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REVIEW

Role of heme oxygenase-1 in tumor immune escape

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Abstract

The inducible enzyme heme oxygenase 1 (HO-1) plays a pivotal role in cell defense against different kind of stressors, from oxidative stress to hypoxia. For this reason, HO-1 overexpression has been correlated to cancer aggressiveness in different tumors, being one of the molecular mechanisms used by tumor cells to become resistant to therapies. In addition, HO-1 has a well-recognized role in restraining immune response and in maintaining tolerance. In this context, the possibility that HO-1 induction in immune cells can reduce immune response to cancer and impair cancer immune therapy becomes a hot topic in cancer research. In this review, the most recent evidence pointing out the role of HO-1 in generating a permissive tumor microenvironment has been discussed as well as the most promising therapeutic approaches to increase effectiveness of immune therapies.

Key Words

- HO-1
- cancer
- tumor microenvironment
- immune escape

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Introduction

The enzyme heme oxygenase (HO), described in two different isoforms (HO-1 and HO-2) in mammals, degrades heme group into biliverdin, carbon monoxide (CO), and free iron. The reaction is carried out in presence of molecular oxygen (O_2) and nicotinamide adenine dinucleotide phosphate (Maines 1988). HO-1 and HO-2 are codified by two different genes; HMOX-1 gene maps on the human chromosome 22q12.3 (Kutty *et al.* 1994), on a region of approximately 13,148 bp, containing five exons and four introns, and codifies for a 32 kD protein (Waza *et al.* 2018). HMOX-2 gene maps on the human chromosome 16p13.3 and encodes for two protein transcripts of 36 kDa. The degree of similarity between HO-1 and HO-2 is about 50% (McCoubrey & Maines 1994). While HO-2 is constitutively expressed in particular in brain and testis, HO-1 is expressed at very low levels under physiological conditions in the most cell types and upregulated (Waza *et al.* 2018) as part of stress response mechanisms (Keyse & Tyrrell 1989, Nitti *et al.* 2022, Sies *et al.* 2022). Indeed, HO-1 is induced in response to various stressors (e.g. oxidative insults or iron overload) in order to maintain redox homeostasis preventing cell damage or transformation, and the products of its enzymatic activity exert the antioxidant and pro-surviving properties of HO-1. Indeed, biliverdin, together with bilirubin derived by the reduction carried out by biliverdin reductase, as well as CO are potent antioxidant and antiapoptotic molecules. Furthermore, the release of free iron is normally quenched by ferritin, which is synthesized in parallel with HO-1, or extruded by cells through ferroportin (Yanatori *et al.* 2020), thus preventing Fenton reaction and cell damage.

It is important to note that HO-1 is considered a key molecule in promoting immune tolerance and immune suppression, acting as major regulator of crosstalk between innate and adaptive immune response (Ozen *et al.* 2015). For instance, it has been well demonstrated that HO-1 induction protects cells and tissues from immunological destruction, promoting the generation of $CD4^+CD25^+$

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regulatory T cells in mouse models of transplantation (Yamashita *et al.* 2006).

One of the main effectors of the anti-inflammatory and immunomodulatory action of HO-1 is CO, as demonstrated in different experimental models in which CO administration mimics the effects of HO-1 induction. HO-1-derived CO increases interleukin-10 (IL-10) production from macrophage (Otterbein *et al.* 2000) and drives maturation and proliferation of T cells toward anti-inflammatory and immunosuppressive phenotype (Song *et al.* 2004). Moreover, HO-1 induction and CO generation act as a safeguard mechanism to prevent inappropriate T cell activation, as demonstrated in monocyte and in naïve $CD4^+$ and $CD8^+$ T cells (Burt *et al.* 2010). In addition, CO is able to inhibit APC maturation and induces Treg proliferation and expansion, ensuring a tolerogenic phenotype (Wegiel *et al.* 2013). Indeed, both in rats and in human, the overexpression of HO-1 increases the refractory of dendritic cells (DCs) to lipopolysaccharide (LPS)-induced maturation (Chauveau *et al.* 2005) and limits antigen presentation, thus impairing the activation of $CD4^+$ T-cell responses (Campbell *et al.* 2018). In addition, HO-1-derived CO promotes immunotolerance at fetal–maternal interface (Sollwedel *et al.* 2005) and maintains maternal DCs in an immature state, leading to the expansion of the peripheral Treg population (Schumacher *et al.* 2012, Solano *et al.* 2015). Furthermore, HO-1 upregulation is involved in the early expansion, differentiation, and maturation of myeloid cells into macrophages (Wegiel *et al.* 2014). HO-1 overexpression was demonstrated to be responsible for the switch to M2 macrophage polarization, and M2 macrophages showed high levels of HO-1 expression (Naito *et al.* 2014). Also, HO-1/CO system inhibits both caspase-1 activation and secretion of pro-inflammatory cytokines IL-1β and IL-18, acting as inflammasome inhibitors (Kim & Lee 2013).

However, the anti-inflammatory activity of HO-1 is mediated not only by CO but also by bilirubin. Indeed, the potent anti-inflammatory activity of bilirubin is well recognized since the pioneering observation of Philip S. Hench concerning the anti-inflammatory effect of jaundice (Hench 1938). Nowadays, the increased blood levels of bilirubin, when limited to modest increments, are considered protective against cardiovascular diseases, aging, and inflammatory diseases, as widely revised by Vitek and Tiribelli (Vitek *et al.* 2023). In addition, HO-1-derived bilirubin can act as potent immune modulator also in local tissue microenvironment, for instance favoring wound healing or regulating acute inflammation (Nitti *et al.* 2020). Indeed, not only bilirubin is able to affect innate immunity by interfering with the complement cascade activation (Basiglio *et al.* 2007) and inducing M2-macrophages polarization (Zhao *et al.* 2021) but is also able to affect adaptive immunity. It has been demonstrated that bilirubin inhibits T-cell proliferation and decreases the production of pro-inflammatory Th1 cytokines IL-2 and interferon-γ (IFNγ) in a dose-dependent manner in experimental model of EAE (Liu *et al.* 2008). In addition, macrophage exposure to bilirubin reduces PD-L1 expression and leads to the expansion of Treg cells (Rocuts *et al.* 2010, Adin *et al.* 2017). Furthermore, both *in vitro* and *in vivo* bilirubin inhibits the production of inflammatory cytokines such as IL-6 and tumor necrosis factor α (TNFα) and reduces leukocyte transmigration via interaction with endothelial adhesion molecules (Keshavan *et al.* 2005), in particular decreasing the expression of P- and E-selectin, VCAM, and ICAM (Mazzone *et al.* 2009, Grochot-Przeczek *et al.* 2012).

HO-1 anti-inflammatory activity is widely recognized, and HO-1 has been proposed as a potential pharmacological target to treat chronic inflammatory diseases, as recently revised (Campbell *et al.* 2021).

Thus, due to the prominent role in cell survival and adaptation to stress, the induction of HO-1 in cancer cells can favor tumor progression and resistance to therapy. Nonetheless, in the last years, HO-1 overexpression ha been demonstrated also in cells of the tumor microenvironment (TME) and proved to be involved in the gain of a tolerogenic phenotype.

In this review, the main aspects of the role of HO-1 in the modification of TMEN and in immune escape will be detailed, highlighting new potential therapeutic approaches in cancer treatment.

Molecular mechanisms of HMOX-1 transcription in cancer cells and in immune cells

HMOX-1 gene promoter contains NRF2, hypoxia inducible factor-1 (HIF-1), Sp1, AP-1, nuclear factor-kappa B (NF-kB), and STAT3-binding sites that enable gene transcription in response to oxidative and electrophilic stressors or hypoxia (Lavrovsky *et al.* 1994, Siow *et al.* 1999, Prawan *et al.* 2005, Alam & Cook 2007) and to different signal transduction pathways, setting up a pivotal mechanisms of cell survival and adaptation. Here, we focus on the main molecular mechanisms involved in HMOX-1 transcription demonstrated in cancer cells and in immune

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cells, especially with regard to cancer-immune recognition (Fig. 1).

HO-1 induction in response to the most oxidative and electrophilic stressors mainly relays on the activation of NRF2 (Loboda *et al.* 2016, Yamamoto *et al.* 2018), and the role played in cancer progression by NRF2-dependent HO-1 induction is well known and characterized (Shibata *et al.* 2008, Na & Surh 2014, Furfaro *et al.* 2016*b*). NRF2 in the cytosol is bound to its inhibitor Kelch-like ECH-associated protein 1 (Keap1) which belongs to the Cullin3 (CUL3)-based ubiquitin 3 ligase complex and targets NRF2 to proteasome degradation. Keap1 modifications due to cysteine oxidation or electrophile binding allow NRF2 to move into the nucleus and bind antioxidant/electrophile response elements (AREs) by dimerizing with small Maf proteins, leading to HO-1 transcription (Kobayashi & Yamamoto 2005, Hirotsu *et al.* 2012).

In cancer cells, NRF2/Keap1 system can be affected by genetic modifications (Mitsuishi *et al.* 2012, Furfaro *et al.* 2016*b*). Its constitutive activation due to gain-of-function mutations of NRF2 or loss-of-function mutations of Keap1 has been identified in different kinds of tumors, including head and neck, lung, esophageal, gastric, liver, bladder, and colorectal cancer (Na & Surh 2014). In addition, epigenetic modifications, such as TET-dependent demethylation of NRF2 promoter, or CUL3 hypermethylation of Keap1, can induce NRF2 constitutive activation in lung, ovarian, and colorectal cancers (Hanada *et al.* 2012, van der Wijst *et al.* 2014, Zhao *et al.* 2015). Recent evidence underlines that NRF2/HO-1 pathways activation works as an oncogenic route that favors murine breast cancer progression modulating immune response in pro-carcinogenic direction (Li *et al.* 2021).

In immune cells, NRF2-dependent HO-1 induction has a prominent role in macrophage polarization. It has been recently demonstrated that in diet-induced obese mice JWH-133, an agonist of cannabinoid receptor 2, regulates its anti-inflammatory and anti-obesity activity by promoting macrophage polarization to M2 in adipose tissue via NRF2/HO-1 pathways (Wu *et al.* 2020). In addition, the activation of NRF2/HO-1 pathway is linked to IL-10 production and to the gain of a pro-fibrotic feature in macrophages, cooperating with the arhyl hydrocarbon receptor in response to the exposure to uremic toxins (Barisione *et al.* 2016). Furthermore, NRF2/HO-1 activation in tumor-associated macrophages (TAMs) reduces the efficacy of anticancer treatment and favors melanoma progression, as better discussed in the next sections (Consonni *et al.* 2021).

HO-1 induction is also observed as a response to the increased intracellular concentration of heme groups (Ogawa *et al.* 2001). The BTB domain and CNC homolog 1 (Bach1) is a heme-binding protein able to bind ARE sequences repressing HMOX-1 transcription (Kikuchi *et al.* 2005, Ryter & Choi 2005, Chapple *et al.* 2016, Piras *et al.* 2017, Zhang *et al.* 2019). Heme groups can bind Bach1 that in turns detaches from ARE sequences enabling HMOX-1 transcription (Ogawa *et al.* 2001, Davudian *et al.* 2016, Nitti *et al.* 2017). The degradation of heme due to the activity of HO-1 stabilizes Bach1 and prevents its further degradation, restoring its level. Thus, when Bach1 levels are restored, HO-1 levels in turns decreases. In cancer cells,

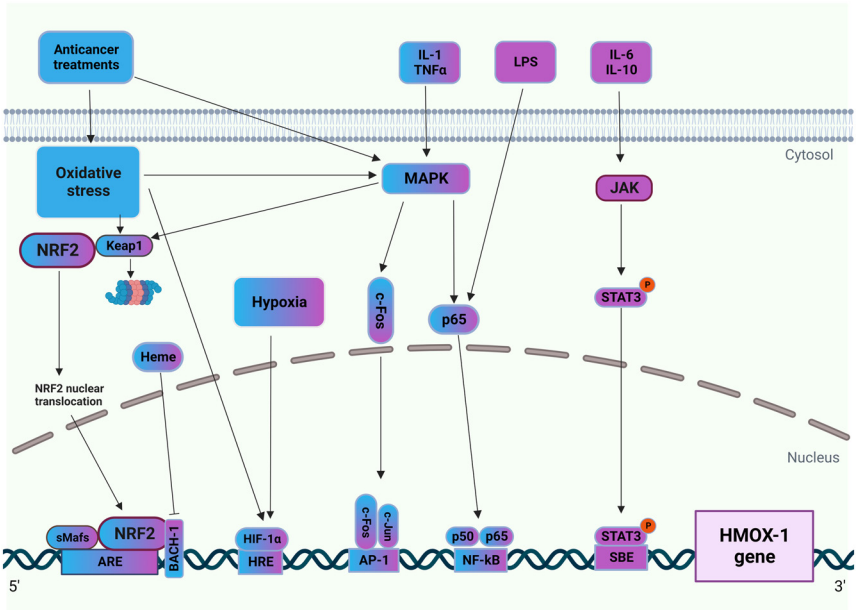

Figure 1
Schematic representation of the main molecular pathways involved in HMOX1 gene transcription, particularly relevant in cancer cells (blue) and immune cells (purple) or in both (degrading). See text for more details. Image created with BioRender.com.

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this mutual regulation between HO-1 and Bach1 seems lost. Indeed, Bach1 stabilization can be observed in the presence of HO-1 expression in lung cancer metastasis and correlates with poor prognosis (Lignitto *et al.* 2019, Wiel *et al.* 2019). The role of heme in Bach1 modification and the correlation with tumor progression has been demonstrated and reviewed elsewhere (Muhseena *et al.* 2021). Considering immune cells, it has been recently well proved that HO-1 can be induced by heme independently by NRF2 but dependently by Bach1, at least in peritoneal macrophages (Zhang *et al.* 2021), and this aspect seems to be particularly relevant in inflammatory response. Moreover, the possibility to induce HO-1 in a Bach1-dependent but NRF2-independent way has been demonstrated for cannabidiol and proved to exert anti-inflammatory effects (Casares *et al.* 2020).

