

**REVIEW**

NRF2 Insufficiency in Chronic Disease

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ABSTRACT: Nuclear factor erythroid 2-related factor 2 (NRF2) orchestrates antioxidant defence, electrophile detoxification, and stress recovery across tissues. This paper frames chronic disease vulnerability through the lens of NRF2 insufficiency, defined as reduced functional output of the antioxidant response element programme. We describe the mechanisms that blunt NRF2 signalling, including genetic variation in NFE2L2 and Keap1, epigenetic repression, post-translational degradation routes, and cumulative exposome pressures. Downstream consequences include impaired glutathione-centred buffering, mitochondrial dysfunction, proteostasis failure, inflammatory amplification, and heightened susceptibility to regulated cell death. Using an organ-by-endotype approach, we map recurring patterns across lung, liver, and metabolic tissues, cardiovascular and renal systems, brain, and barrier interfaces that shape systemic ageing phenotypes. Practical biomarkers are reviewed alongside lifestyle and exposome modification strategies, nutritional and nutraceutical approaches, and pharmacological interventions. Preclinical and human evidence for outcome improvement with NRF2 restoration is evaluated, and a risk-management framework addressing boundary conditions such as cancer progression and therapy resistance is proposed. We close with a research agenda prioritising causal study designs, reproducible scoring, and clinically meaningful endpoints to guide precision modulation of NRF2 in chronic disease.

KEYWORDS: Nuclear factor erythroid 2-related factor 2; Kelch-like ECH-associated protein 1; oxidative stress; antioxidant response element; redox homeostasis; detoxification; inflammation; mitochondrial dysfunction; precision medicine

1 Introduction

NRF2 is a central transcriptional regulator of cellular defence against oxidative and electrophilic stress. By coordinating antioxidant buffering, xenobiotic detoxification, and recovery programs, NRF2 helps tissues maintain function despite continual exposure to metabolic by-products and environmental challenges. This protective role is especially important in barrier epithelia and in highly metabolic organs, where redox fluctuations are frequent and cumulative injury can accrue silently [1].

In healthy physiology, NRF2 signalling is tightly controlled by the Keap1-NRF2 axis and ubiquitin-mediated degradation, which keeps basal activity low while preserving rapid inducibility when stress

risks [2]. When reactive species increase, NRF2 escapes degradation, accumulates, enters the nucleus, and drives an antioxidant response element program that restores redox balance and limits covalent injury. In chronic disease, this inducible program can become blunted, delayed, or poorly resolved, creating a functional deficit that persists even when NRF2 protein remains detectable [3]. Because NRF2 signalling is dynamic and cell-type-specific, interpretation improves when localization, target induction, and functional assays agree. Such triangulation reduces misclassification in heterogeneous clinical samples collected outside peak responses [4].

The clinical relevance of NRF2 insufficiency lies in its ability to connect seemingly disparate disorders through shared pathobiology that repeats across organs and over time. Chronic lung disease, metabolic liver disease, vascular remodelling, kidney decline, neurodegeneration, and barrier dysfunction often converge on oxidative injury, persistent inflammation, mitochondrial stress, and impaired detoxification. These convergent features suggest that a weakened NRF2 response can amplify damage and slow recovery after repeated insults, while also lowering the threshold for flare behaviour after new stressors [5].

At the same time, NRF2 biology is not a linear story in which more activation is always beneficial, because sustained hyperactivation can reshape metabolism and stress tolerance in unintended ways. In some cancers, persistent NRF2 signalling can support survival and therapy resistance, which highlights the need for boundary conditions, patient selection, and careful monitoring during therapeutic translation [6].

Throughout this review, we use the term *endotype* to describe a biologically distinct subtype of a disease or condition defined by a specific underlying mechanism, rather than by clinical presentation alone. This concept, originally developed in immunology and pulmonology, is used here to capture recurring patterns of molecular dysfunction that emerge across different organs when NRF2 signalling is insufficient. We outline mechanistic drivers that reduce NRF2 activity, summarize downstream consequences that emerge at molecular and systems levels, and integrate evidence across tissues using shared endotypes. We discuss practical readouts, including NRF2 target gene panels, oxidative damage biomarkers, and functional proxies that capture mitochondrial function, inflammatory tone, and barrier integrity in accessible samples. We then evaluate strategies to counteract NRF2 reduction, from exposome and lifestyle change to nutritional, pharmacologic, and targeted delivery approaches, with emphasis on benefit, risk, and reproducibility [7]. The literature search underpinning this review was conducted in PubMed and Scopus, covering publications from January 2015 to March 2026, supplemented by seminal earlier works identified through cited reference tracing. Search descriptors included NRF2, NFE2L2, Keap1, antioxidant response element, oxidative stress, chronic disease, redox homeostasis, detoxification, mitochondrial dysfunction, inflammation, ferroptosis, biomarkers, and precision medicine, used individually and in combination with organ-specific terms. Only articles published in peer-reviewed English-language journals were included; preprints were excluded.

2 Scope and Structure of the Review

This review examines NRF2 insufficiency as a unifying biological state that can contribute to chronic disease vulnerability across organ systems. We focus on mechanisms that reduce NRF2 activity, on downstream consequences that emerge from impaired antioxidant buffering and detoxification, and on the clinical patterns that recur across lung, liver, and metabolic tissues, cardiovascular and renal systems, brain, and barrier interfaces. Throughout, we treat NRF2 as a dynamic pathway whose output depends on time, cell type, and tissue context, rather than as a static biomarker measured once. The rationale for this review lies in three converging gaps in the existing literature. First, while many previous reviews have discussed NRF2 activation in individual diseases, none have systematically framed NRF2 insufficiency as a shared mechanistic state that generates recurring endotypes across diverse organ systems, a perspective that carries

direct translational implications for patient stratification and trial design. Second, the field currently lacks a practical and reproducible composite biomarker instrument, and this review proposes a conceptual NRF2-score framework as a step towards addressing that need, while recognising that prospective validation in independent cohorts with hard clinical endpoints remains a necessary future step before it can be considered an operationalised instrument. Third, the rapid expansion of pharmacological NRF2 modulators entering clinical development, alongside emerging evidence on their safety boundaries, calls for an integrated synthesis that covers both the biological rationale and the risk-management framework in a single reference document. This review aims to fill that role.

The structure follows a mechanistic to translational arc. We first define what we mean by low NRF2 activity and how it can be measured, then we outline genetic, epigenetic, and post-translational drivers that depress pathway function under chronic stress. We next summarize the molecular consequences, including redox imbalance, mitochondrial dysfunction, proteostasis failure, inflammatory amplification, and regulated cell death susceptibility.

We then integrate organ-specific vulnerability with cross-cutting endotypes, and we propose biomarker approaches that combine NRF2 target signatures with oxidative damage and functional readouts. Finally, we review interventions that may restore NRF2 toward a physiologic range, and we synthesize evidence from preclinical and human studies with explicit safety boundaries. We close with a research agenda emphasizing causality, stratification, and reproducible study design.

3 Conceptual Framework: What Does “Low NRF2 Activity” Mean?

Before discussing mechanisms and diseases, we need a shared conceptual language for interpreting NRF2 biology across tissues and time. In practice, low NRF2 activity refers to reduced functional output of the antioxidant response element program, measured through localization, target induction, and stress responsiveness, rather than single baseline values. Because NRF2 signalling is pulsatile and cell-type dependent, the same marker can mean different things in airway epithelium, hepatocytes, immune cells, or endothelium. We therefore begin with canonical pathway architecture, then build toward operational readouts, thresholds, and the spectrum that spans insufficient activation and maladaptive persistent activation in disease contexts.

Fig. 1 illustrates how genetic, epigenetic, post-transcriptional, and exposome-related factors converge to reduce functional NRF2 output, leading to impaired activation of the antioxidant response element programme. Attenuated NRF2 signalling compromises glutathione-centred redox buffering, phase II and III detoxification, mitochondrial integrity and stress resolution, while amplifying inflammatory signalling and susceptibility to regulated cell death [8]. These interconnected disturbances propagate across tissues, giving rise to recurring vulnerability patterns in lung, liver, and metabolic organs, cardiovascular and renal systems, and the central nervous system, thereby contributing to shared chronic disease endotypes.

3.1 Canonical NRF2 Biology

Canonical NRF2 biology centres on the Keap1-NRF2 axis, a stress-sensing module that calibrates cellular redox defence. Under basal conditions, Keap1 acts as a substrate adaptor for a Cullin 3 ubiquitin ligase complex, promoting NRF2 ubiquitylation and rapid proteasomal turnover. This constant turnover keeps NRF2 activity low, yet preserves rapid inducibility when electrophiles or oxidants suddenly increase. When reactive species accumulate, critical Keap1 cysteine residues become modified, reducing Keap1 capacity to present NRF2 for degradation. Newly synthesized NRF2 then escapes destruction, accumulates, and translocates into the nucleus, where it engages a broad cytoprotective transcriptional response. In

parallel, several signalling pathways can tune NRF2 stability and localization, adding context-specific control beyond Keap1 alone [9].

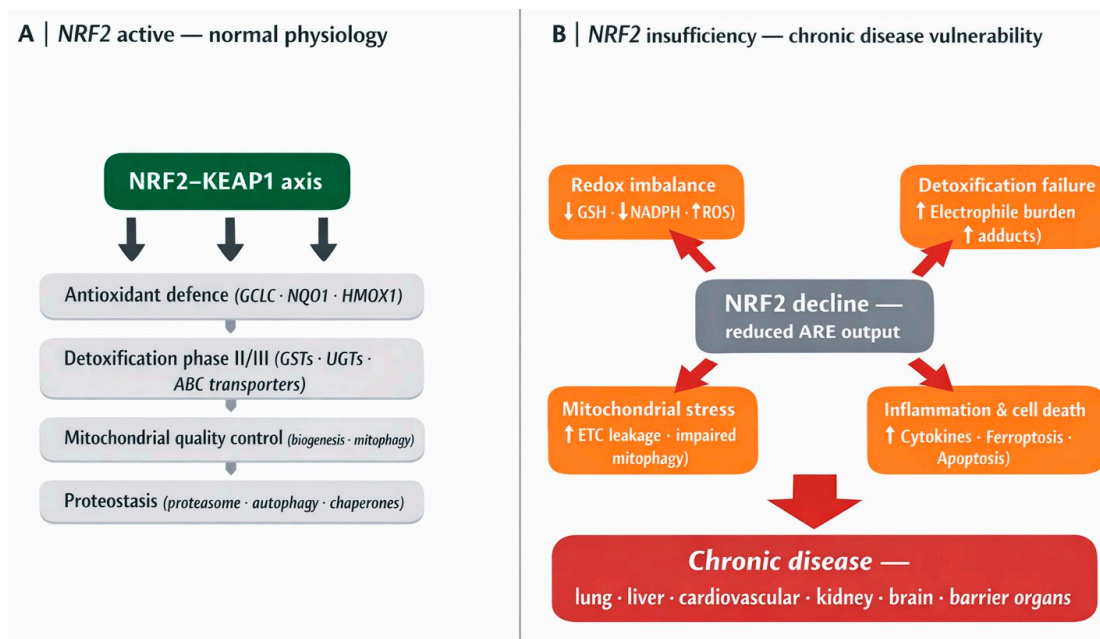


Figure 1: Conceptual model of NRF2 insufficiency as a driver of multiorgan vulnerability in chronic disease. Panel (A) shows the functional output of an intact NRF2-KEAP1 axis, which drives four coordinated cytoprotective programmes: antioxidant defence, phase II/III detoxification, mitochondrial quality control, and proteostasis. Panel (B) illustrates how reduced ARE output dismantles these defences, generating four interconnected failure modules (redox imbalance, detoxification failure, mitochondrial stress, and inflammatory amplification with cell death susceptibility) that reinforce one another through self-sustaining loops and converge on multiorgan chronic disease vulnerability across lung, liver, cardiovascular system, kidney, brain, and barrier organs.

Inside the nucleus, NRF2 heterodimerizes with small Maf proteins and binds antioxidant response elements, often abbreviated as ARE, in promoter and enhancer regions of target genes. This ARE program drives enzymes that restore redox balance, including glutathione synthesis and recycling components such as GCLC, GCLM, SLC7A11, GSR, and thioredoxin-related defences such as TXNRD1. It also induces detoxification and stress response genes, including NQO1 and HMOX1, and expands phase II conjugation systems, including glutathione S-transferases and UDP-glucuronosyltransferases, while supporting NADPH-generating enzymes and xenobiotic efflux transporters. Through these targets, cells not only neutralize reactive molecules, but they also rebuild reducing equivalents and preserve membrane integrity overall. Termination of the response is equally important for homeostasis, since sustained activation can reshape metabolism and cellular behaviour. As stress resolves, Keap1-mediated degradation is restored, nuclear NRF2 diminishes, and transcription returns toward baseline. This reversible on-demand design explains why NRF2 is described as a master regulator of antioxidant and electrophile defence, yet it is tightly controlled in healthy tissues [10].

3.2 What We Mean by “Low NRF2 Activity”: Readouts and Thresholds

In this review, “low NRF2 activity” denotes a functional shortfall in the NRF2-controlled stress response, rather than simply reduced NFE2L2 transcript abundance. We use the term when cells, tissues, or patients

show weakened induction of antioxidant and detoxification genes under conditions that should normally activate the pathway. This distinction matters because NRF2 signalling is dynamic, and a single measurement can miss transient nuclear pulses or compensatory feedback loops that temporarily mask impairment [11].

The most informative readouts and thresholds capture nuclear NRF2 accumulation, DNA binding, and transcriptional output, ideally measured before and after a standardized oxidative or electrophilic stimulus. When nuclear translocation is blunted or when the response fades quickly despite ongoing stress, the inference of impaired pathway activity becomes more robust. Downstream targets provide complementary evidence, and we look for coordinated changes across NQO1, HMOX1, GCLC, GCLM, TXNRD1, SLC7A11, and GSR, rather than isolated shifts in a single marker [12].

Because baseline expression varies across cell types and tissues, thresholds should be anchored to matched controls and expressed as effect sizes, z-scores, or percentile-based pathway scores. In experimental systems, a practical threshold is a reproducible reduction in an ARE reporter signal, or in a predefined NRF2 gene signature, that also translates into greater sensitivity to oxidative challenge. In human studies, where standardized stimuli are rarely feasible, thresholds are often inferred from multi-gene signatures that align with oxidative damage biomarkers, inflammatory tone, and reduced detoxification capacity, while accounting for sampling depth and cell composition [13].

Finally, low activity can occur despite normal NRF2 protein levels, so convergent evidence across localization, transcriptional signatures, and functional assays provides the most defensible overall classification [14].

3.3 Time, Cell-Type, and Tissue Dependence

NRF2 signalling is inherently time-dependent because activation is rapid and reversible, while downstream remodelling can persist longer. Acute oxidative bursts trigger nuclear accumulation, but chronic exposures can produce attenuated pulses, altered feedback, and pathway fatigue. These kinetics mean that a single snapshot may misclassify status, especially when sampling occurs outside the peak response window [15].

Cell-type dependence arises from distinct basal redox loads and transcriptional wiring across lineages. Phagocytes face frequent oxidant flux, and they often couple NRF2 outputs to inflammatory programs, while neurons prioritize proteostasis and mitochondrial protection. Fibroblasts, endothelial cells, and epithelial barriers each integrate NRF2 with repair cues, which shape how strongly ARE targets are induced. This helps explain why the same stimulus yields divergent expression profiles across tissues [16].

