Ex -vivo analysis of **I**mmunogenic cell **DE**ath in **A**birateron e-treated Castrate-Resistant Prostate Cancer (CRPC) patients

Protocol nickname: IDEA

Running title: Immune effects of abiraterone

PROTOCOL TABLE OF CONTENTS

SYNOPSIS

Study Title	Ex-vivo analysis of immunogenic cell death in abiraterone treated CRPC patients
Trial Code	TBD
Type of Study	Non-interventional, translational, prospective, multicenter trial
Number of Patients	48
Duration of Recruitment	1.5 years
Duration of Follow up	0.5 years post-treatment
Aims of the study	To analyze Immunogenic Cell Death-related immune parameters in the peripheral blood of CRPC patients undergoing abiraterone treatment
Primary Endpoints	To investigate and measure selected immunologic parameters in the blood of CRPC patients pre- and post-treatment with abiraterone. To measure the frequency of CD8+ T-cell precursors specific for the HLA-A2-restricted antigenic epitopes of PSMA and PSA antigens by ELISPOT and by tumor-specific dextramers.
Secondary Endpoints	Correlation of immune parameters with clinical characteristics of the patients (age, stage, previous treatments, sites of disease), tumor biology (GS, histology), and with clinical outcome (PSA response rate, time to PSA progression, progression-free survival).

Inclusion Criteria	Age 18-75 Diagnosis of CRPC for whom a decision of treatment with abiraterone have already been made by the attending physician Chemotherapy naïve (not previously treated with docetaxel) PS ECOG score 0-1 HLA-A*0201 positive patients, as assessed by flow cytometry on PBMC. Laboratory criteria for protocol entry: - WBC ≥ 3000/ul or ANC ≥1500/ul (either is sufficient, patients do not need to meet both criteria) - Platelets ≥ 100,000/ul - Estimated creatinine clearance ≥50mL/min by the Jeliffe equation modified for BSA - AST/ALT ≤2X ULN unless due to hepatic metastases in which case levels ≤5xULN are allowed.
	- Bilirubin ≤1.5 ULN. Negative Serology (antibody test) for the following infectious diseases: - Human Immunodeficiency Virus (HIV) type 1 & 2 - Human T-cell Leukemia Virus (HTLV) type 1 and 2 (mandatory in US but optional in Canada and Europe) - Hepatitis B surface antigen - Hepatitis C antibody Completion of a full informed consent process.
Exclusion criteria	Previous treatment with docetaxel PS ECOG ≥2 Chronic treatment with steroids (except for low-dose steroids associated with abiraterone) Autoimmune diseases Any major disease that, in the opinion of the PI, may compromise immune reactivity
Sponsor	ASST Valle Olona, Department of Oncology

Treatment plan	Eligible subjects will be accrued from patient population managed in the Outpatient Clinic of the ASST Valle Olona (which includes three hospitals: Busto Arsizio, Gallarate, Saronno), and from other neighboring hospitals (Varese, Castellanza Santa Maria, Legnano). Blood samples will be taken at day 0, +30 and +60 from all eligible patients, and delivered to San Raffaele Institute at Unit of Biotherapy of Human Tumors for immune analysis. The parameters analysed by flow cytometry will be: T-regs, PD-1+Eomes+ cells in CD8+CD44+ T cells, Ki67+GzmB+ cells in PD-1+Eomes+ T cells, expression of the molecules ICOS, CTLA-4, OX-40 and 4-1BB on PBMCs.
Statistics	Doubling of anti-tumor specific CD8+ T cells at day +30 is expected to be observed after treatment in at least 50% of patients ($H_1 = 0.50$) whereas a percentage equal of inferior to 25% will be rejected ($H_0 = 0.25$). With an alpha error of 0.01 and 1-beta = 0.90, a total of 48 patients will be required to reject the null hypothesis H_0 in favor of H_1 if at least 20 patients will show the doubling of the anti-tumor CD8+ T cells after treatment (A'Hern, Statistics in Medicine 2001).

SIGNATURE PAGE

The following protocol – IDEA version 3.2, dated March 6, 2016 – has been approved by:

Protocol Chief Investigator:	Dr. Marco Bregni Date:
Protocol Sub-Investigator:	Dr. Vincenzo Russo Date:
Coinvestigators:	Dr.ssa Graziella Pinotti Date: Dr. Claudio Verusio Date: Dr. Eraldo Bucci Date: Dr. Sergio Fava Date:
Data Manager:	Dr.ssa Ornella Mesenzani Oncology Unit ASST Valle Olona Date:

Principal Investigator's Statement:

My signature, below, confirms that I understand my responsibilities under the protocol and take responsibility for the conduct of the research in accordance with this version of the protocol, national regulations and the principles of Good Clinical Practice. I understand that I am accountable for this to my employer and, through them to the sponsor of this trial.I will ensure that all persons working on the trial under my supervision are adequately informed about the protocol and their duties.

Name Hospital: ASST Valle Olona

Name Principal Investigator: MARCO BREGNI, M.D.

Signature of Investigator:

Date: June 5, 2017

ABBREVIATION	DEFINITION
ADT	Androgen-Deprivation Therapy
APCs	Antigen-presenting cells
AR	Androgen receptor
AUA	American Urological Association
BSA	Body Surface Area
CRPC	Castration-resistant prostate cancer
DAMPs	Damage-Associated Molecular Patterns
FCM	Flow Cytometry
GM-CSF	Granulocyte macrophage colony-stimulating factor
GS	Gleason Score
ICD	Immunogenic Cell Death
NAIP	Neuronal Apoptosis Inhibitory Protein
PBMC	Peripheral Blood Mononuclear Cells
PC	Prostate Cancer
PSA	Prostate-specific Antigen
PSMA	Prostate-specific Membrane Antigen
TILs	Tumor Infiltrating Lymphocytes
Tregs	Regulatory T-cells
ULN	Upper Normal Limits

