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Titolo della tesi

Family history of cancer as surrogate predictor of benefit from immunotherapy with anti-PD-1/PD-L1 checkpoint inhibitors

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Dottorando

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Sintesi in lingua Italiana

TITOLO PROGETTO DI RICERCA:

Family history of cancer as surrogate predictor of benefit from immunotherapy with PD-1/PD-L1 checkpoint inhibitors.

RAZIONALE

Farmaci Inibitori dei Checkpoint Immunitari (ICI) sono anticorpi monoclonali entrati nella comune pratica clinica del trattamento di pazienti affetti da neoplasie solide ed ematologiche e le loro indicazioni sono in continua espansione. Gli ICI appartengono alla categoria degli immunoterapie, in quanto agiscono non direttamente sulla cellula neoplastica, bensì sul microambiente tumorale, particolarmente andando a stimolare o disinibire il sistema immunitario dell'ospite, al fine di aumentare la risposta immunitaria nei confronti della neoplasia stessa. Gli ICI di maggiore attuale utilizzo in pratica clinica appartengono a due categorie: gli anti-PD-1 come il Nivolumab (Opdivo®; Bristol-Myers Squibb Pharma EEIG, Uxbridge, United Kingdom) ed il Pemrolizumab (Keytruda®, Merck Sharp & Dohme Limited, Hoddesdon, United Kingdome) ed in minor misura perché ancora in fase di sviluppo gli anti-PD-L1 come l'Atezolizumab (Tecenriq®). Il Nivolumab ed il Pembrolizumab sono farmaci simili ed agiscono a livello del recettore programmed death-1(PD1) e ne blocca l'interazione con i ligandi PD-L1 e PD-L2, fisiologicamente espressi dalle cellule presentanti l'antigene e che possono essere espressi dalla cellula tumorale o da altre cellule nel microambiente tumorale. Il recettore PD-1 è un regolatore negativo dell'attività dei linfociti T che porta all'inibizione della proliferazione delle cellule T e della secrezione delle citochine. I farmaci anti-PD-1 ed in maniera analoga i farmaci anti-PD-L1, come l'atezolizumab, potenziano le risposte delle cellule T, incluse le risposte anti-tumorali, attraverso il blocco del legame del PD1 ai ligandi PD-L1 e PD-L2. L'avvento di questi farmaci ha portato ad un rivoluzione degli algoritmi terapeutici, sebbene sia la pratica clinica, che le politiche economiche dei sistemi sanitari, stiano ancora soffrendo della mancanza di criteri di selezione, come biomarcatori, che consentano di selezionare a priori pazienti che si possano beneficiare di questo tipo di trattamenti La valutazione immunoistochimica di PD-L1, sia sulle cellule tumorali che sull'infiltrato infiammatorio, è ad oggi l'unico fattore

parzialmente validato, sebbene abbia dimostrato la sua netta applicabilità solo nel *setting* di prima linea del sa trattamento del carcinoma polmonare non a piccole cellule e con una elevata soglia di espressione. I kit commerciali in uso per la sua valutazione sono inoltre diversi, e nonostante gli studi di "armonizzazione" condotti, differenti aziende farmaceutiche hanno utilizzato come "companion diagnostic" differenti kit per la valutazione del PD-L1, rendendone difficile la riproduzione nella pratica clinica quotidiana. Una più interessante prospettiva per la valutazione di predittività positiva alla risposta è quella che studia il "carico" di mutazioni somatiche del tumore, che è dimostrato essere direttamente proporzionale all'attività dei farmaci anti-PD-1/PD-L1.

Una delle condizioni che è riconosciuta essere associata ad un alto carico di mutazioni è l'instabilità dei micro-satelliti, una condizione di ipermutabilità associata a difetti di riparo del DNA (meccanismo del *mis-match* repair). Questa condizione, oltre che sporadica è nota essere tipica della sindrome di Linch, una patologia di suscettibilità ereditaria allo sviluppo di multiple neoplasie (in particolare colo-rettali ed endometriali). Analogalmente le mutazioni dei geni BRCA 1 e BRCA 2, che sono associate alla sindrome ereditaria del carcinoma della mammella e dell'ovaio (HBOC), conducono ad un alto carico di mutazioni tumorali somatiche. Anche in questo caso inizia ad essere riconosciuto un fenotipo "immuno-sensitivo" per i tumori che nel decorso clinico di questa sindrome possono svilupparsi. La sindrome di Linch e l'HBOC sono solo due delle forme di suscettibilità ereditaria al cancro che attualmente conosciamo.

Partendo quindi dalle osservazioni che le neoplasie correlate a sindromi di suscettibilità ereditaria ai tumori hanno un fenotipo "immuno-sensitivo" e che gran parte della fenomenologia associata a ereditarietà e a predisposizione sia ancora ignota, lo studio si propone di verificare se l'anamnesi familiare positiva per neoplasie possa essere utilizzata come possibili fattori predittivi positivi surrogati per il beneficio clinico al trattamento con farmaci anti-PD-1 e anti-PD-L1.

FASE PRELIMINARE

Il progetto di dottorato prevedeva una prima fase uno studio osservazionale retrospettivo multicentrico, con raccolta dei dati circa l'anamnesi familiare per patologie oncologiche, di pazienti affetti da neoplasie solide avanzate (qualsiasi primitivitá) trattati in monoterapia (qualsiasi linea di trattamento) con farmaci anti-PD-1 ed anti-PD-L1.

Al fine di capire se, nella popolazione oggetto dello studio, l'anamnesi familiare positiva per neoplasie possa essere utilizzata come possibile fattore predittivo positivo surrogato per il beneficio clinico al trattamento con farmaci anti-PD-1 e anti-PD-L1 (tasso di risposte, tasso di controllo di malattia, tempo libero da progressione e sopravvivenza globale), è stata effettuata un'analisi comparativa fra i gruppi di pazienti con familiaritá positiva e negativa per patologie oncologiche. Inoltre è stata effettuata un'analisi in accordo al "carico di familiaritá" distinguendo i pazienti in FHC-high (2 linee parentali affette), FHC-low (una linea parentale) ed FHC-negative, per capire se ci fosse propozionalitá fra outcomes clinici e familiaritá.

La raccolta dati ha coinvolto 21 centri oncologici in tutto il territorio Italiano. I dati oggetto della prima fase del progetto (studio clinico osservazionale) coinvolgono 822 pazienti, di questi il 55,7 % FHC negative, il 35.2% FHC-low ed il 9.1% FHC-high. I pazienti inclusi erano prevalentemente affetti da carcinoma polmonare non a piccole cellule (NSCLC), melanoma, carcinoma renale e carcinomi uroteliali.

Abbiamo dimostrato che i pazienti FHC-high hanno una sopravvivenza libera da progressione ed una sopravvivenza globale significativamente piú lunga dei pazienti FHC-negative, e numericamente piú lunga, ma non significativamente, dei pazienti FHC-negative. Questi risultati sono stati confermati all'analisi multivariata che includeva tutti i fattori prognostici comunemente noti (performance status, carico di malattia, linea di trattamento, etá, sesso) e lo stesso tumore primitivo (melanoma, NSCLC, renal cell carcinoma e altri), al fine di verificare la soliditá del risultato.

Inoltre, pazienti con elevato "burden di familiaritá" hanno anche riportato una maggiore incidenza di eventi avversi immuno-relati se comparati ai pazienti senza familiaritá, risultato confermato in analisi multivariata. Questo risultato supporta ulteriormente la tesi in studio, in quando gli eventi avversi immuno-relati sono effetti farmacodinamici che ampiamente confermati come predittori di efficacia del trattamento con ICI.

La fase iniziale del progetto di ricerca è stata indipendente presentata come poster all' ASCO (america society of clinical oncology) annual meeting 2019:

Alessio Cortellini, Sebastiano Buti, Daniele Santini, Raffaele Giusti, Marcello Tiseo, Federica Zoratto, Paolo Marchetti, Melissa Bersanelli, Federica De Galitiis, Maria Giuseppa Vitale, Francesca Rastelli, Rossana Berardi, Marianna Tudini, Francesco Atzori, Daniela Iacono, Alessandro Inno, Sergio Bracarda, Clara Natoli, Paolo Antonio Ascierto, Corrado Ficorella. Family history of cancer as surrogate predictor for immunotherapy with anti-PD-1/PD-L1 immune checkpoint inhibitors: The FAMI-L1 study. J Clin Oncol 37, 2019 (suppl; abstr 2559). **Poster Presentation** ASCO annual meeting 2019.

Il full text è stato pubblicato sulla rivista Oncoimmunology (Impact Factor: 8.1)

Cortellini A, Buti S, Bersanelli M, Giusti R, Perrone F, Di Marino P, Tinari N, De Tursi M, Grassadonia A, Cannita K, Tessitore A, Zoratto F, Veltri E, Malorgio F, Russano M, Anesi C, Zeppola T, Filetti M, Marchetti P, Botticelli A, Cappellini GCA, De Galitiis F, Vitale MG, Rastelli F, Pergolesi F, Berardi R, Rinaldi S, Tudini M, Silva RR, Pireddu A, Atzori F, Iacono D, Migliorino MR, Gelibter A, Occhipinti MA, Martella F, Inno A, Gori S, Bracarda S, Zannori C, Mosillo C, Parisi A, Porzio G, Mallardo D, Fargnoli MC, Tiseo M, Santini D, Ascierto PA, Ficorella C. Evaluating the role of FAMIly history of cancer and diagnosis of multiple neoplasms in cancer patients receiving PD-1/PD-L1 checkpoint inhibitors: the multicenter FAMI-L1 study. Oncoimmunology. 2020 Jan 7;9(1):1710389. doi: 10.1080/2162402X.2019.1710389.

In considerazione del frequente riscontro di neoplasie multiple nei pazienti affetti da sindromi ereditarie di suscettiblita, fra gli obbiettivi secondari, lo studio si proponeva di verificare se la diagnosi di neoplasie multiple (sincrone o metacrone), potessero essere utilizzate come possibili fattori predittivi positivi surrogati per il beneficio clinico al trattamento con farmaci anti-PD-1 e anti-PD-L1.

In considerazione dell'assenza di un'associazione significativa fra diagnosi di neoplasie multiple e outcome clinici riscontrata nella fase preliminare dello studio, tale obiettivo secondario non è stato ulteriormente esplorato nella fase confermatoria.

FASE CONFERMATORIA E TRASLAZIONALE

La sotto-analisi dei pazienti NSCLC all'interno dello studio FAMI-L1, ha confermato il fatto che l'associazione familiarità-beneficio clinico con immunoterapia, sembrerebbe essere maggiormente espressa proprio nei pazienti affetti da neoplasie polmonari, riscontrando un' associazione con una sopravvivenza globale più lunga ed un trend migliorativo di sopravvivenza libera da progressione e tasso di risposte.

Al fine di discernere fra il possibile ruolo predittivo e quello prognostico della familiaritá positiva per neoplasia, abbiamo portato avanti la progettualitá durante l'anno di fellowship nel Regno Unito presso l'Imperial College London.

Abbiamo raccolto e dati di familiarità per neoplasie in due grandi coorti (di 728 e 652 pazienti, da oltre 30 centri Italiani ed Europei) di pazienti affetti da NSCLC avanzato trattate in prima linea sistemica rispettivamente con immunoterapia anti-PD1 (pembrolizumab) o chemioterapia convenzionale.

Disporre di due coorti del genere ci ha consentito di effettuare un'analisi comparativa sia descrittiva che attraverso un perfect random case-control matching ed una pooled analysis, in modo da pesare il ruolo della familiarità oncologica, sia da un punto di vista qualitativo

che quantitativo, in pazienti trattati con chemioterapia e con immunoterapia con checkpoint inhibitors.

Nel dettaglio, all'analisi delle due coorti matched costitutita di 607 pazienti ciascuna, i pazienti FHC-high hanno riportato un vantaggio significativo in termini di sopravvivenza libera da progressione (HR=0.65[95%CI:0.48-0.89];p=0.0074), sopravvivenza globale (HR=0.67[95%CI:0.46-0.95],p=0.0281), e tasso di controllo di malattia (86.4% vs 67.5%, p=0.0096) rispetto ai pazienti FHC-negative, esclusivamente nella coorte trattata in prima linea con immunoterapia. Di contro, nella coorte matched di pazienti trattati con chemioterapia, la FHC non si è dimostrata associata a nessun outcome oncologico.

Abbiamo anche riportato qualitativamente la distrubuzione di familiaritá per i pazienti FHC-high, notando una prevalenza di familiaritá per tumori polmonari all'interno delle famiglie affatte, sebbene nessun trend di distribuzione sia stato riscontrato, confermando di fatti l'assenza di chiare sindromi di ereditarietá nei pazienti affetti da NSCLC.

I risultati di tale analisi clinica ci hanno consentito di stabilire che l'effetto positivo della familiarità per neoplasie era ristretto ai soli pazienti trattati con immunoterapia, confermandone pertanto il ruolo predittivo per il treattamento immunoterapico.

La nostra ipotesi iniziale consisteva nel fatto che il link biologico che spieghi la correlazione tra familiarità e clinical benefit con immunoterapia, possa essere costituito da alterazioni dei geni DDR (damage and response repair genes), sia somatiche che germinali.

Nel contesto del tumore polmonare non a piccole cellule (NSCLC), esistono giá dati robusti che dimostrano come alterazioni somatiche patogenetiche dei geni DDR siano dei forti predittori di outcome migliorativi con inibitori dei checkpoint immunitari.

Ricciuti B, et al. Clin Cancer Res. 2020 Aug 1;26(15):4135-4142. doi: 10.1158/1078-0432.CCR-19-3529. Epub 2020 Apr 24. PMID: 32332016.

In virtú di ció, abbiamo stabilito di testare la nostra ipotesi circa la correlazione fra storia familiare e alterazione dei geni DDR, a partire dalle alterazioni somatiche.

Inoltre, il tumore polmonare è anche noto per non avere forme sindromiche conosciute, associate a familiaritá specifica ed alterazioni genetiche germinali note.

Abbiamo pertanto raccolto i campioni di 128 pazienti affetti da NSCLC afferenti ad alcuni dei centri coinvolti, precedentemente testati con targeted DNA tumour sequencing (FoundationOne CDx assay

(https://info.foundationmedicine.com/hubfs/FMI%20Labels/FoundationOne_CDx_Label_Technical_Info.pdf).

All'interno del pannello dei 324 geni testati, abbiamo isolato 24 geni di interesse fra quelli testati da Ricciuti et al. categorizzati sulla base del pathway funzionale di interesse:

1.mismatch repair: MLH1, MSH6, PMS2;

2. DNA damage sensing: ATM, ATR, CHEK1, CHEK2;

3. homologous recombination BAP1, BARD1, BRCA1, BRCA2, BRIP1, PALB2, RAD51, RAD51C, RAD52;

4. Fanconi anemia: FANCA, FANCC, FANCG, FANCL;

5. DNA polymerase: POLD1, POLE;

6. nucleotide excision repair: ERCC4;

7. base excision repair: XRCC2).

Abbiamo inoltre valutato l'associazione fra il tumour mutational burden (TMB) mediano, l'espressione tumorale di PD-L1 e la storia familiare di neoplasia.

