Methods Towards Clinical Actionability of Next Generation Sequencing

by Valentin Barsan, MD

Precision medicine necessitates continuously measuring the certainty with which the right patient receives the right treatment at the right time. Common clinical presentations are increasingly binned into detailed diagnoses that precede the selection of targeted therapies. These efforts reduce trial-and-error drug selection and dosing, misdiagnoses, and reactive treatments. For a few but growing number of patients, highly sensitive molecular tests such as next generation sequencing (NGS) have enabled genotypic insights into the interrelated molecular drivers of common clinical phenotypes. Various precision medicine initiatives collectively aim to scale biomarker-informed treatment regimens in the context of regulatory modernization and synergistic public-private partnerships. Clinical diagnoses are thus increasingly sub-classified using data available decades earlier than what becomes clinically apparent into categories that portend the course of disease and imply response to treatment. Considerable challenges remain for widespread clinical adoption of this paradigm for patient management. A case of NGS utilized in the intensive care unit illustrates the need for orthogonal measurements in validating molecular assays for actionable clinical implementation.

The development of precision diagnostics and targeted therapeutics requires a population level understanding of disease at the molecular level through broad and orthogonal clinical measurements (i.e. molecular sequencing, protein assays, imaging, etc.). Conversely, the deployment of precision medicine increasingly requires a series of fit-for-purpose, front-line, and iterative assays. This relationship between exploratory development and actionable deployment underscores the interdependent relationship between laboratory-developed tests (LDTs) and in vitro diagnostics (IVDs), respectively. As ever, the diagnostics laboratory variably knows what (combination of) therapeutic(s) the treating clinician is considering while the treating clinician variably understands what (intricate and complex molecular) tests the laboratory is performing (Figure 1). Regardless, clinical volumes continue to drive data production that in turn results in new clinical insights, democratized patient access, and more efficient treatments. An illustrative clinical case in the application of precision medicine highlights the application of fit-for-purpose assays that aid in specific diagnoses and clinical contexts. The lessons learned from this case imply that multiplex diagnostic and targeted therapeutic co-development will continue to enable a dynamic ecosystem in which feedback loops between the laboratory and clinicians may create value from large complex data sets. Next generation sequencing assays offer a unique opportunity for healthcare systems to share, comment, and curate in defining precision medicine. When coordinately measured in standardized fashion, the aggregation of large cohorts of deeply-phenotyped individuals and their genomic data facilitates precision medicine to safely and responsibly translate what was learned in each patient to a much broader audience of physicians tasked with designing treatment plans for similar diagnostic patterns.

A Case of NGS Applied in Intensive Care

An otherwise healthy 2-year-old girl presented to the pediatric intensive care unit in acute renal failure with symptomatic and diagnostic elements of atypical hemolytic uremic syndrome (aHUS). She arrived after sustaining multiple generalized tonic clonic seizures and immediately required neuroprotective sedation, continuous hemodialysis, and intubation for co-management of metabolic derangements secondary to renal failure and diffuse multi-organ microvascular injury. After medically stabilizing the patient's complications from neurologic, renal, and respiratory compromise, the ICU team next sought to explain why her neurologic symptoms were dramatic and refractory to multi antiepileptic medications.

Classic hemolytic uremic syndrome (HUS) is characterized by microangiopathic hemolytic anemia and thrombocytopenia that primarily results in acute renal failure. Diffuse vascular injury inflames and clots multiple organs through the complement-mediated immunogenic and prothombotic effects of shigatoxin, the veratoxin found in Enterhemorraghic E Coli and Shigella. Atypical HUS (aHUS) has been variably characterized as a complex polygenic disease associated with mutations in complement pathway genes that fail to produce or misshape important effector proteins intended for functional regulation of the extent and nature of the activated complement cascade. In aHUS, therefore, unchecked feed-forward immune reactivity may lead to more profound platelet, leukocyte, and endothelial-cell activation and systemic thrombotic microangiopathy.

