TREATMENT RESISTANCE FOLLOWING ANTI-CANCER THERAPIES

(TRANSLATE)

Therapeutic Area (TA):	Oncology
Protocol Number:	A9001502

Document History

Document	Version Date	Summary of Changes and Rationale
Amendment 2	12 May 2020	Schedule of Activities updated to reflect changes to Informed Consent and Screening process. Section 3. Study design description updated to reflect changes to Informed Consent and Screening process. Figure 1 Study schema updated to reflect changes to Informed Consent and Screening process. Table 1 updated to reflect changes to permitted anti-cancer therapies and estimated cohort size. Inclusion Criterion 1c revised to allow specific axitinib combinations in order to reflect current standard of care therapy. Patient enrollment cohorts 1 and 2 combined and cohort sample size decreased by a total of 50 in order to reflect current standard of care therapy. Prior Protocol Administrative Clarification Letters (PACLs) incorporated (Section 4.1). Pre-screening and main Informed Consent processes merged in order to simplify the consent process (Section 5). Window defined for *de novo* tumor tissue biopsy laboratory shipment in order to allow operational flexibility while maintaining timely return of Next Generation Sequencing analyses (Section 5.2.1.2). Window defined for research blood draws in order to allow operational flexibility while maintaining a collection date contemporaneous with the *de novo* tumor tissue biopsy (Section 5.2.1.3).

		Clarification added regarding screening (Section 5.1), re-screening (Section 5.1.5), enrollment (Section 5.2), and follow-up (Section 5.3) activities. Clarification added regarding allowing patients to participate in other concurrent investigational treatment clinical trials, with prior Sponsor agreement (Section 5.1.2). Clarification added regarding adverse event reporting. Sample size determination re-worded for clarity. CCI [redacted] CCI [redacted] Country-Specific (France) Contrat Unique added to Appendix. Administrative updates made throughout to ensure consistent terminology.
Amendment 1	10 Jun 2019	Added country specific request for Austria to include pregnancy testing in the Schedule of Activities. Added country specific request for Austria to include exclusion criterion for pregnant/breast-feeding patients. Added Section 6.1.3 Pregnancy Testing (Specific to Austria).
Original protocol	03 Aug 2018	Not applicable (N/A).

This amendment incorporates all revisions to date, including amendments made at the request of country health authorities and IRBs/ECs.

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SCHEDULE OF ACTIVITIES

The Schedule of Activities table provides an overview of the protocol visits and procedures. Refer to STUDY PROCEDURES and ASSESSMENTS sections of the protocol for detailed information on each procedure and assessment required for compliance with the protocol.

The investigator may schedule visits (unplanned visits) in addition to those listed in the Schedule of Activities table, in order to conduct evaluations or assessments required to protect the well-being of the patient.

Visit Identifiers	Screening Day -45 to Day 1	Enrollment	
		Biospecimen Collection Day 1	Post-Biospecimen Follow-up ≤30 days after receipt of NGS results at the provider's facility
Informed Consent[1]	X		
Preliminary eligibility assessment[2]	X		
Archival tumor tissue assessment[3]	X		
Confirmation of eligibility[4]	X		
Additional medical and oncological history[5]		X	
Archival tumor tissue submission[6]		X	
Procedures			
De novo tumor biopsy[7]		X	
Research blood collection[8] a. circulating free (cf) DNA sequence analysis; b. germline DNA sequence analysis; c. translational analyses.		X	
Pregnancy test[9]	X	X	X
Clinical Assessments			
Adverse events monitoring[10]		X	X
NGS results consultation[11]			X
Other Assessments			
CCI [redacted]			C
CCI [redacted]			C
Record next anti-cancer treatment following *de novo* biopsy[14]			X

cf = circulating free; DNA=deoxyribonucleic acid; NGS = Next generation sequencing.

1. **Informed Consent**: Study consent is obtained before undergoing study-related activities and should be provided as close as possible to the day of biospecimen collection, optimally the day of, but no more than 45 days before the de novo tumor biopsy or research blood collection
2. **Preliminary Eligibility Assessment:** Confirmation of demographics, histological diagnosis, prior systemic anti-cancer therapy regimens (and discontinuation reasons, if appropriate) or radiation therapy. Patients who have started new anti-cancer therapy **prior** to performing the *de novo* tumor tissue biopsy or research blood collection are not eligible and should be discontinued from the study. Screen failures are defined as participants who consent to participate in the clinical study but do not subsequently undergo biospecimen collection (ie, *de novo* tumor biopsy and research blood collection are not performed).
3. **Archival tumor tissue assessment:** The archival tumor tissue from the most recent primary or metastatic tumor biopsy or resection is checked to confirm availability and adequacy of sample. See protocol Section 5.1.3. If the archival tumor tissue does not meet protocol requirements, the patient must be discontinued and should not proceed with any further enrolment activities; the participant is recorded as a screen failure.
4. **Confirmation of Eligibility**: Confirmation of any changes to systemic anti-cancer therapy regimens, radiation therapy or reasons for treatment discontinuation; confirmation of radiographic evidence of disease progression for the lesion being targeted for the *de novo* tumor tissue biopsy.
5. **Additional medical and oncological history**: Following Enrollment, smoking history, prior cancer surgery, duration and best overall response for each anti-cancer therapy is recorded.
6. **Archival tumor tissue submission:** If the archival tumor tissue is confirmed as meeting protocol requirements (Section 5.1.3), the patient may be considered for enrollment. The archival tumor tissue must be processed and sent to the Central Laboratory as detailed in the laboratory manual.
7. ***De novo* tumor biopsy:** Occurs only if archival tumor tissue meets protocol requirements (see Section 5.1.3). *De novo* biopsy is performed according to institutional guidelines. Pre-biopsy clinical and laboratory assessments per institutional guidelines must be completed and reviewed prior to biopsy procedure. Anti-coagulation therapy needs to be held prior to the biopsy according to institutional guidelines. The biopsy must be performed ≤45 days after the last dose of standard of care (SOC) treatment. Post progression *de novo* biopsy specimens must be shipped no later than 2 weeks after collection. Please refer to Section 5.2.1.2 and the laboratory manual for detailed instructions.
8. **Research blood collection:** Research blood collection may be performed at a separate visit (which may be a home visit, if appropriate) to the *de novo* tumor tissue biopsy but must occur ≤5 days before or ≤1 day (24 hours) after the *de novo* biopsy. Please refer to the laboratory manual for detailed instructions. See Section 8 for details. Research blood specimens must be shipped no later than one week after collection. Please refer to the laboratory manual for detailed collection, processing, storage, and shipment instructions.
9. **Pregnancy Test**: Specific only to Austria. Monthly pregnancy tests per institutional guidelines.
10. **Adverse event monitoring:** Adverse event collection will begin at the time of the *de novo* tumor biopsy and research blood collection, whichever occurs first, through and including 24 hours post-biospecimen collection.
11. **NGS results consultation:** NGS consultation will occur within 30 days of receipt of the *de novo* tumor biopsy or cfDNA NGS results at the provider's facility. These visits should preferably be conducted in person, although phone visits are permitted if the patient is unable or unwilling to travel to the facility.

CCI [redacted]

CCI [redacted]

14. **Next anti-cancer therapy decision**: Next anti-cancer medication following *de novo* tumor biopsy or cfDNA sample collection and whether impacted by the NGS results will be recorded in the appropriate CRF page.

1. INTRODUCTION AND RATIONALE

The overall objective of this study is to better understand why tumors become resistant to treatment with standard of care (SOC) therapies that target critical aspects of tumor biology. Limited availability of post-progression tumor tissue has compromised efforts to rigorously characterize the tumor genomic and microenvironmental factors that drive resistance. Understanding mechanisms of resistance is critical to developing better anti-cancer treatments and treatment combinations. This study specifically addresses the key challenges associated with studying clinical mechanisms of resistance.

Intrinsic or baseline tumor genetic heterogeneity, reflecting varying degrees of both intra-and inter-patient biologic diversity, represents a defining characteristic of cancer biology.[1] In addition, the host response to cancer further defines the tumor microenvironment within which each patient's cancer is subject to further selection, establishing distinct immune landscapes that reflect the complex tumor-immune cell interactions.[1,2] The introduction of targeted therapeutic agents that potently modulate tumor and/or immune cell biology impose additional, very specific, selection pressures that further define the evolving biology of each patient's cancer, frequently resulting in resistance following an initially beneficial treatment. Given the underlying genetic heterogeneity and the diversity of potential immune landscapes in cancer, informative analysis of mechanisms of resistance requires the application of robust platform technologies supporting immuno-genomic profiling of post-progression tumor tissue applied uniformly across a large series of patients.

1.1. Clinical Mechanisms of Resistance

Understanding clinical mechanisms of resistance has proven challenging because obtaining the required clinical tumor samples once resistance is established is difficult and the rationale for patients to undergo a *de novo* tumor biopsy after progression has been limited. As a result, studies of clinical mechanisms of resistance often derive at best from very limited number of studies or case reports, each describing results from analysis of small numbers of patients (see Section 1.1.1 - Section 1.1.4). Furthermore, these independent studies do not employ common or broad molecular profiling technologies, further limiting the utility of the information. This information gap compromises efforts to develop therapeutic agents or combinations of therapeutic agents that might overcome these mechanisms of resistance to initially effective anti-cancer therapies. Despite these limitations initial progress has been made in identification of clinical mechanisms of resistance to different therapeutic agents, and this has been complemented by studies of resistance in preclinical models, as summarized below. The development of high quality clinical Next Generation Sequencing (NGS) tests, together with the availability of a broad spectrum of targeted therapeutic agents that are approved or in clinical development, provides additional rationale to pursue a *de novo* tumor biopsy for patients in whom a biopsy procedure can be safely performed (see Section 1.2). Broad and deep genomic characterization by NGS in larger cohorts increases the probability of identifying clinically relevant resistance mechanisms.

1.1.1. Immune Checkpoint (PD-1/PD-L1) Inhibition

Agents targeting programmed cell death-receptor 1(PD)-1, or programmed cell death-ligand 1 (PD-L1) [PD-1/-L1], have demonstrated significant clinical benefit across a wide range of different tumor types. However, in the majority of settings, responses are limited to a sub-set of patients, and, while responses are highly durable, progression following prolonged response does occur. These phenomena speak to mechanisms of both intrinsic (also referred to as primary or inherent) and acquired (secondary) resistance, which if better understood may allow for more effective treatments.

