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A very usual metabolite, a very unusual pharmacological target, and an unexpected serious adverse effect that combined to halt the development of laquinimod and roquinimex

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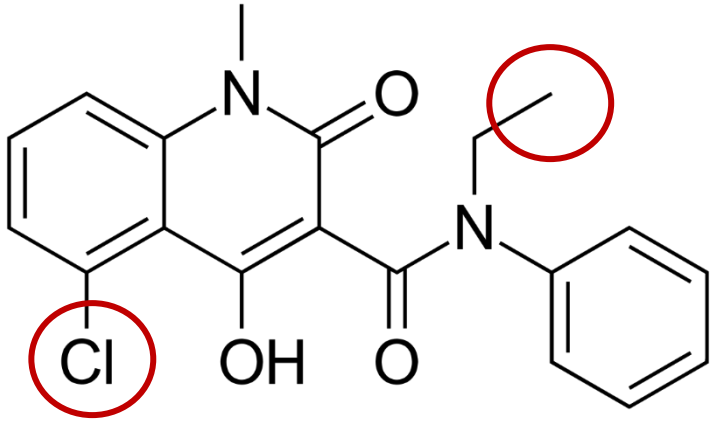
Hypha Discovery Blog

November 2022

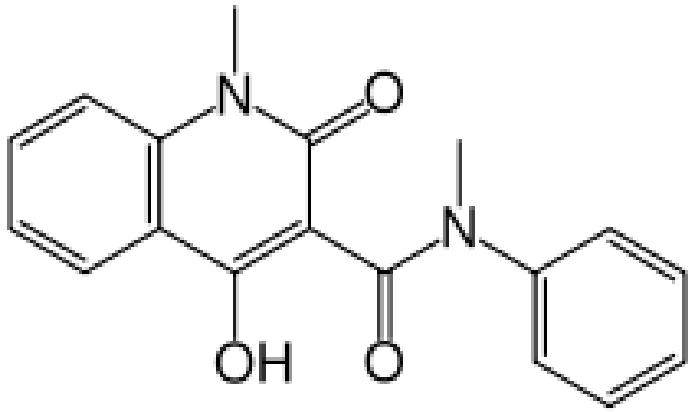
The story of laquinimod is also the story of roquinimex

Company	Teva	Pfizer
Drug	Laquinimod (Nerventra)	Roquinimex (Linomide)
Indication	Multiple sclerosis	Multiple sclerosis

Differences are highlighted in red



Laquinimod (Nerventra) - Teva



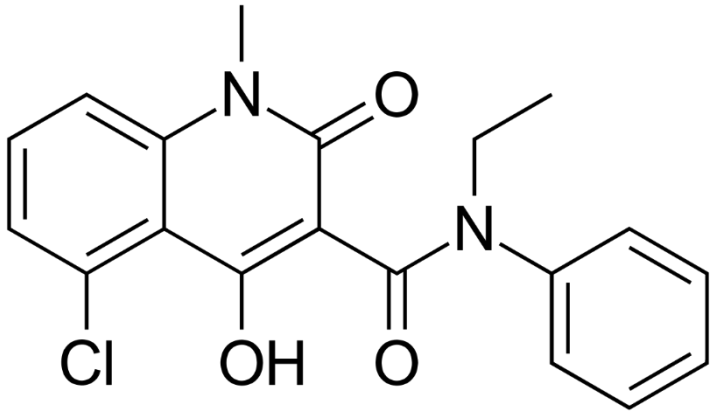
Roquinimex (Linomide) - Pfizer

In both drugs the enol (OH) group is an anionic site with a pKa(A) of ~5

Laquinimod for the treatment of multiple sclerosis

Mechanism of Action: Activation of the Aryl Hydrocarbon Receptor (AhR)

In a mouse model of multiple sclerosis (MS), laquinimod is not efficacious in AhR knock-out mice



Laquinimod (Nerventra)

Teva Pharmaceuticals

Laquinimod arrests experimental autoimmune encephalomyelitis by activating the aryl hydrocarbon receptor

Kaye J, Piryatinsky V, Birnberg T, Hingaly T, Raymond E, Kashi R, Amit-Romach E, Caballero IS, Towfic F, Ator MA, Rubinstein E, Laifenfeld D, Orbach A, Shinar D, Marantz Y, Grossman I, Knappertz V, Hayden MR and Laufer R.

Proc Natl Acad Sci USA. **113**: E6145-E6152, 2016

AhR activation is the mechanism of action of laquinimod for the treatment of multiple sclerosis

Abstract

Objective

MS is an autoimmune demyelinating disease of the CNS, which causes neurologic deficits in young adults and leads to progressive disability. The aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor, can drive anti-inflammatory functions in peripheral immune cells and also in CNS-resident cells. Laquinimod is a drug developed for the treatment of MS known to activate AHR, but the cellular targets of laquinimod are still not completely known. In this work, we analyzed the contribution of AHR activation in astrocytes to its beneficial effects in the experimental autoimmune encephalomyelitis (EAE) preclinical model of MS.

Methods

We used conditional knockout mice, in combination with genome-wide analysis of gene expression by RNA-seq and in vitro culture systems to investigate the effects of laquinimod on astrocytes.

