TNO innovation for life

THE FUTURE OF HUMANIZED IN VITRO AND EX VIVO ADME MODELS

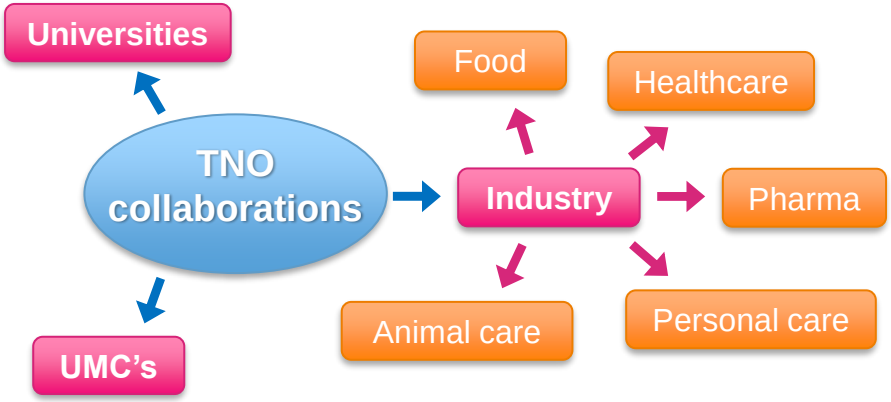
EVITA VAN DE STEEG, PHD

LEIDEN DRUG DEVELOPMENT CONFERENCE (LDDC) – SEPTEMBER 27, 2022

INTRODUCTION & TNO

- Independent research & technology institute
- Applied research
- Many collaborators
  - National and international
  - Industry and academy

- TNO has over **15 years of experience**
  - Drug pharmacokinetics & metabolism
  - Preclinical & early clinical ADME
  - Preclinical efficacy models
  - Functional microbiome assays and read-outs



- MSc –Biomedical Sciences (Maastricht University)
- PhD – Pharmaceutical Sciences (NKI – GSK)

- Joined TNO as scientist ~10 years ago

WE ALWAYS EXPECT
MORE OF MEDICINES

THE PROBLEM IN FIGURES

Expensive, time-consuming and risky development process

13 YEAR

Medicine development

laboratory
to patient

90 %

Chance of failure during costly clinical studies

60% lack of efficacy
40% safety reasons

1.3-2.5 BILLION DOLLARS

Costs for a new drug NME

New Molecular Entity

WE ALWAYS EXPECT
MORE OF MEDICINES

GROWING UNDERSTANDING OF THE PROCESSES OF DISEASES

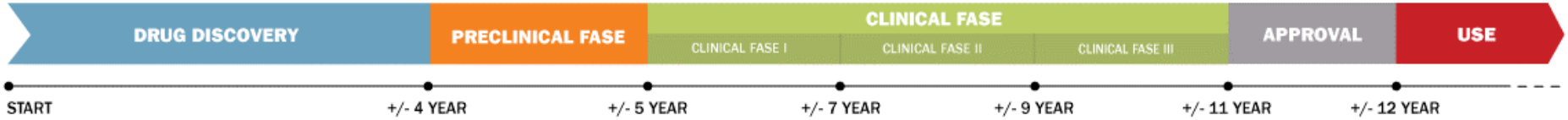
- Possibility of (increasingly) more effective 'tailor-made' medicines
- Medicines that work better for fewer people
- New types of medicines (biologicals, protacs)

SOLUTION

- New technologies and approaches are crucial
- Collaborations are essential

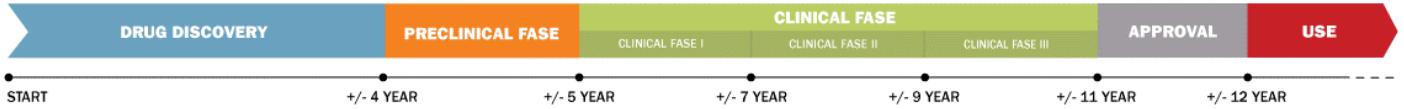


DRUG DEVELOPMENT
DIFFERENT STAGES WITH DIFFERENT CHALLANGES



EFFICACY
- Organ on a chip
- Biomarker research
- InTESTine (on a chip): in vitro human intestinal model
- I-screen: in vivo gut microbiome platform

PHARMACOKINETICS
- Microdosing AMS studies
- Microtracer AMS studies (Phase 0)
- 3D printing of oral dossage forms
- Biomarker research