1.0 PROTOCOL FLOW-CHART

Figure 1: Flow-Chart of the Protocol

	Screening	Treatment with abiraterone

Day	-15	0	30	60	Restaging at 6 months	1 year OS
Test Number	S1	T1	T2	T3		
Protocol Activities						
Informed Consent **1**	x					
Demography **2**	x					
Inclusion/Exclusion Criteria **3**	x					
Previous Medical/Surgical History **4**	x					
Prior and Concomitant Medications **5**	x					
HLA-A2 testing **6**	x					
Clinical Activities*						
Physical Examination **6**	x	x	x	x	x	
Vital Signs **7**	x	x	x	x	x	
Hematology **8**	x	x	x	x	x	
Blood Chemistry **9**	x	x	x	x	x	
Serologies **10**	x					
Tumor Assessments*						
PSA **11**	x	x	x	x	x	
Tumor imaging **12**	x				x	
Survival **13**					x	x
Immune Assessments						
Blood drawings **14**		x	x	x		

* Clinical activities and Tumor Assessments are not included in this Protocol, and are reported here for completeness

Footnotes for the Flow Chart
1. Informed Consent: Every patient must sign the informed consent to participate in this trial before starting any trial related procedures.
2 Demography: Date of birth, sex and date of Informed Consent. Signature must be reported.
3 Inclusion/Exclusion Criteria: to be checked before patient enrollment
4. Previous Medical/Surgical History: A medical oncologic and non-oncologic history will be obtained on each patient to include all prior therapy, surgery, ongoing diseases and disorders.
5.Prior and Concomitant Medications: A medication history will be obtained to include all medications used in the 4 weeks prior to treatment start, with particular attention to steroids.
6. HLA-A2 testing: will be done locally at each participating center before including patients in the study (an HLA different from A2 excludes the patient from the study)
6. Physical Examination: will be done at pretreatment and at each scheduled visit
7. Vital signs: Height (in cm) at baseline only. Blood pressure/pulse (supine), Weight (in kilograms), ECOG performance status
8. Hematology: Hemoglobin, erythrocytes (RBC), white blood cell (WBC) with differential count (neutrophils, lymphocytes, monocytes, eosinophils, basophils, differential other cells), platelets (PLTs). To be done at baseline, at each scheduled visit during treatment, at the end of treatment, and at each follow-up visit
9. Blood Chemistry: including a comprehensive metabolic panel (electrolytes [sodium, potassium, calcium], urea, creatinine, glucose, ALT, AST, alkaline phosphatase, total bilirubin, total protein, and albumin).
10. Serologies: will include HBV surface antigen, surface antibody and core antibody, HCV, and HIV antibodies, to be done at baseline
11. PSA: will be done at baseline and at each visit, and at follow-up
12. Tumor Imaging: CT scan of the chest, abdomen and pelvis with contrast**,** and complete bone scan. To be done at screening and at six months after start of treatment
13. Overall Survival: will be assessed at 1 year from the start of treatment.
14. Immune assessment: Flow Cytometry for CD4+, FOXP3+, CD8, CD44, PD-1, Eomes, Ki67, GzmB, ICOS, CTLA-4, OX-40 and 4-1BB. Frequency of CD8+ T-cell precursors specific for the HLA-A2 restricted antigenic epitopes of PSMA and PSA

2.0 OBJECTIVES

1. To investigate and measure selected immune parameters in blood of CRPC patients pre- and post-treatment with abiraterone.
2. To assess whether abiraterone induces immunogenic cell death (ICD)
3. To correlate the immune parameters referred to above with clinical characteristics and with clinical outcome of the patients

3.0 BACKGROUND AND RATIONALE

Castration-resistant prostate cancer (CRPC): The term 'castration-resistant prostate cancer' (CRPC) identifies a heterogeneous group of both symptomatic and asymptomatic patients with or without clinical metastases. The Prostate Cancer Working Group 2 (PCWG2) recommendations clearly defined CRPC as prostate cancer progressed despite castrate levels of testosterone (<0.5 ng/ml); this progression may be biochemical, radiological or symptomatic. In recent years, the introduction of highly effective novel therapies has significantly changed the treatment landscape of metastatic CRPC (mCRPC) patients, with overall survival (OS) increasing from approximately 9–18 months to >30 months, with associated symptomatic benefits. From 2002 onward, a stepwise improvement in the management of mCRPC patients was observed: in 2002, it was shown that treatment with zoledronic acid could reduce skeletalrelated event (SRE) incidence; in 2004, the TAX 327 study demonstrated that docetaxel improves OS. In 2010, immunotherapy with sipuleucel-T was approved by the US FDA due to prolonged survival experienced by treated patients. In 2011, a novel tubulin-binding taxane, cabazitaxel, demonstrated its efficacy as a second-line chemotherapy, and treatment with denosumab significantly prolonged the median time to the first SRE. Between 2011 and 2012, two new hormonal agents, abiraterone acetate and enzalutamide, showed further OS improvements as second-line therapies. In 2013, radium-223, an α-emitting radium isotope, became available to clinical practice due to its efficacy in prolonging OS and in delaying the time to the first symptomatic skeletal event. Notwithstanding these notable advances, CRPC remains a challenge for physicians and patients; in recent years, the increasing knowledge on the biology of the immune system has brought new concepts of treatment based on elicitation of immune response by old and new drugs.

3.1 Abiraterone acetate: Abiraterone inhibits the androgen synthesis pathway through blockade of the enzymes 17-α-hydroxylase and C17,20-lyase. The COU-AA-301 trial compared abiraterone acetate plus prednisone versus placebo plus prednisone in patients with previous docetaxel therapy. An overall survival advantage was seen for patients taking abiraterone acetate in the interim analysis; on the final analysis, before unblinding and crossover at a median follow-up of 20.2 months, median overall survival with abiraterone acetate remained significantly better than with placebo (15.8 *v* 11.2 months; hazard ratio 0.74, 0.64 to 0.86; P<0.001). The AUA guidelines therefore recommend abiraterone acetate for patients with symptomatic metastatic CRPC with previous docetaxel therapy and good performance status. The randomized double blind COU-AA-302 trial compared 1000 mg abiraterone acetate plus 5 mg prednisone twice daily against placebo plus prednisone in men with asymptomatic or minimally symptomatic metastatic CRPC without previous chemotherapy. The co-primary endpoints were radiographic PFS and overall survival. The final analysis showed a significantly longer median overall survival in the abiraterone acetate group than in the placebo group (34.7 (95% confidence interval 32.7 to 36.8) versus 30.3

(28.7 to 33.3) months; hazard ratio 0.81, 0.70 to 0.93; P=0.0033). Overall, the drug was well tolerated and no significant differences were seen in the occurrence of any adverse events between treatment and placebo in the interim analysis. Low dose oral prednisone is needed with abiraterone, to limit the side effects associated with excess mineralocorticoid. On the basis of the results of this trial, the AUA guidelines recommend abiraterone acetate for patients with asymptomatic or minimally symptomatic metastatic CRPC, as well as patients with symptoms with no previous docetaxel therapy and good performance status (as defined by the Eastern Cooperative Oncology Group).
Taken together, these data indicate that treatment with abiraterone acetate prolong overall survival in patients pre- or post-docetaxel by a margin that is both clinically and statistically significant.

