COMMENTARY

Evolution of Next Generation Therapeutics: Past, Present, and Future of Precision Medicines

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With increasing emphasis on precision approaches to therapeutics, product development has shifted focus to a genome-guided approach to develop novel therapeutics. This Commentary highlights the evolution of next generation precision medicines. We briefly summarize the current state of the science and what lies ahead in terms of opportunities and challenges for development of innovative therapeutic modalities such as antisense oligonucleotides (ASOs) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas 9)-based therapies.

PHARMACOGENETICS

The practice of precision medicine is rooted in resolving the factors that contribute to variability in disease pathways, susceptibility, prognosis, and treatment response, including identification of subsets of patients for whom medical interventions may be optimized. Pharmacogenetics, or the study of genetic variants that predict drug response, has been a major focus of investigations over the years. Despite the decades of genetic research that have contributed to a greater understanding of drug disposition and response, few "classical" pharmacogenomic biomarkers (e.g., CYP450 genotypes) are routinely tested outside of preemptive pharmacogenetic testing programs in academic settings. However, more consumer-centric testing models are gaining popularity, and biotech and pharmaceutical companies are building pharmacogenetic research into their drug development programs. This paradigm shift has facilitated the transition from research to practice, and the pharmacogenetic test results will likely continue to be applied in the clinic.[1]

In tandem with the expansion in testing for pharmacogenetic biomarkers, the prospective development of therapeutic products for patients with certain genetic characteristics has become commonplace. Several examples from therapeutic products approved in 2018 alone can be found in **Table 1**. Targeted drug development and approval, often with a novel companion *in vitro* diagnostic test, is a common feature of oncology drug development, and successes continue to emerge.[2] In fact, as our understanding of the molecular pathology of certain cancers has evolved, drugs that target molecular defects rather than a specific tumor type are now entering the market.[3] Similar successes have been realized in rare diseases, where targeted development is in some cases a necessity. The evolution of novel drugs has required novel approaches to identify potential responders, and nonclinical models have served as the basis for identifying drug-responsive variants, thus defining the patient populations that are eligible for treatment (e.g., as for migalastat and ivacaftor). Because of the prospective design of such programs, barriers to clinical testing of genomic or other biomarkers have been much easier to overcome compared with the application of pharmacogenetic tests to previously approved drugs.

Whether a drug is targeted at the outset or optimized once on the market, infrastructure needs to keep pace with the science. Reliable *in vitro* diagnostic tests that are validated, conducted, and interpreted in a consistent manner across healthcare institutions are important with the continued proliferation of tests. Additionally, in cases of prospective development, expanded investigation beyond the studied population will further help patients access medicines that may benefit them. Such systematic approaches will be necessary to take advantage of the opportunities and address the emerging challenges on the horizon for novel therapeutic modalities.

THE TRANSLATIONAL EVOLUTION OF ASOs: SPINAL MUSCULAR ATROPHY AS A CASE EXAMPLE

Beyond diagnostic testing and pharmacogenetics, the genomics revolution has enabled scientists to characterize the molecular basis of disease and design genetically targeted therapies. One such success was for spinal muscular atrophy (SMA). SMA is one of the fatal monogenic neurodegenerative disorders affecting infants and children, with a US prevalence of about ~10,000 patients. The three predominant types of SMA represent a broad range of phenotypic manifestations and survival, and the onset is predominantly pediatric. SMA is an autosomal recessive disorder caused by deletions or mutations in the survival motor neuron (*SMN1*) gene that results in a deficiency of SMN protein and leads to motor neuron degeneration and progressive muscle atrophy. Although the *SMN1* gene is

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Table 1 Representative examples of targeted drug and biologic new molecular entity approvals in 2018

Drug/biologic	Disease or condition[a]	Biomarker
Patient selection		
Tezacaftor, ivacaftor	Cystic fibrosis	Responsive *CFTR* variant
Migalastat	Fabry disease	Amenable *GLA* variant
Ivosidenib	Acute myeloid leukemia	Susceptible *IDH1* mutation[b]
Binimetinib, encorafenib	Melanoma	*BRAF* V600E/K mutation[b]
Dacomitinib	Non-small cell lung cancer	*EGFR* exon 19 deletion or L858R[b]
Larotrectinib	Solid tumors	*NTRK* gene fusion
Gilteritinib	Acute myeloid leukemia	*FLT3* mutation[b]
Lorlatinib	Non-small cell lung cancer	*ALK* gene rearrangement
Talazoparib	Breast cancer	Germline *BRCA* mutation[b]
Dosing		
Amifampridine	Lambert-Eaton myasthenic syndrome	*NAT2* genotype
Safety		
Avatrombopag	Thrombocytopenia in adult patients with chronic liver disease who are scheduled to undergo a procedure	Factor V Leiden, prothrombin 20210A