Non abbiamo riscontrato associazioni fra alterazioni DDR/TMB e la storia familiare, pertanto pur essendo riusciti a confermare il ruolo predittivo della storia familiare di neoplasia per il trattamento immunoterapico, la nostra ipotesi meccanicistca non é confermata e saranno necessari ulteriori studi per chiarirne i meccanismi sottostanti.

La componente clinical della seconda fase di studio é stata presentata come poster al congress Europeo dell ESMO (European Society of Medical Oncology) dedicato al tumoure polmonare (ELCC – European Lung Cancer Conference):

Cortellini, S. Buti, M. Di Maio, R. Giusti, O. Nigro, L. Cantini, E. Bria, F. Grossi, M. Torniai, M. De Tursi, F. Citarella, F. Mazzoni, A.J. Gelibter, M. Macerelli, M.R. Migliorino, A. Russo, A. Addeo, G. Porzio, C. Ficorella, D.J. Pinato. 117P Family history of cancer and improved outcomes with first-line immunotherapy in NSCLC patients. Journal of Thoracic Oncology Vol. 16 No. 4S. **Poster Presentation.** European Lung Cancer Congress 2021.

La parte traslazionale dello studio sará presentata come poster al prossimo ESMO Immuno-oncology congress (Ginevra 8-11 Dicembre 2021) ed é stata selezionata per un ESMO grant award data la sua innovativitá.

Il full text della seconda fase dello studio é attualmente in considerazione per la pubblicazione (peer-review stage) per la rivista Journal of Hematology and Oncology (Impact Factor 17.3)

Alessio Cortellini, Raffaele Giusti, Marco Filetti, Fabrizio Citarella, Vincenzo Adamo, Daniele Santini, Sebastiano Buti, Olga Nigro, Luca Cantini, Massimo Di Maio, Joachim G.J.V. Aerts, Emilio Bria, Federica Bertolini, Miriam Grazia Ferrara, Michele Ghidini, Francesco Grossi, Annalisa Guida, Rossana Berardi, Alessandro Morabito, Carlo Genova, Francesca Mazzoni, Lorenzo Antonuzzo, Alain Gelibter, Paolo Marchetti, Rita Chiari, Marianna Macerelli, Francesca Rastelli, Luigi Della Gravara, Stefania Gori, Alessandro Tuzi, Michele De Tursi, Pietro Di Marino, Giovanni Mansueto, Federica Pecci, Federica Zoratto, Serena Ricciardi, Maria Rita Migliorino, Francesco Passiglia, Giulio Metro, Gian Paolo Spinelli, Giuseppe L Banna, Alex Friedlaender, Alfredo Addeo, Corrado Ficorella, Giampiero Porzio, Marcello Tiseo, Russano Marco, Alessandro Russo, David James Pinato. High familial burden of cancer correlates with improved outcome from immunotherapy in patients with NSCLC independent of somatic DNA damage response gene status. 2021

Preliminary phase

Introduction

After the advent of Immune Checkpoint Inhibitors (ICI), oncology clinical practice radically changed, leading to an unprecedented improvement of cancer patients clinical outcomes. Nevertheless, we are still a long way from predicting ICIs efficacy in each patient. PD-L1 (programmed death ligand-1) protein expression, evaluated in both tumor and immune cells, is the most investigated predictive biomarker [1], on the other hand, other factors such as tumor mutational burden, body mass index and gut microbiota, have been investigated as predictors of clinical benefit from immunotherapy across different tumor types [2-5].

Mis-match repair (MMR) deficiency, which lead to the condition of genetic hypermutability know as microsatellite instability (MSI), is related to the number of somatic mutations (especially in MSI-high cases); many studies have already confirmed its positive predictive role (MSI-high) for ICIs treatment, particularly with anti-PD-1 (programmed death-1) antibodies [6, 7]. MSI is known to be the hallmark of Lynch syndrome (LS), a familial clustering of colorectal and endometrial cancers. LS is caused by several germ-line mutations, which result in a defective MMR and is inherited as dominant autosomal character. Similarly, *BRCA 1* and *2* (Breast Cancer 1/2) mutations, which are associated with hereditary breast-ovarian cancer syndrome (HBOC), may correlate with the mutational landscape of the tumors, because of the homologous recombination repair deficiency [8]. Moreover, patients with inherited cancer susceptibility syndromes, are more likely to develop multiple primary tumors during their life [9]. “BRCA-like” phenotype may be more sensitive to anti-PD-1/PD-L1 agents [10], thus prospective clinical trials with anti-PD-1 for patients with germ-line *BRCA 1/2* mutations are currently ongoing [11]. Lynch syndrome and HBOC syndrome are just two of the forms of inherited cancer susceptibility. Even though notoriously only about 5% to 10% of all cancers result directly from germ-line mutations [12], we can hypothesize that much about family cancer syndromes and cancer predisposition is still unknown. Starting from this hypothesis and from the suggestion that tumors related to inherited cancer susceptibility syndromes seem to

have an "immune sensitive phenotype", we investigated if positive family history of cancer (FHC) and diagnosis of metachronous and/or synchronous multiple neoplasms (MN), could be somehow related to clinical outcomes with anti-PD-1/PD-L1 treatment.

In the preliminary analysis of the "FAMI-L1" study (211 patients), we found that patients with a positive FHC had higher objective response rate (ORR) and disease control rate (DCR), and prolonged time to treatment failure and overall survival (OS), while patients with diagnosis of MN only had a significantly higher DCR [13]. Our first hypothesis has been that the underlying mechanisms to our findings might be DNA damage repair (DDR) genes alterations [14].

Here we present the updated results of the FAMI-L1 study, implemented in the study population, in order to confirm our preliminary findings [13].

Materials and Methods

Patient Eligibility

This multicenter retrospective observational study evaluated advanced cancer patients consecutively treated with single agent anti-PD-1/PD-L1 immunotherapy from April 2015 to July 2018, regardless of the treatment line, at 17 Italian institutions (**Supplementary file 1**). Patients were eligible if they had histologically confirmed diagnosis of measurable stage IV cancer, with availability of records about FHC and history of eventual metachronous or synchronous MN. All patients provided written, informed consent to the treatment with immunotherapy.

Study design

The primary end-point of this analysis was to confirm the correlations between FHC and clinical outcomes; the secondary end-point was to further investigate the relationships between diagnosis of MN and clinical outcomes. ORR, progression free survival (PFS), OS and incidence of any grade immune-related adverse events (irAEs) of were evaluated. Patients were assessed with radiological imaging every 8-12 weeks using the RECIST (v. 1.0) criteria [15] according to the local clinical practice and national guidelines required by the Agenzia Italiana del Farmaco (AIFA). ORR was defined as the portion of patients experiencing an objective response (complete or

partial response) as best response to immunotherapy. PFS defined as the time from ICI treatment's start to disease progression or death whichever occurred first; OS as the time from the beginning of treatment to death. For PFS as well as for OS, patients without events were considered as censored at the time of the last follow-up.

On the basis of our previous results [13], and what reported in other studies [16-18], we hypothesized that 48% of the evaluated patients were FHC-positive, and 52% were FHC-negative. With a probability of Type I error of 0.05 and of Type II error of 0.20 and assuming a possible survival benefit for FHC-positive patients with a reduction of the risk of death by 70%, 247 total events were necessary and at least 712 patients had to be included. Univariate and multivariate analyses were performed using the following covariates: age (< 70 *vs.* ≥ 70 years old) [19-22], sex (male *vs* female), primary tumor (NSCLC, melanoma, renal cell carcinoma and others), Eastern Cooperative Oncology Group Performance Status (ECOG-PS) (0-1 *vs* ≥ 2), number of metastatic sites (≤ 2 *vs* > 2) and treatment line (first *vs* non-first). $\chi 2$ test was used to correlate ORR and incidence of any grade irAEs with patients features [23]. $\chi 2$ test was also used to evaluate the correlation between FHC (yes vs no) and diagnosis of MN (yes vs no). Logistic regression was used for the multivariate analysis of ORR and incidence of irAEs of any grade [24]. Median PFS and median OS were evaluated using the Kaplan-Meier method [25]. Median period of follow-up was computed according to the reverse Kaplan-Meier method [26]. Cox proportional hazards model [27] was used to evaluate predictor variables in univariate and multivariate analysis for median PFS and median OS. Data cut-off period was October 2018. All statistical analyses were performed using MedCalc Statistical Software version 18.11.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2019).

Definition of family history of cancer and multiple neoplasms

Given to the lack of data availability in medical records, we did not use the traditional designations of first and second degree of relatedness for family history. Family history was collected in lineal (descendants or ascendants) and collateral lines (not-descentants/ascendants) till the second degree of relatedness (grandparents for lineal line and brothers/sisters for the collateral line). FHC was defined as "positive" with at least one diagnosis of cancer among the considered relatives. Patients were also

categorized according to their FHC as follow: FHC-high (in case of cancer diagnoses in both the lineal and collateral family lines), FHC-low (in case of cancer diagnoses in only one family lines, lineal or collateral) and FHC-negative (**Figure 1**). Diagnosis of metachronous and/or synchronous MN was defined according to the international association of cancer registry (IARC/IACR) rules [28]. Patients were also categorized according the diagnosis of MN as follow: MN-high (in case of more than two cancer diagnoses in their medical history), MN-low (in case of two cancer diagnoses in their medical history) and MN-negative. A further analysis was performed categorizing patients into synchronous MN, metachronous MN and MN-negative.

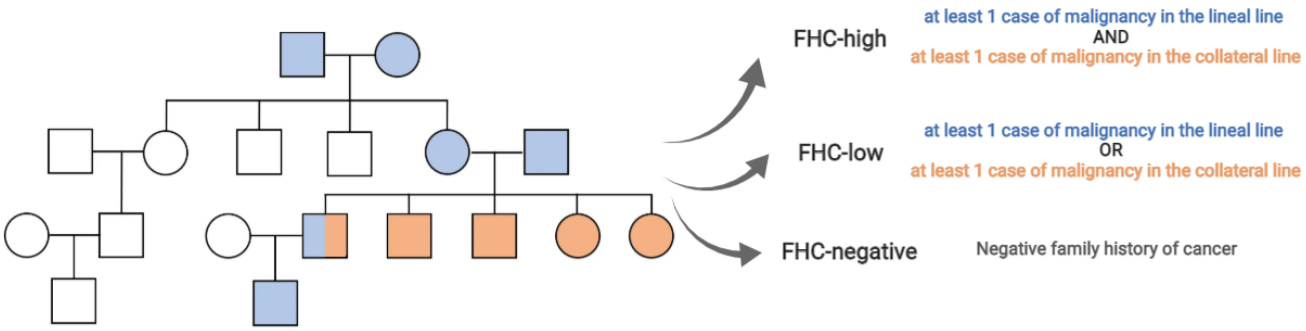

Figure 1: Graphical flow of FHC categories.

Results

Patients' characteristics

822 consecutive, stage IV cancer patients underwent a treatment with anti-PD-1/PD-L1. 458 patients (55.7%) were FHC-negative, while 364 (44.3%) were FCH-positive: 289 (35.2%) FHC-low and 75 (9.1) FHC-high patients, respectively. Among FHC-positive patients 270 (32.8%) were lineal line positive and 167 (20.3%) were collateral line positive. 123 patients (14.9%) had diagnosis of MN: 29 (3.5%) synchronous MN and 94 (11.4%) metachronous MN. 108 patients (13.2%) were MN-low, while 15 (1.8%) were MN-high. All patients features are summarized in **Table 1**

Among FHC-positive and FHC-negative patients, 61 (16.8%) and 62 (13.5%) had diagnosis of MN (p = 0.1987).

	N° (%)
	822
AGE, (years)	
Median	68
Range	21 – 92
Elderly (≥ 70)	359 (43.7)
SEX	
Male	552 (67.1)
Female	270 (32.9)
ECOG PS	
0 - 1	689 (83.8)
≥ 2	133 (16.2)
Primary Tumor	
NSCLC	475 (57.8)
Melanoma	190 (23.1)
Renal cell carcinoma	133 (16.2)
Others	24 (2.9)
No. of metastatic sites	
≤ 2	407 (49.5)
> 2	415 (50.5)
Type of anti-PD-1/PD-L1 agent	
Pembrolizumab	239 (29.1)
Nivolumab	559 (68)
Atezolizumab	24 (2.9)
Treatment line of Immunotherapy	
First	214 (26)
Non-First	608 (74)
FHC	
Negative	458 (55.7)
FHC-low	289 (35.2)
FHC-high	75 (9.1)
FHC-Straight line	270 (32.8)
FHC-Collateral line	167 (20.3)
MN	
Negative	699 (85.1)
MN-low	108 (13.1)
MN-high	15 (1.8)
MN-synchronous	29 (3.5)
MN-methacronous	94 (11.4)

Table 1: Patients features.

Efficacy analysis

Among 822 patient, 775 were evaluable for activity; the other 47 had not yet evaluated the disease at the time of the data cut-off analysis or were lost to follow up/death

without evaluation of clinical response. ORR in the overall population was 34.8% (95%CI: 30.8-39.2, 270 responses). As summarized in **Table 2**, no significant differences was found regarding ORR among subgroups.

ORR analysis			
Variable (comparator)	**Response Ratio**	**ORR (%) (95% CI)**	***p - value***
Overall	270/775	34.8 (30.8 – 39.2)	-
FHC			
Positive	130/347	37.5 (31.3 – 44.5)	*0.1675*
Negative	140/428	32.7 (27.5 – 38.6)	
FHC - Straight line			
Positive	101/256	39.5 (32.1 – 47.9)	*0.0584*
Negative	169/519	32.6 (27.8 – 37.8)	
FHC - Collateral line			
Positive	56/161	34.8 (35.3 – 56.3)	*0.9866*
Negative	214/614	34.9 (26.3 – 45.2)	
FHC (FHC-negative)			
FHC-low	101/275	36.7 (29.9 – 44.6)	*0.3288*
FHC-high	29/72	40.3 (26.9 – 57.8)	
Multiple Neoplasm			
Yes	46/116	39.7 (29.0 – 52.8)	*0.2380*
No	224/659	34.0 (29.6 – 38.7)	
MN (no MN)			
MN-low	41/104	39.4 (28.2 – 53.4)	*0.4922*
MN-high	5/12	41.7 (13.5 – 97.2)	
MN (no MN)			
MN-synchronous	7/27	25.9 (10.4 – 53.4)	*0.1156*
MN-metachronous	39/89	43.8 (31.2 – 59.9)	

Table 2: Activity data for overall population, and subgroups.

The median follow-up was 15.6 months; in the overall population median PFS was 9.2 months (95%CI: 8.2-10.6; 479 events), median OS was 20.5 months (95%CI: 16.2-27.8; 477 censored patients). **Table 3** and **table 4** reported univariate and multivariate analyses of PFS and OS in details.

Median PFS in FHC-negative, FHC-low and FHC-high patients was 9.3 months (95%CI: 7.5-10.6; 277 events), 8.4 months (95%CI: 7-11.4; 166 events) and 20.5 months (95%CI: 8.7-26.4; 36 events), respectively (**Figure 1**). As reported in **Table 3**, FHC-high patients had a significantly longer PFS when compared to FHC-negative patients (HR=0.69 [95%CI: 0.48-0.97], p = 0.0379); at the multivariate analysis, FHC-high was confirmed an independent predictor for PFS (compared to FHC-negative).

