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Title:	Pharmacogenetics Reporting and Analysis Plan for the follow-up PGx investigation of pyrexia by meta-analysis of melanoma subjects from BRF113710, BRF113929, BRF113683 and MEK115306 (PGx7550).

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Description: The study objectives are to follow up previously identified suggestive associations between pyrexia and a genetic variant (*IL28B,* rs8099917), and to explore other genetic associations (candidate gene and genome wide) by meta-analysis of subjects from BRF113710, BRF113929, BRF113683 and MEK115306.

Subject: Meta analysis, dabrafenib, a combination of dabrafenib and trametinib, pharmacogenetics, PGx, genetics, safety, pyrexia, melanoma

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ABBREVIATIONS

AE	Adverse Event
AF	Allele Frequency
Allele	Any 1 of 2 or more alternative forms of a gene or DNA sequence at the same locus. One allele is inherited from each parent
CC	Case-Control
Genetic Marker	Identifiable physical location on a chromosome at which inheritance can be monitored
Genotype	A pair of alleles, one having come from each biological parent, that characterizes an individual at a specified genetic locus. Different combinations can produce different traits/characteristics
GSK	GlaxoSmithKline
HLA	Human Leukocyte Antigen
HWE	Hardy Weinberg Equilibrium
ITT	Intent to Treat
LD	Linkage Disequilibrum
PGx	Pharmacogenetics
PC	Principal Component
Polymorphism	A genetic locus at which there is a difference in DNA sequence among individuals (i.e. which has 2 or more alleles in the population)
QC	Quality Control
RAP	Reporting and Analysis Plan
SE	Standard Error

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1. INTRODUCTION

Dabrafenib (GSK2118436; Tafinlar) is a potent, ATP-competitive and selective inhibitor of mutant BRAF kinase (V600E/K) and trametinib (GSK1120212; Mekinist) is a selective, non-ATP competitive, allosteric inhibitor of MEK1 and MEK2 kinases. The U.S. Food and Drug Administration recently approved dabrafenib and trametinib as single-agent therapies as well as in combination for the treatment of unresectable melanoma or metastatic melanoma in adult patients with the most common type of BRAF mutations: BRAF V600E (dabrafenib) and BRAF V600E/K (trametinib). The BRAF V600E/K mutation is found in 40-60% of melanomas causing constitutive activation of BRAF and, in turn, the MAP kinase pathway.

Pyrexia, or fever, is one of the most common adverse events (AE) in subjects exposed to dabrafenib, and is observed in up to 1/3 of subjects receiving this drug. The incidence of pyrexia is much higher (up to 70%) in subjects treated with a combination of dabrafenib and trametinib. The majority of these AEs are transient and resolve after treatment interruption, while a small proportion (2-5%) of subjects develops serious non-infectious febrile events such as influenza-like illness, cytokine release syndrome, and systemic inflammatory response syndrome which may require extensive management. The underlying mechanism for development of pyrexia on treatment with dabrafenib alone or in combination with trametinib is not clear. A prior PGx investigation of pyrexia (BRF116604/PGx6039) in 3 dabrafenib melanoma studies (BRF113710, BRF113929 and BRF113683) identified suggestive association of a functional variant in *IL28B* with pyrexia.

This study aims to follow up the previously identified suggestive genetic associations with pyrexia AE (BRF116604/PGx6039) and to explore other genetic associations (candidate gene and genome wide) by meta-analysis of subjects from BRF113710, BRF113929, BRF113683, and MEK115306.

2. STUDY OBJECTIVES

The primary objective of this study is to further follow up previously identified suggestive associations between pyrexia and a genetic variant in *IL28B* (rs8099917) by meta-analysis of melanoma subjects from BRF113710, BRF113929, BRF113683 and MEK115306. The exploratory objectives are to investigate other genetic associations (candidate gene and genome wide) with pyrexia by meta-analysis of subjects from BRF113710, BRF113929, BRF113683 and MEK115306, and to assess whether there is any enrichment of genetic associations in a subset of pyrexia cases with SNIFE (Serious Non-infectious Febrile Events) – (if information is available on these cases). The two treatment arms in MEK115306 will be analyzed independently and meta-analyzed with the previous pharmacogenetic data for pyrexia from BRF113710, BRF113929 and BRF113683 studies.

3. STUDY ENDPOINT

The primary endpoint is development of pyrexia (case-control) status, where a

a) **Pyrexia Case** is defined as any metastatic melanoma subject with normal temperature at baseline (< 38 ºC) and developing an AE of pyrexia (grade ≥2* according to *NCI Common Terminology Criteria for Adverse Events (CTCAE) v.4*) while receiving treatment.

