UNDERSTANDING TUMOR AND IMMUNE DYNAMICS AND PREDICTING RESPONSE TO VARIOUS PERIOPERATIVE THERAPIES IN PATIENTS WITH MUSCLE-INVASIVE BLADDER CANCER

Protocol Code

AIRC Investigator Grant (IG) 2022 27746

Version: 01
Date: 02/02/2023

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SIGNATURE PAGE

I declare that this protocol has been read carefully and fully understood. I agree to follow the study procedures as described in this protocol in accordance with Good Clinical Practice and all other regulatory requirements.

Prof. Andrea Necchi		02/02/2023
Principal Investigator	**Signature**	**Date**

Summary

1 INTRODUCTION AND RATIONALE

Urothelial bladder carcinoma (UC) is the fourth most common cancer in the United States with over 60,000 new cases each year.[1] It can usually be treated initially by radical cystectomy (RC) or trans-urethral resection of bladder tumor (TURBT). Unfortunately, over 40% of all patients will develop a cancer recurrence in less than two years and all patients remain at increased risk of recurrence for the remainder of their lives.[2] Neoadjuvant chemotherapy yields Level 1 evidence in the guidelines,[3] but near 50% of the patients cannot receive chemotherapy due to renal function impairment. This study will be conducted in patients with muscle-invasive urothelial carcinoma of the bladder (MIBC), who are ineligible or refuse to receive neoadjuvant chemotherapy, and have RC alone as a standard-of-care with suboptimal survival results. At Ospedale San Raffaele we pioneered the use neoadjuvant immunotherapy in MIBC. A clinical trial (SURE-01) sponsored by our group, with a grant support by AIRC (MFAG-2017-Id.20617), testing pembrolizumab as single agent therapy, given neoadjuvantly in patients with T2-4N0M0 MIBC, resulted in 42% pathologic complete responses (pT0N0 or pCR).[4] Early-phase single-arm trials of neoadjuvant immune-checkpoint inhibition (CPI) prior to RC in MIBC reported a pathologic response-rate ranging 29-40%, but the putative predictive biomarkers of clinical benefit are still inconsistent across trials.[5] While CPI has the potential to induce durable and potent responses, only a minority of patients experience an objective response. There is a gap in our knowledge due to lack of validated predictive biomarkers to determine whether patients with MIBC will respond to CPI or not, thus resulting in unnecessary treatment with CPI in patients who would be inherently resistant to CPI and otherwise candidate to anticipated RC. There is a critical unmet need to better understand the underlying mechanisms of response and resistance to CPI, to enable the selection of patients most likely to respond to CPI and avoid unnecessary toxicity for patients who are predicted to be resistant to CPI. The acknowledgement of the clinical utility of CPI will therefore require a comprehensive understanding of the variety of mechanisms of immune evasion active in MIBC. Our experience with the SURE-01 trial already revealed important information that we will use in order to develop the next step of biomarker studies. To summarize the major findings from the SURE-01 trial: i.) tumors with the highest pre-treatment immune-gene signature scores were associated with the highest probability of benefiting with a complete response from preoperative CPI. These scores were classically recapitulated by the so-called “basal” subtypes or “claudin-low” subtypes according to different classification systems.[6,7] ii.) tumors harboring the highest tumor mutational burden (TMB) levels, or those with the most favorable combination of PD-L1 expression and TMB value, were also likely to experience a pathological response to CPI, although the optimal tool for merging these biomarkers and the most suited cutoff of clinical efficacy are still to be identified.[8] In addition, single-gene alterations or genomic signatures of multiple genes like the homologous recombination repair (HRR) gene alterations did not results to be unequivocally related to the activity of CPI. Such alterations have been also associated to several neoadjuvant therapies including standard cisplatin-based chemotherapy, therefore this overlapping information represents a huge limitation for a patient selection strategy, pending the availability of large randomized clinical trials that are being promoted in MIBC; iii.) by using CPI we could overcome the limitations of the classical histology-based inclusion criteria that characterize the neoadjuvant chemotherapy trials. In fact, SURE-01 data suggested that pembrolizumab was effective also in sarcomatoid histologies or lymphoepithelioid-like tumors.[9] iv.) shifting molecular subtypes may occur when matching the pre-post neoadjuvant CPI tumor samples, meaning that the transcriptome

