

numares
insider



HANDS ON

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INSIDE

Limitations of eGFR

NEWS

15 years of numares

2019

Welcome to *numares insider*



Volker Pfahlert



Philipp Pagel

2019 is a very special year for us: numares was founded in 2004 so we are celebrating 15 years of company history.

Many exciting things have happened in the last 15 years. We have won new customers, engaged in various collaborations, launched new innovative products and built a great team. In this issue, we are presenting a small selection of current activities and topics at numares and we hope to spark your interest.

We are proud to feature *AXINON® Clearance Check* – our new *in vitro* diagnostic product that allows a robust and reliable determination of glomerular filtration rate (GFR). This time, we will give some background on the current state of GFR determination: While traditional GFR estimation based on serum-creatinine works fine for many standard situations, this method is problematic for use in elderly patients or children, in the presence of liver disease or other conditions. *AXINON® Clearance Check* aims to overcome these shortcomings and allow reliable GFR determination in a wide array of non-standard situations without requiring more than a simple serum sample. We believe that this will generate great benefit for patients suffering from kidney disease or at risk of developing it.

numares is about health, and so are our employees: Our team *numaRacers* had a great time at the annual “Landkreislauf” – the most popular running event in the Regensburg area. We are proud to report that our team of people across all kinds of departments finished the 73 km (45.4 miles) in 6 hours and 32 minutes - congratulations.

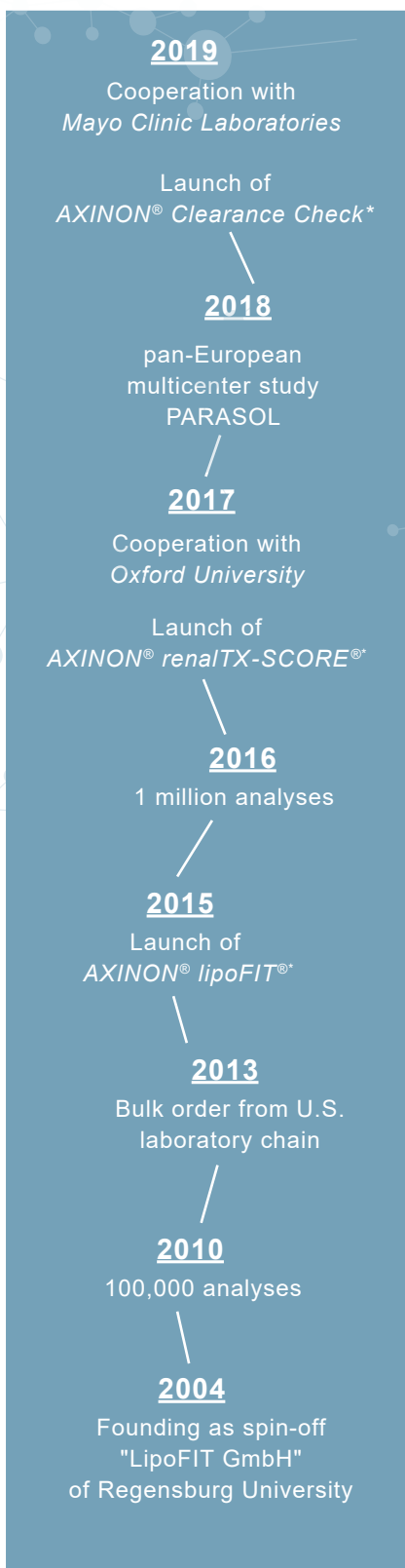
We hope you enjoy this issue of the *numares insider*.

Two handwritten signatures in blue ink. The first signature is for Volker Pfahlert and the second is for Philipp Pagel.

Volker Pfahlert, Chief Executive Officer

Philipp Pagel, Chief Medical Officer

Happy birthday, *numares*! Milestones within 15 years of success



*For Research Use only in the United States. numares' products are not yet available for sale within the United States; they have not yet been approved or cleared by the U.S. Food and Drug Administration.

Kidney function - limitations of estimated glomerular filtration rate



Creatinine is the most commonly used serum marker for GFR estimation.