Studies conducted over the last several years have revealed a complex series of tumor cell intrinsic and extrinsic factors that contribute to the response and resistance to anti-PD-1/-L1 targeting therapies.

1.1.1.1. The Antigenicity of the Tumor

The process of mutation, which is fundamental to the emergence and progression of cancer, also has the potential to generate unique protein sequences that are recognizable by T cells. The frequency of these sequences, called neoepitopes, is proportional to the overall frequency of mutations within a tumor, termed the tumor mutational burden (TMB). Both the number of neoepitopes, and the overall TMB of tumors are emerging as critical determinants of response to anti-PD-1/-L1 therapy in several settings.[51,52,53] Further, a recent study of non-small cell lung cancer (NSCLC) patients indicated that, while changes in the absolute TMB or number of neoepitopes did not correlate with acquired resistance to anti-PD-1, loss of neoepitopes shown to be actively engaging T-cell responses at the time of response was correlated with acquired resistance.[54] This suggests that an evolution of the tumor neoepitope landscape and the overall antigenicity of a tumor, in response to treatment with anti-PD-1/-L1, may also represent a mechanism of resistance.

1.1.1.2. Tumor Cell Intrinsic Immunogenicity

A number of cell intrinsic factors influence how effectively a tumor cell is detected and eliminated by the immune system. These factors include the surface expression of immune stimulatory or inhibitory signaling proteins, such as PD-L1, the ability to process and present antigens via the major histocompatibility complex (MHC), also termed human leukocyte antigen (HLA), and the ability to respond to inflammatory signals. Through their impact on one or more of these factors, mutations in specific genes and pathways can impact tumor cell intrinsic immunogenicity, and response to anti-PD-1/-L1.

Recent work has suggested that loss of heterozygosity (LOH) in the HLA genes can be a mechanism of immune escape during the development and evolution of a tumor.[55] In keeping with this finding, loss of HLA, or other components of the antigen processing and presentation machinery, and presence of specific HLA haplotypes with reduced affinity for the T-cell receptor (TCR) have also been associated with resistance to anti-PD-1/-L1 treatment.[56,57]

The up-regulation of many surface proteins critical to the effective recognition of tumor cells by the immune system, including MHC, is facilitated by the interferon-γ (IFN-γ) response, and mutations in genes that are critical to this response, such as janus kinase (JAK) 1 and 2 and the IFN-γ receptor, have also been associated with resistance to treatment with anti-PD-1/-L1 and anti-cytotoxic T-lymphocyte-associated protein (CTLA)-4.[58,59,60] Preclinically, studies have indicated that epigenetic, as well as mutational, changes can impact the production of key cytokines and chemokines, negatively impacting tumor cell immunogenicity[61] and as such could represent an alternative route to the development of resistance.

Mutations in key oncogenes, as well as mutations in genes associated with immune function, have also been shown to impact tumor immunogenicity and response to anti-PD-1/-L1 treatment. For example, mutations in the epithelial growth factor receptor (EGFR) gene in NSCLC have been associated with reduced immunogenicity and impaired response to anti-PD-1/-L1 treatment.[62] Increased β-catenin signaling has been associated with reduced immunogenicity in melanoma patients, and shown to reduce response to anti-PD-L1 in preclinical models.[63] Similarly loss of phosphatase and tensin homolog (PTEN) in melanoma patients has been associated with reduced T-cell infiltration and reduced tumor shrinkage in response to anti-PD-1. In contrast, mutations in BReast CAncer susceptibility gene (BRCA) 2 have been shown to be enriched among melanoma patients responding to anti-PD-1.[64]

1.1.1.3. Tumor Cell Extrinsic Immunogenicity

The signals and cells present in the microenvironment surrounding the tumor cells represent a third, and likely major, driver of response, and resistance to anti-PD-1/-L1 therapy.

The quantity and quality of the T cells responding to a tumor is likely one important factor related to both response and resistance to anti-PD-1/-L1 treatment. In keeping with this hypothesis, the overall shape of the T cell response, as measured by TCR repertoire sequencing, has been shown to relate to response to anti-PD-1/-L1 therapy in some settings[65,66] and changes in the presence of T cells specific for certain neoepitopes has been associated with acquired resistance.[54] In both NSCLC[67] and squamous cell carcinoma of the head and neck (SCCHN)[68] phenotyping of tumor infiltrating T cells has indicated that the expression of an increasing number of inhibitory cell surface receptors, such as PD-1, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) and lymphocyte activation gene-3 (LAG-3), is associated with a reduced responsiveness to ex vivo antigen stimulation, and in keeping with these findings, preclinical studies in mice have indicated that increased levels of PD-1 high T cells are associated with reduced response to anti-PD-1 treatment.[69]

Immune cells other than T cells have also been shown to impact the response to anti-PD-1/-L1. Most notably an increased level of immunosuppressive myeloid derived suppressor cells (MDSCs) has been shown to correlate to reduced likelihood of response to treatment.[70,71] In addition, broader assessment of transcriptional signatures associated with resistance to anti-PD-1 treatment in melanoma patients has identified an innate anti-PD-1 resistance (IPRES) signature, characterized by genes associated with mesenchymal transition, angiogenesis, hypoxia and wound healing.[64] A similar transcriptomic study of

multiple, differentially responsive lesions within a single patient also identified a signature enriched for genes associated with mesenchymal transition.[72]

Finally, it is worth noting that in addition to the factors described above, the treatment regimen received prior to anti-PD-1 treatment can significantly alter the immunogenicity of a tumor. This is highlighted in recent studies of melanoma patients, in which tumors demonstrating acquired resistance to mitogen-activated protein kinase (MAPK)/BRAF directed therapies also demonstrated a decrease in T cell infiltration, an increase in T-cell exhaustion and a potential shift toward a suppressive, M2 macrophage driven inflammatory microenvironment.[73] In keeping with these findings a separate study indicated that patient outcomes following treatment with the anti-CTLA-4 ipilimumab were poorer in patients who had initially progressed on previous BRAF targeted therapies prior to ipilimumab, as compared to those who had not received these therapies.[74]

1.1.2. Cell Cycle and CDK4/6 Inhibition

The treatment of women with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic breast cancer has been significantly improved by combining a cell cycle inhibitor (eg, palbociclib, abemaciclib, ribociclib) that blocks the function of both cyclin-dependent kinase (CDK) 4 and the redundant CDK6 with an aromatase inhibitor (AI) or fulvestrant.[3] CDK4 or CDK6 complexed with cyclin D1 (CCND1) phosphorylates the retinoblastoma gene product (Rb), releasing the E2F and DP transcription factors that regulate the expression of genes required for entry into the S phase of the cell cycle. CDK4/6 kinase activity and progression through the G1 phase are negatively regulated by CDK4/6 interacting protein-kinase inhibitory protein (Cip-Kip) and by the inhibitor of the cyclin-dependent kinase (INK) 4 families, typified by cyclin-dependent kinase inhibitor 2A (CDKN2A) (gene product p16). The frequent deletion or mutation of CDKN2A in tumor cells suggests that p16 acts as a tumor suppressor. CCND1 is a direct transcriptional target of the estrogen receptor (ER)[4-7] and antiestrogen-induced growth arrest of ER-positive breast cancer cells is accompanied by decreased CCND1 expression,[8] while endocrine resistance is associated with persistent CCND1 expression and Rb phosphorylation.[9] It has been shown that inhibition of CDK4/6 prevents deoxyribonucleic acid (DNA) replication by arresting progression from G1 to S phase during cell division. Thus, interruption of this mechanism should prevent tumor cell proliferation through control of the cell cycle.

1.1.2.1. Preclinical Mechanisms of Resistance

Despite a detailed understanding of the mechanisms underlying cell cycle regulations, based in large part on data from preclinical models, our understanding of the clinical mechanisms of resistance to CDK4/6 inhibitors remains limited. Cancer cell line models have often proven to be limited in their ability to elucidate mechanisms of cell cycle regulation in patients.

Preclinical studies have indicated that increased expression of CCND1, Rb phosphorylation and decreased expression of p16 are associated with response to palbociclib. However, results from biomarker analyses in clinical trials of palbociclib (PALOMA-1, -2, and -3), which included gene expression, fluorescence in situ hybridization (FISH) for CCND1 and

CDKN2A and immunohistochemistry (IHC) for CCND1, p16, and Rb, showed that patients benefitted from palbociclib plus endocrine therapy regardless of differences in the expression of these biomarkers.[10,11]

Additional preclinical studies indicated that in a panel of breast cancer cell lines intact Rb signaling is needed for palbociclib to induce its cytostatic effect, and in ex vivo cultures of human breast tumors Rb loss was associated with resistance to palbociclib.[12] However, although retinoblastoma gene (RB1) mutations are detected in tumors from patients treated with CDK4/6 inhibitors, they are rare and therefore unlikely to represent a dominant response determinant for CDK4/6 inhibition.[13]

Further, preclinical studies showed that sensitivity to CDK4/6 blockade could be mediated by abundant expression of both cyclin D3 and CDK6.[14] However, analysis of Cyclin D3 (CCND3) and CDK6 gene expression in clinical studies of palbociclib (PALOMA-2, -3) demonstrated the efficacy of palbociclib in combination with endocrine therapy did not correlate with expression of these genes.[11,15]

In addition to intrinsic and acquired resistance, rapid adaptation to resist drug pressure, typically by mechanisms other than genetic alterations, has been observed in various preclinical systems treated with different therapeutic agents. Preclinical studies assessing mechanisms of early adaptation and secondary resistance of breast cancer cells to palbociclib treatment highlighted a potential role for either phosphoinositide 3-kinase (PI3K) signaling or CDK2 signaling, which fueled cell-cycle progression despite successful CDK4/6 inhibition.[16] Also, potential secondary resistance was found to be mediated by acquired cyclin E1 gene (CCNE1) amplification. Cyclin E1 functions as an obligate partner complexed with CDK2 to activate its kinase activity which appears to be able to compensate for CDK4/6 inhibition in certain contexts. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Long-term exposure of ER-positive breast cancer cells to CDK4/6 blocking agents resulted in the emergence of acquired resistance after an initial period of cell-cycle arrest; this was mediated by the acquired CDK6 gene amplification and the subsequent overexpression, resulting in therapeutic resistance to abemaciclib treatment. Interestingly, the acquired CDK6 amplification resulted also in reduced ER/progesterone receptor (PgR) expression, and thus reduced sensitivity to endocrine treatment.[17] These results indicated that CDK6 amplification/overexpression could mediate secondary resistance in patients with hormone receptor-positive, HER2-negative breast cancer receiving combined ER-CDK4/6 blockade.[18]

The recent review by Klein et al[79] suggested that tumor cells upon CDK4/6 inhibition could lead to either quiescence or senescence. Such changing in state of cells may depend on the down regulation of MDM2, redistribution of ATRX, and repression of HRAS. In addition, although changes in metabolism after CDK4/6 inhibition are likely to be context specific, cellular metabolism could also lead to non-Rb targets of CDK4/6 in the metabolic machinery including AMPKa2 and GCN5 and/or Rb-dependent manner in the altering glycolytic and oxidative metabolism. Moreover, in preclinical models, both abemaciclib and palbociclib could induce growth arrest and upregulate antigen processing and presentation in tumor cells, changing their tumor microenvironment.