SAFETY
- Target Safety Assessments (TSAs)
- Target identification & evaluation
- TargetTri – Target Profiling

EFFICACY
- Diet-induced NASH models
- Models of Fibrosis
- Cardiovascular/metabolic disease models
- Model of Diabetic Nephropathy

PHARMACOKINETICS
- InTESTine (on a chip), an ex vivo intestinal tissue model
- Drug transporters
- I-screen, a human gut microbiome screening platform
- Ex-vivo liver
- Plasma protein binding (PPB)

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1. **DEVELOPED HUMANIZED EX VIVO ADME MODELS**
    1. Liver
    2. Kidney
    3. Gut
    4. Microbiome

2. **BUILDING THE FUTURE: HUMANIZED IN VITRO ADME MODELS**
    1. Population stratitification
    2. Personalized medicine

DEVELOPED HUMANIZED EX VIVO ADME MODELS

ADME = ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

- ADME describes how drugs eneter and exit the human body and explains how concentrations in the body can change over time
- Liver, kidney, gut and colon microbiota are impartant key players

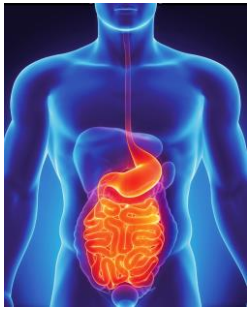
liver

⬇

Normothermic whole organ perfusion models

kidney

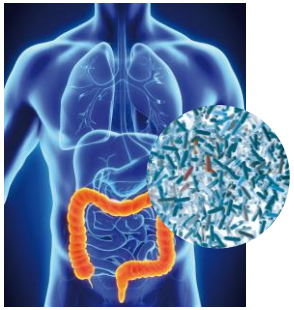
⬇



gut

⬇

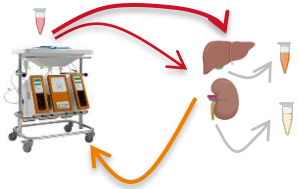
Tissue explant model

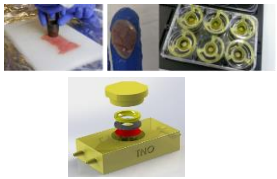


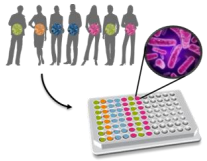
microbiota

⬇

Ex vivo fermentation model







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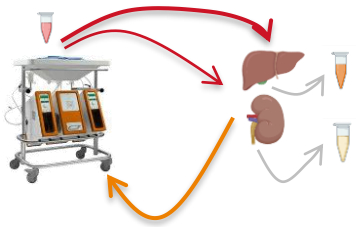
NORMOTHERMIC ORGAN PERFUSION MODELS

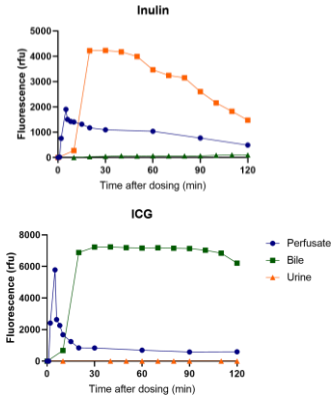
LIVER & KIDNEY

APPLICATION: PREDICTING ORGAN PHARMACOKINETICS & DRUG-DRUG INTERACTIONS

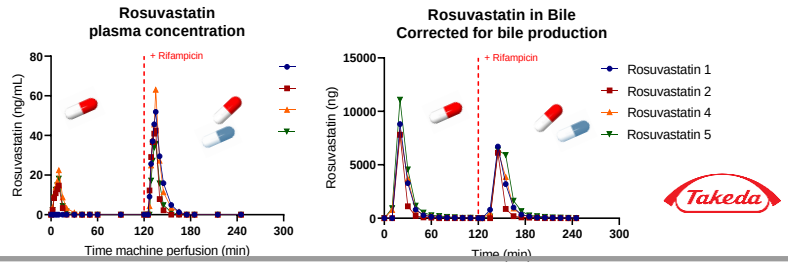
- Hepatic extraction, hepatic metabolism, biliary excretion, renal excretion
- Healthy porcine livers& kidney (slaughterhouse)
- Diseased explanted human organs
  - Insight in effect of disease on PK processes

