The impact of N-nitrosamine impurities on clinical drug development:
Drug-Drug Interaction Studies

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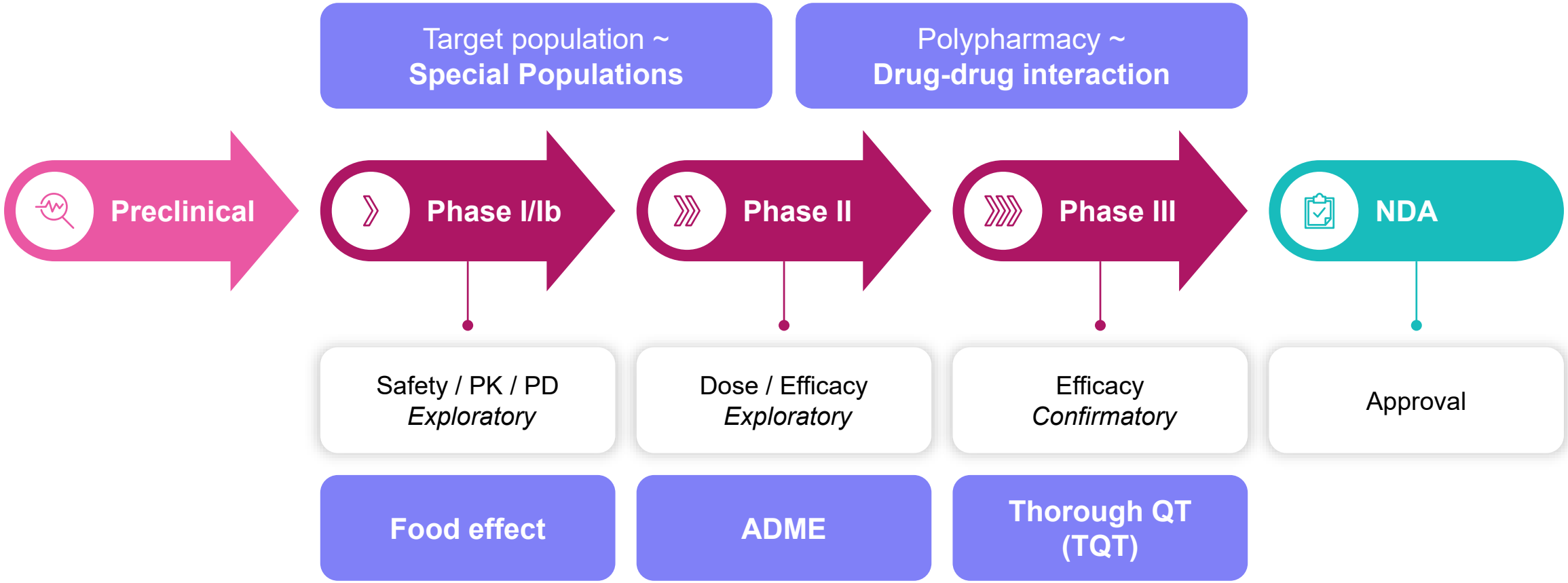
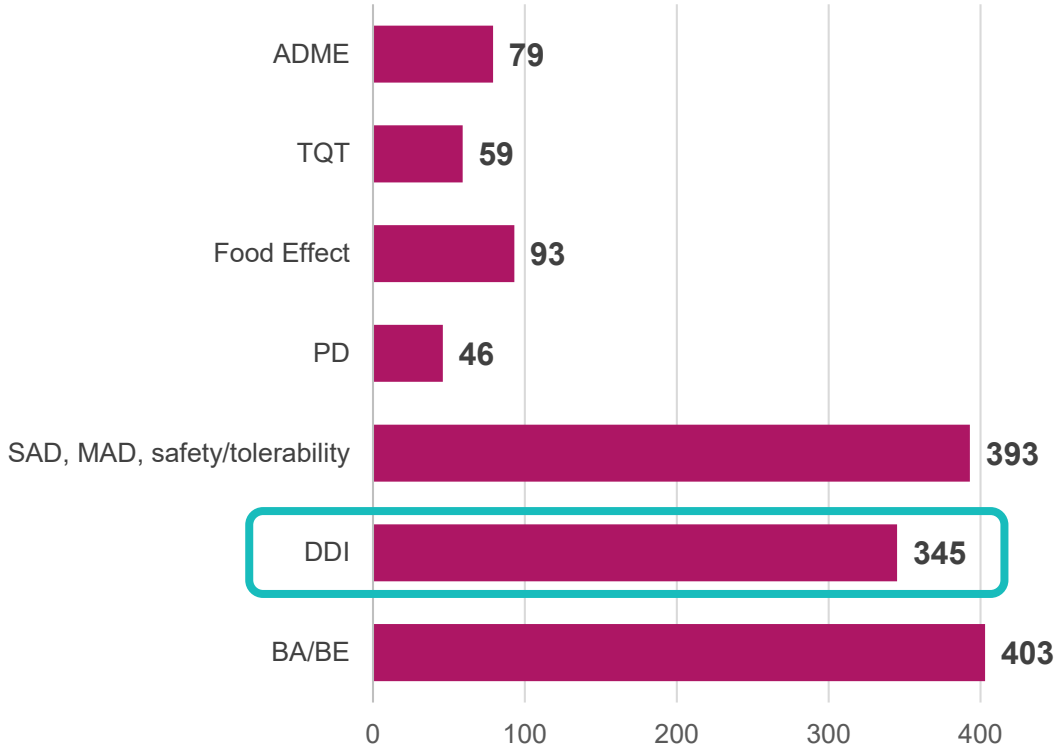
23 March 2023

