The human metabolism of lufotrelvir following intravenous administration

A prodrug for the treatment of SARS-COV2

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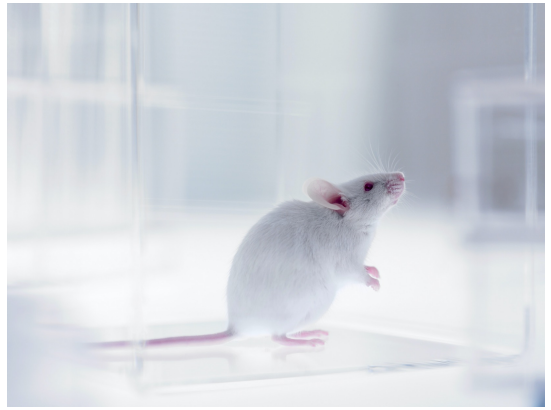
TNO innovation for life

Benefits of early human data in drug development

Some reasons to terminate compound development

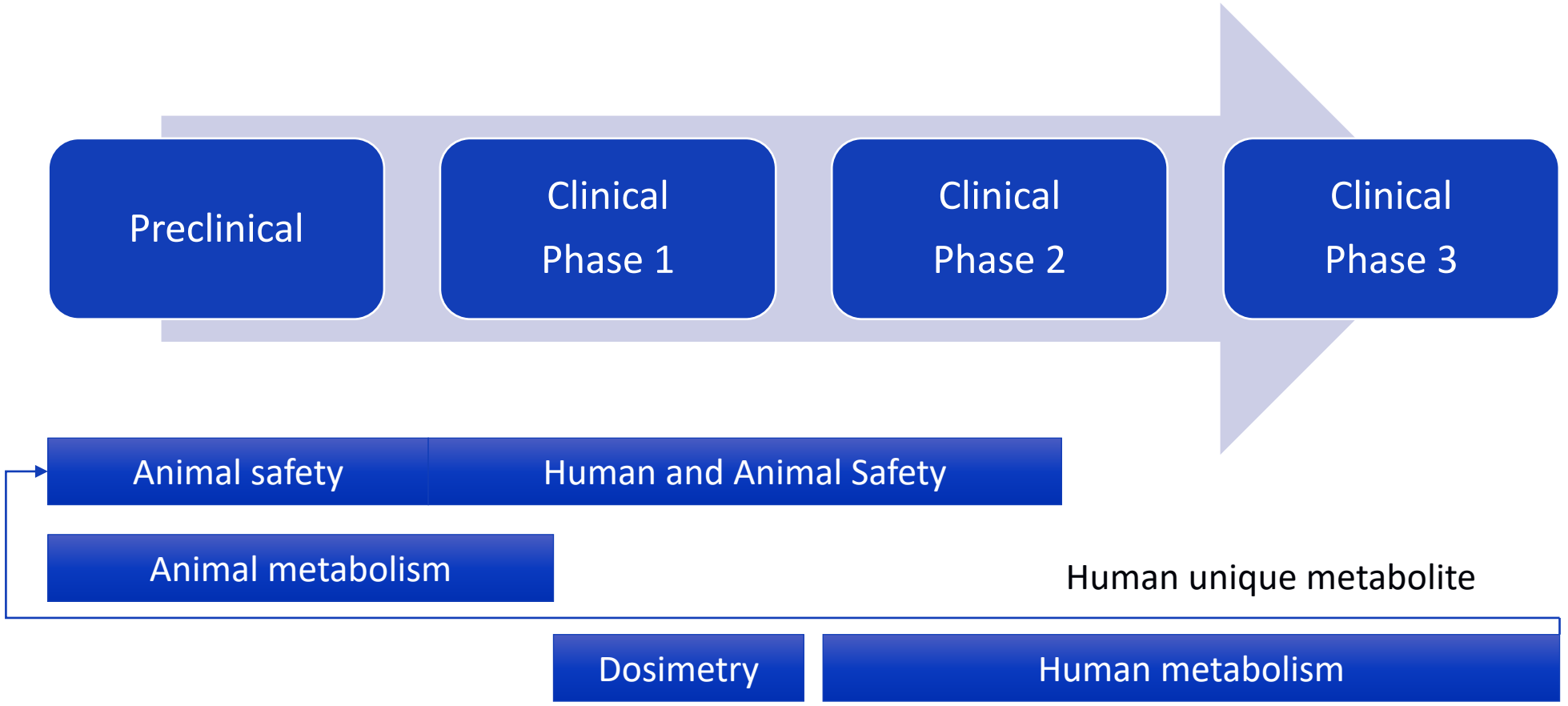
- Undesirable pharmacokinetics
- Insufficient efficacy
- Safety concerns



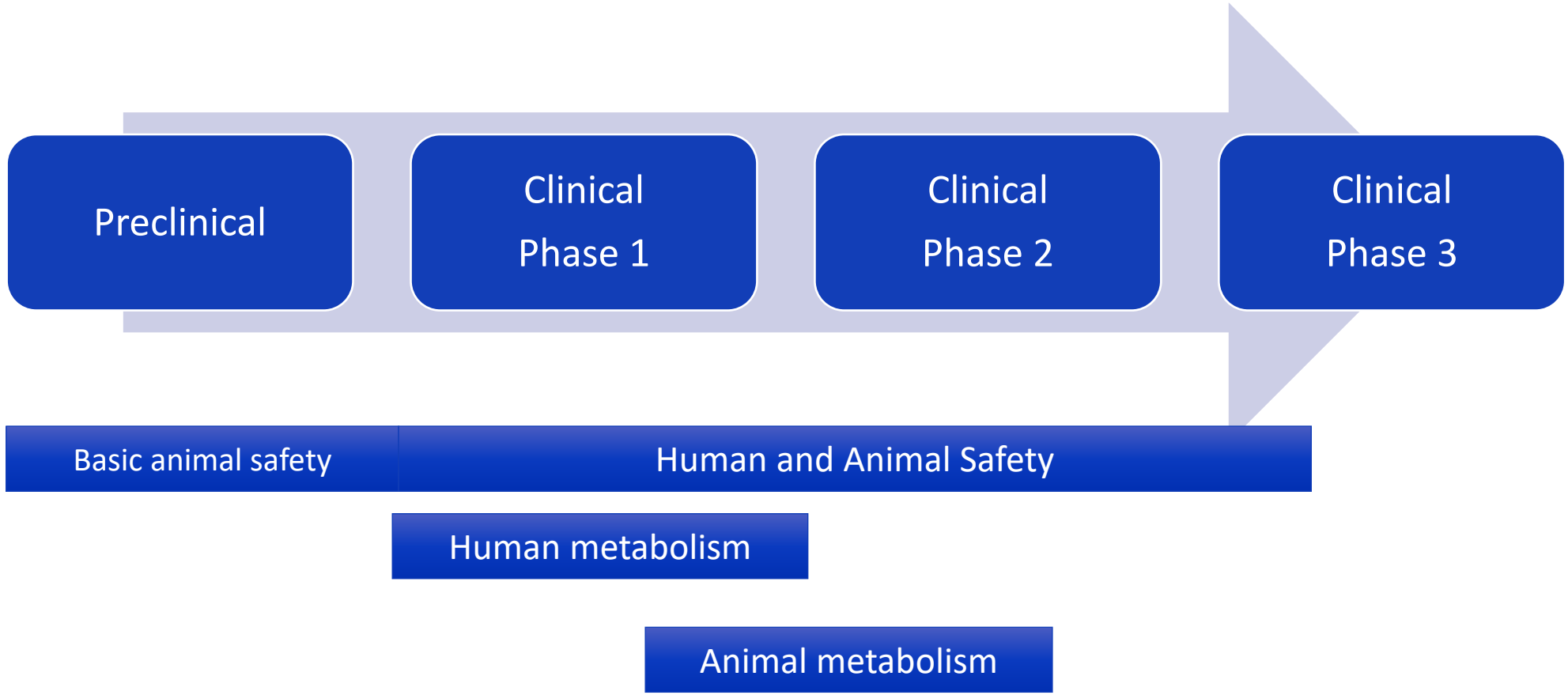
Drug targets become more complicated, ideally the following information is available asap