1.1.2.2. Clinical Mechanisms of Resistance

An increasing body of preclinical and early clinical evidence suggests that cyclin E1 expression is a marker of resistance to CDK4/6 inhibition. High cyclin E1 expression correlates with resistance to CDK4/6 inhibitor treatment in cell line models of breast cancer and ovarian cancer. In triple negative breast cancer cell lines resistant to CDK4/6 inhibition, cyclin E1 expression remains high directly after mitosis, bypassing the restriction point at which CDK4/6 has traditionally been viewed as being required for G1 transition. Upregulation of cyclin E1 expression promoted sufficient CDK2 activity to initiate retinoblastoma phosphorylation thereby bypassing CDK4/6 inhibition. Limited biomarker work from pre-operative palbociclib clinical trials supports cyclin E1 as a biomarker identifying ER-positive, HER2-negative breast cancers that are resistant to CDK4/6 inhibition. In the NeoPalAna neo-adjuvant study, breast cancers that were intrinsically resistant to CDK4/6 had high levels of CCNE1.[19] The baseline gene expression patterns of CDK4/6 inhibitor treatment in naïve or refractory HR+, HER2- advanced breast cancer in the phase Ib study of ribociclib plus everolimus plus exemestane showed that higher levels of CCNE1 expression were observed in patients with progressive disease as compared to those with stable disease.[20] Further, gene expression analysis of baseline breast cancer tissues in the palbociclib PALOMA-3 trial showed that high expression of CCNE1, along with high expression of pathways of E2F activation, predicted for lower benefit from palbociclib.[15] Cancers with high CCNE1 expression were more likely to progress early on treatment. In contrast, there is no evidence that either ER expression or luminal subtype predicts for benefit from palbociclib. These data suggest that the phenotype observed in PALOMA-3 may be present in primary breast cancer, although progression on prior endocrine therapy may conceivably effect CCNE1 expression. Future research will be required to assess to what extent the acquisition of resistance to endocrine therapy could amplify cyclin E1 expression, and also identify the cellular processes that allow cyclin E1 expression to become de-coupled from the requirement for prior CDK4/6 activation.[21]

Using driver mutation targeted sequencing, a longitudinal circulating tumor DNA analysis in 193 pairs of baseline and end of treatment (EOT) plasma samples of PALOMA-3 was conducted. The results show that breast cancer driver mutation landscapes after treatment with either palbociclib + fulvestrant or with fulvestrant alone are largely similar, with acquired PIK3CA and estrogen receptor 1 (ESR1) Y537S mutations likely contributing to fulvestrant resistance. Acquired RB1 mutations are selected, albeit very infrequently, by palbociclib + fulvestrant.[22] These findings demonstrate the critical need to understand the genomic profile of each patient's tumor both before and after treatment in order to decipher potential resistance mechanisms.

1.1.3. Androgen Receptor

Second generation androgen receptor (AR) antagonists (eg, enzalutamide, abiraterone) have demonstrated significant clinical activity in metastatic castration resistant prostate cancer (CRPC), both in the first and second line settings. While responses are observed in the majority of patients treated not all patients respond and many patients eventually relapse. Thus, there is a clear need to identify biomarkers that distinguish responders from non-responders and to identify mechanisms driving acquired resistance to therapeutic inhibition of the androgen receptor.

Resistance to androgen receptor antagonists, such as enzalutamide (Xtandi®), remains poorly understood. However, a number of studies conducted in recent years have begun to elucidate several potential mechanisms of clinical resistance.

1.1.3.1. AR Point Mutations

There is strong evidence that point mutations within the AR increase in frequency in late stage CRPC patients from <1% to 10-20%.[23,24] Moreover, these mutations are also associated with resistance to second generation AR antagonists. For example, F876L results in altered enzalutamide binding such that enzalutamide now functions as a partial AR agonist and increases AR activity.[25] This mutation was found in a patient that no longer responded to apalutamide[25] or to enzalutamide.[26] Additional mutations in AR have been detected in patients progressing on enzalutamide, including L702H, H875Y, and T878A.[27] Importantly, ~8% of patients progressing on enzalutamide harbor multiple AR mutations,[27] emphasizing the difficulty of targeting a specific mutation as a way to combat resistance. While AR mutations driving resistance to enzalutamide may also be associated with resistance to abiraterone (Zytiga®), there are some notable differences which may be attributed to their different modes of action. For example, T878A and L702H are found in abiraterone progressing patients.[26,28,81] These mutations are particularly revealing, as they confer progesterone and, to a lesser extent, estradiol responsiveness to AR suggesting that alternative hormones may be able to bind and activate AR in place of androgens.[29,30] Importantly, CYP17A1 inhibition not only reduces AR activity by decreasing testosterone levels but it also increase progesterone levels, suggesting that these mutations may become less sensitive to CYP17A1 inhibitors, possibly suggesting that enzalutamide may benefit patients harboring a T878A or L702H mutation.[82]

1.1.3.2. AR Amplification

A second emerging mechanism also centers on AR and involves amplification or copy number gain of the gene encoding AR.[26,31] The prevalence of AR amplification has been observed to increase in a number of studies comparing pre-treated samples with tumors/plasma of patients that have progressed on enzalutamide. Interestingly, amplification of AR has been noted to occur to a greater extent in response to enzalutamide compared to abiraterone in two independent studies. In one study amplification of AR was more prevalent in patients progressing on enzalutamide (53%) compared to patients progressing on abiraterone (17%).[26] In a second study, the amplification frequency of AR was 15% at baseline but increased to 30% in patients progressing on enzalutamide; in contrast, no increase was found in patients progressing on abiraterone.[27] Due to the limited number of patients and the number of independent studies examined, it is difficult to conclude if AR amplification status represents a true mechanistic difference in resistance to these agents.

1.1.3.3. AR Splice Variants

Another well described mechanism driving resistance is increased expression of splice variants of the AR, (AR-V's). The most well described of these is AR-V7, which lacks the ligand binding domain of the AR and is constitutively active in the absence of androgens. It should be noted that there are as many as 18 different splice variants of AR[32] with AR-V7 representing the most extensively studied as there are significantly better quality reagents to

detect this variant; thus, the true role of the other variants in driving resistance to AR antagonists remains undetermined. The presence of AR-V7 transcripts in circulating tumor cells (CTCs) has been associated with poor survival[33] and with reduced response to both enzalutamide and abiraterone.[34] However, there is no consensus as to whether these variants are drivers of resistance, or are merely passengers that either mask the true mechanism or act in concert with a second driver.

1.1.3.4. Trans-differentiation as a Mechanism of AR Resistance

Emerging data now suggests that tumors can evade AR modulators by trans-differentiation into a neuroendocrine like phenotype that is characterized by mutations that include loss of RB, p53, or PTEN, and an increase in copy number of N-MYC genes.[35,36] These neuroendocrine prostate cancer (NEPC) tumors are thought to transdifferentiate from a CRPC luminal adenocarcinoma cell by divergent clonal evolution.[35,36] Additionally, NEPCs are often characterized by loss of AR expression and are hormone refractory. This is an especially aggressive form of prostate cancer with a survival of approximately 1 year. In a recent report of 114 metastatic biopsies, tumors progressing on enzalutamide or abiraterone were found to have neuroendocrine like features, including lower AR expression levels and loss of RB and p53.[35] Epigenetic reprogramming involving the histone methyltransferase enhancer of zeste homolog 2 (EZH2) has also been implicated in driving neuroendocrine prostate cancer,[37] suggesting that novel agents such as EZH2 inhibitors may have utility in this setting.

1.1.3.5. Perturbation of Cell Cycle

Emerging data suggests that mutations that increase cell cycle progression from the G1- to the S-phase of the cell cycle are implicated in resistance to enzalutamide. Patients progressing on enzalutamide have mutations in a number of genes involved in regulating this transition, including amplification of CCND1 and CCNE1,[26,27] loss of RB,[26] amplification of CDK6 and an inactivating mutation in the tumor suppressor CDKN2A.[38] All of these chromosomal aberrations converge on proteins involved in regulating the ability of cells to initiate DNA replication, suggesting that drugs such as CDK4/6 inhibitors could have utility in the resistance setting.

In addition to these clinically identified resistance mechanisms, a number of other potential resistance pathways have been proposed based on preclinical models, including loss of AR, ERG gene rearrangements, PTEN deficiency and stabilization of the AR.[39] Further, it is plausible that resistance to androgen receptor antagonists is driven by multiple genomic aberrations. For example, in a recent study 8% of patients progressing on enzalutamide were found to harbor multiple mutations within AR. It's not known if these mutations coexist in the same lesion or if different metastatic sites harbor different AR mutations. Additionally, patients can display either inherent or acquired resistance to enzalutamide that does not have chromosomal aberrations in any of the genes mentioned above or others known to be involved in AR signaling in prostate cancer. Thus, there are other mechanisms that remain unidentified.

1.1.4. DNA Damage Response and PARP Inhibition

Poly (ADP-ribose) polymerase (PARP) inhibitors exert cytotoxic effects by two mechanisms: inhibition of PARP catalytic activity and PARP trapping. Inhibition of PARP catalytic activity results in persistent single-strand DNA breaks that culminate in double-strand DNA breaks, creating a dependency on homologous recombination-mediated DNA damage repair (HR DDR) for cell survival. PARP trapping prevents a PARP inhibitor-bound PARP protein complex from readily dissociating from DNA, thereby inhibiting DNA repair, replication, and transcription, resulting in double-strand DNA breaks and cytotoxicity.[40,41] PARP inhibitors induce synthetic lethality in tumor cells bearing mutations and/or deletions in genes involved in homologous recombination or other DNA repair pathways, most notably BRCA1 and BRCA2.[42]

PARP inhibitors are highly active in patients bearing tumors with HR DDR deficiency. However there is evidence for both intrinsic and acquired resistance, with some patients not responding and others exhibiting transient responses with subsequent disease progression.[42] There has been substantial progress in recent years in elucidating the mechanisms underlying clinical resistance to PARP inhibition. However, as described above and shared by essentially all analyses of clinical mechanisms of resistance, results derive from very small series of patients, typically do not employ uniform or broad molecular profiling technologies, and may be of relevance to only a fraction of patients.