Clinical Studies Enabling a New Drug Application (NDA)

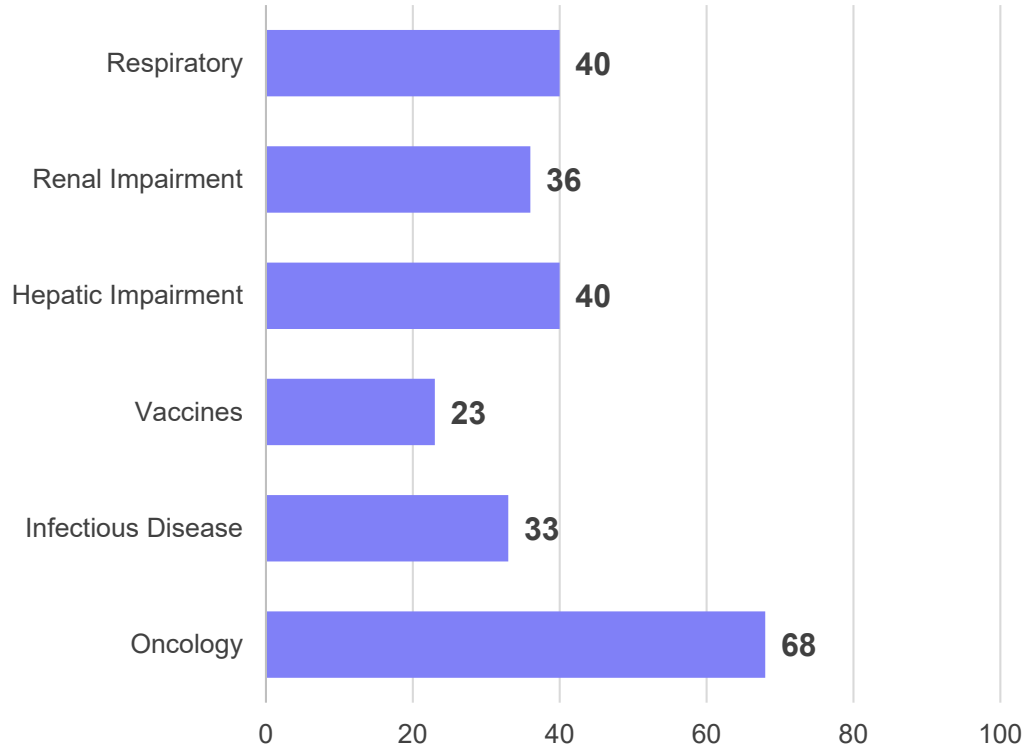


Celerion Study Experience <10 Years

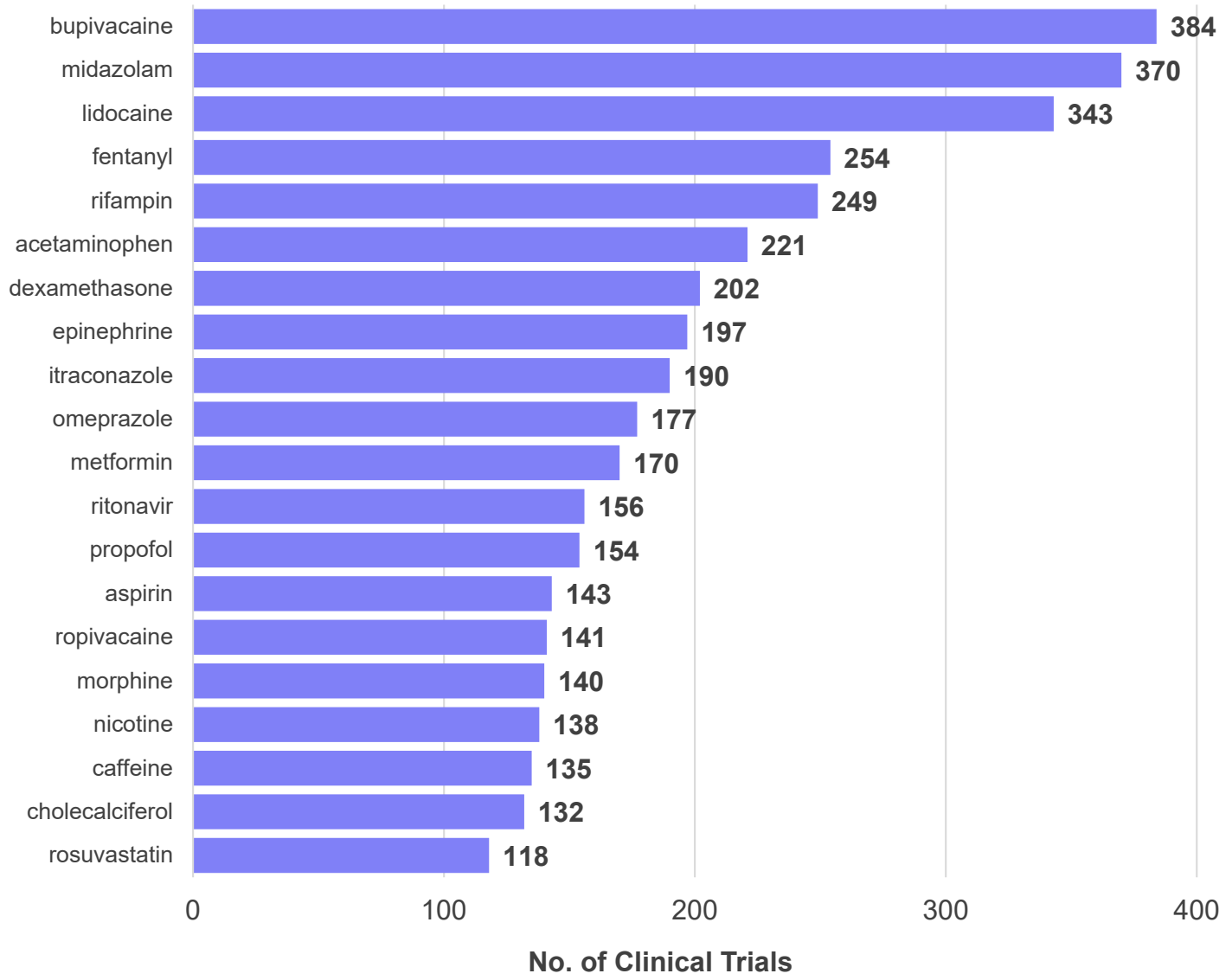
Healthy Volunteers



Studies in Patients
Phase I - IV



Marketed Drugs Used in DDI Studies?



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N-Nitrosamines Impurities in Marketed Medication

Recent history of N-nitrosamine in common medications, resulting in drug recalls

- 2018 – Angiotensin II receptor blockers – NDMA, NDEA, NMBA
- 2019 – Metformin – NDMA
- 2019 – Ranitidine (Zantac) – NDMA
- 2020 – **Rifampin** / Rifapentine – MNP (Rifampin) / CPNP (Rifapentine)
- 2021 – Varenicline (Chantix) – N-nitroso-varenicline

https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications

Source of N-nitrosamine

- Manufacturing process
- Chemical structure
- Storage, package conditions

CPNP, 1-cyclopentyl-4-nitrosopiperazine; **NMBA**, N-nitroso-N-methyl-4-aminobutyric acid; **NDEA**, N-Nitrosoethylisopropylamine; **MNP**, 1-methyl-4-nitrosopiperazine

N-Nitrosamines

- Chemical compounds with a functional N-nitroso group (>N-N=O)
- Present at low levels in water and (processed) foods, and in tobacco smoke