Tissue dependence is strongly driven by exposure, metabolism, and local microenvironmental constraints. The lung epithelium confronts airborne electrophiles, the liver processes xenobiotics and lipids, and the kidney concentrates solutes that can amplify oxidative injury. Oxygen tension, iron handling, and nutrient availability vary by organ, and these factors tune lipid peroxidation risk and detoxification needs [17].

Even within one organ, spatial niches and disease states reshape NRF2 behaviour over time. For example, hypoxic zones, fibrotic regions, and inflamed compartments alter Keap1 abundance, ROS production, and signalling cross-talk. For interpretation, measurements should be matched to the relevant time scale, cell population, and tissue context, rather than assuming one universal threshold [18].

3.4 NRF2 Decline: Cause vs. Consequence vs. Amplifier

The question of whether NRF2 decline is a cause, a consequence, or an amplifier is practical because it guides experiments and informs therapy design. When NRF2 activity is low early, antioxidant buffering

and detoxification capacity fall, and reactive species accumulate more readily during routine metabolic stress. Over time, this biochemical climate can seed mitochondrial dysfunction, inflammatory priming, and molecular damage that gradually pushes tissues toward chronic disease [19].

In many disorders, however, NRF2 decline appears downstream, reflecting chronic inflammation, metabolic overload, persistent infection, or sustained toxic exposure. These pressures can reshape the Keap1-NRF2 axis through epigenetic repression, altered proteostasis, and signalling changes that blunt inducible responses. Senescence programs and impaired autophagy can further shift redox set points, so ARE targets rise less, even when oxidative stress remains substantial. In this framing, low NRF2 becomes a marker of burden, and it may improve when the primary driver is relieved or controlled [20].

Even so, diminished NRF2 can still contribute functionally, because reduced resilience increases sensitivity to later insults and environmental triggers. A third interpretation, often closest to reality, is that NRF2 decline amplifies pathology inside self-reinforcing loops. An initial insult raises oxidative load and inflammatory tone, which suppresses NRF2 outputs and deepens redox imbalance. Reducing NRF2 then worsens mitochondrial injury and detoxification failure, which increases damage signalling and sustains inflammatory programs across cell populations, especially during repeated exposures [21].

Distinguishing cause from consequence demands timing-aware designs that separate association from mechanism, while accounting for tissue heterogeneity, and explicitly for sex, age, and comorbidities. Longitudinal sampling, tissue- or cell-type-specific perturbations, and rescue experiments can show whether NRF2 restoration changes progression rather than only biomarkers. For clinical translation, it is often enough to accept that all three roles can coexist, shifting with tissue, stage, and context [22]. Three clinical observations illustrate how this tripartite framing translates into measurable signals, without yet constituting proof of causation. Several clinical observations support the view that NRF2 deficiency can precede and contribute to tissue damage, rather than merely reflecting it. In chronic obstructive pulmonary disease, lower NRF2 target gene expression in airway epithelium has been documented in smokers who have not yet developed overt obstruction, suggesting that pathway suppression accompanies early exposure burden before clinical disease is established [23]. In metabolic dysfunction-associated steatotic liver disease, reduced hepatic NRF2 activity correlates with disease severity and precedes histological progression in animal models in which dietary stress is controlled, pointing to an association consistent with pathway involvement rather than a purely secondary marker, though causal direction in human disease requires further longitudinal confirmation [24]. In diabetic kidney disease, clinical trials with bardoxolone methyl, an NRF2 activator, improved estimated glomerular filtration rate in patients with stage 3b to 4 chronic kidney disease, providing interventional evidence consistent with NRF2 pathway involvement in a measurable functional endpoint, although the complex haemodynamic and safety profile of bardoxolone methyl means that eGFR improvement alone cannot be attributed solely to NRF2 restoration [25]. Although these examples do not prove that NRF2 deficiency is the sole or initiating event in each context, they collectively suggest that insufficient NRF2 activity may not be simply a bystander, and that interventions targeting this pathway are associated with measurable functional signals, although evidence of disease modification through NRF2 restoration specifically remains to be established in adequately powered causal study designs.

3.5 Systems-Level Vicious Cycles

At a systems level, reduced NRF2 activity rarely acts in isolation, because redox control couples to metabolism, immunity, and tissue repair. When the NRF2 program weakens, reactive oxygen species rise, detoxification slows, and oxidized proteins, lipids, and DNA accumulate. Proteostasis is strained, and

unfolded protein responses often divert resources from recovery. These signals increase danger sensing, which shifts cytokine networks toward persistent, low-grade inflammation [26].

Inflammatory signalling then feeds back onto NRF2 regulation through altered Keap1 function, stress kinase activity, and transcriptional repression in susceptible cell populations. As immune and stromal cells sustain oxidant production, epithelial and endothelial barriers experience repeated microinjury, which promotes recruitment and oxidative burden. In barrier organs, this instability can permit microbial products to enter, sustaining immune activation [27].

Mitochondrial dysfunction forms a reinforcing node because impaired electron transport increases oxidant leakage and reduces NADPH for antioxidant recycling. Damaged mitochondria also release mitochondrial DNA and signals that activate innate sensors and inflammasome pathways, amplifying inflammation and cellular stress [28]. If autophagy and mitophagy are insufficient, dysfunctional organelles persist, and oxidative signalling remains elevated [29].

A third node involves senescence and maladaptive remodelling, where persistent stress drives cell cycle arrest and a senescence-associated secretory phenotype. Senescent cells secrete proinflammatory mediators, matrix modifiers, and oxidant-generating enzymes, which propagate dysfunction into neighbouring tissues and lock the system into self-sustaining loops. This framework explains why NRF2 restoration may interrupt progression, yet timing and tissue-specificity remain critical for clinical benefit [30].

3.6 The Spectrum Model: NRF2 Insufficiency vs. Maladaptive Hyperactivation

Rather than treating NRF2 status as simply high or low, the spectrum model views NRF2 signalling as a continuum shaped by time, tissue context, and selective pressures. NRF2 insufficiency describes a state in which cells fail to mount an adequate ARE program when confronted with oxidative or electrophilic stress, resulting in weaker detoxification, lower glutathione support, and reduced stress tolerance across diverse organ systems. This phenotype often emerges during chronic inflammation, metabolic overload, or aging, where repeated stress pulses blunt inducible NRF2 responses and amplify redox imbalance over time [31].

At the opposite end lies maladaptive hyperactivation, where NRF2 becomes persistently elevated and decoupled from normal resolution cues. In several cancers, mutations in Keap1 or NRF2 regulators can sustain the pathway, enhancing antioxidant buffering, xenobiotic clearance, and survival under hypoxia or chemotherapy exposure. Related patterns may appear in non-malignant tissues under prolonged stress, where chronic activation reshapes metabolism, shifts immune tone, and can support fibrotic remodelling [32].

Clinically, these extremes may look deceptively similar if a single marker is measured at one moment in time. Interpretation should therefore combine nuclear localization, coordinated target gene signatures, and functional stress challenges, while accounting for cell-type composition and disease stage. Therapeutic strategies should aim for restoration toward a physiological range, using dose, timing, and tissue targeting to avoid overshooting into sustained activation and unintended adaptations [33,34].

Fig. 2 represents NRF2 activity as a continuum, in which insufficient signalling is associated with reduced antioxidant response element output and diminished stress resilience, whereas sustained hyperactivation may become maladaptive under specific selective pressures. The central objective for translation is range-seeking restoration, using stratification and biomarker-confirmed engagement to move patients towards a physiological window rather than maximising induction. Boundary conditions are highlighted for contexts in which persistent NRF2 activation could be undesirable, including oncology-related therapy resistance, chronic infection settings, and late-stage fibrosis dominated by remodelling and senescence.

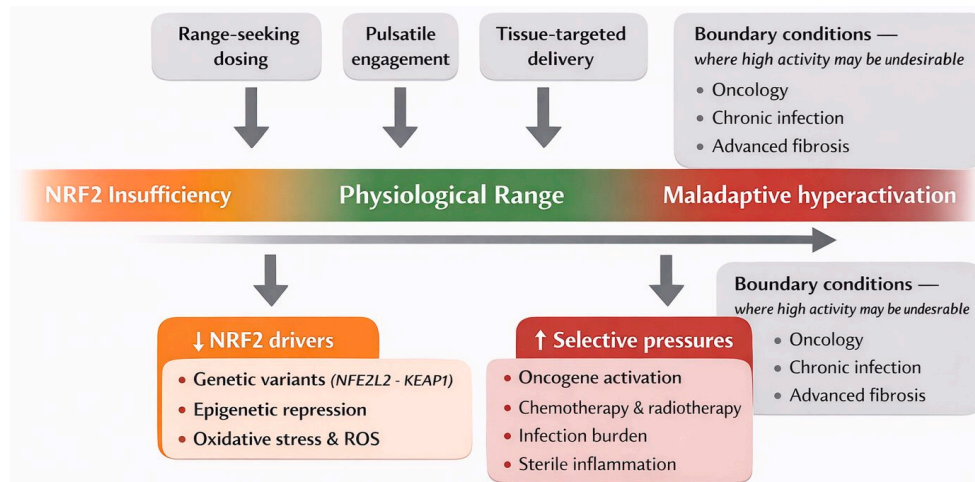


Figure 2: Spectrum model of NRF2 signalling and risk-managed restoration towards a physiological range. The horizontal axis represents the continuum of NRF2 activity, ranging from insufficient signalling on the left to maladaptive hyperactivation on the right, with the physiological window indicated in the centre. Upward arrows (↑) denote increasing pathway output, target gene induction, antioxidant buffering capacity, and stress resilience as activity rises towards the physiological range. Downward arrows (↓) denote declining pathway output, reduced glutathione support, impaired detoxification, and diminished stress tolerance as activity falls below the physiological window. Boundary conditions at the right extreme highlight contexts in which persistent hyperactivation may become maladaptive, including oncology-related therapy resistance, chronic infection settings, and late-stage fibrosis dominated by remodelling and senescence. The central objective for therapeutic translation is range-seeking restoration, using stratification and biomarker-confirmed engagement to move patients towards the physiological window rather than maximising induction.

4 Why NRF2 Activity Declines: Mechanistic Drivers

NRF2 activity can decline through multiple layers of regulation that accumulate over years of exposure and disease progression. Some mechanisms reduce NRF2 abundance, others prevent effective nuclear signalling, and others blunt transcriptional output despite apparently intact protein levels. In many patients, these layers overlap, producing a functional deficit that is difficult to infer from any single assay. To organize the evidence, we group drivers into genetic determinants that shift baseline set points, epigenetic and post-transcriptional repression that locks in lower inducibility, post-translational degradation routes that tune protein stability, and exposome and lifestyle inputs that repeatedly challenge the system until adaptive capacity erodes [8].

4.1 Genetic Determinants

Genetic determinants of NRF2 signalling begin with variation in NFE2L2, the gene encoding NRF2, and in Keap1, its principal cytosolic repressor. Common single-nucleotide polymorphisms can subtly shift baseline expression or inducibility, while rare variants may alter protein stability, nuclear trafficking, or DNA binding. These changes often act as vulnerability modifiers, increasing sensitivity to oxidative burden without causing disease on their own [34,35].

In Keap1, variants that weaken NRF2 binding or reduce Keap1 abundance tend to increase NRF2 activity, whereas variants that strengthen repression can contribute to NRF2 insufficiency in chronically stressed tissues [36]. Additional components of the ubiquitin machinery, including CUL3 and related

adaptor proteins, can also influence turnover rates and response amplitude. In practice, the functional impact depends on allele frequency, tissue expression, and environmental exposures [37].

Beyond the core axis, multiple regulators shape NRF2 through Keap1-independent routes, including kinases that modulate degradation or nuclear export, and transcriptional cofactors that tune ARE selectivity. Genetic differences in these regulators may not map neatly onto a single biomarker, yet they can help explain why individuals exposed to similar stressors diverge in clinical trajectories. Polygenic effects are likely, especially for complex chronic diseases in humans overall [38].

Risk variants therefore deserve interpretation in context, with attention to ancestry, linkage patterns, and gene-by-environment interactions. The most actionable insight is often not a single variant, but a genetic backdrop that shifts the threshold for NRF2 activation, and lowers resilience when inflammatory or metabolic stress accumulates over years, in phenotyped cohorts and designed mechanistic studies [39].

4.2 Epigenetic and Post-Transcriptional Repression

Epigenetic and post-transcriptional repression can lower NRF2 activity without changing the coding sequence, and this mechanism is especially plausible in chronic disease settings where exposures accumulate over time. DNA methylation at regulatory regions of NFE2L2, or at loci encoding NRF2 target genes, can reduce transcriptional responsiveness and flatten inducible expression profiles. In parallel, histone marks that promote chromatin compaction can restrict access to antioxidant response elements, so that even normal nuclear NRF2 levels yield a muted transcriptional program [40].

These layers often interact with inflammation and metabolic stress, which can reshape chromatin through cytokine-driven signalling and altered metabolite availability. Acetyl-CoA, SAM, and NAD plus dependent deacetylase activity influence histone acetylation and methylation landscapes, linking energy state to transcriptional capacity. As a result, the same oxidative stimulus can produce different NRF2 outputs in the same tissue at different disease stages [41].

Post-transcriptional repression by miRNAs adds another level of tuning, because miRNAs can reduce NFE2L2 mRNA stability or translation, and can also target key NRF2 effectors such as detoxification enzymes and glutathione pathway genes [42]. This matters because miRNA profiles shift with aging, senescence, and immune activation, and they can enforce a long-lasting reduction in stress responsiveness. In practical terms, epigenetic and miRNA mediated effects are often reversible, yet reversal may require sustained removal of the upstream stressor, or targeted interventions that restore permissive chromatin and translation capacity [43].

4.3 Post-Translational Control and Degradation

Post-translational regulation is the most immediate way cells tune NRF2, because it determines how long the protein survives and where it localizes. Under basal conditions, Keap1-dependent degradation dominates, with Keap1 recruiting NRF2 to a CUL3 ubiquitin ligase complex and directing rapid proteasomal clearance [4].

Additional post-translational inputs include acetylation and deacetylation that influence DNA binding, as well as phosphorylation events that modulate nuclear import, nuclear export, and transcriptional cofactor recruitment. Because these layers can either stabilize NRF2 or accelerate its turnover, the same tissue may display low NRF2 activity despite normal Keap1 levels, or show high NRF2 signatures driven by altered proteostasis [44].

Stress alters this Keap1-dependent process through covalent modification of Keap1 sensor cysteines, which weakens substrate presentation and allows newly synthesized NRF2 to accumulate. The pathway is

also shaped by competitive interactions, including p62 mediated sequestration of Keap1 during impaired autophagy, which can shift the balance toward higher NRF2 activity in selected contexts [45].

At the same time, Keap1-independent routes provide parallel control, and they often become prominent during chronic signalling or metabolic remodelling. A well-described mechanism involves phosphorylation of NRF2 by kinases such as GSK3, which creates a recognition motif for beta-TrCP, enabling ubiquitination and degradation through alternative E3 ligase machinery. Cuadrado A. Structural and functional characterization of Nrf2 degradation by glycogen synthase kinase 3, β -TrCP [46].

