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To whom it may concern

Bratislava, 13th August 2017

Expert opinion report:

I hereby declare and confirm that I received a detailed written and verbal explanation of the matter regarding the investigation of possible anti-doping rule violations of Mr. Matej TOTH, born on 10.02.1983, a Slovak elite athlete, ABP P111K34. I have received advice and help from assoc. prof. Peter Celec MD, Dipl Ing., Dr. Rer. Nat., DSc., MPH, head of Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University in Bratislava, Slovakia and Dr. Michal Raab, data scientist, HighChem, s.r.o., Bratislava, Slovakia. I confirm that I have not entered into any arrangement where the amount or payment of my fees is in any way dependent on the outcome of the case.
Here, I would like to express my personal opinion.

Sample 7

Even though sample 7 was not considered as the main suspicious feature of the ABP P111K34 in the Joint Expert Opinion dated 8.6.2017, for the sake of clarity some arguments will be given.

The determined concentration of HB for sample 7 (16.9 g/dl) cannot be viewed as a deviation of the individual's haematological profile since it is equal to the upper limit of the expected pattern (16.9 g/dl) and does not, therefore, exceed the range of variation. In addition, the upper limit of the statistically estimated range of variation (the expected pattern) in the Athlete Haematological Passport (ABP) at the time point of sample 7 is the lowest value (16.9 g/dl) in more than a 5-year period (13.8.2009 - 16.5.2015), and can be considered a statistical outlier. A second value determined by a different laboratory could provide more clarification, however, this is not available. In addition, a single HB concentration value prohibits an analytical error estimate or an inter-laboratory interval estimation, such as a confidence interval.

Since it is not known what analytical error has been used in the ABP software to predict the adaptive model, one can only speculate about it. The Laboratory documentation package from the Swiss Laboratory for Doping Analyses (sample 529326) dated from 01.02.2017, exhibits the instrument's calibration variation between 0-0.2 g/dl (Annex N° 8 Check assay sheets - Level 2, e-CHECK XE ASSAY SHEET, Lot No. 11990811). It is standard practice in quantitative analysis to take into account the individual measurement variance for every data point. Utilising the observed instrument's calibration variation of 0.2 g/dl, the HB concentration of sample 7 is well within the expected concentration range.

In addition, sample 7 was collected in the morning, and this fact may contribute to its high HB level based on the findings of the diurnal pattern of Matej Toth presented later in this report.

Sample 20

The experimentally determined concentration of HB for sample 20 in ABP P111K34 was flagged as atypical. The interpretation of values resulting from a statistical model incorporating haematological laboratory results requires careful evaluation and the close scrutiny of several important factors relating to both a single HB concentration and to an observed long term pattern.

Pre-analytical and analytical errors cannot be ruled out

In relation to the range of variation (the expected pattern), the HB concentration of sample 20 is the only outlier in the entire ABP time frame, exhibiting 5% difference between the reported value of 13.5 g/dl and the lower threshold of 14.0 g/dl. In terms of quantitative analysis, a single concentration outlier emerging from a 7-year non-equidistant sampling can be classified as a suspect measurement, and it is very unusual to draw serious conclusions based on a single value without conducting a confirmatory test at an independent analytical facility. Since no other haematological value examined according to WADA ABP Operating Guidelines lies outside the expected range, except a single off-score that is derived from the HB value, from an analytical chemistry standpoint a single HB outlier must be viewed as a suspect measurement that may be attributed to human or instrument error. The official files from IAAF Results Management as sent to the athlete demonstrate that minor human errors are unavoidable. Based on the Lab documentation reports, the file names of samples 17 and 19 that encode the sample number, sample code and date of test (17_ABP_LDP_181465_02022016.pdf and 19_ABP_LDP_181765_27042016.pdf) have been mixed up.

A single concentration value in a haematological profile is inadequate evidence of blood doping

About 20 years ago, new scientific fields emerged that have revolutionised our understanding of the biochemical process in the human body: proteomics and metabolomics. With advancing analytical techniques, proteomic and metabolomic analysis has enabled us to study differences or changes in protein and small molecule concentration patterns of various compartments, including blood. Under several circumstances, a single atypical haematological value resulting from multi-year time-course measurements of biological samples, even if obtained within a subject, is an insignificant predictor of an abnormality or causality regardless of the type of study, especially for an endogenous substance such as haemoglobin which is involved in complex biochemical cascades[1]. In proteomics and metabolomics, biological data is inherently characterised by high variance from both biological variation and analytical errors[2]. Consideration of the expected variation is essential to ensure that experiments will have sufficient power to address the biological questions being addressed[3]. To define the biological variation accurately and validly, large sample sizes are required[4]. It is, therefore, of

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[1] Sankaran, Vijay G., and Mitchell J. Weiss. "Anemia: progress in molecular mechanisms and therapies." *Nature medicine* 21, no. 3 (2015): 221-230.

