

RESEARCH ARTICLE

Soluble biomarkers development in osteoarthritis: from discovery to personalized medicine

Yves Henrotin^{1,2,3,4}, Christelle Sanchez¹, Anne Cornet³, Joachim Van de Put³, Pierre Douette^{4#}, and Myriam Gharbi³

¹Bone and Cartilage Research Unit, Arthropôle Liège, University of Liège, Institute of Pathology, CHU Sart-Tilman, Liège, Belgium,

²Department of Physical Therapy and Rehabilitation, Princess Paola Hospital, Marche-en-Famenne, Belgium, ³GIGA Tower, Liège, Belgium, and

⁴Synolyne Pharma, Herstal, Belgium

Abstract

Context: Specific soluble biomarkers could be a precious tool for diagnosis, prognosis and personalized management of osteoarthritic (OA) patients.

Objective: To describe the path of soluble biomarker development from discovery to clinical qualification and regulatory adoption toward OA-related biomarker qualification.

Methods and results: This review summarizes current guidance on the use of biomarkers in OA in clinical trials and their utility at five stages, including preclinical development and phase 1 to phase 4 trials. It also presents all the available regulatory requirements.

Conclusions: The path through the adoption of a specific soluble biomarker for OA is steep but is worth the challenge due to the benefit that it can provide.

Keywords

Clinical qualification, drug development tool, personalized healthcare/medicine

History

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Introduction

As given by its definition, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention (Biomarkers Definitions Working Group, 2001). Biomarkers are not only essential for the understanding of pathological pathways but also for diagnosis, prognosis and follow up as previously described by Kraus et al. (2011). In addition, they could be a valuable tool in the new era of personalized medicine. They include soluble analytes measured in biospecimens, such as blood and urine, anatomic features detected by radiography and magnetic resonance imaging (MRI), or even functional measurements, such as gait analyses and histological measurements produced as a result of a joint tissue biopsy, such as a synovial biopsy. This paper is focused on soluble biomarkers, specifically to protein biomarkers, and discusses the milestones between biomarker discovery and clinical application.

Biomarkers can provide value at all stages of drug development. Biomarkers may be companion tools for drug

development from *in vitro* screening to phase III clinical trials and also, at the post-marketing stage, for the individual follow-up of response to treatment (Kraus et al., 2015). Therefore, the collection of biospecimens is strongly recommended in all osteoarthritis (OA) clinical trials to determine whether biomarkers are useful in identifying patients most likely to receive clinically important benefits from an intervention; and to determine whether biomarkers are useful for identifying patients at earlier stages of OA in order to institute treatment at a time more amenable to disease modification. In addition, biomarkers might help to select progressors and by this allow reduction of both sample size and duration of clinical trials investigating structural effects.

With respect to the complex nature of the disease, developing new biomarkers in OA represents a major challenge, which requires strong basic research and complementary clinical and regulatory expertise. In such context, it is best relying on a good definition of the intended purpose of the biomarker in qualification and a scientifically sound strategy in order to achieve regulatory/market adoption. For the identification of potentially interesting biomarkers, the discovery process can rely on in-depth understanding of cell biology combined to breakthrough “OMICS” technologies, such as mass spectrometry-based proteomics. Following discovery, efforts have to be made for developing and validating robust assays used to reliably quantify these new biomarkers in complex body fluids. This process is called “validation” and corresponds to verification of analytical performance characteristics (such as precision, accuracy, stability, etc.) as well as clinical correlation of a biomarker with a biological process or clinical outcome. Finally, clinical qualification activities have to be performed in order to

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#Pierre Douette is responsible for statistical design/analysis. E-mail: Pierre.Douette@kiomedpharma.com