- Nitrosamines are metabolized in the liver (CYP2E1) and can produce DNA reacting agents
  - Cytotoxic
  - Mutagenic
  - Carcinogenic
- Probable or possible human carcinogens
- ICH M7: Cohort of concern
- Most potent: NDEA and NDMA

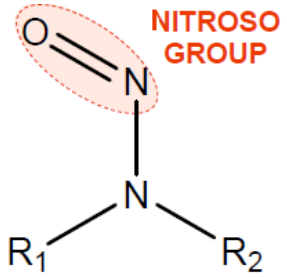
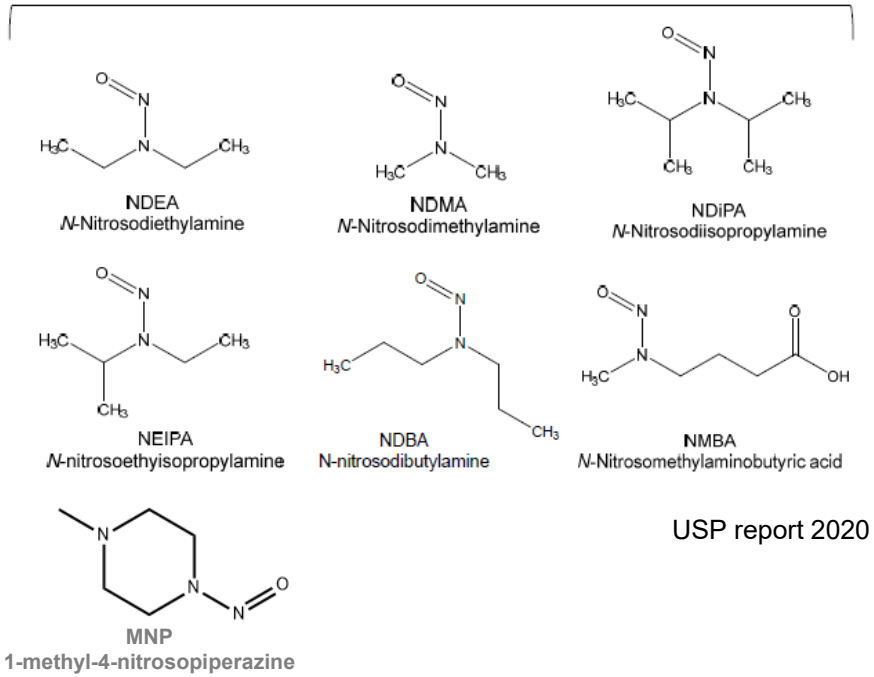


Figure 1. Generic *N*-nitrosamine structure



USP report 2020

DDI Substrates and Perpetrators: N-nitrosamine contaminations

Drug	Nitrosamine Detected	Role in DDI Studies	Impact to DDI Studies	Alternatives for DDI Studies
Ranitidine, Nizatidine	NDMA	Acid reducing agent	Removed from market (ranitidine) or recalled (nizatidine)	Famotidine or proton pump inhibitor (esomeprazole or rabeprazole)
Metformin	NDMA	OCT2, MATE1/2K substrate	No impact	IR-metformin is available for DDI studies and does not contain impurity
Rifampin	MNP	1. Strong CYP3A4 inducer 2. OATP1B1/3 inhibitor (single dose)	Batches available for patients only, use alternatives	1. Carbamazepine, efavirenz, lumacaftor, phenytoin 2. Atazanavir & ritonavir, clarithromycin, cyclosporine, gemfibrozil, lopinavir, ritonavir
Propranolol	nitroso-propranolol	CYP2D6 substrate	Product recalled (CND), but no impact (not an index substrate)	Desipramine, dextromethorphan, nebivolol

