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Opportunities for Nitric Oxide in Potentiating Cancer Immunotherapy

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Abstract——**Despite nearly 30 years of development and recent highlights of nitric oxide (NO) donors and NO delivery systems in anticancer therapy, the limited understanding of exogenous NO's effects on the immune system has prevented their advancement into clinical use. In particular, the effects of exogenously delivered NO differing from that of endogenous NO has obscured how the potential and functions of NO in anticancer therapy may be estimated and exploited despite the accumulating evidence of NO's cancer therapy–potentiating effects on**

the immune system. After introducing their fundamentals and characteristics, this review discusses the current mechanistic understanding of NO donors and delivery systems in modulating the immunogenicity of cancer cells as well as the differentiation and functions of innate and adaptive immune cells. Lastly, the potential for the complex modulatory effects of NO with the immune system to be leveraged for therapeutic applications is discussed in the context of recent advancements in the implementation of NO delivery systems for anticancer immunotherapy applications.

Significance statement——Despite a 30-year history and recent highlights of nitric oxide (NO) donors and delivery systems as anticancer therapeutics, their clinical translation has been limited. Increasing evidence of the complex interactions between NO and the immune system has revealed both the potential and hurdles in their clinical translation. This review summarizes the effects of exogenous NO on cancer and immune cells in vitro and elaborates these effects in the context of recent reports exploiting NO delivery systems in vivo in cancer therapy applications.

I. Introduction

Nitric oxide (NO) is an endogenous gaseous molecule that plays a myriad of biologic and pathophysiological functions involved in cardiovascular homeostasis, neurotransmission, angiogenesis, immune response, and apoptosis (Fukumura et al., 2006; Carpenter and Schoenfisch, 2012). Inspired by its in vivo functions (Fukumura et al., 2006; Carpenter and Schoenfisch, 2012) as well as its biocompatibility (Fukumura et al., 2006; Kim et al., 2014) originating from its role as an endogenous signaling molecule and rapid degradation into nontoxic ions after reaction, there has been continuous effort toward the development of anticancer drugs that leverage NO donors (Hrabie and Keefer, 2002; Wang et al., 2002) that are defined as "NO releasing small molecules or functional groups" (Kim et al., 2014). The most widely explored paradigm of NO donors in anticancer therapy seeks to exert NO's cytotoxic effects on cancer cells by achieving burst intracellular release of NO. For example, O^2-(2,4-dinitrophenyl) 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate) (JS-K) has been intensively explored for this purpose (Ren et al., 2003; Shami et al., 2003; Shami et al., 2006; Udupi et al., 2006; Kiziltepe et al., 2007; Chakrapani, Goodblatt et al., 2008; Chakrapani, Kalathur et al., 2008; Simeone et al., 2008; Kitagaki et al., 2009; Liu et al., 2017; Liu, Huang et al., 2018; Zhao et al., 2019) because it allows the burst release of NO in response to the high intracellular redox environment of cancer cells. In addition, several NO delivery systems have been developed with the goal of delivering a high concentration of NO donors to the tumor microenvironment to elicit NO mediated cytotoxicity as an anticancer therapeutic approach (Kumar et al., 2010; Park, Im et al., 2019; Kim, Suh et al., 2022). Although various NO donors and delivery systems have demonstrated anticancer effects in vitro as well as in several in vivo xenograft tumor models (Ren et al., 2003; Shami et al., 2003; Shami et al., 2006; Udupi et al., 2006; Kiziltepe et al., 2007; Chakrapani, Goodblatt et al., 2008; Chakrapani, Kalathur et al., 2008; Simeone et al., 2008; Kitagaki et al., 2009; Kumar et al., 2010; Stevens et al., 2010; Xu et al., 2015; Fan et al., 2017; Liu et al., 2017; Liu, Huang et al., 2018; Zhao et al., 2019), their clinical translation is currently limited due to observations that at concentrations lower than the threshold that elicits cytotoxicity, NO can also accelerate tumor progression (Mocellin et al., 2007; Kim et al., 2014; Kim et al., 2017). In addition to its cytotoxic functions, NO also has drug sensitizing effects (Mocellin et al., 2007; Fan et al., 2015; Kim et al., 2017; Deng et al., 2018; Hu et al., 2018; Pramanick et al., 2018; Wang et al., 2019; Ding et al., 2019; Feng et al., 2019; Wang et al., 2019; Zhang et al., 2018; Gao et al., 2020), which have accelerated the rapid progress of NO donors or delivery systems for use in combination with other therapeutic modalities including chemotherapy, radiation therapy, photothermal therapy, and photodynamic therapy (Mocellin et al., 2007; Fan et al., 2015; Kim et al., 2017; Deng et al., 2018; Hu et al., 2018; Pramanick et al., 2018; Wang et al., 2019; Ding et al., 2019; Feng et al., 2019; Wang et al., 2019; Zhang et al., 2018; Gao et al., 2020). For example, NO donor RRx-001 [*N*-(bromoacetyl)-3,3-dinitroazetidine] was evaluated for

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its potential as a radiosensitizer in clinical trials (Kim et al., 2016; Oronsky, Scicinski, Cabrales et al., 2016; Oronsky, Scicinski, Ning et al., 2016). However, sensitizing effects as well as cytotoxic functions of NO donors and delivery systems have been investigated almost entirely at an in vitro level or in immune-deficient mouse models. As a result, NO's effects on the immune system have been underexplored.

A substantial body of literature has reported endogenous NO's pivotal role in various immunologic mechanisms. However, the effects of exogenously delivered NO on tumor progression have not summarily been presented. In this review, major NO donor and delivery system classes are described. A synthesis of the existing literature landscape of NO's known direct effects on cancer cells and immune cells, including dendritic cells (DCs), macrophages, B cells, CD8$^+$ T cells, CD4$^+$ T cells, regulatory T cells (T$_{reg}$s), natural killer (NK) cells, and myeloid-derived suppressor cells (MDSCs), based on in vitro studies is then presented. Finally, contemporary studies developing NO delivery systems are summarized, revealing the complex immune networks regulated by NO and its capacity to potentiate anticancer immunotherapy.

II. NO-donors

Numerous NO donors have been developed for medicinal purposes (Hrabie and Keefer, 2002; Wang et al., 2002). The characteristics of such NO donors, which vary in their chemistry and mechanism of NO donation, are herein presented.

A. Nitrates/Nitrites

The oldest class of NO donors is comprised of organic nitrates and nitrites that have a generic structure RONO$_2$ and RONO, respectively. Representative examples include glyceryl trinitrate and amyl nitrite, which have been pharmacologically used to treat angina for more than a century. Organic nitrates/nitrites can be metabolized by reacting with cellular cysteine and glutathione (GSH) (Needleman and Krantz, 1965; Needleman et al., 1971; Ignarro and Gruetter, 1980; Horowitz et al., 1983; Wang et al., 2002; Thatcher et al., 2004). Although decomposition of organic nitrates/nitrites to release NO was reported to be associated with the enzymatic reaction with cytochrome P450 and/or glutathione S-transferase (GST) (Schroder, 1992; Seth and Fung, 1993; McDonald and Bennett, 1993; Meyer et al., 1994; Kenkare et al., 1994; Wang et al., 2002; Thatcher et al., 2004), it is currently believed to be associated with mitochondrial aldehyde dehydrogenase-2, independent of cytochrome P450 (Wenzel et al., 2007; Mayer and Beretta, 2008; Daiber and Munzel, 2015; Neubauer et al., 2015; Lopez et al., 2021). Organic nitrates and nitrites are synthesized by reacting alcohols with nitric acid and nitrous acid, respectively (Wang et al., 2002;

Omar et al., 2012). Because of their easy and simple chemistry, nitrates/nitrites have been used to develop hybrid prodrugs to release NO and nonsteroidal anti-inflammatory drugs (NSAID) (Thatcher et al., 2004; Dunlap et al., 2008), as well as incorporated into NO delivery systems.

B. Amidoximes/Oximes

Amidoximes and oximes, which have the generic structure R$_1$C($=$ N-OH)R$_2$, mimic the N-hydroxy-$_L$-arginine, which is a kind of amidoxime produced as an intermediate of enzymatic reactions between endogenous NO synthases (NOS) and $_L$-arginine substrates (Blatt, 1938; Tavakol and Arshadi, 2009; Belmar et al., 2013; Bohle et al., 2013; Novikov and Bolotin, 2017; Sahyoun et al., 2019).

Amidoximes are synthesized by reacting nucleophile hydroxylamine with nitriles, thioamide, amidine hydrochloride, hydrazide imide, iminoether, or imidoylbenzotriazole (Tiemann, 1884; Warburton, 1966; Bjoklund and Coburn 1980; Katritzky et al., 2006; Ranjbar-Karimi et al., 2018; Sahyoun et al., 2018; Sahyoun et al., 2019), by reacting oximinoether and hydroximic acid chlorides with ammonia (Lossen W, 1889; Sahyoun et al., 2019), or by reducing nitrosolic acids with hydrogen sulfide (Wieland and Bauer, 1906; Sahyoun et al., 2019). Oximes on the other hand are prepared via condensation of aldehydes or ketones with hydroxylamine (Sahyoun et al., 2019) or by introducing hydroxyimino group into the diene moiety using nitrite under acidic conditions (Wang et al., 2002). Amidoximes and oximes can be decomposed to release NO via an oxidation process with singlet oxygens (Öcal and Erden, 2001) and enzymatic reaction with cytochrome P450 enzymes, the reduced form of NADP, and O$_2$ (Andronik-Lion et al., 1992; Boucher et al., 1992; Mantyla et al., 2004). However, NOS does not contribute to the oxidation process of amidoximes and oximes except the $_L$-arginine and N-hydroxy-$_L$-arginine (Moali et al., 2000; Caro et al., 2001). Oximes that have been explored in biomedical applications include NOR-1, NOR-3, and NOR-4.

C. S-nitrosothiols

S-nitrosothiols are classified as analogs of O-nitroso compounds or organic nitrites, which have the generic structure R-S-N $=$ O (RSNO), an electronegative oxygen and sulfur, the latter of which is slightly more electrically negative than nitrogen (Hogg, 2002). Nitrosation makes α-proton and α-carbon downfield shifted in ^{1}H and ^{13}C nuclear magnetic resonance spectra. S-nitrosothiols also exhibit characteristic UV-visible absorbance in 255 to 261 nm ($\pi \rightarrow \pi^*$ transition), 330 to 350 nm (n$_0 \rightarrow \pi^*$ transition), and 550 to 600 nm (n$_N \rightarrow \pi^*$ transition). The third band determines the color of S-nitrosothiol compounds from red to green (Wang et al., 2002; Zhang et al., 2017). In infrared spectra, N-O vibration and N-S vibration

appear in 1430 to 1710 cm^{-1} and 610 to 685 cm^{-1} or 1000 to 1170 cm^{-1}, respectively (Zhang et al., 2017).

Formation and metabolism of S-nitrosothiol regulate numerous physiologic functions. As examples, serum albumin is one of the major forms of S-nitrosothiol in blood plasma, and erythrocytes also can contain a S-nitrosothiol modified hemoglobin. These S-nitrosothiols in blood not only maintain vascular tone homeostasis and exert important vasodilation functions with the help of GSH to be reversely converted to S-nitrosoglutathione (GSNO) (Hogg, 2002), but also transfer S-nitrosothiol functional groups to the cells via cystine-mediated trans-nitrosation (Zhang and Hogg, 2004). On the other hand, GSNO is one of the major intracellular NO donors because GSH is highly abundant in cells, and a thiol in GSH with a simple structure is thermodynamically easier to be reacted with nitronium cation (NO$^+$) produced by nitrosative biologic reactions than thiols in proteins with larger molecular weight and tertiary structures (Massa et al., 2021). Nevertheless, close interactions of NO$^+$, GSNO, and other NO donors with proteins allow S-nitrosylation of proteins (Massa et al., 2021). This post-translational modification plays an important role in signal transduction with the help of dinitrosyl iron complexes–mediated transnitrosation, which modulates the activity and functions of proteins (Konorev et al., 2000; Hogg, 2002; Bosworth et al., 2009; Kevil and Patel, 2010).

S-nitrosothiols can be synthesized via nitrosation by reacting thiol compounds with N-oxides (NOCl, N$_2$O$_4$, N$_2$O$_3$, NO$_2$, HNO$_2$) (Zhang et al., 2017). For example, GSNO can be synthesized by reacting GSH with sodium nitrite (NaNO$_2$) in acidic aqueous conditions (Hart 1985). This aqueous nitrosation method has been the most widely used in synthesizing RSNO and S-nitrosothiol conjugated NO delivery systems, which is beneficial in preventing the unwanted N-nitrosation but is limited for use with material systems that undergo hydrolysis in acidic conditions (Wang et al., 2002; Damodaran et al., 2012; Kim et al., 2014; Zhang et al., 2017). On the other hand, the nonaqueous nitrosation method utilizing tert-butyl nitrite in organic solvents not only can prevent the hydrolysis of synthetic substrates but also restrain spontaneous decomposition of SNO groups during synthesis and purification in aqueous conditions.

The decomposition of SNO groups results in the release of NO, which is dependent on temperature, light, heavy metal ions, and redox conditions. GSH, ascorbic acid, and heavy metal ions including Cu$^+$, Fe^{2+}, Hg^{2+}, and Ag$^+$ induce catalytic release of NO by reducing the RSNO (Wang et al., 2002). Indeed, intracellular concentration of GSH or ascorbic acid leads to a burst NO release by acting as a reducing agent or nucleophile (Wang et al., 2002; Kim et al., 2020). Initial rates of decomposition of RSNO are dependent on its initial concentration because RSH and

RS$^{.}$ produced during decomposition of RSNO contribute to the autocatalytic reaction (de Oliveira et al., 2002). Likewise, Cu$^+$ ions act as a reducing agent to accelerate the decomposition of RSNO (Stamler and Toone, 2002), however, whether nitrosothiols on proteins and peptides are susceptible to copper-catalyzed decomposition remains unclear since free copper is only very lowly abundant in most tissues (Stamler and Toone, 2002). Accordingly, copper-containing biomaterials have been developed to accelerate NO release from endogenous GSNO (Hwang and Meyerhoff, 2008; Zhou et al., 2021). It should be noted that RSNO is not stable at room temperature, as it undergoes thermal decomposition with homolytic cleavage of S-N bond with 20 to 30 kcal mol^{-1} of homolytic bond dissociation energy to release NO$^{.}$ (Bartberger et al., 2001; Wang et al., 2002). In addition, RSNO exhibits photolytic release of NO under UV light due to the absorption band as previously described (Wang et al., 2002; Marazzi et al., 2012). Although their low thermal- and photostability remain to be addressed, S-nitrosothiols have been widely employed in (pre)clinical applications and mechanistic studies due to the ease of this chemical modification on any type of thiol-containing substance (Kim et al., 2014). In addition to NO delivery systems, S-nitrosothiol prodrugs based on α-S-nitroso-α-phenyl acetic acid have been developed to improve its stability for practical therapeutic applications (Lu et al., 2007).

D. Metal Nitrosyl Complexes

Metal nitrosyl complexes have a generic coordination structure of M-NO where NO binds as a ligand to metals (M) including Fe, Ru, Co, Ni, Cu, Cr, MO, etc. (Wang et al., 2002; Wright and Hayton, 2012). An example of metal nitrosyl complexes is sodium nitroprusside (SNP; Na$_2$[Fe(CN)$_5$NO]) which has been used clinically for the treatment of angina for decades. SNP spontaneously releases NO, which can be accelerated under light irradiation and by reductive conditions as well as changes in oxygen levels, pH, and temperature (Wang et al., 2002; Rose and Mascharak, 2008; Fry and Mascharak, 2011; Xiang et al., 2017). However, reduction of SNP results in the release of cyanide, which is cytotoxic (Butler and Megson, 2002; Wang et al., 2002; Rose and Mascharak, 2008; Fry and Mascharak, 2011; Xiang et al., 2017). Other metal nitrosyl examples include Roussin's black salt (RBS; [Fe$_4$S$_3$(NO)$_7$]$^-$) and Roussin's red salt (RRS; [Fe$_2$S$_2$(NO)$_4$]$^{2-}$), a class of iron-sulfur cluster nitrosyls mimicking iron-sulfur clusters in endogenous enzymes (Wang et al., 2002). RBS and RRS spontaneously release NO, which can be accelerated by light exposure, free thiols, and high temperature (Bourassa et al., 1997; Butler and Megson, 2002; Wang et al., 2002; Rose and Mascharak, 2008). Another example is dinitrosyl iron complexes (DNICs) that contain two NO ligands [Fe(NO)$_2$L$_2$]$^{x+}$ where L is a ligand that can be -SR, -NR$_2$, or -OR

(Butler and Megson, 2002; Wang et al., 2002). DNICs also spontaneously release NO, which can be increased by light exposure, free thiols, acids, and elevated temperature (Wang et al., 2002).

Metal nitrosyl complexes have been actively explored in the development of photoresponsive NO delivery systems owing to their distinctive photolytic NO release behaviors via $d_\pi(M) \rightarrow \pi^*(NO)$ transitions (Mascharak, 2012; Ford, 2013; Kim et al., 2014). However, most metal nitrosyl complexes exhibit photolytic NO release in response to the UV and/or visible light that are toxic and/or not appropriate to penetrate to tissues (Mascharak, 2012; Ford, 2013; Xiang et al., 2017). Accordingly, there has been demand for NO donors that are responsive to near-infrared (NIR) light that is relatively biosafe and can penetrate tissues at greater depths relevant to (pre)clinical applications. The wavelength of light required to induce photolytic NO release from metal nitrosyl complexes is dependent on the coordinated ligands and transition metal of the donor (Rose and Mascharak, 2008; Fry and Mascharak, 2011; Xiang et al., 2017). Therefore, several metal nitrosyl complexes have been developed to enable metal nitrosyl complexes to release NO in responsive to the longer wavelength of light by using various polydentate ligands and transition metals to tightly bind NO and ligands (Wecksler et al., 2004; Eroy-Reveles et al., 2008; Rose and Mascharak, 2008; Akl et al., 2016; Xiang et al., 2017). In addition, these novel metal nitrosyl complexes show improved stability of metal nitrosyl complexes during storage and the prevention of ancillary ligand loss to induce nonspecific cytotoxicity during their decomposition (Rose et al., 2008; Kim et al., 2014; Akl et al., 2016; Xiang et al., 2017).

E. Sydnonimines

Sydnonimines are meso-ionic heterocyclic compounds, that include molsidomine and 3-morphorlinosydnonimine (SIN-1). In particular, molsidomine that is used as an oral anti-anginal drug is a prodrug that undergoes deacetylation and ring cleavage to release SIN-1 via enzymatic and nonenzymatic reaction (Wang et al., 2002; Granik, 2010). SIN-1 spontaneously decomposes to release NO to exert its biologic effects by consuming oxygen. In addition, light irradiation can improve the release of NO from SIN-1 in an oxygen-dependent manner (Ullrich et al., 1997). Because of the simultaneous generation of superoxide anion ($O_2^{\cdot-}$) capable of reacting NO during decomposition of SIN-1, sydnonimines are also peroxynitrite ($ONOO^-$) donors. Inspired by therapeutic activity of SIN-1 and clinical use of molsidomine, several SIN-1 prodrugs have been developed by conjugating 5-imines of SIN-1 with substrates responsive to enzymatic degradation or reductive conditions (Cai et al., 2005; Kim, Suh et al., 2022). These SIN-1 prodrugs show high storage stability and exhibit a stimuli-responsive NO release behaviors, which are beneficial characteristics for clinical applications.

F. Diazeniumdiolates

Diazeniumdiolates, the abbreviated name of diazen-1-ium-1,2-diolates and frequently referred to as NONOates, have a structure of X-N(O) = N-O$^-$ with a zwitterionic electropositive diazen group (N = N) and two electronegative oxygen atoms (Hrabie and Keefer, 2002; Kim et al., 2014). A diazeniumdiolate conjugated to the carbon (X = C) is called as C-diazeniumdiolate, whereas its conjugation to nitrogen (X = N) is categorized as N-diazeniumdiolate. N-diazeniumdiolate is a synthetic compound not found in nature, while C-diazeniumdiolate is (Hrabie and Keefer, 2002). Acidified nitrite similar with the aqueous nitrosation method for S-nitrosothiols is generally employed to synthesize C-diazeniumdiolate on primary amine or oximes. However, NO (g) under high pressure and strong basic conditions can produce C-diazeniumdiolate as well as N-diazeniumdiolate. O^1- or O^2-protected diazeniumdiolates can also be developed when the first or second position of oxygen is protected via alkylation, respectively. While O^1-protected diazeniumdiolates are only found in C-diazeniumdiolates, O^2-protected diazeniumdiolates are available in both C- and N-diazeniumdiolate. Despite N-diazeniumdiolate being less stable than C-diazeniumdiolate, only N-diazeniumdiolates have been widely used in the development of NO donors and delivery systems because most C-diazeniumdiolates do not release NO but instead produce nitrous oxide. In addition, N-diazeniumdiolates on primary amines are much less stable than those on secondary amines (Drago and Karstetter, 1961; Hrabie and Keefer, 2002). Accordingly, herein only the N-diazeniumdiolates formed on secondary amines are discussed in detail.

N-diazeniumdiolates are generally synthesized by reacting secondary amine-containing materials with NO (g) under high-pressure (5 − 10 atm) and strong basic conditions using sodium methoxide (NaOCH$_3$) (Hrabie and Keefer 2002; Besson et al., 2009; Nguyen et al., 2010; Kim et al., 2011; Hong et al., 2013). Electron-withdrawing N-diazeniumdiolates make α-proton and α-carbon downfield shifted in ^{1}H and ^{13}C nuclear magnetic resonance spectra (Kim et al., 2011), and N_{1s} and C_{1s} peaks in the X-ray photoelectron spectroscopy (Hong et al., 2013). N-diazeniumdiolates also exhibit a characteristic UV-visible absorbance in 220 to 250 nm (Hrabie and Keefer 2002; Besson et al., 2009; Kim et al., 2011). In the infrared spectra, N-N, N-O, and N = N stretches and NO vibrations appear in 1000 to 1070 cm^{-1}, 1480 to 1540 cm^{-1}, 1390 to 1410 cm^{-1}, 1620 to 1640 cm^{-1}, and 1735 cm^{-1}, respectively (Keefer et al., 2001; Hrabie and Keefer 2002; Besson et al., 2009; Nguyen et al., 2010; Hong et al., 2013). The decomposition of N-diazeniumdiolates is initiated by protonation of secondary amines bearing a diazeniumdiolates group, which follows acid catalyzed first-order kinetics (Davies et al., 2001; Hrabie and Keefer 2002). The NO release kinetics of N-diazeniumdiolates

are significantly affected by the compound's molecular structure and functional groups that influence the hydrogen bonding and hydrophobicity with the diazeniumdiolates (Zhang et al., 2003; Horstmann et al., 2002; Kim et al., 2011; Lu et al., 2011).

The low stability of bare N-diazeniumdiolates during storage and in vivo limits their practical application. In addition to the NO delivery systems that will be discussed in the next section, various O^2-substituted N-diazeniumdiolates have been developed to address this issue. O^2 positions of N-diazeniumdiolates can be protected with the alkyl or aryl groups by using alkyl or aryl halides, alkyl sulfates, and epoxides (Saavedra et al., 1992; Makings and Tsien, 1994; Saavedra et al., 2001; Hrabie and Keefer 2002). The O^2-substituted N-diazeniumdiolates are not only stable under physiologic conditions but also exibit stimuli-responsive NO release behavior if the protecting groups are substrates for specific stimuli including enzymes, redox conditions, etc. Examples include β-lactamase-responsive cephalosporin-3′-diazeniumdiolates (Yepuri et al., 2013), glycosidase-responsive glycosylated diazeniumdiolates (Wu et al., 2001; Valdez et al., 2008), esterase-responsive O^2-acetoxymethylated diazeniumdiolates (Saavedra et al., 2000), and NSAID (Velázquez et al., 2007); UV-responsive O^2-benzyl derivatives-substituted diazeniumdiolates (Bushan et al., 2002; Ruane et al., 2002; Pavlos et al., 2004); and GST- and GSH-responsive O^2-2,4-dinitrophenyl diazeniumdiolates (Saavedra et al., 2001; Chakrapani, Kalathur et al., 2008). Representative O^2-substituted N-diazeniumdiolates include JS-K with O^2-2,4-dinitrophenyl substitution on the diazeniumdiolate group, which has been widely explored as an anticancer agent (Ren et al., 2003; Shami et al., 2003; Shami et al., 2006; Udupi et al., 2006; Kiziltepe et al., 2007; Chakrapani, Goodbatt et al., 2008; Chakrapani, Kalathur et al., 2008; Simeone et al., 2008; Kitagaki et al., 2009; Liu et al., 2017; Liu, Huang et al., 2018; Zhao et al., 2019).

III. Design of NO Delivery Systems

NO delivery systems aim to deliver NO to tissues and cells of interest at bioactive doses in a controlled release manner, which can be accomplished by incorporating NO donors into drug delivery systems (DDSs). NO donors and DDSs have their own intrinsic physicochemical properties, so their combinations facilitate the development of various and diverse NO delivery systems (Riccio and Schoenfisch, 2012; Kim et al., 2014; Kim et al., 2017; Kim et al., 2017; Yang, Zelikin et al., 2018; Wu et al., 2021; Yang et al., 2021). Fundamentally, NO delivery systems are designed not only to prevent the unintended decomposition of NO donors during storage and in vivo outside of the intended tissue or cell target by protecting the direct exposure of NO donors to environments that can trigger NO release, which include aqueous environments, low pH, oxygen, enzymes, and/or light but also to exhibit stimuli-responsive NO-releasing behaviors at the target sites. Therefore, NO release kinetics of NO donors vary with NO delivery system, which differ widely from those of bare NO donors. Therefore, instead of introducing all examples of specific NO delivery systems, the fundamentals in designing NO delivery systems based on the general physicochemical properties of NO donor and DDS combinations is the focus of the ensuing discussion. It is important note that strategies that indirectly increase endogenous NO without utilizing NO donors will not be covered in this discussion, such as NOS gene delivery (Chen et al., 2002; Cooney et al., 2007; Sharif et al., 2012), arginine substrate delivery (Kudo and Nagasaki, 2015; Cao et al., 2016; Fan et al., 2017; Jiang et al., 2018; Wan et al., 2018; Wang et al., 2019; Ma et al., 2020; Tao et al., 2022), copper catalysis delivery (Hwang and Meyerhoff, 2008; Zhou et al., 2021), approaches that interfere with endogenous NO production mechanisms (Sharma et al., 2013; Guo et al., 2015; Zhang, Lai et al., 2019; Costa et al., 2021), and so on. In addition to NO donors discussed in the previous section, NO delivery systems covered in this section will provide a backdrop for the subsequent discussion of their effects on immune system elaborated in subsequent sections.

A. Physical Loading of NO Donors

Physical loading of NO donors to DDSs can be achieved via simple mixing, which however requires the consideration of physicochemical properties of DDSs and NO donors, such as their hydrophobicity/hydrophilicity and electric charge.

