Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: November 30, 2022

Citation: Priya Hays, 2022. Clinical Tier Grading of Cancer Stem Cells According to Clinical Characteristics for Immune Checkpoint Inhibitors Guided by mRNA stemness index, Medical Research Archives, [online] 10(11). https://doi.org/10.18103/mra.v10i11.3373

DOI
https://doi.org/10.18103/mra.v10i11.3373

ISSN: 2375-1924

RESEARCH ARTICLE

Clinical Tier Grading of Cancer Stem Cells According to Clinical Characteristics for Immune Checkpoint Inhibitors Guided by mRNA stemness index

Priya Hays, Hays Documentation Specialists, LLC, San Mateo, CA USA

*priya.hays@outlook.com

ABSTRACT

Cancer stems cells are cells in tumors that have self-renewing capabilities and proliferation, and are partly responsible for tumor growth, metastasis and drug resistance, and have been associated with multidrug resistance and epithelial-mesenchymal transition. mRNA stemness index or mRNAsi is a machine learning tool that uses the application of algorithms to find associations between cancer stemness and tumor prognostic signatures. mRNAsi predicts gene mutation status and identifies tumor signaling pathways. Clinical tier grading is a common feature for stratifying the presenting features and symptoms of patients in several diseases. This study is a review article that summarizes studies in lung cancer, gastric cancer, hepatocellular carcinoma and glioblastoma that use mRNA stemness index machine learning tools to identify differentially expressed genes, characterize the tumor microenvironment and tumor mutational burden, and determine clinical endpoints. A prognostic signature is shown in this paper as determined by mRNAsi high and low values, and a clinical tier grading system is proposed that categorizes cancer stemness presenting characteristics. This clinical grading tier system demonstrates a relationship between cancer stemness and immune checkpoint inhibitor efficacy. This type of tiered system for cancer patients and the accompanying workflow proposed may prove useful to oncologists, and has not been performed before, and is unique in the literature.

Keywords: cancer stemness, mRNA stemness index, lung cancer, gastric cancer, hepatocellular carcinoma, glioblastoma, immune checkpoint inhibitor efficacy

INTRODUCTION

Cancer stems cells are cells in tumors that have self-renewing capabilities and proliferation, and are partly responsible for tumor growth, metastasis and drug resistance. They have also been associated with multidrug resistance and epithelial-mesenchymal transition. Recent studies have shown that cancer stemness is capable of being targeted by immunotherapies that prevent cancer stem cells from escaping from antitumor therapy. The nature of cancer stemness is related to the tumor microenvironment (TME) and its propensity to metastasize. Several studies have been performed to identify associations between cancer stemness and tumor prognosis, and have developed algorithms establishing prognostic signatures.[1,2,3]

mRNA stemness index, or mRNAsi, is a machine learning tool that uses the application of algorithms to find associations between cancer stemness and tumor prognostic signatures. mRNAsi predicts gene mutation status and leads to the identification of tumor signaling pathways. Researchers identified mutated genes from The Cancer Genome Atlas (TCGA), and then found strong associations between mRNAsi and gene mutational subtypes. mRNAsi values were directly proportional to gene subtype: mutant subtype groups had higher mRNAsi values when compared to wild-type subtype groups.[4]

mRNAsi divides normal and tumor samples into high and low mRNAsi values that associate with tumor mutational burden and the TME, and lead to the calculation of risk scores and thus tumor prognosis as well. Differentially expressed genes, or DEIRGs, result from mRNAsi analysis of tumors that also serve as prognostic indicators. Studies on lung adenocarcinoma and lung squamous cell carcinoma, gastric cancer, hepatocellular carcinoma and glioblastoma, and other tumors of varying degrees of prognosis, have formed the focus of mRNAsi tools which ultimately lead to clinical outcome data such as overall survival (OS) and progression-free survival (PFS).[5,6,7,8,9] mRNAsi has been developed to analyze prognostic significance for immunotherapy response for cancer stemness in lung cancer, gastric cancer, hepatocellular carcinoma and glioblastoma that form the focus of this review article.