3.2. Immunotherapy of CRPC: A strong immune rationale supports the development of immunotherapy for PC. The lack of afferent lymphatics and the immunosoppressive properties of seminal fluid confer to the prostate gland an immunologically privileged status. Biologically, the majority of prostate tumors behave like a slow-growing disease, allowing time for a clinically relevant immune response, and thus justifying the high immunogenicity of this tumor. PC cells, in fact, show an abnormal over-expression of several highly immunogenic tumor-associated antigens that represent potential target for immunotherapeutic approaches. Moreover, PC tissue is marked by a large inflammatory infiltrate of T-cells [tumor infiltrating lymphocytes (TILs)] within the tumor and in the surrounded microenvironment. Both the innate and the adaptive branches of the immune system participate in host defense mechanisms against neoplastic prostate cells. Macrophages/antigen-presenting cells (APCs), CD8+ cytotoxic T lymphocytes, CD4+ helper T lymphocytes, and natural killer (NK) cells should recognize and destroy cancer cells. Therefore, dense TILs infiltration seems to have a positive prognostic value, correlating with longer patient survival. Moreover, high grade prostatic adenocarcinomas have significantly less infiltration of T-cells as compared to benign nodular prostatic hyperplasia, underscoring that tumor progression could be associated with defects in cell-mediated immune responses. The inability to mount an efficient immune response that restricts cancer progression is partially due to the presence of non-active effector TILs [lacking markers of functional activity like perforin or gamma interferon (IFNg)], and regulatory T-cells (Tregs) within the inflammatory infiltrate of PC tissue. Tregs is a small subpopulation of CD4+/CD25+ and CD8+/Foxp3 T lymphocytes with suppressive functions on the anti-immune response [directly *via* cell-cell contact or indirectly by secreting anti-inflammatory cytokines, like interleukin-10 (IL-10) or tumor growth factor (TGFb)], supposed to have a negative prognostic role in PC patients, highlighting that blockage of these cells may stimulate the generation of effective CD8+ T-cell immune responses and therefore induce beneficial clinical responses.

3.2.1. Cancer vaccines: The rationale behind vaccines in cancer is to mount a strong and effective immune response against tumor-related antigens, which can lead to the eradication of tumors. There are several approaches to vaccine-based immunotherapy, which mainly include autologous or heterologous cell or peptide vaccines, viral- and DNA-based vaccines.
Sipuleucel-T (Provenge™) is a cell-based vaccine manufactured from the patient's own peripheral blood mononuclear cells, which are obtained by leukapheresis. These cells, which are enriched for antigen-presenting cells (APCs), are subsequently incubated with a recombinant fusion protein

consisting of prostatic acid phosphatase (PAP) and granulocyte macrophage colony-stimulating factor (GM-CSF). This process results in the activation of APCs and the final product is administered back to the patient by intravenous infusion. In the phase III trial, the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial, a total of 512 patients were randomized to receive Sipuleucel-T or placebo. This trial showed a 22% relative reduction in the risk of death [hazard ratio (HR) 0.78], which was translated to a 4.1-month improvement in OS (25.8 and 21.7 months, in the Sipuleucel-T and placebo groups, respectively). A retrospective analysis of the data from the IMPACT trial showed that patients with the lowest tumor burden were likely to obtain greater benefit from Sipuleucel-T. Indeed, patients with lower PSA levels at baseline demonstrated a benefit of 13 months compared to placebo, whereas patients with higher PSA levels showed only a 2.8-month improvement with Sipuleucel-T. These results mirror the degree of immune suppression caused by the tumor (greater in higher tumor burden), as well as the fact that vaccine immunotherapy "may need" time to act and produce a sustained responses. Sipuleucel-T is currently under investigation in several trials in combination with other approved drugs for mCRPC (abiraterone acetate, enzalutamide, or radium-223) or with other forms of immunotherapy (ipilimumab), (NCT01487863, NCT01981122, NCT02463799, NCT01832870 and NCT01804465).

GVAX is a whole cancer cell-based vaccine composed of whole tumor cells, derived from LNCaP and PC3 allogeneic prostate cancer cell lines. Cells are genetically modified to secrete the immune stimulatory cytokine GM-CSF and are also irradiated for safety. Two phase III trials were designed to evaluate GVAX in mCRPC patients. VITAL-1 enrolled asymptomatic patients to receive GVAX or docetaxel and VITAL-2 symptomatic patients to receive GVAX plus docetaxel or docetaxel alone. Unfortunately, a safety review of VITAL-2 showed an increase of deaths in the GVAX and docetaxel combination arm, compared with the docetaxel arm. This finding led to the termination of the VITAL-2 trial. A few months after the closure of VITAL-2, a futility analysis of VITAL-1 showed that there was a <30% chance of meeting its OS endpoint, so this trial was also terminated. GVAX is currently under investigation in combination with several agents in other cancers, such as pancreatic and colorectal (NCT02004262 and NCT01952730).

PROSTVAC is a recombinant viral vaccine, which consists of two poxviruses (vaccinia as priming and fowlpox as boosting agents). The two viruses are genetically engineered to express whole PSA as antigen, as well as three co-stimulatory molecules (B7.1, ICAM-1 and LFA-3; TRICOM) in order to enhance the PSA-targeted response. A large phase III trial, which has completed enrollment of almost 1,300 asymptomatic or minimally symptomatic mCRPC patients is currently ongoing, with OS being the primary endpoint (NCT01322490).