LEIDEN UNIVERSITY MEDICAL CENTER

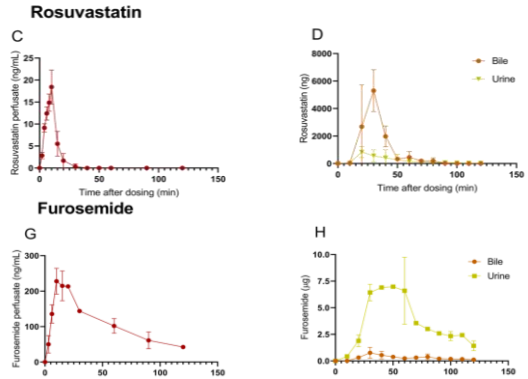




Hepatic extraction, biliary excretion & DDI



Hepatic versus renal clearance



Rosuvastatin is mainly cleared via bile and partly by renal excretion

Furosemide is only cleared by renal excretion

Stevens et al., 2021 Drug Metab Dispos. 49(9):780-789
Stevens et al., 2021 Drug Metab Rev. 52(3):438-45

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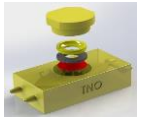
TISSUE EXPLANT MODEL

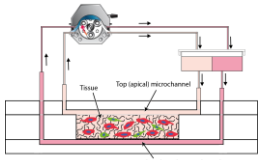
GASTROINTESINAL TRACT

APPLICATION: PREDICTING ORAL ABSORPTION AND INTESTINAL WALL METABOLISM

- Oral absorption in various regions & various formulations, metabolism, drug-drug interactions
- Healthy porcine tissue (left-over or slaughterhouse)
- Human intestinal tissue (left-over from surgeries)

ex vivo intestinal tissue model (inTESTine™)

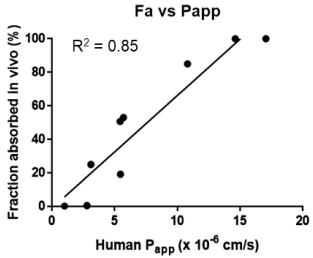


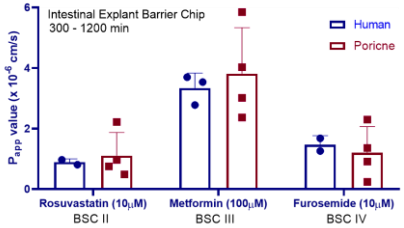


Integrity markers are used in every study
- [^{3}H]-mannitol/atenolol (paracellular transport route)
- [^{14}C]-caffeine /antipyrine (transcellular transport route)
- FD4 , MW 4000 (tissue integrity marker)

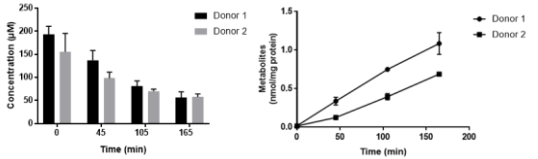
Acceptance criteria:
- P_{app} C/M or A/A > 3 (jejunum)
- Leakage of FD4 < 1% / h

Prediction intestinal absorption

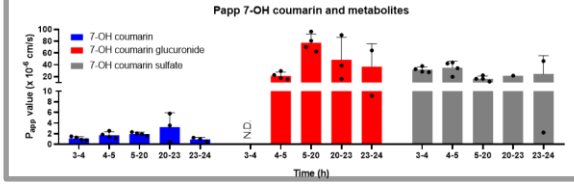




Metabolic activity: testosteron metabolism



CYP3a, UGT & SULT mediated metabolism of coumarin



Stevens et al., 2019 Eur J Pharm Sci
Westerhout et al., 2017, Eur J Pharm Sci
Westerhout et al., 2014, Eur J Pharm Sci
Vaessen et al., 2017 Drug Met Disp
Donkers et al., Lab Chip, 2022,22, 326-342

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EX VIVO FERMENTATION MODEL

COLON MICROBIOTA

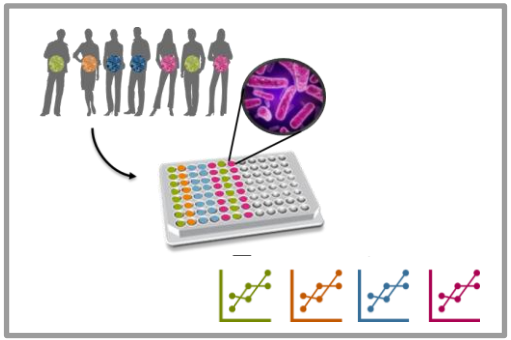
APPLICATION: DRUG METABOLISM BY MICROBIOTA