For interpretation and therapeutic design, it is essential to identify which degradation route dominates in the relevant cell type and time window. This distinction matters in human tissues, where cell mixtures can obscure the dominant regulatory route locally [9].

4.4 Exposome and Lifestyle Inputs

Exposome and lifestyle inputs provide a practical explanation for why NRF2 signalling gradually weakens across the life course, even when genetics remain unchanged. Aging reshapes redox balance, proteostasis, and immune surveillance, and it also increases the burden of senescent cells that maintain inflammatory tone. With repeated low-grade oxidative challenges, inducible NRF2 pulses may fade, so identical triggers later produce lower nuclear accumulation and fewer ARE targets [47].

Among external exposures, smoking/pollution delivers electrophiles and particulates to barrier tissues, while simultaneously promoting systemic cytokine programs that repress cytoprotective transcription. Diet influences NRF2 through micronutrient adequacy, oxidized lipid intake, and microbiome-derived inflammatory cues, and these effects often accumulate quietly. Metabolic stress, especially when paired with insulin resistance, increases mitochondrial oxidant leakage and reduces NADPH availability for antioxidant recycling and tissue repair [48].

Infections can activate NRF2 transiently during acute responses, yet recurrent infections may lock in inflammatory signalling that limits pathway flexibility over time. Drugs/toxins add further complexity, because some agents induce NRF2 briefly, whereas others elevate oxidative burden or impair mitochondrial function and autophagy pathways. Sleep disruption, low physical activity, and harmful alcohol patterns can further reduce NRF2-responsiveness, particularly when combined with obesity and ongoing psychosocial stress in daily life [49]. Industrial and environmental contaminants add a further layer of exposome complexity that deserves separate consideration. Heavy metal exposure represents an additional and clinically relevant exposome input that can bidirectionally perturb NRF2 signalling. Metals such as arsenic, cadmium, lead, and mercury generate reactive oxygen species, modify Keap1 sensor cysteines, and can either transiently induce or chronically suppress NRF2 activity depending on concentration, duration, and cell type. At low acute doses, some heavy metals may activate NRF2 through cysteine modification, whereas sustained or high-level exposure depletes glutathione, overwhelms phase II detoxification, and ultimately impairs inducible pathway responses. This pattern is relevant to occupational and environmental health contexts, particularly in populations with cumulative multi-metal exposure, and it further underscores why exposome characterisation should accompany NRF2 biomarker assessment in epidemiological studies [50].

5 Molecular and Cellular Consequences of NRF2 Decline

Once NRF2 output is persistently reduced, downstream consequences unfold in a coordinated sequence that affects multiple core stress response modules. The most immediate effect is a narrowing of redox buffering capacity, but longer-term effects include impaired detoxification, disrupted mitochondrial quality control, and loss of proteostasis. These changes then feed into inflammation and immune dysregulation, and

they can increase susceptibility to regulated cell death in vulnerable contexts [51]. Because these modules interact, the clinical phenotype often reflects reinforcing loops rather than a single failing pathway. In the sections below, we summarize each consequence as a distinct node, while emphasizing the connections that allow NRF2 decline to amplify chronic disease trajectories across tissues.

Fig. 3 summarises how a sustained functional decline in NRF2 can act as a unifying upstream driver of multiple, partially interdependent cellular failure modes that are repeatedly implicated across chronic disease settings. The central concept is that lower NRF2 transcriptional output reduces the overall capacity for inducible stress responses, which in turn weakens redox buffering, electrophile detoxification, mitochondrial quality control, and proteostasis, while concurrently shifting inflammatory tone and increasing vulnerability to regulated cell death. The layout is intentionally modular because each box represents a mechanistic “module” that can be discussed independently in the text, yet the arrows emphasise that these modules rarely operate in isolation within tissues under chronic stress. The perimeter connections highlight self-reinforcing loops, for example, redox imbalance worsening mitochondrial dysfunction, mitochondrial stress aggravating proteostasis collapse, proteostatic stress sustaining inflammatory signalling, and inflammation feeding back into oxidative stress, thereby consolidating a progressive pathological trajectory.

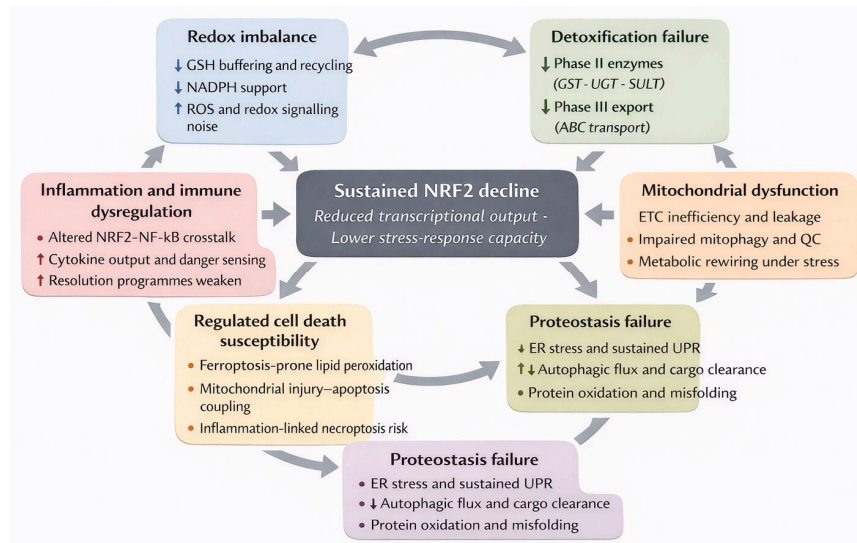


Figure 3: Integrated molecular and cellular consequences of sustained NRF2 decline. The central box represents a sustained reduction in NRF2 transcriptional output, which reduces the overall capacity for inducible stress responses across tissues. Six interconnected modules radiate from this central node: (i) redox imbalance and loss of antioxidant buffering, reflecting erosion of glutathione-centred defences and NADPH availability; (ii) impaired electrophile detoxification, reflecting reduced phase II and phase III conjugation and efflux capacity; (iii) mitochondrial dysfunction and metabolic rewiring, driven by oxidative damage to electron transport chain components and impaired mitophagy; (iv) proteostasis failure, including endoplasmic reticulum stress, autophagy deficits, and accumulation of misfolded proteins; (v) inflammatory amplification and immune dysregulation, driven by oxidised lipids, mitochondrial danger signals, and cytokine network activation; and (vi) susceptibility to regulated cell death, encompassing ferroptosis, apoptosis, and pyroptosis pathways. Upward arrows (↑) indicate processes that increase as a consequence of reduced NRF2 output, such as reactive oxygen species accumulation, electrophile burden, and inflammatory signalling. Downward arrows (↓) indicate processes that decrease, including glutathione synthesis, detoxification enzyme activity, mitochondrial membrane potential, and proteostatic capacity. Perimeter arrows depict self-reinforcing loops that consolidate a progressive pathological trajectory across organ systems.

5.1 Redox Imbalance and Loss of Antioxidant Buffering

When NRF2 activity declines, redox balance becomes fragile, because oxidant production continues while inducible defences no longer scale. Reactive oxygen species persist longer, and they drive chain reactions that injure proteins, lipids, and nucleic acids in vulnerable cells. Even modest inflammatory signaling can then push tissues toward a chronically oxidized and damage-prone state [52].

A central vulnerability is the erosion of glutathione buffering, since NRF2 normally coordinates substrate supply, synthesis, and recycling within GSH systems. As the reduced to oxidized glutathione ratio falls, thiol-sensitive enzymes lose activity, membrane transporters drift, and redox signalling shifts toward persistent stress programs [10].

This shift is not only biochemical, because redox state also shapes proliferation, differentiation, and immune activation trajectories. Because NADPH supply often becomes limiting, antioxidant enzymes like peroxiredoxins and catalase cannot fully reset redox tone [53].

NRF2 decline also weakens enzymatic defences that neutralize electrophiles and redox cycling compounds, with NQO1/HO-1 serving as accessible sentinels of pathway output in many studies. Here, we use the shorthand phrase GSH systems, NQO1/HO-1, etc., to emphasize that coordinated nodes fail together, rather than one marker moving alone. When NQO1 activity drops, quinone redox cycling can intensify, amplifying oxidative flux and depleting reducing equivalents needed for recovery. When HO-1 induction is blunted, adaptive heme handling falters, and pro-oxidant heme species accumulate during inflammation. In parallel, reduced phase II conjugation prolongs reactive intermediate lifetimes, increasing covalent stress signalling locally [54].

Over time, this imbalance becomes self-reinforcing because oxidant stress injures mitochondria, lowers NADPH availability, and slows antioxidant recycling. Clinically, the pattern aligns with elevated oxidative damage biomarkers, reduced stress tolerance, slower recovery after insults, and progressive tissue dysfunction [55].

5.2 Impaired Detoxification and Electrophile Handling

Beyond neutralizing reactive oxygen species, NRF2 coordinates a broad detoxification architecture that limits the lifetime of electrophiles and reactive intermediates. When NRF2 activity is low, phase II conjugation responses decline, and potentially harmful compounds persist longer in cells and tissues. This matters because many environmental toxicants, lipid peroxidation products, and drug metabolites act as electrophiles that covalently modify proteins and disrupt signalling networks [56].

Impaired detoxification and electrophile handling (phase II/III responses) often begin with reduced expression of glutathione S transferases, UDP glucuronosyltransferases, and sulfotransferases, which normally add conjugates that increase solubility and reduce reactivity. These conjugation steps are tightly linked to substrate availability, especially glutathione and activated sulphate or glucuronic acid, so redox imbalance can compound detoxification failure. As a result, electrophile adduct formation increases, altering enzyme activity, mitochondrial proteins, and membrane transport properties, and creating neoantigens that may sustain immune activation [57].

Phase III transport, including ATP binding cassette efflux pumps, also depends on NRF2-regulated transcription, and its weakening restricts export of conjugated metabolites and xenobiotics. Even when conjugation occurs, slower efflux can prolong intracellular exposure and raise the risk of back reactions or compartmental redistribution. In barrier tissues, reduced efflux capacity can intensify local inflammation and can also shift microbiome interactions by changing luminal metabolite composition [58].

Clinically, this combined phase II/III impairment can manifest as heightened sensitivity to air pollution, tobacco smoke, occupational chemicals, and drug-induced stress, especially in older or metabolically compromised individuals. It also creates mechanistic plausibility for multi-organ vulnerability, because detoxification bottlenecks in liver, kidney, lung, and endothelium can amplify systemic exposure and promote chronic inflammatory sequelae [59].

5.3 Mitochondrial Dysfunction and Metabolic Rewiring

Mitochondrial dysfunction is a common consequence of NRF2 decline because redox buffering and quality control become insufficient. Electron transport chain components are especially sensitive to oxidative modification, and small defects can increase electron leak. This leak raises mitochondrial reactive oxygen species, which further damages mitochondrial proteins and membranes [60].

As oxidative stress accumulates, mitochondria may shift toward fragmented networks, reduced membrane potential, and impaired mitophagy. When damaged organelles are not cleared efficiently, cells maintain a population of underperforming mitochondria that continuously generate stress signals [61].

At the same time, reduced NRF2 output can limit NADPH regeneration, lowering the capacity to recycle glutathione and thioredoxin systems that protect mitochondria. Mitochondrial DNA and oxidized cardiolipin can then reach the cytosol, activating innate sensors and sustaining cytokine programs beyond the initiating insult [62].

Metabolic rewiring often follows across chronically stressed cell types, with cells increasing glycolytic flux to compensate for reduced oxidative phosphorylation efficiency. This shift can support short-term survival, yet it also promotes lactate accumulation, alters signalling through redox-sensitive enzymes, and changes substrate use in tissues. Fatty acid oxidation can become inefficient, and lipid intermediates accumulate, which further impairs insulin signalling and mitochondrial dynamics [51].

The net result is a reinforcing loop, because mitochondrial stress amplifies inflammation and lowers cellular resilience to subsequent insults. Over time, energy deficits and redox instability impair repair processes, reduce barrier integrity, and accelerate functional decline across organ systems [63].

5.4 Proteostasis Failure: ER Stress, Autophagy/Mitophagy Deficits

Proteostasis failure becomes more likely when NRF2 signalling is persistently reduced, because redox imbalance increases protein oxidation and misfolding. Chaperone capacity is then consumed by damaged substrates, and the endoplasmic reticulum becomes a bottleneck for secreted and membrane proteins. This pressure promotes ER stress, and it activates adaptive programs that can restore folding, yet they often remain incomplete during sustained inflammatory or metabolic overload [64].

When ER stress persists, unfolded protein response signalling gradually shifts from protective remodelling toward inflammatory outputs that disrupt tissue homeostasis. Oxidized lipids and altered calcium handling destabilize the luminal environment, and they reduce the fidelity of disulphide bond formation. In parallel, reduced NRF2 output limits NADPH availability and phase II defences, which increases reactive aldehydes that covalently modify nascent proteins. These adducts impair trafficking, increase aggregation, and prolong energy intensive quality control, leaving fewer resources for repair [65].

Autophagy is a second pillar of proteostasis, and autophagy/mitophagy deficits can lock in dysfunction once damaged cargo accumulates. When autophagic flux declines, p62 positive aggregates increase, lysosomal acidity falls, and mitochondria with low membrane potential persist longer. Defective mitophagy sustains oxidant leakage and releases mitochondrial DNA, which activates innate sensors and reinforces cytokine programs [66].

Over time, these coupled failures reduce barrier integrity, impair regeneration, and increase susceptibility to secondary insults that would otherwise be tolerated. Therapeutic strategies that restore NRF2 activity may help, but benefits will depend on improving clearance pathways and reducing the upstream stressor in clinical settings [16].

5.5 Inflammation and Immune Dysregulation

Inflammation and immune dysregulation emerge readily when NRF2 signalling is insufficient, because redox imbalance amplifies danger sensing and cytokine release. Oxidized lipids, protein adducts, and mitochondrial stress signals activate innate immune pathways, and they lower the threshold for sustained myeloid activation. Over time, this favours a chronic, low-grade inflammatory milieu that perturbs tissue repair and metabolic homeostasis [67].

In immune cells, NRF2 normally supports resolution by limiting excessive reactive species and by sustaining antioxidant buffering during activation bursts. When that buffering is weak, phagocytes can produce more oxidants, but they also suffer greater self-injury and altered antigen processing. This pattern can skew macrophage polarization, intensify neutrophil extracellular trap formation, and promote endothelial activation that increases leukocyte recruitment [68].

Mechanistically, NRF2-NF- κ B crosstalk provides a useful framework, because both pathways respond to stress cues and co-regulate many inflammatory genes. Reduced NRF2 output can remove constraints on NF- κ B driven transcription, while persistent NF- κ B activity can repress antioxidant programs through chromatin and signalling feedback. The result is a self-sustaining circuit in which inflammation deepens oxidative stress, and oxidative stress further stabilizes inflammatory signalling across compartments [69].

Adaptive immunity can also be affected, since redox tone shapes lymphocyte differentiation, exhaustion, and tolerance programs. In barrier tissues, repeated inflammatory microinjury alters epithelial integrity, increases microbial product exposure, and reinforces immune activation. Clinically, this translates into greater flare susceptibility, slower recovery after insults, and higher risk of multisystem complications when metabolic stress and environmental exposures accumulate. These dynamics help explain why NRF2 restoration may reduce inflammation, yet outcomes depend on timing, specificity, and the underlying trigger [70].

