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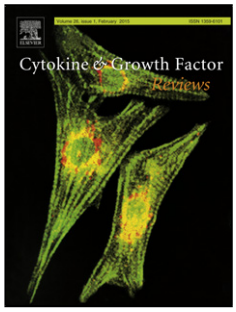

Anticancer Innovative Therapy Congress: Highlights from the 10th Anniversary Edition

Francesca De Santis, Giovanni Fucà, Dirk Schadendorf, Alberto Mantovani, Luca Magnani, Michael Lisanti, Stephen Pettitt, Matteo Bellone, Giannino Del Sal, Saverio Minucci, Alexander Eggermont, Paolo Bruzzi, Silvio Bicciato, Pierfranco Conte, Roberta Noberini, John Hiscott, Filippo De Braud, Michele Del Vecchio, Massimo Di Nicola

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HIGHLIGHTS

- Epigenetics, and in particular, chromatin modifiers;
- Cancer metabolism;
- Cancer stem cells;
- Tumor cell signaling;
- The immune system.

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1. Abstract

During the Tenth Edition of the Annual Congress on "Anticancer Innovative Therapy" [Milan, 23/24 January 2020], experts in the fields of immuno-oncology, epigenetics, tumor cell signaling, and cancer metabolism shared their latest knowledge on the roles of i] epigenetics, and in particular, chromatin modifiers, ii] cancer metabolism, iii] cancer stem cells [CSCs], iv] tumor cell signaling, and iv] the immune system. The novel therapeutic approaches presented included epigenetic drugs, cell cycle inhibitors combined with ICB, antibiotics and other off-label drugs, small-molecules active against CSCs, liposome-delivered miRNAs, tumor-specific CAR-T cells, and T-cell–based immunotherapy. Moreover, important evidence on possible mechanisms of resistance to these innovative therapies were also discussed, in particular with respect to resistance to ICB. Overall, this conference provided scientists and clinicians with a broad overview of future challenges and hopes to improve cancer treatment reasonably in the medium-short term.

2. Introduction

A national and international audience of experts in immuno-oncology and cancer cell signaling gathered for the tenth annual edition of the "Milan Congress on Anticancer Innovative Therapy", organized by Fondazione IRCCS Istituto Nazionale dei Tumori (Fondazione IRCCS INT), in Milan, Italy, on 23/24 January, 2020. The conference presented a detailed overview of the most recent discoveries in the field of immune-oncology and cancer cell signaling, providing a great opportunity to deeply understand new challenges and scenarios of the immunotherapy and targeted therapy. The opening session included novel targets in anticancer treatment, headed by D. Schadendorf, and a lecture by A. Mantovani, entitled "Innate immunity, inflammation and cancer: double edged swords". Session I focused on pre-clinical evidences and new targets, with the main topics of breast cancer genomic analyses, pre-clinical and clinical studies aimed at eradicating cancer stem

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cells (CSCs) with anti-mitochondrial drugs, and synthetic lethal targeting of DNA damage response. After the discussion, a NIBIT (Italian Network for the Tumor Biological Therapy) lecture by M. Bellone, addressed the topic of novel and diagnostic targets in the crosstalk between prostate cancer stem-like cells and the tumor microenvironment. A keynote lecture by G. Del Sal, about the role of p53 at the crossroads of mechano-signaling and metabolic pathways in cancer, closed the morning table.

Session II was mainly based on clinical translation. It started with novel insights into tumor cell metabolism by S. Minucci, and continued with the adjuvant immunotherapy in high-risk melanoma patients by A. Eggermont, new targets discoveries under the statistical point of view by P. Bruzzi, and finally, an extensive overview of integrative bioinformatics in precision oncology by S. Bicciato. In conclusion, P. Conte discussed the present and the future of targeted therapies. The award to the best abstract presented by young researcher closed the proceedings of the conference. Overall, clinicians and basic and translational scientists provided the audience with a broad overview of data supporting the promise of using combinations of immunotherapies and standard treatments, which still presents an intriguing challenge as well as a real opportunity for future therapeutic approaches.