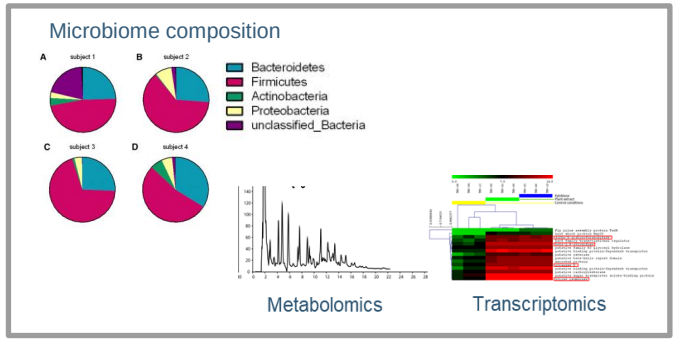
Microtiter plate platform for high throughput human gut fermentations

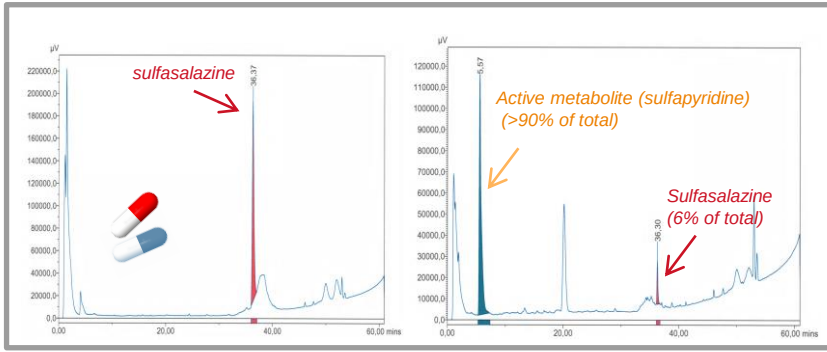
Fully anaerobic culture conditions

Dedicated set-up for stabilizing high density gut microbiota

- Standardized pooled human adult colon microbiota
- Colon (infants, children, teenagers, adults, elderly)
- Healthy versus diseased population (obese, IBD, IBS, Parkinson)







Sulfasalazine as reference pro-drug for anaerobic metabolism

Sulfasalzine metabolized into 5-ASA and sulfapyridine by azoreductases of gut microbiota

Steeg, Schuren et al., Drug Metab Dispos 2018

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COMBINED APPROACH

PBPK MODELING

Drug specific properties:

M_m, logP, B/P, F_{up}, pKa, AT, $P_{t:b}$

System specific properties:

gender, age, length, weight, organ/tissue volumes, blood flows

Ex-vivo models

Intestine:

apparent permeability (P_{app})

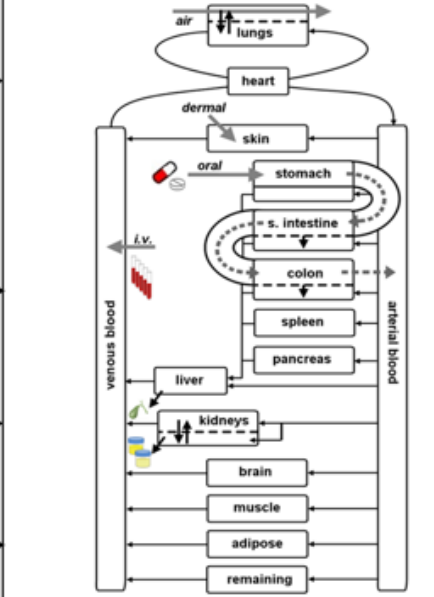
Liver:

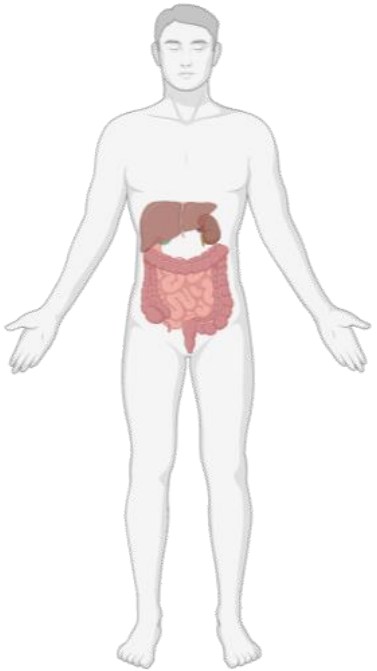
biliary excretion (CL_{bile})

