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THERAPEUTIC TARGETING OF INNATE IMMUNITY IN THE FAILING HEART

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Abstract

Recent studies suggest that the heart possesses an intrinsic system that is intended to delimit tissue injury, as well as orchestrate homoeostatic responses within the heart. The extant literature suggests that this intrinsic stress response is mediated, at least in part, by a family of pattern recognition receptors that belong to the innate immune system, including CD14, the soluble pattern recognition receptor for lipopolysaccharide, and Toll like receptors-2, 3, 4, 5, 6, 7 and 9. Although this intrinsic stress response system provides a short-term adaptive response to tissue injury, the beneficial effects of this phylogenetically ancient system may be lost if myocardial expression of these molecules either becomes sustained and/or excessive, in which case the salutary effects of activation of these pathways is contravened by the known deleterious effects of inflammatory signaling. Herein we present new information with regard to activation of innate immune gene expression in the failing human heart, as well as review the novel TLR antagonists that are being developed for other indications outside of heart failure.

This review will discuss the interesting possibility that the TLR pathway may represent a new target for the development of novel heart failure therapeutics.

Overview of Innate Immunity

The adult heart responds to tissue injury by synthesizing a variety of proteins that delimit myocardial injury through upregulation of cytoprotective factors, as well as by activating mechanisms that facilitate tissue repair. While, the exact mechanisms that are responsible for orchestrating these stress responses within the heart are not known, there is a growing body of literature which suggests that the innate immune system plays an important role in terms of initiating, integrating, and perpetuating an ongoing the myocardial response to tissue injury. Our understanding of the molecular components that regulate innate immunity and inflammation and that lead to the induction of pro-inflammatory cytokines has increased dramatically with the discovery of a family of phylogenetically ancient receptors termed Toll-like receptors (TLRs) [1]. TLRs serve as pattern recognition receptors (PRRs) that recognize conserved motifs on pathogens, so called pathogen-associated molecular patterns (PAMPs). More recently it has become clear that TLRs also recognize molecular signatures

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emanating from endogenous host material that is released during cellular injury or death, referred to as damage associated molecular patterns (DAMPs) [2,3], thereby providing a potential link between tissue injury, activation of inflammatory mediators, and the pathogenesis of heart failure.

Expression and Regulation of Toll Receptors in Animal Models

The heart expresses pattern recognition receptors belonging to the innate immune system, including CD14, the soluble pattern recognition receptor for lipopolysaccharide [4], and Toll like receptors-2, 3, 4, 5, 6, 7 and 9 (TLR-2, TLR-3. TLR-4, TLR-5 and TLR-6, TLR-7, TLR-8, TLR-9 respectively) [5,6]. TLR 2, 4, 5 and 6 are expressed on the cell surface of murine and rat cell types residing within the heart, including TLR2 and TLR4 expression in cardiac myocytes, whereas TLR 3, 7 and 9 are expressed in intracellular compartments, primarily endosomes and the endoplasmic reticulum, with the ligand binding domains facing the lumen of the vesicle. There are three general categories of TLR ligands: proteins (TLR5), nucleic acids (TLR3,7,9) and lipid-based elements (TLR2, TLR4, TLR6, TLR2/TLR6) [7]. At the time of this writing, very little is known with regard to the regulation and/or spatial localization TLR expression within the heart, although TLR4 appears to be upregulated in the failing human heart [8,9].

One of the first TLR signaling pathways to be elucidated was the TLR4 signaling pathway (Figure 1). All TLRs (except for TLR3) interact with an adaptor protein termed myeloid differentiation factor 88 (MyD88) via their Toll Interleukin Receptor (TIR) domains. When stimulated, MyD88 recruits IL-1 receptor associated kinase (IRAK) to the receptor complex. IRAK is then activated by phosphorylation on serine/threonine residues and associates with tumor necrosis receptor associated factor 6 (TRAF6), leading to NF-κB activation.[10] Although the adaptor molecule TIR domain-containing adapter protein (TIRAP) was initially thought to contribute to MyD88 independent signaling, studies have shown that TIRAP is required for TLR2 and TLR4 mediated activation of NF-κB. The exact ligands that activate TLR signaling in the heart are not known. In this regard it is interesting to note it that in addition to activation by the classic pathogen associated molecular patterns (e.g. lipolysaccharide), TLR receptors are activated by damaged proteins released by injured and/or dying cells [2,3]. For example, both heat shock protein 60 and 70 are sufficient to activate TLR signaling in the heart [11,12], whereas fibronectin can activate TLR signaling in non-myocytes [13]. Once these damage associated molecular patterns are recognized by pattern recognition receptors, they activate the components of the innate signaling pathway, including NF-κB, pro-inflammatory cytokines and nitric oxide [14], that in turn provoke immune cell recruitment and activation.

