PRINCIPAL INVESTIGATOR

1. **PRINCIPAL INVESTIGATOR'S FULL NAME AND QUALIFICATION:**
Giuseppe Fornarini, Medical Oncologist, MD

2. PROPOSAL TITLE:
Immune Tumor MicroEnvironment in correlation with peripheral blood immune biomarkers as prognostic factor in metastatic renal cell carcinoma treated with nivolumab: ancillary study of the Meet-URO 15 I-BIO-REC study (Meet-URO 15 I-TME).

3. PRIMARY AREA OF RELEVANCE: Medical Oncology

4. RELEVANCE FOR THE NATIONAL HEALTH SYSTEM:
Prognostic and predictive tumor-associated and peripheral blood biomarkers in pretreated metastatic renal cell carcinoma patients treated with immunotherapy.

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9. PLACE AND DATE: Genova, 17/02/2020 Cristina Grandi

SELF EVALUATION FORM

1. Investigator's full name (PI): Giuseppe Fornarini
2. Total papers: 72 ; Total IF: 596,306
3. Total papers (last 10 years): 58 Total IF (last 10 years): 511,773
4. Total Papers as first/last author or corresponding author: 4
5. Total H-index: 15

ABSTRACT

Rationale of the study: The treatment landscape of metastatic renal cell carcinoma (mRCC) has advanced in recent years with the approval of immune-checkpoint inhibitors (ICI). Nivolumab was the first ICI associated with a survival advantage over the standard therapy Everolimus in pretreated mRCC. Nonetheless, only a small percentage of patients (20-30%) experienced disease response and long-term benefit. There is an unmet but growing need for identification of biomarkers to guide therapeutic choice in clinical practice and to recognize patients most likely to respond to ICIs. Potential predictive biomarkers are under examination, including PD-L1 expression, tumor mutational burden, gene expression profiles, but none of these potential biomarkers has yet translated into clinical practice. Inflammatory biomarkers from peripheral blood, such as neutrophil-to-lymphocyte ratio (NLR), are of high interest for their ready and easy accessibility in clinical practice, so they are easily integrable in therapeutic decision making. Many clinical studies and meta-analysis have shown their prognostic role in many types of solid tumors, including renal cancer. Recently, they have also been studied, both as prognostic and predictive biomarkers, in melanoma, NSCLC and RCC patients treated with ICIs with promising results. Besides peripheral blood biomarkers, also intra-tumoral immune cell infiltrates, including CD8+/CD4+ T cells, regulatory T cells (Treg), macrophages, NK cells, and most recently B cells (especially if organized into tertiary lymphoid structures) have been reported to have inhibiting and promoting effects on cancer and immune system in various types of cancer, including renal cancer, and correlates with patient's clinical outcome. High level of CD8+ T lymphocytes and NK cells are associated with prolonged survival, while high level of Tregs suppress the antitumor response, leading to tumor immune escape and are associated with poor prognosis. Peripheral blood monocytes migrate into tumor tissue (tumor-associated macrophages – TAM) and differentiate into proinflammatory and antitumor phenotype (M1) and immunosuppressive and protumor phenotype (M2). Another point of the complex interactions of the tumor microenvironment (TME) is the mTOR pathway, including the phosphorylated effectors S6K1 and 4E-BP, which is a critical regulator of diverse metabolic inputs and have an important role both in innate and adaptive immune responses. Previous studies have shown that tumor-specific immune signature may affect TKI therapeutic efficacy and could improve patient selection. Recent data have shown that immune TME, especially CD8+ T cell infiltration, can be useful in assessing tumor response with ICIs. Therefore, the identification of immune TME may help better define which patients could benefit from ICIs.

Preliminary results: The role of inflammatory biomarkers in mRCC treated with immunotherapy has not yet been extensively studied, so a multicenter retrospective study (Meet-URO 15 I-BIO-REC), including 35 Italian centers, is ongoing on the correlation of different type of peripheral blood biomarkers both at baseline and during treatment, with survival and response outcomes. The preliminary analysis was conducted on 470 mRCC patients treated with Nivolumab as $\geq 2^{nd}$ line therapy from October 2015 to October 2019. Lower baseline NLR (bNLR<3) was associated with statistically significant longer progression-free survival (PFS), overall survival (OS), with similar overall response rate (ORR) but higher disease control rate (DCR). Lower early change of NLR (ΔNLR<1.1), defined as the difference between NLR at 2^{nd} cycle and bNLR, Lower baseline systemic inflammation index (SII: Platelet x NLR) (bSII<1375) and lower early change of SII (ΔSII<383) were associated with a statistically significant improvement of PFS, OS, ORR and DCR.

Detailed description of the translational value of the research: Due to the success of the Meet-URO 15 study, in terms of national accrual and promising results, a translational ancillary study was designed to assess the correlation of immune TME of primary tumor and metastatic sites, peripheral blood inflammatory biomarkers and survival / response outcomes. The analysis of immune TME will be conducted on two cohorts of mRCC patients treated with Nivolumab as $\geq 2^{nd}$ line therapy: *responders* (PFS $\geq$ 12 months) and *non-responders* (PFS $\leq$ 3 months) (about 100 vs 100 patients). It will consist in the immunohistochemical genomic and transcriptomic analysis of the morphological and immunophenotypic evaluation of tumor-infiltrating lymphocytes (TILs) (CD8+,CD4+, FOXP3 T cells), tumor-associated macrophages polarization (pro-inflammatory M1 and anti-inflammatory M2 macrophages), tissue NLR and LMR, population of NK cells (CD56+) and expression of the phosphorilated mTOR effectors S6K1 and 4E-BP which are associated with innate immune response. All the assessments of the immune TME will be correlated, in terms of response and survival outcomes, with the peripheral blood biomarkers NLR, derived NLR [dNLR, defined as neutrophil / (white blood cells – neutrophils)], Platelet to lymphocyte ratio (PLR), lymphocyte to monocyte ratio (LMR) and SII at baseline and during the first four cycles of treatment.

