ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN CANCER HORIZONS

NEWS

In the literature: October 2021

COMPREHENSIVE GENOMIC AND TRANSCRIPTOMIC ANALYSIS FOR GUIDING THERAPEUTIC DECISIONS IN PATIENTS WITH RARE CANCERS

Precision oncology has improved clinical outcomes across cancer patients changing the central role of histology towards a molecular-based approach. In many cases, however, the treatment response seems to depend on tumor type, making difficult biomarker evaluation for rare cancers. Thus, to accelerate the discovery of effective biomarker-drug combinations, including their tissue dependence, novel adaptive study designs have been implemented for rare cancers.[1,2]

The German Cancer Consortium established a multicenter, prospective observational study (MASTER trial) based on a common workflow for diagnostics, therapeutic decision making, and structured follow-up. The primary aim was to investigate the clinical value of whole-genome/exome sequencing (WGS/WES) and RNA sequencing (RNA-seq) for adults, $<$51 years, with advanced cancers and in patients with advanced rare cancers across age groups.

In an interesting paper recently published in *Cancer Discovery*, Horak et al.[3] presented the molecular and clinical results for the first cohort of patients enrolled in the MASTER trial. Notably, about three-quarters were diagnosed with rare cancers. A total of 1310 (88.3%) metastatic patients, who had progressed to a first line, were discussed in a cross-institutional, multidisciplinary molecular tumor board (MTB). All the patients were analyzed with RNA-seq and DNA sequencing. The results of these analyses were generated automatically thanks to an in-house original bioinformatic workflow that studies (i) alignment, (ii) calling of single-nucleotide variants, small insertions and deletions, somatic copy number alterations (CNAs), structural variants, and gene fusions, (iii) evaluation of gene expression, and (iv) detection of potentially actionable molecular changes. A study of those biomarkers providing information about sensitivity to homologous recombination deficiency-directed therapies, such as poly(ADP-ribose) polymerase (PARP) inhibition, or immune checkpoint blockade was also provided. Based on this workflow, the MTB provided evidence-based management recommendations, including diagnostic reevaluation, genetic counseling, and experimental treatment, in 88% of cases.

Recommended therapies were administered in 362 of 1138 patients (31.8%). Among 181 patients assessable for the best response comparison, the overall response rate and disease control rate with molecularly informed treatment improved from 16.3% to 23.9% and from 46.3% to 55.3%, respectively, compared with the last systemic therapy administered. In particular, most successful implementations were seen in patients with carcinomas of the upper gastrointestinal tract, cancer of unknown primary, non-small-cell lung cancer, and hepatopancreaticobiliary cancers. In the large and heterogeneous soft-tissue sarcoma sub-cohort, 35% of patients had a progression-free survival (PFS) ratio (PFSr) $>$1.3. Synovial sarcoma and gastrointestinal stromal cancer benefited the most from this approach. In contrast, genome- or transcriptome-directed therapies for patients with bone sarcomas were mostly ineffective. When measuring the PFSr across treatment baskets, the highest rates of therapeutic success were associated with drugs targeting the mitogen-activated protein kinase (MAPK) or phosphoinositide 3-kinase-AKT-mammalian target of rapamycin (PI3K-AKT-mTOR) pathways, immune checkpoint inhibitors, and a group of diverse compounds, including androgen receptor antagonists, enhancer of zeste homolog 2 (EZH2) inhibitors, and isocitrate dehydrogenase 1 (IDH1) inhibitors. Overall, this strategy, compared with previous therapies, translated into a progression-free survival ratio $>$1.3 in 35.7% of patients.

In conclusion, the authors remark the feasibility and clinical utility of a structured precision oncology workflow in patients with rare cancers, a population otherwise underrepresented in previous studies. These data highlight the benefit of molecular stratification in rare cancers and promote clinical trial access and drug approvals in this underserved patient population.

