The Effects of Mercaptopurine on Pulmonary Vascular Resistance and BMPR2 Expression in Pulmonary Arterial Hypertension

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Letter to the editor:

Pulmonary arterial hypertension (PAH) is an incurable disease characterized by vascular obliteration and luminal narrowing (1). Current vasodilator therapies have no proven effect on the underlying molecular and cellular changes in the PAH lung, which include the emergence of apoptosis-resistant cell populations (2, 3) and proliferation of endothelial cells (4).

We recently showed that 6-Mercaptopurine (MP, Purinethol©) augmented BMP signaling and reversed abnormal vascular remodeling and right ventricle (RV) hypertrophy in the Sugen-hypoxia rat model of PAH (5). MP has been used in leukaemia and inflammatory bowel disease (IBD) for decades with an acceptable and manageable toxicity profile (6, 7). MP is an immunosuppressant that reduces inflammation and proliferation of vascular cells, which is one of the pathophysiological hallmarks of PAH (4). Here we report the results of an open label, proof-of-concept single center study of MP in PAH patients (EudraCT 2017-000137-31, Dutch Medical Ethical Committee 2017.059). The primary aim was to evaluate the efficacy and safety of MP treatment in PAH patients using a dose of 1.2-1.5 mg/kg (80-100%) once daily for 16 weeks, rounded in tablets of 25mg. The patient population consisted of idiopathic, hereditary or drug-induced PAH, New York Heart Association (NYHA) functional class II, III or IV and stable on current PAH-medication (no dose adjustments in PAH specific therapy for ≥3 months or changes in diuretics for 30 days). Eligible patients were required to show pre-capillary pulmonary hypertension with mean pulmonary artery pressures (mPAP) ≥25 mmHg, pulmonary artery wedge pressure (PAWP) ≤15 mmHg and, in order to enrich the population under study, a PVR of ≥480 $dyn.s^{-1}.cm^{-5}$. Thiopurine methyltransferase (TPMT) activity was tested prior to enrolment to prevent MP toxicity.

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Safety assessments included recording of all adverse events (AE) and blood sampling at weeks 1, 2, 4, 6, 8, 12 and 16. The primary study endpoint was a change in PVR, secondary study endpoints included changes in mPAP and cardiac index (CI), changes in RV volumes measured by cardiac magnetic resonance imaging, NT-proBNP levels, 6-minute walking distance (6MWD), NYHA functional class and quality of life scores. With a statistical power of 80% (α=0.05) we expected to detect a clinically relevant decrease in PVR of >240 $dyn.s^{-1}.cm^{-5}$ (standard deviation of 400 $dyn.s^{-1}.cm^{-5}$). With an expected responder rate of 50% (8) and a dropout rate of 15% we estimated the sample size at 50 patients.

Out of 77 patients that were eligible for inclusion in our center, 60 patients (78%) declined to participate, mainly because of high study burden (time, need to travel and undergo procedures, fear of side-effects). 15 patients were enrolled into the study from 2017-2019. The mean PVR was 878 $dyn.s^{-1}.cm^{-5}$ and was significantly decreased after treatment (p=0.0273 intention-to-treat analysis and p=0.0044 per-protocol analysis in table 1). This decrease in PVR remained significant after correcting for confounding effects of hemoglobin (p=0.0071). mPAP was significantly decreased in the per-protocol analysis (-5 mmHg p=0.04) but not in intention-to-treat analysis. Although this decrease in PVR was significant, the magnitude of reduction was relatively small, leading to no improvement in CI, RV function, NYHA functional class, 6MWD or NT-proBNP concentrations.

Although the dosage of MP used in this study is generally well-tolerated by IBD patients (6), it appeared too burdensome for this PAH population. Two patients stopped the study after 2 (not shown) and 13 weeks because of nausea, vomiting and fatigue. Two other patients were excluded after 6 and 8 weeks because of migraine and myelosuppression. Hemoglobin and leukocyte counts were decreased in almost all patients (hemoglobin -0.81

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g/dL p=0.0399, leukocytes -2.7*10^9/L p=0.0001). Leucopenia was higher than expected (5-25% reported (6) vs. 40% in our study) and quality of life scores were significantly worse. 8 out of 15 patients that finished the protocol (53%) received dose reductions indicating poor tolerability. Interestingly, none of the patients showed liver toxicity. All side effects were dose-dependent and resolved after dose adjustments or treatment discontinuation. Low TPMT enzymatic activity -the best-known and most frequently studied risk factor for thiopurine-induced leukopenia (6)- was not present in our patient cohort.