Clinical tier grading is a common feature for stratifying the presenting features and symptoms of patients in several diseases. Santoro et al presented a clinical tier grading system for Down's regression syndrome that graded patients into clinical categories for management and treatment.[10] The aim of this review is to create a clinical tier grading system that categorizes prognostic signatures and clinical characteristics derived from the application of mRNAsi tools in these studies to predict immune checkpoint inhibitor efficacy. The scope of this type of tiered system for cancer patients and the accompanying workflow that includes determining levels of biomarker expression as well potentially extends to oncologists and the management and treatment of their patients, has not been proposed before, and is unique in the literature.

RESULTS

Lung Adenocarcinoma (LUAD) and Lung Squamous Cell Carcinoma (LUSC)

Li et al analyzed the role of cancer stemness in lung cancer survival data, specifically lung adenocarcinoma (n=452) and lung squamous cell carcinoma (n=363).[5,11] In their study, they reported how differentially expressed genes related to stemness origination, which then led to the dysregulation of the tumor microenvironment and abundance of infiltrating immune cells. The tumor microenvironment included "naïve B cells, B cells memory, plasma cells, T cells CD8, T cells CD4 memory resting, T cells CD4-memory activated, T cells follicular helper, Tregs, NK cells resting, NK cells activated, monocytes, macrophages M0, macrophages M1, macrophages
M2, dendritic cells resting, dendritic cells activated, mast cells resting, mast cells activated, eosinophils, and neutrophils", which were profiled with CIBERSORT. This association was mediated by a machine learning algorithm tool, mRNA stemness index. The authors created an immunogenomic model from the stem cell characteristics in LUAD and LUSC through tools such as ESTIMATE R package, which delivered mathematical derivations such as StromalScore, ImmuneScore and ESTIMATEScore for stromal and immune cell infiltration, which positively associated with tumor purity. High and low mRNAsi values divided lung cancer samples into different prognostic groups. Eight genes ANGPTL5, CD1B, CD1E, CNTFR, CTSG, EDN3, IL12B, and IL2 were prognostic factors that divided lung adenocarcinoma patients into low and high risk categories according to overall survival and tumor microenvironment. CCL1, KLRC3, KLRC4, CCL23, and KLRC1 were the contributing factors analogously for lung squamous cell carcinoma. Moreover, they constructed a network between transcription factors and stemness-related differentially expressed immune-related genes (DEIRGs) that "revealed the potential mechanisms of stemness-related DEIRGs in LUAD and LUSC, respectively."

The patients with high mRNAsi (range from 0 to 1, closer to 1 represented stronger stemness) as derived from X-tile software (n=39) showed significantly poorer prognosis in LUAD (p = 0.047) and LUSC (n=143) (p = 0.021). Lung adenocarcinoma displayed a significantly different distribution of immune cells based on different high and low mRNAsi subtypes ($p<0.05$), including memory B cells (p = 0.002), resting dendritic (p = 0.005) and mast cells (p=0.0008). Likewise, LUSC had distinguishable immune types according to high and low-mRNAsi-subtypes, with the distribution of macrophages, CD4 T cells memory resting, T cells CD4 memory activated, and T cells CD8 significantly higher in low-mRNAsi subtype ($p < 0.05$) and plasma cells higher in high mRNAsi subtype ($p < 0.05$). 34 differentially expressed genes "were identified between high- and low-mRNAsi subtypes in LUAD including 9 upregulated DEIRGs (OBP2A, CALCB, PTH2R, PDIA2, FABP2, CGA, PMP2, LCN8, and RETNLB) and 25 downregulated DEIRGs (CMA1, CRLF2, ELANE, IL2, GLP1R, PRTN3, EDN3, CD1B, ANGPTL5, FCN2, IL12B, CTSG, CCR9, PCSK2, CSF2, CD1A, ANGPTL7, AZU1, CCL17, CNTFR, CD1E, IL22RA2, HTR3A, IL1F7, and BMP7."

Statistical analysis for LUAD showed that 11 DEIRGs were related to overall survival (OS) "including ANGPTL5, ANGPTL7, CCL17, CD1A,CD1B, CD1E, CNTFR, CTSG, EDN3, IL12B, and IL2" with greater predictive accuracy provided by lasso regression of the "eight-immune-related gene-signature model (ANGPTL5, CD1B, CD1E, CNTFR, CTSG, EDN3, IL12B, and IL2)." Differentially related gene expression also was found to be significantly correlated with TNM system that supported their prognostic model.

Risk score analysis was conducted using principal component analysis and LUAD tissue samples were divided according to high and low risk groups which revealed a high degree of difference between the immune character of these groups. Naïve and memory B cells, macrophages, dendritic cells, mast cells, and neutrophils were differentially distinct according to high and low risk categories.

