Legacy Biomarker Qualification Project Status Update[1]

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[1] The content you provide in this completed Status Update will be publicly posted as part of the section 507 transparency provisions.

Legacy Project Background. Rationale and Request

Beth Walton of the FDA requested on March 15, 2018, that Debra Rasmussen of Janssen Research & Development, LLC submit a Status Update for the Biomarker Qualification Project (DDT-BMQ-000006). The Sponsor for this submission process was changed from Janssen R&D to Menarini Silicon Biosystems on October 8, 2018. We are submitting this Update, in response to Beth Walton's previous request, and in order to transition the Biomarker Qualification Project to the 507 regulatory process created by the 21st Century Cures Act.

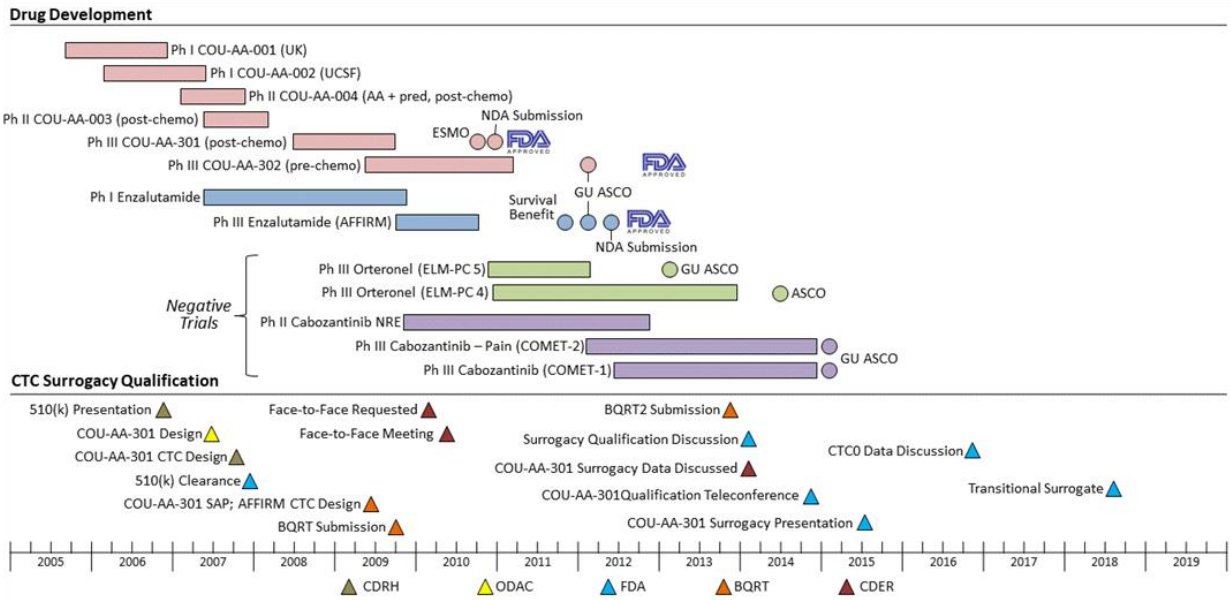
Measures of response that are clinically meaningful and occur early remain a critical unmet need in metastatic castration-resistant prostate cancer (mCRPC) clinical research and practice. New and ongoing molecular profiling studies have led to a more biologically based disease taxonomy identifying subsets of patients likely to respond or not respond to specific classes of drug.[1] Historically, clinical research in the mCRPC population has relied on Prostate-specific antigen (PSA) changes, such as the maximal percent or percent at a fixed time point, as indicators of treatment efficacy, although neither is a strong indicator of Overall Survival (OS).[2,3] Other response end points, such as radiographic measures for bone metastases (the dominant location of metastasis in mCRPC), are problematic because of the difficulty distinguishing whether early unfavorable changes represent worsening or improving disease status. Changes in measurable disease, assessed by Response Evaluation Criteria in Solid Tumors, are also used, although they occur infrequently. With these limitations, along with the increasing number of possible treatment combinations the unmet need for response indicators that reliably reflect survival and that occur early so trials can be completed in a shorter time, has become more urgent.[4]

This lack of clinical endpoints that occur early, began a discussion long ago between the sponsors and the FDA. The premise that drove those discussions was to build on an existing assay; one that was already cleared as being predictive of OS and progression free survival (PFS) and could be used as an aid to monitoring mCRPC. Most metastasizing cancers spread through the blood as single cells or in clusters. At present there are a range of devices and assays that enable the detection, enumeration, and biologic characterization of Circulating Tumor Cells (CTCs).[5,6] Only one, CellSearch (Menarini Silicon Biosystems, Huntingdon Valley, PA), has achieved the level of FDA clearance for the context of use as an "aid in the monitoring of patients with mCRPC…in conjunction with other clinical methods."[7] Studies in patients with mCRPC have shown that the number of CTCs detected is higher in patients with bone disease relative to lymph node disease and that association with disease burden is modest, [8-10] which shows that the ability of a cancer cell to detach, circulate, survive, and colonize a distant site is an intrinsic property of the tumor. It follows that inhibiting the spread of cells through the circulation would represent a therapeutic objective that is clinically meaningful.[10-15]

After demonstrating CTC conversion rates between 35% and 40% in three phase II studies of abiraterone and enzalutamide,[16-18] a collaboration was initiated with the US FDA Center for Disease and Radiologic Health to study post-treatment CTC containing end points as potential surrogates for survival. To do so, the CTC biomarker question was embedded in

a series of phase III registration trials with a primary end point of OS. The studies, and milestones, are summarized in Figure 1, and in detail in Attachment 1.

Figure 1. Biomarker Submission Timeline and Milestones



Three of the phase III drug trials failed to achieve their primary endpoint of improved OS. Regardless, the CTC biomarker appeared to perform well with respect to predicting the favorable and unfavorable responses to experimental treatments. In collaboration with the FDA, and upon their request, the conceptual objective for the biomarker evolved from a surrogate for OS to where it is today, a single factor response biomarker.

A Statistical Analysis Framework was submitted by the sponsors in November of 2013. The Framework proposed analyzing Circulating Tumor Cells (CTCs), previously or currently embedded in 5 Phase III clinical trials, for their ability to serve as an early indicator of response. In September of 2015 the FDA issued a Letter of Support;[19] and on June 3, 2016 the FDA responded favorably regarding the Statistical Analysis Framework:

> "In general, your proposed statistical analysis framework is acceptable for evaluation of the limited context of use (COU) for Circulating Tumor Cells (CTC) as a biomarker endpoint for early evaluation of drug product activity in clinical trials for the metastatic castration resistant prostate cancer (mCRPC) patient population."

All 5 of the Phase III clinical trials have since been completed and summaries of their findings have been published[11-15] (See Attachments 4 thru 8). The statistical analysis of these 5 trials, done in accordance with the Statistical Analysis Framework, has also been completed. This analysis was published by Heller et. al. in the Journal of Clinical Oncology in February of 2018.[4] The Heller analysis explored several ways to define the change in CTCs as an indicator of response. CTC going from detectable at baseline to undetectable at week 13, outperformed all other measures.

The sponsors believe no early biomarker in mCRPC has been as thoroughly validated analytically, or has been so consistently correlated with OS and PFS, as that of CTCs. Our analysis will show the change in CTC numbers occurs within 12 weeks of the start of therapy, is the strongest predictor of a beneficial response to therapy, and accurately foreshadows the traditional measures of response that occur late. This CTC biomarker will, within the limited context of use, enable measurement of responses in many more patients currently classified as having non-measurable disease. As a consequence, it is our hope, and belief, the use of this biomarker will enable rapid readouts of response, from smaller clinical trials, greatly accelerating the ability to generate the data required to support Breakthrough Designation for effective new single or combination therapies.

Menarini Silicon Biosystems and Memorial Sloan Kettering are submitting this update in response to Beth Walton's 2018 request. It addresses the questions requested of an update. An update is typically a forward-looking document, written before the start of data collection. However, this submission which originated under the legacy biomarker program, is atypical because the 5 Clinical studies have been completed[11-15], and the CTC analysis has already been published.[4] Therefore, the sponsors are asking the FDA to allow us to proceed quickly to formally submit the Qualification Plan and Full Qualification Package in order to complete our 507 process application.

I. Context of Use

A. Biomarker Category

Pharmacodynamic/Response Biomarker

B. Intended Use in Drug Development

A critical unmet need in the development of drugs to treat mCRPC is the current lack of a reliable early indicator of a beneficial response to therapy (see Table 1). This prolongs the drug development cycle and deprives patients access to promising, new, life extending treatments. The intent of this biomarker is to address that need within the context of mCRPR clinical trials. We proposed, and our analysis has borne out, that a favorable change in a patient's CTC numbers, from baseline to after 12 weeks of therapy, indicates a positive and beneficial response to treatment[4]. This is because of the strong association between CTC numbers and OS and PFS in this and other cancers.[9,20-23] The intended use of this biomarker therefore is to be used within mCRPC clinical trials as an early indicator of a response to therapy that reflects patient benefit; and which can be used to inform pharmaceutical drug development and regulatory decision-making.

