JCET

Jagiellonian Centre
for Experimental Therapeutics

ENDOTHELIAL PROFILING OF DRUG VASOTOXICITY

JAGIELLONIAN UNIVERSITY IN KRAKOW

PREDICTIVE SAFETY ANALYSIS OF A PANEL OF SMALL-MOLECULE TYROSINE KINASE INHIBITORS

Fundusze Europejskie Inteligentny Rozwój

Rzeczpospolita Polska

FNP Fundacja na rzecz Nauki Polskiej

Unia Europejska
Europejski Fundusz Rozwoju Regionalnego

WHY ENDOTHELIAL PROFILING?

- vascular endothelium is the largest interface for systemic drugs
- **drug-induced endothelial/vascular dysfunction** is an important safety issue – yet currently neglected in pre-clinical drug development
- severe vascular adverse effects have led to clinical failure of a number of diverse drugs
- endothelial dysfunction is an independent predictor for major cardiovascular events
- **evaluation of endothelial function can predict vascular toxicity**

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

PROTEIN KINASE INHIBITORS – THE HOTSPOT OF PHARMA

- >250 kinase inhibitors are currently undergoing clinical trials, **>50 already approved for use by the FDA** [*1*]
- most in oncologic indications (**imatinib - first small molecule KI approved for chemotherapy**)
- emerging KIs for **other indications**, such as RA, IBD, glaucoma and neurodegenerative diseases [*2*]
- one of the most prevalent druggable targets - **about $^1/_3$ of all drug discovery programs** aimed at developing KIs
- however, attrition rate is high due to **severe toxicities** observed in preclinical studies and **in clinical trials** [*3, 4*]

[1] Roskoski R., Jr., Properties of FDA-approved small molecule protein kinase inhibitors: A 2020 update. Pharmacol Res, 2020. 152: p. 104609.

[2] Benn & Dawson, Clinically precedented protein kinases: rationale for their use in neurodegenerative disease. Front Aging Neurosci, 2020. 12: p. 242.

[3] Bhullar et al., Kinase-targeted cancer therapies: progress, challenges and future directions. Mol Cancer, 2018. 17(1): p. 48.

[4] Ferguson & Gray, Kinase inhibitors: the road ahead. Nat Rev Drug Discov, 2018. 17(5): p. 353-377.

THREE GENERATIONS OF TYROSINE KINASE INHIBITORS

Major drug of each gen	FDA approval	Sponsor	Target	Indications	FDA label cardiovascular warnings and precautions*
Imatinib (*1st gen*)	2001	Novartis	Bcr-Abl	First-line Chronic Myelogenous Leukaemia (CML) treatment	severe congestive heart failure and LV dysfunction; cardiogenic shock
Nilotinib (*2nd gen*)	2007	Novartis	Bcr-Abl	Second-line Chronic Myelogenous Leukaemia (CML) treatment	QT prolongation and sudden deaths (***boxed warning***)
Ponatinib (*3rd gen*)	2012	ARIAD Pharmaceuticals	Bcr-Abl, BEGFR, PDGFR, FGFR, EPH, SRC, c-KIT, RET, TIE2, FLT3	T315I-positive Chronic Myelogenous Leukaemia (CML); T315I-positive Acute Lymphoblastic Leukaemia (ALL)	arterial occlusion, venous thromoembolism, heart failure (***black box***); hypertension; cardiac arrhythmias

* Lanmore *et al.* (**2020**) *Chem. Res. Toxicol.* 33, 125−136; see also Moslehi J.J. (**2016**) *N Engl J Med.* 375(15):1457-1467, Alexandre *et al.* (**2020**) J Am Heart Assoc. 9(18):e018403, Manouchehri *et al.* (**2020**) Arterioscler Thromb Vasc Biol. 40(2):301-308

CAN WE PREDICT CV LIABILITIES WELL BEFORE CLINIC?

Major drug of each gen	FDA approval	Sponsor	Target	Indications	FDA label cardiovascular warnings and precautions*
Imatinib (*1st gen*)	2001	Novartis	Bcr-Abl	First-line Chronic Myelogenous Leukaemia (CML) treatment	severe congestive heart failure and LV dysfunction; cardiogenic shock
Nilotinib (*2nd gen*)	2007	Novartis	Bcr-Abl	Second-line Chronic Myelogenous Leukaemia (CML) treatment	QT prolongation and sudden deaths (***boxed warning***)
Ponatinib (*3rd gen*)	2012	ARIAD Pharmaceuticals	Bcr-Abl, BEGFR, PDGFR, FGFR, EPH, SRC, c-KIT, RET, TIE2, FLT3	T315I-positive Chronic Myelogenous Leukaemia (CML) T315I-positive Acute Lymphoblastic Leukaemia (ALL)	arterial occlusion, venous thromoembolism, heart failure (***black box***); hypertension; cardiac arrhythmias

* Lanmore *et al.* (**2020**) *Chem. Res. Toxicol.* 33, 125−136; see also Moslehi J.J. (**2016**) *N Engl J Med.* 375(15):1457-1467, Alexandre *et al.* (**2020**) J Am Heart Assoc. 9(18):e018403, Manouchehri *et al.* (**2020**) Arterioscler Thromb Vasc Biol. 40(2):301-308

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

PREDICTIVE VASCULAR SAFETY STUDIES USING MRI *IN VIVO*

Drug studied	6-year incidence of cardiovascular events*	Hypertension	Abnormal ankle-brachial index (ABI)*	Overall cardiovascular risk	PRECLINICAL ENDOTHELIAL PROFILING
Imatinib (*1st gen*)	**2.5%** (*400 mg once per day*)	**N/A**	**6.3%** (*first-line imatinib*)	**low**	endothelial dysfunction detected in mice at a HIGH DAILY DOSE
Nilotinib (*2nd gen*)	**15.9%** (*400 mg twice per day*)	**6.6%****	**26%** (*first-line nilotinib*)	**medium**	endothelial dysfunction detected in mice at a MEDIUM DAILY DOSE
Ponatinib (*3rd gen*)	**cumulative serious adverse effects: 10%** cardiovascular, **7%** cerebrovascular, **7%** peripheral, **14%** arterial, **3%** venous (*median follow-up 28 months*)	**26%** (*median follow-up 28 months*)*	**N/A** (*first-line trial closed due to* ***significant vascular toxicity***)	**high**	endothelial dysfunction detected in mice at a LOW DAILY DOSE