profiling, like other immune-biomarkers, can change during treatment as a result of a therapeutic pressure.[10] v.) neoadjuvant CPI treatment is medically and surgically safe and does not expose patients with MIBC to an excess risk of adverse events compared to RC upfront.[11] vi.) finally, it is possible to develop radiological tools aimed at predicting the pathological response to treatment, mainly with the use of multiparametric magnetic resonance imaging (mpMRI) of the bladder.[12,13] This tool is well suited for integration with tumor and immune-biomarkers, aiming to a noninvasive strategy of treating and assessing response of MIBC. New molecular entities may raise post-CPI, like the tissues that we classified as “scar-like” subtype according to their mRNA expression profile, thus suggesting novel opportunities for therapeutic sequences. Testing different compounds, including their combination, in the same preoperative setting will enable us continuing our path towards the identification of the mechanisms of action and resistance development of UC cancer cells to treatment. Indeed, besides the possibilities of combining standard chemotherapy with CPI, another wave of revolution in this disease is being represented by the antibody-drug conjugates (ADC). Two ADCs are now approved by the United States Food and Drug Administration (FDA) for the treatment of advanced UC: enfortumab vedotin and sacituzumab govitecan.[14,15] At our center we have developed an umbrella of various neoadjuvant therapies within several phase 2 trials testing the newer single-agents or combination therapies integrated with RC. This platform is offered to continue our path of biomarker discovery and validation. Our research proposal will address the critical gaps in knowledge by identifying predictive immunologic and molecular biomarkers of response and resistance to various neoadjuvant therapies in MIBC that will inform personalized treatment strategies. Our research proposes a rational step in advancing the field of immunotherapy in MIBC.

2 OBJECTIVES

The objective of the present study is to provide a proof-of-concept for the development of new biomarkers from tumor tissue or liquid biopsies that are associated with the response or resistance (seen at the pathological assessment of the radical cystectomy specimen) to various neoadjuvant therapies in patients with MIBC, expanding our knowledge on the mechanisms of response and resistance to novel neoadjuvant therapies including immunotherapy combinations and ADC.

According to the aim of the study, “response” is defined as a complete (ypT0N0) or major response (ypT<2N0) at the radical cystectomy report.

3 DESIGN OF THE STUDY

This is both a prospective and retrospective study aimed to evaluate multiple cohorts of patients already included in ongoing prospective clinical trials (SURE-01, SURE-02, NURE-COMBO), plus an additional cohort of patients prospectively receiving upfront radical cystectomy as routine clinical practice.

As most of the clinical trials representing the core-business of the study are already recruiting patients, we will retrospectively retrieve and analyze the tumor and blood samples of these patients, and in parallel we will prospectively collect the material of newer patients.

The retrospective part of the study will be based on the analysis of tumor and blood samples analyses dating back to January 2022, date of the start of the enrolment of the ongoing clinical trials that are the core business of this project.

MIBC tumor tissues will be collected from MIBC patients undergoing neoadjuvant therapy. We will acquire pre-treatment tissue from TURBT and post-treatment tissue from residual disease at RC (from patients not achieving complete pathologic response).

The umbrella of neoadjuvant studies representing the source of tumor and blood samples will consist of the following studies (Figure 1):