The primary clinically identified mechanism of resistance to PARP inhibition is acquired reversion mutations in BRCA1 and BRCA2. Moreover, in non-BRCA mutated tumors there is anecdotal but compelling evidence that acquired resistance to PARP inhibitors in the clinic is consistently associated with reversion mutations in other HR DDR genes, including Partner and localizer of BRCA2 (PALB2), RAD51C and RAD51D. For example, in ARIEL2 Part 1, a Phase 2 study of rucaparib in platinum-sensitive, relapsed ovarian cancer, core HR pathway genes in 12 pairs of pre-treatment and post-progression tumor biopsy samples were sequenced.[43] In 6 of 12 pre-treatment biopsies, a truncation mutation in BRCA1 (n=4), RAD51C (n=1) or RAD51D (n=1) was identified. In five of six paired post-progression biopsies from these 6 patients, one or more secondary mutations restored the open reading frame.

In another analysis, BRCA1 or BRCA2 reversion mutations were identified in circulating free DNA (cfDNA) from 4 patients with ovarian cancer (21%) and from two patients with breast cancer (40%) who were resistant or refractory to platinum-based chemotherapy or PARP inhibitors (PARPi).[44] Serial analysis of plasma from one breast cancer patient demonstrated the presence of such BRCA2 reversion mutations post carboplatin and prior to talazoparib, consistent with BRCA reversion mutations also being an established mechanism of resistance to platinum-based therapy.[77] This patient did not respond to talazoparib and displayed a greater diversity in BRCA2 reversion mutations post talazoparib.[44]

In an analysis of patients with metastatic prostate cancer,[45] 10 of 16 patients with initial response to the PARPi olaparib had cfDNA samples acquired at the time of resistance and disease progression. In 2 of 2 patients with a germline BRCA2 frameshift mutation, at the time of tumor progression additional somatic BRCA2 mutations restoring the open reading frame (ORF) were detected in cfDNA. In a patient with a somatic BRCA2 mutation,

reversion mutations restoring the ORF were detected at progression. Moreover, in a patient with a somatic PALB2 mutation, reversion mutations restoring the ORF were also detected.

These and similar analyses confirm that such reversion mutations as a mechanism of acquired resistance are not confined to BRCA1 or BRCA2, but also evident in genes encoding other proteins involved in HR DDR. Moreover, such reversion mutations can occur regardless of whether the initial mutations were germline or somatic in origin.

Multiple additional potential mechanisms of intrinsic and/or acquired resistance to PARPi have been identified based on work in preclinical models. These include:

1. Loss of DNA repair proteins 53BP1 or REV7 which results in the restoration of HR-mediated DNA repair.[46,47]

2. Loss of a number of proteins, including PARP1 itself, which are involved in maintaining replication fork stability.[48]

3. Loss of Schlafen 11 (SLFN11) in small cell lung cancer cell lines grown in culture and in vivo. SLFN11 expression was demonstrated to predict responsiveness to talazoparib, while loss of SLFN11 via Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 or short hairpin ribonucleic acid (RNA) conferred resistance to PARPi.[49]

4. Expression of BRCA1 variants. Some BRCA1 truncation mutations result in expression of a RING-less BRCA1 isoform that is associated with resistance to PARPi in cells and in vivo models.[50]

5. Loss of AT-rich interactive domain-containing protein 1A (ARID1A) or GC-Rich Promoter Binding Protein 1 (GPBP1), based on a quantitative chemotherapy genetic interaction map and limited clinical correlative analyses.[78]

6. Mutations in PARP1. A combination of genome-wide and high density CRISPR-Cas9 screens identified point mutations in PARP1 which were associated with resistance to PARPi in nonclinical models. These mutations impacted DNA binding and/or PARP trapping on DNA. Importantly, a similar mutation was observed in a patient with *de novo* resistance to olaparib.

It should be noted that these additional potential mechanisms of resistance have generally not been identified clinically and if identified, prevalence would need to be defined to better understand clinical relevance.

In summary, various potential mechanisms of resistance to therapeutics that are the subject of the current study have emerged from preclinical and clinical assessment in previous efforts, with limitations noted on patient cohort sizes, technology platforms utilized, and preclinical observations only. The current study seeks to confirm and expand upon this early body of work. All previously identified resistance mechanisms summarized above will be captured by the methodologies applied in this study, and with a larger number of patients pairing

pretreatment and post-progression biopsies for analysis. This will afford clinical and independent confirmation and improved frequency assessment of these previously identified resistance mechanisms. In addition, broader reaching approaches (eg, whole exome sequencing, whole transcriptome) will be employed to identify new/unknown potential resistance mechanisms.

1.2. Potential Risks and Possible Benefits

The primary potential risk to patients who participate in the study is associated with the *de novo* tumor biopsy performed as a study-related procedure. To be eligible for the study patients must have a tumor lesion that is considered to be safely accessible to a *de novo* biopsy performed in accord with local institutional practice standards. See Section 4 for details.

Possible benefits for patients who participate in the study derive from the NGS results from the analysis of their tumor tissue and/or blood performed by a third-party laboratory. These results are returned to the patient's physician/ health care provider who will then discuss the results with the patient. To enable sufficient time to thoroughly discuss the results and possible treatment implications, the study also supports the direct interaction between the patient and the physician by incorporating dedicated consultation time (See Schedule of Activities). Further, patients will be able to obtain a personal copy of the NGS results through their health care provider. It is recognized that results from NGS analysis of the patient's tumor tissue and/or blood may not directly impact the subsequent treatment plan. However, only by performing such analyses can low probability treatment opportunities be identified.

2. STUDY OBJECTIVES AND ENDPOINTS

Primary Objective:	**Primary Endpoint:**
• To obtain and analyze archival pre-treatment tumor samples and post-progression tumor biopsies to identify molecular markers of resistance to selected anti-cancer therapies.	• Change in the frequency of gene alterations between pre-treatment tumor samples and post-progression tumor biopsies.
Secondary Objectives:	**Secondary Endpoints:**
• To evaluate the success rate in obtaining paired archival and post-progression tumor biopsies that are adequate to meet the objectives of the study; • To determine concordance in gene alterations identified by NGS analysis of post-progression tumor tissue and blood.	• Proportion of patients with fully evaluable archival and post-progression tumor biopsy (eg, sample sufficient for all intended analyses at all measured time points); • Overall agreement rates of gene alterations between post-progression biopsy tissue and blood NGS results.

Secondary Objectives by Target Biology:	Secondary Endpoints by Target Biology:
PD-1/-L1 inhibition: • To evaluate alterations in genes encoding HLA, β2-Microglobulin, Signal transducer and activator of transcription 1(STAT1), JAK1, JAK2, IFN-γ, and interferon-gamma receptor (IFNGR) as mechanisms of resistance to PD-1/PD-L1 checkpoint blockade.	• Change in the frequency of alterations in genes encoding HLA, β2-Microglobulin, STAT1, JAK1, JAK2, IFN- γ and IFN-γR between pre-treatment archival and post-progression samples; • The frequency of alterations in genes encoding HLA, β2-Microglobulin, STAT1, JAK1, JAK2, IFN-γ and IFNGR in cfDNA.
CDK4/6 inhibition: • To evaluate alterations in the Rb gene as a mechanism of resistance to palbociclib.	• Change in the frequency of RB1 gene alterations between pre-treatment archival and post-progression samples. • The frequency of RB1 gene alterations in cfDNA.
AR inhibition: • To evaluate AR gene alterations as mechanisms of resistance to enzalutamide or abiraterone; • To evaluate changes in expression of nuclear hormone receptor genes as a mechanism of resistance to enzalutamide or abiraterone.	• Change in the frequency of AR gene alterations between pre-treatment archival and post-progression samples; • The frequency of AR gene alterations in cfDNA; • Changes in the expression of nuclear hormone receptors or related RNA signatures reflecting nuclear receptor pathway activity between pre-treatment archival and post-progression samples.
PARP inhibition: • To evaluate somatic reversion of germline BRCA (gBRCA) gene alterations as a mechanism of resistance to monotherapy PARP inhibition.	• Change in the frequency of somatic reversion alterations in gBRCA mutant allele between pre-treatment archival and post-progression samples.
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• CCI [redacted]	CCI [redacted]

3. STUDY DESIGN

This is a multi-national, multi-center study to collect and analyze archival pre-treatment tumor and post-progression biospecimens (*de novo* tumor biopsy and blood) from patients with advanced or metastatic cancer whose disease has progressed on selected SOC therapy. Approximately 500 evaluable patients with different tumor types will participate in the study. Evaluable patients are defined as the ones with both archival tumor tissue and *de novo* tumor biopsy that is sufficient to enable the intended sample analyses.

Following Informed Consent, Screening assessments are performed to confirm availability and adequacy of archival tumor tissue and to confirm preliminary eligibility criteria.

Once disease progression on SOC therapy has occurred, patients who remain eligible are enrolled in the study and undergo post-progression biospecimen collection (*de novo* tumor tissue biopsy and research blood draws) and other study-related activities. Molecular profiling, including NGS analysis, will be performed on the archival and post-progression biospecimens.

As part of study participation, results from the NGS analysis of the *de novo* post-progression biopsy or research blood specimens will be returned to the patient's physician/health care provider. Results will come from tests performed in a College of American Pathologists (CAP) CAP-accredited, Clinical Laboratory Improvements Amendments (CLIA)-certified, and good clinical practice (GCP)-compliant clinical reference third-party laboratory using a commercially available NGS test.

A study-specific follow-up consultation between the treating physician and patient will be conducted to discuss the NGS results and potential treatment options (see Schedule of Activities). All treatment decisions, including potential referral to a clinical trial, will be per the treating physician as part of the patients' clinical care and are not dictated or defined by the study. See Figure 1.

