LETTER TO THE EDITORS

Cancer bio-immunotherapy XVIII annual NIBIT-(Italian network for tumor biotherapy) meeting, October 15–16, 2020

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Abbreviations

AACR	American association for cancer research
ACC	Alleanza contro il cancro
ACE2	Angiotensin-converting enzyme 2
AIOM	Associazione Italiana di oncologia medica
BAL	Bronchoalveolar lavage
ccRCC	Clear-cell renal cell carcinoma
CAR	Chimeric antigen receptor
COVID-19	Coronavirus disease-19
ECOG	Eastern cooperative oncology group
ESMO	European society for medical oncology
FEST	Functional expansion of specific T cells
ICB	Immune checkpoint blockade
MANA	Mutation-associated neoantigens
NIBIT	Italian network for tumor biotherapy
PDAC	Pancreatic ductal adenocarcinoma
RBD	Receptor binding domain
pSTAT3	Phosphorylated STAT3
SARS-Cov-2	Severe respiratory syndrome coronavirus 2
SIICA	Società Italiana di immunologia, immunologia clinica ed allergologia
TERAVOLT	Thoracic cancer international COVID-19 collaboration
TLS	Tertiary lymphoid structures
TME	Tumor microenvironment

Précis: COVID-19 at the intersection between cancer, immunity and immunotherapy was the theme of the XVIII annual meeting of the Italian Network for Tumor Biotherapy (NIBIT), which gathered on-line basic scientists, clinicians and international leaders on October 15–16, 2020.

Introduction

Like a tsunami, the SARS-Cov2 pandemic suddenly changed our programs and perspectives. Because traditional meetings are not allowed in the COVID era, the XVIII international meeting of the Italian Network for Tumor Biotherapy (NIBIT) went virtual on October 15–16, 2020. COVID-19 has been a formidable challenge for oncologists and immunologists. Thus, while maintaining the focus on cancer immunology and immunotherapy, most of the sessions also investigated how COVID-19 impacted these topics. Accordingly, "Coronavirus disease-19 (COVID-19) at the

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intersection between cancer, immunity and immunotherapy" was chosen as theme of the meeting. Plenary sessions were organized in two consecutive afternoons, in the intent to allow convenient time in the morning for usual business. Three poster sessions in the morning also allowed 20 investigators to orally present their most recent and exciting findings. With appreciation of almost 150 attendees, we at NIBIT were very pleased to meet the educational needs of its members and of young investigators committed to cancer immunotherapy.

Session 1. COVID-19 and cancer

The XVIII NIBIT meeting started with a focus on the impact COVID-19 had on cancer patients. This session was jointly organized by the NIBIT and the Associazione Italiana di Oncologia Medica (AIOM), and it was animated by four experts in both oncology and COVID-19: Alessandra Gennari (Novara, Italy), oncologist, also in representation of AIOM; Matteo Bellone (Milan, Italy), immunologist and Secretary and Treasurer of the NIBIT; Giuseppe Di Lucca (Milan, Italy), oncologist with a wide experience in COVID-19 clinic, and Claudio Tripodo (Palermo, Italy), pathologist.

Pathology was the natural start of the session. While several reports have highlighted the relevant pathological findings obtained from postmortem examinations [1–3], few data were available on biopsies taken within the first days since COVID-19 onset [4, 5]. Claudio Doglioni (Milan, Italy) reported on pathology examination of cryobiopsies from the lungs of 12 COVID-19 patients [6]. Transbronchial lung biopsy was taken within 20 days of the first symptom appearance in patients with documented pneumonia but not in need of invasive ventilatory support. When compared to biopsies taken from patients with diffuse parenchymal lung disease (i.e., nonspecific or usual interstitial pneumonia, chronic hypersensitivity pneumonia, sarcoidosis, acute fibrinous and organizing pneumonia, and smoking-related interstitial pneumonia), samples from COVID-19 patients showed signs of acute lung injury, but lacked hyaline membranes, a typical features of diffuse alveolar damage, and only occasionally edema and cellular debris were found in the alveolar space. Conversely, they were characterized by spots of patchy acute lung injury with alveolar type II cell hyperplasia. Most of these cells showed strong nuclear expression of phosphorylated STAT3 (pSTAT3), suggesting the activity of the IL-6-STAT3 pathway in the pathology underlying COVID-19. Endothelial cells lining venules, which were characterized by luminal enlargement, thickened walls and perivascular CD4$^+$ T cell infiltration, also expressed pSTAT3, PD-L1 and IDO. While irregular clusters of mononuclear cells were present within alveolar spaces in 10/12 cases, these cells did not express pSTAT3,

