ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

EDITORIAL

Pancreatic cancer transcriptomes: molecular stratification in the adjuvant setting

Pancreatic ductal adenocarcinoma (PDAC) remains a challenging clinical problem.[1] Unless there are dramatic advances in the management of the disease, it is foreseen that PDAC may become the second cause of cancer deaths in the Western world.[2,3] To avoid this epidemic, progress will be needed through multiple approaches: improved prevention and early diagnostic strategies, molecular tumor stratification for personalized and molecularly targeted antitumor treatments, and development of rational and tolerable multimodal therapies. Furthermore, it is essential to spread the concept in the medical community that a PDAC diagnosis is not always a death sentence. The incremental, but consistent, improvement in long-term survival observed in the last few years proves that the field is moving ahead, much like what happened approximately 20 years ago in the treatment of colorectal cancer.

Since its approval in the late 1990s, gemcitabine has been the main chemotherapeutic drug used in the treatment of PDAC. There has been extensive research on biomarkers predictive of response to the drug, most notably the expression of gemcitabine metabolic enzymes such as CDA and CDK,[4-7] and the transporter hENT.[8,9] However, despite intense efforts, robust predictive biomarkers of response to gemcitabine have not reached routine clinical applicability.[10]

In this issue of *Annals of Oncology*, Nicolle et al. report on a transcriptomic signature to predict response to gemcitabine in the adjuvant setting.[11] Independent component analysis of RNA-seq data was used to identify signatures associated with *in vitro* gemcitabine response and *in vitro* duplication time using 38 cell lines, as well as *in vivo* tumor response using 12 patient-derived xenografts. Notably, there was relatively little overlap between the features associated with sensitivity to gemcitabine *in vivo* and effect on cell proliferation *in vitro*. Results from both models were used to build the final *GemPred* signature, which was found to be specific for sensitivity to gemcitabine.

The signature was then tested on a small ($n = 67$) retrospective cohort of patients treated or not with adjuvant gemcitabine monotherapy. As much 16% of the patients who were treated were identified as *GemPred*$^+$; these patients had a significantly longer overall survival than those who were *GemPred*$^-$. Among those who did not receive adjuvant gemcitabine, the survival of *GemPred*$^+$ patients was nonsignificantly longer compared with the *GemPred*$^-$ group. The signature was then tested in a large multicenter retrospective validation cohort of 368 patients who had undergone surgery with curative intent, 55% of whom had received adjuvant gemcitabine monotherapy. Patients who did not receive adjuvant therapy were older and had lower frequency of lymph node involvement and positive margins. The proportion of patients with a *GemPred*$^+$ tumor signature was again 17%, all of which had 'classical' transcriptomic features. The significantly longer overall survival of these patients was confirmed. A combined analysis of both cohorts ($n = 435$) revealed that the median disease-free survival of patients with *GemPred*$^+$ tumors who had received adjuvant treatment ($n = 41$) was 42.5 months [95% confidence interval (CI) 29.9 to not reached], compared with 13.4 months (95% CI 10.3-15.5) for all other patients. The median overall survival of patients with *GemPred*$^+$ tumors who had received adjuvant treatment was 91.3 months (95% CI 63.1 to not reached), compared with 31.7 (95% CI 24-34.2) and 23.7 (95% CI 18.4-34.9) months for patients with *GemPred*$^-$ tumors, with or without adjuvant gemcitabine, respectively. A Cox proportional hazards regression model showed that the adjuvant gemcitabine $\times$ GemPred$^+$ interaction term was significant with a hazards ratio of 0.44 ($P = 0.025$). The *GemPred* signature compared favorably with other transcriptomic signatures derived from the analysis of gemcitabine sensitivity of PDAC organoids[12] and with messenger RNA expression levels of genes involved in gemcitabine metabolism (e.g. *CDA*, *DCK*) and *hENT*. However, *GemPred* and hENT1 immunohistochemistry fared similarly as predictors of sensitivity.