3. Opening Session

3.1 Novel targets in anticancer treatment

D. Schadendorf (Essen, Germany) challenged the current direction of immunotherapy and discussed new approaches aimed at improving the cure rate of patients. The impact of cancer immunotherapy on clinical cancer care is rapidly growing. However, different immunotherapies have distinct problems in cancer–immune system interactions. Schadendorf illustrated how effective adjuvant options would significantly contribute to the efficiency of these therapies, thereby increasing the survival rates. A framework to improve biomarker research is represented by the cancer immunogram [1]. Specifically, the outcome of cancer–immune interactions are based on a number of unrelated parameters, such as tumor “foreignness” and T-cell–inhibitory mechanisms (Fig. 1).

The information required for this analysis may be obtained from the combination of tumor genomics, immunohistochemistry, and standard assays using the peripheral blood compartment and can differ greatly among patients. Such measurements will be useful to determine which states of the cancer immunogram are most commonly inhibited, both during natural cancer-immune interaction and upon immunotherapy.

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A cancer immunogram should evolve, for instance to incorporate new biomarkers that reflect the capacity for T-cell priming. Several biomarkers for immunotherapy response have been proposed. These biomarkers need to undergo further analyses and larger validations before their translation into clinical practice. A more homogeneous collection of tissue samples in prospective trials, and incorporation of this bias into the interpretation of biomarkers, are warranted. Additionally, some biomarkers may be more dynamic than others and should be monitored closely [2].

3.2 Innate immunity, inflammation, and cancer: Double-edged swords

A. Mantovani (Milan, Italy), presented the lecture for the 10th conference anniversary. The talk was centered on a topic largely discussed in the last 10 years regarding the close relation between inflammation and cancer. In particular, two kinds of pathway categories responsible for this correlation have been identified: the intrinsic one and the extrinsic one. In the intrinsic pathway, genetic events causing neoplasia initiate the expression of inflammation-related programs that guide the construction of an inflammatory microenvironment [3,4]. In contrast, in the extrinsic pathway, inflammatory conditions facilitate the development of cancer. Chronic inflammation triggers cancer risk through infections (e.g. *Helicobacter pylori* for gastric cancer and mucosal lymphoma; papilloma virus, cervical cancer; and hepatitis viruses, liver carcinoma), autoimmune diseases (e.g. inflammatory bowel disease for colon cancer) and inflammatory conditions of uncertain origin (e.g. prostatitis for prostate cancer). From this perspective**,** cancer-related inflammation (CRI) is a key component of tumors and represents the seventh hallmark of cancer (Fig. 2) as well as a target for innovative therapeutic strategies and prevention [5,6]. Different pathways are involved in the interconnection between inflammation and cancer, both as positive as well as negative modulators. Macrophages are a major component of the leukocyte infiltrate that is present in widely different amounts in all tumors [8]. Tumor-associated macrophages (TAMs) are a paradigm for leukocytes and inflammatory mediators present in the tumor context and play a dominant role as orchestrators of CRI. TAMs influence tumor cell–intrinsic properties as well as the tumor microenvironment (Fig. 2). Dissecting TAM diversity at the single cell level now represents a large percentage of the current challenges.

4. Session I: Preclinical evidence and new targets

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4.1 Diving into the dark matter of the breast cancer genome

