INfluenza Vaccine Indication during therapy with Immune checkpoint inhibitors: A transversal challenge. the INVIDIa study

INfluenza **V**accine **I**ndication **D**uring anticancer therapy with **I**mmune checkpoint inhibitors: **a** transversal challenge for patient's counselling – the **INVIDIa** study

Bersanelli Melissa[1#], Giannarelli Diana[2], Castrignanò Paola[1], Fornarini Giuseppe[3], Panni Stefano[4], Mazzoni Francesca[5], Tiseo Marcello[1], Rossetti Sabrina[6], Gambale Elisabetta[7], Rossi Ernesto[8], Papa Anselmo[9], Cortellini Alessio[10], Lolli Cristian[11], Ratta Raffaele[12], Michiara Maria[1], Milella Michele[13], De Luca Emmanuele[14], Sorarù Mariella[15], Mucciarini Claudia[16], Atzori Francesco[17], Banna Giuseppe Luigi [18], La Torre Leonardo[19], Vitale Maria Giuseppa[20], Massari Francesco[21], Rebuzzi Sara Elena[3], Facchini Gaetano[6], Schinzari Giovanni[8], Tomao Silverio[9], Bui Simona[1], Vaccaro Vanja[13], Procopio Giuseppe[12], De Giorgi Ugo[11], Santoni Matteo[22], Ficorella Corrado[10], Sabbatini Roberto[20], Maestri Antonio[19], Natoli Clara[7], De Tursi Michele[7], Di Maio Massimo[14], Rapacchi Elena[1], Pireddu Annagrazia[17], Sava Teodoro[15], Lipari Helga[18], Comito Francesca[21], Verzoni Elena[12], Leonardi Francesco[1], and Buti Sebastiano[1].

[#]**Correspondence to**: Dr. Melissa Bersanelli, University Hospital of Parma, Medical Oncology, Via Gramsci 14, 43126, Parma, Italy; phone: 0521-702316; fax: 0521-992316; e-mail: bersamel@libero.it

[1] University Hospital of Parma, Medical Oncology Unit, Parma, Italy.

[2] Regina Elena National Cancer Institute, Biostatistical Unit, Rome, Italy.

[3] IRCCS Policlinico San Martino Hospital, Medical Oncology Unit 1, Genova, Italy.

[4] ASST- Istituti Ospitalieri Cremona Hospital, Medical Oncology Unit, Cremona, Italy.

[5] University Hospital Careggi, Medical Oncology Unit, Firenze, Italy.

[6] National Cancer Institute 'Fondazione G. Pascale' – IRCCS, Napoli, Italy.

[7] University G. D'Annunzio, Department of Medical, Oral and Biotechnological Science, Chieti-Pescara, Italy.

[8] University Hospital Policlinico Gemelli, Medical Oncology, Roma, Italy.

[9] University “La Sapienza”, Department of Medical and Surgical Science and Biotechnology; Latina, Italy.

[10] University of L’Aquila, Department of Applied Clinical Sciences and Biotechnology, L’Aquila, Italy.

[11] Scientific Institute of Romagna for the Study and Treatment of Tumors (IRST) IRCCS, Medical Oncology, Meldola, Italy.

[12] National Cancer Institute of Milan - IRCCS, Genito-Urinary Oncology Unit, Milano, Italy.

[13] Regina Elena National Cancer Institute, Oncology Unit 1, Rome, Italy.

[14] Ordine Mauriziano Hospital, University of Turin, Medical Oncology, Torino, Italy.

[15] Camposampiero Hospital, Medical Oncology; Padova, Italy.

[16] Ramazzini Hospital, Medical Oncology Unit, Carpi, Italy.

[17] University Hospital and University of Cagliari, Department of Medical Sciences “M. Aresu”, Medical Oncology, Cagliari, Italy.

[18] Cannizzaro Hospital, Medical Oncology, Catania, Italy.

[19] Santa Maria della Scaletta Hospital, Medical Oncology Department, Imola, Italy.

[20] University Hospital of Modena, Medical Oncology Unit, Modena, Italy.

[21] Sant'Orsola-Malpighi Hospital, Division of Oncology, Bologna, Italy.

[22] Macerata Hospital, Medical Oncology, Macerata, Italy.

Acknowledgments:

no acknowledgments to be done. No funding.

Running head:

Flu vaccine and immune checkpoint inhibitors: the INVIDIa study

Prior presentation at ESMO Immuno-Oncology Congress 2017, Geneve (8 of December), as abstract and poster:

58P - Influenza vaccine indication during anticancer therapy with immune-checkpoint inhibitors: A transversal challenge for patient's counselling – preliminary analysis of the INVIDIa study. M Bersanelli, P Castrignanò, E Gambale, A Cortellini, M Tiseo, C Natoli, C Ficorella, S Panni, S Rossetti, A Papa, F Mazzoni, G Facchini, U De Giorgi, G Procopio, F Atzori, T Sava, E De Luca, A Maestri, F Massari, S Buti. Annals of Oncology, Volume 28, Issue suppl_11, 1 December 2017, mdx711.039, https://doi.org/10.1093/annonc/mdx711.039. Published: 11 December 2017.