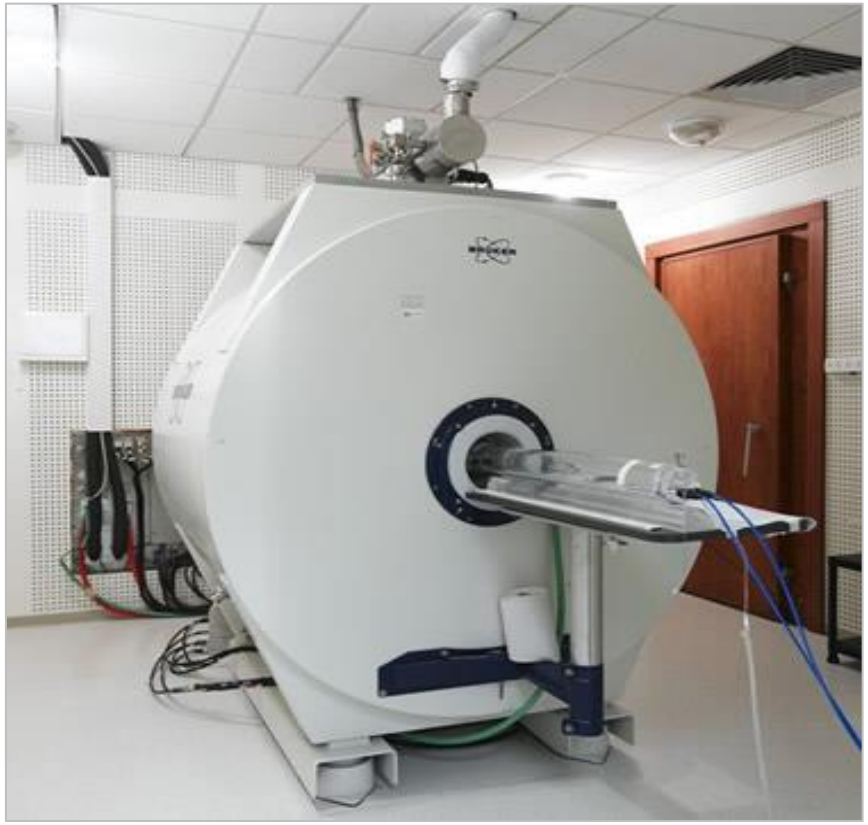
* *compiled from:* Moslehi & Deininger (2015) *J Clin Oncol* **33**:4210-4218; ** *as reported by:* Novo *et al.* (2020) Oncology 98(7):445-451

ENDOTHELIAL PROFILING *IN VIVO*

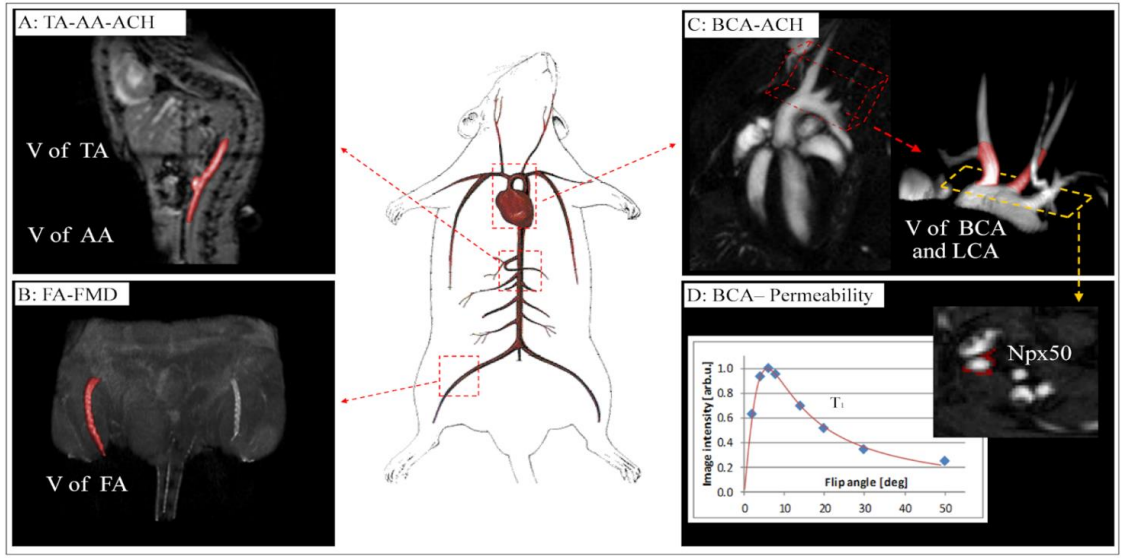
- impact of TKIs on endothelium-dependent vasodilation *in vivo* (C57BL/6 mice)
- 3 series of experiments including:
  1. **endothelium-dependent response to acetylcholine** and evaluation of vasodilatation response assessed in the abdominal (AA) and thoracic (TA) aorta
  2. **endothelium-independent response to sodium nitroprusside** and evaluation of vasodilatation response assessed in the abdominal (AA) and thoracic (TA) aorta
  3. assessment of vasodilation resulting from increased blood flow in the femoral artery (FA) after short-term occlusion using flow-mediated vasodilation (FMD) - **the most widely accepted way to assess endothelial function in clinical condition**
- study used **original MRI-based method to evaluate endothelial function in mice** (*developed by JCET**)

* *Bar et al. (2016) **NMR in Biomedicine**, 29(8):1088*

MAGNETIC RESONANCE IMAGING (MRI)

