Author's response to reviews

Title: Genotyping Panel for Assessing Response to Cancer Chemotherapy

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Author's response to reviews: see over
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Dear Dr. Edmunds:

Thank you for forwarding the comments of the reviewers on our manuscript entitled “Genotyping Panel for Assessing Response to Cancer Chemotherapy” by Dai et al. (266630459167989). We are grateful to the reviewers for their valuable comments and suggestions, and have revised the manuscript accordingly. Specific responses are explained in detail below.

Response to reviewer Dr. Federico Innocenti:

1) The assessment of the cost-effectiveness of this platform should be conducted, in comparison with other available platforms. This point needs to be thoroughly addressed in the discussion, as the cost-effectiveness would be one of the major advantages for using this platform.

The goal of the work is to develop the most informative genotyping panel, increasingly targeting polymorphisms shown to be relevant to disease and therapy. The optimal genotyping platform will evolve over time, maybe even on a yearly basis, so that we do not want to overemphasize a technology comparison that may already be dated by the time of publication. More important are the criteria by which the methodology needs to be selected. To address both of these issues, we have added the following information on page 21.

“The goal is to develop genotyping panels containing polymorphisms shown to be relevant to disease and drug therapy. Therefore, the genotyping platform needs to be flexible to accommodate new findings, while the number of pertinent SNPs remains rather modest at present. In contrast, for discovery of new candidate genes and polymorphisms, very large SNP panels are beginning to be the norm. The SNPlex platform is designed for genotyping assays involving an intermediate number of SNPs (30-500). As each panel is multiplexed to maximally 48 SNPs, multiple panels need to run for larger SNP panel genotyping. As reagent cost is ~$5.00 per run ($0.10/SNP), the method is cost-effective for targeted genotyping of up to 500 to maximally 1000 candidate SNPs. Use of multiple panels permits flexibility in genotyping for specific applications, involving just a few samples or large cohorts. From our experience, for genotyping more than 500-1000 SNPs in any given project, alternative methods such as bead arrays may be more practical because of the increasing number of SNPlex panels needed. However, the optimal method will change rapidly on a yearly basis.”

2) For the reasons already described in my previous review, any genotype-phenotype analysis with either flavopiridol pharmacokinetics in one study and cancer risk in another study should be removed from this paper.
Per request, we have removed the following sentences from the manuscript.

**In the ABSTRACT section:**
“Association analysis revealed several candidate genes potentially associated with colorectal cancer, requiring further study.”

**In the INTRODUCTION section:**
“and have then compared the results with population genotypes available on the Web (HapMap and other tools). While this therefore must be considered a pilot study, we have identified several candidate SNPs that may be associated with cancer risk or progression and require further study.”

**In the MATERIALS AND METHODS section:**
“Association between genotype and clinical data was performed using HelixTree software according to the manufacture’s manual (Golden Helix, Inc. Bozeman, MT, USA). Clinical phenotypes were designated 1 and 0 for cases and controls respectively in a case-control study of colorectal cancer, or pharmacokinetic parameters for the flavopiridol clinical trial.”

**In the RESULTS section:**
“Several polymorphisms in transporter genes did show significant association with flavopiridol pharmacokinetics and are being further evaluated. Detailed analysis of phenotype-genotype correlation will be published separately.”

“Since we did not have access to a control population specifically designed to match the colon cancer patients in this study, we compared genotype frequencies to historical controls, as provided by HapMap and further databases. While this approach incurs the risk of population stratification, it nevertheless provides a first pass at identifying potential risk factors, in particular if more than one database supports potential association with colon cancer.

Among all 231 SNPs analyzed, genotype information for 170 SNPs is available in the HapMap project for 90 samples collected from people living in Utah with ancestry from Northern and Western Europe (retrieved September 2006). Using the data from the HapMap project as control group for a colorectal cancer association study, 27 (15.9%) of 170 SNPs showed adjusted p values < 0.05, and 8 (4.7%) were at p < 0.01. The eight SNPs with p < 0.01 are listed in Table 2. Some of these SNPs had also been genotyped for control cohorts in other projects, such as Perlegen and the NCI’s Cancer Genome Anatomy SNP500Cancer Project. Both of these have been integrated into the NCBI database [37]. The available results are listed in Table 2.

We also searched the literature to obtain further information on SNPs and genes implicated in this analysis. For instance, prohibitin (PHB) rs6917 (C>T) had an adjusted p value of 0.008. Located in the 3’-UTR region of the gene, this polymorphism has been shown to be functional but has yet to be implicated in colorectal cancer. The C allele of the prohibitin 3’-UTR SNP produces a functional RNA serving in a tumor suppressor mode, whereas the T creates a null allele. In our pilot study, the T allele was present
in 42% (C/T 35%, T/T 7%) of 74 Caucasian patients with colorectal cancer, whereas the frequency was only 30% of the 90 HapMap samples taken as a control [22]. The latter distribution is consistent with a previous report that C/T and T/T combined account for 32% in 1046 normal controls, with a majority (84%) being Caucasians [38]. Since the sample size of the current study is small, we will genotype a larger cohort of colorectal cancer patients to validate this intriguing finding, before launching more detailed molecular genetic analysis.”

