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**Recent advances in hypertension and cardiovascular toxicities  
with vascular endothelial growth factor (VEGF) inhibition**

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Physiologically, vascular endothelial growth factors (VEGF) and their receptors (VEGFR) play a critical role in vascular development, neogenesis, angiogenesis, endothelial function and vascular tone. Pathologically, VEGF-VEGFR signaling induces dysregulated angiogenesis, which contributes to the growth and spread of tumors. The development of VEGF-VEGFR inhibitors (VEGFIs) has thus proven to be a valuable strategy in the management of a number of malignancies, yielding improved survival outcomes. Not surprisingly, VEGFIs are now standard of care as first-line monotherapy for some cancers and the scope of this class of drugs is growing. However with the promise of improved outcomes, VEGFIs also led to clinically relevant toxicities, especially hypertension and cardiovascular disease (CVD). As such, cancer patients treated with VEGFIs may have improved cancer outcomes, but at the cost of an increased risk of CVD. Indeed, dose intensity and protracted use of these drugs can be limited by cardiovascular side effects and patients may require dose reduction or drug withdrawal, thus compromising anti-cancer efficacy and survival. Here we summarize the vascular biology of VEGF-VEGFR signaling and discuss the cardiovascular consequences and clinical impact of VEGFIs. New insights into molecular mechanisms whereby VEGFIs cause hypertension and heart disease are highlighted.

### **A primer in vascular biology and signaling of VEGF**

VEGFs, of which there are 4 isoforms (VEGFA, VEGFB, VEGFC, VEGFD), signal through VEGFR tyrosine kinases, and are critically involved in the development and function of the vasculature (1). VEGFs are produced by endothelial cells, fibroblasts, podocytes and cancer cells. Of the 3 VEGFR subtypes (VEGFR1, VEGFR2, VEGFR3), VEGFR2 is the primary receptor through which VEGF, especially VEGFA, signals in endothelial cells. VEGFs also bind to neuropilin receptors and to

heparin sulphate proteoglycans. Ligand-receptor binding promotes receptor dimerization and phosphorylation of receptor tyrosine kinases that trigger intracellular signaling with acute non-genomic effects, such as endothelial permeability and vasodilation, and chronic genomic responses, including cell differentiation, survival and proliferation (1) (figure 1). VEGFR2 signaling is also activated through non-ligand processes, such as shear stress and stretch, that stimulate non-canonical signaling through cytoplasmic tyrosine kinases (e.g. c-Src) (1,2). Multiple mechanisms regulate VEGFRs, including protein expression, ligand availability, co-activators, intracellular tyrosine kinases/phosphatases, intracellular degradation and recycling and cross-talk between VEGFs and VEGFR subtypes (1).

VEGFR2 activation triggers pathways essential for endothelial biology, including PLC-DAG-IP<sub>3</sub> and downstream Ca<sup>2+</sup> and ERK1/2 signaling, important in arteriogenesis, neogenesis and angiogenesis, through regulation of cell migration, fate specification, proliferation and contraction/dilation (1). VEGF-VEGFR2-mediated increase in intracellular free Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) influences calcineurin-induced nuclear translocation of NFAT, which downregulates VEGFR1, thereby further increasing VEGFR2 signaling, because VEGFR1 negatively regulates VEGFR2 (3). VEGFR2 phosphorylation also promotes activation of small GTPases, Src, stress kinases and sphingosine-1-phosphate that influence cytoskeletal organization, cell morphology, adhesion, migration and cell-cell interaction, important in endothelial integrity (1-4).

In addition to regulating vascular development and permeability, VEGF-VEGFR2 influences vascular tone by modulating vasorelaxation. VEGFR2-mediated activation of PI3K-AKT leads to eNOS phosphorylation, increased NO generation and consequent vasodilation (1,5). Other vasodilatory pathways include VEGF-

stimulated COX-stimulated production of the vasodilator prostacyclin I<sub>2</sub> (1) VEGF also inhibits endothelial production of the potent vasoconstrictor endothelin-1 (ET-1) (6). Accordingly, physiological VEGF-VEGFR2 signaling maintains vascular tone by balancing NO- and prostacyclin-induced vasodilation and ET-1-regulated vasoconstriction.