Micelles, liposomes, polymersomes, mesoporous silica nanoparticles (MSN), and polymeric nanoprecipitations are widely used DDSs for physical loading of various drugs, which is generally achieved by hydrophobic or hydrophilic interactions between the DDS matrix and the agent to be incorporated. Accordingly, the hydrophobicity/hydrophilicity of NO donors should be first considered when selecting DDSs for physical loading of NO donors in the design of NO delivery systems. Hydrophobic NO donors include nitrate/nitrite–NSAID, NOR-3, SIN-1 prodrug, and O^2-substituted diazeniumdiolates. However, other hydrophilic NO donors can be chemically modified to exhibit hydrophobicity as needed (Huang X et al., 2019). Micelles comprised of amphiphilic block copolymers have a self-assembled hydrophobic core and hydrophilic shell, which allow the hydrophobic NO donors to be loaded into the hydrophobic core (Kumar et al., 2010; Kaur et al., 2013; Pramanick et al., 2018; Kang et al., 2019). Liposomes and polymersomes comprised of phospholipids or amphiphilic polymers consist of an aqueous core and bilayer lipids or polymers, which allow both hydrophilic (Dinh et al., 2005; Tai et al., 2010; Suchyta and Schoenfisch, 2015; Suchyta and Schoenfisch,

2017; Yoshikawa et al., 2019) and hydrophobic NO donors (Nakanishi et al., 2015) to be loaded into either the aqueous core or hydrophobic bilayers, respectively. MSN also exhibit uniform mesoporous channels that can act as a reservoir for hydrophobic NO donors (Li, Song et al., 2020). Nanoprecipitation is a traditional method to physically encapsulate drugs into nanoparticles (NPs) via emulsion methods, which is used in the physical loading of NO donors as well. In detail, oil-in-water emulsion facilitates the load of hydrophobic NO donors into polymeric particles by sequentially emulsifying hydrophobic NO donors and/or hydrophobic polymers in organic phases into hydrophilic polymers or amphiphilic copolymers in aqueous solutions. In contrast, water-in-oil-in-water emulsions enable the loading of hydrophilic NO donors into polymeric particles by sequentially emulsifying hydrophilic NO donors in aqueous solvents into hydrophobic polymers or amphiphilic copolymers in organic solvents, followed by hydrophilic polymer addition in an aqueous solvent (Yang, Hwang et al., 2018).

Charged NO donors including diazeniumdiolates, SNP, RBS, and RRS can also be loaded into DDSs via electrostatic adsorption. In particular, negatively charged RBS and RRS have been widely explored to develop photoresponsive NO DDSs relevant to in vivo applications by adsorbing to positively charged amine functionalized upconversion NPs (Garcia et al., 2012; Tan et al., 2017; Zhao, Hu et al., 2020) and quantum dots (Tan et al., 2014) that are excited by NIR wavelength light that is capable of penetrating into deep tissues, and then the resulting emitted light induces the decomposition of NO donors that are incorporated into the DDSs.

In addition to nano-particulate systems, hydrogels have been widely used to achieve the sustained release of NO donors and NO by simple physical mixing any types of NO donors into the polymeric hydrogel matrix (Shishido et al., 2003; Halpenny et al., 2009; Pelegrino et al., 2018; Zahid et al., 2019; Liu et al., 2020; Santos et al., 2021; Kim, Francis et al., 2022). The release kinetics of NO donors and NO from hydrogels are dependent on not only hydrogel stability but also hydrophobic, electrostatic, and/or hydrogen-bonding interactions between NO donors and polymer matrix that may influence free diffusion.

B. Chemical Conjugation of NO Donors

There are two fundamental strategies that are employed for the chemical conjugation of NO donors to DDSs in the development of NO delivery systems. First, NO donors with functional groups facile to further chemical reactions can be conjugated to DDSs or materials comprising DDSs. For example, a hydroxyl group of hydrophobic nitrobenzene to release NO in response to UV light exposure was conjugated to a block copolymer, which self-assembled into the core of micelles (Naoki et al., 2010). Another example includes a redox responsive SIN-1 prodrug with a thiol

group, which were conjugated to thiols in DDSs including albumin, 4-arm poly(ethylene glycol) thiols (4-arm PEG-SH), and silica NPs-SH via a disulfide bond (Kim, Suh et al., 2022). However, this strategy is limited to NO donors that are stable and have reactive functional groups for chemical conjugations. On the other hand, another approach is the direct formation of NO donors on the precursors in DDSs and materials comprising DDSs, which account for the majority of developed NO delivery systems and are discussed in the following text.

Various polymers with thiols or secondary amines have been investigated in the formation of S-nitrosothiols or N-diazeniumdiolates, which have been explored for controlled NO release in a form of unstructured polymers (Park et al., 2013; Lu et al., 2014; Ahonen et al., 2019; Maloney et al., 2021), coating materials (Zhang et al., 2002; Reynolds et al., 2006; DeRosa et al., 2007; Wan et al., 2009; Reynolds et al., 2010), or hydrogels/scaffolds (Lipke and West, 2005; Kim et al., 2011; Damodaran et al., 2012; Schanuel et al., 2015; Yao et al., 2015; Hasan et al., 2021). In particular, block copolymers bearing thiols and secondary amines have been exploited for the development of NO delivering micelles and polymersomes. NO donors on hydrophilic segments of block copolymers are exposed to aqueous environments in the self-assembled structures of micelles and polymersomes (Song et al., 2014; Fan et al., 2021). As S-nitrosothiols and N-diazeniumdiolates are polar functional groups that can spontaneously release NO in aqueous solutions, however, they are generally designed to be protected in hydrophobic core or inner layer of micelles or polymersomes to prevent unintended decomposition during storage and in vivo delivery. It can be generally achieved by forming NO donors in the hydrophobic blocks of polymers, by surrounding the NO donors with hydrophobic moieties, or by forming the NO donors at the end of hydrophobic segments in chemical polymer structures (Duong et al., 2013; Gao et al., 2015; Yu et al., 2015; Schudel et al., 2018; Hou et al., 2019; Park, Im et al., 2019; Wu et al., 2020). Interestingly, block copolymers with a hydrophilic segment and a hydrophilic segment containing secondary amines were reported to be self-assembled into a micelle after the formation of N-diazeniumdiolates on the hydrophilic segment containing secondary amines despite the hydrophilic and polar nature of N-diazeniumdiolates (Jo et al., 2009). However, the mechanism behind the hydrophilic-to-hydrophobic conversion in the formation of N-diazeniumdiolates has remained unclear. In addition to synthetic polymers, proteins as natural polymers can be also used for the development of NO delivery systems. Representative examples of protein-based NO delivery systems include albumin-SNO synthesized by converting a thiol in albumin to S-nitrosothiol, which has been explored as a NO delivery system for antitumor therapy (Katayama

et al., 2010; Ishima et al., 2013) owing to the albumin's favorable circulation times that enable tumor accumulation and active tumor targeting capability (Frei, 2011; Hoogenboezem and Duvall, 2018).

Several inorganic metal NPs including gold NPs and superparamagnetic NPs have been under development as a platform for NO delivery systems by modifying the surface of NPs with materials containing precursors for NO donors via self-assembled monolayer chemistry, followed by the formation of NO donors (Rothrock et al., 2005; Polizzi et al., 2007; Duong et al., 2014; Santos et al., 2016). Nevertheless, the most extensively explored inorganic NO delivery systems are silica materials based on well-defined silane chemistry, such as silica NPs, MSN, and xerogels. In brief, S-nitrosothiols or N-diazeniumdiolates can be functionalized on thiols- or amines-functionalized silane-based DDSs that were prepared via a co-condensation of thiolalkoxysilane or aminoalkoxysilane with alkoxysilanes or via a condensation of thiolalkoxysilane or aminoalkoxysilane on the as-prepared silane-based DDSs (Nablo et al., 2001; Zhang et al., 2002; Marxer et al., 2003; Nablo et al., 2005; Hetrick et al., 2007; Shin et al., 2007; Riccio et al., 2009; Carpenter et al., 2011; Riccio et al., 2011; Carpenter et al., 2012; Riccio et al., 2012). In addition, aminoalkoxysilane can be functionalized with N-diazeniumdiolates or O^2-protected N-diazeniumdiolates in advance and then condensed with alkoxysilanes to prepare silane-based NO delivery systems (Hetrick et al., 2008; Shin and Schoenfisch 2008; Hetrick et al., 2009; Stevens et al., 2010; Carpenter et al., 2013; Storm and Schoenfisch, 2013). Furthermore, silane-based NO delivery systems with S-nitrosothiols or N-diazeniumdiolates can be covered with other polymers (Nablo and Schoenfisch 2004; Nablo and Schoenfisch 2005) or calcium phosphate (Choi et al., 2016) or further modified with superhydrophobic fluorine (Storm et al., 2014), which achieve sustained and/or pH-responsive NO release.

NO coating strategies, which result in the modification of materials, nanoparticles, vessel stents, etc., with NO releasing moieties (e.g., NO donors), allow the surface of materials to release NO. Layer-by-layer methods have been employed as one NO coating strategy that is achieved by alternating deposition of positively charged materials containing secondary amine groups and negatively charged materials, followed by formation of N-diazeniumdiolates (Park et al., 2019a; Park et al., 2019b; Tanum et al., 2019). The zwitterionic negative charge of N-diazeniumdiolates contributes to the stabilization of the layers. In addition, hydrogels (Lipke and West, 2005; Kim et al., 2011; Damodaran et al., 2012; Schanuel et al., 2015; Yao et al., 2015; Hasan et al., 2021) and xerogels (Nablo et al., 2001; Marxer et al., 2003; Riccio et al., 2009; Riccio et al., 2012; Storm and Schoenfisch, 2013) chemically conjugating NO donors also exhibit sustained release of

NO owing to the reduced diffusion rates in the polymeric matrix, such as hydrogels physically loaded with NO donors (Shishido et al., 2003; Halpenny et al., 2009; Pelegrino et al., 2018; Zahid et al., 2019; Liu et al., 2020; Santos et al., 2021; Kim, Francis et al., 2022). Polycatecholamine-diazeniumdiolates have also emerged as a versatile NO coating strategy (Hong et al., 2013; Park et al., 2016; Adnan et al., 2018; Sadrearhami Z et al., 2019). Polycatechnolamines are synthesized by oxidative self-polymerization of catecholamines including dopamine, norepinephrine, and so on, which can be formed on the surface of any material substrate including metals, polymers, semiconductors, and ceramics, regardless of shape and size (Lee et al., 2007). Techniques for polycatecholamine–diazeniumdiolates were developed by simply forming N-diazeniumdiolates on the secondary amines of polycatecholamine films on substrate materials. The advantages of polycatecholamine–diazeniumdiolates over hydrogels and xerogels were reported to include material-independent coating ability and thin thickness (nm vs. μm-mm) (Hong et al., 2013).

C. Considerations on the Immunologic Effects of NO Donors and Their Therapeutic Realization Using NO Delivery Systems

Since the cellular and microenvironmental balances of enzymes, singlet oxygen, O_2, and GSH levels decisively regulate a tissue's immune microenvironment (Ghezzi 2011; Gostner et al., 2013; Noman et al., 2015; Chen et al., 2016), their consumption or usage in the decomposition of NO donors, which vary widely in their decomposition mechanism ranging from consuming or using aldehyde dehydrogenase-2, singlet oxygens, cytochrome P450, the reduced form of NADP, O_2, temperature, light, heavy metal ions, GSH, GST, ascorbic acid, or esterase, etc., as previously discussed, also can affect the overall biologic outcomes in addition to the effects of NO itself. Furthermore, even with the similar or same DDS and NO donors, engineering inter- and intramolecular bonding and association between NO donors and materials comprising NO delivery systems can govern kinetics of NO release (Shin et al., 2007; Riccio et al., 2009; Kim et al., 2011; Hong et al., 2013), followed by various concentration- and releasing kinetic-dependent mechanisms of NO (Fukumura et al., 2006; Carpenter and Schoenfisch, 2012; Kim et al., 2014). In particular, numerous materials used in the NO delivery systems also elicit an immune response on their own. As examples, silica, which is widely used for NO releasing xerogels, NPs, and MSNs, have been reported to act as an adjuvant, whose efficacy and pathway are dependent on size, morphology, and surface functional groups (Vallhov et al., 2007; Heidegger et al., 2016; Li et al., 2016; Abbaraju et al., 2017; Nguyen et al., 2019; Zhao, Jiang et al., 2020). How new mechanistic insights into NO's immune regulatory roles that have been largely

generated using NO donors can be realized for therapeutic ends using NO delivery systems more amenable to clinical translation should therefore be carefully considered.

IV. Direct Effects of NO on the Immunogenicity of Cancer Cells In Vitro

Given the myriad of physiologic roles of NO, NO donors and delivery systems have been widely explored for their potential as therapeutics. Herein, activities of NO as potentiating cancer therapy that are relevant to immunotherapy, directly in modulating cancer cell immunogenicity, are discussed while NO's effects on the immune system will be discussed in subsequent sections.

Even without considering its complex effects on the immune system, NO itself has seemingly opposing effects on modulating the proliferation and viability of cancer cells (Mocellin et al., 2007; Kim et al., 2014). High NO concentrations can induce cancer cell apoptosis and necrosis by directly damaging DNA, activating caspases-1 and -10, phosphorylating p53, inhibiting DNA repair mechanisms associated with poly(ADP-ribose) polymerase and DNA-dependent protein kinase, downregulating antiapoptotic protein survivin, and increasing Fas (CD95/APO-1) receptor expression (Ren et al., 2003; Shami et al., 2003; Shami et al., 2006; Udupi et al., 2006; Kiziltepe et al., 2007; Mocellin et al., 2007; Chakrapani, Goodblatt et al., 2008; Chakrapani, Kalathur et al., 2008; Simeone et al., 2008; Kitagaki et al., 2009; Kumar et al., 2010; Stevens et al., 2010; Singh and Gupta 2011; Xu et al., 2015; Fan et al., 2017; Kim et al., 2017; Liu et al., 2017; Liu, Huang et al., 2018; Zhao et al., 2019). Seemingly in complete opposition to this, NO can also augment the proliferation of cancer cells by stabilizing hypoxia inducible factor 1 subunit alpha, activating matrix metalloproteinases, upregulating vascular endothelial growth factor, increasing antiapoptotic proteins including B-cell lymphoma-2 and cyclooxygenase-2, and scavenging cytotoxic radical species (Ren et al., 2003;

Shami et al., 2003; Shami et al., 2006; Udupi et al., 2006; Kiziltepe et al., 2007; Mocellin et al., 2007; Chakrapani, Goodblatt et al., 2008; Chakrapani, Kalathur et al., 2008; Simeone et al., 2008; Kitagaki et al., 2009; Kumar et al., 2010; Stevens et al., 2010; Singh and Gupta 2011; Xu et al., 2015; Fan et al., 2017; Kim et al., 2017; Liu et al., 2017; Liu, Xiao et al., 2018; Zhao et al., 2019). NO has also been shown to synergize with chemotherapy-, radiotherapy-, photothermal therapy– and photodynamic therapy–mediated apoptosis and necrosis effects by affecting multidrug-resistance, hypoxia, autophagy, balance of reactive oxygen species (ROS), and so on (Matthews et al., 2001; Frederiksen et al., 2003; Konovalova et al., 2003; Evig et al., 2004; Riganti et al., 2005; Bonavida et al., 2006; Hirst and Robson, 2007; Mocellin et al., 2007; Bonavida et al., 2008; Bratasz et al., 2008; Huerta et al., 2008; Bonavida and Garban, 2015; Fan et al., 2015; Kim et al., 2017; Deng et al., 2018; Hu et al., 2018; Pramanick et al., 2018; Wan et al., 2018; Ding et al., 2019; Feng et al., 2019; Hays and Bonavida, 2019a; Wang et al., 2019; Zhang, Jin et al., 2019; Bonavida B, 2020; Gao et al., 2020; Pieretti et al., 2020). Taking into account the numerous research and review papers discussing those actions and mechanisms (Ren et al., 2003; Shami et al., 2003; Shami et al., 2006; Udupi et al., 2006; Kiziltepe et al., 2007; Mocellin et al., 2007; Bonavida et al., 2008; Chakrapani, Goodblatt et al., 2008; Chakarapani, Kalathur et al., 2008; Simeone et al., 2008; Kitagaki et al., 2009; Kumar et al., 2010; Stevens et al., 2010; Singh and Gupta, 2011; Kim et al., 2014; Fan et al., 2015; Xu et al., 2015; Fan et al., 2017; Liu et al., 2017; Deng et al., 2018; Hu et al., 2018; Kim et al., 2017; Liu, Xiao et al., 2018; Pramanick et al., 2018; Wan et al., 2018; Ding et al., 2019; Feng et al., 2019; Park, Im et al., 2019; Wang et al., 2019; Zhang et al., 2018; Zhao et al., 2019; Gao et al., 2020), in this section, we focus on the effects of NO donors and delivery systems on the immunogenicity (Mahanty et al., 2015) of cancer cells (Table 1).

TABLE 1
Influences of NO donors on cancer cells in vitro

Types of NO donor	Concentration	Cells (in vitro)	Functions	Reference
DETA-NONOate	200 μM	SKO-007(J3), U266, OPM-2, RPMI-8226, ARK, LP1	PVR/CD155 ↑	Fionda et al., 2015
	>200 μM	A172	PD-L1 ↑	Kiriyama et al., 2020
DNIC	>5 μM	HCA-1, JHH-7	PD-L1 ↓	Sung et al., 2019
GSNO	0.1 μM	B16F10	CRT ↑ and ATP ↑ (when combined with PTX)	Kim et al., 2020
NCX4040	200 μM	SKO-007(J3), U266, OPM-2, RPMI-8226, ARK, LP1	PVR/CD155 ↑	Fionda et al., 2015
SNCEE	600 μM	Jurkat	CRT ↑	Tarr et al., 2010
SNAP	100 μM	HT29-dx	CRT ↑	Kopecka et al., 2011
SNP	100 μM	HT29^{iNOS-}	CRT ↑	De Boo et al., 2009
-SNO NP	0.1 μM	B16F10	CRT ↑ and ATP ↑ (when combined with PTX)	Kim et al., 2020

A172, human glioblastoma cell line; B16F10, murine melanoma cell line; HCA-1, murine hepatocellular carcinoma; HT29, human colon cancer; HT29iNOS-, HT29 cells silenced for iNOS; HG2T-dx, drug-resistant HT29; JHH-7, human hepatocellular carcinoma; Jurkat, human immortalized T lymphocyte; NCX4040, nitric oxide–aspirin; SKO-007(J3), U266, OPM-2, RPMI-8226, ARK, LP1, human multiple myeloma cell lines; SNCEE, S-nitroso-L-cysteine ethyl ester.

A. NO Effects on Ligands of Immune Checkpoints: CD155 and Programmed Cell Death Ligand 1

Immune checkpoints play crucial regulatory functions in the homeostasis of immune system and the prevention of autoimmunity. However, immune checkpoints are also exploited by tumors to evade immune surveillance (Pardoll, 2012). In this regard, modulation or blockade of immune checkpoints and their ligands provide alternative ways to subvert the evasion of cancer cells of immune surveillance, leading to more robust tumor control by cancer immunotherapies (Shin and Ribas, 2015; Burugu et al., 2018; Marin-Acevedo et al., 2018). Despite the expression of various immune checkpoint ligands by cancer cells, such as galectin-9, glucocorticoid-induced tumor necrosis factor (TNF) receptor family-related protein ligands, CD112/CD155, and programmed cell death ligand 1 (PD-L1) (Shin and Ribas, 2015; Burugu et al., 2018; Marin-Acevedo et al., 2018), it has not been well investigated whether, which, or how immune checkpoint ligands on cancer cells are affected by exogenous NO. In this section, how exogenous NO modulates the expression of T cell immunoreceptor with Ig and ITIM domains (TIGIT) and PD-L1 on cancer cells is discussed.

The effects of exogenous NO on TIGIT pathways have been explored in multiple myeloma (MM) cell lines. MM is characterized by abnormal growth of plasma cells in bone marrow, exhibiting various symptoms, such as abnormal proteins from plasma cells, low blood cells, weak immunity, accelerated osteoclasts, and kidney damage (Stoopler et al., 2007), which express the poliovirus receptor (PVR/CD155) that is a ligand of both DNAX accessory molecule-1 and TIGIT (Shin and Ribas, 2015; Zhu et al., 2016; Gao et al., 2017; Burugu et al., 2018; Marin-Acevedo et al., 2018; Stamm et al., 2018; Harjunpää and Guillerey, 2020). TIGIT is an immune checkpoint that acts similar with cytotoxic T-lymphocyte-associated protein-4 (CTLA-4). In brief, the activation of cytotoxic T cells and NK cells is inhibited when TIGIT on T cells or NK cells binds to PVR/CD155 on antigen presenting cells (APCs), while binding of CD226 on T cells or NK cells to PVR/CD155 on APCs leads to the opposite effect (Shin and Ribas, 2015; Zhu et al., 2016; Gao et al., 2017; Burugu et al., 2018; Marin-Acevedo et al., 2018; Stamm et al., 2018; Harjunpää and Guillerey, 2020). In addition, the interaction of DNAX accessory molecule-1 on NK or T cells with PVR/CD155 on cancer cells exerts cytotoxicity via cytotoxic cytokines (Chan et al., 2010) such as interleukin (IL)-2, IL-12, or IL-21. Accordingly, antibodies antagonizing TIGIT have been developed (Gao et al., 2017; Harjunpaa and Guillerey, 2020) and chemotherapeutic drugs that up-regulate PVR/CD155 ligands on MM cells have been explored (Soriani et al., 2009; Chan et al., 2010; Fionda et al., 2013). Interestingly, chemotherapeutic drugs upregulating PVR/CD155 ligands are associated with DNA damage responses (Soriani et al., 2009; Fionda et al., 2013; Fionda et al., 2015) and NO is a radical species capable of exerting genotoxicity. In this regard, the effects of NO donors on the expression of PVR/CD155 ligands followed by NK cell–mediated immunotherapy were investigated (Fionda et al., 2015). Fionda et al. (2015) demonstrated that NO donors (200 μM) including diethylenetriamine NO adduct (DETA-NONOate), NO aspirin (NCX4040), and JS-K induce the upregulation of PVR/CD155 on six different MM cell lines including SKO-007(J3), U266, OPM-2, RPMI-8226, ARK, and LP1 via a DNA damage response pathway, which enhanced the susceptibility of those MM cells to NK cells to induce cytotoxicity in vitro (Fionda et al., 2015) (Fig. 1). Since other cancer cells including melanoma cells also express PVR/CD155 (Mahnke and Enk, 2016; Zhu et al., 2016; Gao et al., 2017; Stamm et al., 2018; Harjunpaa and Guillerey, 2019), the demonstrated potential of NO donors on modulating tumor expression of PVR/CD155 could be explored for its effects in other indications.

Exogenous NO modulates PD-1/PD-L1 pathways as well. Summarizing the major mechanism of PD-1/PD-L1 simplistically, the binding of PD-1 on T cells to PD-L1 on APCs or cancer cells inhibits the antitumor functions of cytotoxic CD8$^+$ T cells [cytotoxic T lymphocytes (CTLs)] (Pardoll, 2012; Shin and Ribas, 2015; Burugu et al., 2018; Marin-Acevedo et al., 2018). As a result, various antagonistic antibodies for PD-1 and PD-L1 have been developed and approved by the US Food and Drug Administration (FDA) for anticancer immunotherapy by allowing CD8$^+$ T cells or cancer cells to be more activated or susceptible to CTLs, respectively (Pardoll, 2012; Shin and Ribas, 2015; Burugu et al., 2018; Marin-Acevedo et al., 2018). In addition, several strategies have been explored to downregulate PD-L1 on cancer cells by exploiting small interfering RNA, microRNA, CRISPR, and
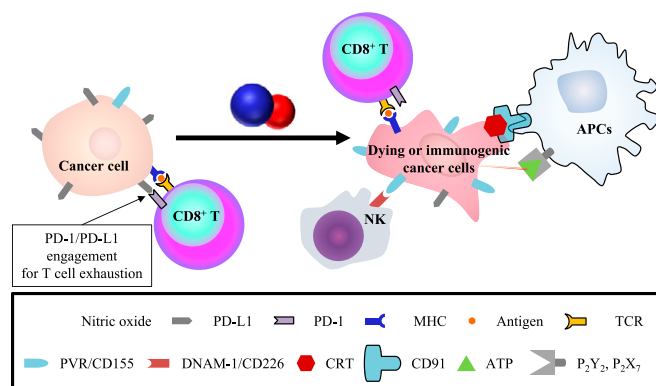
Fig. 1. Direct effects of exogenous NO on cancer cell immunogenicity. Exogenous NO can suppress PD-L1 expression by cancer cells, which enhances the efficacy of anti-tumor CD8$^+$ T cell immunity. It can also upregulate the PVR/CD155 expression by cancer cells, which induces NK cell responses. It can also stimulate the ATP release as well as CRT expression, which recruits APCs. These immunogenic effects on cancer cells mediated by the application of exogenous NO are dependent on the types of cancer cells and NO donors/delivery systems, as well as the dose of NO donors/delivery systems.

CRISPR-associated protein 9 gene-editing techniques or hypoxia-regulating NPs (Zou et al., 2018; Guan et al., 2019; Phung et al., 2019; He et al., 2020). Although it is well-known that PD-L1 expression on cancer cells is governed by hypoxia (Sun et al., 2018; Lequeux et al., 2019) that is regulated by NO via ubiquitous transcription factor Yin Yang 1 and hypoxia-inducible factor 1 subunit alpha (Mateo et al., 2003; Bonavida et al., 2008; Dai et al., 2018; Hays and Bonavida, 2019a,b), the effects of NO on PD-L1 expression began to be investigated only very recently (Sung et al., 2019; Kiriyama et al., 2020). Sung et al. (2019) revealed that NO releasing NPs (>5 μM) regulated the activity of transcription factor SP1 in vitro, which led to the suppression of the PD-L1 expression on hepatocellular carcinoma cells in vitro in a dose-dependent manner (Sung et al., 2019). However, the opposite results were also reported, with DETA-NONOate (>200 μM) upregulating PD-L1 expression on A172 glioblastoma cells in vitro via c-Jun N-terminal kinase pathways (Kiriyama et al., 2020). These results imply that the expression of PD-L1 can depend on the cancer cell type and the concentration and kind of NO donor or delivery system (Fig. 1).

B. NO Effects on Immunogenic Cell Death: Calreticulin, ATP, and High Mobility Group Protein B1

Immunogenic cell death (ICD) is a process in which dying apoptotic cells expose and release damage-associated molecular patterns including calreticulin (CRT), ATP, and high mobility group protein B1 (HMGB1), which activate anticancer immunity (Krysko et al., 2012). ICD is induced by simultaneous actions of ROS and endoplasmic reticulum stress (Krysko et al., 2012). Although NO not only regulates intracellular ROS levels but also modulates the functions of endoplasmic reticulum (Fukumura et al., 2006; Gotoh and Mori, 2006; Mocellin et al., 2007; Carpenter and Schoenfisch, 2012), there have been few reports to investigate the effects of NO donors and delivery systems on ICD by cancer cells.

CRT is called as an "eat-me" signal to be recognized by phagocytosis receptors of CD91 positive APCs (Gotoh and Mori, 2006). Doxorubicin (DOX) is an anticancer chemotherapeutic drug well-known to induce CRT on cancer cells (Gotoh and Mori, 2006). Interestingly, DOX-mediated CRT exposure was not observed in inducible NOS (iNOS) knockout human colon cancer cells lines (HT29$^{\text{iNOS}-}$ cells) in contrast to normal HT29 cells (De Boo et al., 2009). In addition, treatment with a NO donor (SNP; 100 μM) per se upregulated the externalization of CRT on HT29$^{\text{iNOS}-}$ cells in vitro, while treatment with NO scavenger (2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl 3-oxide) diminished the effects of SNP on CRT exposure (De Boo et al., 2009). In particular, SNP significantly improved the phagocytosis of HT29$^{\text{iNOS}-}$ cells by DCs and proliferation of allogenic lymphocytes in a mixed lymphocyte reaction (MLR) (De Boo et al., 2009). An additional study demonstrated that a NO donor [S-nitrosopenicillamine, (SNAP); 100 μM] facilitated the externalization of CRT in both normal and drug-resistant HT29 cells (HT29-dx cells) via soluble cyclic guanosine monophosphate (cGMP) (Kopecka et al., 2011). Similar results were also reported with the increased externalization of CRT on Jurket cell lines (human leukemic T-cell lymphoblast) that were treated with NO donor S-nitroso-L-cysteine ethyl ester (600 μM) (Tarr et al., 2010). In contrast with those results utilizing very high concentrations of NO (>10 μM) sufficient to induce apoptosis of cancer cells, low concentrations of a NO donor (GSNO; ~0.1 μM), which is not enough to exert cytotoxicity but sufficient to facilitate the chemosensitization, exhibited negligible effects on the CRT exposure in B16F10 murine melanoma cells (Kim et al., 2020). However, the low concentrations of nitrosothiols (~0.1 μM) formulated in paclitaxel (PTX)-delivery NPs (pPTX/pCD-pSNO) enhanced the CRT externalization compared with PTX-delivery NPs (pPTX/pCD-pSH) without NO donors (Kim et al., 2020). These results imply that NO donors and delivery systems improved the immunogenicity of cancer cells by upregulating the externalization of CRT when their concentrations are enough to induce apoptosis or when they are combined with other ICD-inducing drugs or DDSs (Fig. 1).