C. Context of Use Statement

CTC0 is a Response Biomarker for the early evaluation of drug product activity in mCRPC clinical trials.

II. Drug Development Need

Barriers to expeditious drug development in prostate cancer are due largely to the limitations inherent in the "early" indicators of efficacy used historically. Imaging modalities used to assess disease in bone, the most common site of spread, have not been standardized and no drug approvals have been based on "favorable changes" in these scans (see Table 1). The directional change in PSA levels, the most frequently altered biomarker in the disease, may not fully reflect the status of disease accurately.[24, 25] For example, up to 20% of men with mCRPC, who eventually respond to a systemic cytotoxic therapy, have an initial PSA increase before the decline;[26, 27] which may not occur for up to 12 weeks.[28] Immunomodulatory agents, postulated to slow disease trajectory through effects on the tumor microenvironment (e.g., sipuleucel-T[29]), have not consistently shown a favorable effect on any disease manifestation making it challenging to determine treatment efficacy for an individual patient. It is therefore not surprising that the association between a given post-therapy change in PSA and survival is modest, and PSA alone is not accepted, appropriately, by regulatory agencies for drug approvals[3,14,15] see Table 1. With these limitations, along with the increasing number of new agents and treatment combinations, the unmet need for response indicators that reliably reflect survival and that occur early has become increasingly urgent.

The endpoints that have been used to support drug approvals in mCRPC and their indications are summarized in Table l. Note that all of the endpoints, with the exception of the control or relief of pain, represent time to event measures that occur late.

Table 1. Endpoints used in prostate cancer drug approvals

Response: Early	Time to Event: Late
Control of Pain • Radiopharmaceuticals • Mitoxantrone + prednisone - docetaxel • Indicated but not approved for: Alpharadin (indicated for patients with symptomatic osseous disease)	***Skeletal Related (SRE) or Symptomatic Skeletal Events (SSE)*** • Zoledronic acid (SRE) • Denosumab (SRE) • Alpharadin *(Shown)* (SSE) ***Metastasis-free survival*** • Apalutamide • Darolutamide
No approvals based on favorable Change in a Disease Manifestation • *PSA decline* • *Tumor shrinkage or regression* • *"Favorable" change in a bone scan*	***Death / Survival*** • Docetaxel • Sipuleucel-T • Cabazitaxel • Abiraterone • Enzalutamide • Alpharadin • Pembrolizumab

Near-term "early" response endpoints that correlate with and predict clinical benefit, are a critical unmet need in mCRPC. Our data, has been summarized briefly in section IX, and

analyzed in greater detail in the paper by Heller et. al.[4] The data demonstrates the CTC0 definition out performs all other early indicators of response, and expands the number of evaluable patients by 25%. It is our belief, the use of this biomarker will enable rapid readouts of response, from smaller clinical trials, greatly accelerating the ability to generate the data required to support Breakthrough Designation for effective new single or combination therapies.

III. Biomarker Information

A. Biomarker Name, Source, Type and Description

The name of the response biomarker for this application is "CTC0." It is a single factor biomarker that registers the change in CTC numbers between two time points, baseline and after 12 weeks of therapy. A favorable response to therapy is observed when patients with a CTC count $\geq$1 in 7.5 mLs of blood at baseline (Detectable CTCs), are later found to have 0 CTCs (Undetectable CTCs) after 12 weeks of therapy.

The source of the CTCs being isolated from the peripheral blood of these patients is from one or more of their primary or metastatic cancer sites. The process of metastasis has been well delineated and results from individual tumor cells, and cell clusters, leaving their original primary or metastatic site where they extravagate into the blood, travel in the circulation, subsequently exit the blood vessels, and establish secondary sites of tumor growth in distant organs. The ability of a CTC to successfully complete this invasion-metastasis cascade is accompanied by several molecular changes resulting, in phenotypic changes that enable the CTC to complete this process.[32]

Type of Biomarker (Check relevant type(s))			
	Molecular		Radiologic/Imaging
	Histologic		Physiologic Characteristic
X	Other (please describe): Cellular analysis from Liquid Biopsy*		

*A **liquid biopsy** is a simple blood draw that allows collection of tumor materials including circulating tumor cells, tumor cell fragments (vesicles), circulating DNA or RNA, exosomes, etc., with the potential to be more informative than a single site biopsy that is invasive, costly, difficult to repeat and may not be as representative of the disease as a whole.*

CTCs are extremely rare cells in the bloodstream and are estimated to account for at most one cell in a hundred million to a billion of the cells circulating in blood.[33] As such, their capture, isolation, enumeration and characterization is challenging. Although a range of CTC technologies are available, the CellSearch CTC Test (Menarini Silicon Biosystems) is the only assay that has received an FDA clearance for CTC enumeration (K031588[34,43] & K073338[7]). This assay captures and enumerates a particular class of CTCs from the total population of all CTCs, by using an Anti-EpCAM conjugated magnetic particle (ferrofluid). CTCs in this assay are strictly defined as those intact cells that are EpCAM+, have a DAPI+ nucleus surrounded

by cytoplasm that is cytokeratin+ (CK+), and the CTC must also be CD45(-).[7,34] The CellSearch assay has been established as analytically validated and highly reproducible.[35] While CTCs with other phenotypes and properties are known to exist, neither their clinical significance, nor their clinical equivalence to the EpCAM+, DAPI+, CK+, CD45(-) CTC population enumerated by CellSearch has ever been established in mCRPC. It is important to emphasize therefore, that what is essential to this biomarker is not the technology used to isolate and enumerate these cells, which can be achieved in multiple ways, but rather, that the cell population isolated by any given technology is phenotypically and numerically the same as those used to qualify the CTC0 biomarker.

The CTC0 endpoint is an indicator that a population of cancer cells, that were previously detectable circulating in the blood, are no longer detectable (in 7.5 mLs of blood), a binary and definitive endpoint that represents a clinically meaningful outcome for the patient, as indicated in the CellSearch CTC Kit Intended use Statement below:

For *in vitro* diagnostic use.

The CELLSEARCH® Circulating Tumor Cell Kit is intended for the enumeration of circulating tumor cells (CTC) of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+, and/or 19+) in whole blood. The presence of CTCs in the peripheral blood, as detected by the CELLSEARCH® CTC Test, is associated with decreased progression-free survival and decreased overall survival in patients treated for metastatic breast, colorectal, or prostate* cancer. The test is to be used as an aid in the monitoring of patients with metastatic breast, colorectal, or prostate cancer. Serial CTC testing should be used in conjunction with other clinical methods for monitoring metastatic breast, colorectal, and prostate* cancer. Evaluation of CTCs at any time during the course of disease allows assessment of patient prognosis and is predictive of progression-free survival and overall survival.

CELLSEARCH® CTC Test results should be used in conjunction with all clinical information derived from diagnostic tests (eg, imaging, laboratory tests), physical examination, and complete medical history, in accordance with appropriate management procedures.

For more information on the full intended use and limitations please see the Instructions for Use

*Metastatic prostate cancer patients were defined as having two consecutive increases in the serum marker prostate-specific antigen above a reference level, despite standard hormonal management. These patients are commonly described as having androgen-independent, hormone-resistant, or castration-resistant prostate cancer. For more information on the intended use and limitations for the CELLSEARCH® Circulating Tumor Cell Test, please refer to the Instructions for Use which can be found at documents.cellsearchctc.com.

The CTC0 endpoint represents a single component biomarker that may be reached shortly after treatment initiation, and provides investigators and practitioners with early, objective, and reliable evidence that the therapy being administered has altered the patient's prognosis in a favorable way.[4]

B. For molecular biomarkers, please provide a unique ID.

N/A

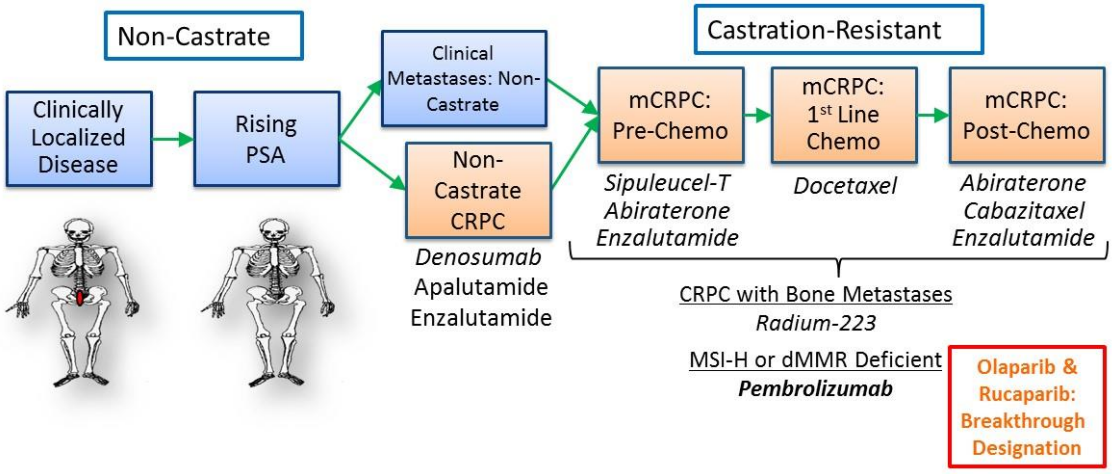
C. Rationale for Biomarker

Natural History of the disease:

The natural history of the Prostate Cancer (PC) is summarized in Figure 2. A patient may present for the first time with localized disease confined to the prostate, or in some men, it may have already spread beyond the prostate and is found in other regions of the body as well (metastatic disease). Typically, early on, the tumor will respond to androgen blocking therapies

(Castration sensitive PC). Later, PC will likely develop resistance to androgen blocking therapies (Castration Resistant, CRPC); and may become metastatic (mCRPC), if it had not previously been metastatic in that patient.

