Gasotransmitters in cancer: from pathophysiology to experimental therapy

Csaba Szabo

Abstract | The three endogenous gaseous transmitters — nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H\textsubscript{2}S) — regulate a number of key biological functions. Emerging data have revealed several new mechanisms for each of these three gasotransmitters in tumour biology. It is now appreciated that they show bimodal pharmacological character in cancer, in that not only the inhibition of their biosynthesis but also elevation of their concentration beyond a certain threshold can exert anticancer effects. This Review discusses the role of each gasotransmitter in cancer and the effects of pharmacological agents — some of which are in early-stage clinical studies — that modulate the levels of each gasotransmitter. A clearer understanding of the pharmacological character of these three gases and the mechanisms underlying their biological effects is expected to guide further clinical translation.

The three small, diffusible gaseous mediators — nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H\textsubscript{2}S) — have multiple roles in normal physiology and in the pathogenesis of many diseases. Although a substantial amount of work has been conducted regarding the roles of NO, CO and H\textsubscript{2}S in cancer, the field is full of paradoxes and controversies that present a considerable obstacle for clinical translation. One of the biggest obstacles to understanding the roles of these gasotransmitters in cancer was the seeming discrepancy between some studies showing that these mediators have pro-tumour effects and others showing that they have antitumour effects. Owing to more-recent research, it is now recognized that these mediators have pro-tumour effects and others showing that they have antitumour effects. Owing to more-recent research, it is now recognized that, in cancer, these three gases exhibit a bell-shaped (often also termed ‘biphasic’, ‘bimodal’ or ‘Janus-faced’) pharmacological character.

A greater appreciation of the complex pharmacological character of these mediators has important implications for a deeper understanding of the pathophysiology of cancer. It also resolves some of the controversies in the field, thereby facilitating the formulation of novel therapeutic concepts that are based on either pharmacologically inhibiting the formation of these mediators or increasing their levels via therapeutic donation.

This Review discusses the major roles of NO, CO and H\textsubscript{2}S in tumour pathophysiology, illustrating how either lower or higher concentrations of these gases can affect tumour growth, angiogenesis and survival. It also highlights the potential therapeutic value in cancer of compounds that modulate gasotransmitter levels.

**Nitric oxide**

NO, a free-radical mediator, has been implicated in a plethora of biological processes. It is produced from L-arginine in various tissues by a family of enzymes called NO synthases (NOSs)\textsuperscript{1-4} (Table 1). Endothelial NOS (eNOS; also known as NOS3) and neuronal NOS (nNOS; also known as NOS1) are constitutive, low-output enzymes, whereas the macrophage-type NOS isoform, known as inducible NOS (iNOS; also known as NOS2), is an inducible, high-output enzyme. NOS enzymes use molecular oxygen and require a number of cofactors for their activity. For instance, calmodulin binds tightly with iNOS such that the enzyme is in a continuously activated state\textsuperscript{2}. NO biosynthesis by the three NOS isoforms can be suppressed using various small-molecule inhibitors, some of which have selectivity for individual NOS isoforms: L-G-methyl-L-arginine (L-NMA) inhibits all NOS isoforms and L-N\textsubscript{G}-nitroarginine methyl ester (L-NAME) has some selectivity for the constitutive NOS isoforms, whereas other inhibitors (for example, aminoguanidine, 1400W and many others) exhibit selectivity for iNOS\textsuperscript{5,6}.

In its classical physiological pathway, NO binds to the haem group of guanylyl cyclase to increase intracellular cyclic GMP levels, leading to downstream effects via cGMP-dependent protein kinases (PKGs). At physiological concentrations, NO also opens ATP-dependent potassium channels (K\textsubscript{ATP} channels) and mediates various post-translational protein modifications via S-nitrosylation (Table 1). At higher concentrations,
**Table 1 | Gasotransmitter properties and effects on tumour cells**

<table>
<thead>
<tr>
<th></th>
<th>NO</th>
<th>CO</th>
<th>H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biological sources</strong></td>
<td>NO synthases</td>
<td>Non-enzymatic processes (for example, via conversion from nitrite)</td>
<td>Produced in mammalian cells from L-cysteine by at least three distinct enzymes</td>
</tr>
<tr>
<td></td>
<td>Conversion from nitrite</td>
<td>Conversion from nitrite by several bacteria (for instance, in the oral cavity)</td>
<td>Produced from D-cysteine in certain tissues (for example, the kidneys)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-enzymatic processes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Produced by enteral bacterial flora (for example, in the oral cavity and intestines)</td>
</tr>
<tr>
<td><strong>Chemical properties</strong></td>
<td>A diffusible and labile free-radical gas</td>
<td>A diffusible and labile gas</td>
<td>A diffusible and labile gas</td>
</tr>
<tr>
<td><strong>Biological half-life</strong></td>
<td>Short (a few seconds)</td>
<td>Long (minutes)</td>
<td>Medium (seconds to minutes)</td>
</tr>
<tr>
<td><strong>Elimination</strong></td>
<td>Mainly via the urine as nitrite and nitrate</td>
<td>Mainly unaltered, in the exhaled air</td>
<td>Via the urine as sulfite, sulfate and thiosulfate</td>
</tr>
<tr>
<td></td>
<td>A small amount is exhaled</td>
<td></td>
<td>A small amount is exhaled</td>
</tr>
<tr>
<td><strong>Key biological reactions</strong></td>
<td>Reacts with haem iron centres in various proteins</td>
<td>Reacts with protein cysteines to initiate S-nitrosylation.</td>
<td>Binds to haem iron centres to yield carboxyhaemoglobin</td>
</tr>
<tr>
<td></td>
<td>Reacts with protein cysteines to initiate S-nitrosylation.</td>
<td>Has multiple reactions with oxygen free radicals (for example, with superoxide, to yield peroxynitrite)</td>
<td>Binds to protein cysteines to initiate sulhydrylation</td>
</tr>
<tr>
<td></td>
<td>Reacts with haemoglobin to yield nitrosyl-haemoglobin and met-haemoglobin</td>
<td></td>
<td>Reacts with oxygen free radicals</td>
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<td></td>
<td></td>
<td></td>
<td>Can form persulfides and polysulfides</td>
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<td></td>
<td></td>
<td></td>
<td>Reacts with haemoglobin to yield sulfaemoglobin</td>
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<tr>
<td><strong>Selected signalling pathways</strong></td>
<td>Activates guanylyl cyclase to increase cGMP levels</td>
<td>Activates guanylyl cyclase (less potently than NO), which then forms cGMP</td>
<td>Post-transcriptional protein modification via sulhydrylation</td>
</tr>
<tr>
<td></td>
<td>Post-transcriptional protein modification via nitrosylation and reactions with haem groups</td>
<td>Reacts with haemoglobin to yield carboxyhaemoglobin</td>
<td>Activates (opens) K⁺s channels</td>
</tr>
<tr>
<td></td>
<td>Activates (opens) K⁺s channels</td>
<td></td>
<td>Inhibits cGMP and cAMP phosphodiesterases</td>
</tr>
<tr>
<td><strong>Vascular effects in tumours</strong></td>
<td>Vasodilation</td>
<td>Vasodilation</td>
<td>Vasodilation</td>
</tr>
<tr>
<td></td>
<td>Maintains basal blood flow to various organs and to tumour tissues</td>
<td>Possibly maintains basal blood flow to some organs and some tumours (to a lesser degree than NO or H₂S)</td>
<td>Maintains basal blood flow to various organs and some tumours (to a lesser degree than NO)</td>
</tr>
<tr>
<td></td>
<td>Stimulates angiogenesis (including tumour angiogenesis)</td>
<td></td>
<td>Stimulates tumour angiogenesis</td>
</tr>
<tr>
<td><strong>Bioenergetic effects in tumour cells</strong></td>
<td>Inhibits cytochrome c oxidase and other mitochondrial enzymes, thus inhibiting cellular energetics</td>
<td>Inhibits cytochrome c oxidase, impairing cellular bioenergetics</td>
<td>Stimulates cellular bioenergetics at low concentration (as it donates electrons at mitochondrial complex II to stimulate aerobic metabolism and sulfhydral GAPDH to stimulate anaerobic metabolism)</td>
</tr>
<tr>
<td></td>
<td>After conversion to peroxynitrite, increases poly(ADP-ribose) polymerase activity, impairing cellular bioenergetics</td>
<td></td>
<td>Has inhibitory effects at higher concentration via inhibition of cytochrome c oxidase and others</td>
</tr>
<tr>
<td><strong>Direct effects on tumour cell viability</strong></td>
<td>Low concentrations mediate antioxidant effects and are thus protective</td>
<td>Low concentrations activate MAPKs and other survival pathways, leading to cytoprotection</td>
<td>Low concentrations mediate antioxidant, signalling and positive bioenergetic mechanisms and are thus cytostatic</td>
</tr>
<tr>
<td></td>
<td>Higher concentrations of NO or, more importantly, secondary species such as peroxynitrite, can be toxic</td>
<td>Higher concentrations inhibit mitochondrial electron transport and protein synthesis and are thus toxic</td>
<td>Higher concentrations inhibit mitochondrial electron transport and have other toxic effects</td>
</tr>
</tbody>
</table>

| cAMP, cyclic AMP; cGMP, cyclic GMP; CO, carbon monoxide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; H₂S, hydrogen sulfide; K⁺s channel, ATP-dependent potassium channel; KCa channel, calcium-dependent potassium channel; MAPK, mitogen-activated protein kinase; NO, nitric oxide. |

Peroxynitrite

A short-lived cytotoxic oxidant species that is the product of the diffusion-controlled reaction between nitric oxide (NO) and a superoxide radical (O₂⁻).