Disclaimers: conflicts of interests have been declared for each author (COI and contribution forms have been collected for each author).

Abstract

PURPOSE: Considering the transversal unmet need for the counselling of advanced cancer patients treated with immune checkpoint inhibitors (CKI) about influenza vaccination, we planned an explorative study to assess the efficacy of influenza vaccine in this population, its potential impact on the severity and mortality of influenza syndrome (IS) and on of anticancer immunotherapy outcome. **METHODS:** INVIDIa was a retrospective, observational, multicenter, explorative study at 21 Italian centers, enrolling consecutive advanced cancer patients receiving treatment with CKI during the Italian influenza vaccinal season 2016-2017. All data regarding influenza vaccination, IS and treatment with CKI were collected. Subgroup analyses were pre-planned for elderly and lung cancer patients. **RESULTS:** The study enrolled 300 patients, 79 of which received flu vaccine; 221 unvaccinated patients constituted the control group. The incidence of IS was of 24.1% among vaccinated, *vs* 11.8% of controls; OR=2.4 (95%CI=1.23-4.59; p=0.009). Severity of IS was mild, with no flu-related deaths and low rates of complications. No differences were seen in terms of treatment outcome, basing on vaccine or IS in the overall study population. Considering the patient population starting CKI treatment closer to the vaccinal season, objective response rate was significantly better for vaccinated. The ineffectiveness of vaccine was more pronounced among elderly, with rate of IS reaching 37.8% of vaccinated patients, *vs* 6.1% of unvaccinated, OR=9.28 (95%CI=2.77-31.14), p<0.0001. **CONCLUSION:** The INVIDIa study suggests that, despite influenza vaccine could be ineffective and unnecessary in advanced cancer patients undergoing treatment with CKI, it seems to not compromise their clinical outcome and to not negatively impact the efficacy of anticancer therapy.

BACKGROUND AND PURPOSE

Prevention of infection is crucial for individuals with impaired immunity. Viral infections in cancer patients often result in high morbidity and mortality rates, the latter reaching 9% for influenza syndrome (IS) [1,2]. Because of tumor-induced and treatment-induced immunosuppression, live vaccines are not used, whilst split vaccines are permitted and often recommended for this population [3-5]. However, the greater the degree of immunosuppression, the less likely the patient is to respond to vaccine immunization [1]. Some evidence in favor of serological response to influenza vaccination in high-risk patients with proliferative diseases has been provided [6,7], but it remains unclear how much this response protects them from flu infection, due to the lack of morbidity measures [8]. The few available data about immunocompromised patients have been summarized in a meta-analysis, showing significantly lower odds of influenza-like illness for vaccinated individuals [9].

Nevertheless, immunogenicity could vary widely basing on different tumor types and treatment regimens. It is generally assumed that the advanced cancer patient, mainly treated with chemotherapy, is a fragile and immunocompromised subject, that regardless of age must be included in the vaccination strategy. Influenza vaccine is recommended in all cancer patients, especially those with lung cancer, considering their susceptibility and high mortality from infectious respiratory diseases [3-5,10].

In the last years, the new immunotherapy with immune checkpoint inhibitors (CKI) revolutionized the systemic treatment of advanced solid tumors such as melanoma, lung cancer, renal carcinoma, urothelial cancers, head and neck carcinoma [11]. This new oncologic population has not been specifically studied for its susceptibility to infections. The goal of such therapies is to restore the cellular immunocompetence, which is involved

against the tumor but also against infectious agents. It is therefore plausible that the patient underwent treatment with CKI could be more immunocompetent than the average cancer patient.

It is only based on the drugs characteristics that flu vaccine has been considered as potentially safe in patients treated with CKI. The phase II study CA184-004, providing the only formal proof of humoral efficacy of vaccines during CKI immunotherapy, demonstrated humoral responses to influenza vaccine administered in melanoma patients treated with ipilimumab [12]. Nevertheless, no data have been published about the clinical efficacy and safety of flu vaccine during CKI, nor about its potential impact on the efficacy of anticancer immunotherapy. A small case-control study, presented at the European Lung Cancer Conference 2017, suggested that the seasonal influenza vaccination may increase the rate of serious immune-related adverse events (irAEs) from CKI [13]. Interestingly, these patients had a greater and more rapid humoral response compared with vaccinated healthy controls. Such data support the hypothesis that CKI-treated patients are effectively more immunocompetent, since immunotherapy enhances cellular and humoral immunity, and that influenza vaccine can result in an overwhelming activation of the immune system, potentially amplifying irAEs of CKI [12-13].