* Subjects with grade 1 (38-39ºC) fever are excluded from the definition of case because of the possible non-specificity of the low-grade fever (related to study drug vs. due to other underlying conditions).

b) **Control** is defined as a metastatic melanoma subject who received dabrafenib or a combination of dabrafenib and trametinib for at least 142 (see Table 1 for details) or 182 (see Table 2 for details) days, respectively (which corresponds to the time by which 90% of the cases have had an AE of pyrexia)**, had normal temperature (< 38.0 ºC) at baseline, and no pyrexia AEs throughout the treatment duration.

** The cumulative duration of exposure to dabrafenib or a combination of dabrafenib and trametinib may vary among subjects treated in MEK115306. To ensure that the subjects in the control population had sufficient cumulative exposure to dabrafenib or a combination of dabrafenib and trametinib and to reduce the risk of including 'hidden' cases (subjects that could have become pyrexia cases had they been exposed to dabrafenib longer), a conservative selection of controls was needed. The 90% percentile for pyrexia onset was adopted from BRF116604/PGx6039 result which was presented to BRAF project team.

The selection process for cases and controls in three dabrafenib melanoma studies (BRF113710, BRF113929 and BRF113683) are available (BRF116604/PGx6039-RAP).

Table 1 Number of control subjects who have cumulative duration of exposure ≥ time to pyrexia onset in cases receiving dabrafenib in MEK115306

Percentile	Time to pyrexia onset in days (for cases)	The number of controls who have cumulative duration of exposure ≥ time to pyrexia onset in cases
95%	271	50
90%	**142**	**89**
75%	67	105
50%	27	117

Table 2 Number of control subjects who have cumulative duration of exposure ≥ time to pyrexia onset in cases receiving a combination of dabrafenib and trametinib in MEK115306

Percentile	Time to pyrexia onset in days (for cases)	The number of controls who have cumulative duration of exposure ≥ time to pyrexia onset in cases
95%	208	55
90%	**182**	**56**
75%	114	66
50%	40.5	76

4. STUDY DESIGN

A brief description of each study is provided in Table 3 below:

Table 3 Description of four clinical studies

Clinical Trial Number	Brief Description of Study	Indication	Phase
BRF113710	A Phase II single-arm, open-label study of dabrafenib in BRAF-mutant metastatic melanoma.	BRAF-mutant metastatic melanoma	II
BRF113929	A Phase II open-label, two-cohort, multicentre study of dabrafenib as a single agent in treatment naïve and previously treated subjects with BRAF mutation-positive metastatic melanoma to the brain (BREAK-MB).	BRAF mutation-positive metastatic melanoma to the brain	II
BRF113683	A Phase III randomized, open-label study comparing dabrafenib to DTIC in previously untreated subjects with BRAF mutation positive advanced (Stage III) or metastatic (Stage IV) melanoma.	BRAF mutation positive advanced or metastatic melanoma	III
MEK115306	A two-arm, randomized, double-blinded, multi-center Phase III study to evaluate efficacy and safety of dabrafenib + trametinib compared to dabrafenib + trametinib-placebo in subjects with unresectable (Stage IIIC) or metastatic (Stage IV) melanoma.	BRAF mutation-positive melanoma	III

Meta-analysis will be conducted on subjects from four metastatic melanoma studies (BRF113710, BRF113929, BRF113683 and MEK115306). The subjects from BRF113710, BRF113929, BRF113683) were analyzed in a prior PGx investigation of pyrexia, BRF116604/PGx6039 (see RAP and result for details). The two arms in MEK115306 will be analyzed independently and meta-analyzed with results from BRF116604/PGx6039. Overall, 132 and 275 subjects meet the strict definition of case

and control, respectively, as described in Section 3, and will be selected for analysis. A brief description of the four clinical studies and the subjects selected for inclusion in this investigation is provided in Table 4.