NO can exert deleterious effects, including: the inhibition of mitochondrial enzymes; the initiation of DNA damage; and the activation of p53 and poly(ADP-ribose) polymerase (TABLE 1). In biological systems, many of these adverse effects are the consequence of the simultaneous production of NO and oxygen-derived reactive oxidative species (ROS); one pathway that has been implicated in this process involves the generation of peroxynitrite (ONOO⁻) from one molecule of NO and one superoxide ion (O₂⁻), followed by the generation of a hydroxyl-radical-type reactive species⁴⁻⁹,¹⁰.

The cellular microenvironment strongly influences the biological profile of NO. For example, acidosis increases the half-life and diffusibility of NO. Under acidic conditions, NO can also be generated from its semi-stable metabolite, nitrite (NO₂⁻). Moreover, when levels of intracellular antioxidants and/or certain NOS cofactors (for instance, tetrahydrobiopterin (BH₄)) are depleted, NOS produces superoxide ions instead of NO. Such effects of environment and concentration must be borne in mind when interpreting the biological roles of NOS and NO⁴⁻⁹,¹⁰.
At low concentrations, NO tends to exert antioxidant-type responses, thereby protecting against oxidative cell injury and cell death\(^1\)\(^{-3}\)\(^,\)\(^6\)\(^,\)\(^10\). It is thought to do this through: neutralization of deleterious redox responses (for example, by inactivating superoxide); cGMP-mediated signalling; phosphorylation of extracellular signal-regulated kinase (ERK); and inhibition of caspase activation. By contrast, higher NO levels lead (directly or indirectly) to DNA damage and impaired cellular metabolism, and can initiate various forms of cell death (including apoptotic, necrotic or mixed-type cell death)\(^1\)\(^{-3}\)\(^,\)\(^11\)\(^,\)\(^12\).

**Antitumour effects of nitric oxide.** Several decades ago, it was observed that the killing of tumour cells by activated macrophages is associated with a massive production of nitrite and nitrate. The underlying pathomechanism for this was not understood until it was noticed that this macrophage-mediated killing depends on the presence of l-arginine in the culture medium\(^13\). Subsequent studies have shown that the NOS inhibitor l-NMA inhibits macrophage-mediated killing of tumour cells, and that NO (or a closely related species) is responsible for lysis of the tumour cell\(^14\)\(^,\)\(^15\). Importantly, the importance of NO in macrophage-mediated cell death is highly dependent on the type of tumour cell\(^16\). The molecular mechanisms involved in NO-mediated cell death are multiple and, as mentioned above, involve high local concentrations or fluxes of NO, which together with ROS induce metabolic inhibition and DNA damage\(^5\)\(^{-16}\) (FIG. 1a).

The *in vivo* correlate of this paradigm is the immune-mediated killing of tumour cells in tumour-bearing immunocompetent (or even immunologically hyperactivated) mice. In a mouse model of *Bacillus Calmette–Guérin* (BCG)-induced tumour resistance, the BCG-induced clearance of a syngeneic ovarian tumour was attenuated by treatment with l-NMA, suggesting that NO contributes to the antitumour immune effector response\(^17\). Likewise, interferon-β (IFNβ)-overexpressing metastatic murine pancreatic adenocarcinoma cells and 3-methylcholanthrene-induced fibrosarcoma lines grew much faster in mice lacking iNOS than in wild-type control hosts\(^18\)\(^,\)\(^19\). Similarly, treatment with the selective iNOS inhibitor 1400W produced a 50% reduction in the antitumour effect of tumour necrosis factor (TNF) therapy against MethA mouse fibrosarcoma\(^20\). The antitumour effect of interleukin-13 (IL-13) against various head and neck tumours was also attenuated by l-NMA\(^21\). Finally, treatment of mice bearing pancreatic adenocarcinoma tumours (which only express low levels of iNOS) with N\(^\text{O}\)\(^{-}\text{-(1-iminoethyl)-L-lysine (l-NIL; another NOS inhibitor with limited selectivity for iNOS)} increased the formation of liver metastases\(^22\).

In *in vivo* work demonstrating the marked variation in the susceptibility of tumour cells to NO-mediated killing\(^23\)\(^,\)\(^25\), several *in vivo* studies have shown that the growth of implanted tumours depends on the type of tumour and the immune status of the host. For instance, the growth of B16-BL6 melanoma and M5076 ovarian sarcoma was enhanced by only 20% in mice lacking iNOS\(^21\), whereas the growth of B16-F1 melanoma cells was in fact slightly reduced in such mice\(^25\), perhaps indicating that the growth of these different tumour types may depend on the presence or relative scarcity of NO.

Interestingly, factors (that have not yet been characterized) in the environment of some tumours can attenuate the host’s NO-mediated antitumour action by suppressing the ability of M2 macrophages to convert into pro-inflammatory M1 macrophages, which produce higher levels of NO\(^25\)\(^,\)\(^26\). This response can protect some tumours from macrophage-mediated cell death\(^25\)\(^,\)\(^26\).

There are several ways in which the antitumour effects of NO can be exploited therapeutically. The first approach involves the on-demand upregulation of intratumour levels of NO (and/or associated reactive nitrogen species) to extremely high — cytotoxic — levels, a strategy that can be used alongside tumour immunotherapy to boost the natural antitumour immune response. The most successful strategy to achieve this relates to the immunotherapy of bladder cancer with, for instance, IFNα or BCG; the upregulation of the local antitumour immune response results in the marked upregulation of iNOS, NO and peroxynitrite, which contribute to the antitumour efficacy of the therapy\(^27\)\(^{-30}\).

As iNOS is a high-output source of NO that relies heavily on substrate availability (as opposed to eNOS and nNOS, which produce lower amounts of NO under more-regulated conditions), the macrophage production of NO by iNOS may also be enhanced by supplementation with its substrate, l-arginine. In preclinical studies, l-arginine treatment was found to stimulate anticancer immune responses and reduce tumour growth\(^30\)\(^{-32}\). In clinical trials, oral l-arginine supplementation was found to counteract tumour-induced immunosuppression and to enhance the antitumour immune response\(^33\)\(^{-37}\). l-Arginine restored antitumour immunity and improved long-term survival in malnourished patients with gastric cancer or head and neck cancer receiving neoadjuvant chemotherapy\(^35\). Moreover, treatment of individuals with colorectal cancer with 30 g l-arginine per day for 3 days reduced the expression of survivin (a nuclear antigen found on proliferating cells), increased iNOS expression in tumour cell biopsies and increased the serum levels of NO metabolites\(^38\). Although these findings suggest that l-arginine supplementation attenuates the development of colorectal tumours by increasing NO levels within the tumour tissue, one must keep in mind that l-arginine is also a secretagogue for growth hormone, insulin-like growth factor 1, insulin and prolactin. Thus, the relative contributions of NO-dependent and NO-independent actions of l-arginine in cancer remain to be dissociated in future studies.

The very fact that cancer develops in the first place proves that the innate immune system is often inadequate to defeat the growth of tumours. One mechanism by which a tumour may evade the NO-mediated immune response of the host involves increasing the expression of the microRNA miR-146a in tumour cells\(^39\). miR-146a inhibits the translation of iNOS in the tumour cells, which, in turn, (via mechanisms that remain to be fully characterized) suppresses the ability of

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**Bacillus Calmette–Guérin**

A live attenuated strain of *Mycobacterium bovis* that is a US-approved therapy for *in situ* bladder carcinoma.

**3-methylcholanthrene**

The most common isomer of a highly carcinogenic polycyclic aromatic hydrocarbon — its topical administration in mice is often used as an experimental cancer model.

**Secretagogue**

A biological substance that induces the secretion of another substance. For example, angiotensin II is a secretagogue for aldosterone.
Angiogenesis

Blood flow and nutrient supply to the tumour

Tumour tissue with tumour-associated macrophages

L-arginine

iNOS

NO

O₂

ONOO−

DNA damage

p53 pathway and caspase activation

Cell cycle arrest

Activation of cell death pathways

Tumour cell death

Tumour cell

Macrophage

NADPH oxidase

Macrophage activation

Endothelial cell

Blood vessels

Blood vessels

Tumour tissue

Tumour cell

Macrophage

Endothelial cell

iNOS

O₂−

NADPH oxidase

Macrophage activation

Endothelial cell migration and proliferation

Smooth muscle relaxation

Angiogenesis

Blood flow and nutrient supply to the tumour

L-arginine

NO

CO

H₂S

↓ Oxygen consumption

↓ Electron flow

↓ ATP turnover

Blood vessels

Tumour cell

Blood vessels

Tumour cell

L-cysteine

CBS

H₂S

COX2

Activation of PI3K pathway and NRF2 pathway

Activation of PI3K pathway and MMPs

Electron donation

Bioenergetic stimulation

Cytoprotective and proliferative pathways

Cytoprotective and proliferative pathways

Cytoprotective and proliferative pathways

↓ Tumour cell proliferation, migration and invasion

↓ Tumour growth and metastasis

• cGMP

• PKG

• PKC

• RAF

• ERK1 and ERK2

• COX2

• HIF1α

• FGF2

• TIMPs

• MMPs

↑ Cell cycle arrest

↑ Activation of cell death pathways

↑ Endothelial cell migration and proliferation

↑ Smooth muscle relaxation

↑ Angiogenesis

↑ Blood flow and nutrient supply to the tumour

↑ Tumour cell proliferation, migration and invasion

↑ Tumour growth and metastasis

↑ cGMP

↑ PKG

↑ PKC

↑ RAF

↑ ERK1 and ERK2

↑ HIF1α

↑ FGF2

↑ TIMPs

↑ MMPs

↑ Electron donation

↑ Bioenergetic stimulation
tumour-infiltrating macrophages to kill tumour cells. Further understanding and pharmacological correction of these evasive actions of tumours may open new therapeutic avenues.