The work of Nicolle et al. has a number of strengths, including the use of RNA isolated from formalin-fixed paraffin-embedded tumor tissue, the robustness derived from the use of two different genome-wide transcriptomic approaches (microarrays and RNA-seq), the validation in an independent cohort, and the availability of a web-based tool for use by the community. Given the enormous advances in molecular diagnostics and bioinformatics, including RNA-based analysis, and the increasing role of routine molecular tumor boards, this approach demonstrates an exciting new road to molecular-based stratified clinical decision making in pancreatic cancer care.

However, there are also several limitations, including the lack of comparison of the two transcriptome analytical platforms, the failure to consider the response to gemcitabine in patients who received the drug upon tumor progression, and the not fully resolved issue of the possible

prognostic component of the signature. Most relevant, the retrospective nature of the cohorts limits evaluation of the *GemPred* signature for outcome. The test cohort was fairly small and efforts should now be made to rigorously test the signature in prospective trials. Evaluation, if possible, in cases from the recently presented large adjuvant trials PRODIGE 24 and APACT may help determine the value of the *GemPred* signature in the context of gemcitabine/nab-paclitaxel combination and FOLFIRINOX-based therapy, respectively. Notably, current adjuvant trial initiatives such as ESPAC-6 have incorporated treatment-specific signatures into the protocol, which will allow prospective evaluation of signature-based therapy stratification.

As with many signature-based stratification approaches, the biological nature of the signature was not explored. Will the *GemPred* signature be of value in the adjuvant and the metastatic setting? Is the signature associated with therapy resistance, in this case gemcitabine, or with invasive/metastatic abilities of remnant tumor cells, or other tumor features? In this regard, the investigated cases show a surprisingly low frequency of the basal-like subtype according to subtyping analysis using the PURIST algorithm.[13] Thus, a better understanding of the association of *GemPred*-based tumor stratification in the context of the previously characterized molecular subtypes and the underlying tumor biology will likely enable further refinement and development of stratified therapeutic approaches.

What are the next steps? Two recently reported trials have shown that combination chemotherapy with gemcitabine plus capecitabine[14] and modified FOLFIRINOX[15] are superior to gemcitabine alone in the adjuvant setting. Therefore, gemcitabine-only adjuvant therapy is becoming a less preferred option in the majority of patients and will likely be applied only to patients in frail condition. It will be interesting to see whether the *GemPred* signature is also predictive of outcome in the context of gemcitabine-based combination therapies that have been evaluated in the adjuvant setting such as gemcitabine/capecitabine[14] and gemcitabine/nab-paclitaxel.[16]

The study also raises several important questions: (i) for application in the clinical context, is global transcriptome RNA-seq preferable to a small gene signature, as the community has adopted for breast cancer?; (ii) will a signature that is predictive of sensitivity in the adjuvant setting also be predictive of response in patients with borderline resectable (neoadjuvant) or metastatic PDAC?; (iii) while a proportion of patients with advanced PDAC continue to be treated with gemcitabine monotherapy, the drug is being used more frequently in combination with nab-paclitaxel. Will the value of the signature remain in that context?

These questions can only be answered through further well-conducted studies. Some retrospective analyses may shed light on these issues but prospective validation is undoubtedly required. However, in a moving field such as this one, a significant risk is that once prospective validation has been performed, it may no longer be helpful because new, better, diagnostic, and therapeutic approaches have come along. For these reasons, it is time for us all to evaluate the potential of such molecular signatures and biomarkers in a concerted approach and to incorporate them in ongoing and upcoming state-of-the-art clinical trials.

F. X. Real[1,2,3]* & J. T. Siveke[4,5,6]
[1]*Epithelial Carcinogenesis Group, Spanish National Cancer Research Centre-CNIO, Madrid;*
[2]*CIBERONC, Madrid;*
[3]*Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain;*
[4]*Institute for Developmental Cancer Therapeutics, West German Cancer Center, University Hospital Essen, Essen;*
[5]*Division of Solid Tumor Translational Oncology, German Cancer Consortium (DKTK, partner site Essen), Essen;*
[6]*German Cancer Research Center, DKFZ, Heidelberg, Germany*
(*E-mail: freal@cnio.es).