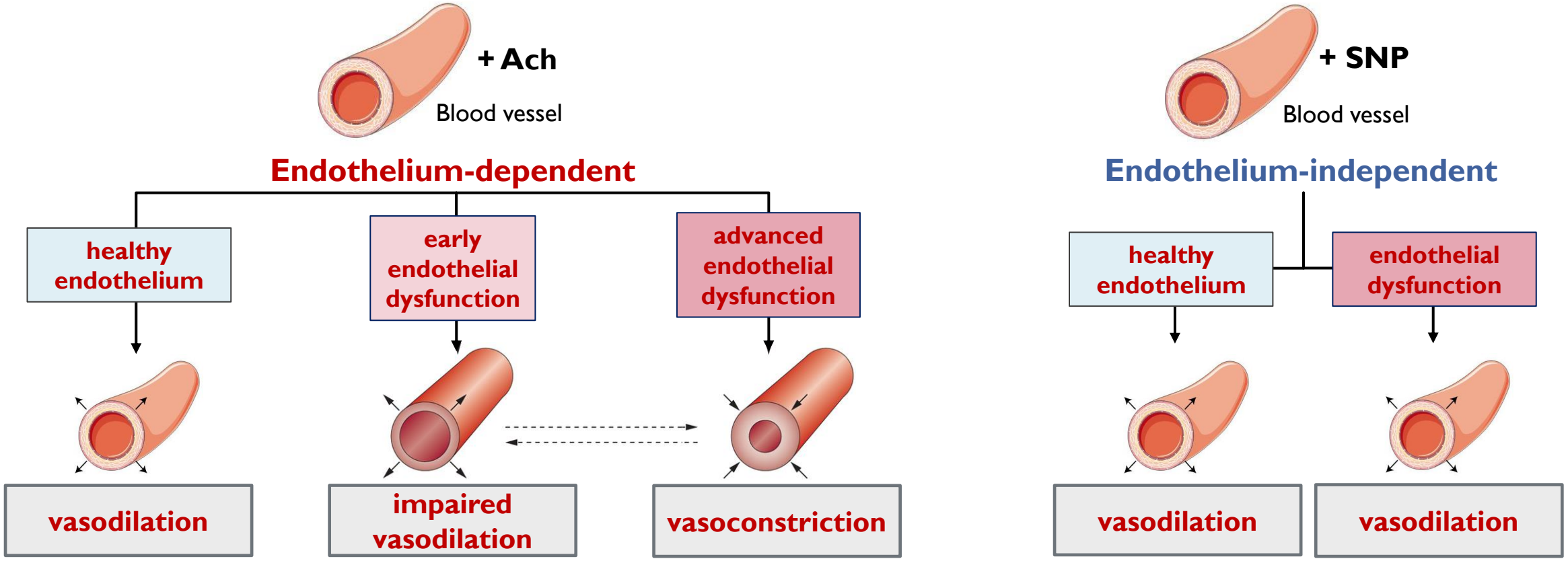


- imaging performed using a 9.4 T scanner (BioSpec 94/20 USR, Bruker) with a 210 mm horizontal bore magnet, equipped with a BFG-113/60-S gradient system and 36 mm ^{1}H quadrature volume resonator