Table 4 Summary of Final PGx Analysis Population

BRAF Study	BRF113710	BRF113929	BRF113683		MEK115306		Total	Cases	Controls
			# treated with dabrafenib	# crossed over from DTIC to dabrafenib	Dabrafenib only	Dabrafenib+ Trametinib			
PGx Population	73	123	146	23	177	184	726		
Patients with Fever									
Missing/elevated temp at baseline or during treatment	1	2	3				6		
No fever	54	84	105	17	118	79	457		275
Grade 1	13	18	19	2	32	47	131		
Grade 2	5	18	16	3	23	46	111	111	
Grade 3		1	3	1	4	12	21	21	
Total								**132**	**275**

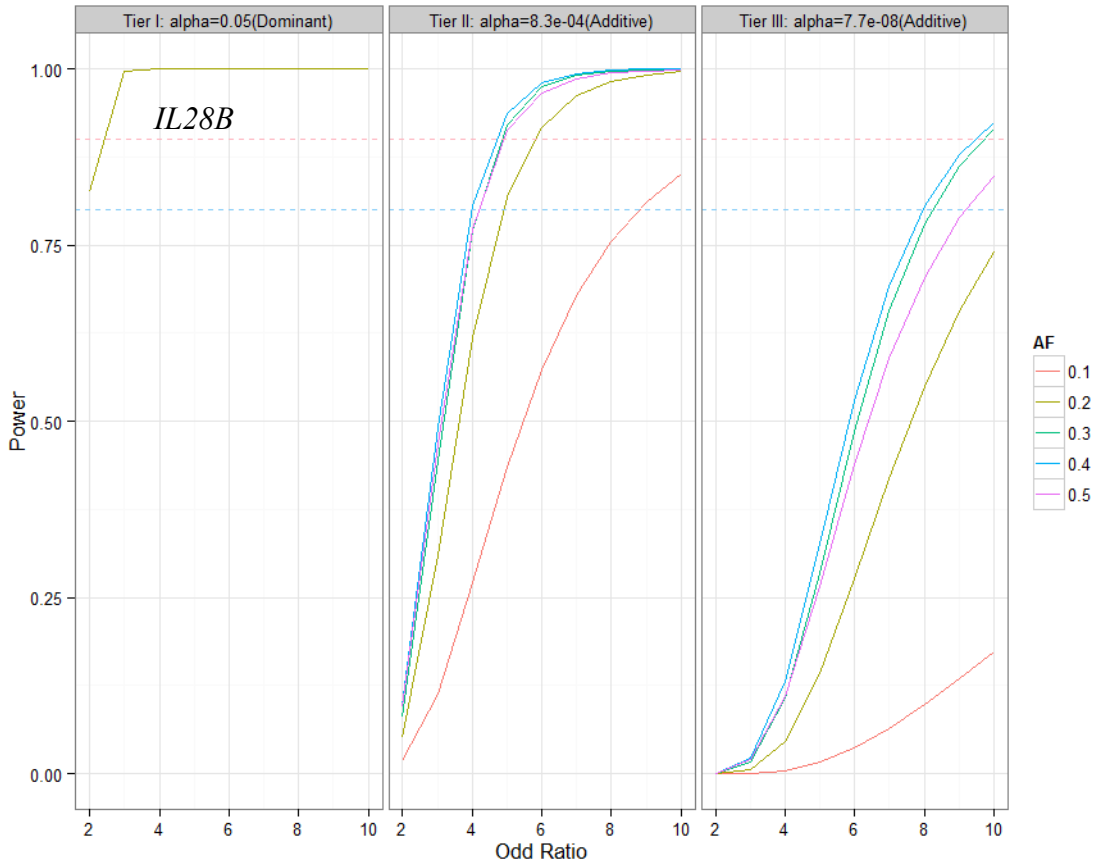
5. SAMPLE SIZE CONSIDERATIONS

The clinical study being examined was not prospectively designed to address PGx research hypotheses and, thus, may not have statistical power to detect moderate genetic effects. Post-hoc assessment of statistical power is necessary to determine the sizes of effects that can be detected given the admissible PGx data. The distribution of genotypes varies considerably from one genetic marker to the next (i.e., the genotype data within each genetic marker will not be balanced), so the statistical power of each analysis cannot be guaranteed. However, power can be estimated by assuming a range of risk SNP allele frequencies and genetic effect sizes in meta-analysis.

Statistical power to detect a genetic effect using all 132 pyrexia cases and 275 controls was evaluated assuming varying frequencies for a potential risk allele, and a range of likely odds ratios (2 to 15) for a genetic effect. These calculations assumed three thresholds for declaring statistical significance: 0.05, 8.3×10^{-4} and 7.7×10^{-8}, which correspond to 3 tiers of SNPs (the *IL28B* SNP in Tier I, 60 Tier II SNPs and GWAS Tier III). For tiers II and III, considering an additive genetic model with varying allele frequencies (10-50%) and OR of 1-15, the statistical power curves are plotted (Figure 1) and the different colour curves represent power estimates for a range of allele frequencies. The light red and light blue dashed lines represent 90% and 80% powers, respectively. For the *IL28B* SNP, rs8099917 in tier I (MAF: 20%), a dominant model and an OR of 2.5 was used for estimating power based on the prior data from BRF116604/PGx6039.