Expected impact on the NHS: Peripheral blood inflammatory biomarkers and the immunohistochemical examination are routinely performed in clinical practice with low costs. Therefore, if their correlation identified patients most likely to benefit from immunotherapy, it could have an important clinical impact, especially considering the cost-effectiveness balance, not only in daily routine but also as useful tools for prospective patient selection for clinical trials.

MAIN PROPOSAL BODY

INTRODUCTION

Renal cell carcinoma (RCC) is the second most common malignancy of the urinary system, responsible for about 805% of all primary renal neoplasms and represents about 4% of all new cancer diagnosis. Around 20% of RCC patients are diagnosed at a locally advanced or metastatic stage [1].
In the past two decades, there has been significant progress in the understanding of the molecular pathogenesis of advanced RCC (aRCC), resulting in the development of multiple agents targeting vascular endothelial growth factors (VEGF) and the mammalian target of rapamycin (mTOR). More recently, the treatment landscape of aRCC has significantly changed due to the approval of immune-checkpoint inhibitors (ICIs), which promote the activation of anti-cancer immune response, and their combination with target therapy or other ICIs [2].
In 2015 Nivolumab, a programmed death receptor-1 (PD-1) antibody, was approved for its survival advantage over the standard therapy Everolimus in pretreated aRCC patients [3].
Despite the survival advantage observed with immunotherapy, most patients do not respond to the treatment from the beginning (overall response rate – ORR – 20-30%) and only a small percentage of them experience a long clinical benefit [4].
Therefore, there is an intense interest in identifying and developing prognostic and predictive biomarkers to better understand and overcome mechanisms of resistance to immunotherapy and to better select patients most likely to benefit from immunotherapy. These will help to optimize clinical decisions, avoid useless toxicities and minimizing costs derived from the use of these innovative but expensive therapies [4].
Inflammatory biomarkers from peripheral blood, like neutrophil-to-lymphocyte ratio (NLR), have shown a prognostic role in many types of solid tumors, including renal cancer, with promising results in recent analysis in patients treated with immunotherapy, especially melanoma and lung cancer [5-7].
An Italian multicenter retrospective study (Meet-URO 15 I-BIO-REC) was designed by Dr Giuseppe Fornarini and Dr Sara Elena Rebuzzi from the Medical Oncology of IRCCS Ospedale Policlinico San Martino of Genova, to investigate the prognostic role of different type of peripheral blood biomarkers both at baseline and during treatment in aRCC patients treated with Nivolumab.
Preliminary results on about 500 patients have shown positive results on the correlation of these biomarkers with response and survival benefit.
The great success of the Meet-URO 15 study, both in terms of national participation (36 Italian centers were involved) and promising results, led to the idea of analysing the immune tumor microenviroment (TME) and its correlation with peripheral blood biomarkers and with immunotherapy benefit.

BACKGROUND AND RATIONALE

In recent years, the therapeutic landscape of aRCC has dramatically changed with the approval of ICIs as single agents or in combination with other agents (target therapy or other ICIs) [4]. ICIs include anti-programmed death receptor-1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors and anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) antibodies. ICI-based combinations are currently the standard treatment of intermediate/poor risk aRCC patients and Nivolumab is one of the main options in pretreated aRCC patients with target therapy [4].
The success of immunotherapy lies on the immunogenic and immune-responsive nature of RCC, because of the high expression of immunosuppressive ligands on tumor cells with a subsequent immunosuppressive action on T lymphocytes and NK cells and immune evasion of the tumor [8]. ICIs interfere with the immunosuppressive action of these tumor ligands abrogating inhibitory signals and augmenting the host antitumor response.
Nivolumab was the first approved ICI for its survival advantage over the standard therapy Everolimus in pretreated mRCC [3]. Despite the improvement in survival outcomes observed with ICIs, only a

small percentage of patients respond to immunotherapy (20-30%) or show long-term clinical benefit [4]. The identification of predictive and/or prognostic biomarkers to identify patients most likely to respond to immunotherapy is still a crucial point of ongoing clinical trials.

Because of the availability of many systemic therapy choices for aRCC patients (tyrosine kinase inhibitors, ICI alone or ICI-based therapy), there is also an unmet need for biomarkers that could help to guide the choice of the most appropriate therapy, avoiding exposure to inadequate treatments, unnecessary toxicity and costs [9].

Potential predictive biomarkers are under examination, including PD-L1 expression, tumor mutational burden, gene expression profiles, but none of these potential biomarkers has yet translated into clinical practice. Cancer-associated inflammation plays a key role in tumour development and progression of cancer, associated with worse outcomes and lower therapeutic response in cancer patients [5].

Different inflammatory biomarkers from peripheral blood have been investigated as potential immune-based markers in various malignancies. Considering the diversity of the host immune system, as a balance between pro-tumour and anti-tumour forces, not only a single biomarker should be used but also combined biomarker, in their relative proportion (ratio) or scores [10,11]. They include mainly neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR). These peripheral blood parameters are of high interest for their ready and easy accessibility and limited costs in clinical practice, so they are easily integrable in therapeutic decision making. Many clinical studies and meta-analysis have shown the prognostic role of these peripheral blood biomarkers in many types of solid tumors, including renal cancer [5,11].

Recently, they have also been studied, both as prognostic and predictive biomarkers, in melanoma and non-small cell lung cancer patients treated with ICIs with promising results on their predictive and prognostic role. These biomarkers have been also integrated into immune-based scores with clinical characteristics [10].

Besides peripheral blood biomarkers, also intra-tumoral immune cell infiltrates have been reported to have inhibiting and promoting effects on cancer and immune system in various types of cancer, including renal cancer, and correlates with patient's clinical outcome [12,13].