- PK
- Metabolism
- Absolute bio-availability
- Mass balance data

Drug development pipeline



Faster human data



Safety assessment principles

B. Identification of Metabolites

Sponsors should identify the metabolic profile of the drug during the drug development process. This identification can be accomplished at different stages of development using in vitro and in vivo methods. In vitro studies can use liver microsomes, liver slices, or hepatocytes from animals and humans; these studies should generally be conducted before initiating clinical trials. In vivo metabolism study results in nonclinical test species generally should be available early in drug development, and their results will either confirm the results obtained from the in vitro studies or reveal quantitative and/or qualitative differences in metabolism across species. It is the latter situation that may pose a safety concern. Usually, sponsors have conducted human in vivo metabolism studies relatively later in drug development, but we strongly recommend that sponsors conduct in vivo metabolic evaluation in humans as early as feasible.

[1]U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), March 2020 Pharmacology/Toxicology Revision 2

Human metabolism data

Generally data are generated late Phase 2 or Phase 3

- Administration of a high ^{14}C dose (not ethical in early phase)

Lower the ^{14}C dose significantly (e.g. 1000 fold) PK

- ALARA
- No ethical concern even if compound is discontinued
- No QWBA

It is not standard practice for drug metabolites to be evaluated separately in a cross-species safety assessment. As a result, their specific contribution to the overall toxicity of the parent drug has often remained unknown. This lack of understanding about the role of metabolites in drug toxicity may partly reflect the inadequate sensitivity of the analytical methods used to detect and characterize metabolites derived from the parent drug. Technological advances, however, have greatly improved the analytical abilities to detect, identify, and characterize metabolites and may allow a better understanding of the role metabolites play in drug safety assessment.

[1]U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), March 2020 Pharmacology/Toxicology Revision 2

Microdosing research

Often ^{14}C dose is between 100nCi-1µCi

- Application to human volunteers at low dose (<0.1 mSv) is justified for research to increase knowledge
- Single dose ≤ 100 µg (or 30 nmol) and (≤ 1/100th NOAEL and ≤ 1/100th pharmacologically active dose)
- Extended 14 day single dose, one species (rodent), intended route or i.v.; SAR (structure activity relationship) study required

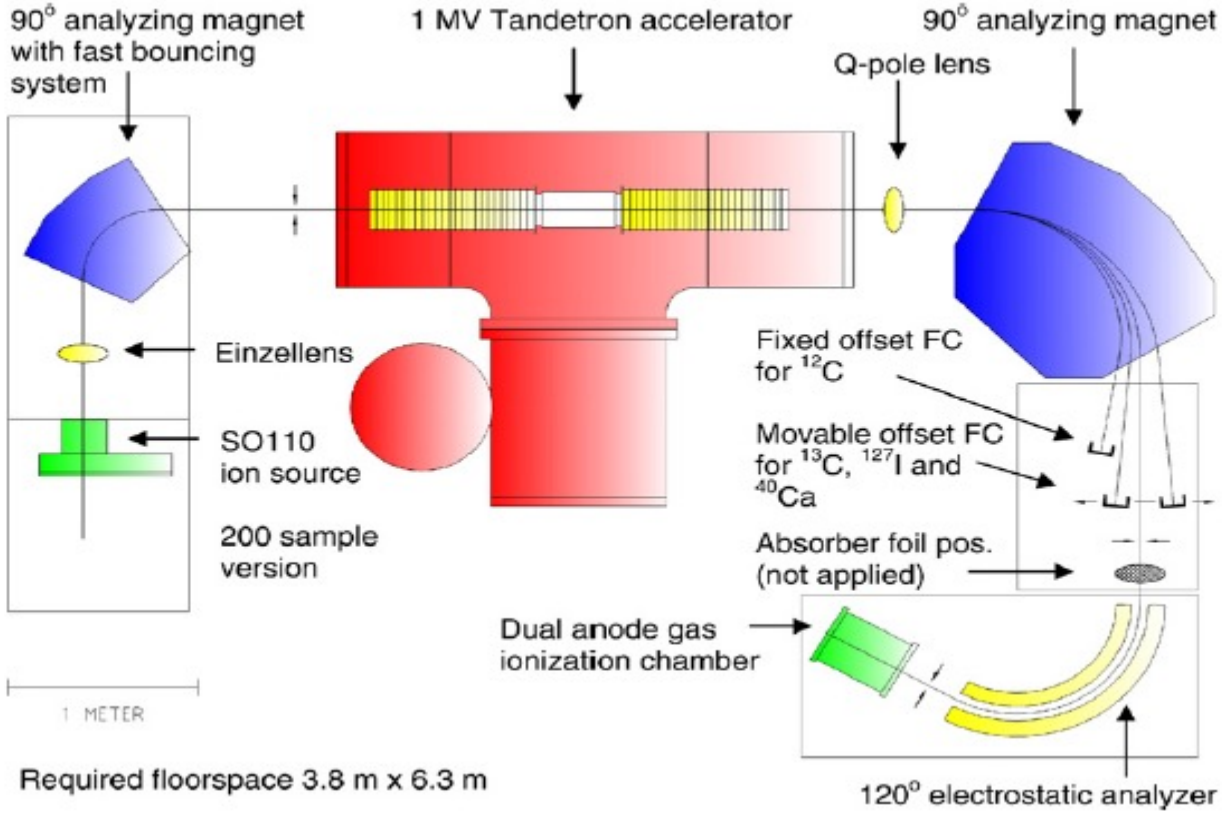
Phase 0 - Microdose: PK, metabolite profile, MB