- In SURE-01, 56 eligible patients will receive neoadjuvant treatment with 10 mg/kg sacituzumab govitecan IV, on days 1, 8, of each 21 day cycle, for a total of 4 cycles.
- In SURE-02, pembrolizumab will be administered in combination with sacituzumab govitecan on day 1, every 21 days, at the standard dose of 200 mg intravenously. A total of 4 cycles is planned before surgery and the study sample size is 48 patients.
- In Nure-Combo trial 29 patients will receive 4 cycles of preoperative nivolumab 360 mg intravenously, every 3 weeks, plus nab-paclitaxel 125 mg/m2 intravenously, on days 1 and 8, every 3 weeks.
- Curative-intent RC + pelvic lymph node dissection will be planned at the time of study inclusion to be done within 4 weeks of the last dose of study drug.
- After surgery, in SURE-01 patients will be managed with surveillance or adjuvant chemotherapy depending on the pathological response, according to the protocol.
- In SURE-02, after surgery patients will receive additional 13 cycles of pembrolizumab at the standard dose of 200 mg, IV, every 3 weeks, according to the protocol. Imaging assessments will be managed according to the standard-of-care.
- In Nure-Combo, after RC, patients will receive an additional 13 cycles of nivolumab monotherapy at the dose of 360 mg, every 3 weeks, according to the protocol.
- After the adjuvant period, there will be a follow-up period lasting for a maximum of 1 year.
- An additional RC cohort will be used as a standard-of-care cohort and will consist of a pre-determined sample size of 20 patients.

All the study procedures and treatments have been already approved by the regulatory authorities and ethical committee and the studies are currently open to accrue patients, therefore all the details regarding the study-specific examinations and timelines are indicated in the corresponding protocol files.

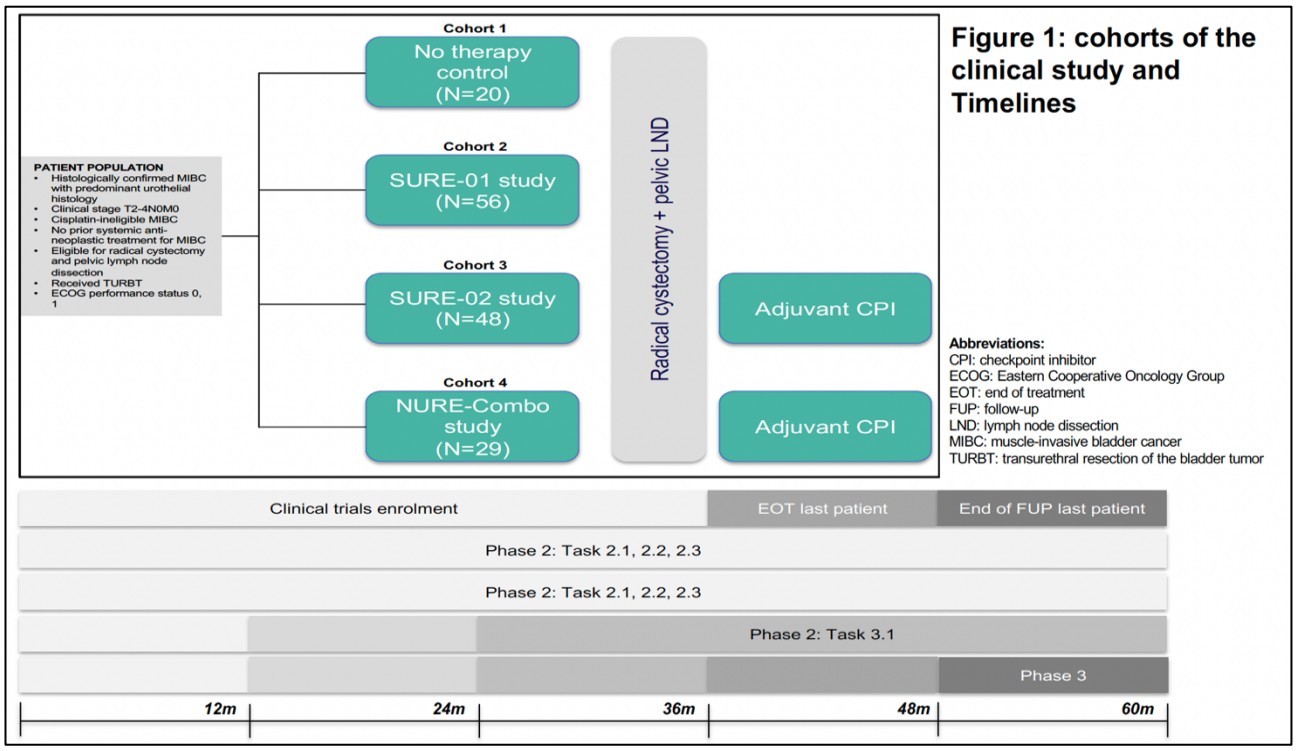


Figure 1: cohorts of the clinical study and Timelines

Regarding the present study, we have identified different phases and tasks to be simultaneously achieved, as detailed in Figure 1.

