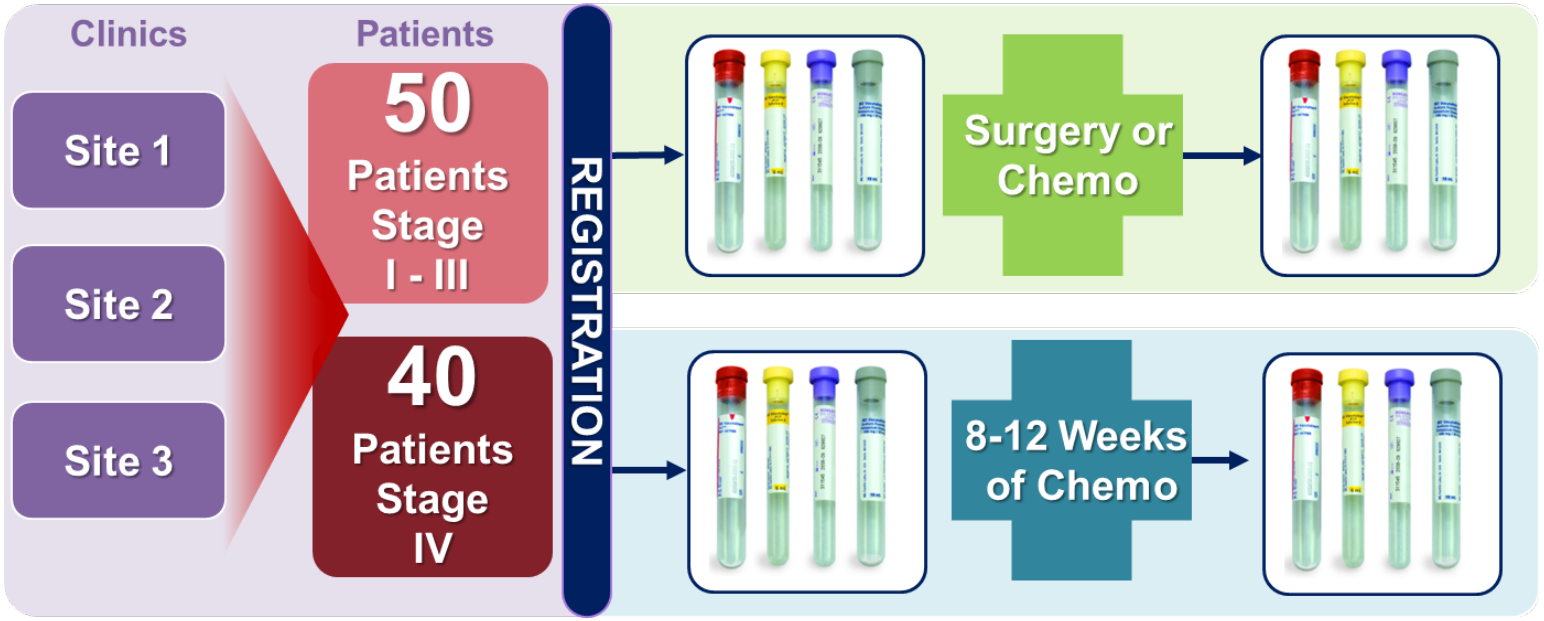
Overview

Goal: To characterize the effect of two pre-analytical variables, namely five blood collection tubes (BCT), Streck, EDTA, Heparin, Citrate ACD and CellSave, and four times-to-assay (TTA) on the bench, 24, 48, 72 and 96 hours, relevant to the enumeration of circulating tumor cells (CTCs) and specific high-content screening (HCS) measurements. Enumeration and characterization of HD-CTC will determine the best combination of BCT and TTA that will subsequently be compared to the CellSearch®

