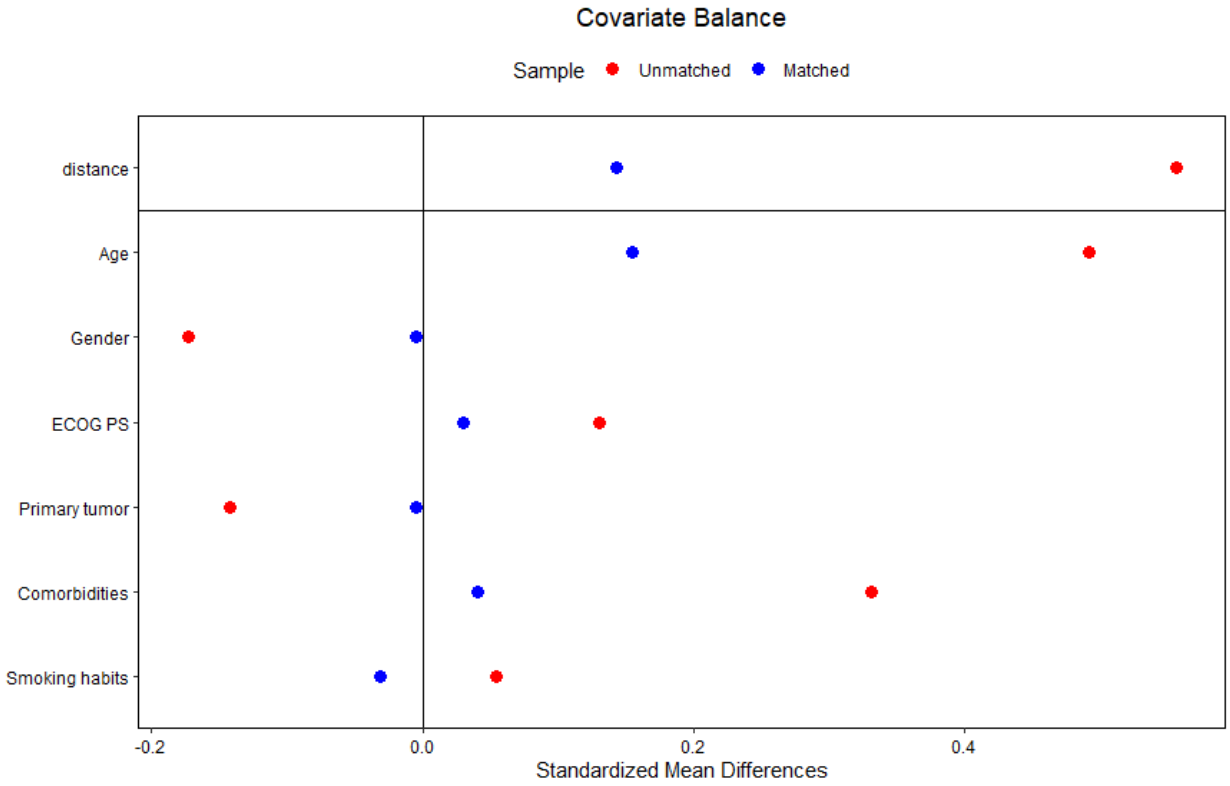
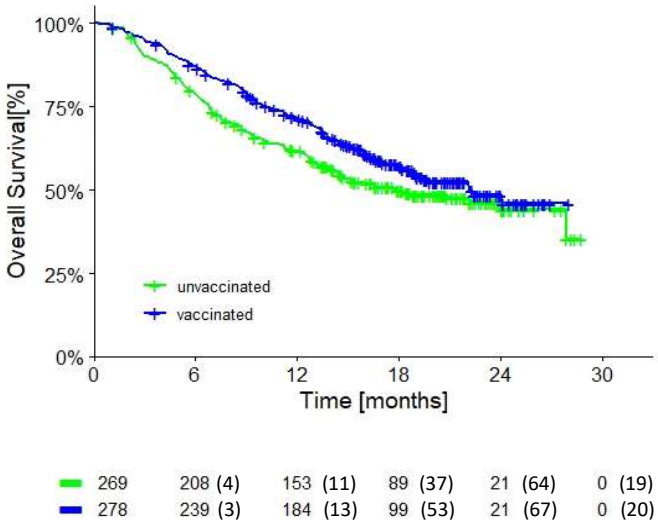
Supplementary Files

Supplementary Figure S1 – Standardized mean differences (SMD) of the propensity score analysis.

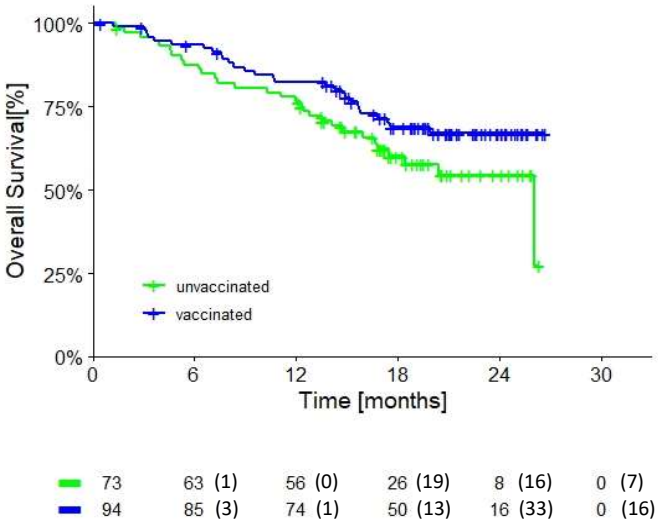


[Greifer, N. (2023) cobalt: Covariate Balance Tables and Plots. R package version 4.4.1.9002]

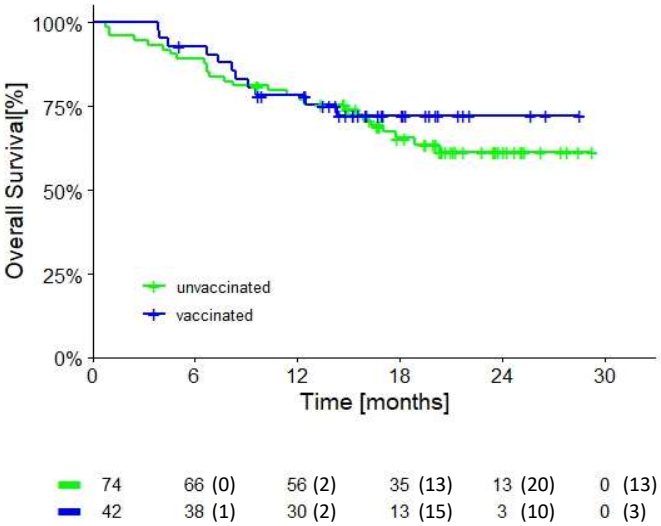
Supplementary Figure S2 – Overall survival (OS) by primary tumor site and vaccination status.



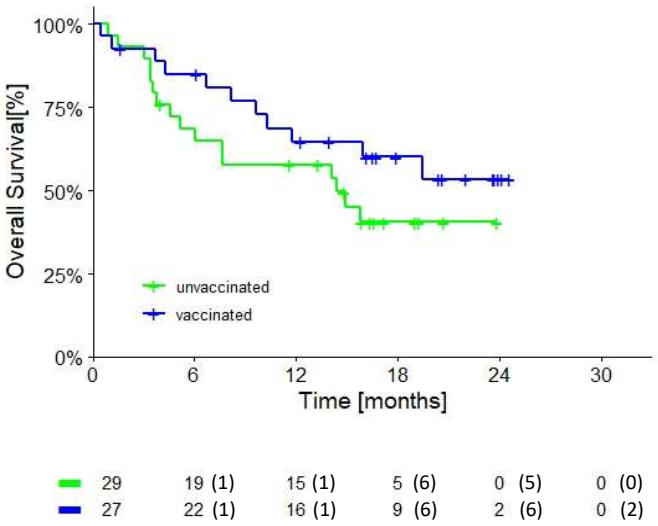
a. Lung cancer



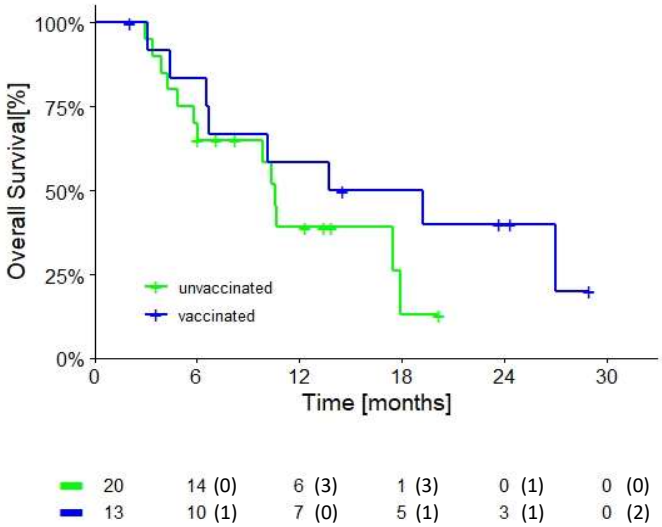
b. Renal cancer



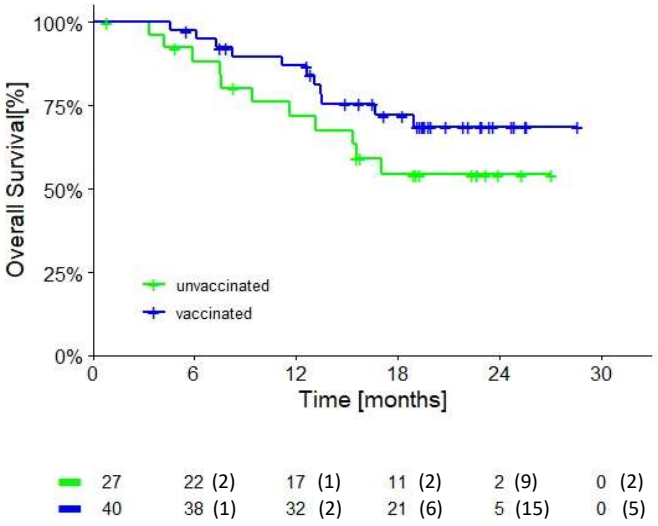
c. Melanoma



d. Urothelial cancer



e. Head&Neck cancer



f. Other primary tumors

Supplementary Table S3 – Median progression-free survival (mPFS) by primary tumor site and vaccination status.

