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A biomarker is any substance, structure or process that can be measured in the human body or its secretions and influence and/or predict the incidence of outcomes of disease, the effects of treatments and interventions [9]. You may come across many different definitions of the word biomarker, but without a doubt, it sounds like the perfect tool to be used against horrible diseases ruining people's lives. And since details make perfection, each biomarker has a long list of criteria to fulfil: candidate discovery, qualification, verification, research assay optimization, biomarker validation and commercialization [6]. Everything starts with an "Eureka", just a tiny snowball on the top of the mountain and it takes many years, great effort from experienced professionals and even greater amounts of money, before a snowball rolls all the way down and becomes big enough to defeat a disease. But when it comes to biomarkers for rare diseases, things get even more complicated.

The definition of a 'rare' disease varies from one region to another. The prevalence figure accepted in the EU is 5 individuals per 10 000 of the population. In the USA - approximately 7.5 per 10 000. In Japan and Australia - no more than 4.2 and 1.1 individuals, respectively, per 10 000[2]. These numbers separately may seem low, but imagine, if all of the people with rare diseases lived in a single country, it would be the world's 3rd most populous (350-400 million)[2]. Even these numbers would not be that critical, if there were enough knowledge, valuable biomarkers, reliable diagnostic and treatment methods, but since general lack of knowledge makes diagnosis difficult, since around 95% of rare diseases have not a single FDA approved drug treatment, since about 50 % of RDs affect children, 30% of which will not reach their 5th birthday[10], RDs represent a huge threat to mankind.

Another point worth noting is that 80% of RDs have genetic origins and due to vague symptoms are mistakenly attributed to common diseases[10]. That's why searching deeper into the molecular basis of disease and discovering potent biomarkers can lead to better diagnosis and treatment, rather than just analyzing the clinical signs.

If we take a look at the concept of personalized medicine we realize that it has a huge potential to translate research results into clinical practice. For rare diseases which have genetic origins: causative genes, disease-causing mutations, polymorphisms, and phenotypic dynamic markers etc. RNA/miRNAs, proteins, and metabolites that can change over time are all considered valuable biomarkers to identify/characterize the disease as well as it's cellular pathophysiology[8]. Quoting Sun Tzu: "Know yourself, know your enemy, and you shall win a hundred battles without loss," The use of integrated "-omics" technologies (genomics, transcriptomics, proteomics, metabolomics, together called GBs), the measuring of biomarkers in biological samples obtained via non-invasive methods, provide scientists with "screenshots" of real ongoing processes in the body, and can help them know each disease inside out. In the era of whole-exome (WES) or whole-genome sequencing (WGS), in the era of newly discovered i-motif DNA, in the era of nuclear magnetic resonance (NMR), mass spectrometry (MS) and a long list of other informative techniques researchers have nothing to do, but to put the greatest effort possible in understanding the basics of diseases and especially RDs. That's the first step in the battle to defeat RDs.

We can name several examples of beneficial uses of biomarker technology. For instance, 7 candidate biomarkers: BDNF, NrCAM, clusterin, adiponectin, apoE, VCAM-1, and myoglobin have been identified in neuronal ceroid lipofuscinoses by using 3 proteomic approaches. This is a rare, polygenic LSD, which primarily affects children and has no cure yet[3]. But biomarker discovery and better image of pathologic process is already a step forward.

As for duchenne muscular dystrophy (another example of a RD), although CK is commonly used as a biomarker to evaluate the level of muscle damage and necrosis, it is highly variable and is frequently affected by stressful conditions such as exercise. That's why searching for a more stable biomarker has become necessary. Latest research suggests that circulating miRNAs might be useful in monitoring muscular regeneration activity in DMD patients between 2 and 6 years of age[5].

MicroRNSs appear to alter glucocerebrosidase activity in Gaucher disease and as such, are thought to potentially act as modifiers[7]. Lyso-Gb1- a downstream metabolic product of

glucosylceramide, has also been identified as a sensitive and reliable biomarker for GD[1].

Another recent research paper offers measuring lyso-GB3 and hsTNT at least once a year in order to facilitate disease staging[4].

Organic acidemias, amino acid disorders, fatty acid oxidation defects, congenital disorders of glycosylation and lysosomal storage diseases can nowadays be routinely screened for by specific mass spectrometry methods[2].

An important method that can speed up the research process by avoiding wasting time on already discovered facts, is the use of biobanks which meet quality assurance criteria. For rare diseases the EuroBioBank was a first well-established network. Now it's the biobank network of RD-connect, which integrates members from all continents, provides researchers with a number of bioinformatic tools, such as "Registry and Biobank Finder" and "Sample Catalogue". This is a promising new way to easily exchange data across the world and has to definitely be improved in the future.