5.6 Regulated Cell Death Susceptibility

Regulated cell death susceptibility (e.g., ferroptosis-prone contexts) becomes more clinically relevant when NRF2 activity is reduced, because the cellular capacity to buffer lipid oxidation is narrowed. In this setting, oxidative stress is not only a damaging by-product, but it becomes a deterministic signal that can push cells toward irreversible loss of viability. The shift is often subtle at first, yet it meaningfully lowers the threshold for injury during inflammation or toxic exposure [71].

Ferroptosis-prone contexts are especially favoured when glutathione availability falls, cystine import is constrained, and lipid peroxides accumulate in iron-rich environments. NRF2 normally supports several of these protective nodes through coordinated regulation of glutathione synthesis, redox recycling, and genes involved in iron sequestration and export [72].

When this support weakens, membranes become vulnerable to peroxidation cascades, and small redox perturbations can trigger runaway damage. In parallel, inflammatory signalling can increase free iron and reactive lipid species, intensifying the local conditions that promote ferroptotic commitment [70].

Other regulated death programs may also become easier to trigger when antioxidant defences are impaired, including apoptosis during mitochondrial injury and necro-inflammatory death when immune

signals are persistent. Importantly, the dominant death mode is tissue and stage-dependent, and it reflects the balance between lipid handling, mitochondrial integrity, and immune activation in each niche [73,74].

This framework helps explain why restoring NRF2 signalling can be protective, while also emphasizing that timing and context determine which cell populations are actually rescued [75].

6 Organ Systems and Clinical Endotypes of NRF2 Insufficiency

In the following organ focused sections, each system is discussed using the same organizing pattern, vulnerability drivers, dominant endotypes, exemplar diseases, biomarkers, evidence, and therapeutic implications. Vulnerability drivers describe why a given tissue is repeatedly stressed, including exposure, metabolic demand, and local immune features. Dominant endotypes summarize the recurring biological patterns that appear when NRF2 responses are insufficient, such as chronic inflammation, lipotoxic injury, or fibrosis-prone remodelling. Exemplar diseases anchor these endotypes in recognizable clinical entities, while biomarkers and evidence highlight the readouts that best capture pathway status and injury burden in that organ. Finally, therapeutic implications translate the biology into practical strategies, emphasizing burden reduction, targeted restoration, and context-specific safety boundaries [76].

6.1 Lung and Airways

In the lung and airways, vulnerability drivers are unusually strong because the epithelium directly confronts inhaled oxidants and electrophiles every minute. Airborne particulate matter, cigarette smoke, ozone, and occupational chemicals challenge antioxidant buffering, while high oxygen tension magnifies reactive chemistry at the airway surface [77].

Dominant endotypes under NRF2 insufficiency often combine epithelial fragility with persistent innate immune activation, which promotes mucus hypersecretion, barrier leak, and structural remodelling. Exemplar diseases include COPD, severe asthma with frequent exacerbations, and heightened susceptibility to acute lung injury that can progress toward ARDS after infections or toxic exposures [78].

Biomarkers and evidence across models and human sampling often converge on reduced expression of NRF2 target genes in airway epithelium, together with higher oxidative damage markers in sputum, breath condensate, or blood. Studies also describe lower glutathione availability, increased lipid peroxidation products, and inflammatory mediators that correlate with airflow limitation, symptom burden, and exacerbation frequency [79].

Therapeutic implications follow this exposure dominated biology, since reducing the oxidative load can restore functional reserve even before any targeted pathway modulation is attempted. Smoking cessation, indoor and outdoor pollution mitigation, improved vaccination coverage, and careful management of recurrent infections can lessen repeated NRF2 suppressing insults and support mucosal recovery [80].

When NRF2-directed interventions are considered, dosing and timing should aim for physiologic restoration, and monitoring should track exacerbations, lung function, and signatures [81].

In parallel, adjunct approaches that improve mucus clearance and reduce airway hyperreactivity may synergize, because they lower oxidative bursts during flares and permit endogenous repair programs to recover steadily [82].

6.2 Liver and Metabolic Tissues

In liver and metabolic tissues, the vulnerability drivers reflect continuous exposure to nutrient flux, xenobiotic metabolism, and lipid handling, all of which generate electrophiles and reactive oxygen species during overload. Hepatocytes are asked to process free fatty acids, lipoproteins, and drugs at high-

throughput, while maintaining redox balance and mitochondrial performance. When NRF2 activity is insufficient, this workload more easily translates into oxidative stress, endoplasmic reticulum pressure, and impaired detoxification, which together destabilize cellular homeostasis [83].

Dominant endotypes along the steatosis/steatohepatitis axis include lipotoxic injury with mitochondrial dysfunction, inflammatory amplification driven by Kupffer cells, and gradual activation of fibrogenic programs in stellate cells [84]. Exemplar diseases include metabolic dysfunction associated steatotic liver disease, progressive steatohepatitis, and the downstream complications of fibrosis and cirrhosis, which can also increase cardiometabolic risk. In this setting, low NRF2 signalling can function as an amplifier, because reduced antioxidant buffering increases lipid peroxidation, and reactive aldehydes sustain inflammatory and stress signalling that further suppresses protective gene programs [85].

Biomarkers and evidence often involve reduced expression of NRF2 target genes in liver tissue, altered glutathione status, and elevated oxidative damage markers that correlate with disease severity. Clinically accessible signals include patterns of insulin resistance, higher transaminases in active injury states, and inflammatory markers that track metabolic stress, although they remain nonspecific without pathway signatures. Experimental models support a mechanistic link through impaired phase II responses and compromised mitochondrial resilience during dietary fat and sugar excess [86].

Therapeutic implications emphasize first reducing metabolic stressors that keep the liver in a high electrophile state, including weight loss, dietary quality improvement, and physical activity that improves insulin sensitivity. Interventions that restore NRF2 signalling may be most useful when paired with strategies that reduce lipotoxic flux, because otherwise the pathway can be overwhelmed. Monitoring should combine metabolic endpoints, fibrosis risk assessment, and a small panel of NRF2-responsive markers when feasible, while remaining attentive to the context where sustained NRF2 activation may become maladaptive [87].

6.3 Cardiovascular System

In the cardiovascular system, reduced NRF2 signalling can convert transient oxidative cues into persistent dysfunction, because vessels face continuous metabolic and inflammatory stress. The endothelium is especially vulnerable, since it must preserve nitric oxide bioavailability while buffering reactive species created by shear forces and circulating mediators. This links endothelium, atherogenesis, and myocardial stress to a shared redox vulnerability when NRF2 responses are blunted [88].

Atherogenesis is promoted when oxidized lipids and electrophilic aldehydes persist longer, because phase II conjugation and efflux responses are less inducible. Oxidized LDL and lipid peroxidation products amplify innate immune activation within the intima, increasing monocyte recruitment and foam cell formation in early lesions. Smooth muscle cells respond with phenotypic switching and extracellular matrix remodelling, which can enlarge plaques and reduce stability during chronic inflammation [89].

Myocardial stress increases under NRF2 insufficiency, since cardiomyocytes require stable mitochondrial function to sustain high ATP demand and tightly regulated calcium handling. Ischemia reperfusion, pressure overload, and insulin resistant metabolism generate reactive bursts that injure mitochondrial proteins and membranes in susceptible hearts. With weaker NRF2 output, mitochondrial recovery is slower, maladaptive hypertrophic signalling persists, and contractile reserve declines progressively [90].

Evidence from preclinical models and human studies aligns with impaired endothelial function measures, higher oxidative damage signatures, and inflammatory markers that track cardiometabolic risk over time. Therapeutic implications emphasize lowering oxidative inputs through smoking cessation, air pollution mitigation, blood pressure control, and improved glycemic and lipid management. If NRF2

modulation is pursued, dose and timing should aim for physiologic restoration, and monitoring should include robust vascular function endpoints, together with coordinated redox biomarker panels [91].

6.4 Kidney

In the kidney, NRF2 insufficiency is especially consequential because renal tubules operate under high metabolic demand while concentrating solutes that can become toxic. Vulnerability drivers include abundant mitochondria in proximal tubular cells, frequent exposure to filtered xenobiotics, and the need to reabsorb nutrients and electrolytes with substantial ATP cost. Even mild systemic inflammation can therefore translate into oxidative injury within the tubule-interstitium [92].

Along the AKI-to-CKD continuum, reduced NRF2 activity can amplify the initial injury and impair recovery, allowing temporary dysfunction to transition into persistent scarring. Exemplar contexts include ischemic injury, nephrotoxic drug exposure, and sepsis related stress, where reactive species rise quickly, and protective transcriptional responses may be too weak. When repair is incomplete, maladaptive fibroblast activation and capillary rarefaction promote progressive chronic kidney disease [93].

Diabetic injury contexts add sustained metabolic pressure, because hyperglycaemia and dyslipidaemia increase mitochondrial oxidant leakage and glycation-derived electrophiles. Endothelial dysfunction and glomerular hyperfiltration further increase oxidative burden, and inflammatory signalling promotes mesangial expansion and matrix remodelling. With blunted NRF2 outputs, phase II detoxification and glutathione-dependent buffering are reduced, and the kidney becomes less tolerant of repeated hemodynamic and inflammatory hits [94].

Evidence from experimental models supports the protective effects of restoring NRF2 signalling on tubular integrity, inflammation, and fibrosis pathways, while human data often show associations between oxidative stress biomarkers and progression risk [95]. Therapeutic implications prioritize glycemic control, blood pressure optimization, and avoidance of nephrotoxic exposures, because these reduce the ongoing stress that suppresses adaptive responses. If NRF2 targeted strategies are tested, endpoints should include both functional measures, such as eGFR trajectories and albuminuria, and molecular markers that reflect pathway engagement in relevant renal compartments [96].

6.5 Brain

In the brain, vulnerability drivers reflect high oxygen consumption, abundant polyunsaturated lipids, and limited regenerative capacity in key neuronal populations. Neurons depend on mitochondrial performance and tight calcium control, so oxidative fluctuations can rapidly impair synaptic function and plasticity. Blood-brain barrier integrity also matters because barrier stress permits peripheral cytokines and immune cells to amplify local injury [97].

With NRF2 insufficiency, dominant endotypes include persistent neuroinflammation in microglia and reduced antioxidant support from astrocytes and oligodendrocytes. Reactive microglia sustain cytokine and oxidant release, which damages synapses and promotes axonal degeneration across vulnerable networks. Astrocytic glutathione handling and lactate shuttling can weaken, lowering neuronal stress tolerance during excitotoxicity and hypometabolism [98].

Exemplar diseases include Alzheimer type neurodegeneration, Parkinsonian syndromes with dopaminergic oxidative burden, and small vessel disease that injures white matter. Across these conditions, lipid peroxidation products and mitochondrial danger signals reinforce inflammatory loops and progressively further reduce circuit level compensation. Evidence often combines diminished NRF2 target gene signatures

in brain tissue or peripheral cells, with higher F2-isoprostanes, 4-HNE adducts, and 8-oxo-dG in CSF or plasma, alongside imaging markers of neurovascular dysfunction and microglial activation [99].

Therapeutic implications prioritize lowering systemic inflammation, optimizing sleep and exercise, and controlling vascular and metabolic risks that impair neurovascular coupling. Adjunct strategies that support vascular health can indirectly stabilize redox tone within neural tissue. When NRF2 activation is tested, earlier stages may benefit most because synaptic networks and glial phenotypes remain more reversible. Monitoring should integrate cognitive and motor outcomes with redox and inflammatory panels, and remain alert to contexts where sustained NRF2 elevation could become maladaptive [100].

6.6 Barrier Organs and Systemic Aging Phenotypes

Barrier organs and systemic aging phenotypes are tightly connected, because the skin and gut are interfaces with the environment. The skin/gut axis sets the tone for immune education and oxidative exposure, and it therefore shapes how frequently NRF2 is challenged. When NRF2 signalling is insufficient, barrier repair slows, and low-grade damage accumulates with daily stressors [101].

In skin, ultraviolet light, pollutants, and irritants increase electrophilic load, while reduced antioxidant buffering favours lipid peroxidation and impaired keratinocyte differentiation. Barrier leak can then promote local inflammation, pruritus, and delayed wound healing, which are common features of aging tissue. In parallel, dermal fibroblasts may adopt profibrotic programs that stiffen the extracellular matrix and reduce resilience [102].

In the gut, impaired NRF2 responses can weaken epithelial tight junction maintenance and mucus defences, allowing microbial products to cross into the circulation. This supports chronic innate immune activation and contributes to inflammaging, which in turn further suppresses adaptive stress responses. Dysbiosis and altered bile acid metabolism can add metabolic stress, reinforcing systemic oxidative burden [103].

Across these interfaces, systemic frailty signals may emerge as a downstream phenotype, reflecting reduced physiologic reserve rather than one organ failure. Clinically, this can align with sarcopenia, slower recovery after infections, and heightened sensitivity to medications or dietary insults. A practical implication is that restoring barrier integrity and reducing exposures may partially normalize NRF2 dynamics, and this may slow progression toward multi system vulnerability overall [104].

6.7 Cross-Cutting Synthesis: Shared Endotypes across Organs

Across organ systems, NRF2 insufficiency tends to generate a limited repertoire of recurring clinical and biological patterns, even when diagnoses and exposures appear very different. A cross-cutting synthesis is helpful because it emphasizes shared mechanisms, and it supports comparable endpoints, biomarkers, and trial designs across tissues that otherwise seem unrelated.

Chronic inflammatory endotypes emerge when weakened antioxidant buffering and impaired detoxification allow oxidant signalling to persist, and this persistence keeps cytokine networks active for longer than intended. Barrier microinjury, endothelial activation, and innate immune recruitment then reinforce each other, so inflammation becomes both a driver and a consequence of declining stress resilience in multiple organs [68].

Metabolic syndrome/diabetes complications represent a second shared trajectory, because nutrient surplus and insulin resistance increase mitochondrial oxidant leakage while simultaneously reducing NADPH availability for antioxidant recycling. When NRF2 output is low, glutathione support and phase II conjugation responses do not scale with this burden, and lipid peroxidation products accumulate, amplifying injury in liver, vasculature, kidney, and brain [105].

Fibrosis-prone states develop when repeated low-grade injury shifts repair toward persistent remodelling rather than full restoration of tissue architecture. Under NRF2 insufficiency, epithelial and endothelial cells are more likely to enter senescence or regulated death, and stromal compartments receive prolonged inflammatory cues that sustain myofibroblast activation and extracellular matrix deposition across organs [106].

Environmental injury and post-stressor syndromes capture the clinical observation that exposures such as smoke, particulate pollution, infections, and certain drugs can leave behind incomplete recovery and heightened reactivity. When adaptive NRF2 responses are blunted, a single insult can create a longer symptom tail, and subsequent challenges trigger exaggerated flares that erode physiologic reserve and accelerate systemic vulnerability over time [107].

Cancer paradox (initiation vs. progression/therapy resistance) highlights the need for careful context, because low NRF2 can increase oxidative DNA damage and malignant initiation in chronically inflamed or exposed tissues. Yet once tumours are established, persistent NRF2 activation, often driven by Keap1 pathway alterations, can enhance redox buffering, detoxification capacity, and survival under therapy pressure, which complicates strategies aimed at indiscriminate pathway activation [108].

