Immunotherapy of Solid Tumours

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Disclosures

- **Honoraria / Consultancies / Speaker:**
  - Astra Zeneca
  - Bayer
  - Bristol Myers Squibb
  - Celgene
  - Clovis
  - Eisai
  - Genentech
  - Glaxo Smith Kline
  - Immunova
  - Jennerex / Transgene
  - Karus Therapeutics (Scientific Advisory Board)
  - Otsuka
  - Roche

The Clinical Problem

- VEGFi – renal cancer, HCC, colon cancer, etc
- Endothelial dysfunction, vascular injury
- Significant cardiovascular toxicity

  hypertension, thrombo-embolic events, ischaemia, LV systolic dysfunction, heart failure
- Understanding the pathogenesis - ? Biomarkers, optimise treatment (especially in the “adjuvant” setting)
- ECMC Research nurse and biomarker protocol

University of Glasgow | Institute of Cardiovascular & Medical Sciences

Microparticles from VEGF Inhibitors (VEGFi)-treated Cancer Patients Mediate Endothelial Cell Signaling and ET-1 Production: Implications in Hypertension

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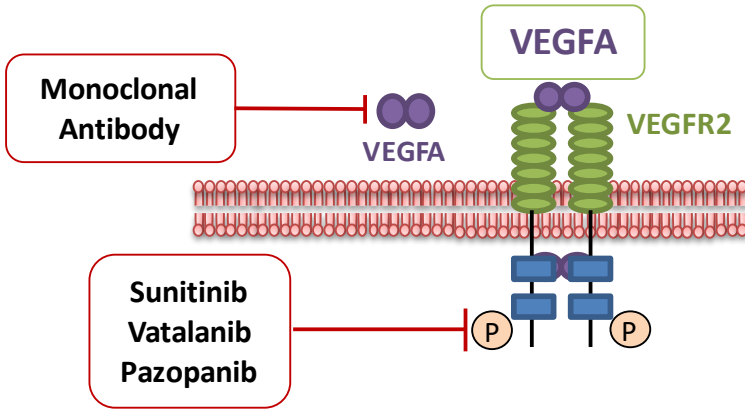
Background

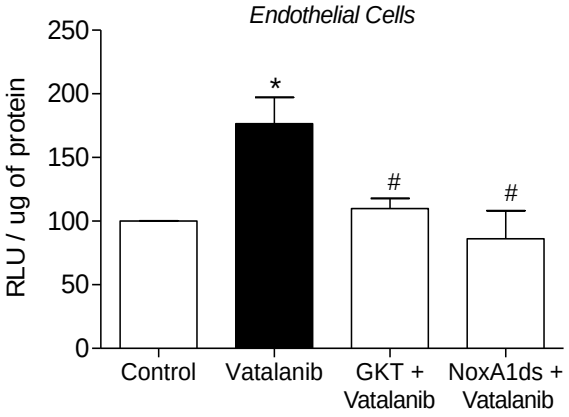
- VEGF: important function in vascular development → tumour progression and metastasis
- VEGF inhibitors (VEGFi): ***first line treatment*** for several types of cancer;

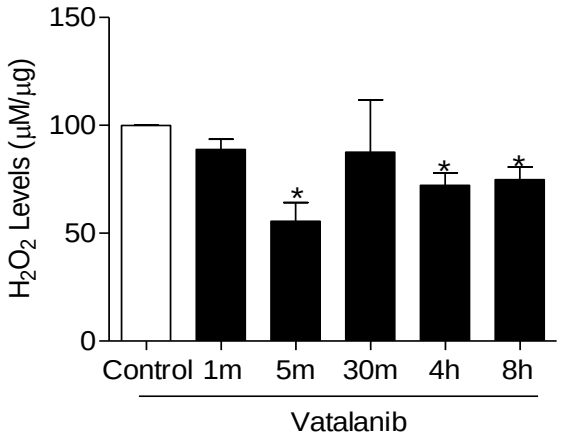
The use of VEGFi is limited by development of cardiovascular toxicity: ***Hypertension***

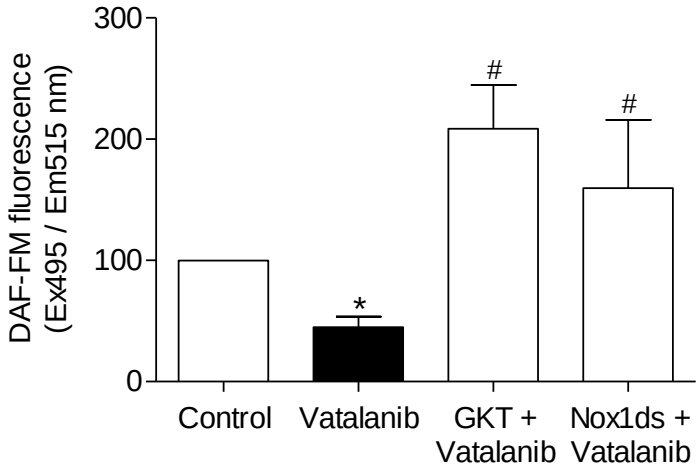


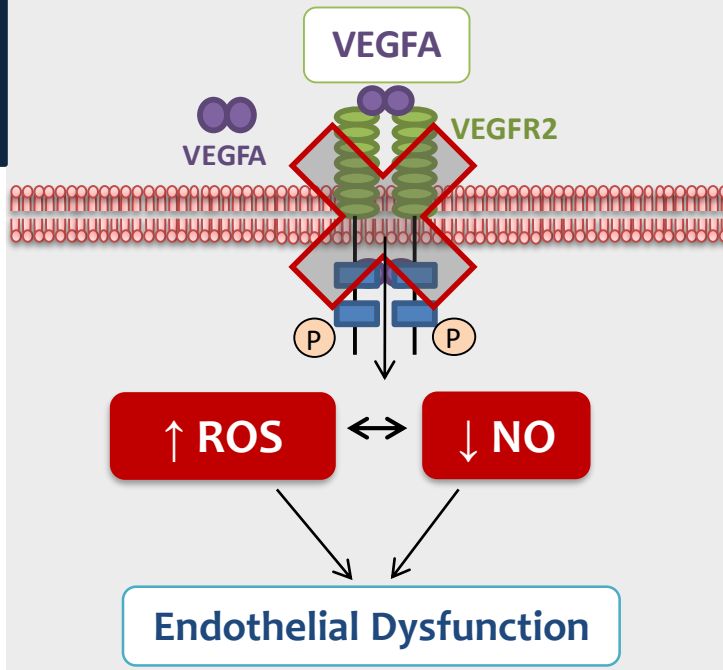
- 40-80% → Increase in BP, which is reduced by the interruption of the treatment;
- **Potential mechanisms:**
  - ↑ **ET-1 levels**
  - RAS activation
  - EC apoptosis
  - Microvascular rarefaction