Functional role of TLR Signaling in Experimental Models

Although the exact role of TLR signaling in the heart is not known, the extant literature supports the point of view that short-term activation of TLR signaling confers cytoprotective responses in the heart, whereas long-term signaling is maladaptive and can lead to cardiac remodeling (see below). For example, activation of TLR4 by LPS in vivo or ex vivo protects the myocardium following myocardial ischemia reperfusion (I/R) injury (reviewed in [15]). Hearts isolated from rats that were pretreated with a low dose of LPS (0.5 mg/kg) 24 hours prior to terminal sacrifice had preserved LV function after I/R injury compared with the saline treated control hearts [16]. The cytoprotective effects of LPS were manifest 12-24 h after the administration of LPS, were sensitive to inhibition with cycloheximide, and required TLR4 signaling [17]. Analogous to ischemic pre-conditioning, the cytoprotective effects of LPS were mediated by NOS2 and Akt [15]. Consistent with these studies, a recent report from our laboratory showed that ischemic preconditioning is mediated via a TLR2-

TIRAP dependent signaling pathway that involves protein kinase C [18]. Interestingly, in these latter studies the phosphorylation of GSK-3β was TLR2-TIRAP dependent, whereas Akt phosphorylation was not. Given that GSK-3β has been implicated in opening of the mitochondrial transition pore and cell death in ischemia reperfusion injury, and that inactivation of GSK3β through phosphorylation leads to pre-conditioning by preventing opening of the mitochondrial transition pore [19], the observation GSK-3β phosphorylation was TLR2-TIRAP dependent suggest that one of the mechanisms whereby innate responses are cytoprotective is through inhibition of the formation of mitochondrial permeability transition pores trigger cell death following ischemia reperfusion injury.

Although the above studies suggest a beneficial role for TLR signaling in the heart, it should be recognized that the signaling pathways evolved in organisms with relatively short life spans (weeks to months), and thus never intended to provide long-term adaptive responses to the host organism. And indeed “loss of function studies” in experimental heart failure models suggest that sustained activation of TLRs is maladaptive and can contribute to adverse cardiac remodeling (Table 1). For example, mice with a missense mutation of TLR4 or targeted disruption of TLR4 [20-22], TLR2 [23], or MyD88 [24] have reduced infarct sizes when compared to wild-type controls. Correspondingly, mice pre-treated with a TLR4 antagonist (Eritoran) [25] had smaller infarct sizes when compared to vehicle treated animals. Mortality and LV remodeling are reduced in mice with targeted disruption of TLR4 or TLR2 [26,27]. Although the mechanism(s) for the deleterious effects of TLR signaling following I/R injury and/or myocardial infarction have not been elucidated, a study in an experimental systemic sepsis model suggested that TLR4 receptors on bone marrow-derived hematopoietic cells were required for the neutrophil recruitment to the myocardium with resultant adverse cardiac remodeling [28]. Whether the mechanisms involved in an experimental model of sepsis will also obtain in experimental models of cardiac injury remains to be determined.

