iCare

Integrated Assessment and
Advanced Characterisation
of Neuro-Nanotoxicity

iCare's Approach towards the Harmonisation of human and ecotox Models for Neurotoxicity

Joint online Workshop: Harmonisation & Standardisation of Test Methods for Nano- and Advanced Materials

22nd November 2023

Funded by
the European Union

Integrated assessment and Advanced Characterisation of Neuro-Nanotoxicity (iCare project)

iCare project

Participants

INL (lead), Portugal
NIA, Belgium
IIT, Italy
Temasol, Switzerland
GAIKER, Spain
ICHB PAN, Poland
O11, The Netherlands
AVAN, Spain
Versarien, UK
AMD, UK
USyD, Australia

Objective

The overarching aim of iCare is to develop a resilient and adaptive set of advanced imaging technologies to quantify Phys Chem properties for ANMC in complex matrices, which in combination with new high throughput and multi-dimensional assays and in vitro and bridging models for neuro-nanotoxicity will deliver more and better-quality data to innovators and industry in a rapid and cost-effective manner.

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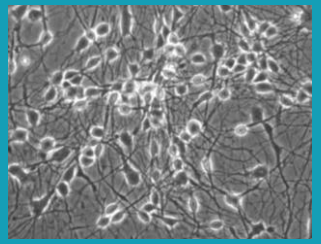
In vitro and bridging models for neuro-nanotoxicity

Human

- *In vitro* human-based blood-brain barrier model for permeability studies
- *In vitro* human-based model to assess neurotoxicity

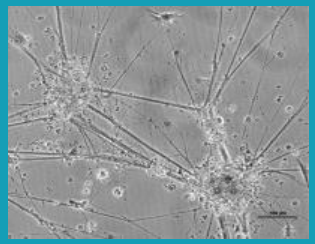


Bridging models

- *C. elegans*
- *Planaria*

Ecotox

- *In vitro fish* cell lines (monotypic and co-cultured with immune cells



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Human health

In vitro human-based BBB model for permeability studies

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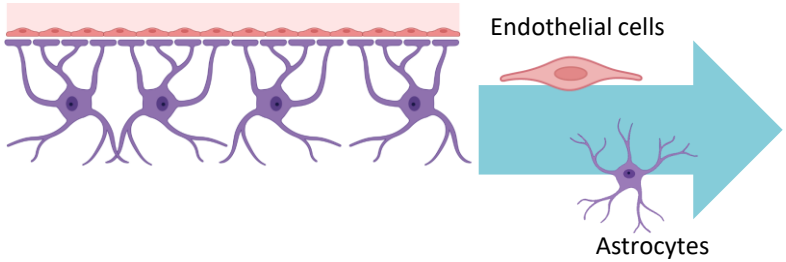
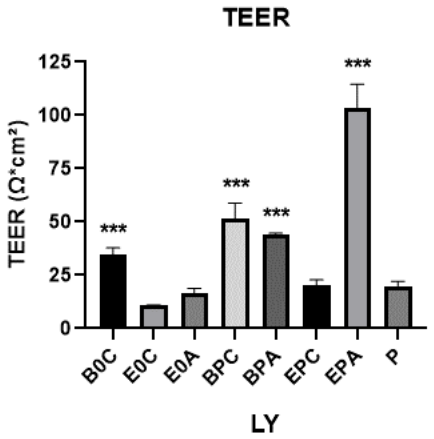
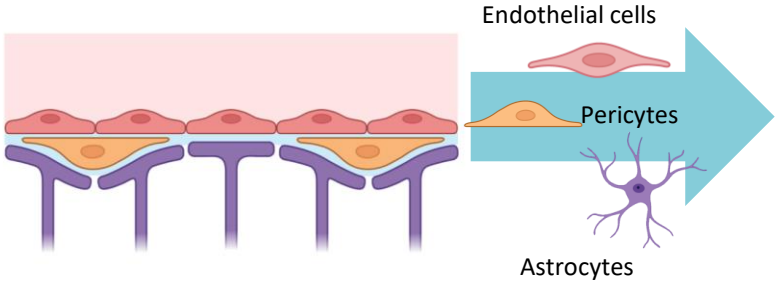
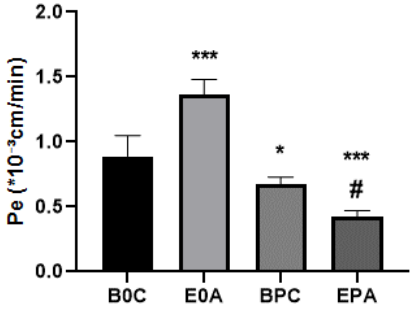
Static BBB model