The composition of the immune microenvironment (TME) is heterogeneous and includes CD8+/CD4+ T, regulatory T cells (Treg), B cells, M1 and M2 Macrophages, neutrophils, NK cells and others. TME has been reported to function as tumor promoters or suppressors in various types of cancer and to have great prognostic value. Many studies have reported that the density, phenotype, and localization of TILs can predict both patient's prognosis and clinical response to diverse treatments, including immune checkpoint inhibitors [14]. High CD8+ T cells and NK cells infiltration has been reported as a prognostic factor of longer survival and a potential marker of response to immunotherapy in many cancers, including RCC [14]. In addition to T cells, the main component of the adaptive immune system consists of B cells. Higher expression of B cells, especially if organized into tertiary lymphoid structures, have been shown to be positively associated with improved survival and responses to different types of therapies and, more recently, also with immunotherapy [15,16].

The TME contemplate also immunosuppressive cells, such as Tregs, which suppress anti–tumor T cell responses, causing tumors to lose their immunogenicity, leading to tumor immune escape. High level of Tregs suppress the antitumor response and are, therefore, associated with poor prognosis [17].

Another important immune population in the immune TME include the so-called tumor-associated macrophages (TAM) [18]. Peripheral blood monocytes migrate into tumor tissue and differentiate into two different sub-population of macrophages: immune stimulating cells (M1 macrophages), linked to a better prognosis, thanks to their antitumoral activities, and immune suppressor cells (M2 macrophages) related to a worse prognosis. It has not yet been cleared if they are two distinct population or just the extremes of a range of functional states but the mutual conversion of M1 and M2 macrophage has been shown to have a prognostic value [18].

Neutrophils constitute an important portion of the immune TME and when are recruited to the TME are called tumor-associated neutrophils (TANs), acquiring either protumor or antitumor function. Many studies have shown the prognostic value of TANs in multiple types of cancer, including RCC [19].

According to immune TME, there is a growing evidence of heterogeneity within the primary tumor and between primary tumor and metastatic sites (intra- and inter-tumor heterogeneity) [20]. The sequential exposure to different therapeutic agents, with consequent selective pressure, can modify the

environment of the tumor and therefore its sensitivity to different treatments.
A continuous modification of the tumor immune context is conceivable over time and may be responsible for modulation of tumor aggressiveness and behavior, influencing clinical outcome and responses to therapy. Intra- and inter-tumor heterogeneity contribute, therefore, to treatment failure and drug resistance [20].
Not only histological features, but also molecular pathways of signalling inside the TME such as the mammalian target of rapamycin (mTOR) pathway, have been studied as prognostic factors. The mTOR pathway, especially the phosphorylated effectors S6K1 and 4E-BP, is a critical metabolic regulator of diverse cell functions in the immune microenvironment (immune homeostasis) and have an important role both in innate and adaptive immune responses. mTOR exists in two distinct complexes, mTOR complex 1 (mTORC1) and mTORC2, which mediate separate but overlapping cellular functions. The downstream activation of mTORC1 leads to the phosphorylation of ribosomal protein S6 kinase (S6K) and eukaryotic translation initiation factor 4E binding proteins (4E-BPs) to stimulate translation initiation with the central function of inducing cellular growth and proliferation [21]. mTOR regulates many immune cells, including neutrophils, NK cells, T cells and macrophages. The mTOR activation has been shown to promote the development of Treg and T cell anergy inducing an immunosuppressive microenvironment.
Immune TME and peripheral blood biomarkers can be useful tools of sensitivity or resistance to ICIs and may help, therefore, to define which patients could benefit from immunotherapy.

EXPERIMENTAL DESIGN

The primary aim of the study is to analyse the immune TME of the primary tumor and metastatic sites on two cohorts of mRCC patients treated with Nivolumab as $\geq 2^{nd}$ line therapy divided as *responders* (PFS $\geq$ 12 months) and *non-responders* (PFS $\leq$ 3 months) (about 100 vs 100 patients).
Patients will be selected from the database of the Meet-URO 15 study on about 500 patients according to the subsequent inclusion criteria:

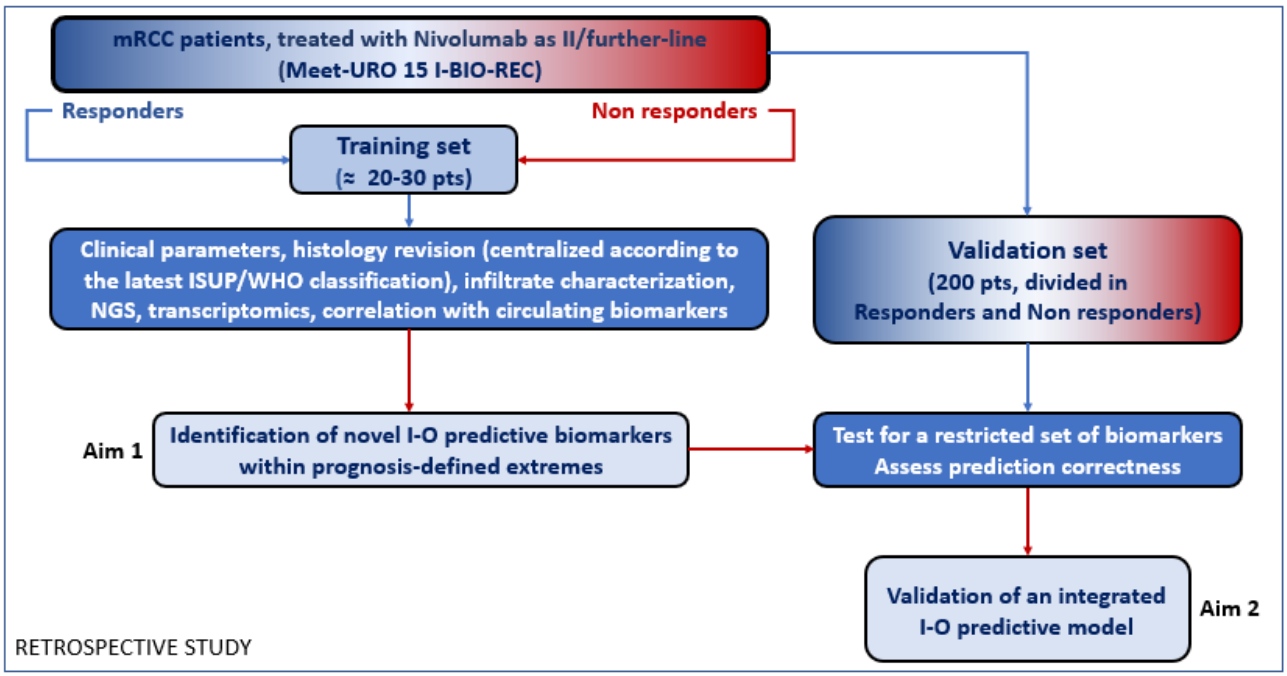
- PFS of $\leq$ 3 months (*non-responder*) or $\geq$ 12 months (*responder*)
- Availability of sufficient tumor tissue from the primary tumor and/or metastasis to perform the planned immunohistochemical analysis
- Availability of all the values of the complete blood count (white blood cells, neutrophil, lymphocyte, platelets, monocytes) at baseline (pre-immunotherapy)
- Availability of the IMDC prognostic score at baseline (pre-immunotherapy)