	Progression Free Survival	
	Univariate Analysis	**Multivariate Analysis**
VARIABLE (Comparator)	**HR (95% CI); *p – value***	**HR (95% CI); *p - value***
FHC Positive vs Negative	0.92 (0.76–1.10); *p=0.3705*	-
FHC - Straight line Positive vs Negative	0.87 (0.72–1.06); *p=0.1790*	-
FHC - Collateral line Positive vs Negative	0.91 (0.73–1.15); *p=0.4722*	-
(FHC-negative) FHC-low FHC-high	 0.98 (0.81–1.19); *p=0.9116* 0.69 (0.48–0.97); *p=0.0379*	 0.94 (0.78–1.14); *p=0.5845* 0.64 (0.45–0.91); *p=0.0148*
Multiple Neoplasm Yes vs No	0.78 (0.61–1.02); *p=0.0771*	-
MN **(MN-negative)** MN-low MN-high	 0.79 (0.60–1.04); *p=0.1060* 0.73 (0.34–1.55); *p=0.4170*	-
MN **(MN-negative)** MN-synchronous MN-metachronous	 0.84 (0.51–1.38); *p=0.4939* 0.77 (0.58–1.04); *p=0.0912*	-
Primary Tumor (NSCLC) Melanoma Kidney Others	 0.60 (0.47-0.76); *p<0.0001* 0.79 (0.62-1.02); *p=0.0716* 1.34 (0.81-2.22); *p=0.2516*	 0.70 (0.54-0.90); *p=0.0053* 0.65 (0.51-0.84); *p=0.0012* 1.11 (0.66-1.84); *p=0.6911*
Sex Male vs Female	1.15 (0.95–1.40); *p=0.1309*	-
Age Elderly vs Non-elderly	1.02 (0.85–1.22); *p=0.7982*	-
Treatment line Non-first vs First	1.46 (1.16–1.84); *p=0.0011*	1.33 (1.03–1.71); *p=0.0261*
N° of metastatic sites >2 vs ≤ 2	1.71 (1.43–2.06); *p<0.0001*	1.62 (1.35–1.95); *p<0.0001*
ECOG PS ≥2 vs 0-1	2.14 (1.72–2.67); *p<0.0001*	2.14 (1.72–2.69); *p<0.0001*

Table 3: Univariate and multivariate analyses for PFS.

Median OS in FHC-negative, FHC-low and FHC-high patients was 18.2 months (95%CI: 14.9-23.9; 250 censored patients), 20.8 months (95%CI: 15.4-20.9; 176 censored patients) and 31.6 months (95%CI: 26.2-31.6; 51 censored patients), respectively (**Figure 1**). As reported in **Table 4**, FHC-high patients had a significantly longer OS when compared to FHC-negative patients (HR=0.61 [95%CI: 0.39-0.93], p = 0.0210); at multivariate analysis, FHC-high was confirmed an independent predictor for OS (compared to FHC-negative).

	Overall Survival	
	Univariate Analysis	**Multivariate Analysis**
VARIABLE (Comparator)	**HR (95% CI); *p – value***	**HR (95% CI); *p - value***
FHC Positive vs Negative	0.81 (0.65–1.01); *p=0.0612*	-
FHC - Straight line Positive vs Negative	0.79 (0.63–1.01); *p=0.0572*	-
FHC - Collateral line Positive vs Negative	0.82 (0.62–1.08); *p=0.8207*	-
(FHC-negative) FHC-low FHC-high	0.87 (0.69–1.10); *p=0.2652* 0.61 (0.39–0.93); *p=0.0210*	0.84 (0.67–1.06); *p=0.1600* 0.57 (0.37–0.88); *p=0.0114*
MN Yes vs No	0.86 (0.63–1.17); *p=0.3403*	-
MN **(MN-negative)** MN-low MN-high	0.83 (0.62–1.15); *p=0.2837* 1.05 (0.49–2.23); *p=0.8909*	-
MN **(MN-negative)** MN-synchronous MN-metachronous	1.01 (0.57–1.75); *p=0.9753* 0.82 (0.58–1.16); *p=0.2624*	-
Primary Tumor (NSCLC) Melanoma Kidney Others	0.46 (0.35-0.62); *p<0.0001* 0.56 (0.44-0.82); *p=0.0014* 1.34 (0.75-2.39); *p=0.3239*	0.54 (0.40-0.74); *p=0.0001* 0.49 (0.36-0.68); *p<0.0001* 1.03 (0.57-1.85); *p=0.9233*
Sex Male vs Female	1.51 (1.19–1.92); *p=0.0006*	1.30 (1.02–1.65); *p=0.0317*
Age Elderly vs Non-elderly	1.15 (0.93–1.42); *p=0.1972*	-
Treatment line Non-first vs First	1.41 (1.07–1.84); *p=0.0129*	1.19 (0.88–1.61); *p=0.2361*
N° of metastatic sites >2 vs ≤ 2	1.66 (1.34–2.06); *p<0.0001*	1.52 (1.22–1.89); *p=0.0001*

ECOG PS ≥2 vs 0-1	3.09 (2.43–3.92); *p<0.0001*	3.05 (2.39–3.89); *p<0.0001*

Table 4: Univariate and multivariate analyses for OS.

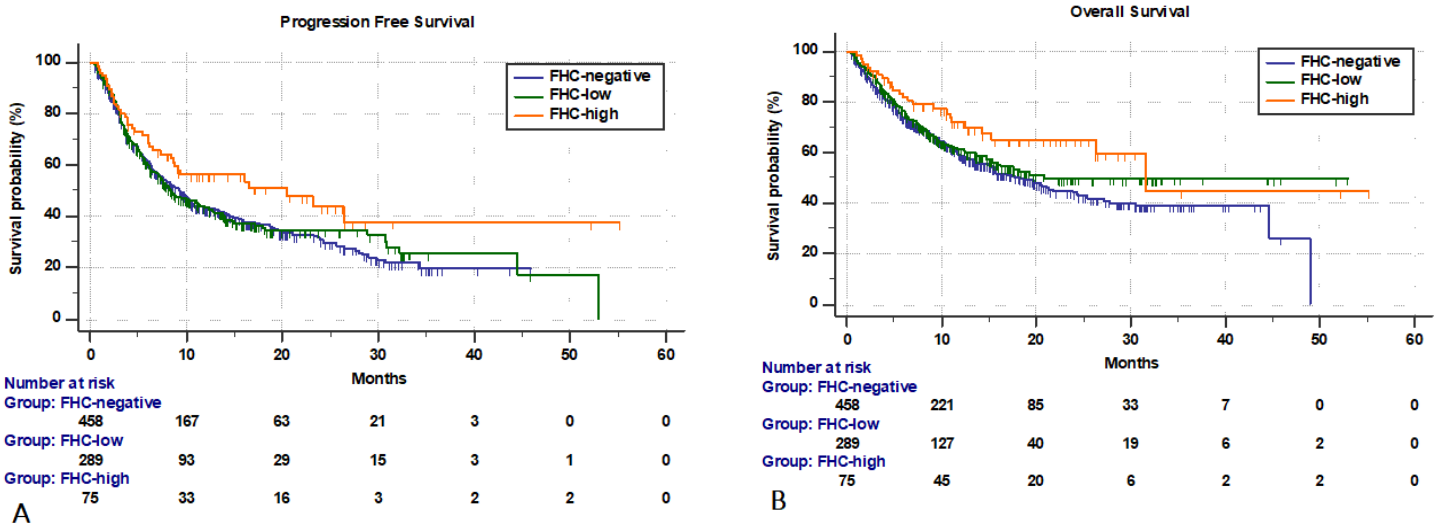

Figure 1: Kaplan-Meier survival curves according to FHC. **(A) Progression Free Survival**. FHC-negative: 9.3 months (95%CI: 7.5-10.6; 277 events); FHC-low: 8.4 months (95%CI: 7-11.4; 166 events); FHC-high: 20.5 months (95%CI: 8.7-26.4; 36 events). **(B) Overall Survival**. FHC-negative: 18.2 months (95%CI: 14.9-23.9; 250 censored patients); FHC-low: 20.8 months (95%CI: 15.4-20.9; 176 censored patients); FHC-high: 31.6 months (95%CI: 26.2-31.6; 51 censored patients).

Median PFS in MN-negative, MN-low and MN-high patients was 8.7 months (95%CI: 7.6-10.2; 414 events), 12.3 months (95%CI: 8.3-28.9; 58 events) and 14.4 months (95%CI: 3.6-14.5; 7 events), respectively (**Figure 2**). As reported in **Table 3**, no significant differences were found regarding PFS, according to MN categories.

Median OS in MN-negative, MN-low and MN-high patients was 20.5 months (95%CI: 15.7-27.1; 43 censored patients), 26.2 months (95%CI: 18.7-48.9; 66 censored patients) and 15.9 months (95%CI: 10.5-15.9; 8 censored patients), respectively (**Figure 2**). As reported in Table 4, no significant differences were found regarding OS, according to MN categories.

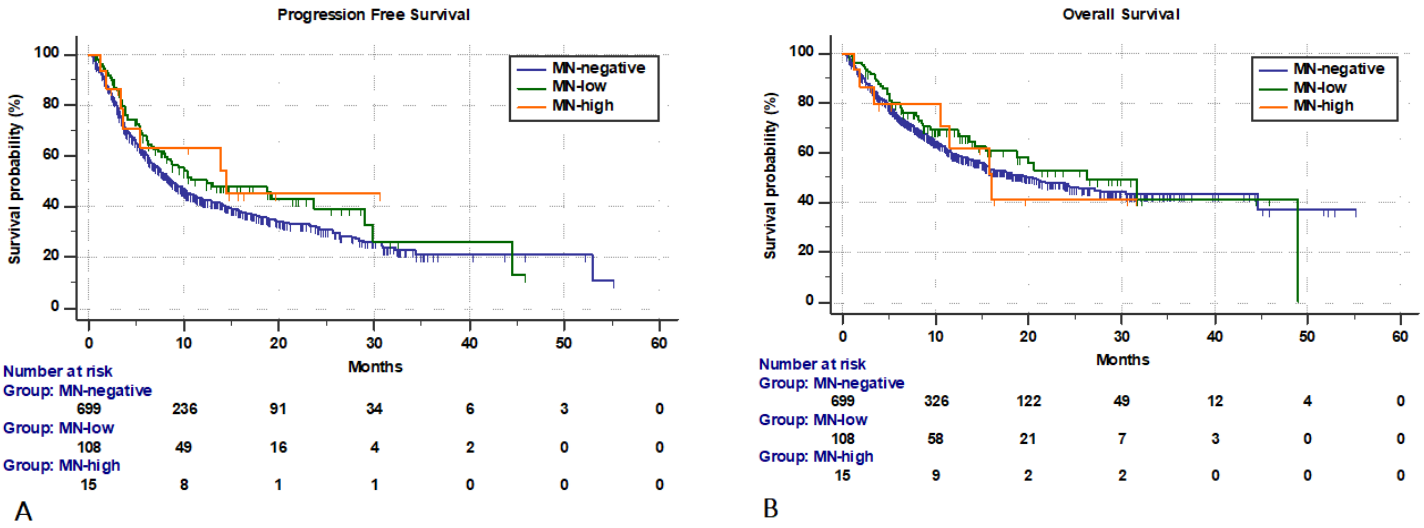

Figure 2: Kaplan-Meier survival curves according to MN. **(A) Progression Free Survival**. MN-negative: 8.7 months (95%CI: 7.6-10.2; 414 events); MN-low: 12.3 months (95%CI: 8.3-28.9; 58 events); MN-high: 14.4 months (95%CI: 3.6-14.5; 7 events). **(B) Overall Survival**. MN-negative: 20.5 months (95%CI: 15.7-27.1; 43 censored patients); MN-low: 26.2 months (95%CI: 18.7-48.9; 66 censored patients); MN-high: 15.9 months (95%CI: 10.5-15.9; 8 censored patients).

Immune-related adverse events

In the overall population, 329 patients experienced any grade irAEs (40%). **Table 5** summarized the univariate and multivariate analysis of irAEs of any grade. Overall, FHC-positive patients had significantly higher incidence of irAEs of any grade (p = 0.0132) compared to FHC-negative patients; this also occur when considering lineal line exclusively (p = 0.0015), but not when considering collateral line exclusively (p = 0.1491). FHC-high patients had a significantly higher incidence of irAEs of any grade, compared to FHC-negative patients (p = 0.0012), while FHC-low did not (p = 0.1240). FHC overall (positive vs negative) and FHC-high (vs negative) were confirmed independent predictor for higher incidence of irAEs of any grade at the multivariate analysis.

irAEs of any grade - UNIVARIATE ANALYSIS			
Variable (comparator)	**Events Ratio**	**Incidence (95% CI)**	***p - value***
Overall	329/822	40.0 (35.8 – 44.6)	
FHC Positive	163/364	44.8 (38.1 – 52.2)	*0.0132*

Negative	166/458	36.2 (30.9 – 42.2)	
FHC - Straight line			
Positive	129/270	47.8 (39.8 – 56.7)	*0.0015*
Negative	200/552	36.2 (31.3 – 41.6)	
FHC - Collateral line			
Positive	75/167	44.9 (35.3 – 56.3)	*0.1491*
Negative	254/655	38.8 (34.1 – 43.8)	
FHC (FHC-negative)			
FHC-low	121/289	41.9 (34.7 – 50.0)	*0.1240*
FHC-high	42/75	56.0 (40.3 – 75.7)	*0.0012*
Multiple Neoplasm			
Yes	49/123	39.8 (46.8 – 86.0)	*0.9634*
No	280/699	40.1 (46.8 – 86.0)	
MN (MN-negative)			
MN-low	43/108	39.8 (28.8 – 53.6)	*0.9619*
MN-high	6/15	40 (14.7 – 87.1)	*0.9964*
MN (MN-negative)			
MN-synchronous	15/29	51.7 (28.9 – 85.3)	*0.2101*
MN-metachronous	34/94	36.2 (25.1 – 50.5)	*0.4697*
Primary Tumor (NSCLC)	170/475	35.8 (30.6 – 41.6)	
Melanoma	95/190	50.0 (40.4 – 61.1)	*0.0007*
Kidney	62/133	46.6 (35.7 – 59.7)	*0.0232*
Others	2/24	8.3 (1.0 – 30.1)	*0.0050*
Sex			
Male	196/552	35.5 (30.7 – 40.8)	*0.0002*
Female	133/270	49.3 (41.2 – 58.3)	
Age			
Elderly	186/463	40.2 (34.6 – 46.3)	*0.9215*
Non-elderly	143/359	39.8 (33.6 – 46.9)	
Treatment line			
First	92/214	43.0 (34.6 – 52.7)	*0.3034*
Non-first	237/608	39.0 (34.1 – 44.2)	
N° of metastatic sites			
>2	150/415	36.1 (30.6 – 42.4)	*0.0203*
≤ 2	179/407	43.9 (37.7 – 50.9)	
ECOG PS			
≥2	34/133	25.6 (17.7 – 35.7)	*0.0002*
0-1	295/689	42.8 (38.1 – 47.9)	
irAEs of any grade - MULTIVARIATE ANALYSIS			
Variable (comparator)	**Coefficient**	**Std. Error**	***p - value***
FHC (yes vs no)	0.3870	0.1498	*0.0098*
Primary Tumor			
(NSCLC)	-	-	-
Melanoma	0.5456	0.1798	*0.0024*
Kidney	0.6012	0.2050	*0.0034*
Others	-1.7464	0.7519	*0.0202*
Sex	-0.4783	0.1563	*0.0022*
N° of metastatic sites	-0.2747	0.1497	*0.0666*
ECOG-PS	-0.6687	0.2213	*0.0025*

			Nagelkerke R^2: 0.0945
irAEs of any grade - MULTIVARIATE ANALYSIS			
Variable (comparator)	**Coefficient**	**Std. Error**	***p - value***
FHC (FHC -negative)			
FHC-low	0.2795	0.1602	*0.0810*
FHC-high	0.7989	0.2624	*0.0023*
Primary Tumor			
(NSCLC)	-	-	
Melanoma	0.5614	0.1803	*0.0019*
Kidney	0.6176	0.2058	*0.0027*
Others	-1.6780	0.7520	*0.0257*
Sex	-0.4594	0.1571	*0.0034*
N° of metastatic sites	-0.2526	0.1505	*0.0932*
ECOG-PS	-0.6865	0.2222	*0.0020*
			Nagelkerke R^2: 0.1003

Table 5: Univariate and multivariate analysis for incidence of irAEs of any grade.