Results

We found that AHR activation in astrocytes by laquinimod ameliorates EAE, a preclinical model of MS. Genome-wide RNA-seq transcriptional analyses detected anti-inflammatory effects of laquinimod in glial cells during EAE. Moreover, we established that the Delaq metabolite of laquinimod dampens proinflammatory mediator production while activating tissue-protective mechanisms in glia.

Conclusions

Taken together, these findings suggest that AHR activation by clinically relevant AHR agonists may represent a novel therapeutic approach for the treatment of MS.

Rothhammer V, Kenison JE, Li Z, Tjon E, Takenaka MC, Chao C-C, Alves de Lima K, Borucki DM, Kaye J and Quintana FJ.

Aryl hydrocarbon receptor activation in astrocytes by laquinimod ameliorates autoimmune inflammation in the CNS.

Neurol Neuroimmunol Neuroinflamm. **8**(2)**:** e946, 2021.

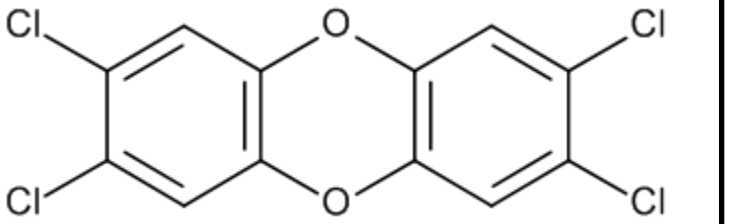
DOI: 10.1212/NXI.0000000000000946

In the case of laquinimod and roquinimex, AhR activation cannot be avoided because this is their mechanism of therapeutic action

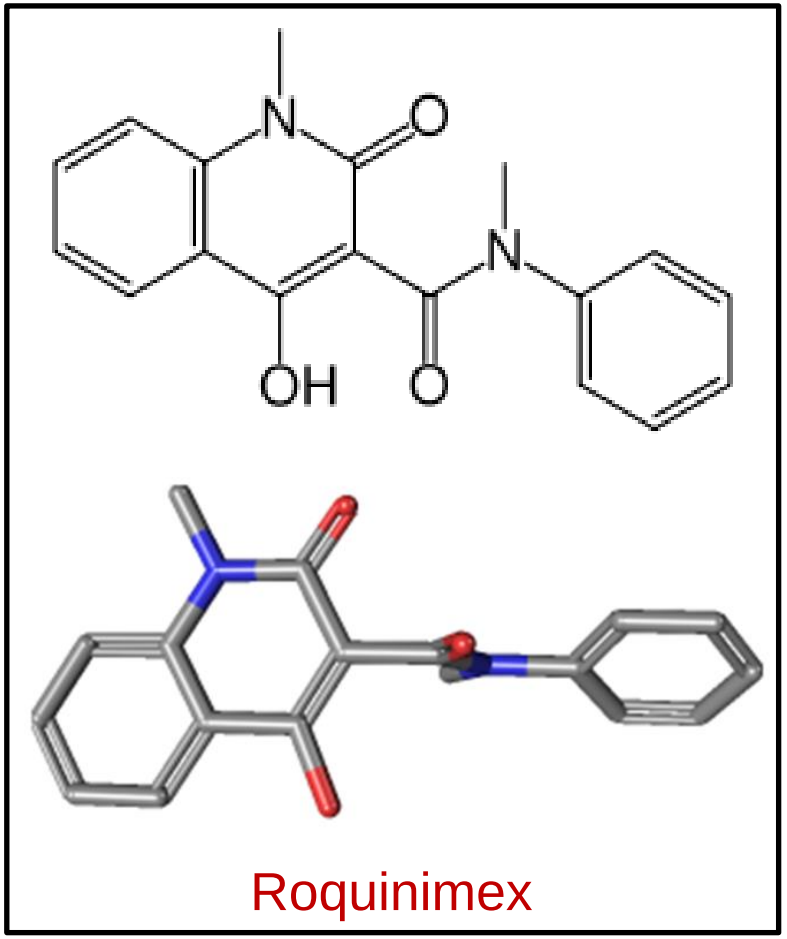
Roquinimex and laquinimod don't resemble TCDD

TCDD (dioxin) is coplanar, non-ionized and highly lipophilic (LogP ~7)

Roquinimex and laquinimod are non-planar, negatively charged (anions) and moderately lipophilic (LogP ~2.6)

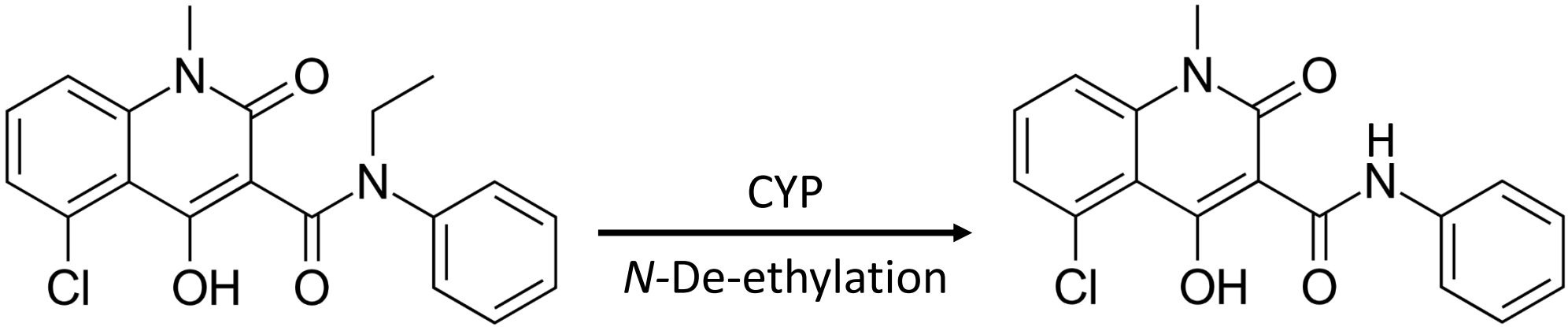


TCDD (dioxin)