Address for correspondence: Yves Henrotin, Bone and Cartilage Research Unit, Institute of Pathology, Level 5, CHU Sart-Tilman, 4000 Liège, Belgium. Tel: +32 424 77 97. E-mail: yhenrotin@ulg.ac.be

confirm the clinical relevance of the biomarker in a particular context (i.e. drug discovery). Qualification is a process applied to a particular biomarker to support its use as a surrogate end-point in drug discovery, development or post-approval and, where appropriate, in regulatory decision-making (Biomarkers Definitions Working Group, 2001). There are many possible qualifying endpoints for an OA-related biomarker including signs (inflammation) and symptoms (pain), structure or functional outcomes in OA. In practice, the qualification process is gradual one correlating changes in a biomarker with change in state of joint. Biomarkers for drug development use can be divided into four categories according to the degree or level that the biomarker can be shown to be associated with the pathophysiological state or the clinical outcome. The four levels are exploratory, demonstration, characterization and finally surrogate level. The use of a surrogate endpoint as the basis for approval of a new drug requires prior agreement with the regulatory agency. Until now, none of the existing biomarker has been considered as a surrogate biomarker. The clinical qualification of biomarkers is a prerequisite in order to better designed clinical trial and to develop efficient therapies (Karsdal et al., 2014; Kraus, 2012).

Existing biomarkers can be categorized according to the OA process targeted as markers of cartilage degradation/synthesis, bone remodeling or synovial tissue degradation/activity. A system first introduced as BIPED by Bauer et al. (2006) and van Spil et al. (2010), and extended to BIPEDS by Kraus et al. (2011) that classify the major types of biomarkers

according their clinical background into six categories corresponding to burden of disease, investigational, prognostic, efficacy of intervention, diagnostic biomarkers and safety biomarkers. In 2011, OARSI/FDA osteoarthritis Biomarkers Working Group has classified biomarkers into four categories (exploration, demonstration, characterization and surrogacy levels) according to their current level of qualification for drug development (Kraus et al., 2011; Wagner et al., 2007). More recently, OARSI RCT Working Group published guidelines for soluble biomarkers assessment in clinical trials (Kraus et al., 2015). This document summarizes current guidance on the use of biomarkers in OA clinical trials and their utility at five stages, including preclinical development and phase I to phase IV trials.

The present review gives an industry perspective of the complex development lifecycle required for regulatory adoption of new biomarkers in the field of OA. It presents the extent of activities and issues associated with OMICS-based biomarker discovery, assay development and validation, and preclinical and clinical qualification (Figure 1).

Biomarker discovery

The discovery of peptide-based biomarker could follow top-down or bottom-up processes. In the top-down approach, the sequences of interest for potential biomarkers are conceptualized using knowledge, computerized methods and/or bioinformatics that allows the prediction at a molecular level of the biological effects occurring during the pathology