Functional role of TLR Signaling in Human Heart Failure

As noted above the experimental literature suggests that sustained activation of TLR signaling following cardiac injury is maladaptive and can lead to a heart failure phenotype, very little is known regarding the innate immune system in the failing human heart. Two studies have shown that expression of TLR4 mRNA and TLR4 protein is increased in the hearts of patients with advanced heart failure [8,9]. However, until recently it has been unclear whether or innate immune genes are expressed differently in human heart failure. To this end, we examined the expression profiles of genes that were involved in innate immune signaling using the Cardiogenomics Consortium data base (http://www.cardiogenomics.med.harvard.edu]), which was obtained from explanted hearts from patients with ischemic cardiomyopathy (ICM), idiopathic dilated cardiomyopathy (DCM), and viral cardiomyopathy (VCM), as well as non-failing (NF) hearts. Expression data for innate immune signaling genes were analyzed by Principal Component Analysis (PCA), a mathematical modeling procedure that transforms a number of possibly correlated variables into a smaller number of uncorrelated variables that are termed principal components[29]. The 3-dimension PCA plot illustrated in Figure 2 shows two important findings with respect to innate immune gene expression in human heart failure. First, the numerical values for the PCA plots for NF hearts were clustered differently than the numerical values for the PCA plots for the ICM, DCM, VCM hearts, which tended to cluster together (best observed in Figure 2A). The second salient finding is that the PCA profiles were different in ICM patients when compared to DCM patients. This raises the intriguing possibility that that the innate immune system is activated differentially in response to the nature of the pathological tissue injury pattern. Inspection of Figure 2A shows that the PCA plots for VCM patients were similar to that observed in DCM. This particular observation is

of interest, insofar as occult and/or persistent viral myocarditis has been suggested as a potential etiology for idiopathic dilated cardiomyopathy [30]. An unsupervised hierarchical clustering analysis of DCM, ICM, VCM and non-failing human hearts confirmed that there were (1) distinct gene expression profiles for innate immune genes in failing and non-failing hearts, and (2) that there were distinct gene expression profiles for innate immune genes in ICM and DCM hearts [31]. Indeed there were 37, 37, and 27 transcripts, respectively, whose expression levels were significantly different in DCM, ICM, and VCM when compared to non-failing hearts. Interestingly there were only 14 transcripts whose changes were similar in all forms of cardiomyopathy, of which there were 3 genes whose expression levels increased and 11 immune genes whose expression decreased in all forms of cardiomyopathy [31]. Although any inferences with respect to a cause and effect relationship vis-à-vis differential expression of innate immune genes in the failing human heart and the pathogenesis of human heart failure must be regarded as provisional until detailed studies of protein levels of TLRs are analyzed in failing human hearts, the observation that NF-κB activation and pro-inflammatory cytokines gene and protein expression are increased in the failing human heart [32-34] raises the interesting possibility that enhanced innate immune signaling may contribute to the pathogenesis of human heart failure by provoking the expression of pro-inflammatory mediators that are sufficient to contribute to a heart failure phenotype (reviewed in [35]).

Translation Potential of TLR Signaling in Human Heart Failure

There has been significant interest in developing TLR antagonists as novel therapeutics in diseases such as sepsis, systemic lupus erythematosis and rheumatoid arthritis, wherein the immune system and inflammatory mediators are inappropriately overactive. Currently, there are a variety of novel antagonists that are being developed for TLR 2, 4, 7, and 9 (reviewed in [7]). Given the focus of the present review on innate immunity in heart failure, we will focus on TLR2 and TL4 antagonism (see Table 2), for which there is the most direct evidence of TLR involvement in heart failure.

TLR2

OPN-305, a TLR2-specific monoclonal anti-body that inhibits TLR2-mediated pro-inflammatory cytokine production, was granted orphan status for the prevention of the ischemia and reperfusion injury associated with organ transplantation. The first human trials as a potential treatment of inflammatory diseases are expected to begin in 2010. Given the role of TLR2 in mediating ischemia reperfusion injury, this molecule is attractive for testing in phase I clinical trials in patients with ischemic cardiomyopathy. AP177, which is a DNA aptamer that was identified by a SELEX (systematic evolution of ligands by exponential enrichment) screen [36], binds to TLR and competitively antagonizes TLR2 ligand binding, thereby inhibiting NF-κB activity and pro-inflammatory cytokine production [7].