Suspecting aHUS as the diagnosis in our young patient, our team initiated targeted therapy (eculizumab) intended to suppress her complex feed-forward immune reaction. Given the critical nature of initiating therapy, the medication was started prior to obtaining the results of a next-generation sequencing aHUS panel. Six days later, a (12-gene immune system complement pathway) NGS test revealed a novel

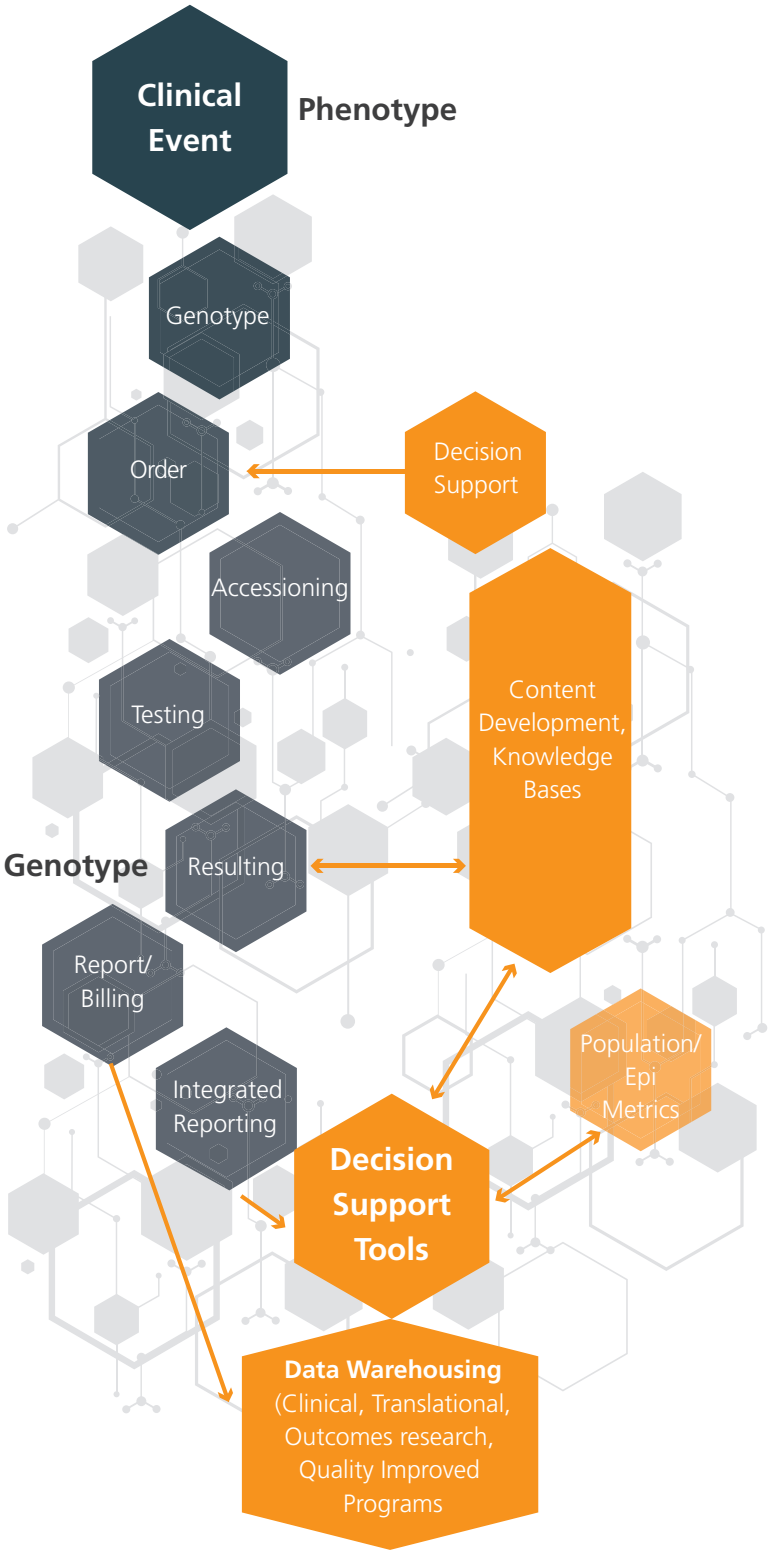


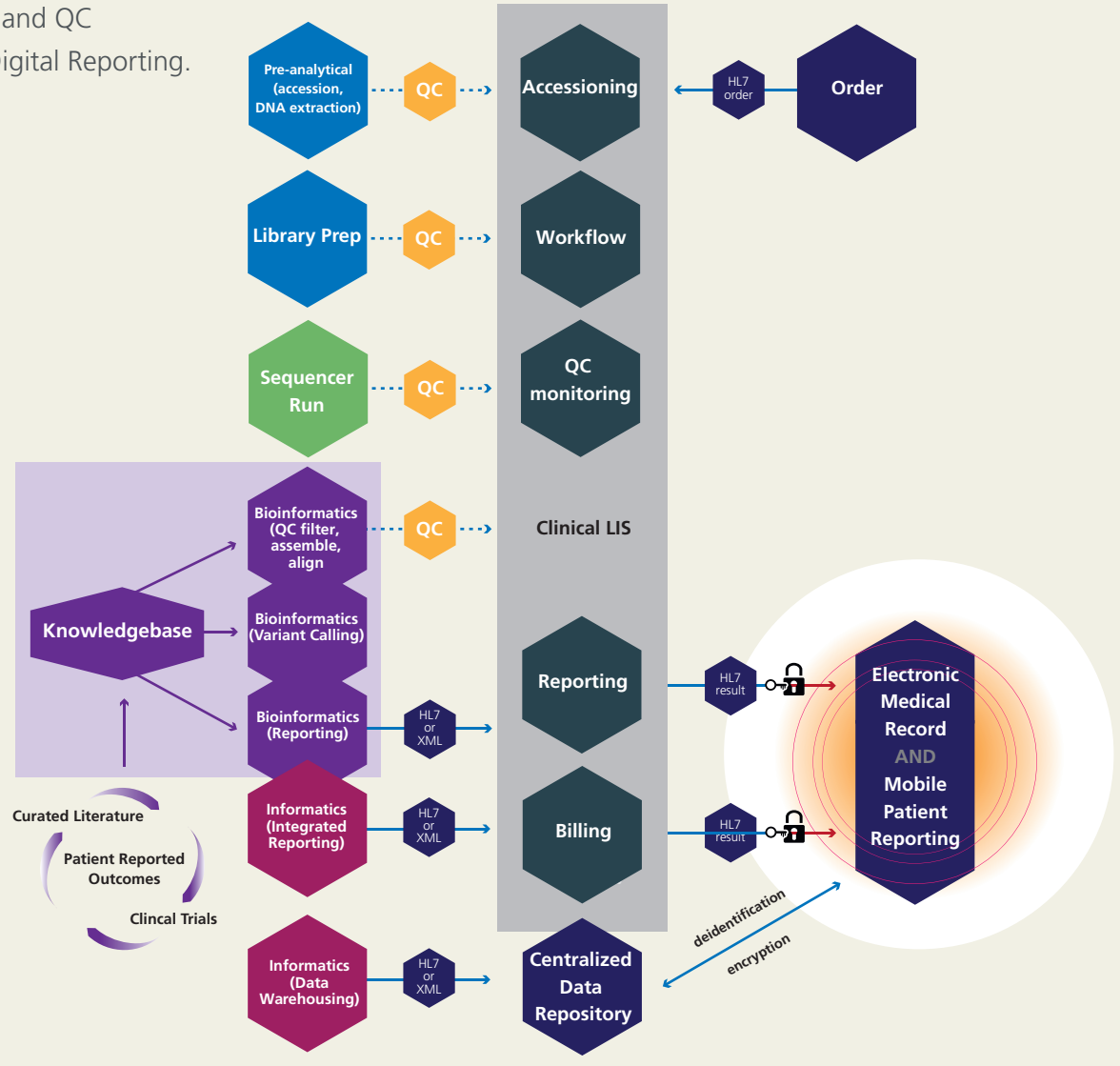
Figure 1. Implementation of Molecular Diagnostic Reporting.