3.2.2. Immune checkpoint inhibition has recently changed clinical practice in tumors such as melanoma, lung cancer and Hodgkin lymphoma, and is now under investigation in the vast majority of solid and hematologic malignancies. Antibodies against CTLA-4, PD-1, and its ligand PD-L1, are enhancing T cell activity by "releasing the brakes" of the T cell-mediated antitumor response. Ipilimumab is a fully human IgG4 monoclonal antibody against CTLA-4. CTLA-4 has been shown to be up regulated upon T cell activation, in order to diminish this response. In the mCRPC setting, ipilimumab was tested in a phase I/II trial, where patients received ipilimumab in several dosing schedules plus radiation to a single bone metastasis. Results of this trial showed that ipilimumab has antitumor activity with tumor control and manageable toxicities. Following these results, two phase III trials were initiated, in mCRPC patients, using the dose of 10 mg/kg every 3 weeks for up to 4 doses plus bone-directed radiotherapy, after docetaxel failure or prior to docetaxel, respectively. The study protocol permitted the administration of ipilimumab as maintenance treatment every 12 weeks after completion of the first 4 doses. Results of the postdocetaxel trial showed no statistical difference in OS between ipilimumab and placebo (median OS 11.2 *vs*. 10.0 months, P=0.053). Nevertheless, a subgroup analysis demonstrated

that ipilimumab offers a survival advantage to patients with favorable baseline characteristics, such as alkaline phosphatase <1.5 times the upper limit of normal, hemoglobin >11.0 g/dL and no visceral metastases. These patients had a median OS of 22.7 months with ipilimumab *vs*. 15.8 months with placebo (P=0.004). At ESMO/ECCO 2015, the updated OS analysis, with an additional year of follow-up, was presented and was consistent with the primary analysis, with the same difference in OS between ipilimumab and placebo (11.2 *vs*. 10.0 months, P=0.030). Also consistent with previous reports, pre-specified subgroup analyses suggest greater activity in patients with lower disease burden. Another, similar in design trial, evaluated ipilimumab in chemotherapy-naïve mCRPC patients but results have not yet been reported (NCT02279862). Several studies have shown that T cells, which infiltrate prostate tumors, express PD-1 in high levels. Surprisingly enough, in a pilot study of nivolumab (anti-PD1 antibody) there were no objective responses among 17 mCRPC patients and all those cases were negative for tumor PD-L1 expression. Nonetheless, these agents are currently under investigation in prostate cancer patients through combinatorial treatment strategies (NCT02601014 and NCT02499835).

3.3. Immune effects of hormone-deprivation therapies: the common view is that hormonal therapies used for PC treatment have immunomodulatory effects. Indeed, anti-androgens can activate thymic regeneration and promote thymopoiesis and B-cell proliferation, reduce intratumoral infiltration of immunosuppressive Tregs, mitigate tolerance to prostatic antigens, increase NK cell infiltrate, and induce high levels of T-cell infiltration (mainly CD4+ cells) within PC tissue, suggesting the potential role of combining hormonal agents with immunotherapy to enhance anticancer immune-based treatments. With regard to new hormonal agents, both enzalutamide and abiraterone render prostate tumor cells more sensitive to T cell-mediated lysis through immunogenic modulation, and these immunomodulatory activities are androgen receptor (AR)-dependent. Expression of the antiapoptotic gene NAIP (NLR family, neuronal apoptosis inhibitory protein) was significantly down-regulated in human prostate tumor cells treated *in vitro* and *in vivo* with enzalutamide. Functional analysis revealed that NAIP played a critical role in inducing CTL sensitivity. Enzalutamide enhances sensitivity to immune-mediated killing of prostate tumor cells that overexpress AR, a major mechanism of resistance to androgen-deprivation therapy (ADT). Most recently, in a mouse model, AR antagonists have been reported to suppress immune response through an off-target effect of GABA-A inhibition, while abiraterone, which acts by inhibiting androgen biosynthesis, was synergic with immunotherapy. The immunomodulatory properties of abiraterone provide a rationale for its use in combination with immunotherapeutic agents in CRPC. However, no in-vivo study in patients with CRPC have assessed yet the immune effects of abiraterone.

3.4. Immunogenic Cell Death (ICD): Following antineoplastic treatment, emission of danger signals or damage-associated molecular patterns (DAMPs) as a part of an elaborate danger signalling module can increase the immunogenicity of dying, stressed or dead cancer cells – a concept pioneered by the labs of Guido Kroemer and Laurence Zitvogel. Operationally, it has been shown that eliciting a cancer cell death associated with the activation of danger signalling pathways evoking the pre-mortem emission of DAMPs markedly increases the immunogenicity of these dying cells. This cell death pathway has been termed as 'immunogenic cell death (ICD). ICD has the ability to convert dying or dead cancer cells into a "vaccine" capable of inducing anticancer immunity in absence of any additional adjuvants. Immunogenic cell death (ICD) involves changes in the composition of the cell surface as well as the release of soluble mediators, occurring in a defined temporal sequence. Such signals operate on a series of receptors expressed by dendritic cells to stimulate the presentation of tumor antigens to T cells. The

concomitant induction of oxidative stress and ER stress is crucial for elicitation of danger signalling pathways mediating the trafficking and emission of danger signals or DAMPs. The first systematic screening for ICD inducers recognized anthracyclines, mitoxantrone, and radiotherapy as potent inducers of ICD in cancer. Since this screening study, other novel and highly efficacious ICD inducers have been identified such as chemotherapeutics, certain targeted therapeutics, various physical modalities, certain components of Chinese traditional medicine, certain oncolytic viruses and hypericin-based photodynamic therapy. Ex vivo studies evaluating whether androgen inhibitors induce immunogenic cell death through de novo generation of TAA-specific CTLs in clinical studies are lacking.

3.5. Rationale for the study: We plan to analyze ICD-related immune parameters in the peripheral blood of CRPC patients undergoing abiraterone treatment. This project aims to provide insights into the immunogenicity of abiraterone, and therefore set the basis for traslational clinical trials combining new hormonal agents with immune potentiating agents such as immune checkpoint inhibitor drugs, the final goal being to develop a chemotherapy-free treatment for CRPC patients. Since low-dose prednisone is utilized in combination with abiraterone, there is concern that steroids may decrease activation of immune system to immunogenic cell death. There is no direct answer to this issue, but we can infer indirect evidence from the clinical experience with immune checkpoint inhibitors in other tumors (e.g., nivolumab, pembrolizumab). Although immune-related adverse effects are observed in patients treated with immune checkpoint inhibitors, when steroids are used to treat such adverse effects they do not abrogate the clinical benefit of treatment. Indeed, a substantial number of patients in a study of ipilimumab plus nivolumab required steroidal immunosuppressive agents to treat toxicity (83.4%); however, of those patients, many responded to the combination (67.5%; 81 out of 120 patients).