Kidney:

renal clearance (CL_{renal})

$$\frac{dA_{tissue}}{dt} = Q_{tissue} * (C_{ab} - \frac{C_{tissue}}{P_{t:b}})$$





COMBINED APPROACH
PBPK MODELING

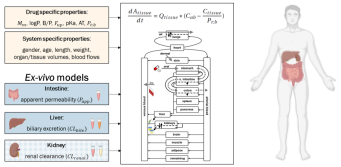
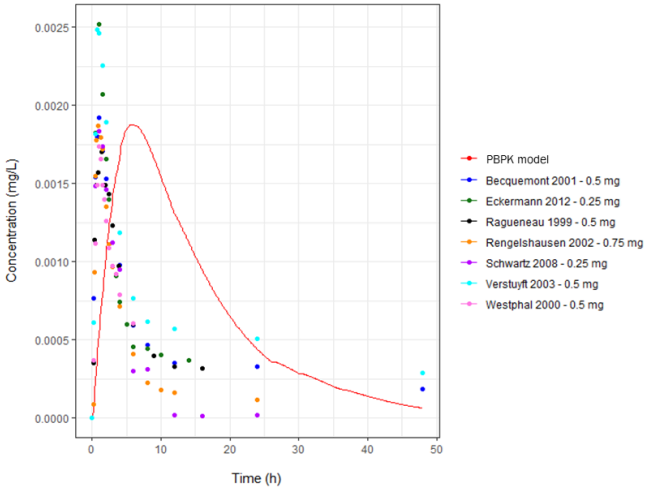


Table. *The simulated vs. clinical pharmacokinetic parameters for single PO dose of 0.5 mg digoxin or 10 mg rosuvastatin*

	Parameter	Simulated	Clinical data
Digoxin	C_{max} *(ng/mL)*	1.90	2.50 ± 0.70
	T_{max} *(h)*	5.80	1.50 (0.8-2.3)
	*AUC (h*ng/mL)*	32.1	28.3 ± 6.3
Rosuvastatin	C_{max} *(ng/mL)*	27.7	25.9 ± 18.77
	T_{max} *(h)*	4.40	3.91 ± 3.73
	*AUC (h*ng/mL)*	255.6	210.2 ± 178.70

Digoxin



Rosuvastatin

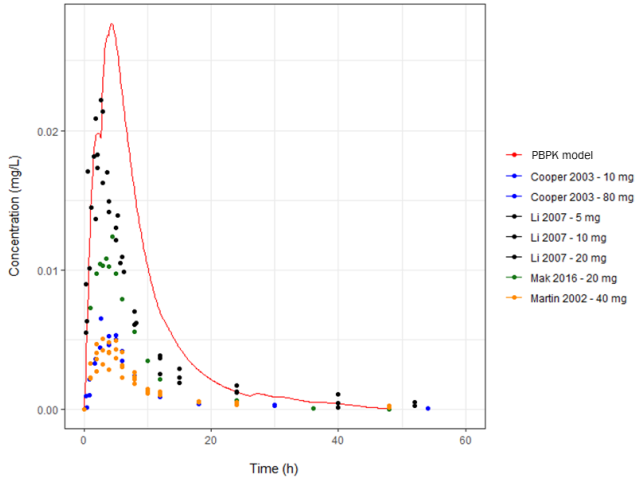


Figure . *Simulated plasma profile of (A) digoxin and (B) rosuvastatin compared to clinical data*