### **VEGF signaling as a target for anti-angiogenic and anti-cancer therapy.**

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is critical for tumor growth and metastasis. This process is regulated by growth factors of which VEGFA-VEGFR2 plays a key role (7). Inhibition of angiogenesis, by targeting VEGF-VEGFR signaling, has revolutionised cancer therapy with improved outcomes in some previously untreatable cancers. Four major classes of VEGFI are currently used clinically, including monoclonal VEGF antibodies (bevacizumab), monoclonal VEGFR antibodies (ramucirumab), soluble decoy receptors (VEGF traps) (afibercept) and small molecule VEGFR tyrosine kinase inhibitors (TKI) (sunitinib, cabozantinib, pazopanib, axitinib, vandetanib, regorafenib) (8,9) (figure 2). Since endothelial cells are physiologically quiescent, no adverse effects during anti-angiogenesis therapy were expected. However, clinical observations demonstrated that VEGFIs are associated with unexpected cardiovascular toxicity, especially hypertension.

### **Cardiovascular toxicities of VEGF inhibition**

VEGF inhibitors are associated with an increased incidence of various cardiovascular pathologies including hypertension, ischaemic heart disease, heart failure, QT-interval prolongation and thromboembolism (10-12). Of these, hypertension is the most commonly reported toxicity in VEGFI trials, an effect that may limit anti-cancer

treatment (9,10). Cardiovascular effects of VEGFIs may be severe with an associated increased risk of reversible leukoencephalopathy and fatal cardiovascular events (13).

Inhibition of VEGF signaling has also been implicated in the pathophysiology of preeclampsia, where there is an increased production of placental factors including a splice variant of VEGFR-1 (soluble Fms-like tyrosine kinase 1 (sFlt-1)) (14). sFlt-1, by binding VEGF, leads to reduced VEGFR activation and consequent endothelial dysfunction.

### **VEGF inhibition and hypertension**

The magnitude of VEGFI-induced hypertension is significant, with almost every trial reporting an increase in blood pressure (BP), often >150/100 mmHg (9,10,15). Development of hypertension is dose-dependent and rises to ≈100% when VEGFIs are combined. Pre-existing hypertension, older age and overweight are important risk factors for development of VEGFI-induced hypertension (16).

The risk of hypertension was found to be 20–30% higher than expected for bevacizumab (17). Home BP monitoring might lead to even higher rates of hypertension (especially of lower grades) than clinic measurements (17). Ambulatory BP monitoring (ABPM) revealed that VEGFI-induced BP increase occurs rapidly, within hours of commencement of therapy, and >90% of patients had an increase in BP.(18,19).In sunitinib-treated renal cell carcinoma patients the average BP increase was 14/11 mmHg in the first and 22/17 mmHg in the second week, a response that was sustained until therapy withdrawal, when BP decreased rapidly (18). ABPM studies further demonstrated that circadian BP patterns are lost or attenuated, a response that increases risk of hypertension-associated cardiovascular events.

In the general population, BP monitoring and treatment are important for primary (and secondary) prevention of CVD and long-term events. Previously this

was not a priority in cancer patients with limited life expectancy. However with improved survival outcomes and chronicity of VEGFI treatment, BP monitoring and management are essential. For example, patients with chronic myeloid leukemia, are treated chronically with ponatinib, exposing them to increased hypertension and CVD.

VEGFI-induced hypertension does not seem to be a risk factor for proteinuria (19). This agrees with experimental studies demonstrating that proteinuria and renal injury, including glomerular ischemia, develop first and mainly as a function of VEGFI dose (20). Hypertension occurs earlier and at lower doses of VEGFI therapy, and these dynamics also suggest that renal dysfunction is not the initial pro-hypertensive insult either (20). However, VEGFI is associated with a rightward shift of the renal pressure-natriuresis curve and impaired sodium excretion with consequent fluid retention and salt-dependent hypertension.

The acuteness of the BP increase caused by VEGFI, and the rapid BP reduction upon drug withdrawal suggest that functional changes in vascular tone (decreased vasodilation/increased vasoconstriction) may be the key trigger of the hypertensive response. These vascular phenomena reflect direct actions of VEGFI and as such are on-target effects. Hypertension may thus serve as a biomarker of efficacy of VEGFI therapy, and indeed, studies have demonstrated better outcomes for cancer patients who develop hypertension with VEGFI treatment (21).