The different kinases (i.e. MAPKs and PI3K/AKT) involved in HO-1 induction in cancer cells can act on NRF2 but also on other transcription factors. In MCF-7 breast cancer cells treated with cadmium chloride, the induction of HO-1 is due to p38 MAPK-dependent NRF2 activation (Alam *et al.* 2000). In human gastric cancers, HO-1 induction is mediated by ERK activation but independent of NRF2 (Liu *et al.* 2004). Also, PI3K/AKT plays a role in HO-1 induction in neuroblastoma and in cholangiocarcinoma treated with guanosine (Dal-Cim *et al.* 2012) or piperlongumine (Talabnin *et al.* 2020), respectively.

HIF-1α (Wang *et al.* 1995, Pugh *et al.* 1997) also induces HO-1 expression in response to cellular stressors, including endogenous ROS and oxygen deprivation (Chin *et al.* 2007, Palazon *et al.* 2014). A specific HIF-1α/HO-1 pathway has been well characterized in halting inflammatory response in lungs (Hu *et al.* 2015) and correlated to the modulation of mitochondrial biogenesis (Yu *et al.* 2016, Shi *et al.* 2021). Importantly, a specific role of HIF-1α-dependent HO-1 induction has been demonstrated to be involved in maintaining Hodgkin lymphoma cells as undifferentiated (Nakashima *et al.* 2021). Notably, the expression of HIF-1α has been proved to be highly interconnected with HO-1 overexpression in order to constitute a highly tolerogenic TME, favoring M2 polarization and Treg recruitment in breast cancer (Duechler *et al.* 2014).

Sp1 and AP-1 have been demonstrated to be responsible of HO-1 induction in mouse brain endothelial cells exposed to prostaglandin 15d-PGJ2 downstream the activation of ROS/PKCδ/JNK1/2 cascade (Yang *et al.* 2022). Moreover, it has been demonstrated that HO-1 is induced through the activation of AP-1 via PKCα/Pyk2/p38α MAPK- or JNK1/2-dependent c-Jun activation, in human pulmonary alveolar cells exposed to mevastatin, and suppresses TNFα-dependent inflammation (Yang *et al.* 2020).

In addition, the relevance of AP-1 and NF-kB in the regulation of HMOX-1 transcription has been described in the inflammatory response (Luu Hoang *et al.* 2021). Indeed, the binding site for NF-kB in the promoter region of HO-1 was identified by Abraham's lab in 1996 (Lavrovsky *et al.* 1994) and confirmed later on as part of the mechanisms underlying HO-1 expression in macrophages exposed to LPS (Kurata *et al.* 1996) or in epithelial cells treated with TGFβ (Lin *et al.* 2007*a*). Interestingly, also the pharmacological modulation of this pathway has been proposed in the treatment of B-cell lymphoma (Huang *et al.* 2016).

With particular relevance to macrophages, IL-10 induces the upregulation of HO-1 via IL-10R, through STAT3 phosphorylation and its binding to the STAT-binding element in the promoter region of HO-1 (Ricchetti *et al.* 2004, Naito *et al.* 2014). Notably, STAT3 can be activated downstream of HO-1 induction, since HO-1 inhibition was able to downregulate STAT3 activation (Magri *et al.* 2022).

It is important to note that the presence of two kinds of polymorphisms such as (GT)n repeats and SNPs in gene promoter can influence HMOX1 inducibility. Indeed, the length of (GT)n repeats (long vs short) correlates with different transcriptional activity (lower vs higher, respectively) and with the development of cardiovascular and pulmonary diseases (Exner *et al.* 2004, Daenen *et al.* 2016). Also, a higher degree of inducibility is associated with SNP413 A>T and with a reduction of cardiovascular disease risk (Ono *et al.* 2004). Yet, few data have been provided so far as far as neoplastic pathology is concerned, as previously reported by us (Nitti *et al.* 2021).

HO-1 expression is regulated by microRNA as well, as elsewhere revised (Cheng *et al.* 2013), directly or through the modulation of NRF2-dependent activation pathway affecting cancer progression. Indeed, the modulation of NRF2/HO-1 by miRNA-155 or miR200a has a role in lung cancer and breast cancer progression, respectively (Eades *et al.* 2011, Gu *et al.* 2017). Also, miR-1254 or miR-193a-5p acts directly on HO-1 and reduces the growth of non-small cell lung cancer (NSCLC) and prostate cancer, respectively (Pu *et al.* 2017, Yang *et al.* 2017), and we proved that miR494 favors neuroblastoma cell survival in oxidative stress condition by inducing HO-1 (Piras *et al.* 2018).

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HO-1 protein structure and localization

HO-1 has an α-helical structure. The heme group is coordinated with His 25 and is accommodated between the distal and the proximal helices generating a closer conformation in the holoenzyme (Rahman *et al.* 2013).

Q11 HO-1 main localization is the endoplasmic reticulum (ER) where co-localizes with cytochrome P450 (CYP450) reductase (Durante 2020), but evidence has been provided also for plasma membrane, where HO-1 co-localizes with caveolin 1 and 2 (Jung *et al.* 2003), mitochondria (Slebos *et al.* 2007), and nuclei (Lin *et al.* 2007*b*). It has been proved that HO-1 prevents apoptosis acting on caveolin 1 through the activity of CO (Kim *et al.* 2005). Localization at the mitochondria seems to be involved in the control of apoptotic pathway, in a mutual relation with HO-2 (Turkseven *et al.* 2007). In addition, mitochondrial HO-1 controls the metabolism of heme groups (Converso *et al.* 2006). However, the role played by the different localization of HO-1 in the progression of tumors has not been evaluated, with the exception of the nuclear localization. Indeed, a truncated form of HO-1, with nuclear localization and no enzymatic activity, derived Q12 by proteolytic activity of peptide peptidase (SSP), has been described (Lin *et al.* 2007*b*, Hsu *et al.* 2015). Being characterized by a transcriptional activity, truncated HO-1 has been hypothesized to be importantly involved in cancer progression. In the acetylated form, truncated HO-1 increases AP-1 transcriptional activity, leading to cancer progression (Hsu *et al.* 2017, Mascaró *et al.* Q13 2021). Contrasting results have been described, both highlighting not only the association between nuclear compartmentalization and disease severity in chronic myeloid leukemia (Tibullo *et al.* 2013) but also opposite observations (Ferrando *et al.* 2011, Degese *et al.* 2012), and the topic has been revised in deep elsewhere (Mascaró *et al.* 2021).

To note, HO-1 has been detected extracellularly, in plasma, serum, milk, cerebrovascular fluids, and urine (Serpero *et al.* 2012, Signorelli *et al.* 2016, Vanella *et al.* 2016), opening to the chance to investigate a potential role of HO-1 as biomarker (Tibullo *et al.* 2013). The mechanisms underlying the extracellular localization are still largely unknown, and both the active secretion and the result of cellular lysis have been hypothesized. In patients with acute myocardial infarction, the plasma level of HO-1 does not correlate with biomarkers of necrosis (Novo *et al.* 2011), and in patients with acute kidney injury, the urinary level of HO-1 mirrors the increased level in renal tissue, as a response to cell damage (Zager *et al.* 2012). Interestingly, a correlation between the serum level of HO-1 and the progression of abdominal aortic aneurysm has been highlighted (Hofmann *et al.* 2021). With regard to cancer, it is to note that HO-1 protein is found in the culture medium of breast, lung, melanoma, and kidney tumor cells in the extracellular vesicles (Hurwitz *et al.* 2016). In this context, HO-1 needs to be taken into consideration as a potential circulating biomarker, especially considering the evident correlation among HO-1 expression levels in cancer tissues and clinical disease score or prognosis, as already reviewed (Nitti *et al.* 2021), even though more investigations are needed.

HO-1 expression and immune escape

During the progression of neoplastic disease, the upregulation of HO-1 can modify TME, decreasing cancer cell immune recognition. This effect can be achieved by different mechanisms. On one hand, cancer cells can upregulate HO-1 and elude immune surveillance modifying the expression of receptors for immune cells or through the generation of immune-suppressive cytokines. On the other hand, immune cells themselves can overexpress HO-1 gaining a less aggressive, tolerant phenotype. These two main mechanisms are detailed below.

HO-1 expression in cancer cells reduces immune recognition

The induction of HO-1 in cancer cells has been related to the progression of disease, and associated with resistance to therapy, invasiveness, metastasis, and angiogenesis, as widely reviewed by us and others (Jozkowicz *et al.* 2007, Was *et al.* 2010, Nitti *et al.* 2017) and not further discussed here.

Nonetheless, in the last years, HO-1 upregulation in cancer cells gained attention for the ability to impair immune recognition (Fig. 2).

We have recently demonstrated that, in $BRAF^{V600}$-mutated melanoma cells treated with vemurafenib (PLX4032), HO-1 overexpression reduces natural killer (NK) recognition impairing the expression of NK ligands (B7-H6 and ULBP3), both under standard culture conditions (Furfaro *et al.* 2020) and under physiological oxygen tension or hypoxia (Furfaro *et al.* 2022). Moreover, the induction of HO-1 in cervical cancer cells reduces the expression of specific markers of NK activation (NKG2D,

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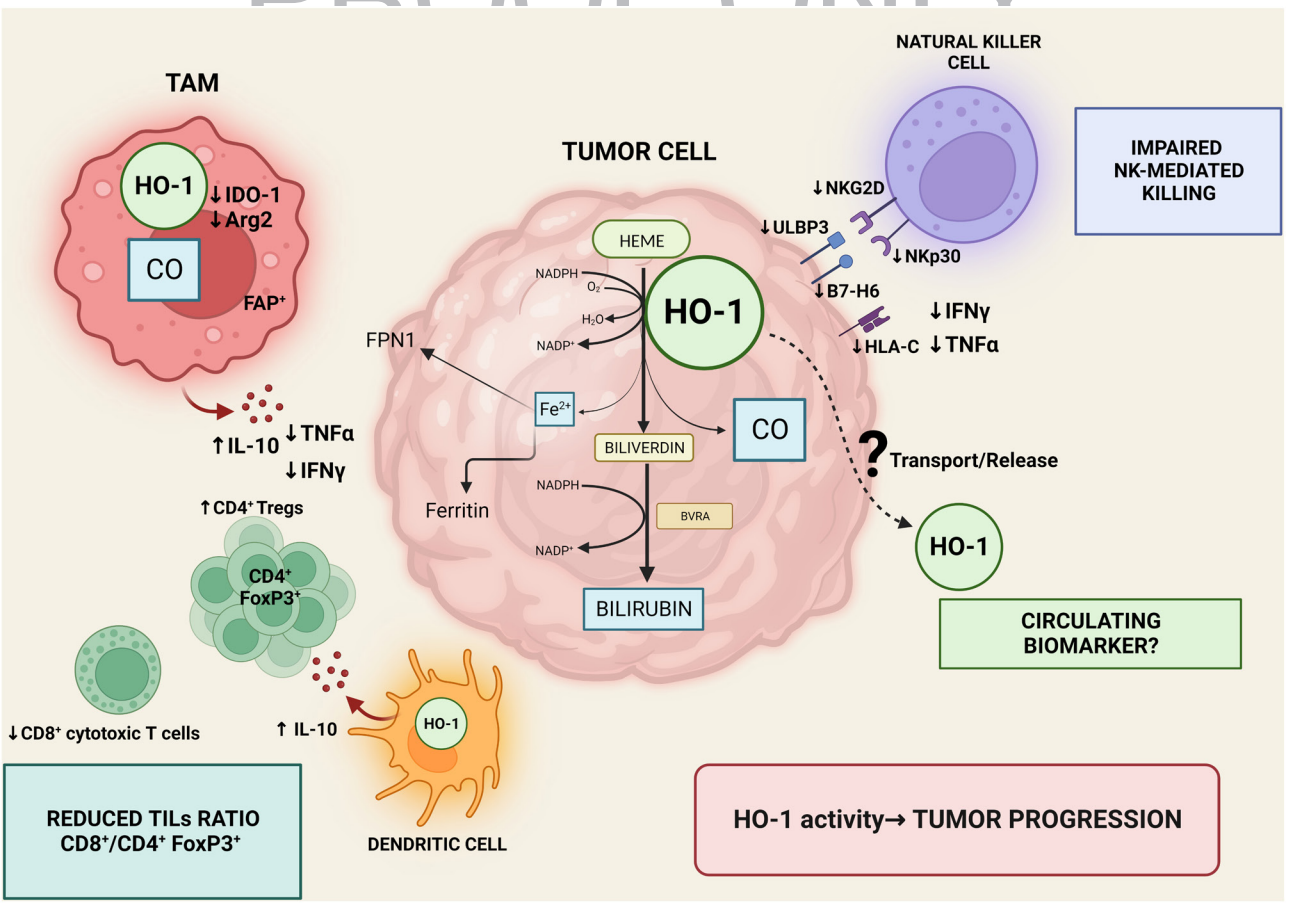

Figure 2
Schematic representation of the immune-suppressive activity induced by HO-1 overexpression in cancer cells, in TAMs, and in DCs and the effects on T and NK cells that lead to immune-escape and cancer progression. Image created with BioRender.com.

NKp30, and NKp46) and the production of IFNγ and TNFα in a co-culture model (Gomez-Lomeli *et al.* 2014). In both experimental systems, the downregulation or the enzymatic inhibition of HO-1 in cancer cells restores the NK antitumor activity restoring the expression of NK ligands on cancer cells (Furfaro *et al.* 2020, 2022) or NK-activating receptors (Gomez-Lomeli *et al.* 2014). In line with these results, HO-1 overexpression in acute myeloid leukemia (AML) cells has been shown to decrease NK cytotoxicity activity by inhibiting CD48-2B4 axis both *in vitro* and *in vivo* and is associated with a poor prognosis in term of overall survival, refractory, and relapse (Zhang *et al.* 2022). More recently, the induction of HO-1 in AML has been proved to reduce the expression of HLA-C, thus favoring tumor escape from NK-mediated killing (Feng *et al.* 2023).