L. Magnani (London, UK) opened Session I by presenting data about the role of the non-coding genome and its potential contribution to drive transcriptional aberrations in breast cancer patients. From a clinical point of view, the question arises: do both coding [9] and non-coding mutations [10,11] represent a driver element toward *de novo* and/or acquired resistance? The outgrowth of primary luminal breast cancer (BC) is driven by a non-mutated estrogen receptor α (ERα), and all patients receive adjuvant endocrine therapy (ET) after curative surgery. This strategy significantly delays clinical relapse but does not abrogate it completely, as about ~3% of the patients come back with overt relapse each year, which inevitably leads to further metastatic development [12,13]. The frequency of relapse remains constant up to 20 years after surgery, making ET resistance the most critical clinical problem for the management of these patients [14]. Recent developments in next-generation sequencing (NGS) revealed that tumors are genetically heterogeneous [15,16], and in some cancer types, heterogeneity correlates with the likelihood of recurrence and development of drug resistance [17,18]. In addition, despite recent studies showing that the majority of the genetic lesions in BC are accumulated during the early phases of tumor development [14,19], researchers have failed to identify any major driver associated to metastasis or resistance, with the exception of a minor fraction of cases showing either ESR1 mutations or CYP19A1 amplification [20,21]. Nonetheless, the transcriptomes of the resistant cells are profoundly heterogeneous and differ from those of the primary tumor [22,23], suggesting a contribution of non-genetic mechanisms [10]. Magnani presented new data obtained through a wide range of techniques, including a combination of live cell imaging, single-cell RNA-sequencing [scRNA-seq], and machine learning, to dissect the phenotypic heterogeneity and plasticity of ERα-positive BC, and leverage this information to identify a subpopulation of rare, pre-adapted cells both *in vitro* and *in vivo*. This model would reconcile why ET are sometime effective for downstaging neo-adjuvant patients, but fail to clear micro-metastatic disease. Nevertheless, single-cell lineage-tracing approaches coupling unambiguous identification of clones to transcriptome mapping are needed to get definitive proof that the progeny of PA cells are those that eventually acquire full resistance. How this bottleneck affects the progression of the tumor also requires further investigation. Future studies on the necessary steps towards resistance, and the timing of occurrence

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during treatment, must be carried out in order to expose potential vulnerabilities of these quiescent cells.

4.2 Eradicating cancer stem cells (CSCs) with anti-mitochondrial therapeutics: Pre-clinical evidence and clinical trials

M. Lisanti (University of Salford, UK) presented data on new possible approaches to counteract CSCs. Mitochondria are the energetic hubs of a cell, and their function and homeostasis are fundamental for cell survival. The "reverse Warburg effect" theory states that cancer cell mitochondria oxidatively metabolize nutrients (i.e. lactate, ketones) provided by adjacent stromal cells that are undergoing aerobic glycolysis [24], the so-called "two-compartment tumor metabolism" model [25]. This metabolic coupling supports mitochondrial ATP production via oxidative phosphorylation (OXPHOS) in the anabolic cancer cells, promoting a stemness phenotype, metastatic potential, and tumor growth [26]. Given the key role of mitochondrial metabolism in CSC maintenance and drug resistance [27], the Lisanti group was interested in finding new approaches to counteract CSC by targeting mitochondria. In particular, treatment with oligomycin A [a known inhibitor of the mitochondrial ATP synthase] produced a detrimental effect on the mammosphere-forming efficiency [28]. Indeed, cells with high mitochondrial mass ("mito-high", as detected by the MitoTracker deep red dye) more closely resembled features of CSCs, such as having higher ALDH activity, mammosphere-formation capacity, tumorigenic potential, and a chemo-resistant phenotype [29]. The possibility to isolate the most therapy-resistant components of a tumor represents a powerful tool for investigating drug sensitivity in the context of personalized cancer treatment. Different classes of FDA-approved antibiotics are known to inhibit mitochondrial biogenesis as an "off-target" effect. Lisanti and collaborators have suggested re-purposing antibiotics (i.e. doxycycline) for CSC eradication as a novel cost-effective cancer treatment approach of broad applicability [30]. Recent data revealed the existence of a subset of CSCs defined as "energetic CSCs", characterized by higher metabolic activity (higher mitochondrial OXPHOS), hyper-proliferation, enrichment in stem cell features (ALDH activity, sphere-forming efficiency, mitochondrial mass), and dependence on a 3D micro-environment. Given these peculiar characteristics, the "energetic CSC" subpopulation could be pharmacologically targeted with OXPHOS inhibitors (i.e. DPI) or CDK4/6 inhibitors (i.e. ribociclib) [31]. Overall, these novel approaches targeting CSC

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mitochondria with repurposed drugs pave the way to more affordable as well as more effective and durable treatments.