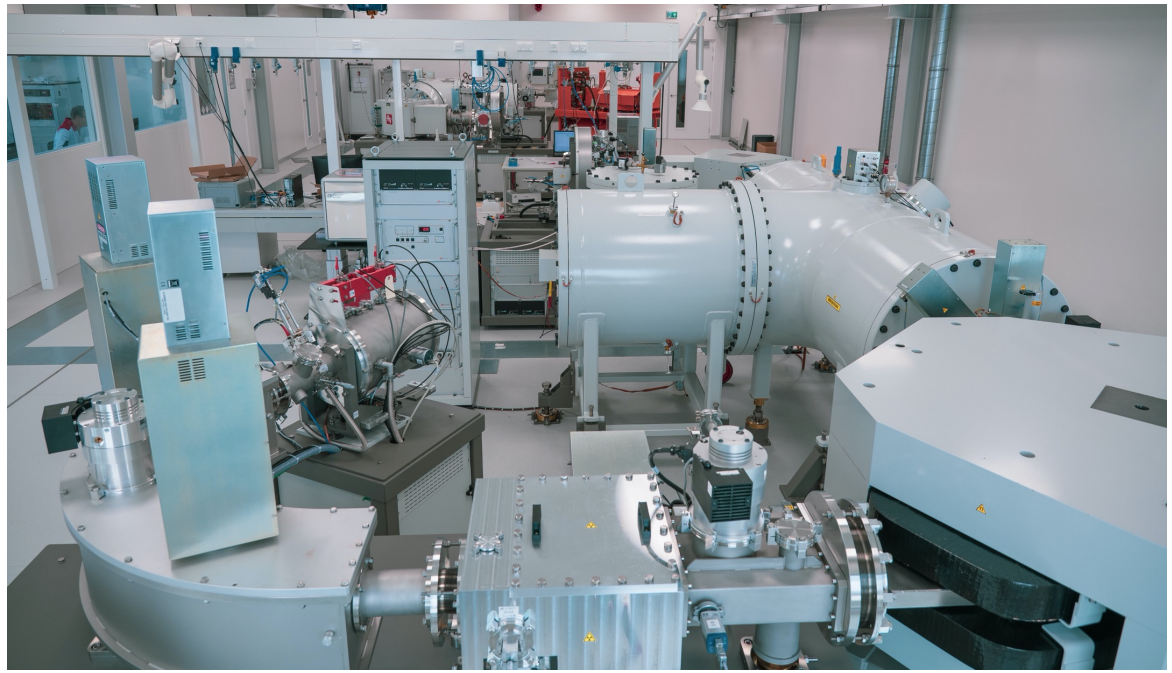
- Low radioactive dose only

Phase 1 - Microtrace: AB, PK, metabolite profile (+ ID), MB, DDI

- Low radioactive dose on top of unlabeled therapeutic dose

Accelerator Mass Spectrometry

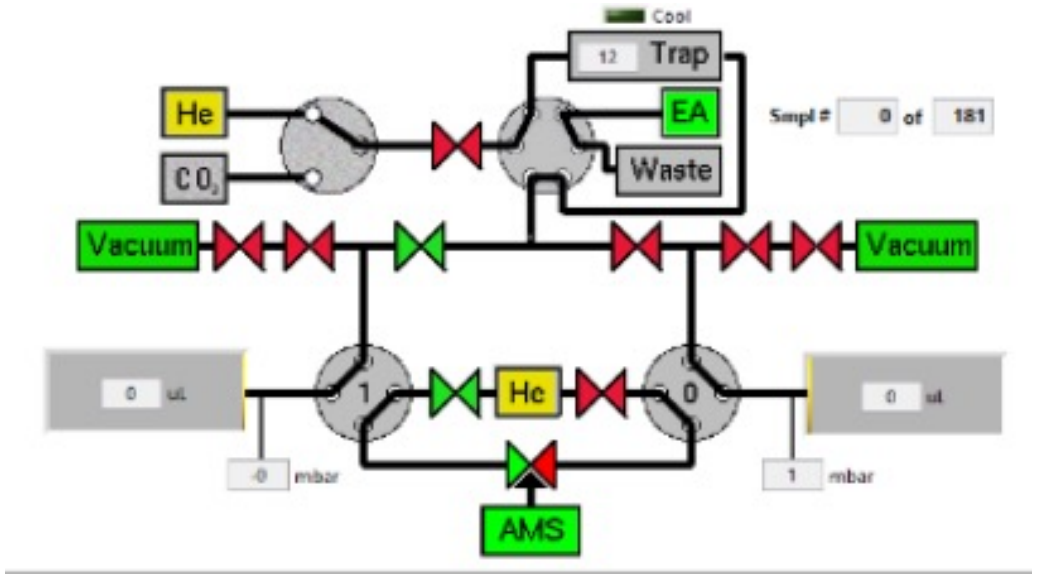




Analysis in the fg/mL range (extreme sensitivity) absolute amounts: attograms

Klein et al., Nuclear Instr and Meth in Phys Res B 2013

AMS with gas accepting ion source

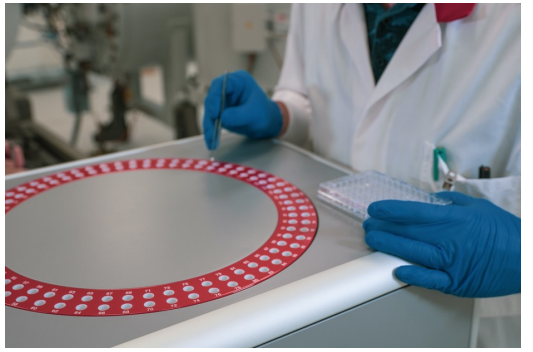


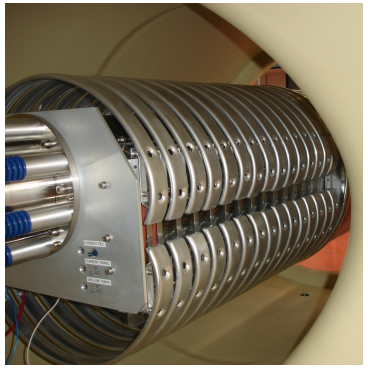
- Elemental analyzer (EA) combusts sample, generated CO_2 is used as a source of carbon
- Rapid analysis time
- Unauthorized analysis up to 200 samples

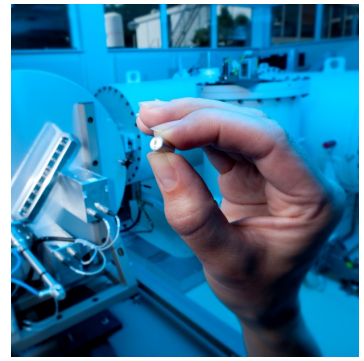
[2]van Duijn E, Sandman H, Grossouw D, Mocking JAJ, Coulier L, Vaes WHJ. Anal.Chem. 2014, *86*, 7635-7641.

Instrument Qualification, total ^{14}C

Matrix	Volume (µL)	Dynamic range (mBq/mL)
Urine	15	1.08-269
Whole blood	5	5.28-2113
Feces	30	2.78-557
Plasma	5	1.51-1004







In plasma for a small molecule with a molecular weight of 400 Da this equals 123 fg/mL (<700 ag)

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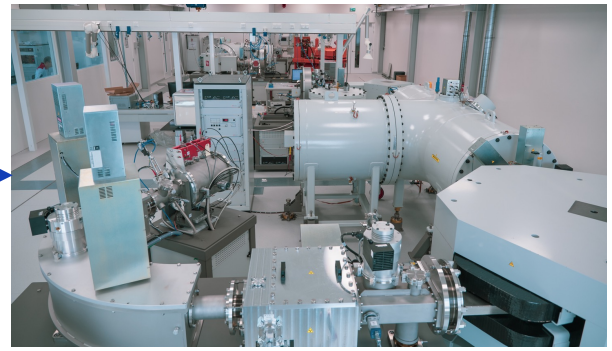
[2]van Duijn E, Sandman H, Grossouw D, Mocking JAJ, Coulier L, Vaes WHJ. Anal.Chem. 2014, *86*, 7635-7641.