Furthermore, breast cancer and melanoma progression has been successfully halted by fasting-mimicking diet that increases the infiltration of cytotoxic CD8+ tumor-infiltrating lymphocytes (TILs) through the downregulation of HO-1 in cancer cells (Di Biase & Longo 2016).

Interestingly, a potent immune-suppressive response is observed in regulatory CD8+ T cells that specifically recognized HO-1 and that crucially contribute to the suppression of T-mediated antitumoral response (Andersen *et al.* 2009). Thus, HO-1 could drive the suppression of T-cell response once recognized by immune cells. In addition, these cells have been detected not only in tumor mass but also in peripheral blood potentially working as biomarkers in cancer patients (Andersen *et al.* 2009).

HO-1 expression in TAMs, TILs, and DCs impairs cancer immune recognition

In recent years, HO-1 upregulation has been described in immune cells of TME, where it plays a role in suppressing antitumor response promoting a permissive environment

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for growth and metastasis. In this context, the most important evidence comes from the analysis of TAMs, TILs, and DCs (Luu Hoang *et al.* 2021).

TAMs are the main source of HO-1 in the TME. They respond to different tumor-derived stimuli and are able to differentiate and reprogram into different subsets that are beyond the classic M1 and M2 dichotomy, and HO-1 has been recently proved to be overexpressed in different specific subgroups of TAMs (Arnold *et al.* 2014, Muliaditan *et al.* 2018*a*, Consonni *et al.* 2021).

Indeed, in two murine models of Lewis lung carcinoma and pancreatic ductal adenocarcinoma, a particular subset of HO-1-positive TAMs co-expressing fibroblast activation protein alpha (FAP$^+$HO-1$^+$ TAMs) has been described (Arnold *et al.* 2014). In these cells, HO-1 conditional ablation or HO-1 pharmacological inhibition decreases tumor growth, confirming the immunosuppressive role of HO-1 in TAMs.

Similar TAM subsets were found in tissue sections of human adenocarcinoma and in the 4T1 orthotopic model of breast adenocarcinoma (Muliaditan *et al.* 2018*a*). They are predominantly located in the perivascular region of the tumor and facilitate trans-endothelial migration and metastatic spread, and HO-1 inhibition completely abrogates this effect (Muliaditan *et al.* 2018*a*).

Q14 Positive staining for HO-1 was also found in CD11b$^+$- and F4/80$^+$-infiltrating macrophages in E.G7-OVA tumor-bearing mice (Alaluf *et al.* 2020). HO-1$^+$ TAMs derive from a subset of Ly6C^{hi} monocytes that gradually differentiate into Ly6C^{lo}MHCII$^+$ TAMs in TME. Compared with HO-1-negative TAMs, this subset shows decreased MHC II expression in line to its immunosuppressive feature.

Recently, another particular subgroup of TAMs expressing high levels of HO-1 (F4/80^{hi}CD115^{hi}C3aR^{hi}CD88^{hi}) has been identified (Consonni *et al.* 2021). This population, phenotypically similar to erythrocyte macrophages, preferentially accumulates at the invasive edge of the tumor, in line with their involvement in neoangiogenesis, epithelial-to-mesenchymal transition, and tumor spread. Furthermore, Q15 M-CSF or C3a-induced differentiation of bone marrow-derived monocytes (BMDMs) upregulates HO-1 in an NRF2-dependent way coordinated by the p50NF-kBi-CSF-R1-C3aR axis and induces TAM phenotype in CO-dependent manner. Importantly, both the deletion of myeloid HO-1 and the inhibition of recruitment pathway of HO-1$^+$ TAMs are able to block metastasis formation and to enhance the effect of immunotherapy, in particular increasing the efficacy of anti-PD-1 therapy. Moreover, in patients with stage III melanoma, HO-1 expression levels in the peripheral monocytes correlate with HO-1 expression in CD163$^+$ cells of metastatic lesions, thus highlighting the correlation between HO-1 expression and poor prognosis (Consonni *et al.* 2021).

Other evidence shows that following chemotherapy-induced phagocytosis of tumor cells, TAMs upregulate HO-1 expression that, in turns, hampers M1 polarization, attenuating the effect of chemotherapeutics. In fact, using HO-1 knockout mice or in the presence of HO-1 inhibitor the response to chemotherapy is efficiently restored (Kim *et al.* 2020, 2021).

In addition, HO-1 plays a role in the metabolic changes of TAMs, such as modification in aminoacid metabolism, driving the establishment of an immunosuppressive microenvironment (Magri *et al.* 2022). In fact, the inhibition of HO-1 in BMDMs significantly reduces the expression of IDO-1 and Arg2 (Magri *et al.* 2022), two essential enzymes involved in the catabolism of L-arginine and L-tryptophane, associated with the immunosuppressive network in cancer (Mondanelli *et al.* 2017).

Notably, HO-1 expression in TAMs modulates the activity of TILs, DCs, and NK cells toward an immune-suppressive feature. Indeed, the inhibition of HO-1 in TAMs affects the production of cytokines involved in T-cell recruitment and regulation, leading to a reduction in the expression of Tregs and increasing the proportion of CD8$^+$ T cells, highlighting the strategic potential associated with TAMs reprogramming by HO-1 inactivation (Kim *et al.* 2021). Importantly, myeloid ablation of HO-1 is able to improve the response toward therapeutic immunization promoting antitumor CD8$^+$ T cell proliferation and cytotoxicity (Alaluf *et al.* 2020).

Q16 Moreover, in glioblastoma tissues, a large presence of CD68$^+$/HO-1$^+$ macrophages and a lower percentage of CD8$^+$ T lymphocytes have recently been shown; HO-1 inhibition, which strongly reduces IL-10 release, is able to drive the complete recovery of T-cell proliferation (Magri *et al.* 2022).

Furthermore, HO-1 deletion, by promoting a phenotypic switch in F4/80^{hi} TAMs, increases the expression of IFNγ and GrzB in CD8$^+$ T cells, leading to a higher frequency of effector memory cells (CD8$^+$CD44$^+$CD62L$^-$ cells) and to an augmented CD8$^+$/CD4$^+$FoxP3$^+$ ratio restoring their antitumor activities (Consonni *et al.* 2021). Notably, the infiltration of HO-1$^+$CD4$^+$CD25$^+$ FoxP3$^+$ Tregs correlates with the progression and grading of glioma (El Andaloussi & Lesniak 2007).

With regard to DCs, it has been demonstrated that HO-1 induction maintains DCs in an immature and

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pro-tolerogenic status (Chauveau *et al.* 2005). Moreover, the immunomodulatory activity of $CD4^+CD25^+$ Tregs is dependent on HO-1 expression in DCs (George *et al.* 2008). Importantly, pro-tolerogenic signature of DCs in TME is achieved in an HO-1-dependent manner (Trojandt *et al.* 2016).

No evidence of HO-1 upregulation in NK cells in TME has been pointed out so far.

HO-1 inhibitors in cancer therapy

The role of HO-1 in tumor progression appears, then, to be related to two crucial aspects: on one hand, HO-1 exerts an antiapoptotic, pro-surviving activity that protects cancer cells from the death induced by therapeutic agents, and this has been widely revised elsewhere by us and others (Podkalicka *et al.* 2018, Nitti *et al.* 2021). However, HO-1 carries out a crucial immune-suppressive activity both in tumor cells, reducing their immune recognition, and in immune cells reducing their antitumoral activity. Thus, the inhibition of HO-1 activity as well as its molecular downregulation reducing the availability of HO-1 bioactive products, CO and bilirubin, can have strategic therapeutic potential acting both as chemosensitizer, increasing the efficacy of traditional anticancer treatments (chemo-, radio-, and photodynamic therapies), and as immune-stimulatory tool improving the efficacy of novel immunotherapy approaches, as explained below.

Different pharmacological compounds as well as genetic tools able to downregulate HO-1 activity have been proposed (Podkalicka *et al.* 2018). Among pharmacological compounds, proto- and mesoporphyrin derivatives and imidazole-based compounds are the most well known (Podkalicka *et al.* 2018).

The first generation of HO inhibitors are metalloporphyrins (Vreman *et al.* 1993). They are structurally similar to heme molecules and strongly inhibit HO-1 activity, although they lack in specificity (Schulz *et al.* 2012). Indeed, they act on other heme-dependent enzymes such as nitric oxide synthase (NOS), soluble guanylate cyclase (sGC), and CYP450 (Appleton *et al.* 1999, Kinobe *et al.* 2008). Moreover, their translational applicability was sometimes limited due to their poor solubility in aqueous solutions. However, the generation of water-soluble compounds by conjugation with specific molecules, for example, polyethylene glycol or amphiphilic styrene–maleic acid copolymer, increased their applicability (Sahoo *et al.* 2002, Iyer *et al.* 2007, Herrmann *et al.* 2012).

Metalloporphyrins showed efficacy both *in vitro* and *in vivo*. The most used are zinc-protoporphyrin IX (ZnPPIX), tin-protoporphyrin IX (SnPPIX), and tin-mesoporphyrin IX (SnMPIX) (Podkalicka *et al.* 2018, Nitti *et al.* 2021).

It has been shown that ZnPPIX treatment enhances the efficacy of cisplatin in hepatoma cancer cells (Liu *et al.* 2014), increases the apoptotic rate in glioma cells treated with arsenic trioxide (Liu *et al.* 2011) and is able to enhance the cytotoxic effect of gemcitabine in urothelial cancer cells (Miyake *et al.* 2010). We have demonstrated that HO-1 inhibition by ZnPPIX sensitizes neuroblastoma cells to glutathione depletion and etoposide (Furfaro *et al.* 2012) and to bortezomib treatments (Furfaro *et al.* 2014). Furthermore, in $BRAF^{V600}$-mutated melanoma cells, SnMPIX increases cell death induced by vemurafenib/PLX4032 (Furfaro *et al.* 2020).

Moreover, ZnPPIX treatment sensitizes A549 NSCLC cells to radiotherapy (Zhang *et al.* 2011) and colon and ovarian cancer cells to photodynamic therapy (Nowis *et al.* 2006), and SnPPIX sensitizes melanoma cells to photodynamic therapy (Frank *et al.* 2007).

Importantly, it has been recently reported that the inhibition of HO-1 by metalloporphyrins reprograms the immune response toward tumor cells and consequently improves the efficacy of immunotherapy. Indeed, $FAP^+HO\text{-}1^+$ TAMs can be therapeutically targeted using SnMPIX, which prevents metastatic spread by blocking HO-1-dependent CO release (Muliaditan *et al.* 2018*a*). Moreover, the inhibition of HO-1 activity by ZnPPIX restores the expression of pro-inflammatory genes such as TNFα and CXCL10 while downregulating the expression of typical anti-inflammatory genes like IL-10 and CCL22, and treatment with CO-releasing molecule, CORM-2, was able to revert these effects, confirming the role played by HO-1-dependent CO in suppressing tumor immune recognition (Consonni *et al.* 2021). In addition, in mice treated with anti-PD-1, HO-1 inhibition with ZnPPIX improves the efficacy of immunotherapy, resulting in a decrease of tumor volume (Consonni *et al.* 2021). More recently, in macrophage derived from glioblastoma patients, it has been proved that treatments with ZnPPIX or SnPPIX decrease PD-L1 expression; on the contrary, HO-1 induction obtained by macrophage exposure to cobalt protoporphyrin IX (CoPPIX) increases PD-L1 expression, indicating that PD-L1 regulation depends on HO-1 activity (Magri *et al.* 2022). Also, in a preclinical model of breast cancer, it has been demonstrated that SnMPIX can be used as immune checkpoint that targeting myeloid-derived HO-1 improves response to chemotherapy, achieving the efficacy of PD-1 blockade (Muliaditan *et al.* 2018*b*).

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Furthermore, the inhibition of HO-1 can be a strategy to improve the adoptive immunotherapy. Indeed, SnMPIX-based HO-1 inhibition significantly increases the generation of WT1 leukemia-specific T cells, from healthy donors, enhancing the effective T-cell immunity in leukemia patients (Schillingmann *et al.* 2019).

The second generation of HO-1 inhibitors, namely the imidazole-based compounds, are water-soluble non-porphyrin molecules. Acting with a non-competitive mechanism, they show a limited inhibitory activity on NOS, sGC, and CYP450 and are selective toward HO-1 (Kinobe *et al.* 2006, Pittala *et al.* 2013). Azalanstat was the first imidazole-based compound described (Vlahakis *et al.* 2005), but other molecules derived from its structural modification have been synthesized and extensively reviewed by the group of Salerno and Pittalà (Salerno *et al.* 2019). Recently, a novel acetamide-based HO1 inhibitor, with potent antiproliferative activity in U87MG glioma cells, has been discovered (Fallica *et al.* 2021).

Among imidazole-based compounds, a small molecule OB-24 shows potent inhibition of HO-1 activity. In particular, OB-24 selectively inhibits HO-1 in prostate advanced cancer cells, leading to a significant decrease of cell proliferation *in vitro* and a reduction of tumor growth and lymph node/lung metastasis *in vivo*, also showing a potent synergistic activity when combined with taxol (Alaoui-Jamali *et al.* 2009). In macrophages from glioblastoma patients, OB-24, similar to ZnPPIX and SnMPIX, reduced PD-L1 expression (Magri *et al.* 2022). Notably, in B16-F0 melanoma-bearing mice, the combination therapy OB-24 plus anti-PD-1 reduces the tumor size compared to monotherapy. The effect seems to be dependent on the inhibition of cytoprotective function of HO-1; the inhibition of HO-1, indeed, renders melanoma cells more susceptible to immune-mediated killing (Khojandi *et al.* 2021). In addition, the same authors demonstrated that OB-24-dependent HO-1 inhibition counteracts immune $CD4^+$ and $CD8^+$ TIL evasion of B16 melanoma cells, leading to a decrease in tumor volume. In fact, HO-1 induction by hemin treatment protects B16 cells from CTL-mediated killing (Kuehm *et al.* 2021).

