

Creating Clinical Target Validation Groups via Quality-Assured Transfer of Robust Clinical Pharmacodynamic (PD) Assays from the National Cancer Institute: Clinical Implementation of a HIF1 α Immunoassay in Tumor Biopsies

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Introduction

Early phase clinical trials of investigational agents benefit from laboratory assays that quantify the pharmacodynamic (PD) target engagement (1st PD) as well as desired changes in biochemical signals (2nd PD), and 3rd PD cellular responses such as apoptosis. Robust PD assay results are valuable for informing go/no-go decisions about continued preclinical and clinical development of new agents and for identifying combinations of targeted agents. Importantly, Phase 0/I clinical trials are reliable sources of tumor biopsies for evaluating PD, whereas Phase II/III trials are not. The National Cancer Institute's Division of Cancer Treatment and Diagnosis (DCTD) develops and validates PD assays to obtain accurate information about drug effect on intended molecular targets in first-in-human clinical trials and inform clinical development decisions. The Pharmacodynamic Assay Development and Implementation Section (PADIS) and National Clinical Target Validation Laboratory (NCTVL) were established at SAIC-Frederick to develop and validate PD assays.

Reading PD assays for clinical use involves validating analytical performance, demonstrating fitness-for-purpose for the clinical protocol, and finalizing companion standard operating procedures (SOPs) for specimen handling and processing. Because clinical PD questions often demand assay performance that meets or even exceeds clinical diagnostic assay standards but key assay reagents are usually R&D- rather than GMP-grade, stringent reagent Quality Control is critical for preventing assay failures due to lot-to-lot variability.

Proven clinical assays are transferred from the NCI to requesting sites in academia, the pharmaceutical industry, and other organizations via laboratory-based certification and training, centralized access to SOPs, assistance with assay transfer, and participation in the assay's Quality Assurance Plan. The result is an assay user group that can rapidly communicate assay issues, solve those issues, and implement required SOP changes while achieving consistent assay results across multiple sites over months to years of clinical studies.

Problem and Approach

Problem: There is no commercial market force driving the availability of diagnostic-quality tests and reagents to demonstrate drug effects of targeted agents; these forces only arise after the targeted agents are shown to be clinically effective.

Approach: Achieve diagnostic-quality tests using materials and reagents from research supply houses that are suitable for the analysis of valuable and limited patient samples.

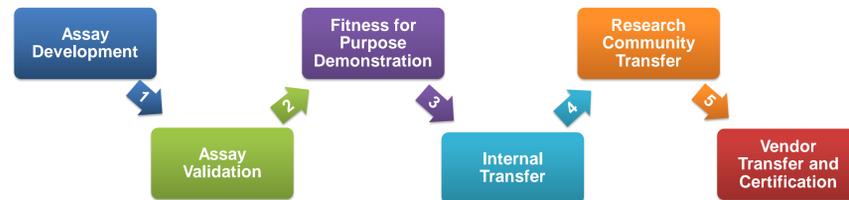
- Develop robust, accurate, and sensitive PD assays for preclinical and clinical use with collaboration between DCTD, PADIS, NCTVL, and NCI clinical staff.
- Use proven, clinically available assay platforms such as immunoassays, circulating tumor cells, microscopy, and qRT-PCR.
- Use assay instrumentation that has broad market availability so assays can be transferred to the community.
- Implement quality assurance strategies borrowed from clinical laboratory medicine and GMP-manufacturing for key reagents.
- Meet rigorous performance standards to be considered clinically ready.

Conclusions

- It is possible to develop robust assays to measure target engagement (1st PD effect) as well as biochemical consequences (2nd PD effect) and cellular responses (3rd PD effect) suitable for analysis of patient specimens, including their use as primary endpoints in Phase 0/exploratory IND trials.
- Applying more stringent production and Internal Quality Control (IQC) specifications is crucial for accepting/rejecting new lots of critical reagents.
- Along with on-site training and SOP-based assay transfers to other laboratories, QC of critical reagent supply chains is key to achieving consistent assay results over several years of clinical drug development.
- If you have a well defined and effective QA plan, it is possible to conduct these PD assays with a network of users to achieve consistent results and quality across sites, users, and times, using R&D-grade resource materials and defined critical reagents.

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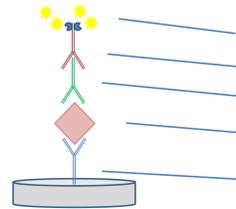
Assay Lifecycle and the Importance of Formal Assay Transfer



Every assay transfer (3, 4, and 5) is a formal, SOP-driven process with the goal of equivalent assay performance at the training laboratory and the new assay site.