Figure 2. The castration resistant prostate cancer therapeutic landscape



Disease Mechanism and Causal Pathway:

The mechanism, or process, by which tumors metastasize has been well delineated[32] and results from individual tumor cells, and cell clusters, leaving their original, primary or metastatic, site where they extravagate into the blood, travel in the circulation, subsequently exit the blood vessels, and establish secondary sites of tumor growth in distant organs. Most cancer deaths actually are due to these metastases. It was postulated therefore, that finding CTCs in blood represents evidence of an aggressive, active metastatic process, versus a slower, more indolent disease progression when they are not found in the blood. Furthermore, a patient should derive unambiguous benefit from any treatment that could eliminate, or substantially lower, CTCs in the blood.

The development of using CTCs as an endpoint originated with the demonstration in patients enrolled in trials of similar design with metastatic breast,[36] colorectal,[22,23] and prostate cancer[20,21] about to start new lines of chemotherapy. In all three of these epithelial cancers, patients could be divided into groups at baseline with a favorable or unfavorable prognosis based on the number of CTCs present prior to treatment. Particularly relevant to this submission was the analysis of the IMMC38 trial[21] that enrolled 276 men with progressive mCRPC; 164 of these patients were starting first-line chemotherapy. In IMMC38 the post-therapy conversion from 5 or more CTCs to 4 or less CTCs per 7.5 ml of blood at 4, 8, and 12 weeks was predictive of improved OS and PFS (see also the intended use statement above); an obvious benefit for the patient. This change in CTC number was found to be more informative than a 50% decline in PSA, and led to the 510(k) clearance in mCRPC as an aid to monitor disease in conjunction with other methods.[21] These results also suggested the possible use of CTCs as an intermediate endpoint of survival in mCRPC clinical trials.

Magnitude, Risk Factors, and Cutoff versus Continuous Variable:

The reanalysis of the IMMC38 trial evaluated both the use of CTC numbers as a continuous variable (versus a discrete cutoff of 5 cells) and whether CTCs could serve as a prognostic marker of survival in mCRPC.[21] This reanalysis also included two other markers, PSA and Lactate dehydrogenase (LDH), alone or in combination with CTCs, as surrogates of OS. CTC number, analyzed as a continuous variable, was more predictive of survival than PSA at baseline and during patient follow-up, and could be used to monitor disease status. Overall Survival prediction models that combined PSA levels with CTC number were no better than models using CTCs alone. High baseline LDH (above 240 IU/mL) was found to be associated with a significant increase in risk of death. An OS prediction model that incorporated a patient's baseline LDH along with their pre- and post-therapy CTC numbers was the most predictive of OS independent of cutoff value. It is important to note that in this study LDH levels were collected only at baseline, and not over time or after therapy. High baseline LDH levels therefore, reflected a patient's risk stratification with respect to OS, but could not be evaluated as a biomarker of response to therapy. The reanalysis did confirm however, that a change in CTC number pre and post-therapy, even as a continuous variable, was the best predictor of OS after 4, 8, and 12 weeks of therapy in mCRPC patients undergoing first line chemotherapy. This was consistent with the OS and PFS predictive claim in the CellSearch intended use statement.

Response Biomarker Rationale and Single versus a Panel Biomarker

Starting in 2004, discussions were begun in order to address the pharmacodynamics dilemma of using PSA levels as a response biomarker in clinical trials. The concern was that post-therapy declines in PSA may only represent a pharmacodynamic effect and not reflect any change in tumor growth or patient benefit. Parties to these discussions were Dr. Howard Scher, a medical oncologist practicing at the Memorial Sloan Kettering Cancer Institute, and representatives of the FDA (including both the Center for Devices and Radiological Health, CDRH and the Center for Drug evaluation and Research, CDER). CTC enumeration was included as a biomarker in the initial development trials of Abiraterone acetate given in combination with prednisone and Enzalutamide. Both of these drugs directly target recognized oncogenic changes in androgen receptor signaling that occur commonly in castration resistant disease and drive tumor growth. In 2007 Dr. Scher and the FDA were joined by representatives from the pharmaceutical development company Cougar, and Veridex LLC, in discussions regarding the design of the COU-AA-301 trial (see Attachment 1). This was a Phase III study to compare the clinical benefit of Abiraterone acetate plus prednisone with placebo plus prednisone in patients with mCRPC in the post-chemotherapy setting. It was proposed to include CTC testing as part of the qualification process for a CTC-based biomarker. Following Dr. Scher's interactions with both CDER and CDRH, the design of the CTC biomarker qualification component of this study was agreed on. The biomarker question was embedded as a secondary objective, with a formal charge to develop a CTC-based biomarker alone or in combination with LDH. The trial met the primary overall survival endpoint leading to drug approval – the first drug showing definitive life prolonging efficacy in mCRPC.

The biomarker panel using CTC count and lactate dehydrogenase (LDH) level was shown to satisfy the four Prentice criteria for individual-level surrogacy.[37] The Prentice criteria are a set

of conditions that specify the conditional independence of the impact of a treatment on the true endpoint, given the surrogate endpoint. Although the Prentice criteria were met, following several additional meetings with the FDA's Biomarker Qualification Review Team (BQRT), it was decided to remove LDH, and to move forward with CTCs as a single component response biomarker instead of as an OS surrogate marker. A Letter of Support, dated September 25, 2015, was issued for "the further development of CTC enumeration as a potential disease activity biomarker for use in clinical trials for metastatic castration-resistant prostate cancer (mCRPC)."[19]

Later, a statistical framework was proposed to use data from five randomized clinical trials (NCT01193244, NCT00638690, NCT00974311, NCT01605227, and NCT01193257)[11-15] to support the Context of Use (COU) "Qualifying CTCs to serve as an endpoint for early evaluation of drug product activity in clinical trials."

The subsequent analysis by Heller et. al. into CTCs as an endpoint demonstrated CTC counts are the strongest measure associated with longer survival following therapy; an unambiguous clinical benefit to patients.[4] Several ways of defining what constitutes a response based on CTC counts were compared to each other, and traditional measures typically followed in clinical trials. Heller et. al.[4] compared comparable percent changes in PSA levels and CTC counts. In addition, they compared two other response measures they called CTC Conversion, and CTC0. All of these measures compared the change seen between the Baseline measurements of the marker, with that seen after 12 weeks of therapy. A positive response as measured by "CTC Conversion" was defined as patients who had $\geq$5 CTCs at baseline but had $\leq$4 CTCs after 12 weeks of therapy. The CTC0 concept was introduced as defining a positive response to therapy as a Patient who had $\geq$1 CTCs at baseline but had 0 CTCs after 12 weeks of therapy. The performance of each of these ways of defining a positive response to therapy was compared to each other for their ability to predict patient benefit.

The power of CTC counts to predict a survival improvement was confirmed using individual patient data from >3,000 men who were evaluable for CTC response.[4] This assessment was consistent across five phase III randomized registration trials powered on survival in which the CTC biomarker question was embedded prospectively (Table 2).

Each of the response measures considered in the individual trials was evaluated independent of the specific intervention under study in the trial and the treatment arm on which a patient was enrolled. The interventions included placebo, prednisone monotherapy, three next-generation androgen receptor-signaling inhibitors administered alone or in combination with prednisone, and a signaling inhibitor. The trials were conducted in three distinct populations of patients with mCRPC patients undergoing first, second and third line treatments, who had been previously exposed to either no, one (docetaxel), or two (docetaxel and an approved androgen receptor signaling inhibitor) life-prolonging therapies, respectively. CTCs were the best predictors of benefit, and the CTC0 definition out performed all other measure of response. Taken together, the consistency of the outcomes across treatments and disease states shows the generalizability of the biomarker and further supports the CTC0 endpoint as an indicator of clinical benefit for use in mCRPC clinical trials. Use of this biomarker will enable efficient clinical trials and more quickly identify clinical benefit for patients. This will help speed the development, and introduction of new therapies.

Table 2. Summary of the five Phase III randomized trials used in the analysis for this submission.