The analysis will be conducted on formalin-fixed and paraffin-embedded (FFPE) specimens from previous radical or partial nephrectomy, biopsies or metastasis resections using immunohistochemical staining and transcriptomic analysis, carried out according to standard methods, on:

- histology revision and subtyping according to the last WHO classification of renal tumors according to morphology, immunohistochemical and molecular characteristics
- qualitative and quantitative assessment of TILs, defined as immune cells within the tumor and at the tumor margin. Immune cells outside the tumor were excluded. We also excluded immune cells in tumor zones with crush artefacts, necrosis or hemorrhage.
    - morphological and immunophenotypic evaluation of lymphocyte subpopulations (CD8+, CD4+, FOXP3 T cells)
    - TILs density: low number of TILs (TILs-L) and high number of TILs (TILs-H).
- qualitative and quantitative assessment of tumor-associated macrophages (CD68+) polarization (pro-inflammatory M1 and anti-inflammatory M2 macrophages)
- tissue NLR and LMR
- quantitative assessment of NK cells (CD56+)
- expression of the phosphorylated mTOR effectors S6K1 and 4E-BP
- Genomic characterization using a focused on Next Generation Sequencing (NGS) panel.
- Transcriptomic characterization using custom panels (e.g *Nanostring*).

The immunohistochemical and transcriptomic findings of the immune TME will be described and correlated, according to response (ORR, DCR, duration of response – DOR) and survival outcomes (PFS, OS):

- between the primary tumor and the metastatic site to identify potential inter-tumor immune heterogeneity.
- between the two cohorts of patients to identify potential differences and specific immune profiles of long responders and true immune-resistant mRCC patients.
- with the peripheral blood biomarkers at baseline and during treatment (1°, 2°, 3°, 4° cycle):
  - NLR
  - derived NLR (dNLR), defined as neutrophil / (white blood cells – neutrophils)
  - PLR
  - LMR
  - SII
- IMDC prognostic score



FURTHER DETAILS ON THE OVERALL METHODS THAT WILL BE USED IN THIS PROJECT

All the FFPE specimens will be collected by each identified center and will be sent to the laboratories of Anatomic pathology of Ospedale Policlinico San Martino of Genova, University of Genova Italy) and to the Department of Diagnostics and Public Health, of Azienda Ospedaliera Universitaria Integrata of Verona (AOUI), University of Verona (Italy).

All collected clinical data will be analysed anonymously by descriptive statistics methods. The descriptive analysis will be carried out using percentages for the binary variables, mean and median for the continuous variables, reporting their respective dispersion values. Survival curves of PFS and OS will be generated using the Kaplan–Meier method. Differences in PFS and OS will be evaluated using the log-rank test (Mantel-Cox) for statistical significance, which is defined at $p<0.05$ level. Two-sided 95% CIs will be provided for the main statistical estimators. Univariate and multivariate analyses are performed to determine the correlation between the inflammatory biomarkers and PFS, ORR, DOR and OS. For the comparison of percentages, means and medians, confidence limits and tests are provided, such as Chi-square test, Wilcoxon test and Fisher test, where indicated.

WORK CARRIED OUT AND PRELIMINARY RESULTS

The role of peripheral blood inflammatory biomarkers in mRCC carcinoma treated with immunotherapy has not yet been extensively studied, so a multicenter retrospective study (Meet-URO 15 I-BIO-REC), including 35 Italian centers, is ongoing on the correlation of different type of peripheral blood biomarkers (NLR, LMR, PLR, SII) both at baseline and during treatment, with survival and response outcomes.

For this project, Dr Giuseppe Fornarini received a funding grant by IRCCS Ospedale Policlinico San Martino from the program of "Ricerca Corrente" of the Italian Health Ministry in November 2018. Moreover, Dr Sara Elena Rebuzzi won the "Associazione Italiana di Oncologia Medica" (AIOM) Liguria award "Giovanni Gardin" as one of the best project on the "molecular iperselection of patients with solid tumors" in September 2019.

The first preliminary analysis of the Meet-URO 15 was conducted in October 2019 on 189 patients treated with Nivolumab as $\geq 2^{nd}$ line from 13 Italian centers. It was accepted as two poster presentation at the 2020 Genitourinary Cancers Symposium – ASCO Annual Meeting, which took place from 13th to 15th of February 2020 at San Francisco. The analysis reported a general mOS and progression-free survival (mPFS) of 30.5 months and 9.5 months. Overall response rate (ORR) and disease control rate (DCR) of 28% and 57%.

