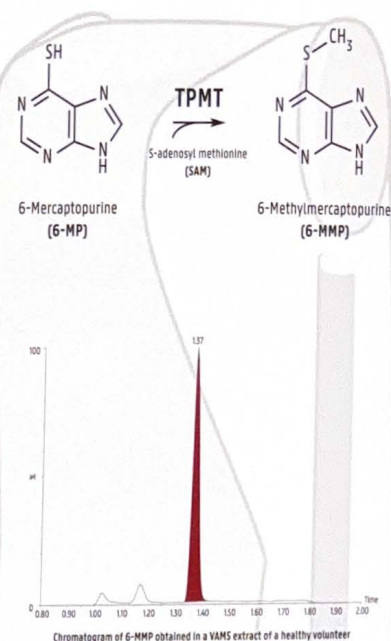
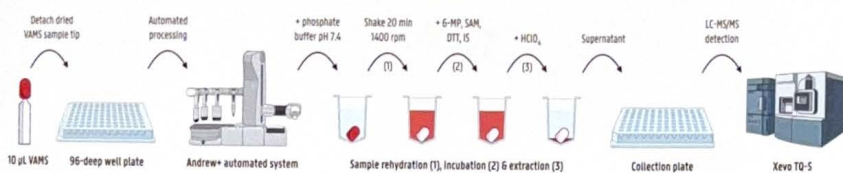


Introduction

Thiopurines (6-mercaptopurine, 6-thioguanine and azathioprine) are purine antimetabolites used for Crohn's disease and other oncologic and autoimmune disorders that may cause severe myelosuppression. Thiopurine S-methyltransferase (TPMT) methylates and inactivates thiopurines and is therefore a major determinant of toxicity. Approximately 11% of Caucasians have reduced TPMT activity and 1 in 300 are completely deficient, making pre-treatment TPMT assessment essential. Phenotyping is often preferred over genotyping due to lower cost and faster turnaround, yet **conventional assays require labour-intensive** red blood cell lysate preparation. We aimed to simplify TPMT phenotyping using **volumetric absorptive microsampling (VAMS)** combined with **automated sample preparation**.

Methods

Rehydrated 10 μ L VAMS samples were incubated with 16 mM 6-MP (substrate) and 2.0 mM SAM (cofactor) for 60 min at 37°C. The reaction was quenched with 40% perchloric acid, followed by extraction and LC-MS/MS quantification of the formed 6-MMP.



Results

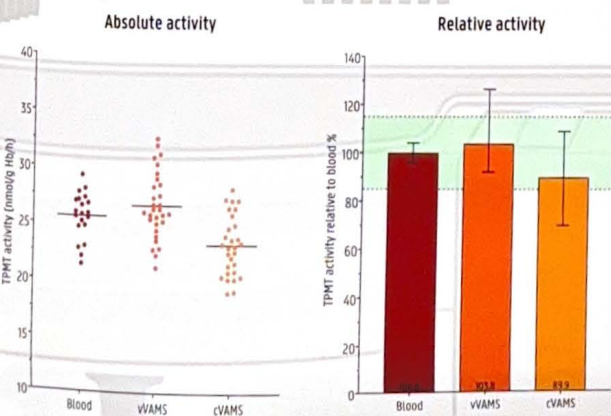
1. Analytical validation

Quantitative determination of 6-MMP in 10 μ L liquid blood and 10 μ L VAMS extracts.

- Accuracy** Bias <8% (blood) and <6% (VAMS)
- Precision** Total imprecision <6% at LLoQ and <4% at all QC levels
- Matrix effect** 100-104% (blood and VAMS), relative matrix effect $\leq 1.2\%$ CV
- Recovery** 97-104% (blood and VAMS), relative recovery $\leq 2.0\%$ CV
- Selectivity** Absence of interferences in blank blood from six donors

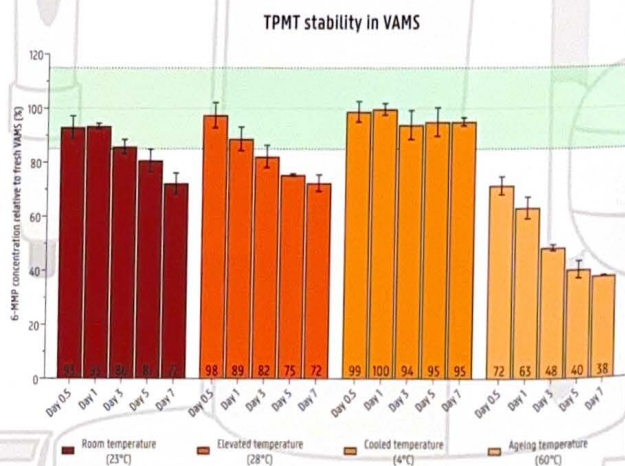
3. Proof-of-concept study

TPMT activity was assessed in 10 healthy volunteers using 10 μ L liquid whole blood (n=2), venous VAMS (vVAMS; n=3), and capillary VAMS (cVAMS; n=3). Activity was expressed as nanomoles of 6-MMP formed per gram of hemoglobin per hour (nmol/g Hb/h). VAMS results within $\pm 15\%$ of whole-blood activity were considered comparable.



2. Stability

TPMT enzyme activity was evaluated in VAMS samples from 3 donors after storage under different temperature conditions. All samples were dried for 3 h at ambient temperature. One replicate was stored at -80°C immediately after drying ("fresh"), while the remaining replicates were stored at 4°C, 23°C, 28°C, or 60°C before transfer to -80°C until analysis.



Conclusions

We developed and analytically validated the first TPMT phenotyping method using dried Mitra® VAMS with automated sample preparation. TPMT activity remained stable during storage at 4°C, and proof-of-concept data showed good agreement with liquid whole blood for both venous and capillary VAMS. This approach may support simplified, automated TPMT phenotyping and facilitate clinical implementation.