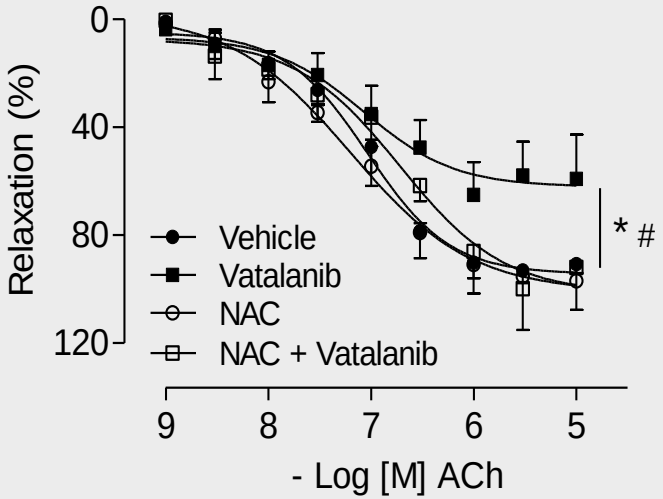
Background





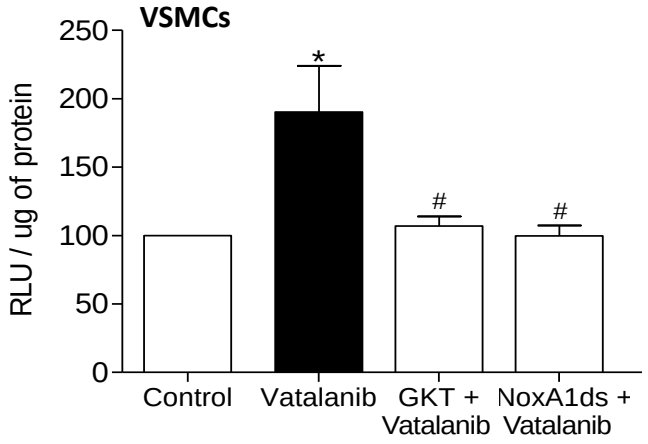


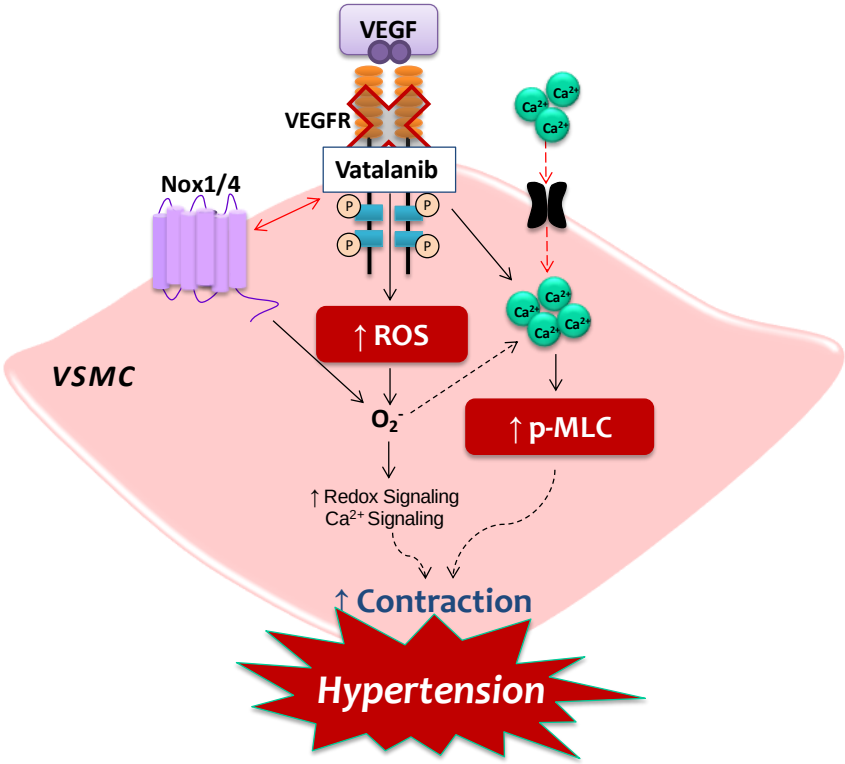


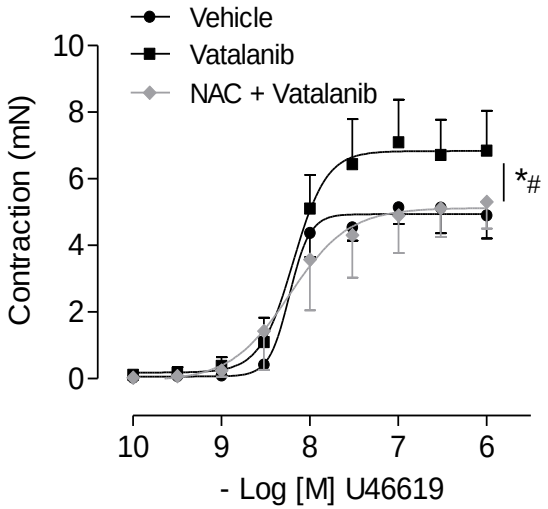


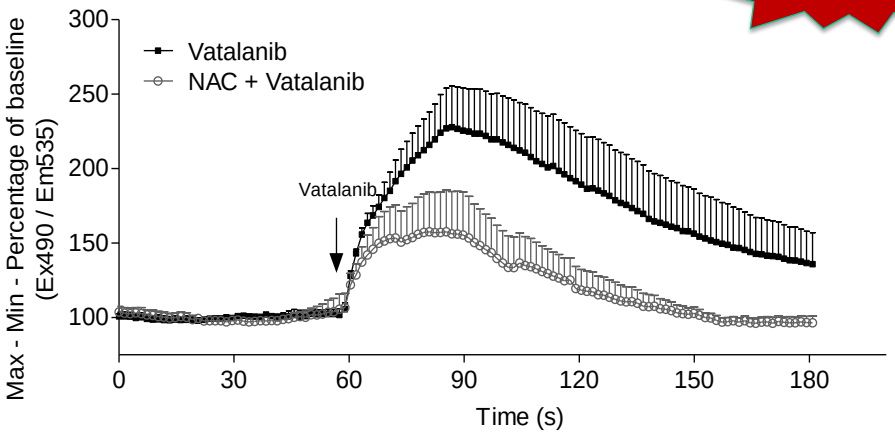
Neves KB et al. *Hypertension* 2018.

Background









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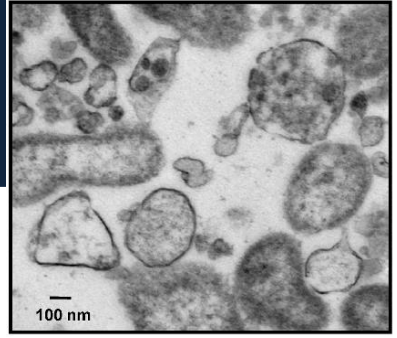
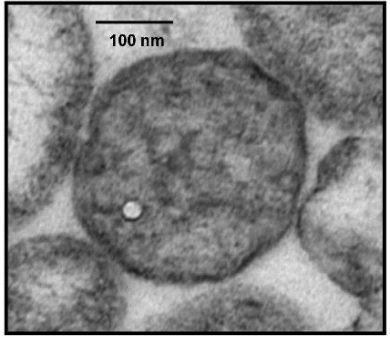
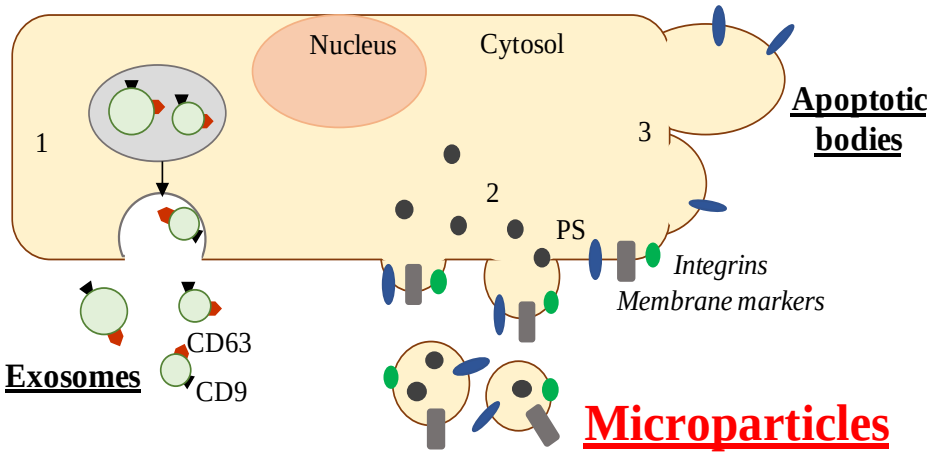


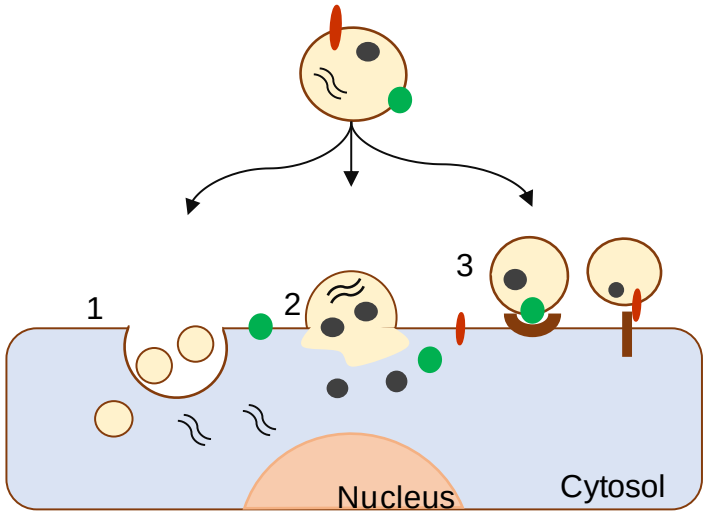


Burger D et al. *ATVB*, 2011

Vascular/Endothelial damage and dysfunction