Trials	ELM-PC-4[9]	COU-AA-301[10]	ELM-PC-5[11]	AFFIRM[12]	COMET-1[13]
ClinicalTrials.gov identifier	NCT01193244	NCT00638690	NCT01193257	NCT00974311	NCT01605227
Patient population	1st line	2nd line Post Chemo	2nd line Post Chemo	2nd line Post-Chemo	2nd line Post Chemo
Experimental treatment	AR target Orteronel + prednisone	AR target Abiraterone + prednisone	AR target Orteronel+ prednisone	AR target Enzalutamide	Cabozantinib
Control treatment	Placebo + Prednisone	Placebo + prednisone	Placebo + Prednisone	Placebo	Prednisone
Number of patients	1034	1191	1099	1560	1028
Randomization Ratio (Study: Control)	2:1	2:1	2:1	1:1	2:1

IV. Biomarker Measurement Information

A. General Description of Biomarker Measurement

The FDA-cleared CELLSEARCH® CTCs referenced in this submission are characterized as EpCAM+, CK+, DAPI+, and CD45(-). Complete details of reagents and process are described in the attached Instructions For Use (Attachment 2).

7.5-mL patient blood is collected in the CELLSAVE® Preservative Tube, in which the CTCs are stable for up to 96 hours (K030596[38]). The sample is transferred to a CELLTRACKS AutoPrep tube, in which 6.5 ml of Dilution Buffer is added and mixed by inversion, and then the sample is centrifuged at 800 x g for 10 minutes. The sample is then placed on the CELLTRACKS AutoPrep System (K040077[39]). After incubation and magnetic separation with anti-EpCAM ferrofluid, the cell suspension (enriched for cells expressing EpCAM) is incubated with Staining Reagent containing phycoerythrin-labeled (PE) anti-cytokeratin monoclonal antibodies, CD45-APC, and DAPI in the presence of a permeabilization buffer. Excess fluorescent material is removed by repeated magnetic washes, and the fluorescently labeled cells are re-suspended in a cell fixative to a final volume of 425 µL and transferred to a sample cartridge.

The sample cartridge is situated inside a magnetic device, called a MagNest®, which orients the magnetically labeled cells into the same focal plane for fluorescence microscopic examination on a CELLTRACKS Analyzer II®(K050145[40]). This automated image analysis system scans the entire surface of the cartridge, acquires images, and displays to the user any event where CK-PE and DAPI fluorescence are co-located. Images are presented to the technologist in a gallery format for final classification and

enumeration. The technologist is trained to classify an object as a tumor cell when its morphological features are consistent with that of a tumor cell and it is EpCAM+, CK+, DAPI+, and CD45(-).

B. Test/Assay Information

Indicate whether the biomarker test/assay is one or more of the following:

i. Laboratory Developed Test (LDT) ○ Yes ● No

ii. Research Use Only (RUO) ○ Yes ● No

iii. FDA Cleared/Approved. ●Yes ○ No
If yes, provide 510(k)/PMA #: K031588[42] (initial clearance for metastatic breast cancer) & K073338[7] (subsequent clearance to expand indications for use to metastatic prostate cancer)

iv. If the biomarker is qualified, will the test/assay be performed in a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory?
● Yes ○ No

v. Is the biomarker test currently under review by the Center for Devices and Radiological Health or the Center for Biologics Evaluation and Research?
○ Yes ● No ○ Don't Know

vi. Is there a standard operating procedure (SOP) for sample collection and storage?
● Yes ○ No

vii. Is there a laboratory SOP for the test/assay methodology?
● Yes ○ No

C. Biomarker Measurement

With the de novo 510(k) clearance of the CellSearch system, the FDA issued a guidance on May 11, 2004, "Class II Special Controls Guidance Document: Immunomagnetic Circulating Cancer Cell Selection and Enumeration System";[41] the guidance document provided FDA recommendations on specific performance characteristics related to reproducibility, interference, limits of detection, reference interval, linearity/reportable range, recovery, and cutoff. Although there are an array of CTC tests available for research, or clinical use as Laboratory Developed Tests, no other CTC test has gained FDA clearance to date.

"The analytical performance of the assay was submitted and cleared in multiple 510(k)s and has been subsequently published.[35] The applicable 510(k)s can be found in references 7, 34, and 38 through 48. Analytical performance includes the following metrics where applicable:

i. Precision/Reproducibility
    a. System reproducibility with CellSearch CTC Control
    b. System Reproducibility with patient samples
    c. Precision study per NCCLS EP-5A
ii. Linearity/assay reportable range
iii. Detection limit

iv. Analytical specificity
v. Carryover
vi. Assay cut-off
vii. Interfering substances

Biomarker Qualification is meant to be agnostic of the technology, used to define the Biomarker. All of the data submitted in support of the CTC0 qualification in mCRPC cancer was generated on the CellSearch system. The CellSearch System is exquisitely sensitive, but is also highly selective of a population of CTCs. Therefore, the data presented is restricted not by the technology, but to the class of CTCs enriched and detected by CellSearch. The class of CTCs addressed by the data in this submission is restricted to those CTCs larger than 4 μM in size, having a cellular morphology consistent with that of an intact cell, and that cell being EpCAM+, CK+, containing a DAPI+ nucleus, and being CD45(-). This phenotype however, is a property of the tumor cell, and is independent of the technology used to isolate and enumerate it. Several technologies may be envisioned that may identify this class of CTCs accurately. However, some technologies may exclude some, or all, of this CTC phenotype; or include this class of CTCs intermixed among CTCs having other phenotypes and characteristics. In this case, it may be necessary to be able to discriminate accurately between the different classes of the CTCs isolated. Sponsors using alternate CTC technologies may need to validate equivalence to the cells detected by CellSearch; or demonstrate analytical and clinical validity for the biomarker if it is to include CTC classes not represented in this data set.

i. Quality Assurance

The assay type of the CELLSEARCH CTC Kit is that of a physiologic measurement, as it quantitatively measures a class of tumor cells circulating in blood. Pre-analytic variables have been studied, including, but not limited to, analytic specificity, cross-reacting substances, interfering substances, and storage conditions. These studies may be found in the CELLSEARCH CTC Kit 510(k).[7, 42, 43]

Samples are collected by drawing blood into a CellSave tube.[38, 45] The CellSave Instructions For Use (IFU) contains detailed instructions for sample collection, mixing, storage, and shipment.

The CELLSEARCH CTC Kit and the CELLSEARCH AutoPrep and CELLTRACKS ANALYZER II IFUs contain detailed instructions regarding the CTC enumeration assay methodology and image interpretation. Following installation of a CELLSEARCH System, users are trained by factory representatives on the use and image interpretation of the instruments and assay that includes extensive education on the theoretical background of the test, as well as hands on operation and proficiency testing. Details regarding the operation and software that comprise the CELLSEARCH System may be found in their respective 510(k) clearances.[7,34,38-48]

The manufacturer of the CELLSEARCH CTC Kit, Menarini Silicon Biosystems Inc., manufactures the CELLSEARCH product line in compliance with FDA 21 CFR 820, Quality Systems Regulation, and ISO 13485:2016 *Medical devices – Quality Management*

Systems – requirements for regulatory purposes. Menarini Silicon Biosystems Inc. is a participant in the Medical Device Single Audit Program (MDSAP) and undergoes routine third party audit for compliance certification.

ii. Quality Control

The FDA-cleared CELLSEARCH CTC test was used to enumerate the circulating tumor cells of the patients enrolled in the clinical studies referenced in this document. The CELLSEARCH Circulating Tumor Cell Control Kit (K040898)[44, 48] is intended for use as an assayed control, to be used when performing the CellSearch CTC test on the CellTracks AutoPrep System. These control cells ensure that the sample detection, and identification systems, are working as intended. In other words, the CTC Control Kit is used to verify the performance of the CELLSEARCH CTC Kit reagents, and instrument systems.

The CELLSEARCH CTC Kit contains reagents and supplies for immunomagnetic selection of rare circulating tumor cells from whole blood. The CELLTRACKS AUTOPREP System is designed to automate and standardize optimal sample preparation when using the CELLSEARCH CTC Kit. Analysis of CTCs is performed using the CELLTRACKS ANALYZER II, a semi-automated fluorescence microscope that is used to enumerate the fluorescently labeled CTCs that have been immunomagnetically captured, stained, and magnetically mounted by the CELLSEARCH AutoPrep system.