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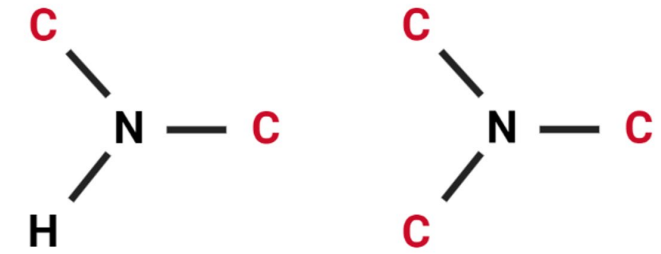
Nitrosamines - NDSRIs

- Multiple routes for nitrosamine formation
  - Both from API and excipients or solvents
- API-derived complex nitrosamines called **NDSRIs**
  - Nitrosamine Drug Substance Related Impurities
- Nitrosamine precursors
  - Secondary amines *(risk depending on basicity of secondary amine)*
  - Tertiary amines *(2-3 orders of magnitude slower nitrosation of amine)*



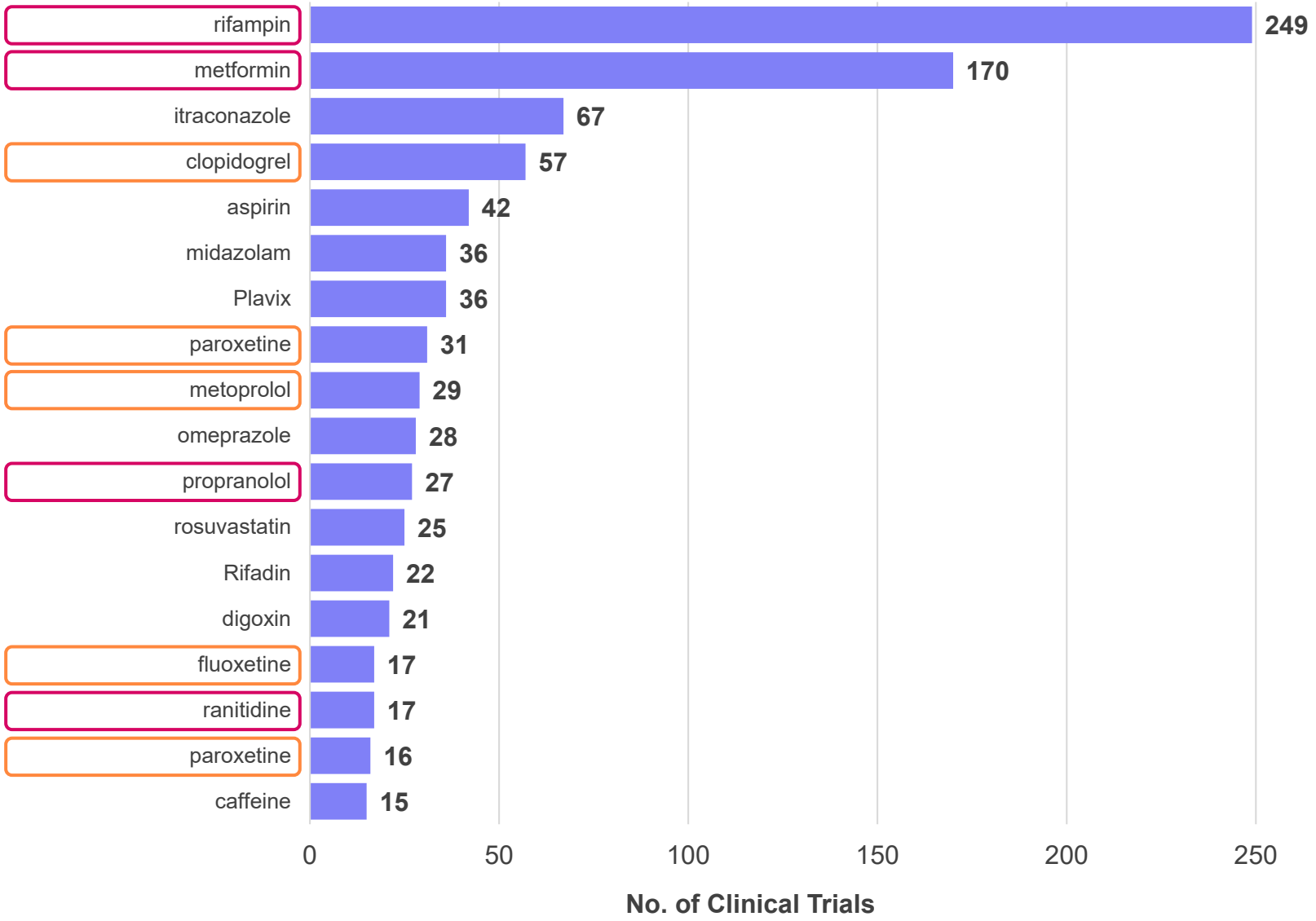
Secondary amine Tertiary amine

DDI Substrates and Perpetrators at Risk

Drug	Amine	Role in DDI Studies	Alternatives for DDI Studies
Metoprolol	Secondary	Moderate CYP2D6 sensitive substrate	Encainide, Propafenone
Fluoxetine, Paroxetine	Secondary	1. Strong CYP2D6 index inhibitors 2. Strong CYP2C19 inhibitor (fluoxetine only)	1. Mirabegron (moderate inhibitor) 2. Fluconazole
Duloxetine	Secondary	1. Sensitive CYP1A2 substrate 2. Moderate CYP2D6 inhibitor	1. Alosetron, Caffeine, Melatonin, Ramelteon, Tasimelteon, Tizanidine 2. Mirabegron
Clopidogrel	Tertiary	Moderate CYP2C8 index inhibitor	Gemfibrozil (strong index inhibitor)
Ticlopidine	Tertiary	Strong CYP2C19 inhibitor	Fluconazole
Desipramine	Tertiary	Sensitive CYP2D6 index substrate	Dextromethorphan, Nebivolol
Imipramine	Tertiary	Moderate CYP2D6 sensitive substrate	Encainide, Propafenone
Venlafaxine	Tertiary	R-venlafaxine sensitive CYP2D6 substrate S-venlafaxine moderate sensitive CYP2D6 substrate	Dextromethorphan