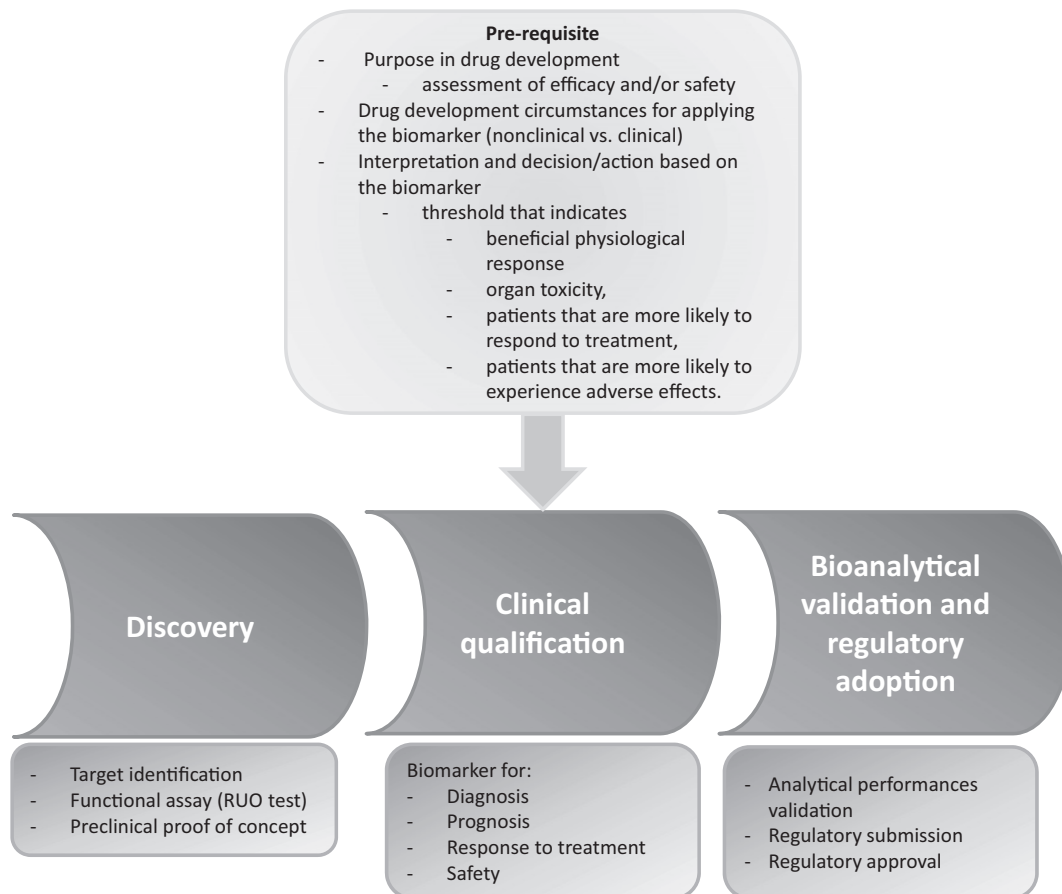


Figure 1. Overview of the path from biomarker discovery to clinical qualification.

progression [e.g. in the cancer field (Baumgartner et al., 2011)]. This approach requires a deep understanding of joint biochemistry as well as of the surrounding tissues (musculoskeletal environment), which contribute to disease initiation and progression (Lotz et al., 2013; Mahjoub et al., 2012). On the other hand, a good knowledge of the pathophysiology of the disease at the molecular level is also required to know which targets are involved and when they act on the joint, leading to a fine cartography of the potential fragments/molecule, which could be used as biomarker (Man & Mologhianu, 2014).

This can be done by epitope mapping of proteins, which is known to be involved in the pathophysiological state being studied. The most popular OA biomarkers (i.e. Coll2-1, C2C, CTX-II) have been conceptualized using this top-down approach. Most of them are epitopes located in type II collagen, one of the most specific molecules of the articular cartilage matrix (Birmingham et al., 2007; Henrotin et al., 2007). Collagen is degraded by enzymatic and non-enzymatic mechanisms in OA. Included in the native form of the parental proteins, biomarkers are very often undetectable but when degradation processes occur, new epitopes become detectable and then can reflect the catabolic level present in the affected joint. Other matrix proteins, such as aggrecan and cartilage oligomeric matrix protein (COMP) have also contributed to get a better integrative view and understanding of the disease (Lohmander et al., 1989, 1994).

For example, C2C corresponds to the c-terminal neo-epitope generated by the collagenase-mediated cleavage of collagen type II triple helix (Poole et al., 2004). Coll2-1 is an amino acid sequence located in the triple helical part of type II collagen that is revealed after triple helix unwinding and cleavage by gelatinases. This sequence shows the particularity to contain one tyrosine, an aromatic amino acid sensitive to nitration, by among others, peroxynitrite (–ONOO). This chemical distinction was used to develop Coll2-INO2, a biomarker of the oxidative-stress related cartilage degradation (Deberg et al., 2005). Coll2-INO2 is well correlated with the c-reactive protein (CRP) indicating that this biomarker could be a marker of joint inflammation (Deberg et al., 2005, 2008).

In this case, the knowledge of the post-transcriptional and/or post-maturation of the proteins has been essential for the identification of such kind of biomarkers. Noteworthy, another limiting aspect of this top-down approach is the field of knowledge in a particular domain at a precise moment.