heterozygous mutation (c.3358AT, p.Ile1120Phe) in the promoter region of complement factor H (CFH). More than 100 different mutations of CFH have been identified in adults and children with sporadic or familial HUS (1) suggesting a defect in the protection of endothelial cells favoring complement system activation and autoimmune collateral damage. Yet we remained unclear as to what degree this novel variant in a genetic promoter region explained our patient's clinical symptoms. We acknowledged that mutations in CFH are the most frequent genetic abnormality in aHUS patients as they account for 20 to 30% of cases (2, 3). We further intended for early initiation of eculizumab to presumably impart protection of host cells by inactivation of the complement system whose potentially-mutant CFH suggested an unchecked prothombotic state. But we were unable to validate this theory. Moreover, the targeted therapy chosen required life-long administration despite known immunosuppressant side effects and exorbitant six figure cost.

Was NGS Helpful?

Clinically, the feed-forward amplification of thrombotic microangiopathy in genetically sensitized patients implied that early recognition of aHUS and immediate hemodynamic and monoclonal therapy correlated strongly with our patient's prognosis. Diagnostically, however, the novel variant discovered left questions unanswered regarding the true correlation between genotype and phenotype. Functional studies exist to analyze the interaction between CFH and its negative regulation of ligands (C3b, glycosaminoglycans, heparin and endothelial cells) (4-6). Some mutations in CFH (type 1 mutations) are associated with a quantitative deficiency in CFH (decreased CFH plasma levels), but many variants are associated with normal plasma levels of CFH, the mutant CFH being functionally deficient (type 2 mutations). Measurable by antibody assays, plasma C3 levels (in fact normal in our patient) are decreased in 30% to 50% of patients with heterozygous mutant CFH, yet C3 plasma levels may be decreased while CFH level is normal and vice versa (7-9). However, functional proteomic assays are not yet standardized or clinically validated.

Figure 2. Security and QC Components of Digital Reporting.



One must still consider how CFH quantity or quality as measured through such assays relates to spatiotemporal variation of cellular proteins throughout the clinical course. The in vivo immune cascade conditions in which CFH operates are technically challenging to replicate through in vitro assays. Similar challenges in clinical validation of inducible biomarkers have been well recognized by the immuno-oncology community as PD-L1 expression variably correlates to patient response to checkpoint inhibitors. Fundamentally, expression-based biomarkers operate along a biologic continuum and are therefore not "present" or "absent." Accordingly, the parallel developments of increasingly sophisticated histological and gene expression techniques have resulted in the separation of imaging from transcriptome analysis. Gene transcripts represent a proxy for protein abundance in proportion to the rates of RNA translation, degradation, and modification that variably correlate with the amount and quality of protein produced from any one transcript. Assays such as reverse phase protein arrays (RPPA) are used to quantitate known proteins and phosphoproteins. NGS assays that merely identify novel variants have limited clinical actionability in evidence-based practice and necessitate additional genomic counseling regarding implications to patients.

NGS and other molecular clinical reports are generated to provide guidance to the ordering physician in optimizing each patient's individualized care analogous to how interpreted radiographs and imaging studies provide but one perspective of the holistic clinical presentation. Molecular reports are thus professional consultations that interpret the observed data in the context of the patient's medical condition, the analytical performance of the assay, and the qualities of the analyzed sample. The digital nature of molecular variants detected does not obviate the downstream clinical need for straightforward conclusions and therapeutic recommendations, where possible. Recognizing where confounders render molecularly-informed recommendations less conclusive, both the issuing laboratory and the treating physician may struggle to convey implications of NGS tests to patients. Actionable NGS reporting must therefore convey the analytic and clinical nuances of results so that reports are not misinterpreted as diagnostic finality when truth depends on a plurality of measurements.