Paglialunga & van Haarst. J Pharm Sci 2023;
Schlingemann et al. J Pharm Sci 2022

(DDI) Studies with Marketed Drugs: Contaminated or at Risk



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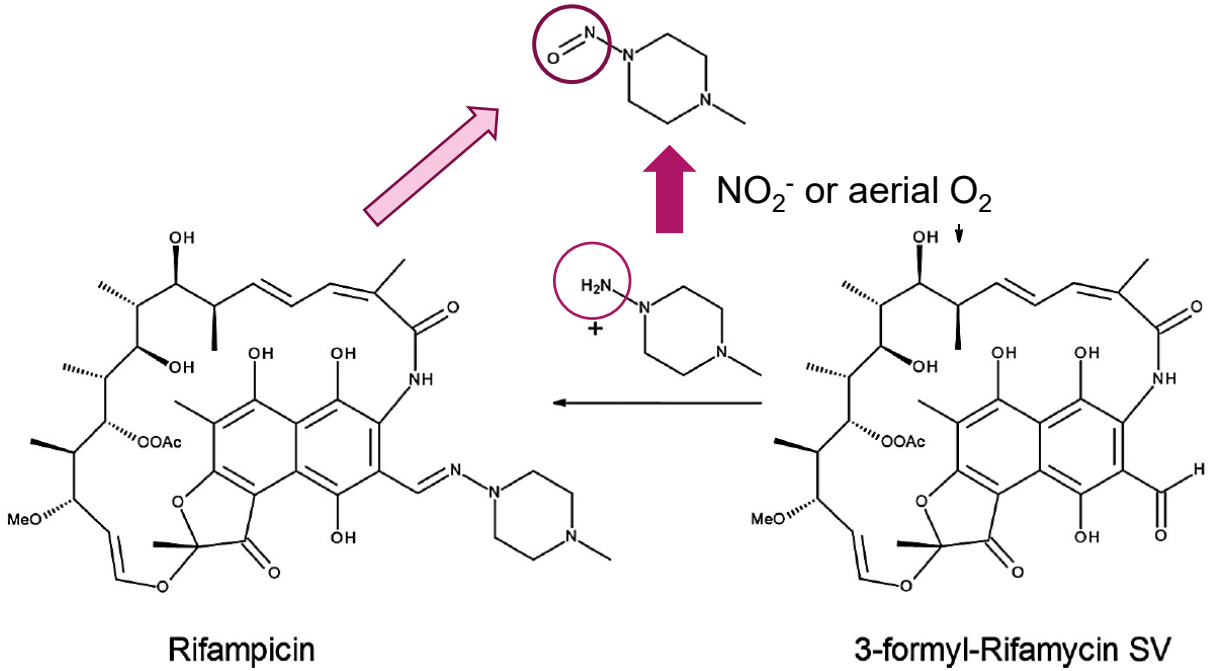
Rifampin Impurity Issue: Implications for DDI Studies?

N-Nitrosamine Source - Manufacturing Risk

- Rifampin is derived from rifamycin B and is used for treating tuberculosis
- During manufacturing, addition of AMP can lead to MNP formation
- Acceptable intake (AI)
  - AI <0.16 ppm MNP
  - MNP ranges across batches: 1.49 – 3.47 ppm
  - AI for use in patients only: <5 ppm

N-nitrosamine:
MNP = 1-methyl-4-nitrosopiperazine



Wohlfart et al. J Pharma Biomed Analysis 2021

AMP = 1-amino-4-methilpiperazine

Rifampin Use in Healthy Volunteers; Regulatory Positions

FDA:
Per Celerion-Sponsor communication:

FDA notified 2 of our Sponsors that ***using rifampin in healthy subjects is NOT acceptable*** and suggested to one of the sponsors to use phenytoin or carbamazepine

EMA:
Per EMA Committee for medicinal products for human use – Meeting minutes 19-22 Apr 2021:

The CHMP noted the question from the PKWP on the use of Rifampicin in Drug Interaction Studies in healthy volunteers and discussed the recommendation from the Nitrosamine Implementation Oversight Group (NIOG) that Rifampicin containing nitrosamine levels above the acceptable intake should not be used in these studies. The CHMP was in agreement with the recommendation and adopted the response to PKWP.