Bar *et al.* (2019) *Journal of the American Heart Association, 8(6)*, e011171

EXPERIMENTAL APPROACH – THE PHARMACOLOGY BEHIND

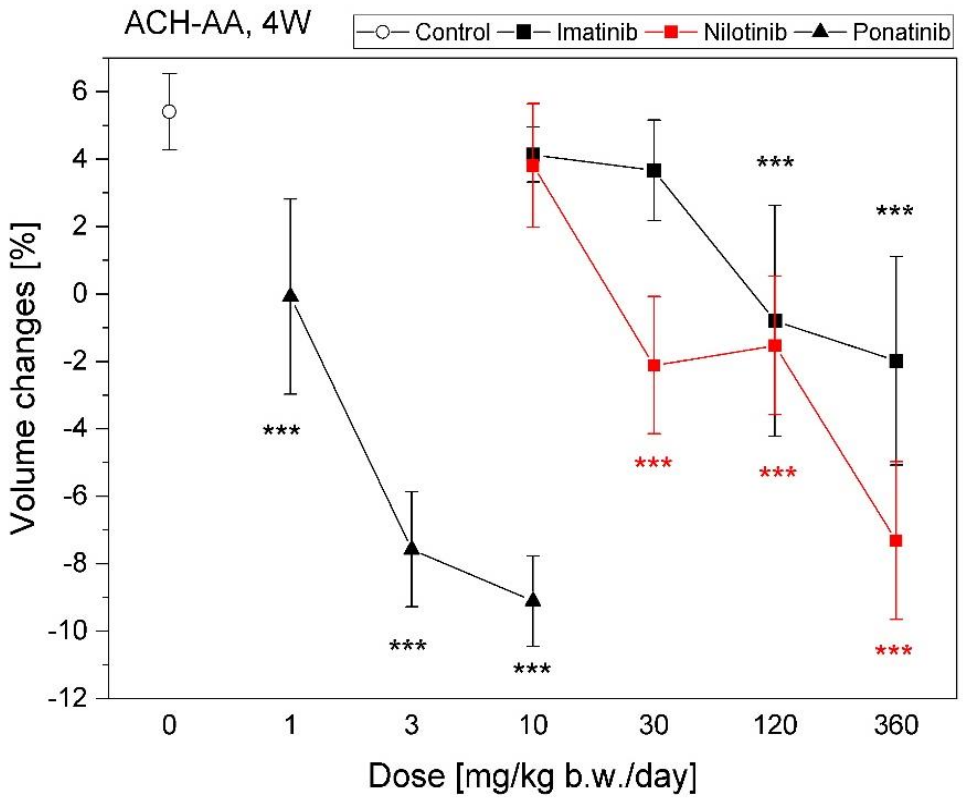


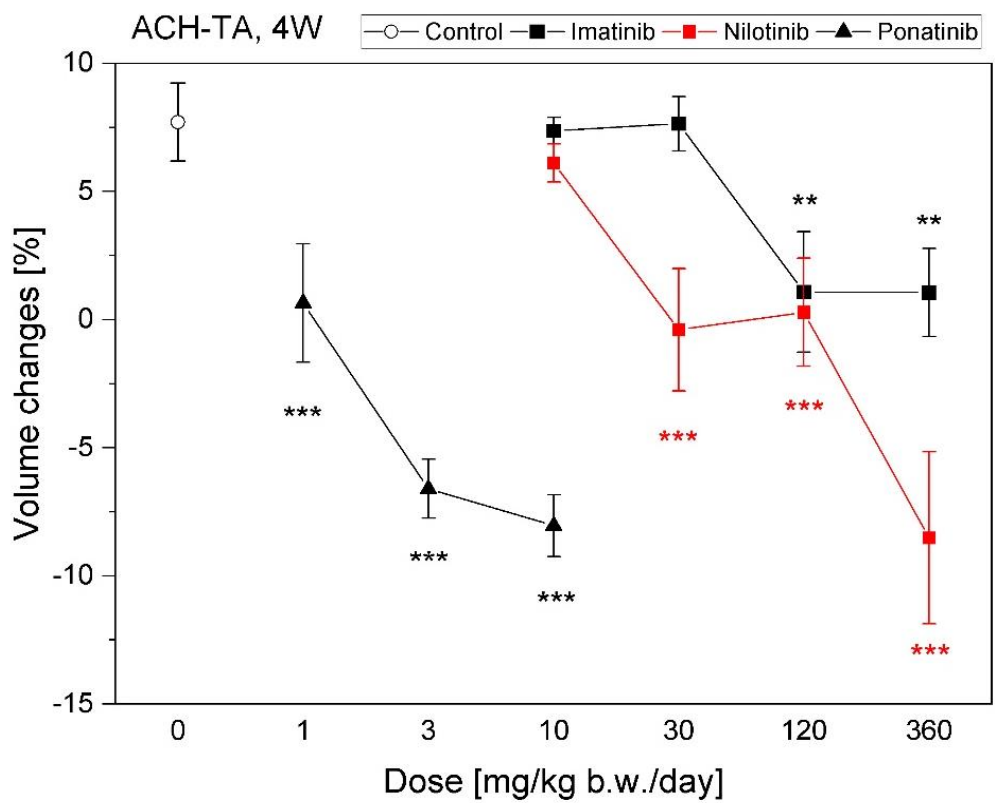
adopted from: Camici *et al.* (2015) *Nat Rev Cardiol* **12,** 48–62

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

ENDOTHELIUM-DEPENDENT FUNCTION IMPAIRED
ACETYLCHOLINE-MEDIATED VASODILATATION OF AORTA (AA, TA)





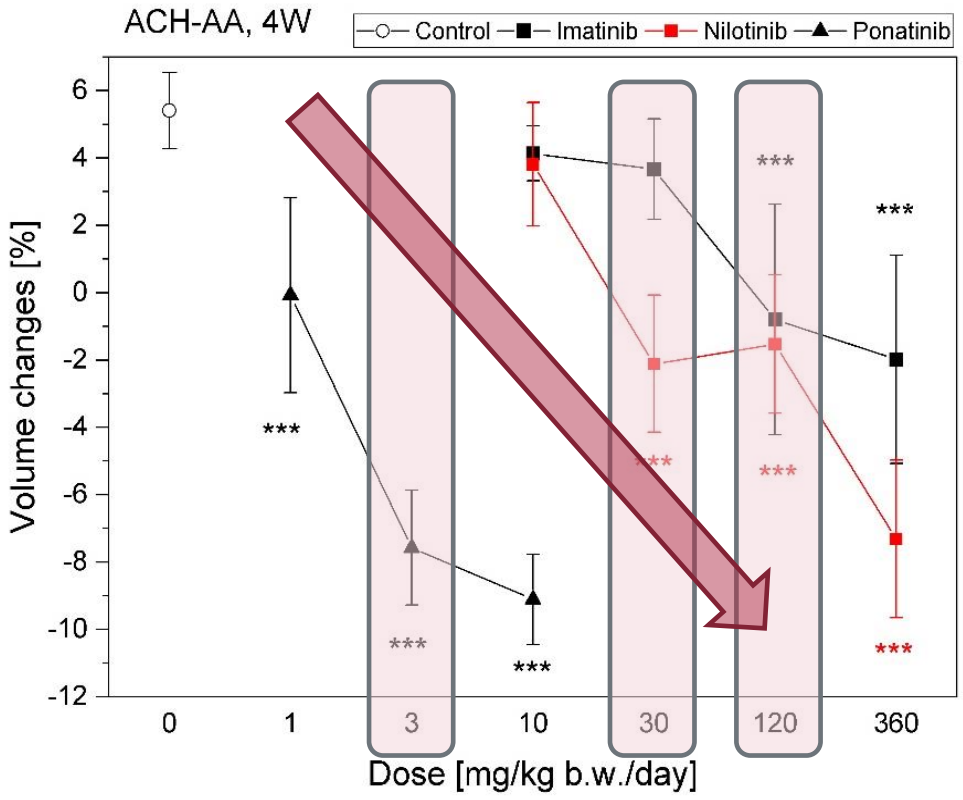
CONFIDENTIAL – *unpublished results from:* Marczyk B. *et al.* (2022), in preparation

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

ENDOTHELIUM-DEPENDENT FUNCTION IMPAIRED

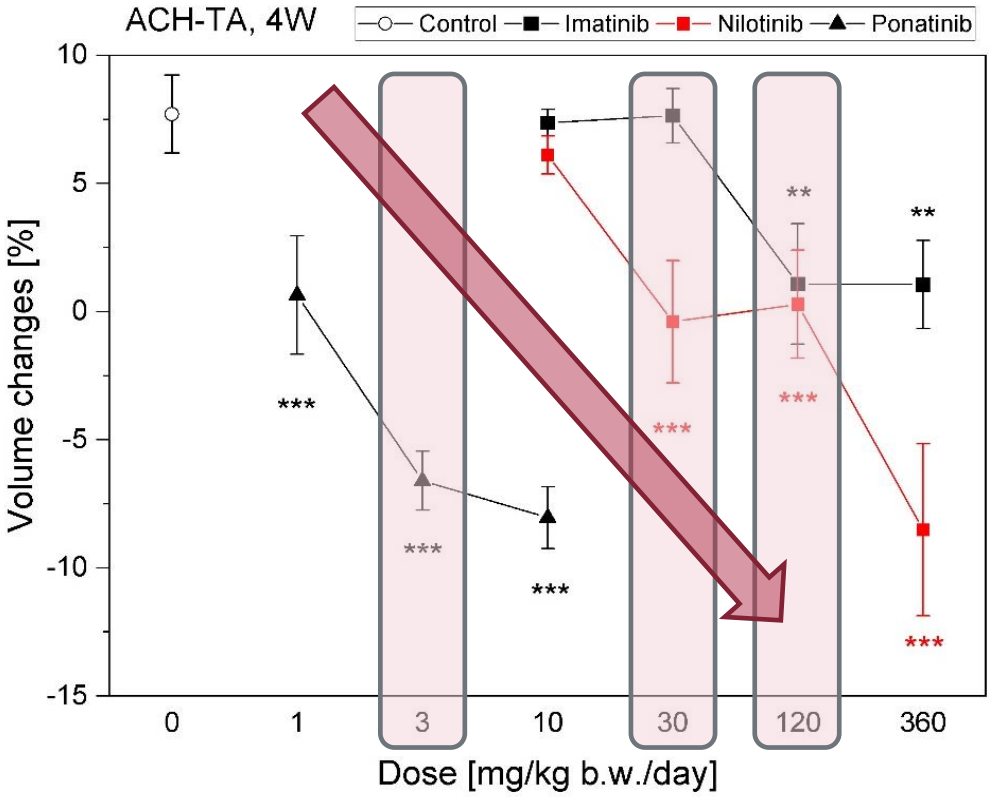
ACETYLCHOLINE-MEDIATED VASODILATATION OF AORTA (AA, TA)