The Need for Orthogonal Measurements

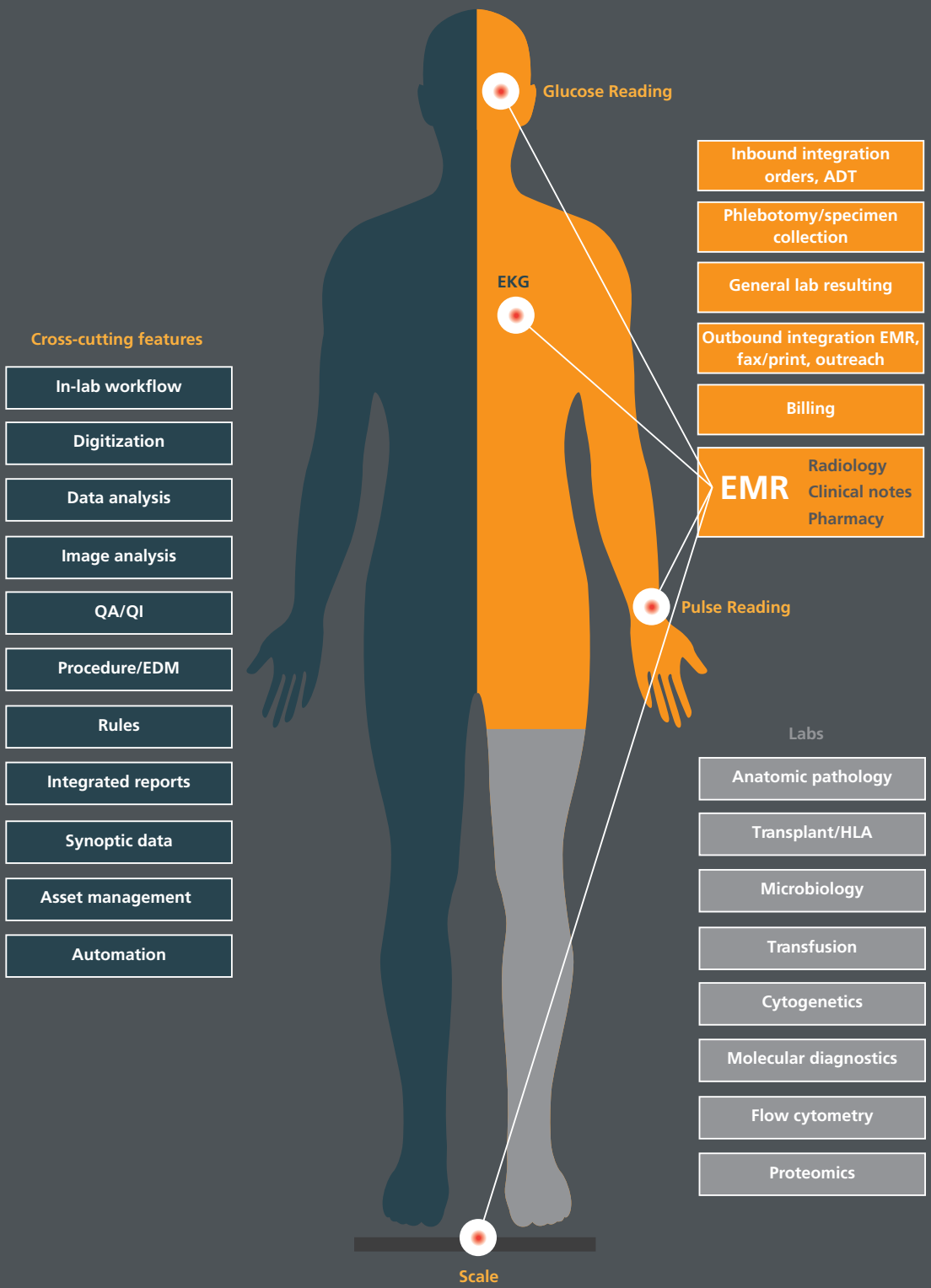
Many assessments of genotypic and phenotypic phenomena have robust analytic validity. Clinical validity and clinical utility, however, predicate on the concatenation of orthogonal measurements for characterizing complex phenotypes. In a version of an illustrative tale, a group of children are asked to describe what they feel as they touch an elephant in the dark. Each one feels a different part, but only one part such as the tusk or the tail. They compare their experiences to learn that they remain in complete disagreement as to the animal in the darkness. Their subjective experiences, while true, remained inherently limited by failure to account for a collective totality of truth. Application of this lesson in clinical diagnostics suggests that, wherever possible, all diagnostic studies must be integrated holistically and qualified by their functional components. Assimilation of orthogonal objective data precedes the precision sought in clinical decision-making. Multiplex robust biomarkers are routine, feasible, simple, inexpensive, rapid,reproducible, quantitative, standardized, and pathology-based. Precision medicine entails integration of such objective measurements into the routine context of subjective impressions and patient-physician preferences. Accounting for a plurality of diagnostic modalities, NGS and other molecular assays afford greatest clinical value when recommendations are worded with finesse and reflex/confirmatory tests defined wherever implications remain ambiguous. value when recommendations are worded with finesse and reflex/confirmatory tests defined wherever implications remain ambiguous. Treatment decisions are based on many pieces of information beyond genetic alterations, most of which are not available to the laboratory issuing the report. Molecular data, notwithstanding, enable patients and physicians to define and target the drivers of disease rather than merely eliminate symptoms.

