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INVITED REVIEW

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Structural basis of glucocorticoid receptor signaling bias

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Abstract

Dissociation between the healthy and toxic effects of cortisol, a major stress-responding hormone has been a widely used strategy to develop anti-inflammatory glucocorticoids with fewer side effects. Such strategy falls short when treating brain disorders as timing and activity state within large-scale neuronal networks determine the physiological and behavioral specificity of cortisol response. Advances in structural molecular dynamics posit the bases for engineering glucocorticoids with precision bias for select downstream signaling pathways. Design of allosteric and/or cooperative control for the glucocorticoid receptor could help promote the beneficial and reduce the deleterious effects of cortisol on brain and behavior in disease conditions.

KEYWORDS
allostery, biased signaling, cooperativity, drug discovery, nuclear receptors

1 | INTRODUCTION

Cortisol is a human steroid hormone (corticosterone in rodents) released from the adrenal gland that uses nuclear receptors, firmly characterized molecularly, as well as hypothetical membrane receptors to adjust demands of the internal and external milieu. The receptors use an arsenal of rapid (seconds-to-minutes) nongenomic and slow (hours-to-days) acting genomic actions within cell ensembles in the brain and body affecting development, growth, metabolism and behavior.[1] The low affinity nuclear glucocorticoid receptor (GR) is the most widespread in the body while the high affinity mineralocorticoid receptor (MR) is more cell-type specific and often coexpressed with GR.[2] Both receptor types are well established soluble and diffusible signaling entities unlike their membrane-bound counterparts supposed to transduce the rapid activities.[3] Even if rapid steroid effects are unequivocal, the nature, or even existence of membrane-associated GR and MR is still debated due to the indirect pharmacological and genetic evidence.[4–8]

Glucocorticoids are mainstay of treatment for many inflammatory and immune conditions, which however comes with side effects, particularly with chronic administration and high doses. Adverse and dissuasive effects of treatment include a diabetic-like state, peptic ulcer, gastrointestinal bleeding, increased blood pressure, iatrogenic Cushing's syndrome, osteoporosis, skin atrophy, delayed wound healing, glaucoma, cataract, cognitive impairment and other neurological dysfunctions.[9,10] Alternate treatments with fewer side effects targeting cortisol release, inflammation or immunosuppression may however display inferior therapeutic efficacy. Glucocorticoids are the most powerful treatment for a wide range of diseases, remaining indispensable, despite the seriousness of the side effects associated with chronic administration. Therefore, alleviating the adverse effects of glucocorticoids while retaining the desired property among a large array of functions (e.g., anti-inflammatory, immunosuppressant, cognitive enhancer, neuroprotective, proapoptotic…) remains a major focus for innovation. Topical administration for the skin, the eyes and aerosol delivery for the lungs are well tolerated but inadequate for most targeted diseases of the brain and inner body. Moreover, inhalation steroids have—epidemiologically—been associated with side effects.[11,12] Finally, the risks of using glucocorticoids in neurological disorders despite the benefits currently precludes clinical applications beyond the experimental.

The unravelling of the molecular bases of ligands recognition in the cortisol binding pocket prompted the synthesis of more potent glucocorticoids but side effects remain in spite of improved selectivity.[13,14] Development of new activity-modifying ligands outside of the cortisol binding domain could promote the graded or biased responses that are compatible with lesser side effects for treating conditions of resistance and hypersensitivity to glucocorticoids.[15] A major question

remains how to screen for such selective and dissociative activities. Here, we apply to GR the concepts of allosteric cooperativity and biased signaling (see Box 1 for definitions) as developed originally for G-protein coupled receptors, to promote the design of glucocorticoids dissociating the beneficial from the detrimental effects.[16]