3.6. Design of Trial: CRPC patients for whom a decision of treatment with abiraterone have already been made by the attending physician, will be followed according to standard clinical practice. A blood draw will be obtained before, and 30 and 60 days after the beginning of treatment with abiraterone, for immune assessments. This is a non-interventional study, no changes in the clinical practice is planned according to this study. In our clinical practice, we prescribe either abiraterone or enzalutamide on the basis of standard clinical criteria, taking into account the predicted side effects of either drug (cardiac and neurological among others). In this study we will consider only patients on abiraterone treatment, planning to extend this analysis also to patients on enzalutamide in due time.

4.0 ENDPOINTS

4.1 **Primary endpoints:**

a. To investigate and measure selected immunologic parameters in the blood of CPRC patients pre- and post-treatment with abiraterone. Peripheral Blood Mononuclear Cells (PBMCs) will be evaluated for the presence of CD4+FOXP3+ T-regulatory cells (T-regs), in order to detect possible variations induced by the treatment. Moreover, PBMCs will be stained by CD8, CD44, PD-1, Eomes, Ki67 and GzmBmAbs. This staining will allow evaluating the percentage of PD-l + Eomes+ cells in CD8+CD44+ T cells, and Ki67+GzmB+ cells in PD-l+Eomes+ T cells, the latter predicting a better clinical outcome in melanoma patients treated with a combination of RT plus Ipilimumab. Additionally, we will analyze by flow cytometry the expression of the molecules ICOS, CTLA-4, OX-40 and 4-1BB

on PBMCs collected before and after abiraterone treatment. We will evaluate costimulatory molecule profiles during the course of the treatment and analyze whether possible changes correlate with the clinical outcome of treated patients.

b. To measure the frequency of CD8+ T-cell precursors specific for the HLA-A2-restricted antigenic epitopes of PSMA and PSA antigens by ELISPOT and by tumor-specific dextramers. These assays will be performed on T-cells collected before and after treatment. We will evaluate whether PSA- and/or PSMA-specific CD8+ T-cell precursors will occur during the treatment course. Possible increase of CD8+ T-cell precursors against the above-mentioned antigens will be then correlated with the clinical outcome. These analyses will be performed on HLA-A*0201 positive patients, who represent 44% of patients population.

4.2 **Secondary Endpoints:**

Correlation of immune parameters described above with clinical characteristics of the patients and with clinical outcome. Clinical characteristics taken in account will be: age, Gleason score, histology, disease stage, sites of metastatic disease. Clinical outcome parameters will include the PSA response rate (defined as the proportion of patients with a decrease of ≥50% in the PSA concentration from the pretreatment baseline PSA value, confirmed after at least 4 weeks by an additional PSA evaluation).

Other secondary end points include time to PSA progression according to pre-specified criteria (in patients in whom the PSA level had not decreased, PSA progression will be defined as a 25% increase over the baseline and an increase in the absolute-value PSA level by at least 5 ng per milliliter, confirmed by a second value; in patients in whom the PSA had decreased but had not reached response criteria [PSA =50%], progressive disease will be considered to occur when the PSA level increase 25% over the nadir, provided that the increase is a minimum of 5 ng per milliliter and is confirmed; and if at least a 50% decrease in the PSA level had been achieved, PSA progression would be an increase of 50% above the nadir at a minimum of 5 ng per milliliter), and radiographic evidence of progression-free survival according to clinical practice (defined as soft-tissue disease progression according to modified Response Evaluation Criteria in Solid Tumors [RECIST] [with a baseline lymph node of =2.0 cm considered to be a target lesion], or progression according to bone scans showing two or more new lesions not consistent with tumor flare).

5.0 SUBJECT ELIGIBILITY

The following conditions must be met for all patients treated on this study:

5.1 **Inclusion Criteria:**

1. CRPC patients (testosterone levels <20 ng/mL in presence of LHRH analog therapy) for whom a decision of treatment with abiraterone have already been made by the attending physician.
2. Chemotherapy-naïve (not previously treated with docetaxel)
3. ECOG score <2,
4. HLA-A*0201 positive patients as assessed by flow cytometry on PBMC.

5. Laboratory criteria for protocol entry:
   a. WBC ≥ 3000/ul or ANC ≥1500/ul (either is sufficient, patients do not need to meet both criteria)
   b. Platelets ≥ 100,000/ul
   c. Estimated creatinine clearance ≥50mL/min by the Jeliffe equation modified for BSA
   d. AST/ALT ≤2X ULN unless due to hepatic metastases in which case levels ≤5xULN are allowed.
   e. Bilirubin ≤1.5 ULN.
6. Negative Serology (antibody test) for the following infectious diseases:
   a. Human Immunodeficiency Virus (HIV) type 1 and 2
   b. Human T-cell Leukemia Virus (HTLV) type 1 and 2 (mandatory in US but optional in Canada and Europe)
   c. Hepatitis B surface antigen
   d. Hepatitis C antibody
7. Completion of a full informed consent process.

5.2 **Exclusion Criteria**

1. ECOG PS≥2
2. Prior treatment with chemotherapy
3. Autoimmune diseases
4. Any major disease that, in the opinion of the PI, may compromise immune reactivity

6.0 RECRUITMENT PLAN

Eligible patients will be recruited by the appropriate service at each participating institution. Participation is voluntary. The consenting physician will inform patients of the non-interventional nature of this study and will obtain a signed informed consent. The duration of accrual will be 18 months, and the follow-up period 6 months.

7.0 PRETREATMENT EVALUATION

Subjects will undergo the following assessments during screening before enrollment:

7.1 **Medical History:** A medical history will be obtained on each patient to include all prior therapy, ongoing diseases and disorders.

7.2 **Prior and concomitant medications:** Medications, treatments and therapies used in the prior 4 weeks as well as those currently being taken, with particolar

renard to steroids. A low dose steroid medication (e.g., prednisone 5 mg twice per day) is required during abiraterone treatment. LHRH analogs are allowed with abiraterone.