Discussion

It is well known that a small percentage (5 - 10%) of cancers are related to inherited mutations, which usually occurs with typical familial patterns [11]. Syndromes of inherited cancer predisposition are also one of the underlying mechanisms of MN development [9]. In our population 44.3% and 14.9% of the patients had a positive FHC and diagnosis of MN, respectively; these findings are quite aligned to what previously reported among cancer patients [9, 16-18].

In the preliminary analysis of the FAMI-L1 study, including the first 211 patients, FHC-positive patients had significantly higher ORR/DCR (disease control rate), significantly longer time to treatment failure and OS, when compared to FHC-negative patients [13]. No significant association was found between diagnosis of MN (all metachronous tumors) and clinical outcomes, with the exception of a higher DCR compared to MN-negative patients [13]. In this update, no significant associations were found between FHC, MN and ORR; however from a speculative point of view, looking at the ORRs for FHC-negative, FHC-low and FHC-high (32.7%, 36.7% and 40.3%, respectively), we can noticed that there is a trend to a direct proportionality, between the number of the positive familial lines and the ORR. Moreover, we can now confirm that MN does not affect PFS and OS, even considering the different analysis according to "burden of MN" and to synchronous/metachronous diagnosis of MN. Interestingly, only FHC-high patients had a significantly longer PFS and OS, when compared to FHC-negative patients, while no significant differences were found

between FHC-low and FHC-negative, nor between FHC-positive and FHC-negative patients (analyzed overall, for lineal line only and for collateral line only, see Tables 3 and 4). The aim of the preliminary analysis was exploratory and purely descriptive. We did not compute the sample size nor performed subgroup analyses according to the "FHC burden". In our opinion, present results are more reliable, thanks to the bigger sample size and to the more appropriate analysis.

Although our preliminary results seem now mitigated [13], this update seems to confirm our hypothesis that there is at least an association between the "FHC burden" and immunotherapy clinical outcomes, as if the more positive family lines, the greater the benefits. Looking to the hazard ratios, it is noticeable that they are concordantly higher in each comparison between FHC-high and FHC-negative patients, than in those between FHC-low and FHC-negative. Intriguingly, adding the irAEs analysis we found a significantly higher incidence of any grade irAEs among FHC-positive patients (overall and for lineal line only), when compared to FHC-negative patients. Moreover, FHC-high patients had a significantly higher incidence of irAEs of any grade, when compared to FHC-negative patients, while not FHC-low patients. It is also noticeable that the highest incidence of irAEs of any grade was reported among FHC-high patients (56%). In light of the emerging association between the development of irAEs and improved clinical outcomes with ICIs across different tumor types [29-32], these findings would bear our hypothesis.

As previously stated, a history of MN is one of the clinical hallmark of inherited cancer susceptibility, just as a positive FHC. Despite that, in our population FHC and diagnosis of MN are not significantly related, and this is reflected in the different correlation that they have with clinical outcomes. Nevertheless, it is noticeable that patients with metachronous MN and MN-high ones had the highest ORRs (43.8% and 41.5%, respectively, see Table 2). Moreover, MN-high patients had in the same time the longest PFS and the shortest OS (compared to MN-negative and MN-low). We can thus speculate that a history of MN may underlie a kind of "immune sensitiveness", demonstrated by good ORR and PFS to treatment, which is however outclassed by the prognostic weight that further malignancies have. We could assume that underlying mechanisms of MN and FHC are the same and lead to the same "immune sensitiveness", but on the other hand, patients developing MN surely have some negative prognostic features compared to FHC-positive patients.

The possible relationships between somatic alterations of genes belonging to DNA repair systems (such as homologous recombination, MMR, nucleotide excision repair, cell cycle checkpoints, Fanconi anemia DNA repair pathway, and others), "immune-sensitiveness", and ICIs clinical outcomes have been already explored [33, 34]. Teo et al. reported a significant association between better clinical outcomes and somatic DDR genes alterations in a cohort of advanced urothelial cancer patients treated with atezolizumab [35]. Importantly, a higher response rate was found not only in patients whose tumors harbored known or likely deleterious DDR genes alterations but also in patients with DDR alterations of unknown significance when compared to patients whose tumors were wild type for DDR genes [35]. In a study of single-agent pembrolizumab in docetaxel-refractory metastatic castration-resistant prostate cancer patients (mCRP), those with somatic mutations in *BRCA1/2* or *ATM* (ataxia telangiectasia mutated) had higher responses rates [36].

That being said, if we are demonstrating that there is a proportional relationship between better clinical outcomes with anti-PD-1/PD-L1 inhibitors and "burden of familiarity", we are allowed to think that DDR genes alterations (even of unknown clinical significance) might represent the underlying mechanism, which would make the cancer more "immune-sensitive", maybe throughout an increased production of neo-antigens. However, assuming that FHC is a surrogate of DDR genes alterations, such alterations should not be found exclusively with somatic assays (on the tumor specimen), but also with germ-line assays. In a recent study of mCRP patients, treated with Durvalumab (an anti-PD-L1checkpoint inhibitor) and olaparib, patients harboring somatic DDR genes alterations were more likely to benefit from the treatment [37]. Interestingly, four out of nine responders harbored germ-line alterations in DDR genes: one had a known deleterious mutation in NBN (nibrin) and 3 had frameshift *BRCA2* indels [37]. We must however recognize that prostate cancer might be associated to specific syndromes of inherited cancer susceptibility [38], thus it does not represents the most appropriate model to be extended to all other cancers. Nevertheless, it is conceivable that in case of a non-specific high "burden of familiarity", even without a peculiar familial pattern of cancers (as in the Lynch and HBOC syndromes), germ-line DDR genes alterations might be the substrate which explains the better outcomes with immunotherapy.

Among limitations of the present study, we must cite the retrospective design, which expose to selection biases, and the lack of centralized data review (imaging and toxicities). Our cohort was made of patients who received anti-PD-1/PD-L1 as different treatment line, thus we are not able to balance the expected immunosuppression induced by previous treatments. Even if the discussion about Lynch and HBOC syndromes was only a presupposition for our study, which "generated the hypothesis", we must recognize that our patients were not affected by breast/ovarian cancers nor by colorectal cancer. Moreover, we do not have sufficient data for a proper counselling (e.g. age at diagnosis, and type/number of malignancies among the relatives), nor regarding inherited cancer predisposition syndromes diagnosis and DDR genes alteration (including germ-line BRCA mutations or Lynch syndrome diagnosis).

Collecting the family history is one of the first steps in filling each patient medical record. Even though the role of this information is often underestimated, it should be taken into consideration to properly evaluate development risk of a wide range of disease, including cancer [39, 40]. We are long way from saying that FHC could be used as a selection method for anti-PD-1/PD-L1 treatments. However, our study it gives rise to interesting insights, which we intend to validate prospectively.

Thanks to the great sample size, this update confirms our preliminary findings. Particularly, FHC-high patients seem to benefit more than FHC-negative patients from PD-1/PD-L1 checkpoint inhibitors, suggesting that FHC might be the surrogate of some biological features related to the immune-sensitiveness. However, further investigations on the topic are still required.

Institution	**Department**
St. Salvatore Hospital, University of L'Aquila, L'Aquila	Medical Oncology
SS Annunziata Hospital, Chieti	Medical Oncology
University Hospital of Parma, Parma	Medical Oncology
St. Camillo Forlanini Hospital, Rome	Pulmonary Oncology
University Hospital of Cagliari, Cagliari	Medical Oncology
S Maria Goretti Hospital, Latina	Medical Oncology
St. Andrea Hospital, Rome	Medical Oncology

“Istituto Dermopatico Dell’Immacolata” Hospital, Rome	Medical Oncology
Campus Bio-Medico University, Rome	Medical Oncology
“Ospedali Riuniti” Hospital, Ancona	Medical Oncology
Policlinico Umberto I, Rome	Medical Oncology
“Santa Maria” Hospital, Terni	Medical Oncology
Hospital of Fabriano, Fabriano	Medical Oncology
“Augusto Murri” Hospital, Fermo	Medical Oncology
“G. Mazzini” Hospital, Teramo	Medical Oncology
IRCCS – Istituto Nazionale Tumori, Fondazione “G. Pascale”, Napoli	Medical Oncology
IRCCS Sacro Cuore Don Calabria, Negrar	Medical Oncology

Supplementary file 1: list of the oncological institutions of the study

Ethics approval and consent to participate

All patients provided written, informed consent to treatment with immunotherapy. All patients alive at the time of data collection provided an informed consent for the present retrospective analysis. The procedures followed were in accordance with the precepts of Good Clinical Practice and the declaration of Helsinki. The study was approved by the respective local ethical committees on human experimentation of each institution, after previous approval by the coordinating center (University of L’Aquila, Internal Review Board protocol number 32865, approved on July 24th, 2018).

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Confirmatory phase and translational study

Introduction.

Familial distribution of cancer diagnoses, especially in first-degree relatives, is an epidemiological hallmark of cancer risk [1]. Accurate reconstruction of family history of cancer (FHC) is often the first step towards tailored screening approaches and genetic counselling, even though hereditary cancer syndromes are eventually identified only in 5-10% of cases [2]. Alongside its important value in determining cancer susceptibility, a positive FHC has also emerged as a predictor of outcome in certain malignancies, including for instance gastric and colorectal cancer, where patients with FHC displayed improved survival following curative treatment [3-6]. The biologic foundations underlying the improved prognosis of patients with a positive FHC remains however subject of contention, with a variety of genetic, behavioral, and environmental risk factors being postulated for their role in determining cancer susceptibility and survival [7].

Among the few underlying mechanisms of inherited cancer susceptibility, the presence of high penetrance, pathogenic germline mutations of tumour suppressor genes are the most frequently involved traits, especially variants affecting the DNA damage response and repair (DDR) genes. However, clear inheritable defects like those leading to mis-match repair (MMR) deficiency in the Lynch syndrome [8] and homologous recombination deficiency (HRD) in the hereditary breast-ovarian cancer (HBOC) syndrome [9], explain only a limited part of the FHC usually seen in clinical practice. The genetic architecture of cancer risk is in fact more complex and largely polygenic, where combinations of common polymorphic variants of low penetrance shape risk of cancer in the general population [10].

Acknowledging the immune-sensitive phenotype of cancers related to DDR genes defects [11-12], we postulated that FHC can be linked to immune checkpoint inhibitors (ICIs) efficacy in solid tumours. In a previous study, we demonstrated that a high burden of FHC (FHC-high) is an independent, tumour-agnostic predictor of prolonged overall survival (OS) and progression free survival (PFS) in a large multicenter cohort

of patients treated with programmed death-1/programmed death ligand-1 (PD-1/PD-L1) [13-14], a finding that led us to hypothesize that the underlying biological mechanism to this incremental benefit may relate to pathogenetic DDR genes alterations [15].

In non-small cell lung cancer (NSCLC), an oncological indication where single agent ICIs have led to significant improvements in disease management, somatic DDR genes alterations have been already established as an independent predictor of response and survival to PD-1/PD-L1 inhibitors [16], whereas the role of FHC has never been comprehensively evaluated, probably as a result of limited evidence of an hereditary component to NSCLC risk and no solid linkage to hereditary syndromes [17-18].

To investigate whether FHC correlates with outcomes from immunotherapy in NSCLC, we designed this study including two large, matched cohorts of patients with metastatic NSCLC treated with either first-line single agent pembrolizumab or chemotherapy. In addition, we explored the relationship between FHC and DDR genes alterations as a potential mechanism to explain the difference in clinical outcomes across groups.

Materials and Methods

Study design

The main aim of this study was to evaluate the role of FHC in a cohort of patients with metastatic NSCLC with a PD-L1 tumour expression ≥ 50%, treated with first-line pembrolizumab monotherapy [19-26]. Following a data request update, 29 institutions participated to the study (**Supplementary Table 1**) and retrospectively included patients treated from January 2017 to May 2020.

In order to assess the potential different impact of FHC depending on the treatment strategy, we evaluated a second cohort of patients with metastatic *EGFR* (Epidermal Growth Factor Receptor) wild type NSCLC (ALK and ROS-1 unknown) treated with first line chemotherapy at 13 of the participating institutions from January 2013 to May 2020; the censoring date was 30 September 2020.

Study endpoints included objective response rate (ORR), disease control rate (DCR), PFS and OS. Patients were assessed with radiological imaging at participating institutions, with a frequency ranging from 8 to 12 weeks; investigator-assessed disease response followed Response Evaluation Criteria in Solid Tumors (RECIST)

criteria v1.1. PFS and OS were measured from treatment initiation to disease progression and/or death. Patients without documented disease progression at the data cut off were censored on the date of last clinical follow-up and radiological assessment for OS and PFS, respectively. Methodology of PD-L1 tumour expression is reported in the **Supplementary Methods**.

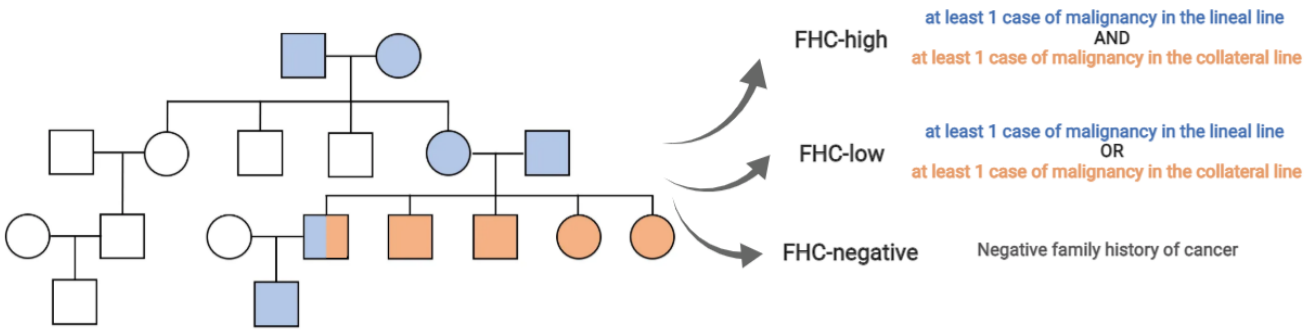
To estimate the differential impact of the FHC across the two populations, we evaluated the impact of FHC on clinical outcomes after a perfect random case-control matching between the two cohorts. Cases and controls were randomly paired on the basis of the FHC, age (< 70 vs. ≥ 70 years old), ECOG-PS (0-1 vs ≥ 2), and burden of disease (≥ 2 vs < two metastatic sites).