Another distinct but related therapeutic approach involves increasing NO levels in the tumour microenvironment in ways that are independent of the host immune system. Traditionally, NO could be delivered using NO donors, but additional approaches may involve therapeutic overexpression of iNOS in the tumour (for instance, through gene therapy). In some cases, these approaches can be combined with antitumour chemotherapy, whereas in other cases, they rely on ‘hybrid’ or ‘multifunctional’ NO donor molecules in which a NO donor group is linked to an existing drug (such as a non-steroidal anti-inflammatory drug). In yet other cases, NO donors can be used in combination or sequentially with radiotherapy. The use of therapeutic donation of NO in cancer is discussed further in specialized reviews.64–66

Killing tumour cells with high concentrations of NO donors in vitro is relatively straightforward. Moreover, in vivo, depending on the dose and type of the NO donor and type of tumour tissue involved, some degree of NO-mediated vasodilatation (Fig. 1b) may help with the delivery of cytotoxic drugs to the tumour. However, as NO potently induces vasodilatation at concentrations that are well below those required to elicit cytotoxicity, one of the central challenges associated with NO donor therapies is to deliver high concentrations of NO into the tumour without ‘spilling’ too much NO into the circulation, where it induces a dose-limiting systemic hypertensive side effect. There are several approaches to selectively direct NO to tumour cells: one approach involves exploiting the specific metabolic activity of cancer cells by using glutathione-S-transferase (GST)-activated NO donors such as para-aminobenzoic acid (PABA)—NO and JS-K64,67—in some cases, in combination with multi-arm polymeric nanocarriers.64,68. Another strategy is to use photoactivated NO donors, which in some cases could be applied as supramolecular complexes.66,67 One study tested a combination of the Escherichia coli enzyme nitroreductase and NO-producing prodrugs directed to the tumour,65 as well as NO donors activated by tumour-cell esterase or DT-diaphorase.62

Another strategy exploits the hypoxic nature of the tumour cell by delivering NO in the form of S-nitrosohaemoglobin (SNO-haemoglobin) or nitrite, both of which preferentially release NO in hypoxic or acidic environments62. The structures of some non-tumour-targeted and tumour-targeted NO donors are shown in Fig. 2a.

Although the upregulation of NO levels can be used as an antitumour approach, in many forms of cancer immunotherapy (for example, TNF, IFN or IL-2 therapies) it represents an undesirable side effect, because these immunotherapies induce an increase in NO production in the systemic vasculature (BOX 1). In these instances, therefore, the inhibition of systemic NO production could be an approach to mitigate the dose-limiting adverse haemodynamic effects that is, systemic vasodilatation and hypotension of these therapies.

Pro-tumour effects of tumour-derived nitric oxide. Several tumours (including gastrointestinal cancers, breast cancer, ovarian cancer, bladder cancer and glioma) express high levels of iNOS and produce increased amounts of NO35–37 (Table 2), and this may affect the profiles of these tumours (Fig. 1b), iNOS-overexpressing colon adenocarcinoma tumours implanted into nude mice grew markedly faster, exhibited a more invasive phenotype and showed a higher degree of intratumoural and peritumoural vascularization than did wild-type control tumours. In vitro, the growth of many iNOS-overexpressing tumours can be reduced by NO inhibitors (for example, l-NMA) or by the iNOS inhibitor aminoguanidine, suggesting that endogenous, tumour-derived NO can support tumour growth66—a view supported by the results of clinical trials.69–71

Intra-tumoral administration of NO to tumours 38–42 has been shown to improve the efficacy of anti-tumour immunotherapies, although further studies are needed to 40 determine the mechanisms involved. One study noted that delivery of NO to tumours 31,34 using a NO-releasing prodrug reduced the growth of metastases in mice implanted with 33 tumours that were not responsive to anti-tumour 39 immunotherapy. Further work is needed to determine the mechanisms involved.

Figure 1 | Effects of the three gasotransmitters on tumour survival and growth. a | Nitric oxide (NO)-mediated mechanisms of killing of tumour cells by tumour-associated macrophages. Upregulation of inducible NO synthase (iNOS) in activated tumour-associated macrophages leads to the production of high local levels of NO. At the same time, macrophages also produce superoxide (O2-) from NADPH oxidase and other cellular sources. Together, NO and superoxide form peroxynitrite (ONOO-), a reactive oxidant species (ROS). The resulting combination of nitrosative and oxidative stress can be cytostatic or cytotoxic to certain tumour cell types (the NO-associated component of cell killing is tumour-cell-type dependent). In susceptible tumour cells, the NO-mediated killing of cells involves the inhibition of mitochondrial activity, DNA damage and activation of downstream pathways such as the p53 and caspase-activation pathways, culminating in tumour cell lysis. These mechanisms can be enhanced by various immunostimulatory therapies and/or by supplementation of L-arginine, the substrate of NOS. b | Pro-tumour effects of low levels of endogenously produced NO, carbon monoxide (CO) and hydrogen sulfide (H2S). The survival and proliferation of the tumour cell are stimulated by the production of gasotransmitters in the tumour. Here, induction of iNOS and consequently elevated levels of NO (left tumour cell), induction of haem oxygenase 1 (HO1) and elevated levels of CO (middle tumour cell) or induction of cystathionine-β-synthase (CBS) and elevated levels of H2S (right tumour cell) can exert pro-survival and pro-proliferative effects. Depending on the gasotransmitter, these signalling mechanisms can culminate in upregulation of fibroblast growth factor 2 (FGF2), activation of matrix metalloproteinases (MMPs), upregulation of tissue inhibitors of metalloproteinases (TIMPs), activation of phosphoinositide 3-kinase (PI3K) and/or the stimulation of the inducible isoform of cyclooxygenase (COX2). In addition to tumour-autonomous effects, each gasotransmitter can diffuse out from the tumour cells and can stimulate intratumour and peritumour angiogenesis through paracrine actions on endothelial cells, for instance through: stimulation of various pro-angiogenic pathways (including the cyclic GMP–cGMP-dependent protein kinase (PKG) signalling pathway; activation of protein kinase C (PKC), RAF, extracellular signal-regulated kinase 1 (ERK1) and ERK2; and stabilization of hypoxia-inducible factor 1a (HIF1a). Although the signalling mechanisms are gasotransmitter- and condition-dependent, the ultimate result is the stimulation of peritumour angiogenesis and an increase in tumour blood flow, NRF2, nuclear factor erythroid 2-related factor 2.

Glutathione-S-transferase (GST): A soluble protein with a molecular mass of ~50 kDa. GSTs represent a major group of detoxification enzymes and catalyse the conjugation of the reduced form of glutathione (GSH) to various cellular substrates.

Multi-arm polymeric nanocarriers: Branched, globular, nanoscale materials exhibiting a large surface area. They are commonly used for targeted drug delivery.

Prodrugs: Inactive precursors of active therapeutic agents. The conversion from the inactive to the active form occurs through normal metabolic processes, often involving the hydrolysis of an ester group.
### a) Classic NO donors

- Classic NO donors
  - Sodium nitroprusside
  - Glyceryl trinitrate

### b) L-arginine-based NOS inhibitors

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>n</th>
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<tbody>
<tr>
<td>NH2</td>
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<tr>
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</tr>
<tr>
<td>NH2</td>
<td>NOH</td>
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</tr>
</tbody>
</table>

### c) Porphyrin-based HO inhibitors

- Metal: Mesoporphyrin (R = –CH2–CH3)
- Protoporphyrin (R = –CH=CH2)
- Metal: Zinc, Tin, Chromium, Manganese
- ZnMP, SnMP, CrMP, MnMP

### d) HO1 inhibitors

- OB-24
- Azalanstat

### e) CORMs

<table>
<thead>
<tr>
<th>Formula</th>
<th>Structure</th>
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<tbody>
<tr>
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<tr>
<td>CORM-3</td>
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</table>

### f) CBS inhibitors

- Aminoxyacetic acid
- Hydroxylamine
- GYY4137

### g) H2S donors

- Diallyl trisulfide
- S-propargyl-cysteine
and assume a migratory and epithelial cells lose their cell (EMT). A process by which
Epithelial–mesenchymal component.
chorioallantoic membranes cells are grown on chick model in which melanoma

hydrogen sulfide (H₂S) donors. Sodium nitroprusside and glyceryl trinitrate are considered ‘classic NO donors’ and have been
injected at least in part, to effects of NO that extend outside the tumour cell — such as induction of angiogenesis, which
would enhance tumour perfusion and supply of nutrients to the tumour (Fig. 1b). These effects are consistent with the well-established role of NO as an endogenous pro-angiogenic mediator.