The glomerular filtration rate (GFR) is the volume of primary urine produced by all renal glomeruli per time unit. It is considered the best parameter to assess kidney health and function, e.g. for diagnosing different stages of chronic kidney disease (CKD). In clinical practice, GFR is usually estimated (eGFR) using the serum concentration of an endogenous filtration marker, most commonly creatinine. The requirements for an ideal GFR marker were defined by Homer Smith in 1951: It is a molecule that is freely filtered by the glomeruli into the urine without being reabsorbed or secreted (1).

Non-GFR determinants of creatinine

Although creatinine is the most commonly used serum marker to estimate GFR, its use has several limitations. In addition to glomerular filtration, its serum levels are influenced by other physiological processes such as tubular secretion or extra-renal excretion (2, 3). Tubular secretion seems to increase as GFR declines. On the other hand, secretion is inhibited by certain drugs (e.g. trimethoprim, fenofibrate and cimetidine)

(3, 4). Factors affecting the extra-renal elimination of creatinine include the inhibition of gut creatininase by antibiotics and large volume losses (3). The generation of creatinine is mainly driven by skeletal muscle cells, but also influenced by diet. Endogenous creatinine production is highly dependent on muscle mass and correlates with age, sex, body size and race (5).

Creatinine is not a reliable marker for GFR in some clinical situations

False GFR estimates can be obtained in athletes or in patients who are malnourished, with severe hepatic disease or low muscle mass due to muscle wasting diseases or limb amputation (3, 4). While cooking, the creatine contained in meat is converted into creatinine, which can raise serum levels considerably. Creatinine levels can thus be influenced by a high protein diet or creatine supplements (3). GFR estimation is not reliable at non-steady state conditions of renal function as in acute kidney injury (AKI) (3). Finally, at higher GFR levels, estimates are less precise due to higher biological variability in non-GFR determinants and higher measurement error (3). Thus, creatinine can be considered a suboptimal marker in certain circumstances.

Numerous equations for GFR estimation were developed to compensate some of these limiting factors using demographic factors including age, body size and race (6). These equations such as the Chronic Kidney disease Epidemiology Collaboration (CKD-EPI) equation (7) perform reasonably well in many clinical situations, but have their limitations, e.g. in individuals with muscle mass not typical for their age and sex. In addition to the “physiological” limitations, as for every analyte, there exist analytical issues such as interference (e.g. bilirubin), imprecision and bias (3, 4).

Does accuracy matter?

Given the diverse sources of variation, the fraction of creatinine-based eGFR results within 30% of measured GFR (mGFR) ranges between 60-90% (3). In most situations, eGFR is sufficient for clinical decision making. However, there can be circumstances, where it is critical to have a very accurate measure of a patient's GFR, because inaccurate estimates may have adverse consequences (2). As described above, creatinine-based estimates are inaccurate in persons with abnormal levels of muscle mass. This is particularly relevant for chronically ill (e.g. liver disease, malnutrition, neuromuscular disease) or hospitalized patients with reduced muscle mass. In these patients, eGFR systematically overestimates the true GFR which might lead to overdosing of medications or other medical problems. In patients with liver disease, this overestimation might delay the diagnosis of the hepatorenal syndrome or the timely start of therapies and affect decisions on simultaneous liver kidney (SLK) transplantation (8).

An accurate GFR is also important for several aspects of pharmacotherapy such as drug selection, dosing and monitoring of toxicity. As some drugs are contraindicated at low GFR, an accurate assessment of kidney function might be advisable to determine a patient's eligibility for therapy or to select an alternative substance before administration of drugs such as platinum-based chemotherapeutics, iodine- or gadolinium-based contrast agents as well as certain antibiotics, anti-hyperglycemic or anti-hypertensive medications (9, 10). Kidney function is a major determinant for drug dosing. Overdosing drugs with a narrow therapeutic window could lead to toxicity, whereas underdosing could reduce efficacy in treatment (9, 10). Drugs can either be directly nephrotoxic or affect kidney function indirectly. Thus, monitoring for signs of toxicity is important, especially after initiation of agent, change in dose or change in symptoms (2). In the context of kidney transplantation, more accurate determination is crucial for potential living kidney donors and should be considered for monitoring of kidney transplant recipients (2).