4.3 Synthetic lethal targeting of the DNA damage response: PARP inhibitors and beyond

The DNA damage response comprises a diverse range of pathways that detect, signal, and repair damage to the DNA. Some cancers have defects in the DNA damage response, such as BRCA1/2-deficient tumors, which are defective in homologous recombination (HR). S. Pettitt (London, UK), previously showed that BRCA1/2–defective cells are exquisitely sensitive to inhibition of poly (ADP-ribose) polymerase (PARP). However, development of resistance has been observed in the clinic, and a number of laboratory studies have also investigated mechanisms of PARP inhibitor resistance, suggesting new therapeutics aimed at specifically targeting resistance mechanisms. The most well-described clinical mechanism of PARP inhibitor resistance is reversion mutations that restore function of the defective HR gene – in most cases BRCA1 or BRCA2. Analysis of these mutations indicated that there may be differences in the DNA repair pathways responsible for generating reversions – BRCA1 reversions have a greater number of substitution and wild-type reversions than BRCA2 reversions, which tend to be larger deletions with more extensive microhomology at junctions. Other mechanisms of PARP inhibitor resistance have also been previously described, including overexpression of drug efflux pumps that export PARPi from the cell, and loss of 53BP1 in BRCA1 mutant cells, which restores resection at DNA ends and allows HR to proceed even in the absence of BRCA1. Pettitt and colleagues carried out genome-wide CRISPR-Cas9 mutagenesis screens for PARPi resistance in the BRCA1 mutant breast cancer cell line SUM149. The major hit from these screens was loss of PARP1 itself, consistent with a trapping mechanism of toxicity in which inhibited PARP1 remains bound to sites of DNA damage and cannot complete its catalytic cycle. Importantly, development of PARP inhibitor resistance via these different mechanisms leads to different secondary vulnerabilities. Mechanisms restoring HR will likely cause cross-resistance to platinum, whereas PARPi-specific methods [such as drug efflux or PARP1 mutations] may retain platinum sensitivity. These results have implications for the management of clinical PARP inhibitor resistance.

4.4 NIBIT LECTURE: Novel diagnostic and therapeutic targets in the crosstalk between prostate cancer stem-like cells and the tumor microenvironment

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In 2020, more than 190,000 new cases of prostate cancer, and more than 72,000 related deaths, are expected to have occurred in the United States, making prostate cancer the most frequently diagnosed cancer, and the second-leading cause of cancer-related death in men, (following basal cell and squamous cell skin cancers) and in situ carcinomas (following urinary bladder) [32]. In the last 30 years, prostate-specific antigen screening has likely been responsible for the observed 50% decrease in cases of metastatic prostate cancer [33]. However, at first diagnosis, more than 10% of contemporary prostate cancer patients already show lymph node involvement, and about 5% have distant metastasis [33]. Additionally, more than 10% of patients who have undergone radical prostatectomy for prostate cancer will have recurrence within about 2 years after surgery, many of whom will eventually die of the disease [34]. Altogether, these data highlight the urgent unmet clinical needs of identification and validation of novel markers, and using imaging tools for early diagnosis of metastatic prostate cancer are. These arguments were the focus the 2020 NIBIT Lecture, given by M. Bellone (Milan, Italy), on the biology of prostate cancer and how to identify novel markers and imaging tools for early diagnosis of metastatic prostate cancer. According to the model of hierarchical evolution in cancer, cancer stem-like cells (CSCs) are the cells endowed with tumorigenic potential within the tumor bulk, and they drive tumor growth, metastasis, and relapse [35]. The same might apply to prostate cancer [36]. Bellone and colleagues have recently reported that phenotypically and functionally identical prostate CSCs can be found both in oncogene-driven prostate intraepithelial neoplasia [PIN] lesions and in histopathologically negative prostate draining lymph nodes in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice at about 10-12 weeks of age [37], thus demonstrating that lymph node invasion may already occur at the stage of PIN in TRAMP mice. Prostate CSCs, which are both target of adaptive and innate immunity [38], migrate early on to the draining lymph nodes, where they generate an immunosuppressive environment that eventually favors CSC persistence [39], likely in a quiescent state. Importantly, TNC is expressed in advanced PIN lesions and in metastatic lymph nodes also in humans. Moreover, the Bellone group found that galectin 3 [Gal-3], an extracellular matrix glycan-binding protein that has been described to exert immunosuppressive and pro-tumor functions [40], is over-expressed in CSCs from PIN lesions [41] and contributes to prostate CSC-mediated immune suppression. Thus, Gal-3 is as an additional key molecule in prostate CSCs, and it represents a potential biological

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marker and a therapeutic target already in the early phases of prostate cancer progression and metastasis. Taken together, these findings support a role for prostate CSCs in lymph node metastasis, and they identify actionable molecules in early prostate cancer.

