

To investigate differences between 1-PP concentrations that either inhibited CYP1A1 activity alone (10^{-7} M) or the activities of CYP1A1 and CYP1B1 (10^{-6} M) in the experiments with CYP1 cell lines (Figure 12), human activated PBMCs were treated with these two different 1-PP concentrations. PBMCs were treated with 1-PP alone, 1-PP in the presence of FICZ (5×10^{-10} M) or 1-PP, FICZ and CH-223191 (3×10^{-6} M) for 5 days.

3.4.1. 1-PP-dependent transcription of genes related to the AHR pathway

The transcription of genes related to the AHR pathway and of cytokines were analysed by qRT-PCR. C_t-values were normalised to *EF1A* transcription and relative expression levels to medium-only control were calculated. Among the transcription factors involved in the AHR pathway and in oxidative stress response *AHR*, *NRF2* and *ARNT* were slightly but significantly up-regulated with 10^{-6} M 1-PP in PBMCs treated with 1-PP alone. An increased 1-PP concentration did not change *NRF2*, *AHR*, *ARNT* and *HIF1A* transcription levels in co-treated PBMCs (Figure 39). Mean fold changes are summarised in Table 40. Among target genes related to the AHR pathway, *KIT* transcription was induced with a 1-PP concentration of 10^{-6} M. Significant effects were detected in FICZ co-treated cells and when PBMCs were treated with 1-PP alone. Neither *ABCG2*, *NQO1* nor *PTGS2* were significantly up-regulated in a concentration-dependent manner. A concentration of 10^{-6} M 1-PP significantly decreased *ABCG2* transcription in 1-PP, FICZ and CH-223191 co-treated PBMCs (Figure 39). Mean fold changes are summarised in Table 40. Both *CYP1A1* and *CYP1B1* genes were significantly induced in FICZ and 1-PP co-treated cells whereas *CYP2S1* was unaffected by higher 1-PP concentrations. 1-PP with a concentration of 10^{-6} M additionally induced the transcription of *CYP1A1* when CH-223191 was added into the culture medium. Mean fold changes are summarised in Table 40.

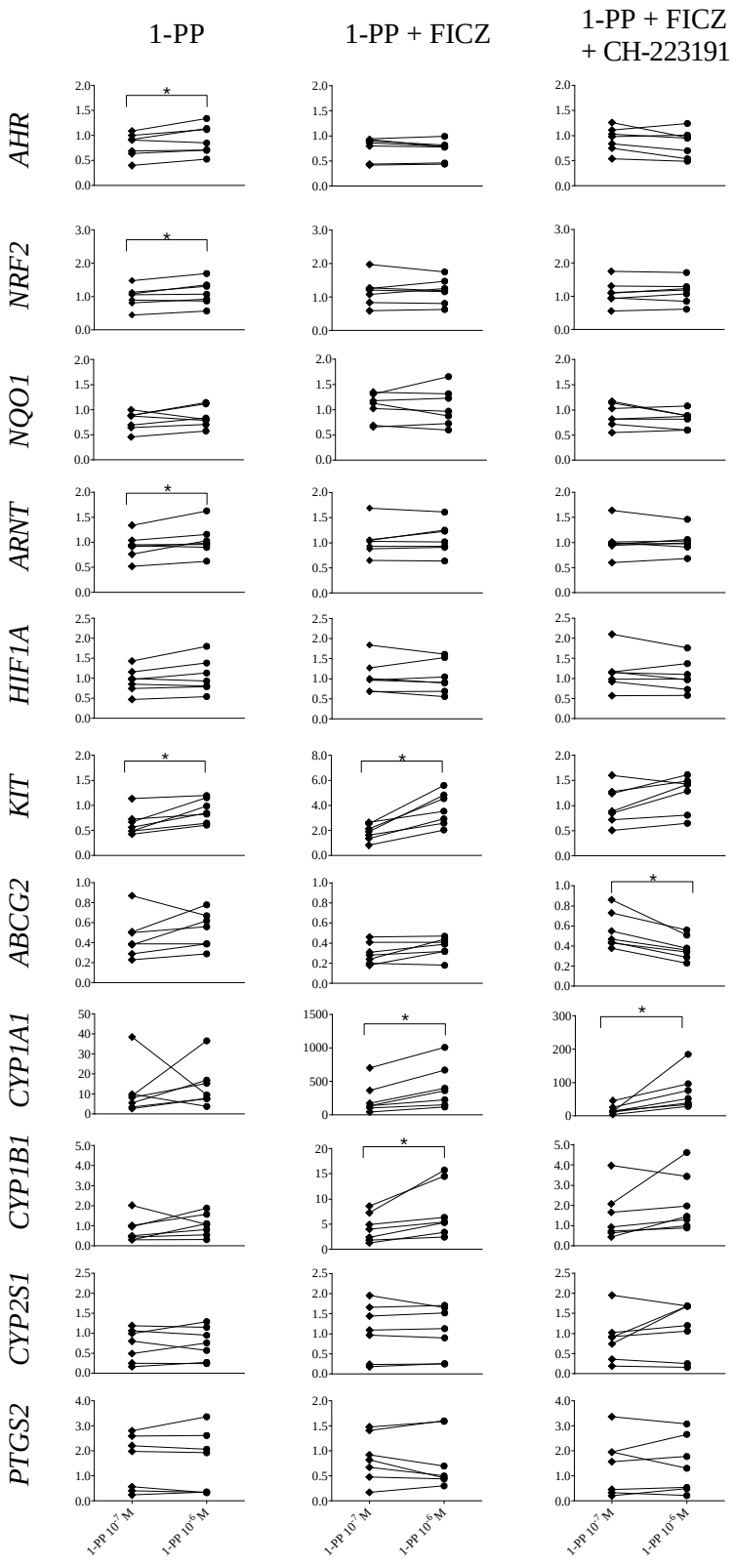


Figure 39. 1-PP-dependent regulation of genes involved in the xenobiotic response– RNA level
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. RNA expression levels of target genes related to the AHR pathway (*NRF2*, *AHR*, *NQO1*, *ARNT*, *HIF1A*, *KIT*, *ABCG2*, *CYP1A1*, *CYP1B1*, *CYP2S1* and *PTGS2*) were analysed by qRT-PCR. Target gene expression was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. Significant differences between 1-PP concentrations were calculated with Wilcoxon rank test for paired samples (*$p < 0.05$).

3.4.2. 1-PP-dependent transcription of *IL22*, *IFNγ*, *IL17* and *IL26*

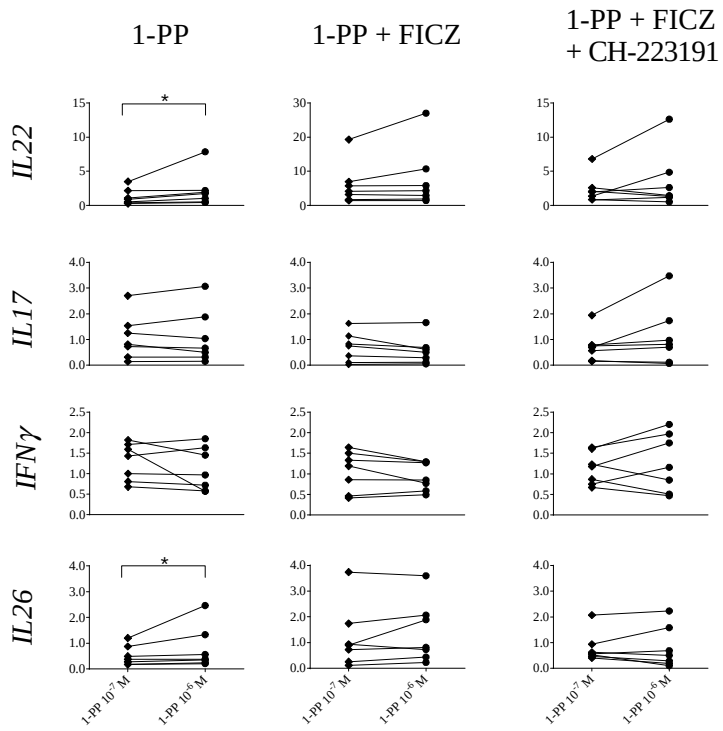


Figure 40. 1-PP-dependent cytokine transcription
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Transcription levels of genes coding for cytokines were analysed by qRT-PCR, target gene expression was normalised to *EF1A* as a housekeeping gene and relative expression was calculated to medium-only control. Significant differences between 1-PP concentrations were calculated with Wilcoxon rank test for paired samples (*$p < 0.05$).

Regarding the 1-PP-dependent regulation of different cytokines, transcription of *IL22* and *IL26* was significantly induced by 10^{-6} M compared with 10^{-7} M 1-PP in PBMCs treated with 1-PP alone. Positive trends for the transcription of the *IL22* gene were also detected in co-treated PBMCs. Conclusively, induction of the *CYP1A1*, *CYP1B1*, *KIT*, *IL22* and *IL26* genes by a higher 1-PP concentration underlined previous results that an augmented CYP1 inhibition increased AHR-regulated targets in human immune cells (Figure 40 and Figure 39). Mean fold changes are summarised in Table 40.

3.4.3. 1-PP-dependent expression of IL-22, IL-17, IFN-γ, TNF-α and IP-10

Human activated PBMCs were treated with FICZ, 1-PP, FICZ and 1-PP or FICZ, 1-PP and CH-223191 for 5 days. Cytokines in the supernatants were analysed by ELISA, data were normalised by proliferation and fold expressions related to medium-only control are shown.

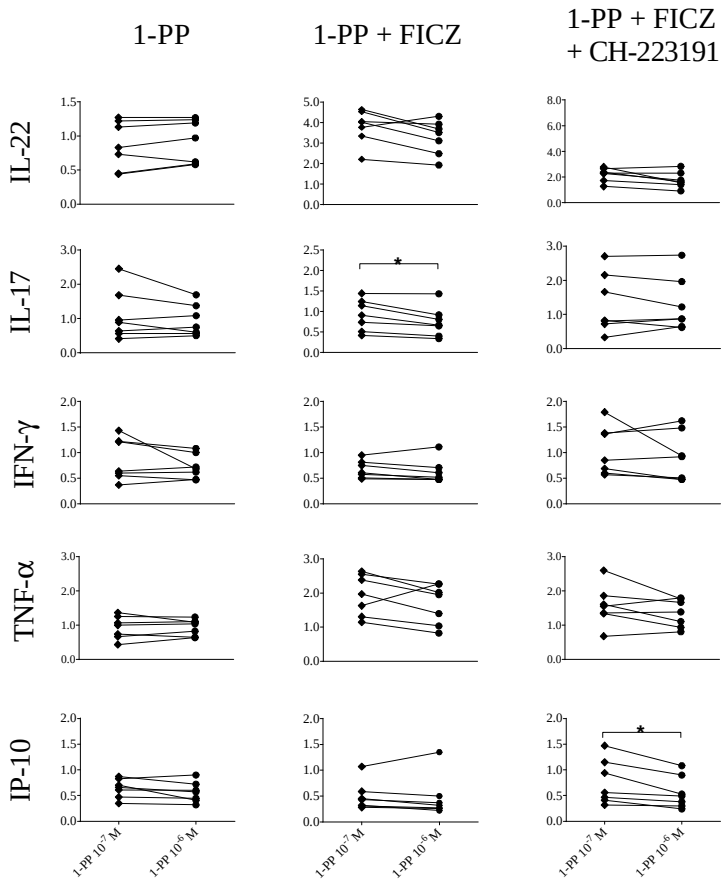


Figure 41. **1-PP-dependent cytokine production**
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Significant differences between 10^{-6} M 1-PP and 10^{-7} M 1-PP were calculated with Wilcoxon signed-rank test for paired samples (*$p < 0.05$).

Compared with 10^{-7} M 1-PP, a concentration of 10^{-6} M 1-PP slightly decreased the expression of IL-17 in 1-PP and FICZ co-treated cells and of IP-10 in the 1-PP, FICZ and CH-223191 co-treatment. Other investigated cytokines were not significantly regulated by 1-PP on protein level. However, IL-22 and TNF-α were down-regulated by trends with a concentration of 10^{-6} M 1-PP in the 1-PP and FICZ, and in the 1-PP, FICZ and CH-223191 co-treatments (Figure 41). Mean fold changes are summarised in Table 41.

3.4.4. 1-PP-dependent expression of c-Kit and IL-22 on $CD3^+$ and $CD3^-$ PBMCs

Activated PBMCs were treated with 1-PP, 1-PP and FICZ, or FICZ, 1-PP and CH-223191 for 5 days. The 1-PP-dependent regulation of c-Kit and IL-22 was determined by flow cytometry.

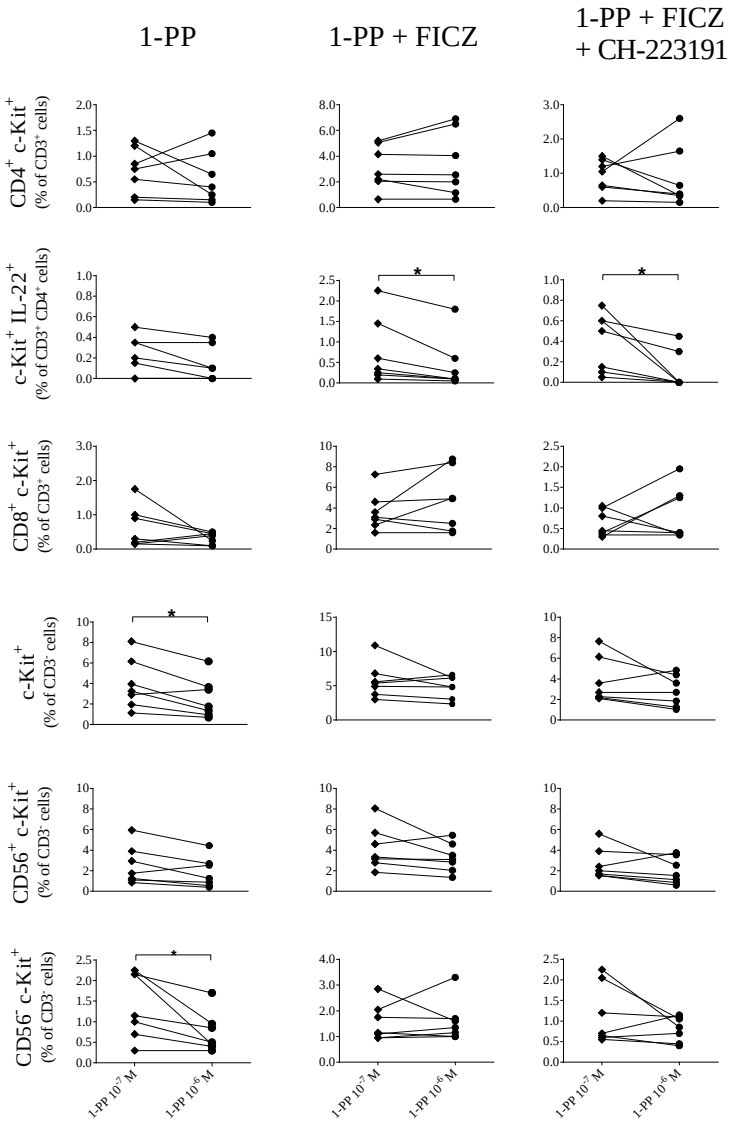


Figure 42. 1-PP-dependent regulation of c-Kit in $CD3^+$ and $CD3^-$ PBMCs
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M) and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Frequencies of c-Kit^+ and IL-22^+ cells were analysed by flow cytometry. Significant differences between 1-PP concentrations were calculated with Wilcoxon signed-rank test for paired samples (n=7) (*$p < 0.05$).

A significant concentration-dependent regulation of the c-Kit protein on $CD3^+CD4^+$ or $CD3^+CD8^+$ T cells was not detected. The frequency of c-$Kit^+IL\text{-}22^+$ cells was significantly reduced with a concentration of 10^{-6} M 1-PP in the 1-PP and FICZ, and in the 1-PP, FICZ and CH-223191 co-treatments. In single treated cells, a higher 1-PP concentration significantly reduced c-Kit on $CD3^-$ and on $CD3^-CD56^-$ PBMCs. In the other treatments, c-Kit on $CD3^-$ cells showed similar but not significant tendencies (Figure 42). Mean fold changes are summarised in Table 41.

3.4.5. 1-PP-dependent viability and proliferation

To analyse toxic or proliferative effects of FICZ and 1-PP treatments, viability was determined by LDH assay and flow cytometry. For LDH assay, medium control was set to 100%. Proliferation of cells was analysed by ^{3}H-thymidine assay.

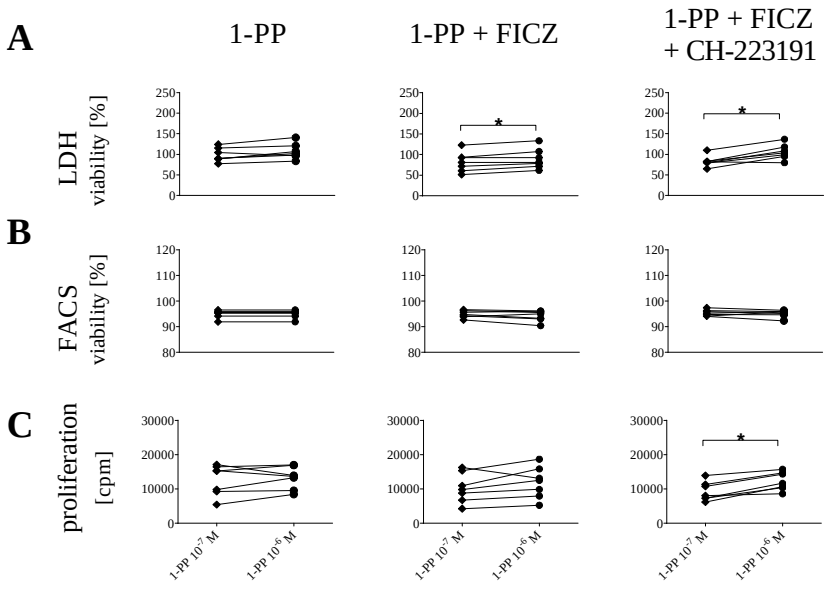


Figure 43. Concentration-dependent regulation of viability and proliferation
PBMCs (3 x 10^5 cells/well) activated with anti-CD3/CD28 antibodies were treated with the AHR agonist FICZ (5 x 10^{-10} M), the CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M) and the AHR antagonist CH-223191 (3x10^{-6} M) for 5 days. Viability was determined by LDH or with flow cytometry (FACS). Significant differences between 1-PP concentrations were calculated with Wilcoxon signed-rank test for paired samples (n=7) (*$p < 0.05$).

The LDH experiments indicated an increased viability with a higher 1-PP concentration. No changes in viability were detected by flow cytometry. Proliferation was slightly increased with a concentration of 10^{-6} M 1-PP in 1-PP, FICZ and CH-223191 co-treated cells. These data indicated that a higher 1-PP concentration was less toxic in the conducted experiments.

3.5. Characterisation of splenocytes from *Cyp1a2* knockout mice and C57BL/6 mice

The data indicated that CYP1 inhibition in human PBMCs increased AHR target gene expression. Thus, CYP1-mediated AHR activation takes place in human PBMCs treated with 1-PP in the presence of a low FICZ concentration. To test whether genomic deletion of CYP expression also affects IL-22 expression in mice, splenocytes from *Cyp1a2* knockout mice were treated with FICZ alone or in the presence of CH-223191.

3.5.1. CYP-dependent IL-22 regulation in *Cyp1a2* knockout mice and C57BL/6 mice

To investigate the mechanism of CYP1-mediated AHR activation in murine immune cells, splenocytes of either *Cyp1a2* knockout or C57BL/6 (wt) mice were investigated for IL-22 expression. Splenocytes were activated with anti-CD3/CD28 and treated with FICZ (5×10^{-10} M) or FICZ and CH-223191 (3×10^{-6} M) for 3 days. IL-22 and IFN-γ were determined in $CD4^+$ and $CD8^+$ splenocytes by flow cytometry.

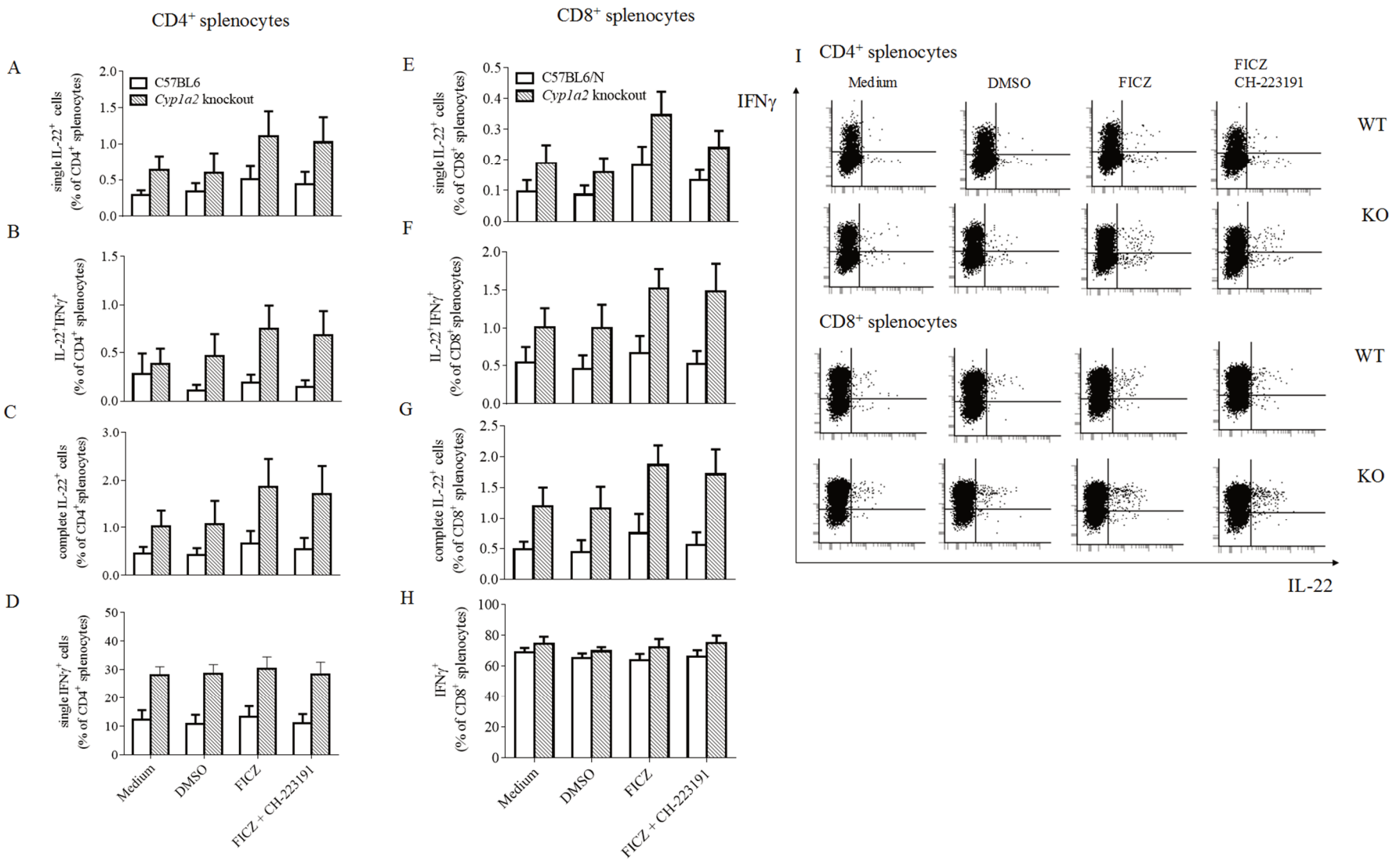


Figure 44. IL-22 and IFN-γ expression in CD4⁺ and CD8⁺ splenocytes isolated from *Cyp1a2* knockout mice

Murine splenocytes were activated with anti-CD3/CD28 antibodies. The AHR agonist FICZ (5×10^{-10} M) was used alone or together with the AHR antagonist CH-223191 (3×10^{-6} M) for 72 h. Percentages of IL-22⁺ and IFNγ⁺ cells in CD4⁺ and CD8⁺ splenocytes were analysed by flow cytometry (n=3). Means ± s.e.m are shown.

