Mini-Review

Theme: Emerging Role of Pharmacogenomics (PGx) and Big Data on Development of Biologics

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Recent Advances in Application of Pharmacogenomics for Biotherapeutics

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Abstract. Biotherapeutics (BTs), one of the fastest growing classes of drug molecules, offer several advantages over the traditional small molecule pharmaceuticals because of their relatively high specificity, low off-target effects, and biocompatible metabolism, in addition to legal and logistic advantages. However, their clinical utility is limited, among other things, by their high immunogenic potential and/or variable therapeutic efficacy in different patient populations. Both of these issues, also commonly experienced with small molecule drugs, have been addressed effectively in a number of cases by the successful application of pharmacogenomic tools and approaches. In this introductory article of the special issue, we review the current state of application of pharmacogenomics to BTs and offer suggestions for further expansion of the field.

KEY WORDS: biologics; biotherapeutics; genetic variability; pharmacogenomics.

INTRODUCTION

Protein therapeutics, sometimes also referred to as “biologics” are generally defined as therapeutic protein(s) derived from a biological source (1). Historically, biologics mainly constituted blood and blood products, and were extracted directly from organisms that produced them naturally. However, in recent times, a number of novel biotherapeutics have been invented and approved for clinical use. This is a result of the advent first, and, convergence later, of several independently developed technologies such as genetic engineering, monoclonal antibody production, heterologous transfer, and expression of genes in multicellular eukaryotes, bioinformatics, as well as large-scale production and characterization of recombinant proteins (2–4). Together, these technologies are also called “biotechnology”. Arrival of the “omics” era has only accelerated the pace and number of novel recombinant therapeutic proteins invented, and broadened their applications (5,6). Thus, in recent years, the terms “biologics”, “biopharmaceuticals”, or “biotherapeutics” have been used interchangeably to refer to polypeptide drugs produced using one or more of these biotechnological approaches (7,8). For the sake of consistency, in this review article, the term “biotherapeutics” (BTs) is used to describe various recombinant protein drugs.

Our goal for this article is to provide the readers an overview of the current status of application of pharmacogenomics (PGx) for improving the health outcomes of BTs. We will begin with a brief background on BTs and PGx applications for small drug molecules, highlighting the lessons learnt with reference to BTs. This will be followed by description of select examples of BTs with actionable pharmacogenomic information that is resulting in improved efficacy reduced adverse reactions of those BTs. We will conclude with some thoughts on the challenges and opportunities for PGx application to this growing class of drug substances.

BACKGROUND

Although biologic products such as blood proteins have been used as drugs for centuries (2,4), recombinant insulin (Humulin®), the first FDA-approved BT, was marketed only in 1982 (9). Since then, tremendous advances have been made in the discovery and development of this class of drug molecules (2–4). As a result, currently over 200 biotherapeutics are available in the US and European markets (10). The anti-inflammatory BT Humira® has claimed a spot among the top three best-selling drugs for the past 4 years in a row (11). As summarized in Table I, in 2014, eight of the top ten drugs sold globally were BTs, and these eight drugs collectively generated revenues of over 61 billion (12), with several BTs acquiring the “blockbuster drug” status. This extraordinary growth in the number of BTs is expected to continue for the foreseeable future, as reflected by increasing number of new BTs licensed over the past 14 years (Fig. 1), as well as by entry of many “biosimilars” or

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Table I. Biotherapeutics Global Sales for 2014*

Rank**	Drug	Active ingredient	Sales (Billions)
1	Humira®	(Adalimumab)	13.021
3	Remicade®	(Infliximab)	10.151
4	Enbrel®	(Eternacept)	9.12
5	Lantus®	(Insulin glargine)	8.152
6	MabThera®/Rituxan®	(Rituximab)	7.356
7	Avastin®	(Bevacizumab)	6.841
9	Herceptin®	(Trastuzumab)	6.69
			61.331

*Adopted from Philipidis, A. (ref. (2))

**Rank based on global sales of all types of drugs in 2014

“biobetters” into this market segment (13–16). Several reasons that account for the rapid expansion of this class of drugs are outlined below.

- A. High specificity: In addition to the obvious differences in molecular composition and dynamic structure, this important functional characteristic primarily distinguishes BTs from their low molecular weight counterparts. Proteins carry out a unique (or relatively few) well-defined function(s) in living organisms, limiting their involvement largely to those reactions/pathways. This specificity in itself is sufficient to improve the therapeutic efficacy of BTs when compared to small molecules.
- B. Low off-target effects: A consequence of the high functional specificity of BTs is the low off-target effect as these molecules are not likely to interact with pathways that they do not normally participate in.
- C. Biocompatible metabolism: Many BTs (e.g., enzymes or receptors) are used to essentially replace a defective or non-functional biomolecule. Thus, their interactions are more compatible with other biomolecules and organelles associated with the metabolic reactions they are expected to “repair”. Additionally, pathways naturally utilized to metabolize cellular proteins will also metabolize most BTs into “normal” metabolites such as the constituent amino acid and carbohydrate metabolites (exception: conjugated

small molecule drugs). In most instances, these metabolites are recycled to the body’s normal metabolic flux, further reducing possibility of any adverse effects.