	Vaccine yes mPFS in months (C·I)	Vaccine no mPFS in months (C·I)	HR (95% CI) (vaccinated vs unvaccinated)
Lung	11·8 (9·5-14·1)	8·5 (7·0-10·0)	0.84 (0.68-1.04)
Kidney	10·1 (3·6-16·7)	11·1 (7·4-14·8)	0.87 (0.59-1.28)
Melanoma	20·0 (15·8-24·2)	Not reached	1.23 (0.71-2.15)
Urothelial	8·3 (0·3-16·2)	7·7 (3·9-11·4)	0.76 (0.39-1.47)
H&N	5·7 (0·0-16·3)	6·6 (5·2-7·9)	0.70 (0.31-1.61)
other	Not reached	11·9 (0·0-25·2)	0.66 (0.33-1.30)

mPFS = median progression-free survival; H&N = head and neck; NE = not evaluable

Supplementary Table S4 – Objective response rate by primary tumor site and vaccination status.

	Vaccine yes N° of CR + PR (ORR)	Vaccine no N° of CR + PR (ORR)	OR (95% CI) (vaccinated vs unvaccinated)
Lung	100 (35·5%)	86 (30·9%)	1.23 (0.86-1.75)
Kidney	31 (33·0%)	24 (32·4%)	1.03 (0.53-1.96)
Melanoma	17 (37·8%)	27 (36·5%)	1.06 (0.49-2.27)
Urothelial	14 (50·0%)	7 (24·1%)	3.14 (1.02-9.71)
H&N	5 (38·5%)	5 (25·0%)	1.87 (0.41-8.47)
other	19 (47·5%)	9 (33·3%)	1.81 (0.66-4.98)

CR = complete response; PR = partial response; ORR = objective response rate; H&N = head and neck

Supplementary Table S5 – Disease control rate (DCR) by primary tumor site and vaccination status.

	Vaccine yes N° of CR + PR + SD (DCR)	Vaccine no N° of CR + PR + SD (DCR)	OR (95% CI) (vaccinated vs unvaccinated)
Lung	210 (74·5%)	181 (65·1%)	1.56 (1.09-2.25)
Kidney	71 (75·5%)	51 (68·9%)	1.39 (0.71-2.75)
Melanoma	36 (80·0%)	51 (68·9%)	1.80 (0.75-4.35)
Urothelial	19 (67·9%)	17 (58·6%)	1.49 (0.50-4.41)
H&N	8 (61·5%)	13 (65·0%)	0.86 (0.20-3.66)
other	31 (77·5%)	21 (77·8%)	0.98 (0.31-3.18)

CR = complete response; PR = partial response; SD = stable disease; DCR = disease control rate; H&N = head and neck

Indication for Influenza Vaccination During Cancer Immunotherapy with immune checkpoint inhibitors. A multicentre, prospective, observational study: **INVIDIa-2**

Study code:	**INVIDIa-2**
Version and date	Version 1.1 of 08.05.2019
Sponsor	FICOG Federation of Italian Cooperative Oncology Groups *c/o* AIOM - Via Enrico Nöe, 23 - 20133 - Milan Taxcode 97718100155 - VAT no. 09892430969 segreteria.ficog@gmail.com – ficog@messaggipec.it
Principal Investigator	Dr Melissa Bersanelli MedicalOncology
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AUTHORISATION:

I have read the study protocol and approve its content and corresponding procedures. I hereby declare that I will conduct the study in compliance with all applicable regulations and the ethical and scientific aspects set forth in the Declaration of Helsinki and applicable standards of good clinical practice.

Coordinating investigator
Dr Melissa Bersanelli

Signature
[signed]

Investigator of the satellite centre

Dr

Signature

INDEX

SYNOPSIS

Study title	Indication for Influenza Vaccination During Cancer Immunotherapy with immune checkpoint inhibitors. A multicentre, prospective, observational study: INVIDIa-2
Sponsor	FICOG - Federation of Italian Cooperative Oncology Groups
Version and date	Version 1.1 of 08.05.2019
Objectives of the study	**The primary objective** of the study is therefore to investigate the utility of vaccine administration in a specific subgroup of cancer patients, by evaluating the impact of vaccination on the incidence of influenza-like illness in vaccinated subjects compared to patients who are not vaccinated. **The secondary objectives** of this study are: • to describe vaccine cover in this population of cancer patients • to describe the vaccine's impact in reducing the severity and fatality of influenza-like illness when developed in vaccinated compared to non-vaccinated subjects • to describe the impact of vaccination on the activity and efficacy of the cancer therapy and on the course of the disease, by comparing vaccinated and non-vaccinated patients • to describe the impact of vaccination on the frequency of irAEs during cancer immunotherapy, by comparing vaccinated and non-vaccinated patients • to describe the incidence and severity of local adverse reactions to the vaccine during therapy with CKI • to describe the frequency with which pharyngeal and/or nasal swabs are used in clinical practice to confirm the diagnosis of the influenza-like illness in the cancer patients enrolled (regardless of vaccination) and the laboratory confirmation rate for the cases for which the test was performed.

Study design	The INVIDIa-2 study is a multicentre, prospective, observational study with a transverse nature: all patients with advanced solid tumours are eligible for enrolment, provided they are candidates for treatment with CKIs, either as a single agent or in combination with other CKIs and/or other medicinal products, including in clinical trials on medicinal products.
Number of patients and centres	The study will involve approximately 100 clinical sites in Italy and is expected to enrol 974 patients
Inclusion and exclusion criteria	The inclusion criteria for the study are: • > 18 years of age • Signing of the informed consent form for the study • Subject is cooperative and compliant vis-à-vis influenza-like illness monitoring, and is willing to provide the date of any influenza vaccination (which the patient may have at his/her discretion) and the type of vaccine used • Diagnosis of advanced or metastatic solid tumour • Indication to start systemic therapy with CKIs (PD-1 inhibitors, PD-L-1 inhibitors, or CTLA-4 inhibitors) as a single agent or in combination with other CKIs or with other cancer treatments (including chemotherapy agents, tyrosine kinase inhibitors, other monoclonal antibodies or compounds with different mechanisms of action) by 31 January 2020, as part of routine clinical practice or clinical studies with an immunotherapy agent, OR systemic immunotherapy already in progress at the time of enrolment, provided it was started no earlier than 1 April 2019 The exclusion criteria for the study are: • Blood cancers and/or lymphomas • Immunotherapy treatment commenced prior to 1 April 2019 • Treatment as part of a blinded clinical trial in which an immunotherapy arm is compared with an arm not receiving immunotherapy (patients treated in blinded studies are eligible provided immunotherapy is administered in all treatment arms, either alone or in combination with other agents)

Endpoints	**The primary endpoint** is the **incidence of influenza-like illness,**weighted according to the time of exposure and calculated as *"*time to influenza-like illness" (TTI), compared in the group of vaccinated patients with patients who are not vaccinated. *"*Influenza free survival" (IFS) will also be calculated as an indirect estimate of the incidence of influenza-like illness. A sub-analysis of influenza-like illness will focus on the peak outbreak period, which will be identified *a posteriori* through the National Monitoring Centre of the *IstitutoSuperiore di Sanità* [Italian National Institute for Health] during the influenza season considered, in order to restrict the possibility of the misclassification of cases of influenza-like illness, thereby increasing the likelihood of the pertinence of the symptoms observed to actual influenza infection during the epidemic peak. The **secondary endpoints** regarding vaccination and the severity and fatality of influenza will be measured in terms of: ■ vaccine cover rate (% of patients vaccinated in the study population) ■ incidence of bacterial complications (as shown by culture tests) ■ incidence of infection recrudescence (recurrence of influenza symptoms following the complete resolution of influenza-like illness)

	■ incidence of clinically diagnosed complications/ superinfections during the influenza episode (otitis, pneumonia, pharyngo-tonsillitis, other) ■ incidence of hospital admissions for influenza-like illness or associated complications ■ need for intravenous therapy for influenza-like illness ■ duration of the influenza symptoms ■ influenza-like illness fatality rate ■ usage rate for the quick test / nasal/pharyngeal swab or viral serology in subjects with influenza-like illness ■ confirmation rate using the quick test/ nasal/pharyngeal swab or with viral serology of cases of clinically-diagnosed influenza-like illness. In addition, the following secondary endpoints regarding the cancer treatment will also be measured: • disease control rate (DCR); • objective response rate(ORR) • time to treatment failure(TTF) • progression-free survival (PFS) • overall survival (OS). The efficacy endpoints will be explored with subgroup analysis, more specifically on the basis of the major disease groups, such as lung cancer, melanoma, renal cell cancers, others, and in the group of patients who started immunotherapy no earlier than 1 August 2019, in order to assess any interference of the vaccine with the onset of immune-mediated response to the cancer treatment. For each of the endpoints described, the group of vaccinated subjects will be compared with the non-vaccinated group.