Gastric Cancer

Mao et al conducted a study also proposing similar associations between mRNAsi and prognosis of tumors in training and validation cohorts.[6,12,13] Their case example was gastric cancer. They reported Kaplan-Meier survival curves with OS, progression-free survival, disease free survival outcomes (OS: $P<0.05$, $HR<1$; DFS: $P<0.05$, $HR<1$). Prognosis based on microsatellite high instability was also determined: "There was also a close relationship between mRNAsi values and MSI, and mRNAsi values were higher in the MSI-H and MSI-L groups than in the MSS group ($P<0.001$)."[14] They also determined relationships between differentially expressed genes and the specific immune cell infiltration of the tumor microenvironment using ESTIMATE. Higher mRNAsi values also correlated with tumor purity, ($P<0.0001$, $r=0.5976$). They also used Pearson's correlation analysis to compute TME with mRNAsi, demonstrating negative correlation with immune scores ($P<0.0001$, $r=-0.3421$), stromal scores ($P<0.0001$, $r=-0.7561$) and ESTIMATE scores ($P<0.0001$, $r=-0.5980$), and identified prognostic signatures through mRNAsi evaluations in gastric cancers using lasso regression. They additionally reported gene mutation status through mRNAsi, including p53. Based on their TCGA cohort that included the top 10 mutated genes, they reported correlations between mRNAsi groups and mutation status. Wild-type groups associated with lower mRNAsi values compared to the mutant subtype group ($P < 0.05$). mRNAsi values had lower value for T2, T3 and T4 stages versus T1 stage ($P<0.01$). Stage II, III and IV had lower mRNAsi value than stage I groups as well ($P<0.05$). Their analysis also indicated that mRNAsi values positively correlated with PD-L1 expression and negatively correlated with macrophages and CAFs. Substitutions, deletions, coding errors, insertions could be calculated to derive a measure of TMB, since they found that mRNAsi values of high TMB groups "were also increased significantly ($P<0.0001$)."

According to the investigators, "[w]e know that macrophages, especially M2 macrophages, and CAFs [cancer associated fibroblasts] play important roles in driving the progression of GC. By evaluating the numbers of these two types of cells, the outcome of GC can be better predicted. The above results suggest that mRNAsi values themselves can serve as a novel predictive biomarker of immunotherapy response."

Hepatocellular Carcinoma

Xu et al also conducted a study on the associations between cancer stemness, mRNAsi, differential gene expression, and TME to identify prognostic signatures in hepatocellular carcinoma (HCC).[15,16] ESTIMATE, immune and stromal scores were negatively correlated with mRNAsi and positively correlated with tumor purity. According to Kaplan-Meier curves, longer OS times were observed with

lower mRNAsi values. Their analysis led them to the elucidation of differentially expressed genes between normal and tumor tissues based on mRNAsi analysis. 7,273 genes were upregulated and 394 genes were downregulated between HCC and normal samples. Nine genes correlated with good prognosis with p value < 0.005 according to univariate regression, and lasso Cox regression was performed on these nine genes, creating an eight-gene (N4BP3, NRGN, ITGB5, FAM110D, LPCAT1, CASQ2, UNC5B, and SLCO2A1) prognostic signature, which further indicates how differentially gene expression can find associations between stemness and prognosis. Risk scores were then obtained for the HCC samples: "In single-factor regression analysis, the risk score was discovered to be significantly correlated with OS (HR = 3.635, 95% CI = 2.406–5.491, p < 0.001). After correction for other confounding factors, the risk score still was an independent predictor for OS in the multivariate Cox regression analysis (HR = 2.722, 95% CI = 1.735–4.270, p < 0.001)." The role of the mRNAsi in the diversity and complexity of the TME led the investigators to elucidate that high-risk scores had negative correlations with abundant mast cells, neutrophils, but positively associated with CAFs, M2 macrophages, resting mast cells and regulatory T cells. Additionally, Spearman correlation analysis was performed and revealed that risk scores stratified the samples characterized by anti-tumor effects or immunosuppression. Low-risk groups (n=183)were characteristic of the presence of antitumor lymphocyte cells, which might enhance anti-tumor effect. In contrast, the high-risk samples (n=182) were characterized by immunosuppressive cells, such that the authors observed that "risk score may act as a determining factor in the regulation of immune response further predicting immunotherapeutic efficacy." Further analysis revealed that the risk scores indicated TMB status, since the TMB value was higher in the high-risk score subgroup. Subgroups based on low and high risk had mutational analysis performed on them to reveal that "CTNNB1 (30 vs. 21%) experienced higher somatic mutation rates in the low-risk score subtype, while TP53 (16 vs. 40%) possessed higher somatic mutation rates in the high-risk score subgroup", indicating how somatic mutations had a significantly complex relationship to stemness and could form the basis for diagnosis, therapy and prognosis. The investigators also suggested that CTNNB1 could serve as a predictive indicator in HCC since it was associated with the "immune-excluded phenotype."