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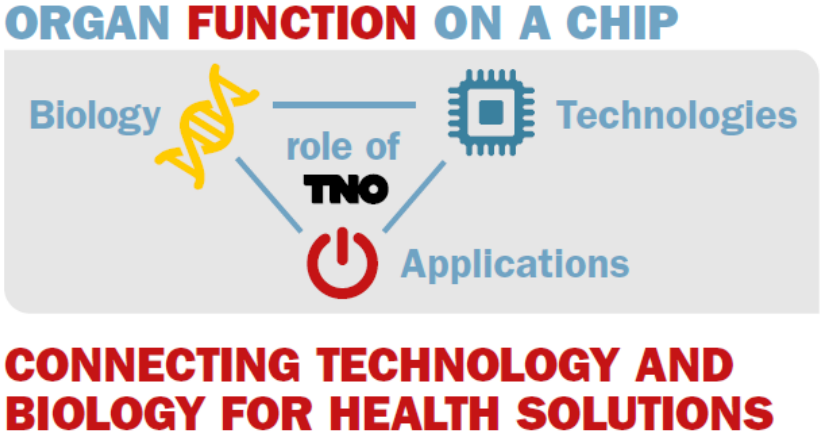
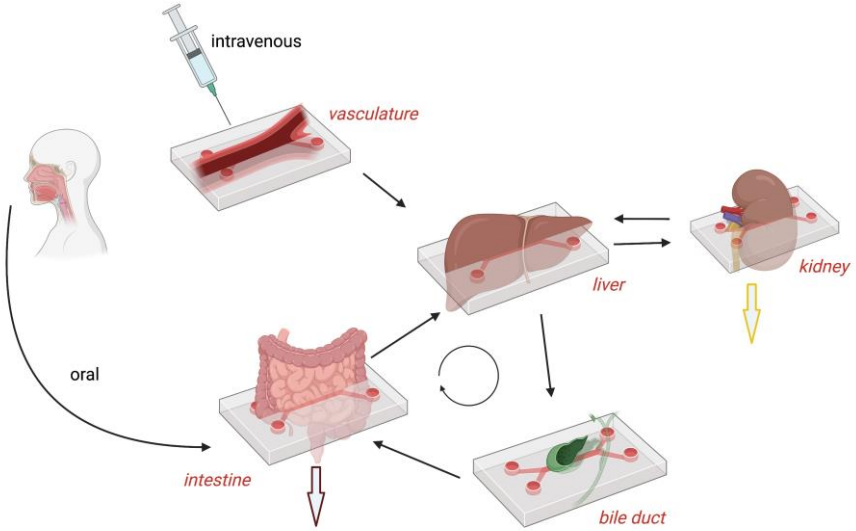
BUILDING THE FUTURE: HUMANIZED IN VITRO ADME MODELS

- Human tissue based models
- Human stem-cell based models to represent:
  - Populational differences (pediatric vs adult, disease vs healthy)
  - Interindividual variations (donor-donor variation)

- Microphysiological systems (MPS) to mimic blood flow and nutrient/oxygen exchange



DDOC: DRUG DISPOSITION ON-A-CHIP TO MIMIC ADME IN VITRO



Created with BioRender.com

Utrecht University

Roos Masereeuw: Faculty of Science, Department of Pharmaceutical Sciences
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Marit Keuper-Navis (started 1 May 2021)

NOVARTIS

Birk Poller
Markus Waller
Olivier Kretz

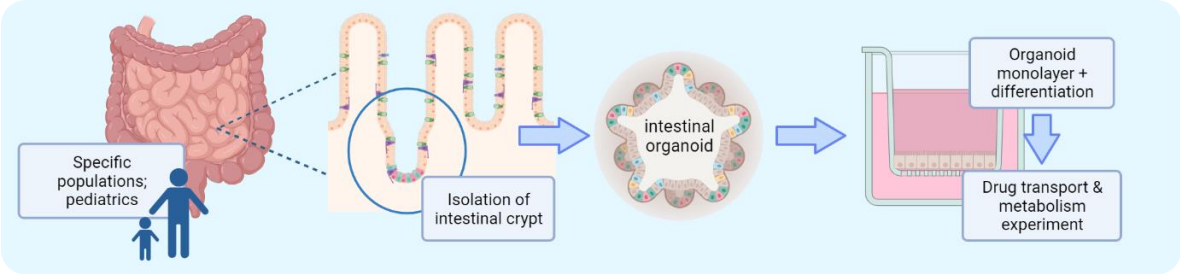
AZAR innovations

Hossein Eslamni Amirabdi

Health~Holland
SHARED CHALLENGES, SMART SOLUTIONS

Towards Pediatric Organoids to elucidate Intestinal Drug Transport (POINTeR project)

proof-of-concept study to assess the potential of liver and intestinal organoids for addressing pediatric drug exposure and safety



Roche

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Evita van de Steeg
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Institute for Health Sciences
Radboudumc