HD-SCA assay

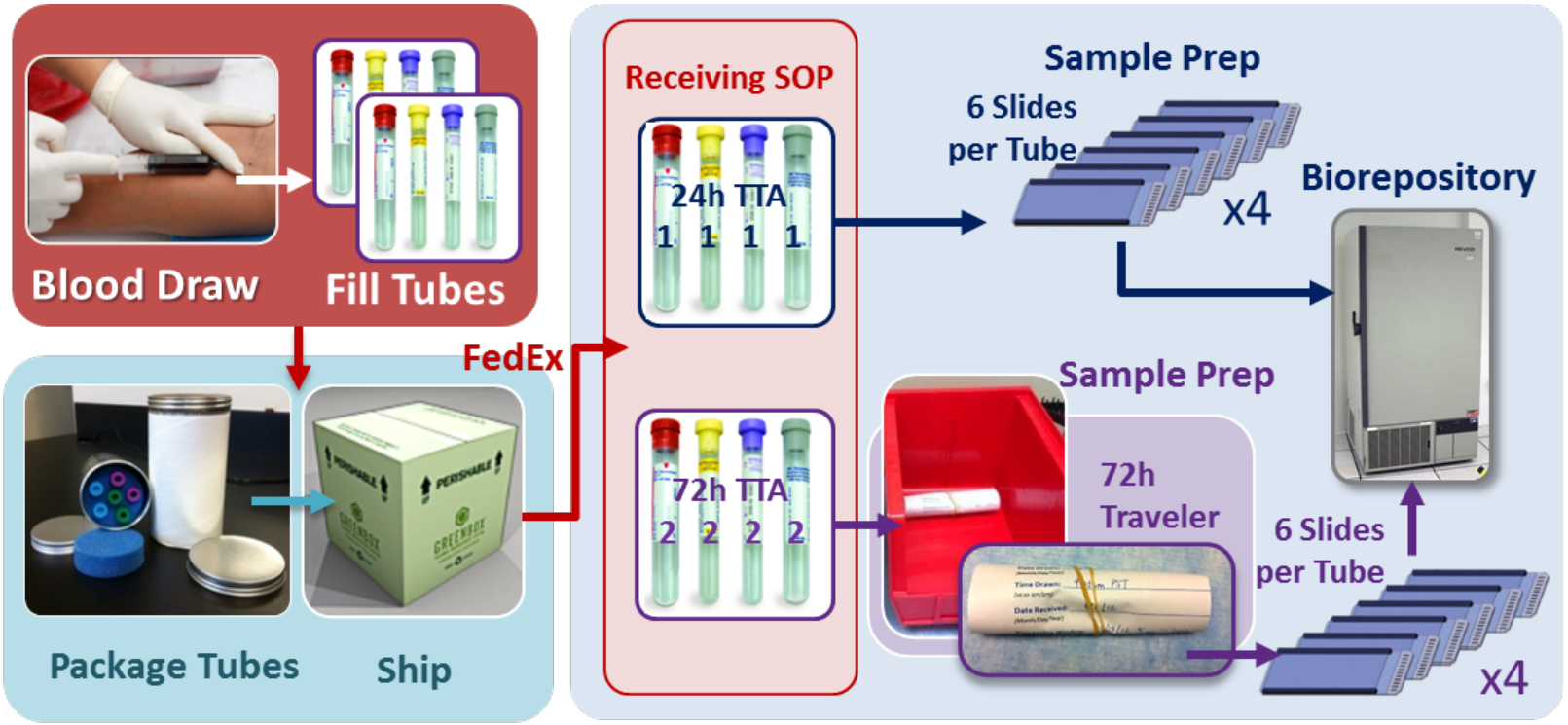
A high-content screening (HCS) methodology is being used to characterize multiple parameters at the single cell level in conjunction with a HCS triaging concept to identify events of interest. Developed at the Kuhn laboratory of the University of Southern Califnornia, the HD-SCA (high definition – single cell assay) is an inherently non-enrichment assay providing multiple overall opportunities as a fluid biopsy. Its expanded use requires a detailed understanding of pre-analytical variables.