The notion that tumour-derived, iNOS-mediated overproduction of NO supports tumour angiogenesis and tumour growth in vivo (Fig. 1b) has been confirmed using selective iNOS inhibitors. 1400W, a NO
inhibitor with high selectivity for iNOS (approximately 5,000-fold over other isozymes), reduced the growth rate of iNOS-overexpressing mammary adenocarcinoma in nude mice, as well as the growth rate of other tumours that spontaneously expressed high levels of iNOS64. Moreover, 1-NAME inhibited the proliferation of L3.6pl human pancreatic cancer cells implanted into nude mice65 and the proliferation of melanoma cells in a chorioallantoic membrane model66; aminoguanidine inhibited the growth of subcutaneously implanted mouse forestomach carcinoma cells in athymic mice67; 1-NIL and 1,3-PBIT slowed the growth of human melanoma in immunodeficient mice68; and aminoguanidine (1-NAME) slowed growth of oestrogen-receptor-negative breast cancers in mice69,70. In most cases, the antitumour responses of the NOS inhibitors were associated with a suppression of tumour angiogenesis, further supporting the notion that the inhibition of the paracrine effects of tumour-derived NO underlies the action of

Selective inhibition of tumour iNOS may therefore help to combat the actions of tumour-iNOS-derived NO by reducing the tumour-cell-autonomous actions of NO (including cytoprotection and the stimulation of proliferation and migration) as well as by reducing the paracrine actions of iNOS-derived NO (such as stimulation of tumour angiogenesis) (Fig. 1b). Although such an approach can only be expected to exert anticancer effects in tumour cells with high levels of iNOS expression, iNOS-dependent tumours are fairly common (Table 2). Inhibition of iNOS and/or scavenging of NO can have stand-alone effects in these susceptible tumour types but are more likely to be used to enhance the antitumour effect of other chemotherapies56–70.

However, these approaches would probably not be used with radiotherapy, as NO inhibitors can produce tumour ischaemia or hypoxia, which can create tumour radioresistance71,72. The measurement of NO metabolite levels in the blood of people with cancer and/or the immunohistochemical detection of iNOS in resected primary tumours may be used in the future to identify patients who would be likely to respond to iNOS inhibitor therapy.

iNOS inhibition has long been clinically investigated for its potential to treat various forms of local inflammation (such as arthritis, colitis and asthma) and systemic inflammation (such as circulatory shock)73–75. The potential for iNOS inhibition in treating cancer only emerged later on. Preclinical efficacy studies of iNOS inhibitors in cancer have been carried out for 1400W76, a research compound synthesized by Glaxo Wellcome; aminoguanidine77, a several-decade-old compound with mixed pharmacological actions (which, nevertheless, includes some selectivity for iNOS, especially in vivo); and 1-NIL78, another 1-arginine-based NO inhibitor that is not a clinical development candidate. Of these, the only compound that may be immediately available for clinical trials is aminoguanidine, which has already been tested in humans for non-oncological indications (including diabetic nephropathy and chronic obstructive pulmonary disease) and shows an acceptable safety profile78,79 (Box 2). However, the intellectual property status of aminoguanidine (which is not a ‘novel structure of matter’) may diminish industry’s interest in developing it.
Cancer immunotherapy — for instance, with interleukin-2 (IL-2) — is associated with a severe, often dose-limiting hypotension that some studies have suggested is partly attributable to systemic overproduction of nitric oxide (NO). Although initial observations suggested that inducible NO synthase (iNOS), which is expressed in vascular smooth muscle, may have a role in this hypotension\(^{11,12,13}\), more-recent work has implicated endothelial NOS (eNOS)-derived NO\(^{14,15}\). The formation of peroxynitrite has also been implicated in mediating part of the IL-2-induced organ-damaging side effects\(^{16}\). These findings indicate that inhibition of systemic NO synthesis and/or the neutralization of peroxynitrite may be used to reduce the systemic toxicity of cancer immunotherapy. In a Phase I clinical trial, the non-isof orm-selective NOS inhibitor N\(^{6}\)-methyl-L-arginine (L-NMA) was tested in 23 patients with cancer\(^{16}\), the majority of whom had developed hypotension in response to IL-2. L-NMA exhibited marked antihypotensive activity at all dose levels (3–36 mg per kg), and the duration of the effect was proportional to the dose of the NOS inhibitor used. At the highest dose tested (36 mg per kg), adverse effects of NOS inhibition, such as an increase in pulmonary vascular resistance and a decrease in cardiac output, were also observed. These observations suggest that NOS inhibition may be useful to alleviate the hypotensive effects of high-dose IL-2 therapy (or of other immunotherapies) in individuals with cancer. According to preclinical data, non-isof orm-selective or eNOS-selective NOS inhibition does not interfere with the anticancer effect of IL-2 (REF. 202).

Generally, the interest of the pharmaceutical industry in NOS inhibitors has diminished over the past decade. Historically, this may be related to the failure of L-NMA in Phase III clinical trials in patients with circulatory shock\(^{25}\); however, L-NMA is not a selective inhibitor of iNOS, and newer-generation NOS inhibitors that are more selective for iNOS may have markedly different safety and efficacy profiles. GlaxoSmithKline has completed several small clinical trials with GW273629 (another selective iNOS inhibitor)\(^{26}\); the compound failed to show clinical efficacy in migraine\(^{27}\) and asthma\(^{28}\), but exhibited some efficacy in rheumatoid arthritis\(^{29}\). Pfizer’s iNOS inhibitor cindunistat (which is structurally closely related to GW273629) failed to show efficacy in a 2-year trial in osteoarthritis\(^{30}\).

There are no published data on GW273629 or cindunistat in preclinical or clinical cancer studies. According to a recent patent filing\(^{31}\), Astellas’ iNOS inhibitor FK330 (also known as FR260330) shows antitumour efficacy in combination with taxol; however, the development stage of this compound has not been publicly disclosed. The structures of some NOS-inhibiting compounds are shown in Fig. 2b.

**Pro-angiogenic effects of host-derived nitric oxide.** The inhibitory effect of selective iNOS inhibitors on tumour growth is not universal. For example, whereas amino- guanidine, aminoethyl isothiourea and 1400W did not inhibit the growth of rat carcinomas, the eNOS-selective NOS inhibitors l-NAMe and N\(^{3}\)-nitro-L-arginine (L-NNA), as well as various ruthenium-based NO scavengers, did\(^{32,33}\). These observations may indicate that although some tumour tissues do not overproduce NO, and although NO that emanates from the tumour tissue may not always be essential for tumour angiogenesis, eNOS-derived NO that is produced by the blood vessels of the host can, nevertheless, increase tumour blood flow and/or peritumour angiogenesis. In such instances, therapeutic inhibition of host eNOS may be of potential therapeutic benefit. The fact that l-NAMe exerts marked reductions in tumour blood flow and consequently induces tumour hypoxia has long been established\(^{34,35,36}\), and subsequent studies have also demonstrated that l-NAMe can reduce tumour angiogenesis\(^{37}\). Melanomas that were grown in eNOS-deficient mice had decreased vessel numbers (but increased vessel perimeters and numbers of endothelial nuclei per vessel cross-section) compared with those grown in wild-type animals\(^{38}\). By contrast, in the same experimental model, the genetic deletion of iNOS (instead of eNOS) in the host stromal cells did not affect angiogenesis or vessel morphology\(^{39}\). Similarly, the deficiency of host eNOS reduced the growth of pancreatic cancer\(^{40}\).

Thus, regardless of the level of NOS expression or NO production by the tumour tissue, inhibition of host eNOS may be a stand-alone target for antitumour therapy. Nevertheless, in most cases both the tumour tissue and the host tissue are likely to contribute to NO levels in the tumour microenvironment. Moreover, eNOS and eNOS that are localized to the tumour cell (TABLE 2) may also contribute to the elevation of NO levels in the tumour microenvironment\(^{40,41}\). Thus, NOS inhibition needs to be tailored and adjusted to the relevant source or sources of NO within the specific tumour type.

Several early-stage clinical trials have now been conducted to target host eNOS or to non-selectively inhibit all NOS isoforms in order to suppress tumour angiogenesis. Advantages of these approaches include their broad applicability for vascularized tumours and the fact that some of the NOS inhibitors (for instance, l-NMA) have already undergone human clinical trials. In a Phase I clinical study in 19 patients with non-small-cell lung cancer, prostate cancer or cervical cancer, l-NMA (0.1–0.9 mg per kg) was administered intravenously and caused a notable decrease in tumour blood flow that was maintained for 24 hours\(^{41}\). The side effects of this treatment were relatively minor: mild bradycardia and slight hypertension\(^{42}\). Although these findings are encouraging, if eNOS inhibitors were to be used chronically, the haemodynamic side effects may become problematic. This concern, coupled with the fact that the typical non-isof orm-selective or eNOS-selective inhibitors (including l-NAMe and l-NMA, among others) no longer have proprietary patent status, means that the clinical translation of this approach will be less likely to ultimately succeed.