Splenocytes from *Cyp1a2* knockout mice had a higher percentage of IL-22$^+$ cells than splenocytes from C57BL/6 mice. In *Cyp1a2* knockout mice, treatment with FICZ increased the frequency of IL-22$^+$ and of IL-22$^+$IFN-γ^+ cells in both the CD4$^+$ and the CD8$^+$ compartment. CD4$^+$ splenocytes from *Cyp1a2* knockout mice showed a higher frequency of single IFNγ^+ cells than splenocytes from C57BL/6 mice. This effect was not detected in the CD8$^+$ compartment. However, the percentage of IFNγ^+ cells was higher in CD8$^+$ splenocytes than in CD4$^+$ splenocytes. Treatment of murine splenocytes with FICZ did not affect the frequency of IFNγ^+ cells, and IL-22$^+$ cells were only marginally reduced by the addition of the AHR antagonist CH-223191 (Figure 44).

3.6. AHR and CYP1-induced feedback pathways were active in PBMCs from CMCD patients

3.6.1. High-dose FICZ induced *CYP1A1*, *KIT* and *IL22* in CMCD

IL-22 and IL-17 levels deviate in patients suffering from a *STAT1*-dependent CMC disease (CMCD) compared with healthy donors. In the following experiments, FICZ in a high concentration was tested for the possibility to induce the AHR pathway and the cytokine IL-22 in PBMCs from CMCD patients.

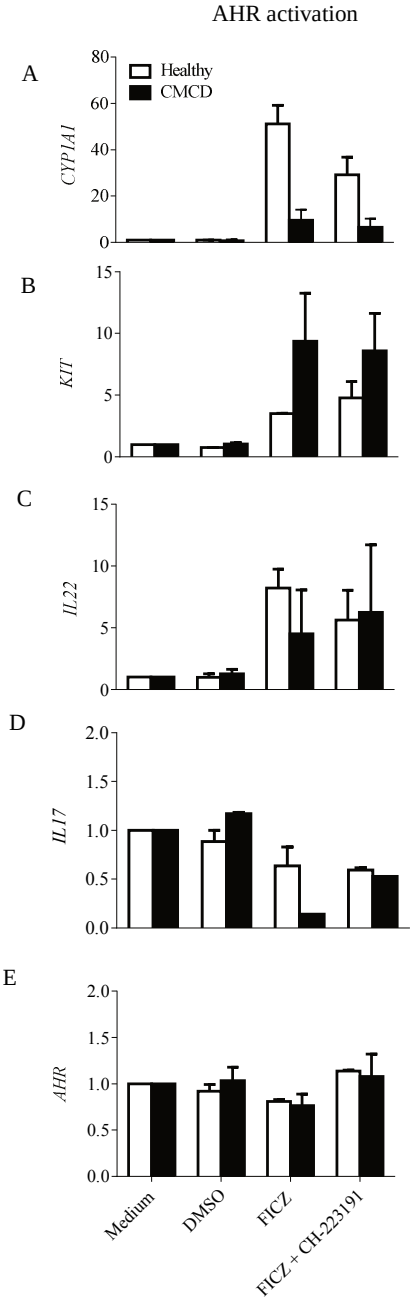


Figure 45. FICZ regulated *CYP1A1*, *KIT* and cytokine transcription in PBMCs from CMCD patients
PBMCs (3 x 10^5 cells/well) were activated with anti-CD3/CD28 antibodies with the AHR agonist FICZ (10^{-7} M) or with FICZ in the presence of the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Transcription levels were analysed by qRT-PCR. Means ± s.e.m. are shown (n=2).

Human activated PBMCs from healthy subjects and CMCD patients were treated with a high concentration of FICZ (10^{-7} M) or with FICZ in the presence of the AHR antagonist CH-223191 for 5 days. DMSO in all treatments was adjusted to the same concentration and transcription of genes was analysed with qRT-PCR. On mRNA level, *CYP1A1*, *KIT* and *IL22* were induced in healthy subjects and in CMCD patients by FICZ, but not by the vehicle control (DMSO) (Figure 45 A-C). Compared with healthy subjects, *CYP1A1*, *KIT*, *IL22* and *IL17* transcription levels inclined to be differently regulated in CMCD patients. *CYP1A1* and *IL22* were lower, whereas *KIT* transcription showed a higher induction in CMCD patients than in healthy donors. *IL17* was strongly down-regulated by FICZ in CMCD patients and to a lesser extent in healthy subjects. The expression of *AHR* was marginally reduced by the addition of FICZ in both healthy subjects and CMCD patients and differences between healthy subjects and CMCD patients were not detected. The addition of the AHR antagonist CH-223191 to FICZ changed the FICZ effects only marginally (Figure 45 A-E). Fold changes are summarised in Table 42.

3.6.2. High-dose FICZ induced IL-22 but not IL-17 and IP-10 in CMCD

The induction of AHR-regulated genes with a high FICZ concentration showed that the AHR pathway could be activated in PBMCs from CMCD patients. After 5 days, supernatants of activated PBMCs were analysed for IL-22, IL-17 and IP-10 expression levels by ELISA. Raw data of cytokine release were normalised by proliferation.

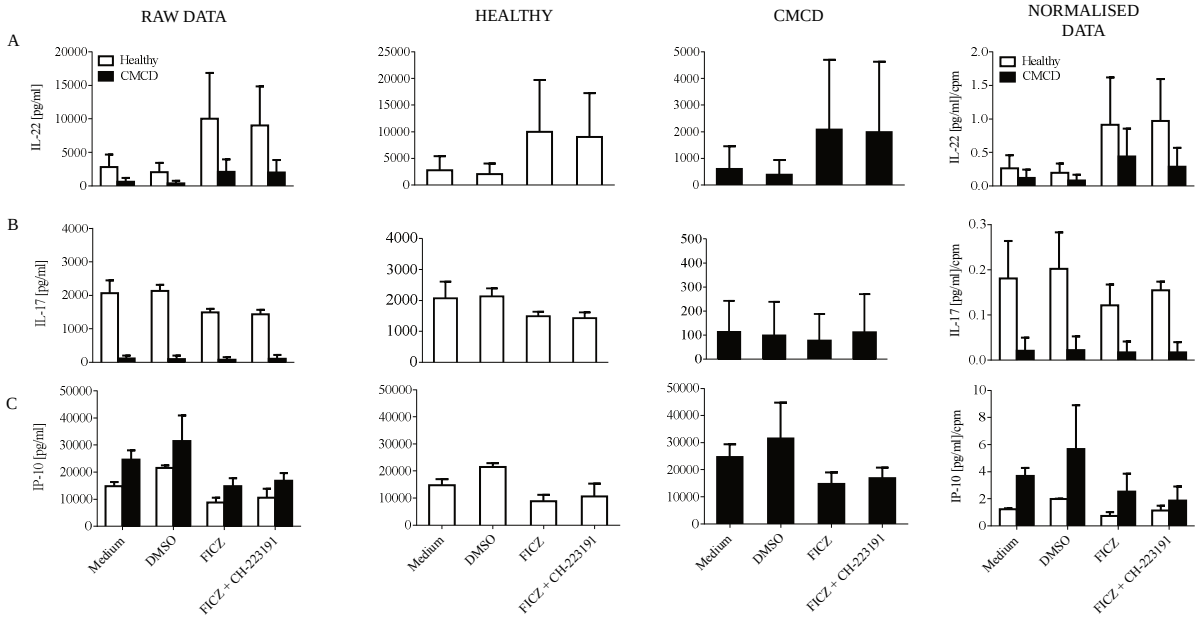


Figure 46. FICZ regulated IL-22, IL-17 and IP-10 in PBMCs from CMCD patients
PBMCs (3 x 10^5 cells/well) were activated with anti-CD3/CD28 antibodies in presence of the AHR agonist FICZ (10^{-7} M) alone or together with the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Cytokine expression was determined by ELISA. Means ± s.e.m. are shown (n=2).

In all treatments, IL-17 and IL-22 were determined with lower concentrations in CMCD patients than in healthy donors. This was shown in both raw and normalised data. The addition of FICZ induced IL-22 expression in CMCD patients and in healthy controls. The normalised data showed that IP-10, a cytokine downstream of STAT1, was detected with higher levels in CMCD patients than in healthy individuals. Similar to the RNA data, the addition of CH-223191 has less effects on PBMCs treated with a high dose of FICZ. Fold changes are summarised in Table 43.

3.6.3. High-dose FICZ induced c-Kit in CMCD

Following the results of *KIT* induction on mRNA level, FICZ-dependent regulation of c-Kit protein in PBMCs from CMCD patients and healthy donors was examined. Activated PBMCs were treated with the AHR agonist FICZ and the AHR antagonist CH-223191 for 5 days. c-Kit expression was analysed by flow cytometry.

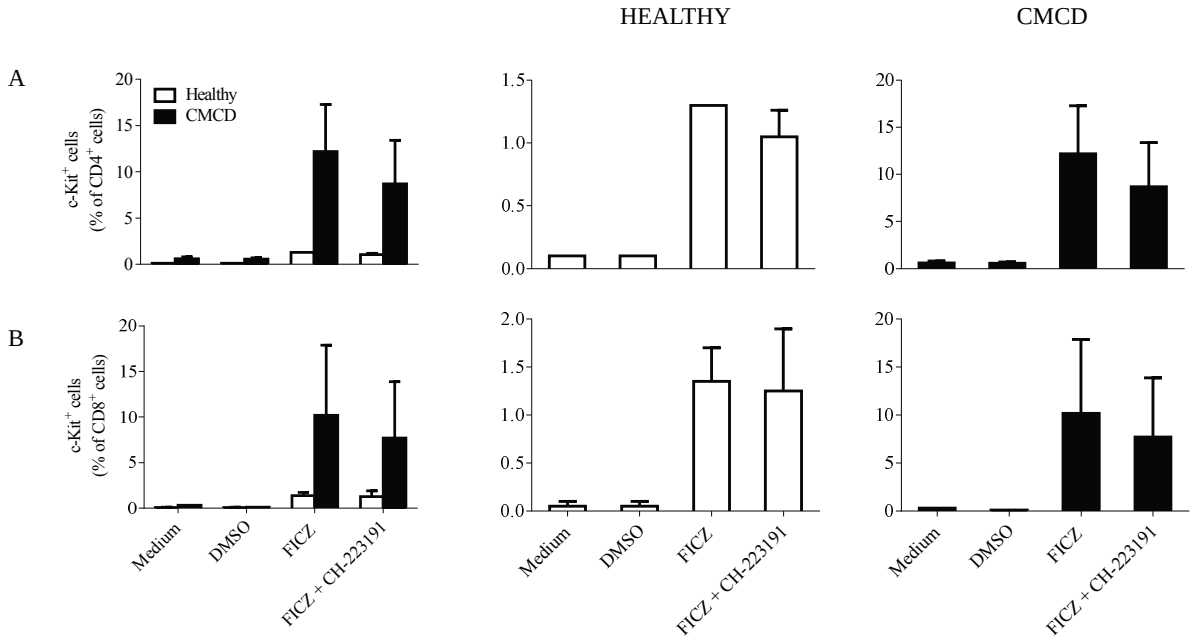


Figure 47. FICZ induced c-Kit expression in PBMCs from CMCD patients
PBMCs (3 x 10^5 cells/well) were activated with anti-CD3/CD28 antibodies in presence of the AHR agonist FICZ (10^{-7} M) alone or together with the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. c-Kit expression was analysed by flow cytometry in **A.** $CD4^+$ and **B.** $CD8^+$ lymphocytes. Means ± s.e.m. are shown (n=2).

The AHR agonist FICZ up-regulated c-Kit in PBMCs from healthy and from CMCD subjects. However, in the $CD4^+$ and the $CD8^+$ compartments c-Kit was more strongly induced in CMCD patients than in healthy donors. The AHR antagonist CH-223191 inhibited the FICZ effects only marginally (Figure 47).

3.6.4. CYP1 inhibition up-regulated *CYP1A1*, *KIT* and *IL22* in CMCD

The AHR targets *CYP1A1*, IL-22 and c-Kit were induced with a high FICZ concentration in PBMCs from CMCD patients, indicating that the AHR pathway was inducible in these subjects. Therefore, the AHR feedback regulation by inhibition of CYP1 activity in PBMCs from CMCD patients and in comparison to healthy donors was tested in following experiments. RNA expression in human activated PBMCs treated with a low dose of FICZ (5×10^{-10} M), with 1-PP (10^{-6} M), FICZ and 1-PP or FICZ, 1-PP and the AHR antagonist CH-223191 (3×10^{-6} M) was analysed with qRT-PCR.

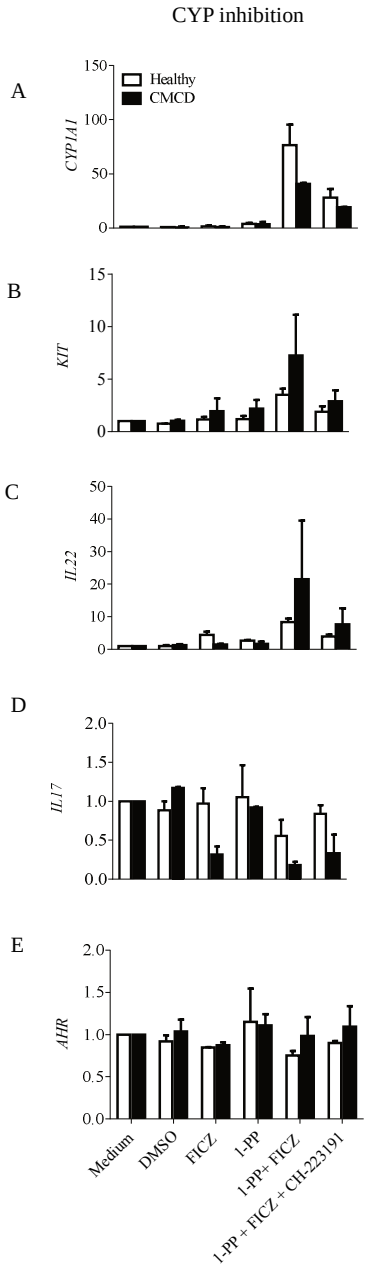


Figure 48. CYP1 inhibition regulated *CYP1A1, KIT* and cytokine transcription in PBMCs from CMCD patients

PBMCs (3×10^5 cells/well) were activated with anti-CD3/CD28 antibodies in the presence of the AHR agonist FICZ (5×10^{-10} M) alone, together with the CYP1 inhibitor 1-PP (10^{-6} M) or with 1-PP, FICZ and the AHR antagonist CH-223191 (3×10^{-6} M) for 5 days. Means ± s.e.m. are shown (n=2).

Inhibition of CYP1 activity up-regulated the transcription of *CYP1A1*, *IL22* and *KIT*. In co-treatments, *KIT* was higher and *CYP1A1* was lower transcribed in PBMCs from CMCD patients than in PBMCs from healthy donors. This was similar to the stimulations with a high FICZ concentration. In the 1-PP and FICZ co-treatment, the transcription of *IL17* confirmed the results with the high FICZ concentration. The cytokine *IL17* was more strongly down-regulated in CMCD patients than in healthy donors. Antagonising AHR with CH-223191 inverted all 1-PP-induced effects in both CMCD and healthy PBMCs. Fold changes are summarised in Table 44.

3.6.5. CYP1 inhibition up-regulated IL-22 but repressed IL-17 and IP-10 in CMCD

The RNA data indicated that inhibition of CYP1 activity by 1-PP up-regulated AHR pathway genes in PBMCs from CMCD patients. To study the effects of CYP1-induced AHR activation on cytokine expression, supernatants of treated PBMCs were analysed by ELISA. Determined concentrations were normalised to proliferation.

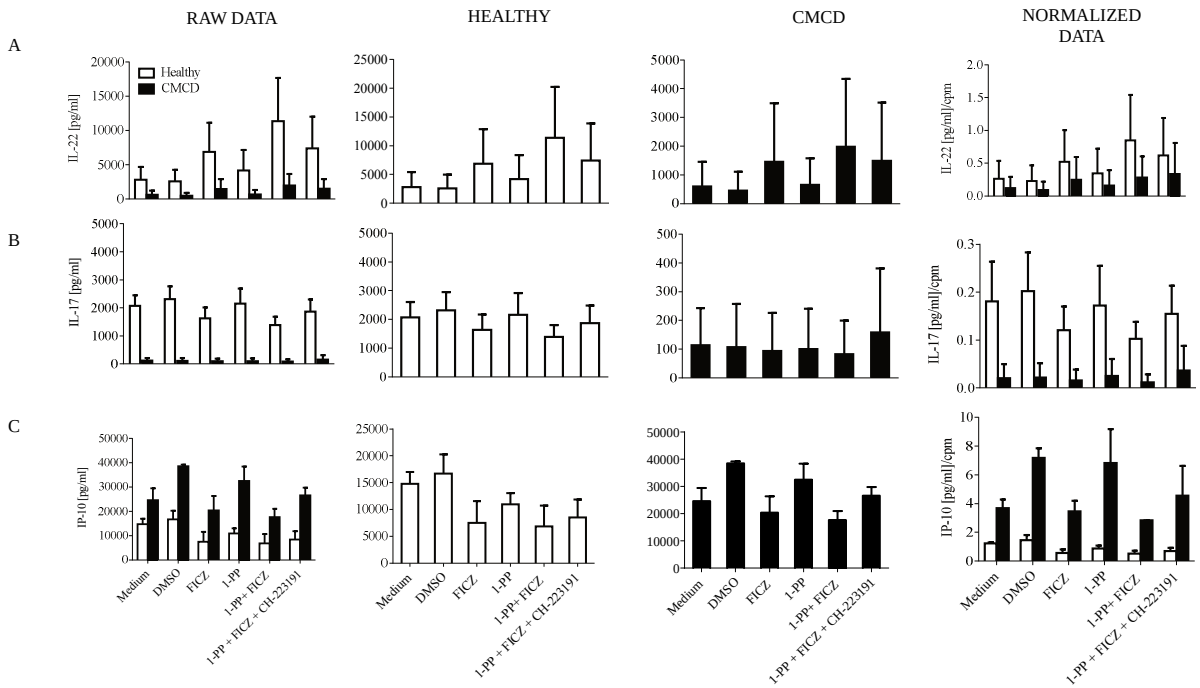


Figure 49. CYP1 inhibition regulated cytokine expression in PBMCs from CMCD patients
PBMCs (3 x 10^5 cells/well) were activated with anti-CD3/CD28 antibodies in presence of the AHR agonist FICZ (5 x 10^{-10} M) alone, together with the CYP1 inhibitor 1-PP (10^{-6} M), or 1-PP, FICZ and the AHR antagonist CH-223191 (3 x 10^{-6} M) for 5 days. Means ± s.e.m. are shown (n=2).

In all treatments, diseased PBMCs had lower IL-22 and IL-17 levels and a higher concentration of IP-10 than PBMCs from healthy subjects. As described previously, a low dose of the AHR agonist FICZ induced IL-22 in PBMCs from healthy controls and from CMCD patients. CYP1 inhibition by 1-PP enhanced FICZ-induced IL-22 expression. In healthy subjects, IL-17 and IP-10 were decreased with FICZ alone and in co-treated cells compared with the vehicle control. IL-17 in CMCD patients was low expressed and marginally regulated. IP-10 showed a high expression level in CMCD patients and was reduced with FICZ alone and in co-treated cells. Addition of the AHR antagonist CH-223191 decreased the IL-22 expression and tended to induce IL-17 and IP-10. Fold changes are summarised in Table 45.

3.6.6. CYP1 inhibition up-regulated c-Kit in CMCD

A high concentration of FICZ up-regulated c-Kit in PBMCs from CMCD patients, and a 1-PP-dependent AHR activation was present in these cells. Therefore, CYP1-dependent c-Kit regulation in PBMCs from CMCD patients and healthy volunteers was investigated. PBMCs were activated and treated with a low concentration of FICZ (5×10^{-10} M), with 1-PP (10^{-6} M), a combination of both, or with FICZ, 1-PP and CH-223191 (3×10^{-6} M) for 5 days. c-Kit expression was analysed on $CD4^+$ and $CD8^+$ PBMCs by flow cytometry.

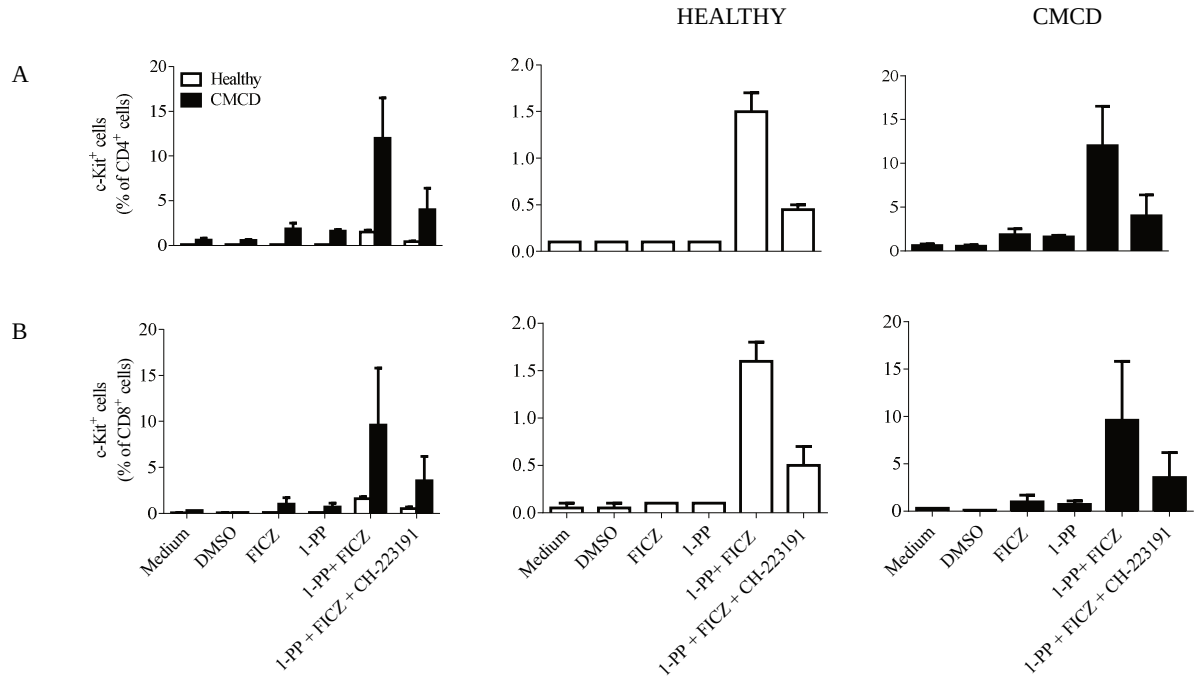


Figure 50. CYP1 inhibition induced c-Kit expression on PBMCs from CMCD patients
PBMCs (3×10^5 cells/well) were activated with anti-CD3/CD28 antibodies in presence of the AHR agonist FICZ (5×10^{-10} M) alone, together with the CYP1 inhibitor 1-PP (10^{-6} M) or with 1-PP, FICZ and the AHR antagonist CH-223191 (3×10^{-6} M) for 5 days, n=2. Means ± s.e.m. are shown (n=2).

c-Kit was induced on PBMCs from CMCD patients in single treatments with a low dose of FICZ or 1-PP. These effects were lower in cells from healthy subjects. Additionally, FICZ and 1-PP co-treated cells clearly up-regulated c-Kit compared with single incubations. Similar to the incubations with a high FICZ concentration, c-Kit was more strongly induced in PBMCs from CMCD patients than in PBMCs from healthy subjects in both the $CD4^+$ and the $CD8^+$ PBMCs. Contrary to the high FICZ concentration, the addition of the AHR inhibitor CH-223191 clearly reduced CYP1-induced c-Kit expression.