Roquinimex

Laquinimod is converted by CYP-dependent *N*-de-ethylation to *N*-desethyl-laquinimod (DELAQ)



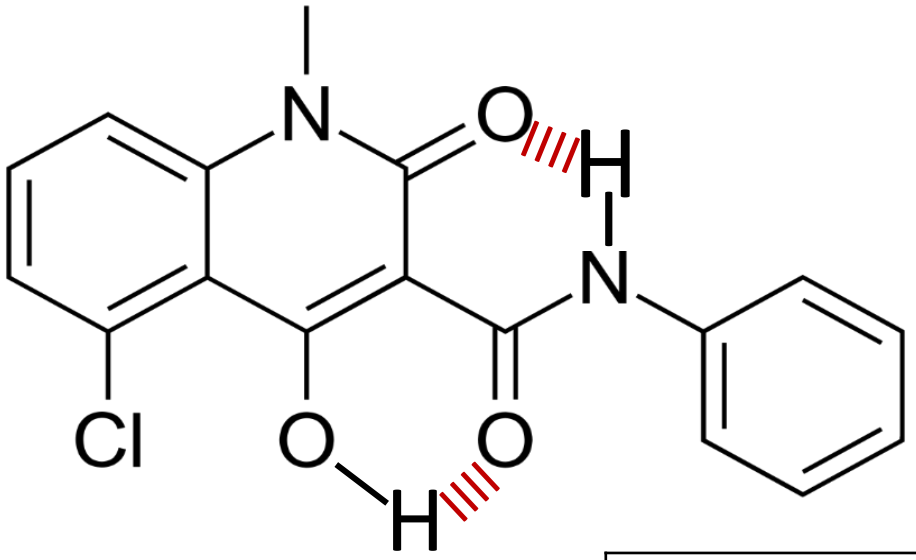
Laquinimod

DELAQ

Circulating levels of DELAQ are low compared with parent drug

Despite this, DELAQ is responsible for the therapeutic effects of laquinimod

Due to internal hydrogen bonding, DELAQ is coplanar, nonionized, and very lipophilic (like TCDD)



DELAQ and laquinimod have very different physicochemical properties

Unlike laquinimod, DELAQ activates AhR

Property	Laquinimod (parent)	DELAQ (metabolite)
Configuration	Non-planar	Coplanar
Charge in blood	Negative	Neutral
LogP	2.6	7
AhR agonist	No	Yes

DELAQ is a potent AhR agonist

Mahiout S, Lindén J, Esteban J, Sánchez-Pérez I, Sankari S, Pettersson L, Håkansson H and Pohjanvirta R.

Toxicological characterisation of two novel selective aryl hydrocarbon receptor modulators in Sprague-Dawley rats.

Toxicol Appl Pharmacol. **326**: 54-65, 2017.

The following slide is complicated but the take-home point is:

DELAQ activates AhR with a potency comparable to TCDD

S. Mahiout et al. / Toxicology and Applied Pharmacology 326 (2017) 54–65

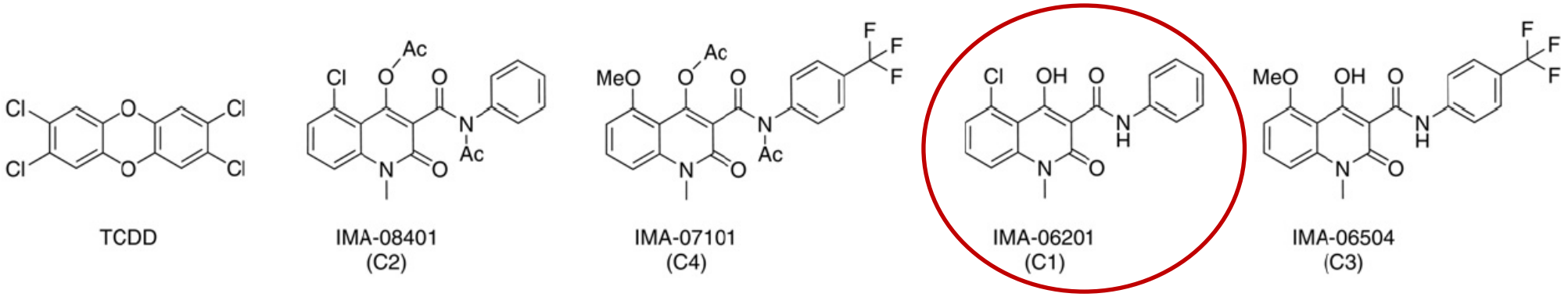
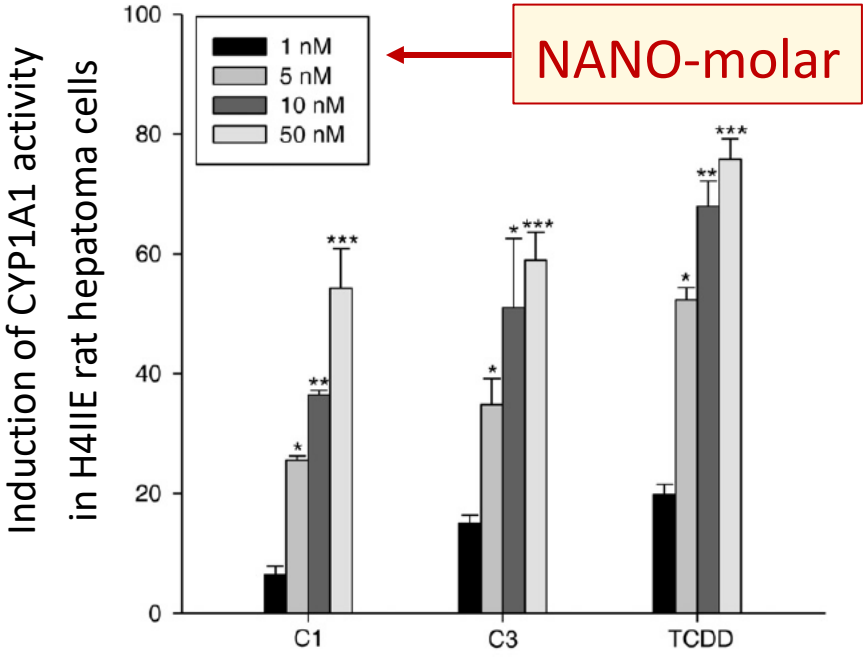


Fig. 2. Chemical structures of TCDD, C2 and C4, and those of the respective deacetylated metabolites C1 and C3 (used in *in vitro* assays).



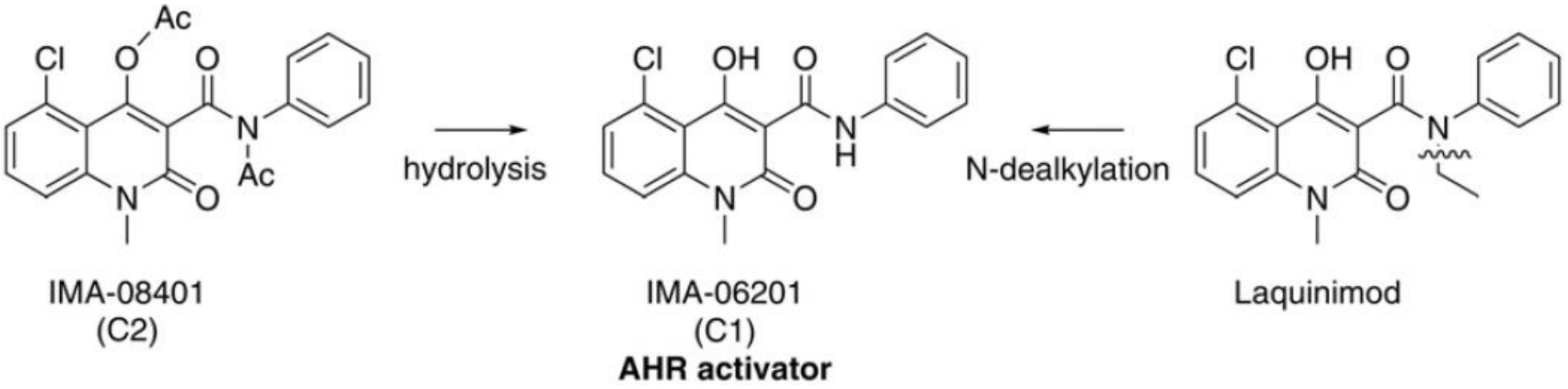
NANO-molar

C2 → C1, a **potent** CYP1A1 inducer

C4 → C3, a **potent** CYP1A1 inducer

C1 is DELAQ

The potent AhR activator C1 is the *N*-desethyl metabolite of laquinimod



IMA-08401
(C2)

IMA-06201
(C1)
AHR activator

Laquinimod

Remove the chlorine atom and C1 is the *N*-desmethyl metabolite of roquinimex

A similar study was published in 2022

Rikken G, van den Brink NJM, van Vlijmen-Willems IMJJ, van Erp PEJ, Pettersson L, Smits JPH, van den Bogaard EH. **Carboxamide derivatives are potential therapeutic AhR ligands for restoring IL-4 mediated repression of epidermal differentiation proteins**. *Int J Mol Sci*. **23**:1773, 2022.

The cardiotoxicity of laquinimod

The first two clinical studies (ALLEGRO and BRAVO) were conducted with an oral dose of 0.6 mg laquinimod. There were no signs of cardiotoxicity

The third clinical study (CONCERTO) included an oral dose of 1.2 mg laquinimod (twice the previous study). Five cases of myocardial infarction were reported.

Comi G, Dadon Y, Sasson N, Steinerman JR, Knappertz V, Vollmer TL, Boyko A, Vermersch P, Ziemssen T, Montalban X, Lublin FD, Rocca MA, Volkinshtein R, Rubinchick S, Halevy N, Filippi M.