Implementation

An expanding selection of molecularly targeted therapies is remarkably efficacious but only in the right genetic contexts. Accordingly, NGS gene panels deployed as fit-for-purpose diagnostic tests simplify the burden of interpretation while focusing on reporting solely results (both positive or negative) that change patient management. Expanding beyond gene panels to elucidate the networked relationships of novel variants requires aggregation of curated standardized biomarker and biosensor data. Actionability of molecular data can be defined at the hardware level (gene content) or software level (tiered reporting). In either iteration, the digital integration of molecular data with compatible electronic health records will most meaningfully advance evidence-based point-of-care clinical decision-making.

Access to NGS data in the medical record in a structured digital fashion (i.e. through the use of HL7 messaging standards and Logical Observation Identifiers Names and Codes (10)) must accompany the aesthetic allure of fully customized reports (for the clinician's and patient's comprehension of diagnostic findings) as standardized meta-analyses are sought (Figure 2). To make such discrete data elements fully computable, HL7 Clinical Document Architecture offers guidelines for the integrity of a carefully designed report accommodating digital integration into electronic medical records that cannot handle complex text or graphical formatting. Because it is not routinely feasible for electronic medical records to extract granular data from nuanced

Figure 3. Components of the Laboratory Information Management System (LIMS).



molecular reports, it remains critical to digitize all discrete molecular data elements via a standard HL7 message. Much as standard blood-based laboratory tests and imaging modalities integrate into a common electronic medical record, so too seamless merger of actionable NGS data (and interpretive reports) will enable ordering clinicians to readily consolidate pertinent patient data in a holistic manner for meaningful use.

Improved health economics have decentralized diagnostic testing, enabling additional laboratories to adopt diagnostic technologies such as NGS. Myriad biosensors worn by patients further enable continuous digital phenotyping. Biosensors provide a wealth of objective information inevitably sought clinically when patients present with symptoms conveyed though subject to recollection bias and practitioner interview variation. The individual resolution of patients' clinical descriptions is widely variable (Figure 3). Standardization of methods in precision medicine benefits everyone by enabling portability, security, reliability, accessibility, and readiness for clinical use. Thus, our collective ability to translate diagnostic and biosensor data into potentially breakthrough information predicates on its uniform interoperable standardization. Precision medicine cannot rely on shooting the arrow of molecular testing such as NGS and then hastily painting a bullseye at the spot where it lands.

As methods are expected to continuously evolve, precision measurements must strive to communicate in the same language. A sensitivity/specificity that is acceptable today may be inadequate in the future consequent to new clinical discoveries, changes in technology, and refined algorithms. Therefore, transparent (i.e. publically available) communication of assay parameters is fundamental to generating shared utility from NGS data and orthogonal clinical correlates. Further empowering patients to manage their own health record by annotating their health data with symptomatology, feedback on intervention effectiveness, and integration of wearable biosensor data would serves to add more subjective truths to the collective quantified self. Acknowledging and implementing this principle may further strengthen team-based approaches to health care.