We then explored the impact of the FHC within the pembrolizumab and chemotherapy cohorts using univariable analyses. A fixed regression model including major determinants of clinical outcome within the study population [19-26] was used for the multivariable analysis of the pembrolizumab cohort. Additionally, to further evaluate the role of FHC depending on the treatment modality (immunotherapy vs chemotherapy), we performed a pooled analysis of both the cohorts, with and without the interaction term between the FHC and the therapeutic modality (pembrolizumab vs chemotherapy).

Definition of family history of cancer

Family history data was collected from medical records as previously described, and all oncological disease with malignant potential, both hematological and solid, were screened [14]. Lineal line (descendants or ascendants) and collateral line (non-descentants/ascendants e.g., brothers/sisters) were screened till the second degree (grandparents for lineal line and brothers/sisters for the collateral line). Patients were categorized as follow: FHC-high (in case of at least one cancer diagnosis in both lineal and collateral family lines), FHC-low (in case of at least one cancer diagnosis in either the lineal or collateral line) and FHC-negative (**Figure 1**). On the basis of our previous findings [14], FHC-high was considered the group of interest for all analyses.

Figure 1: Graphical flow of FHC categories.



DDR genes exploratory analysis - FDx cohort.

We used a parallel cohort of patients with NSCLC from 4 of the participating institutions (reported in **Supplementary Table 1**), in order to explore the putative role of DDR genes alterations as underlying mechanism to the effect of FHC on clinical outcomes. Relevant baseline clinico-pathologic data and tumour genomic information were gathered. The targeted DNA tumour sequencing was performed with the FoundationOne CDx assay (Detailed information on the variant calling and functional evaluation are available at https://info.foundationmedicine.com/hubfs/FMI%20Labels/FoundationOne_CDx_Label_Technical_Info.pdf).

The panel of 53 genes related to DNA damage response and repair defined by Ricciuti et al. in a large cohort of patients with NSCLC [16] was used as reference, to verify their possible association with FHC. We identified 24 genes of interest among the 324 cancer-related genes and selected rearrangement detected by the FoundationOne CDx assay (1. mismatch repair: *MLH1, MSH6, PMS2;* 2. DNA damage sensing: *ATM, ATR, CHEK1, CHEK2;* 3. homologous recombination *BAP1, BARD1, BRCA1, BRCA2, BRIP1, PALB2, RAD51, RAD51C, RAD52;* 4. Fanconi anemia: *FANCA, FANCC, FANCG, FANCL;* 5. DNA polymerase: *POLD1, POLE;* 6. nucleotide excision repair: *ERCC4;* 7. base excision repair: *XRCC2*). We also explored the associations between FHC, median tumor mutational burden (TMB), smoking status and PD-L1 tumour expression.

Statistical Analysis

The sample size was estimated for the pembrolizumab cohort only, on the basis of the expected number of FHC high patients. According to the subgroup analysis on NSCLC patients evaluated within our previous study [14], we hypothesized a 11% prevalence of FHC high patients and assumed a survival benefit for FHC high patients compared to non-FHC high, with a reduction of the risk of death by 56%. With a probability of Type I error of 0.05 and of Type II error of 0.20, 238 total events were necessary and at least 633 patients had to be recruited overall from the original cohort. Baseline patients' characteristics were reported with summative descriptive statistics (means, medians and proportions) as appropriate. $\chi 2$ test and Fisher's exact test were used to compare categorical variables as appropriate. The Kruskal-Wallis test was used to compare median TMB according to the FHC. One-way analysis of variance was used to evaluate the associations between FHC and PD-L1 tumor expression among the pembrolizumab cohort. Median PFS and median OS were evaluated using the Kaplan-Meier method and the log-rank test. Median period of follow-up was calculated according to the reverse Kaplan-Meier method. Logistic regression was used for the multivariable analysis of DCR and to compute odds radios (OR) with 95% confidence intervals (Cis). Cox proportional hazards regression was used for the multivariable analysis of PFS and OS and to compute all the hazard ratios (HR) for disease progression and death with 95% CIs. Considering that all the selected variables were categorical, a caliper width of < 1 for the standard deviation was used for the random case-control matching. The alpha level for all analyses was set to $p<0.05$. All statistical analyses were performed using MedCalc Statistical Software version 19.3.1 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020).

Results

Patient' characteristics

Overall, 167 (18.7%) out of 890 patients and 88 (11.9%) out of 740 patients were excluded from the pembrolizumab and chemotherapy cohort respectively, due to missing FHC data. The final study population consisted of 723 and 652 patients respectively included in the pembrolizumab and chemotherapy cohorts, respectively. Within the chemotherapy cohort, 564 patients (86.5%) were treated with platinum-based doublets, whilst 88 patients with single-agent chemotherapy (13.5%). After

disease progression, 315 of them (48.3%) received PD-1/PD-L1 checkpoint inhibitors. There was a significantly higher proportion of elderly patients in the pembrolizumab cohort, compared to the chemotherapy cohort (49.0% vs 43.4%, p = 0.0391). Among the pembrolizumab treated patients, 222 (30.7%) and 49 (6.8%) were FHC-low and FHC-high, respectively, while among the chemotherapy treated patients 202 (31%) and 61 (9.4%) were FHC-low and FHC-high, respectively. Patients' characteristics are summarized in **Table 1**. The absolute value of PD-L1 expression was available for 536 patients (74.1%) and the median value was 70%. The mean PD-L1 expression for FHC-negative, FHC-low and FHC-high patients were 71.6% (standard deviation [sd]: 13), 72.2% (sd: 14) and 72.5% (sd: 13) respectively; the analysis of variance confirmed that FHC did not significantly influence the PD-L1 expression [F (2,533) = 0.139, p = 0.871].

	PEMBROLIZUMAB COHORT 723 N° (%)	**CHEMOTHERAPY COHORT 652 N° (%)**	
Age, (years)			**χ2 test**
Median Range Elderly (≥ 70)	69 28 – 92 354 (49.0)	68 31 – 92 283 (43.4)	P = 0.0391
Gender			
Female Male	255 (35.3) 468 (64.7)	205 (31.4) 447 (68.6)	P = 0.1332
ECOG-PS			
0 - 1 ≥ 2	596 (82.4) 127 (17.6)	544 (85.1) 97 (14.9)	P = 0.1778
Histology			
Squamous Non-squamous	174 (24.1) 549 (75.9)	140 (21.5) 512 (78.5)	P = 0.2527
Smoking status			
Never smokers Current/Former smokers	90 (12.4) 633 (87.6)	82 (12.6) 570 (87.4)	P = 0.1011
CNS metastases			
No Yes	589 (81.5) 134 (18.5)	544 (83.4) 108 (16.6)	P = 0.3385
Liver metastases			
No Yes	601 (83.1) 122 (16.9)	561 (86.0) 91 (14.0)	P = 0.1356
Bone metastases			
No Yes	490 (67.8) 233 (32.2)	453 (69.5) 199 (30.5)	P = 0.4965
FHC			
Negative Low High	452 (62.5) 222 (30.7) 49 (6.8)	389 (59.7) 202 (31.0) 61 (9.4)	P = 0.1907

Table 1: patients' characteristics for both the pembrolizumab and chemotherapy cohorts. ECOG-PS: Eastern Cooperative Oncology Group-Performance Status; CNS: Central Nervous System; FHC: Family History of Cancer.

Supplementary Table 2 provides a breakdown of patients' characteristic according to FHC grouping across both the cohorts. None of the baseline characteristics, including smoking status, were significantly associated to the FHC category in either the pembrolizumab or the chemotherapy cohorts. **Supplementary Figure 1** and **Supplementary Table 3** provide detailed FHC information regarding the 49 FHC-high patients from the pembrolizumab cohort. Lung cancer was the most frequent malignancy reported, however, no specific family clusters could be defined.

The median follow-up was 23.3 months (95%CI: 21.8-38.0) for the pembrolizumab cohort and 38.4 months (95%CI: 33.1- 86.7) for the chemotherapy cohort. Median OS and PFS of the entire pembrolizumab cohort were 15.4 months (95%CI: 12.8 – 17.3; 421 events) and 6.9 months (95%CI: 5.8 – 7.9; 523 events), respectively, whilst for the chemotherapy cohort were 14.4 months (95%CI: 12.9 – 16.6; 466 events) and 5.9 months (95%CI: 5.3 – 6.3; 594 events), respectively.

The effect of FHC on clinical outcome is restricted to pembrolizumab treated patients.

After the random case-control matching, 607 patients from the pembrolizumab cohort and 607 patients from the chemotherapy cohort were perfectly paired, with no significant differences between the characteristics of matched subjects. **Table 2** summarizes the clinical outcomes analysis across the two matched cohorts.

	PEMBROLIZUMAB MATCHED COHORT (607 patients)			**CHEMOTERAPY MATCHED COHORT (607 patients)**		
FHC	**Response/ratio**	**ORR (95%CI)**	**χ2 test**	**Response/ratio**	**ORR (95%CI)**	**χ2 test**
HIGH NON-HIGH	20/44 220/508	45.5% (27.7-70.2) 43.3% (37.7-49.4)	P = 0.7830	7/33 163/458	21.2% (8.5-43.7) 35.6% (30.3-41.5)	P = 0.0940
	Disease control/ratio	**DCR (95%CI)**	**χ2 test**	**Disease control/ratio**	**DCR (95%CI)**	**χ2 test**
HIGH NON-HIGH	38/44 343/508	86.4% (61.1-118.5) 67.5% (60.5-75.1)	P = 0.0096	23/33 289/458	69.7% (44.1-104.5) 63.1% (56.0-70.1)	P = 0.4475
	PFS (months) (95%CI) [events]	**log-rank**	**HR (95%CI)**	**PFS (months) (95%CI) [events]**	**log-rank**	**HR (95%CI)**

HIGH NON-HIGH	17.2 (8.6 – 28.2) [28] 6.5 (5.4 – 28.3) [405]	P = 0.0074	0.65 (0.48-0.89)	5.9 (3.9 – 6.9) [44] 5.9 (5.3 – 6.4) [509]	P = 0.7039	1.06 (0.77-1.46)
	OS (months) (95%CI) [events]			**OS (months) (95%CI) [events]**		
HIGH NON-HIGH	31.3 (15.2-31.3) [21] 15.3 (12.8 –17.5) [327]	P = 0.0281	0.67 (0.46-0.95)	16.9 (12.1 – 34.5) [29] 13.8 (12.3 – 15.8) [408]	P = 0.0866	0.75 (0.54-1.04)

Table 2: Summary the clinical outcomes analysis across the two matched cohorts. ECOG-PS: Eastern Cooperative Oncology Group-Performance Status; CNS: Central Nervous System; FHC: Family History of Cancer; ORR: Objective Response Rate; DCR: Disease Control Rate; PFS; Progression Free Survival; OS: Overall Survival; HR: Hazard Ratio; CI: Confidence Interval.

As compared to FHC-low/negative patients, FHC high were confirmed to have a significantly longer OS (HR=0.67 [95%CI: 0.46-0.95], p = 0.0281; **Figure 2A**), a significantly longer PFS (HR=0.65 [95%CI: 0.48-0.89]; p = 0.0074; **Figure 2B**) and a higher DCR (86.4 vs 67.5, p = 0.0096; **Figure 2C**), within the pembrolizumab cohort. On the contrary, no significant associations were found between FHC and OS (HR = 0.75 [95%CI: 0.54 – 1.04], p = 0.0866; **Figure 2D**), PFS (HR = 1.06 [95%CI: 0.77 – 1.46], p = 0.7039; **Figure 2E**), and DCR (69.7% vs 63.1%, p = 0.1202; **Figure 2F**), within the chemotherapy cohort. FHC was not associated with ORR in either of the matched cohorts.

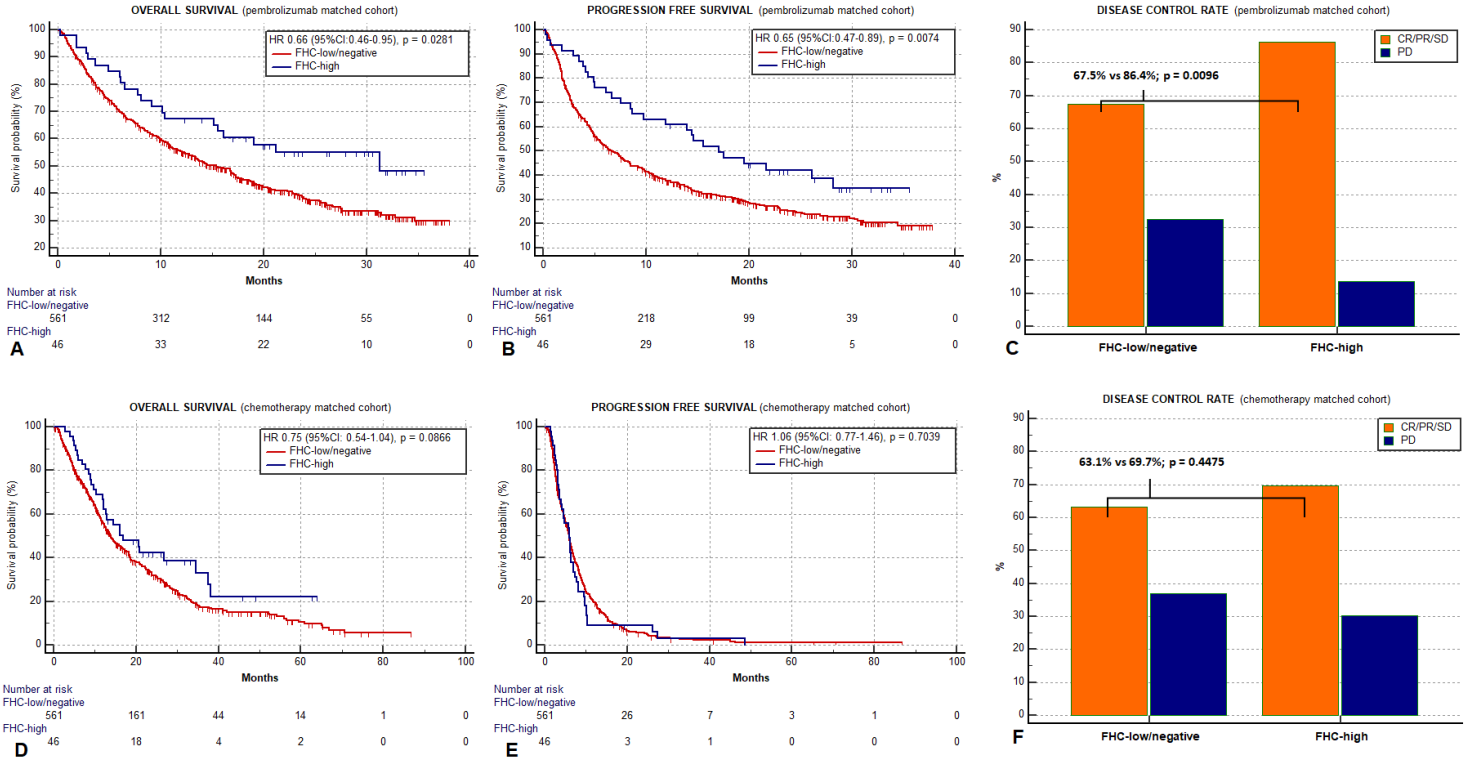


Figure 2: Clinical outcomes analysis according to the FHC the pembrolizumab and chemotherapy matched cohorts (also reported in Table 2). (**A**) Pembrolizumab cohort, Kaplan-Meier survival estimate for Overall Survival. **(B)** Pembrolizumab cohort, Kaplan-Meier survival estimate for Progression Free Survival. (**C**) Pembrolizumab cohort, Frequency chart for disease control rate. (**D**) Chemotherapy cohort, Kaplan-Meier survival estimate for Overall Survival. (**E**) Chemotherapy cohort, Kaplan-Meier survival estimate for Progression Free Survival. (**F**) Chemotherapy cohort, Frequency chart for disease control rate.