	Lastly, the following will be included as further secondary endpoints: o immune-related toxicity, in terms of irAEs during therapy with CKIs, comparing vaccinated and non-vaccinated subjects o incidence of local adverse reactions to the vaccine at the administration site, assessed in the vaccinated subject group. **Subgroup analyses** will be conducted, on the basis of the characteristics listed below: > primary tumour (in particular, lung cancer) > age (with various cut-offs: 71 years, as identified by the retrospective study; 65 years, the age after which vaccination is recommended and provided by the Italian National Health Service) > type of cancer treatment (more specifically, immunotherapy alone vs chemo-immunotherapy combinations) > performance status (ECOG PS 0, 1, 2, 3) > co-administration of pneumococcal vaccine > timing of the respective administration of the vaccine and the immunotherapy (in terms of both sequence - vaccine followed by the start of the immunotherapy or vaccine administered when the immunotherapy is already in progress - and intervening interval)
Sample size and statistical analysis	On the basis of the data obtained in the INVIDIa retrospective study,considering an expected influenza-like illness incidence of 12% for non-vaccinated subjects, having established a 50% reduction in the incidence of influenza-like illness (ILI) in vaccinated patients as the desirable objective of vaccination, having established a statistical power of 80% and statistical significance with $p < 0.05$, and hypothesising vaccine cover of 75% (WHO recommendations for at-risk categories), an overall study sample of **974** patients was calculated. Time to influenza-like illness (TTI) will measure the impact of vaccination on the incidence of ILI. A log-rank test comparing the two curves (TTI in the group of vaccinated versus not vaccinated patients) <0.05 will be considered as significant. Other tests related to the secondary endpoints will be interpreted in an explorative approach. Influenza-free survival (IFS) will also be calculated as an indirect estimate of the incidence of ILI. The times to the events will be analysed using the Kaplan-Meier method and the curves will be compared using the log-rank test.

ABBREVIATIONS

European Centre for Disease Prevention and Control (ECDC)

Inactivated influenza vaccines (IIV)

Trivalent influenza vaccines (TIV)

Adverse event (AE)

Adverse reaction (AR)

Immune-related adverse events (irAEs)

Immune checkpoint inhibitors (CKI)

Disease control rate (DCR)

Objective response rate (ORR)

Time to treatment failure (TTF)

Progression-free survival (PFS)

Overall survival (OS)

Performance Status (PS)

Common Terminology Criteria for Adverse Events (CTCAE)

Serious adverse event (SAE)

Immune-related serious adverse event (irSAEs)

Influenza-like illness (ILI)

Time toinfluenza-like illness(TTI)

Influenza free survival (IFS)

1. INTRODUCTION AND RATIONALE

1.1 Influenza and vaccination

1.1.1 Influenza epidemiology in Italy

Influenza is an acute respiratory disease caused by influenza virus infection transmitted by airborne droplets. It is a seasonal illness that, in the Western hemisphere, occurs during the winter. Influenza virus was first isolated in humans in 1933 in England (although influenza had already been isolated from both chickens and pigs). Since, three different types, constituting the Orthomixovirus genus, have been identified: type A and type B viruses, which are responsible for classic influenza symptoms, and type C, which is of little clinical relevance (usually asymptomatic).

Influenza epidemiology is based on the marked tendency of all influenza viruses to acquire variations in their surface proteins. As a result, the composition has to be updated every year and surveillance is fundamental to preparing the vaccine for the following season, on the basis of the strains that were most prevalent during the last epidemic period.

Influenza virus, which is usually acquired through contact with other infected individuals, is found in both saliva and in respiratory tract mucus and can penetrate into the body through the mucosae (mouth, eyes and nose). The virus can be transmitted, through airborne droplets, from the time of contagion to three-four days after the first symptoms, which present one to four days after infection. This means that the virus can also be transmitted by apparently healthy individuals. It spreads very readily in crowded places.

The frequency with which cases of influence occur in Italy, despite being considerably different from one season to the next, is on average approximately 8% (range: 4-12%) of the general population, every year, whereas in the 0-14 years range, that most affected, the average incidence is about 22%. On the other hand, the least-affected age range is that of the elderly (over 64), probably due to their reduced social exposure[1-2]; however, this age range is that most prone to complications, where severe cases and mortality peak between the age of 64 and 74 years[3].

In Italy, influenza occurs during the winter, mainly between December and March; there is usually a peak epidemic period, lasting 1-2 weeks, in which there is the highest incidence of case reports (usually between late December and early January). Influenza-like illness usually resolves in five-seven days, although cough and general malaise can persist for two weeks or more. Influenza is characterised by a sequence of systemic and respiratory symptoms: fever (lasting approximately three days), with a sudden onset, accompanied by shivers, bone and muscle pain, headache, severe general malaise, sore throat, cold, dry cough and conjunctivitis. Fever is usually higher in type A infections and remains lower in those caused by type B influenza. In the elderly (over 75 years), fever usually remains low, the onset of symptoms is gradual and above all involves weakness, joint pain and, in some cases, mental confusion[4].

The diagnosis of influenza is usually based on clinical symptoms. In inpatients or outpatients at risk of complications, the clinician may decide, at his/her discretion, to perform a pharyngeal and/or nasal swab for laboratory confirmation (PCR method, quick quality test for type A/B influenza), despite its low predictive capacity[5-6].

The complications of influenza range from bacterial pneumonia, to dehydration, to a worsening of existing conditions. They are more common in the elderly and in the presence of risk conditions or chronic illness, such as tumours, especially when in the advanced stage and when the patient is subjected to immunosuppressive treatments, such as chemotherapy[4].

Each year, the epidemiology of infection in Italy is closely monitored. The 2015/16 influenza season was characterised by a relatively low cumulative incidence (82 cases per 1,000 patients). 89 severe cases and 32 serologically-confirmed deaths were reported by 13 regional and autonomous provincial authorities. The average age of the severe cases was 57 years (range 0-95); whereas the average age amongst the deaths was 59 years (0-88). 76% of the severe cases and 63% of the deaths reported presented at least one existing chronic condition, for which influenza vaccination is recommended and just 9.7% had been vaccinated[5].
During the following influenza season, 2016/17, the cumulative incidence observed was 93 cases per 1,000 patients.

This influenza season was therefore characterised by an average cumulative incidence (considering that there were 116 cases per 1,000 during the 2004-2005 season and 99 cases per 1,000 patients during the 2009-2010 pandemic season). As usual, the incidence dropped with age, and reached the lowest value in the elderly (87 cases per 1,000 patients amongst individuals of between 15 and 64 years, and 51 cases amongst those over 65). The impact of this season, in terms of the number of severe cases and deaths due to confirmed influenza, was of average entity, and therefore, more severe cases and deaths were reported than in the previous season. More specifically, during the 2016/17 season, 162 severe cases and 68 confirmed deaths from influenza were reported by 11 regional and autonomous provincial authorities. The median age of reported cases was 71 years (range 3-94). 83% of severe cases and 100% of the deaths reported presented at least one existing chronic medical condition. The most common conditions were chronic respiratory diseases (48.3%), followed by cardiovascular diseases (46.7%), diabetes (31.7%) and obesity (18.3%). The annual report does not specifically mention cancers[6-7].
The 2017/18 season influenza epidemic in Italy was of a very high intensity, in terms of both total incidence and number of severe cases and deaths. The epidemiology was characterised by dominant type B strains; more than half of all the severe and serious cases, and especially the deaths, were however caused by virus A/H1N1. When the severe cases in subjects under and over 65 years are considered separately, a significant difference can be observed in the viral types: in subjects under 65 years of age, approximately 75% of cases were caused by type A and just 25% by type B; over 65 years of age, the situation is reversed with 75% of cases caused by type B and 25% by type A[8].

1.1.2 Clinical definition of influenza-like illness

Since the 2014-2015 season, the definition of "*influenza-like illness*" has been changed in line with that adopted in Europe by the European Centre for Disease Prevention and Control (ECDC), defined according to the European Commission Decision of 28/04/2008. In order to guarantee the greatest possible homogeneity in detection, a clinical definition of "influenza-like illness" is provided that includes the acute presentations with general and respiratory symptoms[9].

A subject can be considered as having influenza-like illness when he/she presents a **sudden and rapid** onset of **one or more** of the following general symptoms:

- fever or low-grade fever
- malaise and/or listlessness
- headache
- myalgia

and at **least one** of the following respiratory symptoms:

- cough
- sore throat
- dyspnoea.

This is the definition adopted for the purposes of this study

1.1.3 Influenza vaccination

Vaccination is the most efficacious form of influenza prevention. The World Health Organisation (WHO) and the 2017-2019 Italian National Vaccination Plan consider 75% influenza vaccination cover as the minimum target and 95% as the optimum target in subjects over 65 years of age and in groups at risk[10].

The influenza vaccines available in Italy are inactivated* and therefore do not contain any whole active viral particles. They can be classified into the following types:

> inactivated influenza vaccines (IIV): The inactivated influenza vaccines currently authorised for use in Italy are a mixture of split virus vaccines and subunit vaccines. In split vaccines, the virus has been disrupted by a detergent. In subunit vaccines, the HA and NA proteins have been further purified with the removal of other viral components. Trivalent influenza vaccines (TIV) containing two type A viruses (H1N1 and H3N2) and one type B virus and quadrivalent vaccines containing two type A viruses (H1N1 and H3N2) and two type B viruses are currently available in Italy. They do not contain

adjuvants.

>Adjuvanted inactivated influenza vaccines: one of the trivalent products contains the adjuvant MF59, an oil-in-water emulsion in which squalene constitutes the oily phase. The seasonal vaccines adjuvanted with MF59 are currently authorised for the immunisation of subjects > 64 years of age. The function of adjuvants is to boost the immune response to the vaccine, making them particularly indicated for the immunisation of elderly and poorly-respondent subjects.

