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From biomarker strategies to biomarker activities and back

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The pharmaceutical industry must find ways to improve the unacceptably high attrition rate during drug development. Clearly, pharma has moved away from treat-and-see testing of new drugs in patients, with a strong current focus on generating translational biomarkers early in the research process to enable more predictive evaluation of drug action in clinical trials. Underlying such a translational medicine approach is the intensive search for and use of high-quality biomarkers indicative of successful drug target engagement, pharmacological effects, efficacy or safety. This review outlines our rational question-based drug development strategy in which biomarker data drive decisions on which drug candidates to progress to clinical testing.

The issues at hand

The development of a novel drug is an extremely challenging task. On average, the attrition during clinical development is 90%, meaning that only one in ten projects being pursued through clinical testing in patients is successful (Fig. 1). An analysis of various therapeutic areas has illustrated that there are differences in success rate in different therapeutic areas; drugs treating CNS, oncology and women's health are the most difficult to develop [1,2]. The major cause for this high attrition is the proof-of-concept phase, during which the attrition rate is as high as 80% because of a lack of efficacy and/or unacceptable safety liabilities. This leads one to surmise that preclinical studies in pharmaceutical research are insufficient to predict drug action in patients.

The pressure is now on the pharmaceutical industry to improve this unsustainable situation. Drug developers need to be more successful in avoiding costly late-stage attrition by selecting the best compounds as early as possible. The question at hand is how to achieve this in the context of conventional drug discovery and development infrastructure. This review addresses the potential utility of biomarkers and the requirement that biomarkers are considered by early-stage discovery scientists in conjunction with clinical colleagues to ensure a successful strategy is implemented to guide the clinical development program.

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Biomarker strategies

Translational medicine

In the 2004 white paper 'Innovation or stagnation', the FDA emphasized that 'a new product development toolkit – containing powerful new scientific and technology methods such as animal or computer-based predictive models, biomarkers for safety and effectiveness and new clinical evaluation techniques – is urgently needed to improve predictability and efficacy along the critical path from laboratory concept to commercial product' [3]. The concept of translational medicine captures this ethos, which in pharma is realized by a close collaboration between drug discovery and drug development scientists in a reciprocal interaction. Indeed, discovery researchers need to view successful clinical development as the objective of their efforts, whereas clinical developers need to provide feedback on early discovery regarding clinical needs and possibilities (Fig. 2). Consequently, in early research, when selecting biomarkers to support mechanistic studies in cellular or animal models, one should keep in mind that those selected biomarkers should also be measurable in human samples and/or subjects. Conversely, when designing a clinical trial protocol during drug development, one should consider explorative biomarker approaches that will benefit discovery research through, for example, the collection of clinical biosamples or clinical interrogation of mechanisms targeted in discovery research. Interestingly, such a translational mindset may result in

1359-6446/06/$ - see front matter © 2009 Published by Elsevier Ltd. doi:10.1016/j.drudis.2009.11.004

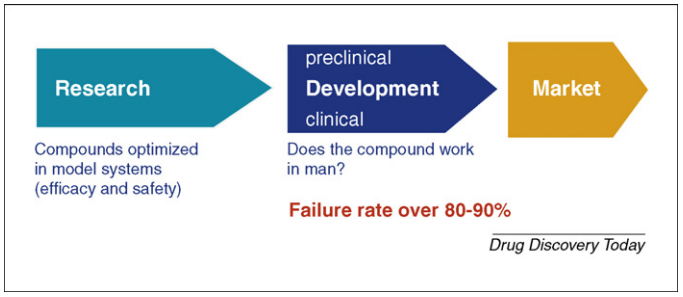

FIGURE 1

The problem at hand: high attrition in clinical development of pharmaceutical drugs.

valuable extensions of biomarker use. Indeed, biomarkers could function as efficacy read-outs in one project and as safety biomarkers in another study [3,4]. In addition, biomarker data can be used to elucidate alternative therapeutic applications and label extensions as a result of drug repositioning discovery [5].