Human health

In vitro human-based BBB model for permeability studies

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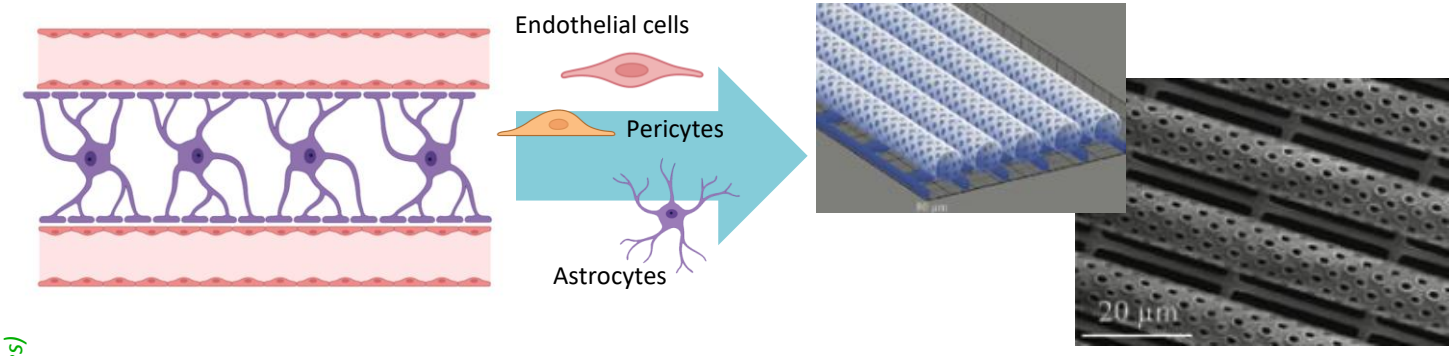
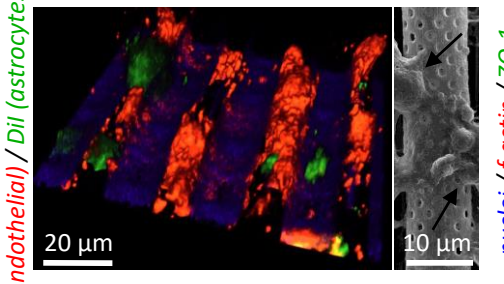
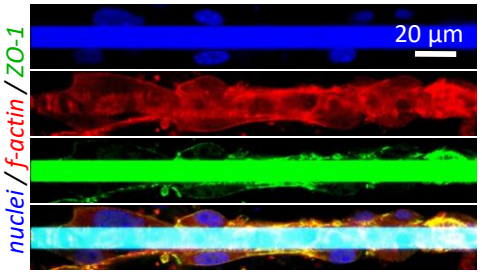
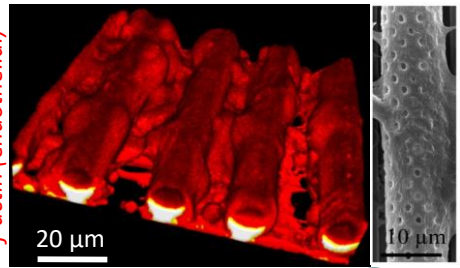
Microfluidic BBB model