For example, with 132 cases and 275 controls, power to detect less common genetic variants (MAF ≤ 10%) that may confer small or moderately large genetic effects (OR<10) is very limited in Tiers II and III. However, the power estimate for the *IL28B* SNP, rs809917 in Tier I would be over 90% with OR > 2.5 under a dominant genetic model. In addition, if the risk allele is relatively common in the study population (i.e., MAF ≥ 20%) and odd ratio is greater than 6.0, then the power is more than 80% and 50% for Tier II and Tier III, respectively, under an additive genetic model.

Figure 1 **Power for CC analysis. The power is calculated assuming 132 cases and 275 controls**



6. PGX ANALYSIS POPULATIONS

The PGx analysis population will consist of the patients enrolled in MEK115306 who provided written informed consent for PGx research, provided a blood sample for genotyping and were successfully genotyped for at least one of the genetic markers under study, have valid phenotype data and pass genotyping QC.

The initial PGx analysis will focus on the metastatic melanoma subjects from MEK115306 who met the definition of a case or control (as described in Section 3). These subjects will be meta-analyzed along with the 3 study populations from BRF116604/PGx6039.

7. GENERAL CONSIDERATIONS FOR DATA ANALYSES

It is anticipated that after an initial review of the results of the analyses described here, there may be a need for additional follow-up analyses to be conducted. These will be discussed, defined, and agreed by the authors of this RAP and other relevant parties at that time. This RAP will not be updated to include such a case; any additional follow-up analyses will be described in the PGx report.

7.1. Dependent Variables (Endpoint Variables)

The primary endpoint will be Pyrexia case/control status.

7.2. Independent Variables

Variables which were listed in BRF116604/PGx6039 RAP as potential covariates will be evaluated for inclusion into the analysis model as covariates. The following variables will be tested:

- Dabrafenib Formulation (gelatine vs. HPMC)
- Genetic Ancestry Estimates (see Section 7.5 for details)

Since the biological basis of the effect of baseline temperature on treatment-related fever could not be established, baseline temperature will not be included as a covariate in the analysis. However, for the purpose of sensitivity assessment, we will look into correlating baseline temperature with markers that show some evidence of association with pyrexia while on treatment.

7.3. Genetic Markers

The following sections describe the genetic markers that will be evaluated in this analysis.

7.3.1. Genetic Data Available for Analysis

PGx subjects from MEK115306 (n=380) will be genotyped for all markers on the Affymetrix Axiom Biobank plus GSK Custom array panel. After genotype QC, approximately 650K SNPs across the genome, are expected to be available for analysis. While all 650K markers will be used for Principal Component Analysis to characterize the genetic ancestry of the PGx population, the following markers will be selected and stratified into tiers for genetic association analysis with the study endpoints:

- Tier I: *IL28B* candidate gene variant (see Table 5).
- Tier II: Functional polymorphisms in 29 candidate genes (N=60)* encoding for:
  - Pro- and anti-inflammatory cytokines released in cytokine release syndrome
  - Prostaglandin (PG) synthesis and signaling
  - Proteins/receptors regulating cytokine, PG levels/activity
  - Proteins involved in absorption, distribution, metabolism, and excretion (ADME) of dabrafenib

*see Appendix Table 7 for details

- Tier III: All the markers on Affymetrix Atom (N=650,000 SNPs in total)

Table 5 Tier I: Functional variants in 1 candidate gene (N=1), which are on the Axiom Biobank/Custom

rsID	Chr	Gene	Variant	Function
rs8099917	19	*IL28B*/IFN-λ3	8 kb upstream	Il28B contributes to immune/inflammatory responses, viral clearance. The variant associated with sustained virologic response (SVR) in HCV patients treated with PEG-IFN-α/RBV

7.3.1.1. Imputation for missing markers

In order to have a common set of markers between the two sets of subjects (PGx6039 and MEK115306) for meta-analysis, the markers that will not be available on Biobank for MEK115306 subjects will be imputed from the 1000 Genomes Project whole genome sequence data using an in-house software pipeline developed based on Minimac (Howie, et al. 2012). For example, of the markers in the candidate gene list available on the OEE, there are 5 that are not available on the Biobank. In addition, most of the proxy markers available on the OEE are not available on the Biobank (see Appendix Table 7 for details). These missing markers on the Biobank will be imputed for meta-analysis.