The second step and probably the most challenging one is the achievement of the concept by developing a functional

bioassay from the theoretical sequence, and especially by using as reference in the immunoassay the peptide or protein fragments as close as possible than the actual form present in the biological fluids.

The proof-of-concept of these top-down biomarkers is achieved by identification and quantification of the biomarkers in biological fluids of representative patient population once the appropriate bioassay has been developed.

In the bottom-up approach (i.e. fibulin-3 fragments), biological fluids of representative patient population (i.e. OA patients) are compared to those of healthy population or of patients suffering of another disease (i.e. rheumatoid arthritis (RA) patients or osteoporosis) using ‘‘OMICs’’ (i.e. proteomic, lipidomic, metabolomic) approaches (Henrotin et al., 2012). For example, comparative proteomic tools, such as two-dimensional differential gel electrophoresis (2D-DIGE) allow the identification of proteins and related degradation products that are significantly modified between groups (Gharbi et al., 2011). Other techniques, such as immunoaffinity enrichment, depletion, protein chip array methods have also allowed the identification and/or the verification of many proteins that are directly or indirectly involved in the pathological processes of OA (Hsueh et al., 2014). The main limitation of this bottom-up approach comes from the limitation of the OMIC techniques, more particularly, the sample processing involving enzymatic digestion and the range of molecular weight or predefined proteins investigated by these methods. A prototype assay is then developed from a small number of samples leading to the proof of concept.

From preclinical proof of concept to clinical qualification

Qualification of the biomarker is related to its meaning – the evidentiary process of linking a biomarker with biological processes and clinical endpoints – while the validation is related to analytical performances of the assay, regardless of particular clinical context.

Four levels of qualification were defined (Wagner et al., 2007). Biomarkers are qualified through their use from *in vitro*/preclinical studies to clinical trials. Preclinical evidence or proof-of-concept qualifies the biomarker at the exploratory level for its use in R&D as development tool. When associated with clinical outcome, biomarker is at the demonstration level. If the demonstration is performed reproducibly in several prospective clinical studies, the biomarker is at the characterization level. Highest level of

Table 1. Intended use of biomarkers in clinical trial.

Intended use	Clinical endpoint	Clinical benefit
Diagnosis	Threshold values related to OA phase (early/late) Burden of disease	Stratification of patients with phase-characterized OA
Prognosis	Intra-subject variation related to OA risk factor	Stratification of patients based on risk of progression
Clinical surrogacy: Response to treatment	Intra-subject variation related to DMOAD treatment	Drug efficacy and compliance, companion
Clinical surrogacy: Safety	Intra-subject variation related to (serious) adverse events	Early signs of toxicity during treatment and drug safety Note: This step requires agreement with regulatory authorities as an FDA registerable endpoint.

Table 2. Five phases of drug development.

Stage of drug development	Purpose
Preclinical	Assess drug safety Assist with selection of animal models and lead compounds
Phase I trial	Assess drug mechanism of action Assessing mode of action Assist with dose finding and selection
Phase II trials	Assess safety via surveillance of effects on joint metabolism Potentially early objective indicator of drug effect Assist in identifying the minimal effective dose and dose response profile Multiple Comparison Procedure Modeling (MCP-Mod) – how modeling and simulation during Phase II can aid dose selection for Phase III clinical trials in OA
Phase III trials	Increase study power through enrichment of an appropriate target population Shorten duration of trial Clinical trial simulations based on biomarkers to gain approval for different dose range not in original trial
Phase IV	Enrichment for progressors to reduce trial sample size and increase power Cost-effective surveillance of safety post-marketing of drug Likely mandated if conditional approval is granted on the basis of a surrogate Identification of subgroup of patients which are responders or non-responders Monitoring of drug effectiveness and safety in real life condition

qualification is surrogacy wherein the biomarker is able to substitute a clinical endpoint. At this time, there are no surrogate biomarkers.