Alternatives - cystatin C and mGFR

Thus, additional tests (such as cystatin C or a clearance measurement) are recommended in



*Inaccurate, creatinine-based GFR estimation can be obtained in several groups of people, e.g. in patients with low muscle mass.**

specific circumstances when eGFR based on serum creatinine is known to be less accurate (3). Cystatin C is an alternative endogenous filtration marker. As with creatinine, sources of error in GFR estimation from serum cystatin C remain and include non-steady state conditions (AKI), factors affecting cystatin C generation (race, disorders of thyroid function, corticosteroid therapy) and extra-renal elimination (increased by severe decrease in GFR), measurement error at higher GFR, and interferences with the cystatin C assays (e.g. by heterophilic antibodies) (3). An equation using the combination of creatinine and cystatin C showed higher accuracy and improved the classification of CKD patients compared to an eGFR based on creatinine alone (11). *The Kidney Disease: Improving Global Outcomes (KDIGO) initiative* suggests measuring cystatin C in adults with eGFR (creatinine) of 45-59 ml/min/1.73 m² who do not have markers of kidney damage if confirmation of CKD is required (3).

Measured GFR (mGFR) can be obtained via determination of the renal (urinary) or plasma clearance of an exogenous marker. Urinary clearance requires that the patient's urine is collected precisely over a defined time period, in some cases even via a bladder catheter. During the urine collection period, plasma is

→



Assessment of kidney function for drug dosing is important.

sampled. After quantitating the marker concentration in the urine and blood, mGFR is calculated as the urine concentration of the filtration marker, multiplied by the volume of the timed urine sample, and divided by the average plasma concentration during the same time period. Obtaining accurate urine collections can prove difficult, e.g. in patients with urinary incontinence or retention. This is a major reason for the rising interest in plasma clearance methods to measure GFR. The markers can usually be injected as a single bolus intravenously or subcutaneously. Measurement of the urinary clearance of inulin, an inert fructose polymer meeting all requirements for an ideal filtration marker, is considered the “gold standard” for the determination of GFR (5). However, inulin for human use is currently unavailable in the United States and measurement procedures for inulin are no longer available at most clinical laboratories (5). In addition, in most protocols, the marker needs to be continuously infused intravenously to reach steady-state. Two frequently used markers for measuring GFR are iothalamate and iohexol. An overview of GFR measurement methods and markers is given in the reviews by Stevens and Levey (2) and Seegmiller et al. (5). In summary, mGFR methods are highly elaborate and time-consuming.

There is an ongoing search for both new markers and new formulas to improve estimation of GFR in different patient populations (12). However, a single filtration marker might be unlikely to be successful because of variables other than GFR (5, 11, 12). The use of a panel of markers could improve GFR assessment by reducing errors due to variation in non-GFR determinants of each marker (13).

Novel multi-parametric test on GFR

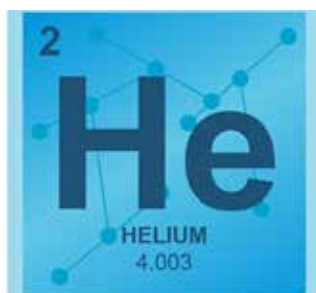
numares developed a novel multi-parametric test based on a metabolic constellation analyzed by *Magnetic Group Signaling (MGS®)* empowered nuclear magnetic resonance (NMR) spectroscopy. This serum test, *AXINON® Clearance Check®*, combines the accuracy of renal plasma clearance methods with the convenience of creatinine-based eGFR and obviates the need for laborious and invasive tracer measurements. The metabolites used in the newly identified metabolic constellation reflect different aspects of underlying kidney pathology, such as metabolic acidosis or oxidative stress. By taking these markers into account, in addition to GFR, physicians gain a much deeper insight into kidney function. This technology is available worldwide for clinical laboratories and available on the European market as CE-marked in vitro diagnostic test. □