**In the DISCUSSION section:**

“We have launched two pilot projects with the genotyping panels, one related to a Phase-1 trial of flavopiridol in the treatment of CLL, primarily conducted to establish and validate the genotyping panels. The pharmacogenetic results will be reported elsewhere. The other study employed our cancer biology panels involving genes in apoptosis and cell growth, and DNA repair. This was applied to a colorectal cancer cohort and the results compared to historical control groups previously genotyped on a large scale and accessible on the Web. It is noted that these public databases are continuously growing, allowing more accurate control experiments for pilot studies. For follow-up, we still must establish prospective controls matched for age, sex, ethnicity, etc. Nevertheless, a few leads were identified, including the gene encoding prohibitin, containing a known functional SNP in the 3’ untranslated region. The significance of the mutation to colorectal cancer requires further study.”

“**Table 2.** Eight SNPs with adjusted p < 0.01 using the data from the HapMap project as control group for a colorectal cancer association study” is deleted. The original table 3 is table 2 in the revised manuscript.

3) **A more stringent cut-off (p<0.01) should be used for the HWE deviation.**

The authors modified the results using the more stringent cut-off (p<0.01). The results have been incorporated on page 19, “Over 95% (136 out of 146) of the SNPs follow Hardy-Weinberg equilibrium (Chi-squared test, P>0.01)” and on page 20, “Chi-squared test indicates all SNPs follow Hardy-Weinberg equilibrium (P>0.01).”

4) **The fact that the platform does not include, in addition to the functional variants, also htSNPs needs to be addressed as a limitation of this study in the discussion. The purpose of including htSNPs is not to discover new functional polymorphism (as stated by the authors as a justification for not including the htSNPs), but to comprehensively survey the variation in a gene without missing the information that is not captured by the inclusion of single functional variants in the platform.**
The following sentences were added on page 23. “In addition, the selection of SNPs was not designed to optimize haplotype tagging – commonly used to survey variation in a gene, and this may represent a limitation of the present panels. Rather, the intent was to genotype a maximum number of SNPs either known or suspected of being functionally relevant – with newly discovered functional variants to be added in additional panels in the future.” We also moved the following sentences to here to make the flow better. “Moreover, most of the SNPs are in the transcribed regions. We can use these SNPs as markers for analysis of allelic mRNA expression imbalance, a powerful means for discovering regulatory SNPs that alter gene expression and RNA stability. It is estimated that regulatory SNPs are more abundant than nonsynonymous polymorphisms that alter the amino acids [29, 30, 40, 41].”

5) As stated in my previous review, studies from genome-wide associations of cancer risk have been published in prestigious scientific journals; in some of these studies, findings have been independently replicated. Hence, the authors did not take advantage of those GWAS for selecting cancer-risk variants in the platform. This aspect should be regarded as a limitation of the present study.

We have added a statement on page 22. “One limitation of the targeted SNP approach is that the panels fall short of covering all functional SNPs. Novel genetic polymorphisms associated with complex diseases, such as cancer, are identified in an increasing pace. For example, results from genome-wide association studies (GWAS) continue to reveal new polymorphisms that suggest the presence of functional variants in candidate genes [38, 39]. However, the odds ratios of implicated polymorphisms in these case control studies usually range at or below 1.5, insufficient for inclusion with the intended genotyping panels that are eventually geared towards establishing clinical biomarkers for therapy. Yet, we expect that functional polymorphisms with high odds ratios with respect to specific phenotypes (e.g., treatment outcomes) will emerge from GWAS and its follow-up studies, then to be incorporated into the SNPlex panels. Since the SNPlex platform is flexible and expandable, a small subset of genetic polymorphisms, especially SNPs, could be easily added to the panel established in the current study.”

6) The authors report an LD cut-off of >0.7. Is this for $r^2$ or $D’$?

We used D’, and the sentence on page 9 was modified. “SNPs in high LD ($D’>0.7$) with another SNP already in the panel were generally excluded, although in some case we added such SNPs for the assays design, to assure that either one was represented in the panel design.”

Response to reviewer Dr. Michael Gottesman:
The authors have provided clear and thorough answers for each and every comment made by the reviewers. The manuscript was appropriately revised and can be considered now ready for publication.

The authors appreciated the reviewer’s positive comments on our manuscript.

We would like to thank the reviewers for their thoughtful comments, and we appreciate your consideration and look forward to hearing your decision.

Sincerely,

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