The effect of introducing a new antigen in the immune system of individuals treated with CKI is unknown. Viral antigens, which according to the concept of the “foreignness” are much more immunogenic than tumor antigens [14-19], could deviate T cell response induced by CKI, potentially weakening the antitumor response in favor of the anti-viral reaction. Thus, the effectiveness of anticancer treatment might be undermined, especially if vaccination occurs at the beginning of the immunotherapy, when the immune response is still not established.

Considering the transversal unmet need for the counselling of CKI-treated cancer patients about flu vaccination, we planned an explorative study to assess the efficacy of influenza

vaccine in this population, its potential impact on the severity and mortality of IS and on the outcome of anticancer immunotherapy.

PATIENTS AND METHODS

INVIDIa was a retrospective, observational, multicenter, explorative study, involving Medical Oncology Units from 21 Italian centers, approved by the Local Ethical Committees. Records of consecutive advanced cancer patients receiving treatment with CKI during the Italian influenza vaccinal season 2016-2017, namely from November 1th, 2016 to May 30, 2017, were collected in an anonymized database. Any primary tumor and any systemic treatment with anti-PD-1, anti-PD-L1 (anti-PD-ligand 1) or anti-CTLA-4 antibodies, with at least one administration in the observation period, were allowed. Detailed information regarding influenza vaccination, IS occurrence and treatment with CKI was investigated, also contacting the primary care physician of each patient. Patients for which exhaustive data about influenza vaccine administration and IS development were not available were excluded.

IS, according to the Italian Ministry of Health, was defined as illness of likely viral origin characterized by fever ≥ 38°C and the presence of at least one respiratory symptom (cough, dyspnea or rhinorrhea) and general symptoms (headache, myalgias, bone or joint pains) [20].

With the aim of assessing the efficacy of influenza vaccine in this population, by comparing the morbidity of IS among vaccinated and unvaccinated patients, the primary endpoint of the study was the incidence of IS.

The explorative secondary endpoints were the severity and lethality of IS, and the impact of influenza vaccine and of IS on the outcome of patients.

The first were evaluated assessing: flu-related death rate (defined as the proportion of patients which death was attributable to IS or its complications); flu-relapse rate (the proportion of patients experiencing at least a recurrence of the flu episode); need for hospitalization due to flu-illness; need for intravenous therapy in day-hospital regimen due to flu-related symptoms; incidence of documented bacterial superinfections; flu-syndrome duration.

The latter was investigated in terms of objective response rate (ORR) obtained with CKI (defined as the rate of complete and partial responses assessed by RECIST 1.1 [21]), of disease control rate (DCR, defined as the rate of stable diseases, partial and complete responses), of time to treatment failure (TTF, defined by the time from CKI treatment initiation to permanent discontinuation for any reason) and of median overall survival (mOS, defined from therapy starting to death for any reason). Follow-up for OS is still ongoing, with data cut-off provided on May 30, 2018; only preliminary survival data will be reported herein.

Preplanned subgroup analyses

Hypothesizing higher risk of interaction with immunotherapy when flu vaccine is administered in its early phase, the analyses for ORR, DCR, TTF and OS were planned to be repeated in the selected subgroup of patients initiating CKI therapy closer to the vaccinal season, namely after the 1st of August 2016.

Further subgroup analyses were pre-planned for lung cancer patients and for elderly, using as pre-specified cut-off the median age of the vaccinated subgroup at the time of flu vaccine administration. Age for the control group was calculated at the start of the observation period.

Statistical analyses

Demographic variables were reported using descriptive statistics. Median values were associated with their interquartile range (IQR) or 95% confidence intervals (95%CI) and compared using the Mann-Whitney t-test. χ^2 test was used to evaluate the association between categorical variables. Survival curves were estimated with the Kaplan–Meier method and the outcomes in groups were compared using the log-rank test. Cox proportional hazard model was used to estimate hazard ratio and 95%CI for each factor and in a multivariate analysis, including all evaluable parameters, using a forward stepwise selection method.

RESULTS

Overall INVIDIa study population

Characteristics of patients

The INVIDIa study enrolled 300 advanced cancer patients; their characteristics are reported in **Table 1**. Median OS (**Supplementary Figure S1a**) was not reached at the median follow-up of 12.2 months (95%CI=11.2-13.3; 232 censored). Immunotherapy was still ongoing for 149 patients at the data cut-off; median TTF (mTTF) was of 11.7 months (95%CI=9.0-14.5). Response to treatment was significantly related to OS (p<0.0001, **Supplementary Figure S1b**).