but showed an unusual hybrid phenotype (CD68, CD11c, CD14, CD205, CD206, CD123/IL3AR, and PD-L1). Type I pneumocyte necrosis, alveolar hemorrhage, granulocytic infiltration, and hyaline membrane were absent in all cases of early COVID-19 pneumonia analyzed by Doglioni. Scattered microthrombi were observed in only two cases. Thus, the pattern of interstitial pneumonia in early COVID-19 patients is rather different from the other interstitial pathologies of the lung examined by Doglioni et al. [6] as well as from the postmortem diffuse alveolar damage found in COVID-19 patients [1–3]. These data have been recently reviewed [7].

Valter Torri (Milan, Italy) investigated if cancer patients were more prone to get infected by severe respiratory syndrome coronavirus 2 (SARS-Cov-2) and/or to develop a more aggressive disease. An exhaustive review of the literature clearly showed that cancer patients, and those under immunotherapy in particular [8], were among those with the highest probability of hospitalization and severe disease. He also introduced the Thoracic Cancer International COVID-19 Collaboration (TERAVOLT), a network aimed at measuring the real impact of the pandemic on thoracic cancer patients and at identifying prognostic factors in these subjects [9]. Preliminary data suggested that Eastern Cooperative Oncology Group (ECOG) performance status ≥ 2, smoking history, stage IV, age > 65, steroids > 10 mg, and chemotherapy or no treatment were significantly associated with worst outcome [10]. These preliminary results demonstrate the importance in rapidly react to global health threats with international multidisciplinary networks and patient registries like TERAVOLT.

Maria Rescigno (Milan, Italy) switched gear at the meeting, and, after a short and preliminary report on SARS-Cov-2 antibody titration in cohorts of Lombardy residents during the COVID-19 pandemics, she focused her talk on the delicate balance between gut microbiota and the host during intestinal tumorigenesis. Her group identified *Faecalibaculum rodentium* and its human homologue *Holdemanella biformis* as anti-tumorigenic strains of the intestinal microbiota [11]. The two strains were poorly represented in mouse and human intestinal cancer, likely due to altered composition of the intestinal mucus that impeded their colonization. Through the production of short chain fatty acids, both strains inhibited calcineurin and NFATc3 activation, thus contributing to control protein acetylation and tumor cell proliferation. *Faecalibaculum rodentium* or its metabolic products delayed tumor growth in a genetically engineered model of colorectal cancer. Interestingly, both the number of regulatory T cells and IL-17-producing CD4$^+$ T cells, which are relevant populations in the crosstalk between gut microbiota and the host in cancer [12], were not modulated by *F. rodentium* colonization, thus suggesting that these cells

do not contribute to the beneficial effect exerted by gut microbiota modulation in this context. However, treatment with *F. rodentium* associated with reduced circulating Ly6G$^+$CD11b$^+$ inflammatory monocytes, which might contribute to carcinogenesis. Butyrate appeared the most important SCFA in the antitumor activity of *F. rodentium* and other butyrate-producing bacteria as well as treatment with butyrate itself mimicked these effects [11]. Thus, the antitumor activity of *F. rodentium* is not unique and could be extended to SCFA-producing bacteria that are able to colonize the gut and locally produce butyrate.

