NANOphotonic DEvice for Multiple therapeutic drug monitoring („NANODEM“)

SEVENTH FRAMEWORK PROGRAMME

1 Oct 2012 - 30 Sept 2016

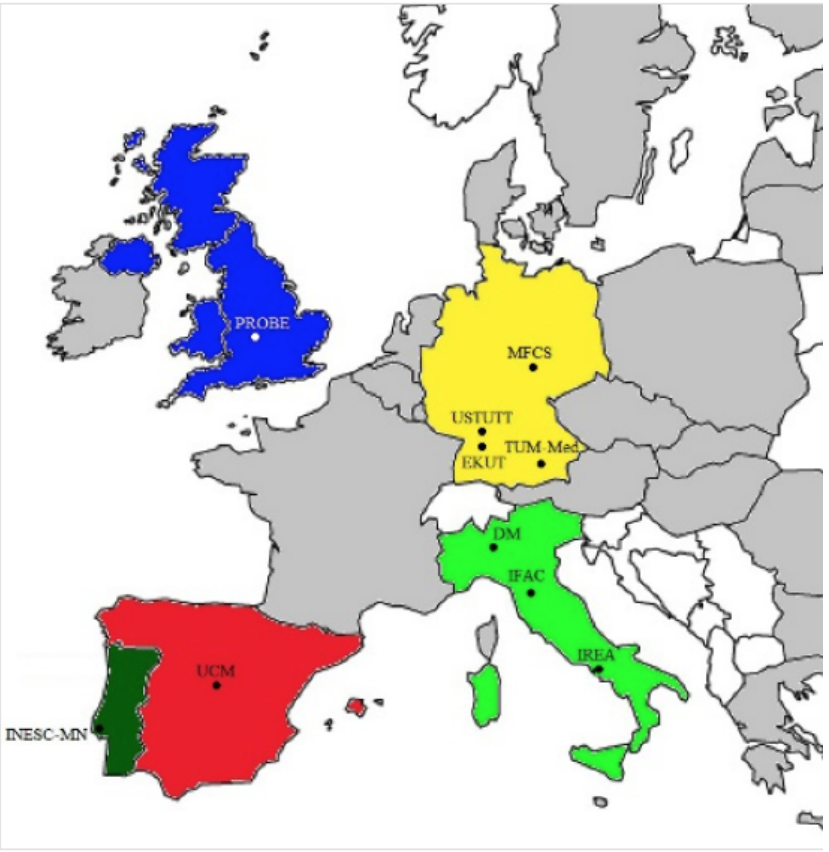
Peter B. Luppa Institute for Clinical Chemistry, TU Munich, Germany

SEVENTH FRAMEWORK PROGRAMME

NANODEM

Goal and consortium

Consiglio Nazionale delle Ricerche a)Institute of Applied Physics b)Institute for Electromagnetic Sensing of the Environment	**CNR** IFAC IREA
University of Tübingen Institute of Physical and Theoretical Chemistry	**EKUT**
Datamed S.r.L.	**DM**
Klinikum rechts der Isar der Technischen Universität München Institut für Klinische Chemie und Pathobiochemie	**TUM-Med**
Probe Scientific Ltd	**PROBE**
Universidad Complutense de Madrid Chemical Optosensors and Applied Photochemistry Group	**UCM**
University of Stuttgart Institute for Photovoltaics	**USTUTT**
INESC Microsystems and Nanotechnologies	**INESC-MN**
Microfluidic ChipShop GmbH	**MFCS**



EC contribution: 3,983,000 (STREP Collaborative project)
Starting date: 1 October 2012
Coordinator: Institute of Applied Physics, Florence, Italy

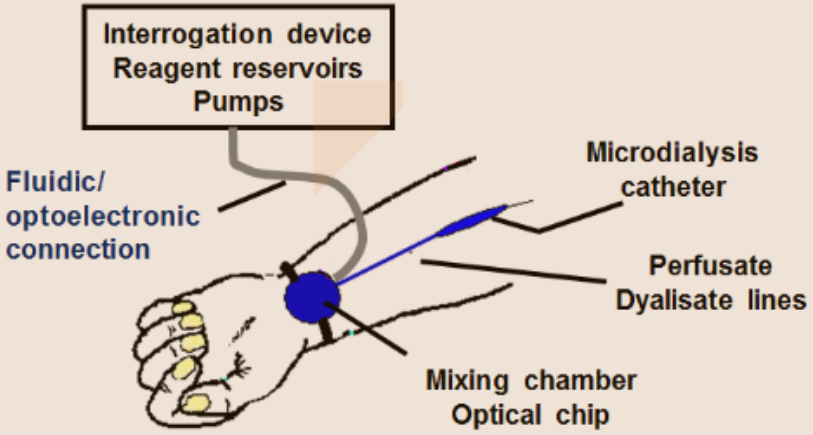
User´s needs and unique value

- Continuous monitoring of therapeutic drugs and metabolites ("TDM") is a strong requirement coming from transplantation surgeons and critical care medicine
- Drugs of interest are therapeutics with a narrow therapeutic window, which regularly pose considerable problems in initial and ongoing dosing. Therefore there is a strong requirement of TDM of immunosuppressants
- Strategies based on sparse sampling have been developed for clinical purposes, estimating the area under the concentration time curve (AUC). These strategies have been shown to substantially improve patient outcome
- Project aim: development of a new TDM point-of-care testing (POCT) device for in-line & in-time immunosuppressant measurements

User´s needs and unique value

- Substantial progress for newly transplanted patients might be:
- Continuous monitoring in the early phase of medication after trans-plantation
- Patient-near operation mode ("POCT")
- Benefits for patients after heart, lung, kidney or liver transplantation.

The patient will be connected to the POCT device by an intravenous microdialysis catheter to allow 48 - 72 h online measurements. The free fraction of the immunosuppressants and related metabolites will be monitored at short time interval.



Innovation process, road to exploitation

Patent investigation - overview completed by every partner

Commercialization questionnaire (driven by MFCS, Probe, and Datamed)

Inputs from project partners → updated consecutively in a living Mini-business plan

Companies involved in the project, able to manufacture the different modules of the whole device

- **Microfluidic ChipShop**

key player in the currently massively growing field of microfluidics, with own products as well as OEM (Original Equipment Manufacturer) activities.

- **Probe Scientific**

first producer of a vascular body interface.

- **Datamed**

industrial expert in the area of optoelectronic instrumentation and information technologies for medical applications and OEM of small series of medical devices fulfilling CE mark regulations

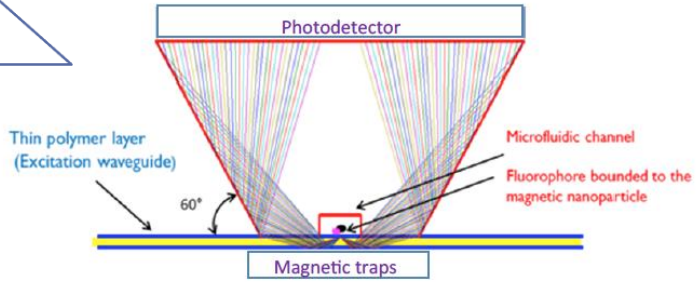
Innovation process, road to exploitation

General agreement for an IP exploitation in the consortium.