Overall, 79 patients received influenza vaccine (26.3%), as classical intramuscular injectable flu shot, with trivalent (two type A viruses, H1N1 and H3N2, and one type B virus, B/Brisbane) or quadrivalent (adding a type B virus, B/Phuket) inactivated virus vaccine (split vaccine), according to the National and International recommendations [22-23]. The remaining 221 patients did not receive flu vaccination, constituting the control group.

Of the 79 vaccinated patients, 31 received the flu shot before starting immunotherapy, with median time interval (mTI) of 2.6 months (IQR=1.0-4.5), while 38 patients were vaccinated after prior immunotherapy initiation, with mTI of 8.1 months (IQR=3.4-10.0). The patient population that started CKI treatment closer to the vaccinal season (after the 1st of August 2016) was of 184 cases, with median follow-up of 8.5 months (95%CI=7.2-9.7). Their mOS was of 15.3 months (95%CI=10.3-20.3; 131 censored); mTTF was of 5 months (95%CI=3.3-6.6; 80 censored).

Of the 45 patients with occurrence of IS during the study observation period, 40 developed the illness after immunotherapy initiation, with mTI of 7.3 months (IQR=2.4-12.8), whilst 5 had the IS before starting CKI treatment, with mTI of 3.1 months (IQR=2-3.5). Almost all vaccinated patients developing IS (18/19) had the occurrence of the illness after the administration of the flu shot, with mTI of 2.6 months (IQR=1-5-4.8).

Influenza syndrome morbidity

The incidence of IS in the overall study population was of 15% (45/300). It was higher in the lung cancer subgroup, reaching 20% of cases.

The incidence of IS was of 24.1% among patients receiving the vaccine (19/79), compared to those of 11.8% of the unvaccinated control group (26/221); odds ratio (OR)=2.4 (95%CI=1.23-4.59), *p*=0.009 (**Figure 1**).

Influenza syndrome lethality and severity

No cases of flu-related death were reported. Overall, 8 patients had at least one influenza recurrence: 4 (19.1%) among vaccinated and 4 (15.4%) of unvaccinated cases (*p*=0.62). Three vaccinated patients required intravenous therapy in day-hospital regimen due to flu-related symptoms (15.8%) *versus* 2 cases in the control group (7.7%), *p=0.39.* Five vaccinated patients required hospitalization due to IS (26.3%) *versus* 4 among unvaccinated (15.4%), *p*=0.36*.* No cases of documented bacterial superinfections were

reported. Mean duration of IS was similar among vaccinated and unvaccinated patients (respectively 8.2 days +/-3.1 and 8.3 days +/-4.1).

Anticancer treatment outcome

No statistically significant differences were seen in terms of ORR, DCR or TTF with CKI therapy, respectively, between vaccinated and control patients or between patients developing IS or not in the overall study population. Nevertheless, considering the patient population starting CKI treatment closer to the vaccinal season (184 cases), ORR was significantly better for vaccinated patients compared to unvaccinated controls, OR=2.56 (95%CI=1.12-5.88), *p*=0.03. Moreover, the presence of at least one variable among flu vaccine administration and IS development was positively related to ORR in this subgroup (OR=2.0, 95%CI=1.02-4.0, *p*=0.05). No statistically significant differences were seen in terms of DCR or of TTF. With the limit of immature data, results of preliminary univariate and multivariate analyses for OS are reported in **Table 2**.

Lung cancer subgroup

In the population of 103 non-small cell lung cancer (NSCLC) patients, mOS was not reached (76 censored); mTTF was of 10.2 months (95%CI=6.8-13.6; 40 censored).
Flu incidence of vaccinated patients reached 27% of cases (9/33) versus 17% of unvaccinated patients (12/70); OR=1.81 (95%CI=0.67-4.86), *p*=0.29.
No statistically significant differences were seen in terms of ORR, DCR or TTF according to the vaccinal status and/or to IS occurrence. Despite immature data for OS, a statistically significant positive correlation with flu vaccine administration and/or IS development was demonstrated in this subgroup: 1-year OS 86.7% (95%CI=75.7-97.7) for vaccine/IS group *versus* 66.7% (95%CI=53.6-79.8), *p*=0.02 (**Figure 2**). The correlation was maintained at the multivariate analysis (**Table 3**).

Elderly subgroup

Pre-specified subgroup analysis of IS incidence basing on age, with the calculated cut-off of 71 years, demonstrated similar rates of flu infections, of 14.3% and 14.1%, respectively for vaccinated and unvaccinated patients, in the younger subgroup (≤71 years, 198 patients). The incidence of IS was instead significantly different according to the vaccinal status in the subgroup of elderly (>71 years, 102 patients), with a rate of IS reaching 37.8% of vaccinated patients, compared to those of 6.1% of unvaccinated cases, OR=9.28 (95%CI=2.77-31.14), p<0.0001 (**Figure 3**).