[a]For US Food and Drug Administration–approved indications, please refer to product labeling. [b]Co-approved with an *in vitro* companion diagnostic for patient selection.

responsible for producing the bulk of functional SMN protein, in SMA where there is no functioning copy of *SMN1*, rescue of an otherwise lethal phenotype is possible due to the presence of a "backup" paralogous *SMN2* gene, which produces a small amount of the identical SMN protein. The number of copies of the *SMN2* gene modifies the severity of the condition and is a strong predictive biomarker of the severity of the phenotype.

Drug development for SMA has been aided by (i) the generation of informative knock-in and knock-out animal models; (ii) therapeutic strategies and options for developing viable treatments such as replacing the *SMN1* gene or modulating splicing of *SMN2* to promote inclusion of exon 7; and (iii) establishment of disease biomarkers, including genomic biomarkers (e.g., *SMN2* copy number and SMN protein level in blood), and electrophysiological biomarkers (e.g., compound motor action potential).

There has been long-term interest in the development of oligonucleotide technologies for the treatment of genetic diseases. Early studies pioneered by Hua and colleagues[4] generated numerous ASOs that modulate the activity of exon splicing inhibitors and enhancers at the *SMN2* exon/intron 7 junction. The 2016 approval of the ASO nusinersen for the treatment of SMA is a milestone for both SMA treatment as well as for oligonucleotide technologies to be considered as a viable treatment option. Nusinersen displaces inhibitory hnRNPs at a unique sequence that regulates exon splicing, permitting binding of a U1snRNP, which in turn promotes inclusion of exon 7 into the *SMN2* mRNA transcript and elicits an increase in production of full-length SMN protein.

Dose-ranging studies with nusinersen in a transgenic mouse model of SMA demonstrated remarkable improvement in survival and motor function. Nusinersen came into the clinic in 2011 and progressed rapidly through dose-finding, safety, and proof-of-concept studies. Postmortem tissue of three treated patients in a phase II study provided an opportunity to confirm drug delivery to target motor neurons in the brainstem and spinal cord, with a greater than twofold increase in exon 7 inclusion in the *SMN2* transcript and an increase in SMN protein in these cells.[5] Pivotal randomized controlled trials of repeated intrathecal delivery of nusinersen in infants and children with SMA confirmed safety and clinical efficacy and supported regulatory approval in 2016 as the first US Food and Drug Administration (FDA) approved drug for SMA.[6] Nusinersen has now been used to treat thousands of patients with SMA, with sustained improvement in survival and motor function.

Modulation of splicing of pre-mRNA by ASOs is an attractive strategy for other genetic disorders and has the advantage of high specificity, low risk of off-target effects, and a long half-life. Given that the ASO technology is <50 years old, with the approval of a handful of drugs and several hundred drugs in development, ASOs are poised to accelerate drug development by targeting diverse sets of targets and diseases.

REALIZING THE POTENTIAL FOR CRISPR TO TRANSFORM DRUG DEVELOPMENT

Whereas modulating genetic disturbances directly through RNA interference has proven effective, we are now entering an era where it is possible to directly change the DNA sequences that result in human disease or "genetically edit" related biologic pathways. CRISPR/Cas 9 gene editing is a powerful new tool not only to enable basic research but also in the development of novel drug therapies.[7] It is a novel gene editing method that, by targeting previously undruggable targets or targets for which significant resistance mechanisms prevail, has the potential to transform health care by facilitating development of gene-based therapeutics for unmet medical needs. Such therapies include genetically engineering cells *ex vivo* for subsequent administration to patients or conducting gene editing *in vivo* by delivering the CRISPR/Cas 9 reagents to target tissues. The development of autologous and allogeneic chimeric antigen receptor (CAR)-T cell therapies is a promising and active example of *ex vivo* CRISPR/Cas 9-based drug therapies[8] (**Figure 1**).