MHRA:
Per general Celerion inquire to MHRA:

The Commission on Human Medicines has advised that that rifampin should, at present, ***not be used in Drug-Drug-Interaction studies healthy volunteers***. Alternative suitable PK-inducers, such as Rifabutin, may be used instead.

Selection of Rifampin Alternatives

- ICH Harmonized Guidance: M12 DDI Studies (Draft '22)
  - https://www.fda.gov/media/161199/download
  - https://www.ema.europa.eu/en/documents/scientific-guideline/draft-ich-guideline-m12-drug-interaction-studies-step-2b_en.pdf

- "When evaluating the investigational drug as a substrate, the first clinical DDI studies should, in general, determine the effects of a strong index inhibitor and a strong index inducer"

- "Moderate index inhibitors or inducers can be used if strong index inhibitors or inducers are not available for a particular enzyme"

CYP Enzyme	Strong inducers	Moderate inducers
CYP3A	Carbamazepine, Phenytoin, Rifampin,	Efavirenz

Rifampin Replacement Candidates

Perpetrator	Drug Type & Indication	Comments	Suitable?
Apalutamide	Nonsteroidal antiandrogen	Increased risk of seizure and incidence of fall and fractures	✗
Carbamazepine	Sodium channel blocker	Dose titration to mitigate AEs and black box warning	✓
Efavirenz	Nonnucleoside rt inhibitor	Listed in ICH M12 Guidance; only moderate inducer	?
Enzalutamide	Nonsteroidal antiandrogen	Increased risk of seizure and incidence of fall and fractures	✗
Ivosidenib	Mutant isocitrate-DH1 inhibitor	Multiple doses of ivosidenib not studied in healthy participants (lack of data)	✗
Lumacaftor	CFTR modulator	Only in combination with ivacaftor (Orkambi). Favorable safety profile	?
Mitotane	Adrenal cytotoxic agent	Occurrence of common AEs >15%	✗
Phenytoin	Sodium channel blocker	Narrow therapeutic window, yet preferred perpetrator	✓ ✓
Rifabutin	Antimicrobial	MHRA recommendation; not an option listed by FDA	✗
St. John's wort	Herbal supplement	Effect varies widely and is preparation-dependent	✗

Adapted from FDA (www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers) Table 3-3.

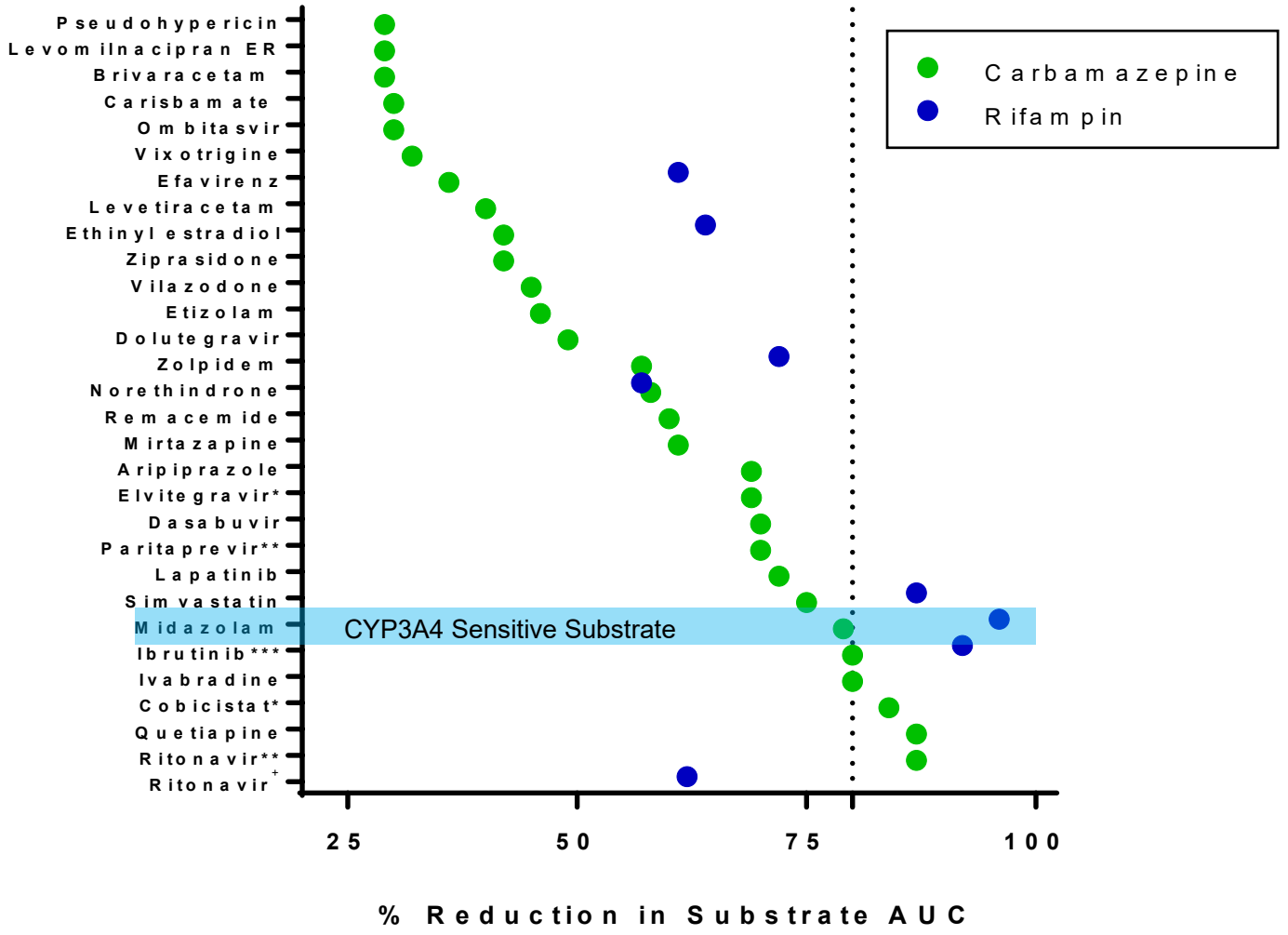
Carbamazepine as Alternative?