- D. Broad therapeutic potential: Because BTs are designed based on their biological functions, they often fill unmet medical needs, be those in the area of complex metabolic disorders, or genetic defects.
- E. Logistic and legal advantages: Rapid technological advances in discovery, development, and manufacturing of BTs, coupled with streamlined regulatory approvals and extended patent positions are expected to add to this growing list of drug molecules entering the market.

Despite these advantages, BTs also face significant challenges such as high costs, immunogenicity, and variable efficacy. While the cost factor may be addressed through the market forces such as logistical improvements and competition, the later two issues, unless addressed scientifically, may severely limit applications of BTs. Immunogenicity, an issue predominantly affecting therapeutic outcomes of BTs, is a subject of extensive research over the past few decades and has been reviewed in detail recently (17–22). However, variable efficacy is an issue common to both the small molecule drugs as well as BTs. During the past few years and especially since the completion of the human genome project in 2003, PGx has helped address issues related to the variable efficacy as well as the adverse effects of several small molecule drugs and some BTs. Specifically, the successful application of PGx to small molecule drugs offers important lessons. As discussed below, a brief summary would be useful for further enhancing therapeutic outcomes of BTs.

Lessons Learned from PGx of Small Molecule Therapeutics

Although an elegant argument for the concept of “chemical individuality” was made as early as 1902 (23), the actionable applications of genetic variability for managing drug safety were reported only in the middle of the twentieth century for the small molecule antimalarial primaquine (24,25) and the anti-tuberculosis drug isoniazid ((26), references therein). A major web-based PGx resource, PharmGKB (27), lists over 200 examples where genetic information is included in the labels of drugs approved by

*Data adapted from ref. 6; Numbers for 2015 up to April 30.

Fig. 1. Biotherapeutics licenses during 2001–2015*. *Data adapted from ref. (6); Numbers for 2015 up to April 30

one or more of the four international organizations viz. the US Food and Drug Administration or FDA (28), the European Medicines Agency or EMA (29), the Pharmaceuticals and Medical Devices Agency or PMDA, Japan (30), and Health Canada/Santé Canada or HCSC (31). Of these, 105 examples exhibit associations with haplotypes-multiple variations that are inherited together (27).

However, just listing genetic associations with a specific drug is not enough. Translation of the genetic data must be made to establish and implement a decision-making process for the clinicians. Therefore, as a next step, the dosing guidelines/recommendations are defined through a collaborative effort of the Clinical Pharmacogenomics Implementation Consortium in collaboration with the Pharmacogenomics Research Network (PGRN). These guidelines and recommendations have been designed mainly to assist clinicians in using the genetic information while making decisions regarding the drug choice and or drug dosing (27). Four levels of evidence (A through D) have been established. Evidence levels A and B are required to recommend a clinical action such as choice of a different dose of the same drug or choice of an alternative drug. Evidence ranked at level C does not lead to any changes in prescription recommendations because either (i) the genetics based recommendation does not make a difference in the therapeutic outcome; or (ii) therapeutic alternatives do not exist, are less effective, or are more toxic than the original drug. Evidence level D indicates that the published results are inconclusive, weak, even conflicting, and warrant no further clinical recommendation. To date, 68 examples of drug labels that contain dosing guidelines and recommendations based on evidence ranked as A or B have also been published (ref. (27) and references therein).

From PGx of Small Molecule Therapeutics to PGx of BTs

Analysis of these 200+ examples reveals that majority of these recommendations are for small molecule drugs. Furthermore, variations affecting therapeutic outcomes of these drugs are in genes required for their absorption, distribution, metabolism, or excretion. Thus, most of the early targets of this search for variations for Phase I enzymes (32–35) or Phase II enzymes (36,37) transporters (36,38–44) and receptors (45–53). However, there is a small but significant number of examples of application of PGx to BTs (Table II). Of the 14 examples of genomic biomarkers shown in Table II, over 85% represent BTs used for cancer treatment. Two of these (Adcetris® and Zevalin®) are antibody drug conjugates, and one (Ontak®) represents a chimeric BT- recombinant human IL-2 fused in frame with diphtheria toxin. Benlysta® and Krystexxa® are used to treat systemic lupus erythematosus and treatment refractory gout, respectively. Notable by the absence in this group shown in Table II are examples of BTs used for replacement therapy in genetic defects associated with metabolic enzymes (e.g., glucocerebrosidases), peptide hormones (e.g., growth hormone), or blood protein components (e.g., Factor VIII).