The talk given by Andrea Alimonti (Bellinzona, Switzerland) shifted the audience's attention away from colon cancer into prostate cancer and back to COVID-19. The viral spike protein of SARS-Cov-2 binds to angiotensin-converting enzyme 2 (ACE2) on the cell surface, and it gets cleaved by the enzyme TMPRSS2 to allow fusion of the viral and cellular membranes. TMPRSS2 is expressed in prostate cancer but also in non-prostatic tissues, including lung, and its expression is regulated by androgens. Alimonti and colleagues hypothesized that androgen deprivation therapy (ADT), by reducing expression of TMPRSS2, protected patients affected by prostate cancer from SARS-Cov-2 infection. They analyzed a population of 9280 COVID-19 patients and found that males developed more severe complications were more frequently hospitalized and showed a worse clinical outcome than females [13]. In the male population, patients affected by cancer showed a higher risk of being infected by SARS-Cov-2. Although the number of prostate cancer patients analyzed (i.e., 118) was small, patients undergoing ADT for prostate cancer had a significantly lower risk of SARS-Cov-2 infection compared to prostate cancer patients not receiving this treatment. Among 5273 patients receiving ADT, only four developed COVID-19 and none of them died. Similar results were obtained comparing prostate cancer patients under ADT with patients affected by other type of cancer [13]. ADT-induced downregulation of TMPRSS2 might not be the only mechanism, as androgens can increase the production of pro-inflammatory cytokines [14]. Additionally, in vitro culture in the presence of antiandrogenic drugs reduces ACE2 expression and protects from SARS-Cov-2 infection [15].

Modulation of the renin-angiotensin axis might have additional implications on clinical manifestations related to COVID-19 as suggested by Matteo Bellone, who underlined the delicate balance of the renin-angiotensin axis and the pro-inflammatory activity of ACE2 [16]. The section closed with and interesting comment by Alessandra Gennari on the OnCOVID registry [17]. Analyzing data from a cohort of 890 cancer patients affected by COVID-19, they found a worsening gradient of mortality from breast cancer to hematological malignancies. They also confirmed that male gender, older age, and number of co-morbidities identified a subset of patients with significantly worse mortality rates from COVID-19 [17].

Session 2. B cells in COVID-19 and cancer

Session 2 was dedicated to the role of B cells both in cancer and in COVID-19. The session was animated by Vincenzo Barnaba (Rome, Italy), Mario Paolo Colombo (Milan, Italy) and Paolo Dellabona (Milan, Italy).

Antonio Lanzavecchia (Bellinzona, Switzerland) discussed about specificity and kinetics of antibody responses to the SARS-CoV-2 receptor binding domain (RBD), domain A, S2 subunit, S and N proteins based on analysis of 4726 serum samples from hospitalized, symptomatic, and asymptomatic COVID-19 patients, as well as pre-pandemic healthy donors [18]. Hospitalized individuals presented higher titers of serum IgG and detectable levels of IgA compared to non-hospitalized or asymptomatic subjects, with binding titers correlating with the severity of clinical course. RBD was the primary target of neutralizing Abs, accounting for the 90% of the neutralizing activity present in SARS-CoV-2 serum samples. Notably, a map of the major RBD antigenic sites through structural studies provided a new way to perfectly engineer receptor-fitted antibodies, which are not permissive to pathogen escape, opening to new vaccine and therapeutic design strategies [19].

Catherine Sautès-Fridman (Paris, France) discussed the density of B cells, particularly in TLSs, in several human cancers [20]. Her findings on the analysis of the tumor microenvironment in different human cancers, from breast to pancreatic cancer patients, highlighted a correlation between the presence of B cells, mainly compartmentalized in CXCL13-rich milieu of the TLS, and a favorable prognostic impact. On the other hand, tumor B cells also display a regulatory role, by locally inhibiting T cell activation and negatively affecting prognosis. In conclusion, B cells may act as double player promoting either tumor destruction or growth [21]. Nowadays, the knowledge of the heterogeneity and diversity of B cell subsets in tumors is still lacking, identifying in this emerging field a novel potential source of prognostic and predictive markers, as well as new targets in oncology treatments.

Session 3. Innate immunity in COVID-19 and cancer

NIBIT and the Società Italiana di Immunologia, Immunologia Clinica ed Allergologia (SIICA) jointly organized Session 3, which was animated by Giorgio Cassatella, Next SIICA President (Verona, Italy), Giulia Casorati (Milan, Italy), and Vincenzo Russo and Antonio Sica as

members of the NIBIT Board of Directors. The session delved into the role of innate immune cells in COVID-19 and cancer.