**Carbon monoxide**

CO is a stable, diffusible, gaseous mediator that, similar to NO, has many physiological roles. It is produced in various mammalian cells and tissues by a family of enzymes called haem oxygenases (HOs) (TABLE 1) that catalyse the oxidative degradation of haem (reviewed in REFs 92–96). The inducible HO isoform (haem oxygenase 1 (HO1)) can be upregulated in response to various stimuli, including haem, oxidative stress, ultraviolet irradiation, heat shock, hypoxia and NO. The constitutive HO isoform HO2 is expressed in several tissues, including the brain, kidney, liver and spleen.
Table 2 | Changes in the expression of various gasotransmitter-producing enzymes in various forms of cancer

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>NO</th>
<th>CO</th>
<th>H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biliary tract carcinoma</td>
<td>iNOS ↑</td>
<td>–</td>
<td>CBS ↑</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>iNOS ↑ ↕️</td>
<td>HO1 ↑</td>
<td>CBS ↑</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>iNOS ↑ ↔️</td>
<td>HO1 ↑</td>
<td>CBS and CSE ↑</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>iNOS ↑</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glioma</td>
<td>nNOS and iNOS ↑</td>
<td>HO1 ↑</td>
<td>MST ↑</td>
</tr>
<tr>
<td>Hepatic cholangiocarcinoma</td>
<td>iNOS ↑</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>iNOS ↓ or iNOS ↑</td>
<td>HO1 ↑</td>
<td>–</td>
</tr>
<tr>
<td>Leukaemia or lymphoma</td>
<td>iNOS ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>nNOS and iNOS ↑</td>
<td>HO1 ↑</td>
<td>CSE and MST ↑</td>
</tr>
<tr>
<td>Myeloma</td>
<td>iNOS and nNOS ↑</td>
<td>–</td>
<td>CBS ↑</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>iNOS ↑</td>
<td></td>
<td>CBS ↑</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>eNOS ↑</td>
<td>HO1 ↑</td>
<td>–</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>iNOS ↑</td>
<td>HO1 ↑</td>
<td>CBS and CSE ↑</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>eNOS and iNOS ↑</td>
<td>HO1 ↑</td>
<td>CBS ↑</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>eNOS and iNOS ↑</td>
<td>HO1 ↑</td>
<td>–</td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>iNOS ↑</td>
<td>HO1 ↑</td>
<td></td>
</tr>
</tbody>
</table>

CBS, cystathionine-β-synthase; CO, carbon monoxide; CSE, cystathionine-β-lyase; eNOS, endothelial NOS; H₂S, hydrogen sulfide; HO1, haem oxygenase 1; iNOS, inducible NOS; MST, 3-mercaptopyruvate sulfurtransferase; nNOS, neuronal NOS; NO, nitric oxide; NOS, NO synthase.

The enzymatic activity of HOs depends on NADPH and requires oxygen. Importantly, HO-dependent CO production can be inhibited with various small-molecule HO-inhibitor protoporphyrins (PPs), such as tin PP (SnPP) and zinc PP (ZnPP), and mesoporphyrins, such as zinc deuteroporphyrin (ZnDP).

The classical pathways of the physiological actions of CO involve the stimulation of the guanylyl cyclase–cGMP pathway, although the affinity of CO for the haem group of guanylyl cyclase is much lower than that of NO. Low CO concentrations also activate (open) Kᵥ₅ᵢ channels and influence various intracellular kinase pathways, including the PI3K–AKT and p38 mitogen-activated protein kinase (MAPK) signalling pathways (TABLE 1).

At higher concentrations, CO exerts adverse biological effects, which, in vitro, are mainly attributed to the binding of CO to haemoglobin; the resulting carboxyhaemoglobin reduces the oxygen-carrying capacity of the blood and leads to tissue hypoxia. In vitro, CO inhibits mitochondrial electron transport by irreversibly inhibiting cytochrome c oxidase (also known as complex IV). The combination of these deleterious actions is considered the central mode of CO inhalation poisoning, a relatively common clinical problem.

Some of the best-characterized physiological effects of CO include anti-inflammatory, antiproliferative, anti-apoptotic and anticoagulative responses; by contrast, at higher concentrations, CO becomes cytotoxic (TABLE 1). In contrast to NO, the cytoprotective and cytotoxic effects of CO are intimately intertwined. For example, a low level of CO-mediated inhibition of mitochondrial activity followed by a slight increase in intracellular ROS production has been shown to be important in CO-mediated cytoprotective signalling events (such as activation of kinase pathways and stabilization of hypoxia-inducible factor 1α (HIF1α))⁹²–⁹⁴. In a way, the cytoprotective effects of CO resemble the protective effects of pharmacological preconditioning, whereby a short, relatively mild (and survivable) insult triggers a secondary cytoprotective phenotype, for example via activation of the prototypical antioxidant response element nuclear factor erythroid 2-related factor 2 (NRF2). Thus, a protective cellular phenotype is maintained in the cell for a long time after CO has already been cleared from the biological system.

**Pro-tumour effects of carbon monoxide.** Several tumour types (including prostate cancer, renal cell carcinoma, hepatocellular carcinoma, glioblastoma, melanoma, Kaposi sarcoma, pancreatic cancer and chronic myeloid leukaemia) contain high levels of HO1 either in the tumour cells themselves or in the tumour-infiltrating macrophages⁹⁵,⁹⁶ (TABLE 2).

The functional importance of intratumour CO is well illustrated by studies showing that small interfering RNA (siRNA)-mediated suppression of HO1 expression reduces the viability and the rate of proliferation of pancreatic cancer cells in vitro and in vivo⁹⁷–⁹⁹, as well as reducing cellular survival and increasing apoptosis in mouse hepatocellular carcinoma cell lines⁹⁸. Tumours in which HO1 was genetically silenced grew slower than did tumours expressing normal levels of the enzyme, and this reduced growth was associated with a reduced microvessel density, consistent with the notion that HO1 (and CO) facilitates intratumour and peritumour angiogenesis⁹⁸,⁹₉. Likewise, when implanted into severe combined immunodeficient (SCID) mice, prostate tumours in which HO1 was silenced using short hairpin RNA
Box 2 | Drug repurposing in the field of gasotransmitters and cancer

A substantial remaining challenge for translational and clinical work is the identification of suitable clinical development candidates. For each gasotransmitter, future clinical trials may be made possible through the revitalization or repurposing of various clinical-stage drugs. Compared with most of the indications previously considered for these compounds, the regulatory guidelines for cancer require a relatively small regulatory ‘package’; thus, it can be hoped that clinical work with such compounds will be feasible in the future. Repurposing is an approach that is therefore often advocated, both for the pharmaceutical industry and for academic clinical translational efforts, and it has been successfully used for the experimental therapy of cancer, as demonstrated by the cases of topoisomerase inhibitors, metformin and others.208–209. In the area of gasotransmitter research, the production of each of the three gasotransmitters may be modulated by compounds that have already been in clinical trials for different indications.

For the inhibition of inducible nitric oxide synthase (iNOS), the use of aminoguanidine is a possibility. Although this compound does not have a high degree of selectivity for iNOS, it has a reasonably good inhibitory potency for this enzyme. Its use in cancer is supported by in vivo data that show that its use led to a marked reduction of tumour growth in mammary adenocarcinoma models.209 Aminoguanidine has previously been used in clinical trials (in the experimental therapy of diabetic complications), both for its iNOS inhibitory actions and for its NOS-independent actions as an inhibitor of the formation of advanced glycation end-products210,211.

For the inhibition of carbon monoxide (CO) production by haem oxygenase 1 (HO1), tin mesoporphyrin (SnMP; a porphyrin-based HO1 inhibitor) has already been in clinical trials for the experimental therapy of infant hyperbilirubinemia and acute porphyrinic crisis and may be available for future trials in cancer.

For the inhibition of hydrogen sulfide ($H_2S$) production, the cystathionine-β-synthase (CBS) inhibitor aminooxyacetic acid (AOAA) has already been tested in humans in the contexts of Huntington disease and tinnitus.212–214. Although the intended target in these trials was not CBS, but the γ-amino butyric acid aminotransferase GABA-AT (a pyridoxal phosphate-dependent enzyme involved in the biosynthesis of GABA in the nervous system), these trials have yielded useful human safety and tolerability information on AOAA.215,216.

(shRNA) grew considerably slower, exhibited a less invasive phenotype and showed a lower degree of metastatic activity than did control tumours.217,218. Studies investigating the effects of HO1 inhibitors on tumour angiogenesis and growth have further confirmed the role of CO overproduction in cancer.219

Treatment of tumour-bearing mice with ZnPPIX reduced tumour growth in several different studies with ovarian, pancreatic and colon carcinoma cell lines,219–221 and OB-24, an imidazole-based inhibitor of HO1, inhibited the growth of prostate tumours implanted in mice.222,223 OB-24 also exerted additive or synergistic effects when administered in combination with taxol therapy,224 possibly indicating that the inhibition of CO biosynthesis may be therapeutically applicable in combination with antimetron chemotherapy.

Despite the evidence that HO1-derived CO has cytoprotective and pro-angiogenic effects, it should be noted that, in a few reports, HO1 silencing increased rather than decreased tumour growth,211,212 implying that the role of HO1 and CO in cancer may be very much dependent on tumour type. As discussed elsewhere,213–215, the biological effects of inhibition or genetic silencing of HO1 cannot be equated to the pharmacological inhibition of biological CO production, as the roles of HO1 go beyond CO production and involve the modulation of cellular levels of bilirubin and haem, with consequent changes in cellular redox status. Moreover, the selectivity of the most commonly used HO1 inhibitor, ZnPPIX, is limited (as with most currently known HO inhibitors); the pharmacological actions of such compounds extend well beyond HO1 inhibition.