HMGB1 attracts immune cells to stimulate the production of proinflammatory cytokines (Krysko et al., 2012). To date, the effects of NO donors and delivery systems on the release of HMGB1 are not fully established. A study demonstrated that SNP (2 mM) suppressed the expression of HMGB1 in lysates of papillary thyroid cancer cells obtained from patients with tyroid cancer (Mardente et al., 2010). However, DETA-NONOate (500 μM or 1–10 μM) significantly increased the release of HMGB1 from noncancer cells, such as rat insulinoma (RINm5F cell lines) or murine macrophages (RAW 264.7 cell lines), respectively (Jiang and Pisetsky, 2006; Steer et al., 2006). Accordingly, further investigations are required with different types and concentrations of NO donors and delivery systems to clarify the effects and mechanisms of NO on the HMGB1-mediated ICDs by various cancer cells.

ATP is a representative "find-me" signal released from apoptotic cells, which facilitates the P_2Y_2- and P_2X_7-mediated phagocytosis by APCs (Martins et al., 2014; Elliott et al., 2009; Krysko et al., 2012). Although there have been several reports exploring the effects of NO on red blood cells and astrocytes, it is unclear whether NO promotes or inhibits the release of ATP due to diversity in experimental conditions, such as oxygen level and types and concentrations of NO donors (Bal-Price et al., 2002; Olearczyk, Ellsworth, et al., 2004; Olearczyk, Stephenson et al., 2004; Cao et al., 2009). Likewise, the effects of NO donors and

delivery systems on ATP release from cancer cells have not been clearly evaluated (Fig. 1). Our group recently investigated the effects of low NO donor concentration (~0.1 μM) on ATP release from B16F10 murine melanoma cells (Kim et al., 2020). Like CRT exposure, the low concentration of the NO donor (GSNO; ~0.1 μM) exhibited negligible effects on ATP release, while pPTX/pCD-pSNO enhanced the ATP release compared with pPTX/pCD-pSH (Kim et al., 2020). There are two representative mechanisms in the release of ATP; caspase activation and autophagy (Martins et al., 2014). However, pPTX/pCD-pSNO failed to enhance autophagy process compared with pPTX/pCD-pSH, whereas free PTX and PTX/pCD-pSH led to enhanced autophagy compared with PBS and GSNO (Kim et al., 2020). These results imply that the modulation of ATP by low concentrations of NO in PTX codelivery is not associated with autophagy mechanisms (Kim et al., 2020). On the other hand, high concentrations of JS-K (1–5 μM), which are enough to elicit cytotoxicity to cancer cells, improved the autophagy in A2780 and SKOV3 ovarian cancer cell lines (Liu et al., 2019) and MDA-MB-453 breast cancer cell lines (McMurtry et al., 2011) in vitro. However, these results cannot guarantee that NO induces autophagy in a concentration-dependent manner because there have been reports of oppositive effects, demonstrating that different types and concentrations of NO donors and NOS inhibitors differentially suppress autophagy in various cell types (Sarkar et al., 2011; Benavides et al., 2013; Shen et al., 2014; Zhang, Jin et al., 2019). In addition, whether NO mediated changes in autophagy indeed regulate ATP release also need to be further investigated.

In addition to the ligands of immune checkpoints and ICD mechanisms as previously discussed, it was reported that NO enhances the degradation of antiapoptotic survivin in cancer cells, which sensitizes cancer cells to DC-induced cell death mediated by Fas–Fas ligand–independent as well as dependent pathways (Huang et al., 2005). These reports imply the potential presence of additional mechanisms that exogenous NO has on the immunogenicity of cancer cells.

In conclusion, NO donors and NO delivery systems have potential to improve the immunogenicity of cancer cells, although the types and dose are required to elicit these favorable effects remain to be further optimized.

V. Direct Effects of NO on Immune Cells In Vitro

Endogenous NO synthesized via an enzymatic reaction with endogenous NOSs and L-arginine substrates modulates the functions and populations of multiple immune cell subtypes in autocrine, paracrine, and endocrine manners, expertly summarized by Bogdan (2001, 2015). However, in several cells, exogenously delivered NO can act differently from endogenously generated NO (Gansauge et al., 1997; Nakano et al., 2000).

Moreover, the immune system functions through the coordinated effects among various immune cells that govern tumor progression (Burkholder et al., 2014; King et al., 2017; Navarro-Tableros et al., 2018). These complexities make it difficult to understand the mechanism behind the physiologic outcomes in NO-based anticancer therapy. Accordingly, in this section, we discuss how NO donors and delivery systems directly regulate immune cells in vitro (Table 2 and 3).

A. Dendritic Cells

DCs are a specialized APC subtype that play pivotal roles in shaping innate and adaptive immune response (Wculek et al., 2020). Endogenous NO is also reported to directly modulate the functions of DCs as represented by TNF- and iNOS-producing DCs (Tip-DCs) to be involved in the innate immune response, priming of T cells, and modulation of CD8^{+} T cell activity (Shimamura et al., 2002; Serbina et al., 2003; Nicolas et al., 2007; Serbina et al., 2008; Marigo et al., 2016; Xue et al., 2018). Functions of Tip-DCs are reported to be significantly modulated by NO, which have emphasized the crucial roles of NO in the actions of DCs (Shimamura et al., 2002; Serbina et al., 2003; Nicolas et al., 2007; Serbina et al., 2008; Marigo et al., 2016; Xue et al., 2018). Likewise, there have been significant efforts to investigate the effects of exogenous NO donors on DCs. DC-mediated antitumor immunity is comprised of a cascade pathway: differentiation of immature DCs, antigen uptake by immature DCs in peripheral tissues, migration of the maturating DCs into secondary lymphoid organs, and antigen presentation of DCs to T cells (Palucka and Banchereau, 2012). Accordingly, the effects of NO on each aspect of DC-regulated immune response are discussed stepwise in the following text.

NO donors such as SNP (50 nM), SIN-1 (50 μM), DEA-NO (50 μM), and DETA-NONOate (50 μM) were reported to differentiate hematopoietic stem cells and monocytes into DCs (Fernández-Ruiz et al., 2004; Tiribuzi et al., 2013). Interestingly, DCs expanded by NO donors (SNAP; 500 nM) seem to consist of IL-6–, IL-12–, and TNF-producing effector DCs and IL-10– and PD-1–expressing regulatory DCs (Si et al., 2016). An iNOS-inhibitor [L-N[6]-(1-iminoethyl)lysine; 40 μM] selectively induced the differentiation of effector DCs, as contrasted with opposite results observed for NO donor-treated (SNAP; 500 μM) DCs in vitro (Si et al., 2016).

DCs matured by TNF-α have a suppressed endocytic ability via intracellular accumulation of the lipid messenger ceramide (Sallusto et al., 1996). However, the DETA-NONOate (100 μM) and SNAP (200 μM) significantly improved the endocytic ability of TNF-α-treated DCs by inhibiting intracellular accumulation of ceramide via the cGMP pathway (Paolucci et al., 2000). Uptaken antigens are digested and processed by DCs and are then presented on the DC cell surface. Interestingly, a NO donor [3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-

TABLE 2
Influences of NO donors on dendritic cells and B cells in vitro

Types of NO donor	Concentration	Cells (in vitro)	Functions	Reference
DEA-NO	50 μM	Human monocytes from PBMC	Differentiation to DCs ↑ (under GM-CSF and IL-4)	Fernández-Ruiz et al., 2004
DETA-NONOate	50 μM	Human monocytes from PBMC	Differentiation to DCs ↑ (under GM-CSF and IL-4)	Fernández-Ruiz et al., 2004
	100 μM	moDCs from PBMC	Endocytosis activity ↑ (when combined with TNF-α)	Paolucci et al., 2000
	50 μM	moDCs from PBMC	Negligible effects on maturation, maturation ↑ and T cell prime ↑ (when combined with TNF-α)	Paolucci et al., 2003
DTPA-NONOate	600 μM	moDCs from PBMC	IL-12p70 ↓ (when combined with LPS)	Obregon et al., 2015
GSNO	1–50 μM	Mice BMDC	Negligible effects on Activation of DCs and T cell prime in MLR, T cell prime ↑ (when combined with PTX)	Kim et al., 2020
NOR-4	25–100 μM	moDCs from PBMC	IL-12p70 ↓ (when combined with LPS), migration to CCL19 and CXCL12 ↑ (when combined with LPS)	Giordano et al., 2006
SIN-1	50 μM	Human monocytes from PBMC	Differentiation to DCs ↑ (under GM-CSF and IL-4)	Fernández-Ruiz et al., 2004
SNP	50 nM	Hematopoietic stem cells from PBMC	Differentiation to DCs ↑	Tiribuzi et al., 2013
	100 μM	moDCs from PBMC	Endocytosis activity ↑ (when combined with TNF-α)	Paolucci et al., 2000
	100–1000 μM	Mice BMDCs	IL-12p40 ↓ (when combined with LPS)	Xiong et al., 2004
	100 μM	Mice BMDCs	TNF-α ↓ IL-6 ↓ and IL-12 ↓ (when combined with LPS/IFN-γ)	Si et al., 2016
SPER/NO	100–200 μM	A20 B cells	Intracellular antigen process ↓	Lemaire et al., 2009
PAPA-NO	20 μM	DC2.4	Intracellular antigen process ↓	Siddiqui et al., 2011
-SNO NP	1–50 μM	Mice BMDCs	Activation ↑ and T cell prime ↑ in MLR (when combined with PTX)	Kim et al., 2020

BMDCs, bone marrow–derived dendritic cells; A20 B cells, murine B lymphoma cell line; moDC, monocyte-derived dendritic cells; PAPA-NO, 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine; PBMC, peripheral blood mononuclear cell; SPER/NO, N-[4-[1-(3-aminopropyl)-2-hydroxy-2-nitrosohydrazino]butyl]1,3-propanediamine.

propanamine; 20 μM] reduced the presentation of antigens to the major histocompatibility complex class I by DCs when a protein antigen to be digested was loaded, whereas it had no effect on antigen presentation compared with control when a peptide antigen was used (Siddiqui et al., 2011). These results suggested that NO hampers the intracellular antigen processing step by inhibiting proteolysis (Siddiqui et al., 2011).

During antigen processing, DCs undergo maturation and migrate into the secondary lymphoid organs where they initiate the adaptive immune response. GSNO (1–50 μM) and DETA-NONOate (50 μM) have been shown to exert negligible effects on the maturation of DCs, respectively (Paolucci et al., 2003; Kim et al., 2020). However, NO donors showed unpredictable results when they are treated with other agents or DDSs to induce the maturation of DCs. While DETA-NONOate (50 μM) and S-nitrosothiol (1–50 μM) improved the maturation of TNF-α- or pPTX/pCD-SH–treated DCs (Paolucci et al., 2003; Kim et al., 2020), SNAP (100–1000 μM), dipropylenetriamine NONOate (600 μM), or N-(-4-ethyl-2-hydroxyimino-5-nitrohex-3-enyl)pyridine-3-carboxamide (NOR-4; 25–100 μM) suppressed the expression of IL-6, IL-12, and TNF-α in DCs matured by lipopolysaccharide (LPS; 100 or 500 μg/mL)

(Xiong et al., 2004; Giordano et al., 2006; Obregon et al., 2015; Si et al., 2016).

The effects of NO donors on the migration of DCs have been also investigated (Giordano et al., 2006). NOR-4 (25–100 μM) was reported to improve LPS-matured DC responses to CCL19 in a dose-dependent manner. NOR-4 slightly enhanced CCR7 expression in LPS-treated DCs, implying that NOR-4–mediated enhanced migration of mature DCs toward CCL19 was not simply associated with the increase of CCR7 but achieved by a mechanism unrelated to receptor expression. LPS promoted expression and activity of cGMP kinase that phosphorylates vasodilator-stimulated phosphoprotein, which disrupted focal adhesion and inhibited cell migration. Interestingly, NOR-4 reversed the effects of LPS on cGMP kinase, which facilitated the enhanced migration of DCs toward CCL19. Furthermore, NOR-4 increased the expression of CXCR4 in LPS-treated DCs, which were correlated to the enhanced migration of the matured DCs toward CXCL12. The enhanced migration toward CCL19 and CXCL12 by NOR-4 was not observed in the immature DCs, suggesting the requirement of maturation status in exogenous NO-mediated enhancement of DC migration toward secondary lymphoid tissues.

TABLE 3
Functions of NO donors on T cells and NK cells in vitro

Types of NO donor	Concentration	Cells (in vitro)	Functions	Reference
DETA-NONOate	50–200 μM	CD4 T, moDCs from PBMC	Proliferation of CD4 T $\downarrow$ in MLR	Markowitz et al., 2017
	100–100 μM	OT-II CD 4 T	Differentiation to Th1 $\uparrow$ (under OVA peptide, splenic DC and TGF-β)	Lee et al., 2011
	5–10 μM	CD4 T from human PBMCs	Differentiation to Th1 $\uparrow$ (under IL-12 and anti-IL-4)	Niedbala et al., 2002
	10–100 μM	CD4 T from human PBMCs	Differentiation to Th1 $\downarrow$ (under IL-12 and anti-IL-4)	Niedbala et al., 2002
	100–200 μM	CD4 T from human PBMCs	Proliferation of CD4 $\downarrow$	Obermajer et al., 2013
	50–200 μM	Th17	Proliferation of CD4 $\downarrow$, IL-17 $\downarrow$, IL-22 $\downarrow$	Niedbala et al., 2011
	100 μM	CD4 T	Differentiation to Th9 $\uparrow$ (under anti-CD3, IL-6, TGF-β, IL-1β, IL-23, anti-IFN-γ, anti-IL-4)	Niedbala et al., 2014
	50–200 μM	CD4$^+$CD25$^-$ T under anti-CD3	Differentiation to NO T$_{reg}$s $\uparrow$	Niedbala et al., 2007
	10–100 μM	OT-II CD 4 T	Differentiation to T$_{reg}$ $\downarrow$ (under OVA peptide, splenic DC and TGF-β)	Lee et al., 2011
	25 μM	CD4$^+$CD25$^+$T under anti-CD3 and anti-CD28	Differentiation to T$_{reg}$ $\downarrow$	Brahmachari and Pahan, 2009
SNP	1 mM	ST4, PF382, Jurkat	Proliferation $\downarrow$	Allione et al., 1999
	1–100 μM	EL4, D10.G4, CDC25	IL-4 $\uparrow$ (under Con A or TPA/A2318716)	Chang et al., 1997
GSNO	100–500 μM	SupT1	Proliferation $\downarrow$	Henson et al., 1999
SIN-1	1–10 mM	CD45.1 OT-1 CD8 T	Tyrosine nitrosylation of the TCR-CD8 complex	Nagaraj et al., 2007
NOC12	100 μM	ST4, PF382, Jurkat	Proliferation $\downarrow$	Allione et al., 1999
SNAP	50–200 μM	CD4 T, moDCs from PBMC	Proliferation of CD4$^+$ T $\downarrow$ in MLR	Markowitz et al., 2017
	1–10 μM	CD4 T from OVA TCR-transgenic mice	Differentiation to Th1 $\uparrow$ (when combined with OVA peptide, IL-12 and anti-IL-4)	Niedbala et al., 1999
	50–500 μM	CD4 T from OVA TCR-transgenic mice	Differentiation to Th1 $\downarrow$ (under OVA peptide, IL-12 and anti-IL-4)	Niedbala et al., 1999
	10–100 μM	OT-II CD 4 T	Differentiation to Th1 $\uparrow$ (under OVA peptide, splenic DC and TGF-β)	Lee et al., 2011
	10–100 μM	39A1	Differentiation to Th1 $\downarrow$ (under anti-CD3)	van der Veen et al., 1999
	20–200 μM	EL4, D10.G4, CDC25	IL-4 $\uparrow$ (under Con A or TPA/A23187)	Chang et al., 1997
	10–200 μM	CD4 T under TGF-β1, IL-6, IL-23, and anti-IL-4	Differentiation to Th17 $\downarrow$	Yang et al., 2013
	10–100 μM	OT-II CD 4 T	Differentiation to T$_{reg}$ $\downarrow$ (under OVA peptide, splenic DC and TGF-β)	Lee et al., 2011
	100 μM	NK cells from human PBMCs	Nitrosylated the tyrosine residues $\uparrow$, ADCC functions $\downarrow$	Stiff et al., 2018
Peroxynitrite	0.1–2.4 mM	CD3 T cells from human PBMCs	CXCR4 expression $\downarrow$	Kasic et al., 2011

39A1, murine Th1 clone; A23187, calcium ionophore to activate T cells; ADCC, antibody-dependent cellular cytotoxicity; CDC25, murine Th2 cell clone; Con A, concanavalin A; D10.G4, murine Th1 cell clone; EL4, murine T lymphoma cell line; moDC, monocyte-derived dendritic cells; NOC12, N-ethyl-2-(1-ethyl-2-hydroxy-2-nitrosohydrazino)ethanamine; PF382, human T acute lymphoblastic leukemia; ST4, human T cell lymphoma; SupT1, human T cell lymphoma; TPA, O-tetradecanoylphorbol 13-acetate.

Mature and activated DCs that migrate into secondary lymphoid tissues prime and instruct T cells to manifest antigen-specific adaptive immune response. NO donors (GSNO, 1–20 μM, and DETA-NONOate, 50 μM) themselves have been shown to exhibit negligible effects or slightly enhance the proliferation of CD3$^+$ or CD8$^+$ T cells in MLR using DCs or DC-containing splenocytes as stimulating cells (Paolucci et al., 2003; Obregon et al., 2015; Kim et al., 2020). However, the proliferation of T cells was significantly improved when cells were cotreated with NO donors in addition to PTX or TNF-α (Paolucci et al., 2003; Obregon et al., 2015; Kim et al., 2020).

Overall, exogenous NO has complex and seemingly opposing effects on DCs (Table 2). Delivered NO appears to promote the differentiation into the DCs, improve the endocytic ability of mature DCs, improve the migration of mature DCs toward secondary lymphoid tissues, and induce negligible proliferation of primed T cells in vitro (Fig. 2). In addition, T cell

activation and proliferation induction by DCs can be improved when NO donors are used in combination with other agents including TNF-α, PTX, and PTX-containing DDS in vitro. However, exogenously supplied NO seems to also expand regulatory DCs, suppress the intracellular antigen processing of DCs, and exert negligible effects on DC activation, that can only marginally enhance T cell proliferation in vitro (Fig. 2). Accordingly, further investigations and optimizations are required to preferentially exploit the DC-stimulatory functions of NO delivery for antitumor immunotherapy.

B. Macrophages

Macrophages not only serve as T cell instructors but also play an important role in innate immunity in vivo by taking up pathogens and apoptotic cells (Chaperot et al., 2000; Hume, 2008; Ferenbach and Hughes, 2008; Gottschalk and Kurts, 2015). Macrophages are largely subcategorized into M1 and M2, based on their polarization states (Ley, 2017; Russell et al., 2019). Classically, M1 is defined as CD11b$^+$F4/80$^+$MHCII$^+$ macrophages to produce NO via an enzymatic reaction using $_L$-arginine and iNOS, while M2 is defined as CD11b$^+$F4/80$^+$CD206$^+$ macrophages

that avidly consume $_L$-arginine by arginase-1 (Ley, 2017; Russell et al., 2019; Nath and Kashfi, 2020). The proinflammatory M1 secrets high levels of cytokines including IL-12, IL-1β, and IL-23 to foster polarization of T lymphocytes to a T helper (Th) cell 1 type as well as DC maturation, whereas anti-inflammatory M2 produces IL-4, IL-10, and TGF-β (Ley, 2017; Russell et al., 2019; Nath and Kashfi, 2020). Tumor-associated macrophages are believed to accelerate the growth, angiogenesis, and metastasis of tumors, as well as contribute to the acquired resistance to chemotherapeutic drugs and immune checkpoint blockade by certain cancers (Ley, 2017; Lin et al., 2019; Russell et al., 2019; Nath and Kashfi, 2020). Because M2 is the dominant phenotype of tumor-associated macrophages, there have been continued efforts to reprogram M2 macrophages toward an M1 phenotype (Zanganeh et al., 2016; Rodell et al., 2018). Despite the potential antitumor effects of M1 macrophages (Zanganeh et al., 2016; Pang et al., 2018; Rodell et al., 2018), the functions of NO produced by M1 macrophages are debated as NO released from iNOS of M1 macrophages has been reported to not only exert direct cytotoxicity but also suppress the proliferation and activation of T cells (Bingisser et al., 1998; Lu et al., 2015; van der Veen et al., 2000).

In line with the pivotal roles of endogenous NO in macrophage-mediated cancer progression and response to therapy, the effects of exogenous NO on the polarization of macrophages have been investigated (Lu et al., 2015; Lee et al., 2020) (Fig. 3A). The solution containing NO generated by microwave plasma generator (NO$_2^-$ 316.7 μM + NO$_3^-$ 24.2 μM) induced M1 polarization with increased iNOS and decreased arginase-1 in RAW 264.7 macrophages (Lee et al., 2020). However, SNAP (100–1000 μM) suppressed iNOS expression by RAW 264.7 macrophages polarized to M1 by interferon (IFN)-γ (Lu et al., 2015). These results imply a potential feedback loop associated with NO in macrophage polarization.
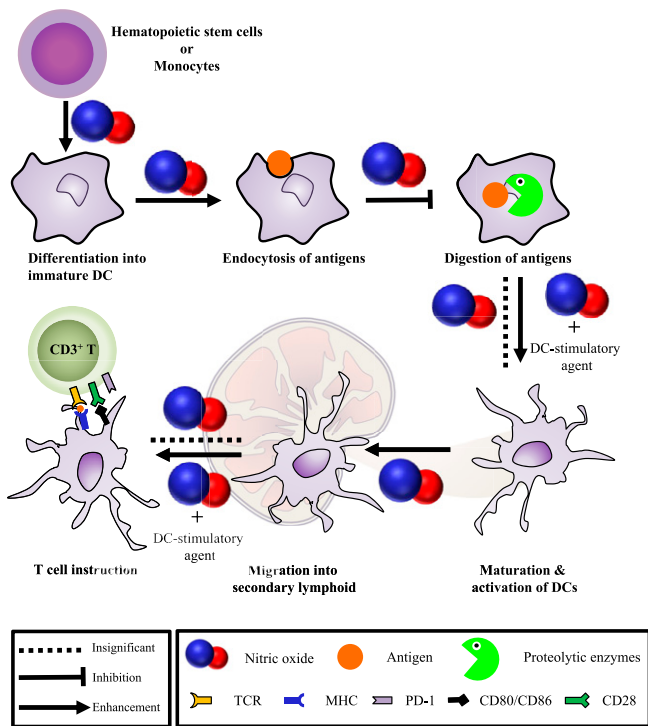
Fig. 2. Direct effects of exogenous NO on DCs. Exogenous NO improves differentiation of hematopoietic stem cells and monocytes to DCs. It enhances the endocytic functions of DCs, while hampering processing of intracellular antigen. Although exogenous NO itself has negligible effects on or inhibits the maturation of DCs, it can improve the effects of DC stimulating agents. It can also promote CCL19-mediated migration of DCs into the secondary lymphoid tissues. Although exogenous NO itself has an insignificant effect on or slightly enhances T cell proliferation, it can improve the efficacy of DC-stimulating agents on induction of T cell proliferation. These exogenous NO effects on DCs can be dependent on DC state as well as the types and dose of NO donors/delivery systems.

Fig. 3. Direct effects of exogenous NO on macrophages and B cells. (A) Exogenous NO promotes M1 polarization over M2, while the cotreatment with IFN-γ exerts the opposite effect. (B) Exogenous NO impairs the intracellular antigen processing functions of B cells by interfering with lysozyme activity.

C. B Cells

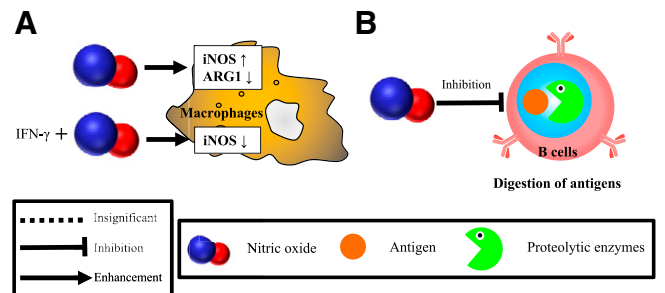B cells are not only APCs but also specialized immunoglobulin (Ig)-producing cells (Nelson, 2010; Tsou et al., 2016; Yuen et al., 2016; Sarvaria et al., 2017; Wennhold et al., 2019; Petitprez et al., 2020). Moreover, B cells promote antigen presentation by other APCs (Kurt-Jones et al., 1988), which in turn modulate T cell priming and response to immune checkpoint blockade therapy (Candolfi et al., 2011; Wang et al., 2016; Griss et al., 2019; Cabrita et al., 2020; Helmink et al., 2020; Petitprez et al., 2020). In addition to their pivotal roles in adaptive immunity, B cells also have innate functions as B cells activated by IFN-α or TLR agonist (CpG-containing oligodeoxynucleotides) can directly elicit cytotoxicity against cancer cells (Kemp et al., 2004). Owing to their versatile functions, adoptive immunotherapies with activated and primed B cells have been under development, with demonstrated antitumor efficacy (Li et al., 2009; Ren et al., 2014; Wennhold et al., 2019).

B cells produce NO, implying a role for NO in B cell immune functions (Mannick et al., 1994; Zhao et al., 1998; Bogdan, 2001; Jayasekera et al., 2006; Tumurkhuu et al., 2010; Saini et al., 2014; Bogdan 2015). As with DCs, exogenous NO (N-[4-[1-(3-aminopropyl)-2-hydroxy-2-nitrosohydrazino]butyl]1,3-propanediamine; 100–200 μM) impaired intracellular antigen processing functions of B cells in vitro (Lemaire et al., 2009) (Fig. 3B). These results are correlated with in vivo reports of a negligible effect of GSNO on B cell populations (Kim et al., 2020) and higher production of antibody in iNOS-deficient mice than wild-type mice against influenza virus infection (Jayasekera et al., 2006). Beyond what is known with respect to effects on intracellular antigen processing (Table 2), further investigations are required to elucidate the effects of exogenous NO on the functions and differentiation of B cells.