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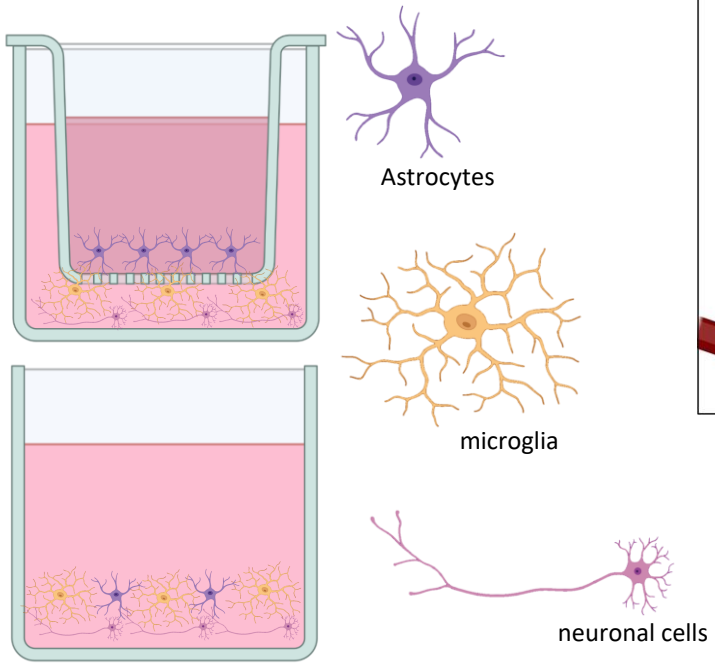
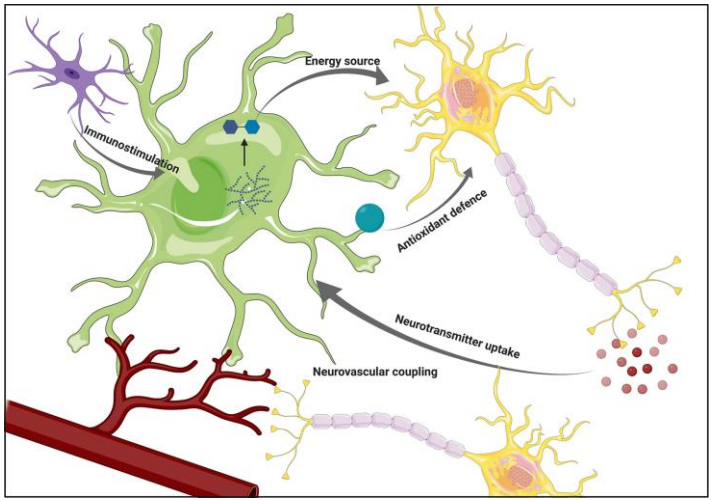
Human health

In vitro human-based model for neuro-nanotoxicity studies

3D co-culture model





Mulica et al. 2021 Frontiers

Characterisation:

- inflammatory responses (Luminex)
- Glutamate
- Amyloid beta

Bridging models
Alternative species for neuro-nanotoxicity studies

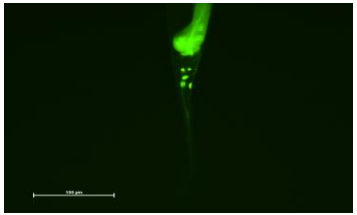
Nematodes (C. elegans)

- Life span: ~ 2.5 weeks
- The majority of human genes, including disease genes (65%), are conserved
- **Bridging model** between *in vitro* to *in vivo*
- **Bridging model** between environmental hazards and human health hazards assessment

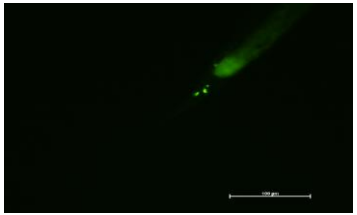
Establishment of Alzheimer's disease strain

Establishment of Transgenic (GFP expressing) neurological strains to visualize the neuronal degeneration

Normal condition



Advanced Degeneration



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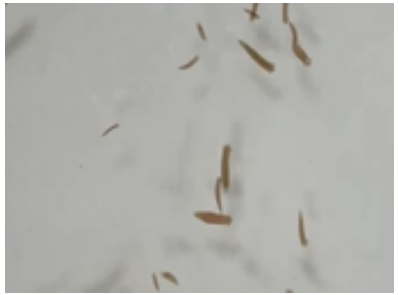
Bridging models

Alternative species for neuro-nanotoxicity studies

Planarians (D. japonica)



- Validated alternative model in nanomedicine
- Considered the earliest extant example of evolution of a mammalian-like brain
- Widely used to evaluate toxic of metals and organic contaminants
- **Bridging model** between *in vitro* to *in vivo*
- **Bridging model** between environmental hazards and human health hazards assessment

Neuro-behaviour evaluation

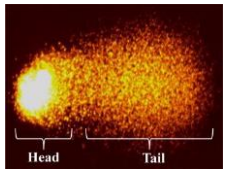


Photo credit: Dr. Albert W.