4.5 KEYNOTE LECTURE: p53 at the crossroads of mechano-signaling, and metabolic pathways in cancer

G. Del Sal (Trieste, Italy) provided the audience with a keynote lecture regarding novel aspects of p53 role in cancer. Although having been the object of extensive studies, the p53 network, both wild-type (wt) and mutant, retains relevant aspects that are still unclear. *TP53*, which encodes the p53 protein, is the most frequently altered gene in human tumors [42]. Its mutations are associated with poor prognosis in several cancers [42]. Furthermore, germline *TP53* mutations are causative of the Li Fraumeni (LF) syndrome, a rare familial cancer predisposition [43].

The majority of *TP53* mutations are missense, producing single-residue substitutions within the protein's DNA-binding domain. p53 missense mutant proteins [mutp53] lose the ability to activate canonical p53 target genes, and some mutants exert trans-dominant repression over the wild-type counterpart. Beyond this, cancer cells appear to gain selective advantages by retaining only the mutant form of the p53 protein. On this basis, specific missense p53 mutants have been reported to subvert crucial cellular pathways and to foster cancer cell proliferation and survival, and to promote invasion, migration, metastasis, and chemoresistance [44, 45]. Several pieces of evidence indicate that mutp53 provides cancer cells with the ability to face challenging conditions related to tumorigenesis, such as hyperproliferation-related DNA damage, oxidative and proteotoxic stress, nutrient fluctuations, physical constraints, stromal cues, and the anti-tumor immune response. Reprogramming of cell metabolism is a hallmark of cancer, required to sustain tumor cells' biosynthetic needs for continuous growth and proliferation [46]. Not surprisingly, cell metabolism is affected by multiple oncogenic conditions, including expression of mutp53. One widespread metabolic adaptation of cancer cells is represented by increased glucose uptake accompanied by aerobic glycolysis (known as Warburg effect), which feeds tumor growth in hypoxic conditions and contributes to suppressing immune surveillance through extracellular acidification [47]. Depending on the specific context, mutp53 can also promote oxidative phosphorylation, as shown in pre-neoplastic thymus and spleen of LF mouse models and in muscles of LF patients [48]. Moreover, many solid tumors undergo alterations

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of lipid metabolism; in particular, synergistic interactions of mutp53 with SREBPs, which are master regulators of fatty acids and cholesterol biosynthesis, lead to transcriptional induction of the mevalonate pathway (MVP) [49]. Several oncogenic outcomes stem from this activity of mutp53, including dismantling of normal mammary tissue architecture to facilitate tumor invasion [49], and promoting the aberrant mechano-responsiveness of tumor cells. The ability of cancer cells to actively shape a permissive microenvironment is thus crucial for cancer progression. Increasing evidence indicates that mutp53 can remodel the tumor microenvironment, and thereby enhancing cancer cell adaptation to hostile extracellular conditions by favoring tumor neo-angiogenesis, through the upregulation of ID4, a member of the ID family proteins. The impact of mutp53 on the inflammatory tumor microenvironment is largely dependent on a functional interaction with the transcriptional regulator NF-κB. In particular, mutp53 is able to promote p65 RelA nuclear translocation and to amplify NF-κB transcriptional activity in cancer cells treated with TNFα [50,51].
To conclude, the numerous *TP53* missense mutations have several functional effects in different tumor contexts and specific multi-omic approaches, and their precise characterization is still needed to determine a panel of ideal therapeutic targets.