After defining the correlation between PD-L1[+] c-FLIP[+] monocytes, serum levels of IL-6 and the negative prognosis of patients with pancreatic ductal adenocarcinoma (PDAC)[22], Vincenzo Bronte (Verona, Italy) discussed the inhibitory action elicited by the JAK1 and JAK2 inhibitor 'baricitinib' on the serum levels of inflammatory cytokines (IL-6, IL-1β and TNFα) in COVID-19 patients [23]. Noteworthy, this anti-inflammatory action was paralleled by a rapid recovery of circulating T and B cell frequencies and increased antibody production against the SARS-CoV-2 spike protein. Bronte also discussed the relationship between COVID-19 severity and myelopoietic alterations. By integrating data from single-cell RNA-seq analysis performed in blood and bronco-alveolar lavage fluids with clinical, immunological and functional ex vivo data, they found that naïve lymphoid cells accumulated in the lung, while activated myeloid cells expanded systemically [24]. He also pointed out that circulating low density neutrophils, expressing an immature CEACAM8 and DEFA3 phenotype, suppress T cell proliferation. Further, by scRNA-seq analysis, he unveiled a cluster of immature CD14^{+} monocytes, expressing MPO, PLAC8, IL1R2 and low HLA-DR levels, as an immune-related hallmark of COVID-19 patients [24]. These myeloid changes occur in parallel with compromised health conditions of patients, a condition characterized by an increased frequency of bronchoalveolar lavage (BAL)-associated naïve T cells, in spite of CD4^{+} and CD8^{+} effector memory T cells and are representative of worst clinical outcome.

Karin De Visser (Amsterdam, the Netherlands) discussed about cancer-cell-intrinsic mechanisms that dictate the heterogeneity in systemic neutrophilic inflammation observed in breast cancer patients. She first introduced the concept of inter-patient heterogeneity in immune composition and functions, as well as the negative association between high systemic neutrophil-to-lymphocyte ratios and overall patients survival [25]. Moreover, she revealed that loss of p53 in cancer cells induces secretion of WNT ligands that stimulate tumor-associated macrophages to produce IL-1β, thus driving systemic inflammation. Of relevance, distinct p53 mutations differentially shaped the immune landscape of the host, establishing a 'hot' or 'cold' immune microenvironment. Within this scenario, autophagy is required for response of immune-enriched p53 mutant tumors to anti-PD-1 therapy, while pharmacological or genetic blockade of WNT secretion in p53-null cancer cells reversed macrophage production of IL-1β and subsequent neutrophilic inflammation, resulting in reduced metastasis formation. These observations indicate possible strategies to convert 'cold' p53 mutants into 'hot' mutants.

Session 3 ended with the NIBIT Keynote Lecture by Giorgio Trinchieri (Bethesda, USA). Trinchieri discussed the role of the microbiota in cancer, linking the intestinal metagenome to immunotherapeutic responses [26]. Trinchieri defined the composition of the microbiota in patients undergoing immunotherapy, demonstrating the prevalence of some enterotypes with the disease-free progression phase and with the response to anti-PD-1 immunotherapy. His speech was then focused on the discovery of microbiome-related biomarkers for response prediction and patient stratification; identification of favorable microbiomes for fecal transfer from responder patients and healthy donors; the identification of perturbations (i.e., diet, probiotics, antibiotics) to maintain a favorable composition of the microbiome. These studies led to fecal microbiota transplant in cancer patients to overcome resistance to anti-PDL-1 therapy [27].

Session 4. Cytokines and complement in cancer and COVID-19

Section 4 focuses on cytokines and complement as drivers of both cancer and COVID-19. This was a NIBIT-Società Italiana di Cancerologia (SIC) session and saw Paola Allavena (Milan, Italy), Giulio Cavalli (Milan, Italy) and Claudio Tripodo (Palermo, Italy) as discussants.