- Anticonvulsant drug
- **Strong inducer of CYP3A** and CYP2B6;
  - Also weak inducer of CYP2C9
- Safety concerns:
  - Risk of severe cutaneous adverse reactions with high starting doses (**Black Box warning**)
  - Risk of aplastic anemia & agranulocytosis
- Risk mitigation measures
  - Exclude anyone with positive HLAB*1502 allele (~ risk of CBZ hypersensitivity reactions)
  - Dose titration to mitigate AEs
    - 3+3 days 100 / 200 mg BID; ≥7 days 300 mg BID
  - Monitor platelet and WBC counts
- Sufficient experience with DDI trials in healthy volunteers

> In study A, 7 out of twelve subjects completed the study according to protocol. Five subjects discontinued the study due to the emergence of generalized exanthema, a well-known and common side effect of carbamazepine. In three

Sitsen et al., Eur. J. Drug Metab. Pharmacokinet. 2001

Carbamazepine- and Rifampin-Induced %AUC Reduction

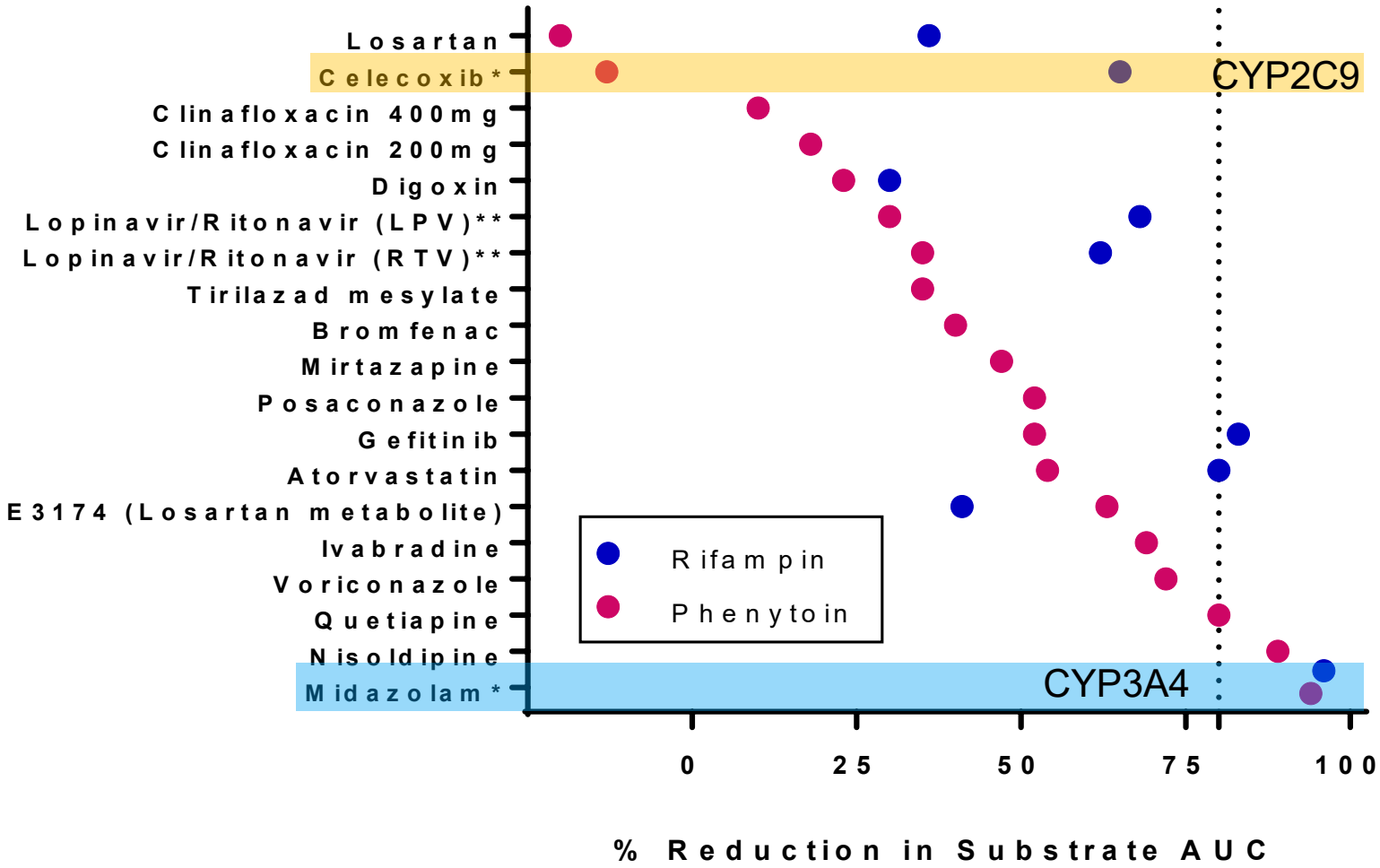


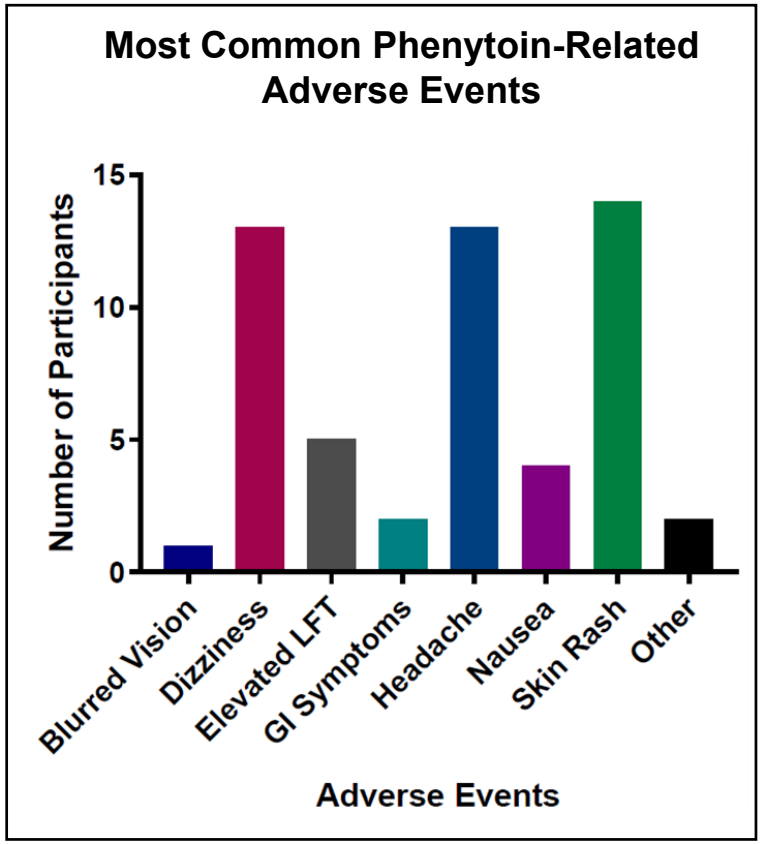
*Combination cobicistat and elvitegavir therapy.**Combination of ritonavir and paritaprevir therapy. ***Simulated ibrutinib+carbamazepine data. +Combination of ritonavir and lopinavir therapy in patients. Dashed line represents 80% reduction.

Phenytoin as Alternative?

- Anticonvulsant drug
- **Strong inducer of CYP3A**
  - Also moderate inducer of CYP1A2 and CYP2C19
- Narrow therapeutic window
  - Safety concerns (e.g. risk of seizures & neurological events)
- Long half-life, requiring time to reach Css and maximal CYP3A induction
- Risk mitigation measures
  - Genotyping 2C9 and 2C19 poor metabolizers
  - Exclude history of seizures, neurological conditions and suicide ideation
  - Exclude WCBP because of prenatal risks
  - If substrate may increase phenytoin levels, monitor phenytoin levels
- Recommended phenytoin regimen: 100 mg TID phenytoin for ≥14 days

Phenytoin- and Rifampin-Induced %AUC Reduction





- Mild-moderate AEs, transient in nature