Supplementary Table 4 summarizes all the univariable analyses according to the FHC across the entire pembrolizumab and chemotherapy cohorts. When considering the whole population, FHC-high patients achieved a longer median OS (HR = 0.65 [95%CI: 0.43-0.99], p = 0.0438; **Supplementary Figure 2A**) and PFS (HR = 0.62 [95%CI: 0.43-0.89], p = 0.0080; **Supplementary Figure 2B**), as compared to FHC low/negative patients among the pembrolizumab cohort. Additionally, a significantly higher DCR was reported for the FHC-high group compared FHC-low/negative patients (87.0% vs 65.6%, p = 0.0029; **Supplementary Figure 2C**). Among the entire chemotherapy cohort, FHC-high patients achieved a longer OS (HR = 0.69 [95%CI: 0.49-0.97], p = 0.0307; **Supplementary Figure 1D**), as compared to FHC-low/negative. No differences in term of PFS (HR = 1.05 [95%CI: 0.80-1.38], p =

0.6905; **Supplementary Figure 1E**) and DCR (p = 0.3838; **Supplementary Figure 1F**) were reported.

Supplementary Table 5 summarises the multivariable analyses for DCR, PFS and OS of the pembrolizumab. FHC-high patients were confirmed to have a significantly higher probability of achieve a disease control (OR = 3.17 [95%CI: 1.29 – 7.68], p = 0.0113) and a significantly lower probability of experience disease progression (HR = 0.68 [95%CI: 0.47 – 0.99], p = 0.0444), as compared to the FHC-low/negative group, while only a trend towards a reduced risk of death was reported (HR = 0.75 [95%CI: 0.49 – 1.14]; p = 0.1791).

The pooled analysis including both the cohorts with and without the interaction term between the FHC and therapeutic modality is reported in **Table 3**. At the pooled analysis, a higher DCR (OR = 1.88 [95%CI: 1.09 – 3.27]; p = 0.0233) and a longer OS (HR = 0.67 [95%CI: 0.51 – 0.87]; p = 0.0028). were reported for the FHC-high group, while no effect on PFS was found (HR = 0.87 [95%CI: 0.70-1.08]; p = 0.2306). With the inclusion of the interaction, the Chi-squared statistic of the overall model fit improved for the DCR (94.4 vs 97.2) and the PFS (210.6 vs 216.6), while no changes were reported for the OS (176.1 vs 176.1). Although the interaction term was not significant (p = 0.1020), FHC-high patients did not show a significantly higher DCR after including it in the model (HR = 1.23 [95%CI: 0.60 – 2.52]; p = 0.5707). On the contrary, the interaction was statistically significant (p = 0.0170) with respect to the PFS and did not affect the PFS analysis according to the FHC (HR = 1.09 [95%CI: 0.83 – 1.43]; p = 0.4937). Lastly, the interaction term between the FHC and the treatment modality was not statistically significant with respect of the OS.

	POOLED ANALYSIS (without interaction)		
	DISEASE CONTROL RATE	**PROGRESSION FREE SURVIVAL**	**OVERALL SURVIVAL**
VARIABLE	**OR (95% CI);** ***p - value***	**HR (95% CI);** ***p - value***	**HR (95% CI);** ***p - value***
Cohort Pembrolizumab vs Chemotherapy	1.16 (0.90-1.50); p = 0.2298	0.61 (0.54-0.69); p < 0.0001	0.91 (0.79-1.04); p = 0.1876
FHC High vs Non-high	1.88 (1.09-3.27); p = 0.0233	0.87 (0.70-1.08); p = 0.2306	0.67 (0.51-0.87); p = 0.0028
Gender Male vs Female	0.95 (0.72-1.26); p = 0.7373	1.13 (0.99-1.29); p = 0.0687	1.13 (0.97-1.31); p = 0.1005
Age Elderly vs Non-elderly	0.94 (0.72-1.21); p = 0.6444	1.08 (0.96-1.22); p = 0.1775	1.25 (1.09-1.43); p = 0.0011
ECOG PS ≥2 vs 0-1	0.34 (0.24-0.47); p < 0.0001	1.95 (1.66-2.28); p < 0.0001	2.44 (2.06-2.89); p < 0.0001
Smoking status Never vs Current/former	0.72 (0.48-1.08); p = 0.1198	1.31 (1.09-1.58); p = 0.0040	1.07 (0.86-1.33); p = 0.4912
CNS metastases Yes vs No	1.20 (0.84-1.70); p = 0.3125	1.11 (0.94-1.30); p = 0.1877	1.22 (1.03-1.46); p = 0.0208
Bone metastates Yes vs No	0.58 (0.44-0.75); p = 0.0001	1.42 (1.25-1.61); p < 0.0001	1.41 (1.22-1.62); p < 0.0001
Liver metastases Yes vs No	0.52 (0.37-0.72); p = 0.0001	1.56 (1.33-1.83); p < 0.0001	1.38 (1.16-1.65); p = 0.0003
Chi-squared statistic for the overall model fit	94.4, DF: 9; p < 0.0001	210.6, DF 9; p < 0.0001	176.1, DF 9; p < 0.0001
	POOLED ANALYSIS (with interaction)		
Cohort Pembrolizumab vs Chemotherapy	1.10 (0.85-1.43); p = 0.4413	0.63 (0.56-0.72); p < 0.0001	0.90 (0.79-1.04); p = 0.1798
FHC High vs Non-high	1.23 (0.60-2.52); p = 0.5707	1.09 (0.83-1.43); p = 0.4937	0.65 (0.46-0.91); p = 0.0129
Interaction FHC*Cohort	**p = 0.1020**	**p = 0.0170**	**p = 0.7923**
Gender Male vs Female	0.95 (0.72-1.26); p = 0.7646	1.12 (0.98-1.28); p = 0.0864	1.13 (0.97-1.31); p = 0.0985
Age Elderly vs Non-elderly	0.94 (0.73-1.22); p = 0.6648	1.09 (0.96-1.23); p = 0.1597	1.25 (1.09-1.43); p = 0.0011
ECOG PS ≥2 vs 0-1	0.33 (0.24-0.47); p < 0.0001	1.95 (1.67-2.28); p < 0.0001	2.45 (2.07-2.89); p < 0.0001
Smoking status Never vs Current/former	0.73 (0.49-1.10); p = 0.1406	1.30 (1.08-1.57); p = 0.0047	1.07 (0.86-1.34); p = 0.4896
CNS metastases Yes vs No	1.21 (0.85-1.72); p = 0.2843	1.10 (0.94-1.29); p = 0.2243	1.22 (1.03-1.46); p = 0.0206
Bone metastates Yes vs No	0.57 (0.44-0.75); p < 0.0001	1.43 (1.25-1.62); p < 0.0001	1.41 (1.22-1.62); p < 0.0001
Liver metastases Yes vs No	0.52 (0.37-0.73); p = 0.0002	1.55 (1.33-1.82); p < 0.0001	1.38 (1.16-1.65); p = 0.0003
Chi-squared statistic for the overall model fit	97.2, DF: 10; p < 0.0001	216.6, DF 10; p < 0.0001	176.1, DF 10; p < 0.0001

Table 3: Summary of the pooled multivariable analysis for DCR, PFS and OS within the pembrolizumab cohort without and with the interaction term FHC*Cohort. ECOG-PS: Eastern Cooperative Oncology Group-Performance Status; CNS: Central Nervous System;

FHC: Family History of Cancer; DCR: Disease Control Rate; PFS; Progression Free Survival; OS: Overall Survival; OR: Odd Ratio; HR: Hazard Ratio; CI: Confidence Interval.

DDR genes analysis (FDx cohort).

Overall, 118 patients were included in the FDx cohort, of which 20 FHC-high (16.9%) and 98 FHC-low/negative (83.1%). Relevant baseline clinic-pathologic characteristics and the DDR genes profile data are summarized in the OncoPrint plot provided in **Figure 3**. The prevalence of at least one DDR genes somatic mutations was 20% (4/20) and 24.5% (24/74) for FHC-low/negative and FHC-high patients, respectively (p = 0.6684). The sample of one patient among the FHC-high harboured two or more DDR genes alterations (5%), compared to 5 patients among the FHC-low/negative patients (5.1%) (p = 8977). The median TMB for FHC-high was 6 Mut/Mb (range: 1 – 18), whilst for the FHC-low/negative was 7.6 Mut/Mb (range: 0 – 42.8) (p = 0.6018) (**Supplementary Figure 3A**) and no association between FHC and PD-L1 tumour expression was reported (**Supplementary figure 3B**). No significant association was found between the FHC and other mutations known to affect clinical outcomes to immunotherapy in NSCLC (e.g. TP53, KEAP1, STK11, KRAS - data not shown).

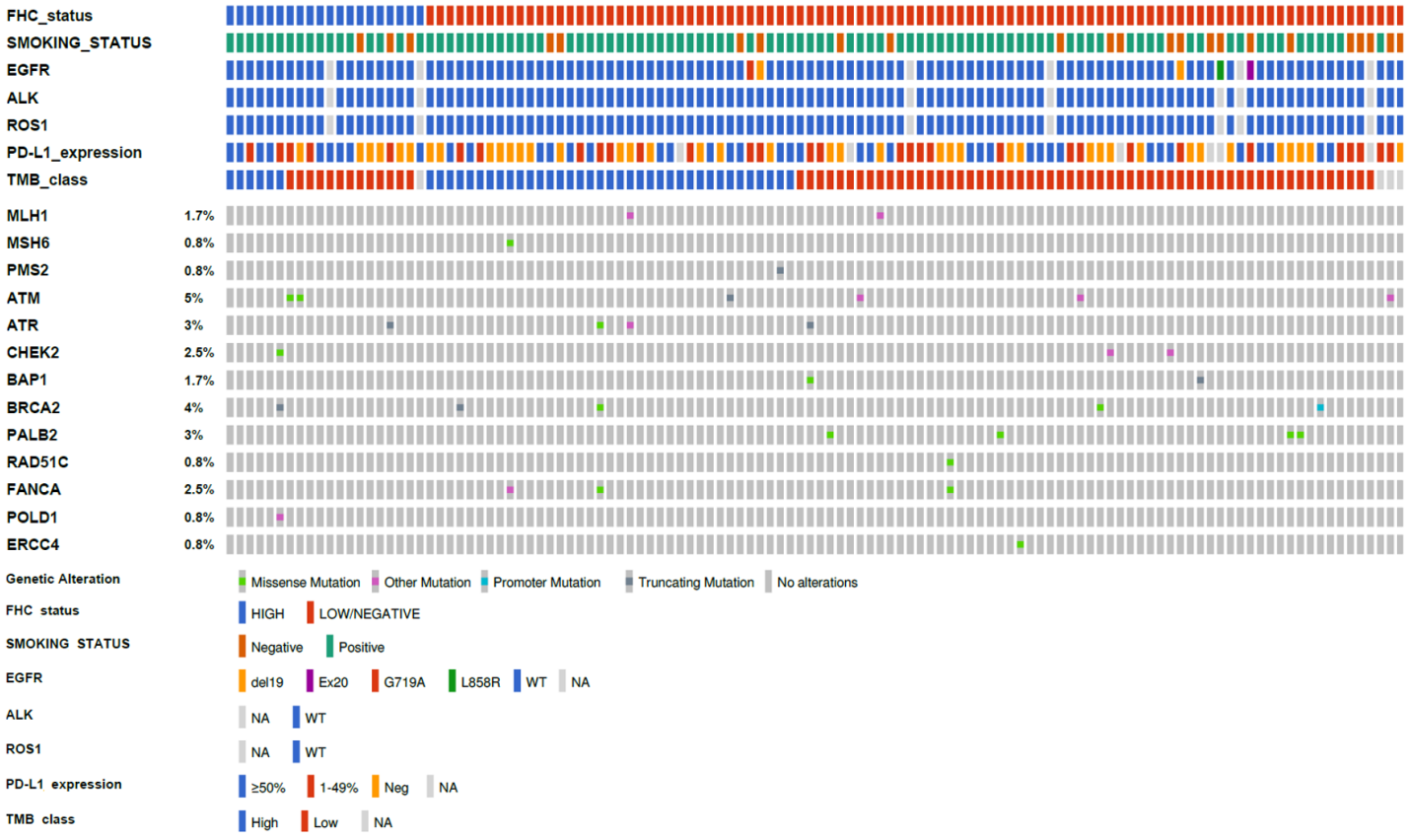


Figure 3: OncoPrint plot summarizing relevant baseline clinic-pathologic characteristics and the DDR genes profile of the FDx cohort. Patients are clustered according to the FHC status (first row) and in the upper section the smoking status, common actionable biomarkers (including EGFR, ALK and ROS-1), the PD-L1 tumour expression and the TMB category (with a cut off of $\geq$ vs $<$ 10 mutations/megabase) are reported. The mutational status and its prevalence of selected DDR genes is reported with different colours according to the mutation's type. Made with cBioPortal oncoprinter, available at: https://www.cbioportal.org/oncoprinter.

Discussion.

Response to immunotherapy is underscored by a complex interplay of host and tumoral factors. In the context of metastatic NSCLC, where the treatment landscape is continuously evolving, additional predictive biomarkers beyond PD-L1 tumour expression are the focus of intense scrutiny [27-28].

In our large multi-centre study of >1300 patients with NSCLC, we identified a subgroup of patients with high burden of FHC who were characterised by significantly increased therapeutic benefit from pembrolizumab as shown by the improved DCR and prolonged PFS following checkpoint inhibition. In an attempt to differentiate prognostic from predictive role of FHC, we utilised random case control matching and pooled analysis including a chemotherapy-treated cohort. These supplementary analyses highlight the positive effect of FHC on DCR and PFS to be restricted to pembrolizumab-treated patients with no significant effect seen in the chemotherapy control cohort, strengthening its putative role as a predictive correlate of outcome from PD-1 inhibition in previously untreated NSCLC patients. This finding contributes to the ongoing debate around therapeutic decision making in PD-L1 selected patients with metastatic NSCLC [29], lending FHC as a clinically useful and inexpensive predictor of benefit to immunotherapy that can integrate currently available biomarkers in the context of PD-1 blockade.

Interestingly, the role of FHC as a predictor of outcome was independent of age, gender, ECOG PS, smoking status and disease burden, suggesting the true independence of FHC from common prognostic predictors of adverse outcome in NSCLC.