2 | GLUCOCORTICOID RECEPTOR STRUCTURAL ALLOSTERY

Allostery is an activity-modifying process defined as the structural changes propagated from one site to another, often distal, functional site like the ligand-binding pocket (Figure 1A). Crystallography and nuclear magnetic resonance (NMR) revealed mechanisms common to most ligands (agonists and antagonists) with a similar pharmacophore (i.e., acting at the classical binding pocket) that transduces conformational surface changes of the activation function-2 (AF-2) coregulator-binding surface. This is conserved across most species and in many nuclear receptors.[13,17–19] For example, molecular dynamics of helix-12 in the ligand binding domain predict docking of either corepressors or coactivators,[20] serving as structural basis for screening ligands that would stabilize conformations desired for agonistic or antagonistic effects.[21,22] Mutations in or surrounding the helix-12 often cause GR signaling defects and glucocorticoid resistance.[23,24] A recent study indicates how ligand-driven conformational shifts of helix-12 permit signal transmission from the ligand binding-domain to the transactivation domain and beyond to downstream GR-docking effectors.[25] Conformational flexibility can explain how ligands with distinct chemical scaffolds and pharmacophores could elicit graded responses (full/partial agonism) and biased signaling (pathway specificity). Screening of GR ligands that bias the equilibrium toward the antagonist conformational state produced dissociative responses with anti-inflammatory effects devoid of transactivation responses.[26] However, dissociative compounds have been disappointing in clinical situations because transactivation of anti-inflammatory compounds is also needed for full efficacy.[27] Synthetic ligands were also designed to favor the transactivation of target genes over transrepression.[28] Such ligands also have intrinsic limitations as the desired response often consists of a mixture of activated and suppressed genes in target tissues.[29] Additionally, high-affinity GR modulators were characterized as partial agonists in some tissues and antagonists in others, permitting the dissociation between beneficial and deleterious effects of excessive and sustained levels of endogenous corticosterone in a model of chronic stress.[30] These modulators were identified based on differential GR-coregulator interactions.

Combining NMR with surface plasmon resonance revealed that different coregulators with distinct amino acid sequence motifs vary in their ability to propagate ligand-induced allostery to downstream protein interaction networks. For example, direct binding of GR to either the nuclear receptor coactivator-2 (NCOA2) or the peroxisome proliferative activated receptor, gamma, coactivator 1 (PRGC1) depends on subtle but distinct conformational changes activable with selective ligands.[25] Mutational analyses previously revealed the importance of AF-2 binding to proteins like the heat shock protein-90 (HSP90) and 14–3-3 for controlling the equilibrium between alternative GR conformational states adopted upon ligand binding.[31,32] GR may adopt a high or low affinity state for effector substrates depending on mutations, chemical scaffolds of ligands, protons and cofactors.[33] Concentrations of salts, pH and titrations of ligands may also affect allosteric transmission between GR functional domains. Taken together, allosteric coupling of the ligand-binding pocket with the coregulator-binding sites opens the possibility to design drugs and modulators that can exert desired effects via specific downstream pathways.[34]

BOX 1 Key terms

Orthosteric ligands: interact with the pharmacophore of the endogenous ligand.

Allosteric ligands: bind to a site other than the pharmacophore of the natural ligand.

Bivalent ligands: consist of two molecules connected by a linker and binding to distinct sites in the same protein or partners proximal in structure.

Cooperativity: occurs when binding to a site influences binding to another.

Biased signaling: is a differential transduction cascade assigned to peculiar receptor conformations.