* Phenytoin and/or anti-seizure medication co-administration in patients.

** Combination ritonavir and lopinavir therapy. Dashed line represents 80% reduction

Paglialunga & van Haarst, 2022 ACCP Poster Presentation

Phenytoin vs Carbamazepine – Induced %AUC Reduction

Substrate	Phenytoin	Carbamazepine	Concordance
Rivaroxaban (NCA)	90%	58%	Stronger PHT effect
Quetiapine	80%	87%	✓
Albendazole metabolites	66-78%	49-67%	✓
Ivabradine	69%*	80%	Slightly stronger CBZ effect, however PHT was not dosed to steady state
Quinidine	56%	61%	✓
Mirtazapine	47%	61%	Slightly stronger CBZ effect

* 5 days of PHT administration

CBZ, carbamazepine; **NCA**, noncompartmental analysis; **PHT**, phenytoin

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Conclusion

- Various commercial drug batches contaminated with nitrosamines, including drugs used in DDI studies
  - Rifampin: contaminations across all batches of main concern for drug development
- Rifampin to be replaced by alternative perpetrators in DDI studies until sufficient and uncontaminated batches become available
- Phenytoin and carbamazepine most viable options for healthy volunteer DDI studies
- Phenytoin and carbamazepine have been shown to be effective inducers of CYP3A
- A recommended total daily dose of 300 mg phenytoin for ≥14 days was found to be generally safe and well tolerated in healthy subjects.
  - Carbamazepine would require dose titration (altogether ≥13 days)

Thank You!

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Journal of Pharmaceutical Sciences (2023, Epub ahead of press):
The impact of N-nitrosamine impurities on clinical drug development
Sabina Paglialunga & Aernout van Haarst

Clinical Pharmacology & Therapeutics (2023, 113: 816-821) *(Epub Date: 21 May 2022):*
Rifampin drug-drug-interaction studies; reflections on the nitrosamine impurities issue
Aernout van Haarst, Stephen Smith, Clare Garvin, Natacha Benrimoh, Sabina Paglialunga