Microparticles (MPs)





Rios FJ, Unpublished

*Released by **activated / stressed** cells*

- ❖ *Cytoskeletal Reorganization*
- ❖ *Membrane Blebbing*
- ❖ *Shedding of Membrane Fragments*

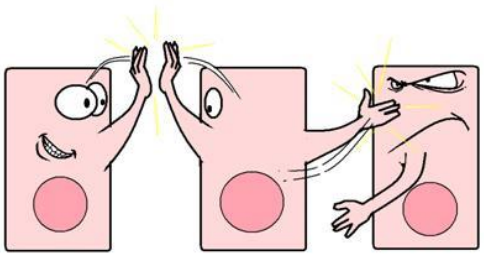
1) Endocytosis
2) Membrane fusion
3) Receptor interaction

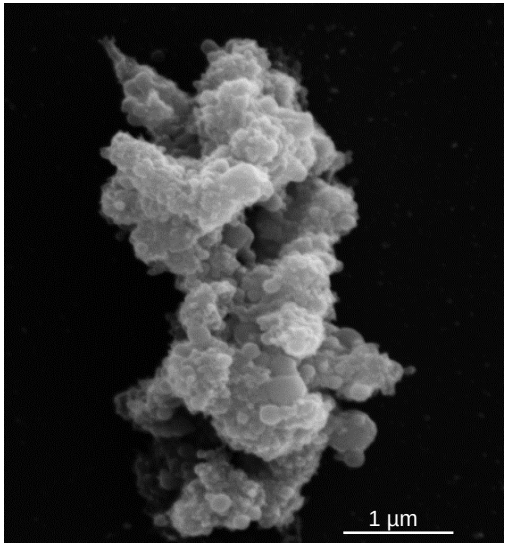
Background

Endothelial **Microparticles (ECMPs)**

Cell-cell communication

VSMC injury





Endothelial cells:

↓ NO production;

↑ endothelial oxidative stress and inflammation;

Platelet and macrophage adhesion to ECs.