Furthermore, different genetic tools such as RNA interference and CRISPR/Cas9 technology have been tested to modulate HO-1 activity in cancer therapy.

Small interfering RNA and short hairpin RNA act by targeting HO-1 at the transcriptional level, leading to a decrease of protein synthesis, and the efficacy of HO-1 silencing in sensitizing cancer cells to therapy has been reported widely. We have demonstrated that HO-1 silencing overcomes cancer cell resistance in neuroblastoma cells exposed to bortezomib (Furfaro *et al.* 2014, 2016*a*) and in melanoma cancer cells exposed to target therapy (Furfaro *et al.* 2020). HO-1 silencing sensitizes pancreatic cancer cell lines to gemcitabine or radiation treatment, leading to a significant inhibition of tumor growth (Berberat *et al.* 2005). Silencing of HO-1 significantly increased apoptosis as demonstrated in lung (Kim *et al.* 2008) and colon cancer cells (Busserolles *et al.* 2006). In the orthotopic model of hepatocellular carcinoma, siHO-1 results in diminished tumor growth (Sass *et al.* 2008). Moreover, siHO-1 sensitizes human urothelial and cervical cancer cells to 5-aminolevulinic acid-based photodynamic therapy (Miyake *et al.* 2009, Ohgari *et al.* 2011) and mediated the photodynamic cytotoxicity, increasing the responsiveness, in C-26 colon adenocarcinoma, in MDAH2774 human ovarian carcinoma (Nowis *et al.* 2006) and in WM451Lu human metastatic melanoma cells (Frank *et al.* 2007).

In addition, silencing of HO-1 significantly enhanced the sensitivity of HL-60R AML cell line to chemotherapy (Zhe *et al.* 2015) and induced apoptosis and cell growth arrest in acute lymphocytic leukemia (Cerny-Reiterer *et al.* 2014) as well as in chronic lymphocytic leukemia, where the silencing also enhanced the effects of the combined therapy fludarabine plus entinostat (Zhou *et al.* 2019).

CRISPR/Cas9 editing system through the genetic ablation of HO-1 leads to a stable knockdown and to a high efficiency of protein inhibition. In BRAF-WT melanoma cells, HO-1 CRISPR/Cas9 editing decreased clone formation and tumor cell growth (Liu *et al.* 2019) and in pancreatic ductal adenocarcinoma suppressed cell proliferation and increased, under hypoxia condition, the efficacy of gemcitabine treatment (Abdalla *et al.* 2019). Moreover, in T47D breast cancer cells, HO-1 CRISPR/Cas9-mediated knockdown decreased both proliferation and migration and increased cisplatin-induced apoptosis (Evazi Bakhshi *et al.* 2022).

Importantly, *in vivo* experimental models on HO-1 ablation leads to important findings on the role played by HO-1 in response to immunotherapy. Indeed, it has been demonstrated that myeloid specific ablation of HO-1 in MN/MCA1 fibrosarcoma enhances the efficacy of anti-PD-1 therapy in decreasing tumor volume and the percentage of metastatic area (Consonni *et al.* 2021).

In xenograft mouse models of AML, HO-1 gene knockout enhances the antitumor effect of PD-1 inhibition by reducing tumor growth and increasing survival. In this context, HO-1 knockout inhibits the immunosuppressive function of both polymorphonuclear and monocytic/myeloid-derived suppressor cell populations (Zhou *et al.* 2018). Moreover, it has been demonstrated that HO-1

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Table 1 Efficacy of HO-1 inhibitors in cancer therapies.

Inhibitor	Experimental models		Effect	Reference
	In vitro	*In vivo*		
ZnPPIX	Hepatoma cells (HepG2)	Xenograft mouse model of liver cancer	ZnPPIX enhances cellular sensitivity to cisplatin by increasing ROS production	Liu *et al.* (2014)
	Human glioma cell lines (U251MG and A172)		ZnPPIX increases cell death and ROS generation in glioma cells potentiating the effects of arsenic trioxide	Liu *et al.* (2011)
	UC lines (T24 and MGHU3)	Xenograft mouse model of urothelial cancer	ZnPPIX sensitizes UC to gemcitabine and irradiation treatment. In *in vivo* model, ZnPPIX decreases the number of proliferating cells and increases the apoptotic rate. In addition, siHO-1 sensitizes to 5-ALA-based photodynamic therapy	Miyake *et al.* (2010)
	Neuroblastoma cells (GIMEN)		ZnPPIX sensitizes GIMEN cells to GSH depletion and etoposide treatment increasing ROS production	Furfaro *et al.* (2012)
	Neuroblastoma cells (HTLA-230)		ZnPPIX improves the pro-apoptotic effect of proteasome inhibitor-based therapy (bortezomib) in high-risk neuroblastoma cells. HO-1 silencing also sensitizes neuroblastoma cells to bortezomib treatment	Furfaro *et al.* (2014)
	Human NSCLC cells (A549)		ZnPPIX in combination with irradiation decreases cell viability and clonogenicity and enhances the apoptotic index as well as the percentage of cells in G1 phase.	Zhang *et al.* (2011)
	Human NSCLC cells (A549)		ZnPPIX and siHO-1 increase apoptosis of A549 cells exposed to cisplatin	Kim *et al.* (2008)
	Human ovarian carcinoma cells (MDAH2774) and murine colon adenocarcinoma cells (C-26)		ZnPPIX treatment enhances photodynamic mediated cytotoxicity. HO-1 silencing also sensitizes carcinoma cells to photodymamic therapy	Nowis *et al.* (2006)
		Mouse 4T1 breast tumor model	Cotreatment with ZnPPIX and paclitaxel decreases tumor growth and restores the proportion of infiltrating $CD8^+$ cytotoxic T lymphocytes	Kim *et al.* (2020)
		Mouse 4T1 breast tumor model	ZnPPIX increases the expression of CD86-M1 polarization marker in mice treated with paclitaxel and decreases the expression of IL-10 in $CD11b^+$ myeloid cells	Kim *et al.* (2021)
	Ex vivo HO-1 deleted TAMs	B-16 melanoma-bearing mice	ZnPPIX treatment decreases tumor growth, improves the effect of anti-PD1 immunotherapy, and reduces the percentage of metastatic area in lung. Myeloid ablation HO-1 also blocks metastasis formation and increases the efficacy of anti PD-1 therapy. *Ex vivo* deletion of HO-1 in TAMs restores CD8+ T-cell antitumor activity	Consonni *et al.* (2021)
	Ex vivo BMDM derived from glioblastoma patients		ZnPPIX treatment decreases PD-L1 expression in macrophages derived from glioblastoma patients and enables the recovery of $CD8^+$ T lymphocytes. Similar results were obtained using OB-24 as HO-1 inhibitor	Magri *et al.* (2022)

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SnMPIX	Primary BRAF^{V600} melanoma cells (MeOV-1, MeTA, and MeMi)		SnMPIX increases cell death induced by vemurafenib in BRAF^{V600}-mutated melanoma cells and increases NK cell recognition and killing. siHO-1 also improves the efficacy of vemurafenib, further reducing cell viability and restoring NK ligand expression	Furfaro *et al.* (2020)
		4T1 orthotopic model breast carcinoma	SnMPIX treatment prevents trans-endothelial migration and metastatic dissemination of cancer cells, blocking CO release	Muliaditan *et al.* (2018*a*)
		Spontaneous murine model of mammary adenocarcinoma (MMTV-PyMT)	SnMPIX targets myeloid HO-1 activity favoring CD8+ T-cell response	Muliaditan *et al.* (2018*b*)
	Ex vivo PBMC from healthy donors		SnMPIX increases the generation of WT1-specific T cells used in adoptive immunotherapy to improve T-cell-based therapy for leukemia patients	Schillingmann *et al.* (2019)
OB-24	Human prostate advance cancer cells (PCA) and resistant cells (PCA-R)	Mouse model of human prostate cancer PC3M	HO-1 inhibition leads to a significant decrease of cell proliferation *in vitro* and a reduction of tumor growth and lymph-node metastasis *in vivo.* OB-24 + Taxol shows potent therapeutic effect yielding >90% reduction in tumor growth *in vivo*. Short hairpin HO-1 shows similar results both *in vitro* and *in vivo.*	Alaoui-Jamali *et al.* (2009)
		B16-F0 melanoma-bearing mice	Combined therapy OB-24 + anti-PD-1 reduces tumor size compared to monotherapy	Khojandi *et al.* (2021)
		B16-F0 melanoma-bearing mice	HO-1 inhibition counteracts immune CD4$^+$ and CD8$^+$ TIL evasion and leads to a decrease in tumor volume	Kuehm *et al.* (2021)
siHO-1	Human pancreatic cancer cells (Panc-1, MIa PaCa-2, SU8686, and Colo 357)		HO-1 silencing sensitizes pancreatic cancer cells to gemcitabine or radiation treatment and leads to a significant decrease in cancer cell growth	Berberat *et al.* (2005)
	Colon cancer cells (Caco-2)		HO-1 silencing increases apoptosis	Busserolles *et al.* (2006)
	Human epithelial cervical cancer cells (HeLa)		SnPPIX and HO-1 silencing sensitize to 5-ALA-based photodynamic therapy	Ohgari *et al.* (2011)
	Ex vivo primary ALL cells		HO-1 silencing induces apoptosis and cell growth arrest in cell treated with imatinib. Polyethylene glycol ZnPPIX and styrene maleic acid ZnPPIX also sensitize cells to imatinib	Cerny-Reiterer *et al.* (2014)
	CLL cells		HO-1 silencing enhances the effects of the combined therapy fludarabine plus entinostat	Zhou *et al.* (2019)
HO-1 CRSPR/ Cas9 editing	Melanoma cells (A375) and HO-1 knockout A375 cells	SCID mice injected with A375 cells	HO-1 stable knockdown decreases *in vitro* clone formation and *in vivo* tumor growth	Liu *et al.* (2019)
	Pancreatic ductal adenocarcinoma cell lines (CD18/HPAF, COLO 357, Capan-1, and MIA PaCa-2)	PDAC cell-derived xenograft tumors	HO-1 editing suppresses cell proliferation and increases the efficacy of gemcitabine treatment in hypoxia condition. HO-1 inhibition (ZnPPIX and SnPPIX) suppresses PDAC proliferation, increases susceptibility to gemcitabine, and induces apoptosis under hypoxia	Abdalla *et al.* (2019)

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	T47D breast cancer cells	HO-1 editing decreases cell proliferation and migration and increases cisplatin-induced apoptosis	Evazi Bakhshi *et al.* (2022)
HO-1 ablation	C57/BL6 HO-1 knockout mice injected with HL-60 cells	HO-1 gene knockout enhances the antitumor effect of PD-1 inhibition, leading to a reduction of tumor growth and increasing overall survival	Zhou *et al.* (2018)
	Hmox1$^{\Delta M}$ mice	*Hmox1* gene deletion in myeloid cells improves the response to therapeutic immunization by enhancing antitumor CD8+T cell proliferation and cytotoxicity	Alaluf *et al.* (2020)

5-ALA, 5-aminolevulinic acid; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BMDMs, bone marrow-derived monocytes; GSH, glutathione; NK, natural killer; NSCLC, human non-small cell lung cancer; PBMCs, peripheral blood mononuclear cells; PDAC, pancreatic ductal adenocarcinoma cell; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; ROS, reactive oxygen species; SnMPIX, tin-mesoporphyrin IX; TAMs, tumor-associated macrophages; TILs, tumor-infiltrating lymphocytes; UC, urothelial cells; WT-1, Wilms' tumor protein 1; ZnPPIX, zinc-protoporphyrin IX.

myeloid ablation strongly improves the response to therapeutic immunization by enhancing antitumor CD8+ T-cell proliferation and cytotoxicity (Alaluf *et al.* 2020).

The main strategies used for HO-1 inhibition/downregulation are summarized in Table 1.

Conclusions and future perspectives

Although the precise molecular mechanisms involved in the induction of HO-1 in cancer cells and in cells from TME are far from be clearly understood, increasing evidence points out the role played by HO-1 in halting cancer immune recognition. On one hand, the overexpression of HO-1 in tumor cells, as a result of cell adaptation to therapy or to hypoxia, reduces tumor antigenicity; on the other hand, the overexpression of HO-1 in immune cells, in particular TAMs, induces tolerogenic and immune-suppressive phenotype. The two aspects converge toward a common goal, namely favoring tumor progression. Notably, the role of HO-1 in increasing tumor cell resistance to chemo-, radio-, and photodynamic therapies was already recognized, but the role of HO-1 in immune escape opens a new scenario where HO-1 inhibition could efficiently enhance the outcome of immune therapies as well, reducing therapeutic failure or disease relapses.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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MN, JO, and ALF conceived the study. MN, JO, and ALF wrote the original draft. MN and ALF reviewed the final version. All authors have read and agreed to the published version of the manuscript.