Glioblastoma

Wang et al performed a stemness index study on glioblastoma, or GBM. They divided GBM patients into two subtypes Stemness Subtype I (233 patients, 45.0%) and Stemness Subtype II (285 patients, 55.0%) based on stemness index and conducted comparisons of clinicopathological parameters to address correlations between stemness subtype and clinical features. The found that Stemness Subtype I patients with high values for mRNAsi had greater efficacy with immunotherapies, versus temozolomide or standard of care. The gene expression profiles obtained of 518 GBM patients were calculated and categorized and ranked from low to high in order to explore how mRNAsi related to clinical features. mRNAsi had significant associations with gene mutation status. Higher mRNAsi scores were significantly associated with elderly patients, while patients less than 40 years of age had lower mRNAsi scores. PTEN-mutant subtypes had lower mRNAsi subtypes than PTEN wildtype samples (P=0.037). Likewise TP53 mutant (P<0.001), IDH-mutant (P=0.004) and ATRX-mutant samples (P < 0.001) had lower mRNAsi value when compared with wild-type samples. Stemness Subtype I patients associated with tumor mutational burden, and Stemness Subtype I also had a higher number of copy number amplifications and deletions. TP53 (42%) was frequently mutated gene in Stemness Subtype I and PTEN (39%) was frequently mutated in Stemness Subtype II. Stemness Subtype II had lower mutation frequencies of less than 10% of ATRX and IDH1 compared to Stemness Subtype I where they were observed to be significantly higher (I versus II, 15.5 versus 6.7%; P = 0.001) and ATRX (I versus II, 16.8% versus 2.3%; P<0.001). As the authors conclude on immunotherapy effectiveness: "No significant difference was found regarding the mutation frequencies of BRAF, PTEN, EGFR and TERT between the two subtypes. In addition, patients in the Stemness Subtype I group had significantly higher TMB than those in the Stemness Subtype II group (P=4.5×10–13). All these findings could suggest underlying differences in the immunotherapy response of the two stemness subtypes."

TME was evaluated through ESTIMATE algorithms, and although stemness had no significant correlation with tumor purity, it was associated with infiltration of immune and stromal cells which decreased with elevation of stemness in GBM. mRNAsi values decreased while tumor purity increased in high immunity versus low immunity groups. CIBERSORT algorithms were applied to further quantify the abundance of 22 types of immune cells: "the

stemness index was significantly positively correlated with T cell subsets [including follicular helper (R = 0.63, P<0.001), naive CD4(R = 0.38, P = 0.002), and memory activated CD4 (R = 0.32, P = 0.041) T cells], activated natural killer (NK) cells (R = 0.49, P<0.001), memory B cells (R = 0.48, P<0.001), and plasma cells (R = 0.35, P<0.001);meanwhile the stemness index was significantly negatively correlated with M2 macrophages (R =−0.47, P<0.001) and monocytes (R =−0.32, P = 0.047)."

CIBERSORT was also utilized to quantify infiltration abundances and it was shown that CD4 and CD8 T cell subsets, natural killer cells, monocytes, macrophages and neutrophils were more abundant in Stemness group II while plasma cells, follicular helper T cells, dendritic cells and mast cells were "significantly more abundant" in Stemness Subtype I. Stemness Subtype I were characterized by higher proportions of high and medium immunity tumors while Stemness Subtype II had low immunity tumors, and it could be demonstrated that Stemness Subtype I had low immune infiltration while having high tumor purity, such that the it possesses relatively high immunity.

