Response to "Plasma Uracil as a DPD Phenotyping Test: Pre-analytical Handling Matters"

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RESPONSE LETTER TO THE EDITOR

Response to "Plasma Uracil as a DPD Phenotyping Test: Pre-analytical Handling Matters"

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We thank Thomas *et al*. for the comments on our publication[1] and continued discussion regarding the feasibility and clinical validity of plasma uracil concentration as a marker for DPD deficiency. The authors request the number of patients per hospital included in the analysis, which we provided in **Figure 1a**. Thomas *et al*. propose that the reliability of our conclusion is undermined, because we used uracil data irrespective of sample origin. We tend not to agree with this, as our conclusion focusses on the feasibility of using uracil for DPD phenotyping in routine clinical practice, across a range of treatment centers.

Additional analyses excluding the six centers with significantly higher uracil concentrations are suggested. However, this would not be a methodologically justified approach, being a non-preplanned analysis and without further justification of why to exclude certain sites. Nevertheless, we performed this analysis and this showed similar results, with no association with severe toxicity (**Figure 1b**). In addition, an analysis using the reference center alone showed similar negative results (**Figure 1c**).

As Thomas *et al*. mentioned, French authorities recommend a maximum blood storage of 1.5 hours at room temperature. However, there is no consensus for the maximum time of blood storage and several studies have demonstrated consistent increases in uracil concentrations when whole blood is stored at room temperature before centrifugation.[2–4] More specifically, average uracil concentrations were found to be increased by 27% after 1 hour,[2] 21% after 1.5 hours,[3] and ~25% after 2 hours.[4] Our unpublished data shows an increase of 12.7% after 2 hours in whole blood and at room temperature. Therefore, delayed processing could potentially result in misclassification of patients. The instability of uracil makes it a highly complex marker for predicting DPD deficiency accurately.

In addition, Thomas *et al*. provided data from three academic laboratories. We acknowledge that these data look more re-assuring. Nevertheless, for two of these laboratories, they also show significant differences in uracil concentrations between centers, despite the relatively large sample sizes.

Aside from remaining questions around pre-analytical processing, the clinical validation of uracil as a biomarker to guide fluoropyrimidine dosing is also incomplete. In our recent Alpe2U study, we aimed to validate this method.[5] We gave a 50% dose reduction advice for *DPYD* wild type patients with uracil levels > 16 ng/mL. Despite this, these patients had a 56% lower AUC of 5-FU than expected, indicating underdosing.[5]

In conclusion, we deem that there are outstanding concerns around the feasibility, clinical validation, and usefulness of uracil testing in clinical practice.