Sabine Norkauer, Product Management

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Helium - The hidden star in NMR-Spectroscopy



Helium is the second most abundant element in the universe. Roughly 25% of all existing matter is Helium which was created some 14 billion years ago. The creation process started three minutes after the Big

Bang when the universe cooled down to about one billion Kelvin. It then took only two minutes to create more or less all of this inconceivably large amount of Helium. Unfortunately, it's a rare commodity here on Earth because it simply floats away into space if released. The only source for Helium is the natural decay of radioactive elements deep below the surface. After its generation the Helium accumulates in natural gas cavities to a concentration of up to 7 vol-%. The usual production method is to first separate it from the natural gas and pollutants by fractional distillation and liquefy it afterwards. Liquefying Helium is quite difficult due to its very low boiling point of 4.2K (-269°C, -452°F) which is the lowest of all elements.

In **nuclear magnetic resonance (NMR) spectroscopy**, liquid Helium with its boiling point close to absolute zero is perfectly suited to cool the magnet coil of the spectrometer below the temperature where the wire becomes super-conductive. Superconductivity is needed to generate the strong magnetic field required for NMR measurements. Once charged, the electric current keeps flowing inside the coil as there is no resistance which would lower the current in the coil. Thus, it is very important that the coil is always covered with liquid Helium. When even a tiny part of it becomes warmer than the superconductivity threshold, the coil develops a finite resistance and all

the energy stored in the magnetic field is immediately released as heat. This causes a sudden, nearly explosive boil-off of Helium, which is called a quench. Besides being dangerous, such a magnet quench can be quite expensive as large amounts of Helium are needed to re-cool the magnet or, even worse, the coil gets damaged and the magnet needs to be replaced.



In case of a quench, the room with the spectrometer has to be evacuated immediately as evaporated Helium can displace all oxygen in the room resulting in a high risk of suffocation.

Also, the technical support of *numares* and *Bruker* should be informed as soon as possible:

numares: technicalsupport@numares.com

Bruker: center@bruker.com

In order to avoid quenches due to too low levels of Helium certain precautions are in place. First of all, there are several layers of insulation around the Helium vessel. Secondly, liquid Nitrogen is used to keep as much heat away from the liquid Helium as possible. It is important that the user always monitors the Helium and Nitrogen level and refills as soon as the volume reaches the limit of the specified range. Helium and Nitrogen filling levels and the specified range can be easily checked on the Bruker workstation using the "MICS" software (Magnet Information and Control System).

MICS - BRUKER Magnet Information and Control System

Overview Magnet Helium Nitrogen Service Event Log Notification Settings Help Administration

Magnet

Name: **BZH 102'600'70H** ...

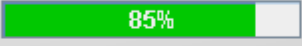

Type: **BZH 102'600'70H**

Field: **600 MHz**

Bore: **54 mm**

Installed: **2013-01-23**

Helium

Level:  **85%** 

Measurement date: **2019-11-14 04:15**

Last refill: **<unknown>**

Estimated next refill: **2020-02-10** *in 87 days*

Screenshot of the MICS - Software from Bruker showing the liquid Helium and liquid Nitrogen levels.

The table below shows some important facts for the "Bruker Ascend™ 600" magnet used in our AXINON® Systems.

Helium vessel total volume	90 liters
Helium refill volume	58 liters
Helium evaporation rate	0.4 liters per day
Helium hold time (maximum)	150 days
Helium refill (including amount for cooling used equipment)	80 liters (every 3 month)

When it comes to helium filling, two points are important to consider:

- Due to the low temperatures of liquid Helium even Nitrogen and Oxygen immediately begin to condensate.
- Therefore, it is important to use special, vacuum isolated transfer lines and to make sure the Helium Dewar is not opened longer than five seconds to the atmosphere during the procedure. □

Michael Grasse, Head of Service USA



Bruker offers a **Helium filling service** as part of their service contracts that can also help to deal with Helium shortages on the market.