Although this seems straightforward, it is not so easy to implement. The development of a drug from target discovery to market introduction spans on average 14 years and requires many disciplines, including molecular biology, (molecular) pharmacology, chemistry, informatics, toxicology, pharmaceutics, clinical development, registration and marketing, to name but a few. Only very few drug hunters have been exposed to all these stages of the drug development process. Hence, the smooth transition of projects through the different stages of pharma R&D requires that scientists from different backgrounds, but all with a translational mindset, work together to understand the limitations or challenges within each discipline and work as one team to design an optimal trajectory for successfully developing a novel drug.

Question-based drug development

We believe that an important strategy to support translational medicine is to monitor drug action at all crucial stages through a rational and consistent application of key decision-making biomarkers. Although there are multiple ways to classify and apply biomarkers, within Schering–Plough we have chosen a biomarker strategy that is based on translational questions. In this so-called 'question-based drug development approach', a set of basic but crucial translational questions are considered in discovery and a strategy to answer these questions in clinical testing is defined. The answers are provided by biomarkers, enabling data-driven decisions during the development of the drug. The questions in this question-based drug development approach include:

Does the compound get to the site of action? For a drug to work, it should reach its target. Routinely, a pharmacokinetic (PK) analysis of drug and/or metabolite levels in blood is performed, but this does not always reflect the site of action of the drug target. This is particularly true for the development of neuroscience and oncology drugs, in which it is imperative to demonstrate that the compound engages its drug target in the brain or tumor. The use of noninvasive imaging biomarkers, such as positron emission tomography (PET) tracers, has proved useful in assessing drug exposure after dosing [6].

Does the compound cause its intended pharmacological and functional effects? Most drugs inhibit or stimulate their target, with downstream functional consequences. To verify that the drug has the same effect on the pathway in patients as in the preceding cellular or animal models, clinically applicable biomarkers indicative of signaling pathways are required. For example, for a kinase drug target active in blood cells, one can monitor the phosphorylated substrates and/or downstream gene and protein expression patterns. If the drug is found not to modulate its mechanism of action in patients, one can rapidly decide to switch to an improved drug candidate. If the drug does modulate the mechanism as predicted but there is no effect on disease parameters, the concept of targeting this drug target in these patients can be abandoned.

Does the compound have beneficial effects on disease or clinical pathophysiology? Biomarkers that reflect disease mechanisms