In accordance with our preclinical study (5), this proof-of-concept study provides first evidence that MP decreases PVR coinciding with an increased *BMPR2* mRNA expression in PBMCs of 10/11 patients after MP treatment ($p<0.006$ in table 1 and BMPR2 expression per patient in figure 1). Impaired BMP signaling is observed in both HPAH and IPAH lungs and pulmonary vascular remodeling can be attenuated by enhancing *BMPR2* activity (9). Therefore, targeting the *BMPR2* pathway has repeatedly emerged as a novel treatment strategy for PAH (10) and improvement of BMPR2 expression in circulating PBMCs could have contributed to the decrease in PVR.

In addition to the small inclusion capacity of a single center, this study suffered from patient reluctance to participate after sharing of negative experiences by study participants in the PAH patient community (*e.g.* social media, patient supportive groups). High toxicity rates together with declining inclusion led us to prematurely end the study. We did not measure thiopurine end-products 6-TGN or 6-MMP, which are known to generate the cytotoxic and apoptotic effects of thiopurines and give rise to an increased risk for thiopurine-induced leucopenia. Literature shows that alternative thiopurine analogs, such as azathiopurine or thioguanine (Thiosix©), or a (metabolite-sensed) step-up dosing may result

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in less toxicity (6, 7). This is also shown by the fact that patients who started with 1.2mg/kg (80%) all completed the study because of lower side-effects (figure 1). As myelosuppression is dose-dependent, we hypothesize that a lower starting dosage in a step-up scheme increases the tolerability of MP treatment (6), but at the same time still effectively reduces pulmonary pressures.

The observed decrease in PVR confirms that targeting excessive vascular remodeling by inhibiting cell proliferation and inducing apoptosis with MP is of therapeutic interest. As frequency and severity of side effects were higher than reported and expected in the current design, we conclude that anti-proliferative therapy with MP as add-on treatment for hereditary and idiopathic PAH has an unfavourable risk/benefit ratio. However, improvements in dosing schemes and/or use of other thiopurine analogues deserve further study.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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Legends

Figure 1: Percentage difference of pulmonary vascular resistance and mean pulmonary artery pressure depicted per patient. Patients with T-box transcription factor (TBX4) mutations depicted in green. Patients with a bone morphogenetic protein receptor type II (BMPR2) mutation depicted in blue. Idiopathic and drug-induced patients depicted in red. ◊ indicate patients that pre-maturely ended the study. Right panel depicts the used dosage per patient during the time course of the study. Starting dose was 80-100% (1.2-1.5mg/kg rounded to tablets of 25mg), light colour means dose reduction.

Table 1: Intention to treat analysis on all patients that started therapy in white, and per protocol analysis on patients that finished 16-week treatment in blue. Data are presented as mean (SD). NYHA: New York Heart Association; HPAH: hereditary pulmonary arterial hypertension; IPAH: idiopathic pulmonary arterial hypertension; DIPAH: drug-induced pulmonary arterial hypertension; BMPR2: Bone morphogenetic protein receptor type II; TBX4: T-box transcription factor; NT-proBNP: N-terminal brain natriuretic peptide; p-value in paired-Test. [1]: non-parameteric data depicted as median [interquartile range] and Wilcoxon non-parametric test. [2]: Generalized Estimation Equation analysis of PVR corrected for decrease in hemoglobin.