According to manufacturer's recommendations, the CELLSEARCH Circulating Tumor Cell Controls should be run each day of patient testing, or when starting a new lot of the CELLSEARCH CTC Kit. The CELLSEARCH CTC Control Kit contains single-use bottles of fixed cells from a breast carcinoma cell line (SK-BR-3). Each single use bottle of Control cells contains two populations of SK-BR-3 cells at different concentrations (low and high). The two cell populations are distinguished from each other by use of fluorescent dyes that are specific to each population. They express epithelial cell markers recognized by the antibodies in the CELLSEARCH CTC Kit and are used to control for the reproducibility of the assay and instruments. The numbers of high and low control cells are determined by the manufacturer. The average number of high and low control cells that should be found, and their respective acceptance ranges, are provided for each lot. The control sample is run on the CELLTRACKS AUTOPREP system, using the same kit or kit lot to be used for patient analysis. The control is then analyzed by the CELLTRACKS ANALYZER II and the operator determines the high and low control cell counts obtained. The full CELLSEARCH system is functioning properly when the high and low control cell counts both fall within the acceptance range provided for that lot. The detailed description of the use of the CELLSEARCH CTC Control Kit can be found in the instructions for use (see Attachment 3).

iii. Limits Sources and Quantification of Measurement Error

The system reproducibility of CTC detection and enumeration using the CELLSEARCH CTC Assay has been studied[35] and evaluated as part of the assay's 510(k) clearance.[7,42] Details of the studies can be found in the CELLSEARCH CTC Assay's Instructions for

use (See Attachment 2). The clinical research staff responsible for conducting all the studies used to support the CTC0 Biomarker submission were fully trained on the manufacturer's instructions regarding the recommended clinical practice for blood draw order, sample processing, and data interpretation. All tests were processed in CLIA certified laboratories to maximize data integrity, and minimize process and measurement variability.

CTCs captured and enumerated by the CellSearch System, when using the IVD CELLSEARCH CTC Kit reagents, have a narrowly defined phenotype (EpCAM+, CK+, DAPI+, and CD45(-) and measurement of CTCs is restricted to only those CTCs that conform to this phenotype.

D. Additional Considerations for Radiographic Biomarkers

N/A

VIII. Assessment of Benefits and Risks

CTC0 is a Response Biomarker for the early evaluation of drug product activity in mCRPC clinical trials. The stated context of use is as an early indicator of response, in mCRPC pharmaceutical clinical trials. A favorable change in CTC0 occurs early in the response, and is the biomarker that correlates best with improved OS and/or PFS outcomes. Improved OS and PFS are obvious benefits for the patients. For study sponsors, they benefit by having a rapid and reliable response biomarker in pivotal clinical studies to indicate treatment benefit and improved OS or PFS for patients with mCRPC.

A. Benefits

The proposed context of use for CTC0 is as a mCRPC clinical trial response biomarker, measured shortly after the start of treatment that can indicate a favorable response to the therapy being administered. A favorable change in the CTC0 biomarker is the strongest known early indicator that a patient is likely to derive greater OS from their current treatment; an unequivocal benefit to the patient. A reliable early indicator of a favorable response to treatment is a critical unmet need in mCRPC. For clinical trial study sponsors, CTC0 can provide early, reliable, non-invasive, and easily accessible evidence of a favorable response to therapy, and the likelihood of a new therapy to achieve improved OS in a treated population, over that of the current standard of care. This can enable faster drug development and regulatory decision making, and speed the transition of effective new therapies from the laboratory to the clinic. Early assessment of response, and smaller clinical trials, may accelerate the ability to generate the data required to support Breakthrough Designation for effective new single or combination therapies.

B. Risks

There are no negative consequences for, or harm to, any patients, if the interpretation of the biomarker measurement is mistaken within the limited context of use. The biomarker

is a single factor biomarker (CTC count only) but requires measurements at two time points, baseline and after 12 weeks of therapy. Evaluation of the response using this biomarker can only happen therefore, after a patient has been enrolled in a trial, and has received 12 weeks of therapy. The biomarker is an indicator of response and a predictor of improved outcomes. It is not a predictor of who will respond, or eligibility to receive a given therapy.

Patients will be eligible for a trial based on their clinical and treatment history. A patient who is enrolled in a trial, but is then found to have 0 CTCs at baseline, would fall into the category of patients whose baseline CTC are undetectable. We know from the Heller paper[4] that approximately 25% of patients at baseline will have 0 CTCs/7.5 mLs. Whereas the CTC0 response biomarker cannot be evaluated for these patients, these patients would still be eligible to participate in the clinical trial. For patients having 0 CTCs at baseline any positive effect of treatment would be evaluable only by changes in the late events that are traditionally measured.

It is conceivable that CTC0 could be incorporated into a study design that requires all patients have detectable CTCs at baseline in order to enroll. In this case, a finding of 0 CTCs would prevent such a patient from participation in the trial. However, this is not a mistaken interpretation of the biomarker, simply an inability to evaluate the biomarker in that patient. Furthermore, the patient would of course continue to receive standard of care, or may qualify for other clinical trials where this is not an inclusion criteria.

When conducting clinical trials, blood samples may be drawn from patients at multiple centers and shipped to a central laboratory for CTC testing. Because CTCs are fragile and can be damaged in shipment, steps must be taken to preserve their integrity. The CTC0 biomarker performance could be affected if CTCs are disrupted or destroyed in transit. If CTC samples are to be shipped, the integrity of the count, and the length of time for which that count is stable, must be validated.

C. Risk Mitigation Strategy

For all of the studies on which the CTC0 biomarker performance is based, blood was collected in CellSave tubes. CellSave tubes are validated, and FDA cleared, for use in drawing blood, and preserving CTC counts for up to 96 hours. They have been used extensively, and since 2003, have been used in many prospective, peer reviewed, clinical research, and registration trials. All clinical sites were trained in the proper use of these tubes.

Although CellSave was used exclusively in the studies used for CTC0, other cell preservation tubes are known to exist, and are commercially available. Some CTC technologies process the blood sample soon after draw, at or near by the site of draw, and then ship the processed (and stabilized CTC sample) to a central facility for analysis. Therefore, as with the CellSearch Assay technology itself, other technical solutions may be used to mitigate the risk of CTC loss during transit. Other tubes are commercially available, technologies for affixing and mounting cells to special slides before shipping

them exist, and still others may be envisioned. Importantly, whatever solution is used, it must be validated to perform with the CTC enumeration technology.

D. Conclusions

There are no negative consequences for, or harm to, any patients, if the interpretation of the biomarker measurement is mistaken within the limited context of use. A favorable response to treatment, as measured by the CTC0 biomarker, is predictive of improved OS for patients, and an indicator of a patient population's favorable or unfavorable response to therapy. For clinical trial study sponsors, CTC0 can provide early, reliable, non-invasive, easily accessible evidence of a favorable response to therapy. The early readout and possibly smaller clinical trials resulting from more patients with measurable disease, may lead to faster trials and the collection of data that may support Breakthrough Status for new mono and combination therapies.

The risk associated with transporting blood from the site of draw and the fragility of CTCs was successfully mitigated by using CellSave tubes for collecting and transporting blood. Although CellSave was used in these studies, here too other technical solutions exist to mitigate this risk.

To date, no other early biomarker in mCRPC has been investigated to the degree of rigor as has been done for CTC0. The well-validated, FDA-cleared CellSearch System and reagents has allowed definitive demonstration of the CTC0 metric as a potential early efficacy response indicator in mCRPC trials. The use of CTC0 as a validated response biomarker should accelerate both the elimination of ineffective treatments and the advance of beneficial pharmaceutical candidates in our fight against mCRPC.

IX. Evaluation of Biomarker in Data Collection:

A. Completed Pre-Clinical Information, as appropriate

The pre-clinical information of biomarker measurement has been discussed above in Section **IV C**. Pre-clinical information is also available in multiple references and 510(k) submissions.[7, 35,42]

B. Ongoing Data Collection (pre-clinical and clinical)

The establishment of CTC0 as a response biomarker is based on analysis of >3000 men with mCRPC that took part in the 5 Phase III clinical trials mentioned in Table 2. All 5 of these trials have been completed, and all follow-up outcomes data has been collected. No additional clinical data is anticipated at this time.

C. Summary of Ongoing Information Collection/Analysis Efforts

Over the years, Veridex, Janssen, and Memorial Sloan Kettering, along with their partners, have conducted multiple clinical trials where CTCs were embedded as a Secondary or Experimental end point. These studies have repeatedly shown that CTCs are useful, early predictors of OS and PFS across multiple trials, multiple cancers, and multiple drugs.[8-10, 16-18, 20-23, 36]

Early studies towards demonstrating CTC as a Qualified Biomarker in patients with mCRPC starting chemotherapy, showed that non-0 pre-treatment CTC counts below the cutoff of 5 CTC were associated with a better prognosis when compared to patients at or above the 5 CTC cutoff, however their OS and PFS where slightly worse than that for patients having a baseline CTC count of 0. An apparent foreshadowing of our finding that CTC counts are indeed a continuous variable. Importantly, post-treatment conversion from Unfavorable ($\geq$5 CTCs) to Favorable (< 5 CTCs) counts or from favorable (<5 CTCs) to Unfavorable ($\geq$ 5 CTCs) were better predictors of superior or inferior OS respectively, than was a >50% decline in post-treatment PSA.[2, 20, 37]

The conventional approach to determining whether a new biomarker adds value to current models is to establish its association with survival when combined with other known prognostic factors. The association analysis is often developed through a proportional hazards model, using the hazard ratio and P value affiliated with the new marker to establish its clinical importance. These association analyses alone, however, are not sufficient to assess the magnitude of the added value of a biomarker. Thus, we went beyond the standard association analyses by assessing whether CTC enumeration before and after treatment improves risk classification and the prediction of survival time for patients with mCRPC. To do so, we compared models that contained or excluded CTCs for their ability to discriminate and calibrate survival times.