(* *with the exception of a quadrivalent influenza vaccine constituted by live attenuated influenza viruses, known as LAIV, which is administered by the nasal route and that, although it is authorised in Italy, it is rarely used. This vaccine will not be considered for the purposes of this study, because it is contraindicated in potentially immunodepressed subjects and therefore, by definition, cannot be administered to subjects eligible for inclusion in this protocol).*

The definition of *"**vaccinated subject**"* adopted for this study is a patient who has received an inactivated influenza vaccine approved for clinical use in the 2019/2020 influenza season, regardless of the type of vaccine (trivalent, tetravalent, adjuvanted or non-adjuvanted), during the vaccination season considered, regardless of when the vaccine was administered in relation to the patient's enrolment in this study and in relation to the start of the cancer immunotherapy treatment.

In Italy, vaccination is offered actively and gratuitously to those individuals who, on account of their personal conditions, have a greater risk of encountering complications if they get influenza. According to the Italian Ministry of Health Circular "Prevention and control of influenza: recommendations for the 2018-2019 season", cancer patients are specifically mentioned amongst those subjects for whom influenza vaccination is recommended[10-11].
The period identified for influenza vaccination campaigns is, on account of the climatic situation in Italy and the time trends observed for influenza epidemics, the autumnal period, between late October and the end of December[12].
However, the administration period can vary due to delays in the effective distribution of the vaccine to all areas of the country and the availability of the vaccine in pharmacies and from general practitioners' surgeries and may also include later months[15].

In the general population, vaccine coverage for 2017/18 was 15.3% (15.1% for the 2016/17 season). Cover amongst subjects over 65 years of age rose from 52.0% in the previous season to 52.7% for the most recent data. In order to considerably reduce influenza-induced morbidity, complications and mortality, it was considered necessary to achieve vaccine cover of at least 75% in the target population groups, especially in elderly subjects over 65 years of age and in high-risk subjects of all ages. Vaccine-induced protection commences two weeks after inoculation and persists for a period of six-eight months[16].

Immunodepression does not constitute a contraindication to the administration of influenza vaccination. As the seasonal influenza vaccine does not contain live viruses, rather only surface antigens of the influenza virus, it is also recommended in immunodepressed individuals because of the effect of immunosuppressive therapies or the effect of other medical conditions (including cancer). The administration of influenza vaccine is considered to be safe in immunodepressed patients and the Ministerial Circular regarding the prevention of seasonal influenza explicitly mentions those individuals with illnesses that compromise antibody production and those with drug-induced immunodepression amongst those to whom vaccination must be offered[16].

In the case of autoimmune diseases, in which there is an overactivation of the immune system, the ministerial recommendation is for the specialist to evaluate each patient thoroughly on a case-by-case basis. The recommendation in these cases is to use alternative prophylaxis, such as the vaccination of the subject's immediate family, the use of antivirals and behavioural prophylaxis.

The vaccine is usually well tolerated, but is subject to close pharmacovigilance.

Influenza vaccination can also be associated with a number of undesirable effects, the frequency of which depends on the type of vaccine, how it is administered and the age of the person vaccinated. Inactivated vaccines, which are administered by intramuscular injection, often cause local reactions such as tenderness and redness around the injection site and, less frequently, fever, muscle or joint pain orheadache. These symptoms are usually modest and do not require medical treatment, as they resolve spontaneously, or with symptomatic treatment.

An adverse event is considered as any undesired effect that may present during or after the administration of the vaccine with which it does not necessarily have a causal relationship.

An adverse reaction (AR), on the other hand, is generally taken to mean any harmful or unintended reaction to a medicinal product used at the doses usually administered in humans for prophylactic, diagnostic or therapeutic purposes or to restore, correct or modify physiological functions. Unlike adverse events, adverse reactions to the vaccine are characterised by a suspected causal relationship between the vaccine and the event. This is the definition adopted for the purpose of this study for adverse reactions at the injection site (local AR), probably associated with the influenza vaccine. As systemic adverse events to the vaccine are immune-related (they are triggered by an immune stimulation mechanism), in this study they will be included in the definition of immune-related adverse events (irAEs) that are potentially attributable to the cancer immunotherapy (see below).

In rare cases, influenza vaccines containing inactivated virus can cause allergic reactions such as urticaria, rapid swelling at the inoculation site, asthma or severe systemic (generalised) allergic reactions, due to hypersensitivity to certain components of the vaccine.

Inactivated vaccines contain dead whole viruses or parts of viruses (the surface antigens hemagglutinin and neuraminidase, viral subunits) that cannot cause any illness. The influenza vaccine in itself can cause short-term side effects characterised by influenza-like symptoms, but that are very mild.

Local reactions usually present within the few days immediately after vaccination. The most common systemic reactions (such as general malaise, fever and myalgia) usually present within 6-12 hours of vaccine administration and last 1 to 2 days. Serious adverse events following influenza vaccine administration are reported every year (approximately 20-24% of all reports), but a certain causal relationship with the vaccine has not been demonstrated[13].

1.2 Cancer immunotherapy

The main mechanism by which tumours evade the immune system is by using immune checkpoints, that by the transduction of molecular signals activated by the bond between cell surface molecules, are physiologically able to reduce the activation of the T-cells and their effector functions in the lymphatic and peripheral tissues[17].

The advent of novel cancer immunotherapy, involving the development of antibodies directed against these specific immune checkpoints, and therefore able to reactivate the endogenous cell-mediated immune response against the tumour, has undoubtedly changed and will continue to modify the classification paradigm for the immunological risk of cancer patients.

Indeed, whereas most chemotherapy treatments cause (often direct) immunodepression, through medullary suppression, and contribute to the already debilitated condition in which tumour patients find themselves from the time they are diagnosed with advanced cancer, novel immunotherapy aims, on the contrary, to reactivate immune response, by preventing T-cell inhibition and anergy. The immune system is therefore normalised, which no longer justifies the univocally "immunosuppressed" vision of cancer patients.

The first immunotherapy that was seen to improve survival in advanced disease, in the case of melanoma, was the monoclonal antibody ipilimumab, a cytotoxic T lymphocyte antigen-4 inhibitor (CTLA-4 inhibitor). PD-1 (programmed death receptor) inhibitor antibodies such as nivolumab and pembrolizumab, and PD-L1 (PD-1 ligand, also known as CD274 or B7-H1) inhibitors, such as atezolizumab, durvalumab and avelumab were subsequently also tested and approved for clinical practice in lung cancer, melanoma, renal cell carcinoma, urothelial tumours, cancers of the head and neck and Merkel cell carcinoma. Further indications in various solid tumours are currently being studied for these and other recently-developed antibodies, as single agents or in combinations, with or without chemotherapy.

The mechanism of action of these new medicinal products consists in removing T cell inhibition by blocking the inhibiting checkpoints such as CTLA-4/CD80, CTLA-4/CD86 and PD-1/PD-L1. By interrupting these molecular pathways, the T cells are able to activate, proliferate, produce cytokines and exert their cytotoxic effector functions inside the tumour microenvironment. The side effects of immune checkpoint inhibitors are associated with immunological activation and present as autoimmune reactions towards normal tissues[17].

The assessment of disease response to the treatment in the case of immunotherapy and the main criterion for evaluating the outcome of therapy are parameters that are still under discussion as the immune checkpoint inhibitor response patterns differ from those of cytotoxic treatments. More specifically, the progression-free survival and response rate assessed using standard radiological criteria do not appear to constitute reliable predictors of survival, the most reliable treatment outcome indicator, which is, however, in turn, burdened by very long timeframes and can be influenced by the subsequent treatments received by the patient for the disease.

Some patients experience pseudo-progression, which has been reported in 10-20% of cases, which justifies waiting for a delayed response after initial apparent disease progression and therefore warrants continuing the treatment even after radiological progression in those cases in which the patient obtains clinical benefit, defined as remission or absence of disease symptoms in the presence of good treatment tolerance. Alternative radiological response assessment criteria, known as immune-related response criteria, have been tested and, in some cases, validated. The time to treatment failure can, in turn, be used as a surrogate efficacy endpoint, representing in particular all those cases in which the clinician decides to continue the treatment even after presumed progression, on the basis of clinical benefit[18].

1.2.1 Immune checkpoint inhibitors and influenza vaccination

The prevention of influenza infection and, consequently, influenza-like illness, is fundamental for individuals who are immuno-deficient because of cancer or its immunosuppressing treatment. Viral infections in cancer patients often have high morbidity and mortality rates and influenza has fatality rates reported as being as high as 9% in this population[19-20]. Although live vaccines are not used, due to the risk of reactivation in immunodepressed subjects, split vaccines are permitted and often recommended[21-23]. However, the greater the degree of immunosuppression, the lower the probability that the patient will respond adequately to immunisation[19]. Some data demonstrating serological response to influenza vaccination in high-risk cancer patients have been reported[24-25]; however, it is still unknown to what extent this serological response protects the individual from infection, as these studies did not evaluate morbidity clinically[26]. The limited clinical data available for immunocompromised patients were summarised in a meta-analysis that shows significantly lower percentages of influenza-like illness amongst vaccinated individuals[27].

However, the immunocompetence of cancer patients can vary greatly depending on the type of tumour and the treatment regimens they receive. It is usually presumed that a patient with advanced cancer treated with chemotherapy is a frail and immunocompromised patient who, regardless of age, should be included in the vaccination strategy. Indeed, influenza vaccination is currently recommended for all cancer patients, especially those with lung cancer, considering the susceptibility to and high mortality from infectious respiratory diseases[21-23,28].

In recent years, novel immunotherapy with immune checkpoint inhibitors (CKI) has constituted a fundamental innovation in the systemic treatment of advanced solid tumours, such as melanoma, lung cancer and renal

cancer, for which it constitutes current clinical practice in Italy, as well as in urothelial tumours, cancer of the head and neck and certain gastrointestinal cancers, for which it is still currently being tested[29]. This new oncological population has not yet been specifically studied with regard to susceptibility to infections. The aim of the new immunotherapy is to restore cellular immunocompetence. It is therefore plausible that patients treated with CKIs may be more immunocompetent than those treated with chemotherapy.