In conclusion of the combined experiments in 3.6, IL-22 and IL-17 were only marginally expressed in PBMCs from CMCD patients with an overactive STAT1 protein. The AHR agonist FICZ promoted the AHR pathway as shown by the elevated transcription of the AHR target *CYP1A1*. The results indicated an active AHR pathway in patients with a *STAT1*-dependent CMCD. FICZ treatment increased IL-22 and c-Kit and reduced IL-17 and IP-10 levels. IP-10 and c-Kit were higher expressed in PBMCs from CMCD patients than in PBMCs from healthy subjects. Inhibition of CYP1 activity by 1-PP presumably led to an accumulation of FICZ and an enhanced AHR-dependent *CYP1A1*, *KIT* and *IL22* up-regulation. Although IL-22 expression did not reach the same level as detected in healthy subjects after treatment with a high FICZ concentration or with FICZ and 1-PP, both treatments induced IL-22 expression in CMCD. The AHR pathway was only partially inhibited by CH-223191 and this inhibition was less efficient in PBMCs treated with a high dose of FICZ than in PBMCs treated with a low FICZ concentration. Noteably, for all experiments only limited numbers of CMCD patients with different disease phenotypes and *STAT1* mutations were available. However, the results need to be interpreted with caution. Higher numbers of patients are needed to confirm these findings.

3.7. Differential expression of xenobiotic-metabolising enzyme (XME) genes in human immune cell subpopulations

An AHR-dependent and CYP1-mediated mechanism in primary human immune cells was elaborated in this thesis. IL-22 and c-Kit, immunological targets for AHR activation, were induced by CYP1 inhibition in human immune cells when a low dose of the AHR agonist FICZ was present. This implies a modulating function of CYP enzymes in human immune cells. To map this environmentally driven pathway and because evidence of CYP transcription in human primary immune cells is rare, different immune cells were screened for their expression of genes encoding xenobiotic-metabolising enzymes. These are involved in the metabolism of exogenous or endogenous small molecules. Constitutive expression of these genes was studied in lymphoid- or myeloid-derived immune cell subpopulations. That the level was constitutive was guranteed by the RNA isolation immediately upon finishing cell isolations. RNA was transcribed into cDNA and analysed by TaqMan Low Density Arrays (TLDAs) spotted with custom-made primer and probes for phase I, phase II and phase III genes. Data were normalised by the housekeeping gene *GAPDH* and log2-transformed relative expression to *HPRT1* as

a second housekeeping gene in each cell type was plotted. As human primary mast cells and basophils were in a special focus of this project in the beginning (Effner, 2008), different mast cell marker genes were additionally used as a transcriptional control.

3.7.1. Proband characteristics, cell purities and RNA qualities for TLDAs

Tables are summarised in supplemental material (Table 46 to Table 52).

3.7.2. XME transcription profile in various human and primary immune cells

To analyse the xenobiotic-metabolising capacity of different primary immune cell subpopulations, RNAs were immediately isolated after cell purifications, transcribed into cDNA and analysed by TLDAs. In the shown heatmaps, rows represent subjects and colums represent distinct genes. Black squares indicated not-detected transcripts.

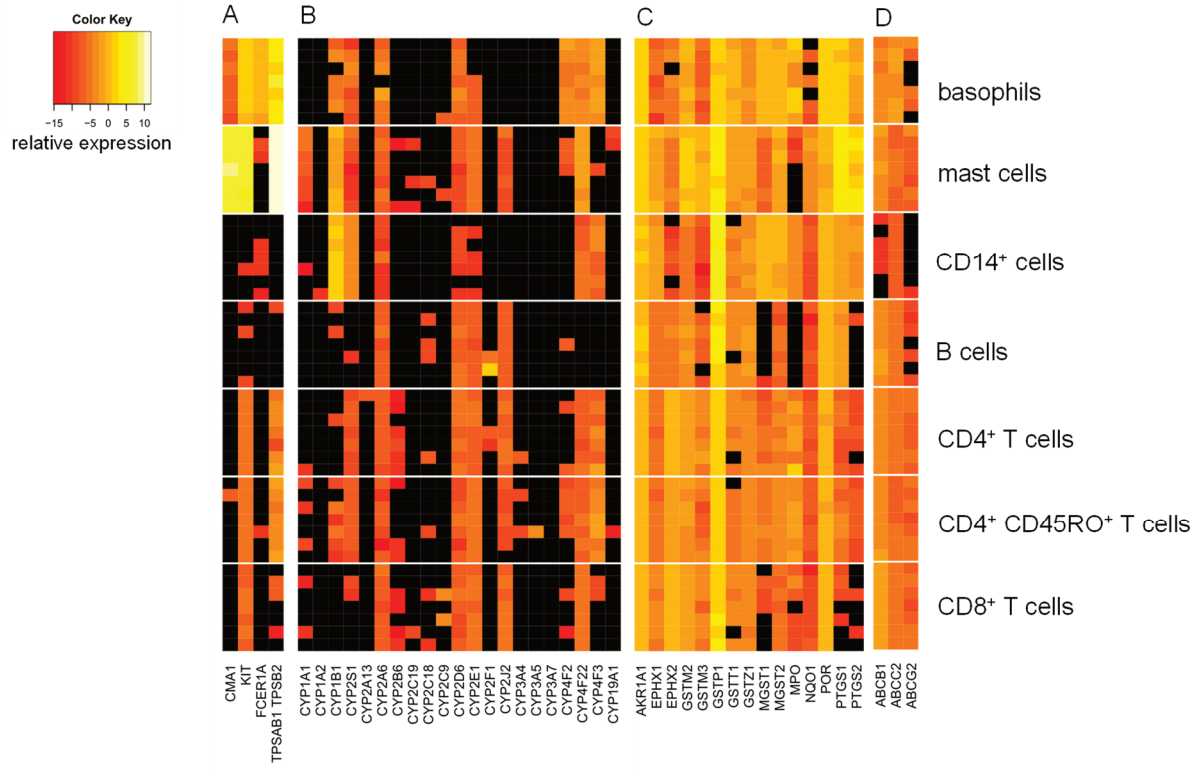


Figure 51. CYP and XME transcription profile in different human immune cell subtypes

cDNA isolated from different human immune cell subpopulations was screened with TLDAs for the presence of **A**, mast cell marker genes, **B**, phase I (cytochrome P450), **C**, phase I and II and **D**, phase III transcripts. Ct-values were normalised with the housekeeping gene *GAPDH*. Log2-transformed data relative to *HPRT1* expression in each cell type are shown. Each row represents an individual donor, each column a distinct gene and black squares represent not detected transcripts. Abbreviations are summarised in Table 15.

Due to their close functional and phenotypic proximity, human primary mast cells and primary basophils express the mast cell marker genes *CMA1, KIT* and tryptase. However, mast cells showed a higher relative transcription level of these control genes than basophils. The alpha chain of the high affinity IgE receptor (*FcER1A*) was most abundantly detected in basophils, but detected with a very low transcriptional level and frequency in primary mast cells. Besides the high transcription of the *KIT* gene in mast cells and basophils, *KIT* transcription was also detected in $CD4^+$ Th cells, $CD4^+CD45RO^+CD45RA^-$ memory Th cells and $CD8^+$ Tc cells, but not in B lymphocytes. The mast cell marker gene coding for tryptase was also detected among T lymphocytes, especially with a higher frequency in the $CD4^+$ compartment.

Each immune cell subpopulation showed a typical gene transcription pattern for xenobiotic-metabolising enzymes. Genes encoding CYP enzymes were more variable transcribed than genes coding for phase II or phase III proteins, which were homogeneously and abundantly expressed in human immune cell subpopulations. The transcription of *CYP* genes was only basal and less frequent than the transcription of phase II and phase III coding genes. Differently expressed *CYP* genes include enzymes involved in the metabolism of exogenous and endogenous (*CYP1A1, CYP1B1, CYP4F2, CYP4F3, CYP2J2*) and of unknown (*CYP4F22*) substrates. The *CYP2D6, CYP2A6* and *CYP2E1* transcripts were detected with the highest frequencies. The RNA for the phase II enzyme GSTP1 indicated the highest transcription level among all cell populations. The phase III transporter proteins were ubiquitously expressed except *ABCG2*, which lacks a transcription in $CD14^+$ monocytes. Simultaneous transcription of the three investigated AHR-regulated CYP enzymes, *CYP1A1, CYP1B1* and *CYP2S1* was only detected in human primary foreskin mast cells. *CYP1B1* was well transcribed in myeloid cells with the highest transcription level in $CD14^+$ monocytes. It was also found in human primary foreskin mast cells and showed a basal transcription in basophils and in $CD4^+$ memory Th cells. Noteworthy, the frequency of *CYP1B1* was higher in $CD4^+$ memory Th cells than in complete $CD4^+$ Th cells. As a hepatic and lung expressed CYP, *CYP1A2* was not detected in immune cells. The AHR target *CYP1A1* was detected with a very low frequency among T cells. Here, $CD4^+$ memory Th cells tended to have a higher frequency than complete $CD4^+$ Th cells. Mast cells and all investigated lymphocytes including B cells transcribed *CYP2J2*, which was not detected in $CD14^+$ monocytes and basophils. T lymphocytes and myeloid-derived cells transcribed *CYP4F22*. $CD4^+$ Th cells, $CD14^+$ monocytes and basophils were positive for the *CYP4F3* RNA. $CD4^+$ memory Th cells and basophils showed the highest frequency of *CYP4F2* transcription. In conclusion, various immune cell subtypes were characterised by a specific CYP transcription pattern while 17 genes coding for XMEs were transcribed in all investigated immune cell populations. Among these, only three CYP1-coding phase I genes (*CYP2A6, CYP2D6* and *CYP2E1*) were transcribed in nearly all cell types and the majority of transcribed genes belong to the phase II and the phase III metabolism (*AKAR1A1, EPHX1, EPHX2, GSTM2, GSTM3, GSTP1, GSTT1, GSTZ1, MGST2, NQO1, POR, PTGS1, ABCG1, ABCC2*). Relative transcription values are summarised in Table 53 and Table 54.

4 Discussion

Evidence is increasing that the ligand-activated transcription factor AHR impacts cell differentiation and mediates diverse cellular reactions in the immune system (Di Meglio et al., 2014; Duarte et al., 2013; Stockinger et al., 2014; Quintana et al., 2010; Veldhoen et al., 2008). Previous studies also indicated that the degradation of AHR ligands by CYP1 enzymes limits AHR activation and, *vice versa*, that CYP1 inhibition prolongs receptor induction (Chang and Puga, 1998; Chiaro et al., 2007; Puga et al., 1990; RayChaudhuri et al., 1990; Wincent et al., 2012). This study sheds light on the function of CYP1 enzymes acting downstream of the AHR in human immune cells. Thus, it extends the observations of a CYP1-dependent AHR feedback regulation to human immune cells.

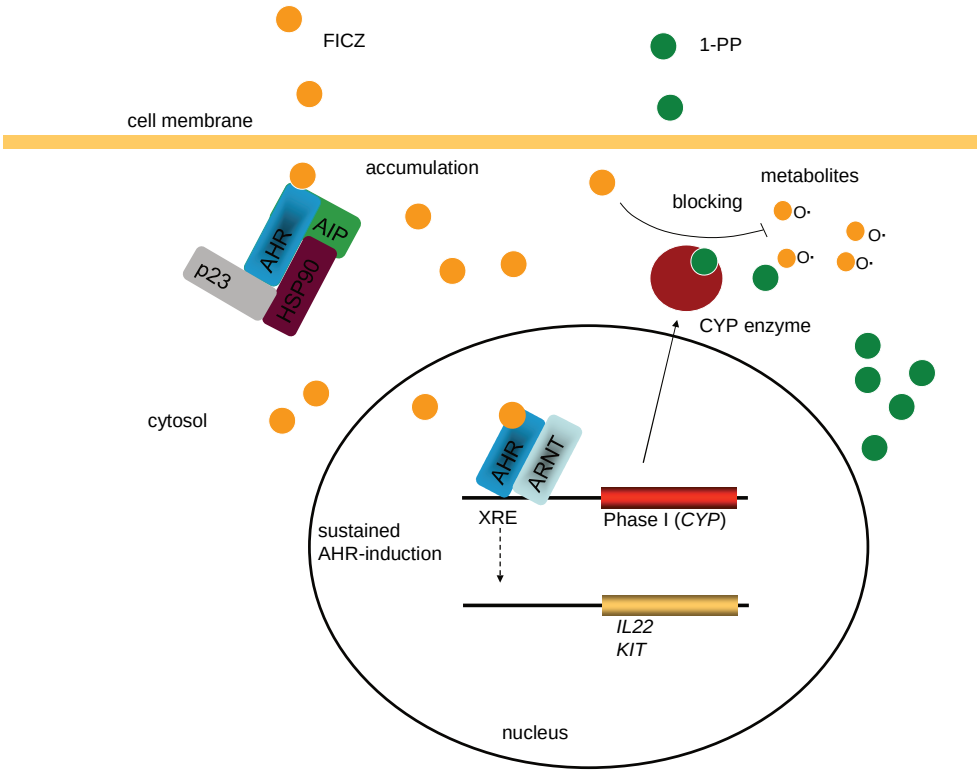


Figure 52. Induction of immunological AHR targets by feedback regulation

Due to the induction of the cytokine IL-22 and the predominant regulation of the surface receptor c-Kit, the intracellular AHR feedback activation by inhibition of CYP1 enzymes can gain increasing physiological relevance. The expression of the cytokines IL-22, IL-17, IFN-γ, TNF-α, *IL26* and of other targets related to the AHR pathway (c-Kit, *ABCG2*, *NRF2*, *NQO1*) was regulated by CYP1 activity through the metabolism of the endogenous AHR agonist and CYP1 substrate FICZ in human immune cells. Additional studies with *Cyp1a2*-deficient mice showed a higher percentage of IL-22-producing splenocytes after FICZ treatment and underlined these observations. Correlation analyses demonstrated

that the transcription of the *CYP1A1* gene and the expression of the c-Kit protein were negatively correlated with the *AHR* transcription. The correlations with the *CYP1B1* transcription showed the opposite effects. Thus, the ratio of the *CYP1A1* and *CYP1B1* transcripts also correlated with the expression of target compounds related to the AHR pathway. These results imply that the AHR expression level and presumably the balance of CYP1 expression impact the regulation of cytokines and/or of c-Kit in human immune cells. Following these observations, a transcription profile of several drug-metabolising enzymes finally deciphered a cell-specific transcription pattern in various immune cell subpopulations.

This study additionally observed increased levels of IL-22 and c-Kit in PBMCs from CMCD patients after AHR activation by FICZ treatment or by CYP1 inhibition. CMCD patients suffer from an isolated, chronic and recurrent infection with the yeast *Candida*, mostly *Candida albicans*. To a large extent, these patients have a lack in the production of Th17 cytokines including IL-22. The study demonstrated that the AHR pathway was active in CMCD patients and could provide a way for IL-22 production. IL-22 controls barrier functions and induces innate defence mechanisms. Following these results, inhibition of CYP1 activity in these patients might enhance innate immunity at epithelial tissues. The study also observed the surface receptor c-Kit as a susceptible target for a reduced CYP1 activity especially in human T cells.

4.1. Inhibition of CYP1 activity in V79 Chinese hamster cells

Before investigating the CYP1-dependent AHR activation in human immune cells, specificity of two CYP inhibitors had been tested in V79 Chinese hamster cell lines that express human CYP1 enzymes. The PAH 1-PP is a strong mechanism-based (suicide) inhibitor for the CYP1A1 enzyme (Shimada et al., 2007; Shimada et al., 1998; Zhu et al., 2011b). Consistent with this, 1-PP strongly and completely inhibited CYP1A1, but CYP1A2 and CYP1B1 activities to lesser extents. 1-PP acts as a suicide inhibitor for CYP1A1, is considered as a competitive inhibitor of CYP1A2 and it is likely metabolised by CYP1B1 (Shimada et al., 2007; Shimada et al., 1998). These effects might explain the different outcomes of 1-PP on various CYP1 enzymes. However, long-term CYP1 inhibition with testing and monitoring a regained CYP1 activity during 1-PP treatment was not analysed here. Whereas 1-PP inhibits CYP1 family enzymes selectively (Shimada et al., 1998), a selective inhibition of CYP1A1, CYP1B1 and CYP1A2 by 1-ABT was not detected. This underlines the non-specific inhibitory character of 1-ABT. Contrary to 1-PP, which inhibited all investigated human CYP1 activities completely at indicated concentrations, residual CYP1 activities were still measurable in cells treated with high 1-ABT concentrations. This is in line with a previous study where 1-ABT is effective in eliminating CYP2A6 and CYP3A4 activities but lacking a complete inhibition of other CYP enzymes (Linder et al., 2009). According to these results, 1-PP was considered as an appropriate inhibitor of CYP1 activity in human immune cells and used to study CYP1-induced AHR activation.

4.2. Effects of CYP1 inhibition in human PBMCs

4.2.1. Effects of CYP1 inhibition on the expression of *CYP1*, cytokines and c-Kit

The study´s results imply an intracellular accumulation of FICZ and a prolonged AHR activation by a reduced CYP1 metabolism in human immune cells. This also supports the existence of an endogenous way for a sustained AHR activation. In preliminary experiments with PBMCs, inhibition of CYP1 activity by 1-PP alone slightly increased IL-22 and c-Kit when 1-PP was used at high concentrations. Previous studies demonstrated that tryptophan-derived AHR ligands are formed in cell culture media by UV light (Öberg et al., 2005; Veldhoen et al., 2009; Wincent et al., 2012). Therefore, background levels of AHR ligands in the culture medium that are also CYP1 substrates probably explain the IL-22- and c-Kit-inducing effects with the CYP1 inhibitor 1-PP alone. In order to examine whether tryptophan-derived molecules are factors for agonistic and intrinsic AHR effects and responsible for the induction of IL-22 and c-Kit in single 1-PP treatments, medium devoid of tryptophan but supplemented with freshly reconstituted tryptophan should be investigated.

The 1-PP concentrations 10^{-7} M and 10^{-6} M potently inhibited CYP1A1 or CYP1A1 and CYP1B1 activities in CYP1 cell lines, increased IL-22 and c-Kit in 1-PP and FICZ co-treated PBMCs and did not have any cytotoxic effect in 48 h treatments. Therefore, these concentrations were used in combination with a low FICZ concentration in commercial RPMI for studying CYP1-induced AHR activation.

In the 5 day experiments, the transcription of the AHR-regulated genes *CYP1A1*, *CYP1B1*, *KIT*, *IL22*, the expression of the cytokine IL-22, and of the c-Kit surface receptor were induced in FICZ and 1-PP co-treatments compared with single treatments. IL-17 and IFN-γ were only slightly regulated and confirmed the AHR response in co-treated human PBMCs. The AHR-dependent regulation was proven by the addition of the AHR antagonist CH-223191 to co-treated cells. c-Kit is an AHR-regulated surface receptor (Kadow et al., 2011; Kiss et al., 2011) and in this study CYP1 inhibition induced c-Kit on human lymphocytes. Human T cells responded with a sustained c-Kit expression indicating that lymphoid-derived immune cells are susceptible targets for CYP1-induced AHR activation. Up-regulation of c-Kit was present on both $CD3^+$ and $CD3^-$ cells, and c-$Kit^+IL\text{-}22^+$ lymphocytes were most frequent among Th cells.

Besides IL-22, TNF-α is a cytokine produced by Th22 cells but as well promotes Th22 differentiation (Duhen et al., 2009; Eyerich et al., 2009; Trifari et al., 2009). Similar to IL-22, a low concentration of FICZ alone increased TNF-α production. At 48 h, however, TNF-α was not regulated by CYP1 inhibition indicating that the induction of c-Kit and of IL-22 is independent of TNF-α. On the contrary, co-treated cells showed an increased production of TNF-α after 5 days. The time-delayed effects in TNF-α release may indicate that TNF-α is induced in naïve T cells whereas IL-22 alone is already up-regulated in memory T cells when CYP1 activity is reduced. Whether IL-22 and TNF-α are produced by enriched Th22 cells has not been determined in this study.

The gene encoding the Th17 cytokine IL26 is located nearby the *IL22* gene locus in humans (Dumoutier et al., 2000a; Goris et al., 2002). In this study, *IL26* was regulated similar to IL-22 but differently than IL-17 and IFN-γ. The origin of IL-26 production in treated PBMCs was not determined here, however, the *IL26* gene could be considered as a new AHR-regulated target in human PBMCs. The detected down-regulation of IL-17 and the increase of IL-22, TNF-α and *IL26* imply that a reduced CYP1 activity could promote c-Kit$^+$ IL-22$^+$IL-26$^+$ TNF-α$^+$ IL-17$^-$ T cells via AHR activation in humans. Whether these cells are related to Th22 cells, or whether *IL26* and TNF-α are produced by various innate cell populations should be investigted in future. Up to now, it is still elusive whether AHR activation or CYP1 inhibition facilitates the formation of human Th22 cells, which express c-Kit or have stem cell-like features, and whether c-Kit could switch T cell plasticity in local chemical microenvironments. Furthermore, c-Kit-dependent mechanisms for IL-22 regulation in T cells contributing to immune and tissue cell interactions remain to be determined. The c-Kit ligand is produced by diverse cell types including epithelial cells and may affect intraepithelial localised T cell populations. Similar to IL-22, c-Kit induction was already detected after 48 h. This also suggests that memory T cells are susceptible for responding to CYP1 inhibition. However, whether CYP indeed could shift the T cell pool towards Th22 cells or changes T cell plasticity and memory among diverse subpopulations is still ambiguous. In the present study, CYP1-dependent AHR activation was studied in human PBMCs. In future studies, a detailed characterisation of CYP metabolism in isolated immune cell subpopulations might reveal triggers for metabolism-dependent c-Kit and cytokine regulations.