Exploitation will take place on different levels:

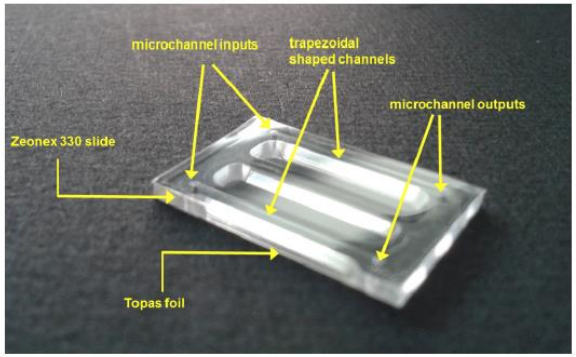
1. Commercialization of the POCT system, being developed
   - Instrument
   - Chip
   - Assay
   - Microfuidics/body interface
2. Commercialization of modules under development
3. Commercialization of technologies and fabrication processes being developed
4. Sales channels
   - modules/technologies/fabrication processes by the companies within the consortium
   - final device: identification of and contacts to large IVD companies

The heterogeneous IA-based chip

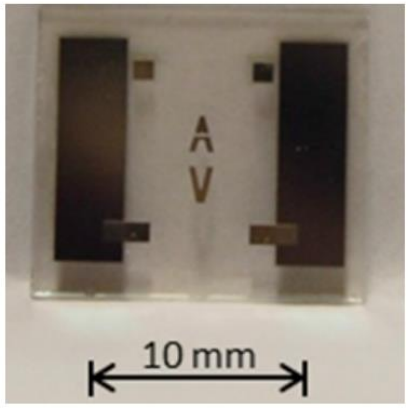


Chip design: transversal section

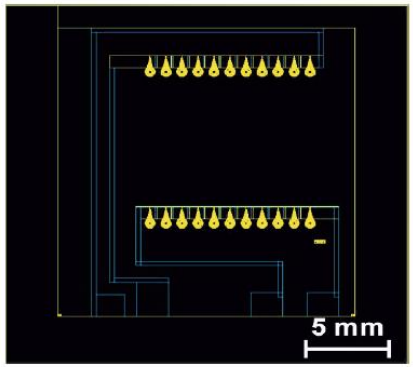
Multichannel configuration (10 parallel microchannels) for the simultaneous detection of the immunosuppressants



First prototype of the two-channel heterogeneous chip



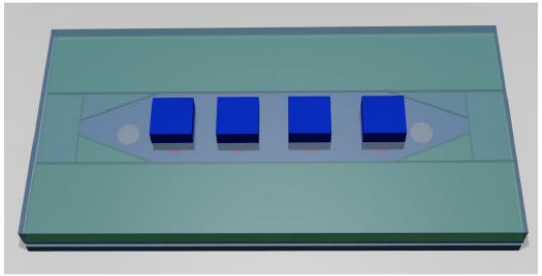
Thin Film Photodetectors for the two-channel heterogeneous chip



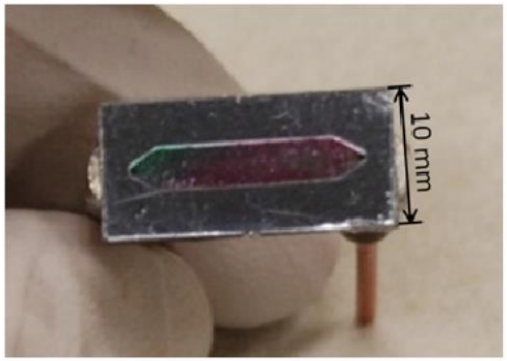
Magnetic traps for the two-channel heterogeneous chip

The homogeneous IA-based chip

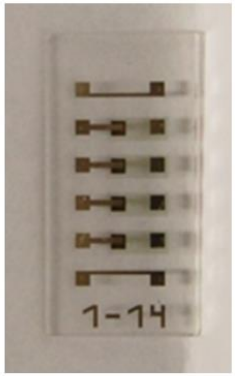
Single microchannel configuration for the sequential detection of the immunosuppressants



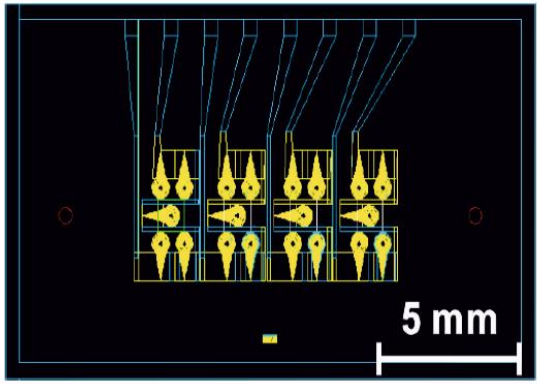
Chip design: top view



First prototype of the homogeneous chip



Thin Film Photodetectors for the homogeneous chip



Magnetic traps for the homogeneous chip

Distance to market

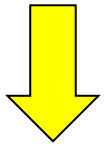
Non-technical steps

- **Clinical steps**
- need of a clinical assessment (verification of the sensing approach with the immunuppressants measured in the dialysate)
- clinical trial defined at the last year of the project: at least 25 adult patients for each drug during the first 2 weeks after organ transplantation, as well as additional 25 patients during the maintenance period (trial may go chronologically beyond the end of the funding period)
- **Market step**
- need of the identification of a clinical/market access partner

Distance to market

Technical steps

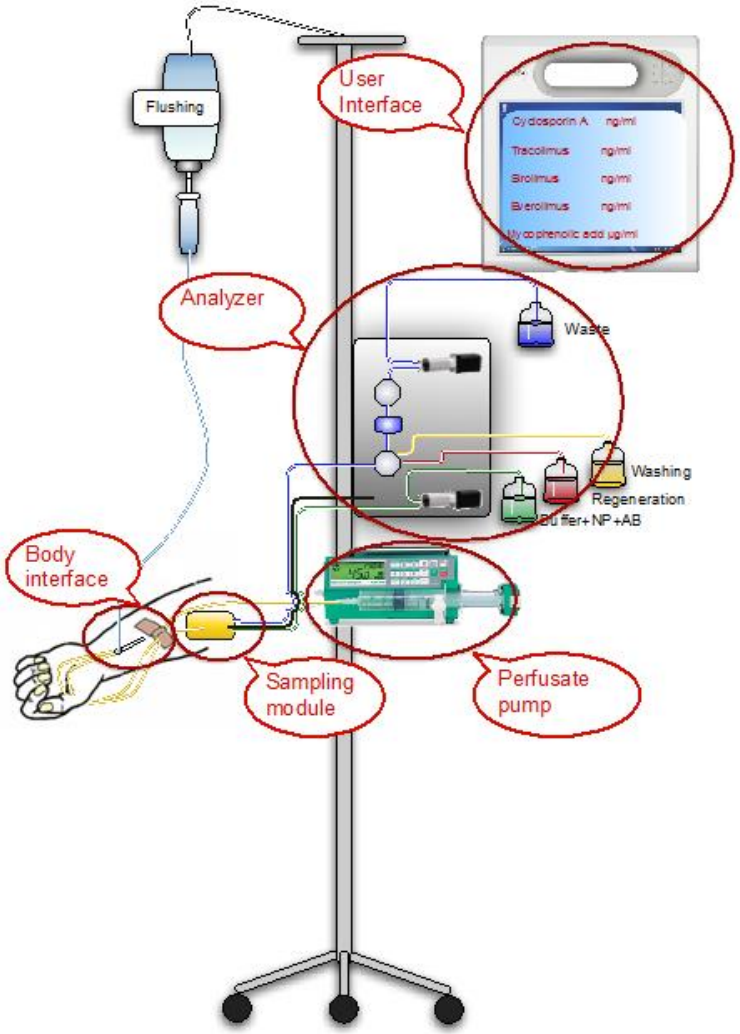
- Combination of magnetic traps/magnetic nanoparticles and fluorescence detection within the same assay chip



need to adopt particular chip configuration which must be compatible with injection mould production

- Design of microfluidic circuit which interfaces the intravascular microdialysis catheter and the optical biochip, which must be able to perform the bioassay as frequent as possible for a period of 24 - 48 hours

Thanks for your attention!