### **Mechanism of hypertension during VEGF inhibition**

Mechanisms underlying VEGFI-induced hypertension remain unclear. Carter et al. showed that administration of four different multi-targeted tyrosine kinase inhibitors (RTKIs) in conscious rats induced a BP rise accompanied by significant vasoconstriction in the mesenteric and hindquarter vascular beds, but not in the

kidney (22). These findings support previous observations that vasoconstriction rather than cardiosuppression or renal dysfunction underlies the VEGFI-induced BP rise. The vasoconstrictive response to VEGFIs likely relates to reduced levels of the vasodilator NO due to inhibition of endothelial nitric oxide synthase (eNOS)-induced NO production. In support of this, in healthy volunteers, intra-arterial infusion of bevacizumab in the forearm acutely reduced the vasodilator response to acetylcholine (23). In conscious rats the dose-dependent increase in sunitinib-induced BP elevation was associated with a dose-dependent decrease in urinary excretion of the NO effector molecule cGMP (24). However some clinical studies failed to show a role of decreased NO availability as an underlying cause of the vasoconstriction and BP rise caused by VEGFIs (5).

Another mechanism that has been implicated in VEGFI-induced hypertension is increased production of the potent vasoconstrictor ET-1. Sunitinib is associated with raised levels of circulating ET-1, both in patients and in rats (5,25). Increased plasma ET-1 concentration has also been reported in patients treated with regorafenib as well as in preeclamptic patients with elevated sFlt-1 levels (25). Proof that an activated ET-1 system is instrumental, at least during the initial sunitinib-induced BP rise, has been obtained with ET-1 receptor (ETR) antagonists. In conscious rats, co-administration of the ETR antagonist macitentan prevented hypertension caused by sunitinib (26). In this same model sildenafil, a specific inhibitor of the enzyme phosphodiesterase 5, responsible for the breakdown of cGMP to 5'-GMP, did not lower BP. In swine exposed to sunitinib for one week the elevated BP could be returned to pre-sunitinib levels with the ETR antagonist tezosentan (27). Unexpectedly, in this same model acute administration of the NOS inhibitor L-NMMA was associated with an aggravation of BP elevation. Furthermore, anti-



oxidants given after sunitinib for one week were associated with an attenuated BP decline compared to their response pre-sunitinib administration, questioning a potential role for oxidative stress in VEGFI-induced hypertension (27). However not all studies have shown an ET-1-dependent effect. Bosentan, the ETAR/ETBR blocker failed to reduce BP in cediranib-treated rats (22).

Another potential mechanism whereby VEGFIs influence vascular tone and BP responses might relate to changes in  $[Ca^{2+}]_i$ , a key player regulating vascular contraction. VEGFI may inhibit  $Ca^{2+}$  channel activation, leading to increased  $[Ca^{2+}]_i$  (1-4), which is critically involved in triggering vascular contraction.

Rarefaction (decreased microvessel number) is another putative mechanism underlying the rise in vascular resistance and BP associated with VEGFI (28). The fact that BP increases within hours after initiation of VEGFI argues against a pathophysiological role for structural rarefaction in the developmental phase of hypertension. Since rarefaction is reversible upon drug withdrawal (28), it may be a functional phenomenon due to profound vasoconstriction, at least acutely. Structural rarefaction may contribute to hypertension during prolonged VEGFI. It should be highlighted that for a relatively small increase in vascular resistance an extensive degree of rarefaction is required. Furthermore, hypertension itself may cause rarefaction (29).