Saskia de Wildt
Rick Greupink
Frans Russel

UMCG

Sven Ijzendoorn

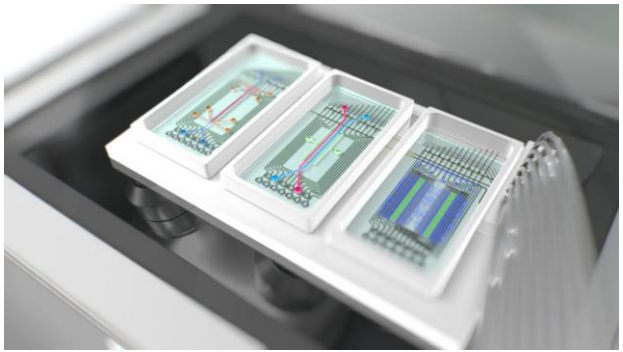
TOWARDS STANDARDIZATION OF OOC

TTW SMART PROJECT

SMART OoC

SMART Organ-on-Chip

Standardized open Modular Approach to Recapitulate Tissues



SMART OoC

TU/e Eindhoven University of Technology · University of Twente. · TU Delft

Universiteit Leiden · UMC Utrecht · Wageningen University & Research

Amsterdam UMC University Medical Centers · Maastricht University · Maastricht UMC+ · DMT Human Organ and Disease Model Technologies

300MICRONS · BioLamina · Hy2Care · iba · Lipogoat · PolyVation · provio · SPEKTRAX · USHIO

applikon · convergence · DEMCON · ibidi · Life Science Methods · micronit microtechnologies · poietis · TISSUSE

Galapagos · Genmab · imec · OnePlanet Research Center · rivm · TNO innovations for life · Prinses Máxima Centrum · ReumaNederland · Unilever

AstraZeneca · Bayer · Beiersdorf

gsk GlaxoSmithKline · Boehringer Ingelheim

PHILIPS · Roche

NWO

Nationaal Groeifonds

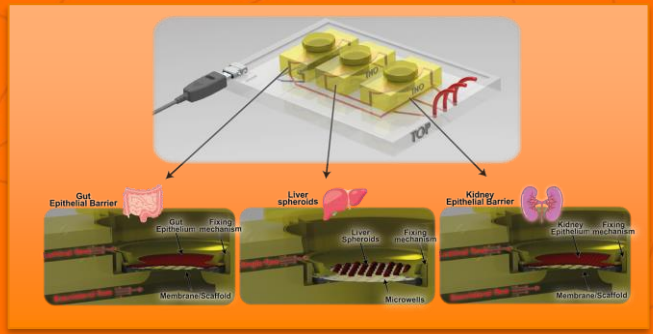
Voor economische groei en welvaart, ook voor komende generaties

TNO innovation for life | Ministerie van Economische Zaken en Klimaat | High Tech NL | FME POWERED BY DUTCH TECHNOLOGY

NXTGEN HIGHTECH | BIOMEDICAL PRODUCTION TECHNOLOGIES

A new generation hightech equipment – for a new generation

Democase: ADME on-a chip (standardized design) with integrated read-outs and sensors



Domain lead: dr. ir. Berend van Meer
LUMC | Utwente | hDMT Dutch Organ-on-Chip Consortium

NL

hDMT 5 HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES 2015 2020

Holland High Tech
Global Challenges, Smart Solutions

VITALTISSUE INITIATIF

HUMAN TISSUE IS NEEDED FOR DEVELOPMENT &IMPLEMENTATION OF NOVEL ANIMAL FREE MODELS

INITIATIEF

- Waarom: Beperkte beschikbaarheid van vitaal humaan weefsel voor wetenschappelijk onderzoek
- Doel: Opzetten van een nationaal platform (not for profit) om vitaal humaan restweefsel uit ziekenhuizen beschikbaar te maken voor research (in brede toepassing)
- Fase: Haalbaarheidsstudie & pilots, December 2018 – Maart 2021.

CONSORTIUM

HOGESCHOOL UTRECHT, Galapagos, UMC Utrecht, BBMRI.nl, Erasmus MC, TNO, umcg, brand doctors, Sanquin, LOGIQOL, rijksuniversiteit groningen, Med Law consult, charles river

MEDE MOGELIJK GEMAAKT DOOR

STICHTING PROEFDIER & MAATSCHAPPIJ

ZonMw, PROEFDIERVRIJ, Samenwerkende GezondheidsFondsen SGF

WETENSCHAPPELIJK ONDERZOEK KAN MENSELIJK(ER)