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Figure 1. Study Schema

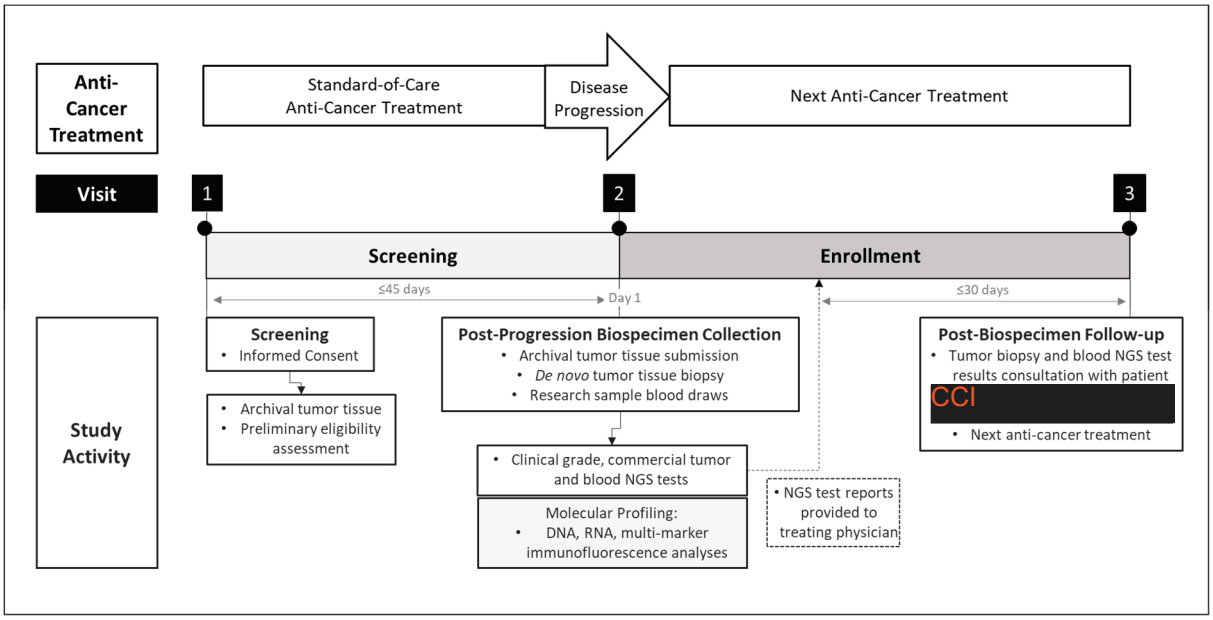

Table 1 describes the target areas of biology, disease cohorts, prior SOC therapy, indications and cohort sizes. All cohorts will enroll concurrently. Individual cohorts and cohort sizes may be adjusted based upon emerging data. Cohort size refers to evaluable patients as defined above.

Table 1. Targeted Biology and Disease Cohorts

Target Biology	Tumor Type	Most Recent Anti-Cancer Therapy	Indication	Cohort number	Estimated Cohort Size
Checkpoint Inhibition	NSCLC	Anti-PD-1/-L1 monotherapy	1st line	1	100
		Anti-PD-1/-L1 plus platinum-containing regimen	1st line	2	
	RCC with clear cell component	Anti-PD-1/-L1 monotherapy *or*	2nd line	3	100
		Doublet anti-PD-1/-L1 plus anti-CTLA-4 *or*	1st line		
		Pembrolizumab plus axitinib *or* Avelumab plus axitinib	1st line		
CDK4/6 inhibition	Breast (HR+/HER2-)	Palbociclib + Hormonal therapy	1st line	4	100
Androgen receptor Inhibition	Prostate (castrate-resistant)	Enzalutamide	Any line	5	50
		Abiraterone plus prednisone	Any line	6	50
PARP Inhibition	Breast (gBRCAm)	Olaparib or talazoparib monotherapy	gBRCAm, HER2-	7	100

CDK = Cyclin-dependent kinase; CTLA-4 = cytotoxic T-lymphocyte-associated protein 4; gBRCAm = germline mutated BRCA; HER2 = human epidermal growth factor receptor 2; NSCLC = non-small cell lung cancer; PARP = Poly (ADP-ribose) polymerase; PD-1/L1 = programmed cell death receptor 1 or programmed cell death ligand 1; RCC = renal cell carcinoma.

4. PATIENT ELIGIBILITY CRITERIA

This study can fulfill its objectives only if appropriate patients are enrolled. The following eligibility criteria are designed to select patients for whom participation in the study is considered appropriate. All relevant medical and nonmedical conditions should be taken into consideration when deciding whether a particular patient is suitable for this protocol.

4.1. Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for enrollment in the study:

1. Histological diagnosis of locally advanced (primary or recurrent) or metastatic solid tumors treated as follows (See Table 1 for cohorts):

   a. NSCLC monotherapy (Cohort 1):

      - Disease progression (PD) on 1st-line monotherapy anti-PD-1/-L1.

   b. NSCLC combination (Cohort 2):

      - PD on 1st-line anti-PD-1/-L1 plus standard doublet platinum-containing regimen; or
      - PD on 1st-line anti-PD-1/-L1 plus standard doublet platinum-containing regimen followed by continuation of single agent anti-PD-1/-L1).

   c. Renal cell carcinoma (RCC) with clear cell component (Cohort 3):

      - PD on 2nd-line monotherapy anti-PD-1/-L1; or
      - PD on 1st-line combination of doublet anti-PD-1/-L1 with anti-CTLA-4; or
      - PD on 1st-line combination of avelumab with axitinib or pembrolizumab with axitinib.

   d. HR+ HER2- adenocarcinoma of the breast (Cohort 4):

      - PD on 1st-line combination of doublet palbociclib with hormonal therapy.

   e. Castrate-resistant adenocarcinoma of the prostate (Cohort 5):

      - PD on enzalutamide monotherapy.

   f. Castrate-resistant adenocarcinoma of the prostate (Cohort 6):

      - PD on abiraterone in combination with prednisone.

g. germline mutated BRCA (gBRCAm), HER2- adenocarcinoma of the breast (Cohort 7):

- PD on a PARP inhibitor monotherapy in patients previously treated with chemotherapy in the neoadjuvant, adjuvant, or metastatic setting.

2. Radiographic evidence of PD, including the target lesion being subjected to biopsy for the study, on the most recent regimen that requires a change in anti-cancer treatment.

3. Medically stable for a biopsy procedure as defined by the local institutional guidelines.

4. A tumor lesion for the *de novo* biopsy that meets the following criteria:

a. Is safely accessible to a biopsy procedure (ie, core needle, excisional) performed in accordance with local institutional practice standards;

b. Is anticipated to yield an amount of tumor tissue sufficient to meet the objectives of the study (see Section 2);

c. Not previously irradiated;

d. Does not require decalcification for subsequent processing. (ie, no bone lesions).

5. Availability and adequacy of an archival, formalin-fixed, paraffin embedded (FFPE) tumor tissue block confirmed during Screening as containing sufficient tumor tissue to allow for sectioning of up to 25 slides each containing tissue sections that are 5 microns thick with a minimum tissue cross-sectional area of approximately 5 mm^2 and containing a minimum of approximately 20-40% tumor by ratio of tumor nuclei to benign nuclei. Fewer slides may be required for FFPE tissue blocks containing tissue that is greater than 5 mm^2 in cross-sectional area or greater than 40% tumor. Where local or regional regulations prevent submission of the archival tumor tissue block, a designated number of unstained slides each containing the indicated minimum amount of tissue must be submitted (refer to the laboratory manual for the required number of slides based on cross sectional area of tissue available in the FFPE tissue block) (See Section 5.1.3).

6. Post-progression biospecimen collection can be performed within 45 days of obtaining informed consent for the study.

7. Post-progression biospecimen collection can be performed within 45 days of the last dose of the most recent anti-cancer regimen.

8. Age ≥18 years at the time of informed consent.

9. Patients who are willing and able to comply with scheduled visits and study procedures.

10. Evidence of a personally signed and dated informed consent document indicating that the patient has been informed of all pertinent aspects of the study.

4.2. Exclusion Criteria

Patients with any of the following characteristics/conditions will not be included in the study:

1. Discontinuation of current or most recent anti-cancer therapy due to toxicity and not progressive disease.

2. Initiation of new anti-cancer therapy after PD prior to planned biopsy.

3. Any medical condition that, in the investigator's judgement, unacceptably increases risk associated with the tumor biopsy or blood sampling procedures (eg, evidence of inadequate wound healing, significant neutropenia or thrombocytopenia, recent history of clinically significant bleeding or tumor hemorrhage).

4. Cohorts 1, 2 and 3 (NSCLC monotherapy, NSCLC combination, RCC with clear cell component) only: Treatment with an anti-PD-1/-L1 agent prior to current or most recent anti-PD-1/-L1 therapy.

5. Cohort 4 (HR+ HER2- breast cancer) only: Treatment with a CDK 4/6 inhibitor prior to current or most recent CDK 4/6 inhibitor.

6. Cohort 5 (castrate-resistant prostate cancer) only: Treatment with an agent that blocks adrenal androgen synthesis (eg, abiraterone acetate) and a second-generation AR antagonist other than enzalutamide (eg, apalutamide).

7. Patients who are investigator site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the investigator, or patients who are Pfizer employees, including their family members, directly involved in the conduct of the study.

8. Other acute or chronic medical or psychiatric condition including recent (within the past year) or active suicidal ideation or behavior or laboratory abnormality that may increase the risk associated with study participation or may interfere with the interpretation of study results and, in the judgment of the Investigator, would make the patient inappropriate for entry into this study.

9. Austria only: Pregnant female patients; breastfeeding female patients.

5. STUDY PROCEDURES

5.1. Screening (Study Day -45 to Day 1)

5.1.1. Informed Consent

Informed Consent will be obtained prior to any study-related activities. Consent should be obtained as close as possible to the day of the biospecimen collection, optimally the day of, but within 45 days before the *de novo* biopsy or research blood collection (whichever is sooner).

5.1.2. Preliminary Eligibility Assessment

Prior to progressing to archival tumor tissue assessment, a preliminary eligibility assessment should be made by confirming the following:

- Demographics (Inclusion Criterion 8);
- Histological diagnosis of locally advanced or metastatic solid tumors and most recent standard of care anti-cancer therapy (Inclusion Criterion 1);
- Prior systemic anti-cancer therapy regimens or radiation therapy (Inclusion Criteria 1, 4c);
- New anti-cancer therapy which has not already been started (Exclusion Criterion 2);
- Reasons for discontinuation of current or most recent anti-cancer therapy (Exclusion Criterion 1).