Immunogenicity analysis also revealed that "expression levels of PD1/PD-L1/PD-L2 and CTLA/CD80/CD86 were exactly reversed in the two stemness subtypes. The expression levels of PD1 and its ligands (PD-L1 and PD-L2) were significantly higher in Stemness Subtype I (all P<0.001), and those of CTLA and its ligands (CD80 and CD86) were significantly higher in Stemness Subtype II (all P<0.05)" and the authors showed that , "the proportion of responders to immunotherapy in the Stemness Subtype I group was more than two times that in the Stemness Subtype II group (44.6 versus 21.8%, P<0.001)."

Kaplan-Meier survival curves showed that high mRNAsi groups had better OS, but contradictorily, had low PFS, which correlated with stratification by distinct clinical variables. The Stemness Subtype I group presented with better OS (HR=0.606) and poorer PFS (HR = 1.349) compared to the Stemness Subtype group II. "The median OS time of Stemness Subtype I group patients was longer than that of Stemness Subtype II group patients (1.21 versus 1.05 years), whereas the median PFS time of Stemness Subtype I group patients was markedly shorter than that of Stemness Subtype II group patients (0.48 versus 0.71 years)." Stemness subtype I served as an indicator of favorable OS and unfavorable PFS in GBM, further providing evidence that stemness is a prognostic factor for predicting both OS and PFS. "The stemness index of patients with Stemness Subtype I (0.43±0.12) was significantly higher than that of patients with Stemness Subtype II (0.27±0.08),with P<2.0×10–16. This demonstrated that patients in the Stemness Subtype I group had higher levels of neoplastic stemness, which suggested stronger potential for the self-renewal, differentiation, and proliferation of tumor cells and might explain their poorer PFS."

Proposed Clinical Tier Grading of Cancer Stemness and Workflow

The significance of these studies outlined potentially arrive at a proposed workflow and clinical tier grading system for predicting immune checkpoint inhibitor efficacy from algorithms measuring cancer stemness. In Figure 1, a workflow is presented that begins with the identification of mutated genes inTCGA, the application of the mRNAsi tool to determine high and low subtypes, which in turn can identify differentially immune-related expressed genes. After revealing biomarker expression (PD-L1 and CTLA-4) and clinical presentation, the tumor microenvironment, tumor mutational burden and Kaplan-Meier survival curves are also characterized, leading to prognosis of ICI efficacy.

In Table 1, a clinical tier grading system is proposed whereby patients, particularly those presenting with metastasis, present with signatures based on mRNAsi values and biomarker expression levels. Clinical characteristics such as risk scores, OS, and PFS, can predict immune checkpoint inhibitor (ICI) efficacy. As Table 1 proposes, those in Tier 1 have high mRNAsi values, low PD-1 or low CTLA-4, high risk and low OS and high TMB or mutational status, and thus would be predicted to have low ICI efficacy. Putatively, patients with tumors present with these characteristics are administered pembrolizumab, nivolumab, and ipilimumab, their prognosis would be poorer and patients would be stratified to Tier 1. In Tier 2, low mRNAsi values, high PD-L1 and CTLA-4, and low OS and low to moderate risk would be predicted to respond well to ICI treatments.