5. Session II: Clinical translation

5.1 Novel insights into tumor cell metabolism

S. Minucci (Milan, Italy) opened the second session of the conference with a talk covering i) metabolic plasticity of tumor cells, and ii) the lysine-specific demethylase 1 (LSD1/KDM1A) small molecule inhibitors and their roles in different cancer cell types. Tumor cells may adapt to metabolic challenges by alternating between glycolysis and oxidative phosphorylation (OXPHOS). A strategy to target metabolic plasticity is to combine intermittent fasting (IF), a clinically feasible approach that reduces glucose availability, with the OXPHOS inhibitor metformin. In particular, in pre-clinical models, metformin impaired tumor growth only when administered during fasting-induced hypoglycemia. In particular, the Breakfast Trial is currently active at Istituto Nazionale dei Tumori (INT) in Milan under the supervision of Minucci, F. De Braud and C. Vernieri. It is investigating the efficacy of a fasting-mimicking diet together with chemotherapy and a metformin combination treatment in triple-negative breast cancer (TNBC) patients. The second part of the talk dealt with the role of the histone demethylase LSD1 and the deregulation of its activity in several tumors, including leukemias, providing the rationale for the clinical use of LSD1 inhibitors. In

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acute promyelocytic leukemia [APL], pharmacological doses of retinoic acid (RA) induce differentiation of APL cells, triggering degradation of the PML-RAR oncogene. APL cells are resistant to LSD1 inhibition or knockout, but targeting LSD1 sensitizes them to physiological doses of RA without altering of PML-RAR levels, and extends survival of leukemic mice upon RA treatment [52]. In addition, pharmacological inhibition of CDK4/6, following palbociclib administration, sensitizes to LSD1 inhibition in a p21-dependent fashion. Thus, LSD1 has emerged as an interesting target for cancer therapy, and LSD1 inhibitors have entered clinical trials for treatment of several cancer types, including acute myeloid leukemia (AML). Only a minority of AML cells are sensitive to LSD1 inhibition as single treatment [53]; a strong cooperative action of LSD1i and RA can be, however, measured even in those AML subtypes that are not responsive to either drug alone [54], justifying a clinical investigation of this approach.

5.2 Immunotherapy in melanoma, from advanced to adjuvant to neoadjuvant

After an almost 20-year era of interferon (IFN)-based adjuvant therapies with marginal benefits for patients with high-risk stage II–III melanoma, A. Eggermont (Gustave Roussy, France), showed that a new epoch of effective adjuvant therapies for this disease has just begun. Following the FDA approval of a number of therapies for advanced-stage melanoma [55], the results from four randomized controlled trials (RCT) demonstrated substantial improvements in recurrence-free survival [RFS] in patients with resected melanoma who received adjuvant ipilimumab [56], nivolumab [57], dabrafenib plus trametinib [58], or pembrolizumab [59]—which have given strikingly consistent outcomes. Indeed, ipilimumab, nivolumab, or dabrafenib plus trametinib have been approved in the adjuvant setting. Ipilimumab has a modest but statistically significant RFS benefit. At 5 years, ipilimumab treatment increased both RFS and overall survival (OS) by 11%. Nivolumab, pembrolizumab, or dabrafenib plus trametinib treatment all seem to provide a greater degree of clinical benefit together with a significantly better toxicity profile than ipilimumab. Of note, data for nivolumab and pembrolizumab are from interim analyses [57,59], with most patients censored after 12–18 months, whereas data for the ipilimumab and dabrafenib plus trametinib [58] are reported after the pre-specified number of RFS events had occurred. Thus, a new adjuvant therapy landscape for high-risk melanoma has emerged with the advancement of pembrolizumab and nivolumab into this setting, with the additional option of dabrafenib–trametinib for BRAFV600E/K-mutant disease. The effects of these treatments

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on RFS are so substantial that OS benefits are expected. Moreover, the neoadjuvant use of pembrolizumab, nivolumab ± ipilimumab, or combo-targeted therapy can permit a less demolitive surgical approach, by improving locoregional disease control and impacting favorably on the RFS and desirably OS. A. Eggermont concluded his talk evidencing how adjuvant therapy with anti-PD-1 antibodies [nivolumab or pembrolizumab], regardless of mutational status, or with dabrafenib plus trametinib for patients with BRAFV600E/K-mutant disease, currently represent the standard of care in stage III and stage IV NED (with no evidence of disease after metastasectomy, only for nivolumab) melanomas.