CONCERTO: A randomized, placebo-controlled trial of oral laquinimod in relapsing-remitting multiple sclerosis. Mult Scler. 28: 608-619, 2022.

doi: 10.1177/13524585211032803. Epub 2021 Aug 11. PMID: 34378456.

What is the mechanism of cardiotoxicity?

Although clinical development of laquinimod and roquinimex were halted due to serious cardiovascular effects, the mechanism of this adverse effect is not known.

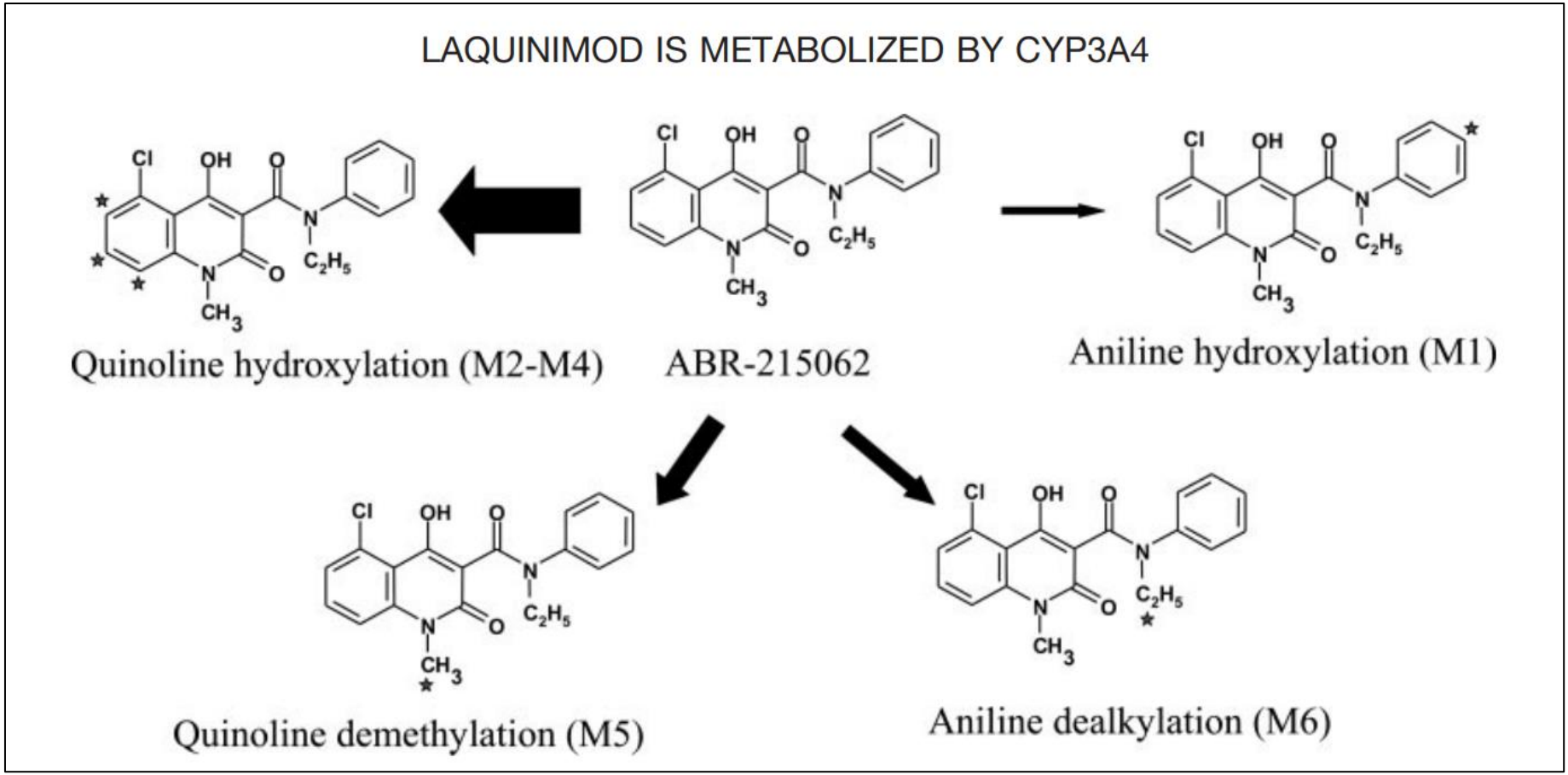
It *may* be a consequence of AhR activation.

But then again, it *may not*.

Laquinimod is converted to several metabolites. In fact, DELAQ is a minor metabolite of laquinimod (see next slide)

Parent drug and metabolites that do not activate AhR are all possible causes of cardiotoxicity.

DELAQ was not detected when laquinimod (1.5 mM) was incubated with human liver microsomes (2 mg/mL) for 60 min



Tuvesson H, Hallin I, Persson R, Sparre B, Gunnarsson PO and Seidegård J. **Cytochrome P450 3A4 is the major enzyme responsible for the metabolism of laquinimod, a novel immunomodulatory**. *Drug Metab Dispos*. **33**: 866-872, 2005.