Aim: to investigate whether MP status is altered in cancer patients treated with VEGFRi and whether MPs influence endothelial cell function associated with vascular dysfunction.

Patients (clinical information)

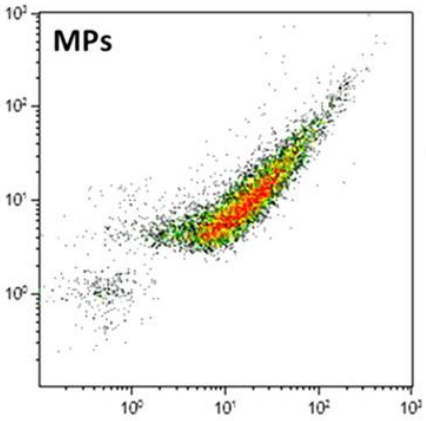
	Male	Female
Sample size	30	9
Number of patients with renal cancer	24	8
Number of patients with other cancer (colorectal, oesophagus, etc)	6	1
Number of patients on sunitinib	3	1
Number of patients on pazopanib	21	7
Number of patients on other VEGFi (sorafenib, etc)	4	1
Number of patients on other drugs	2	-

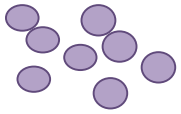
Clinical Condition	Number of Patients
Nephrectomy	17
Transarterial chemo-embolization (TACE)	03
Prior cytotoxic chemotherapy	01
Ischemic heart disease	06
Atrial Fibrillation with no Ischemic Heart Disease	02
No history of heart diseases	26
Prior Hypertension	17
Unknown	05

Methods

Blood samples from **cancer patients** pre- and post-treatment with **VEGFi**

- n = 30-39
- Renal Cancer
- Sunitinib, Sorafenib & Pazopanib

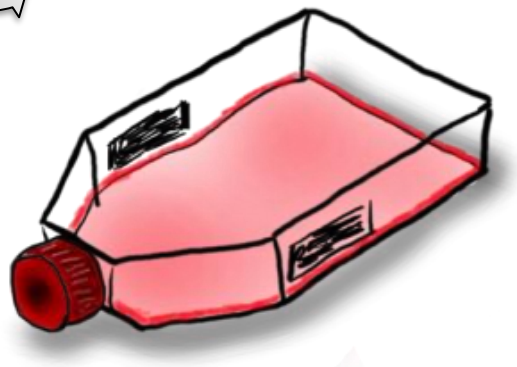




MPs

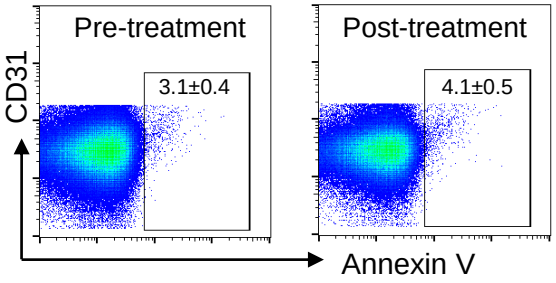
Flow Cytometry:
Annexin V (+)
CD31(+) CD42 (+) – Platelets
CD31(+) CD42 (-) – EC

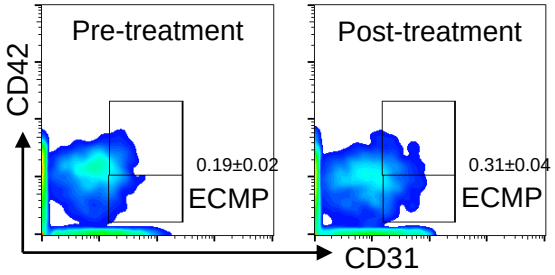
HAEC - Human Aortic Endothelial cells

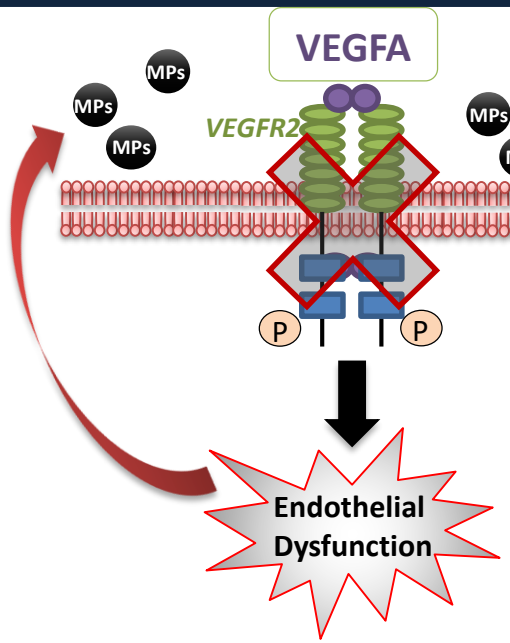


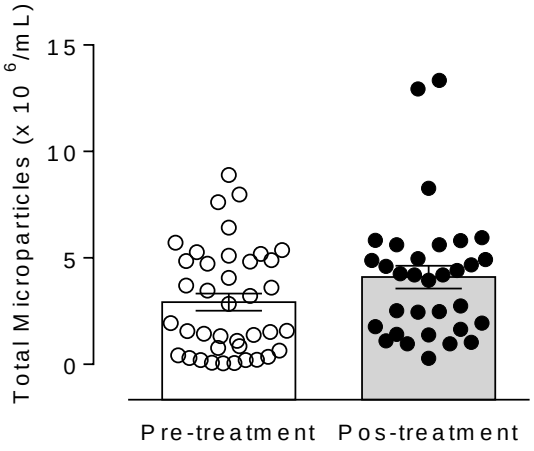
- ROS production
- Proinflammatory markers
- p-eNOS / NO production