D. T Cells

CD8$^+$ T cells are one of the central effector cells in adoptive immune response to elicit antigen-specific cytotoxicity, and in many respects represent the holy grail in anticancer immunotherapy (Pardoll, 2012; Shin and Ribas, 2015; Burugu et al., 2018; Marin-Acevedo et al., 2018; Waldman et al., 2020). Although the differentiation, activation, and priming of CD8$^+$ T cells is mainly shaped by the APCs with which they interact, CD4$^+$ T cells including Th and T$_{reg}$s also influence the fate of CTLs by interacting with various immune cells and secreting cytokines and chemokines (Pardoll, 2012; Shin and Ribas, 2015; Burugu et al., 2018; Marin-Acevedo et al., 2018; Waldman et al., 2020). To improve the activation and differentiation of CD8$^+$ T cells, various antagonistic immune checkpoints blockade approaches have been developed with great clinical success and FDA approval (Pardoll, 2012; Shin and Ribas, 2015; Burugu et al., 2018; Marin-Acevedo et al., 2018; Waldman et al.,

2020). In addition, numerous adoptive T cell transfer therapies utilizing chimeric antigen receptor T cells have been under clinical investigation worldwide, resulting in the FDA approval of KYMRIAH and YESCARTA for treatment of B cell lymphoma (June et al., 2018; Waldman et al., 2020).

NO is also one of the key molecules in the function and differentiation of T cells via signal transduction and post-translational modification via S-nitrosylation (Garcia-Ortiz and Serrador, 2018). T cells also express NOSs to produce endogenous NO to modulate T cell metabolism and CD4$^+$ T cell differentiation, implying the pivotal role of NO in T phenotype (Garcia-Ortiz and Serrador, 2018). Interestingly, NO released from Tip-DCs improves the activity and proliferation of CD8$^+$ T cell in vivo, while NO generated from cancer cells is immunosuppressive against T cells (Shimamura et al., 2002; Serbina et al., 2003; Nicolas et al., 2007; Serbina et al., 2008; Marigo et al., 2016; Garcia-Ortiz and Serrador, 2018; Xue et al., 2018). These results imply yet again seemingly paradoxical functions of NO in T cell–mediated immune response.

The direct in vitro effects of exogenous NO on T cells have been widely investigated (Table 3). GSNO (100–500 μM), SNP (1 mM), and N-ethyl-2-(1-ethyl-2-hydroxy-2-nitrosohydrazino)ethanamine (100 μM) inhibited the proliferation of SupT1 (human T cell lymphoma), ST4 (human T cell lymphoma), PF382 (human T acute lymphoblastic leukemia), and Jurkat (human acute lymphoblastic line) cells (Allione et al., 1999; Henson et al., 1999). Treatment of SIN-1 (1–10 mM) resulted in tyrosine nitrosylation of the T cell receptor (TCR)–CD8 complex, which inhibited the proliferation of antigen-specific CD8$^+$ T cell by impairing the binding of peptide major histocompatibility complex (pMHC) to CD8$^+$ T cells, an effect that occurred without affecting T cell expression of CD8 and TCR (Nagaraj et al., 2007) (Fig. 4A). In addition, DETA-NONOate and SNAP (50–200 μM) inhibited the proliferation of CD4$^+$ T cells in a MLR assay using DCs as APC (Markowitz et al., 2017) (Fig. 4A).

NO donors govern the differentiation of CD4$^+$ T cells in a concentration-dependent manner. Low SNAP [1–0 μM (Niedbala et al., 1999) 10–100 μM (Lee et al., 2011)] and DETA-NONOate [10–100 μM (Lee et al., 2011); 5–10 μM (Niedbala et al., 2002)] concentration enhanced the differentiation of CD4$^+$ T cells toward a Th1 phenotype, while higher SNAP [50–500 μM (Niedbala et al., 1999) 10–100 μM (van der Veen et al., 1999)] or DETA-NONOate [10–100 μM (Niedbala et al., 2002)] suppressed Th1 differentiation (Niedbala et al., 1999; Niedbala et al., 2002) as well as the proliferation of Th1 and Th2 cells (van der Veen et al., 1999) (Fig. 4B). On the other hand, SNP (1–100 μM) and SNAP (20–200 μM) increased the production of IL-4 from Th2 clones and EL4 cells activated by concanavalin A or
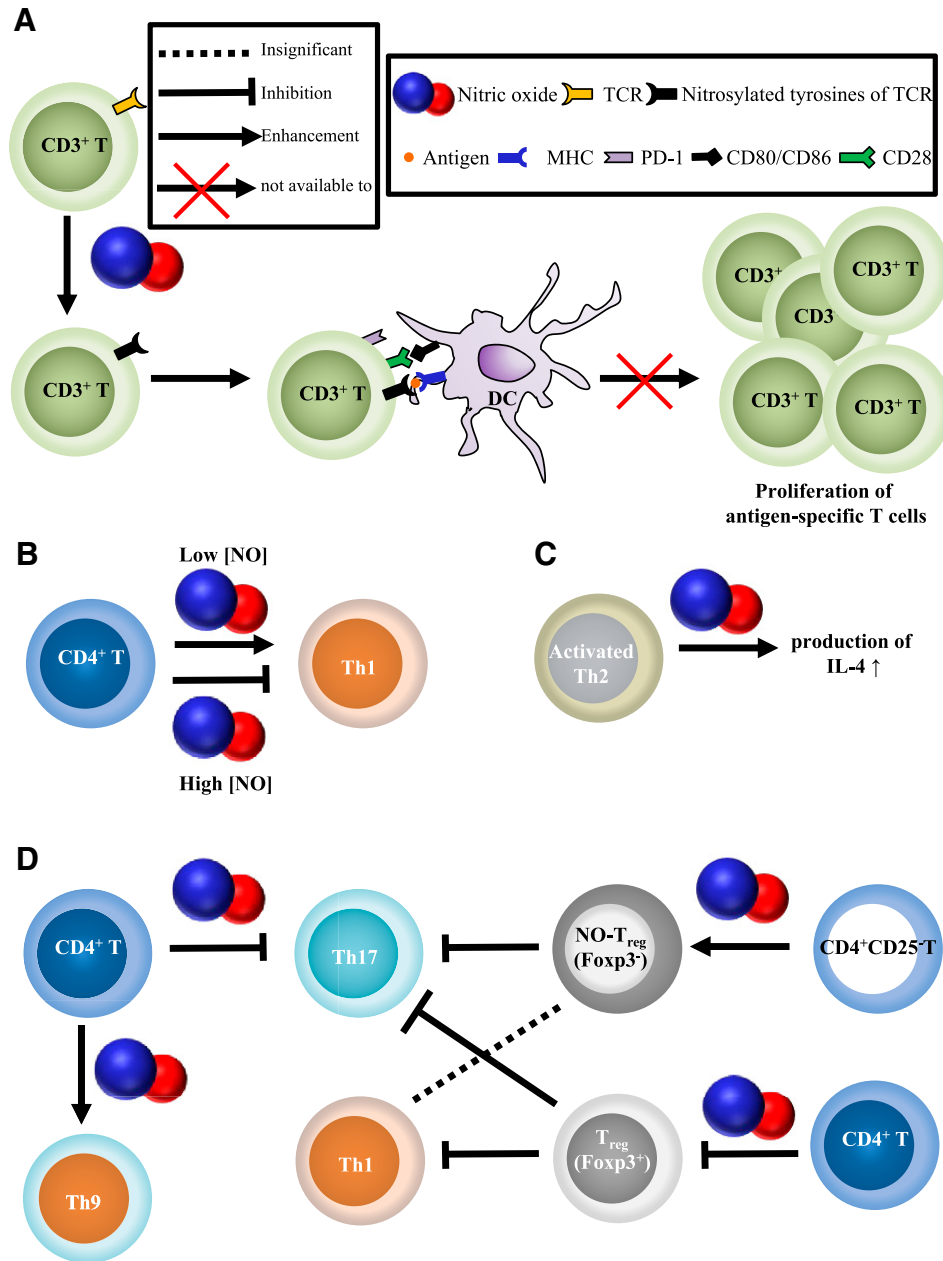
Fig. 4. Direct effects of exogenous NO on T cells. (A) Exogenous NO nitrosylates the TCR of T cells, which suppress the proliferation of T cells in response to antigen recognition. (B) Low concentrations of exogenous NO promotes the differentiation of CD4$^+$ T cells to a Th1 type, while high concentrations suppresses it. (C) Exogenous NO enhances the production of IL-4 from activated Th2 cells. (D) Exogenous NO suppresses Th17 differentiation from CD4$^+$ T cells partially by expansion of NO T$_{reg}$s. In addition, exogenous NO suppresses T$_{reg}$ differentiation from CD4$^+$ T cells and improves the proliferation of Th9 type cells.

O-tetradecanoylphorbol 13-acetate/A23187 (Chang et al., 1997) (Fig. 4C). The polarization and functions of Th17 cells were also found to be controlled by NO donors (Niedbala et al., 2011; Obermajer et al., 2013; Yang et al., 2013). Physiologic concentrations of DETA-NONOates (10–25 μM), which are comparable to the NO level produced by MDSCs induced Th17 differentiation with insignificant effects on CD4$^+$ T cell proliferation, whereas higher concentrations (100–200 μM) suppressed the proliferation of CD4$^+$ T cells (Obermajer et al., 2013) (Fig. 4D). This is in line with reports that DETA-NONOate (50–200 μM) decreases the size and proliferation of preestablished Th17

cell pools with the suppressed production of IL-17 and IL-22 in dose-dependent manner (Niedbala et al., 2011) (Fig. 4D). Likewise, SNAP (10–200 μM) suppressed the differentiation of Th17 from naïve CD4$^+$ T cells isolated from wild type as well as iNOS knockout mice (Yang et al., 2013). DETA-NONOate (100 μM), which suppressed Th17 cells, resulted in the enhanced differentiation of Th9 cells that are associated with anti-inflammatory responses to infection and allergy (Niedbala et al., 2014) (Fig. 4D). Interestingly, DETA-NONOate (50–200 μM) induced the differentiation of NO T$_{reg}$s defined as CD4$^+$

$CD25^+Foxp3^-$ converted from $CD4^+CD25^-Foxp3^-$ when exposed to NO (Niedbala et al., 2007), while SNAP [10–100 μM (Lee et al., 2011)] and DETA-NONOate [10–100 μM (Lee et al., 2011), 25 μM (Brahmachari and Pahan, 2009)] significantly suppressed the differentiation of normal T_{reg}s ($CD4^+CD25^+Foxp3^+$) (Fig. 4D). The NO T_{reg}s inhibited the proliferation of effector $CD4^+CD25^-$ T cells (Niedbala et al., 2007) and the polarization of Th17 without interfering the differentiation and functions of Th1 (Niedbala et al., 2013) (Fig. 4D). These NO T_{reg}s are distinguished from normal T_{reg}s to suppress the polarization of both Th1 and Th17 cells (Niedbala et al., 2013) (Fig. 4D). In addition, peroxynitrite ($ONOO^-$; 0.1–2.4 mM), a major product by superoxide and NO, inhibited the CCL-2 mediated T cell migration by suppressing the expression of CXCR4 (Kasic et al., 2011).

Overall, very high concentrations (>100 μM) of exogenous NO appear to inhibit the differentiation, proliferation, and migration of T cells, whereas low NO concentrations help the differentiation of $CD4^+$ T cells into Th1, Th2, Th17, and NO T_{reg} cells.

E. Natural Killer Cells

NK cells are mainly responsible for the innate immunity by utilizing granzyme B and perforin (Guillerey et al., 2016). Although NK cells lack antigen specificity, their rapid cytotoxicity against target cells have promoted the progress and development of genetic engineering and surface modification of NK cells for the treatment of various cancers (Kim et al., 2019; Rezvani et al., 2017). Endogenous NO production has been reported to be closely linked to the function and activation of NK cells (Cifone et al., 1994; Cifone et al., 2001; Lamas et al., 2012). Depletion of arginine also led to the suppression of proliferation, activation, and cytotoxic functions of NK cells (Lamas et al., 2012). Arginine supply increased the cytotoxic ability of NK cells, while NOS inhibitors suppressed it (Cifone et al., 1994; Cifone et al., 2001). In addition, the enhanced production of endogenous NO was accompanied with the activation of NK cells by IL-2 (Cifone et al., 1994). In contrast to endogenous NO, exogenously supplied NO inhibited the functions of NK cells via nitrosylation of functional components of NK cells, similar to effects seen in T cells (Stiff et al., 2018). SNAP (100 μM) treatment nitrosylated the tyrosine residues in NK cells, which impaired Fc receptor-mediated NK cell functions in vitro, such as production of IFN-γ and antibody dependent cellular cytotoxicity of NK cells on the cancer cells (Stiff et al., 2018) (Table 3). Further investigations are required to understand the roles of exogenous NO on the functions and differentiation of NK cells.

F. Myeloid-Derived Suppressor Cells

MDSCs play a central role regulating $CD4^+$ T, $CD8^+$ T, NK cells, and macrophage functions and are generally considered to promote tumor growth and metastasis (Burkholder et al., 2014; Kumar et al., 2016). Importantly, the functions of MDSCs are highly dependent on the NO produced by NOSs (Liao et al., 2014; Hirano et al., 2015; Yang et al., 2016). In detail, NO released from MDSCs nitrosylates the tyrosines in TCR, which suppresses the expansion of antigen specific $CD4^+$ and $CD8^+$ T cells in vivo (Nagaraj et al., 2007; Nagaraj et al., 2010; Nagaraj et al., 2013; Markowitz et al., 2017). In addition, iNOS is expressed by various cancer cells, which directly provokes the recruitment and expansion of MDSCs in tumor microenvironment by modulating vascular endothelial growth factor secretion in tumor and upregulating STAT3 and ROS in MDSCs (Jayaraman et al., 2012; Kim et al., 2020). That is, NO from tumors recruits and expands MDSCs, which in turn fosters a high reactive nitrogen species environment in the tumor to accelerate further infiltration of MDSCs (Jayaraman et al., 2012; Kim et al., 2020). The resultant iNOS-dependent positive feedback loop seems to result in nitrosylation of the chemokine CCL2 within tumors, which impedes the infiltration of T cells with antitumor functions into the tumor microenvironment (Molon et al., 2011; Kim et al., 2020). Considering that MDSCs promote the expansion of T_{reg}s through CD40–CD40 ligand interactions, NO-mediated expansion of MDSCs may contribute to increasing local T_{reg} populations (Pan et al., 2010). MDSCs furthermore restrain the Fc receptor–mediated functions of NK cells in antibody-coated target cells via iNOS expression (Stiff et al., 2018).

Several in vitro results discussed in the sections of T cells and NK cells are in line with the immunosuppressive functions of NO produced by MDSCs in vivo (Nagaraj et al., 2007; Kasic et al., 2011; Obermajer et al., 2013; Yang et al., 2013; Markowitz et al., 2017; Stiff et al., 2018). In particular, the immunosuppressive functions of MDSCs and exogenous NO donors were reversed in iNOS knockout animals (Nagaraj et al., 2007) and when used in combination with NOS inhibitors in wild-type animals (Yang et al., 2013; Markowitz et al., 2017; Stiff et al., 2018), emphasizing the role that exogenously delivered NO plays in mimicking the immunosuppressive functions of NO that is endogenously produced by MDSCs. Unfortunately, the direct effects of exogenous NO donors and delivery systems on the functions, differentiation, and expansion of MDSCs have not been well investigated in vitro.

VI. Recent Advances in Antitumor Immunotherapy Leveraging NO-Delivery Systems In Vivo

Although traditional strategies using NO donors and delivery systems focus on exerting NO's cytotoxicity directly on cancer cells, which exhibit powerful anticancer effects against various cancer cells in vitro, there have been conflicting reports of whether these strategies

really work (Shami et al., 2003; Kiziltepe et al., 2007; Duan et al., 2012; Fan et al., 2017; Yin et al., 2017; Deng et al., 2018; Park, Im et al., 2019; Zhao et al., 2019; You et al., 2020) or not (Feng et al., 2018; Ding et al., 2019; Li et al., 2019; Zhang, Lai et al., 2019; Zhang Jin et al., 2019; Chen et al., 2020) even in complex xenograft tumor models that use immune-deficient animals. Likewise, using NO donors and delivery systems without any help of other therapeutic agents has produced conflicting reports in allograft tumor models using conventional mouse models (Fan et al., 2015; Liu, Xiao et al., 2018; Studenovsky et al., 2018; Dong et al., 2019; Feng et al., 2019; Kang et al., 2019; An et al., 2020; Kim et al., 2020). S-nitrosothiol conjugated upconversion NPs (Fan et al., 2015), SNP (Feng et al., 2019), 1,3-bis-(2,4,6-trimethylphenyl)imidazolylidene NO-loaded micelles (Kang et al., 2019), GSNO containing zeolitic imidazolate framework with cancer cell membrane (An et al., 2020), nitrate-conjugated polymers (Studenovsky et al., 2018), nitrate-conjugated bovine serum albumin–protected gold nanocluster (Liu et al., 2018), and S-nitrosothiol–conjugated mesoporous silica NPs (Dong et al., 2019) exhibited negligible antitumor effects on murine breast 4T1 (Fan et al., 2015; Liu, Xiao et al., 2018; Dong et al., 2019; Feng et al., 2019; Kang et al., 2019; An et al., 2020), murine melanoma B16F10 (Kim et al., 2020), and EL4 murine T-cell lymphoma (Studenovsky et al., 2018) tumor models. Intratumoral GSNO treatment (570 μg/kg, one-time injection) even led to increased B16F10 tumor growth compared with saline treatment (Kim et al., 2020). In contrast, there are several reports demonstrating therapeutic effects of NO in vivo (Sung et al., 2019; Lee et al., 2020; Li, Ji et al., 2020). A solution containing NO generated by microwave plasma generator (Lee et al., 2020) and an implantable and wireless powered NO release system that stimulates more NO release from GSNO under wirelessly powered light-emitting diode to irradiate 335 or 545 nm (Li, Ji et al., 2020), elicited antitumor effects in allograft tumor-bearing normal mouse, specifically in B16F10-bearing C57BL/6 and luciferase expressing CT26 murine colorectal carcinoma cells (CT26-luc)-bearing Balb/C mouse, respectively. It is unclear how a solution containing NO generated by microwave plasma generator (Lee et al., 2020) and wireless-powered NO release system (Li et al., 2020) can lead to the antitumor effects as contrasted with other reports (Fan et al., 2015; Liu, Xiao et al., 2018; Studenovsky et al., 2018; Dong et al., 2019; Feng et al., 2019; Kang et al., 2019; An et al., 2020; Kim et al., 2020). These conflicting claims may be ascribed to the different kinds of NO donors/delivery systems, dose, administration routes, and tumor types used in those studies. Indeed, very high concentrations of NO were frequently administered or implanted into and near the tumor with a solution

containing NO generated by microwave plasma generator (Lee et al., 2020) or implantable and wireless-powered NO release system (Li, Ji et al., 2020), which might exert efficient direct cytotoxicity to tumor cells regardless of the complex interactions with the in vivo immune system. These reports suggest the potential of intratumoral administration of NO delivery systems to deliver very high concentrations of NO in the treatment of primary tumors.

As discussed, NO has both immune stimulatory and suppressive functions, which are highly dependent on NO concentration. Accordingly, it is natural to try to exploit immune-stimulatory functions more selectively by minutely controlling NO concentration. Sung et al. (2019) hypothesized that low NO dose could normalize the abnormal tumor vessel as NO plays a pivotal role in blood vessel homeostasis (Kim et al., 2011; Carpenter and Schoenfisch, 2012; Godo and Shimokawa, 2017) and vasodilation (Carpenter and Schoenfisch, 2012; Deepagan et al., 2018; An et al., 2020), while high doses of NO can eradicate the tumor by destroying the vessel of tumor as high concentration of NO can induce cytotoxicity to cells including blood vessel endothelial cells as well as cancer cells (Mocellin et al., 2007; Kim et al., 2017). Indeed, a very high dose of DNIC [Fe(μ-SEt)$_2$(NO)$_4$] containing lipid-PLGA hybrid NPs (DNIC equivalent to 0.5–1 mg/kg, six times intravenous injection) facilitated the suppression of tumor (murine hepatocellular carcinoma HCA-1 tumor model) growth with decreased mean vessel density in the tumor (Sung et al., 2019). However, CD4$^+$ and CD8$^+$ T cells were not expanded in the tumor. On the other hand, a low dose of DNIC (0.1 mg/kg, 6 times intravenous injection) NPs significantly enhanced perfusion and functional perfused vessel. Interestingly, the low dose of DNIC NPs selectively polarized M1 (F4/80$^+$CD86$^+$) over M2 (F4/80$^+$CD206$^+$), which significantly expanded CD4$^+$ and CD8$^+$ T cells in the tumor. In particular, DNIC NPs suppressed the PD-L1 expression on HCA-1 tumor cells in a dose-dependent manner in vivo. As a result, a low dose of DNIC NPs allowed the efficient T cell–mediated antitumor effects in the codelivery of a vaccine despite the insignificant antitumor effects of a vaccine or low dose of DNIC NPs used alone. Furthermore, a low dose of DNIC NPs suppressed the distal metastasis with negligible effects on the primary tumor.

Our group shed light on the issue of how high-dose NO suppresses the expansion of T cells (Kim et al., 2020) using a B16F10-OVA dual mouse tumor model. Melanomas were established by implanting B16F10-OVA cells in the left dorsal skin as a primary (1°) tumor on day 0 and in the right dorsal skin as a secondary (2°) tumor to investigate both the local and systemic anticancer effects as well as immune response as a result of NO donor treatment. One-time intratumoral administration of GSNO (570 μg/kg)

into the 1° tumor on day 7 increased 1° tumor size without affecting the size of the 2° tumor. MDSCs numbers were found to be significantly increased in both the 1° and 2° tumors as well as the lymph nodes draining each tumor seven days after one-time treatment of the primary tumor by intratumoral administration of GSNO (570 μg/kg). However, the number of total $CD8^+$, antigen-experienced $CD8^+$ (PD-1$^+$ CD8$^+$), and antigen-specific $CD8^+$ (tetramer$^+$ CD8$^+$) T cells were negligibly changed in both the 1° and 2° tumor, spleen, and both tumor-draining lymph nodes despite increased levels of activated and expanded DCs in the 2° tumor and spleen, ratios of M1 (CD11b$^+$F4/80$^+$CD86$^+$) to M2 (CD11b$^+$ F4/80$^+$CD206$^+$) macrophages in the spleen and lymph nodes draining the 2° tumor (2° dLNs), and NK cells (CD3$^-$NK1.1$^+$) in 2° dLNs. These results suggest that NO treatment induced the expansion of MDSCs (Nagaraj et al., 2007; Nagaraj et al., 2010; Jayaraman et al., 2012; Nagaraj et al., 2013; Markowitz et al., 2017), which contribute to preventing the instruction (Nagaraj et al., 2007; Nagaraj et al., 2010; Nagaraj et al., 2013; Markowitz et al., 2017) and infiltration (Molon et al., 2011) of $CD8^+$ T cell, and M1 macrophage- and NK cell–mediated (Stiff et al., 2018) antitumor response. Taken together, both studies suggest that high concentrations of NO prevent the development of robust antitumor immunity despite the presence of expanded and activated DCs, as well as M1-polarized macrophages.

The development of combined NO delivery systems with other therapeutic methodologies including chemotherapy, radiation therapy, photothermal therapy, photodynamic therapy, and immunotherapy can be an alternative to overcome the previously discussed dose-associated problems of NO delivery systems (Fan et al., 2015; Liu, Xiao et al., 2018; Studenovsky et al., 2018; Dong et al., 2019; Feng et al., 2019; Kang et al., 2019; Sung et al., 2019; An et al., 2020; Kim et al., 2020) because therapeutic effects of combined NO delivery systems are primarily not dependent on NO but rather on other therapeutic methodologies including chemotherapy, radiation therapy, photothermal therapy, and photodynamic therapy. S-nitrosothiol–conjugated upconversion NP enhanced the therapeutic effects of radiation therapy (Fan et al., 2015), and docetaxel- and SNP-containing mesoporous Prussian blue NPs improved the therapeutic effects of docetaxel and photothermal therapy (Feng et al., 2019). The enhanced perfusion by a low dose of DNIC NPs facilitated the improved penetration of DOX and tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) protein to the tumor, which enhanced the therapeutic effects of DOX and TRAIL although the low dose of DNIC NPs did not elicit antitumor effects by themselves (Sung et al., 2019). 1,3-Bis-(2,4,6-trimethylphenyl)imidazolylidene nitric oxide and DOX-loaded micelles enabled to enhance the therapeutic effects of DOX by promoting vasodilation of tumor blood vessels (Kang

et al., 2019). GSNO and chlorine e6-containing zeolitic imidazolate framework with cancer cell membrane exhibited the enhanced therapeutic effects compared with control groups (An et al., 2020). Nitrate-conjugated polymers promoted the antitumor effects of DOX-conjugated polymers (Studenovsky et al., 2018) and PTX-containing nitrate conjugated bovine serum albumin–protected gold nanocluster facilitated to encourage the antitumor effects of chemo and photothermal therapy (Liu, Xiao et al., 2018). DOX-loaded and S-nitrosothiol–conjugated mesoporous silica NPs showed higher antitumor effects than each DOX-loaded or S-nitrosothiol–conjugated one (Dong et al., 2019). Despite the therapeutic potential of combined NO delivery systems in allograft tumor models, most did not evaluate the associated immune response through immune phenotyping studies (Fan et al., 2015; Liu, Xiao et al., 2018; Studenovsky et al., 2018; Dong et al., 2019; Feng et al., 2019; Kang et al., 2019; Sung et al., 2019; An et al., 2020).

Our group demonstrated the potent immune response achieved through the combination of chemo- and NO-mediated cancer therapy by employing a B16F10-OVA dual tumor model (Kim et al., 2020). Interestingly, intratumoral administration of free PTX + free GSNO and pPTX/pCD-pSNO into the 1° tumor (PTX contents equivalent to 10 mg/kg PTX and NO contents equivalent to 570 μg/kg GSNO) showed similar antitumor effects on the 1° tumor compared with free PTX and pPTX/pCD-pSH, respectively. On the other hand, the intratumoral administration of free GSNO, free PTX+free GSNO, and pPTX/pCD-pSNO into the 1° tumor exhibited negligible antitumor effects on the 2° tumor compared with saline, while free PTX and pPTX/pCD-pSH improved the growth of 2° tumor. These results imply that NO mitigates systemic immune suppression induced by chemotherapeutic PTX but does not enhance its cytotoxic effects. Specifically, in response to pPTX/pCD-pSNO administration into the 1° tumor, MDSCs were expanded in the 1° tumor and its draining lymph nodes (1° dLN), and antigen-specific $CD8^+$ T cells (tetramer$^+$ CD8$^+$ T) were significantly suppressed in the 1° dLN despite the activation (CD86$^+$) and expansion of DCs (CD11b$^+$ CD11c$^+$) in the 1° dLN, spleen, and 2° tumor, and expansion of NK cells (CD3$^-$NK1.1$^+$) in the dLN and M1 (CD11b$^+$F4/80$^+$CD86$^+$) in spleen and 2° tumor.

Binding of CD28 on T cells to CD80/CD86 on APCs results in activation of T cells, while that of CTLA-4 on T cells to CD80/CD86 dampens the activations of T cells (Pardoll, 2012; Shin and Ribas, 2015; Burugu et al., 2018; Marin-Acevedo et al., 2018). As CTLA-4 has much higher affinity to CD80/CD86 than CD28, the antagonistic monoclonal antibodies to CTLA-4 facilitates the activation and expansion of antigen-specific $CD8^+$ T cells (Pardoll, 2012; Shin and Ribas, 2015; Burugu

et al., 2018; Marin-Acevedo et al., 2018). Based on the observed locoregional expansion of CD86-activated DCs resulting from administration of pPTX/pCD-pSNO into the 1° tumor (Kim et al., 2020), our group exploited antagonistic aCTLA-4 to overcome the attenuated expansion and antigen-presentation of $CD8^+$ T cell by activated DCs and M1 macrophages (Kim et al., 2020). In so doing, intratumoral administration in the 1° tumor of dual melanoma-bearing animals with pPTX/pCD-pSNO in combination with aCTLA-4 showed improved control of both 1° and 2° (abscopal) tumors, which led to prolonged animal survival, whereas aCTLA-4 alone elicited marginal and negligible therapeutic benefits.