Low bNLR (bNLR < 3) correlated with statistically significant longer PFS [11.5 vs 5.6 months; HR 1.61 (1.09-2.39), p = 0.017] and OS [NR vs 22.4 months; HR 2.61 (1.53-4.46), p < 0.001], with similar ORR (32% vs 32%) but higher DCR (66% vs 55%) compared to NLR ≥ 3. Lower early change of NLR (ΔNLR< 0.3), defined as the as the difference between NLR at 2nd cycle and bNLR, correlated with statistically significant longer PFS [17.1 vs 8.5 months; HR 1.57 (1.02-2.43) p = 0.04] and OS [medians not reached; HR 1.91 (1.04-3.51) p = 0.038], with similar ORR (39% vs 32%) but higher DCR (73% vs 56%) compared to ΔNLR ≥ 0.3. Univariate and multivariate analyses adjusted for IMDC group, line of therapy and metastatic sites, confirmed the statistically significant correlation between ΔNLR with PFS and OS and NLR with OS but not with PFS.

Higher baseline LMR (bLMR ≥ 3) correlated with statistically significant longer OS [NR vs 26.8 months, HR 0.50 (0.29-0.88); p = 0.016] but not PFS (10.3 vs 9.9 months, HR 0.87 (0.58-1.31); p = 0.5) with similar ORR (34% vs 33%) and DCR (60% vs 63%) compared to LMR < 3. Lower baseline SII (bSII < 1375) correlated with statistically significant longer PFS [11.5 vs 3.4 months; HR 2.16 (1.36-3.43), p = 0.001] and OS [NR vs 9.5 months; HR 3.87 (2.21-6.78), p <0.001] with higher ORR (35% vs 20%) and DCR (65% vs 40%) compared to SII ≥ 1375. Univariate and multivariate analyses adjusted for IMDC group, line of therapy and metastatic sites, confirmed the statistically significant correlation between SII with PFS and OS, but not for LMR.

The second preliminary analysis was conducted in January 2020 on 470 mRCC patients treated with Nivolumab as $\geq 2^{nd}$ line therapy from October 2019 in 30 Italian centers. Two abstracts on NLR and SII results have been submitted to the 2020 ASCO Annual Meeting which will take place from 29th May to 2nd June in Chicago. The analysis reported a general mOS and mPFS of 34.8 and 7.5 months, ORR and DCR of 30% and 61%.

Lower baseline NLR (bNLR < 3) were associated with statistically significant longer PFS [11.4 *vs* 5.4 months; HR 1.69 (1.33-2.15)] and OS [46.2 *vs* 17.2 months; HR 2.37 (1.72-3.26)] (both p<0.001), with similar ORR (35% *vs* 30%, p=0.28) but higher DCR (71% vs 52%, p<0.001). Lower early change of NLR (ΔNLR< 1.1) was associated with a statistically significant improvement of PFS [11.2 *vs* 4.9 months; HR 1.53 (1.16-2.03), p=0.03], OS [Not-Reached *vs* 19.7 months; HR 1.83 (1.28-2.61), p=0.001], ORR (37% *vs* 23%, p=0.011) and DCR (68% *vs* 53%, p=0.008). Multivariate analyses adjusted for IMDC group, line of therapy and metastatic sites, confirmed the statistically significant correlation of bNLR and ΔNLR with OS, PFS and DCR.

Lower baseline SII (bSII < 1375) was associated with statistically significant improvement of PFS [10.2 *vs* 4.1 months, HR 2.06 (1.54-2.76), p<0.001], OS [46.2 *vs* 9.5 months, HR 3.16 (2.23-4.49), p<0.001], ORR (35% vs 21%, p=0.035) and DCR (67% *vs* 40%, p<0.001). Lower early change of SII (ΔSII < 383), defined as the difference between SII at 2nd cycle and bSII, was associated with

statistically significant improvement of PFS [11.3 *vs* 4.7 months; HR 1.64 (1.23-2.18), *p*=0.001] and OS [NR *vs* 21.1 months; HR 1.76 (1.21-2.56), *p*=0.003], ORR (37% *vs* 24%, *p*=0.023) and DCR (68% *vs* 53%, *p*=0.01). Multivariate analyses adjusted for IMDC group, line of therapy and metastatic sites, confirmed the statistically significant correlation of bSII and ΔSII with OS, PFS and DCR.
Due to the success of the Meet-URO 15 study, in terms of the participation of the most important Italian expert on genito-urinary cancer, the massive national accrual and the promising results, a translational ancillary study was born and designed from the close collaboration of the University Hospitals of Genova, Verona and Parma.
With the funding received by IRCCS Ospedale Policlinico San Martino ("Ricerca Corrente" of the Italian Health Ministry) a training set of of the translational study will be stared on about 20-30 patients divided in *responder* and *non-responder*. The LILT project will concern a wider population (Validation set) on about 200 patients, divided in *responder* and *non-responder* patients.