Rat studies have shown that the VEGFI-induced BP increase is aggravated by a high sodium diet and accompanied by a leftward shift of the pressure-natriuresis curve, indicating that the BP rise is salt-sensitive (30). Decreased NO production in renal proximal tubular cells, impairing vasa recta dilatation and consequently pressure-natriuresis may underlie this salt-sensitivity. In response to a high salt diet, sodium and chloride accumulate in the skin in excess of water, leading to a hypertonic

interstitial fluid compartment. In response to this hypertonicity, dermal mononuclear phagocytes system (MPS) cells produce the transcription factor tonicity-responsive enhancer-binding protein that initiates expression and production of VEGF-C (31). VEGF-C in turn stimulates formation of lymph vessels to clear excessive electrolytes. Interruption of this pathway in rodents is associated with salt-sensitive hypertension (30). Hence, impairment of lymphangiogenesis, a feature of most VEGFIs, may contribute to salt-sensitive hypertension following VEGFI. In high salt-fed normotensive rats sodium and chloride accumulation in the skin was associated with an exaggerated sunitinib-induced BP elevation. Skin MPS cell density increased during high salt diet and sunitinib administration, but no further increment was seen with combined salt and sunitinib. A high salt diet also increased skin lymphangiogenesis, while sunitinib mildly reduced lymphangiogenesis, both during a normal and high salt diet. These data suggest that sunitinib induces salt-sensitive hypertension, but that impaired skin lymphangiogenesis is unlikely to play an important role therein. The clinical significance of these findings awaits clarification.

### **Renal injury, hypertension and VEGF inhibition**

VEGF inhibition is associated with kidney injury, commonly reflected by proteinuria and occasionally by an increased serum creatinine. Different mechanisms account for the BP rise and kidney injury (32). In sunitinib-induced hypertensive rats, there was a discrepancy between the BP-lowering and antiproteinuric effects of antihypertensive agents (26). Captopril and sildenafil prevented proteinuria without effect on BP, whereas amlodipine prevented development of hypertension without antiproteinuric effect. In this model the ETR antagonist macitentan prevented hypertension and proteinuria, supporting recent studies suggesting that ET-1 not only plays a role in VEGFI-induced hypertension but also in kidney injury (26).

Glomerular endotheliosis, also seen in preeclampsia, and thrombotic microangiopathy (TMA) are well-established histological abnormalities associated with VEGFI. In ≈50% of patients, TMA is restricted to the kidney (33). Kidney biopsies of 100 patients with proteinuria and renal dysfunction following VEGFI showed TMA in 73 and minimal change and collapsing focal segmental glomerulosclerosis in 27 patients (33). Almost all patients with TMA were treated with bevacizumab or VEGF-trap, whereas all but one patient with the alternative renal histopathology were treated with RTKIs. A high salt intake exacerbates the severity of VEGFI-associated glomerular injury

### **VEGF inhibition, sFlt-1 and preeclampsia**

Preeclampsia is associated with increased production of placental factors, including sFlt-1, soluble endoglin, and inflammatory cytokines (34), which cause activation of endothelial cells and generalized endothelial dysfunction. sFlt-1, a soluble form of VEGF and PlGF receptors, binds to free VEGF and PlGF, thereby reducing their bioavailability for their respective membrane receptors. This leads to inhibition of VEGF and PlGF signaling and disruption in the balance between pro- and anti-angiogenic factors, which not only affects the growth and development of the placenta and fetus, but also the function of endothelial cells and the kidney (34). It thus offers an explanation for both hypertension and renal dysfunction occurring in preeclampsia. The hypertension, proteinuria and glomerular endotheliosis occurring during VEGFI is similar to that observed in preeclampsia and accordingly, some authors have termed the condition as a 'preeclampsia-like' syndrome (35). In support of this, preeclamptic women and VEGFI-treated cancer patients exhibit hypertension with elevated ET-1 levels, which correlate with the degree of VEGF inhibition, represented by serum sFlt-1 levels or VEGF inhibitor dose. Animal models replicating the various aspects

of human preeclampsia support the sFlt-1/ET-1 upregulation, and show that ETR antagonism alleviates preeclampsia symptoms to the same degree as it prevents the preeclampsia-like symptoms during VEGFI (25,36). The exact cause(s) of the ET-1 rise remain unclear, but in addition to VEGF inhibition per se, placenta-derived soluble endoglin has been suggested to induce endothelial ET-1 production (37). The net clinical effect of VEGF inactivation (excess sFlt-1 in preeclampsia) and VEGF inhibition (VEGF inhibitors) is similar .

### **Cardiotoxic effects of VEGF inhibitors**

Although hypertension is the most common cardiovascular side effect of VEGFIs, these drugs are also associated with cardiac toxicity.