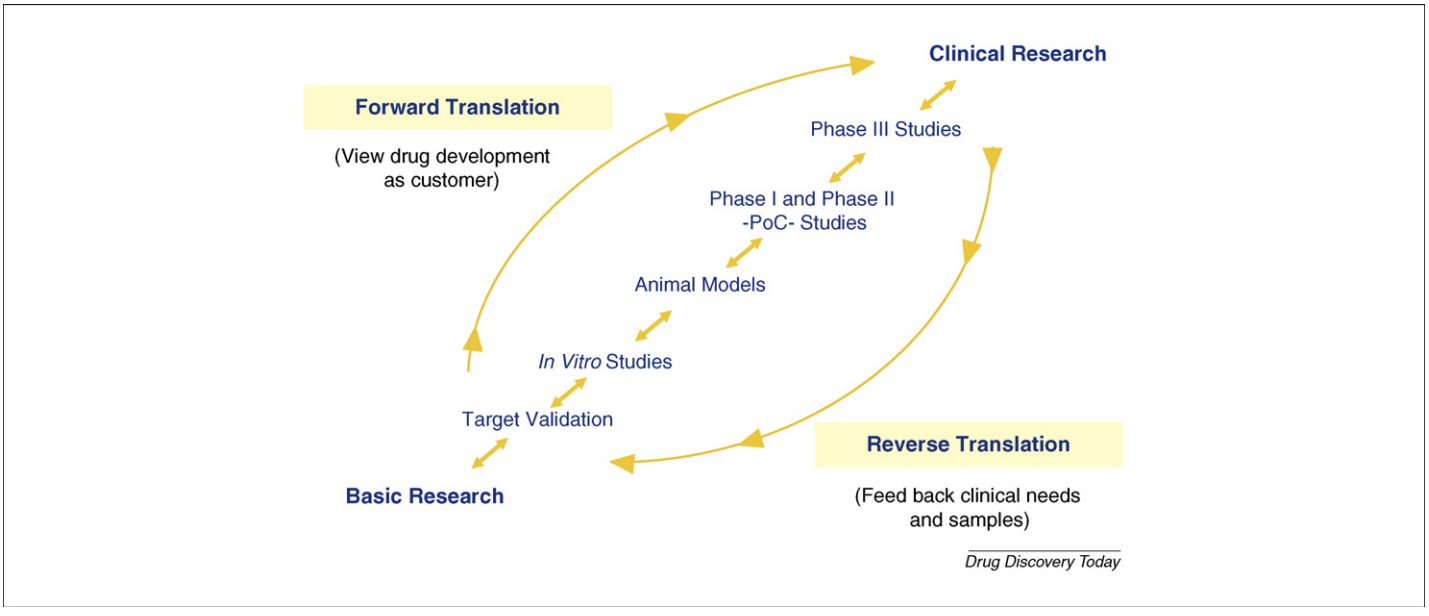

FIGURE 2

A working model for translational medicine in pharmaceutical industry.

underlying clinical pathophysiology are useful tools for demonstrating early efficacy read-outs in a limited number of patients. Such biomarkers would enable early decisions to progress to drug testing in larger patient cohorts. An example of such a biomarker is ^{18}fluorodeoxyglucose (FDG)-PET in monitoring drug efficacy in oncology [6]. Potentially, these biomarkers have the potential to mature into surrogate endpoints, provided there is sufficient evidence from clinical studies to gain regulatory recognition.

What is the therapeutic window (*i.e. how safe is the drug*)? The safety liability of a drug can be the result of two events: toxicity issues related to the inhibition or stimulation of the therapeutic target, or off-target effects of the compound itself. For example, the former can be seen with kinases or membrane receptors when excessive inhibition of the receptor leads to exaggerated pharmacology with downstream adverse or toxic effects [7]. To monitor this, one needs biomarkers that reflect not only the pathway to be modulated but also other pathways that might be affected at high drug levels.

How do sources of variability in drug response in target population affect efficacy and safety? Patient stratification biomarkers have great potential for enabling the preselection of patients who might have a greater chance of responding beneficially to the drug treatment. During clinical drug development, this can decrease the size of the clinical trial, leading to lower costs and faster decision cycles. Ultimately, and after a thorough qualification process, such theranostic stratification biomarkers might enable personalized medicine, whereby patients are advised on specific drug regimens after a biomarker-enabled prescreening.

The emphasis on specific questions will differ for each project, depending on the therapeutic area and mode of action of the drug target. To optimally apply the question-based drug development approach for translational medicine in pharmaceutical R&D, one needs to start asking these questions early in the process. Optimally, this should be done at the start of lead optimization, when a candidate drug is identified that still needs to be optimized in cellular and pharmacological models. The biomarkers used for this assessment should be selected with future clinical application in mind. We have found that the question-based drug development approach helps R&D scientists with different backgrounds to focus on the key issues associated with each project and to define a common biomarker strategy to work on, thus facilitating cross-functional teamwork across projects and therapeutic areas.

The biomarkers used to answer the questions posed above serve mainly for internal decision-making until clinical proof-of-concept is reached (i.e. until demonstration of clinical efficacy in the target population with an acceptable safety risk). Only a few of these biomarkers will continue to later clinical stages as theranostic biomarkers for patient stratification or as surrogate endpoints. These types of biomarkers require a much more stringent level of qualification and a dialogue between the pharmaceutical industry, diagnostic industry and regulatory agencies.

Biomarker activities

A good project's biomarker strategy will indicate the strengths and weaknesses of the available biomarkers for developing the drug in the selected therapeutic area. Implementation of this strategy will result in biomarker activities that focus on either biomarker discovery (if good biomarkers are lacking) or biomarker validation and assay development (if good candidate biomarkers are available). Whether the biomarker of interest is conserved across species that are being used to test drug action is important here because this greatly improves the translational character of the studies.

In pharmaceutical R&D, the focus of biomarkers in pharma is on known biomarkers that are biologically well annotated and reported in literature because these provide a scientific rationale to question-based drug development. Innovation in pharma resides mostly in new drug entities and less in the tools used to monitor their action. By contrast, the biomarker focus is different in an academic environment in which the emphasis is on the discovery of novel biomarkers that can be published. These distinct focal points provide a great opportunity for fruitful collaborations between pharmaceutical, academic and clinical researchers whereby the combination of interests, scientific backgrounds and expertise enables mutually beneficial interactions.