These analyses strengthened earlier observations by showing that CTC number, analyzed as a continuous variable, was independently associated with survival and added value to historical prognostic factors, including PSA and LDH.[2,37] A model including LDH levels and baseline and post-treatment CTC, independent of discrete cutoff values was the model that was most predictive of OS.[2,37] The discrimination analysis in the COU-AA-301 marker data demonstrated that, for defining a low-risk cohort, the addition of CTC number to the risk model produced higher survival rates relative to a risk model developed without CTC enumeration. This finding was validated using an independent cohort of patients treated in the ELM-PC4 trial, where the addition to CTCs to the model showed even greater separation in the survival curves among the low-risk cohort developed with and without CTCs. In this study, we found that CTC enumeration measured at baseline and early in the treatment phase, regardless of the treatment received, provided incremental value to the clinical factors, and laboratory test results, acquired in the course of routine clinical practice.[2]

The CTC-conversion endpoint was studied prospectively in the development of abiraterone and enzalutamide in the post-chemotherapy treated phase I and phase II trials independently at Royal Marsden Hospital (UK) and The Memorial Sloan Kettering Cancer

Center (MSKCC, NY USA), which showed comparable CTC numbers with similar results.[17,18] In the phase III registration trial that led to the approval of abiraterone and prednisone, a biomarker composite of CTC count (favorable/unfavorable) with LDH (normal/abnormal), when measured at 13-weeks post-therapy initiation, satisfied all of the Prentice criteria for predicting survival.[37]

In the analyses of all previous relevant data, it was noted that only 50% of patients had unfavorable counts at baseline (≥5 CTC), limiting the number of patients for whom a CTC based response indicator endpoint could be studied. To expand the utility of CTC as a response biomarker, a newly proposed endpoint was defined - CTC0, representing a change from the detection of any (≥1) CTC pretreatment to none (0) post-therapy at week 13. Defining detectable CTC as ≥1 instead of the ≥5 used for conversion, meant the number of patients that could be evaluated using the CTC0 biomarker jumped from 50% to 75% of those enrolled in the study.[4]

Collectively, these observations formed the basis of subsequent efforts, in collaboration with CDRH, to develop CTCs as a surrogate endpoint, and specifically CTC0, as a qualified Early Response Biomarker in five phase III registration trials (COU-AA-301 [ClinicalTrials.gov identifier NCT00638690], AFFIRM [NCT00974311], ELM-PC5 [NCT01193257], ELM-PC4 [NCT01193244], and COMET-1 [NCT01605227])[11–15] which included over 5000 patients, using the weighted c-index.[4]

These five trials form the core of data supporting CTC0 as an early response biomarker. Succinct summaries of each trial can be found in Attachment 4-8. Collectively, our studies over the last 14 years have developed a body of evidence regarding the clinical importance of tumor cells in blood, and how best to capture their diagnostic value for patient benefit. Our recent analysis and published data[4], indicates that within the context of a clinical trial, CTC0 can address the critical unmet need for a response biomarker. CTC0 is the best, and most rigorously studied mCRPC biomarker, that early in the course of treatment can document a response to an experimental therapy in a way that is strongly correlated with, and predictive of, traditional measures of clinical benefit.

X. Knowledge Gaps in Biomarker Development

A. List and describe any knowledge gaps, including any assumptions, that exist in the application of the biomarker for the proposed COU

Knowledge gaps and assumptions that may influence the application of the CTC0 response biomarker are:

1) A significant knowledge gap is whether the inclusion of CTC having phenotypes and properties other than, or in addition to, being EpCAM+, CK+, Nucleated (DAPI+), and CD45 negative, add to or detract from, the predictive power of the CTC0 biomarker.

2) An underlying assumption within this analysis was that the CellSearch assay had sufficient sensitivity, and adequate precision, to meaningfully discriminate between 0 and 1 CTC per 7.5 mLs of blood in a mCRPC patient population.

The knowledge gap (1) is not directly relevant to the CTC0 biomarker, because all of the data generated is restricted to EpCAM+, CK+, CD45(-), nucleated cells of 4 μM size or larger. EpCAM(-) CTCs were not captured by the assay used in these studies. Cytokeratin negative CTCs may have been captured, if they were sufficiently EpCAM+, but they would not have been counted because they would have been CK(-). Therefore, cells having phenotypes other than as classically defined above, do not contribute to any of our CTC0 biomarker data. Furthermore, in a study of 108 men with mCRPC, and 22 women with metastatic breast cancer, where the number of both EpCAM+ and EpCAM(-), CK+, CD45(-), nucleated cells were examined, the number of EpCAM(+) cells were a statistically significant predictor of OS, but in contrast, the EpCAM(-), CK+, CD45(-), nucleated cell population from the same patients, showed no correlation with OS. Inclusion of both EpCAM+ and EpCAM(-) cells together from the same patient increased the number of patients with detectable CTCs but surprisingly revealed this "Total CTC Count" also failed to predict, or correlate with OS.[49] A similar finding was published from the same laboratory, in a small study of non-small cell lung cancer.[50] It is not known at this time if, or how, the EpCAM(-) CTC population's numbers respond between baseline and week 13.

The important working assumption (2) was that the CellSearch CTC assay could meaningfully discriminate between 0 and 1 CTC; a difference of only 1 single cell per 7.5 mLs of blood. With the FDA cleared CellSearch CTC kit in mCRPC, a patient who has 4 CTCs/7.5 mLs represent a relatively good prognosis, whereas 5 CTCs or more represents a relatively poor prognosis for that person. The difference between 4 and 5 is the same 1 cell per 7.5 mLs of blood as between 0 and 1. The FDA cleared cutoff in Breast Cancer (BC) is also between 4 and 5 cells. In Colorectal Cancer (CRC), the same CTC kit has a FDA cleared cutoff of between 2 and 3 cells/7.5 mLs of blood. The prognostic and predictive power of these tests has been challenged in numerous independent, peer reviewed studies over all three of these cancers, and have withstood the test of time. Therefore, if in 7.5 mLs of blood, the CellSearch test can successfully, and meaningfully discriminate in mCRPC and BC between 4 and 5 cells, or in the case of CRC, between 2 and 3 cells, it is difficult to argue, it could not discriminate meaningfully between 0 and 1 cell.

The demonstrated LoD of the CellSearch CTC assay is 1.2 cells/7.5 mLs[7,35,42]. This further suggest the assay is fully capable of discriminating between a true CTC count of 0 or 1 approximately 80% of the time. It was understood and accepted that there would be analytical uncertainty surrounding a cutoff of 0 and 1, but with a LoD of 1.2 cells/7.5 mLs of blood, that uncertainty would not be substantially greater than the uncertainty surrounding the well-established cutoffs of 2 and 3 or more, and particularly between that of 4 and 5 or more cells/7.5 mLs.

The assumption that the analytical uncertainty between 0 and 1 was operationally similar to that between 4 and 5 is confirmed by the analysis of Heller, et. al.[4] In that analysis the

performance of the CTC0 as a response biomarker was compared to the performance of CTC conversion (≥5 CTCs at baseline dropping to ≤4 CTCs after therapy) as a biomarker. The weighted c index between these two potential response indicators was very nearly identical, and the small difference between them was not statistically significant. If the analytical uncertainty surrounding a call of 0 or 1 were sufficiently large to affect the ability of CTC0 to accurately ascertain a response to therapy within the limited context of use, we would have expected CTC0's performance to compare poorly against that of CTC conversion. If the CTC0 cutoff of ≥1 mistakenly placed too many patients into the wrong Favorable versus Unfavorable response categories compared to the extensively validated cutoff of ≥5, then CTC0 should have had a lower and possibly statistically significant difference in its weighted c index compared to that of CTC Conversion. In contrast however; CTC0's weighted c index was higher, albeit not statistically significantly higher, than that of CTC conversion.[4]

B. List and describe the approach/tools you propose to use to fill in the above-named gaps when evidence is unknown or uncertain, (i.e., statistical measures and models, meta-analysis from other clinical trials).

The inclusion of CTC phenotypes other than EpCAM+, CK+, CD45(-), nucleated cells is not relevant to the instant submission. "Total CTC" counts, or CTCs having other phenotypes from those classically defined as EpCAM(+), CK(+) and CD45(-) are not represented in any of the response biomarker data collected. Their clinical relevance, although a subject of much speculation, has never been conclusively established in liquid biopsies of mCRPC. No additional studies related to other CTC phenotypes are planned for CTC0 at this time.

We believe 16 years of clinical experience in BC, CRC and mCRPC, the extensively validated performance, and unprecedented LoD of the FDA cleared CellSearch CTC kit speaks convincingly to the analytical performance of the assay at extremely low target cell numbers. Within the limited context of use, the analysis performed by Heller et. al.[4] firmly establishes the performance of the assay and thereby CTC0, as the best and strongest early response biomarker in mCRPC that can be associated with classic measures of clinical benefit. We conclude therefore, our assumption regarding the acceptable performance of the assay, even at the threshold of 0 or 1 CTCs per 7.5 mLs of blood, has been operationally confirmed.