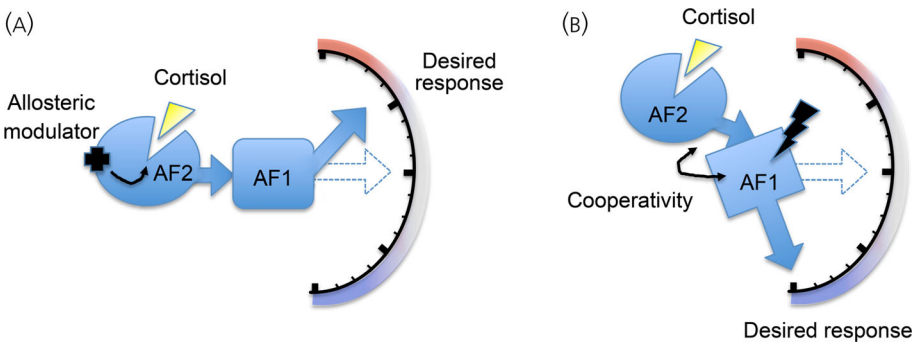

FIGURE 1 Modulation of cortisol response. (A) Allosteric modulation transforms cortisol response. (B) Cooperativity between functional domains synergize with cortisol response

3 | GLUCOCORTICOID RECEPTOR STRUCTURAL COOPERATIVITY

Cooperativity is defined by the structural changes propagated between two or more protein sequences that act dependently on each other (Figure 1B). In particular, the folding of intrinsically disordered protein sequences abundant in the N-terminal domain harboring the activation domain-1 (AF-1) sets the basis for cooperative coupling between distinct functional domains in GR.[35] Direct proof that ligand-induced binding and folding of the intrinsically disordered parts of AF-1 can cooperatively influence the binding to a natively structured domain came from studies of the AF-1 binding with coregulators like the tata-box binding protein (TBP) and the tumor susceptibility-101 (TSG101) that increase the affinity of the DNA-binding domain for palindromic responsive elements.[36,37] Some allosteric ligands act as agonists under certain conditions and as antagonists in others for a given function which contrasts with the orthosteric ligands that typically stabilize one or the other conformation. Switching between positive and negative allosteric modulation of a given function does not necessarily require that the interaction between cortisol and its pharmacophore differs.[33] Instead, the cooperativity between structural domains prior to ligand stimulation could set the evoked response. Adopting conformational surfaces prone to functional cooperativity between domains has an energetic cost that varies between the agonistic and antagonistic folding states.[33] Remarkably, the energy threshold needed to fold the AF-1 can be reduced by cortisol-independent mechanisms.

Post-translational modifications influence the energy requirement for structural cooperativity.[38] For example, phosphorylation sites are abundant in the intrinsically disordered parts of GR and have the competence to switch between dynamic conformations or to stabilize a transition state cued to the biochemical environment.[39] The chemical, steric and electrostatic attributes of the phosphoryl moiety can modify intramolecular interactions like those between Ser211 and Arg214. Folding of the AF-1 is more pronounced when three well-established cortisol-dependent sites (Ser203, Ser211, Ser226) are phosphorylated simultaneously rather than independently corresponding to greater loss-of-function in the triple mutant compared to single mutants.[40] Indeed, Ser211 also makes hydrogen bound to Trp213 when Ser203 and Ser226 are also phosphorylated, forming docking sites for direct binding with coregulators.[41]

Isoforms of GR that differ in length in the intrinsically disordered parts of the N-terminal domain due to alternative translational start codons also exhibit differential activities,[42] DNA-binding affinities,[35] transcriptomes and tissue expressions.[43,44] Moreover, shedding parts of the intrinsically disordered sequences harboring multiple GR phosphorylation sites in the N-terminal domain via a caspase-1 cleavage site blunts the transcriptional response to glucocorticoid therapy, which is associated with disease progression and poor prognosis in patients with acute lymphoblastic leukemia.[45] Often, a proline next to the phosphorylated residue can adopt cis or trans conformations in the amino acid chain to direct protein–protein interactions. This is the case for Pro526 when Thr524 and Ser617 are phosphorylated in the AF-2 domain. The proline isomer then allows the carbonyl oxygen of Leu525 to form a hydrogen bond with amino groups Lys120 and Asn173, stimulating subsequent intermolecular interaction with the cytosolic scaffold protein 14–3-3, a negative regulator of GR transcriptional activity.[32] Mutation of both Thr524 and Ser617 phosphosites in the AF-2 domain impairs GR binding to 14–3-3 but incompletely (by 35%) given the dependency on Ser134 in the AF-1 domain.[46] The phosphorylation of Ser134 is not dependent on glucocorticoid binding, suggesting that cooperativity between the AF-1 and AF-2 domains must be guided by the biochemical context of the cells. Of note, the cortisol-independent phosphorylation sites locate next to predicted α-helices of the N-terminal domain (Figure 2A), unlike cortisol-dependent sites that reside in its most disordered parts (Figure 2B). Therefore, it is paramount to consider how phosphorylation events could promote structural order from entropy for screening new generations of glucocorticoid modulators.