In Fig. 4, to make cross-disease comparisons tractable, we organise organ-specific sections around a limited repertoire of endotypes that repeatedly emerge when NRF2 functional output is reduced. Fig. 4 provides an integrative map linking each organ system to its dominant shared endotypes, and it aligns these patterns with convergent biomarker domains that jointly capture pathway capacity and cumulative injury burden. This representation supports a practical translational logic in which stratification, engagement confirmation, and burden reduction are matched to the endotype, enabling range-seeking restoration while preserving interpretability across heterogeneous cohorts. The cross-cutting perspective has a direct analytical implication: because the same endotype recurs across different diagnoses and organs, it becomes possible to design clinical studies that recruit patients by biological profile rather than by diagnostic label alone. Chronic inflammatory endotypes, metabolic endotypes, and fibrosis-prone endotypes each carry distinct biomarker signatures, different optimal windows for intervention, and different safety boundaries, and recognising this convergence is what separates a precision NRF2 strategy from an indiscriminate one. The shared endotype framework therefore does not merely organise existing knowledge; it generates testable predictions about which patient subgroups should respond to NRF2 restoration and under what conditions the intervention could be counterproductive.

Table 1 proposes a set of core reporting domains for NRF2-focused clinical studies, ranging from sampling matrix and clinical context to cell composition and functional proxies, together with the proposed NRF2-score framework, a composite index that weights target gene expression against oxidative damage readouts, presented here as a conceptual instrument pending prospective validation. Standardising these components will support cross-study comparability, improve patient stratification, and strengthen the credibility of trials claiming to have restored NRF2 signalling towards a physiological range.

Table 1: Organ-specific mechanistic drivers and dominant endotypes of NRF2 insufficiency.

Organ	Principal Vulnerability Drivers	Dominant Mechanistic Consequence	Dominant Endotype	Exemplar Disease
Lung	Direct inhalation of oxidants and electrophiles; high oxygen tension	Epithelial fragility, mucus hypersecretion, impaired mucosal repair	Chronic innate immune activation with structural remodelling	COPD, severe asthma, ARDS

Table 1: *Cont.*

Organ	Principal Vulnerability Drivers	Dominant Mechanistic Consequence	Dominant Endotype	Exemplar Disease
Liver	High-throughput xenobiotic and lipid metabolism; nutrient flux overload	Lipotoxic injury, impaired phase II/III detoxification, ER stress	Steatohepatitis with fibrogenic progression	MASLD, steatohepatitis, cirrhosis
Cardiovascular	Shear stress, circulating mediators, dyslipidaemia; cardiomyocyte high ATP demand	Endothelial dysfunction, oxidised LDL persistence, mitochondrial injury in cardiomyocytes	Atherogenic inflammation, maladaptive hypertrophy	Atherosclerosis, heart failure, ischaemia-reperfusion injury
Kidney	High proximal tubule metabolic demand; solute concentration; glycation-derived electrophiles	Tubular oxidative injury, impaired repair, maladaptive fibroblast activation	AKI-to-CKD transition, diabetic nephropathy	CKD, diabetic nephropathy, nephrotoxic AKI
Brain	High O ₂ consumption, abundant polyunsaturated lipids, limited neuronal regeneration	Neuroinflammation, weakened astrocytic glutathione support, synaptic degeneration	Persistent microglial activation, progressive circuit failure	Alzheimer's disease, Parkinson's disease, small vessel disease
Barrier organs (skin/gut)	UV radiation, pollutants, microbial products; continuous environmental challenge	Barrier leak, impaired tight junction maintenance, dysbiosis-driven innate activation	Inflammaging, systemic frailty, barrier-driven immune priming	Atopic dermatitis, IBD, sarcopaenia, post-infection vulnerability

Note: NRF2, nuclear factor erythroid 2-related factor 2 (NFE2L2); COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome; MASLD, metabolic dysfunction-associated steatotic liver disease; ER, endoplasmic reticulum; LDL, low-density lipoprotein; AKI, acute kidney injury; CKD, chronic kidney disease; O₂, oxygen; IBD, inflammatory bowel disease; UV, ultraviolet.

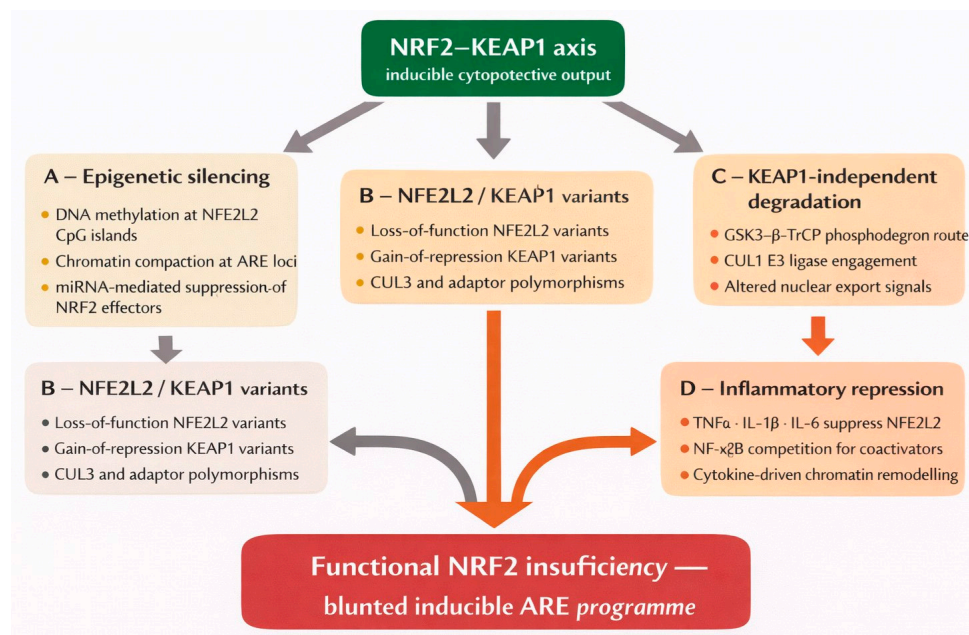


Figure 4: Cross-organ endotype map of NRF2 insufficiency with aligned biomarker domains and translational levers. Each row of the map represents one organ system (lung, liver and metabolic tissues, cardiovascular system, kidney,

brain, and barrier interfaces) and its dominant shared endotype when NRF2 functional output is reduced. The central column lists the principal mechanistic consequences that recur across organs, including oxidative epithelial fragility, lipotoxic injury, atherogenic inflammation, tubular oxidative stress, neuroinflammation, and barrier leak. The right-hand columns align each organ-endotype pair with convergent biomarker domains that jointly capture pathway capacity and cumulative injury burden, and with translational levers indicating the intervention classes most relevant to each context. Colour coding distinguishes the three broad intervention categories: burden-reduction strategies (lifestyle and exposome modification), pathway-engaging approaches (nutritional and nutraceutical), and pharmacological or targeted delivery options. The map is intended to support patient stratification and range-seeking restoration by matching endotype to intervention rather than treating NRF2 insufficiency as a uniform target across all disease settings.

7 Biomarkers and Stratification

Biomarkers and stratification are essential for moving NRF2 biology from plausible mechanism to testable clinical endotypes. Because NRF2 signalling is dynamic and tissue dependent, a useful biomarker strategy should capture both pathway capacity and the burden of oxidative injury, while remaining feasible for repeated sampling in humans. We therefore emphasize convergent measurement, combining transcriptional or proteomic indicators of NRF2 output with redox damage readouts and functional proxies that reflect resilience at the organ level. This section also highlights practical confounders, including timing and cell composition, and it motivates a minimal reporting set that supports cross study comparability. We begin with pathway proximal signatures, since target gene panels and integrated scores provide the most direct estimate of NRF2 activity in complex samples [109].

Fig. 5 outlines a translational workflow that integrates sampling metadata and the collection phase with convergent biomarker domains, including NRF2 target gene or protein panels, oxidative damage readouts, inflammatory tone, mitochondrial performance, and organ-reserve proxies. These inputs are combined into a proposed composite NRF2-score framework to support patient stratification, selection of lifestyle, nutritional, or pharmacological interventions, and iterative monitoring to restore NRF2 signalling towards a physiological range while avoiding overshoot under predefined boundary conditions.

7.1 NRF2 Target-Gene Panels and Transcriptomic/Proteomic Scores

NRF2 target-gene panels and transcriptomic/proteomic scores provide the most scalable way to quantify pathway status in complex tissues. Rather than relying on a single marker, these approaches capture coordinated regulation across canonical antioxidant and detoxification programs [13].

A practical panel typically includes genes such as NQO1, HMOX1, GCLC, GCLM, TXNRD1, SLC7A11, and GSR, sometimes complemented by phase II and transporter genes. The key principle is coherence, because truly engaged NRF2 produces a concerted shift, while noise often affects isolated transcripts [110].

Transcriptomic scores can be computed from bulk RNA sequencing, microarrays, or targeted platforms, using z-score aggregation, principal component methods, or curated gene set enrichment. Single-cell profiles can refine interpretation, but they require careful batch correction and gene detection to avoid biased scores in rare cell states. Thresholds should be anchored to matched controls and adjusted for cell composition, since inflammation can change the apparent signal by altering cellular proportions [111].

Proteomic scores add functional proximity, because protein abundance and post-translational modification may better reflect enzymatic capacity than mRNA levels alone. Mass spectrometry and multiplex immunoassays can quantify targets, yet sensitivity, antibody specificity, and dynamic range still limit broad clinical deployment [112].

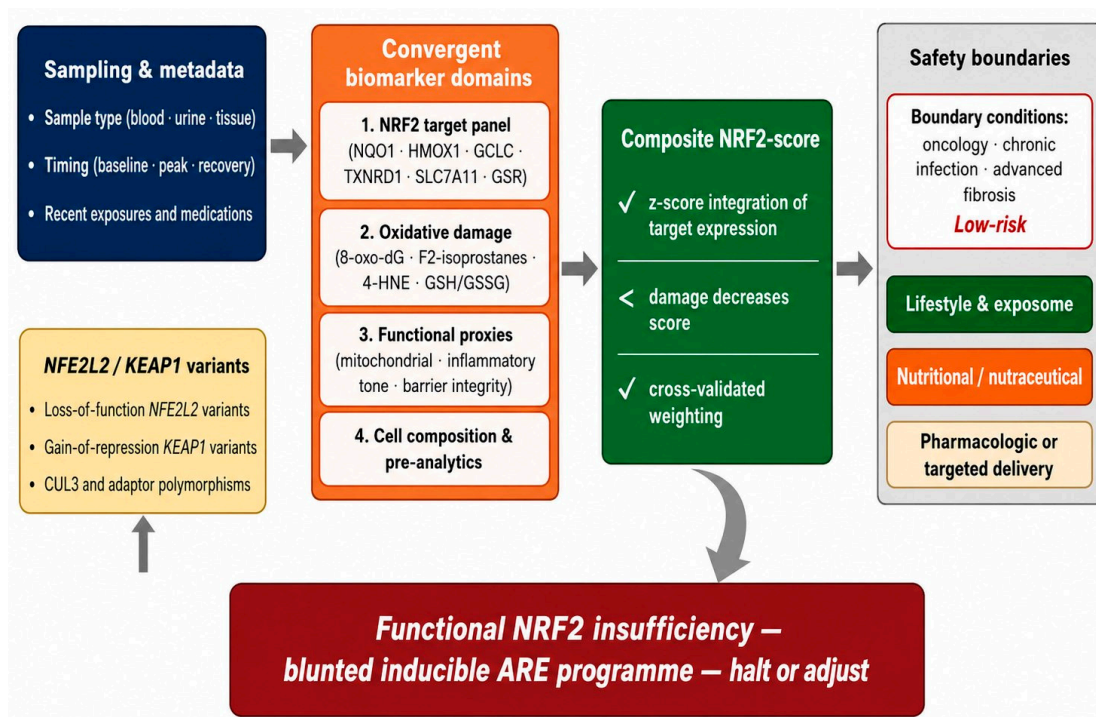


Figure 5: Biomarker-guided framework for precision modulation of NRF2 with explicit safety boundaries. The workflow proceeds from left to right through five sequential stages. Stage 1 integrates sampling metadata and collection phase information, including tissue type, time point relative to stress or treatment, and cell composition estimates. Stage 2 combines convergent biomarker domains: NRF2 target gene or protein panels (NQO1, HMOX1, GCLC, GCLM, TXNRD1, SLC7A11, GSR), oxidative damage readouts (8-OHdG, 4-HNE, isoprostanes), inflammatory tone markers, mitochondrial performance proxies, and organ-reserve indicators. Stage 3 derives a proposed composite NRF2-score by weighting target gene expression against oxidative damage readouts, adjusted for cell composition, using an algorithm that requires prospective validation before clinical implementation. Stage 4 uses the score to stratify patients and select the appropriate intervention class. Stage 5 applies iterative monitoring to confirm pathway engagement and detect overshoot, supported by predefined boundary conditions and stopping rules that are activated when signals suggest maladaptive sustained activation, poor engagement, or worsening clinical trajectory. The letter B visible in the figure indicates the biomarker integration panel and will be removed or relabelled in the revised figure file as advised by the reviewer.

For translational use, panels should be pre-registered, cross-validated across cohorts, and linked to functional endpoints such as oxidative damage markers or stress challenge responses. Reporting should include assay platform, normalization strategy, and a minimal set of targets, so that NRF2 status can be compared across studies and disease contexts [113].

7.2 Redox and Oxidative-Damage Biomarkers (DNA/Lipid/Protein Damage Readouts)

Redox and oxidative-damage biomarkers (DNA/lipid/protein damage readouts). Complement NRF2 transcriptional signatures by capturing the biochemical consequences of insufficient buffering. While gene panels estimate pathway capacity, damage readouts reflect the integrated burden of oxidant flux, detoxification failure, and repair efficiency across time [114].

For DNA injury, 8-oxo-dG and related oxidized nucleosides are commonly measured in blood, urine, or tissue, and they provide a window into oxidative pressure on genomic and mitochondrial compartments.

Interpretation should consider renal clearance, diet, and inflammation, since these factors can influence circulating levels and urinary excretion. When feasible, pairing DNA damage with mitochondrial markers strengthens inference, because mitochondrial stress often precedes broader cellular dysfunction [115].

Lipid peroxidation readouts are particularly informative in ferroptosis-prone contexts and in organs rich in polyunsaturated membranes. F2-isoprostanes, malondialdehyde, and 4-HNE adducts can indicate sustained membrane oxidation, and they often track metabolic stress and inflammatory activity. Because these markers can rise during acute infections or intense exercise, timing relative to recent stressors should be documented carefully [116].

Protein oxidation provides another layer, because carbonylated proteins, nitrotyrosine, and specific adducts report covalent damage that can impair enzymes and structural proteins. These signals can be measured in plasma, sputum, CSF, or tissue homogenates, and they can support tissue-specific interpretation when sampling is targeted. However, preanalytical handling, storage time, and assay chemistry can introduce variability, so standardized protocols are essential [117].