Inspired by reports that NO controls transcription factor AP-1 (Tabuchi et al., 1994) that potentially modulates metabolism of CTLA-4 (Valk et al., 2008), we further unveiled a mechanism associated with immunosuppressive functions of exogenous NO (Kim, Francis et al., 2022). Subcutaneous administration of GSNO (570 μg/kg) into naïve mice was found to upregulate the extra- and intracellular expression of CTLA-4 by $F4/80^-$cDCs ($CD11b^+CD11c^+F4/80^-$), CD11c− macrophages ($CD11b^+CD11c^-F4/80^+$), and MDSCs in lymph nodes draining the injection site. It is important note that CTLA-4 can be expressed on cancer cells, DCs, and MDSCs in addition to T cells, which contributes to the suppression of T cell priming, differentiation, and function (Liu et al., 2009; Halpert et al., 2016; Chen et al., 2017; Hargadon, 2020; Kim, Francis et al., 2022). Considering that GSNO expanded and activated DCs as well (Kim et al., 2020; Kim, Francis et al., 2022), these results are interestingly in line with previous reports that NO donors expanding both effector and regulatory DCs (Si et al., 2016). However, intratumor administration of GSNO (570 μg/kg) in the B16F10-OVA dual tumor model resulted in the slight acceleration of both treated (1°) and untreated abscopal (2°) tumor growth, despite no effects on tumor cell proliferation and immunogenicity including CRT, CTLA-4, PD-1, and PD-L1 by tumor cells themselves in vitro and in vivo, respectively. These results imply that protumoral effects of GSNO may be attributed to the immunosuppression mediated by CTLA-4 expressing $F4/80^-$ cDCs, $CD11c^-$ macrophages, and MDSCs, which are also associated with the suppressed expansion, activation, and priming of $CD8^+$ T cell despite the presence of activated and expanded DCs that result from the administration of GSNO. Accordingly, our group hypothesized that the cotreatment of aCTLA-4 could unleash the functions of DCs activated by GSNO if immunosuppressive functions of CTLA-4–expressing immune cells induced by GSNO were antagonistically suppressed (Fig. 5). In testing this hypothesis by coadministration of GSNO intratumorally into the 1° tumor and aCTLA-4 intraperitoneally, growth of both 1° and 2° tumors was
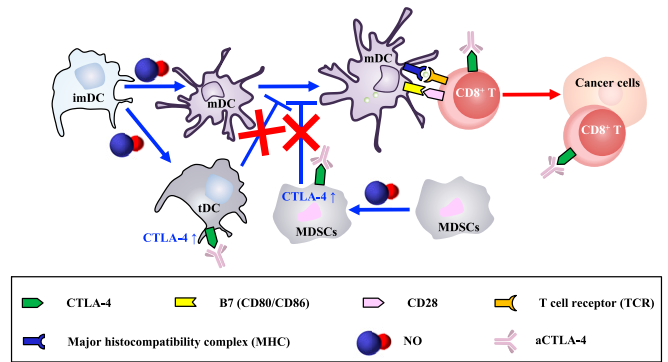

Fig. 5. The effects of exogenous NO on CTLA-4 expression and its potential to potentiate the effects of aCTLA-4 immunotherapy. Exogenous NO induces not only the maturation and activation of DCs but also the elevated expression of CTLA-4 on DCs, macrophages, and MDSCs, which suppresses $CD8^+$ T cell priming and expansion. Cotreatment with aCTLA-4 inhibits CTLA-4-mediated immunosuppression, which results in increased efficiency of DC-mediated $CD8^+$ T cell instruction that is otherwise suppressed by CTLA-4 expressing immune cells. Figure adapted from Kim, Francis et al. (2022).

observed that corresponded with significant expansions of total, activated $CD25^+$ and $LAG3^+$, antigen-experienced $PD-1^+$, antigen-specific $tetramer^+$ $CD8^+$ T cells, NK cells, and NK T cells in the blood. Given their potent immunotherapeutic synergies, we sought to explore the potential for sustained corelease of NO and aCTLA-4 antitumor immunotherapy (Kim, Francis et al., 2022). F127-grafted gelatin (F127-g-Gelatin) polymer was developed as a lower critical solution temperature polymer to exhibit a gelation at low polymer concentrations ($4.0-7.0$ wt%) at temperatures above 29°C to 31°C. The thermosensitive hydrogel could load GSNO and aCTLA-4 via a simple physical mixing, which allowed to release drugs over sustained times (days) both in vitro and in vivo. As hypothesized, the intratumoral sustained release of GSNO and aCTLA-4 with F127-g-Gelatin hydrogel significantly improved the therapeutic index in the dual B16F10-OVA melanoma model as well as a dual 4T1 breast tumor model, compared with treatment with free GSNO and aCTLA-4 (Kim, Francis et al., 2022).

Synthesizing what can be concluded from the three aforementioned investigations (Sung et al., 2019; Kim et al., 2020; Kim, Francis et al., 2022) (Table 4), NO delivered to the tumor and/or its dLNs promoted the local expansion of macrophages and DC populations, which consist of both effector and regulatory phenotypes (Si et al., 2016; Sung et al., 2019; Kim et al., 2020; Kim et al., 2022). However, MDSCs and T_{reg}s were expanded as well (Kim et al., 2020; Kim et al., 2022), which contributed to fostering an immunosuppressive environment (Nagaraj et al., 2007; Nagaraj et al., 2010; Pan et al., 2010; Molon et al., 2011; Jayaraman et al., 2012; Nagaraj et al., 2013; Markowitz et al., 2017; Stiff et al., 2018; Kim et al., 2020). Accordingly, delivery of high concentrations of NO failed to facilitate effective cancer immunotherapy

TABLE 4
Immunotherapeutic effects of delivered NO in preclinical in vivo tumor models

NO donors/delivery system	NO donor dose + other therapeutics	Tumor model	Immune profiles	Reference
DNIC/Lipid-PLGA NPs	0.1 mg/kg 6X i.v.	HCA-1 allograft tumor models	M1 ↑ PD-L1 ↓	Sung et al., 2019
	0.1 mg/kg 6X i.v. + vaccine (mitomycinc-C treated cGM-CSF-overexpressing HCA-1) 4X i.p.	HCA-1 allograft tumor models	Granzyme B$^+$CD8 T ↑	Sung et al., 2019
	Tumor suppression: NO releasing NP+vaccine > NO releasing NP			
-SNO/pPTX/pCD NPs	570 μg/kg + PTX 10 mg/kg 1X i.t.	B16F10 allograft dual tumor model	DC activation ↑, M1 ↑, MDSC ↑, NK ↑, antigen-specific CD8 T ↓	Kim et al., 2020
	570 μg/kg 1X i.t. + PTX 10 mg/kg 1X i.t. + aCTLA-4 100 μg/mice 3X i.p.	B16F10 allograft dual tumor model	Not determined	Kim et al., 2020
	Tumor suppression: PTX/-SNO NP + aCTLA-4 > PTX/-SNO NP > PTX NP, PTX/-SNO NP > free PTX + free GSNO			
GSNO	570 μg/kg 1X i.d.	Naïve mice	CTLA-4 ↑ on F4/80⁻cDCs, CD11c- macrophages, and MDSCs, DC activation ↑, Negligible effects on CD8 T	Kim, Francis et al., 2022
	570 μg/kg 1X i.t.	B16F10 allograft dual tumor model	Negligible changes on CRT, CTLA-4, PD-1, PD-L1 on B16F10 tumor cells	Kim, Francis et al., 2022
	570 μg/kg 1X i.d. + aCTLA-4 100 μg/mouse 3X i.p.	B16F10 allograft dual tumor model	CD8 T ↑, antigen-specific CD8 T ↑ NK ↑, NK T ↑	Kim, Francis et al., 2022
GSNO/hydrogel	570 μg/kg + aCTLA-4 100 μg/mouse in hydrogel 1X i.t.	B16F10 and 4T1 allograft dual tumor model	Not determined	Kim, Francis et al., 2022
	Tumor suppression: GSNO + aCTLA-4 in hydrogel > free GSNO + free aCTLA-4 > aCTLA-4 > Saline > GSNO			

Hydrogel, herein thermosensitive hydrogel comprised of gelatin and pluronic F127; PLGA, poly(lactic-co-glycolic acid); 4T1, murine breast cancer line.

(Sung et al., 2019; Kim et al., 2020; Kim, Francis et al., 2022). However, lower NO concentrations appeared to exert lower immunosuppressive functions, which allowed the CD8$^+$ T- and M1 macrophage-mediated antitumor immunotherapy in a codelivery with a vaccine (Sung et al., 2019). In particular, aCTLA-4 codelivery with NO led to efficient tumor control by rescuing the immunosuppressive environments fostered by CTLA-4 expressing DCs, macrophages, and MDSCs induced by NO, indicating controlled delivery of NO donors with synergistic agents in combination represents a new potential strategy in NO mediated anticancer immunotherapy (Kim, Francis et al., 2022). Lastly, sustained release of aCTLA-4 and NO through the development of a novel drug-laden micelle-releasing thermosensitive hydrogel not only improved local and systemic therapeutic effects of the drug combination but also achieved dose sparing and obviated the need for repeated administration (Kim, Francis et al., 2022), further demonstrating the utility and potential of sustained delivery systems in NO mediated antitumor therapy.

VII. Conclusions and Perspective

In summary, exogenous NO directly modulates the immunogenicity of cancer cells and the functions, activation, and differentiation of multiple immune cell subtypes that influence their complex interdependent immune stimulatory or immune-suppressive interactions relevant to antitumor therapy. The application of exogenous sources of NO improves the immunogenicity of cancer cells, expands the populations of activated DCs and M1-polarized macrophages, and helps to differentiate CD4$^+$ T cells into Th1, Th2, Th17, and NO T$_{reg}$s, while simultaneously manifesting immunosuppressive effects as well that include expansion of regulatory DCs, suppression of intracellular antigen process of DCs and B cells, inhibition of CTL differentiation, proliferation, and migration, and expansion of MDSCs. Three recent studies suggest new ways to exploit these immune-stimulatory functions more selectively; one involves the control of NO dose using a DDS (Sung et al., 2019), and the others explore combination therapy strategies that leverage NO donors used in combination with other immune-modulatory agents (Kim et al., 2020; Kim, Francis et al., 2022).

Due to the concentration-dependent paradoxical functions of NO, there are currently two strategies in antitumor therapy based on NO delivery systems. High concentrations of NO have the potential to induce direct tumor toxicity while simultaneously inducing systemic immune suppression. Accordingly, DDSs capable of delivering high concentrations of NO have been developed to result in direct killing of tumor cells (Lee et al., 2020; Li, Ji et al., 2020). An unintended consequence of such an approach, however, is accelerated tumor progression resulting from immunosuppression (Kim et al., 2020; Kim, Francis et al., 2022) elicited by NO treatment since current NO delivery systems are limited in their capacity in delivering high concentrations of NO only to

target sites (Kim et al., 2014; Kim et al., 2017; Park, Im et al., 2019; Kim, Suh et al., 2022). In addition, high systemic dose of NO has a significant risk of arterial pressure reduction due to the vasodilation effects of NO (Carpenter and Schoenfisch, 2012; Kim, Francis et al., 2022). Low NO concentrations on the other hand appear to induce an immune stimulatory environment but do so to extents insufficient to elicit direct antitumor effects as monotherapies (Sung et al., 2019). In this regard, combined NO delivery systems that deliver low concentrations of NO with other conventional agents with antitumor effects have been explored and have demonstrated promising potential.

Another one of the biggest hurdles to the broader utilization of NO in antitumor immunotherapy is the lack of stability and targeting ability of current NO delivery systems. Although a variety of NO donors are physically loaded or chemically conjugated to the DDSs, the resultant NO delivery systems are primarily based on SNP, S-nitrosothiols, N-diazeniumdiolates, and nitrates, which spontaneously release NO under physiologic conditions (Hrabie and Keefer, 2002; Wang et al., 2002; Riccio and Schoenfisch, 2012; Kim et al., 2014; Kim et al., 2017; Yang, Zelikin et al., 2018; Wu et al., 2021; Yang et al., 2021). To surmount this issue, redox-responsive O^2-protected N-diazeniumdiolates (Kumar et al., 2010; Park, Im et al., 2019), SIN-1 (Kim, Suh et al., 2022), UV-responsive NO donors (Pramanick et al., 2018), and ultrasound-responsive NO donors (Kang et al., 2019) have been used to develop stimuli-responsive NO delivery systems that do not spontaneously release NO during storage and blood circulation but does so under specific stimuli. In addition, several systems to use cascade reactions have been developed to not only attenuate NO release from NO donors before use but also to facilitate the target-specific NO release (Garcia et al., 2012; Choi et al., 2016). Other alternative strategies are to deliver L-Arg, a substrate of NOSs, to the tumor microenvironment (Kudo and Nagasaki, 2015; Cao et al., 2016; Fan et al., 2017; Jiang et al., 2018; Wan et al., 2018; Wang et al., 2019; Ma et al., 2020; Tao et al., 2022) or to deliver NOS gene (Chen et al., 2002; Cooney et al., 2007; Sharif et al., 2012), which enable the acceleration of endogenous NO production while preventing unintended NO release during storage and blood circulation.

Overall, the understanding of NO's effects on the immune system has rapidly expanded in recent years, which opens new opportunities to leverage its therapeutic potential. With the continuous efforts in optimizing dose, use in combination with other synergistic agents, and the development of controlled NO donors and delivery systems, NO's therapeutic potential marches closer to being fully realized.

References

Abbaraju PL, Meka AK, Song H, Yang Y, Jambhrunkar M, Zhang J, Xu C, Yu M, and Yu C (2017) Asymmetric silica nanoparticles with tunable head-tail structures enhance hemocompatibility and maturation of immune cells. *J Am Chem Soc* 139:6321–6328.
Adnan NNM, Sadrearhami Z, Bagheri A, Nguyen T-K, Wong EHH, Ho KKK, Lim M, Kumar N, and Boyer C (2018) Exploiting the versatility of polydopamine-coated nanoparticles to deliver nitric oxide and combat bacterial biofilm. *Macromol Rapid Commun* 39:e1800159.
Ahonen MJR, Dorrier JM, and Schoenfisch MH (2019) Antibiofilm efficacy of nitric oxide-releasing alginates against cystic fibrosis bacterial pathogens. *ACS Infect Dis* 5:1327–1335.
Akl J, Sasaki I, Lacroix PG, Hugues V, Vicendo P, Bocé M, Mallet-Ladeira S, Blanchard-Desce M, and Malfant I (2016) Trans- and cis-(Cl,Cl)-[Ru^II(FT)Cl_2 (NO)](PF_6): promising candidates for NO release in the NIR region. *Photochem Photobiol Sci* 15:1484–1491.
Allione A, Bernabei P, Bosticardo M, Ariotti S, Forni G, and Novelli F (1999) Nitric oxide suppresses human T lymphocyte proliferation through IFN-γ-dependent and IFN-γ-independent induction of apoptosis. *J Immunol* 163:4182–4191.
An J, Hu Y-G, Li C, Hou X-L, Cheng K, Zhang B, Zhang R-Y, Li D-Y, Liu S-J, Liu B, et al. (2020) A pH/Ultrasound dual-response biomimetic nanoplatform for nitric oxide gas-sonodynamic combined therapy and repeated ultrasound for relieving hypoxia. *Biomaterials* 230:119636.
Andronik-Lion V, Boucher JL, Delaforge M, Henry Y, and Mansuy D (1992) Formation of nitric oxide by cytochrome P450-catalyzed oxidation of aromatic amidoximes. *Biochem Biophys Res Commun* 185:452–458.
Bal-Price A, Moneer Z, and Brown GC (2002) Nitric oxide induces rapid, calcium-dependent release of vesicular glutamate and ATP from cultured rat astrocytes. *Glia* 40:312–323.
Bartberger MD, Mannion JD, Powell SC, Stamler JS, Houk KN, and Toone EJ (2001) S-N dissociation energies of S-nitrosothiols: on the origins of nitrosothiol decomposition rates. *J Am Chem Soc* 123:8868–8869.
Belmar J, Quezada J, Jimenez CA, Diaz-Gallifa P, Pasan J, and Ruiz-Perez C (2013) Synthesis, crystal structures and tautomerism in novel oximes based on hydroxyalkylpyrazolones. *New J Chem* 37:2002–2010.
Benavides GA, Liang Q, Dodson M, Darley-Usmar V, and Zhang J (2013) Inhibition of autophagy and glycolysis by nitric oxide during hypoxia-reoxygenation impairs cellular bioenergetics and promotes cell death in primary neurons. *Free Radic Biol Med* 65:1215–1228.
Besson E, Amalric J, Mehdi A, and Mutin PH (2009) Synthesis of a NO releasing lamellar silsesquioxane by topotactic exchange of CO_2 for NO. *J Mater Chem* 19:5723–5725.
Bingisser RM, Tilbrook PA, Holt PG, and Kees UR (1998) Macrophage-derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/STAT5 signaling pathway. *J Immunol* 160:5729–5734.
Bjorklund MD and Coburn MD (1980) 3,3-Dinitrobutyl-1.2.4-oxadiazoles (1). *J Heterocycl Chem* 17:819–821.
Blatt AH (1938) The tautomerism of oximes. *J Org Chem* 3:91–98.
Bogdan C (2001) Nitric oxide and the immune response. *Nat Immunol* 2:907–916.
Bogdan C (2015) Nitric oxide synthase in innate and adaptive immunity: an update. *Trends Immunol* 36:161–178.
Bohle DS, Chua Z, Perepichka I, and Rosadiuk K (2013) E/Z oxime isomerism in PhC(NOH)CN. *Chemistry* 19:4223–4229.
Bonavida B (2020) Sensitizing activities of nitric oxide donors for cancer resistance to anticancer therapeutic drugs. *Biochem Pharmacol* 176:113913.
Bonavida B, Baritaki S, Huerta-Yepez S, Vega MI, Chatterjee D, and Yeung K (2008) Novel therapeutic applications of nitric oxide donors in cancer: roles in chemo- and immunosensitization to apoptosis and inhibition of metastases. *Nitric Oxide* 19:152–157.
Bonavida B and Garban H (2015) Nitric oxide-mediated sensitization of resistant tumor cells to apoptosis by chemo-immunotherapeutics. *Redox Biol* 6:486–494.
Bonavida B, Khineche S, Huerta-Yepez S, and Garbán H (2006) Therapeutic potential of nitric oxide in cancer. *Drug Resist Updat* 9:157–173.
Bosworth CA, Toledo JC Jr, Zmijewski JW, Li Q, and Lancaster JR Jr (2009) Dinitrosyliron complexes and the mechanism(s) of cellular protein nitrosothiol formation from nitric oxide. *Proc Natl Acad Sci USA* 106:4671–4676.
Boucher J-L, Genet A, Vadon S, Delaforge M, Henry Y, and Mansuy D (1992) Cytochrome P450 catalyzes the oxidation of N^ω-hydroxy-L-arginine by NADPH and O_2 to nitric oxide and citrulline. *Biochem Biophys Res Commun* 187:880–886.
Bourassa J, DeGraff W, Kudo S, Wink DA, Mitchell JB, and Ford PC (1997) Photochemistry of Roussin's red salt, Na_2[Fe_2S_2(NO)_4], and of Roussin's black salt, NH_4[Fe_4S_3(NO)_7]. *in situ* nitric oxide generation to sensitize γ-radiation induced cell death. *J Am Chem Soc* 119:2853–2860.
Brahmachari S and Pahan K (2009) Suppression of regulatory T cells by IL-12p40 homodimer via nitric oxide. *J Immunol* 183:2045–2058.
Bratasz A, Selvendiran K, Wasowicz T, Bobko A, Khramtsov VV, Ignarro LJ, and Kuppusamy P (2008) NCX-4040, a nitric oxide-releasing aspirin, sensitizes drug-resistant human ovarian xenograft tumors to cisplatin by depletion of cellular thiols. *J Transl Med* 6:9.
Burkholder B, Huang R-Y, Burgess R, Luo S, Jones VS, Zhang W, Lv Z-Q, Gao C-Y, Wang B-L, Zhang Y-M, et al. (2014) Tumor-induced perturbations of cytokines and immune cell networks. *Biochim Biophys Acta* 1845:182–201.
Burugu S, Dancsok AR, and Nielsen TO (2018) Emerging targets in cancer immunotherapy. *Semin Cancer Biol* 52:39–52.

Bushan KM, Xu H, Ruane PH, D'Sa RA, Pavlos CM, Smith JA, Celius TC, and Toscano JP (2002) Controlled photochemical release of nitric oxide from *O(2)*-naphthylmethyl- and *O(2)*-naphthylallyl-substituted diazeniumdiolates. *J Am Chem Soc* 124:12640–12641.

Butler AR and Megson IL (2002) Non-heme iron nitrosyls in biology. *Chem Rev* 102:1155–1166.

Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, Johansson I, Phung B, Harbst K, Vallon-Christersson J, et al. (2020) Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature* 577:561–565.

Cai TB, Lu D, Tang X, Zhang Y, Landerholm M, and Wang PG (2005) New glycosidase activated nitric oxide donors: glycose and 3-morphorlinosydnonimine conjugates. *J Org Chem* 70:3518–3524.

Candolfi M, Curtin JF, Yagiz K, Assi H, Wibowo MK, Alzadeh GE, Foulad D, Muhammad AKMG, Salehi S, Keech N, et al. (2011) B cells are critical to T-cell-mediated antitumor immunity induced by a combined immune-stimulatory/conditionally cytotoxic therapy for glioblastoma. *Neoplasia* 13:947–960.

Cao Y, Feng Y, Zhang Y, Zhu X, and Jin F (2016) L-Arginine supplementation inhibits the growth of breast cancer by enhancing innate and adaptive immune responses mediated by suppression of MDSCs in vivo. *BMC Cancer* 16:343.

Cao Z, Bell JB, Mohanty JG, Nagababu E, and Rifkind JM (2009) Nitrite enhances RBC hypoxic ATP synthesis and the release of ATP into the vasculature: a new mechanism for nitrite-induced vasodilation. *Am J Physiol Heart Circ Physiol* 297:H1494–H1503.

Caro AA, Cederbaum AI, and Stoyanovsky DA (2001) Oxidation of the ketoxime acetoxime to nitric oxide by oxygen radical-generating systems. *Nitric Oxide* 5:413–424.

Carpenter AW, Reighard KP, Saavedra JE, and Schoenfisch MH (2013) *O2*-Protected diazeniumdiolate-modified silica nanoparticles for extended nitric oxide release from dental composites. *Biomater Sci* 1:456–459.

Carpenter AW and Schoenfisch MH (2012) Nitric oxide release: part II. Therapeutic applications. *Chem Soc Rev* 41:3742–3752.

Carpenter AW, Slomberg DL, Rao KS, and Schoenfisch MH (2011) Influence of scaffold size on bactericidal activity of nitric oxide-releasing silica nanoparticles. *ACS Nano* 5:7235–7244.

Carpenter AW, Worley BV, Slomberg DL, and Schoenfisch MH (2012) Dual action antimicrobials: nitric oxide release from quaternary ammonium-functionalized silica nanoparticles. *Biomacromolecules* 13:3334–3342.

Chakrapani H, Goodblatt MM, Udupi V, Malaviya S, Shami PJ, Keefer LK, and Saavedra JE (2008) Synthesis and in vitro anti-leukemic activity of structural analogues of JS-K, an anti-cancer lead compound. *Bioorg Med Chem Lett* 18:950–953.

Chakrapani H, Kalathur RC, Maciag AE, Citro ML, Ji X, Keefer LK, and Saavedra JE (2008) Synthesis, mechanistic studies, and anti-proliferative activity of glutathione/glutathione S-transferase-activated nitric oxide prodrugs. *Bioorg Med Chem* 16:9764–9771.

Chan CJ, Andrews DM, McLaughlin NM, Yagita H, Gilfillan S, Colonna M, and Smyth MJ (2010) DNAM-1/CD155 interactions promote cytokine and NK cell-mediated suppression of poorly immunogenic melanoma metastases. *J Immunol* 184:902–911.

Chang R-H, Feng M-HL, Liu W-H, and Lai M-Z (1997) Nitric oxide increased interleukin-4 expression in T lymphocytes. *Immunology* 90:364–369.

Chaperot L, Chokri M, Jacob M-C, Drillat P, Garban F, Egelhofer H, Molens J-P, Sotto J-J, Bensa J-C, and Plumas J (2000) Differentiation of antigen-presenting cells (dendritic cells and macrophages) for therapeutic application in patients with lymphoma. *Leukemia* 14:1667–1677.

Chen AF, Ren J, and Miao C-Y (2002) Nitric oxide synthase gene therapy for cardiovascular disease. *Jpn J Pharmacol* 89:327–336.

Chen X, Jia F, Li Y, Deng Y, Huang Y, Liu W, Jin Q, and Ji J (2020) Nitric oxide-induced stromal depletion for improved nanoparticle penetration in pancreatic cancer treatment. *Biomaterials* 246:119999.

Chen X, Shao Q, Hao S, Zhao Z, Wang Y, Guo X, He Y, Gao W, and Mao H (2017) CTLA-4 positive breast cancer cells suppress dendritic cells maturation and function. *Oncotarget* 8:13703–13715.

Chen X, Song M, Zhang B, and Zhang Y (2016) Reactive oxygen species regulate T cell immune response in the tumor microenvironment. *Oxid Med Cell Longev* 2016:1580967.

Choi HW, Kim J, Kim J, Kim Y, Song HB, Kim JH, Kim K, and Kim WJ (2016) Light-induced acid generation on a gatekeeper for smart nitric oxide delivery. *ACS Nano* 10:4199–4208.

Cifone MG, Festuccia C, Cironi L, Cavallo G, Chessa MA, Pensa V, Tubaro E, and Santoni A (1994) Induction of the nitric oxide-synthesizing pathway in fresh and interleukin 2-cultured rat natural killer cells. *Cell Immunol* 157:181–194.

Cifone MG, Ulisse S, and Santoni A (2001) Natural killer cells and nitric oxide. *Int Immunopharmacol* 1:1513–1524.

Cooney R, Hynes SO, Sharif F, Howard L, and O'Brien T (2007) Effect of gene delivery of NOS isoforms on intimal hyperplasia and endothelial regeneration after balloon injury. *Gene Ther* 14:396–404.

Costa AR, França RN, Silva-Jardim I, Silva RJS, Lima-Santos J, Salay LC, and Santos RLSR (2021) Self-assembling micellar system based on Pluronic and pyrazole-dithiocarbazate-conjugate stimulates production of nitric oxide from macrophages. *Colloid Interface Sci Commun* 41:100378.

Dai X, Pi G, Yang SL, Chen GG, Liu L-P, and Dong H-H (2018) Association of PD-L1 and HIF-1α coexpression with poor prognosis in hepatocellular carcinoma. *Transl Oncol* 11:559–566.

Daiber A and Münzel T (2015) Organic nitrate therapy, nitrate tolerance, and nitrate-induced endothelial dysfunction: emphasis on redox biology and oxidative stress. *Antioxid Redox Signal* 23:899–942.

Damodaran VB, Joslin JM, Wold KA, Lantvit SM, and Reynolds MM (2012) S-nitrosated biodegradable polymers for biomedical applications: synthesis, characterization and impact of thiol structure on the physicochemical properties. *J Mater Chem* 13:5990–6001.

Davies KM, Wink DA, Saavedra JE, and Keefer LK (2001) Chemistry of the diazeniumdiolates. 2. Kinetics and mechanism of dissociation to nitric oxide in aqueous solution. *J Am Chem Soc* 123:5473–5481.