In previous studies investigating the prognostic role of FHC, the positive relationship between FHC and survival might have been at least in part influenced by FHC-related behavioural bias (e.g., changes in lifestyle, pro-active surveillance with earlier diagnosis, improved rates of relapse detection), stemming from the patient's or clinician's-perceived risk of worse outcome. This type bias does not apply to our study, which included only stage IV patients, whose palliative treatment planning was not informed or modified by knowledge of FHC [3-6]. Our study reports a significant effect of FHC as predictor of OS following chemotherapy, a finding that can be explained by the fact that half of patients treated with first-line chemotherapy subsequently received immune checkpoint inhibitors post-progression, indicating a possible influence of second-line immunotherapy exposure on OS.

In an attempt to elucidate the mechanisms underlying the protective role of FHC in immunotherapy recipients, we focused on DDR defects as a primary putative mechanism. DDR gene aberrations have become an increasingly studied and translationally appealing tumorigenic mechanism across malignancies because their presence confers sensitivity to poly-(ADP ribose) polymerase inhibitors. With the decreasing costs of next generation sequencing, the recommendation to screen for DDR gene defects has become stronger across tumour types [30, 31], although the ratio of germline:somatic alterations varies significantly across malignancies and individual DDR defect considered [32].

In the context of immunotherapy, DDR gene defects result in accumulation of DNA damage that can ultimately lead to the increase of mutation-associated neoantigens, leading to a high immunogenic potential [33]. Furthermore, DDR deficiency can also promote tumour immunogenicity in response to DNA damage through neoantigen-independent mechanisms, such as the activation of the cGAS-STING pathway [33]. Considering that somatic DDR gene alterations are established predictors of response and survival to checkpoint inhibitors in patients with NSCLC [16], and in view of their role as major hallmarks of familial cancers, we utilised a highly reproducible next generation sequencing platform to detect alterations of DDR defects in relationship with FHC status.

Interestingly, results from our translational work suggests that the distribution of DDR defects was not significantly enriched in FHC-high patients, arguing against our original hypothesis. Although germline testing is accepted as core methodology in the

context of FHC, we can safely assume that somatic screening detects most germline variants. Although some discrepancy in the tumour-germline DDR genes status have been described [34], most studies documenting prevalence of DDR pathway alterations do not distinguish the germline or somatic origins of the variants identified [35]. Despite DDR gene defects represented a highly plausible candidate mechanism to explain our findings, the lack of association between somatic DDR genes and FHC status in the FDx cohort clearly highlights the complexity of the mechanisms involved, that may go beyond single-hit germline tumour-suppressor genes mutations, as we are used to see in HBOC and LS. For example, it has been recently showed that the accumulation of low-risk variants may explain some familial and/or early-onset MMR-proficient colorectal cancer [36].

Interestingly, lung cancer was the most frequently reported malignancy among FHC-high patients from the pembrolizumab cohort, a finding that leads us to postulate whether unrecognised germline factors that may be responsible for familial clustering of NSCLC may also be responsible for the increased response to checkpoint inhibitors [37]. Given there are no recognised genetic syndromes that shape the risk of lung cancer and limited evidence to address inheritable factors associated with a diagnosis of NSCLC [17-18], our study reinforces the importance for a broad and prospective evaluation of the immunogenetics of lung cancer, mirroring ongoing efforts in other solid tumours [38].

For instance, polymorphic variation of inheritable human leukocyte antigen (HLA) haplotypes may be a plausible unifying factor linking familial clustering of cancer and response to immunotherapy. Functional variations of the major histocompatibility complex (MHC) influence lung cancer susceptibility, and HLA status may influence anti-tumour T-cell response and immune escape [39-40]. Amongst alternative explanations to our findings it should be emphasised that a significant proportion of cancer inheritability not explained by DNA sequence variation can be mediated by epigenetic dysregulation [41-42], a mechanism that can broadly affect lung tumorigenesis [43] and tumour immunogenicity through expression cancer–testis antigens (CTAs) and human endogenous retroviruses (ERVs) [44].

Whilst we were able to confirm a lack of association between DDR status and FHC, we lack germline genetic data and more comprehensive somatic immune profiling from patients recruited to our pembrolizumab cohort, suggesting the need for further

mechanistic investigation of the molecular and immunologic factors underpinning the positive association between FHC and clinical outcomes.

Interestingly, the present results are aligned to our previous findings in a large cohort of unselected malignancies [14]. The tumour agnostic role of high burden of FHC may point towards a shared immunogenetic mechanism across malignancies, which should be investigated in prospective translational studies.

Our study acknowledges several limitations, including the retrospective design and selection bias. Despite the case-control matching and the pooled analysis, the clinical cohorts were inherently different, with a higher proportion of elderly patients within the pembrolizumab cohort. Additionally, although we did not find any association between the FHC and the PD-L1 status, we were not able to much the two cohorts according to PD-L1 tumour expression, and we have to assume that only 30% of the chemotherapy recipients had a high PD-L1 [45]. In addition, our study was not powered to explore familial risk of cancer and our methodology was designed to analyse FHC in a quantitative way for its prognostic value rather than to describe family risk. Lastly, we did not collect clinical outcomes of the FDx cohort, as we used it only to parallelly explore the FHC distribution according to the DDR status and patients were treated with different regimens/strategies, across different lines. Furthermore, the FDx cohort was collected retrospectively, characterised by a relatively small sample size, and was not matched to the immunotherapy cohort.

Conclusion

Careful documentation of family history is of paramount importance in every oncological first consultation, however several issues might affect the accuracy of the process, including recall bias and misclassification [46-47]. It is therefore important to acknowledge that this might have affected the reliability of our data collection as well, considering the missing FHC data in 18.7% and 11.9% of the two cohorts. Moreover, the possible influence of behavioral/environmental factors within families have always made the FHC/survival topic controversial and debatable [48]. Despite these concerns, we provide clinical evidence confirming that FHC-high patients with NSCLC achieve better outcomes to first-line pembrolizumab as compared to FHC-low/negative patients. Further prospective studies incorporating germline and somatic mutational screening of immunotherapy recipients are required to fully elucidate the mechanisms

underlying the association between FHC and improved outcomes from immunotherapy in NSCLC. As the treatment landscape of NSCLC evolves and the number of predictive biomarkers of benefit from PD-1/PD-L1 checkpoint inhibitors increases [49], our study qualifies FHC as an easy-accessible, inexpensive and reproducible information to integrate clinical decision making in routine practice.

Supplementary Table 1: list of the participating Institutions. * Intitutions which provided data for the chemotherapy cohort. ¥ Institution which provided data for the FDX cohort.

Institution
St. Salvatore Hospital, University of L'Aquila, L'Aquila *
SS Annunziata Hospital, Chieti *
University Hospital of Parma, Parma
St. Camillo Forlanini Hospital, Rome
University Hospital of Modena, Modena
S Maria Goretti Hospital, Latina *
St. Andrea Hospital, Rome * ¥
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan *
Campus Bio-Medico University, Rome * ¥
AOU Papardo, Messina * ¥
"Ospedali Riuniti" Hospital, Ancona *
Policlinico Umberto I, Rome
Azienda Ospedaliera Santa Maria, Terni
AUSL Latina, Aprilia *
"Augusto Murri" Hospital, Fermo
IRCCS – Istituto Nazionale Tumori, Fondazione "G. Pascale", Napoli
IRCCS Sacro Cuore Don Calabria, Negrar
University Hospital of Udine, Udine
ASST-Sette Laghi, Varese *
University Hospital "A.Gemelli", Rome
"Madre Teresa Di Calcutta" Hospital Padova Sud, Monselice

“F. Spaziani” Hospital, Frosinone
“Careggi” University Hospital, Florence ¥
“Monaldi” Hospital, Naples *
Erasmus Medical Center, Rotterdam, the Netherlands
Imperial College London, United Kingdome
“Santa Maria della Misericordia” Hospital, Perugia *
University Hospital of Geneva, Geneva *
IRCCS, Policlinico San Martino, Genova

	PEMBROLIZUMAB COHORT			**CHEMOTHERAPY COHORT**		
AGE, (years)	**FHC-high**	**FHC-low/negative**	**χ2 test**	**FHC-high**	**FHC-low/negative**	**χ2 test**
Median Range Elderly (≥ 70)	69 56 – 80 27 (46.9)	69 28 – 92 331 (49.1)	P = 0.7693	69 51 – 84 27 (44.3)	68 31 – 92 256 (43.3)	P = 0.8872
Gender						
Female Male	18 (36.7) 31 (63.3)	237 (35.2) 437 (64.8)	P = 0.8242	17 (27.9) 44 (72.1)	188 (31.8) 403 (68.2)	P = 0.5282
ECOG PS						
0 - 1 ≥ 2	42 (85.7) 7 (14.3)	554 (82.2) 120 (17.8)	P = 0.5323	52 (85.2) 9 (14.8)	503 (85.1) 88 (14.9)	P = 0.9774
Histology						
Squamous Non-squamous	15 (30.6) 34 (69.4)	159 (23.6) 515 (76.4)	P = 0.2673	8 (13.1) 53 (86.9)	132 (22.3) 459 (77.7)	P = 0.0952
Smoking status						
Never smokers Current/Former smokers	4 (8.2) 45 (91.8)	86 (12.8) 588 (87.2)	P = 0.3470	54 (88.5) 7 (11.5)	535 (90.5) 56 (9.5)	P = 0.6150
CNS metastases						
No Yes	44 (89.8) 5 (10.2)	545 (80.9) 129 (19.1)	P = 0.1204	49 (80.3) 12 (19.7)	495 (83.8) 96 (16.2)	P = 0.4932
Liver metastases						
No Yes	45 (91.8) 4 (8.2)	556 (82.5) 118 (17.5)	P = 0.0920	54 (88.5) 7 (11.5)	507 (85.8) 84 (14.2)	P = 0.5572
Bone metastases						
No Yes	36 (73.5) 13 (26.5)	454 (67.4) 220 (32.6)	P = 0.3772	47 (77.0) 14 (23.0)	406 (68.7) 185 (31.3)	P = 0.1778

Supplementary Table 2: breakdown of patients’ characteristic according to FHC grouping across both the cohorts. ECOG-PS: Eastern Cooperative Oncology Group-Performance Status; CNS: Central Nervous System; FHC: Family History of Cancer.

PATIENT N°	FAMILY HISTORY OF CANCER DESCRIPTIVE
1	**Mother:** lung cancer; **Brother:** lung cancer
2	**Father:** unknown/do not remember; **Sister:** endometrial cancer
3	**Father:** CNS tumor; **Mother:** colon cancer; **Sister:** lung cancer
4	**Mother:** colon cancer; **Sister:** breast cancer
5	**Mother:** leukemia; **Sister:** gastric cancer; **Sister:** lymphoma
6	**Father:** colon cancer; **Sister:** melanoma
7	**Father:** Head and Neck cancer; **Brother**: bladder cancer
8	**Mother:** unknown/do not remember; **Brother:** colon cancer
9	**Mother:** pancreatic cancer; **Sister**: lung cancer
10	**Mother:** endometrial cancer; **Brother:** renal cancer/Thyroid, Lung cancer
11	**Mother**: leukemia; **Sister:** cervical cancer
12	**Father:** lung cancer; **Brother:** melanoma
13	**Mother**: colon cancer; **Sister:** lymphoma
14	**Father:** Head and Neck cancer; **Brother:** colon cancer
15	**Father:** lung cancer; **Brother:** colon cancer
16	**Father:** prostate cancer; **Brother:** lung and Head and Neck cancer
17	**Daughter:** breast cancer; **Sister:** ovarian cancer; **Brother:** melanoma; **Sister:** Head and Neck cancer
18	**Mother:** biliary tract cancer; **Father:** Head and Neck cancer; **Sister:** Thyroid cancer
19	**Mother:** ovarian cancer; **Sister:** ovarian cancer
20	**Grandfather:** GI not specified; **Brother:** unknown/do not remember
21	**Mother:** breast cancer; **Sister:** unknown/do not remember
22	**Grandfather:** Head and Neck cancer; **Sister:** unknown/do not remember
23	**Mother:** breast cancer; **Father:** colon cancer; **Brother:** colon cancer
24	**Mother:** ovarian cancer; **Sister:** breast cancer
25	**Father:** lung cancer; **Brother**: colon cancer
26	**Mother:** Head and Neck cancer; **Grandson:** renal cancer; **Brother:** CNS neoplasm
27	**Gran**dfather: lung cancer; **Father**: lung cancer; **Brother**: lung cancer
28	**Grandfather:** lung cancer; **Brother**: unknown/do not remember
29	**Father:** lung cancer; **Mother:** unknown/do not remember; **Sister:** colon cancer
30	**Mother:** breast cancer; **Brother:** unknown/do not remember
31	**Mother:** breast cancer; **Sister:** unknown/do not remember
32	**Father:** lung cancer; **Brother:** lung cancer
33	**Mother:** hepatocellular carcinoma; **Father:** leukemia; **Sister:** ovarian cancer
34	**Father:** lung cancer; **Brother**: lung cancer; **Brother**: hepatocellular carcinoma
35	**Mother:** unknown/do not remember; **Brother**: unknown/do not remember
36	**Father:** colon cancer; **Brother:** unknown/do not remember
37	**Father:** unknown/do not remember; **Daughter:** breast cancer
38	**Father:** lung cancer; **Sister**: leukemia
39	**Mother:** Head and Neck carcinoma; B**rother**: hepatocellular carcinoma
40	**Mother**: colon cancer; **Father:** prostate cancer; **Sister**: breast cancer
41	**Father:** gastric cancer; **Son:** bladder cancer; **Sister:** leukemia
42	**Father:** unknown/do not remember; **Sister:** breast cancer
43	**Son:** penile cancer; **Daughter:** breast cancer; **Brother:** colon cancer; **Brother:** prostate cancer
44	**Father:** Head and Neck cancer; **Brother:** gastric cancer
45	**Mother:** GI cancer; **Son:** unknown/do not remember; **Brother:** unknown/do not remember
46	**Grandfather:** unknown/do not remember; **Son:** lymphoma; **Sister:** unknown/do not remember
47	**Father:** lung cancer; **Brother:** bladder cancer
48	**Mother:** pancreatic cancer; **Brother:** prostate cancer
49	**Father:** Head and Neck cancer; **Sister**: breast cancer

Supplementary Table 3: Detailed FHC information for FHC-high patients of the pembrolizumab cohort.