Despite the positive recommendation, in actual fact there are no certain data supporting the use of split vaccines during cancer immunotherapy, as its safety with immune checkpoint inhibitors has not been proven. The counselling of patients in this care setting is not based on scientific evidence, but so far only on clinical common sense and on the general classification of the cancer patient as a subject at risk of infectious complications. It is merely on the basis of the pharmacological characteristics of CKIs that influenza vaccination has been considered potentially safe in patients receiving cancer immunotherapy.

The phase 2 CAI84-004 study, which provides the only formal demonstration of the serological efficacy of the vaccines during immunotherapy with CKI, showed humoral response to influenza vaccine in melanoma patients treated with ipilimumab[30]. However, no data was provided on the actual clinical efficacy of influenza vaccination during CKI therapy, in terms of the incidence of influenza-like illness in vaccinated subjects, or on the vaccine's potential impact on the efficacy of the cancer immunotherapy. The cited study involved the administration of tetanus vaccine ten days before and influenza and pneumococcal vaccination one week after the start of cancer therapy with ipilimumab in patients with advanced melanoma. Humoral response was tested at baseline and in week 7 of treatment. Most patients experienced an increase in the levels of specific antibodies (humoral response) directed against the antigens of influenza virus B, A/H1N1 and A/H3N2, which were absent in the patients who were not vaccinated. No difference was observed in terms of antibody response in the group receiving 3 mg/kg of ipilimumab compared to the group receiving 10 mg/kg[30].

There are also very limited data available regarding nivolumab: influenza vaccination was permitted at any time during the treatment programme in the phase 1 CA209-003 study; however the corresponding endpoints were not included in the published study results[31].

Some serious adverse events following administration of the influenza vaccine during treatment with nivolumab and ipilimumab were reported in clinical studies, but there is no certain evidence of a direct relationship between these adverse events and the vaccine, or of the fact that these reactions were caused or exacerbated by the concomitant immunotherapy. More specifically, a review of the manufacturer's safety database identified 4 cases in which serious adverse events were reported for patients who received the influenza vaccine during combination immunotherapy (nivolumab-ipilimumab combination): three patients with melanoma died following fatal myocarditis, these same patients also developed myositis in one case and rhabdomyolysis in two cases; one patient with renal carcinoma had severe rhabdomyolysis that, however, resolved positively[32].

One recently-published, small, prospective, case-control study suggested that vaccination against seasonal influenza may increase the rate of serious irAEs in patient receiving immunotherapy. This evidence, which is undeniably limited by the small sample size (23 patients compared with a control cohort of 10 individuals), provides the first doubt regarding a possible contraindication to influenza vaccination in patients treated with CKI. The vaccine was associated with a higher rate of irAEs than expected on the basis of the literature, the most common being: rash (13%), joint pain (13%), colitis (9%), encephalitis (9%), hypothyroidism (4%), pneumonia (4%) and neuropathy (4%). Overall, more than half of all patients reported an irAEs and 6 patients (26%) reported a serious irAE. The researchers suggested that, in patients treated with immune checkpoint inhibitors, due to the overactivation of the immune system, the vaccine may induce excessive responses, thereby triggering more frequent and more severe irAEs than anticipated[33].

It is interesting to note that the patients treated with CKIs described in this publication had a greater and more rapid serological response than the vaccinated control subjects. These data support the hypothesis that patients treated with CKIs are more immunocompetent, as the immunotherapy improves cell and humoral immunity. In these subjects, the influenza vaccine may cause considerable immune system overactivation, thereby also amplifying the adverse events caused by the CKI[32-33].

Another recent retrospective study conducted on a prospective population of 127 patients with lung cancer treated with nivolumab as part of an expanded access programme in Holland, did not reveal any significant differences in terms of irAEs between the vaccinated cohort and the cohort of non-vaccinated patients. The authors report an irAE incidence of 26% and 22% for vaccinated and non-vaccinated patients, respectively (odds ratio = 1.20, 95% confidence interval [CI] 0.51-2.65) and a serious irAE rate of 7% and 4 %, respectively (odds ratio = 2.07, 95% CI 0.28-15.43). In this population, in which the clinical efficacy of the vaccine was not explored, vaccine administration did not show any correlation with the treatment discontinuation rate or with the objective response to treatment[34].

Although, on the one hand, the safety of the vaccine is an essential requirement for the clinical recommendation to administer it, on the other hand, this kind of patient is known to be frail and prone to infections, especially when they have had surgery for lung cancer and have had disease relapse resulting in respiratory impairment. Negative counselling regarding vaccination in these subjects would undoubtedly require more certain evidence, considering the potential fatality of influenza infection and associated complications in these vulnerable conditions. The weight of the risk/benefit assessment in these cases must still be weighed up, as it calls for a case-by-case evaluation without any solid supporting literature. These controversial findings demonstrate the need for greater evidence regarding both the safety of vaccine administration during immunotherapy and the appropriateness of advising against the vaccine in a population in which its utility and need have not been specifically proven. Indeed, the impact that the decision of not administering the seasonal vaccine to the subgroup of cancer patients treated with medicinal products that stimulate the immune system is unknown.

Lastly, from the cancer therapy standpoint, certain considerations could theoretically discourage the use of the vaccine, with the intent to maintain unaltered the potential efficacy of the immune checkpoint inhibitor therapy. Indeed, as these cancer treatments act by activating the CD8+ cytotoxic T cells, the interference of the vaccine, which triggers CD4+ T cell (T-helper)-mediated response, is plausible with regard to the cytokine-induced, cell-mediated immune interactions. The effect of introducing a new viral antigen, albeit a dead one, into the immune system of individuals treated with CKI is, to a large extent, unknown. According to the concept of *"*foreignness*"*, viral antigens are thought to be more immunogenic that tumour antigens[35-40], and they could, therefore,divertT-cells response and potentially weaken anti-tumour response in favour of the antiviral reaction. The efficacy of the cancer treatment could therefore be reduced, especially if vaccination takes place during the early stages of CKI therapy, before immune response has been established.

1,3 Rationale

Considering the transverse unmet need for the counselling of outpatients receiving CKI therapy with regard to influenza vaccination, the Medical Oncology Unit of Azienda Ospedaliero-Universitaria di Parma sponsored a preliminary, exploratory study on a multicentre, retrospective sample, to analyse the efficacy of the influenza vaccine in this population, its potential impact on the severity and mortality of influenza-like illness and on the outcome of the cancer immunotherapy (the INVIDIa study)[41].

The INVIDIa retrospective study enrolled 300 patients with advanced cancer, primarily lung cancer, renal cell carcinoma and melanoma, during treatment with immune checkpoint inhibitors (PD-1-, PD-L1- or CTLA-4 inhibitors) during the 2016/17 influenza season. These patients, who were treated in 21 Italian cancer facilities, received or did not receive the vaccine in accordance with routine clinical practice, individual patient decision or the general practitioner's decision. The data were collected retrospectively at the end of the influenza observation season.

The results of the INVIDIa study, which were presented at various international events[42-43] and subsequently published in full, are unexpected and controversial: the incidence of influenza-like illness was seen to be morethan doubled amongst vaccinated patients (constituting 26.3% of the sample), reaching 24.1%, compared to 11.8% in the non-vaccinated patients ($p = 0.009$).

The clinical inefficacy of the vaccine was even greater in the subgroup of elderly patients (> 71 years, cut-off established on the basis of the median age of patients at the time the seasonal vaccine was administered), whereas the incidence of influenza-like illness was 6.1% amongst non-vaccinated subjects (in line with expectations) and 37.8% in vaccinated elderly subjects ($p < 0.0001$).

The vaccine appeared to be ineffective also with regard to the severity and fatality of the illness: although the study population had a serious medical condition (advanced cancer), the influenza-like illness did not cause significant complications, hospitalisations or relapses, duration was similar for both groups and it did not cause any deaths amongst either vaccinated or non-vaccinated subjects, as if the patients in the study were more

immunocompetent than expected.

As regards potential interactions with the immunotherapy, the study did not reveal any differences in disease response in the vaccinated compared to the non-vaccinated patients, with similar response rates, time to treatment failure and disease control rate. On the contrary, certain subgroups, such as that of elderly patients, appeared to obtain a better outcome from the cancer treatment if vaccinated (in terms of disease control rate, 84% vs 65%, $p = 0.04$) and even if they contracted influenza-like illness regardless of vaccination (53% response rate in those who contracted influenza vs 29% in those who did not, $p = 0.05$). Lastly, despite being immature, the preliminary survival analyses showed a statistically-significant difference, confirmed by the multivariate analysis, in favour of a better survival in patients who contracted influenza-like illness ($p = 0.02$), regardless of the vaccine, in the whole population.

Vaccine inefficacy was less pronounced in the lung cancer subgroup, with an incidence of 27% in vaccinated subjects compared to 17% in non-vaccinated subjects, but did not reach statistical significance ($p = 0.29$); furthermore, in this subgroup, both the univariate and the multivariate analysis showed a positive correlation between patient survival and the development of influenza-like illness and/or administration of the vaccine (presence of at least one of the variables; $p = 0.008$), suggesting that the antigen stimulation generated by the vaccine and/or infection may have a favourable role in patients receiving immunotherapy.

In conclusion, the INVIDIa study provided a number of interesting stimuli for consideration. Firstly, it can be hypothesised that the vaccine is not required in this cancer population (incidence of influenza-like illness higher in vaccinated patients rather than lower); secondly, it can be hypothesised that influenza-like illness is not a potentially compromising event in this subpopulation, given the modest severity and the null fatality of the influenza-like illness observed, regardless of vaccination status. Lastly, the study provides comforting hypotheses regarding the cancer treatment, whose efficacy does not appear to be affected in any way by the vaccine, and patient outcomes, which even seem to improve with vaccination and/or natural infection.