TLR4

There are a number of strategies that have been undertaken to inhibit TLR4 activation [7]. Eritoran (E5564), which reduces the binding of lipid-A (the biologically active part of the lipolysaccharide molecule), reduced mortality by 6.4% compared with the placebo group in a phase II sepsis trial, and is currently undergoing evaluation in Phase III sepsis trials (NCT00334828). Given that the pharmacodynamic profile of Eritoran requires administration as a continuous infusion or by repeated intravenous injections, this TLR4 antagonist may not be practical for treating chronic heart failure. However, it may be useful during myocardial inflammatory states or in the setting of an acute coronary syndromes that lead to the development of heart failure. Alternative approaches have been to develop variations of lipid-A that bind TLR4, but have reduced agonist activity (e.g., CRX-527, lipid

▪ IVa). TAK-242 also targets TLR4- dependent signaling, although the precise target is not known. Development of this compound was discontinued during a Phase III sepsis clinical trial because the drug's profile did not meet the criteria required to support continued development, not because of drug safety issues (NCT00633477). Ibudilast (AV411) is another TLR4 antagonist, that suppresses pro-inflammatory cytokines such as TNF and IL-6, and may induce the anti-inflammatory cytokine IL-10, is undergoing phase II trials for opioid dependence (NCT00723177). OPN-401, is a viral protein-derived peptide that inhibits TLR4-dependent signaling is also in preclinical development.

Direction of Future Research

The foregoing review suggests that activation of TLR signaling in the heart confers short-term benefits in the heart when activated acutely, but that the beneficial effects of TLR signaling are lost in the chronic setting, wherein ongoing tissue damage can lead to sustained TLR signaling that is sufficient to provoke a heart failure phenotype. Thus, analogous to the renin angiotensin system and the adrenergic nervous system, activation of the innate immune system can provoke disparate responses, depending on the context, as well as duration of activation of this phylogenetically conserved signaling system. Although the data linking increased TLR signaling to heart failure is provisional at the time of this writing, the extant literature suggests that increased TLR signaling, perhaps secondary to DAMPs, may contribute to adverse cardiac remodeling secondary to increased NF-κB activation and increased expression of pro-inflammatory cytokines. Whereas the initial clinical heart failure trials that employed targeted anti-inflammatory approaches yielded disappointing results [35,37,38], targeting the TLR signaling pathway in heart failure may offer a more rationale therapeutic approach, insofar as the TLR signaling pathway modulates a much broader portfolio of inflammatory mediators, and acts as a important upstream nodal mechanism for activating inflammatory signaling in response to tissue injury. Moreover, targeting specific TLR pathways may allow for tailoring anti-inflammatory strategies to specific subsets of heart failure patients. Indeed a recent consensus statement from the Translation Research Committee of the Heart Failure Association of the European Society of Cardiology suggested that there may not be a common inflammatory pathway that characterizes all of the different forms of heart failure, and that going forward it would be important to design specific anti-inflammatory approaches for different types and stages of heart failure, as well as to determine the specific inflammatory pathways that are activated in different forms of heart failure [39]. Based on the extant literature, targeting TLR signaling in the setting of chronic ischemic cardiomyopathy, wherein TLR signaling has been implicated in plaque rupture in atherosclerotic coronary arteries, as well as adverse cardiac remodeling, would appear to be attractive. As with all therapeutic approaches in heart failure, the only way to really answer the question of whether these types of anti-inflammatory strategies will have any added value in heart failure is through well designed clinical trials. However, it bears emphasis that at present our knowledge of the role of TLR signaling is not sufficient to support the evaluation of this therapeutic target in phase I clinical trials. Additional studies in clinical heart failure samples that demonstrate increased protein levels and/or activation of the signal transduction pathways that are downstream from TLR signaling will be required to complement the studies on human heart failure gene expression. Further, it will also be important to determine whether blocking TLR2, TLR4 or TLR2 and TLR4 with antibodies or small molecule inhibitors is more effective with respect to preventing the development of a heart failure phenotype in small animal models, insofar as Eritoran is the only molecule that has been tested in experimental heart failure models. Lastly, it remains to be determined whether antagonizing TLR signaling in heart failure will lead to worsening heart failure because of the potential loss of the beneficial effects of TLR signaling in the heart. Despite these cautionary notes, drug development in this area will benefit from the explosive growth in knowledge that has occurred over the past decade with

respect to the biology of innate immune responses in the heart, which in turn will facilitate choosing the appropriate biomarkers and surrogate end-points that would support enrolling patients in well-designed phase I –II clinical trials to test the efficacy of this therapeutic target in heart failure.