4.2.2. Effects of CYP1 inhibition on the expression of genes related to the AHR pathway

To get a more detailed insight into the regulation of genes that are related to the AHR pathway, the transcription of *NRF2*, *HIF1A* and *ARNT*, of *PTGS2* (coding for COX-2), *CYP2S1* and the phase III transporter *ABCG2* during CYP1 inhibition was studied.

The genes *CYP2S1*, *PTGS2*, *NQO1* and *ABCG2* were selected because they are XMEs regulated by the AHR pathway (Deb and Bandiera, 2010; Fritsche et al., 2007; Prud'homme et al., 2010; Tan et al., 2010; Tijet et al., 2006). Similar to CYP1A1 and CYP1B1, CYP2S1 has functions in the extrahepatic metabolism (Ding and Kaminsky, 2003; Rylander et al., 2001; Saarikoski et al., 2005). However, whereas CYP1 family enzymes are typical members of the AHR gene battery and metabolise lipophilic environmental compounds most effectively, polyunsaturated fatty acids derived from arachidonic acid are the preferred substrates for CYP2S1 (Bui et al., 2011). In the current study, the transcription of *CYP2S1* was higher in the FICZ and 1-PP co-treatment than in single stimulations.

Contrary to *CYP* but similar to *AHR* transcription, the *PTGS2* gene (coding for COX-2) tended to be down-regulated by combinatorial treatment. This is contrary to a previous study with human keratinocytes where COX-2 was induced (Fritsche et al., 2007). Products of COX-2, such as prostaglandins, are described as CYP2S1 substrates (Madanayake et al., 2012). Following these results, AHR activation or CYP1 inhibition could interfere with prostaglandin turnover in human immune cells. Furthermore, the COX-2 enzyme is up-regulated during hypoxia and the *CYP2S1* gene contains HRE binding sites (Rivera et al., 2007; Xing et al., 2015). However, whether oxidative stress or hypoxia occurs during CYP1 inhibition and FICZ treatment in the conducted experiments, and whether this impacts *CYP2S1* and *PTGS2* expression levels in human immune cells is still elusive.

Conversly to *AHR*, the genes encoding the transcription factor NRF2 and the phase I enzyme NQO1 were slightly up-regulated during FICZ and 1-PP co-treatment. This hints at a FICZ-dependent regulation of *NRF2* and *NQO1* or at the formation of reactive intermediates. The transcription of *HIF1A* and *ARNT* was neither regulated by FICZ nor by CYP1-induced AHR activation indicating that the genes coding for hypoxic transcription factors are not directly controlled by the AHR in the conducted experiments. However, downstream targets of the HIF1A/ARNT dimer were not investigated here.

The phase III membrane transporter ABCG2 is responsible for multidrug resistance in cell lines and it was investigated because it is considered to define a side-population of bone marrow cells containing immature hematopoietic stem cells (Scharenberg et al., 2002; Zhou et al., 2001). Contrary to *KIT* transcription, *ABCG2* transcription was attenuated in human PBMCs after FICZ and 1-PP co-treatment. These results are contrary to previous findings where ABCG2 was up-regulated by AHR ligands in diverse cell types (Tan et al., 2010). Like *AHR*, *ABCG2* transcription was slightly down-regulated by the 1-PP and FICZ co-treatment. In these connections, it could be expected that FICZ treatment or AHR activation differently affect the regulation of AHR pathway genes and the expression of stem cell

markers in human PBMCs. However, a detailed phenotypical characterisation of the ABCG2 protein on c-Kit-expressing immune cell subtypes was not investigated in this study.

The study´s results imply an importance of AHR activation and CYP1 inhibition for clinical drug resistance in hematopoietic cell types by regulating the efflux transporter ABCG2 on immune cells. Interestingly, imatinib, a c-Kit tyrosine kinase inhibitor used in anti-cancer therapies, is considered to change ABCG2 activity and several studies have indicated that this therapeutic molecule is transported by ABCG2 (Brendel et al., 2007; Burger et al., 2004; Kosztyu et al., 2014; Robey et al., 2009). Following the assumption of endogenous pathways for ABCG2-dependent c-Kit signalling, *ABCG2* down-regulation may retain small chemicals intracellularly, which then could interfere with c-Kit signalling.

FICZ is a substrate for CYP1 enzymes and it is effectively metabolised by CYP1A1. The metabolic breakdown of FICZ results in various mono- or di-hydroxylated phase I products. Phase II enzymes for increasing water solubility of FICZ intermediates are sulfotransferases, and FICZ metabolites found in the urine of humans are conjugated sulfates (Bergander et al., 2004; Wincent et al., 2009). ABCG2 is characterised by a wide substrate specificity including sulfate conjugates (Imai et al., 2003; Mizuno et al., 2004). Although phase III metabolism of FICZ is less investigated, a recent study indicated that ABCG2 transports the tryptophan metabolite kynurenic acid (Dankers et al., 2013). It is still elusive in detail to what extent FICZ metabolites or other tryptophan derivatives are substrates for, or inhibitors of ABCG2. Analyses of overlapping substrate specificities of the CYP1 family enzymes with the ABCG2 transporter protein are lacking so far to my knowledge. Although this study pointed out that the stem cell receptors c-Kit and *ABCG2* were AHR- and CYP1-regulated targets on human peripheral blood cells from healthy subjects, a detailed phenotyping of additional stem cell markers and transcription factors in human immune cells on protein level is lacking.

4.2.3. Correlations between AHR pathway targets

The AHR is a transcription factor interacting with a wide range of partner proteins and small chemicals. One important stress-related pathway regulating AHR downstream anti-oxidative responses is the NRF2 pathway (Köhle and Bock, 2007). Analyses of *NRF2* regulation were included in this study as FICZ and CYP enzymes contain the capacity to produce reactive intermediates that likely regulate NRF2 activity (Costa et al., 2010; Marchand et al., 2004; Morel et al., 1999; Radjendirane and Jaiswal, 1999; Sibilano et al., 2012). *NRF2* was up-regulated in 1-PP and FICZ co-treated PBMCs, whereas *AHR* transcription tended to be down-regulated. Following these data, correlations in the investigated individuals were analysed in detail. The strong positive correlations of the *AHR* transcription with the transcription of *NRF2*, *HIF1A* and *NQO1* in most treatments, including vehicle control, mirror the intimately related regulation of these genes. In all investigated treatments, the transcription of *AHR* was positively correlated with the transcription of *HIF1A* and *NQO1*. Interestingly, the transcription of the cytokines *IL17*, *IFNγ* and *IL26* was positively correlated with the *AHR* gene transcription in this study. In previous studies, HIF1α is highly expressed in Th17 cells (Dang et al., 2011). This is similar to AHR expression (Veldhoen et al., 2008). Contrary, low levels of HIF1α are detected during Treg differentiation (Shi et al., 2011). It was also observed that HIF1α binds FOXP3 and mark it for proteasomal degradation (Dang et al., 2011). Additionally, an interaction of AHR and HIF1α was demonstrated during Tr1 cell differentiation (Mascanfroni et al., 2015). Both AHR and HIF1α act as heterodimers and share the same partner ARNT that is also referred to as HIF1β. Thus, AHR activation during hypoxia and HIF1α-mediated limitations of AHR-induced toxic effects are well investigated (Gassmann et al., 1997; Schults et al., 2010). The ligand-dependent impact of AHR activation on Th17 and Treg differentiation is still under investigation. AHR may affect this dichotomy via regulating immune cells´ oxidative status by the regulation of XMEs, ARNT, HIF1α or NRF2. Noteworthy, reactive oxygen species (ROS) could regulate HIF1 expression (Chandel et al., 2000). Furthermore, HIF1α expression is tightly coupled to glycolytic metabolism and regulates energy balance (Cheng et al., 2014; Semenza, 2007; Shi et al., 2011). Therefore, the significant and strong positive correlations between *HIF1A* and *NRF2* transcriptions, found in all treatments including vehicle control, probably indicated that these relations are partly independent of FICZ treatment and reflect a more basal regulation of the oxygen homeostasis to the supplied cell culture conditions. As HIF1α is activated by low oxygen levels and could be stabilised by ROS, it has to be elaborated in future whether the formation of certain CYP1-derived reactive intermediates could interfere with energy metabolism, and whether this CYP1 metabolites could switch the balance between Treg, Th17 cells and Th22 cells.

The *AHR* transcription was positively correlated with the majority of analysed transcripts, except for *CYP1A1*. Individuals with an unregulated *AHR* had a lower *CYP1A1* induction than individuals with a down-regulated *AHR*. The inverse effect was demonstrated for the *CYP1B1* gene in human PBMCs. Additionally, negative correlations were also detected when *AHR* transcription was correlated with c-Kit expression. These results indicated a negative co-regulation of *CYP1A1* and c-Kit with *AHR* expression. Likely due to time-dependent effects, *AHR* did not correlate with *KIT* and *ABCG2* transcription levels but with the expression of the c-Kit protein. Recent studies showed that a lack of AHR favours stem cell and leukemic cell expansion, and that AHR-deficient mice have a higher frequency of $L^-S^+K^+$ cells (Boitano et al., 2010; Pabst et al., 2014; Singh et al., 2009) than wild type mice. $L^-S^+K^+$ cells lack lineage defining receptors, are positive for c-Kit and possess a high capacity to self-renewal. Additionally, a low AHR activity is reciprocally related to Oct-4 expression, a transcription factor important for maintaining stem cell differentiation and pluripotency (Kim et al., 2009). Tranilast and 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), two tryptophan derivatives and AHR agonists, suppress Oct-4 expression and regulate differentiation of stem cells and stem-like cancer cells (Cheng et al., 2015; Kang and Wang, 2015; Prud'homme et al., 2010). In the current study, *AHR* transcription tended to be down-regulated in FICZ and 1-PP co-treated cells. The results obviously showed that genes related to the AHR pathway were reorganised in individuals by adding a low FICZ concentration and 1-PP. As the expression of *CYP1A1* and c-Kit was negatively correlated with the transcription of *AHR*, an AHR down-regulation may favour the expression of c-Kit and *CYP1A1*.

Collective results of these studies imply that the expression of AHR pathway compounds depends on various factors such as AHR activation, *AHR* expression and probably also on the oxygen tension. Here, additional studies for analysing factors that either compete with, or that regulate AHR in defined cell types would be required.

4.2.4. Correlations between *CYP1* ratio and AHR targets

An inverse correlation of *CYP1A1* gene transcription with *AHR* transcription was determined in this study. Individuals with an unregulated *AHR* showed a lower *CYP1A1* induction than individuals with a down-regulated *AHR*. *CYP1* ratios for all treatments and correlations with the transcription of target genes in all individuals were analysed. In all individuals, FICZ and 1-PP co-treatment increased the *CYP1* ratio by a stronger *CYP1A1* than *CYP1B1* induction.

The transcription of the *AHR* gene did not correlate with the expression of IL-22, IL-17, IFN-γ, IP-10 and TNF-α proteins, however, trends in decline were detected in the correlations with the *CYP1* ratio. A high *CYP1* ratio correlated with a low Th17 cytokine transcription including *IL22*, *IL17* and *IL26* and

a high c-Kit protein expression. Therefore, the immunological outcomes of AHR could either depend on AHR activation by ligands such as FICZ or on *AHR* expression itself.

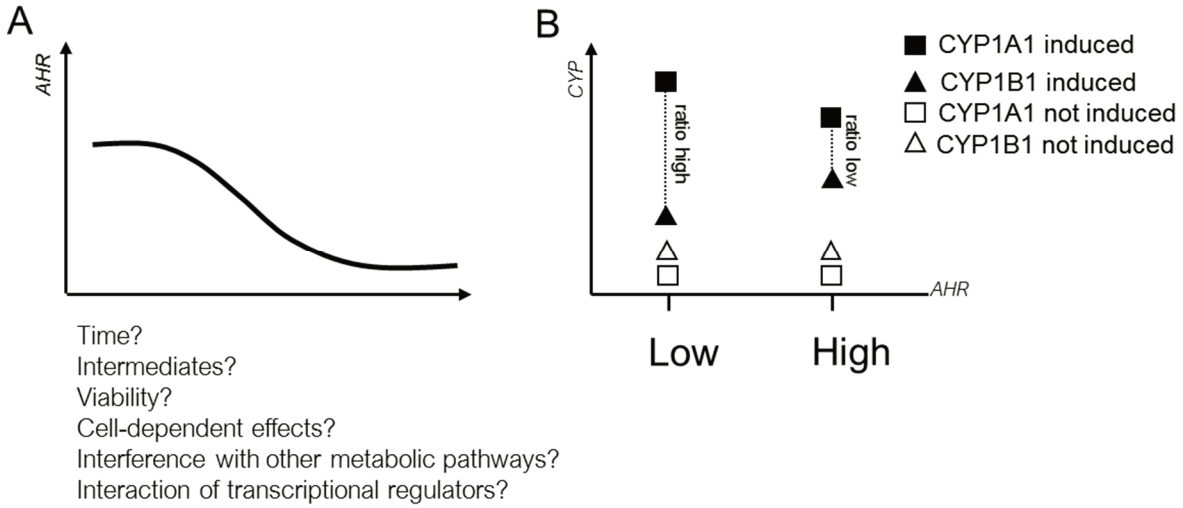


Figure 53. Hypothetical schemes for CYP1 ratio dependent on AHR expression
A. During CYP1 inhibition *AHR* expression was slightly decreasing. The variables for down-regulating *AHR* are not claryfied so far. **B.** A low/reduced *AHR* transcription correlated with a high *CYP1* ratio, whereas a high/unregulated transcription of *AHR* correlated with a low *CYP1* ratio.

The correlations of the *CYP1* ratio with the viability and with the transcription of *NRF2* and *NQO1* imply less cytotoxic effects with a high *CYP1* ratio, whereas a reduced ratio tended to favour cells stress. Both *NRF2* and *NQO1* are sensitive to oxidative stress and were slightly induced by FICZ and 1-PP co-treatment. Although significant correlations of the viability with the *CYP1* ratio were not detected, positive trends with a high ratio imply that an environment with a strong *CYP1A1* induction increases viability through a reduced formation of cytotoxic and/or reactive FICZ intermediates. In accordance with these results, *NRF2* and *NQO1* transcription levels were low in individuals with a high *CYP1* ratio. This was similar to cytokine transcriptions. These findings are contrary to a previous study where CYP1A1 overexpression increases NQO1 expression (Marchand et al., 2004).

The current data imply that the AHR expression level and the balance of CYP1 expression could contribute to the formation of CYP1-specific FICZ intermediates with adverse effects in human immune cells. These putative intermediates may regulate redox-sensitive transcription factors or induce cytokine expression, and seem to be higher in donors with a low *CYP1* ratio.

CYP1 ratio high	*CYP1* ratio low
NRF2 ↓	*NRF2* ↑
HIF-1A ↓	*HIF-1A* ↑
AHR↓	*AHR* ↑
ARNT ↓	*ARNT* ↑
CYP2S1 ↓	*CYP2S1* ↑
PTGS2 ↓	*PTGS2* ↑
viability ↑	viability ↓
c-Kit ↑	c-Kit ↓
cytokines ↓	cytokines ↑

Figure 54. Overview of CYP1 ratio and expression of AHR pathway compounds

Ligand-independent CYP gene regulation by CYP1A1 activity or by an oxygen imbalance was described previously (Morel and Barouki, 1998; Morel et al., 2000; Morel et al., 1999; Schults et al., 2010). But whether CYP1A1 enzyme activity could directly regulate *CYP1B1* transcription or *vice versa* e.g by certain FICZ or reactive intermediates in this system has to be elaborated in future. Additionally, it remains to be seen whether AHR acts differently on *CYP1A1* and *CYP1B1* expression in human immune cells during FICZ treatment e.g. by interacting with additional partner proteins.

The formation of FICZ metabolites by using rat liver microsomes was analysed in previous studies. Here, CYP1B1 further metabolises CYP1A1/1A2-derived FICZ metabolites. Although the major metabolites of FICZ could be detected in studies with human and rat liver microsomes, both display different metabolic profiles (Bergander et al., 2003; Bergander et al., 2004). These results imply that FICZ is a CYP1 substrate with a species-specific selectivity. Human urine contains several sulfated FICZ metabolites. This may hint at an individual response to FICZ, propably by CYP1-metabolism (Wincent et al., 2009). In the current study, *CYP1A2* was not detected in human immune cells and it was neither induced by FICZ nor by FICZ and 1-PP co-treatment (data not shown). Following these findings, the major FICZ metabolites in human immune cells would be derived from CYP1A1 and CYP1B1 metabolism and the metabolic profile of FICZ in human immune cells might be different to that detected with human liver microsomes. It was not investigated here what FICZ metabolites occured in human immune cells, however, differential CYP1 activity may shifts the concentration of FICZ intermediates to more or less toxic molecules.

Although the endogenous function of CYP1 family enzymes in human hematopoietic diseases has not yet been fully identified (Zhuo et al., 2012a; Zhuo et al., 2012b), the presence of Cyp1b1 in mice enhances the generation of lymphomas. *Cyp1b1*-null mice are resistant to treatments with a PAH used for cancer induction. The authors asserted a less contribution of the CYP1A1 enzyme to the chemical-induced carcinogenesis (Buters et al., 1999; Shimada and Fujii-Kuriyama, 2004). In accordance with this, the current study implies that CYP1A1 activity is less cytotoxic than CYP1B1 activity in human immune cells. This is probably depended on the nature of the CYP substrate, however, the positive correlations of an increased viability with a high *CYP1* ratio support these findings.
Conclusively, in the current study the *CYP1* ratio changed with the AHR level, and both may impact diverse processes in human immune cells such as oxidative status, viability, cytokine and c-Kit expression levels. These regulations occured already when a low concentration of the amino acid derivative FICZ was present in the culture medium. In this context, tissue-specific CYP1A1, CYP1A2 and CYP1B1 expression levels and functional outcomes of CYP activities in various immune cell subtypes would be additional factors to study. Neither DNA- or RNA-sequencing, determination of copy number and genetic variants nor epigenetic modifications in *CYP1*-encoding genes were conducted. The questions remain, whether the regulation of immunological marker proteins such as cytokines and c-Kit depends on CYP1 activity *in vivo*, and whether CYP could contribute to reprogramming differentiated immune cells. Additionally, collective effects of FICZ and 1-PP treatment indicate that several and probably overlapping mechanisms in the AHR pathway take part for regulating AHR-dowstream gene expression in human immune cells.

4.3. Concentration-dependent 1-PP effects in human PBMCs

The inhibition of CYP1 activity in the presence of a low FICZ concentration induced *CYP1A1*, *CYP1B1*, IL-22 and c-Kit and enhanced the *CYP1* ratio. These results extend previous findings of the CYP1-dependent AHR activation to human immune cells, and imply that the balance of CYP1A1 and CYP1B1 could shift immunological processes. To distiguish between 1-PP concentrations, which either inhibited CYP1A1 activity alone or the activities of CYP1B1 and CYP1A1 in the experiments with CYP1 cell lines, human activated PBMCs were treated with two different 1-PP concentrations (10^{-6} M or 10^{-7} M) alone or in the presence of FICZ. In accordance with the preferential regulation of *CYP1* family genes by the AHR, a higher 1-PP (10^{-6} M) concentration up-regulated *CYP1A1* and *CYP1B1* transcription levels but did not induce CYP2S1. Although evidence of the enzymatic CYP1 activity in human PBMCs is lacking in this study, the results underline the existence of a CYP1-dependent AHR regulation in human immune cells.

The results of the *KIT* gene transcription, of c-Kit protein expression, and of the cytokine expressions are unexpected. c-Kit expression on $CD3^-$ cells and the frequency of $CD4^+$c-$Kit^+IL\text{-}22^+$ cells decreased with 10^{-6} M 1-PP in co-treated cells compared with 10^{-7} M. It was supposed that the higher 1-PP concentration inhibits both CYP1A1 and CYP1B1 activities in PBMCs. The decline of $IL\text{-}22^+$ Th cells by the treatment with a high 1-PP concentration supports the previously postulated opinion that the *CYP1* ratio could impact IL-22 production. According to these results CYP1B1 activity seems to be more responsible for IL-22 production than CYP1A1. Noteworthy, similar to c-Kit, IL-22 expression was inverse regulated on RNA and protein level. These divergent results may occur due to time-dependent effects on RNA and on protein level, due to cell type-specific effects or due to metabolic processing of FICZ. Additionally, viability and proliferation slightly increased with a higher 1-PP concentration. May this distrubs the CYP1 balance and supports the view that CYP1B1 activity enhances cytotoxic effects.

However, further observations are needed to study overlapping effects of CYP1 activities in human lymphatic cells by a targeted down-regulation of specific CYP1 enzymes. In the current study, PBMCs were used and it should be examined whether purified immune cells react similar to CYP1 inhibition.

4.4. IL-22 regulation in *Cyp1a2* knockout and C57BL/6 mice

The inhibition of CYP1 activity changed the expression of immunological target proteins such as IL-22 and c-Kit in human PBMCs. Therefore, IL-22 expression in *Cyp1a2* knockout mice was investigated. CYP1A2 is constitutively expressed only in the human liver and to a limited extent detected in the lung (Wei et al., 2001). A transcription of *CYP1A2* was not detected in human immune cells. The treatment of murine splenocytes from both *Cyp1a2* knockout mice and C57BL/6 mice with a low dose of FICZ increased the percentage of $IL\text{-}22^+$ cells in the $CD4^+$ as well as in the $CD8^+$ compartment showing that FICZ treatment is intact in these mice. *Cyp1a2* knockout mice had a higher frequency of $IL\text{-}22^+$ and of IFN-γ^+ cells in the $CD4^+$ compartment than wild type mice, however, significant differences between *Cyp1a2* knockout and C57BL/6 mice were not detected. This may give a hint to an endogenous cytokine regulator in *Cyp1a2* knockout mice.

In comparison to human PBMCs treated with a low FICZ concentration, the AHR antagonist CH-223191 decreased FICZ-induced IL-22 expression in mice only marginally. CH-223191 is a selective AHR antagonist and the data indicated that CH-223191 probably exhibits species-specific properties on AHR activity (Zhao et al., 2010 -a). Although the data are not significant, the findings of a higher frequency of $IL\text{-}22^+$ splenocytes in *Cyp1a2* knockout mice during FICZ treatment are consistent with the human results. Although the results are unexpected concerning *Cyp1a2* expression in immune cells, they imply that an attenuated degradation of potential AHR ligands by a reduced CYP1 activity, in this case Cyp1a2, could facilitate IL-22 production.