7.3.1.2. Coding of Genotypes

The majority of the genetic markers evaluated in this analysis will be SNPs with two possible alleles and therefore three possible genotypes. For the primary analyses, directly measured genotypes will be coded as 0, 1, or 2, to indicate the number of copies of the minor allele. If there are markers with many more than two possible alleles, a pseudo-genotype will be coded for each allele present in the sample to be analyzed and each of these pseudo-genotypes will be analyzed separately. The pseudo-genotype will be coded by taking each possible allele and determining the number of copies of the allele present (0, 1, or 2). For imputed markers, an estimate for the number of copies of the minor allele (between 0 and 2) will be calculated. For these markers, the dosage value will be used as a continuous variable in the statistical modelling.

7.3.2. HLA Markers

As there is no prior evidence for HLA genetic variants to be associated with general mechanism of fever or drug induced fever, an exploratory approach will be undertaken. The pyrexia cases and controls will be imputed to HLA 4-digit resolution using HIBAG (an R Package for HLA Genotype Imputation with Attribute Bagging, Zheng, et al. 2014). These results will be meta-analyzed along with the previous HLA results obtained by the 3 study populations from BRF116604/PGx6039.

7.4. Marker Map

The GRCh 37 map will be used.

7.5. Examination of Racial Subgroups & Genetic Ancestry Estimates

When a study sample contains individuals who vary in ancestry, population substructure is said to exist. Confounding due to population substructure needs to be addressed in the analysis of the genetic contribution to a particular endpoint, as it can inflate type 1 error, increasing the probability of generating a false positive result, and in some circumstances reducing the power to identify true effects. This confounding occurs when the genetic variant being assessed, as well as the risk for the endpoint, vary in frequency across ethnic subgroups. Self-reported ancestry can be useful in stratifying patients into more homogenous subgroups and a common approach is to conduct separate analyses (inferential or descriptive) within each ethnic subgroup. However, even within purported 'homogeneous' subgroups, stratification can occur.

Subjects for these clinical trials were recruited from multiple countries resulting in diverse samples of patients with different self-identified ethnicities. Analyses will be conducted using all subjects from the primary PGx analysis population, regardless of ethnicity. To adjust for genetic ancestry differences, the first few principal components generated from Axiom panel, will be included in the model, as appropriate. Also, individual patients whose genetic ancestry estimates differ greatly from other patients in any subgroup being investigated may be excluded from genetic analysis

7.6. Multiple Comparisons and Multiplicity

This is an exploratory experiment and results from analysis may be hypothesis generating for future safety studies. Although adjustment for multiple testing will be applied to declare statistical significance, markers providing suggestive evidence for association will be further explored.

Multiple testing corrections will be made while assessing markers for association within each tier (Table 6). Per-marker significance levels will be calculated using a Bonferroni adjustment and will (conservatively) assume independence of the markers within tiers. For the HLA polymorphisms (subset) and the whole genome markers (tier III), it will be assumed that there are approximately 125 (based on BRF116604/PGx6039) and 650,000 independent tests, respectively.

Table 6 *P*-value thresholds for tests on Tier I, II, III and HLA markers

Tier	# SNPs	Alpha	Threshold for Significance
I	1	0.05	0.05
II	60	0.05	0.0008
III	650000	0.05	7.7×10^{-8}
HLA	125	0.05	0.0004

8. DATA HANDLING CONVENTIONS

8.1. Premature Withdrawal and Missing Data

If a patient contributed data to any of the analysis populations and also provided a DNA sample, then the patient will be included in the analyses outlined here. If a patient withdrew from the study but did not withdraw consent for pharmacogenetic research, the patient's information may still be used in these analyses.

Missing values for clinical data will not be imputed in the analysis.

9. STUDY POPULATION

9.1. Disposition of Subjects

The number of patients included in each of the analysis populations will be summarized overall and may be summarized more specifically by relevant independent variables.

In general, categorical data will be summarized using frequency counts and percents, and continuous data will be summarized using means, standard deviations, minimums, medians, and maximums, overall, and by relevant independent variables. These summary statistics will be inspected visually for any concerning imbalances. If any statistically significant imbalances that may affect the analysis are present, these factors will be accounted for in the analysis models.

10. PHARMACOGENETIC ANALYSIS

10.1. Data Quality Control

Prior to the PGx analysis, quality control (QC) will be conducted on the genetic markers themselves and on the subjects utilizing the genetic marker data.