The INVIDIa study did not explore immune-related toxicity or the adverse reactions to the vaccine, and the hypothesis of a higher incidence of adverse events due to the concomitance of influenza vaccination and immunotherapy with CKI is yet to be confirmed. The study's main limitation lies in its retrospective nature, which makes it impossible the draw definitive conclusions with which to guide clinical practice.

On the basis of the questions generated and the hypotheses posed by the INVIDIa study, we designed a prospective study, named INVIDIa-2, to clarify how the counselling of cancer patients receiving immunotherapy regarding influenza vaccination should be directed.

1.4 Expected results

With this study we expect to obtain data of true clinical utility for **guiding, with scientific evidence, the counselling of patients with advanced cancer regarding the indication for influenza vaccination during cancer immunotherapy with immune checkpoint inhibitors.**

More specifically, we expect to be able to establish the vaccine's impact in reducing the morbidity and mortality of influenza-like illness in this subgroup of cancer patients, based on administration safety in terms of adverse reactions and immune-related adverse events and in terms of any impact on the outcome of the cancer immunotherapy in the study population.

On the basis of these data, we therefore expect to reach consensus regarding the appropriateness of recommending or not recommending the vaccine in this population.

2, OBJECTIVES AND ENDPOINTS

The main purpose of the INVIDIa-2 study will be the prospective description of the efficacy and safety of influenza vaccine in patients with advanced cancer, regardless of the primary site, during immunotherapy with CKIs.

The **primary objective** of the study is therefore to explore the utility of administering the vaccine in this specific subgroup of cancer patients, by evaluating the impact of vaccination on the incidence of influenza-like illness in vaccinated subjects compared to patients who are not vaccinated.

The **secondary objectives** of the study are:

- 1. to describe vaccine cover in this population of cancer patients
- 2. to describe the vaccine's impact in reducing the severity and fatality of influenza-like illness when developed in vaccinated compared to non-vaccinated subjects
- 3. to describe the impact of vaccination on the activity and efficacy of the cancer therapy and on the course of the disease, by comparing vaccinated and non-vaccinated patients
- 4. to describe the impact of vaccination on the frequency of irAEs during cancer immunotherapy, by comparing vaccinated and non-vaccinated patients
- 5. to describe the incidence and severity of local adverse reactions to the vaccine during therapy with CKI
- 6. to describe the frequency with which pharyngeal and/or nasal swabs are used in clinical practice to confirm the diagnosis of influenza-like illness in the cancer patients enrolled (regardless of vaccination) and the laboratory confirmation rate for the cases for which the test was performed.

In order to achieve the objectives listed above, the study endpoints are as described below.

The primary endpoint consists of the **incidence of influenza-like illness,** weighted according to the time of exposure and therefore calculated as the time to influenza-like illness (TTI), comparing the group of patients who receive the vaccine with those who did not.
TTI is defined as the interval time between the date of enrollment, and the date of first symptoms of ILI; for patients not experiencing ILI and still on treatment at the cut-off date of 31.04.2020 time will be censored at this date otherwise it will be censored at the date of death. The TTI for vaccinated and not vaccinated patients will be compared by the log-rank test.

*"*Influenza-free survival" (IFS) will also be calculated as an indirect estimate of the incidence of influenza-like illness. IFS is defined as the TTI but considering death as an event. IFS will be a secondary endpoint related to the Primary Objective.
A sub-analysis of the incidence of influenza-like illness will focus on the peak outbreak period, which will be identified *a posteriori* by the National Monitoring Centre of the *Istituto Superiore di Sanità* [Italian National Institute for Health] during the influenza season considered, in order to restrict the possibility of the misclassification of cases of influenza-like illness, thereby increasing, during the peak outbreak period, the likelihood of the pertinence of the symptoms observed to actual influenza infection.

The other **secondary endpoints,** regarding vaccination and the severity and fatality of influenza, will be measured in terms of:

- vaccine cover rate (% of patients vaccinated in the study population) – related to the secondary objective (1)
- incidence of bacterial complications (as shown by culture tests) – related to the secondary objective (2)
- incidence of infection recrudescence (recurrence of influenza symptoms following the complete resolution of influenza-like illness) – related to the secondary objective (2)
- incidence of clinically-diagnosed complications/ superinfections during the influenza episode (otitis, pneumonia, pharyngo-tonsillitis, other) – related to the secondary objective (2)
- incidence of hospital admissions for influenza-like illness or its complications – related to the secondary objective (2)
- need for intravenous therapy to treat the influenza-like illness – related to the secondary objective (2)
- duration of the influenza symptoms – related to the secondary objective (2)
- influenza-like illness fatality rate – related to the secondary objective (2)
- usage rate for the quick test / nasalorpharyngeal swab or viral serology in subjects with influenza-like illness – related to the secondary objective (6)

- confirmation rate using the quick test/nasal or pharyngeal swab or with viral serology of cases of influenza diagnosed clinically (confirmed influenza) – related to the secondary objective (6)

In addition, the following secondary endpoints regarding the cancer treatment will also be measured:

- disease control rate (DCR) – related to the secondary objective (3)
- objective response rate(ORR) – related to the secondary objective (3)
- time to treatment failure(TTF) – related to the secondary objective (3)
- progression-free survival (PFS) – related to the secondary objective (3)
- overall survival (OS) – related to the secondary objective (3)

The efficacy endpoints will be explored also in the group of patients who started immunotherapy no earlier than 1 August 2019, in order to assess any interference of the vaccine with the onset of immune-mediated response to the cancer treatment.

For each of the endpoints described, the group of vaccinated subjects will be compared with the non-vaccinated group.

Lastly, the following will be included as further secondary endpoints:

- immune-related toxicity, in terms of irAEs during therapy with CKIs, comparing the vaccinated and non-vaccinated subjects – related to the secondary objective (4)
- incidence of local adverse reactions to the vaccine at the administration site, assessed in the vaccinated subject group – related to the secondary objective (5)

Subgroups analyses for all endpoints will be conducted, on the basis of the characteristics listed below:

> primary tumour (in particular, lung cancer)
> age (with various cut-offs: 71 years, as identified by the retrospective study; 65 years, the age after which vaccination is recommended and provided by the Italian National Health Service)
> type of cancer treatment (more specifically, immunotherapy alone vs chemo-immunotherapy combinations)
> performance status (ECOG PS 0, 1, 2, 3)
> co-administration of pneumococcal vaccine
> timing of the respective administration of the vaccine and the immunotherapy (in terms of both sequence - vaccine followed by the start of the immunotherapy or vaccine administered when the immunotherapy was already in progress - and the intervening intervals)

3. STUDY DESIGN

The INVIDIa-2 study is a multicentre, prospective, observational study with a transverse nature: indeed, all patients with advanced solid tumours are eligible for enrolment, provided they are candidates for treatment with CKIs, either as single agents or in combination with other CKIs and/or other medicinal products, including in clinical trials on medicinal products. It is anticipated that the study will involve approximately 100 clinical centres in Italy, with an enrolment target of 974 patients (details regarding the calculation of the sample size are provided below).

Despite the risk of a bias regarding the recruitment of the patients to be vaccinated according to the doctor's clinical judgement, it was decided that the study should have an observational nature as it would be unethical to randomise patients to vaccine administration, considering, on the one hand, the lack of adequate evidence for considering vaccine administration during immunotherapy safe (and therefore an interventional arm imposing it arbitrarily) and, on the other, the common existence of further individual conditions that may pose an indication for vaccination for the patient regardless of the cancer therapy, such as age, comorbidity and functional respiratory deficits. The high frequency of these conditions in the population considered would prevent many patients from being randomised, therefore preventing adequate enrolment and causing even greater recruitment bias.

In order to measure the entity of the recruitment bias deriving from the study's observational nature, and to take it into due account when interpreting the results, the characteristics of the population in the two groups of vaccinated and non-vaccinated patients, respectively, will be collected and compared, in particular in terms of comorbidity, age and primary tumour.

The **vaccine season** to be studied, which will coincide with the enrolment period, will be from 1 October 2019 to 31 January 2020.