Precision medicine cannot push possibilities ahead of quality science when it comes to patient care: analytic and clinical validation are essential, to provide physicians with confidence in their therapeutic decisions. Data interoperability, portability, and security are fundamental for quality. Cloud-based encryption, firewalls, and extensive auditing capabilities are commonplace in online commerce and are evolving to meet the elastic and expanding needs of secure health data networks. The scale of studies required to enumerate multiple rare genetic signatures that will inform management of common chronic conditions remains prohibitively expensive unless data can be collected and shared from diverse deeply annotated clinical encounters.

Our understanding and treatment of illness has become an increasingly rational science whose myriad phenotypic complexities are manifestations of interrelated DNA (genome), RNA (transcriptome), protein (proteome), and posttranslational (epigenomic) modifications. The accumulation and sharing of molecular diagnostic evidence in standardized fashion accelerates translation of findings from hypothesis testing to robust value demonstration. The definitive value of integrated fit-for-purpose NGS assays and orthogonal clinical measurements derives not from which genes are key in which pathologies, but rather in what molecular or cellular contexts their rational co-targeting elicits durable and sustained clinical responses in patients.

References

1. Saunders RE, Abarrategui-Garrido C, Fremeaux-Bacchi V, et al: The interactive Factor H-atypical hemolytic uremic syndrome mutation database and website: update and integration of membrane cofactor protein and Factor I mutations with structural models. Hum Mutat 2007, 28:222-234.
2. Caprioli J, Noris M, Brioschi S, et al. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. Blood 2006;108:1267-79.
3. Loirat C, Noris M, Fremeaux-Bacchi V: Complement and the atypical hemolytic uremic syndrome. Pediatr Nephrol 2008, 23:1957-1972.
4. Ferreira VP, Herbert AP, Cortes C, et al: The binding of factor H to a complex physiological polyanions and C3b on cells is impaired in atypical hemolytic uremic syndrome. J Immunol 2009, 182:70097018.
5. Lehtinen MJ, Rops AL, Isenman DE, et al: Mutations in factor H impair regulation of surface-bound C3b by three mechanisms in atypical hemolytic uremic syndrome. Biol Chem 2009, 284:15650-15658.
6. Roumenina LT, Loirat C, Dragon-Durey MA, et al: Alternative complement pathway assessment in patients with atypical HUS. J Immunol Methods 2011, 365:8-26.
7. Noris M, Caprioli J, Bresin E, et al: Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol 2010, 5:1844-1859.
8. Loirat C, Noris M, Fremeaux-Bacchi V: Complement and the atypical hemolytic uremic syndrome. Pediatr Nephrol 2008, 23:1957-1972.
9. Neumann HP, Salzmann M, Bohnert-Iwan B, et al: Haemolytic uraemic syndrome and mutations of the factor H gene: a registry-based study of German speaking countries. J Med Genet 2003, 40:676-681.
10. Regenstrief Institute. LOINC from Regenstrief website. http://www.loinc.org

Dr. Valentin Barsan is a bioengineer and physician at UC San Diego. He applied his engineering training from UC San Diego to scale genotyping, expression, and next generation sequencing consumables processes at Illumina. He then completed his medical training at Baylor College of Medicine in the Texas Medical Center alongside translational molecular pathology research and patient care at MD Anderson Cancer Center. In the lab of Dr. Sendurai Mani, Dr. Barsan helped investigate molecular drivers of epithelial-to-mesenchymal transition (EMT) using complementary molecular approaches. He also saw patients at MD Anderson Cancer Center in the weekly lymphoma clinic of Dr. Larry Kwak. Dr. Barsan is currently a resident physician in the Department of Pediatrics at UC San Diego and clinical consultant in oncology at Illumina. His translational clinical interests focus on molecular diagnostics to advance our understanding of immunity and cancer.