4.5. AHR and CYP1-induced feedback pathway in CMCD

As a cytokine acting on surface tissues, IL-22 has important functions in the epithelial homeostasis. In the skin, IL-22 enhances wound healing, epithelial barrier formation and the induction of antimicrobial peptides (Eyerich et al., 2011; Liang et al., 2006; Wolk et al., 2006). Patients suffering from chronic infections with the yeast *Candida*, mostly *Candida albicans*, have impaired innate and adaptive mechanisms for defending against this pathogen (Puel et al., 2012). A subpopulation of patients is recurrently infected with *Candida* predominantly at mucosal sites without having other primary or secondary immunodeficiencies. An impaired Th17 response is recognised as one cause for this isolated form of candidiasis that is also referred to as chronic mucocutaneous candidiasis disease (CMCD). Most of these patients carry GOF mutations in the *STAT1* gene, a negative regulator of Th17 response. Therefore, CMCD patients are considered to have an impaired or a failed production of Th17 cytokines such as IL-17 and IL-22 (Liu et al., 2011). It was investigated in this study whether AHR activation by FICZ treatment or by CYP1 inhibition could induce IL-22, c-Kit and other genes regulated by the AHR in CMCD patients´ PBMCs. AHR activation with a high FICZ concentration increased *CYP1A1*, IL-22 and c-Kit expression levels similar to the experiments with the CYP1 inhibitor 1-PP and a low FICZ concentration. Addition of the AHR antagonist CH-223191 inverted the FICZ-induced effects in the experiments with a low FICZ concentration more effectively than in experiments with a high FICZ concentration. These results are consistent with previous reporter gene studies using various AHR ligands. Here, CH-223191 has ligand-selective properties (Zhao et al., 2010 -a). Thus, the AHR antagonist CH-223191 might be not sufficient enough at the used concentration to counteract the high affinity FICZ competitively, when FICZ is used with a high concentration.

IL-22 and c-Kit were up-regulated by a high FICZ concentration and by inhibition of CYP1 activity in the presence of a pharmacological low FICZ concentration. These results demonstrated an intact AHR pathway and a CYP1-induced feedback regulation in CMCD patients with an overactive STAT1 protein. The activation of the AHR is a potential way to up-regulate IL-22 in patients with a STAT1-dependent defect in producing this cytokine. Contrary to the c-Kit induction, which was more strongly induced in PBMCs from CMCD patients than in PBMCs from healthy individuals, the IL-22 concentration reached only a level that was detected in healthy PBMCs in the non-treated controls. These observations imply that several AHR pathways for IL-22 regulation exist and that one of these is blocked in PBMCs from CMCD patients but active in healthy individuals. Furthermore, it could be expected that c-Kit and IL-22 are differently regulated by AHR activation in human PBMCs.

Nevertheless, the results of the study indicate a mechanism for inducing IL-22 in CMCD patients either by AHR activation or by CYP1 inhibition. Activation of the AHR favours Th17 differentiation by blocking STAT1 protein in mice (Kimura et al., 2008). However, as expected and shown in healthy subjects, IL-17 was down-regulated whereas IL-22 was induced in PBMCs from CMCD patients after FICZ treatment. Although the IFN-γ-regulated cytokine IP-10 was down-regulated in cells treated with

FICZ alone and in 1-PP and FICZ co-treated cells, to prove a direct interaction of STAT1 and AHR was not the aim of this study.

A role of AHR in the differentiation of Th22 cells in humans and of Th17 cells in mice is under discussion (Trifari et al., 2009; Veldhoen et al., 2008). To date and my knowledge it is unknown to what extent STAT1 is involved in human Th22 cell differentiation. Th22 cells are located in close proximity to barrier tissues, the sites where endogenous AHR ligands have been proposed to be produced (Esser et al., 2013; Eyerich et al., 2009; Fritsche et al., 2007; Jux et al., 2011). Thus, the question arises whether Th22 could originate in the tissues under the influence of a tissue-specific chemical and cytokine microenvironment.

Supposing that AHR induces Th22 cells with stem cell-like features, CYP-dependent IL-22 and c-Kit induction could provide a way for drug-induced immune cell regulation in CMCD. Noteworthy, FICZ and AHR activation lead to an increased genomic instability (Aitken et al., 2008; Okudaira et al., 2010; Okudaira et al., 2012; Rannug, 2010). Therefore, a detailed characterisation of epigenetic phenomena likely leading to pre-cancerogenic effects should be investigated when AHR ligands are considered as therapeutics. A huge amount of drugs and plant-derived molecules have AHR agonistic or CYP inhibitory potential and could be considered as alternatives for AHR activation in CMCD patients (Denison and Nagy, 2003; Wincent et al., 2012).

In the current study, PBMCs from CMCD patients responded more strongly with c-Kit expression than healthy subjects. This implies that CMCD patients are more susceptible for inducing c-Kit in response to environmental stimuli than healthy individuals. Compared with healthy subjects, *AHR* was similar transcribed whereas *CYP1A1* and *IL17* were lower expressed in CMCD patients. Upon now, it is only rudimentary investigated to what extent STAT1 signalling interferes with CYP expression or activity in human immune cells. Interestingly, STAT1 is a downstream target of the c-Kit signalling cascade and is activated by the c-Kit ligand (Deberry et al., 1997). Recent studies reported that the c-Kit inhibitor imatinib dampens the STAT1 pathway in human prostata cell lines and that IFN-γ-induced STAT1 activity favours expansion of HPCs (Imura et al., 2012 ; Zhao et al., 2010 -b). However, whether STAT1-regulated c-Kit expression takes place in the recurrent infections with *Candida* or whether c-Kit inhibitor imatinib could have beneficial or adverse effects in CMCD patients has to be investigated in future. A comparison of c-Kit expression in non-cultured PBMCs would provide an insight into whether CMCD patients have a higher frequency of c-Kit-positive cells than healthy individuals. Unfortunately, due to a limited number of patients and difficulties in obtaining cell material, this study could only provide preliminary results.

4.6. Transcription profile of genes coding for xenobiotic-metabolising enzymes

The study´s results strongly indicated a mechanistic function for CYP enzymes in human immune cells of healthy subjects and of CMCD patients and emphasised the need to screen different immune cells for the expression of genes coding for xenobiotic-metabolising enzymes. Following this idea a specific transcription pattern of xenobiotic-metabolising phase I, II and III enzymes was assigned to seven immune cell subtypes. Although the AHR expression in immune cell subtypes is well characterised (Esser and Rannug, 2015; Frericks et al., 2007; Veldhoen et al., 2008), a comprehensive overview of the xenobiotic-metabolising enzymes in different immune cells has been lacking so far.

4.6.1. Differential expression of XMEs in various human immune cell subpopulations

This study showed that immune cell subtypes were characterised by a specific, constitutive transcription pattern of genes coding for phase I, phase II and phase III metabolising enzymes in non-cultured, freshly isolated and untreated human immune cell subtypes from healthy donors. Several studies characterised CYP expression in PBMCs or isolated immune cell populations after incubation with CYP-inducing agents (summarised in (Siest et al., 2008)). The AHR-regulated genes *CYP1A1*, *CYP1B1* and *NQO1* were all clearly detected in human mast cells indicating a potential sensitivity of mast cells for AHR activation. According to their transcription profile, human mast cells seem to be active in metabolising AHR ligands, as *CYP1A1* and *CYP1B1* were transcribed. CYP1A1 and CYP1B1 are typically extrahepatic expressed enzymes and as described previously it was found that *CYP1B1* was highly transcribed in myeloid-derived cells (Baron et al., 1998). The highest frequency of constitutive expression of AHR-regulated genes was determined in mast cells, basophils, $CD14^+$ monocytes and among lymphocytes in $CD4^+$ memory Th cells. Whereas *CYP1A1* transcription was only clearly detected in human primary forskin mast cells, *CYP1B1* expression showed a more frequent and constitutive expression in myeloid-derived cells than in lymphoid-derived cells.

Basophils are functional closely related to mast cells and the data here shown indicated that mast cell marker proteins were also transcribed in basophils. The unexpected finding that the gene coding for FcεR1α (*FcER1A*) was transcribed in basophils and not in mast cells is likely hinting at an alternative promoter usage in these both cell types (Hasegawa et al., 2003; Nishiyama et al., 2002; Nishiyama et al., 2001). c-Kit is a survival receptor for mast cells, however, the current study indicated a basal transcription of the *KIT* gene also in T cells. This finding additionally underlines the previous results that lymphocytic T cells are susceptible for a rapid c-Kit induction.

Regarding the expression of *GSTP1, CYP2J2, CYP2D6, CYP2A6, CYP3A5, CYP3A4*, the data confirm previous studies of metabolising enzymes in human lymphocytes from healthy donors, however, a clear transcription of *CYP1A1, CYP2C9, CYP2C18* and *CYP2C19* could not be found (Krovat et al., 2000; Siest et al., 2008). These effects might be explained by different cell isolation methods. Here,

bead-based methods were used to avoid culturing of blood cells and, as characterised by reproducible data among different donors, very clean cell populations were reached.

As described previously, *CYP1B1* is transcribed in human lymphocytes (Hakkola et al., 1997) but the current study indicated an exculsive transcription of *CYP1B1* in memory Th cells. The CYP1B1 enzyme is constitutively expressed in extrahepatic tissues, is overexpressed in human tumors and activates a variety of pre-cancerogens (Murray et al., 2001; Murray et al., 1997). However, its function in adaptive immunity is rudimentary known at present. Additionally, the unexpected results that complete Th cells and memory Th cells could be cleary distinguished according to the transcription of *CYP1B1*, *CYP2B6* and *CYP4F2* hint at a different phase I metabolism in these cell types.

In the conducted experiments with the CYP1 inhibitor 1-PP and the AHR agonist FICZ, *CYP1A1* was more strongly induced than *CYP1B1* in each healthy subject. However, whether the presence of CYP1B1 alone could affect the cytokine expression particularly IL-22 expression in human memory Th cells remains to be clarified in future. Th cells are the major producers of IL-22 in the adaptive immunity. These lymphocytes also up-regulated IL-22 and c-Kit after FICZ treatment in the current study. On CYP level Th cells could be discriminated from other lymphocytes according to the transcription of *CYP2S1* and *CYP4F3*. As arachidonic acid metabolites are proposed as endogenous AHR inducers, metabolism of eicosanoids by CYP2S1 may contribute to pro- or anti-inflammatory immune reactions in T cells (Bui et al., 2011; Madanayake et al., 2012). As the *PTGS2* gene was also transcribed, the data imply that Th cells are active in the metabolism of polyunsaturated fatty acids.

Additionally, transcription of tryptase genes, encoding soluble α- and β-tryptases (Pallaoro et al., 1999), was detected in Th cells. Tryptases are major enzymes in mast cells and elevated levels are found in patients with allergies or mast cell diseases (Lin et al., 2000; Schwartz et al., 2003). Tryptases enhance proliferation of fibroblasts and epithelial cells and are stimulating factors for the synthesis of type I collagen in fibroblasts (Cairns and Walls, 1996, 1997; Gruber et al., 1997). Functions of tryptases in T cells are less investigated, but the current findings underline an involvement of Th cells in tissue homeostasis.

The liver is the major organ of drug- and xenobiotic-metabolism. It modulates systemic levels of potential ligands on xeno-sensing receptors, and hepatic metabolism maintains plasma levels of small chemicals (Buters, 2008). However, lipophilic molecules acting as ligands on xeno-sensing receptors could accumulate in specific tissues (Müllerová and Kopecký, 2007). The CYP1-dependent regulations, shown in this study, imply that a cell type-specific CYP expression may impact organ-specific immunity in a context of a certain chemical microenvironment. Variants of CYP genes could be up-coming modulators of immunological disorders such as mucosal disorders, allergies, autoimmune diseases or hematopoietic malignancies.

Although the study provides only data on transcriptional level and biological functions have to be proven, the study indicated that each immune cell subpopulation is equipped with a unique metabolic response to chemicals.

4.7. CYP and AHR in allergy

The current study pointed out that c-Kit, IL-22 and IL-17 were susceptible targets for CYP1-dependent AHR activation particularly in lymphoid-derived cells. With the effects on IL-17, IL-22, c-Kit, and on Ig production (Kadow et al., 2011; Kiss et al., 2011; Trifari et al., 2009; Yoshida et al., 2012), AHR is probably a critical modifier of allergic diseases. IL-22 is considered to modulate allergic inflammation (Besnard et al., 2011; Pennino et al., 2013). IL-22, AHR together with the c-Kit ligand SCF are detected with elevated levels in patients with allergic asthma (Lei et al., 2008; Makowska et al., 2009; Oliveira et al., 2002; Zhu et al., 2011a). More studies should clarify whether CYP1 activity could aggravate or attenuate immune responses during sensitisation or chronification of allergic diseases.
A microenvironment with elevated levels of IL-22 generated by a reduced CYP1 activity possibly reduces the release of pro-allergic cytokines from epithelial cells that contribute to allergy by enhancing group 2 ILC (ILC2). IL-22 reduces differentiation of ILC2 and allergic inflammation (Klein Wolterink et al., 2012; Mjösberg et al., 2011; Takahashi et al., 2011). Additionally, it was shown that exposure to AHR ligands during pregnancy increases postnatal infections and reduces the prevalence of allergies (Weisglas-Kuperus et al., 2000). A possible anti-allergic effect by shifting Th2 cell differentiation into Th1 direction by AHR activation was already supposed in cell culture experiments (Negishi et al., 2005). The function of CYP in allergy has been rudimentary investigated, however, polymorphisms in genes encoding XMEs are found to be associated with a risk for bronchial asthma (Polonikov et al., 2014; Polonikov et al., 2009; Polonikov et al., 2007). As described previously, AHR also induces immune-regulatory mechanisms, however, neither regulation of IL-10 nor of FOXP3 by CYP1 inhibition was considered in this study. A detailed cell phenotyping of *Cyp1* knockout mice especially during sensitisation and environmental exposure is currently ongoing.
During the last decades prevalence and incidence of allergic diseases have been progressively rising in industrialised societies where 20 - 30 percent of the population is affected (Zheng et al., 2011).
Typical environmental pollutants such as cigarette smoke or anthropogenic combustion particles are a source of PAHs, many being AHR ligands and CYP substrates at the same time. DEPs or smoking are generally accepted as adjuvant factors for allergy, however, divergent studies with protective effects of AHR activation are emerging (Givi et al., 2013; Jeong et al., 2012; Negishi et al., 2005). These inconsistent results might be explained by different cell-specific and ligand-dependent AHR pathways or by various chemical features among AHR ligands that could also result in immunosuppressive effects. Several toxicological studies indicated that PAH mixtures such as coal tar or urban dust particulate matter decrease toxic effects of single PAHs and could inhibit CYP1 activity (Courter et al., 2007a; Courter et al., 2007b; Mahadevan et al., 2007). In the context of the current study, a crude PAH mixture, prolonged applicated in low doses, may disturb cytokine production and cell differentiation by CYP1 inhibition and amplified AHR activation. Thus, possibly other immune cells at epithelial interfaces, which express AHR, c-Kit and IL-22 such as mast cells, γδ T cells, ILCs or T cells, may shift the immune response according to the chemical microenvironment. These collective findings suggest

that polymorphisms in CYP genes might offer explanations why some individuals are more susceptible to the immunological effects of environmental chemicals than others. Ubiquitous polymorphisms in *CYP* genes could result in reduced or absent catalytic activities and give a good opportunity to study their role in immunology including allergic diseases.

5 Summary

5.1. Summary

The ligand-activated transcription factor aryl hydrocarbon receptor (AHR) mainly responds to environmental toxins. AHR induces cytochrome P4501 (CYP1) enzymes that degrade lipophilic xenobiotics and control the metabolism of endogenous chemicals. By modifying cytokine expression and differentiation of immune cells, AHR is increasingly recognised as an immune-modulating factor. Several studies have reported that AHR regulates the dichotomous development of either suppressive T regulatory T cells (Treg) or pro-inflammatory T helper (Th) 17 cells. The production of the Th17 cytokines interleukin (IL)-22 and IL-17 is impaired in patients suffering from an isolated form of chronic mucocutaneous candidiasis (CMCD). CMCD patients are characterised by recurrent infections with the yeast *Candida*, mostly *Candida albicans*. To a large extent, these patients have gain-of-function mutations in the signal transducer and activator of transcription 1 *(STAT1)* gene that lead to an overactive STAT1 protein. STAT1 is a negative regulator of the Th17 response. An interaction of AHR with STAT1 has been recently indicated and various AHR ligands induce the exclusive up-regulation of IL-22 but not of IL-17A and IFN-γ in humans. Additionally, recent studies emphasised that the stem cell factor receptor gene (*KIT*) is an AHR target in mice. The receptor tyrosine kinase cellular (c)-Kit is important for driving survival and division of bone marrow-derived hematopoietic stem cells. Although AHR in immunity has been intensively studied, to date the role of CYP metabolism in immunity is unclear. However, inhibition of CYP1 activity provides a mechanism for AHR activation. The present study hypothesised that CYP could navigate immune response by degradation of ligands on xeno-sensing transcription factors and thus contribute as metabolic keys to immunological reactions.

The aim of this thesis was to investigate the impact of CYP1 activity on the AHR pathway in human immune cells. Besides other toxicologically relevant genes and target genes related to the AHR pathway, the Th17 cytokines and c-Kit were in the focus of this study. The importance of the AHR feedback activation was also investigated using *Cyp1a2* knockout mice. In addition, CMCD patients were studied to confirm the relevance of the AHR pathway for IL-22 induction in a human immunlogical disease. Furthermore, the study investigated the expression pattern of toxicologically important genes in human immune cell subtypes.

Activated peripheral blood mononuclear cells (PBMCs) from healthy donors and CMCD patients were treated with 1-(1-propynyl)-pyrene (1-PP), a suicide inhibitor for CYP1 in the presence of a low dose of the AHR ligand 6-formylindolo[3,2-*b*]carbazole (FICZ) alone or in combination with the AHR antagonist CH-223191. As control, PBMCs from CMCD patients and healthy subjects were additionally treated with a high concentration of FICZ. Cytokine, *CYP1* and c-Kit expression levels were analysed by quantitative real time-polymerase chain reaction (qRT-PCR), enzyme-linked immunosorbent assay

(ELISA) and flow cytometry (FACS). Viability and proliferation were analysed by flow cytometry, lactate dehydrogenase (LDH) activity and ^{3}H-thymidine assays. Expression of genes encoding xenobiotic-metabolising enzymes in different human immune cells was examined by TaqMan Low Density Arrays (TLDAs).

Inhibition of CYP1 activity by 1-PP in the presence of a low concentration of the AHR agonist FICZ increased *CYP1*, c-Kit and IL-22 expression levels but dampened IL-17 production in activated PBMCs from healthy subjects as well from CMCD patients. Inhibition of the AHR inverted these effects. In particular, human T cells responded highly sensitive to CYP1 inhibition with an up-regulation of c-Kit, and Th cells that co-express c-Kit and IL-22 were selected in healthy subjects. Furthermore, c-Kit and IL-22 were up-regulated in activated PBMCs from CMCD patients after AHR activation or inhibition of CYP1 activity. However, both Th and cytotoxic T cells (Tc) from CMCD patients responded with a stronger induction of c-Kit than cells from healthy subjects. Unexpectedly, data of correlation analyses in healthy subjects indicated that the *CYP1A1* gene transcription was negatively correlated, whereas *CYP1B1* transcription correlated positively with the *AHR* transcription. Following these observations, the ratio of *CYP1A1* and *CYP1B1* transcription levels correlated differently with the expression of AHR pathway compounds. Additionally, splenocytes from *Cyp1a2* knockout mice had a higher percentage of IL-22^{+} cells than wild type mice after AHR activation and in control treatments. This further confirmed a function of CYP1 in immune cells. Finally, major immune cell subtypes could be grouped according to their *CYP* expression. Although 17 of the studied genes were transcribed in all investigated immune cells, genes encoding XMEs fingerprinted human monocytes, Th cells, memory Th cells, Tc cells, B cells, human primary foreskin mast cells and basophils.

Conclusively, the present study demonstrates an active CYP1-dependent AHR activation in a range of human immune cells and depicts a mechanism for c-Kit and IL-22 induction in both PBMCs from healthy subjects and CMCD patients. The surface receptor c-Kit was used as a susceptible target for a CYP1-dependent AHR activation especially in human T cells. In addition, the results highlight peripheral Th cells that co-express IL-22 and c-Kit. Although IL-22 and c-Kit are induced by inhibition of CYP1 activity, both are probably differently regulated. The selective expression of CYP-coding genes in human immune cells hints at cell type-specific functions of these enzymes and suggests that similar mechanisms are present in multiple immune cells. Therefore, this model could be used to study the environmentally induced etiologies of immunological diseases such as CMCD or allergic diseases.

5.2. Zusammenfassung

In seiner klassischen Rolle erfüllt der ligandenabhängige Transkriptionsfaktor Arylhydrokarbon-Rezeptor (AhR) wichtige Funktionen im Fremdstoffmetabolismus. Der AhR reguliert Fremdstoff-metabolisierende Enzyme wie Cytochrom P450 (CYP). CYP Enzyme sind notwendig, um lipophile Fremdstoffe effektiv aus dem Körper auszuscheiden. CYP Enzyme der Familie 1 (CYP1) unterliegen der direkten Regulation des AhRs. Neuere Studien zeigen zudem, dass AhR immun-modulierende und immun-regulierende Funktionen besitzt, und sowohl die Entwicklung von T-Helferzellen vom Subtyp 17 (Th17), Th22 und auch von regulatorischen T-Zellen (Treg) beeinflusst. Als ein Mechanismus, der die Bildung von Th17 Zellen im murinen System fördert, wird die Interaktion des AhRs mit *Signal Transducer and Activator of Transcription 1* (STAT1) vermutet. Das STAT1 Protein hemmt den Th17 Signalweg und ist in Patienten, die an einer isolierten und chronischen Form der *Candida*-Infektion (*Chronic Mucocutaneous Candidiasis Disease* (*CMCD*)) leiden, häufig überaktiv. Diese Patienten sind gekennzeichnet durch autosomal-dominant vererbte Mutationen im *STAT1* Gen und einer meist verringerten Bildung der Th17 Zytokine Interleukin (IL)-22 und IL-17. Sowohl im humanen als auch im murinen System kann die Produktion von IL-22 durch verschiedene AhR Liganden erhöht werden. Neben der Induktion von IL-22 sind die weitreichenden Funktionen des AhRs im Immunsystem durch die Regulation des Stammzellfaktor-Rezeptors (c-Kit) gegeben. c-Kit ist eine Rezeptor-Tyrosinkinase und an der Entwicklung verschiedener hämatopoetischer Zellpopulationen beteiligt. Aufgrund der vielfältigen und meist ligandenabhängigen Funktionen des AhRs wurde in dieser Studie angenommen, dass AhR-regulierte Fremdstoff-metabolisierende Enzyme ebenfalls eine Aufgabe im Immunsystem haben können. Die Hemmung der CYP1-Aktivität kann den Abbau eines potentiellen AhR Liganden verringern und AhR dadurch aktivieren. Die Aktivierung von AhR durch diesen Rückkopplungsmechanismus in humanen Immunzellen war bisher nicht untersucht.

Ziel der vorliegenden Arbeit war es, eine CYP1-abhängige Aktivierung des AhRs in humanen Immunzellen zu untersuchen. Zur Überprüfung dieses Mechanismus dienten als wichtigste Endpunkte, neben anderen mit dem AhR-Signalweg assoziierten Genen, die Regulation der CYP1-kodierenden Gene und die Expression von c-Kit, IL-22 und IL-17. Weitere Schwerpunkte lagen in der Analyse des AhR-Signalweges in CMCD Patienten, dem Nachweis von IL-22 in Splenozyten aus *Cyp1a2* knock-out Mäusen und in der Charakterisierung der Fremdstoff-metabolisierenden Kapazität in unterschiedlichen humanen Immunzellpopulationen.