PHASE 1 (Months 1-60):

- Task 1, neoadjuvant trials accrual, follow-up completion and sample collection.

PHASE 2 (Months 1-60): Planned translational analyses.

- Task 2.1 (Months 1-60): *To define local and peripheral evolution of the immune response to experimental combination therapies in MIBC*.

These analyses will be conducted at the laboratory facilities of Ospedale San Raffaele S.r.l.

The proposed therapeutic strategies will impact systemic and local cancer immunity to a different extent and with potential synergy. Whereas sacituzumab govitecan treatment in MIBC patients often associates with neutropenia and leukopenia, topoisomerase 1 inhibitors may enhance antitumor immunity.[17] Also nab-paclitaxel supports the cancer-immunity cycle by enhancing antigen presentation ability of antigen presenting cells, promoting T cell activation, reversing immune suppression in the TME, and cooperating with cytotoxic T lymphocytes in killing tumor cells.[18] Thus, sacituzumab govitecan and nab-paclitaxel are expected to further improve the therapeutic efficacy of pembrolizumab in patients with MIBC by acting on several steps of the cancer-immunity cycle.

Objective of this task of the proposal is to assess and quantitate the impact of the proposed treatments on cancer immunity in MIBC. Two complimentary approaches will be implemented to fulfill this task: spatial distribution of the immune infiltrate within tumor lesions by multiplex

fluorescence immunohistochemistry, and single cell analysis of peripheral blood mononuclear cells by multiparametric flow cytometry. This strategy applied to both biopsies and surgical specimens will allow investigating, on the one hand, the whole tissue and appreciate inter- and intraindividual diversities and commonalities among immune cells infiltrating tumor lesions as well as the peritumoral stroma. An additional advantage of this strategy is that the whole samples will be available for pathology examination, including expression of PD-L1. Considering MIBC multifocality, this is essential. On the other hand, single cell analysis of circulating T lymphocytes, neutrophils, monocytes and myeloid derived suppressor cells (MDSCs) will provide several snapshots of immune cell reactivity during the whole therapeutic regimen. The two pieces of information will be combined to obtain a dynamic image of immune cell reactivity to the selected treatments in homogenous populations of MIBC patients. As designed, the two immunoanalytical strategies will provide results easily comparable among the three clinical trials. This will be an unprecedented opportunity to understand the dynamics of the local and systemic immune response under the pressure of different immunomodulating agents.

For example, a high number of T-cells infiltrating the tumor microenvironment (TME) indicates an inflamed MIBC subtype with a very good prognosis.[19] Our experimental design has the power to confirm and extend this observation to patients with MIBC that will undergo either RC alone or combined with different neoadjuvant treatments. We will also define distance between effector CD8 T cells and tumor cells and either regulatory T cells or myeloid cells, both populations with immunosuppressive activity in bladder cancer.[20] This will provide mechanistic insights on the role of effector T cells in MIBC and how their activity is potentially impaired by immunosuppressive cells. Importantly, the same immunosuppressive cells and their precursors will be investigated in peripheral blood before treatment, during neoadjuvant therapies and after RC in search of circulating tumor-promoting immune cells *en route* to the TME. We will also be able to find phenotypic and functional correlates in tumor infiltrating and peripheral blood T cells, thus, hopefully providing clinicians with peripheral proxy of the immune response in the TME. Finally, immunological data will be matched with genomic data to provide a more comprehensive picture of the disease and its capacity to respond to the proposed neoadjuvant treatments.