Wolf Fridman (Paris, France) discussed the role of B cells in cancer [28], particularly in soft tissue sarcomas, which encompass more than 50 histological subtypes. He underlined the fact that both the clinical presentation and responses to therapy, including immune checkpoint blockers, of patients with different subtypes of soft tissue sarcomas are often heterogenous. Fridman and colleagues investigated gene expression profiles in 608 tumors across subtypes of soft tissue sarcoma, and they established an immune-based classification based on the composition of the tumor microenvironment [29]. They identified five distinct phenotypes: immune-low (A and B), immune-high (D and E), and highly vascularized (C) groups. Of note, they showed that class E was characterized by the presence of tertiary lymphoid structures (TLS), which were particularly enriched in B cells and containing T cells and follicular dendritic cells. In this context, B cells were the strongest prognostic factor, independently of high or low CD8^{+} T cells. Patients belonging to the class E group experienced improved overall survival and a high response rate to PD1 blockade. Thus, B cell-rich TLS could be potential predictors of immunotherapy-sensitive tumors [29]. He showed similar findings in patients affected by melanoma and renal cell carcinoma [30]. Finally, he discussed how the complement cascade affects the clinical outcome of patients affected by clear-cell Renal Cell Carcinoma (ccRCC). He showed that ccRCC tumors infiltrated with high densities of C1q-producing TAMs exhibited an

immunosuppressed microenvironment, characterized by high expression of immune checkpoints (i.e., PD-1, Lag-3, PD-L1, and PD-L2), and experienced poor prognosis [31]. At the end of his presentation, Catherine Sautès-Fridman and Wolf Fridman were awarded the NIBIT Career Award for their outstanding scientific contribution to cancer immunology and for their continuous participation to the NIBIT activities.

Paolo Antonio Ascierto (Napoli, Italy) discussed the analogies between immune-related adverse events in patients undergoing immunotherapy and cytokine storm in patients affected by COVID-19. He discussed emerging data indicating parallels between elevated cytokine levels in COVID-19 and cytokine release syndrome associated with chimeric antigen receptor T cell therapy [32]. In particular, he showed that severe COVID-19 disease is characterized by a respiratory distress syndrome accompanied by elevated levels of several systemic cytokines. This cytokine profile resembles that observed in known inflammatory pathologies such as hemophagocytic lymphohistiocytosis and cytokine release syndrome secondary to chimeric antigen receptor (CAR) T cell therapy. In this context, the modulation of inflammatory cytokines, particularly IL-6, was proposed as a strategy to blunt severe disease. He showed preliminary encouraging data with the anti-IL-6 mAb tocilizumab [33]. These findings have been confirmed in large studies [34, 35]. Moreover, he discussed the use of steroids and other agents, which were successfully used to manage adverse events of cancer patients treated with immunotherapy.

At the end of Session 4, Enzo Galligioni the President of the Pezcoller Foundation, based in Trento (Italy), briefly summarized the many activities promoted by the Foundation in favor of cancer research, which also include sponsoring the prestigious international AACR-Pezcoller prize. NIBIT is very pleased to host a Pezcoller lecture delivered this year by Drew Pardoll (Baltimore, MD, USA) with great audience appreciation. The lecture title—Applying the MANAFEST platform to analyze neoantigen-specific and SARS-CoV2-specific T cell responses at the clonal level—perfectly fitted the meeting aim exploring the immune intersection between cancer and COVID-19. Mutation-Associated NeoAntigens (MANA) are target of antitumor T cells whose antigen-specific repertoire can be identified through functional expansion of specific T cells (FEST) and T cell receptor sequencing of short-term, peptide-stimulated cultures coupled with a bioinformatic platform able to identify antigen-specific clonotypic amplifications [36]. This assay can be adapted for all types of antigens, including those from Corona viruses. Indeed, Pardoll showed that COVID-19 convalescent patients possess SARS-CoV2-spike reactive T cells that do and do not cross-react with spike for seasonal coronaviruses. Their data confirm the existence of unique memory CD4^{+} T cell clonotypes cross-recognizing SARS-CoV-2

and common cold coronaviruses [37]. Work in progress will establish whether such cross-reaction can impact on titer and duration of neutralizing antibodies, the role of cross-reactive CD8 responses and the difference between natural infection and vaccination.

Session 5. Updates in cancer immunotherapy

Relevant updates in cancer immunotherapies are given in Session 5. This session was jointly organized by NIBIT and Alleanza Contro il Cancro (ACC), and it was animated by Concetta Quintarelli (Rome, Italy) for ACC, Ann Leen (Houston, USA) and Marco Bregni (Milan, Italy) and Pier Francesco Ferrucci (Milan, Italy) for the NIBIT Board of Directors.