The DCR of elderly patients (102 cases) was higher when vaccinated, respectively 83.8% *versus* 64.6%, OR=2.83 (95%CI=1.03-7.78), p=0.039. IS occurrence did not impact on DCR. No statistically significant differences were seen in terms of ORR according to the vaccinal status in the elderly patients, but better ORR, of 52.6%, was demonstrated for patients developing IS *vs* 28.9% of unaffected patients (irrespective of the vaccinal status), p=0.048, OR=2.73 (95%CI=0.99-7.56). mTTF was of 11.3 months among elderly (95%CI=5.12-17.44), and it remained unaffected by vaccination and/or IS. Preliminary analyses for OS were not significant (mOS not reached, 82 censored).

DISCUSSION

To our knowledge, this is the first published study addressing the issue of influenza vaccine administration in cancer patients undergoing immune checkpoint blockade.

Flu vaccine effectiveness

According to the INVIDIa study results, it seems that flu vaccine is not effective in advanced cancer patients treated with CKI. The incidence of IS occurrence is much higher for the vaccinated group than for the non-vaccinated one (**Figure 1**). This phenomenon

cannot be justified by vaccine-derived flu-like syndromes, because of the prolonged time frame from vaccine administration to IS occurrence and the unequivocal definition of IS.
The ineffectiveness of influenza vaccine seems to be even more pronounced among elderly, relating to the highest flu morbidity (**Figure 3**). This is not justified by a hypothetically greater expected morbidity of influenza in the elderly: flu incidence is contrariwise generally lower in elderly populations [24-26].
During the 2016-2017 flu season in Italy, the cumulative incidence of IS was around 6.8%; incidence rates among elderly (>65 years) ranged from 0.3% to 6.3%; vaccinal coverage was of 22%; 95% of serious cases and 100% of deaths had pre-existing chronic disease [24-25].
In our study population, the severity of IS seems to be quite mild, with no flu-related deaths and low rates of complications. The increased morbidity of influenza seems to not negatively impact the clinical outcome of affected patients. We hypothesized that CKI treatment may have been responsible of a greater immunocompetence against viral infections, preserving patients from severity and lethality of IS. This assumption is supported by preclinical evidence, demonstrating that the therapeutic inhibition of PD-1/PD-L1 binding in the airways can prevent the T cell impairment provoked by influenza virus and enhances CD8+ T cell function and viral clearance, reducing viral titers, mitigating airway dysfunction and accelerating infection recovery in mice [27-28].
Of note, in the INVIDIa population, similar times of duration of symptoms and similar rates of recurrence and hospitalizations and have been demonstrated among patients with IS irrespective of the flu shot administration, suggesting that the vaccine does not even work to further mitigate the severity of IS in case of its occurrence.
A possible scientific rationale for an even increased incidence of infection in vaccinated CKI treated patients comes from the evidence that an enhanced aberrant T cell response could paradoxically contribute to the IS immunopathology [29]. After initial influenza virus

contagion, T cells become responsible of detrimental effects if immune response is excessive, contributing to damage (probably mediated by TNF-α) and to the flu development [30]. A detrimental role of CD8+ T cells was already demonstrated in the 1980s, when nude mice showed a delay in pathology, morbidity and mortality from influenza virus infection compared with wild type animals [31]. Interestingly, the adoptive transfer of specific T cells or of viral peptide-loaded dendritic cells on mice models respectively caused lethal lung injury and elicited a strong T CD8+ response in the absence of viral infection [32,28]. This suggests that also the only vaccination could trigger the detrimental effect. Immune checkpoint blockade might upset the critical balance between the number and function of T cells required for virus clearance and that responsible for harmful effects, increasing the flu morbidity even more in vaccinated patients, who received an additional antigenic stimulus.

Considering the study results, the counselling of CKI treated cancer patients about influenza vaccine should not exempt from notifying them of its possible ineffectiveness and of its potential uselessness.

A separated reflection should be deserved for lung cancer patients, showing high influenza morbidity and less pronounced inefficacy of flu vaccine. For them, vaccination and/or IS development inexplicably result in a better survival, irrespective of the anticancer treatment outcome. Precautionary vaccinal measures, although possibly not useful, but obviously not harmful, could be undertaken with prudent intent in this subgroup.

Flu vaccine impact on anticancer therapy

The second key issue of the study seems to find a reassuring reply. Flu vaccine does not negatively affect the treatment outcome, in terms of ORR, DCR or TTF. Conversely, in some cases it even potentially correlates with better treatment efficacy.