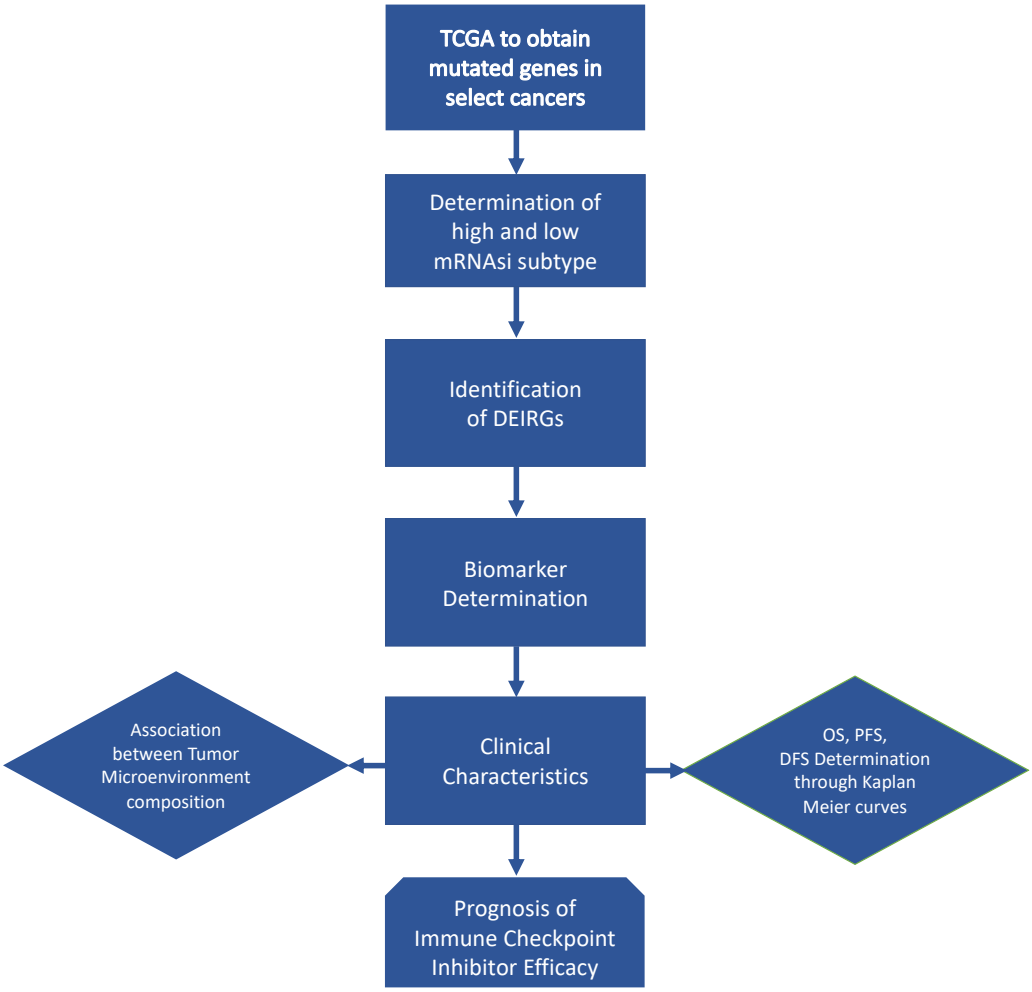

Figure 1 Proposed Workflow for Determination of ICI Efficacy and Presenting Clinical Characteristics Associated with Cancer Stemness. DEIRG (differentially expressed immune-related genes)

Tier 1		
Signature	**Clinical Characteristics**	**ICI Efficacy**
High-mRNAsi	High risk	Low
Low PD-L1	Low OS	
Low PD-1	High TMB	
Low CTLA-4		
Tier 2		
Signature	**Clinical Characteristics**	**ICI Efficacy**
Low-mRNAsi	Low to moderate risk	High
High PD-L1	High OS	
High PD-1		
High CTLA-4		

Table 1 Proposed Clinical Tier Grading System for ICI Efficacy and mRNAsi Analysis and Clinical Characteristics ICI(immune checkpoint inhibitor), mRNAsi (mRNA stemness index)

CONCLUSION

In conclusion, clinical outcomes are predicted based on mRNAsi indicators. OS and DFS with statistically significant p values are plotted on Kaplan-Meier curves. Progression-free survival and disease-specific survival were also affected by mRNAsi (HR<1). mRNAsi-guided tools determined differentially expressed genes in tumors and generated prognostic signatures which in turn reflected clinical characteristics that could be grouped into a tiered list, creating grading categories for ICI treatment efficacy. Low-risk and high-risk survival groups, tumor mutational burden, TNM pathological stages, overall survival were generated from prognostic gene signatures through mRNAsi.

These results on the prediction of ICI efficacy stem from the studies described that have a common theme: cancer stemness is a characteristic of these tumors with poor prognosis and variable degrees of responsive to ICIs. mRNAsi is a tool that when applied to normal and tumor samples can reveal the nature of the TME, clinical outcomes, such as OS and DFS, and further complex statistical analysis such as Cox regression and univariate and multivariate analysis can reveal either low or high risk for these tumors.

The tumors discussed are lung adenocarcinoma, lung squamous cell carcinoma, gastric cancer, hepatocellular carcinoma and glioblastoma. The prevalence of these tumors are high among cancer patients, and they present with poor prognosis in many cases, especially when identified at later stages. Each of them has differential responses to immune checkpoint inhibitors based on immunogenicity.