Additional issues

IPR Committee

Formed by one representative of each partner, with the task of ensuring protection of results achieved during the project, of implementing the two mini business plans during the project and of defining an on-time common strategy for the exploitation of the results

Manufacture strategy

The manufacture of the prototype device, to be tested on transplanted patients in the final year of the project and beyond its end, is the main task of Datamed.

In the case of "clinical" success, this will be the basis for the manufacture of the device for the market

Market dissemination activities

Pitching of the project at relevant commercial events such as COMPAMED forum, AACC, BIOMEDevice

Organisation of training events on microfluidics by microfluidic ChipShop

NANODEM commercialization workshop organized by microfluidic ChipShop in the period months 39-48, possibly in conjunction with a major event

Innovation process, road to exploitation

- Generation of a two-staged mini business plan, collection of market data/reports
- First level exploitation of functional modules (e.g. mixers, optofluidic components) through microfluidic ChipShop's catalogue: easy and fast access for first (typically academic) users
- Second level exploitation of technologies (e.g. hybrid integration) by including them in the technology portfolio of microfluidic ChipShop, Probe and Datamed, thus strengthening their market position
- Third level exploitation of complete system by search and selection of clinical/market access partner during months 30-36 (Probe, Datamed, MFCS)

Motivation & Project Aim

In the transplanted patients treatment

strong requirements (from physicians)

- Continuos measurement of immunosuppressants;
- Fast & reliable response for the identification of the right dosage;

Critical aspects:

- narrow therapeutic window for the drugs of interest (problems in initial & ongoing dosing);
- Drug polymorphic metabolism & their individual variability require a correct dosage & monitoring;

- **Therapeutic Drug Monitoring (TDM)** based on the measurement of the trough level before the next dose (information loss about the kinetics);
- Novel strategies based on sparse sampling and estimating the area under the concentration time curve (AUC);

Project Aim:

Development of a new therapeutic drug monitoring (TDM) point-of-care testing (POCT) device for the **in-line & in-time** immunosuppressant measurements

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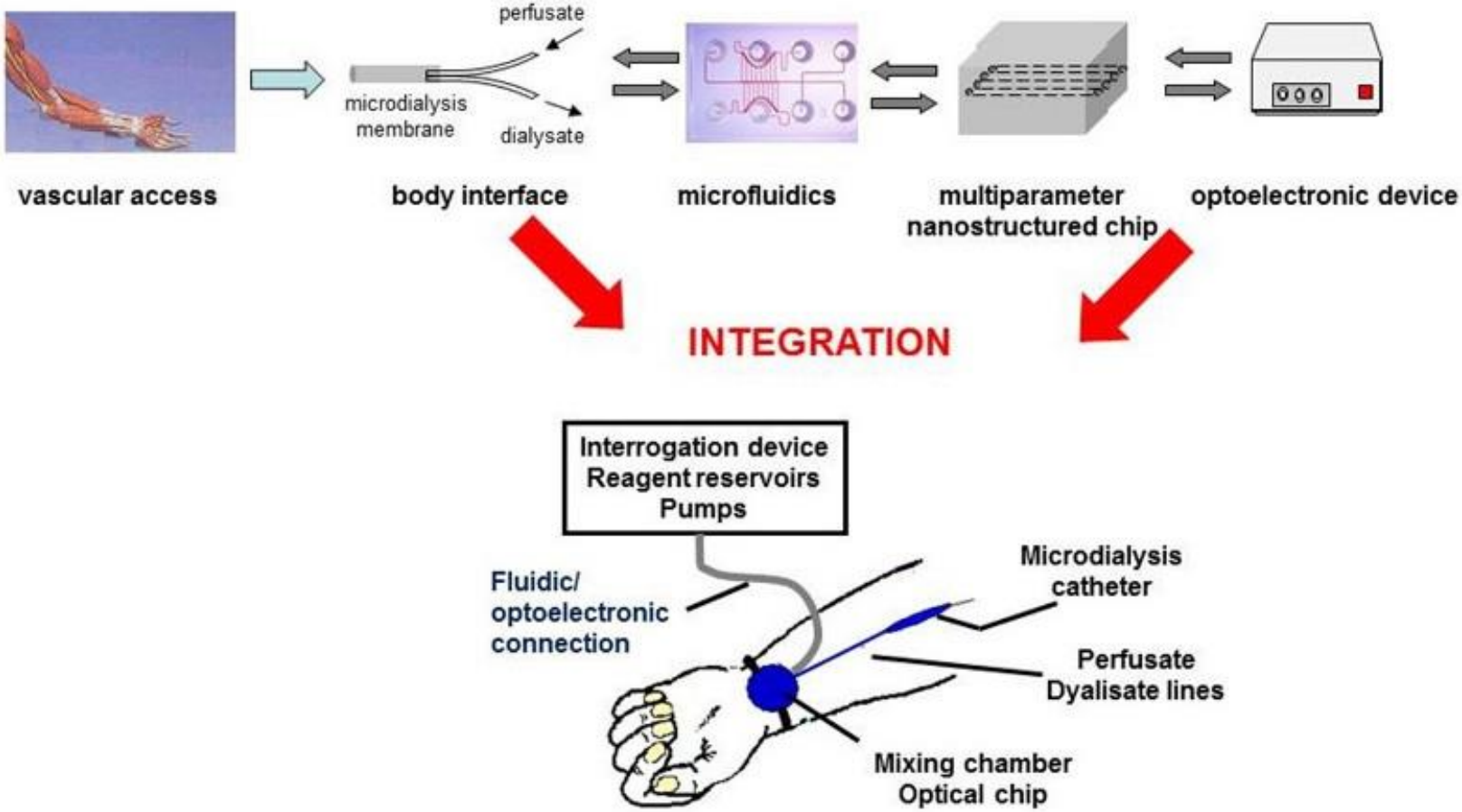
Therapeutic drug monitoring of Immunosuppressive drugs