4 | GLUCOCORTICOID RECEPTOR SIGNALING BIAS

Signaling bias is defined as the dynamic structural cooperativity that propagates parts of the full spectrum allosteric transmission between functional domains evoked by cortisol (Figure 3A). Biased signaling of GR results from a large repertoire of folded transitional structures that dynamically switch, via allostery and cooperativity, from a native state to stimulated states. Here, the native state is predetermined at any given time by intracellular biochemical circumstances prior to ligand exposure while the stimulated states are glucocorticoid-bound. A dynamic equilibrium between alternative conformations permits differential binding to downstream effectors.[49] Cooperativity between cortisol′s pharmacophore and effector sites sets the basis for signaling pathway specificity expected to vary between different tissues and cell types.[50] GR relies on dimerization and palindromic DNA ligands—the glucocorticoid-responsive elements (GRE) and rare nonpalindromic negative regulatory elements (nGRE)—to drive transcriptional activation and repression.[51,52] Specific interactors affect the direction of the response as for example, AP-1 and NFκB for transrepression and CREB1, SRC1-3 and CBP for transactivation.[2,53,54]

Timing is also an important factor as the biochemical state of cell activity guides GR access to its coregulators through reversible post-translational modifications and subcellular dynamic distributions (Figure 3B,[55]). For example, GR phosphorylation status has been linked to a GR interaction network with specific coregulators that signal via differential pathways in various cell types.[1,56] GR is phosphorylated in absence of cortisol binding, albeit it is enhanced by agonists but not by antagonists.[57] This means that dephosphorylation of GR is more than an off-switch. Dephosphorylation was previously showed to be a necessary step for other transcription factors to traffic into the nucleus and to bind to DNA.[58] The stoichiometry of phosphorylation at cortisol-dependent and -independent sites reflects a balance between kinases and phosphatases activities available for GR at a given time and space.[2] For instance, in neurons, unliganded GR binds