MIST Study Sample Analysis

One UPLC injection

Metabolite identification



Metabolite quantification

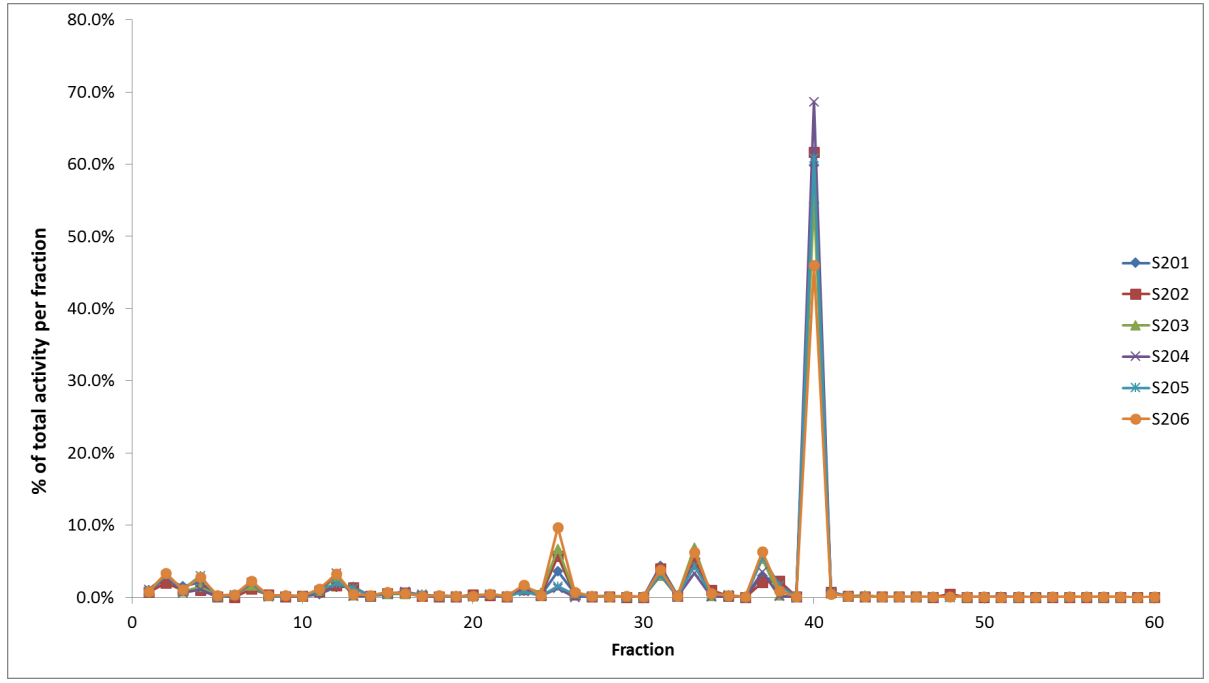
- Combine UPLC, fraction collection and AMS with high resolution MS/MS-identification
- Quantification and compound identification data generated from one single injection

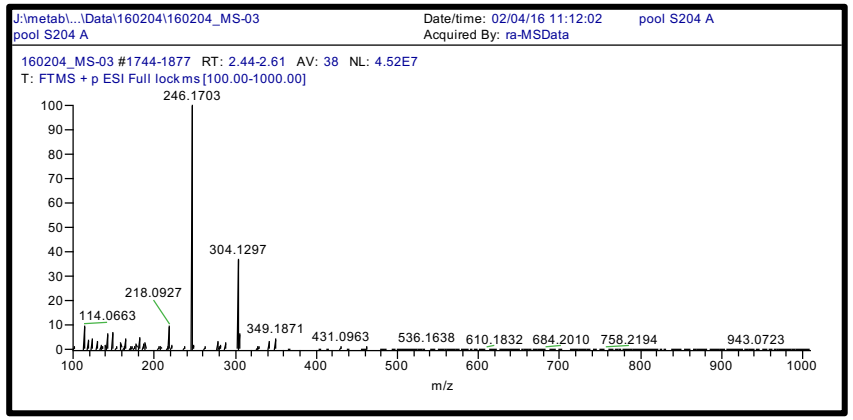
Typical Early MIST Study Design

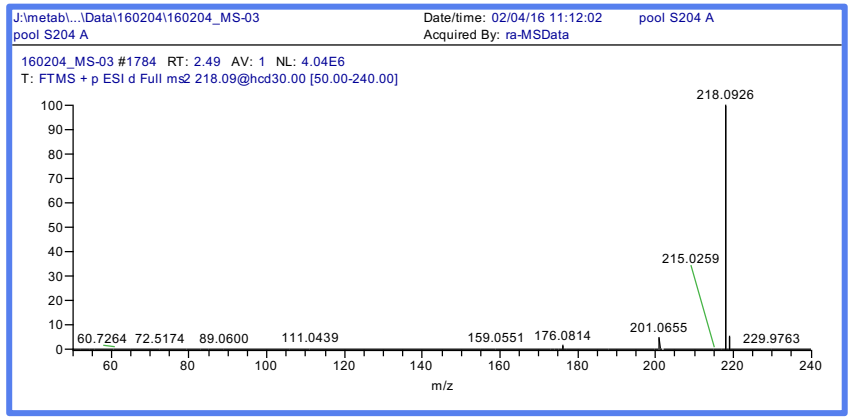
- No dosimetry data needed prior to study
- No separate clinical trial needed (ICRP class 1 study)
- Include in early clinical trials
- Close to intended therapeutic dose
- Include 100 nanoCi to 1 microCi of ^{14}C labelled drug
- Sampling blood, urine, faeces
- Profile samples with chromatography
- Count fractions (AMS) and identify with LC-hrMS/MS

- Often combined with mass balance study (discharge from clinic based on AMS data)