De Boo S, Kopecka J, Brusa D, Gazzano E, Matera L, Ghigo D, Bosia A, and Riganti C (2009) iNOS activity is necessary for the cytotoxic and immunogenic effects of doxorubicin in human colon cancer cells. *Mol Cancer* 8:108.

Deepagan VG, Ko H, Kwon S, Rao NV, Kim SK, Um W, Lee S, Min J, Lee J, Choi KY, et al. (2018) Intracellularly activatable nanovasodilators to enhance passive cancer targeting regime. *Nano Lett* 18:2637–2644.

Deng Y, Jia F, Chen S, Shen Z, Jin Q, Fu G, and Ji J (2018) Nitric oxide as an all-rounder for enhanced photodynamic therapy: Hypoxia relief, glutathione depletion and reactive nitrogen species generation. *Biomaterials* 187:55–65.

de Oliveira MG, Shishido SM, Seabra AB, and Morgon NH (2002) Thermal stability of primary S-nitrosothiols: roles of autocatalysis and structural effects on the rate of nitric oxide release. *J Phys Chem A* 106:8963–8970.

DeRosa F, Kibbe MR, Najjar SF, Citro ML, Keefer LK, and Hrabie JA (2007) Nitric oxide-releasing fabrics and other acrylonitrile-based diazeniumdiolates. *J Am Chem Soc* 129:3786–3787.

Ding Y, Du C, Qian J, and Dong C-M (2019) NIR-responsive polypeptide nanocomposite generates NO gas, mild photothermia, and chemotherapy to reverse multidrug-resistant cancer. *Nano Lett* 19:4362–4370.

Dinh B, Dove K, Jappar D, Hrabie JA, and Davies KM (2005) Effect of hydrophobic structure on the catalysis of nitric oxide release from zwitterionic diazeniumdiolates in surfactant and liposome media. *Nitric Oxide* 13:204–209.

Dong X, Liu H-J, Feng H-Y, Yang S-C, Liu X-L, Lai X, Lu Q, Lovell JF, Chen H-Z, and Fang C (2019) Enhanced drug delivery by nanoscale integration of a nitric oxide donor to induce tumor collagen depletion. *Nano Lett* 19:997–1008.

Drago RS and Karstetter BR (1961) The reaction of nitrogen(II) oxide with various primary and secondary amines. *J Am Chem Soc* 83:1819–1822.

Duan S, Cai S, Yang Q, and Forrest ML (2012) Multi-arm polymeric nanocarrier as a nitric oxide delivery platform for chemotherapy of head and neck squamous cell carcinoma. *Biomaterials* 33:3243–3253.

Dunlap T, Abdul-Hay SO, Chandrasena RE, Hagos GK, Sinha V, Wang Z, Wang H, and Thatcher GRJ (2008) Nitrates and NO NSAIDs in cancer chemoprevention and therapy: in vitro evidence querying the NO donor functionality. *Nitric Oxide* 19:115–124.

Duong HTT, Kamarudin ZM, Erlich RB, Li Y, Jones MW, Kavallaris M, Boyer C, and Davis TP (2013) Intracellular nitric oxide delivery from stable NO polymeric nanoparticle carriers. *Chem Commun (Camb)* 49:4190–4192.

Duong HTT, Adnan NNM, Barraud N, Basuki JS, Kutty SK, Jung K, Kumar N, Davis TP, and Boyer C (2014) Functional gold nanoparticles for the storage and controlled release of nitric oxide: applications in biofilm dispersal and intracellular delivery. *J Mater Chem B Mater Biol Med* 2:5003–5011.

Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, Park D, Woodson RI, Ostankovich M, Sharma P, et al. (2009) Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* 461:282–286.

Eroy-Reveles AA, Leung Y, Beavers CM, Olmstead MM, and Mascharak PK (2008) Near-infrared light activated release of nitric oxide from designed photoactive manganese nitrosyls: strategy, design, and potential as NO donors. *J Am Chem Soc* 130:4447–4458.

Evig CB, Kelley EE, Weydert CJ, Chu Y, Buettner GR, and Burns CP (2004) Endogenous production and exogenous exposure to nitric oxide augment doxorubicin cytotoxicity for breast cancer cells but not cardiac myoblasts. *Nitric Oxide* 10:119–129.

Fan W, Bu W, Zhang Z, Shen B, Zhang H, He Q, Ni D, Cui Z, Zhao K, Bu J, et al. (2015) X-ray radiation-controlled NO release for on-demand depth-independent hypoxic radiosensitization. *Angew Chem Int Ed Engl* 54:14026–14030.

Fan W, Lu N, Huang P, Liu Y, Yang Z, Wang S, Yu G, Liu Y, Hu J, He Q, et al. (2017) Glucose-responsive sequential generation of hydrogen peroxide and nitric oxide for synergistic cancer starving-like/gas therapy. *Angew Chem Int Ed Engl* 56:1229–1233.

Fan Y, Wu W, Luo T, Hu Y, Zhang Q, Zhang J, and Xia X (2021) Cross-linking of S-nitrosothiolated AIEgens inside cancer cells to monitor NO release and reverse chemo-resistance. *Chem Commun (Camb)* 57:12520–12523.

Feng Q, Li Y, Yang X, Zhang W, Hao Y, Zhang H, Hou L, and Zhang Z (2018) Hypoxia-specific therapeutic agents delivery nanotheranostics: A sequential strategy for ultrasound mediated on-demand tritherapies and imaging of cancer. *J Control Release* 275:192–200.

Feng T, Wan J, Li P, Ran H, Chen H, Wang Z, and Zhang L (2019) A novel NIR-controlled NO release of sodium nitroprusside-doped Prussian blue nanoparticle for synergistic tumor treatment. *Biomaterials* 214:119213.

Ferenbach D and Hughes J (2008) Macrophages and dendritic cells: what is the difference? *Kidney Int* 74:5–7.

Fernández-Ruiz V, González A, and López-Moratalla N (2004) Effect of nitric oxide in the differentiation of human monocytes to dendritic cells. *Immunol Lett* 93:87–95.

Fionda C, Abruzzese MP, Zingoni A, Soriani A, Ricci B, Molfetta R, Paolini R, Santoni A, and Cippitelli M (2015) Nitric oxide donors increase PVR/CD155 DNAM-1 ligand expression in multiple myeloma cells: role of DNA damage response activation. *BMC Cancer* 15:17.

Fionda C, Malgarini G, Soriani A, Zingoni A, Cecere F, Iannitto ML, Ricciardi MR, Federico V, Petrucci MT, Santoni A, et al. (2013) Inhibition of glycogen synthase kinase-3 increases NKG2D ligand MICA expression and sensitivity to NK cell-mediated cytotoxicity in multiple myeloma cells: role of STAT3. *J Immunol* 190:6662–6672.

Ford PC (2013) Photochemical delivery of nitric oxide. *Nitric Oxide* 34:56–64.

Frederiksen LJ, Siemens DR, Heaton JP, Maxwell LR, Adams MA, and Graham CH (2003) Hypoxia induced resistance to doxorubicin in prostate cancer cells is inhibited by low concentrations of glyceryl trinitrate. *J Urol* 170:1003–1007.

Frei E (2011) Albumin binding ligands and albumin conjugate uptake by cancer cells. *Diabetol Metab Syndr* **3**:11.

Fry NL and Mascharak PK (2011) Photoactive ruthenium nitrosyls as NO donors: how to sensitize them toward visible light. *Acc Chem Res* **44**:289–298.

Fukumura D, Kashiwagi S, and Jain RK (2006) The role of nitric oxide in tumour progression. *Nat Rev Cancer* **6**:521–534.

Gansauge S, Gansauge F, Nussler AK, Rau B, Poch B, Schoenberg MH, and Beger HG (1997) Exogenous, but not endogenous, nitric oxide increases proliferation rates in senescent human fibroblasts. *FEBS Lett* **410**:160–164.

Gao J, Zheng Q, Xin N, Wang W, and Zhao C (2017) CD155, an onco-immunologic molecule in human tumors. *Cancer Sci* **108**:1934–1938.

Gao M, Liu S, Fan A, Wang Z, and Zhao Y (2015) Nitric oxide-releasing graft polymer micelles with distinct pendant amphiphiles. *RSC Advances* **5**:67041–67048.

Gao S, Zhang W, Wang R, Hopkins SP, Spagnoli JC, Racin M, Bai L, Li L, Jiang W, Yang X, et al. (2020) Nanoparticles encapsulating nitrosylated maytansine to enhance radiation therapy. *ACS Nano* **14**:1468–1481.

Garcia JV, Yang J, Shen D, Yao C, Li X, Wang R, Stucky GD, Zhao D, Ford PC, and Zhang F (2012) NIR-triggered release of caged nitric oxide using upconverting nanostructured materials. *Small* **8**:3800–3805.

García-Ortiz A and Serrador JM (2018) Nitric oxide signaling in T cell-mediated immunity. *Trends Mol Med* **24**:412–427.

Ghezzi P (2011) Role of glutathione in immunity and inflammation in the lung. *Int J Gen Med* **4**:105–113.

Giordano D, Magaletti DM, and Clark EA (2006) Nitric oxide and cGMP protein kinase (cGK) regulate dendritic-cell migration toward the lymph-node-directing chemokine CCL19. *Blood* **107**:1537–1545.

Godo S and Shimokawa H (2017) Divergent roles of endothelial nitric oxide synthases system in maintaining cardiovascular homeostasis. *Free Radic Biol Med* **109**:4–10.

Gostner JM, Becker K, Fuchs D, and Sucher R (2013) Redox regulation of the immune response. *Redox Rep* **18**:88–94.

Gotoh T and Mori M (2006) Nitric oxide and endoplasmic reticulum stress. *Arterioscler Thromb Vasc Biol* **26**:1439–1446.

Gottschalk C and Kurts C (2015) The debate about dendritic cells and macrophages in the kidney. *Front Immunol* **6**:435.

Granik VG (2010) Biotransformations of drugs belonging to nitrogen heterocycles. *Russ Chem Bull* **59**:1–36.

Griss J, Bauer W, Wagner C, Simon M, Chen M, Grabmeier-Pfistershammer K, Maurer-Granofszky M, Roka F, Penz T, Bock C, et al. (2019) B cells sustain inflammation and predict response to immune checkpoint blockade in human melanoma. *Nat Commun* **10**:4186.

Guan X, Lin L, Chen J, Hu Y, Sun P, Tian H, Maruyama A, and Chen X (2019) Efficient PD-L1 gene silence promoted by hyaluronidase for cancer immunotherapy. *J Control Release* **293**:104–112.

Guillerey C, Huntington ND, and Smyth MJ (2016) Targeting natural killer cells in cancer immunotherapy. *Nat Immunol* **17**:1025–1036.

Guo C, Xia Y, Niu P, Jiang L, Duan J, Yu Y, Zhou X, Li Y, and Sun Z (2015) Silica nanoparticles induce oxidative stress, inflammation, and endothelial dysfunction in vitro via activation of the MAPK/Nrf2 pathway and nuclear factor-κB signaling. *Int J Nanomedicine* **10**:1463–1477.

Halpenny GM, Steinhardt RC, Okialda KA, and Mascharak PK (2009) Characterization of pHEMA-based hydrogels that exhibit light-induced bactericidal effect via release of NO. *J Mater Sci Mater Med* **20**:2353–2360.

Halpert MM, Konduri V, Liang D, Chen Y, Wing JB, Paust S, Levitt JM, and Decker WK (2016) Dendritic cell-secreted cytotoxic T-lymphocyte-associated protein-4 regulates the T-cell response by downmodulating bystander surface B7. *Stem Cells Dev* **25**:774–787.

Hargadon KM (2020) Tumor microenvironmental influences on dendritic cell and T cell function: A focus on clinically relevant immunologic and metabolic checkpoints. *Clin Transl Med* **10**:374–411.

Harjunpää H and Guillerey C (2020) TIGIT as an emerging immune checkpoint. *Clin Exp Immunol* **200**:108–119.

Hart TW (1985) Some observations concerning the *S*-nitroso and *S*-phenylsulphonyl derivatives of ʟ-cysteine and glutathione. *Tetrahedron Lett* **26**:2013–2016.

Hasan N, Lee J, Kwak D, Kim H, Saparbayeva A, Ahn H-J, Yoon I-S, Kim M-S, Jung Y, and Yoo J-W (2021) Diethylenetriamine/NONOate-doped alginate hydrogel with sustained nitric oxide release and minimal toxicity to accelerate healing of MRSA-infected wounds. *Carbohydr Polym* **270**:118387.

Hays E and Bonavida B (2019a) Nitric oxide-mediated enhancement and reversal of resistance of anticancer therapies. *Antioxidants* **8**:407.

Hays E and Bonavida B (2019b) YY1 regulates cancer cell immune resistance by modulating PD-L1 expression. *Drug Resist Updat* **43**:10–28.

He X-Y, Ren X-H, Peng Y, Zhang J-P, Ai S-L, Liu B-Y, Xu C, and Cheng S-X (2020) Aptamer/peptide-functionalized genome-editing system for effective immune restoration through reversal of PD-L1-mediated cancer immunosuppression. *Adv Mater* **32**: e2000208.

Heidegger S, Gössl D, Schmidt A, Niedermayer S, Argyo C, Endres S, Bein T, and Bourquin C (2016) Immune response to functionalized mesoporous silica nanoparticles for targeted drug delivery. *Nanoscale* **8**:938–948.

Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, Yizhak K, Sade-Feldman M, Blando J, Han G, et al. (2020) B cells and tertiary lymphoid structures promote immunotherapy response. *Nature* **577**:549–555.

Henson SE, Nichols TC, Holers VM, and Karp DR (1999) The ectoenzyme γ-glutamyl transpeptidase regulates antiproliferative effects of S-nitrosoglutathione on human T and B lymphocytes. *J Immunol* **163**:1845–1852.

Hetrick EM, Prichard HL, Klitzman B, and Schoenfisch MH (2007) Reduced foreign body response at nitric oxide-releasing subcutaneous implants. *Biomaterials* **28**: 4571–4580.

Hetrick EM, Shin JH, Paul HS, and Schoenfisch MH (2009) Anti-biofilm efficacy of nitric oxide-releasing silica nanoparticles. *Biomaterials* **30**:2782–2789.

Hetrick EM, Shin JH, Stasko NA, Johnson CB, Wespe DA, Holmuhamedov E, and Schoenfisch MH (2008) Bactericidal efficacy of nitric oxide-releasing silica nanoparticles. *ACS Nano* **2**:235–246.

Hirano K, Hosoi A, Matsushita H, Iino T, Ueha S, Matsushima K, Seto Y, and Kakimi K (2015) The nitric oxide radical scavenger carboxy-PTIO reduces the immunosuppressive activity of myeloid-derived suppressor cells and potentiates the antitumor activity of adoptive cytotoxic T lymphocyte immunotherapy. *Oncoimmunology* **4**:e1019195.

Hirst D and Robson T (2007) Targeting nitric oxide for cancer therapy. *J Pharm Pharmacol* **59**:3–13.

Hogg N (2002) The biochemistry and physiology of S-nitrosothiols. *Annu Rev Pharmacol Toxicol* **42**:585–600.

Hong S, Kim J, Na YS, Park J, Kim S, Singha K, Im G-I, Han D-K, Kim WJ, and Lee H (2013) Poly(norepinephrine): ultrasmooth material-independent surface chemistry and nanodepot for nitric oxide. *Angew Chem Int Ed Engl* **52**:9187–9191. >

Hoogenboezem EN and Duvall CL (2018) Harnessing albumin as a carrier for cancer therapies. *Adv Drug Deliv Rev* **130**:73–89.

Horowitz JD, Antman EM, Lorell BH, Barry WH, and Smith TW (1983) Potentiation of the cardiovascular effects of nitroglycerin by N-acetylcysteine. *Circulation* **68**:1247–1253.

Horstmann A, Menzel L, Gäbler R, Jentsch A, Urban W, and Lehmann J (2002) Release of nitric oxide from novel diazeniumdiolates monitored by laser magnetic resonance spectroscopy. *Nitric Oxide* **6**:135–141.

Hou L, Zhang Y, Yang X, Tian C, Yan Y, Zhang H, Shi J, Zhang H, and Zhang Z (2019) Intracellular NO generator based on enzyme trigger for localized tumor-cytoplasm rapid drug release and synergetic cancer therapy. *ACS Appl Mater Interfaces* **11**:255–268.

Hrabie JA and Keefer LK (2002) Chemistry of the nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its oxygen-substituted derivatives. *Chem Rev* **102**:1135–1154.

Hu C, Cun X, Ruan S, Liu R, Xiao W, Yang X, Yang Y, Yang C, and Gao H (2018) Enzyme-triggered size shrink and laser-enhanced NO release nanoparticles for deep tumor penetration and combination therapy. *Biomaterials* **168**:64–75.

Huang J, Tatsumi T, Pizzoferrato E, Vujanovic N, and Storkus WJ (2005) Nitric oxide sensitizes tumor cells to dendritic cell-mediated apoptosis, uptake, and cross-presentation. *Cancer Res* **65**:8461–8470.

Huang X, Xu F, Hou H, Hou J, Wang Y, and Zhou S (2019) Stimuli-responsive nitric oxide generator for light-triggered synergistic cancer photothermal/gas therapy. *Nano Res* **12**:1361–1370.

Huerta S, Chilka S, and Bonavida B (2008) Nitric oxide donors: novel cancer therapeutics (review). *Int J Oncol* **33**:909–927.

Hume DA (2008) Macrophages as APC and the dendritic cell myth. *J Immunol* **181**:5829–5835.

Hwang S and Meyerhoff ME (2008) Polyurethane with tethered copper(II)-cyclen complex: preparation, characterization and catalytic generation of nitric oxide from S-nitrosothiols. *Biomaterials* **29**:2443–2452.

Ignarro LJ and Gruetter CA (1980) Requirement of thiols for activation of coronary arterial guanylate cyclase by glyceryl trinitrate and sodium nitrite: possible involvement of S-nitrosothiols. *Biochim Biophys Acta* **631**:221–231.

Ishima Y, Kragh-Hansen U, Maruyama T, and Otagiri M (2013) Poly-s-nitrosated albumin as a safe and effective multifunctional antitumor agent: characterization, biochemistry and possible future therapeutic applications. *BioMed Res Int* **2013**: 353892.

Jayaraman P, Parikh F, Lopez-Rivera E, Hailemichael Y, Clark A, Ma G, Cannan D, Ramacher M, Kato M, Overwijk WW, et al. (2012) Tumor-expressed inducible nitric oxide synthas controls induction of functional myeloid derived suppressor cells (MDSC) through modulation of VEGF release. *J Immunol* **188**:5365–5376.

Jayasekera JP, Vinuesa CG, Karupiah G, and King NJC (2006) Enhanced antiviral antibody secretion and attenuated immunopathology during influenza virus infection in nitric oxide synthase-2-deficient mice. *J Gen Virol* **87**:3361–3371.

Jiang L, Ding Y, Xue X, Zhou S, Li C, Zhang X, and Jiang X (2018) Entrapping multifunctional dendritic nanoparticles into a hydrogel for local therapeutic delivery and synergetic immunochemotherapy. *Nano Res* **11**:6062–6073.

Jiang W and Pisetsky DS (2006) The role of IFN-alpha and nitric oxide in the release of HMGB1 by RAW 264.7 cells stimulated with polyinosinic-polycytidylic acid or lipopolysaccharide. *J Immunol* **177**:3337–3343.

Jo YS, van der Vlies AJ, Gantz J, Thacher TN, Antonijevic S, Cavadini S, Demurtas D, Stergiopulos N, and Hubbell JA (2009) Micelles for delivery of nitric oxide. *J Am Chem Soc* **131**:14413–14418.

June CH, O'Connor RS, Kawalekar OU, Ghassemi S, and Milone MC (2018) CAR T cell immunotherapy for human cancer. *Science* **359**:1361–1365.

Kang Y, Kim J, Park J, Lee YM, Saravanakumar G, Park KM, Choi W, Kim K, Lee E, Kim C, et al. (2019) Tumor vasodilation by N-Heterocyclic carbene-based nitric oxide delivery triggered by high-intensity focused ultrasound and enhanced drug homing to tumor sites for anti-cancer therapy. *Biomaterials* **217**:119297.

Kasic T, Colombo P, Soldani C, Wang CM, Miranda E, Roncalli M, Bronte V, and Viola A (2011) Modulation of human T-cell functions by reactive nitrogen species. *Eur J Immunol* **41**:1843–1849.

Katayama N, Nakajou K, Ishima Y, Ikuta S, Yokoe J, Yoshida F, Suenaga A, Maruyama T, Kai T, and Otagiri M (2010) Nitrosylated human serum albumin (SNO HSA) induces apoptosis in tumor cells. *Nitric Oxide* **22**:259–265.

Katritzky AR, Khashab NM, Kirichenko N, and Singh A (2006) Microwave-assisted preparations of amidrazones and amidoximes. *J Org Chem* **71**:9051–9056.

Kaur I, Terrazas M, Kosak KM, Kern SE, Boucher KM, and Shami PJ (2013) Cellular distribution studies of the nitric oxide-generating antineoplastic prodrug O(2) -(2,4-dinitrophenyl)1-((4-ethoxycarbonyl)piperazin-1-yl)diazen-1-ium-1,2-diolate formulated in Pluronic P123 micelles. *J Pharm Pharmacol* **65**:1329–1336.

Keefer LK, Flippen-Anderson JL, George C, Shanklin AP, Dunams TM, Christodoulou D, Saavedra JE, Sagan ES, and Bohle DS (2001) Chemistry of the diazeniumdiolates.

I. Structural and spectral characteristics of the [N(O)NO]- functional group. *Nitric Oxide* **5**:377–394.

Kemp TJ, Moore JM, and Griffith TS (2004) Human B cells express functional TRAIL/Apo-2 ligand after CpG-containing oligodeoxynucleotide stimulation. *J Immunol* **173**:892–899.

Kenkare SR, Han C, and Benet LZ (1994) Correlation of the response to nitroglycerin in rabbit aorta with the activity of the mu class glutathione S-transferase. *Biochem Pharmacol* **48**:2231–2235.

Kevil CG and Patel RP (2010) S-Nitrosothiol biology and therapeutic potential in metabolic disease. *Curr Opin Investig Drugs* **11**:1127–1134.

Kim J, Francis DM, Sestito LF, Archer PA, Manspeaker MP, O'Melia MJ, and Thomas SN (2022) Thermosensitive hydrogel releasing nitric oxide donor and anti-CTLA-4 micelles for anti-tumor immunotherapy. *Nat Commun* **13**:1479.

Kim J, Lee Y, Singha K, Kim HW, Shin JH, Jo S, Han D-K, and Kim WJ (2011) NONOates—polyethylenimine hydrogel for controlled nitric oxide release and cell proliferation modulation. *Bioconjug Chem* **22**:1031–1038.

Kim J, Saravanakumar G, Choi HW, Park D, and Kim WJ (2014) A platform for nitric oxide delivery. *J Mater Chem B Mater Biol Med* **2**:341–356.

Kim J, Sestito LF, Im S, Kim WJ, and Thomas SN (2020) Poly(cyclodextrin)-polydrug nanocomplexes as synthetic oncolytic virus for locoregional melanoma chemoimmunotherapy. *Adv Funct Mater* **30**:1908788.

Kim J, Yung BC, Kim WJ, and Chen X (2017) Combination of nitric oxide and drug delivery systems: tools for overcoming drug resistance in chemotherapy. *J Control Release* **263**:223–230.

Kim K-S, Han J-H, Park J-H, Kim H-K, Choi SH, Kim GR, Song H, An HJ, Han DK, Park W, et al. (2019)Multifunctional nanoparticles for genetic engineering and bioimaging of natural killer (NK) cell therapeutics. *Biomaterials* **221**:119418.

Kim MM, Parmar H, Cao Y, Pramanik P, Schipper M, Hayman J, Junck L, Mammoser A, Heth J, Carter CA, et al. (2016) Whole brain radiotherapy and RRx-001: two partial responses in radioresistant melanoma brain metastases from a phase I/II clinical trial: a TITE-CRM phase I/II clinical trial. *Transl Oncol* **9**:108–113.

Kim T, Suh J, Kim J, and Kim WJ (2022) Lymph-directed self-immolative nitric oxide prodrug for inhibition of intractable metastatic cancer. *Adv Sci (Weinh)* **9**:2101935.

King J, Mir H, and Singh S (2017) Association of cytokines and chemokines in pathogenesis of breast cancer. *Prog Mol Biol Transl Sci* **151**:113–136.

Kiriyama Y, Tani A, Kadoya M, Okamoto R, and Nochi H (2020) Induction of PD-L1 by nitric oxide via JNK activation in A172 glioblastoma cells. *Biol Pharm Bull* **43**:1020–1022.

Kitagaki J, Yang Y, Saavedra JE, Colburn NH, Keefer LK, and Perantoni AO (2009) Nitric oxide prodrug JS-K inhibits ubiquitin E1 and kills tumor cells retaining wild-type p53. *Oncogene* **28**:619–624.

Kiziltepe T, Hideshima T, Ishitsuka K, Ocio EM, Raje N, Catley L, Li C-Q, Trudel LJ, Yasui H, Vallet S, et al. (2007) JS-K, a GST-activated nitric oxide generator, induces DNA double-strand breaks, activates DNA damage response pathways, and induces apoptosis in vitro and in vivo in human multiple myeloma cells. *Blood* **110**:709–718.

Konorev EA, Kalyanaraman B, and Hogg N (2000)Modification of creatine kinase by S-nitrosothiols: S-nitrosation vs. S-thiolation. *Free Radic Biol Med* **28**:1671–1678.

Konovalova NP, Goncharova SA, Volkova LM, Rajewskaya TA, Eremenko LT, and Korolev AM (2003) Nitric oxide donor increases the efficiency of cytostatic therapy and retards the development of drug resistance. *Nitric Oxide* **8**:59–64.

Kopecka J, Campia I, Brusa D, Doublier S, Matera L, Ghigo D, Bosia A, and Riganti C (2011) Nitric oxide and P-glycoprotein modulate the phagocytosis of colon cancer cells. *J Cell Mol Med* **15**:1492–1504.

Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, and Vandenabeele P (2012) Immunogenic cell death and DAMPs in cancer therapy. *Nat Rev Cancer* **12**:860–875.

Kudo S and Nagasaki Y (2015) A novel nitric oxide-based anticancer therapeutics by macrophage-targeted poly(l-arginine)-based nanoparticles. *J Control Release* **217**:256–262.

Kumar V, Hong SY, Maciag AE, Saavedra JE, Adamson DH, Prud'homme RK, Keefer LK, and Chakrapani H (2010) Stabilization of the nitric oxide (NO) prodrugs and anticancer leads, PABA/NO and double JS-K, through incorporation into PEG-protected nanoparticles. *Mol Pharm* **7**:291–298.

Kumar V, Patel S, Tcyganov E, and Gabrilovich DI (2016) The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol* **37**:208–220.

Kurt-Jones EA, Liano D, HayGlass KA, Benacerraf B, Sy MS, and Abbas AK (1988) The role of antigen-presenting B cells in T cell priming in vivo. Studies of B cell-deficient mice. *J Immunol* **140**:3773–3778.

Lamas B, Vergnaud-Gauduchon J, Goncalves-Mendes N, Perche O, Rossary A, Vasson M-P, and Farges M-C (2012) Altered functions of natural killer cells in response to L-Arginine availability. *Cell Immunol* **280**:182–190.

Lee CB, Seo IH, Chae M-W, Park JW, Choi EH, Uhm HS, and Baik KY (2020) Anticancer activity of liquid treated with microwave plasma-generated gas through macrophage activation. *Oxid Med Cell Longev* **2020**:2946820.

Lee H, Dellatore SM, Miller WM, and Messersmith PB (2007) Mussel-inspired surface chemistry for multifunctional coatings. *Science* **318**:426–430.