7.3 **Physical Examination:** including vital signs, height, weight (calculation of body surface area).

7.4 **Basic laboratory Studies:** including a comprehensive metabolic panel (electrolytes, BUN [urea], creatinine, glucose, calcium, ALT, AST, alkaline phosphatase, total bilirubin, total protein, and albumin), and complete blood count.

7.5 **Serologies:** will include HBV surface antigen, surface antibody, and core antibody, HCV, and HIV antibodies.

7.6 **Serum tumor marker studies:** includes PSA and free PSA fraction.

7.7 **HLA-A2 testing:** a FCM assay on PBMC will be done for assessing the HLAA2 haplotype.

7.8 **CT scan of the chest, abdomen and pelvis with contrast and bone scan:** For patients with a contraindication to intravenous iodinated contrast, MRI of the abdomen and pelvis with contrast and CT scan of the chest without contrast can be used.

7-9 **Overall survival**: will be assessed during follow-up until 6 months from the end of treatment.

8.0 STUDY PROCEDURES

8.1 **Assessing eligibility**: HLA-A2 testing will be performed at each participating Center prior to the inclusion in the study. An HLA haplotype different from HLA-A2 excludes the patient from the study.

8.2 **PBMC samples**: A sample of heparinized peripheral blood of 30-mL volume will be drawn at defined intervals (time 0, +30, +60: refer to the Flow Chart). Samples will be delivered at room temperature at the San Raffaele Institute, Laboratory of Biotherapy and Immunotherapy of Human Tumors, via DHL courier. Mention the code xxxx when calling the courier. Samples will be delivered the same day by 4:00 pm at the laboratory, where they will be processed and cryopreserved for successive analysis.

8.3 **Analysis of immune parameters**: immunologic parameters analyzed in the blood of CPRC patients pre- and post-treatment with abiraterone will be:

8.3.1 The frequency of CD4+FOXP3+ T-regulatory cells (T-regs), in order to detect possible variations induced by the treatment. Moreover, PBMCs will be stained

by CD8, CD44, PD-1, Eomes, Ki67 and GzmBmAbs. This staining will allow evaluating the percentage of PD-l + Eomes+ cells in CD8+CD44+ T cells, and Ki67+GzmB+ cells in PD-l+Eomes+ T cells, the latter predicting a better clinical outcome in melanoma patients treated with a combination of RT plus Ipilimumab.

8.3.2 The expression of the molecules ICOS, CTLA-4, OX-40 and 4-1BB on PBMCs collected before and after abiraterone treatment. We will evaluate costimulatory molecule profiles during the course of the treatment and analyze whether possible changes correlate with the clinical outcome of treated patients.

8.3.3 The frequency of CD8+ T-cell precursors specific for the HLA-A2-restricted antigenic epitopes of PSMA and PSA antigens by ELISPOT and by tumorspecific dextramers. We will evaluate whether PSA- and/or PSMA-specific CD8+ T-cell precursors will occur during the treatment course. Possible increase of CD8+ T-cell precursors against the above-mentioned antigens will be then correlated with the clinical outcome.

8.4 **Correlation of immune parameters with clinical data:**

Immune parameters described above will be correlated with clinical characteristics of the patients and with clinical outcome.
Clinical characteristics taken in account will be: age, Gleason score, histology, disease stage, sites of metastatic disease.
Clinical outcome parameters will include the PSA response rate (defined as the proportion of patients with a decrease of ≥50% in the PSA concentration from the pretreatment baseline PSA value, confirmed after at least 4 weeks by an additional PSA evaluation).
Other secondary end points include time to PSA progression according to pre-specified criteria (in patients in whom the PSA level had not decreased, PSA progression will be defined as a 25% increase over the baseline and an increase in the absolute-value PSA level by at least 5 ng per milliliter, confirmed by a second value; in patients in whom the PSA had decreased but had not reached response criteria [PSA =50%], progressive disease will be considered to occur when the PSA level increase 25% over the nadir, provided that the increase is a minimum of 5 ng per milliliter and is confirmed; and if at least a 50% decrease in the PSA level had been achieved, PSA progression would be an increase of 50% above the nadir at a minimum of 5 ng per milliliter), and radiographic evidence of progression-free survival according to clinical practice (defined as soft-tissue disease progression according to modified Response Evaluation Criteria in Solid Tumors [RECIST] [with a baseline lymph node of =2.0 cm considered to be a target lesion], or progression according to bone scans showing two or more new lesions not consistent with tumor flare).

9.0 STATISTICAL ANALYSIS

9.1 Brief Statistical Rationale:

The selected immunologic parameters, as they are mentioned at points 4.0 and 4.1 of the section "Endpoints", will be analysed using paired t-test in order to check whether any significant difference between the timepoints 0, +30 and +60 exists. Also, p-value for trend will be provided if applicable.

The correlation of immune parameters with clinical characteristics of the patients and with clinical outcome will be tested using uni- and multivariate Cox regression; when needed, some immunological variables will be treated as time-dependent covariates.

9.2 **Statistical Power Calculations:**

Doubling of anti-tumor CD8+ T cells is expected to be observed at the evaluation at day +30 after treatment in at least 50% of patients ($H_1 = 0.50$) whereas a percentage equal of inferior to 25% will be rejected ($H_0 = 0.25$). With an alpha error of 0.01 and 1-beta = 0.90, a total of 48 patients will be required to reject the null hypothesis H_0 in favor of H_1 if at least 20 patients will show the doubling of the anti-tumor CD8+ T cells after treatment (A'Hern, Statistics in Medicine 2001).

9.3 **Primary Analysis** (includes planned analysis of the primary endpoint):

Paired t-test will performed on blood samples taken at day 0, +30 and +60 for all evaluable patients. As mentioned above, the parameters analysed will be: T-regs, PD-1+Eomes+ cells in CD8+CD44+ T cells, Ki67+GzmB+ cells in PD-1+Eomes+ T cells, expression (by flow cytometry) of the molecules ICOS, CTLA-4, OX-40 and 4-1BB on PBMCs. A p-value < 0.05 will be considered as significant.

10.0 EVALUATION DURING STUDY

A minimal essential data (MED) form with demographics, referring Center, clinical data will be kept at the coordination Center.