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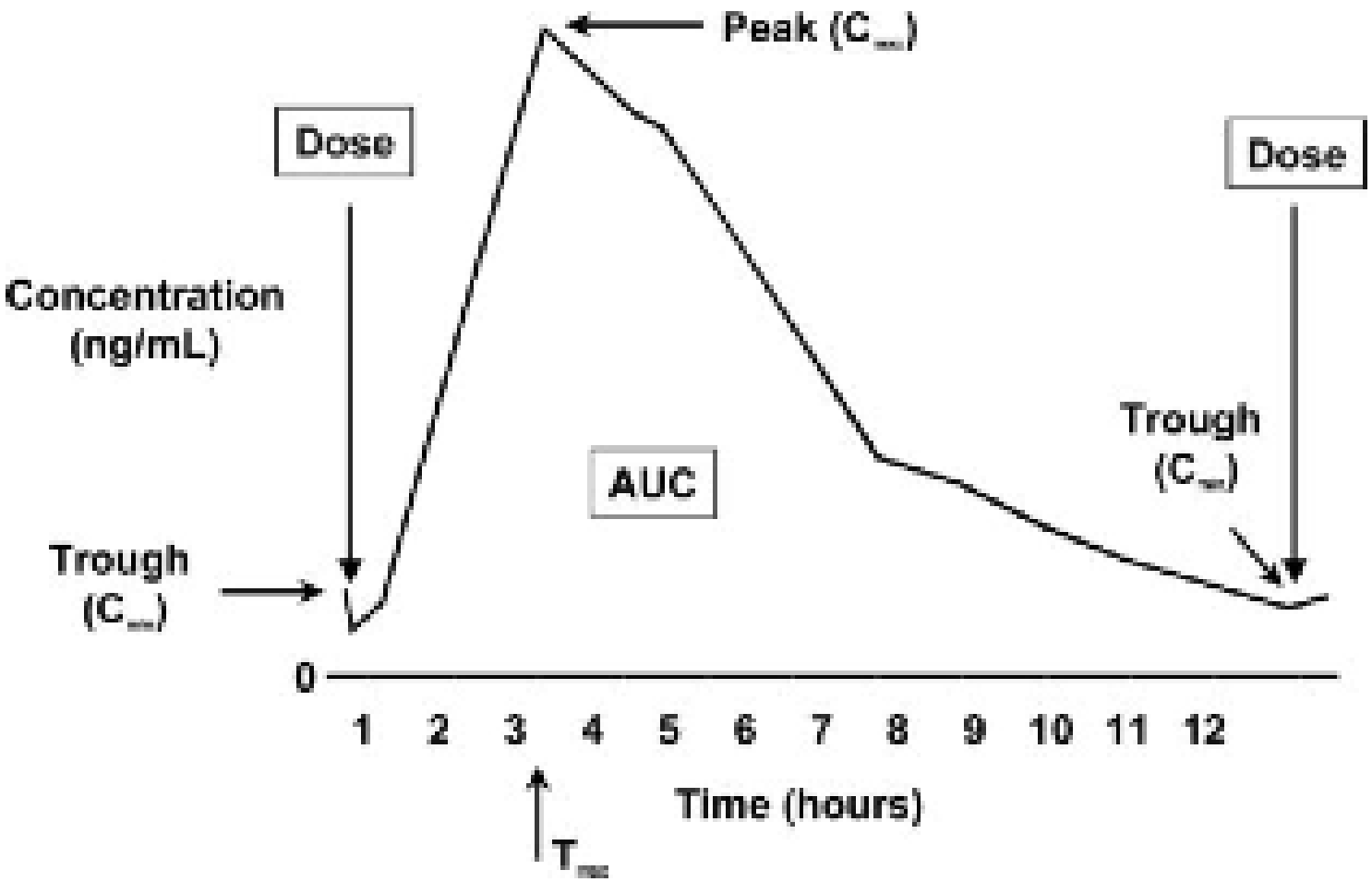
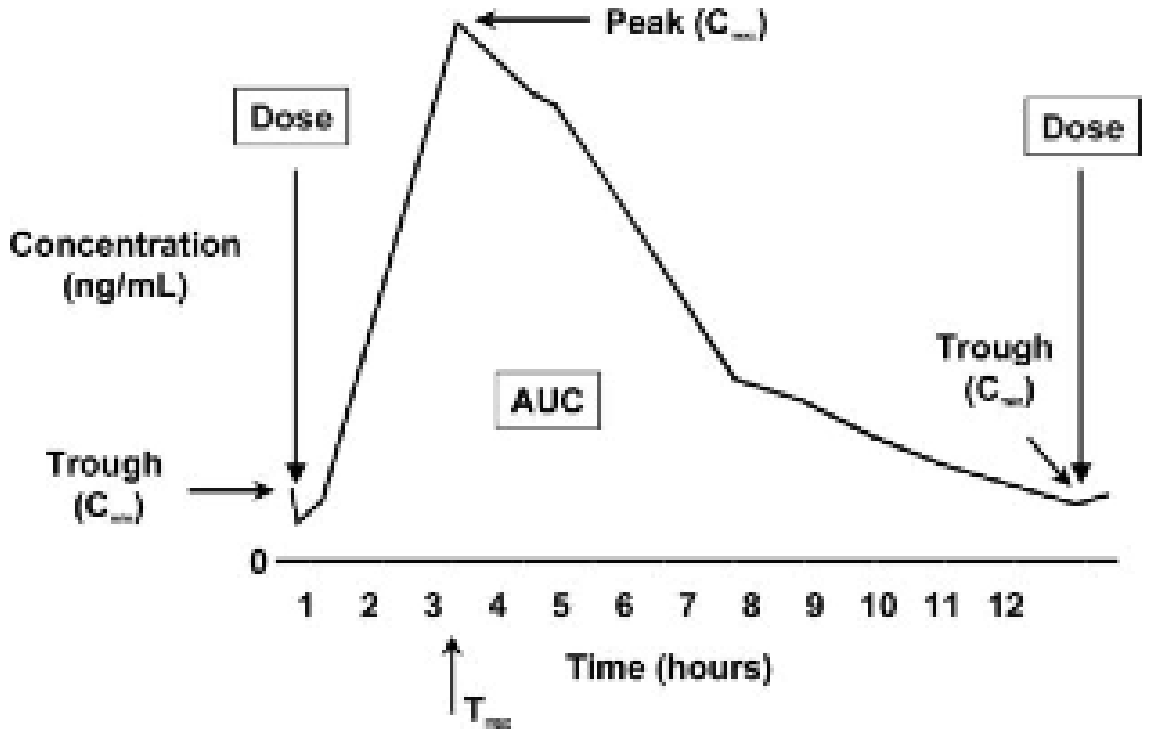


AIM: development of a novel therapeutic drug monitoring POCT device for the in-line measurement of immunosuppressants and related metabolites in transplanted patients, by means of the use of an intravascular microdialysis catheter

J Schiff et al. Clin J Am Soc Nephrol 2: 374–384, 2007.



Drug levels during the course of a dosing interval. The drug concentration is lowest (C_{min}) just before the dose is taken, the rises to a peak level (C_{max}) at a certain time after the dose (T_{max}). The concentration then falls back to C_{min} before the next dose. The area under the concentration-time curve (AUC) describes total drug exposure during the entire dosing interval.

J Schiff et al. Clin J Am Soc Nephrol 2: 374–384, 2007.

TDM in clinical practice

- In general, each therapeutic drug is distributed in various body compartments in different concentrations.
- The blood compartment is best available for monitoring.
- The levels of the drug – either in whole blood or in plasma/serum or in a dialysate – have to be correlated thoroughly to the therapeutic effects.
- As a consequence, target therapeutic ranges (TR) have to be defined according to the respective matrix or compartment.
- These TR are established empirically and may also vary for different organ transplantations.

State-of-the-art measurement of CyA and Tac in whole blood

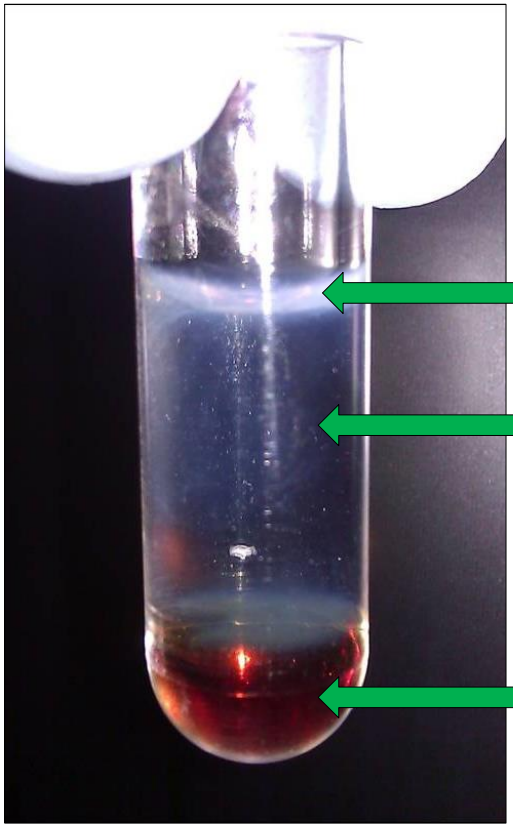
- Total concentration of the respective mother drug in whole blood is measured by use of LC-MS/MS. Anticoagulation is guaranteed by addition of EDTA.