Enzymatic activity (i.e. AChE, ATPase, MAO-A)

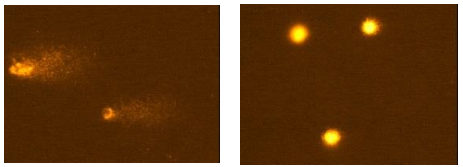


Photo credit: Dr. Bernardeschi M., PhD

Cytotox and genotox assessment

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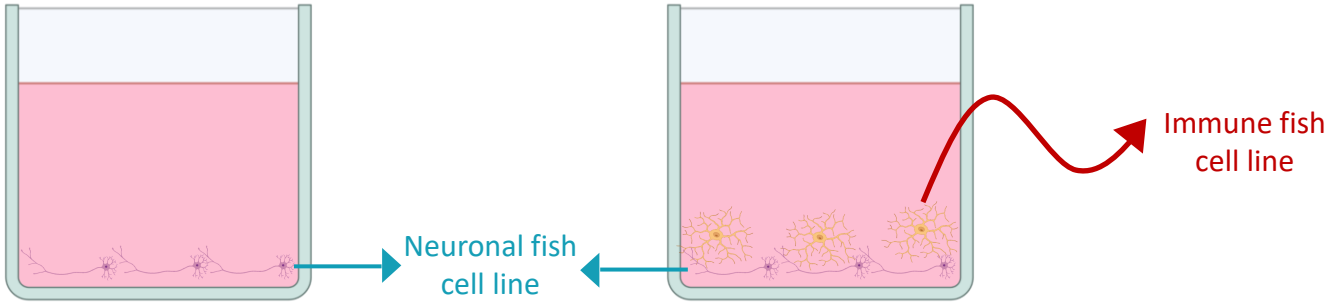
Ecotox
In vitro fish cell lines for neuro-nanotoxicity studies



- Cell viability
- Plasma membrane integrity
- Cellular uptake
- Oxidative stress

Step forward

- Inflammatory responses
- antioxidant enzymes
- lipid peroxidation
- AChE
- Etc.

Positive controls and materials' library

Positive controls:

- **Barrier integrity (i.e. triton-X)**
- **Cell viability (i.e. triton-X)**
- **Inflammation (i.e. LPS, TNF-α)**
- **Etc.**

Benchmark NMs:

- **SiO_2 (i.e. JRC NM203)**
- **Ag (i.e. JRC NM300K)**
- **Graphene (i.e. JRCNM48001)**
- **Etc.**

AdMa:

- **GO**
- **rGO**
- **GO-Ag**
- **Etc.**

Roadmap towards harmonisation

Integrated assessment and Advanced Characterisation of Neuro-Nanotoxicity (iCare project)

1. Developers

- Set up models/assays
- Intralaboratory
- Development of SOPs

2. Verification

- Interlaboratory
- SOP verification

3. Validation

- Interlaboratory
- SOP validation

NETVAL

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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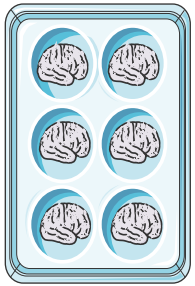
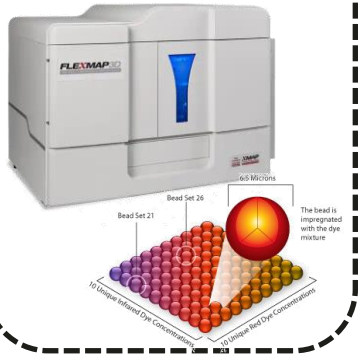
High-content assays
Inflammation, genotoxicity, epigenetics