Patient phenotyping is critical to the success of biomarker qualification. OA is a tremendously heterogeneous group of different phenotypes of disease at different joint locations and different combinations thereof. It is possible that biomarkers will perform very differently in these different phenotypes, e.g. markers for early onset post-traumatic knee OA might be very different from valid biomarkers for erosive hand OA.

The subject sample needs not only to be carefully detailed with respect to conventional demographic characteristics, i.e. age, sex, body mass index, comorbidities, etc., but also the targeted OA phenotype, joint location(s) and disease stage depending on study purpose, such as diagnostic/burden of disease, prognosis, monitoring efficacy or safety. As demonstrated by the experience with prostate cancer biomarkers, there is a need for a standardized procedure to qualify new biomarkers to achieve comparability (Schalken, 2010). In addition to comprehensive phenotyping, very precise conditions are also required for sample collection, handling and storage.

Thus, when using a biomarker as a substitute for a clinically meaningful endpoint, one must first be clear about the clinically meaningful endpoint for which the biomarker is a proposed surrogate (Fleming & Powers, 2012). The objectives pursued in such trials should be clearly defined as to demonstrate the clinical benefits in relation to the intended use of the biomarker (Table 1).

For instance, a biomarker qualified for OA progression, and modified by interventions that block progression, might be used as drug development and perhaps someday, drug approval as a chondroprotective agent.

In 2013, the Qualification Process Working Group (FDA) (FDA et al., 2013) has published guidance for the qualification process for drug development tools (DDT). DDTs are methods, materials, or measures that aid drug development including biomarkers, clinical outcome and animal models. Biomarker qualification has been recognized by the FDA as a significant area of interest, either as a single biomarker or as composite biomarker, the latter consisting of several

individual biomarkers combined in a stated algorithm to obtain an easily interpretable readout. The guidance also contains an indication on how sponsor should formulate the so-called “context of use” (COU). These include a “use statement” (the name of the biomarker and the specific purpose for use in drug development) and a description of conditions for the biomarker to be used in the qualified setting that are termed “condition for qualified use” (the conditions for the use of the biomarker in the qualified setting).

Biomarkers could be involved and provide value in OA-related drug development at five levels, from preclinical development to phase 1–4 clinical trials. Table 2 summarizes considerations for each of these phases of drug development and trial work.

In addition to clinical trials intended to show clinical benefits of the biomarker in development, some studies must be set up to evaluate the influence of environmental factors on the reliability of the kit, such as circadian rhythm and seasonality of biomarkers in individuals, the impact of the sampling and sample preparation procedures on biomarker levels in biological fluids.

Consequently the clinical methodology described in the study protocol should address the following elements:

- Sound trial/statistical design and clear objectives.
- Primary and secondary outcomes, including, if soluble biomarkers, the analytical performance of the measurement method and the context of use of the biomarker.
- Representative population (inclusion/exclusion = bias).
- Solid procedures for data collection and biological sample collection and analysis.
- Fully-characterized cohorts and samples.
- Robust statistics and modeling.

In conclusion, from design to acceptance as clinical endpoint, a large number of (pre)clinical trials are needed to support the clinical qualification of innovative biomarkers.

Regulatory affairs and quality concerns around biomarkers including bioanalytical validation

This section presents key regulatory considerations and quality certifications/guidance for use of OA biomarkers in

Table 3. FDA classification for medical device.

	Control level	Related Codes of Federal Regulation (CFR)
Class I low to moderate risk	general controls	21 CFR 860 section 501, 502, 510, 516, 518, 519 and 520
Class II moderate to high risk	general controls and special controls	As mentioned above + device specific controls
Class III high risk	As mentioned above + premarket approval (PMA)	As mentioned above + premarket approval (21 CFR 814, 21 CFR 860 section 513, 515)

Table 4. List (non-exhaustive) of changes in the EC guidance.