Multiplex immunofluorescence allows defining the immune contexture of a lesion, which might be predictive of response to therapy and correlate with overall survival. Our analysis will focus on T cells, B cells, NK cells and myeloid cells. Several panels have been already set up that investigate the spatial distribution of these populations.[21] More in details, panel A (CD20, CD3, CD68, CD56 and NE) identifies B lymphocytes, T cells, macrophages, NK cells and neutrophils, respectively. Panel B investigates regulatory T cells (CD4, Foxp3), cytotoxic T cells (CD8+, Granzyme B+) and putative M2 macrophages (CD163+). Both panels also contain DAPI to identify nuclei and an epithelial marker. A third panel (panel C: CD20, CD3, DC-Lamp, CD21, CD23 and CXCL13) will investigate the existence and density of early (CD21-), primary (CD21+) and secondary (CD23+) lymphoid structures as described by Silina K et al.[22] We expect that germinal centers (CD23+) will identify those patients that will more likely respond to the proposed therapies. It will also be interesting to assess if chemotherapy disrupts such structures.[22]

- Task 2.2 (Months 1-60): *whole transcriptome expression analyses.*

We will rely on an established collaboration with Veracyte scientists, extensively pursued in SURE-01 trial.[4,6,9,10]

Specimen collection and sample processing will be conducted using Veracyte (former Decipher Biosciences Laboratory, San Diego, CA, USA), a clinical-grade whole transcriptome assay, as described in our previous work.[6,10]

Unsupervised consensus clustering: For unsupervised clustering analysis (R package ConsensusClusterPlus), normalized expression data will be pre-processed by filtering low-variance genes, selecting the 2,000 genes with the highest median absolute deviation. The expression clustering analysis will be performed by consensus partitioning around medoids (PAM) approach, using Pearson correlations as the similarity metric, the Ward algorithm for clustering, running 1,000 iterations with 0.875 random fraction of samples used in each iteration (pItem) and 0.95 random fraction of gene features used in each iteration (pFeature).

Classification of tumors into molecular mRNA subtypes: To assign tumors to the consensus bladder cancer and The Cancer Genome Atlas (TCGA) 2017 molecular subtypes,[23] we will download and apply the centroid-based models as described previously. The Decipher subtypes will be assigned by identifying neuroendocrine (NE)-like patients and then classifying the remaining tumors using Seiler *et al.* model.[24] The Lund (2012)[25] model will be applied as previously described.[26] The consensus classifier will provide the standard classification of the NE-like subtype.[26]

Gene expression analysis: Heatmaps and boxplots will be used to visualize differences between samples from unsupervised consensus clusters (CCs). Sample purity will be calculated by the ESTIMATE algorithm and the other signature score calculations have been previously described.[6,9]

- Task 2.3 (Months 1-60): *Immune-monitoring by multiparametric flow cytometry*

Multiparametric flow cytometry allows the phenotypic and functional analysis of peripheral blood cells at single cell level. Blood samples will be collected at baseline, at the end of neoadjuvant therapy and post-surgery and frozen. Longitudinal analysis of peripheral blood mononuclear cells might represent a proxy of the dynamics of the immune response within the TME and in the secondary lymphoid organs. Multiparametric flow cytometry will be implemented to look at absolute count and frequency of CD8+, CD4+ and CD4 regulatory T cells. The latter population will be identified as CD25high and CD127low cells. Differentiation, activation or exhaustion status of CD4+ and CD8+ T cell populations will be determined by key markers. CCR7 and CD45RA expression will define the four main sub-populations of T cells: naive (CCR7+CD45RA+), central memory (CCR7+CD45RA−), effector memory (CCR7−CD45RA−) and effector memory RA (CCR7−CD45RA+). The homeostasis marker CD127 will identify long-term surviving T cells. The CD137 and ICOS co-receptors will define the activation status of T cells, and PD-1, TIM-3 and LAG-3 co-inhibitory receptors we will identify exhausted T cells. On frozen/thawed samples, we will also study the myeloid compartment (CD3-CD56-CD19- cells) focusing on myeloid derived suppressor cells (MDSCs). According to standard classification, we will determine the absolute count and frequency of monocytic-MDSCs (CD11b+CD14+HLA-DRlow/− CD15−) and early-stage MDSC (CD15-CD14-HLA-DR− CD33+). All these panels have been already set up and are running in the lab.