The workflow and clinical tiered system proposed here on the basis of these robust studies analyzing these tumors could be considered a synthesis on the literature associating mRNAsi with the prognosis of ICIs for the purpose of aiding oncologists in determining ICI efficacy and clinical outcomes based on clinical signatures and clinical characteristics, as shown in Table 1. Future studies could provide empirical evidence for these hypothetical set of proposals. Based on this clinical presentation, guidance for ICI therapy could be developed for further proof-of-concept studies.

DATA AVAILABILITY

Not applicable

AUTHOR CONTRIBUTION

P.H. contributed to the entirety of this manuscript.

ACKNOWLEDGEMENTS

P.H. would like to thank L. Smith MD of the European Society of Medicine and the Society for Immunotherapy of Cancer for their exceptional support and guidance in the writing of this manuscript.

REFERENCES

1. Qin P, Li Q, Liu S, Ning N, Zhang X, Xu Y, Chang AE, Wicha MS. Targeting cancer stem cells using immunologic approaches. *Stem Cells*. 2015 July ; 33(7): 2085–2092.
2. López-Lázaro M. The Stem Cell Division Theory of Cancer. *Crit Rev Oncol Hematol* (2018) 123:95–113.
3. Lathia J, Liu H, Matei D. The Clinical Impact of Cancer Stem Cells. *Oncologist*. (2020) 25(2):123–31.
4. Malta TM, Sokolov A, Gentles AJ, Burzykowski T et al. Machine learning identifies stemness features associated with oncogenic differentiation. 2018; Cell 173, 338-354.
5. Li N, Li Y, Zheng P, Zhan Z. Cancer Stemness-Based Prognostic Immune-Related Gene Signatures in Lung Adenocarcinoma and Lung Squamous Cell Carcinoma. *Front. Endo*. 2021.
6. Mao D, Zhou Z, Song S, Li D, He Y, Wei Z, Zhang C. Identification of Stemness Characteristics Associated With the Immune Microenvironment and Prognosis in Gastric Cancer. *Front Onc* 2021.
7. Xu Q, Xu H, Chen S and Huang W. Immunological Value of Prognostic Signature Based on Cancer Stem Cell Characteristics in Hepatocellular Carcinoma. *Front Cell Dev Biol*. 2021;9:7102-7.
8. Wang Z, Wang Y, Yang T, Xing H, et al. Machine learning revealed stemness features and a novel stemness-based classification with appealing implications in discriminating the prognosis, immunotherapy and temozolomide responses of 906 glioblastoma patients. *Brief Bion* 22(5) 2021; 1-20.
9. Shi X, Liu Y, Cheng, S, Hu H, Zhang J, Wei M, Lin Zhao L, Xin S. Cancer Stemness Associate With Prognosis and the Efficacy of Immunotherapy in Adrenocortical Carcinoma. *Front Onc*. 2021,
10. Santoro, S. Clinical Phenotype and Management Data in Down Syndrome Regression Disorder. American College of Medical Genetics and Genomics Annual Meeting 2022.
11. Heng WS, Gosens R, Kruyt FAE. Lung Cancer Stem Cells: Origin, Features, Maintenance Mechanisms and Therapeutic Targeting. *Biochem Pharmacol*. (2019) 160:121–33.
12. Yang YC, Wang SW, Hung HY, Chang CC, Wu IC, Huang YL, et al. Isolation and characterization of human gastric cell lines with stem cell phenotypes. *J Gastroenterol Hepatol* ((2007) 22:1460–8.
13. Chen XL, Chen XZ, Wang YG, He D, Lu ZH, Liu K, et al. Clinical significance of putative markers of cancer stem cells in gastric cancer: A retrospective cohort study. *Oncotarget* (2016) 7:62049–69.
14. Pietrantonio F, Miceli R, Raimondi A, Kim YW, Kang WK, Langley RE, et al. Individual Patient Data Meta-Analysis of the Value of Microsatellite Instability As a Biomarker in Gastric Cancer. *J Clin Oncol* (2019) 37:3392–400.
15. Nishino M, Ramaiya N, Hatabu H, and Hodi R. (2017). Monitoring immune checkpoint blockade response evaluation and biomarker development. *Nat Rev Clin Oncol* 14, 655-668.
16. El-Khoueiry A, Sangro B, Yau T and Crocenzi T. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase ½ dose escalation and expansion trial. *Lanc*. 2017;389:2492-2502.