***Left Ventricular Systolic Dysfunction.*** While cardiac toxicity of VEGFIs may manifest as arrhythmia and atherothrombosis, a more pressing concern is their association with left ventricular systolic dysfunction (LVSD) and heart failure (38,39). Most cardiotoxic effects of VEGFIs were neither predicted nor observed prior to their clinical introduction. This may reflect a relative lack of robust assessment in early clinical trials and an under-appreciation that many patients with cancer, who might not have been eligible for inclusion in trials, have factors predisposing them to potential cardiotoxicity, such as pre-existing hypertension, diabetes and coronary artery disease. Furthermore, many patients have had prior treatment with other cancer chemotherapy agents or radiotherapy predisposing to cardiotoxicity.

The clinical spectrum of VEGFI-associated myocardial toxicity ranges from asymptomatic LVSD to heart failure or cardiogenic shock and death (38-40). Unfortunately, obtaining an accurate incidence of LVSD or heart failure with VEGFI is challenging and the reported incidence may actually underestimate the true scale of

the problem. There is often overlap between symptoms that potentially reflect heart failure with those related to cancer and not the heart. For example, breathlessness, fatigue and edema are often reported by patients and attributed to cancer and not the heart (38). In addition, imaging of left ventricular function is not a consistent feature in VEGFI trials. Consequently, asymptomatic LVSD during the time-limited trial period is usually not reported or assessed.

The largest meta-analysis of TKI trials included 10,647 patients (39). The relative risk for the development of asymptomatic LVSD or heart failure was 2.69 for those treated with TKI versus those not receiving these agents (39,40). Notably, the risk of developing LVSD or heart failure for specific TKIs (e.g. axitinib) was similar to relatively non-specific TKIs (sunitinib, sorafenib, vandetanib and pazopanib) (40,41).

A meta-analysis of 3,784 patients receiving bevacizumab for breast cancer revealed an overall incidence of heart failure of 1.6% in bevacizumab-treated patients versus 0.4% in placebo-treated individuals (42) and similar findings were observed for sunitinib (41). Retrospective data from VEGFI-treated patients revealed a similar incidence of heart failure. These ‘real world’ patients were also screened for LV dysfunction or rise in brain natriuretic peptide. 27% of patients had elevated NT-proBNP and/or decline in left ventricular ejection fraction during treatment (43), a proportion similar to the observed 28% incidence of reduced LV ejection in a randomised controlled trial with sunitinib.

***Acute Coronary Syndrome.*** Over and above the pro-coagulant effects of malignancy, thrombosis risk associated with VEGFI is increased, with greater tendency to arterial thrombosis than venous thrombo-embolism. In a meta-analysis of 38,078 patients (72

trials), there was a 3.5-fold increase in myocardial infarction risk (44). Although this relative increase in risk may appear striking, this has to be taken in context and the absolute increase in risk for myocardial infarction for these patients was only 0.8%. Bevacizumab was associated with a 2.1-fold increase in the risk of 'high-grade' cardiac ischemia and arterial thrombotic events have been reported consistently (45). These bevacizumab-associated effects are particularly notable in patients >65 years and with a history of thromboembolism (45). In a phase I/II sunitinib trial, 1% of patients suffered myocardial infarction whilst 1% suffered cardiac death (44). In a randomised trial of sorafenib-treated patients 3% suffered myocardial ischaemia or infarction (46). The fact that VEGFIs are also associated with a paradoxical increase in the bleeding risk poses a challenging dilemma when considering anti-platelet therapy to reduce risk of myocardial ischaemia in patients treated with VEGFIs.

Risk factors for the development of VEGFI-associated LV dysfunction are poorly understood, but a history of heart failure or coronary artery disease, pre-existing hypertension, increasing age, lower body mass index and previous or concurrent treatment with other cardiotoxic agents may be important (47). Neither dose nor treatment duration has been shown to influence cardiotoxicity risk (48).