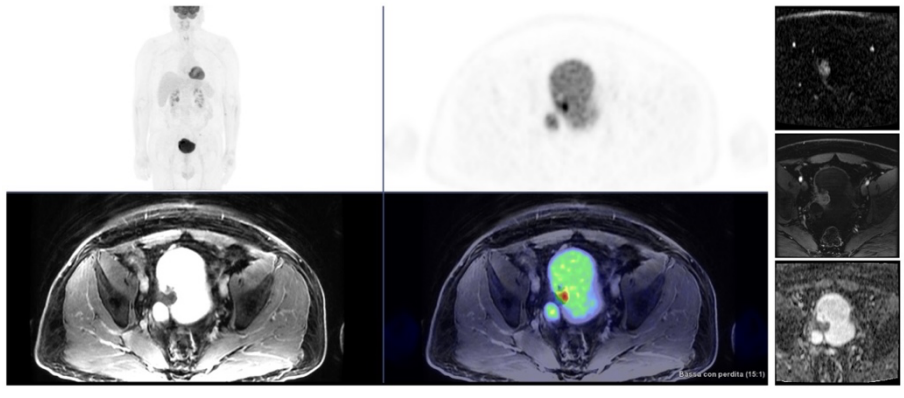
Because freezing of samples leads to loss of polymorphonuclear MDSCs,[27] this population will be investigated in fresh blood samples with a dedicated panel (CD11b, CD14, CD15, CD16, CD33, CD45, CD66b, CD163, HLA-DR, LOX1, CD62L and 7AAD). Preliminary results on the first 3 patients enrolled in the Nure-Combo trial are reported in Figure 4a.

- Task 3.1 (Months 24-60): *Noninvasive prediction of tumor response to neoadjuvant treatment.*

Every tumor has a slightly different appearance on mpMRI of the bladder. This appearance translates into different imaging features that can be extrapolated through dedicated software and are the object of artificial intelligence algorithms that can be aimed to predict response to treatments. At our center, a formal academic collaboration with the Department of Radiology at Columbia University, New York, is ongoing (see details on the corresponding Protocol: 118/INT/2021, Prof. Montorsi) whereby we aim to identify radiomics feature that help predicting response to neoadjuvant immunotherapy. Besides this ongoing study, in order to expand our knowledge and potentially improve the prediction performance, we are implementing our previous findings reported with the use of multiparametric MRI of the bladder within the PURE-01 trial (Necchi A, et al. J Clin Oncol. 2018) with the use of Fluorodeoxyglucose Positron Emission Tomography (FDG-PET)/MRI of the bladder within the ongoing NureCombo, SURE-01 and SURE-02 trials.[12] PET-MRI sessions will consist of anatomic, functional and metabolic imaging. Anatomical: T1-weighted and T2-weighted MRI; Functional: Diffusion-weighted (DW) and dynamic-contrast enhanced (DCE) MRI; Metabolic: 18F-FDG PET. An example of pre-treatment, baseline PET-MRI performed at our center in a patient included in Nure-Combo trial is shown in Figure 2.

Clinicians will contour bladder tumors depicted on PET/MRI scans. Quantitative parameters from PET (lesion maximum standardized uptake value (SUV) and SUV mean) and MRI (lesion size, apparent diffusion coefficient from DW MRI, transfer constant Ktrans from DCE MRI) will be recorded. Tumor response will be defined as percentage change in these parameters between baseline and post-neoadjuvant therapy. Tumor response will be evaluated with bladder MRI using the Vesical Imaging-Reporting And Data System (VI-RADS, PMID: 29755006). VI-RADS categories will be associated with the pathological response seen at radical cystectomy.

Figure 2



PHASE 3 (Months 48-60): *Integrating results: key hypotheses.*

A comprehensive characterization of the tumor-associated immune compartment in MIBC is currently lacking but would have important implications for predicting determinants of response and resistance to both immunotherapy and other therapies in MIBC. We expect to compare peripheral and local immune contexture in MIBC patients from 3 unique cohorts receiving different neoadjuvant therapies, and compare the findings with untreated tumor models. The findings generated in this study could potentially be expanded to significantly larger MIBC datasets currently available, comprised of whole tissue gene expression data with limited to no immunological data.
Overall, we anticipate identifying patterns of peripheral immune response and/or tumor immune infiltration with prognostic and predictive significance in the context of neoadjuvant therapy, which provides the opportunity to study patient-matched pre- and post-therapy specimens from the same anatomic site allowing deeper characterization of features specifically associated with treatment exposure itself. Furthermore, by integrating the immune signature obtained with transcriptome analyses and IHC with radiological imaging data we could potentially provide investigators with a useful tool to inspire newer bladder-sparing approaches in the near future. The value of the clinical data source relies mainly in the use of advanced treatments that anticipate the use of the best therapeutic options available at present (CPI-chemotherapy combination, ADC and CPI+ADC combination).