TERTIARY LYMPHOID STRUCTURES: A PROMISING PREDICTIVE BIOMARKER FOR CHECKPOINT INHIBITORS RESPONSE IN SOLID TUMORS

Tertiary lymphoid structures (TLSs) are ectopic and organized aggregates of B cells that develop in non-lymphoid tissues exposed to chronic inflammatory signals. TLSs are crucial for the development of an effective adaptive immune response. Their strategic location, near or within the proper lesion, results in the efficient presentation of neighbors' antigens and generation of effector memory B cells and antibody-producing plasma cells. The presence of follicular dendritic cells (FDCs) and germinal centers within TLSs define a subset of well-developed and mature TLSs (mTLSs), usually adjoined to a smaller T-cell zone containing a mixture of CD4+ and CD8+ T cells, plasma cells, and high endothelial venules (HEVs), resembling secondary lymphoid organs.[4]

In solid tumors, TLSs have been demonstrated as a promoter of an efficacious immune response, linked to increased density of tumor-infiltrating CD8+ T cells and better overall survival and disease-free survival. Moreover, among patients with a high density of activated CD8+ lymphocytes, TLSs define a subset of patients with a higher survival advantage. This suggests that B cells

cooperation is mandatory to obtain an effective antitumor immune response even in high CD8+ T-cell settings. Evidence supporting TLSs as an independent prognostic factor is convincing, and its value as a predictor of response to conventional chemotherapy has been addressed in a few specific cancer types. In breast cancer, according to results from Prabhakaran et al.,[5] TLSs are more frequently encountered in high-grade, hormone receptor-negative and human epidermal growth factor receptor 2 (HER2)-positive tumors, and the presence of high TLSs content is linked to better survival and partial complete response (pCR) to neoadjuvant chemotherapy in these specific groups of tumors.[5] In colon cancer, the presence of TLSs is associated to mismatch repair defective proteins and BRAF-mutated status and defines a very low-risk group of tumors.[6]

Spontaneous remission of prostatic adenocarcinoma, an extremely unfrequented event, has been associated with a dense concentration of TLSs and CD8+ T cells. Additionally, the positive prognostic impact of TLS in metastatic colorectal, breast, and ovarian cancer seems to be similar to that found in primary tumors.

In their recent work published in *Nature Cancer*, Vanhersecke et al.[7] demonstrated the presence of mTLSs as a valuable tool for prediction of response to immune checkpoint inhibitor therapy in a multicentric and retrospective large cohort of solid tumors. Although TLSs were previously suggested as a predictor of response to immunotherapy in specific tumor types, Vanhersecke et al.[7] analyzed both the expression of programmed death-ligand 1 (PD-L1) and the TLSs content, making very interesting observations. According to their results, the presence of mTLSs in samples taken before treatment plays a crucial role as a predictive biomarker of patients' response to anti-programmed cell death protein 1 (PD-1)/PD-L1 treatment, and this association was independent on PD-L1 expression as it was yielded in both PD-L1-positive and PD-L1-negative patients in all tumor types. The exact mechanism of this protective effect is not fully understood, but the presence of FDCs within mTLSs seems to define the group of cases prone for anti PD-1/PD-L1 treatment, and those tumors in which FDCs were absent behave in the same manner as the TLSs-negative group. FDCs play a main role in B-cell maturation and their sole presence within TLSs is linked to better prognosis. This association is probably explained by the role of FDCs as an amplifier of the antitumor signal through the internalization of antigen-antibody complexes generated by plasma cells, promoting B-cell development, class switching, and maturation within the TLSs, particularly in the context of immune checkpoint blockade.

TLSs are easily recognized during the pathological analysis, a significant advantage in comparison to PD-L1 immunohistochemistry which highly depends on preanalytical conditions, antibody clone, storing systems, and interpretation. The histopathological assessment of TLSs is the most robust method and allows not only their *in situ* recognition on routine pathological evaluation, but also the study of their composition. The presence of FDCs and the development of germinal centers, the main defining characteristics of mTLSs are well recognized in the pathological evaluation. Various methods for *in situ* assessment of TILs have been proposed, ranging from morphological isolated hematoxylin and eosin evaluation and immunohistochemistry to cell-specific dual or multiplex immunofluorescence to highlight B cells (CD20), FDCs (CD23, CD21), follicular helper T cells (T_{FH}) (CD4, BCL6, PD1, ICOS), germinal centers (BCL6), plasma cells (CD138, CD38), and HEVs (PNAd, MECA79). The histopathological evaluation also allows recording the specific location of TILs, a feature that may elicit different antitumor responses. The prognostic advantage effect of TLSs seems to be more pronounced when they are located within the tumor and not in the peritumoral area.