Translating such evidence in terms of counselling, patients could be reassured about the likely innocuousness of the vaccine against the effectiveness of anticancer therapy. Interestingly, also IS occurrence, in some cases, seems to be related with a better treatment outcome. It appears that vaccine-induced or influenza-induced antigen stimulation may similarly positively impact the cell-mediated immune response to CKI treatment, acting as analogous immunogenic stimuli. This concept is anything but new: at the end of the 19th century, William Coley provoked anti-tumor immune responses by injecting cancer patients with bacteria or bacterial products [33]. Moreover, a relationship between feverish infection and concurrent spontaneous remission from cancer has been known for a very long time [34]. In our case, the immune activation given by the viral particles of the split vaccine, and furthermore by the infection itself, might be synergistic to CKI, contributing to the final antitumor effect.

Study limits

The main limit of the study is represented by its retrospective nature. The inclusion criteria, considering patients receiving therapy in a prespecified time lapse, irrespective of treatment starting, potentially represents a positive selection bias: nevertheless, the study endpoints have been compared within the study population, preserving the internal validity, also considering the relatively good balance of the two groups (see **Table 1**). The limit of not including irAEs and vaccine-related AEs among endpoints was due to the expected scarce reliability of such retrospective data. Finally, the relatively limited sample size for subgroup analyses suggests caution in their interpretation. Survival data are still immature.

Conclusion

The INVIDIa study suggests that, despite influenza vaccine could be ineffective and unnecessary in advanced cancer patients undergoing treatment with CKI, it seems to not

compromise their clinical outcome and to not negatively impact the efficacy of anticancer therapy.

References

1. Hibberd PL, Rubin RH: Approach to immunization in the immunosuppressed host. Infect Dis Clin North Am 4(1):123-42, 1990
2. Cooksley CD, Avritscher EB, Bekele BN, et al: Epidemiology and outcomes of serious influenza-related infections in the cancer population. Cancer 1;104(3):618-28, 2005
3. Rubin LG, Levin MJ, Ljungman P, et al (Infectious Diseases Society of America): 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis 58(3):e44-100, 2014
4. Pedrazzoli P, Baldanti F, Donatelli I, et al: Vaccination for seasonal influenza in patients with cancer: recommendations of the Italian Society of Medical Oncology (AIOM). Ann Oncol 25(6):1243–1247, 2014
5. Centers for Disease Control and Prevention (CDC): Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices--United States, 2013-2014. MMWR Recomm Rep 62(RR-07):1-43, 2013
6. Brydak LB, Całbecka M: Immunogenicity of influenza vaccine in patients with hemato-oncological disorders. Leuk Lymphoma 32(3-4):369-74, 1999
7. Anderson H, Petrie K, Berrisford C, et al: Seroconversion after influenza vaccination in patients with lung cancer. Br J Cancer 80(1-2):219-20, 1999
8. Mahmoud AS, Nagla AK. Influenza Vaccination in Cancer Patients Undergoing Systemic Therapy. Clin Med Insights Oncol 8: 57–64, 2014
9. Beck CR, McKenzie BC, Hashim AB, et al [University of Nottingham Influenza and the ImmunoCompromised (UNIIC) Study Group]: Influenza vaccination for

immunocompromised patients: systematic review and meta-analysis by etiology. J Infect Dis 206(8):1250-9, 2012

10. Kohno S, Koga H, Oka M, et al: The pattern of respiratory infection in patients with lung cancer. Tohoku J Exp Med 173(4):405-11, 1994
11. Bersanelli M, Buti S: From targeting the tumor to targeting the immune system: Transversal challenges in oncology with the inhibition of the PD-1/PD-L1 axis. World J Clin Oncol 8(1):37-53, 2017
12. Weber JS, Hamid O, Chasalow SD, et al: Ipilimumab increases activated T cells and enhances humoral immunity in patients with advanced melanoma. J Immunother 35:89-97, 2012
13. Rothschild S, Balmelli C, Kaufmann L, et al: Immune response and adverse events to influenza vaccine in cancer patients undergoing PD-1 blockade. Ann Oncol (28), 2017 (suppl 2). Presented at European Lung Cancer Conference, Geneva, CH, April 25, 2017 (Abstract 112P_PR)
14. Tauber JW: "Self": standard of comparison for immunological recognition of foreignness. Lancet 2(7980):2913, 1976
15. Maurer PH: Immunogenicity and the nature of antigens. Med Clin North Am 49(6):1505-16, 1965
16. Jaravine V, Raffegerst S, Schendel DJ, et al: Assessment of cancer and virus antigens for cross-reactivity in human tissues. Bioinformatics 33(1):104-111, 2017
17. van den Berg HA, Rand DA: Foreignness as a matter of degree: the relative immunogenicity of peptide/MHC ligands. J Theor Biol 231(4):535-48, 2004

18. Kanduc D: Immunogenicity in peptide-immunotherapy:
from self/nonself to similar/dissimilar sequences. Adv Exp Med Biol 640:198-207, 2008