EXPECTED RESULTS AND RELEVANT CORRESPONDING MILESTONES

Anti-tumor immune response is a heterogeneous process that involves many TME cells and pathways, organically affecting each other, which are also involved in the sensitivity to different treatments including ICIs.
A comprehensive analysis, according to morphology, genetic and transcriptomic analysis, of the underlined process of response and resistance to ICIs may help to better define the biological and clinical significance of immune biomarkers in correlation to response and survival patients' outcomes. If a distinct immune infiltration profile of two deeply different population according to response to ICI (true resistant patients and long-responders patients) can be identified, a patient's immune infiltration could help clinicians to evaluate potential cancer outcomes, predict immunotherapy response and, therefore, the need of different type of treatment.
Peripheral blood inflammatory biomarkers and the immunohistochemical examination are routinely performed in clinical practice without any additional effort or equipment, and so with low costs. Therefore, if their correlation identified patients most likely to benefit from immunotherapy, it could have an important clinical impact, especially considering the cost-effectiveness balance, not only in daily routine but also as valuable resources for research to improve patients' selection and immunotherapy benefit.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2020. CA Cancer J Clin. 2020 Jan;70(1):7-30.
2. Gulati S, Vaishampayan U. Current State of Systemic Therapies for Advanced Renal Cell Carcinoma. Curr Oncol Rep. 2020 Feb 11;22(3):26.
3. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373(19):1803 – 1813.
4. Rebuzzi SE, Perrone F, Bersanelli M, et al. Prognostic and predictive molecular biomarkers in metastatic renal cell carcinoma patients treated with immune checkpoint inhibitors: a systematic review. Expert Rev Mol Diagn. 2019 Oct 24:1-17.
5. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014 May 29;106(6):dju124.
6. Rosner S, Kwong E, Shoushtari AN, et al. Peripheral blood clinical laboratory variables associated with outcomes following combination nivolumab and ipilimumab immunotherapy in melanoma. Cancer Med. 2018;7(3):690-697.
7. Tanizaki J, Haratani K, Hayashi H, et al. Peripheral Blood Biomarkers Associated with Clinical Outcome in Non – Small Cell Lung Cancer Patients Treated with Nivolumab. J Thorac Oncol. 2018;13(1):97-105.
8. Drake CG, Stein MN. The immunobiology of kidney cancer. J Clin Oncol. 2018;36:3547 – 3552JCO2018792648.
9. Putzu C, Cortinovis DL, Colonese F, et al. Blood cell count indexes as predictors of outcomes in advanced non-small-cell lung cancer patients treated with Nivolumab. Cancer Immunol Immunother. 2018;67(9):1349-1353.
10. Mezquita L, Auclin E, Ferrara R, et al. Association of the Lung Immune Prognostic Index With Immune Checkpoint Inhibitor Outcomes in Patients With Advanced Non-Small Cell Lung Cancer. JAMA Oncol 2018;4(3):351-357.
11. Templeton AJ, Ace O, McNamara MGet al. Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 2014;23(7):1204-1212.
12. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012;12:298 – 306.
13. Zhu G, Pei L, Yin H, et al. Profiles of tumor-infiltrating immune cells in renal cell carcinoma and their clinical implications. Oncol Lett. 2019 Nov;18(5):5235-5242.
14. Li J, Byrne KT, Yan F, Yamazoe T, Chen Z, Baslan T, et al. Tumor Cell-Intrinsic Factors Underlie Heterogeneity of Immune Cell Infiltration and Response to Immunotherapy. Immunity. 2018;49(1):178-93 e7.
15. Helmink, B.A., Reddy, S.M., Gao, J. et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature 2020;577:549–555.
16. Cabrita, R., Lauss, M., Sanna, A. et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature 2020;577:561–565.
17. Shang B, Liu Y, Jiang Sj , et al. Prognostic value of tumor-infiltrating FoxP3 + regulatory T cells in cancers: A systematic review and meta-analysis. Sci Rep 2015;5:15179
18. Dannenmann SR, Thielicke J, Stockli M, et al. Tumor-associated macrophages subvert T-cell function and correlate with reduced survival in clear cell renal cell carcinoma. Oncoimmunology 2013;2:e23562.
19. Shaul ME, Fridlender ZG. Cancer-related circulating and tumor-associated neutrophils - subtypes, sources and function. FEBS J. 2018 Dec;285(23):4316-4342.
20. Bersanelli M, Gnetti L, Varotti E, et al. Immune context characterization and heterogeneity in primary tumors and pulmonary metastases from renal cell carcinoma. Immunotherapy. 2019 Jan;11(1):21-35.
21. Jones RG1, Pearce EJ. MenTORing Immunity: mTOR Signaling in the Development and Function of Tissue-Resident Immune Cells. Immunity. 2017 May 16;46(5):730-742.