Lee S-W, Choi H, Eun S-Y, Fukuyama S, and Croft M (2011) Nitric oxide modulates TGF-β-directive signals to suppress Foxp3+ regulatory T cell differentiation and potentiate Th1 development. *J Immunol* **186**:6972–6980.

Lemaire G, Guittet O, Vesin M-F, Lepoivre M, and Cottet M-H (2009) Glutathione depletion reveals impairment of antigen processing and inhibition of cathepsin activity by nitric oxide in antigen-presenting cells. *Mol Immunol* **46**:1100–1108.

Lequeux A, Noman MZ, Xiao M, Sauvage D, Van Moer K, Viry E, Bocci I, Hasmim M, Bosseler M, Berchem G, et al. (2019) Impact of hypoxic tumor microenvironment and tumor cell plasticity on the expression of immune checkpoints. *Cancer Lett* **458**:13–20.

Ley K (2017) M1 means kill; M2 means heal. *J Immunol* **199**:2191–2193.

Li B, Ji P, Peng S-Y, Pan P, Zheng D-W, Li C-X, Sun Y-X, and Zhang X-Z (2020) Nitric oxide release device for remote-controlled cancer therapy by wireless charging. *Adv Mater* **32**:e2000376.

Li J, Jiang R, Wang Q, Li X, Hu X, Yuan Y, Lu X, Wang W, Huang W, and Fan Q (2019) Semiconducting polymer nanotheranostics for NIR-II/Photoacoustic imaging-guided photothermal initiated nitric oxide/photothermal therapy. *Biomaterials* **217**:119304.

Li Q, Teitz-Tennenbaum S, Donald EJ, Li M, and Chang AE (2009) In vivo sensitized and in vitro activated B cells mediate tumor regression in cancer adoptive immunotherapy. *J Immunol* **183**:3195–3203.

Li S, Song X, Zhu W, Chen Y, Zhu R, Wang L, Chen X, Song J, and Yang H (2020) Light-switchable yolk-mesoporous shell UCNPs@MgSiO3 for nitric oxide-evoked multidrug resistance reversal in cancer therapy. *ACS Appl Mater Interfaces* **12**:30066–30076.

Li WA, Lu BY, Gu L, Choi Y, Kim J, and Mooney DJ (2016) The effect of surface modification of mesoporous silica micro-rod scaffold on immune cell activation and infiltration. *Biomaterials* **83**:249–256.

Liao J, Wang X, Bi Y, Shen B, Shao K, Yang H, Lu Y, Zhang Z, Chen X, Liu H, et al. (2014) Dexamethasone potentiates myeloid-derived suppressor cell function in prolonging allograft survival through nitric oxide. *J Leukoc Biol* **96**:675–684.

Lin Y, Xu J, and Lan H (2019) Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. *J Hematol Oncol* **12**:76.

Lipke EA and West JL (2005) Localized delivery of nitric oxide from hydrogels inhibits neointima formation in a rat carotid balloon injury model. *Acta Biomater* **1**:597–606.

Liu B, Huang X, Li Y, Liao W, Li M, Liu Y, He R, Feng D, Zhu R, and Kurihara H (2019) JS-K, a nitric oxide donor, induces autophagy as a complementary mechanism inhibiting ovarian cancer. *BMC Cancer* **19**:645.

Liu H, Zhu X, Guo H, Huang H, Huang S, Huang S, Xue W, Zhu P, and Guo R (2020) Nitric oxide released injectable hydrogel combined with synergistic photothermal therapy for antibacterial and accelerated wound healing. *Appl Mater Today* **20**:100781.

Liu L, Huang Z, Chen J, Wang J, and Wang S (2018) Protein phosphatase 2A activation mechanism contributes to JS-K induced caspase-dependent apoptosis in human hepatocellular carcinoma cells. *J Exp Clin Cancer Res* **37**:142.

Liu R, Xiao W, Hu C, Xie R, and Gao H (2018) Theranostic size-reducible and no donor conjugated gold nanocluster fabricated hyaluronic acid nanoparticle with optimal size for combinational treatment of breast cancer and lung metastasis. *J Control Release* **278**:127–139.

Liu Y, Yu Y, Yang S, Zeng B, Zhang Z, Jiao G, Zhang Y, Cai L, and Yang R (2009) Regulation of arginase I activity and expression by both PD-1 and CTLA-4 on the myeloid-derived suppressor cells. *Cancer Immunol Immunother* **58**:687–697.

Liu Z, Li G, Gou Y, Xiao D, Luo G, Saavedra JE, Liu J, and Wang H (2017) JS-K, a nitric oxide prodrug, induces DNA damage and apoptosis in HBV-positive hepatocellular carcinoma HepG2.2.15 cell. *Biomed Pharmacother* **92**:989–997.

Lopez M, Malacarne PF, Gajos-Draus A, Ding X, Daiber A, Lundberg JO, Offermanns S, Brandes RP, and Rezende F (2021) Vascular biotransformation of organic nitrates is independent of cytochrome P450 monooxygenases. *Br J Pharmacol* **178**:1495–1506.

Lossen W (1889) Structural formulae of hydroxylamine and its derivatives. *Justus Liebigs Ann Chem* **252**:170–224.

Lu D, Nadas J, Zhang G, Johnson W, Zweier JL, Cardounel AJ, Villamena FA, and Wang PG (2007) 4-Aryl-1,3,2-oxathiazolylium-5-olates as pH-controlled NO donors: the next generation of S-nitrosothiols. *J Am Chem Soc* **129**:5503–5514.

Lu G, Zhang R, Geng S, Peng L, Jayaraman P, Chen C, Xu F, Yang J, Li Q, Zheng H, et al. (2015) Myeloid cell-derived inducible nitric oxide synthase suppresses M1 macrophage polarization. *Nat Commun* **6**:6676.

Lu Y, Slomberg DL, and Schoenfisch MH (2014) Nitric oxide-releasing chitosan oligosaccharides as antibacterial agents. *Biomaterials* **35**:1716–1724.

Lu Y, Sun B, Li C, and Schoenfisch MH (2011) Structurally diverse nitric oxide-releasing poly(propylene imine) dendrimers. *Chem Mater* **23**:4227–4233.

Ma Z, Liu S, Ke Y, Wang H, Chen R, Xiang Z, Xie Z, Shi Q, and Yin J (2020) Biomimetic nano-NOS mediated local NO release for inhibiting cancer-associated platelet activation and disrupting tumor vascular barriers. *Biomaterials* **255**:120141.

Mahanty S, Prigent A, and Garraud O (2015) Immunogenicity of infectious pathogens and vaccine antigens. *BMC Immunol* **16**:31.

Mahnke K and Enk AH (2016) TIGIT-CD155 interactions in melanoma: a novel co-inhibitory pathway with potential for clinical intervention. *J Invest Dermatol* **136**:9–11.

Makings LR and Tsien RY (1994) Caged nitric oxide: stable organic molecules from which nitric oxide can be photoreleased. *J Biol Chem* **269**:6282–6285.

Maloney SE, McGrath KV, Ahonen MJR, Soliman DS, Feura ES, Hall HR, Wallet SM, Maile R, and Schoenfisch MH (2021) Nitric Oxide-Releasing Hyaluronic Acid as an Antibacterial Agent for Wound Therapy. *Biomacromolecules* **22**:867–879.

Mannick JB, Asano K, Izumi K, Kieff E, and Stamler JS (1994) Nitric oxide produced by human B lymphocytes inhibits apoptosis and Epstein-Barr virus reactivation. *Cell* **79**:1137–1146.

Mäntylä A, Rautio J, Nevalainen T, Vepsäläinen J, Juvonen R, Kendrick H, Garnier T, Croft SL, and Järvinen T (2004) Synthesis and antileishmanial activity of novel buparvaquone oxime derivatives. *Bioorg Med Chem* **12**:3497–3502.

Marazzi M, López-Delgado A, Fernández-González MA, Castaño O, Frutos LM, and Temprado M (2012) Modulating nitric oxide release by S-nitrosothiol photocleavage: mechanism and substituent effects. *J Phys Chem A* **116**:7039–7049.

Mardente S, Zicari A, Consorti F, Mari E, Di Vito M, Leopizzi M, Della Rocca C, and Antonaci A (2010) Cross-talk between NO and HMGB1 in lymphocytic thyroiditis and papillary thyroid cancer. *Oncol Rep* **24**:1455–1461.

Marigo I, Zilio S, Desantis G, Mlecnik B, Agnellini AHR, Ugel S, Sasso MS, Qualls JE, Kratochvill F, Zanovello P, et al. (2016) T cell cancer therapy requires CD40-

CD40L activation of tumor necrosis factor and inducible nitric-oxide-synthase-producing dendritic cells. *Cancer Cell* 30:377–390.

Marin-Acevedo JA, Dholaria B, Soyano AE, Knutson KL, Chumsri S, and Lou Y (2018) Next generation of immune checkpoint therapy in cancer: new developments and challenges. *J Hematol Oncol* 11:39.

Markowitz J, Wang J, Vangundy Z, You J, Yildiz V, Yu L, Foote IP, Branson OE, Stiff AR, Brooks TR, et al. (2017) Nitric oxide mediated inhibition of antigen presentation from DCs to CD4+ T cells in cancer and measurement of STAT1 nitration. *Sci Rep* 7:15424.

Martins I, Wang Y, Michaud M, Ma Y, Sukkurwala AQ, Shen S, Kepp O, Métivier D, Galluzzi L, Perfettini J-L, et al. (2014)Molecular mechanisms of ATP secretion during immunogenic cell death. *Cell Death Differ* 21:79–91.

Marxer SM, Rothrock AR, Nablo BJ, Robbins ME, and Schoenfisch MH (2003) Preparation of nitric oxide (NO)-releasing Sol–gels for biomaterial applications. *Chem Mater* 15:4193–4199.

Mascharak PK (2012) NO and CO-donors: an emerging class of pharmaceuticals in current medicine. *Indian J Chem Sect A* 51:99–107.

Massa CM, Liu Z, Taylor S, Pettit AP, Stakheyeva MN, Korotkova E, Popova V, Atochina-Vasserman EN, and Gow AJ (2021) Biological mechanisms of *S*-nitrosothiol formation and degradation: how is specificity of *S*-nitrosylation achieved? *Antioxidants* 10:1111.

Mateo J, García-Lecea M, Cadenas S, Hernández C, and Moncada S (2003) Regulation of hypoxia-inducible factor-1α by nitric oxide through mitochondria-dependent and -independent pathways. *Biochem J* 376:537–544.

Matthews NE, Adams MA, Maxwell LR, Gofton TE, and Graham CH (2001) Nitric oxide-mediated regulation of chemosensitivity in cancer cells. *J Natl Cancer Inst* 93:1879–1885.

Mayer B and Beretta M (2008) The enigma of nitroglycerin bioactivation and nitrate tolerance: news, views and troubles. *Br J Pharmacol* 155:170–184.

McDonald BJ and Bennett BM (1993) Biotransformation of glyceryl trinitrate by rat aortic cytochrome P450. *Biochem Pharmacol* 45:268–270.

McMurtry V, Saavedra JE, Nieves-Alicea R, Simeone A-M, Keefer LK, and Tari AM (2011) JS-K, a nitric oxide-releasing prodrug, induces breast cancer cell death while sparing normal mammary epithelial cells. *Int J Oncol* 38:963–971.

Meyer DJ, Kramer H, and Ketterer B (1994) Human glutathione transferase catalysis of the formation of S-nitrosoglutathione from organic nitrites plus glutathione. *FEBS Lett* 351:427–428.

Moali C, Brollo M, Custot J, Sari M-A, Boucher J-L, Stuehr DJ, and Mansuy D (2000) Recognition of α-amino acids bearing various C=NOH functions by nitric oxide synthase and arginase involves very different structural determinants. *Biochemistry* 39:8208–8218.

Mocellin S, Bronte V, and Nitti D (2007) Nitric oxide, a double edged sword in cancer biology: searching for therapeutic opportunities. *Med Res Rev* 27:317–352.

Molon B, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D, De Palma A, Mauri P, Monegal A, Rescigno M, et al. (2011) Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. *J Exp Med* 208:1949–1962.

Nablo BJ, Chen T-Y, and Schoenfisch MH (2001) Sol-gel derived nitric-oxide releasing materials that reduce bacterial adhesion. *J Am Chem Soc* 123:9712–9713.

Nablo BJ, Prichard HL, Butler RD, Klitzman B, and Schoenfisch MH (2005) Inhibition of implant-associated infections via nitric oxide release. *Biomaterials* 26:6984–6990.

Nablo BJ and Schoenfisch MH (2004) Poly(vinyl chloride)-coated sol-gels for studying the effects of nitric oxide release on bacterial adhesion. *Biomacromolecules* 5:2034–2041.

Nablo BJ and Schoenfisch MH (2005) In vitro cytotoxicity of nitric oxide-releasing sol-gel derived materials. *Biomaterials* 26:4405–4415.

Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, Herber DL, Schneck J, and Gabrilovich DI (2007) Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nat Med* 13:828–835.

Nagaraj S, Schrum AG, Cho H-I, Celis E, and Gabrilovich DI (2010) Mechanism of T cell tolerance induced by myeloid-derived suppressor cells. *J Immunol* 184:3106–3116.

Nagaraj S, Youn J-I, and Gabrilovich DI (2013) Reciprocal relationship between myeloid-derived suppressor cells and T cells. *J Immunol* 191:17–23.

Nakanishi K, Koshiyama T, Iba S, and Ohba M (2015) Lipophilic ruthenium salen complexes: incorporation into liposome bilayers and photoinduced release of nitric oxide. *Dalton Trans* 44:14200–14203.

Nakano A, Liu GS, Heusch G, Downey JM, and Cohen MV (2000) Exogenous nitric oxide can trigger a preconditioned state through a free radical mechanism, but endogenous nitric oxide is not a trigger of classical ischemic preconditioning. *J Mol Cell Cardiol* 32:1159–1167.

Naoki K, Kazuhiro Y, and Yukio N(2010) PEGylated polymer micelle-based nitric oxide (NO) photodonor with NO mediated antitumor activity. *Chem Lett* 39:1008–1009.

Nath N and Kashfi K (2020) Tumor associated macrophages and 'NO'. *Biochem Pharmacol* 176:113899.

Navarro-Tableros V, Gomez Y, Camussi G, and Brizzi MF (2018) Extracellular vesicles: New players in lymphomas. *Int J Mol Sci* 20:41.

Needleman P, Blehm DJ, Harkey AB, Johnson Jr EM, and Lang S (1971) The metabolic pathway in the degradation of glyceryl trinitrate. *J Pharmacol Exp Ther* 179:347–353.

Needleman P and Krantz Jr JC (1965) The biotransformation of nitroglycerin. *Biochem Pharmacol* 14:1225–1230.

Nelson BH (2010) CD20+ B cells: the other tumor-infiltrating lymphocytes. *J Immunol* 185:4977–4982.

Neubauer R, Wölkart G, Opelt M, Schwarzenegger C, Hofinger M, Neubauer A, Kollau A, Schmidt K, Schrammel A, and Mayer B (2015) Aldehyde dehydrogenase-independent bioactivation of nitroglycerin in porcine and bovine blood vessels. *Biochem Pharmacol* 93:440–448.

Nguyen JG, Tanabe KK, and Cohen SM (2010). Postsynthetic diazeniumdiolate formation and NO release from MOFs. *CrystEngComm* 12:2335–2338.

Nguyen TL, Choi Y, and Kim J (2019) Mesoporous silica as a versatile platform for cancer immunotherapy. *Adv Mater* 31:e1803953.

Nicolas A, Cathelin D, Larmonier N, Fraszczak J, Puig P-E, Bouchot A, Bateman A, Solary E, and Bonnotte B (2007) Dendritic cells trigger tumor cell death by a nitric oxide-dependent mechanism. *J Immunol* 179:812–818.

Niedbala W, Alves-Filho JC, Fukada SY, Vieira SM, Mitani A, Sonego F, Mirchandani AS, Nascimento DC, Cunha FQ, and Liew FY (2011) Regulation of type 17 helper T-cell function by nitric oxide during inflammation. *Proc Natl Acad Sci USA* 108:9220–9225.

Niedbala W, Besnard A-G, Jiang HR, Alves-Filho JC, Fukada SY, Nascimento D, Mitani A, Pushparaj P, Alqahtani MH, and Liew FY (2013) Nitric oxide-induced regulatory T cells inhibit Th17 but not Th1 cell differentiation and function. *J Immunol* 191:164–170.

Niedbala W, Besnard A-G, Nascimento DC, Donate PB, Sonego F, Yip E, Guabiraba R, Chang H-D, Fukada SY, Salmond RJ, et al. (2014) Nitric oxide enhances Th9 cell differentiation and airway inflammation. *Nat Commun* 5:4575.

Niedbala W, Cai B, Liu H, Pitman N, Chang L, and Liew FY (2007) Nitric oxide induces CD4+CD25+ Foxp3 regulatory T cells from CD4+CD25 T cells via p53, IL-2, and OX40. *Proc Natl Acad Sci USA* 104:15478–15483.

Niedbala W, Wei X-Q, Campbell C, Thomson D, Komai-Koma M, and Liew FY (2002) Nitric oxide preferentially induces type 1 T cell differentiation by selectively up-regulating IL-12 receptor β 2 expression via cGMP. *Proc Natl Acad Sci USA* 99:16186–16191.

Niedbala W, Wei X-Q, Piedrafita D, Xu D, and Liew FY (1999) Effects of nitric oxide on the induction and differentiation of Th1 cells. *Eur J Immunol* 29:2498–2505.

Noman MZ, Hasmim M, Messai Y, Terry S, Kieda C, Janji B, and Chouaib S (2015) Hypoxia: a key player in antitumor immune response. a review in the theme: cellular responses to hypoxia. *Am J Physiol Cell Physiol* 309:C569–C579.

Novikov AS and Bolotin DS (2017) Tautomerism of amidoximes and other oxime species. *J Phys Org Chem* 31:e3772.

Obermajer N, Wong JL, Edwards RP, Chen K, Scott M, Khader S, Kolls JK, Odunsi K, Billiar TR, and Kalinski P (2013) Induction and stability of human Th17 cells require endogenous NOS2 and cGMP-dependent NO signaling. *J Exp Med* 210:1433–1445.

Obregon C, Graf L, Chung KF, Cesson V, and Nicod LP (2015) Nitric oxide sustains IL-1β expression in human dendritic cells enhancing their capacity to induce IL-17-producing T-cells. *PLoS One* 10:e0120134.

Öcal N and Erden I (2001) Oxidative cleavage of the C=N bond during singlet oxygenations of amidoximes. *Tetrahedron Lett* 42:4765–4767.

Olearczyk JJ, Ellsworth ML, Stephenson AH, Lonigro AJ, and Sprague RS (2004) Nitric oxide inhibits ATP release from erythrocytes. *J Pharmacol Exp Ther* 309:1079–1084.

Olearczyk JJ, Stephenson AH, Lonigro AJ, and Sprague RS (2004) NO inhibits signal transduction pathway for ATP release from erythrocytes via its action on heterotrimeric G protein Gi. *Am J Physiol Heart Circ Physiol* 287:H748–H754.

Omar SA, Artime E, and Webb AJ (2012) A comparison of organic and inorganic nitrates/nitrites. *Nitric Oxide* 26:229–240.

Oronsky B, Scicinski J, Cabrales P, and Minchinton A (2016) RRx-001, an epigenetic-based radio- and chemosensitizer, has vascular normalizing effects on SCCVII and U87 tumors. *Clin Epigenetics* 8:53.

Oronsky B, Scicinski J, Ning S, Peehl D, Oronsky A, Cabrales P, Bednarski M, and Knox S (2016) RRx-001, a novel dinitroazetidine radiosensitizer. *Invest New Drugs* 34:371–377.

Palucka K and Banchereau J (2012) Cancer immunotherapy via dendritic cells. *Nat Rev Cancer* 12:265–277.

Pan P-Y, Ma G, Weber KJ, Ozao-Choy J, Wang G, Yin B, Divino CM, and Chen S-H (2010) Immune stimulatory receptor CD40 is required for T-cell suppression and T regulatory cell activation mediated by myeloid-derived suppressor cells in cancer. *Cancer Res* 70:99–108.

Pang L, Zhu Y, Qin J, Zhao W, and Wang J (2018) Primary M1 macrophages as multifunctional carrier combined with PLGA nanoparticle delivering anticancer drug for efficient glioma therapy. *Drug Deliv* 25:1922–1931.

Paolucci C, Burastero SE, Rovere-Querini P, De Palma C, Falcone S, Perrotta C, Capobianco A, Manfredi AA, and Clementi E (2003) Synergism of nitric oxide and maturation signals on human dendritic cells occurs through a cyclic GMP-dependent pathway. *J Leukoc Biol* 73:253–262.

Paolucci C, Rovere P, De Nadai C, Manfredi AA, and Clementi E (2000) Nitric oxide inhibits the tumor necrosis factor α -regulated endocytosis of human dendritic cells in a cyclic GMP-dependent way. *J Biol Chem* 275:19638–19644.

Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 12:252–264.

Park D, Im S, Saravanakumar G, Lee YM, Kim J, Kim K, Lee J, Kim J, and Kim WJ (2019) A cyotosol-selective nitric oxide bomb as a new paradigm of an anticancer drug. *Chem Commun (Camb)* 55:14789–14792.

Park D, Kim J, Lee YM, Park J, and Kim WJ (2016) Polydopamine hollow nanoparticle functionalized with N-diazeniumdiolates as a nitric oxide delivery carrier for antibacterial therapy. *Adv Healthc Mater* 5:2019–2024.

Park J, Kim J, Singha K, Han D-K, Park H, and Kim WJ (2013) Nitric oxide integrated polyethylenimine-based tri-block copolymer for efficient antibacterial activity. *Biomaterials* 34:8766–8775.

Park K, Jeong H, Tanum J, Yoo J-C, and Hong J (2019a) Developing regulatory property of gelatin-tannic acid multilayer films for coating-based nitric oxide gas delivery system. *Sci Rep* 9:8308.

Park K, Jeong H, Tanum J, Yoo J-C, and Hong J (2019b) Poly-L-lysine/poly-L-glutamic acid-based layer-by-layer self-assembled multilayer film for nitric oxide gas delivery. *J Ind Eng Chem* 69:263–268.

Pavlos CM, Xu H, and Toscano JP (2004) Controlled photochemical release of nitric oxide from O2-substituted diazeniumdiolates. *Free Radic Biol Med* 37:745–752.

Pelegrino MT, De Araujo Lima B, Do Nascimento MHM, Lombello CB, Brocchi M, and Seabra AB (2018) Biocompatible and antibacterial nitric oxide-releasing pluronic F-127/chitosan hydrogel for topical applications. *Polymers (Basel)* 10:452.

Petitprez F, de Reyniès A, Keung EZ, Chen TW-W, Sun C-M, Calderaro J, Jeng Y-M, Hsiao L-P, Lacroix L, Bougoüin A, et al. (2020) B cells are associated with survival and immunotherapy response in sarcoma. *Nature* 577:556–560.

Phung CD, Nguyen HT, Choi JY, Pham TT, Acharya S, Timilshina M, Chang J-H, Kim J-H, Jeong J-H, Ku SK, et al. (2019) Reprogramming the T cell response to cancer by simultaneous, nanoparticle-mediated PD-L1 inhibition and immunogenic cell death. *J Control Release* 315:126–138.

Pieretti JC, Pelegrino MT, Nascimento MHM, Tortella GR, Rubilar O, and Seabra AB (2020) Small molecules for great solutions: Can nitric oxide-releasing nanomaterials overcome drug resistance in chemotherapy? *Biochem Pharmacol* 176:113740.

Polizzi MA, Stasko NA, and Schoenfisch MH (2007) Water-soluble nitric oxide-releasing gold nanoparticles. *Langmuir* 23:4938–4943.

Pramanick S, Kim J, Kim J, Saravanakumar G, Park D, and Kim WJ (2018) Synthesis and characterization of nitric oxide-releasing platinum(IV) prodrug and polymeric micelle triggered by light. *Bioconjug Chem* 29:885–897.

Ranjbar-Karimi R, Davodian T, and Mehrabi H (2018) Survey reactivity of some substituted quinazolinones with pentafluoro(chloro)pyridine. *J Heterocycl Chem* 55:475–480.

Ren H, Zhao S, Li W, Dong H, Zhou M, Cao M, Hu H-M, and Wang L-X (2014) Therapeutic antitumor efficacy of B cells loaded with tumor-derived autophagasomes vaccine (DRibbles). *J Immunother* 37:383–393.

Ren Z, Kar S, Wang Z, Wang M, Saavedra JE, and Carr BI (2003) JS-K, a novel non-ionic diazeniumdiolate derivative, inhibits Hep 3B hepatoma cell growth and induces c-Jun phosphorylation via multiple MAP kinase pathways. *J Cell Physiol* 197:426–434.

Reynolds MM, Hrabie JA, Oh BK, Politis JK, Citro ML, Keefer LK, and Meyerhoff ME (2006) Nitric oxide releasing polyurethanes with covalently linked diazeniumdiolated secondary amines. *Biomacromolecules* 7:987–994.

Reynolds MM, Saavedra JE, Showalter BM, Valdez CA, Shanklin AP, Oh BK, Keefer LK, and Meyerhoff ME (2010) Tailored synthesis of nitric oxide-releasing polyurethanes using O-protected diazeniumdiolated chain extenders. *J Mater Chem* 20:3107–2114.

Rezvani K, Rouce R, Liu E, and Shpall E (2017) Engineering natural killer cells for cancer immunotherapy. *Mol Ther* 25:1769–1781.

Riccio DA, Coneski PN, Nichols SP, Broadnax AD, and Schoenfisch MH (2012) Photoinitiated nitric oxide-releasing tertiary S-nitrosothiol-modified xerogels. *ACS Appl Mater Interfaces* 4:796–804.

Riccio DA, Dobmeier KP, Hetrick EM, Privett BJ, Paul HS, and Schoenfisch MH (2009) Nitric oxide-releasing *S*-nitrosothiol-modified xerogels. *Biomaterials* 30:4494–4502.

Riccio DA, Nugent JL, and Schoenfisch MH (2011) Stöber Synthesis of Nitric Oxide-Releasing S-Nitrosothiol-Modified Silica Particles. *Chem Mater* 23:1727–1735.

Riccio DA and Schoenfisch MH (2012) Nitric oxide release: part I. Macromolecular scaffolds. *Chem Soc Rev* 41:3731–3741.

Riganti C, Miraglia E, Viarisio D, Costamagna C, Pescarmona G, Ghigo D, and Bosia A (2005)Nitric oxide reverts the resistance to doxorubicin in human colon cancer cells by inhibiting the drug efflux. *Cancer Res* 65:516–525.

Rodell CB, Arlauckas SP, Cuccarese MF, Garris CS, Li R, Ahmed MS, Kohler RH, Pittet MJ, and Weissleder R (2018) TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated macrophages to enhance cancer immunotherapy. *Nat Biomed Eng* 2:578–588.

Rose MJ, Fry NL, Marlow R, Hinck L, and Mascharak PK (2008) Sensitization of ruthenium nitrosyls to visible light via direct coordination of the dye resorufin: trackable NO donors for light-triggered NO delivery to cellular targets. *J Am Chem Soc* 130:8834–8846.

Rose MJ and Mascharak PK (2008) Photoactive ruthenium nitrosyls: effects of light and potential application as NO donors. *Coord Chem Rev* 252:2093–2114.

Rothrock AR, Donkers RL, and Schoenfisch MH (2005) Synthesis of nitric oxide-releasing gold nanoparticles. *J Am Chem Soc* 127:9362–9363.

Ruane PH, Bushan KM, Pavlos CM, D'Sa RA, and Toscano JP (2002) Controlled photochemical release of nitric oxide from O2-benzyl-substituted diazeniumdiolates. *J Am Chem Soc* 124:9806–9811.