References

Abdalla MY, Ahmad IM, Rachagani S, Banerjee K, Thompson CM, Maurer HC, Olive KP, Bailey KL, Britigan BE & Kumar S 2019 Enhancing responsiveness of pancreatic cancer cells to gemcitabine treatment under hypoxia by heme oxygenase-1 inhibition. *Translational Research: the Journal of Laboratory and Clinical Medicine* **207** 56–69. (https://doi.org/10.1016/j.trsl.2018.12.008)

Adin CA, VanGundy ZC, Papenfuss TL, Xu F, Ghanem M, Lakey J & Hadley GA 2017 Physiologic doses of bilirubin contribute to tolerance of islet transplants by suppressing the innate immune response. *Cell Transplantation* **26** 11–21. (https://doi.org/10.3727/096368916X692096)

Alaluf E, Vokaer B, Detavernier A, Azouz A, Splittgerber M, Carrette A, Boon L, Libert F, Soares M, Le Moine A, *et al.* 2020 Heme oxygenase-1 orchestrates the immunosuppressive program of tumor-associated macrophages. *JCI Insight* **5**. (https://doi.org/10.1172/jci.insight.133929)

Alam J & Cook JL 2007 How many transcription factors does it take to turn on the heme oxygenase-1 gene? *American Journal of Respiratory Cell and Molecular Biology* **36** 166–174. (https://doi.org/10.1165/rcmb.2006-0340TR)

Alam J, Wicks C, Stewart D, Gong P, Touchard C, Otterbein S, Choi AM, Burow ME & Tou J 2000 Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells. Role of p38 kinase and Nrf2 transcription factor. *Journal of Biological Chemistry* **275** 27694–27702. (https://doi.org/10.1074/jbc.M004729200)

Alaoui-Jamali MA, Bismar TA, Gupta A, Szarek WA, Su J, Song W, Xu Y, Xu B, Liu G, Vlahakis JZ, *et al.* 2009 A novel experimental heme oxygenase-1-targeted therapy for hormone-refractory prostate cancer. *Cancer Research* **69** 8017–8024. (https://doi.org/10.1158/0008-5472.CAN-09-0419)

Andersen MH, Sorensen RB, Brimnes MK, Svane IM, Becker JC & thor Straten P 2009 Identification of heme oxygenase-1-specific regulatory CD8+ T cells in cancer patients. *Journal of Clinical Investigation* **119** 2245–2256. (https://doi.org/10.1172/jci38739)

Appleton SD, Chretien ML, McLaughlin BE, Vreman HJ, Stevenson DK, Brien JF, Nakatsu K, Maurice DH & Marks GS 1999 Selective inhibition of heme oxygenase, without inhibition of nitric oxide synthase or soluble guanylyl cyclase, by metalloporphyrins at low concentrations.

Drug Metabolism and Disposition: the Biological Fate of Chemicals **27** 1214–1219.

Arnold JN, Magiera L, Kraman M & Fearon DT 2014 Tumoral immune suppression by macrophages expressing fibroblast activation protein-alpha and heme oxygenase-1. *Cancer Immunology Research* **2** 121–126. (https://doi.org/10.1158/2326-6066.CIR-13-0150)

Barisione C, Garibaldi S, Furfaro AL, Nitti M, Palmieri D, Passalacqua M, Garuti A, Verzola D, Parodi A, Ameri P, *et al.* 2016 Moderate increase of indoxyl sulfate promotes monocyte transition into profibrotic macrophages. *PLoS One* **11** e0149276. (https://doi.org/10.1371/journal.pone.0149276)

Basiglio CL, Arriaga SM, Pelusa HF, Almará AM, Roma MG & Mottino AD 2007 Protective role of unconjugated bilirubin on complement-mediated hepatocytolysis. *Biochimica et Biophysica Acta* **1770** 1003–1010. (https://doi.org/10.1016/j.bbagen.2007.03.005)

Berberat PO, Dambrauskas Z, Gulbinas A, Giese T, Giese N, Kunzli B, Autschbach F, Meuer S, Buchler MW & Friess H 2005 Inhibition of heme oxygenase-1 increases responsiveness of pancreatic cancer cells to anticancer treatment. *Clinical Cancer Research* **11** 3790–3798. (https://doi.org/10.1158/1078-0432.CCR-04-2159)

Burt TD, Seu L, Mold JE, Kappas A & McCune JM 2010 Naive human T cells are activated and proliferate in response to the heme oxygenase-1 inhibitor tin mesoporphyrin. *Journal of Immunology* **185** 5279–5288. (https://doi.org/10.4049/jimmunol.0903127)

Busserolles J, Megias J, Terencio MC & Alcaraz MJ 2006 Heme oxygenase-1 inhibits apoptosis in Caco-2 cells via activation of Akt pathway. *International Journal of Biochemistry and Cell Biology* **38** 1510–1517. (https://doi.org/10.1016/j.biocel.2006.03.013)

Campbell NK, Fitzgerald HK & Dunne A 2021 Regulation of inflammation by the antioxidant haem oxygenase 1. *Nature Reviews. Immunology* **21** 411–425. (https://doi.org/10.1038/s41577-020-00491-x)

Campbell NK, Fitzgerald HK, Malara A, Hambly R, Sweeney CM, Kirby B, Fletcher JM & Dunne A 2018 Naturally derived heme-Oxygenase 1 inducers attenuate inflammatory responses in human dendritic cells and T cells: relevance for psoriasis treatment. *Scientific Reports* **8** 10287. (https://doi.org/10.1038/s41598-018-28488-6)

Casares L, García V, Garrido-Rodríguez M, Millán E, Collado JA, García-Martín A, Peñarando J, Calzado MA, de la Vega L & Muñoz E 2020 Cannabidiol induces antioxidant pathways in keratinocytes by targeting BACH1. *Redox Biology* **28** 101321. (https://doi.org/10.1016/j.redox.2019.101321)

Cerny-Reiterer S, Meyer RA, Herrmann H, Peter B, Gleixner KV, Stefanzl G, Hadzijusufovic E, Pickl WF, Sperr WR, Melo JV, *et al.* 2014 Identification of heat shock protein 32 (Hsp32) as a novel target in acute lymphoblastic leukemia. *Oncotarget* **5** 1198–1211. (https://doi.org/10.18632/oncotarget.1805)

Chapple SJ, Keeley TP, Mastronicola D, Arno M, Vizcay-Barrena G, Fleck R, Siow RCM & Mann GE 2016 Bach1 differentially regulates distinct Nrf2-dependent genes in human venous and coronary artery endothelial cells adapted to physiological oxygen levels. *Free Radical Biology and Medicine* **92** 152–162. (https://doi.org/10.1016/j.freeradbiomed.2015.12.013)

Chauveau C, Remy S, Royer PJ, Hill M, Tanguy-Royer S, Hubert FX, Tesson L, Brion R, Beriou G, Gregoire M, *et al.* 2005 Heme oxygenase-1 expression inhibits dendritic cell maturation and proinflammatory function but conserves IL-10 expression. *Blood* **106** 1694–1702. (https://doi.org/10.1182/blood-2005-02-0494)

Cheng X, Ku CH & Siow RC 2013 Regulation of the Nrf2 antioxidant pathway by microRNAs: new players in micromanaging redox homeostasis. *Free Radical Biology and Medicine* **64** 4–11. (https://doi.org/10.1016/j.freeradbiomed.2013.07.025)

Chin BY, Jiang G, Wegiel B, Wang HJ, Macdonald T, Zhang XC, Gallo D, Cszimadia E, Bach FH, Lee PJ, *et al.* 2007 Hypoxia-inducible factor 1alpha stabilization by carbon monoxide results in cytoprotective preconditioning. *Proceedings of the National Academy of Sciences of the United States of America* **104** 5109–5114. (https://doi.org/10.1073/pnas.0609611104)

Consonni FM, Bleve A, Totaro MG, Storto M, Kunderfranco P, Termanini A, Pasqualini F, Alì C, Pandolfo C, Sgambelluri F, *et al.* 2021 Heme catabolism by tumor-associated macrophages controls metastasis formation. *Nature Immunology* **22** 595–606. (https://doi.org/10.1038/s41590-021-00921-5)

Converso DP, Taille C, Carreras MC, Jaitovich A, Poderoso JJ & Boczkowski J 2006 HO-1 is located in liver mitochondria and modulates mitochondrial heme content and metabolism. *FASEB Journal* **20** 1236–1238. (https://doi.org/10.1096/fj.05-4204fje)

Daenen KEL, Martens P & Bammens B 2016 Association of HO-1 (GT)n promoter polymorphism and cardiovascular disease: a reanalysis of the literature. *Canadian Journal of Cardiology* **32** 160–168. (https://doi.org/10.1016/j.cjca.2015.06.006)

Dal-Cim T, Molz S, Egea J, Parada E, Romero A, Budni J, Martín de Saavedra MD, del Barrio L, Tasca CI & López MG 2012 Guanosine protects human neuroblastoma SH-SY5Y cells against mitochondrial oxidative stress by inducing heme oxigenase-1 via PI3K/Akt/GSK-3β pathway. *Neurochemistry International* **61** 397–404. (https://doi.org/10.1016/j.neuint.2012.05.021)

Davudian S, Mansoori B, Shajari N, Mohammadi A & Baradaran B 2016 BACH1, the master regulator gene: a novel candidate target for cancer therapy. *Gene* **588** 30–37. (https://doi.org/10.1016/j.gene.2016.04.040)

Degese MS, Mendizabal JE, Gandini NA, Gutkind JS, Molinolo A, Hewitt SM, Curino AC, Coso OA & Facchinetti MM 2012 Expression of heme oxygenase-1 in non-small cell lung cancer (NSCLC) and its correlation with clinical data. *Lung Cancer* **77** 168–175. (https://doi.org/10.1016/j.lungcan.2012.02.016)

Di Biase S & Longo VD 2016 Fasting-induced differential stress sensitization in cancer treatment. *Molecular and Cellular Oncology* **3** e1117701. (https://doi.org/10.1080/23723556.2015.1117701)

Duechler M, Peczek L, Zuk K, Zalesna I, Jeziorski A & Czyz M 2014 The heterogeneous immune microenvironment in breast cancer is affected by hypoxia-related genes. *Immunobiology* **219** 158–165. (https://doi.org/10.1016/j.imbio.2013.09.003)

Durante W 2020 Targeting heme Oxygenase-1 in the arterial response to injury and disease. *Antioxidants (Basel)* **9**. (https://doi.org/10.3390/antiox9090829)

Eades G, Yang M, Yao Y, Zhang Y & Zhou Q 2011 miR-200a regulates Nrf2 activation by targeting Keap1 mRNA in breast cancer cells. *Journal of Biological Chemistry* **286** 40725–40733. (https://doi.org/10.1074/jbc.M111.275495)

El Andaloussi A & Lesniak MS 2007 CD4+ CD25+ FoxP3+ T-cell infiltration and heme oxygenase-1 expression correlate with tumor grade in human gliomas. *Journal of Neuro-Oncology* **83** 145–152. (https://doi.org/10.1007/s11060-006-9314-y)

Evazi Bakhshi S, Mohammadi Roushandeh A, Habibi Roudkenar M, Shekarchi S & Bahadori MH 2022 CRISPR/Cas9-mediated knockout of HO-1 decreased the proliferation and migration of T47D cells and increased cisplatin-induced apoptosis: an in vitro study. *Medical Oncology* **39** 175. (https://doi.org/10.1007/s12032-022-01773-1)

Exner M, Minar E, Wagner O & Schillinger M 2004 The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radical Biology and Medicine* **37** 1097–1104. (https://doi.org/10.1016/j.freeradbiomed.2004.07.008)

Fallica AN, Sorrenti V, D'Amico AG, Salerno L, Romeo G, Intagliata S, Consoli V, Floresta G, Rescifina A, D'Agata V, *et al.* 2021 Discovery of novel acetamide-based heme Oxygenase-1 inhibitors with potent in vitro antiproliferative activity. *Journal of Medicinal Chemistry* **64** 13373–13393. (https://doi.org/10.1021/acs.jmedchem.1c00633)

Feng C, Zhang T, Pan C, Kang Q, Wang L, Liu X, Shang Q, Chen S, Hu T & Wang J 2023 Heme oxygenase-1 inhibits the cytotoxicity of natural killer cells to acute myeloid leukemia by downregulating human leukocyte antigen-C. *Cytotherapy* **0**. (https://doi.org/10.1016/j.jcyt.2023.02.001)

Ferrando M, Gueron G, Elguero B, Giudice J, Salles A, Leskow FC, Jares-Erijman EA, Colombo L, Meiss R, Navone N, *et al.* 2011 Heme oxygenase 1 (HO-1) challenges the angiogenic switch in prostate cancer. *Angiogenesis* **14** 467–479. (https://doi.org/10.1007/s10456-011-9230-4)

Frank J, Lornejad-Schafer MR, Schoffl H, Flaccus A, Lambert C & Biesalski HK 2007 Inhibition of heme oxygenase-1 increases responsiveness of melanoma cells to ALA-based photodynamic therapy. *International Journal of Oncology* **31** 1539–1545. (https://doi.org/10.3892/ijo.31.6.1539)

Furfaro AL, Loi G, Ivaldo C, Passalacqua M, Pietra G, Mann GE & Nitti M 2022 HO-1 limits the efficacy of vemurafenib/PLX4032 in BRAFV600E mutated melanoma cells adapted to physiological normoxia or hypoxia. *Antioxidants (Basel)* **11** 1171. (https://doi.org/10.3390/antiox11061171)

Furfaro AL, Macay JR, Marengo B, Nitti M, Parodi A, Fenoglio D, Marinari UM, Pronzato MA, Domenicotti C & Traverso N 2012 Resistance of neuroblastoma GI-ME-N cell line to glutathione depletion involves Nrf2 and heme oxygenase-1. *Free Radical Biology and Medicine* **52** 488–496. (https://doi.org/10.1016/j.freeradbiomed.2011.11.007)

Furfaro AL, Ottonello S, Loi G, Cossu I, Piras S, Spagnolo F, Queirolo P, Marinari UM, Moretta L, Pronzato MA, *et al.* 2020 HO-1 downregulation favors BRAFV600 melanoma cell death induced by vemurafenib/PLX4032 and increases NK recognition. *International Journal of Cancer* **146** 1950–1962. (https://doi.org/10.1002/ijc.32611)

Furfaro AL, Piras S, Domenicotti C, Fenoglio D, De Luigi A, Salmona M, Moretta L, Marinari UM, Pronzato MA, Traverso N, *et al.* 2016*a* Role of Nrf2, HO-1 and GSH in neuroblastoma cell resistance to bortezomib. *PLoS One* **11** e0152465. (https://doi.org/10.1371/journal.pone.0152465)

Furfaro AL, Piras S, Passalacqua M, Domenicotti C, Parodi A, Fenoglio D, Pronzato MA, Marinari UM, Moretta L, Traverso N, *et al.* 2014 HO-1 up-regulation: a key point in high-risk neuroblastoma resistance to bortezomib. *Biochimica et Biophysica Acta* **1842** 613–622. (https://doi.org/10.1016/j.bbadis.2013.12.008)