Patients who have started new anti-cancer therapy **prior** to performing the *de novo* tumor tissue biopsy or research blood collection are not eligible and should be discontinued.

Screen failures are defined as participants who consent to participate in the clinical study but do not subsequently undergo biospecimen collection (ie, *de novo* tumor biopsy and research blood collection are not performed).

Patients *are permitted* to participate in interventional treatment clinical trials at the same time as participating in Study A9001502, with prior Sponsor agreement.

5.1.3. Archival Tumor Tissue Assessment

Assessment of availability and adequacy of an archival, formalin-fixed, paraffin embedded (FFPE) tumor tissue block (or equivalent slides; Inclusion Criterion 5) from the most recent primary or metastatic tumor biopsy or resection should be performed.

The archival tumor tissue assessment involves inspection by local qualified personnel of the available archival FFPE tumor tissue block and an associated H&E-stained slide to confirm the following: 1) the presence of tissue that is at least 5 mm^2 in cross-sectional area on inspection of the cut face of the FFPE block, and 2) the presence of tumor tissue containing at least 20-40% tumor by ratio of tumor nuclei to benign nuclei based on review of an H&E-stained slide previously generated from the associated FFPE tissue block.

The FFPE tissue block must contain sufficient tumor tissue to allow for sectioning of up to 25 slides each containing tissue sections that are 5 microns thick with a minimum tissue cross-sectional area of approximately 5 mm^2 and containing a minimum of approximately 20-40% the sample by ratio of tumor nuclei to benign nuclei. Fewer slides may be required for FFPE tissue blocks containing tissue that is greater than 5 mm^2 in cross-sectional area or greater than 40% tumor.

Questions regarding archival tumor tissue sample requirements should be emailed to CCI

Archived tumor tissue from cytological sampling (eg, fine needle aspiration, pleural effusion, including FFPE cell pellet material), or from biopsies of bone metastasis that require decalcification are not adequate for trial participation and should not be submitted.

If an archival FFPE tissue block cannot be provided due to documented local or institutional regulations, a designated number of unstained slides each containing tissue sections that are 5 microns thick must be submitted. Freshly cut slides should be sectioned as close as possible to the post-progression biopsy and must be shipped no more than 21 calendar days after sectioning. The number of slides required is based on the estimated cross-sectional area of the available tissue (refer to the laboratory manual for a guide defining the required number of slides that must be submitted).

5.1.4. Confirmation of Eligibility

Prior to Enrollment, medical and oncological history should be reviewed to confirm if there have been any changes to systemic anti-cancer therapy regimens or radiation therapy or reasons for treatment discontinuation.

Radiographic evidence of PD for the lesion being targeted for the *de novo* tumor tissue biopsy must also be confirmed.

The patient should be discontinued and should not undergo further study-related activities (including *de novo* tumor tissue biopsy or research blood collection) if new anti-cancer therapy has already been started following disease progression. The participant should be recorded as a screen failure in the case report form (CRF).

5.1.5. Re-Screening

Re-screening is permitted on a case-by-case basis and only with prior agreement from the study Sponsor.

5.2. Enrollment (Study Day 1)

Following confirmation of eligibility, the patient may be enrolled in the study and undergo study-related procedures.

5.2.1. Biospecimen Collection

Biospecimen collection may occur on different days; however, *de novo* tumor biopsy or research blood collection must occur within 45 days of obtaining study Informed Consent.

Day 1 of the study is the date on which the *de novo* tumor biopsy or research blood collection occurs (whichever is sooner, if occurring on different dates). Research blood collection must occur ≤5 days before, or ≤1 day (24 hours) after, the *de novo* biopsy.

5.2.1.1. Archival Tumor Tissue Submission

Eligible archival FFPE tumor tissue (or equivalent) from the most recent primary or metastatic tumor biopsy or resection (see Section 5.1.3) should be submitted for analyses. Please refer to the laboratory manual for detailed collection, processing, storage, and shipment instructions.

All submitted FFPE tissue blocks (or equivalent slides) will be returned as soon as possible after the required number of sections are obtained and analyzed.

5.2.1.2. *De Novo* Tumor Biopsy

Performing a *de novo* biopsy should be compatible with the overall patient treatment plan. Pre-biopsy clinical and laboratory assessments per institutional guidelines must be completed and reviewed prior to biopsy procedure. It is expected that these pre-biopsy assessments include but are not limited to hematology and coagulation profiles. Safe values for biopsy are per institutional standard operational procedures (SOP) and normal lab ranges. Anti-coagulation therapy needs to be held prior to the biopsy according to institutional guidelines.

De novo tumor biopsies (ie, core needle, excisional) should be conducted according to institutional guidelines. For core biopsies, a minimum 18 gauge core needle and 4 separate cores are recommended to ensure, to the greatest extent possible, that the quality and amount of tumor tissue obtained is sufficient to enable the intended sample analyses. Tumor tissue from cytologic sampling (eg, fine needle aspiration, pleural effusion, including FFPE cell pellet material) is not adequate and should not be submitted.

The *de novo* tumor biopsy should be processed locally by formalin fixation and paraffin embedding, following specific processing guidelines described in the laboratory manual. The resulting FFPE tissue block should be shipped in its entirety as part of the tumor tissue specimen collection kit (room temperature).

Any remaining post-progression biopsy material that is not used for molecular analyses as part of the study will be returned to the site where the biopsy was conducted within approximately 6 months of collection.

The post-progression *de novo* biopsy must be obtained within 45 days of the last dose of SOC treatment but prior to initiation of any subsequent anti-cancer therapy.

Post-progression *de novo* biopsy specimens must be shipped as soon as possible after the appropriate processing but no later than 2 weeks after collection to facilitate timely return of Next Generation Sequencing analyses. Please refer to the laboratory manual for detailed collection, processing, storage, and shipment instructions.

There will be no banking of tumor biopsy samples.

5.2.1.3. Research Blood Specimens

Research blood specimens will be drawn into separate blood collection tubes as follows:

a. Three 10-mL tubes of blood will be collected in specially provided tubes containing blood stabilizer (ie, Streck tubes, room temperature) for cfDNA analysis using a NGS panel approach.

b. One 8.5-mL blood specimen will be collected in a specially provided tube containing blood stabilizer (eg, Paxgene blood DNA tube; Streck tube) for germline DNA sequence analysis. Results will be used as a control to compare with tumor DNA sequence results in order to identify tumor-specific (ie, somatic) gene alterations, and/or to investigate the potential contribution of specific gene alterations to mechanisms of resistance. Remaining DNA material may also be used for translational analyses as indicated below (item c).

c. One 8.5 mL blood specimen will be collected in a specially provided tube containing blood stabilizer (eg, Paxgene blood DNA tube; Streck tube) for additional translational analyses which may include, but may not be limited to, T and/or B cell receptor sequence analysis or epigenetic profiling by NGS.

Research blood collection may be performed at a separate visit (which may be a home visit, if appropriate) to the *de novo* tumor tissue biopsy but **must occur ≤5 days before or ≤1 day (24 hours) after the *de novo* biopsy**.

The post-progression research blood specimens must be obtained within 45 days of the last dose of SOC treatment and prior to initiation of any subsequent anti-cancer therapy.

The research blood specimens must be **shipped no later than one week after collection**. Please refer to the laboratory manual for detailed collection, processing, storage, and shipment instructions.

There will be no banking of blood samples.

5.2.2. Additional Medical and Oncological History

Smoking history, prior surgery, duration and best overall response for each therapy should be recorded in the CRF.

5.2.3. Pregnancy Testing (*Specific to Austria*)

Monthly pregnancy tests will be performed per institutional guidelines.

5.3. Post-Biospecimen Collection Follow-Up (≤30 days after receipt of NGS results at the provider's facility)

5.3.1. NGS Results Consultation

NGS results will be reviewed at a post-biospecimen follow-up consultation visit, which should occur within 30 days of receipt of the *de novo* tumor biopsy or cfDNA NGS results at the provider's facility. These visits should be conducted in-person; however, phone visits are permitted if the patient is unable or unwilling to travel to the facility.

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5.3.3. Next Anti-Cancer Therapy

The patient's next anti-cancer therapy following *de novo tumor* biopsy or cfDNA sample collection, and whether it was impacted by the NGS results, will be recorded in the case report form (CRF).

5.4. Patient Withdrawal

Patients may withdraw from the study at any time at their own request, or they may be withdrawn at the discretion of the Investigator or Sponsor for safety or behavioral reasons, or the inability of the patient to comply with the protocol-required schedule of study visits or procedures.

Reasons for withdrawal from the study may include:

- Study terminated by Sponsor;
- Lost to follow-up;
- Refused further follow-up;
- Death.

If the patient withdraws from the study, and also withdraws consent for disclosure of future information, no further procedures, visits or assessments should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

Withdrawal of consent:

Patients should notify the investigator in writing of the decision to withdraw consent from post-biospecimen follow-up, whenever possible. The withdrawal of consent should be explained in detail in the medical records by the investigator, as to the reason and entered on the appropriate case report form (CRF) page.

Lost to follow-up:

All reasonable efforts must be made to locate patients to determine post-biospecimen follow-up. Lost to follow-up is defined by the inability to reach the patient after a minimum of 2 documented phone calls, faxes, or e-mails as well as lack of response by the patient to 1 registered mail letter. All attempts should be documented in the patient's medical records.

6. ASSESSMENTS

Every effort should be made to ensure that the protocol-required procedures, visits and assessments are completed as described. However, it is anticipated that from time to time there may be circumstances, outside of the control of the Investigator that may make it unfeasible to perform the procedure(s), visits or assessments within the designated times. In these cases, the Investigator will take all steps necessary to ensure the safety and well-being of the patient. The study team will be informed of these incidents in a timely fashion.

For samples being collected and shipped, detailed collection, processing, storage, and shipment instructions and contact information will be provided to the investigator site prior to initiation of the study in the study laboratory manual.

Patients should follow the approved package insert for any ongoing medications.

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6.3. Pregnancy Testing *(Specific to Austria)*

Pregnancy tests will be performed at the times listed in the Schedule of Activities. Pregnancy tests may also be repeated if requested by institutional review boards (IRBs)/ethics committees (ECs) or if required by local regulations.

7. SAFETY

7.1. Adverse Events

An AE is defined as any untoward medical occurrence and can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease, whether or not related to the patient's participation in the study.