For clinical and translational studies, the strongest approach combines at least one DNA, one lipid, and one protein damage marker, alongside a redox capacity readout such as GSH/GSSG. This composite strategy improves specificity, supports mechanistic triangulation with NRF2 target gene panels, and helps distinguish transient oxidative bursts from sustained redox dysregulation [118].

7.3 Functional Readouts

Functional readouts (mitochondrial function, inflammatory tone, barrier integrity proxies).bridge molecular signatures with meaningful physiology. They show whether NRF2-related resilience becomes energy production, restrained immunity, and stable endothelial interfaces under stress. When aligned with symptoms, these measures help distinguish adaptive compensation from loss of reserve overall [119].

Mitochondrial function can be assessed through oxygen consumption and respiratory capacity in cells, or through circulating surrogates such as lactate kinetics and acylcarnitine patterns. In tissues, respirometry and imaging can reveal limited oxidative phosphorylation and increased reactive species when demand rises, especially during a standardized challenge. Assays of membrane potential and mitochondrial DNA release can strongly support interpretation [120].

Inflammatory tone is captured with cytokine panels, acute phase proteins, and cell activation markers, yet interpretation improves when networks are considered rather than single analytes. Persistent elevation of IL 6, TNF, or CRP, together with myeloid activation signatures, can indicate a redox driven inflammatory set point that might respond to NRF2 restoration over time. Serial sampling reduces misclassification during intercurrent infection, vaccination, or acute metabolic stress [121].

Barrier integrity proxies include transepithelial resistance *in vitro*, markers of endothelial activation, and gut permeability signals such as zonulin or microbial product translocation measures. In airway and skin contexts, mucus properties and wound healing kinetics can serve as functional outputs that reflect redox buffering and repair capacity. Standardized pre-analytics and harmonized units are essential for cross-cohort reliable [122].

7.4 Tissue vs. Blood Sampling: Feasibility and Interpretability

Tissue vs. blood sampling, feasibility and interpretability, is a central practical issue for NRF2-focused biomarker work. Blood is easy to obtain repeatedly, but it reflects a mixed systemic signal rather than the microenvironment where injury unfolds [123].

Tissue sampling can capture cell-specific NRF2 target expression, oxidative damage patterns, and local immune activation with higher resolution. It also reveals compartmental gradients, such as epithelial versus stromal signatures, that are diluted in circulation. However, biopsies are invasive, ethically constrained, and often unavailable in early disease stages [124].

Blood-based readouts are attractive for screening and longitudinal monitoring, yet they require careful interpretation. Peripheral signatures can be driven by leukocyte composition shifts, acute infections, recent exercise, or medication changes, which may mimic NRF2-related changes. Even when transcriptomic scores are informative, they should be paired with damage markers and clinical timing metadata [125]. A critical and frequently overlooked preanalytical constraint is that NRF2 protein is subject to rapid proteasomal degradation following blood collection. For this reason, processing must begin immediately after venepuncture and samples must be maintained under strict refrigeration throughout handling. If the blood collection protocol is not standardised with respect to time-to-processing, anticoagulant choice, and temperature chain, any NRF2 protein measurement or NRF2-score derived from such samples will be irreproducible. Studies intending to quantify NRF2 protein, the NRF2/Keap1 ratio, or NRF2 nuclear localisation in peripheral blood mononuclear cells should specify and adhere to a validated stabilisation protocol, and should report deviation from protocol as a quality control metric.

An intermediate strategy uses minimally invasive tissue adjacent sampling, including nasal brushings, sputum, urine, stool, or skin tape strips. These matrices can approximate local biology and provide organ relevant signals, while allowing repeated collection in real-world settings. Standardized handling is essential, because oxidative markers are sensitive to storage and processing delays [126].

For translation, the best approach is triangulation across compartments, using tissue when it changes decisions and blood when it enables scale. Studies should report feasibility metrics, sampling success rates, and patient burden alongside assay performance. Interpretability improves when tissue informed signatures guide which blood markers are expected to move clinically, and when results are modelled with cell composition adjustments [127]. Two emerging approaches deserve mention as they may help bridge the gap between invasive tissue sampling and the limited resolution of bulk blood readouts. First, organ-specific extracellular vesicles and exosomes are increasingly recognised as carriers of cell-type-enriched cargo, including mRNAs, miRNAs, and proteins that reflect the redox and inflammatory state of their tissue of origin. Exosomal NRF2 target transcripts or oxidative damage adducts isolated from plasma may eventually provide organ-proximal information without the need for biopsy, and several proof-of-concept studies have explored this approach in liver and lung disease contexts. Second, imaging tracers for oxidative stress, including certain positron emission tomography probes designed to detect reactive oxygen species or lipid peroxidation *in vivo*, represent a non-invasive route to assessing tissue-level redox burden. Although these PET-based approaches remain largely experimental and are not yet validated for routine NRF2 pathway assessment, their development directly addresses the invasiveness limitation of tissue biopsies and may eventually complement biomarker panels in stratification and monitoring workflows.

7.5 Confounders and Pitfalls

Confounders and pitfalls (timing, cell composition, acute stress effects) can easily distort NRF2-related biomarker interpretation, especially in heterogeneous clinical cohorts. NRF2 signalling is dynamic, so a single sample may miss transient nuclear pulses or delayed transcriptional waves. Sampling shortly after a flare can therefore exaggerate pathway suppression or activation signals [128].

Timing is particularly sensitive to recent infections, vaccinations, surgery, or intense exercise, because each can induce short lived oxidative and inflammatory programs. These acute stress effects may elevate

oxidative damage markers while simultaneously inducing compensatory transcripts, producing a confusing mixed picture. Recording recent exposures and repeating measurements during stable periods improves reliability substantially [129].

Cell composition is another major confounder, because bulk blood and tissue assays reflect shifting proportions of leukocytes, stromal cells, and stressed epithelial subsets. A rise in neutrophils or activated monocytes can inflate inflammatory tone and obscure NRF2 target gene patterns. Whenever possible, studies should adjust for differential counts, use deconvolution, or analyse sorted populations [130].

Preanalytical variation adds further pitfalls, because oxidative biomarkers are sensitive to handling, freeze-thaw cycles, and storage duration. Drug effects also matter, since many agents influence detoxification enzymes and redox balance indirectly. Robust study design therefore overall requires harmonized protocols, careful metadata capture, and prespecified analytic plans that reduce false mechanistic inferences [131].

7.6 Minimum Reporting Set and Proposed NRF2-Score

A Minimum reporting set and proposed NRF2-score can turn pathway claims into comparable results across studies. Without shared reporting, investigators may label a cohort NRF2 low from one marker and one time point, which weakens synthesis [132].

The minimum reporting set should describe the sampling compartment, the clinical context, and the timing relative to acute stressors. It should note recent infection, vaccination, medication changes, and strenuous exercise, because these exposures can shift NRF2 targets and oxidative damage markers transiently [133].

It should also document cell composition and sample handling, because bulk assays are sensitive to population shifts and pre-analytics. Differential blood counts, tissue cellularity estimates, storage conditions, and freeze-thaw cycles should be stated, since oxidative biomarkers can drift during processing [134].

For pathway engagement, studies should report a predefined NRF2 target panel, the assay platform, normalization steps, and an explicit thresholding approach. A compact panel including NQO1, HMOX1, GCLC, GCLM, TXNRD1, SLC7A11, and GSR can be sufficient when measured consistently, and when complemented by damage readouts from DNA, lipids, and proteins [135].

The proposed NRF2-score can integrate capacity and burden, combining a standardized target gene signature with damage markers and a functional resilience measure. A practical approach uses z-scores, where higher target expression increases the score, and higher damage decreases it, with weights derived from cross-validated prediction of outcomes [112]. It is important to recognise that measuring nuclear translocation alone does not guarantee that the NRF2 pathway is functionally active. Nuclear NRF2 must also bind antioxidant response element sequences and activate downstream transcription to exert its protective role. DNA-binding assays, such as electrophoretic mobility shift assays or ELISA-based transcription factor binding kits, provide an additional layer of confirmation that translocated NRF2 is indeed engaging its target promoters. Where feasible, these assays should complement immunofluorescence or subcellular fractionation data in research settings. Furthermore, given that NRF2 regulation is predominantly post-translational and proteasomal rather than purely transcriptional, the NRF2/Keap1 protein ratio measured in peripheral blood mononuclear cells offers a more direct readout of pathway engagement than mRNA levels alone. Studies aiming for robust NRF2-score framework construction are encouraged to incorporate this ratio alongside gene expression panels, provided that strict preanalytical stabilisation conditions are met.

To support adoption, the score should be reported with confidence intervals, missingness rules, and sensitivity analyses across compartments. When these elements are present, NRF2 biology becomes easier

to compare, and intervention studies can more credibly claim pathway restoration. This transparency also reduces publication bias, because null results remain interpretable and scientifically useful [136].

Table 2 summarizes a minimum set of measures to assess NRF2 activation in humans, combining expression of NRF2 target genes with oxidative stress biomarkers. It also proposes a conceptual “NRF2-score framework” to enable comparisons across interventions, tissues, and clinical outcomes between studies.

Table 2: Minimum reporting set and NRF2-score components for NRF2-focused clinical studies.

Domain	What to Report (Minimum)	Notes/Examples
Sampling matrix	Compartment(s) sampled; collection protocol	Blood for scale; tissue or adjacent matrices when they change decisions.
Clinical context	Diagnosis, stage/severity, baseline stability window	Include key comorbidities and relevant exposures (e.g., smoking, metabolic status).
Timing & recent changes	Time since infection, vaccination, surgery, intense exercise; medication/supplement changes	Acute stressors and drug changes can transiently shift NRF2 targets and damage markers.
Cell composition & pre-analytics	Differential counts/deconvolution; tissue cellularity; processing time; storage; freeze-thaw cycles	Bulk assays are sensitive to population shifts and handling-related drift.
NRF2 engagement	Predefined NRF2 target panel; assay platform; normalization; thresholds	Example compact panel: NQO1, HMOX1, GCLC, GCLM, TXNRD1, SLC7A11, GSR.
Oxidative burden	DNA, lipid, and protein damage readouts; redox capacity	E.g., 8-oxo-dG; F2-isoprostanes or 4-HNE; protein carbonyls; GSH/GSSG.
Functional proxies	Organ-relevant mitochondrial/inflammatory/barrier measures	Choose endpoints that track reserve (e.g., eGFR slope, exacerbations, permeability markers).
NRF2-score & QC	Score definition (directionality, weighting/scaling), CI, missingness, sensitivity analyses	Higher target expression increases score; higher damage decreases score; report batch-effect handling.

Note: NRF2, nuclear factor erythroid 2–related factor 2 (NFE2L2); NRF2-score, composite score reflecting NRF2 pathway engagement (↑ NRF2 target gene expression) and/or oxidative burden (↓); NQO1, NAD(P)H: quinone oxidoreductase 1; HMOX1 (HO-1), heme oxygenase 1; GCLC, glutamate–cysteine ligase catalytic subunit; GCLM, glutamate–cysteine ligase modifier subunit; TXNRD1, thioredoxin reductase 1; SLC7A11 (xCT), solute carrier family 7 member 11; GSR, glutathione reductase; 8-oxo-dG, 8-oxo-2'-deoxyguanosine; F2-isoprostanes, F2-isoprostanes; 4-HNE, 4-hydroxynonenal; GSH/GSSG, reduced glutathione/oxidized glutathione (glutathione disulphide); eGFR, estimated glomerular filtration rate; QC, quality control; CI, confidence interval; DNA, deoxyribonucleic acid.

8 Therapeutic Strategies

Therapeutic strategies for NRF2 insufficiency should be framed as restoration of the adaptive range, rather than indiscriminate activation. In many patients, the most direct leverage comes from reducing the ongoing exposures and metabolic pressures that keep the pathway suppressed, because persistent burden can overwhelm any attempt to pharmacologically increase NRF2 output. Interventions, therefore, span lifestyle and exposome modification, nutritional and nutraceutical approaches, and pharmacologic or advanced delivery strategies that can provide more specific engagement. Across these categories, translation depends on matching therapy to endotype, confirming pathway engagement with predefined biomarkers, and monitoring for boundary conditions where sustained NRF2 activation may be unhelpful. We begin with lifestyle and exposome measures because they are scalable, low-cost, and often necessary to make downstream modulation biologically meaningful [137].

Fig. 6 provides a practical translational structure for the therapeutic section by organising NRF2-directed strategies into a decision-oriented taxonomy. The schematic distinguishes burden-reduction interventions from pathway-engaging interventions, and it clarifies how lifestyle and exposome modification, nutritional or nutraceutical approaches, and pharmacologic or targeted delivery options can be selected and combined depending on the dominant driver of NRF2 suppression. By explicitly aligning intervention classes with biomarker confirmation steps and predefined safety boundaries, the figure illustrates the range-seeking principle and supports reproducible strategy design across heterogeneous cohorts.

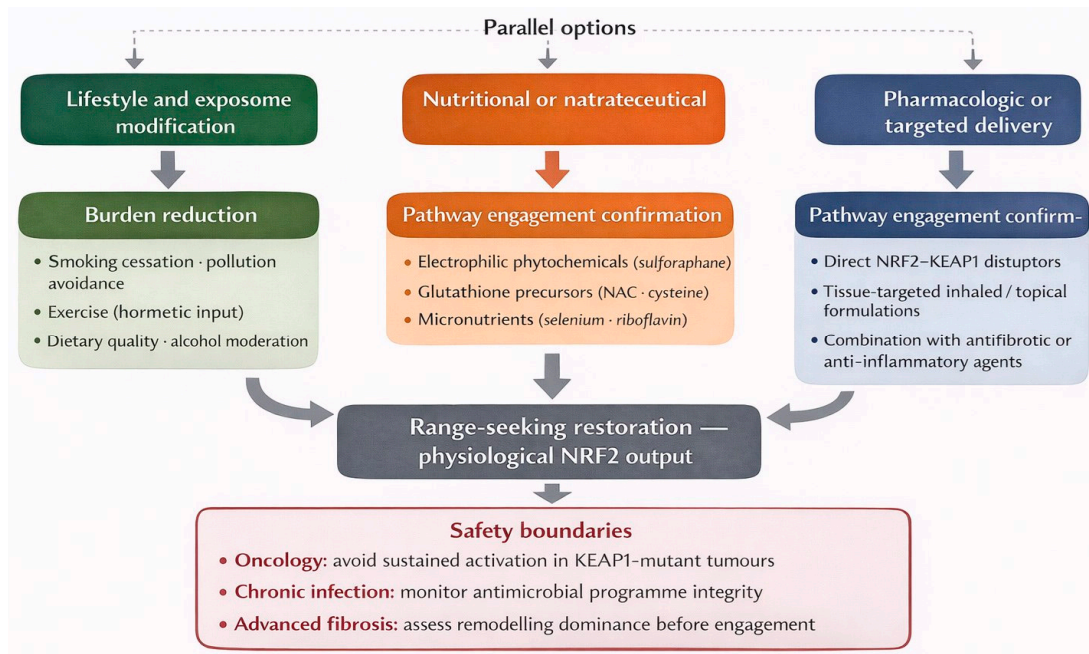


Figure 6: Intervention taxonomy for range-seeking restoration of NRF2 function, aligned with driver reduction, engagement confirmation, and safety boundaries. The taxonomy organises NRF2-directed strategies into two principal tiers. The first tier comprises burden-reduction interventions that address the upstream suppressors of NRF2 activity, including smoking cessation, pollutant and electrophile avoidance, alcohol moderation, sleep optimisation, and medication review to reduce iatrogenic oxidative load. The second tier comprises pathway-engaging interventions, subdivided into nutritional and nutraceutical approaches (dietary sulforaphane precursors, omega-3 fatty acids, curcumin, resveratrol, and related phytochemicals), and pharmacological or targeted delivery options (bardoxolone methyl and related CDDO derivatives, dimethyl fumarate, sulforaphane-loaded nanoparticles, and emerging gene therapy approaches). Across both tiers, engagement confirmation via predefined biomarker panels is required before escalating to pharmacological intervention, and explicit safety boundaries are indicated for contexts where sustained NRF2 activation may be undesirable, including active malignancy, chronic infection with intracellular pathogens, and advanced fibrosis with dominant remodelling and senescence phenotypes.