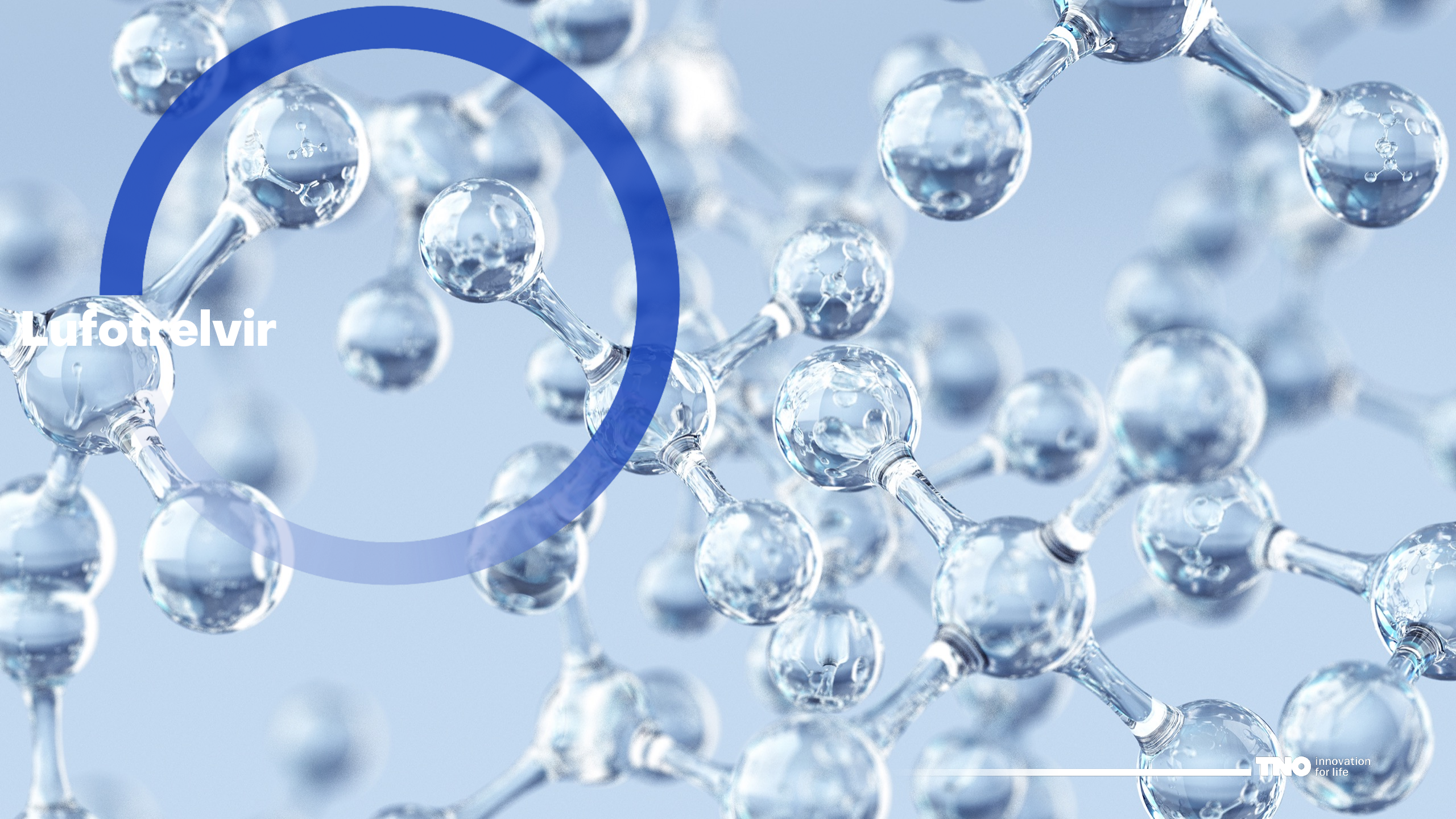
Radioactivity profiles for individual AUC plasma pools







Lufotrelvir



TNO innovation for life

Lufotrelvir

- Novel phosphate prodrug of PF-00835231
- Designed for SARS-COV1 (~20 years ago)
- Targets viral protein essential for replication and propagation.
- Inhibitor of main protease A (very similar beween COV-1 and COV-2)
- Prodrug required for solubilisation