Any AE that occurs from the time the patient undergoes the tumor biopsy and research blood collection, whichever occurs first, through and including 24 hours post tumor biopsy and blood collection must be recorded. The investigator is required to assess whether the AE may be related to the patient's participation in the study.

The investigator must pursue and obtain information adequate to determine the outcome of the AE and to assess whether it meets the criteria for classification as a research related injury requiring immediate notification to Pfizer as described below.

7.1.1. Research Related Injury

Should a patient, in the investigator's opinion, suffer a medically important research related injury caused by their participation in the study, the designated Pfizer clinician or medical monitor must be notified immediately by emailing CCI [redacted]

A medically important research related injury is any untoward medical occurrence that:

- Results in death;
- Is life-threatening (immediate risk of death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity (substantial disruption of the ability to conduct normal life functions);
- Results in congenital anomaly/birth defect.

Medical and scientific judgment is exercised in determining whether an injury is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above, the important medical event should be reported as a research related injury.

An investigator may be requested by the designated Pfizer clinician or medical monitor to obtain specific additional follow-up information in an expedited fashion. In general, this will include a description of the injury in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant treatments, vaccines, and/or illnesses must be provided. In the case of a patient death, a summary of available autopsy findings must be submitted as soon as possible to Pfizer or its designated representative.

8. DATA ANALYSIS/STATISTICAL METHODS

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a statistical analysis plan (SAP), which will be maintained by Pfizer.

This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint and/or its analysis will also be reflected in a protocol amendment.

8.1. Analysis Population

All the endpoints will be analyzed in the evaluable population. The evaluable population is defined as the patients who have both archival tumor tissue and *de novo* tumor biopsy that is sufficient to enable the intended sample analyses.

8.2. Sample Size Determination

The primary objective of this study is to obtain and analyze archival and post-progression tumor biopsies to identify molecular markers of resistance to selected standard-of-care anti-cancer therapies.

There is no standard definition of clinically meaningful change in the frequency of gene alteration between resistant and sensitive samples. Therefore, characterization of the difference of the frequency of alterations between archival and post-progression tumor in this study is considered hypothesis-generating. The precision of estimates (eg, 95% confidence intervals) are provided in Table 2 for different sample sizes (50, 75, 100) and observed changes in the frequency of gene alteration (10%, 20% and 30%). This covers the sample sizes considered for different tumor types in Table 1.

Table 2 provides general guidance about the sample size per cohort. The final sample size per cohort may change depending on the outcome of interim analysis.

Table 2. Sample Size And 95% Score Based Confidence Intervals For Change In The Frequency Of Gene Alterations After Disease Progression

Number of patients	Observed Difference	95% CI
100	10%	(6%, 17%)
100	20%	(13%, 29%)
100	30%	(22%, 40%)
75	10%	(5%, 19%)
75	20%	(13%, 30%)
75	30%	(21%, 41%)
50	10%	(2%, 21%)
50	20%	(11%, 33%)
50	30%	(19%, 44%)

8.3. Efficacy Analysis

Formal efficacy analyses will not be performed for this study.

8.4. Safety Analysis

Formal safety analyses will not be performed for this study.

8.5. Analysis of Other Endpoints

8.5.1. Analysis of Primary Endpoint

The primary endpoint is the change in the frequency of gene alterations associated with disease progression. As the goal is to compare the differences in frequency of mutation rate between pre-treatment archival and post-progression samples, a score based 95% CI for matched pairs will be calculated for each genomic alteration.[75,76] Estimates of the frequency of mutations, the difference of the frequency of mutations between archival and post-progression samples and associated 95% CIs will be generated.

8.5.2. Analysis of Secondary Endpoints

Summary statistics will be generated for the secondary endpoints. For the proportion of patients with fully evaluable archival and post-progression tumor biopsies, proportions and 95% CI will be calculated. For the overall agreement rates of gene alterations between post-progression biopsy tissue and blood NGS results, the frequency of mutations detected from post-progression biopsy tissue, blood and the corresponding 95% CIs will be generated. The concordance between both matched tissue biopsy tissue and blood samples and the 95% CI will also be calculated to evaluate the agreement.

8.5.3. Analysis of Asset Specific Secondary Endpoints

Table 3. Analysis of Asset Specific Secondary Endpoints

Asset	Endpoint	Analysis
PD-1/-L1 inhibition	• Change in the frequency of alterations in genes encoding HLA, β2-Microglobulin, STAT1, JAK1, JAK2, IFN-γ and IFNGR between pre-treatment archival and post-progression samples. • The frequency of alterations in genes encoding HLA, β2-Microglobulin, STAT1, JAK1, JAK2, IFN- γ and IFNGR in cfDNA.	• For each alteration, the estimate of the frequency and the 95% CI at pre-treatment archival and post-progression will be generated; A score based 95% CI will be generated for the difference in frequency between pre-treatment archival and post-progression samples. • For each alteration in cfDNA, the estimate of the frequency and the 95% CI will be generated.
CDK4/6 inhibition	• Change in the frequency of RB1 gene alterations between pre-treatment archival and post-progression samples. • The frequency of RB1 gene alterations in cfDNA.	• For each alteration, the estimate of the frequency and the 95% CI at pre-treatment archival and post-progression will be generated. A score based 95% CI will be generated for the difference in frequency between pre-treatment archival and post-progression samples. • For each alteration in cfDNA, the estimate of the frequency and the 95% CI will be generated.
AR inhibition	• Change in the frequency of AR gene alterations between pre-treatment archival and post-progression samples. • The frequency of AR gene alterations in cfDNA. • Changes in the expression of nuclear hormone receptors or related RNA signatures reflecting nuclear receptor pathway activity between pre-treatment archival and post-progression samples.	• For each alteration in cfDNA, the estimate of the frequency and the 95% CI at pre-treatment archival and post-progression will be generated. A score based 95% CI will be generated for the difference in frequency between pre-treatment archival and post-progression samples. • For each alteration, the estimate of the frequency and the 95% CI will be generated. • Estimates of mean expression and 95% CIs at pre-treatment archival and post progression will be generated; Estimates of the difference in expression and the 95% CI between pre-treatment archival and post-progression samples will be generated.
PARP inhibition	• Change in the frequency of somatic reversion alterations in gBRCA mutant allele between pre-treatment archival and post-progression samples.	• For each somatic reversion alteration, the estimate of the frequency and the 95% CI at pre-treatment archival and post-progression will be generated. A score based 95% CI will be generated for the difference in frequency between

Asset	Endpoint	Analysis
		pre-treatment archival and post-progression samples.

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

8.6. Interim Analysis

A formal interim analysis will not be performed for this study. However, multiple snapshots of the data will be taken for data review based on accrual (eg, at 25%, 50% and 75% of cohort and/or study accrual).

9. QUALITY CONTROL AND QUALITY ASSURANCE

Pfizer or its agent will conduct periodic monitoring visits during study conduct for studies conducted at non-Pfizer investigator sites, to ensure that the protocol and Good Clinical Practices (GCPs) are being followed. The monitors may review source documents to confirm that the data recorded on CRFs are accurate. The investigator and institution will allow Pfizer monitors/auditors or its agents and appropriate regulatory authorities direct access to source documents to perform this verification. This verification may also occur after study completion.

During study conduct and/or after study completion, the investigator site may be subject to review by the Institutional Review Board (IRB)/Ethics Committee (EC,) and/or to quality assurance audits performed by Pfizer, or companies working with or on behalf of Pfizer, and/or to inspection by appropriate regulatory authorities.

The investigator(s) will notify Pfizer or its agents immediately of any regulatory inspection notification in relation to the study. Furthermore, the investigator will cooperate with Pfizer or its agents to prepare the investigator site for the inspection and will allow Pfizer or its agent, whenever feasible, to be present during the inspection. The investigator site and

investigator will promptly resolve any discrepancies that are identified between the study data and the patient's medical records. The investigator will promptly provide copies of the inspection findings to Pfizer or its agent. Before response submission to the regulatory authorities, the investigator will provide Pfizer or its agents with an opportunity to review and comment on responses to any such findings.

For studies conducted at non-Pfizer investigator sites, it is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

10. DATA HANDLING AND RECORD KEEPING

10.1. Case Report Forms/Data Collection Tools/Electronic Data Record

As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this study.

A CRF is required and should be completed for each included patient. The completed original CRFs are the sole property of Pfizer and should not be made available in any form to third parties, except for authorized representatives of Pfizer or appropriate regulatory authorities, without written permission from Pfizer. The investigator shall ensure that the CRFs are securely stored at the study site in encrypted electronic form and will be password protected or secured in a locked room to prevent access by unauthorized third parties.

The investigator has ultimate responsibility for the collection and reporting of all clinical, safety, and laboratory data entered on the CRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring, and available when required. The CRFs must be signed by the investigator or by an authorized staff member to attest that the data contained on the CRFs are true. Any corrections to entries made in the CRFs or source documents must be dated, initialed, and explained (if necessary) and should not obscure the original entry.

In most cases the source documents are the hospital or the physician's chart. In these cases, data collected on the CRFs must match those charts.

In some cases, the CRF may also serve as the source document. In these cases, a document should be available at the investigator site and at Pfizer that clearly identifies those data that will be recorded on the CRF, and for which the CRF will stand as the source document.

10.2. Record Retention

To enable evaluations and/or inspections/audits from regulatory authorities or Pfizer, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records, eg, CRFs and hospital records), all original signed informed consent documents, copies of all CRFs, safety reporting forms, source documents, detailed records of treatment disposition, and adequate documentation of relevant correspondence (eg, letters, meeting minutes, and telephone call reports). The records should be retained by the investigator according to the International Conference on Harmonisation

(ICH) guidelines, according to local regulations, or as specified in the clinical study agreement (CSA), whichever is longer. The investigator must ensure that the records continue to be stored securely for so long as they are retained.

If the investigator becomes unable for any reason to continue to retain study records for the required period (eg, retirement, relocation), Pfizer should be prospectively notified. The study records must be transferred to a designee acceptable to Pfizer, such as another investigator, another institution, or to an independent third party arranged by Pfizer.

Investigator records must be kept for a minimum of 15 years after completion or discontinuation of the study or for longer if required by applicable local regulations.

The investigator must obtain Pfizer's written permission before disposing of any records, even if retention requirements have been met.

11. ETHICS

11.1. Institutional Review Board/Ethics Committee

It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, informed consent documents, and other relevant documents, eg, recruitment advertisements, if applicable, from the IRB/EC. All correspondence with the IRB/EC should be retained in the investigator file. Copies of IRB/EC approvals should be forwarded to Pfizer.