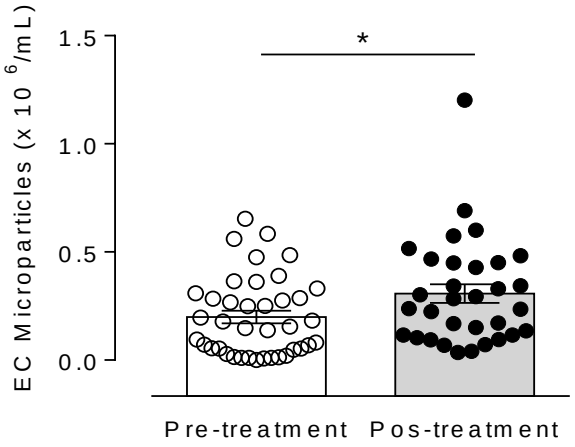
VEGFR inhibition increases ECMPs release







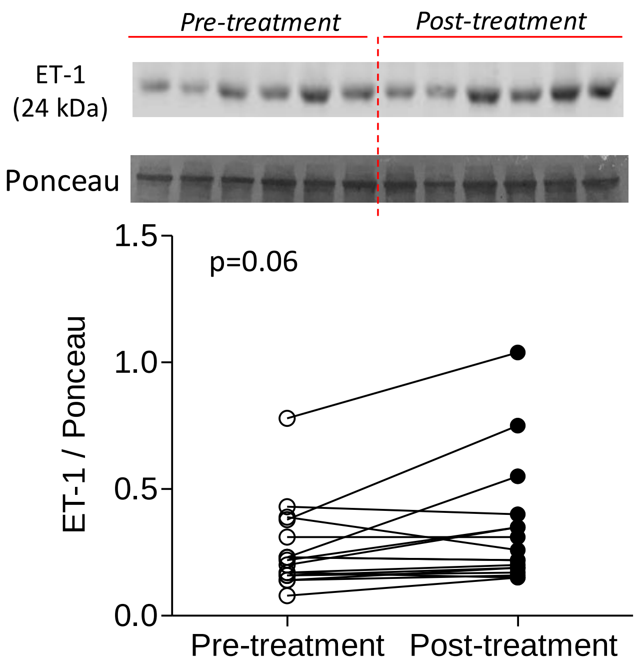


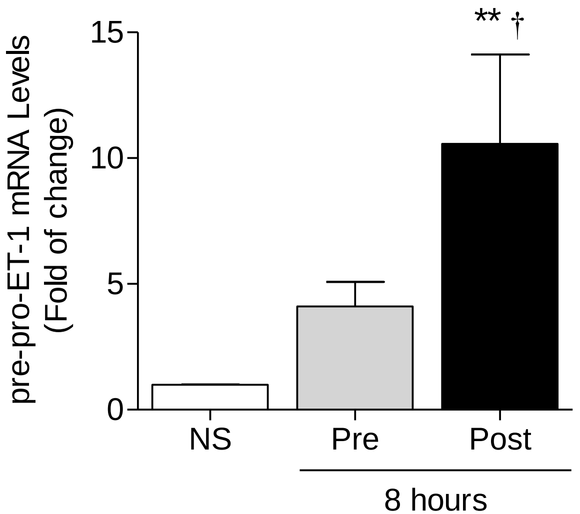


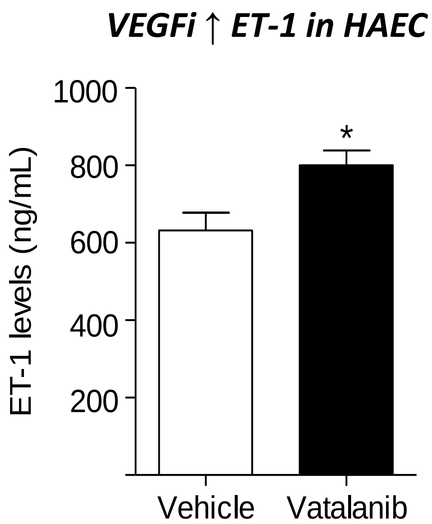
* *vs.* Pre-treatment

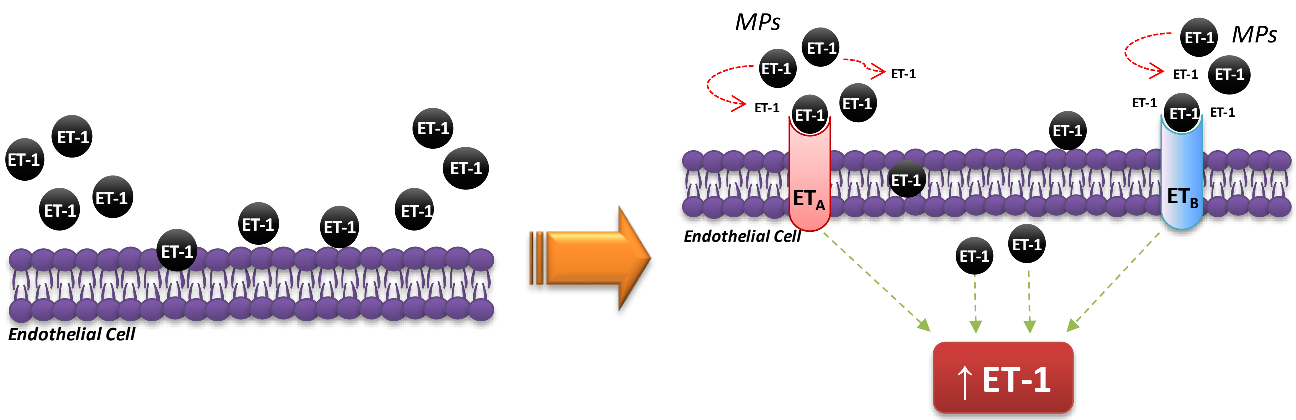
* ECMPs: $CD31^{+}$ $CD42^{-}$
* Platelets: $CD31^{+}$ $CD42^{+}$