Classification	As device class increases from A to D the regulatory controls also increase. In class D evaluation is made by Medical Device Coordination Group. Companion kits are automatically classified in C.
Economical operators	From manufacturers to distributors, they all have legal responsibility in case of non-conformities.
Batch release	A Qualified Person (QP) has to be designated in order to validate the release of a new product lot.
Traceability	Unique Device Identifier (also called UDI code) on each product component.
Post Marketing Surveillance (PMS) and vigilance	Inspired by ISO13485. All severe incidents have to be communicated into a European Portal on the web offering an easy access to everybody.
Notified bodies	Extension of competencies. Unexpected audit frequency depending on product classification.
Clinical evidence	Correlation between test results and patient health modifications is mandatory.

Table 5. Summary of the current guidance on analytical validation processes specific to biomarkers.

Performance	Description
Selectivity/specificity	Is the study of interferences from substances physico-chemically similar to the analyte (cross-reactivity) and the study of matrix effects which should be evaluated by parallelism between diluted samples and diluted standards, which is also called dilution linearity.
Accuracy	Expresses the closeness of agreement between the value that is accepted either as the conventional true value or an accepted reference value and the value found (Kraus et al., 2011). It is also called trueness.
Precision	Expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (Kraus et al., 2011). Precision may be considered at three levels defined here below (repeatability, intermediate precision and reproducibility).
Repeatability	Expresses the precision under the same operating conditions over a short interval of time and is also termed intra-assay precision or within-run precision.
Intermediate precision	Expresses within-laboratories variations: different days, different operators, different equipment, different lots of reagents, etc. It is also called between-run precision or inter-assay precision.
Reproducibility	Expresses the precision between laboratories and is also called beta-test.
Recovery	Is the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method (FDA et al., 2013).
Sensitivity	Is defined as the lowest analyte concentration that can be measured specifically by an analytical procedure. This definition also includes Limit Of Quantitation (LOQ), Limit Of Detection (LOD) and Limit Of Blank (LOB).
Limit of Quantitation (LOQ)	Is the lowest amount of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy (FDA et al., 2013). It is also called Lower Limit Of Quantitation (LLOQ).
Limit of detection (LOD)	Is the lowest amount of analyte in a sample that can be detected and reliably distinguished from the LOB.
Limit Of Blank (LOB)	Is the highest apparent analyte concentration expected to be found when replicates of a sample containing no analyte are tested (Armbruster & Pry, 2008).
Robustness	Measures the capacity of a bioanalytical method to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage (Kraus et al., 2011).
Stability	The chemical stability of an analyte in a given matrix under specific conditions for given time intervals (FDA et al., 2013). It is assessed in several days. Stability evaluations should cover the expected sample handling and storage conditions, including conditions at the clinical site, during shipment, and all other secondary sites.

humans in the scope of product commercialization with a focus on *in vitro* diagnostic (IVD) kits.

In fact regulation requirements for IVD kits are much more stringent than those for Research Use Only (RUO) kits due to their intended uses. Following the FDA definition, IVD Products are reagents, instruments and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. RUO kits are devices not intended for use in the diagnosis of disease or other

conditions. They are intended for use in research or investigations on human samples and they also may be marketed for and used in the research and investigation of other regulated products. They do not need to comply with safety and efficiency regulatory requirements but they all need to be labeled with the mention telling that the intended use is for research only.

International regulatory framework is complex. Classification and regulation still need to be harmonized. Currently, three regions lead the debate and their own

regulations are internationally recognized: the USA (with Food and Drug Administration, FDA), Europe (with European Commission, EC) and Japan (with Pharmaceuticals and Medical Devices Agency, PMDA). IVD products are part of medical devices (MD). Nevertheless IVDs are submitted to a specific regulatory system.

Official attempt for harmonization is led by International Medical Device Regulators Forum (IMDRF), which is formerly called as Global Harmonization Task Force; <http://www.imdrf.org/> (GHTF) and created by representatives from the MD regulatory authorities of Australia, Brazil, Canada, China, the European Union, Japan and the United States, as well as the World Health Organization (WHO). This forum provides guidance for international harmonized good practices in the MD sector.