11.0 SAFETY DATA AND COMPLAINT COLLECTION AND REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of patients, physicians, and the sponsor, and are mandated by regulatory agencies worldwide. All clinical studies will be conducted in accordance with established procedures and regulatory requirements to ensure appropriate reporting of safety information.

Given the observational nature of this study the Reference Safety Information is the approved and up-to-date Summary of Product Characteristics.

11.1 Definitions and Classifications – see Appendix 2

11.2 Procedures

In this non-interventional study, Abiraterone Acetate is the product under study. Each investigator participating in the study assumes responsibility for appropriate reporting of serious and non serious adverse drug reactions originating from the data collected for medicinal products to the regulatory authorities. All collected adverse events will be summarized in the final study report.

The sponsor will provide appropriate pharmacovigilance training to the participating sites' personnel.

The AE ,SAE and Product Quality Complaints (PQC) must be sent to the Sponsor Contact:

Name: Dr. Marco Bregni

Phone: 0331 699489

Fax: 0331 699295

11.2.1 All Adverse Events

Adverse Events Systematically Collected (Solicited Adverse Events)

All adverse events and special situations, following exposure to Abiraterone Acetate as the product under study are to be systematically recorded in the CRF and the patient's source records, regardless of seriousness or causality. Adverse event collection should start with the first use of the product under study and will apply to all adverse events, regardless of seriousness, that occur within 30 days after a patient's last use within the study. All adverse events following exposure to the product under study should be assessed by the physician to document his opinion concerning the relationship of the event to the product under study; the causal relationship of the adverse event must be recorded in the CRF. An adverse event will be considered as an adverse drug reaction (ADR) if there is at least a reasonable possibility of a causal relationship (ie, a causal relationship is possible, probable or very likely).

All adverse events should be followed-up in accordance with clinical practice, regardless of seriousness. This follow-up should be recorded in the patient's source records.

11.2.2 Serious Adverse Events

All serious adverse events following exposure to the products relevant for the study should be reported directly by the participating physician, within 24 hours of them becoming aware, to the sponsor using a Serious Adverse Event Report Form (or local equivalent).

For reports of hospitalization, it is the sign, symptom or diagnosis which led to hospitalization that is the serious event for which details must be provided.

Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (eg, social reasons such as pending placement in long-term care facility).

- Surgery or procedure(s) planned before entry into the study (should be documented in the CRF). [Note: Hospitalizations that were planned before the start of data collection, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.]

Disease progression should not be recorded as an adverse event or serious adverse event term; instead, signs and symptoms of clinical sequelae resulting from disease progression will be reported in the CRF if they fulfill the serious adverse event definition. Since a fatal outcome is part of the study outcomes, adverse events with fatal outcome will only be recorded as serious in the CRF and should not be reported as a serious adverse event.

The cause of death of a patient in a study within 30 days of the last use of a product relevant fo the study, whether or not the event is expected or associated with the product relevant for the study, is considered a serious adverse event.

11.2.3 Non Serious adverse events

The sponsor will extract listings of non-serious AEs from the CRF annually and at the end of the study. He will evaluate the causality with the study drug for regulatory authority reporting, if related.

11.2.4 Special Situations

Even if not considered AE, the following events have to be collected:

- Drug exposure during pregnancy (maternal/paternal)
- Exposure to a medicinal product from breastfeeding
- Off label use of a medicinal product
- Overdose of a medicinal product
- Suspected abuse/misuse of a medicinal product
- Inadvertent or accidental exposure to a medicinal product (eg. occupational exposure)
- Any failure of expected pharmacological action (lack of effect)
- Medication error involving a medicinal product (with or without patient exposure to the medicinal product e.g. name confusion)
- Suspected transmission of any infectious agent via administration of a medicinal product

- Unexpected therapeutic or clinical benefit from use of a medicinal product

Special situations for the product under study should be recorded in the CRF. Any special situation that meets the criteria of a serious adverse event should be recorded on a Serious Adverse Event Report Form, reported to the sponsor within 24 hours of the investigator becoming aware and reported to the HA if related.

11.2.5 Pregnancy

Since the population in study includes male castrated subjects, no cases of pregnancies in partners following exposure to AA are expected. Therefore, reporting of pregnancy is not applicable.

11.2.6 Product Quality Complaints (PQC)

A PQC may have an impact on the safety and effectiveness of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of patients, physicians, and the sponsor, and are mandated by regulatory agencies worldwide. . Since the product under study is a marketed product, the reporting of the PQC follows the normal clinical practice.

11.2.7 Adverse Events Not Systematically Collected (Spontaneous Adverse Events)

For adverse events and special situations that are not systematically collected (eg, for a medicinal product other than the product(s) relevant for the study, and where the participating physician considers there is a possible, probable or very likely relationship to a medicinal product (ie, spontaneous ADRs), the participating physician is requested to notify the appropriate regulatory/competent authority through the national spontaneous reporting system as soon as possible.
Where available, reports of spontaneous ADRs will be summarised in the clinical study report.

12.0 ESSENTIAL REFERENCES

Ritch CR, Cookson MS. Advances in the management of castration resistant prostate cancer. BMJ. 2016 Oct 17;355:i4405

de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, Chi KN, Jones RJ, Goodman OB Jr, Saad F, Staffurth JN, Mainwaring P, Harland S, Flaig TW, Hutson TE, Cheng T, Patterson H, Hainsworth JD, Ryan CJ, Sternberg CN, Ellard SL, Fléchon A, Saleh M, Scholz M, Efstathiou E, Zivi A, Bianchini D, Loriot Y, Chieffo N, Kheoh T, Haqq CM, Scher HI; COU-AA301 Investigators. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011 May 26;364(21):1995-2005. doi: 10.1056/NEJMoa1014618.

Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, Fizazi K, Mainwaring P, Piulats JM, Ng S, Carles J, Mulders PF, Basch E, Small EJ, Saad F, Schrijvers D, Van Poppel H, Mukherjee SD, Suttmann H, Gerritsen WR, Flaig TW, George DJ, Yu EY, Efstathiou E, Pantuck A, Winquist E, Higano CS, Taplin ME, Park Y, Kheoh T, Griffin T, Scher HI, Rathkopf DE; COU-AA-302 Investigators. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 2013 Jan 10;368(2):138-48

Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PF, Sternberg CN, Miller K, Logothetis CJ, Shore ND, Small EJ, Carles J, Flaig TW, Taplin ME, Higano CS, de Souza P, de Bono JS, Griffin TW, De Porre P, Yu MK, Park YC, Li J, Kheoh T, Naini V, Molina A, Rathkopf DE; COU-AA-302 Investigators. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 2015 Feb;16(2):152-60.

Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013;31:51-72. doi: 10.1146/annurev-immunol-032712 -100008. Epub 2012 Nov 12.

Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol. 2008 Jan;8(l):59-73.

Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, Benci JL, Xu B, Dada H, Odorizzi PM, Herati RS, Mansfield KD, Patsch D, AmaravadiRK, Schuschter LM, Ishwaran H, Mick R, Pryma DA, Xu X, Feldman MD, Gangadhar TC, Hahn SM, Wherry EJ, Vonderheide RH, Minn AJ. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature. 2015 Apr 16;520(7547):373-7. doi: 10.1038/naturel4292. Epub 2015 Mar 9.

Ardiani A, Gameiro SR, Kwilas AR, Donahue RN, Hodge JW. Androgen deprivation therapy sensitizes prostate cancer cells to T-cell killing through androgen receptor dependent modulation of the apoptotic pathway. Oncotarget. 2014 Oct 15;5(19):9335-48.

Larkin, J. et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N. Engl.J. Med. http://dx.doi.org/10.1056/nejmoa1504030 (2015).

Postow, M. A. et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N. Engl. J. Med. 372, 2006–2017 (2015).

Pardoll D. Cancer and the immune system: Basic concepts and targets for intervention. Semin Oncol. 2015 Aug;42(4):523-38. doi: 10.1053/j.seminoncol.2015.05.003.

Modena A, Ciccarese C, Iacovelli R, et al. Immune checkpoint inhibitors and prostate cancer: a new frontier? Oncology Reviews 2016; volume 10:293

Sutherland JS, Goldberg GL, Hammett MV, et al. Activation of Thymic Regeneration in Mice and Humans following Androgen Blockade, J Immunol 2005, 175: 2741–2753.

Pu Y, Meng X, Liang Y, et al. Androgen receptor antagonists compromise T cell response against prostate cancer leading to early tumor relapse. Science Transl Med 2016;8:333

APPENDIX A: CENTERS

Oncology Unit, Dept. of Oncology, ASST Valle Olona, via A. Da Brescia 1, 21052 Busto Arsizio, Ospedale di Circolo di Busto Arsizio (Coordinating Center)

Oncology Unit, Dept. of Oncology, ASST Valle Olona, via A. Da Brescia 1, 21052 Busto Arsizio, Ospedale Sant'Antonio, Gallarate

Oncology Unit, Dept. of Oncology, ASST Valle Olona, via A. Da Brescia 1, 21052 Busto Arsizio, Ospedale di Saronno

Oncology Unit, Dept. of Oncology, ASST Sette Laghi, Via Borri 52, Varese, Ospedale di Circolo Fondazione Macchi

Oncology Unit, ASST Milano Ovest, Ospedale di Legnano

Oncology Unit, Multimedica, Viale Piemonte, 70, 21053 Castellanza VA

APPENDIX 2: DEFINITIONS and CLASSIFICATIONS

Adverse Event

An adverse event is any untoward medical occurrence in a patient administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can be any unfavorable and unintended sign (including an abnormal finding or lack of expected pharmacological action), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition based on International Conference on Harmonization [ICH]).

This includes any occurrence that is new in onset or aggravated in severity from the baseline condition, or abnormal results of any diagnostic procedures that are conducted within clinical practice.

Adverse Drug Reaction

An adverse drug reaction (ADR) is defined as a response to a medicinal (investigational or non-investigational) product that is noxious and unintended. The phrase “response to a medicinal product” means that a causal relationship between a medicinal product and an adverse event is possible, probable or very likely.

An ADR, in contrast to an adverse event, is characterized by the fact that a causal relationship between the medicinal product and the occurrence is suspected. All adverse events judged by either the reporting physician or the sponsor as having a reasonable causal relationship to a medicinal product qualify as ADRs.

Serious Adverse Event

A serious adverse event based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use, is any untoward medical occurrence that at any dose:
Results in death
Is life-threatening
(the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)
Requires inpatient hospitalization or prolongation of existing hospitalization
Results in persistent or significant disability/incapacity
Is a congenital anomaly/birth defect
Is medically important*
* Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life threatening or result in death or hospitalization but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed above.

Product Quality Complaint

A product quality complaint (PQC) is any written, electronic, or oral communication that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance of a product after it is released for distribution.

Attribution Definitions

An adverse event is considered associated with the use of the product under study if the attribution is possible, probable, or very likely according to the definitions listed below:

Not Related

An adverse event that is not related to the use of the product under study.

Doubtful

An adverse event for which an alternative explanation is more likely, eg, concomitant drug(s), concomitant disease(s), and/or the relationship in time suggests that a causal relationship is unlikely.

Possible

An adverse event that might be due to the use of the product under study. An alternative explanation, eg, concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Probable

An adverse event that might be due to the use of the product under study. The relationship in time is suggestive (eg, confirmed by dechallenge). An alternative explanation is less likely, eg, concomitant drug(s), concomitant disease(s).

Very Likely

An adverse event that is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, eg, concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (eg, it is confirmed by dechallenge and rechallenge).

Special Situations

Safety events of interest for a product under study that require reporting for safety evaluation include, but are not limited to:

- Drug exposure during pregnancy (maternal/paternal)
- Exposure to a medicinal product from breastfeeding
- Off label use of a medicinal product
- Overdose of a medicinal product
- Suspected abuse/misuse of a medicinal product
- Inadvertent or accidental exposure to a medicinal product (eg. occupational exposure)
- Any failure of expected pharmacological action (lack of effect)
- Medication error involving a medicinal product (with or without patient exposure to the medicinal product e.g. name confusion)

- Suspected transmission of any infectious agent via administration of a medicinal product
- Unexpected therapeutic or clinical benefit from use of a medicinal product

These safety events may not meet the definition of an adverse event; however, they are treated in the same manner as adverse events.

Special situations for a product under study should be recorded in the CRF. Any special situation that meets the criteria of a serious adverse event should be recorded on a Serious Adverse Event Report Form and reported to the sponsor within 24 hours of them becoming aware of the event.