Furfaro AL, Traverso N, Domenicotti C, Piras S, Moretta L, Marinari UM, Pronzato MA & Nitti M 2016*b* The Nrf2/HO-1 axis in cancer cell growth and chemoresistance. *Oxidative Medicine and Cellular Longevity* **2016** 1958174. (https://doi.org/10.1155/2016/1958174)

George JF, Braun A, Brusko TM, Joseph R, Bolisetty S, Wasserfall CH, Atkinson MA, Agarwal A & Kapturczak MH 2008 Suppression by CD4+CD25+ regulatory T cells is dependent on expression of heme oxygenase-1 in antigen-presenting cells. *American Journal of Pathology* **173** 154–160. (https://doi.org/10.2353/ajpath.2008.070963)

Gomez-Lomeli P, Bravo-Cuellar A, Hernandez-Flores G, Jave-Suarez LF, Aguilar-Lemarroy A, Lerma-Diaz JM, Dominguez-Rodriguez JR, Sanchez-Reyes K & Ortiz-Lazareno PC 2014 Increase of IFN-gamma and TNF-alpha production in CD107a+NK-92 cells co-cultured with cervical cancer cell lines pre-treated with the HO-1 inhibitor. *Cancer Cell International* **14** 100. (https://doi.org/10.1186/s12935-014-0100-1)

Grochot-Przeczek A, Dulak J & Jozkowicz A 2012 Haem oxygenase-1: non-canonical roles in physiology and pathology. *Clinical Science* **122** 93–103. (https://doi.org/10.1042/CS20110147)

Gu S, Lai Y, Chen H, Liu Y & Zhang Z 2017 miR-155 mediates arsenic trioxide resistance by activating Nrf2 and suppressing apoptosis in lung cancer cells. *Scientific Reports* **7** 12155. (https://doi.org/10.1038/s41598-017-06061-x)

Hanada N, Takahata T, Zhou Q, Ye X, Sun R, Itoh J, Ishiguro A, Kijima H, Mimura J, Itoh K, *et al.* 2012 Methylation of the KEAP1 gene promoter region in human colorectal cancer. *BMC Cancer* **12** 66. (https://doi.org/10.1186/1471-2407-12-66)

Hench PS 1938 Effect of jaundice on rheumatoid arthritis. *British Medical Journal* **2** 394–398. (https://doi.org/10.1136/bmj.2.4050.394)

Herrmann H, Kneidinger M, Cerny-Reiterer S, Rülicke T, Willmann M, Gleixner KV, Blatt K, Hörmann G, Peter B, Samorapoompichit P, *et al.* 2012 The Hsp32 inhibitors SMA-ZnPP and PEG-ZnPP exert major growth-inhibitory effects on D34+/CD38+ and CD34+/CD38- AML progenitor cells. *Current Cancer Drug Targets* **12** 51–63. (https://doi.org/10.2174/156800912798888992)

Hirotsu Y, Katsuoka F, Funayama R, Nagashima T, Nishida Y, Nakayama K, Engel JD & Yamamoto M 2012 Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. *Nucleic Acids Research* **40** 10228–10239. (https://doi.org/10.1093/nar/gks827)

Hofmann A, Müglich M, Wolk S, Khorzom Y, Sabarstinski P, Kopaliani I, Egorov D, Horn F, Brunssen C, Giebe S, *et al.* 2021 Induction of heme Oxygenase-1 is linked to the severity of disease in human abdominal aortic aneurysm. *Journal of the American Heart Association* **10** e022747. (https://doi.org/10.1161/JAHA.121.022747)

Hsu FF, Chiang MT, Li FA, Yeh CT, Lee WH & Chau LY 2017 Acetylation is essential for nuclear heme oxygenase-1-enhanced tumor growth and invasiveness. *Oncogene* **36** 6805–6814. (https://doi.org/10.1038/onc.2017.294)

Hsu FF, Yeh CT, Sun YJ, Chiang MT, Lan WM, Li FA, Lee WH & Chau LY 2015 Erratum: Signal peptide peptidase-mediated nuclear localization of heme oxygenase-1 promotes cancer cell proliferation and invasion independent of its enzymatic activity. *Oncogene* **34** 2410–2411. (https://doi.org/10.1038/onc.2014.464)

Hu R, Zhang Y, Yang X, Yan J, Sun Y, Chen Z & Jiang H 2015 Isoflurane attenuates LPS-induced acute lung injury by targeting miR-155-HIF1-alpha. *Frontiers in Bioscience (Landmark Edition)* **20** 139–156. (https://doi.org/10.2741/4302)

Huang J, Guo P, Ma D, Lin X, Fang Q & Wang J 2016 Overexpression of heme oxygenase-1 induced by constitutively activated NF-κB as a potential therapeutic target for activated B-cell-like diffuse large B-cell lymphoma. *International Journal of Oncology* **49** 253–264. (https://doi.org/10.3892/ijo.2016.3529)

Hurwitz SN, Rider MA, Bundy JL, Liu X, Singh RK & Meckes DG 2016 Proteomic profiling of NCI-60 extracellular vesicles uncovers common protein cargo and cancer type-specific biomarkers. *Oncotarget* **7** 86999–87015. (https://doi.org/10.18632/oncotarget.13569)

Iyer AK, Greish K, Seki T, Okazaki S, Fang J, Takeshita K & Maeda H 2007 Polymeric micelles of zinc protoporphyrin for tumor targeted delivery based on EPR effect and singlet oxygen generation. *Journal of Drug Targeting* **15** 496–506. (https://doi.org/10.1080/10611860701498252)

Jozkowicz A, Was H & Dulak J 2007 Heme oxygenase-1 in tumors: is it a false friend? *Antioxidants and Redox Signaling* **9** 2099–2117. (https://doi.org/10.1089/ars.2007.1659)

Jung NH, Kim HP, Kim BR, Cha SH, Kim GA, Ha H, Na YE & Cha YN 2003 Evidence for heme oxygenase-1 association with caveolin-1 and -2 in mouse mesangial cells. *IUBMB Life* **55** 525–532. (https://doi.org/10.1080/15216540310001620968)

Keshavan P, Deem TL, Schwemberger SJ, Babcock GF, Cook-Mills JM & Zucker SD 2005 Unconjugated bilirubin inhibits VCAM-1-mediated transendothelial leukocyte migration. *Journal of Immunology* **174** 3709–3718. (https://doi.org/10.4049/jimmunol.174.6.3709)

Keyse SM & Tyrrell RM 1989 Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. *Proceedings of the National Academy of Sciences of the United States of America* **86** 99–103. (https://doi.org/10.1073/pnas.86.1.99)

Khojandi N, Kuehm LM, Piening A, Donlin MJ, Hsueh EC, Schwartz TL, Farrell K, Richart JM, Geerling E, Pinto AK, *et al.* 2021 Oxidized lipoproteins promote resistance to cancer immunotherapy independent of patient obesity. *Cancer Immunology Research* **9** 214–226. (https://doi.org/10.1158/2326-6066.CIR-20-0358)

Kikuchi G, Yoshida T & Noguchi M 2005 Heme oxygenase and heme degradation. *Biochemical and Biophysical Research Communications* **338** 558–567. (https://doi.org/10.1016/j.bbrc.2005.08.020)

Kim HP, Wang X, Nakao A, Kim SI, Murase N, Choi ME, Ryter SW & Choi AMK 2005 Caveolin-1 expression by means of p38beta mitogen-activated protein kinase mediates the antiproliferative effect of carbon monoxide. *Proceedings of the National Academy of Sciences of the United*

States of America **102** 11319–11324. (https://doi.org/10.1073/pnas.0501345102)

Kim HR, Kim S, Kim EJ, Park JH, Yang SH, Jeong ET, Park C, Youn MJ, So HS & Park R 2008 Suppression of Nrf2-driven heme oxygenase-1 enhances the chemosensitivity of lung cancer A549 cells toward cisplatin. *Lung Cancer* **60** 47–56. (https://doi.org/10.1016/j.lungcan.2007.09.021)

Kim SH, Kim SJ, Park J, Joe Y, Lee SE, Saeidi S, Zhong X, Kim SH, Park SA, Na HK, *et al.* 2021 Reprograming of tumor-associated macrophages in breast tumor-bearing mice under chemotherapy by targeting heme Oxygenase-1. *Antioxidants (Basel)* **10** 470. (https://doi.org/10.3390/antiox10030470)

Kim SH, Saeidi S, Zhong X, Gwak SY, Muna IA, Park SA, Kim SH, Na HK, Joe Y, Chung HT, *et al.* 2020 Breast cancer cell debris diminishes therapeutic efficacy through heme oxygenase-1-mediated inactivation of M1-like tumor-associated macrophages. *Neoplasia* **22** 606–616. (https://doi.org/10.1016/j.neo.2020.08.006)

Kim SJ & Lee SM 2013 NLRP3 inflammasome activation in D-galactosamine and lipopolysaccharide-induced acute liver failure: role of heme oxygenase-1. *Free Radical Biology and Medicine* **65** 997–1004. (https://doi.org/10.1016/j.freeradbiomed.2013.08.178)

Kinobe RT, Dercho RA & Nakatsu K 2008 Inhibitors of the heme oxygenase - carbon monoxide system: on the doorstep of the clinic? *Canadian Journal of Physiology and Pharmacology* **86** 577–599. (https://doi.org/10.1139/y08-066)

Kinobe RT, Vlahakis JZ, Vreman HJ, Stevenson DK, Brien JF, Szarek WA & Nakatsu K 2006 Selectivity of imidazole-dioxolane compounds for in vitro inhibition of microsomal haem oxygenase isoforms. *British Journal of Pharmacology* **147** 307–315. (https://doi.org/10.1038/sj.bjp.0706555)

Kobayashi M & Yamamoto M 2005 Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxidants and Redox Signaling* **7** 385–394. (https://doi.org/10.1089/ars.2005.7.385)

Kuehm LM, Khojandi N, Piening A, Klevorn LE, Geraud SC, McLaughlin NR, Griffett K, Burris TP, Pyles KD, Nelson AM, *et al.* 2021 Fructose promotes cytoprotection in melanoma tumors and resistance to immunotherapy. *Cancer Immunology Research* **9** 227–238. (https://doi.org/10.1158/2326-6066.CIR-20-0396)

Kurata S, Matsumoto M, Tsuji Y & Nakajima H 1996 Lipopolysaccharide activates transcription of the heme oxygenase gene in mouse M1 cells through oxidative activation of nuclear factor kappa B. *European Journal of Biochemistry* **239** 566–571. (https://doi.org/10.1111/j.1432-1033.1996.0566u.x)

Kutty RK, Nagineni CN, Kutty G, Hooks JJ, Chader GJ & Wiggert B 1994 Increased expression of heme oxygenase-1 in human retinal pigment epithelial cells by transforming growth factor-beta. *Journal of Cellular Physiology* **159** 371–378. (https://doi.org/10.1002/jcp.1041590221)

Lavrovsky Y, Schwartzman ML, Levere RD, Kappas A & Abraham NG 1994 Identification of binding sites for transcription factors NF-kappa B and AP-2 in the promoter region of the human heme oxygenase 1 gene. *Proceedings of the National Academy of Sciences of the United States of America* **91** 5987–5991. (https://doi.org/10.1073/pnas.91.13.5987)

Li R, Zeng X, Yang M, Feng J, Xu X, Bao L, Ye T, Wang X, Xue B & Huang Y 2021 Antidiabetic DPP-4 inhibitors reprogram tumor microenvironment that facilitates murine breast cancer metastasis through interaction with cancer cells via a ROS-NF-κB-NLRP3 axis. *Frontiers in Oncology* **11** 728047. (https://doi.org/10.3389/fonc.2021.728047)

Lignitto L, LeBoeuf SE, Homer H, Jiang S, Askenazi M, Karakousi TR, Pass HI, Bhutkar AJ, Tsirigos A, Ueberheide B, *et al.* 2019 Nrf2 activation promotes lung cancer metastasis by inhibiting the degradation of Bach1. *Cell* **178** 316–329.e18. (https://doi.org/10.1016/j.cell.2019.06.003)

Lin CC, Chiang LL, Lin CH, Shih CH, Liao YT, Hsu MJ & Chen BC 2007*a* Transforming growth factor-beta1 stimulates heme oxygenase-1 expression via the PI3K/Akt and NF-kappaB pathways in human lung epithelial cells. *European Journal of Pharmacology* **560** 101–109. (https://doi.org/10.1016/j.ejphar.2007.01.025)

Lin Q, Weis S, Yang G, Weng YH, Helston R, Rish K, Smith A, Bordner J, Polte T, Gaunitz F, *et al.* 2007*b* Heme oxygenase-1 protein localizes to the nucleus and activates transcription factors important in oxidative stress. *Journal of Biological Chemistry* **282** 20621–20633. (https://doi.org/10.1074/jbc.M607954200)

Liu L, Wu Y, Bian C, Nisar MF, Wang M, Hu X, Diao Q, Nian W, Wang E, Xu W, *et al.* 2019 Heme oxygenase 1 facilitates cell proliferation via the B-Raf-ERK signaling pathway in melanoma. *Cell Communication and Signaling* **17** 3. (https://doi.org/10.1186/s12964-018-0313-3)

Liu Y, Li P, Lu J, Xiong W, Oger J, Tetzlaff W & Cynader M 2008 Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis. *Journal of Immunology* **181** 1887–1897. (https://doi.org/10.4049/jimmunol.181.3.1887)

Liu Y, Liang Y, Zheng T, Yang G, Zhang X, Sun Z, Shi C & Zhao S 2011 Inhibition of heme oxygenase-1 enhances anti-cancer effects of arsenic trioxide on glioma cells. *Journal of Neuro-Oncology* **104** 449–458. (https://doi.org/10.1007/s11060-010-0513-1)

Liu Y, Wei R & Hong TP 2014 Potential roles of glucagon-like peptide-1-based therapies in treating non-alcoholic fatty liver disease. *World Journal of Gastroenterology* **20** 9090–9097. (https://doi.org/10.3748/wjg.v20.i27.9090)

Liu ZM, Chen GG, Ng EKW, Leung WK, Sung JJY & Chung SCS 2004 Upregulation of heme oxygenase-1 and p21 confers resistance to apoptosis in human gastric cancer cells. *Oncogene* **23** 503–513. (https://doi.org/10.1038/sj.onc.1207173)

Loboda A, Damulewicz M, Pyza E, Jozkowicz A & Dulak J 2016 Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cellular and Molecular Life Sciences: CMLS* **73** 3221–3247. (https://doi.org/10.1007/s00018-016-2223-0)

Luu Hoang KN, Anstee JE & Arnold JN 2021 The diverse roles of heme Oxygenase-1 in tumor progression. *Frontiers in Immunology* **12** 658315. (https://doi.org/10.3389/fimmu.2021.658315)

Magri S, Musca B, Pinton L, Orecchini E, Belladonna ML, Orabona C, Bonaudo C, Volpin F, Ciccarino P, Baro V, *et al.* 2022 The immunosuppression pathway of tumor-associated macrophages is controlled by heme oxygenase-1 in glioblastoma patients. *International Journal of Cancer* **151** 2265–2277. (https://doi.org/10.1002/ijc.34270)

Maines MD 1988 Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB Journal* **2** 2557–2568. (https://doi.org/10.1096/fasebj.2.10.3290025)

Mascaró M, Alonso EN, Alonso EG, Lacunza E, Curino AC & Facchinetti MM 2021 Nuclear Localization of Heme Oxygenase-1 in Pathophysiological Conditions: Does It Explain the Dual Role in Cancer? *Antioxidants (Basel)* **10** 87.