10.1.1. Genotype Quality Control

The objective of genotype QC is to define the set of markers that are of high quality and suitable for use in genetic association analyses. The QC steps performed prior to statistical analysis are to:

- Remove markers that are monomorphic in all patients (i.e, all patients carry the same genotype for marker)

- Remove markers identified by the generating lab as failures.

10.1.2. Subject Quality Control

The genotype data from a patient will be thoroughly reviewed and if the percentage of markers successfully genotyped for the patient is less than 97%, the patient may be removed from analysis if it cannot be concluded that the available genotypes for the patient are likely to be of high quality.

Patients will be removed from the analysis if their genotypes for the sex chromosomes do not agree with their reported gender.

An assessment will be done on the patients, at the genetic level, to identify patients that may be highly-related. Samples will be removed if they are identified as unintentional duplicates.

10.2. Hardy-Weinberg Equilibrium (HWE) Analysis

Departure from Hardy-Weinberg equilibrium (HWE) is a measure of the association between two alleles at an individual locus. Departure from HWE may indicate a laboratory genotyping error or population selection bias. HWE analysis will be applied to all markers and will be conducted using the ethnic group with the largest sample size – the Whites. Departure from HWE will be tested using an exact test, by considering the distribution of genotypes conditional on observed allele frequencies. No markers will be removed prior to the analysis based on their HWE P-values. However, markers showing substantial evidence of departure from HWE ($p<1x10^{-8}$) will be investigated thoroughly for laboratory errors or other causes of departure from equilibrium and, if the cause remains undetermined, they may be removed from the list of associated markers. For markers identified as associated with pyrexia ($p<0.05$) but show strong departure from HWE expectations ($1x10^{-4}<p<1x10^{-8}$), this information will be presented along with their results for interpretation.

10.3. Linkage Disequilibrium Analysis

LD examination is important in evaluating the relative independence of information from the SNPs within a gene and across the genes. In some instances, LD can indicate that a chromosomal segment contains polymorphisms that are highly correlated with each other (i.e. a "haplotype block"), but not with polymorphisms outside the segment. LD analyses will be conducted to assist in assessing significant association results. As with the HWE analysis, the LD analysis will be conducted using the ethnic group with the largest sample size – the White patients. Pairwise LD in the form of r^2 (the squared correlation of genotypes of a pairwise SNP combination) will be visualized along with the association analysis results in an integrated manner along with a tabular summaries, as necessary, for significant markers.

10.4. Ancestry Estimates

Genetic ancestry estimates will be obtained by principal components analysis (PCA) in the SNPRelate R package (Zheng, et al., 2012). Plots will be created using the principal

components overlaid with self-reported ethnic groups to aid in visualization of the data clustering and to determine the number of principal components required to infer genetic ancestry. These plots will be examined to determine adjustment for ancestry estimates as covariates in the genetic model of the primary analysis including all subjects.

10.5. Genotypic Association Analyses

Genotype association tests will be performed assuming an additive genetic model. Other optimal genetic models will be explored once significant markers are identified.

Logistic regression

A likelihood logistic regression method with covariate(s) or Fisher exact test without covariate will be used to assess the effect of marker genotypes on the case-control status using PLINK V1.07. After screening, penalized likelihood ratio tests will be performed again to obtain more accurate regression estimates and standard errors for the screened polymorphisms. Prior to the PGx analysis, a model which includes necessary non-genetic independent variables (described in Section 7.2.) that may influence the endpoints will be identified. This analysis will be conducted using SAS software and the "Firth" option within PROC LOGISTIC.

For significantly associated markers, the "risk genotype" will be identified by calculating the odds ratio for each genotype against the other possibilities, and the genotype having the greatest odds ratio will be referred to as the risk genotype. In order to control for cells missing observations in the calculation of odds ratios, the Haldane correction that adds 0.5 to each cell of the contingency table will be implemented.

HLA polymorphisms

The majority of the genetic markers evaluated in this analysis will be single nucleotide polymorphisms (SNPs) with two possible alleles and therefore three possible genotypes. Analyses will be optimized to accommodate this type of genetic marker. There are some markers that will have more than two possible alleles, such as those from the HLA genes. For such markers, a pseudo-genotype will be coded for each allele present in the sample to be analyzed. "+,+" will indicate that two copies of the allele are present, "+,-" that one copy is present, and "-,-" that no copy is present. Each of these pseudo-genotypes will be analysed separately. This analysis will be conducted using SAS software and the "Firth" option within PROC LOGISTIC.