Assay Quality Control and Analyte Stabilization Lessons Learned from the HIF1 α Immunoassay

- Develop and validate robust assays
 - Develop custom sample collection, extraction and analyte preservation methods that can be applied clinically
 - Identify critical reagents
- Develop reagent quality control and distribution program
 - Lot-to-lot reagent evaluations
 - Production and management of control materials
- Customize data analysis process and data quality control criteria



Case Study: Qualification of Commercially Available Kits

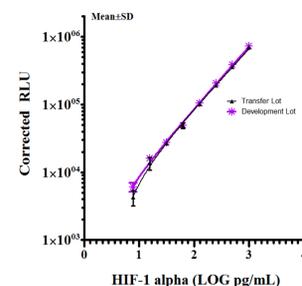
Issue

The HIF1 α immunoassay utilizes a commercial kit with a slightly modified protocol. Each new lot of kits are subject to qualification testing prior to acceptance.

Resolution

The kit includes lyophilized antibodies and calibrator that have a long shelf life, allowing for qualification of large lots of kits for use in clinical trials with long accrual times.

Assay controls (high, mid, and low) were produced from extracts of HEK293T cells transfected with a HIF1 α expression vector and PC-3 cells.



IQC Methodology Established

New kit lots are subject to analysis using a proficiency panel. Kit acceptance criteria are 25% variance from a previous passing lot. The panel includes quality control specimens that are also included in each clinical assay run.

Case Study: Pre-defined Data Analyses and Quality Control Evaluation Criteria

QA Methodology Established

Pre-defined data analyses and quality control evaluation criteria are used to perform a semi-automated process to analyze data and evaluate acceptability of performance for the assay run control and clinical specimens. This process allows the operator and laboratory supervisor to rapidly identify assay and sample failures, and monitor assay performance over time. In addition, the process allows the data to be tracked from the initial raw values to the reported clinical value for QA purposes.

Background Wells	Run 1				Run 2				
	Mean RLU	CV%	Criteria	Pass/Fail	Mean RLU	CV%	Criteria	Pass/Fail	
Background	13108	9.4	<20% CV	Pass	16120	11.1	<20% CV	Pass	
Background + STD	16885				21479				
Calibrator Wells	HIF-1 α (pg)	Mean RLU	S/B	Criteria	Pass/Fail	Mean RLU	S/B	Criteria	Pass/Fail
STD 1	0.78	18600	1.42	>1.1 S/B	Fail	20824	1.29	>1.1 S/B	Fail
STD 2	1.56	26726	2.04	>3SD Back	Pass - LIQ	28908	1.79	>3SD Back	Pass - LIQ
STD 3	3.13	37719	2.88			39602	2.46		
STD 4	6.25	66022	5.04			67629	4.2		
STD 5	12.5	116395	8.88			119864	7.44		
STD 6	25	215712	16.46			215927	13.4		
STD 7	50	389867	29.75			397559	24.66		
STD 8	100	666066	59.81	>20 S/B	Pass - LIQ	697204	43.25	>20 S/B	Pass - LIQ

Case Study: The Oxygen Dilemma – Custom Extraction Process

Issue

HIF1 α protein low abundance and instability in the presence of oxygen limits reliable measurement in samples that are processed under normoxic conditions. We tested various strategies for HIF1 α stabilization in solid tumors including nitrogen gas-purged lysis buffer, addition of proteasome inhibitors or the prolyl hydroxylase inhibitor 2-hydroxyglutarate, sonication, and ceramic bead homogenization. A customized extraction process for HIF1 α was developed and optimized using 18-g needle biopsies from mouse xenografts to mimic tissue collection and handling procedures that would occur in the clinic.

Resolution

Degassing and adding 2-hydroxyglutarate to the processing buffer increased HIF1 α recovery, while bead-homogenization in sealed tubes both improved HIF1 α recovery and reduced sample variability.

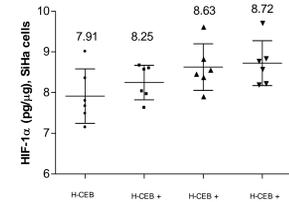


Figure: HIF1 α levels in SiHa cell lysates incubated with 1 μ M bortezomib for 4 hours then lysed with buffer alone or containing 100 μ M bortezomib, MG132 or 2-hydroxyglutarate (2-HG).

