

## 3<sup>rd</sup> AC21 International Research Festival

From Genes to Patients: New Perspectives on Personalised Medicines

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### Poster Abstracts:

#### 16. Preliminary Phenotyping Studies in the TARGET Project: Thiopurine Methyltransferase (TPMT) Measurements and Azathioprine Metabolite Profiling

Jane E Alder<sup>1</sup>, Gary P. Sidgwick<sup>1</sup>, William Newman<sup>1&2</sup>, Sudeep P. Pushpakom<sup>3</sup>, Karen Tricker<sup>1</sup>, Katherine Payne<sup>1</sup>, Emily A Fargher<sup>1</sup>, Kay Poulton<sup>2</sup>, Faieza Qasim<sup>2</sup>, J Brian Houston<sup>1</sup>, Rachel A Elliott<sup>1</sup>, Robert G Elles<sup>3</sup>, David W Ray<sup>1</sup>, John Shaffer<sup>4</sup>, Christopher E Griffiths<sup>1</sup>, Ian N Bruce<sup>1</sup>, Steven A Roberts<sup>1</sup>, Julie Andrews<sup>1</sup> and William E Ollier<sup>1</sup>

<sup>1</sup>The University of Manchester, Oxford Road, Manchester M13 9PL; <sup>2</sup>Central Manchester & Manchester Children's University Hospitals NHS Trust; <sup>3</sup>National Genetics Reference Laboratory, St Mary's Hospital, Manchester; <sup>4</sup>Salford Royal Hospitals NHS Trust

**Introduction:** The **TARGET** Project (TPMT:Azathioprine Response to Genotype and Enzyme Testing) is a prospective randomised controlled trial (funded by the Department of Health, UK) to determine the cost effectiveness of introducing a pharmacogenetic test (genotyping of common TPMT alleles) with a view to reducing the number of adverse drug reactions (ADRs) associated with azathioprine treatment. Certain allelic variants in the TPMT gene (e.g. \*2, \*3A and \*3C) significantly reduce enzyme activity and hence raise thioguanine nucleotide (TGN) metabolite levels in patients who are heterozygous (~11% UK population) or homozygous (~0.3% UK population) for mutant alleles. This is important because elevated TGN levels can lead to severe, life-threatening, myelo-suppression. Prediction of TPMT activity is therefore likely not only to reduce the incidence of ADRs, but may also be beneficial in optimising personalised drug dosage.

**Methodology:** In addition to genotyping, all blood samples will be phenotyped by measurement of red blood cell: (i) TPMT activity, using HPLC- fluorescence detection of the conversion of thioguanine (6-TG) to methyl-thioguanine (6-MTG) (ii) azathioprine metabolite levels, using HPLC-UV/Vis detection of methyl-mercaptopurine (6-MMP) and thioguanine (6-TG). Metabolite levels will also provide a measure of patient compliance.

**Results:** Preliminary studies were carried out to see which published method (for determination of TPMT activity) gave the most reproducible data. This was found to be the method of Ford and Berg, 2003 [1]. Since both TPMT activity and metabolite levels are given per gram haemoglobin (Hb), the accuracy and precision of Hb measurements in the red blood cell (rbc) lysate is paramount. Imprecision of Hb measurements showed that the coefficient of variation (CV) was 2.3% within batch (n= 7) and 3.4% between batches (n=3). The distribution of TPMT activity, within a sample set of 33 patient blood samples, was 2 patients with values between 10-20 nmol 6-MTG/gHb/h (subsequently confirmed as heterozygotes by genotyping) and 31 patients with values between 21-50 nmol 6-MTG/gHb/h. A sample from a known homozygous-deficient patient had a value of <1 nmol 6-MTG/gHb/h. The CV for individual TPMT activity measurements was



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5.7% (n=11). These values and frequencies are consistent with previously published data [1].

**Conclusion:** The TARGET study is designed to provide robust data concerning therapeutic outcomes, costs, and service users' and providers' preferences to inform the introduction of a pharmacogenetic test into the NHS. However, phenotyping studies of the type described here, will provide additional information on TPMT genotype-phenotype correlations and may also help to elucidate the role of other polymorphic genes in the thiopurine metabolic pathway.

1. Ford, L.T. and J.D. Berg, *J Chromatogr B Analyt Technol Biomed Life Sci*, 2003. **798**(1): p. 111-5.