Guidelines for Studies of Host Determinants of Drug Response and Toxicity

Overview

The potential for personalized approaches to cancer treatment requires a comprehensive understanding of both host and tumor factors that determine response. Host determinants of drug response and toxicity are often understudied in oncology. An increasing number of studies have highlighted the importance of host variation in determining both drug efficacy and toxicity. The information contained in this document is intended to provide basic information related to potential host biomarkers that could be explored in clinical studies. An overview of phenotype data, sample collection and processing, and estimated costs are provided for pharmacogenomic, epigenomic, metabolomic and metagenomic analyses. Model language for protocols incorporating host determinant studies is also provided. Study specific input for correlative studies is available from Deanna Kroetz (deanna.kroetz@ucsf.edu).

Pharmacogenomics

Genetic variation in drug response and toxicity has been validated for numerous drugs used in oncology. A recent analysis identified 12 oncology drugs with clinically actionable pharmacogenetic associations (Table 1). Additional genetic associations reported for drugs used in oncology require validation.

Table 1. Clinically Actionable Gene-Drug Pairs for Oncology

Drug(s)	Clinically Actionable Gene(s)/Variants	Gene(s)	Phenotype	Effect Size	Recommended Clinical Action	Refs
Asparaginase	rs6021191, rs17885382	*NFATC2*, *HLA-DRB1*	Hypersensitivity	OR, 3.1	Closer monitoring for hypersensitivity strongly recommended for those carrying any risk alleles at either of the specified loci	[1]
Capecitabine/ fluorouracil	DPD deficient	*DPYD*	Systemic toxicity		For those with decreased DPD activity, decrease dose by 25-50% For those with complete DPD deficiency, avoid use of fluoropyrimidine drugs	[2, 3]
Cisplatin	rs1872328	*ACYP2*	Ototoxicity	HR, 4.5	Closer monitoring for ototoxicity is strongly recommended for patients carrying the risk allele	[4, 5]
Doxorubicin	rs1883112	*NCF4*	Cardiotoxicity	OR, 2.5	For carriers of 1 risk allele, no modifications are warranted. For carriers of 2 risk alleles, closer monitoring for cardiotoxicity is strongly recommended	[6, 7]
	rs8187710	*ABCC2*		OR, 4.3	Closer monitoring for cardiotoxicity is strongly recommended for patients carrying the risk allele	[7, 8]
	rs13058338	*RAC2*		OR, 2.8	Closer monitoring for cardiotoxicity is strongly recommended for patients carrying the risk allele	[7, 8]
Irinotecan	*UGT1A1**28	*UGT1A1*	Toxicity (primarily diarrhea and neutropenia)		For those carrying 1 risk allele, monitor closely for toxicity. For those homozygous for the risk allele, reduce starting dose.	[9]
Lapatanib	*HLA-DQA1**02:01	*HLA*	ALT elevation	OR, 14.1	Closer monitoring for hepatotoxicity is strongly recommended for patients carrying the risk allele	[10-12]
Mercaptopurine /thiogunaine	Intermediate or poor metabolizer (IM/PM)	*TPMT*	Myelosuppress-ion		For IMs, decrease starting dose by 30-70% and adjust doses based on degree of myelosuppression. For PMs, drastically decrease starting dose	[13, 14]

					and dosing frequency and adjust doses based on degree of myelosuppression	
Sunitinib	rs307826 rs776746	*VEGFR2* CYP3A5	PFS, RR Toxicity-induced dose reduction	HR, 8.8 HR, 3.8	Not recommended for clinical implementation at this time For those carrying the risk allele, closer monitoring for toxicities is strongly recommended; earlier dose reduction may be required.	[15]
Tamoxifen	Ultrarapid/normal/ intermediate/poor metabolizer (UM/NM/IM/PM)	*CYP2D6*	Recurrence, DFS, RFS, DRFS, BCSS, OS		For IMs, consider an alternative drug (e.g., an aromatase inhibitor) or an increased tamoxifen dose if aromatase inhibitors are contraindicated. For PMs, an alternative drug is recommended (e.g. an aromatase inhibitor); an increased tamoxifen dose could be considered if AIs are contraindicated.	[16, 17]
Vincristine	rs924607	CEP72	Peripheral neuropathy	OR, 4.3	For carriers of 1 risk allele, no modifications are warranted. For carriers of 2 risk alleles, closer monitoring for neuropathy is strongly recommended	[18, 19]