MPs from VEGFi-treated patients carry ET-1 and also increases pre-pro-ET-1 gene expression in HAEC



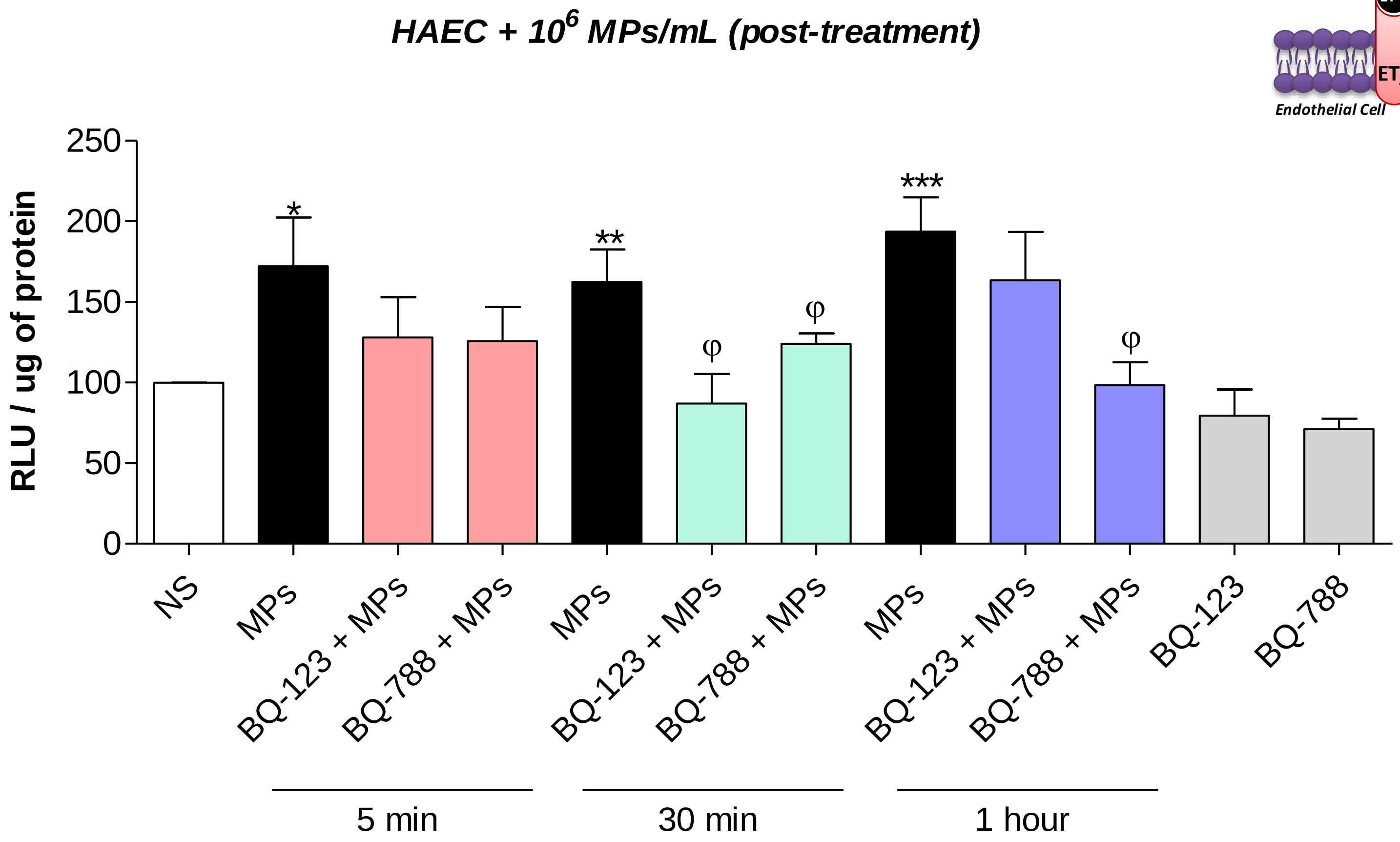


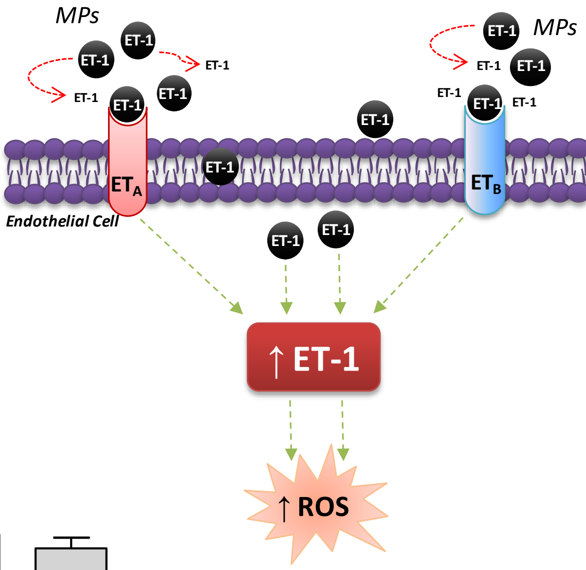




* *vs.* NS or vehicle
† *vs.* Pre-treatment

ETA and ETB receptor antagonism prevents increase in ROS production induced by post-treatment MPs