Genetic biomarkers associated with these BTs are applicable for patient stratification and/or for improving efficacy. Thus, CD20 positive status has been used to improve efficacy of Bexxar® and Gazyva® for treatment of non-Hodgkin lymphoma patients (27), *ERBB2* overexpression has

been used to treat breast cancer patients with Herceptin® and *EGFR* or *KRAS* expression status has been applied to classify and treat cancer patients with Erbitux® or Vectibix®. On the other hand, *G6PD* status has been used to screen out patients from treatment with Elitek® or Krystexxa® to avoid adverse effects such as severe hemolysis.

Table III presents three examples of BTs where PGx may be applied to drug-dosing decisions. Thus, CPIC guidelines for Elitek® recommend using *G6PD* variant status to decide if the patient receives Elitek® therapy or the alternative therapy with allopurinol (54). Similarly, CPIC guidelines have been published for Pegasys® and Pegitron®, BTs used for hepatitis C virus treatment (55). Thus, Muir *et al.* offer a “strong recommendation” for using pegylated interferon alpha -2a or -2b therapy for hepatitis C patients with the ‘favorable response’ allele CC at rs12979860; an equally strong recommendation is offered against using the pegylated interferon alpha-2a or -2b therapy for the unfavorable response alleles CT or TT of rs12979860 (55). These two examples also point to the general trend seen with PGx of small molecules, where initial focus has been on finding variations in genes associated with the metabolic and/or signaling pathways involved in the action of the therapeutic agents.

In summary, the examples outlined in Tables II and III certainly expand the applications of PGx to BTs. However, these drugs represent a small fraction of the current and growing list of BTs on the market (10–12). Several technical, logistic, and regulatory factors have been cited as possible barriers (56–63). Some of these challenges appear to be common both small and large molecule drugs. For example, observations from the retrospective studies must be supported by well-designed prospective studies inclusive of randomized study cohorts as well as validation cohorts. On the other hand, some challenges are unique to protein therapeutics because of their unique pharmacokinetic and/or pharmaco-dynamic properties (57,58). Compared to somatic variation studies, large-scale investigations on association of germline variations with therapeutic outcomes of BTs are difficult to conduct due to limited patient populations. Quantitative (high vs. low antibody titer) as well as qualitative (neutralizing vs. non-neutralizing antibodies) heterogeneity of the immune response seen in a population of patients to a BT adds another dimension of complexity (59–63). The use of biosimilars and biobetters may further complicate data analysis and interpretation when addressing immunogenicity of the BTs. Finally, as seen with various small molecule drugs (64), multiple variations, each with only marginal influence on the therapeutic outcome of a BT, may not justify practical implementation of the results.

CONCLUDING REMARKS

Current trends project a significant expansion in the role of BTs for treating various metabolic, immunologic, and genetic disorders (2–6). However, despite many advantages outlined above, a large body of evidence has also accumulated which underscores limitations in clinical application of BTs (reviewed in refs. (17–22)). Given the complex and multifactorial nature of the immune responses to BTs (65), additional efforts and resources would have to be devoted to