If you need more information, please contact us or Bruker.

numares: technicalsupport@numares.com
Bruker: center@bruker.com

numares elects Kathy L. Bates as new member of the supervisory board

numares appoints Kathy L. Bates, MBA, as new member of the Supervisory Board with immediate effect. Ms. Bates is Senior Director, Laboratory Services and Partnership Development at Mayo Clinic, in Rochester, Minnesota.

“We are delighted to welcome Kathy to the board,” says Dr. Volker Pfahlert, Chief Executive Officer of *numares*. “As we continue our focus on North American expansion, the depth of knowledge and relevant experience in the US healthcare will be invaluable to *numares*.” Ms. Bates is the first member of the Supervisory Board from the US.

After starting her career in 1994, Ms. Bates completed various leading positions at *Mayo Clinic* and its partnership organizations. Her competence profile is supplemented by a large pool of experiences serving on the board of directors of *Oncospire Genetics Inc.* and on a Scientific Advisory Board for *Roche Diagnostics Switzerland*.

Since 2005, Kathy Bates is Senior Director, Laboratory Services and Partnership Development at *Mayo Clinic*, leading technology evaluations and strategic business development activities in laboratory diagnostics.

Collaboration on nuclear magnetic resonance

Recently, *numares* and *Mayo Clinic Laboratories* announced their collaboration to develop new diagnostic tests using the “*numares* model” – meaning a distinctive nuclear magnetic resonance (NMR) technology, to identify metabolite constellations for diagnostics.

At the annual general meeting Ms. Bates was elected to follow Dr. Gerd Grenner, who supported *numares*



Kathy L. Bates, MBA. Senior Director, Laboratory Services at Mayo Clinic
Picture: Mayo Clinic

as member of the Supervisory Board since 2014 and left his position due to private reasons.

The other Board Members, Dr. Schirmers, Dr. Ries and Dr. Kutzner were reelected. Dr. Bernhard Schirmers, Chairman of the Supervisory Board, thanked Dr. Grenner in special recognition of his many years of commitment for his achievements on behalf of the Supervisory Board. □

Julia Hertlein, Public Relations

University Hospital Regensburg and numares present results for detection of acute kidney transplant rejection based on a simple urine test

Early non-invasive detection of kidney rejection after transplantation was the central aim of a collaboration between Prof. Dr. Bernhard Banas, Chairman of Nephrology at the *University Hospital Regensburg* (UKR) and the medical diagnostics company, *numares*. The results of their joint clinical trial "UMBRELLA" were just published in *EBioMedicine* and will be presented right now at the *American Society of Nephrology's* "Kidney Week", November 5-10, in Washington DC.

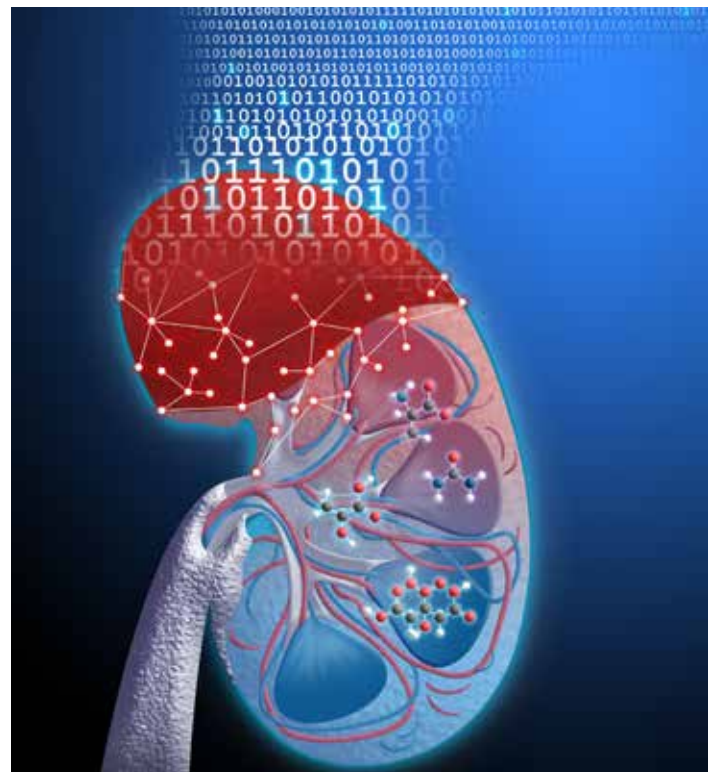
Last year, in partnership, UKR and *numares* published a joint method article¹ describing a test for graft rejection based on clinical samples from UKR and the unique machine learning biomarker discovery platform pioneered by *numares*. Urine samples from patients with and without rejection were measured with nuclear magnetic resonance spectroscopy (NMR) and subsequently analyzed by machine learning algorithms in order to identify a metabolite constellation that facilitates diagnostic decisions.