Zum Nachweis wurden humane periphere mononukleäre Blutzellen (*Peripheral Blood Mononuclear Cells* (PBMCs)) aus nicht-atopischen Spendern und CMCD Patienten mit anti-CD3/CD28 aktiviert und mit einem spezifischen CYP1 Inhibitor 1-(1-Propynyl)-pyren (1-PP) alleine oder in Kombination mit dem hoch-affinen AhR Agonisten 6-Formylindolo[3,2-b]carbazol (FICZ) in niedrigen Konzentrationen behandelt. PBMCs isoliert aus CMCD Patienten und gesunden Probanden wurden zusätzlich mit FICZ

in hoher Konzentration stimuliert. Die AhR-abhängige Regulation wurde durch die Zugabe des AhR Antagonisten CH-223191 getestet. Die Zytokinexpression mittels ELISA, FACS und qRT-PCR gemessen. c-Kit und *CYP1*-kodierende Gene wurden durch FACS und qRT-PCR analysiert. Die Expression von IL-22 in *Cyp1a2* knock-out Mäusen konnte nach Stimulation mit FICZ mittels FACS analysiert werden. *TaqMan Low Density Arrays* dienten dem Nachweis der konstitutiven Expression von Genen, die für Fremdstoff-metabolisierende Enzyme in $CD4^+$ T-Helferzellen, $CD4^+$ Gedächtnis-T-Helferzellen, $CD8^+$ zytotoxischen T-Zellen, basophilen Granulozyten, B-Zellen, $CD14^+$ Monozyten und humanen primären Vorhautmastzellen kodieren.

Die Hemmung der CYP1 Enzyme in PBMCs von gesunden Probanden verstärkte sowohl die Expression von *CYP1A1* und *CYP1B1* als auch die des Oberflächenrezeptors c-Kit und des Zytokins IL-22. IL-17, welches durch die AhR-Aktivierung im humanen System unterdrückt wird, wurde entsprechend zu IL-22 entgegengesetzt reguliert. Sowohl $CD4^+$ als auch $CD8^+$ T-Zellen zeigten eine höhere Frequenz von c-Kit^+ Zellen nach CYP1 Hemmung. PBMCs, die gleichzeitig positiv für c-Kit und IL-22 waren und CYP1- bzw. AhR-abhängig induziert wurden, konnten in gesunden Spendern den T-Helferzellen zugewiesen werden. Die Zugabe des AhR Antagonisten verringerte diese Effekte. Ein weiteres Ergebnis dieser Studie war die negative Korrelation von c-Kit und *CYP1A1* mit der *AHR* Transkription. Im Gegensatz dazu korrelierte *CYP1B1* positiv. In PBMCs von CMCD Patienten wurden sowohl IL-22 als auch c-Kit durch die Hemmung der CYP1-Aktivität bzw. durch die Aktivierung des AhRs verstärkt gebildet. Im Gegensatz zu gesunden Spendern wurden jedoch eine stärkere Induktion von c-Kit und eine geringere Konzentration von IL-22 gemessen. Splenozyten von *Cyp1a2* knock-out Mäusen zeigten im Vergleich zu Wildtyp Mäusen ebenfalls eine höhere Frequenz von IL-22^+ Zellen sowohl im $CD4^+$ als auch im $CD8^+$ Zellkompartment und bestätigten zudem eine Funktion von CYP1 in Immunzellen. In unterschiedlichen humanen Immunzelltypen konnte eine basale Expression von 17 verschiedenen Genen gemessen werden, die für Fremdstoff-metabolisierende Enzyme kodieren. Diese ließen sich jedoch aufgrund spezifischer CYP Muster unterscheiden.

Zusammenfassend zeigt diese Arbeit, dass immunologische Proteine wie c-Kit und IL-22 in humanen PBMCs von gesunden Probanden und von CMCD Patienten über Fremdstoff-metabolisierende Enzyme reguliert werden können. Obwohl beide gewählten immunologischen Zielproteine durch eine verstärkte AhR- bzw. eine reduzierte CYP1-Aktivität induziert wurden, werden c-Kit und Zytokine möglicherweise über unterschiedliche AhR-abhängige Mechanismen reguliert. Mit dem Nachweis spezifischer Transkriptionsprofile in unterschiedlichen Immunzellen konnte zudem ein Hinweis auf eine zellspezifische Funktion von CYP erarbeitet werden. Ob die CYP1-induzierte AhR-Aktivierung physiologisch relevant ist und CMCD oder allergische Erkrankungen beeinflusst, wird in zukünftigen Studien weiter untersucht.

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IX. Dictionary of Molecules

AHR: Aryl hydrocarbon receptor

AHR is a cytosolic, ligand-activated transcription factor mostly investigated in the context of environmentally induced xenobiotic metabolism and toxicology (Nebert et al., 1993). AHR-mediated toxicity to exogenous compounds is partially dependent on oxidative stress, genotoxic and proteinotoxic effects and manifested in diverse organs and multiple cancers (Nebert, 1989; Nebert and Dalton, 2006; Nebert et al., 2004). AHR belongs to the basic helix-loop-helix (bHLH)/Per-ARNT-Sim (PAS) transcription factor family and was initially considered as a regulator of lipophilic, exogenous molecules (xenobiotics) (Burbach et al., 1992; Ema et al., 1994; Ema et al., 1992; Gu et al., 2000; Kewley et al., 2004; McIntosh et al., 2010, Nebert, 1989; Nebert et al., 1993; Poland and Knutson, 1982). The receptor is highly expressed in the liver, in barrier organs and diverse immune cell subpopulations (Dolwick et al., 1993; Esser and Rannug, 2015; Li et al., 1994).

ARNT: AHR nuclear translocator

ARNT is the nuclear dimerisation partner of AHR in the canonical AHR pathway (Reyes et al., 1992; Soshilov and Denison, 2011; Whitelaw et al., 1993). Like AHR, ARNT belongs to the basic helix-loop-helix (bHLH)/Per-ARNT-Sim (PAS) transcription factor family (Gu et al., 2000; Kewley et al., 2004; McIntosh et al., 2010). AHR and ARNT bind to xenobiotic response elements (Bacsi et al., 1995; Whitelaw et al., 1993).

ABCG2/BCRP: ATP-binding casette subfamily G member 2/Breast cancer resistant protein

ABCG2 is a transmembrane transporter using adenosine triphosphate (ATP) as energy donor for transporting substrates against a concentration gradient through the membrane. ABCG2 is one important multidrug resistant (MDR) protein expressed on stem cells and malignant cells. Efflux of therapeutics through ABCG2 promotes drug resistance (Kosztyu et al., 2014; Tan et al., 2010; Zhou et al., 2001)

CYP: Cytochrome Pigment-450

CYP proteins are heme-containing and mixed-functioned mono-oxygenases. The enzymes are involved in the oxidative hydroxylation and metabolism of exogenous and endogenous lipophilic chemicals including drugs. The enzymes are ubiquitously expressed and embedded in the endoplasmatic reticulum, where they use molecular oxygen and reducing equivalents for substrate oxidation. During the CYP catalytic cycle mutagenic molecules and reactive oxygen metabolites can be generated (Bondy and Naderi, 1994; Guengerich, 1992, 2006; Nebert, 1991; Nebert and Dalton, 2006; Nebert and Karp, 2008; Nebert and Russell, 2002; Puntarulo and Cederbaum, 1998). The name cytochrome P-450 originates from the observation that in the presence of a reducing environment carbon monoxide (CO) forms a complex with CYP. This complex display a characteristic absorption maximum at 450 nm. This behaviour was crucial for the name cytochrome pigment-450 (CYP) (Klingenberg, 1958; Omura and

Sato, 1964). CYP enzymes, according to their amino acid sequence identities, are structured into families and subfamilies, whereas a sequence homology of more than 40% determines for the same family (e.g. CYP1) and enzymes of a same subfamily display a sequence identity of at least 55% (e.g. CYP1A) (Nelson et al., 1996). The human genome presumably encodes for 57 functional CYP proteins. These are structured according to their sequence identities in 18 families (Lewis, 2004; Nelson, 2002; Nelson et al., 2004). Most of them are polymorphic and display a complex organ-, development- and sex-specific expression. CYP enzymes are most abundantly expressed in the liver, the intestine and in tissues with close proximity to the environment such as lung, skin and gastrointestinal tract (Ding and Kaminsky, 2003; Gandhi et al., 2004; Gundert-Remy et al., 2014; Macé et al., 1998; Nebert, 2000; Swanson, 2004; Waxman and Holloway, 2009; Zanger et al., 2014; Zanger and Schwab, 2013). CYP1 family enzymes are the most important downstream targets of the AHR (Ma, 2001; Nebert et al., 2004; Nebert and Jones, 1989).

HIF1A/ HIF1α: Hypoxia-inducible factor 1 alpha

Together with ARNT and AHR, HIF1α belongs to the PAS protein family of transcription factors that are cellular sensors for environmental stressors including oxygen (Gu et al., 2000; Kewley et al., 2004; McIntosh et al., 2010; Wang et al., 1995). HIF1α is activated during hypoxia, and HIF1α-regulated genes such as erythropoietin (EPO) and vascular endothelial growth factor (VEGF) contain hypoxia responsive elements (HRE) (Salceda and Caro, 1997; Wenger et al., 2005). Both AHR and HIF1α act as heterodimers and share the same partner ARNT that is also referred to as HIF1β. Thus, interference between AHR activation during hypoxia and HIF1α-mediated limitations of AHR-induced toxic effects are well investigated (Bacsi et al., 1995; Gassmann et al., 1997; Schults et al., 2010).

IL22/IL-22 : Interleukin-22

IL-22 is a tissue-regulatory cytokine produced by many innate and adaptive lymphocytes including Th17, γδ T cells and ILCs. IL-22 is the signature cytokine of Th22 cells (Colonna, 2009; Martin et al., 2009; Spits and Di Santo, 2011; Trifari et al., 2009; Witte et al., 2010). The *IL22* gene is clustered within a conserved genomic region together with the *IL26* and the *IFNG* genes on the human chromosome 12q14-15 (Dumoutier et al., 2000a; Goris et al., 2002). The cytokine belongs to the family of IL-10-related cytokines and its biological function is mediated through binding to the IL-10R2/IL-22R1 receptor chains, mainly expressed on tissue cells (Dumoutier et al., 2000b; Kotenko et al., 2001; Wolk et al., 2004; Xie et al., 2000). IL-22 has both pro- and anti-inflammatory properties depending on the microenvironmental settings (Besnard et al., 2011; Liang et al., 2006; Sonnenberg et al., 2010). In the skin, IL-22 is an essential factor for skin homeostasis and regulates epithelial barrier functions and host defence. The cytokine supports the innate defence mechanisms by inducing antimicrobial peptides in epithelial cells (Duhen et al., 2009; Eyerich et al., 2009; Liang et al., 2006; Wolk et al., 2004; Wolk et al., 2006).

KIT/c-Kit:

Normal cellular (c)-Kit protein, is a receptor tyrosine kinase important for survival and division of bone marrow-derived hematopoietic stem cells (HSC) and other c-Kit-expressing cells. It was discovered as a homolog of the feline retroviral oncogen v-Kit. Mutations in the *KIT* gene result in various forms of cancers and mark it as a protooncogene. While c-Kit expression on progenitor cells is necessary for sustained immune cell progression, the receptor is down-regulated in most immune cell populations during lineage commitment (Ashman et al., 1991; Besmer et al., 1986; Chabot et al., 1988; Ogawa et al., 1991). c-Kit provides response to the cytokine stem cell factor (SCF) (Blechman et al., 1993; Huang et al., 1990; Nocka et al., 1990; Williams et al., 1990; Yarden et al., 1987; Zsebo et al., 1990). It is closely related to other growth factor receptors like platelet-derived growth factor receptor or the receptor for the macrophage colony-stimulating factor 1 (Qiu et al., 1988; Yarden et al., 1987). Certain differentiated cells have kept or retained c-Kit expression and c-Kit is expressed on mast cells, γδ T cells, eosinophils, on subpopulations of innate lymphoid cells (ILCs) (including natural killer (NK) cells) and on non-hematopoietic cells (such as melanocytes) (Kadow et al., 2011; Kirshenbaum et al., 1999; Kiss et al., 2011; Matos et al., 1993; Spits and Di Santo, 2011; Yoshida et al., 2001).

NRF2/*NRF2* : Nuclear factor erythroid 2 like 2

NRF2 is a basic leucin zipper transcription factor and sequestered in the cytoplasm by Kelch-like-ECH-associating-protein 1 (Keap1). Keap1 senses oxidative and electrophilic stress and is an adapter protein of an E3 ubiquitin ligase. When intracellular oxidative burden is low, interaction of Keap1 with NRF2 induces a permanent NRF2 degradation. Rising concentrations of reactive oxygen species or electrophiles stabilise and release NRF2 from Keap1 allowing the translocation into the nucleus (Itoh et al., 2003; Kang et al., 2004; Kobayashi et al., 2004; McMahon et al., 2003; Nguyen et al., 2003b). NRF2 binds onto antioxidant response elements (AREs) in gene batteries coding for phase I and for phase II enzymes. NQO1, aldo-keto reductases (AKRs) and gluthation-s-transferases (GSTs) are NRF2 targets acting downstream of CYP enzymes (Chanas et al., 2002; Jaiswal, 2004; McMahon et al., 2001; Nguyen et al., 2003b; Radjendirane and Jaiswal, 1999; Venugopal and Jaiswal, 1996). NRF2 co-operates with AHR for inducing detoxification of potentially harmful intermediates and for regulating oxidative defence. Expression of both transcription factors is regulated mutually and activation of both factors control partly overlapping signalling pathways and XME batteries (Köhle and Bock, 2007; Ma et al., 2004; Miao et al., 2005; Wang et al., 2013).

NQO1/*NQO1*: NAD(P)H dehydrogenase quinone 1

NQO1 is an oxidoreductase that protects against the formation of reactive quinone intermediates. The enzyme reduces quinones by a two-electron transfer to hydroquinones and thus prevents generation of very reactive oxygen species. Reactive semiquinone intermediates occur during phase I metabolism and contain unpaired electrons resulting in various forms of toxic cell damage. The *NQO1* gene contains both AREs and XREs and could be regulated by AHR and NRF2 (Nioi and Hayes, 2004).

PTGS2/COX-2: Prostaglandin-endoperoxide synthase 2/ Cyclooxygenase-2

Cyclooxygenase-2 (COX-2) is an inducible membrane-located enzyme encoded by the prostaglandin-endoperoxide synthase 2 (*PTGS2*) gene. COX-2 converts arachidonic acid into precursors of prostaglandins such as prostaglandin E_2 (PGE_2). These mediators are important for diverse intracellular and intercellular pathways and processes including cell proliferation, inflammation and pain (Brock et al., 1999).

X. Supplemental Material

Table 30. Characteristics of PBMC donors

PBMCs	gender	age	IgE/kU/l
1	F	29	10.40
2	M	33	7.50
3	F	48	9.11
4	F	53	9.26
5	F	35	9.13
6	F	23	19.90
7	F	29	3.9
Mean		**35.71**	**9.89**
Standard deviation		**10.07**	**4.53**

Table 31. Characteristics of $CD14^+$ cell donors

$CD14^+$ cells	gender	age	total IgE (kU/l)
CD14.1	F	28	3.90
CD14.2	F	28	6.10
CD14.3	M	25	22.90
CD14.4	F	28	10.40
CD14.5	M	32	7.50
CD14.6	F	32	14.50
CD14.7	F	28	8.02
Mean		**28.71**	**10.47**
Standard deviation		**2.312**	**5.57**

Table 32. Characteristics of $CD4^+CD45RO^+CD45RA^-$ T cell donors

$CD4CD45RO^+CD45RA^-$ T cells	gender	age	total IgE (kU/l)
CD4CD45RO.1	F	28	3.90
CD4CD45RO.2	F	28	6.10
CD4CD45RO.3	F	23	18.40
CD4CD45RO.4	M	25	22.90
CD4CD45RO.5	M	32	7.50
CD4CD45RO.6	F	32	14.50
CD4CD45RO.7	F	28	8.02
Mean		**28.83**	**11.62**
Standard deviation		**2.48**	**6.14**

Table 33. Characteristics of B cell donors

B cells	gender	age	total IgE (kU/l)
Bcell.1	M	32	7.50
Bcell.2	F	28	10.40
Bcell.3	F	28	43.30
Bcell.4	F	29	2.53
Bcell.5	F	28	3.90
Bcell.6	M	25	22.90
Bcell.7	F	25	25.50
Mean		**27.86**	**16.58**
Standard deviation		**2.231**	**12.81**

Table 34. Characteristics of CD8$^+$ T cell donors

CD8+ T cells	gender	age	total IgE (kU/l)
CD8.1	F	28	10.40
CD8.2	M	30	58.60
CD8.3	F	28	43.30
CD8.4	F	30	5.19
CD8.5	F	29	2.53
CD8.6	F	28	3.90
CD8.7	F	25	25.50
Mean		**28.28**	**21.35**
Standard deviation		**1.57**	**20.45**

Table 35. Characteristics of CD4$^+$ T cell donors

CD4+ T cells	gender	age	total IgE (kU/l)
CD4.1	F	28	10.40
CD4.2	M	30	58.60
CD4.3	F	28	43.30
CD4.4	F	30	5.19
CD4.5	F	29	2.53
CD4.6	F	28	3.90
CD4.7	M	25	22.90
Mean		**28.29**	**20.97**
Standard deviation		**1.578**	**19.07**

Table 36. Characteristics of basophil donors

Basophils	gender	age	total IgE (kU/l)
Bas.1	M	21	22.90
Bas.2	F	30	34.1
Bas.3	F	28	14.50
Bas.4	F	25	56.6
Bas.5	F	36	78.40
Bas.6	F	25	42.10
Bas.7	M	26	58.60
Mean		**28.14**	**43.89**
Standard deviation		**4.196**	**19.28**

Table 37. Mean fold changes of AHR target gene transcription

PBMCs activated with anti-CD3 and anti-CD28 were treated with the AHR agonist FICZ ($5x10^{-10}$ M), CYP1 inhibitor 1-PP (10^{-6} M) and the AHR antagonist CH-223191 ($3x10^{-6}$ M) for 5 days. **A-C,** Relative RNA expression of AHR-regulated cytochrome P450s (*CYP1A1*, *CYP1B1*, *CYP2S1*), **D-G,** other AHR-regulated genes (*KIT*, *ABCG2*, *NQO1*, *PTGS2*), **H-K**, transcription factors (*AHR*, *ARNT*, *NRF2*, *HIF1A*) and **L-O,** cytokines (*IL22*, *IL17*, *IFNγ*, *IL26*) was analysed with qRT-PCR. Mean fold changes of compared conditions are shown. Bold values indicate significant changes.

Comparison of conditions Fold changes		FICZ vs. DMSO	1-PP vs. DMSO	1-PP + FICZ vs. DMSO	1-PP FICZ vs. FICZ	1-PP FICZ vs. 1-PP	1-PP + FICZ vs. 1-PP + FICZ + CH-223191
A	*CYP1A1*	**12.42**	**12.01**	**365.45**	**44.47**	**36.04**	**5.97**
B	*CYP1B1*	**1.61**	**1.84**	**14.02**	**8.60**	**8.33**	**3.68**
C	*CYP2S1*	1.29	1.17	1.69	**1.24**	**1.37**	1.04
D	*KIT*	1.24	**1.30**	**5.42**	**4.46**	**4.09**	**3.22**
E	*ABCG2*	**0.58**	**0.67**	**0.47**	0.82	**0.71**	0.99
F	*NQO1*	1.00	0.97	1.19	1.20	**1.22**	1.26
G	*PTGS2*	0.98	1.00	0.67	0.76	0.77	**0.75**
H	*NRF2*	1.05	1.12	**1.19**	**1.13**	1.08	1.04
I	*AHR*	0.88	0.94	**0.74**	**0.85**	**0.81**	0.88
J	*ARNT*	0.88	0.94	0.74	0.85	0.81	0.88
K	*HIF1A*	1.00	1.04	1.02	1.02	1.00	0.97
L	*IL22*	**4.32**	2.94	**9.45**	**2.41**	**3.59**	**2.66**
M	*IFNγ*	0.84	1.17	0.98	**1.19**	0.89	0.83
N	*IL17*	0.71	0.91	**0.50**	**0.77**	**0.54**	**0.61**
O	*IL26*	1.25	1.02	1.96	**1.71**	**2.06**	**2.14**

Table 38. Mean fold changes of protein expression

PBMCs activated with anti-CD3 and anti-CD28 were incubated for 5 days with the AHR agonist FICZ ($5x10^{-10}$ M), CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M) and the AHR antagonist CH-223191 ($3x10^{-6}$ M). **A-E,** Cytokine release (IL-22, IL-17, IFN-γ , TNF-α and IP-10) was determined with ELISA. **F-H**, c-Kit$^+$ expression on CD3$^+$CD4$^+$, on CD3$^+$CD8$^+$ lymphocytes and on **J-L,** complete CD3$^-$, CD3$^-$CD56$^+$ and CD3$^-$CD56$^-$ PBMCs was measures by FACS analysis. Bold values indicate significant changes.