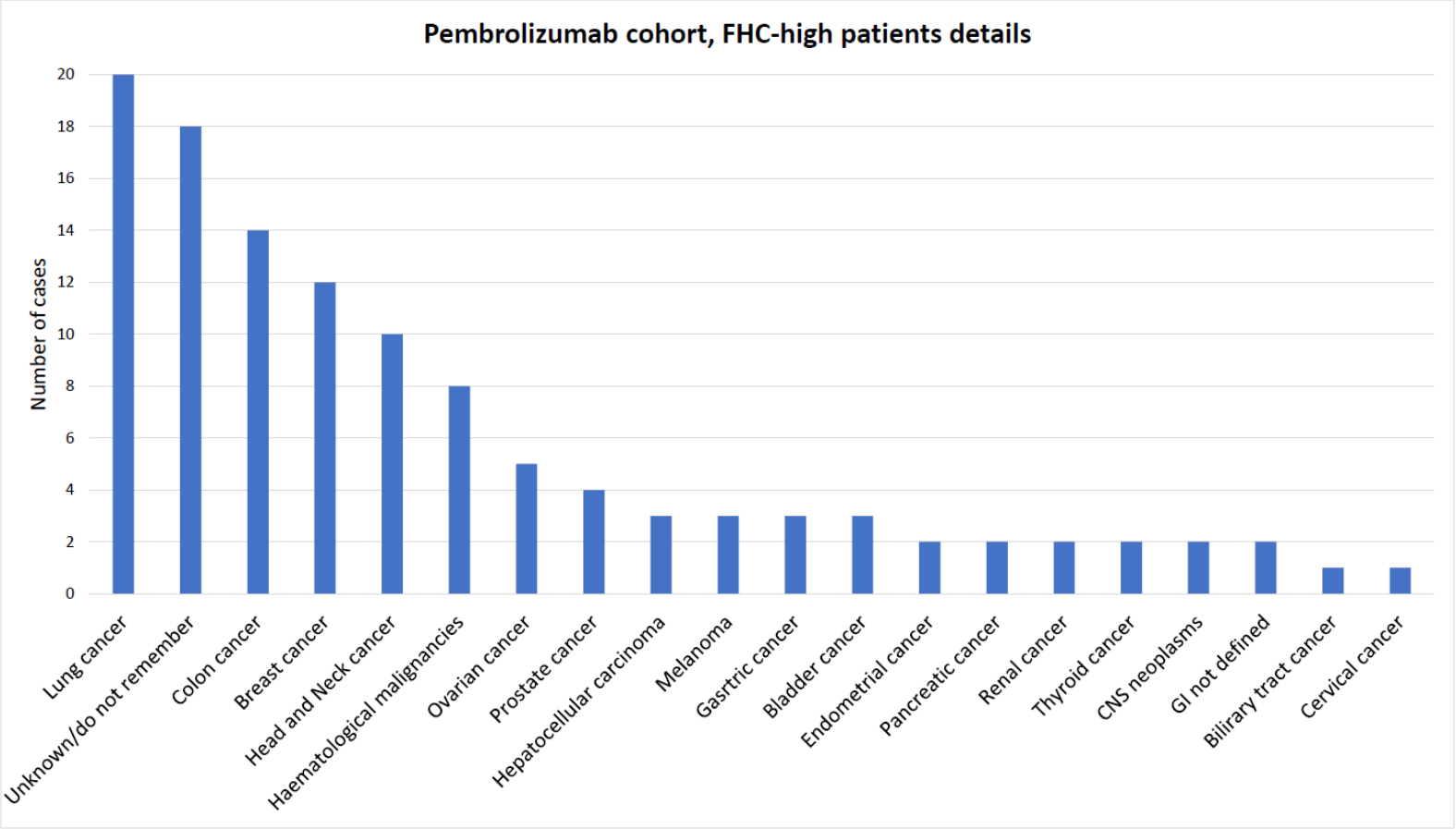
	PEMBROLIZUMAB COHORT			CHEMOTERAPY COHORT		
FHC	**Response/ratio**	**ORR (95%CI)**	**χ2 test**	**Response/ratio**	**ORR (95%CI)**	**χ2 test**
HIGH NON-HIGH	21/46 259/613	45.7% (28.2-69.7) 42.3% (37.2-47.7)	P = 0.6529	9/41 176/477	22.0% (10.0-41.7) 36.9% (31.6-42.7)	P = 0.0555
	Disease control/ratio	**DCR (95%CI)**	**χ2 test**	**Disease control/ratio**	**DCR (95%CI)**	**χ2 test**
HIGH NON-HIGH	40/46 402/613	87.0% (62.1-118.4) 65.6% (59.3-72.3)	P = 0.0029	29/41 305/477	70.7% (47.4-101.0) 63.9% (56.9-71.5)	P = 0.3838
	PFS (months) (95%CI) [events]	**log-rank**	**HR (95%CI)**	**PFS (months) (95%CI) [events]**	**log-rank**	**HR (95%CI)**
HIGH NON-HIGH	15.6 (8.6 – 28.2) [31] 6.3 (5.4 – 7.5) [492]	P = 0.0100	0.62 (0.43-0.89)	6.0 (4.0 – 48.6) [59] 5.9 (5.3 – 6.4) [535]	P = 0.6905	1.05 (0.80-1.38)
	OS (months) (95%CI) [events]			**OS (months) (95%CI) [events]**		
HIGH NON-HIGH	31.3 (15.2 – 31.3) [24] 14.3 (12.0 – 17.1) [397]	P = 0.0438	0.65 (0.43-0.99)	20.8 (12.7 – 34.5) [38] 13.9 (12.6 – 16.2) [428]	P = 0.0307	0.69 (0.49-0.97)

Supplementary Table 4: Summary the clinical outcomes analysis across the two cohorts. ECOG-PS: Eastern Cooperative Oncology Group-Performance Status; CNS: Central Nervous System; FHC: Family History of Cancer; ORR: Objective Response Rate; DCR: Disease Control Rate; PFS; Progression Free Survival; OS: Overall Survival; HR: Hazard Ratio; CI: Confidence Interval.

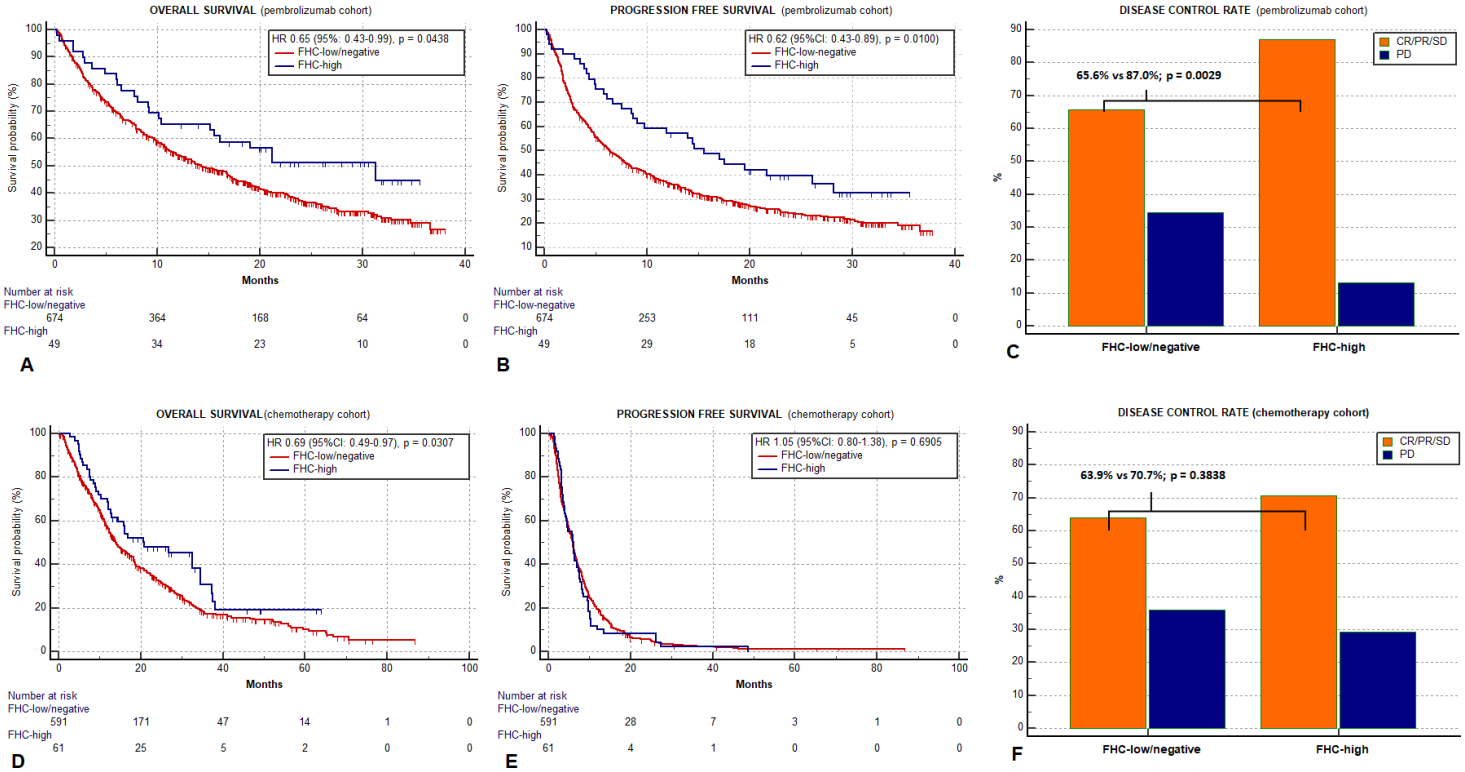
	PEMBROLIZUMAB COHORT MULTIVARIABLE ANALYSIS		
	DISEASE CONTROL RATE	**PROGRESSION FREE SURVIVAL**	**OVERALL SURVIVAL**
VARIABLE	**OR (95% CI); *p - value***	**HR (95% CI); *p - value***	**HR (95% CI); *p - value***
FHC High vs Non-high	3.17 (1.29-7.68); p = 0.0113	0.68 (0.47-0.99); p = 0.0444	0.75 (0.49-1.13); p = 0.1791
Gender Male vs Female	0.95 (0.65-1.38); p = 0.7930	1.09 (0.90-1.31); p = 0.3582	1.14 (0.92-1.42); p = 0.2011
Age Elderly vs Non-elderly	1.08 (0.76-1.52); p = 0.6570	1.05 (0.88-1.25); p = 0.5537	1.16 (0.95-1.42); p = 0.1325
ECOG PS ≥2 vs 0-1	0.36 (0.23-0.56); p < 0.0001	1.87 (1.51-2.32); p < 0.0001	2.40 (1.91-3.02); p < 0.0001
Smoking status Never vs Current/former	0.51 (0.29-0.88); p = 0.0157	1.60 (1.24-2.06); p = 0.0002	1.57 (1.19-2.09); p = 0.0014
CNS metastases Yes vs No	1.38 (0.87-2.19); p = 0.1628	1.12 (0.89-1.40); p = 0.3006	1.17 (0.91-1.50); p = 0.2074
Bone metastases Yes vs No	0.77 (0.53-1.11); p = 0.1715	1.34 (1.11-1.62); p = 0.0019	1.35 (1.10-1.66); p = 0.0040
Liver metastases Yes vs No	0.53 (0.34-0.84); p = 0.0065	1.75 (1.40-2.19); p < 0.0001	1.78 (1.40-2.27); p < 0.0001

Supplementary Table 5: Summary of the multivariable analysis for DCR, PFS and OS within the pembrolizumab cohort. ECOG-PS: Eastern Cooperative Oncology Group-Performance Status; CNS: Central Nervous System; FHC: Family History of Cancer; DCR: Disease Control Rate; PFS; Progression Free Survival; OS: Overall Survival; OR: Odd Ratio; HR: Hazard Ratio; CI: Confidence Interval.

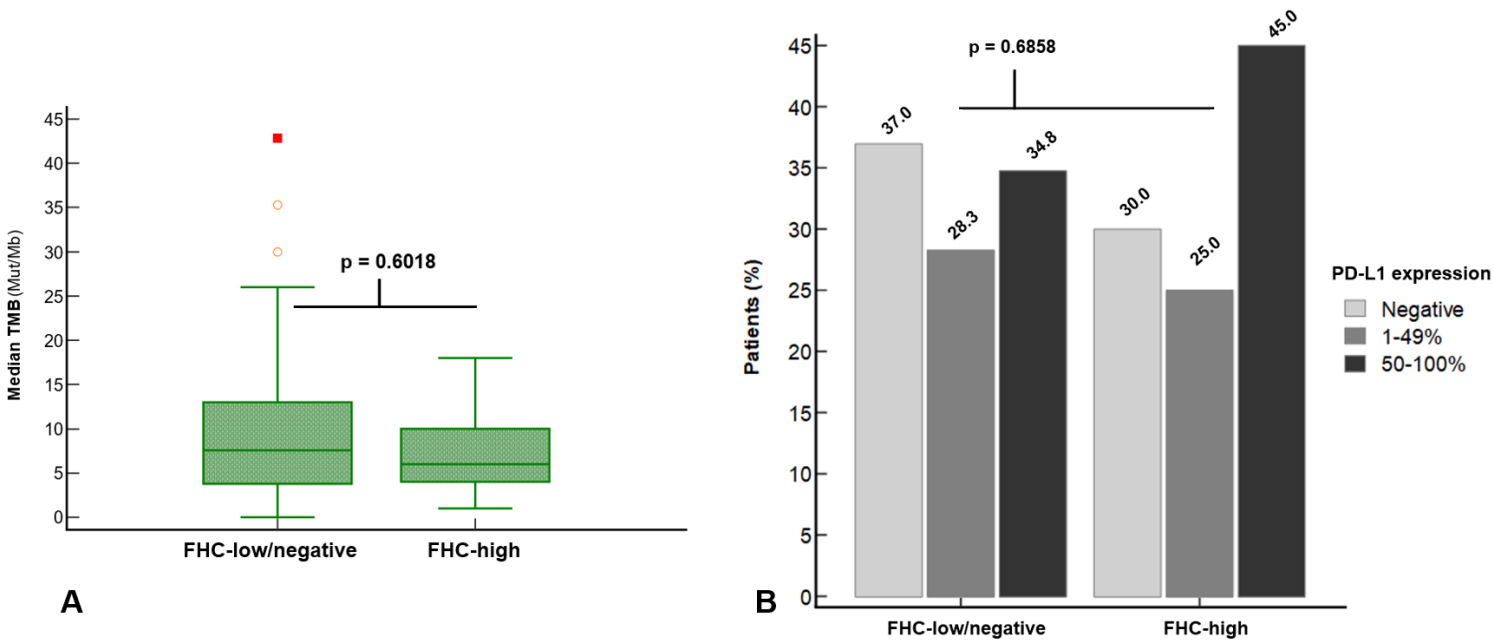
Supplementary Figure 1: Detailed FHC information for FHC-high patients of the pembrolizumab cohort. CNS: Central Nervous System; GI: Gastro-Intestinal.



Supplementary Figure 2: Clinical outcomes analysis according to the FHC the pembrolizumab and chemotherapy entire cohorts (also reported in **Supplementary Table 4**). (A) Pembrolizumab cohort, Kaplan-Meier survival estimate for Overall Survival. (B) Pembrolizumab cohort, Kaplan-Meier survival estimate for Progression Free Survival. (C) Pembrolizumab cohort, Frequency chart for disease control rate. (D) Chemotherapy cohort, Kaplan-Meier survival estimate for Overall Survival. (E) Chemotherapy cohort, Kaplan-Meier survival estimate for Progression Free Survival. (F) Chemotherapy cohort, Frequency chart for disease control rate.



Supplementary Figure 3: FDx cohort (A) Multiple comparison graph for the median TMB according to the FHC grouping. (B) Frequency chart for the PD-L1 expression distribution according to the FHC grouping. Frequencies are reported in %.



Supplementary Figure 3

Ethics approval and consent to participate

All patients provided written, informed consent to treatment with immunotherapy. The procedures followed were in accordance with the precepts of Good Clinical Practice and the declaration of Helsinki. The study was approved by the respective local ethical committees on human experimentation of each institution, after previous approval by the coordinating center (Comitato Etico per le province di L'Aquila e Teramo, verbale N.15 del 28 Novembre 2019).

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