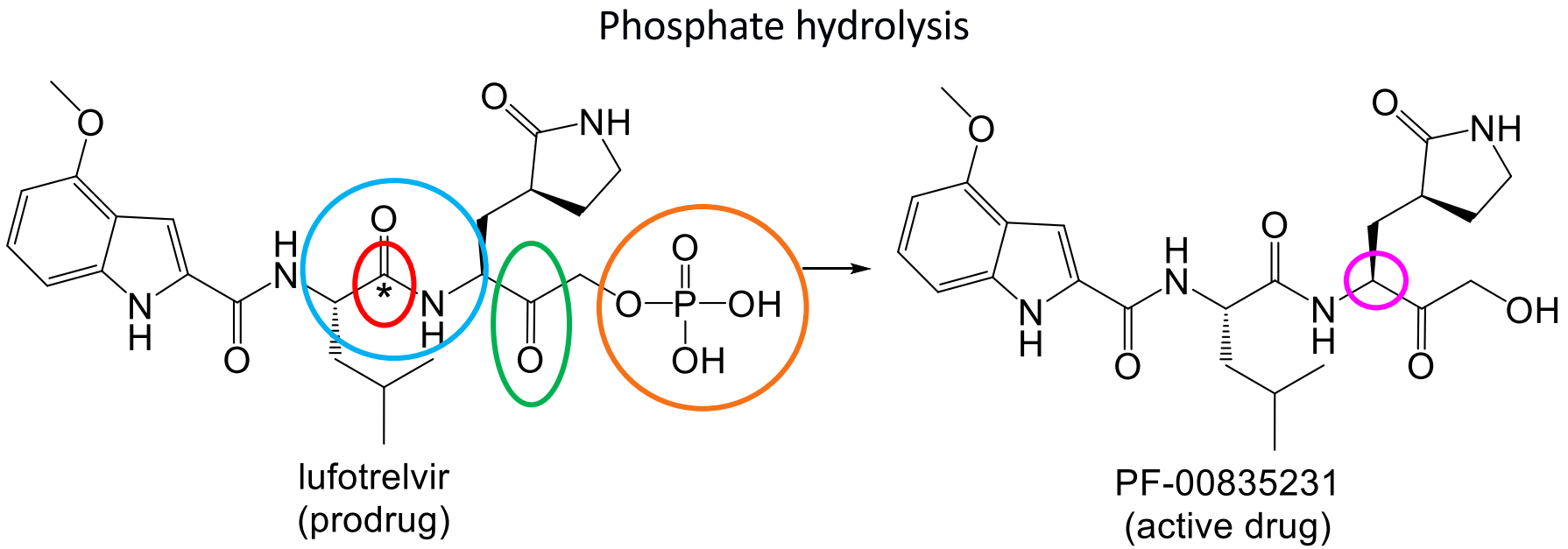
Phosphate group

P1-P2 amide bond

14C label

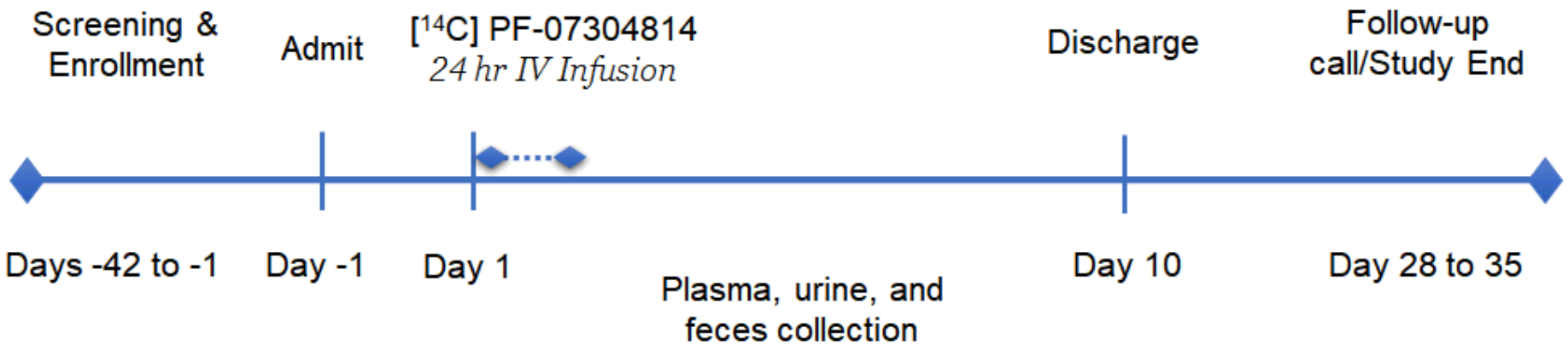
Keton

Epimerisation site

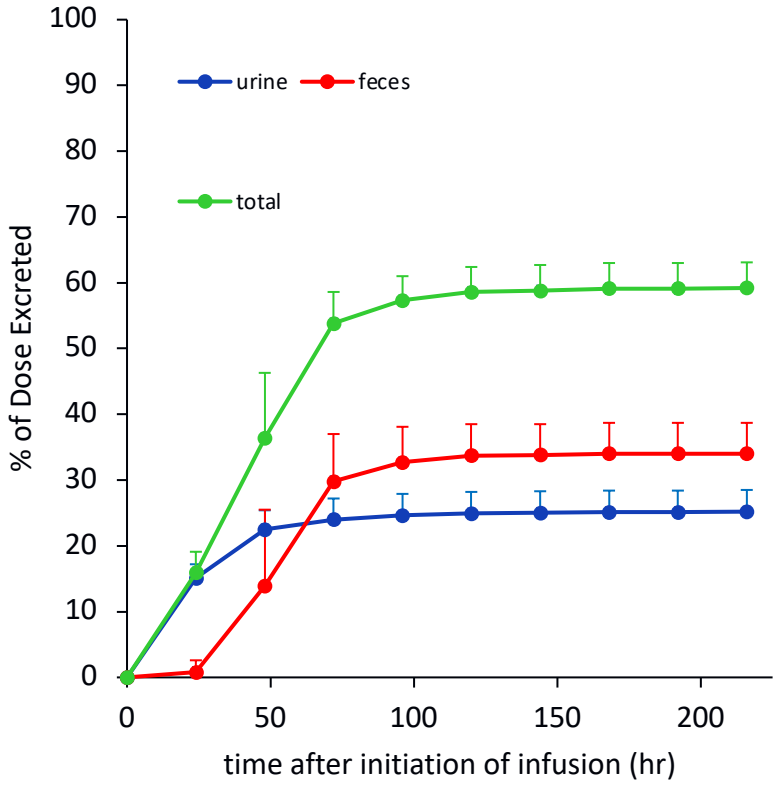


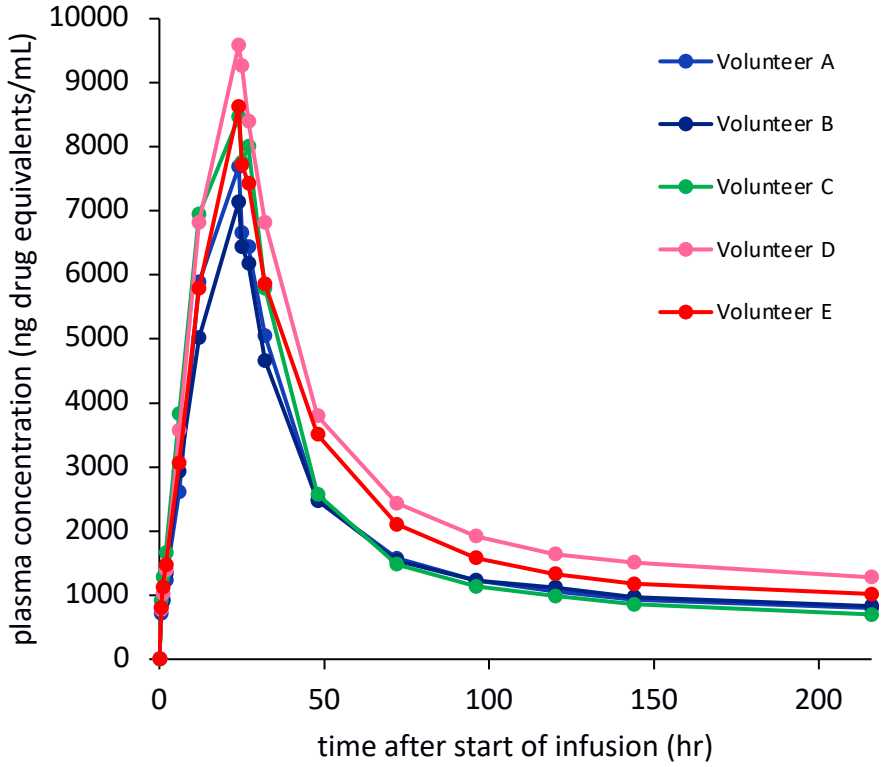
Study Design

- Metabolism and excretion of lufotrelvir after iv administration
  - Designed for hospital treatment
- Extremely rapid drug development timelines due to urgency of the pandemic
- Microtracer approach ideal, it obviates the need for
  - Tissue distribution
  - Tissue dosimetry estimations for humans
- N=5 subjects ^{14}C-lufotrelvir (500 mg containing 419 nCi)



Excretion and half life in plasma



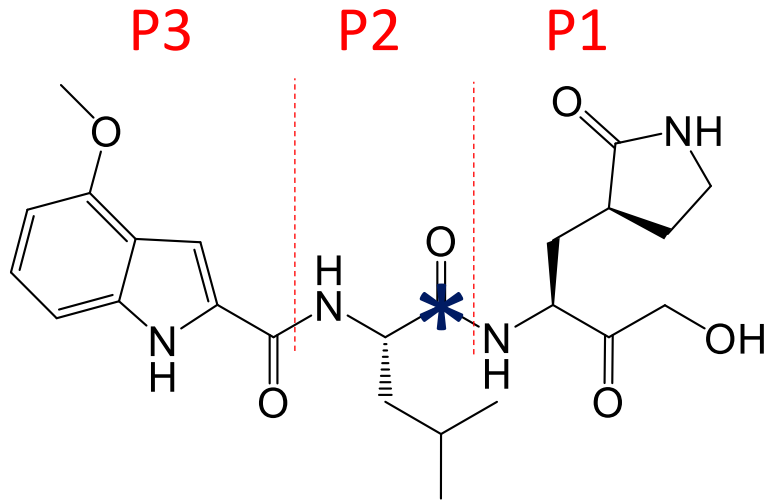


- Rapid decline after end of infusion up to 48h
- Prolonged terminal phase with T1/2 > 200h