Table II. Biotherapeutics with Associated Genetic Markers in the Labels

Drug name	Active ingredient	Genetic biomarker	Clinical use	Comments
ADCETRIS®	Brentuximab Vedotin	<i>TNFRSF8</i>	Oncology	An antibody drug conjugate of chimeric anti-CD30 IgG1 coupled to a microtubule disrupting agent MMAE
ARZERRA®	Ofatumumab	<i>TNFSF13B</i>	Oncology	Efficacy in CD20 (product of <i>MS4A1</i>) expressing B cell chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma
BENLYSTA®	Belimumab	<i>TNFSF13B</i>	Autoimmune disorders	<i>TNFSF13B</i> also known as BLyS or BAFF, a B-lymphocyte stimulator protein overexpressed in systemic lupus erythematosus patients
BEXXAR®	Tositumomab	<i>MS4A1</i>	Oncology	Efficacy in CD20 (product of <i>MS4A1</i>) positive non-Hodgkin lymphoma patients
ERBITUX®	Cetuximab	<i>EGFR, KRAS</i>	Oncology	Efficacy in <i>EGFR</i> negative, <i>KRAS</i> wild type cancers
GAZYVA®	Afutuzumab or Obinutuzumab	<i>MS4A1</i>	Oncology	Efficacy in CD20 (product of <i>MS4A1</i>) positive non-Hodgkin lymphoma patients
HERCEPTIN®	Trastuzumab	<i>ERBB2</i>	Oncology	Efficacious only in patients overexpressing <i>ERBB2</i> (<i>HER-2</i>) gene product
KADCYLA®	Ado-Trastuzumab Emtansine	<i>ERBB2</i>	Oncology	Efficacious only in patients overexpressing <i>ERBB2</i> (<i>HER-2</i>) gene product
KRYSTEXXA®	Pegloticase	<i>G6PD</i>	Rheumatology	Individuals negative for <i>G6PD</i> may experience severe hemolysis
ONTAK®	Denileukin Diftitox	<i>CD25 (IL2RA)</i>	Oncology	Chimeric protein containing enzymatically active and membrane translocation domain of diphtheria toxin linked to human IL-2; Efficacy in CD25 positive T cell lymphoma patients
PERJETA®	Pertuzumab	<i>ERBB2</i>	Oncology	Efficacious only in patients overexpressing <i>ERBB2</i> (<i>HER-2</i>) gene product
RITUXAN®	Rituximab	<i>MS4A1</i>	Oncology	Efficacy in CD20 (product of <i>MS4A1</i>) positive non-Hodgkin lymphoma patients
VECTIBIX®	Panitumumab	<i>EGFR, KRAS</i>	Oncology	Efficacy in <i>EGFR</i> negative, <i>KRAS</i> wild type cancers
ZEVALIN®	Ibritumomab tiuxetan	<i>MS4A1</i>	Oncology	An antibody drug conjugate of anti-CD-20 IgG with Yttrium-90 radionuclide; efficacy in CD20 (product of <i>MS4A1</i>) positive patients particularly those with relapsed or refractory, low-grade or follicular B cell non-Hodgkin's lymphoma

MS4A1, membrane-spanning 4-domains, subfamily A, member 1; CD20, B-lymphocyte antigen CD20; *CYB5R1-4*, cytochrome b5 reductase 1; *G6PD*, glucose-6-Phosphate dehydrogenase; *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *ERBB2*, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2; *HER-2*, human epidermal growth factor receptor 2; CD25 (*IL2RA*), interleukin 2 receptor, alpha; *IL28B* (INF-λ-3), interleukin 28 B (Interferon lambda-3); MMAE, monomethyl auristatin E; TNFSF, tumor necrosis factor receptor superfamily. Information in this table adapted and compiled from ref. (27,50)

Table III. Biotherapeutics with PGx-Based Dosing Guidelines

Drug name	Description	Genetic biomarker	Dosing guidelines	Ref.
ELITEK®	Rasburicase	<i>CYB5R1-4</i> , <i>G6PD</i>	Individuals negative for <i>G6PD</i> may experience severe hemolysis; <i>CYB5R</i> variants with reduced <i>CYB5R</i> activity may also exhibit increased hemolysis	(54)
PEGASYS®	Peginterferon alpha 2a	<i>IL28B</i> rs12979860	Hepatitis C genotype 1 patients with the rs12979860 CC genotype have increased likelihood of response (higher SVR rate) to peginterferon-alpha-containing regimens as compared to patients with rs12979860 CT or TT genotypes	(55)
PEGINTRON®	Peginterferon alpha 2b	<i>IL28B</i> rs12979860	Hepatitis C genotype 1 patients with the rs12979860 CC genotype have increased likelihood of response (higher SVR rate) to peginterferon-alpha-containing regimens as compared to patients with rs12979860 CT or TT genotypes	(56)

understand the genetic determinants of the immunogenic response of BTs. Recently, this approach has been applied to understand genetic basis of immunogenicity to recombinant Factor VIII, a BT used for treatment of hemophilia A (66–70). A growing body of literature points to influence of human genetic variability on efficacy as well as tolerability/adverse effects of a number of vaccines (reviewed in ref. (59,63,71,72)). Specifically, associations of variations in human genes for HLA, cytokines, or cytokine receptors, molecules associated with mounting immune response, have been implicated in determining response to hepatitis B vaccine (73–76), hepatitis C vaccine (77–79) as well as a number of childhood vaccines including rubella (80), mumps (81,82), and measles (81–83), as well as malaria (84). However, further scrutiny with prospective studies would be needed to translate this information into clinical recommendations. Application of newer genomic as well as bioinformatics tools for collecting and analyzing data on large-scale (85,86) would prove useful for discovering and conclusively proving association of genetic variations (somatic or germline) to efficacy and/or adverse effects of BTs. Readers are directed to the accompanying articles in this issue for a detailed discussion of these and similar approaches.

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