RELEVANT PUBLICATIONS BY THE RESEARCH GROUP ON THE TOPIC OF THE PROJECT

- **Rebuzzi SE**, Perrone F, **Bersanelli M**, Bregni G, Milella M, **Buti S**. Prognostic and predictive molecular biomarkers in metastatic renal cell carcinoma patients treated with immune checkpoint inhibitors: a systematic review. Expert Rev Mol Diagn. 2019 Oct 24:1-17. doi: 10.1080/14737159.2019.1680286. PubMed PMID: 31608727.
- De Giorgi U, Procopio G, Giannarelli D, Sabbatini R, Bearz A, **Buti S**, Basso U, Mitterer M, Ortega C, Bidoli P, Ferraù F, Crinò L, Frassoldati A, Marchetti P, Mini E, Scoppola A, Verusio C, **Fornarini G**, Cartenì G, Caserta C, Sternberg CN. Association of Systemic Inflammation Index and Body Mass Index with Survival in Patients with Renal Cell Cancer Treated with Nivolumab. Clin Cancer Res. 2019 Jul 1;25(13):3839-3846. doi: 10.1158/1078-0432.CCR-18-3661. Epub 2019 Apr 9. PubMed PMID: 30967420.
- **Bersanelli M**, Gnetti L, Varotti E, Ampollini L, Carbognani P, Leonardi F, Rusca M, Campanini N, Ziglioli F, Dadomo CI, Pilato FP, Cortellini A, Rapacchi E, Caruso G, Silini EM, Maestroni U, **Buti S**. Immune context characterization and heterogeneity in primary tumors and pulmonary metastases from renal cell carcinoma. Immunotherapy. 2019 Jan;11(1):21-35. doi: 10.2217/imt-2018-0097.
- Santoni M, **Buti S**, Conti A, Porta C, Procopio G, Sternberg CN, Bracarda S, Basso U, De Giorgi U, Rizzo M, Derosa L, Ortega C, Massari F, Milella M, **Bersanelli M**, Cerbone L, Muzzonigro G, Burattini L, Montironi R, Santini D, Cascinu S. Prognostic significance of host immune status in patients with late relapsing renal cell carcinoma treated with targeted therapy. Target Oncol. 2015 Dec;10(4):517-22. doi: 10.1007/s11523-014-0356-3. Epub 2015 Jan 6. PubMed PMID: 25559290.
- **Buti S**, Rovere RK, Donini M, Passalacqua R, Pezzuolo D, Buzio C. Changes in lymphocyte count induced by repeated cycles with low-dose interleukin-2 and interferon-α in 146 patients with renal cell carcinoma. Tumori. 2012 Jan-Feb;98(1):45-52. doi: 10.1700/1053.11499. PubMed PMID: 22495701.
- Iacovelli R, De Giorgi U, Galli L, Zucali P, Nolè F, Sabbatini R, Fraccon AP, Basso U, Mosca A, Atzori F, Santini D, Facchini G, **Fornarini G**, Pasini F, Masini C, Massari F, **Buti S**, Sava T, Sacco C, Ricotta R, Sperduti I, Tortora G, Porta C. Is It Possible to Improve Prognostic Classification in Patients Affected by Metastatic Renal Cell Carcinoma With an Intermediate or Poor Prognosis? Clin Genitourin Cancer. 2018 Oct;16(5):355-359.e1. doi: 10.1016/j.clgc.2018.04.007. Epub 2018 May 7. PubMed PMID: 29803346.
- **Bersanelli M**, Gnetti L, Vaglio A, Sverzellati N, Campanini N, Incerti M, Galetti M, Varotti E, Corrado M, Parziale R, Bottarelli L, Azzoni C, Silini EM, Leonardi F, **Buti S**. Correlations between tumor-infiltrating and circulating lymphocyte subpopulations in advanced renal cancer patients treated with nivolumab. Acta Biomed. 2019 Dec 23;90(4):468-474. doi: 10.23750/abm.v90i4.7057. PubMed PMID: 31910171.
- **Bersanelli M, Buti S.** From targeting the tumor to targeting the immune system: Transversal challenges in oncology with the inhibition of the PD-1/PD-L1 axis. World J Clin Oncol. 2017 Feb 10;8(1):37-53. doi: 10.5306/wjco.v8.i1.37. Review. PubMed PMID: 28246584; PubMed Central PMCID: PMC5309713.
- **Rebuzzi SE**, Leonetti A, Tiseo M, Facchinetti F. Advances in the prediction of long-term effectiveness of immune checkpoint blockers for non-small-cell lung cancer. Immunotherapy. 2019 Aug;11(12):993-1003. doi: 10.2217/imt-2019-0107. Epub 2019 Jul 19. PubMed PMID: 31319742.
- Prelaj A, Ferrara R, **Rebuzzi SE**, Proto C, Signorelli D, Galli G, De Toma A, Randon G, Pagani F, Viscardi G, Brambilla M, Trevisan B, Ganzinelli M, Martinetti A, Gallucci R, Di Mauro RM, Molino G, Zilembo N, Torri V, de Braud FM, Garassino MC, Lo Russo G. EPSILoN: A Prognostic Score for Immunotherapy in Advanced Non-Small-Cell Lung Cancer: A Validation Cohort. Cancers (Basel). 2019 Dec 5;11(12). pii: E1954. doi: 10.3390/cancers11121954. PubMed PMID: 31817541; PubMed Central PMCID: PMC6966664.
- **Bersanelli M**, Gnetti L, Azzoni C, Bottarelli L, Sverzellati N, Campanini N, Varotti E, Corrado M, Parziale R, Rapacchi E, Caruso G, Leonardi F, Silini EM, **Buti S.** Loss of heterozygosity of key tumor suppressor genes in advanced renal cancer patients treated with nivolumab. Immunotherapy. 2018 Jul;10(9):743-752. doi: 10.2217/imt-2017-0160.
- Facchinetti F, Veneziani M, **Buti S**, Gelsomino F, Squadrilli A, Bordi P, **Bersanelli M**, Cosenza A, Ferri L, Rapacchi E, Mazzaschi G, Leonardi F, Quaini F, Ardizzoni A, Missale G, Tiseo M. Clinical and hematologic parameters address the outcomes of non-small-cell lung cancer patients treated with nivolumab. Immunotherapy. 2018 Jun;10(8):681-694.

- Tiseo M, Facchinetti F, **Buti S**, Gelsomino F, Veneziani M, Squadrilli A, Bordi P, **Bersanelli M**, Cosenza A, Ferri L, Rapacchi E, Mazzaschi G, Leonardi F, Quaini F, Ardizzoni A, Missale G. Circulating immune-profile as predictor of outcome in advanced NSCLC patients treated with Nivolumab. Ann Oncol. 2017 Oct;28 Suppl 6:vi60.
- Santoni M, **Buti S**, Conti A, Porta C, Procopio G, Sternberg CN, Bracarda S, Basso U, De Giorgi U, Rizzo M, Derosa L, Ortega C, Massari F, Milella M, **Bersanelli M**, Cerbone L, Muzzonigro G, Burattini L, Montironi R, Santini D, Cascinu S. Prognostic significance of host immune status in patients with late relapsing renal cell carcinoma treated with targeted therapy. Target Oncol. 2015 Dec;10(4):517-22.
- Signorelli D, Giannatempo P, Grazia G, Aiello MM, Bertolini F, Mirabile A, **Buti S**, Vasile E, Scotti V, Pisapia P, Cona MS, Rolfo C, Malapelle U; Immune-Oncology YOUNG Group. Patients Selection for Immunotherapy in Solid Tumors: Overcome the Naïve Vision of a Single Biomarker. Biomed Res Int. 2019 Apr 24;2019:9056417.
- De Giorgi U, Procopio G, Giannarelli D, Sabbatini R, Bearz A, **Buti S**, Basso U, Mitterer M, Ortega C, Bidoli P, Ferraù F, Crinò L, Frassoldati A, Marchetti P, Mini E, Scoppola A, Verusio C, **Fornarini G**, Cartenì G, Caserta C, Sternberg CN. Association of Systemic Inflammation Index and Body Mass Index with Survival in Patients with Renal Cell Cancer Treated with Nivolumab. Clin Cancer Res. 2019 Jul 1;25(13):3839-3846.
- Mazzaschi G, Facchinetti F, Missale G, Canetti D, Madeddu D, Zecca A, Veneziani M, Gelsomino F, Goldoni M, **Buti S**, Bordi P, Aversa F, Ardizzoni A, Quaini F, Tiseo M. The circulating pool of functionally competent NK and CD8+ cells predicts the outcome of anti-PD1 treatment in advanced NSCLC. Lung Cancer. 2019 Jan;127:153-163.
- Massari F, Ciccarese C, Caliò A, Munari E, Cima L, Porcaro AB, Novella G, Artibani W, Sava T, Eccher A, Ghimenton C, Bertoldo F, Scarpa A, Sperandio N, Porta C, Bronte V, Chilosi M, Bogina G, Zamboni G, Tortora G, Samaratunga H, Martignoni G, **Brunelli M**. Magnitude of PD-1, PD-L1 and T Lymphocyte Expression on Tissue from Castration-Resistant Prostate Adenocarcinoma: An Exploratory Analysis. Target Oncol. 2016 Jun;11(3):345-51.
- Carbognin L, Pilotto S, Nortilli R, **Brunelli M**, Nottegar A, Sperduti I, Giannarelli D, Bria E, Tortora G. Predictive and Prognostic Role of Tumor-Infiltrating Lymphocytes for Early Breast Cancer According to Disease Subtypes: Sensitivity Analysis of Randomized Trials in Adjuvant and Neoadjuvant Setting. Oncologist. 2016 Mar;21(3):283-91.
- Prelaj A, Ferrara R, **Rebuzzi SE**, Proto C, Signorelli D, Galli G, De Toma A, Randon G, Pagani F, Viscardi G, Brambilla M, Trevisan B, Ganzinelli M, Martinetti A, Gallucci R, Di Mauro RM, Molino G, Zilembo N, Torri V, de Braud FM, Garassino MC, Lo Russo G. EPSILoN: A Prognostic Score for Immunotherapy in Advanced Non-Small-Cell Lung Cancer: A Validation Cohort. Cancers (Basel). 2019 Dec 5;11(12). pii: E1954.