Activation of AhR and induction of CYP1A1 and CYP1A1 often raise safety concerns in the pharmaceutical industry and regulatory agencies

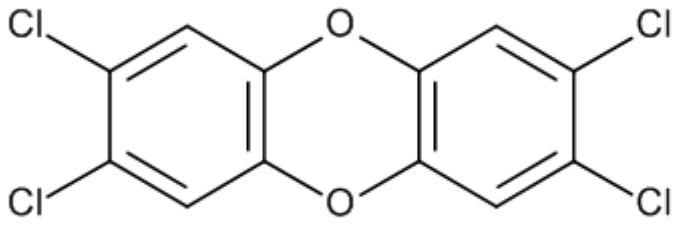
What are these concerns and why are they misplaced?

Concern 1. AhR mediates the toxicity of dioxin (TCDD) and dioxin-like compounds (DLC). In some species, dioxin is one of the most toxic compounds known

Concern 2. AhR mediates the induction of CYP1 enzymes, which can activate carcinogenic aromatic hydrocarbons and aromatic amines. When omeprazole was identified as a CYP1A inducer in human hepatocytes, the article and an editorial in *Gastroenterology* raised concerns that this proton pump inhibitor would predispose to chemical carcinogenesis

Activation of AhR raises safety concerns - Part 1

Concern 1. AhR mediates the toxicity of dioxin (TCDD) and dioxin-like compounds (DLC). In some species, dioxin is one of the most toxic compounds known. Adverse effects are species-dependent but include wasting syndrome (lethal), thymic atrophy, hepatic porphyria, chloracne and others.



2,3,7,8-Tetrachlorobenzo-*p*-dioxin

Okey AB. **An aryl hydrocarbon receptor odyssey to the shores of toxicology: The Deichmann Lecture, International Congress of Toxicology-XI.** *Toxicological Sciences*, **98**: 5-38, 2007. https://doi.org/10.1093/toxsci/kfm096

Mitigation: Non-dioxin-like compounds (non-DLCs) do not cause wasting syndrome or many of the other symptoms of dioxin toxicity

Examples: Omeprazole, β-naphthoflavone, indole-3-carbinol and various polycyclic aromatic hydrocarbons (as well as laquinimod and roquinimex)

Activation of AhR raises safety concerns - Part 2

Concern 2. Numerous *in vitro* studies established that CYP1A1 and CYP1A2 can convert numerous procarcinogens (such as polycyclic aromatic hydrocarbons [PAHs] and aromatic/heterocyclic amines/ amides) to mutagenic/carcinogenic (DNA-reactive) metabolites.

From these *in vitro* studies, it is commonly assumed that induction of CYP1 enzymes *in vivo* would *potentiate* chemical carcinogenesis by PAHs and aromatic/ heterocyclic amines.

Mitigation: Contrary to expectation, there is no *in vivo* evidence to support this inference but there is an abundance of nonclinical and clinical data to support the very opposite; namely that CYP1 induction *protects* against chemical carcinogenesis.

Activation of AhR raises safety concerns - Part 2

Why are these concerns misplaced?

Three separate lines of evidence support the conclusion that increasing CYP1 enzyme activity protects against, rather than predisposes to, chemical carcinogenesis:

1. CYP1 induction in rodents protects against chemical carcinogenesis.
2. Chemical carcinogenesis is potentiated in mice lacking CYP1A1 or CYP1A2 (so-called knockout mice).
3. Gain of-function genetic polymorphisms in human CYP1A1 do not potentiate cigarette-smoking related cancers of the lung or upper aerodigestive tract (UADT).

Activation of AhR raises safety concerns - Part 2

Why are these concerns misplaced?

Mitigation 1: CYP1 induction in rodents protects against chemical carcinogenesis

Numerous investigators have repeatedly shown that induction of CYP1 enzymes in rodents decreases the tumorigenicity of chemical carcinogens.

Treatment of rodents with 3-methylcholanthrene (3-MC), benzo[a]pyrene, β-naphtho-flavone (BNF) or TCDD, all of which induce CYP1 enzymes, provides protection against the carcinogenic effects of polycyclic aromatic hydrocarbons, aminoazo dyes, arylamines, aflatoxin, urethane and/or dimethylnitrosamine (DMN) (Anderson and Seetharam, 1985; Cohen et al., 1979; Conney, 1982; Conney and Burns, 1972; Fujioka et al., 2016; Kouri et al., 1978; Wattenberg, 1978, 1980, 1985; Wattenberg and Loub, 1978).

Anderson LM and Seetharam S Protection against tumorigenesis by 3-methylcholanthrene in mice by beta-naphthoflavone as a function of inducibility of methylcholanthrene metabolism. Cancer Res 45: 6384-6389, 1985.

Chen Z, Li Z, Niu X, Ye X, Yu Y and Lu S The effect of CYP1A1 polymorphisms on the risk of lung cancer: a global meta-analysis based on 71 case-control studies. Mutagenesis 26: 437-446, 2011.

Cohen GM, Bracken WM, Iyer RP, Berry DL, Selkirk JK and Slaga TJ Anticarcinogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene tumor initiation and its relationship to DNA binding. Cancer Res 39: 4027-4033, 1979.

Conney AH Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. Cancer Res 42: 4875-4917, 1982.

Conney AH and Burns JJ Metabolic interactions among environmental chemicals and drugs. Science 178: 576-586, 1972.