The current publication in *EBioMedicine* titled "A urinary metabolite constellation to detect acute rejection in kidney allografts"² presented the results of a clinical validation study of the metabolite constellation, marketed by *numares* as *AXINON® renalTX-SCORE®*. The researchers analyzed almost 1,000 urine samples from 109 patients, which were collected over a period of one year after kidney transplantation. Afterwards, they were evaluated against kidney biopsies. The biomarker constellation was able to detect rejection up to one week prior to

clinical symptoms in some cases. Overall, the study established a promising diagnostic performance.

Early warning system allows to react quickly

"We are pleased to publish the results of our work and to present them in Washington DC. We reached a true milestone on the way to a completely new rejection diagnostics and hope to successfully bring



The results of the "UMBRELLA" study were published at "Kidney Week" in Washington.

the *renalTX-test* into clinical routine practice together with *numares*", says Prof. Dr. Banas, Chairman of the *Transplantation Center Regensburg* and President of the *German Transplantation Society*. Dr. Miriam Banas, private lecturer and lead author of the study adds: "*numares*' allograft rejection test has several advantages. First, it allows close non-invasive kidney allograft monitoring. We get an early-warning system and can react quickly, e.g. by optimizing the therapy. Second, we naturally try to reduce the number of unnecessary biopsies with appropriate diagnostic support." *AXINON® renalTX-SCORE®* is available in the European Union as a CE-marked in-vitro test for use in clinical routine.

"We would like to thank the UKR and Professor Banas and his team for the successful cooperation", says Dr. Philipp Pagel, Chief Medical Officer of *numares*. "It is important for us to give a positive example of a cooperation between hospitals and industry. Regensburg is a great location for this kind of collaborative work." *numares* was founded in 2004 as a spinoff from the *University of Regensburg* and is located in *BioPark*, just one kilometer away from the UKR.

Next study already started

Based on the success of this collaborative partnership, UKR and *numares* have already taken the next step: The PARASOL study is a pan-European, multicentric, prospective observational study that evaluates the test in an extended setting. In addition to Regensburg, the partners participating in the study are located in Vienna, Prague, Grenoble and Barcelona. By the end of 2019, already more than 1,000 patients will be recruited. Initial results are expected in the first quarter of 2020. □

Julia Hertlein, Public Relations



Prof. Dr. Bernhard Banas, Chairman of Nephrology at the University Hospital Regensburg

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doi: [10.1016/j.ebiom.2019.10.007](https://doi.org/10.1016/j.ebiom.2019.10.007)

numaRacers at their best



numares' running team, from left to right: Doris Zeugner (Product Management), Christian Karger (Product Management), Andreas Faltermeier (Software Development), Emily Agasa (Finance), Eric Schiffer (Clinical Development), Veronika Bobinger (Medical Affairs), Andreas Wolf (Software Development / IT), Torsten Winkler (Product Management), Floxie van der Sterren (HR).

numares is dedicated to supporting our local community. Since 2013, the Landkreislaf event has been an integral part of our community involvement. This past Sept. 14 the *numares* running team "numaRacers" took part in the regional cross country trail competition "Mittelbayerische Landkreislaf."

Since 2013, participation in the "Landkreislaf" event has been one of the highlights of the *numares* calendar. Every year the team prepares meticulously, trains together and cheers each other on to success.