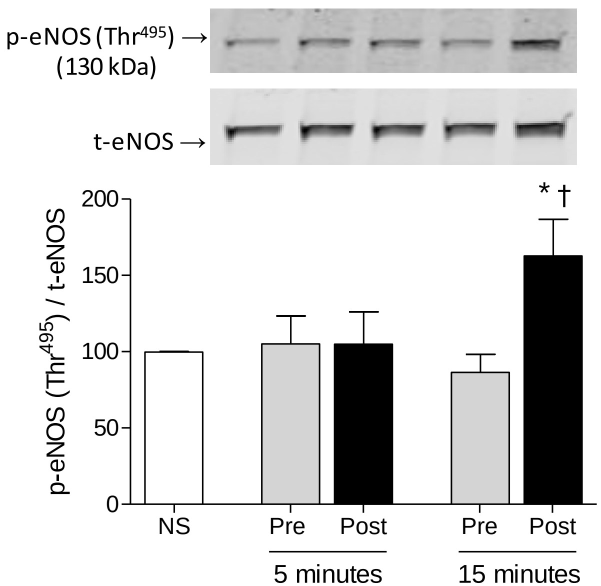


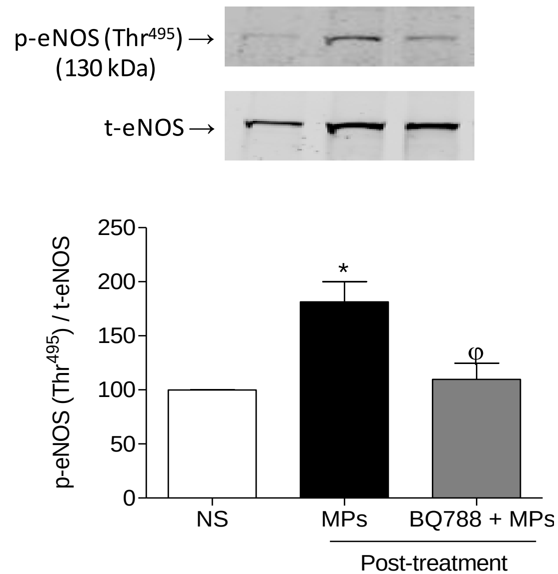
BQ123: ETA antagonist
BQ788: ETB antagonist

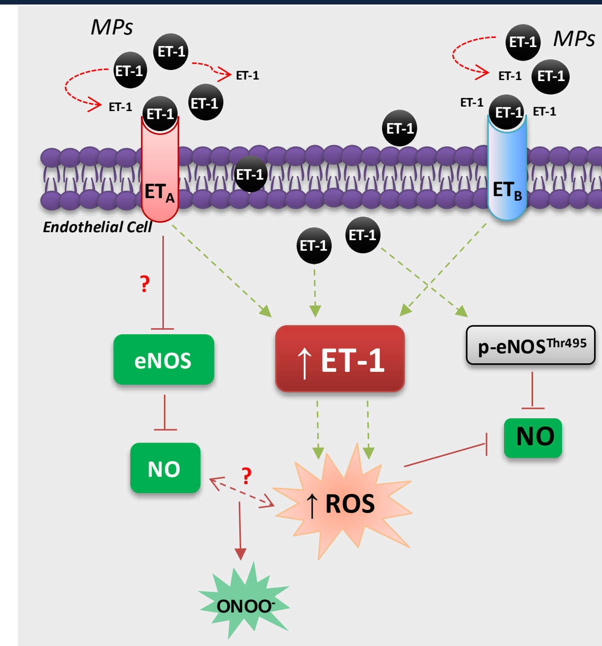
* *vs.* NS
Φ vs. MPs Post-treatment

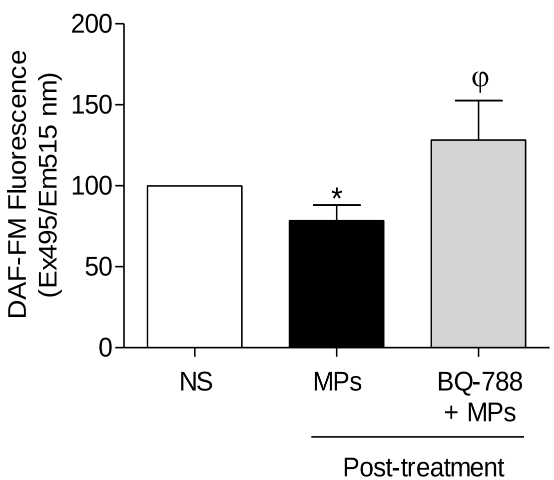
*No effects in HAEC stimulated with pre-treatment MPs

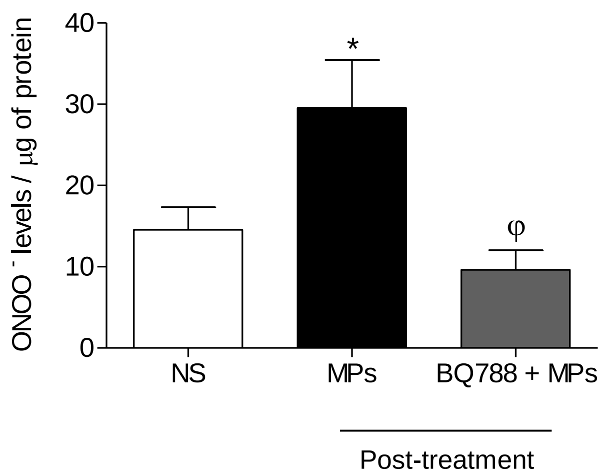
MPs from VEGFi-treated patients increase phosphorylation of the inhibitory site (Thr^{495}) of eNOS and decrease NO levels in HAEC