- CyA is found 70% intracellular (erys, lymphos) and 30% in plasma. Tac is found 80% intracellular and 20% in plasma.
- In plasma, CyA and Tac are bound to lipoproteins, serum albumin and alpha-1-acid glycoprotein.
- Approx. 3% of the CyA fraction and 1% of the Tac fraction are free and dialysable.

Monitoring of free drug fraction vs. whole blood immunosuppressant concentrations

- There‘s a lack of relevant references available dealing with the significance of measuring the free drug concentrations in plasma instead of the total drug concentration.
- One of the reasons is that the analysis of the free fraction is more complex than the measurement of the total level (lower conc, temperature dependence etc.).
- In theory, the free fraction might be closely correlated to the total concentration.
- It is worth mentioning, however, that the free drug concentration will change significantly if other therapeutics in high concentrations interfere with the plasma protein binding of the immunosuppressants. As a consequence, the levels of the free drugs increase.

Determination of free drug fraction in human serum
– establishment of an adequate ultracentrifugation technique –

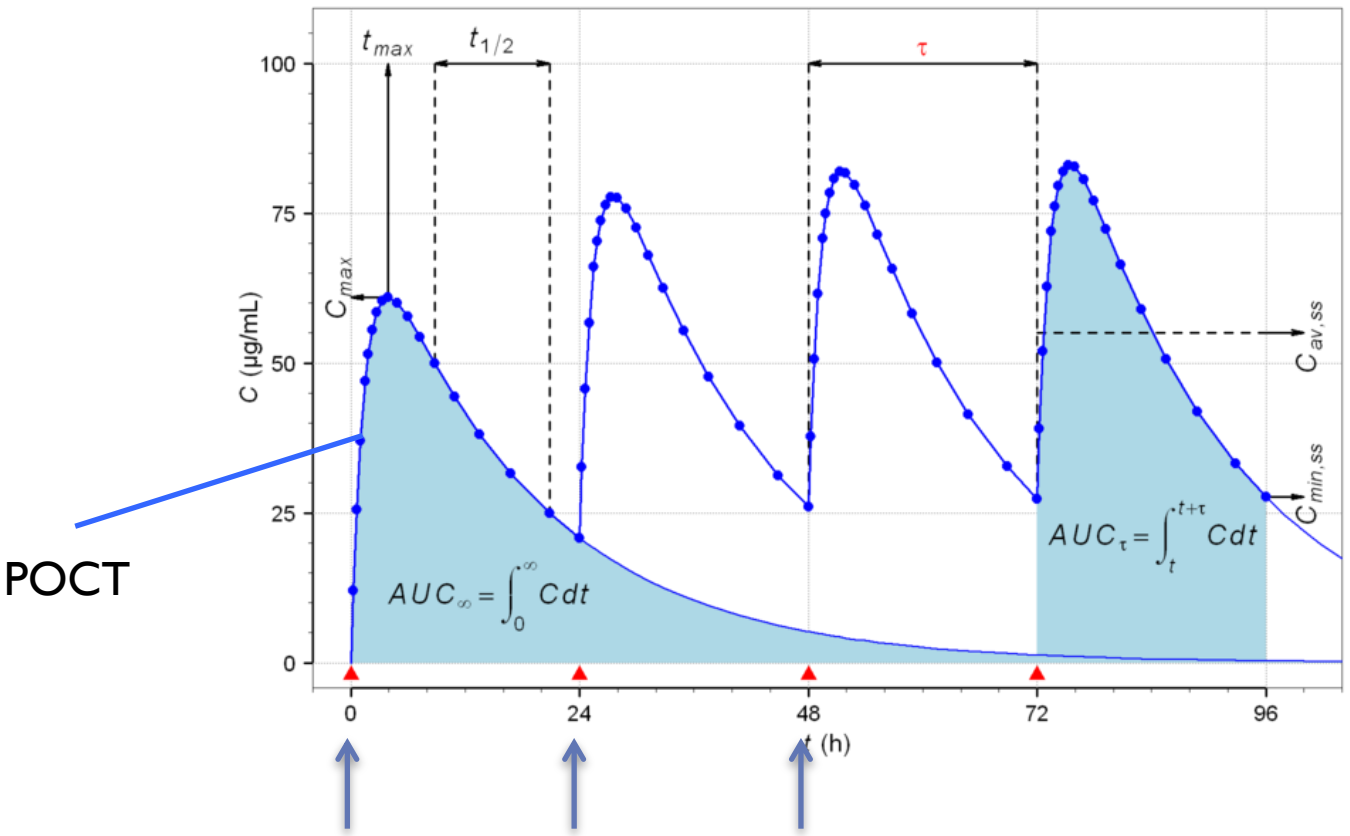


Test conditions:
6.0 hours ultracentrifugation at 160 000 x g
(according to W. Piekoszewski & W.J. Jusko, 1993)

lipid layer: low-density lipoproteins (LDL), chylomicrons

protein-free supernatant : water, salts, hormones, drugs
Quality check:
determination of protein content via Bradford assay
Result: ~ 0,5% of native serum protein is found in the supernatant

Sediment: albumin, immunoglobulins, free Hb, free bilirubin, drug protein complexes etc.



POCT

LC-MS/MS

When establishing POCT measurements of CyA and Tac, the quantification of the dialysable fraction will be calibrated against the LC-MS/MS method in whole blood, e.g. in a 24h period of time.

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Microdialysis of immunosuppressants

Contribution to WP3 (body interface and microfluidics)

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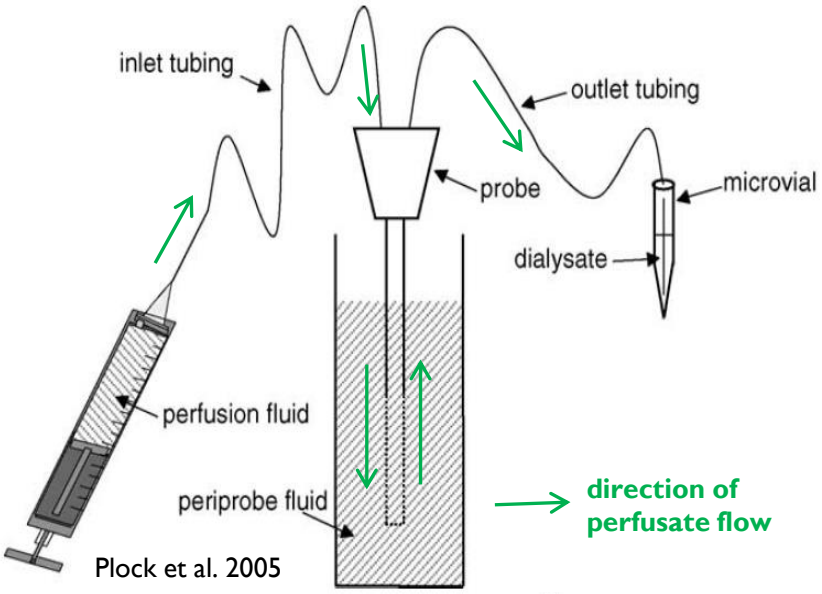
Microdialysis Workflow