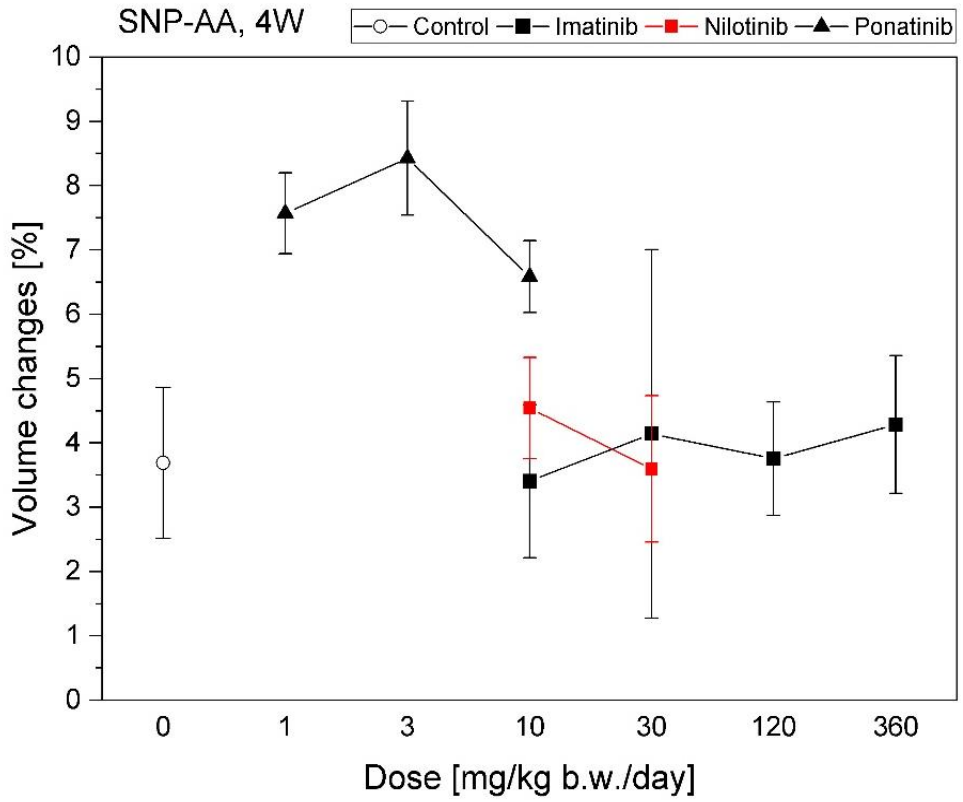
I: 120 mg /kg b.w./day

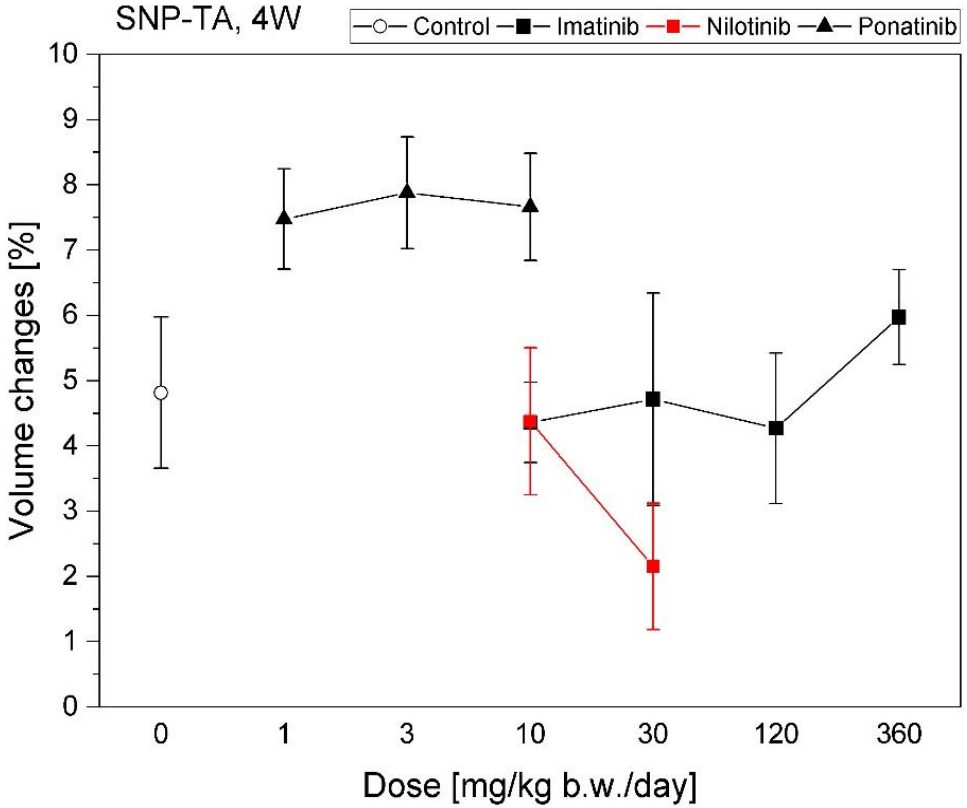
N: 30 mg /kg b.w./day

P: 3 mg /kg b.w./day



ENDOTHELIUM-INDEPENDENT FUNCTION UNAFFECTED
SODIUM NITROPRUSSIDE-MEDIATED DILATATION OF AORTA (AA, TA)