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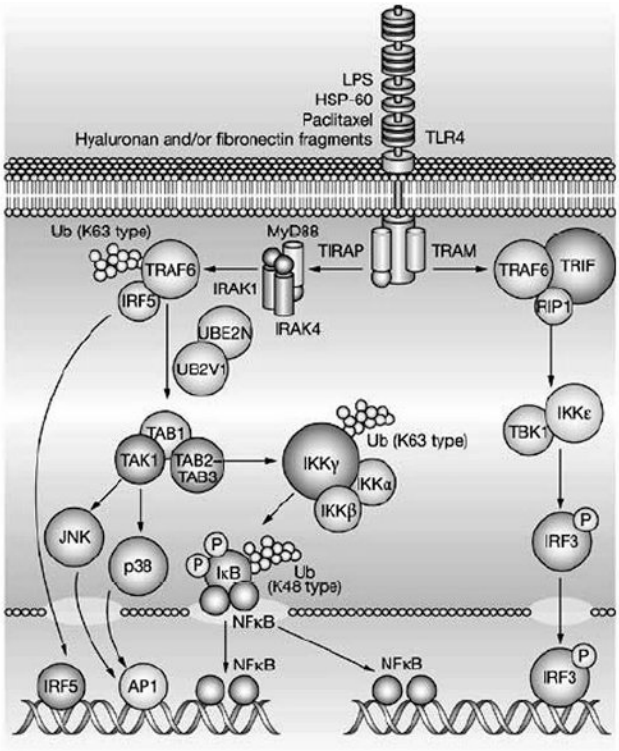

Figure 1.

The Toll-like receptor signaling pathway. (Key: AP1, activator protein 1; HSP-60, heat shock protein 60; IκB, inhibitor of nuclear factor κB; IKKα, inhibitor of nuclear factor κ-B kinase α; IKKß, inhibitor of nuclear factor κB kinase-ß; IKKε, inhibitor of nuclear factor κ-B kinase ε; IKKγ, inhibitor of nuclear factor κ-B kinase γ; IRAK1, interleukin 1 receptor-associated kinase 1; IRAK4, interleukin 1 receptor-associated kinase 4; IRF3, interferon regulatory factor 3; IRF5, interferon regulatory factor 5; JNK, c-jun N-terminal kinase; LPS, lipopolysaccharide; MyD88, myeloid differentiation primary response protein; NF-κB, nuclear factor κB; RIP1, receptor-interacting protein 1; TAB1, TAK1 - binding protein 1; TAB2-TAB3, TAK1 -binding proteins 2 and 3; TAK1 (M3K7), transforming growth factor-ß-activated kinase 1; TBK1, serine-threonine-protein kinase; TIRAP, TIR domain-containing adaptor protein; TLR4, Toll-like receptor 4; TRAF6, tumor necrosis factor receptor-associated factor 6; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adaptor inducing interferon ß; Ub, ubiquitin; UB2V1, ubiquitin-conjugating enzyme E2 variant 1; UBE2N, ubiquitin-conjugating enzyme E2N (Reproduced with permission from Frantz, S., Ertl, G., & Bauersachs, J. Mechanisms of disease: Toll-like receptors in cardiovascular disease. *Nat.Clin.Pract.Cardiovasc.Med.* 4, 444-454, 2007).

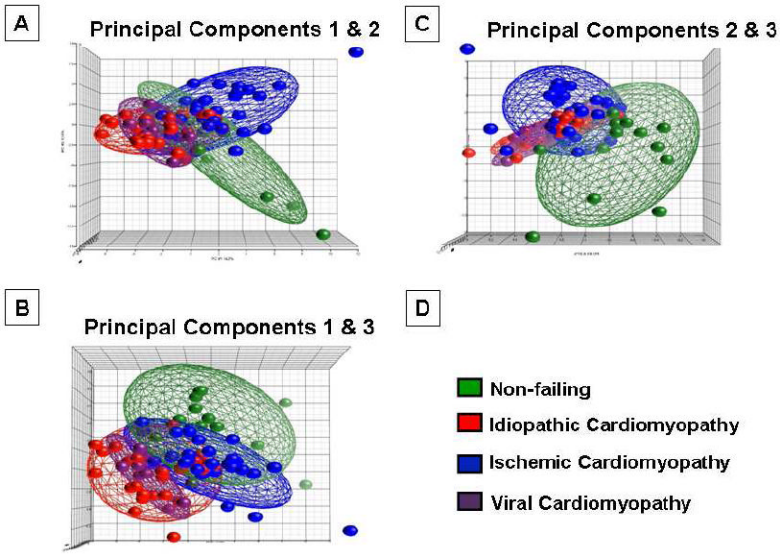

Figure 2.