Recruitment plan

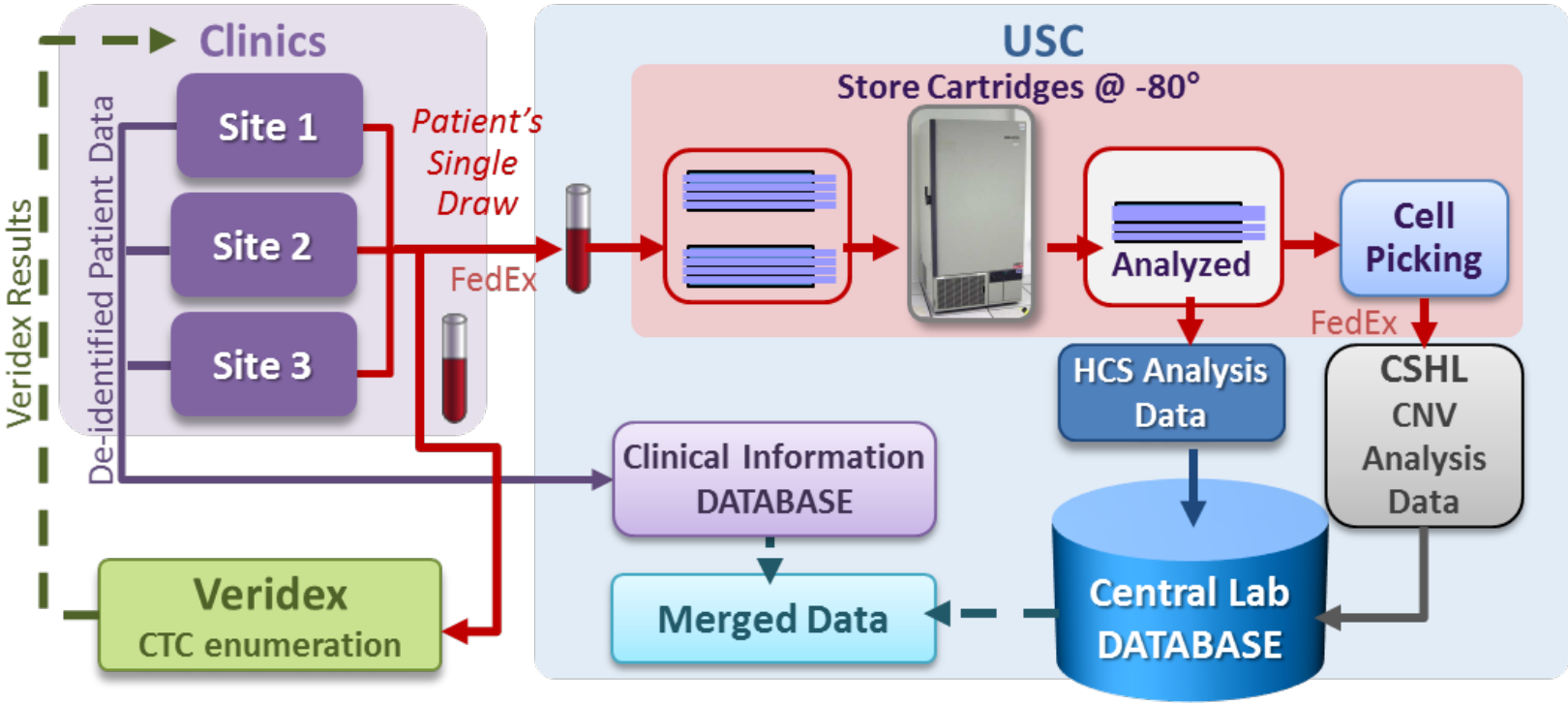


A total of 90 breast cancer patients will be enrolled in the study from three clinical sites. Patients with early stage disease will have 2 study related blood draws, whereas up to 12 study related blood draws will be obtained for patients with metastatic disease over a 2-year period.

Experimental Design



Blood from patients is drawn into a syringe and distributed amongst the different blood collection tube types. Variables such as anatomic draw location, needle gauge and tube fill number are tracked with a standardized questionnaire. All BCTs are then packaged and sent to the central laboratory. The 24h TTA tubes are processed and stored in the -80°C biorepository upon arrival. The 72h TTA tubes are stored in a designated location until 68-76 hours after the time of draw. Six slides are created from each BCT and all slides are stored in the biorepository until analysis.



Blood specimens from research subjects are sent by FedEx overnight to USC for sample preparation and storage into the -80°C biorepository. At the time of draw at the clinical site, information about the blood draw parameters is collected into OpenClinica-based case report forms. After sample preparation and analysis, all HD-SCA derived data is uploaded to the laboratory central database. For Veridex specimens, samples collected in CellSave™ tubes will be forwarded to a commercial laboratory for CellSearch® analysis and results will be entered into a database. Experiments for copy number variation (CNV) will require cell picking at USC with the individual cells being shipped to Cold Spring Harbor Laboratory for analysis. CNV data will then be returned to the laboratory central database. The pre-analytical draw data (including CellSearch® results) will be merged with the laboratory central database so that HD-SCA data (including CNV results) and clinical data can be simultaneously accessed.