Fujioka N, Fritz V, Upadhyaya P, Kassie F and Hecht SS. Research on cruciferous vegetables, indole-3-carbinol, and cancer prevention: A tribute to Lee W. Wattenberg. Mol Nutr Food Res. 60: 1228-1238, 2016.

Kouri RE, Rude TH, Joglekar R, Dansette PM, Jerina DM, Atlas SA, Owens IS and Nebert DW 2,3,7,8-tetrachlorodibenzo-p-dioxin as cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in mice genetically "nonresponsive" at Ah locus. Cancer Res 38: 2777-2783, 1978.

Sykora P The discrepancy between in vivo and in vitro experiments with polycyclic aromatic hydrocarbon(PAH) carcinogens: a hypothetical explanation. J Theor Biol 110: 59-66, 1984.

Wattenberg LW Effects of dietary constituents on the metabolism of chemical carcinogens. Cancer Res 35: 3326-3331, 1975.

Wattenberg LW Inhibition of chemical carcinogenesis. J Natl Cancer Inst 60: 11-18, 1978.

Wattenberg LW Inhibitors of chemical carcinogens. J Environ Pathol Toxicol 3: 35-52, 1980.

Wattenberg LW Chemoprevention of cancer. Cancer Res 45: 1-8, 1985.

Wattenberg LW and Loub WD Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. Cancer Res 38: 1410-1413, 1978.

Zhan P, Wang Q, Qian Q, Wei SZ and Yu LK CYP1A1 MspI and exon7 gene polymorphisms and lung cancer risk: an updated meta-analysis and review. J Exp Clin Cancer Res 30: 99, 2011.

Activation of AhR raises safety concerns - Part 2

Why are these concerns misplaced?

Mitigation 2: Chemical carcinogenesis is potentiated in mice lacking CYP1A1 or CYP1A2 (knockout mice)

Deletion of CYP1A1 *potentiates* the toxicity of oral benzo[a]pyrene, which causes increased spleen and thymus weight, leukocytopenia and extreme hypercellularity in the bone marrow (and death within 30 days) in CYP1A1 knockout mice but not in wild-type mice.

Likewise, deletion of CYP1A2 similarly potentiates the toxicity and liver tumorigenicity of aromatic/heterocyclic amines. Deletion of CYP1A2 increased the toxicity of 4-aminobiphenyl (methemoglobinemia), adduct formation in liver and bladder (two targets of arylamine carcinogenicity in rodents) and the incidence of hepatocellular tumors and preneoplastic foci. Deletion of CYP1A2 likewise increased adduct and tumor formation by the cooked food mutagens (heterocyclic amines) known as PhIP and IQ.

These studies establish that metabolism of aromatic/heterocyclic amine/amide by CYP1 enzymes is protective (Ma and Lu, 2007; Nebert and Dalton, 2006; Shimada, 2006).

Ma Q and Lu AY CYP1A induction and human risk assessment: an evolving tale of in vitro and in vivo studies. Drug Metab Dispos 35: 1009-1016, 2007.

Nebert DW and Dalton TP The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat Rev Cancer 6: 947-960, 2006.

Shimada T Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. Drug Metab Pharmacokinet 21: 257-276, 2006.

Activation of AhR raises safety concerns - Part 2

Why are these concerns misplaced?

Mitigation 3: Gain of-function genetic polymorphisms in human CYP1A1 do not potentiate cigarette-smoking related cancers of the lung or upper aerodigestive tract.

A large number of studies have examined the impact of genetic polymorphisms in CYP1A1 on the incidence of lung and upper aerodigestive tract (UADT) cancers in cigarette smokers and, in some cases, non-smokers.

Two meta-analysis studies, one based on 18,397 subjects (Zhan et al., 2011) and the other based on 30,368 subjects (Chen et al., 2011), both concluded that the overall risk of cigarette smoking lung cancer posed by the gain-of-function polymorphism in CYP1A1 was low (odds ratio ~1.2) but that slightly higher risks could be discerned in subgroups based on ethnicity.

Chen Z, Li Z, Niu X, Ye X, Yu Y and Lu S The effect of CYP1A1 polymorphisms on the risk of lung cancer: a global meta-analysis based on 71 case-control studies. Mutagenesis 26: 437-446, 2011.

Zhan P, Wang Q, Qian Q, Wei SZ and Yu LK CYP1A1 MspI and exon7 gene polymorphisms and lung cancer risk: an updated meta-analysis and review. J Exp Clin Cancer Res 30: 99, 2011.

Additional references about concerns over CYP1 induction and reasons why these concerns are misplaced

Diaz D, Fabre I, Daujat M, Saint Aubert B, Bories P, Michel H and Maurel P. Omeprazole is an aryl hydrocarbon-like inducer of human hepatic cytochrome P450. *Gastroenterology* **99**: 737-747, 1990.

Editorial about the previous paper
Farrel GC and Murray M. Human cytochrome P450 isoforms. *Gastroenterology* **99**: 885-889, 1990.

Letter to Gastroenterology (with 116 references)
Parkinson A and Hurwitz A. Omeprazole and the induction of human cytochrome P-450: A response to concerns about potential adverse effects. *Gastroenterology* **100**: 1157-1164, 1991.

Why do we *think* CYP1A induction will promote chemical carcinogenesis?