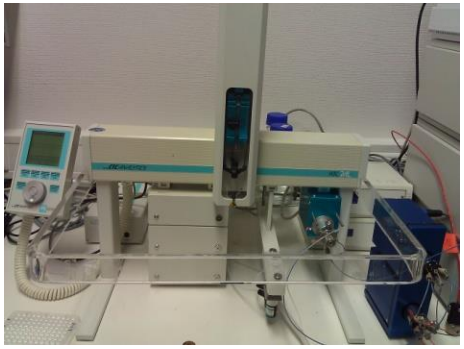
microdialysis
of free fraction of immunosuppressants

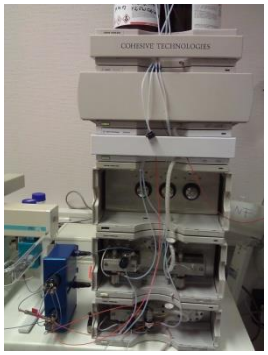
sample preparation

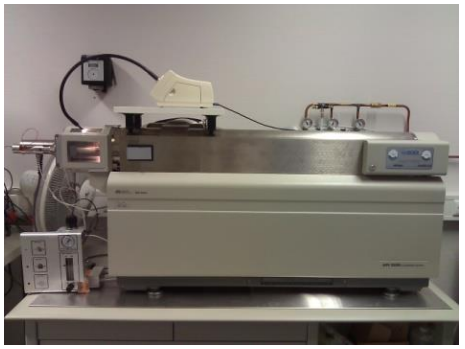
determination of immunosuppressant concentration via LC-MS/MS

$$\text{Relative Recovery [\%]} = \frac{c_{dial}}{c_{periprobe\ fluid}} * 100$$

LC-MS/MS system at Klinikum rechts der Isar







System 1

Autosampler:	**HTC PAL (CTC Analytics)**
LC columns:	**HTLC column (Cyclon, 50 µm, 1 x 50 mm, Cohesive)**
	HPLC column (Luna Phenyl-Hexyl, 5 µm, 2 x 50 mm, Phenomenex)
Mass Spectrometer:	**Tandem Mass Spectrometer API 3000 (AB SCIEX)**
Software:	**Analyst 1.5.1 (AB SCIEX)**

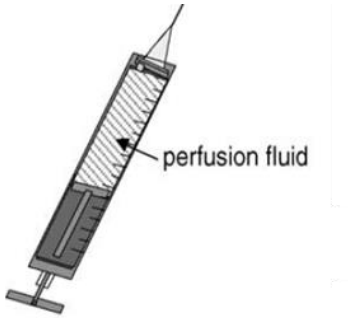
System 2

Autosampler:	**Prominence Autosampler UFLC (Shimadzu)**
LC column:	**HPLC column (Luna Phenyl-Hexyl, 5 µm, 2 x 50 mm, Phenomenex)**
Mass Spectrometer:	**Tandem Mass Spectrometer QTRAP® 5500 (AB SCIEX)**
Software:	**Analyst 1.5.1 (AB SCIEX)**

Perfusate 1 : 20% (v/v) Lipofundin® in saline

sterile fatty emulsion used for parenteral feeding

\+ 0.9 % NaCl →



perfusate

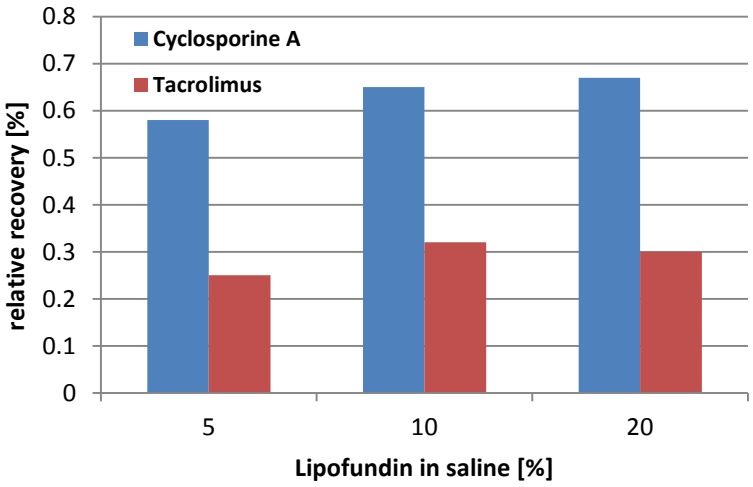
1000 ml Lipofundin® contents 200.0 g Soybean Oil, 12.0 g Egglecithin, 25.0 g Glycerol
Other ingredients: Alpha-Tocopherol, Sodiumoleat, Water for Infusion
Physicochemical properties: 350-380 mOsm/kg, pH 6.5-8.5

- **Shipment of Lipofundin® to PROBE for microdialysis of Mycophenolic acid**
- **Shipment of perfusate/dialysate to bioassay developers EKUT and CNR-IFAC for a first check on applicability**
  → No interference with antibodies and no disturbing light scattering when diluted

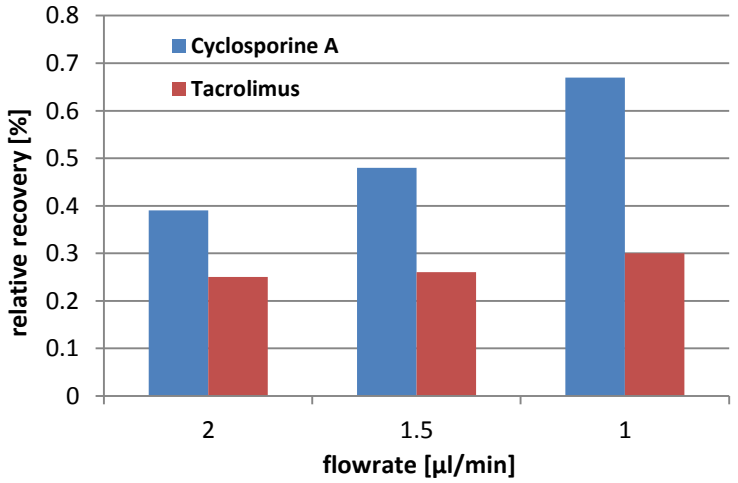
Microdialysis with Lipofundin® perfusate

Surrounding medium: spiked EDTA blood, 37° C

Influence of Lipofundin® concentration in perfusate



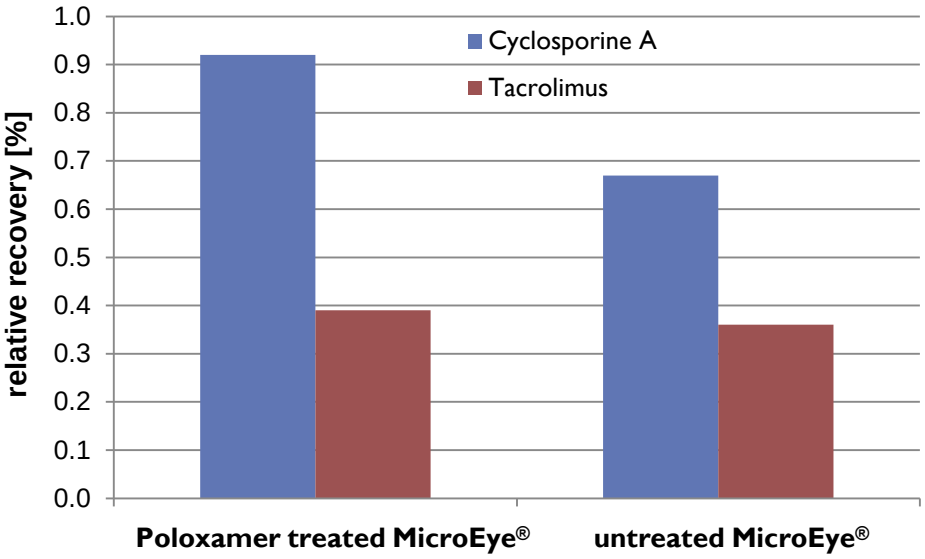
Influence of flowrate on relative recovery
Perfusate: 20% Lipofundin in saline