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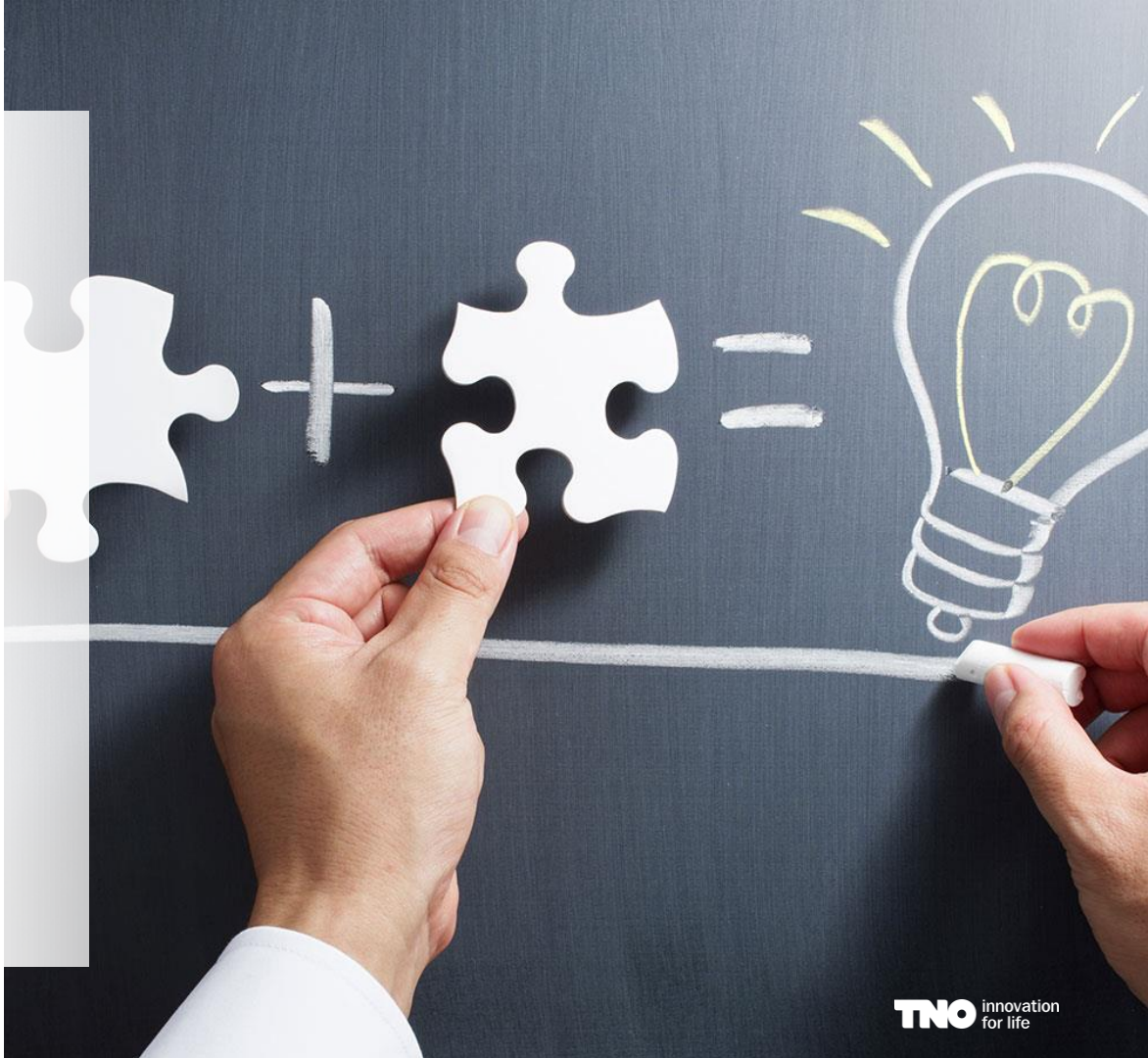
1. **DEVELOPED** HUMANIZED EX VIVO ADME MODELS
2. BUILDING THE FUTURE: HUMANIZED IN VITRO ADME MODELS
    1. Population stratitification
    2. Personalized medicine
    3. Standardization of platforms

TNO DEVELOPS TOGETHER WITH (INTER)NATIONAL PARTNERS PRECLINICAL MODELS FOR STUDYING TOMORROWS MEDICINE



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LACDR

UMCG

UNIVERSITY OF TWENTE.

Utrecht University

AZAR innovations

DMT/5
HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES 2015 2020

Cell4Pharma

NWO

Health~Holland
SHARED CHALLENGES, SMART SOLUTIONS

PROEFDIERVRIJ

Takeda

Pfizer

Roche

NOVARTIS

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THANKS FOR THE ATTENTION

EVITA VAN DE STEEG, PHD

EVITA.VANDESTEEG@TNO.NL

LEIDEN DRUG DEVELOPMENT CONFERENCE (LDDC) – SEPTEMBER 27, 2022