8.1 Lifestyle and Exposome Interventions

Lifestyle and exposome interventions focus on two complementary levers, remove suppressors and hormetic inputs, which together can restore a more physiological NRF2 response. This approach treats low NRF2 activity as partially reversible, because exposures and habits continuously shape cellular stress tone [138].

Removing suppressors begins with reducing inhaled and ingested electrophile burden through smoking cessation, cleaner indoor air, and lower particulate exposure when feasible. Medication review can also

matter, because some drugs increase oxidative load, impair mitochondria, or disrupt sleep and recovery. Alcohol moderation and consistent sleep schedules reduce sympathetic activation, which otherwise sustains inflammatory signalling and oxidative bursts [139].

Dietary patterns influence NRF2 indirectly by changing lipid peroxidation substrates, micronutrient sufficiency, and postprandial oxidative peaks. A nutrient-dense pattern with adequate protein, fibre, and polyphenol-rich plants can lower oxidative pressure, while limiting ultra-processed fats and excess sugars that sustain redox stress. In metabolic syndrome, modest weight loss and improved glycemic variability can reduce mitochondrial oxidant leakage, supporting endogenous antioxidant recycling [140].

Hormetic inputs use controlled, time limited stress to train adaptive defences, with regular aerobic and resistance exercise as the most consistent example in humans. Heat exposure, cold exposure, and time restricted eating may add benefit for selected individuals, provided they are introduced gradually and aligned with clinical stability [141].

Implementation should be individualized and staged, because abrupt intensity increases can trigger acute stress effects that obscure true pathway recovery. Longitudinal monitoring of symptoms, sleep, and simple redox linked biomarkers can help confirm that resilience improves, while adverse signals remain absent [142].

8.2 Nutritional/Nutraceutical Approaches

Nutritional/nutraceutical approaches (mechanistic classes; standardization issues) are attractive because they can modulate NRF2 signalling gently, yet their evidence base is heterogeneous. Mechanistic classes include electrophilic phytochemicals that modify Keap1 cysteines, polyphenols that influence redox signalling and inflammatory tone, and micronutrients that support glutathione and thioredoxin recycling. Some compounds act indirectly by improving mitochondrial function or by reducing postprandial oxidative peaks, which can preserve NRF2-responsiveness over time [143].

In food-based strategies, cruciferous vegetables and other plant-rich patterns provide a mixture of precursors and mild stressors that can engage adaptive pathways. Adequate protein intake supports glutathione synthesis through cysteine availability, while selenium and riboflavin support redox enzyme function in selected deficiency settings. The challenge is that diet effects are rarely isolated, because weight change, microbiome shifts, and medication adjustments can confound interpretation [144].

Standardization issues are a major barrier for nutraceuticals, because preparations vary in bioactive content, stability, and bioavailability. Label doses do not guarantee tissue exposure, and many compounds undergo rapid metabolism that differs across individuals. Batch testing, third-party verification, and clear reporting of formulation are therefore necessary in interventional studies [145].

For translation, the most credible approach combines mechanistic plausibility with reproducible formulations, dose justification, and biomarker-based confirmation of pathway engagement. Studies should track adverse effects and drug interactions, because induction of detoxification enzymes can change medication levels. Without these safeguards, apparent NRF2 benefits may reflect uncontrolled confounding rather than true biological restoration [146].

8.3 Pharmacologic and Advanced Approaches

Pharmacologic and advanced approaches (direct/indirect activators, targeted delivery, combination strategies), aim to restore NRF2 activity with greater potency and predictability than lifestyle measures alone. Direct or indirect activators can increase NRF2 nuclear signalling by modifying Keap1, altering degradation pathways, or shifting upstream kinase inputs that regulate turnover and localization. The clinical rationale

is strongest when oxidative injury and detoxification failure are persistent drivers, and when measurable NRF2 engagement can be demonstrated alongside functional benefit [147]. Table 3 summarises the principal NRF2-directed therapeutic strategies reviewed in this section, including their target organ applicability, bioavailability considerations, level of clinical evidence, and current regulatory status.

Targeted delivery is an important evolution because systemic NRF2 activation may not be desirable in every tissue or disease stage. Inhaled formulations for airway disease, local delivery for skin and barrier injury, or nanoparticle-based carriers that enrich specific cell types can reduce off-target exposure. Tissue-targeted strategies also support lower doses, which may help avoid sustained activation patterns that could become maladaptive [148].

Combination strategies can improve efficacy by pairing NRF2 activation with downstream support, such as mitochondrial protectants, anti-inflammatory agents, or precursors for glutathione synthesis. In metabolic disease, combining modest NRF2 activation with insulin-sensitizing measures can reduce the ongoing oxidant burden that otherwise overwhelms adaptive programs. In fibrotic contexts, pairing NRF2 engagement with antifibrotic pathways may improve remodelling outcomes [149].

For translation, pharmacologic programs should include predefined biomarkers of target engagement, careful safety monitoring, and stopping rules. Dose and timing should be optimized for physiologic restoration rather than maximal induction. The most convincing trials will stratify participants by baseline NRF2 status, so that treatment is tested in the endotype most likely to respond [12].

9 Evidence: Does NRF2 Restoration Improve Outcomes?

Evidence that NRF2 restoration improves outcomes must be evaluated across experimental control and human complexity. Preclinical models allow precise loss and rescue manipulations, yet they can over-estimate benefit when exposures are simplified and disease duration is short. Human studies bring clinical relevance, but they introduce heterogeneity in genetics, comorbidities, medications, and sampling, which can obscure pathway engagement. We therefore organize this section by tiers of evidence, starting with mechanistic preclinical experiments and then moving to observational and interventional human data. Throughout, we prioritize endpoints that reflect disease trajectory, and we highlight limitations that inform safety and future trial design [150].

9.1 Preclinical Evidence

Preclinical work has been essential for clarifying whether NRF2 insufficiency is merely associative or truly causal. The strongest evidence comes from models that manipulate pathway components under controlled stress, then quantify redox injury, inflammatory tone, and functional decline across organs and stages [151].

Loss-of-function studies, including NFE2L2 knockout animals and cell-specific deletions, consistently show reduced antioxidant buffering and heightened sensitivity to toxins, infection, and metabolic overload. These models often exhibit accelerated fibrosis, impaired barrier repair, and exaggerated inflammatory signalling, which together mirror key features of chronic disease vulnerability [152].

Rescue experiments strengthen causal inference because they test reversibility within the same biological context. Genetic background and diet can shift phenotypes, so replication across laboratories and both sexes is essential. Many studies model acute injury, so translating results to chronic trajectories requires follow-up and staging [24].

Table 3: Comparative overview of NRF2-directed therapeutic strategies: target organ applicability, bioavailability considerations, clinical evidence, and current status.

Intervention Class/Example	Principal Target Organs	Bioavailability/Delivery	Current Clinical Evidence	Level of Evidence	Clinical Status
Lifestyle: exercise and exposome reduction	Systemic (all organs)	Indirect; systemic hormetic effect	RCTs support NRF2 induction by aerobic exercise; pathway engagement partially reversed in older adults	Moderate (RCT, indirect endpoints)	Recommended standard of care
Nutraceutical: sulforaphane (cruciferous vegetables)	Lung, liver, brain, gut	Oral; variable bioavailability; formulation-dependent	Small RCTs; null result in COPD (BROCCOLI trial); positive signals in autism and metabolic disease	Low-moderate (small RCTs, heterogeneous assays)	Investigational/dietary
Pharmacologic: dimethyl fumarate	CNS (MS), immune	Oral; systemic; GI tolerability an issue	Phase 3 RCTs (DEFINE, CONFIRM) confirmed NRF2 engagement and relapse reduction in MS	High (Phase 3 RCT)	Approved (MS)
Pharmacologic: bardoxolone methyl	Kidney, liver, lung	Oral; potent NRF2/Keap1 disruptor; fluid retention risk requires patient selection	Phase 2/3 RCTs (TSUBAKI); improved eGFR in diabetic CKD; halted in heart failure (BEACON)	Moderate-high (Phase 2/3 RCT)	Investigational (CKD, COPD); not approved for Friedreich ataxia (omaveloxolone/Skyclarys is the approved agent for that indication)
Pharmacologic: omaveloxolone (Skyclarys)	CNS (Friedreich ataxia)	Oral; hepatotoxicity monitoring required (aminotransferase elevations); restricted to adults aged ≥ 16	MOXIe Part 2 RCT: significant improvement in modified Friedreich Ataxia Rating Scale vs. placebo in patients aged ≥ 16	High (Phase 3 RCT)	Approved (Friedreich ataxia, adults ≥ 16 ; Skyclarys/FDA 2023)
Targeted delivery: inhaled NRF2 activators	Lung, airway epithelium	Inhaled; high local concentration; reduced systemic exposure	Preclinical and early phase; proof-of-concept in pulmonary hypertension models	Low (preclinical/Phase 1)	Investigational
Combination: NRF2 activator + glutathione precursor (e.g., NAC)	Liver, kidney, metabolic	Oral; synergistic substrate + induction approach	Early phase studies; mechanistic rationale supported by preclinical data	Low (Phase 1/2)	Investigational

Note: NRF2, nuclear factor erythroid 2-related factor 2 (NFE2L2); RCT, randomised controlled trial; COPD, chronic obstructive pulmonary disease; MS, multiple sclerosis; GI, gastrointestinal; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; NAC, N-acetylcysteine; NRF2/Keap1, nuclear factor erythroid 2-related factor 2/Kelch-like ECH-associated protein 1.

Genetic re-expression of NRF2, reduction of Keap1 activity, or pharmacologic engagement that restores canonical targets can partially normalize glutathione handling and phase II responses, while improving tissue outcomes in many injury paradigms. Temporal activation designs address a frequent translational concern, namely that timing may determine benefit or harm [153].

When NRF2 is activated early or during recovery windows, protection is often stronger than when activation is delayed into advanced remodelling, where inflammation and stromal programs may already be entrenched. Tissue-specific models further reveal that pathway needs differ between epithelium, endothelium, immune cells, and fibroblasts. These approaches clarify which compartments drive systemic phenotypes, and they help prioritize targeted delivery strategies that maximize benefit while limiting maladaptive hyperactivation [154].

9.2 Human Evidence

Human evidence for NRF2 insufficiency has expanded, yet it remains uneven across organs and diseases in both depth and rigor. Most datasets reflect chronic exposure and comorbidity, so causal direction often requires careful interpretation and triangulation. Across studies, harmonized sampling times and cell composition adjustment are rarely reported, limiting comparability [11].

Observational links frequently show lower NRF2 target gene expression in accessible tissues, alongside higher oxidative damage markers and inflammatory signatures in blood. Genetic studies add supportive signals, because variants in NFE2L2 or Keap1 pathways can correlate with susceptibility, severity, or faster progression [23].

Interventional trials are fewer, and they vary widely in mechanism, dosing, and participant selection across conditions. Pharmacologic agents, nutraceuticals, and structured lifestyle programs have each been tested, but many studies lack confirmed pathway engagement in the target tissue. Even when trials report benefit, effects often concentrate in subgroups with higher oxidative burden and NRF2 suppression [155].

Endpoints and limitations should be considered together, because symptom scores alone rarely capture redox biology or tissue repair. Stronger trials combine clinical outcomes with NRF2 target signatures, redox markers, and functional readouts such as exacerbation rates, eGFR slopes, or vascular reactivity [25].

Key limitations include short follow-up, small sample sizes, heterogeneous assays, and confounding by medications, smoking, and acute intercurrent stress. Future human evidence will be most persuasive when participants are stratified by baseline NRF2 status, and when predefined biomarkers verify biologic response [156].

9.3 Safety, Boundary Conditions, and a Risk-Management Framework for NRF2 Reactivation

Safety, Boundary Conditions, and a Risk-Management Framework for NRF2 Reactivation should be explicit, because NRF2 sits at a biological tipping point. Too little signalling permits cumulative oxidative injury, yet sustained activation can reshape metabolism and stress tolerance. A practical framework targets restoration toward a physiologic range, rather than maximal induction everywhere [157].

Boundary conditions are clearest in oncology, where persistent NRF2 can support tumour survival and therapy resistance. In chronic infection, excessive cytoprotection may blunt antimicrobial programs, potentially prolonging pathogen persistence. In advanced fibrosis, late activation may have limited benefit, because matrix remodelling and senescence dominate trajectories [158].

A risk-management framework begins with stratification, using baseline NRF2 signatures and oxidative damage markers to identify responders. Dosing should favour pulsatile or time-limited engagement, with tissue targeting when feasible to reduce systemic exposure. Drug interaction review matters because

induction of detoxification pathways can alter pharmacokinetics and efficacy. Where uncertainty remains, conservative titration and short exposure windows help clarify benefit, while limiting long-term adaptive rewiring biologically [159].

Monitoring should combine clinical endpoints with biomarkers of engagement, oxidative burden, and inflammatory tone at predefined intervals. Stopping rules should be prespecified, based on adverse signals, lack of engagement, or maladaptive activation patterns. With these safeguards, NRF2 reactivation can be tested responsibly, while preserving interpretability and patient safety.

10 Research Agenda and Translational Roadmap

A research agenda and translational roadmap are needed because NRF2 biology is compelling, yet many claims remain difficult to compare across diseases and cohorts. The next phase should prioritize questions that clarify causal direction, identify the cell types that drive self-sustaining loops, and define which readouts best predict clinical benefit when NRF2 is restored. Equally important, translation requires practical rules for stratification, dosing dynamics, and safety monitoring, especially in contexts where NRF2 hyperactivation may be maladaptive. In the sections below, we focus on the most actionable gaps, then propose study designs that can close them with reproducible methods and clinically meaningful endpoints. We conclude with a path toward precision modulation that integrates patient selection, combination strategies, and boundary conditions [5].

Fig. 7 summarizes a stepwise framework for translating NRF2 modulation into clinically meaningful interventions. The roadmap integrates causal study design, patient stratification based on dynamic NRF2 status, confirmation of pathway engagement through convergent biomarker panels, and selection of composite clinical endpoints that capture both benefit and recovery. Critical confounders that influence NRF2 signalling in real-world settings are highlighted, together with explicit stopping rules and safety boundaries, to support range-seeking restoration of NRF2 activity while minimizing the risk of maladaptive sustained activation.