4 STUDY DURATION

The study duration, and corresponding funding, will be of 5 years for the period 2023-2028.

5 POPULATION/ELIGIBILITY CRITERIA

5.1 Sample size and justification

The study population and corresponding sample size will be determined by what is already established within the ongoing NureCombo, SURE-01, and SURE-02 clinical trials.
An additional cohort of 20 patients undergoing radical cystectomy without any neoadjuvant therapy will constitute the standard-of-care arm. The same eligibility criteria will apply to this cohort of patients (as detailed below). For this cohort we have planned to accrue a pre-determined to allow the generation of biomarkers data from this population, without any aim to make comparative analyses between arms.

5.2 Elegibility criteria

5.2.1 Inclusion criteria

Key inclusion criteria, which apply to all the above ongoing studies, are the following:

1. Patients enrolled in NureCombo, SURE-01, and SURE-02 clinical trials or candidates to radical cystectomy as per routine clinical practice.
2. Histopathologically-confirmed UC.
3. ECOG performance status 0-1.
4. Clinical stage T2-T4aN0M0, assessed by thorax-abdomen CT scan and combined whole-body FDG-PET scan plus bladder mpMRI.

5. The patient accepts to undergo RC as per clinical practice.
6. Ineligibility to receive cisplatin-based neoadjuvant chemotherapy based on poor renal function (glomerular filtration rate according to Cochroft-Gault formula <60 ml/min) OR refusal to receive neoadjuvant cisplatin-based chemotherapy

5.2.2 Exclusion criteria

Patients unwilling to partecipate to the study and unwilling to sign the informed consent.

6 PROCEDURES

There are no special procedures scheduled for this study in addition to those already planned within the NureCombo, SURE-01, SURE-02, and the routine clinical practice.

7 STUDY VARIABLES

All the clinical variables of the patients will be collected from the CRF of the corresponding studies. The same body of information will be collected from paper source data for the patients included in the standard-of-care radical cystectomy cohort as reported in the list of variables in attachment.

8. HANDLING OF ADVERSE EVENTS AND ADVERSE REACTIONS

Adverse events will be treated as reported in the reference studies (SURE-01, SURE-02, NURE-COMBO) and in accordance with clinical practice for the cohort doing clinical practice cystectomy, as there are no procedures performed *ad hoc* for the present study.

9. DATA MANAGEMENT

The data of patients included in the study-corresponding trials are already gathered and collected within electronic or paper source, depending on the trial. For the standard-of-care arm the data will be collected via paper source of clinical charts.

10. HANDLING OF BIOLOGICAL SAMPLES

Tumor tissue samples used for biomarker analyses will be obtained from archival, paraffin-embedded blocks that are stored at the Department of Pathology of IRCCS Ospedale San Raffaele or in other institutions/hospitals, according to the routine clinical practice. Therefore, there is no study-specific legislation regulating the storage and handling of such samples. Regarding the liquid biopsy samples, these samples will be collected and stored, limited to the duration of study analysis, at the Laboratory of Prof. Matteo Bellone a the Laboratory of Cellular Immunology Unit of IRCCS San Raffaele Hospital. At the end of the study samples will be dismissed and destroyed and no further storage is expected. Regarding the imaging repository, the imaging data are collected, stored and anonymized according to the routine practice.

11. STATISTICAL ANALYSES

The study is aimed to provide a proof-of-principle to inform further prospective studies aimed at establishing new biomarkers of response to neoadjuvant therapies in MIBC.