Biomarker discovery

A wide variety of disease-related and pathway-related biomarkers and biomarker panels have been described for various therapeutic areas and drug targets. Part of these biomarkers is currently being applied in standardized clinical tests. When available biomarkers do not fulfil the exact criteria that are needed for a drug development project, additional biomarker discovery efforts are needed. The objective of these biomarker discovery efforts is to enrich panels of known biomarkers with novel validated biomarkers exhibiting higher specificity for the site of action, to ensure that the drug target and/or disease status can be assayed more robustly. Discovery might focus on identifying novel biomarkers or validate reported biomarkers in the model systems used to test drug action.

There are several approaches for identifying, selecting and validating novel biomarkers, including molecular profiling, non-invasive imaging, informatics and pharmacokinetic/pharmacodynamic (PD) modeling and simulation. Here, we review the potential of the first two approaches.

Molecular profiling

The terminology 'molecular profiling' captures the genetics, genomics, proteomics and metabolomics technologies, also known as the 'omics'. In the past ten years, considerable technological advancements have been made in these fields, enabling standardized application of these content-rich profiling approaches in translational medicine.

Genetics, analyzing normal and disease genomes through genome-wide association studies by sequencing and microarrays, has identified many genome variants, including amplified DNA regions, single-nucleotide polymorphisms and insertion–deletions. The presence or absence of such genetic markers and their correlation with disease state has yielded candidate biomarkers that enable the identification of the potential underlying mechanism and, thus, enable the prescription of selective drugs targeting this specific mechanism [8]. The earliest and most well-known example is Herceptin; only those breast cancer patients that test positive for HER2 amplification are subjected to trastuzumab treatment [9]. Tests for several other genetic biomarkers for other diseases and applications have become available and are being applied in clinical research to determine their added value [10].

Genomics, measuring the transcribed genes in the transcriptome, has benefited greatly from the sequencing of the human genome and the standardization of gene expression microarray platforms and downstream bioinformatics [11]. Within pharma, pharmacogenomics has evolved as a major application for biomarker discovery and has delivered decision-making biomarkers [12]. By exposing biological systems (cells, tissues and animals) to different drugs and subsequently analyzing gene expression, drug developers are able to pinpoint genes and pathways that are specifically modulated by some drugs but not by others. These profiles facilitate clustering and (de)selection of candidate drugs in lead optimization, as well as investigative toxicology based on a mechanistic assessment of drug action [13]. Selected genes can be taken forward for validation and subsequent biomarker assay development using an assay format detecting either the transcript itself or the encoded protein [14,15].

Proteomics is an attractive and seemingly more direct approach to the discovery of mechanistic and functional biomarkers. The proteome is more complex than the genome and the transcriptome and reflects protein abundance, post-translational modifications, localization and interactions with other macromolecules in the cell. These aspects often define the homeostasis in a biological system, and proteomic biomarkers directly reflect drug action on such systems. However, this complexity, together with the large dynamic range and the transient nature of protein modifications and interactions, makes it difficult to identify protein biomarkers and develop stable biomarker assays [16]. Consequently, proteomics approaches in pharma have switched from nontargeted top-down approaches, such as gel electrophoresis and mass spectrometry (MS), to targeted approaches, whereby large panels of known candidate protein biomarkers are analyzed using high-throughput multiplex technologies such as protein arrays and multiple-reaction-monitoring MS [17–19]. After validation, there are several assay formats by which the protein biomarkers can be analyzed in (pre)clinical samples; ELISA-based immunoassays are still the gold standard [20].

Metabolomics studies small-molecule biochemicals that result from the activation or inactivation of biological pathways. Several biomarkers that are part of standard clinical chemistry testing, including glucose and cholesterol, are metabolomic biomarkers, suggesting that novel biomarkers that result from metabolomics profiling can be applied directly in clinical testing. In addition, because metabolites are generally very similar across species, metabolomics represents an easily translatable and system-wide biomarker discovery approach [21,22]. Although the quantitative analytics of metabolomics is powerful, based on NMR and MS platforms, the field is challenged to increase the coverage of the known metabolome, both in detection and in identification.