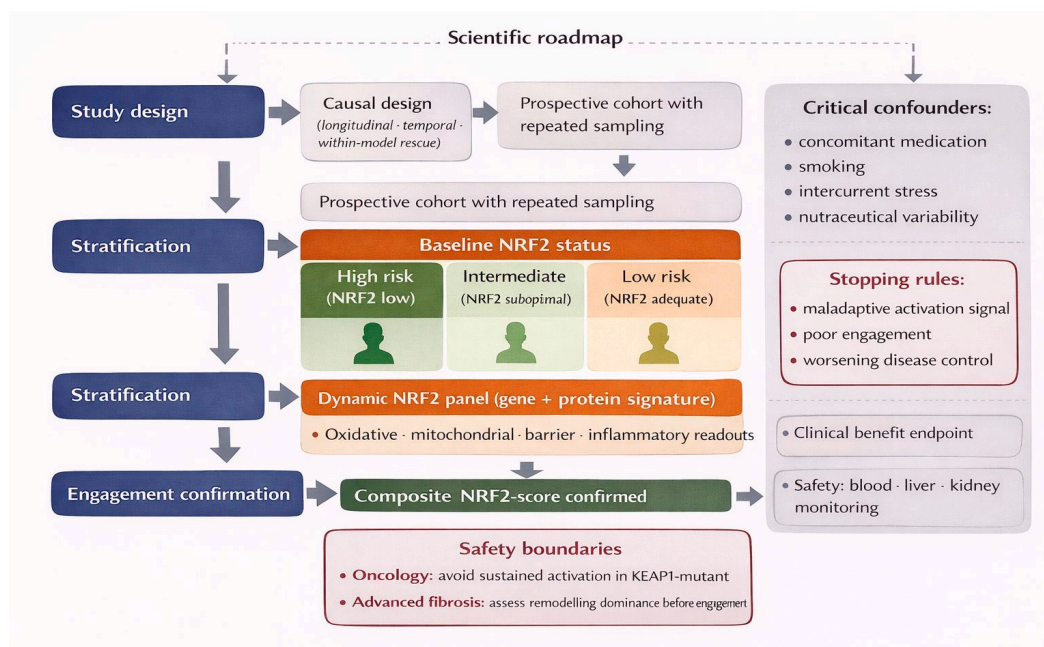


Figure 7: Translational roadmap for NRF2-directed intervention in chronic disease. The roadmap is structured as

a sequential decision framework progressing through six steps. Step 1 (Causal study design) identifies the optimal study architecture, ranging from observational longitudinal cohorts for hypothesis generation to platform trials for confirmatory evidence. Step 2 (Patient stratification) applies dynamic NRF2 status assessment using composite biomarker panels to define low-activity subgroups that are most likely to benefit. Step 3 (Pathway engagement confirmation) requires pre-specified biomarker responses confirming that the intervention has meaningfully shifted NRF2 target gene output before proceeding to efficacy evaluation. Step 4 (Composite clinical endpoint selection) captures both biological benefit (oxidative damage reduction, organ-reserve preservation) and patient-relevant outcomes (disease progression, quality of life, hospitalisation). Step 5 (Confounder management) highlights critical variables that must be controlled, including sex, age, comorbidity burden, cell composition, and sampling timing relative to stress or treatment windows. Step 6 (Safety monitoring with stopping rules) defines explicit criteria for dose reduction or trial pause when signals of maladaptive activation, off-target effects, or worsening clinical trajectory are detected. The roadmap is designed to support range-seeking restoration of NRF2 activity while minimising the risk of overshooting into sustained hyperactivation.

10.1 Key Gaps and Highest-Yield Mechanistic Questions

Key gaps remain in how NRF2 insufficiency is defined mechanistically across real human tissues and disease stages. Many studies infer low activity from single markers, yet we still lack shared thresholds that link NRF2 target capacity, oxidative burden, and clinically meaningful loss of reserve [13].

A first highest yield question asks whether NRF2 decline initiates pathology, or whether it mainly reflects accumulated stress from inflammation and exposure. Answering it will require time-resolved designs, with longitudinal sampling, and with within-model rescue that demonstrates altered trajectories rather than temporary biomarker shifts [1].

A second gap concerns compartment specificity, because immune cells, epithelia, endothelium, and fibroblasts can show different NRF2 trajectories within the same organ. We still do not know which cell types must be corrected to interrupt vicious cycles, and which compartments simply report the burden of disease [160].

A third question is how NRF2 integrates with metabolism, iron handling, and mitochondrial quality control during chronic nutrient excess and toxicant exposure. We need causal maps that explain when lipid peroxidation, ferroptosis susceptibility, and NF- κ B driven inflammation become dominant, and when epigenetic repression and proteostasis failure lock the system into low inducibility [53].

Finally, the field needs translation rules that connect pathway engagement to outcomes, not only to intermediate readouts. Priority problems include standardized perturbation assays, reproducible NRF2 scores across tissues and blood, dosing dynamics that favour physiologic restoration, and boundary conditions in cancer and infection that demand risk-managed precision at scale [7].

10.2 Recommended Study Designs

Recommended study designs should be built to separate causality from correlation, because NRF2 signatures often reflect both disease burden and adaptive capacity. The most informative designs align mechanistic readouts with longitudinal clinical endpoints, and they include predefined analysis plans that reduce flexible interpretation [161].

For causality, studies should combine prospective cohorts with repeated sampling and exposure tracking, alongside mechanistic perturbation in parallel models. In humans, short standardized challenges, such as controlled exercise bouts or carefully selected dietary probes, can reveal inducibility and recovery kinetics more clearly than resting measurements alone. In experimental systems, tissue-specific knock-

down and rescue, with temporal control, can test whether restoring NRF2 changes progression rather than merely shifting biomarkers [162].

Endpoints should be selected for biological relevance and clinical meaning, and they should include both intermediate and hard outcomes. Examples include exacerbation frequency, eGFR slope, fibrosis progression metrics, vascular reactivity, and validated neurocognitive measures, paired with redox damage panels and NRF2 target signatures. Predefining a minimal biomarker set improves comparability and reduces the risk of cherry picking [163].

Stratification should be planned from the start, because NRF2 biology is unlikely to be uniform across patients. Baseline NRF2 scores, oxidative damage burden, genetic background, age, sex, and comorbidity profiles can identify endotypes that are most likely to respond. Tissue informed blood markers can support scalable screening, while targeted tissue sampling in subsets can validate mechanistic alignment [164].

Reproducibility requires harmonized sampling protocols, rigorous pre-analytics, and transparent reporting of missingness and batch effects. Multi-site replication, blinded outcome adjudication, and open code for scoring pipelines should become standard, especially for transcriptomic and proteomic measures. When these elements are present, NRF2-focused trials can be compared, meta-analysed, and translated with greater confidence across disease domains [165].

10.3 Path to Precision NRF2 Modulation

Precision NRF2 modulation begins with patient selection that matches biology, because indiscriminate activation risks weak benefit or unintended adaptation. A practical entry criterion is an NRF2 low signature combined with oxidative damage and baseline inflammatory tone measures, measured during a stable baseline window. Stratification should consider age, metabolic status, smoking exposure, and cancer history, since these factors shift the benefit and risk [166].

Dosing dynamics should aim for restoration rather than maximal induction, because NRF2 biology often follows a U-shaped response. Pulsatile schedules or stepwise titration can engage the pathway while limiting chronic rewiring of metabolism and detoxification. Whenever possible, tissue-targeted delivery, such as inhaled airway approaches or topical barrier formulations, can reduce systemic exposure and ease monitoring [167].

Combination therapy is often necessary, since NRF2 activation alone may be overwhelmed by ongoing stressors. Pairing NRF2 engagement with insulin-sensitizing strategies, antifibrotic agents, or anti-inflammatory control can reduce the burden that suppresses inducibility. Adjunct support for glutathione precursors, mitochondrial quality control, and sleep recovery can strengthen response durability [168].

A precision path also requires predefined monitoring and stopping rules that integrate clinical endpoints with engagement biomarkers. Serial NRF2 target gene panels, redox damage markers, and organ measures should be collected with consistent pre-analyses. If signals suggest maladaptive activation, poor engagement, or worsening disease control, dosing should be reduced or paused promptly.

Fig. 8 defines a minimum reporting framework for studies assessing NRF2 functional output in human or experimental settings. The workflow integrates sampling and metadata annotation, a core panel of NRF2 target genes, orthogonal readouts of oxidative damage and functional impairment, and stepwise construction of a composite NRF2 score. Quality control elements, including longitudinal tracking, confidence interval estimation, and explicit handling of missing data, are incorporated to support reproducibility, interpretability, and cross-study comparison.

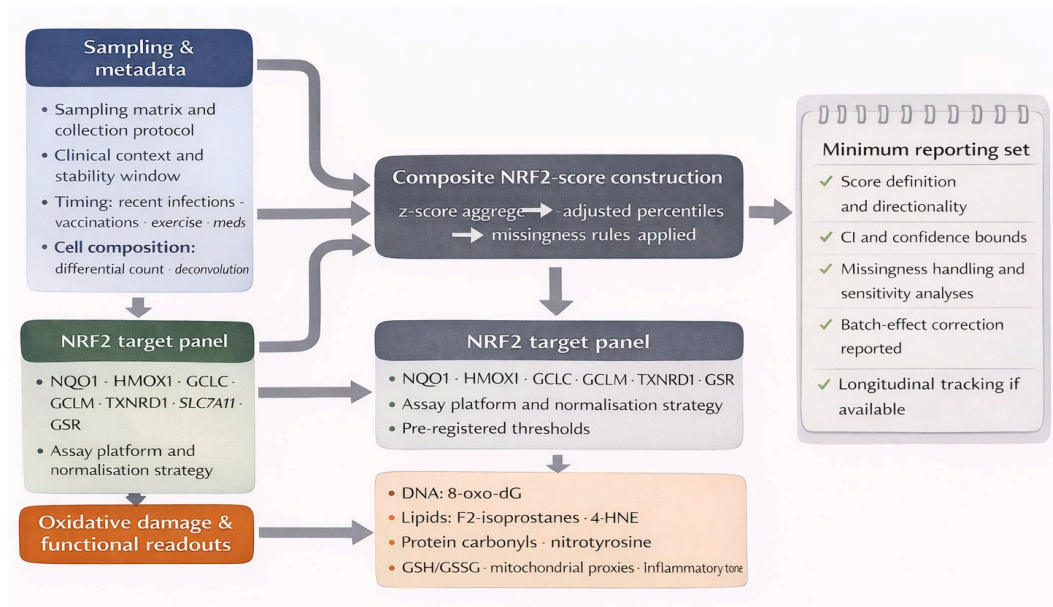


Figure 8: Minimum reporting set and workflow for the construction of a composite NRF2 score. The workflow proceeds from left to right through five integrated components. Component 1 (Sampling and metadata annotation) specifies minimum requirements for tissue source, collection timing relative to stress or treatment, clinical context, and cell composition estimates. Component 2 (Core NRF2 target gene panel) defines a reproducible set of canonical target genes, including NQO1, HMOX1, GCLC, GCLM, TXNRD1, SLCA11, and GSR, measured by a pre-specified platform with batch correction applied. Component 3 (Orthogonal readouts) incorporates oxidative damage markers in DNA, lipids, and proteins, plus functional impairment proxies capturing mitochondrial performance, inflammatory tone, and organ reserve. Component 4 (NRF2-score construction) describes the stepwise derivation of the composite index, weighting pathway output against damage burden and adjusting for cell composition, with confidence interval estimation and explicit handling of missing data. Component 5 (Quality control and longitudinal tracking) specifies pre-specified thresholds for score interpretation, inter-batch reproducibility checks, and serial sampling requirements to support cross-study comparison and validation in independent cohorts.

11 Conclusions

Across chronic diseases, NRF2 stands out as a regulator of cellular resilience because it coordinates antioxidant buffering, electrophile detoxification, and recovery after stress. In this review, we defined low NRF2 activity as a functional deficit in inducible protection, rather than as a single static marker measured at rest. This framing links molecular biology to clinical vulnerability, and it clarifies why timing, cell identity, and tissue context shape interpretation.

Mechanistically, reduced NRF2 output erodes glutathione-centred redox control and limits the coordinated induction of phase II and phase III detoxification programs that normally shorten the lifetime of reactive intermediates. Mitochondrial quality control and proteostasis also suffer, and ER stress responses can drift from adaptive remodelling toward inflammatory outputs that impair regeneration and repair. These deficits rarely remain local because oxidized lipids, protein adducts, and mitochondrial danger signals amplify immune activation, propagate microinjury, and sustain cytokine networks that further suppress adaptive stress responses.

Our integrated organ synthesis shows that shared endotypes recur across the lung, the liver, the cardiovascular system, the kidney, the brain, and barrier interfaces that communicate with systemic immunity. Chronic inflammatory patterns, metabolic and diabetic complications, fibrosis-prone remodelling,

and post-stressor vulnerability appear repeatedly, even when clinical labels and initiating triggers differ. This recurring logic supports biomarker strategies that combine NRF2 target gene panels, oxidative damage readouts in DNA, lipids, and proteins, and functional proxies that track mitochondrial performance, inflammatory tone, and barrier integrity.

Therapeutically, NRF2 restoration should be viewed as range seeking rather than maximization, because maladaptive hyperactivation can become problematic, particularly in oncology and in selected infectious contexts. Lifestyle and exposome interventions can remove suppressors and introduce hormetic inputs, while nutritional, pharmacologic, and targeted delivery approaches may provide more direct engagement when disease burden is high. The translational priority is to match intervention to endotype, confirm engagement with predefined signatures, titrate dosing dynamics, and pair modulation with burden reduction and careful interaction review.

Future progress will depend on study designs that establish causality, standardize pathway scores, and link engagement to outcomes that matter to patients and clinicians. Longitudinal cohorts and stratified trials, supported by tissue-specific perturbation models, should converge on rules for who benefits, when to intervene, and how to monitor safety with explicit stopping criteria. With these advances, the NRF2 insufficiency hypothesis can progress from an attractive unifying concept towards a testable clinical framework for precision prevention and therapy, provided that causal study designs and validated biomarker instruments are in place, while respecting boundary conditions and preserving interpretability. Several specific findings and open questions are worth highlighting as priorities for the next research cycle. First, the NRF2-score proposed in this review has not yet been prospectively validated in an independent cohort with hard clinical endpoints; doing so is the single most impactful step toward clinical adoption, and we encourage collaborative data sharing across existing biobank initiatives to accelerate this validation. Second, the bardoxolone methyl experience in diabetic kidney disease demonstrated both the feasibility and the complexity of NRF2-directed pharmacology in humans, including fluid retention signals that required careful patient selection; future trials should build on this experience by embedding mechanistic substudies that distinguish on-target from off-target effects. Third, the intersection of heavy metal exposure and NRF2 suppression remains underexplored in chronic disease cohorts, despite clear mechanistic plausibility and global public health relevance; integrating metal speciation into exposome-aware NRF2 studies represents an accessible and impactful research direction [50]. Fourth, single-cell and spatial transcriptomic technologies now allow NRF2 target gene heterogeneity to be mapped at cellular resolution within diseased tissue, and applying these tools to human biopsy archives from well-phenotyped cohorts could definitively resolve which cell compartments must be corrected to interrupt self-reinforcing pathological cycles. Finally, the development of organ-targeted NRF2 activators with well-characterised pharmacokinetic and safety profiles remains the principal unmet need for therapeutic translation, and investment in this area should be viewed as a strategic priority for precision redox medicine over the coming decade.

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