[2] Sriyudthsak, Kansuporn, Fumihide Shiraishi, and Masami Yokota Hirai. "Mathematical Modeling and Dynamic Simulation of Metabolic Reaction Systems Using Metabolome Time Series Data." *Frontiers in molecular biosciences* 3 (2016).

[3] Karp, Natasha A., Paul S. McCormick, Matthew R. Russell, and Kathryn S. Lilley. "Experimental and statistical considerations to avoid false conclusions in proteomics studies using differential in-gel electrophoresis." *Molecular & Cellular Proteomics* 6, no. 8 (2007): 1354-1364.

[4] Dunn, W.B., Broadhurst, D., Begley, P., Zelena, E., Francis-McIntyre, S., Anderson, N., Brown, M., Knowles, J.D., Halsall, A., Haselden, J.N. and Nicholls, A.W., 2011. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nature protocols*, 6(7), p.1060.

paramount importance that the method of examination is based on sufficient data points that support the declared outcomes to ensure valid conclusions.

In line with accepted proteomics and metabolomics standards, Sottas et al. (2008)[5] in their central publication upon which the Athlete Biological Passport is based, demonstrate in Figure 2. the longitudinal effects of a prohibited substance on an ABPS profile where a test is considered as positive if a measured value is higher than the corresponding threshold which "happened seven times out of eight" in the given example. In another study[6], a male endurance athlete tested positive to a homologous blood transfusion (BT) based on 13 tests carried out during a four-year period, while indirect markers of blood doping exceeded individual upper and lower limits of the ABP a number of times (2x HB, 5x off-score, 4x RET%, 5x ABPS).

In both cases, a distinct trend of multiple threshold outliers was conclusive evidence for blood manipulation. In contrast to the above, a single HB concentration along with a single off score, derived from the same HB concentration, during the entire ABP P111K34 history is used as the main argument for a blood doping scenario, while all other values, including those of RET%, remain in the expected range. Such a strict measure seems to be not only inconsistent with scientific literature, it goes far beyond the reference publication of "fathers" of ABP.

Applying objective scientific criteria adopted from peer-reviewed literature leads to the conclusion that a single concentration value in a haematological profile is not indicative of an abnormal situation in regard to blood doping.

Training cessation may decrease HB concentration

Plasma volume contributes significantly to variation in haemoglobin concentration in healthy volunteers, and across a variety of disease states[7]. According to the seminal paper of Costill and Fink (1974)[8], cited several thousand times, the mean relative change in plasma volume exceeded 10% after 3 h exercise, leading to HB concentration changes between 0.8-2.2 d/dl, while the highest difference of 2.2 g/dl was observed in two out of six runners.

According to the Joint Expert Opinion, "training suspension is usually associated with plasma volume contraction, mild haemoconcentration and relative HB increase, while HB can decrease as a consequence of the haemodilution which is caused by intense training or prolonged endurance competition". However, with regard to training suspension, Shaskey and Green argue in their extensive review article *"Sports Haematology"*[9] the opposite case. The stress of exhaustive exercise causes an initial volume contraction due to fluid loss, which is then followed by plasma volume expansion. Expansion may be 6 to 25% greater than baseline and the greatest plasma volume increase occurs in elite, endurance athletes following the cessation of training. This observation correlates with Matej Toth's training and HB profile. At the end of the well-documented training cessation period caused by

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[5] Sottas, Pierre-Edouard, Neil Robinson, Martial Saugy, and Olivier Niggli. "A forensic approach to the interpretation of blood doping markers." *Law, Probability & Risk* 7, no. 3 (2008): 191-210.

[6] Giraud, Sylvain, Pierre-Edouard Sottas, Neil Robinson, and Martial Saugy. "Blood transfusion in sports." In *Doping in Sports: Biochemical Principles, Effects and Analysis*, pp. 295-304. Springer Berlin Heidelberg, 2010.