Transcriptomic signatures based on the expression of chemokines or cytotoxic markers have been developed to recognize enriched TLS tumors. Chemokine signatures include genes related with a T_{FH} phenotype as CXCL13, CCL19, CCL21, and their usefulness has been tested in colorectal cancer, melanoma, and breast cancer to identify high-TLSs tumors with better prognosis. A comparison of the expression profile generated from mRNA extracted from TLSs-positive cancer tissue, however, reveals some heterogeneity. Besides the range of the methods used in different series to study TLSs, their prognostic value remains.

In summary, TLSs are strongly related to effective antitumor immune response and better survival in solid tumors. The role of TLSs as a predictive biomarker of immunotherapy is a promising feature to explore and may increase the number of patients who are more likely to benefit from immune checkpoint inhibition, especially among those PD-L1-negative, the most urgently needed group for accurate predictive biomarkers. Introducing TLSs to the few approved tools for the prediction of immunotherapy response will require a better understanding of the mechanisms associated with the regulation of immune suppression through TLSs and the development of a consensus, practical and robust algorithm for TLSs evaluation in clinical trials and routine practice.

MULTI-OMIC PROFILING OF PERITONEAL METASTASES IN GASTRIC CANCER IDENTIFIES MOLECULAR SUBTYPES AND THERAPEUTIC VULNERABILITIES

One of the great challenges in gastric cancer (GC) is to understand why there are limited responses to treatment in peritoneal lesions, suggesting intrinsic drug resistance. Peritoneal carcinomatosis is a frequent site of GC metastasis and it is characterized by a poor prognosis, generally evolving to intestinal occlusion and preventing any type of treatment.[8,9]

Tanaka and colleagues[10] have recently published an interesting work focusing on the identification of molecular therapeutic vulnerabilities in patients with GC and peritoneal metastasis in *Nature Cancer*. They analyzed malignant ascites and their corresponding tumor cell lines

from 98 patients, with a multi-omics approach including whole-genome sequencing, RNA-seq, DNA methylation and enhancer landscape with some interesting findings.

CDH1, TP53, ARID1A, RHOA, KRAS, and PIGR were found as significant drivers supported by three complementary algorithms for driver gene detection. Interestingly, PIGR mutations, ($n = 9$) coexisted with CDH1 mutations, RHOA mutations, or ARHGAP fusions. A high number of genetic alterations in the growth signaling pathway affecting receptor tyrosine kinase (RTK) and MAPK pathways were found in peritoneal metastasis (69%). Moreover, a high degree of gene amplification ($>5\times$ ploidy) in the RTK-Ras pathway was also observed in 45% of cases, including KRAS (19.4%), FGFR2 (11.2%), MET (7.1%), ERBB2 (5.1%), and EGFR (4.1%). Amplification of FGFR2 and MET was observed in both primary GC tissues and ascitic samples. Furthermore, the CNA profiles of distant metastases (liver and lymph node) and the peritoneal cancer cells revealed a unique pattern of genetic alterations with increased frequencies of KRAS and FGFR2 amplification and decreased frequency of ERBB2 amplification. Interestingly, amplification of FGFR2 and MET was observed in both primary GC tissues and ascitic cancer cells. Another genomic characteristic of this cohort was the high frequency of TP53 pathway alterations (61%).

Based on the gene expression profiles, hierarchical clustering separated the 59 GC cell lines into two distinct clusters founding epithelial mesenchymal transition (EMT) as the gene set most differentially expressed between the two groups. In a further analysis, clustering of these cell lines according to the expression profile of 200 genes belonging to the EMT gene set was done. The group with active EMT, mostly with diffuse GCs (59%), presented the worst prognosis. A significant elevated expression of SMAD3/7 and the ligands of transforming growth factor-β (TGF-β) receptors was found in the EMT group. Moreover, non-coding RNA was found mainly regulated by EMT.