PERSONNEL INVOLVED IN THE RESEARCH

Name	Date of birth	Role on project	Fellowship required	Effort on project (%)	Present position
Giuseppe Fornarini	18/03/1968	Principal Investigator		100%	Medical Oncologist, MD
Sara Elena Rebuzzi	27/05/1989	Sub-investigator	Yes	100%	Medical Oncology Resident, MD
Matteo Brunelli	10/04/1974	Sub-investigator		100%	Anatomo-pathologist, MD, PhD
Valerio Vellone	06/09/1978	Sub-investigator		100%	Anatomo-pathologist, MD, PhD
Sebastiano Buti	28/06/1977	Sub-investigator		80%	Medical Oncologist MD, PhD
Andrea Zivi	07/11/1979	Sub-investigator		80%	Medical Oncologist, MD
Melissa Bersanelli	27/05/1984	Sub-investigator		80%	Medical Oncologist, MD
Gabriele Gaggero	02/04/1976	Sub-investigator		80%	Anatomo-pathologist, MD
Bruno Spina	20/11/1958	Sub-investigator		80%	Anatomo-pathologist, MD
Guido Martignoni	18/07/1963	Sub-investigator		80%	Anatomo-pathologist, MD, PhD
Anna Caliò	01/05/1985	Sub-investigator		80%	Anatomo-pathologist, MD
Veronica Murianni	27/05/1991	Sub-investigator		50%	Medical Oncology Resident, MD
Fabio Catalano	23/05/1992	Sub-investigator		50%	Medical Oncology Resident, MD
Valentino Martelli	30/08/1991	Sub-investigator		50%	Medical Oncology Resident, MD
Chiara Casertelli	11/10/1988	Sub-investigator		50%	Medical Oncology Resident, MD
Sara Merler	01/10/1990	Sub-investigator		50%	Medical Oncology Resident, MD
Ilaria Zampiva	16/12/1989	Sub-investigator		50%	Medical Oncology Resident, MD

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

1. **Oncologists and sub-investigators**:
   - collection and shipping of histological blocks
   - collection clinical data
   - statistical analysis
2. **Anatomo-pathologists**: immunohistochemical and transcriptomic analysis on histological blocks

BUDGET FORM /YEAR

1. **Research costs:** 10000 €
2. **Instruments:** 20000 €
3. **Indirect costs:** 4000 €

Sub-total: 34000 €

5. Overheads 2000 €
6. Fellowships: 30.000,00 € for one year

Total:
- Investigator Grant (IG): 36000 €
- Borsa di studio: 30000 €, which could start at the end of the Medical Oncology residency period

Justifications, Itemized research costs None

EXISTING/PENDING SUPPORT None

SUGGESTED REVIEWERS (MAX 3)

- Ugo De Giorgi, Oncologia Medica, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori di Meldola
- Umberto Basso, Oncologia Medica, Istituto Oncologico Veneto IRCCS di Padova
- Luca Galli, Oncologia Medica, Azienda Ospedaliero Universitaria di Pisa

BIOETHICAL REQUIREMENT

1. Human experimentation: NOT – the study is going to be submitted to the competent Ethical Committee as the analysis will be performed on the archival histological blocks.
2. Animal experimentation: NOT

DECLARATION

I shall confirm to the Declaration of Helsinki in its latest version.
I shall also apply the Bioethics Convention of the Council of Europe.
In implementing the proposed research, I shall adhere most strictly to all existing ethical and safety provisions applicable.
Before start of the research, I shall obtain clearance from the competent ethical committee in case of involvement of human subjects in the research and /or in case of other ethical implications.
I shall conform with all regulations protecting the animals used for research purpose.

Date: 17/02/2020

Name of PI: Giuseppe Fornarini Signature:

IRCCS - Az. Osp. Universitaria San Martino
Ist. Naz. per la Ricerca sul Cancro
U.O. ONCOLOGIA MEDICA 1
Dott. GIUSEPPE FORNARINI
Dirigente Medico - O.M. GE n° 15591
FRN GPP 68C18 H501M

Principal investigator's signature .....

Authorized Administrative Official's signature dott.ssa Cristina Grandi

Date: 17/02/2020

Si autorizza al trattamento dei dati ai sensi del d.lgs. 196/2003