[7] James M. Otto, James O. M. Plumb, Eleri Clissold, Shriya Kumar, Denis J.Wakeham, Walter Schmidt, Michael P.W. Grocott, Toby Richards, Hugh Montgomery "Hemoglobin concentration, total hemoglobin mass and plasma volume in patients: implications for anemia." *Haematologica* Jun (2017)

[8] Dill, D.B. and Costill, D.L., 1974. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of applied physiology*, 37(2), pp.247-248.

[9] Shaskey, David J., and Gary A. Green. "Sports haematology." Sports Medicine 29, no. 1 (2000): 27-38

inflammation and injury, the HB level reaches its minimum (sample 20). After resuming intensive training on 6.5.2016 the HB concentration subsequently increased, well in accordance with Shaskey and Green. A variety of studies using different types of exercise protocol have already demonstrated that all the analytes measured herein suffer some kind of modulation by exercise and the effects of detraining on HB concentration remains in some cases ambiguous since both plasma volume and haemoglobin mass play a role[10].

Complex dependency of the training activity on the HB concentration was probably the notion that led Sottas et al. (2008) to the following consideration: "although both HB and ABPS can theoretically be used to estimate the prevalence, ABPS is a better candidate since this marker offers a higher discriminative power and is more resistant to haemodilution and to strong physical activity".

A comparison of ABP software results show a normal physiological range

To my knowledge, software used for generation of ABP is not publicly available. According to fundamental principles of scientific publishing and according to Good Laboratory Practice (GLP), a gold standard in clinical, forensic, environmental and other application areas designed by OECD decades ago[11], all results must be verifiable. It is not the purpose of this report to discuss prospects of transparent verification of expected HB range predicted by ABP software but an attempt to compare source data with published profiles may provide valuable insights into the normal physiological variations of HB concentrations in elite athletes during prolonged period of time.

Schumacher and Pottgiesser (2011)[12] reported profiles of HB concentration for 5 male athletes (2 soccer players and 3 cyclists) observed over 3 years period calculated using the ABP software with defined upper and lower individual limits for HB concentration. When projecting the Matej Toth's HB profile during the last 3 years (samples 13-24) onto the above HB profiles of 5 athletes in the same time domain (Figure 1.), no deviation of expected profile can be observed. Two out of five expected profiles (athletes 3, 5) fully accommodate Matej Toth's HB profile. For the remaining three profiles (athletes 1, 2, 4), an adaptive HB offset has been applied due to the progressive adjustment of the adaptive model to the initial HB concentrations for an individual athlete. In the published study (Schumacher and Pottgiesser 2011) the specificity levels were set at 99.9%, in ABP P111K34 at 99%. The reference publication of adaptive model (Sottas et al. 2008) displays calculated threshold limit on longitudinal HB data using specificity of 99.9% along with longitudinal HB data of a female elite endurance athlete, aged 31 years, living at low altitude. In other example provided by Sottas et al. (2008), a test was presented as positive since seven values of ABPS were higher than the corresponding threshold returned by the Bayesian approach for a specificity of 99.9% after administration of prohibited substance (rHuEPO) to a volunteer amateur athlete.

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[10] Eastwood, A., P. C. Bourdon, K. R. Snowden, and C. J. Gore. "Detraining decreases Hbmass of triathletes." *International journal of sports medicine* 33, no. 04 (2012): 253-257.

[11] Merz, Wolfgang, and Rolf Wittlinger. "Is good laboratory practice necessarily good analytical practice? Is GLP necessarily GAP?." *Microchimica Acta* 105, no. 1 (1991): 11-16.

[12] Schumacher, Y. O., and T. Pottgiesser. "The Impact of Acute Gastroenteritis on Haematological Markers Used for the Athletes Biological Passport–Report of 5 cases." *International journal of sports medicine* 32, no. 02 (2011): 147-150.