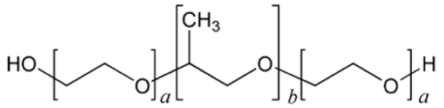


$$\text{Relative Recovery [\%]} = \frac{c_{dial}}{c_{periprobe\ fluid}} * 100$$

Poloxamer treatment of inner MicroEye® tubing material

Surrounding medium: spiked EDTA blood, 37° C
flowrate: 1µl/min, perfusate: 20% (v/v) Lipofundin® in saline



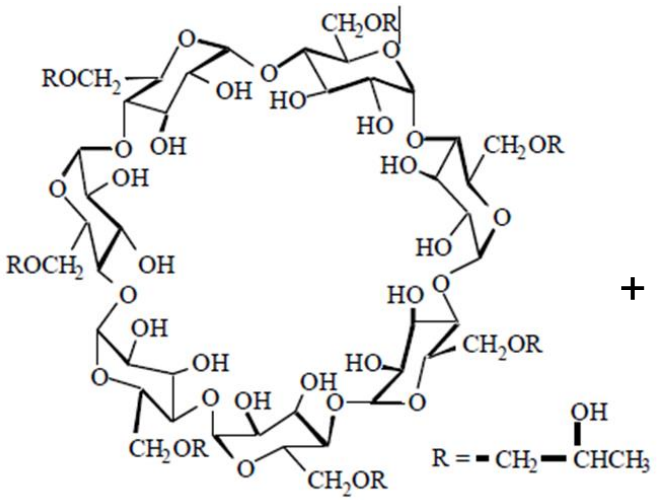


Poloxamer 407

Treatment conditions:

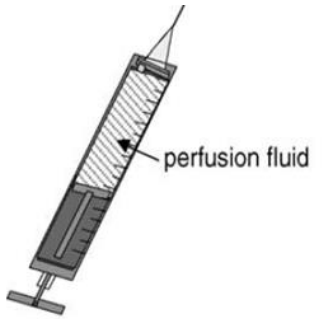
1. **24 h flushing of MicroEye® device with 5% Poloxamer (flow rate 0.5 µl/min)**
2. **14 h flushing of MicroEye® device with Millipor water (flow rate 0.5 µl/min)**

Perfusate 2 : 10% 2-Hydroxypropyl-ß-Cyclodextrin (HPCD) in saline



HPCD

+ 0.9 % NaCl →

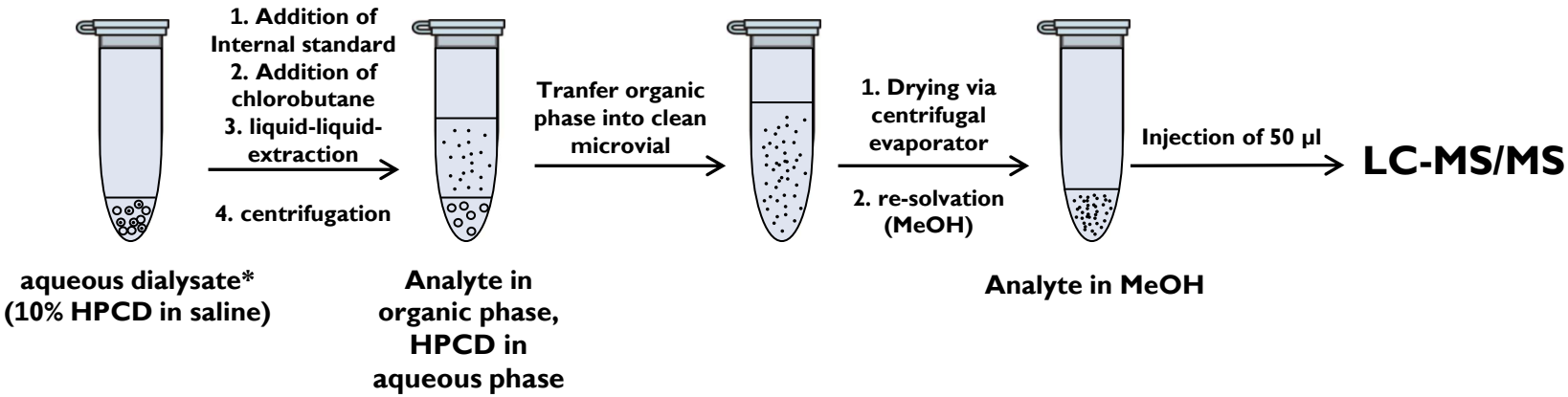


perfusate

- cyclic oligosaccharide
- hydrophobic cavity
- guest-host type complex with lipohilic molecules
- „non toxic solubizer“ (FDA approved)
- clear and colorless solution with water

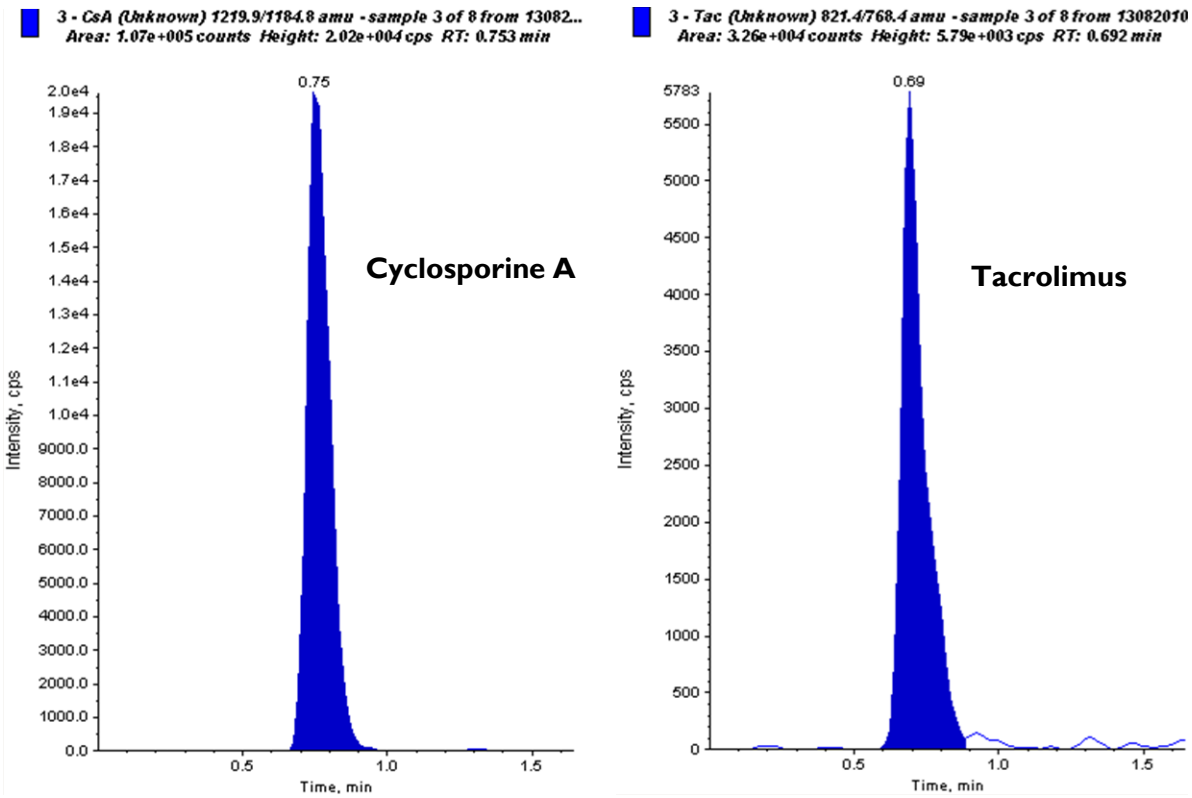
→ **Transfer of perfusate/dialysate to CNR-IFAC and of HPCD to EKUT and UCM for applicability testings**