Sample No.	Cohort 1		Cohort 2		Cohort 3		Cohort 4	
	Sonic.	Homog.	Sonic.	Homog.	Sonic.	Homog.	Sonic.	Homog.
1	14.6	27.6	35.2	37.7	38.1	37	35.8	21.1
2	39	29	22.2	28.2	28.5	36.4	43.6	32.2
3	31.4	27.9	30.9	27.4	32.4	36.9	31.4	28.4
4	32.3	29.1	28.7	36.6	14.2	29.4	39.3	31.9
5					31	33.7	49.8	39.1
6					13.3	24.7	24.8	36.2
7					31.2	27	42.5	38.7
8					18.5	24.7	17.9	22.9
Mean	29.3	28.4	29.3	32.5	25.9	31.2	35.6	31.3
SD	10.4	0.8	5.4	5.4	9.3	5.4	10.5	6.8
%CV	35.4	2.7	18.5	16.7	35.8	17.3	29.5	21.7

Table: HIF1 α levels from PC3 xenograft lysates processed using the Precellys24 bead homogenizer (Homog.) and an ultrasonic homogenizer (Sonic.). Frothing of samples inherent in sonication will introduce oxygen into the buffer and likely caused the increased variance observed in HIF-1 α readouts.

Assay Methodology Established

Clinical implementation of HIF1 α analysis in tumor biopsies from patients treated with angiogenesis inhibitors required consideration of many factors. In total, five SOPs have been developed to define procedures from collection and freezing of the needle biopsies in the radiology suite to data analyses and reporting. Together, these defined procedures are being used to perform PD analyses as key parts of two clinical trial evaluations at NCI and will be launched to the community once clinical utility is demonstrated.

Internal Assay Transfer for First-In-Human Use

Validated assays are transferred from the development laboratory (PADIS) to the clinical laboratory (NCTVL) for first-in-human use of the assay to measure target engagement by drug. Assay transfer is conducted using identical lots of critical reagents, calibrators, and controls from an established supply chain; identical samples, blinded, representing the dynamic range of the assay; identical instrumentation at both laboratories; and SOPs that have been signed and approved by the assay development laboratory.

Prior to assay transfer, a minimum of two familiarization runs are performed between PADIS and NCTVL to ensure that all key reagents and equipment are in place and SOP procedures are well understood. During proficiency testing, the assay is run at least five times at both laboratories using identical equipment and the same specimen and critical reagent sets to verify that assay precision and performance are matched to performance claims and are comparable between laboratories.

Method	Platform	Target(s)
Immunoassay	ELISA	Total MET, pY1234/Y1235-MET, pY1235-MET and pY1356-MET
Multiplex IFA	Bond-Max, Definiens	γ H2AX, Ki67, Cleaved Caspase 3

Community Transfer for Early Phase DCTD Trials

Validated Assays Currently Available for Research Community Training and Certification; DCTD Biomarkers Web site: <http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm>

DCTD offers training and certification in the use of its validated assays that have undergone peer review through the publication process. Training is conducted at the Frederick National Laboratory for Cancer Research in Frederick, Maryland, by senior scientific staff.

Method	Platform	Target(s)
Circulating tumor cells (CTCs)	CellSearch	γ H2AX
Immunofluorescent assay (IFA)	Bond-Max, Image-Pro	γ H2AX
Immunoassay	ELISA	Total Top1

Completion of on-site, laboratory-based training classes at the Frederick National Laboratory of Cancer Research triggers assay transfer to the new site along with the supply of critical reagents to sites participating in DCTD-sponsored clinical trials for which the assay was designed. Laboratories with Certified Assay Operators can provide assay calibrators and control readouts so that assay performance can be monitored and QA issues addressed and resolved as a group.

Establish Procedure for Notifying Certified Assay Sites of High Priority Assay or Reagent Updates

While Certified Assay Operators are expected to check the DCTD Biomarkers Web site to verify that they are using the most recent SOPs, the Assay Trainee List is used to notify Certified Operators about high priority changes to the SOPs.

Emerging Validated Assays for Research Community Training and Certification

Method	Platform	Target(s)
Circulating tumor cells (CTCs)	CellSearch	p16 ^{INK4a}
Immunoassay	ELISA	HIF1 α

Vendor Certification for Late Phase DCTD Trials

- Following community transfer, assays are transferred (via the same process as community transfer) to interested clinical laboratories and companies (vendors). These vendors can offer the clinical assay on a fee basis under DCTD's Cancer Pharmacodynamics (caPD) Certification Mark (shown on left).
- DCTD will support PD applications by Certified Vendors through the provision of human tumor xenograft specimens for production of reference material and master lots of calibrators.
- PD specimens from DCTD clinical trial sites can be analyzed by the Certified Vendor and/or on-site Certified Assay Operators.
- Certified Vendors are encouraged to pursue other commercial applications of the assay; for example, diagnostic and theranostic assays.



Method	Platform	Target(s)
Immunoassay	ELISA	Poly(ADP-Ribose)

References

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