Susanne Topalian (Baltimore, USA) reviewed the advances in neoadjuvant immunotherapy as an important next step for enhancing the response of locally advanced tumors to immune checkpoint blockade (ICB). The mechanistic rationale suggests a clinical utility not only for debulking tumors preoperatively, but also for enhancing the systemic T cell response to available tumor antigens in respect to the adjuvant setting. This includes the known immunological effects of the PD-1 pathway on T cell priming, effector functions, and exhaustion. Systemic response is predicted by pathological complete responses, to result in enhanced detection and killing of micrometastatic disease, thus avoiding postsurgical relapse. Essentially, the high tumor antigen load in the context of neoadjuvant therapy hypothetically results in presentation to and thus priming of more tumor-specific T cells. There are two potential mechanisms for the enhancement of systemic antitumor T cell immunity after neoadjuvant PD-1 blockade. One could result in the in situ expansion of tumor-specific T cell clones already within the tumor microenvironment, which is largely driven by PDL1– and PDL2–expressing dendritic cells in the tumor. Dendritic cells originating in the tumor pick up tumor antigens and traffic to the tumor-draining lymph nodes, where they present antigens either ineffectively or in a tolerogenic fashion to tumor-specific T cells. PD-1 blockade could also act at this point, enhancing productive stimulation of tumor-specific T cells or partially reversing tolerance induction.

At present, more than 100 clinical neoadjuvant trials using anti-PD-1 s, as monotherapy or in combination, are ongoing or planned in different diseases, which may help to assign patients to postsurgical observation or intervention depending on the degree of pathological response at surgery. Furthermore, pathological assessment criteria of response could then act as a surrogate marker for relapse-free and overall survival. Interestingly, tumors resected after neoadjuvant immunotherapy provide sufficient materials for

in-depth scientific analysis in order to better understand the mechanisms of response and resistance, revealing pathways and molecules that can be cotargeted in new treatment combinations to increase the efficacy of this approach [38].

A pressing question in the field of cancer immunotherapy is whether CAR T cell therapy can be made effective in treating solid tumors. This unique challenge includes three steps for engineered cells to reach their goal: finding, entering, and surviving in the tumor. Cliona Rooney (Houston, USA) explored the current landscape, starting from how to solve poor in vivo persistence of CAR T cells, and how to overcome multiple inhibitory pathways simultaneously without affecting safety. A plethora of strategies to increase persistence in a tolerable manner are being evaluated as costimulatory signal integration, alleviation of inhibitory signaling, and selection of optimal T cell subsets. Probably, CAR-T therapies require further engineering to achieve their potential against solid tumors. Facilitating cytokine signaling appears to be essential in achieving better responses. The structure and costimulatory domains chosen for the CAR play an important role in their overall function in the tumor microenvironment, and "armored" CARs that secrete cytokines and third- and fourth-generation CARs with multiple costimulatory domains offer ways to enhance their function. Moreover, the immunosuppressive tumor microenvironment has implications for T cell function in terms of differentiation and exhaustion. Combining CARs with ICB or depletion of other suppressive factors in the microenvironment has shown very promising results. The use of dual CAR designs that recognize multiple antigens at once and local administration of CAR T cells are both strategies that have been used to solve the hurdle of localization to the tumor. The potentially unchecked proliferation and potency raises the question of whether the simultaneous combination of enhancements will prove safe, necessitating continued monitoring and efficient schemes to eventually switch off these engineered cells. New small adaptive trials are warranted to address these relevant issues [39].

Session 6: At the forefront of technology

The last session of the meeting was dedicated to novel technological strategies in the field of cancer immunology and immunotherapy, with a discussion panel from the NIBIT Board of Directors: Paola Nisticò, Massimo Di Nicola, Vincenzo Bronte e Antonio Rosato.

Raza Ali (Cambridge, UK) reported on the use of imaging mass cytometry as a tool in advanced tumor pathology to dissect the complexity of multicellular ecosystems such as tumor microenvironment (TME). In particular, human formalin-fixed paraffin-embedded breast cancer tissue samples have been analyzed, and in situ single-cell phenotyping

uncovered fourteen TME modules associated with distinct clinical outcomes [40]. Therefore, multiparametric spatial biomarkers have the potential to become part of precision immuno-oncology.