5.3 New targets: new endpoints?

P. Bruzzi (Genova, Italy), provided new insights regarding the better understanding of cancer biology and the shift in cancer drug development. This requires novel approaches in clinical trial design by academia and industry, and development of new assessment tools by regulatory authorities. Pharmaceutical industry is developing new targeting agents and generating many clinical studies, including target combinations. This requires improved operational efficiency by development of innovative trial designs, strategies for early-stage decision making, and early selection of candidate drugs with a high likelihood of success.
In particular, it is necessary to have new endpoints to assess the activity of a drug, to predict the outcome and the efficacy of a treatment, and to measure the efficacy of a treatment. From this point of view, endpoints could be defined as qualitative or quantitative variables used to assess the consequences of an exposure or an intervention on a group of study participants.
In addition, patient awareness and ethical considerations necessitate that agents will be rapidly available to patients. Regulatory authorities such as the European Medicine Agency and national agencies recognize that these changes require a different attitude towards benefit–risk analysis for drug approval. The gold standard of randomized confirmatory phase III trials is not always ethical or feasible when developing drugs for treatment of small cancer populations. Alternative strategies comprise accelerated approval via conditional marketing approval, which can be granted in the EU based on small, non-randomized phase II trials.
In conclusion, novel innovative trial designs, with the efforts of pharmaceutical industry and regulatory authorities to deal with the paradigm shift, are needed.

5.4 Integrative bioinformatics in precision oncology

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S. Bicciato (Modena, Italy) introduced the role of bioinformatics in precision systems medicine, highlighting that it plays an essential role by providing the elements required to process patients´ multi-omics profiles and then to integrate these profiles with clinical data; this is required to gain a mechanistic understanding of diseases, which will facilitate more personalized treatments. Integrating heterogeneous molecular data, captured by different technologies under different conditions, is a fundamental component of -omics and provides a convergence framework, which integrates experiments and enables technologies and computation to generate new ideas, discoveries, and innovation. In general, integration of multi-dimensional data from different datasets, bio-samples, or modalities can be broadly categorized into three approaches: concatenation-based, which combines multiple data for each sample before performing the analysis; transformation-based, which first transforms each data type into an intermediate form and then merges all transformations to perform the analysis; or model-based integration, in which multiple models are generated using the different types of data, which are then combined to generate a final model. These approaches were described using examples of their application in the integrative analysis of different -omics data obtained from tumor profiling experiments.

Finally, Bicciato discussed the integrative analysis of -omics data generated by single-cell technologies. Single-cell genomics is a pristine, exploding field that is flooding biologists with a new wave of data, each with its own specificities in terms of pre-processing, normalization, and downstream analysis. In this context, data integration is emerging as an essential component, although the combination of different single-cell genomic signals is computationally challenging for experimental, technical, and biological reasons. As for bulk experiments, multi-view single-cell data are high-dimensional and comprise many distinct yet interdependent signals, each with specific characteristics, dimensionality, and noise. Across modalities, data are commonly collected in different genomic locations (genes, genomic regions), scales, and formats (levels, states). Moreover, datasets vary widely in the number and type of profiled cells, for investigated biological samples (e.g., treatments, individuals) and for technical aspects (such as sample processing, library preparation, and sequencing depth). All these heterogeneities pose additional computational challenges to the application of methods previously developed for bulk experiments. An overview of computational methods for the integrative analysis of single-cell data was presented in the context of different types of single-cell data integration problems.

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5.5 Targeted therapies: present and future

P. Conte (Padua, Italy) addressed the present and future targeted therapies in BC. Targeted therapies affecting specific molecular target that is expressed preferentially by neoplastic cells have been shown to block cancer growth. Current targets are represented by cell-surface trans-membrane proteins, intracellular proteins, and growth factors. Targeted therapies exist for most commonly diagnosed types of human cancers, and they are often combined with chemotherapy but sometimes used as a monotherapy. Targeted therapies are emerging topics in clinical cancer research, since it is now commonly accepted that hitting the right target could be equivalent to finding the right patient. However**,** targeted therapies in BC has fueled several challenges, such as the comparison between immunohistochemical (IHC) and genomic analyses, as well as between primary tumor *vs* metastasis, PD-L1 testing strategy, the evaluation of novel immune biomarkers (TILs, immune signatures, microbioma), BRCA testing, and multigene vs multiple single gene testing. The analysis of these aspects is not easy due to the occurrence of a huge amounts of variables. Through an analysis of several clinical trials, Conte defined a roadmap for facing all the challenges of current targeted therapy in BC.