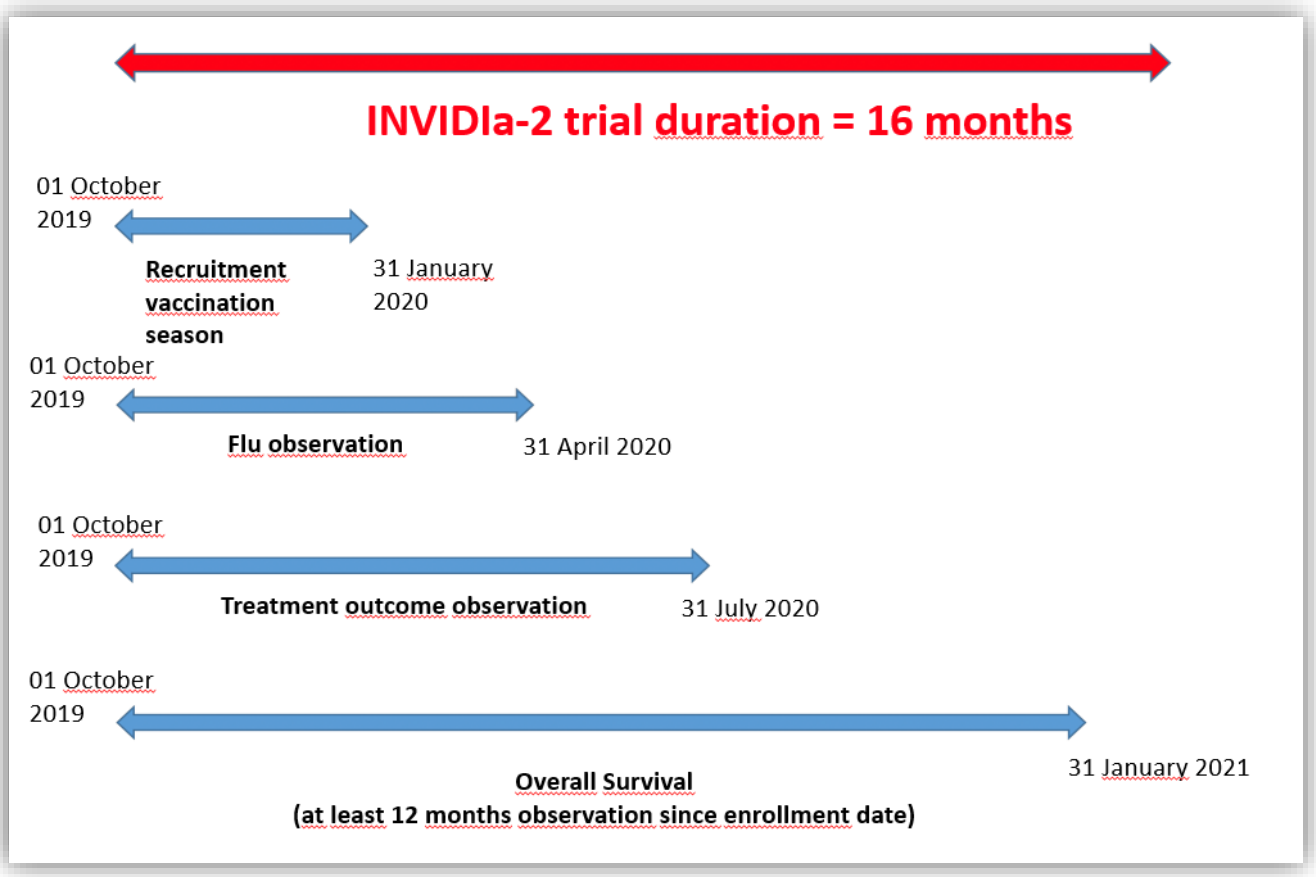
The corresponding **influenza season,** from October 2019 to April 2020 included, will constitute the influenza observation period for the enrolled patients, with a cut-off date of 30 April 2020 for the primary endpoint and the secondary endpoints regarding the severity and fatality of influenza-like illness, immune-related toxicity and adverse reactions to the vaccine.

The secondary endpoints regarding treatment outcomes will be investigated from October 2019 during the outcome observation period with a cut-off date of 31 July 2020, in order to guarantee at least 6 months' follow-up for all enrolled patients, considering the expected progression-free survival times and the timing of the radiological disease assessments during treatment.

The cut-off date for overall survival requires a period of at least 12 months from enrolment, with a cut-off date of 31 January 2021, given the forecast overall survival expected on the basis of the medical conditions included in the study and epidemiological data. The study is expected to last a total of 16 months, with a 4-month enrolment window. The extension of the survival observation period will be evaluated on the basis of the

number of cases recorded at the cut-off set.

Study schedule



3.1 Sample size

On the basis of the data obtained in the INVIDIa[41] retrospective study, considering an expected influenza-like illness incidence of 12% for non-vaccinated subjects, having established a 50% reduction in the incidence of influenza-like illness in vaccinated patients as the desirable objective of vaccination, having established a statistical power of 80% and statistical significance with $p < 0.05$, and based on WHO recommendations fora preferable vaccine cover of 75% for at-risk categories, an overall study sample of **974** patients was calculated[44]. This sample size was based on the use of the two-tailed log-rank test for the comparison of the two time to influenza-like illness curves hypothesising a hazard rate of 50%, which requires the occurrence of 65 events, a four-month rate of 88%, a loss to follow-up rate of 5% and adopting the most conservative situation (which coincides with that requiring the greater sample size) of vaccine cover of 75%.

4. INCLUSION AND EXCLUSION CRITERIA

Study enrolment will be proposed, prospectively and consecutively at all involved centres, to all advanced cancer patients, regardless of the primary tumour site, who are eligible for (or already being treated with) immunotherapy with CKIs (CTLA-4-, PD-1/PD-L1 inhibitors, either as single agents or combined with other CKIs or other medicinal products, even as part of a clinical trial on medicinal products), between 1 October 2019 and 31 January 2020.

During this enrolment period, it will also be possible to include in the study patients already receiving immunotherapy, provided they started the CKI recently, i.e. since 1 April 2019, in order to exclude patients who were already receiving immunotherapy during the 2018-2019 vaccination season *(with a margin of two months from any influenza vaccine they may have received during the previous season, which was theoretically possible up to 31 January 2018)*

Patients will be enrolled regardless of the decision to have or not have the influenza vaccine, which will be left to clinical practice, namely the individual patient's decision and general practitioner's advice.

Enrolment, which will start on 1 October 2019, will envisage the following **inclusion** criteria:

- Age > 18 years
- Signing of the informed consent form for the study
- Subject is cooperative and compliant vis-à-vis influenza-like illness monitoring, and is willing to provide the date of any influenza vaccination (which the patient may have at his/her discretion) and the type of vaccine used
- Diagnosis of advanced or metastatic solid tumour
- Indication to start systemic therapy with CKIs (PD-1 inhibitors, PD-L-1 inhibitors, or CTLA-4 inhibitors) as a single agent or in combination with other CKIs or with other cancer treatments (including chemotherapy agents, tyrosine kinase inhibitors, other monoclonal antibodies or compounds with different mechanisms of action) by 31 January 2020, as part of routine clinical practice or clinical studies with an immunotherapy agent, OR systemic immunotherapy already in progress at the time of enrolment provided it was started no earlier than 1 April 2019

The **exclusion** criteria for the study are:

- Blood cancers or lymphomas
- Immunotherapy treatment commenced prior to 1 April 2019
- Treatment as part of a blinded clinical trial in which an immunotherapy arm is compared with an arm not receiving immunotherapy (patients treated in blinded studies are eligible provided immunotherapy is administered in all treatment arms, either alone or in combination with other agents)

5. STUDY PROCEDURES

Starting from the **enrolment start date, 1 October 2019,** the supervising oncologists and the enrolment centres will propose the study to all patients about to start immunotherapy, or patients who have already started immunotherapy, in compliance with the inclusion and exclusion criteria. They may also propose the study to patients already on immunotherapy, who commenced treatment before the start of the study, in compliance with the time exclusion criteria above.
During the screening visit, once the patient has given informed consent, we will proceed with the consecutive enrolment of all those patients who meet the enrolment criteria, and therefore the collection of the clinical data of the enrolled subjects, which will continue for the duration of the study, including the follow-up period, until January 2021. A full medical history will be recorded during the screening visit, in order to collect information on the patient's cancer, clinical history and co-morbidities. Information will also be recorded regarding the immunotherapy administered, the concomitant treatments received by the patient (in particular, radiotherapy, antibiotic therapy, therapy with corticosteroids or infliximab, supportive therapy with bisphosphonates or denosumab), as they could influence the patient's immunotherapy outcome and could have an impact on the efficacy of the influenza vaccine[48] and on the clinical course of the influenza-like illness[47-48-49] and any influenza vaccine administration.

With regard to this last point, patients will be asked to cooperate by providing information regarding the date and type of any influenza vaccine they receive; in order to obtain accurate information on vaccination, for vaccinated cases, the enrolment centre will contact the patient's general practitioner, who administered the vaccine and will have been duly informed with a dedicated letter regarding patient enrolment in the protocol, in order to confirm the date on which the vaccine was actually administered and the type of vaccine used.

During the subsequent visits, which will be scheduled according to routine clinical practice during the study's observation period, data will also be collected regarding influenza-like illness, its duration and symptoms and any complications of infection. Patients will be duly instructed by the supervising oncologists of the enrolment centres to report and record any sign or symptom that may be associated with influenza-like illness. In the

event of outpatient appointments or hospitalisation for an episode of influenza and/or its complications, information will be collected regarding the diagnostic and therapeutic procedures undertaken in accordance with routine clinical practice on these occasions (in particular, the performance of a pharyngeal or nasal swab to confirm respiratory infection, serological confirmation of influenza infection, the finding of bacterial complications using culture tests, the administration of antibiotic and/or intravenous supportive therapies associated with influenza-like illness and/or associated complications).

5.1 Flow chart

	Screening visit between 1 2019 and 31 January 2020	**Observation Period (Influenza-like illness) between 1 October and 30 April 2020**	**Observation Period (Overall Survival) between 1 October 2019 and 31 January 2021**
Informed consent	X		
History	X		
Details of Vaccine administration	X		
Infectious and other complications	X	X	
Episodes of influenza	X	X	
ECOG PS	X		
Concomitant pharmacological treatments	X	X	X
Cancer assessment	X	X	X
irAEs AEs to be added in cases with associated TT/CT	X	X	X

6. ADVERSE EVENTS

Any adverse event occurring after the patient has signed the informed consent form and throughout the observation period up to 31 July 2020 must be recorded on the appropriate sheet of the e-CRF to be used to report adverse events and monitored until the resolution of the adverse event. Laboratory test abnormalities will be recorded on the adverse event report form using the appropriate diagnostic description.

The spontaneous reporting of adverse events to the deputy regulatory entity (AIFA, Agenzia Italiana del Farmaco), as required by law, is recommended. Guidelines for Good PharmacoVigilance Practices (GVP) and for Good Pharmacoepidemiology Practices (GPP) will be followed within the present protocol.

For the purposes of this study, the following four types of adverse event will be considered, as described below:

■ Local adverse reaction to the vaccine: defined as a local reaction at the injection site, characterised by the suspicion of a causal relationship between the vaccine and the event; the type, entity and duration of the reaction must be recorded in the e-CRFs provided.