Comparison of conditions Fold changes		FICZ vs. DMSO	1-PP vs. DMSO	1-PP+ FICZ vs. DMSO	1-PP FICZ vs. FICZ	1-PP FICZ vs. 1-PP	1-PP+ FICZ vs. 1-PP + FICZ + CH-223191
A	IL-22	**2.39**	1.26	**4.64**	**2.00**	**3.80**	**1.94**
B	IL-17	0.81	0.88	0.78	0.88	0.77	**0.64**
C	IFNγ	**0.68**	0.83	0.79	1.06	0.88	0.77
D	TNF-α	1.15	0.98	**1.55**	**1.28**	**1.45**	1.29
E	IP-10	**0.57**	0.74	0.61	0.94	0.68	0.82
F	CD3$^+$CD4$^+$c-Kit$^+$	1.47	1.52	**9.62**	**8.80**	**7.57**	**4.66**
G	CD3$^+$CD4$^+$c-Kit$^+$ IL-22$^+$	0.70	1.15	**3.27**	**4.47**	**2.54**	3.00
H	CD3$^+$CD8$^+$c-Kit$^+$	2.83	2.57	**38.57**	**16.56**	**16.52**	**6.17**
J	CD3$^-$c-Kit$^+$	1.03	0.90	**2.19**	**2.22**	**2.65**	**2.01**
K	CD3$^-$CD56$^+$c-Kit$^+$	1.30	1.05	**2.39**	**1.97**	**2.46**	**1.92**
L	CD3$^-$CD56$^-$c-Kit$^+$	**0.71**	0.80	1.94	**2.77**	**2.90**	**2.03**

Table 39. Spearman´s correlation coefficients and p-values

***AHR* vs**	**DMSO**		**FICZ**		**1-PP**		**1-PP + FICZ**		**1-PP + FICZ + CH-223191**	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**
NRF2	0.86	**0.0238***	0.8571	**0.0238***	0.955	**0.0032****	0.8469	**0.0238***	0.8929	**0.0123***
NQO1	0.7748	**0.0492***	0.8214	**0.0341***	0.8108	**0.0349***	0.7388	0.0667	0.9274	**0.0079****
ARNT	0.8214	**0.0341***	0.4643	0.3024	0.6307	0.1429	0.7748	**0.0492***	0.75	0.0663
HIF1A	0.955	**0.0032****	0.8571	**0.0238***	0.8829	**0.0151***	0.8469	**0.0238***	0.8571	**0.0238***
IL22	0.3214	0.4976	0.4286	0.3536	0.5225	0.2349	0.6307	0.1413	0.6786	0.1095
IL17	-0.1622	0.6984	0.8289	**0.0302***	0.8571	**0.0238***	0.6667	0.1167	0.8571	**0.0238***
IFNγ	0.5225	0.2349	0.9643	**0.0028***	0.8469	**0.0246***	0.8364	**0.027***	0.9643	**0.0028****
IL26	-0.07207	0.8587	0.75	0.0663	0.8469	**0.0246***	0.8469	**0.0238***	0.9286	**0.0067****
ABCG2	0.4643	0.3024	0.07143	0.9063	0.2818	0.5317	-0.02727	0.931	0.4643	0.3024
KIT	0.6365	0.1349	-0.03571	0.9635	0.1081	0.819	-0.3243	0.4587	0.6429	0.1389
CYP1A1	-0.6429	0.1389	-0.6786	0.1095	-0.1802	0.6698	-0.8108	**0.0278***	-0.3571	0.4444
CYP1B1	0.3964	0.381	0.6071	0.1667	0.3784	0.4008	0.8289	**0.0278***	0.9286	**0.0067****
CYP2S1	0.03571	0.9635	0.8571	**0.0238***	0.7568	0.0579	0.5406	0.2206	0.8154	**0.0381***
PTGS2	0.4286	0.3536	0.4643	0.3024	0.4144	0.3571	0.7928	**0.0397***	0.5357	0.2357
IL-22 protein	0.1442	0.7571	0.1071	0.8397	0.4144	0.3571	-0.1261	0.7571	0.6071	0.1667
IL-17 protein	0.7027	0.0897	0.2342	0.623	0.2857	0.556	0.1081	0.8206	0.4144	0.3595
IFN-γ protein	0.25	0.5948	-0.07143	0.9063	0.3424	0.4579	-0.1683	0.6381	0.4144	0.3556
IP-10 protein	0.3571	0.4444	0.07143	0.9063	0.6126	0.1579	0.1081	0.8206	0.4286	0.3536
TNF-α	-0.1982	0.6373	-0.1786	0.7131	0.1802	0.7032	-0.1261	0.7571	0.3214	0.4976
$CD4^+$ $c\text{-}Kit^+$	-0.2594	0.531	-0.9274	**0.0048****	-0.8289	**0.0246***	-0.8108	**0.0278***	-0.7568	0.0532
$CD8^+$ $c\text{-}Kit^+$	0.5092	0.2492	-0.7456	0.0556	-0.6881	0.0857	-0.7568	**0.0492***	-0.5559	0.1786
$CD3^-$ $c\text{-}Kit^+$	-0.1429	0.7825	-0.9286	**0.0067****	-0.7748	**0.0397***	-0.8829	**0.0095****	-1	**0.0004****
$CD3^-$ $CD56^+$ $c\text{-}Kit^+$	-0.3243	0.4563	-0.9286	**0.0067****	-0.7748	**0.0397***	-0.7928	**0.0349***	-1	**0.0004****
$CD3^-$ $CD56^-$ $c\text{-}Kit^+$	-0.1429	0.7825	-0.6786	0.1095	-0.7748	**0.0397***	-0.1818	0.6587	-0.7143	0.0881
NRF2 vs	**DMSO**		**FICZ**		**1-PP**		**1-PP + FICZ**		**1-PP + FICZ + CH-223191**	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**
NQO1	0.8289	**0.0278***	0.6071	0.1667	0.7143	0.0881	0.5	0.2667	0.8729	**0.019***
ARNT	0.9643	**0.0028***	0.7143	0.0881	0.6429	0.1389	0.9286	**0.0067***	0.8571	**0.0238***
HIF1A	0.955	**0.0032****	1	**0.0004*****	0.9286	**0.0067****	1	**0.0004*****	0.9643	**0.0028****
IL22	0.1786	0.7131	0.3929	0.3956	0.5	0.2667	0.3571	0.4444	0.5714	0.2
IL17	-0.4685	0.2778	0.5714	0.2	0.6786	0.1095	0.3929	0.3956	0.6071	0.1667
IFNγ	0.3784	0.4008	0.8214	**0.0341***	0.7857	**0.048***	0.6487	0.1262	0.9643	**0.0028****
IL26	-0.1622	0.6984	0.4286	0.3536	0.7857	**0.048***	0.5714	0.2	0.75	0.0663
ABCG2	0.2857	0.556	-0.1071	0.8397	0.1261	0.8	-0.2703	0.5373	0.2143	0.6615
KIT	0.6547	0.1254	0.1254	0.6615	0.3214	0.4976	-0.2143	0.6615	0.7143	0.0881
CYP1A1	-0.6071	0.1667	-0.6429	0.1389	-0.1071	0.8397	-0.7143	0.0881	-0.4643	0.3024
CYP1B1	0.5766	0.1857	0.5714	0.2	0.3214	0.4976	0.5357	0.2357	0.8214	**0.0341***
CYP2S1	0.2143	0.6615	0.5714	0.2	0.6429	0.1389	0.2857	0.556	0.593	0.1786
PTGS2	0.6786	0.1095	0.5357	0.2357	0.4286	0.3536	0.5357	0.2357	0.4286	0.3536
IL-22 protein	0.2883	0.5286	0.07143	0.9063	0.5357	0.2357	0.1429	0.7825	0.7143	0.0881
IL-17 protein	0.6847	0.1016	0.4286	0.3536	0.3214	0.4976	0.4643	0.3024	0.5946	0.1698
IFNg protein	0.4643	0.3024	0.1429	0.7825	0.4643	0.3024	0.2594	0.5905	0.5946	0.173
IP-10 protein	0.5357	0.2357	0.2143	0.6615	0.6429	0.1389	0.4643	0.3024	0.6071	0.1667
TNF-α	0.1802	0.7032	-0.25	0.5948	0.3214	0.4976	0.1429	0.7825	0.4286	0.3536
$CD4^+$ $c\text{-}Kit^+$	-0.1853	0.6238	-0.7456	0.0556	-0.75	0.0663	-0.7143	0.0881	-0.5946	0.1579
$CD8^+$ $c\text{-}Kit^+$	0.291	0.5365	-0.5637	0.181	-0.6547	0.1079	-0.6429	0.1389	-0.4077	0.3143
$CD3^-$ $c\text{-}Kit^+$	0	> 0.9999	-0.8571	**0.0238***	-0.6786	0.1095	-0.5714	0.2	-0.8929	**0.0123***
$CD3^-$ $CD56^+$ $c\text{-}Kit^+$	-0.07207	0.8571	-0.8571	**0.0238***	-0.6786	0.1095	-0.4643	0.3024	-0.8929	**0.0123***
$CD3^-$ $CD56^-$ $c\text{-}Kit^+$	-0.1071	0.8397	-0.5357	0.2357	-0.6786	0.1095	0.2703	0.5508	-0.6071	0.1667

viability vs	**DMSO**		**FICZ**		**1-PP**		**1-PP + FICZ**		**1-PP + FICZ + CH-223191**	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**
AHR	0.4643	0.3024	-0.6786	0.1095	-0.6847	0.0865	-0.5225	0.2206	-0.75	0.0663
NRF2	0.6429	0.1389	-0.6429	0.1389	-0.6429	0.1389	-0.4286	0.3536	-0.6429	0.1389
NQO1	0.7568	0.0571	-0.75	0.0663	-0.75	0.0663	-0.75	0.0663	-0.7638	**0.0476***
ARNT	0.7143	0.0881	-0.07143	0.9063	-0.07143	0.9063	-0.2857	0.556	-0.3571	0.4444
HIF1A	0.5225	0.2349	-0.6429	0.1389	-0.5357	0.2357	-0.4286	0.3536	-0.6786	0.1095
IL22	0.4643	0.3024	-0.03571	0.9635	-0.07143	0.9063	0.1071	0.8397	-0.2143	0.6615
IL17	-0.6307	0.123	-0.6786	0.1095	-0.75	0.0663	-0.6429	0.1389	-0.7857	**0.048***
IFNγ	-0.3604	0.4008	-0.6429	0.1389	-0.75	0.0663	-0.2162	0.6135	-0.6786	0.1095
IL26	-0.5766	0.1698	-0.2857	0.556	-0.75	0.0663	-0.3929	0.3956	-0.8214	**0.0341***
ABCG2	-0.25	0.5948	-0.4286	0.3536	-0.4505	0.2897	-0.3063	0.477	-0.4286	0.3536
KIT	0.1455	0.7619	-0.3929	0.3956	-0.3571	0.4444	0.2857	0.556	-0.4643	0.3024
CYP1A1	-0.4643	0.3024	0.3571	0.4444	0.03571	0.9635	0.6786	0.1095	0.4286	0.3536
CYP1B1	0.3784	0.4008	-0.2143	0.6615	-0.1071	0.8397	-0.6786	0.1095	-0.75	0.0663
CYP2S1	0.1786	0.7131	-0.6786	0.1095	-0.7143	0.0881	-0.6429	0.1389	-0.9266	**0.0024****
PTGS2	0.7143	0.0881	-0.07143	0.9063	0.1786	0.7131	-0.5714	0.2	-0.25	0.5948
IL-22 protein	-0.1802	0.6667	-0.2143	0.6615	-0.5	0.2667	-0.3571	0.4444	-0.8571	**0.0238***
IL-17 protein	0.1802	0.7008	-0.3929	0.3956	-0.3929	0.3956	-0.1071	0.8397	-0.7027	0.077
IFN-γ protein	0	> 0.9999	-0.1429	0.7825	-0.6786	0.1095	-0.07412	0.8405	-0.6667	0.1016
IP-10 protein	-0.03571	0.9635	-0.1786	0.7131	-0.7143	0.0881	-0.1071	0.8397	-0.6786	0.1095
TNF-α	0.1261	0.8	0.3571	0.4444	-0.07143	0.9063	-0.3571	0.4444	-0.6071	0.1667
$CD4^+$ $c\text{-}Kit^+$	0.3706	0.4429	0.4546	0.3048	0.6071	0.1667	0.7143	0.0881	0.5406	0.2135
$CD8^+$ $c\text{-}Kit^+$	0.5274	0.2365	0.09092	0.846	0.491	0.2698	0.6071	0.1667	0.07412	0.8929
$CD3^-$ $c\text{-}Kit^+$	0.1429	0.7825	0.7143	0.0881	0.8571	**0.0238***	0.5	0.2667	0.75	0.0663
$CD3^-$ $CD56^+$ $c\text{-}Kit^+$	0.2342	0.6135	0.7143	0.0881	0.8571	**0.0238***	0.6429	0.1389	0.75	0.0663
$CD3^-$ $CD56^-$ $c\text{-}Kit^+$	-0.2143	0.6615	0.1429	0.7825	0.8571	**0.0238***	0.05406	0.919	0.2143	0.6615
***CYP1* ratio vs**	**DMSO**		**FICZ**		**1-PP**		**1-PP + FICZ**		**1-PP + FICZ + CH-223191**	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**
AHR	-0.4286	0.3536	-0.9286	**0.0067****	-0.3424	0.4262	-0.7748	**0.0397***	-0.8571	**0.0238***
NRF2	-0.7857	**0.048***	-0.8929	**0.0123***	-0.2857	0.556	-0.5714	0.2	-0.8571	**0.0238***
NQO1	-0.6667	0.1	-0.6429	0.1389	-0.5	0.2667	-0.8214	**0.0341***	-0.982	**< 0.0001****
ARNT	-0.75	0.0663	-0.7143	0.0881	-0.1429	0.7825	-0.3929	0.3956	-0.7857	**0.048***
HIF1A	-0.6307	0.1222	-0.8929	**0.0123***	-0.5714	0.2	-0.5714	0.2	-0.9286	**0.0067****
IL22	0.03571	0.9635	-0.3571	0.4444	-0.2143	0.6615	-0.4286	0.3536	-0.6071	0.1667
IL17	0.3424	0.4587	-0.6786	0.1095	-0.4643	0.3024	-0.75	0.0663	-0.7143	0.0881
IFNγ	-0.1622	0.7032	-0.8571	**0.0238***	-0.2857	0.556	-0.7027	0.0778	-0.8214	**0.0341***
IL26	-0.03604	0.919	-0.6071	0.1667	-0.2857	0.556	-0.7143	0.0881	-0.8214	**0.0341***
ABCG2	0.03571	0.9635	0.2143	0.6615	0.2703	0.5587	0.07207	0.8889	0	> 0.9999
KIT	-0.2364	0.581	0.1786	0.7131	0.1786	0.7131	0	> 0.9999	-0.3929	0.3956
CYP2S1	-0.6071	0.1667	-0.6786	0.1095	-0.5714	0.2	-0.8214	**0.0341***	-0.6671	0.1048
PTGS2	-0.7143	0.0881	-0.6786	0.1095	-0.9286	**0.0067****	-0.6429	0.1389	-0.4643	0.3024
IL-22 protein	-0.2523	0.5579	-0.07143	0.9063	-0.25	0.5948	-0.3214	0.4976	-0.75	0.0663
IL-17 protein	-0.3784	0.3778	-0.3571	0.4444	-0.3214	0.4976	-0.3214	0.4976	-0.4685	0.2778
IFN-γ protein	-0.5357	0.2357	-0.03571	0.9635	0.03571	0.9635	-0.07412	0.8405	-0.4505	0.2873
IP-10 protein	-0.5	0.2667	-0.1071	0.8397	-0.2857	0.556	-0.3214	0.4976	-0.4286	0.3536
TNF-α	-0.3424	0.4262	0.03571	0.9635	-0.5357	0.2357	-0.3214	0.4976	-0.3571	0.4444
$CD4^+$ $c\text{-}Kit^+$	0.2594	0.6024	0.9092	**0.0079****	0.6786	0.1095	0.6429	0.1389	0.7928	**0.0397***
$CD8^+$ $c\text{-}Kit^+$	-0.07274	0.8524	0.8001	**0.0413***	0.3637	0.4222	0.4643	0.3024	0.5189	0.2452
$CD3^-$ $c\text{-}Kit^+$	0	> 0.9999	0.8214	**0.0341***	0.1786	0.7131	0.7857	**0.048***	0.8571	**0.0238***
$CD3^-$ $CD56^+$ $c\text{-}Kit^+$	0	0.9881	0.8214	**0.0341***	0.1786	0.7131	0.8571	**0.0238***	0.8571	**0.0238***
$CD3^-$ $CD56^-$ $c\text{-}Kit^+$	0.0238	0.6615	0.6786	0.1095	0.1786	0.7131	0.1081	0.8222	0.6429	0.1389
Viability	-0.5357	0.2357	0.4643	0.3024	-0.1429	0.7825	0.75	0.0663	0.7143	0.0881

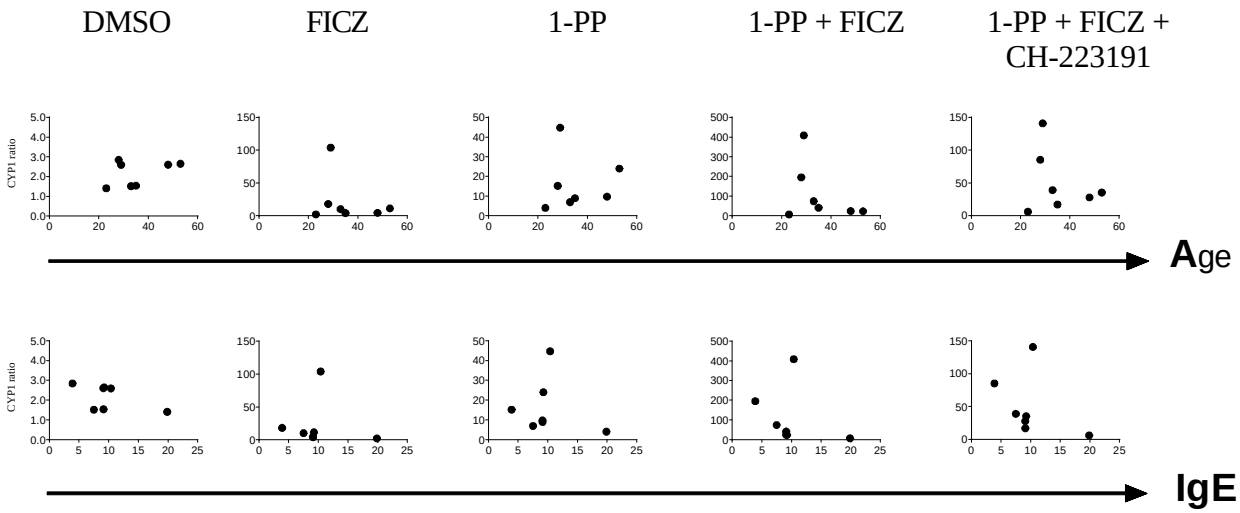

Figure 55. Correlation of *CYP1* ratio with age and IgE level

Table 40. Mean fold changes of 1-PP-dependent target gene transcription

With anti-CD3 and anti-28 activated PBMCs were treated for 5 days with the AHR agonist FICZ ($5x10^{-10}$ M), CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M) and the AHR antagonist CH-223191 ($3x10^{-6}$ M). 1-PP-dependent transcription of AHR-regulated cytochrome P450s (*CYP1A1*, *CYP1B1*, *CYP2S1*) other AHR-regulated genes (*KIT*, *ABCG2*, *NQO1*, *PTGS2*), transcription factors (*AHR*, *ARNT*, *NRF2*, *HIF1A*) and cytokines (*IL22*, *IL17*, *IFNγ*, *IL26*) was analysed with qRT-PCR. Bold values showed 1.5-fold up- or down-regulation. * marked significant values.

Comparison of conditions	10^{-6} M 1-PP vs. 10^{-7} M 1-PP		
Fold changes	1-PP	1-PP + FICZ	1-PP + FICZ + CH-223191
CYP1A1	**2.06**	**2.03***	**5.05***
CYP1B1	**1.66**	**1.81***	**1.69**
CYP2S1	1.15	1.06	1.26
C-KIT	**1.46***	**2.06***	1.26
ABCG2	1.24	1.28	0.69*
NQO1	1.11	1.00	0.94
PTGS2	1.01	0.99	1.20
NRF2	1.13*	1.02	1.04
AHR	1.14*	0.97	0.90
ARNT	1.13*	1.05	1.02
HIF1A	1.100	0.973	0.947
IL22	**1.661***	1.151	1.423
IFNγ	0.871	0.955	1.078
IL17	0.97	0.95	1.28
IL26	1.40*	1.39	0.87

Table 41. Mean fold changes in 1-PP-dependent cytokine and c-Kit regulation
PBMCs activated with anti-CD3- and anti-CD28 were incubated for 5 days with the AHR agonist FICZ ($5x10^{-10}$ M), CYP1 inhibitor 1-PP (10^{-6} M or 10^{-7} M) and the AHR antagonist CH-223191 ($3x10^{-6}$ M). Cytokine release (IL-22, IL-17, IFNγ, TNF-α and IP-10) were determined with ELISA. c-Kit$^+$ expression on $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes and on complete $CD3^-$, $CD3^-CD56^+$ and $CD3^-CD56^-$ PBMCs was analysed by flow cytometry. Wilcoxon rank test for paired samples was used to compare significant differences (*p < 0.05). Bold values showed 1.5-fold up- or down-regulation. * marked significant values.

Comparison of conditions	10^{-6} M 1-PP vs. 10^{-7} M 1-PP		
Fold changes/ protein	1-PP	1-PP + FICZ	1-PP + FICZ + CH-223191
IL-22	1.10	0.87	0.80
IL-17	0.95	0.81*	1.09
IFNγ	0.93	0.92	0.90
TNF-α	1.06	0.87	0.91
IP-10	0.86	0.80	0.79*
$CD3^+CD4^+$c-KIT$^+$	0.85	1.01	0.93
$CD3^+CD4^+$c-KIT$^+$ IL-22$^+$	0.65	**0.47***	**0.19***
$CD3^+CD8^+$c-KIT$^+$	1.01	1.31	**1.73**
$CD3^-$c-KIT$^+$	0.64*	0.88	0.77
$CD3^-CD56^+$c-KIT$^+$	0.72	0.81	0.76
$CD3^-CD56^-$c-KIT$^+$	0.605*	1.072	0.864

Table 42. Mean fold changes of RNA expression in FICZ-treated PBMCs from CMCD patients
Bold values showed 1.5-fold up- or down-regulation**.**

Fold changes RNA	Healthy		
Comparison of conditions	FICZ vs. DMSO	FICZ + CH-223191 vs. DMSO	FICZ + CH-223191 vs. FICZ
CYP1A1	**53.953**	**30.871**	**0.572**
KIT	**4.61**	**6.25**	1.36
IL22	**8.20**	**5.63**	**0.69**
IL17	0.72	0.67	0.93
AHR	0.88	1.24	1.41
	CMCD		
Comparison of conditions	FICZ vs. DMSO	FICZ + CH-223191 vs. DMSO	FICZ + CH-223191 vs. FICZ
CYP1A1	**13.24**	**9.06**	0.68
KIT	**8.98**	**8.25**	0.92
IL22	**3.56**	**4.93**	1.38
IL17	**0.12**	**0.45**	**3.77**
AHR	0.74	1.04	1.41
	CMCD vs Healthy		
	DMSO	FICZ	FICZ + CH-223191
CYP1A1	0.77	**0.19**	**0.22**
KIT	1.37	**2.66**	**1.80**
I-22	1.27	**0.55**	1.11
IL17	1.32	**0.22**	0.89
AHR	1.13	0.94	0.95

Table 43. Mean fold changes of cytokine expression in FICZ-treated PBMCs from CMCD patients
Bold values showed 1.5-fold up- or down-regulation.

Comparison of conditions /raw data		Healthy				Comparison of conditions /normalised to proliferation		Healthy			
Fold changes/protein		DMSO vs Medium	FICZ vs DMSO	FICZ + CH-223191 vs DMSO	FICZ + CH-223191 vs FICZ	Fold changes/protein		DMSO vs Medium	FICZ vs DMSO	FICZ + CH-223191 vs DMSO	FICZ + CH-223191 vs FICZ
A	IL-22	0.73	**4.90**	**4.43**	0.90	A	IL-22	0.89	**3.91**	**4.17**	1.07
B	IL-17	1.03	0.70	**0.67**	0.96	B	IL-17	1.12	**0.60**	0.76	1.28
C	IP-10	1.46	**0.41**	**0.49**	1.20	C	IP-10	**1.61**	**0.37**	**0.57**	**1.56**
		CMCD						CMCD			
Fold changes/protein		DMSO vs Medium	FICZ vs DMSO	FICZ + CH-223191 vs DMSO	FICZ + CH-223191 vs FICZ	Fold changes/protein		DMSO vs Medium	FICZ vs DMSO	FICZ + CH-223191 vs DMSO	FICZ + CH-223191 vs FICZ
A	IL-22	0.65	**5.35**	**5.10**	0.95	A	IL-22	0.76	**4.78**	**3.12**	**0.65**
B	IL-17	0.87	0.79	1.13	1.44	B	IL-17	1.05	0.78	0.77	0.98
C	IP-10	1.28	**0.47**	**0.54**	1.14	C	IP-10	**1.54**	**0.45**	**0.33**	0.74
		CMCD vs Healthy						CMCD vs Healthy			
Fold changes/protein		Medium	DMSO	FICZ	FICZ + CH-221319	Fold changes/protein		Medium	DMSO	FICZ	FICZ + CH-223191
A	IL-22	**0.21**	**0.19**	**0.21**	**0.22**	A	IL-22	**0.46**	**0.40**	**0.48**	**0.30**
B	IL-17	**0.05**	**0.05**	**0.05**	**0.08**	B	IL-17	**0.11**	**0.11**	**0.14**	**0.11**
C	IP-10	**1.67**	1.46	**1.68**	**1.59**	C	IP-10	**2.98**	**2.84**	**3.46**	**1.63**

Table 44. Mean fold changes in 1-PP and FICZ-treated PBMCs from CMCD patients
Bold values showed 1.5-fold up- or down-regulation.