Molecular profiling approaches have successfully yielded candidate biomarkers for application in translational drug discovery, but in as many other occasions they have failed to do so. This success or failure is largely driven by the biology behind the disease and drug action, and in some situations, drug effects on a disease can be observed using one methodology (e.g. genomics) but not another (e.g. metabolomics). In that respect, there is great potential value in parallel molecular profiling analyses and combining datasets through data fusion to yield a more complete mechanistic picture of the biological system under investigation. For instance, by combining pharmacogenomics, phosphoproteomics and analysis of secreted proteins and metabolites, drug researchers can identify drug-selective pathways and derive the most promising biomarkers. Importantly, the discovery and validation of such biomarkers should be performed across different species and disease states to demonstrate the true translatability of the biomarkers. Such a 'systems biology' approach, if supported by a well-defined experimental design, can provide validation of the biomarkers simultaneously with their discovery.

Imaging

Noninvasive imaging is a powerful translational tool for enabling data-driven decisions in both preclinical and clinical drug discovery and development; indeed, imaging was recognized as 'a key technology for assessing, accelerating the development of and guiding the use of new therapeutic options' by the FDA in the Critical Path Opportunities Report 2006 [23,24]. This is further demonstrated by the increased use of noninvasive imaging to support New Drug Applications and approvals of new drugs [25]. As part of the drug discovery and development process, noninvasive imaging biomarkers can be used to provide answers to the question-based drug development approach through evaluation of biodistribution, target engagement, pharmacological/functional effects, efficacy, patient stratification or side-effect profiling.

Given the breadth of imaging modalities, with their application across various therapeutic areas, a full review is outside the scope of this article (the subject has been reviewed extensively elsewhere [6,26]). However, several imaging methodologies are of particular note given both their translational nature and their ability to generate decision-making data within drug discovery and development programs.

Recently, the nuclear medicine techniques of PET or single photon emission computed tomography (SPECT) have seen particular interest from pharma. PET has been predominantly used as a diagnostic tool (e.g. FDG-PET is used to investigate tumor metastasis). Preclinical and clinical PET and SPECT studies, however, are increasingly used by pharma to assist in decision-making. For example, the ability to label small molecules or biologicals with PET or SPECT isotopes to investigate biodistribution or to empirically confirm target engagement provides invaluable PK and PD data. This enables the selection of the most promising drug and the establishment of the optimal drug dosing regimen. Also, these data provide confidence in progressing a compound to a larger scale efficacy study with the knowledge that the mechanism of action or clinical hypothesis in a Phase II or III study will be effectively tested.

Given the potential limitations of nuclear imaging regarding tracer development, magnetic resonance imaging (MRI) or magnetic resonance spectroscopy, which does not rely on the use of a specific tracer, permits the noninvasive determination of structure, function and levels of neurotransmitters or metabolites in both preclinical and clinical studies. Structural MRI has a multitude of uses and, for example, is currently employed in oncology studies to evaluate tumor progression or remission [27] and in neuroscience to investigate the relationship between brain atrophy and disease progression (e.g. in Alzheimer's disease) [28]. It is clear that establishing such baseline data is important before the

deployment of imaging in therapeutic intervention trials. Functional MRI (fMRI) is being employed to interrogate the mechanistic hypothesis or to explore pharmacological/functional effects, efficacy or side-effect profiling. In the field of neuroscience, the use of fMRI to investigate drug responses or to design sophisticated human experimental model systems in volunteers or patients is beginning to impact early-phase clinical studies. Further developments in MRI methodologies (e.g. arterial spin labeling and resting-state MRI) should provide additional MR-based methodologies to aid decision-making in drug discovery programs.

Recent developments have improved the routine deployment of imaging into drug discovery and development projects through increased in-house expertise (bringing together a wide range of disciplines), better access to expensive and specialized equipment (often only available through a small number of academic centers), and improved throughput and timelines associated with inclusion of imaging protocols within study designs. However, as with all biomarker activities, it is crucial to consider early planning of imaging studies to ensure an imaging strategy can be implemented and validated in time, and at the correct phase of the project, to enable the generation of data to drive decisions (e.g. novel PET tracers should be validated before Phase I studies).