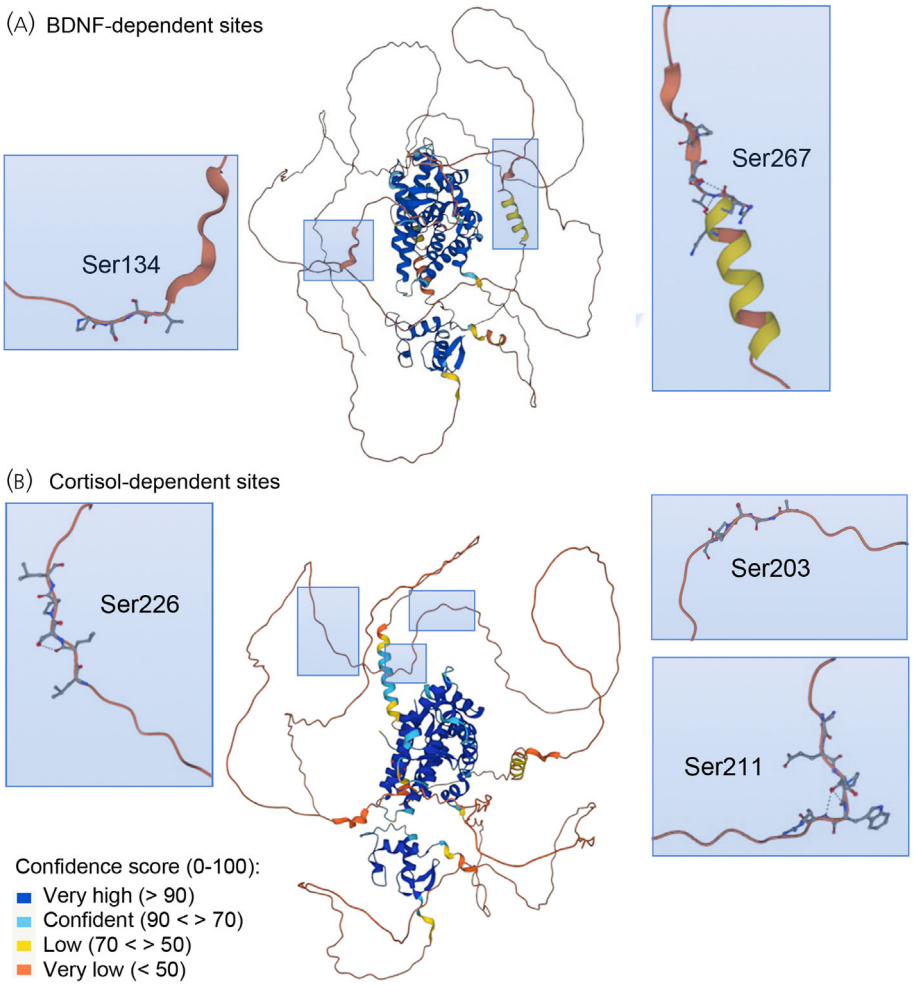

FIGURE 2 Predicited structure of the glucocorticoid receptor distinguishes the cortisol-dependent from the -independent phosphorylation sites. (A) BDNF-dependent sites are adjacent to predicted α-helices in the N-terminal domain. (B) Cortisol-dependent sites locate in the highly disordered unfolded parts of the N-terminal domain. Structure confidence was determined with the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk)[47,48]

to its preferred phosphatase, PP5 ensuring low levels of constitutive phosphorylation, whereas corticosterone binding triggers the release of PP5 from GR as a permissive act for any kinases to take the stage.[59] AKT, CDK, ERK, P38, JNK are all phosphorylating GR at particular sites, which means that cortisol binding concurrent with (one or more) pathways that activate specific kinases may help present GR with multiple conformations in different parts of the cell (Figure 3C). One good example is neurotrophic signaling that transforms cortisol response in neuronal cells[60] and in neuronal networks[61] given that the release of brain-derived neurotrophic factor (BDNF) is restricted by neural activity and behavioral experience (Figure 3D). Yet, such context-dependence is not limited to BDNF as cytokines, neurotransmitters and peptide hormones that can concur with cortisol binding may also transform the glucocorticoid output response.[62]

5 | ENGINEERING OF DESIGNER DRUGS FOR BIASING CORTISOL RESPONSE

To customize the next generation of GR ligands to a desirable effect, one must not only integrate the concepts of allostery, cooperativity and biased signaling into drug discovery programs but also take into consideration the outcome measures for screening that effect. Innovation shall be directed toward the unmet needs, that is not only the tissue or cellular specificity but also the dissociation between the nongenomic and the genomic glucocorticoid effects.[3] Better assays are needed for screening the nongenomic effects because they might have better therapeutic potential than the genomic effects in particular settings. In the brain, rapid nongenomic effects alter neurotransmission with an arsenal of trans-synaptic messengers (retrograde and anterograde) that must impinge on the expression of the slow genomic effects through homeostatic scaling of neuronal networks,[63] coincidence of signaling pathways[62] and epigenomic priming.[64] A possible direction to overcome prior bottlenecks and pitfalls of drug discovery would be to focus on protein–protein interactions between GR and a synaptic (rapid) and/or a nuclear (slow) effector to screen for dissociative modulators. New structural studies of GR interaction with select coactivators better defines the cavities to target in future high throughput screening for dissociative modulators.[65–67]