From Wellmann et al. [20]

Pharmacogenetic correlative studies should be considered for any of the drugs listed above. In addition, if drugs are known to have dose-limiting toxicity, germline DNA samples could be collected for discovery studies. In some cases, GI, dermatological, neurological and cardiovascular toxicities might be shared across a drug class and these samples could be included in future cross-study pharmacogenomics analyses.

Phenotype data:

Pharmacogenomic associations with drug response would utilize typically collected measures of response (PFS, OS, RECIST etc). It is important that appropriate measures of drug toxicity are collected for association analyses. A representative list is shown here, although other criteria might be appropriate for a specific drug.

- NCI-CTCAE grading, including Grades 1 and 2.
- Relevant laboratory values (e.g., baseline and on treatment BP, liver and renal function tests)
- Where appropriate, relevant baseline and on treatment diagnostic tests for cardiac output.
- Co-morbidities such as diabetes, alcoholism, HIV, Crohns etc. that could influence drug toxicity
- Cumulative dose at time of dose reduction or Grade 3 or higher toxicity
- Dose delays due to toxicity

Samples for genomic DNA:

- 6 ml blood sample in an EDTA tube obtained at baseline
- Document time of draw, processing, aliquoting and storage
- Blood sample should be stored prior to genomic DNA extraction at -80ºC if possible, but -20ºC is also acceptable
- SOP for collection, processing and storage:
    - Collect blood into lavender top K_2EDTA Vacutainer tube
    - Invert tube 8 times immediately after collection
    - In a biosafety cabinet, aliquot the blood into 3-4 1.8 ml cryovials
    - Label with patient ID and 'WB' for whole blood
    - Store at -80ºC
- Alternative protocol if you cannot aliquot immediately after collection:
    - Collect blood into lavender top K_2EDTA Vacutainer tube
    - Invert tube 8 times immediately after collection
    - Store upright at 4ºC for no longer than 24 hrs
    - The following day, remix blood by inverting 8 times
    - In a biosafety cabinet, aliquot the blood into 3-4 1.8 ml cryovials
    - Label with patient ID and 'WB' for whole blood
    - Store at -80ºC

Estimated costs:

- CRS research nursing rates for research outpatient procedures – direct venipuncture ≤4 tubes = $27.77
- CRS mobile research nursing rates for outpatient procedures - direct venipuncture ≤4 tubes = $111.10
- CTSI sample processing and aliquoting - $11.24/tube
- CTSI sample storage per vial per month (fridge or freezer) - $1.28
- CTSI shipment, ambient or cold packs - $8.83/box
- Genomics core DNA extraction - ~$35/sample (will require storage at 4ºC after isolation)
- PCR genotyping - ~$1/SNP/sample not including personnel
- Genome-wide genotyping array
    - Affymetrix Precision Medicine Research Array - $29/sample includes reagents, array and consumables; sold in 96-well units; UCSF genomics core charge of $28.10/plate for processing the arrays
    - Affymetrix UK Biobank Array - $59/sample includes reagents, array and consumables; sold in 96-well units; UCSF genomics core charge of $28.10/plate for processing the arrays
    - Illumina Genotyping Arrays range from $49-$298/sample (reagents, array and consumables); sold in 96-well units; arrays require processing at the UC Davis Genomics Core (costs negotiable on a per project basis)
        - Most commonly used arrays for clinical focus are $49-$119/sample
- Exome sequencing – per sample costs: ~$300 for 50X coverage, $400 for 100X coverage; $50 for analysis (Novogene)

Epigenomics

There is increasing interest in epigenomic markers of drug response and toxicity. For germline variation in epigenetic marks, samples should be collected prior to drug treatment. To determine if drug treatment affects epigenetic regulation, blood samples for DNA extraction should also be collected during or after treatment. Genome-wide methylation arrays are commonly used for discovery of epigenomic variation associated with drug response.