Obviously advances in technologies mean nothing without professionals who are able to interpret them in a correct and efficient manner. Encouragement and education of researchers is crucial in biomarker identification and development.

Despite the fact that GBs represent informative tools, several complicating factors do exist. Complex disorders caused by multiple genetic and environmental factors are characterized by high population prevalence, lack of clear Mendelian patterns of transmission, etiologic and phenotypic heterogeneity. For example there are three forms of Gaucher Disease and within these forms clinical effects can be manifested in different ways and at different rates. Therefore, prior to widespread clinical application of a GB, multiple scientific and clinical studies must be completed[6].

Turning a biomarker into a sensitive and specific test, or an orphan drug is a bigger headache than anyone can imagine. According to experts, double blind, controlled and randomized trials are pretty challenging to design. Firstly, due to the lack of qualifying patients able to be enrolled in a clinical trial, not only because of the rarity of the disease itself, but also because of geographic dispersion. Imagine a patient from the developing world where lack of professionals or equipment makes conducting a trial unfeasibly expensive and transporting the patient is far too difficult due to the severity of the disease in question. It goes without saying that these are issues which may lead to uncertainties in the data analysis.

On the other hand, as most RDs affect pediatric populations and are rapidly fatal, conducting placebo-controlled trials has become controversial. "They are only rare until they happen to you, or a loved one" – I've read more than once. You don't realize the severity of a condition, before you somehow get in touch with it. Loads of different RD stories have conquered the internet and social media. This all leads to the view that PCTs are in some ways unethical, however we have to realize that placebo groups have a very significant effect on the analytical power of the studies in question and are invaluable to future generations. Obviously risks of those in the placebo arm of these studies should be minimized and if not so, PCT should be the method of choice, when scientists merely have no other way left.

Unfortunately, trial design issues are merely on the top of the list. The estimated costs of new drug development have steadily increased. Commercially, orphan drug costs are disproportionately high, because the expected returns are too low. In 1983, the USA became the first country to introduce orphan drug legislation. This encouraged a number of other countries (Japan, Australia, etc.). In 2000, the European Union joined this process. Nowadays multiple microgrants are available for RD studies, however these may only cover the costs of initial stages of research. The only way to solve the problem of funding is to simply make the process global. International Rare Disease Research Consortium, promoting international collaboration is a promising example. IRDiRC achieved it's goal of developing 200 new treatments in 2017, 3 years earlier than predicted. Next step is 1000 new drugs by 2027.

Last but not least, let me ask this, can anyone imagine liquid biopsy without relevant biomarkers? Can CART cells work without a target? Could CRISPR/Cas-9 be applied without deep knowledge of bacterial and human genomes? No! Biomarkers have made a revolutionary impact in the management of diseases. The time for RDs has come. We are standing on a road leading us to the future of personalized medicine, future of early diagnosis and completed treatment. And we have no right to get lost.

1. Elstein, Mellgard, Dinh, (2017). Reductions in glucosylsphingosine (lyso-Gb1) in treatment-naïve and previously treated patients receiving velaglucerase alfa for type 1 Gaucher disease: Data from phase 3 clinical trials. Mol Genet Metab 122(1-2):113-120.
2. Gülbakan, Özgül, Yüzbaşıoğlu. (2016) Discovery of biomarkers in rare diseases: innovativeapproaches by predictive and personalized medicine. EPMA J. 7(1): 24
3. Hersrud, Geraets, Weber (2017) Plasma Biomarkers for Neuronal Ceroid Lipofuscinosis. FEBS J. 283(3): 459–471.
4. Kramer, Weidemann.(2018).Biomarkers for Diagnosing and Staging of Fabry Disease.Curr Med Chem. 25(13):1530-1537.
5. Li, Zhao, Zhang, (2014) Circulating Muscle-specific miRNAs in Duchenne Muscular Dystrophy Patients Mol Ther Nucleic Acids.3(7): e177.
6. Novelli, Ciccacci, Borgiani. (2008 ). Genetic tests and genomic biomarkers: regulation, qualification and validation Clin Cases Miner Bone Metab5(2): 149–154
7. Siebert, Westbroek,Chen, (2014) Identification of miRNAs that Modulate Glucocerebrosidase Activity in Gaucher Disease Cell. RNA Biol.1(10):1291-300.
8. Strimbu, Tavel, (2011). What are biomarkers? Curr Opin HIV AIDS. 5(6): 463–466.
9. WHO International Programme on Chemical Safety Biomarkers in Risk Assessment: Validity and Validation. 2001.
10. www.globalgenes.org/rarefacts/