The conformational states adopted by select cooperative residues can tailor coregulator identity and bias the signaling response between the desired therapeutic and the unwanted side effects. GR interactomes provides a resource for future clinical and biological discovery, and drug design based on ligand/coregulator dependencies.[68,69] With

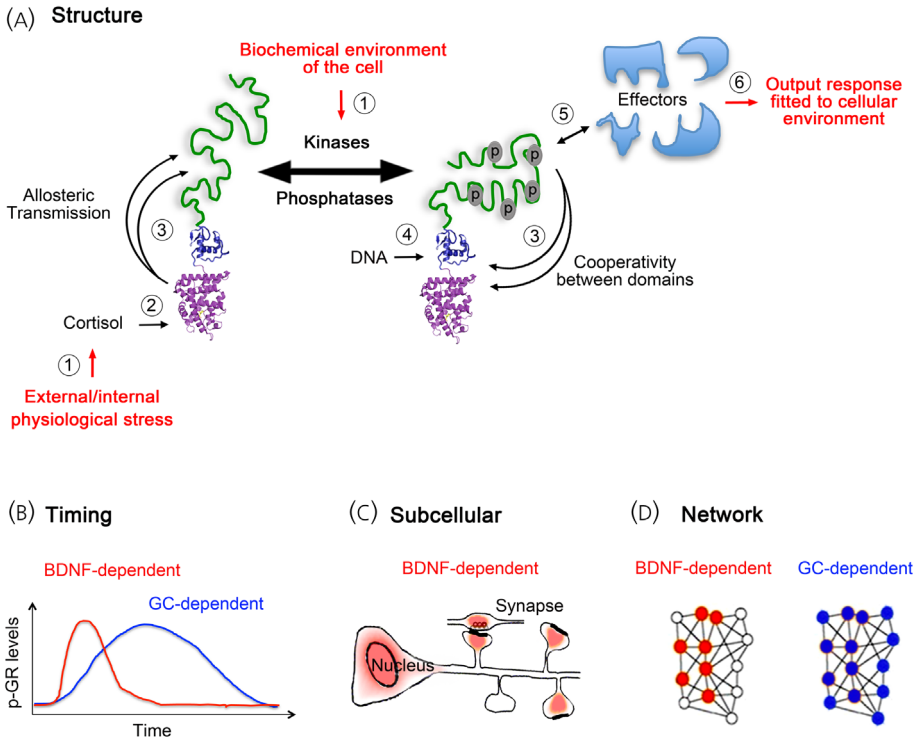

FIGURE 3 Signaling bias of the glucocorticoid receptor on multiple scales. (A) Molecular dynamics in glucocorticoid receptor (GR) domains are influenced by the concentrations of salts, pH and ligands available in the cellular environment (1). The state of cell activity alters GR post-translational modifications mostly in its N-terminal domain made of intrinsically disordered sequences contrary to the natively folded ligand-binding and DNA-binding domains. Reversible phosphorylation sets the range of cooperativity between domains induced by the allosteric transmission of cortisol binding (2) through the shifting of the α-helices (3). Consensus DNA sequences up to 15 base pairs binds to GR zinc fingers (4). Through allostery and cooperativity, GR adopts docking sites for specific effectors within the available range (5). The structural bias determined by phosphorylation prior to cortisol stimulation scales the range of output responses fitted to the cellular environment (6). (B) Distinct temporal domains of GR phosphorylation at BDNF-dependent and GC-dependent sites, rapid (minutes) and slow (minutes to hours), respectively. (C) GR phosphorylation at BDNF-dependent sites distributes within the cytoplasm and synapses, unless cortisol binding forces GR into the nucleus. (D) GR phosphorylation at BDNF-dependent sites is specific of cells responding to BDNF, a neurotrophic factor released at the synapse in an activity-dependent fashion. It is detected in task-allocated cell ensembles expressing c-fos. In contrast, GR phosphorylation at GC-dependent sites is more widespread between cell types and tissues