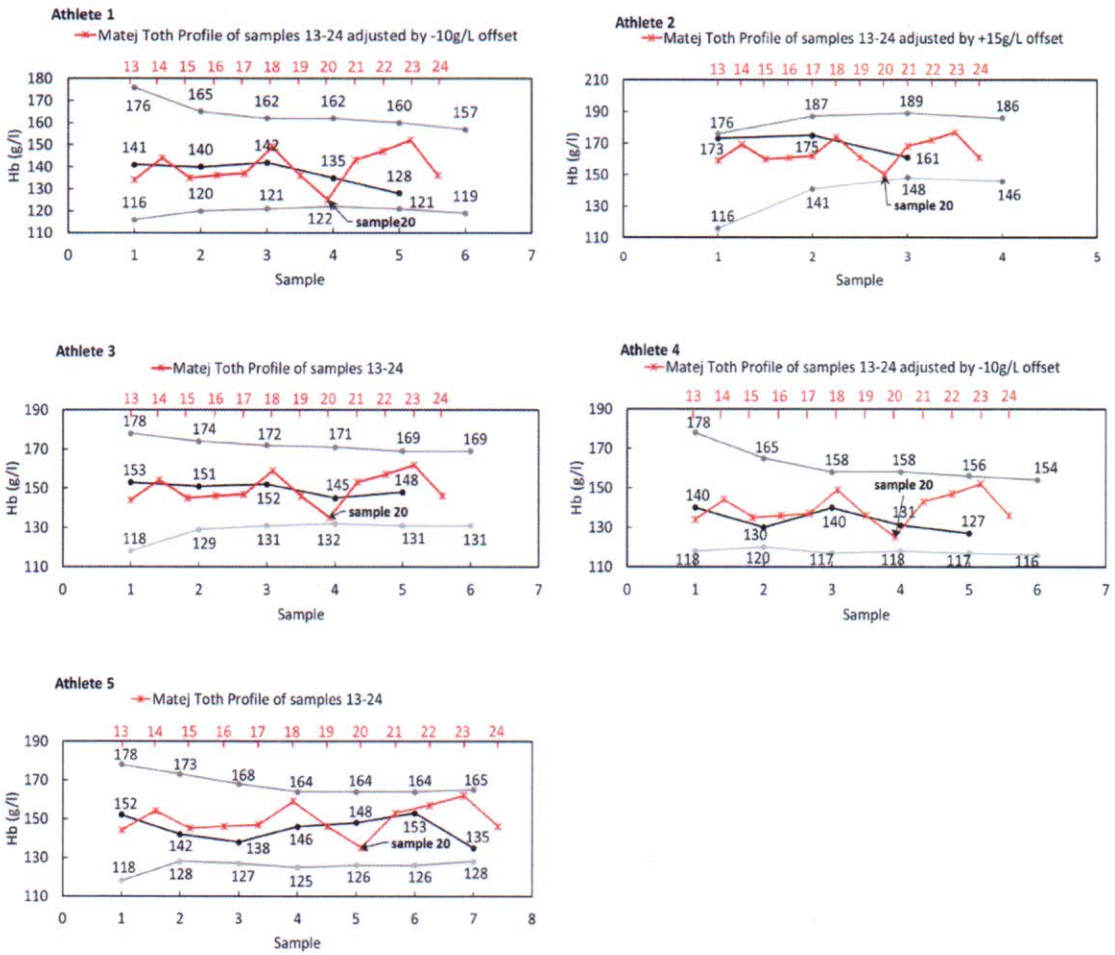

Figure 1. Comparison of individual HB concentration profiles (Athlete 1-5) calculated for each athlete using the ABP software as reported in Schumacher and Pottgiesser (2011) with Matej Toth ABP profile of samples 13-24. The upper and lower individual limit for HB concentration were calculated using specificity of 99.9%.

It is evident from Figure 1. that HB concentration profile of ABP P111K34 lies well within the 99.9% specificity range. WADA imposes specificity of 99% to identify atypical haematological values, however, the proof of doping beyond a reasonable doubt poses a different situation. According to literature studies, specificity of 99.9% is a reliable discriminatory range to recognise normal physiological variation and all of Matej Toth's samples appear to be situated within this range. In the hypothetical case of profile deviation, the chance that this is due to normal physiological variation would be 1:1 000 or less (99.9% specificity). In other words, there is a 1:1 000 chance or less of false conviction if specificity of 99.9% would be applied and if the case would be built solely on HB profile. If specificity is set to 99%, the chance is 1:100. Potential detection of individual athletes abusing blood boosting methods could use typical error and associated probabilities, where a 1:1 000 likelihood of a false positive might be acceptable[13].

[13] Sharpe, Ken, Michael J. Ashenden, and Yorck O. Schumacher. "A third generation approach to detect erythropoietin abuse in athletes." *Haematologica* 91, no. 3 (2006): 356-363.