Biomarker development

To make decisions in drug development based on biomarker data, robust biomarker assays are required to quantify biomarkers in preclinical and clinical samples. After clearly defining what quality level the biomarker data should have and what kind of a response is expected, a suitable analytical method should be selected and the level of validation should be defined. The analytical method is driven by the nature of the biomarker, and many assay formats can be considered, as reviewed extensively elsewhere [20,29]. Initially, easily accessible commercial tools and methods that generate merely qualitative biomarker data will suffice for preclinical decision-making. Hereafter, there is a requirement for the biomarker assay to become more robust for clinical application and, as such, more extensive method validation has to be performed, following a 'fit for purpose' principle [29]. The recent FDA Guidance for Industry outlined three types of biomarkers: probable valid biomarker, known valid biomarker and valid biomarker; these definitions further help to define the objectives of biomarker assay development and validation toward clinical application [30].

When progressing pharmaceutical drug development projects, the majority of biomarkers used will be exploratory in nature, aiming to support internal decision-making. The biomarker data should be sufficiently reliable to enable this, supported by PK/PD modeling and simulation combining drug bioavailability and multiple biomarker read-outs. Consequently, the robustness and validation of the biomarker assays should be brought to such a level. In all cases, however, the biomarker assay should deliver reliable data that can be used for making data-driven decisions at each stage of the question-based drug development process.

Collaborations

It is clear that high-quality biomarker R&D cannot be performed in isolation. The identification, development and qualification of clinical biomarkers is an ambitious process that involves multiple disciplines and expertise and requires collaboration between several parties.

First, the recent investments in the development of advanced biomarker technologies have created a shift in the way in which such technologies and approaches are accessed and applied in pharmaceutical R&D. Because different biomarker projects require different technology platforms, it is a costly and unsustainable model for most pharma to have all the required high-end equipment and operating knowledge in house, especially in cases in which such technologies are not employed full time. Consequently, in the past decade, many pharmaceutical companies have increasingly outsourced those biomarker activities that depend on high-end technologies, while maintaining a limited internal core of experts to manage the outsourcing process. The external outsourcing partners could be fee-for-service technology providers, academic technology centers or diagnostic companies that execute well-defined and focused biomarker discovery or development activities as needed to support the pharma project.

Second, for larger projects, the participation in multipartner science-driven consortia is attractive because it brings together participants whose combined expertise is the only way to achieve the often ambitious project objectives. Partners in such consortia can include pharma, biotech, diagnostics companies, academia, governmental agencies, device manufacturers and regulators, facilitated by national or international funding programs such as EU-FP6 in Europe or PhRMA in the USA. Consortia project objectives could include the discovery of disease-related biomarkers using various technological approaches or the qualification of candidate biomarkers in larger patient cohorts to enable clinical disease management or progression to surrogate regulatory endpoints.

Opportunities to develop biomarkers as companion diagnostics or patient stratification tools are also areas of great common interest because such biomarkers would enable personalized medicine and improved healthcare. The synergistic collaboration between pharmaceutical and diagnostic industry would provide a mutually attractive model whereby pharma's investment in biomarker discovery could be returned through the licensing of diagnostic intellectual property.

Future developments

There has already been a substantial improvement in the pharmaceutical industry's trial-and-error approach to progressing new compounds to the clinic. Today, much more attention is given for measuring target engagement and pharmacological effects in early clinical trials to make data-driven decisions on drug and dose selection. Nonetheless, there is room for improvement, and future developments should encompass the following: first, an even more stringent application of biomarker read-outs in early clinical trials to prove target engagement and mechanistic drug effects to better assess therapeutic potential and safety of new compounds. Second, the further development of clinical experimental medicine models for different diseases that can predict the efficacy of a new drug in the indication of interest. This will require, in many cases, further in-depth understanding of the pathophysiology of these diseases. Third, increasing interactions between preclinical pharmaceutical scientists involved at the early drug discovery phase and their clinical and translational medicine counterparts.

Fourth, the validation and development of translational models and clinically applicable biomarkers through substantial concerted effort of multidisciplinary consortia. This will benefit greatly from the availability of well-annotated clinical samples, through shared comprehensive clinical biorepositories. And finally, the further development of patient stratification biomarkers. Among others, it is to be expected that deep genome sequencing on an individual basis is coming within reach of use in clinical trials, opening new avenues to patient stratification biomarkers and truly personalized medicine.

Acknowledgements

We thank Terri McClanahan, Eric Gustafson and Jacob de Vlieg for their constructive comments.

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