cortisol on board all the time at naturally occurring oscillating levels,[70,71] the allosteric/cooperative approach to new biased glucocorticoid drugs appears like an added value compared to molecules with constitutive agonistic or antagonistic activities.[72] Bivalent ligand strategy (see Box 1 for definition) is also possible to integrate the allostery of the pharmacophore with the cooperativity of coregulator docking, allowing to bypass unfavorable cellular environments (Figure 4A). This type of approach opens the possibility of using structure-based drug optimization strategies to customize therapeutic effects while minimizing adverse effects.[49] Separating GR transrepression from transactivation activities resulted in glucocorticoids with selective response at proinflammatory gene targets but in vivo applications are still limited by some opposing effects of post-translational modifications and cell type specificities.[10]

Engineering of an allosteric control of protein function is often used to make sensors (e.g., metabolites, neurotransmitters, ions or pH) and modifiers (e.g., chemogenetics and optogenetics).[73] Mutational analysis of a protein prototype revealed that only 5% of residues participate in its core allosteric transmission while a far larger amount of residues enriched at the protein surface and targeted by post-translational modifications act as allosteric modifiers.[74] This suggests more potential for screening drugs targeting the allosteric sites (allo-drug) compared to orthosteric compounds (ortho-drugs). Given that allosteric sites are less conserved than cortisol's pharmacophore, allo-drugs would potentially be more specific relative to other nuclear receptors and offer lesser side effects than ortho-drugs. Validated algorithms can now integrate protein entropy with physical dynamics and perturbation propagation to predict allosteric transmission pathways.[75] Future research will determine if these tools can be applied to GR for developing dissociative ligands. Drugs engineered with a photo-sensitive cage (e.g., photoswitch or photolytic, Figure 4C) have proven superior to parent molecules in terms of spatiotemporal precision and dosage to reach desired effects.[76] Light-switchable nanobodies (camelid single chain antibodies) whose binding to the target surface of interest in a protein could be enhanced or suppressed by light illumination is also an area for future glucocorticoid research.[77] Additionally, phosphorylation within the epitope binding of nanobodies offers the possibility to design phosphorylation-state locked

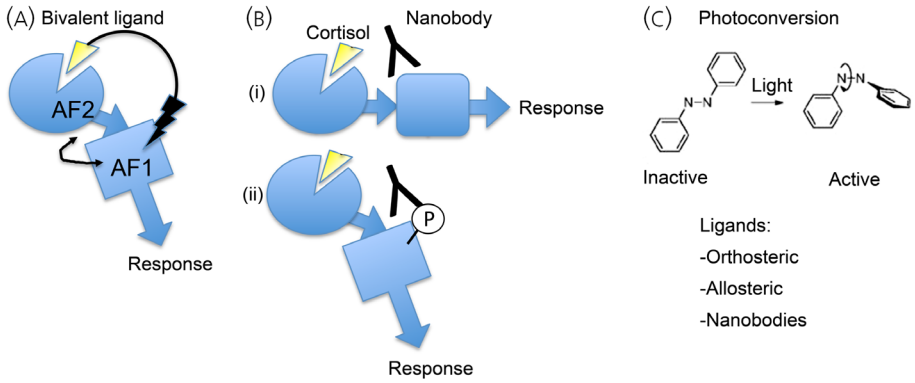

FIGURE 4 Next generation of glucocorticoid modulators. (A) Bivalent ligands could target both the orthosteric and allosteric sites to promote biased signaling. (B) Nanobodies can be selected to promote the activation or suppression of the protein target function, acting at the orthosteric or allosteric sites. Nanobody can be selected to recognize either the (i) nonphosphorylated or (ii) phosphorylated form of the epitope. (C) Glucocorticoid receptor (GR) ligands (orthosteric or allosteric) and nanobodies could be conjugated with a photoconvertible scaffold known as photoswitch or photolytic (chemical or protein) to gain temporal and spatial precision of action thereby limiting side effects. Here, the red-shifted fast-relaxed azobenzene scaffold is presented but many others exist with distinct attributes