19. Larsen MV, Lundegaard C, Lamberth K, et al:
An integrative approach to CTL epitope prediction:
a combined algorithm integrating MHC class I binding, TAP transport efficiency, and proteasomal cleavage predictions. Eur J Immunol 35(8):2295-303, 2005

20. Italian Ministry of Health: Influnet. Rete Italiana Sorveglianza Influenza. Sistema di Sorveglianza Sentinella dell'influenza basata su Medici di Medicina Generale e Pediatri di Libera Scelta. Sorveglianza Epidemiologica e Virologica. Protocollo Operativo. Stagione influenzale 2012 – 2013.
http://www.salute.gov.it/imgs/C_17_pubblicazioni_1853_allegato.pdf (Page 7)

21. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur. J. Cancer 45, 228–247 (2009).

22. World Health Organization (WHO): Recommended composition of influenza virus vaccines for use in the 2016-2017 northern hemisphere influenza season.
http://www.who.int/influenza/vaccines/virus/recommendations/2016_17_north/en/

23. Italian Ministry of Health: Prevenzione e controllo dell'influenza. Raccomandazioni per la stagione 2016-2017.
http://www.igienistionline.it/docs/2016/30circinfluenzale.pdf

24. Italian Inter-University Research Center on Influenza and other Transmissible Infections (CIRI-IT): Morbosità per sindrome influenzale.
https://www.cirinet.it/dati_prec/epi/risultati_2016_2017/ili_nazionale.html

25. Italian Ministry of Health: FluNews. Rapporto Epidemiologico Settimanale. http://www.salute.gov.it/portale/influenza/documenti/flunews/FluNews_2017_14.pdf
26. Fowlkes A, Dasgupta S, Chao E, et al: Estimating influenza incidence and rates of influenza-like illness in the outpatient setting. Influenza Other Respir Viruses 7(5):694-700, 2013
27. McNally B, Ye F, Willette M, et al: Local blockade of epithelial PDL-1 in the airways enhances T cell function and viral clearance during influenza virus infection. J Virol 87(23):12916-24, 2013
28. Erickson JJ, Gilchuk P, Hastings AK, et al: Viral acute lower respiratory infections impair CD8+ T cells through PD-1. J Clin Invest 122, 2967–2982, 2012
29. Hillaire ML, Rimmelzwaan GF, Kreijtz JH. Clearance of influenza virus infections by T cells: risk of collateral damage? Curr Opin Virol 3(4):430-7, 2013
30. Srikiatkhachorn A, Chintapalli J, Liu J, et al: Interference with intraepithelial TNF-α signaling inhibits CD8(+) T-cell-mediated lung injury in influenza infection. Viral Immunol 23(6):639-45, 2010
31. Wells MA, Albrecht P, Ennis FA: Recovery from a viral respiratory infection. I. Influenza pneumonia in normal and T-deficient mice. J Immunol 126(3):1036-41, 1981
32. Small BA, Dressel SA, Lawrence CW, et al: CD8(+) T cell-mediated injury in vivo progresses in the absence of effector T cells. J Exp Med 194(12):1835-46, 2001
33. Orange M, Reuter U, Hobohm U: Coley's Lessons Remembered: Augmenting Mistletoe Therapy. Integr Cancer Ther 15(4):502-511, 2016.Hobohm U: Fever and cancer in perspective. Cancer Immunol Immunother 50(8):391-6, 2001

Tables

Table 1 – Characteristics of patients from the INVIDIa study.

	N° of patients (%) Overall population	**N° of patients (%) Vaccinated group**	**N° of patients (%) Control group**
Median age* **68 year** (range 36-85)			
Age < 68 years	149 (49.7)	29 (36.7%)	120 (54.3%)
Age ≥ 68 years	151 (50.3)	50 (63.3%)	101 (45.7%)
Gender			
Male	207 (69%)	59 (74.7%)	148 (67%)
Female	93 (31%)	20 (25.3%)	73 (33%)
Primary tumor			
Non-small cell lung cancer	103 (34.3%)	33 (41.8%)	70 (31.7%)
Renal cell carcinoma	112 (37.3%)	24 (30.4%)	88 (39.8%)
Melanoma	55 (18.3%)	13 (16.4%)	42 (19%)
Other[#]	30 (10%)	9 (11.4%)	21 (9.5%)
Treatment line			
I line	43 (14.3%)	10 (12.7%)	33 (14.9%)