Comparison of conditions		Healthy					
Fold changes/RNA		FICZ vs. DMSO	1-PP vs. DMSO	1-PP + FICZ vs. DMSO	1-PP FICZ vs. FICZ	1-PP FICZ vs. 1-PP	1-PP + FICZ vs. 1-PP + FICZ + CH-223191
A	*CYP1A1*	**1.57**	**3.83**	**80.75**	**51.52**	**21.06**	**2.73**
B	*KIT*	**1.52**	**1.57**	**4.60**	**3.03**	**2.94**	**1.85**
C	*IL22*	**4.39**	**2.69**	**8.39**	**1.91**	**3.12**	**2.13**
D	*IL17*	1.09	1.19	**0.63**	**0.57**	**0.53**	**0.66**
E	*AHR*	0.92	1.25	0.82	0.89	**0.65**	0.83
		CMCD					
Fold changes/RNA		FICZ vs. DMSO	1-PP vs. DMSO	1-PP + FICZ vs. DMSO	1-PP FICZ vs. FICZ	1-PP FICZ vs. 1-PP	1-PP + FICZ vs. 1-PP + FICZ + CH-223191
A	*CYP1A1*	1.25	**4.83**	**56.28**	**44.88**	**11.66**	**2.15**
B	*KIT*	**1.87**	**2.12**	**6.98**	**3.73**	**3.29**	**2.50**
C	*IL22*	1.21	1.34	**17.00**	**14.07**	**12.72**	**2.82**
D	*IL17*	**0.27**	0.79	**0.16**	**0.57**	**0.20**	**0.55**
E	*AHR*	0.84	1.07	**0.95**	1.13	0.89	0.90
		CMCD vs Healthy					
Fold changes/RNA			DMSO	FICZ	1-PP	1-PP + FICZ	1-PP + FICZ + CH-223191
A	*CYP1A1*		0.77	**0.61**	0.96	**0.53**	0.68
B	*KIT*		1.37	**1.68**	**1.85**	**2.07**	**1.54**
C	*IL22*		1.27	**0.35**	0.63	**2.57**	**1.95**
D	*IL17*		1.32	**0.33**	0.88	**0.33**	**0.40**
E	*AHR*		1.13	1.03	0.96	1.31	1.22

Table 45. Fold changes of cytokine expression in FICZ- and 1-PP-treated PBMCs from CMCD patients
Bold values showed 1.5-fold up- or down-regulation.

Comparison of conditions/ raw data		Healthy						Comparison of conditions/normalised to proliferation		Healthy					
Fold changes/protein		FICZ vs. DMSO	1-PP vs. DMSO	1-PP + FICZ vs. DMSO	1-PP FICZ vs. FICZ	1-PP FICZ vs. 1-PP	1-PP + FICZ vs. 1-PP + FICZ + CH-223191	Fold changes/protein		FICZ vs. DMSO	1-PP vs. DMSO	1-PP + FICZ vs. DMSO	1-PP FICZ vs. FICZ	1-PP FICZ vs. 1-PP	1-PP + FICZ vs. 1-PP + FICZ + CH-223191
A	IL-22	**2.70**	**1.63**	**4.44**	**1.65**	**2.72**	**1.53**	A	IL-22	2.24	1.49	3.65	1.63	2.44	1.37
B	IL-17	0.71	0.93	**0.60**	0.85	**0.64**	0.74	B	IL-17	**0.60**	0.85	**0.51**	0.85	**0.60**	0.67
C	IP-10	**0.45**	0.66	**0.41**	0.91	**0.62**	0.80	C	IP-10	**0.39**	**0.60**	**0.35**	0.91	**0.59**	0.72
		CMCD								CMCD					
Fold changes/protein		FICZ vs. DMSO	1-PP vs. DMSO	1-PP + FICZ vs. DMSO	1-PP FICZ vs. FICZ	1-PP FICZ vs. 1-PP	1-PP + FICZ vs. 1-PP + FICZ + CH-223191	Fold changes/protein		FICZ vs. DMSO	1-PP vs. DMSO	1-PP + FICZ vs. DMSO	1-PP FICZ vs. FICZ	1-PP FICZ vs. 1-PP	1-PP + FICZ vs. 1-PP + FICZ + CH-223191
A	IL-22	**3.19**	1.43	**4.32**	1.35	**3.02**	1.32	A	IL-22	2.70	1.78	3.10	1.15	1.74	0.84
B	IL-17	0.88	0.93	0.77	0.88	0.83	**0.52**	B	IL-17	0.74	1.16	**0.54**	0.73	**0.47**	**0.32**
C	IP-10	**0.53**	0.84	**0.46**	0.87	**0.54**	0.67	C	IP-10	**0.48**	0.95	**0.39**	0.82	**0.41**	**0.62**
		CMCD vs Healthy								CMCD vs Healthy					
Fold changes/protein			DMSO	FICZ	1-PP	1-PP + FICZ	1-PP + FICZ + CH-223191	Fold changes/protein			DMSO	FICZ	1-PP	1-PP + FICZ	1-PP + FICZ + CH-223191
A	IL-22		**0.18**	**0.21**	**0.16**	**0.17**	**0.20**	A	IL-22		**0.40**	**0.48**	**0.47**	**0.34**	**0.54**
B	IL-17		**0.05**	**0.06**	**0.05**	**0.06**	**0.08**	B	IL-17		**0.11**	**0.13**	**0.15**	**0.11**	**0.24**
C	IP-10		**2.31**	**2.71**	**2.96**	**2.58**	**3.12**	C	IP-10		**4.95**	**6.18**	**7.89**	**5.55**	**6.44**

Table 46. Cell purity and RIN - $CD14^+$ cells

$CD14^+$ cells	**$CD14^+/CD8^-$ cells**	**Isotype control**	**Purity**	**RIN value**
CD14.1	95.68	0.41	95.27	9.60
CD14.2	97.71	1.24	96.47	10.00
CD14.3	97.85	1.39	96.46	9.90
CD14.4	95.80	0.86	94.94	9.40
CD14.5	98.28	2.83	95.45	9.70
CD14.6	95.06	0.89	94.17	8.80
CD14.7	98.27	0.33	97.94	9.90
Mean			**95.81**	**9.61**
Standard deviation			**1.15**	**0.36**

Table 47. Cell purity and RIN – $CD4^+CD45RO^+CD45RA^-$ T cells

$CD4CD45RO^+$ $CD45RA^-$ T cells	**$CD45RO^+/CD45RA^-$ cells**	**Isotype control**	**Cell purity**	**RIN value**
CD4CD45RO.1	94.31	0.40	93.91	10.00
CD4CD45RO.2	96.63	0.49	96.14	9.90
CD4CD45RO.3	97.15	1.47	95.68	10.00
CD4CD45RO.4	85.43	1.07	84.36	9.50
CD4CD45RO.5	94.41	0.55	93.86	9.90
CD4CD45RO.6	94.58	2.38	92.20	9.80
CD4CD45RO.7	92.59	2.05	90.54	10.00
Mean			**92.38**	**9.87**
Standard deviation			**3.73**	**0.16**

Table 48. Cell purity and RIN - B-cells

B cells	**$CD4^-/CD19^+$ cells**	**Isotype control**	**Cell purity**	**RIN value**
Bcell.1	89.06	0.33	88.73	9.50
Bcell.2	96.58	0.13	96.45	9.70
Bcell.3	96.26	0.09	96.17	9.20
Bcell.4	98.13	0.17	97.96	N/A
Bcell.5	98.72	0.12	98.60	9.00
Bcell.6	96.75	0.19	96.56	8.70
Bcell.7	96.54	0.24	96.30	8.50
Mean			**95.82**	**9.10**
Standard deviation			**3.02**	**0.39**

Table 49. Cell purity and RIN - $CD8^+$ T cells

$CD8^+$ T cells	$CD4^-/CD8^+$ cells	Isotype control	Cell purity	RIN value
CD8.1	95.56	0.58	94.98	9.60
CD8.2	92.11	0.28	91.83	9.90
CD8.3	92.35	0.33	92.02	9.80
CD8.4	94.96	0.83	94.13	9.50
CD8.5	96.61	0.44	96.17	9.90
CD8.6	95.89	0.08	95.81	9.80
CD8.7	92.35	0.16	92.19	9.7
Mean			**93.88**	**9.74**
Standard deviation			**1.72**	**0.13**

Table 50. Cell purity and RIN - $CD4^+$ T cells

$CD4^+$ T cells	$CD8^-CD4^+$ cells	Isotype control	Cell purity	RIN value
CD4.1	95.79	0.12	95.67	9.50
CD4.2	97.35	0.00	97.35	9.50
CD4.3	96.74	0.12	96.62	9.40
CD4.4	97.89	0.21	97.68	9.20
CD4.5	98.13	0.17	97.96	9.60
CD4.6	98.86	0.01	98.85	9.50
CD4.7	97.37	0.02	97.35	9.60
Mean			**97.35**	**9.47**
Standard deviation			**0.93**	**0.12**

Table 51. Cell purity and RIN - Basophils

Basophils	$CD123^+/CD303^-$	Isotype control	Cell purity	RIN value
Bas.1	98.04	0.40	97.64	2.40
Bas.2	96.91	0.56	96.35	N/A
Bas.3	97.99	0.57	97.42	8.20
Bas.4	95.78	1.36	94.42	N/A
Bas.5	96.22	0.24	95.98	N/A
Bas.6	94.54	0.95	93.59	N/A
Bas.7	97.80	0.43	97.37	8.60
Mean			**96.11**	**6.40**
Standard deviation			**1.46**	**2.45**

Table 52. Cell purity and RIN - human primary forskin mast cells

Human primary forskin mast cells	$CD117^+$ cells	Isotype control	Cell purity	RIN value
hPMC I	95.24	1.46	93.78	N/A
hPMC II	97.75	0.98	96.77	8.70
hPMC V	97.75	0.37	97.38	8.90
hPMC VI	97.89	0.52	97.37	9.30
hPMC VII	97.24	3.15	94.09	9.10
hPMC XI	95.82	2.15	93.67	8.70
hPMC XII	95.17	2.08	93.09	9.00
Mean			**95.16**	**8.95**
Standard deviation			**1.77**	**0.21**

Table 53. Target gene fold expression in primary basophils, PMCs and CD14$^+$ cells relative to HPRT
NA: not available

Relative fold expression related to HPRT1	basophils			PMCs			CD14$^+$ cells		
housekeeping gene	Mean	SD		Mean	SD		Mean	SD	
GAPDH	68.00742	13.43541	7/7	28.63046	7.08689	7/7	33.60008	2.42672	7/7
HPRT1	1.00000	0.00000	7/7	1.00000	0.00000	7/7	1.00000	0.00000	7/7
control genes									
KIT	2.39707	0.36401	7/7	122.81383	61.25353	7/7	0.00048	NA	1/7
CMA1	0.01157	0.00809	7/7	189.08330	66.58531	7/7	NA	NA	0/7
FCER1A	0.89528	0.31362	7/7	0.00144	0.00045	2/2	0.00063	0.00027	4/7
TPSAB1 TPSB2	19.16383	27.41442	7/7	2542.20381	677.21057	7/7	NA	NA	0/7
Phase I									
CYP1A1	NA	NA	0/7	0.01146	0.01555	7/7	0.00009	NA	1/7
CYP1A2	NA	NA	0/7	NA	NA	0/7	0.00043	NA	1/7
CYP1B1	0.03853	0.02701	5/7	0.50175	0.36715	7/7	2.53602	0.96017	7/7
CYP2S1	0.01846	0.02299	7/7	0.00577	0.00948	7/7	0.09765	0.02321	7/7
CYP2A13	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7
CYP2A6	0.08849	0.11602	6/7	0.01335	0.00995	7/7	0.00334	0.00509	7/7
CYP2B6	NA	NA	0/7	0.00012	0.00009	2/7	NA	NA	7/7
CYP2C19	NA	NA	0/7	0.00071	0.00034	3/7	NA	NA	7/7
CYP2C18	NA	NA	0/7	0.00117	NA	1/7	NA	NA	7/7
CYP2C9	0.01103	NA	1/7	0.00148	NA	1/7	NA	NA	7/7
CYP2D6	0.01892	0.01764	7/7	0.00329	0.00176	7/7	0.00106	0.00030	5/7
CYP2E1	0.01049	0.00209	4/7	0.03271	0.01169	7/7	0.00069	0.00024	4/7
CYP2F1	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7
CYP2J2	NA	NA	0/7	0.00471	0.00494	7/7	NA	NA	0/7
CYP3A4	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7
CYP3A5	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7
CYP3A7	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7
CYP4F2	0.11809	0.14738	7/7	0.00718	0.00333	3/7	NA	NA	0/7
CYP4F22	0.08413	0.02125	7/7	0.23953	0.07593	7/7	0.01321	0.00619	7/7
CYP4F3	0.20647	0.13277	7/7	0.00173	NA	1/7	0.02653	0.01734	7/7
CYP19A1	NA	NA	0/7	0.00185	0.00096	2/7	NA	NA	0/7
Phase II									
AKR1A1	2.75901	0.33626	7/7	5.44286	1.02939	7/7	1.82786	0.22969	7/7
EPHX1	0.03053	0.01414	7/7	0.37512	0.18508	7/7	0.30761	0.09222	7/7
EPHX2	0.04157	0.02338	6/7	0.06633	0.06934	7/7	0.00193	0.00125	5/7
GSTM3	0.03079	0.00692	7/7	0.79242	0.22879	7/7	0.00186	0.00134	7/7
GSTP1	6.78866	1.56577	7/7	10.33044	2.54310	7/7	29.76137	3.24525	7/7
GSTT1	0.76358	0.36542	7/7	0.35290	0.12723	7/7	0.05102	0.01809	6/7
GSTZ1	0.14185	0.04667	7/7	0.24494	0.07937	7/7	0.15198	0.02538	7/7
MGST1	1.02029	0.17666	7/7	0.01602	0.01383	7/7	0.77401	0.16011	7/7
MGST2	0.76444	0.15269	7/7	0.50055	0.22211	7/7	0.61222	0.06141	7/7
MPO	1.82260	2.12796	7/7	0.00280	0.00181	3/7	0.10963	0.01950	7/7
NQO1	0.00767	0.00408	4/7	0.11367	0.03819	7/7	0.00404	0.00146	7/7
POR	3.80535	0.90758	7/7	0.89370	0.32733	7/7	1.49809	0.11452	7/7
PTGS1	2.11581	0.60472	7/7	9.88015	1.77273	7/7	0.24111	0.02620	7/7
PTGS2	0.36557	0.20872	7/7	7.62997	4.88036	7/7	0.19526	0.10564	7/7
Phase III									
ABCB1	0.12122	0.04351	7/7	0.16601	0.11079	7/7	0.00060	0.00023	4/4
ABCC2	0.15704	0.06074	7/7	0.01669	0.00796	7/7	0.00707	0.00211	7/7
ABCG2	0.02426	0.01794	4/7	0.02709	0.03985	7/7	0.00083	NA	1/7

Table 54. Target gene fold expression in primary B cells $CD4^+CD45RO^+CD45RA^-$, $CD4^+$ and $CD8^+$ T cells

Relative fold expression related to HPRT1	B cells			$CD4^+CD45RO^+$ $CD45RA^-$			$CD4^+$			$CD8^+$		
housekeeping gene	mean	SD		mean	SD		mean	SD		mean	SD	
GAPDH	7.64330	2.00594	7/7	14.59289	2.23419	7/7	10.69655	0.96475	7/7	8.93241	1.35958	7/7
HPRT1	1.00000	0.00000	7/7	1.00000	0.00000		1.00000	0.00000	7/7	1.00000	0.00000	7/7
control genes												
KIT	0.00234	0.00116	3/7	0.03637	0.01035	7/7	0.02434	0.00700	7/7	0.01809	0.01157	7/7
CMA1	NA	NA	0/7	0.00627	NA	1/7	NA	NA	0/7	NA	NA	0/7
FCER1A	NA	NA	0/7	0.00111	NA	1/7	NA	NA	0/7	NA	NA	0/7
TPSB2 TPSAB1	NA	NA	0/7	0.15957	0.13644	6/7	0.09167	0.08057	7/7	0.00608	0.00536	3/7
Phase I												
CYP1A1	NA	NA	0/7	0.00199	0.00097	3/7	0.00090	NA	1/7	0.00035	0.00008	2/7
CYP1A2	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7
CYP1B1	0.00179	0.00040	2/7	0.01384	0.01761	6/7	0.00200	NA	1/7	NA	NA	0/7
CYP2S1	0.00046	NA	1/7	0.00707	0.00289	7/7	0.00343	0.00221	7/7	0.00093	0.00048	2/7
CYP2A13	NA	NA	0/7	NA	NA	0/7	0.00923	NA	1/7	NA	NA	0/7
CYP2A6	0.00847	0.00418	7/7	0.00604	0.00496	7/7	0.00494	0.00085	7/7	0.00803	0.00512	7/7
CYP2B6	NA	NA	0/7	0.00060	0.00041	3/7	0.00098	0.00115	6/7	0.00029	0.00018	4/7
CYP2C19	NA	NA	0/7	NA	NA	7/7	NA	NA	0/7	0.00139	NA	1/7
CYP2C18	0.00152	0.00027	3/7	0.00155	NA	1/7	0.00471	NA	1/7	0.00077	0.00030	2/7
CYP2C9	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7	0.08344	0.01477	2/7
CYP2D6	0.01772	0.01007	7/7	0.00603	0.00498	7/7	0.02006	0.00903	7/7	0.00769	0.00467	7/7
CYP2E1	0.00670	0.00318	7/7	0.03251	0.00752	7/7	0.01574	0.00901	7/7	0.01880	0.01037	7/7
CYP2F1	2.28299	2.25169	2/7	NA	NA	0/7	0.00247	0.00198	2/7	NA	NA	0/7
CYP2J2	0.00994	0.00287	7/7	0.00304	0.00149	6/7	0.01563	0.00851	7/7	0.01699	0.00495	7/7
CYP3A4	NA	NA	0/7	0.00253	0.00044	2/7	0.00406	NA	1/7	NA	NA	0/7
CYP3A5	NA	NA	0/7	0.15336	NA	1/7	NA	NA	0/7	NA	NA	0/7
CYP3A7	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7	NA	NA	0/7
CYP4F2	0.00873	NA	1/7	0.00450	0.00212	5/7	0.00632	0.00490	2/7	0.00133	0.00114	2/7
CYP4F22	NA	NA	0/7	0.01097	0.00469	7/7	0.00867	0.00372	7/7	0.02580	0.00725	7/7
CYP4F3	NA	NA	0/7	0.11413	0.06160	7/7	0.07207	0.10577	7/7	0.00178	0.00052	2/7
CYP19A1	NA	NA	0/7	0.00010	NA	1/7	NA	NA	0/7	NA	NA	0/7
Phase II												
AKR1A1	1.78452	0.41857	7/7	0.80493	0.08567	7/7	0.80338	0.05629	7/7	0.87536	0.15686	7/7
EPHX1	0.02769	0.00920	7/7	0.02271	0.00774	7/7	0.02185	0.00829	7/7	0.06869	0.02758	7/7
EPHX2	0.03221	0.01242	7/7	0.39976	0.09905	7/7	1.18403	0.26312	7/7	0.74463	0.13955	7/7
GSTM2	0.15077	0.06317	7/7	0.14045	0.03586	7/7	0.41974	0.07800	7/7	0.34339	0.04818	7/7
GSTM3	0.00776	0.00464	5/7	0.03590	0.03554	7/7	0.10237	0.06997	7/7	0.07780	0.04140	7/7
GSTP1	7.06289	0.95155	7/7	4.38841	0.81269	7/7	4.61844	0.89554	7/7	5.69392	1.34676	7/7
GSTT1	0.07945	0.01627	6/7	0.03197	0.01210	6/7	0.06621	0.02438	6/7	0.05930	0.01568	6/7
GSTZ1	0.16393	0.03121	7/7	0.05547	0.00642	7/7	0.06477	0.01887	7/7	0.06634	0.02029	7/7
MGST1	0.00101	NA	1/7	0.02203	0.01173	7/7	0.02367	0.03101	7/7	0.00302	0.00203	3/7
MGST2	0.01278	0.00605	7/7	0.09837	0.01586	7/7	0.04661	0.01306	7/7	0.01778	0.00597	7/7
MPO	NA	NA	0/7	0.25592	0.15811	7/7	0.51283	0.76108	7/7	0.01763	0.03042	6/7
NQO1	0.00276	0.00164	7/7	0.00578	0.00250	7/7	0.00404	0.00208	7/7	0.00268	0.00153	7/7
POR	0.84278	0.19496	7/7	0.69479	0.13439	7/7	0.76750	0.15515	7/7	1.02215	0.37257	7/7
PTGS1	0.21231	0.03114	7/7	0.02814	0.01585	7/7	0.01295	0.01253	7/7	0.00470	0.00460	4/7
PTGS2	0.00119	NA	1/7	0.00469	0.00380	7/7	0.00322	0.00123	6/7	0.00135	0.00032	2/7
Phase III												
ABCC2	0.02379	0.00540	7/7	0.01464	0.00413	7/7	0.02353	0.00577	7/7	0.02180	0.00518	7/7
ABCG2	0.00171	0.00078	5/7	0.01703	0.01057	7/7	0.01331	0.00814	7/7	0.01891	0.01474	7/7
ABCB1	0.14239	0.02446	7/7	0.14026	0.03067	7/7	0.08128	0.02606	7/7	0.34866	0.09160	7/7

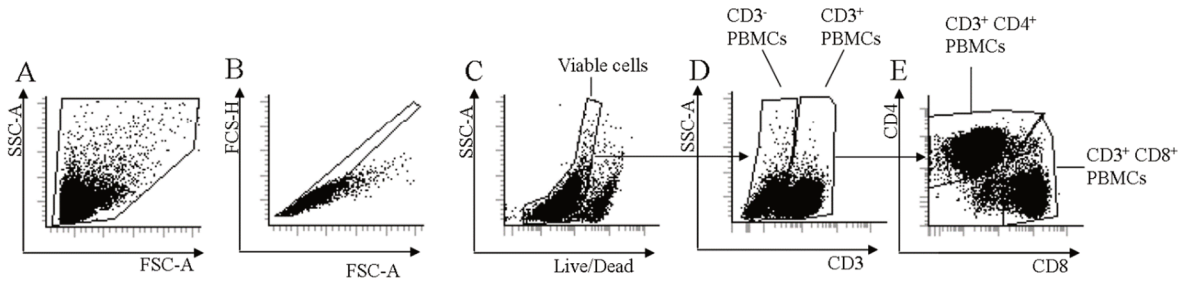

Figure 56. Gating strategy for 5 day FACS analysis