Cristophe Mollet (Promega Corporation, Madison, USA) presented data on the relevance of testing microsatellite instability (MSI) as a prognostic biomarker in colorectal cancer and on the evidence that microsatellite instability-high tumors, irrespective of their primary location, respond better to immune checkpoint inhibitor therapies. According to the "European Society for Medical Oncology (ESMO) recommendations on microsatellite instability testing for immunotherapy in cancer" released in 2019 [41], the first test of choice is immunohistochemistry analysis of four mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2); in case of doubt, confirmatory molecular analysis by PCR amplification of at least five specific markers is mandatory. Combinatory use of both methods can increase sensitivity to over 99%. Next-generation sequencing represents an increasingly adopted type of molecular test to assess MSI which allows concomitant determination of tumor mutational burden.

Dario Armando Leone (Miltenyi Biotec, Bergisch Gladbach, Germany) gave an overview of the main integrated solutions to identify biomarkers for cancer and inflammation therapy developed by Miltenyi Biotec. These include the MACSima Imaging Platform, a fully automated iterative fluorescent staining system, which uses a broad spectrum of recombinant ready-to-use fluorochrome-conjugated antibodies, permitting the analysis of hundreds of markers on a single fixed sample. The MACSQuant Tyto cell sorter that operates with a closed, disposable cartridge with a microfluidic chip to collect the sorted and the negative fractions, reducing the risk of contamination [42]. The microchip-based technology reduces cell sorter-induced-cell stress, resulting in high cell viabilities and preserved cell functionality. To visualize the identified biomarkers, Miltenyi Biotec proposes the UltraMicroscope Blaze for imaging large cleared samples at subcellular resolution in 3D.

Alexandre Darmoise (NanoString, Seattle, USA) introduced the GeoMx Digital Spatial Profiler for proteomics and transcriptomics analyses with preservation of the spatial context in formalin-fixed paraffin-embedded tissues, fresh frozen, and fixed frozen tissues [43]. The technology combines immunofluorescence with digital barcoded antibodies or oligonucleotides, making high-plex protein or RNA expression profiling with spatial correlation possible. The analysis can be performed on user-defined regions of interests within the sample, such as areas enriched with normal epithelial, tumor, stromal or immune cells. The GeoMx Digital Spatial Profiler workflow is flexible, enabling analysis from selected targeted oncology and immune pathways to the whole transcriptome. This technique has the potential to

support the prediction of prognosis [44], offering an unprecedented view of the composition, function and location of immune cells within the microenvironment in primary tumors and in sentinel lymph nodes [45].

At the end of the last session of the meeting, recipients of the NIBIT awards were announced by Mario Colombo and Pier Francesco Ferrucci. NIBIT Awards were sponsored this year by the Fondazione Grazia Focacci. Claudia Enriquez (Milan, Italy) won the NIBIT Basic Science Award for her work on the role of the matrix protein SPARC in neuroendocrine differentiation of prostate cancer. Abbass Darwich (Milan, Italy) received the NIBIT Translational Science Award for his work on chitinase 3 like-1 protein as a soluble immune checkpoint inhibitor of NK function in cancer. For her work on the impact of lipid metabolism in non-mall lung cancer patients treated with immune checkpoint blockade, Giulia Galli (Milan, Italy) won the NIBIT Clinical Science Award.

Conclusions

The XVIII NIBIT meeting although narrowed by the web format was well attended with satisfaction for the scientific sections in which speakers and discussion panelists had a live chat for discussion also delivering audience write messages. Of course, we missed all beauty and social activities that were planned in Padua like the Giotto painted Scrovegni Chappell to cite one, but it will retain the good memory of a vibrant meeting to be repeated, as a format, should the COVID-19 pandemic will require participation from remote.

Declarations

Conflict of interest PFF has received honorarium for advisory board participation from Bristol Meyers Squibb, Novartis, MSD, Pierre Fabre and Roche. VB reports relationship with IoBiotech Aps and Codiak BioScience (personal fees), outside the submitted work. All other authors have no conflict of interest to declare.

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