US regulations

Marketing IVD products in USA requires FDA approval. FDA classifies MD into three categories depending on patient and public health risks. As device class increases from Class I, to Class II to Class III, the regulatory controls also increase (Table 3). Classification procedure of Medical Devices is described in Code of Federal Regulation 21 part 860 (21 CFR 860).

Other codes of federal regulation related to MD have to be taken into account:

- 21 CFR 820 for quality system regulation of MD: Requirements for Good Manufacturing Practices are described in this part. These requirements govern the methods used in, and the facilities and controls used for, the design, manufacture, packaging, labeling, storage, installation and servicing of all finished devices intended for human use.
- 21 CFR 807 Establishment registration and device listing for manufacturers and initial importers of devices: This part provides Medical Device registration procedures (dossier “510k”).
- And many other FDA guidelines on companion IVD, IVD per therapeutic indication.
- European regulations.

Currently in Europe, for biomarkers used as IVD MD in routine, regulation is described by EC Directive 98/79/EC on CE-marking. IVDs are classified into two groups: list A (high risk and including HIV, HTLV I and II, Hepatitis B, C and D) and list B (moderate risk). List B IVDs are marketed following self-conformity assessment while list A IVDs are overseen by notified bodies. The European Commission is about to release new directives, which will make a better matching between IVD and Medical Device regulations while maintaining their own specificities. A list of changes is presented in Table 4. This new directive is submitted to amendments. Then this list represents the current status and is non-exhaustive.

A common part in the US and CE regulation is the establishment of analytical performances of the IVD in order to achieve the device suitability for the purposes specified by the manufacturer. Validation refers to the measurement performance characteristics of a biomarker. Validation of a bioanalytical method is needed to demonstrate that it is reliable and

reproducible for the intended quantitative measurement of the biomarker(s) in a given biological matrix (e.g. blood, plasma, serum or urine). During the development and validation phases, many factors must be investigated to achieve appropriate assay robustness and to guarantee continuous performance. Depending on the technique developed to reliably measure the biomarker, the method used to assess these performances can vary. Table 5 is an attempt to define bioanalytical method performances and is adapted from FDA guidance for industry (FDA et al., 2013), European Directive 98/79/EC on *in vitro* diagnostic medical devices and published articles (Armbruster & Pry, 2008; Kraus et al., 2011), which refer to commonly used definitions in the market.

In addition to regulatory guidelines, manufacturers shall comply with quality standards which are provided by International Standardization Organization (ISO) and well represented by ISO9001:2008 Quality Management System, ISO13485 Design & Development and manufacturing of medical devices, ISO17025/GLP for clinical labs, ISO15189/CLIA for medical labs, ISO 15198 Validation of user quality control procedures by the manufacturer.

Each manufacturer must pay attention to change in regulations. Regulatory intelligence is the process of monitoring the current regulatory environment and anticipating the shape of future regulations, guidelines, policies and legislations. It requires expertise and quality/regulatory oversight.

Conclusion

The path through the clinical qualification, acceptance by regulatory authorities and market of a biomarker is steep. However, the benefit that biomarker can provide in the understanding of the pathogenesis process and in treatment development is worth the challenge. The extensive use of biomarkers in clinical trials could lead to a faster biomarker qualification step. Therefore, it is strongly recommended to collect biological fluid in clinical trials. There is now a panel of biomarkers that can be measured in urine or serum with well validated techniques and demonstrated to be associated with imaging OA features. These biomarkers, even if they cannot be considered as surrogate biomarkers, can be used as drug development tools at all stages. They investigate the effects of a drug on joint tissues metabolism and then are indicative of its biological activity on joint tissue. Besides the symptomatic and structural effects, the metabolic effect of an intervention should be considered, mainly in subject with high risk of OA. Of course, this requires the demonstration that a metabolic response prevents the onset or progression of the disease.

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Declaration of interest

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Royal Canin, Expanscience, AC, JVDP and MG are employees of Artialis S.A.

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