Mazzone GL, Rigato I, Ostrow JD, Bossi F, Bortoluzzi A, Sukowati CHC, Tedesco F & Tiribelli C 2009 Bilirubin inhibits the TNFalpha-related induction of three endothelial adhesion molecules. *Biochemical and Biophysical Research Communications* **386** 338–344. (https://doi.org/10.1016/j.bbrc.2009.06.029)

McCoubrey WK & Maines MD 1994 The structure, organization and differential expression of the gene encoding rat heme oxygenase-2. *Gene* **139** 155–161. (https://doi.org/10.1016/0378-1119(94)90749-8)

Mitsuishi Y, Motohashi H & Yamamoto M 2012 The Keap1-Nrf2 system in cancers: stress response and anabolic metabolism. *Frontiers in Oncology* **2** 200. (https://doi.org/10.3389/fonc.2012.00200)

Miyake M, Fujimoto K, Anai S, Ohnishi S, Nakai Y, Inoue T, Matsumura Y, Tomioka A, Ikeda T, Okajima E, *et al.* 2010 Inhibition of heme oxygenase-1 enhances the cytotoxic effect of gemcitabine in urothelial cancer cells. *Anticancer Research* **30** 2145–2152.

Miyake M, Ishii M, Kawashima K, Kodama T, Sugano K, Fujimoto K & Hirao Y 2009 siRNA-mediated knockdown of the heme synthesis and degradation pathways: modulation of treatment effect of 5-aminolevulinic acid-based photodynamic therapy in urothelial

cancer cell lines. *Photochemistry and Photobiology* **85** 1020–1027. (https://doi.org/10.1111/j.1751-1097.2009.00543.x)
Mondanelli G, Ugel S, Grohmann U & Bronte V 2017 The immune regulation in cancer by the amino acid metabolizing enzymes ARG and IDO. *Current Opinion in Pharmacology* **35** 30–39. (https://doi.org/10.1016/j.coph.2017.05.002)
Muhseena K, Mathukkada S, Das SP & Laha S 2021 The repair gene BACH1 - a potential oncogene. *Oncology Reviews* **15** 519. (https://doi.org/10.4081/oncol.2021.519)
Muliaditan T, Caron J, Okesola M, Opzoomer JW, Kosti P, Georgouli M, Gordon P, Lall S, Kuzeva DM, Pedro L, *et al.* 2018*a* Macrophages are exploited from an innate wound healing response to facilitate cancer metastasis. *Nature Communications* **9** 2951. (https://doi.org/10.1038/s41467-018-05346-7)
Muliaditan T, Opzoomer JW, Caron J, Okesola M, Kosti P, Lall S, Van Hemelrijck M, Dazzi F, Tutt A, Grigoriadis A, *et al.* 2018*b* Repurposing tin mesoporphyrin as an immune checkpoint inhibitor shows therapeutic efficacy in preclinical models of cancer. *Clinical Cancer Research* **24** 1617–1628. (https://doi.org/10.1158/1078-0432.CCR-17-2587)
Na HK & Surh YJ 2014 Oncogenic potential of Nrf2 and its principal target protein heme oxygenase-1. *Free Radical Biology and Medicine* **67** 353–365. (https://doi.org/10.1016/j.freeradbiomed.2013.10.819)
Naito Y, Takagi T & Higashimura Y 2014 Heme oxygenase-1 and anti-inflammatory M2 macrophages. *Archives of Biochemistry and Biophysics* **564** 83–88. (https://doi.org/10.1016/j.abb.2014.09.005)
Nakashima M, Watanabe M, Nakano K, Uchimaru K & Horie R 2021 Differentiation of Hodgkin lymphoma cells by reactive oxygen species and regulation by heme oxygenase-1 through HIF-1α. *Cancer Science* **112** 2542–2555. (https://doi.org/10.1111/cas.14890)
Nitti M, Furfaro AL & Mann GE 2020 Heme oxygenase dependent bilirubin generation in vascular cells: a role in preventing endothelial dysfunction in local tissue microenvironment? *Frontiers in Physiology* **11** 23. (https://doi.org/10.3389/fphys.2020.00023)
Nitti M, Ivaldo C, Traverso N & Furfaro AL 2021 Clinical significance of heme oxygenase 1 in tumor progression. *Antioxidants* **10** 789. (https://doi.org/10.3390/antiox10050789)
Nitti M, Marengo B, Furfaro AL, Pronzato MA, Marinari UM, Domenicotti C & Traverso N 2022 Hormesis and oxidative distress: pathophysiology of reactive oxygen species and the open question of antioxidant modulation and supplementation. *Antioxidants (Basel)* **11** 1613. (https://doi.org/10.3390/antiox11081613)
Nitti M, Piras S, Marinari UM, Moretta L, Pronzato MA & Furfaro AL 2017 HO-1 induction in cancer progression: a matter of cell adaptation. *Antioxidants (Basel)* **6**. (https://doi.org/10.3390/antiox6020029)
Novo G, Cappello F, Rizzo M, Fazio G, Zambuto S, Tortorici E, Marino Gammazza A, Corrao S, Zummo G, De Macario EC, *et al.* 2011 Hsp60 and heme oxygenase-1 (Hsp32) in acute myocardial infarction. *Translational Research: the Journal of Laboratory and Clinical Medicine* **157** 285–292. (https://doi.org/10.1016/j.trsl.2011.01.003)
Nowis D, Legat M, Grzela T, Niderla J, Wilczek E, Wilczynski GM, Glodkowska E, Mrowka P, Issat T, Dulak J, *et al.* 2006 Heme oxygenase-1 protects tumor cells against photodynamic therapy-mediated cytotoxicity. *Oncogene* **25** 3365–3374. (https://doi.org/10.1038/sj.onc.1209378)
Ogawa K, Sun J, Taketani S, Nakajima O, Nishitani C, Sassa S, Hayashi N, Yamamoto M, Shibahara S & Fujita H 2001 Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1. *EMBO Journal* **20** 2835–2843. (https://doi.org/10.1093/emboj/20.11.2835)
Ohgari Y, Miyata Y, Miyagi T, Gotoh S, Ohta T, Kataoka T, Furuyama K & Taketani S 2011 Roles of porphyrin and iron metabolisms in the delta-aminolevulinic acid (ALA)-induced accumulation of protoporphyrin and photodamage of tumor cells. *Photochemistry and Photobiology* **87** 1138–1145. (https://doi.org/10.1111/j.1751-1097.2011.00950.x)
Ono K, Goto Y, Takagi S, Baba S, Tago N, Nonogi H & Iwai N 2004 A promoter variant of the heme oxygenase-1 gene may reduce the incidence of ischemic heart disease in Japanese. *Atherosclerosis* **173** 315–319. (https://doi.org/10.1016/j.atherosclerosis.2003.11.021)
Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA & Choi AM 2000 Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nature Medicine* **6** 422–428. (https://doi.org/10.1038/74680)
Ozen M, Zhao H, Lewis DB, Wong RJ & Stevenson DK 2015 Heme oxygenase and the immune system in normal and pathological pregnancies. *Frontiers in Pharmacology* **6** 84. (https://doi.org/10.3389/fphar.2015.00084)
Palazon A, Goldrath AW, Nizet V & Johnson RS 2014 HIF transcription factors, inflammation, and immunity. *Immunity* **41** 518–528. (https://doi.org/10.1016/j.immuni.2014.09.008)
Piras S, Furfaro AL, Brondolo L, Passalacqua M, Marinari UM, Pronzato MA & Nitti M 2017 Differentiation impairs Bach1 dependent HO-1 activation and increases sensitivity to oxidative stress in SH-SY5Y neuroblastoma cells. *Scientific Reports* **7** 7568. (https://doi.org/10.1038/s41598-017-08095-7)
Piras S, Furfaro AL, Caggiano R, Brondolo L, Garibaldi S, Ivaldo C, Marinari UM, Pronzato MA, Faraonio R & Nitti M 2018 microRNA-494 favors HO-1 expression in neuroblastoma cells exposed to oxidative stress in a Bach1-independent way. *Frontiers in Oncology* **8** 199. (https://doi.org/10.3389/fonc.2018.00199)
Pittala V, Salerno L, Romeo G, Modica MN & Siracusa MA 2013 A focus on heme oxygenase-1 (HO-1) inhibitors. *Current Medicinal Chemistry* **20** 3711–3732. (https://doi.org/10.2174/0929867311320300003)
Podkalicka P, Mucha O, Józkowicz A, Dulak J & Łoboda A 2018 Heme oxygenase inhibition in cancers: possible tools and targets. *Contemporary Oncology* **22** 23–32. (https://doi.org/10.5114/wo.2018.73879)
Prawan A, Kundu JK & Surh YJ 2005 Molecular basis of heme oxygenase-1 induction: implications for chemoprevention and chemoprotection. *Antioxidants and Redox Signaling* **7** 1688–1703. (https://doi.org/10.1089/ars.2005.7.1688)
Pu M, Li C, Qi X, Chen J, Wang Y, Gao L, Miao L & Ren J 2017 MiR-1254 suppresses HO-1 expression through seed region-dependent silencing and non-seed interaction with TFAP2A transcript to attenuate NSCLC growth. *PLOS Genetics* **13** e1006896. (https://doi.org/10.1371/journal.pgen.1006896)
Pugh CW, O'Rourke JF, Nagao M, Gleadle JM & Ratcliffe PJ 1997 Activation of hypoxia-inducible factor-1; definition of regulatory domains within the alpha subunit. *Journal of Biological Chemistry* **272** 11205–11214. (https://doi.org/10.1074/jbc.272.17.11205)
Rahman MN, Vukomanovic D, Vlahakis JZ, Szarek WA, Nakatsu K & Jia Z 2013 Structural insights into human heme oxygenase-1 inhibition by potent and selective azole-based compounds. *Journal of the Royal Society, Interface* **10** 20120697. (https://doi.org/10.1098/rsif.2012.0697)
Ricchetti GA, Williams LM & Foxwell BMJ 2004 Heme oxygenase 1 expression induced by IL-10 requires STAT-3 and phosphoinositol-3 kinase and is inhibited by lipopolysaccharide. *Journal of Leukocyte Biology* **76** 719–726. (https://doi.org/10.1189/jlb.0104046)
Rocuts F, Zhang X, Yan J, Yue Y, Thomas M, Bach FH, Czismadia E & Wang H 2010 Bilirubin promotes de novo generation of T regulatory cells. *Cell Transplantation* **19** 443–451. (https://doi.org/10.3727/096368909X484680)
Ryter SW & Choi AM 2005 Heme oxygenase-1: redox regulation of a stress protein in lung and cell culture models. *Antioxidants and Redox Signaling* **7** 80–91. (https://doi.org/10.1089/ars.2005.7.80)
Sahoo SK, Sawa T, Fang J, Tanaka S, Miyamoto Y, Akaike T & Maeda H 2002 Pegylated zinc protoporphyrin: a water-soluble heme oxygenase inhibitor with tumor-targeting capacity. *Bioconjugate Chemistry* **13** 1031–1038. (https://doi.org/10.1021/bc020010k)
Salerno L, Floresta G, Ciaffaglione V, Gentile D, Margani F, Turnaturi R, Rescifina A & Pittalà V 2019 Progress in the development of selective

heme oxygenase-1 inhibitors and their potential therapeutic application. *European Journal of Medicinal Chemistry* **167** 439–453. (https://doi.org/10.1016/j.ejmech.2019.02.027)

Sass G, Leukel P, Schmitz V, Raskopf E, Ocker M, Neureiter D, Meissnitzer M, Tasika E, Tannapfel A & Tiegs G 2008 Inhibition of heme oxygenase 1 expression by small interfering RNA decreases orthotopic tumor growth in livers of mice. *International Journal of Cancer* **123** 1269–1277. (https://doi.org/10.1002/ijc.23695)

Schillingmann DA, Riese SB, Vijayan V, Tischer-Zimmermann S, Schmetzer H, Maecker-Kolhoff B, Blasczyk R, Immenschuh S & Eiz-Vesper B 2019 Inhibition of heme Oxygenase-1 activity enhances Wilms Tumor-1-Specific T-cell responses in cancer immunotherapy. *International Journal of Molecular Sciences* **20** 482. (https://doi.org/10.3390/ijms20030482)

Schulz S, Wong RJ, Vreman HJ & Stevenson DK 2012 Metalloporphyrins - an update. *Frontiers in Pharmacology* **3** 68. (https://doi.org/10.3389/fphar.2012.00068)