Principal component analysis of changes in innate immune gene expression in failing and non-failing human hearts. Innate immune genes were subjected to a principal component analysis (PCA), and the first, second and third principal components were displayed in a 3-D graphic format. (From Mann DL, Topkara VK, Evans S, Barger PM. Innate immunity in the adult Mammalian heart: for whom the cell tolls. *Trans Am Clin Climatol Assoc* 2010;121:34-50).

Table 1

TLR signaling Modulation of Myocardial Ischemia Reperfusion Injury and Cardiac Remodeling

Mice	Infarct Models	Effects in Knockout Mice
TLR2 signaling		
TLR2$^{-/-}$	I/R (30' I/60R')[23]	smaller infarct sizes, reduced neutrophil recruitment, reduced ROS and cytokines
TLR2$^{-/-}$	Permanent coronary ligation[26]	Improved survival rate, attenuated remodeling, but same infarct sizes at 4 wk
TLR4 signaling		
C57 BL/10 ScCr * C3H/HeJ**	I/R (60' I/24 h R)[22]	Smaller infarct sizes, reduced MPO activity and complement 3 deposition
C3H/HeJ**	I/R (60' I/120' R)[20]	Smaller infarct sizes, decreased cardiac expression of TNF, MCP-1, and ILs
C3H/HeJ**	I/R (60' I/24 h R)[21]	Smaller infarct sizes, but no gain in LV function
WT with eritoran	I/R (30' I/120' R)[25]	Smaller infarct sizes, reduced pJNK, reduced cytokine expression
C3H/HeJ**	Permanent coronary ligation[40]	Reduced LV remodeling, improved systolic function, reduced cytokine expression
C57 BL/10 ScCr*	Permanent coronary ligation[27]	Improved LV function on day 6 after infarction, improved survival rate, reduced LV remodeling and apoptosis at 4 wk.
MyD88$^{-/-}$	I/R (30' I/24 h R)[24]	Smaller infarct sizes, improved LV function, and attenuated cytokine expression and neutrophil recruitment

* C57 BL/10 ScCr mice contain a null mutation in the *Lps* gene and

** C3H/HeJ mice contain a missense point mutation (Pro → His) in the *Lps* gene, rendering these mice hyporesponsive to lipoplysaccharide.

(Key: MPO, myeloperoxidase; MCP-1, monocyte chemoattractant protein-1; MyD88, myeloid differentiation primary-response gene 88; pJNK, phosphorylated JNK; TLR, Toll-like receptor; ROS, reactive oxygen species)

(Modified from Chao W, Am. J. Heart Circ Physiol 296: H1-H12, 2009)

Table 2

Development Status of TLR2 and TLR4 antagonists

Compound	Indications	Target	Drug Class	Clinical Phase
OPN-305	Inflammation, autoimmunity, ischemia/reperfusion	TLR2 antagonist	Antibody	Orphan status for prevention of ischemia reperfusion injury
OPN-401	IBD, rheumatoid arthritis	TLR2/TLR4 antagonist	viral-derived peptide	Preclinical
AP177	NS	TLR2 antagonist	DNA aptamer	Preclinical
Eritoran (E5564)	Sepsis	TLR4 antagonist	Synthetic lipodisaccharide	Phase III
Lidid-IVa	NS	TLR4 antagonist	Lipid A partial mimetic	Preclinical
TAK-242	Sepsis	TLR4 antagonist	Small molecule inhibitor	Suspended in phase III
1A6	Colitis	TLR4 antagonist	Antibody	Preclinical
CPG-52364	SLE	PolyTLR antagonist	Quinazoline derivative	Phase I
Ibudilast (AV411)	Pain management, withdrawal	TLR4 antagonist	Small-molecule phosphodiesterase inhibitor	Phase II

Modified from Hennessy et al., Nat. Rev Drug Discov. 9, 293-307; 2010