Developing gene editing therapies is in some ways similar to small molecule development, but certainly with additional unique considerations. Toxicology studies are often unable to address "on-target" safety because the gene edited therapies are not pharmacologically active in animals due to the differences in the human vs. animal genome. However,
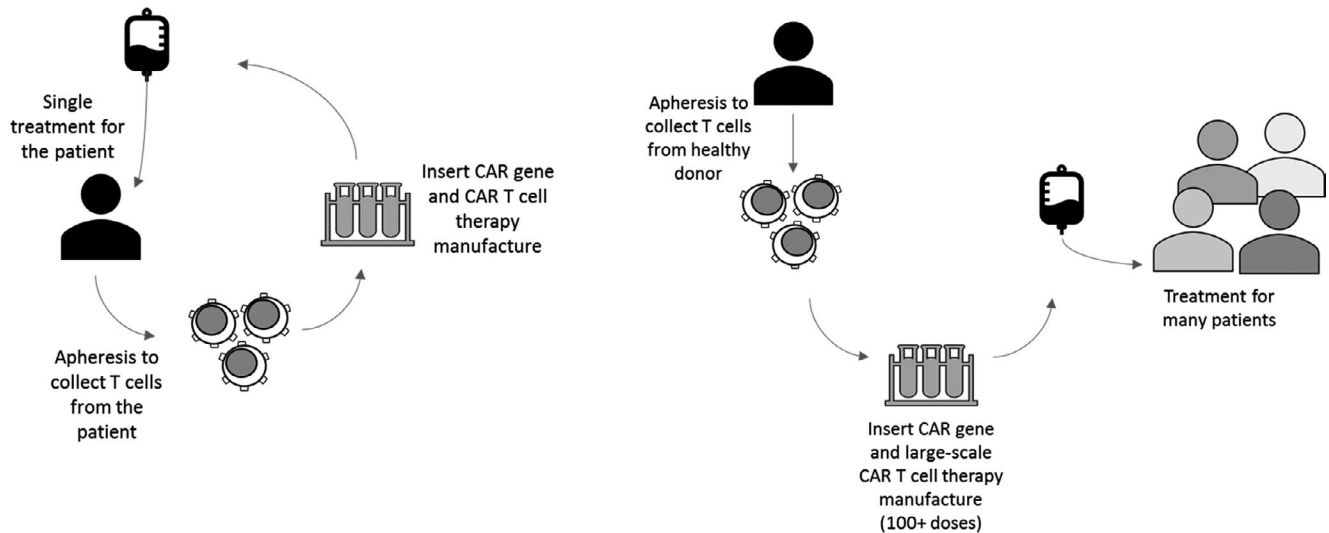
Figure 1 Overview of autologous and allogeneic chimeric antigen receptor (CAR)-T manufacturing. Patient's (autologous; left) or healthy donor's (allogeneic; right) T cells can be extracted through apheresis. These cells can be reprogrammed to recognize cancer cells or cells expressing a specific antigen. These newly created CAR-cells undergo expansion and can be subsequently used to treat patient(s).

understanding the propensity for immune responses like graft vs. host disease (for allogeneic therapies) and characterizing the safety of "off-target" gene edits are important novel considerations. In the allogeneic cell therapy setting, the screening and selection of healthy donor starting materials (i.e., T cells) is important, yet with novel and still emerging considerations relative to other allogeneic transplant settings. The pharmacokinetic disposition of CAR-T cells (for example) typically involve expansion of the administered T cells ("living drugs") followed by cell expansion, clearance, and, in some patients, a durable low level of cell persistence.[9] Thus, pharmacokinetic assays include application of polymerase chain reaction–based or flow cytometry–based technologies to detect cells. The study of biomarkers and other factors predicting response to CAR-T cell therapy is an emerging area of interest. Although the CAR-T cell dose tends not to predict response, expansion of cells *in vivo* does.[9] Furthermore, emerging literature suggests that other biomarkers related to the starting T cells (naïve "memory-like" immunophenotypes, for example) also predict response.[10]

The rapid evolution of gene editing technologies has led to the approval of two CAR-T treatments. Continued rapid advancement of these technologies along with improved understanding of the diseases (e.g., cancer, as well as autoimmune, infectious, and inflammatory diseases) will enable the translation of these innovative therapeutic options from the laboratory to the clinic.

SUMMARY

Advances in genomics have offered deeper insights into understanding diseases, understanding of variability in drug exposure and response, and identification of new drug targets. This has led to the development and approval of tailored therapeutic products (some innovative and some old),

such as ASOs, CAR-T cells, and CRISPR/Cas 9. Clinical development and approval of next generation therapies is an intensive process due to the inherent novelty and heterogeneity of these treatment modalities. The product development life cycle for these and any new technologies usually follows a succession of exploratory research, followed by controlled validation and product development, and then adoption in clinic practice. Clinicians, scientists, drug developers, and regulators alike continue to focus on ensuring a proper balance of benefit and risk for new and existing treatments when subgroups that respond well or seem to be more susceptible to adverse events emerge.

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