Schumacher A, Wafula PO, Teles A, El-Mousleh T, Linzke N, Zenclussen ML, Langwisch S, Heinze K, Wollenberg I, Casalis PA, *et al.* 2012 Blockage of heme oxygenase-1 abrogates the protective effect of regulatory T cells on murine pregnancy and promotes the maturation of dendritic cells. *PLoS One* **7** e42301. (https://doi.org/10.1371/journal.pone.0042301)

Serpero LD, Frigiola A & Gazzolo D 2012 Human milk and formulae: neurotrophic and new biological factors. *Early Human Development* **88**(Supplement 1) S9–S12. (https://doi.org/10.1016/j.earlhumdev.2011.12.021)

Shi J, Yu T, Song K, Du S, He S, Hu X, Li X, Li H, Dong S, Zhang Y, *et al.* 2021 Dexmedetomidine ameliorates endotoxin-induced acute lung injury in vivo and in vitro by preserving mitochondrial dynamic equilibrium through the HIF-1a/HO-1 signaling pathway. *Redox Biology* **41** 101954. (https://doi.org/10.1016/j.redox.2021.101954)

Shibata T, Ohta T, Tong KI, Kokubu A, Odogawa R, Tsuta K, Asamura H, Yamamoto M & Hirohashi S 2008 Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. *Proceedings of the National Academy of Sciences of the United States of America* **105** 13568–13573. (https://doi.org/10.1073/pnas.0806268105)

Sies H, Belousov VV, Chandel NS, Davies MJ, Jones DP, Mann GE, Murphy MP, Yamamoto M & Winterbourn C 2022 Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nature Reviews. Molecular Cell Biology* **23** 499–515. (https://doi.org/10.1038/s41580-022-00456-z)

Signorelli SS, Li Volsi G, Fiore V, Mangiafico M, Barbagallo I, Parenti R, Rizzo M & Li Volti G 2016 Plasma heme oxygenase-1 is decreased in peripheral artery disease patients. *Molecular Medicine Reports* **14** 3459–3463. (https://doi.org/10.3892/mmr.2016.5644)

Siow RC, Sato H & Mann GE 1999 Heme oxygenase-carbon monoxide signalling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide? *Cardiovascular Research* **41** 385–394. (https://doi.org/10.1016/s0008-6363(98)00278-8)

Slebos DJ, Ryter SW, van der Toorn M, Liu F, Guo F, Baty CJ, Karlsson JM, Watkins SC, Kim HP, Wang X, *et al.* 2007 Mitochondrial localization and function of heme oxygenase-1 in cigarette smoke-induced cell death. *American Journal of Respiratory Cell and Molecular Biology* **36** 409–417. (https://doi.org/10.1165/rcmb.2006-0214OC)

Solano ME, Kowal MK, O'Rourke GE, Horst AK, Modest K, Plösch T, Barikbin R, Remus CC, Berger RG, Jago C, *et al.* 2015 Progesterone and HMOX-1 promote fetal growth by CD8+ T cell modulation. *Journal of Clinical Investigation* **125** 1726–1738. (https://doi.org/10.1172/JCI68140)

Sollwedel A, Bertoja AZ, Zenclussen ML, Gerlof K, Lisewski U, Wafula P, Sawitzki B, Woiciechowsky C, Volk HD & Zenclussen AC 2005 Protection from abortion by heme oxygenase-1 up-regulation is associated with increased levels of Bag-1 and neuropilin-1 at the fetal-maternal interface. *Journal of Immunology* **175** 4875–4885. (https://doi.org/10.4049/jimmunol.175.8.4875)

Song R, Mahidhara RS, Zhou Z, Hoffman RA, Seol DW, Flavell RA, Billiar TR, Otterbein LE & Choi AMK 2004 Carbon monoxide inhibits T lymphocyte proliferation via caspase-dependent pathway. *Journal of Immunology* **172** 1220–1226. (https://doi.org/10.4049/jimmunol.172.2.1220)

Talabnin C, Talabnin K & Wongkham S 2020 Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. *Oncology Letters* **20** 2483–2492. (https://doi.org/10.3892/ol.2020.11784)

Tibullo D, Barbagallo I, Giallongo C, La Cava P, Parrinello N, Vanella L, Stagno F, Palumbo GA, Li Volti G & Di Raimondo F 2013 Nuclear translocation of heme oxygenase-1 confers resistance to imatinib in chronic myeloid leukemia cells. *Current Pharmaceutical Design* **19** 2765–2770. (https://doi.org/10.2174/1381612811319150012)

Trojandt S, Bellinghausen I, Reske-Kunz AB & Bros M 2016 Tumor-derived immuno-modulators induce overlapping pro-tolerogenic gene expression signatures in human dendritic cells. *Human Immunology* **77** 1223–1231. (https://doi.org/10.1016/j.humimm.2016.08.014)

Turkseven S, Drummond G, Rezzani R, Rodella L, Quan S, Ikehara S & Abraham NG 2007 Impact of silencing HO-2 on EC-SOD and the mitochondrial signaling pathway. *Journal of Cellular Biochemistry* **100** 815–823. (https://doi.org/10.1002/jcb.21138)

van der Wijst MG, Brown R & Rots MG 2014 Nrf2, the master redox switch: the Achilles' heel of ovarian cancer? *Biochimica et Biophysica Acta* **1846** 494–509. (https://doi.org/10.1016/j.bbcan.2014.09.004)

Vanella L, Barbagallo I, Tibullo D, Forte S, Zappalà A & Li Volti G 2016 The non-canonical functions of the heme oxygenases. *Oncotarget* **7** 69075–69086. (https://doi.org/10.18632/oncotarget.11923)

Vitek L, Hinds TD, Stec DE & Tiribelli C 2023 The physiology of bilirubin: health and disease equilibrium. *Trends in Molecular Medicine* **29** 315–328. (https://doi.org/10.1016/j.molmed.2023.01.007): (S1471-4914(23)00031-X)

Vlahakis JZ, Kinobe RT, Bowers RJ, Brien JF, Nakatsu K & Szarek WA 2005 Synthesis and evaluation of azalanstat analogues as heme oxygenase inhibitors. *Bioorganic and Medicinal Chemistry Letters* **15** 1457–1461. (https://doi.org/10.1016/j.bmcl.2004.12.075)

Vreman HJ, Ekstrand BC & Stevenson DK 1993 Selection of metalloporphyrin heme oxygenase inhibitors based on potency and photoreactivity. *Pediatric Research* **33** 195–200. (https://doi.org/10.1203/00006450-199302000-00021)

Wang GL, Jiang BH, Rue EA & Semenza GL 1995 Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proceedings of the National Academy of Sciences of the United States of America* **92** 5510–5514. (https://doi.org/10.1073/pnas.92.12.5510)

Was H, Dulak J & Jozkowicz A 2010 Heme oxygenase-1 in tumor biology and therapy. *Current Drug Targets* **11** 1551–1570. (https://doi.org/10.2174/1389450111009011551)

Waza AA, Hamid Z, Ali S, Bhat SA & Bhat MA 2018 A review on heme oxygenase-1 induction: is it a necessary evil. *Inflammation Research* **67** 579–588. (https://doi.org/10.1007/s00011-018-1151-x)

Wegiel B, Hanto DW & Otterbein LE 2013 The social network of carbon monoxide in medicine. *Trends in Molecular Medicine* **19** 3–11. (https://doi.org/10.1016/j.molmed.2012.10.001)

Wegiel B, Hedblom A, Li M, Gallo D, Csizmadia E, Harris C, Nemeth Z, Zuckerbraun BS, Soares M, Persson JL, *et al.* 2014 Heme oxygenase-1 derived carbon monoxide permits maturation of myeloid cells. *Cell Death and Disease* **5** e1139. (https://doi.org/10.1038/cddis.2014.97)

Wiel C, Le Gal K, Ibrahim MX, Jahangir CA, Kashif M, Yao H, Ziegler DV, Xu X, Ghosh T, Mondal T, *et al.* 2019 BACH1 stabilization by antioxidants stimulates lung cancer metastasis. *Cell* **178** 330–345.e22. (https://doi.org/10.1016/j.cell.2019.06.005)

Wu Q, Ma Y, Liu Y, Wang N, Zhao X & Wen D 2020 CB2R agonist JWH-133 attenuates chronic inflammation by restraining M1 macrophage polarization via Nrf2/HO-1 pathway in diet-induced obese mice. *Life Sciences* **260** 118424. (https://doi.org/10.1016/j.lfs.2020.118424)

Yamamoto M, Kensler TW & Motohashi H 2018 The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. *Physiological Reviews* **98** 1169–1203. (https://doi.org/10.1152/physrev.00023.2017)

Yamashita K, Ollinger R, McDaid J, Sakahama H, Wang H, Tyagi S, Csizmadia E, Smith NR, Soares MP & Bach FH 2006 Heme oxygenase-1 is essential for and promotes tolerance to transplanted organs. *FASEB Journal* **20** 776–778. (https://doi.org/10.1096/fj.05-4791fje)

Yanatori I, Richardson DR, Toyokuni S & Kishi F 2020 The new role of poly (rC)-binding proteins as iron transport chaperones: proteins that could couple with inter-organelle interactions to safely traffic iron. *Biochimica et Biophysica Acta. General Subjects* **1864** 129685. (https://doi.org/10.1016/j.bbagen.2020.129685)

Yang CC, Hsiao LD, Shih YF, Chang CI & Yang CM 2022 Induction of heme Oxygenase-1 by 15d-prostaglandin J2 mediated via a ROS-dependent Sp1 and AP-1 cascade suppresses lipopolysaccharide-triggered interleukin-6 expression in mouse brain microvascular endothelial cells. *Antioxidants (Basel)* **11** 719. (https://doi.org/10.3390/antiox11040719)

Yang CM, Lin CC, Yang CC, Cho RL & Hsiao LD 2020 Mevastatin-induced AP-1-dependent HO-1 expression suppresses vascular cell adhesion Molecule-1 expression and monocyte adhesion on human pulmonary alveolar epithelial cells challenged with TNF-α. *Biomolecules* **10** 381. (https://doi.org/10.3390/biom10030381)

Yang Z, Chen JS, Wen JK, Gao HT, Zheng B, Qu CB, Liu KL, Zhang ML, Gu JF, Li JD, *et al.* 2017 Silencing of miR-193a-5p increases the chemosensitivity of prostate cancer cells to docetaxel. *Journal of Experimental and Clinical Cancer Research* **36** 178. (https://doi.org/10.1186/s13046-017-0649-3)

Yu J, Shi J, Wang D, Dong S, Zhang Y, Wang M, Gong L, Fu Q & Liu D 2016 Heme Oxygenase-1/Carbon monoxide-regulated mitochondrial dynamic equilibrium contributes to the attenuation of endotoxin-induced acute lung injury in rats and in lipopolysaccharide-activated macrophages. *Anesthesiology* **125** 1190–1201. (https://doi.org/10.1097/ALN.0000000000001333)

Zager RA, Johnson ACM & Becker K 2012 Plasma and urinary heme oxygenase-1 in AKI. *Journal of the American Society of Nephrology* **23** 1048–1057. (https://doi.org/10.1681/ASN.2011121147)

Zhang A, Suzuki T, Adachi S, Naganuma E, Suzuki N, Hosoya T, Itoh K, Sporn MB & Yamamoto M 2021 Distinct regulations of HO-1 gene expression for stress response and substrate induction. *Molecular and Cellular Biology* **41** e0023621. (https://doi.org/10.1128/MCB.00236-21)

Zhang H, Zhou L, Davies KJA & Forman HJ 2019 Silencing Bach1 alters aging-related changes in the expression of Nrf2-regulated genes in primary human bronchial epithelial cells. *Archives of Biochemistry and Biophysics* **672** 108074. (https://doi.org/10.1016/j.abb.2019.108074)

Zhang T, Fang Q, Liu P, Wang P, Feng C & Wang J 2022 Heme oxygenase 1 overexpression induces immune evasion of acute myeloid leukemia against natural killer cells by inhibiting CD48. *Journal of Translational Medicine* **20** 394. (https://doi.org/10.1186/s12967-022-03589-z)

Zhang W, Qiao T & Zha L 2011 Inhibition of heme oxygenase-1 enhances the radiosensitivity in human nonsmall cell lung cancer a549 cells. *Cancer Biotherapy and Radiopharmaceuticals* **26** 639–645. (https://doi.org/10.1089/cbr.2010.0939)

Zhao XQ, Zhang YF, Xia YF, Zhou ZM & Cao YQ 2015 Promoter demethylation of nuclear factor-erythroid 2-related factor 2 gene in drug-resistant colon cancer cells. *Oncology Letters* **10** 1287–1292. (https://doi.org/10.3892/ol.2015.3468)

Zhao YZ, Huang ZW, Zhai YY, Shi Y, Du CC, Zhai J, Xu HL, Xiao J, Kou L & Yao Q 2021 Polylysine-bilirubin conjugates maintain functional islets and promote M2 macrophage polarization. *Acta Biomaterialia* **122** 172–185. (https://doi.org/10.1016/j.actbio.2020.12.047)

Zhe N, Wang J, Chen S, Lin X, Chai Q, Zhang Y, Zhao J & Fang Q 2015 Heme oxygenase-1 plays a crucial role in chemoresistance in acute myeloid leukemia. *Hematology* **20** 384–391. (https://doi.org/10.1179/1607845414Y.0000000212)

Zhou Z, Fang Q, Li P, Ma D, Zhe N, Ren M, Chen B, He Z, Wang J, Zhong Q, *et al.* 2019 Entinostat combined with fludarabine synergistically enhances the induction of apoptosis in TP53 mutated CLL cells via the HDAC1/HO-1 pathway. *Life Sciences* **232** 116583. (https://doi.org/10.1016/j.lfs.2019.116583)

Zhou Z, Fang Q, Ma D, Wei D, Hu X, Liao Y & Wang J 2018 Knockout tumor microenvironment HO-1 neutralizes myeloid-derived suppressor cells and enhances the antitumor effect of PD-1 inhibition in murine models of acute myeloid leukemia. *Blood* **132** 2782. (https://doi.org/10.1182/blood-2018-11-884247)

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