### **Pathophysiology of VEGF inhibitor-associated cardiotoxicity**

Pathophysiological mechanisms underpinning myocardial toxicity of VEGFI have not been fully investigated. While hypertensive effects of VEGFI are likely important, alterations in cardiac energy generation through AMP-kinase inhibition and mitochondrial dysfunction appear central (49), as well as effects related to inhibition of VEGF, PDGFR and c-Kit pathways (40). These changes either evoke reduced contractility or render the myocardium more vulnerable to effects of subsequent or co-existent insults, including hypertension. TKIs act at a raft of different pathways

both ‘on-target’ (i.e. present in both the cancer and the heart) and ‘off-target’ bystander kinases, which are unintended drug targets resulting from limited selectivity of these drugs (40). On-target effects may lead to direct cardiac toxicity or an inadequate compensatory response to myocardial stress. VEGF is critically involved in the compensatory hypertrophic response to myocardial stress, including that provoked by hypertension. Interruption of normal VEGF function with VEGFI accelerates development of left ventricular dilation in response to aortic constriction-related pressure overload in mice (50) and murine VEGF<sub>120</sub> gene knockout models have impaired cardiac contractile function and decreased myocardial angiogenesis (50). VEGF-A mRNA and protein are decreased in myocardial biopsy specimens from patients with non-ischaemic cardiomyopathy and reduced VEGF-A mRNA expression is also associated with rarefaction in these samples thus contributing to tissue hypoxia and activation of hypoxia-inducible factors.

Electron microscopy of endomyocardial biopsies from patients with VEGFI-associated heart failure demonstrated mitochondrial abnormalities but not apoptosis or fibrosis, suggesting a reversible process (43). Cardiotoxic effects of VEGFI are now considered to be at least partially reversible and routine monitoring of LV function in patients receiving these drugs should become routine clinical practice.

## **Conclusions**

The scale of the clinical problem of VEGFI-induced hypertension and CVD is growing with an increasing number of cancer patients requiring these anti-angiogenic drugs for treatment. Moreover, cancer usually occurs in older individuals who are more susceptible to hypertension and CVD. Consequently increased usage of VEGFI, coupled with older cancer patients already at risk for cardiovascular events, means that the associated increased risk will not only negatively influence quality of life, but

also represents a potentially significant economic and health-care burden that will impact the emerging ‘epidemic of cancer and cardiovascular disease’ (51). Currently, exact causes for VEGFI-induced hypertension and cardiotoxicity remain unclear, although advances in the field have elucidated the potential role of impaired vasorelaxation/vasoconstriction, renal dysfunction, rarefaction and ET-1 hyperactivation. Further research is urgently needed to fully understand molecular mechanisms whereby inhibition of VEGF signaling induces cardiovascular injury, with the hope that disease-targeted approaches will prevent hypertension and CVD without negatively impacting on beneficial anti-cancer VEGFI therapeutic effects.

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## Figure legends

### Figure 1.

Diagram demonstrating signalling pathways induced by VEGFR activation. VEGFR is activated by VEGF binding and by non-ligand mechanisms (shear stress, stretch). Both genomic and non-genomic pathways are stimulated leading to endothelial cell growth, differentiation, migration, adhesion and vasodilation. p, phosphorylation site of VEGFR tyrosine kinase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide.

### Figure 2.

Schematic illustrating possible pathophysiological processes whereby VEGF-VEGFR inhibition contributes to the development of hypertension and preeclampsia. Four major classes of VEGF-VEGFR inhibitors, including monoclonal VEGF antibodies, anti-VEGFR2 antibodies, soluble decoy receptors (VEGF-traps) and small molecule VEGFR tyrosine kinase inhibitors (TKI) are used clinically as anti-angiogenesis drugs in cancer. In pregnancy, placenta-derived soluble fms-like tyrosine kinase 1 (s-Flt), acts as a VEGF-trap reducing free VEGF availability for binding to VEGFR2R. These processes result in reduced VEGFR signaling and consequent reduction in production of vasodilators (NO and PGI<sub>2</sub>), increased production of vasoconstrictors (ET-1), oxidative stress and rarefaction, resulting in increased vascular tone and arterial remodeling. Reduced pressure natriuresis and impaired lymphatic function contribute to volume overload. p, phosphorylation site of tyrosine kinase; ROS, reactive oxygen species; NO, nitric oxide; ET-1, endothelin-1; Ab, antibody; ECF, extracellular fluid; CVD, cardiovascular disease, PGI<sub>2</sub>, prostaglandin I<sub>2</sub>