C. Describe the status of other work currently underway and planned for the future toward qualification of this biomarker for the proposed context of use.

All 5 of the Phase 3 pharmaceutical clinical trials that form the basis of our CTC0 analysis have been completed. No other trials are currently on going, neither are any anticipated at this time in pursuit of qualifying this biomarker for the proposed context of use.

Attachments

Attachment 1: Summary overview of CTC Biomarker interactions with FDA.
All associated documents may be made upon request.

Date	Parties Involved	Activity / Action
06Nov2007	MSKCC, FDA, JDx	Dr. Scher presented "Toward Qualification of Circulating Tumor Cells as a biomarker in Prostate Cancer"
28Apr2009	Veridex, MSK to FDA	Letter to request a meeting with FDA (Dr. Goodsaid) to provide update on the status of Cougar study, and present new AFFIRM trial.
21Aug2009	Veridex, FDA	Email exchanges to schedule a teleconference premeeting in advance of video conference on 27Aug2009
25Aug2009	Veridex, FDA	Letter to frame the topics discussed during videoconference on 27Aug2009. The proposed COU is "Circulating Tumor Cell enumeration as a biomarker in CRPC patients in the context of use as an efficacy-response biomarker in trials of AR signaling inhibitors."
27Aug2009	Veridex, MSK	Draft briefing documents.
01Dec2009	Veridex, MSK to FDA	Submitted briefing document to FDA to request a meeting
22Mar2010	FDA to Veridex, MSK	Comments to Dec2009 proposal
27Apr2010	FDA to Veridex, MSK	Comments to Dec2009 proposal
07May2010	Veridex, MSK, Medivation	BQRT face-to-face meeting regarding a proposed clinical study plan for an efficacy response biomarker using CTC.
08Nov2013	JDx, MSK	Submittal of Briefing document - request for BQRT Advice "Submission for CTC as an efficacy-response biomarker in CRPC"
11Feb2014	FDA to JDx, MSK	FDA Comments Regarding the Memorial Sloan-Kettering Cancer Center/ Medivation Inc./Janssen Diagnostics LLC Briefing Document, “Use of Circulating Tumor Cells as an Efficacy Response Biomarker in Castration-Resistant Prostate Cancer Biomarkers” submitted to the Biomarker Qualification Program submitted 13Nov2013.
18Feb2014	FDA, JDx, MSK	FTF Meeting to discuss proposal of statistical analyses
08Aug2014	FDA Qualification Review Team, JDx	Request for Qualification Review Team Advice: CTC as an efficacy-response biomarker in castration-resistant prostate cancer
06Nov2014	FDA Qualification Review Team, JDx	FDA provided written premeeting comments for Biomarker Qualification Teleconference
14Nov2014		Teleconference to discuss COU, clarifications, and LDH.

	FDA Qualification Review Team, JDx, MSKCC	
22Jun2015	FDA, JDx	Initial Request for Letter for Support
25Sep2015	FDA, JDx	Biomarker Letter of Support
05Apr2016	FDA, JDx	JDx submitted "Request for feedback on statistical analysis framework" for "Qualifying CTCs to serve as an endpoint for early evaluation of drug product activity in clinical trials."
03Jun2016	FDA, JDx	Initial comments on the statistical analysis framework: "In general, your proposed statistical analysis framework is acceptable for evaluation of the limited context of use (COU) for Circulating Tumor Cells (CTC) as a biomarker endpoint for early evaluation of drug product activity in clinical trials for the metastatic castration resistant prostate cancer (mCRPC) patient population. Please note that this updated COU does not address the predictive accuracy of a CTC derived short-term response biomarker for survival time in the specified patient population. We await submission of your detailed statistical plan and may have more comments once you provide this to us."
10Jun2016	FDA, JDx	Confirmation of July 20 face-to-face meeting. Explanation of predictive accuracy.
20Jul2016	FDA, JDx	Face to face meeting with FDA was not held due to scheduling challenges.
08Jun2017	FDA, JDx	FDA to JDx, "Process for Qualification of Drug Development Tools under New FD&C Act Section 507. DDTBMQ000006.
26Jun2017	FDA, JDx	JDx to FDA, acknowledge the communication and consent to follow Section 507.
27Jun2017	FDA, JDx	JDx to FDA, accepts the opportunity for meeting with BQ program staff to clarify the submission process.
10Jul2017	FDA, JDx	FDA to JDx, confirms telecon for August 1, 2017.
01Aug2017	FDA, JDx, MSKCC	Telecon to discuss the status of the Janssen/MSK Biomarker Qualification submission in light of the changes to the FDA biomarker qualification program as a result of the 21st Century Cures Act.
04Aug2017	FDA, JDx, MSKCC	JDx provides FDA with Clinical Summary of 5 studies, also Heller/Scher/McCormack's abstract at ASCO in 2017.
07Aug2017	FDA, JDx	FDA acknowledges receipt of information.
11Aug2017	FDA, JDx	FDA sends announcement on BQP update.
22Sep2017	FDA, JDx	FDA informs updated contact information following JDx's attempt to follow up on next step.

1Nov2017	JDx	Prepared draft Biomarker Qualification Submission (dated in May 2017) based on previous program guidance.
28Nov2017	FDA, JDx	JDx requests a status update from new BQ director. Requests possibility for meeting
20Dec2017	FDA, JDx	Sarah left VM for Beth Walton about the CTC BQ project.
22Dec2017	FDA, JDx	FDA confirms 8/7 amendment documents were received, intends to contact JDx with a meeting date.
04Jan2018	FDA, JDx	FDA informs JDx of a planned outreach communication to biomarker submitters.
15Mar2018	FDA, JDx	Deb received FDA email requesting for project status update using new template.
17Jul2018	FDA, JDx	Sarah had a phone call with Beth Walton, FDA BQ project manager, to inform her that Janssen would no longer sponsor the project and to discuss how best to transfer the project. Beth Walton asked that Janssen write a transfer letter to inform FDA of the new sponsorship for the project
9Oct2018	FDA, JDx, MSKCC, MSBUS	Sarah formally notified Beth Walton of the sponsorship transition, and was acknowledged by FDA.

Attachment 2: IFU for the CellSearch CTC Kit

e631600006_EN_cell search CTC kit IFU.p

Attachment 3: IFU for the CellSearch CTC Control Kit

LBL-0022_EN_cellsea rch CTC control kit I

Attachment 4: Clinical Trial NCT00638690

NCT00638690_COU-AA-301_deBono J_Al

Attachment 5: Clinical Trial NCT00974311

NCT00974311_AFFI
RM_Scher H_mCRPC

Attachment 6: Clinical Trial NCT01193257

NCT01193257_ELM-
PC-5_Fizazi K_Ortero

Attachment 7: Clinical Trial NCT01193244

NCT01193244_ELM-
PC 4_Saad F_Orteroı

Attachment 8: Clinical Trial NCT01605227

NCT01605227_COM
ET-1_Smith M_Cabo:

Attachment 9: Heller et. al. (2018). Analysis of CTCs over the 5 Phase III Clinical Trials

Heller_2018_CTC
Response Prolonge

APPENDIX I. References

1. Robinson D, Van Allen EM, Wu Y-M, et al. Integrative clinical genomics of advanced prostate cancer. *Cell*. 2015;161(5):1215-1228.

2. Heller G, Fizazi K, McCormack R, et al. The Added Value of Circulating Tumor Cell Enumeration to Standard Markers in Assessing Prognosis in a Metastatic Castration-Resistant Prostate Cancer Population. Clin Cancer Res Off J Am Assoc Cancer Res. 2017;23(8):1967-1973

3. Scher HI, Morris MJ, Stadler WM, et al. Trial Design and Objectives for Castration-Resistant Prostate Cancer: Updated Recommendations From the Prostate Cancer Clinical Trials Working Group 3. J Clin Oncol. 2016;34(12):1402-1418. doi:10.1200/JCO.2015.64.2702

4. Heller G, McCormack R, Kheoh T, et al. Circulating Tumor Cell Number as a Response Measure of Prolonged Survival for Metastatic Castration-Resistant Prostate Cancer: A Comparison With Prostate-Specific Antigen Across Five Randomized Phase III Clinical Trials. *J Clin Oncol Off J Am Soc Clin Oncol*. 2018;36(6):572-580. doi:10.1200/JCO.2017.75.2998

5. Paoletti C, Hayes DF. Circulating tumor cells. Adv. Exp. Med. Biol. 2016 ;882:235-238

6. Parkinson DR, Dracopoli N, Pett BG, et al : Considerations in the development of circulating tumor cell technology for clinical use. J Transl. Med. 2012 ;10 :138.

7. 510(k) Premarket Notification, K073338, CELLSEARCH CIRCULATING TUMOR CELL KIT. FDA 510(k) Premarket Notification. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm?ID=K073338. Accessed November 12, 2018.

8. Danila DC, Heller G, Gignac GA, et al: Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin. Cancer Res. 2007;13:7053-7058.