Meta-Analysis

The random effects approach is often used as a mildly conservative test under the assumption that the studies are not designed in exactly the same way, and there is likely to be some study-to-study variability in the genetic effects that could be observed. In this study, we do have at least some substantial differences such as mono versus combination therapy to consider. Thus, the random effect approach will be applied to this study. The analysis will be conducted using new random effects model optimized to detect associations when some studies have an effect and some studies do not in METASOFT (Han and Eskin, 2011).

Before meta-analysis, the results obtained by GWAS data and HLA markers from the Illumina Omni Express Exome (OEE) beadchip in BRF116604/PGx6039 will be realigned by a list of markers to flip strand and a list of new coordidates which are different with the GRCh 37 map.

11. INTERPRETATION OF ASSOCIATION ANALYSES AND REPORTING OF RESULTS

11.1. Focus on Strongest Exploratory Results

Some of the most supported, highly significant associations will be focussed on in the reporting of this exploratory study. Beyond assessment of p-values, questions will be asked to identify the genetic associations with the strongest support, including the following:

- Are the association trends observed consistent with commonly observed genetic models (i.e. dominant, additive, or recessive)?
- Are there sufficient numbers of patients driving the genotype association to yield strong statistical support?
- Is there further genetic or biological support?
    - Is there support from other genetic variants within the gene in LD with the associated SNP?
    - Are the results consistent across multiple trials?
    - Are the results consistent across multiple treatments?
    - Is the known function of the allele or genotype plausible with respect to the observed effect?
    - Is there another probable functional SNP in LD with the associated SNP?

11.2. Reporting of Significant Results for Future Evaluation

This analysis plan covers only one study contributing towards meeting an overall PGx objective that may require additional confirmation and demonstration of potential clinical utility in an independent dataset.

12. REFERENCES

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13. APPENDIX

Table 7 List of Tier II Candidate Gene Variants for pyrexia analysis

Gene	SNP	Position	On OEE	Proxy available on OEE or Exome Chip (rsID)	LD Score between a Candidate SNP and its Available Proxy on OEE or Exome Chip	On Biobank
Cytokines (Anti and Pro-Inflammatory)						
IL1A	rs17561	4845 G/T (A114S)	YES			YES
IL1A	rs1800587	-889 C/T	YES			YES
IL1B	rs1143627	-31 T/C (TATA Box)	YES			NO‡
IL1B	rs1143634	+3962 C/T (F105F)-? same as +3953/54 C/T ?	YES			YES
IL1B	rs16944	-511C/T	YES			YES
IL-6	rs10499563	-6331 T>C	YES			YES
IL-6	rs1800796	-572 G>C	YES			YES
IL-6	rs1800797	nt565 A>G	YES			YES
TNFA	rs1799724	–857 C/T	YES			NO‡
TNFA	rs1799964	–1031 T/C	YES			YES
TNFA	rs1800629	-308*A/G	YES			YES
TNFA	rs1800630	–863 C/A	YES			YES
IL-10	rs1800871	-819 C/T was rs3021097	YES			YES

Gene	SNP	Position	On OEE	Proxy available on OEE or Exome Chip (rsID)	LD Score between a Candidate SNP and its Available Proxy on OEE or Exome Chip	On Biobank
IL-10	rs1800896	-1082G/A	YES			YES
IL28B	rs8099917		YES			YES
IL28B	rs12979860		YES*			YES
IL-8	rs1126447	2767A>T	YES			NO‡
IFNL4	ss469415590	dinucleotide variant comprised of two SNPs, rs11322783 and rs74597329, which are side-by-side	see below			see below
IFNL4	rs11322783		NO			YES
IFNL4	rs74597329		NO			YES
TLR Pathway						
TLR4	rs1554973		YES			YES
TLR4	rs7856729		YES			YES
TIRAP (Toll-interleukin-1 receptor domain-containing adaptor protein), also known	rs7932766	C558T	YES			NO‡

Gene	SNP	Position	On OEE	Proxy available on OEE or Exome Chip (rsID)	LD Score between a Candidate SNP and its Available Proxy on OEE or Exome Chip	On Biobank
as MAL (MYD88 adaptor-like)						
TIRAP	rs8177374	S180L	YES			YES
Eicosanoid Synthesis						
PTGS2	rs20417	-765G/C	YES			NO‡
ALOX5	rs2228065	Glu>Lys (exon 6; G>A)	YES			YES
GSK'436 ADME						
CYP2C8	rs11572103	*2 (I269F)	YES			YES
CYP2C8	rs10509681	*3 (K399R)	YES			YES
NR1I2	rs3814055	-25385C>T; 5'end	YES			YES
NR1I3	rs2307424		YES			YES
NR1I3	rs2307418		YES			YES
NR1I3	rs4073054		YES			YES
ABCB1	rs1045642	3435C>T	YES			YES
ABCB1	rs1128503	1236T>C	YES			YES
ABCB1	rs2032582	2677G>T/A (A893S/T)	YES			YES
SLCO1B1	rs2306283	N130D; *1B	YES			YES