Russell DG, Huang L, and VanderVen BC (2019) Immunometabolism at the interface between macrophages and pathogens. *Nat Rev Immunol* 19:291–304.

Saavedra JE, Dunams TM, Flippen-Anderson JL, and Keefer LK (1992) Secondary amine/nitric oxide complex ions, R₂N[N(0)NO]-. O-functionalization chemistry. *J Org Chem* 57:6134–6138.

Saavedra JE, Shami PJ, Wang LY, Davies KM, Booth MN, Citro ML, and Keefer LK (2000) Esterase-sensitive nitric oxide donors of the diazeniumdiolate family: in vitro antileukemic activity. *J Med Chem* 43:261–269.

Saavedra JE, Srinivasan A, Bonifant CL, Chu J, Shanklin AP, Flippen-Anderson JL, Rice WG, Turpin JA, Davies KM, and Keefer LK (2001) The secondary amine/nitric oxide complex ion R(2)N[N(O)NO](-) as nucleophile and leaving group in S9N)Ar reactions. *J Org Chem* 66:3090–3098.

Sadrearhami Z, Shafiee FN, Ho KKK, Kumar N, Krasowska M, Blencowe A, Wong EHH, and Boyer C (2019) Antibiofilm nitric oxide-releasing polydopamine coatings. *ACS Appl Mater Interfaces* 11:7320–7329.

Sahyoun T, Arrault A, and Schneider R (2019) Amidoximes and oximes: synthesis, structure, and their key role as NO donors. *Molecules* 24:2470.

Sahyoun T, Gaucher C, Zhou Y, Ouaini N, Schneider R, and Arrault A (2018) Synthesis of novel mono and bis nitric oxide donors with high cytocompatibility and release activity. *Bioorg Med Chem Lett* 28:3329–3332.

Saini AS, Shenoy GN, Rath S, Bal V, and George A (2014) Inducible nitric oxide synthase is a major intermediate in signaling pathways for the survival of plasma cells. *Nat Immunol* 15:275–282.

Sallusto F, Nicolò C, De Maria R, Corinti S, and Testi R (1996) Ceramide inhibits antigen uptake and presentation by dendritic cells. *J Exp Med* 184:2411–2416.

Santos MC, Seabra AB, Pelegrino MT, and Haddad PS (2016) Synthesis, characterization and cytotoxicity of glutathione- and PEG-glutathione-superparamagnetic iron oxide nanoparticles for nitric oxide delivery. *Appl Surf Sci* 367:26–35.

Santos MI, da Silva LCE, Bomediano MP, Catori DM, Gonçalves MC, and de Oliveira MG (2021) 3D printed nitric oxide-releasing poly(acrylic acid)/F127/ cellulose nanocrystal hydrogels. *Soft Matter* 17:6352–6361.

Sarkar S, Korolchuk VI, Renna M, Imarisio S, Fleming A, Williams A, Garcia-Arencibia M, Rose C, Luo S, Underwood BR, et al. (2011) Complex inhibitory effects of nitric oxide on autophagy. *Mol Cell* 43:19–32.

Sarvaria A, Madrigal JA, and Saudemont A (2017) B cell regulation in cancer and anti-tumor immunity. *Cell Mol Immunol* 14:662–674.

Schanuel FS, Raggio Santos KS, Monte-Alto-Costa A, and de Oliveira MG (2015) Combined nitric oxide-releasing poly(vinyl alcohol) film/F127 hydrogel for accelerating wound healing. *Colloids Surf B Biointerfaces* 130:182–191.

Schröder H (1992) Cytochrome P-450 mediates bioactivation of organic nitrates. *J Pharmacol Exp Ther* 262:298–302.

Schudel A, Sestito LF, and Thomas SN (2018) S-nitrosated poly(propylene sulfide) nanoparticles for enhanced nitric oxide delivery to lymphatic tissues. *J Biomed Mater Res A* 106:1463–1475.

Serbina NV, Jia T, Hohl TM, and Pamer EG (2008) Monocyte-mediated defense against microbial pathogens. *Annu Rev Immunol* 26:421–452.

Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, and Pamer EG (2003) TNF/ iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* 19:59–70.

Seth P and Fung HL (1993) Biochemical characterization of a membrane-bound enzyme responsible for generating nitric oxide from nitroglycerin in vascular smooth muscle cells. *Biochem Pharmacol* 46:1481–1486.

Shami PJ, Saavedra JE, Bonifant CL, Chu J, Udupi V, Malaviya S, Carr BI, Kar S, Wang M, Jia L, et al. (2006) Antitumor activity of JS-K [O2-(2,4-dinitrophenyl) 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate] and related O2-aryl diazeniumdiolates in vitro and in vivo. *J Med Chem* 49:4356–4366.

Shami PJ, Saavedra JE, Wang LY, Bonifant CL, Diwan BA, Singh SV, Gu Y, Fox SD, Buzard GS, Citro ML, et al. (2003) JS-K, a glutathione/glutathione S-transferase-activated nitric oxide donor of the diazeniumdiolate class with potent antineoplastic activity. *Mol Cancer Ther* 2:409–417.

Sharif F, Hynes SO, McCullagh KJA, Ganley S, Greiser U, McHugh P, Crowley J, Barry F, and O'Brien T (2012) Gene-eluting stents: non-viral, liposome-based gene delivery of eNOS to the blood vessel wall in vivo results in enhanced endothelialization but does not reduce restenosis in a hypercholesterolemic model. *Gene Ther* 19:321–328.

Sharma A, Muresanu DF, Patnaik R, and Sharma HS (2013) Size- and age-dependent neurotoxicity of engineered metal nanoparticles in rats. *Mol Neurobiol* 48:386–396.

Shen C, Yan J, Erkocak OF, Zheng X-F, and Chen X-D (2014) Nitric oxide inhibits autophagy via suppression of JNK in meniscal cells. *Rheumatology (Oxford)* 53:1022–1033.

Shimamura H, Cumberland R, Hiroishi K, Watkins SC, Lotze MT, and Baar J (2002) Murine dendritic cell-induced tumor apoptosis is partially mediated by nitric oxide. *J Immunother* 25:226–234.

Shin DS and Ribas A (2015) The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? *Curr Opin Immunol* 33:23–35.

Shin JH, Metzger SK, and Schoenfisch MH (2007) Synthesis of nitric oxide-releasing silica nanoparticles. *J Am Chem Soc* 129:4612–4619.

Shin JH and Schoenfisch MH (2008) Inorganic/Organic Hybrid Silica Nanoparticles as a Nitric Oxide Delivery Scaffold. *Chem Mater* 20:239–249.

Shishido SM, Seabra AB, Loh W, and Ganzarolli de Oliveira M (2003) Thermal and photochemical nitric oxide release from *S*-nitrosothiols incorporated in Pluronic F127 gel: potential uses for local and controlled nitric oxide release. *Biomaterials* 24:3543–3553.

Si C, Zhang R, Wu T, Lu G, Hu Y, Zhang H, Xu F, Wei P, Chen K, Tang H, et al. (2016) Dendritic cell-derived nitric oxide inhibits the differentiation of effector dendritic cells. *Oncotarget* 7:74834–74845.

Siddiqui S, Alatery A, Kus A, and Basta S (2011) TLR engagement prior to virus infection influences MHC-I antigen presentation in an epitope-dependent manner as a result of nitric oxide release. *J Leukoc Biol* 89:457–468.

Simeone A-M, McMurtry V, Nieves-Alicea R, Saavedra JE, Keefer LK, Johnson MM, and Tari AM (2008) TIMP-2 mediates the anti-invasive effects of the nitric oxide-releasing prodrug JS-K in breast cancer cells. *Breast Cancer Res* 10:R44.

Singh S and Gupta AK (2011) Nitric oxide: role in tumour biology and iNOS/NO based anticancer therapies. *Cancer Chemother Pharmacol* 67:1211–1224.

Song Q, Tan S, Zhuang X, Guo Y, Zhao Y, Wu T, Ye Q, Si L, and Zhang Z (2014) Nitric oxide releasing d-α-tocopheryl polyethylene glycol succinate for enhancing antitumor activity of doxorubicin. *Mol Pharm* 11:4118–4129.

Soriani A, Zingoni A, Cerboni C, Iannitto ML, Ricciardi MR, Di Gialleonardo V, Cippitelli M, Fionda C, Petrucci MT, Guarini A, et al. (2009) ATM-ATR-dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. *Blood* 113:3503–3511.

Stamler JS and Toone EJ (2002) The decomposition of thionitrites. *Curr Opin Chem Biol* 6:779–785.

Stamm H, Wellbrock J, and Fiedler W (2018) Interaction of PVR/PVRL2 with TIGIT/DNAM-1 as a novel immune checkpoint axis and therapeutic target in cancer. *Mamm Genome* 29:694–702.

Steer SA, Scarim AL, Chambers KT, and Corbett JA (2006) Interleukin-1 stimulates beta-cell necrosis and release of the immunological adjuvant HMGB1. *PLoS Med* 3:e17.

Stevens EV, Carpenter AW, Shin JH, Liu J, Der CJ, and Schoenfisch MH (2010) Nitric oxide-releasing silica nanoparticle inhibition of ovarian cancer cell growth. *Mol Pharm* **7**:775–785.

Stiff A, Trikha P, Mundy-Bosse B, McMichael E, Mace TA, Benner B, Kendra K, Campbell A, Gautam S, Abood D, et al. (2018) Nitric oxide production by myeloid-derived suppressor cells plays a role in impairing Fc receptor–mediated natural killer cell function. *Clin Cancer Res* **24**:1891–1904.

Stoopler ET, Vogl DT, and Stadtmauer EA (2007) Medical management update: multiple myeloma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **103**:599–609.

Storm WL and Schoenfisch MH (2013) Nitric oxide-releasing xerogels synthesized from N-diazeniumdiolate-modified silane precursors. *ACS Appl Mater Interfaces* **5**:4904–4912.

Storm WL, Youn J, Reighard KP, Worley BV, Lodaya HM, Shin JH, and Schoenfisch MH (2014) Superhydrophobic nitric oxide-releasing xerogels. *Acta Biomater* **10**:3442–3448.

Studenovsky M, Sivak L, Sedlacek O, Konefal R, Horkova V, Etrych T, Kovar M, Rihova B, and Sirova M (2018) Polymer nitric oxide donors potentiate the treatment of experimental solid tumours by increasing drug accumulation in the tumour tissue. *J Control Release* **269**:214–224.

Suchyta DJ and Schoenfisch MH (2015) Encapsulation of N-Diazeniumdiolates within Liposomes for Enhanced Nitric Oxide Donor Stability and Delivery. *Mol Pharm* **12**:3569–3574.

Suchyta DJ and Schoenfisch MH (2017) Controlled release of nitric oxide from liposomes. *ACS Biomater Sci Eng* **3**:2136–2143.

Sun C, Mezzadra R, and Schumacher TN (2018) Regulation and function of the PD-L1 checkpoint. *Immunity* **48**:434–452.

Sung Y-C, Jin P-R, Chu L-A, Hsu F-F, Wang M-R, Chang C-C, Chiou S-J, Qiu JT, Gao D-Y, Lin C-C, et al. (2019) Delivery of nitric oxide with a nanocarrier promotes tumour vessel normalization and potentiates anti-cancer therapies. *Nat Nanotechnol* **14**:1160–1169.

Tabuchi A, Sano K, Oh E, Tsuchiya T, and Tsuda M (1994) Modulation of AP-1 activity by nitric oxide (NO) in vitro: NO mediated modulation of AP-1. *FEBS Lett* **351**:123–127.

Tai L-A, Wang Y-C, and Yang C-S (2010) Heat-activated sustaining nitric oxide release from zwitterionic diazeniumdiolate loaded in thermo-sensitive liposomes. *Nitric Oxide* **23**:60–64.

Tan L, Huang R, Li X, Liu S, and Shen Y-M (2017) Controllable release of nitric oxide and doxorubicin from engineered nanospheres for synergistic tumor therapy. *Acta Biomater* **57**:498–510.

Tan L, Wan A, Zhu X, and Li H (2014) Visible light-triggered nitric oxide release from near-infrared fluorescent nanospheric vehicles. *Analyst (Lond)* **139**:3398–3406.

Tanum J, Jeong H, Heo J, Choi M, Park K, and Hong J (2019) Assembly of graphene oxide multilayer film for stable and sustained release of nitric oxide gas. *Appl Surf Sci* **486**:452–459.

Tao Y, Li X, Wu Z, Chen C, Tan K, Wan M, Zhou M, and Mao C (2022) Nitric oxide-driven nanomotors with bowl-shaped mesoporous silica for targeted thrombolysis. *J Colloid Interface Sci* **611**:61–70.

Tarr JM, Young PJ, Morse R, Shaw DJ, Haigh R, Petrov PG, Johnson SJ, Winyard PG, and Eggleton P (2010) A mechanism of release of calreticulin from cells during apoptosis. *J Mol Biol* **401**:799–812.

Tavakol H and Arshadi S (2009) Theoretical investigation of tautomerism in N-hydroxy amidines. *J Mol Model* **15**:807–816.

Thatcher GRJ, Nicolescu AC, Bennett BM, and Toader V (2004) Nitrates and NO release: contemporary aspects in biological and medicinal chemistry. *Free Radic Biol Med* **37**:1122–1143.

Tiemann F (1884) Effect of hydroxylamine on nitriles. *Chem Ber* **17**:126–129.

Tiribuzi R, Crispoltoni L, Tartacca F, Orlacchio A, Martino S, Palmerini CA, and Orlacchio A (2013) Nitric oxide depletion alters hematopoietic stem cell commitment toward immunogenic dendritic cells. *Biochim Biophys Acta* **1830**:2830–2838.

Tsou P, Katayama H, Ostrin EJ, and Hanash SM (2016) The Emerging Role of B Cells in Tumor Immunity. *Cancer Res* **76**:5597–5601.

Tumurkhuu G, Koide N, Dagvadorj J, Noman ASM, Khuda II-E, Naiki Y, Komatsu T, Yoshida T, and Yokochi T (2010) B1 cells produce nitric oxide in response to a series of toll-like receptor ligands. *Cell Immunol* **261**:122–127.

Udupi V, Yu M, Malaviya S, Saavedra JE, and Shami PJ (2006) JS-K, a nitric oxide prodrug, induces cytochrome c release and caspase activation in HL-60 myeloid leukemia cells. *Leuk Res* **30**:1279–1283.

Ullrich T, Oberle S, Abate A, and Schröder H (1997) Photoactivation of the nitric oxide donor SIN-1. *FEBS Lett* **406**:66–68.

Valdez CA, Saavedra JE, Showalter BM, Davies KM, Wilde TC, Citro ML, Barchi Jr JJ, Deschamps JR, Parrish D, El-Gayar S, et al (2008) Hydrolytic reactivity trends among potential prodrugs of the O^2-glycosylated diazeniumdiolate family: targeting nitric oxide to macrophages for antileishmanial activity. *J Med Chem* **51**:3961–3970.

Valk E, Rudd CE, and Schneider H (2008) CTLA-4 trafficking and surface expression. *Trends Immunol* **29**:272–279.

Vallhov H, Gabrielsson S, Strømme M, Scheynius A, and Garcia-Bennett AE (2007) Mesoporous silica particles induce size dependent effects on human dendritic cells. *Nano Lett* **7**:3576–3582.

van der Veen RC, Dietlin TA, Pen L, and Gray JD (1999) Nitric oxide inhibits the proliferation of T-helper 1 and 2 lymphocytes without reduction in cytokine secretion. *Cell Immunol* **193**:194–201.

van der Veen RC, Dietlin TA, Pen L, Gray JD, and Hofman FM (2000) Antigen presentation to Th1 but not Th2 cells by macrophages results in nitric oxide production and inhibition of T cell proliferation: interferon-γ is essential but insufficient. *Cell Immunol* **206**:125–135.

Velázquez CA, Praveen Rao PN, Citro ML, Keefer LK, and Knaus EE (2007) O2-acetoxymethyl-protected diazeniumdiolate-based NSAIDs (NONO NSAIDs):

synthesis, nitric oxide release, and biological evaluation studies. *Bioorg Med Chem* **15**:4767–4774.

Waldman AD, Fritz JM, and Lenardo MJ (2020) A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* **20**:651–668.

Wan A, Gao Q, and Li H (2009) Preparation and characterization of diazeniumdiolate releasing ethylcellulose films. *J Mater Sci Mater Med* **20**:321–327.

Wan S-S, Zeng J-Y, Cheng H, and Zhang X-Z (2018) ROS-induced NO generation for gas therapy and sensitizing photodynamic therapy of tumor. *Biomaterials* **185**:51–62.

Wang J-Z, Zhang Y-H, Guo X-H, Zhang H-Y, and Zhang Y (2016) The double-edge role of B cells in mediating antitumor T-cell immunity: Pharmacological strategies for cancer immunotherapy. *Int Immunopharmacol* **36**:73–85.

Wang L, Chang Y, Feng Y, Li X, Cheng Y, Jian H, Ma X, Zheng R, Wu X, Xu K, et al. (2019) Nitric oxide stimulated programmable drug release of nanosystem for multidrug resistance cancer therapy. *Nano Lett* **19**:6800–6811.

Wang PG, Xian M, Tang X, Wu X, Wen Z, Cai T, and Janczuk AJ (2002) Nitric oxide donors: chemical activities and biological applications. *Chem Rev* **102**:1091–1134.

Warburton WK (1966) The reaction of benzoyldicyandiamide [PhCO·NH·C(NH2): N·CN] with hydroxylamine hydrochloride to give oxadiazoles. *J Chem Soc C Org* **1966**:1522–1524.

Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, and Sancho D (2020) Dendritic cells in cancer immunology and immunotherapy. *Nat Rev Immunol* **20**:7–24.

Wecksler S, Mikhailovsky A, and Ford PC (2004) Photochemical production of nitric oxide via two-photon excitation with NIR light. *J Am Chem Soc* **126**:13566–13567.

Wennholm K, Shimabukuro-Vornhagen A, and von Bergwelt-Baildon M (2019) B cell-based cancer immunotherapy. *Transfus Med Hemother* **46**:36–46.

Wenzel P, Hink U, Oelze M, Seeling A, Isse T, Bruns K, Steinhoff L, Brandt M, Kleschyov AL, Schulz E, et al. (2007) Number of nitrate groups determines reactivity and potency of organic nitrates: a proof of concept study in ALDH-2$^{-/-}$ mice. *Br J Pharmacol* **150**:526–533.

Wieland H and Bauer H (1906) Benzenylnitrosolic acid. *Berichte Dtsch Chem Ges* **39**:1480–1488.

Wright AM and Hayton TW (2012) Recent developments in late metal nitrosyl chemistry. *Comments Inorg Chem* **33**:207–248.

Wu M, Lu Z, Wu K, Nam C, Zhang L, and Guo J (2021) Recent advances in the development of nitric oxide-releasing biomaterials and their application potentials in chronic wound healing. *J Mater Chem B Mater Biol Med* **9**:7063–7075.

Wu W, Chen M, Luo T, Fan Y, Zhang J, Zhang Y, Zhang Q, Sapin-Minet A, Gaucher C, and Xia X (2020) ROS and GSH-responsive S-nitrosoglutathione functionalized polymeric nanoparticles to overcome multidrug resistance in cancer. *Acta Biomater* **103**:259–271.

Wu X, Tang X, Xian M, and Wang PG (2001) Glycosylated diazeniumdiolates: a novel class of enzyme-activated nitric oxide donors. *Tetrahedron Lett* **42**:3779–3782.

Xiang H-J, Guo M, and Liu J-G (2017) Transition-metal nitrosyls for photocontrolled nitric oxide delivery. *Eur J Inorg Chem* **2017**:1586–1595.

Xiong H, Zhu C, Li F, Hegazi R, He K, Babyatsky M, Bauer AJ, and Plevy SE (2004) Inhibition of interleukin-12 p40 transcription and NF-kappaB activation by nitric oxide in murine macrophages and dendritic cells. *J Biol Chem* **279**:10776–10783.

Xu J, Zeng F, Wu H, and Wu S (2015) A mitochondrial-targeting and NO based anticancer nanosystem with enhanced photo-controllability and low dark-toxicity. *J Mater Chem B Mater Biol Med* **3**:4904–4912.

Xue Q, Yan Y, Zhang R, and Xiong H (2018) Regulation of iNOS on immune cells and its role in diseases. *Int J Mol Sci* **19**:3805.

Yang C, Hwang HH, Jeong S, Seo D, Jeong Y, Lee DY, and Lee K (2018) Inducing angiogenesis with the controlled release of nitric oxide from biodegradable and biocompatible copolymeric nanoparticles. *Int J Nanomedicine* **13**:6517–6530.

Yang F, Li Y, Wu T, Na N, Zhao Y, Li W, Han C, Zhang L, Lu J, and Zhao Y (2016) TNFα-induced M-MDSCs promote transplant immune tolerance via nitric oxide. *J Mol Med (Berl)* **94**:911–920.

Yang J, Zhang R, Lu G, Shen Y, Peng L, Zhu C, Cui M, Wang W, Arnaboldi P, Tang M, et al. (2013) T cell–derived inducible nitric oxide synthase switches off Th17 cell differentiation. *J Exp Med* **210**:1447–1462.

Yang T, Zelikin AN, and Chandrawati R (2018) Progress and promise of nitric oxide-releasing platforms. *Adv Sci (Weinh)* **5**:1701043.

Yang Y, Huang Z, and Li L-L (2021) Advanced nitric oxide donors: chemical structure of NO drugs, NO nanomedicines and biomedical applications. *Nanoscale* **13**:444–459.

Yao X, Liu Y, Gao J, Yang L, Mao D, Stefanitsch C, Li Y, Zhang J, Ou L, Kong D, et al. (2015) Nitric oxide releasing hydrogel enhances the therapeutic efficacy of mesenchymal stem cells for myocardial infarction. *Biomaterials* **60**:130–140.

Yepuri NR, Barraud N, Mohammadi NS, Kardak BG, Kjelleberg S, Rice SA, and Kelso MJ (2013) Synthesis of cephalosporin-3'-diazeniumdiolates: biofilm dispersing NO donor prodrugs activated by β-lactamase. *Chem Commun (Camb)* **49**:4791–4793.

Yin M, Tan S, Bao Y, and Zhang Z (2017) Enhanced tumor therapy via drug co-delivery and in situ vascular-promoting strategy. *J Control Release* **258**:108–120.

Yoshikawa T, Mori Y, Feng H, Phan KQ, Kishimura A, Kang J-H, Mori T, and Katayama Y (2019) Rapid and continuous accumulation of nitric oxide-releasing liposomes in tumors to augment the enhanced permeability and retention (EPR) effect. *Int J Pharm* **565**:481–487.

You C, Li Y, Dong Y, Ning L, Zhang Y, Yao L, and Wang F (2020) Low-temperature trigger nitric oxide nanogenerators for enhanced mild photothermal therapy. *ACS Biomater Sci Eng* **6**:1535–1542.

Yu SH, Hu J, Ercole F, Truong NP, Davis TP, Whittaker MR, and Quinn JF (2015) Transformation of RAFT polymer end groups into nitric oxide donor moieties: en route to biochemically active nanostructures. *ACS Macro Lett* **4**:1278–1282.

Yuen GJ, Demissie E, and Pillai S (2016) B lymphocytes and cancer: a love-hate relationship. *Trends Cancer* **2**:747–757.

Zahid AA, Ahmed R, Raza Ur Rehman S, Augustine R, Tariq M, and Hasan A (2019) Nitric oxide releasing chitosan-poly (vinyl alcohol) hydrogel promotes angiogenesis in chick embryo model. *Int J Biol Macromol* **136**:901–910.

Zanganeh S, Hutter G, Spitler R, Lenkov O, Mahmoudi M, Shaw A, Pajarinen JS, Nejadnik H, Goodman S, Moseley M, et al. (2016) Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. *Nat Nanotechnol* **11**:986–994.

Zhang C, Biggs TD, Devarie-Baez NO, Shuang S, Dong C, and Xian M (2017) S-nitrosothiols: chemistry and reactions. *Chem Commun (Camb)* **53**:11266–11277.

Zhang H, Annich GM, Miskulin J, Osterholzer K, Merz SI, Bartlett RH, and Meyerhoff ME (2002) Nitric oxide releasing silicone rubbers with improved blood compatibility: preparation, characterization, and in vivo evaluation. *Biomaterials* **23**:1485–1494.

Zhang H, Annich GM, Miskulin J, Stankiewicz K, Osterholzer K, Merz SI, Bartlett RH, and Meyerhoff ME (2003)Nitric oxide-releasing fumed silica particles: synthesis, characterization, and biomedical application. *J Am Chem Soc* **125**:5015–5024.

Zhang H, Lai L, Wang Y, Ye B, Deng S, Ding A, Teng L, Qiu L, and Chen J (2019) Silk Fibroin for CpG Oligodeoxynucleotide Delivery. *ACS Biomater Sci Eng* **5**:6082–6088.

Zhang X, Du J, Guo Z, Yu J, Gao Q, Yin W, Zhu S, Gu Z, and Zhao Y (2018)Efficient near infrared light triggered nitric oxide release nanocomposites for sensitizing mild photothermal therapy. *Adv Sci (Weinh)* **6**:1801122.

Zhang X, Jin L, Tian Z, Wang J, Yang Y, Liu J, Chen Y, Hu C, Chen T, Zhao Y, et al. (2019) Nitric oxide inhibits autophagy and promotes apoptosis in hepatocellular carcinoma. *Cancer Sci* **110**:1054–1063.

Zhang Y and Hogg N (2004) The mechanism of transmembrane S-nitrosothiol transport. *Proc Natl Acad Sci USA* **101**:7891–7896.

Zhao H, Dugas N, Mathiot C, Delmer A, Dugas B, Sigaux F, and Kolb J-P (1998) B-cell chronic lymphocytic leukemia cells express a functional inducible nitric oxide synthase displaying anti-apoptotic activity. *Blood* **92**:1031–1043.

Zhao H, Jiang Z, Lv R, Li X, Xing Y, Gao Y, Lv D, Si Y, Wang J, Li J, et al. (2020) Transcriptome profile analysis reveals a silica-induced immune response and fibrosis in a silicosis rat model. *Toxicol Lett* **333**:42–48.

Zhao J, Hu Y, Lin SW, Resch-Genger U, Zhang R, Wen J, Kong X, Qin A, and Ou J (2020) Enhanced luminescence intensity of near-infrared-sensitized upconversion nanoparticles via Ca^{2+} doping for a nitric oxide release platform. *J Mater Chem B Mater Biol Med* **8**:6481–6489.

Zhao X, Cai A, Peng Z, Liang W, Xi H, Li P, Chen G, Yu J, and Chen L (2019) JS-K induces reactive oxygen species-dependent anti-cancer effects by targeting mitochondria respiratory chain complexes in gastric cancer. *J Cell Mol Med* **23**:2489–2504.

Zhou Y, Yang T, Namivandi-Zangeneh R, Boyer C, Liang K, and Chandrawati R (2021) Copper-doped metal-organic frameworks for the controlled generation of nitric oxide from endogenous S-nitrosothiols. *J Mater Chem B Mater Biol Med* **9**:1059–1068.

Zhu Y, Paniccia A, Schulick AC, Chen W, Koenig MR, Byers JT, Yao S, Bevers S, and Edil BH (2016) Identification of CD112R as a novel checkpoint for human T cells. *J Exp Med* **213**:167–176.

Zou M-Z, Liu W-L, Li C-X, Zheng D-W, Zeng J-Y, Gao F, Ye J-J, and Zhang X-Z (2018) A multifunctional biomimetic nanoplatform for relieving hypoxia to enhance chemotherapy and inhibit the PD-1/PD-L1 Axis. *Small* **14**:e1801120.