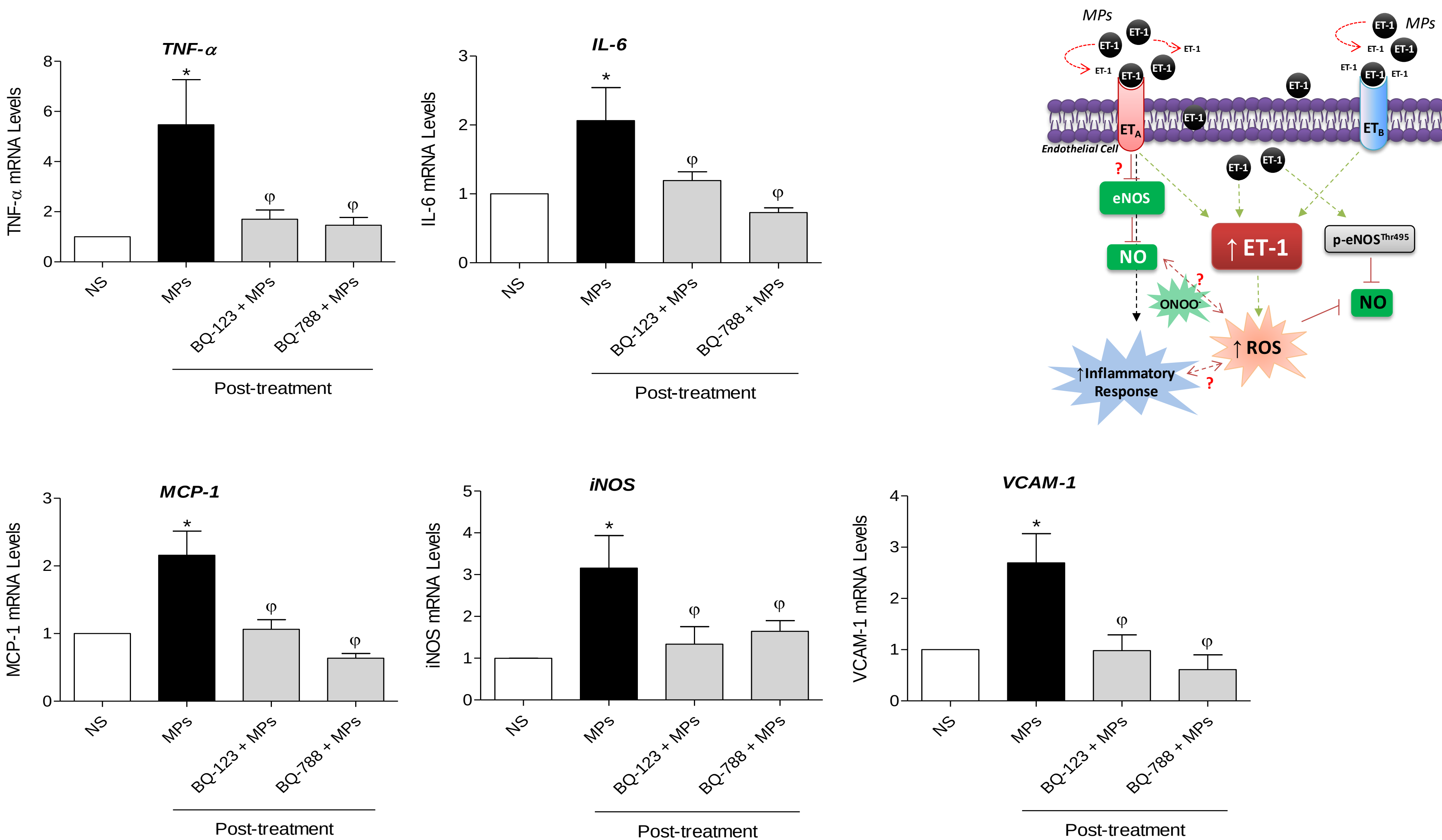






* *vs.* NS
† *vs.* Pre-treatment
Φ vs. MPs Post-treatment

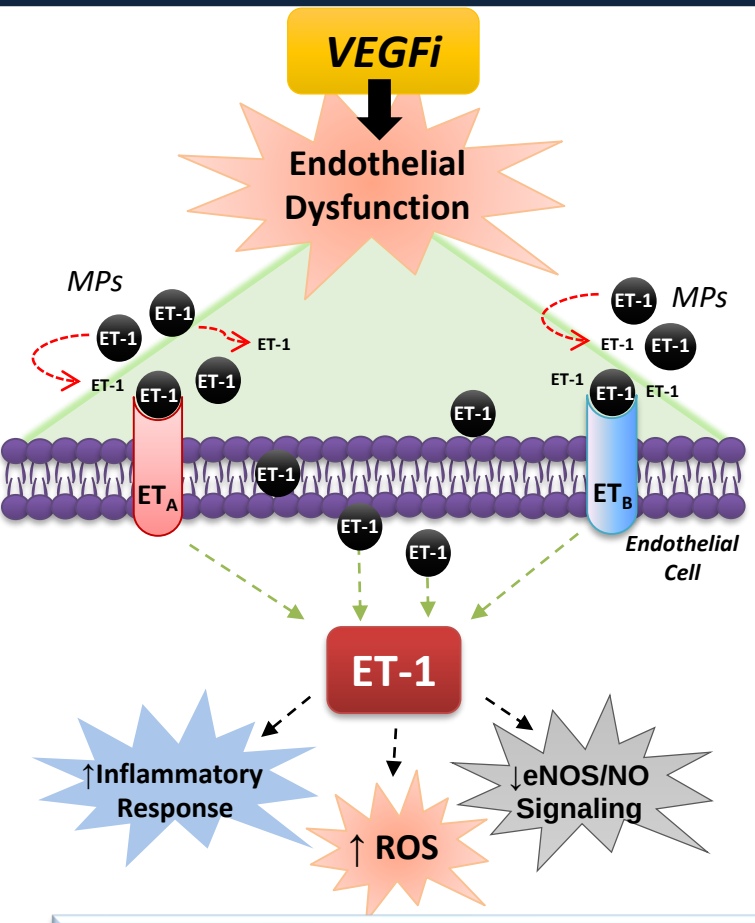
ET-1 receptor antagonism reduces proinflammatory markers levels in HAEC exposed to MPs post VEGFi treatment



* *vs.* NS

Φ vs. Post-treatment MPs

Conclusions



- MPs released under VEGFi treatment in cancer patients, are important vehicles for cell communication and play a role in abnormal EC function;
- ET-1-containing MPs underlie endothelial injury and may be involved in the pathophysiology of VEGFi-induced cardiovascular toxicity.

Perspectives

ECMPs are biomarkers of VEGFi-induced endothelial injury and mediators of ET-1-sensitive redox-regulated EC signalling.

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