The authors also tested GC cell lines enriched with the molecular abnormalities found in this cohort of patients in the RTK pathway and they were tested using six molecularly targeted drugs, tackling FGFR, MET, ALK, EGFR, ERBB2, and MEK1/2, according to their profile, with a positive effect of FGFR2 and MET inhibition as well as EGFR inhibition, respectively. ALK inhibition was efficacious exclusively for two patients with EML4-ALK fusions. Inhibition of ERBB2 was not seen, however, for ERBB2 amplification. None of KRAS amplification, KRAS mutations, or MAP2K1 mutations were found reliable biomarkers predicting the efficacy of MEK1/2 inhibitors. Interestingly, TEAD inhibition was also explored, finding positive results for the EMT group with a synergistic effect of TEAD and MEK1/2 inhibition, suggesting a new potential molecular-guided therapeutic strategy for this subtype.

This work highlights the dynamics of molecular alterations in GC patients, and the potential benefit of a personalized approach relative to conventional chemotherapy. By contrast, it may contribute to the discovery of new therapeutic targets through the inhibition of the TEAD pathway, that could be a promising approach to overcome intrinsic therapeutic resistance in GC patients with an EMT trait.

V. Gambardella[1,2], C. Martínez-Ciarpaglini[2,3], T. Fleitas[1,2] & A. Cervantes[1,2]*

[1]*Department of Medical Oncology, Hospital Clínico Universitario, INCLIVA Biomedical Research Institute, University of Valencia, Valencia;*
[2]*CIBERONC, Instituto de Salud Carlos III, Madrid;*
[3]*Department of Pathology, Hospital Clínico Universitario, INCLIVA Biomedical Research Institute, University of Valencia, Valencia, Spain*
(*E-mail: andres.cervantes@uv.es).

Available online xxx

https://doi.org/10.1016/j.esmoop.2021.100285

FUNDING

This paper was supported by grants from the Instituto de Salud Carlos III [grant number PI18/01909] to AC. VG was supported by Rio Hortega contract [grant number CM18/00241] from the Carlos III Health Institute. TF was supported by Juan Rodés contract [grant number 17/00026].

DISCLOSURE

AC declares institutional research funding from Genentech, Merck Serono, Bristol Myers Squibb, Merck Sharp & Dohme, Roche, Beigene, Bayer, Servier, Lilly, Novartis, Takeda, Astellas, Takeda and Fibrogen and advisory board or speaker fees from Amgen, Merck Serono, Roche, Bayer, Servier and Pierre Fabre in the last 5 years. All remaining authors have declared no conflicts of interest.

REFERENCES

1. Schwaederle M, Zhao M, Lee JJ, et al. Association of biomarker-based treatment strategies with response rates and progression-free survival in refractory malignant neoplasms: a meta-analysis. *JAMA Oncol*. 2016;2:1452-1459.
2. Petak I, Kamal M, Dirner A, et al. A computational method for prioritizing targeted therapies in precision oncology: performance analysis in the SHIVA01 trial. *NPJ Precis Oncol*. 2021;5:59.
3. Horak P, Heining C, Kreutzfeldt S. Comprehensive genomic and transcriptomic analysis for guiding therapeutic decisions in patients with rare cancers. *Cancer Discov*. 2021;11:2780-2795.
4. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Cancer*. 2019;19:307-325.
5. Prabhakaran S, Rizk V, Ma Z, et al. Evaluation of invasive breast cancer samples using a 12-chemokine gene expression score: correlation with clinical outcomes. *Breast Cancer Res*. 2017;19:71.

6. Posch F, Silina K, Leibl S, et al. Maturation of tertiary lymphoid structures and recurrence of stage II and III colorectal cancer. *Oncoimmunology*. 2017;7:e1378844.
7. Vanhersecke L, Brunet M, Guégan J, et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. *Nat Cancer*. 2021;2:794-802.
8. Thomassen I, van Gestel YR, van Ramshorst B, et al. Peritoneal carcinomatosis of gastric origin: a population-based study on incidence, survival and risk factors. *Int J Cancer*. 2014;134:622-628.
9. Smyth EC, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold A. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2016;27(suppl V):v38-v49.
10. Tanaka Y, Chiwaki F, Kojima S, et al. Multi-omic profiling of peritoneal metastases in gastric cancer identifies molecular subtypes and therapeutic vulnerabilities. *Nat Cancer*. 2021;2:962-977.