There will not be a formal statistical comparison of findings between the study cohorts, as the treatment characteristics and the overall design will prevent doing such comparisons. Therefore, statistical analyses will be applied within each trial arm to measure the effects between biomarker cohorts.

The primary endpoint of interest for each of the clinical trials is the pathological complete response (pT0N0). Patient, tumor characteristics and pT0N0 rates will be compared between subgroups by using X^2 tests and two-sided Wilcoxon rank-sum tests.

Descriptive statistics with medians and IQR, or frequencies and proportions were presented as appropriate. Fischer's exact and Wilcoxon rank-sum tests will be used to analyze the associations between laboratory or clinical variables, e.g., molecular subtypes or single biomarker tests, and the pathological response. Kaplan-Meier curves and log-rank tests will be used to analyze the relapse-free survival (RFS) and overall survival (OS) outcomes in relation to gene signatures, molecular subtypes, or single gene expressions. Continuous-scale analyses will evaluate single-gene or signatures expression, and will be analyzed in Cox proportional hazards models that will include biomarker and the clinical T-stage.

Logistic regression models will analyze pre- and post-pembro VI-RADS against pT≤1N0 and pT0N0 pathological response. VI-RADS scores were dichotomized between 0-3 and 4-5.

All statistical tests will be two-sided, and will be reported with a significance level of 0.05. Analyses willbe performed in R v3.4.1 (R Foundation, Vienna, Austria).

12. ETHICAL ASPECTS AND INFORMED CONSENT

The final study protocol, including the final version of the Informed Consent Form, have been approved by the Independent Ethical Committee (IEC) of IRCCS Ospedale San Raffaele.

The Principal Investigator will be responsible for informing the IEC of any amendment to the current study file in compliance with the local requirements. In addition, the IEC will approve all advertising used to recruit subjects for the study. The protocol will be re-approved by the IEC upon receipt of amendments.

For biomarker samples of retrospective patients included in NureCombo, SURE-01 and SURE-02 trial, the patients will be asked to sign the informed consent for the present study. In case the patients died or they are unable to provide consent, provided that they have already consented for the use of their biological samples for research purposes (refer to Consenso URI), the corresponding material will be anonymized and it will be assigned a unique code for case identification.

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate.

The Principal Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

13. DATA PRIVACY AND ANONYMIZATION

All information related to the subjects into the study will be kept confidential and handled acording to the applicable legislation (D.L.196/30 giugno 2003 e s.m.i. e regolamento UE 679/2016; Linee Guida del Garante della Privacy del 24/07/2008).
All patients will be assigned a unique identification number. All the clinical data related to each patient will be matched to this identification code. The attribution of each code to the single patient will be allowed only to the study physician and any other authorized people.

Information collected in this clinical study is subject to the Health Insurance Portability and Accountability Act of 1996 (HIPAA) as described in 45 CFR 160 and 45 CFR 164, as well as the REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 April 2016 (on the protection of natural persons with regard to the processing of personal data and on the free movement of such data). The study Investigator is responsible for informing subjects of their rights under HIPAA and General Data Protection and obtaining any necessary HIPAA authorizations. Any clinical study information referred to in this section is understood to be compliant with the provisions of the Privacy Act. The information obtained during the conduct of this study is confidential, and disclosure to third parties other than those noted below is prohibited.

14. INSURANCE AND COSTS

There is no need for an ad-hoc insurance coverage based on the nature of the present study. All the clinical trials included in the present study are already covered and corresponding costs of trial management and drug supply are supplied by the collaborating Companies (Merck Inc, Gilead, BMS).

All the costs related to the biomarker analyses included in the present study are covered by a research grant of the Italian Association for the Research on Cancer (AIRC).

15. DATA PROPERTY AND PUBLICATION RULES

The owner of the data will be the academic sponsor of this study, Vita-Salute San Raffaele University.

The principal investigator of the study will be in charge of disseminating the results in compliance with the data privacy legislation and all the applicable laws.

Data will be presented as congress presentations and manuscripts, either as interim analyses or final analyses.

In addition, the conditions regulating dissemination of the information derived from NureCombo, SURE-01, and SURE-02 are described in the corresponding Clinical Study Agreements.

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