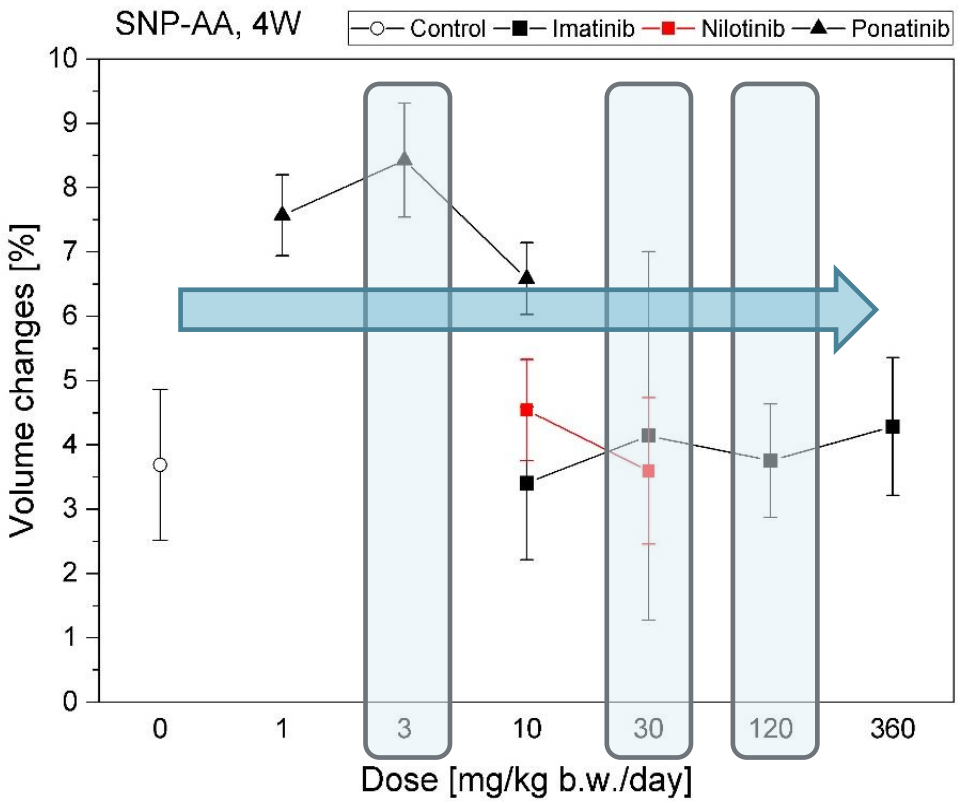


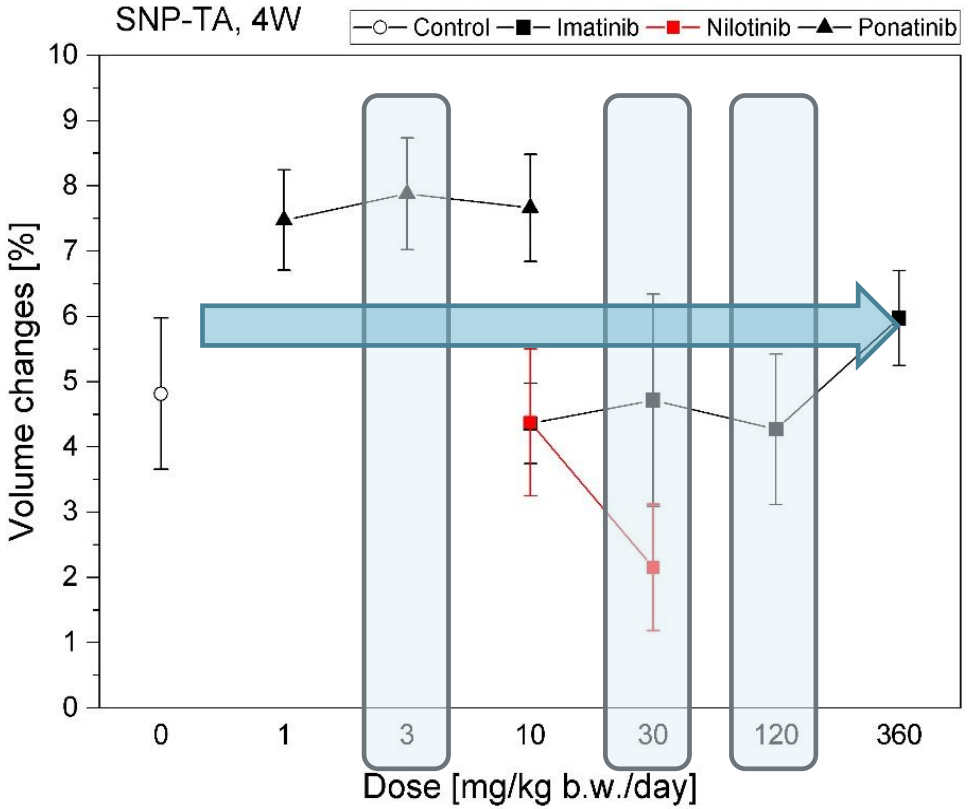
JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

ENDOTHELIUM-INDEPENDENT FUNCTION UNAFFECTED

SODIUM NITROPRUSSIDE-MEDIATED DILATATION OF AORTA (AA, TA)





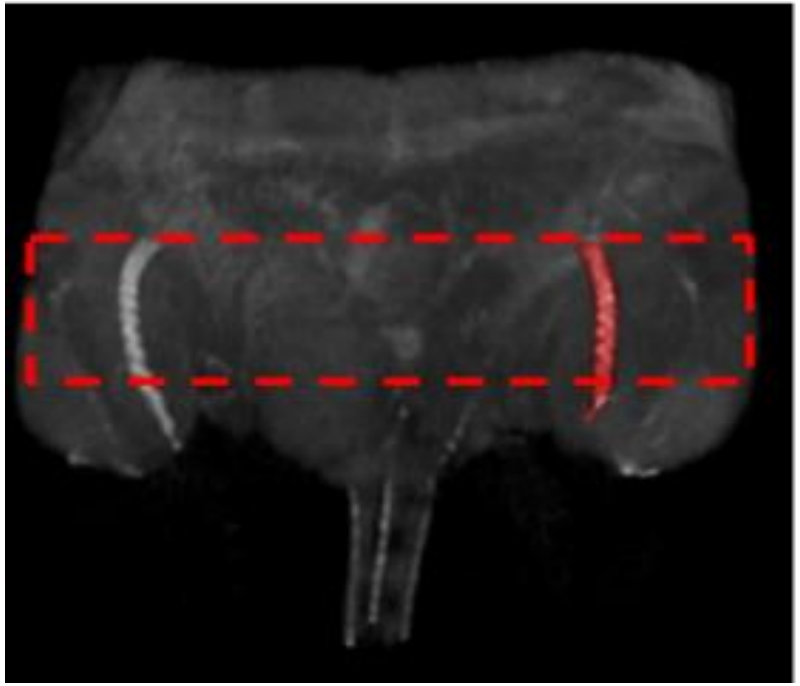
CONFIDENTIAL – *unpublished results from:* Marczyk B. *et al.* (2022), in preparation