Nevertheless, the validity of the approach of therapeutically inhibiting HO1 to reduce the protective actions (cytoprotection, stimulation of proliferation and migration) and the paracrine actions (stimulation of tumour angiogenesis) of CO (FIG. 1b) is supported by preclinical data from several studies. Confirmation of HO1 overexpression in the tumour tissue of a patient before therapy would be expected to increase the likelihood of therapeutic success. The clinical or translational progression of HO1 inhibition for cancer would require a HO1 inhibitor of suitable potency, selectivity and safety for clinical development. Tin mesoporphyrin (SnMP), an HO1 inhibitor with a reasonably good potency (BOX 2), was tested clinically in the experimental therapy of hyperbilirubinemia and acute porphyrinic crisis and may be a potential candidate for clinical repurposing for cancer. Infacare Pharmaceutical Corporation currently holds the intellectual property rights for the compound (under the brand names Stannsoporfin and Stanate).

Another compound, a polyethylene glycol (PEG)-ylated form of ZnPPIX, showed improved pharmacological properties in cancer models compared with the non-PEGylated ZnPPIX molecule;226 further improvements to its structure were later published.227,228. OB-24 has also been shown to exert antitumour effects in vivo against prostate cancer.229 Additional potential avenues may include the discovery and development of novel HO1 inhibitors and approaches focusing on silencing HO1 or suppressing its induction. The structures of several novel HO1 inhibitors have recently been disclosed, including that of azalanstat.230 However, these agents have not yet been evaluated in cancer models. The structures of selected HO1 inhibitors are shown in FIG. 2c,d.

Antitumour effects of carbon monoxide on metabolism. Beyond a certain threshold, high levels of CO (owing to, for instance, CO gas, high concentrations of CO-releasing molecules (CORMs) or overexpression of HO1) can be detrimental to cell viability. At such high concentrations (which can typically be produced by millimolar concentrations of CORMs in vitro), CO reduces mitochondrial activity, triggers the generation of mitochondrial ROS, inhibits cellular protein synthesis and decreases cell viability, proliferation and survival.231–233. Accordingly, in vivo exposure of tumour-bearing mice to inhaled CO (250 parts per million (ppm) for 1 hour per day) suppressed the growth of prostate cancer xenografts, and this effect was associated with increased tumour cell apoptosis and reduced tumour vascularization.234 Similar effects of CO were observed in two models of spontaneously developed tumours (in the transgenic adenocarcinoma mouse prostate cancer model [TRAMP cancer model] and in the lung tumour KRAS mouse model).235 Moreover, inhaled CO (500 ppm, 1 hour per day) attenuated the growth rate and the peritumour angiogenic response of Capan-2 pancreatic cancer cells.
in CD1 athymic mice; the effects of inhaled CO were recapitulated by CORM2 (35 mg per kg per day, through intraperitoneal injection).

The above data raise the notion of using therapeutic CO donation for experimental therapy in cancer. Although CO has a bad reputation with physicians owing to its well-known toxicity profile in the context of CO poisoning, over the past decade experimental therapeutic CO administration for many conditions — from transplant rejection to pulmonary diseases — has been explored in some detail. However, recently, the development of inhaled CO (Covox) by Ikaria has stopped in Phase II, and the CORMs developed by HemoCORM/Alfama have not yet entered clinical testing. The reasons for clinical development hurdles have previously been discussed elsewhere and include regulatory issues and potential concerns related to therapeutic indices, as well as (real or perceived) issues around clinicians’ willingness to use an ‘obviously highly toxic’ molecule therapeutically. Examples of various CORMs (that are currently only used as preclinical experimental tools) are shown in FIG. 2e.

Inhaled CO gas is widely available in the hospital environment — it is used in pulmonary function tests that are based on the measurement of the diffusing capacity of CO and thus it is available for small, investigator-initiated trials, similar to ones previously conducted in Europe and Japan. For instance, CO inhalation at doses that exhibit efficacy in murine models of cancer (250–500 ppm for 1 hour per day) has already been tested in humans and seems to be well tolerated, at least in short-term regimens.

Hydrogen sulfide

H₂S is produced in various mammalian cells and tissues by three principal enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST) (reviewed in REFS 133–137; TABLE 1). CBS and CSE are pyridoxal phosphate (PLP)-dependent enzymes that use l-cysteine and homocysteine as their substrates, whereas the substrate of MST is 3-mercaptopyruvate, which is produced from l-cysteine by cysteine aminotransferase. An additional enzymatic pathway for H₂S production that involves d-amino acid oxidase has recently been identified in the kidney. CBS, CSE and MST are constitutive enzymes and have different expression levels in different tissues and organs, but their levels can also be upregulated or downregulated in various conditions. Importantly, CBS and CSE can be inhibited with small molecules of varying degrees of selectivity; propargylglycine (PAG) is the most commonly used research tool to inhibit CSE, and aminooxyacetic acid (AOAA) and hydroxylamine are research compounds that are most often used to inhibit CBS (FIG. 2f).

H₂S activates many intracellular signalling pathways; it opens K<sub>ATP</sub> channels and indirectly stimulates the guanylyl cyclase–cGMP pathway by inhibiting cGMP phosphodiesterases — actions that promote vasodilation and angiogenesis. In addition, at low concentrations, H₂S stimulates the cytoprotective PI3K–AKT, p38–MAPK and NRF2 pathways. Many of the biological actions of H₂S (including K<sub>ATP</sub> channel opening) occur, at least in part, via sulphydrylation, a post-translational modification of specific protein cysteines. At physiological concentrations, H₂S can also stimulate cellular bioenergetic function by donating electrons to the mitochondrial electron transport chain at complex II and by increasing mitochondrial levels of cyclic AMP. At higher concentrations, H₂S inhibits cytochrome c oxidase, thus disrupting mitochondrial electron transport; it can also exert pro-oxidant and DNA-damaging effects.

Similar to NO and CO, at low concentrations (owing to low production rates, low fluxes or a shorter duration of exposure), H₂S tends to exert cytoprotective, antioxidant-type responses, whereas higher concentrations can lead to mitochondrial inhibition or poisoning and cell death. Importantly, whereas low concentrations of H₂S are generally anti-inflammatory, higher concentrations can stimulate various pro-inflammatory pathways.

Pro-tumour effects of CBS-derived hydrogen sulfide

Colon cancer cells, ovarian cancer cells, prostate cancer cells and breast cancer cells exhibit high expression levels of CBS and produce increased amounts of H₂S compared with adjacent non-cancerous tissue or non-transformed cells (TABLE 2). The functional role of CBS-derived H₂S in the regulation of proliferation, migration and invasion in colon cancer and ovarian cancer has been studied in vitro using a combination of genetic approaches (such as siRNA-mediated stable silencing of CBS) and pharmacological agents (such as AOAA). Downregulation or inhibition of CBS inhibited cell proliferation and, at higher concentrations, AOAA reduced tumour cell metabolism and viability. Mechanistically, downregulation or inhibition of CBS suppresses cellular bioenergetics (both through mitochondrial electron transport and through oxidative phosphorylation and glycolysis), reduces intracellular levels of the antioxidant glutathione (as shown in ovarian cancer models) and triggers apoptotic cascades through modulation of the nuclear factor-κB (NF-κB) and p53 pathways.

Another important consequence of silencing or inhibiting CBS is an increase in cellular ROS levels, which may be secondary to intracellular antioxidant depletion. This mechanism may contribute to the sensitization of tumour cells to macrophage-mediated killing after silencing of tumour CBS, as demonstrated in breast cancer cells in vitro.

Subsequent studies in nude mice transplanted with colon cancer or ovarian cancer xenografts extended the findings into in vivo models. shRNA-mediated stable silencing of CBS expression in tumour cells before their implantation into the mice reduced in vivo tumour growth by approximately 40–50%, led to a marked decrease in the size and number of tumour nodules and inhibited peritumour angiogenesis. These effects were recapitulated by AOAA; indeed, the efficacy of AOAA was superior to that of CBS silencing, probably
reflecting additional, CBS-independent pharmacological actions of this compound. Importantly, inhibition or silencing of tumour CBS also sensitized the cancer cells to concomitant chemotherapy.

The findings above suggest that CBS-derived H₂S creates a supportive environment for the tumour cell (FIG. 1b). Nevertheless, it must be pointed out that, in a glioma model, CBS silencing increased rather than decreased tumour growth, illustrating the different tumour-cell-type dependent roles of H₂S. Notably, because CBS activity affects the cellular levels of cysteine and homocysteine and modulates oxidative status, the biological effects of CBS inhibition or silencing cannot be simply equated to the pharmacological inhibition of H₂S production. Moreover, the pharmacological effects of the most commonly used CBS inhibitor, AOAA, go well beyond CBS inhibition.

The literature on the functional role of CSE and MST in cancer is less developed than that on the role of CBS. Upregulation of CSE has been demonstrated in several tumours — including melanoma, prostate cancer and lung cancer — whereas MST upregulation has been reported in astrocytoma and melanoma. CSE silencing suppressed tumour cell proliferation in vitro and in vivo in a colon cancer model, but CSE inhibition or CSE silencing failed to affect tumour cell proliferation in melanoma. The functional role of changes in the levels of the various H₂S-producing enzymes in most other types of cancer has not yet been explored.