Phenotype data: same as for pharmacogenomics

Samples for genomic DNA:

- Same as for pharmacogenomics
- Additional samples collected during or after treatment if drug effects on methylation are of interest

Estimated costs:

- See above for sample collection and processing
- Genome-wide methylation arrays
    - Illumina EPIC array - $260/sample includes reagents, array and consumables; sold in 96-well units; arrays require processing at the UC Davis Genomics Core (costs negotiable on a per project basis)

Metabolomics

Plasma markers of metabolites have been associated with variable response to antidepressants, antihypertensives and oncology drugs. In most cases, changes in plasma biomarkers in response to treatment are linked to variability in response and toxicity. Such analyses require both baseline and treatment samples. Discovery studies typically use large panels of 1000+ metabolites; lipid panels and targeted panels are also available. ***Sample processing and storage is critical for maintaining sample integrity (0.5-1 ml plasma samples required).***

Phenotype data: same as for pharmacogenetics

Sample collection and processing:

- Collect two 6 ml blood samples in an EDTA tube
- Document time of draw, processing, aliquoting and storage
- SOP for collection, processing and storage:
    - Collect blood into lavender top K_2EDTA Vacutainer tubes
    - Centrifuge at least 15 min at 2200-2500 rpm
    - In a biosafety cabinet, immediately aliquot the plasma into chilled polypropylene tubes with external threading (2.0 ml cryovials or 1.5 ml Eppendorfs)
    - Label with patient ID and 'PL' for plasma
    - Flash freeze in liquid nitrogen if possible. If liquid nitrogen is not available, immediately place at -80ºC or in a dry ice/ethanol bath.
    - Store at -80ºC

Estimated costs:

- See above for sample collection and processing
- Metabolon costs vary depending on sample size but generally ~$100/sample including all analytical and bioinformatics analyses

Metagenomic Analyses

The gut microbiome has been linked to drug bioavailability and toxicity. In oncology, an emerging example of this is irinotecan. Glucuronide metabolites of the active SN-38 metabolite of irinotecan can undergo enterohepatic recirculation where they are involved in dose-limiting diarrhea. Genetic variation in *UGT1A1* has been recognized for a while to contribute to variation in irinotecan-induced diarrhea [21]. More recently, individual differences in gut bacterial β-glucuronidase activity has been linked to this toxicity [22, 23]. Metagenomic biomarkers of drug efficacy and toxicity is likely to be important for effective personalized approaches to dosing irinotecan and other drugs with significant glucuronidation. Orally active targeted therapies can also be metabolized by gut bacteria and it will be important to characterize variability in these processes which can greatly impact drug bioavailability and therefore efficacy.

Phenotype data:

Associations between gut microbiome species and metabolic activity with drug response and toxicity would utilize the measures described for the pharmacogenomic studies. In addition, where gut bacteria are suspected of limiting drug bioavailability it would be critical that plasma samples are collected for quantification of drug and metabolite levels.

Sample collection and processing:

For microbiome studies fecal samples should be collected at baseline. In some cases, additional samples during or after treatment should be considered to characterize the effect of therapy on the gut microbiome.