9. Hayes DF, Cristofanilli M, Budd GT, et al. Circulating Tumor Cells at Each Follow-up Time Point during Therapy of Metastatic Breast Cancer Patients Predict Progression-Free and Overall Survival. Clin Cancer Res. 2006;12(14):4218-4224. doi:10.1158/1078-0432.CCR-05-2821

10. Goodman OB Jr, Symanowski JT, Loudyi A, et. al.: Circulating tumor cells as a predictive biomarker in patients with hormone-sensitive prostate cancer. Clin. Genitourin. Can. 2011;9:31-38

11. Saad F, Fizazi K, Jinga V, et al. Orteronel plus prednisone in patients with chemotherapy-naive metastatic castration-resistant prostate cancer (ELM-PC 4): a double-blind, multicentre, phase 3, randomised, placebo-controlled trial. Lancet Oncol. 2015;16(3):338-348.

12. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and Increased Survival in Metastatic Prostate Cancer. N Engl J Med. 2011;364(21):1995-2005. doi:10.1056/NEJMoa1014618

13. Fizazi K, Jones R, Oudard S, et al. Phase III, Randomized, Double-Blind, Multicenter Trial Comparing Orteronel (TAK-700) Plus Prednisone With Placebo Plus Prednisone in Patients With Metastatic Castration-Resistant Prostate Cancer That Has Progressed During or After Docetaxel-Based Therapy: ELM-PC 5. J Clin Oncol. 2015;33(7):723-731. doi:10.1200/JCO.2014.56.5119

14. Scher HI, Fizazi K, Saad F, et al. Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy. N Engl J Med. 2012;367(13):1187-1197. doi:10.1056/NEJMoa1207506

15. Smith M, De Bono J, Sternberg C, et al. Phase III Study of Cabozantinib in Previously Treated Metastatic Castration-Resistant Prostate Cancer: COMET-1. J Clin Oncol. 2016;34(25):3005-3013. doi:10.1200/JCO.2015.65.5597

16. Danila DC, Morris MJ, de Bono JS, et. al: Phase II multicenter study of abiraterone acetate plus prednisone therapy in patients with docetaxel-treated castration-resistant prostate cancer. J Clin. Oncol. 2010;28:1496-1510.

17. Reid AHM, Attard G, Danila DC, et al. Significant and Sustained Antitumor Activity in Post-Docetaxel, Castration-Resistant Prostate Cancer With the CYP17 Inhibitor Abiraterone Acetate. J Clin Oncol. 2010;28(9):1489-1495. doi:10.1200/JCO.2009.24.6819

18. Scher HI, Beer TM, Higano CS, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. *Lancet*. 2010;375(9724):1437-1446. doi:10.1016/S0140-6736(10)60172-9

19. Woodcock J. Biomarker Letter of Support. September 2015. https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/UCM605359.pdf. Accessed November 12, 2018.

20. Bono JS de, Scher HI, Montgomery RB, et al. Circulating Tumor Cells Predict Survival Benefit from Treatment in Metastatic Castration-Resistant Prostate Cancer. *Clin Cancer Res*. 2008;14(19):6302-6309. doi:10.1158/1078-0432.CCR-08-0872

21. Scher HI, Jia X, de Bono JS, et al. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. *Lancet Oncol*. 2009;10(3):233-239. doi:10.1016/S1470-2045(08)70340-1

22. Cohen SJ, Punt CJA, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008;26(19):3213-3221.

23. Cohen SJ, Punt CJA, Iannotti N, et al. Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol Off J Eur Soc Med Oncol*. 2009;20(7):1223-1229.

24. Scher HI, Morris MJ, Kelly WK, Schwartz LH, Heller G. Prostate Cancer Clinical Trial End Points: "RECIST"ing a Step Backwards. *Clin Cancer Res*. 2005;11(14):5223-5232. doi:10.1158/1078-0432.CCR-05-0109

25. Newling D. Issues with the Use of Prostate-Specific Antigen as a Surrogate End Point in Hormone-Resistant Prostate Cancer. Eur Urol. 2009;8:13-19.

26. Berthold DR, Pond GR, Roessner M, et al. Treatment of hormone-refractory prostate cancer with docetaxel or mitoxantrone: relationships between prostate-specific antigen, pain, and quality of life response and survival in the TAX-327 study. Clin Cancer Res Off J Am Assoc Cancer Res. 2008;14(9):2763-2767.

27. Madan RA, Pal SK, Sartor O, Dahut WL. Overcoming chemotherapy resistance in prostate cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2011;17(12):3892-3902.

28. Fleming MT, Morris MJ, Heller G, Scher HI. Post-therapy changes in PSA as an outcome measure in prostate cancer clinical trials. Nat Clin Pract Oncol. 2006;3(12):658-667.

29. Gulley JL, Drake CG. Immunotherapy for prostate cancer: recent advances, lessons learned, and areas for further research. Clin Cancer Res Off J Am Assoc Cancer Res. 2011;17(12):3884-3891.

30. Bubley GJ, Carducci M, Dahut W, et al. Eligibility and Response Guidelines for Phase II Clinical Trials in Androgen-Independent Prostate Cancer: Recommendations From the Prostate-Specific Antigen Working Group. J Clin Oncol. 1999;17(11):3461-3467. doi:10.1200/JCO.1999.17.11.3461

31. Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol Off J Am Soc Clin Oncol. 2008;26(7):1148-1159.

32. Valastyan S, Weinberg RA. Tumor Metastasis: Molecular Insights and Evolving Paradigms. *Cell*. 2011;147(2):275-292. doi:10.1016/j.cell.2011.09.024

33. Miller MC, Doyle GV, Terstappen LWMM. Significance of Circulating Tumor Cells Detected by the CellSearch System in Patients with Metastatic Breast Colorectal and Prostate Cancer. *J Oncol*. 2010;2010:617421. doi:10.1155/2010/617421

34. Device Classification under Section 513(f)(2)(de novo), K031588, CELLSEARCH EPITHELIAL CELL ENRICHMENT KIT; ELLSPOTTER SYSTEM.

https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/denovo.cfm?ID=DEN040001. Accessed May 23, 2019

35. Allard WJ, Matera J, Miller MC, et. al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with non-malignant diseases. Clin. Cancer Res. 2004;10:6897-6904

36. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer. *N Engl J Med*. 2004;351(8):781-791. doi:10.1056/NEJMoa040766

37. Scher HI, Heller G, Molina A, et al. Circulating Tumor Cell Biomarker Panel As an Individual-Level Surrogate for Survival in Metastatic Castration-Resistant Prostate Cancer. *J Clin Oncol*. 2015;33(12):1348-1355. doi:10.1200/JCO.2014.55.3487

38. 510(k) Premarket Notification, K030596, CELLSAVE PRESERVATIVE TUBE. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=K030596. Accessed April 2, 2019.

39. 510(k) Premarket Notification, K040077, CELLTRACKS AUTOPREP SYSTEM. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=K040077. Accessed May 23, 2019.

40. 510(k) Premarket Notification, K050145, CELLTRACKS ANALYZER II. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=K050145. Accessed May 23, 2019

41. Health C for D and R. Class II Special Controls Guidance Document: Immunomagnetic Circulating Cancer Cell Selection and Enumeration System - Guidance for Industry and FDA Staff. /regulatory-information/search-fda-guidance-documents/class-ii-special-controls-guidance-document-immunomagnetic-circulating-cancer-cell-selection-and. Published February 9, 2019. Accessed May 23, 2019.

42. Device Classification under Section 513(f)(2)(de novo), K031588, CELLSEARCH EPITHELIAL CELL ENRICHMENT KIT; CELLSPOTTER SYSTEM. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/denovo.cfm?ID=DEN040001 Accessed May 23,2019

43. 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY, K031588. https://www.accessdata.fda.gov/cdrh_docs/reviews/K031588.pdf. Accessed April 2, 2019.

44. 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY, K040898. https://www.accessdata.fda.gov/cdrh_docs/reviews/K040898.pdf. Accessed April 2, 2019.

45. 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY, K030596. https://www.accessdata.fda.gov/cdrh_docs/reviews/K030596.pdf. Accessed April 2, 2019.

46. 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY, K040077. https://www.accessdata.fda.gov/cdrh_docs/reviews/K040077.pdf. Accessed April 2, 2019.

47. 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY, K050145. https://www.accessdata.fda.gov/cdrh_docs/reviews/K050145.pdf. Accessed April 2, 2019.

48. 510(k) Premarket Notification, K040898, CELLSEARCH EPITHELIAL CELL CONTROL KIT. FDA 510(k) Premarket Notification. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm?ID=K040898. Accessed November 12, 2018

49. de Wit S, et. al. EpCAM^{high} and EpCAM^{low} circulating tumor cells in metastatic prostate and breast cancer patients. Oncotarget 2018; 9(86): 35705-35716

50. de Wit S, et. al. The detection of EpCAM+ and EpCAM- circulating tumor cells. Sci. Rep. 2015; 5:12270 https://doi.org/10.1038/srep12270