Inhibition of CBS, CSE or both is expected to exert antitumour effects, although therapeutic inhibition of CBS in cancer is expected to induce less ‘collateral damage’ than inhibition of CSE, as CSE is broadly expressed in the cardiovascular system, whereas CBS is restricted to a smaller number of organs (including the liver and the brain). Ideally, patients with tumours that produce high levels of H₂S should be identified as probable responders to CBS-inhibiting treatments. Exhaled H₂S is increased in many cancer patients, and measurement of exhaled H₂S levels (or quantification of the intratumour expression of CBS) could be combined with CBS inhibition in a theranostic approach. The most potent CBS inhibitor identified to date is AOAA, with a half-maximal inhibitory concentration (IC₅₀) of approximately 3–10 μM for human recombinant CBS; however, AOAA also inhibits other transaminases. Preclinical data from tumour-bearing nude mice demonstrate that AOAA prodrugs have better cellular uptake and higher anticancer potency than the parent compound, AOAA.

Future medicinal chemistry efforts could be targeted to identify new CBS inhibitor scaffolds with higher potency and/or selectivity for CBS, to test and develop as drugs. AOAA was in clinical trials several decades ago for non-oncological indications (including Huntington disease and tinnitus) (BOX 2) and as such could potentially be repurposed. As CBS is the main enzyme involved in the biological degradation of homocysteine, chronic CBS inhibition is expected to induce hyperhomocysteinemia. This could be viewed as a systemic side effect of CBS inhibition (which, in the short-to-mid-term, is purportedly reasonably well tolerated), or it could be used as a biomarker of CBS inhibition to confirm therapeutic target engagement.

**Antitumour effects of hydrogen sulfide donors.** Similar to NO and CO, elevation of cellular H₂S levels beyond a certain threshold (typically achieved by millimolar concentrations of H₂S donors) becomes detrimental to cell viability. Accordingly, H₂S donors — either as stand-alone agents or as H₂S-donating functional moieties of pharmacologically active base scaffolds — have been investigated as potential cancer therapies. It is relatively easy to kill cancer cells with high concentrations of H₂S donors in vitro; however, such experiments are of limited value in predicting the in vivo utility of the compound. Therefore, the discussion below focuses on in vivo studies. At low or medium concentrations, GYY4137 — a ‘slow-release’ H₂S donor (TABLE 2) — induces vasodilation, hypotension, cytoprotection and anti-inflammatory effects; however, at higher concentrations, it is antiproliferative and becomes detrimental to the viability of cells through various mechanisms — including mitochondrial inhibition, activation of cell death signalling and intracellular acidification — culminating in the activation of caspase 9 and apoptosis.

In SCID mice, daily administration of up to 300 mg per kg GYY4137 attenuated the growth of subcutaneous tumours. GYY4137 also exerted antitumour efficacy in a subcutaneous hepatic carcinoma xenograft model in mice. GYY4137 is currently the most specific H₂S donor that has confirmed in vivo anticancer effects.

Many studies have also demonstrated the anticancer effect of the naturally occurring H₂S donor compounds diallyl sulfide, diallyl disulfide and diallyl trisulfide in vivo. These compounds generate H₂S in the cellular environment through glutathione-dependent release processes and elevate intracellular and circulating H₂S levels. The pharmacological actions of these compounds extend beyond H₂S donation, making the interpretation of the findings in terms of gasotransmitter biology difficult. Nevertheless, these compounds have shown anticancer effects in vivo in mice bearing glioblastoma, melanoma, gastric cancer, osteosarcoma, pulmonary adenocarcinoma or colon cancer. Additional H₂S donor compounds with in vivo anticancer actions include S-propargyl-cysteine and various H₂S-donating acetylsalicylic acid derivatives. Multifunctional H₂S donors — which contain an H₂S-donating moiety conjugated to a previously known drug (such as a non-steroidal anti-inflammatory drug) — have been reviewed elsewhere.

Despite the large body of preclinical work investigating various naturally occurring polysulfides in cancer, and the fact that these compounds can be considered to be natural supplements (as they are abundant in, for instance, garlic oil), the pharmacology of these compounds is complex, and H₂S donation represents only one of their many modes of action. Future drug discovery efforts could exploit the environment of tumours to produce specific H₂S donors that are selectively bioactivated in tumour tissues.
Summary and future directions

Three decades of preclinical studies in the field of the three gasotransmitters NO, CO and H₂S have identified several pathophysiological paradigms and associated experimental therapeutic approaches that may be ultimately suitable for use in clinical translation. Specifically in cancer, the initially confusing concept in which, looking superficially at the literature, gasotransmitter donors and gasotransmitter synthesis inhibitors seem to have anticancer effects can be explained by the complex biology and bell-shaped pharmacology of NO, CO and H₂S (FIG. 3), and should not be viewed as a barrier for translation.

With several caveats in mind, therapeutic inhibition of gasotransmitter biosynthesis can generally be warranted if the following three conditions are met. First, the tumour should express high levels of gasotransmitter-producing enzymes. Second, the tumour should produce substantial amounts of the gasotransmitter, and use it to defend itself from the host and foster its own growth, proliferation and angiogenesis. Third, the gasotransmitter that is targeted should not constitute a major component of the host’s own antitumour immune defence mechanism. This can be conceptualized by shifting the dose–response curve in FIG. 3 to the left. By contrast — and fairly independently from the expression level of gasotransmitter-producing enzymes in the tumour — donation of cytotoxic levels of gasotransmitter may be warranted in some forms of tumours, akin to shifting the dose–response curve in FIG. 3 to the right. The relatively younger fields of CO donors and H₂S donors may thus receive inspiration from the
field of NO and may consider creating tumour-specific donors that rely on tumour-associated enzymes for tumour-specific bioactivation.

It must be stressed that, with each of the three gaseous transmitters discussed in this article, inhibition or genetic silencing of each of the enzymes that produce the transmitters can have biological effects because the intervention will influence l-arginine metabolism (in the case of NO), haem metabolism (in the case of the HO isozymes) or l-cysteine metabolism (in the case of the H$_2$S-producing enzymes). These effects (or pseudophenomena) must be dissected from the biological effects mediated by NO, CO or H$_2$S through careful control experiments — for example, by pharmacological replacement of the gaseous transmitter after inhibition of the enzyme that produces it or by comparing the results of enzyme inhibitor experiments with studies that test the effects of scavengers of the gaseous transmitters.

Another point to consider is that the selection of the cancer model used (for instance, using an immunocompetent versus an immunosuppressed host into which the tumour is implanted) markedly influences the conclusions of preclinical studies. For instance, in immunocompetent (or immunologically hyperstimulated) hosts, the host macrophage iNOS-derived antitumour component can be considerable, whereas the results of experiments that use immunosuppressed hosts (for example, nude mice) will highlight the biological character of the implanted tumour, often at the expense of recognizing relevant properties of the host organism.

We must remain realistic about how much stand-alone antitumour action can be expected from gasotransmitter-related therapeutic approaches. Inhibiting gasotransmitter production by the tumour cell will create a less nourishing environment for the tumour but may not induce overt cell death (as illustrated by the examples of partial, but not complete, suppression of tumour growth after silencing of tumour iNOS, HO1 or CBS). Likewise, although high local concentrations of NO, CO and H$_2$S can be cytostatic or cytotoxic, the intrinsic toxicity of these molecules is much lower than that of most ‘professional’ chemotherapeutic agents. Therefore, most of the success in gasotransmitter modulation in cancer is expected to be from uses in combination with chemotherapy, radiotherapy or targeted therapies.

Indeed, one area of particular translational promise is radiosensitization through the use of NO donors (Box 3). For each gasotransmitter, the most recent developments (for instance, the tumour-targeted NO donors, intermittent low-dose CO inhalation and slow-acting H$_2$S donors) hold the most substantial scientific novelty and translational promise. Nevertheless, much preclinical work remains to be conducted to further establish dose–response relationships, to identify drugs that might be repurposed (Box 2) and the most likely responder populations, and to develop suitable combinations with other therapies.

One must also bear in mind that the three gasotransmitters do not act in isolation, but rather, in concert, sometimes by using overlapping signalling pathways (for example, both NO and CO stimulate the guanylyl cyclase pathway)\textsuperscript{177}, and other times by enhancing each other’s action (for example, NO directly stimulates the guanylyl cyclase pathway, and H$_2$S concurrently blocks the degradation of cGMP via inhibition of cGMP phosphodiesterases)\textsuperscript{141}. These interactions remain to be studied in the context of cancer and may be exploited in the future for therapeutic benefit. As one such effort, a recent study demonstrated the in vitro and in vivo anticancer effect of a combined NO- and H$_2$S-donating compound, NOSH-aspirin\textsuperscript{178}.