In vitro models of the BBB and 3D models

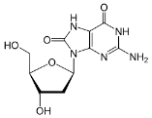
Inflammation

e.g. IL-1β, TNF-α, IL-6, IFN-γ, MCP-1…

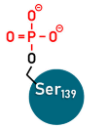


Genotoxicity

DNA damage

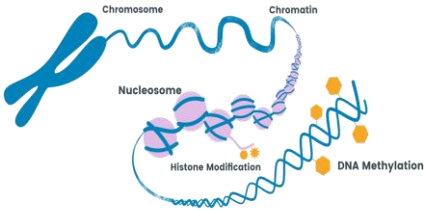


8-OHdG



γ-H2AX

Epigenetics



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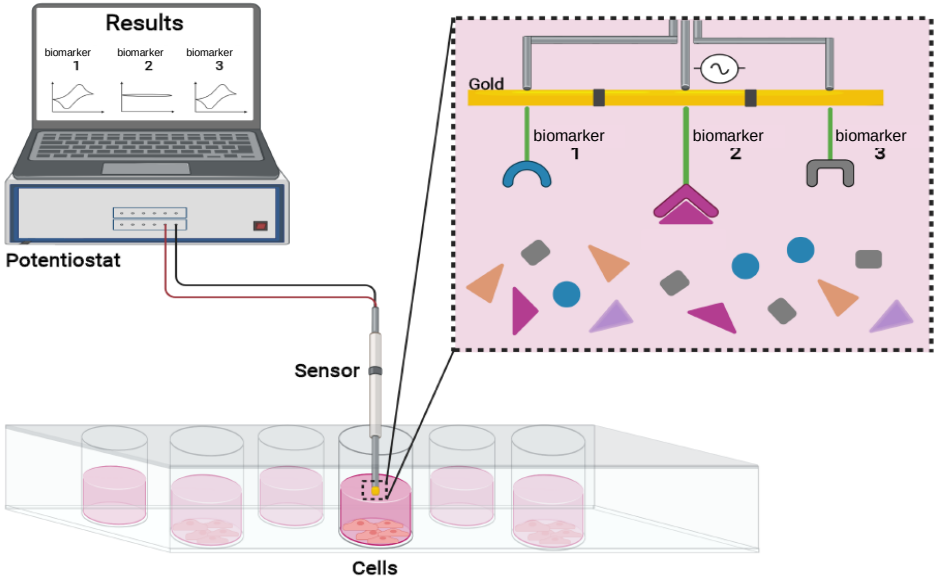
High-content assays

Real-time multiplex sensors for multibiomarkers



Miniaturised system

Biomarkers of:

- ✓ Oxidative stress
- ✓ Inflammation
- ✓ Cell death

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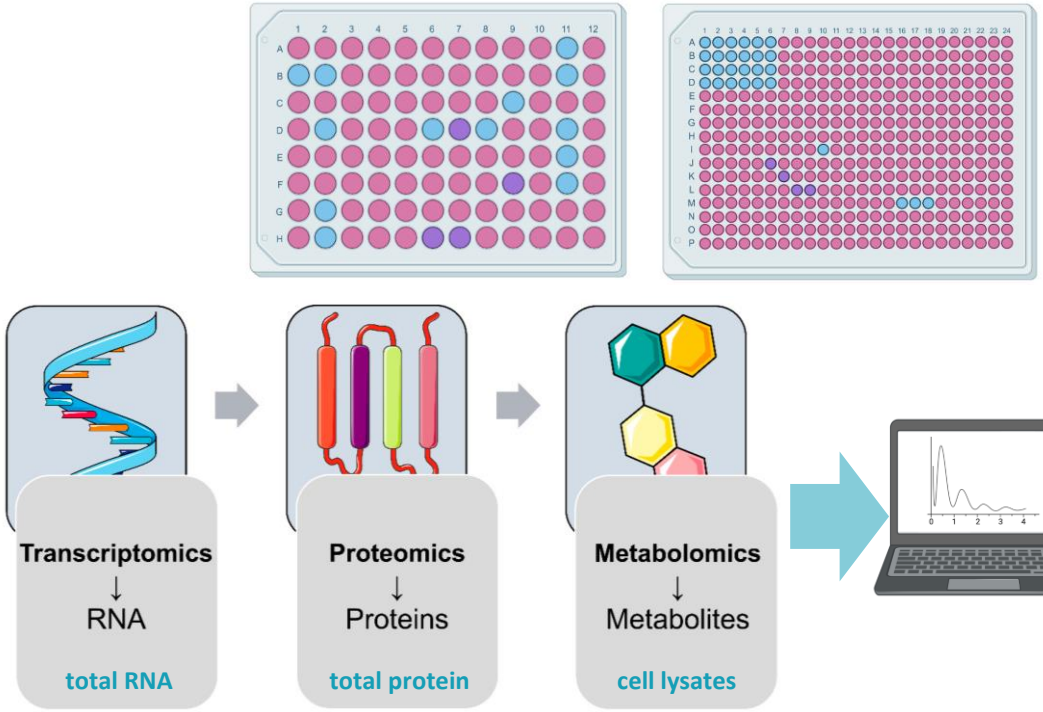
High-content assays
Integrated omics analysis