6. **Award for the best abstract:** ***Epi-proteomics profiling of clinical samples reveals novel hallmarks of cancer and biomarkers for breast cancer patient stratification***

Although cancer has been traditionally considered to be the result of an accumulation of genetic defects, striking evidence has now shown that epigenetic changes also contribute to cancer initiation and progression, as discussed in the abstract from R. Noberini (Milan, Italy), which was honored as the best abstract. Aberrations in histone post-translational modifications (PTMs) are hallmarks of cancer and can be prognostic markers, and many inhibitors of histone modifying enzymes are currently being test for use as cancer treatments. Therefore, profiling histone PTMs in cancer could have important implications for the discovery of biomarkers for patient stratification as well as for novel epigenetic targets. Noberini and co-workers developed a battery of mass spectrometry–based approaches, with which they then profiled more than 200 cancer patient tissues of different origin. By comparing tumor and normal tissues for various cancer models, they identified histone modification changes that represent general hallmarks of cancer, in addition to those previously reported. Moreover, they carried out the MS- profiling of histone PTMs in different breast cancer subtypes, with a special focus on TNBCs. This allowed them to

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identify a panel of epigenetic marks that distinguish TNBCs from other BC subtypes, and that differentiate TNBC patients with and without relapse after chemotherapy. The histone marks could represent potential biomarkers that are useful for BC patient stratification as well as for prediction of response to therapy. Noberini and co-workers are now currently intersecting histone PTM profiling data with global proteomics information and ChIP-seq analyses, to investigate potential epigenetic mechanisms underlying BC in general, and TNBC in particular, and to identify possible novel epigenetic pathways targetable for therapy.

7. Conclusion

Cancer research is taking important steps towards understanding the molecular mechanisms involved in cancer development, maintenance, invasiveness, and metastasis, as well as the mechanisms underlying anti-tumor therapy resistance. Tumors are no longer considered as merely “compositions of cancer cells”; rather, it is increasing evident that the tumor microenvironment [TME], including stromal cells as well as the anti-tumor/pro-tumor immune system, plays a critical and regulatory role. Novel therapeutic approaches should therefore take into account all these aspects to succeed in improving patient care.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure legends

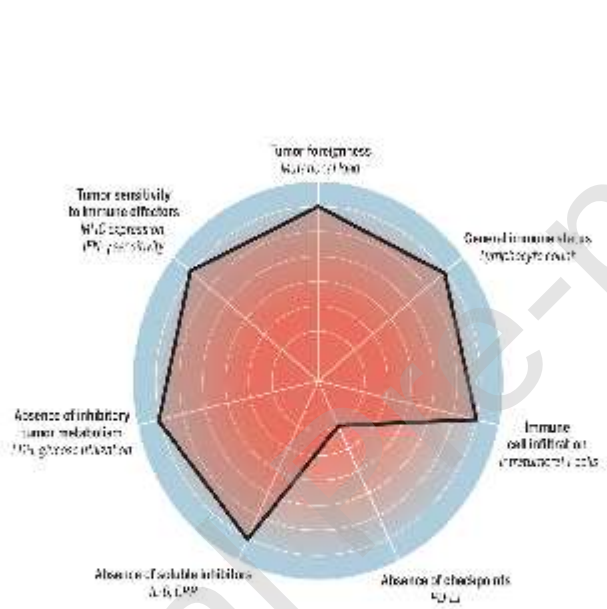

Figure 1. *The "Cancer Immunogram".* Radar plot depicting the seven parameters, that characterize aspects of cancer–immune interactions for which biomarkers have been identified or are plausible. Taken from Blank UC et al, Science 2016 May 6;352[6286]:658-60.

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Figure 2

Figure 2. *Inflammation as the seventh hallmark of cancer.* Taken from Hanahan and Weinberg [7] and Mantovani [4]

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