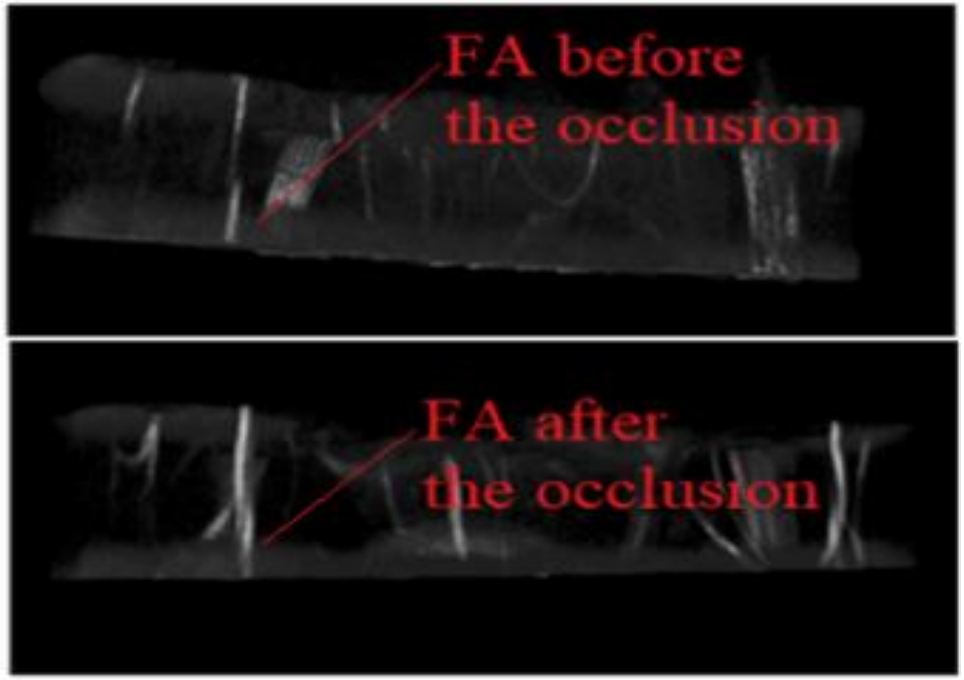
JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

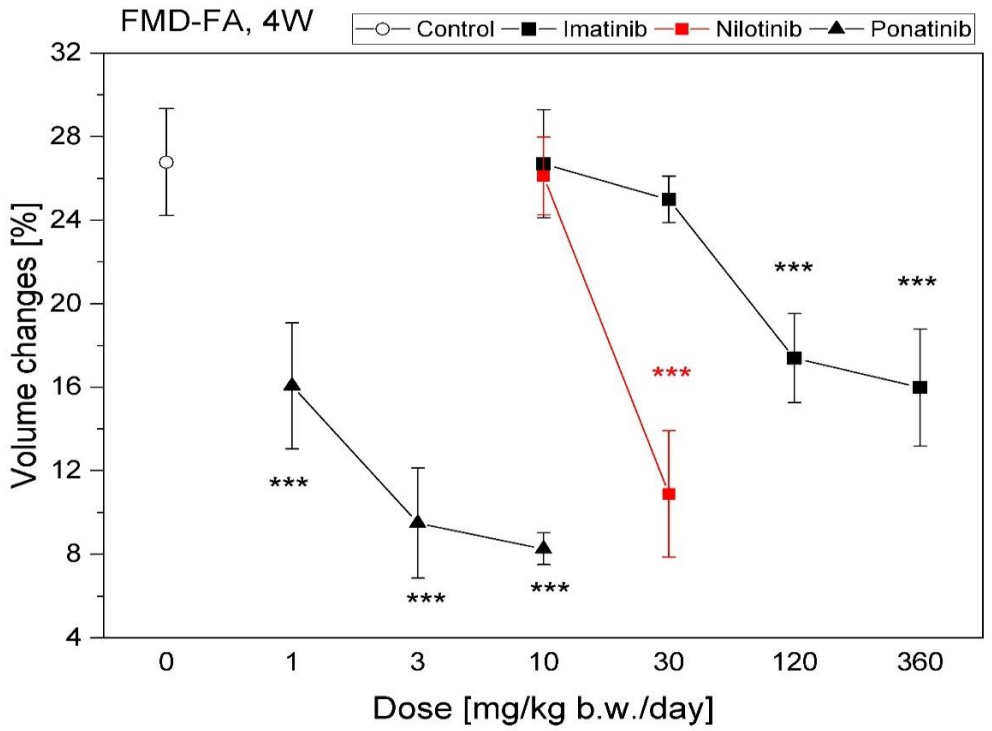
EXPERIMENTAL APPROACH – THE PHARMACOLOGY BEHIND

- **flow-mediated vasodilation (FMD)** – dilation of the femoral artery (FA) resulting from increased blood flow after short-term occlusion
- **clinically-relevant**, used in humans





ENDOTHELIUM-DEPENDENT FUNCTION IMPAIRED
FLOW MEDIATED DILATATION OF FEMORAL ARTERY (FA)