drugs for cooperative modulation of glucocorticoids (Figure 4B). Prototypical light-sensitive therapeutics are molecules targeting membrane receptors like G protein-coupled receptors[78] but photoswitchable ligands for nuclear receptors are emerging.[79,80] This increases prospects for the design of new glucocorticoids with better benefit/risk ratio. Prospective applications of phototherapeutics derived from animal studies include metastatic cancer, rheumatoid arthritis, microbial infections, stroke, neurological and psychiatric diseases.[76]

6 | PERSPECTIVES FOR THERAPEUTIC MANIPULATION OF GLUCOCORTICOID RECEPTOR

There are three major disturbances of GR signaling in diseases: (i) ligand availability, (ii) receptor/effector levels, and (iii) signal transduction. A major difficulty in targeting GR is to respect the diversity of responses across contexts, cells and tissues.[64] In most therapeutic contexts, only a limited number of tissues or cell types would be the actual target, and even then, it may be beneficial to only modulate a part of the cellular effects of glucocorticoids (e.g., leaving effects mitochondrial function intact). Improving the benefit/risk ratio of glucocorticoids would require targeting diseased cells, tissues and organs while preserving the healthy ones that also express GR.

Congenital or acquired glucocorticoid resistance is a common feature of cortisol secretion defects, loss-of-function mutations, GR haploinsufficiency or ectopic levels of the decoy GR-β isoform (lacking cortisol-binding) and feedforward MAP-Kinases signaling.[9,81] One difficult issue with acquired glucocorticoid resistance is that it is uneven throughout the cells and tissues of the organism. Correction of glucocorticoid resistance in patients with acute lymphoblastic leukemia is possible with Caspase inhibitors because the ectopic activation of caspase-1 in leukocytes shades the intrinsically disordered parts of GR that promote cooperativity between domains.[45] Inhibitors of P38 kinase restore cortisol sensitivity by acting on GR phosphorylation in airways smooth muscle cells that could benefit patients with asthma and chronic obstructive pulmonary dysfunctions without side effects of chronic glucocorticoid therapy.[82] Activators of the dual-specificity phosphatase DUSP1, a GR-inducible immediate early gene poorly expressed in conditions of glucocorticoid-resistance (e.g., inflammatory bone disorders, atherosclerosis, pulmonary disease,[83] major depression, Alzheimer's disease and others[84]) suppress MAPK activity, reduce GR phosphorylation and accelerate the resolution of inflammation without side effects of chronic glucocorticoid therapy. Moreover, a dissociative compound modifying the phosphorylation status of GR (lack of Ser211 phosphorylation) displays anti-inflammatory properties in mice without inducing hyperglycemia as a side effect.[85] Therefore, structural cavities involved in allosteric modulation of a given GR function via cell-type specific coregulators provide an entry point to design glucocorticoids with lesser pleiotropic effects.[72]

7 | CONCLUSION

GR presents itself in multiple conformations throughout the cells of our bodies depending on ligand titration, receptor/effector forms and levels, and feedback/feedforward signaling at the time of treatment. Existing strategies for biased signaling rely on the presence of either interacting (proinflammatory) transcription factors, or on the presence of specific coregulators molecules. An alternative strategy is to make use of allosteric modulation of GR to change functions, perhaps even at a subcellular resolution.[86] Such a next generation of glucocorticoids should take advantage of GR signaling bias for tailoring safety and efficacy.

AUTHOR CONTRIBUTIONS

Marie-Pierre Moisan: Writing – review and editing. **freddy jeanneteau:** Conceptualization; funding acquisition; project administration;

resources; writing – original draft; writing – review and editing. **Onno C. Meijer:** Writing – review and editing.

CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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