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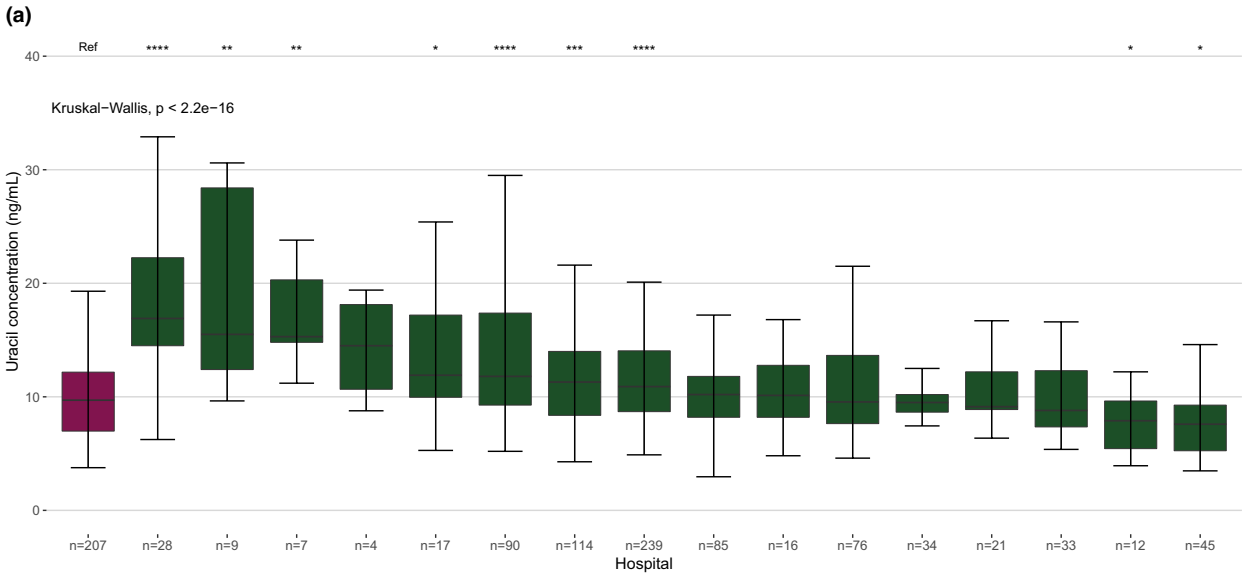
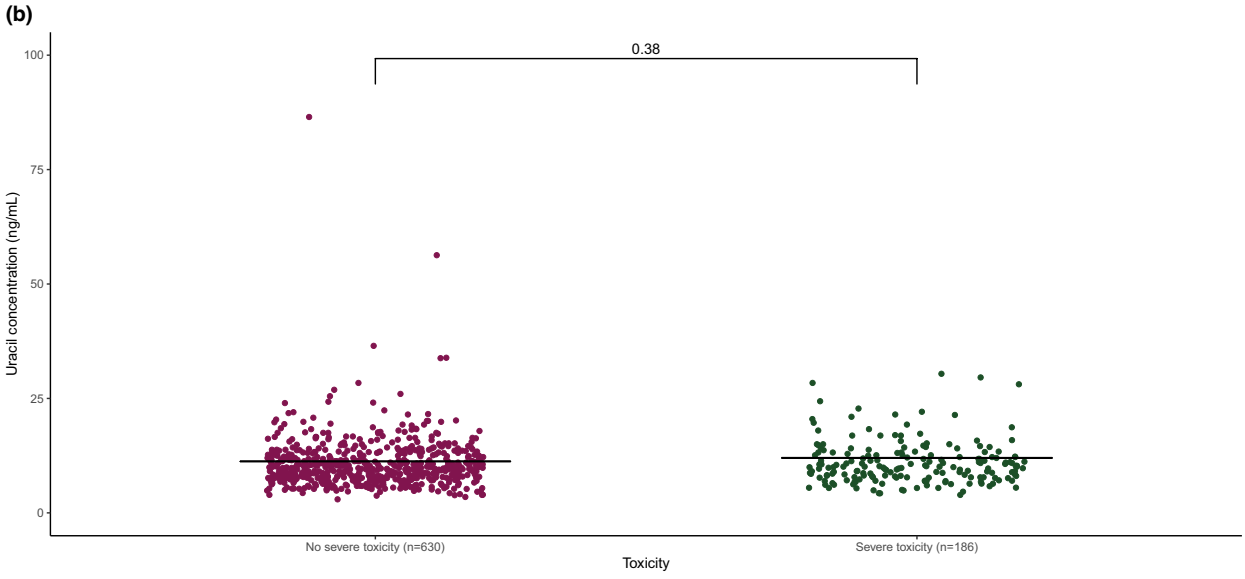
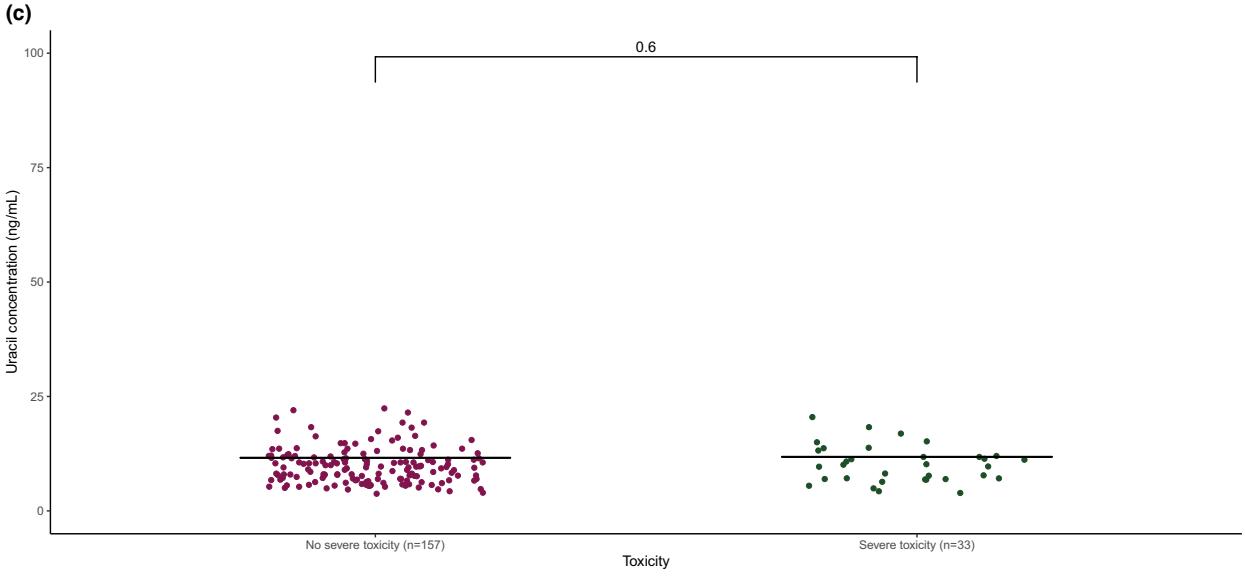
CONFLICT OF INTEREST

The authors declare no conflict of interest. Jan Schellens is a (part time) employee, stock- and patent holder of Modra Pharmaceuticals, a spin out company developing oral taxane formulations; Jan Schellens is also a part-time employee of Byondis bv and received consultancy fees from Debiopharm all not related to the contents of the manuscript. Didier Meulendijks is a current full-time employee and shareholder of AstraZeneca, not related to the contents of the manuscript.

Figure 1 Differences in measured pretreatment uracil levels between hospitals. (a) Differences in uracil concentrations (ng/mL) among the 17 participating hospitals in 955 *DPYD* wild type patients (clinicaltrials.gov identifier NCT02324452). All the samples were measured centrally, and therefore the central hospital was chosen to be the reference hospital (indicated in red). Differences between medians were determined using one-way analysis of variance (Kruskal-Wallis). The following symbols indicate a *P* value of: *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$. (b) Comparison of uracil concentration between patients experiencing no severe toxicity (grade 0–2) and patients who did experience severe toxicity (grade 3–5) excluding the six hospitals with the highest median uracil concentrations. (c) Comparison of uracil concentration between patients experiencing no severe toxicity (grade 0–2) and patients who did experience severe toxicity (grade 3–5) in the reference hospital.







(For caption see page 473).

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