An area in which substantial progress can be expected is the field of multifunctional compounds: clinically used drugs with added NO- or H$_2$S-donating moieties. The pharmacology of some of these compounds is challenging. One reason for this is that the amount of the gaseous transmitter released from them is very small. Another is that the contributions of different parts of the molecule may need defining: for example, in the case of NO-aspirin, the NO serves only as a leaving group and the spacer group has been shown to be responsible for some of the added pharmacological action\textsuperscript{151}. Despite these challenges, further work in this field is expected to produce additional pharmacologically active compounds and potential drug development candidates. Some of the multifunctional NO donors with notable anticancer effects in vitro and in vivo include GIT-27NO, which is an NO-donating version of the isoxazoleacetic acid derivative VGX-1027 (REFS 179, 180), and the NO-donating derivative of the protease inhibitor saquinavir\textsuperscript{181, 182}. Multifunctional CO-donating agents have not yet been characterized. There are, however, multiple examples of multifunctional H$_2$S-donating donors with direct anticancer effects (see above)\textsuperscript{174–176}, as well as an H$_2$S-releasing version of naproxen\textsuperscript{183}.
Table 3 | Advantages and disadvantages of approaches to modulate gasotransmitter levels in cancer

<table>
<thead>
<tr>
<th>Biological target or mechanism</th>
<th>Therapeutic approaches</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NO</strong></td>
<td>Upregulation of the antitumour immune response, by inducing NOS activity (for example, using cytokines or non-specific immunostimulation) to promote NO production, or by supplementing a NOS substrate (for example, L-arginine)</td>
<td>• Exploits an endogenous ‘natural’ mechanism</td>
<td>• Only effective against some forms of tumours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• The antitumour effects of NO work in synergy with other effectors of the immune system</td>
<td>• Overstimulation of the system can be associated with side effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Tumours may develop mechanisms to evade the host immune response</td>
<td>• Tumours may develop mechanisms to evade the host immune response</td>
</tr>
<tr>
<td>NO at high local concentrations can kill tumour cells</td>
<td>NO donation (systemic or tumour-cell-targeted)</td>
<td>• Effects are independent of the host’s immunological response and the iNOS status of the tumour</td>
<td>• Non-tumour-cell targeted approaches can have systemic effects (such as hypotension)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Tumours with the highest degree of vascularization would be the most likely responders to this therapy</td>
<td>• Less likely to be used as a stand-alone approach and more likely to be used to enhance the effect of chemotherapy</td>
</tr>
<tr>
<td>NO overproduction in certain tumour cells (usually owing to iNOS overexpression) exerts cytoprotective, pro-proliferative and pro-angiogenic effects</td>
<td>Selective inhibition of iNOS, via small-molecule iNOS inhibitors</td>
<td>• Semi-selective to tumour cells</td>
<td>• iNOS inhibition is only expected to be effective against tumour types that express iNOS and use NO to create a nourishing local environment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Systemic side effects are expected to be relatively mild (for instance, there would be no haemodynamic side effects)</td>
<td>• Systemic side effects may include immunosuppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Can be used in combination with chemotheraphy</td>
<td>• Systemic side effects of non-isoform-selective or eNOS-selective NOS inhibition include: vasoconstriction and hypertension; loss of the vascular protective effects of NO (including increased platelet and mononuclear cell adhesion and activation); and perhaps enhancement of cardiovascular adverse events</td>
</tr>
<tr>
<td>NO produced by eNOS in the host vasculature contributes to the maintenance of tumour blood supply and stimulates tumour angiogenesis</td>
<td>Inhibition of eNOS or scavenging of NO in the host organism</td>
<td>• No need for positive identification of the NOS status of the tumour</td>
<td>• Systemic side effects of NO donation therapy may be narrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Tumours with the highest degree of vascularization would be the most likely responders to this therapy</td>
<td>• Systemic side effects are possible</td>
</tr>
<tr>
<td>CO overproduction within the tumour (usually owing to HO1 overexpression in the tumour cell and/or the tumour-infiltrating immune cells) exerts cytoprotective, pro-proliferative and pro-angiogenic effects</td>
<td>Selective inhibition of HO1 using small-molecule HO inhibitors</td>
<td>• Semi-selective to tumour cells</td>
<td>• HO inhibition is only expected to be effective against those tumour types that express HO1 and use CO to create a nourishing local environment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Systemic side effects are expected to be relatively mild</td>
<td>• Systemic toxicity needs to be monitored (for example, by measuring carboxyhaemoglobin levels in the blood)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Can be used in combination with chemotherapy</td>
<td>• CO may be perceived as toxic by physicians</td>
</tr>
<tr>
<td>High tumour levels of CO impair tumour cell metabolism and exert antitumour effects</td>
<td>Donation of CO (via CO gas inhalation or parenteral CORM administration)</td>
<td>• No need for positive identification of the CO status of the tumour</td>
<td>• The therapeutic index of CO inhalation or CO donation therapy may be narrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Many tumours expected to respond</td>
<td>• Systemic toxicity needs to be monitored (for example, by measuring carboxyhaemoglobin levels in the blood)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• CO is highly diffusible and so can easily enter the tumour tissue</td>
<td>• CO may be perceived as toxic by physicians</td>
</tr>
<tr>
<td>H₂S</td>
<td>Selective inhibition of CBS using small-molecule inhibitors</td>
<td>• Semi-selective to tumour cells</td>
<td>• Systemic side effects of CBS inhibition may include elevation of circulating homocysteine levels, a known cardiovascular risk factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Systemic side effects are expected to be relatively mild</td>
<td>• Therapeutic index may be narrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Can be used in combination with chemotherapy</td>
<td>• H₂S may be perceived as toxic by physicians</td>
</tr>
<tr>
<td>H₂S overproduction in the tumour cells (usually owing to CBS overexpression) stimulates tumour cell bioenergetics, activates growth and proliferation and exerts cytoprotective and pro-angiogenic effects</td>
<td>Selective inhibition of CBS using small-molecule inhibitors</td>
<td>• Semi-selective to tumour cells</td>
<td>• Systemic side effects of CBS inhibition may include elevation of circulating homocysteine levels, a known cardiovascular risk factor</td>
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<td></td>
<td></td>
<td>• Can be used in combination with chemotherapy</td>
<td>• H₂S may be perceived as toxic by physicians</td>
</tr>
<tr>
<td>H₂S donation impairs tumour cell metabolism and exerts antitumour effects</td>
<td>Treatment with H₂S donor compounds</td>
<td>• H₂S is highly diffusible and so can easily enter the tumour tissue</td>
<td>• Therapeutic index may be narrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• CBS inhibition may include elevation of circulating homocysteine levels, a known cardiovascular risk factor</td>
<td>• H₂S may be perceived as toxic by physicians</td>
</tr>
</tbody>
</table>

CBS, cystathionine-β-synthase; CO, carbon monoxide; CORM, CO-releasing molecule; eNOS, endothelial NOS; H₂S, hydrogen sulfide; HO, haem oxygenase; iNOS, inducible NOS; nNOS, neuronal NOS; NO, nitric oxide; NOS, NO synthase.
21 As NO, CO and H₂S can leave the tumour (sometimes to form new compounds) and can even be measured in the exhaled breath, future gasotransmitter inhibition therapy may be combined with measurement of the levels of these mediators. Indeed, there are several reports of increased circulating and exhaled NO levels in patients with cancer[14,18], increased carboxyhaemoglobin levels in colon cancers [19] and increased exhaled H₂S levels in patients with cancer (reviewed in REF. 148).

There are several further reasons to continue research into the cancer biology of each of the three gasotransmitters covered here. Some of the finer-detailed changes in intracellular localization of the various gasotransmitter-producing enzymes in tumours — such as the nuclear translocation of HO1 in certain forms of cancer [20] or the mitochondrial translocation of CBS in others [21,22] — may further modify the complex role of each gasotransmitter in tumour biology. Moreover, emerging in vitro evidence suggests that the upregulation of gasotransmitter production in tumours can also be a reactive phenomenon that occurs in response to chemotherapy or radiotherapy, conferring tumour cell resistance and/or dedifferentiation [23]. Further, there is increasing evidence for the involvement of the three gasotransmitters in the expansion and proliferation of tumour stem cells [24,25], thereby providing potential additional future therapeutic targets. There is also much to be considered about the potential role of these gasotransmitters in carcinogenesis or tumorigenesis (which may occur in part owing to the pro-inflammatory effects of high concentrations of each of them) and in chemoprevention; these stand-alone fields are the subjects of dedicated review articles [26,27].

Taken together, gasotransmitter biology offers an opportunity for a diverse set of therapeutic approaches (TABLE 3). Each of these approaches has advantages and potential disadvantages, and should be tailored to the biological character of the tumour to be targeted. Hopefully, this Review will help to define distinct pathophysiological and therapeutic paradigms that characterize the roles of these three gaseous transmitters in cancer, and may stimulate the formulation of novel therapeutic concepts and the revitalization of drug discovery efforts and drug development programmes in this area.
The role of tumour-derived iNOS in tumour progression and angiogenesis.


This study defines the role of NO as a pro-angiogenic mediator.


This study shows that NOS inhibition suppresses tumour blood flow in patients with cancer.


This study provides evidence that modulation of NO homeostasis can affect tumor radiation response.


This study shows that silencing of HO1 suppresses tumour growth in vivo.


Li, Y. et al. PTEF inhibition and heme oxygenase-1 overexpression cooperatively promote cancer progression and are associated with adverse clinical outcome. J. Pathol. 224, 90–110 (2011).


This study shows that inhibition of HO1 suppresses tumour growth in vivo.
Quoth the Raven: carbon monoxide byproduct carbon monoxide in term newborns with direct Coombs-positive ABO incompatibility.

This study introduces the concept of CO-releasing molecules (ET-CORMs) reveals quantitative differences in biological activities in terms of toxicity and inflammation. This paper shows evidence for cooperative signalling between NO and H2S in the control of angiogenesis and vascular relaxation.

This study shows that silencing CBS or inhibiting CBS suppresses colon cancer bioenergetics and growth in vivo.

This study shows that silencing CBS or inhibiting CBS suppresses ovarian cancer bioenergetics and growth in vivo.


186. This paper presents an approach of measuring patient-exhaled levels of NO for the detection of lung cancer.
201. This study shows that inhibition of NOS production by the non-isof orm-selective NOS inhibitor -NMA reduces the hypotensive side effects of IL-2 during the immunotherapy of patients with cancer.

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Competing interests statement
The author declares competing interests: see Web version for details.