Gene	SNP	Position	On OEE	Proxy available on OEE or Exome Chip (rsID)	LD Score between a Candidate SNP and its Available Proxy on OEE or Exome Chip	On Biobank
SLCO1B1	rs4149056	V174A; *5	YES			YES
SLCO1B1	rs11045853	R253Q	YES			YES
SLCO1B3	rs4149117	334T>G Ser112Ala	YES			YES
SLCO1B3	rs3764006	1833G>A Gly611Gly	YES			YES
Renin-Angiotensin System						
AGT	rs4762	T174M	YES			YES
AGT	rs699	M235T	YES			YES
AGT1R	rs5186	A1166C- 3'UTR poly	YES			YES
Immune Enhancers						
CTLA4	rs231775	+49A>G;Thr17Ala	YES			YES
TNFSF4	rs3850641		YES			YES
Candidate Gene Variants with Proxy Available						
Cytokines (Anti and Pro-Inflammatory)						
IL1A	rs1894399	intronic A/G	NO	rs2856837	0.96	NO‡ (proxy)
IL1A	rs2856836	3' UTR	NO	rs4848300	1.00	NO‡ (proxy)
IL1R1	rs2041748	1622 A/G (Hinf1 site)-intron 2	NO	rs4141631	1.00	NO‡ (proxy)
IL-6	rs1800795	-174 G>C	NO	rs1800797	0.97	YES (proxy)†
IFN-	rs243056	874 T/A; was	NO	rs2069727	1.00	NO‡

Gene	SNP	Position	On OEE	Proxy available on OEE or Exome Chip (rsID)	LD Score between a Candidate SNP and its Available Proxy on OEE or Exome Chip	On Biobank
gamma	1	rs61923114				(proxy)
IL-10	rs1800872	-590 A/C	NO	rs1800871	1.00	YES (proxy)†
IL1RN	rs2234678		NO	rs4251985	1.00	NO‡ (proxy)
IL-8	rs4073	-251T>A	NO	rs1951242	0.97	NO‡ (proxy)
TLR Pathway						
TLR4	rs4986790	896A/G (D299G)	NO	rs4986791	1.00	YES (proxy)
Eicosanoid Synthesis						
PTGER2	rs708494	uS5 (5'UTR)	NO	rs1254606	0.87	NO‡ (proxy)
ALOX5	rs12762303	-557 T>C	NO	rs6593482	0.85	NO‡ (proxy)
Dabrafenib ADME						
CYP2C8	rs11572080	*3 (R139K)	NO	rs10509681	1.00	YES (proxy)†
CYP3A4	rs2740574	-392A>G	NO	rs2023548	1.00	NO‡ (proxy)
SLCO1B1	rs11045819	P155T	NO	rs10841753	1.00	NO‡ (proxy)
SLCO1B3	rs2053098		NO	rs4578447	1.00	NO‡ (proxy)
SLCO1B3	rs60140950		NO	rs7306033	0.94	NO‡ (proxy)
SLCO1B3	rs7311358		NO	rs1910188	1.00	NO‡ (proxy)
Renin-Angiotensin System						

Gene	SNP	Position	On OEE	Proxy available on OEE or Exome Chip (rsID)	LD Score between a Candidate SNP and its Available Proxy on OEE or Exome Chip	On Biobank
NOS3	rs2070744	Promoter poly -786T>C	NO	rs3807370	0.93	NO‡ (proxy)
Immune Enhancers						
CTLA4	rs5742909	-318C>T	NO	rs4335928	0.88	NO‡ (proxy)
CTLA4	rs1024161		NO	rs11571315	1.00	NO‡ (proxy)
TNFSF4 (OX40L)	rs45454293	-921 T>C	NO	rs3861953	1.00	YES (proxy)
TNFRSF4	rs2298212		NO	rs3813199	1.00	NO‡ (proxy)

* TaqMan assay for PGx6039
† Proxy in original available SNP list
‡ The marker will be imputed based on 1000 genome reference panel