Because CYP1A enzymes catalyze the detoxication of chemical carcinogens more than they catalyze activation, but *in vitro* test systems tend to measure only the latter. Here is an analogy

100 kids are each given a voucher for a free Happy Meal at McDonald's. Another 100 kids are given a voucher for a free meal at a fancy seafood restaurant (McLobster).

Only complaints are measured.

On the first day, 5 kids complain about McDonald's, but only 2 complain about McLobster. The next day, no kids complain about McDonald's, but 2 more kids complain about McLobster.

It turns out all 100 kids ate at McDonald's on the first day, and only 5 of them complained (5% adverse reaction). But only 4 kids ate McLobster each day. The complaints came in slowly, but after all the kids had eaten at McLobster 50 of them had complained (50% adverse reaction). Rate and extent tell two different stories.

CYP1A enzymes are McDonald's. Complaints (reactive metabolites) look bad based on rate. But they look really good based on extent. When everyone is served (when all of the carcinogen is metabolized) you have far greater satisfaction (detoxication) than complaints (activation). That's why CYP1A enzymes protect against chemical carcinogenesis.

One fun slide

One serious slide

And one speculative slide

Is there any other case where two related things that *should* be alike turn out to be completely different? Yes there is

Parent
(Dr. Parkinson #1)

Metabolite (Son)
(Oliver or Dr. Parkinson #2)

The aryl hydrocarbon receptor (AhR)

Old view — AhR = a toxicological receptor

New view — AhR = a potential pharmacological target

An endogenous aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress experimental autoimmune encephalomyelitis.

Quintana FJ, Murugaiyan G, Farez MF, Mitsdoerffer M, Tukpah AM, Burns EJ and Weiner HL. *Proc Natl Acad Sci USA.* **107**: 20768-20773, 2010

The aryl hydrocarbon receptor: A molecular pathway for the environmental control of the immune response.

Quintana FJ. *Immunology* **138**: 183-189, 2013

Aryl hydrocarbon receptor control of adaptive immunity.

Quintana FJ and Sherr DH. *Pharmacol Rev* **65**: 1148-1161, 2013

The CONCERTO study reported that laquinimod causes pancreatitis

Quote from the publication cited below

Similarly, there was an apparent dose-related increase in risk of pancreatitis, with 5 cases (0.7%) in the 0.6-mg treatment group, 11 cases (1.5%) in the 1.2-mg treatment group, and 4 cases (0.5%) in the placebo group.

Dose-related pancreatitis

Placebo 0.5%

0.6 mg 0.7%

1.2 mg 1.5%

What does this have to do with Ukraine?

Comi G, Dadon Y, Sasson N, Steinerman JR, Knappertz V, Vollmer TL, Boyko A, Vermersch P, Ziemssen T, Montalban X, Lublin FD, Rocca MA, Volkinshtein R, Rubinchick S, Halevy N, Filippi M.

CONCERTO: A randomized, placebo-controlled trial of oral laquinimod in relapsing-remitting multiple sclerosis. Mult Scler. 28: 608-619, 2022.

doi: 10.1177/13524585211032803. Epub 2021 Aug 11. PMID: 34378456.

Viktor Yushchenko, the ex president of Ukraine

In 2004, someone tried to assassinate the president of Ukraine with TCDD

He developed chloracne (a toxic effect of TCDD) but he survived

His first symptom was backache. His doctor suspected pancreatitis. He was right.

One of the lab tests that established Viktor Yushchenko had pancreatitis was a low level of melatonin

Melatonin is a substrate for CYP1A1 and CYP1A2, the enzymes induced by AhR agonists

If you develop an AhR agonist, you might consider coadministering melatonin

Who knows? It might be cardioprotective. Although that's pure speculation on my part, the references on the last slide suggest it might have some merit.

Melatonin protects against pancreatitis

Chen HM, Chen JC, Ng CJ, Chiu DF and Chen MF. Melatonin reduces pancreatic prostaglandins production and protects against caerulein-induced pancreatitis in rats. *J Pineal Res.* **40**: 34-9, 2006.

Jaworek J, Szklarczyk J, Jaworek AK, Nawrot-Porąbka K, Leja-Szpak A, Bonior J and Kot M, Protective Effect of Melatonin on Acute Pancreatitis. *Int J Inflam*, 1-8, 2012 (doi:10.1155/2012/173675).

Jin Y, Lin CJ, Dong LM, Chen MJ, Zhou Q and Wu JS. Clinical significance of melatonin concentrations in predicting the severity of acute pancreatitis. *World J Gastroenterol.* **19**: 4066-4071, 2013.

Jaworek J, Leja-Szpak A, Kot M, Jaworek A, Nawrot-Porbka K, Bonior J and Szklarczyk J. The role of melatonin in pancreatic protection: could melatonin be used in the treatment of acute pancreatitis? *Curr Pharm Des.* **20**: 4834-4840, 2014.

Jaworek J, Leja-Szpak A, Nawrot-Porąbka K, Szklarczyk J, Kot M, Pierzchalski P, Góralska M, Ceranowicz P, Warzecha Z, Dembinski A and Bonior J. Effects of melatonin and its analogues on pancreatic inflammation, enzyme secretion, and tumorigenesis. *Int J Mol Sci.* **18**: 1-13, 2017.