Sampling outside morning hours accounts for lower HB concentrations

The HB levels in the ABP P111K34 show relatively large but consistent variances around the mean value of 15.2g/dl during the whole period of monitoring that can be attributed to the normal physiological variation. A study on biological variation performed by Nunes et al. (2010)[14] show relatively large intra and inter-individual variations of HB concentration of 56 healthy physically active individuals during a period of 4 months (see Figure 2.). It is very important to stress that this study was conducted following a standard operating procedure. Blood samples were collected after 2 days of rest to avoid the effects of haemodynamic variations and in the morning after 12 hours of fasting, in line with broadly accepted notion to minimise the effect of plasma volume changes, recent dietary intake and diurnal variation. Disobeying some of the generally accepted principles of sample collection may provide sufficient grounds for rejection of the publication in peer-reviewed journals across the biochemical fields since absence of uniformly standardised conditions can easily compromise the integrity of reported results. Ideally, sample collection for longitudinal comparison of athlete's data and for use in the athlete biological passport should hence be standardised, and samples preferably collected early in the morning (Lippi et al. 2013)[15].

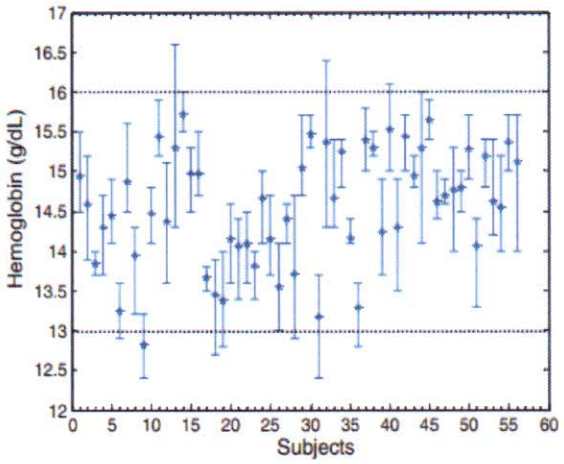

Figure 2. Biological variations of HB concentration for 56 physically active male subjects reported by Nunes et al (2010). Values shown represent the mean, minimum and maximum values for each individual at four time points. Dotted horizontal lines represent the reference interval (2.5% lower percentile and 97.5% upper percentile) obtained with a larger number of subjects (n = 300) from the same physically active population.

Touitou et al. (1986)[16] studied circadian and seasonal rhythms in HB concentration and observed the lowest values at 23:45 h or 03:45 h, the highest at 07:45 h, whereas the largest drop was around 5%. The seasonal patterns of HB concentration revealed that highest drop occurred between January and June (5.9%-6.7%). In another study, mentioned by Joint Expert Opinion, the diurnal rhythm of HB concentration in 24 young males has been shown to range between about 13.7 and 14.5 g/dl, with peaks around 12:00 h and the lowest values around 00:00 h.

To investigate the influence of sampling time on overall HB results in case of Matej Toth, the exact times of sample collection were extracted from his Blood Sampling Forms and statistically evaluated.

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[14] Nunes, Lázaro Alessandro S., René Brenzikofer, and Denise Vaz de Macedo. "Reference change values of blood analytes from physically active subjects." *European journal of applied physiology* 110, no. 1 (2010): 191-198.

[15] Lippi, Giuseppe, Camilla Mattiuzzi, and Giuseppe Banfi. "Controlling sources of preanalytical variability in doping samples: challenges and solutions." *Bioanalysis* 5, no. 12 (2013): 1571-1582.

[16] Touitou, Y., C. Touitou, A. Bogdan, A. Reinberg, A. Auzeby, H. Beck, and P. Guillet. "Differences between young and elderly subjects in seasonal and circadian variations of total plasma proteins and blood volume as reflected by hemoglobin, hematocrit, and erythrocyte counts." *Clinical chemistry* 32, no. 5 (1986): 801-804.

When separating samples collected in the morning (blue in Figure 3.) and outside the morning times (red), a sizeable effect of diurnal rhythm on HB concentrations emerges. A difference in HB concentration between means of morning (15.61 g/dl; n=14) and remaining samples (14.82 g/dl; n=10) amounts to 0.79 g/dl (see Figure 3.). After outlier removal (sample 7 and 20), a difference between means of morning (15.52 g/dl; n=13) and remaining samples (14.97 g/dl; n=9) still yields a considerable value of 0.55 g/dl (not shown).