- Patient is provided with an “at-home” Microbiome Collection Kit.
- Microbiome Collection Kits are customized for the desired amount of timepoints.
- Microbiome Collection Kits include:
    - Stool collection toilet adapters, preservative-free plastic collection vials with attached scoop, FOBT (Fecal Occult Blood Test) Cards.
    - Hygienic consumables (such as gloves, wipes, and ziplock bags).
    - Shipping materials (ice packs, thermal bubble mailers) and prepaid postage for express delivery to the laboratory.
- For Stool Sample: Patient will defecate into the toilet adapter and collect 1-2 grams of stool in a preservative-free plastic collection vial using the attached scoop.
- For FOBT Card Sample: Patient will take a small portion of stool and smear the sample across the area of the FOBT card.
- Patient will document their Patient ID and date/time of collection using label provided on the collection vial and FOBT card.
- Patient will seal the stool sample & FOBT card in a Ziploc bag and place into a thermal bubble mailer with an ice pack to prepare for immediate shipping.
- Patient will ship the samples directly to the research laboratory using the provided mailing materials prepaid express postage.
- Upon receipt at the laboratory, the patient samples will be immediately banked by laboratory personnel and stored at -80°C.
- Collection of additional stool samples during or after treatment will be collected using the aforementioned SOP.

Estimated costs:

- Microbiome Collection Kit Consumables (priced per unit, per sample submission):
    - FOBT Card: $3.03
    - Stool Collection Toilet Adapter: $1.17
    - Stool Collection Vial: $1.98
    - Thermal Bubble Mailer: $1.36
    - Ice Pack: $1.20
    - Collection Box & Hygienic Consumables: $7.00
- Prepaid express postage (per sample): $26.20+
- DNA extraction reagents: $20 per sample
- Multiplex amplicon sequencing (16S/ITS): $20 per sample
- Metagenomic sequencing: $200 or more per sample, depending on depth of coverage

Model Protocol Language for Host Genetic Studies

Pharmacogenomic correlative studies have been included in many CALGB/Alliance cooperative group studies. In each case, a candidate gene or SNP approach has been proposed as a primary hypothesis but language is included so that additional analyses can be conducted at a later date, including genome wide analyses using genotyping and sequencing. ***Below is an example from A0131201 that examines abiraterone pharmacogenetics.*** This language has been approved by NCI and can be adopted for local studies and expanded to include other host markers of variability.

Background and hypothesis
Abiraterone is a selective inhibitor of androgen biosynthesis that potently blocks CYP17A1, which is critical to testosterone synthesis by the adrenals, testes, and within the prostate tumor. Genetic variation in expression of CYP17A1 is regulated in part by promoter sequence variation, including a T>C change at nucleotide -34.

The primary statistical objective for this companion study is to investigate a drug by *CYP17A1* interaction with respect to overall survival. Specifically, we hypothesize that patients on the enzalutamide/abiraterone arm (Arm B) with a homozygous −34T genotype (Genotypic Group 1, 43% of the population) will have an inferior overall survival to patients with a heterozygous or homozygous −34C genotype (Genotypic Group 2, 57% of population). No genotype effect is expected in Arm A (enzalutamide alone).

As secondary objectives, we will assay candidate variants and loci hypothesized to be associated with other clinical phenotypes (e.g., progression-free survival or toxicity) or other eQTLs.

In addition, we may use the DNA collected to consider other candidate SNPs or to conduct a genome-wide association study (GWAS) to validate other or identify novel candidates, or, as next generation sequencing platforms become more cost effective, consider exome or whole- genome sequencing. As a randomized trial with uniform assessment and follow-up this represents an important opportunity to better understand the relationships among germline genetic variation, disease, and treatment-response phenotypes. At this time, the Population Pharmacology and Pharmacogenomics Committee of Alliance is conducting genomewide analyses of previously completed CALGB trials using The Illumina 1M platform in collaboration with the RIKEN Center for Genomic Medicine. More than 1,000,000 SNPs are simultaneously genotyped. In addition, there are 4,300 SNPs in regions of copy number variations (CNVs), thus allowing for the detection of CNVs as well. Near the completion of this clinical trial, specific hypotheses based on currently evolving findings will be formulated and a detailed investigational plan will be submitted as an amendment to this investigation. This flexible, anticipatory design is not only conventional, but also necessary in cancer pharmacogenomics.

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