The only circumstance in which an amendment may be initiated prior to IRB/EC approval is where the change is necessary to eliminate apparent immediate hazards to the patients. In that event, the investigator must notify the IRB/EC and Pfizer in writing immediately after the implementation.

11.2. Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, legal and regulatory requirements, and the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), ICH Guideline for Good Clinical Practice, and the Declaration of Helsinki.

11.3. Patient Information and Consent

All parties will comply with all applicable laws, including laws regarding the implementation of organizational and technical measures to ensure protection of patient personal data. Such measures will include omitting patient names or other directly identifiable data in any reports, publications, or other disclosures, except where required by applicable laws.

The personal data will be stored at the study site in encrypted electronic form and/or paper form and will be password protected or secured in a locked room to ensure that only authorized study staff have access. The study site will implement appropriate technical and organizational measures to ensure that the personal data can be recovered in the event of disaster. In the event of a potential personal data breach, the study site shall be responsible

for determining whether a personal data breach has in fact occurred and, if so, providing breach notifications as required by law.

To protect the rights and freedoms of natural persons with regard to the processing of personal data, when study data are compiled for transfer to Pfizer and other authorized parties, patient names will be removed and will be replaced by a single, specific, numerical code, based on a numbering system defined by Pfizer. All other identifiable data transferred to Pfizer or other authorized parties will be identified by this single, patient-specific code. The investigator site will maintain a confidential list of patients who participated in the study, linking each patient's numerical code to his or her actual identity. In case of data transfer, Pfizer will maintain high standards of confidentiality and protection of patients' personal data consistent with the Clinical Study Agreement and applicable privacy laws.

The informed consent documents and any patient recruitment materials must be in compliance with ICH GCP, local regulatory requirements, and legal requirements, including applicable privacy laws.

The informed consent documents used during the informed consent process and any patient recruitment materials must be reviewed and approved by Pfizer, approved by the IRB/EC before use, and available for inspection.

The investigator must ensure that each study patient is fully informed about the nature and objectives of the study, the sharing of data relating to the study and possible risks associated with participation, including the risks associated with the processing of the patient's personal data. The investigator further must ensure that each study patient is fully informed about his or her right to access and correct his or her personal data and to withdraw consent for the processing of his or her personal data.

The investigator, or a person designated by the investigator, will obtain written informed consent from each patient before any study-specific activity is performed. The investigator will retain the original of each patient's signed consent document.

12. PUBLICATIONS BY INVESTIGATORS

Pfizer supports the exercise of academic freedom and has no objection to publication by the principal investigator (PI) of the results of the study based on information collected or generated by the PI, whether or not the results are favorable to the Pfizer product. However, to ensure against inadvertent disclosure of confidential information or unprotected inventions, the investigator will provide Pfizer an opportunity to review any proposed publication or other type of disclosure of the results of the study (collectively, "publication") before it is submitted or otherwise disclosed.

The investigator will provide any publication to Pfizer at least 30 days before it is submitted for publication or otherwise disclosed. If any patent action is required to protect intellectual property rights, the investigator agrees to delay the disclosure for a period not to exceed an additional 60 days.

The investigator will, on request, remove any previously undisclosed confidential information before disclosure, except for any study- or Pfizer product-related information necessary to the appropriate scientific presentation or understanding of the study results.

If the study is part of a multicenter study, the investigator agrees that the first publication is to be a joint publication covering all investigator sites, and that any subsequent publications by the PI will reference that primary publication. However, if a joint manuscript has not been submitted for publication within 12 months of completion or termination of the study at all participating sites, the investigator is free to publish separately, subject to the other requirements of this section.

For all publications relating to the study, the institution will comply with recognized ethical standards concerning publications and authorship, including Section II - "Ethical Considerations in the Conduct and Reporting of Research" of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, http://www.icmje.org/index.html#authorship, established by the International Committee of Medical Journal Editors.

Publication of study results is also provided for in the CSA between Pfizer and the institution. In this section entitled Publications by Investigators, the defined terms shall have the meanings given to them in the CSA.

If there is any conflict between the CSA and any attachments to it, the terms of the CSA control. If there is any conflict between this protocol and the CSA, this protocol will control as to any issue regarding treatment of study patients, and the CSA will control as to all other issues.

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Appendix 1. Abbreviations

This following is a list of abbreviations that may be used in the protocol.

Abbreviation	Term
AE	adverse event
AI	Aromatase inhibitor
ALK	anaplastic lymphoma kinase
AR	Androgen receptor
ARID1A	AT-rich interactive domain-containing protein 1A
AR-V	Androgen receptor splice variant
BRCA	BReast CAncer susceptibility gene
cfDNA	circulating free DNA
CAP	College of American Pathologists
CCND1	Cyclin D1
CCND3	Cyclin D3
CCNE1	cyclin E1 gene
CDK	Cyclin-dependent kinase
CDKN2A	Cyclin-dependent 2A gene
CI	Confidence interval
CLIA	Clinical Laboratory Improvements Amendments
CRF	Case report form
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRPC	Castrate resistant prostate cancer
CSA	clinical study agreement
CSR	clinical study report
CTC	Circulating tumor cell
CTCAE	Common terminology criteria for adverse events
CTLA4	cytotoxic T-lymphocyte-associated protein 4
DCT	data collection tool
DNA	deoxyribonucleic acid
EC	ethics committee
EGFR	epidermal growth factor receptor
EOT	End of treatment
ER	Estrogen receptor
ESR1	estrogen receptor 1
EZH2	Enhancer of zeste homolog 2
FISH	Fluorescent in situ hybridization
FFPE	Formalin fixed paraffin embedded
FSH	follicle-stimulating hormone
gBRCAm	Germline mutated BRCA
GCP	Good Clinical Practice
GPBP1	GC-Rich Promoter Binding Protein 1
H&E	Hematoxylin and Eosin
HER2	human epidermal growth factor receptor 2

Abbreviation	Term
HLA	human leukocyte antigen
HR	Hormone receptor
HR DDR	homologous recombination-mediated DNA damage repair
ICH	International Conference on Harmonisation
IFN-γ	interferon-γ
IFNGR	interferon-gamma receptor
IHC	Immunohistochemistry
INK	inhibitor of the cyclin-dependent kinase
IPRES	innate anti-PD-1 resistance signature
IRB	institutional review board
JAK	janus kinase
K2EDTA	dipotassium ethylenediaminetetraacetic acid
LAG-3	lymphocyte activation gene-3
LOH	Loss of heterozygosity
MAPK	mitogen-activated protein kinase
MDSC	myeloid derived suppressor cell
MHC	Major histocompatibility
N/A	not applicable
NCI	National Cancer Institute
NEPC	Neuro-endocrine prostate cancer
NGS	Next generation sequencing
NSCLC	Non-small cell lung cancer
ORF	Open reading frame
PACL	Protocol administrative clarification letter
PALB2	Partner and localizer of BRCA2
PARP	Poly (ADP-ribose) polymerase
PARPi	PARP inhibitor
PD	Progressive disease
PD-1	programmed cell death receptor 1
PD-L1	programmed cell death-ligand 1
PD-1/-L1	programmed cell death receptor 1 or programmed cell death-ligand 1
PDx	PD-1/-L1
PI	principal investigator
PI3K	phosphoinositide 3-kinase
PK	Pharmacokinetics
PGx	Pharmacogenomics
PgR	Progesterone receptor
PTEN	phosphatase and tensin homolog
RB1	Retinoblastoma - gene
Rb	Retinoblastoma – gene product
RCC	Renal cell carcinoma
RNA	Ribonucleic acid
ROS1	c-ros oncogene 1

Abbreviation	Term
SAP	Statistical Analysis Plan
SCCHN	squamous cell carcinoma of the head and neck
SLFN11	Loss of Schlafen
SoA	Schedule of Activities
SOC	Standard of care
SOP	Standard operational procedures
STAT1	Signal transducer and activator of transcription 1
TA	therapeutic area
TCR	T-cell receptor
TIM-3	T-cell immunoglobulin and mucin-domain containing-3
TMB	Tumor mutational burden
WES	Whole Exome Sequencing

CCI [redacted]

[redacted]

CCI [redacted]

CCI [redacted]

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CCI [redacted]

CCI [redacted]

[redacted]

○ Yes ○ No

[redacted]

○ Not at all ○ A little bit ○ Somewhat ○ Quite a bit ○ Very much

[redacted]

○ Not at all ○ A little bit ○ Somewhat ○ Quite a bit ○ Very much

[redacted]

○ Not at all ○ A little bit ○ Somewhat ○ Quite a bit ○ Very much

C
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Not at all | A little bit | Somewhat | Quite a bit | Very much

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[redacted]

CCI [redacted]

[redacted]

[redacted]

[redacted]

- [redacted]

  ○ Yes ○ No

- [redacted]

  ○ Not at all ○ A little bit ○ Somewhat ○ Quite a bit ○ Very much

- CCI [redacted]

  ○ Not at all ○ A little bit ○ Somewhat ○ Quite a bit ○ Very much

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  ○ Not at all ○ A little bit ○ Somewhat ○ Quite a bit ○ Very much

- [redacted]

  ○ Not at all ○ A little bit ○ Somewhat ○ Quite a bit ○ Very much

- [redacted]

  ○ Not at all ○ A little bit ○ Somewhat ○ Quite a bit ○ Very much

CCI [redacted]

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Not at all | A little bit | Somewhat | Quite a bit | Very much

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CCI [redacted]

Appendix 4. Country-Specific Requirements

France *Contrat Unique*

1. GCP Training.

   Before enrolling any participants, the investigator and any subinvestigators will complete the Pfizer-provided Good Clinical Practice training course ("Pfizer GCP Training") or training deemed equivalent by Pfizer. Any investigators who later join the study will do the same before performing study-related duties. For studies of applicable duration, the investigator and subinvestigators will complete Pfizer GCP Training or equivalent every 3 years during the term of the study, or more often if there are significant changes to the ICH GCP guidelines or course materials.

2. Study Intervention.

   No participants or third-party payers will be charged for study intervention.

3. Urgent Safety Measures.

   In addition, the investigator will inform Pfizer immediately of any urgent safety measures taken by the investigator to protect the study participants against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the investigator becomes aware of.

4. Termination Rights.

   Pfizer retains the right to discontinue the A9001502 study at any time. There is no Investigational Medicinal Product in the study.