Dialysate work-up scheme



*** Microdialysis conditions:**
37°C, flow rate: 1 µl/min , spiked EDTA blood (~ 10fold greater drug concentrations than in patient blood)

LC-MS/MS chromatogram screenshot



Test conditions:
10% HPCD in saline (perfusate), spiked EDTA blood, dialysate volume: 60 µl, 37°C, flow rate: 1µl/min

Targeted limits of quantification (LOQ) for LC-MS/MS measurement of immunosuppressants in dialysate

	Target trough whole blood levels in kidney transplant patients	Free drug fraction [%]	Relative microdialysis recovery[3] [%]	Targeted LOQ
Cyclosporine A	200-400 ng/ml [1]	3	e.g. 30	= 200 ng/ml x 0.03 x 0.3 = **1.800 ng/ml**
Tacrolimus	15-20 ng/ml [2]	1	e.g. 30	= 15 ng/ml x 0.01 x 0.3 = **0.045 ng/ml**
Sirolimus	4-12 ng/ml [1]	8	e.g. 30	= 4 ng/ml x 0.08 x 0.3 = **0.096 ng/ml**
Everolimus	3-8 ng/ml	5-10	e.g. 30	= 3 ng/ml x 0.05 x 0.3 = **0.045 ng/ml**
Mycophenolic Acid	1-3.5 µg/ml	1-3	e.g. 30	= 1 µg/ml x 0.01 x 0.3 = **3.0 ng/ml**

[1] first months after transplantation [2] first two weeks after transplantation [3]Related to free drug fraction

Current estimate of LOQs achieved by the LC-MS/MS method: < 0.5 ng/ml (Cyclosporine A) and 0.1 ng/ml (Tacrolimus)

Next Steps

- Further method development and validation of sample preparation and LC-MS/MS analysis (HPCD assay)
- LC-MS/MS method development for detection of free drug fraction
- Switch from „common“ internal standards to deuterated internal standards for exact determination of drug concentrations in HPCD derived dialysates
- Applying to ethics committee to obtain approval for collection of transplant patient blood samples for ex-vivo testing

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Nanodem
1st Year Review Meeting
WP 8 – Clinical assessment
Ex-vivo trial

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Heike Eggert and Peter B. Luppa (TUM-Med), 28th November 2013, Florence

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WP 8 Objectives

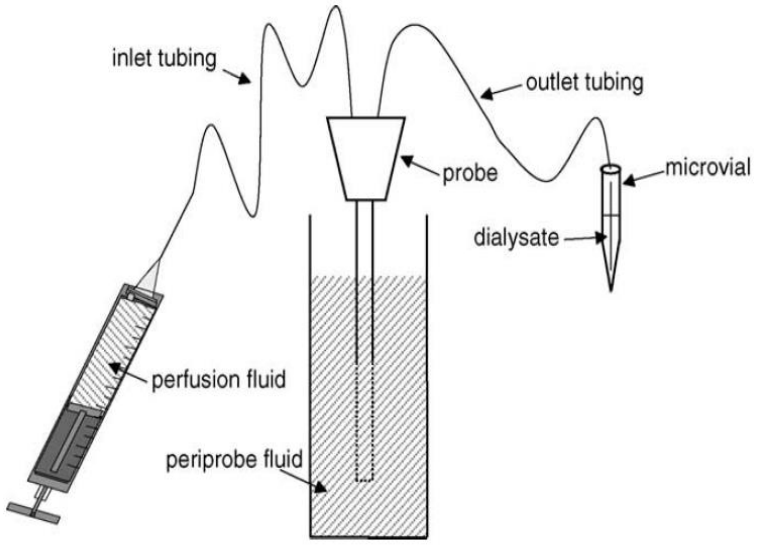
- The first part of the planned study should evaluate analytical aspects of the novel POCT device under clinical circumstances.

- The second part of the clinical study has to clarify the question whether the integrated POCT system provides the opportunity to apply a clinically viable AUC assessment for the respective immunosuppressive drug.

- The clinical trial needs authentic human samples. These patient specimen collection will be performed according to the FDA guidance entitled "…IVD Device Studies using Leftover Human Specimens"(http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm078384.htm). Human blood leftover specimens are remnants of specimens collected for routine clinical analysis by the core lab that would have been discarded. Specimens are not individually identifiable, i.e., the identity of the subject is not known to and may not readily be ascertained by the investigator or any other individuals associated with the study.

Clinical assessment I – Analytical aspects

When the preparatory work is done, we will start the *Ex vivo* study – test of MicroEye® with LC-MS/MS using real patient samples



When the NANODEM POCT device is ready to use, we will start the

- **Clinical assessment II – AUC assessment of immunosuppressive drugs**

  Scheduled for year 4

Workplan

- **Clinical requirements for bioassays**
  - Requirements reported in D8.1
  - Literature overview for TDM given in D8.3

- **Clinical assessment I – Analytical aspects**
  - Evaluation of the study by the ethics committee (D8.2) delayed by six month due to issues with organ allocation at TUM-Med
  - Final optimisation of perfusate
  - Final optimisation of LC-MS/MS assay
  - ➢ Most likely no delay for D8.4 (collection of patient samples) and D8.5 (first results achieved using routine lab methods)

We submit the request for permission of the patient sample measurements to the ethical review committee of the Medical faculty (TU München) in January 2014.

The Ethical review committee is strictly following the declaration of Helsinki and the ICH-GCP guidelines. ICH is the „International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use“.

The ICH topics are divided into four categories and ICH topic codes are assigned according to these categories.

Quality Guidelines

Harmonisation achievements in the Quality area include pivotal milestones such as the conduct of stability studies, defining relevant thresholds for impurities testing and a more flexible approach to pharmaceutical quality based on Good Manufacturing Practice (GMP) risk management.

Safety Guidelines

ICH has produced a comprehensive set of safety Guidelines to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity. A recent breakthrough has been a non-clinical testing strategy for assessing the QT interval prolongation liability: the single most important cause of drug withdrawals in recent years.

Efficacy Guidelines

The work carried out by ICH under the Efficacy heading is concerned with the design, conduct, safety and reporting of clinical trials. It also covers novel types of medicines derived from biotechnological processes and the use of pharmacogenetics/genomics techniques to produce better targeted medicines.

Multidisciplinary Guidelines

Those are the cross-cutting topics which do not fit uniquely into one of the Quality, Safety and Efficacy categories. It includes the ICH medical terminology (MedDRA), the Common Technical Document (CTD) and the development of Electronic Standards for the Transfer of Regulatory Information (ESTRI).

If patient samples have to be recruted from other centers for liver transplantation, we will contact also the ethical review committees from:

- Ludwig-Maximilians Universität München, Klinikum Großhadern
- Klinikum der Universität Regensburg

Clinical assessment I – Analytical aspects

- **Optimisation of LC-MS/MS assay**

  Derivatives of the drugs are currently used as internal standard. This is not optimal due to different extraction rates during sample preparation. Therefore the use of deuterated drug as internal standard is advisable.

- Measurements of free fractions of the drug are currently under development
  - Recovery rates proportional to free fraction.
  - Need to evaluate effect of changes in free fraction on recovery.
  - Free fraction as a better measure than total concentration?

Thanks for your attention!