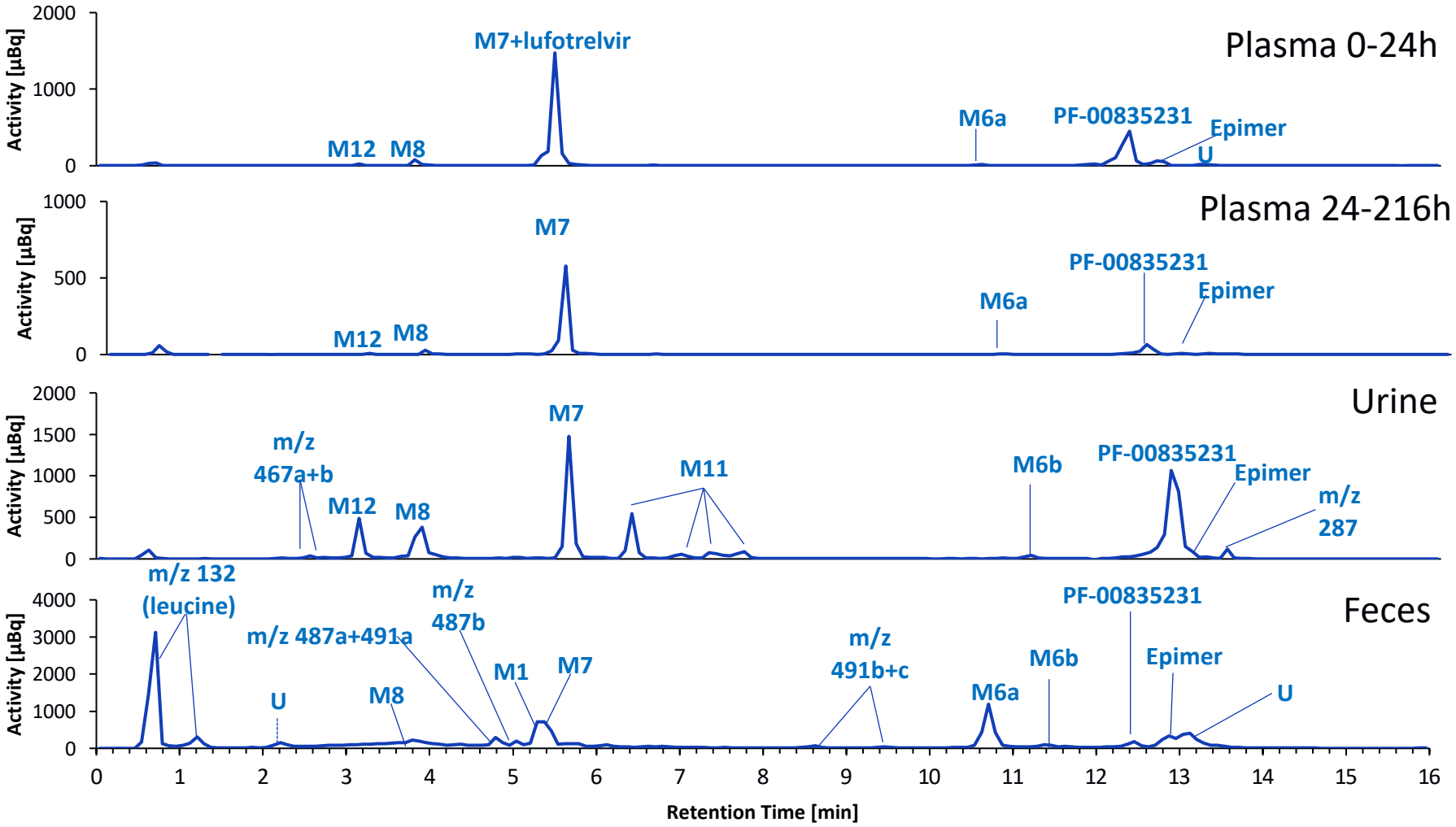
Excretion results

- Overall recovery ~63%

Volunteer	% of dose in urine	% of dose in feces	% of dose excreted
A	28.7	33.0	61.7
B	23.1	41.2	64.2
C	22.0	52.6	74.6
D	28.9	28.5	57.3
E	23.2	35.3	58.5
Mean	25.2	38.1	63.3
SD	3.3	9.3	6.9