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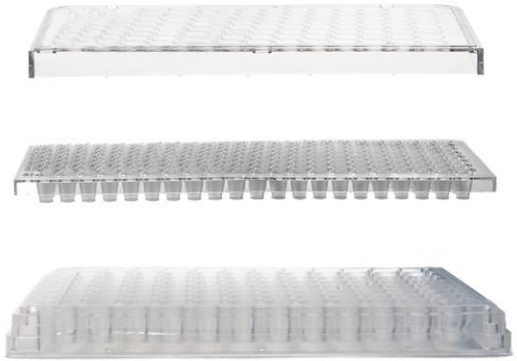
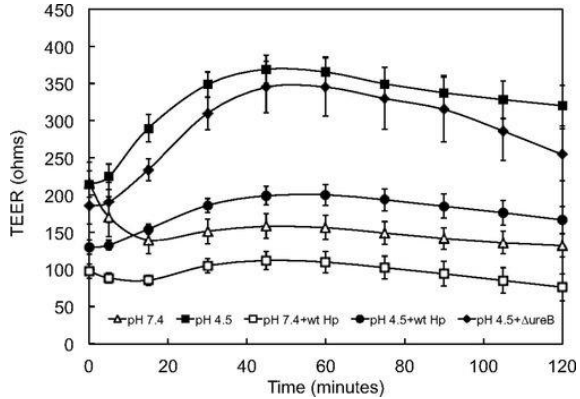
High-throughput screening
Real-time miniaturised TEER measurement





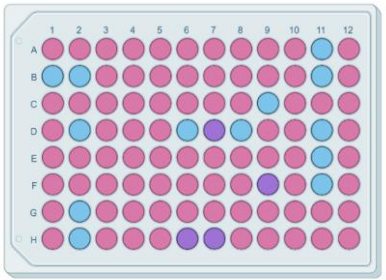
High-throughput screening
Integrated omics analysis

Validation of sensing platforms

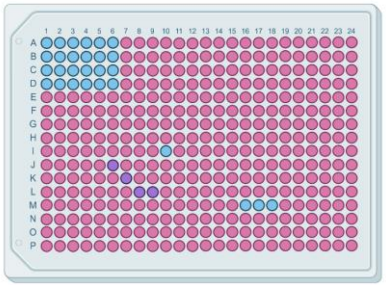
fluorescence/bioluminescence-based assays detecting the same parameters will be performed in parallel

Assay types

- ✓ Biochemical assays:
  - Absorbance
  - Fluorescence and derivatives
  - Luminescence
- ✓ Cell based assays:
  - Target based (ex. Reporter assay)
  - Phenotypic (ex. Toxicity)
  - High Content Screening
- ✓ Format: 384 well plates



Screening and high-throughput assays



high-throughput ✓
affordable ✓
easy-to-use ✓

Thanks

Do you have any questions?

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