Available online 21 November 2020

https://doi.org/10.1016/j.annonc.2020.11.012
DOI of original article: https://doi.org/10.1016/j.annonc.2020.10.601

ACKNOWLEDGEMENTS

We thank N. Malats for valuable discussions and comments.

FUNDING

Work in the laboratory of FXR is supported by a grant from the Ministerio de Ciencia e Innovación (Madrid, Spain; grant number RTI2018-101071-B-I00). JTS is supported by funding from the German Cancer Consortium (DKTK), the German Cancer Aid [grant number 70112505 (PIPAC) and grant number 70113834 (PREDICT-PACA)], and the German Research Foundation (DFG) through grant number SI1549/3-1 (Clinical Research Unity KFO337).

DISCLOSURE

The authors have declared no conflicts of interest.

REFERENCES

1. Mizrahi JD, Surana R, Valle JW, Shroff RT. Pancreatic cancer. *Lancet*. 2020;395(10242):2008-2020.
2. Rahib L, Smith BD, Aizenberg R, et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res*. 2014;74(11):2913-2921.
3. Malvezzi M, Bertuccio P, Levi F, et al. European cancer mortality predictions for the year 2014. *Ann Oncol*. 2014;25(8):1650-1656.
4. Fujita H, Ohuchida K, Mizumoto K, et al. Gene expression levels as predictive markers of outcome in pancreatic cancer after gemcitabine-based adjuvant chemotherapy. *Neoplasia*. 2010;12(10):807-817.
5. Serdjebi C, Seitz JF, Ciccolini J, et al. Rapid deaminator status is associated with poor clinical outcome in pancreatic cancer patients treated with a gemcitabine-based regimen. *Pharmacogenomics*. 2013;14:1047-1051.

6. Giovannetti E, Del Tacca M, Mey V, et al. Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. *Cancer Res*. 2006;66(7):3928-3935.
7. Maréchal R, Mackey JR, Lai R, et al. Deoxycitidine kinase is associated with prolonged survival after adjuvant gemcitabine for resected pancreatic adenocarcinoma. *Cancer*. 2010;116(22):5200-5206.
8. Greenhalf W, Ghaneh P, Neoptolemos JP, et al. Pancreatic cancer hENT1 expression and survival from gemcitabine in patients from the ESPAC-3 trial. *J Natl Cancer Inst*. 2014;106(1):djt347.
9. Elander NO, Aughton K, Ghaneh P, et al. Expression of dihydropyrimidine dehydrogenase (DPD) and hENT1 predicts survival in pancreatic cancer. *Br J Cancer*. 2018;118(7):947-954.
10. Raffenne J, Nicolle R, Puleo F, et al. hENT1 testing in pancreatic ductal adenocarcinoma: are we ready? A multimodal evaluation of hENT1 status. *Cancers*. 2019;11(11):1808.
11. Nicolle R, Gayet O, Duconseil P, et al. A transcriptomic signature to predict adjuvant gemcitabine sensitivity in pancreatic adenocarcinoma. *Ann Oncol*. 2021;32(2):250-260.
12. Tiriac H, Belleau P, Engle DD, et al. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. *Cancer Discov*. 2018;8(9):1112-1129.
13. Rashid NU, Peng XL, Jin C, et al. Purity Independent Subtyping of Tumors (PurIST), a clinically robust, single-sample classifier for tumor subtyping in pancreatic cancer. *Clin Cancer Res*. 2020;26(1):82-92.
14. Neoptolemos JP, Palmer DH, Ghaneh P, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. *Lancet*. 2017;389(10073):1011-1024.
15. Conroy T, Hammel P, Hebbar M, et al. FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. *N Engl J Med*. 2018;379(25):2395-2406.
16. Tempero MA, Reni M, Riess H, et al. APACT: phase III, multicenter, international, open-label, randomized trial of adjuvant nab-paclitaxel plus gemcitabine (nab-P/G) vs gemcitabine (G) for surgically resected pancreatic adenocarcinoma. *J Clin Oncol*. 2019;37(15_suppl):4000.