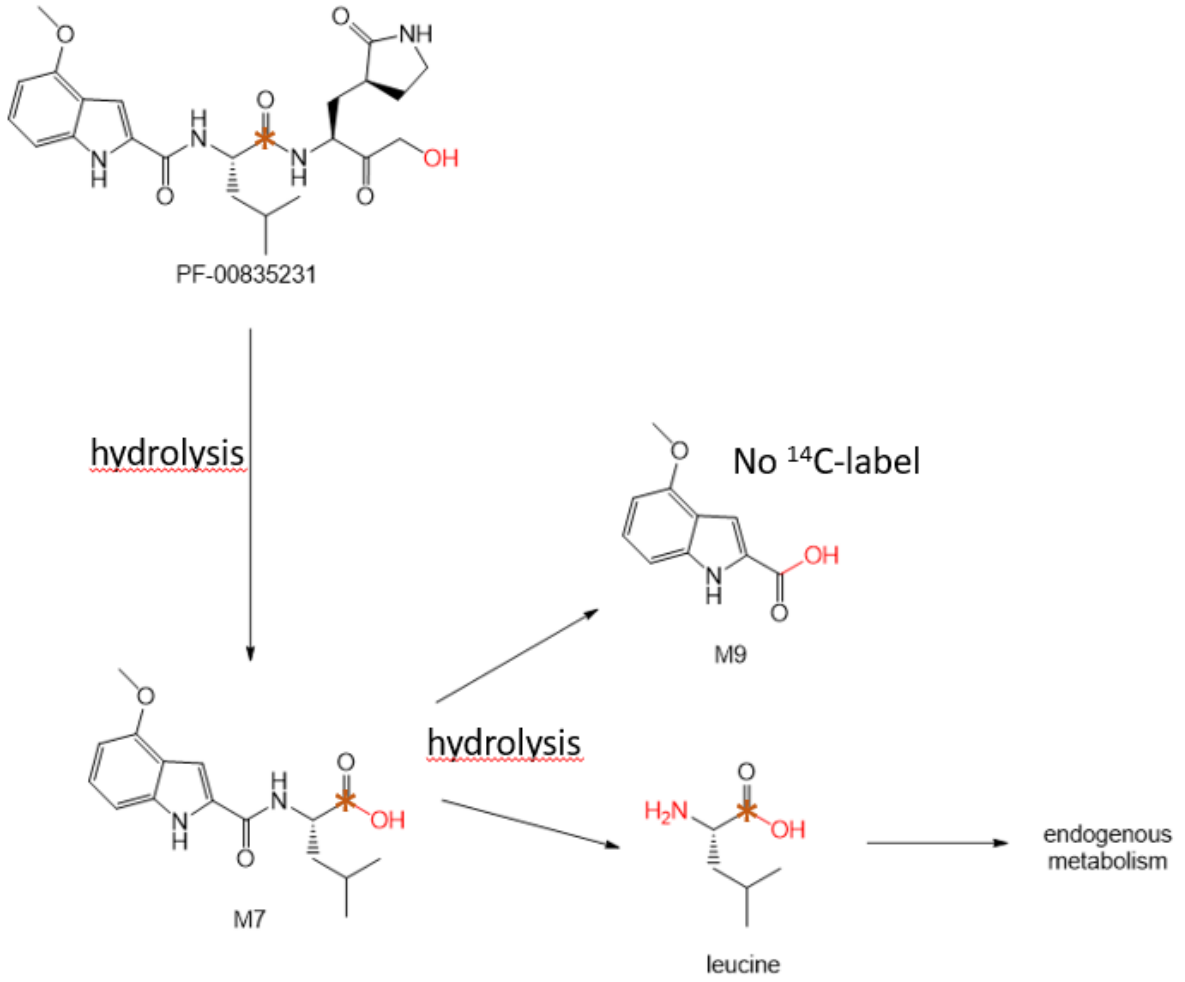


Metabolite profiles



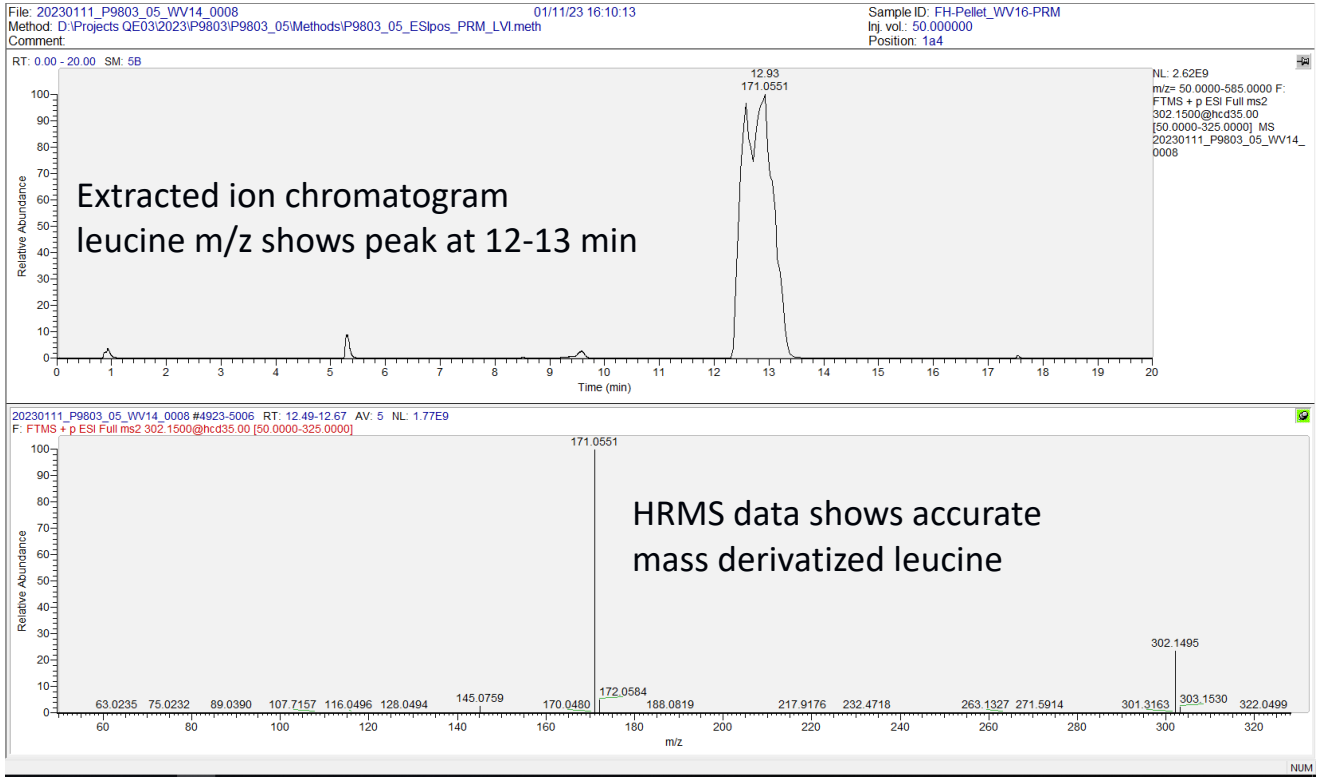
Unextractable material

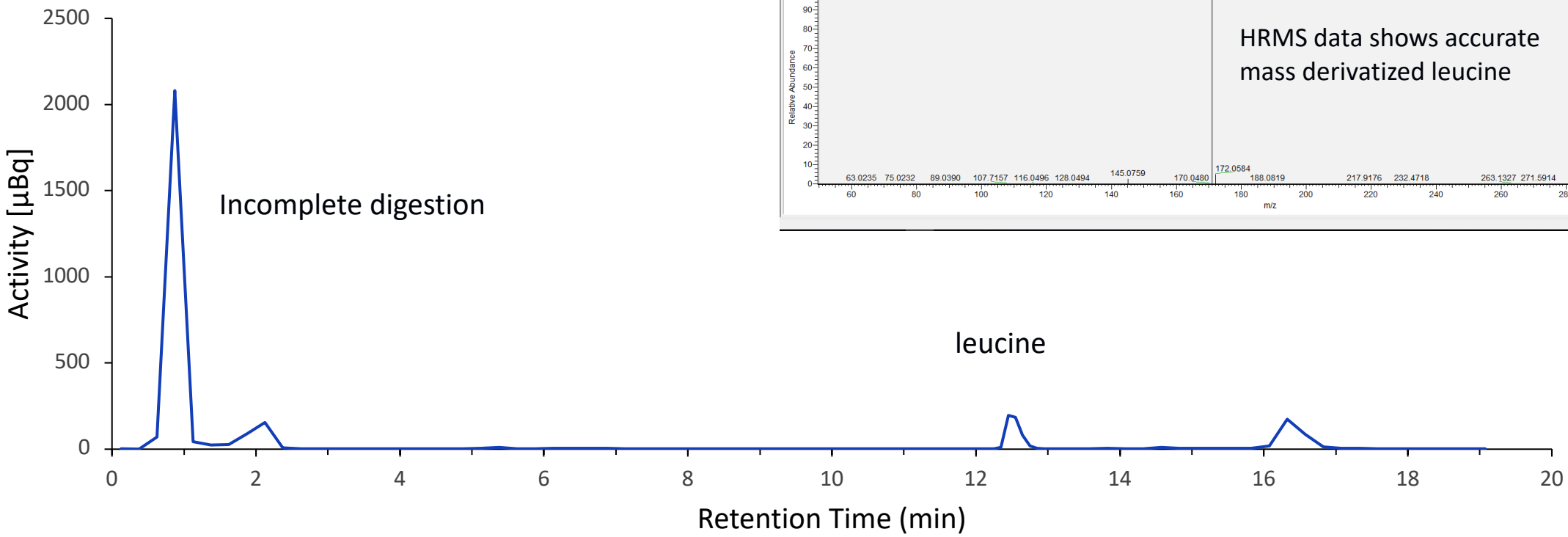
- Low mass, balance
- Extended half life of ^{14}C in plasma
- Unextractable ^{14}C in plasma and feces (activity remained in pellet)
- Significant portion of ^{14}C in void and just after void (plasma and fecal profile)
  - HRMS shows an ion of 132.1019 m/z: $C_6H_{14}NO_2$ ((iso)leucine)
  - Reconstructed ion chromatogram for this m/z shows peaks at void and at 1.2 min
  - Injection of a standard of leucine shows an elution time of 1.2 min
- Formation of leucine is possible via M7 hydrolysis to M9



Pellet profiling

- Pellet digestion (pronase)
- Amino acid derivatision (AccQtag)
- UPLC+AMS





Drug disposition

- Hydrolysis major pathway for clearance active drug (60-70%)
- hADME study had to be carried out in parallel with Phase I
- AMS enabled the tight deadlines of the project (due to pressure pandemic)

- ^{14}C not at ideal location; carbonyl carbon of the leucine (P2)
  - Expedited ^{14}C synthesis, leucine is a natural amino acid, can be purchased as ^{14}C-leu
  - risk of leucine formation via metabolism and incorporation into endogeneous material
  - Hydrolysis risk was not supported by in vitro data

- Placement of label in other portion would have prevented complicated ^{14}C profiles

Thank you for your attention

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