The "Landkreislaf" is run through the beautiful Regensburg area. This year's route was 73 km long and divided into 10 relay sections of different distances and difficulties, so there was a suitable route for each type of runner. The race started at 9 o'clock in a small village called Aufhausen with numaRacer Christian Karger. After 9.6 km and an incredible time of 38:32 minutes

he handed the baton over to his *numares* team mate. Finally after 9 baton changes, *numares'* last runner Felix Klinger reached the finish line in a time of 6 hours and 32 minutes, which secured a historic best result for the *numares* team. The *numaRacers* finished 52nd of 125 competing mixed teams.

Congratulations to a great team, thanks for all your effort and we are looking forward to more of this great teamwork next year.

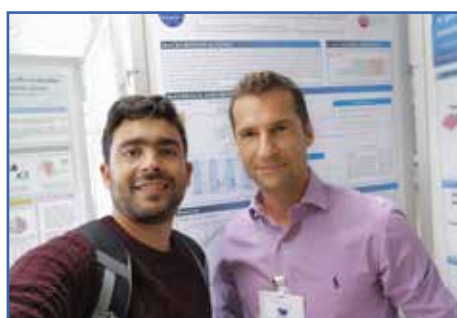
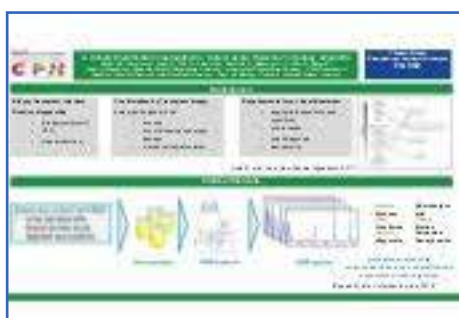
The income generated by the entry fees is donated for a good cause. This year the money was given to a project which supports terminally ill people in fulfilling their last wishes (for example visiting the concert of their favorite band or taking part at a family reunion) by providing medical transportation and personnel necessary for these special occasions. □

Doris Zeugner, Product Management

#numares@Social Media

Welcome to the social media world of *numares*! By following the LinkedIn ([.com/company/numares-ag](https://www.linkedin.com/company/numares-ag)) profile of *numares*, you will get a continuous flow of

news around our products, developments and the company itself. Here is an excerpt from the latest news:



numares' clinical cooperation partner *University Hospital Regensburg* presented results of the joint prospective observational study *UMBRELLA* at this year's congress of the *European Society for Organ Transplantation (ESOT)* in Copenhagen.

The developed risk score detects acute allograft rejection after kidney transplantation by a novel, **NON-INVASIVE** diagnostic test (CE-marked) based on metabolomics.

If you missed the talk, get the paper here: <https://lnkd.in/dqtjVzk>.

Every patient deserves the best and fastest treatment based on a diagnosis that provides reliable data from individual and comprehensive status acquisition.

numares is at the *26th Meeting of the European Section of Urological Research (ESUR)*! Our colleagues Marouane Kdadra and mentor Eric Schiffer are on site in lovely, sunny Porto! Marouane shares his research results about "Assessment of prostate cancer aggressiveness using metabolomics evaluation of urine by NMR spectroscopy" in the ESUR poster session.

As Ph.D. candidate of the TransPot program he is working on biomarker discovery for prostate cancer, doing fundamental work for the development of novel diagnostics options based on metabolite constellations, Machine Learning and NMR. Get to know *numares* and our efforts in Cancer Research to create novel diagnostics here:

https://lnkd.in/d-Xe_QE

numares is in *Newport News*! We are working on the distribution of the *AXINON®* system, offering clinical laboratories access to diagnostic testing based on metabolomics and *Magnetic Group Signaling (MGS®)*.

The tests are evaluating a disease-specific metabolite constellation – a combination of several biomarkers in blood or urine to address diagnostic questions in the areas of cardiovascular disease, kidney disease, kidney transplant, multiple sclerosis and cancer. Learn more at

<https://lnkd.in/gGMf9EW>



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