II line	124 (41.3%)	38 (48.1%)	86 (38.9%)
III line	89 (29.7%)	23 (29.1%)	66 (29.9%)
≥ IV line	44 (14.7%)	8 (10.1%)	36 (16.3%)
Type of treatment**			
Nivolumab	239 (79.7%)	61 (77.2%)	178 (80.5%)
Pembrolizumab	25 (8.3%)	8 (10.1%)	17 (7.7%)
Atezolizumab	22 (7.3%)	6 (7.6%)	16 (7.2%)
Avelumab	6 (2%)	3 (3.8%)	3 (1.4%)
Ipilimumab	2 (0.7%)	0 (0%)	2 (0.9%)
Immunotherapy combinations	4 (1.3%)	1 (1.3%)	3 (1.4%)
Chemo-Immunotherapy	2 (0.7%)	0 (0%)	2 (0.9%)
Best response§			
Complete response	7 (2.3%)	0 (0%)	7 (3.1%)
Partial response	94 (31.3%)	23 (29.1%)	71 (32.1%)
Stable disease	107 (35.7%)	37 (46.8%)	70 (31.7%)
Progressive disease	82 (27.3%)	19 (24%)	63 (28.5%)
Not evaluable	10 (3.3%)	0 (0%)	10 (4.5%)
Cause of death°			

Cancer related	58 (85.3%)	12 (75%)	46 (88.5%)
Treatment related	0 (0%)	0 (0%)	0 (0%)
Other/unknown	10 (14.7%)	4 (25%)	6 (11.5%)

* In this case, age was calculated at the time of immunotherapy initiation.

§ Best response was evaluated by RECIST 1.1 criteria, according to the local clinical practice of all the participating centers.

Other primary malignancies were represented by head & neck carcinoma, urothelial cancer, gastric cancer and colon adenocarcinoma.

** Immune checkpoint inhibitors were used as monotherapy when not otherwise expressly specified.

° 232 patients were censored at the data cut-off (May 30, 2017); 68 events.

Table 2 – Results of preliminary univariate and multivariate analyses for overall survival in the overall study population.

	Univariate analysis	Multivariate analysis
GENDER **(male vs female)**	1.25 (0.73-2.14) *P*=0.42	--
AGE **(years)**	1.00 (0.97-1.03) *P*=0.99	--

LINE **(>=3 vs 1-2)**	0.82 (0.51-1.33) *P*=0.42	--
VACCINE **(yes vs no)**	0.75 (0.43-1.32) P=0.32	--
IS **(yes vs no)**	0.48 (0.21-1.12) *P*=0.09	0.35 (0.15-0.84) *P*=0.02
VACCINE a/o FLU **(yes vs no)**	0.69 (0.41-1.16) *P*=0.16	--
ORR (yes vs no)	0.06 (0.02-0.20) *P*<0.0001	0.15 (0.04-0.50) *P*=0.002
DCR (yes vs no)	0.08 (0.04-0.13) *P*<0.0001	0.11 (0.06-0.21) *P*<0.0001

ORR = objective response rate; DCR = disease control rate

Table 3 – Results of preliminary univariate and multivariate analysis for overall survival in the lung cancer subgroup (103 patients).

	Univariate analysis	Multivariate analysis
GENDER	1.31 (0.49-3.46)	--

(male vs female)	*P* = 0.59	
AGE **(years)**	1.03 (0.98-1.08) *P* = 0.20	--
TREATMENT LINE **(≥ 3 vs 1-2)**	0.74 (0.30-1.83) *P* = 0.51	0.29 (0.11-0.78) *P* = 0.01
FLU VACCINE **(yes vs no)**	0.43 (0.16-1.13) *P* = 0.08	--
IS **(yes vs no)**	0.31 (0.07-1.30) *P* = 0.10	--
VACCINE and/or IS **(yes vs no)**	0.39 (0.16-0.92) *P* = 0.03	0.29 (0.11-0.74) *P* = 0.008
ORR (yes vs no)	0.02 (0.01-0.68) *P* = 0.03	*NE*
DCR (yes vs no)	0.08 (0.04-0.19) *P* < 0.0001	0.06 (0.03-0.15) *P* < 0.0001

ORR = objective response rate; DCR = disease control rate; *NE* = not evaluated

FIGURES' LEGEND

Figure 1 – Incidence of IS of patients receiving influenza vaccine compared to those of the unvaccinated control group.

Figure 2 – Overall Survival in lung cancer patients according to vaccine and/or IS status (103 patients).

Figure 3 – Incidence of IS according to the vaccinal status in the younger patients' subgroup and in the elderly patients' subgroup, respectively.

SUPPLEMENTARY MATERIALS

Legend of Supplementary Figure

Figure S1 - S1a. Overall survival of whole study population (300 patients). 1-year overall survival was 79.3% (95% CI: 74.2-84.4). S1b. Overall survival of whole population according to response to treatment (300 patients). At 1-year survivals were 100%, 100%, 88.4% and 40.5%, respectively for CR (complete responses), PR (partial responses), SD (stable disease) and PD (progression of disease).