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	Intention to treat Before n=15 mean (SD)	**Intention to treat After n=14** mean (SD)	**p-value**	**Per protocol Before n=11** mean (SD)	**Per protocol After n=11** mean (SD)	**p-value**
Patient characteristics						
Age (years)	42 (15)					
NYHA (2/3/4)	8 / 5 / 2					
Diagnosis (HPAH / IPAH / DIPAH)	11 / 3 /1					
BMPR2 mutation	9					
TBX4 mutation	2					
PAH therapy (Mono / duo / triple)	2 / 9 / 4					
Weight (kg)	65.6 (11)	65.3 (11)	0.2589	69.6 (11)	67.4 (10.8)	0.2183
6 minute walking distance (m)	499 (127)	506 (114)	0.4511	510 (116)	513 (107)	0.7130
NTproBNP (pg/mL)	626 [156-169] [1]	413 [168-859] [1]	0.9999[1]	626 [156 – 782] [1]	340 [176-561] [1]	>0.999[1]
Quality of Life questionnaire score (0-84)	36 (18)	39 (16)	**0.0432**	29 (17)	35 (15)	**0.0096**
Hemodynamic characteristics						
Mean right atrial pressure (mmHg)	8 (3)	8 (3)	0.2880	8 (3)	8 (3)	0.5024
Mean pulmonary artery pressure (mmHg)	58 (12)	53 (9)	0.1181	59 (14)	53 (9)	**0.0411**
Pulmonary arterial wedge pressure (mmHg)	11 (3)	12 (4)	0.2715	11 (3)	12 (4)	0.2392
Pulmonary vascular resistance (dyn.s-1.cm-5)	878 (345)	757 (308)	**0.0273**	879 (354)	759 (342)	**0.0044** **0.0071[2]**
Systemic mean arterial blood pressure (mmHg)	81 (13)	82 (9)	0.3200	78 (13)	81 (10)	0.5316
Heart rate (bpm)	76 (10)	72 (11)	0.2526	72 (7)	69 (11)	0.4068
Peripheral blood cell count				**n=11**	**n=11**	
Peripheral blood mononuclear cells (*10^9/L)				10.9 (7.3)	5.7 (2.4)	**0.0390**
Monocytes (*10^9/L)				3.0 (2.2)	1.3 (0.6)	**0.0350**
Neutrophils (*10^9/L)				1.2 (0.9)	0.8 (0.6)	0.1910
Interleukin-6 (pg/mL)				4.5 (3.8)	5.5 (5.9)	0.4420
Tumor necrosis factor-α (pg/mL)				1.3 (1.1)	1.3 (0.9)	0.7595
Relative BMPR2 expression				1.001 (0.59)	2.027 (0.63)	**0.0057**

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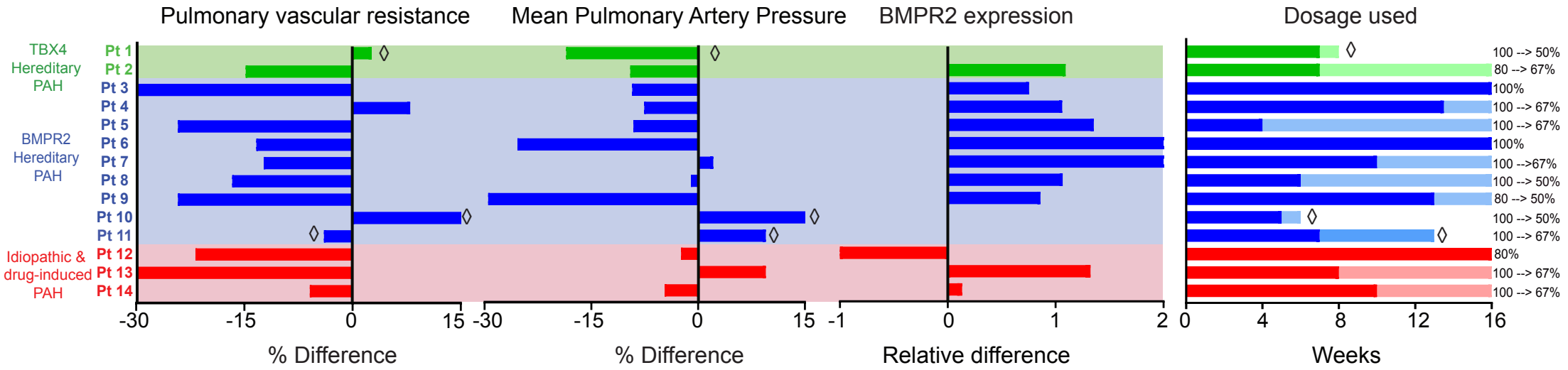
Figure 1:



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