■ Immune-related adverse event (irAE): an irAE is any undesirable event that may occur during or after the administration of the immunotherapy, even when there is no evidence of a causal relationship with it; it will be recorded according to system organ class as indicated in **Table 1** below, and in the e-CRF, where it will be classified according to type, entity in accordance with the Common Terminology Criteria for Adverse Events (CTCAE)[45] Version 5 and duration. By definition, any adverse event that occurs in a subject receiving immunotherapy can be immune-related until proven to the contrary. Considering the secondary endpoints of this study, which focus on irAEs, any adverse event must be recorded as such for subjects who are not receiving any other non-immunotherapy cancer treatments in combination, and should also preferably be recorded as irAEs even in patients receiving other treatments, provided they can be classified using the system organ classes indicated in Table 1.
If the patient receives other non-immunotherapy cancer treatments in combination and the event cannot be classified as described in Table 1, it will be classified as a simple adverse event **(Table 2)** and recorded as such in the dedicated e-CRF. If the event cannot be classified in Table 1 but the patient is not receiving any cancer treatments other than immunotherapy, the event will be reported as an irAE and classified as "Other", providing a description and specifying its grade.

■ Adverse event (AE): for the purpose of this study, an adverse event is any event that cannot be classified in any of the irAE classes indicated in Table 1, provided it presents in subjects who are receiving chemotherapy or other cancer treatments (e.g. molecular targeted therapy) in combination with the immunotherapy; in these cases, it will be classified in accordance with CTCAE v.5[45] and recorded in the dedicated section of the e-CRF.

■ Serious Adverse Event (SAE): a serious adverse event is defined as an untoward medical occurrence or affect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalization, results in persistent or significant disability, or is a congenital anomaly or birth defect. For the purpose of this study, SAEs are considered according to whether they are immune-related (irSAEs) or not (simple SAEs) with the same criteria described above, respectively, and will be reported in the appropriate section together with the related AE or irAEs.

Table 1 Classification of immune-related adverse events (irAEs)

System/organ	Immune-related adverse events
Skin	Maculo-papular rash Pruritus Psoriasis Vitiligo DRESS/Stevens-Johnson syndrome
Intestine	Enterocolitis/Diarrhoea Pancreatitis Gastritis
Endocrine system	Hyper-/hypothyroidism Hypopituitarism Diabetes Adrenal insufficiency
Lungs	Pneumonia Pleuritis Sarcoid-like granulomatosis
Eye	Uveitis Conjunctivitis Scleritis/ episcleritis Blepharitis Retinitis
Skeletal system	Arthritis Synovitis
Cardiovascular system	Myocarditis Pericarditis Vasculitis
Liver	Hepatitis
Kidneys	Nephritis Renal insufficiency

Nervous system	Neuropathy Myelopathy Guillain-Barré syndrome Encephalitis/ meningitis Myasthenia
Blood	Haemolytic anaemia Thrombocytopenia Neutropenia Haemophilia
Muscles	Myositis
General	Fatigue
Other	(specify)

Table 2 - Simple adverse events (AE) (classifiable for subjects receiving non-immunotherapy cancer treatments in combination with immunotherapy).

AEs
HAEMATOLOGICAL TOXICITY
Anaemia
Neutropenia
Febrile neutropenia
Thrombocytopenia
NON-HAEMATOLOGICAL TOXICITY
Nausea
Vomiting
Diarrhoea
Stomatitis
Neurotoxicity
Alopecia
Hand-foot syndrome
Asthenia

Loss of appetite
Fever without neutropenia
EGFR inhibitor-induced rash
Electrolyte abnormalities
Renal insufficiency
Hepatic insufficiency
Hypertension
Other (specify)

From a temporal point of view, adverse events will be recorded from the time of enrolment in the protocol regardless of when immunotherapy was started, in order to guarantee prospective data collection.

7. STATISTICAL ANALYSIS

Time to influenza-like illness (TTI) will measure the impact of vaccination on the incidence of ILI. A log-rank test comparing the two curves (TTI in the group of vaccinated versus not vaccinated patients) <0.05 will be considered as significant.
All other tests related to the following endpoints will be interpreted in an explorative approach.

Regarding the Primary Objective, Influenza-free survival (IFS) will also be calculated as an indirect estimate of the incidence of influenza-like illness for a more conservative approach to the data. The times to the events in general will be analysed using the Kaplan-Meier method and the curves will be compared using the log-rank test.

As far as the secondary objectives are concerned these are of two kinds: incidence and time to events; the incidences and response rates will be calculated as the ratio between events and the number of patients enrolled and they will be compared according to the characteristics identified in the subgroups indicated in section 2, using both Fisher's exact test or the X^2 test, as appropriate, and using a logistic regression model to allow the adjusted estimates of the odds ratio. Survival and all times to events will be analysed using the Kaplan-Meier method. The differences between the curves will be evaluated using the log-rank test; once again for these types of endpoint, a proportional risk model will be used to adjust the hazard ratio estimates. The results for all the endpoints considered will be reported together with the corresponding 95% confidence interval.

All patients satisfying eligibility criteria will be considered for analysis.

7.1 Definitions

TTI is defined as the interval time between the date of enrollment, and the date of first symptoms of ILI; for patients not experiencing ILI and still on treatment at the cut-off date of 31.04.2020 time will be censored at this date otherwise it will be censored at the date of death.
IFS is defined as TTI but considering death as an event.
PFS will be defined as the time between the start of the treatment and the occurrence of disease progression or death for all causes, whichever occurs sooner. The observation time-points of patients who have not had disease progression and are not deceased when the results are analysed will be recorded with the date of the most recent information on live status. OS will be calculated as the time from the start of treatment to death for all causes. The observation time-points of patients who are not deceased when the results are analysed will be recorded with the date of the most recent information on live status. ORR will be defined as the objective response rate considering the best response obtained to the immunotherapy treatment, regardless of the patient enrolment date, considering partial and complete responses in accordance with the radiological criteria commonly used in oncology (RECIST1.1[46]). DCR will be defined as ORR but considering also Stable Disease as a favourable outcome.
TTF will be defined at the time of exposure to the treatment with CKI, from its start to its definitive discontinuation for whatever cause. The observation time-points of patients who are still receiving treatment when the results are analysed will be recorded with the date of the most recent information on treatment status. Toxicity will be described and classified in accordance with CTCAE v.5[45]. The definition of simple AE and irAE is provided in the dedicated section. On the other hand, an AR will be defined as any local reaction observed in the influenza vaccine inoculation site, characterised by the suspicion of a causal relationship between the vaccine and the event. As systemic adverse events to the vaccine are immune-related events (they are triggered by an immune stimulation mechanism), they will be included in the definition of immune-related adverse events (irAEs) that are potentially attributable to the cancer immunotherapy (see Tables 1 and 2).

Influenza-like illness (ILI) will be defined as a sudden and rapid onset of one or more of the following general symptoms: fever or low-grade fever, malaise and/or listlessness, headache, myalgia; and at least one of the following respiratory symptoms: cough, sore throat, dyspnoea. “Sudden and rapid” is taken to mean to the extent that the date of symptom onset can be clearly identified. Confirmed influenza refers to cases confirmed by pharyngeal/ nasal swab or serology tests. Influenza vaccination is an inactivated influenza vaccine (trivalent, tetravalent, adjuvanted or non-adjuvanted) approved for clinical use in the 2019/2020 influenza season and received during the vaccination season considered, regardless of when the vaccine was administered in relation to the patient’s enrolment in this study and in relation to the start of the cancer immunotherapy treatment.

8. PATIENT REGISTRATION PROCEDURES

Patients may only be registered in the study by authorised investigators. Patients will be registered through a web-based platform, once the study centre has confirmed that the patient is eligible. Patient data will be recorded pseudo-anonymously in a dedicated database, by each enrolment centre, on a centralised web-based platform.

Study patients' data will be entered in compliance with applicable personal data processing regulations and processed for the statistical and descriptive analysis in compliance with privacy regulations.

9. DATA COLLECTION AND MANAGEMENT

Patients' original data must be kept in the medical records of the study centre. The data required for the individual patients must be reported by the investigators of the individual centres involved in the study on the electronic case report form, e-CRF. The patient data entered in the e-CRFs during the study will be recorded pseudo-anonymously and the patient will be identified by a code alone. The investigators will be under obligation to check that all the information required by the protocol is recorded in both the patient's medical record and the e-CRF.

10. ETHICAL ASPECTS

The coordinators of this study guarantee under their own responsibility that the study will be conducted in accordance with the Declaration of Helsinki, as amended.
Informed consent will be obtained from all patients. Any amendment to this protocol will be submitted for the approval of the local ethics committees and the Regulatory Authorities, pursuant to applicable regulations.

The investigator must make sure that patients have been duly informed about the study and understand the risks and benefits associated with their participation, including the fact that they can ask questions regarding study participation. The risks and benefits of study participation must be explained in a clear and straight-forward manner before the patient is enrolled. Patients must be informed that they may withdraw from the study at any time and will continue to receive the treatment they need, without any disadvantage.

Any change to this protocol requires a written amendment that must be approved by the Steering Committee before it can be implemented. Before implementation, amendments must receive additional approval by the ethics committees of all the participating centres, in compliance with the regulations applicable in Italy.
The approval obligation shall not, in any case, prevent any immediate action needed to protect the safety of all subjects enrolled in the study.

11. ADMINISTRATIVE PROCEDURES

The study's coordinating centre will be responsible for drafting the protocol, reviewing and discussing the data entered in the eCRFs by the investigators and the subsequent publication of the results. The coordinating centre is also authorised to answer any questions and queries from the other investigators regarding the study's eligibility criteria, processing procedures and patient assessment.

12. SPONSOR, PATRONAGE AND PUBLICATION

The final publication regarding the results of the study will be drafted by the coordinators of the study on the basis of the statistical analysis provided by the statistical support team. The manuscript must be ready for publication within 6-12 months of the final processing of the data collected. The manuscript will be submitted to a scientific journal once it has been reviewed by the coordinating centre and the co-authors. The investigators and co-investigators will be mentioned as authors according to their contribution to the study conduct and in compliance with publishing rules.

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13. REFERENCES