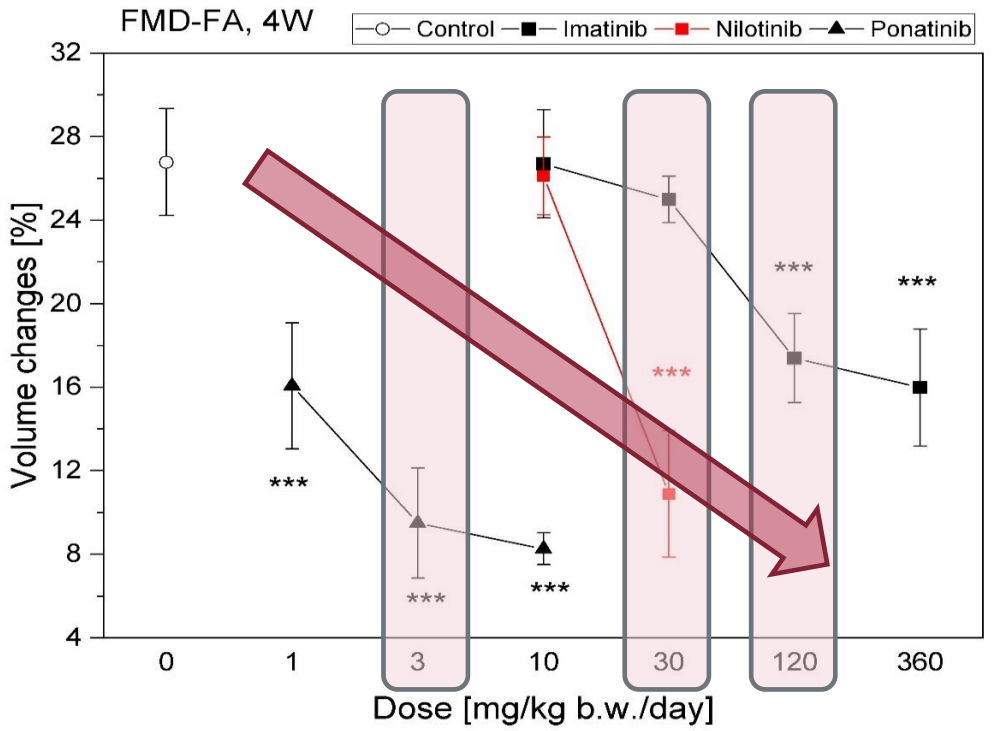


JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

ENDOTHELIUM-DEPENDENT FUNCTION IMPAIRED

FLOW MEDIATED DILATATION OF FEMORAL ARTERY (FA)



I: 120 mg /kg b.w./day

N: 30 mg /kg b.w./day

P: 3 mg /kg b.w./day

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

ENDOTHELIAL PROFILING
PREDICTIVE VALUE – CLINICAL TOXICITIES VS. PRECLINICAL RESULTS

Drug studied	6-year incidence of cardiovascular events*	Hypertension	Abnormal ankle-brachial index (ABI)*	Overall cardiovascular risk	Endothelial profiling *in vivo*
Imatinib (*1st gen*)	**2.5%** (*400 mg once per day*)	**N/A**	**6.3%** (*first-line imatinib*)	**low**	**LOW RISK** endothelial function impaired at 120 mg/kg b.w./day
Nilotinib (*2nd gen*)	**15.9%** (*400 mg twice per day*)	**6.6%****	**26%** (*first-line nilotinib*)	**medium**	**MEDIUM RISK** endothelial function impaired at 30 mg/kg b.w./day
Ponatinib (*3rd gen*)	**cumulative serious adverse effects: 10%** cardiovascular, **7%** cerebrovascular, **7%** peripheral, **14%** arterial, **3%** venous (*median follow-up 28 months*)	**26%** (*median follow-up 28 months*)*	**N/A** (*first-line trial closed due to* ***significant vascular toxicity***)	**high**	**HIGH RISK** endothelial function impaired at 3 mg/kg b.w./day

* *compiled from:* Moslehi & Deininger (2015) *J Clin Oncol* **33**:4210-4218; ** *as reported by:* Novo *et al.* (2020) Oncology 98(7):445-451

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

ENDOTHELIAL PROFILING
SIGNIFICANCE FOR DRUG DISCOVERY AND DEVELOPMENT

- first demonstration of NO-dependent endothelial function impaired by TKI *in vivo* in mice
- direct evidence of **subtle, yet differentiating** endothelium-dependent response in the aorta, both induced by acetylcholine and by increased flow
- dose-dependent endothelial dysfunction at dosing relevant to human CML treatment
- first kinase inhibitor study based on our state-of-the-art 3D MRI method *in vivo*
- **your compound could be next!**

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

BEYOND KINASE INHIBITORS – ENDOTHELIAL PROFILING AT JCET

- scientific expertise - **centre of excellence in endothelial research**, 40+ top-tier publications in 2021
- original experimental approach based on **internal know-how and experienced team** [*1*]
- methodology successfully used and validated in a **number of indications/animal models** in large vessels [*2-7*], as well as in recently-developed approach to study **microvasculature** [*8*]

[1] Bar et al. (2016) ***NMR in Biomedicine***, *29(8):1088*
[2] Sternak et al. (2018) ***Frontiers in Pharmacology***, *9, 178*
[3] Bar et al. (2019) ***Vascular Pharmacology***, *106581*
[4] Bar et al. (2019) ***Journal of the American Heart Association***, *8(6), e011171*
[5] Bar et al. (2020) ***Journal of the American Heart Association***, *9(21), e016929*
[6] Proniewski et al. (2021) ***Cells***, *10(6), 1448*
[7] Mohaissen et al. (2021) ***Cardiovascular research***, *cvab306*
[8] Kwiatkowski et al. (2021) ***Scientific reports***, *11(1):18915*

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

USE CASES FOR VASOTOXICITY PROFILING *IN VIVO*

- risk assessment for new compounds
- understanding the mechanisms of toxicity
- lead selection and candidate nomination
- managing clinical development risk
- improved out-licensing data package
- deeper in-licensing due diligence

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

MORE THAN TOXICITY: ENDOTHELIAL/VASCULAR PROFILING *IN VIVO*

- risk assessment for new compounds
- understanding the mechanisms of toxicity
- lead selection and candidate nomination
- managing clinical development risk
- improved out-licensing data package
- deeper in-licensing due diligence

- detection of **vasoprotective profile**
- understanding the protective mechanisms
- exploring new therapeutic areas
- drug repurposing/repositioning
- improved out-licensing data package
- deeper in-licensing due diligence

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKOW

CONTACT

- **Prof. Stefan Chłopicki**
  Director of JCET
  stefan.chlopicki@jcet.eu

- **Dr. Dominik Czaplicki**
  Industrial Liaison Officer
  dominik.czaplicki@jcet.eu