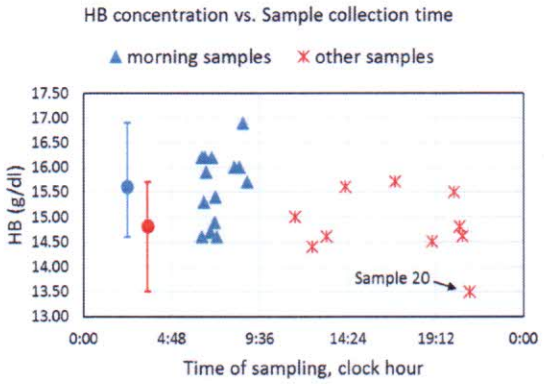


Figure 3. Effect of diurnal variation on HB concentration in ABP P111K34. Samples were divided into two groups, samples collected in morning hours are in blue, remaining samples in red.

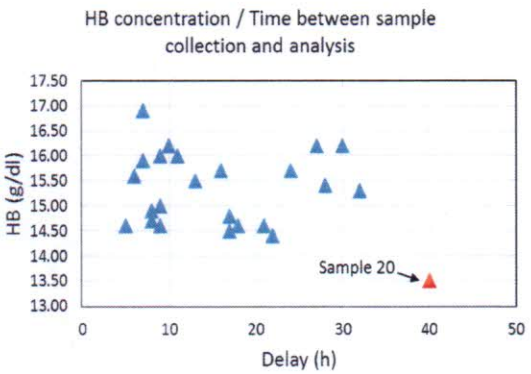


Figure 4. Dependency on HB concentration as compared to delay between blood sample collection. No correlation can be observed, however, sample 20 appears to be a suspect outlier in terms of delay.

An average drop of 0.79 g/dl in ABP P111K34 for samples collected outside morning hours along with cumulative effects of other confounding factors that are hardly predictable but known to lower HB concentration, such as natural physiological variability, potential haemolysis (Figure 4.), haemodilution, inflammation, or detraining, could possibly explain the difference of 0.5 g/dl that separates the sample 20 (13.5 g/dl) collected at 21:04 h from the lower range of expected ABP P111K34 profile of 14.0 g/dl.

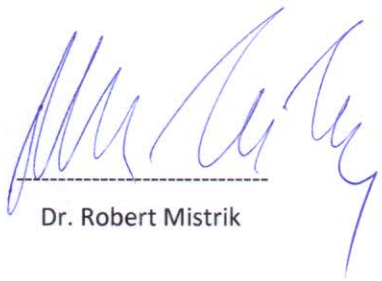
In regard to antidoping tests, Lippi et al. (2013) even argue that "it is hence clear that data obtained on samples collected during the night and especially after eating a meal should be considered unreliable and thereby discarded".

Conclusion

After careful evaluation of analytical, statistical and biochemical aspects, I conclude that available scientific evidence supports the view that data related to ABP P111K34 with associated information is not indicative of blood manipulation and does not prove any type of doping beyond reasonable doubt.

Curriculum Vitae

Robert Mistrik received a Masters degree from the Slovak Technical University, Bratislava, Slovakia in 1991 and a Ph.D. from the University of Vienna, Austria in 1994. Between 1995 and 1997, he held a postdoctoral position at the National Institute of Standards and Technology, Gaithersburg, MD, USA working in the Mass spectrometry data center where he participated in the development of the NIST library. Back in Slovakia in 1998, he founded HighChem, s.r.o., a privately owned company focused on analytical technologies, biomedical research and software development, and since then he has held the position of CEO. He is the founding developer of Mass Frontier software which has been licensed to more than 2,000 laboratories around the world, including leading pharmaceutical companies, universities, forensic agencies and environmental institutions. Together with leading experts in metabolomics he is co-author of SPLASH, a hashed identifier for mass spectra that was recently published in Nature Biotechnology. In 2009, he was awarded the Head of the Year prize, a national award for exceptional achievement in science and technology. Dr. Mistrik was a member of the scientific steering committee in the METAcancer consortium aiming to identify small molecule biomarkers in breast cancer tissue. In 2012, he was elected, and in 2014 re-elected, onto the Board of Directors of the international Metabolomics Society. In 2013 he initiated - and subsequently has led - the development of the mzCloud library, the world's largest database of LCMS mass spectra used for the identification of natural products, human endogenous metabolites, food additives, drugs of abuse, doping agents, environmental contaminants, and other important compound classes. In 2016 Dr. Mistrik and his team launched a project mapping and spectral fingerprinting the small molecules in the human body, which one day may help to decipher the molecular mechanisms of various diseases.



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